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# Advancing the public health applications of *Chlamydia trachomatis* serology

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**Genital *Chlamydia trachomatis* infection is the most commonly diagnosed sexually transmitted infection. Trachoma is caused by ocular infection with *C trachomatis* and is the leading infectious cause of blindness worldwide. New serological assays for *Chlamydia trachomatis* could facilitate improved understanding of *C trachomatis* epidemiology and prevention. *C trachomatis* serology offers a means of investigating the incidence of chlamydia infection and might be developed as a biomarker of scarring sequelae, such as pelvic inflammatory disease. Therefore, serological assays have potential as epidemiological tools to quantify unmet need, inform service planning, evaluate interventions including screening and treatment, and to assess new vaccine candidates. However, questions about the performance characteristics and interpretation of *C trachomatis* serological assays remain, which must be addressed to advance development within this field. In this Personal View, we explore the available information about *C trachomatis* serology and propose several priority actions. These actions involve development of target product profiles to guide assay selection and assessment across multiple applications and populations, establishment of a serum bank to facilitate assay development and evaluation, and development of technical and statistical methods for assay evaluation and analysis of serological findings. The field of *C trachomatis* serology will benefit from collaboration across the public health community to align technological developments with their potential applications.**

## Introduction

*Chlamydia trachomatis* is an obligate intracellular bacterium that can cause both genital and ocular infections. Genital *C trachomatis* infection is the most common, curable STI worldwide, with an estimated 131 million new cases each year.[1] Most infections are asymptomatic, but if left untreated can cause scarring of the upper reproductive tract in women and lead to serious complications in women, including pelvic inflammatory disease, ectopic pregnancy, and tubal infertility.[2] Trachoma is caused by ocular infection with *C trachomatis* and is the leading infectious cause of blindness worldwide.[3] WHO estimates that around 190 million people are at risk of blindness from trachoma, with most of these people living in Africa. There has been substantial investment in the past decade in public health programmes to control both genital and ocular infection due to *C trachomatis*. Control efforts for genital chlamydia have largely focused on enhanced detection and treatment of *C trachomatis* among young adults, and for trachoma control efforts have focused on improved access to water, sanitation, and hygiene, and mass drug administration with azithromycin in affected communities.[3] However, several important questions remain about *C trachomatis* epidemiology, the most effective means of control, and optimal models of surveillance.[4–6] Given the ongoing control efforts and the promise of new *C trachomatis* vaccines,[7] robust methods are needed to allow monitoring of and insight into the prevalence and incidence of chlamydia infection,[8] and the progression to scarring sequelae. Measures of current infection based on DNA or RNA detection (ie, using nucleic acid amplification tests) provide an inadequate understanding of these features of *C trachomatis* infection. Therefore, alternative approaches are required, and in recent years interest in the use of serological assays within the fields of genital chlamydia and trachoma has been increasing.

Methods to detect *C trachomatis* antibodies in serum have been available for several decades.[9] However, use of serological assays has been limited by *C trachomatis*' cross-reactivity with other *Chlamydia* spp,[10] suboptimal sensitivity of many assays,[11,12] an incomplete underst`anding about the longevity and clinical implications of *C trachomatis* antibodies, and the relationship between *C trachomatis* infection and antibody response.[11] Consequently, the widespread use of chlamydia seroepidemiology decreased among researchers for several years, as did funding. Following the development of novel, sensitive, and more specific *C trachomatis* serological assays,[13–17] there is now growing interest in the use of these assays as an epidemiological tool. For example, assays have been developed with the capability to detect antibodies against a range of *C trachomatis* antigens, lateral flow assays are being evaluated for field use, and dried blood spots have been used to facilitate specimen collection, transport, and storage (table 1).

The current understanding of mucosal immunity and *C trachomatis* immunology suggests that urogenital and ocular infections with *C trachomatis* lead to detectable IgG response with use of appropriate serological assays in most confirmed infections.[13–15,23] Several factors affect the magnitude of IgG response and the ability of serological assays to detect a previous *C trachomatis* infection, including the target antibody, assay used, time since infection, and patient characteristics such as age, sex, race, and the number of previous *C trachomatis* infections.[13,14,16,58] In a UK-based study[13,15] that compared several assays in the same population, the sensitivity to detect a previous known chlamydia infection ranged

