Evaluation of 2-SP transport medium for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by two automated amplification systems and culture for chlamydia

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Abstract

Aims—To assess the performance of 2-sucrose-phosphate based transport medium (2-SP) for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by an automated commercial polymerase chain reaction (PCR) and ligase chain reaction (LCR) compared to centrifugation culture on McCoy cells for *C trachomatis*. Second, to compare both amplification systems for initial diagnostic testing of a low prevalence population for sexually transmitted diseases.

Methods—Four hundred and eighty one consecutive urogenital and conjunctival specimens were examined. All tests were performed on the same specimen collected with a dacron swab and transported in 2-SP medium. Samples that were positive by culture or by both PCR and LCR were considered to be true positives.

Results—The prevalences of *C trachomatis* and of *N gonorrhoeae* were 2.7% and 0.4%, respectively. PCR had a resolved sensitivity and specificity of 100% and 99.8%, respectively, for *C trachomatis*, and 100% and 98.9%, respectively, for *N gonorrhoeae*. LCR was 100% sensitive and specific for both pathogens. The resolved sensitivity of the shell vial assay was 85%. No culture positive sample would have been missed by PCR or LCR. The inhibition rate for PCR was 4.8%.

Conclusions—2-SP medium proved to be suitable for both PCR and LCR. It is not limited to any one test manufacturer and allows the performance of amplification techniques and viral and chlamydia culture from the same specimen. The LCR was more reliable than PCR on initial testing. However, hands on time is longer, and no amplification control is available for LCR.

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Keywords: 2-sucrose-phosphate medium; polymerase chain reaction; ligase chain reaction

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the most frequent agents causing sexually transmitted diseases worldwide.^{1,2} In Sweden and Germany the incidence of gonococcal infections is only about 10 cases per 100 000 and the situation is similar in Switzerland.³⁻⁵ In

the past decade, *C trachomatis* has become the most common bacterium causing sexually transmitted disease in Europe, although data concerning the incidence are scarce.^{6,7}

In recent years, the introduction of polymerase chain reaction (PCR) and ligase chain reaction (LCR) based methods has resulted in a substantial increase in sensitivity and (compared with most other non-culture methods) specificity for the detection of *C trachomatis* and *N gonorrhoeae* in urogenital swab specimens and urine.⁸⁻¹⁶ The development of commercial, partially automated assays further simplified and shortened these procedures and made them available to a broader range of diagnostic laboratories.^{10,17}

One of our concerns as a diagnostic laboratory was the increasing number of manufacturer and test specific specimen collection and transport systems, each one with its particular handling directions and variable compatibility with other assays. The same specimen collection and transport kit should be suitable for amplification techniques and for viral and chlamydial culture. In this study, we evaluated the performance of 2-sucrose-phosphate (2-SP) based transport medium for the detection of *C trachomatis* and *N gonorrhoeae* in urogenital and ocular swab specimens with automated LCR and PCR techniques compared with culture for chlamydia. *N gonorrhoeae* culture was not an integral part of the study because it cannot be performed on samples transported in 2-SP, but culture results from a second swab were available for most patients. In addition, we wanted to determine which of the automated amplification systems would be more suitable for initial testing in our population with a low prevalence of sexually transmitted disease.

Materials and methods

POPULATION

Four hundred and ten consecutive cervical, 49 urethral (30 male, 19 female), and 22 conjunctival specimens sent to our laboratory for diagnostic purposes during a period of four months were tested prospectively by LCR and PCR for *C trachomatis* and *N gonorrhoeae* plus culture for chlamydia. Only the specimens collected with a dacron swab in 2-SP medium were included in the study (> 90% of all submitted specimens). The Obstetrics and Gynaecology University Hospital of Berne provided 74.2%

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of the specimens, 10.6% were obtained from the Obstetrics and Gynaecology Clinic of the Bürgerspital at Solothurn, 3.5% from the Department of Internal Medicine, and 2.3% from the Pediatric Clinics of the University Hospital of Berne, 2.1% were obtained from urologists, 3.3% from other laboratories, and 4% from other physicians or hospitals. Three hundred and sixty one swabs were available for *N gonorrhoeae* culture.

