

Advancing the Public Health Applications of *Chlamydia trachomatis* serology

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SUMMARY

New serological tests for *Chlamydia trachomatis* (Ct) present promising options to improve understanding of Ct epidemiology and prevention. Ct serology offers a means of investigating Ct incidence and may be developed as a biomarker of scarring disease. Serological assays therefore have potential as epidemiological tools to quantify unmet need, inform service planning and evaluate interventions including Ct screening, treatment and new vaccine candidates. However, questions about their performance characteristics and interpretation remain, which must be addressed to advance development in this field. In this personal view, we explore the current state of knowledge related to Ct serology and propose several priority actions. These are: i) development of 'target product profiles' to guide assay selection and evaluation across multiple applications and populations; ii) establishment of a serum bank to facilitate assay development and evaluation and iii) development of technical and statistical methods for assay evaluation and analysis of serological findings.

Introduction

There has been substantial investment over the last decade in public health programmes to control both genital¹ and ocular² infection with *Chlamydia trachomatis* (Ct). However, several important questions about Ct epidemiology, the most effective means of control, and optimum models of surveillance remain.³⁻⁵ Given ongoing control efforts, and the promise of Ct vaccines on the horizon,⁶ robust methods are needed to allow monitoring of and insight into Ct prevalence, incidence,⁷ and the progression to scarring sequelae. Measures of current infection based on DNA/RNA detection provide a limited understanding of these features of Ct infection. Alternative approaches are therefore required, and in recent years we have seen a revival in the use of Ct serology in the fields of genital chlamydia and trachoma.

Methods to detect Ct antibodies in serum have been available for several decades.⁸ However, use of Ct serology has been hampered by cross-reactivity with other chlamydia species,⁹ suboptimal sensitivity of many assays,^{10,11} an incomplete understanding about the longevity and clinical implications of Ct antibodies and the relationship between Ct infection and antibody response.¹⁰ Consequently, chlamydia seroepidemiology fell out of widespread use among researchers and funding bodies for several years. Following the development of novel, sensitive and more specific Ct serologic assays,¹²⁻¹⁶ there is now growing interest in the use of Ct serology as an epidemiological tool. For example, assays have been developed with capability to detect antibodies against a range of Ct 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).

Our understanding of mucosal immunity and Ct immunology suggest that urogenital and ocular infections with Ct lead to detectable IgG response using appropriate serological assays in the majority of confirmed infections.^{12-14,17} Several factors affect the magnitude of response and the ability of serological tests to detect a previous infection, including the target antibody, the assay used, time since infection and patient characteristics such as age, sex, race and prior Ct infection.^{12,13,15,18} In a UK-based study that compared several assays in the same population, the sensitivity to detect a previous known infection ranged from 46% (Medac) to 83% (Pgp3 double-antigen) in women and 40% (SeroCT) to 54% (Pgp3 double-antigen) in men when compared to a previous Ct diagnosis by NAAT.^{12,14} Seroreversion (loss of detectable antibodies) has been demonstrated in some cases but varies by infection history and assay,¹⁸ with minimal loss of detectable antibody reported in one study using a double-antigen Pgp3 ELISA.¹⁴ In a study of Ct seroprevalence in the context of mass azithromycin treatment for trachoma prevention in a high prevalence area, no instances of seroreversion were observed after six months.¹⁹ Ct serological tests can therefore be used to measure age-specific cumulative incidence,^{10,20} albeit representing a lower bound estimate due to potentially incomplete seroconversion and loss of detectable antibodies over time. Ct antibody response has also been found to correlate with known history of scarring sequelae, with antibody titres found to be higher in those with tubal factor infertility²¹ and detection of specific antibodies being more common in those with known disease.²² Thus, serological assays may also offer the promise of use as a biomarker of disease.

