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Journal

Sexually Transmitted Diseases, 48(12)

ISSN

0148-5717

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Publication Date

2021-12-01

DOI

10.1097/olq.0000000000001558

Peer reviewed

HHS Public Access

Author manuscript

Sex Transm Dis. Author manuscript; available in PMC 2022 December 01.

Published in final edited form as:

Sex Transm Dis. 2021 December 01; 48(12): e215–e222. doi:10.1097/OLQ.0000000000001558.

Pooled 3-anatomic site testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: A systematic review and meta-analysis

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Abstract

Background: Pooled testing for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) may be a cost-saving solution to increase screening by simplifying testing procedures and reducing resource burdens. We conducted a systematic review and meta-analysis to examine the performance of pooled three-anatomical site testing (pharyngeal, rectal, and urogenital sites) for CT and NG in comparison to single-anatomical site testing.

Methods: We conducted a systematic literature search in PubMed, Embase, and Web of Science to identify original evaluation studies of the performance of pooled testing for CT and NG infections and identified 14 studies for inclusion. Each study was systematically evaluated for bias. We conducted bivariate fixed-effect and random-effects meta-analyses using a full Bayesian method of the positive percent agreement and negative percent agreement.

Results: The combined positive percent agreement for CT was 93.11% (95% Confidence interval (CI): 91.51%, 94.55%) and the negative percent agreement was 99.44% (95% CI: 99.18%, 99.65%). For NG, the combined positive percent agreement was 93.80% (95% CI: 90.26%, 96.61%) and the negative percent agreement was 99.73% (95% CI: 99.30%, 99.97%).

Conclusion: We found that pooled three-anatomic site tests performed similarly to single-site anatomical tests for the detection of CT and NG. The pooled three-anatomic site tests have the added potential benefit of reduced cost and resource requirement, which could lead to improved testing access and screening uptake.

Short Summary:

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Conflict of Interest Statement: The authors report no conflicts of interest.

We conducted a systematic review and meta-analysis to examine the performance of pooled three-anatomical site testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in comparison to single-anatomical site testing.

Keywords

pooled testing; Chlamydia trachomatis (CT); Neisseria gonorrhoeae (NG); meta-analysis

Introduction

Rates of sexually transmitted infections (STIs) in the United States have doubled over the past 20 years.(1, 2) The two most prevalent and treatable bacterial STIs are caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG), which accounted for an estimated 213 million new STI cases worldwide in 2016.(3) There have been drastic increases in cases of CT and NG infections in the United States cases since 2014, with a 19% increase in CT infections and a 63% increase in NG infections.(1) Testing for CT and NG infections requires specimens from the anatomic sites of possible infection (pharyngeal, rectal and urogenital anatomic sites). The cost of testing for CT and NG infections, particularly at multiple anatomic sites per individual, can be a barrier to screening.

In traditional settings, samples are collected from three separate anatomical sites of possible infection, then each specimen is tested for CT and NG separately using a nucleic acid amplification test (NAAT).(4) NAATs for the detection of CT and NG are highly sensitive and are recommended by the United States Centers for Disease Control and Prevention (CDC) for screening at genital and extra-genital anatomic sites. Some NAAT assays such as the Aptima Combo 2 Assay (Hologic Inc., San Diego, CA) and the Xpert CT/NG (Cepheid, Sunnyvale, CA) are FDA cleared for use with extragenital specimen types.(5) Pooled testing for CT and NG infections involves collecting specimens from three anatomic sites and combining the three specimens into one sample to be tested. This reduces the cost and resource requirements for three separate tests to a single test. As long as the sensitivity and specificity of pooled sampling for different anatomic sites in the same individual is not significantly lower than separate site screening, it may be a cost-saving solution to increase screening for CT and NG, particularly in settings with a low prevalence that would spend less on any confirmatory single-site testing.(6) In a 2018 survey,(7) 83.7% of surveyed sexual health clinicians in England acknowledged the potential cost-saving benefits of pooled testing and 76.9% wanted additional research conducted to assess the sensitivity and specificity of pooled sampling. Furthermore, pooled testing has the potential to identify asymptomatic infections of CT and NG that may otherwise be missed by clinicians if 3 site testing is not conducted.

