Table S1. Serological assays used to detect urogenital *Chlamydia trachomatis* infections

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
Micro-immunofluorescence assay (MIF)[a]	1974[1]	• Uses formalin-fixed *C. trachomatis* elementary bodies, primarily outer membrane protein A (OmpA) • Indirect fluorescent antibody assay that captures antibodies specific to *Chlamydia* elementary bodies • EBs act as antigen and are fixed on glass slides[2] • Usually measures IgG and IgM antibodies but can also measure IgA • Requires standardization of antigen preparations and subjective microscopic interpretation, making it labor-intensive[3,4,5]	• Labor intensive • Subjective reading • Most versions are cross-reactive with *Chlamydia pneumoniae*[6]	MRL-MIF Assay (MRL Diagnostics)[7]	IgG, IgA, IgM[7]	IgG: 79.2, 83.1	Nucleic acid amplification test (NAAT)	*Population*[7] • 149 females aged 20-29 years participating in a cohort study in Denmark[8] who were screened using NAAT *Cases:* 43 females with positive NAAT results *Controls:* 106 females with negative NAAT results
				MIF (Ani Labsystems Ltd.) • detects antibodies to *C. trachomatis, C. pneumoniae*, and *C. psittac*i and is species-specific[9,10]	IgG, IgA, IgM[9,10,11]	IgG: 44, 89	NAAT	*Population*[9] • 25 patients with acute urogenital chlamydia aged 17-65 years • 19 healthy blood donors aged 20-39 years • ~30% female in each group *Cases:* Participants with positive NAATs *Controls:* Participants with negative NAATs
						IgG/IgM: 100, 92	Labsystems and Focus MIFs	*Population*[10] • 101 females who received a laparoscopy during an infertility workup at a fertility clinic in Belgium between Sept. 2005 and May 2007 • 40 had tubal damage detected during laparoscopy *Cases:* Females with positive results from both Labsystems and Focus MIFs *Controls*: Females with negative results from both Labsystems and Focus MIFs
						IgG: 83.3, 95.6	5 tests (MIF, 2 MOMP ELISAs, HSP60 ELISA, AEI)	*Population*[11] • 405 females presenting to a miscarriage clinic in the United Kingdom (UK) • 251 females with miscarriage • 154 females without miscarriage *Cases*: Females with 4 or 5 tests positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI) *Controls*: Females with 0 or 1 tests of positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI)
				MIF (Focus Diagnostics) • uses 2 step sandwich method[10]	IgG, IgA, IgM[10]	IgG: 100, 98	Labsystems and Focus MIFs	*Population*[10] • 101 females who received a laparoscopy during an infertility workup at a fertility clinic in Belgium between Sept. 2005 and May 2007 • 40 had tubal damage detected during laparoscopy *Cases:* Females with positive results from both Labsystems and Focus MIFs *Controls*: Females with negative results from both Labsystems and Focus MIFs
				MIF (bioMérieux)[12,13]	IgG, IgA, IgM[12,13]	IgG: 63.6, 81	Laparoscopy	*Population*[12] • Female OBGYN patients: 76 subfertile, 150 pregnant, 220 controls in the Netherlands *Cases*: Subfertile females with tubal pathology screened through X-ray with contrast or laparoscopy *Controls*: Controls or pregnant females with no known tubal pathology
						IgG: 71, 74	Laparoscopy	*Population*[13] • 315 females who sought treatment for subfertility and received laparoscopy in the Netherlands without previous pelvic surgery other than appendectomy or Caesarean section *Cases:* Females with tubal pathology screened via laparoscopy (extensive periadnexal adhesions and/or distal occlusion of both tubes) *Controls*: Females without tubal pathology *Notes* • IgG titer cutoff of 64 used for the sensitivity and specificity reported here, but other cutoffs were also examined
				MIF (LabSystems)[13]	IgG, IgA, IgM[13]	IgG/IgM: 47, 95	Laparoscopy	*Population*[13] • 315 females who sought treatment for subfertility and received laparoscopy in the Netherlands without previous pelvic surgery other than appendectomy or Caesarean section *Cases:* Females with tubal pathology screened via laparoscopy (extensive periadnexal adhesions and/or distal occlusion of both tubes) *Controls*: Females without tubal pathology *Antibodies:* IgG *Notes* • IgG titer cutoff of 64 used for the sensitivity and specificity reported here, but other cutoffs were also examined
Whole cell inclusion Immunofluorescence assay (WIF)[a]	1975[14]	• single antigen immunofluorescence assay where McCoy cells are treated with cytochalasin B then infected with an LGV type 2 strain of CT and plated in wells on slides coated with polytetrafluoroethylene • *C. trachomatis* acts as antigen • detects *Chlamydia* genus-specific LPS antibody and *C.*	• Potentially easier to read than MIF • labor intensive • subjective reading • cross-reactive with *C. pneumoniae*[15, 16]	Shirley Richmond & E. O. Caul[15]	IgG, IgA, IgM[15]	IgG/IgA/IgM: 100, 19.3	NAAT	*Population*[17] • 45 females who received an endometrial biopsy in the UK *Cases:* Females with positive NAAT results Controls: Females with negative NAAT results *Notes* • Richmond & Caul's method was modified by using L2 CT serovars to infect McCoy cell monolayers • Sera with a reactivity >=1:64 was considered a positive result
				BAG-Chlamydia-EIA (Biologische	IgG, IgA[12]	*Subfertile, IgG/IgA:* 66.7, 84.6	MIF	*Population*[12] • Female OBGYN patients: 76 subfertile, 150 pregnant, 220 controls in the Netherlands *Cases*: Females with a "starry sky" MIF result (fluorescent green spots on a red background)

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
