Swab-yourself trial with economic monitoring and testing for infections collectively (SYSTEMATIC): Part 2. A diagnostic accuracy, and cost-effectiveness, study comparing rectal, pharyngeal and urogenital samples analysed individually, versus as a pooled specimen, for the diagnosis of gonorrhoea and chlamydia

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Summary of main points

Pooling someone's rectal, pharyngeal and FCU/VVS samples into one container was as accurate as individual samples for diagnosing gonorrhoea but 3% less sensitive for chlamydia. Pooled specimens were more accurate, and cost-effective, at diagnosing gonorrhoea and chlamydia than FCU/VVS alone.

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Abstract

Background:

Sexual history does not accurately identify those with extragenital *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) so universal extragenital sampling is recommended. Nucleic acid amplification tests (NAATs) are expensive. If urogenital, plus rectal and pharyngeal, samples are analysed the diagnostic cost is trebled. Pooling samples into one NAAT container would cost the same as urogenital samples alone. We compared clinician triple samples analysed individually with self-taken pooled samples for diagnostic accuracy, and cost, in MSM and females.

Methods:

Prospective, convenience, sample in UK sexual health clinic. Randomised order of clinician and self-samples from pharynx, rectum, plus first catch urine (FCU) in MSM and vulvovaginal swabs (VVS) in females, for NG and CT detection.

Results:

Of 1793 participants (1284 females, 509 MSM), 116 had NG detected (75 urogenital, 83 rectum, 72 pharynx). 276 had CT detected (217 urogenital, 249 rectum, 63 pharynx). There was no difference in sensitivities between clinician triple samples and self-pooled specimens for NG (99.1%, 98.3%) but clinician samples analysed individually identified 3% more chlamydia infections than pooled (99.3%, 96.0%; p=0.027). However, pooled specimens identified more infections than VVS/FCU alone. Pooled specimens missed 2 NG and 11 CT infections, whereas VVS/FCU missed 41 NG and 58 CT infections. Self-taken pooled specimens were the most cost-effective.

Conclusions:

Just FCU/VVS testing missed many infections. Self-taken pooled samples were as sensitive as clinician triple samples for identifying NG, but clinician samples analysed individually identified 3% more CT infections than pooled. The extragenital sampling was achievable at no additional diagnostic cost to the FCU/VVS.

Trial Registration:

ClinicalTrials.gov NCT02371109

Background

Neisseria gonorrhoeae (NG) and *Chlamydia trachomatis* (CT) are the most common bacterial sexually transmitted infections (STIs) worldwide.[1] Both infections can be asymptomatic in males and females, but lead to serious sequelae if untreated.[2,3] In addition to infecting the urogenital tract, both can infect the rectum and pharynx (extragenital sites), usually with no symptoms. Infections may be present in urogenital and/or extragenital sites.

Most guidelines[2-5] suggest extragenital screening in men who have sex with men (MSM) and females based on reported sexual history of receptive anal sex and giving oral sex. However, several studies indicate that sexual history does not accurately identify those with extragenital infections so universal extragenital sampling is being recommended.[6-11]

Nucleic acid amplification tests (NAATs) are expensive. If rectal and pharyngeal samples are taken with urogenital samples (first-catch urine [FCU] in MSM and vulvovaginal swab [VVS] in females), the diagnostic cost is trebled. This increased cost in all females and MSM is unaffordable for most publicly funded sexual health services.[12-17]

Self-taken extragenital samples are as accurate at detecting NG and CT as clinician-taken samples, and are more cost-effective. [Wilson JD, Wallace HE, Loftus-Keeling M, et al. Swab-yourself trial with economic monitoring and testing for infections collectively (SYSTEMATIC): Part 1. A diagnostic accuracy, and cost-effectiveness, study comparing clinician-taken versus self-taken rectal and pharyngeal samples for the diagnosis of gonorrhoea and chlamydia. Accepted by Clin Infect Dis.] These can be performed by individuals along with FCU/VVS samples. If rectal, pharyngeal, FCU/VVS, samples were pooled into one NAAT container and analysed together, laboratory testing costs for triple-site samples would cost the same as urogenital samples alone. However, NAAT sensitivity and

specificity may be affected by the pooling process. Six studies in MSM have compared pooled triple-site samples with triple-site samples analysed individually to detect NG and CT.[12-17] All identified reduced sensitivity of NG and CT in pooled specimens and no optimum pooling technique was reported. No studies have compared pooled specimens in females, yet their prevalence of rectal chlamydia is as high as MSM.[11]

A pooling technique of self-taken, triple-site, pooled-samples that was as accurate as clinician-samples analysed individually, would enable testing of all potentially infected sites without increasing diagnostic costs. This complete testing would prevent the false reassurance of negative FCU/VVS where extragenital infections were missed.

We therefore performed a study comparing clinician-taken rectal and pharyngeal swabs, plus FCU/VVS, analysed individually versus self-taken rectal, pharyngeal, and FCU/VVS samples pooled together for the diagnosis of NG and CT in MSM and females, and their cost-effectiveness.

Methods

The full methods are described elsewhere. [Wilson JD, Wallace HE, Loftus-Keeling M, et al. Swab-yourself trial with economic monitoring and testing for infections collectively (SYSTEMATIC): Part 1. A diagnostic accuracy, and cost-effectiveness, study comparing clinician-taken versus self-taken rectal and pharyngeal samples for the diagnosis of gonorrhoea and chlamydia. Accepted by Clin Infect Dis.] Briefly, females and MSM, 16 years and over presenting to Leeds Sexual Health between January 2015 and September 2016 to be tested for NG and CT, and willing to perform self-taken swabs in addition to standard clinician performed swabs, were invited to participate. Exclusion criteria were antibiotics in the preceding 28 days, and/or rectal symptoms. Written consent was obtained with inclusion allowed only once. Details of age, sex, and urogenital symptoms suggestive of a bacterial

STI (vaginal discharge, intermenstrual/post-coital bleeding, deep dyspareunia, lower abdominal pain in females; urethral discharge, dysuria, testicular pain in MSM), were collected.

