Factors that influence confirmation of Neisseria gonorrhoeae positivity by molecular methods

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Abstract

Several *Neisseria gonorrhoeae* (NG) nucleic acid amplification tests (NAATs) exist with high sensitivity. However, specificity of NG NAAT testing may be suboptimal, particularly in extra-genital biospecimens. Consequently, confirmation with a second NAAT is common, although represents a burden on resources. Furthermore, the rationale for confirmation is contentious.

The objective of this work was to assess NG confirmation in over 13,000 NG screen-positive samples representing various biospecimens and three separate screening assays: the Abbott Real Abbott RealTime CT/NG (Abbott Molecular Inc., Des Plaines, IL), the Cobas CT/NG test (Roche Diagnostics, Indianapolis, IN) and the BD ProbeTec ET CT/GC Amplified DNA assay (Becton Dickinson and Company, Sparks, MD). Factors predictive of confirmation were determined via logistic regression involving: sex, year, whether the samples was formally validated and sample site.

Level of confirmation varied according to screening assay (96.2%, 86.0% and 73.9%) for the Abbott, Roche and BD test respectively in sample types formally included according to manufacturers instructions (i.e.-validated). Sex did not affect confirmation for 2/3 assays and the likelihood of confirmation of samples not formally included in manufacturer instructions (ie non validated) was 89.1%, 82.1% and 59.2% for the Abbott Roche and BD test respectively. Rectal swabs, which are non validated samples, confirmed in 91.5%, 90.1% and 87.4% of samples initially tested with the respective assays

The requirement to confirm NG in validated samples is not required for all NAATs although initial, assay-specific evaluation is justified given observed variability. Rectal samples

represent robust biospecimens for NG NAAT testing and do not require confirmation when screened with the assays described.

Introduction

The management of *Neisseria gonorrhoeae* (NG) infection represents an increasing and significant clinical burden, in various settings including the UK. ln Scotland in 2017 there were 2610 episodes of gonorrhoea, in comparison with only 1073 cases in 2008 (1). Similar observations of an increase over the last decade have also been noted in England. (2)
Quantifying the magnitude of the increase as a result of changed sexual behaviours and/or shortfalls in management approaches is confounded by variability in indications for testing and the increased use of highly sensitive nucleic acid amplification tests (NAAT). Consistency of recommendations for testing-practice and application of robust NAATs can support the more accurate measurement of genuine trends. Certainly, NAAT rather than culture of NG is recommended as a first line test and an increasing diversity of molecular tests exists. Assay-driven influences in NAAT confirmation of NG have been reported including in a recent UK study where 27% of samples from females did not confirm with a second line test (3). These data reconcile with those from a study by Vyth et al (2016) who reported that assay-choice significantly influenced the likelihood of culture confirmation (4).

Guidance for the application of NG NAATs is available with the purpose of harmonising approach to support practically achievable, meaningful and technically-robust testing. Such guidance includes that from Public Health England (PHE) which recommends confirmatory testing of all extra-genital samples, with an assay that targets a different sequence from the initial assay in order to enhance specificity (5). The same guidelines stipulate that "*laboratories should only issue positive test results that are confirmed by supplementary*

testing or, for genital samples, where the positive predictive value (PPV) of the initial NAAT has been validated in the local laboratory as being ≥90%". The British Association for Sexual Health and HIV (BASHH, 2011) offer similar advice on confirmatory testing including the requirement for confirmation of extra-genital samples (6). Guidance on confirmatory testing has also been issued by the Centre for Disease Control CDC (2014) which stipulate that repeat testing of NAAT-positive genital tract specimens is not recommended (if the screening assay is FDA approved) "because the practice does not improve the positive predictive value of the test" (7). For extra-genital sites the guidance indicates that in-house validation to CLIA standards is sufficient.

