Diagnostic Microbiology and Infectious Disease 93 (2019) 293–298

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Optimization of a rapid test for antibodies to the *Chlamydia trachomatis* antigen Pgp3

Sarah Gwyn [a], Harran Mkocha [b], Jessica Morgan Randall [a], Mabula Kasubi [c], Diana L. Martin [a,*]

[a] Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, USA
[b] Kongwa Trachoma Project, Kongwa, Tanzania
[c] Muhimbili University, Dar es Salaam, Tanzania

ARTICLE INFO

Article history:
Received 7 July 2018
Received in revised form 2 November 2018
Accepted 3 November 2018
Available online 10 November 2018

Keywords:
Trachoma
Pgp3
Lateral flow assay
Antibodies
Chlamydia trachomatis

ABSTRACT

Serological surveillance for trachoma could allow monitoring of transmission levels in areas that have achieved elimination targets. Platforms that allow testing in basic laboratories or testing of easy-to-manage samples such as dried blood spots would contribute to the feasibility of serologic testing. Blood from 506 1–12-year-olds in 2 villages in Kongwa district, Tanzania, was tested for antibodies against the antigen Pgp3. Whole blood, plasma, and dried blood spots (DBS) were tested in lab and field settings using a cassette-enclosed Pgp3 lateral flow assay (LFA-cassette) and a pared-back "dipstick" assay (LFA-dipstick). DBS were also tested with a bead-based multiplex assay (MBA). There was no significant difference in antibody positivity between the MBA and either LFA format (ranging from 42.5% to 48.4%). Interrater agreement between an expert rater and 3 different raters in field and lab settings was uniformly good, with Cohen's kappa >0.81 in all cases.

1. Introduction

Trachoma, resulting from repeated infection with the bacterium *Chlamydia trachomatis* (*Ct*), is the leading infectious cause of blindness. Trachoma is targeted for elimination as a public health problem by 2020 using the SAFE strategy: Surgery for trichiasis (lashes rubbing against the eye), Antibiotics to treat infection, Facial cleanliness to reduce the spread of infection, and Environmental improvement through provision of greater access to sanitation and water to disrupt transmission. District-wide distribution of azithromycin to affected populations is carried out through annual mass drug administration (MDA). Programmatic decision-making for stopping annual MDA is based on trachomatous inflammation—follicular (TF) prevalence in 1–9-year-olds. After the recommended number of annual rounds of MDA, an impact survey is conducted and MDA is stopped if the district-wide prevalence of TF is <5% in 1–9-year-olds. However, after multiple rounds of MDA, the correlation between TF and active ocular infection is less certain at both individual and population levels (Ramadhani et al., 2016).

A sensitive and specific measure of *Ct* transmission would benefit trachoma programs as they approach and reach elimination targets. Antibody responses to the *Ct* antigens Pgp3 and CT694 increase with age in trachoma-endemic settings, allowing estimates of seroconversion rates as a measure of transmission (Cama et al., 2017; Martin et al., 2015a, Pinsent et al., submitted for publication). Data on antibody responses have primarily been collected using bead-based assays (such as the multiplex bead assay [MBA]) (Goodhew et al., 2012; Gwyn et al., 2018; Martin et al., 2015a, 2015b; Sun et al., 2017) or enzyme-linked immunosorbent assay (ELISA) (Cama et al., 2017; Migchelsen et al., 2017a, 2017b). For reasons of throughput, cost, and ease of use, it would be valuable to be able to test for anti-*Ct* antibodies using a rapid test.

We previously adapted antibody tests for anti-*Ct* antigens to a field-friendly, rapid, lateral-flow-based assay (LFA) which was housed in a plastic cassette (Pgp3 LFA-cassette (Gwyn et al., 2016)). The Pgp3 LFA-cassette has high sensitivity and specificity compared to the Pgp3 MBA and Pgp3 ELISA based on latent class analysis (Wiegand et al., 2018), although the sensitivity of the LFA is lower with whole blood than with serum and inclusion of whole blood testing in the latent class model decreases the goodness of fit of the model (Wiegand et al., 2018). Whole blood samples were more likely to test negative than serum samples on the Pgp3 LFA-cassette in a separate study as well (Gwyn et al., 2016). However, the whole blood samples used in that study were artificially manufactured in the lab, and blood and serum tested by Pgp3 LFA-cassette have never been directly compared in the field. Additionally, early prototypes of the Pgp3 LFA-cassette worked with serum and whole blood samples but not dried blood spots (DBS), which are increasingly being used for field studies and program evaluations (Corran et al., 2008; Meesters and Hooff, 2013; Vazquez-Moron et al., 1858). Adapting the Pgp3 LFA-cassette to allow testing of DBS

* Corresponding author at: 1600 Clifton Rd NE, Atlanta GA 30329, 404-718-4147
E-mail address: hzx3@cdc.gov (D.L. Martin).

https://doi.org/10.1016/j.diagmicrobio.2018.11.001

would expand the sample sets available for analysis and allow programs to use stored samples from a wider variety of surveys to monitor transmission of ocular *Ct* after validation of the elimination of trachoma as a public health problem.