Participants had three sets of samples: 1. Clinician-taken rectal and pharyngeal swabs analysed individually (Clinician); 2. Self-taken rectal, pharyngeal and VVS/FCU analysed individually (Self); 3. Self-taken rectal, pharyngeal and VVS/FCU analysed as pooled specimen (Pooled). This paper reports the results of clinician-rectal and pharyngeal swabs, with VVS/FCU, analysed individually versus pooled self-taken rectal and pharyngeal swabs, plus VVS/FCU. Results of clinician compared with self-taken extragenital samples analysed individually are reported elsewhere. [Wilson JD, Wallace HE, Loftus-Keeling M, et al. Swab-yourself trial with economic monitoring and testing for infections collectively (SYSTEMATIC): Part 1. A diagnostic accuracy, and cost-effectiveness, study comparing clinician-taken versus self-taken rectal and pharyngeal samples for the diagnosis of gonorrhoea and chlamydia. Accepted by Clin Infect Dis.]

Instructions were given on swab-taking and insertion into the NAAT specimen containers. MSM produced one FCU sample in a 20ml universal container; 2ml were pipetted into a NAAT transport medium container for individual analysis and 2ml were pipetted into the pooled specimen container. Sampling order was randomised before the study with separate randomisation for females and MSM. Laboratory staff were blinded to which were clinician and self-taken extragenital samples and the participant origin of the pooled specimens.

Sample pooling

With Aptima assays (Hologic, San Diego, USA), only one swab shaft can remain in NAAT transport medium containers to avoid interference with probe movement. For female pooled specimens, the pharyngeal swab was inserted into a VVS transport medium container, agitated for 5 seconds, squeezed against the container side during removal to extract as

much material as possible and discarded. Cell lysis is almost instantaneous when swabs are inserted into NAAT transport medium so 5 seconds agitation was deemed adequate. A rectal swab was inserted into the same container, agitated, squeezed, removed and discarded. Lastly, a VVS was inserted into the same container and the stem broken off leaving the swab inside. Without inhibition from the other samples, no reduction in VVS sensitivity was expected, but with additional detection of rectal and pharyngeal infections. For MSM pooled specimens, the pharyngeal swab was processed as above in a NAAT transport medium container for urine. A rectal swab was inserted into the same container and the stem broken off leaving the swab inside. Lastly, 2ml FCU were pipetted into the same container so the volume of fluid was between the recommended fill lines. Without inhibition/dilution from the other samples, no reduction in rectal sample sensitivity was expected, but with additional detection of urethral and pharyngeal infections.

Microbiological analysis

The samples were tested for NG and CT using Aptima Combo 2 (AC2). To ensure high specificity, equivocal or positive tests were tested further using Aptima-GC or Aptima-CT for confirmation. Positive CT samples of MSM were also tested for lymphogranuloma venereum (LGV) specific DNA using an in-house PCR. Some participants had clinician-taken cultures for NG from urogenital and extragenital sites if clinically indicated.

The patient infected status (PIS) for gonorrhoea or chlamydia was defined as at least two confirmed positive NG or CT NAAT samples (one could be pooled specimen). The site-infected status (SIS) was at least two positive site samples, or one positive site sample and positive pooled specimen. A positive NG culture from any site conferred a positive NG PIS and SIS for the sites positive. The pooled infected status was defined as pooled sample positive and at least one other positive sample.

Clinician samples were assumed to have sensitivities of 99%. Sensitivity of 80% or less for pooled specimens would be rejected as too low. Fifty NG and CT positive samples would detect a significant difference between sensitivities of 80% and 99% with 80% power and 95% probability. The sensitivity and specificity of clinician-taken rectal and pharyngeal swabs plus FCU/VVS analysed individually, and the pooled specimen of self-taken rectal and pharyngeal swabs with FCU/VVS, were determined using the PIS and SIS and sub-classified for sex and urogenital symptoms. Any differences between sensitivities in those infected, and specificities in those without infection, were determined using McNemar's test.[18]

Health Economics analysis

Within-study cost-effectiveness analyses following the National Institute of Health and Care Excellence recommendations were undertaken from the perspective of the National Health Service (NHS).[19] Cost and outcome data were combined to produce a deterministic and probabilistic incremental cost-effectiveness ratio (ICER) based on the correct test result from clinician, self-taken and pooled samples. The analyses assumed the estimated cost of performing the triple tests analysed individually was £60 and a willingness to pay would not exceed this threshold.

The grade of clinician, any urogenital symptoms, time taken to perform clinician swabs, and the participant's first set of swabs, were recorded. NHS unit costs were used.[20] Diagnostic kit and processing costs were obtained from the Department of Microbiology. Swab collection costs were calculated for females and MSM. The analyses used a price year of 2016.

Results

A STARD diagram of participant recruitment is shown in Figure 1. In total, 1793 participants (1284 females, 509 MSM) were recruited by 5 clinicians.

In females, the mean age was 25 years (range 16-71; median 23 years) and 489 (38.1%) had urogenital symptoms in keeping with a bacterial STI. In MSM, the mean age was 33 years (range 18-77; median 29 years) and 66 (13.0%) had urogenital symptoms in keeping with a bacterial STI.

***Neisseria* gonorrhoeae and *Chlamydia trachomatis* results**

The numbers fulfilling the PIS for NG were 116 (9%); 64 (5%) females and 52 (10.2%) MSM. The numbers fulfilling the PIS for CT were 276 (15.4%); 237 (18.5%) females and 39 (7.7%) MSM.