SPECIMEN COLLECTION, TRANSPORT, AND PROCESSING

All specimens were collected with dacron swabs in 2-SP tubes. After arriving at our laboratory, the specimens were immediately split into two separate 1 ml aliquots for amplification techniques and culture. For LCR and PCR 100 µl was used and for culture 200 µl was used. All tests were performed within three days. Before testing, the material was stored at 4°C. The remaining material was frozen at -80°C.

2-SP MEDIUM

The 2-SP medium contained 0.2 M sucrose, 0.0146 M $K_2HPO_4 \cdot 3H_2O$, 0.054 M KH_2PO_4 (all from Merck, Darmstadt, Germany), 2.5 mg/l amphotericin B (Squibb, Baar, Switzerland), 100–120 mg/l gentamicin (Seromed, Berlin, Germany), 10 g/l bovine serum albumin (Sigma, Buchs, Switzerland), and 0.0025% phenol red solution (Flow Laboratories, Baar, Switzerland).

AMPLIFICATION TECHNIQUES

Sample preparation and amplification/detection were performed in distinct laboratory areas. Standard precautions to avoid cross-contamination were adopted.¹⁸

LIGASE CHAIN REACTION

LCR was performed with the LCx system (Abbott Laboratories, Delkenheim, Germany). Sample preparation followed a modified urine processing protocol. After vortexing briefly, 100 µl samples were transferred to a 1.5 ml conical microcentrifuge tube (Sarstedt, Sevelen, Switzerland) and centrifuged for 15 minutes at 9000 ×g in a microcentrifuge. The supernatant was discarded and the pellet resuspended in 1 ml urine resuspension buffer from the kit. The microcentrifuge tube was heated at 97°C for 15 minutes in a heatblock and left for another 15 minutes to cool to room temperature. Aliquots of 100 µl were transferred to "ready for use" unit dose tubes from the kit containing 100 µl of mastermix with four oligonucleotide probes specific for the *C trachomatis* multicopy cryptic plasmid or the *N gonorrhoeae* Opa-1 gene, respectively. Further processing was performed according to the manufacturer's instructions.

POLYMERASE CHAIN REACTION

PCR was performed with the COBAS AmpliCor system (Roche Molecular Diagnostic Systems, Basel, Switzerland). All steps of the sample preparation, amplification, and detection were performed according to the manufac-

turer's instructions. Briefly, 100 µl of 2-SP medium was added to 100 µl of lysis buffer from the kit. After 10 minutes of incubation at room temperature, 200 µl of specimen diluent from the kit was added, followed by another 10 minutes of incubation at room temperature. An aliquot of 50 µl of the processed sample was transferred to the previously prepared amplification tubes, containing a mastermix with biotinylated primers for the cryptic plasmid of *C trachomatis*, the putative cytosine DNA methyltransferase gene of *N gonorrhoeae*, and the amplification control (DNA plasmid with primer binding regions identical to *C trachomatis* but unique probe binding region). A positive control for *C trachomatis* (a negative control for *N gonorrhoeae*) and for *N gonorrhoeae* (a negative control for *C trachomatis*) were run with each batch. The detection of the optional amplification control was applied to all samples. Amplification and detection are fully automated procedures with no intervening manipulations. Samples with a negative amplification control were retested after 10-fold dilution with the specimen diluent from the kit and 10 minutes heating in a heatblock at 95°C.¹⁹

CELL CULTURE

Single pass cell culture was performed as a centrifugation culture (shell vial assay; SVA) on McCoy cell monolayers according to standard procedures.^{20, 21} Briefly, 200 µl sonicated sample was inoculated on to McCoy cell shell vials and centrifuged for 45 minutes at 700 ×g at room temperature. The supernatant was discarded and Eagle's minimal essential medium supplemented with 10% inactivated fetal bovine serum and cycloheximide was added. The shell vials were incubated for 72 hours at 36°C in an atmosphere with 5% CO₂. After incubation they were fixed with 4°C cold acetone/methanol and stained with chlamydia genus specific monoclonal antibodies (Argene, Varilhes, France) and fluorescein labelled monoclonal antimouse F(ab')₂ fragments (BioScience, Emmenbrücke, Switzerland). The number of inclusions was counted on a fluorescence microscope with a mercury 100 lamp at a magnification of ×200. One or more typical inclusions were considered to be positive.

