for 7 different antigens (35,000 assays) with a single operator performing 10 ELISA plates (5 sera per plate) a day for 5 days a week would take 140 weeks (2.6 years) to be completed. If the same antigens are tested simultaneously, the same operator can complete the study in 20 weeks (0.4 years).

HIGH-THROUGHPUT ASSAYS THAT MEASURE ANTIBODY QUANTITY

In this section, we discuss specific examples of high-throughput assays that can be found in the evaluation of Abs to Men and Pnc Ps antigens (Table 1). For example, for IgG Ab concentrations, microsphere-based assays can measure simultaneously the Ab to different antigens. One of these technologies uses the Luminex™ (Austin, Texas, U.S.) format for capturing mean fluorescence units that can be converted into Ab concentrations if a reference serum with Ab assignments or a calibrator is available. Initially, this technique was widely used for the measurement of cytokine concentrations (58). Since then, several assays have been developed for the evaluation of Pnc Ab, with various degrees of success in terms of validation. One of the first assays described for Pnc antigens was described by Pickering et al. (48) with a companion flow cytometric assay for the quantitation of Ab to tetanus, diphtheria, and Hib (59). Biagini et al. described the covalent linkage of each of 23 Pnc Ps to amino groups in the microspheres using sodium periodate to oxidize the Ps (49). However, this assay requires great care in the oxidation step, with periodate making this technique difficult to implement in a reproducible manner. A modification of this assay is currently being evaluated at the Centers for Disease Control and Prevention (CDC) in the United States (60). Schlottmann et al. described a modified assay that uses Ps conjugation via the carboxyl functional groups in the microspheres using DMTMM (4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium) (52). The nanoplex assay developed by Lal et al., which has been validated at the Health Protection Agency in the United Kingdom (51), uses a modification of the poly-L-lysine conjugation technique described by Pickering (48). The multiplex assay gave high limits of detection (between 32.3 pg/mL for serotype 1 and 109.7 pg/mL for serotype 19F) and correlated well with ELISA-derived Ab concentrations with r values between 0.95 and 0.98. This assay has currently been validated for the measurement of 12 different anticapsular Abs to Pnc and has outperformed other microsphere-based assays in an interlaboratory comparison (54). The nanoplex assay has the capacity of being combined in a 13-plex format along with a tetraplex assay for quantitation of Abs to Men Ps, A, C, Y, and W_{135} (50). This multiplex assay can reduce the amount of sera needed for the evaluation of both meningococcal and pneumococcal antibodies.

Another example of multiplex determinations of Ab to Pnc anticapsular Ps is the electrochemiluminescent assays developed and standardized by Marchese et al. (55) using Meso Scale Discovery (MSD™) technology. This technology allows for multiplex ELISA determinations with a high degree of reproducibility. This novel technique uses carbon-coated electroplates, which do not require a chemical modification of the Ps for antigen coating. In general, multiplex assays greatly reduce wastage of materials, the amount of serum sample, reagents, and operator time while increasing the throughput of assay determinations by a single operator and the limits of detection (Table 1). Some drawbacks of these technologies are the cost of more sophisticated instruments such as flow cytometers and electrochemiluminescent readers that require

Table 1 Multiplex Assays for Measuring Specific Antibodies to *Neisseria Meningitidis* or *Streptococcus Pneumoniae*

Assay/method	Bacterial antigens	Single-plex correlation	Limit of detection	Standardized / validated	Clinical trials use	Reference/year
IgG/Luminex Microspheres	Pnc 14 types	r values 0.92–0.98	NA	Yes/Yes	Yes	Pickering et al., 2002 (48)
IgG/Luminex Microspheres	Pnc 23 types	Evaluated only 89S-2	20–600 pg/mL	Yes/No	No	Biagini et al., 2003 (49)
IgG/Luminex Microspheres	Men groups A, C, Y, W135	r values 0.86–0.97	260–650 pg/mL	Yes/Yes	Yes	Lal et al., 2004 (50)
IgG/Luminex Microspheres	Pnc 9 types	r values 0.95–0.98	32–110 pg/mL	Yes/Yes	Yes	Lal et al., 2005 (51)
IgG/Luminex Microspheres	Pnc 12–15 types	NA	0.6–53 ng/mL	Yes/Yes	NA	Schlottmann et al., 2006 (52)
IgG/XMAP	Pnc/7–15 types	$r \geq 0.931$[a]	NA	Yes/No	No	53
IgG/FlowAps	Pnc/7-22 types	$r = 0.992$[a]	NA	Yes/No	No	Whaley et al. 2008 (54)
IgG/MSD Electro plates	Pnc 8 types	$r = 0.994$	8–66 pg/mL	Yes/Yes	No	Marchese et al., 2008 (55)
OPA/Fluorospheres	Men groups A, C, Y, W135	Mean r_{SBA} = 0.96	Titer of 8	Yes/Yes	Yes	Martinez et al., 2002 (56)
OPA/Fluorospheres/ Bact.	Pnc, panels of 3–4 for 7 types	r values 0.61–0.91	Titer of 8	Yes/Yes	Yes	Martinez et al., 2006 (57)
OPKA/ Anti-microbials	Pnc 7 types	NA	Titer of 10	Yes/No	NA	Bogaert et al., 2004 (43)
OPKA/Fluorescence	Pnc 7 types	r values 0.76–0.97	Titer of 8	Yes/Yes	Limited	Bieging et al., 2005 (44)
OPKA/ Anti-microbials	Pnc, panels of 4 for 13 types	$r \geq 0.97$	Titer of 4	Yes/Yes	Yes	Burton et al., 2006 (42)

[a]Compared with Lal et al. (51), not to a single-plex ELISA. ELISA QC panel concordance was 42% to 55% (54).