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| | Platform or format | Antigens detected (antibody class or subclass) | Examples of public health applications | | | |
|---|---|---|---|---|---|---|
| | | | Measure seroprevalence or estimate incidence | Investigate association with disease | Evaluate control interventions | |
| | | | | | Genital *Chlamydia trachomatis* | Trachoma |
| **ELISAs** | | | | | | |
| Wills et al[13] | Indirect ELISA | Pgp3 (IgG) | 18 | .. | 18 | .. |
| Geisler et al[14] | Indirect ELISA | Elementary bodies from *Chlamydia trachomatis* serovars D/UW-3, F/ICCal-13, and J/UW-36 (IgG1, IgG3) | 14,19 | 20,21 | .. | .. |
| Horner et al[15] | Double-antigen ELISA | Pgp3 (IgG, IgA, IgM) | 15,18,22 | .. | 18 | .. |
| Winstanley et al[16] | Indirect ELISA | Pgp3 (IgG) | .. | .. | .. | .. |
| Albritton et al[23] | Indirect ELISA | Elementary bodies from *Chlamydia trachomatis* serovars D/UW3/Cx and E/UW5/Cx (IgG, IgA) | .. | 23 | .. | .. |
| Migchelsen et al[24] | Indirect ELISA on dried blood spots | Pgp3 (IgG) | 24, 25 | .. | .. | .. |
| Menon et al[26] | Multi-peptide indirect ELISA | 12-mer peptides derived from HtrA, hsp60, and Ct443[27] (IgG) | .. | 26,28 | .. | .. |
| Commercially available* | ELISA or EIA | MOMP and hsp60 (IgG, IgA) | 29,30,31 | 32,21, 33-39 | .. | .. |
| **Multiplex bead arrays** | | | | | | |
| Goodhew et al[17] | Multiplex bead array | Pgp3 and CT694 (IgG, IgA) | 17 | .. | .. | 40–42,43 |
| Willhauck-Fleckenstein et al[44] | Multiplex bead array | MOMP A/D/L2, PorB, TARP, hsp60-1, and Pgp3 (IgG, or IgG, IgA, IgM) | .. | .. | .. | .. |
| **Near-patient testing** | | | | | | |
| Gwyn et al[45] | Lateral flow | Pgp3 (IgG, IgA, IgM) | 45,46 | .. | .. | .. |
| **Whole proteome microarray** | | | | | | |
| Budrys et al[47] | ELISA-based proteome array | Representing 908 proteins of *Chlamydia trachomatis* strain D/UW-3/Cx (IgG) | .. | 47 | .. | .. |
| Lu et al[48] | Whole proteome microarray | Representing 908 genomic and plasmid open reading frames of *Chlamydia trachomatis* strain D/UW3 (IgG, IgA, IgM) | .. | 48,49 | .. | .. |
| Hufnagel et al[50] | Whole proteome microarray | Representing 895 proteins of *Chlamydia trachomatis* strain D/UW-3/Cx (IgG, or IgG, IgA, IgM) | .. | 50 | .. | .. |
| **Immunofluorescence** | | | | | | |
| Chernesky et al[51] | Whole-cell inclusion immunofluorescence | L2 serovar (IgG, IgA, IgM) | .. | 52,53 | .. | .. |
| Commercially available† | Micro-immunofluorescent assay | Elementary bodies from *Chlamydia trachomatis* serovars D-K (IgG, IgA, IgM) | .. | 54 | .. | .. |
| Wang[55] | Modified micro-immunofluorescent assay protocol | Elementary bodies from *Chlamydia trachomatis* (IgG) | 56 | 57 | .. | .. |

This table is not intended to be a comprehensive review. ELISA=enzyme-linked immunosorbent assays. HtrA=high temperature requirement A protease. hsp60=heat shock protein 60. EIA=enzyme immunoassay. MOMP=major outer membrane protein. TARP=translocated actin-recruiting phosphoprotein. *Chlamydien IgG rELISA medac assay, medac, Wedel, Germany; *Chlamydia trachomatis* EIA assay kit, Pathtech, Melbourne, Australia; and SeroCT RT kit, Savyon Diagnostics, Ashdod, Israel. † Chlamydia MIF IgG, Focus Diagnostics, Cypress, USA and SeroFIA, Savyon Diagnostics, Ashdod, Israel.

***Table 1: Chlamydia trachomatis* serological assays and examples of their public health applications to date**

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from 46% (IgG pELISA plus medac assay, medac, Wedel, Germany) to 83% (Pgp3 double-antigen) in women and 40% (SeroCT; Savyon Diagnostics, Ashdod, Israel) to 54% (Pgp3 double-antigen) in men when compared with a previous *C trachomatis* diagnosis by nucleic acid amplification tests. Seroreversion (ie, loss of detectable antibodies) has been shown in some cases but varies by infection history and assay,[58] with minimal loss of detectable antibody reported in one study using a Pgp3 double-antigen ELISA.[15] In a study[59] of *C trachomatis* seroprevalence in the context of mass azithromycin treatment for trachoma prevention in a high prevalence area, no instances of seroreversion were observed after 6 months. *C trachomatis* serological assays can therefore be used to measure age-specific cumulative incidence,[11,18] despite representing a lower bound estimate due to potentially incomplete seroconversion and loss of detectable antibodies over time. *C trachomatis* antibody response has also been found to correlate with a known history of scarring sequelae. For example, titres of *C trachomatis* antibodies have been found to be higher in women with tubal factor infertility than in women without this disease and some specific antibodies have been found to be more common in women with known disease than in those without disease.[47] Thus, serological assays might be used as a potential biomarker of disease.

Because the sensitivity of serological assays is inadequate, *C trachomatis* serology has limited diagnostic value; in the absence of genetic diagnostic methods it is an accepted tool for presumptive diagnosis of lymphogranuloma venereum,[60] but it is not used for diagnosis of other biovars. Instead, *C trachomatis* serological assays have a potential role in monitoring and surveillance by providing a measure of history of *C trachomatis* exposure among individuals tested. When applied to appropriate samples, such measures might be useful to inform resource allocation and possible clinical need by indicating the size of the population affected, and the effect of population-based interventions such as screening or mass drug administration.

Therefore in this Personal View, we explore the potential public health applications of *C trachomatis* serology, discuss key challenges of its use, and finally propose priorities for research and development that might help advance the field of *C trachomatis* control. This work grew out of an expert meeting convened by Public Health England in September, 2016 (The Public Health applications of *Chlamydia trachomatis* serology; London, UK), and subsequent discussions of studies relating to different assays presented at the 2016 European Society for Chlamydia Research meeting (Oxford, Sept 6–9, 2016).

## Public health applications of *Chlamydia trachomatis* serology

*C trachomatis* serology provides a means of quantifying the prevalence and incidence of chlamydia infection. A thorough understanding of population-level *C trachomatis* prevalence and incidence is crucial to identify unmet needs for screening and treatment services and to evaluate the impact of *C trachomatis* control interventions. However, obtaining reliable estimates of *C trachomatis* prevalence and incidence is challenging. In the case of genital chlamydia, most *C trachomatis* infections are asymptomatic, so an increase in screening would lead to an increase in reported diagnoses.[61] As a result, surveillance is often centred on only case-based reporting, with scarce information about numbers of patients tested, resulting in an underestimate of the true incidence of *C trachomatis* infection.[61] Furthermore, comparability of surveillance data between countries is limited by differences in testing recommendations, performance characteristics of diagnostic tests, and reporting policies and practices.[62] Even when the total number of people tested in a given country is known, interpreting estimates of the proportion of people who test positive for chlamydia infection is difficult, because the tested population has a different underlying risk from the general population. Therefore, the proportion testing positive will often present a biased measure of prevalence.[61,63] Few countries have undertaken surveys of prevalence in samples of the general population, and when they have been done,[64–66] the surveys were resource intensive and unlikely to be feasible in many countries, because of the costs involved.

