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Evaluation of Automated COBAS AMPLICOR PCR System for Detection of Several Infectious Agents and Its Impact on Laboratory Management

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We evaluated the COBAS AMPLICOR (CA) PCR system (Roche Diagnostic Systems) designed for automated PCR amplification and detection of nucleic acids from infectious agents in clinical samples. The Roche AMPLICOR microwell plate (MWP) PCR was the reference method. CA amplifies target nucleic acid, captures the biotinylated amplification products by using magnetic particles coated with specific oligonucleotide probes, and detects the bound products colorimetrically. For *Mycobacterium tuberculosis*, the correlation of the results of CA tests with those of MWP tests was 100% with 230 samples, including 20 culture-positive samples. For hepatitis C virus, the correlation was 100% with 214 samples, including 60 positive samples. MultiPlex CA analysis of 199 cervical specimens for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and the internal control gave 100% concordance. These samples included 19 *C. trachomatis* and 3 *N. gonorrhoeae* culture-positive samples. Overall, the agreement between PCR methods for all 842 comparisons was 100%. Compared with culture, the sensitivities of the assays for *C. trachomatis* and *M. tuberculosis* were ≥95%. After spiking alternating amplification tubes in the CA system with 10^{14} copies of the *Chlamydia* amplicon per ml, we were unable to demonstrate any carryover cross-contamination of negative samples. Using the criteria of the College of American Pathologists workload recording method, we found that the total hands-on time to produce CA PCR results was 4.4, 7.9, and 3.3 min for *M. tuberculosis*, hepatis C virus, and the MultiPlexed assay for chlamydia plus gonorrhea and an internal control, respectively. The CA system brings true PCR automation to laboratories. In addition to the accuracy of automated results, the CA system provides labor savings, provides containment of the amplification and detection components of PCR, and supports both MultiPlex amplification and sequential algorithm (ReFlex) detection of analytes.

PCR holds the potential for allowing automation of parts of the clinical microbiology laboratory in much the same way that labor-intensive sections of the clinical chemistry laboratory were automated during the last three decades. PCR technology offers a sensitive and specific means of identifying difficult to culture organisms such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, and hepatitis C virus (HCV). The first clinical PCR kits were the AMPLICOR microwell plate (MWP) assays (Roche Diagnostic Systems, Branchburg, N.J.). These were designed for amplification in the Perkin-Elmer (Norwalk, Conn.) 9600 thermal cycler, with enzyme-linked immunosorbent assay (ELISA)-like colorimetric detection taking place in microwell plates coated with an oligonucleotide designed to capture the biotinylated amplicon product. The first PCR test approved by the U.S. Food and Drug Administration was the AMPLICOR *Chlamydia* MWP assay (2). In Europe and Japan, other AMPLICOR MWP kits became available for clinical use. Among these were tests for *N. gonorrhoeae*, *M. tuberculosis*, and HCV. The sensitivity and specificity of the MWP assays have been established clinically in Europe, Japan, and the United States (1, 3, 6, 10, 12–15). The purpose of the present study was to compare the first-generation MWP assay with an automated COBAS AMPLICOR (CA) PCR system, also from Roche Diagnostic Systems. The CA system amplifies target DNA, captures the biotinylated amplification products with magnetic particles coated with specific oligonucleotide probes, and detects the bound products colorimetrically. The study was designed as a preclinical trial, with emphasis on determining the degree of concordance between the MWP assay and the CA assay systems when tested with separate aliquots from clinical samples. The following were chosen for testing: a single analyte, *M. tuberculosis*; an RNA analyte HCV; and MultiPlexed analytes including *C. trachomatis*, *N. gonorrhoeae*, and an internal control (IC). The IC monitors the possible inhibition of the PCR. A second goal of the study was to use the method of the College of American Pathologists (CAP; Northfield, Ill.) workload recording system to determine the workload units for three typical tests performed with the CA system (4). A limited study of the laboratory management aspects of the CA system was made. Finally, we tested the operational characteristics of the instrument to evaluate the potential for cross-contamination of samples. A detailed comparison of the PCR results with culture or other test results and clinical history was not a primary goal of the present preclinical study. Those issues will be addressed in expanded clinical trials for each analyte.