RESOLUTION OF DISCREPANT SAMPLES

Samples positive by either culture alone or with both amplification techniques were considered to be true positives. Samples positive or equivocal by just one amplification technique were considered discrepant. For *C trachomatis*, all discrepant results could be resolved by repeating LCR and PCR from new aliquots, whereas for *N gonorrhoeae* most discrepant results could be resolved this way. One repeatedly discrepant specimen for *N gonorrhoeae* was tested by a confirmatory PCR (16S rRNA; Roche, Basel, Switzerland).

Table 1 Comparison of results by PCR, LCR, and SVA for *Chlamydia trachomatis*

Material	Total	LCR +ve	PCR +ve	SVA +ve	True +ve (%)
Cervical swabs	410	11	12*	10	11 (2.7)
Urethral swabs	49	2	2	1	2 (4.1)
Conjunctival swabs	22	0	0	0	0
Total	481	13	14	11	13 (2.7)

*one cervical swab was not reproducibly positive by PCR only and therefore was considered as false positive.

STATISTICAL ANALYSIS
Descriptive statistics were performed with the StatView II, version 4.01 software (Abacus Concepts, Berkeley, California, USA).

Results
Of the 410 cervical and 49 urethral specimens, 11 (2.7%) and two (4.1%), respectively, were positive for *C trachomatis* by LCR and PCR. Ten (91%) and one, respectively, of these positive specimens were also positive by SVA. Both positive urethral specimens were from men with urethritis. One cervical swab was initially equivocal by PCR with a negative amplification control but positive by both LCR and SVA. This sample turned out to be PCR positive on retesting after eliminating inhibitors with pretreatment (10-fold dilution and heating for 10 minutes at 95°C). One culture and LCR negative sample was not reproducibly positive for *C trachomatis* by PCR and, therefore, was considered to be a false positive (table 1).

One cervical and one male urethral specimen were positive for *N gonorrhoeae* by both amplification techniques. The cervical specimen was initially negative by PCR with a negative amplification control and turned out to be positive on retesting, after elimination of inhibitors with the sample pretreatment described above. Four cervical samples were positive initially for *N gonorrhoeae* by PCR only and negative on retesting. Three additional LCR negative samples were equivocal by PCR, one of them reproducibly. This sample was negative by the backup PCR of the manufacturer and, therefore, it was considered negative in our evaluation (table 2). As mentioned earlier, *N gonorrhoeae* culture was not an integral part of this study. Nevertheless, *N gonorrhoeae* culture on supplemented GC medium (Difco Laboratories, Detroit, Michigan, USA) in a 5% CO₂ atmosphere at 35°C could be performed with specimens collected at the same examination for 361 patients, and in particular, for all patients with positive or equivocal initial amplification results. Of the two confirmed positive samples the urethral swab was culture positive and the cervical specimen negative. None of the negative, equivocal, and initially discrepant samples was positive by culture.

Table 2 Comparison of results by PCR and LCR for *Neissereria gonorrhoeae*

Material	Total	LCR +ve	PCR +ve	Culture +ve*	True +ve
Cervical swabs	410	1	5†	0/325	1
Urethral swabs	49	1	1	1/24	1
Conjunctival swabs	22	0	0	0/12	0
Total	481	2	5	1/361	2 (0.4%)

*positive culture results/total culture results available.
†four cervical swabs were not reproducibly positive by PCR only and therefore were considered as false positives.

Despite the expectancy of dual infections no specimen was positive for both pathogens.²

Due to the lack of a built in amplification control, no data concerning inhibition with the LCR assay were available. The overall inhibition rate of the PCR assay assessed with the amplification control of the COBAS Amplicor was 4.8%. The individual inhibition rates for cervical, urethral, and conjunctival swabs were 5.1%, 6.1%, and 0.0%, respectively.