This all said, how closely real-life practice follows guidance is not documented. Confirmatory testing of validated genital samples occurs and this is not exclusive to those laboratories where the initial NAAT has been confirmed to have a PPV of <90%. Furthermore, as described, NAATs continue to evolve and improve; whether confirmatory testing, including that associated with samples from extra-genital sites is needed requires evidence-based assessment. This consideration is made relevant by the fact that confirmatory testing does not represent a trivial resource implication for the laboratory.

The evolution of NG NAATs, the increased diversity of biospecimens tested in service laboratories and the disconnectedness and vintage of the guidelines justifies further detailed interrogation of the value of NG NAAT confirmation in large, population based, data series. The objective of the present work was to determine the level of NG confirmation in over 13,000 samples representing various biospecimen types from males and females and sent to a reference laboratory facility for routine confirmation via in-house PCR. Variables which influenced confirmation were subsequently detemined.

Material and Methods

Context and dimensions of sample

Currently, NG testing in Scotland is recommended only for groups where the prevalence is likely to be greater or equal to 1%, in line with national guidelines. The Scottish Bacterial STI reference laboratory provides a centralised, national service of NG NAAT confirmation for Scottish Health Boards (8). The timeframe under study relates to samples received between Jan 2009 to June 2016.

Confirmation testing

The reference, confirmatory test was based on an in house approach which involved two multiplex real-time PCR methods, each incorporating an extraction inhibition control (phocine herpes virus-1) and a target specific for *N. gonorrhoeae* (*porA* and 16S rRNA genes). Samples (urine or swab eluate) were concentrated by centrifugation of 1 ml at 20,000 *g* for 5 min, then discarding 800 µl supernatant. Extraction of the samples was by NucliSENS easyMAG (Biomerieux, Marcy-l'Etoile, France) automated extractor using the Generic 2.0.1 protocol, adding 200 µl and eluting in 100 µl. In the event of inhibition the Qiagen QIAamp DNA Mini kit was utilised, again with the addition of 200 µl sample and a final elution volume of 100 µl.

The first line assay targeted the *porA* gene (Whiley, 2005), with a limit of detection of around 10 copies/ml in swab eluate and 100 c/ml in urine samples (9). The second line assay targeted the 16S rRNA gene to detect *porA*-negative strains. This method was developed in-house. Although the primers were found to bind to other non-gonococcal *Neisseria* species,

the probe was specific to *N. gonorrhoeae*. Therefore this assay may lead to false-negative results where non-gonococcal *Neisseria* are present, such as the pharynx Note that no further adjudication with .an additional assay was performed if there was discordance between the screening assay and the reference test.

Measuring agreement of screening vs confirmation test and identification of significant predictors of confirmation.

Level of confirmation was performed according to screening assay, specimen type (individual anatomical site), sex and the year of screening test through comparison with the reference laboratory confirmation test. Confirmation was also assessed according to whether a sample was considered validated (urine or genital swab) or non validated (all other samples) by the manufacturer of the screening test.

To determine what factors were predictive of confirmation for each screening assay individually, a logistic regression was performed using sex (male/female), whether the samples was validated (yes/no) and specific sample site/type. Year the samples was submitted (2009-2016 - treated as a continuous variable) was also included to serve as a proxy for the potential influence of assay version-change over time which was not available in the data set. Additionally, arguably sample year provides potential insight into the significance other factors within general lab testing environment that may have changed over time. Where any variables were found to not to be statistically significant they were sequentially removed to generate a final model containing only those variables which reached statistical significance at the 5% level. For the purpose of the regression, any sample with unknown sex and/or whether unvalidated vs validated status could not be

confirmed was removed. The small number eye swabs (n=11) led to their removal for the regression analysis.