MATERIALS AND METHODS

Specimens. For evaluations for *C. trachomatis* and *N. gonorrhoeae* three prospectively collected endocervical swabs were collected from each of 199 women from an obstetrics and gynecology clinic or a family planning clinic. The first swab was for *N. gonorrhoeae* culture. The second and third swabs were randomized and used for either *C. trachomatis* culture or a *C. trachomatis-N. gonorrhoeae* combination PCR. The specimens were frozen at −75°C until testing by PCR.

For *M. tuberculosis*, 230 sputum specimens were collected, processed, and cultured as previously reported as part of our normal clinical laboratory testing procedure for patients for whom *M. tuberculosis* cultures were ordered (3). These included 203 prospectively collected samples and 27 previously saved samples

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TABLE 1. Comparison of detection step of CA and MWP tests for *C. trachomatis-N. gonorrhoeae* PCR[a]

Reagent	CA system		MWP system	
	Vol (μl)	Incubation	Vol (μl)	Incubation
Denaturation	100	10 min at 25°C	100	10 min at 25°C
Hybridization	100	15 min at 37°C	100	60 min at 37°C
Conjugate	200	15 min at 37°C	100	15 min at 37°C
Substrate	300	15 min at 37°C	100	15 min at 37°C
Stop solution			100	10 min at 25°C

[a] The solid phases of the CA and MWP systems are magnetic microparticles (Dynal magnetic particles) and the bottom of the microwell plate, respectively. The optical densities used by the two systems are 660 and 450 nm, respectively.

that were known to be from *M. tuberculosis*-positive patients. Leftover sputum concentrates were frozen at −75°C until they were used for analysis by PCR.

For HCV, sera sent to the clinical laboratory for HCV ELISA testing were refrigerated at 4°C for no longer than 6 h. Multiple aliquots of these sera were transferred into clean tubes and frozen at −75°C. The aliquots were used to avoid repeated freezing-thawing. Of the 214 prospective specimens sent to our laboratory for antibody testing with the Abbott HCV ELISA system (Abbott Diagnostics, Abbott Park, Ill.), 76 were ELISA positive for HCV antibody and 138 were negative.

***C. trachomatis-N. gonorrhoeae* combination PCR test.** Specimen collection with the AMPLICOR transport medium, the MWP test, and the CA test were performed as recommended by the manufacturer. PCR amplification with duplicate detections was performed with each sample by both PCR methods. The first PCR result was used in our comparisons. Pretreated specimen was added to a master mixture containing two sets of biotinylated oligonucleotide primers (one pair for *C. trachomatis* and one pair for *N. gonorrhoeae*), *Taq* polymerase, and excess deoxynucleoside triphosphates. The *C. trachomatis* primers amplified regions of the cryptic plasmid of *C. trachomatis*. For *N. gonorrhoeae* the primers amplified regions of the cytosine methyltransferase gene M:Ngo P11 that is unique to *N. gonorrhoeae*. The master mixture contained AmpErase to ensure the selective amplification of target DNA. The amplified products were detected with specific oligonucleotide probes (for *C. trachomatis* or *N. gonorrhoeae*) coated onto MWPs or magnetic particles for the MWP test or the CA test, respectively. Signal generation in both PCR assays uses avidin-horseradish peroxidase conjugate and then color development with H_2O_2 and tetramethylbenzidine. To avoid DNA carryover in the MWP system, separate dedicated areas were used for reagent preparation, sample preparation, and detection; and plugged (aerosol-resistant) pipette tips were used. For the CA system, a bidirectional flow of personnel was allowed, except that laboratory coats and gloves were changed between areas. To prevent contamination of previously amplified DNA into a new sample, uracil-*N*-glycosylase (UNG), which recognizes and destroys uracil-containing DNA, was used with both PCR systems. Only previously amplified product contained dUTP.

