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Alarming poor performance in *Chlamydia trachomatis* point of care testing

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Key words

Point of care test, *Chlamydia trachomatis*, NAAT, sensitivity and specificity

Key messages

1. Point-of-care tests could potentially diagnose *Chlamydia trachomatis* genital infections rapidly enough to enable the immediate start of therapy.
2. The point-of-care tests evaluated in this study are not ready for widespread use, due to lack of sensitivity.
3. Our results underline the need for good quality assurance of POC tests, especially in view of Internet based trading possibilities.

Abstract (word count: 230)

Objectives Infection by *Chlamydia trachomatis* (CT) is the most prevalent sexually transmitted infection (STI) worldwide. The most frequently used diagnostic test for CT is a nucleic acid amplification test (NAAT), which is highly sensitive and specific. To further shorten time delay until diagnosis has been made, in order to prevent CT spread, the use of point of care (POC) tests

could be the way forward. Three POC tests, Handilab-C, Biorapid CHLAMYDIA Ag test and QuickVue Chlamydia test, were evaluated regarding diagnostic performance in comparison with NAAT.

Methods All women, above the age of 16 years old, consulting at an STI clinic between September 2007 and April 2008, were asked to participate. Women were asked to complete a short questionnaire and to collect 6 self-taken vaginal swabs (SVS). SVS 2 was used for NAAT and SVS 3 to 5 were randomized for the different POC tests. SVS 1 and 6 were used for determining quantitative CT load to validate the use of successive SVS. All POC tests were performed without knowledge of NAAT results. NAAT was used as the 'gold standard'.

Results 772 women were included. CT prevalence was 11% in our population. Sensitivities of the Biorapid CHLAMYDIA Ag test, QuickVue Chlamydia and Handilab-C test were 17%, 27% and 12% respectively.

Conclusions In our opinion, the evaluated POC tests, due to the very low sensitivities, are not ready for widespread use. Our results underline the need for good quality assurance of POC tests, especially in view of Internet possibilities.

Article (2980 words)

INTRODUCTION

Worldwide, *Chlamydia trachomatis* (CT) remains the most prevalent bacterial sexually transmitted infection (STI), with increased incidence in Europe over the past decade.¹ CT infection is, a major cause of reproductive morbidity^{2,3}, bacterial conjunctivitis in neonates⁴, and may facilitate human immunodeficiency virus (HIV) transmission.⁵ The use of Nucleic Acid Amplification Tests (NAAT) with self-taken vaginal swabs (SVS) or urine have made CT testing more sensitive, specific and acceptable.⁶ Nevertheless, case finding and case recognition is hampered by the limited willingness of patients at risk to undergo STI testing because of fear of pelvic examination and stigmatisation, and secondly due to the frequently asymptomatic nature of these infections.⁷ Moreover, with the use of NAAT, there is still a time delay between first consultation and treatment, usually around one to two weeks.⁸ Although some infections may resolve during this period, secondary transmission can take place and infection can progress. Therefore, a point of care (POC) test with proven diagnostic accuracy may well help limit the spread of and morbidity associated with CT.

Over the past few years, an increase in availability of POC tests in drug stores and on the Internet has been noticeable. In general, there appears to be a trend of producing diagnostics, which are faster and easier to use. The World Health Organization has formulated criteria by which a POC test should suffice⁹: a new STI diagnostic test should be affordable by those at risk, sensitive (sensitivity between 43-65%), specific (specificity of 98%), user-friendly, rapid and robust, equipment-free and deliverable to those in need (ASSURED criteria; www.who.int/std_diagnostics). We have selected three widely available POC CT diagnostic tests, which could meet these criteria and have not been evaluated thoroughly yet. We assessed laboratory performance and the potential acceptability, when used in optimal conditions compared with NAAT, to maximize POC test results before evaluation in non-laboratory and/or less developed settings. Moreover, the use of successive SVS was validated using a quantitative CT NAAT.