The first programmatic evaluation of the Pgp3 LFA-cassette showed excellent agreement between the LFA-cassette and MBA when measuring district-level seroprevalence (1.5% versus 1.6% seropositive, respectively (Sun et al., 2017)) but poor agreement at the individual level. While the poor individual-level agreement between tests in low-prevalence settings (where the percent antibody-positive falls within the range of specificity of the assay) may not be important for programmatic use, the collective data from these studies suggested that further field testing and optimization of the Pgp3 LFA were desired.

In the current study, we evaluated the performance of Pgp3 LFA-cassette in the field using blood and plasma. We also evaluated an LFA that had been adapted to an open-system "dipstick" format (LFA-dipstick) for testing DBS in the lab at CDC compared the Pgp3 LFA-cassette. Finally, the results from all LFA testing—field with blood and plasma, and lab with DBS on cassette and dipstick LFAs—were compared to results obtained using the MBA.

2. Methods

2.1. Study population

The study was conducted in 2 villages in the Kongwa district of the Dodoma Region of Tanzania in September 2016. A simple random sample of 506 1–9-year-olds was generated based on a previous census of the 2 communities: the population of Iduo was 1353 (September 2015 census) and that of Chilinjilizi was 1345 (August 2014 census). A total of 506 children were enrolled, and the self-reported age in the study was 1 to 12.

2.2. Ethics statement

Parental permission was obtained for all enrolled children, and oral assent was obtained for ≥6-year-olds. Ethical approval for the study was granted by the Tanzanian National Institute of Medical Research. CDC investigators were not considered to be engaged in human subjects research and did not have interactions with study participants or access to identifying information.

2.3. TF grading

Each child was evaluated for TF by trained staff from the Kongwa Trachoma Project (KTP) using the WHO simplified grading scheme (Thylefors et al., 1987). Data were recorded into mobile devices using the LINKS system (Pavluck et al., 2014).

2.4. Ocular swab collection

Polyester-tipped swabs were passed 3 times (with a 120°-turn between each pass) over the right conjunctiva. The examiner and specimen manager took precautions to avoid cross-contamination between participants or swabs in the field. Control air swabs were obtained every 50th child by passing the swab approximately 4 inches in front of the eye.

2.5. Ocular swab testing for Ct infection

Dry ocular swabs were rehydrated with 1–1.2 mL of sterile molecular-grade diethylpirocarbonate water (Quality Biological Inc., Gaithersburg, MD) 20 min prior to testing. Pools were created prior to running by combining 200 μL from each of 4 samples and adding 3 mL of collection medium. Each pool was vortexed for 30 s, and then 1 mL was added to the GeneXpert CT/NG cartridge (Cepheid Inc., Sunnyvale, CA Version 1.0 cartridge). The cartridge was loaded onto the GeneXpert module and analyzed using the GeneXpert CT/NG Assay Version 1.0 software. Results were reported by the computer as positive or negative for *Ct* DNA or indeterminate (invalid, error, or no result). If the initial GeneXpert result was indeterminate, the specimen was retested 1 time using a new aliquot of specimen, if available, and a new GeneXpert cartridge. If the pool tested positive, the individual samples comprising the pools were tested individually to determine the individual positive specimen within those pools. If the pool tested negative, then all the samples that made up the pool were presumed negative.

2.6. Blood collection

Fingerpick blood was collected into EDTA-coated microtubes (Ram Scientific, Nashville, TN) and onto filter paper containing 6 circular extensions calibrated to absorb 10 μL of whole blood (TropBio Pty Ltd., Townsville, Queensland, Australia). Plasma was separated from red blood cells by letting whole blood sit at 4 °C for 4–24 h and then removing the top plasma layer.

2.7. Pgp3 lateral flow assay

2.7.1. Manufacturing

Pgp3 LFAs in cassettes for the previously published LFA-cassette were manufactured at CDC as previously described (Gwyn et al., 2016). LFA-dipstick tests were manufactured at CDC as follows: Pgp3 protein (1.5 mg/mL) and biotinylated bovine serum albumin (BSA-biotin, 1.5 mg/mL; Arista Biologicals, Allentown, PA) were dispensed onto a nitrocellulose membrane (Millipore, Billerica, MA) at a rate of 0.1 μL/mm using an IsoFlow Dispenser (Imagene Technology, Hannover, NH). An absorbent pad (Arista Biologicals) and the nitrocellulose membrane were placed on a backing card (DCN Diagnostics, Carlsbad, CA) with a 1–2-mm overlap to facilitate sample flow. LFA dipsticks (4-mm strips) were produced using a guillotine cutter and stored at room temperature in sealed foil pouches with desiccant prior to use.

