

WORLD HEALTH ORGANIZATION
STRATEGIC AND TECHNICAL ADVISORY GROUP
FOR NEGLECTED TROPICAL DISEASES
WORKING GROUP ON MONITORING AND EVALUATION

**TRACHOMA ALTERNATIVE INDICATORS STUDY
DATA REVIEW**

31 AUGUST – 1 SEPTEMBER 2016
WORLD HEALTH ORGANIZATION, GENEVA, SWITZERLAND



**World Health
Organization**

Trachoma Alternative Indicators Study

Data review

31 August – 1 September 2016

World Health Organization, Geneva, Switzerland

Strategic and Technical Advisory Group for Neglected Tropical Diseases

Working Group on Monitoring and Evaluation



This report contains the collective views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization.

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Abbreviations

CDC	United States Centers for Disease Control and Prevention
ELISA	enzyme-linked immunosorbent assay
PCR	polymerase chain reaction
Pgp	plasmid gene product 3
TF	trachomatous inflammation—follicular
ROC	receiver operating characteristic

1. Background

1.1 Trachoma causes blindness through repeated conjunctival infection with *Chlamydia trachomatis* (1). To eliminate trachoma as a public health problem (2), the World Health Organization (WHO) recommends use of the SAFE strategy (that is, surgery for advanced disease; mass drug administration of antibiotics to clear *C. trachomatis* infection; and facial cleanliness and environmental improvement to reduce transmission) (3). Current (2006) guidelines (4) on the implementation of the A, F and E components are based on the prevalence of the sign “trachomatous inflammation—follicular”, or TF (5), in children aged 1–9 years.

1.2 As the prevalence of TF in 1–9-year-olds declines towards the elimination threshold of 5%, so too does the positive predictive value of TF for conjunctival *C. trachomatis* infection at both the individual and community levels (6–9). Consequently, implementation of interventions against trachoma (particularly mass drug administration of antibiotics¹) could, in some contexts, continue for longer than necessary to meet trachoma-related public-health goals.

1.3 A further consequence of declines in the prevalence of TF in 1–9-year-olds is that it becomes progressively more difficult to train graders to recognize TF (10, 11), and to prove that they can do so accurately through formal inter-grader agreement exercises (12).

1.4 The Trachoma Alternative Indicators Study was initiated in 2014 to examine, in a variety of settings, the relationships between the district-level prevalence of TF and (i) the district-level prevalence of conjunctival *C. trachomatis* infection, and (ii) the district-level prevalence of antibodies to *C. trachomatis*-derived antigens, in order to determine whether one or both should be used as adjuncts or alternatives for deciding whether to stop mass drug administration of antibiotics in trachoma elimination programmes. The study responds, in part, to recommendations made at a 2014 Technical Consultation on Trachoma Surveillance (13).

1.5 The purpose of this meeting was to undertake an objective, open review of data generated by the study to date, consider implications for global policy and plan further work. The meeting agenda is presented as Annex 1. Participants are listed in Annex 2.

2. Trachoma grading, tests for infection, and tests for antibodies

2.1 In discussions of the advantages and disadvantages of the potential programmatic use of tests for *C. trachomatis* infection or for anti-*C. trachomatis* antibodies (7, 8), it is often implicitly assumed that (i) the current method for characterizing a population’s requirements for interventions – namely, examination by trained graders of the conjunctivae of 1–9-year-olds for the presence or absence TF – costs nothing other than the cost of fieldwork to undertake the actual surveys, while (ii) deploying an assay would add the cost of assays to the same fieldwork costs. This comparison is flawed. Training and certifying trachoma graders costs money.

