Development and Validation of a Multiplex Bead Assay and Seroprevalence of IgG Antibodies against 13 Pneumococcal Serotypes in United Kingdom Children

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Declaration

With the exception of any statements to the contrary, all data presented in this thesis are the results of my own efforts. No part of this thesis has been plagiarised from other sources. In addition, no portion of the work included in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

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List of Abbreviations

%	percent
% CV	% coefficient of variation
% Rec	percentage recovery
µg	microgram(s)
µL	microlitre(s)
µm	micrometre(s)
ads+22F	adsorbent buffer containing Pn22F Ps
bead(s)	fluorescent polystyrene microspheres
CD4+ T_H17	CD4+ Interleukin 17A producing T cells
CI	confidence interval
C-Ps	pneumococcal cell wall polysaccharide
DoH	Department of Health
ECL	electrochemiluminescence
EDC	1-ethyl-3-(-3-dimethylaminopropyl)-carbodiimide hydrochloride
ELISA	enzyme linked immunosorbent assay
EMA	European Medicines Agency
FDA	Food and Drug Administration
FI	fluorescent intensity
GMC	geometric mean concentration
hr(s)	hour(s)
Hi	*Haemophilus influenzae*
IgG	immunoglobulin G
IgG-	immunoglobulin G deficient
LLQ	lower limit of quantitation
LOD	limit of detection

M	Molar
MFI	median fluorescent intensity
mg	milligram(s)
min(s)	minute(s)
mL	millilitre(s)
NHS	N-hydroxysulphosuccinimide
PBS	phosphate buffered saline
PCV13	13-valent pneumococcal conjugate vaccine
PCV7	7-valent pneumococcal conjugate vaccine
PHE	Public Health England
PLL	poly-l-lysine
Pn	*Streptococcus pneumoniae* capsular serotype
Pneumococcus/Pneumococcal	*Streptococcus pneumoniae*
PPV23	23-valent pneumococcal polysaccharide vaccine
PS	polysaccharide
Psp	pneumococcal surface protein
QC	quality control
rpm	revolutions per minute
RT	room temperature
SEU	Sero-Epidemiology Unit
UK	United Kingdom
USA	United States of America
v/v	volume/volume
VEU	Vaccine Evaluation Unit
w/v	weight/volume
WHO	World Health Organization

xg	gravitational force

Abstract

Streptococcus pneumoniae causes significant global morbidity and mortality and is responsible for many cases of septicaemia, meningitis and pneumonia. Protective antibodies against capsular polysaccharides (> 90 serotypes) can be acquired naturally or induced by vaccination.

In April 2010, a 7-valent conjugate vaccine (PCV7; serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) was replaced by a 13-valent conjugate vaccine (PCV13; PCV7 serotypes plus 1, 3, 5, 6A, 7F and 19A) in the United Kingdom immunisation schedule. Response to vaccine can be evaluated by determining serotype-specific immunoglobulin G (IgG) antibody concentrations. The Vaccine Evaluation Unit of Public Health England currently use a multiplex fluorescent bead based assay to determine IgG concentrations for serotypes in PCV13 (excluding serotype 6A).

In this thesis, this assay has been developed to include serotype 6A and successfully validated in terms of specificity, accuracy, reproducibility, linearity and stability. A seroprevalence study was conducted to test the assay, using sera from children aged < 4 years old in 2009 and 2012 (pre and post PCV13 introduction, respectively). Results showed no differences in antibody concentrations against serotypes present in both vaccines between 2009 and 2012. Additional serotypes in PCV13 had significantly higher geometric mean concentrations of IgG in 2012 compared to 2009, except for serotype 3, and an increase in the proportion of individuals with a protective antibody concentration ≥ 0.35 μg/mL.

The 13-plex assay is a useful tool in assessing the effectiveness of PCV13.

1. Introduction

The World Health Organization (WHO) describes *Streptococcus pneumoniae* (*S. pneumoniae*; pneumococcus) as a significant cause of morbidity and mortality worldwide, accounting for many cases of bacterial meningitis, especially in young children and infants (WHO, 2011). Vaccines against a limited number of serotypes of *S. pneumoniae* have reduced the threat caused by this bacterium, but not eliminated it (Musher, 2013). Higher valency conjugate vaccines are therefore required to further protect against disease caused by *S. pneumoniae*. Laboratory methods for the evaluation of vaccines against *S. pneumoniae* include enzyme linked immunosorbent assays (ELISA), opsonophagocytic killing assays (OPA), fluorescent polystyrene microsphere (bead) based immunoassays and electrochemiluminescent (ECL) immunoassays (Romero-Steiner *et al.*, 1997; Pickering *et al.*, 2002; Wernette *et al.*, 2003; Lal *et al.*, 2005; Marchese *et al.*, 2009). The aim of this project was to adapt the multiplexed microsphere assay used in the Vaccine Evaluation Unit (VEU) of Public Health England (PHE) by optimising and adding a 13th antigen, to validate the assay and to undertake a seroprevalence study for all 13 antigens in children from the United Kingdom (UK).

1.1. *Streptococcus pneumoniae*

1.1.1. Disease

S. pneumoniae is implicated in a range of diseases from relatively innocuous conditions such as conjunctivitis, through more painful illnesses such as *otitis media* and sinusitis to serious diseases such septicaemia, meningitis and pneumonia (Carvalho *et al.*, 2003; Dowson, 2004; WHO, 2008).

S. pneumoniae is the most common cause of bacterial pneumonia and it is estimated that pneumonia accounts for 18% of all deaths worldwide in those under 5 years old (Andrews *et al.*, 2003; WHO, 2010).

S. pneumoniae are also implicated in a number of diseases that are grouped under the heading 'invasive pneumococcal disease' (IPD) which is defined by the WHO as "any condition in which *Streptococcus pneumoniae* is present in blood, cerebrospinal fluid or another normally sterile body site," (WHO, 2008). Examples of IPD thus include meningitis and septicaemia.

The early symptoms of meningitis include stiff neck, fever, disorientation, and sensitivity to light and patients may go on to suffer shock, seizures and coma (Rehm *et al.*, 2009; de Jonge *et al.*, 2010). It has been estimated that case fatality rates for pneumococcal meningitis in the United States of America (USA) are around 12.5 % (Tsai *et al.*, 2008).

In addition to the symptoms of meningitis, patients with septicaemia may also have joint pain and case fatality rates for septicaemia are estimated at 15%-20% in adults, or higher in the elderly, despite appropriate treatment (WHO, 2008).

The most common method for diagnosis of *S. pneumoniae* is by growth in culture media, although this is easiest in cases of IPD where bacteria can be isolated from normally sterile sites (WHO, 2008). It has also been suggested that agglutination, antigen detection and PCR methods are useful tools for case confirmation where culture methods have not been effective (Smith *et al.*, 2009).

Treatment for pneumococcal disease is normally in the form of a broad spectrum antibiotic, followed by more targeted antibiotic(s) once any susceptibility has been determined during typing. *S. pneumoniae* is,

however, known to be increasingly resistant to a number of antibiotics, including β-lactams (such as penicillin and cephalosporins) and macrolides (such as erythromycin and clarithromycin).

Whilst most patients suffering from community acquired pneumonia can expect to make a full recovery after 6 months many patients with IPD have long term sequelae as a result of invasive infection by pneumococcus (El Moussaoui *et al.*, 2006; de Jonge *et al.*, 2010).

1.1.2. The Organism

S. pneumoniae is an encapsulated Gram-positive diplococcus and common commensal of the human upper respiratory tract (Obaro & Adegbola, 2002; WHO, 2008). Its surface structure briefly consists of a plasma membrane, cell wall and capsule; some, but not all, pneumococci also express a pilus.

A schematic representation of the structure of pneumococci is shown in Figure 1.1. The cell wall is highly conserved amongst all pneumococci, predominantly composed of teichoic acid and lipoteichoic acid; these are also known as cell wall polysaccharide, C-polysaccharide or C-Ps (Fischer, 2000).

In contrast, the pneumococcal capsule is highly variable, consisting of repeating units of a variety of sugars, which allows for serotyping of pneumococci according to which capsular polysaccharide is expressed. With the recent discoveries of serotypes 9D and 11E there are currently 94 serotypes of pneumococci described (Yother, 2004; Bratcher *et al.*, 2010; Calix & Nahm, 2010).

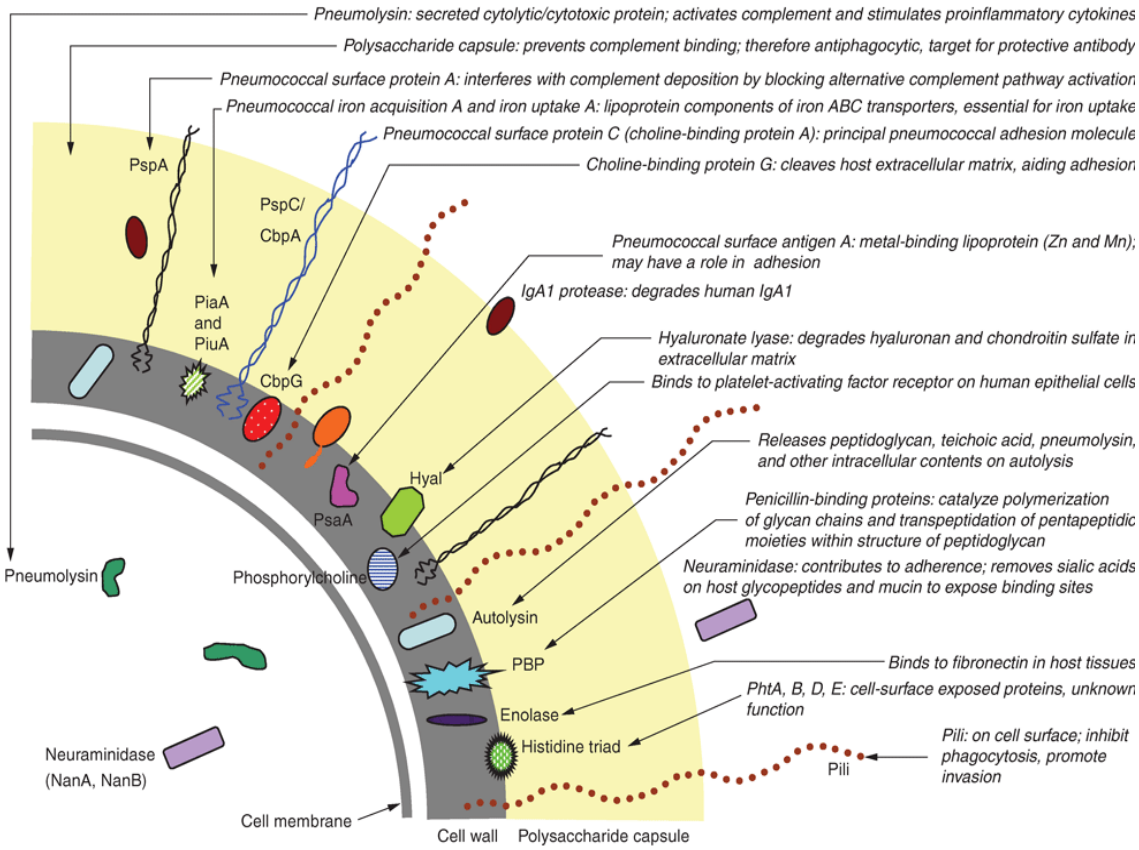
1.1.3. Carriage, Virulence Factors and Pathogenesis

S. pneumoniae is usually found within the nasopharynx of the human host, with colonisation occurring after host contact with droplet secretions

expelled during breathing, coughing or sneezing; pneumococci in the nasopharynx elicit no inflammatory immune response in the host and may persist for a number of weeks (asymptomatic carriage) before being cleared by the host's immune response (Tuomanen, 2000; O'Brien & Nohynek, 2003). Carriage rates among children are high, with one in three children carrying at least one serotype of pneumococcus (Tuomanen, 2000).

Acquisition of a new serotype, rather than persistence of carriage of a serotype, is associated with onset of pneumococcal disease, both in children and adults (Gray *et al.*, 1980; Musher, 1992).

Figure 1.1: *S. pneumonia* surface components



This figure illustrates the pneumococcal cell membrane, wall and capsule and includes various virulence factors; image reproduced from Longo *et al.* (2011).

The capsule is essential to the organism's ability to colonise the host, probably by protecting the pneumococcus on entry to the nasopharynx by reducing agglutination of the bacteria and clearance by mucus, allowing the organism to survive long enough to adhere (Magee & Yother, 2001; Nelson *et al.*, 2007). Pneumococci can regulate the amount of capsule produced to adapt to different conditions, reducing the capsule in order to facilitate adherence to epithelial cells (Weiser *et al.*, 1994; Cundell, Weiser, *et al.*, 1995; Kim & Weiser, 1998; Fischer, 2000; Hammerschmidt *et al.*, 2005).

Once colonisation has occured a number of attributes contribute to the ability of the pneumococcus to convert from a commensal to a pathogen, many of these virulence factors are shown in Figure 1.1. Absence of virulence factors may prevent the organism from becoming pathogenic, as in the case of the capsule, or reduce the pathogenicity of the organism, as in the case of the pilus (Magee & Yother, 2001; Aguiar *et al.*, 2008). The virulence factors of pneumococci have a number of roles, from facilitating adherence and internalisation to protecting the bacterium from the host defences (Cundell, Gerard, *et al.*, 1995; Schneider *et al.*, 1999; Yoshimura *et al.*, 1999; Braun *et al.*, 2002; Hoffmann *et al.*, 2007; McCullers *et al.*, 2008; Orihuela *et al.*, 2009; Thornton *et al.*, 2010).

1.1.4. Immunology

Upon invasion of the host by *S. pneumoniae* the primary immune response is mediated by the complement cascade (Winkelstein, 1981). Both the classical and alternative complement pathways, but not the mannose-binding lectin (MBL) pathway, are involved in this response although it may be that the alternative pathway is more important (Winkelstein, 1981; Brown *et al.*, 2002; Jarva *et al.*, 2003).

Evidence showing that pneumococcal serotypes differ in their ability to protect against complement mediated phagocytosis suggests that an important role of the capsule may be in masking, reducing or inhibiting the deposition of complement on the bacterial surface (Winkelstein, 1981; Melin *et al.*, 2010; Hyams *et al.*, 2010). It has been demonstrated that the capsule of *S. pneumoniae* inhibits the deposition of C3b/iC3b from both the classical and alternative pathways through a number of mechanisms and that other pneumococcal structures, including pneumococcal surface protein (Psp) A, Psp C binding of Factor H and C-Ps recruitment of C-reactive protein, are also involved in defence against complement opsonisation (Jarva *et al.*, 2003; Hyams *et al.*, 2010).

It is known that antibodies produced against pneumococcal capsular polysaccharides during infection or after vaccination are able to protect against reoccurrence of diseases caused by the bacterium (Austrian, 1977; Black *et al.*, 2000; Siber *et al.*, 2007). Similarly, it has been shown that antibodies against capsular polysaccharides, and other pneumococcal virulence factors, are induced as a result of carriage of the organism in the nasopharynx (Goldblatt *et al.*, 2005; Weinberger *et al.*, 2008). This has led to the assumption that the primary mechanism of acquired immunity against pneumococci is the generation of anti-capsular antibodies (Malley, 2010). However, there is some evidence that an acquired immunity begins to cause a decrease in the incidence of carriage and diseases caused by pneumococci prior to there being a detectable anti-capsular antibody presence in vaccine naive children and so there remain some questions as to the mechanisms of immunological protection (Lipsitch *et al.*, 2005). Work to determine the mechanism of this acquired immunity has led to the

hypothesis that CD4+ Interleukin 17A producing T (CD4+ T_H17) cells that recognise pneumococcal antigens and recruit phagocytes are implicated in the reduction of acquisition of carriage and therefore disease. As a result it has been proposed that acquired immunity to the pneumococcus has two components, the earlier CD4+ T_H17 cell component followed by the anti-capsular antibody component (Malley, 2010).

All currently licensed vaccines against pneumococci aim to elicit an immune response against capsular polysaccharides. Polysaccharides are T-cell independent antigens, and the immune response to them is both complement and B-cell dependent (Breukels *et al.*, 2005). The CD21 receptor on B cells appears to be key in this immune response and the CD21 receptor is found in greater quantity on B cells in the splenic marginal zone but is not expressed as highly in neonates and infants which may explain the observation that these patients do not respond well to plain polysaccharide vaccines (Breukels *et al.*, 2005; Timens *et al.*, 1989).

Polysaccharide vaccines have also been associated with hypo-responsiveness, an attenuated response by the patient to repeated doses of vaccine especially when the doses are given within a short space of time, in older age groups (Jackson & Janoff, 2008; Poolman & Borrow, 2011).

While both young children and the elderly are at increased risk of pneumococcal disease, no vaccine was available prior to 2000 that was effective in the younger age group (especially those under 2 years of age) (WHO, 2011). After the success in infants of other conjugate vaccines, such as the *Haemophilus influenzae* (Hi) type B and meningococcal serogroup C conjugate vaccines, efforts were made to formulate a pneumococcal

conjugate vaccine (Peeters *et al.*, 1991; Vella *et al.*, 1992; Kelly *et al.*, 2004; Campbell *et al.*, 2009).

In contrast to plain polysaccharide vaccines, conjugate vaccines induce an immune response in infants as they elicit both a B and T cell response. The conjugation of the polysaccharide to the protein carrier allows antigen presenting B cells to recruit CD4+ T helper cells, inducing both immunogenicity in infants and immunological memory (Poolman & Borrow, 2011).

Interestingly, the issue of hypo-responsiveness has not been completely resolved by the use of conjugate pneumococcal vaccines. There is increasing evidence that the administration of vaccine while the recipient is carrying a vaccine serotype leads to a blunted immune response to the carried serotype, although not to the non-carried types (Dagan *et al.*, 2010; Vakevainen *et al.*, 2010). This phenomenon puts patients at risk of contracting IPD despite receiving a full course, or even additional doses, of vaccine (Borrow *et al.*, 2008; Stanford *et al.*, 2012).

The mechanism for this blunted response is hypothesised to be most likely associated with B-cell fatigue, where the circulating capsular polysaccharide from the carried serotype causes a reduction in responsiveness by the B-cell pool to the normally immunogenic conjugate vaccine (Dagan *et al.*, 2010).

1.1.5. Pneumococcal Vaccines

A 14-valent polysaccharide vaccine was licensed in the 1970s after effectiveness data showed that polysaccharide vaccines protected South African miners from pneumococcal diseases (Smit *et al.*, 1977; Hilleman *et al.*, 1978). This vaccine was replaced by a 23-valent polysaccharide vaccine

(PPV23) which has been available for use since 1983 (Robbins *et al.*, 1983). The formulation of PPV23 contains pneumococcal capsular serotypes (Pn) 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F (Merck and Co., 2011).

As PPV23 is ineffective in infants under 2 years a 7-valent conjugate vaccine (PCV7) containing Pn 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to CRM_{197}, a non-toxic variant of the diphtheria toxin, was developed and licensed in 2000 in the United States of America and subsequently in many other countries worldwide (ACIP, 2000; Pfizer, 2009).

Licensure of PCV7 was based on efficacy data generated in vaccine naive populations (Black *et al.*, 2000; ACIP, 2000). As it would not be ethical, or even possible, to perform similar studies with newer formulations of vaccine recommendations were made to facilitate licensure of new vaccines based on non-inferiority to the current vaccines as demonstrated by IgG concentration measured by ELISA, with a correlate of protection suggested of ≥ 0.35 µg/mL; as a secondary, supporting, end-point the recommendations were that functional antibody should be determined by OPA and that immunological memory should be demonstrated (WHO, 2005).

These recommendations were used to inform the licensure of conjugate vaccines which protect against increased numbers of serotypes. A 10-valent (PCV10) and 13-valent (PCV13) conjugate vaccine have both been licensed (Hausdorff *et al.*, 2009; ACIP, 2010).

The formulation of PCV10 contains Pn 1, 4, 5, 6B, 7F, 9V, 14, 19F and 23F conjugated to Protein D, a protein derived from non-typeable Hi, Pn 18C

conjugated to Tetanus Toxoid and Pn 19F conjugated to Diphtheria Toxoid (GlaxoSmithKline, 2011).

PCV13 contains Pn 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM_{197} (Pfizer, 2011).

Following licensure of these vaccines the UK childhood vaccine schedule was amended in April 2010 so that PCV13 was used instead of PCV7 (PHE, 2013c)

1.1.6. Incidence and Epidemiology

Whilst the epidemiology of IPD varies according to geography, age and by disease manifestation, it is well known that the very young (less than 5 years and especially less than 2 years of age) and the elderly (over 65 years of age) are most at risk of serious IPD, and this was certainly the case prior to the introduction of PCV7 in England and Wales (Kaye *et al.*, 2009). It has been estimated that serious pneumococcal disease accounts for 11% of all deaths globally in children less than 5 years old (O'Brien *et al.*, 2009).

A review reported that PCV7 has had a significant impact on the recent epidemiology of pneumococcal serotypes and disease; this effect has been twofold, firstly in the target patient group (those less than two years old) where there has been a highly significant reduction in disease caused by vaccine serotypes, and secondly with an originally unanticipated reduction in disease caused by vaccine serotypes across all age groups. The data demonstrating this in the studies cited in the review disagree on the effects of PCV7 on the older age groups (Weinberger *et al.*, 2011). Data from England and Wales, and the USA have shown evidence of this effect (ACIP, 2005; Kaye *et al.*, 2009; Miller *et al.*, 2011a).

This reduction in disease caused by vaccine serotypes outside the vaccinated population is due to ‘herd protection’; a phenomenon whereby vaccination of the target group prevents carriage of the vaccine serotypes and so the serotypes are prevented from spreading from the target group to other age groups, providing an indirect protection. While this herd protection is mostly a positive benefit of vaccination there are concerns about serotype replacement, where serotypes that previously caused little disease replace the eliminated serotypes, causing disease which cannot be prevented by the vaccine in use. There is evidence to show that this phenomenon has occurred in the UK, Europe and the USA with Pn 7F, 19A and 22F all observed as potential replacement serotypes (Kaye *et al.*, 2009; Hanage *et al.*, 2010; Miller *et al.*, 2011a; Weinberger *et al.*, 2011).

The licensure of PCV10 and PCV13 is expected to further reduce the incidence of IPD and will reduce the concerns raised by serotype replacement as some replacement serotypes are contained within these new formulations (GlaxoSmithKline, 2011; ACIP, 2010).

Data available from the UK shows that introduction of PCV13 has reduced the incidence of disease from the additional 6 serotypes significantly in both vaccinees and in older children and adults (PHE, 2013b).

Changes to the intranasal flora of children affected by otitis media have been attributed the introduction of PCV7 and it has been shown that the main cause of otitis media has shifted from pneumococci to Hi since the introduction of the vaccine (Brunton, 2006). The inclusion of a component to induce immunity to non-typeable Hi in a 10-valent pneumococcal conjugate vaccine is anticipated to further reduce the incidence of otitis media (Schuerman *et al.*, 2009).

1.2. Pneumococcal Assays

A number of assays have been developed to assist in the evaluation of vaccines against pneumococci. These can assay for functional antibody (OPA) or for concentration of an immunoglobulin class (ELISA, bead based and electrochemiluminescence assays)

1.2.1. Detection of functional antibodies

Different forms of opsonophagocytic assays (OPA), including viability/killing, up-take and multiplexed assays, have been described (Romero-Steiner *et al.*, 1997; Martinez *et al.*, 1999; Nahm *et al.*, 2000; Martinez *et al.*, 2006).

A workshop on OPA standardisation in 2007 came to the consensus that the preferred 'gold standard' OPA should be a viability assay and the importance of OPA as a correlate of protection in vaccine evaluation was later highlighted, although a protective threshold is not yet identified due to standardisation issues (WHO, 2007; WHO, 2009).

1.2.2. Determination of immunoglobulin concentration

1.2.2.1. ELISA

As early as 1978 an ELISA for detection of anti-capsular antibodies was described, although it was later observed that antigen preparations used for serotype-specific ELISA were contaminated with C-Ps leading to false positives (Berntsson *et al.*, 1978; Koskela, 1987).

A method was later described which incorporated a C-Ps adsorption step to remove this nonspecific binding (Quataert *et al.*, 1995). Efforts were made to standardise this methodology to allow data generated from multiple sites to be used in the licensure of vaccines. To facilitate this, a standard reference serum was produced, 89-SF, and weight based antibody concentrations determined, a panel of 12 sera identified to be used to

calibrate the assay between laboratories and 2 reference laboratories set up (Quataert *et al.*, 1995; Wernette *et al.*, 2003; Quataert *et al.*, 2004). A set of guidelines and recommendations has been suggested so that laboratories can demonstrate that their ELISA matches the operating characteristics of the laboratories used in a multicentre study (Plikaytis *et al.*, 2000).

Another modification of the method was subsequently made to further enhance the specificity of the ELISA by adding a different pneumococcal capsular polysaccharide, different methods for providing this extra adsorption have been developed using either serotype 22F polysaccharide, serotypes 25 and 72 polysaccharides or an extra C-Ps purified from 22F (Concepcion & Frasch, 2001; Marchese *et al.*, 2006; Skovsted *et al.*, 2007). The current WHO accepted protocol uses 22F as the extra adsorption component (WHO, 2000).

In brief the WHO ELISA is performed using pneumococcal capsular polysaccharide bound to the wells, serially diluted 89-SF as the standard curve (pre-adsorbed with C-Ps alone), QC and test sera (pre-adsorbed with C-Ps and Pn22F Ps) are added to the wells, followed by an enzyme linked anti-human antibody, and finally a substrate. Colour development is measured in a spectrophotometer with an increased colour corresponding to a greater anti-capsular antibody present in the sample (WHO, 2000).

Currently, the ELISA is the primary correlate of protection used in vaccine evaluation (WHO, 2009), with a threshold of ≥ 0.20 to ≥ 0.35 µg/mL used as the threshold concentration demonstrating protection.

Concerns that have been raised about the ELISA are that it is time consuming, laborious and utilises a large serum volume (Pickering *et al.*, 2002; Lal *et al.*, 2005).

Due to depletion of stocks of the 89-SF reference standard efforts were made to develop and characterise an alternative, resulting in the publication of data validating a new reference serum, 007sp, for use in the pneumococcal ELISA with adsorption using both C-Ps and 22F polysaccharide (Goldblatt, Plikaytis, *et al.*, 2011).

1.2.2.2. Bead based assays

As an alternative to the ELISA a multiplexed bead based assay was described allowing multiple (14) serotypes to be assayed in the same well simultaneously by utilising beads, instead of the plastic plate surface, as the base on which to conjugate antigen (Pickering *et al.*, 2002)..

A fluorescent dye is used as the reporter which allows an adapted flow cytometer (Luminex or Bio-Plex) to quantify the amount of antibody bound to the beads by comparing to a reference standard (Pickering *et al.*, 2002).

A similar 9-plex bead assay used in the Vaccine Evaluation Unit (VEU) of Public Health England (PHE) has previously been published (Lal *et al.*, 2005) and comprises capsular polysaccharides from Pn 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F coupled to fluorescent microspheres. Since publication the assay has been expanded to include capsular polysaccharides from serotypes 3, 7F and 19A to make a total of 12 serotypes (Yaro *et al.*, 2013).

1.2.2.3. ECL assay

In 2009 a new mulitiplex method for detecting antibodies against pneumococcal serotypes was described which uses ECL as the detection method (Marchese *et al.*, 2009). ECL uses a chemical reaction that is

initiated by an electrical current to generate light (Meso Scale Discovery, 2012). Antigen is attached to specific spots within a well of a plate, with up to 10 spots per well. When reporter is added and electrical current passed through the plate the magnitude of light generated, relative to the reference standard, is used to quantify the antibody concentration in the sample (Marchese *et al.*, 2009).

This methodology has been compared to the WHO ELISA with the conclusion that although the new methodology produced higher results in some cases it was generally in agreement with the WHO ELISA (Goldblatt, Ashton, *et al.*, 2011).

1.3. Aims

This thesis aims to demonstrate the adaptation of the PHE multiplex bead assay by optimising Pn6A PS for inclusion in the assay as the 13th serotype included in PCV13. Validation parameters including specificity, accuracy, reproducibility, linearity and stability will be tested to show the assay is fit for use. Finally, a seroprevalence study on 1.00 to 3.99 year old infants from the UK in 2009 and 2012 will be performed for al 13 serotypes in to determine the effect of introduction of PCV13 in 2010 on antibody levels in the target group.

2. Methods

2.1. Conjugation of pneumococcal polysaccharides to poly-l-lysine

Pn 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F polysaccharides (PS) (LGC Standards, Middlesex, UK) were covalently linked to poly-l-lysine (PLL) as described by Lal *et al.*, with minor modifications (Lal *et al.*, 2005).

Reconstituted PS was added to alkalising buffer (0.01 % weight/volume [w/v] sodium hydroxide [Sigma-Aldrich Co. LLC, Gillingham, UK] containing 0.001 % w/v phenolphthalein [Sigma]) as summarised in Table 2.1. This was added to 12.5 milligrams (mg) cyanuric chloride (Sigma) and vortex mixed until transparent, followed by the addition of 50 microlitres (µL) of 250 µg/mL PLL (Sigma), vortex mixing and incubation at 2 to 8 ºC for 16 to 20 hours (hrs).

A PD-10 Sephadex column (GE Healthcare, Buckinghamshire, UK) was primed with 25 millilitres (mL) of filter sterilised, using a 0.2 micrometre (µm) filter (Nalgene, Rochester, USA), phosphate buffered saline (PBS; 0.14 Molar [M] sodium chloride, 0.021 M monobasic potassium phosphate, 0.013 M dibasic sodium phosphate, 0.0021 M potassium chloride pH 7.3 [all Sigma]).

Following this, PS/PLL was passed through the column and eluted using 3.5 mL of PBS. The eluent was collected and aliquots prepared which were stored at -20 ºC (-15 to -30 ºC) until needed.

2.2. Conjugation of *PS/PLL* to beads

Unconjugated carboxylated beads (500 µL) $1.25x10^7$ beads/mL (Luminex Corporation, Austin, Texas, USA) were washed by centrifugation at 16,060 gravitational force (xg) for 2 minutes (mins), supernatant removed and

beads re-suspended by vortex mixing in 200 µL PBS. This procedure was repeated. Beads were then centrifuged as above, supernatant removed and beads re-suspended by vortex mixing in a final volume of 500 µL of bead activation buffer containing 5 µg/mL 1-ethyl-3-(-3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC; Pierce Protein Research Products, Perbio Science UK Ltd., Northumberland, UK) and 5 µg/mL N-hydroxysulphosuccinimide (NHS; Perbio Science UK Ltd.) and incubated on an orbital shaker (Polymax 1040; Heidolph UK, Essex, UK) at 50 revolutions per minute (rpm) for 20 mins in the dark.

Beads were then washed by centrifugation as previously, but re-suspended in a final volume of 100 µL of *PS/PLL* and incubated in the dark for 1.25 to 3 hrs depending on the pneumococcal serotype being conjugated (Table 2.1) at room temperature (RT). A different 'region' of beads was used for each different serotype.

Following this incubation beads were washed by centrifugation as previously, but after the final supernatant was removed the beads were re-suspended in 200 µL PBS and centrifuged at 16060 xg for 30 seconds (s) counted from the point the centrifuge reached the required xg), the supernatant was removed and the beads re-suspended in 1 mL of bead storage buffer (PBS containing 0.1% w/v bovine serum albumin and 0.05% w/v sodium azide [both Sigma], filter sterilised [0.22 µm filter; milipore]), vortex mixed and enumerated using a FastRead counting chamber (Immune Systems Ltd., Devon, UK) under a Labophot 2 upright microscope (Nikon UK Ltd., Surrey, UK) at 10x magnification.

2.3. A multiplexed bead based assay for the detection of IgG to pneumococcal serotypes.

2.3.1. Preparation of the standard reference serum, lot 89-SF, quality control (QC) and sample sera for use in the multiplex assay

The standard serum used in the assay was the pneumococcal reference serum, lot 89-SF (89-SF; Food and Drug Administration, Maryland, USA). A 7 point serially diluted standard curve was prepared (starting dilution 1/20, dilution factor 1/4). Serum was diluted in adsorbent buffer (PBS containing 0.05 % volume/volume [v/v] Tween-20 [Sigma], 1 % v/v newborn bovine serum [NBBS; Labtech International Ltd, East Sussex, UK] and 4 µg/mL C-Ps [Statens Serum Institut, Copenhagen, Denmark]) to give final dilutions at 1/80, 1/320, 1/1280, 1/5120, 1/20480 and 1/81920.

Table 2.1: Conditions for bead preparation

Serotype of pneumococcal capsular polysaccharide	Concentration of reconstituted PS (ug/mL)	Volume of reconstituted PS (mL)	Volume of alkalising buffer (mL)	Incubation time of beads with *PS/PLL* (mins)
1	2	1.75	1.25	90
3	3	1.25	1.25	90
4	2	1.25	1.25	90
5	2	1.25	1.25	90
6B	2	1.5	1.25	180
7F	2	1.25	1.25	90
9V	2	1.25	1.25	90
14	2	1.25	1.25	75
18C	2	1.5	1.25	120
19A	2	1.25	1.25	90
19F	2	1.75	1.25	180
23F	2	1.25	1.25	180

This table shows the concentration and volume of reconstituted Pn PS used in *PS/PLL* and the incubation times of *PS/PLL* with beads.

Three QC sera were used QCHigh, at a final dilution of 1/1000, and QCMed (both Pfizer Ltd., Kent, UK) and IgG deficient serum (IgG-; Sigma), at a final dilution 1/100. QC sera were diluted in adsorbent buffer containing 5 µg/mL Pn 22F PS (ads+22F).

Test serum samples were also diluted in ads+22F, at 1/100; if necessary higher dilutions were prepared at 1/500, 1/1000, 1/5000 or 1/10000.

2.3.2. Assay procedure

2.3.2.1. Dilution of beads prior to use in the assay.

Stock beads for each serotype required were sonicated for 30 s, vortex mixed for 30 s and then the volume containing 5000 beads per well for each serotype was pooled together to create a 'bead cocktail' of all the required serotypes. The bead cocktail was centrifuged at 16060 xg for 2 mins, supernatant removed and beads re-suspended in 200 µL PBS, followed by brief vortex mixing to re-suspend the beads. Centrifugation was repeated as above and supernatant removed after which the beads were re-suspended in the total volume of PBS required.

2.3.2.2. Addition of sample to filter plates and addition of beads to the sample

A 96 well MultiScreen HTS filter plate (Millipore UK Ltd., Watford, UK) was prepared by adding 50 µL per well of PBS containing 0.05% v/v Tween-20 (PBS-T) followed by gentle vacuum filtration (pressure ≤ 5 mm/Hg) using a MultiScreen HTS Vacuum Manifold (Millipore).

Adsorbent buffer (used as a blank), 89-SF dilutions, QC sera and test samples were prepared as previously described (section 2.3.1) and assayed in duplicate by adding 25 µL per well. An example plate layout is given in

. Following this, 25 µL of bead cocktail was added to each well. The plate was then incubated on a plate shaker at 500 rpm in the dark for 20 mins.

2.3.2.3. Filter Plate Wash

Plate washing was as follows: Liquid was removed from wells by vacuum filtration as described previously. Well contents were re-suspended in 100 µL PBS-T. Vacuum filtration and re-suspension were repeated followed by a final vacuum filtration.

2.3.2.4. Addition of R-Phycoerythrin conjugated anti-human goat Immunoglobulin G (conjugate)

Conjugate (Stratech Scientific Ltd., Suffolk, UK) was diluted 1/200 with PBS and 100 µL added to each well. The plate was incubated on a plate shaker at 500 rpm in the dark for 20 mins.

Figure 2.1: Example plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	89-SF 1:1280	QCHigh	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22	Sample 26	Sample 30	Sample 34
B	Blank	89-SF 1:1280	QCHigh	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22	Sample 26	Sample 30	Sample 34
C	89-SF 1:81920	89-SF 1:320	QCMed	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19	Sample 23	Sample 27	Sample 31	Sample 35
D	89-SF 1:81920	89-SF 1:320	QCMed	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19	Sample 23	Sample 27	Sample 31	Sample 35
E	89-SF 1:20480	89-SF 1:80	IgG-	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20	Sample 24	Sample 28	Sample 32	Sample 36
F	89-SF 1:20480	89-SF 1:80	IgG-	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20	Sample 24	Sample 28	Sample 32	Sample 36
G	89-SF 1:5120	89-SF 1:20	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21	Sample 25	Sample 29	Sample 33	Sample 37

H	89-SF 1:5120	89-SF 1:20	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21	Sample 25	Sample 29	Sample 33	Sample 37

This figure shows an example of the layout of and plate for an assay run with the position of duplicate wells for the blank, 89-S, QC and teast sample dilutions.

2.3.2.5. Re-suspension of beads for acquisition

Following this the plate was washed as previously described (section 2.3.2.3). The contents of the wells were re-suspended in 125 µL PBS-T and incubated in the dark as above for a minimum of 5 mins.

2.3.2.6. Data Acquisition

The plate was read using a Bio-Plex 100 or 200 plate reader (Bio-Plex; Bio-Rad Laboratories, Inc, Hertfordshire, UK) using Bio-Plex Manager version 4.1.1 software (Bio-Rad).

2.3.2.7. Data Analysis

The standard curve was generated using a four parameter logistic (4PL) by Bio-Plex Manager on the median FI (MFI) data for the duplicate standard points minus the MFI of the blank. Alternative data analysis

software were assessed, but none were found to be superior to Bio-Plex Manager 4.1.1, the currently used software.

For all serotypes except Pn14 the most dilute standard curve dilution (1/81920) were masked as standard. The limit of detection, based on the lowest available curve point, varied by serotype from 0.01 µg/mL for Pn3 to 0.09 µg/mL for Pn19A. The lower limit of quantitation (LLQ) was set at 0.1 µg/mL for all serotypes. The ½ LLQ for all serotypes, assigned to any result below the LLQ, was 0.05 µg/mL. Further blank, standard curve and QC result rejection criteria were applied as applicable (Table 2.2).

The sample results were accepted provided the %CV between duplicates was ≤ 25% and the MFI fell in the linear phase of the curve. Samples which

fell outside the linear phase of the curve were repeated at dilution factors more dilute or more concentrated as applicable (section 2.3.1). Samples at a 1/100 dilution which were below the lower limit of the curve or samples which produced a result below the LLQ were assigned the ½ LLQ value (0.05 µg/mL).

2.4. Optimisation of conditions for inclusion of Pn6A capsular polysaccharide conjugated beads in the multiplex assay

2.4.1. Optimisation of Pn6A PS used in bead preparation.

Pneumococcal serotype 6A polysaccharide lots CPS6A-2, CPS6A-5 (both SSI) and 59508355 (LGC) were reconstituted and diluted to 0.01, 0.1, 0.25, 0.5, 1, 2, 3 or 6 mg/mL and used to prepare beads as described previously (sections 2 and 2.2) with a 1.5 hr incubation of beads with *PS/PLL*.

Beads were run in monoplex with 89-SF and QC serum only as previously described (section 2.3).

Table 2.2: Assay rejection criteria.

Rejection criteria for standard curve points and QC results	Action taken on rejection
Blank value > 200 MFI	Serotype failed
Standard Curve Point %CV ≥ 30 %	Curve point masked
Standard Curve Point Obs/Exp < 80 %	Curve point masked
Standard Curve Point Obs/Exp > 120 %	Curve point masked
< 5 Standard Curve Points	Serotype failed
Either QCHigh or QCMed %CV ≤ 25 %	Serotype failed
Either QCHigh or QCMed result outside ± 2 SD of historical data	Serotype failed

IgG- > LLQ	Serotype failed

This table shows the rejection criteria applied to assays during data analysis and the action taken on rejection.

For CPS6AM-1 bead preparations (2 to 5 per concentration) were produced at 0.25, 0.5, 1, 2, 3 and 6 µg/mL PS with up to 3 replicates (Day 1, 20 to 23 or 43 post conjugation) generated per bead preparation.

For CPS6AM-5 bead preparations (1 to 3 per concentration) were produced at 0.01, 0.1, 1, 2 and 3 µg/mL PS with up to 2 replicates (Day 1, 23 or 77 post conjugation) generated per bead preparation.

For 59508355 bead preparations (1 to 3 per concentration) were produced at 0.01, 0.1, 1, 2 and 3 µg/mL PS with up to 3 replicates (Day 1, 23 or 77 post conjugation) generated per bead preparation.

2.4.1.1. Final preparation method for beads prepared using Pn6A PS

The method for producing Pn6A beads was as described previously (sections 2 and 2.2) using a 1 mg/mL concentration of Pn6A PS to prepare *PS/PLL* and a 1.5 hr incubation of beads with *PS/PLL*.

2.4.2. Assessment of specificity of beads prepared using different PS using inhibition analyses

The impact of mixing beads prepared using different serotypes was assessed by running beads for each serotype in a monoplex and a duplex with beads of each other serotype. These tests were performed using surplus volume from anonymised test samples sourced from the pneumococcal serology service provided by the laboratory. An ethical opinion was sought on 05/12/2011 from Professor Simon Mitchell, chair of the Northwest 7 Research Ethics Committee – Greater Manchester Central,

who replied to inform that formal ethical review was not required for use of these samples to validate the assay.

Three test samples with known high positive results and with sufficient volume for multiple tests was sourced and prepared at a 1/100 dilution as described previously (section 2.3). The mean percentage recovery (% Rec) for the three samples was calculated for each of the duplex combinations.

Secondly, beads of each serotype were run in monoplex with or without inhibition with PS from each of the 13 pneumococcal serotypes from the assay giving homologous and heterologous inhibition. A test sample with known high positive results and with sufficient volume for multiple tests was sourced and incubated 1/1 with water for injections (uninhibited result) or 1/1 with 100 µg/mL of pneumococcal PS for 1 hour. Each mixture was diluted to 1/100 final dilution of test sample serum in ads+22F before assaying as described previously (section 2.3.2). Two data points were generated for each combination and the mean % Rec calculated for each of the inhibitions. A further replicate from each of 11 different test samples using beads prepared with serotypes 4, 18C, 19A and 19F PS inhibited with Pn5 PS were generated and the results incorporated into the mean % Rec data.

2.4.3. Separation of the 13-plex assay into two panels

In order to limit potential cross reactivity, serotypes were separated into 2 panels as follows: Panel A contained Pn4, Pn6B, Pn9V, Pn14, Pn19A and Pn23F and panel B contained Pn1, Pn3, Pn5, Pn6A, Pn7F, Pn18C and Pn19F. The panels were run as separate plates, with no mixing of serotypes between panels (i.e. no panel A serotype to be run with any panel

B serotype), but within a panel any mixture of serotypes was permitted (e.g. serotypes could be run as monoplex or up to hexaplex in panel A).

2.4.4. Modifications to assay method

Once panels were defined, bead cocktails were prepared as previously described (section 2.3.2.1) but only using beads from within each panel (section 2.4.3). In order to test a sample for all thirteen serotypes two identical filter plates were prepared (section 2.3.2.2) and a bead cocktail (panel A or B) was added, each to a different plate and incubated as described in section 2.3.2.2 from which point the plates were treated as per section section 2.3.2.3 onwards.

2.5. Validation of the 13 plex assay

2.5.1. Sample selection

Initial investigation of 603 anonymised test samples sourced from the clinical pneumococcal serology service provided by the laboratory resulted in 74 samples being selected for use in the assay validation. Samples were tested using the assay as described previously (section 2.4.4) and compared to previous data (except for Pn6A). Samples with > 50 % CV and/or a greater than 4-fold difference in concentration between results were excluded. Investigation of a further 3818 samples found 43 potential negative samples of which only 5 had no detectable antibody for all 13 serotypes. A total of 79 samples were available for the validation.

2.5.2. Bead preparation

Except where specifically mentioned different operators (myself, Helen Flynn, Jake Mills, Sarah Frankland, Sarah Martin, Ros Warrington and

Michael Norman) were involved in preparing beads, using all 13 serotypes, for use in all parameters of the validation of the assay.

2.5.3. Inhibition

Positive and low positive samples (5 of each) were identified for each serotype and inhibited by addition of homologous and heterologous inhibitor. The heterologous inhibitors used were the other 12 Pn PS used in the assay and *Neisseria meningitidis* group A and C PS.

Aliquots of the sample were incubated 1:1 with either water for injections (without inhibition) or with a 100 µg/mL solution of pneumococcal or meningococcal PS for 60 mins at RT with vortex mixing every 15 mins. Each mixture was diluted 1/50 in ads+22F, to give a final test sample dilution of 1/100, before being assayed as described previously (section 2.4.4) using either Panel A or Panel B beads.

Results from each serotype were generated, by 1 operator, for each of the uninhibited and inhibited test samples with results required from the same assay.

The criteria for passing the inhibition analysis were that ≥ 80 % of the samples had more than 70 % Rec for the heterologous inhibitors and less than 50 % Rec for the homologous inhibitor.

2.5.4. Spiking

Five negative test samples and one positive test sample were selected for each serotype. The negative test samples were spiked with the positive sample at dilutions of 1/2, 1/4 and 1/8. The negative test sample, the three spiked dilutions and the undiluted positive test sample were prepared as

described previously (section 2.3.1) and the assay performed as described previously (section 2.4.4) with all five preparations run on the same assay.

Results from each serotype were generated for each of the positive test sample, the three dilutions and the negative test sample from the same assay.

The acceptance criteria for the spiking analysis were that for ≥ 80% of the test sample results the negative (un-spiked) sample produced a negative result and the spiked test samples produced values within ± 30 % of the positive test sample once adjusted for the dilution factor.

2.5.5. Accuracy

Accuracy is normally determined by running test samples with known concentrations to demonstrate that the assay produces the correct result. No such test samples are available for all 13 serotypes using 89-SF as the standard serum. As such the accuracy of the assay was assessed using a replicate spiking assay which was performed as described previously (section 2.5.4) except that five replicates from separate assays were produced for each serotype.

The acceptance criteria were as described previously (2.5.4) with the addition that ≥ 80 % of the results for each of the spiked test samples fell within ± 30 % of the median value for that dilution.

2.5.6. Reproducibility

For all the reproducibility parameters, except the low end reproducibility, 15 positive test samples across the whole range of the standard curve and 5 negative test samples were selected for each serotype. Where possible the same samples were used across multiple serotypes and multiple parameters.

For the low end reproducibility the criteria were for 15 samples from the bottom 20 % of the standard curve of each serotype and five negative sera for each serotype.

The results generated from negative sera were excluded from the acceptance criteria for each parameter.

2.5.6.1. Inter assay reproducibility

The test samples were prepared and assay performed described previously (section 2.4.4).

Results from each serotype were generated for each test sample with 5 results each generated from a different assay required.

The acceptance criteria were that for ≥ 80 % of the samples the % CV between the replicate results was ≤ 25 %.

2.5.6.2. Intra assay reproducibility

The test samples were prepared and assay performed as described previously (section 2.4.4).

Results from each serotype were generated for each test sample with 5 results all generated from the same assay required.

The acceptance criteria were that for ≥ 80 % of the samples the % CV between the replicate results was ≤ 25 %.

2.5.6.3. Inter operator reproducibility

The samples were prepared and assay performed as described previously (section 2.4.4).

Results from each serotype were generated, by 3 different operators (myself, Sarah Frankland and Katy Gray), for each test sample with 2 results from each operator generated from a different assay required.

The acceptance criteria were that for ≥ 80 % of the samples the % CV between the replicate results was ≤ 25 %.

2.5.6.4. Inter operator analyte reproducibility

The samples were prepared and assay performed as described previously (section 2.4.4) using 3 different bead preparations each prepared by the same operator (Helen Flynn) as described previously in sections 2.2 and 2.4.1.1.

In order to limit the variability of the assay, as much as possible, to the different beads the assay was performed across up to 6 plates (3 plates each for panel A and panel B) with the plates sourced from the same bag to reduce variability due to transport and storage. Sufficient volumes of 89-SF, QC and sample serum were prepared to plate the same preparation across the 6 plates.

Results from each serotype were generated for each test sample with 1 result from each bead preparation (3 results per serotype) required from the same assay except for beads prepared using Pn19A PS for which results were generated from 2 assays.

The acceptance criteria were that for ≥ 80 % of the samples the % CV between the replicate results was ≤ 25 %.

2.5.6.5. Intra operator analyte reproducibility

The samples were prepared and assay performed as described previously (section 2.4.4) using 4 different bead preparations each prepared by a different operator as described previously in sections 2.2, 2.4.1.1 and 2.5.2.

In order to limit the variability of the assay as much as possible to the different beads the assay was initially performed across up to eight plates (four plates each for panel A and panel B) with the plates sourced from the

same bag to reduce variability due to transport and storage. Sufficient volumes of 89-SF, QC and sample serum were prepared to plate the same preparation across the 8 plates.

Results from each serotype were generated for each test sample with 1 result from each bead preparation (4 results per serotype) required from the same assay except for beads prepared using Pn23F, Pn3 and Pn5 PS for which results were generated from 2 assays.

The acceptance criteria were that for ≥ 80 % of the samples the % CV between the replicate results was ≤ 25 %.

2.5.6.6. Low end reproducibility

The samples were prepared and assay performed as described previously (section 2.4.4).

Results from each serotype were generated for each test sample with 5 results each generated from a different assay required.

The acceptance criteria were that for ≥ 80 % of the samples the % CV between the replicate results was ≤ 25 %.

2.5.7. Linearity

For each serotype ten positive samples were selected and prepared in a similar manner to section 2.5.4 except that only 1/4 and 1/8 dilutions were prepared and the dilutions were prepared using a commercially available IgG deficient (IgG-) serum (Sigma).

Results from each serotype were generated for each of the positive test sample, 1/4 and 1/8 dilutions from the same assay.

The acceptance criteria were that for ≥ 80 % of sample results the diluted samples produced values within ± 30 % of the positive sample once adjusted for the dilution factor.

2.5.8. Stability

For the stability parameters 15 positive test samples that covered the range of the standard curve and 5 negative test samples for each serotype were selected. The test samples were pre-aliquoted on selection into 2 µL aliquots.

The results generated from negative test samples were excluded from the acceptance criteria for each parameter.

2.5.8.1. Freeze/thaw stability

Aliquots for each sample were thawed at RT until fully thawed and re-frozen for a minimum of twelve hours at – 80 ºC (-65 to -95 ºC) until there were aliquots available that had been freeze-thawed 1 (the original aliquoting), 5, 10 and 15 times.

The samples were prepared and assay performed as described previously (section 2.4.4).

Results from each serotype were generated for each test sample from all 4 different freeze/thaw cycle points from the same assay.

The acceptance criteria were that for ≥ 80 % of sample the % CV between the four freeze/thaw cycle levels was ≤ 25 %

2.5.8.2. Short term temperature stability

An aliquot of each test sample was thawed at RT for a minimum of 5 hrs. This was then prepared as described previously (section 2.4.4) alongside another aliquot that had been thawed for less than 1 hr.

Results from each serotype were generated for each test sample with results for both incubation periods required from the same assay.

The acceptance criteria were that for ≥ 80 % of samples the % CV between the results from the time points was less ≤ 25 %.

2.5.8.3. Post preparative stability

An aliquot of each test sample was thawed and prepared as described previously (section 2.4.4), the prepared test sample was incubated at RT for 6 hours. A second aliquot of each test sample was thawed and prepared in the same manner but not allowed to stand at RT for greater than 1 hr. Both samples were assayed together as described previously (section 2.4.4).

Results from each serotype were generated for each test sample at with results for both incubation periods required from the same assay.

The acceptance criteria were that for ≥ 80% of the samples the % CV between the two preparations was ≤ 25 %.

2.6. Seroprevalence of antibodies against 13 serotypes in UK infants in 2009 and 2012 aged between 1.00 to 3.99 years.

2.6.1. Samples

Serum samples provided by the Sero-Epidemiology Unit (SEU) of PHE from 2009 (n= 200) and 2012 (n = 203) were selected for testing from children aged between 1.00 and 3.99 years of age. Sample testing was covered by SEU ethics as part of testing for a larger seroprevalence survey being undertaken (Appendix I).

2.6.2. Assay

Except where specifically mentioned different operators (myself, Helen Flynn, Katy Gray and Michael Norman) were involved in preparing beads, using all 13 serotypes, for use in all testing the seroprevalence samples.

All 403 samples were tested (by myself, Helen Flynn, Katy Gray, Joanna Gregson, Ruksana Isaji, Ros Warrington, Sarah Frankland or Sarah Martin)

as described previously (section 2.4.4), or using an assay similar to that described previously (section 2.3) but using 007sp as the standard serum, until 1 result for each sample from all 13 serotypes was acquired.