METHODS

Study setting, specimen collection and population

Women above the age of 16 applying for STI consultation between September 2007 and April 2008 were included in the study. The medical ethics committee of the Maastricht University Medical Centre approved of this study (MEC LLL06srs) and all participants signed a written consent form. At the STI clinic, each patient was asked to take 6 number-marked SVS in the order of number (SVS 1 to 6). Patients were shown how to introduce the vaginal swab by approximately 4 to 5 cm insertion and with 10-seconds vaginal rotation and rubbing time and how to position the swab into each capped tube. During the consultation, demographic and behavioural data were collected and, if indicated, samples were collected for other STI diagnostics. All data and SVS were anonymized and transported to the hospital while refrigerated. Patients who tested positive for CT were treated with a single dose of 1000 mg azithromycin. CT prevalence was expected to be 11% in this population with no loss to follow-up.⁶

Point of care tests

SVS 3 to 5 were used for the POC-tests. The three point-of-care tests that were validated were the Handilab-C (Zonda Incorporated, Dallas, USA), Biorapid CHLAMYDIA Ag test (Biokit, S.A., Barcelona, Spain) and QuickVue Chlamydia test (Quidel Corporation, San Diego, USA). All POC tests had a CE mark and were commercially available. In order to control for possible differences in CT load in successive taken SVS, the POC tests were randomized before distribution, into SVS groups (named A, B and C) with Handilab-C, Biorapid CHLAMYDIA Ag test and QuickVue Chlamydia tests being performed on SVS 3-4-5 in group A, SVS 4-5-3 in group B and SVS 5-3-4 in group C, respectively. The Handilab-C is an enzymatic test with a detection limit of 16 inclusion bodies/test (package insert). The Biorapid CHLAMYDIA Ag test and QuickVue Chlamydia test are antigen tests; the detection limit of the Biorapid CHLAMYDIA Ag test is 57-570 elementary bodies/test and the QuickVue Chlamydia should have a sensitivity of 81% when less than 100 inclusion forming units (IFU)/mL are present (package inserts). All POC tests were stored and performed under optimal conditions in the medical microbiology laboratory, after training provided by the suppliers, and according to the manufacturers' instructions. One exception was the use of an SVS instead of an endocervical specimen with the Biorapid CHLAMYDIA Ag test and QuickVue Chlamydia test. The POC-tests were performed in the medical microbiology laboratory, but the Handilab-C test was started at the STI clinic: 'fluid A' was allowed to mix with the specimen and left standing for 10 minutes. After transportation to the laboratory, the swab was pushed through the foil in order to make a short contact with 'fluid B'. This procedure was discussed and supported by the manufacturer. The Handilab-C cannot be used during menstruation and the second step of the

test performance must be completed within 24 hours (definition of an ‘on time’ result). Both the Biorapid CHLAMYDIA Ag test and QuickVue Chlamydia test had to be performed within 72 hours after collecting the SVS (definition of an ‘on time’ result). POC tests were performed and read by LvD and 3 fully qualified microbiological technicians. NAAT results and clinical data were linked to the POC test results no sooner than at the end of the study. Stratification by menstruation and time to test performance was therefore done retrospectively.

NAAT tests

The COBAS Amplicor CT/NG (Roche Diagnostics Systems, Basel, Switzerland) on SVS 2 was used as gold standard for determining CT presence. Although the COBAS Amplicor CT/NG is not licensed for SVS, previous studies have demonstrated no significant difference in performance between the use of SVS and that of endocervical swabs.^{10,11} SVS 2 was placed in 1 ml lysis buffer and after rotation for 10 seconds the swab was squeezed by pressing against the plastic tube and then removed. Next, 1 ml diluent was added, mixed, centrifuged and 50 µl of the supernatant was added to 50 µl PCR Mix. The sample was processed further according to the Standard Operating Procedure for CT PCR. A result of more than 10,000 DNA copies was considered positive. All low positive samples between 2000 and 9999 copies of CT DNA were retested to confirm the presence of CT. Samples with a repeatedly borderline (n=1) or inhibited (n=8) NAAT results were excluded from analysis.

