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Chlamydia trachomatis seroassays used in epidemiologic research: a narrative review and practical considerations

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Abstract

Chlamydia trachomatis (CT) is a sexually transmitted infection that can lead to adverse reproductive health outcomes. CT prevalence estimates are primarily derived from screening using nucleic acid amplification tests (NAATs). However, screening guidelines in the United States only include particular subpopulations, and NAATs only detect current infections. In contrast, seroassays identify past CT infections which are important for understanding the public health impacts of CT, including pelvic inflammatory disease and tubal factor infertility. Older seroassays have been plagued by low sensitivity and specificity and have not been validated using a consistent reference measure, making it challenging to compare studies, define the epidemiology of CT and determine the effectiveness of control programs. Newer seroassays have better performance characteristics. This narrative review summarizes the "state of the science" for CT seroassays that have been applied in epidemiologic studies and provides practical considerations for interpreting the literature and employing seroassays in future research.

Keywords

Chlamydia trachomatis ; serology; antibodies; epidemiology

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Author's contributions

M.B.W. conducted the literature review, abstracted the data, and wrote the first draft of the manuscript. K.H., R.I., and B.K. critically reviewed abstracted laboratory data, and L.E.M. and K.M.K critically reviewed epidemiologic data. C.M.K conceptualized the review, oversaw the literature review and abstraction, and critically reviewed all abstracted data. All authors critically reviewed and approved the manuscript.

Conflicts of interest

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INTRODUCTION

Prior to SARS-CoV-2, *Chlamydia trachomatis* (CT) was the most common nationally notifiable condition in the United States (US)[1], with an estimated 4 million incident cases of CT in 2018[2], leading to $691 million in direct medical costs[3]. CT, which infects epithelial cells of the oropharynx, eye, urogenital tract, and gastrointestinal tract, causes substantial reproductive tract morbidity, including pelvic inflammatory disease (PID), chronic pelvic pain, ectopic pregnancy, and infertility[4,5]. Despite longstanding CT control programs in the US, rates of CT infections have increased in the past 10 years and reached an all-time high in 2019[1].

Most CT infections are asymptomatic and only detected through screening. Current US guidelines recommend annual CT screening for sexually active women under age 25, sexually active women 25 years and older who are at increased risk[6], and men who have sex with men (MSM) at sites of contact[4]. National guidelines also recommend more frequent screening for MSM who are at higher risk and recommend screening transgender populations based on anatomy[4]. However, only an estimated 50% of sexually active women under age 25 in the US are screened annually for CT[7], and other populations (e.g., heterosexual men) are not systematically screened. This inability to comprehensively capture CT cases has led to a substantial underestimate of the burden of CT in the US[8]. Further, clinical screening guidelines for CT recommend nucleic acid amplification tests (NAAT), which detect DNA or RNA from urine samples or vaginal, rectal, urethral, or eye swab specimens[1]. While these tests allow for *current* infections to be detected, they are unable to detect *past* infections[9]. As most CT infections are transient, the utility of NAATs to monitor population-level burden of CT is limited.

This limitation of NAATs has motivated the use of seroassays in epidemiologic studies that aim to identify the prevalence of “lifetime” infection (i.e., whether or not someone has ever been infected with CT). This application of CT seroassays is critical to understanding correlates of protection and associations between past CT infections and adverse reproductive tract outcomes that can lead to infertility in females. Relatively recently, there have been several assays developed that utilize novel combinations of CT antigens[10,11]. These newer assays – which often have improved sensitivity and specificity compared to older assays – have permitted a better understanding of the timing of seroconversion of antibodies to CT[12–16] and present an opportunity to estimate lifetime prevalence of CT infection more accurately.

The goal of this narrative review is to summarize and compare CT seroassays that have been used in epidemiologic studies (i.e., studies that quantify population-level burden of CT or the association between CT and reproductive health outcomes) and to present practical considerations for interpreting the literature and applying CT seroassays in future studies. First, we describe the “state of the science” of CT seroassays, including assay function, validation, and use in epidemiologic studies. Next, we offer some considerations for investigators applying CT seroassays in epidemiologic studies, including laboratory resources, what is currently known about the human host antibody response to CT, and the

potential for misclassification when using antibodies to CT as a marker of past infection. We conclude by discussing future applications for CT seroassays.

