

Target Product Profile

Trachoma Surveillance Diagnostic

Use case: Post-elimination surveillance

Platform: Lateral flow test

Biomarker: Antibody

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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 **S**urgery, **A**ntibiotics, **F**acial cleanliness, and **E**nvironmental 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 accurate **post-elimination surveillance**. 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 antibodies.

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)	Post-elimination surveillance after stopping mass drug administration (MDA).	Post-elimination surveillance after stopping mass drug administration (MDA).
1.3 Target populations	Children 1 to 5 years old, born after transmission interruption.	Children 6 months to 10 years old, born after transmission interruption.
1.4 Target countries/geographic coverage	Trachoma-endemic countries where MDA for trachoma has recently stopped.	Trachoma-endemic countries where MDA for trachoma has recently stopped.
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 team 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 workflow. Linkage to action will be assessing disease exposure and determining MDA effectiveness.	Direct replacement of WHO clinical exams, limited to no impact on workflow. Linkage to action will be assessing disease exposure and determining MDA effectiveness.
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.

Attribute	Acceptable	Ideal
2. Design		
2.1 Analyte (diagnostic marker)	<i>Chlamydia trachomatis</i> antibodies , species species-specific.	<i>Chlamydia trachomatis</i> antibodies , ocular trachoma serovar-specific
2.2 Sample type and volume	Finger stick whole blood sample, 100µL.	Finger stick whole blood sample, 15µL.
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, less than 1 day.	Minimal, less than 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)	To be determined.	To be determined.

Attribute	Acceptable	Ideal
3.2 Analytical specificity	Species-specific <i>Chlamydia trachomatis</i> antibodies, which include all serovars A through K.	<i>Chlamydia trachomatis</i> ocular serovars only, serovars A–C.
3.3 Clinical sensitivity	> 75%.	> 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	Standardized immunoassay.	Standardized immunoassay.
4. Commercialization		
4.1 Desired end-user price	< \$2.00 per test.	< \$1.00 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 test 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 test is required to monitor progress toward the GET 2020 goals of eliminating trachoma by the 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 improved diagnostic tools to inform the stopping decision of antibiotic distribution and subsequent surveillance for disease exposure.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 PCR 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 viable targets and create diagnostics specific to ocular trachoma that will enable the development of a high-performing RDT immune-based assay.^{9,10} The immune targets for monitoring disease exposure post-MDA completion are antibodies, in contrast to antigens that indicate active infection and would be used for MDA stopping decisions. In theory, if MDA has indeed ceased the transmission of *C. trachomatis*, then those children born close to or post the MDA stopping decision point should test negative for an antibody response as they will not have been exposed to the bacteria. The term post-elimination is used for the period after stopping MDA.

This TPP focuses on a technology that enables accurate post-elimination surveillance of persistent disease exposure based on an immunoassay platform that uses antibody targets.

1.2 Intended use (use case)

Acceptable: Post-elimination surveillance after stopping MDA.

Ideal: Post-elimination surveillance after stopping MDA.

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*).

After stopping MDA, trachoma control programs must monitor for recrudescence of disease transmission, requiring conclusive data that transmission has been interrupted, or at least reduced to the point where trachoma no longer represents a public health problem. The RDT is expected to 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 100–300 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, though in some countries they survey children in schools or clinics. Exam findings are recorded and the data are 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 finger stick blood samples for on-site processing and determination of post-elimination incidence of disease exposure.

1.3 Target populations

Acceptable: Children 1 to 5 years old, born after transmission interruption.

Ideal: Children 6 months to 10 years old, born after transmission interruption.

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 starting 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. Notably, if a species-level antibody target is utilized, then children greater than five years old should not be included in the study population, as they may have been exposed to a sexually transmitted *C. trachomatis* strain. While neonatal transmission is still a risk, it is believed that this would be a small, insignificant portion of positive cases.

1.4 Target countries/geographic coverage

Acceptable: Trachoma-endemic countries where MDA for trachoma has recently stopped.

Ideal: Trachoma-endemic countries where MDA for trachoma has recently stopped.

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.) This test will specifically be used in areas where MDA has been stopped and programs are currently performing post-elimination surveillance activities. These activities will therefore only be performed in countries in which trachoma is or has been endemic.