		trachomatis species-specific major outer membrane protein (MOMP) antibody (IgG and IgM)[15]		Analysensystem GmbH)[12]		*Pregnant, IgG/IgA:* 77.1, 91.3 *Control, IgG/IgA:* 76.8, 89.4		*Controls*: Females without a "starry sky" MIF result *Notes* • Sensitivities/specificities are stratified by subfertile, pregnant, and control groups
Reticulate & elementary body enzyme-linked immunosorbent assay (ELISA)	1983[18]	• Reticulate bodies from a type C and elementary bodies from a type L2 strain of *C. trachomatis* used as antigens[18]	• No commercial versions[18] • ELISAs are less labor intensive than MIF and have more objective readings[9]	Jones et al.[18]	IgG[18]	*Males*: 65, 50 *Females*: 92, 76	MIF	*Population[18]* • Patients with nongonococcal urethritis (NGU) who tested negative for gonorrhea recruited from an STI clinic in the U.S. *Cases:* 42 males and 42 females with NGU *Controls:* 14 nuns (female) and 10 children
Major Outer Membrane Protein-peptide/OmpA ELISA (MOMP or OmpA peptide ELISA)[a]	1985[19]	• Synthetic major outer membrane protein is encoded by the OmpA gene in Chlamydia DNA • most commercial versions include an IgG and IgA version (both can be used in combination) • conventional indirect ELISA • antibodies typically detected using anti-human-IgG-horseradish peroxidase and anti-human-IgA-HRP with tertramethyl benzidine as a chromogen • IgM versions also exist[20]	• ELISAs are less labor intensive than MIF and have more objective readings[9] • Cost-effective compared to MIF[11,21] • Some versions are cross-reactive with *C. pneumoniae* and *C. psittaci*[11]	CT IgG-IgA ELISA (Labsystems OY)[7,9,10,15,20,22]	IgG, IgA[7,9,10,15,20,22]	IgG: 84.7, 98.6	NAAT	*Population*[7] • 149 females aged 20-29 years participating in a cohort study in Denmark[8] who were screened for CT using NAAT *Cases:* 43 females with positive NAAT results *Controls:* 106 females with negative NAAT results
						IgG: 58, 68	NAAT	*Population*[9] • 45 patients with acute urogenital CT infection aged 17-65 years • 31 healthy blood donors aged 20-39 years • ~30% female in each group *Cases:* Participants with positive NAAT results *Controls:* Participants with negative NAAT results
						IgG: NA, 94.4	WIF	*Population*[15] • 36 people who were diagnosed with *Chlamydia psittaci* or *C. pneumoniae* using WIF *Controls:* Participants with negative WIF results (for *C. psittaci* or *C. pneumoniae*) *Notes* • The assay was only tested in participants who were negative for *C. psittaci* and *C. pneumoniae*. No information on *C. trachomatis* antibodies according to WIF are provided. Thus, only specificity was reported.
						IgG: 69[b], 84[b] IgA: 38[b], 74[b]	NAAT	*Population*[20] • 424 patients from an STI clinic in the Netherlands *Cases*: 324 patients with positive NAAT results *Controls*: 100 patients without active *C. trachomatis* infection
						IgG: 13.4, 84.4[b] IgA: 2.4, 99.3[b] IgG+IgA: 68.3, 71[b]	culture	*Population*[22] • Females from an STI clinic in Finland with suspected chlamydia infection who had *C. trachomatis* culture results and serum samples, females who had *C. trachomatis* serum antibodies from an OBGYN clinic in Japan, and patients with serum samples who were suspected to have chlamydia infections and suspected to be female from another clinic in Japan *Cases*: 82 females who were culture positive for *C. trachomatis* *Controls*: 148 females who were culture negative for *C. trachomatis*
						IgG: 10, 84.2[b] IgA: 5, 89.2[b] IgG+IgA: 46.3, 88.3[b]	culture	*Population*[22] • Males from an STI clinic in Finland with suspected chlamydia infection who had *C. trachomatis* culture results and serum samples *Cases*: 80 males who were culture positive for *C. trachomatis* *Controls*: 120 males who were culture negative for *C. trachomatis*
						IgG: 100, 96	Labsystems and Focus MIFs	*Population[10]* • 101 females who received a laparoscopy during an infertility workup at a fertility clinic in Belgium between Sept. 2005 and May 2007 • 40 had tubal damage detected during laparoscopy *Cases:* Females with positive results from both Labsystems and Focus MIFs *Controls*: Females with negative results from both Labsystems and Focus MIFs
				CT IgG/IgM/IgA ELISA (Vircell SL)[10,15]	IgG, IgA, IgM[15]	IgG/IgM: NA, 58.3	WIF	*Population*[15] • 36 people who were diagnosed with *Chlamydia psittaci* or *C. pneumoniae* using WIF *Controls:* Participants with negative WIF results (for *C. psittaci* or *C. pneumoniae*) *Notes* The assay was only tested in participants who were negative for *C. psittaci* and *C. pneumoniae*. No information on *C. trachomatis* antibodies according to WIF are provided. Thus, only specificity was reported.

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
						IgG/IgM: 90, 98	Labsystems and Focus MIFs	*Population*[10] • 101 females who received a laparoscopy during an infertility workup at a fertility clinic in Belgium between Sept. 2005 and May 2007 • 40 had tubal damage detected during laparoscopy *Cases:* Females with positive results from both Labsystems and Focus MIFs *Controls*: Females with negative results from both Labsystems and Focus MIFs
				C tracho^{pep} ELISA-IgG (PBS Orgenics)[15]	IgG[15]	IgG: NA, 94.4	WIF	*Population*[15] • 36 people who were diagnosed with *Chlamydia psittaci* or *C. pneumoniae* using WIF *Controls:* Participants with negative WIF results (for *C. psittaci* or *C. pneumoniae*) *Notes* The assay was only tested in participants who were negative for *C. psittaci* and *C. pneumoniae*. No information on *C. trachomatis* antibodies according to WIF are provided. Thus, only specificity was reported.