We conducted a systematic review and meta-analysis of evaluations of pooled 3 anatomic site testing for CT and NG and report the clinical positive and negative percent agreement in each and calculated a combined estimate of the sensitivity and specificity of pooled testing.

Materials and Methods

Overall Study Design

We followed Cochrane guidelines and used the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement for report the results (Figure 1)(8). We searched for and compiled studies of pooled NAAT for CT and NG infections.

Literature Search Strategy

We conducted a multistep literature search in PubMed, Embase, and Web of Science to identify original studies examining the performance of pooled testing for CT and NG infections published in English from database inception to 17 August 2021. The systematic search terms are included in the supplemental file.

Study Selection

We screened articles on the basis of titles and abstracts using the following inclusion criteria: (i) a study on pooled anatomic site testing for CT and NG, (ii) included data required to calculate positive and negative percent agreement (specificity and sensitivity) and (iii) used a NAAT assay for testing. The exclusion criteria consisted of the following: (i) not an evaluation of pooled anatomic site testing for CT and NG infection and/or (ii) was an overlapping dataset to other included studies. When two studies presented overlapping datasets (i.e. analyses of the same subjects/samples) we contacted the study authors to confirm overlap and used the most recent publication.

Data Extraction Methods and Measures

We extracted data on methodology of pooled testing evaluation including (i) study population, (ii) study location, (iii) sample collection methods for both the individual and pooled specimens, (iv) NAAT assays for both individual and pooled tests, (v) pooling methods, and (vi) number of true positives and negatives as well as the number of false positives and negatives.

Quality Assessment of Diagnostic Accuracy Studies (QUADAS)

The QUADAS-2 tool was used by 2 independent investigators (JA and JP) to systematically evaluate each study for bias by answering questions in the tool based on four qualities: patient selection, index test, reference standard, and flow and timing.(9) All discrepancies were resolved by consensus. Studies that are judged as “low” on all domains relating to bias are classed as having a low risk of bias and studies judged as “high” or “unclear” in one or more domains may be deemed as at risk of bias.

Data analysis

We calculated the positive percent agreement, negative percent agreement and the exact 95% confidence intervals for each included study. Using data from all of the included studies, we conducted bivariate fixed-effect and random-effects meta-analyses using a full Bayesian method of positive percent agreement and negative percent agreement with a SAS software (Cary, NC) procedure (PROC MCMC)(10) and we present combined estimates of each.

We selected the fixed-effect model for presentation of the combined estimate of positive and negative percent agreement over the random effects model if its deviance information criterion (DIC) was less than 10 greater than the DIC of the random-effects model; that is, if (DIC_{FIXED} - DIC_{RANDOM} <10).(11)

Results

Our search yielded a total of 71 publications, 31 of which were in duplicate from another database. 14 met our inclusion criteria(12-25) and 26 were excluded because they were not studies evaluating pooled testing, were overlapping datasets, or were a commentary, meta-analysis or review article [Figure 1]. Table 1 lists the included studies that were published between 2016 and 2021 with data from Australia, Belgium, West Africa, Germany, Indonesia, Japan, the UK, and the United States. In total, the studies included results from over 4,000 pooled samples. Most studies used pharyngeal swabs, rectal swab, and urine specimens, however three studies(24-26) used vaginal swabs rather than urine and one study(12) used gargle samples in place of a pharyngeal swab. The Ando study collected the pharyngeal specimen by gargle, as it is considered more feasible for self-collection and its use is widespread in Japan. All studies used a range of nucleic acid amplification test (NAAT) assays as the gold standard comparison test: Aptima Combo 2 (Hologic Inc., San Diego, CA), Abbott Real Time CT/NG Test (Abbott Laboratories, Abbott Park, Illinois), Xpert CT/NG Assay (Cepheid, Sunnyvale, CA), and Roche Cobas 4800 CT/NG Assay (Roche Diagnostics, Indianapolis, IN). Some studies only included positive specimens, thus negative percent agreement could not be calculated for those studies.(27-30) One study only tested for CT infection.(20)

Our bias investigation using the QUADAS-2 tool generally found a low risk of bias across the 14 included studies (Table 2).

