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## Current Opinion

## Development of a new platform for neglected tropical disease surveillance

Patrick J. Lammie<sup>a,\*</sup>, Delynn M. Moss<sup>b</sup>, E. Brook Goodhew<sup>a</sup>, Katy Hamlin<sup>a</sup>, Alejandro Krolewiecki<sup>c</sup>, Sheila K. West<sup>d</sup>, Jeffrey W. Priest<sup>b</sup><sup>a</sup> Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, USA<sup>b</sup> Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA<sup>c</sup> Instituto de Investigaciones en Enfermedades Tropicales, Sede regional Orán, Universidad Nacional de Salta, San Ramón de la Nueva Orán, Argentina<sup>d</sup> Dana Center for Preventive Ophthalmology, Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD, USA

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## ABSTRACT

An expanded global focus on the control and elimination of neglected tropical diseases (NTDs) has called attention to the need to develop and validate surveillance strategies that are cost effective and can be integrated across diseases. Here, we describe a multiplex tool for the sensitive detection of antibody responses to NTDs as well as vaccine preventable diseases, malaria, and waterborne and zoonotic infections. The assay platform is robust, can be performed with either serum or dried blood spots and can be adapted to local epidemiological conditions and public health priorities. Multiplex assays open the door to conducting routine serosurveillance for NTDs through demographic health surveillance or malaria indicator surveys.

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## 1. Introduction

Diagnostic tools are essential for the development and evolution of public health programs to control and eliminate neglected tropical diseases (NTDs). From the initial mapping to define the geographic distribution of infections through the various stages of program implementation and post-treatment follow-up, high quality prevalence data are needed to focus and to refine program strategies so as to maximize public health impact. Simple clinical or parasitological tools are typically adequate in early stages of NTD programs, but as lower levels of infection prevalence are reached, tools with greater sensitivity and specificity are required to detect residual infections (Bergquist et al., 2009). Global elimination targets have been established for lymphatic filariasis and trachoma, and ambitious elimination goals are now being considered for onchocerciasis and schistosomiasis (WHO, 2010). Over the last decade, remarkable success has been achieved, both in terms of scaling up mass drug administration (MDA) campaigns and in reducing the burden of the targeted NTD infections (Ottesen et al., 2008; WHO, 2010, 2011a).

Programmatic decisions on when to stop MDA are based on surveys to document that infection levels have been reduced below a given threshold; however, conclusive demonstration that trans-

mission has been interrupted (or reduced to the point where it no longer represents a public health problem) requires that surveillance be carried out for recrudescence of transmission (WHO, 2011b). If MDA interventions have achieved the desired objectives, children born since the initial MDA should, in theory, have been protected from infection; consequently, young children represent the ideal sentinel population for post-MDA surveillance activities. Diagnostic tools used for surveillance must be capable of detecting incident infections in children with great sensitivity and specificity. At a recent workshop sponsored by the World Health Organization (WHO), experts concluded that antibody tests offered the greatest promise for detection of recrudescence transmission of NTDs after interventions had stopped (Solomon et al., 2012). Due to the relatively high costs of surveillance and the overlapping nature of NTDs, development of an integrated surveillance platform was considered to be the ultimate goal. In this paper, an available diagnostic platform that can be used to integrate surveillance for NTDs with the monitoring and evaluation of other public health efforts, including immunization and malaria programs is described.

## 2. Assay design

Luminex platforms have been used for many years to measure levels of multiple analytes in biological samples including cytokines, nucleic acids and antibodies (Jones et al., 2002; de Jager et al., 2003; Dunbar, 2006). Fluorescent microspheres can be readily conjugated with protein ligands, and the different spectral

\* Corresponding author. Address: 1600 Clifton Road, NE, Mail stop D-65, Atlanta, GA 30329, USA. Tel.: +1 404 718 4135; fax: +1 404 718 4193.