Three NG pooled (two PIS positive) and three CT pooled (available samples supporting negative PIS) were missing. Four NG pooled (two categorised true positives, two as false positives) and five CT pooled samples (four categorised true positives, one as false positive) were indeterminate (see supplementary figures s1 and s2). Details of the other missing and indeterminate results are described elsewhere, [Wilson JD, Wallace HE, Loftus-Keeling M, et al. Swab-yourself trial with economic monitoring and testing for infections collectively (SYSTEMATIC): Part 1. A diagnostic accuracy, and cost-effectiveness, study comparing clinician-taken versus self-taken rectal and pharyngeal samples for the diagnosis of gonorrhoea and chlamydia. Accepted by Clin Infect Dis.] and in Supplementary Figures 3 and 4.

Figure 2 shows sensitivities, and Table 1 specificities, positive predictive values (PPV) and negative predictive values (NPV) of clinician-taken rectal, pharyngeal, with VVS/FCU samples, analysed individually; self-taken rectal, pharyngeal, plus VVS/FCU samples,

analysed individually; VVS/FCU samples only, and self-taken pooled specimens, for the detection of NG and CT as defined by PIS. Clinician-samples, and self-taken samples analysed individually identified over 99% of NG and CT infections, and pooled specimens over 98% of NG with no significant difference in all participants, females and MSM. However, CT pooled specimen sensitivity of 96% was significantly lower than clinician-taken samples of 99%, which identified nine more infected people. Pooled specimens missed 13 infected people. In 9 females these were one NG urogenital; and for CT, 3 urogenital+rectal, one rectal+pharyngeal, one pharyngeal and 3 rectal infections. In 4 MSM these were one NG rectal; and for CT, one pharyngeal and 2 rectal infections.

Of the 39 MSM with chlamydia, LGV results were unavailable in 4/39. Two of 35 (0.4% of total CT positive and LGV tested) MSM had LGV; one positive on rectal and pooled specimen; one positive on rectal and equivocal on pooled specimen.

The sensitivities of self-taken swabs analysed individually (98.28%) and self-taken pooled swabs (98.25%) for gonorrhoea were almost identical indicating that pooling did not introduce inhibition or dilution. However, self-taken swabs analysed individually identified ten additional people with chlamydia than pooled specimens, giving a significant reduction in sensitivity from 99.6% to 96.0%, The reduction in pooled sensitivity was greater in MSM (92.3%) than females (96.6%), suggesting urine did cause dilution leading to false-negative results, but due to small numbers of chlamydia in MSM, was not significant (p= 0.19; Fisher's exact test).

However, if analysing only one NAAT sample, pooled specimens were significantly better at identifying people with gonorrhoea and chlamydia than VVS/FCU samples. The VVS missed seven NG (sensitivity 88.7%) and 31 CT infections (sensitivity 86.9%) and FCU missed 35 NG (sensitivity 33.3%) and 27 CT infections (sensitivity 29.7%).

There were more false positive results in self-taken than clinician-taken samples indicating that nucleic acid contamination can occur when people take their own swabs.[21,22] However, there were fewer false positive results with pooled specimens, than self-taken swabs analysed individually, suggesting our pooling process itself did not increase the risk of contamination.

At least one urogenital symptom was reported by 555 (31%) of the participants; 53 (9.5%) and 94 (16.9%) were infected with NG and CT respectively. Table 2 shows the sensitivities, specificities, PPV and NPV of clinician-samples analysed individually, and pooled specimens, for the detection of NG and CT in those with, and without, urogenital symptoms. There were no differences between sensitivities of clinician-taken or self-taken samples analysed individually versus pooled specimens in NG and CT detection in those with and without symptoms. There were also no differences in pooled sensitivities for NG detection (OR 1.12, [95%CI 0.07-19.60], p=1.0), or for CT (OR 0.72 [95%CI 0.19-2.77], p=0.75) between those with and without symptoms.

Health Economics analysis

Clinicians performed the swabs in an average of 3 minutes irrespective of grade, whereas participants required an average of 4 minutes. Females took longer than MSM (4.2 versus 3.7 minutes) but clinicians performed the swabs quicker in females (2.9 versus 3.1). Symptomatic participants took longer to take their swabs than those asymptomatic (4.3 versus 3.9) and clinicians took longer to perform swabs on those with symptoms than those without (3.7 versus 2.7). Estimated costs of self-taken pooled samples and clinician-taken rectal, pharyngeal and FCU/VVS analysed individually are shown in Table 3. Results of the cost-effectiveness analysis are shown in Table 4. Pooled specimens were the most cost-effective method in every scenario, mainly due to savings of £16 on NAAT diagnostic costs. The results were robust as the probability of cost-effectiveness was high. Greater than 25% reduction of pooled specimen effectiveness would be needed for clinician-samples to have

an ICER of £60. Clinician samples for symptomatic participants were the most expensive as this included time for symptom assessment.

Discussion

This trial of clinician-taken samples analysed individually versus pooled self-taken rectal, pharyngeal, and urogenital samples in females and MSM demonstrated good concordance for the detection of gonorrhoea. The pooled sensitivity for chlamydia at 96% was significantly lower than clinician-taken samples analysed individually with sensitivity >99%. Despite this reduced chlamydia sensitivity, all sensitivities were over the recommended 90% for NAATs.[23] If budgets allow only one NAAT to be analysed, pooled specimens were significantly better at identifying people with gonorrhoea and chlamydia than a VVS/FCU.