Given the imperfect sensitivity of serological tests, Ct serology has limited diagnostic value; in the absence of genetic diagnostic methods it is an accepted tool for presumptive diagnosis of lymphogranuloma venereum (LGV),²³ yet is not utilized for diagnosis of other biovars. The real potential value of Ct serology is instead in the realm of surveillance. Similar to the use of HIV antibody tests within recent infection test algorithms (RITA) to support and monitor recent HIV infection,²⁴ Ct serology is unlikely to aid in the diagnosis or management of Ct infection. However, it may prove invaluable in monitoring history of Ct exposure to inform resource allocation and possible clinical need as well as the impact of population-based interventions.

In this personal view, we explore the potential public health applications of Ct serology, discuss key challenges of using Ct serology in these ways and finally propose priorities for research and development to progress the field. This work grew out of an expert meeting convened by Public Health England in 2016 and subsequent discussions, and has been supplemented by a rapid review of the literature, using searches of two electronic databases (MEDLINE and EMBASE, see Table 1). We hope that our collective thoughts help create a direction for research and programmatic activities for this exciting field.

Public health applications of *Chlamydia trachomatis* serology

Ct serology provides a means of quantifying the prevalence and incidence of Ct infection. A more thorough understanding of population-level Ct prevalence and incidence is critical to identify unmet need for screening and treatment services and to evaluate the impact of Ct control interventions. Obtaining reliable estimates of these measures is, however, challenging. In the case of genital chlamydia, the

majority of Ct infections are asymptomatic and increases in screening leads to increases in reported diagnoses.²⁵ As a result, surveillance is often based on case-based reporting alone with no or limited information on numbers tested, which is believed to produce a gross underestimate of the true population level of disease.²⁵ Furthermore, comparability between countries is limited by differences in testing recommendations, performance characteristics of diagnostic tests, and reporting policies and practices.²⁶ Even where testing denominators are available, interpreting positivity as a measure of prevalence is difficult as the tested population has a different underlying risk from the general population.^{25,27} Few countries have undertaken surveys of prevalence in general population samples and where surveys have been done,²⁸⁻³⁰ they were resource intensive and are unlikely to be feasible in many settings.

Ct seroprevalence as a marker of cumulative incidence has been used in several countries^{14,20,31-34} as a means of exploring Ct epidemiology and in some cases to investigate population impact of control interventions. In the field of trachoma, mass drug administration (MDA) programmes have been hugely successful in reducing Ct infection and Ct-related ocular disease.³⁵⁻³⁷ Longitudinal Ct serology monitoring has strong potential as a tool for post-elimination surveillance,³⁸⁻⁴⁰ 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.⁴¹ With suitable statistical approaches, Ct serology has the potential to be used to measure annual incidence of Ct (at any site of infection) using repeated, cross-sectional measurement over time (Ades, personal communication) or to detect step changes in exposure by birth-cohort expected in the context of

control measures.^{38,42} Distinguishing between recent and past or longstanding infections would also help to inform understandings of incidence and methods are already being developed to enable the use of Ct serology in this manner.⁴³

The second potential application of Ct serology is as a measure of Ct-related disease, such as pelvic inflammatory disease (PID) or ectopic pregnancy (EP). As the ultimate goal of Ct control is to reduce incidence of disease, monitoring biomarkers of disease (and not just infection) would allow an improved understanding of whether Ct control is leading to a reduction in reproductive sequelae, even in the absence of substantial reductions in transmission. A Ct-specific biomarker of disease would be especially useful, because Ct is not the only cause of long-term reproductive complications such as PID, EP, and tubal factor infertility (TFI), and because Ct-related conditions may occur many years after the causative infection.⁴⁴ Measures of the proportion of long-term sequelae that are attributable to Ct infection (the '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 Ct infection and sequelae and to estimate the proportion of long-term sequelae attributable to genital Ct infection.^{21,45-48} Novel approaches also offer some promise in this area. For example, Ades *et al* have developed a method using finite mixture modelling of antibody titre to estimate the population excess fraction of TFI caused by chlamydia.²¹ Additionally, proteomic arrays are also being explored as a means of identifying 'serological fingerprints' to indicate the presence of disease related to

genital Ct infection (personal communication, Katrin Hufnagel, DKFZ) and scarring following ocular infection.⁴⁹