Results

Description of samples

Data were excluded if the technology of the sender was undefined, or if the screening test was NG negative. A total of 13803 specimens were subsequently included in the analysis; 69.7% were from males and 54.9% of samples were manufacturer-validated. The most common screening assay accounted for 73.5% of samples (n=10,139) and was the Abbott RealTime CT/NG assay (Abbott Laboratories, Abbott Park, IL, USA) compared to 13.2% (n=1824) and 13.3% (n=1841) samples screened by the Cobas 4800 (Roche Diagnostics, Pleasanton, CA, USA), and BD ProbeTec™ ET GC Amplified DNA Assay (Franklin Lakes, NJ, USA) respectively. One sample was tested with both platforms, Abbott and Roche. Key variables that relate to the data set under assessment are presented in Table 1.

Overall agreement between screening vs confirmatory assay

Overall, the confirmatory assay was positive in 12,184/13803 samples 88.3%; (95% CI: 87.7 – 88.8). Overall agreement was 93.1%; (95% CI: 92.6 – 93.6) with the Abbott assay, 84.0% (95% CI: 82.2 – 85.6) with the Roche assay and 65.9% (95% CI: 63.7 – 68.1) with the BD assay (Table 2).

Table 3 shows level of confirmation with the screening and confirmatory assay according to sex, sampling year, validation status of sample and specific sample type, according to each assay. A small effect on confirmation according to sampling year was observed; with a

reduced likelihood for confirmation over time/sampling year. Confirmation according to gender was 93.6% for female samples vs 92.9% in male samples for the Abbott assay. Respective values for the Roche assay were 80.5% and 85.5% for female and male samples. Finally for the BD assay 58% of female samples confirmed vs 70.1% of male samples.

As expected specimen type/site and validation status did influence likelihood of confirmation. For formally validated (by the manufacturer) specimens, confirmation was evident in 96.2%; (95% CI: 95.7 – 96.6), 86.0% (95% CI: 83.7 – 88.1) and 73.9% (95% CI: 70.8 – 76.7) of specimens initially tested with the Abbott, Roche and BD assays respectively. In relation to unvalidated specimens 89.1%; (95% CI: 88.1 – 89.9), 82.1%; (95% CI: 79.4 – 84.4) and 59.2% (95% CI: 56.0 – 62.2) confirmed with the Abbott, Roche and BD test respectively. The most common unvalidated specimens were throat swabs (n=2817) and rectal swabs (n=2207). Confirmation of throat swabs was evident in 87.1%; (95% CI: 85.6 – 88.4), 78.1%; (95% CI: 74.4 – 81.3) and 49.5%; (95% CI 45.8 – 53.2) of specimens tested with the Abbott, Roche and BD assays respectively. Equivalent figures for confirmation of rectal swabs according to assay were higher at 91.5%; (95% CI: 90.2 – 92.7), 90.1%; (95% CI: 86.1 – 93.0) and 87.4%; (95% CI: 82.7 – 91.0) according to respective assay; similar to confirmation levels associated with validated biospecimens.

The sample type, least likely to confirm was an eye swab at 54.5%; (95% CI: 28.0 – 78.7) with the caveat that only 11 were submitted for confirmation.

<u>Which factors are significantly associated with confirmation?</u>

Variables that were identified as significant are included in Table 4. Factors influencing confirmation of the Abbott assay were that samples from females were more likely to confirm. Additionally a small year on year reduction in likelihood of confirmation was observed. Finally, cervical, rectal, urethral and urine samples were all more likely to confirm compared to vulval samples.

The only factor that was associated with confirmation with the Roche assay was sample year, however 2009 (which related to only 24 samples of which only one confirmed) may represent an anomaly and partially account for this observation. A greater number of factors were found to significantly affect confirmation of the BD assay, namely that female samples were less likely to confirm. With respect to specific sample types, cervical samples were less likely to confirm compared to vulval samples throat samples were less likely to confirm compated to vulval samples and urethral samples were more likely to confirm compared to vulval samples. Additionally a small year on year reduction in likelihood of confirmation was observed.

