

Target Product Profile

Trachoma Surveillance Diagnostic

Use case: Preventive chemotherapy
reduction or stopping decision

Platform: Lateral flow test

Biomarker: Antigen

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Executive Summary

Trachoma is the leading cause of infectious blindness in the world. The infectious agent of trachoma is the bacteria *Chlamydia trachomatis* that spreads by contact with an infected person's hands or clothing. Infection leads to conjunctival inflammation that produces trachoma follicles visible on physical exam. Yet it is the repeated episodes of reinfection and inflammation that lead to scarring, distortion of the eyelid, and in-turning of the lid with the eyelashes touching the cornea, called trichiasis, that leads to blindness.¹ Infectious spread is prevented by good hygiene practices, including hand and face cleanliness, and environmental improvements. Antibiotics, namely oral azithromycin or topical tetracycline, are an effective treatment of active trachoma infections, while surgery is indicated to manage trichiasis.

The Alliance for Global Elimination of Blinding Trachoma by 2020 (GET 2020), led by the World Health Organization (WHO), developed the SAFE strategy to reach their goal of eliminating trachoma by 2020 through Surgery, Antibiotics, Facial cleanliness, and Environmental improvement.² The most commonly used antibiotic for trachoma, oral azithromycin, is donated free of charge by the pharmaceutical company Pfizer and is given to entire communities. In order to assess the impact of the community-wide antibiotic distribution, commonly known as mass drug administration (MDA), trachoma surveillance is performed with a physical exam of the eye. This method of diagnosis is acceptable for early control programs; however, as we move closer to elimination of trachoma, more sensitive and specific diagnostics are needed.

This report proposes a target product profile (TPP) for the development of a new diagnostic technology that facilitates an accurate **stopping decision** phase for MDA. Each attribute has an “acceptable” standard that must be met and an “ideal” standard that, if met, would maximize the target product's value. This TPP focuses on the development of a lateral flow rapid diagnostic test (RDT) that detects trachoma antigens.

As reference, for a description of the currently available nucleic acid amplification tests for trachoma, please see Appendices A-1 and A-2.

Overview of Target Product Profile

Attribute	Acceptable	Ideal
1. Context (Use Case)		
1.1 Clinical and/or surveillance need (value proposition)	Current diagnostic practices are not sufficiently accurate and a new diagnostic test is required to monitor progress toward the GET 2020 goals of eliminating trachoma by the year 2020.	Current diagnostic practices are not sufficiently accurate and a new diagnostic test is required to monitor progress toward the GET 2020 goals of eliminating trachoma by the year 2020.
1.2 Intended use (use case)	Monitoring prevalence following MDA and informing the decision to adjust the treatment strategy to support elimination.	Monitoring prevalence following MDA and informing the decision to adjust the treatment strategy to support elimination.
1.3 Target populations	Children 1 to 5 years old.	Children 6 months to 9 years old.
1.4 Target countries/ geographic coverage	Trachoma-endemic countries.	Trachoma-endemic countries.
1.5 Location of use (infrastructure level)	Tier 2 facility, household or school setting at the community level, minimal or no infrastructure requirements.	Tier 2 facility, household or school setting at the community level, minimal or no infrastructure requirements.
1.6 Target user	Health care professional, trained in eye exams.	Surveillance teams made up of individuals such as community health workers with minimal training.
1.7 Fit with clinical workflow/ linkage to action	Direct replacement of WHO clinical exams, limited to no impact on current workflow. Linkage to action unchanged.	Direct replacement of WHO clinical exams, limited to no impact on current workflow. Linkage to action unchanged.
1.8 Desired stability, storage, and cold chain requirements	Up to 40°C. Able to withstand daily temperature fluctuations from 25°C to 40°C and relative humidity levels of 40% to 88%. No cold chain required.	Up to 45°C. Able to withstand daily temperature fluctuations from 25°C to 40°C and relative humidity levels of 20% to 88%. No cold chain required.
2. Design		
2.1 Analyte (diagnostic marker)	<i>Chlamydia trachomatis</i> antigens , species specific.	<i>Chlamydia trachomatis</i> antigens , ocular trachoma serovar specific.

Attribute	Acceptable	Ideal
2.2 Sample type and volume	Dry ocular conjunctival swab; only one swab required.	Dry ocular, oral, nasopharyngeal, or tear swab; only one swab required.
2.3 Sample preparation	Minimal collection or processing step.	No sample preparation required.
2.4 Sample transport stability	No sample transport.	No sample transport.
2.5 Waste management (hazardous materials/chemicals)	Minimal or no hazardous materials, per WHO and country standards.	Minimal or no hazardous materials, per WHO and country standards.
2.6 Nature of result	Qualitative.	Qualitative.
2.7 Time to result	Same-day result, < 24 hours.	Same-day result, ≤ 15 minutes.
2.8 Throughput	> 50 samples per day.	> 100 samples per day.
2.9 Instrumentation format and complexity level	Simple lateral flow test with minimal user steps.	Simple lateral flow test with minimal user steps.
2.10 Infrastructure requirements	Minimal, consistent with Tier 2 facility.	Minimal, consistent with Tier 2 facility.
2.11 Test-specific training requirements	Minimal, 1 day.	Minimal, 1/2 day.
2.12 Instrumentation size and weight	Small, easily deployable in the field.	Small, easily deployable in the field.
2.13 Ancillary supplies	Minimal supplies to ensure optimal test performance, packaged as a kit.	None.
2.14 Mean time between failures	Not applicable.	Not applicable.
2.15 Quality control	Internal control line, industry standards for positive and negative external controls.	Internal control line, industry standards for positive and negative external controls.
2.16 Calibration	Minimal, not required in the field.	None.
2.17 Product shelf life	12 months.	36 months; packaging should include thermal indicator.
3. Performance		
3.1 Analytical limit of detection (LOD)	≤ 2 x 10 ⁴ elementary bodies (EBs)/mL.	≤ 2 EBs/mL.

Attribute	Acceptable	Ideal
3.2 Analytical specificity	<i>Chlamydia trachomatis</i> species-specific Ag (serovars A–K).	<i>Chlamydia trachomatis</i> ocular serovars only Ag (A–C).
3.3 Clinical sensitivity	> 70%.	> 90%.
3.4 Clinical specificity	> 95%.	> 99%.
3.5 Reproducibility and robustness	Replicate determinations of weak positive samples classify the same \geq 95% of the time.	Replicate determinations of weak positive samples classify the same \geq 95% of the time
3.6 Comparative reference method	Performance comparable to a current regulatory-approved NAAT.	Performance comparable to a current regulatory-approved NAAT.
4. Commercialization		
4.1 Desired end-user price	< \$2 per test.	< \$1 per test.
4.2 Channels to market	To be determined.	To be determined.
4.3 Supply, service, and support	To be determined.	To be determined.
4.4 Product registration path and WHO prequalification	Not required for surveillance tests.	Not required for surveillance tests.

Rationale

1. Context (Use Case)

1.1 Clinical and/or surveillance need (value proposition)

Acceptable: Current diagnostic practices are not sufficiently accurate and a new diagnostic tool is required to monitor progress toward the GET 2020 goals of eliminating trachoma by the year 2020.