Six studies have compared pooled triple-site samples with individually analysed samples.[12-17] The details are shown in table 5. Pooled sensitivities were all reduced to approximately 90%. All confirmed positive NAATs were considered true positives. This is likely to overestimate some pooled sensitivities if, as in our study, there were more false positive self-taken than clinician-taken swabs (we required at least one other confirmed positive sample for the pooled specimen to be categorized true positive). Three studies pooled the triple-site specimens in the laboratory.[14,15,17] The others, as in this study, pooled the samples immediately after being taken using different techniques; Sultan used two techniques within one study.[12]

Our pooled NG and CT sensitivities at 98% and 96% were high compared with these studies. Two also used AC2[12,16] but others used different NAATs, and sensitivities vary between NAATs[24]. AC2 has high sensitivity for CT and NG which might explain the differences. However, Sultan and Durukan[12,16] reported NG sensitivities of 90% and 91% compared with our 98% and CT sensitivities of 92% and 86% compared with our 96%. We

believe our pooling method and retention of VVS (female) and rectal swab (MSM) within the transport medium increased sensitivity. Also, previous authors suggest reduced pooled sensitivity may be from urine volume dilution on single site infections with low bacterial loads.[13,14,16] Our results support this suggestion, as pooled sensitivities were higher in females (no urine added) than MSM. All our MSM false negative pooled specimens had single site infections. These were possibly low bacterial-load infections that dropped below the level of detection as urine diluted the pooled samples. We used 2ml FCU in MSM pooling whereas some studies used more. To improve sensitivity, future MSM pooling studies should use the minimal volume of FCU or another urethral sample, such as meatal swabs, to avoid this dilution.[25]

In every scenario, pooled specimens were much more cost-effective than samples analysed individually. Pooling could be utilised for self-taken samples in asymptomatic testing, and clinician-taken samples when assessing those with urogenital symptoms, as there was no difference in sensitivities between pooled specimens in those with and without symptoms.

To optimise specificity, our self-swabbing written instructions included details about reducing environmental contamination. [Wilson JD, Wallace HE, Loftus-Keeling M, et al. Swab-yourself trial with economic monitoring and testing for infections collectively (SYSTEMATIC): Part 1. A diagnostic accuracy, and cost-effectiveness, study comparing clinician-taken versus self-taken rectal and pharyngeal samples for the diagnosis of gonorrhoea and chlamydia. Accepted by Clin Infect Dis.] Even with these there were more false positive self-taken than clinician swabs, indicating that nucleic acid contamination occurs when people take their own swabs. Reassuringly, pooling itself did not increase nucleic acid contamination. In fact, the dilution of pooling may reduce low levels of environmental contamination to below the limits of detection. In the end, all specificities, of all samples, were over 99%.

Limitations of our study are that it was single-centred and assessed only one NAAT. Our results cannot necessarily be extrapolated to other NAATs as sensitivities and specificities vary between them, and some are affected by inhibitors.[24] The planned allocation of female to MSM, to achieve 50 cases of each infection at each site, gave only 39 CT infections in MSM which underpowered the MSM CT sub-analyses.

A limitation of a positive pooled specimen is that it does not identify the infected site. This only matters if different treatments are recommended for different infected sites, because pooled specimens can be used for tests of cure. The same first-line gonorrhoea treatment is recommended for all infected sites.[3,5] Azithromycin 1g single dose has been used for urogenital chlamydia but its estimated efficacy (82.9%) is lower than doxycycline (99.6%) for rectal chlamydia so its use has been questioned.[26,27] Consequently, many guidelines no longer recommend single dose azithromycin for chlamydia treatment.[28] Unless single dose azithromycin is being used, it is not necessary to know the sites of chlamydia infection for most people, as doxycycline is effective at all sites.[28] However, MSM with rectal symptoms, and without symptoms if living with HIV, should be LGV tested if CT positive.[29] We found only two LGV infections (but MSM with rectal symptoms had been excluded from the study); one pooled specimen was positive, the other equivocal. These numbers are clearly too small to draw any conclusions on the sensitivity of pooled specimens to detect LGV. This should be assessed in future studies.

In summary, if affordable, triple-site samples analysed individually were most sensitive for diagnosing gonorrhoea and chlamydia. There was no significant difference in diagnostic accuracy between triple-site samples analysed individually, and those pooled, for the diagnosis of gonorrhea but pooling samples reduced chlamydia sensitivity by 3% in females and 5% in MSM. However, gonorrhoea and chlamydia detection was higher in pooled NAATs than VVS/FCU NAATs, for the same diagnostic cost. The small reduction in pooled chlamydia sensitivity was more than offset by the large reduction in cost of triple NAATs,

meaning that pooled specimens were the most cost-effective way of diagnosing all gonorrhoea and chlamydia infections.

These findings have important implications for policy makers and providers of STI services. Even in high-income countries, publicly funded health systems struggle to fund triple NAATs in MSM, let alone in females, even though evidence of their benefit in identifying additional infections is undisputed. However, pooling enables triple-site testing, for the same diagnostic cost as urogenital testing, with a large increase in detection of gonorrhoea and chlamydia infections.

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Conflicts of Interest

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Table 1. Sensitivities, specificities, PPV, and NPV of clinician-taken rectal, pharyngeal, plus VVS/FCU samples, analysed individually; self-taken rectal, pharyngeal plus VVS/FCU samples, analysed individually; VVS/FCU samples only, and self-taken pooled samples, for the detection of gonorrhoea and chlamydia in all participants, females, and MSM.