The third potential application of Ct serology is in the development and evaluation of Ct vaccines. The need for an effective Ct vaccine has been set out in the joint WHO-NIH STI vaccine roadmap.⁵⁰ Substantial progress towards a Ct vaccine has been made in recent years, with candidate vaccines now in the preclinical and clinical testing phases.⁶ Several 'priority action areas' set out in the roadmap may be addressed through serology. These include obtaining better epidemiological data, improving understanding of the natural history of Ct and burden of sequelae, expediting clinical development and evaluation and encouraging investment in Ct vaccine development. Specifically, Ct serology could be used to obtain more complete and precise estimates of the global burden of Ct-associated sequelae, which are critical for establishing the public health rationale for vaccination and for potential investors who need to assess the likely impact of investing in any successful vaccine candidate.⁶

When a safe vaccine candidate does come to Phase III clinical trial, there is also a clear role for serology in identifying Ct-naïve participants for recruitment and for developing 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, given current diagnostic inaccuracy for Ct-related outcomes such as PID.⁴⁴ The time and resources needed to power a clinical trial of candidate Ct vaccines with PID or TFI as outcomes may also be prohibitive. It is not yet clear whether serology will be able to

provide such a marker, perhaps in combination with cellular markers or radiologic findings, but this is an important area of interest given the need for such measures in any future vaccine evaluation. As Ct infections (with the exception of LGV) 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 Ct,¹⁷ which may complement serological investigations. Assessing vaccine-induced immune responses will depend on the vaccine's mechanism of action. Assays that distinguish between natural and vaccine-induced antibody response will therefore be needed.

Key challenges to the use of Ct serology in Public Health

While progress has been made in recent years, there remain some important challenges within the field of Ct serology that need to be addressed to improve the utility and value of these methods in a public health context. Interpreting Ct seroprevalence is not straightforward. Complexities include that: not everyone exposed to Ct will become infected; some individuals with Ct infection will not necessarily develop antibodies; women are more likely to develop detectable antibodies than men following urogenital Ct infection;^{12,20,51} Ct antibodies are not infection-site (i.e. ocular/urogenital) specific; and seroprevalence can vary with number of previous infections and time since infection (as antibodies develop or wane).¹⁸ These complexities require careful consideration when planning studies and undertaking statistical analysis. However, we imagine many of these limitations will bias estimates of Ct seroprevalence to the same degree over time, allowing for particular utility in monitoring trends over time.

Assay sensitivity and specificity determination in the absence of an agreed-upon gold standard is also a challenge. Careful consideration of the application and population in which a test is to be used will be needed when selecting negative and positive controls and determining assay thresholds. For example, studies exploring previous Ct infection versus a specific Ct-associated complication may dictate different analytic needs in terms of sensitivity and specificity and choice of controls. Choice of thresholds for distinguishing Ct 'positive' from 'negative' antibody responses in different populations presents a further challenge, as assays may be affected by differences in cross-reactivity and background antibody levels, which can vary (e.g. by country and/or ethnicity).

The relative performance of different tests cannot easily be determined without evaluation against the same reference sera. Some laboratory-developed assays have been compared to commercial assays or other laboratory-developed assays,^{12,14,52,53} but there is relatively little data available to show how different assays perform within the same population. In order to establish performance characteristics of assays for different applications and populations, large numbers of sera which are well-characterised in terms of clinical and demographic information are needed. Serum collections from previous studies (e.g. HPV vaccine trials,⁵⁴ HIV unlinked anonymous testing⁵⁵) or residual samples from clinical testing³¹ could be of use, but these often have limited clinical or demographic information, and varying access arrangements mean that assays have not been evaluated on comparable samples.

Optimal test characteristics for one application may vary from those needed in another, meaning that different characteristics may be prioritised. For example, a test to measure if someone has had Ct infection will need to detect antibodies that persist over time at relatively low levels with high specificity. However, a test which is used to estimate the population excess fraction would ideally be able to distinguish between complicated and uncomplicated Ct infections (e.g. by identifying high versus low levels of antibody in serum,²¹ subclass of antibody,¹³ or antibodies specifically associated with complications²²).