The combined positive percent agreement from a fixed effect model for CT was 93.11% (95% CI: 91.51%, 94.55%) and the negative percent agreement was 99.44% (95% CI: 99.18%, 99.65%) (Figure 2, Table 3). The combined positive percent agreement from a random effects model for NG was 93.80% (95% CI: 90.26%, 96.61%) and the negative percent agreement was 99.73% (95% CI: 99.30%, 99.97%) (Table 4).

Discussion

We conducted an evaluation of pooled three anatomic-site tests for the detection of CT and NG infection and found that pooled tests performed similarly to single anatomic site tests. For both CT and NG detection the positive percent agreement of pooled testing with single site testing was over 93% using fixed effect and random effects models and negative percent agreement was over 99% for both infection types. Our findings suggest that implementation of pooled testing for CT and NG infection should be considered in clinical settings where cost and efficiency savings could improve testing access and improve screening uptake. Future research is needed on the potential impact and cost effectiveness of pooled testing. In some settings it may be necessary to know the anatomic site of infection. Testing protocols

could be developed to conduct individual anatomic site testing when a positive pooled test result is obtained.

Our meta-analysis had some limitations including the differing methodologies of pooled testing used in the 14 included studies, which may affect both the values for percent agreement as well as the generalizability of the study. In addition, the relative volume of the three specimen types and buffer solution in the specimen transport kits could contribute to missed infections if the dilution was too great. Another limitation was that some studies in the meta-analysis did not use the same specimen types. For example, in three studies(24-26) vaginal swabs were used in place of a first pass urine sample, and one(12) collected a gargle sample instead of a pharyngeal swab. Vaginal swabs may have a higher sensitivity than first pass urine samples and future research should address which specimen types may provide the highest sensitivity for pooled testing.(31) The studies included in our meta-analysis differed in whether the individual specimens were self-collected or clinician-collected; prior studies have found similar sensitivities between clinician-collected and self-collected samples.(32) Self-collected specimens may also have the added benefit of reducing access barriers such as stigma and privacy concerns, and they have been shown to increase testing uptake.(32) An additional limitation was that the different studies used varying reference tests as the comparison to pooled testing. However, the fixed effect and random effects models were used to account for some of the heterogeneity between the studies. To further strengthen the reliability and validity of the data, we suggest further clinical study on varied populations. In addition, further research is needed to optimize pooling techniques and methodologies to improve sensitivity and workflow.

The high positive percent agreement and negative percent agreement for pooled three-anatomic site testing for NG and CT suggests that pooled testing can be used in the same clinical settings as single-site anatomical testing without a significant sacrifice in the sensitivity of the screening. Additionally, pooled testing for the detection of NG and CT may reduce the cost-burden and resource requirement of typical single-site testing and could potentially reduce barriers to screening.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

We are thankful to founder of Bali Peduli, Dr Frank Stephen Wignall who provided expertise that greatly assisted the research conducted by Romyco et al. that is included in this meta-analysis and Prof. David Lewis who provided advice during that course of that study.

Sources of Support:

This study was funded by National Institute of Allergy and Infectious Diseases (K01AI136725) and the University of California Center for AIDS Research under award number P30 AI036214.