E-mail address: [plammie@cdc.gov](mailto:plammie@cdc.gov) (P.J. Lammie).

signatures of the labeled beads permit up to 100 distinct antigens to be included in a single assay well for quantitative determinations of bound antibody. Luminex-based antibody assays are generally as sensitive as conventional ELISAs, have a wide dynamic range and are highly reproducible from assay to assay (Waterboer et al., 2005). Fig. 1 shows the results of two seven-antigen multiplex assays run in our laboratory 2 weeks apart. The dynamic range spanned more than three orders of magnitude and the Spearman rank order correlation coefficient for these data was 0.999. These results were superior to those obtained previously for our recombinant protein-based *Cryptosporidium parvum* 27-kDa antigen ELISA (correlation coefficient >0.969; Priest et al., 2001). More importantly from the standpoint of testing children in NTD-endemic regions, such multiplex assays only require a small sample volume (1  $\mu$ l of serum or less) and can be conducted with dried blood spots, thus facilitating the collection and shipping of samples from the field to a reference laboratory.

Although candidate antigens that are potentially suitable for post-MDA surveillance have previously been described for lymphatic filariasis and onchocerciasis, and more recently for trachoma (Lobos et al., 1991; Chandrashekar et al., 1994; Goodhew et al., unpublished data), other NTDs currently targeted by MDA do not yet have well characterized and validated antigens that could be used for monitoring of antibody responses at the population level. Beyond the infections targeted by preventive chemotherapy, drugs used for MDA also provide important collateral benefits through their effects on other infections. Ivermectin, for example, provides therapeutic benefit beyond filarial parasites through its effect on *Strongyloides* and scabies (Gann et al., 1994; Heukelbach et al., 2004). Other secondary therapeutic benefits have been described for other drugs used for MDA (Porco et al., 2009; Smith and Brooker, 2010; Coles et al., 2011). In general, these additional benefits of MDA have not been well captured by NTD programs, leading to underestimates of the public health gains associated with NTD control. If appropriate antigens can be identified from non-targeted infections, their use in the multiplex assay could provide additional opportunities to measure the impact of NTD programs.

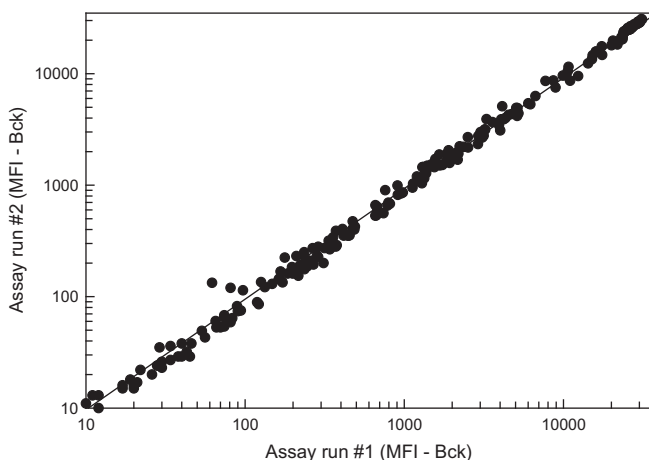
Collection of NTD data would be more useful and therefore a higher priority for Ministries of Health if data on the impact of other public health programs could be generated in parallel. Major

public health programs, including immunization, bednet and water and sanitation programs, assess program performance through coverage assessments, often collected through periodic population-based surveys. Household level surveys are valuable tools for monitoring the public health status and are carried out periodically to assess demographic trends as well as vaccine coverage and access to health programs. Recognizing the significant costs associated with demographic health surveys (DHS) and other population-based surveys, there are important potential benefits of including the collection of serum or dried blood spots in parallel to provide biomarker data. In principle, the NTD multiplex could also be expanded to include vaccine and malaria antigens as well as antigens derived from other infections of public health importance such as waterborne or zoonotic infections. Such a tool would, thus, represent an integrated surveillance platform that could be used across the public health spectrum for monitoring universal coverage (e.g. vaccines) and program effectiveness (e.g. NTDs).