For quantitative CT load determination, a Real Time PCR (TaqMan assay) targeting the cryptic plasmid of *C. trachomatis* (sensitivity of 0.01 IFU as compared to 1 IFU for the COBAS Amplicor and able to detect the recently reported Swedish variant of *C. trachomatis*) or the human HLA was developed with Primer Express v2.0 (Applied Biosystems, Foster City, CA, USA), described previously by Catsburg *et al.*¹² Real Time PCR reactions were performed in a volume of 30 µl PCR volume, consisting of TaqMan Mastermix (Applied Biosystems), 300 nM of each primer, 150 nM of each probe and 5 µl prepared sample. Amplification and detection was performed with an ABI Prism 7000 sequence detection system (Applied Biosystems) by standard PCR conditions of the manufacturer, with 45 cycles. By using a chlamydial and a human target, the average chlamydial/human cell load ratio, and IFU/swab were calculated. All samples were spiked with an optimal amount of internal control to validate the sample preparation as well as the RT-PCR procedure.

Statistical analysis

Sensitivity, specificity, negative (NPV) and positive (PPV) predictive values of the different POC tests compared to gold standard PCR were calculated. Categorical variables were analysed with Pearsons’ Chi-Square test for independence and with Fishers’ exact test where appropriate. Binary

logistic regression was used to determine the influence of different variables (including randomisation) on NAAT and POC tests outcome. A p value <0.1 was used for selecting variables and a p value <0.05 was used to determine significant adjusted OR. Quantitative CT results were compared by using the T-test for paired samples. A p value <0.05 was considered statistically significant. Analyses were performed with the SPSS package version 14.0 (SPSS, Inc., Chicago, IL).

Role of POC test providers

None of the POC test providers had any role in study design, collecting or interpretation of data or writing the manuscript.

RESULTS

Population and questionnaire

Between September 2007 and April 2008, 772 women were included with a median age of 23 years (range 16-64 years). Over 95% of all clients filled in the questionnaire. The median age of first sexual contact was 16 years (range 6-36 years). The median lifetime number of sexual partners was 9 (range 1– more than 99) and almost half of these contacts were considered as unsafe sexual contact. During the last six months, the median number of newly acquired sexual partners was 3 (mean 4; range 0- more than 99). Only 2 out of 772 women were co-infected with *Neisseria gonorrhoeae*. No syphilis or HIV cases were detected. In the month prior to visiting the outpatient clinic, 13% (99/772) of the clients had used antibiotics, 5 of whom were CT positive with NAAT. The CT positive clients could not recall which antibiotic they had used.

POC tests compared with NAAT

Chlamydia trachomatis testing by COBAS Amplicor resulted in a CT prevalence of 11% in our population (84/772 clients). Sensitivities, specificities, NPV and PPV of the different POC-tests compared with NAAT are presented in table 1. Results are presented according to time between collecting the SVS and performance of the POC test and subdivided for women with self-reported symptoms. Due to logistical limitations, 49% of the Handilab-C results were performed in time. On time Handilab-C results are depicted for non-menstruating clients, since this test is not validated in case of menstruation. Sensitivities of the Biorapid CHLAMYDIA Ag test, QuickVue Chlamydia test and Handilab-C were 17%, 27% and 12% respectively. The failure rate (meaning an invalid or missing test result) of 5% when including all Handilab-C results is mainly caused by presence of blood on the SVS, which hinders interpretation of the test result; self-reported menstruation was the probable cause of 85% (23/27) of the bloody samples. If all POC tests were included, sensitivity only decreased significantly in the QuickVue Chlamydia test. Binary logistic regression was performed using all POC test results, taking in account factors that might influence diagnostic test

results^{13,14} (details on the binary logistic regression are available in the appendix, table 2). This assessment suggested no relevant influences.

	N	Sensitivity % (95% C.I.)	Specificity % (95% C.I.)	PPV %	NPV %	Failure %
Biorapid CHLAMYDIA Ag test						
- Performed within 72 hours	737	17.3 (8.8-25.9)	93.5 (91.6-95.4)	23.2	90.9	1.2
○ Clients with symptoms	359	17.0 (6.3-27.8)	92.6 (89.7-95.5)	25.8	88.1	0.8
- All results	763	17.1 (8.9-25.2)	93.7 (91.9-95.5)	24.6	90.4	1.2
QuickVue Chlamydia test						
- Performed within 72 hours	737	27.3 (17.3-37.2)	99.7 (99.3-100)	91.3	92.2	1.2
○ Clients with symptoms	357	28.6 (15.9-41.2)	99.7 (99.0-100)	93.9	89.8	1.4
- All results	763	25.0 (15.7-34.3)	99.7 (99.3-100)	91.3	91.5	1.2
Handilab-C						
- Performed within 24 hours in non- menstruating females	378	11.6 (2.0-21.2)	91.9 (89.0-94.9)	15.6	89.0	1.0
○ Clients with symptoms	180	11.1 (0.0-23.0)	91.5 (87.1-95.9)	18.8	85.4	0.6
- All results	735	22.5 (13.3-31.7)	88.9 (86.4-91.3)	19.8	90.4	4.8

Table 1. Performance of the different POC tests.