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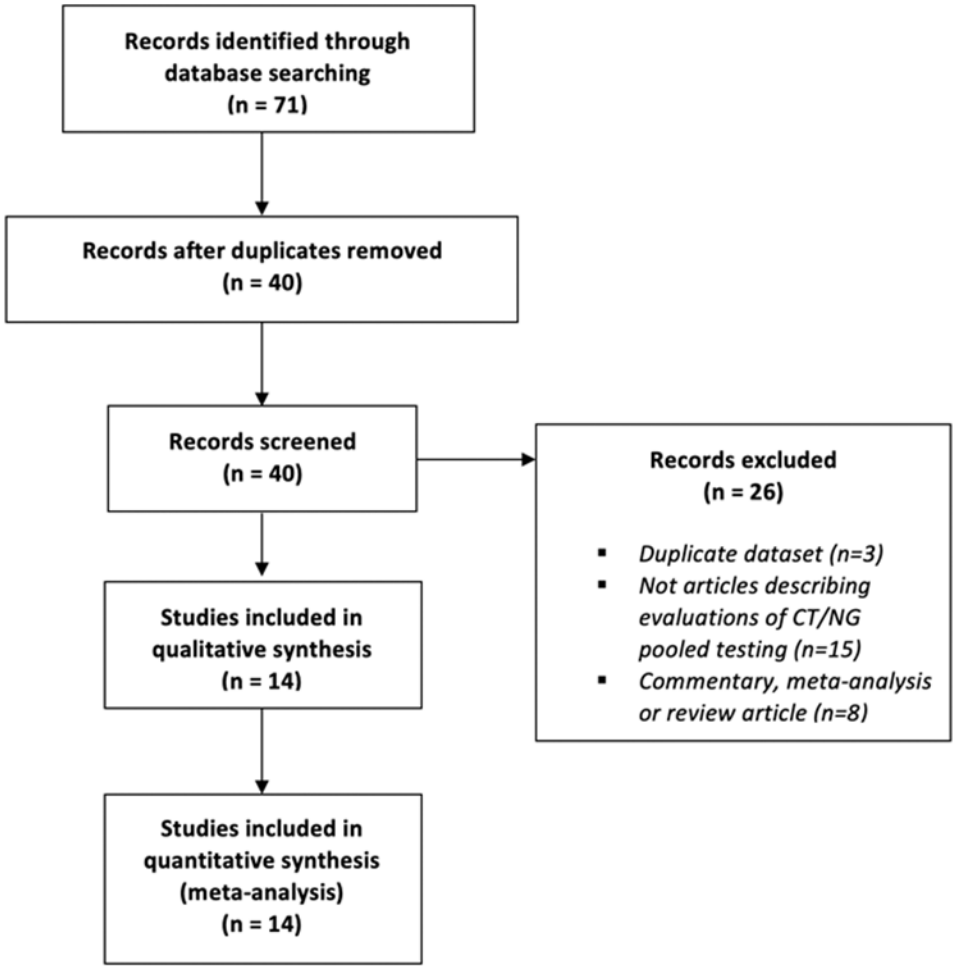

Fig. 1.
PRISMA flow diagram for systematic search.