2.6.3. Data Analysis

Geometric mean concentrations (GMC) and 95 % confidence intervals (CI) were calculated for results within 1 year age bands (i.e. 1.00 to 1.99, 2.00 to 2.99 and 3.00 to 3.99 years of age). In addition the percentage of samples with an antibody concentration ≥ 0.35 µg/mL was calculated for each serotype in each age band by year of collection. Independent t-tests were performed between the results for each year of collection in each age band to determine any significant differences with a p value of ≤ 0.05 considered as significant.

Additional analyses were performed on 2 sub-sets within this data. The first sub-set was for those samples from the 2012 collection for which the known date-of-birth of the patient was after 01/05/2010 and who were at least 1.25 years of age at the time of sample collection, the second sub-set was samples from the 2012 collection for which the known date of birth of the patient was between 01/04/2009 and 30/11/2009. In both cases samples of corresponding age were selected from the 2009 collection for comparison using GMC, 95 % CI, % ≥ 0.35 µg/mL and t-tests as described above.

3. Results

3.1. Optimisation of conditions for inclusion of pneumococcal serotype 6A polysaccharide in the multiplex assay.

3.1.1. Optimisation of concentration of pneumococcal serotype 6A PS used to prepare beads

Beads were prepared from three Pn6A PS lots (CPS6AM-1, 509508355, CPS6AM-5) using different concentrations as previously described (section 2.4.1). Blanks, standard curves and QC sera were assayed in monoplex as described previously (section 2.4.4) from 2 to 12 times depending on lot and coating concentration as specified previously (section 2.4.1). Statistical analysis was performed using an independent t-test to compare data from each coating concentration with $p < 0.05$ considered as significant. Data from CPS6AM-1, for which the lowest PS concentration used was 0.25 µg/mL, showed no significant difference between blank values, the standard curves or QC results by reduction of PS concentration (data not shown).

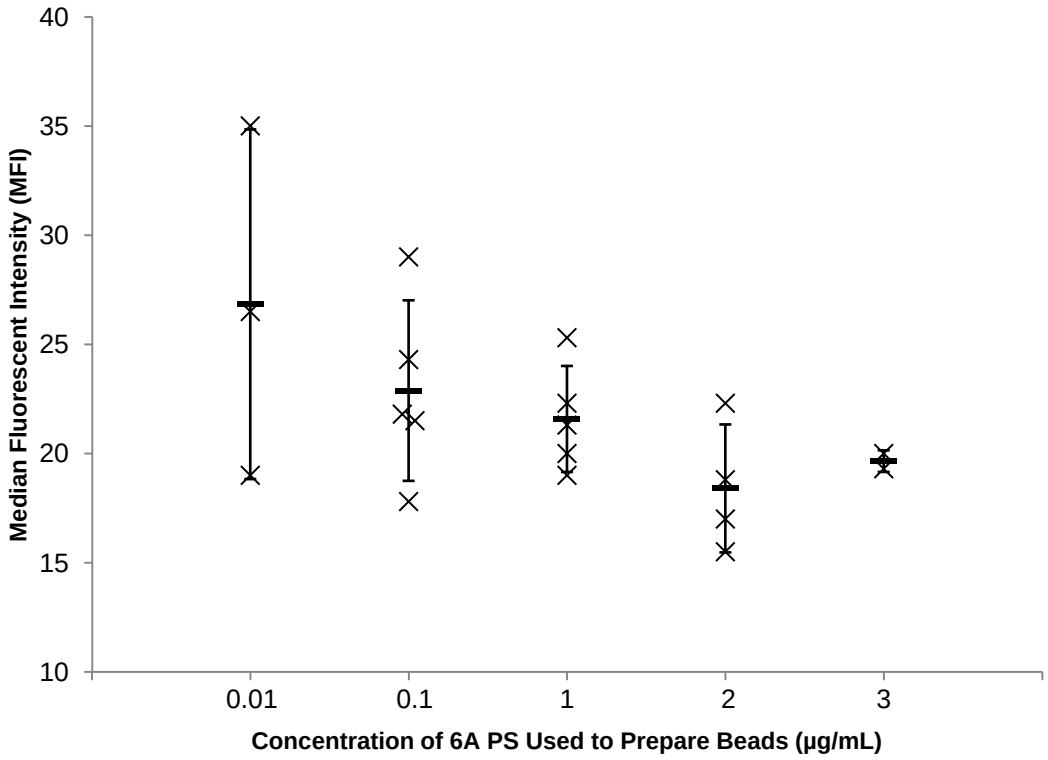
The other 2 PS lots were prepared at lower PS concentrations (0.1 µg/mL and 0.01 µg/mL) to determine if there was an effect of reducing PS concentration further. Data including the values and means of the blanks (Figure 3.1) and a representative standard curve from each of the 5 coating concentrations (Figure 3.2) are shown for beads prepared using lot 59508355.

The means of the QC sera results for each coating concentration are shown for PS lot 509508355 (Figure 3.3). Different numbers of results were obtained for this lot at the different coating concentrations of 0.01 (n = 3), 0.1 (n = 5), 1 (n = 5), 2 (n = 4) and 3 µg/mL (n = 2).

No significant difference was noted between the blank values obtained using beads conjugated with any concentration of Pn6A PS (Figure 3.1), although possible increased variability was noted in the lower coating concentrations. An increase in MFI values for all standard curve dilutions was observed from a coating concentration of 0.01 mg/mL to 0.1 mg/mL, but no further increase was seen at higher coating concentrations for both lot 509508355 (Figure 3.2) and CPS6AM-5 (data not shown) demonstrating that using at 0.01 mg/mL the beads had not been optimally coated. Significant differences were found between results obtained from both QC sera for a coating concentration of 0.01 mg/mL compared to 0.1, 1 and 2 mg/mL, suggesting that at this concentration results obtained are not optimal. Additionally a significant difference between beads coated at 0.1 mg/mL and 1 mg/mL was noted for QcMedium for lot 509508355 (Figure 3.3). The statistical differences observed for this lot were not seen at any concentration in lot CPS6AM-5, although it should be noted that the number of results available for analysis in this lot was reduced by 1 for each concentration.

The concentration which produced the most optimal results was 1 mg/mL and so this concentration was selected for preparation of *PS/PLL* and used to prepare beads for analysis and testing from this point forward.

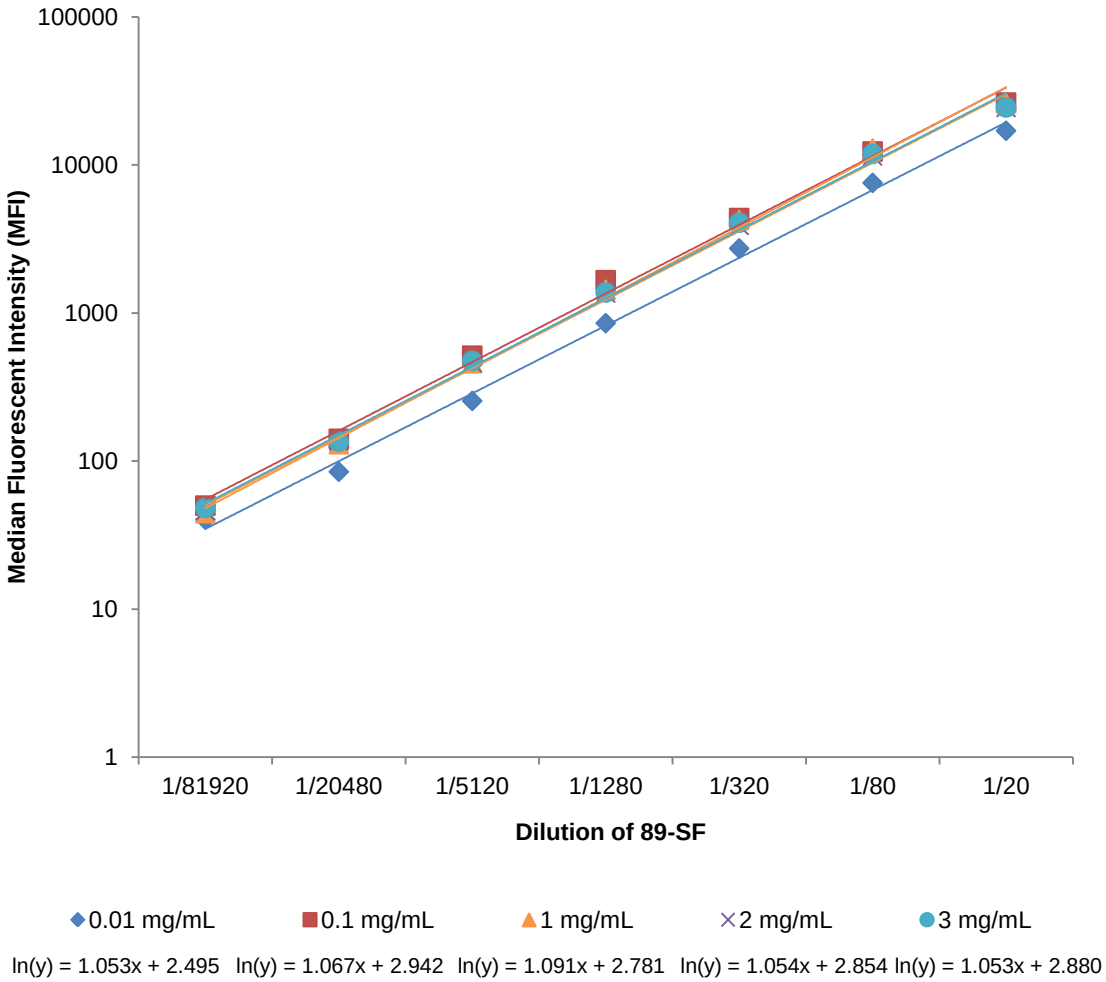
Figure 3.1: Blank values generated at different Pn 6A PS coating concentrations



The blank values generated (x), the mean values of the blanks (**–**) and ± 1 standard deviation (SD) are plotted for beads prepared using lot 59508355. Different numbers of results were obtained at the different coating concentrations: 0.01 (n = 3), 0.1 (n = 5), 1 (n = 5), 2 (n = 4) and 3 µg/mL (n = 2). No significant difference in blank values was noted at any of the coating concentrations.

This figure shows that blank values at all coating concentrations are low and appears to show a reduction in blank values and variability as coating concentration increases.

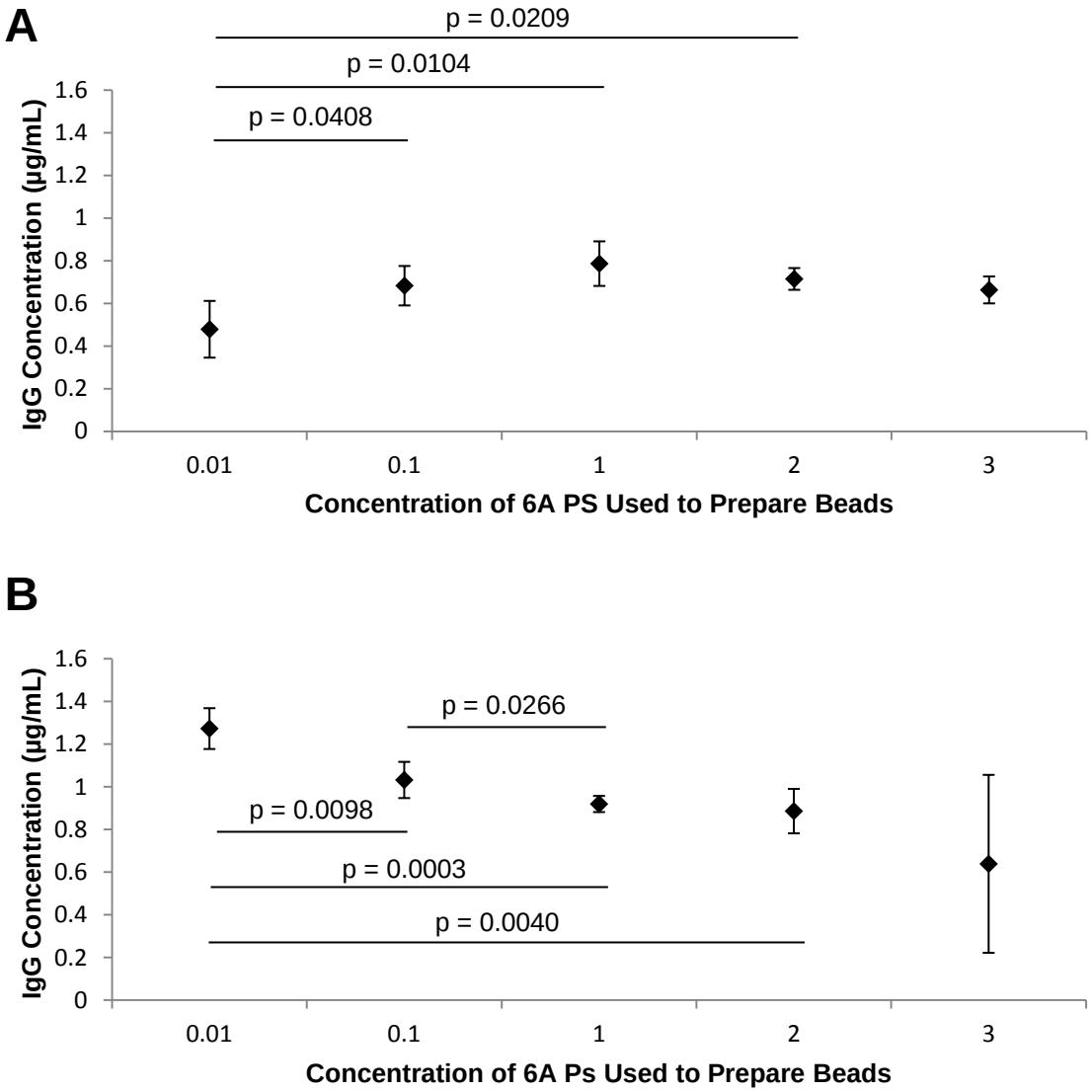
Figure 3.2: Standard curves generated at different Pn 6A PS coating concentrations



One representative standard curve for each coating concentration using beads prepared with Pn6A PS lot 59508355 are plotted and show an increase in MFI at all curve points from beads prepared using Pn6A PS at 0.01 mg/mL to 0.1 mg/mL but no further increase in MFI with any further PS concentration increase.

This figure shows that the optimal standard curve is achieved using a coating concentration of 0.1 mg/mL or higher.

Figure 3.3: Mean QC serum concentrations generated at different coating concentrations



The mean values ± 1 SD for all results at the different coating concentrations for QcHigh (**A**) and QcMedium (**B**) from beads prepared using PS lot 59508355 are plotted. Different numbers of results were obtained at the different coating concentrations: 0.01 (n = 3), 0.1 (n = 5), 1 (n = 5), 2 (n = 4) and 3 µg/mL (n = 2). A significant difference between the mean results at 0.01 µg/mL and 0.1, 1 and 2 µg/mL was observed for QcHigh ($p < 0.05$) and QcMedium ($p < 0.01$). Additionally, a significant difference was observed between the 0.01 and 1 µg/mL results for QcMedium ($p < 0.05$).

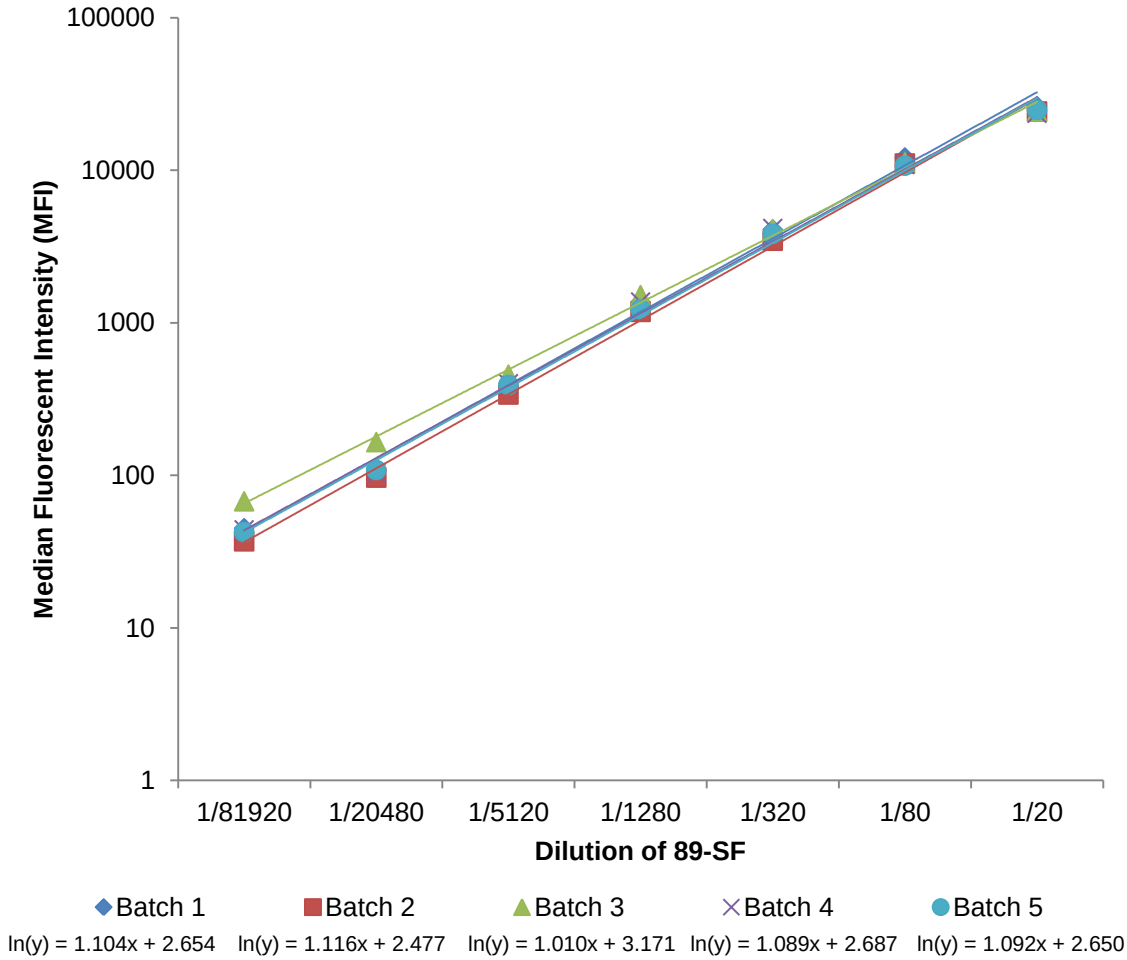
This figure shows that the optimal QC results are obtained using a coating concentration of 1 mg/mL or higher.

3.1.2. Consistency of beads preparations produced using the same PS lot

Results were obtained from 5 different bead preparations prepared using Pn6A PS lot CPA6AM-1 as described previously (section 2.4.1.1) and were used to analyse consistency of bead preparations as the most bead preparations were produced for this PS lot. All 5 bead preparations were tested the day after preparation as described previously (section 2.4.4).

The highest MFI observed for blank values for the 5 preparations was 54.3 MFI, below the maximum of 200 MFI. Standard curves generated by the 5 preparations are shown in Figure 3.4 and % CV was calculated between the replicates at each dilution with results less than 25 % for all curve dilutions except the 1/81920 dilution (25.02 %). QC sera results showed good consistency between bead batches with % CV between QcHigh and Qc Medium results for all 5 batches of 15.1 % and 12.6 %, respectively. These results demonstrated that the coating concentration of 1 mg/mL produced blank values in the expected range, consistent MFI values for all standard curve dilutions and consistent concentration results for QC sera across multiple bead preparations.

Figure 3.4: Standard curves generated from multiple bead preparations



Curves from all 5 bead preparations using PS lot CPS6AM-1, and tested the day following preparation, are shown. The % CV between the points were all below 25 % except at the 1/81920 dilution where the % CV was 25.02 %.

This figure demonstrates that curves generated by different bead preparations give have consistent MFI values.

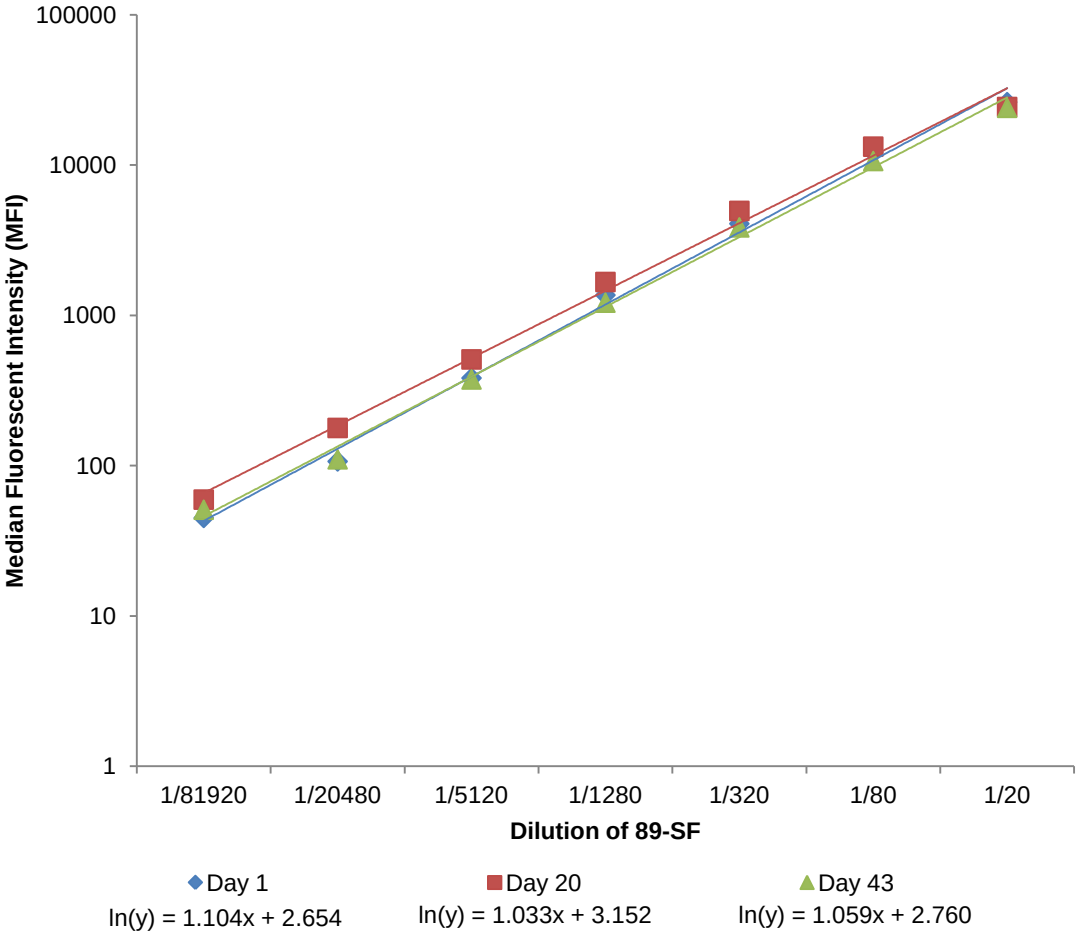
3.1.3. Stability of beads up to 77 days post conjugation

Bead preparations were produced for all PS lots and assayed on three occasions up to 43 to 77 days post conjugation as described previously (section 2.4.1). Standard curve MFI values from 1 representative bead preparation from lot CPS6AM-1, which was tested on day 1, 20 and 43 post-preparation, are shown in Figure 3.5.

The highest MFI value for the blank wells from beads prepared using any of the PS lots was 66.3, well below the maximum 200 MFI threshold (Figure 3.6). The average % CV between standard curve dilutions across the 3 time points was 18.8 % and between QcHigh and QcMedium results was 16.3 % and 12.8 % respectively. Similar results were observed in other bead preparations (data not shown).

These results demonstrated that the coating concentration of 1 mg/mL produced beads, for any lot of PS, which showed consistent results for blank, standard curve MFI and QC sera in the first 77 days post-preparation and suggest that bead preparations are stable over time after preparation.

Figure 3.5: Standard curves generated up to 43 days post preparation



Curves from 1 representative bead preparation produced using PS lot CPS6AM-1 and assayed 1, 20 and 43 days after preparation are shown. The % CV between the 3 time points from most dilute to most concentrated curve point are 14.1, 30.6, 17.9, 16.3, 13.6, 10.9 and 4.8 %, respectively.

This figure demonstrates that there is little variation between standard curve MFI values produced over time suggesting that bead preparations are stable post-preparation.

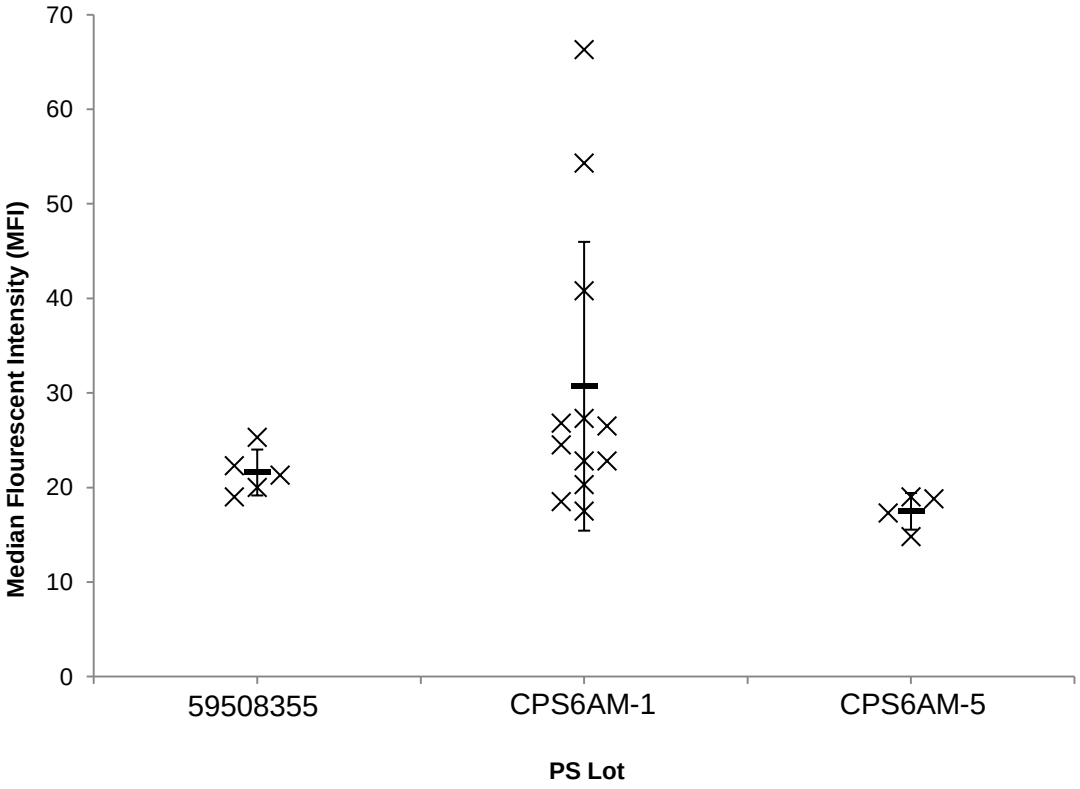
3.1.4. Different lots of 6A PS from different suppliers produce similar blank, standard curve and QC results

Results from beads prepared using different lots of Pn6A Ps, CPS6AM-1 and CPS6AM-5 from SSI and 59508355 from LGC, and assayed as described previously (section 2.4.1) were compared. Statistical analysis was performed using an independent t-test to compare data from each coating concentration with $p < 0.05$ considered as significant.

The maximum blank value for any PS lot was 66.3 MFI as reported previously (Figure 3.6). The means of all curves generated for each lot are shown (Figure 3.7) and the % CV between the means was low (a maximum of 20.0 %). Mean results for each QC sera are shown in Figure 3.8 with only one comparison between the lots showing a significant difference, namely the mean concentration for 59508355 was significantly lower than that for CPS6AM-1 ($p = 0.010$). No other significant differences were seen.

As such, no clear advantage could be seen to using any one supplier, however, the variation in results for lot 59508355 appeared to be slightly less, at least for the blanks and QcMedium, and so this lot was chosen for use in the assay during validation.

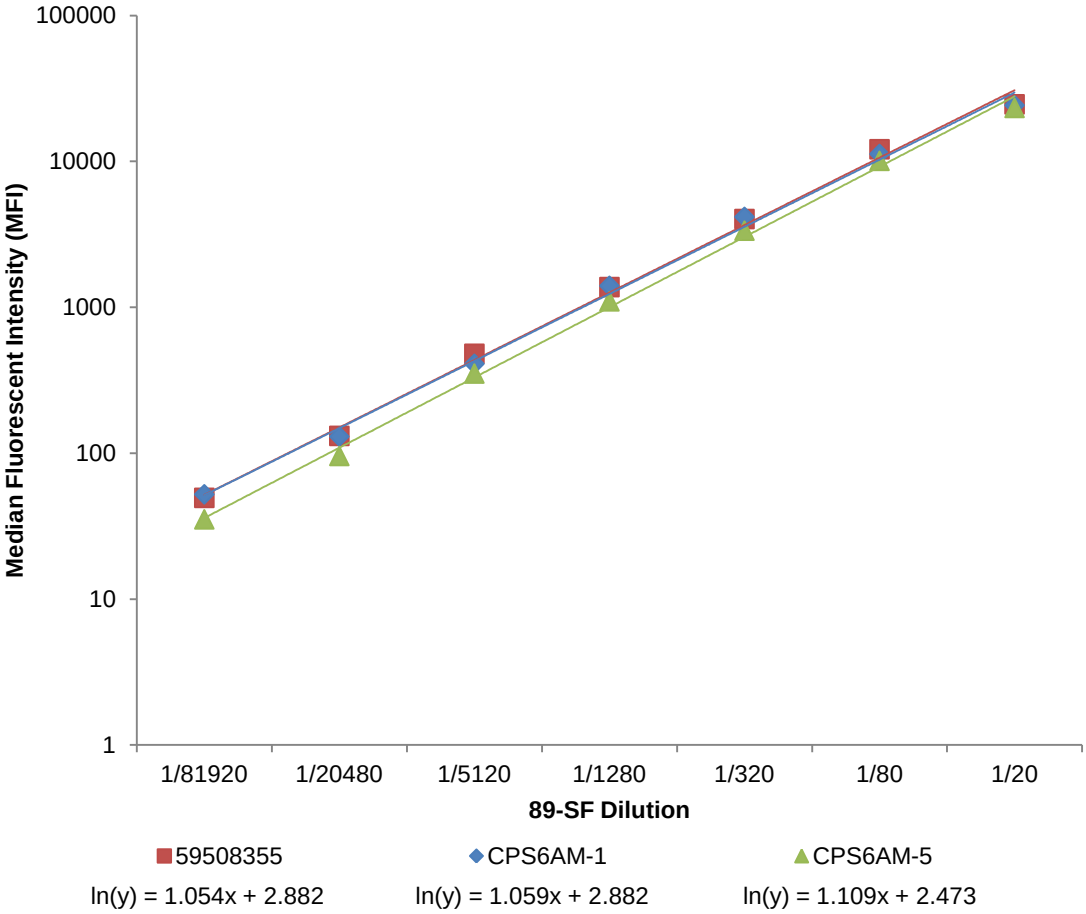
Figure 3.6: Blank values generated using lots of Pn6A PS



The blank values generated (x), the mean values of the blanks (–) and the standard deviation (I) are plotted for PS lots from LGC (59508355) and SSI (CPS6AM-1 and CPS6AM-5). The maximum observed blank value was 66.3 MFI, below the maximum acceptable value of 200 MFI. Lots 59508355 and CPS6AM-5 showed less variation ion results, but had fewer results, than CPS6AM-1.

This figure shows that all 3 PS lots produced blank values below the acceptable limit.

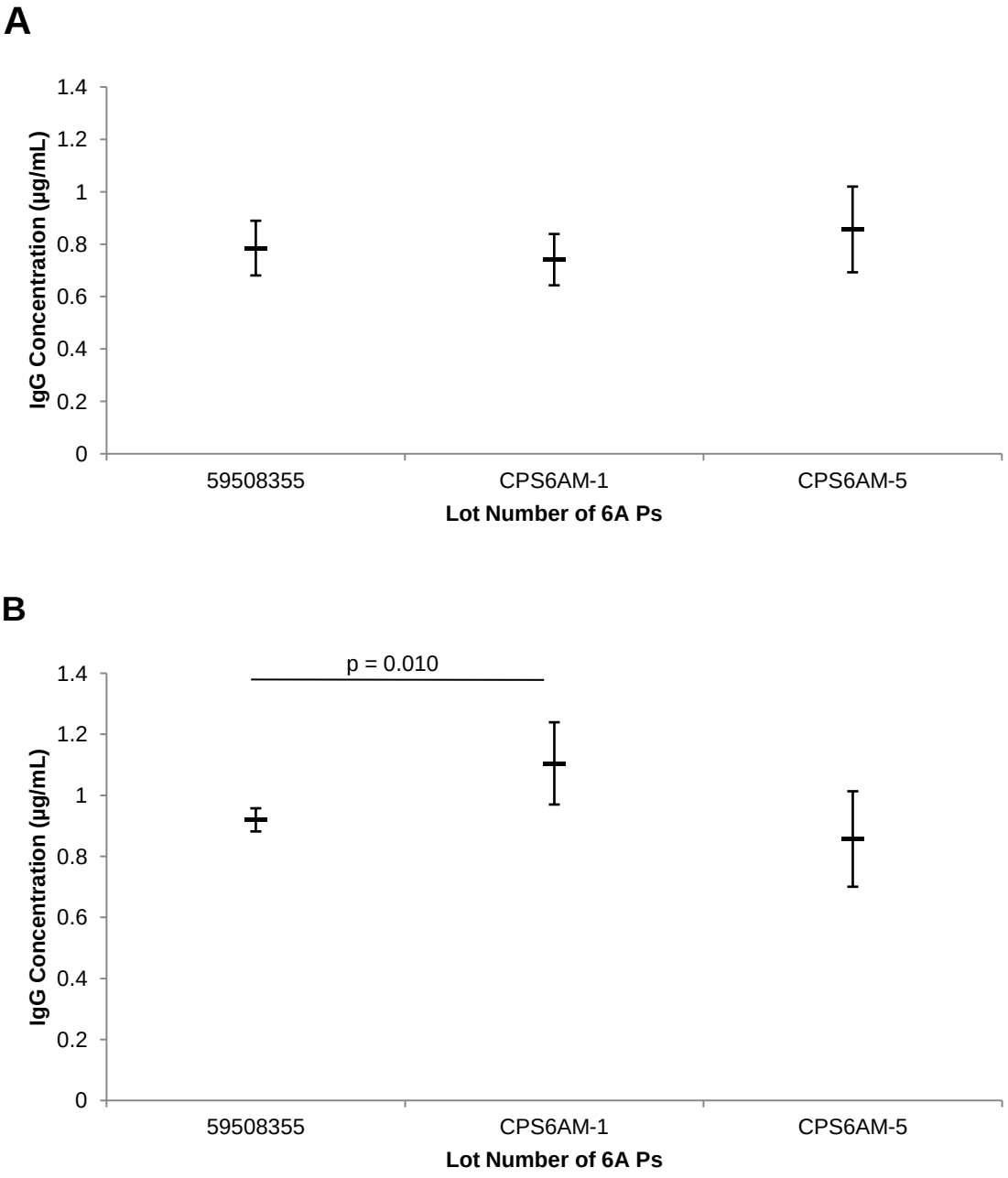
Figure 3.7: Mean values of standard curves generated using different Pn6A PS lots



The mean values of all curves generated for each PS lot from LGC (59508355) and SSI (CPS6AM-1 and CPS6AM-5) are plotted. The % CV between the mean values for the 3 PS lots from most dilute to most concentrated curve point are 20.0, 17.0, 15.5, 13.0, 11.3, 9.1 and 2.9 %, respectively.

This figure shows that there is little variation between standard curve MFI values produced between lots.

Figure 3.8: Mean concentrations generated for QcHigh and QcMedium using different Pn6A PS lots



This figure shows the mean values ± 1 SD for all results the different PS lots from LGC (59508355) and SSI (CPS6AM-1 and CPS6AM-5) for QcHigh (**A**) and QcMedium (**B**). Different numbers of results were obtained for each lot: 59508355 (n = 5), CPS6AM-1 (12) and CPS6AM-5 (4). Following calculation of the independent t-test statistic, no significant difference was observed between the results for QcHigh for any lot. For QcMedium a significant difference was found between lots 59508355 and CPS6AM-1 (p=0.010) but not between any other combination of lots.

This figure shows that for 1 lot of LGC PS, but not the other, there was a significant difference in results generated in the assay compared to the ATCC lot.

3.2. Inclusion of beads prepared with Pn6A PS with beads prepared using other Pn PS.

A total of 14 test samples were selected from samples submitted for clinical testing as required for the following parts of the optimisation. Samples were chosen that were positive for the 12 serotypes tested in the original assay (section 2.3.2) in order to show a clear inhibition if any were observed.

3.2.1. Comparison of beads assayed as monoplex or as duplex with beads prepared using any other serotype.

Results from 3 test samples generated for beads from each serotype run in monoplex or duplex with each other serotype as described previously (section 2.4.2) were compared to provide a mean percentage recovery (% Rec) for each serotype. A change in % Rec of ± 20 % was used as the threshold for a change as a result of combining the beads.

No impact of running a bead preparation from any serotype with and other bead preparation was observed except combining beads prepared with Pn23F and Pn3 together saw an increase in the mean % Rec of 21.42 % over the monoplex result for the results for Pn23F, but no impact was observed the result for beads prepared using Pn3 when run with beads prepared using Pn23F (Table 3.1). Of the other duplex combinations 148/156 results, including serotypes expected to cross react, had a % Rec within ± 10 %.

These results suggest that there is no impact on the specificity of the assay as a result of combining bead preparations in multiplex.

Table 3.1: Mean % Rec of beads assayed in duplex combinations

Monoplex bead preparation (serotype)	Duplex bead preparation (serotype)												
	Pn1	Pn3	Pn4	Pn5	Pn6A	Pn6B	Pn7F	Pn9V	Pn14	Pn18C	Pn19A	Pn19F	Pn23F
Pn1		105.35%	107.93%	104.68%	108.92%	106.43%	106.42%	105.02%	98.37%	103.68%	104.39%	103.95%	100.88%
Pn3	99.10%		96.46%	96.87%	96.48%	92.39%	94.54%	93.41%	94.18%	88.11%	94.66%	87.70%	93.99%
Pn4	108.57%	98.09%		95.75%	101.05%	96.03%	104.91%	97.79%	97.28%	95.67%	99.97%	96.93%	101.50%
Pn5	103.23%	104.43%	98.31%		104.43%	106.47%	101.90%	101.18%	96.62%	99.04%	99.12%	99.22%	87.39%
Pn6A	105.09%	105.81%	104.22%	109.81%		94.99%	97.52%	95.73%	94.89%	97.10%	97.49%	95.36%	95.56%
Pn6B	100.66%	97.03%	100.81%	110.83%	98.73%		99.29%	100.87%	101.82%	93.91%	97.51%	98.57%	95.30%
Pn7F	101.63%	98.29%	99.74%	102.62%	99.57%	98.53%		98.06%	98.43%	95.60%	96.14%	97.51%	97.20%
Pn9V	98.17%	95.47%	96.15%	103.34%	99.42%	100.74%	98.84%		99.50%	96.64%	97.39%	97.15%	99.81%
Pn14	99.43%	108.05%	104.01%	98.25%	96.72%	101.95%	98.66%	98.58%		96.29%	91.94%	96.26%	97.17%
Pn18C	100.11%	94.81%	98.65%	101.12%	101.29%	97.36%	99.76%	100.05%	99.63%		98.81%	98.19%	98.37%
Pn19A	93.30%	97.15%	93.97%	101.59%	98.45%	97.64%	98.29%	97.80%	96.04%	99.95%		99.98%	94.44%
Pn19F	99.22%	90.71%	97.43%	106.66%	102.66%	102.36%	103.33%	101.68%	103.48%	98.60%	101.45%		86.15%
Pn23F	115.13%	121.42%	119.32%	102.88%	104.62%	102.95%	107.84%	105.68%	106.63%	102.48%	97.88%	91.29%	

Three samples were tested and mean % Rec calculated between beads preparations in monoplex or as a duplex for all serotype combinations. One duplex combination (Pn3 with Pn23F) had a % Rec greater than ± 20 %.

This table shows that there does not appear to be an impact on results as a result of combining beads prepared with one serotype with beads prepared with another.

3.2.2. Inhibition of beads prepared using all serotypes with homologous and heterologous inhibitors

Inhibition of beads in monoplex was performed using polysaccharides as homologous and heterologous inhibitors and assayed as described previously (section 2.4.2). The results for un-inhibited and inhibited samples were combined to give a mean % Rec for each bead preparation inhibited with each PS (Table 3.2).

Inhibition using homologous PS showed a maximum % Rec of 6.04 and so homologous inhibition occurred as expected.

Inhibition revealed cross reactivity between beads prepared using Pn6A PS with Pn6B PS and *vice versa* and beads prepared using Pn19A PS with Pn19F PS and *vice versa*. Inhibition of beads prepared using Pn18C PS by Pn19A Ps and beads prepared using Pn18C and Pn19A Ps by Pn5 PS was also noted.

These result showed that there was some cross reactivity between serotype. Of concern following these results was the requirement of the assay to pass the specificity validation parameter, measured using this type of inhibition analysis, and so the assay was split into 2 panels as previously described in section 2.4.3 to limit the cross reactivity revealed by the heterologous inhibition.

Table 3.2: Mean % Rec of beads inhibited with homologous or heterologous polysaccharides

		Bead preparation (serotype)												
		Pn1	Pn3	Pn4	Pn5	Pn6A	Pn6B	Pn7F	Pn9V	Pn14	Pn18C	Pn19A	Pn19F	Pn23F
Inhibitor (PS)	Pn1	1.61%	100.51%	103.74%	101.05%	100.41%	100.80%	103.24%	98.52%	103.31%	107.92%	98.57%	100.71%	111.00%
	Pn3	96.21%	2.52%	80.05%	97.44%	105.52%	99.28%	103.76%	99.67%	104.91%	106.52%	96.46%	98.33%	121.40%
	Pn4	96.83%	99.71%	2.25%	99.87%	103.78%	101.16%	102.72%	101.18%	102.93%	108.29%	98.80%	100.88%	119.50%
	Pn5	98.19%	94.77%	88.08%	0.55%	105.14%	104.53%	103.20%	99.94%	107.96%	74.58%	73.53%	101.35%	116.52%
	Pn6A	94.25%	94.44%	93.88%	45.06%	2.56%	41.18%	100.47%	96.14%	102.46%	102.39%	93.06%	88.39%	112.76%
	Pn6B	94.92%	94.75%	96.21%	92.41%	11.17%	1.87%	99.88%	97.34%	102.77%	104.04%	96.94%	96.96%	80.28%
	Pn7F	94.24%	91.20%	96.51%	96.51%	102.09%	99.01%	6.04%	97.45%	78.93%	99.62%	93.02%	102.13%	115.95%
	Pn9V	94.45%	89.03%	98.94%	97.86%	101.38%	101.33%	98.09%	5.02%	102.42%	82.32%	96.13%	95.71%	116.60%
	Pn14	93.81%	89.83%	89.75%	101.26%	65.40%	73.71%	99.64%	91.80%	2.73%	99.20%	84.14%	99.25%	115.99%
	Pn18C	95.97%	100.11%	96.06%	97.36%	66.44%	69.76%	101.59%	94.51%	98.57%	2.76%	95.29%	99.48%	112.24%
	Pn19A	88.87%	74.18%	84.11%	94.46%	64.04%	70.00%	97.01%	91.52%	92.59%	2.52%	2.52%	65.52%	105.35%
	Pn19F	96.68%	101.55%	102.48%	105.69%	97.19%	98.68%	98.59%	94.53%	103.42%	90.21%	78.24%	1.41%	98.21%
	Pn23F	95.35%	104.61%	98.62%	105.34%	93.06%	90.38%	98.57%	93.22%	104.83%	108.55%	98.98%	108.25%	2.24%

This table highlights results for which a mean % Rec of less than 80 % was achieved (light blue). Homologous inhibition occurred as expected (highlighted in grey). Of particular note are the inhibition of the beads prepared using Pn18C PS by Pn19A Ps, the cross reactivity between Pn6A PS and Pn6B PS and between Pn19A PS and Pn19F PS and the inhibition by Pn5 PS of beads prepared using Pn18C and Pn19A PS.

This table demonstrates that specificity between related, and some unrelated, serotypes is not sufficient to pass validation and formed the basis for splitting the assay into 2 panels

3.2.3. Summary of assay optimisation

Beads prepared using different lots Pn6A PS were investigated to determine the optimal coating concentration. The outcome of the investigations was that a concentration of 1 mg/mL was found to be optimal (section 3.1.1) giving good consistency of blank, standard curve and QC values between bead preparations (section 3.1.2) and over time (section 3.1.3). No lot to lot variation was observed (section 3.1.4).

Investigation of the performance of the beads in multiplex identified a number of serotypes that showed evidence of cross reactivity (section 3.2.2), as a result the assay was split into 2 panels (panel A contained beads prepared using Pn 4, 6B, 9V, 14, 19A and 23F and panel B contained 1, 3, 5, 6A, 7F, 18C and 19F) to separate cross reacting serotypes.

3.3. Validation of the 13-plex Assay

Seventy nine test samples were selected from samples submitted to the VEU for clinical testing as previously described (section 2.5.1). Samples were selected to cover the range of the standard curve for all serotypes and, where possible, to be used across multiple serotypes; in addition samples that were completely negative for all serotypes were selected for the spiking analyses. At least 80 % of results within each serotype were required to meet or exceed the pre-determined acceptance criteria of that parameter in order for the parameter to be considered as validated for that serotype.

3.3.1. Specificity

The specificity parameter comprised both inhibition and spiking tests.

3.3.1.1. Inhibition

Ten samples (5 low positive and 5 positive) were prepared with or without homologous and heterologous inhibitors as described previously (section 2.5.3). Inhibited results were compared to uninhibited results to give % Rec for each serotype (Appendix II).

A summary of the inhibition results show that a minimum of 80 % of samples met or exceeded the pre-determined criteria of % Rec < 50 % for homologous inhibition and % Rec > 70 % for heterologous inhibition (Table 3.3).

These result demonstrated that the serotypes, when tested within the assigned panels, showed cross reactivity within permitted boundaries and so the cross reactivity seen in the assay previously in section 3.2.2 had been circumvented following the split of the assay into 2 panels.

3.3.1.2. Spiking

A sample with a high concentration was spiked into each of 5 negative samples previously described (section 2.5.4). Negative samples produced negative results for 100 % of the results analysed (Appendix II). Spiked results, adjusted for dilution factor, were compared to see if they fell within ± 30 % of the un-spiked test sample result (Appendix II).

A summary of the results of the spiking tests show that a minimum of 86.67 % of results met or exceeded the pre-determined criteria (Table 3.3).

The results of both the inhibition and spiking analyses show that the specificity of the assay was validated for all serotypes.

Table 3.3: Summary of results for the specificity parameter.

	Homologous Inhibition	Heterologous Inhibition	Spiking
	% Rec < 50 (%)	% Rec > 70 (%)	Results within ± 30 % of un-spiked result (%)
Pn4	100.0	90.0 to 100.0	86.67
Pn6B	100.0	100.0	93.33
Pn9V	90.0	100.0	100.00
Pn14	100.0	100.0	86.67
Pn19A	100.0	90.0 to 100.0	86.67
Pn23F	100.0	80.0 to 100.0	100.00
Pn1	80.0	90.0 to 100.0	100.00
Pn3	100.0	100.0	100.00
Pn5	90.0	90.0 to 100.0	100.00
Pn6A	90.0	90.0 to 100.0	100.00
Pn7F	80.0	100.0	93.33
Pn18C	90.0	80.0 to 100.0	100.00
Pn19F	90.0	80.0 to 100.0	100.00

A summary of the results obtained for the specificity parameter of the assay validation is shown. The percentage of samples meeting or exceeding the acceptance criteria of each test are shown, with at least 80 % of samples required in order for the parameter to pass.

This table shows that the specificity parameter was passed for beads prepared using all serotypes.

3.3.2. Accuracy

A sample with a high concentration was spiked into each of 5 negative samples as previously described (section 2.5.5). For each serotype 5 replicated results were generated. Spiked results, adjusted for dilution factor, were compared to see if they fell within ± 30 % of the un-spiked test sample result from the same run and the results from each dilution factor were compared to see if they fell within ± 30 % of the median result for that dilution factor (Appendix III).

A summary of the results for all serotypes show that for Pn 4, 6B, 9V, 14, 23F, 1, 3, 5, 6A, 18C and 19F show that the accuracy parameter met or exceeded the pre-determined criteria (Table 3.4). For Pn7F the parameter exceeded the pre-determined criteria for percentage of results within ± 30 % of the median (98.67 %) but did not meet the pre-determined criteria for results within ± 30 % of the positive sample (76.00 %) and for Pn19A the results did not meet the pre-determined criteria for both criteria (77.33 and 54.67 %, respectively).

Table 3.4: Summary of results for the accuracy parameter

Serotype	Results within ± 30 % of un-spiked result (%)	Results within ± 30 % of median result (%)
Pn4	89.33	94.67
Pn6B	86.67	90.67
Pn9V	89.04	97.26
Pn14	91.76	94.52
Pn19A	54.67	77.33
Pn23F	94.67	96.00
Pn1	89.33	90.67
Pn3	81.33	89.33
Pn5	85.33	97.33
Pn6A	94.67	93.33
Pn7F	76.00	98.67
Pn18C	94.67	96.00
Pn19F	85.33	94.67

A summary of the results obtained for the accuracy parameter of the assay validation is shown. The percentage of results meeting or exceeding the acceptance criteria of each test are shown, with at least 80 % required in both tests for the parameter to pass.

This table shows that the pre-determined criteria were met or exceeded for the accuracy parameter using beads prepared with Pn 4, 6B, 9V, 14, 23F, 1, 3, 5, 6A, 18C and 19F, but using beads prepared with Pn 19A and 7F.

3.3.3. Reproducibility

For each serotype 15 positive and 5 negative test samples were selected using historical data to provide a spread across the whole standard curve range for all reproducibility tests except for the low end reproducibility where the 15 positive test samples were selected to be in the bottom 20 % of the range of the standard curve. On testing in the reproducibility analysis some samples that were selected to be positive gave negative results and, rarely, test samples that were expected to be negative gave positive results. Only positive results from test samples were included in the analyses of the tests. Raw data for each of the reproducibility tests can be found in Appendix IV.

3.3.3.1. Inter assay

The test samples were assayed to give 5 results each from a different assay as described previously (section 2.5.6.1). A summary of results for this test show that beads prepared using all serotypes except Pn19A and Pn3 (both 66.67 %) met or exceeded the pre-determined criteria (Table 3.5).

3.3.3.2. Intra assay

The test samples were assayed to give 5 results from the within the same assay as previously described (section 2.5.6.2). A summary of results for this test show that beads prepared using all serotypes met or exceeded the pre-determined criteria (Table 3.5).

3.3.3.3. Inter operator

The test samples were assayed by 3 operators to give 2 results from each operator as described previously (section 2.5.6.3). A summary of results for this test show that beads prepared using all serotypes except Pn23F (78.57

%) and Pn3 (73.33 %) met or exceeded the pre-determined criteria (Table 3.5)

3.3.3.4. Inter operator analyte

The test samples were assayed using 3 bead preparations each produced by the same operator as described previously (section 2.5.6.4). A summary of results for this test show that beads prepared using all serotypes except Pn1 (78.57 %) met or exceeded the pre-determined criteria (Table 3.5)

3.3.3.5. Intra operator analyte

The test samples were assayed using 4 bead preparations each produced by a different operator as previously described (section 2.5.6.5). A summary of results for this test show that beads prepared using all serotypes met or exceeded the pre-determined criteria (Table 3.5)

3.3.3.6. Low end

The test samples were assayed to give 5 results each from a different assay as described previously (section 2.5.6.6). A summary of results for this test shown that beads prepared using all serotypes met or exceeded the pre-determined criteria except Pn 19A and 3 which had only 71.43 and 60.00 %, respectively (Table 3.5).