*C trachomatis* seroprevalence as a marker of cumulative incidence has been used in several countries[15,18,22,29,30,67] to assess *C trachomatis* epidemiology, and in some cases to investigate population effect of control interventions. In the field of trachoma, mass drug administration programmes have been successful in reducing *C trachomatis* infection and *C trachomatis*-related ocular disease.[24,68,69] Longitudinal *C trachomatis* serology monitoring has strong potential as a tool for post-elimination surveillance,[40–42] and so provides an opportunity to evaluate programme effectiveness and possibly a further understanding of the public health response needed in countries where trachoma has not been eliminated.[70] Subject to certain assumptions about the population sampled, *C trachomatis* incidence can be estimated from repeated, cross-sectional *C trachomatis* seroprevalence surveys,[71] although it would be necessary to adjust for imperfect assay sensitivity and specificity (Ades AE, personal communication). *C trachomatis* serology can also be used to detect step changes in exposures by birth cohort, which would be expected in the context of control measures.[40,72] Distinguishing between recent and past or long-standing infections would also help determine the incidence of disease, and methods are already being developed to enable *C trachomatis* serology to be used for this purpose.[73]

Another potential application of *C trachomatis* serology is as a measure of diseases related to chlamydia infections, such as pelvic inflammatory disease or ectopic pregnancy. Because the end goal of *C trachomatis* control is to reduce the incidence of disease, monitoring of disease biomarkers and not just of infection might improve understanding of whether *C trachomatis* control is leading to a reduction in reproductive sequelae, even in the absence of substantial reductions in transmission. A *C trachomatis*-specific biomarker of disease would be particularly useful, because *C trachomatis*-related diseases might occur many years after the causative infection and *C trachomatis* is not the only cause of long-term reproductive complications such as pelvic inflammatory disease, ectopic pregnancy, and tubal factor infertility.[2] Measures of the proportion of long-term sequelae that are attributable to *C trachomatis* infection (the so-called population excess fraction) are also essential to determine the need for and cost effectiveness of control interventions.

Serological methods have been used to investigate the relationship between *C trachomatis* infection and sequelae as well as to estimate the proportion of long-term sequelae attributable to genital *C trachomatis* infection.[20,21,32,52,74] Novel approaches also offer some promise in this area; for example, Ades and colleagues[52] have developed a method using finite mixture modelling of antibody titre to estimate the population excess fraction of tubal factor infertility caused by chlamydia

infection. Additionally, proteomic arrays are also being assessed as a means of identifying serological fingerprints to indicate the presence of disease related to genital *C trachomatis* infection[50] and scarring following ocular infection.[49]

Another potential application of *C trachomatis* serology is development and evaluation of *C trachomatis* vaccines. The joint WHO and National Institutes of Health (NIH) sexually transmitted infection vaccine roadmap[75] has set out the need for an effective *C trachomatis* vaccine. Substantial progress towards this vaccine has been made in recent years, with candidate vaccines now in the preclinical and clinical testing phases.[7] Several priority action areas set out in the WHO and NIH roadmap might be addressed through the development and application of serological assays. This includes using serological assays to obtain better epidemiological data, improve understanding of the natural history of *C trachomatis* and burden of sequelae, and expedite clinical development and evaluation of candidate vaccines, thereby encouraging investment in *C trachomatis* vaccine development. Specifically, if acting as biomarkers for disease *C trachomatis* serological assays could be used to obtain more complete and precise estimates of the global burden of *C trachomatis*-associated sequelae, which are important for establishing the public health rationale for vaccination and for potential investors to assess the possible effect of investing in any successful vaccine candidate.[7]

When a safe vaccine candidate does enter a phase 3 clinical trial, *C trachomatis* serology could help identify *C trachomatis*-naive participants for recruitment, and help develop vaccination strategies through an understanding of age-specific exposure. Vaccine evaluation would also benefit from a biomarker of tubal damage for use as part of a clinical endpoint for assessing vaccine efficacy, because of the current diagnostic inaccuracy for *C trachomatis*-related outcomes such as pelvic inflammatory disease.[2] The time and resources needed for a clinical trial of candidate *C trachomatis* vaccines with pelvic inflammatory disease or tubal factor infertility as outcomes might also be prohibitive. It is unclear whether serology will be able to provide such a biomarker of tubal damage, and serological methods might need to be used in combinaton with cellular markers or radiological findings. However, serology is an important area of research given the need for such measures in any future vaccine evaluation. Because *C trachomatis* infections (with the exception of lymphogranuloma venereum) are localised in the columnar epithelium, detection of antibodies from genital secretions has been proposed as a means of investigating correlates of immune protection against *C trachomatis*,[23] which might complement serological investigations. Assessment of vaccine-induced immune responses will depend on the vaccine's mechanism of action. Assays that distinguish between the natural and vaccine-induced antibody response will therefore be needed.