SEROASSAYS TO DETECT ANTIBODIES TO CT

Overview of Seroassays

Over the past five decades, several seroassays have been developed to detect IgA, IgM, and IgG anti-CT serum antibodies. These assays are not recommended for clinical diagnoses of CT infections, and commercial versions of these assays are not approved by regulatory bodies such as the United States Food and Drug Administration. Additionally, use of these assays outside of epidemiologic CT studies is fairly limited, although some argue that seroassays may be useful when evaluating patients with suspected PID[17].

To identify CT seroassays to include in this review, we reviewed English-language publications that either (1) described the development and validation of CT seroassays, (2) compared various types of CT seroassays, or (3) estimated seroprevalence of antibodies to CT and/or the association of seroprevalence of antibodies with adverse reproductive health outcomes in a population. We searched PubMed and Google between October 1, 2021, and August 1, 2022, using the search terms “chlamydia serological assays”, “*Chlamydia trachomatis* serological assays”, and “*Chlamydia trachomatis* serology”. We reviewed references from published papers yielded in this search to identify papers that may have been missed in the initial search.

We identified 26 distinct types of validated CT seroassays (further sub-divided into 28 commercially-available versions and 25 non-commercial versions of these assays) that have been reported in 55 publications. A full description of the function, validation, and strengths and weaknesses of these assays is provided in Supplementary Table 1. Because the goal of this review is to focus on CT seroassays that have been used in epidemiologic studies, we provide no further information on the CT seroassays that have been developed and validated but not applied in an epidemiologic context. From the 26 types of assays that we initially identified, 10 have been used or are currently being used in epidemiologic studies to measure seroprevalence of anti-CT antibodies in various populations or the association between seroprevalence and other health outcomes. The remainder of this narrative review focuses on these 10 seroassays (described in detail in Table 1): the microimmunofluorescence assay (MIF), the whole cell inclusion immunofluorescence assay (WIF), the major outer membrane protein ELISA (MOMP ELISA), the Heat Shock Protein 60 ELISA (cHSP60 ELISA), the lipopolysaccharide recombinant ELISA (LPS rELISA), Plasmid Gene Protein 3 ELISA (Pgp3 ELISA), the Luminex MAGPIX multiplex bead array (MBA), the elementary body ELISA (EB ELISA), the mixed peptide ELISA, and the Pgp3 luciferase immunosorbent assay (Pgp3 LISA).

Seroassay Methods of Detection

The MIF, WIF, and MBA use immunofluorescence for the detection of antibodies to CT. The MIF was developed by Wang and Grayston in the early 1970s[18] and has historically been the “gold standard” of CT seroassays[19–26]. Many versions use CT elementary bodies,

primarily formalin-fixed outer membrane protein A (OmpA) as the antigen on glass slides to detect IgG and IgM antibodies to CT, but other antigen preparations may also be used, including those to detect IgA[23,27,28]. Similar to the MIF, the WIF assay uses fluorescence to detect IgG and IgM antibodies to CT LPS IgG and MOMP, respectively, and in contrast to the MIF, the WIF uses the entire CT inclusion as antigen rather than elementary bodies[29]. The MBA also uses fluorescence to detect IgG antibodies[30,31].

The Pgp3 LISA uses a luciferase immunoprecipitation system[10], where the presence of IgG antibody is detected via luminescence[32]. The remaining assays that we focus on are classified as ELISAs, which use colorimetric substrates and enzyme amplification to detect antibodies[30]. In terms of antibody detection, the MOMP ELISA uses major outer membrane protein, which is encoded by the *ompA* gene of CT[33] and has been used to detect IgG, IgM, or IgA antibodies. The LPS rELISA[21], EB ELISA[16,34–37], and the mixed peptide ELISA[11] can be used to detect IgG and IgA CT antibodies, while the cHSP60 ELISA[19,20,38,39] and the Pgp3 ELISA[19,26,40] exclusively detect IgG in a manner similar to the MBA.