Table 3.5: Summary of results for the reproducibility parameter

	Inter Assay % CV between replicates ≤ 25.00 (%)	Intra Assay % CV between replicates ≤ 25.00 (%)	Inter Operator % CV between replicates ≤ 25.00 (%)	Inter operator Analyte % CV between replicates ≤ 25.00 (%)	Intra Operator Analyte % CV between replicates ≤ 25.00 (%)	Low End % CV between replicates ≤ 25.00 (%)
Pn4	93.75	93.33	100.00	86.67	100.00	92.86
Pn6B	93.33	100.00	93.33	100.00	100.00	93.33
Pn9V	100.00	93.33	100.00	86.67	100.00	100.00
Pn14	93.33	100.00	80.00	100.00	93.33	80.00
Pn19A	66.67	92.86	100.00	86.67	93.33	71.43
Pn23F	100.00	100.00	78.57	85.71	100.00	100.00
Pn1	85.71	85.71	87.50	78.57	87.50	84.62
Pn3	66.67	86.67	73.33	92.86	66.67	60.00
Pn5	100.00	100.00	94.12	100.00	93.75	100.00
Pn6A	93.33	100.00	100.00	100.00	100.00	93.33
Pn7F	100.00	100.00	80.00	100.00	87.50	93.33
Pn18C	93.33	93.33	100.00	100.00	100.00	100.00
Pn19F	100.00	100.00	93.33	100.00	93.33	86.67

A summary of the results obtained for the reproducibility parameter of the assay validation is shown. The percentage of results meeting or exceeding the acceptance criteria of each test are shown, with at least 80 % required in all tests for the parameter to pass.

This table shows that the reproducibility results met or exceeded the pre-determined criteria beads prepared using Pn4, Pn6B, Pn9V, Pn14, Pn5, Pn6A, Pn7F, Pn18C and Pn19F, but not for beads prepared using Pn19A, Pn23F, Pn1 and Pn3.

3.3.4. Linearity

For the linearity parameter 10 test samples were spiked into commercial IgG- sera at a dilution of 1/4 and 1/8 as described previously (section 2.5.7). For beads prepared using each serotype 1 result for the un-spiked positive sample and the 2 dilutions was generated from the same assay. Spiked results, adjusted for dilution factor, were compared to see if they fell within ± 30 % of the un-spiked test sample result, results from test samples that were negative or which fell below the limit of detection (LOD) of the curve were excluded from the analysis (Appendix V).

A summary of the results shows that > 80 % of results met or exceeded the pre-determined acceptance criteria for beads prepared using all serotypes except Pn4 and Pn1.

Table 3.6: Summary of results for the linearity parameter

Serotype	Results within ± 30 % of unspiked result (%)
Pn4	78.95
Pn6B	93.75
Pn9V	95.00
Pn14	95.00
Pn19A	94.74
Pn23F	100.00
Pn1	75.00
Pn3	80.00
Pn5	95.00
Pn6A	100.00
Pn7F	80.00
Pn18C	100.00
Pn19F	100.00

A summary of the results obtained for the linearity parameter of the assay validation is shown. The percentage of results meeting or exceeding the acceptance criteria of each test are shown, with at least 80 % required in all tests for the parameter to pass.

This table shows that the linearity results met or exceeded the pre-determined criteria for beads prepared using all serotypes except Pn4 and Pn1.

3.3.5. Stability

For the stability parameter sub-aliquots of test samples were prepared in advance. For each serotype 15 positive test samples from across the range of the standard curve and 5 negative samples were selected. Only positive results from the test samples were included in the analyses of the tests. Raw data for the stability tests can be found in 0.

3.3.5.1. Freeze/thaw

Test samples were thawed and re-frozen up to 15 times as described previously (section 2.5.8.1). Results for each test sample were obtained for 1, 5, 10 and 15 freeze/thaw cycles from the same assay and the % CV between the 4 time points calculated. A summary of the results for this test shows that the pre-determined criteria were met or exceeded for beads prepared using all serotypes except Pn6A (78.57 %; Table 3.7).

3.3.5.2. Short term temperature

Test samples were thawed and incubated at RT <1 hr or 5 hr as described previously (section 2.5.8.2). Both aliquots of the test samples were assayed together and the % CV between results at the two time points was calculated. A summary of the results for this test shows that the pre-determined criteria were met or exceeded for beads prepared using all serotypes (Table 3.7).

3.3.5.3. Post preparative

Test samples were thawed and diluted in ads+22F before incubation at RT <1 hr for or 6 hr as described previously (section 2.5.8.3). Both aliquots of the test samples were assayed together and the % CV between results at the two time points was calculated. A summary of the results for this test

shows that the pre-determined criteria were met or exceeded for beads prepared using all serotypes except for Pn3 (73.33%; Table 3.7).

Table 3.7: Summary of results for the stability parameter

	Freeze/ Thaw % CV between replicates ≤ 25.00 (%)	Short Term % CV between replicates ≤ 25.00 (%)	Post Preparative % CV between replicates ≤ 25.00 (%)
Pn4	100.00	93.33	92.86
Pn6B	92.86	93.33	100.00
Pn9V	100.00	100.00	100.00
Pn14	93.33	100.00	93.33
Pn19A	93.75	86.67	93.33
Pn23F	93.33	100.00	93.75
Pn1	84.62	100.00	91.67
Pn3	86.67	86.67	73.33
Pn5	81.25	86.67	93.33
Pn6A	78.57	93.33	93.33
Pn7F	87.50	87.50	93.75
Pn18C	86.67	100.00	100.00
Pn19F	81.25	100.00	93.75

A summary of the results obtained for the linearity parameter of the assay validation is shown. The percentage of results meeting or exceeding the acceptance criteria of each test are shown, with at least 80 % required in all tests for the parameter to pass.

This table shows that of the stability results met or exceeded the pre-determined criteria for beads prepared using all serotypes except Pn6A (freeze/thaw) and Pn3 (post preparative).

3.3.6. Summary of assay validation

The performance of the assay was compared to pre-determined criteria for specificity, accuracy, reproducibility, linearity and stability in order to demonstrate validity of the results (sections 3.3.1, 3.3.2, 3.3.3, 3.3.4 and 3.3.5). The pre-determined criteria were met or exceeded for all parameters for beads prepared using Pn 6B, 9V, 14, 5, 18C and 19F. For beads prepared using Pn 4, 23F 6A and 7F the pre-determined criteria were not met for results from only one test and for beads prepared using Pn 19A, 1 and 3 the results from multiple tests did not meet the pre-determined criteria.

The results of the validation are discussed further below (section 4) with the conclusion that the minimum requirements for validation were met for all serotypes.

3.4. Seroprevalence of antibodies against 13 serotypes in UK infants 1.00 to 3.99 years of age.

A total of 403 samples from patients aged 1 to < 4 years were selected for testing for the seroprevalence study of which 200 were from 2009 and 203 were from 2012. Of these, 1 sample from 2009 was found to be insufficient which left 199 samples available to be tested for this year.

Samples were tested as previously described (section 2.6.2) with the first accepted result for each serotype accepted as the correct result.

3.4.1. Overall seroprevalence of antibodies

Samples were grouped by year of sample collection and then by age at sample collection (i.e. 1.00 to 1.99, 2.00 to 2.99 and 3.00 to 3.99 years of age) and geometric mean concentrations (GMC), 95 % confidence interval (CI) calculated and the % of samples with concentration ≥ 0.35 µg/mL was calculated for all serotypes at each age band (Table 3.8). The independent t-test statistic was calculated between the years of collection for each serotype (Table 3.8).

No statistical difference in GMC titres was observed between the years for any of the serotypes in PCV7 except for Pn4 in the age 1.00 to 1.99 year group and Pn18C in the age 2.00 to 2.99 year group. In both cases the 2012 cohort had a lower GMC than 2009, however, the p values were only just significant (0.045 and 0.049, respectively) and 95 % CI overlap completely in both instances suggesting these results are due to natural variations in the data sets and are not genuine differences. Similarly % samples with concentrations ≥ 0.35 µg/mL were observed between the two years.

These results suggest that there has been no effect of the introduction of PCV13 on the serotypes that were present in both vaccines.

For serotypes present in PCV13 but not PCV7 significant increases were observed from 2009 to 2012 for all serotypes except Pn3 in both the age 1.00 to 1.99 and 2.00 to 2.99 year groups. Significant increases for serotypes Pn5 and Pn7F were also noted in the age 3.00 to 3.99 year group. Very few children aged > 3 in 2012 are likely to have received a dose of PCV13 as they were born and vaccinated prior to introduction of PCV13, as such it is likely that these increases are due to individual samples in the cohorts with antibodies obtained naturally by contact with those serotypes. The samples with concentrations ≥ 0.35 µg/mL greatly increased for non-PCV7 serotypes samples from those aged 1.00 to 2.99 years regardless of serotype, and there was a slight increase in all serotypes in those aged 3.00 to 3.99 years.

These results indicate that the introduction of PCV13 has significantly increased the antibody concentrations in infants for serotypes that were not present in PCV7, and that these increases in antibody concentration result in an increased proportion of vaccinees protected from infection by those serotypes.

Table 3.8: Seroprevalence of antibodies in 2009 and 2012

Age	Serotype	2009			2012			p (GMC)
		GMC (µg/mL)	95% CI	> 0.35 µg/mL (%)	GMC (µg/mL)	95% CI	> 0.35 µg/mL (%)	
1.00 to 1.99 (n = 40 [2009] and 70 [2012])	Pn4	1.99	(1.38,2.61)	85.00	1.55	(0.26,2.85)	81.43	**0.045**
	Pn6B	1.12	(0.77,1.46)	67.50	0.64	(-0.55,1.83)	61.43	0.102
	Pn9V	1.04	(0.72,1.37)	67.50	0.76	(-0.07,1.58)	71.43	0.212
	Pn14	2.47	(1.71,3.24)	90.00	2.30	(0.55,4.05)	88.57	0.900
	Pn18C	1.06	(0.73,1.39)	77.50	0.91	(-0.36,2.19)	78.57	0.702
	Pn19F	1.57	(1.08,2.05)	87.50	2.13	(-1.52,5.77)	91.43	0.507
	Pn23F	1.05	(0.73,1.38)	65.00	1.09	(-0.93,3.11)	72.86	0.881
	Pn1	0.09	(0.06,0.12)	10.00	0.78	(-0.78,2.34)	67.14	**0.019**
	Pn3	0.09	(0.06,0.12)	10.00	0.36	(0.00,0.72)	52.86	0.233
	Pn5	0.06	(0.04,0.07)	2.50	0.60	(0.07,1.14)	65.71	**< 0.001**
	Pn6A	0.20	(0.13,0.26)	27.50	1.90	(-2.47,6.27)	82.86	**0.013**
	Pn7F	0.13	(0.09,0.17)	10.00	1.42	(0.08,2.77)	90.00	**0.002**
	Pn19A	0.20	(0.14,0.27)	40.00	1.00	(-1.67,3.67)	64.29	**0.026**
2.00 to 2.99 (n = 59 [2009] and 100 [2012])	Pn4	1.41	(1.05,1.77)	83.05	0.75	(-0.77,2.27)	73.00	0.818
	Pn6B	0.98	(0.73,1.23)	79.66	0.63	(-1.53,2.79)	68.00	0.884
	Pn9V	0.83	(0.62,1.05)	72.88	0.41	(-1.03,1.85)	53.00	0.709
	Pn14	1.85	(1.38,2.32)	93.22	0.88	(-1.61,3.38)	76.00	0.583
	Pn18C	0.90	(0.67,1.13)	74.58	0.48	(-0.03,0.99)	66.00	**0.049**
	Pn19F	1.78	(1.33,2.24)	79.66	0.98	(-1.29,3.25)	79.00	0.058
	Pn23F	1.24	(0.92,1.55)	83.05	0.82	(-1.37,3.01)	67.00	0.290
	Pn1	0.09	(0.07,0.11)	6.78	0.42	(-0.33,1.17)	53.00	**0.016**
	Pn3	0.09	(0.07,0.11)	11.86	0.27	(-0.40,0.95)	44.00	0.074
	Pn5	0.06	(0.04,0.07)	1.69	0.39	(-0.56,1.35)	59.00	**0.047**
	Pn6A	0.26	(0.19,0.33)	38.98	0.99	(-1.10,3.08)	78.00	**0.030**
	Pn7F	0.10	(0.08,0.13)	13.56	0.88	(0.23,1.53)	80.00	**< 0.001**
	Pn19A	0.23	(0.17,0.29)	33.90	0.62	(-5.58,6.82)	67.00	**0.049**
3.00 to 3.99 (n = 100 [2009] and 33 [2012])	Pn4	0.50	(0.40,0.59)	66.00	0.53	(-0.75,1.82)	66.67	0.453
	Pn6B	0.50	(0.40.0.60)	52.00	1.03	(-0.61,2.68)	84.85	0.673
	Pn9V	0.39	(0.32,0.47)	50.00	0.38	(0.15,0.62)	57.58	0.356
	Pn14	0.65	(0.52,0.77)	64.00	0.51	(-0.45,1.48)	60.61	0.445
	Pn18C	0.55	(0.44,0.66)	60.00	0.39	(0.01,0.76)	54.55	0.129
	Pn19F	1.06	(0.85,1.27)	64.00	0.80	(0.30,1.31)	78.79	0.108
	Pn23F	0.54	(0.43,0.64)	54.00	1.26	(-5.36,7.91)	75.76	0.068
	Pn1	0.11	(0.09,0.13)	11.00	0.15	(0.02,0.29)	21.21	0.508
	Pn3	0.16	(0.13,0.19)	28.00	0.18	(-1.50,1.86)	30.30	0.484
	Pn5	0.06	(0.04,0.07)	1.00	0.11	(-0.01,0.24)	18.18	**< 0.001**
	Pn6A	0.31	(0.25,0.37)	39.00	0.45	(-0.28,1.17)	57.58	0.518
	Pn7F	0.15	(0.12,0.18)	19.00	0.43	(-0.42,1.28)	51.52	**0.003**
	Pn19A	0.29	(0.23,0.34)	42.00	0.24	(-0.36,0.84)	33.33	0.743

GMC, 95% CI and % samples with concentration ≥ 0.35 µg/mL for each serotype at different age ranges in each of the 2009 and 2012 cohorts are shown. The independent t-test statistic between 2009 and 2012 is also shown (P < 0.05 in bold typeface).

This table shows no effect of PCV13 introduction on PCV7 serotypes and significant increase in antibody concentrations for non PCV7 serotypes, except Pn3, for infants aged 1.00 to 2.99, along with increases in the proportion of patient reaching at least 0.35 µg/mL in all additional age groups.

3.4.2. Comparison of 3 doses of PCV7 to 3 doses of PCV 13

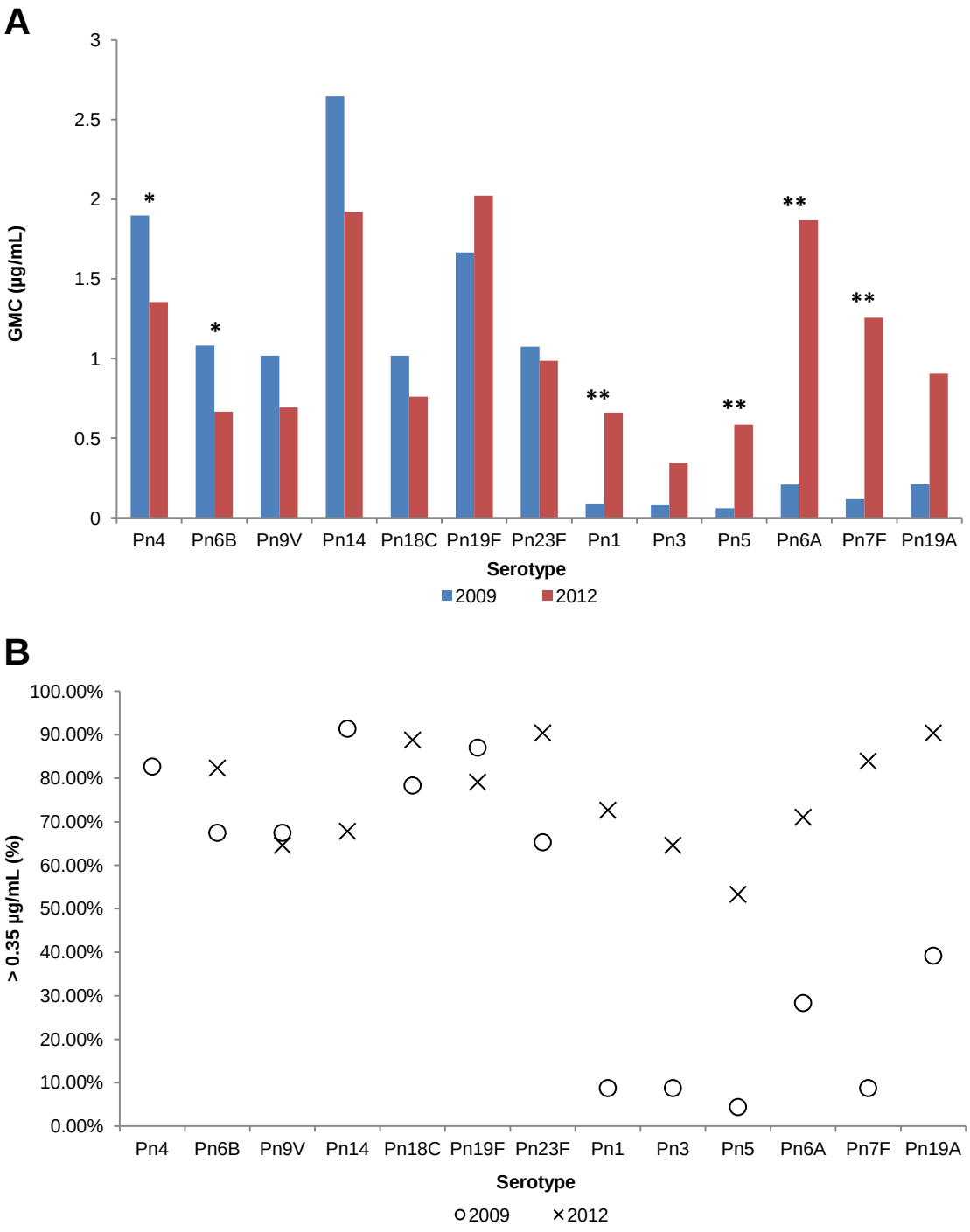
Results were selected out of the total data set for samples from the 2012 cohort whose date of birth could be determined to be between 01/05/2010 and who were at least 15 months old at the time the sample was collected which resulted in selection of 62 samples. These samples were expected to have received the full 3 dose course of PCV13. Samples (n = 46) from the 2009 cohort with the same age profile at time of sample collection were also selected. GMCs, 95 % CI were calculated for the 2 years, and independent t-test calculations were performed on the results between the years, for each serotype.

Significant increases in GMC were observed for all serotypes in PCV13 but not in PCV7, except for Pn3 and Pn19A (Figure 3.9). Although there was an apparent increase in GMC for both serotypes from 2009 to 2012 this was not significant ($p = 0.215$ [Pn3] and 0.060 [Pn19A]). The proportion of samples with antibody concentration ≥ 0.35 µg/mL increased for all serotypes not in PCV7 by 2 to 10 fold (Figure 3.9).

No differences were observed for the serotypes present in PCV7 except that a significant decrease in GMC was noted for Pn4 and Pn6B from 2009 to 2012 ($p = 0.011$ [Pn4] and 0.027 [Pn6B]; Figure 3.9) although no difference was seen in the proportion of samples with ≥ 0.35 µg/mL antibody concentration for any PCV7 serotypes (Figure 3.9).

These results demonstrate that the full 3 dose course of PCV 13 produces robust increases in GMC for all the extra serotypes, including Pn3, without any adverse effect on the serotypes present in PCV7.

Figure 3.9: GMC and percentage ≥ 0.35 µg/mL from patients receiving 3 doses of PCV7 (2009) or PCV13 (2012)



The GMC (**A**) and percentage results ≥ 0.35 µg/mL (**B**) are shown for each serotype for patients who had received either 3 doses of PCV7 (2009) or PCV13 (2012). Significant differences in GMC are denoted by * ($P < 0.05$) and ** ($P < 0.01$).

These figures show significant increases in GMC for serotypes not in PCV7 except Pn3 and Pn19A, 2 to 10 fold increases in the proportion of non PCV7 serotype results ≥ 0.35 µg/mL, and no reduction in any PCV7 serotype proportion ≥ 0.35 µg/mL despite significant reductions in Pn4 and Pn6B GMC.

3.4.3. Comparison of 3 doses of PCV7 with 2 doses of PCV7 followed by 1 dose of PCV13

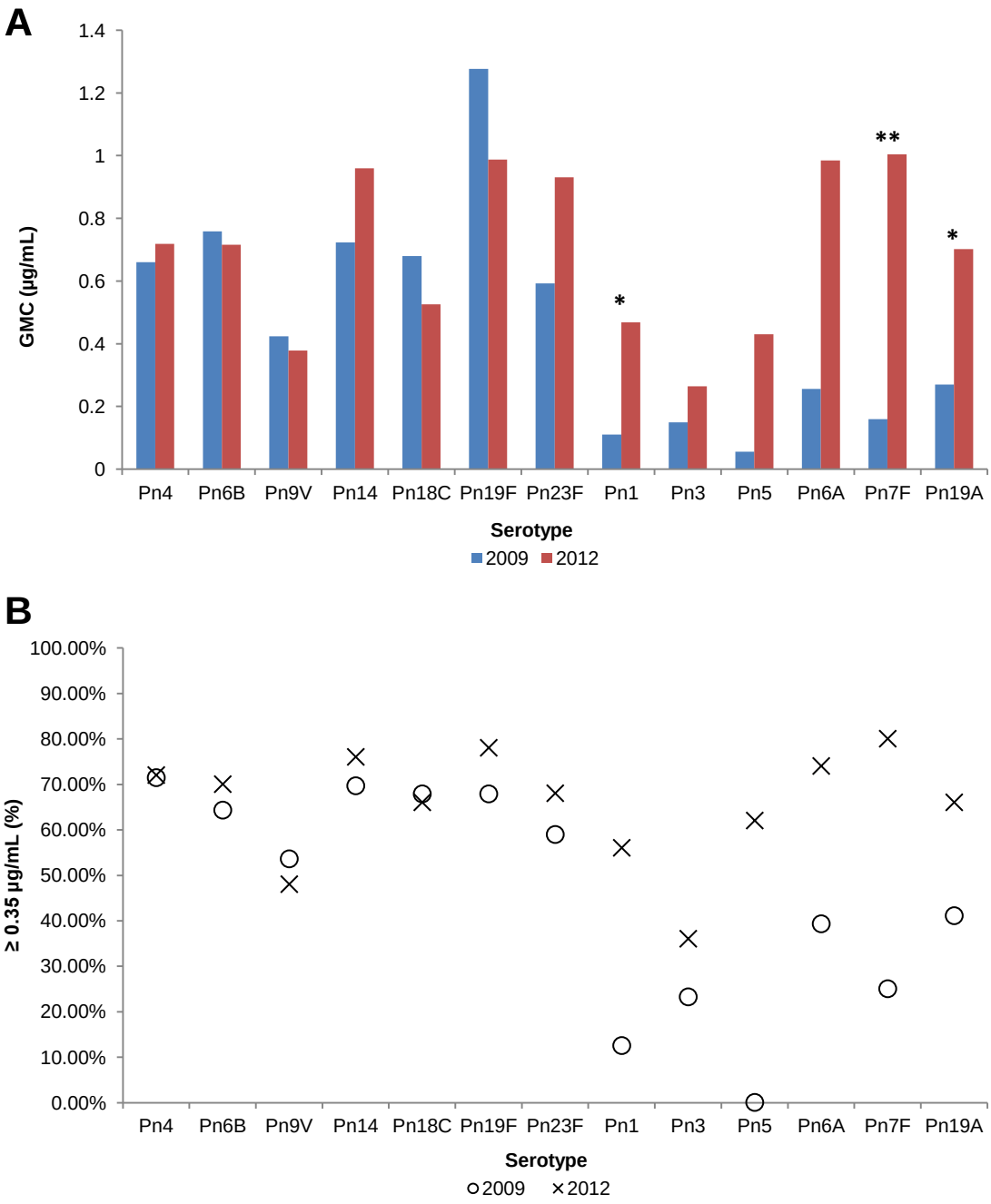
Results were also selected out of the total data set for samples from the 2012 cohort were selected whose date of birth could be determined to be after 01/04/2009 and 30/11/2009 resulting in selection of 50 samples. These samples were expected to have received the 2 doses of PCV7 as they were born before introduction of PCV 13 but to have received the booster dose of PCV13 as they were 13 months old at least one month after introduction of PCV13. Samples from the 2009 cohort with the same date of birth range, but 3 years earlier, were also selected resulting in 56 samples being selected. GMCs, 95 % CI were calculated for the 2 years, and independent t-test calculations were performed on the results between the years, for each serotype.

Increases in GMC were observed for all non PCV7 serotypes, although significance was only found for Pn1, Pn7F and Pn19A (Figure 3.10). Increases in the proportion of samples achieving ≥ 0.35 µg/mL of at least 1.5 fold were seen in all non PCV7 serotypes (Figure 3.10).

No significant difference was noted in GMC and the proportion of results ≥ 0.35 µg/mL was similar for all serotypes present in PCV7 (Figure 3.10).

These results demonstrate that increased protection for all extra serotypes in PCV13 was achieve with only 1 dose of PCV13 after 12 months of age, without detriment to protection of PCV7 serotypes.

Figure 3.10: GMC and percentage ≥ 0.35 µg/mL from patients receiving 3 doses of PCV7 (2009) or 2 doses of PCV7 plus 1 dose of PCV13 (2012)



The GMC (**A**) and percentage results ≥ 0.35 µg/mL (**B**) are shown for each serotype for patients who had received either 3 doses of PCV7 (2009) or 2 doses of PCV7 followed by one dose of PCV13 (2012). Significant differences in concentrations are denoted by * ($P < 0.05$) and ** ($P < 0.01$).

These figures show significant increases in GMC for serotypes Pn1, Pn7F and Pn19A, at least 1.5 fold increases in the proportion of non PCV7 serotype results ≥ 0.35 µg/mL, and no adverse impact on any PCV7 serotype.

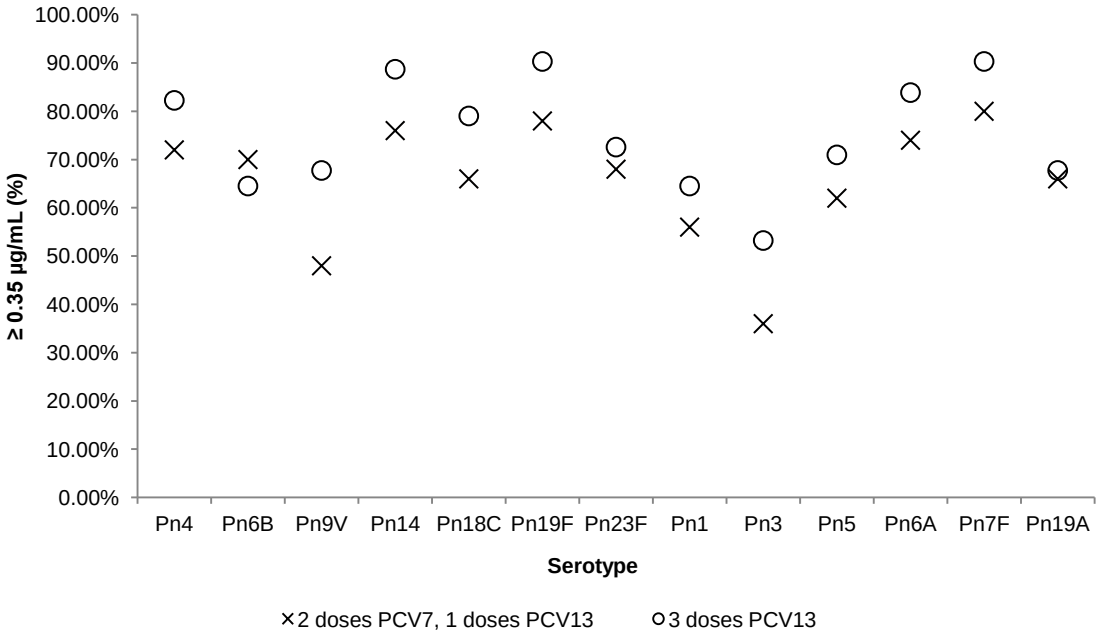
3.4.4. Comparison of 3 doses of PCV13 with 2 doses of PCV7 followed by 1 dose of PCV13

In addition, independent t-tests were performed between the results from 2012 of patients who were eligible to have received 2 doses of PCV7 and 1 of PCV13 and those who were eligible to have received 3 doses of PCV13.

No significant difference in concentration was seen for any serotype (data not shown), although in general a 10 % higher proportion of results ≥ 0.35 µg/mL was seen across all serotypes (Figure 3.11).

This result indicates that, although there is no apparent difference in concentrations, protection is more likely to be achieved after 3 doses of PCV13 than after 1 booster dose.

Figure 3.11: Percentage ≥ 0.35 µg/mL from patients receiving either 2 doses of PCV7 plus 1 dose of PCV13 or 3 doses of PCV13.



The percentage of results with concentration ≥ 0.35 µg/mL for patients receiving either 2 doses of PCV7 followed by a booster dose of PCV13 or a full 3 dose course of PCV13 are shown for each serotype.

This figure shows that, in general, approximately 10 % more patients achieved ≥ 0.35 µg/mL after 3 doses of PCV13 than after 2 PCV7 and 1 PCV13.

3.4.5. Summary of seroprevalence study

Comparison of results from 2012 with 2009 show that there has been a significant rise in IgG GMC against most of the serotypes present in PCV13 but not in PCV7 (Pn 1, 5, 6A, 7F, and 19A but not 3) in the target age group following introduction of PCV13 in April 2010. In addition the proportion of patients with IgG concentrations ≥ 0.35 µg/mL substantially increased for all additional serotypes (sections 3.4.1 and 3.4.2). No impact was seen on the IgG GMC against serotypes in both vaccines or on the proportion of patients with ≥ 0.35 µg/mL (sections 3.4.1 and 3.4.2).

Further, patients who were expected to have received only one dose of PCV13 showed good increases in both IgG GMC and proportion ≥ 0.35 µg/mL (sections 3.4.3 and 3.4.4).

These results showed that patients who were expected to receive at least 1 dose of PCV13 had good responses to vaccination.

4. Discussion

Streptococcus pneumoniae continues to be a significant global cause of pneumonia, septicaemia, meningitis (WHO, 2008). Antibodies against *S. pneumoniae* serotype-specific capsular polysaccharides, acquired naturally or by vaccination with PPV23, PCV7, PCV10 or PCV13, protect patients from pneumococcal infection and disease (Austrian, 1977; Nielsen *et al.*, 1993; Black *et al.*, 2000; Vesikari *et al.*, 2009; Snape *et al.*, 2010). Infants, young children and the elderly are at increased risk of pneumococcal disease but young children do not respond well to plain polysaccharide vaccines (Timens *et al.*, 1989; Balmer *et al.*, 2007a).

Current vaccination strategies rely on eliciting a response to each capsular polysaccharide in order to provide protection; in order to increase coverage it is necessary to increase the number of PS contained in the vaccine (Kaye *et al.*, 2009; Rodgers & Klugman, 2011). In order to circumvent this some groups have explored alternative vaccination strategies using surface expressed protein or whole cell vaccines with varying degrees of success (Miyaji *et al.*, 2013).

Following introduction of PCV7 a vaccine which is effective in infants, there has been a significant decrease in disease caused by vaccine serotypes in both the vaccinated age groups (0 to 2 years) and in all other age groups (Isaacman *et al.*, 2010; Flasche *et al.*, 2011; Miller *et al.*, 2011a; Fitzwater *et al.*, 2012). The mechanism for this broad effect is that the vaccine prevents acquisition of vaccine serotypes, preventing the capsular types from being transmitted within the infant age group and therefore more widely (Flasche *et al.*, 2011). An increase in disease caused by capsular serotypes not included in the vaccine, especially Pn19A, has been noted; a phenomenon

termed ‘serotype replacement’ (Hanage *et al.*, 2010; Miller *et al.*, 2011a; Weinberger *et al.*, 2011). It has, therefore, been necessary to increase the number of serotypes included in a paediatric conjugate vaccine to combat the serotype replacement (Gladstone *et al.*, 2011).

Measurement of IgG antibodies against pneumococcal capsular types for licensure of vaccines has been undertaken using an ELISA method; however, this is time and labour intensive, requires increasingly large volumes of sera as more serotypes are tested and is expensive, so multiplex bead based assays have been developed as alternatives to the ELISA (Wernette *et al.*, 2003; Pickering *et al.*, 2002; Lal *et al.*, 2005; Marchese *et al.*, 2009).

A previously optimised assay in use in this laboratory which tested for antibodies against 9 (Lal *et al.*, 2005), and later against 12 (Yaro *et al.*, 2013), pneumococcal capsular serotypes was optimised and validated to include Pn6A. The optimisation included examination of the coating concentration of Pn6A Ps, preliminary consistency and stability investigations and analysis of cross-reactivity between serotypes and resulted in a 2 panel assay testing for antibodies against all 13 capsular serotypes.

The beads used in the assay were MicroPlex® beads. An alternative bead type, SeroMAP® beads, which are designed to reduce non-specific binding in serological assays (Luminex, 2006) showed no advantage in preliminary investigations (data not shown).

Beads were washed by centrifugation and re-suspension, or by vacuum filtration and re-suspension, raising the possibility of bead loss during pipetting or of damage to filter plates resulting in leaking wells. An

alternative magnetic bead type (MagPlex®, Luminex) may circumvent these issues and improve the specificity and signal-to-noise ratio (Luminex, 2012). The assay in use in the VEU has not historically had issues with bead loss or leaking plates and the noise (background) of the assay is normally low. In addition, switching to magnetic beads would require additional equipment and assay optimisation and so was not investigated.

In order to conjugate the PS to the beads a 2 step process is used, firstly binding of the PS to PLL then the binding of PLL to the beads (Gray, 1979; Pickering *et al.*, 2002; Lal *et al.*, 2005). This process means there is the potential for an insufficient amount of binding to take place at either step leaving some binding sites, either on the beads or on PLL, available for non-specific binding. The quantity of PLL used in the reaction has been shown previously to be sufficient to coat the beads and so it was decided not to investigate this step (Pickering *et al.*, 2002; Lal *et al.*, 2005).

Attachment of PS/PLL to beads was performed using carbodiimide chemistry utilising EDC and NHS. This chemistry is well established although others have noted that it is a complex multistep process which can be circumvented by using an alternative linker molecule, 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium (DMTMM), which may be simpler than the PLL/EDC/NHS method as it requires only one step (Staros *et al.*, 1986; Schlottmann *et al.*, 2006; Farkas *et al.*, 2013). However, changing the chemistry of bead conjugation would be a major change to the current method in use in the VEU, requiring re-optimisation of the method for all serotypes, and as the remit of this project was to optimise Pn6A into the current assay method this was not investigated.

Measurements were made using Bioplex 100 or 200 devices. These are first generation devices which can identify up to 100 regions simultaneously (Luminex, 2013). A subsequent generation of equipment, the FlexMAP 3D, can measure up to 500 analytes simultaneously and has an increased dynamic range (Luminex, 2008). The additional benefits of using this next generation equipment would lie in the increased dynamic range, the additional capacity for analytes is not required, and this is limited as few of the serotypes saturate at the most concentrated curve points.

In order to generate readings the Bioplex instrument samples each well of the plate in sequence, utilising the same needle for each sample. This raises the possibility of beads from the previous well(s) being carried over into subsequent readings. In order to limit this, the needle is flushed with an instrument buffer (sheath fluid, Bio-Rad) when the well contents are ejected. In preliminary tests, up to 20 beads, compared to up to 1000 beads recovered from the expected region, were carried over in up to 2 subsequent wells following sampling (data not shown). This may affect the variability of results generated and is a disadvantage of this technology compared to both the ELISA and ECL assays as these do not involve physical sampling of the wells (Wernette *et al.*, 2003; Hanley, 2008; Marchese *et al.*, 2009). As part of the validation guidelines carryover is, however, anticipated and previous data from the VEU has shown that carryover does not have an significant impact on the data produced in the assay (data not shown), and so this issue was not of concern in the optimisation and validation of the assay (FDA, 2001; EMA, 2011).

Software used for calculation of standard curve and sample results values was Bioplex Manager 4.1.1, the current version available from Bio-Rad is

Bioplex Manager 6.1. During the optimisation of the assay conditions this newer version of Bioplex Manager, along with xPonent (Luminex), Masterplex QT (Miraibio Group, CA) and Statlia (Brendan Technologies, Inc., CA) were assessed but none were sufficiently superior in terms of standard curve generation, and downstream result management, to warrant replacement of old version of Bioplex Manager.

A Pn6A PS concentration of 1 mg/mL was found to be optimal for preparing beads; in contrast, the other PS used to prepare beads for use in the assay are prepared using 2 or 3 mg/mL. Other assays, using similar methodology, have used PS concentrations from 1 mg/mL to 5 mg/mL in the preparation of beads (Pickering *et al.*, 2002; Schlottmann *et al.*, 2006). No advantage was identified in using a PS concentration higher than 1 mg/mL for Pn6A in the work presented in this thesis, and it may be that optimisation of the concentrations used for the other serotypes identifies that they can be lowered without impact.

For coating concentrations less than 1 mg/mL statistical differences were seen between QC samples. The 0.1 mg/mL result showed a significant difference against only a coating concentration of 2 mg/mL, which had very low variability in results, but not any other concentration and it may be that low numbers of results available in the different experiments made it difficult to properly determine significance (Witt & McGrain, 1985). The 0.01 mg/mL results in the same analysis are clear; there was a difference noted against all other concentrations except 3 mg/mL (which had a low n) giving confidence that the significance was true.

Samples were selected, following a favourable ethical opinion, for both optimisation and validation from samples submitted for clinical testing.

Although these samples were anonymised samples are normally submitted to the clinical service to ensure determine the immunological status of older adults, although some infant sera are submitted, and so the samples used are most likely to be from adult samples with naturally acquired antibodies or antibodies produced in response to PPV23 vaccination. Naturally acquired antibodies are broad spectrum, lower avidity antibodies, whereas vaccinated individuals produce serotype-specific, high avidity antibodies (Baxendale *et al.*, 2008; Ekstrom *et al.*, 2013). Avidity is an important factor in pneumococcal immunity as there is an inverse relationship between avidity of antibodies and the concentration required for protection, however, antibody binding assays - such as ELISA, bead based and ECL assays – rely on avidity and results will give some indication of the strength of antibody binding (Usinger & Lucas, 1999; Sun *et al.*, 2001; Ekstrom *et al.*, 2013). Within the validation it is impossible to identify samples with natural, low affinity antibodies or vaccine-related high avidity antibodies, or assess the impact on results of the 2 antibody types.

89-SF was the serum used as the standard curve for assay optimisation and validation. This reference serum is well characterised and was the WHO approved international standard serum until stocks depleted. A new international standard reference serum, 007sp, has been produced and characterised (Goldblatt, Plikaytis, *et al.*, 2011).

89-SF was prepared by pooling serum from a large number of adults who had been vaccinated with PPV23 and weight based antibody levels against each serotype were determined using an ELISA based method (Quataert *et al.*, 1995; Quataert *et al.*, 2004; Goldblatt, Plikaytis, *et al.*, 2011). An ELISA method for detection of pneumococcal antibodies has been used since at

least 1978, although the current WHO consensus method has undergone revisions over time as a significant degree of non specific binding was observed due to the presence of CPs in capsular polysaccharide preparations, pre-adsorption of sera with CPs alleviates this issue (Berntsson *et al.*, 1978; Goldblatt *et al.*, 1992; Wernette *et al.*, 2003). It was noted, however, that some non-specific binding remained in the assay, and this was alleviated by pre-adsorption of sera with capsular polysaccharide from an un-assayed serotype, most commonly Pn22F although other capsular PS or CWPS2 – a product derived from the cell wall of a de-capsulated Pn22F strain - have also been used (Soininen *et al.*, 2000; Concepcion & Frasch, 2001; Marchese *et al.*, 2006; Skovsted *et al.*, 2007).

Interestingly, pre-adsorption of 89-SF with 22F is not recommended in the WHO consensus ELISA method as the weight based antibody calculations were performed without this step (Quataert *et al.*, 1995; Plikaytis *et al.*, 2000; Quataert *et al.*, 2004; Poolman *et al.*, 2010). It has been shown that a reduction in reported results occurs if 89-SF is pre-adsorbed (Henckaerts *et al.*, 2006).

The minimum test sample dilution used was 1/100, the minimum sample dilution accepted in the WHO consensus ELISA was 1/50 (Marchese *et al.*, 2006). At a 1/50 dilution the assay presented in this thesis would be more sensitive at the lower end. During the linearity assessment samples were diluted up to 1/800 without indication of any issues as a result of dilution. Higher dilutions were not assessed although the assay is used with a sample dilution of up to 1/10,000 as samples requiring dilutions at these higher factors are highly positive and not regularly required.

QC sera were sampled in each assay to give confidence in the assay performance in each run. In order for the assay to be acceptable for beads prepared using any given serotype the result for the QC sample is compared to, and must fall within ± 2 SD of, historical results. Ideally QC sera would fall on different points of the curve, giving confidence in the performance across the whole curve. In practice this not the case as results for beads prepared with some serotypes produce QC results on similar parts of the curve (data not shown).

Both QC and test samples are determined using a single point, in contrast to the ELISA which determines results from a serial dilution of QC and samples (Wernette *et al.*, 2003). In order for a single point determination to be used there must be confidence that the sample, on dilution, has a curve parallel to that of the standard sera (Plikaytis *et al.*, 1994). In validating the assay both accuracy, in which 1 sample was spiked into different unknown sera, and linearity, in which multiple samples were spiked into known IgG negative sera, showed no evidence of non-parallelism giving confidence that a single point determination is acceptable in this format.

The LLQ assigned to the assay was 0.1 µg/mL; this value is slightly above the concentration of the most dilute accepted standard curve point for the serotype with the highest antibody levels present in 89-SF (Pn19A; (Quataert *et al.*, 1995; Quataert *et al.*, 2004). It would be possible, with further analysis and optimisation, to assign LLQ values lower than this for many of the serotypes. The LLQ assigned is below the 0.2 to 0.35 µg/mL antibody concentrations that correlate to protection in adults (Henckaerts *et al.*, 2006; WHO, 2009).

During the optimisation of the assay inhibition analyses observed that both serotypes 6A/6B and 19A/19B PS inhibited each other. This was not an unexpected result due to the similarities in the structure of these serotypes (Grant *et al.*, 2013). In validating specificity of assays the required validation method is an inhibition analysis as this shows that the presence of a heterologous analyte does not impact on the result generated by the assay (FDA, 2001; EMA, 2011). The inhibition analysis performed in the optimisation showed that in a 13-plex assay the required level of specificity was unlikely to be met for 6A/6B and 19A/19F pairs and so the decision was made to split the assay into 2 panels. This decision allowed the specificity parameter to be passed for all analytes in the validation of the assay.

Examination of the literature revealed only one other current assay using all 13 serotypes present in the new vaccine, in which assay all 13 serotypes were performed on one plate (Elberse *et al.*, 2010). This study reported that increased antibodies against Pn6A were reported after administration of PCV7 which they attributed to cross reactive Pn6B antibodies (others have noted that cross reactive antibodies may be of low avidity, but that antibodies against Pn6A induced by PCV7 are likely to be high avidity) but did not comment on Pn19A/Pn19F cross reactivity nor on the impact of cross reactivity on the assay (Elberse *et al.*, 2010; Grant *et al.*, 2013; Licciardi *et al.*, 2010). Whilst at high antibody concentrations cross reactivity is unlikely to be an issue at low concentrations, especially around the correlate of protection, antibodies which are cross reactive against multiple serotypes may make a difference between an un-protected or protected outcome. This issue will also be important for ECL assays as they are

validated, although cross reactive serotypes such as Pn6A/6B and Pn19A/19F have not yet been assayed on the same plate using this method (Marchese *et al.*, 2009).

Further, this issue raises the concern that what is measured in these assays is not functional antibody. Although an IgG concentration of ≥ 0.35 µg/mL has been proposed as a correlate of protection previous observations have noted that naturally occurring high IgG concentrations occur in ageing populations alongside an increase in IPD (WHO, 2005; Balmer *et al.*, 2007b). As the mechanism of defence against pneumococci is opsononophagocytosis and ideal surrogate of protection would be measured by a standard OPA method able to determine functional antibody levels against pneumococcal strains, however this has proved difficult (Hu *et al.*, 2005; Balmer *et al.*, 2007b; Wang *et al.*, 2008; Rose *et al.*, 2011).

Assay validation is required to ensure that the results obtained using an assay during a clinical trial can be interpreted in a way that gives confidence in the outcome of the trial (Shah *et al.*, 2000; DoH, 2004). Prior to performing the validation criteria were assigned to each parameter to determine whether a parameter had met the requirements of validation. In determining these criteria reference was made to guidelines for validation of bioanalytical methods (FDA, 2001; EMA, 2011). In many cases, however, the criteria set for the validation of this assay were more stringent than the requirements in the guidelines.

The criteria for all parameters were set so that a minimum 80 % of results were to pass the requirements of any given test in order to be validated. Within the guidelines it is suggested that a pass rate of 75 % is sufficient for validation. In many cases where the 80 % pre-determined criteria was not

met in the validation of this assay ≥ 75 % of results did meet the criteria. This was true for results from:

- Accuracy for Pn 19A ± 30 % of median result (77.33 %) and 7F within ± 30 % of un-spiked result (76.00 %),
- Inter operator reproducibility for Pn 23F (78.57 %),
- Inter operator analyte reproducibility for Pn 1 (78.57 %),
- Linearity for Pn 4 (78.54 %) and 1 (75.00 %) and
- Freeze/Thaw stability for Pn 6A (78.54 %).

For each of these results, although the pre-determined criteria were not met, the outcomes for the tests met the minimum requirements of the guidelines and were considered to be validated.

The Pn19A accuracy results within ± 30 % of un-spiked result (54.67 %) did not meet the pre-determined criteria, nor were ≥ 75 %. The sample used for this test gave very high results, close to the upper limit of quantitation, for Pn 19A. The Pn 19A curve also saturates at the top end and as a result diluting the high sample produced highly variable results within each of the assays performed for the accuracy. This means that the outcome for Pn19A in this part of the accuracy validation is most likely due to the sample used and not a true reflection of the accuracy of beads prepared using Pn19A.

The remaining results which did not meet the pre-determined criteria were all reproducibility results, except the Pn 3 post-preparative stability, and for all these tests the pre-determined criteria were that results from 15 samples (across the required range of the curve) should be gained. Within the guidelines the minimum requirement is that results should be gained from 3 test samples (at different concentration levels) for the reproducibility and from 2 test samples (low and high) for the stability (FDA, 2001). A minimum

of 9 test samples gave results which passed the acceptance criteria for any these tests and so, although less than 75 % of results met the criteria, the minimum requirements to demonstrate validity were achieved.

Of interest were the results for the reproducibility parameter for Pn 3 as these were unexpectedly variable. Examination of the results shows an interesting phenomenon as reproducibility tests for Pn 3 met the pre-determined criteria when the assay took place on the same day using the same standard curve (for the intra assay and inter-operator analyte reproducibility) but for other reproducibility tests where data were required from multiple assays on different days the outcome was that beads prepared using Pn 3 did not meet the criteria. Also, in other parameters the results for Pn 3 were obtained from the same assay, except for the replicate spiking test where a different method of assessing the closeness of the results was used (a range around the median, not % CV).

Variability for beads prepared using Pn 3 PS may well be due to the physical properties of the PS as beads prepared using Pn3 produce the lowest MFIs in the standard curve, typically 6,000 to 9,000 MFI at the highest standard concentration compared to > 18,000 MFI for beads prepared using all other serotypes. This means that for this serotype the curve stops well before the maximum point in the dynamic range of the instrument and before the curve has reached a plateau. As a result relatively small differences in the curve MFI could have a great impact on the slope of the curve and may have resulted in higher variability in the results from different assays. Also many of the samples with high % CV for beads prepared using Pn 3 had low concentration. These samples are likely to be from un-immunised patients with low levels of naturally acquired,

broad spectrum, low avidity antibody. Whilst this did not appear to be an issue for other serotypes it may be that this, coupled with the greater curve variability, added to the reduced reproducibility of the assay for this serotype.

As the purpose of this project was to introduce beads prepared using Pn6A into the current assay, without assessing the other serotypes prior to validation, this was not observed until the validation was performed. It may be that this is an issue which cannot be easily solved as it will likely require alteration to the method for diluting the standard curve, require more standard serum (of which stocks are now depleted) and so require re-optimisation of the curves for all serotypes. As beads prepared using Pn3 met the minimum requirements of the guidelines and insufficient time was available to properly carry out this optimisation this investigation was not performed.

Accuracy of low samples was not assessed as part of the accuracy parameter in validation of the assay, due mainly to the method employed to assess accuracy which would be best assessed using a panel of reference sera of known concentrations. Such a panel was not available for use in this validation as historical samples from a previous multicentre trial only had results for 7 of the capsular serotypes tested generated using the ELISA (Plikaytis *et al.*, 2000). A new panel was available using 007sp as the standard sera with no data available for those samples using 89-SF as the standard sera, and again data were generated using the ELISA (Goldblatt, Plikaytis, *et al.*, 2011). As a result it was unclear if the results generated as part of the validation would be able to match the results generated in these studies and so the decision was made to use the replicate spiking method

to assess accuracy. A spiking method using low samples would be at risk of diluting samples so results are below the curve and so high positive samples were used to assess accuracy. Some lower samples were used in the linearity validation, although all were above the 0.35 µg/mL threshold, and of these samples only Pn 1 results for 1 sample were, on spiking, were below 0.35 µg/mL when the un-spiked sample was above. Low end reproducibility was also assessed as part of the validation, using samples above and below the 0.35 µg/mL, and showed that this assay had good reproducibility at the low end indicating that the assay is likely to be accurate even around the protective threshold.

The criteria determined for validating the assay were met or exceeded by the majority of serotypes for all, or all but 1, of the tests performed and so it is likely that this level of stringency was appropriate for the validation of the assay. For some tests, particularly the Pn3 reproducibility, it may be that a lower level of stringency, still meeting the minimum requirements of the guidelines, was more appropriate. Overall, the entire validation of the assay met, and often exceeded, the minimum requirements for validation as set out in published guidelines (FDA, 2001; EMA, 2011).

In 2007 a study was published in which the 9-plex assay was used to assess the seroprevalence of antibodies against pneumococci prior to introduction of PCV7 (Lal *et al.*, 2005; Balmer *et al.*, 2007b). They observed that in general a small rise in GMC levels occurred at around age 1 followed by a second rise in GMC around age 20, probably coinciding with parenthood and exposure to pneumococci in infants (Balmer *et al.*, 2007b). The study also observed that IPD in the elderly increased despite relatively high GMCs (Balmer *et al.*, 2007b).

There is a great deal of evidence to show that introduction of PCV7 resulted in a reduction in disease caused by the vaccine serotypes in both the targeted age group (0 to 2 years) and in the wider population (Isaacman *et al.*, 2010; Gladstone *et al.*, 2011; Myint *et al.*, 2013; PHE, 2013a). This protection of the wider population is a result of the vaccine preventing carriage of the vaccine serotype in immunised infants and so preventing the exposure of others to the pneumococci by contact with a colonised individual, this effect is termed 'herd protection' (Weinberger *et al.*, 2008; Flasche *et al.*, 2011; Rodrigo *et al.*, 2013).

In order to broaden coverage of pneumococcal vaccines to cover other serotypes in circulation and to combat serotype replacement PCV13 was introduced to the UK schedule in 2010. Results from the seroprevalence study undertaken here have shown that high GMC levels are maintained for the PCV7 serotypes with the introduction of the new vaccine, although some significantly lower values were noted (Pn4 and Pn18C) the percentage of vaccinees with ≥ 0.35 μg/mL was similar in both groups. This is in line with other immunogenicity data reported during the licensure and introduction of PCV13 (Kieninger *et al.*, 2010; Weckx *et al.*, 2012).

Significant increases in GMC were noted for most of the non-PCV7 serotypes in the 1.00 to 1.99 age group, with only Pn3 failing to make a significant response, and this significant increase was continued into the 2.00 to 2.99 age group for all serotypes except Pn3 and Pn19A. Again these results are similar to previous observations (Nunes & Madhi, 2011; Miller *et al.*, 2011b). Pn3 is known to be less immunogenic than the other serotypes; there is a good response to initial vaccination but failure to make a response to the booster dose in most patients, although it is unclear if this

has any clinical significance (Nunes & Madhi, 2011; Poolman & Borrow, 2011; EMA, 2013).

Epidemiology in the UK, and in Norway, has noted that the impact of PCV13 introduction on IPD caused by the new vaccine serotypes has been rapid, reducing in the vaccinated age groups within 1 year of introduction, and evidence of herd protection has also been noted 2 years after introduction (PHE, 2013a). This epidemiological data shows that the increases in GMC and % ≥ 0.35 µg/mL seen in this study have translated into reduced disease.