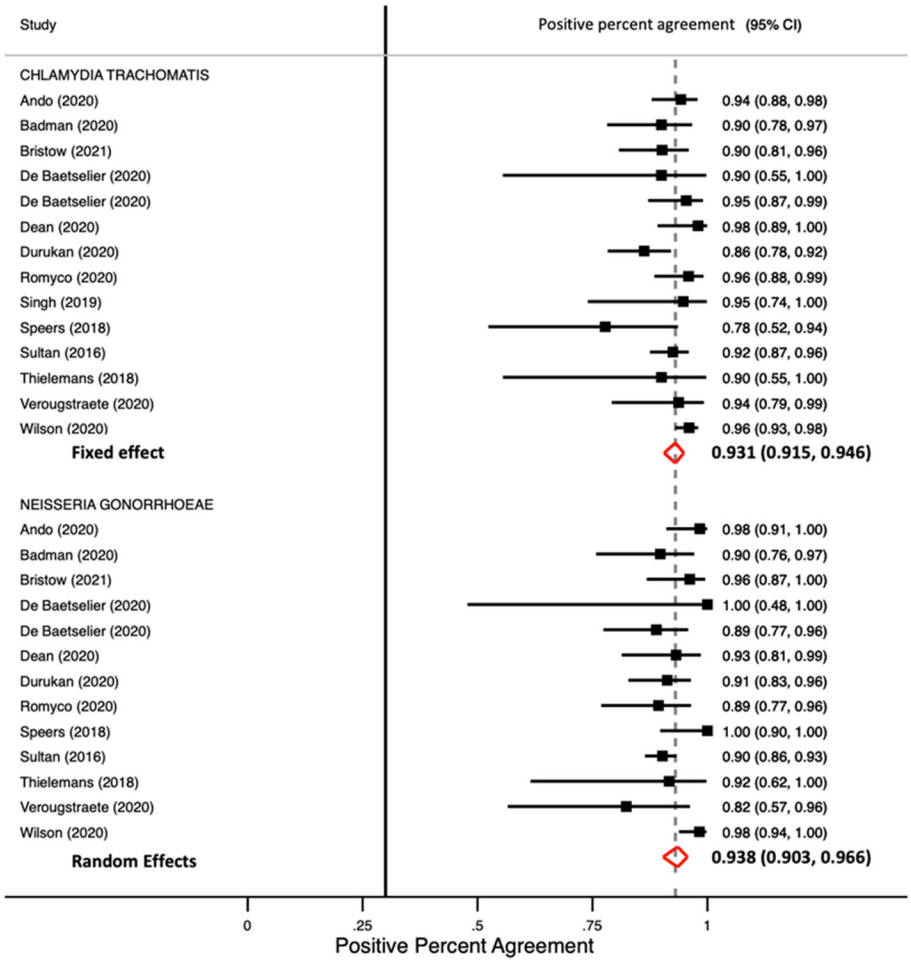

Fig. 2.

Meta-analysis of Positive Percent Agreement of 3 anatomic site pooled specimen testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Table 1.

Studies meeting the inclusion criteria of the systematic review and meta-analyses.

Author (year)	Study Population	Study Location	Individual test pharyngeal and rectal specimen collection (self-collected or clinician-collected)	Pooled test pharyngeal and rectal specimen collection (self-collected or clinician-collected)	Test assay for individual tests	Pooled test assay	Pooling methodology
Ando (2020)	MSM	Japan	Self	Self	Aptima Combo 2	Aptima Combo 2	Rectal swab, 1mL gargle sample & 1mL first catch urine sample were combined into 1 Aptima Combo 2 transport tube for pooled testing.
Badman (2020)	≥ 16 years of age	Brisbane, AU	Self	Self	Xpert CT/NG Assay	Xpert CT/NG Assay	1mL from each remnant pharyngeal and rectal swab specimen Cepheid transport tubes was placed in an empty Cepheid urine collection tube, 7mL urine added. The pooled specimen was inverted 10 times before extraction of pooled specimen for testing.
Bristow (2021)	MSM	CA, USA	Self	Self	Xpert CT/NG Assay	Xpert CT/NG Assay	Equal volume of remnant pharyngeal and rectal swab specimens and urine specimens from Cepheid transport tubes was combined in a dry tube and vortexed for 30 seconds before extraction of pooled specimen for testing.
De Baetselier (2020)	MSM	Belgium	Clinician (pharyngeal), self (anorectal)	Clinician (pharyngeal), self (anorectal)	Abbott Real Time CT/NG test with positive samples being confirmed on an in-house PCR	Abbott Real Time CT/NG test	Equal volume of pharyngeal and anorectal swabs eluates (eluted in diluted phosphate-buffered saline) were added to an equal volume of urine.
De Baetselier (2020)	MSM	West Africa	Clinician	Clinician	Abbott Real Time CT/NG test with positive samples being confirmed on an in-house PCR	Xpert CT/NG Assay	Equal volume of pharyngeal and rectal swab specimen from Eswab (Coban Diagnostics) collection kits was combined with equal volume of urine type was combined and vortexed before extraction of pooled specimen for testing.
Dean (2020)		Australia	Not stated	Not stated	Xpert CT/NG Assay	Xpert CT/NG Assay	Equal volume of remnant pharyngeal and rectal swab specimens and urine specimens from Cepheid transport tubes was combined in a dry tube before extraction of pooled specimen for testing.
Durukan (2020)	MSM	Australia	Clinician	Clinician	Aptima Combo 2	Aptima Combo 2	Anorectal swab added to urine specimen tube, vigorously agitated in the buffer for 15 seconds, rolled firmly on the side of the tube to expel fluid, and discarded. Pharyngeal swab was added to the tube along with 2mL of first catch urine.
Romyco (2019)	MSM	Bali, IDN	Not stated	Not stated	Xpert CT/NG Assay	Xpert CT/NG Assay	Throat, anal swabs, and first catch urine pooled into one cartridge. No further detail provided.
Singh (2019)	Cisgender women	Germany	Self or clinician	Self or clinician	Aptima Combo 2	Aptima Combo 2	Not stated