Discussion

The increase in NG infection and the implications of this necessitate swift and accurate diagnosis to support and inform both epidemiological assessment and crucially, appropriate clinical support and management. The need for confirmation requires review so that resources can be used equitably and efficiently. In this evaluation we observed that the two most commonly applied first line tests for NG in Scotland showed high concordance with a secondary reference test performed in a centralised reference laboratory and exceeded (Abbott) or approached (Roche) 90%. This puts into question the additional value that is

gained via confirmation of samples initially tested with these assays particularly with respect to validated samples (10,11)

Of interest confirmation of rectal swabs (the second most common non-validated specimen received), exceeded 90% for the Roche and Abbott assay and was 87% for the BD, which was actually higher than confirmation associated with certain validated samples for the latter assay. These data are consistent with those in an earlier study by Perry et al (2014) who concluded that rectal NG positives generated by the Roche 4800 assay did not require confirmation when compared to an opa/pap duplex assay (12). While the conclusions of Perry and colleagues were on the basis of less than 100 samples they are nevertheless validated in the present study where over 2000 rectal NG screen positive samples were assessed. Abandoning confirmation of screen positive rectal samples may represent a significant efficiency; in Scotland the rise in NG in men, is thought to be largely driven by men who have sex with men (MSM) and in 2017, 37% of episodes in men were diagnosed from a rectal swab positive for gonorrhoea (1). The most common non validated sample type; the throat swab, was associated with a lower level of confirmation overall compared to rectal swabs, although the level of confirmation varied considerably depending on assay and ranged from ~50% (BD Assay) to ~90% (Abbott Assay). The greater likelihood of cross-reacting non *gonorrhoeae Neisseria* species in the throat (compared to other anatomical sites) explains this issue of analytical specificity and these observations are consistent with the information in the BD assay product insert. Consequently, expectations on likelihood of confirmation of throat samples the should be managed and the type of screening assay taken into account.

Certainly the present work has demonstrated that assay type can drive discordance, so specific evaluation of other and novel assays is warranted when considering whether confirmation is required. This said, it would appear that the current state of the art of commercial NG assays is promising. A recent article by Causer et al (2018) showed a reassuring level of concordance between point of care (POC) testing with the Xpert CT/NG assay (Cepheid, Wooburn Gree, UK) vs standard of care. In this study, overall concordance for NG detection was 99.9% with positive concordance of 100.0% and negative concordance of 99.9% (13). Clearly, POC testing does not rely on confirmation of the result at a reference laboratory and growing interest in POC is evident in new technologies and quality processes to support this approach (14 - 16).

There are limitations to the study, as indicated, we accept that there are an increasing number of NG assays available and in the present study, we evaluated only three platforms. Furthermore, the most common screening assay applied was the Abbott test by some margin meaning that as a function of its larger sample size, smaller differences (in confirmation) were more likely to be significant when compared to the other two assays. In addition we accept that any reference/confirmatory test is not itself impervious to error. However the strengths of the study are the large overall denominator which allowed assessment of concordance according to several variables including specific sample site/biospecimen type - and the fact that the demographic was population-based given that samples were sent from across Scotland as part of a routinely offered service.

To conclude, the level of confirmation with NG screening assays and an in house reference assay was high and exceeded or approached 90% for the two most commonly used assays in Scotland in validated samples. The rationale for confirmatory testing of validated samples

tested by the Abbott and Roche assays is therefore not supported. In addition rectal swabs appear to constitute robust bio-specimens for NG testing and confirmatory testing may not be justified.