Seroassay “validation” studies

There is currently no agreed-upon reference standard to evaluate CT seroassays. Most assays included in this review used NAAT at the time serum was drawn as a reference standard for validation. However, using NAAT as a reference standard may not be appropriate when the goal is to estimate history of CT infection. Using NAAT as a comparison indicates whether the assay is sensitive and specific at detecting antibodies during a *current* infection, but not whether it is a valid assay to detect antibodies from a *prior* infection. The MIF has also been used as a reference standard for validating several CT seroassays[19,20,22,23,25,26]. However, due to the lower sensitivity of the MIF in comparison to newer CT seroassays such as the MOMP and mixed peptide ELISAs as well as the Pgp3 LISA and MBA, using the MIF alone may no longer be the best choice for a reference standard.

Comparisons of Seroassays

Despite the lack of a reference standard for CT seroassays, the seroassays we reviewed provided published estimates of “sensitivity” and “specificity”. Although this terminology may not be accurate (i.e., true sensitivity would be a measure of how many individuals were seropositive of all those infected, which is unknown), these published values permit comparisons across assays that used the same reference (e.g., NAAT). Here, we present these values as positive percent agreement (PPA) and negative percent agreement (NPA), which represent the percent of people with current infection who are seropositive and percent without current infection who are seronegative, respectively. In Table 1 we report the mean and range of these percentages for each assay in the detection of IgG when NAAT was used as the reference standard (except where otherwise noted, see footnotes). The mean PPA across the assays ranged from 52.4% to 100%. The mean NPA ranged from 5.9% to 100%. A detailed description of the populations included in these validation studies and the raw sensitivity and specificity values for assays with multiple validation studies where we present means and ranges are provided in Supplementary Table 1.

The assays with mean PPA >80% were the WIF, LPS rELISA, MBA, mixed peptide ELISA, and the Pgp3 LISA, with the highest PPA (~93% or higher) noted for the WIF, MBA, and the Pgp3 LISA. A mean NPA of 80% or higher was noted for all seroassays except the WIF, LPS rELISA, and the MBA. The EB ELISA, mixed peptide ELISA, and the Pgp3 LISA all reported a mean NPA >98%. However, these results for the WIF, Pgp3 ELISA, MBA, mixed peptide ELISA, and the Pgp3 LISA are each based on only one validation study. Additionally, the validation study for the WIF tested IgG, IgM, and IgA together while the validation studies for the other assay types only tested IgG. Although comparing the performance of different assays is difficult due to inconsistent reference standards used, there have been improvements in these agreements between NAAT results and these assays in recent years. Most notably, the mixed peptide ELISA[11] (composite reference standard of commercial seroassays) and the Pgp3 LISA (NAAT as reference)[10] both have a PPA >85% and NPA >98%.

Use in Epidemiologic Studies

Table 1 details how CT seroassays have been applied in epidemiologic research. The assays with commercially-available versions have been used in epidemiologic studies considerably more often than laboratory-developed assays. The MIF has been used to estimate CT seroprevalence among select populations in the Netherlands[41–43], Japan[44,45], and Jamaica[46], and an in-house version of the WIF examined CT seroprevalence among select populations in Finland[47]. Laboratory-developed EB ELISAs[16] have been used to measure CT prevalence from key populations in the US[37], and have been used to examine the association between CT seropositivity and gastroschisis[36], pregnancy outcomes[37], and tubal factor infertility (TFI)[48]. The MOMP ELISA has been applied to explore the correlation between seroprevalence of anti-CT antibodies and subfertility or infertility in females in the Netherlands[43], Rwanda[49], Samoa[50], and Iran[51s]. The one commercial version of the cHSP60 ELISA was used among females who were subfertile, infertile, had TFI, or had a male partner who was infertile[41,50,52s,53s]. The Pgp3 ELISA has measured seroprevalence in population-based samples of women in the United Kingdom[54s] and the U.S.[55s] and to predict the CT-attributable population fraction of TFI by race in U.S. females[56s]. The Pgp3 LISA was applied to estimate seroprevalence of antibodies to CT in adults in Northern China[10]. The single commercial version of the LPS rELISA was applied in Germany to measure the association between CT seropositivity and infertility in males[21]. The mixed peptide ELISA is currently being used to estimate the lifetime prevalence of CT in U.S. men and characterize factors associated with recent versus past infection[57s].