In general, no difference was noted between the 3.00 to 3.99 age group between the 2 years, a significant increase in Pn 5 and 7F results is likely to be due to a difference in numbers of results between the groups (2009 had 100 results and 2012 only 33 in this age group), this result was expected as those aged over 3 in 2012 are expected to have been vaccinated prior to introduction of PCV13.

No advantage, in terms of GMC achieved, was noted in patients who receive a full three doses of PCV13 compared to those who only received PCV13 as a booster. It is known, however, that in infants the risk of IPD is higher amongst those aged < 1 year and reduces with age, and so it is likely that the doses received at 4 and 8 weeks of age will be important in protecting younger infants (Kaye *et al.*, 2009; Ardanuy *et al.*, 2009; Link-Gelles *et al.*, 2013).

The seroprevalence study presented here is limited, only a few samples from a narrow age range were analysed. The samples were submitted to the SEU following consent after collection by clinicians in various regions of the UK, the reason for collection and patient history of the samples is

unknown, and so it has been assumed that these samples have undergone 'normal' immunisation as per the UK schedule. Also, the number of samples in each age band varied considerably (33 to 100 samples), in comparison more than 100 samples was used in each of these age bands in previous work (Balmer *et al.*, 2007b). Also, older populations are at increased risk of IPD and this study was unable to examine seroprevalence of antibodies in this group.

5. Future work

In order to confirm that the accuracy results for beads prepared using Pn 19A did not meet the pre-determined validation criteria due to sample selection the replicate spiking assay will be repeated for these beads using a sample that is on the linear part of the curve, rather than at the extreme of the curve.

Both long-term sample stability and analyte (bead) preparation stability experiments will be performed up to one year to demonstrate stability of these components after storage up to this point.

Stocks of 89-SF are depleted, and so a new reference serum, 007sp, has been produced and characterised to replace 89-SF (Goldblatt, Plikaytis, *et al.*, 2011). As the assay described in this thesis was optimised and validated using 89-SF the assay will need to be re-optimised to make the change to using 007sp as the reference serum. Further, a partial validation will be performed to ensure the new reference serum can be used without concern.

Further work should be carried out to analyse the seroprevalence of antibodies in the UK population, Balmer, *et al.* (2007b) comment that it

would be interesting to follow an age group through life to see changes to seroprevalence over time, seroprevalence of samples from older ages may give some insight into this question. Also, duration of protection is a question that could be assessed for patients immunised with PCV7 using a seroprevalence study; how long do protective levels of antibodies last for? Data could be examined for vaccines up to 7 years post PCV7 introduction in the UK to increase knowledge in this area.

6. Conclusion

This thesis has presented the optimisation of beads prepared using Pn6A PS and the subsequent validation of a 13-plex assay for detection of antibodies to pneumococcal serotypes. While the pre-determined criteria were not met for all serotypes in some parameters, the minimum requirements as described in published guidelines were met and the assay deemed fit for purpose.

Seroprevalence analysis showed that levels of protection remain similar for the PCV7 serotypes following the switch to PCV13 and that GMC levels increase significantly for 5/6 of the additional serotypes. Patients who only received 1 dose of PCV13 as a booster do not appear to be at a disadvantage to those who completed the whole course.

The assay described in this thesis is a useful tool to examine the effectiveness of PCV13 vaccination.

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Appendices

Appendix I. Ethical approval for testing seroprevalence samples

The Joint UCL/UCLH Committees on the Ethics of Human Research (Committee A)
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RM/cw/05A223

27 May 2005

Dr Richard Pebody
Consultant Epidemiologist
Health Protection Agency, Centre for Infections
61 Colindale Avenue
London NW9 5EQ

Dear Dr Pebody

Full title of study: ***Sero-epidemiological surveillance of the National Immunisation programme of England and Wales***
REC reference number: ***05/Q0505/45***

The Research Ethics Committee reviewed the above application at the meeting held on 26th May 2005.

Ethical opinion

Approval requested for anonymous harvesting of routinely collected blood samples for national surveillance of immunisation programmes.

Study approved with no time limit specified. There is no requirement to provide an annual report for this study.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation.

"No local investigator" status

The Committee agreed with your declaration that this is a "no local investigator" study. Site-specific assessment is not required for sites involved in the research and no information about the study needs to be submitted to Local Research Ethics Committees. However, you should arrange for the R&D Departments of all relevant NHS care organisations to be notified that the research will be taking place before the research commences.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Appendix II. Assay Validation: Specificity

Appendix II-1: Inhibition results for beads prepared using Pn4 Ps

Sample	Inhibitor							
	Pn4	Pn6B	Pn9V	Pn14	Pn19A	Pn23F	MnA	MnC
13-Plex 05	13.89%	155.56%	152.78%	147.22%	133.33%	158.33%	138.89%	133.33%
13-Plex 08	2.73%	102.19%	98.36%	119.67%	106.01%	120.22%	119.13%	107.65%
13-Plex 09	6.61%	85.96%	85.15%	113.46%	109.51%	124.94%	85.85%	108.58%
13-Plex 12	10.42%	114.58%	97.92%	120.83%	93.75%	100.00%	95.83%	87.50%
13-Plex 18	24.56%	115.79%	106.35%	110.53%	54.39%	100.00%	117.46%	122.81%
13-Plex 19	2.42%	113.04%	106.76%	97.10%	97.10%	111.11%	113.04%	110.63%
13-Plex 28	8.62%	70.69%	77.59%	93.10%	77.59%	94.83%	84.48%	100.00%
13-Plex 39	39.30%	99.13%	112.23%	95.63%	98.25%	103.93%	117.90%	111.79%
13-Plex 49	22.73%	95.45%	113.64%	104.55%	90.91%	90.91%	109.09%	118.18%
13-Plex 54	2.48%	111.39%	106.44%	93.07%	95.05%	93.07%	90.10%	96.04%
Total no. of results	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	10	-	-	-	-	-	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	100	-	-	-	-	-	-	-
No. of results with > 70 % Recovery with heterologous inhibition	-	10	10	10	9	10	10	10
Percentage of sample results with > 70 % Recovery with heterologous inhibition	-	100	100	100	90	100	100	100

Appendix II-2: Inhibition results for beads prepared using Pn6B PS.

Sample	Inhibitor							
	Pn4	Pn6B	Pn9V	Pn14	Pn19A	Pn23F	MnA	MnC
13-Plex 05	112.50%	12.50%	110.00%	107.50%	105.00%	100.00%	110.00%	112.50%
13-Plex 08	106.55%	1.21%	100.24%	119.42%	95.87%	93.20%	108.25%	99.51%
13-Plex 09	97.52%	1.80%	103.75%	115.92%	114.11%	119.89%	101.58%	107.73%
13-Plex 19	99.56%	2.18%	124.02%	108.30%	107.42%	95.20%	106.99%	103.49%
13-Plex 28	117.59%	4.63%	107.41%	114.81%	109.26%	117.59%	110.19%	111.11%
13-Plex 39	86.49%	45.95%	112.16%	100.68%	104.05%	70.27%	117.57%	117.57%
13-Plex 42	103.70%	18.52%	111.11%	107.41%	107.41%	88.89%	118.52%	114.81%
13-Plex 46	115.22%	5.43%	110.87%	113.04%	113.04%	109.78%	114.13%	122.83%
13-Plex 50	100.00%	3.40%	91.84%	76.19%	100.68%	74.15%	95.24%	95.24%
13-Plex 54	114.23%	1.87%	105.62%	92.13%	96.25%	79.78%	93.63%	97.38%
Total no. of results	10	10	10	10	10	10	10	10
No. of results with <50% Recovery with homologous inhibition	-	10	-	-	-	-	-	-
Percentage of sample results with <50% Recovery with homologous inhibition	-	100	-	-	-	-	-	-
No. of results with >70% Recovery with heterologous inhibition	10	-	10	10	10	10	10	10
Percentage of sample results with >70% Recovery with heterologous inhibition	100	-	100	100	100	100	100	100

Appendix II-3: Inhibition results for beads prepared with Pn9V PS

Sample	Inhibitor							
	Pn4	Pn6B	Pn9V	Pn14	Pn19A	Pn23F	MnA	MnC
13-Plex 05	116.33%	113.27%	5.10%	106.12%	106.12%	105.10%	113.27%	113.27%
13-Plex 08	110.11%	103.75%	1.87%	111.99%	95.51%	103.37%	105.62%	101.12%
13-Plex 09	97.25%	107.14%	12.64%	111.54%	112.09%	115.38%	102.75%	99.45%
13-Plex 19	106.97%	116.09%	1.34%	102.41%	115.28%	113.94%	113.67%	110.99%
13-Plex 28	95.79%	77.89%	35.79%	108.42%	75.79%	104.21%	108.42%	109.47%
13-Plex 39	100.00%	107.14%	17.86%	102.38%	115.48%	104.76%	121.43%	119.05%
13-Plex 46	107.69%	98.46%	24.62%	110.77%	106.15%	106.15%	104.62%	113.85%
13-Plex 49	100.00%	96.55%	55.17%	89.66%	86.21%	100.00%	113.79%	120.69%
13-Plex 50	102.70%	95.95%	6.76%	74.32%	102.70%	82.43%	91.89%	91.89%
13-Plex 54	115.99%	117.47%	1.86%	96.65%	102.23%	103.35%	94.05%	98.88%
Total no. of results	10	10	10	10	10	10	10	10
No. of results with <50% Recovery with homologous inhibition	-	-	9	-	-	-	-	-
Percentage of sample results with <50% Recovery with homologous inhibition	-	-	90	-	-	-	-	-
No. of results with >70% Recovery with heterologous inhibition	10	10	-	10	10	10	10	10
Percentage of sample results with >70% Recovery with heterologous inhibition	100	100	-	100	100	100	100	100

Appendix II-4: Inhibition results for beads prepared with Pn14 PS

Sample	Inhibitor							
	Pn4	Pn6B	Pn9V	Pn14	Pn19A	Pn23F	MnA	MnC
13-Plex 05	113.79%	112.23%	111.29%	1.57%	106.58%	104.70%	108.15%	120.06%
13-Plex 08	102.56%	100.00%	98.60%	2.33%	97.67%	101.86%	104.65%	99.07%
13-Plex 28	116.51%	111.49%	105.45%	4.01%	104.97%	114.42%	107.05%	105.61%
13-Plex 29	107.69%	94.87%	102.56%	6.41%	97.44%	102.56%	105.13%	98.72%
13-Plex 32	109.09%	106.82%	97.73%	25.00%	111.36%	111.36%	109.09%	118.18%
13-Plex 39	97.92%	110.07%	112.15%	4.51%	111.81%	104.17%	119.44%	114.58%
13-Plex 46	111.18%	99.41%	108.24%	12.35%	107.06%	112.94%	111.76%	114.12%
13-Plex 49	100.00%	100.00%	100.00%	31.25%	93.75%	100.00%	112.50%	125.00%
13-Plex 50	104.21%	106.32%	92.63%	5.26%	104.21%	85.26%	94.74%	93.68%
13-Plex 54	112.41%	118.44%	106.03%	1.77%	99.29%	98.58%	91.84%	96.10%
Total no. of results	10	10	10	10	10	10	10	10
No. of results with <50% Recovery with homologous inhibition	-	-	-	10	-	-	-	-
Percentage of sample results with <50% Recovery with homologous inhibition	-	-	-	100	-	-	-	-
No. of results with >70% Recovery with heterologous inhibition	10	10	10	-	10	10	10	10
Percentage of sample results with >70% Recovery with heterologous inhibition	100	100	100	-	100	100	100	100

Appendix II-5: Inhibition results for beads prepared with Pn19A PS

Sample	Inhibitor							
	Pn4	Pn6B	Pn9V	Pn14	Pn19A	Pn23F	MnA	MnC
13-Plex 05	153.03%	164.77%	133.66%	137.18%	0.98%	139.33%	145.40%	114.87%
13-Plex 08	99.18%	99.45%	97.16%	101.26%	0.55%	100.11%	98.74%	95.19%
13-Plex 09	91.98%	100.00%	98.85%	108.40%	11.83%	108.02%	104.20%	103.05%
13-Plex 19	104.64%	111.26%	102.65%	98.01%	6.62%	106.62%	109.27%	106.62%
13-Plex 29	97.37%	92.11%	92.11%	102.63%	13.16%	100.00%	105.26%	89.47%
13-Plex 37	103.92%	92.16%	88.24%	100.00%	47.06%	94.12%	100.00%	109.80%
13-Plex 39	93.77%	110.28%	125.86%	105.61%	36.45%	101.87%	129.60%	133.64%
13-Plex 49	98.07%	101.50%	101.93%	100.00%	10.30%	102.15%	116.95%	123.61%
13-Plex 54	100.96%	112.50%	100.96%	91.35%	25.00%	96.15%	93.27%	102.88%
13-Plex 58	100.00%	105.13%	107.69%	12.82%	12.82%	100.00%	112.82%	105.13%
Total no. of results	10	10	10	10	10	10	10	10
No. of results with <50% Recovery with homologous inhibition	-	-	-	-	10	-	-	-
Percentage of sample results with <50% Recovery with homologous inhibition	-	-	-	-	100	-	-	-
No. of results with >70% Recovery with heterologous inhibition	10	10	10	9	-	10	10	10
Percentage of sample results with >70% Recovery with heterologous inhibition	100	100	100	90	-	100	100	100

Appendix II-6: Inhibition results for beads prepared with Pn23F Ps

Sample	Inhibitor							
	Pn4	Pn6B	Pn9V	Pn14	Pn19A	Pn23F	MnA	MnC
13-Plex 05	117.95%	85.47%	105.13%	102.14%	91.03%	2.14%	111.97%	128.21%
13-Plex 08	123.68%	97.37%	107.89%	126.32%	115.79%	13.16%	121.05%	121.05%
13-Plex 09	113.18%	82.17%	89.15%	120.16%	121.71%	17.83%	91.47%	117.05%
13-Plex 12	122.07%	87.59%	115.17%	127.93%	117.59%	10.00%	104.48%	98.62%
13-Plex 18	81.65%	43.58%	98.17%	80.73%	94.04%	5.05%	99.08%	94.50%
13-Plex 19	106.32%	87.36%	126.95%	114.31%	107.62%	0.93%	111.90%	106.13%
13-Plex 46	74.14%	65.52%	67.24%	75.86%	75.86%	8.62%	67.24%	79.31%
13-Plex 54	111.33%	82.44%	103.11%	94.22%	102.89%	3.78%	92.89%	94.44%
13-Plex 58	94.12%	78.43%	111.76%	111.76%	94.12%	29.41%	103.92%	90.20%
13-Plex 78	101.52%	98.48%	102.70%	107.76%	119.90%	8.60%	114.84%	114.00%
Total no. of results	10	10	10	10	10	10	10	10
No. of results with <50% Recovery with homologous inhibition	-	-	-	-	-	10	-	-
Percentage of sample results with <50% Recovery with homologous inhibition	-	-	-	-	-	100	-	-
No. of results with >70% Recovery with heterologous inhibition	10	8	9	10	10	-	9	10
Percentage of sample results with >70% Recovery with heterologous inhibition	100	80	90	100	100	-	90	100

Appendix II-7: Inhibition results for beads prepared with Pn1 PS

Sample	Inhibitor								
	Pn1	Pn3	Pn5	Pn6A	Pn7F	Pn18C	Pn19F	MnA	MnC
13-Plex 10	35.71%	92.86%	85.71%	92.86%	92.86%	92.86%	114.29%	92.86%	85.71%
13-Plex 12	1.88%	125.86%	124.80%	134.04%	111.62%	112.03%	126.84%	108.10%	115.88%
13-Plex 16	4.95%	102.64%	90.76%	105.45%	105.12%	98.84%	110.73%	106.11%	114.19%
13-Plex 18	27.78%	105.56%	161.11%	105.56%	122.22%	94.44%	97.22%	102.78%	122.22%
13-Plex 37	86.11%	108.33%	127.78%	116.67%	119.44%	111.11%	111.11%	116.67%	97.22%
13-Plex 39	54.55%	99.24%	95.45%	88.64%	93.18%	96.97%	99.24%	92.42%	102.27%
13-Plex 42	33.99%	97.80%	104.16%	100.24%	97.07%	97.80%	98.29%	95.84%	106.60%
13-Plex 46	31.50%	108.06%	118.32%	108.06%	119.78%	127.84%	137.36%	121.61%	106.59%
13-Plex 50	24.00%	97.33%	104.67%	98.67%	116.00%	112.67%	71.33%	66.67%	67.33%
13-Plex 54	33.52%	106.82%	103.4 %	102.27%	157.39%	157.39%	159.66%	148.86%	117.61%
Total no. of results	10	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	8	-	-	-	-	-	-	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	80	-	-	-	-	-	-	-	-
No. of results with > 70 % Recovery with heterologous inhibition	-	10	10	10	10	10	10	9	9
Percentage of sample results with > 70 % Recovery with heterologous inhibition	-	100	100	100	100	100	100	90	90

Appendix II-8: Inhibition results for beads prepared with Pn3 PS

Sample	Inhibitor								
	Pn1	Pn3	Pn5	Pn6A	Pn7F	Pn18C	Pn19F	MnA	MnC
13-Plex 08	102.96%	1.64%	83.09%	97.21%	94.42%	88.51%	92.61%	89.82%	75.37%
13-Plex 10	108.93%	8.93%	96.43%	103.57%	101.79%	96.43%	107.14%	96.43%	96.43%
13-Plex 12	102.63%	2.19%	104.39%	103.07%	109.21%	110.53%	112.28%	124.56%	103.07%
13-Plex 27	104.76%	7.94%	109.52%	87.30%	119.05%	114.29%	103.17%	103.17%	115.87%
13-Plex 37	97.12%	12.95%	116.55%	110.79%	117.99%	120.86%	112.23%	105.04%	89.21%
13-Plex 39	87.50%	35.42%	104.17%	97.92%	100.00%	102.08%	95.83%	91.67%	97.92%
13-Plex 40	127.27%	9.09%	120.00%	130.91%	141.82%	130.91%	90.91%	96.36%	136.36%
13-Plex 42	102.09%	34.84%	110.80%	100.00%	97.91%	103.83%	101.05%	99.30%	108.01%
13-Plex 46	147.01%	6.72%	109.33%	96.64%	141.98%	154.10%	141.79%	126.68%	97.76%
13-Plex 49	90.32%	6.45%	93.55%	90.32%	103.23%	87.10%	96.77%	87.10%	93.55%
Total no. of results	10	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	-	10	-	-	-	-	-	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	-	100	-	-	-	-	-	-	-
No. of results with > 70 % Recovery with heterologous inhibition	10	-	10	10	10	10	10	10	10
Percentage of sample results with > 70 % Recovery with heterologous inhibition	100	-	100	100	100	100	100	100	100

Appendix II-9: Inhibition results for beads prepared with Pn5 PS

Sample	Inhibitor								
	Pn1	Pn3	Pn5	Pn6A	Pn7F	Pn18C	Pn19F	MnA	MnC
13-Plex 08	102.73%	103.64%	1.14%	96.36%	108.64%	90.23%	93.86%	94.55%	90.00%
13-Plex 12	108.00%	109.45%	5.45%	116.00%	102.91%	112.73%	111.64%	103.27%	102.55%
13-Plex 18	97.74%	112.78%	3.76%	106.02%	120.30%	98.50%	109.02%	106.02%	116.54%
13-Plex 39	80.00%	98.95%	73.68%	88.42%	98.95%	94.74%	98.95%	98.95%	103.16%
13-Plex 42	101.89%	100.94%	4.72%	96.23%	98.11%	98.11%	97.17%	96.23%	97.17%
13-Plex 43	117.74%	103.23%	16.13%	103.23%	114.52%	100.00%	129.03%	109.68%	96.77%
13-Plex 46	107.04%	102.82%	18.31%	104.23%	114.08%	114.08%	130.99%	121.13%	105.63%
13-Plex 49	78.85%	84.62%	34.62%	82.69%	90.38%	80.77%	82.69%	80.77%	84.62%
13-Plex 50	104.11%	96.21%	2.37%	99.84%	120.54%	112.95%	61.61%	58.77%	60.35%
13-Plex 54	101.47%	111.03%	8.09%	102.21%	159.56%	166.91%	168.38%	157.35%	161.03%
Total no. of results	10	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	-	-	9	-	-	-	-	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	-	-	90	-	-	-	-	-	-
No. of results with > 70 % Recovery with heterologous inhibition	10	10	-	10	10	10	9	9	9
Percentage of sample results with > 70 % Recovery with heterologous inhibition	100	100	-	100	100	100	90	90	90

Appendix II-10: Inhibition results for beads prepared with Pn6A PS

Sample	Inhibitor								
	Pn1	Pn3	Pn5	Pn6A	Pn7F	Pn18C	Pn19F	MnA	MnC
13-Plex 08	103.31%	101.93%	89.24%	2.76%	107.45%	90.21%	93.93%	96.69%	89.24%
13-Plex 12	100.00%	109.09%	100.00%	45.45%	100.00%	109.09%	109.09%	100.00%	109.09%
13-Plex 18	93.64%	110.00%	180.91%	4.55%	120.91%	96.36%	110.91%	106.36%	120.91%
13-Plex 39	79.17%	98.21%	94.64%	76.79%	96.43%	94.05%	97.62%	95.83%	98.21%
13-Plex 40	85.71%	128.57%	92.86%	17.86%	96.43%	85.71%	92.86%	89.29%	135.71%
13-Plex 42	106.58%	104.61%	115.13%	6.58%	102.63%	103.29%	100.00%	103.29%	102.63%
13-Plex 43	118.75%	103.13%	109.38%	21.88%	112.50%	96.88%	131.25%	109.38%	100.00%
13-Plex 46	100.56%	101.11%	101.11%	11.11%	108.89%	113.33%	130.00%	112.78%	98.89%
13-Plex 50	101.06%	92.61%	103.69%	1.32%	111.08%	106.60%	66.49%	62.53%	64.91%
13-Plex 54	100.00%	106.76%	104.05%	12.16%	155.41%	163.51%	160.81%	155.41%	144.59%
Total no. of results	10	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	-	-	-	9	-	-	-	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	-	-	-	90	-	-	-	-	-
No. of results with > 70 % Recovery with heterologous inhibition	10	10	10	-	10	10	9	9	9
Percentage of sample results with > 70 % Recovery with heterologous inhibition	100	100	100	-	100	100	90	90	90

Appendix II-11: Inhibition results for beads prepared with Pn7F PS

Sample	Inhibitor								
	Pn1	Pn3	Pn5	Pn6A	Pn7F	Pn18C	Pn19F	MnA	MnC
13-Plex 08	106.94%	112.15%	96.18%	103.47%	4.17%	98.96%	93.75%	100.35%	88.89%
13-Plex 10	95.58%	93.81%	85.84%	95.58%	14.16%	92.92%	102.65%	92.92%	95.58%
13-Plex 12	123.40%	112.77%	110.64%	104.26%	34.04%	114.89%	110.64%	112.77%	110.64%
13-Plex 18	95.51%	107.87%	133.71%	95.51%	15.73%	96.63%	104.49%	104.49%	114.61%
13-Plex 37	100.00%	91.43%	102.86%	91.43%	68.57%	82.86%	97.14%	105.71%	91.43%
13-Plex 39	89.33%	102.67%	103.33%	89.33%	50.00%	99.33%	100.00%	100.00%	104.00%
13-Plex 40	98.25%	119.30%	94.74%	84.21%	40.35%	82.46%	94.74%	96.49%	124.56%
13-Plex 42	109.97%	95.95%	110.59%	102.18%	15.89%	101.25%	102.80%	99.07%	101.25%
13-Plex 50	94.70%	96.97%	100.00%	100.76%	9.85%	106.06%	79.55%	75.00%	75.00%
13-Plex 54	103.56%	110.44%	102.00%	101.33%	8.67%	163.11%	157.78%	142.00%	122.44%
Total no. of results	10	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	-	-	-	-	8	-	-	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	-	-	-	-	80	-	-	-	-
No. of results with > 70 % Recovery with heterologous inhibition	10	10	10	10	-	10	10	10	10
Percentage of sample results with > 70 % Recovery with heterologous inhibition	100	100	100	100	-	100	100	100	100

Appendix II-12: Inhibition results for beads prepared with Pn18C Ps

Sample	Inhibitor								
	Pn1	Pn3	Pn5	Pn6A	Pn7F	Pn18C	Pn19F	MnA	MnC
13-Plex 10	101.49%	97.01%	53.73%	98.51%	95.52%	28.36%	100.00%	94.03%	95.52%
13-Plex 12	106.94%	105.56%	74.31%	113.19%	100.00%	16.67%	100.69%	104.17%	105.56%
13-Plex 18	97.22%	97.22%	37.50%	84.72%	108.33%	6.94%	44.44%	95.83%	93.06%
13-Plex 39	82.81%	98.44%	78.13%	89.06%	98.44%	65.63%	101.56%	96.88%	104.69%
13-Plex 40	116.77%	132.30%	84.16%	120.50%	124.22%	1.55%	100.00%	102.48%	127.02%
13-Plex 42	100.77%	104.62%	73.08%	97.69%	103.08%	5.38%	93.08%	98.46%	101.54%
13-Plex 46	113.66%	112.42%	107.45%	111.80%	126.09%	10.56%	138.51%	126.71%	104.97%
13-Plex 49	79.62%	82.80%	84.08%	86.62%	95.54%	29.30%	91.72%	82.17%	82.17%
13-Plex 50	104.11%	96.58%	82.19%	104.79%	117.81%	8.90%	68.49%	67.12%	69.18%
13-Plex 54	100.00%	106.52%	73.91%	100.00%	147.83%	15.22%	145.65%	143.48%	136.96%
Total no. of results	10	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	-	-	-	-	-	9	-	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	-	-	-	-	-	90	-	-	-
No. of results with > 70 % Recovery with heterologous inhibition	10	10	8	10	10	-	8	9	9
Percentage of sample results with > 70 % Recovery with heterologous inhibition	100	100	80	100	100	-	80	90	90

Appendix II-13: Inhibition results for beads prepared with Pn19F PS

Sample	Inhibitor								
	Pn1	Pn3	Pn5	Pn6A	Pn7F	Pn18C	Pn19F	MnA	MnC
13-Plex 08	110.12%	100.86%	85.25%	100.17%	108.40%	93.14%	1.72%	94.34%	90.39%
13-Plex 12	107.59%	115.19%	108.86%	121.52%	106.33%	117.72%	13.92%	113.92%	112.66%
13-Plex 18	97.02%	115.59%	189.67%	102.80%	129.25%	101.23%	0.88%	109.81%	120.49%
13-Plex 27	109.52%	100.00%	114.29%	90.48%	109.52%	109.52%	23.81%	104.76%	123.81%
13-Plex 37	98.73%	101.27%	103.80%	105.06%	110.13%	102.53%	20.25%	105.06%	91.14%
13-Plex 42	104.31%	102.01%	106.90%	102.87%	100.57%	100.57%	11.21%	94.54%	100.86%
13-Plex 43	113.95%	102.33%	120.93%	106.98%	111.63%	93.02%	34.88%	127.91%	100.00%
13-Plex 49	69.09%	78.18%	67.27%	74.55%	80.00%	70.91%	58.18%	69.09%	76.36%
13-Plex 50	101.71%	96.83%	87.32%	101.22%	112.44%	107.32%	2.44%	67.32%	70.49%
13-Plex 54	96.85%	104.50%	97.75%	100.90%	153.15%	159.46%	18.92%	145.50%	105.86%
Total no. of results	10	10	10	10	10	10	10	10	10
No. of results with < 50 % Recovery with homologous inhibition	-	-	-	-	-	-	9	-	-
Percentage of sample results with < 50 % Recovery with homologous inhibition	-	-	-	-	-	-	90	-	-
No. of results with > 70 % Recovery with heterologous inhibition	9	10	9	10	10	10	-	8	10
Percentage of sample results with > 70 % Recovery with heterologous inhibition	90	100	90	100	100	100	-	80	100

Appendix II-14: Spiking results for beads prepared using panel A serotypes

Pn4				
Positive sample		13-Plex 08		
		Result	-30%	+30%
		2.56	1.79	3.33
Negative sample	Neat	Spiked result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	2.38	2.37	1.72
13-Plex 67	0.05	1.86	2.42	3.06
13-Plex 70	0.05	2.13	2.38	2.60
13-Plex 74	0.05	1.75	2.36	2.92
13-Plex 76	0.05	2.90	2.40	3.03
Total number or results				15
Number of results within ± 30 % of neat positive sample				13
Percentage of results within ± 30 % of neat positive sample				86.67%

Pn6B				
Positive sample		13-Plex 08		
		Result	-30%	+30%
		3.64	2.55	4.73
Negative sample	Neat	Spiked result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	3.12	2.15	3.54
13-Plex 67	0.05	3.68	2.66	4.04
13-Plex 70	0.05	3.79	2.73	3.48
13-Plex 74	0.05	3.72	2.60	2.64
13-Plex 76	0.05	3.59	2.90	3.20
Total number or results				15
Number of results within ± 30 % of neat positive sample				14
Percentage of results within ± 30 % of neat positive sample				93.33%

Pn9V				
Positive sample		13-Plex 08		
		Result	-30%	+30%
		1.78	1.25	2.31
Negative sample	Neat	Spiked result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	1.50	1.80	2.02
13-Plex 67	0.05	1.78	1.84	1.89
13-Plex 70	0.05	1.59	1.64	1.75
13-Plex 74	0.05	1.40	1.75	1.80
13-Plex 76	0.05	1.51	1.73	1.81
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%

Pn14				
Positive sample		13-Plex 08		
		Result	-30%	+30%
		3.23	2.26	4.20
Negative sample	Neat	Spiked result adjusted for dilution factor		
		1/2	1/4	1/8

Appendix II-14: Spiking results for beads prepared using panel A serotypes (continued)

13-Plex 64	0.05	2.74	3.10	3.69
13-Plex 67	0.05	3.18	3.39	3.50
13-Plex 70	0.05	2.88	3.03	3.12
13-Plex 74	0.05	2.59	3.23	3.45
13-Plex 76	0.05	2.78	3.22	3.29
Total number or results				15
Number of results within ± 30 % of neat positive sample				13
Percentage of results within ± 30 % of neat positive sample				86.67%
Pn19A				
Positive sample		13-Plex 08		
		Result	-30%	+30%
		18.24	12.77	23.71
Negative sample	Neat	Spiked result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	19.51	13.95	16.87
13-Plex 67	0.05	17.94	10.86	15.09
13-Plex 70	0.05	16.03	13.95	17.05
13-Plex 74	0.05	16.85	11.70	14.38
13-Plex 76	0.05	18.13	15.28	15.97
Total number or results				15
Number of results within ± 30 % of neat positive sample				13
Percentage of results within ± 30 % of neat positive sample				86.67%
Pn23F				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		4.78	3.35	6.21
Negative sample	Neat	Spiked result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	4.11	4.33	4.75
13-Plex 67	0.05	4.69	5.18	5.67
13-Plex 70	0.05	4.42	4.56	4.35
13-Plex 74	0.05	4.52	5.22	5.69
13-Plex 76	0.05	4.30	4.57	5.84
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%

Appendix II-15: Spiking results for beads prepared using panel B serotypes

Pn1				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		3.00	2.10	3.90
Negative sample	Neat	Spiked Result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	2.73	2.72	3.04
13-Plex 67	0.05	3.02	3.07	3.29
13-Plex 70	0.05	2.47	2.64	2.73
13-Plex 74	0.05	2.83	3.06	3.10
13-Plex 76	0.05	2.70	2.67	3.32
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%

Pn3				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		2.48	1.74	3.22
Negative sample	Neat	Spiked Result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	2.17	2.44	2.79
13-Plex 67	0.05	2.55	2.50	2.93
13-Plex 70	0.05	2.09	2.18	2.34
13-Plex 74	0.05	2.40	2.43	2.71
13-Plex 76	0.05	2.24	2.34	2.94
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%

Pn5				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		3.31	2.32	4.30
Negative sample	Neat	Spiked Result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	3.12	3.28	3.92
13-Plex 67	0.05	3.50	3.67	4.28
13-Plex 70	0.05	2.80	3.18	3.60
13-Plex 74	0.05	3.17	3.54	4.04
13-Plex 76	0.05	3.16	3.28	4.29
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%

Pn6A				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		10.02	7.01	13.03
Negative sample	Neat	Spiked Result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	8.81	8.48	9.67

Appendix II-15: Spiking results for beads prepared using panel B serotypes (continued)

13-Plex 67	0.05	10.01	9.72	10.96
13-Plex 70	0.05	7.78	8.45	9.31
13-Plex 74	0.05	9.19	9.23	10.11
13-Plex 76	0.05	8.82	8.64	10.68
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%
Pn7F				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		1.13	0.79	1.47
Negative sample	Neat	Spiked Result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	1.01	1.34	1.35
13-Plex 67	0.05	1.03	1.13	1.30
13-Plex 70	0.05	1.09	1.35	1.58
13-Plex 74	0.05	1.00	1.26	1.24
13-Plex 76	0.05	1.10	1.04	1.34
Total number or results				15
Number of results within ± 30 % of neat positive sample				14
Percentage of results within ± 30 % of neat positive sample				93.33%
Pn18C				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		2.71	1.90	3.52
Negative sample	Neat	Spiked Result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	2.29	2.35	2.82
13-Plex 67	0.05	2.60	2.55	3.09
13-Plex 70	0.05	2.08	2.39	2.48
13-Plex 74	0.05	2.51	2.54	2.75
13-Plex 76	0.05	2.34	2.32	2.99
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%
Pn19F				
Positive sample		13-Plex 19		
		Result	-30%	+30%
		5.49	3.84	7.14
Negative sample	Neat	Spiked Result adjusted for dilution factor		
		1/2	1/4	1/8
13-Plex 64	0.05	5.33	5.46	6.64
13-Plex 67	0.05	4.97	5.68	5.96
13-Plex 70	0.05	5.19	5.46	6.52
13-Plex 74	0.05	5.31	5.89	6.66
13-Plex 76	0.05	5.30	5.87	6.68
Total number or results				15
Number of results within ± 30 % of neat positive sample				15
Percentage of results within ± 30 % of neat positive sample				100.00%

Appendix III. Assay Validation: Accuracy

Appendix III-1: Accuracy results for beads prepared using Pn4 PS

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	2.56	1.79	3.33	2.38	2.37	1.72
	2	0.05	2.06	1.44	2.68	2.02	1.88	1.71
	3	0.05	2.27	1.59	2.95	2.22	2.33	2.48
	4	0.05	1.98	1.39	2.57	1.84	1.77	1.81
	5	0.05	1.78	1.25	2.31	1.75	1.72	1.87
	Median				Result	2.02	1.88	1.81
					- 30 %	1.41	1.32	1.27
					+ 30 %	2.63	2.44	2.35
13-Plex 67	1	0.05	2.56	1.79	3.33	1.86	2.42	3.06
	2	0.05	2.06	1.44	2.68	1.95	2.00	2.78
	3	0.05	2.27	1.59	2.95	1.99	2.25	2.32
	4	0.05	1.98	1.39	2.57	1.74	1.91	1.61
	5	0.05	1.78	1.25	2.31	2.08	2.15	2.43
	Median				Result	1.95	2.15	2.43
					- 30 %	1.37	1.51	1.70
					+ 30 %	2.54	2.80	3.16
13-Plex 70	1	0.05	2.56	1.79	3.33	2.13	2.38	2.60
	2	0.05	2.06	1.44	2.68	2.00	2.09	2.74
	3	0.05	2.27	1.59	2.95	2.11	2.20	2.51
	4	0.05	1.98	1.39	2.57	1.82	1.85	1.97
	5	0.05	1.78	1.25	2.31	1.92	1.88	2.11
	Median				Result	2.00	2.09	2.51
					- 30 %	1.40	1.46	1.76
					+ 30 %	2.60	2.72	3.26
13-Plex 74	1	0.05	2.56	1.79	3.33	1.75	2.36	2.92
	2	0.05	2.06	1.44	2.68	2.24	1.88	2.78
	3	0.05	2.27	1.59	2.95	1.96	2.28	2.41
	4	0.05	1.98	1.39	2.57	1.71	1.68	1.55
	5	0.05	1.78	1.25	2.31	1.94	1.82	2.30
	Median				Result	1.94	1.88	2.41
					- 30 %	1.36	1.32	1.69
					+ 30 %	2.52	2.44	3.13
13-Plex 76	1	0.05	2.56	1.79	3.33	2.90	2.40	3.03
	2	0.05	2.06	1.44	2.68	1.60	1.94	2.66
	3	0.05	2.27	1.59	2.95	2.12	2.18	2.61
	4	0.05	1.98	1.39	2.57	1.56	1.65	1.86
	5	0.05	1.78	1.25	2.31	2.39	2.24	2.55
	Median				Result	2.12	2.18	2.61
					- 30 %	1.48	1.53	1.83
					+ 30 %	2.76	2.83	3.39
Total number of results						75		
Number of results within ± 30 % of neat positive						67		
Percentage of results within ± 30 % of neat positive						89.33 %		
Number of results within ± 30 % of median						71		
Percentage of results within ± 30 % of median						94.67 %		

Appendix III-2: Accuracy results for beads prepared using Pn6B PS

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	3.64	2.55	4.73	3.12	2.15	3.54
	2	0.05	4.71	3.30	6.12	4.13	3.96	2.92
	3	0.05	3.59	2.51	4.67	3.25	3.08	3.89
	4	0.05	4.01	2.81	5.21	3.06	2.99	3.25
	5	0.05	3.67	2.57	4.77	3.67	3.29	3.52
	Median				Result	3.25	3.08	3.52
					- 30 %	2.28	2.16	2.46
					+ 30%	4.23	4.00	4.58
13-Plex 67	1	0.05	3.64	2.55	4.73	3.68	2.66	4.04
	2	0.05	4.71	3.30	6.12	3.19	3.93	5.07
	3	0.05	3.59	2.51	4.67	3.65	3.33	4.74
	4	0.05	4.01	2.81	5.21	3.12	3.32	3.03
	5	0.05	3.67	2.57	4.77	4.57	4.18	4.41
	Median				Result	3.65	3.33	4.41
					- 30 %	2.56	2.33	3.09
					+ 30%	4.75	4.33	5.73
13-Plex 70	1	0.05	3.64	2.55	4.73	3.79	2.73	3.48
	2	0.05	4.71	3.30	6.12	3.59	4.05	4.22
	3	0.05	3.59	2.51	4.67	3.42	3.72	4.67
	4	0.05	4.01	2.81	5.21	3.34	3.29	3.53
	5	0.05	3.67	2.57	4.77	4.08	3.62	4.06
	Median				Result	3.59	3.62	4.06
					- 30 %	2.51	2.53	2.84
					+ 30 %	4.67	4.71	5.28
13-Plex 74	1	0.05	3.64	2.55	4.73	3.72	2.60	2.64
	2	0.05	4.71	3.30	6.12	3.04	4.03	5.41
	3	0.05	3.59	2.51	4.67	3.85	3.22	5.04
	4	0.05	4.01	2.81	5.21	3.05	2.97	3.16
	5	0.05	3.67	2.57	4.77	4.18	3.62	4.43
	Median				Result	3.72	3.22	4.43
					- 30 %	2.60	2.25	3.10
					+ 30 %	4.84	4.19	5.76
13-Plex 76	1	0.05	3.64	2.55	4.73	3.59	2.90	3.20
	2	0.05	4.71	3.30	6.12	5.12	4.06	5.22
	3	0.05	3.59	2.51	4.67	3.04	3.32	4.83
	4	0.05	4.01	2.81	5.21	2.68	2.90	3.18
	5	0.05	3.67	2.57	4.77	5.03	4.29	4.63
	Median				Result	3.59	3.32	4.63
					- 30 %	2.51	2.32	3.24
					+ 30 %	4.67	4.32	6.02
Total number of results						75		
Number of results within ± 30 % of neat positive						65		
Percentage of results within ± 30 % of neat positive						86.67 %		
Number of results within ± 30 % of median						68		
Percentage of results within ± 30 % of median						90.67 %		

Appendix III-3: Accuracy results for beads prepared using Pn9V Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	1.78	1.25	2.31	1.50	1.80	2.02
	2	0.05	1.96	1.37	2.55	1.69	1.62	1.99
	3	0.05	1.48	1.04	1.92	1.77	1.92	2.16
	4	0.05	2.09	1.46	2.72	1.86	1.57	2.14
	5	0.05	1.88	1.32	2.44	1.59	1.39	2.09
	Median				Result	1.69	1.62	2.09
					- 30 %	1.18	1.13	1.46
					+ 30 %	2.20	2.11	2.72
13-Plex 67	1	0.05	1.78	1.25	2.31	1.78	1.84	1.89
	2	0.05	1.96	1.37	2.55	1.86	1.86	1.95
	3	0.05	1.48	1.04	1.92	1.51	1.85	1.88
	4	0.05	2.09	1.46	2.72	2.82	-	1.96
	5	0.05	1.88	1.32	2.44	2.82	-	2.26
	Median				Result	1.86	1.85	1.95
					- 30 %	1.30	1.30	1.37
					+ 30 %	2.42	2.41	2.54
13-Plex 70	1	0.05	1.78	1.25	2.31	1.59	1.64	1.75
	2	0.05	1.96	1.37	2.55	1.73	1.65	1.68
	3	0.05	1.48	1.04	1.92	1.71	1.86	2.16
	4	0.05	2.09	1.46	2.72	1.57	1.66	1.93
	5	0.05	1.88	1.32	2.44	1.62	1.70	1.87
	Median				Result	1.62	1.66	1.87
					- 30 %	1.13	1.16	1.31
					+ 30 %	2.11	2.16	2.43
13-Plex 74	1	0.05	1.78	1.25	2.31	1.40	1.75	1.80
	2	0.05	1.96	1.37	2.55	1.66	1.36	1.84
	3	0.05	1.48	1.04	1.92	1.71	1.69	2.18
	4	0.05	2.09	1.46	2.72	1.77	1.72	2.25
	5	0.05	1.88	1.32	2.44	1.61	1.67	2.00
	Median				Result	1.66	1.69	2.00
					- 30 %	1.16	1.18	1.40
					+ 30 %	2.16	2.20	2.60
13-Plex 76	1	0.05	1.78	1.25	2.31	1.51	1.73	1.81
	2	0.05	1.96	1.37	2.55	1.55	1.79	1.89
	3	0.05	1.48	1.04	1.92	1.66	1.61	2.01
	4	0.05	2.09	1.46	2.72	1.59	1.45	2.31
	5	0.05	1.88	1.32	2.44	1.66	1.54	1.74
	Median				Result	1.59	1.61	1.89
					- 30 %	1.11	1.13	1.32
					+ 30 %	2.07	2.09	2.46
Total number of results						73		
Number of results within ± 30% of neat positive						65		
Percentage of results within ± 30% of neat positive						89.04 %		
Number of results within ± 30% of median						71		
Percentage of results within ± 30% of median						97.26 %		

Appendix III-4: Accuracy results for beads prepared using Pn14 Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	025	0.05	3.23	2.26	4.20	2.74	3.10	3.69
	026	0.05	3.54	2.48	4.60	2.86	2.71	3.06
	027	0.05	3.11	2.18	4.04	3.44	3.77	4.14
	028	0.05	3.66	2.56	4.76	3.15	2.79	3.52
	029	0.05	3.13	2.19	4.07	2.51	2.18	3.69
	Median				Result	2.86	2.79	3.69
					- 30 %	2.00	1.95	2.58
					+ 30 %	3.72	3.63	4.80
13-Plex 67	025	0.05	3.23	2.26	4.20	3.18	3.39	3.50
	026	0.05	3.54	2.48	4.60	3.23	2.95	3.23
	027	0.05	3.11	2.18	4.04	2.97	3.77	3.85
	028	0.05	3.66	2.56	4.76	5.21	-	3.23
	029	0.05	3.13	2.19	4.07	4.48	-	3.48
	Median				Result	3.23	3.39	3.48
					-30 %	2.26	2.37	2.44
					+ 30 %	4.20	4.41	4.52
13-Plex 70	025	0.05	3.23	2.26	4.20	2.88	3.03	3.12
	026	0.05	3.54	2.48	4.60	3.18	2.75	2.58
	027	0.05	3.11	2.18	4.04	3.39	3.62	3.83
	028	0.05	3.66	2.56	4.76	2.81	2.65	3.27
	029	0.05	3.13	2.19	4.07	2.68	2.58	2.98
	Median				Result	2.88	2.75	3.12
					- 30 %	2.02	1.93	2.18
					+ 30 %	3.74	3.58	4.06
13-Plex 74	025	0.05	3.23	2.26	4.20	2.59	3.23	3.45
	026	0.05	3.54	2.48	4.60	2.97	2.35	3.31
	027	0.05	3.11	2.18	4.04	3.37	3.28	4.30
	028	0.05	3.66	2.56	4.76	3.22	3.04	3.73
	029	0.05	3.13	2.19	4.07	2.75	2.79	3.36
	Median				Result	2.97	3.04	3.45
					- 30 %	2.08	2.13	2.42
					+ 30 %	3.86	3.95	4.49
13-Plex 76	025	0.05	3.23	2.26	4.20	2.78	3.22	3.29
	026	0.05	3.54	2.48	4.60	2.82	2.83	3.10
	027	0.05	3.11	2.18	4.04	3.22	3.10	3.68
	028	0.05	3.66	2.56	4.76	2.74	2.25	3.90
	029	0.05	3.13	2.19	4.07	2.61	2.35	2.84
	Median				Result	2.78	2.83	3.29
					- 30 %	1.95	1.98	2.30
					+ 30 %	3.61	3.68	4.28
Total number of results						73		
Number of results within ± 30 % of neat positive						67		
Percentage of results within ± 30 % of neat positive						91.78%		
Number of results within ± 30 % of median						69		
Percentage of results within ± 30 % of median						94.52%		

Appendix III-5: Accuracy results for beads prepared using Pn19A Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	18.24	12.77	23.71	19.51	13.95	16.87
	2	0.05	17.00	11.90	22.10	12.99	10.65	7.74
	3	0.05	14.11	9.88	18.34	10.72	8.84	10.57
	4	0.05	17.48	12.24	22.72	14.94	11.45	10.97
	5	0.05	21.08	14.76	27.40	35.45	24.60	22.09
	Median				Result	14.94	11.45	10.97
					- 30 %	10.46	8.02	7.68
					+ 30 %	19.42	14.89	14.26
13-Plex 67	1	0.05	18.24	12.77	23.71	17.94	10.86	15.09
	2	0.05	17.00	11.90	22.10	9.48	10.66	13.64
	3	0.05	14.11	9.88	18.34	12.26	9.81	12.61
	4	0.05	17.48	12.24	22.72	13.28	11.96	10.31
	5	0.05	21.08	14.76	27.40	37.34	32.31	24.80
	Median				Result	13.28	10.86	13.64
					- 30 %	9.30	7.60	9.55
					+ 30 %	17.26	14.12	17.73
13-Plex 70	1	0.05	18.24	12.77	23.71	16.03	13.95	17.05
	2	0.05	17.00	11.90	22.10	10.64	10.93	11.19
	3	0.05	14.11	9.88	18.34	10.82	10.81	13.09
	4	0.05	17.48	12.24	22.72	16.39	11.52	11.57
	5	0.05	21.08	14.76	27.40	36.86	26.23	20.68
	Median				Result	16.03	11.52	13.09
					- 30 %	11.22	8.06	9.16
					+ 30 %	20.84	14.98	17.02
13-Plex 74	1	0.05	18.24	12.77	23.71	16.85	11.70	14.38
	2	0.05	17.00	11.90	22.10	8.71	10.74	13.30
	3	0.05	14.11	9.88	18.34	12.82	9.22	13.30
	4	0.05	17.48	12.24	22.72	13.26	10.46	10.39
	5	0.05	21.08	14.76	27.40	38.25	25.40	25.98
	Median				Result	13.26	10.74	13.30
					- 30 %	9.28	7.52	9.31
					+ 30 %	17.24	13.96	17.29
13-Plex 76	1	0.05	18.24	12.77	23.71	18.13	15.28	15.97
	2	0.05	17.00	11.90	22.10	18.12	11.18	13.56
	3	0.05	14.11	9.88	18.34	9.11	8.95	12.89
	4	0.05	17.48	12.24	22.72	11.43	10.61	10.59
	5	0.05	21.08	14.76	27.40	37.45	23.91	22.66
	Median				Result	18.12	11.18	13.56
					- 30 %	12.68	7.83	9.49
					+ 30 %	23.56	14.53	17.63
Total number of results						75		
Number of results within ± 30% of neat positive						41		
Percentage of results within ± 30% of neat positive						54.67%		
Number of results within ± 30% of median						58		
Percentage of results within ± 30% of median						77.33%		

Appendix III-6: Accuracy results for beads prepared using Pn23F Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	4.78	3.35	6.21	4.11	4.33	4.75
	2	0.05	4.69	3.28	6.10	4.93	4.93	5.49
	3	0.05	5.07	3.55	6.59	4.33	3.66	6.76
	4	0.05	5.99	4.19	7.79	4.71	4.23	7.44
	5	0.05	5.07	3.55	6.59	3.91	3.86	4.31
	Median				Result	4.33	4.23	5.49
					- 30 %	3.03	2.96	3.84
					+ 30 %	5.63	5.50	7.14
13-Plex 70	1	0.05	4.78	3.35	6.21	4.69	5.18	5.67
	2	0.05	4.69	3.28	6.10	4.48	5.19	5.12
	3	0.05	5.07	3.55	6.59	4.57	4.14	5.39
	4	0.05	5.99	4.19	7.79	4.19	5.71	8.19
	5	0.05	5.07	3.55	6.59	3.58	3.86	5.00
	Median				Result	4.48	5.18	5.39
					- 30 %	3.14	3.63	3.77
					+ 30 %	5.82	6.73	7.01
13-Plex 72	1	0.05	4.78	3.35	6.21	4.42	4.56	4.35
	2	0.05	4.69	3.28	6.10	4.44	4.48	5.75
	3	0.05	5.07	3.55	6.59	4.87	4.88	5.24
	4	0.05	5.99	4.19	7.79	4.88	5.64	6.74
	5	0.05	5.07	3.55	6.59	4.26	4.59	5.23
	Median				Result	4.44	4.59	5.24
					- 30 %	3.11	3.21	3.67
					+ 30 %	5.77	5.97	6.81
13-Plex 74	1	0.05	4.78	3.35	6.21	4.52	5.22	5.69
	2	0.05	4.69	3.28	6.10	4.79	5.07	5.65
	3	0.05	5.07	3.55	6.59	4.29	4.71	5.73
	4	0.05	5.99	4.19	7.79	4.71	5.61	7.64
	5	0.05	5.07	3.55	6.59	3.60	3.34	4.89
	Median				Result	4.52	5.07	5.69
					- 30 %	3.16	3.55	3.98
					+ 30 %	5.88	6.59	7.40
13-Plex 76	1	0.05	4.78	3.35	6.21	4.30	4.57	5.84
	2	0.05	4.69	3.28	6.10	4.94	5.21	5.48
	3	0.05	5.07	3.55	6.59	5.05	4.70	6.46
	4	0.05	5.99	4.19	7.79	3.98	6.98	6.11
	5	0.05	5.07	3.55	6.59	3.96	5.77	5.29
	Median				Result	4.30	5.21	5.84
					- 30 %	3.01	3.65	4.09
					+ 30 %	5.59	6.77	7.59
Total number of results						75		
Number of results within ± 30% of neat positive						71		
Percentage of results within ± 30% of neat positive						94.67%		
Number of results within ± 30% of median						72		
Percentage of results within ± 30% of median						96.00%		

