TRACHOMA: Analysis of diagnostic gaps and a proposed path forward
This report was written by PATH and supported in whole or part by a grant from the Bill & Melinda Gates Foundation. The views expressed herein are solely those of the authors and do not necessarily reflect the views of the Foundation.

Suggested citation

PATH. TRACHOMA: Analysis of diagnostic gaps and a proposed path forward. Seattle: PATH; 2015.

Contact information

Tala de los Santos
Director, Diagnostics
PATH
Email: tdelossantos@path.org

PATH is the leader in global health innovation. An international nonprofit organization, we save lives and improve health, especially among women and children. We accelerate innovation across five platforms—vaccines, drugs, diagnostics, devices, and system and service innovations—that harness our entrepreneurial insight, scientific and public health expertise, and passion for health equity. By mobilizing partners around the world, we take innovation to scale, working alongside countries primarily in Africa and Asia to tackle their greatest health needs. Together, we deliver measurable results that disrupt the cycle of poor health.

For more information on PATH’s work in diagnostic technologies, visit: [http://sites.path.org/dx/](http://sites.path.org/dx/)

Copyright © 2015, PATH. All rights reserved. The material in this document may be freely used for educational or noncommercial purposes, provided that the material is accompanied by an acknowledgment.

Cover photo: Orbis UK/Raul Vasquez
# Table of Contents

1. Executive summary .................................................................................................................. 4
2. Disease overview ...................................................................................................................... 5
3. Research gaps .......................................................................................................................... 7
4. Risks and mitigation .................................................................................................................. 8
5. Diagnostic landscape .............................................................................................................. 11
6. Use case statement ................................................................................................................. 13
7. Conclusion .............................................................................................................................. 14
8. References .............................................................................................................................. 16
   Appendices .............................................................................................................................. 20
1. Executive summary

A key component of the global effort to eliminate trachoma has been reducing transmission using mass drug administration (MDA) with azithromycin, made possible by the large-scale drug donation by Pfizer. Currently, clinical examination to assess the prevalence of disease indicators is the sole method used to inform control program decisions regarding MDA. However, there has been increasing recognition within the international trachoma community of the need for improved diagnostic tools to support control program decisions given the limitations of using clinical disease indicators to accurately inform late-stage decisions, such as when to stop MDA.

For the Endgame Portfolio project, PATH is seeking to assess diagnostic needs, landscape potential solutions, and determine an appropriate strategy to support improvement of diagnostic testing in support of trachoma elimination efforts. Based upon previous input from experts in the trachoma community, including input obtained during the Diagnostics Working Group convened by the Bill & Melinda Gates Foundation in London in 2013, two diagnostic use cases for trachoma were prioritized for further exploration—informing MDA stopping decisions and post-MDA surveillance. To this end, PATH has refined Target Product Profiles (TPPs) for trachoma diagnostics that are applicable to these use cases. However, while candidate biomarkers and current prioritized diagnostics have been identified in this report, a decision to move forward with major investments to develop new diagnostic products for trachoma may not yet be warranted due to:

- Uncertainty of the timeline and support of key stakeholders needed for the amendment of World Health Organization (WHO) guidelines that will be required for the use of new diagnostics;
- The need for further delineation on how new measures and diagnostics based on infection will be shaped into guidelines to effectively inform control program decisions regarding MDA;
- The current availability of diagnostic solutions including commercial products that are likely to meet immediate needs and are prioritized for evaluation and implementation by key stakeholders (i.e., lab-based nucleic acid amplification testing (NAAT) platforms);
- The early stage of research on new diagnostic biomarkers assessing antibody responses.

Thus, in order to address urgent needs for new diagnostic tools to impact elimination efforts across Endgame portfolio diseases, it is our opinion that the initiation of product development efforts for new trachoma diagnostics are of lower priority at this time compared to soil-transmitted helminths and schistosomiasis. In the meantime, PATH will continue to maintain engagement with trachoma stakeholders to assess and support emerging needs related to diagnostics including later product development efforts, if warranted.
2. Disease overview

Trachoma is the leading preventable cause of blindness in the world. An estimated 7.2 million people are already suffering from the disease while as many as 320 million are at risk for infection—primarily concentrated in populations living in impoverished areas of the world where conditions promote the spread of infection [1-3]. Trachoma is a painful and debilitating disease where inflammation in response to repeated infections with the disease-causing bacteria, *Chlamydia trachomatis*, can lead to progressive damage from active trachoma to scarring of the eyelid, trachomatous trichiasis (TT), corneal opacity, and ultimately blindness. In response to this global epidemic, the WHO Global Alliance for the Elimination of Trachoma set a goal to eliminate trachoma as a public health problem by the year 2020. To accomplish this goal, the alliance works to mobilize resources to support the Surgery, Antibiotics, Facial cleanliness and Environmental improvement (SAFE) strategy. A key element of SAFE is reducing transmission and the risk of trachoma disease through the use of MDA campaigns, enabled through the global donation by Pfizer of more than 340 million doses of the antibiotic azithromycin (Zithromax®) to date, distributed through the International Trachoma Initiative[2-5].

Accurate surveillance to inform decisions by trachoma control programs remains critical to the success of the interventions outlined in the SAFE strategy, in particular MDA. Currently, clinical examination of the eye for signs of disease using the WHO simplified grading system is the only method approved for use by national trachoma control programs. Decisions on whether or not to start or stop MDA are made based on the prevalence of the clinical signs for trachomatous inflammation-follicular (TF) in children 1 to 9 years old [6]. If the prevalence of TF in this group is above 10%, MDA is initiated (or re-started, if previously stopped). Once prevalence falls below 10%, more targeted interventions (including MDA) and surveillance may be considered at the discretion of the program. Finally, a TF threshold of less than 5% signals MDA should be stopped and post-MDA surveillance should be initiated. Elimination may eventually be considered if TF stays below 5% in children 1 to 9 years old and the burden of severe disease in the adult population is reduced to a maximum of 1/1000 as measured by the prevalence of TT [1, 3, 6].