				IgG/IgM ELISA (Eurimmun)[24]	IgG, IgM	NA	NA	*No published validation found.*
				C-IgG/IgA-pELISA (Medac Diagnostica GmbH)[7,12,15]	IgG, IgA[7,12,15]	IgG: 71.4, 97.3	NAAT	*Population*[7] • 149 females aged 20-29 years participating in a cohort study in Denmark[8] who were screened for CT using NAAT *Cases:* 43 females with positive NAAT results *Controls:* 106 females with negative NAAT results
						Subfertile, IgG/IgA: 58.3, 96.2 *Pregnant IgG/IgA:* 77.1, 91.3 *Control, IgG/IgA:* 76.8, 89.4	MIF	*Population*[12] • Female OBGYN patients: 76 subfertile, 150 pregnant, 220 controls in the Netherlands *Cases*: Females with a "starry sky" MIF result (fluorescent green spots on a red background) *Controls*: Females without a "starry sky" MIF result *Notes* • Sensitivities/specificities are stratified by subfertile, pregnant, and control groups
						IgG: NA, 97.2	WIF	*Population*[15] • 36 people who were diagnosed with *Chlamydia psittaci* or *C. pneumoniae* using WIF *Controls:* Participants with negative WIF results (for *C. psittaci* or *C. pneumoniae*) *Notes* • The assay was only tested in participants who were negative for *C. psittaci* and *C. pneumoniae*. No information on *C. trachomatis* antibodies according to WIF is provided. Thus, only specificity was reported.
						IgG: 63, 95	Composite reference standard (see Validation)	*Population*[4] • Females aged 18-38 years: 125 females with a positive NAAT, 18 low-risk females with no past chlamydia diagnoses, and 31 female blood donors who self-reported being chlamydia free *Cases:* Females with a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) Controls: Females without a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) *Notes* • A composite reference standard was created by combining the results of NAAT, four commercial ELISAs that test for *C. trachomatis* serum antibodies (GenWay ELISA, Serion ELISA, Savyon ELISA, and Medac ELISA), and three "in-house" mixed peptide ELISAs. Rather than testing the specificities of each assay, specificities were computed using receiver operating curve (ROC) analysis based on the sensitivities.
						IgG: 93.3, 96.9	all 5 tests (MIF, 2 MOMP ELISAs, HSP60 ELISA, AEI)	*Population*[11] • 405 females presenting to a miscarriage clinic in the United Kingdom (UK) • 251 females with miscarriage • 154 females without miscarriage *Cases*: Females with 4 or 5 tests positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI) *Controls*: Females with 0 or 1 tests of positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI)
						IgG: 87, 95 IgA: 32, 95	NAAT/past infection	*Population*[25] • Females attending an STI clinic in the Netherlands who were tested for chlamydia using NAAT during an STI consultation *Cases:* 33 females with a positive NAAT or probable history of chlamydia *Controls:* 83 females with a negative NAAT and no history of chlamydia
						IgG: 75[b], 83[b] IgA: 45[b], 83[b]	NAAT	*Population*[20] • 424 patients from an STI clinic in the Netherlands *Cases*: 324 patients with positive NAAT results *Controls*: 100 patients without active *C. trachomatis* infection
				CT pELISA (R-Biopharm)[11]	IgG[11]	IgG: 96.7, 99.7	all 5 tests (MIF, 2	*Population*[11] • 405 females presenting to a miscarriage clinic in the United Kingdom (UK)

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
							MOMP ELISAs, HSP60 ELISA, AEI)	• 251 females with miscarriage • 154 females without miscarriage *Cases*: Females with 4 or 5 tests positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI) *Controls*: Females with 0 or 1 tests of positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI)
				SeroCT IgG and IgA ELISA (Orgenics, Savyon Diagnostics Ltd)	IgG, IgA	IgG: 84.7, 98.6	NAAT	*Population*[7] • 149 females aged 20-29 years participating in a cohort study in Denmark[8] who were screened for CT using NAAT *Cases:* 43 females with positive NAAT results *Controls:* 106 females with negative NAAT results
						IgG: NA, 91.7	WIF	*Population*[15] • 36 people who were diagnosed with *Chlamydia psittaci* or *C. pneumoniae* using WIF *Controls:* Participants with negative WIF results (for *C. psittaci* or *C. pneumoniae*) *Notes* • The assay was only tested in participants who were negative for *C. psittaci* and *C. pneumoniae*. No information on *C. trachomatis* antibodies according to WIF are provided. Thus, only specificity was reported.
						IgG: 63.2, 95	Composite reference standard (see Validation)	*Population*[4] • Females aged 18-38 years: 125 females with a positive NAAT, 18 low-risk females with no past chlamydia diagnoses, and 31 female blood donors who self-reported being chlamydia free *Cases:* Females with a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) Controls: Females without a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) *Notes* • A composite reference standard was created by combining the results of NAAT, four commercial ELISAs that test for *C. trachomatis* serum antibodies (GenWay ELISA, Serion ELISA, Savyon ELISA, and Medac ELISA), and three "in-house" mixed peptide ELISAs. Rather than testing the specificities of each assay, specificities were computed using receiver operating curve (ROC) analysis based on the sensitivities.