Sample extraction, control preparation, and preamplification steps were the same for both the MWP and the CA tests. Reagents were allowed to equilibrate to room temperature prior to use. In the reagent preparation area, 50 μl of the master mixture containing deoxyribonucleotide triphosphates (dATP, dCTP, dUTP, and dGTP), *Taq* DNA polymerase, UNG, and two sets of biotinylated oligonucleotide primers (one for *C. trachomatis* and one for *N. gonorrhoeae*) were added to the appropriate number of PCR tubes needed for patient specimen and control testing. For the MWP test, two *C. trachomatis*-positive and two *N. gonorrhoeae*-positive controls were tested per PCR tray. For the CA test, one *C. trachomatis*-positive control and one *N. gonorrhoeae*-positive control were tested per 12-tube amplification ring (A ring) during the course of the study. The *C. trachomatis*-positive control served as the negative control for *N. gonorrhoeae*, and the *N. gonorrhoeae*-positive control served as the negative control for *C. trachomatis*. The *C. trachomatis*-positive control and *N. gonorrhoeae*-positive control were prepared by adding 750 μl of specimen diluent (Tween 20 and magnesium) to each control vial. The vial was recapped and vortexed. One milliliter of specimen diluent was added to each specimen transport tube. The tube was recapped and vortexed for 10 s. The controls and specimens were then incubated at room temperature for 10 min. A 50-μl aliquot of patient sample or control was added to the appropriate PCR tube. Tubes of the PCR tray for the MWP test and A rings for the CA test were capped. The sample tray was placed into a thermal cycler (9600; Perkin-Elmer) for amplification. The A rings were placed in the CA onboard thermal cyclers and amplified. The CA system contains a thermal cycler with two segments capable of amplifying 12 samples each. Each segment can run independent cycles simultaneously.

AMPLICOR MWP detection. The steps of the detection assays are summarized in Table 1. After coamplification the PCR sample tray was removed from the thermal cycler and 100 μl of denaturation solution (EDTA with sodium hydroxide) was immediately added to each PCR tube with a multichannel pipette. The amplicon was incubated at room temperature for 10 min to allow complete separation of the double-stranded DNA. Two separate 96-microwell plates were used for the detection of amplified *C. trachomatis* and *N. gonorrhoeae* DNAs. A 100-μl aliquot of hybridization buffer was pipetted into each well of both plates. To the appropriate wells of each of the detection plates 25 μl of denatured amplified sample or control was added. The plates were covered and gently tapped to mix the contents, and the plates were incubated for 1 h at 37°C. The plates were washed five times with wash buffer with an ELP 35 MWP washer (Biotek Instruments, Winooski, Vt.) to remove unhybridized material. Each wash consisted of the addition of 400 μl of wash solution per well and a 30-s soak and then aspiration. Avidin-horseradish peroxidase conjugate (100 μl) was pipetted into the wells, and the plates were covered and incubated for 15 min at 37°C. The plates were washed as described above. A 100-μl aliquot of substrate containing tetramethylbenzidine and hydrogen peroxide (1:4 parts) was added to each well. The plates were incubated in the dark at room temperature for 10 min to allow color development. Stop reagent (100 μl) was pipetted into the wells, and the optical density of the reaction was measured at 450 nm in a Biotek EL 311 MWP auto-reader. An absorbance reading greater than 0.250 optical density units was considered positive for the presence of *C. trachomatis* or *N. gonorrhoeae.*

Automated (CA) detection. With the benchtop CA analyzer, the amplification, addition of denaturation reagent, and detection of the amplified product were automated. Level sensing, pipetting of reagents and samples, and movement of detection (D) cups to and from the D-cup racks, incubator, wash station, and photometer were accomplished by a transfer probe mechanism that was belt driven on the *x* and *y* axes and elevator gear driven on the *z* axis. This resulted in a robotic probe arm that could move in three dimensions.

