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Research paper

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ABSTRACT

We describe here a lateral flow-based assay (LFA) for the detection of antibodies against immunodominant antigen Pgp3 from *Chlamydia trachomatis*, the causative agent of urogenital chlamydia infection and ocular trachoma. Optimal signal detection was achieved when the gold-conjugate and test line contained Pgp3, creating a dual sandwich capture assay. The LFA yielded positive signals with serum and whole blood but not with eluted dried blood spots. For serum, the agreement of the LFA with the non-reference multiplex assay was 96%, the specificity using nonendemic pediatric sera was 100%, and the inter-rater agreement was $\kappa = 0.961$. For whole blood, the agreement of LFA with multiplex was 81.5%, the specificity was 100%, and the inter-rater agreement was $\kappa = 0.940$. The LFA was tested in a field environment and yielded similar results to those from laboratory-based testing. These data show the successful development of a lateral flow assay for detection of antibodies against Pgp3 with reliable use in field settings, which would make antibody-based testing for trachoma surveillance highly practical, especially after cessation of trachoma elimination programs.

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1. Introduction

Trachoma, resulting from repeated infection with the bacterium *Chlamydia trachomatis* (Ct), is the leading global infectious cause of blindness. Blinding trachoma as a public health problem is targeted for elimination by 2020 using the SAFE strategy: Surgery for trichiasis, Antibiotics to treat infection, Facial cleanliness to prevent spread of infection, and Environmental improvement. Antibiotic distribution (Zithromax™, donated by Pfizer) is carried out through annual mass drug administration (MDA) (Solomon et al., 2006). Current decision-making for starting and stopping MDA programs are based on the prevalence of the clinical indicator trachomatous inflammation–follicular (TF) among 1–9 year olds. An impact assessment is conducted after 1, 3, 5, or 7 rounds of MDA, depending on the baseline TF level, and MDA is stopped if the district-wide prevalence of TF is <5%. Two years after stopping MDA, a survey is done to show continued TF levels <5%, which will provide the evidence for a dossier for validation of elimination of trachoma as a public health problem (“pre-validation survey”). However, in post-MDA settings where trachoma prevalence is low, the correlation between TF and active ocular infection breaks down (Mabey et al., 2003; Burton et al., 2011), in part because other

pathogens can cause similar clinical signs (Dean et al., 2008; Capriotti et al., 2009; Dean et al., 2013).

The program end point for trachoma does not require complete elimination of the bacterium causing trachoma. Therefore, a sensitive and specific measure of Ct exposure will be critical at the end of programs and in post-validation settings for trachoma surveillance to ensure lack of recrudescence of infection. We have recently shown that antibody responses against Ct antigens Pgp3 and CT694 show high sensitivity and specificity for ocular Ct infection (Goodhew et al., 2012), suggesting antibodies are good markers of exposure to Ct. Antibody responses appear to be long-lived and act as indicators of historical rather than active infection (Goodhew et al., 2014). Analysis of antibody responses from young children born after cessation of MDA shows that seroprevalence represents a good indicator of low to no transmission (Martin et al., 2015a; Pant et al., 2016; West et al., 2016). Initial serological studies used multiplex bead array (MBA) testing, which is highly sensitive and can be integrated with other serological tests. However this technology is not feasible for many trachoma-endemic countries due to the high initial cost of the instrumentation and the need to maintain high technical capacity and strong infrastructure for analysis and maintenance of the instrument. We therefore sought to develop a field-deployable lateral flow-based assay for measuring anti-Ct antibodies and chose Pgp3 based on the large amount of data on this protein as an immunodominant Ct antigen (Comanducci et al., 1994; Ghaem-Maghami et al., 2003; Li et al., 2008; Wills et al., 2009).

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2. Methods

2.1. Samples

Serum samples were collected from a community in Nepal in 2000, 2002, or 2014 as part of a study of trachoma post-MDA serosurveillance (N = 432; age range 1–90 Gwyn et al., manuscript in preparation) that had been tested on a previously-described multiplex bead array (MBA) assay for antibodies against the Ct antigen Pgp3 (Goodhew et al., 2012, 2014; Martin et al., 2015b). Two extensively studied Pgp3-positive control samples from Haiti were used for test optimization. For specificity testing, a set of serum samples from 4 to 9 year olds from three communities in central Bolivia collected as part of a study of Chagas disease in 2010 were used as non-trachoma endemic controls (N = 78). Whole blood specimens (N = 301) from a subset of the Nepal serum samples described above were manufactured by spiking an equal volume of study serum into red blood cells depleted of plasma from negative control blood (Mei et al., 2001). For testing of dried blood spots, serum was eluted from dried blood spots from 1 to 9 year olds in Tanzania as previously described (Goodhew et al., 2012). Samples were collected under protocols approved by local IRBs, and CDC staff were determined to be not engaged in the research study by the CDC human subjects coordinator.