Appendix III-7: Accuracy results for beads prepared using Pn1 Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	3.00	2.10	3.90	2.73	2.72	3.04
	2	0.05	3.23	2.26	4.20	3.24	2.99	3.77
	3	0.05	2.95	2.07	3.84	2.93	2.34	4.09
	4	0.05	3.82	2.67	4.97	3.03	2.93	5.19
	5	0.05	3.68	2.58	4.78	5.48	4.51	4.63
	Median				Result	3.03	2.93	4.09
					- 30 %	2.12	2.05	2.86
					+ 30 %	3.94	3.81	5.32
13-Plex 70	1	0.05	3.00	2.10	3.90	2.47	2.64	2.73
	2	0.05	3.23	2.26	4.20	2.99	3.05	3.57
	3	0.05	2.95	2.07	3.84	3.07	2.95	3.31
	4	0.05	3.82	2.67	4.97	3.35	3.97	4.43
	5	0.05	3.68	2.58	4.78	2.52	2.52	3.22
	Median				Result	2.99	2.95	3.31
					- 30 %	2.09	2.07	2.32
					+ 30 %	3.89	3.84	4.30
13-Plex 74	1	0.05	3.00	2.10	3.90	2.83	3.06	3.10
	2	0.05	3.23	2.26	4.20	3.16	3.40	3.58
	3	0.05	2.95	2.07	3.84	2.66	2.97	3.32
	4	0.05	3.82	2.67	4.97	3.08	3.40	4.59
	5	0.05	3.68	2.58	4.78	3.02	3.92	4.37
	Median				Result	3.02	3.40	3.58
					- 30 %	2.11	2.38	2.51
					+ 30 %	3.93	4.42	4.65
13-Plex 75	1	0.05	2.95	2.07	3.84	3.15	2.66	3.92
	2	0.05	3.82	2.67	4.97	3.29	2.95	5.58
	3	0.05	3.68	2.58	4.78	3.96	4.05	4.28
	4	0.05	3.70	2.59	4.81	3.36	4.33	9.86
	5	0.05	3.75	2.63	4.88	3.45	3.72	4.28
	Median				Result	3.36	3.72	4.28
					- 30 %	2.35	2.60	3.00
					+ 30 %	4.37	4.84	5.56
13-Plex 76	1	0.05	3.00	2.10	3.90	2.70	2.67	3.32
	2	0.05	3.23	2.26	4.20	3.05	3.33	3.80
	3	0.05	2.95	2.07	3.84	3.08	3.06	3.46
	4	0.05	3.82	2.67	4.97	2.89	4.91	4.00
	5	0.05	3.68	2.58	4.78	3.91	4.14	9.42
	Median				Result	3.05	3.33	3.80
					- 30 %	2.14	2.33	2.66
					+ 30 %	3.97	4.33	4.94
Total number of results						75		
Number of results within ± 30% of neat positive						67		
Percentage of results within ± 30% of neat positive						89.33%		
Number of results within ± 30% of median						68		
Percentage of results within ± 30% of median						90.67%		

Appendix III-8: Accuracy results for beads prepared using Pn3 Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	2.48	1.74	3.22	2.17	2.44	2.79
	2	0.05	2.18	1.53	2.83	2.29	2.28	2.79
	3	0.05	2.01	1.41	2.61	2.20	2.06	3.68
	4	0.05	2.68	1.88	3.48	2.46	2.53	4.37
	5	0.05	2.49	1.74	3.24	3.37	3.00	3.20
	Median				Result	2.29	2.44	3.20
					- 30 %	1.60	1.71	2.24
					+ 30 %	2.98	3.17	4.16
13-Plex 70	1	0.05	2.48	1.74	3.22	2.09	2.18	2.34
	2	0.05	2.18	1.53	2.83	1.97	2.31	2.64
	3	0.05	2.01	1.41	2.61	2.20	2.33	3.09
	4	0.05	2.49	1.74	3.24	2.44	2.44	3.77
	5	0.05	2.35	1.65	3.06	1.78	1.89	2.67
	Median				Result	2.09	2.31	2.67
					- 30 %	1.46	1.62	1.87
					+ 30 %	2.72	3.00	3.47
13-Plex 74	1	0.05	2.48	1.74	3.22	2.40	2.43	2.71
	2	0.05	2.18	1.53	2.83	2.03	2.42	2.63
	3	0.05	2.01	1.41	2.61	1.92	2.19	3.21
	4	0.05	2.68	1.88	3.48	2.19	2.91	3.68
	5	0.05	2.49	1.74	3.24	2.09	2.55	2.97
	Median				Result	2.09	2.43	2.97
					- 30 %	1.46	1.70	2.08
					+ 30 %	2.72	3.16	3.86
13-Plex 75	1	0.05	2.01	1.41	2.61	2.30	2.12	3.24
	2	0.05	2.68	1.88	3.48	2.42	2.50	4.54
	3	0.05	2.35	1.65	3.06	2.46	2.61	3.29
	4	0.05	2.30	1.61	2.99	2.24	2.94	7.00
	5	0.05	2.49	1.74	3.24	2.40	2.58	2.91
	Median				Result	2.40	2.58	3.29
					- 30 %	1.68	1.81	2.30
					+ 30 %	3.12	3.35	4.28
13-Plex 76	1	0.05	2.48	1.74	3.22	2.24	2.34	2.94
	2	0.05	2.18	1.53	2.83	2.12	2.43	2.78
	3	0.05	2.01	1.41	2.61	2.22	2.52	2.68
	4	0.05	2.68	1.88	3.48	2.37	3.77	3.20
	5	0.05	2.35	1.65	3.06	2.61	2.98	6.94
	Median				Result	2.24	2.52	2.94
					- 30 %	1.57	1.76	2.06
					+ 30 %	2.91	3.28	3.82
Total number of results						75		
Number of results within ± 30% of neat positive						61		
Percentage of results within ± 30% of neat positive						81.33%		
Number of results within ± 30% of median						67		
Percentage of results within ± 30% of median						89.33%		

Appendix III-9: Accuracy results for beads prepared using Pn5 Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	3.31	2.32	4.30	3.12	3.28	3.92
	2	0.05	3.42	2.39	4.45	3.40	3.45	4.50
	3	0.05	2.97	2.08	3.86	3.16	2.75	4.86
	4	0.05	3.94	2.76	5.12	3.29	3.46	5.91
	5	0.05	3.68	2.58	4.78	3.31	3.37	3.97
	Median				Result	3.29	3.37	4.50
					- 30 %	2.30	2.36	3.15
					+ 30 %	4.28	4.38	5.85
13-Plex 67	1	0.05	3.31	2.32	4.30	3.50	3.67	4.28
	2	0.05	3.42	2.39	4.45	3.14	3.81	3.94
	3	0.05	2.97	2.08	3.86	2.95	3.09	4.01
	4	0.05	3.94	2.76	5.12	3.10	4.10	5.71
	5	0.05	3.68	2.58	4.78	2.95	3.47	4.02
	Median				Result	3.10	3.67	4.02
					- 30 %	2.17	2.57	2.81
					+ 30 %	4.03	4.77	5.23
13-Plex 70	1	0.05	3.31	2.32	4.30	2.80	3.18	3.60
	2	0.05	3.42	2.39	4.45	3.34	3.61	4.38
	3	0.05	2.97	2.08	3.86	3.14	3.37	4.38
	4	0.05	3.94	2.76	5.12	3.56	4.26	5.32
	5	0.05	3.68	2.58	4.78	3.26	3.87	4.70
	Median				Result	3.26	3.61	4.38
					- 30 %	2.28	2.53	3.07
					+ 30 %	4.24	4.69	5.69
13-Plex 74	1	0.05	3.31	2.32	4.30	3.17	3.54	4.04
	2	0.05	3.42	2.39	4.45	3.29	3.86	4.37
	3	0.05	2.97	2.08	3.86	2.80	3.36	4.34
	4	0.05	3.94	2.76	5.12	3.30	4.08	5.50
	5	0.05	3.68	2.58	4.78	3.12	3.15	3.90
	Median				Result	3.17	3.54	4.34
					- 30 %	2.22	2.48	3.04
					+ 30 %	4.12	4.60	5.64
13-Plex 76	1	0.05	3.31	2.32	4.30	3.16	3.28	4.29
	2	0.05	3.42	2.39	4.45	3.24	3.85	4.54
	3	0.05	2.97	2.08	3.86	3.16	3.49	4.10
	4	0.05	3.94	2.76	5.12	3.10	5.00	4.75
	5	0.05	3.68	2.58	4.78	3.18	4.19	4.21
	Median				Result	3.16	3.85	4.29
					- 30 %	2.21	2.70	3.00
					+ 30 %	4.11	5.01	5.58
Total number of results						75		
Number of results within ± 30% of neat positive						64		
Percentage of results within ± 30% of neat positive						85.33%		
Number of results within ± 30% of median						73		
Percentage of results within ± 30% of median						97.33%		

Appendix III-10: Accuracy results for beads prepared using Pn6A Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	10.02	7.01	13.03	8.81	8.48	9.67
	2	0.05	10.28	7.20	13.36	9.53	8.96	11.36
	3	0.05	8.24	5.77	10.71	8.40	7.12	12.57
	4	0.05	11.90	8.33	15.47	9.37	9.27	15.81
	5	0.05	10.91	7.64	14.18	8.99	8.73	10.02
	Median				Result	8.99	8.73	11.36
					- 30 %	6.29	6.11	7.95
					+ 30 %	11.69	11.35	14.77
13-Plex 67	1	0.05	10.02	7.01	13.03	10.01	9.72	10.96
	2	0.05	10.28	7.20	13.36	8.66	9.89	10.25
	3	0.05	8.24	5.77	10.71	8.26	7.64	10.18
	4	0.05	11.90	8.33	15.47	8.61	11.20	15.23
	5	0.05	10.91	7.64	14.18	8.02	9.16	10.42
	Median				Result	8.61	9.72	10.42
					- 30 %	6.03	6.80	7.29
					+ 30 %	11.19	12.64	13.55
13-Plex 70	1	0.05	10.02	7.01	13.03	7.78	8.45	9.31
	2	0.05	10.28	7.20	13.36	8.88	8.98	11.30
	3	0.05	8.24	5.77	10.71	8.73	8.78	11.11
	4	0.05	11.90	8.33	15.47	9.90	12.10	14.63
	5	0.05	10.91	7.64	14.18	8.67	10.43	12.34
	Median				Result	8.73	8.98	11.30
					- 30 %	6.11	6.29	7.91
					+ 30 %	11.35	11.67	14.69
13-Plex 74	1	0.05	10.02	7.01	13.03	9.19	9.23	10.11
	2	0.05	10.28	7.20	13.36	9.28	10.20	10.86
	3	0.05	8.24	5.77	10.71	7.63	8.84	11.27
	4	0.05	11.90	8.33	15.47	9.21	11.30	15.07
	5	0.05	10.91	7.64	14.18	8.54	8.24	9.97
	Median				Result	9.19	9.23	10.86
					- 30 %	6.43	6.46	7.60
					+ 30 %	11.95	12.00	14.12
13-Plex 76	1	0.05	10.02	7.01	13.03	8.82	8.64	10.68
	2	0.05	10.28	7.20	13.36	8.73	9.78	11.16
	3	0.05	8.24	5.77	10.71	8.78	8.99	9.90
	4	0.05	11.90	8.33	15.47	8.80	14.29	12.70
	5	0.05	10.91	7.64	14.18	8.23	11.10	11.24
	Median				Result	8.78	9.78	11.16
					- 30 %	6.15	6.85	7.81
					+ 30 %	11.41	12.71	14.51
Total number of results						75		
Number of results within ± 30% of neat positive						71		
Percentage of results within ± 30% of neat positive						94.67%		
Number of results within ± 30% of median						70		
Percentage of results within ± 30% of median						93.33%		

Appendix III-11: Accuracy results for beads prepared using Pn7F Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	0.97	0.68	1.26	0.94	1.06	1.16
	2	0.05	1.17	0.82	1.52	1.31	1.21	1.69
	3	0.05	1.06	0.74	1.38	1.14	1.11	1.97
	4	0.05	1.13	0.79	1.47	1.01	1.34	1.35
	5	0.05	1.12	0.78	1.46	1.01	1.52	1.51
	Median				Result	1.01	1.21	1.51
					- 30 %	0.71	0.85	1.06
					+ 30 %	1.31	1.57	1.96
13-Plex 67	1	0.05	0.97	0.68	1.26	1.10	1.23	1.36
	2	0.05	1.17	0.82	1.52	1.06	1.34	1.54
	3	0.05	1.06	0.74	1.38	1.17	1.08	1.46
	4	0.05	1.13	0.79	1.47	1.03	1.13	1.30
	5	0.05	1.12	0.78	1.46	1.16	0.94	1.32
	Median				Result	1.10	1.13	1.36
					- 30 %	0.77	0.79	0.95
					+ 30 %	1.43	1.47	1.77
13-Plex 70	1	0.05	0.97	0.68	1.26	0.84	1.07	1.18
	2	0.05	1.17	0.82	1.52	1.02	1.35	1.67
	3	0.05	1.06	0.74	1.38	1.20	1.23	1.48
	4	0.05	1.13	0.79	1.47	1.09	1.35	1.58
	5	0.05	1.12	0.78	1.46	1.12	1.41	1.70
	Median				Result	1.09	1.35	1.58
					- 30 %	0.76	0.95	1.11
					+ 30 %	1.42	1.76	2.05
13-Plex 74	1	0.05	0.97	0.68	1.26	1.12	1.20	1.28
	2	0.05	1.17	0.82	1.52	1.33	1.32	1.62
	3	0.05	1.06	0.74	1.38	1.07	1.22	1.66
	4	0.05	1.13	0.79	1.47	1.00	1.26	1.24
	5	0.05	1.12	0.78	1.46	1.09	1.10	1.40
	Median				Result	1.09	1.22	1.40
					- 30 %	0.76	0.85	0.98
					+ 30 %	1.42	1.59	1.82
13-Plex 76	1	0.05	0.97	0.68	1.26	1.02	1.10	1.48
	2	0.05	1.17	0.82	1.52	1.22	1.35	1.59
	3	0.05	1.06	0.74	1.38	1.21	1.13	1.54
	4	0.05	1.13	0.79	1.47	1.10	1.04	1.34
	5	0.05	1.12	0.78	1.46	1.06	1.02	1.23
	Median				Result	1.10	1.10	1.48
					- 30 %	0.77	0.77	1.04
					+ 30 %	1.43	1.43	1.92
Total number of results						75		
Number of results within ± 30% of neat positive						57		
Percentage of results within ± 30% of neat positive						76.00%		
Number of results within ± 30% of median						74		
Percentage of results within ± 30% of median						98.67%		

Appendix III-12: Accuracy results for beads prepared using Pn18C Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	2.71	1.90	3.52	2.29	2.35	2.82
	2	0.05	2.94	2.06	3.82	2.76	2.59	3.21
	3	0.05	2.44	1.71	3.17	2.31	1.97	3.49
	4	0.05	3.57	2.50	4.64	2.64	2.55	4.31
	5	0.05	3.10	2.17	4.03	2.75	2.64	2.90
	Median				Result	2.64	2.55	3.21
					- 30 %	1.85	1.79	2.25
					+ 30 %	3.43	3.32	4.17
13-Plex 67	1	0.05	2.71	1.90	3.52	2.60	2.55	3.09
	2	0.05	2.94	2.06	3.82	2.60	2.99	2.96
	3	0.05	2.44	1.71	3.17	2.31	2.22	2.92
	4	0.05	3.57	2.50	4.64	2.29	3.01	4.18
	5	0.05	3.10	2.17	4.03	2.50	2.72	3.15
	Median				Result	2.50	2.72	3.09
					- 30 %	1.75	1.90	2.16
					+ 30 %	3.25	3.54	4.02
13-Plex 70	1	0.05	2.71	1.90	3.52	2.08	2.39	2.48
	2	0.05	2.94	2.06	3.82	2.73	2.65	3.02
	3	0.05	2.44	1.71	3.17	2.36	2.49	2.99
	4	0.05	3.57	2.50	4.64	2.74	3.29	3.71
	5	0.05	3.10	2.17	4.03	2.71	3.17	3.64
	Median				Result	2.71	2.65	3.02
					- 30 %	1.90	1.86	2.11
					+ 30 %	3.52	3.45	3.93
13-Plex 74	1	0.05	2.71	1.90	3.52	2.51	2.54	2.75
	2	0.05	2.94	2.06	3.82	2.69	2.91	3.13
	3	0.05	2.44	1.71	3.17	2.15	2.42	3.29
	4	0.05	3.57	2.50	4.64	2.67	3.07	4.14
	5	0.05	3.10	2.17	4.03	2.78	2.54	2.98
	Median				Result	2.67	2.54	3.13
					- 30 %	1.87	1.78	2.19
					+ 30 %	3.47	3.30	4.07
13-Plex 76	1	0.05	2.71	1.90	3.52	2.34	2.32	2.99
	2	0.05	2.94	2.06	3.82	2.66	2.85	3.22
	3	0.05	2.44	1.71	3.17	2.48	2.57	2.99
	4	0.05	3.57	2.50	4.64	2.41	3.96	3.59
	5	0.05	3.10	2.17	4.03	2.44	3.12	3.25
	Median				Result	2.44	2.85	3.22
					- 30 %	1.71	2.00	2.25
					+ 30 %	3.17	3.71	4.19
Total number of results						75		
Number of results within ± 30% of neat positive						71		
Percentage of results within ± 30% of neat positive						94.67%		
Number of results within ± 30% of median						72		
Percentage of results within ± 30% of median						96.00%		

Appendix III-13: Accuracy results for beads prepared using Pn19F Ps

Negative sample	Replicate	Negative Sample	Positive sample 13-Plex 08			Spiked Result adjusted for dilution factor		
		Neat	Result	- 30 %	+ 30 %	1/2	1/4	1/8
13-Plex 64	1	0.05	5.22	3.65	6.79	4.80	5.03	5.70
	2	0.05	5.49	3.84	7.14	5.33	5.46	6.64
	3	0.05	5.20	3.64	6.76	4.96	4.35	7.80
	4	0.05	6.78	4.75	8.81	5.51	5.48	9.40
	5	0.05	5.07	3.55	6.59	7.55	6.47	7.03
	Median				Result	5.33	5.46	7.03
					- 30 %	3.73	3.82	4.92
					+ 30 %	6.93	7.10	9.14
13-Plex 70	1	0.05	5.22	3.65	6.79	4.55	4.96	5.35
	2	0.05	5.49	3.84	7.14	5.19	5.46	6.52
	3	0.05	5.20	3.64	6.76	5.46	5.49	6.53
	4	0.05	6.78	4.75	8.81	5.71	7.02	8.44
	5	0.05	5.07	3.55	6.59	3.39	3.53	4.78
	Median				Result	5.19	5.46	6.52
					- 30 %	3.63	3.82	4.56
					+ 30 %	6.75	7.10	8.48
13-Plex 74	1	0.05	5.22	3.65	6.79	5.24	5.51	6.02
	2	0.05	5.49	3.84	7.14	5.31	5.89	6.66
	3	0.05	5.20	3.64	6.76	4.71	5.54	6.84
	4	0.05	6.78	4.75	8.81	5.34	6.42	8.79
	5	0.05	5.07	3.55	6.59	4.31	5.65	6.43
	Median				Result	5.24	5.65	6.66
					- 30 %	3.67	3.96	4.66
					+ 30 %	6.81	7.35	8.66
13-Plex 75	1	0.05	5.20	3.64	6.76	5.49	4.62	7.25
	2	0.05	6.78	4.75	8.81	5.72	5.44	9.61
	3	0.05	5.07	3.55	6.59	5.26	5.66	6.03
	4	0.05	5.28	3.70	6.86	4.68	5.92	13.70
	5	0.05	5.16	3.61	6.71	5.03	5.16	6.09
	Median				Result	5.26	5.44	7.25
					- 30 %	3.68	3.81	5.08
					+ 30 %	6.84	7.07	9.43
13-Plex 76	1	0.05	5.22	3.65	6.79	4.94	5.18	6.26
	2	0.05	5.49	3.84	7.14	5.30	5.87	6.68
	3	0.05	5.20	3.64	6.76	5.47	5.51	6.55
	4	0.05	6.78	4.75	8.81	4.96	8.41	7.61
	5	0.05	5.07	3.55	6.59	5.56	5.99	13.43
	Median				Result	5.30	5.87	6.68
					- 30 %	3.71	4.11	4.68
					+ 30 %	6.89	7.63	8.68
Total number of results						75		
Number of results within ± 30% of neat positive						64		
Percentage of results within ± 30% of neat positive						85.33%		
Number of results within ± 30% of median						71		
Percentage of results within ± 30% of median						94.67%		

Appendix IV. Assay Validation: Reproducibility

Appendix IV-1 Inter assay reproducibility results for beads prepared using Pn4 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 05	0.65	0.78	0.82	0.73	0.74	0.74	0.06	8.53 %
13-Plex 06	0.26	0.26	0.28	0.18	0.19	0.23	0.05	19.49 %
13-Plex 08	1.62	2.00	2.30	2.14	1.99	2.01	0.25	12.53 %
13-Plex 12	0.47	0.36	0.50	0.40	0.44	0.43	0.06	12.79 %
13-Plex 17	0.05	0.10	0.10	0.05	0.05	0.10	0.00	0.00 %
13-Plex 18	0.41	0.57	0.69	0.52	0.49	0.54	0.10	19.37 %
13-Plex 20	0.80	0.82	0.92	0.72	0.65	0.78	0.10	13.12 %
13-Plex 21	0.55	0.55	0.60	0.45	0.43	0.52	0.07	14.08 %
13-Plex 28	0.86	0.99	0.88	0.85	0.84	0.88	0.06	6.91 %
13-Plex 32	0.20	0.29	0.27	0.26	0.25	0.25	0.03	13.23 %
13-Plex 39	1.56	1.88	3.44	2.12	1.92	2.18	0.73	33.44 %
13-Plex 42	1.35	1.51	1.79	1.68	1.60	1.59	0.17	10.56 %
13-Plex 46	0.47	0.58	0.72	0.63	0.65	0.61	0.09	15.25 %
13-Plex 59	0.11	0.15	0.16	0.13	0.11	0.13	0.02	17.28 %
13-Plex 60	0.44	0.40	0.47	0.41	0.35	0.41	0.05	10.88 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.16	0.15	0.15	0.13	0.13	0.14	0.01	9.32 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								16
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								93.75 %

Appendix IV-2: Inter assay reproducibility results for beads prepared using Pn6B PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.25	0.26	0.20	0.20	0.18	0.22	0.03	16.02 %
13-Plex 05	0.37	0.44	0.28	0.27	0.29	0.33	0.07	22.16 %
13-Plex 08	4.04	5.05	6.48	4.98	4.87	5.08	0.88	17.31 %
13-Plex 09	9.43	16.06	16.02	12.33	12.28	13.22	2.83	21.37 %
13-Plex 10	3.28	3.16	3.76	3.04	3.20	3.29	0.28	8.45 %
13-Plex 11	2.87	2.66	3.27	2.45	2.72	2.79	0.31	10.95 %
13-Plex 18	1.10	1.50	1.85	1.23	1.29	1.39	0.29	21.02 %
13-Plex 20	0.34	0.55	0.49	0.37	0.35	0.42	0.09	22.46 %
13-Plex 21	1.23	1.51	1.61	1.03	1.06	1.29	0.26	20.35 %
13-Plex 25	0.20	0.29	0.19	0.20	0.18	0.21	0.04	20.94 %
13-Plex 28	1.04	1.12	1.16	1.04	1.02	1.08	0.06	5.64 %
13-Plex 31	6.47	10.33	14.08	9.35	8.86	9.82	2.77	28.25 %
13-Plex 42	0.44	0.64	0.58	0.43	0.45	0.51	0.10	18.85 %
13-Plex 46	0.69	0.86	0.91	0.70	0.74	0.78	0.10	12.72 %
13-Plex 50	1.35	1.53	2.11	1.34	1.43	1.55	0.32	20.69 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.13	0.05	0.05	0.05	0.07	0.04	
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-3: Inter assay reproducibility results generated for beads prepared using Pn9V PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.28	0.31	0.27	0.38	0.23	0.29	0.06	19.03%
13-Plex 05	1.09	1.07	1.24	1.35	1.07	1.16	0.13	10.83%
13-Plex 06	0.52	0.56	0.58	0.55	0.49	0.54	0.04	6.55%
13-Plex 08	2.89	2.96	3.68	2.96	2.54	3.01	0.42	13.81%
13-Plex 10	4.23	5.26	5.17	3.94	3.61	4.44	0.74	16.65%
13-Plex 11	1.18	1.18	1.28	1.41	1.11	1.23	0.12	9.46%
13-Plex 16	0.66	0.55	0.64	0.60	0.53	0.60	0.06	9.39%
13-Plex 17	0.17	0.15	0.15	0.16	0.16	0.16	0.01	5.30%
13-Plex 28	0.70	0.63	0.72	0.70	0.63	0.68	0.04	6.33%
13-Plex 31	0.44	0.41	0.54	0.50	0.46	0.47	0.05	10.85%
13-Plex 42	1.42	1.01	1.57	1.47	1.28	1.35	0.22	16.07%
13-Plex 46	0.58	0.51	0.60	0.58	0.50	0.55	0.05	8.23%
13-Plex 50	0.75	0.67	0.77	0.79	0.69	0.73	0.05	7.05%
13-Plex 51	0.21	0.19	0.24	0.23	0.18	0.21	0.03	12.14%
13-Plex 52	0.18	0.18	0.18	0.15	0.12	0.16	0.03	16.56%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.13	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with %CV of less than or equal to 25.00								15
Percentage of sample with %CV of less than or equal to 25.00								100.00 %

Appendix IV-4: Inter assay reproducibility results for beads prepared using Pn14 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.89	0.81	0.72	0.66	0.90	0.80	0.11	13.19 %
13-Plex 08	4.39	4.67	3.89	3.58	4.25	4.16	0.43	10.28 %
13-Plex 10	5.50	6.10	5.18	4.90	4.98	5.33	0.49	9.15 %
13-Plex 11	0.82	0.65	0.68	0.60	0.71	0.69	0.08	11.89 %
13-Plex 12	2.96	3.63	3.36	2.89	3.26	3.22	0.30	9.39 %
13-Plex 16	1.37	1.27	1.28	1.16	1.14	1.24	0.09	7.60 %
13-Plex 18	1.08	0.99	0.76	0.83	0.95	0.92	0.13	13.83 %
13-Plex 25	0.40	0.27	0.23	0.34	0.23	0.29	0.07	25.29 %
13-Plex 28	8.05	8.59	6.76	7.19	6.55	7.43	0.87	11.68 %
13-Plex 31	0.95	0.92	0.77	0.83	0.70	0.83	0.10	12.42 %
13-Plex 32	0.58	0.44	0.43	0.37	0.42	0.45	0.08	17.53 %
13-Plex 42	2.03	2.09	1.99	1.84	2.00	1.99	0.09	4.65 %
13-Plex 44	1.83	1.86	1.71	1.44	2.01	1.77	0.21	12.04 %
13-Plex 51	1.16	1.11	0.97	1.01	1.03	1.06	0.08	7.32 %
13-Plex 60	0.38	0.27	0.26	0.23	0.28	0.28	0.06	20.01 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-5: Inter assay reproducibility results for beads prepared using Pn19A PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	1.17	1.05	0.88	1.42	0.82	1.07	0.24	22.52%
13-Plex 05	7.51	7.50	5.69	7.28	6.16	6.83	0.85	12.39%
13-Plex 06	0.46	0.41	0.38	0.59	0.34	0.44	0.10	22.15%
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 10	21.55	16.69	13.53	19.92	18.99	18.14	3.12	17.19%
13-Plex 11	6.26	5.16	3.81	11.64	7.47	6.87	2.99	43.54%
13-Plex 16	4.26	3.70	2.96	4.95	3.49	3.87	0.76	19.67%
13-Plex 17	1.31	1.15	0.94	1.41	1.01	1.16	0.20	16.96%
13-Plex 18	10.93	7.52	5.32	8.56	5.60	7.59	2.30	30.36%
13-Plex 21	0.65	0.56	0.47	0.78	0.47	0.59	0.13	22.46%
13-Plex 25	1.25	1.18	0.94	1.41	1.02	1.16	0.19	16.07%
13-Plex 32	0.65	0.63	0.47	0.33	0.35	0.49	0.15	30.99%
13-Plex 33	0.54	0.42	0.33	0.51	0.45	0.45	0.08	18.26%
13-Plex 34	1.07	0.86	0.71	1.13	0.80	0.91	0.18	19.61%
13-Plex 44	0.44	0.34	0.26	0.44	0.26	0.35	0.09	25.89%
13-Plex 51	0.23	0.16	0.15	0.32	0.23	0.22	0.07	31.35%
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								10
Percentage of sample with % CV of less than or equal to 25.00								66.67 %

Appendix IV-6: Inter assay reproducibility results for beads prepared using Pn23F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 05	2.63	2.70	2.34	2.39	2.77	2.57	0.19	7.44 %
13-Plex 09	1.84	2.01	1.78	1.81	1.80	1.85	0.09	5.04 %
13-Plex 10	6.29	6.96	6.34	5.94	5.21	6.15	0.64	10.41 %
13-Plex 11	5.57	5.58	5.32	5.42	4.78	5.33	0.33	6.15 %
13-Plex 12	2.63	3.87	3.07	3.33	3.12	3.20	0.45	14.08 %
13-Plex 16	0.23	0.20	0.20	0.18	0.17	0.20	0.02	11.75 %
13-Plex 18	1.90	2.49	1.81	1.92	1.66	1.96	0.32	16.14 %
13-Plex 20	2.14	2.57	1.93	1.81	2.21	2.13	0.29	13.72 %
13-Plex 21	3.73	4.21	3.25	3.30	3.56	3.61	0.39	10.75 %
13-Plex 28	0.52	0.52	0.45	0.46	0.53	0.50	0.04	7.62 %
13-Plex 34	0.48	0.47	0.40	0.38	0.43	0.43	0.04	10.01 %
13-Plex 42	0.78	0.91	0.71	0.71	0.62	0.75	0.11	14.46 %
13-Plex 46	0.70	0.74	0.60	0.65	0.69	0.68	0.05	7.87 %
13-Plex 50	1.55	2.13	1.64	1.69	1.61	1.72	0.23	13.49 %
13-Plex 59	0.36	0.34	0.31	0.31	0.45	0.35	0.06	16.30 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-7: Inter assay reproducibility results for beads prepared using Pn1 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.15	0.15	0.15	0.18	0.15	0.16	0.01	8.60 %
13-Plex 08	23.02	25.16	29.96	24.43	24.91	25.50	2.63	10.31 %
13-Plex 10	0.11	0.09	0.11	0.07	0.07	0.09	0.02	22.22 %
13-Plex 11	4.53	5.5	3.45	2.92	3.19	3.92	1.07	27.44 %
13-Plex 12	4.76	3.62	5.66	4.21	4.54	4.56	0.75	16.48 %
13-Plex 16	5.51	4.83	4.51	3.83	3.42	4.42	0.82	18.63 %
13-Plex 18	0.33	0.31	0.31	0.25	0.26	0.29	0.03	11.96 %
13-Plex 20	0.12	0.12	0.09	0.08	0.07	0.10	0.02	23.98 %
13-Plex 21	0.16	0.16	0.13	0.12	0.13	0.14	0.02	13.36 %
13-Plex 28	0.14	0.10	0.10	0.09	0.09	0.10	0.02	19.94 %
13-Plex 31	0.87	1.79	1.61	1.35	1.27	1.38	0.35	25.50 %
13-Plex 32	0.68	0.72	0.71	0.49	0.56	0.63	0.10	16.11 %
13-Plex 34	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	1.91	1.92	2.15	1.95	1.80	1.95	0.13	6.55 %
13-Plex 50	0.79	0.65	0.70	0.63	0.59	0.67	0.08	11.45 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								14
Number of samples with % CV of less than or equal to 25.00								12
Percentage of sample with % CV of less than or equal to 25.00								85.71 %

Appendix IV-8: Inter assay reproducibility results for beads prepared using Pn3 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	%CV
13-Plex 03	0.27	0.31	0.27	0.30	0.37	0.30	0.04	13.48%
13-Plex 08	2.96	3.49	2.41	3.28	5.35	3.50	1.11	31.80%
13-Plex 10	1.04	1.03	1.02	0.77	0.86	0.94	0.12	12.94%
13-Plex 16	0.71	0.69	0.67	0.78	0.95	0.76	0.11	15.00%
13-Plex 18	3.56	3.91	3.07	3.68	5.02	3.85	0.72	18.80%
13-Plex 20	1.69	2.12	1.80	2.19	2.46	2.05	0.31	15.11%
13-Plex 21	3.86	5.33	3.30	5.41	6.74	4.93	1.37	27.73%
13-Plex 28	0.23	0.20	0.27	0.14	0.14	0.20	0.06	29.00%
13-Plex 32	0.34	0.53	0.51	0.43	0.73	0.51	0.14	28.54%
13-Plex 34	1.70	1.51	1.60	1.06	1.50	1.47	0.25	16.63%
13-Plex 44	0.60	0.57	0.72	0.49	0.64	0.60	0.09	14.08%
13-Plex 46	7.41	11.19	8.71	12.29	9.71	9.86	1.94	19.65%
13-Plex 51	0.60	0.77	0.77	0.71	0.96	0.76	0.13	17.15%
13-Plex 52	0.38	0.45	0.40	0.36	0.40	0.40	0.03	8.41%
13-Plex 59	0.23	0.28	0.32	0.17	0.19	0.24	0.06	26.14%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with %CV of less than or equal to 25.00								10
Percentage of sample with %CV of less than or equal to 25.00								66.67 %

Appendix IV-9: Inter assay reproducibility results for beads prepared using Pn5 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 08	4.19	4.95	5.38	4.78	5.03	4.87	0.44	8.97 %
13-Plex 09	0.61	0.63	0.67	0.50	0.58	0.60	0.06	10.67 %
13-Plex 10	0.21	0.25	0.22	0.20	0.21	0.22	0.02	8.82 %
13-Plex 11	2.85	2.74	2.87	2.51	2.56	2.71	0.16	6.09 %
13-Plex 12	3.08	2.87	3.60	3.11	3.33	3.20	0.28	8.68 %
13-Plex 18	1.78	1.84	2.12	1.54	1.76	1.81	0.21	11.51 %
13-Plex 20	1.13	1.37	1.51	1.12	1.21	1.27	0.17	13.27 %
13-Plex 28	0.25	0.27	0.34	0.24	0.24	0.27	0.04	15.70 %
13-Plex 31	0.19	0.17	0.26	0.15	0.19	0.19	0.04	21.60 %
13-Plex 33	0.15	0.17	0.26	0.16	0.18	0.18	0.04	23.88 %
13-Plex 44	1.28	1.40	1.62	1.25	1.40	1.39	0.15	10.47 %
13-Plex 46	0.76	0.84	1.13	0.72	0.80	0.85	0.16	19.15 %
13-Plex 50	6.45	9.27	9.49	8.18	8.23	8.32	1.20	14.46 %
13-Plex 51	0.51	0.41	0.57	0.41	0.46	0.47	0.07	14.56 %
13-Plex 62	1.20	1.24	1.41	1.12	1.22	1.24	0.11	8.59 %
13-Plex 59	0.05	0.05	0.11	0.05	0.05	0.06	0.03	-
13-Plex 71	0.10	0.10	0.15	0.05	0.11	0.12	0.02	20.70 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								16
Number of samples with % CV of less than or equal to 25.00								16
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-10: Inter assay reproducibility results for beads prepared using Pn6A PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.27	0.31	0.26	0.29	0.30	0.29	0.02	7.25 %
13-Plex 05	1.35	1.67	1.76	1.31	1.71	1.56	0.21	13.64 %
13-Plex 08	6.25	6.62	6.28	4.37	6.22	5.95	0.90	15.08 %
13-Plex 10	4.35	3.71	3.64	3.47	2.81	3.60	0.55	15.34 %
13-Plex 11	4.02	4.58	4.95	3.96	4.77	4.46	0.45	10.00 %
13-Plex 17	0.19	0.19	0.19	0.18	0.14	0.18	0.02	12.18 %
13-Plex 18	1.70	1.78	1.88	1.60	1.68	1.73	0.11	6.16 %
13-Plex 21	1.75	2.18	1.83	1.66	1.83	1.85	0.20	10.67 %
13-Plex 31	1.41	1.66	1.58	1.26	1.75	1.53	0.20	12.85 %
13-Plex 34	0.17	0.19	0.28	0.13	0.19	0.19	0.05	28.62 %
13-Plex 42	1.73	2.18	2.07	1.60	2.03	1.92	0.25	12.76 %
13-Plex 46	1.90	1.73	1.86	1.62	1.51	1.72	0.16	9.45 %
13-Plex 50	4.12	5.47	6.14	4.89	4.53	5.03	0.79	15.79 %
13-Plex 51	0.41	0.52	0.63	0.43	0.43	0.48	0.09	19.03 %
13-Plex 52	0.11	0.14	0.14	0.11	0.11	0.12	0.02	13.47%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-11: Inter assay reproducibility results for beads prepared using Pn7F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 05	1.29	1.18	1.29	1.05	1.07	1.18	0.12	9.80 %
13-Plex 08	4.56	4.93	5.04	5.12	5.16	4.96	0.24	4.86 %
13-Plex 12	0.72	0.50	0.75	0.55	0.60	0.62	0.11	17.28 %
13-Plex 16	1.47	1.49	1.66	1.24	1.29	1.43	0.17	11.80 %
13-Plex 17	0.89	0.59	0.74	0.65	0.79	0.73	0.12	16.06 %
13-Plex 18	0.92	0.99	1.08	0.77	0.83	0.92	0.12	13.46 %
13-Plex 20	0.46	0.47	0.51	0.36	0.42	0.44	0.06	12.80 %
13-Plex 28	6.33	5.88	5.94	7.13	6.18	6.29	0.50	7.99 %
13-Plex 31	0.89	1.00	1.21	0.93	1.07	1.02	0.13	12.40 %
13-Plex 34	3.93	3.68	3.38	3.26	3.44	3.54	0.27	7.55 %
13-Plex 39	1.99	1.67	2.67	1.73	1.75	1.96	0.41	21.11 %
13-Plex 42	3.16	3.03	3.58	2.93	3.04	3.15	0.25	8.10 %
13-Plex 46	1.79	1.50	1.98	1.49	1.60	1.67	0.21	12.57 %
13-Plex 51	0.24	0.21	0.30	0.18	0.20	0.23	0.05	20.66 %
13-Plex 52	0.14	0.10	0.15	0.10	0.10	0.12	0.02	21.10 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-12: Inter assay reproducibility results for beads prepared using Pn18C PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.18	0.18	0.23	0.20	0.18	0.19	0.02	11.29 %
13-Plex 05	2.37	2.91	2.73	2.54	2.67	2.64	0.20	7.68 %
13-Plex 06	1.11	1.28	1.31	1.11	1.09	1.18	0.11	8.97 %
13-Plex 09	4.62	4.19	3.93	4.02	4.06	4.16	0.27	6.52 %
13-Plex 10	0.60	0.76	0.57	0.60	0.64	0.63	0.07	11.78 %
13-Plex 16	2.01	2.45	2.48	2.28	2.20	2.28	0.19	8.43 %
13-Plex 17	0.47	0.54	0.63	0.53	0.58	0.55	0.06	10.83 %
13-Plex 18	0.65	0.83	0.88	0.67	0.72	0.75	0.10	13.43 %
13-Plex 21	3.14	3.30	2.94	2.54	2.61	2.91	0.33	11.32 %
13-Plex 28	4.24	4.34	4.55	4.74	4.36	4.45	0.20	4.47 %
13-Plex 34	4.69	5.43	5.14	5.49	4.78	5.11	0.37	7.15 %
13-Plex 39	0.88	0.92	1.36	0.90	0.97	1.01	0.20	19.95 %
13-Plex 46	1.72	2.09	2.30	1.94	2.06	2.02	0.21	10.53 %
13-Plex 50	1.41	1.86	2.03	1.57	1.68	1.71	0.24	14.19 %
13-Plex 62	0.44	0.47	0.53	0.43	0.45	0.46	0.04	8.57 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-13: Inter assay reproducibility results for beads prepared using Pn19F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	9.36	10.37	11.43	14.34	10.83	11.27	1.88	16.67 %
13-Plex 05	2.83	3.39	3.34	2.79	3.03	3.08	0.28	9.09 %
13-Plex 08	4.64	5.48	6.18	5.35	5.65	5.46	0.56	10.19 %
13-Plex 10	9.15	11.47	7.68	10.96	10.03	9.86	1.51	15.29 %
13-Plex 11	6.84	7.35	7.48	6.93	6.76	7.07	0.32	4.56 %
13-Plex 12	1.01	0.85	1.26	0.90	1.01	1.01	0.16	15.73 %
13-Plex 16	1.45	1.62	1.87	1.40	1.54	1.58	0.18	11.72 %
13-Plex 18	5.38	7.10	7.58	5.56	5.84	6.29	0.99	15.66 %
13-Plex 20	2.09	2.52	2.77	2.12	2.35	2.37	0.28	12.00 %
13-Plex 25	0.16	0.20	0.25	0.19	0.17	0.19	0.04	18.08 %
13-Plex 28	0.74	0.79	0.99	0.80	0.75	0.81	0.10	12.49 %
13-Plex 32	0.58	0.47	0.50	0.43	0.54	0.50	0.06	11.62 %
13-Plex 44	0.89	1.04	1.29	0.84	1.00	1.01	0.18	17.31 %
13-Plex 50	3.62	5.05	5.85	4.26	4.58	4.67	0.84	17.96 %
13-Plex 51	0.32	0.25	0.35	0.23	0.26	0.28	0.05	17.98 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with %CV of less than or equal to 25.00								15
Percentage of sample with %CV of less than or equal to 25.00								100.00 %

Appendix IV-14: Intra assay reproducibility results for beads prepared using Pn4 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 05	0.66	0.72	0.75	0.85	0.77	0.75	0.07	9.29 %
13-Plex 06	0.55	0.23	0.29	0.25	0.24	0.31	0.13	43.26 %
13-Plex 08	2.14	1.96	1.94	2.32	2.17	2.11	0.16	7.50 %
13-Plex 12	0.40	0.55	0.49	0.46	0.49	0.48	0.05	11.40 %
13-Plex 17	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 18	0.45	0.51	0.49	0.44	0.44	0.47	0.03	6.89 %
13-Plex 20	0.75	0.44	0.69	0.71	0.63	0.64	0.12	18.94 %
13-Plex 21	0.33	0.31	0.36	0.37	0.44	0.36	0.05	13.73 %
13-Plex 22	1.50	1.43	1.53	1.47	1.51	1.49	0.04	2.62 %
13-Plex 28	0.58	0.59	0.69	0.61	0.64	0.62	0.04	7.14 %
13-Plex 32	0.20	0.24	0.24	0.27	0.23	0.24	0.03	10.64 %
13-Plex 42	1.24	1.24	1.44	1.24	1.33	1.30	0.09	6.81 %
13-Plex 46	0.50	0.50	0.51	0.42	0.46	0.48	0.04	7.88 %
13-Plex 59	0.10	0.12	0.11	0.11	0.11	0.11	0.01	6.43 %
13-Plex 60	0.32	0.30	0.29	0.32	0.31	0.31	0.01	4.23 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.10	0.11	0.10	0.10	0.11	0.10	0.01	5.27 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-15: Intra assay reproducibility results for beads prepared using Pn6B PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.16	0.14	0.15	0.18	0.18	0.16	0.02	11.04 %
13-Plex 05	0.22	0.28	0.27	0.33	0.27	0.27	0.04	14.28 %
13-Plex 08	4.78	4.56	4.14	4.96	5.16	4.72	0.39	8.32 %
13-Plex 10	2.66	2.72	3.24	3.26	2.93	2.96	0.28	9.50 %
13-Plex 18	1.31	1.43	1.43	1.34	1.31	1.36	0.06	4.51 %
13-Plex 20	0.44	0.27	0.40	0.42	0.39	0.38	0.07	17.33 %
13-Plex 21	0.95	0.95	1.11	1.18	1.38	1.11	0.18	16.12 %
13-Plex 22	4.23	4.54	4.66	4.69	4.35	4.49	0.20	4.43 %
13-Plex 25	0.17	0.20	0.20	0.18	0.18	0.19	0.01	7.21 %
13-Plex 28	0.76	0.80	0.89	0.83	0.89	0.83	0.06	6.81 %
13-Plex 31	10.30	9.26	10.42	9.85	10.20	10.01	0.47	4.68 %
13-Plex 42	0.49	0.51	0.56	0.52	0.51	0.52	0.03	5.00 %
13-Plex 46	0.58	0.60	0.57	0.51	0.58	0.57	0.03	6.02 %
13-Plex 50	1.32	1.37	1.34	1.43	1.40	1.37	0.04	3.24 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.17	0.19	0.16	0.17	0.18	0.17	0.01	6.55 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-16: Intra assay reproducibility results for beads prepared using Pn9V PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.29	0.28	0.28	0.33	0.38	0.31	0.04	13.86 %
13-Plex 05	0.99	1.18	1.17	1.29	1.12	1.15	0.11	9.47 %
13-Plex 06	0.86	0.46	0.50	0.42	0.45	0.54	0.18	33.88 %
13-Plex 08	3.31	3.32	2.67	3.49	3.41	3.24	0.33	10.09 %
13-Plex 10	3.85	4.18	4.96	4.38	3.80	4.23	0.47	11.12 %
13-Plex 16	0.48	0.58	0.61	0.62	0.77	0.61	0.10	17.04 %
13-Plex 20	3.16	1.99	3.15	3.13	2.93	2.87	0.50	17.48 %
13-Plex 22	1.81	1.80	1.89	1.85	1.80	1.83	0.04	2.15 %
13-Plex 28	0.49	0.52	0.55	0.50	0.60	0.53	0.04	8.34 %
13-Plex 31	0.56	0.46	0.55	0.58	0.54	0.54	0.05	8.56 %
13-Plex 42	1.26	1.38	1.46	1.42	1.35	1.37	0.08	5.53 %
13-Plex 46	0.54	0.55	0.56	0.48	0.51	0.53	0.03	6.20 %
13-Plex 50	0.75	0.66	0.70	0.75	0.74	0.72	0.04	5.47 %
13-Plex 51	0.14	0.17	0.21	0.19	0.16	0.17	0.03	15.53 %
13-Plex 52	0.13	0.13	0.14	0.12	0.14	0.13	0.01	6.34 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-17: Intra assay reproducibility results for beads prepared using Pn14 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.65	0.59	0.61	0.70	0.75	0.66	0.07	9.94 %
13-Plex 08	4.34	4.00	3.65	4.42	4.33	4.15	0.32	7.75 %
13-Plex 10	4.40	4.58	5.20	5.01	5.19	4.88	0.37	7.51 %
13-Plex 12	2.65	3.72	3.31	3.23	3.56	3.29	0.41	12.44 %
13-Plex 16	0.74	0.82	0.84	0.93	1.06	0.88	0.12	13.91 %
13-Plex 18	0.79	0.84	0.84	0.77	0.75	0.80	0.04	5.12 %
13-Plex 22	2.66	2.89	2.97	2.94	2.89	2.87	0.12	4.26 %
13-Plex 25	0.19	0.22	0.22	0.20	0.19	0.20	0.02	7.43 %
13-Plex 28	4.82	5.22	5.75	5.69	6.34	5.56	0.58	10.34 %
13-Plex 31	0.89	0.77	0.89	0.91	0.91	0.87	0.06	6.75 %
13-Plex 32	0.33	0.36	0.38	0.39	0.36	0.36	0.02	6.32 %
13-Plex 42	1.09	1.17	1.34	1.23	1.20	1.21	0.09	7.57 %
13-Plex 44	1.08	1.31	1.25	1.18	1.10	1.18	0.10	8.25 %
13-Plex 51	0.67	0.71	0.83	0.75	0.69	0.73	0.06	8.66 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.18	0.17	0.19	0.19	0.18	0.18	0.01	4.60 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-18: Intra assay reproducibility results for beads prepared using Pn19A PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.59	0.49	0.54	0.68	0.73	0.61	0.10	16.28 %
13-Plex 05	7.51	8.34	9.37	11.22	8.62	9.01	1.40	15.56 %
13-Plex 06	2.83	0.29	0.45	0.27	0.29	0.83	1.12	135.91 %
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 10	26.32	26.40	30.78	26.69	25.28	27.09	2.13	7.86 %
13-Plex 16	3.94	4.58	5.08	5.54	6.81	5.19	1.08	20.87 %
13-Plex 17	0.58	0.77	0.66	0.72	0.77	0.70	0.08	11.56 %
13-Plex 18	9.33	9.26	9.04	8.75	7.19	8.71	0.88	10.12 %
13-Plex 21	0.39	0.44	0.43	0.43	0.52	0.44	0.05	10.78 %
13-Plex 22	2.42	2.16	2.37	2.31	2.39	2.33	0.10	4.43 %
13-Plex 25	1.12	1.28	1.29	1.16	1.09	1.19	0.09	7.75 %
13-Plex 32	0.37	0.42	0.45	0.46	0.41	0.42	0.04	8.44 %
13-Plex 33	0.53	0.54	0.60	0.51	0.53	0.54	0.03	6.31 %
13-Plex 34	0.64	0.62	0.60	0.66	0.74	0.65	0.05	8.29 %
13-Plex 46	1.90	2.09	1.77	1.67	1.73	1.83	0.17	9.12 %
13-Plex 51	0.05	0.05	0.10	0.05	0.05	0.06	0.02	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								14
Number of samples with % CV of less than or equal to 25.00								13
Percentage of sample with % CV of less than or equal to 25.00								92.86 %

Appendix IV-19: Intra assay reproducibility results generated for beads prepared using Pn23F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 05	2.31	2.51	2.47	2.94	2.79	2.60	0.26	9.80 %
13-Plex 10	5.91	5.53	6.91	7.01	6.40	6.35	0.64	10.01 %
13-Plex 12	2.74	3.63	3.53	3.02	3.71	3.33	0.42	12.74 %
13-Plex 16	0.15	0.16	0.17	0.18	0.22	0.18	0.03	15.35 %
13-Plex 18	1.74	1.90	2.02	1.73	1.80	1.84	0.12	6.65 %
13-Plex 20	2.12	1.37	2.10	2.01	1.89	1.90	0.31	16.27 %
13-Plex 21	2.50	2.40	2.88	2.70	3.78	2.85	0.55	19.31 %
13-Plex 22	4.63	4.66	4.90	4.73	4.51	4.69	0.14	3.07 %
13-Plex 28	0.32	0.36	0.40	0.37	0.36	0.36	0.03	7.91 %
13-Plex 34	0.36	0.35	0.33	0.34	0.37	0.35	0.02	4.52 %
13-Plex 42	0.82	0.86	0.95	0.90	0.93	0.89	0.05	5.90 %
13-Plex 46	0.56	0.58	0.58	0.50	0.50	0.54	0.04	7.53 %
13-Plex 50	1.64	1.53	1.55	1.70	1.69	1.62	0.08	4.84 %
13-Plex 59	0.23	0.28	0.25	0.26	0.26	0.26	0.02	7.10 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								14
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-20: Intra assay reproducibility results for beads prepared using Pn1 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	%CV
13-Plex 03	0.13	0.15	0.14	0.14	0.14	0.14	0.01	5.05 %
13-Plex 08	32.08	31.43	30.68	30.05	30.68	30.98	0.78	2.53 %
13-Plex 10	0.05	0.10	0.05	0.05	0.13	0.12	0.02	18.45 %
13-Plex 12	5.61	4.99	4.01	4.8	5.08	4.90	0.58	11.85 %
13-Plex 16	5.30	5.99	5.86	6.18	5.77	5.82	0.33	5.65 %
13-Plex 18	0.69	0.39	0.51	0.33	0.34	0.45	0.15	33.42 %
13-Plex 20	0.12	0.05	0.13	0.13	0.12	0.13	0.01	4.62 %
13-Plex 21	0.15	0.27	0.16	0.17	0.20	0.21	0.08	40.41 %
13-Plex 22	2.55	2.58	2.44	2.72	2.75	2.61	0.13	4.89 %
13-Plex 28	0.11	0.14	0.15	0.13	0.75	0.13	0.02	15.61 %
13-Plex 31	1.51	1.47	1.45	1.51	1.49	1.49	0.03	1.75 %
13-Plex 32	0.36	0.51	0.53	0.46	0.49	0.47	0.07	14.19 %
13-Plex 34	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	2.08	2.25	2.27	2.34	2.26	2.24	0.10	4.29 %
13-Plex 50	0.79	0.79	0.81	0.84	0.80	0.81	0.02	2.57 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								14
Number of samples with %CV of less than or equal to 25.00								12
Percentage of sample with %CV of less than or equal to 25.00								85.71 %

Appendix IV-21: Intra assay reproducibility results for beads prepared using Pn3 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 08	5.01	8.79	8.27	5.66	5.21	6.59	1.80	27.29 %
13-Plex 10	0.58	0.65	0.55	0.56	0.79	0.63	0.10	15.92 %
13-Plex 12	1.65	2.35	1.45	1.50	1.74	1.74	0.36	20.78 %
13-Plex 16	0.70	0.95	0.94	1.06	0.96	0.92	0.13	14.44 %
13-Plex 18	4.24	5.04	5.13	3.98	3.93	4.46	0.58	12.99 %
13-Plex 20	2.30	1.36	2.19	2.33	2.21	2.08	0.41	19.52 %
13-Plex 21	5.86	5.97	6.00	6.92	8.45	6.64	1.10	16.53 %
13-Plex 28	0.11	0.12	0.13	0.11	0.21	0.14	0.04	31.02 %
13-Plex 32	0.11	0.16	0.17	0.14	0.15	0.15	0.02	15.77 %
13-Plex 34	0.97	1.10	0.88	0.97	1.00	0.98	0.08	8.02 %
13-Plex 44	0.30	0.39	0.38	0.35	0.35	0.35	0.04	9.91 %
13-Plex 46	5.50	5.85	6.55	4.20	6.01	5.62	0.88	15.66 %
13-Plex 51	0.91	0.93	1.07	1.05	0.83	0.96	0.10	10.50 %
13-Plex 52	0.23	0.23	0.31	0.23	0.27	0.25	0.04	14.09 %
13-Plex 59	0.18	0.15	0.14	0.17	0.17	0.16	0.02	10.14 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								13
Percentage of sample with % CV of less than or equal to 25.00								86.67 %