Ideal: Current diagnostic practices are not sufficiently accurate and a new diagnostic tool is required to monitor progress toward the GET 2020 goals of eliminating trachoma by year 2020.

Ocular trachoma is currently diagnosed by WHO clinical examination criteria that rely upon physical exam findings of the eye. GET 2020, led by the WHO, developed the SAFE strategy to reach their goal of eliminating trachoma by 2020 through **S**urgery, **A**ntibiotics, **F**acial cleanliness, and **E**nvironmental improvement.² Trachoma, with its corresponding disease stage, is currently diagnosed by the following WHO criteria:³

- Trachomatous inflammation, follicular (TF) — Five or more follicles of >0.5mm on the upper tarsal conjunctiva.
- Trachomatous inflammation, intense (TI) — Papillary hypertrophy and inflammatory thickening of the upper tarsal conjunctiva obscuring more than half the deep tarsal vessels.
- Trachomatous scarring (TS) — Presence of scarring in tarsal conjunctiva.
- Trachomatous trichiasis (TT) — At least one ingrown eyelash touching the globe, or evidence of epilation (eyelash removal).
- Corneal opacity (CO) — Corneal opacity blurring part of the pupil margin.

The current diagnostic method, clinical evaluation of the eye, is sufficient during the mapping phase when disease prevalence is high; however, this approach has proven inaccurate for monitoring and stopping decisions when prevalence is presumably low.⁴⁻⁷ Evidence of disease by clinical exam does not always correlate to active infection as determined by laboratory-based polymerase chain reaction (PCR) for *C. trachomatis* nucleic acids. At any given time, only 18% to 40% of individuals with less severe active disease (defined by finding TF on physical exam) will be PCR-positive; 50% to 70% of those with severe inflammation (defined by finding TI on physical exam) will be PCR-positive. Additionally, clinical signs of trachoma can persist long after infection has cleared and DNA is undetectable.¹ Therefore, given the difficulty identifying active disease, there is a need for an improved diagnostic tool to inform the stopping decision of antibiotic distribution.³

Technologies for a laboratory diagnosis of ocular trachoma exist, often adapted from those developed for urogenital *C. trachomatis* infections that cause the sexually transmitted disease chlamydia. The available assays include microscopy of conjunctival scrapings, isolation in cell culture, direct fluorescent antibody,

enzyme immunoassay, serology, nucleic acid hybridization probes, and nucleic acid amplification tests (NAAT). Highest-accuracy testing is currently in the form of nucleic acid amplification tests that are used in research (see Appendix A-1 and A-2 for a list of commercially available NAAT tests used for trachoma). These are highly technical, lab-based diagnostics that enable batch testing and high throughput but require significant infrastructure investment and advanced personnel training. Consequently, their role in MDA management in rural, underdeveloped communities is undetermined. Purchasing NAAT technologies for in-country use is currently underway with the goal of cross-application to other neglected tropical disease (NTD) programs. To achieve the GET 2020 goals, however, some experts advocate for the development of a low-cost, field-deployable RDT.

While PCR-based RDTs are in early experimental stages, a number of enzyme-linked immunosorbent assay (ELISA)-based rapid point-of-care (POC) tests are commercially available for urogenital chlamydia (e.g., Clearview® Chlamydia by Alere, QuickVue Chlamydia by Quidel). The consensus on these tests, however, seems to be that they sacrifice sensitivity for speed.⁸ Thus, efforts are currently underway to identify targets and technologies specific to ocular trachoma that will enable the development of a high-performing RDT immune-based assay.^{9,10} The immune targets for a stopping decision are antigens that indicate active infection, in contrast to an antibody-based assay that would be more applicable for monitoring exposure post-MDA reduction.

This TPP focuses on a technology that enables accurate MDA stopping decisions based on an immunoassay platform that uses antigen targets.

1.2 Intended use (use case)

Acceptable: Monitoring prevalence following MDA and informing the decision to adjust the treatment strategy to support elimination.

Ideal: Monitoring prevalence following MDA and informing the decision to adjust the treatment strategy to support elimination.

The strategy for trachoma control and subsequent elimination is through the following stages:

1. Determining the pre-intervention prevalence (*mapping*).
2. Assessing the community after three to five years of community-based SAFE interventions (*impact monitoring*).
3. Determining the appropriate time to stop MDA (*MDA stopping decision*).
4. Continued surveying post-MDA reduction to ensure continued infection suppression (*post-elimination surveillance*).

The RDT will be used during the MDA stopping decision phase. It will replace the clinical diagnostic exam conducted by surveyors. Currently, annual surveys are conducted by health workers, preferably eye specialists, nurses, or medical assistants trained in the WHO eye exam criteria. Approximately 100 to 300 people per day are examined from a random sample population within a district, the sample number varying based on population size and anticipated prevalence. Surveyors go to individual homes to evaluate all household members, regardless of age. Exam findings are recorded and the data is collected

for evaluation. A ‘prevalence’ of trachoma is calculated based on TF and TT cases and action decisions (i.e., repeat MDA or surveillance) are made.^{11,12} With use of the RDT, surveyors will instead obtain conjunctival swab samples for on-site processing.

1.3 Target populations

Acceptable: Children 1 to 5 years old.

Ideal: Children 6 months to 9 years old.

Rigorous epidemiological studies have already been conducted for trachoma to identify highest burden of disease.¹³ Prevalence of ocular chlamydia trachoma infection is highest in children under the age of ten years, with most significant reservoirs in children less than five years old.^{3,14} Thus implementation of trachoma control activities is prioritized in communities where the prevalence of active trachoma in children aged one to nine years is 10% or higher.² It has been noted, however, that children under 12 months of age can be significant reservoirs of trachoma infection.³ If confirmed as a high-prevalence age group, testing the infant population would also be of interest in the ideal case and test accuracy for this unique population must be verified.

1.4 Target countries/geographic coverage

Acceptable: Trachoma-endemic countries.

Ideal: Trachoma-endemic countries.

Trachoma is endemic in 53 countries across the world including countries in Africa, Asia, Central and South America, Australia, and the Middle East.¹³ Worldwide in 2011, it was estimated that 325 million people live in trachoma-endemic areas. However, this could be an underestimate, since not every endemic country has done a complete assessment of the burden of disease. There is currently underway a Global Trachoma Mapping Project (GTMP) by a consortium of nongovernmental organizations (NGOs) and academic institutions that began in 2012 and is scheduled for completion in March 2015.¹⁵ In order to meet WHO’s definition of global elimination, *all* endemic regions must be controlled and thus the test must be applicable across a broad array of geographies. (See Appendix B for more prevalence information.)