## Key challenges to the use of *Chlamydia trachomatis* serology in public health

Although progress has been made in recent years, some important challenges remain within the field of *C trachomatis* serology that need to be addressed to improve the use and value of new serological assays in a public health context. Interpretation of *C trachomatis* seroprevalence is difficult; several complexities exist and include the following: not everyone exposed to *C trachomatis* will become infected, some individuals with this infection might not develop antibodies, women are more likely to develop detectable antibodies than men following urogenital *C trachomatis* infection,[13,18,76] *C trachomatis* antibodies are not specific to the infection site (ie, ocular or urogenital), and seroprevalence can vary with the number of previous infections and time since infection as antibodies develop or wane.[58] These complexities require careful consideration when planning studies and doing statistical analyses.

Determining assay sensitivity and specificity in the absence of universal guidelines is also challenging. The population that will be tested should be carefully considered when selecting positive and negative controls (ie, those with or without infection or disease) and setting assay thresholds. For example, a study aimed at investigating *C trachomatis* infection might need a different definition of positive and negative controls than a study aimed at investigating *C trachomatis*-associated disease. A further challenge involves the choice of thresholds that define *C trachomatis* antibody response in different populations, because serological assays might be affected by differences in cross-reactivity and background antibody concentrations, which can vary for example by country or ethnicity, or both.

The relative performance of different tests cannot easily be determined without evaluation against the same reference sera. Some laboratory-developed assays have been compared with commercial assays or other laboratory-developed assays,[13,15,45,46] but there are few data available to show how different assays perform within the same population. To establish performance characteristics of *C trachomatis* serological assays for different applications and populations, large numbers of serum samples linked to well characterised clinical and demographical information are needed. Serum samples from previous studies (eg, human papillomavirus vaccine trials[77] and HIV unlinked anonymous testing[78]) or residual samples from clinical testing[67] could be used, but they often have limited clinical or demographical information, and varying access arrangements mean that assays have not been evaluated on comparable samples.

Optimal test characteristics might vary between different applications of *C trachomatis* serology,

suggesting that different characteristics might be prioritised. For example, a test to measure whether someone has had *C trachomatis* infection will need to detect antibodies that persist over time at relatively low concentrations with high specificity. However, a test that is used to estimate the population excess fraction would ideally be able to distinguish between complicated and uncomplicated *C trachomatis* infections (eg, by identifying high versus low concentrations of antibody in serum,[52] the antibody subclass,[14] or antibodies specifically associated with complications[47]).

Similarly, the context in which an assay is to be deployed will influence prioritisation. For example, in a research setting, tests could be more operator-intensive and less cost-effective than tests used for ongoing surveillance given limited government budgets. Furthermore, a test requiring high volume of sera might be acceptable in a setting where additional blood can be collected from consenting patients, whereas surveillance systems relying on leftover sera from routine testing might have an inadequate volume available. Applications of *C trachomatis* serological assays in a surveillance context might be more tolerant to some reduced precision than when used within a vaccine trial, where previous infection needs to be ruled out to precisely define populations for inclusion in any efficacy analysis.

## Priority actions for research and development

To address these challenges, we suggest three priority actions for research and development (panel).

### Generating target product profiles

Target product profiles originated in the field of drug development to focus discussions between regulatory authorities and research sponsors. They allow the drug development process to be directed with the end goal in mind, so that both patient and market needs are met.[79] The process of establishing target product profiles is now commonly used in drug and vaccine development and their use has also extended into the field of diagnostics—eg, for tuberculosis[80] and point-of-care diagnostics for sexually transmitted infections.[81] Target product profiles for *C trachomatis* serological assays should establish the minimal and optimal assay requirements for the different applications previously described. Table 2 sets out some of the initial considerations that can be used to inform target product profiles. A target product profile requires broad technical consultation across clinical, microbiological, and epidemiological fields, as well as representation from vaccine and diagnostic development companies, research groups, public health agencies, and funders.

### Establishing a serum bank

A well defined serum bank focused on the evaluation of *C trachomatis* serology will be an invaluable resource. The value of serum banks in research of infectious diseases was recently set out in an Editorial[82] in *The Lancet*, which highlighted the role serological studies could play in understanding worldwide distribution of disease and argued for the establishment of a World Serology Bank. The development of a C trachomatis-specific serum bank would enable clear and fair access to specimens and relevant epidemiological and clinical data (eg, age, sex, and a history of *C trachomatis* diagnosis). A serum bank would have the potential to support the development and evaluation of serological assays, and to facilitate identification of potential vaccine targets and correlates of protection. The bank should include sera from women and men of a variety of countries, ages, and ethnicities. These individuals would also have a range of characteristics that include varying histories of *C trachomatis* diagnosis, incorporating different times since treatment and different numbers of known infections; those with and without reproductive complications; and those exposed to potentially cross-reactive pathogens such as *Chlamydia pneumoniae*.

***Panel:*** **Priority actions to further develop the public health applications of *Chlamydia trachomatis* serological assays**

**Generate target product profiles for C trachomatis serological assays**

- What are the minimal or optimal characteristics of *C trachomatis* serological assays for different purposes (eg, design and evaluation of genital *C trachomatis* control programmes, design and evaluation of trachoma or ocular *C trachomatis* control programmes, or vaccine development and evaluation) and measures (eg, seroprevalence of *C trachomatis* antibodies as a measure of prevalence and incidence, measure of population excess fraction of disease such as pelvic inflammatory disease or tubal factor infertility, biomarker of disease either alone or in combination with other measures, measure of being *C trachomatis*-naive, or measure of vaccine-induced immune response)?
- What are the minimal or optimal characteristics of the aforementioned purposes and measures in different countries?

**Establish a serum bank**

- Adequate volumes of well characterised serum samples from individuals who have had *C trachomatis* infection and sera from those who have not had this infection should be included. Samples should be stored from individuals of a variety of ages and ethnicities, including children who might still have the maternal antibody, with different characteristics including the numbers of known infections, time since treatment, and presence of known reproductive tract or ocular complications.
- Standardised assessments of clinical outcome and epidemiological data should be incorporated
- The serum bank should be established with appropriate access arrangements.