Clinical grading is not without its problems. It can be somewhat subjective; is prone to false positives (in relation to ocular *Chlamydia trachomatis* infection), particularly after interventions have begun; and misses some cases of subclinical infection. Additionally, MDA is intended to treat the reservoir of active infection in populations in order to reduce further transmission. As trachoma prevalence becomes low, which may occur after multiple rounds of MDA, the correlation between clinical signs and active infection becomes weaker [7-11]. Research studies have shown disease measures do not always correlate to active infection as determined by sensitive molecular assays for *C. trachomatis*. Results of previous studies have demonstrated that only 18% to 40% of individuals with less severe active disease (defined by TF) were positive by NAAT while 50% to 70% of those with severe inflammation (defined by TI) were positive[12]. Additionally, clinical signs of trachoma can lag long after infection has cleared and DNA is undetectable[10]. Thus, clinical signs may lead to an over- or underestimation of trachoma prevalence and ultimately prolong or prematurely halt MDA. Given these limitations and the current availability of high
performance laboratory tests to directly measure infection, there has been growing debate on the need to use improved methods to monitor the impact of MDA, particularly during the final stages of elimination when prevalence is low and key decisions to halt MDA are considered[7-9, 13-16].

The goal of this report is to identify candidate diagnostic options for trachoma, determine what gaps may exist, and make recommendations for next steps related to product development of new diagnostics for trachoma. The following considerations were important in this diagnostic landscape evaluation:

- **Intended use is for population-based surveillance.** For trachoma surveillance, diagnostic tools will be used primarily to inform decisions by control programs on whether to treat entire populations with MDA and not for individual diagnosis and patient management. Thus, operational requirements such as the ability to perform the test at the point of care (POC) and provide a rapid result may take on lesser importance IF specimens can be transported effectively to allow for testing within an external location. Conversely, the capacity to easily batch test large numbers of samples collected during surveys may increase the value of a specific option [17-19].

- **Targeted use cases.** Clinical examination and monitoring of disease indicators is considered by stakeholders to be adequate for monitoring trachoma when prevalence is high, such as when determining baseline prevalence (mapping) or assessing effects of early rounds of MDA in reducing burden (impact monitoring). Thus, the targeted use cases for new diagnostic tools do not prioritize the early stages of trachoma control, but rather informing late-stage decisions on when to stop MDA and then monitor for possible re-emergence after MDA has been halted. [17] Additionally, it is likely that the measure (infection or exposure) used in tools for informing both these late-stage use cases will need to be the same or extremely well-correlated since data will be utilized longitudinally in these stages.

- **New diagnostic tools for trachoma must be able to offer substantial improvement over current methods.** If new diagnostic tools do not provide substantial benefit over clinical examination for informing decisions by control programs, justifying their approval by WHO and use by control programs for surveillance will be difficult. Ideally, a new diagnostic test will offer improvement in terms of accuracy and operational characteristics to justify the increased cost versus relatively inexpensive clinical exams. Molecular tests for detection of trachoma infection remain a prioritized option due to their superior analytical performance as demonstrated through prior research. As operationalizing and implementing current NAAT options still raises legitimate concern, alternative platforms with lower cost and complexity, such as immunoassays for antigen or antibody detection, may still warrant further consideration. However, many older, outdated methodologies and technologies that have been used in prior trachoma research, such as those based on classic microbiologic methodology, were beyond the scope of this landscape report and were omitted in detailed analysis. Their cost, complexity, and, in many cases, inferior performance compared to newer tests make them unlikely to be viable solutions for late-stage uses. A comprehensive review of these methods is also already publically available [18].
3. Research gaps

A change from using the WHO simplified clinical grading method for assessment of disease indicators to the use of direct or indirect measures of infection has been the topic of growing debate within the international trachoma community. While there is general consensus on the superior performance of NAATs over clinical examination for assessing infection, it is not yet clear how measures of infection will be used to accurately inform population-based decisions in all endemic settings. Ultimately, decisions based on new indicators will need to ensure the risk of continued transmission, re-emergence, and/or continued disease burden is minimal prior to a decision to halt MDA. Results using infection markers have demonstrated great potential to improve assessments of the impact of MDA in many other research studies either prior to or after multiple rounds of MDA [5, 8, 14-16, 18, 20, 21]. However, the complexity of using infection indicators for informing MDA decisions in all endemic settings (and why concern remains among key stakeholders) is exemplified by results from recent studies in hyperendemic regions of Ethiopia. Studies that examined both clinical and infection indicators, as measured by sensitive NAATs, after multiple rounds of MDA in Ethiopia showed infection prevalence had been successfully reduced to 0% or very low levels in treated districts even though the prevalence of clinical disease continued be well above a prevalence threshold of the 10% needed to alter MDA strategies. However, once MDA was discontinued based on the absence of infection, both infection and clinical disease prevalence increased in previously-treated districts, including some reaching near pre-MDA levels after two years [9, 22]. This result exemplifies why research may be needed to further delineate the correlation of infection indicators in different endemic settings and to determine how infection measures can successfully improve decisions by control programs. This evidence and the availability of appropriate diagnostic tools will likely be required to garner the support of WHO advisory members to amend current recommendations to allow the use of new diagnostic tools.