1.5 Location of use (infrastructure level)

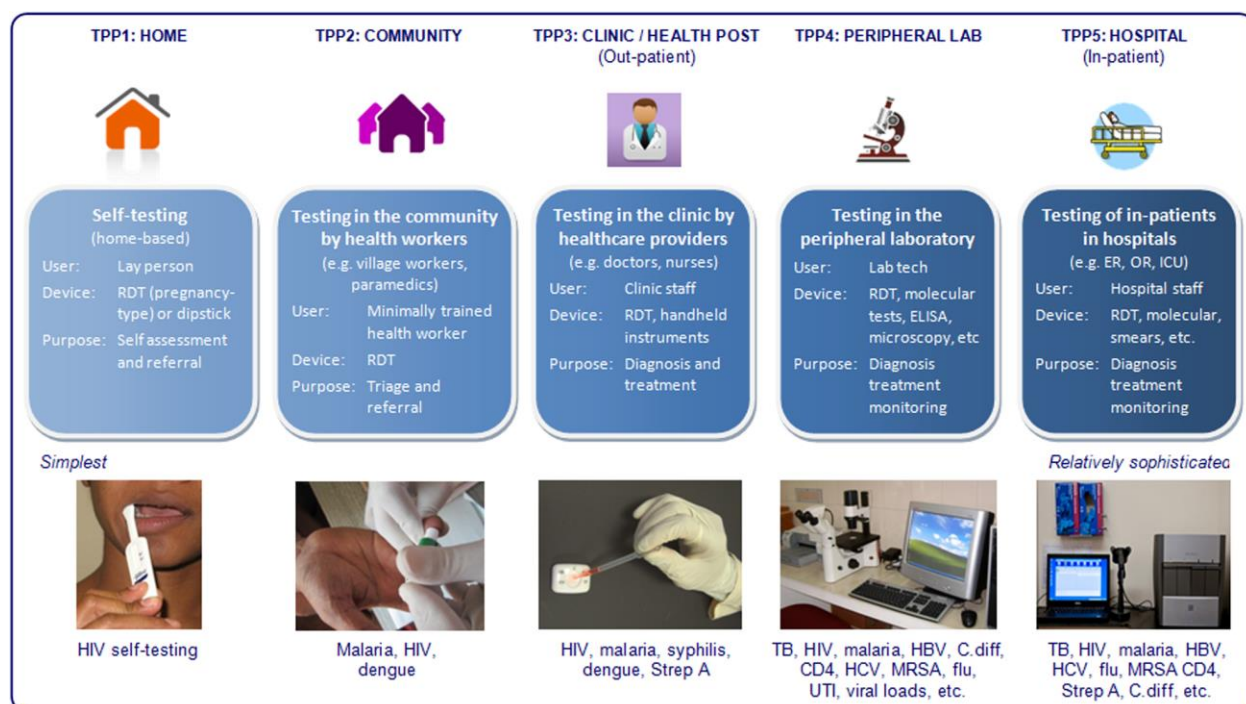
Acceptable: Tier 2 facility, household or school setting at the community level, minimal or no infrastructure requirements.

Ideal: Tier 2 facility, household or school setting at the community level, minimal or no infrastructure requirements.

Trachoma surveillance activities occur in individual households, schools, and/or clinics, depending on the country.¹² Households are ideal as they capture children younger than school age and those of lower socioeconomic families who do not attend school. They also allow for testing of adults who unknowingly may be disease reservoirs. The lateral flow test must be usable in such settings which, as displayed below

in Figure 1, is consistent with a Tier 2 (T2)-level facility (notably not a Tier 1 facility that emphasizes self-testing).

Figure 1: The spectrum of POC testing sites for TPPs.¹⁶



To maximize efficiency and use of limited resources, centralizing efforts at a school may be acceptable if a minimum threshold for school attendance is determined. School-based programs may also provide synergy with other NTD programs, such as schistosomiasis and soil-transmitted helminthes. Ideally, NTD control activities could be harmonized across diseases to increase population compliance, simplify overall survey procedures, and decrease costs.¹⁷

1.6 Target user

Acceptable: Health care professionals trained in eye exams.

Ideal: Surveillance teams made up of individuals such as community health workers with minimal training.

Surveillance workers for trachoma are health professionals, often ophthalmic or general nurses and medical assistants, who undergo training for the clinical eye exam.^{11,12} Ideally, minimally trained field-surveillance teams could administer and interpret the RDT. This would allow for integration of trachoma surveillance into other NTD surveillance programs that use RDTs, such as lymphatic filariasis.

1.7 Fit with clinical workflow/linkage to action

Acceptable: Direct replacement of WHO clinical exams, limited to no impact on current workflow.

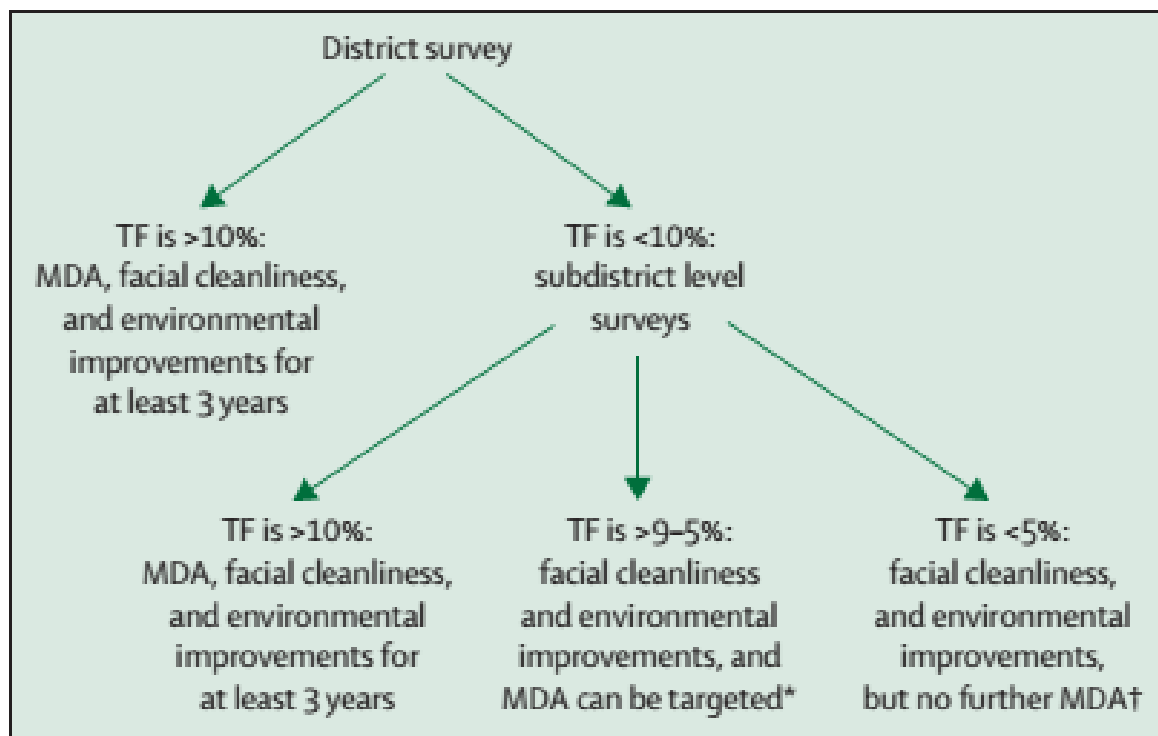
Linkage to action unchanged.

Ideal: Direct replacement of WHO clinical exams, limited to no impact on current workflow. Linkage to action unchanged.

The test results will replace clinical exam findings, thereby maintaining the same clinical workflow for MDA reduction and stopping decisions.

In order to eliminate infectious transmission, the WHO advises antibiotic distribution to an entire community if disease prevalence, currently defined as prevalence of TF per surveyed area, reaches a threshold value. This approach is called mass drug administration (MDA).¹⁴ See Figure 2.