2.2. Antigen preparation

Methods for selection and isolation of the Ct antigen Pgp3 have been previously described (Goodhew et al., 2012).

2.3. Optimization of lateral flow conditions

The optimal conditions for the LFA were determined using the “wet test”. Briefly, nitrocellulose membranes were dipped into wells containing assay buffer, positive and negative control serum, and conjugate. Conditions that yielded the strongest test-line signal, when tested with positive control samples and no test-line signal when tested with negative control samples, were chosen. Conjugate reagents tested were: anti-human IgG colloidal gold, protein A-colloidal gold and Pgp3-colloidal gold. To determine the optimal conditions for the capture reagent, recombinant Pgp3 was dispensed onto a nitrocellulose membrane at various concentrations: 2.0 mg/mL, 1.75 mg/mL, 1.50 mg/mL, 1.25 mg/mL and 1.00 mg/mL.

2.4. Preparation of lateral flow assay

Conjugate reagents and capture reagents were prepared with optimal conditions. For the capture reagents, Pgp3 protein (1.5 mg/mL) and biotinylated bovine serum albumin (BSA-biotin, 1.5 mg/mL; Arista Biologicals, Allentown, PA, USA) were dispensed onto the test and control lines, respectively, of a nitrocellulose membrane (Millipore, Billerica, MA) at a rate of 0.1 μ L/mm using an IsoFlow Dispenser (Imagene Technology, Hannover, NH). The detection reagent (conjugate) was prepared by conjugating Pgp3 to InnovaCoat Gold (Innova Biosciences, Cambridge, UK) following the manufacturer's instructions. The conjugate pad was treated with a buffer containing 20 mM phosphate-buffered saline (PBS) pH 7.4, 2% BSA, 2.5% sucrose, 0.3% polyvinylpyrrolidone, 1% Tween-20 and dried overnight at room temperature. Pgp3-gold conjugate and streptavidin-gold (SA-gold) conjugate (Arista Biologicals) were washed and suspended in buffer containing 2 mM borate pH 7.4, 5% sucrose and 5% trehalose. Pgp3-gold and SA-gold conjugates were mixed at a 4:1 ratio and sprayed onto the pre-treated conjugate pad at a rate of 0.6 μ L/mm using the IsoFlow Dispenser. The blood separation membrane (Lydall, Manchester, CT) was treated with a buffer containing 10 mM PBS pH 7.4, 5% BSA, 0.05% Tween and dried at room temperature overnight.

2.5. Assembly of lateral flow assay device

An absorbent pad (Arista Biologicals), nitrocellulose membrane, conjugate pad, blood separation membrane, and buffer pad (Millipore) were placed on a backing card (DCN Diagnostics, Carlsbad, CA) with a 1–2 mm overlap between each material to facilitate sample flow. A guillotine cutter (Arista Biologicals) was used to produce 4.5 mm strips, which were then placed in plastic housing (DCN Diagnostics). All LFA cassettes were stored at room temperature in a desiccator cabinet with a relative humidity of <15%.

2.6. LFA protocol

Serum (10 μ L) or whole blood (20 μ L) was applied to the sample applicator port, followed by 200 μ L of chase buffer (10 mM PBS, 0.3% Tween 20) added to the buffer applicator port, forcing sample migration onto the conjugate pad. Antibodies specific for Pgp3 present in the sample bind to the Pgp3-gold conjugate and continue to travel up the nitrocellulose membrane. Anti-Pgp3 antibodies bound to the Pgp3-gold conjugate then bind to Pgp3 present on the test line, producing a visible test line signal by accumulation of gold particles on the test line. SA-gold conjugate bound to BSA-biotin on the control line produces a signal irrespective of the presence of anti-Pgp3 antibodies in the sample. Excess liquid is absorbed by the absorbent pad (Fig. 1).

A test was considered valid only if it produced a signal on the control line. Tests were scored as either positive when a clear test line was present (recorded as + or ++ depending on the intensity of the line), or negative when no test line (–) or a faint but difficult to distinguish test line (\pm) was observed. Samples that received a positive score by one user and a negative score by the other user were categorized as “inconclusive”.