2.7.2. Training of field staff to read LFA

All raters were trained in reading LFA results and completed competency testing. A panel of 30 serum samples was run on the LFA and read as positive, negative, or invalid by the trainer and the raters. Trainees were expected to have achieved a Cohen's kappa of ≥0.80 to participate in the survey.

2.7.3. Field LFA testing using blood and plasma

Fingerprick blood was collected into tubes and transported on cold packs to KTP, where (1) blood was tested on LFA-cassettes and (2) plasma was isolated by allowing blood cells to settle and then taking the top layer to test on LFA-cassettes as previously described (Gwyn et al., 2016). Briefly, whole blood (20 μL) or plasma (10 μL) was added to the sample applicator port and chased with 200 μL phosphate-buffered saline (PBS) containing 0.3% Tween 20 (PBST). After 30 min, results were read and recorded as positive, negative, or invalid (if no control line was present). All LFAs were read by rater 1 (expert rater), and a subset was read by 2 other raters (rater 2 and rater 3) based on their availability.

2.7.4. Laboratory LFA-cassette and LFA-dipstick testing, DBS

For LFA-cassette testing, 1 DBS extension was eluted in 60 μL of PBST at 4 °C for 4–24 h. The entire DBS eluate was added to the sample applicator port on the LFA-cassette and chased with 200 μL PBST. Results were read and recorded as positive, negative, or invalid after 30 min. For LFA-dipstick testing, 1 DBS extension was eluted in 60 μL of 0.5% BSA, 0.3% Tween, and 0.02% sodium azide in 1× PBS (LFA buffer) for 4–24 h at 4 °C in a flat-bottom 96-well plate (USA Scientific, Ocala, FL). The conjugate mastermix was prepared at a volume proportional to the number of wells to test, and 20 μL of conjugate mixture (1 μL

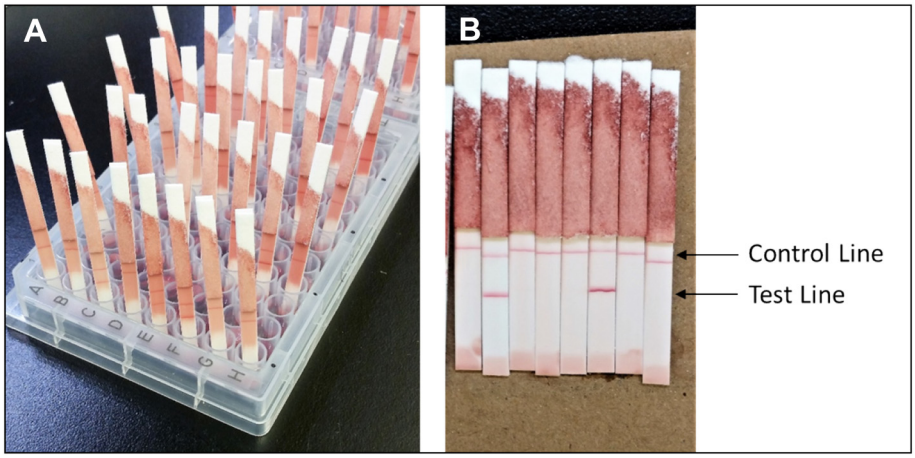

Fig. 1. Pgp3 LFA-dipstick test. DBS were eluted in 96-well flat-bottom plates, and conjugate was added to wells. After approximately 4 h, the LFA-dipstick was inserted into the well for 15–20 min, and then LFA buffer was added (A). LFAs were read when the membrane was completely cleared of background staining (B).

Pgp3-gold + 1 μL SA-gold + 18 μL LFA buffer) was added to each well no more than 4 h prior to testing. LFA-dipsticks were inserted into each well until the DBS eluate–conjugate mixture was absorbed, which took approximately 15–20 min. Then, 40 μL of LFA buffer was added to each well to remove the background caused by hemolyzed red blood cells. LFAs were read as positive, negative, or invalid after the membrane was completely cleared (5–10 min). The LFA-dipstick procedure is pictured in Fig. 1. Only 458 LFA-cassettes were read by rater 1, as the remaining were inadvertently discarded too soon—and there were no more DBS extensions available for testing.