Figure 2: WHO-recommended interventions according to prevalence of active trachoma.¹



TF = active trachoma.

MDA = mass drug administration.

* Targeted means that no further survey is needed, but by use of the best available information, villages, or aggregates of villages, are treated where trachoma rates are suspected to be high.

† Precision for < 5% is 4±2.

The WHO recommends continuing MDA annually for three years prior to reassessment of trachoma prevalence if the community starting prevalence is 10% to 30%. For areas with starting prevalence rates of 30% to 50%, reassessment can be delayed for five years, while for prevalence > 50%, seven years of treatment may be required. Stopping MDA occurs when TF prevalence is < 5% in subdistricts or community clusters.¹

GET 2020 defines its goal of trachoma elimination as follows:¹⁸

1. Observing a reduction in trachomatous follicular (TF) prevalence to less than 5% in children between one and nine years of age.
2. Having a maximum trachomatous trichiasis (TT) burden of 1/1,000 in the total population.

3. Improving facial and environmental cleanliness.

Practices on the initiation or reduction of MDA vary by country. In Mali and Ghana, for example, children are screened and treated if positive for TF. Their close contacts are subsequently screened. If prevalence is noted to be > 5% in the village or community, then MDA is initiated annually for three years, as per WHO guidelines, before a repeat survey is conducted.¹¹ Neighboring villages are also screened, and if TF prevalence is again > 5%, then subdistrict or district MDA commences. If prevalence is below the treatment threshold level on repeat surveys, then MDA is not continued. In contrast, Brazil and Ethiopia conduct national surveys to determine MDA initiation and stopping points. Up to a year may lapse before MDA decisions are made.¹²

1.8 Desired stability, storage, and cold chain requirements

Acceptable: Up to 40°C. Able to withstand daily temperature fluctuations from 25°C to 40°C and relative humidity levels of 40% to 88%. No cold chain required.

Ideal: Up to 45°C. Able to withstand daily temperature fluctuations from 25°C to 40°C and relative humidity levels of 20% to 88%. No cold chain required.

There are notable temperature fluctuations in the areas this test would serve, ranging from roughly 25°C to 45°C on a daily basis (source: internal PATH data). It has also been noted that ocular trachoma is more prevalent in areas with high heat and low relative humidity.⁵ Such variability is unavoidable without cold chain support, so the test must be robust enough to endure these fluctuations long enough to preserve a usable shelf life. Additionally, it would be ideal for the test to have an on-board temperature and humidity indicator alerting extreme conditions exposure.

2. Design

2.1 Analyte (diagnostic marker)

Acceptable: *Chlamydia trachomatis* **antigens**, species specific (potentially pgp3 and CT 694).

Ideal: *Chlamydia trachomatis* **antigens**, ocular trachoma serovar specific.

Chlamydia is a genus of bacteria that are obligate intracellular parasites. Blinding trachoma and urogenital chlamydia are caused by the same *C. trachomatis* species, but differ in serovars (See Appendix D). Serovars A, B, Ba, and C cause ocular trachoma and are localized to epithelial surfaces in the eye, while serovars D through K localize to epithelial surfaces in the genital tract and thus cause chlamydia urogenital infections, though they are also implicated in bacterial conjunctivitis. Recently reclassified as a separate genus, *Chlamydophila* and its associated species, *pneumonia* and *psittaci*, share many molecular similarities with *Chlamydia* and thus diagnostic technologies must appropriately differentiate between these species.

C. trachomatis has a unique life cycle. It is an obligate intracellular bacterium that is found in two forms: an elementary body (EB) and a reticulate body (RB). The EB is the infectious particle responsible for the

bacteria's ability to spread from person to person, analogous to a spore, and is released when the bacterium's host cell ruptures. It is covered by a cell wall and contains, among other cellular structures, a single DNA genome and cryptic DNA plasmids. EBs induce endocytosis into target cells and then differentiate into RBs which are responsible for intracellular replication, multiplying by binary fission. After division, RBs transform back into EBs and are released by the host cell by exocytosis (see Appendix C for a graphical representation of the *Chlamydia* life cycle).³

Current targets for ocular trachoma diagnostics include *C. trachomatis* antibodies, antigens, and nucleic acid, either from the DNA genome, cryptic DNA plasmid, or ribosomal RNA. Notably, the identified analytes to date only detect *C. trachomatis* at the species level and are not ocular serovar specific. This does present the possibility of detecting non-ocular trachoma infections. For an antigen or nucleic acid test that uses conjunctival swabs, the assumption moving forward is that the prevalence of urogenital conjunctivitis in the main survey population (i.e., children less than ten years old) is low and likely represents an insignificantly small portion of positive cases.

The RDT must detect *C. trachomatis* antigens. While ideally the targets would be ocular trachoma serovar specific, current research has only identified species-specific targets. Two antigens have been identified as candidates for a lateral flow immunoassay: *C. trachomatis* antigens pgp3 (pCT03) and CT694. PGP3 is encoded as an ORF5 of the eight total ORFs on the highly conserved cryptic plasmid and is rarely found in *C. pneumonia* isolates.¹⁹ CT694 is a secreted protein involved in pathogenesis that manipulates host proteins by acting as a T3S-dependent substrate.²⁰ These two antigens were first identified as part of a chlamydia antigen-mapping project that assessed antibody responses in women with urogenital chlamydia infections. They were two of the 27 antigenic proteins that were recognized by more than 50% of women's antisera, thereby receiving the designation *immunodominant antigens*.¹⁰ They were then reported to elicit antibody responses in blood samples taken from children in trachoma-endemic regions, with stronger antibody responses elicited from children more than three years old with evidence of active infection or PCR-positive results, thereby suggesting they may play an active role in ocular trachoma.⁹

Using both antigens as targets, as opposed to one alone, may improve performance. For example, in cryptosporidium tests, two antigens are used: one creating a long-lived and one a short-lived antibody response. For malaria RDTs, adding a second antigen boosts sensitivity, while for the lymphatic filariasis RDT, adding a second antigen improves specificity to rule out false positives.

2.2 Sample type and volume

Acceptable: Dry ocular conjunctival swab; only one swab required.

Ideal: Dry ocular, oral, nasopharyngeal, or tear swab; only one swab required.

C. trachomatis is known to invade mucosal epithelial cells. For antigen-detection methods, epithelial cell specimens should be collected by vigorous swabbing of the involved sites. Purulent discharges that lack infected epithelial cells are inappropriate and should be cleaned from the site before the sample is collected. For ocular trachoma, the only known appropriate sample site is the conjunctiva.²¹ Only one swab should be required.

Ideally, the test will be compatible with a variety of swabs including nasopharyngeal areas, such as the mouth or nose, as well as tears so that samples may be obtained from a variety of areas for patients that may be experiencing too much pain to effectively swab the conjunctiva. Research on antigen detection in these areas is still required.

A more acceptable sample type would be blood from a fingerstick. Surveillance teams are more accustomed to this collection method, and moreover ocular swabs do not allow for harmonization with other NTD sampling. Yet blood specimens are currently not recommended for an antigen-detection test. The immune responses detected for *C. trachomatis* mucous membrane infections are often short-lived or due to past infections, making serologic samples less reliable.²⁰

2.3 Sample preparation

Acceptable: Minimal collection or processing step.