2.7. Field comparison to laboratory testing

A set of 200 serum samples previously tested by LFA in the CDC laboratory was shipped to the Kongwa Trachoma Project in Kongwa, Tanzania, where trachoma is endemic. Lateral flow assays were transported at ambient temperatures to Tanzania in foil pouches (DCN Diagnostics) containing desiccant, and were stored at ambient temperatures for 5–8 days prior to use.

2.8. Statistical analysis

The agreement of the LFA with the Pgp3 MBA was determined as the percentage of LFA-positive of the number of positive samples by MBA. Specificity of the assay was determined against a group of serum samples from children from non-trachoma endemic settings. Inter-rater agreement was calculated using Cohen's kappa coefficient with GraphPad Prism.

3. Results

3.1. Optimization of LFA

Optimization of Pgp3 concentration on the test line and capture reagents conjugated to colloidal gold using positive controls showed the strongest test line signal with the combination of Pgp3-colloidal gold and 1.5 mg/mL of Pgp3 as the capture reagent (Fig. 1). Representative positive and negative tests are shown in Fig. 2. Time courses were run on each new lot of LFAs using negative, high positive, and low positive control sera. The optimal read time based on a time course of 15, 20, 30, 60, and 120 min was 30 or 60 min; 30 min was chosen for initial testing.

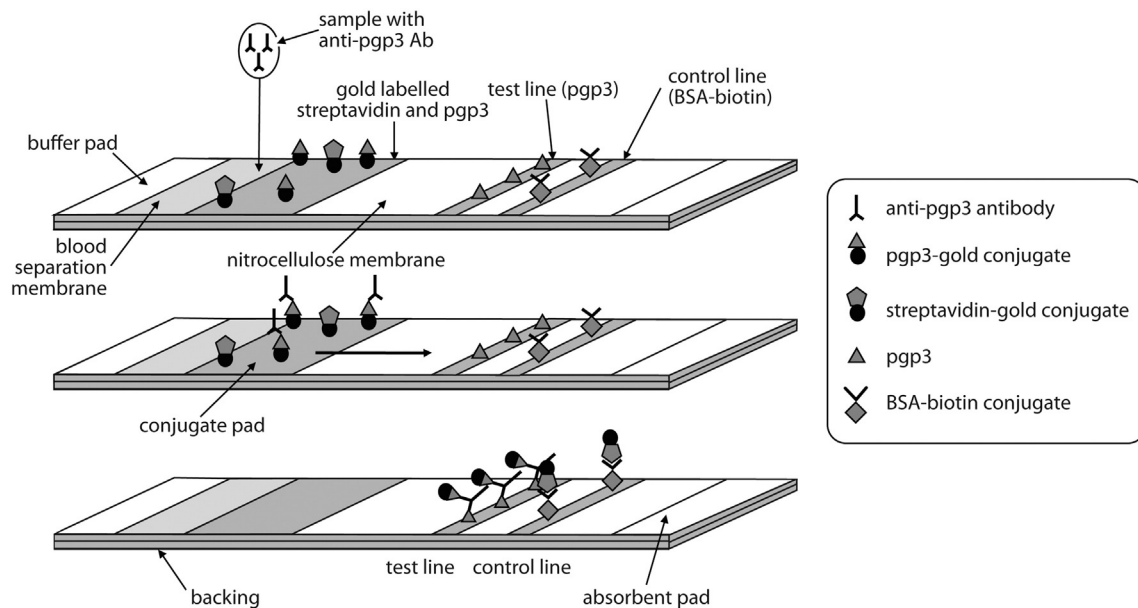


Fig. 1. Schematic of Pgp3 lateral flow assay.



Fig. 2. LFA test. Shown are results of the LFA run with positive (left) and negative (right) control sera.

3.2. Performance of LFA with serum

Serum (N = 512) was tested on the LFA for anti-Pgp3 antibodies and read by two independent graders.

The agreement of LFA with MBA-positive samples was 96% and the specificity was 100% (Table 1). Inter-rater agreement was very good, with a Cohen's kappa coefficient of 0.961 (95% CI 0.937–0.985, data not shown).

3.3. Performance of LFA with whole blood

The agreement of LFA with MBA-positive samples was 81.3% and the specificity was 100% (Table 1). Inter-rater agreement (κ) was 0.940 (95% CI 0.900–0.981). Out of 301 paired whole blood and serum samples, 94 (31.3%) were both scored as positive, 174 (57.8%) were both scored as negative, 14 (4.6%) were scored as inconclusive with one sample type and 19 (6.3%) were scored as positive with one sample type but negative with the other (Table 2). The complete set of readings for all samples by each user for serum and whole blood is shown in Supplementary Table 1.