After on-system amplification, the instrument probe added 100 μl of denaturation reagent to each PCR tube. After 10 min at room temperature the probe added 100 μl of a reagent containing amplicon-specific oligonucleotide probe bound to magnetic microparticles (Dynal AS, Oslo, Norway) to the D cups, and then the probe added 25 μl of denatured sample or control to the D cups. The D cups were then incubated for 15 min at 37°C. The transfer system then moved the appropriate D cups to the wash station, where 350 μl of wash solution was delivered and aspirated four times. The D cups were then transferred to the incubator, and the probe added a 200-μl aliquot of avidin-horseradish peroxidase conjugate to each D cup, and the cups were incubated for 15 min at 37°C. The system washed each D cup as described above. The D cups were then transferred to the incubator, where the probe added 300 μl of substrate to the D cups, which were then incubated at 37°C for 15 min. The reaction in each D cup was measured at 660 nm in a dark chamber. An absorbance reading greater than 0.250 optical density units was considered positive for the presence of *C. trachomatis* or *N. gonorrhoeae* DNA.

MWP and CA PCRs for *M. tuberculosis*. PCR testing steps for *M. tuberculosis* was performed according to the manufacturer's instructions for each PCR system. Specimen preparation and the test cutoff value were as described in the report by Beavis et al. (3). Most other steps were similar to those described for the *C. trachomatis-N. gonorrhoeae* PCR except that the cycling parameters and reagents were optimized for *M. tuberculosis*. Genus-specific primers located in a highly conserved region of the 16S rRNA gene of *Mycobacterium* spp. were used to amplify a 584-bp sequence. A species-specific oligonucleotide capture probe coated onto the surface of an MWP, or in the case of CA, onto magnetic beads, was used to capture an amplicon specific for *M. tuberculosis*. The *M. tuberculosis* IC was the plasmid pSYC21, which has a sequence insert that differs from that of the *M. tuberculosis* target region only by randomization of a small internal region. This ensured the equal efficiency of amplification with the primers developed for the amplification of *M. tuberculosis*. The *Mycobacterium* spp. IC DNA plasmid was introduced into each PCR mixture. The IC was coamplified together with the clinical sample and was detected separately, at the user's option, with an oligonucleotide probe specific for the resulting IC amplicon.

MWP and CA tests for HCV. The PCR testing steps for HCV were performed according to the manufacturer's instructions for each PCR system. Most steps were similar to those described for the *C. trachomatis-N. gonorrhoeae* PCR except that the cycling parameters, reagents, and positive cutoff point were optimized for HCV. For RNA extraction, 400 μl of lysis buffer was added to a 1.5-ml screw-cap microtube. A total of 100 μl of specimen that had been thawed and vortexed was added to each tube, and the tubes were incubated at 60°C for 10 min. A total of 500 μl of isopropanol was added to each tube, and the contents were mixed and incubated for 2 min. The tubes were centrifuged in a microcentrifuge at approximately 16,000 × *g*, and the supernatant was discarded. One milliliter of 70% ethanol was added to each tube, and the contents were mixed and again microcentrifuged. The supernatant was discarded, and 1 ml of specimen diluent containing an IC matched to the HCV target was added to the sample pellet. After mixing, the specimens were analyzed immediately, or in some cases they were frozen at −20°C until they were processed. A total of 50 μl of each processed specimen was added to each PCR tube. Because a single enzyme with both DNA polymerase and reverse transcriptase activities (r*Tth*) was used in the master mixture, no separate reverse transcriptase step was necessary. Detection of the amplicon in both systems was similar to the process described for the *C. trachomatis-N. gonorrhoeae* PCR, except that specific oligonucleotide capture probes specific for the HCV amplicon and the IC were used.