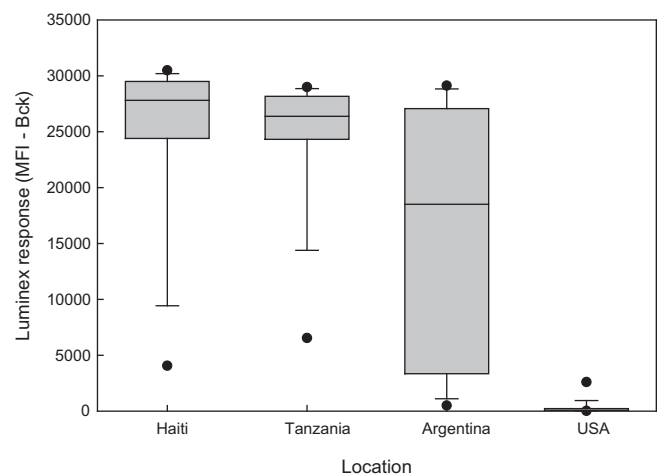
### 3. Proof of principle

Different multiplex assays have been described to assess antibody responses to antigens from parasites, vaccines and waterborne pathogens (Pickering et al., 2002; Reder et al., 2008; Priest et al., 2010; Griffin et al., 2011; Moss et al., 2011). A pilot version of the multiplex assay was developed to analyze the development of antibody responses to filarial antigens in a longitudinal cohort of Haitian children living in an area of intense filarial transmission (Hamlin et al., unpublished data). The assay included more than 20 antigens including *Cryptosporidium*, *Giardia*, *Toxoplasma*, norovirus, tetanus, measles and malaria, as well as filarial antigens and the *Wolbachia* surface protein. The longitudinal nature of the follow-up of these children facilitated the monitoring of the evolution of antifilarial responses as well as the incidence of *Toxoplasma* and malaria (Hamlin et al., unpublished data and unpublished observations). The assay is now being expanded to cover other NTDs, including schistosomiasis and trachoma.

That serological data can provide a measure of the intensity of transmission of infections of interest, and a snapshot of the epidemiological context in which children live is illustrated in Fig. 2.



**Fig. 1.** Interassay comparison using seven antigens. A multiplex bead assay using seven recombinant antigens was performed as previously described (Moss et al., 2011). Samples from 35 Haitian children were assayed twice in the same laboratory approximately 2 weeks apart, and the mean fluorescent intensities minus background values (MFI – Bck) from the two runs were plotted on a logarithmic scale. The regression line from a least-squares analysis of the data is included in the graph.



**Fig. 2.** Enterotoxigenic *Escherichia coli* (ETEC) responses among children <6 years old. A multiplex bead assay using a native *E. coli* heat labile toxin  $\beta$  subunit was performed as previously described (Moss et al., 2011) on sera from children from Haiti ( $n = 115$ ), Tanzania ( $n = 31$ ), Argentina ( $n = 86$ ) and the US ( $n = 107$ ). Samples were collected from children under 6 years of age in the course of studies approved by the relevant human subjects' protection offices. Boxes indicate the 25th and 75th percentiles, bars indicate the 10th and 90th percentiles, and dots indicate the 5th and 95th percentiles. Median values are indicated by a line within the box.

**Table 1**

Potential uses for multiplex tools for serosurveillance.

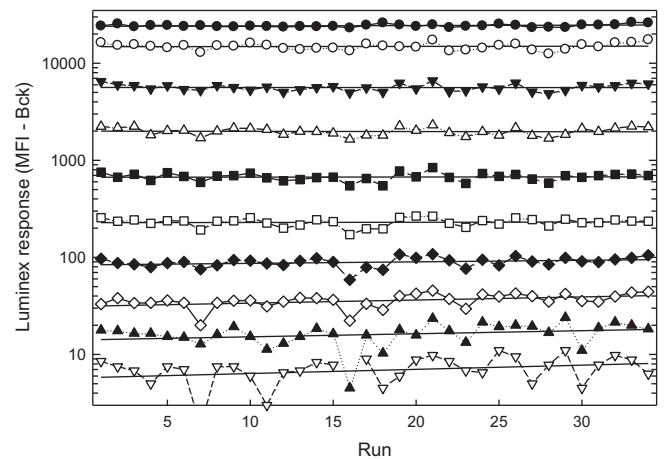
Uses for multiplex tools
<ul style="list-style-type: none"> <li>• Mapping the geographic distribution of multiple infections in humans and animals</li> <li>• Monitoring secular trends (e.g., improved access to water)</li> <li>• Monitoring program impact</li> <li>• Coverage assessments</li> <li>• Post-MDA surveillance</li> <li>• Outbreak investigations</li> </ul>