***Neisseria gonorrhoeae* All Participants 116/1793 (6.5%)**						***Neisseria gonorrhoeae* All Females 64/1284 (5%)**						***Neisseria gonorrhoeae* All MSM 52/509 (10.2%)**					
Total =1793	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**P value**	Total =1284	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**P value**	Total =509	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**P value**
	True positive	False positive	True negative	False negative			True positive	False positive	True negative	False negative			True positive	False positive	True negative	False negative	
Accuracy against PIS						**Accuracy against PIS**						**Accuracy against PIS**					
Clinician swabs plus VVS/FPU N = 116/1793	**99.1** (95.3-100.0)	**99.9** (99.6-100.0)	**98.3** (93.5-99.6)	**99.9** (99.6-100.0)	Sens* 1.00	**Clinician swabs plus VVS** N = 64/1284	**100.0** (94.4-100.0)	**100.0** (99.7-100.0)	**100.0**	**100.0**	Sens* 1.00	**Clinician swabs plus FPU** N = 52/509	**98.1** (89.7-100.0)	**99.6** (98.4-100.0)	**96.2** (86.5-99.0)	**99.8** (98.5-100.0)	Sens* 0.48
	115	2	1675	1	Spec* 1.00		64	0	1220	0	Spec* 1.00		51	2	455	1	Spec* 0.48
Self-taken triple swabs N = 116/1793	**98.3** (93.9-99.8)	**99.5** (99.0-99.8)	**92.7** (86.8-96.1)	**99.9** (99.5-100.0)	Sens* 0.62	**Self-taken triple swabs** N = 64/1284	**98.4** (91.6-100.0)	**99.6** (99.1-99.9)	**92.7** (84.0-96.8)	**99.9** (99.4-100.0)	Sens* 0.48	**Self-taken triple swabs** N = 52/509	**98.1** (89.7-100.0)	**99.1** (97.8-99.8)	**92.7** (82.8-97.1)	**99.8** (98.5-100.0)	Sens* 0.48
	114	9	1668	2	Spec* 0.11		63	5	1215	1	Spec* 0.22		51	4	453	1	Spec* 0.62
VVS/FCU N = 113/1788 (5 missing; 3 PIS pos)	**63.7** (54.1-72.6)	**100.00** (99.8-100.00)	**100.00**	**97.6** (97.0-98.1)	**Sens* <0.001**	**VVS** N = 62/1282 (2 missing; 2 PIS pos)	**88.7** (78.1-95.3)	**100.0** (99.7-100.0)	**100.0**	**99.5** (98.9-99.8)	Sens* 0.13	**FCU** N = 51/506 (3 missing; 1 PIS pos)	**33.3** (20.8-47.9)	**100.0** (99.2-100.0)	**100.0**	**93.1** (91.7-94.2)	**Sens* <0.001**
	72	0	1675	41	Spec* 0.25		55	0	1220	7	Spec* 1.00		17	0	455	34	Spec* 0.48
Pooled N = 114/1790 (3 missing; 2 PIS pos)	**98.3** (93.8-99.8)	**99.8** (99.5-100.0)	**97.4** (92.3-99.1)	**99.9** (99.5-100.0)	N/A	**Pooled** N = 62/1281 (3 missing; 2 PIS pos)	**98.4** (91.3-100.0)	**99.9** (99.5-100.0)	**98.4** (89.6-99.8)	**99.9** (99.4-100.0)	N/A	**Pooled** N = 52/509	**98.1** (89.7-100.0)	**99.6** (98.4-100.0)	**96.2** (86.5-99.0)	**99.8** (98.5-100.0)	N/A
	112	3	1673	2			61	1	1218	1			51	2	455	1	
***Chlamydia trachomatis* All Participants 276/1793 (15.4%)**						***Chlamydia trachomatis* All Females 237/1284 (18.5%)**						***Chlamydia trachomatis* All MSM 39/509 (7.7%)**					
Total =1793	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**P value**	Total =1284	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**P value**	Total =509	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**P value**
	True positive	False positive	True negative	False negative			True positive	False positive	True negative	False negative			True positive	False positive	True negative	False negative	
Accuracy against PIS						**Accuracy against PIS**						**Accuracy against PIS**					
Clinician swabs plus VVS N=276/1793	**99.3** (97.4-99.9)	**99.7** (99.3-99.9)	**98.6** (96.6-99.5)	**99.9** (99.5-100.0)	**Sens* *0.027**	**Clinician swabs plus VVS** N=237/1284	**99.6** (97.7-100.0)	**99.7** (99.2-99.9)	**98.7** (96.2-99.6)	**99.9** (99.3-100.0)	**Sens* 0.046**	**Clinician swabs plus FCU** N = 39/509	**97.4** (86.5-99.9)	**99.8** (98.8-100.0)	**97.4** (84.3-99.6)	**99.80** (98.6-100.0)	Sens*0 .62
	274	4	1513	2	Spec* 0.55		236	3	1044	1	Spec 0.50		38	1	469	1	Spec 0.48
Self-taken triple swabs N=276/1793	**99.6** (98.0-100.0)	**99.5** (99.1-99.8)	**97.5** (94.9-98.8)	**99.9** (99.5-100.0)	**Sens* *0.009**	**Self-taken triple swabs** N=237/1284	**99.6** (97.7-100.0)	**99.4** (98.8-99.8)	**97.5** (94.7-98.9)	**99.9** (99.3-100.0)	**Sens* 0.046**	**Self-taken triple swabs** N = 39/509	**100.0** (91.0-100.0)	**99.8** (98.8-100.0)	**97.5** (84.6-99.6)	**100.0**	Sens*0 .25
	275	7	1510	1	Spec* 0.79		236	6	1041	1	Spec 0.77		39	1	469	0	Spec 0.48
VVS/FCU N = 274/1788 (5 missing; 2 PIS pos)	**79.2** (73.9-83.9)	**99.9** (99.6-100.0)	**99.5** (96.8-99.9)	**96.4** (95.5-97.1)	**Spec* <0.001**	**VVS** N = 237/1282 (2 missing)	**86.9** (82.0-90.9)	**99.9** (99.5-100.0)	**99.5** (96.7-99.9)	**97.1** (96.0-97.9)	**Sens* <0.001**	**FCU** N = 37/506 (3 missing; 2 PIS pos)	**29.7** (15.9-47.0)	**100.0** (99.2-100.0)	**100.0**	**94.8** (93.6- 95.7)	**Sens* <0.001**
	217	1	1513	57	Spec 0.08		206	1	1044	31	Spec 0.13		11	0	469	26	Spec* 1.00
Pooled N = 276/1790 (3 missing)	**96.0** (93.0-98.0)	**99.5** (99.1-99.8)	**97.4** (94.8-98.8)	**99.3** (98.7-99.6)	N/A	**Pooled** N = 237/1281 (3 missing)	**96.6** (93.5-98.5)	**99.4** (98.8-99.8)	**97.5** (94.5-98.8)	**99.2** (98.5-99.6)	N/A	**Pooled** N = 39/509	**92.3** (79.1-98.4)	**99.80** (98.8-100.0)	**97.3** (83.5-99.6)	**99.4** (98.1-99.8)	N/A
	265	7	1507	11			229	6	1038	8			36	1	469	3	