2.2 Between December 2012 and January 2016, the Global Trachoma Mapping Project undertook standardized training of 611 field teams to complete trachoma prevalence surveys

¹ Health promotion (to achieve – among other objectives – facial cleanliness) and provision of safe water and sanitation (to achieve environmental improvement) may be advantageous regardless of whether trachoma is a public health problem or not.

in 29 countries (14). Each team was composed of one grader and one data recorder. Training consisted of an intensive five-day standardized programme (15). The readiness of trainee graders to participate in fieldwork was assessed through live-subject inter-grader agreement exercises involving 50 eyes of 50 children, with the assessments of an internationally-certified grader trainer used as the gold standard; a kappa score of ≥ 0.70 was required to pass (12). Trainee recorders' readiness to participate in fieldwork was assessed through a similarly standardized test (12). Data on costs were collected from training of 553 teams for 17 countries. Costs included in the analysis were participants' per diems, accommodation, training venue hire, vehicle hire and fuel for fieldwork, and training supplies; costs of international flights, per diems for international trainers and epidemiologist salaries were included in global support costs rather than training costs, because they were not exclusively attributable to training. The mean cost of training was US\$ 1953 per team (16), approximately one quarter of which could be attributed to training graders to reliably diagnose TF, and to prove that they were able to do so. Each team mapped a mean of 1.2 evaluation units of which each required examination of approximately 1019 1–9-year-olds (12). This translates to a training cost (excluding costs of international support) amounting to US\$ 0.39 per 1–9-year-old examined. This cost is likely to rise considerably as TF elimination thresholds are reached, because of the need to transport trainee graders to settings in which TF is still sufficiently abundant to facilitate training and testing (10, 11).

2.3 A variety of types of assay are available for the detection of conjunctival *C. trachomatis* infection: microscopy using Giemsa or fluorescent antibodies, culture, enzyme immunoassay, nucleic acid hybridization and nucleic acid amplification-based tests. The latter, which include commercial and in-house tests that employ the polymerase chain reaction (PCR), have the highest sensitivity and specificity and are therefore the best tests to detect *C. trachomatis*. Their cost (usually US\$ 8–16 per test) can be somewhat contained by pooling samples (17, 18), although this increases specimen handling time and may reduce sensitivity for detection of low-load infections. Children do not necessarily relish having swabs rubbed on their everted conjunctivae.

2.4 A variety of types of assay are available for the detection of antibodies to *C. trachomatis* antigens in serum. Blood can be collected via venepuncture or finger-prick and stored as whole blood, serum or dried blood spots on filter paper. Tests can be conducted using enzyme-linked immunosorbent assay (ELISA) (19), lateral flow-based rapid tests (20, 21) or bead-based immunoassays (22–27); the latter facilitates multiplex testing, potentially allowing integrated surveillance for multiple different diseases simultaneously (28, 29). Studies to date have measured antibodies directed against the *C. trachomatis* antigens Pgp3 and CT694 (24). Seroprevalence curves of anti-*C. trachomatis* antibodies against age in children are believed to reflect the intensity of *C. trachomatis* transmission over time. Unfortunately, no currently available test measures antibodies specific for exposure to trachoma-associated (as opposed to urogenital infection-associated) *C. trachomatis* strains. A longitudinal study of individuals in a high prevalence community in Kongwa District, United Republic of Tanzania, did not detect seroreversion in 1–6-year-olds during the 6 months after mass antibiotic treatment (25). In low prevalence settings, at 12 months after treatment, 2–6% of previously seropositive 1–9-year-olds are seronegative. There may be an association between the amount of antibody present in serum at baseline and the likelihood of seroreversion.

2.5 There are currently no international reference standards for undertaking or interpreting *C. trachomatis* serological assays (19).

3. Data from the Trachoma Alternative Indicators Study

3.1 The data available to date are summarized in the Table below.

3.2 Infection and antibody data were collected as research adjuncts of planned trachoma elimination programme activities: baseline surveys in the Lao People's Democratic Republic; impact surveys in Malawi and Uganda; and pre-validation surveillance surveys in Gambia, Ghana, Nepal and the United Republic of Tanzania.

3.3 The prevalence of TF in 1–9-year-olds was below the 5% elimination threshold in all surveyed evaluation units, except for three in Malawi.