3.4. Field performance of LFA

The agreement of LFA with Pgp3-positive samples by MBA was 92% when tested with serum at a field site in Tanzania (Table 1). Inter-rater agreement was 0.890 (95% CI 0.827–0.953). Out of 200 samples evaluated by two readers each in both the laboratory and the field, 173 (86.5%) were graded the same in both sites (89 [44.5%] were scored as positive at both sites and 84 [42%] were scored as negative) (Table 3). Seventeen (8.5%) were scored as inconclusive at one site but either positive or negative at the other (Table 3). Ten (5%) were scored as positive at one site but negative at another site (Table 3). Ratings by each user in the laboratory and in the field is shown in Supplementary Table 1.

3.5. Stability of test line

Tests run in the field were shipped back to CDC and read again 4 months later. Agreement between readings were good (κ = 0.728, CI 0.640–0.816). The majority of positives at the original 30 min read being called positive 4 months later (87/89, 97.7%, Table 4). Of the 78

Table 1
Specificity of the lateral flow assay (LFA) and agreement with multiplex bead array (MBA) testing. Serum specimens were run on the MBA and LFA. Samples were run on LFA in a laboratory at CDC (“Lab”, N = 322) or at Kongwa Trachoma Project (“Field”, N = 93). A subset of serum samples were added to plasma-depleted bloods cells (“Whole Blood”, N = 190). n.d. = not done. 95% confidence intervals (CI) are shown in brackets.

Sample type	LFA result	MBA-positives (percent [95% CI])	Non-endemic children (percent [95% CI])
Serum (lab)	Positive	235 (96.3) [93.9–98.7]	0
	Inconclusive	4 (1.6) [0–3.2]	0
	Negative	5 (2.0) [0.24–3.8]	78 (100) [100–100]
	Total	244	78
Whole blood	Positive	91 (81.3) [74.1–88.5]	0
	Inconclusive	7 (6.2) [1.7–10.7]	0
	Negative	14 (12.5) [6.4–18.6]	78 (100) [100–100]
	Total	112	78
Serum (field)	Positive	85 (91.4) [85.7–97.1]	n.d.
	Inconclusive	3 (3.2) [0–6.8]	n.d.
	Negative	5 (5.4) [0.81–10.0]	n.d.
	Total	93	n.d.

specimens negative at baseline, 67 (85.7%) remained negative at 4 months (Table 4). Most (13/16, 81.3%) of the specimens read as inconclusive at 30 min were read as positive at 4 months. Eight tests were unable to be confidently read at 4 months due to the test line being obscured; most of these (6/8) were negative at the original 30 min read (Table 4).

4. Discussion

We describe here a lateral flow assay for detection of antibodies against the *Ct* antigen Pgp3 with good agreement compared to results from Pgp3 multiplex bead array testing and specificity against a panel of serum samples from children from a non-endemic country. Inter-rater agreement was also very strong with a κ of 0.961 in the laboratory site and 0.89 at the field site. The LFA tests were also very stable, performing well in dry, arid conditions in a trachoma-endemic district of Tanzania after approximately one week at ambient temperatures.

The dual Pgp3 capture of serum antibodies in this LFA add an extra dimension to this assay compared to the ELISA or MBA because the LFA does not distinguish between antibody isotypes. We have shown that IgA antibodies are also present in serum (Goodhew et al., 2014) and IgM antibody may also be present. This may increase the sensitivity of the LFA by detecting the presence of multiple isotypes.

While we undertook this work to develop a rapid antibody test for *Ct* to facilitate country-based serosurveillance for trachoma, there are potential applications for use of this test for urogenital infection as well. Serologic testing provides information on past as well as current exposure to *Ct* and, therefore provides a broader picture of population exposure to *Ct* than active case detection for either ocular trachoma or urogenital chlamydia. The seroepidemiology of urogenital *Ct* has already been evaluated in some settings (Johnson and Horner, 2008; Lyytikäinen et al., 2008; Horner et al., 2013) and a low-cost test such as what we have presented here (currently <1.50 USD per test) may

allow this type of serosurvey for urogenital chlamydia to be feasible in settings with limited laboratory capacity.