Ideal: No sample preparation required.

As the target locations are either individual households or schools, the sample preparation must be minimal and appropriate for the available infrastructure and personnel on-site.

There are RDTs commercially available for urogenital chlamydia that do not require sample preparation. The endocervical or vaginal swab is inserted directly into the test reagents and then applied to the lateral flow strip.²² A similar method for ocular swabs would be ideal.

2.4 Sample transport stability

Acceptable: No sample transport.

Ideal: No sample transport.

This technology should be field-deployable with samples tested on-site. No sample transportation is anticipated.

2.5 Waste management (hazardous materials/chemicals)

Acceptable: Minimal or no hazardous materials, per WHO and country standards.

Ideal: Minimal or no hazardous materials, per WHO and country standards.

The test should not contain hazardous reagents per WHO and in-country safety, environmental, and transport requirements. Any hazardous waste in the form of biologic specimens should be contained on the diagnostic device and disposed of appropriately.

2.6 Nature of result

Acceptable: Qualitative.

Ideal: Qualitative.

The lateral flow platform is traditionally a qualitative test based on a specific limit of detection. A qualitative result is sufficient to achieve the goal of making accurate MDA stopping decisions.

2.7 Time to result

Acceptable: Same-day result, < 24 hours.

Ideal: Same-day result, \leq 15 minutes.

This test is primarily focused on informing public health-based decision-making rather than clinical case management. Thus time to result is not necessarily bound to the logistics of the clinical intervention, but instead should be compatible with the workflow of surveillance teams. Ideally the total time needed from sample collection to result should fit within the team's workflow such that the team may meet the daily testing goals. The need for a quick turnaround time is based on those countries whose teams use positive cases to determine if continued surveillance is needed during a same-day site visit. As noted above, countries like Mali and Ghana screen small samples and, based on the sample prevalence, determine if continued same-day surveillance in the area is required.

2.8 Throughput

Acceptable: > 50 samples per day.

Ideal: > 100 samples per day.

The RDT should match or exceed the throughput of existing practices. An average of 100 people are screened per WHO clinical exam criteria daily. Sampling frequency is expected to decline slightly given the unique challenges of obtaining ocular swabs. Thus, the RDT should process approximately 50 to 100 samples per day. For comparison purposes, during evaluations on an RDT for onchocerciasis based on the Ov16 antigen, which uses a finger stick blood sample, current throughput was approximately 75 samples per day (source: internal PATH data).

2.9 Instrumentation format and complexity level

Acceptable: Simple lateral flow assay with minimal user steps.

Ideal: Simple lateral flow assay with minimal user steps.

The format should be a lateral flow test. The test strip should be one small, single-use device. An additional component, such as a reader, may be acceptable pending size and ease of use. The level of complexity should be consistent with the site where it is used and the end-user. It should consist of only a few timed steps, ideally only one, and not require highly technical skill steps such as precision pipetting. Results should be simple to interpret.

2.10 Infrastructure requirements

Acceptable: Minimal, consistent with Tier 2 facility.

Ideal: Minimal, consistent with Tier 2 facility.

Trachoma is endemic in low-resource and underdeveloped regions where access to general health infrastructure is very limited. Therefore, to access the desired target populations, any field-based test should not depend on any infrastructure beyond basic shelter in a community environment. There may be no access to consistent electrical power, and clean water may be limited.

2.11 Test-specific training requirements

Acceptable: Minimal, 1 day.

Ideal: Minimal, 1/2 day.

Based on the target user and location of use, any test-specific training needs to be minimal and not technical in nature.

2.12 Instrumentation size and weight

Acceptable: Small, easily deployable in the field.

Ideal: Small, easily deployable in the field.

The RDT itself should be small, light-weight, and easily portable for field-surveillance teams. Standard Diagnostic's (SD) current lateral flow test for urogenital chlamydia is approximately 7cm x 2cm x 0.5cm and weighs 4g. This is an acceptable size for an ocular trachoma RDT. There should ideally not be an instrument required beyond the test itself. If there is an additional instrument required, such as a reader, it should be small and easily deployable.

2.13 Ancillary supplies

Acceptable: Minimal supplies to ensure optimal test performance, packaged as a kit.

Ideal: None.

A testing platform that is field-deployable requires that ancillary supplies be minimal. If supplies are necessary to ensure optimal sensitivity, such as specimen concentration, or quality control, such as verification cartridges, this may be acceptable. Ideally, no instruments or other supplies are required.

SD's commercially available chlamydia RDT kits include the test device, two reagents, sterile swabs, transport tube, and a disposable dropper.²¹ This would be an acceptable kit for an ocular chlamydia RDT.

2.14 Mean time between failures

Acceptable: Not applicable.

Ideal: Not applicable.

This attribute is not applicable for a single-use lateral flow test.

2.15 Quality control

Acceptable: Internal control line, industry standards for positive and negative external controls.

Ideal: Internal control line, industry standards for positive and negative external controls.

The lateral flow test should include an internal control with a visible control line to ensure accuracy of the test results. The manufacturer should maintain appropriate industry-quality standards for external controls. Positive and negative controls are necessary for each test or batch of tests.

2.16 Calibration

Acceptable: Minimal, not required in field.

Ideal: None.

Ideally, no calibration would be required, especially if there is no ancillary instrument in addition to the test itself. If required, the interval between calibrations should be sufficiently long to not burden surveillance teams.

2.17 Product shelf life

Acceptable: 12 months.

Ideal: 36 months; packaging should include thermal indicator.

In-country experience has shown that a shelf life less than six months is insufficient as the time frame post-manufacturing but prior to purchase and delivery could be three months or more. It is suggested that a shelf life of one year is acceptable, and as many as three years would be closer to ideal.

3. Performance

3.1 Analytical limit of detection

Acceptable: $\leq 2 \times 10^4$ elementary bodies (EBs)/mL.

Ideal: ≤ 2 EBs/mL.

Analytical limit of detection is the lowest level of target analyte that an assay will detect. Acceptable limit of detection would be dependent on the correlation between limit of detection and clinical sensitivity, which would be specific to the test design. Acceptable levels, therefore, would achieve the desired clinical sensitivity needed to detect cases that are positive for active trachoma infection.

As reported in the 2004 Solomon et al paper published in *Clinical Microbiology Reviews*,⁸ the following lower limits of detection of the bacterium's main infectious particle, the EB, were identified for various assays. These assays detected dilutions of purified *C. trachomatis* EBs from urogenital serovar K spiked into urine, peripheral blood, and peripheral blood leukocytes:

Figure 3. Lower limits of elemental body (EB) detection by assay type.²³

Assay type	Brand name ^a	Urine EBs/mL	Peripheral blood EBs/mL
PCR	N/A (in-house)	2	100
Direct fluorescent antibody	MicroTrak	2×10^3	2×10^7
Enzyme immunoassay	ChlamydiaEIA	2×10^3	UD
Nucleic acid hybridization probe	IDEIA	2×10^4	UD
Immunoassay	PACE 2	2×10^4	UD

UD = undetectable

^a PCR (in-house), target is chlamydia MOMP gene-specific DNA sequence. MicroTrak (Syva Co., Palo Alto, Calif.), target is chlamydia MOMP. ChlamydiaEIA (Syva Co., San Jose, Calif.), target is chlamydia LPS. IDEIA (Dako Diagnostics Ltd., Cambridge, England), target is chlamydia LPS. PACE 2 (Gen-Probe Inc., San Diego, Calif.), target is chlamydia rRNA.