3.4 Where data were available, the prevalence of conjunctival *C. trachomatis* positivity, as determined using a variety of methodologies, was generally low. In only one site (Kilosa District, United Republic of Tanzania) did infection prevalence exceed 1%, and here it was only slightly above 1%, at 1.1%. In Uganda, testing in pools showed 0% infection; these were re-run as individual samples and infection prevalence remained very low at 0.3%.

3.5 ELISA testing for antibodies to Pgp3 was conducted in laboratories in Ghana, Malawi and the United Kingdom. Data from the Gambia, the Lao People's Democratic Republic and Uganda were used to evaluate different methodologies, in order to determine thresholds for seropositivity. The ELISA testing done in Ghana will be compared to the multiplex bead array testing done at the United States Centers for Disease Control and Prevention (CDC); the testing done at CDC will also evaluate antibody responses against other neglected tropical diseases and waterborne diseases.

3.6 Significant delays in some study sites have resulted from a faulty batch of laboratory consumables, inadequate access to appropriately equipped laboratories, limited laboratory throughput and other restrictions.

Table. Data available from the Trachoma Alternative Indicators Study, August 2016

Study site	Study population	Prevalence		
		Trachomatous inflammation—follicular	Conjunctival <i>C. trachomatis</i> infection	Anti- <i>C. trachomatis</i> antibodies
Agago and Pader districts, Uganda (impact survey)	2884 children aged 1–9 years in post-intervention districts	2.6%	0.3% (Hologic Aptima CT)	17.1% (anti-Pgp3, determined by ELISA, cut-off methodology: mixture model)
Lao People's Democratic Republic (baseline survey)	951 children aged 1–9 years in 3 groups of 3 "potential hot-spot" villages (10)	1.5%	≥ 0.6% [3/14 children with TF; 1 child with TF and 936 children without TF not tested] (droplet digital PCR)	15.6% (anti-Pgp3, determined by ELISA, cut-off methodology: mixture model)
Chikwawa (C) and Mchinji (M) districts, Malawi (impact survey)	6076 children aged 1–9 years in 6 evaluation units after three annual rounds of high-coverage azithromycin MDA	4.7% in Chapananga (C) 4.6% in Ngabu Ngokwe (C) 4.4% in Kasisi (C) 7.4% in Mkanda Gumba (M) 6.0% in Luzi Kochilira (M) 5.2% in Nkwazi (M)	No data available yet because of 2-year delay in processing samples caused by lack of laboratory equipment in country	13.6% in Chikwawa, 21.7% in Mchinji (anti-Pgp3, determined by ELISA, cut-off methodology: mixture model)
Nine districts of Ghana (pre-validation surveillance surveys)	12 098 children aged 1–9 years in 18 evaluation units	< 5% for each of 18 evaluation units	~ 0.1% of 2225 samples tested to date (Cepheid GeneXpert)	10.6% of 7056 dried blood spots analysed to date (anti-Pgp3, determined by ELISA, cut-off methodology: mixture model); 3.5% of 4746 samples sent to CDC (anti-Pgp3, bead-based immunoassay, cut-off methodology: ROC J-index)
Kilosa District, United Republic of Tanzania (pre-validation surveillance survey)	1474 children aged 1–9 years in a simple random sample of 30/522 hamlets (22)	0.4%	1.1% (Hologic Aptima Combo2)	7.5% (anti-Pgp3, determined by bead-based immunoassay, cut-off methodology: ROC J-index)
Dang and Dailekh districts, Nepal (pre-validation surveillance surveys)	2021 children aged 1–9 years in 15–20 villages of each district (30)	0.1% in Dang, 0.2% in Dailekh	< 1% (Cepheid GeneXpert)	2% in 1–4-year-olds (anti-Pgp3, determined by bead-based immunoassay, cut-off methodology: ROC J-index)
Lower River Region, Gambia (pre-validation surveillance survey)	383 children aged 1–9 years in 20 randomly-selected enumeration areas	1.8% in 2013 (31)	< 1% (Roche Amplicor) in 2006 (32)	6.5% (anti-Pgp3, determined by ELISA, cut-off methodology: mixture model) in 2014
Upper River Region, Gambia (pre-validation surveillance surveys)	359 children aged 1–9 years in 20 randomly-selected enumeration areas	0.4% in 2013 (31)	No data collected	6.1% (anti-Pgp3, determined by ELISA, cut-off methodology: mixture model) in 2014
Kongwa District, United Republic of Tanzania (impact survey with follow-up)	2111 children aged 1-9 years in 50 randomly-selected communities followed for one year	5.2%	4.6%	30.9% (anti-Pgp3, determined by bead-based immunoassay, cut-off methodology: ROC J-index); seroconversion rate: 9.8%, seroreversion rate: 6.4%

