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Performance of the MagNA Pure LC Robot for Extraction of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA from Urine and Swab Specimens

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DNA from uncentrifuged urines ($n = 195$ and $n = 202$) and cervical swabs ($n = 221$ and $n = 601$) was extracted by the MagNA Pure LC robot and the Amplicor method to validate the robot's extraction for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing by Roche PCR. The robot provided a highly sensitive and specific method of DNA extraction.

Chlamydia trachomatis and *Neisseria gonorrhoeae*, which cause two of the most prevalent bacterial sexually transmitted infections both in the United States and worldwide, are becoming more widely diagnosed by nucleic acid amplified tests (NAATs), of which PCR was the first to be commercially available (2, 5, 8, 9, 12, 14, 16). The Centers for Disease Control and Prevention recommends NAATs as the most sensitive way to test for *C. trachomatis* and *N. gonorrhoeae* (1). Many individuals that are infected with either *C. trachomatis* or *N. gonorrhoeae* are concurrently infected (6, 10). From 32 to 50% of women and 20% of men infected with gonorrhea are also infected with chlamydia (6, 10). Many adverse health consequences have been linked to untreated chlamydia and gonorrhea infections in women, such as infertility, pelvic inflammatory disease, and ectopic pregnancy (2). The annual cost in the United States for treating these diseases and sequelae is estimated to be $5 billion; therefore, public health programs should focus on detecting and treating these infections (11, 13). Since infections may be asymptomatic, screening programs, which use noninvasive specimens tested by NAATs, deserve high priority. Use of NAATs for *C. trachomatis* and *N. gonorrhoeae* in urine specimens, as well as for the more conventional cervical or urethral samples, has shown that these tests are not only very sensitive and specific for the detection of both *C. trachomatis* and *N. gonorrhoeae* but widely accepted by patients for screening (3, 4, 7, 12, 15, 16).

Preparation of samples for PCR testing requires rigorous preparation steps, including multiple centrifugation steps for urine. Use of an automated robotic DNA extraction method could enable faster, hands-free extraction. The purpose of our study was to compare the manual processing method for the DNA extraction process recommended by the manufacturer for the Amplicor PCR test for chlamydia and gonorrhea (Roche Diagnostic Systems, Indianapolis, Ind.) with an automated robotic DNA processing method (Roche MagNA Pure LC) to demonstrate the accuracy of the robotics method.

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Urine and swab samples submitted for routine chlamydia and gonorrhea testing were stripped of patient identifiers after routine PCR testing (Roche). The samples were collected from school-based, family planning, and sexually transmitted disease clinics. These populations included symptomatic and asymptomatic males and females representing teenage and young adult age groups.

Swabs were collected in M-4 (Micro Test, Remel, Liburn, Ga.) transport medium, and all samples were transported to the laboratory from the clinic within 4 days of collection and kept between 2 and 8°C for processing within 7 days. Samples unable to be processed within this time frame were stored in their original tubes at −70°C. DNA was first extracted from urine and swab samples according to the Amplicor lysis protocols, and subsequently, DNA was extracted using the MagNA Pure robot. There were no centrifugation steps performed on the urine samples. An aliquot of 200 μl of the well-mixed sample was added to the sample plate, and extraction proceeded according to the MagNA Pure high-performance DNA extraction protocol. Known *C. trachomatis* and *N. gonorrhoeae* positive processing controls, stored in 2-sucrose phosphate chlamydia transport buffer or phosphate-buffered saline for gonorrhea, were used as extraction controls for each batch of robot extractions. Similarly, negative processing controls, M-4 transport medium, or phosphate-buffered saline, was included in every robotic extraction run.

PCR was performed on the extracted samples from both extraction assays according to the Amplicor protocols, but with a slight modification for the robot-extracted samples, which included the addition of $MgCl_2$ solution to the master mix (final concentration of 1.5 mM/PCR) before samples were amplified. Ordinarily, the $MgCl_2$ is in the Amplicor manufacturer's processing reagents. The PCR kit positive and negative controls were used for every amplification run, as were the robotic processing controls. The amplified samples were subsequently denatured, hybridized, and analyzed according to the manufacturer's directions.

The manufacturer's instructions were used for the determination of positive and negative results. Discordant samples that were PCR positive by the robot extraction methodology

TABLE 1. Sensitivity and specificity for MagNA Pure LC robot processing compared to processing by Amplicor method for PCR testing for *C. trachomatis* and *N. gonorrhoeae* in urine and cervical swabs

Sample and organism (*n*)[a]	MagNA pure result	No. of samples with Amplicor test result[b]		% Sensitivity	% Specificity
		Positive	Negative		
Urine CT (202)	+	25	5*	89.3	97.1
	−	3	169		
Urine NG (195)	+	33	3**	97.1	98.1
	−	1	158		
Swab CT (601)	+	46	2***	97.9	99.6
	−	1	552		
Swab NG (221)	+	6	1****	85.7	99.5
	−	1	213		