With a similar dilution series, and assuming the presence of ten plasmids per organism, another study compared performances of separate commercially available PCR assays using urethral and endocervical swab specimens. It concluded the detection limits of an in-house PCR versus the COBAS® Amplicor (F. Hoffmann-La Roche AG, Basel, Switzerland), the Amplicor plate kit (F. Hoffmann-La Roche AG), and the LCx (Abbot Laboratories, Chicago, Illinois) PCR assays to be approximately 1, 1 to 2, 2 to 4, and 2 EBs (per tested aliquot), respectively.²⁴

These results show the tissue selectivity of *C. trachomatis* with only the PCR and direct fluorescent antibody assays detecting EBs in peripheral blood. They also show the superior limit of detection of the NAAT assay. Recognizing the potential for a moderate performance loss with a field-deployable RDT, the acceptable minimum level of detection for an immunoassay-based RDT is assumed to be equivalent to the laboratory-based immunoassay, approximately $\leq 2 \times 10^4$. Note that whether an RDT can achieve such limits of detection is unknown at this time. The ideal assay would match the NAAT sensitivity.

3.2 Analytical specificity

Acceptable: *Chlamydia trachomatis* species-specific Ag (serovars A–K).

Ideal: *Chlamydia trachomatis* ocular serovars only Ag (A–C).

Analytical specificity is defined as how well the assay detects specific analyte and not closely related analytes. The assay must be able to distinguish *C. trachomatis* versus *C. psittaci* and *C. pneumonia*. As noted above in the Analyte section, a biovar-level target found across serovars A through K may be acceptable, though ideally an ocular trachoma-specific serovar can be utilized.

3.3 Clinical sensitivity

Acceptable: > 70%.

Ideal: > 90%.

Clinical sensitivity is the true positive rate which is the probability that a diseased individual gives a positive test result. Clinical sensitivity is affected by prevalence levels. For disease mapping or MDA monitoring, a relatively lower sensitivity may be sufficient as prevalence is likely high and missed cases will not significantly impact outcomes. However, as the stopping decision approaches and prevalence is significantly reduced, presumably below 5%, the test's clinical sensitivity must be high. Priority at the stopping decision is to identify as many cases as possible since there will likely be few and each positive is of increased importance.

The RDT must out-perform the current standard, which in the field is the WHO clinical diagnostic criteria. Studies show that TF findings on physical exam maintain sensitivities around 80% to 90%, though may decrease to 30% to 70% post MDA when prevalence is presumably low.^{7,25,26} These results again highlight the lack of correlation between clinical findings and active infection. Thus an acceptable minimum is a sensitivity greater than 70%.

The ideal sensitivity would compare favorably to NAAT assays that maintain sensitivities greater than 90%. See Figure 4 for a performance comparison of assays.

Figure 4. Comparison of assays for diagnosis of C. trachomatis infection. (Adapted from Solomon et al 2004)^{a,8}

Test	Detection target	Specimen	Sensitivity (%)	Specificity (%)
Culture	Infectious organism	Conjunctival swab	50–70	100
Enzyme immunoassay				
Lab-based	Antigen	Conjunctival swab	60–85	80–95
Rapid test²⁷	Antigen	Vaginal, cervical, urethral swabs and first void urine	50–80 ^b	97–99
Nucleic acid hybridization	DNA	Conjunctival swab	60–80	95–100
Nucleic acid amplification	DNA or RNA	Conjunctival swab	90–100	95–100

^a Performance compared against a reference standard of culture and / or nucleic acid amplification test.

^b One study showed that sensitivity of a urogenital chlamydia RDT decreased from 65% to 25% when conducted in a high-prevalence population versus a low-prevalence population.²⁸

3.4 Clinical specificity

Acceptable: > 95%.

Ideal: > 99%.

Clinical specificity is the true negative rate which is the probability that a healthy individual gives a negative test result. This also becomes increasingly important as prevalence is reduced. At high prevalence, a 5% false-positive level is not a barrier, but as the stopping decision is approached, the level of false positives should be some fraction of the prevalence level. It is presumed that all positives detected

at the stopping-decision phase will be investigated and retested with the same or an alternative testing method. However, to avoid overburdening the surveillance program, samples requiring follow-up should be kept to a minimum.

TF findings vary widely in specificity, ranging from 37% in one study to over 98% in another, again emphasizing the need for a better diagnostic tool.^{7,25,26} The majority of the assays listed above in Figure 4 maintain a specificity > 95%, including the rapid tests. This is thus the minimum acceptable limit. The ideal is equivalent to a second-generation rRNA-based NAAT which performs at > 99% specificity.²⁶

3.5 Reproducibility and robustness

Acceptable: Replicate determinations of weak positive samples classify the same $\geq 95\%$ of the time.

Ideal: Replicate determinations of weak positive samples classify the same $\geq 95\%$ of the time.

A lateral flow RDT requires the user to interpret results. While a bright, clearly demarcated line mirroring the control is consistently interpreted as a positive test, faint lines (i.e., “weak positives”) may be misinterpreted as negative results. This is a particular concern in field settings where users have varying degrees of training and on-site conditions can affect vision and test readability. It is thus critical that the test maintains a high level of robustness, absorbing potential technician-to-technician and site-to-site variability while not impacting accuracy of interpretation. The test must produce a result that maintains a reproducibility of $\geq 95\%$ whereby weak positives are consistently identified as positive.

3.6 Comparative reference method

Acceptable: Performance comparable to a current regulatory-approved NAAT.

Ideal: Performance comparable to a current regulatory-approved NAAT.

The gold standard for performance metrics is the reference lab-based NAAT. Researchers differ on whether a DNA versus RNA and qualitative versus quantitative test is the true standard. Regardless, the RDT must be compared to a current traditional regulatory-approved NAAT. Outside of the research community, in-country trachoma surveillance teams still use the WHO’s clinical criteria. Although as noted above, this approach is neither sufficiently sensitive nor specific for MDA stopping decisions, particularly in low-prevalence areas, all diagnostic studies developed to date have been assessed against clinical exam performance. Thus, this too must be assessed.

4. Commercialization

Research on the commercialization attributes is ongoing. Further detail will be added as it is available.

4.1 Desired end-user price

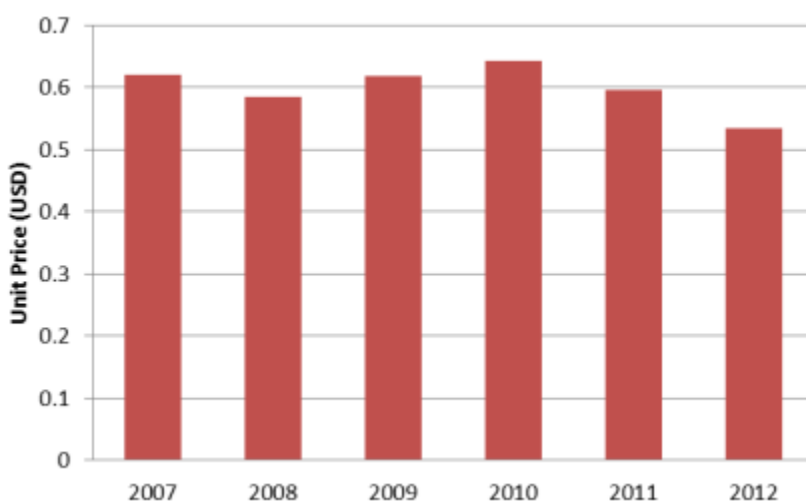
Acceptable: < \$2.00 per lateral test strip.