Author (year)	Study Population	Study Location	Individual test pharyngeal and rectal specimen collection (self-collected or clinician-collected)	Pooled test pharyngeal and rectal specimen collection (self-collected or clinician-collected)	Test assay for individual tests	Pooled test assay	Pooling methodology
Speers (2018)	MSM	Australia	Clinician (pharyngeal), self (anorectal)	Clinician (pharyngeal), self (anorectal)	Roche Cobas 4800 CT/NG Assay	Xpert CT/NG Assay	Pharyngeal and rectal swabs were added to Cepheid urine transport tubes along with 7mL of urine and vortexed for 30 seconds before extraction of pooled specimen for testing.
Sultan (2016)	MSM	U.K.	Clinician	Self	Aptima Combo 2 with positive results confirmed using Aptima single-analyte assays	Aptima Combo 2	Two methods of pooling were used and no significant difference in performance between the two was found. Self-collected swabs added to first catch urine sample OR compressed to wall to release into urine tube, 2mL urine added to AC2 tube.
Thielemans (2018)	MSM	Brussels, BE	Self (anorectal), clinician (pharyngeal)	Self (anorectal), clinician (pharyngeal)	Abbott RealTime CT/NG test	Abbott RealTime CT/NG test	Pharyngeal and anorectal swabs put into a single Abbott multi-Collect specimen tube, first catch urine was added until the liquid level fell within the fill window on the tube label. All samples were vortexed prior to testing. One swab was removed before the assay run to avoid instrument failure.
Verougstraete (2020)	FSW	Belgium	Clinician (pharyngeal), self or clinician (vaginal and rectal)	Clinician (pharyngeal), self or clinician (vaginal and rectal)	Abbott RealTime CT/NG test	Abbott RealTime CT/NG test	Each sample vortexed then equal volume from each Abbott multi-Collect specimen kit was pooled into empty sterile tube.
Wilson (2020)	MSM & females	U.K.	Clinician (rectal and pharyngeal), self (vulvavaginal or first catch urine)	Self	Aptima Combo 2	Aptima Combo 2	Females: pharyngeal and rectal swabs were inserted into transport tube, agitated, squeezed, and discarded, then a vaginal swab was added and left in the transport tube. Males: pharyngeal swab was inserted into transport tube, agitated, squeezed, and discarded. A rectal swab was added and left in the transport tube. 2 ML of first catch urine was added.

Table 2.

Quadas results for specified article for bias.

	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Ando(12)	Low	Unclear	Low	Low
Badman(13)	Low	Low	Low	Low
Bristow(14)	Low	Low	Low	Low
De Baetselier(15)	Unclear	Low	Low	Unclear
De Baetselier(16)	Low	Low	Low	Low
Dean(17)	Unclear	Low	Low	Unclear
Durukan(18)	Low	Low	Low	Low
Romyco[*](19)	Unclear	Unclear	Low	Unclear
Singh[*](20)	Low	Unclear	Low	Unclear
Speers(21)	Low	Low	Low	Low
Sultan(22)	Low	Low	Low	Low
Thielemans(23)	Low	Low	Low	Low
Verougstraete(24)	Low	Low	Low	Low
Wilson(25)	Low	Low	Low	Low

[*]Conference abstract

Bias was evaluated based on patient selection, index test, reference standard, and flow and timing.(8) Classification as “low” indicates a low risk of bias in that domain, and classification as “high” or “unclear” indicates a high or unclear risk of bias for that domain.

Table 3.