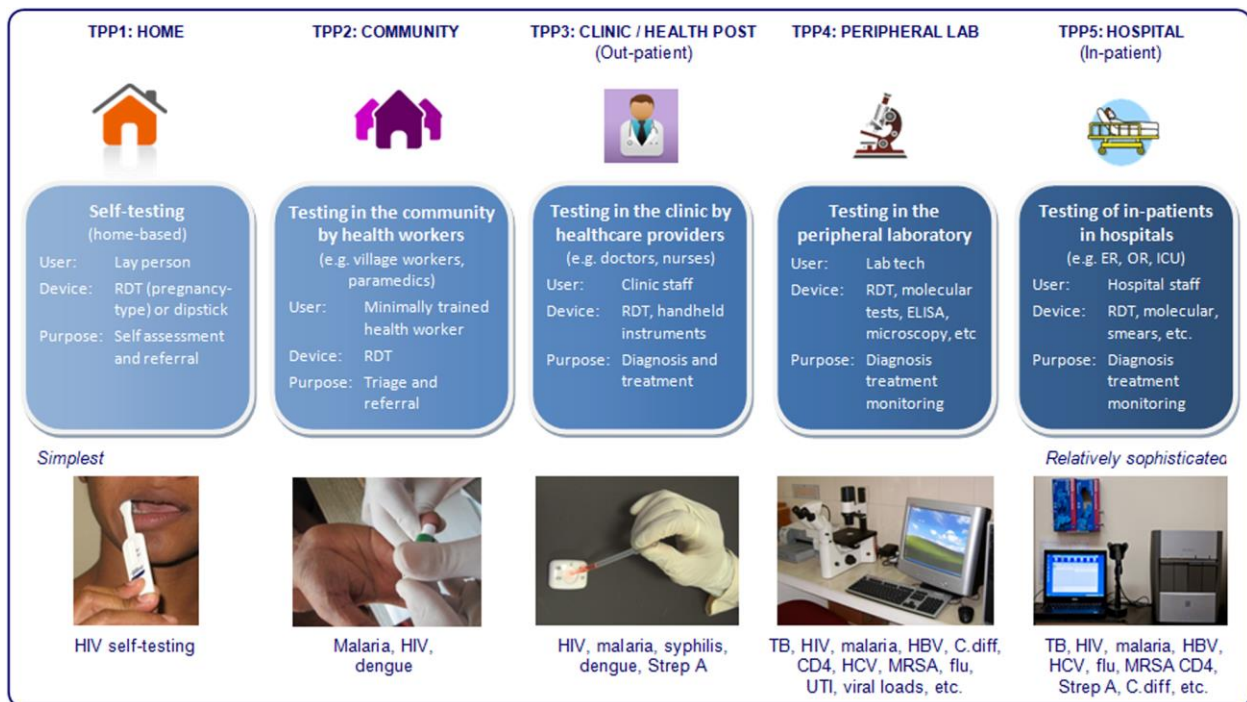
1.5 Location of use (infrastructure level)

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 professional trained in eye exams.

Ideal: Surveillance team 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

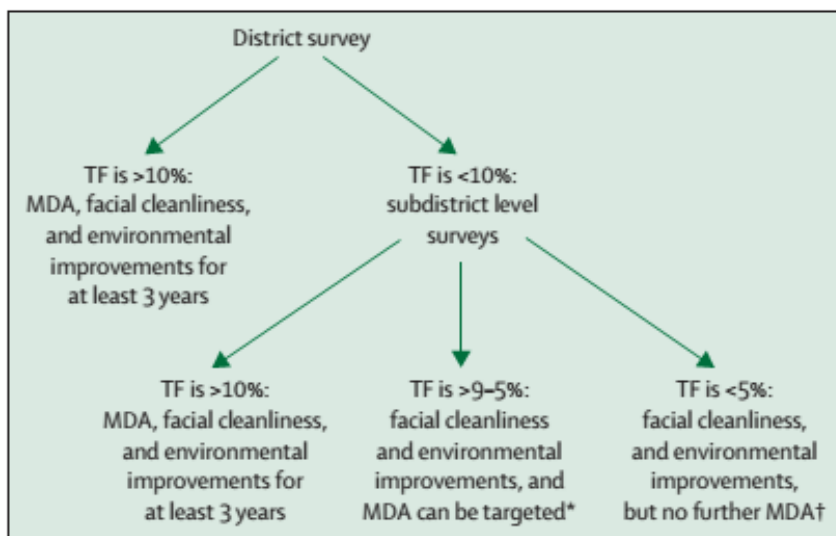
Acceptable: Direct replacement of WHO clinical exams, limited to no impact on workflow. Linkage to action will be assessing disease exposure and determining MDA effectiveness.