2.8. Multiplex bead assay

One DBS extension was eluted overnight at 4 °C in 1.6 mL of PBS containing 0.5% casein, 0.3% Tween 20, 0.5% polyvinyl alcohol, 0.8% polyvinylpyrrolidone, 0.02% sodium azide, and 3 μg/mL *E. coli* extract (Buffer B). DBS eluates were screened in duplicate with Pgp3-coupled beads on the MBA as previously described (Goodhew et al., 2012). Briefly, beads (approximately 2500 per antigen) were added to each well of a 96-well filter plate (Millipore, Billerica, MA) and washed with 0.05% tween in PBS (PBST2). DBS eluates were added at 40 μL per well and incubated with antigen-coupled beads for 1.5 h. Wells were washed and incubated with 50 ng biotinylated mouse anti-human IgG (SouthernBiotech, Birmingham, AL) and 20 ng biotinylated mouse anti-human IgG4 (SouthernBioTech) for 45 min to detect bound IgG. Wells were washed 3 times with PBST2 and incubated with 250 ng phycoerythrin-labeled streptavidin (Invitrogen, South San Francisco, CA) for 30 min. After 3 washes with PBST2, wells were incubated for 30 min with 0.5% BSA, 0.05% Tween 20, and 0.02% sodium azide in PBS to remove any nonspecific binding. After 1 wash with PBST2, wells were suspended in 125 μL of PBS and read on a Bio-Plex 200 instrument (Bio-Rad, Hercules, CA) equipped with Bio-Plex manager 6.0 software (Bio-Rad). The median fluorescence intensity (MFI) with the background from the blank well (Buffer B alone) subtracted out (MFI-bg) was recorded for each antigen for each sample. The cutoff for positivity was established as an MFI-bg

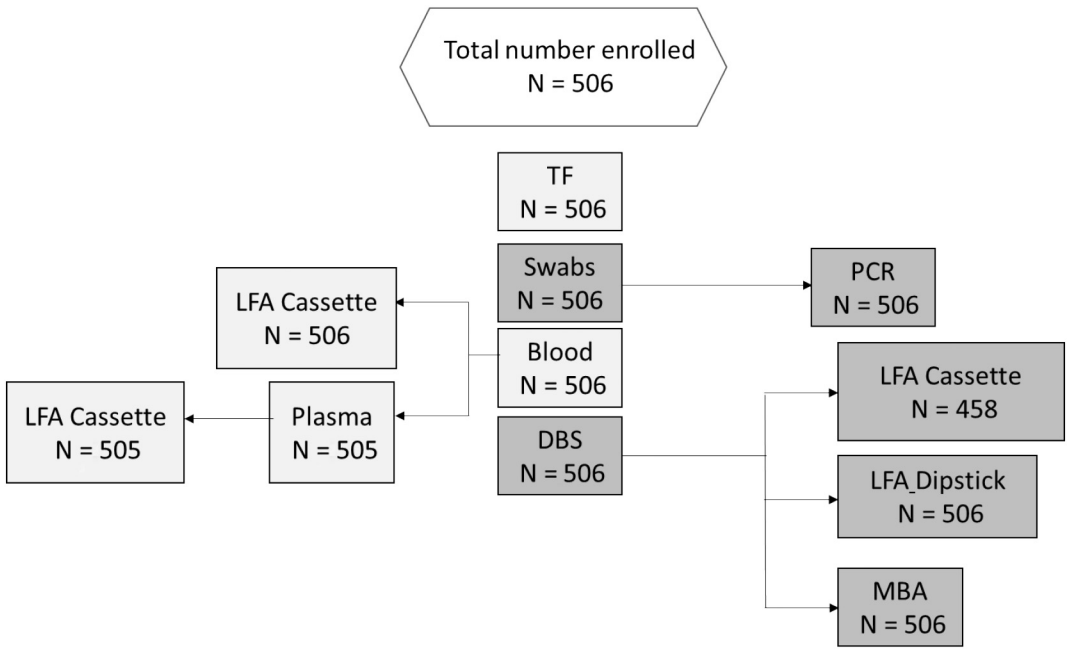

Fig. 2. Sample collection and testing flowchart. The number of samples collected and run for each evaluation is shown. TF = trachomatous inflammation—follicular. PCR = polymerase chain reaction. LFA = Pgp3 lateral flow assay. DBS = dried blood spots. MBA = multiplex bead assay. Lightly shaded boxes represent data collected at the field site and KTP; darkly shaded boxes represent data analyzed in laboratories at Muhimbili University (PCR) or CDC (DBS antibody).

Table 1
Percent of samples testing positive for a given indicator.

				LFA-cassette			LFA-dipstick
	TF	PCR	MBA	Blood field	Plasma field	DBS lab	DBS lab
Percent positive	2.8%	7.5%	45.5%	42.5%	48.4%	44.1%	44.3%
Sensitivity	23.7%	N.A.	94.7%	84.2%	94.7%	97.3%	94.7%

TF = trachomatous inflammation—follicular; DBS = dried blood spot; PCR = polymerase chain reaction; MBA = multiplex bead assay; LFA = lateral flow assay. NA = not applicable.

of 1090 by using receiver operator characteristic curve analysis on a panel of 83 samples previously classified by MBA as positive or negative.