In concert with the growing body of evidence being generated by academic research groups, a current multi-site study led by the Task Force for Global Health (TFGH) in conjunction with the International Trachoma Initiative has been designed to help fill critical information gaps needed to address this issue. A strength of the current study is its multi-country approach, with data and sample collection occurring in Uganda, Malawi, Tanzania, Niger, and Ethiopia in order to provide more standardized data collection from a representative cross-section of trachoma-endemic settings. A primary aim of the study, which is targeting completion of sample collection in 2015, is to compare and correlate standard clinical assessment (trachoma grading to obtain the prevalence of follicular trachoma), infection (obtaining ocular swabs), and antibody (collecting dried blood spots through finger sticks to understand historical exposure) in population-based surveys of children 1 to 9 years old. This data will be evaluated to compare the performance of each methodology in the context of informing decisions following impact assessments after 3 to 5 rounds of MDA or when stopping MDA is being considered.
4. Risks and mitigation

1. A newly-developed diagnostic tool will not be implemented and used by national trachoma control programs to support elimination effort.

   a. **Problem:** Current barriers limit the potential application of newly-developed diagnostic tools to support trachoma elimination efforts.

      i. **WHO guidelines have not been amended:** It is unlikely for any new diagnostic tool to be widely implemented and used outside of research without a recommendation from WHO. Such approval has not yet occurred, and there is currently no assured timeline for when the necessary evidence and support by key stakeholders will be obtained.

      ii. **Current availability of other trachoma diagnostic solutions:** Unlike many of the other diseases targeted under the London Declaration, there are diagnostic tools readily available that can be adapted for use for trachoma, including a number of widely-distributed commercial products (See Appendix 1: Diagnostics Landscape for Trachoma). Emphasis within the trachoma community remains on building evidence to support the uptake and use of nucleic acid tests for direct detection of Chlamydia infection. Current focus and advocacy remains on evaluating commercial, high-throughput lab-based Chlamydia NAAT platforms and developing funding and operational strategies that will successfully expand their access to trachoma control programs. Thus, initiating a parallel product development strategy for new trachoma diagnostics that are not prioritized as a need by key stakeholders and implementation partners within the international trachoma community remains a strong risk [17, 23, 24].

      iii. **Early stage of research for antibody detection tools measures:** Direct measures of infection, such as detection of *C. trachomatis* nucleic acid, continue to be prioritized due in part to the evidence base that has been generated from more than a decade of use in trachoma research. However, research into the use and utility of indirect measures of infection (i.e., exposure based on reactivity of host antibodies against *C. trachomatis* antigens) is still at a relatively early stage of exploration. Further research is still needed to further define the kinetics of antibody responses in different endemic settings, including the variation that might be expected within and among different populations and at different stages of elimination. Also, unless a serovar-specific biomarker is used (which is currently not prioritized by test developers), it will be important to determine the effect that background reactivity against urogenital *C. trachomatis* serovars has on trachoma prevalence estimates. Nonetheless, the potential operational advantages of using immunoaassays for antibody detection remain attractive, as well as the potential to readily multiplex assays for other diseases using a single blood specimen [17]. However, the timeline for obtaining sufficient evidence to support their use in trachoma control programs makes current product development using candidate biomarkers likely better suited for later efforts after implementation of infection detection tests.
b. **Mitigation:** Postpone the initiation of new product development efforts until clarity is obtained on timeline and likelihood of success in amending WHO recommendations. Maintain engagement with trachoma stakeholders to assess the status of current opinion regarding a change in guidelines. Additionally, it will be important to remain up to date on the status of ongoing research to build evidence to support changes, including the outcomes of the current multi-site study led by the TFGH. If and when support for amending WHO guidelines becomes more certain, PATH will work with stakeholders to assess if gaps remain that warrant the initiation of new product development efforts. For instance, if antibody testing becomes a preferred option, PATH could play a translational role supporting the commercialization of the immunoassays developed by the United States Centers for Disease Control and Prevention (CDC).

2. A newly-developed trachoma diagnostic will not have the proper characteristics to meet the needs of control programs.

a. **Problem:** Research is still needed to fully delineate optimal test characteristics for new diagnostics intended for use by trachoma control programs in different endemic settings.

i. **Operational Characteristics:** Lab-based NAATs remain the prioritized option strongly-advocated by key stakeholders within the trachoma community. This is due in part to superior analytical performance, current availability of lab-based platforms needed for testing in many trachoma endemic countries due their use for other diseases, and potential for high-throughput testing. Less certain is how well these tests will meet the needs of country programs in terms of cost, access, and usability. Questions remain in terms of:

1. **Ensuring access to testing:** A secondary objective of the current multi-site study led by TFGH is a comparative evaluation of multiple platforms (Abbott m2000, Hologic Aptima, and Cepheid Xpert) that are commonly used in testing for other diseases such as HIV and tuberculosis in many trachoma-endemic countries. The hope is to ensure availability of at least one verified platform for trachoma surveillance testing in each country. However, ensuring adequate access to these tests may still pose challenges. Even within a country where a platform is available, access for trachoma testing may not be guaranteed, particularly if resources (including laboratory capacity) are siloed within a different disease-specific program. In countries where limited infrastructure, volume of testing needed, and/or access to required diagnostic test equipment and materials make lab-based NAAT testing unfeasible, alternative testing strategies may be required. Development of regional testing capacity to support testing needs for control programs from neighboring countries may be one solution, although country-specific regulations regarding specimen export could delay or prohibit testing. Ultimately, the availability of alternative test options may need to be considered.

2. **Test cost:** Currently, both fundraising and programmatic strategies like specimen pooling are being considered to address cost-related issues associated with implementing and sustaining the large scale expansion of expensive NAAT testing.
for trachoma surveillance. However, with an estimated cost of $10-40USD per test, the sustainability of expensive NAAT testing may pose a significant challenge, particularly during post-elimination surveillance when maintaining donor support for test costs may become more difficult[1, 23]. Ultimately, less expensive alternatives may be required, particularly if country programs eventually become responsible for greater ownership of test costs.