Ideal: Direct replacement of WHO clinical exams, limited to no impact on workflow. Linkage to action will be assessing disease exposure and determining MDA effectiveness.

It is expected that obtaining finger stick blood samples and processing RDTs immediately on-site will replace current practices of on-site clinical exams, thereby minimizing impact on clinical workflow in the field.

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. Provision of the antibiotic to an entire community is referred to as 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 decision to initiate or stop 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* **antibodies**, species-specific.

Ideal: *Chlamydia trachomatis* **antibodies**, 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. This proves a greater challenge for serologic antibody-detection technologies as both neonatal and sexual transmission of urogenital chlamydia are possible and can falsely impact study results.

The RDT must detect *C. trachomatis* antibodies. While ideally the targets would be ocular trachoma serovar specific, current research has only identified species-specific targets. Two antigens have been identified as potential 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 *Chlamydia pneumonia* isolates.¹⁹ CT694 is a secreted protein involved in pathogenesis that manipulates host proteins by acting as a T3S-dependent substrate. 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 over 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 a species-level target, however, presents the possibility of inappropriately including results from past urogenital and neonatal *C. trachomatis* transmission. A serovar-specific target is preferred in order to capture only ocular trachoma exposures. Further research is required to identify such targets.

2.2 Sample type and volume

Acceptable: Finger stick whole blood sample, 100µL.

Ideal: Finger stick whole blood sample, 15µL.

During an active infection, *C. trachomatis* invades mucosal epithelial cells. Thus for antigen and nucleic acid detection technologies, sampling requires conjunctival swabs. For an antibody-based immunoassay, however, a serologic sample is required. This is most easily obtained by a capillary finger stick collection method that obtains one droplet of whole blood. Surveillance teams are already accustomed to collecting finger stick samples, and this method allows for easier harmonization with other NTD programs that also rely on finger sticks. Ideally, a small-volume sample, such as approximately 15µL, would be sufficient.

2.3 Sample preparation

Acceptable: Minimal collection or processing step.

Ideal: No sample preparation required.

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

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 meet programmatic needs, specifically to determine the level of disease exposure following the MDA stopping decision point.

2.7 Time to result

Acceptable: Same-day result, < 24 hours.

Ideal: Same-day result, ≤ 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. Results should be the same day to expedite surveillance team workflow and travel, but could take hours if throughput is still reasonable. Since this test is primarily focused on surveillance rather than clinical case management, time to result is not necessarily bound to the logistics of the clinical intervention.

2.8 Throughput

Acceptable: > 50 samples per day.

Ideal: > 100 samples per day.

On average, surveyors currently screen 100 people per day by the WHO clinical exam criteria. The need for a finger stick blood sample is not anticipated to delay this throughput. Thus, ideally the RDT would match or even exceed this throughput, though at minimum should allow for at least 50 samples per day. For comparison purposes, during evaluations on an RDT for onchocerciasis based on the Ov16 antigen, which also 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 test with minimal user steps.

Ideal: Simple lateral flow test 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, less than 1 day.

Ideal: Minimal, less than 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 instrument 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 must 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.

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. 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: To be determined.

Ideal: To be determined.

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. Further research is required to determine acceptable and ideal levels of detection for a *C. trachomatis* immunoassay.

3.2 Analytical specificity

Acceptable: Species-specific *Chlamydia trachomatis* antibodies, which include all serovars A through K.

Ideal: *Chlamydia trachomatis* ocular serovars only, serovars 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 between species, specifically *C. trachomatis* versus *C. psittaci* and *C. pneumoniae*. Ideally, ocular trachoma-specific antibody targets can be identified and

utilized. This would exclude the possibility of picking up neonatal and sexual transmission of urogenital *C. trachomatis* strains.

3.3 Clinical sensitivity

Acceptable: > 75%.

Ideal: > 90%.

Clinical sensitivity is the true positive rate which is the probability that a diseased individual gives a positive test result. The desired clinical sensitivity will be affected by prevalence levels. For disease mapping, a relatively lower sensitivity may be sufficient as prevalence is likely high and missed cases will not significantly impact outcomes. However, prevalence is significantly reduced, presumably < 5%, when MDA is stopped. Thus the test's clinical sensitivity must be high.

The precise clinical sensitivity an immunoassay-based RDT must maintain is still to be determined. Multiple studies have been conducted to determine the performance metrics of various assays in diagnosing active disease. These results are summarized in Figure 3. Further research is still required, however, to determine the necessary performance of immunoassays in detecting prior disease exposure. Additional data are not currently available for this attribute.