ELISA, enzyme-linked immunosorbent assay; MDA, mass drug administration; PCR, polymerase chain reaction; ROC, receiver operating characteristic.

4. Discussion and conclusions

4.1 There is not yet sufficient information from this series of investigations to draw definitive conclusions about the routine use of tests to identify either *C. trachomatis* infection or anti-*C. trachomatis* antibodies, for the purpose of decision-making in trachoma elimination programmes. Part of this uncertainty stems from the uncertainty over the true biological significance of the 5% TF threshold for either stopping mass administration of antibiotics or defining re-emergence of trachoma. (Even assuming that a TF prevalence of 5% marked a true epidemiological watershed between populations at risk of future incident trachomatous blindness and populations without that risk, our ability to estimate prevalence is subject to uncertainty that can be measured, and is not negligible.)

4.2 Potential alternative or adjunct clinical indicators of conjunctival *C. trachomatis* transmission intensity (such as the prevalence of trachomatous inflammation—intense (5)) were not considered as part of the data presented at this meeting. Several participants considered that such indices may yet have a role, although it was noted that they are likely to have similar drawbacks as estimates of the prevalence of TF, notably (i) difficulties in finding sufficient numbers of cases to train graders, (ii) difficulties in finding a sufficient number of cases to prove (in inter-grader agreement exercises) that graders have been trained well, and (iii) declining positive predictive value for conjunctival *C. trachomatis* infection as the elimination end-point is approached.

4.3 Similarly, the use of conjunctival photography was not considered in detail at this meeting. No formal evaluation of the use of photographs for remote diagnosis of active trachoma (33, 34) has been undertaken since high resolution digital photography became widely available.

4.4 Laboratory capacity in many trachoma-endemic areas is inadequate. This causes delays in sample processing in studies such as this, and would currently be problematic for programmes if a recommendation was made to adopt the use of PCR or serology for routine programme use. In this context, assays (such as those using lateral flow technology) that can be performed reliably after minimal training hold particular promise. The role of an international system for quality assurance should also be considered, taking into account the legal requirements of and cultural issues in trachoma-endemic countries.

4.5 Studies to date have dichotomized antibody responses as positive or negative; more information may yet be obtained by considering antibody titres quantitatively.

4.6 The lack of an international reference standard for evaluating seropositivity is limiting, for several reasons. First, it makes it more difficult to establish cut-offs for positivity – in other words, to distinguish signal from noise. This is a critical issue, illustrated by a recent comparison of different methodologies for determining the cut-off (19). In Uganda, for example, use of a receiver operating characteristic (ROC) J-index resulted in an estimated prevalence of seropositivity of 6.8%, whereas using a fixed mixture model the estimated prevalence was 17.1%. A reference standard would provide an independent mark to resolve this inconsistency, allowing for clearer comparison between study sites and faster progress towards determining biologically important thresholds. Second, a reference standard would allow direct comparison of quantitative data obtained on ELISAs and bead-based assays. Quantitative data from these assays differ based on the assay readout: ELISA optical density data reflect the amount of light absorbed by the solution, while bead-based assays utilize fluorescent detection. A reference standard that could be used as an internal control would allow conversion of optical density and fluorescent readouts to a common unit, facilitating comparison between platforms and laboratories. Data from the Trachoma

Alternative Indicators Study will compare ELISA seropositivity data generated in Ghana to data from the same samples run using the bead-based immunoassay at CDC, but comparisons will be limited by the lack of a reference standard. Finally, a reference standard would aid quality control and test development.