CONSIDERATIONS FOR USING CT SEROASSAYS IN EPIDEMIOLOGIC STUDIES

Laboratory Resources Required for Implementation

There are substantial differences between these assays regarding their ease of implementation and reproducibility. In general, MIFs are harder to implement and less reproducible. The MIF requires subjective microscopic interpretation which makes

it more labor-intensive than ELISAs, and this subjective interpretation can impact reproducibility[26]. Across all the assays, the availability of commercial versions generally allows for easier use and greater reliability. When no commercial version is available, labs must recreate the assays themselves based on the protocols of other research groups. The MIF, WIF, EB ELISA, MOMP ELISA, cHSP60 ELISA, LPS rELISA, and the MBA all have at least one commercial version available, while the mixed peptide ELISA, Pgp3 ELISA, and the Pgp3 LISA do not. In summary, the use of ELISAs and newer techniques like LISA and MBA may be less labor-intensive and more reproducible than older assays[58s].

Timing of Seroconversion and Seroreversion of Antibodies

When designing studies and interpreting results, researchers should consider what is currently known about antibody isotypes and the timing of antibody development.[14–16,59s,60s].The majority of people (61–90%) appear to experience IgG seroconversion within 3 months of a positive NAAT result, though a small proportion will develop antibodies between 3 months and several years after an initial positive NAAT.[14–16] This wide range of estimates of the timing of seroconversion is likely due to several factors, including host genotype[61s,62s], number of previous infections[15,61s], and uncertainty about when an individual actually acquired a CT infection versus when they first tested positive. Another unknown is antibody persistence, which may vary based on the anatomic site of infection. Among children likely exposed to ocular CT, anti-CT IgG levels have been shown to remain stable for 3 years[12]. Öhman and colleagues found that the proportion of people with IgG antibodies from urogenital CT declined from 65.5% to 34.5% 3–10 years after baseline[14], and Alexiou and colleagues found that only 42% of women who were IgG seropositive at the time of a positive NAAT for a urogenital CT infection were still positive 6 years later[60s]. For IgA, one study found that anti-CT IgA seroprevalence declined from 73% to 61% within 6 months of a positive NAAT[16]. Another found that 32% of female participants who were positive for IgA at the time of a positive NAAT no longer had detectable IgA 20–400 days post-NAAT[13].

Investigators using CT seroassays to estimate CT prevalence should take care to understand the timing of serum collection relative to when an individual may have been exposed and/or infected with CT. Due to the variability in timing of seroconversion across individuals, if serum is drawn from participants too proximal to when they acquired an infection, they may not have developed antibodies yet (even if they test positive for CT by NAAT)[14–16]. Likewise, if serum is drawn from participants several years after their infection, it is possible that no serum antibodies would be detected. In both of these situations, there is a high likelihood of underestimating CT seroprevalence, since participants would be misclassified as never being infected with CT when they truly were.

Additionally, the ability to establish timing of infection is a desired attribute when using these seroassays in epidemiologic studies. Although none of the assays can reliably estimate timing of infection, the mixed peptide ELISA is the only assay that we focus on with the purported ability to distinguish between past and recent infection[11]. Rahman and coauthors suggest that this may be possible by testing serum for IgG1 and IgG3, with

the presence of IgG3 indicating a recent infection due to fairly quick seroconversion and seroreversion[34].

Cross-reactivity to other *Chlamydia* Species

When selecting a CT seroassay for use in epidemiologic research, assays that have cross-reactivity with other *Chlamydia* species should be avoided if possible. This is especially relevant when working with populations that may have been exposed to *C. pneumoniae* or other *Chlamydia* species. This cross-reactivity is due to the highly conserved genomes of members of the *Chlamydia* genus[63s]. The MIF, WIF[26], LPS rELISA[21], and some versions of the MOMP ELISA are cross-reactive with *Chlamydia pneumoniae* as shown in Table 1[20]. Other versions of the MOMP ELISA and the cHSP60 ELISA, the Pgp3 ELISA, and the Pgp3 LISA have little cross-reactivity with *C. pneumoniae*, but they are cross-reactive with other *Chlamydia* species that cause zoonotic diseases such as *C. psittaci*[20]. Notably, the mixed peptide ELISA[34] and MBA[64s] have little to no cross-reactivity with other human and veterinary *Chlamydia* species, including species such as *C. suis* and *C. avium*[65s] in addition to *C. pneumoniae* and *C. psittaci*[11]. Assays developed after 2008 tend to be less cross-reactive with other *Chlamydia* species compared to assays developed earlier, and thus recent epidemiologic studies have used these newer assays.