Figure 3. Comparison of assays for the diagnosis of active *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 test²⁰	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.²¹

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 is a critical parameter, as false positives may artificially inflate the prevalence levels reported within a population, particularly as prevalence is reduced. As the prevalence is reduced to a level where an MDA stopping decision is made ($< 5\%$), the level of false positives should be of minimal contribution to the reported prevalence level.

As above, the precise clinical specificity an immunoassay-based RDT must maintain is still to be determined. Additional data are not currently available for this attribute.

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: Standardized immunoassay.

Ideal: Standardized immunoassay.

The gold standard to diagnose an active *C. trachomatis* infection is a NAAT. To determine past exposure, however, a reference laboratory-based immunoassay should be used. Currently an accepted comparative technology used across studies has not been determined but once chosen should be used. 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 post-MDA surveillance, 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

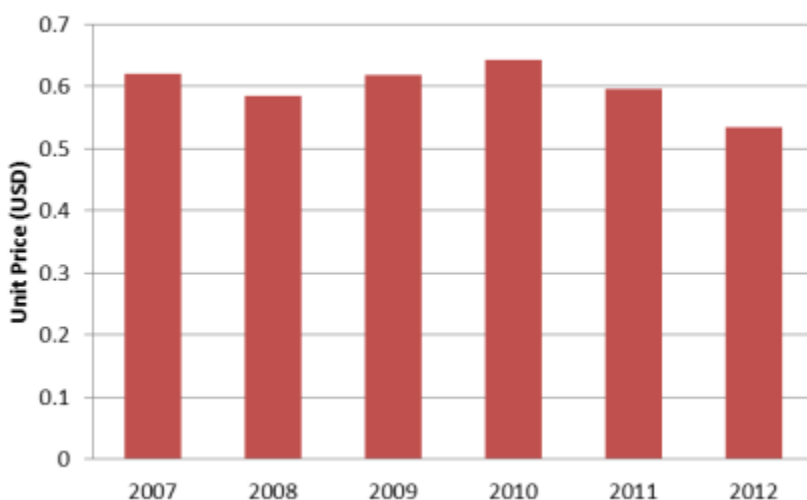
Acceptable: $< \$2.00$ per test.

Ideal: < \$1.00 per test.

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 4 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 2012.²² 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.²³

Figure 4. Average unit prices of malaria RDTs, 2007–2012.²²



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 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 type[‡]	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 extraction[^]	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 / Detection[^]	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 Speciment 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

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

[^]Semi-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 population in endemic areas and 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