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 may be acceptable in a setting where additional blood can be collected from consenting patients whereas surveillance systems relying on leftover sera from routine testing may have a limited volume available. Applications in a surveillance context may be more tolerant to some reduced precision than if used within a vaccine trial, where previous infection needs to be ruled out to precisely define populations for efficacy analysis.

Next Steps

In order to address the challenges set out above, we propose a number of actions to address these research and development priorities as follows:

1. Generate target product profiles for Ct serological tests

Target product profiles (TPPs) originated in the field of drug development as a means of focussing 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.⁵⁶ The process of establishing TPPs is now widespread in drug and vaccine development and their use has also extended into the field of diagnostics, for example for tuberculosis⁵⁷ and point of care diagnostics for STI.⁵⁸

TPPs for Ct serological tests should establish the minimal and optimal assay requirements for the different applications described above. Table 2 sets out some of the initial considerations that can be used to inform TPPs. A TPP requires broad technical consultation across clinical, microbiological and epidemiological science, representation from vaccine and diagnostic development companies, research groups, public health agencies and funders.

2. Establish a serum bank of adequate and well-characterised sera, with standardised clinical outcome assessment and epidemiological data and appropriate access arrangements.

A well-defined serum bank with the evaluation of Ct serology in mind will be an invaluable resource. The value of serum banks in infectious diseases research was recently set out in an editorial in the Lancet.⁵⁹ The development of a Ct-specific serum bank would facilitate clear and fair access to specimens and relevant epidemiologic and clinical data (i.e. age, sex, Ct diagnosis history). A serum bank would have enormous potential to support 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 with a range of characteristics, including: varying histories of Ct diagnosis, incorporating individuals with varying time since treatment and numbers of known infections; those with and without reproductive complications; exposure to potentially cross-reactive pathogens such as *C. pneumoniae*; and from a range of populations across the world. Some applications such as identifying biomarkers of scarring sequelae, or developing serological test to distinguish between infection and exposure may also benefit from simultaneous assessment of cellular immunity. Thus, collections that incorporate both serum and whole blood specimens would be particularly valuable, although would require different regulatory permissions in some settings and would incur additional expense arising from arrangements for collection and storage.

3. Develop methods for assay evaluation and analysis of serological findings.

Shared protocols to guide assay evaluation will allow for comparability of estimates using Ct 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 Ct 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 Ct serological findings.

Summary & conclusion

As reported Ct rates remain high or continue to rise in many developed countries, Ct serology holds much promise in several areas of public health and is already being used to gain further insight into Ct epidemiology and natural history. We explored the current state of knowledge related to Ct serology and identified several priority actions that we believe would directly benefit public health and advance knowledge within the Ct field (Panel 1).

As public health agencies continue to be pressed to address rates of Ct and the considerable accompanying morbidity arising from infections, a more data-driven approach to programmatic decision making at the country, state, and municipality level is critical. Promising interventions, including vaccines, do and will need robust measures for estimating the at-risk population and determining the potential impact of prevention measures. Ct serology holds much promise as an additional public health tool to help better understand populations at risk for Ct and develop novel and effective interventions.

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Contributors

In September 2016, Public Health England convened a multidisciplinary expert meeting entitled 'The Public Health applications of *Chlamydia trachomatis* serology'. Participants included representatives from national and international public health organisations, academia and other research institutions. Individuals based in Europe, the US and Australia from the fields of genital Ct and trachoma attended. The discussions at this meeting and subsequently with the writing group informed this personal view. SW, RG, KD and KB organised and delivered the expert meeting, which was attended by all the authors. SW and SM carried out the literature searches. SW wrote the first draft of the manuscript. All authors commented on the manuscript and approved the final version.

Conflict of interest

Dr. Geisler reports grants from Genocea Biosciences, grants from *Moderna Therapeutics*, outside the submitted work. Dr. Huston has a patent *Chlamydia trachomatis* diagnostic peptide and method, PCT/AU2013/001333 pending. Dr. Horner reports personal fees from Crown Prosecution service, personal fees from British Association for Sexual Health and HIV, grants from Mast Group Ltd, non-financial support from Hologic, outside the submitted work. In addition, Dr. Horner has a patent for a sialidase spot test to diagnose bacterial vaginosis issued to University of Bristol. Other authors report no conflicts of interest.