PCR had a resolved sensitivity, specificity, and positive predictive value of 100%, 99.8%, and 92.9% for *C trachomatis*, and 100%, 98.9%, and 33.3% for *N gonorrhoeae*, respectively. The LCR was 100% sensitive and specific for both pathogens. For *N gonorrhoeae*, only the 361 patients with available culture results were taken into consideration. The resolved sensitivity of the SVA was 85%.

Discussion
LCx and COBAS Amplicor are partially automated devices. For both assays the complete performance time varies between four and five hours, depending on the number of samples tested. As the COBAS Amplicor is a multiplex PCR and automation is more advanced, hands on time is shorter and the daily maximum possible sample through put for a skilled technician is higher than for the LCx (96 v 48 samples).

The performance of 2-SP with the Amplicor PCR has already been assessed, and the use of 2-SP as a transport medium is approved by the manufacturer.¹⁹⁻²² For the LCx system very few data concerning this subject are available.²² We demonstrated that 2-SP was also suitable for LCR. The only disadvantage of 2-SP is that it has to be stored at 4–8°C. The shelf life of 2-SP kept at room temperature should, therefore, be evaluated for amplification techniques.

As a historical comparison, the prevalence of 2.7% for *C trachomatis* and 0.4% for *N gonorrhoeae* is similar to the prevalence we experienced before in a similar population (3.1% for *C trachomatis*, 0.6% for *N gonorrhoeae*) with 755 samples collected in Roche Amplicor transport medium (334 tested with COBAS Amplicor, 421 tested with the manual Amplicor method).

The overall inhibition rate of 4.8% with COBAS Amplicor was clearly below the rate previously experienced by us (~18%) and described by others with the Amplicor transport medium.¹⁹ For the LCx system no amplification control is available. Therefore, we have no data assessing the inhibition rate with the LCx, but others have demonstrated an inhibition rate for cervical samples collected in LCx transport medium and spiked with elementary bodies of 4.4%.²³

According to recently published evaluations of the amplification techniques—which are more sensitive than the former established gold standard of the culture—we used an extended gold standard.²⁴ Samples positive by culture or by both amplification techniques were considered to be true positives. Both amplification techniques amplify a different sequence of the

C. trachomatis cryptic plasmid and sequences from different genes for *N. gonorrhoeae* and, therefore, can be considered to be independent methods.^{6 25 26}

Sensitivity, specificity, and positive and negative predictive values of commercial LCR and PCR methods in male and female populations with various prevalences of sexually transmitted disease have been studied extensively for the diagnosis of *C. trachomatis*, and to a lesser extent for the diagnosis of *N. gonorrhoeae*, in different large scale studies.^{8 11 16 27-29} Due to the low prevalence of 2.7% for *C. trachomatis* infections in our study population (which is similar to that reported by Ninet *et al.* in Geneva),²³ and of less than 1% for *N. gonorrhoeae*, our data regarding sensitivity and positive predictive value should be interpreted with caution. Sensitivity of PCR depends upon the application of the optional amplification control to all samples. The reason for the isolated, not reproducibly positive PCR result for *C. trachomatis* remains unclear, although contamination during the sample processing is the most likely explanation. The high rate of false positive and indeterminate results by the *N. gonorrhoeae* PCR which resulted in a positive predictive value of only 33.3% could be explained by the known cross-amplification of non-pathogenic neisseria species (*N. cinerea*, *N. subflava*) which is described in the manufacturer's instructions. Unless the lack of specificity for *N. gonorrhoeae* is improved, the *N. gonorrhoeae* PCR with the COBAS Amplicor should not be considered to be a suitable tool for testing a population with a low prevalence of *N. gonorrhoeae* infections.

In conclusion, the most efficient combination in our laboratory setting was 2-SP with the LCx system. The ligase chain reaction was more reliable than PCR on initial testing, although the automation is not as fully developed as for the COBAS Amplicor, and no amplification control is available. 2-SP phosphate is a transport medium that allows amplification techniques, and viral and chlamydial culture to be carried out on the same specimen; this use of the same specimen collection and transport kit for both techniques will simplify sample collection for the clinician.

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