						IgG: 52.8, 98.9	Composite reference standard (see Validation)	*Cases:* 125 females with a positive NAAT *Controls:* 87 people without detectable anti-*C. trachomatis* IgG antibodies by any of four commercial ELISAs (GenWay, Serion, Savyon, Medac MOMP ELISA) or the two chemiluminescence "in-house" assays[26]
						IgG: 68[b], 74[b] IgA: 48[b], 86[b]	NAAT	*Population*[20] • 424 patients from an STI clinic in the Netherlands *Cases*: 324 patients with positive NAAT results *Controls*: 100 patients without active *C. trachomatis* infection
						IgG: 86.7, 37.3 IgA: 33.3, 77.1	NAAT	*Population*[33] • 314 female sex workers attending an STI clinic in Germany: 199 had urogenital symptoms, 48 had confirmed *Treponema pallidum* infections *Cases:* Females with a positive NAAT from a cervical swab or urinalysis *Controls:* Females with a negative NAAT from a cervical swab or urinalysis
				IgG/IgA ELISA (Serion)[4,26]	IgG, IgA[4,26]	IgG: 61.2, 95	Composite reference standard (see Validation)	*Population*[4] • Females aged 18-38 years: 125 females with a positive NAAT, 18 low-risk females with no past chlamydia diagnoses, and 31 female blood donors who self-reported being chlamydia free *Cases:* Females with a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) Controls: Females without a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) *Notes* • A composite reference standard was created by combining the results of NAAT, four commercial ELISAs that test for *C. trachomatis* serum antibodies (GenWay ELISA, Serion ELISA, Savyon ELISA, and Medac ELISA), and three "in-house" mixed peptide ELISAs. Rather than testing the specificities of each assay, specificities were computed using receiver operating curve (ROC) analysis based on the sensitivities.
						IgG: 57.6, 98.9	Composite reference standard (see Validation)	*Cases:* 125 females with a positive NAAT *Controls:* 87 people without detectable anti-*C. trachomatis* IgG antibodies by any of four commercial ELISAs (GenWay, Serion, Savyon, Medac MOMP ELISA) or the two chemiluminescence "in-house" assays[26]
				SeroELISA Chlamydia TRUE-IgM (Savyon Diagnostics Ltd)[27]	IgM[27]	IgM: 97, 89	MIF	*Population*[27] • 223 infants and children aged 4 days to 15 years with pneumonia admitted to a hospital in Japan *Cases:* 48 children with *C. trachomatis*-positive MIFs *Controls*: 175 children with *C. trachomatis*-negative MIFs

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
				IPAzyme Chlamydia TRUE-IgM/IgA (Savyon Diagnostics Ltd)[17,27]	IgM, IgA[17,27]	IgM: 89, 84	MIF	*Population*[27] • 223 infants and children aged 4 days to 15 years with pneumonia admitted to a hospital in Japan *Cases:* 48 children with *C. trachomatis*-positive MIFs *Controls*: 175 children with *C. trachomatis*-negative MIFs
						IgA: 57.1, 93.6[b]	NAAT	*Population*[17] • 45 females who received an endometrial biopsy in the UK *Cases:* Females with positive NAAT results Controls: Females with negative NAAT results
				IgG ELISA (Mikrogen GmbH)[28]	IgG	IgG: 93.6, 100	NAAT	*Cases:* 125 females with positive NAAT recruited from a hospital in China *Controls:* 125 children aged 1-6 years at low risk of chlamydia recruited from a hospital in China[28]
				IgM-Capture-ELISA[29]	IgM	IgM: 88[b], 100[b]	Western blot	*Population*[29] • People with suspected chlamydia infections in France with detectable serum IgG and/or IgM by indirect immunofluorescence or Western blot, people with active or treated genital infections, pulmonary infections, or confirmed chlamydial infections (*C. trachomatis* or *C. psittaci*) *Cases*: 17 people who had IgM antibodies detectable by Western blot *Controls*: 22 people without IgM antibodies detectable by Western blot
				de Haro-Cruz et al.[30]	IgG	IgG: 100[b], 58.3[b]	VIRGO Chlamydia trachomatis IgG Immunofluorescence assay	*Population*[30] • 40 infertile females in Mexico *Cases*: 16 females with positive IgG immunofluorescence test *Controls*: 24 females with negative IgG immunofluorescence test
Heat Shock Protein 60 ELISA (cHSP60 ELISA)[a]	1993[31]	• cHSP60 is used as an antigen to detect IgG • cHSP60 presents as a fusion with 26-kDa glutathione S-transferase (GST) of Schistosoma japonicum • cHSP60 or GST is purified using affinity chromatography • Average OD against GST is subtracted from the OD against cHSP60[31]	• cHSP60 is cross-reactive with *C. psittaci* and *Parachlamydia acanthamoebae*[11] • ELISAs are less labor intensive than MIF and have more objective readings[9]	cHSP60-IgG-ELISA (Medac Diagnostica GmbH)[11]	IgG	IgG: 93.3, 87.4	all 5 tests (MIF, 2 MOMP ELISAs, HSP60 ELISA, AEI)	*Population*[11] • 405 females presenting to a miscarriage clinic in the United Kingdom (UK) • 251 females with miscarriage • 154 females without miscarriage *Cases*: Females with 4 or 5 tests positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI) *Controls*: Females with 0 or 1 tests of positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI)
				Bas et al.[9]	IgG	IgG: 62, 80	NAAT	*Population*[9] • 45 patients with acute urogenital CT infection aged 17-65 years • 30 healthy blood donors aged 20-39 years • ~30% female in each group *Cases:* Participants with positive NAAT results *Controls:* Participants with negative NAAT results
				Chernesky et al.[17]	IgG[17]	IgG: 42.9, 100	NAAT	*Population*[17] • 45 females who received an endometrial biopsy in the UK *Cases:* Females with positive NAAT results Controls: Females with negative NAAT results
				Dutta et al.[32]	IgG[32]	*primary infertility*: 50.0, 73.1 *secondary infertility*: 90.91, 89.47 *vaginal discharge*: 52.63, 88.41 *chronic cervicitis*: 79.17, 77.78	IgG MOMP ELISA	*Population*[32] • 198 females aged 16-50 years who were outpatients with gynecological complaints at a hospital in India *Cases*: Females with a positive IgG MOMP ELISA result *Controls*: Females with a negative IgG MOMP ELISA result *Notes* • Sensitivities/specificities are stratified by groups presenting with primary infertility, secondary infertility, vaginal discharge, and chronic cervicitis.