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Panel 1: Research priorities to further develop the public health applications of *Chlamydia trachomatis* serological assays

1. Generate target product profiles (TPP) for Ct serological assays.
 - What are the minimal/optimal characteristics of Ct serological assays for different purposes (design and evaluation of genital Ct control programmes; design and evaluation of trachoma/ocular Ct control programmes; vaccine development and evaluation) and measures (seroprevalence Ct antibodies as a measure of Ct prevalence and incidence; measure of population excess fraction of disease, e.g. PID, tubal factor infertility; biomarker of disease, alone or in combination with other measures; measure of being Ct-naïve; measure of vaccine-induced immune response)?
 - What are the minimal/optimal characteristics for the above purposes and measures in different countries?
2. Establish a serum bank
 - To include adequate volumes of well-characterised sera from women and men who have and have not had Ct infection of a variety of ages (including children who may still have maternal antibody) and ethnicities, with a range of characteristics including: number of known infections, time since treatment and presence of known reproductive tract/ocular complications.
 - To incorporate standardised clinical outcome assessment and epidemiological data.
 - Established with appropriate access arrangements.
3. Develop methods for assay evaluation and analysis of serological findings
 - How should sensitivity and specificity be estimated for different purposes?
 - How should positive and negative controls be selected for different test applications?
 - What assay thresholds should be used for each assay for different applications/test settings?
 - How should head to head comparison studies be carried out?
 - What statistical methods should be used for measuring epidemiological parameters (e.g. incidence, population excess fraction)?

Table 1: *Chlamydia trachomatis* serological assays*

Assays/group	Platform/format	Antigen(s) (antibody class/subclass detected)	Examples of public health applications to date*		
			Measure seroprevalence/estimate incidence	Investigate association with disease	Evaluate control intervention(s) Genital Ct Trachoma
ELISAs					
Wills et al ¹²	Indirect ELISA	Pgp3 (IgG)	○ ²⁰		○ ²⁰
Horner et al ¹⁴	Double-antigen ELISA	Pgp3 (IgG, IgA, IgM)	○ ^{14,20,34}		○ ²⁰
Winstanley et al ¹⁵	Indirect ELISA	Pgp3 (IgG)			
Albritton et al ¹⁷	Indirect ELISA	Elementary bodies (EBs) from Ct serovars D/UW3/Cx and E/UW5/Cx (IgG, IgA)		○ ¹⁷	
Menon et al ⁶⁰	Multi-peptide indirect ELISA	12-mer peptides derived from HtrA, hsp60 and Ct443 ⁶¹ (IgG)		○ ^{60,62}	
Migchelsen et al ³⁵	Indirect ELISA on dried blood spots	Pgp3 (IgG)	○ ^{35,63}		
Geisler et al ¹³	Indirect ELISA	Ct EBs of serovars <u>D</u> /UW-3, <u>F</u> /ICCa1-13, and <u>J</u> /UW-36 (IgG1, IgG3)	○ ^{13,64}	○ ^{45,48}	
Commercially available ⁶⁵⁻⁶⁷	ELISA/EIA	MOMP, hsp60 (IgG, IgA)	○ ^{32,33,68}	○ ^{46,48,69-75}	
Multiplex bead arrays					
Goodhew et al ¹⁶	Multiplex bead array	Pgp3, CT694 (IgG, IgA)	○ ¹⁶		○ ^{38-40,76}
Willhauck-Fleckenstein et al ⁷⁷	Multiplex bead array	MOMP A/D/L2, PorB, TARP, hsp60-1, Pgp3 (IgG, or IgG, IgA, IgM)			
Near-patient testing					
Gwyn et al ⁵²	Lateral flow	Pgp3 (IgG, IgA, IgM)	○ ^{52,53}		
Whole proteome microarray					
Lu et al ⁷⁸	Whole proteome microarray	Representing 908 genomic and plasmid ORFs of Ct strain D/UW3 (IgG, IgA, IgM)		○ ^{49,78}	
Hufnagel et al ⁷⁹	Whole proteome microarray	Representing 895 proteins of Ct strain D/UW-3/Cx (IgG, or IgG, IgA, IgM)		○ ⁷⁹	
Budrys et al ²²	ELISA-based proteome array	Representing 908 proteins of Ct strain D/UW-3/Cx (IgG)		○ ²²	
Immunofluorescence					
Chernesky et al ⁸⁰	Whole cell inclusion immunofluorescence (WIF)	L2 serovar (IgG, IgA, IgM)		○ ^{21,81}	
Commercially-available ^{82,83}	Micro-immunofluorescent assay (MIF)	Ct EBs of serovars D-K (IgG, IgA, IgM)		○ ⁸⁴	
Wang ⁸⁵	Modified MIF protocol	Ct EBs (IgG)	○ ⁸⁶	○ ⁸⁷	