Missing samples are from negative PIS unless stated from positive PIS

Sens = Sensitivity; Spec = Specificity

[a]P values compared with pooled using two-tailed McNemar's test

Table 2. Sensitivities, specificities, PPV, NPV, of the clinician-taken samples analysed individually and the self-taken pooled specimens for the detection of gonorrhoea and chlamydia in those with and without urogenital symptoms

Gonorrhoea Symptomatic						Gonorrhoea Asymptomatic					
Total = 53/555	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	[b]Two-tailed McNemar	Total = 63/1238	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	[b]Two-tailed McNemar
	True positive	False positive	True negative	False negative			True positive	False positive	True negative	False negative	
Clinician swabs plus VVS/FPU N = 53/555	**100.0** (93.3-100.0)	**100.0** (99.3-100.0)	**100.0**	**100.0**		**Clinician swabs plus VVS/FPU** N = 63/1238	**98.4** (91.5-100.0)	**99.8** (99.4-100.0)	**96.9** (88.6-99.2)	**99.9** (99.4-100.0)	
	53	0	502	0			62	2	1173	1	
Pooled N = 52/554 ([a]1 missing; 1 PIS pos)	**98.1** (89.7-100.0)	**100.0** (99.3-100.0)	**100.0**	**99.8** (98.6-100.0)	P = 1.0	**Pooled** N = 62/1236 ([a]2 missing; 1 PIS pos)	**98.4** (91.3-100.0)	**99.7** (99.3-100.0)	**95.3** (86.8-98.4)	**99.9** (99.4-100.0)	P = 0.48
	51	0	501	1			61	3	1172	1	
Chlamydia Symptomatic						**Chlamydia Asymptomatic**					
Total = 94/555	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	[b]Two-tailed McNemar	Total =182/1238	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	[b]Two-tailed McNemar
	True positive	False positive	True negative	False negative			True positive	False positive	True negative	False negative	
Clinician swabs plus VVS/FPU N = 94/555	**100.0** (96.2-100.0)	**100.0** (99.2-100.0)	**100.0**	**100.0**		**Clinician swabs plus VVS/FPU** N = 182/1238	**98.9** (96.1-99.9)	**99.6** (99.0-99.9)	**97.8** (94.4-99.2)	**99.8** (99.3-100.0)	
	94	0	461	0			180	4	1052	2	
Pooled N = 94/554 ([a]2 missing; 0 PIS pos)	**97.9** (92.5-99.7)	**99.4** (98.1-99.9)	**96.8** (90.9-99.0)	**99.6** (98.3-99.9)	P = 0.48	**Pooled** N = 182/1237 ([a]1 missing; 0 PIS pos)	**95.1** (90.8-97.7)	**99.6** (99.0-99.9)	**97.7** (94.2-99.1)	**99.2** (98.4-99.6)	P = 0.07
	91	3	456	3			174	4	1051	8	

[a]Missing samples are from negative PIS unless stated from positive PIS;
[b]P value comparing clinician swabs plus VVS/FPU with pooled using two-tailed McNemar's test

Table 3. Estimated costs of self-taken pooled samples and clinician-taken rectal, pharyngeal and FCU/VVS analysed individually

Population	Group	Cost of clinician time	Cost of test kit[b]	Processing cost of one pooled NAAT[c]	Total cost of tests
Self-taken pooled samples					
All	**MSM**	£22.25	£5.16	£8	£35.41
	Women	£25.87			£39.03
Asymptomatic	**MSM**	£18.71	£5.16	£8	£31.87
	Women	£17.17			£30.33
Symptomatic	**MSM**	£34.10	£5.16	£8	£47.26
	Women	£37.05			£50.21
Asymptomatic *Band 3 HCA*[a]	**MSM**	£12.53	£5.16	£8	£25.69
	Women	£9.76			£22.92

Population	Group	Cost of clinician time	Cost of test kit[b]	Processing cost of three NAAT samples analysed individually[c]	Total cost of tests
Clinician-taken samples analysed individually					
All	**MSM**	£21.45	£5.16	£24	£50.61
	Women	£25.35			£54.51
Asymptomatic	**MSM**	£17.05	£5.16	£24	£46.20
	Women	£14.54			£43.70
Symptomatic	**MSM**	£36.15	£5.16	£24	£65.31
	Women	£39.28			£68.43
Asymptomatic *Band 3 HCA not applicable*					

[a]Assumes self-taken samples overseen by Band 3 Health Care Assistants (Nursing Assistants)
[b]£1.72 per test kit, three test kits are still needed for the swabs for the pooled processed tests
[c]£8.00 to processing each NAAT

Table 4. The cost-effectiveness analysis of the clinician-taken swabs individually analysed versus the self-taken pooled swabs for the diagnosis of gonorrhoea and chlamydia in those with and without urogenital symptoms.