Ideal: < \$1.00 per lateral test strip.

The end-user price for a lateral flow RDT should be comparable to the prices of other commercially available RDTs in low-resource settings. These prices reflect the current market value of POC tests within the global health market place.

Per a 2012 United States Agency for International Development (USAID) report, the average malaria RDT price is \$0.60 per test. Figure 5 displays the changing RDT price over time as found in this report. Although the prices of individual tests decreased over time, the procurement of new, more expensive tests offset the decrease in the older tests, keeping the overall average somewhat constant. The average unit price decreased in 2012 because this particular USAID project did not procure any new types of tests. The reported unit price maximum was \$1.05/RDT and the minimum was \$0.29/RDT from 2007 to 2012.²⁹ Unit prices did vary based on order volume. The RDT for HIV also serves as a potential benchmark. A 2009 report on HIV diagnostic pricing from the Clinton Foundation lists three suppliers with unit prices under \$1, and one outlier charging \$1.60 per test.³⁰

Figure 5. Average unit prices of malaria RDTs, 2007–2012.²⁹



The low cost of an RDT is one strong reason for adopting such a technology and thus must be upheld. This is in contrast to the more expensive NAAT- and ELISA-based laboratory assays. The negotiated average price for a NAAT test in low-resource settings is approximately \$10 to \$11 per test, including ancillary supplies. However, additional costs are incurred with this assay, including but not limited to sample transportation, laboratory staff, and technology delivery and maintenance. ELISA tests may vary, but one manufacturer agreement is \$2.50 for a single test, and then \$0.20 for each additional test (source: internal PATH data).

4.2 Channels to market

Acceptable: To be determined.

Ideal: To be determined.

No data are currently available.

4.3 Supply, service, and support

Acceptable: To be determined.

Ideal: To be determined.

No data are currently available.

4.4 Product registration path and WHO prequalification

Acceptable: Not required for surveillance tests.

Ideal: Not required for surveillance tests.

No data are currently available.

Appendices

Appendix A-1: List of commercially available NAAT tests for ocular trachoma.

Test Brand Name	Manufacturer / Location	Target
COBAS® TaqMAN® Analyzer*	Roche Diagnostics Indianapolis, IN USA	DNA
Aptima Gen-probe	Gen-Probe San Diego, CA USA	RNA
RealTime CT / NG	Abbott Molecular Abbott Park, IL USA	DNA
Abbott's m2000	Abbott Molecular Abbott Park, IL USA	DNA
Cepheid GeneXpert®	Cepheid Sunnyvale, CA USA	DNA
ProbeTec™ ET System	Becton, Dickinson and Company (BD) Franklin Lakes, New Jersey	DNA

**NOTE: The COBAS® replaced the Roche Amplicor PCR which is no longer in production.*

Appendix A-2: Parameters of commercially available NAATs for ocular trachoma diagnosis.

	COBAS CT/GC test	Abbott Realtime CT/GC on the m2000 System	Gen-Probe Aptima	ProbeTec ET System	GeneXpert
Manufacturer	Roche, USA	Abbott, USA	Gen-Probe, USA	BD, USA	Cepheid, USA
Technology	NAAT Real-time PCR	NAAT Real-time PCR	NAAT TMA ¹	NAAT SDA ²	NAAT Real-time PCR
Location of use	reference laboratory	reference laboratory	reference laboratory	reference laboratory	reference laboratory
User	trained lab technician	trained lab technician	trained lab technician	trained lab technician	trained lab technician
Diagnostic target	DNA (Cryptic Plasmid and Chromosomal Gene)	DNA (Cryptic Plasmid)	RNA (Ribosomal)	DNA (Cryptic Plasmid)	DNA (Chromosomal Gene)
Sample type³	conjunctival swab	conjunctival swab	conjunctival swab	conjunctival swab	conjunctival swab
Sample volume	dry swab	dry swab	dry swab	dry swab	dry swab
Sample preparation and extraction⁴	Semi-automated	Semi-automated	Manual or Automated (automated systems available [Tigris DTS and Panther])	Manual or Automated (automated systems available [Viper])	Fully-integrated (using GeneXpert System)
Amplification / Detection⁴	Automated (uses COBAS z 480 Analyzer)	Automated (uses m2000rt instrument)	Semi-Automated or Automated (Leader HC + Luminometer or Tigris DTS or Panther)	Semi-Automated to Automated (Heat Block +ProbeTec ET instrument or Viper)	Fully-integrated (using GeneXpert System)
Level of detection	100-1000 EB/mL	100-1000 EB/mL	< 10 EB/mL	100-1000 EB/mL	< 100 EB/mL
Result type	qualitative	qualitative	qualitative	qualitative	qualitative
Time to results (Sample to Answer)	~4 hours	~4 hours	~4.5 hours	~4 hours	90 minutes
Hardware / ancillary supplies	1) Amplicor CT/NG Sample Prep Kit 2) COBAS x 480 for sample prep 3)COBAS z 480 TaqMAN Analyzer 4) Vortex 5) Pipettes 6) Centrifuge	1) Abbott collection kit; 2) m2000sp instrument; 3) m2000rt instrument; 4) Vortex; 5) Pipettes, 6) Centrifuge	1) GeneProbe Aptima collection kit 2) SB100 Dry Heater/Dry Bath 3) Target Capture System, 4) Leader HC+ Luminometer 5) Vortex 6) Pipettes 7) Centrifuge [Automated systems available: Panther or Tigris TDS]	1) Sample collection kit 2) Lysing rack and heater, 3) Priming well and heater, 4) ProbeTEC amplification and detection instrument 5) Pipettes 6) Centrifuge [Automated systems available: Viper]	1)Cepheid Xpert CT/GC Specimen Collection kit 2) GeneXpert Cartridge 3) GeneXpert System
Commercially available	Yes	Yes	Yes	Yes	Yes
Stability, storage, and cold chain requirements	Up to 18 months/2°C–25°C	18 months at -10°C/18 months at 15°C – 30°C;	18 months/Refrigeration required for some reagents (2°C–8°C)	18 months/Room temperature (2°C – 33°C)	Up to 2 years/Room Temperature (2°C – 33°C)
End-user price (\$ / test)*	Unknown	\$11	\$9.50	Unknown	\$10

*Negotiated price for low resource settings.