3. **Ensuring quality testing.** To date, the majority of studies evaluating laboratory tests for trachoma, including NAATs, have been performed as part of well-controlled research studies involving international research groups. If highly-sensitive NAATs are implemented for routine use by country programs, it will be paramount to have rigorous protocols and quality assurance practices in place throughout the entire test process. Upstream processes related to specimen collection and handling can have huge ramifications on test results. False negative results may occur due to improper specimen collection or degradation due to improper handling during transport and storage [25]. Issues of cross-contamination between samples may result in false positives and can occur at almost any point during the testing process. Other issues include ensuring proper training and the performance of test procedures by end users, maintenance and proper use of analytical equipment, as well as infrastructure issues – all of which may present challenges that could delay testing or result in inaccurate findings that can be costly to programs. Additionally, cost-reduction strategies involving specimen pooling require special consideration as these also present additional opportunities to incur process and testing errors. While leveraging the laboratory capacity and expertise of existing HIV and tuberculosis programs may prove to be valuable in many ways, even rigorous testing programs are susceptible to these issues, particularly with more complex tests [26-28].

ii. **Performance Characteristics:** A determination on what test performance thresholds are needed to properly inform public health decisions will be useful if new products are needed. The ability of NAATs to offer analytical performance that is superior to other formats such as antigen-detection tests is not questioned. However, since the primary purpose of trachoma testing will not be to inform individual treatment, a determination of what level of performance is needed (rather than possible) could be useful. The need for high test specificity once true prevalence has reached low levels will remain a requirement, given the need to limit the impact of false positives on decisions to either prolong MDA or restart MDA (if resurgence is detected). However, further determination of the meaningful sensitivity thresholds remains an important gap. Surveys are conducted on 1 to 9 year old children which typically harbor the highest burden of infection. In terms of defining sensitivity thresholds, it may be important in determining what level of infection is important to detect to ensure the reservoir for transmission is accurately assessed. This could include determining if it is important or detrimental for a test to detect cases with low level infection, including asymptomatic individuals, if these cases do not contribute
significantly to the reservoir responsible for maintaining transmission in the population. Ultimately, decisions on what performance thresholds are needed will determine the feasibility of exploring alternative technologies such as simple antigen detection tests which may sacrifice analytical performance compared to NAATs but could offer operational and/or cost advantages.

b. **Mitigation:** In-depth user needs assessment research, including end users within country programs, could be informative on the feasibility of the successful uptake and use of the current prioritized lab-based NAAT options. Additionally, the further delineation of programmatic (cost, access to platforms, specimen export) and operational (training, usability, infrastructure requirements, environmental conditions for test storage/performance, etc.) needs for a test intended for use in different endemic countries could further inform early planning for implementation and scale up of testing in country programs. Combining information from needs assessment with results from research more clearly defining *required* performance thresholds (versus *possible* with NAATs) could help support the identification or development of alternative diagnostic tools to fill gaps and ensure broad access to quality testing.

### 5. Diagnostic landscape

*C. trachomatis* is the causative agent of ocular trachoma and Chlamydia, which is one of the most prevalent sexually transmitted infections in the world. The majority of current tests used for trachoma are diagnostics developed for the detection of urogenital chlamydia infection that have been adapted for use with ocular specimens. For chlamydia detection tests, there are a multitude of different test types used, from highly-technical microbiologic and molecular assays to low-complexity rapid immunoassay tests intended for use at or near the POC. Previous assays used in trachoma research have included microscopy of conjunctival scrapings, isolation in cell culture, direct fluorescent antibody tests, enzyme immunoassays, serology, nucleic acid hybridization probes, and nucleic acid amplification tests [18].

While there is currently no designated gold standard laboratory test for trachoma, NAATs are generally regarded as being most sensitive and specific due to their superior performance when compared to other methodologies [18, 19, 29]. However, only moderate to high complexity chlamydia NAATs have been evaluated for trachoma. Many of these tests, including commercial assays prioritized for implementation and use by international trachoma stakeholders, allow some level of automation for hands off batch testing, but often require significant infrastructure investment and advanced personnel training [14, 29, 30]. Consequently, their successful implementation, use, and sustainability for use by control programs in the low resource settings where trachoma remains endemic is yet to be determined.

Diagnostic tests used for trachoma detection typically contain biomarkers that are either genus or species-specific. The exception are serologic and genotypic research methods designed specifically to type *C. trachomatis* serovars using specific reagents typically targeted against serovar-specific peptide regions within the Major Outer Membrane Protein (MOMP) or the genetic regions encoding it (*ompA* or *omp1*). Serovars of *C. trachomatis* are considered to be tissue-selective rather than specific with trachoma
serovars A, B, Ba, and C mainly localized to epithelial surfaces in the eye, while serovars D through K localize to epithelial surfaces in the genital tract and cause Chlamydia. Although there are cases where trachoma serovars have been detected in urogenital infections and conjunctivitis caused by Chlamydia serovars[1, 18]. However, these infections may not be critical for surveillance tests using ocular swabs given the vast predominance of trachoma serovars expected with chlamydial eye infections in endemic settings. While not yet fully determined, antibody tests against species-specific antigens are currently considered to be sufficient unless evidence later shows high background prevalence of urogenital chlamydial or other exposures confound survey results and interpretation. More importantly, biomarkers should be able to adequately discriminate against other common bacterial pathogens that may be found in ocular samples and, in the case of antibody tests, able to exclude other closely-related chlamydial species, particularly C. pneumo"miniae, which is a highly prevalent infection globally [31].