Gonorrhoea	Sex	Test	Costs	Effectiveness	Incremental cost	Incremental effectiveness	[a]ICER	Net monetary benefit (£60 threshold)	[b]Probability of CE at a WTP of £60
Symptomatic	MSM	**Pooled**	**£4,483**	**94.00**				**£1,157**	**1.0**
		Individually	£6,002	94.43	£1,519	0.43	£3,515	-£336	0.0
	Women	**Pooled**	**£27,545**	**545.97**				**£5,213**	**1.0**
		Individually	£36,304	536.99	£8,759	-8.98	-£974	-£4,085	0.0
Asymptomatic	MSM	**Pooled**	**£13,178**	**411.96**				**£11,539**	**1.0**
		Individually	£19,802	408.95	£6,623	-3.00	-£2,201	£4,735	0.0
	Women	**Pooled**	**£22,321**	**734.83**				**£21,769**	**1.0**
		Individually	£34,091	733.95	£11,770	-0.88	-£13,352	£9,945	0.0
Chlamydia	**Sex**	**Test**	**Costs**	**Effectiveness**	**Incremental cost**	**Incremental effectiveness**	**[a]ICER**	**Net monetary benefit (£60 threshold)**	**[b]Probability of CE at a WTP of £60[b]**
Symptomatic	MSM	**Pooled**	**£18,024.96**	**504.98**				**£12,274**	**1.0**
		Individually	£25,754.59	507.98	£7,729.63	3.00	£2,574.53	£4,724	0.0
	Women	**Pooled**	**£50,118.00**	**1266.87**				**£25,894**	**1.0**
		Individually	£70,655.31	1262.95	£20,537.32	-3.93	-£5,227.21	£5,121	0.0
Asymptomatic	MSM	**Pooled**	**£13,178.91**	**411.09**				**£11,486**	**1.0**
		Individually	£19,802.37	413.17	£6,623.46	2.08	£3,182.48	£4,988	0.0
	Women	**Pooled**	**£22,321.42**	**724.94**				**£21,175**	**1.0**
		Individually	£34,091.83	722.95	£11,770.40	-1.99	-£5,912.90	£9,285	0.0

[a]ICER = incremental cost-effectiveness ratio;
[b]Probability of CE at a WTP of £60 = Probability of cost-effectiveness at a willingness to pay of £60
Negative ICER represents the alternative is dominated as it is costlier and less effective
Rows in bold represent the cost-effective strategy at a £60 per correct test detected assumed. Cost-effective strategies have a higher net monetary benefit

Table 5. Details of published studies comparing triple-site swabs taken for pooled samples with swabs for individually analysed samples, plus results in MSM and females from this study.

Author and reference	Sultan[12]	Thielemans[13]	Speers[14]	De Baetselier[15]	Durukan[16]	De Baetselier[17]	Wilson	Wilson
Participants	MSM only	MSM only	MSM only	MSM only	MSM only	MSM only	MSM	Women
Total participants	1064	100	107	98 giving 117 sample sets	162 all with recent positive individual site sample	497	509	1284
Swab order	Randomised	Individual first	Together, dual-headed swabs	Samples for pooling randomly chosen by laboratory following collection	Alternated with recruitment order	Samples for pooling randomly chosen by laboratory following collection	Randomised	Randomised
Individual swab taker	Pharyngeal clinician Rectal clinician	Pharyngeal clinician Rectal participant	Pharyngeal clinician Rectal participant	Pharyngeal clinician Rectal participant	Pharyngeal clinician Rectal clinician	Pharyngeal clinician Rectal clinician	Pharyngeal clinician Rectal clinician	Pharyngeal clinician Rectal clinician
Pooled swab taker	Pharyngeal participant Rectal participant	Pharyngeal clinician Rectal participant	Pharyngeal clinician Rectal participant	Pharyngeal clinician Rectal participant	Pharyngeal clinician Rectal clinician	Pharyngeal clinician Rectal clinician	Pharyngeal participant Rectal participant	Pharyngeal participant Rectal participant
Timing of pooling	As soon as swabs taken	As soon as swabs taken	On reaching laboratory	On reaching laboratory	As soon as swabs taken	On reaching laboratory	As soon as swabs taken	As soon as swabs taken
Swab left in pooled specimen	None	Both	Both but pooled in laboratory	Both but pooled in laboratory	Pharyngeal	Both but pooled in laboratory	Rectal in MSM	VVS in women
Volume of urine in pooled sample	Method A 1->20ml Method B 2ml	3ml	7ml	1.7ml	2ml	4µL	2ml	None
NAAT used for pooled samples	Hologic AC2	Abbott Real Time CT/GC test	Cepheid GeneXpert	Abbott Real Time CT/GC test	Hologic AC2	Cepheid GeneXpert	Hologic AC2	Hologic AC2
NAAT used for individual samples	Hologic AC2	Abbott Real Time CT/GC test	Roche cobas 4800	Abbott Real Time CT/GC test	Hologic AC2	Abbott Real Time CT/GC test	Hologic AC2	Hologic AC2
Number (prevalence) with NG	292 (27%)	12 (12%)	34 (32%)	8 (7%)	80 (49%)	57 (11.5%)	52 (10%)	64 (5%)
Number (prevalence) with CT	168 (16%)	10 (10%)	20 (19%)	11 (9%)	109 (67%)	72 (14.5%)	39 (8%)	276 (19%)
Sensitivity (95% CI) pooled NG	**89.9% (85.8-93.1)**	**89.5% (68.6-97.1)[a]**	**100% (89.7-100.0)**	**100% (63.1-100.0)**	**91.3% (83.0-95.1)**	**88.9% (77.4-95.8)**	**98.1% (89.7-100)**	**98.4% (91.3-100)**
Sensitivity (95% CI) pooled CT	**91.9% (86.5- 95.6)**	**89.5% (68.6-97.1)[a]**	**77.8% (52.4-93.6)**	**81.8% (48.2-97.7)**	**86.2% (78.5-91.5)**	**95.4% (87.1-99.0)**	**92.3% (79.1-98.4)**	**96.6% (93.5-98.5)**