2.9. Statistical analysis

The percent positive for all lateral flow assay testing was determined as the number of tests read as positive by rater 1 divided by the total number of valid tests. Agreement among readers was compared between an expert rater (rater 1) and 3 raters who were trained during the study. Percent agreement for all lateral flow assay testing was calculated as the number of tests given the same rating by rater 1 divided by the total number of valid tests. For comparisons between LFA and MBA test outcomes, the results from the rater 1 were used. Interrater agreement as measured by Cohen's kappa (κ) and 95% confidence intervals (CIs) were calculated using GraphPad Prism. Sensitivity was determined as the number of PCR-positive samples positive by each test divided by the total number of samples testing positive by PCR. The complete dataset used for analysis is provided in Supplemental Fig. 1.

3. Results

3.1. Training and competency testing for LFA

Interrater agreement between each rater and the expert rater was $\kappa = 0.81$ or above on a 30-panel competency set. Raters 2 and 3 had interrater agreements (κ) of 0.87 and 0.80, respectively, with rater 1 during competency testing using LFA-cassettes. For laboratory-based DBS testing, rater 4 had an interrater agreement of $\kappa = 0.87$ with rater 1 during competency testing using LFA-dipstick.

3.2. Demographic, TF, and PCR data

A total of 506 1–12-year-olds were enrolled in the study. A flowchart of the data collected for each sample type and test is presented in Fig. 2. Of 506 enrolled individuals, 302 were from village 1, 204 were from village 2, and 247 (48.8%) were male. The percentage of children with TF was 2.8 [95% CI 1.6–4.7] (Table 1). Of 506 children tested by PCR, 2 samples (0.4%) had invalid results and were excluded from further analysis, and 38 (7.5%, 95% CI: 5.4–10.2) tested positive.

3.3. Field evaluation of LFA-cassette using whole blood

Out of 506 blood samples tested by LFA-cassette at KTP, 2 (0.4%) were invalid and excluded from further analysis. The percent positive was 42.5% (95% CI 38.2–46.8) (Table 1). The sensitivity was 84.2% (95% CI 68.8–94.0) (Table 1). Interrater agreement between rater 1 and rater 2 was $\kappa = 0.92$ (95% CI 0.89–0.96, $N = 495$). Interrater agreement between rater 1 and rater 3 was $\kappa = 0.80$ (0.74–0.86, $N = 349$).

3.4. Field evaluation of LFA-cassette using plasma

Of 505 plasma samples tested by LFA-cassette at KTP (1 sample had insufficient blood collected to obtain plasma), 1 (0.2%) was invalid and excluded from further analysis. The percent positive was 48.4% (95% CI 44.0–52.9) (Table 1), and the sensitivity was 94.7% (95% CI 82.3–99.4) (Table 1). Interrater agreement between rater 1 and rater 2 was $\kappa = 0.92$ (95% CI 0.83–0.96, $N = 336$). Interrater agreement between rater 1 and rater 3 was $\kappa = 0.88$ (95% CI 0.83–0.94, $N = 294$).

The percent agreement between LFAs run with blood and plasma was 91.2%.

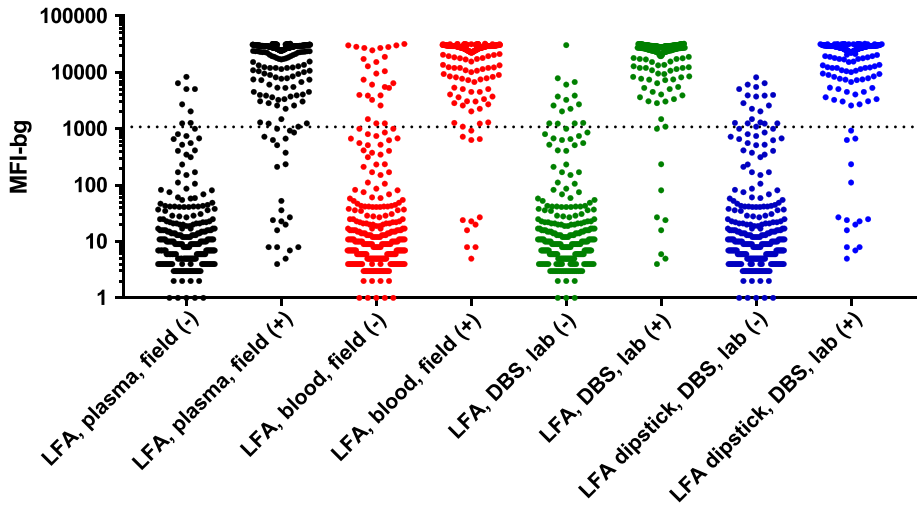

Fig. 3. Intensity of anti-Pgp3 antibody responses by MBA compared to LFA outcome. *X*-axis shows the LFA testing type (LFA-cassette or LFA-dipstick), type of sample used (plasma, blood, or DBS), and site of testing, (field or lab). *Y*-axis shows median fluorescence intensity with background subtracted out (MFI-bg) shown on a log scale. (−) = negative by LFA. (+) = positive by LFA. DBS = dried blood spot. LFA = lateral flow assay. MBA = multiplex bead assay. Horizontal dotted line = MBA cutoff.