5. Recommendations

5.1 Existing and future data from these studies should be pooled and evaluated by modellers with expertise in trachoma, in order to extract as much information as possible and evaluate how to meaningfully relate indices based on nucleic acid amplification-based tests or antibodies to existing indicators. This work should include examination of quantitative antibody data in longitudinal studies. The robustness of any conclusions should be tested through the use of a number of different thresholds determined by different methods.

5.2 As soon as is practicable, appropriately de-identified data should be made available on collaborative data platforms, in order to encourage contributions from the widest possible scientific audience.

5.3 Efforts to collect primary data should continue. Such efforts should focus on the collection of longitudinal data through serial evaluation of the same evaluation units, and collection of data in countries that have already successfully undergone validation of elimination of trachoma as a public health problem, where well-documented serial estimates of active trachoma prevalence are available. Samples collected from individuals outside of the key indicator group are required to characterize the long-term serological and antibody patterns. WHO should play a role in encouraging national trachoma elimination programmes to participate in this work as implementation research, allied to the routine conduct of impact and surveillance surveys.

5.4 An international reference standard for serology is needed. A recombinant humanized antibody against Pgp3 should be commissioned by CDC.

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Annex 1. Meeting agenda

Wednesday, 31 August 2016

<i>Time</i>	<i>Topic</i>	<i>Speakers</i>
09:00–09:30	Welcome Introductions Purpose, outcome and outputs of meeting Adoption of agenda Administrative matters	Anthony Solomon Patrick Lammie Pamela Mbabazi Patrick Lammie Anthony Solomon
09:30–10:00	Background to the Trachoma Alternative Indicators Study	PJ Hooper
10:00–10:10	Systematic review of data on infection and active trachoma	Anthony Solomon
11:10–10:30	How much does it cost to train people to diagnose active trachoma?	Anthony Solomon
10:30–11:00	<i>Coffee</i>	
11:00–11:30	Testing for ocular <i>Chlamydia trachomatis</i> infection	Jeanne Moncada
11:30–12:00	PCR data from Uganda	Jeanne Moncada
12:00–14:00	<i>Lunch</i>	
14:00–14:30	PCR data from the Lao People's Democratic Republic	Steph Migchelsen
14:30–15:00	PCR data from Malawi	Sarah Burr
15:00–15:30	<i>Coffee</i>	
15:30–16:00	PCR data from Ghana	Laura Senyonjo
16:00–16:30	PCR data from the United Republic of Tanzania and Nepal	Sheila West
16:30–17:00	Potential programmatic use of PCR for impact and surveillance surveys	All

Thursday, 1 September 2016

<i>Time</i>	<i>Topic</i>	<i>Speakers</i>
09:00–09:30	Principles of and tools for sero-surveillance in trachoma elimination	Diana Martin
09:30–10:00	How should we determine cut-offs?	Steph Migchelsen
10:00–10:30	Antibody data from the United Republic of Tanzania and Nepal	Sheila West
10:30–11:00	<i>Coffee</i>	
11:00–11:30	Antibody data from Ghana	Laura Senyonjo
11:30–12:00	Antibody data from Malawi	Sarah Burr
12:00–14:00	<i>Lunch</i>	
14:00–14:30	Antibody data from Uganda, the Lao People's Democratic Republic and the Gambia	Steph Migchelsen
14:30–15:00	How should we put these data together to aid policy development?	Tom Lietman
15:00–15:30	<i>Coffee</i>	
15:30–17:00	Next steps, meeting feedback and close	All

Annex 2. List of participants

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