				Dutta et al.[32]	IgG[32]	*primary infertility*: 60.0, 76.0 *secondary infertility*: 67.33, 90.67 *vaginal discharge*: 54.55, 85.37	IgG MOMP ELISA	*Population*[32] • 198 females aged 16-50 years who were outpatients with gynecological complaints at a hospital in India *Cases*: Females with a positive IgG MOMP ELISA result *Controls*: Females with a negative IgG MOMP ELISA result *Notes* • Sensitivities/specificities are stratified by groups presenting with primary infertility, secondary infertility, vaginal discharge, and chronic cervicitis.

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
						chronic cervicitis: 61.0, 92.57		
MOMP IgM western blot	1997[29]	• Separates proteins by size and gel electrophoresis • uses MOMP as an antigen to detect IgG or IgM antibodies[29,30]	• No commercial versions[29,30]	Poussin et al.[29]	IgM[29,30]	IgM: 100[b], 92[b]	IgM-Capture-ELISA	*Population*[29] • People with suspected chlamydia infections in France with detectable serum IgG and/or IgM by indirect immunofluorescence or Western blot, people with active or treated genital infections, pulmonary infections, or confirmed chlamydial infections (*C. trachomatis* or *C. psittaci*) *Cases*: 15 people who had IgM antibodies detectable by IgM-Capture-ELISA *Controls*: 24 people without IgM antibodies detectable by IgM-Capture-ELISA
Lipopolysaccharide recombinant ELISA (LPS rELISA)[a]	2000[33]	• Uses total lipopolysaccharide that is Chlamydia genus-specific 3-deoxy-D-manno-2-octulopyranosonic acid as an antigen[9]	• ELISAs are less labor intensive than MIF and have more objective readings[9]	Chlamydia rLPS (Medac GmbH)[9,26,33]	IgG, IgA, IgM[33]	IgG: 84, 48	NAAT	*Population*[9] • 45 patients with acute urogenital CT infection aged 17-65 years • 31 healthy blood donors aged 20-39 years • ~30% female in each group *Cases:* Participants with positive NAAT results *Controls:* Participants with negative NAAT results
						IgG: 60.8, 98.9	Composite reference standard (see validation)	*Cases:* 125 females with a positive NAAT *Controls:* 87 people without detectable anti-*C. trachomatis* IgG antibodies by any of four commercial ELISAs (GenWay, Serion, Savyon, Medac MOMP ELISA) or the two chemiluminescence "in-house" assays[26]
						IgG: 93.3, 11.6 IgA: 83.3, 39.4 IgM: 16.7, 80.6	NAAT	*Population*[33] • 314 female sex workers attending an STI clinic in Germany: 199 had urogenital symptoms, 48 had confirmed *Treponema pallidum* infections *Cases:* Females with a positive NAAT from a cervical swab or urinalysis *Controls:* Females with a negative NAAT from a cervical swab or urinalysis
Chlamydia Bivalent ELISA Immunocomb	2000[34]	• Solid-phase ELISA that uses LPS-extracted L2 from *C. trachomatis* and elementary bodies from *C. pneumoniae* • measures both IgG and IgA from *C. trachomatis* infection and IgG from *C. pneumoniae* infection[34]	• Distinguishes between *C. trachomatis* and *C. pneumoniae* infections (not cross-reactive)[34] • ELISAs are less labor intensive than MIF and have more objective readings[9]	Orgenics Ltd/Savyon Diagnostics Ltd[34]	IgG, IgA[34]	*NA*	*NA*	*No published validation found.*
MOMP + Pgp3 ELISA	2001[9]	• Uses both MOMP and Pgp3 as antigens to detect IgG antibodies[9]	• ELISAs are less labor intensive than MIF and have more objective readings[9]	Bas et al.[9]	IgG[9]	IgG: 71, 67	NAAT	*Population*[9] • 45 patients with acute urogenital CT infection aged 17-65 years • 30 healthy blood donors aged 20-39 years • ~30% female in each group *Cases:* Participants with positive NAAT results *Controls:* Participants with negative NAAT results
cHSP60 + Pgp3 ELISA	2001[9]	• Uses both cHSP60 and Pgp3 as antigens to detect IgG antibodies[9]	• cHSP60 is cross-reactive with *C. psittaci* and *Parachlamydia acanthamoebae*[11] • ELISAs are less labor intensive than MIF and have more objective readings[9]	Bas et al.[9]	IgG[9]	IgG: 76, 77	NAAT	*Population*[9] • 45 patients with acute urogenital CT infection aged 17-65 years • 30 healthy blood donors aged 20-39 years • ~30% female in each group *Cases:* Participants with positive NAAT results *Controls:* Participants with negative NAAT results

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
Inactivated organism ELISA	2003[15]	*Unknown, despite published use by Jones et al.*[15]	• Chlamydia genus-specific only • cross-reactive with other Chlamydia species[15] • ELISAs are less labor intensive than MIF and have more objective readings[9]	CT ELISA (Genzyme Virotech GmbH)	*Unknown*	*NA*, 5.6	WIF	*Population*[15] • 36 people who were diagnosed with *Chlamydia psittaci* or *C. pneumoniae* using WIF *Controls:* Participants with negative WIF results (for *C. psittaci* or *C. pneumoniae*) *Notes* • The assay was only tested in participants who were negative for *C. psittaci* and *C. pneumoniae*. No information on *C. trachomatis* antibodies according to WIF are provided. Thus, only specificity was reported.
				Platelia Chlamydia IgG (Sanofi Diagnostics Pasteur Ltd)[15]	IgG[15]	IgG: *NA*, 0	WIF	*Population*[15] *Controls:* Participants with negative WIF results (for *C. psittaci* or *C. pneumoniae*) *Notes* • The assay was only tested in participants who were negative for *C. psittaci* and *C. pneumoniae*. No information on *C. trachomatis* antibodies according to WIF are provided. Thus, only specificity was reported.