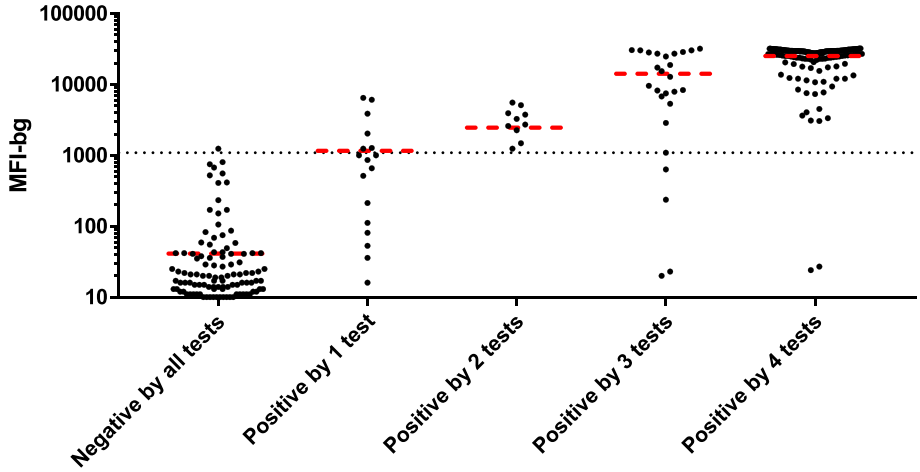

Fig. 4. Intensity of antibody responses to Ppg3 by MBA stratified by the number of positive LFA tests. The median fluorescence intensity with background subtracted out (MFI-bg) is shown on the *Y*-axis. The *X*-axis shows the number of LFA tests (i.e., different iterations of test and sample type) the indicated sample was positive by. Each dot represents an individual sample. Dashed red lines represent the mean of each group. Dotted horizontal line represents the MBA cutoff value. *P* values for each comparison of MFI-bg values are <0.01, except for the comparison of MFI-bg values between the groups "positive by 1 test" and "positive by 2 tests."

3.5. Lab evaluation of LFA using DBS

Of 458 and 506 DBS tested by LFA-cassette and LFA-dipstick in the lab, 7 (1.53%) and 3 (0.59%), respectively, were deemed invalid and excluded from further analysis. The percent agreement between LFA-cassette and LFA-dipstick was 93.8%. The percent positive was 44.1% (95% CI 39.4–48.8) for LFA-cassette and 44.3% (95% CI 39.9–48.8) for LFA-dipstick (Table 1). The sensitivity was 97.3% (95% CI 85.8–99.9) for LFA-cassette and 94.7% (95%CI 82.3–99.4) for LFA-dipstick. The interrater agreement between rater 1 and rater 4 was $\kappa = 0.88$ (0.84–0.93) for LFA-cassette ($N = 357$) and $\kappa = 0.95$ (0.92–0.98) for LFA-dipstick ($N = 506$).

3.6. Comparison of MBA and LFA testing

The percent agreement between the MBA and each LFA format and sample type ranged from 92.3 to 94.7%. The percent positive by MBA was 45.5% (95 CI 41.1–49.9), and the sensitivity was 94.7% (95% CI 82.3–99.4) (Table 1). MFI-bg values tended to be lower for samples testing negative by LFA than samples testing positive by LFA (Fig. 3). Unlike what was seen with plasma or DBS, many blood samples ($N = 13$) testing negative by LFA in the field had an MFI-bg value of above 10,000 (Fig. 3). MFI-bg values tended to be higher for samples positive by all 4 LFA tests than for samples positive by 0, 1, 2, or 3 LFA tests (Fig. 4).

4. Discussion

The data presented here show excellent agreement between 2 iterations of a lateral flow test to measure antibodies against the *Ct* antigen Pgp3 on various sample types (whole blood, plasma, and dried blood spots) and excellent agreement between each of these tests with antibody data obtained using MBA analysis. These data suggest that these are robust assays that will yield equivalent seroprevalence data regardless of sample type or testing type, and may offer a flexible option for using should serology be adopted as a surveillance platform for trachoma programs.