For infection detection, ribosomal RNA (either 16SrRNA or 23SrRNA) which is the target of the commercial Aptima tests (Hologic) represents the most sensitive biomarker for trachoma with a theoretical limit of detection of less than one elementary body per test given the high target copy number per cell. However, molecular tests using DNA detection have been shown to be adequate for accurate detection of trachoma infection in prior research and may be provide for a less costly approach. Among DNA targets, the cryptic plasmid (pCT) may be preferable given it is often present in multiple copies per cell and thus may impart improved sensitivity [18, 19]. Alternatively, the use of chromosomal gene targets, such as ompA or omp1, either alone or in-conjunction with pCT could be advantageous in the event of the emergence and widespread circulation of trachoma variants that are missing the plasmid. To date, no trachoma strains have been described that lack pCT, although it has been reported for urogenital chlamydial strains [32, 33]. For antigen detection, monoclonal antibodies are used to detect genus-specific regions within the chlamydial LPS including rapid diagnostic tests produced by Alere (Quickview) and Quidel (Clearview) as well as the low cost POC rapid test developed by Diagnostics for the Real World (Chlamydia Rapid Test). However, potential performance issues have been noted when current tests have been evaluated under field conditions in trachoma endemic regions [34, 35]. MOMP has also been used in for antigen detection tests with the potential added advantage of being able to use monoclonal antibodies that may target serovar-specific epitopes in the antigen [18, 36, 37]. Until recently, serological tests were generally not considered a useful tool for the diagnosis of trachoma infection due in part to the long-lived nature of serum antibodies elicited by chlamydial infection and thus the inability to distinguish active and past infections [38, 39]. However, the potential to incorporate trachoma diagnostic tests into low cost test formats and/or highly-multiplexed serologic assays has renewed interest, particularly for post-elimination surveillance activities [17]. A recent array-based study identified promising candidate antigens based on their specific immunodominance in cases of severe disease (trachomatis trichiasis) [40]. Among the 10 chlamydial antigens that were selectively reactive in more than 50% of trachoma patient sera, three were prioritized for further assay development and evaluation by CDC—CPAF, pgp3, and CT694 [41]. CPAF was later de-prioritized by the CDC for further evaluation due to technical difficulties incorporating into the current microsphere immunassay (D. Martin [CDC] personal communication). Thus pgp3 and CT694 have been carried forward and evaluated
in multiple studies within trachoma endemic countries, including the current multi-site study led by TFGH [39, 42, 43]. Recently-published studies have already demonstrated the potential value of using these serologic markers as a more standardized tool for assessing transmission during post-MDA surveillance, particularly in the key indicator group of 1-9 year olds. [43] Development of these immunoassays in other formats such as ELISA and lateral flow tests are also currently being explored (D. Martin [CDC] personal communication). Finally, although MOMP is not currently being evaluated in the CDC-developed assays, it has been shown in previous research to be an immunodominant antigen [18]. It has the added advantage of potentially providing target regions that could be used to provide further specificity for trachoma serovars if background seroreactivity with urogenital chlamydia serovars is determined to be problematic. However, given the current efforts to characterize pgp3 and CT694 in trachoma epidemiologic research, it is likely that if antibody tests are later prioritized then these biomarkers would be the strongest candidates based upon relevant evidence and their stage of development.

Although the majority of current research efforts are primarily focused on collecting evidence to further support the adoption and expansion of existing lab-based NAATs, the development of more field-deployable test options for trachoma may still have value to ensure the testing needs of all country programs can be met. While no field-deployable POC molecular test is currently widely-available for chlamydia, new assays and platforms are emerging with potential opportunities for development of future test solutions for trachoma. Field deployable NAAT platforms are becoming available including the Alere-i and -q and Quidel’s Savanna. Polymerase chain reaction assays for chlamydia are already available and multiple isothermal assays have been described in the literature that use isothermal chemistries that are proprietary to the commercial developers of these platforms such as Recombinase Polymerase Amplification (RPA) and Helicase-Dependent Amplification (HDA) [44, 45]. Additionally other test developers including Ustar Diagnostics (http://www.bioustar.com/en/) and Atlas Genetics (http://atlasgenetics.com/systems/io-system) are currently developing molecular assays for C.trachomatis detection for STDs using their own proprietary technology. The tests may one day have utility for trachoma detection as well. Finally, while NAATs remain prioritized, it is important to remember advances in technology and methodology with enzyme immunoassays may warrant further consideration for the use of antigen and antibody-detection tests. Simple, low-cost readers like the Veritor (BD) have shown ability to improve the performance over RDTs for other diseases as well as simply interpretation of test results [46]. Meanwhile, the potential of immunoassays to be highly-multiplexed to integrate testing for many diseases and conditions, sometimes using the same sample, continues to be an attractive quality that could be leveraged for trachoma along with many other priority neglected tropical diseases [17, 39, 47].

6. Use case statement

While clinical grading continues to be invaluable, particularly during the early stages of trachoma control, its ability to accurately inform control decisions (including when to stop MDA) becomes less clear when
prevalence is low, as often occurs following multiple rounds of MDA. MDA is used to treat infection, and clinical disease measures have been shown to become more discordant with markers of infection particularly at when prevalence reaches low levels. Clinical disease can lag long after infection is cleared, as well as be absent in cases of asymptomatic infection. This can lead to an over- or underestimation of prevalence and ultimately to additional unnecessary rounds of MDA that may be costly to donors, control programs, and, ultimately, to the morale of the frontline health care workers and the affected populations. Given the current limitations of clinical diagnosis, a diagnostic tool capable of more accurately informing late-stage decisions by control programs on whether to stop or continue MDA has potential value by reducing unnecessary rounds of MDA and with it the use of limited resources. From a cost/benefit perspective, improved decisions regarding MDA could provide significant value since the estimated cost per treatment cost is $0.35USD per person, with some estimates as high as $1.50USD per person for remote locations [48]. Thus, reducing even one unnecessary round of MDA for a district of 250,000 would provide substantial cost savings ($80,000USD at $0.35/person), and given that up to five rounds of MDA may be required depending on trachoma prevalence, the cost of decisions based on inaccurate survey data can be very significant. Globally, as more countries move toward reaching elimination goals, the enhanced ability to more accurately identify and target remaining reservoirs of infections that either persist or re-emerge could further expedite global elimination goals and with them the winding down and conclusion of the massive drug donation and control program costs.

7. Conclusion

Clinical grading to assess disease indicators will remain the WHO-recommended method for trachoma surveillance until required evidence is generated that clearly demonstrates how diagnostic tools measuring alternative indicators (namely infection) will significantly improve decision-making by control programs. Currently, the continued use of clinical grading remains preferred by key WHO scientific advisors due in part to its low cost, particularly against the more expensive molecular diagnostic tools used in research, and its longstanding use by control programs.