[a] Sample type and organism tested for. *n*, no. of samples tested. CT, *C. trachomatis;* NG, *N. gonorrhoeae.*

[b] *, three of the five samples were positive by LCX testing for CT. **, one of the three samples was positive for NG by LCX. ***, one of the two samples was CT positive by LCX. ****, this specimen was positive for NG by LCX.

and negative by the manufacturer's Amplicor extraction method were additionally tested by another NAAT, LCX (Abbott Laboratories, Abbott Park, Il.). LCX positive results were interpreted as confirming the presence of *C. trachomatis* or *N. gonorrhoeae* within the sample as a true positive. All results positive by the Amplicor manufacturer DNA processing method were considered to be true positives. Test results were separated according to urine and swab samples as well as by organism, and the sensitivity and specificity were calculated.

Because the samples were stripped of identifiers, there was no way to tell whether the urine samples were from male or female patients. All swab samples, however, were collected from females. A total of 29 samples yielded inhibited results or equivocal results after multiple testing. Because of either a lack of adequate volume for retesting or concurrent equivocal results, these samples were omitted from the final analysis. Not all samples were tested for both chlamydia and gonorrhea. There were 202 urines tested for *C. trachomatis* and 195 urines tested for *N. gonorrhoeae*. For female cervical swab specimens, 601 were tested for *C. trachomatis* and 221 were tested for *N. gonorrhoeae*. The prevalence of *C. trachomatis* within the population tested was 9.3%, and the prevalence of *N. gonorrhoeae* was 9.8%.

Of the 202 urine samples tested for *C. trachomatis*, 25 agreed as positive for both processing methods and 169 were negative by both processing methods. Robot processing missed 3 samples that were positive by Amplicor processing, for a sensitivity of 89.3% (25 of 28). Of five samples that were robot positive and negative by Amplicor (specificity of 97.1%), LCX was positive for three (Table 1). Of the 195 urine samples tested for *N. gonorrhoeae*, 33 agreed as positive and 158 were negative for both processing methods. The robot missed 1 sample that was positive by Amplicor processing, for a sensitivity of 97.1% (33 of 34). Of three samples that were positive by robot processing and negative by Amplicor processing (specificity of 98.1%), one was positive by LCX. (Table 1).

Of 601 swabs tested for *C. trachomatis*, there were 46 that agreed as positive for both processing methods and 552 that were negative by both methods. There was 1 sample that was positive by Amplicor processing and negative by the robot, for a sensitivity of 97.9% (46 of 47). Of two samples that were positive by the robot that were negative by Amplicor processing (specificity of 99.6%), one was LCX positive. (Table 1). Of the 221 swabs tested for *N. gonorrhoeae*, there were 6 that were positive by both processing methods and 213 that were negative by both processing methods. There was one Amplicor-processed positive sample that was missed by robot processing, for a sensitivity of 85.7% (six of seven). For the one sample that was negative by Amplicor but positive by the robot method (specificity of 99.5%), the LCX was positive (Table 1).

Testing of genital samples by PCR has been shown to be both sensitive and specific for detection of *C. trachomatis* and *N. gonorrhoeae*, with sensitivity ranging from 93 to 97% and specificity higher than 99% (5, 9, 12, 14, 16). In this study the PCR test results from sample aliquots processed by the robot compared very favorably to those from aliquots processed according to the package insert, yielding high concordance of results for both urine and cervical samples as well as for both *C. trachomatis* and *N. gonorrhoeae*. The samples that were missed by the robot processing may have been missed due to the additional storage time for samples between routine processing, which was performed first, and robotic processing, leading to loss of target of initial low copy number.

The few additional samples that were positive only by the robot processing and were additionally positive by LCX may indicate a slight superiority of the robotic DNA extraction chemistry, which is guanadinium based (package insert), combined with a magnetic bead DNA capture process. The use of the magnetic beads may also serve to decrease inhibitors in the samples. We concluded that the use of the robot to extract DNA from genital samples for Amplicor PCR for *C. trachomatis* and *N. gonorrhoeae* has been satisfactorily validated in that the sensitivity and specificity are both very high.

Use of the robot can decrease processing time without substantially decreasing the sensitivities and specificities of the test. The MagNA Pure LC method is unique in that it does not require a technician to extract the DNA from the samples. Collected samples can be put directly into the robot for DNA extraction. There is no need to centrifuge urine. The approximate technician time that can be saved is about 2.5 to 3 h for urine samples and 0.5 to 1 h for swabs. Importantly, the machine allows hands-free manipulation of samples and may decrease the chance for potential laboratory cross-contamination. It is potentially a cost-saving alternative processing method, since technician time for the sample preparation is greatly decreased. A cost-producing limitation, however, is the initial high cost ($84,000) of the robot. Use of the MagNA Pure LC robot can be recommended as an accurate method for DNA extraction to prepare both urine and cervical swab samples for PCR testing for *C. trachomatis* and *N. gonorrhoeae*.

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