Variable	Description	N (n=13803)	%
Sex	Male	9609/13780	69.7
Sample year	2009	284	2.1
	2010	1417	10.3
	2011	1896	13.8
	2012	2264	16.4
	2013	1724	12.5
	2014	2190	15.9
	2015	2798	20.3
	2016	1230	8.9
Validated biospecimen?		7572/13609	55.6
Biospecimen site/type	Cervical Swab	1012	7.4
	Endocervical	10	0.1
	Eye	11	0.1
	High vaginal swab	39	0.3
	Lower vaginal swab	73	0.5
	Rectal	2427	17.8
	Throat	3599	26.4
	Unknown	194	1.4
	Urethral Swab	286	2.1
	Urine	4285	31.5
	Vaginal Swab (not defined)	322	2.4
	Vulval Swab	1545	11.4
Senders/screening Assay	Abbott RealTime CT/NG assay[1]	10,139	73.5
	Roche Cobas 4800 CT/NG[2]	1824	13.2
	BD ProbeTec™ ET GC[3]	1841	13.3

Table 1: Key characteristics/variables of 13803 samples, received for *Neisseria gonorrhoeae* confirmatory testing included in the analysis.

1: https://www.molecular.abbott/sal/en-us/staticAssets/ctng-8l07-91-us final.pdf

2:https://pimeservices.roche.com/eLD_SF/gb/en/Documents/GetDocument?documentId=4d186450-4bf2-e811-edbb-00215a9b3428.

3:http://moleculardiagnostics.bd.com/syndromic-solutions/womens-health-stis/ct-gc-tv

Screening Assay	**Number confirmation test positive**	**Number positive by assay**	**% confirmed by reference test (95% CI)**
Abbott RealTime CT/NG	9439	10139	93.1 (92.6 – 93.6)
Roche Cobas 4800 CT/NG	1532	1824	84.0 (82.2 – 85.6)
BD ProbeTec™ ET GC	1214	1841	65.9 (63.7 – 68.1)
All	12184	13803	88.3 (87.7 – 88.8)

- **Table 2:** Overall confirmation according to screening assay

		Abbott RealTime CT/NG assay			Roche Cobas 4800 CT/NG			BD ProbeTec™ ET GC			Overall		
		Confirmatory test positive n=9439	% Confirmatory test positive	Abbott positive n=10139	Confirmatory test positive n=1532	% Confirmatory test positive	Roche positive n=1824	Confirmatory test positive n=1214	% Confirmatory test positive	BD positive n=1841	Confirmatory test n=12184	% Confirmatory test positive	All positive by any assay n=13803
Sex	Female	2819	93.6	3011	438	80.5	544	357	58.0	616	3614	86.6	4171
	Male	6605	92.9	7110	1093	85.5	1279	856	70.1	1221	8553	89.0	9609
	Unknown	15	83.3	18	1	100.0	1	1	25.0	4	17	73.9	23
Sample year	2009	221	97.8	226	1	4.2	24	29	82.9	35	250	88.0	284
	2010	1143	96.1	1190	74	82.2	90	94	68.6	137	1311	92.5	1417
	2011	1462	97.3	1502	245	94.2	260	101	75.4	134	1808	95.4	1896
	2012	1726	96.2	1795	193	74.8	258	144	68.2	211	2063	91.1	2264
	2013	1162	93.8	1239	204	86.1	237	152	61.3	248	1518	88.1	1724
	2014	1372	91.2	1505	283	85.8	330	214	60.3	355	1869	85.3	2190
	2015	1579	87.9	1796	383	85.1	450	364	65.9	552	2326	83.1	2798
	2016	774	87.4	886	149	85.1	175	116	68.6	169	1039	84.5	1230
Validated biospecimen?	No	3757	89.1	4218	705	82.1	859	568	59.2	960	5030	83.3	6037
	Unknown	132	86.8	152	14	70.0	20	11	50.0	22	157	80.9	194
	Yes	5550	96.2	5769	813	86.0	945	635	73.9	859	6997	92.4	7572
Biospecimen site/type	Cervical Swab	729	96.2	758	105	76.1	138	68	58.6	116	902	89.1	1012
	Endocervical	5	83.3	6	3	100.0	3	0	0.0	1	8	80.0	10
	Eye	4	100.0	4	0	0.0	1	2	33.3	6	6	54.5	11
	High vaginal	21	95.5	22	8	80.0	10	5	71.4	7	34	87.2	39