**Develop methods for assay evaluation and analysis of serological findings**

- Define how sensitivity and specificity be estimated for different purposes
- Define how positive and negative controls should be selected for different test applications?
- Better understand what thresholds should be used for each assay for different applications or test settings
- Define how head-to-head comparison studies be done
- Explore and assess what statistical methods should be used to measure epidemiological parameters (eg, incidence of infection and population excess fraction)?

| | Measuring infection | Measuring disease | For use in vaccine evaluation |
|---|---|---|---|
| Performance requirements | High sensitivity and specificity; ability to distinguish first from repeat infection; ability to measure recent infection; and lower precision than in vaccine studies would be sufficient for monitoring and surveillance applications | Ability to distinguish between complicated and uncomplicated infections; ability to identify disease-specific antigens or combinations of antigens or the magnitude of response associated with the disease; and high specificity for sequelae to prevent over investment in resource-poor environments arising from over estimation of the incidence of *Chlamydia trachomatis* sequelae | Ability to quantify burden of infection and disease; high precision and high sensitivity for determining *Chlamydia trachomatis*-naive status for trials and distinguishing between exposure and infection; to measure vaccine efficacy, availability of markers of tubal involvement, potentially in combination with other measures (eg, cellular markers, radiological measures); and markers of vaccine-induced immune response will depend on the mechanism of action of vaccine and will need to distinguish between vaccine-induced and natural responses |
| Dependencies | Appropriate collections of population-based sera | Appropriate collections of population-based sera to estimate disease incidence; and availability of reliable cases and controls with clear case definition to estimate population excess fraction | Vaccine trial design; and mechanism of action of vaccine candidate |
| Statistical methods | *Chlamydia trachomatis* serological assay thresholds appropriate to the application and population; and relation between seroprevalence and cumulative or annual incidence should consider the impact of time since infection and repeat infections on *Chlamydia trachomatis* antibodies | *Chlamydia trachomatis* serological assay thresholds appropriate to the application and population | *Chlamydia trachomatis* serological assay thresholds appropriate to the application and population |
| Technical requirements | High throughput, low volume, and low resource use methods would be valued | High throughput, low volume, and low resource use methods for monitoring and surveillance applications; and should tolerate methods requiring higher specimen volume and operator intensive methods | Should tolerate methods requiring higher specimen volume, and operator intensive methods |

***Table 2:*** **Initial considerations that can be used to inform target product profiles for *Chlamydia trachomatis* serological assays**

Some applications of *C trachomatis* serology, such as the identification of biomarkers of scarring sequelae or developing serological assays to distinguish between infection and exposure, might also benefit from simultaneous assessment of cellular immunity. Therefore, collections that incorporate both serum and whole blood specimens would be particularly valuable, although it would require different regulatory permissions in some settings and would incur additional expenses for collection and storage arrangements.

### Developing methods for assay evaluation and analysis

Shared protocols to guide assay evaluation would allow for comparability of estimates with use of *C trachomatis* serological methods across assays and increase consistency of reporting. Evaluation protocols should incorporate a consensus position on optimum methods of estimating sensitivity and specificity of *C trachomatis* serological assays, and recognise the need for selection of controls and assay thresholds to be determined according to the intended application while also considering the potential for cross-reactivity. Future efforts should also focus on development and application of statistical methods to appropriately analyse *C trachomatis* serological findings.

## Conclusions

As the reported incidence of reported cases of *C trachomatis* infection remains high or continues to increase in many developed countries, the use of *C trachomatis* serology in several areas of public health is likely to be effective, and has already provided further insight into *C trachomatis* epidemiology and natural history. We explored the available information about *C trachomatis* serology and identified three priority actions that we believe would directly benefit public health and advance knowledge within the *C trachomatis* field.

As public health agencies continue to address the high rates of *C trachomatis* infection and the considerable morbidity that arises as a result, a more data-driven approach to programmatic decision making at the country, state, and municipality level is essential. Promising interventions, including vaccines, do and will need robust measures for estimating the population at risk and for determining the potential effect of prevention measures. *C trachomatis* serology is a promising additional tool for public health that could help improve the understanding of the populations at risk and could support the development of novel and effective interventions.

**Contributors**

SCW, RJG, JKD, and KB organised and delivered the expert meeting, which was attended by all the authors. SCW and SJM did the literature searches. SCW wrote the first draft of the manuscript. All authors commented on the manuscript and approved the final version.

**Declaration of interests**

WMG has received grants from Genocea Biosciences and Moderna Therapeutics, outside the submitted work. WMH has a pending patent for *Chlamydia trachomatis* diagnostic peptide and method (PCT/AU2013/001333). PJH has received personal fees from the Crown Prosecution Service, the British Association for Sexual Health and HIV, grants from Mast Group, and non-financial support from Hologic, outside the submitted work. Additionally, PJH has a patent for a sialidase spot test to diagnose bacterial vaginosis issued to the University of Bristol, Bristol, UK. All other authors declare no competing interests.

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**References**

1 Newman L, Rowley J, Vander Hoorn S, et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS One* 2015; **10:** e0143304.

2 European Centre for Disease Prevention and Control. Chlamydia control in Europe: literature review. Feb 12, 2014. https://ecdc.europa.eu/en/publications-data/chlamydia-control-europe-literature-review (accessed Nov 1, 2017).

3 WHO. Trachoma factsheet. Geneva: World Health Organization, 2017. http://www.who.int/mediacentre/factsheets/fs382/en/ (accessed Nov 1, 2017).

4 Chow JM, Bauer HM. What data are really needed to evaluate the population impact of chlamydia screening programs? *Sex Transm Dis* 2016; **43:** 9–11.