*Assays listed are provided as examples within the assay type and examples of public health applications to date are included; the absence of a study does not indicate absence of potential use; this is not intended to be a comprehensive review. Assays and studies were identified by participants of the expert meeting, including newly-developed assays presented at the ESCR 2016 meeting and a subsequent search of Embase and Pubmed from September 2016 using the terms 'Chlamydia trachomatis', 'serology' and 'antibodies' to identify emerging evidence and applications. Ct, *Chlamydia trachomatis*; EB, elementary body; ELISA, enzyme-linked immunosorbent assays; EIA, enzyme immunoassay; HtrA, high temperature requirement A protease; MOMP, major outer membrane protein; TARP, translocated actin-recruiting phosphoprotein; hsp60, heat shock protein 60; ORF, open reading frame.

Table 2: Considerations for different applications of *Chlamydia trachomatis* serological assays

	Measuring infection	Measuring disease	For use in vaccine evaluation
Performance requirements	<p>High sensitivity and specificity in relation to infection.</p> <p>Ability to distinguish first from repeat infection and ability to measure recent infection would be beneficial.</p> <p>Monitoring and surveillance applications could likely tolerate lower precision than needed for vaccine studies.</p>	<p>Able to distinguish between complicated and uncomplicated infections.</p> <p>Relies on identifying disease-specific antigens or combinations of antigens AND/OR Magnitude of response associated with disease.</p> <p>High specificity for sequelae to prevent over-investment in resource poor environments arising from over-estimation of the incidence of Ct sequelae.</p>	<p>To quantify burden of infection/disease As for measuring infection/disease</p> <p>For determining Ct-naïve status for trials High precision High sensitivity Distinguish between exposure and infection</p> <p>For vaccine efficacy measurement Marker of tubal involvement potentially in combination with other measures (e.g., cellular markers, radiologic measures) would be valuable. Marker of vaccine-induced immune response will depend on mechanism of action of vaccine; will need to distinguish vaccine-induced vs natural responses.</p>
Dependencies	Appropriate panel(s) of population-based sera.	<p>To estimate disease incidence Appropriate panel(s) of population-based sera.</p> <p>To estimate PEF Availability of reliable cases and controls with clear case definition.</p>	Vaccine trial design. Mechanism of action of vaccine candidate.
Statistical method considerations	<p>Establishing Ct serological assay threshold/cutoffs appropriate to the application and population.</p> <p>Relationship between seroprevalence and cumulative/annual incidence needs consideration of impact of time since infection and repeat infections on Ct antibodies.</p>	Establishing threshold/cutoffs appropriate to the application and population.	Establishing threshold/cutoffs appropriate to the application and population.
Technical requirements	High throughput, low volume, and low resource utilization methods would be valued.	<p>Monitoring and surveillance applications would value high throughput, low volume, and low resource utilization methods.</p> <p>Research applications may tolerate methods requiring higher specimen volume/operator intensive methods.</p>	Could likely tolerate methods requiring higher specimen volume/ operator intensive methods.

Ct: *Chlamydia trachomatis*; PEF: population excess fraction (the proportion of long-term sequelae that are attributable to Ct infection)

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