	swab												
	Lower vaginal swab	29	87.9	33	11	78.6	14	13	50.0	26	53	72.6	73
	Rectal	1727	91.5	1887	264	90.1	293	216	87.4	247	2207	90.9	2427
	Throat	2026	87.1	2327	441	78.1	565	350	49.5	707	2817	78.3	3599
	Unknown	132	86.8	152	14	70.0	20	11	50.0	22	157	80.9	194
	Urethral Swab	196	99.0	198	21	84.0	25	61	96.8	63	278	97.2	286
	Urine	3328	97.7	3405	486	89.5	543	281	83.1	338	4094	95.5	4285
	Vaginal Swab	267	92.4	289	19	95.0	20	8	61.5	13	294	91.3	322
	Vulval Swab	975	92.2	1058	160	83.3	192	199	67.5	295	1334	86.3	1545

Table 3: Confirmation stratified by screening assay, sex, year, validation status and anatomical site

Odds Ratio Estimates				
Assay	**Effect**	**Point Estimate**	**95% Wald Confidence Limit lower**	**95% Wald Confidence Limit upper**
Abbott RealTime CT/NG assay	**Sex F vs M**	1.955	1.398	2.733
	SAMPLE YEAR	0.747	0.713	0.782
	SAMPLE Cervical vs Vulval	1.579	1.023	2.437
	SAMPLE Rectal vs Vulval	1.843	1.225	2.774
	SAMPLE Throat vs Vulval	1.147	0.771	1.706
	SAMPLE Urethral vs Vulval	15.429	3.621	65.739
	SAMPLE Urine vs Vulval	7.423	4.707	11.708
	SAMPLE Vaginal vs Vulval	1.481	0.936	2.344
Roche Cobas 4800 CT/NG	**Sex F vs M**	0.901	0.580	1.401
	SAMPYEAR	1.101	1.024	1.185
	SAMPLE Cervical vs Vulval	0.742	0.426	1.290
	SAMPLE Rectal vs Vulval	1.584	0.812	3.090
	SAMPLE Throat vs Vulval	0.633	0.361	1.111
	SAMPLE Urethral vs Vulval	0.915	0.271	3.093
	SAMPLE Urine vs Vulval	1.610	0.862	3.006
	SAMPLE Vaginal vs Vulval	1.292	0.503	3.315
BD ProbeTec™ ET GC	**Sex F vs M**	0.458	0.315	0.664
	SAMPLE YEAR	0.927	0.872	0.984
	Genital_validated N vs Y	0.247	0.161	0.377
	SAMPLE Cervical vs Vulval	0.604	0.385	0.947
	SAMPLE Rectal vs Vulval	6.594	4.385	9.915
	SAMPLE Urethral vs Vulval	6.130	1.396	26.914
	SAMPLE Urine vs Vulval	1.125	0.674	1.877
	SAMPLE Vaginal vs Vulval	0.630	0.335	1.187

Table 4: Summary of logistic regression models by assay showing variables identified as associated with NG confirmation.

References

1. Cullen BL, Wallace LA, Glancy ME, Shepherd J, Templeton K, Goldberg DJ. 2018. Health Protection Scotland surveillance report: genital chlamydia and gonorrhoea infection in Scotland: laboratory diagnoses 2008-17. Available from https://www.hps.scot.nhs.uk/resourcedocument.aspx?id=6721

2. Public Health England. Sexually transmitted infections and Chlamydia screening in England, 2017. Available from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/713944/hpr2018_AA-STIs_v5.pdf

3. Bennett A, Jeffery K, O'Neill E, Sherrard J. Outbreak or illusion: consequences of 'improved' diagnostics for gonorrhoea. 2017. Int J STD AIDS.28(7):667-671.