5 Davies B, Ward H. A pathway to chlamydia control: updated ECDC guidance. *Sex Transm Infect* 2016; **92:** 483–84.

6 Price MJ, Ades AE, Soldan K, et al. The natural history of *Chlamydia trachomatis* infection in women: a multi-parameter evidence synthesis. *Health Technol Assess* 2016; **20:** 1–250.

7 Poston TB, Gottlieb SL, Darville T. Status of vaccine research and development of vaccines for *Chlamydia trachomatis* infection. *Vaccine* 2017; published online Jan 19. DOI:10.1016/j.vaccine.2017.01.023.

8 The Lancet Infectious Diseases. Time to take sexually transmitted infections seriously. *Lancet Infect Dis* 2016; **16:** 981.

9 Wang SP, Grayston JT. Human serology in *Chlamydia trachomatis* infection with microimmunofluorescence. *J Infect Dis* 1974; **130:** 388–97.

10 Persson K. The role of serology, antibiotic susceptibility testing and serovar determination in genital chlamydial infections. *Best Pract Res Clin Obstet Gynaecol* 2002; **16:** 801–14.

11 Johnson AM, Horner P. A new role for *Chlamydia trachomatis* serology? *Sex Transm Infect* 2008; **84:** 79–80.

12 Peeling RW, Wang SP, Grayston JT, Blasi F, Boman J, Clad A. Chlamydia serology: inter-laboratory variation in microimmunofluorescence results. In: Stephens RS, Byrne GI, Al E, eds. Chlamydial infections proceedings of the 9th International Symposium on human chlamydial infections. Napa, CA: International Chlamydia Symposium, 1998: 159–62.

13 Wills GS, Horner PJ, Reynolds R, et al. Pgp3 antibody enzyme-linked immunosorbent assay, a sensitive and specific assay for seroepidemiological analysis of *Chlamydia trachomatis* infection. *Clin Vaccine Immunol* 2009; **16:** 835–43.

14 Geisler WM, Morrison SG, Doemland ML, et al. Immunoglobulin-specific responses to *Chlamydia* elementary bodies in individuals with and at risk for genital chlamydial infection. *J Infect Dis* 2012; **206:** 1836–43.

15 Horner PJ, Wills GS, Righarts A, et al. *Chlamydia trachomatis* Pgp3 antibody persists and correlates with self-reported infection and behavioural risks in a blinded cohort study. *PLoS One* 2016; **11:** e0151497.

16 Winstanley CE, Ramsey KH, Marsh P, Clarke IN. Development and evaluation of an enzyme-linked immunosorbent assay for the detection of antibodies to a common urogenital derivative of *Chlamydia trachomatis* plasmid-encoded PGP3. *J Immunol Methods* 2017; **445:** 23–30.

17 Goodhew EB, Priest JW, Moss DM, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. *PLoS Negl Trop Dis* 2012; **6:** e1873.

18 Woodhall SC, Wills GS, Horner PJ, et al. *Chlamydia trachomatis* Pgp3 antibody population seroprevalence before and during an era of widespread opportunistic chlamydia screening in England (1994–2012). *PLoS One* 2017; **12:** e0152810.

19 Muzny CA, Kapil R, Austin EL, Brown L, Hook EW 3rd, Geisler WM. *Chlamydia trachomatis* infection in African American women who exclusively have sex with women. *Int J STD AIDS* 2016; **27:** 978–83.

20 Steiner AZ, Diamond MP, Legro RS, et al. *Chlamydia trachomatis* immunoglobulin G3 seropositivity is a predictor of reproductive outcomes in infertile women with patent fallopian tubes. *Fertil Steril* 2015; **104:** 1522–26.

21 Gorwitz RJ, Wiesenfeld HC, Chen PL, et al. Population-attributable fraction of tubal factor infertility associated with chlamydia. *Am J Obstet Gynecol* 2017; **217:** 336.e1–16.

22 Righarts AA, Morgan J, Horner PJ, Wills GS, McClure MO, Dickson NP. *Chlamydia trachomatis* incidence using self-reports and serology by gender, age period, and sexual behavior in a birth cohort. *Sex Transm Dis* 2017; **44:** 344–50.

23 Albritton HL, Kozlowski PA, Lillis RA, et al. A novel whole-bacterial enzyme linked-immunosorbant assay to quantify *Chlamydia trachomatis* specific antibodies reveals distinct differences between systemic and genital compartments. *PLoS One* 2017; **12:** e0183101.

24 Migchelsen SJ, Martin DL, Southisombath K, et al. Defining seropositivity thresholds for use in trachoma elimination studies. *PLoS Negl Trop Dis* 2017; **11:** e0005230.

25 Cocks N, Rainima-Qaniuci M, Yalen C, et al. Community seroprevalence survey for yaws and trachoma in the Western Division of Fiji. *Trans R Soc Trop Med Hyg* 2016; **110:** 582–87.

26 Menon S, Stansfield SH, Logan B, et al. Development and evaluation of a multi-antigen peptide ELISA for the diagnosis of *Chlamydia trachomatis* related infertility in women. *J Med Microbiol* 2016; **65:** 919–22.

27 Stansfield SH, Patel P, Debattista J, et al. Proof of concept: a bioinformatic and serological screening method for identifying new peptide antigens for *Chlamydia trachomatis* related sequelae in women. *Results Immunol* 2013; **3:** 33–39.

28 Menon S, Stansfield SH, Walsh M, et al. Sero-epidemiological assessment of *Chlamydia trachomatis* infection and sub-fertility in Samoan women. *BMC Infect Dis* 2016; **16:** 175.

29 Lyytikäinen E, Kaasila M, Hiltunen-Back E, et al. A discrepancy of *Chlamydia trachomatis* incidence and prevalence trends in Finland 1983–2003. *BMC Infect Dis* 2008; **8:** 169.

30 van Aar F, de Moraes M, Morré SA, et al. *Chlamydia trachomatis* IgG seroprevalence in the general population of the Netherlands in 1996 and in 2007: differential changes by gender and age. *Sex Transm Infect* 2014; **90:** 434–40.