Serum Antibodies to CT Could Represent Exposure at Various Anatomic Sites

An additional challenge in using these seroassays to measure CT seroprevalence is that they do not provide information about the anatomic site of infection. Most studies of CT seroprevalence and implementation of CT seroassays have focused on urogenital or ocular CT infections, but there is growing recognition that rectal CT infections are common in both males and females[66s–69s]. Researchers attempting to distinguish between past infections at different anatomic sites may choose to pair serology data with sexual history data to understand which anatomic sites may have exposed prior to drawing conclusions about infections at the urogenital site. This may be of particular relevance to populations where ocular CT infections are endemic. In these populations, a positive CT serology result may not necessarily indicate a CT infection in the genital tract, and studies that aim to examine the association between CT and adverse reproductive health outcomes in trachoma-endemic areas should interpret their results with this limitation in mind[70s].

FUTURE DIRECTIONS

We conclude by providing areas for future research involving CT seroassays to optimize their use and implementation.

First, given there is no “gold” standard to determine whether or not someone has had a prior CT infection, seroassays could benefit from validation using a reference standard that attempts to capture any past infection rather than current infection (with NAAT). A more accurate reference standard could consist of a combination of methods, including NAAT, electronic health records (EHR), self-report of previous infection, and another seroassay or combination of seroassays with previously published sensitivity and specificity values >75% when compared to NAAT (MOMP ELISA, mixed peptide ELISA, or Pgp3 LISA). Although

this reference standard does not capture asymptomatic infections, it is more accurate than NAAT alone, and EHR records are more readily available and complete than in past decades. This method would not necessarily need to be applied in all studies, but rather only in studies validating new CT seroassays.

Second, seroassays do not provide us with information about the quality or durability of the immune response. Prior studies examining the association between CT antibodies and adverse reproductive health outcomes have been unable to address the key research gap about how the quality of the immune response impacts reproductive health. The development of assays that estimate the quality of the immune response in CT infection may help us explore the biologic mechanisms that underlie the development of PID, TFI, and ectopic pregnancy.

Third, although these assays have been used to study the immune response to CT infections[13–16,71s], more work is needed to apply these seroassays to better define correlates of protection. At present, our lack of understanding about the timing of anti-CT seroconversion and reversion (described above) make it challenging to properly study how the presence of antibody relates to future protection from CT (or lack thereof). Additionally, CT seroassays alone are somewhat limiting, in that they simply detect the presence of antibody and do not examine any antibody functions, which could be important in distinguishing protective versus non-protective antibodies[72s–76s].

Fourth, most seroconversion studies have examined anti-CT IgG[14–16,59s,60s] and IgA, [16,59s] but IgM seroconversion remains poorly understood and is an important area for future work. Incorporating IgM could potentially allow for improved sensitivity of CT seroassays as it could capture the time when someone may be recently NAAT-negative but not yet IgA and IgG positive, since IgM antibodies are the first antibodies generated during the immune response and wane fairly quickly after the onset of infection[77s]. This could be helpful in more fully understanding immune responses to CT in the research context. Cohort studies that carefully incorporate timing of multiple serum draws following a positive NAAT result would better help us understand the timing of sero-conversion and reversion.

Finally, as the development of a CT vaccine progresses, it is important to consider how these seroassays can be incorporated into vaccine trials. Well-validated CT seroassays can be used to identify individuals who are CT-naïve and may be eligible for inclusion in CT vaccine trials, and to monitor the presence of a local and systemic immune response generated by vaccine candidates[78s].

CONCLUSION

CT seroassays are a valuable tool that have the potential to further elucidate CT epidemiology, explore mechanisms of anti-CT immunity, and examine associations between CT infections and reproductive health outcomes. We believe that improvements in CT seroassay function and implementation have created new opportunities to use these assays in epidemiologic research and, by extension, in studies of CT immunology and future CT vaccine studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Seroassays used to detect urogenital *Chlamydia trachomatis* infections in epidemiologic studies