Quantitative CT NAAT results

Quantitative CT NAAT (qNAAT) was used on 70 out of 84 positive CT samples. The qNAAT was inhibited in 6 paired samples and in a single SVS 6; all other samples tested CT positive. Almost 30% of the bacterial loads were identical between the first and sixth swab taken. Higher bacterial loads were observed in SVS 1 (mean: 445678 IFU/swab, median: 19410 IFU/swab: this is excluding extreme values with Grubbs' test for outlier detection¹⁵) compared to SVS6 (mean: 29963 IFU/swab, median: 12180 IFU/swab: excluding extreme values). The CT load was <100 (but above 20 CFU/mL) in one paired sample and in 3 single SVS 1 and 2 single SVS 6. Statistical analysis demonstrated no significant difference in POC test performance in relation to CT load for the different tests (data not shown). On average 14.6×10^6 HLA targets per swab were observed in SVS1 (median: 5.0×10^6 HLA targets/swab), compared to an average of 706.7×10^6 HLA targets/swab in SVS6 (median: 167.9×10^6 HLA targets/swab). The Grubbs' test was used to detect and remove outliers. The average bacterial load per cell was higher in SVS 1 than in SVS6, probably due to mucus removal by the immediately preceding five SVS.

DISCUSSION

The development and marketing of POC tests for CT has occurred as an answer to the demand for more rapid diagnosis, with the obvious goal of earlier treatment and prevention of secondary cases. In this study, three POC tests have been evaluated under optimal laboratory conditions, in a population with a high CT prevalence (11%). Overall, our data show that all POC tests perform alarmingly poorly.

Few limitations in our study should be mentioned. First, choosing solely for a western laboratory setting, limits direct translation of our results to other settings. However, in this regard the poor performance of POC tests in our setting is unlikely to improve under conditions with lower resources. Second, reproducibility of POC tests could not be assessed, since each swab could only be used for one POC test. Third, the COBAS Amplicor does not detect the Swedish variant of CT (swCT or new variant nvCT) and POC-test results could therefore be worse since CT positive samples could have been missed. The swCT however has been detected in The Netherlands in only one case yet and directly linked to a swCT variant positive Swedish women (personal correspondence dr. S.A. Morré).^{16,17} Finally, for the Biorapid CHLAMYDIA Ag test and QuickVue Chlamydia test a SVS was used instead of an endocervical swab as stated in the package insert. As we have shown, the CT loads in the SVS were almost all above the detection limit of the different POC tests and statistical analysis demonstrated no significant influence of CT load on test performance. Moreover, the bacterial loads found in our study using SVS, are comparable to results found for endocervical swabs in a previous study.¹⁸

The strengths of our study are the large study population, the comparison of three POC tests in one and the same study, the experiments performed to control for CT load differences in successive taken SVS and, finally, the use of the ASSURED criteria as a reference enabling objective reviewing of results.

In our experience, all POC tests were easy to perform with respect to laboratory handling, but the Handilab-C was difficult to interpret, even after multiple tests had been performed. Regarding previously published evaluations, a small-scale Norwegian study¹⁹ has already raised questions concerning the performance of the Handilab-C. In this study, 50% of all participating women, who were asked to perform the test themselves, were not certain how to interpret their Handilab-C result. Sixteen out of 157 participating women were CT positive with NAAT (used as gold standard). The Handilab-C result was interpreted as positive by only 4, and as uncertain by 9 clients, which resulted in sensitivity between 25-57%. Michel et al. recently evaluated the Handilab-C in a group of 231 women (38/231 CT NAAT positive), again demonstrating a low sensitivity, and discussing this in view of the value of a CE-mark.²⁰