31 Honkila M, Wikström E, Renko M, et al. Probability of vertical transmission of *Chlamydia trachomatis* estimated from national registry data. *Sex Transm Infect* 2017; **93:** 416–20.

32 Price MJ, Ades AE, Welton NJ, et al. How much tubal factor infertility is caused by *Chlamydia*? Estimates based on serological evidence corrected for sensitivity and specificity. *Sex Transm Dis* 2012; **39:** 608–13.

33 Hoenderboom BM, van Oeffelen AA, van Benthem BH, et al. The Netherlands Chlamydia Cohort Study (NECCST) protocol to assess the risk of late complications following *Chlamydia trachomatis* infection in women. *BMC Infect Dis* 2017; **17:** 264.

34 Łój B, Brodowska A, Ciecwiez S, et al. The role of serological testing for *Chlamydia trachomatis* in differential diagnosis of pelvic pain. *Ann Agric Environ Med* 2016; **23:** 506–10.

35 Sattari M, Ghiami Rad M, Ghasemzadeh A, Mohammadoghli Reihan Z. Frequency of anti-*Chlamydia trachomatis* antibodies in infertile women referred to Tabriz Al-Zahra hospital. *Int J Reprod Biomed (Yazd)* 2017; **15:** 17–20.

36 Olaleye O, Olamijulo JA. The value of chlamydial antibody level for predicting tubal blockage among women undergoing hysterosalpingography in Lagos, Nigeria. *Int J Gynaecol Obstet* 2016; **134:** 33–36.

37 Singh S, Bhandari S, Agarwal P, Chittawar P, Thakur R. Chlamydia antibody testing helps in identifying females with possible tubal factor infertility. *Int J Reprod Biomed (Yazd)* 2016; **14:** 187–92.

38 Rantsi T, Joki-Korpela P, Wikström E, et al. Population-based study of prediagnostic antibodies to *Chlamydia trachomatis* in relation to adverse pregnancy outcome. *Sex Transm Dis* 2016; **43:** 382–87.

39 Tošić-Pajić J, Šeklić D, Radenković J, et al. Augmented oxidative stress in infertile women with persistent chlamydial infection. *Reprod Biol* 2017; **17:** 120–25.

40 Martin DL, Bid R, Sandi F, et al. Serology for trachoma surveillance after cessation of mass drug administration. *PLoS Negl Trop Dis* 2015; **9:** e0003555.

41 West SK, Munoz B, Weaver J, et al. Can we use antibodies to *Chlamydia trachomatis* as a surveillance tool for national trachoma control programs? Results from a district survey. *PLoS Negl Trop Dis* 2016; **10:** e0004352.

42 Zambrano AI, Sharma S, Crowley K, et al. The World Health Organization recommendations for trachoma surveillance, experience in Nepal and added benefit of testing for antibodies to *Chlamydia trachomatis* Pgp3 protein: NESTS Study. *PLoS Negl Trop Dis* 2016; **10:** e0005003.

43 Pant BP, Bhatta RC, Chaudhary JS, et al. Control of trachoma from Achham District, Nepal: a cross-sectional study from the Nepal National Trachoma Program. *PLoS Negl Trop Dis* 2016; **10:** e0004462.

44 Hufnagel K, Lueong, S Willhauck-Fleckenstein M, et al. Immunoprofiling of *Chlamydia trachomatis* using whole-proteome microarrays generated by on-chip in situ expression. *Sci Rep* 2018; **8:** 7503.

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

46 Sun MJ, Zambrano AI, Dize L, et al. Evaluation of a field test for antibodies against *Chlamydia trachomatis* during trachoma surveillance in Nepal. *Diagn Microbiol Infect Dis* 2017; **88:** 3–6.

47 Budrys NM, Gong S, Rodgers AK, et al. *Chlamydia trachomatis* antigens recognized in women with tubal factor infertility, normal fertility, and acute infection. *Obstet Gynecol* 2012; **119:** 1009–16.

48 Lu C, Holland MJ, Gong S, et al. Genome-wide identification of *Chlamydia trachomatis* antigens associated with trachomatous trichiasis. *Invest Ophthalmol Vis Sci* 2012; **53:** 2551–59.

49 Pickering H, Burr SE, Derrick T, et al. Profiling and validation of individual and patterns of *Chlamydia trachomatis*-specific antibody responses in trachomatous trichiasis. *Parasit Vectors* 2017; **10:** 143.

50 Hufnagel K, Lueong S, Willhauck-Fleckenstein M, et al. Immunoprofiling of Chlamydia trachomatis using whole-proteome microarrays generated by on-chip in situ expression. *Sci Rep* 2018; published online May 14. DOI: 10.1038/s41598-018-25918-3.

51 Chernesky M, Luinstra K, Sellors J, et al. Can serology diagnose upper genital tract *Chlamydia trachomatis* infections? Studies on women with pelvic pain, with or without chlamydial plasmid DNA in endometrial biopsy tissue. *Sex Transm Dis* 1998; **25:** 14–19.

52 Ades AE, Price MJ, Kounali D, et al. Proportion of tubal factor infertility due to chlamydia: finite mixture modeling of serum antibody titers. *Am J Epidemiol* 2017; **185:** 124–34.

53 Akande VA, Hunt LP, Cahill DJ, Caul EO, Ford WC, Jenkins JM. Tubal damage in infertile women: prediction using chlamydia serology. *Hum Reprod* 2003; **18:** 1841–47.

54 Claman P, Toye B, Peeling RW, Jessamine P, Belcher J. Serologic evidence of *Chlamydia trachomatis* infection and risk of preterm birth. *CMAJ* 1995; **153:** 259–62.