One drawback of the LFA is that the inter-rater agreement was slightly lower at the field site than in the laboratory. This may be due in part to variations in natural lighting conditions in the field depending on the position of the reader, such as facing a window or with the back to a window. Using an artificial light source, such as a flashlight, to illuminate the strip in a field setting may facilitate reading the strip and improve inter-rater agreement. Disagreement between readers tended to occur on samples for which the MBA values fell near the cut-off (data not shown). However, the stability of the test line – 85.7% of negative samples at the 30 min reading were read as negative and 97.7% of positives were read as positive 4 months later – potentially negates this drawback, as without the restriction of a time-sensitive test, consensus readings can be used. There was a trend towards an increase calling samples positive at the later reading. Eleven samples read as negative at 30 min were called positive or inconclusive at 4 months. It is unclear if trend towards positive readings at 4 months is because the test lines become more clear over time or if the reader was more likely to call a faint line positive because of increased comfort in reading the test at the later time point. An automated reader for lateral flow tests would be useful to address the issue of reader subjectivity for this test.

An additional limitation is the lack of a true gold standard to indicate historical infection with *Ct*, thereby hindering determination of the sensitivity of the LFA. Sensitivity of the Pgp3 MBA was determined from a set of sera from children in endemic countries with nucleic acid amplification test (NAAT)-positive ocular swabs (Goodhew et al., 2012). For the sensitivity determination of this LFA prototype, we are limited to a set of serum samples that lack ocular infection data, thereby precluding determination of sensitivity testing using the gold-standard NAAT. Dried blood spots – the specimen type uniformly used in trachoma studies – cannot be used with the current iteration of the test due to high background signal. Additionally, it is important to note that NAAT

Table 2
Comparison of performance of the LFA with serum and whole blood, N = 301 paired specimens.

	Serum positive	Serum inconclusive	Serum negative
Blood positive	94	1	2
Blood inconclusive	7	1	0
Blood negative	17	5	174

Table 3
Performance of the LFA in the laboratory (“Lab”) and field setting, N = 200, matched serum specimens.

	Lab positive	Lab inconclusive	Lab negative
Field positive	89	3	3
Field inconclusive	3	0	8
Field negative	7	3	84

Table 4

Comparison of readings at 30 min (reading 1) and four months (reading 2) after sample testing.

Reading 1	Reading 2			
	Pos	Neg	Inconclusive	Unreadable
Pos	87	1	0	1
Neg	5	61	6	6
Inconclusive	13	1	1	1

and antibody tests are measuring different analytes, so using NAAT-positive samples to define the sensitivity of a test for antibody is somewhat limiting. Ocular infection will resolve even in the absence of treatment following 2–6 weeks (Bailey et al., 1999), whereas the longevity of anti-pgp3 responses is not currently known, although it is certainly months (Goodhew et al., 2014) and most likely years or decades, likely depending on the number of infections an individual incurs. As such, the usage of antibody tests and by extension the lateral flow assay presented here is population-level surveillance and not diagnosis of trachoma in an individual.

Serosurveillance for trachoma following validation of elimination of trachoma will be critical since the end point for trachoma program is the elimination of blinding trachoma as a public health problem and not the interruption of transmission of ocular *Ct* infection. There therefore remains a chance of recrudescence of disease. Funding for post-elimination surveillance is virtually nonexistent, so the ability to integrate trachoma surveillance with ongoing public health surveys will be crucial, having low-cost tools that require little-to-no technical expertise and can use blood specimens potentially collected for other purposes will be the most practical way to accomplish post-validation surveillance of trachoma.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jim.2016.05.008>.

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References

Bailey, R., Duong, T., Carpenter, R., Whittle, H., Mabey, D., 1999. The duration of human ocular *Chlamydia trachomatis* infection is age dependent. *Epidemiol. Infect.* 123 (3), 479–486.