In terms of diagnostic solutions, the current prioritized diagnostic technology is a NAAT with commercial, lab-based high-throughput platforms, preferred due to their longstanding evaluation in trachoma research, the availability of commercialized products, and the strong advocacy amongst key stakeholders within the trachoma community. As potential concerns relating to cost, access, and usability of current lab-based NAAT options remain, the development of low cost, field-deployable test options for trachoma are likely to still have value to ensure testing needs of control programs across all endemic settings are adequately met. POC test options could take on increased importance for some programs, particularly if a targeted “test and treat” approach becomes necessary to eliminate remaining reservoirs of infection that have potential to prolong transmission within a community. Additionally, tests such as the serologic assays currently under development by the US CDC could play an important role during extended post-elimination surveillance activities as a standardized, lower-cost tool that may also allow integrated surveillance testing for other diseases through multiplexing. If new tests are developed,
prioritized biomarkers would include: the multi-copy cryptic plasmid DNA (pCT) for molecular assays; LPS and/or MOMP for antigen-detection tests due to ease of production and prior evaluation in trachoma research; and pgp3 and CT694 for use in antibody detection assays due to evidence they are immunodominant during chlamydial infection and the enhanced stage of research for their application in trachoma being conducted by TFGH. However, as barriers including lack of WHO recommendations currently exist, there is significant risk that newly-developed trachoma diagnostic products would not achieve intended impact for elimination efforts.

Thus, PATH recommends the following at this time:

1. Remain informed on the status of policy changes at WHO on the use of new trachoma diagnostics
2. Remain informed of the status and findings of key trachoma research
   a. Evidence generated for correlating current clinical disease indicators to infection and antibody indicators (TFGH/ITI, Academic Researchers)
   b. Operational research: Evaluation of lab-based NAAT platforms (ITI)
   c. Test development
      i. Evaluation of new biomarkers for use in serologic tests (Diana Martin, CDC)
      ii. Development of new tests (digital polymerase chain reaction, new research assays, commercial products)
3. Remain engaged with trachoma stakeholders
   a. Assess evolving opinions, needs, and potential diagnostic solutions for use in trachoma
   b. Status of test development: If the antibody test being developed by CDC is determined to be a preferred diagnostic solution, then PATH could play a commercialization role
   c. Maintain a network of support for resources to support future product development (if needed)
4. Explore potential avenues for technical/research collaboration
   a. Make findings of TPPs, impact models, and landscaping available to support efforts
   b. Conduct more in-depth user needs assessment research
      i. Map survey processes in different priority endemic countries
      ii. Provide evidence to support or assess gaps in current prioritized diagnostic solutions (i.e., lab-based NAATs, POC solutions, sample collection and transport)
   c. Identify and evaluate promising new/pipeline POC molecular Chlamydia (STI) tests for potential trachoma diagnostic applications (isothermal assays and platforms)
   d. Assess opportunities for integrating trachoma tests with diagnostics for other priority endgame diseases (multiplexing on immunoassay tests or platforms, evaluating trachoma assays on POC molecular platforms evaluated for other endgame diseases)
8. References


10. Keenan Jd Fau - Lakew, T., et al., Slow resolution of clinically active trachoma following successful mass antibiotic treatments. (1538-3601 (Electronic)).


25. Puren, A., et al., Laboratory operations, specimen processing, and handling for viral load testing and surveillance. (1537-6613 (Electronic)).


36. Frost, E.H., et al., Variation outside variable segments of the major outer membrane protein distinguishes trachoma from urogenital isolates of the same serovar of Chlamydia trachomatis. (0266-4348 (Print)).

37. Kuipers, J.G., et al., Sensitivities of PCR, MicroTrak, ChlamydiaEIA, IDEIA, and PACE 2 for purified Chlamydia trachomatis elementary bodies in urine, peripheral blood, peripheral blood leukocytes, and synovial fluid. (0095-1137 (Print)).


46. Hassan, F., et al., Comparison of the BD Veritor System for Flu A+B with the Alere BinaxNOW influenza A&B card for detection of influenza A and B viruses in respiratory specimens from pediatric patients. (1098-660X (Electronic)).


## Appendix 1: Biomarker landscape for Trachoma

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Surveillance Measure</th>
<th>Description of priority candidates</th>
<th>Sample Type</th>
<th>Format</th>
<th>Use with in Trachoma Diagnostics</th>
<th>Stage of Product Development</th>
<th>Prioritized Use Case</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td>• Clinical examination and grading of disease severity using the WHO simplified grading scheme.</td>
<td>Exam</td>
<td>Clinical Exam</td>
<td>WHO-approved method</td>
<td>Not applicable</td>
<td></td>
<td>• Mapping&lt;br&gt;• Impact Monitoring&lt;br&gt;• MDA Stopping&lt;br&gt;• Post MDA Surveillance&lt;br&gt;<strong>PRIORITIZED</strong>&lt;br&gt;• Note: Currently only approved method for trachoma surveillance.&lt;br&gt;• Near Term: Track research and opinion of trachoma community&lt;br&gt;• Long-term: Same</td>
</tr>
<tr>
<td><strong>Bacterial Nucleic Acid</strong></td>
<td></td>
<td>DNA&lt;br&gt;• Cryptic plasmid (pCT): Extrachromosomal plasmid target; multiple copies per cell; absent in some urogenital C. trachomatis strains and other Chlamydial species&lt;br&gt;• <em>ompA</em> or <em>omp1</em>: chromosomal targets encoding MOMP; used in commercial assays to ensure detection of urogenital CT variants missing pCT; contains highly variable regions that can be used for genotyping&lt;br&gt;RNA&lt;br&gt;• 16S or 23S Chlamydia specific rRNA sequences: encode subunits of the bacterial ribosome; multiple copies per cell</td>
<td>Ocular Swab</td>
<td>Lab or Field-based Molecular Test</td>
<td>Realtime CT/NG for m2000 (Abbott)&lt;br&gt;• ProbeTec ET System (BD)&lt;br&gt;• COBAS and Amplicor CT/GC Test (Roche)&lt;br&gt;• Xpert CT/GC (Cepheid)&lt;br&gt;• Aptima CT and Aptima Combo 2 (Hologic)</td>
<td>Commercial products available</td>
<td>MDA Stopping&lt;br&gt;• Post-MDA Surveillance&lt;br&gt;<strong>PRIORITIZED</strong>&lt;br&gt;• Note: Evidence and support for amending WHO recommendations for use of non-clinical measure remains in critical path to uptake and use.&lt;br&gt;• Near Term: Track progress and provide support as needed for lab-based NAAT options. Commercial lab-based NAAT test options are currently-available and are prioritized as diagnostic solution for trachoma surveillance&lt;br&gt;• Long-term: Development of evaluation of NAATs with lower cost and complexity may be considered. Operational and cost limitations associated with lab-based NAATs by trachoma programs could have potential to result in both accuracy and sustainability issues for testing in some endemic settings. Thus, development of a low cost NAAT test (isothermal, etc.) appropriate for use in lower tier healthcare settings could have benefit in improving access and test performance. Academic research groups have already described homebrew isothermal assays (RPA, HDA) for <em>C.trachomatis</em> detection for STDs using these biomarkers which could which could warrant downstream consideration.</td>
<td></td>
</tr>
</tbody>
</table>
### Appendix 1: Biomarker landscape for Trachoma