Antibody responses of Haitian children were compared with those of children from the United States and from impoverished communities in northern Argentina (Krolewiecki et al., 2010) and central Tanzania (Goodhew et al., unpublished data). Clear differences in the distributions of IgG antibody responses to the enterotoxigenic *Escherichia coli* (ETEC) heat labile toxin  $\beta$  subunit can be seen, arguing for different exposure levels among these communities (Brusow et al., 1990; Flores et al., 2008). In principle, such comparisons could be made across communities within a country or over time in a selected location to assess changes in transmission dynamics following the introduction of public health interventions. Other potential uses of multiplex assays are listed in Table 1.

#### 4. Technical considerations

The quality of the antigen attached to the microspheres is one of the most important determinants of assay performance. Poor quality antigens are likely to generate poor quality data. It is possible to compensate, to some extent, for the presence of contaminating bacterial proteins in semi-purified recombinant antigen preparations by including *E. coli* lysate in the assay diluent (Moss et al., 2011). Experience has shown that coupling procedures are robust but are influenced by commercial sources of coupling reagent. Multiple lots of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) should be tested to identify a reagent that yields the lowest possible background values with negative control sera. Coupling procedures must also be adapted (e.g., through the use of peptide linkers) to permit coupling of carbohydrate antigens to the microspheres.

Once coupled, antigen-coated microspheres may be stable for extended periods of time; cross-linked beads have been successfully stored at 4 °C in the presence of a protease inhibitor cocktail for periods of more than 1 year (Moss et al., 2011). However, because some proteins may be inherently unstable or may have functional protease activity even after conjugation to the microspheres, the stability of each coupled antigen should be monitored closely over time by using a positive control on each assay plate. For example, see Fig. 3 which shows representative data from a 5 month study using recombinant *Brugia malayi* Bm14 antigen that included a 10 point, twofold serial dilution of a strong positive control on each of 34 assay plates and verifies the stability of the coupled antigen (Fig. 3). Other factors that play a role in the reliability and reproducibility of multiplex assay values are: the monoclonal mouse anti-human IgG secondary antibody used, the level of secondary antibody biotinylation, the level of R-phycoerythrin labeling of the streptavidin reporter molecule, and the use of reagents in the sample diluent to suppress non-specific antibody binding to the microspheres (Waterboer et al., 2006; Moss et al., 2011). As with any serological assay, comparison of data generated at different times within a laboratory or between laboratories will require standardization of assays by including appropriate controls and reference reagents. Such quality assurance efforts will be particularly important for assessing vaccine coverage or changes in



**Fig. 3.** Stability of *B. malayi* Bm14 antigen coated microspheres. *B. malayi* Bm14 recombinant antigen was coupled to microspheres and stored at 4 °C with protease inhibitors as previously described (Moss et al., 2011). A twofold serial dilution of a strong positive control (10 samples) was included on each of 34 multiplex assay plates over the course of a 5 month study. Plates were run as previously described beginning with plate 1 (Moss et al., 2011). Mean fluorescent intensity minus background values (MFI – Bck) from each dilution are plotted against plate numbers in the time series (run). Each symbol represents data from a given dilution of the control over time.

antibody responses over time. Although intended as an epidemiological tool rather than as a diagnostic assay per se, it is important to note that bead-based assays do not capture some important functional attributes of the antibodies such as their neutralization capacity.

For testing at the end stage of NTD programs or for less common infections, the specificity of the test is arguably more important than the sensitivity; a test with 99% specificity will generate more false positive results than true positives when the prevalence declines to below 1%. It is especially important to bear this in mind because the greater sensitivity of the multiplex assays relative to other serological assays can introduce more challenges with specificity. The ability to include more than one antigen for each infection of interest provides an option to generate confirmatory data, increase overall assay specificity and validate survey results. As a caveat, the inclusion of closely related antigens in the multiplex should be evaluated because the potential competition between antigens may interfere with antibody determinations.