Assay name	Year introduced[a]	Antibodies that can be detected	Advantages & disadvantages	Mean Positive Percent Agreement (Range) [b]	Mean Negative Percent Agreement (Range) [b]	No. of commercial versions	Summary of epidemiologic studies
Microimmunofluorescence assay (MIF)	1975 [18]	IgG, IgM, IgA	• Labor intensive • Subjective reading • Most versions are crossreactive with *Chlamydia pneumoniae*[10]	61.6 (44, 79.2) [19,79s]	86.0 (83.1, 89) [19,79s]	5	CT *antibody seroprevalence among...* • 1005 adults and children (20%) [44] and 223 pediatric pneumonia inpatients up to age 15 years in Japan (21.5%) [45] • 64 female commercial sex workers aged 17–52 (95%) and 435 blood donors aged 19–59 in Jamaica (53%) [46] • Subfertile females presenting to fertility clinics at 38 hospitals in the Netherlands (23%) [42] *Association between* CT *seropositivity and...* • Conception rates in ovulatory subfertile females without visible tubal pathology in the Netherlands (group with CT seropositivity had 35% lower pregnancy chances) [42] • Epithelial ovarian tumors between 1993–2008 at a hospital system in Sweden (no association) [41] *Used CT seropositivity to predict...* • Risk for CT-induced PID in 280 female sex workers in Kenya (OR 2.6, 95% CI 1.1–6.2) [79s] • Tubal factor subfertility in 315 females who sought treatment for subfertility at a clinic in the Netherlands[43]
Whole cell inclusion immunofluorescence assay (WIF)	1975 [80s]	IgG, IgM, IgA	• Potentially easier to read than MIF [16,29] • Labor intensive • Subjective reading • Cross-reactive with *C. pneumoniae*	100 [38]	19.3 [38]	1	CT *antibody seroprevalence among...* • 128 females presenting to a fertility clinic in Finland and pregnant controls [47]
Major Outer Membrane Protein-peptide/ OmpA Enzyme-linked Immunosorbent Assay (MOMP ELISA)	1985 [81s]	IgG, IgM, IgA	• Less labor intensive • Objective reading • Cost-effective compared to MIF [20,82s] • Some versions are crossreactive with *C pneumoniae* and *C. psittaci* [20]	75.5 (58, 93.6) [10,19,33, 78s]	84.1 (62, 100) [10,19,33, 78s]	11	CT *antibody seroprevalence among...* • 223 pediatric pneumonia inpatients up to age 15 years at two hospitals in Japan (21.5%) [45] • 133 females of infertile couples presenting to a fertility clinic in Finland [83s] • Subfertile females presenting to fertility clinics at 38 hospitals in the Netherlands (23%) [42] and to a hospital in Rwanda [49] • 100 infertile and 125 fertile females aged 18–49 years in Iran [51s] • 473 females with past CT infections and paired serum controls in the Finnish Maternity Cohort [14] *Association between CT seropositivity and...*

Assay name	Year introduced[a]	Antibodies that can be detected	Advantages & disadvantages	Mean Positive Percent Agreement (Range) [b]	Mean Negative Percent Agreement (Range) [b]	No. of commercial versions	Summary of epidemiologic studies
							• TFI in 212 females presenting to a fertility clinic [84s] and in 162 females undergoing in-vitro fertilization (IVF) treatment in Denmark [53s] • Conception rates in ovulatory subfertile females without visible tubal pathology in the Netherlands (group with CT seropositivity had 35% lower pregnancy chances) [42] • Gastroschisis in a case-control study of 99 pregnant females recruited from a hospital in the Western United States (US) [35] *Used* CT *seropositivity to predict...* • Tubal factor subfertility in 315 subfertile females in the Netherlands [43] and in 258 subfertile females in Finland [85s] • CT-associated subfertility in 239 sexually active, non-pregnant females aged 18–29 years in Samoa [50] • Population-attributable fraction of TFI by race in a case-control study of 107 Black and 620 non-Black infertile females aged 19–42 years in the US [48] • CT incidence and reinfection rate among 774 adults aged 18–65 years in Northern China [10]
Heat Shock Protein 60 ELISA (cHSP60 ELISA)	1993 [86s]	IgG	• Cross-reactive with *C. psittaci* and *Parachlamydia acanthamoebae* [84s]	52.4 (42.8, 62) [19,38]	90 (80, 100) [19,38]	1	CT *antibody seroprevalence among...* • Females receiving IVF along with infertile and pregnant controls in Canada [86s] *Association between* CT *seropositivity and...* • TFI in 162 females undergoing in-vitro fertilization (IVF) treatment in Denmark [53s] • Epithelial ovarian tumors between 1993–2008 at a hospital system in Sweden (no association) [41] *Used* CT *seropositivity to predict...* • Risk for CT-induced PID in 280 female sex workers in Kenya [87s] • Tubal factor subfertility/infertility in 251 females [52s] and 258 subfertile females in Finland [85s] • CT-associated subfertility in 239 sexually active, non-pregnant females aged 18–29 years in Samoa [50]
Lipopolysaccharide recombinant ELISA (LPS rELISA)	2000 [88s]	IgG, IgA	• Cross-reactive with other *Chlamydiae* species [88s]	88.7 (84, 93.3) [19,88s]	29.8 (11.6, 48) [19,88s]	1	*Association between* CT *seropositivity and...* • Males of infertile couples in Germany aged 19–58 years [21]
Plasmid Gene Protein 3 ELISA (Pgp3 ELISA)	2009 [89s]	IgG	• Little crossreactivity with *C. pneumoniae* • Cross-reactive with	53 [19]	80 [19]	0	CT *antibody seroprevalence among...* • Females aged 16–24 years from 1994–2012 in the United Kingdom [54s] • 1725 females aged 18–39 years who participated in