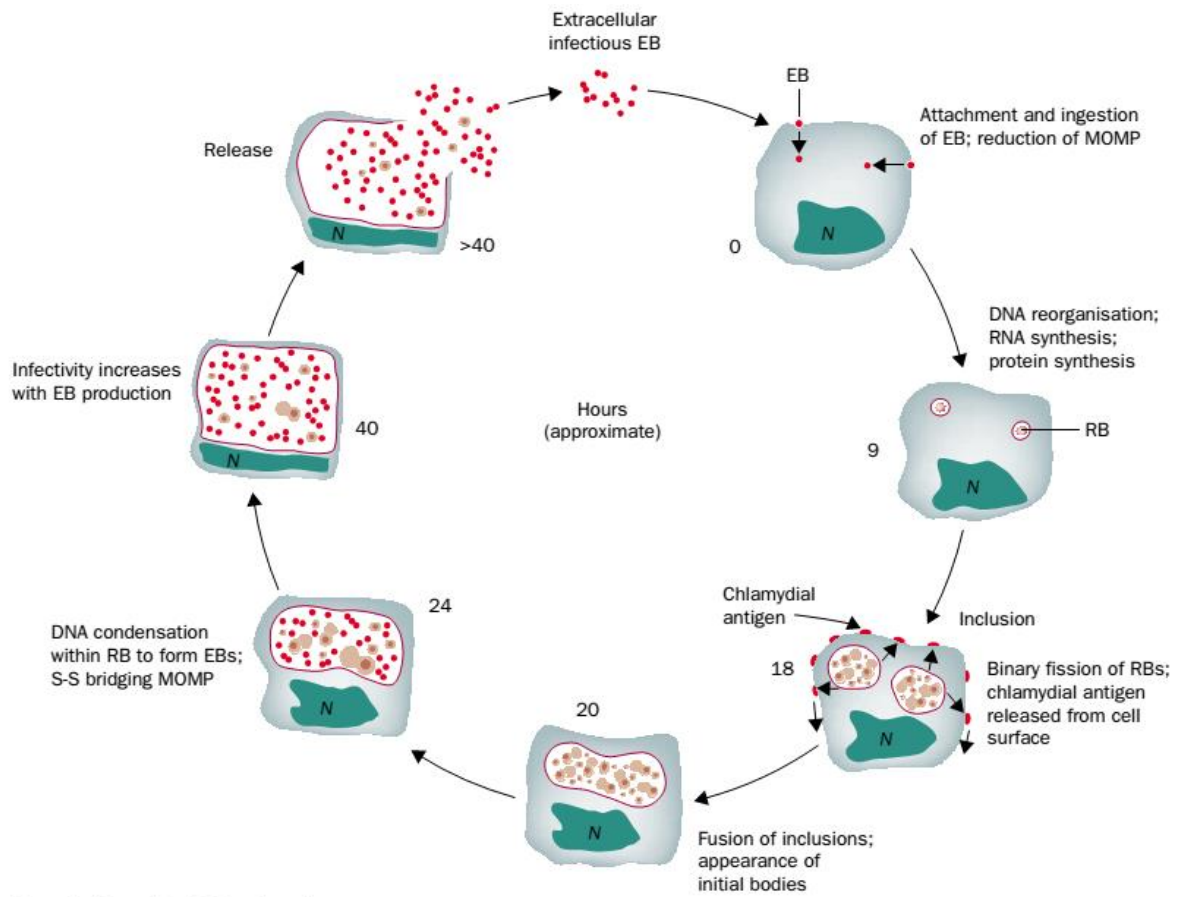
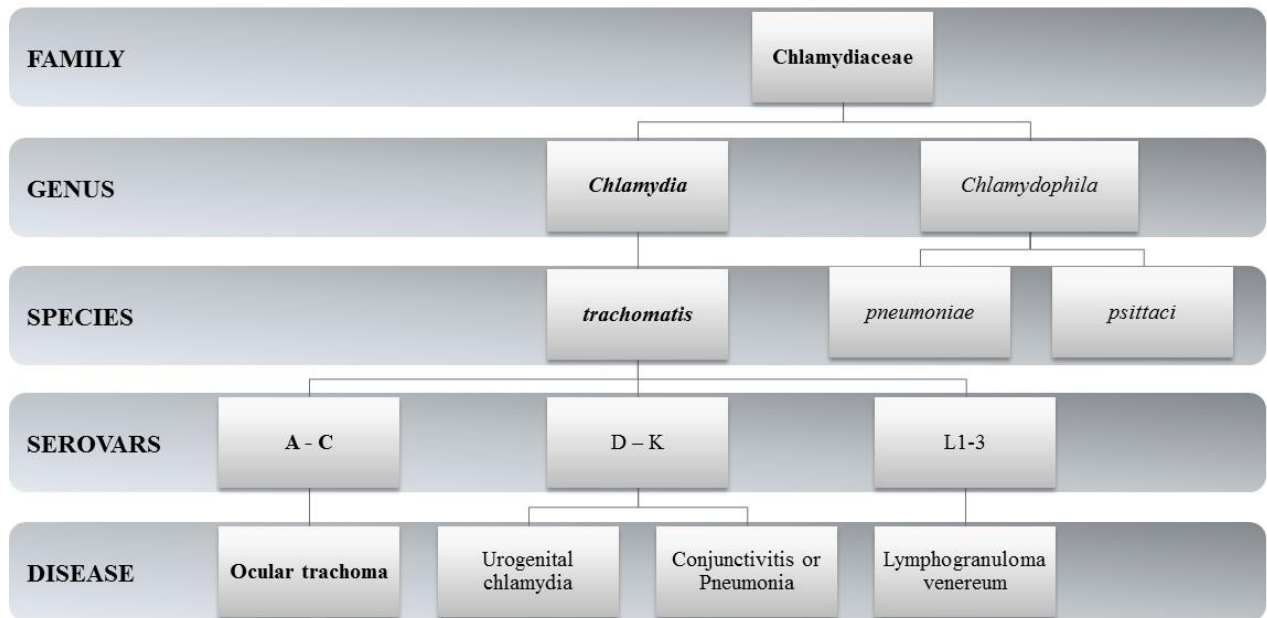


Figure 1: **Life-cycle of *C trachomatis***

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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