#### 5. Summary

In an era of fierce competition for global health dollars, surveillance for single diseases is increasingly difficult to defend as a priority. Working in the NTD arena, where limitations in financial resources have prevented countries from taking full advantage of donated drugs, it is clear that post-MDA surveillance will be a challenge to implement. This Current Opinion describes an assay that can be used to develop alternative approaches for NTD surveillance based on the ability to generate data of value to many public health programs. Such an assay can provide an important complement to DHS or malaria indicator surveys at a relatively low cost compared with the value of the data generated or the cost of stand-alone surveys. Assays can be modified to meet local needs by selecting different sets of antigens according to the epidemiological profiles and the specific interests of program managers. Libraries of antigens representing panels of antigens to cover NTDs, vaccines, waterborne, vectorborne, foodborne and zoonotic infections of public health importance should be pursued as a common, collaborative effort.

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## References

- Bergquist, R., Vang Johansen, M., Utzinger, J., 2009. Diagnostic dilemmas in helminthology: what tools to use and when? *Trends Parasitol.* 25, 151–156.
- Brussow, H., Sidoti, J., Link, H., Hoang, Y.K., Barclay, D., Dirren, H., Freire, W.B., 1990. Age-specific prevalence of antibody to enterotoxigenic *Escherichia coli* in Ecuadorian and German children. *J. Infect. Dis.* 162, 974–977.
- Chandrashekar, R., Curtis, K.C., Ramzy, R.M., Liftis, F., Li, B.-W., Weil, G.J., 1994. Molecular cloning of *Brugia malayi* antigens for diagnosis of lymphatic filariasis. *Mol. Biochem. Parasitol.* 64, 261–271.
- Coles, C.L., Seidman, J.C., Levens, J., Mkocho, H., Munoz, B., West, S., 2011. Association of mass treatment with azithromycin in trachoma-endemic communities with short-term reduced risk of diarrhea in young children. *Am. J. Trop. Med. Hyg.* 85, 691–696.
- de Jager, W., te Velthuis, H., Prakken, B.J., Kuis, W., Rijkers, G.T., 2003. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. *Clin. Diagn. Lab. Immunol.* 10, 133–139.
- Dunbar, S.A., 2006. Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. *Clin. Chim. Acta* 363, 71–82.
- Flores, J., DuPont, H.L., Jiang, Z.-D., Belkinid-Gerson, J., Mohamad, J.A., Carlin, L.G., Padda, R.S., Paredes, M., Martinez-Sandoval, J.F., Villa, N.A., Okhuysen, P.C., 2008. Enterotoxigenic *Escherichia coli* heat-labile toxin seroconversion in US travelers to Mexico. *Int. J. Travel Med.* 15, 156–161.
- Gann, P.H., Neva, F.A., Gam, A.A., 1994. A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. *J. Infect. Dis.* 169, 1076–1079.
- Griffin, S.M., Chen, I.M., Fout, G.S., Wade, T.J., Egorov, A.I., 2011. Development of a multiplex microsphere immunoassay for the quantitation of salivary antibody responses to selected waterborne pathogens. *J. Immunol. Methods* 364, 83–93.
- Heukelbach, J., Winter, B., Wilcke, T., Muehlen, M., Albrecht, S., de Oliveira, F.A., Kerr-Pontes, L.R., Liesenfeld, O., Feldmeier, H., 2004. Selective mass treatment with ivermectin to control intestinal helminthiases and parasitic skin diseases in a severely affected population. *Bull. World Health Organ.* 82, 563–571.
- Jones, L.P., Zheng, H.-Q., Karron, R.A., Peret, T.C.T., Tsou, C., Anderson, L.J., 2002. Multiplex assay for detection of strain-specific antibodies against the two variable regions of the G protein of respiratory syncytial virus. *Clin. Diagn. Lab. Immunol.* 9, 633–638.