The QuickVue Chlamydia has been evaluated twice thus far. In a 1997 publication, the QuickVue Chlamydia was evaluated in a population of 724 women divided in two high risk and one low risk population.²¹ Sensitivity and specificity were on average 90.1% and 99.5%, respectively, in the high-risk populations (n=366, CT prevalence 14.1%). Performance of the QuickVue Chlamydia in this study was compared with culture. Samples with a false positive QuickVue Chlamydia results however, were retested with NAAT and added to the true positive results if found positive with NAAT. In contrast, culture negative samples with a negative QuickVue Chlamydia result, were not retested with NAAT despite a sensitivity of culture of only 65%.²² Therefore, false negative QuickVue Chlamydia test results would not have been detected, and performance of the QuickVue Chlamydia in this study has been overestimated. In 2002, a second evaluation was published comparing QuickVue Chlamydia with NAAT in two groups of 100 women.²³ In the high risk population, sensitivity and specificity were 65% and 100%, respectively, with 16 women being positive with NAAT. In the low risk population however, the sensitivity was only 25% (1/4). If both groups in the study by Rani et al. are taken into account, the CT prevalence in their study is 10% (20/200), which is comparable to the CT prevalence of 11% in our population. Recalculating sensitivity and specificity when using both populations of Rani et al., rendered a sensitivity of 55.0% (CI 33.3%-76.8%) and a specificity of 100%, which is not significantly different from our results. As can be extrapolated from our results, a POC test with excellent performance may make a difference; assuming a primary CT transmission of 65% (without further transmission) when having sexual contact²⁴, a treatment delay of 2 weeks^{8,25} and a POC test sensitivity of 100%, 8 additional new CT cases would have been avoided compared with NAAT. In contrast, when applying the same calculation to our data, the result is negative compared with NAAT and due to false-positive results,

participants would have been treated unnecessary, especially in case of the Biorapid CHLAMYDIA Ag and Handilab-C test. In a recent evaluation, the *Chlamydia* Rapid Test showed promising results²⁶; this POC test primarily would have detected less CT cases compared with NAAT, but due to instant treatment prevent more CT cases resulting in equal outcome in our model. A sensitivity of 83.5% is not sufficient to replace NAAT in a setting with minimal loss to follow-up; cost benefit analysis therefore may determine if combining NAAT and a POC test is beneficial to avert additional CT cases.

In summary, results of this study, performed in a large population, show poorer laboratory performance of the different POC tests than has previously been described. The ASSURED criteria for POC testing including a sensitivity 43-65% and a specificity 98%⁹, are not met by any of the POC tests. The poor performance of all POC tests evaluated in our study has implications for public health, since the Handilab-C test remained commercially available via the Internet (29.95 euro) during the entire inclusion period. The distributor has claimed a reliability of 98.15% (not further specified) on his website while, for instance, sensitivity in our study population was only 12%. Our results underline the need for good quality assurance of POC tests, especially in view of internet possibilities.²⁷ Although excellent guidelines on CT POC test evaluation exist²⁸, these guidelines are regularly ignored, and thus tighter regulations are urgently needed to prevent unrestrained marketing.⁹ In our opinion, the CT POC tests we have evaluated, are not ready for widespread use.

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Competing interests

All authors declare that the answer to the questions on your competing interest form are all No and therefore have nothing to declare.

Contributors

LvD was involved in study design, performed all POC tests, was responsible for the statistical analysis and writing the manuscript; FHvT was involved in study design and contributed to critical revision and writing of the manuscript; SO was involved in statistical analysis and quantitative PCR assays; EEHGB was involved in study design, coordinated patient inclusion at the STI clinic and was involved in collection data from the STI clinic; PHWT was extensively involved in statistical

analysis; PHMS designed the quantitative PCR assay; SAM was involved in study design, quantitative PCR assays and contributed to revision of the manuscript; CAB was involved in study design and revision of the manuscript; CJPAH was involved in study design, supervised the staff at the STI clinic, was involved in data collection at the STI clinic and contributed to critical revision and writing of the manuscript.

Ethical approval

This study was approved by the Medical Ethics Committee of Maastricht University Medical Centre. All participants gave informed consent before taking part in this study.

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Independence of researchers

All authors were independent, and worked independently from the distributors of the tests.

Access to data

All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Data sharing statement

Data sharing: technical appendix, statistical code, and dataset available from the corresponding author at: f.van.tiel@mumc.nl

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