Appendix IV-22: Intra assay reproducibility results for beads prepared using Pn5 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 08	3.81	4.54	4.40	3.87	3.84	4.09	0.35	8.53 %
13-Plex 10	0.24	0.24	0.20	0.20	0.24	0.22	0.02	9.78 %
13-Plex 12	2.91	2.64	2.34	2.77	2.55	2.64	0.22	8.20 %
13-Plex 18	1.81	1.80	1.88	1.49	1.45	1.69	0.20	11.87 %
13-Plex 20	1.16	0.79	1.21	1.18	1.15	1.10	0.17	15.82 %
13-Plex 22	0.91	0.97	0.98	0.96	0.94	0.95	0.03	2.91 %
13-Plex 28	0.24	0.25	0.27	0.26	0.28	0.26	0.02	6.08 %
13-Plex 31	0.19	0.17	0.17	0.18	0.20	0.18	0.01	7.16 %
13-Plex 33	0.16	0.17	0.19	0.16	0.17	0.17	0.01	7.20 %
13-Plex 44	1.11	1.20	1.38	1.24	1.21	1.23	0.10	7.97 %
13-Plex 46	0.63	0.70	0.79	0.68	0.69	0.70	0.06	8.32 %
13-Plex 50	6.94	6.65	7.00	6.75	6.87	6.84	0.14	2.08 %
13-Plex 51	0.41	0.43	0.46	0.45	0.40	0.43	0.03	5.93 %
13-Plex 59	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.19	0.18	0.18	0.20	0.19	0.19	0.01	4.45 %
13-Plex 62	1.04	1.10	1.17	1.21	1.07	1.12	0.07	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								14
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-23: Intra assay reproducibility results for beads prepared using Pn6A PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.20	0.26	0.25	0.23	0.24	0.24	0.02	9.75 %
13-Plex 05	1.49	1.47	1.37	1.33	1.55	1.44	0.09	6.25 %
13-Plex 08	4.85	6.06	6.04	5.03	4.96	5.39	0.61	11.28 %
13-Plex 10	3.14	3.28	2.83	2.72	3.78	3.15	0.42	13.29 %
13-Plex 17	0.19	0.24	0.23	0.23	0.23	0.22	0.02	8.70 %
13-Plex 18	1.75	1.81	1.72	1.42	1.42	1.62	0.19	11.64 %
13-Plex 21	1.85	2.02	2.03	2.17	2.31	2.08	0.17	8.34 %
13-Plex 22	5.35	5.73	5.58	5.65	5.94	5.65	0.22	3.81 %
13-Plex 31	1.37	1.35	1.38	1.45	1.51	1.41	0.07	4.71 %
13-Plex 34	0.19	0.19	0.14	0.17	0.18	0.17	0.02	11.92 %
13-Plex 42	1.58	1.62	1.70	1.75	1.74	1.68	0.07	4.47 %
13-Plex 46	1.46	1.57	1.85	1.64	1.62	1.63	0.14	8.75 %
13-Plex 50	4.85	4.62	4.81	4.87	4.87	4.80	0.11	2.20 %
13-Plex 51	0.49	0.52	0.56	0.58	0.50	0.53	0.04	7.31 %
13-Plex 52	0.12	0.11	0.15	0.12	0.14	0.13	0.02	12.84 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with %CV of less than or equal to 25.00								15
Percentage of sample with %CV of less than or equal to 25.00								100.00 %

Appendix IV-24: Intra assay reproducibility results for beads prepared using Pn7F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	%CV
13-Plex 05	1.11	0.98	1.06	1.03	1.10	1.06	0.05	5.04%
13-Plex 08	3.35	4.20	3.88	3.58	3.29	3.66	0.38	10.40%
13-Plex 12	0.56	0.65	0.47	0.54	0.52	0.55	0.07	12.06%
13-Plex 16	1.13	1.29	1.26	1.22	1.21	1.22	0.06	4.96%
13-Plex 17	0.51	0.71	0.65	0.59	0.58	0.61	0.08	12.44%
13-Plex 18	0.88	0.89	0.87	0.77	0.79	0.84	0.06	6.63%
13-Plex 20	0.39	0.28	0.39	0.39	0.36	0.36	0.05	13.16%
13-Plex 21	0.80	0.75	0.87	0.94	0.99	0.87	0.10	11.29%
13-Plex 28	4.13	4.36	5.15	4.92	5.08	4.73	0.46	9.65%
13-Plex 31	1.29	1.21	1.22	1.29	1.30	1.26	0.04	3.43%
13-Plex 34	2.81	3.19	2.51	2.79	3.28	2.92	0.32	10.84%
13-Plex 42	2.08	2.29	2.41	2.58	2.33	2.34	0.18	7.79%
13-Plex 46	1.22	1.39	1.64	1.33	1.40	1.40	0.15	11.03%
13-Plex 51	0.25	0.24	0.26	0.27	0.23	0.25	0.02	6.32%
13-Plex 52	0.10	0.10	0.12	0.10	0.12	0.11	0.01	10.14%
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with %CV of less than or equal to 25.00								15
Percentage of sample with %CV of less than or equal to 25.00								100.00 %

Appendix IV-25: Intra assay reproducibility results generated for beads prepared using Pn18C PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.12	0.15	0.16	0.15	0.15	0.15	0.02	10.39 %
13-Plex 05	2.58	2.67	2.42	2.37	2.54	2.52	0.12	4.82 %
13-Plex 06	2.35	0.93	1.15	1.08	1.12	1.33	0.58	43.64 %
13-Plex 10	0.63	0.63	0.55	0.54	0.69	0.61	0.06	10.30 %
13-Plex 16	1.96	2.11	2.17	2.42	2.10	2.15	0.17	7.83 %
13-Plex 17	0.43	0.54	0.53	0.51	0.57	0.52	0.05	10.22 %
13-Plex 18	0.87	0.85	0.89	0.71	0.70	0.80	0.09	11.39 %
13-Plex 21	1.58	1.77	1.68	1.93	2.00	1.79	0.17	9.68 %
13-Plex 22	0.95	1.06	1.04	0.97	1.00	1.00	0.05	4.60 %
13-Plex 28	2.73	2.89	3.15	2.92	3.28	2.99	0.22	7.32 %
13-Plex 34	4.32	4.75	4.10	4.42	4.71	4.46	0.27	6.11 %
13-Plex 46	1.33	1.48	1.70	1.52	1.56	1.52	0.13	8.82 %
13-Plex 50	1.26	1.30	1.25	1.32	1.29	1.28	0.03	2.24 %
13-Plex 52	0.19	0.20	0.23	0.22	0.21	0.21	0.02	7.53 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.00 %
13-Plex 62	0.34	0.36	0.40	0.41	0.37	0.38	0.03	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-26: Intra assay reproducibility results for beads prepared using Pn19F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	7.94	11.03	10.98	10.07	9.30	9.86	1.29	13.09 %
13-Plex 05	3.52	3.53	3.26	3.25	3.63	3.44	0.17	5.02 %
13-Plex 08	4.69	6.11	6.28	5.06	5.05	5.44	0.71	13.05 %
13-Plex 10	9.88	10.82	9.02	9.25	12.59	10.31	1.45	14.08 %
13-Plex 12	1.06	1.42	0.84	0.97	0.90	1.04	0.23	22.04 %
13-Plex 16	1.62	1.79	1.86	1.96	1.84	1.81	0.12	6.88 %
13-Plex 18	7.92	8.13	8.08	6.68	6.38	7.44	0.84	11.28 %
13-Plex 20	2.84	1.99	2.83	2.81	2.79	2.65	0.37	13.97 %
13-Plex 22	5.55	6.32	5.56	5.68	5.82	5.79	0.32	5.49 %
13-Plex 25	0.21	0.22	0.24	0.22	0.24	0.23	0.01	5.94 %
13-Plex 28	0.74	0.82	0.84	0.81	1.00	0.84	0.10	11.40 %
13-Plex 32	0.30	0.40	0.39	0.36	0.36	0.36	0.04	10.77 %
13-Plex 44	1.03	1.12	1.27	1.11	1.09	1.12	0.09	7.90 %
13-Plex 50	4.87	4.55	4.79	4.86	4.98	4.81	0.16	3.34 %
13-Plex 51	0.20	0.19	0.22	0.22	0.19	0.20	0.02	7.43 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with %CV of less than or equal to 25.00								15
Percentage of sample with %CV of less than or equal to 25.00								100.00 %

Appendix IV-27: Inter operator reproducibility results for beads prepared using Pn4 PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 05	0.65	0.78	0.72	0.76	0.80	0.68	0.73	0.06	8.04 %
13-Plex 06	0.26	0.26	0.27	0.31	0.25	0.22	0.26	0.03	11.19 %
13-Plex 08	1.62	2.00	2.16	3.20	2.21	2.23	2.24	0.52	23.41 %
13-Plex 11	4.36	5.46	5.28	4.97	3.77	4.58	4.74	0.63	13.26 %
13-Plex 12	0.47	0.36	0.44	0.41	0.42	0.47	0.43	0.04	9.73 %
13-Plex 17	0.05	0.10	0.05	0.05	0.05	0.05	0.06	0.02	-
13-Plex 18	0.41	0.57	0.56	0.66	0.71	0.58	0.58	0.10	17.65 %
13-Plex 20	0.80	0.82	0.92	1.00	0.86	0.81	0.87	0.08	8.99 %
13-Plex 21	0.55	0.55	0.51	0.49	0.54	0.54	0.53	0.02	4.62 %
13-Plex 28	0.86	0.99	0.98	0.93	0.85	0.95	0.93	0.06	6.43 %
13-Plex 32	0.20	0.29	0.23	0.22	0.22	0.22	0.23	0.03	13.47 %
13-Plex 42	1.35	1.51	1.49	1.59	1.57	1.50	1.50	0.08	5.63 %
13-Plex 46	0.47	0.58	0.51	0.46	0.55	0.56	0.52	0.05	9.50 %
13-Plex 59	0.11	0.15	0.15	0.12	0.14	0.13	0.13	0.02	12.25 %
13-Plex 60	0.44	0.40	0.35	0.45	0.43	0.43	0.42	0.04	8.81 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.16	0.15	0.15	0.11	0.16	0.15	0.15	0.02	12.69 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									15
Percentage of sample with % CV of less than or equal to 25.00									100.00 %

Appendix IV-28: Inter operator reproducibility results for beads prepared using Pn6B PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	0.25	0.26	0.22	0.21	0.21	0.23	0.23	0.02	9.12 %
13-Plex 05	0.37	0.44	0.39	0.30	0.37	0.48	0.39	0.06	15.94 %
13-Plex 08	4.04	5.05	4.38	3.93	4.20	4.26	4.31	0.40	9.19 %
13-Plex 10	3.28	3.16	3.24	3.06	3.01	3.58	3.22	0.20	6.31 %
13-Plex 11	2.87	2.66	4.00	3.49	3.8	3.25	3.35	0.52	15.60 %
13-Plex 18	1.10	1.50	1.29	1.38	1.48	1.62	1.40	0.18	13.11 %
13-Plex 20	0.34	0.55	0.42	0.38	0.37	0.40	0.41	0.07	17.99 %
13-Plex 21	1.23	1.51	1.74	1.49	1.70	1.63	1.55	0.19	11.99 %
13-Plex 28	1.04	1.12	1.38	1.09	1.23	1.29	1.19	0.13	10.95 %
13-Plex 31	6.47	10.33	7.68	7.73	6.70	6.18	7.52	1.52	20.21 %
13-Plex 42	0.44	0.64	0.37	0.27	0.39	0.41	0.42	0.12	29.12 %
13-Plex 44	0.24	0.29	0.30	0.26	0.27	0.34	0.28	0.04	12.36 %
13-Plex 46	0.69	0.86	0.73	0.68	0.68	0.95	0.77	0.11	14.85 %
13-Plex 50	1.35	1.53	1.59	1.44	1.65	1.71	1.55	0.13	8.66 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.22	0.27	0.26	0.19	0.24	0.26	0.24	0.03	12.64 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.13	0.05	0.05	0.05	0.05	0.06	0.03	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									14
Percentage of sample with % CV of less than or equal to 25.00									93.33 %

Appendix IV-29: Inter operator reproducibility results for beads prepared using Pn9V PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	0.28	0.31	0.31	0.30	0.32	0.34	0.31	0.02	6.45 %
13-Plex 05	1.09	1.07	1.10	1.06	0.99	1.25	1.09	0.09	7.86 %
13-Plex 06	0.52	0.56	0.46	0.45	0.48	0.45	0.49	0.04	9.16 %
13-Plex 08	2.89	2.96	2.79	2.61	3.18	2.83	2.88	0.19	6.59 %
13-Plex 10	4.23	5.26	3.70	3.33	3.61	4.09	4.04	0.68	16.92 %
13-Plex 11	1.18	1.18	1.14	1.48	1.02	0.93	1.16	0.19	16.24 %
13-Plex 16	0.66	0.55	0.61	0.74	0.53	0.59	0.61	0.08	12.57 %
13-Plex 20	3.02	2.55	2.80	3.05	2.80	2.73	2.83	0.19	6.61 %
13-Plex 28	0.70	0.63	0.58	0.71	0.76	0.75	0.69	0.07	10.21 %
13-Plex 31	0.44	0.41	0.37	0.44	0.33	0.36	0.39	0.05	11.58 %
13-Plex 42	1.42	1.01	1.40	1.29	1.65	1.60	1.40	0.23	16.56 %
13-Plex 46	0.58	0.51	0.56	0.57	0.50	0.72	0.57	0.08	13.76 %
13-Plex 50	0.75	0.67	0.65	0.70	0.82	0.82	0.74	0.07	10.06 %
13-Plex 51	0.21	0.19	0.16	0.18	0.18	0.22	0.19	0.02	11.53 %
13-Plex 52	0.18	0.18	0.12	0.13	0.17	0.18	0.16	0.03	17.23 %
13-Plex 62	0.05	0.05	0.05	0.05	0.10	0.10	0.10	0.00	0.00 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.12	0.06	0.03	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									16
Number of samples with % CV of less than or equal to 25.00									16
Percentage of sample with % CV of less than or equal to 25.00									100.00 %

Appendix IV-30: Inter operator reproducibility results for beads prepared using Pn14 PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	0.89	0.81	0.92	0.98	0.83	0.88	0.89	0.06	6.96 %
13-Plex 08	4.39	4.67	4.73	6.74	4.88	5.05	5.08	0.84	16.63 %
13-Plex 10	5.50	6.10	4.49	3.75	3.95	4.62	4.74	0.91	19.14 %
13-Plex 11	0.82	0.65	0.56	0.42	0.55	0.52	0.59	0.14	23.21 %
13-Plex 12	2.96	3.63	2.79	2.50	2.77	2.50	2.86	0.42	14.64 %
13-Plex 16	1.37	1.27	0.92	0.72	0.91	0.88	1.01	0.25	24.86 %
13-Plex 18	1.08	0.99	1.07	1.04	1.23	1.21	1.10	0.10	8.69 %
13-Plex 25	0.40	0.27	0.21	0.18	0.25	0.21	0.25	0.08	31.05 %
13-Plex 28	8.05	8.59	7.63	6.93	6.77	7.35	7.55	0.69	9.12 %
13-Plex 31	0.95	0.92	0.88	0.74	0.91	0.87	0.88	0.07	8.38 %
13-Plex 32	0.58	0.44	0.40	0.33	0.41	0.43	0.43	0.08	19.07 %
13-Plex 42	2.03	2.09	1.29	0.91	1.32	1.36	1.50	0.46	30.89 %
13-Plex 44	1.83	1.86	1.33	1.33	1.34	1.30	1.50	0.27	17.96 %
13-Plex 51	1.16	1.11	0.81	0.62	0.77	0.82	0.88	0.21	23.76 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.38	0.27	0.20	0.19	0.25	0.24	0.26	0.07	26.80 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									12
Percentage of sample with % CV of less than or equal to 25.00									80.00 %

Appendix IV-31: Inter operator reproducibility results for beads prepared using Pn19A PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	1.17	1.31	1.23	1.14	1.12	1.28	1.21	0.08	6.40 %
13-Plex 05	7.51	9.34	9.81	8.55	7.66	9.49	8.73	0.98	11.21 %
13-Plex 06	0.46	0.31	0.49	0.44	0.41	0.45	0.43	0.06	14.72 %
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 10	21.55	17.54	19.76	17.89	17.13	19.14	18.84	1.66	8.82 %
13-Plex 11	6.26	6.81	8.10	6.44	6.57	5.29	6.58	0.91	13.85 %
13-Plex 16	4.26	3.66	5.45	5.37	4.71	4.47	4.65	0.68	14.66 %
13-Plex 17	1.31	1.19	1.09	0.96	1.09	0.97	1.10	0.13	12.10 %
13-Plex 18	10.93	7.86	10.64	10.84	11.53	11.53	10.56	1.37	12.99 %
13-Plex 21	0.65	0.59	0.66	0.54	0.66	0.71	0.64	0.06	9.49 %
13-Plex 25	1.25	1.15	1.30	1.24	1.31	1.09	1.22	0.09	7.08 %
13-Plex 32	0.65	0.65	0.74	0.61	0.74	0.79	0.70	0.07	10.01 %
13-Plex 33	0.54	0.31	0.58	0.52	0.54	0.56	0.51	0.10	19.53 %
13-Plex 34	1.07	0.71	1.02	0.99	1.05	1.23	1.01	0.17	16.78 %
13-Plex 46	2.49	2.54	2.98	2.91	2.96	3.58	2.91	0.39	13.48 %
13-Plex 51	0.23	0.20	0.25	0.18	0.23	0.30	0.23	0.04	17.99 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.10	0.06	0.02	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									15
Percentage of sample with % CV of less than or equal to 25.00									100.00 %

Appendix IV-32: Inter operator reproducibility results for beads prepared using Pn23F PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 05	2.63	2.70	2.57	2.79	2.72	2.43	2.64	0.13	4.84 %
13-Plex 10	6.29	6.96	5.27	4.68	5.82	5.84	5.81	0.79	13.60 %
13-Plex 11	5.57	5.58	5.64	2.45	4.84	5.14	4.87	1.23	25.18 %
13-Plex 12	2.63	3.87	3.29	1.82	2.96	3.17	2.96	0.69	23.38 %
13-Plex 16	0.23	0.20	0.20	0.40	0.18	0.20	0.24	0.08	35.06 %
13-Plex 18	1.90	2.49	1.91	1.81	2.34	2.01	2.08	0.27	13.18 %
13-Plex 20	2.14	2.57	2.24	1.51	1.91	1.76	2.02	0.38	18.57 %
13-Plex 21	3.73	4.21	4.21	1.59	4.02	3.68	3.57	1.00	27.93 %
13-Plex 28	0.52	0.52	0.58	0.32	0.52	0.51	0.50	0.09	18.06 %
13-Plex 34	0.48	0.47	0.44	0.28	0.49	0.44	0.43	0.08	17.97 %
13-Plex 42	0.78	0.91	0.75	0.82	0.76	0.75	0.80	0.06	7.83 %
13-Plex 46	0.70	0.74	0.62	0.52	0.67	0.71	0.66	0.08	12.08 %
13-Plex 50	1.55	2.13	1.64	1.39	1.70	1.73	1.69	0.25	14.67 %
13-Plex 59	0.36	0.34	0.37	0.24	0.37	0.32	0.33	0.05	14.90 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									14
Number of samples with % CV of less than or equal to 25.00									11
Percentage of sample with % CV of less than or equal to 25.00									78.57 %

Appendix IV-33: Inter operator reproducibility results for beads prepared using Pn1 PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	0.15	0.15	0.17	0.16	0.17	0.21	0.17	0.02	13.24%
13-Plex 08	23.02	25.16	31.40	28.56	31.77	27.61	27.92	3.44	12.31%
13-Plex 10	0.11	0.05	0.27	0.25	0.29	0.30	0.24	0.08	31.69%
13-Plex 11	4.53	5.5	5.34	5.36	5.17	4.91	5.14	0.36	6.99%
13-Plex 12	4.76	3.62	6.81	6.75	6.91	7.49	6.06	1.51	24.98%
13-Plex 16	5.51	4.83	5.50	6.04	5.74	4.92	5.42	0.47	8.65%
13-Plex 18	0.33	0.31	0.43	0.44	0.52	0.51	0.42	0.09	20.79%
13-Plex 20	0.12	0.12	0.18	0.16	0.18	0.19	0.16	0.03	19.74%
13-Plex 21	0.16	0.16	0.21	0.17	0.22	0.27	0.20	0.04	21.96%
13-Plex 28	0.14	0.10	0.19	0.13	0.18	0.20	0.16	0.04	25.10%
13-Plex 31	0.87	1.79	1.26	1.19	1.19	1.10	1.23	0.30	24.70%
13-Plex 32	0.68	0.72	0.60	0.60	0.67	0.71	0.66	0.05	7.90%
13-Plex 34	0.05	0.05	0.11	0.05	0.10	0.10	0.10	0.01	5.59%
13-Plex 42	1.91	1.92	2.06	2.33	2.17	2.45	2.14	0.22	10.25%
13-Plex 50	0.79	0.65	0.79	0.77	0.84	0.89	0.79	0.08	10.22%
13-Plex 62	0.05	0.05	0.12	0.11	0.13	0.13	0.12	0.01	7.82%
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									16
Number of samples with % CV of less than or equal to 25.00									14
Percentage of sample with % CV of less than or equal to 25.00									87.50 %

Appendix IV-34: Inter operator reproducibility results for beads prepared using Pn3 PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 10	1.04	1.03	0.97	0.97	0.94	0.95	0.98	0.04	4.25 %
13-Plex 11	1.02	1.3	0.80	0.68	0.76	0.84	0.90	0.23	25.14 %
13-Plex 12	4.39	5.02	4.45	5.43	4.05	4.23	4.60	0.52	11.40 %
13-Plex 16	0.71	0.69	0.87	1.00	0.77	0.83	0.81	0.11	14.16 %
13-Plex 18	3.56	3.91	3.07	3.76	3.27	3.16	3.46	0.34	9.86 %
13-Plex 20	1.69	2.12	1.77	1.98	1.40	1.47	1.74	0.28	16.15 %
13-Plex 21	3.86	5.33	3.44	4.29	2.83	3.09	3.81	0.91	23.96 %
13-Plex 28	0.23	0.20	0.32	0.20	0.39	0.28	0.27	0.08	27.91 %
13-Plex 32	0.34	0.53	0.30	0.38	0.25	0.30	0.35	0.10	28.11 %
13-Plex 34	1.70	1.51	1.45	1.27	1.63	1.32	1.48	0.17	11.40 %
13-Plex 44	0.60	0.57	0.66	0.64	0.59	0.56	0.60	0.04	6.52 %
13-Plex 46	7.41	11.19	6.63	7.32	5.76	6.00	7.39	1.98	26.82 %
13-Plex 51	0.60	0.77	0.59	0.60	0.47	0.55	0.60	0.10	16.48 %
13-Plex 52	0.38	0.45	0.35	0.37	0.33	0.30	0.36	0.05	14.11 %
13-Plex 59	0.23	0.28	0.25	0.21	0.25	0.22	0.24	0.03	10.54 %
13-Plex 62	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.02	0.05	0.05	0.05	0.05	0.05	0.01	-
13-Plex 69	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.02	0.05	0.05	0.05	0.05	0.05	0.01	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									11
Percentage of sample with % CV of less than or equal to 25.00									73.33 %

Appendix IV-35: Inter operator reproducibility results for beads prepared using Pn5 PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 06	0.34	0.31	0.45	0.39	0.38	0.39	0.38	0.05	12.75 %
13-Plex 08	4.19	4.95	5.50	6.30	5.65	6.03	5.44	0.77	14.10 %
13-Plex 10	0.21	0.25	0.25	0.26	0.25	0.29	0.25	0.03	10.18 %
13-Plex 11	2.85	2.74	3.44	3.29	3.49	3.70	3.25	0.38	11.65 %
13-Plex 12	3.08	2.87	3.16	3.31	3.20	3.34	3.16	0.17	5.43 %
13-Plex 18	1.78	1.84	2.25	2.39	2.54	2.19	2.17	0.30	13.90 %
13-Plex 20	1.13	1.37	1.60	1.68	1.53	1.58	1.48	0.20	13.55 %
13-Plex 28	0.25	0.27	0.42	0.33	0.40	0.40	0.35	0.07	21.12 %
13-Plex 31	0.19	0.17	0.30	0.36	0.40	0.38	0.30	0.10	33.00 %
13-Plex 33	0.15	0.17	0.22	0.22	0.23	0.18	0.20	0.03	16.77 %
13-Plex 44	1.28	1.40	1.48	1.35	1.41	1.39	1.39	0.07	4.81 %
13-Plex 46	0.76	0.84	0.90	0.95	0.98	1.14	0.93	0.13	14.04 %
13-Plex 50	6.45	9.27	7.23	7.88	7.92	7.58	7.72	0.93	12.07 %
13-Plex 51	0.51	0.41	0.38	0.27	0.37	0.34	0.38	0.08	20.92 %
13-Plex 59	0.05	0.05	0.10	0.05	0.11	0.05	0.11	0.01	6.73 %
13-Plex 62	1.20	1.24	1.24	1.21	1.28	1.24	1.24	0.03	2.28 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.12	0.11	0.11	0.12	0.12	0.01	5.02 %
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									17
Number of samples with % CV of less than or equal to 25.00									16
Percentage of sample with % CV of less than or equal to 25.00									94.12 %

Appendix IV-36: Inter operator reproducibility results for beads prepared using Pn6A PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	0.27	0.31	0.32	0.29	0.28	0.30	0.30	0.02	6.34 %
13-Plex 05	1.35	1.67	1.75	1.75	1.60	1.49	1.60	0.16	9.85 %
13-Plex 08	6.25	6.62	6.73	7.99	6.98	6.36	6.82	0.63	9.22 %
13-Plex 10	4.35	3.71	3.62	3.91	3.36	3.79	3.79	0.33	8.74 %
13-Plex 11	4.02	4.58	4.23	4.08	4.1	3.54	4.09	0.34	8.23 %
13-Plex 17	0.19	0.19	0.28	0.21	0.24	0.20	0.22	0.04	16.24 %
13-Plex 18	1.70	1.78	1.50	1.69	1.77	1.53	1.66	0.12	7.20 %
13-Plex 21	1.75	2.18	2.54	2.52	2.60	2.07	2.28	0.34	14.73 %
13-Plex 31	1.41	1.66	1.18	1.20	1.21	1.01	1.28	0.23	17.68 %
13-Plex 34	0.17	0.19	0.19	0.18	0.18	0.21	0.19	0.01	7.32 %
13-Plex 42	1.73	2.18	1.73	1.72	1.69	1.56	1.77	0.21	11.98 %
13-Plex 46	1.90	1.73	1.98	2.07	1.99	1.96	1.94	0.12	5.98 %
13-Plex 50	4.12	5.47	4.12	4.83	4.53	4.01	4.51	0.56	12.45 %
13-Plex 51	0.41	0.52	0.68	0.57	0.67	0.66	0.59	0.11	18.24 %
13-Plex 52	0.11	0.14	0.16	0.14	0.17	0.13	0.14	0.02	15.08 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									15
Percentage of sample with % CV of less than or equal to 25.00									100.00 %

Appendix IV-37: Inter operator reproducibility results for beads prepared using Pn7F PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 05	1.29	1.18	1.68	1.63	1.58	1.20	1.43	0.23	15.98 %
13-Plex 08	4.56	4.93	7.12	8.62	7.59	6.67	6.58	1.57	23.82 %
13-Plex 12	0.72	0.50	1.03	0.97	1.04	1.07	0.89	0.23	25.76 %
13-Plex 16	1.47	1.49	2.62	2.57	2.80	2.20	2.19	0.58	26.68 %
13-Plex 17	0.89	0.59	1.00	0.92	1.01	1.05	0.91	0.17	18.43 %
13-Plex 18	0.92	0.99	1.23	1.38	1.47	1.32	1.22	0.22	18.02 %
13-Plex 20	0.46	0.47	0.68	0.64	0.68	0.65	0.60	0.10	17.31 %
13-Plex 28	6.33	5.88	15.69	11.13	12.27	8.23	9.92	3.80	38.34 %
13-Plex 31	0.89	1.00	0.99	1.01	1.03	1.04	0.99	0.05	5.43 %
13-Plex 34	3.93	3.68	5.71	5.64	5.43	4.44	4.81	0.90	18.78 %
13-Plex 42	3.16	3.03	4.27	4.28	4.38	4.29	3.90	0.63	16.08 %
13-Plex 44	0.23	0.24	0.38	0.34	0.39	0.38	0.33	0.07	22.38 %
13-Plex 46	1.79	1.50	2.02	1.96	1.98	2.50	1.96	0.33	16.72 %
13-Plex 51	0.24	0.21	0.22	0.19	0.22	0.26	0.22	0.02	10.85 %
13-Plex 52	0.14	0.10	0.18	0.15	0.18	0.18	0.16	0.03	20.71 %
13-Plex 60	0.05	0.05	0.05	0.05	0.10	0.05	0.06	0.02	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									12
Percentage of sample with % CV of less than or equal to 25.00									80.00 %

Appendix IV-38: Inter operator reproducibility results for beads prepared using Pn18C PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	0.18	0.18	0.18	0.16	0.17	0.18	0.18	0.01	4.78 %
13-Plex 05	2.37	2.91	2.85	2.85	2.45	2.36	2.63	0.26	10.03 %
13-Plex 06	1.11	1.28	1.20	1.18	1.04	0.96	1.13	0.12	10.28 %
13-Plex 10	0.60	0.76	0.52	0.56	0.53	0.57	0.59	0.09	14.93 %
13-Plex 16	2.01	2.45	2.43	2.30	2.38	2.04	2.27	0.20	8.63 %
13-Plex 17	0.47	0.54	0.56	0.58	0.63	0.43	0.54	0.07	13.72 %
13-Plex 18	0.65	0.83	0.71	0.75	0.82	0.76	0.75	0.07	8.99 %
13-Plex 21	3.14	3.30	2.82	2.44	2.91	2.81	2.90	0.30	10.26 %
13-Plex 25	0.22	0.29	0.25	0.26	0.28	0.23	0.26	0.03	10.74 %
13-Plex 28	4.24	4.34	5.10	4.17	4.58	3.79	4.37	0.44	10.09 %
13-Plex 31	0.48	0.57	0.64	0.59	0.68	0.56	0.59	0.07	11.79 %
13-Plex 34	4.69	5.43	5.80	6.36	5.58	5.43	5.55	0.55	9.83 %
13-Plex 46	1.72	2.09	1.67	1.75	1.75	1.70	1.78	0.15	8.70 %
13-Plex 50	1.41	1.86	1.50	1.59	1.70	1.55	1.60	0.16	9.92 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.44	0.47	0.41	0.39	0.44	0.38	0.42	0.03	8.13 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									15
Percentage of sample with % CV of less than or equal to 25.00									100.00 %

Appendix IV-39: Inter operator reproducibility results for beads prepared using Pn19F PS.

Sample	Operator 1		Operator 2		Operator 3		Mean	SD	% CV
13-Plex 03	9.36	10.37	10.24	8.99	8.30	8.38	9.27	0.89	9.61 %
13-Plex 05	2.83	3.39	3.66	3.60	3.37	2.90	3.29	0.35	10.64 %
13-Plex 08	4.64	5.48	5.86	6.57	6.18	5.59	5.72	0.66	11.58 %
13-Plex 10	9.15	11.47	8.74	8.75	7.80	8.50	9.07	1.26	13.87 %
13-Plex 11	6.84	7.35	4.75	4.41	4.86	3.99	5.37	1.38	25.75 %
13-Plex 12	1.01	0.85	0.95	0.85	1.01	0.95	0.94	0.07	7.72 %
13-Plex 16	1.45	1.62	1.89	1.74	1.93	1.57	1.70	0.19	11.05 %
13-Plex 18	5.38	7.10	7.14	7.73	8.23	6.85	7.07	0.97	13.70 %
13-Plex 20	2.09	2.52	2.61	2.72	2.53	2.02	2.42	0.29	11.96 %
13-Plex 25	0.16	0.20	0.22	0.18	0.21	0.18	0.19	0.02	11.63 %
13-Plex 28	0.74	0.79	0.72	0.54	0.69	0.58	0.68	0.10	14.32 %
13-Plex 32	0.58	0.47	0.44	0.40	0.49	0.44	0.47	0.06	13.18 %
13-Plex 44	0.89	1.04	1.14	1.06	1.12	0.92	1.03	0.10	10.00 %
13-Plex 50	3.62	5.05	4.43	4.67	5.12	4.42	4.55	0.55	11.98 %
13-Plex 51	0.32	0.25	0.27	0.20	0.27	0.29	0.27	0.04	15.12 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)									15
Number of samples with % CV of less than or equal to 25.00									14
Percentage of sample with % CV of less than or equal to 25.00									93.33 %

Appendix IV-40: Inter operator analyte reproducibility results for beads prepared using Pn4 PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 05	0.66	0.62	0.70	0.66	0.04	6.06 %
13-Plex 06	0.19	0.24	0.18	0.20	0.03	15.81 %
13-Plex 08	1.44	1.27	1.51	1.41	0.12	8.77 %
13-Plex 12	0.26	0.37	0.32	0.32	0.06	17.39 %
13-Plex 17	0.10	0.10	0.05	0.10	0.00	0.00 %
13-Plex 18	0.38	0.40	0.47	0.42	0.05	11.34 %
13-Plex 20	0.61	0.75	0.66	0.67	0.07	10.54 %
13-Plex 21	0.34	0.48	0.42	0.41	0.07	16.99 %
13-Plex 28	0.76	0.99	0.80	0.85	0.12	14.46 %
13-Plex 31	0.49	0.33	0.59	0.47	0.13	27.90 %
13-Plex 32	0.22	0.16	0.23	0.20	0.04	18.62 %
13-Plex 42	1.03	0.93	1.32	1.09	0.20	18.53 %
13-Plex 46	0.51	0.27	0.66	0.48	0.20	40.98 %
13-Plex 59	0.10	0.05	0.11	0.11	0.01	6.73 %
13-Plex 60	0.24	0.29	0.27	0.27	0.03	9.44 %
13-Plex 62	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						13
Percentage of sample with % CV of less than or equal to 25.00						86.67 %

Appendix IV-41: Inter operator analyte reproducibility results for beads prepared using Pn6B PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	0.16	0.16	0.15	0.16	0.01	3.69 %
13-Plex 05	0.34	0.33	0.33	0.33	0.01	1.73 %
13-Plex 08	3.09	3.23	3.29	3.20	0.10	3.20 %
13-Plex 10	2.64	2.75	2.82	2.74	0.09	3.32 %
13-Plex 18	1.01	0.99	1.12	1.04	0.07	6.73 %
13-Plex 20	0.34	0.34	0.35	0.34	0.01	1.68 %
13-Plex 21	1.34	1.37	1.43	1.38	0.05	3.32 %
13-Plex 22	3.45	4.00	4.08	3.84	0.34	8.92 %
13-Plex 25	0.23	0.26	0.21	0.23	0.03	10.79 %
13-Plex 28	1.09	0.87	1.15	1.04	0.15	14.22 %
13-Plex 31	7.01	7.74	8.06	7.60	0.54	7.08 %
13-Plex 42	0.28	0.24	0.26	0.26	0.02	7.69 %
13-Plex 46	0.61	0.63	0.65	0.63	0.02	3.17 %
13-Plex 50	1.19	1.30	1.29	1.26	0.06	4.83 %
13-Plex 52	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.22	0.20	0.21	0.21	0.01	4.76 %
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						15
Percentage of sample with % CV of less than or equal to 25.00						100.00 %

Appendix IV-42: Inter operator analyte reproducibility results for beads prepared using Pn9V PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	0.27	0.29	0.33	0.30	0.03	10.30 %
13-Plex 05	0.84	0.97	0.93	0.91	0.07	7.29 %
13-Plex 06	0.43	0.50	0.51	0.48	0.04	9.08 %
13-Plex 08	2.26	2.54	2.54	2.45	0.16	6.61 %
13-Plex 10	3.34	4.35	3.69	3.79	0.51	13.52 %
13-Plex 16	0.44	0.44	0.53	0.47	0.05	11.06 %
13-Plex 20	2.33	2.77	2.76	2.62	0.25	9.59 %
13-Plex 28	0.71	0.62	0.50	0.61	0.11	17.27 %
13-Plex 31	0.53	0.29	0.28	0.37	0.14	38.60 %
13-Plex 34	0.99	1.26	1.18	1.14	0.14	12.13 %
13-Plex 42	1.14	1.23	1.37	1.25	0.12	9.30 %
13-Plex 46	0.46	0.38	0.66	0.50	0.14	28.84 %
13-Plex 50	0.49	0.62	0.58	0.56	0.07	11.82 %
13-Plex 51	0.16	0.13	0.13	0.14	0.02	12.37 %
13-Plex 52	0.11	0.11	0.12	0.11	0.01	5.09 %
13-Plex 62	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						13
Percentage of sample with % CV of less than or equal to 25.00						86.67 %

Appendix IV-43: Inter operator analyte reproducibility results for beads prepared using Pn14 PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	1.12	1.05	1.11	1.09	0.04	3.46 %
13-Plex 05	3.65	3.57	3.38	3.53	0.14	3.93 %
13-Plex 08	4.82	4.44	4.46	4.57	0.21	4.68 %
13-Plex 10	4.39	4.19	3.86	4.15	0.27	6.45 %
13-Plex 12	2.76	2.81	2.46	2.68	0.19	7.07 %
13-Plex 16	0.73	0.69	0.64	0.69	0.05	6.57 %
13-Plex 18	0.97	0.91	0.87	0.92	0.05	5.49 %
13-Plex 25	0.20	0.20	0.19	0.20	0.01	2.94 %
13-Plex 28	6.80	6.51	5.30	6.20	0.80	12.83 %
13-Plex 31	0.71	0.63	0.45	0.60	0.13	22.32 %
13-Plex 32	0.31	0.29	0.20	0.27	0.06	21.97 %
13-Plex 42	0.62	0.68	0.70	0.67	0.04	6.24 %
13-Plex 44	1.27	1.16	1.21	1.21	0.06	4.54 %
13-Plex 51	0.74	0.67	0.65	0.69	0.05	6.88 %
13-Plex 52	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.20	0.20	0.17	0.19	0.02	9.12 %
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						15
Percentage of sample with % CV of less than or equal to 25.00						100.00 %

Appendix IV-44: Inter operator analyte reproducibility results for beads prepared using Pn19A PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	0.80	0.67	0.69	0.72	0.07	9.72%
13-Plex 05	10.11	9.55	7.57	9.08	1.33	14.70%
13-Plex 06	0.46	0.43	0.45	0.45	0.02	3.42%
13-Plex 07	0.05	0.05	0.05	0.05	0.00	-
13-Plex 10	12.38	18.50	22.24	17.71	4.98	28.11%
13-Plex 16	4.21	4.63	4.29	4.38	0.22	5.10%
13-Plex 17	0.95	0.92	0.97	0.95	0.03	2.66%
13-Plex 18	11.66	7.94	7.54	9.05	2.27	25.11%
13-Plex 21	0.51	0.45	0.48	0.48	0.03	6.25%
13-Plex 25	1.36	1.04	1.17	1.19	0.16	13.52%
13-Plex 32	0.38	0.49	0.34	0.40	0.08	19.26%
13-Plex 33	0.49	0.45	0.46	0.47	0.02	4.46%
13-Plex 34	0.94	0.75	0.79	0.83	0.10	12.12%
13-Plex 46	2.02	2.05	1.68	1.92	0.21	10.72%
13-Plex 50	2.36	2.00	1.88	2.08	0.25	12.01%
13-Plex 51	0.15	0.15	0.10	0.13	0.03	21.65%
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						13
Percentage of sample with % CV of less than or equal to 25.00						86.67 %

Appendix IV-45: Inter operator analyte reproducibility results for beads prepared using Pn23F PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 05	2.48	2.88	2.22	2.53	0.33	13.16 %
13-Plex 10	4.69	4.88	4.70	4.76	0.11	2.25 %
13-Plex 12	2.57	2.43	2.73	2.58	0.15	5.83 %
13-Plex 16	0.22	0.31	0.17	0.23	0.07	30.41 %
13-Plex 18	1.88	2.22	1.95	2.02	0.18	8.90 %
13-Plex 20	1.96	1.89	2.01	1.95	0.06	3.09 %
13-Plex 21	2.24	1.76	2.96	2.32	0.60	26.03 %
13-Plex 22	4.60	5.44	4.61	4.88	0.48	9.87 %
13-Plex 28	0.45	0.35	0.47	0.42	0.06	15.19 %
13-Plex 34	0.38	0.33	0.37	0.36	0.03	7.35 %
13-Plex 42	0.71	0.79	0.73	0.74	0.04	5.60 %
13-Plex 46	0.51	0.59	0.51	0.54	0.05	8.61 %
13-Plex 50	1.57	1.67	1.32	1.52	0.18	11.86 %
13-Plex 59	0.29	0.26	0.31	0.29	0.03	8.78 %
13-Plex 60	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						14
Number of samples with % CV of less than or equal to 25.00						12
Percentage of sample with % CV of less than or equal to 25.00						85.71 %

Appendix IV-46: Inter operator analyte reproducibility results for beads prepared using Pn1 PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	0.12	0.14	0.14	0.13	0.01	8.66 %
13-Plex 05	1.60	1.54	1.67	1.60	0.07	4.06 %
13-Plex 08	30.58	21.80	29.24	27.21	4.73	17.39 %
13-Plex 10	0.10	0.17	0.10	0.12	0.04	32.77 %
13-Plex 12	10.58	3.21	8.17	7.32	3.76	51.34 %
13-Plex 16	5.19	4.35	4.56	4.70	0.44	9.30 %
13-Plex 18	0.31	0.21	0.25	0.26	0.05	19.61 %
13-Plex 20	0.14	0.16	0.14	0.15	0.01	7.87 %
13-Plex 21	0.12	0.18	0.14	0.15	0.03	20.83 %
13-Plex 28	0.18	0.18	0.17	0.18	0.01	3.27 %
13-Plex 31	1.35	0.52	0.60	0.82	0.46	55.61 %
13-Plex 32	0.49	0.74	0.56	0.60	0.13	21.62 %
13-Plex 34	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	1.93	1.29	1.90	1.71	0.36	21.16 %
13-Plex 50	0.80	0.54	0.76	0.70	0.14	20.00 %
13-Plex 62	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						14
Number of samples with % CV of less than or equal to 25.00						11
Percentage of sample with % CV of less than or equal to 25.00						78.57 %

Appendix IV-47: Inter operator analyte reproducibility results for beads prepared using Pn3 PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 08	3.49	2.86	2.73	3.03	0.41	13.43 %
13-Plex 10	0.76	0.88	0.89	0.84	0.07	8.58 %
13-Plex 12	4.03	3.82	4.24	4.03	0.21	5.21 %
13-Plex 16	0.77	0.68	0.58	0.68	0.10	14.05 %
13-Plex 18	3.26	3.39	3.35	3.33	0.07	2.00 %
13-Plex 20	1.91	1.87	1.7	1.83	0.11	6.10 %
13-Plex 21	4.04	3.44	3.51	3.66	0.33	8.96 %
13-Plex 28	0.19	0.2	0.2	0.20	0.01	2.94 %
13-Plex 32	0.33	0.23	0.22	0.26	0.06	23.40 %
13-Plex 34	1.13	1.37	1.33	1.28	0.13	10.07 %
13-Plex 44	0.05	0.05	0.05	0.05	0.00	-
13-Plex 46	11.23	6.63	5.76	7.87	2.94	37.33 %
13-Plex 51	0.7	0.6	0.53	0.61	0.09	14.01 %
13-Plex 52	0.35	0.3	0.27	0.31	0.04	13.18 %
13-Plex 59	0.19	0.2	0.18	0.19	0.01	5.26 %
13-Plex 62	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						14
Number of samples with % CV of less than or equal to 25.00						13
Percentage of sample with % CV of less than or equal to 25.00						92.86 %

Appendix IV-48: Inter operator analyte reproducibility results for beads prepared using Pn5 PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 08	3.87	4.73	3.81	4.14	0.51	12.44 %
13-Plex 10	0.21	0.21	0.21	0.21	0.00	0.00 %
13-Plex 12	2.65	2.74	2.65	2.68	0.05	1.94 %
13-Plex 18	1.48	2.00	1.41	1.63	0.32	19.78 %
13-Plex 20	1.21	1.50	1.14	1.28	0.19	14.87 %
13-Plex 28	0.29	0.34	0.26	0.30	0.04	13.62 %
13-Plex 31	0.14	0.19	0.17	0.17	0.03	15.10 %
13-Plex 33	0.14	0.16	0.14	0.15	0.01	7.87 %
13-Plex 42	0.85	1.02	0.83	0.90	0.10	11.60 %
13-Plex 44	1.07	1.13	1.00	1.07	0.07	6.10 %
13-Plex 46	0.82	0.71	0.60	0.71	0.11	15.49 %
13-Plex 50	7.00	7.98	6.07	7.02	0.96	13.61 %
13-Plex 51	0.36	0.26	0.32	0.31	0.05	16.06 %
13-Plex 59	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.16	0.13	0.13	0.14	0.02	12.37 %
13-Plex 62	1.05	0.99	0.91	0.98	0.07	7.14 %
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						15
Percentage of sample with % CV of less than or equal to 25.00						100.00 %

Appendix IV-49: Inter operator analyte reproducibility results for beads prepared using Pn6A PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	0.26	0.25	0.26	0.26	0.01	2.25 %
13-Plex 05	1.47	1.51	1.43	1.47	0.04	2.72 %
13-Plex 08	5.65	6.21	6.17	6.01	0.31	5.20 %
13-Plex 10	3.21	3.80	3.67	3.56	0.31	8.71 %
13-Plex 17	0.24	0.25	0.26	0.25	0.01	4.00 %
13-Plex 18	1.18	1.24	1.33	1.25	0.08	6.04 %
13-Plex 21	2.18	2.00	2.27	2.15	0.14	6.39 %
13-Plex 22	4.74	4.58	4.65	4.66	0.08	1.72 %
13-Plex 31	0.96	1.01	1.08	1.02	0.06	5.93 %
13-Plex 34	0.17	0.18	0.19	0.18	0.01	5.56 %
13-Plex 42	1.20	1.30	1.31	1.27	0.06	4.79 %
13-Plex 46	1.73	1.68	1.67	1.69	0.03	1.90 %
13-Plex 50	3.41	3.67	3.63	3.57	0.14	3.92 %
13-Plex 51	0.59	0.57	0.59	0.58	0.01	1.98 %
13-Plex 52	0.13	0.16	0.15	0.15	0.02	10.41 %
13-Plex 62	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						15
Percentage of sample with % CV of less than or equal to 25.00						100.00 %

Appendix IV-50: Inter operator analyte reproducibility results for beads prepared using Pn7F PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 05	1.70	1.85	1.66	1.74	0.10	5.77 %
13-Plex 08	7.40	7.56	7.84	7.60	0.22	2.93 %
13-Plex 12	0.90	0.80	0.89	0.86	0.06	6.38 %
13-Plex 16	2.42	2.27	2.25	2.31	0.09	4.02 %
13-Plex 17	0.39	0.32	0.37	0.36	0.04	10.02 %
13-Plex 18	1.32	1.37	1.33	1.34	0.03	1.97 %
13-Plex 20	0.65	0.65	0.67	0.66	0.01	1.76 %
13-Plex 21	1.68	1.49	1.67	1.61	0.11	6.63 %
13-Plex 28	13.16	11.17	12.37	12.23	1.00	8.19 %
13-Plex 31	0.70	0.88	0.74	0.77	0.09	12.22 %
13-Plex 34	5.65	6.19	6.23	6.02	0.32	5.38 %
13-Plex 42	3.87	4.36	4.22	4.15	0.25	6.08 %
13-Plex 46	2.72	3.35	2.40	2.82	0.48	17.12 %
13-Plex 51	0.24	0.24	0.23	0.24	0.01	2.44 %
13-Plex 52	0.16	0.16	0.15	0.16	0.01	3.69 %
13-Plex 60	0.10	0.05	0.05	0.07	0.03	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						15
Percentage of sample with % CV of less than or equal to 25.00						100.00 %

Appendix IV-51: Inter operator analyte reproducibility results for beads prepared using Pn18C PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	0.14	0.14	0.14	0.14	0.00	0.00 %
13-Plex 05	2.37	2.85	2.36	2.53	0.28	11.08 %
13-Plex 06	1.18	1.24	1.11	1.18	0.07	5.53 %
13-Plex 10	0.56	0.67	0.60	0.61	0.06	9.13 %
13-Plex 16	2.36	2.45	2.14	2.32	0.16	6.88 %
13-Plex 17	0.43	0.47	0.45	0.45	0.02	4.44 %
13-Plex 18	0.66	0.75	0.70	0.70	0.05	6.41 %
13-Plex 21	2.32	2.15	2.48	2.32	0.17	7.12 %
13-Plex 22	0.96	1.00	0.94	0.97	0.03	3.16 %
13-Plex 28	4.00	4.20	3.94	4.05	0.14	3.36 %
13-Plex 34	4.59	5.14	4.73	4.82	0.29	5.93 %
13-Plex 46	1.65	1.72	1.61	1.66	0.06	3.35 %
13-Plex 50	1.50	1.59	1.51	1.53	0.05	3.22 %
13-Plex 52	0.20	0.20	0.21	0.20	0.01	2.84 %
13-Plex 60	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.44	0.42	0.43	0.43	0.01	2.33 %
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						15
Percentage of sample with % CV of less than or equal to 25.00						100.00 %

Appendix IV-52: Inter operator analyte reproducibility results for beads prepared using Pn19F PS.