Chlamydiae Western blot	2007[35]	• Recombinant antigen Western blot that uses 4 recombinant antigens from *C. trachomatis* and *C. pneumoniae* and 1 antigen from *C. psittaci* to detect IgG and IgA antibodies[35]	• Can detect antibodies from *C. trachomatis*, *C. pneumoniae*, and/or *C. psittaci*[35]	Chlamycheck Chlamydia Western blot assay (AllDiag)[35]	IgG, IgA[35]	IgG/IgA; 82.1, 51.1	MIF	Population[35] • 32 males and 56 females aged 17-55 years with past chlamydia infection (negative NAAT) Cases: Participants with a positive MIF result Controls: Participants with a negative MIF result
Heat Shock Protein 10 ELISA (CHSP10 ELISA)	2008[32]	• Heat Shock Protein 10 is used as an antigen to detect IgG	• ELISAs are less labor intensive than MIF and have more objective readings[9]	Dutta et al.[32]	IgG[32]	*primary infertility*: 60.0, 88.4 *secondary infertility*: 75.6, 73.87 *vaginal discharge*: 50.0, 79.20 *chronic cervicitis*: 68.18, 88.89	IgG MOMP ELISA	*Population*[32] • 198 females aged 16-50 years who were outpatients with gynecological complaints at a hospital in India *Cases*: Females with a positive IgG MOMP ELISA result *Controls*: Females with a negative IgG MOMP ELISA result *Notes* • Sensitivities/specificities are stratified by groups presenting with primary infertility, secondary infertility, vaginal discharge, and chronic cervicitis.
						primary infertility: 45.45, 78.05 *secondary infertility*: 60.0, 79.37 *vaginal discharge*: 20.0, 72.0 *chronic cervicitis*: 55.0, 71.2	NAAT	*Population*[32] • 198 females aged 16-50 years who were outpatients with gynecological complaints at a hospital in India *Cases*: Females with a positive IgG MOMP ELISA result *Controls*: Females with a negative IgG MOMP ELISA result *Notes* • Sensitivities/specificities are stratified by groups presenting with primary infertility, secondary infertility, vaginal discharge, and chronic cervicitis.
Plasmid Gene Protein 3 ELISA (Pgp3 ELISA)[a]	2009[36]	• Detects IgG antibody to a specific *C. trachomatis* Pgp3 protein[36]	• Little cross-reaction with *C. pneumoniae*[36] • Cross-reactive with other Chlamydia strains (non-human)[5] • ELISAs are less labor intensive than MIF and have more objective readings[9]	Pgp3 indirect ELISA, Horner et al.[37]	IgG	IgG: 58.4[b], 97.8	NAAT	*Cases*: 340 patients presenting to a genitourinary clinic in the United Kingdom who were diagnosed with chlamydia using NAAT at least one month prior to serum being drawn (182 males, 158 females) *Controls*: 494 children aged 2 to 13 years in the United Kingdom with negative MIFs[37] *Notes* • The sensitivity was originally reported separately for males and females, but we calculated a weighted average of the two presented values.
				Bas et al.[9]	IgG	IgG: 53, 80	NAAT	*Population*[9] • 45 patients with acute urogenital CT infection aged 17-65 years • 30 healthy blood donors aged 20-39 years • ~30% female in each group *Cases:* Participants with positive NAAT results *Controls:* Participants with negative NAAT results
				Pgp3 ELISA double	IgG	IgG: 67.6[b], 97.8	NAAT	*Cases*: 340 patients presenting to a genitourinary clinic in the United Kingdom who were diagnosed with chlamydia using NAAT at least one month prior to serum being drawn (182 males, 158 females) *Controls*: 494 children aged 2 to 13 years in the United Kingdom with negative MIFs

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
				antigen/sandwich[37]				*Notes* • The sensitivity was originally reported separately for males and females, but we calculated a weighted average of the two presented values.[37]
Automated epifluorescence assay (AEI)	2010[11]	• automated epifluorescence immuno-assay for simultaneous detection of *C. trachomatis*, *C. pneumoniae* and *C. psittaci* antibodies • uses nanoliter spots for 3 specific antigens from whole bacteria which then detect IgG and IgA antibodies to each *Chlamydia* species • analyzed using fluorescent camera analyzer[11]	• Not cross-reactive • can test for antibodies for multiple *Chlamydia* species at once • cost-saving[11]	MuST Chlamydiae (InoDiag)	IgG, IgA[11]	IgG/IgA: 100, 89.5	all 5 tests (MIF, 2 MOMP ELISAs, HSP60 ELISA, AEI	*Population*[11] • 405 females presenting to a miscarriage clinic in the United Kingdom (UK) • 251 females with miscarriage • 154 females without miscarriage *Cases*: Females with 4 or 5 tests positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI) *Controls*: Females with 0 or 1 tests of positive of the 5 tests (MIF, MOMP ELISAs, HSP60 ELISA, AEI)
Luminex Magpix Multiplex Bead Assay (MBA) Pgp3 assay[a]	2012[38]	• Biotinylated mouse anti-human total IgG is used to detect total IgG antibodies to *C. trachomatis* • Beads are suspended in PBS and read on a BioPlex 200 instrument (Bio-Rad)[38]	• Can be used to detect antibodies from serum or dried blood • Requires BioPlex 200 for reading[38]	Luminex Magpix MBA (Luminex Corporation)[38,39]	IgG[38,39]	IgG: 92.6[b], 5.88[b]	NAAT	*Population*[39] • 118 females aged 13-25 years diagnosed with mild-moderate pelvic inflammatory disease recruited from outpatient clinics and emergency departments in Baltimore, Maryland (United States) *Cases:* 27 females with positive NAAT *Controls*: 91 females with negative NAAT
Elementary body enzyme-linked immunosorbent assay (ELISA) (EB ELISA)[a]	2012[16]	• CT-specific IgG antigen is used to measure serum antibodies (plates are coated with antigen, serum antibodies bind to antigens on plate, enzyme conjugate binds to antibody-antigen complex, substrate is added, enzyme conjugate hydrolyzes substrate during incubation, color intensity correlates with amount of IgG antibody present)[4] • Elementary bodies of *C. trachomatis* serovars D/UW-3, F/IC-Cal-13, and J/UW-36 (from each of the 3 CT serogroups: B, intermediate, and C, respectively), and *C. pneumoniae* strain AR39 were used as antigens for IgG, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2 antibodies[16]	• ELISAs are less labor intensive than MIF and have more objective readings[9]	Chlamydia Trachomatis IgG ELISA (GenWay Biotech, Inc.)[4]	IgG[4]	IgG: 72.6, 95	Composite reference standard (see Validation)	*Population*[4] • Females aged 18-38 years: 125 females with a positive NAAT, 18 low-risk females with no past chlamydia diagnoses, and 31 female blood donors who self-reported being chlamydia free *Cases:* Females with a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) Controls: Females without a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) *Notes* • A composite reference standard was created by combining the results of NAAT, four commercial ELISAs that test for *C. trachomatis* serum antibodies (GenWay ELISA, Serion ELISA, Savyon ELISA, and Medac ELISA), and three "in-house" mixed peptide ELISAs. Rather than testing the specificities of each assay, specificities were computed using receiver operating curve (ROC) analysis based on the sensitivities.