4. Vyth R, Leval A, Eriksson B, Ericson EL, Marions L, Hergens MP. 2016. Gonorrhoea Diagnostic and Treatment Uncertainties: Risk Factors for Culture Negative Confirmation after Positive Nucleic Acid Amplification Tests. PLoS One. 6;11(5):e0155017.

5. Public Health England. 2018. Guidance for the detection of gonorrhoea in England. 2014. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/405293/170215_Gonorrhoea_testing_guidance_REVISED__2_.pdf [Accessed 13 Aug. 2018].

6. UK national guideline for the management of gonorrhoea in adults (2011). BASHH. UK national. Available at: https://www.bashhguidelines.org/media/1044/gc-2011.pdf [Accessed 13 Aug. 2018].

7. CDC.gov Laboratory Recommendations | CDC. [online] Available at: https://www.cdc.gov/std/laboratory/2014labrec/default.htm [Accessed 13 Aug. 2018].

8. Scottish Microbiology Forum, British Association for Sexual Health and HIV, and Health Protection Scotland. Guidance on the user of molecular testing for Neisseria

gonorrhoeae in diagnostic laboratories 2011. Molecular testing for gonorrhoea working group, Edinburgh, Scottish Bacterial Sexually Transmitted Infections Laboratory. 2012. Available from https://www.hps.scot.nhs.uk/resourcedocument.aspx?resourceid=190

9. Whiley & Sloots 2005 Comparison of three in-house multiplex PCR assays for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using real-time and conventional detection methodologies. Pathology 37(5):364-370

10. Hopkins MJ, Smith G, Hart IJ, Alloba F. 2012. Screening tests for Chlamydia trachomatis or Neisseria gonorrhoeae using the cobas 4800 PCR system do not require a second test to confirm: an audit of patients issued with equivocal results at a sexual health clinic in the Northwest of England, U.K. Sex Transm Infect. 88(7):495-7.

11. Schachter J, Chernesky MA. Routine confirmation of positive nucleic acid amplification test results for Neisseria gonorrhoeae is not necessary. J Clin Microbiol. 2012 Jan;50(1):208; author reply 209-10. doi: 10.1128/JCM.06004-11. PubMed PMID: 22203751; PubMed Central PMCID: PMC3256691

12. Perry MD, Jones RN, Corden SA. 2014. Is confirmatory testing of Roche cobas 4800 CT/NG test Neisseria gonorrhoeae positive samples required? Comparison of the Roche cobas 4800 CT/NG test with an opa/pap duplex assay for the detection of N gonorrhoeae. Sex Transm Infect. 90(4):303-8.

13. Causer LM, Guy RJ, Tabrizi SN, Whiley DM, Speers DJ, Ward J, Tangey A, Badman SG, Hengel B, Natoli LJ, Anderson DA, Wand H, Wilson D, Regan DG, Shephard M, Donovan B, Fairley CK, Kaldor JM. 2018. Molecular test for chlamydia and gonorrhoea used at point of care in remote primary healthcare settings: a diagnostic test evaluation. Sex Transm Infect. 94(5):340-345.

14. Badman SG, Causer LM, Guy R, Wand H, Donovan B, Tabrizi SN, Speers D, Shephard MD, Vallely A, Whiley D; TTANGO investigators. 2018. A reliable and easy to transport quality control method for chlamydia and gonorrhoea molecular point of care testing. Pathology. 50(3):317-321.

15. Horst AL, Rosenbohm JM, Kolluri N, Hardick J, Gaydos CA, Cabodi M, Klapperich CM, Linnes JC. 2018. A paperfluidic platform to detect Neisseria gonorrhoeae in clinical samples. Biomed Microdevices. 11;20(2):35.

16. Speers DJ, Chua IJ, Manuel J, Marshall L. Detection of Neisseria gonorrhoeae and Chlamydia trachomatis from pooled rectal, pharyngeal and urine specimens in men who have sex with men. 2018. Sex Transm Infect. 94(4):293-297