Overall, the performance of the LFA when using blood was less robust than with other sample types. The percent positive was slightly higher for plasma than blood, but these percentages had overlapping CIs. While the interrater agreements were good for all tests, with Cohen's kappa consistently >0.81, the field teams reported that the tests run with plasma were subjectively easier to read. As an example, the interrater agreement between rater 1 (the expert rater) and rater 3 was higher with plasma compared to blood. Additionally, the whole blood tests detected fewer PCR-positives than the other sample types. These data support previous observations that Pgp3 LFA testing using whole blood showed lower agreement with other tests than when using plasma (Gwyn et al., 2016, 2017; Wiegand et al., 2018). While in theory decreased performance of the test when using blood as the sample is not promising for a rapid test, in this instance, it may not be critical for the assay. The usage for this assay would not be "test and treat" as a true point-of-care test would be. Since the anti-*Ct* antibody response is measuring exposure, not a current disease or infection, a positive antibody test does not necessitate treatment. The study is limited by not having the full complement of LFA tests read by all 3 raters in the field at KTP and by both raters in the lab. At KTP, the blood was processed at the end of the day's field collection, and since this went into the night, the field staff occasionally had to leave before all samples were processed. At the time of the field work, the test had not been optimized for DBS, so we tested blood and serum the same day as collection. In the lab, there were not always DBS left to do all tests, which is why some of those comparisons are missing. However, the number of samples tested for interrater agreement is sufficient for obtaining a valid κ score.

Operationally, there are other benefits to having a laboratory-based rather than a field-based rapid antibody test. With a 30-min read time, running this test in the field may slow down field teams or provide logistical challenges for teams moving from house to house. The primary objective for this test is to be fast, low cost, and easy to use, allowing national programs flexibility to conduct postvalidation surveillance for trachoma—not to be a point-of-care test. The optimization of the test shown here would allow the test to be used on DBS collected for other purposes, saving on the cost of having to execute a trachoma-specific survey for surveillance.

One limitation of this work is that we do not have a true gold standard for antibody positivity, and so we have been reporting sensitivity as the percent of samples from individuals PCR-positive ocular swabs who also test positive for antibody. This approach is less than ideal but has been used to determine sensitivity for other antibody tests for urogenital chlamydia (Horner et al., 2013; Wills et al., 2009). Blood specimens from children with ocular *Ct* infection tend to have very high levels of antibody (Goodhew et al., 2012, 2014), so this metric does not tell us about the test performance with lower antibody titers (i.e., the limit of detection of the assay), the scenario we would anticipate in low-prevalence or postendemic settings. Additionally, there may be PCR-positive samples from a very recent infection for which an individual may not have yet developed an antibody response. A standard control human anti-Pgp3 monoclonal antibody would be valuable for obtaining better estimates of the sensitivity of these tests.

While there is no gold standard assay for measuring anti-Pgp3 antibodies, trachoma serology work was piloted on and has primarily utilized multiplex bead assay, which is a robust platform with excellent repeatability (Gwyn et al., manuscript submitted, Kaur et al., 2018). Preliminary work has shown good agreement between MBA and LFA (Gwyn et al., 2017; Wiegand et al., 2018). The current study confirms some of these initial findings of high levels of agreement between MBA and LFA and extends these observations to other sample types (DBS) and an alternative iteration of the LFA (LFA-dipstick), which is cheaper, is faster to manufacture, and uses less plastic than the LFA-cassette. The samples that tested LFA negative/MBA positive usually had lower MFI-bg values (below 10,000) for all sample types except the LFA/blood/field. MFI-bg values increase proportionally with the number of positive LFA tests, which may indicate that discordant results between LFA types are due to "low-positive" samples, i.e., those with lower concentrations of antibody, giving very faint test lines that may sometimes be missed when reading the LFA. One explanation for why some samples test negative by MBA but positive by LFA is that the LFA is detecting total immunoglobulin (including IgA), whereas the MBA protocol used specifically detects IgG.

Despite the minor differences in individual tests run, there were no significant differences in the percentage of positive samples as determined by MBA or any LFA tests. This concurs with population-level comparisons of MBA and LFA seen in postelimination settings (Sun et al., 2017). The data suggest that the LFA, in any number of iterations, and MBA could potentially be used interchangeably in a population-level, programmatic setting based on the program needs.

Acknowledgments

The authors would like to thank the KTP field teams and the community members of Kongwa district who participated in the study.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Funding

This work was supported by a grant to the Neglected Tropical Diseases Support Center (NTD-SC) by the United States Agency for International Development (USAID) and through an interagency agreement with CDC.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2018.11.001.

References

Cama A, Muller A, Taoaba R, Butcher RMR, Itibita I, Migchelsen SJ, et al. Prevalence of signs of trachoma, ocular Chlamydia trachomatis infection and antibodies to Pgp3 in residents of Kiritimati Island, Kiribati. PLoS Negl Trop Dis 2017;11(9), e0005863.