Assay name	Year introduced[a]	Antibodies that can be detected	Advantages & disadvantages	Mean Positive Percent Agreement (Range) [b]	Mean Negative Percent Agreement (Range) [b]	No. of commercial versions	Summary of epidemiologic studies
			other *Chlamydia* strains (non-human) [28]				NHANES 2015–2016 in the US [55s] *Used* CT *seropositivity to predict...* • CT population-attributable fraction of TFI by race in a case-control study of 107 Black and 618 non-Black females in the US [56s]
Luminex Magpix Multiplex Bead Array Pgp3 assay (MBA)	2012 [64s]	IgG	• Little crossreactivity with other *Chlamydia* species [64s]	92.6[c] [90s]	5.9[c] [90s]	1	CT *antibody seroprevalence among...* • 1725 females aged 18–39 years who participated in NHANES 2015–2016 [55s]
Elementary body enzyme-linked immunosorbent assay (EB ELISA)	2012 [16]	IgG, IgA	• Little crossreactivity with *C.* pneumoniae [16]	67.4 (64.8, 70.1)[d] [11,34]	98.0 [d] [11,34]	1	CT *antibody seroprevalence among...* • African American women aged 16+ years presenting to an STI clinic in the Southern US [37] *Association between* CT *seropositivity and...* • Gastroschisis in a case-control study of 99 pregnant females recruited from a hospital in the Western US [35] • Pregnancy among 1251 infertile females with tubal patency from hospitals across the US [36] *Used* CT *seropositivity to predict...* • Population-attributable fraction of tubal factor infertility by race in a case-control study of 107 Black and 620 non-Black infertile females aged 19–42 years in the US [48]
Mixed peptide ELISA	2018 [11]	IgG, IgA	• Less labor-intensive than older assays • No crossreactivity with other *Chlamydiae* spp. • Can distinguish between recent and past infection[11]	85.6[d] [11]	98.9[d] [11]	0	CT *antibody seroprevalence among...* • Males in the US and characterize factors associated with recent versus past infection among males and females in NHANES 2017–2018 [57s]
Pgp3 Luciferase Immunosorbent assay (Pgp3 LISA)	2021 [10]	IgG	• Little crossreactivity with *C. pneumoniae* Cross-reaction with other *Chlamydia* strains (non-human)[28]	92.8 [10]	100 [10]	0	*Used* CT *seropositivity to predict...* • CT incidence and reinfection rate among 774 adults aged 18–65 years in Northern China [10]

[a] Year of earliest publication found that used assay to measure CT antibodies

[b] Mean of published sensitivity and specificity values for IgG (except WIF, see [e]) that use NAAT as a gold standard (where cases are individuals with a positive NAAT and controls are individuals with a negative NAAT), except for EB ELISA and Mixed Peptide ELISA, see [d]

[c] Value calculated from published values

[d] Used a composite reference standard: cases were females with a positive NAAT and controls were people without detectable antibodies using four other commercial ELISAs

[e] Measured IgG, IgA, and IgM together