- Burton, M.J., Hu, V.H., Massae, P., Burr, S.E., Chevallier, C., Afwamba, I.A., et al., 2011. What is causing active trachoma? The role of nonchlamydial bacterial pathogens in a low prevalence setting. *Investig. Ophthalmol. Vis. Sci.* 52 (8), 6012–6017.
- Capriotti, J.A., Pelletier, J.S., Shah, M., Caivano, D.M., Ritterband, D.C., 2009. Normal ocular flora in healthy eyes from a rural population in Sierra Leone. *Int. Ophthalmol.* 29 (2), 81–84.
- Comanducci, M., Manetti, R., Bini, L., Santucci, A., Pallini, V., Cevenini, R., et al., 1994. Humoral immune response to plasmid protein pgp3 in patients with *Chlamydia trachomatis* infection. *Infect. Immun.* 62 (12), 5491–5497.
- Dean, D., Kandel, R.P., Adhikari, H.K., Hessel, T., 2008. Multiple Chlamydiaceae species in trachoma: implications for disease pathogenesis and control. *PLoS Med.* 5 (1), e14.
- Dean, D., Rothschild, J., Ruettger, A., Kandel, R.P., Sachse, K., 2013. Zoonotic Chlamydiaceae species associated with trachoma, Nepal. *Emerg. Infect. Dis.* 19 (12), 1948–1955.
- Ghaem-Maghami, S., Ratti, G., Ghaem-Maghami, M., Comanducci, M., Hay, P.E., Bailey, R.L., et al., 2003. Mucosal and systemic immune responses to plasmid protein pgp3 in patients with genital and ocular *Chlamydia trachomatis* infection. *Clin. Exp. Immunol.* 132 (3), 436–442.
- Goodhew, E.B., Priest, J.W., Moss, D.M., Zhong, G., Munoz, B., Mkocho, H., et al., 2012. CT694 and pgp3 as serological tools for monitoring trachoma programs. *PLoS Negl. Trop. Dis.* 6 (11), e1873.
- Goodhew, E.B., Morgan, S.M., Switzer, A.J., Munoz, B., Dize, L., Gaydos, C., et al., 2014. Longitudinal analysis of antibody responses to trachoma antigens before and after mass drug administration. *BMC Infect. Dis.* 14, 216.
- Horner, P., Soldan, K., Vieira, S.M., Wills, G.S., Woodhall, S.C., Pebody, R., et al., 2013. *C. trachomatis* Pgp3 antibody prevalence in young women in England, 1993–2010. *PLoS ONE* 8 (8), e72001.
- Johnson, A.M., Horner, P., 2008. A new role for *Chlamydia trachomatis* serology? *Sex. Transm. Infect.* 84 (2), 79–80.
- Li, Z., Zhong, Y., Lei, L., Wu, Y., Wang, S., Zhong, G., 2008. Antibodies from women urogenitally infected with *C. trachomatis* predominantly recognized the plasmid protein pgp3 in a conformation-dependent manner. *BMC Microbiol.* 8, 90.
- Lyytikäinen, E., Kaasila, M., Koskela, P., Lehtinen, M., Patama, T., Pukkala, E., et al., 2008. *Chlamydia trachomatis* seroprevalence atlas of Finland 1983–2003. *Sex. Transm. Infect.* 84 (1), 19–22.
- Mabey, D.C., Solomon, A.W., Foster, A., 2003. Trachoma. *Lancet* 362 (9379), 223–229.
- Martin, D.L., Bid, R., Sandi, F., Goodhew, E.B., Massae, P.A., Lasway, A., et al., 2015a. Serology for trachoma surveillance after cessation of mass drug administration. *PLoS Negl. Trop. Dis.* 9 (2), e0003555.
- Martin, D.L., Wiegand, R., Goodhew, B., Lammie, P., Black, C.M., West, S., et al., 2015b. Serological measures of trachoma transmission intensity. *Sci. Rep.* 5, 18532.
- Mei, J.V., Alexander, J.R., Adam, B.W., Hannon, W.H., 2001. Use of filter paper for the collection and analysis of human whole blood specimens. *J. Nutr.* 131 (5), 1631S–1636S.
- Pant, B.P., Bhatta, R.C., Chaudhary, J.S., Awasthi, S., Mishra, S., Sharma, S., et al., 2016. Control of trachoma from Achham District, Nepal: a cross-sectional study from the Nepal National Trachoma Program. *PLoS Negl. Trop. Dis.* 10 (2), e0004462.
- Solomon, A., Kuper, H., Buchan, J., Mabey, D., Foster, A., 2006. Trachoma Control: A Guide for Program Managers. World Health Organization, Geneva.
- West, S.K., Munoz, B., Weaver, J., Mrango, Z., Dize, L., Gaydos, C., et al., 2016. Can we use antibodies to *Chlamydia trachomatis* as a surveillance tool for national trachoma control programs? Results from a district survey. *PLoS Negl. Trop. Dis.* 10 (1), e0004352.
- Wills, G.S., Horner, P.J., Reynolds, R., Johnson, A.M., Muir, D.A., Brown, D.W., et al., 2009. Pgp3 antibody enzyme-linked immunosorbent assay, a sensitive and specific assay for seroepidemiological analysis of *Chlamydia trachomatis* infection. *Clin. Vaccine Immunol.* 16 (6), 835–843.