Sample	Operator 1	Operator 2	Operator 3	Mean	SD	% CV
13-Plex 03	9.95	11.37	9.23	10.18	1.09	10.69 %
13-Plex 05	3.01	3.62	2.84	3.16	0.41	12.99 %
13-Plex 08	5.29	5.80	5.39	5.49	0.27	4.92 %
13-Plex 10	8.94	11.59	10.13	10.22	1.33	12.99 %
13-Plex 12	1.07	0.94	1.10	1.04	0.09	8.20 %
13-Plex 16	1.43	1.45	1.30	1.39	0.08	5.85 %
13-Plex 18	5.72	6.42	5.49	5.88	0.48	8.24 %
13-Plex 20	2.58	2.93	2.42	2.64	0.26	9.87 %
13-Plex 22	5.92	5.89	5.30	5.70	0.35	6.13 %
13-Plex 25	0.27	0.23	0.28	0.26	0.03	10.18 %
13-Plex 28	0.79	0.89	0.84	0.84	0.05	5.95 %
13-Plex 32	0.10	0.05	0.05	0.07	0.03	-
13-Plex 44	1.02	1.08	1.03	1.04	0.03	3.08 %
13-Plex 50	4.58	5.38	4.39	4.78	0.53	10.98 %
13-Plex 51	0.21	0.19	0.22	0.21	0.02	7.39 %
13-Plex 62	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)						15
Number of samples with % CV of less than or equal to 25.00						15
Percentage of sample with % CV of less than or equal to 25.00						100.00 %

Appendix IV-53: Intra operator analyte reproducibility results for beads prepared using Pn4 PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 05	0.77	0.81	0.77	0.72	0.77	0.04	4.80 %
13-Plex 06	0.25	0.25	0.28	0.30	0.27	0.02	9.07 %
13-Plex 08	1.92	2.08	1.86	1.72	1.90	0.15	7.87 %
13-Plex 11	4.69	4.54	4.70	5.04	4.74	0.21	4.46 %
13-Plex 12	0.46	0.46	0.45	0.50	0.47	0.02	4.74 %
13-Plex 17	0.05	0.05	0.05	0.13	0.07	0.04	-
13-Plex 18	0.56	0.53	0.65	0.59	0.58	0.05	8.80 %
13-Plex 20	0.87	0.80	0.98	0.94	0.90	0.08	8.84 %
13-Plex 21	0.55	0.52	0.62	0.59	0.57	0.04	7.71 %
13-Plex 28	0.89	0.83	0.86	0.98	0.89	0.06	7.28 %
13-Plex 32	0.28	0.29	0.25	0.29	0.28	0.02	6.82 %
13-Plex 42	1.55	1.59	1.62	1.66	1.61	0.05	2.90 %
13-Plex 46	0.47	0.47	0.45	0.45	0.46	0.01	2.51 %
13-Plex 59	0.14	0.14	0.15	0.16	0.15	0.01	6.49 %
13-Plex 60	0.37	0.39	0.42	0.49	0.42	0.05	12.58 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.13	0.13	0.16	0.19	0.15	0.03	18.83 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							15
Number of samples with % CV of less than or equal to 25.00							15
Percentage of sample with % CV of less than or equal to 25.00							100.00 %

Appendix IV-54: Intra operator analyte reproducibility results for beads prepared using Pn6B PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	0.19	0.18	0.19	0.20	0.19	0.01	4.30 %
13-Plex 05	0.39	0.40	0.42	0.40	0.40	0.01	3.13 %
13-Plex 08	3.36	3.22	3.31	3.03	3.23	0.15	4.50 %
13-Plex 10	3.72	3.35	3.67	4.09	3.71	0.30	8.18 %
13-Plex 11	3.33	3.46	3.15	3.47	3.35	0.15	4.45 %
13-Plex 18	1.08	0.88	1.23	1.04	1.06	0.14	13.60 %
13-Plex 20	0.36	0.32	0.43	0.37	0.37	0.05	12.29 %
13-Plex 21	1.72	1.67	1.99	1.78	1.79	0.14	7.86 %
13-Plex 28	1.18	1.09	1.17	1.19	1.16	0.05	3.95 %
13-Plex 31	7.58	6.67	6.54	6.58	6.84	0.49	7.23 %
13-Plex 42	3.12	2.69	3.00	2.82	2.91	0.19	6.55 %
13-Plex 44	0.27	0.26	0.29	0.29	0.28	0.02	5.41 %
13-Plex 46	0.82	0.69	0.71	0.70	0.73	0.06	8.29 %
13-Plex 50	1.00	1.22	1.37	1.29	1.22	0.16	13.03 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.22	0.21	0.24	0.25	0.23	0.02	7.94 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							15
Number of samples with % CV of less than or equal to 25.00							15
Percentage of sample with % CV of less than or equal to 25.00							100.00 %

Appendix IV-55: Intra operator analyte reproducibility results for beads prepared using Pn9V PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	0.29	0.25	0.29	0.24	0.27	0.03	9.83%
13-Plex 05	1.42	1.43	1.50	1.29	1.41	0.09	6.21%
13-Plex 06	0.59	0.60	0.64	0.50	0.58	0.06	10.14%
13-Plex 08	2.59	2.62	3.00	2.30	2.63	0.29	10.93%
13-Plex 10	4.55	4.39	4.79	4.00	4.43	0.33	7.49%
13-Plex 16	0.69	0.65	0.75	0.60	0.67	0.06	9.43%
13-Plex 20	3.10	3.21	3.51	3.14	3.24	0.19	5.73%
13-Plex 28	0.63	0.55	0.58	0.51	0.57	0.05	8.91%
13-Plex 31	0.45	0.38	0.45	0.37	0.41	0.04	10.54%
13-Plex 34	1.27	1.31	1.36	1.20	1.29	0.07	5.26%
13-Plex 42	1.29	1.21	1.40	1.16	1.27	0.10	8.28%
13-Plex 46	0.58	0.53	0.57	0.50	0.55	0.04	6.78%
13-Plex 50	0.72	0.70	0.79	0.64	0.71	0.06	8.68%
13-Plex 51	0.24	0.20	0.22	0.21	0.22	0.02	7.85%
13-Plex 52	0.19	0.16	0.18	0.16	0.17	0.01	8.70%
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.10	0.05	0.05	0.10	0.10	0.00	0.00%
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							16
Number of samples with % CV of less than or equal to 25.00							16
Percentage of sample with % CV of less than or equal to 25.00							100.00 %

Appendix IV-56: Intra operator analyte reproducibility results for beads prepared using Pn14 PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	0.70	0.66	0.72	0.73	0.70	0.03	4.41 %
13-Plex 08	3.72	3.73	3.61	3.68	3.69	0.05	1.48 %
13-Plex 10	5.58	5.16	5.11	5.65	5.38	0.28	5.20 %
13-Plex 11	0.45	0.44	0.46	0.45	0.45	0.01	1.81 %
13-Plex 12	3.10	2.70	2.72	2.64	2.79	0.21	7.51 %
13-Plex 16	1.09	1.19	1.04	1.05	1.09	0.07	6.27 %
13-Plex 18	0.76	0.68	0.90	0.82	0.79	0.09	11.78 %
13-Plex 25	0.27	0.26	0.29	0.28	0.28	0.01	4.69 %
13-Plex 28	8.07	7.21	7.28	7.61	7.54	0.39	5.20 %
13-Plex 31	0.73	0.68	0.71	0.71	0.71	0.02	2.91 %
13-Plex 32	0.36	0.34	0.36	0.33	0.35	0.02	4.32 %
13-Plex 42	1.62	1.60	1.64	1.71	1.64	0.05	2.91 %
13-Plex 44	1.52	1.49	1.51	1.46	1.50	0.03	1.77 %
13-Plex 51	0.82	0.44	0.84	0.85	0.74	0.20	26.95 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.26	0.25	0.28	0.27	0.27	0.01	4.87 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							15
Number of samples with % CV of less than or equal to 25.00							14
Percentage of sample with % CV of less than or equal to 25.00							93.33 %

Appendix IV-57: Intra operator analyte reproducibility results for beads prepared using Pn19A PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	1.38	1.17	1.22	1.22	1.25	0.09	7.33 %
13-Plex 05	7.75	8.54	7.65	7.49	7.86	0.47	5.95 %
13-Plex 06	0.46	0.45	0.48	0.45	0.46	0.01	3.07 %
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 10	19.79	19.73	19.02	19.30	19.46	0.37	1.88 %
13-Plex 11	6.65	5.77	5.13	3.80	5.34	1.20	22.47 %
13-Plex 16	4.66	4.88	4.04	4.61	4.55	0.36	7.87 %
13-Plex 17	1.38	1.51	1.46	1.22	1.39	0.13	9.11 %
13-Plex 18	7.37	6.78	9.20	7.87	7.81	1.03	13.21 %
13-Plex 21	0.76	0.69	0.88	0.68	0.75	0.09	12.25 %
13-Plex 25	1.32	1.34	1.38	1.52	1.39	0.09	6.49 %
13-Plex 32	0.37	0.39	0.32	0.21	0.32	0.08	24.98 %
13-Plex 33	0.58	0.55	0.58	0.61	0.58	0.02	4.22 %
13-Plex 34	1.06	0.94	0.90	1.05	0.99	0.08	8.07 %
13-Plex 46	2.59	2.39	2.33	2.13	2.36	0.19	8.02 %
13-Plex 51	0.33	0.17	0.29	0.22	0.25	0.07	28.26 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							15
Number of samples with % CV of less than or equal to 25.00							14
Percentage of sample with % CV of less than or equal to 25.00							93.33 %

Appendix IV-58: Intra operator analyte reproducibility results for beads prepared using Pn23F PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 05	2.80	2.48	3	2.74	2.76	0.21	7.78%
13-Plex 10	6.24	6.08	5.53	5.25	5.78	0.46	8.03%
13-Plex 12	3.16	2.84	2.66	2.8	2.87	0.21	7.37%
13-Plex 16	0.19	0.16	0.22	0.16	0.18	0.03	15.74%
13-Plex 18	2.02	1.69	1.88	1.88	1.87	0.14	7.26%
13-Plex 20	2.34	2.09	1.88	2.18	2.12	0.19	9.04%
13-Plex 21	2.99	3.72	2.47	3.55	3.18	0.57	17.86%
13-Plex 28	0.46	0.42	0.45	0.48	0.45	0.02	5.52%
13-Plex 33	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 34	0.41	0.37	0.39	0.42	0.40	0.02	5.58%
13-Plex 42	0.77	0.74	0.72	0.67	0.73	0.04	5.80%
13-Plex 44	3.87	3.38	3.29	4.01	3.64	0.36	9.78%
13-Plex 46	0.50	0.41	0.45	0.48	0.46	0.04	8.51%
13-Plex 50	1.21	1.57	1.48	1.54	1.45	0.16	11.33%
13-Plex 59	0.33	0.31	0.31	0.33	0.32	0.01	3.61%
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							14
Number of samples with % CV of less than or equal to 25.00							14
Percentage of sample with % CV of less than or equal to 25.00							100.00 %

Appendix IV-59: Intra operator analyte reproducibility results for beads prepared using Pn1 PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	0.27	0.18	0.27	0.23	0.24	0.04	17.99 %
13-Plex 05	1.25	1.50	1.47	1.56	1.45	0.14	9.36 %
13-Plex 08	19.85	21.99	18.30	18.57	19.68	1.68	8.55 %
13-Plex 10	0.23	0.19	0.17	0.27	0.22	0.04	20.63 %
13-Plex 12	14.15	10.59	7.07	8.24	10.01	3.12	31.19 %
13-Plex 16	3.37	4.13	4.68	4.06	4.06	0.54	13.23 %
13-Plex 18	0.26	0.22	0.32	0.24	0.26	0.04	16.62 %
13-Plex 20	0.21	0.15	0.16	0.22	0.19	0.04	18.98 %
13-Plex 21	0.26	0.25	0.24	0.32	0.27	0.04	13.44 %
13-Plex 28	0.30	0.22	0.23	0.32	0.27	0.05	18.66 %
13-Plex 31	0.69	0.89	1.87	0.60	1.01	0.58	57.72 %
13-Plex 32	0.51	0.79	0.72	0.82	0.71	0.14	19.68 %
13-Plex 34	0.23	0.20	0.24	0.26	0.23	0.02	10.75 %
13-Plex 42	0.05	0.05	0.11	0.05	0.07	0.03	-
13-Plex 50	0.61	0.70	0.82	0.63	0.69	0.09	13.75 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							14
Number of samples with % CV of less than or equal to 25.00							12
Percentage of sample with % CV of less than or equal to 25.00							87.50 %

Appendix IV-60: Intra operator analyte reproducibility results for beads prepared using Pn3 PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 08	2.74	2.76	1.51	1.52	2.13	0.71	33.44%
13-Plex 10	1.02	0.93	0.83	0.89	0.92	0.08	8.69%
13-Plex 12	4.27	3.96	3.63	3.58	3.86	0.32	8.32%
13-Plex 16	0.57	0.60	0.43	0.67	0.57	0.10	17.76%
13-Plex 18	2.94	2.87	2.26	1.97	2.51	0.47	18.81%
13-Plex 20	1.84	1.83	1.29	1.20	1.54	0.34	22.25%
13-Plex 21	3.39	3.66	2.41	2.67	3.03	0.59	19.42%
13-Plex 28	0.22	0.19	0.26	0.41	0.27	0.10	36.16%
13-Plex 32	0.29	0.32	0.55	0.12	0.32	0.18	55.26%
13-Plex 34	1.26	1.17	1.33	2.20	1.49	0.48	32.07%
13-Plex 44	0.64	0.58	0.50	0.50	0.56	0.07	12.26%
13-Plex 46	5.60	5.18	4.54	5.16	5.12	0.44	8.53%
13-Plex 51	0.31	0.28	0.26	0.35	0.30	0.04	13.05%
13-Plex 52	5.38	5.87	3.51	0.29	3.76	2.53	67.20%
13-Plex 59	0.22	0.18	0.18	0.22	0.20	0.02	11.55%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							15
Number of samples with % CV of less than or equal to 25.00							10
Percentage of sample with % CV of less than or equal to 25.00							66.67 %

Appendix IV-61: Intra operator analyte reproducibility results for beads prepared using Pn5 PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 08	5.33	5.19	4.70	3.47	4.67	0.85	18.10%
13-Plex 10	0.31	0.26	0.32	0.21	0.28	0.05	18.42%
13-Plex 12	4.28	3.73	3.59	2.74	3.59	0.64	17.77%
13-Plex 18	2.08	2.10	2.18	1.46	1.96	0.33	17.02%
13-Plex 20	1.60	1.53	1.63	1.13	1.47	0.23	15.77%
13-Plex 28	0.35	0.34	0.39	0.34	0.36	0.02	6.71%
13-Plex 31	0.23	0.17	0.26	0.20	0.22	0.04	18.01%
13-Plex 33	0.24	0.20	0.26	0.14	0.21	0.05	25.20%
13-Plex 42	1.08	1.13	1.24	0.07	0.88	0.54	61.83%
13-Plex 44	1.69	1.72	1.72	1.38	1.63	0.17	10.18%
13-Plex 46	0.82	0.85	0.91	0.78	0.84	0.05	6.52%
13-Plex 50	11.06	10.51	9.47	6.22	9.32	2.17	23.25%
13-Plex 51	0.05	0.05	0.05	0.45	0.15	0.20	-
13-Plex 59	0.11	0.07	0.10	0.08	0.09	0.02	20.29%
13-Plex 60	0.21	0.20	0.22	0.20	0.21	0.01	4.61%
13-Plex 62	1.38	1.47	1.47	1.27	1.40	0.09	6.80%
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.11	0.10	0.12	0.10	0.11	0.01	8.91%
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							16
Number of samples with % CV of less than or equal to 25.00							15
Percentage of sample with % CV of less than or equal to 25.00							93.75 %

Appendix IV-62: Intra operator analyte reproducibility results for beads prepared using Pn6A PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	0.29	0.27	0.30	0.30	0.29	0.01	4.88 %
13-Plex 05	1.20	1.30	1.24	1.47	1.30	0.12	9.14 %
13-Plex 08	4.30	4.28	4.17	4.56	4.33	0.17	3.82 %
13-Plex 10	4.68	4.04	4.62	4.23	4.39	0.31	7.02 %
13-Plex 12	0.12	0.12	0.12	0.13	0.12	0.01	4.08 %
13-Plex 17	0.39	0.35	0.39	0.38	0.38	0.02	5.01 %
13-Plex 18	1.00	0.98	0.92	1.05	0.99	0.05	5.45 %
13-Plex 21	2.44	2.49	2.52	2.54	2.50	0.04	1.74 %
13-Plex 31	0.84	0.82	0.82	0.95	0.86	0.06	7.28 %
13-Plex 34	0.29	0.28	0.28	0.30	0.29	0.01	3.33 %
13-Plex 42	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 46	2.12	2.25	2.14	2.29	2.20	0.08	3.77 %
13-Plex 50	2.89	3.16	2.99	3.19	3.06	0.14	4.65 %
13-Plex 51	0.65	0.65	0.66	0.70	0.67	0.02	3.58 %
13-Plex 52	0.17	0.16	0.16	0.19	0.17	0.01	8.32 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							14
Number of samples with % CV of less than or equal to 25.00							14
Percentage of sample with % CV of less than or equal to 25.00							100.00 %

Appendix IV-63: Intra operator analyte reproducibility results for beads prepared using Pn7F PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 05	1.40	1.49	1.50	1.69	1.52	0.12	8.02 %
13-Plex 08	4.76	5.27	5.14	5.63	5.20	0.36	6.91 %
13-Plex 12	0.79	0.94	0.90	1.04	0.92	0.10	11.27 %
13-Plex 16	1.56	2.24	2.39	2.70	2.22	0.48	21.66 %
13-Plex 17	0.66	0.57	0.67	0.70	0.65	0.06	8.61 %
13-Plex 18	1.01	1.04	1.06	1.14	1.06	0.06	5.23 %
13-Plex 20	0.56	0.65	0.66	0.77	0.66	0.09	13.03 %
13-Plex 21	1.77	2.53	2.90	3.07	2.57	0.58	22.49 %
13-Plex 28	7.25	10.55	11.42	10.90	10.03	1.89	18.82 %
13-Plex 31	1.42	0.67	0.66	0.57	0.83	0.40	47.70 %
13-Plex 34	4.39	5.41	5.63	5.53	5.24	0.57	10.95 %
13-Plex 42	0.28	0.29	0.30	0.34	0.30	0.03	8.69 %
13-Plex 46	3.42	2.11	2.06	2.08	2.42	0.67	27.66 %
13-Plex 51	0.22	0.20	0.20	0.19	0.20	0.01	6.21 %
13-Plex 52	0.15	0.18	0.21	0.23	0.19	0.04	18.18 %
13-Plex 60	0.05	0.12	0.13	0.15	0.13	0.02	11.46 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							16
Number of samples with % CV of less than or equal to 25.00							14
Percentage of sample with % CV of less than or equal to 25.00							87.50 %

Appendix IV-64: Intra operator analyte reproducibility results for beads prepared using Pn18C PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	0.17	0.17	0.15	0.17	0.17	0.01	6.06 %
13-Plex 05	2.59	2.63	2.83	2.72	2.69	0.11	3.96 %
13-Plex 06	1.28	1.43	1.49	1.32	1.38	0.10	7.03 %
13-Plex 10	0.75	0.66	0.68	0.63	0.68	0.05	7.50 %
13-Plex 16	1.98	2.35	2.47	2.36	2.29	0.21	9.33 %
13-Plex 17	0.67	0.59	0.63	0.59	0.62	0.04	6.18 %
13-Plex 18	0.84	0.66	0.72	0.68	0.73	0.08	11.12 %
13-Plex 21	1.57	2.26	2.50	2.45	2.20	0.43	19.56 %
13-Plex 28	3.18	3.95	3.80	4.08	3.75	0.40	10.62 %
13-Plex 34	5.39	5.63	4.99	5.06	5.27	0.30	5.66 %
13-Plex 42	1.37	1.20	1.20	1.21	1.25	0.08	6.70 %
13-Plex 46	1.33	1.67	1.58	1.48	1.52	0.15	9.62 %
13-Plex 50	1.65	2.00	1.97	1.72	1.84	0.18	9.59 %
13-Plex 52	1.05	0.86	0.87	0.84	0.91	0.10	10.77 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.41	0.40	0.41	0.42	0.41	0.01	1.99 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							15
Number of samples with % CV of less than or equal to 25.00							15
Percentage of sample with % CV of less than or equal to 25.00							100.00 %

Appendix IV-65: Intra operator analyte reproducibility results for beads prepared using Pn19F PS.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Mean	SD	% CV
13-Plex 03	11.46	13.30	12.21	12.52	12.37	0.76	6.16 %
13-Plex 05	3.33	3.13	3.11	2.91	3.12	0.17	5.50 %
13-Plex 08	5.32	5.02	5.19	4.66	5.05	0.29	5.67 %
13-Plex 10	11.43	12.98	12.27	13.15	12.46	0.78	6.29 %
13-Plex 12	1.30	1.13	1.26	1.26	1.24	0.07	5.99 %
13-Plex 16	1.62	1.60	1.57	1.61	1.60	0.02	1.35 %
13-Plex 18	6.36	5.65	5.34	5.32	5.67	0.49	8.57 %
13-Plex 20	2.91	2.61	2.84	2.65	2.75	0.15	5.28 %
13-Plex 25	0.21	0.23	0.22	0.25	0.23	0.02	7.51 %
13-Plex 28	0.73	0.71	0.84	0.87	0.79	0.08	10.07 %
13-Plex 32	0.14	0.15	0.26	0.30	0.21	0.08	37.52 %
13-Plex 44	1.22	1.19	1.23	1.28	1.23	0.04	3.04 %
13-Plex 46	3.80	3.88	4.29	3.87	3.96	0.22	5.63 %
13-Plex 50	5.85	5.45	5.79	5.26	5.59	0.28	5.02 %
13-Plex 51	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.12	0.07	0.04	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)							15
Number of samples with % CV of less than or equal to 25.00							14
Percentage of sample with % CV of less than or equal to 25.00							93.33 %

Appendix IV-66: Low end reproducibility results for beads prepared using Pn4 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.38	0.37	0.44	0.41	0.38	0.40	0.03	7.28 %
13-Plex 05	0.65	0.78	0.82	0.73	0.74	0.74	0.06	8.53 %
13-Plex 06	0.26	0.26	0.28	0.18	0.19	0.23	0.05	19.49 %
13-Plex 10	0.34	0.44	0.49	0.38	0.37	0.40	0.06	14.91 %
13-Plex 12	0.47	0.36	0.50	0.40	0.44	0.43	0.06	12.79 %
13-Plex 20	0.80	0.82	0.92	0.72	0.65	0.78	0.10	13.12 %
13-Plex 21	0.55	0.55	0.60	0.45	0.43	0.52	0.07	14.08 %
13-Plex 28	0.86	0.99	0.88	0.85	0.84	0.88	0.06	6.91 %
13-Plex 31	0.25	0.49	0.55	0.46	0.46	0.44	0.11	25.67 %
13-Plex 32	0.20	0.29	0.27	0.26	0.25	0.25	0.03	13.23 %
13-Plex 50	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 51	0.21	0.23	0.26	0.20	0.21	0.22	0.02	10.75 %
13-Plex 59	0.11	0.15	0.16	0.13	0.11	0.13	0.02	17.28 %
13-Plex 60	0.44	0.40	0.47	0.41	0.35	0.41	0.05	10.88 %
13-Plex 69	0.16	0.15	0.15	0.13	0.13	0.14	0.01	9.32 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								14
Number of samples with % CV of less than or equal to 25.00								13
Percentage of sample with % CV of less than or equal to 25.00								92.86 %

Appendix IV-67: Low end reproducibility results for beads prepared using Pn6B PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.25	0.26	0.20	0.20	0.18	0.22	0.03	16.02 %
13-Plex 05	0.37	0.44	0.28	0.27	0.29	0.33	0.07	22.16 %
13-Plex 16	0.14	0.22	0.15	0.13	0.13	0.15	0.04	24.56 %
13-Plex 18	1.10	1.50	1.85	1.23	1.29	1.39	0.29	21.02 %
13-Plex 20	0.34	0.55	0.49	0.37	0.35	0.42	0.09	22.46 %
13-Plex 21	1.23	1.51	1.61	1.03	1.06	1.29	0.26	20.35 %
13-Plex 25	0.20	0.29	0.19	0.20	0.18	0.21	0.04	20.94 %
13-Plex 28	1.04	1.12	1.16	1.04	1.02	1.08	0.06	5.64 %
13-Plex 34	0.13	0.19	0.14	0.10	0.11	0.13	0.04	26.17 %
13-Plex 42	0.44	0.64	0.58	0.43	0.45	0.51	0.10	18.85 %
13-Plex 44	0.24	0.29	0.21	0.19	0.19	0.22	0.04	18.83 %
13-Plex 46	0.69	0.86	0.91	0.70	0.74	0.78	0.10	12.72 %
13-Plex 50	1.35	1.53	2.11	1.34	1.43	1.55	0.32	20.69 %
13-Plex 51	1.34	1.44	1.70	1.30	1.40	1.44	0.16	10.94 %
13-Plex 59	0.22	0.27	0.19	0.19	0.18	0.21	0.04	17.50 %
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 12	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-68: Low end reproducibility results for beads prepared using Pn9V PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.28	0.31	0.27	0.38	0.23	0.29	0.06	19.03%
13-Plex 06	0.52	0.56	0.58	0.55	0.49	0.54	0.04	6.55%
13-Plex 12	0.54	0.49	0.65	0.60	0.52	0.56	0.06	11.50%
13-Plex 16	0.66	0.55	0.64	0.60	0.53	0.60	0.06	9.39%
13-Plex 17	0.17	0.15	0.15	0.16	0.16	0.16	0.01	5.30%
13-Plex 18	0.78	0.70	0.80	0.70	0.60	0.72	0.08	11.07%
13-Plex 28	0.70	0.63	0.72	0.70	0.63	0.68	0.04	6.33%
13-Plex 31	0.44	0.41	0.54	0.50	0.46	0.47	0.05	10.85%
13-Plex 32	0.46	0.48	0.55	0.55	0.53	0.51	0.04	8.09%
13-Plex 44	0.14	0.15	0.15	0.15	0.11	0.14	0.02	12.37%
13-Plex 46	0.58	0.51	0.60	0.58	0.50	0.55	0.05	8.23%
13-Plex 50	0.75	0.67	0.77	0.79	0.69	0.73	0.05	7.05%
13-Plex 51	0.21	0.19	0.24	0.23	0.18	0.21	0.03	12.14%
13-Plex 52	0.18	0.18	0.18	0.15	0.12	0.16	0.03	16.56%
13-Plex 59	0.11	0.16	0.14	0.11	0.05	0.13	0.02	18.84%
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-69: Low end reproducibility results for beads prepared using Pn14 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.89	0.81	0.72	0.66	0.90	0.80	0.11	13.19%
13-Plex 11	0.82	0.65	0.68	0.60	0.71	0.69	0.08	11.89%
13-Plex 17	0.19	0.13	0.14	0.13	0.12	0.14	0.03	19.54%
13-Plex 18	1.08	0.99	0.76	0.83	0.95	0.92	0.13	13.83%
13-Plex 25	0.40	0.27	0.23	0.34	0.23	0.29	0.07	25.29%
13-Plex 31	0.95	0.92	0.77	0.83	0.70	0.83	0.10	12.42%
13-Plex 32	0.58	0.44	0.43	0.37	0.42	0.45	0.08	17.53%
13-Plex 38	0.25	0.25	0.25	0.21	0.29	0.25	0.03	11.31%
13-Plex 48	0.85	0.91	0.86	0.84	0.63	0.82	0.11	13.26%
13-Plex 49	0.31	0.25	0.30	0.23	0.19	0.26	0.05	19.45%
13-Plex 51	1.16	1.11	0.97	1.01	1.03	1.06	0.08	7.32%
13-Plex 56	0.95	3.30	0.96	0.86	0.80	1.37	1.08	78.51%
13-Plex 59	0.28	0.19	0.16	0.27	0.17	0.21	0.06	26.56%
13-Plex 60	0.38	0.27	0.26	0.23	0.28	0.28	0.06	20.01%
13-Plex 62	1.26	1.05	0.85	1.00	1.02	1.04	0.15	14.20%
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								12
Percentage of sample with % CV of less than or equal to 25.00								80.00 %

Appendix IV-70: Low end reproducibility results for beads prepared using Pn19A PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	1.17	1.05	0.88	1.42	0.82	1.07	0.24	22.52%
13-Plex 06	0.46	0.41	0.38	0.59	0.34	0.44	0.10	22.15%
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 17	1.31	1.15	0.94	1.41	1.01	1.16	0.20	16.96%
13-Plex 20	1.07	1.13	0.90	0.87	0.84	0.96	0.13	13.46%
13-Plex 21	0.65	0.56	0.47	0.78	0.47	0.59	0.13	22.46%
13-Plex 25	1.25	1.18	0.94	1.41	1.02	1.16	0.19	16.07%
13-Plex 28	0.70	0.45	0.40	0.42	0.55	0.50	0.12	24.57%
13-Plex 31	0.44	0.88	0.72	0.85	0.86	0.75	0.18	24.59%
13-Plex 32	0.65	0.63	0.47	0.33	0.35	0.49	0.15	30.99%
13-Plex 33	0.54	0.42	0.33	0.48	0.45	0.44	0.08	17.49%
13-Plex 34	1.07	0.86	0.71	1.13	0.80	0.91	0.18	19.61%
13-Plex 44	0.44	0.34	0.26	0.44	0.26	0.35	0.09	25.89%
13-Plex 51	0.23	0.16	0.15	0.32	0.23	0.22	0.07	31.35%
13-Plex 52	0.18	0.33	0.25	0.36	0.27	0.28	0.07	25.36%
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								14
Number of samples with % CV of less than or equal to 25.00								10
Percentage of sample with % CV of less than or equal to 25.00								71.43 %

Appendix IV-71: Low end reproducibility results for beads prepared using Pn23F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 08	0.44	0.46	0.45	0.45	0.41	0.44	0.02	4.35%
13-Plex 16	0.23	0.20	0.20	0.18	0.17	0.20	0.02	11.75%
13-Plex 25	0.25	0.18	0.18	0.17	0.21	0.20	0.03	16.52%
13-Plex 28	0.52	0.52	0.45	0.46	0.53	0.50	0.04	7.62%
13-Plex 29	1.07	0.83	1.00	0.98	0.69	0.91	0.15	16.71%
13-Plex 31	0.39	0.39	0.29	0.32	0.33	0.34	0.04	12.94%
13-Plex 34	0.48	0.47	0.40	0.38	0.43	0.43	0.04	10.01%
13-Plex 38	0.27	0.23	0.28	0.24	0.19	0.24	0.04	14.73%
13-Plex 42	0.78	0.91	0.71	0.71	0.62	0.75	0.11	14.46%
13-Plex 46	0.70	0.74	0.60	0.65	0.69	0.68	0.05	7.87%
13-Plex 48	0.66	0.61	0.59	0.67	0.46	0.60	0.08	14.06%
13-Plex 49	0.61	0.51	0.61	0.50	0.49	0.54	0.06	11.15%
13-Plex 51	0.45	0.48	0.38	0.39	0.41	0.42	0.04	9.97%
13-Plex 52	0.26	0.20	0.20	0.18	0.25	0.22	0.03	16.02%
13-Plex 59	0.36	0.34	0.31	0.31	0.45	0.35	0.06	16.30%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-72: Low end reproducibility results for beads prepared using Pn1 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.15	0.15	0.15	0.18	0.15	0.16	0.01	8.60 %
13-Plex 10	0.11	0.05	0.11	0.05	0.05	0.11	0.00	0.00 %
13-Plex 20	0.12	0.12	0.05	0.05	0.05	0.12	0.00	0.00 %
13-Plex 21	0.16	0.16	0.13	0.12	0.13	0.14	0.02	13.36 %
13-Plex 28	0.14	0.10	0.10	0.05	0.05	0.11	0.02	20.38 %
13-Plex 29	0.13	0.27	0.32	0.23	0.18	0.23	0.07	32.90 %
13-Plex 31	0.87	1.79	1.61	1.35	1.27	1.38	0.35	25.50 %
13-Plex 32	0.68	0.72	0.71	0.49	0.56	0.63	0.10	16.11 %
13-Plex 34	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 48	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 49	0.18	0.17	0.20	0.15	0.18	0.18	0.02	10.32 %
13-Plex 50	0.79	0.65	0.70	0.63	0.59	0.67	0.08	11.45 %
13-Plex 58	0.17	0.19	0.21	0.17	0.18	0.18	0.02	9.09 %
13-Plex 59	0.11	0.10	0.05	0.05	0.05	0.11	0.01	6.73 %
13-Plex 60	0.11	0.12	0.13	0.05	0.05	0.12	0.01	8.33 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								13
Number of samples with % CV of less than or equal to 25.00								11
Percentage of sample with % CV of less than or equal to 25.00								84.62 %

Appendix IV-73: Low end reproducibility results for beads prepared using Pn3 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.27	0.31	0.27	0.30	0.37	0.30	0.04	13.48%
13-Plex 06	0.13	0.18	0.19	0.11	0.18	0.16	0.04	22.56%
13-Plex 15	0.34	0.37	0.33	0.41	0.43	0.38	0.04	11.53%
13-Plex 16	0.71	0.69	0.67	0.78	0.95	0.76	0.11	15.00%
13-Plex 27	0.76	1.02	0.70	0.76	0.59	0.81	0.14	20.63%
13-Plex 28	0.23	0.20	0.27	0.14	0.14	0.20	0.06	29.00%
13-Plex 29	0.45	0.94	0.63	0.64	0.57	0.65	0.18	28.01%
13-Plex 32	0.34	0.53	0.51	0.43	0.73	0.51	0.14	28.54%
13-Plex 44	0.60	0.57	0.72	0.49	0.64	0.60	0.09	14.08%
13-Plex 49	0.26	0.39	0.24	0.30	0.28	0.29	0.06	19.77%
13-Plex 51	0.60	0.77	0.77	0.71	0.96	0.76	0.13	17.15%
13-Plex 52	0.38	0.45	0.40	0.36	0.40	0.40	0.03	8.41%
13-Plex 56	0.50	1.82	1.20	0.73	0.83	1.02	0.52	50.74%
13-Plex 59	0.23	0.28	0.32	0.17	0.19	0.24	0.06	26.14%
13-Plex 60	0.58	0.13	0.18	0.10	0.14	0.23	0.20	88.47%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								9
Percentage of sample with % CV of less than or equal to 25.00								60.00 %

Appendix IV-74: Low end reproducibility results for beads prepared using Pn5 PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 06	0.34	0.31	0.42	0.28	0.28	0.33	0.06	17.83 %
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 09	0.61	0.63	0.67	0.50	0.58	0.60	0.06	10.67 %
13-Plex 10	0.21	0.25	0.22	0.20	0.21	0.22	0.02	8.82 %
13-Plex 20	1.13	1.37	1.51	1.12	1.21	1.27	0.17	13.27 %
13-Plex 28	0.25	0.27	0.34	0.24	0.24	0.27	0.04	15.70 %
13-Plex 31	0.19	0.17	0.26	0.15	0.19	0.19	0.04	21.60 %
13-Plex 32	0.27	0.21	0.26	0.19	0.23	0.23	0.03	14.43 %
13-Plex 33	0.15	0.17	0.26	0.16	0.18	0.18	0.04	23.88 %
13-Plex 34	0.27	0.26	0.33	0.23	0.23	0.26	0.04	15.53 %
13-Plex 42	1.13	1.12	1.43	1.01	1.10	1.16	0.16	13.75 %
13-Plex 44	1.28	1.40	1.62	1.25	1.40	1.39	0.15	10.47 %
13-Plex 46	0.76	0.84	1.13	0.72	0.80	0.85	0.16	19.15 %
13-Plex 51	0.51	0.41	0.57	0.41	0.46	0.47	0.07	14.56 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.20	0.19	0.25	0.18	0.18	0.20	0.03	14.58 %
13-Plex 62	1.20	1.24	1.41	1.12	1.22	1.24	0.11	8.59 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-75: Low end reproducibility results for beads prepared using Pn6A PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.27	0.31	0.26	0.29	0.30	0.29	0.02	7.25 %
13-Plex 05	1.35	1.67	1.76	1.31	1.71	1.56	0.21	13.64 %
13-Plex 12	0.13	0.16	0.19	0.13	0.10	0.14	0.03	24.09 %
13-Plex 17	0.19	0.19	0.19	0.18	0.14	0.18	0.02	12.18 %
13-Plex 18	1.70	1.78	1.88	1.60	1.68	1.73	0.11	6.16 %
13-Plex 20	0.08	0.11	0.14	0.08	0.10	0.10	0.02	24.41 %
13-Plex 21	1.75	2.18	1.83	1.66	1.83	1.85	0.20	10.67 %
13-Plex 28	0.12	0.12	0.16	0.13	0.10	0.13	0.02	17.39 %
13-Plex 31	1.41	1.66	1.58	1.26	1.75	1.53	0.20	12.85 %
13-Plex 32	0.18	0.22	0.22	0.17	0.25	0.21	0.03	15.73 %
13-Plex 34	0.17	0.19	0.28	0.13	0.19	0.19	0.05	28.62 %
13-Plex 42	1.73	2.18	2.07	1.60	2.03	1.92	0.25	12.76 %
13-Plex 46	1.90	1.73	1.86	1.62	1.51	1.72	0.16	9.45 %
13-Plex 51	0.41	0.52	0.63	0.43	0.43	0.48	0.09	19.03 %
13-Plex 52	0.11	0.14	0.14	0.11	0.11	0.12	0.02	13.47 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-76: Low end reproducibility results for beads prepared using Pn7F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.13	0.11	0.16	0.13	0.11	0.13	0.02	16.01 %
13-Plex 06	0.09	0.09	0.12	0.07	0.07	0.09	0.02	23.29 %
13-Plex 07	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 12	0.72	0.50	0.75	0.55	0.60	0.62	0.11	17.28 %
13-Plex 17	0.89	0.59	0.74	0.65	0.79	0.73	0.12	16.06 %
13-Plex 18	0.92	0.99	1.08	0.77	0.83	0.92	0.12	13.46 %
13-Plex 20	0.46	0.47	0.51	0.36	0.42	0.44	0.06	12.80 %
13-Plex 21	1.17	0.96	1.13	0.81	0.88	0.99	0.16	15.76 %
13-Plex 31	0.89	1.00	1.21	0.93	1.07	1.02	0.13	12.40 %
13-Plex 32	1.17	0.97	1.09	0.92	1.16	1.06	0.11	10.60 %
13-Plex 33	0.63	0.31	0.55	0.43	0.50	0.48	0.12	25.12 %
13-Plex 44	0.23	0.24	0.33	0.19	0.21	0.24	0.05	22.44 %
13-Plex 51	0.24	0.21	0.30	0.18	0.20	0.23	0.05	20.66 %
13-Plex 52	0.14	0.10	0.15	0.10	0.10	0.12	0.02	21.10 %
13-Plex 59	0.11	0.12	0.17	0.10	0.10	0.12	0.03	24.30 %
13-Plex 62	0.41	0.28	0.40	0.26	0.30	0.33	0.07	21.21 %
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								14
Percentage of sample with % CV of less than or equal to 25.00								93.33 %

Appendix IV-77: Low end reproducibility results for beads prepared using Pn18C PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 03	0.18	0.18	0.23	0.20	0.18	0.19	0.02	11.29%
13-Plex 06	1.11	1.28	1.31	1.11	1.09	1.18	0.11	8.97%
13-Plex 10	0.60	0.76	0.57	0.60	0.64	0.63	0.07	11.78%
13-Plex 15	0.23	0.23	0.22	0.23	0.21	0.22	0.01	3.99%
13-Plex 17	0.47	0.54	0.63	0.53	0.58	0.55	0.06	10.83%
13-Plex 18	0.65	0.83	0.88	0.67	0.72	0.75	0.10	13.43%
13-Plex 25	0.22	0.29	0.33	0.27	0.24	0.27	0.04	15.93%
13-Plex 31	0.48	0.57	0.72	0.53	0.55	0.57	0.09	15.84%
13-Plex 38	0.65	0.79	0.61	0.67	0.74	0.69	0.07	10.44%
13-Plex 44	1.00	1.33	1.38	1.00	1.22	1.19	0.18	15.13%
13-Plex 48	0.39	0.40	0.34	0.36	0.37	0.37	0.02	6.42%
13-Plex 52	0.24	0.24	0.28	0.21	0.23	0.24	0.03	10.62%
13-Plex 58	0.46	0.48	0.39	0.45	0.52	0.46	0.05	10.31%
13-Plex 59	0.46	0.51	0.60	0.48	0.46	0.50	0.06	11.65%
13-Plex 62	0.44	0.47	0.53	0.43	0.45	0.46	0.04	8.57%
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								15
Percentage of sample with % CV of less than or equal to 25.00								100.00 %

Appendix IV-78: Low end reproducibility results for beads prepared using Pn19F PS.

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD	% CV
13-Plex 12	1.01	0.85	1.26	0.90	1.01	1.01	0.16	15.73%
13-Plex 16	1.45	1.62	1.87	1.40	1.54	1.58	0.18	11.72%
13-Plex 17	0.36	0.27	0.36	0.29	0.34	0.32	0.04	12.84%
13-Plex 21	0.63	0.90	0.88	0.58	0.63	0.72	0.15	21.14%
13-Plex 25	0.16	0.20	0.25	0.19	0.17	0.19	0.04	18.08%
13-Plex 28	0.74	0.79	0.99	0.80	0.75	0.81	0.10	12.49%
13-Plex 31	0.62	0.72	0.93	0.55	0.67	0.70	0.14	20.65%
13-Plex 32	0.58	0.47	0.50	0.43	0.54	0.50	0.06	11.62%
13-Plex 33	0.12	0.14	0.23	0.13	0.17	0.16	0.04	28.09%
13-Plex 34	0.60	0.62	0.75	0.54	0.58	0.62	0.08	12.86%
13-Plex 44	0.89	1.04	1.29	0.84	1.00	1.01	0.18	17.31%
13-Plex 49	0.32	0.43	0.27	0.32	0.20	0.31	0.08	27.30%
13-Plex 51	0.32	0.25	0.35	0.23	0.26	0.28	0.05	17.98%
13-Plex 52	0.11	0.10	0.12	0.08	0.09	0.10	0.02	15.81%
13-Plex 59	0.12	0.12	0.17	0.10	0.10	0.12	0.03	23.47%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 66	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 69	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 73	0.05	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples tested (not including negative samples)								15
Number of samples with % CV of less than or equal to 25.00								13
Percentage of sample with % CV of less than or equal to 25.00								86.67 %

Appendix V. Assay Validation: Linearity

Appendix V-1: Linearity results using beads prepared from all panel A serotypes

Pn4					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	0.69	0.48	0.90	0.79	0.98
13-Plex 08	2.37	1.66	3.08	2.23	2.24
13-Plex 11	5.42	3.79	7.05	3.69	3.52
13-Plex 12	0.36	0.25	0.47	0.33	0.32
13-Plex 18	0.55	0.39	0.72	0.58	0.63
13-Plex 20	0.76	0.53	0.99	0.74	0.76
13-Plex 22	1.67	1.17	2.17	1.76	1.61
13-Plex 42	1.75	1.23	2.28	1.36	1.40
13-Plex 46	0.60	0.42	0.78	0.48	0.44
13-Plex 53	0.31	0.22	0.40	0.18	N/A
Total number of samples included in analysis				19	
Number of samples with adjusted result within ± 30 %				15	
Percentage of results within acceptance criteria				78.95 %	
Pn6B					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 08	5.26	3.68	6.84	5.22	5.53
13-Plex 11	3.09	2.16	4.02	2.49	2.04
13-Plex 12	0.05	0.04	0.07	N/A	N/A
13-Plex 18	1.60	1.12	2.08	1.66	1.81
13-Plex 20	0.42	0.29	0.55	0.42	N/A
13-Plex 22	5.41	3.79	7.03	5.30	4.99
13-Plex 31	9.20	6.44	11.96	8.09	8.47
13-Plex 42	0.60	0.42	0.78	0.46	N/A
13-Plex 46	0.84	0.59	1.09	0.66	0.65
13-Plex 50	1.49	1.04	1.94	1.70	1.45
Total number of samples included in analysis				16	
Number of samples with adjusted result within ± 30 %				15	
Percentage of results within acceptance criteria				93.75%	
Pn9V					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	1.14	0.80	1.48	1.37	1.43
13-Plex 08	2.79	1.95	3.63	2.97	2.95
13-Plex 11	1.34	0.94	1.74	1.09	0.94
13-Plex 12	0.51	0.36	0.66	0.42	0.35
13-Plex 18	0.70	0.49	0.91	0.67	0.68
13-Plex 20	2.98	2.09	3.87	2.75	2.51
13-Plex 22	2.00	1.40	2.60	1.97	1.73
13-Plex 42	1.63	1.14	2.12	1.48	1.49
13-Plex 46	0.58	0.41	0.75	0.47	0.47
13-Plex 50	0.66	0.46	0.86	0.80	0.64
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				19	
Percentage of results within acceptance criteria				95.00%	
Pn14					

Appendix V-1: Linearity results using beads prepared from all panel A serotypes (continued)

Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	3.37	2.36	4.38	3.48	3.54
13-Plex 08	4.20	2.94	5.46	4.28	4.41
13-Plex 11	0.73	0.51	0.95	0.55	0.44
13-Plex 12	2.96	2.07	3.85	2.65	2.56
13-Plex 18	0.86	0.60	1.12	0.93	0.97
13-Plex 20	3.76	2.63	4.89	3.51	3.16
13-Plex 22	3.17	2.22	4.12	3.44	3.08
13-Plex 42	2.10	1.47	2.73	1.65	1.56
13-Plex 46	1.74	1.22	2.26	1.47	1.56
13-Plex 50	0.88	0.62	1.14	1.00	0.75
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				19	
Percentage of results within acceptance criteria				95.00%	

Pn19A

Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	9.71	6.80	12.62	7.46	8.15
13-Plex 11	5.22	3.65	6.79	4.00	3.70
13-Plex 18	9.26	6.48	12.04	6.63	7.11
13-Plex 20	1.04	0.73	1.35	1.07	0.94
13-Plex 22	2.71	1.90	3.52	2.78	2.54
13-Plex 31	0.99	0.69	1.29	0.82	4.36
13-Plex 42	0.87	0.61	1.13	0.69	N/A
13-Plex 46	2.96	2.07	3.85	2.47	2.57
13-Plex 50	2.67	1.87	3.47	3.14	2.71
13-Plex 53	8.74	6.12	11.36	7.98	7.74
Total number of samples included in analysis				19	
Number of samples with adjusted result within ± 30 %				18	
Percentage of results within acceptance criteria				94.74%	

Pn23F

Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	2.61	1.83	3.39	3.08	3.31
13-Plex 11	4.74	3.32	6.16	4.04	3.86
13-Plex 12	2.80	1.96	3.64	2.46	2.55
13-Plex 18	1.99	1.39	2.59	1.94	2.07
13-Plex 20	2.11	1.48	2.74	1.93	1.89
13-Plex 22	5.08	3.56	6.60	5.36	4.29
13-Plex 42	0.78	0.55	1.01	0.73	0.70
13-Plex 46	0.69	0.48	0.90	0.59	0.58
13-Plex 50	1.62	1.13	2.11	1.87	1.62
13-Plex 53	0.39	0.27	0.51	N/A	N/A
Total number of samples included in analysis				18	
Number of samples with adjusted result within ± 30 %				18	
Percentage of results within acceptance criteria				100.00%	

Appendix V-2: Linearity results using beads prepared from all panel B serotypes

Pn1					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	1.99	1.39	2.59	1.60	1.62
13-Plex 08	31.88	22.32	41.44	27.61	25.44
13-Plex 11	5.27	3.69	6.85	3.89	3.83
13-Plex 18	0.41	0.29	0.53	0.30	0.27
13-Plex 22	3.07	2.15	3.99	3.25	2.82
13-Plex 31	1.92	1.34	2.50	0.87	0.72
13-Plex 42	3.30	2.31	4.29	2.52	2.51
13-Plex 46	3.98	2.79	5.17	4.60	4.64
13-Plex 50	0.90	0.63	1.17	0.89	0.65
13-Plex 53	1.68	1.18	2.18	0.80	0.72
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				15	
Percentage of results within acceptance criteria				75.00%	
Pn3					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	8.43	5.90	10.96	6.29	6.35
13-Plex 12	4.40	3.08	5.72	3.24	2.96
13-Plex 18	4.76	3.33	6.19	3.61	3.66
13-Plex 20	2.46	1.72	3.20	2.12	1.94
13-Plex 22	8.58	6.01	11.15	7.15	6.16
13-Plex 31	4.92	3.44	6.40	1.98	3.27
13-Plex 42	8.76	6.13	11.39	4.19	3.70
13-Plex 46	4.09	2.86	5.32	4.17	4.72
13-Plex 50	9.77	6.84	12.70	7.13	10.71
13-Plex 53	8.84	6.19	11.49	4.67	4.79
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				16	
Percentage of results within acceptance criteria				80.00%	
Pn5					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 08	4.90	3.43	6.37	5.70	5.68
13-Plex 11	3.01	2.11	3.91	2.31	2.05
13-Plex 12	3.14	2.20	4.08	3.01	2.91
13-Plex 18	1.99	1.39	2.59	2.07	2.21
13-Plex 20	1.34	0.94	1.74	1.35	1.31
13-Plex 22	1.06	0.74	1.38	1.31	1.24
13-Plex 42	1.35	0.95	1.76	1.32	1.37
13-Plex 46	0.90	0.63	1.17	0.83	0.83
13-Plex 50	7.55	5.29	9.82	6.62	7.12
13-Plex 53	0.54	0.38	0.70	0.50	0.52
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				19	
Percentage of results within acceptance criteria				95.00%	
Pn6A					

Appendix V-2: Linearity results using beads prepared from all panel B serotypes (continued)

Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	2.00	1.40	2.60	1.89	1.97
13-Plex 08	7.20	5.04	9.36	7.90	7.73
13-Plex 11	4.79	3.35	6.23	3.68	3.46
13-Plex 18	2.02	1.41	2.63	1.88	1.94
13-Plex 20	0.05	0.04	0.07	-	-
13-Plex 22	6.04	4.23	7.85	6.61	6.76
13-Plex 31	2.11	1.48	2.74	1.36	2.23
13-Plex 42	2.80	1.96	3.64	2.45	2.48
13-Plex 46	2.18	1.53	2.83	1.88	1.81
13-Plex 50	4.92	3.44	6.40	5.75	5.32
Total number of samples included in analysis				18	
Number of samples with adjusted result within ± 30 %				18	
Percentage of results within acceptance criteria				100.00%	
Pn7F					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	1.06	0.74	1.38	1.31	1.32
13-Plex 08	3.85	2.70	5.01	4.75	5.21
13-Plex 11	4.73	3.31	6.15	3.39	3.06
13-Plex 12	0.57	0.40	0.74	0.49	0.39
13-Plex 18	0.84	0.59	1.09	0.90	0.94
13-Plex 22	2.99	2.09	3.89	3.40	3.75
13-Plex 42	2.97	2.08	3.86	3.26	3.31
13-Plex 46	1.84	1.29	2.39	1.55	1.39
13-Plex 50	0.94	0.66	1.22	1.36	1.09
13-Plex 53	0.94	0.66	1.22	0.72	0.77
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				16	
Percentage of results within acceptance criteria				80.00%	
Pn18C					
Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	3.27	2.29	4.25	2.78	3.12
13-Plex 08	2.97	2.08	3.86	2.99	2.90
13-Plex 11	5.88	4.12	7.64	3.76	3.71
13-Plex 12	2.05	1.44	2.67	1.95	2.01
13-Plex 18	0.81	0.57	1.05	0.77	0.87
13-Plex 22	1.06	0.74	1.38	1.14	1.18
13-Plex 42	1.59	1.11	2.07	1.39	1.60
13-Plex 46	2.02	1.41	2.63	1.56	1.66
13-Plex 50	1.46	1.02	1.90	1.48	1.44
13-Plex 53	0.84	0.59	1.09	0.90	1.02
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				20	
Percentage of results within acceptance criteria				100.00%	
Pn19F					

Appendix VI-2: Linearity results using beads prepared from all panel B serotypes (continued)

Positive Sample	Result	Result -30%	Result + 30%	Spiked result adjusted for dilution factor	
				1/4	1/8
13-Plex 05	3.50	2.45	4.55	3.68	3.90
13-Plex 08	5.25	3.68	6.83	5.61	5.42
13-Plex 11	6.94	4.86	9.02	5.67	5.43
13-Plex 12	0.96	0.67	1.25	0.93	0.78
13-Plex 18	6.42	4.49	8.35	6.16	6.38
13-Plex 20	2.33	1.63	3.03	2.21	2.09
13-Plex 22	5.63	3.94	7.32	6.48	6.62
13-Plex 42	3.26	2.28	4.24	3.04	3.37
13-Plex 46	4.24	2.97	5.51	3.91	4.06
13-Plex 53	1.72	1.20	2.24	1.65	1.74
Total number of samples included in analysis				20	
Number of samples with adjusted result within ± 30 %				20	
Percentage of results within acceptance criteria				100.00%	

Appendix VI. Assay Validation: Stability

Appendix VI-1: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn4 PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	0.36	0.36	0.34	0.30	0.34	0.03	8.32 %
13-Plex 06	0.22	0.20	0.24	0.23	0.22	0.02	7.68 %
13-Plex 08	1.75	1.78	1.66	1.62	1.70	0.07	4.41 %
13-Plex 10	0.37	0.34	0.32	0.37	0.35	0.02	7.00 %
13-Plex 11	5.70	5.32	5.00	5.19	5.30	0.30	5.58 %
13-Plex 12	0.38	0.39	0.45	0.44	0.42	0.04	8.46 %
13-Plex 18	0.65	0.44	0.52	0.51	0.53	0.09	16.52 %
13-Plex 19	1.99	2.13	1.99	2.34	2.11	0.17	7.83 %
13-Plex 20	0.60	0.65	0.68	0.74	0.67	0.06	8.77 %
13-Plex 21	0.35	0.43	0.40	0.38	0.39	0.03	8.63 %
13-Plex 28	0.72	0.66	0.82	0.75	0.74	0.07	9.02 %
13-Plex 31	0.37	0.39	0.39	0.41	0.39	0.02	4.19 %
13-Plex 39	2.00	2.45	2.68	2.26	2.35	0.29	12.28 %
13-Plex 41	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	2.01	2.07	2.19	1.94	2.05	0.11	5.16 %
13-Plex 44	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.27	0.32	0.31	0.26	0.29	0.03	10.15 %
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							15
Number of samples with % CV ≤ 25 %							15
Percentage of samples with % CV ≤ 25 %							100.00 %