Meta-analysis of pooled testing for detection of *Chlamydia trachomatis* using exact confidence intervals.

First author	Year of publication	TP	FN	FP	TN	PPA	(95% CI)	NPA	(95% CI)
Ando	2020	98	6	3	406	94.2	(87.9, 97.9)	99.3	(97.9, 99.9)
Badman	2020	45	5	0	26	90.0	(78.2, 96.7)	100	(86.8, 100)
Bristow	2021	64	7	4	523	90.1	(80.7, 95.9)	99.2	(98.1, 99.8)
De Baetselier (Belgium)	2020	9	1	.	.	90.0	(55.5, 99.8)	.	
De Baetselier (West Africa)	2020	62	3	5	372	95.4	(87.1, 99.0)	98.7	(96.9, 99.6)
Dean	2020	48	1	.	.	98.0	(89.2. 100)	.	
Durukan	2020	94	15	.	.	86.2	(78.3, 92.1)	.	
Romyco	2019	70	3	0	171	95.9	(88.5, 99.1)	100	(97.9, 100)
Singh	2019	18	1	0	80	94.7	(74.0, 99.9)	100	(95.5, 100)
Speers	2018	14	4	2	89	77.8	(52.4, 93.6)	97.8	(92.3, 99.7)
Sultan	2016	160	13	.	.	92.5	(87.5, 95.9)	.	
Thielemans	2018	9	1	0	90	90.0	(55.5, 99.8)	100	(96.0, 100)
Verougstraete	2020	30	2	0	457	93.8	(79.2, 99.2)	100	(99.2, 100)
Wilson	2020	265	11	7	1507	96.0	(93.0, 98.0)	99.5	(99.1, 99.8)
Fixed Effect model						**93.11**	**(91.51, 94.55)**	**99.44**	**(99.18, 99.65)**
DICfixed= 99.552									
DICrandom= 94.347									

*Used exact confidence intervals.

Abbreviations: TP, true positive; FN, false negative; FP, false positive; TN, true negative; PPA, positive percent agreement; NPA, negative percent agreement; DIC, deviance information criterion.

Table 4:

Meta-analysis of pooled testing for detection of Neisseria gonorrhoeae using exact confidence intervals.

First author	Year of publication	TP	FN	FP	TN	PPA	(95% CI)	NPA	(95% CI)
Ando	2020	59	1	3	450	98.3	(91.1, 100)	99.3	(98.1, 99.9)
Badman	2020	35	4	0	37	89.7	(75.8, 97.1)	100	(90.5, 100)
Bristow	2021	50	2	1	545	96.2	(86.8, 99.5)	99.8	(99.0, 100)
De Baetselier (Belgium)	2020	5	0	.	.	100	(47.8, 100)	.	
De Baetselier (West Africa)	2020	48	6	9	381	88.9	(77.4, 95.8)	97.7	(95.7, 98.9)
Dean	2020	41	3	.	.	93.2	(81.3, 98.6)	.	
Durukan	2020	73	7	.	.	91.3	(82.8, 96.4)	.	
Romyco	2019	42	5	0	197	89.4	(76.9, 96.5)	100	(98.1, 100)
Speers	2018	34	0	0	75	100	(89.7, 100)	100	(95.2, 100)
Sultan	2016	286	31	.	.	90.2	(86.4, 93.3)	.	
Thielemans	2018	11	1	0	88	91.7	(61.5, 99.8)	100	(95.9, 100)
Verougstraete	2020	14	3	0	472	82.4	(56.6, 96.2)	100	(99.2, 100)
Wilson	2020	112	2	3	1673	98.3	(93.8, 99.8)	99.8	(99.5, 100.0)
Random Effects model						**93.80**	**(90.26, 96.61)**	**99.73**	**(99.30, 99.97)**
DICfixed= 102.897									
DICrandom= 80.474									

* Used exact confidence intervals

Abbreviations: TP, true positive; FN, false negative; FP, false positive; TN, true negative; PPA, positive percent agreement; NPA, negative percent agreement; DIC, deviance information criterion.