¹ Transcription mediated amplification

² Strand displacement DNA amplification

³ Use of conjunctival swabs have been strictly RUO. Currently, all commercial tests are only approved for use with urogenital specimens

⁴Semi-automated indicates minimal manual steps such as pipetting and vortexing may be required

Appendix B-1: Distribution of trachoma worldwide, as of 2010.¹³

Map 1 **Distribution of trachoma worldwide, 2010**
Carte 1 **Distribution du trachome dans le monde, 2010**



Appendix B-2: Global estimates of total populations in endemic areas and trachoma cases, by WHO region, 2011.¹³

Region – Région	Population living in endemic areas (millions) – Population vivant en zone d'endémie (en millions)	Active trachoma cases (thousands) – Cas de trachome évolutif (en milliers)	Trachomatous trichiasis a cases (thousands) ^a – Cas de trichiasis trachoma- teux a (en millier) ^a
African – Afrique	231.27	18 287.05	3 202.20
Americas – Amériques	0.28	3.04	48.89
South-East Asia – Asie du Sud-Est	6.21	196.19	497.67
Eastern Mediterranean – Méditerranée orientale	82.31	2 847.82	1 091.79
Western Pacific – Pacifique occidental	4.78	101.08	2 420.42
Global – Monde	324.85	21 435.18	7 260.96

^a Trachomatous trichiasis: ≥1 eyelash rubbing on the eyeball or signs of removal of the eyelash – Trichiasis trachomateux: ≥1 cil frottant le globe oculaire ou signes d'élimination du cil

Appendix C: *Chlamydia* life cycle.³

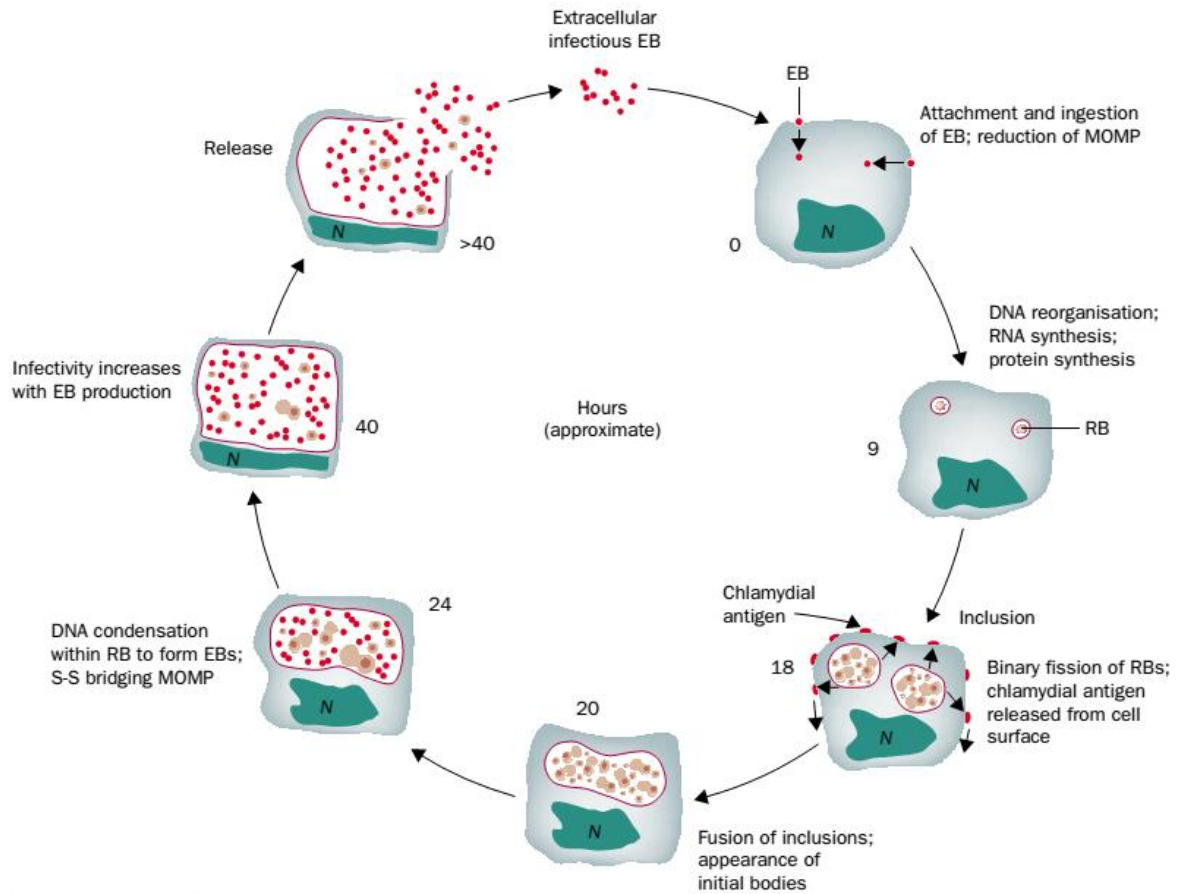
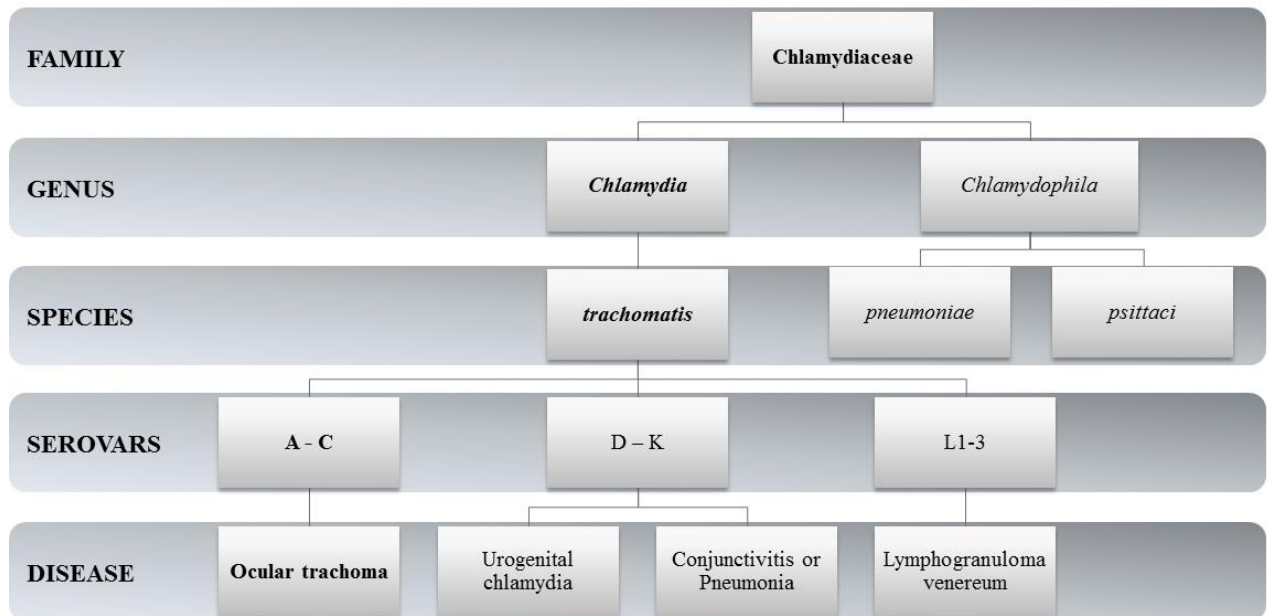


Figure 1: **Life-cycle of *C. trachomatis***

EB=elementary body; MOMP=major outer membrane protein; RB=reticulate body. Adapted from Barron.¹⁰

Appendix D: *Chlamydia* classification, human biovars only.



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