Appendix VI-2: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn6B PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 05	0.35	0.35	0.33	0.32	0.34	0.02	4.44 %
13-Plex 08	3.65	3.83	3.83	3.51	3.71	0.16	4.19 %
13-Plex 10	2.74	2.51	2.41	2.64	2.58	0.14	5.62 %
13-Plex 11	3.35	3.08	3.00	3.07	3.13	0.15	4.93 %
13-Plex 17	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 18	1.67	1.12	1.32	1.35	1.37	0.23	16.67 %
13-Plex 19	2.98	2.81	2.65	3.13	2.89	0.21	7.19 %
13-Plex 21	1.31	1.60	1.35	1.39	1.41	0.13	9.15 %
13-Plex 25	0.22	0.16	0.18	0.21	0.19	0.03	14.31 %
13-Plex 28	0.99	0.86	1.03	1.02	0.98	0.08	8.05 %
13-Plex 31	7.38	7.54	7.09	8.49	7.63	0.61	7.95 %
13-Plex 33	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 34	0.05	0.05	0.05	0.10	0.06	0.03	-
13-Plex 39	1.18	1.37	1.44	1.21	1.30	0.13	9.63 %
13-Plex 41	0.44	0.45	0.54	0.37	0.45	0.07	15.50 %
13-Plex 50	1.26	1.68	1.53	2.67	1.79	0.62	34.46 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.20	0.21	0.20	0.16	0.19	0.02	11.52 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							14
Number of samples with % CV ≤ 25 %							13
Percentage of samples with % CV ≤ 25 %							92.86 %

Appendix VI-3: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn9V PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	0.28	0.31	0.27	0.25	0.28	0.02	9.01 %
13-Plex 05	0.92	0.99	0.89	0.93	0.93	0.04	4.50 %
13-Plex 08	2.56	2.53	2.56	2.38	2.51	0.09	3.44 %
13-Plex 10	3.64	3.35	2.98	3.65	3.41	0.32	9.27 %
13-Plex 11	1.01	0.98	0.89	0.90	0.95	0.06	6.26 %
13-Plex 12	0.58	0.57	0.67	0.65	0.62	0.05	8.08 %
13-Plex 18	0.79	0.54	0.65	0.65	0.66	0.10	15.58 %
13-Plex 19	4.38	4.51	4.03	4.75	4.42	0.30	6.80 %
13-Plex 20	2.26	2.50	2.44	2.78	2.50	0.22	8.64 %
13-Plex 28	0.50	0.45	0.53	0.51	0.50	0.03	6.84 %
13-Plex 31	0.28	0.29	0.30	0.31	0.30	0.01	4.38 %
13-Plex 33	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 34	1.01	0.96	0.95	1.23	1.04	0.13	12.63 %
13-Plex 39	0.66	0.77	0.82	0.65	0.73	0.08	11.51 %
13-Plex 42	1.49	1.83	1.65	1.97	1.74	0.21	12.07 %
13-Plex 52	0.13	0.16	0.14	0.14	0.14	0.01	8.83 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							15
Number of samples with % CV ≤ 25 %							15
Percentage of samples with % CV ≤ 25 %							100.00 %

Appendix VI-4: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn14 PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	0.85	0.89	0.80	0.72	0.82	0.07	8.99 %
13-Plex 08	4.14	4.35	4.28	3.95	4.18	0.18	4.22 %
13-Plex 10	3.25	3.28	3.10	3.43	3.27	0.14	4.14 %
13-Plex 12	2.32	2.42	2.69	2.78	2.55	0.22	8.53 %
13-Plex 16	0.65	0.55	0.61	0.61	0.61	0.04	6.82 %
13-Plex 17	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 18	1.28	0.88	1.04	1.06	1.07	0.16	15.44 %
13-Plex 19	1.98	1.96	1.89	2.13	1.99	0.10	5.08 %
13-Plex 21	3.85	4.38	3.71	3.95	3.97	0.29	7.27 %
13-Plex 28	5.34	4.99	5.56	5.62	5.38	0.28	5.30 %
13-Plex 33	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 39	3.86	3.32	3.44	3.44	3.52	0.24	6.74 %
13-Plex 41	0.22	0.21	0.27	0.19	0.22	0.03	15.30 %
13-Plex 42	1.64	1.69	1.69	1.55	1.64	0.07	4.02 %
13-Plex 50	0.61	0.82	0.77	1.38	0.90	0.34	37.49 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.23	0.18	0.19	0.20	0.20	0.02	10.80 %
13-Plex 62	1.01	0.88	1.14	0.94	0.99	0.11	11.26 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							15
Number of samples with % CV ≤ 25 %							13
Percentage of samples with % CV ≤ 25 %							93.33 %

Appendix VI-5: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn19A PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	0.91	0.89	0.85	0.75	0.85	0.07	8.37 %
13-Plex 04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 05	7.40	8.71	7.70	7.97	7.95	0.56	7.06 %
13-Plex 06	0.46	0.44	0.48	0.46	0.46	0.02	3.55 %
13-Plex 10	13.04	12.67	12.61	12.61	12.73	0.21	1.63 %
13-Plex 18	11.66	7.98	10.20	10.15	10.00	1.52	15.17 %
13-Plex 19	1.71	1.75	1.58	1.86	1.73	0.12	6.70 %
13-Plex 20	0.78	0.85	0.83	0.93	0.85	0.06	7.36 %
13-Plex 21	0.48	0.60	0.51	0.51	0.53	0.05	9.90 %
13-Plex 25	1.48	1.11	1.19	1.39	1.29	0.17	13.29 %
13-Plex 33	0.57	0.44	0.46	0.49	0.49	0.06	11.66 %
13-Plex 34	0.65	0.65	0.64	0.83	0.69	0.09	13.25 %
13-Plex 39	4.06	3.43	3.63	3.62	3.69	0.27	7.23 %
13-Plex 41	0.14	0.10	0.11	0.05	0.12	0.02	17.84 %
13-Plex 47	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 50	2.44	3.16	2.91	6.08	3.65	1.65	45.21 %
13-Plex 51	0.30	0.31	0.29	0.37	0.32	0.04	11.32 %
13-Plex 62	6.15	4.91	7.58	5.61	6.06	1.13	18.67 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							16
Number of samples with % CV ≤ 25 %							15
Percentage of samples with % CV ≤ 25 %							93.75 %

Appendix VI-6: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn23F PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	4.30	4.64	4.06	3.63	4.16	0.42	10.21 %
13-Plex 08	0.31	0.33	0.33	0.30	0.32	0.02	4.72 %
13-Plex 11	3.92	3.59	3.36	3.52	3.60	0.24	6.55 %
13-Plex 12	2.27	2.28	2.57	2.49	2.40	0.15	6.28 %
13-Plex 16	0.25	0.21	0.22	0.23	0.23	0.02	7.51 %
13-Plex 18	2.60	1.82	2.17	2.18	2.19	0.32	14.55 %
13-Plex 19	6.26	6.40	5.96	6.83	6.36	0.36	5.68 %
13-Plex 20	1.50	1.64	1.51	1.78	1.61	0.13	8.18 %
13-Plex 28	0.33	0.33	0.36	0.36	0.35	0.02	5.02 %
13-Plex 31	0.60	0.63	0.60	0.64	0.62	0.02	3.34 %
13-Plex 33	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 34	0.23	0.26	0.24	0.31	0.26	0.04	13.69 %
13-Plex 39	1.50	1.85	1.91	1.58	1.71	0.20	11.73 %
13-Plex 41	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	0.88	1.05	0.95	1.14	1.01	0.11	11.33 %
13-Plex 44	2.90	4.03	2.92	2.11	2.99	0.79	26.40 %
13-Plex 52	0.18	0.22	0.19	0.18	0.19	0.02	9.83 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							15
Number of samples with % CV ≤ 25 %							15
Percentage of samples with % CV ≤ 25 %							93.33 %

Appendix VI-7: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn1 PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	0.14	0.12	0.14	0.14	0.14	0.01	7.41 %
13-Plex 05	1.38	1.14	1.58	1.38	1.37	0.18	13.14 %
13-Plex 08	28.28	27.65	26.52	26.98	27.36	0.77	2.82 %
13-Plex 10	0.10	0.13	0.10	0.12	0.11	0.01	13.33 %
13-Plex 11	7.48	7.43	7.95	6.21	7.27	0.74	10.22 %
13-Plex 12	16.12	17.88	19.08	20.71	18.45	1.94	10.50 %
13-Plex 16	3.95	4.99	4.06	4.00	4.25	0.50	11.66 %
13-Plex 18	0.71	0.30	0.19	0.29	0.37	0.23	61.86 %
13-Plex 19	3.30	3.26	2.43	3.53	3.13	0.48	15.39 %
13-Plex 20	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 21	0.11	0.14	0.05	0.10	0.12	0.02	17.84 %
13-Plex 31	1.65	1.80	1.56	1.02	1.51	0.34	22.54 %
13-Plex 34	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	1.75	1.77	1.68	1.39	1.65	0.18	10.68 %
13-Plex 44	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 50	0.37	0.70	0.76	0.71	0.64	0.18	28.13 %
13-Plex 51	0.05	0.05	0.05	0.15	0.08	0.05	-
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							13
Number of samples with % CV ≤ 25 %							11
Percentage of samples with % CV ≤ 25 %							84.62 %

Appendix VI-8: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn3 PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	0.29	0.25	0.30	0.29	0.28	0.02	7.85%
13-Plex 05	6.90	5.22	7.86	6.95	6.73	1.10	16.35%
13-Plex 10	0.64	0.93	0.73	0.75	0.76	0.12	15.93%
13-Plex 16	0.66	0.85	0.70	0.68	0.72	0.09	11.98%
13-Plex 18	9.40	3.79	2.46	3.89	4.89	3.08	63.05%
13-Plex 19	2.82	2.89	2.01	2.94	2.67	0.44	16.49%
13-Plex 20	1.81	2.16	1.38	2.14	1.87	0.37	19.52%
13-Plex 21	5.73	7.54	4.26	6.10	5.91	1.35	22.81%
13-Plex 28	0.12	0.13	0.12	0.12	0.12	0.01	4.08%
13-Plex 33	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 34	0.73	0.95	0.83	1.05	0.89	0.14	15.68%
13-Plex 39	7.09	10.11	11.66	10.93	9.95	2.01	20.18%
13-Plex 41	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 44	0.50	0.56	0.47	0.52	0.51	0.04	7.37%
13-Plex 50	10.98	10.37	13.08	11.22	11.41	1.17	10.23%
13-Plex 51	0.55	0.68	0.66	1.08	0.74	0.23	31.27%
13-Plex 59	0.17	0.15	0.16	0.16	0.16	0.01	5.10%
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 04	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							15
Number of samples with % CV ≤ 25 %							13
Percentage of samples with % CV ≤ 25 %							86.67 %

Appendix VI-9: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn5 PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 10	0.17	0.21	0.18	0.19	0.19	0.02	9.11 %
13-Plex 11	3.38	3.50	3.51	3.39	3.45	0.07	2.02 %
13-Plex 12	2.92	3.21	3.84	3.42	3.35	0.39	11.56 %
13-Plex 18	3.05	1.71	0.85	1.68	1.82	0.91	49.94 %
13-Plex 19	4.48	4.55	3.20	4.84	4.27	0.73	17.07 %
13-Plex 20	0.97	1.22	0.75	1.24	1.05	0.23	22.19 %
13-Plex 28	0.21	0.21	0.22	0.21	0.21	0.01	2.35 %
13-Plex 31	0.12	0.13	0.12	0.05	0.12	0.01	4.68 %
13-Plex 33	0.14	0.16	0.13	0.16	0.15	0.01	10.17 %
13-Plex 39	1.64	1.84	1.75	1.82	1.76	0.09	5.12 %
13-Plex 41	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	1.15	1.09	1.07	0.91	1.06	0.10	9.71 %
13-Plex 44	1.10	1.32	1.11	1.12	1.16	0.11	9.06 %
13-Plex 50	3.32	6.23	7.46	6.58	5.90	1.79	30.43 %
13-Plex 51	0.34	0.41	0.38	0.66	0.45	0.14	32.30 %
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.88	0.98	0.86	0.99	0.93	0.07	7.23 %
13-Plex 71	0.10	0.10	0.05	0.05	0.10	0.00	0.00 %
Total number of samples included in analysis							16
Number of samples with %CV ≤ 25 %							14
Percentage of samples with %CV ≤ 25 %							87.50 %

Appendix VI-10: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn6A PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 03	0.22	0.19	0.22	0.23	0.22	0.02	8.06 %
13-Plex 05	1.60	1.37	1.90	1.70	1.64	0.22	13.42 %
13-Plex 08	7.56	7.22	7.00	6.94	7.18	0.28	3.91 %
13-Plex 10	4.09	5.08	4.64	4.89	4.68	0.43	9.19 %
13-Plex 11	4.24	4.43	4.63	4.12	4.36	0.22	5.13 %
13-Plex 12	0.05	0.05	0.10	0.05	0.05	0.00	-
13-Plex 18	3.67	1.57	0.81	1.54	1.90	1.23	64.97 %
13-Plex 19	11.44	12.21	8.45	13.14	11.31	2.03	17.94 %
13-Plex 21	2.09	2.85	1.74	2.29	2.24	0.46	20.71 %
13-Plex 31	1.00	1.11	0.98	0.62	0.93	0.21	22.95 %
13-Plex 34	0.05	0.13	0.11	0.15	0.13	0.02	15.38 %
13-Plex 39	1.64	1.74	1.83	1.78	1.75	0.08	4.61 %
13-Plex 41	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 42	1.68	1.54	1.54	1.28	1.51	0.17	11.06 %
13-Plex 50	2.30	4.01	4.67	4.39	3.84	1.06	27.67 %
13-Plex 51	0.45	0.56	0.51	0.86	0.60	0.18	30.64 %
13-Plex 59	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							14
Number of samples with % CV ≤ 25 %							11
Percentage of samples with % CV ≤ 25 %							73.33 %

Appendix VI-11: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn7F PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 08	6.43	5.94	5.69	5.47	5.88	0.41	7.01 %
13-Plex 10	1.87	2.36	1.97	2.21	2.10	0.22	10.62 %
13-Plex 12	0.74	0.80	0.89	0.87	0.83	0.07	8.31 %
13-Plex 17	1.20	1.14	1.17	1.26	1.19	0.05	4.30 %
13-Plex 18	2.08	1.25	0.79	1.22	1.34	0.54	40.40 %
13-Plex 21	1.93	2.43	1.67	2.16	2.05	0.32	15.83 %
13-Plex 28	15.73	16.55	16.45	15.96	16.17	0.39	2.42 %
13-Plex 31	0.64	0.71	0.62	0.40	0.59	0.13	22.62 %
13-Plex 39	1.72	1.89	1.96	1.89	1.87	0.10	5.48 %
13-Plex 41	0.87	0.81	0.92	0.83	0.86	0.05	5.66 %
13-Plex 42	3.43	3.47	3.54	2.66	3.28	0.41	12.60 %
13-Plex 44	0.30	0.35	0.27	0.28	0.30	0.04	11.86 %
13-Plex 50	1.01	1.51	1.85	1.71	1.52	0.37	24.18 %
13-Plex 51	0.24	0.29	0.28	0.46	0.32	0.10	30.69 %
13-Plex 52	0.15	0.16	0.14	0.15	0.15	0.01	5.44 %
13-Plex 59	0.12	0.12	0.11	0.11	0.12	0.01	5.02 %
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							16
Number of samples with % CV ≤ 25 %							13
Percentage of samples with % CV ≤ 25 %							81.25 %

Appendix VI-12: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn18C PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	% CV
	1	5	10	15			
13-Plex 04	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 06	1.17	1.25	1.36	1.06	1.21	0.13	10.48 %
13-Plex 08	3.07	2.94	2.62	2.64	2.82	0.22	7.92 %
13-Plex 10	0.45	0.56	0.49	0.53	0.51	0.05	9.43 %
13-Plex 11	9.58	8.72	9.15	7.98	8.86	0.68	7.70 %
13-Plex 16	2.01	2.25	1.96	1.96	2.05	0.14	6.78 %
13-Plex 17	0.69	0.63	0.66	0.67	0.66	0.03	3.77 %
13-Plex 19	3.29	3.37	2.42	3.59	3.17	0.51	16.23 %
13-Plex 21	2.43	2.98	1.90	2.52	2.46	0.44	18.02 %
13-Plex 25	0.24	0.27	0.17	0.27	0.24	0.05	19.86 %
13-Plex 31	0.56	0.62	0.54	0.31	0.51	0.14	26.79 %
13-Plex 33	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 34	3.69	4.89	4.28	5.41	4.57	0.75	16.32 %
13-Plex 39	1.08	1.11	1.20	1.14	1.13	0.05	4.52 %
13-Plex 44	0.95	1.14	1.01	0.98	1.02	0.08	8.20 %
13-Plex 50	0.76	1.29	1.55	1.42	1.26	0.35	27.62 %
13-Plex 51	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.33	0.38	0.32	0.37	0.35	0.03	8.41 %
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							15
Number of samples with % CV ≤ 25 %							13
Percentage of samples with % CV ≤ 25 %							86.67 %

Appendix VI-13: Stability results after different numbers of freeze/thaw cycles for beads prepared using Pn19F PS.

Sample ID	Number of Freeze Thaw Cycles				Mean	SD	%CV
	1	5	10	15			
13-Plex 05	3.14	2.66	3.65	3.19	3.16	0.40	12.81%
13-Plex 10	8.91	11.29	9.65	10.81	10.17	1.08	10.66%
13-Plex 11	3.11	3.17	3.33	2.83	3.11	0.21	6.70%
13-Plex 12	0.93	1.04	1.20	1.09	1.07	0.11	10.53%
13-Plex 16	1.32	1.51	1.32	1.34	1.37	0.09	6.71%
13-Plex 18	14.62	6.63	3.18	6.50	7.73	4.86	62.87%
13-Plex 19	7.01	7.48	5.16	7.61	6.82	1.13	16.63%
13-Plex 20	2.17	2.76	1.72	2.70	2.34	0.49	20.95%
13-Plex 21	0.69	0.92	0.52	0.76	0.72	0.17	22.95%
13-Plex 25	0.17	0.17	0.11	0.19	0.16	0.03	21.65%
13-Plex 34	0.40	0.50	0.43	0.54	0.47	0.06	13.68%
13-Plex 39	2.06	2.23	2.22	2.23	2.19	0.08	3.82%
13-Plex 42	2.58	2.50	2.37	2.00	2.36	0.26	10.87%
13-Plex 50	2.47	4.28	4.94	4.61	4.08	1.10	27.08%
13-Plex 51	0.19	0.25	0.23	0.40	0.27	0.09	34.31%
13-Plex 52	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 59	0.10	0.10	0.05	0.05	0.10	0.00	0.00%
13-Plex 60	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.05	0.05	0.00	-
Total number of samples included in analysis							16
Number of samples with %CV ≤ 25%							12
Percentage of samples with %CV ≤ 25%							75.00 %

Appendix VI-14: Short term temperature stability results for Panel A serotypes

Pn 4					
Sample ID	<1 Hour	5 Hours	Mean	SD	% CV
13-Plex 03	0.30	0.35	0.33	0.04	10.88%
13-Plex 06	0.22	0.22	0.22	0.00	0.00%
13-Plex 08	2.23	2.13	2.18	0.07	3.24%
13-Plex 10	0.32	0.28	0.30	0.03	9.43%
13-Plex 11	7.09	6.93	7.01	0.11	1.61%
13-Plex 12	0.30	0.22	0.26	0.06	21.76%
13-Plex 18	0.41	0.53	0.47	0.08	18.05%
13-Plex 19	2.19	2.34	2.27	0.11	4.68%
13-Plex 20	0.50	0.71	0.61	0.15	24.54%
13-Plex 21	0.35	0.34	0.35	0.01	2.05%
13-Plex 28	0.73	0.84	0.79	0.08	9.91%
13-Plex 31	0.48	0.46	0.47	0.01	3.01%
13-Plex 39	6.81	9.69	8.25	2.04	24.68%
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 42	2.94	3.04	2.99	0.07	2.36%
13-Plex 44	0.05	0.05	0.05	0.00	-
13-Plex 52	0.10	0.05	0.08	0.04	-
13-Plex 60	0.23	0.16	0.20	0.05	25.38%
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					93.33%
Pn 6B					
Sample ID	<1 Hour	5 Hours	Mean	SD	% CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 05	0.38	0.38	0.38	0.00	0.00%
13-Plex 08	3.75	3.68	3.72	0.05	1.33%
13-Plex 10	3.38	2.86	3.12	0.37	11.79%
13-Plex 11	3.65	3.41	3.53	0.17	4.81%
13-Plex 17	0.05	0.05	0.05	0.00	-
13-Plex 18	0.81	1.02	0.92	0.15	16.23%
13-Plex 19	2.45	2.62	2.54	0.12	4.74%
13-Plex 21	1.36	1.26	1.31	0.07	5.40%
13-Plex 25	0.19	0.19	0.19	0.00	0.00%
13-Plex 28	0.95	1.11	1.03	0.11	10.98%
13-Plex 31	5.52	5.38	5.45	0.10	1.82%
13-Plex 33	0.05	0.10	0.08	0.04	-
13-Plex 34	0.12	0.05	0.09	0.05	58.23%
13-Plex 39	1.49	1.96	1.73	0.33	19.27%
13-Plex 41	0.54	0.69	0.62	0.11	17.25%
13-Plex 50	1.39	1.44	1.42	0.04	2.50%
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 59	0.16	0.20	0.18	0.03	15.71%
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15

Appendix VI-14: Short term temperature stability results for Panel A serotype (continued)

Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					93.33%
Pn 9V					
Sample ID	<1 Hour	5 Hours	Mean	SD	% CV
13-Plex 03	0.23	0.28	0.26	0.04	13.86%
13-Plex 05	1.12	1.08	1.10	0.03	2.57%
13-Plex 08	3.28	3.01	3.15	0.19	6.07%
13-Plex 10	4.49	3.78	4.14	0.50	12.14%
13-Plex 11	0.89	0.89	0.89	0.00	0.00%
13-Plex 12	0.55	0.43	0.49	0.08	17.32%
13-Plex 18	0.47	0.58	0.53	0.08	14.82%
13-Plex 19	4.11	4.32	4.22	0.15	3.52%
13-Plex 20	2.03	2.69	2.36	0.47	19.78%
13-Plex 28	0.57	0.67	0.62	0.07	11.40%
13-Plex 31	0.25	0.25	0.25	0.00	0.00%
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 34	1.23	0.97	1.10	0.18	16.71%
13-Plex 39	0.90	1.12	1.01	0.16	15.40%
13-Plex 42	1.97	2.01	1.99	0.03	1.42%
13-Plex 52	0.17	0.14	0.16	0.02	13.69%
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					100.00%
Pn 14					
Sample ID	<1 Hour	5 Hours	Mean	SD	% CV
13-Plex 03	0.76	0.89	0.83	0.09	11.14%
13-Plex 08	5.07	5.02	5.05	0.04	0.70%
13-Plex 10	3.61	3.17	3.39	0.31	9.18%
13-Plex 12	2.06	1.67	1.87	0.28	14.79%
13-Plex 16	0.45	0.51	0.48	0.04	8.84%
13-Plex 17	0.05	0.05	0.05	0.00	-
13-Plex 18	0.78	0.98	0.88	0.14	16.07%
13-Plex 19	1.77	1.86	1.82	0.06	3.51%
13-Plex 21	3.84	3.58	3.71	0.18	4.96%
13-Plex 28	5.19	5.86	5.53	0.47	8.57%
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 39	2.31	3.00	2.66	0.49	18.38%
13-Plex 41	0.23	0.30	0.27	0.05	18.68%
13-Plex 42	1.95	1.93	1.94	0.01	0.73%
13-Plex 50	0.73	0.76	0.75	0.02	2.85%
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 59	0.15	0.19	0.17	0.03	16.64%

Appendix VI-14: Short term temperature stability results for Panel A serotype (continued)

13-Plex 62	1.06	1.30	1.18	0.17	14.38%
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					100.00%
Pn 19A					
Sample ID	<1 Hour	5 Hours	Mean	SD	% CV
13-Plex 03	0.73	0.84	0.79	0.08	9.91%
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 05	10.40	6.29	8.35	2.91	34.83%
13-Plex 06	0.43	0.42	0.43	0.01	1.66%
13-Plex 10	12.84	14.17	13.51	0.94	6.96%
13-Plex 18	11.22	10.02	10.62	0.85	7.99%
13-Plex 19	1.72	1.65	1.69	0.05	2.94%
13-Plex 20	0.83	0.87	0.85	0.03	3.33%
13-Plex 21	0.59	0.59	0.59	0.00	0.00%
13-Plex 25	1.25	1.05	1.15	0.14	12.30%
13-Plex 33	0.49	0.63	0.56	0.10	17.68%
13-Plex 34	0.79	0.61	0.70	0.13	18.18%
13-Plex 39	2.79	3.80	3.30	0.71	21.67%
13-Plex 41	0.05	0.10	0.08	0.04	-
13-Plex 47	0.05	0.05	0.05	0.00	-
13-Plex 50	2.45	2.56	2.51	0.08	3.11%
13-Plex 51	0.21	0.32	0.27	0.08	29.35%
13-Plex 62	8.61	12.27	10.44	2.59	24.79%
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					13
Percentage of positive sample with % CV ≤ 25 %					86.67%
Pn 23F					
Sample ID	<1 Hour	5 Hours	Mean	SD	% CV
13-Plex 03	3.99	4.73	4.36	0.52	12.00%
13-Plex 08	0.43	0.40	0.42	0.02	5.11%
13-Plex 11	4.70	4.23	4.47	0.33	7.44%
13-Plex 12	2.56	1.87	2.22	0.49	22.03%
13-Plex 16	0.13	0.14	0.14	0.01	5.24%
13-Plex 18	1.66	2.12	1.89	0.33	17.21%
13-Plex 19	5.78	5.83	5.81	0.04	0.61%
13-Plex 20	1.42	1.86	1.64	0.31	18.97%
13-Plex 28	0.38	0.45	0.42	0.05	11.93%
13-Plex 31	0.31	0.30	0.31	0.01	2.32%
13-Plex 33	0.05	0.10	0.08	0.04	-
13-Plex 34	0.29	0.24	0.27	0.04	13.34%
13-Plex 39	1.55	1.93	1.74	0.27	15.44%
13-Plex 41	0.05	0.13	0.09	0.06	-

Appendix VI-14: Short term temperature stability results for Panel A serotype (continued)

13-Plex 42	1.21	1.13	1.17	0.06	4.83%
13-Plex 44	2.18	2.97	2.58	0.56	21.69%
13-Plex 52	0.24	0.20	0.22	0.03	12.86%
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					100.00%

Appendix VI-15: Short term temperature stability results for Panel B serotypes

Pn 1					
Sample ID	<1 Hour	5 Hours	Mean	SD	%CV
13-Plex 03	0.19	0.22	0.21	0.02	10.35%
13-Plex 05	1.70	1.53	1.62	0.12	7.44%
13-Plex 08	28.43	29.50	28.97	0.76	2.61%
13-Plex 10	0.05	0.05	0.05	0.00	-
13-Plex 11	5.30	5.02	5.16	0.20	3.84%
13-Plex 12	6.27	4.94	5.61	0.94	16.78%
13-Plex 16	4.99	5.55	5.27	0.40	7.51%
13-Plex 18	0.31	0.40	0.36	0.06	17.93%
13-Plex 19	3.55	3.25	3.40	0.21	6.24%
13-Plex 20	0.05	0.12	0.09	0.05	58.23%
13-Plex 21	0.13	0.12	0.13	0.01	5.66%
13-Plex 31	1.86	1.73	1.80	0.09	5.12%
13-Plex 34	0.05	0.05	0.05	0.00	-
13-Plex 42	4.45	3.62	4.04	0.59	14.55%
13-Plex 44	0.05	0.05	0.05	0.00	-
13-Plex 50	1.25	0.97	1.11	0.20	17.84%
13-Plex 51	0.10	0.10	0.10	0.00	0.00%
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					14
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					100.00%
Pn 3					
Sample ID	<1 Hour	5 Hours	Mean	SD	%CV
13-Plex 03	0.25	0.29	0.27	0.03	10.48%
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 05	7.89	6.79	7.34	0.78	10.60%
13-Plex 10	0.73	0.62	0.68	0.08	11.52%
13-Plex 12	3.56	2.33	2.95	0.87	29.53%
13-Plex 16	0.55	0.60	0.58	0.04	6.15%
13-Plex 18	3.36	4.35	3.86	0.70	18.16%
13-Plex 19	2.66	2.69	2.68	0.02	0.79%
13-Plex 20	1.46	2.22	1.84	0.54	29.21%
13-Plex 21	6.17	6.14	6.16	0.02	0.34%
13-Plex 28	0.13	0.14	0.14	0.01	5.24%
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 39	10.12	12.99	11.56	2.03	17.56%
13-Plex 41	0.05	0.12	0.09	0.05	-
13-Plex 44	0.65	0.50	0.58	0.11	18.45%
13-Plex 50	9.40	9.60	9.43	0.04	0.45%
13-Plex 51	0.88	0.87	0.88	0.01	0.81%
13-Plex 59	0.19	0.20	0.20	0.01	3.63%
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-

Appendix VI-15: Short term temperature stability results for Panel B serotype (continued)

Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					13
Percentage of positive sample with % CV ≤ 25 %					86.67 %
Pn 5					
Sample ID	<1 Hour	5 Hours	Mean	SD	%CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 10	0.24	0.21	0.23	0.02	9.43%
13-Plex 11	3.46	3.55	3.51	0.06	1.82%
13-Plex 12	2.71	1.89	2.30	0.58	25.21%
13-Plex 18	1.69	2.08	1.89	0.28	14.63%
13-Plex 19	4.45	4.21	4.33	0.17	3.92%
13-Plex 20	0.93	1.12	1.03	0.13	13.11%
13-Plex 28	0.20	0.29	0.25	0.06	25.98%
13-Plex 31	0.34	0.26	0.30	0.06	18.86%
13-Plex 33	0.17	0.20	0.19	0.02	11.47%
13-Plex 39	1.83	1.90	1.87	0.05	2.65%
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 42	1.34	1.23	1.29	0.08	6.05%
13-Plex 44	1.23	1.10	1.17	0.09	7.89%
13-Plex 50	11.61	8.49	10.05	2.21	21.95%
13-Plex 51	0.45	0.45	0.45	0.00	0.00%
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 59	0.05	0.10	0.08	0.04	-
13-Plex 62	1.15	1.07	1.11	0.06	5.10%
13-Plex 71	0.05	0.10	0.08	0.04	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					13
Percentage of positive sample with % CV ≤ 25 %					86.67%
Pn 6A					
Sample ID	<1 Hour	5 Hours	Mean	SD	%CV
13-Plex 03	0.20	0.22	0.21	0.01	6.73%
13-Plex 05	1.87	1.62	1.75	0.18	10.13%
13-Plex 08	7.49	7.52	7.51	0.02	0.28%
13-Plex 10	4.47	3.94	4.21	0.37	8.91%
13-Plex 11	4.19	4.37	4.28	0.13	2.97%
13-Plex 12	0.10	0.05	0.08	0.04	47.14%
13-Plex 18	1.30	1.60	1.45	0.21	14.63%
13-Plex 19	12.15	11.32	11.74	0.59	5.00%
13-Plex 21	1.75	1.79	1.77	0.03	1.60%
13-Plex 31	1.04	0.99	1.02	0.04	3.48%
13-Plex 34	0.16	0.13	0.15	0.02	14.63%
13-Plex 39	1.70	1.75	1.73	0.04	2.05%
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 42	1.87	1.49	1.68	0.27	15.99%
13-Plex 50	5.91	4.70	5.31	0.86	16.13%
13-Plex 51	0.58	0.57	0.58	0.01	1.23%

13-Plex 59	0.05	0.05	0.05	0.00	-

Appendix VI-15: Short term temperature stability results for Panel B serotype (continued)

13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					93.33%

Pn 7F					
Sample ID	<1 Hour	5 Hours	Mean	SD	%CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 08	7.04	6.84	6.94	0.14	2.04%
13-Plex 10	1.96	1.71	1.84	0.18	9.63%
13-Plex 12	0.74	0.62	0.68	0.08	12.48%
13-Plex 17	0.92	0.90	0.91	0.01	1.55%
13-Plex 18	0.96	1.22	1.09	0.18	16.87%
13-Plex 21	1.44	1.33	1.39	0.08	5.62%
13-Plex 28	7.73	11.50	9.62	2.67	27.73%
13-Plex 31	1.03	0.97	1.00	0.04	4.24%
13-Plex 39	2.13	2.13	2.13	0.00	0.00%
13-Plex 41	0.75	0.75	0.75	0.00	0.00%
13-Plex 42	5.02	3.86	4.44	0.82	18.47%
13-Plex 44	0.35	0.29	0.32	0.04	13.26%
13-Plex 50	2.49	2.10	2.30	0.28	12.02%
13-Plex 51	0.36	0.34	0.35	0.01	4.04%
13-Plex 52	0.10	0.15	0.13	0.04	28.28%
13-Plex 59	0.12	0.14	0.13	0.01	10.88%
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					16
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					87.50%

Pn 18C					
Sample ID	<1 Hour	5 Hours	Mean	SD	%CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 06	1.36	1.22	1.29	0.10	7.67%
13-Plex 08	3.05	3.06	3.06	0.01	0.23%
13-Plex 10	0.61	0.56	0.59	0.04	6.04%
13-Plex 11	5.08	5.12	5.10	0.03	0.55%
13-Plex 16	1.95	2.47	2.21	0.37	16.64%
13-Plex 17	0.63	0.59	0.61	0.03	4.64%
13-Plex 19	3.29	3.18	3.24	0.08	2.40%
13-Plex 21	2.32	2.37	2.35	0.04	1.51%
13-Plex 25	0.28	0.27	0.28	0.01	2.57%
13-Plex 31	0.60	0.57	0.59	0.02	3.63%
13-Plex 33	0.05	0.05	0.05	0.00	-

Appendix VI-15: Short term temperature stability results for Panel B serotype (continued)

13-Plex 34	4.47	3.85	4.16	0.44	10.54%
13-Plex 39	1.14	1.14	1.14	0.00	0.00%
13-Plex 44	1.24	1.08	1.16	0.11	9.75%
13-Plex 50	2.22	1.73	1.98	0.35	17.54%
13-Plex 51	0.05	0.05	0.05	0.00	-
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.42	0.40	0.41	0.01	3.45%
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15

Percentage of positive sample with % CV ≤ 25 %					100.00%
Pn 19F					
Sample ID	<1 Hour	5 Hours	Mean	SD	%CV
13-Plex 05	4.07	3.50	3.79	0.40	10.65%
13-Plex 10	10.83	9.53	10.18	0.92	9.03%
13-Plex 11	2.54	2.53	2.54	0.01	0.28%
13-Plex 12	1.00	0.73	0.87	0.19	22.07%
13-Plex 16	1.35	1.59	1.47	0.17	11.54%
13-Plex 18	5.88	7.38	6.63	1.06	16.00%
13-Plex 19	7.52	7.11	7.32	0.29	3.96%
13-Plex 20	2.00	2.53	2.27	0.37	16.55%
13-Plex 21	0.72	0.74	0.73	0.01	1.94%
13-Plex 25	0.21	0.21	0.21	0.00	-
13-Plex 34	0.55	0.47	0.51	0.06	11.09%
13-Plex 39	2.75	2.69	2.72	0.04	1.56%
13-Plex 42	3.91	3.33	3.62	0.41	11.33%
13-Plex 50	7.36	6.02	6.69	0.95	14.16%
13-Plex 51	0.24	0.23	0.24	0.01	3.01%
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 59	0.09	0.11	0.10	0.01	14.14%
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					100.00%

Appendix VI-16: Post preparative temperature stability results for Panel A serotypes

Pn 4					
Sample ID	<1 Hour	6 Hours	Mean	SD	% CV
13-Plex 03	0.32	0.30	0.31	0.01	4.56 %
13-Plex 06	0.05	0.05	0.05	0.00	-
13-Plex 08	1.44	1.29	1.37	0.11	7.77 %
13-Plex 10	0.26	0.28	0.27	0.01	5.24 %
13-Plex 11	6.43	7.07	6.75	0.45	6.70 %
13-Plex 12	0.23	0.18	0.21	0.04	17.25 %
13-Plex 18	0.42	0.39	0.41	0.02	5.24 %
13-Plex 19	0.96	1.30	1.13	0.24	21.28 %
13-Plex 20	0.22	0.28	0.25	0.04	16.97 %
13-Plex 21	0.23	0.22	0.23	0.01	3.14 %
13-Plex 28	0.23	0.23	0.23	0.00	0.00 %
13-Plex 31	1.40	1.12	1.26	0.20	15.71 %
13-Plex 39	5.89	4.10	5.00	1.27	25.34 %
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 42	2.09	1.70	1.90	0.28	14.55 %
13-Plex 44	0.05	0.05	0.05	0.00	-
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 60	0.17	0.19	0.18	0.01	7.86 %
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					14
Number of positive samples with % CV ≤ 25 %					13
Percentage of positive sample with % CV ≤ 25 %					92.86%
Pn 6B					
Sample ID	<1 Hour	6 Hours	Mean	SD	% CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 05	0.31	0.29	0.30	0.01	4.71 %
13-Plex 08	4.18	3.88	4.03	0.21	5.26 %
13-Plex 10	2.80	3.31	3.06	0.36	11.80 %
13-Plex 11	2.93	2.54	2.74	0.28	10.08 %
13-Plex 17	0.05	0.05	0.05	0.00	-
13-Plex 18	1.28	1.15	1.22	0.09	7.57 %
13-Plex 19	1.86	2.44	2.15	0.41	19.08 %
13-Plex 21	1.30	1.21	1.26	0.06	5.07 %
13-Plex 25	0.18	0.18	0.18	0.00	0.00 %
13-Plex 28	0.81	0.91	0.86	0.07	8.22 %
13-Plex 31	8.54	8.06	8.30	0.34	4.09 %
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 34	0.13	0.11	0.12	0.01	11.79 %
13-Plex 39	1.66	1.52	1.59	0.10	6.23 %
13-Plex 41	0.54	0.54	0.54	0.00	0.00 %
13-Plex 50	1.38	1.50	1.44	0.08	5.89 %

Appendix VI-16: Post preparative temperature stability results for Panel A serotypes (continued)

13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 59	0.18	0.20	0.19	0.01	7.44 %
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					100.00 %
Pn 9V					
Sample ID	<1 Hour	6 Hours	Mean	SD	% CV
13-Plex 03	0.25	0.27	0.26	0.01	5.44 %
13-Plex 05	0.95	0.91	0.93	0.03	3.04 %
13-Plex 08	2.44	2.60	2.52	0.11	4.49 %
13-Plex 10	3.64	3.65	3.65	0.01	0.19 %
13-Plex 11	0.75	0.78	0.77	0.02	2.77 %
13-Plex 12	0.51	0.49	0.50	0.01	2.83 %
13-Plex 18	0.47	0.46	0.47	0.01	1.52 %
13-Plex 19	3.03	3.33	3.18	0.21	6.67 %
13-Plex 20	2.54	2.30	2.42	0.17	7.01 %
13-Plex 28	0.46	0.49	0.48	0.02	4.47 %
13-Plex 31	0.22	0.23	0.23	0.01	3.14 %
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 34	1.17	1.02	1.10	0.11	9.69 %
13-Plex 39	1.01	0.93	0.97	0.06	5.83 %
13-Plex 42	1.58	1.55	1.57	0.02	1.36 %
13-Plex 52	0.11	0.11	0.11	0.00	0.00 %
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					100.00 %
Pn 14					
Sample ID	<1 Hour	6 Hours	Mean	SD	% CV
13-Plex 03	0.70	0.73	0.72	0.02	2.97 %
13-Plex 08	4.66	4.28	4.47	0.27	6.01 %
13-Plex 10	4.04	4.51	4.28	0.33	7.77 %
13-Plex 12	2.91	2.33	2.62	0.41	15.65 %
13-Plex 16	0.66	0.79	0.73	0.09	12.68 %
13-Plex 17	0.05	0.05	0.05	0.00	-
13-Plex 18	0.90	0.27	0.59	0.45	76.15 %
13-Plex 19	1.28	1.23	1.26	0.04	2.82 %
13-Plex 21	5.48	4.29	4.89	0.84	17.23 %
13-Plex 28	5.80	6.11	5.96	0.22	3.68 %
13-Plex 33	0.05	0.05	0.05	0.00	-

Appendix VI-16: Post preparative temperature stability results for Panel A serotypes (continued)

13-Plex 39	2.85	2.98	2.92	0.09	3.15 %
13-Plex 41	0.23	0.22	0.23	0.01	3.14 %
13-Plex 42	1.47	1.54	1.51	0.05	3.29 %
13-Plex 50	0.82	0.92	0.87	0.07	8.13 %
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 59	0.16	0.18	0.17	0.01	8.32 %
13-Plex 62	0.90	1.02	0.96	0.08	8.84 %
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					93.33 %
Pn 19A					
Sample ID	<1 Hour	6 Hours	Mean	SD	% CV
13-Plex 03	0.95	1.03	0.99	0.06	5.71 %
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 05	8.79	7.93	8.36	0.61	7.27 %
13-Plex 06	0.65	0.69	0.67	0.03	4.22 %
13-Plex 10	13.52	14.04	13.78	0.37	2.67 %
13-Plex 18	9.40	5.94	7.67	2.45	31.90 %
13-Plex 19	1.60	1.80	1.70	0.14	8.32 %
13-Plex 20	1.08	1.09	1.09	0.01	0.65 %
13-Plex 21	0.69	0.71	0.70	0.01	2.02 %
13-Plex 25	1.44	1.43	1.44	0.01	0.49 %
13-Plex 33	0.52	0.55	0.54	0.02	3.97 %
13-Plex 34	0.72	0.68	0.70	0.03	4.04 %
13-Plex 39	3.35	3.03	3.19	0.23	7.09 %
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 47	0.05	0.05	0.05	0.00	-
13-Plex 50	2.42	2.46	2.44	0.03	1.16 %
13-Plex 51	0.25	0.18	0.22	0.05	23.02 %
13-Plex 62	6.99	7.48	7.24	0.35	4.79 %
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					93.33 %
Pn 23F					
Sample ID	<1 Hour	6 Hours	Mean	SD	% CV
13-Plex 03	7.94	8.56	8.25	0.44	5.31 %
13-Plex 08	0.72	0.66	0.69	0.04	6.15 %
13-Plex 11	2.70	3.43	3.07	0.52	16.84 %
13-Plex 12	1.74	1.99	1.87	0.18	9.48 %
13-Plex 16	0.91	0.80	0.86	0.08	9.10 %

Appendix VI-16: Post preparative temperature stability results for Panel A serotypes (continued)

13-Plex 18	2.39	2.23	2.31	0.11	4.90 %
13-Plex 19	8.47	10.23	9.35	1.24	13.31 %
13-Plex 20	1.42	1.45	1.44	0.02	1.48 %
13-Plex 28	0.46	0.31	0.39	0.11	27.55 %
13-Plex 31	1.68	1.76	1.72	0.06	3.29 %
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 34	0.36	0.36	0.36	0.00	0.00 %
13-Plex 39	3.00	2.82	2.91	0.13	4.37 %
13-Plex 41	0.19	0.15	0.17	0.03	16.64 %
13-Plex 42	1.48	1.19	1.34	0.21	15.36 %
13-Plex 44	2.52	2.37	2.45	0.11	4.34 %
13-Plex 52	0.28	0.25	0.27	0.02	8.00 %
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					16
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					93.75 %

Appendix VI-17: Post preparative temperature stability results for Panel B serotypes

Pn 1					
Sample ID	<1 Hour	6 Hours	Mean	SD	%CV
13-Plex 03	0.13	0.11	0.12	0.01	11.79%
13-Plex 05	1.22	1.29	1.26	0.05	3.94%
13-Plex 08	29.57	28.90	29.24	0.47	1.62%
13-Plex 10	0.05	0.05	0.05	0.00	-
13-Plex 11	4.83	5.11	4.97	0.20	3.98%
13-Plex 12	8.26	5.99	7.13	1.61	22.53%
13-Plex 16	3.52	4.02	3.77	0.35	9.38%
13-Plex 18	0.28	0.26	0.27	0.01	5.24%
13-Plex 19	2.02	2.70	2.36	0.48	20.37%
13-Plex 20	0.05	0.11	0.08	0.04	-
13-Plex 21	0.12	0.11	0.12	0.01	6.15%
13-Plex 31	0.91	0.61	0.76	0.21	27.91%
13-Plex 34	0.06	0.05	0.06	0.01	-
13-Plex 42	2.66	2.07	2.37	0.42	17.64%
13-Plex 44	0.05	0.05	0.05	0.00	-
13-Plex 50	0.76	0.73	0.75	0.02	2.85%
13-Plex 51	0.05	0.05	0.05	0.00	-
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					12
Number of positive samples with % CV ≤ 25 %					11
Percentage of positive sample with % CV ≤ 25 %					91.67%
Pn 3					
Sample ID	<1 Hour	6 Hours	Mean	SD	%CV
13-Plex 03	0.21	0.20	0.21	0.01	3.45%
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 05	3.72	6.43	5.08	1.92	37.76%
13-Plex 10	0.90	0.96	0.93	0.04	4.56%
13-Plex 12	5.23	6.93	6.08	1.20	19.77%
13-Plex 16	0.71	0.69	0.70	0.01	2.02%
13-Plex 18	4.51	2.99	3.75	1.07	28.66%
13-Plex 19	2.88	3.24	3.06	0.25	8.32%
13-Plex 20	2.09	2.74	2.42	0.46	19.03%
13-Plex 21	5.15	5.15	5.15	0.00	0.00%
13-Plex 28	0.18	0.12	0.15	0.04	28.28%
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 39	11.75	2.48	7.12	6.55	92.13%
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 44	0.62	0.62	0.62	0.00	0.00%
13-Plex 50	10.39	10.27	10.33	0.08	0.82%
13-Plex 51	0.80	0.80	0.80	0.00	0.00%
13-Plex 59	0.19	0.18	0.19	0.01	3.82%

Appendix VI-17: Post preparative temperature stability results for Panel B serotypes (continued)

13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					11
Percentage of positive sample with % CV ≤ 25 %					73.33 %
Pn 5					
Sample ID	<1 Hour	6 Hours	Mean	SD	%CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 10	0.24	0.26	0.25	0.01	5.66%
13-Plex 11	3.14	3.94	3.54	0.57	15.98%
13-Plex 12	2.80	3.55	3.18	0.53	16.70%
13-Plex 18	2.43	1.78	2.11	0.46	21.83%
13-Plex 19	3.56	4.26	3.91	0.49	12.66%
13-Plex 20	1.50	1.34	1.42	0.11	7.97%
13-Plex 28	0.39	0.26	0.33	0.09	28.28%
13-Plex 31	0.34	0.33	0.34	0.01	2.11%
13-Plex 33	0.21	0.25	0.23	0.03	12.30%
13-Plex 39	2.08	2.00	2.04	0.06	2.77%
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 42	1.35	1.37	1.36	0.01	1.04%
13-Plex 44	1.11	1.31	1.21	0.14	11.69%
13-Plex 50	8.86	8.60	8.73	0.18	2.11%
13-Plex 51	0.31	0.26	0.29	0.04	12.41%
13-Plex 52	0.05	0.05	0.05	0.00	-
13-Plex 59	0.05	0.05	0.05	0.00	-
13-Plex 62	0.89	1.06	0.98	0.12	12.33%
13-Plex 71	0.05	0.10	0.08	0.04	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					93.33%
Pn 6A					
Sample ID	<1 Hour	6 Hours	Mean	SD	%CV
13-Plex 03	0.22	0.26	0.24	0.03	11.79%
13-Plex 05	1.23	1.74	1.49	0.36	24.28%
13-Plex 08	7.62	5.30	6.46	1.64	25.39%
13-Plex 10	4.03	4.83	4.43	0.57	12.77%
13-Plex 11	3.27	3.74	3.51	0.33	9.48%
13-Plex 12	0.11	0.13	0.12	0.01	11.79%
13-Plex 18	1.77	1.34	1.56	0.30	19.55%
13-Plex 19	9.74	12.15	10.95	1.70	15.57%
13-Plex 21	1.96	2.03	2.00	0.05	2.48%
13-Plex 31	1.60	1.57	1.59	0.02	1.34%
13-Plex 34	0.14	0.11	0.13	0.02	16.97%
13-Plex 39	1.17	1.28	1.23	0.08	6.35%
13-Plex 41	0.05	0.05	0.05	0.00	-
13-Plex 42	1.45	1.30	1.38	0.11	7.71%

Appendix VI-17: Post preparative temperature stability results for Panel B serotypes (continued)

13-Plex 50	3.89	3.72	3.81	0.12	3.16%
13-Plex 51	0.59	0.59	0.59	0.00	0.00%
13-Plex 59	0.05	0.05	0.05	0.00	-
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					14
Percentage of positive sample with % CV ≤ 25 %					93.33%
Pn 7F					
Sample ID	<1 Hour	6 Hours	Mean	SD	%CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 08	5.81	4.16	4.99	1.17	23.40%
13-Plex 10	1.86	1.96	1.91	0.07	3.70%
13-Plex 12	0.79	0.86	0.83	0.05	6.00%
13-Plex 17	0.75	0.90	0.83	0.11	12.86%
13-Plex 18	1.11	0.81	0.96	0.21	22.10%
13-Plex 21	1.22	1.20	1.21	0.01	1.17%
13-Plex 28	8.18	5.59	6.89	1.83	26.60%
13-Plex 31	1.53	1.39	1.46	0.10	6.78%
13-Plex 39	1.99	1.83	1.91	0.11	5.92%
13-Plex 41	0.80	0.90	0.85	0.07	8.32%
13-Plex 42	3.25	3.36	3.31	0.08	2.35%
13-Plex 44	0.26	0.27	0.27	0.01	2.67%
13-Plex 50	1.48	1.64	1.56	0.11	7.25%
13-Plex 51	0.35	0.25	0.30	0.07	23.57%
13-Plex 52	0.13	0.11	0.12	0.01	11.79%
13-Plex 59	0.13	0.12	0.13	0.01	5.66%
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
13-Plex 77	0.05	0.05	0.05	0.00	-
Total number of positive samples					16
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					93.75%
Pn 18C					
Sample ID	<1 Hour	6 Hours	Mean	SD	%CV
13-Plex 04	0.05	0.05	0.05	0.00	-
13-Plex 06	0.85	1.06	0.96	0.15	15.55%
13-Plex 08	2.96	2.14	2.55	0.58	22.74%
13-Plex 10	0.59	0.63	0.61	0.03	4.64%
13-Plex 11	5.09	5.59	5.34	0.35	6.62%
13-Plex 16	1.76	1.85	1.81	0.06	3.53%
13-Plex 17	0.54	0.67	0.61	0.09	15.19%
13-Plex 19	2.81	3.27	3.04	0.33	10.70%
13-Plex 21	1.43	1.58	1.51	0.11	7.05%
13-Plex 25	0.23	0.21	0.22	0.01	6.43%

Appendix VI-17: Post preparative temperature stability results for Panel B serotypes (continued)

13-Plex 31	0.83	0.86	0.85	0.02	2.51%
13-Plex 33	0.05	0.05	0.05	0.00	-
13-Plex 34	3.91	3.95	3.93	0.03	0.72%
13-Plex 39	1.82	1.42	1.62	0.28	17.46%
13-Plex 44	0.72	0.85	0.79	0.09	11.71%
13-Plex 50	1.27	1.28	1.28	0.01	0.55%
13-Plex 51	0.05	0.05	0.05	0.00	-
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.28	0.32	0.30	0.03	9.43%
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					15
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					100.00%
Pn 19F					
Sample ID	<1 Hour	6 Hours	Mean	SD	%CV
13-Plex 05	2.38	3.51	2.95	0.80	27.13%
13-Plex 10	10.33	12.07	11.20	1.23	10.99%
13-Plex 11	2.25	2.67	2.46	0.30	12.07%
13-Plex 12	1.03	1.33	1.18	0.21	17.98%
13-Plex 16	1.62	1.63	1.63	0.01	0.44%
13-Plex 18	6.78	5.04	5.91	1.23	20.82%
13-Plex 19	5.94	7.31	6.63	0.97	14.62%
13-Plex 20	2.49	2.16	2.33	0.23	10.04%
13-Plex 21	0.78	0.83	0.81	0.04	4.39%
13-Plex 25	0.24	0.21	0.23	0.02	9.43%
13-Plex 34	0.57	0.58	0.58	0.01	1.23%
13-Plex 39	2.48	2.48	2.48	0.00	0.00%
13-Plex 42	2.90	2.94	2.92	0.03	0.97%
13-Plex 50	4.75	5.07	4.91	0.23	4.61%
13-Plex 51	0.25	0.21	0.23	0.03	12.30%
13-Plex 52	0.10	0.05	0.08	0.04	-
13-Plex 59	0.11	0.12	0.12	0.01	6.15%
13-Plex 60	0.05	0.05	0.05	0.00	-
13-Plex 62	0.05	0.05	0.05	0.00	-
13-Plex 71	0.05	0.05	0.05	0.00	-
Total number of positive samples					16
Number of positive samples with % CV ≤ 25 %					15
Percentage of positive sample with % CV ≤ 25 %					93.75%