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Surveillance Measure</th>
<th>Description of priority candidates</th>
<th>Sample Type</th>
<th>Format</th>
<th>Use with in Trachoma Diagnostics</th>
<th>Stage of Product Development</th>
<th>Prioritized Use Case</th>
<th>Recommendations</th>
</tr>
</thead>
</table>
| **Bacterial Antigens** | • Infection | • MOMP: Immunodominant outer membrane protein encoded by omp; contains species-and serovar-specific variable regions  
• Lipopolysaccharide (LPS): Monoclonal antibodies developed that target regions of this common bacterial outer membrane component that are specific for the Chlamydia genus | Ocular Swab | • Immunoassay (RDT, ELISA), Culture | • MOMP: MicroTrek EIA  
• LPS: Chlamydia Rapid Test (Diagnostic for the Real World), MicroTrek II EIA(trinity Biotech); Quickview RDT (Quidel) | Commercial products available | • MDA Stopping  
• Post-MDA Surveillance | NOT PRIORITIZED AT THIS TIME  
• Near term: No action needed at this time. Performance limitations of currently available test is a concern based on limited evaluations of Chlamydia RDTs for use in trachoma.  
• Long term: Track progress and landscape of new, improved test options. If minimal required LOD of trachoma infection detection test is further delineated and a technical solution can be used to allow an RDT to meet this (optimization, use of a reader, etc), then operational and cost aspects of an RDT could potentially make this an attractive option. |
| **Host Antibody** | • Exposure | • pgp3 and CT694 selected for further development from panel of CT antigens that were reactive in screen of sera from human cases of trachoma trichiasis (TT).  
• Pgp3: 28kDA secreted glycoprotein encoded by Open Reading Frame 5 (ORF 5) of the extrachromosomal plasmid isolated from Chlamydia trachomatis which is missing in C. pneumonia strains.  
• CT694: Hypothetical Type III secretion effector; Modulates host cytoskeletal protein organization during EB invasion  
• MOMP: Immunodominant outer membrane protein; Protein contains species- and serovar-specific variable regions and is used in serotyping  
• CPAF: Serine protease that is secreted via sec-dependent pathway for manipulating host signaling pathways; one on major immunodominant antigens used in serologic assays for chlamydia | Blood, Serum, Dried Blood Spots | • Immunoassay (Lab-based MIA; ELISA; RDT) | • Microarray: Immunodominant antigen identified through screening CT protein array of chlamydial antigens against sera of TT patients (UT-Houston)  
• Immunoassays (MIA and ELISA): Current ongoing research and development evaluating potential application for surveillance immunoassays (US CDC) | Research and Development | • MDA Stopping  
• Post-MDA Surveillance | NOT PRIORITIZED AT THIS TIME  
• Near term: Track progress and maintain communication with CDC. Currently pgp3 is the prioritized antigen for further study. Still in exploratory stage and is a relatively new indicator under consideration for use in trachoma. Minimal evidence exists on interpretation of antibody responses in the context of trachoma control.  
• Long term: Potential role translating biomarkers to a commercial product. If long term evidence demonstrates utility of this biomarker in informing decision-making and it becomes an approved option, then CDC will likely either need to support lab-based surveillance test option or seek a commercialization partner to translate test to a commercial platform. If data and further support for antibody testing becomes available, then benefits of using this marker could include potential of an inexpensive RDT or ELISA format and/or ability to multiplex with serologic tests for other diseases. |
## Appendix 2: Diagnostics landscape for Trachoma

<table>
<thead>
<tr>
<th>Company</th>
<th>Diagnostic</th>
<th>Biomarker</th>
<th>Surveillance Measure</th>
<th>Availability</th>
<th>Description</th>
<th>Current Status for use in Trachoma Surveillance</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>Who Simplified Grading, clinical eye exam</td>
<td>Clinical</td>
<td>Disease</td>
<td>Developed</td>
<td>• Simplified disease grading system based on clinical examination of the eye for signs of trachoma.</td>
<td></td>
<td>• Approved for use by WHO</td>
<td>• Subjective interpretation</td>
</tr>
<tr>
<td>WHO</td>
<td>Consensus fine grading, clinical eye exam</td>
<td>Clinical</td>
<td>Disease</td>
<td>Developed</td>
<td>• Expanded grading system based on clinical examination of the eye for signs of trachoma and disease severity.</td>
<td></td>
<td>• Research use only</td>
<td>• Findings can be correlated with simplified grading system</td>
</tr>
<tr>
<td>Abbott</td>
<td>Realtime CT/GC on the m2000 System</td>
<td>DNA</td>
<td>Infection</td>
<td>Commercialized</td>
<td>• Amplification Technology: Realtime PCR Target: Cryptic Plasmid High-throughput, lab-based NAAT system approved for urogenital chlamydial and gonorrhea detection Adapted for use with ocular samples</td>
<td></td>
<td>• Research use only for trachoma Prioritized platform undergoing current validation by TFGH/ITI in multi-site study. Potential for use by control programs contingent upon further evaluation</td>
<td>• Good accuracy even at low prevalence</td>
</tr>
<tr>
<td>Hollogic/Gen-probe</td>
<td>Aptima CT (ACT) or Aptima Combo 2 (AC2)</td>
<td>RNA</td>
<td>Infection</td>
<td>Commercialized</td>
<td>• Amplification Technology: Transcription Mediated Amplification (TMA) Target for trachoma: 23S rRNA (AC2) or 16rRNA (ACT) High-throughput, lab-based NAAT system approved for urogenital chlamydial and gonorrhea detection Adapted for use with ocular samples</td>
<td></td>
<td>• Research use only for trachoma Prioritized platform undergoing current validation by TFGH/ITI in multi-site study. Potential for use by control programs contingent upon further evaluation</td>
<td>• Considered most sensitive test available</td>
</tr>
<tr>
<td>Cepheid</td>
<td>GeneXpert CT/GC</td>
<td>DNA</td>
<td>Infection</td>
<td>Commercialized</td>
<td>• Amplification Technology: Realtime PCR Target for trachoma: Chromosomal Fully-integrated process Lab-based NAAT system with single to batch testing capacity Approved for urogenital chlamydial and gonorrhea detection Adapted for use with ocular samples</td>
<td></td>
<td>• Research use only for trachoma Prioritized platform undergoing current validation by TFGH/ITI in multi-site study. Potential for use by control programs contingent upon further evaluation</td>
<td>• Good accuracy even at low prevalence</td>
</tr>
</tbody>
</table>
## Appendix 2: Diagnostics landscape for Trachoma