						IgG: 60.8, 98.9	Composite reference standard (see Validation)	*Cases:* 125 females with a positive NAAT *Controls:* 87 people without detectable anti-*C. trachomatis* IgG antibodies by any of four commercial ELISAs (GenWay, Serion, Savyon, Medac MOMP ELISA) or the two chemiluminescence "in-house" assays[26]
				Gupta et al.[40]	IgG[40]	IgG: 98.6, NA	NAAT	*Cases:* 150 females with a positive NAAT who returned to an STI clinic in the U.S. for treatment *Notes* • The assay was only tested in participants with positive NAATs. Thus, only sensitivity was reported.[40]
Pgp3 lateral flow assay	2016[41]	• Pgp3 used as antigen • serum added to Pgp3 LFA cassette followed by chase buffer (PBS) • more commonly used for ocular trachoma antibody detection[39,41]	• Can be used in the field, quick[39] • No known commercial versions	Dize et al. adapted from Gwyn et al.[39,41]	IgG[39]	IgG: 74.1[b], 36.3[b]	NAAT	*Population*[39] • 118 females aged 13-25 years diagnosed with mild-moderate pelvic inflammatory disease recruited from outpatient clinics and emergency departments in Baltimore, Maryland (U.S.) *Cases*: 27 females with positive NAAT *Controls*: 91 females with negative NAAT
High Temperature Requirement Protein ELISA (HtrA ELISA)	2017[42]	• Genes encoding HtrA are amplified from *C. trachomatis* serovar D using primers (BamHI and NotI recognition sequences) • HtrA protein is used as antigen[42]	• HtrA is particularly expressed during persistant *C. trachomatis* infections so potential for distinguishing between different types of infection[42]	Hokynar et al.[42]	IgG[42]	IgG: 28.8, 86.8[b]	NAAT	*Population*[42] • 156 patients presenting to an STI clinic in Finland with suspected chlamydia *Cases*: 80 patients with positive NAAT *Controls*: 76 patients with negative NAAT

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
			• Little cross-reaction with Chlamydia Pneumoniae[6] • ELISAs are less labor intensive than MIF and have more objective readings[9]					
High Temperature Requirement Protein ELISA (TroA ELISA)	2017[42]	• Genes encoding TroA are amplified from *C. trachomatis* serovar D using primers (EcoRI and XhoI recognition sequenes) • TroA protein is used as antigen[42]	• TroA is particularly expressed during persistant *C. trachomatis* infections so potential for distinguishing between different types of infection[42] • Little cross-reaction with *C. pneumoniae*[6] • ELISAs are less labor intensive than MIF and have more objective readings[9]	Hokynar et al.[42]	IgG, IgA[42]	IgG: 8.8, 86.8[b]	NAAT	*Population*[42] • 156 patients presenting to an STI clinic in Finland with suspected chlamydia *Cases*: 80 patients with positive NAAT *Controls*: 76 patients with negative NAAT
IgG1 + IgG3 + IgA1 ELISA	2018[4]	• uses 11 CT peptide antigens (including OmpA, IncE, PmpD, CT143, and CT442 proteins) to detect IgG1, IgG3, and IgA1 antibodies[4]	• Little cross-reaction with any *Chlamydia* strains because peptide antigens are *C. trachomatis* specific[5] • Labor-intensive because it uses one well per peptide[4] • ELISAs are less labor intensive than MIF and have more objective readings[9]	IgG1 + IgG3 + IgA1 ELISA[4]	IgG, IgA[4]	IgG/IgA: 95.2, 95	Composite reference standard (see Validation)	*Population*[4] • Females aged 18-38 years: 125 females with a positive NAAT, 18 low-risk females with no past chlamydia diagnoses, and 31 female blood donors who self-reported being chlamydia free *Cases:* Females with a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) Controls: Females without a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) *Notes* A composite reference standard was created by combining the results of NAAT, four commercial ELISAs that test for *C. trachomatis* serum antibodies (GenWay ELISA, Serion ELISA, Savyon ELISA, and Medac ELISA), and three "in-house" mixed peptide ELISAs. Rather than testing the specificities of each assay, specificities were computed using receiver operating curve (ROC) analysis based on the sensitivities.