Corran PH, Cook J, Lynch C, Leendertse H, Manjurano A, Griffin J, et al. Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. Malar J 2008;7:195.

Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis 2012;6(11), e1873.

Goodhew EB, Morgan SM, Switzer AJ, Munoz B, Dize L, Gaydos C, et al. Longitudinal analysis of antibody responses to trachoma antigens before and after mass drug administration. BMC Infect Dis 2014;14:216.

Gwyn S, Mitchell A, Dean D, Mkocha H, Handali S, Martin DL. Lateral flow-based antibody testing for Chlamydia trachomatis. J Immunol Methods 2016;435:27–31.

Gwyn S, Cooley G, Goodhew B, Kohlhoff S, Banniettis N, Wiegand R, et al. Comparison of platforms for testing antibody responses against the Chlamydia trachomatis antigen Pgp3. Am J Trop Med Hyg 2017;97(6):1662–8.

Gwyn SE, Xiang L, Kandel RP, Dean D, Gambhir M, Martin DL. Prevalence of Chlamydia trachomatis-specific antibodies before and after mass drug administration for trachoma in community-wide surveys of four communities in Nepal. Am J Trop Med Hyg 2018;98(1):216–20.

Horner PJ, Wills GS, Reynolds R, Johnson AM, Muir DA, Winston A, et al. Effect of time since exposure to Chlamydia trachomatis on chlamydia antibody detection in women: a cross-sectional study. Sex Transm Infect 2013;89(5):398–403.

Kaur H, Dize L, Munoz B, Gaydos C, West SK. Evaluation of the reproducibility of a serological test for antibodies to Chlamydia trachomatis pgp3: a potential surveillance tool for trachoma programs. J Microbiol Methods 2018;147:56–8.

Martin DL, Wiegand R, Goodhew B, Lammie P, Black CM, West S, et al. Serological measures of trachoma transmission intensity. Sci Rep 2015a;5, 18532.

Martin DL, Bid R, Sandi F, Goodhew EB, Massae PA, Lasway A, et al. Serology for trachoma surveillance after cessation of mass drug administration. PLoS Negl Trop Dis 2015b;9(2), e0003555.

Meesters RJ, Hooff GP. State-of-the-art dried blood spot analysis: an overview of recent advances and future trends. Bioanalysis 2013;5(17):2187–208.

Migchelsen SJ, Martin DL, Southisombath K, Turyaguma P, Heggen A, Rubangakene PP, et al. Defining seropositivity thresholds for use in trachoma elimination studies. PLoS Negl Trop Dis 2017a;11(1), e0005230.

Migchelsen SJ, Sepulveda N, Martin DL, Cooley G, Gwyn S, Pickering H, et al. Serology reflects a decline in the prevalence of trachoma in two regions of The Gambia. Sci Rep 2017b;7(1), 15040.

Pavluck A, Chu B, Mann Flueckiger R, Ottesen E. Electronic data capture tools for global health programs: evolution of LINKS, an Android-, web-based system. PLoS Negl Trop Dis 2014;8(4), e2654.

Ramadhani AM, Derrick T, Macleod D, Holland MJ, Burton MJ. The relationship between active trachoma and ocular Chlamydia trachomatis infection before and after mass antibiotic treatment. PLoS Negl Trop Dis 2016;10(10), e0005080.

Sun MJ, Zambrano AI, Dize L, Munoz B, Gwyn S, Mishra S, et al. Evaluation of a field test for antibodies against Chlamydia trachomatis during trachoma surveillance in Nepal. Diagn Microbiol Infect Dis 2017;88(1):3–6.

Thylefors B, Dawson CR, Jones BR, West SK, Taylor HR. A simple system for the assessment of trachoma and its complications. Bull World Health Organ 1987;65(4):477–83.

Vazquez-Moron S, Ryan P, Ardizone-Jimenez B, Martin D, Troya J, Cuevas G, et al. Evaluation of dried blood spot samples for screening of hepatitis C and human immunodeficiency virus in a real-world setting. Sci Rep 2018;8(1):1858.

Wiegand RE, Cooley G, Goodhew B, Banniettis N, Kohlhoff S, Gwyn S, et al. Latent class modeling to compare testing platforms for detection of antibodies against the Chlamydia trachomatis antigen Pgp3. Sci Rep 2018;8(1):4232.

Wills GS, Horner PJ, Reynolds R, Johnson AM, Muir DA, Brown DW, et al. Pgp3 antibody enzyme-linked immunosorbent assay, a sensitive and specific assay for seroepidemiological analysis of Chlamydia trachomatis infection. Clin Vaccine Immunol 2009;16(6):835–43.