<table>
<thead>
<tr>
<th>Company</th>
<th>Diagnostic</th>
<th>Biomarker</th>
<th>Surveillance Measure</th>
<th>Availability</th>
<th>Description</th>
<th>Current Status for use in Trachoma Surveillance</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Roche Molecular Diagnostics   | COBAS CT or CT/GC; Amplicor             | DNA       | Infection            | Commercialized     | • Amplification Technology: Real-time PCR  
  • Target for trachoma: Cryptic Plasmid and/or chromosomal  
  • High-throughput, lab-based NAAT system approved for urogenital chlamydial and gonorrhea detection  
  • Adapted for use with ocular samples  
  • Research use only for trachoma  
  • Not currently prioritized for validation by TFGH/ITI  
  • Potential for use by control programs contingent upon further evaluation |                                           | • Good accuracy even at low prevalence  
  • Direct detection of infection  
  • High-throughput platform available  
  • Ease in test interpretation | • Not WHO-approved  
  • Cost  
  • Infrastructure requirements  
  • High-Complexity |
| Becton Dickinson              | ProbeTec ET System                      | DNA       | Infection            | Commercialized     | • Amplification Technology: Strand Displacement Amplification  
  • Target for trachoma: Cryptic Plasmid  
  • High-throughput, lab-based NAAT system approved for urogenital chlamydial and gonorrhea detection  
  • Adapted for use with ocular samples  
  • Research use only for trachoma  
  • Not currently prioritized platform for validation by TFGH/ITI  
  • Potential for use by control programs contingent upon further evaluation |                                           | • Good accuracy even at low prevalence  
  • Direct detection of infection  
  • High-throughput platform available  
  • Ease in test interpretation | • Not WHO-approved  
  • Cost  
  • Infrastructure requirements  
  • High-Complexity |
| London School of Tropical Medicine and Hygiene | Droplet Digital PCR                     | DNA       | Infection            | Research and Development | • Homebrew assay developed by LSTMH  
  • Assay developed using BioRad X100 system  
  • Allows for quantitation.  
  • Research use only for trachoma  
  • Not currently prioritized platform for validation by TFGH/ITI  
  • Potential for use by control programs more downstream since not commercialized |                                           | • Research method  
  • Not WHO-approved  
  • High test/equipment cost  
  • Infrastructure requirements  
  • High-Complexity | |
| Ustar Biotechnologies Ltd.    | FASTeasy CT Isothermal Amplification Test | DNA       | Infection            | Research and Development | • Amplification Technology: Cross-priming amplification (CPA)  
  • Target for trachoma: Chromosomal  
  • Amplification test currently being developed for use in non-central laboratories  
  • Not on market  
  • Intended use is for detection of urogenital Chlamydia infection.  
  • Performance limitations observed in PATH’S very limited initial evaluation using purified EB from trachoma serovars. |                                           | • Performance may not be equal NAATs for trachoma (PATH internal evaluation)  
  • Not Commercialized  
  • Still requires some lab infrastructure to perform  
  • Limited evaluation on Trachoma | |
| Trinity Biotech               | MicroTrak II Chlamydia EIA              | Chlamydia LPS | Infection            | Commercialized     | • Immunoassay for antigen detection  
  • Approved for use with ocular specimens, but limited information on use in trachoma surveillance |                                           | • Lower infrastructure requirements than NAAT  
  • Allows for batch testing  
  • Analyzed with ocular swabs | • Performance limitations compared to NAATs  
  • Still requires lab infrastructure including specialized equipment  
  • Cost  
  • Limited evaluation on Trachoma |
| Diagnostics for the Real World / University of Cambridge | Chlamydia Rapid Test                   | Chlamydia LPS | Infection            | Commercialized     | • Research use only  
  • POC enzyme immunoassay in a dipstick format for detection of Chlamydia LPS  
  • Primary intended use is for detection of urogenital Chlamydia infection.  
  • Evaluated for use as trachoma diagnostic |                                           | • Low cost  
  • Low Complexity  
  • Familiar RDT format  
  • Potential for Field use | • Performance limitations when used under field conditions  
  • Limited availability | |
| CDC                           | Serologic assay for detection of host Igg and Iga responses against pgp3 and CT0694 | Host antibody to C. trachomatis antigens | Exposure            | Research and Development | • Research use only.  
  • Current format is a microsphere immunoassay analyzed on a Luminex platform  
  • Research stage of development  
  • Potential use by control programs pending further evaluation  
  • Alternative immunoassay formats being considered  
  • Potential for high degree of multiplexing with other diseases  
  • Batch testing potential  
  • Potential for low cost/low complexity format including field deployable RDT  
  • Interpretation may not be straightforward due to antibody kinetics  
  • Not commercialized |                                           | |