- Krolewiecki, A.J., Ramanathan, R., Fink, V., McAuliffe, I., Won, K., Cajal, S.P., Juarez, M., Di Paolo, A., Tapia, L., Acosta, N., Lee, R., Lammie, P., Abraham, D., Nutman, T.B., 2010. Improved diagnosis of *Strongyloides stercoralis* using recombinant antigen-based serologies in a community-wide study in northern Argentina. *Clin. Vac. Immunol.* 17, 1624–1630.
- Lobos, E., Weiss, N., Karam, M., Taylor, H.R., Ottesen, E.A., Nutman, T.B., 1991. An immunogenic *Onchocerca volvulus* antigen: a specific and early marker for infection. *Science* 251, 1603–1605.
- Moss, D.M., Priest, J.W., Boyd, A., Weinkopff, T., Kucerova, Z., Beach, M.J., Lammie, P.J., 2011. Multiplex bead assay on sera from Haitian children enrolled in a drug study for the treatment of lymphatic filariasis. *Am. J. Trop. Med. Hyg.* 85, 229–237.
- Ottesen, E.A., Hooper, P.J., Bradley, M., Biswas, G., 2008. The global programme to eliminate lymphatic filariasis: health impact after 8 years. *PLoS Negl. Trop. Dis.* 2, e317.
- Pickering, J.W., Martins, T.B., Schroder, M.C., Hill, H.R., 2002. Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for quantitation of antibodies to tetanus, diphtheria, and *Haemophilus influenzae* Type b. *Clin. Diagn. Lab. Immunol.* 9, 872–876.
- Porco, T.C., Gebre, T., Ayele, B., House, J., Keenan, J., Zhou, Z., Hong, K.C., Stoller, N., Ray, K.J., Emerson, P., Gaynor, B.D., Lietman, T.M., 2009. Effect of mass distribution of azithromycin for trachoma control on overall mortality in Ethiopian children: a randomized trial. *JAMA* 302, 962–968.
- Priest, J.W., Li, A., Khan, M., Arrowood, M.J., Lammie, P.J., Ong, C.S., Roberts, J.M., Isaac-Renton, J., 2001. Enzyme immunoassay detection of antigen-specific immunoglobulin G antibodies in longitudinal serum samples from patients with cryptosporidiosis. *Clin. Diagn. Lab. Immunol.* 8, 415–423.
- Priest, J.W., Moss, D.M., Visvesvara, G.S., Jones, C.C., Li, A., Isaac-Renton, J.L., 2010. Multiplex assay detection of immunoglobulin G antibodies that recognize *Giardia intestinalis* and *Cryptosporidium parvum* antigens. *Clin. Vaccine Immunol.* 11, 1695–1707.
- Reder, S., Riffelmann, M., Becker, C., von König, C.H.W., 2008. Measuring immunoglobulin G antibodies to tetanus toxin, diphtheria toxin, and pertussis toxin with single-antigen enzyme-linked immunosorbent assays and a bead-based multiplex assay. *Clin. Vaccine Immunol.* 15, 744–749.
- Smith, J.L., Brooker, S., 2010. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. *Trop. Med. Int. Health* 15, 776–795.
- Solomon, A.W., Engels, D., Bailey, R.L., Blake, I.M., Brooker, S., Chen, J.-X., Chen, J.-H., Churcher, T., Drakely, C.J., Edwards, T., Fenwick, A., French, K.M., Gabrielli, A., Grassly, N.C., Harding-Esch, E.M., Holland, M.J., Koukounari, A., Lammie, P.J., Leslie, J., Mabey, D.C.W., Rhajaoui, M., Secor, W.E., Stothard, R.J., Wei, H., Willingham, A.L., Xiao, Zhou, N., Peeling, R.W., 2012. A diagnostics platform for the integrated mapping, monitoring and surveillance of neglected tropical diseases: rationale and target product profiles. *PLoS Negl. Trop. Dis.* 6, e1746.
- Waterboer, T., Sehr, P., Michael, K.M., Franceschi, S., Nieland, J.D., Joos, T.O., Templin, M.F., Pawlita, M., 2005. Multiplex human papillomavirus serology based on in situ-purified glutathione S-transferase fusion proteins. *Clin. Chem.* 51, 1845–1853.
- Waterboer, T., Sehr, P., Pawlita, M., 2006. Suppression of non-specific binding in serological Luminex assays. *J. Immunol. Methods* 309, 200–204.
- World Health Organization, 2010. Working to overcome the global impact of neglected tropical disease.
- World Health Organization, 2011a. Global programme to eliminate lymphatic filariasis: progress on mass drug administration, 2010. *Weekly Epid. Record* 35, 377–388.
- World Health Organization, 2011b. Global programme to eliminate lymphatic filariasis: Monitoring and epidemiological assessment of mass drug administration.