55 Wang SP, Grayston JT, Kuo CC, Alexander ER, Holmes KK. Serodiagnosis of *Chlamydia trachomatis* infection with the micro-immunofluorescence test. In: Hobson D, Holmes KK, eds. Nongonococcal urethritis and related infections. Washington DC: American Society for Microbiology, 1977: 237–248.

56 Frisse AC, Marrazzo JM, Tutlam NT, et al. Validity of self-reported history of *Chlamydia trachomatis* infection. *Am J Obstet Gynecol* 2017; **216:** 393.e1–7.

57 Moore KR, Smith JS, Cole SR, Dittmer DP, Schoenbach VJ, Baird DD. *Chlamydia trachomatis* seroprevalence and ultrasound-diagnosed uterine fibroids in a large population of young African-American women. *Am J Epidemiol* 2018; **187:** 278–86.

58 Horner PJ, Wills GS, Reynolds R, et al. Effect of time since exposure to *Chlamydia trachomatis* on chlamydia antibody detection in women: a cross-sectional study. *Sex Transm Infect* 2013; **89:** 398–403.

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

60 de Vries HJ, Zingoni A, Kreuter A, et al. 2013 European guideline on the management of lymphogranuloma venereum. *J Eur Acad Dermatol Venereol* 2015; **29:** 1–6.

61 Miller WC. Epidemiology of chlamydial infection: are we losing ground? *Sex Transm Infect* 2008; **84:** 82–86.

62 European Centre for Disease Prevention and Control. Chlamydia—annual epidemiological report 2016 [2014 data]. Dec 30, 2016. http://ecdc.europa.eu/en/healthtopics/chlamydia/Pages/Annual-epidemiological-report-2016.aspx (accessed Nov 1, 2017).

63 Satterwhite CL, Grier L, Patzer R, Weinstock H, Howards PP, Kleinbaum D. Chlamydia positivity trends among women attending family planning clinics: United States, 2004–2008. *Sex Transm Dis* 2011; **38:** 989–94.

64 Sonnenberg P, Clifton S, Beddows S, et al. Prevalence, risk factors, and uptake of interventions for sexually transmitted infections in Britain: findings from the National Surveys of Sexual Attitudes and Lifestyles (Natsal). *Lancet* 2013; **382:** 1795–806.

65 Torrone E, Papp J, Weinstock H, and the Centers for Disease Control and Prevention (CDC). Prevalence of *Chlamydia trachomatis* genital infection among persons aged 14–39 years—United States, 2007–12. *MMWR Morb Mortal Wkly Rep* 2014; **63:** 834–38.

66 Klavs I, Rodrigues LC, Wellings K, Kese D, Hayes R. Prevalence of genital *Chlamydia trachomatis* infection in the general population of Slovenia: serious gaps in control. *Sex Transm Infect* 2004; **80:** 121–23.

67 Horner P, Soldan K, Vieira SM, et al. *C trachomatis* Pgp3 antibody prevalence in young women in England, 1993–2010. *PLoS One* 2013; **8:** e72001.

68 Solomon AW, Harding-Esch E, Alexander ND, et al. Two doses of azithromycin to eliminate trachoma in a Tanzanian community. *N Engl J Med* 2008; **358:** 1870–71.

69 Harding-Esch EM, Sillah A, Edwards T, et al. Mass treatment with azithromycin for trachoma: when is one round enough? Results from the PRET Trial in the Gambia. *PLoS Negl Trop Dis* 2013; **7:** e2115.

70 Admassu F, Bayu S, Bejiga A, Amare B. Active trachoma two years after three rounds of azithromycin mass treatment in Cheha District Gurage Zone, Southern Ethiopia. *BMC Pediatr* 2013; **13:** 199.

71 Becker NG. Analysis of infectious disease data. London: Chapman & Hall, 1989.

72 Sepulveda N, Stresman G, White MT, Drakeley CJ. Current mathematical models for analyzing anti-malarial antibody data with an eye to malaria elimination and eradication. *J Immunol Res* 2015; **2015:** 738030.

73 Bakshi RK, Gupta K, Jordan SJ, et al. Immunoglobulin-based investigation of spontaneous resolution of *Chlamydia trachomatis* infection. *J Infect Dis* 2017; **215:** 1653–56.

74 Price MJ, Ades AE, Welton NJ, Simms I, Macleod J, Horner PJ. Proportion of pelvic inflammatory disease cases caused by *Chlamydia trachomatis*: consistent picture from different methods. *J Infect Dis* 2016; **214:** 617–24.

75 Gottlieb SL, Deal CD, Giersing B, et al. The global roadmap for advancing development of vaccines against sexually transmitted infections: update and next steps. *Vaccine* 2016; **34:** 2939–47.

76 Wang J, Zhang Y, Lu C, Lei L, Yu P, Zhong G. A genome-wide profiling of the humoral immune response to *Chlamydia trachomatis* infection reveals vaccine candidate antigens expressed in humans. *J Immunol* 2010; **185:** 1670–80.

77 Deleré Y, Wichmann O, Klug SJ, et al. The efficacy and duration of vaccine protection against human papillomavirus: a systematic review and meta-analysis. *Dtsch Arztebl Int* 2014; **111:** 584–91.

78 Datta J, Wellings K, Kessel A. 'Once the stuff's left my body, it's not me': service users' views on unlinked anonymous testing of blood for HIV. *Cult Health Sex* 2013; **15:** 896–909.

79 US Food and Drug Administration. Guidance for industry and review staff. Target product profile—a strategic development process tool. March, 2007. https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm080593.pdf (accessed Nov 1, 2017).

80 WHO. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva: World Health Organization, 2014.

81 WHO. STI Point of Care Tests Meeting Report. Annecy: World Health Organization, 2014.

82 Metcalf CJ, Farrar J, Cutts FT, et al. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. *Lancet* 2016; **388:** 728–30.