				IgG1 + IgG3 ELISA[4]	IgG[4]	IgG: 94.1, 95	Composite reference standard (see Validation)	*Population*[4] • Females aged 18-38 years: 125 females with a positive NAAT, 18 low-risk females with no past chlamydia diagnoses, and 31 female blood donors who self-reported being chlamydia free *Cases:* Females with a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) Controls: Females without a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) *Notes* A composite reference standard was created by combining the results of NAAT, four commercial ELISAs that test for *C. trachomatis* serum antibodies (GenWay ELISA, Serion ELISA, Savyon ELISA, and Medac ELISA), and three "in-house" mixed peptide ELISAs. Rather than testing the specificities of each assay, specificities were computed using receiver operating curve (ROC) analysis based on the sensitivities.
				IgG3 + IgA1 ELISA[4]	IgG, IgA[4]	IgG/IgA: 87.3, 95	Composite reference standard (see Validation)	*Population*[4] • Females aged 18-38 years: 125 females with a positive NAAT, 18 low-risk females with no past chlamydia diagnoses, and 31 female blood donors who self-reported being chlamydia free *Cases:* Females with a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) Controls: Females without a positive NAAT or a positive serum antibody ELISA (from any of the four commercial assays or three "in-house" assays) *Notes*

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
								• A composite reference standard was created by combining the results of NAAT, four commercial ELISAs that test for *C. trachomatis* serum antibodies (GenWay ELISA, Serion ELISA, Savyon ELISA, and Medac ELISA), and three "in-house" mixed peptide ELISAs. Rather than testing the specificities of each assay, specificities were computed using receiver operating curve (ROC) analysis based on the sensitivities.
Mixed peptide ELISA[a]	2018[26]	• uses 12 CT peptides as antigens including OmpA, IncE, PmpD CT143, and CT442 proteins to detect IgG1, IgG3, and IgA antibodies[26]	• ELISAs are less labor intensive than MIF and have more objective readings[9] • only requires 1 microtiter well[26]	Ctr Mix1 ELISA[26]	IgG, IgA[26]	IgG: 85.6, 98.9 IgG3: 70.4, 98.9 IgA: 60.0, 98.9	Composite reference standard (see Validation)	*Cases:* 125 females with a positive NAAT *Controls:* 87 people without detectable anti-*C. trachomatis* IgG antibodies by any of four commercial ELISAs (GenWay, Serion, Savyon, Medac MOMP ELISA) or the two chemiluminescence "in-house" assays[26]
				Ctr Mix2 ELISA[26]	IgG, IgA[26]	IgG: 63.2, 98.9 IgG3: 41.6, 98.9 IgA: 30.4, 98.9	Composite reference standard (see Validation)	*Cases:* 125 females with a positive NAAT *Controls:* 87 people without detectable anti-*C. trachomatis* IgG antibodies by any of four commercial ELISAs (GenWay, Serion, Savyon, Medac MOMP ELISA) or the two chemiluminescence "in-house" assays[26]
Outer membrane complex protein B (OmcB) ELISA	2018[40]	• Serum IgG1 were detected using alkaline phosphatase-labeled mouse anti-human IgG1 and HP6069 at an optical density of 405 nm[40]	• ELISAs are less labor intensive than MIF and have more objective readings[9] • No known commercial versions	Gupta et al.[40]	IgG[40]	IgG: 79.3, NA	NAAT	*Cases:* 150 females with a positive NAAT who returned to an STI clinic in the U.S. for treatment *Notes* • The assay was only tested in participants with positive NAATs. Thus, only sensitivity was reported.[40]
Plasmid Gene Protein 3 Luciferase Immunosorbent assay (Pgp3 LISA)[a]	2021[28]	• *C. trachomatis* Pgp3 gene are amplified from serovar E • Pgp3 gene is subcloned into the pNLF1-N luciferase expression vector (downstream of Nluc luciferase gene) • Recombinant plasmid is transferred to HEK-293T cells and then cells with Nluc-pgp3 protein are collected • These cells are plated to be used antigen along with Protein G or monoclonal mouse anti-human IgG3 • Nano-Glo Luciferase assay reagent is used to determine luciferase light units[28]	• Little cross-reaction with *C. pneumoniae* • Cross-reaction with other Chlamydia strains (non-human)[5]	Shui et al.[28]	IgG[28]	IgG: 92.8, 100	NAAT	*Cases:* 125 females with positive NAAT recruited from a hospital in China *Controls:* 125 children aged 1-6 years at low risk of chlamydia recruited from a hospital in China[28]
Elementary-body immunofluorescence assay (EB IFA)[43]	*Unknown*	• Purified elementary bodies are fixed to slide wells to serve as antigen • Serum is incubated with antigen • Fluorescein-conjugate anti-human IgG, IgA, or IgM is added and incubated • Slides are dried and mounted then examined with fluorescence microscopy • Bright "apple-green" fluorescent elementary bodies are identified against a dark background[43]	*Unknown*	SeroFIA *C. trachomatis* (Savyon Diagnostics Ltd.)	IgG, IgA, IgM[43]	IgG: 90.3, 95.8	MIF	*Population*[43] • 100 patients with suspected chlamydia *Cases*: 52 patients with a positive MIF *Controls*: 48 patients with a negative MIF
IgG indirect fluorescent antibody test[44]	*Unknown*	• Indirect fluorescent antibody staining method is used to detect IgG with lyophilized, inactivated goat anti-human IgG as the counterstain • bright green fluorescence is observed in cells that have *C.*	*Unknown*	VIRGO Chlamydia trachomatis IgG IFA test (Hemagen Diagnostics, Inc.)[44]	IgG[44]	NA	NA	*No published validation found.*

Assay name	Year introduced	Method	Advantages & disadvantages	Versions	Antibodies that can be detected	Positive & negative percent agreements (%)	Gold standard	Validation
		trachomatis IgG in their cytoplasm[44]						

[a] Names of assays used in epidemiologic studies, displayed in Table 1

[b] calculated sensitivity/specificity value

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