[a]NG and CT combined analysis due to small numbers but pooled in laboratory

Figure 1. STARD Diagram SYSTEMATIC

SYSTEMATIC Part 2 version 2

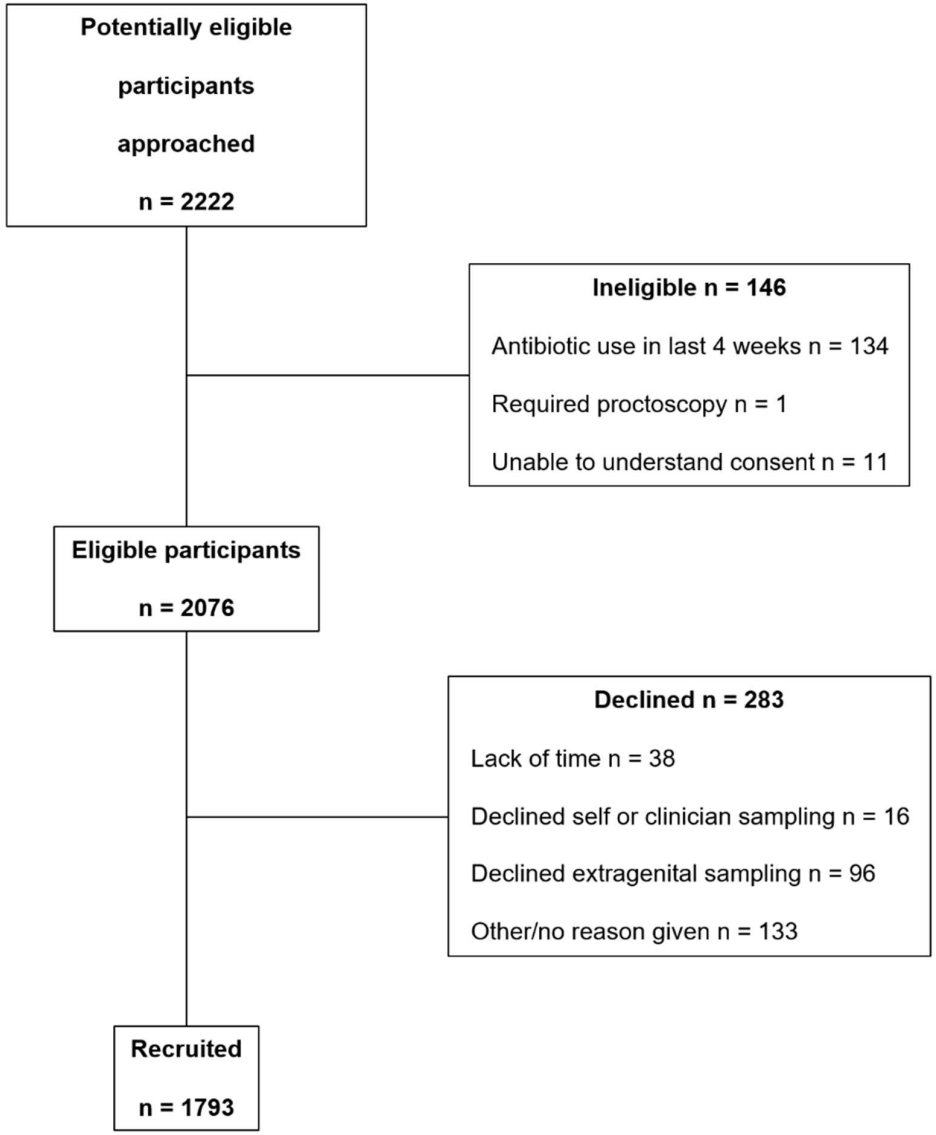
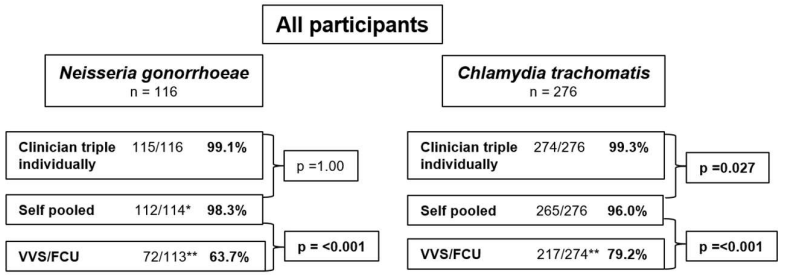
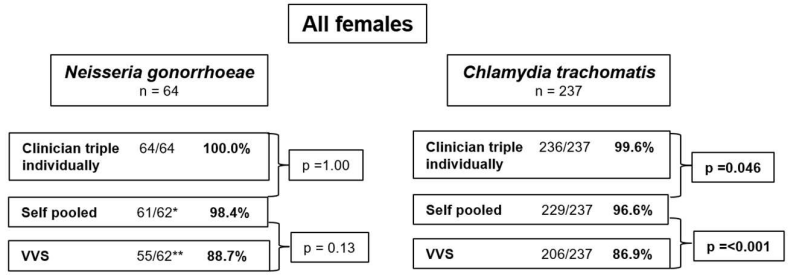
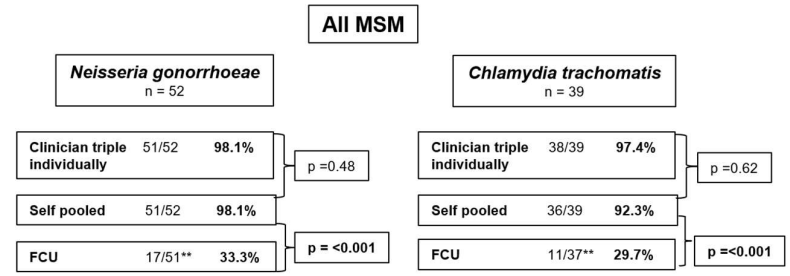


Figure 2. Sensitivities of clinician-taken rectal, pharyngeal, plus VVS/FCU samples, analysed individually; self-taken triple-site pooled samples; and single site VVS/FCU only; for the detection of gonorrhoea and chlamydia in all participants, females, and MSM.







*Two missing female pooled samples where infected status was NG positive
**Two missing VVS and one FCU sample where infected status was NG positive
P values are compared with pooled samples using two-tailed McNemar's test