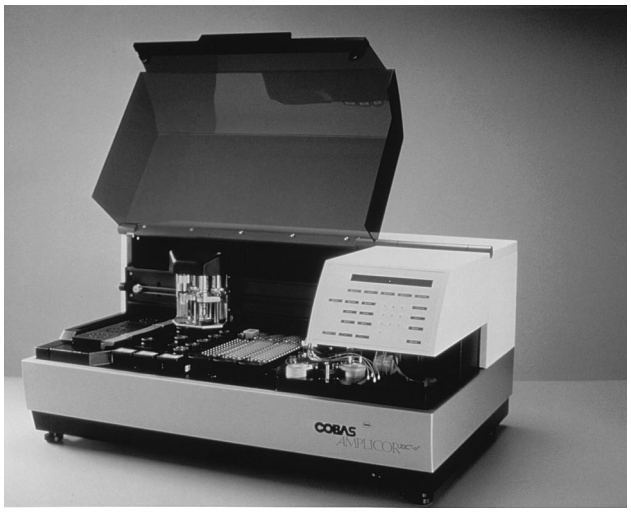

FIG. 1. Automated CA PCR system showing the self-contained unit with lid enclosing the amplification, reagent, and detection areas plus associated robotics in one benchtop instrument.

RESULTS

Figure 1 shows the tabletop CA PCR system. Table 1 compares the amplicon detection process for both the MWP and the CA systems for *C. trachomatis-N. gonorrhoeae*. Of the 398 PCR tests initially performed for *C. trachomatis* and *N. gonorrhoeae*, 396 were in agreement with both PCR systems and 2 were not. The two false-positive results with the CA system were the *C. trachomatis* and *N. gonorrhoeae* results for a single sample. The specimen transport medium for that sample exhibited gross precipitation. After allowing the precipitate to settle out of solution for approximately 1 min, the supernatant of the sample transport medium was retested for *C. trachomatis* and *N. gonorrhoeae* by both the MWP and the CA tests. All repeat results were negative. Moreover, the specimen was culture negative for both *C. trachomatis* and *N. gonorrhoeae*. The precipitate may have been trapped in the magnetic microparticle mixture or clogged the needle probe, causing a false-positive result on the CA analyzer. The CA and MWP tests had 100% concordance for the *C. trachomatis-N. gonorrhoeae* combination PCR test when care was taken to avoid the use of precipitated samples in the CA system. The final correlation of MWP and CA PCR results for the detection of *C. trachomatis* and *N. gonorrhoeae* after performing the revised procedure to avoid introducing precipitated samples into the assay was as follows. The results are for a total of 199 specimens. Each specimen produced two results, one for *C. trachomatis* and one for *N. gonorrhoeae*. Therefore, a total of 398 tests were performed with the 199 specimens. Both tests detected 23 samples positive for *C. trachomatis* or *N. gonorrhoeae*. A total of 376 samples tested negative by both tests. No sample testing positive by one test was negative by the other test. The PCR results compared with the results of culture for *C. trachomatis* are as follows. The results are for a total of 199 specimens. Because the MWP and CA PCR systems gave identical PCR results, the classification of PCR positive or negative applies to both the MWP and the CA tests. Eighteen specimens were culture positive and PCR positive, 1 specimen was culture negative and PCR positive, 1 specimen was culture positive and PCR negative, and 179 specimens were both culture and PCR positive.

For *N. gonorrhoeae*, only 3 of the 199 samples were culture positive. Both PCR systems detected all of these positive specimens plus one additional specimen that was positive by both PCR systems. No attempt was made to arbitrate the results for this sample. There was complete agreement for 195 negative samples.

The correlation of the MWP and the CA tests for *M. tuberculosis* are as follows. Thirty-one samples were positive by both tests, and 199 samples were negative by both tests. No sample testing positive by one test was negative by the other test. Therefore, the tests had 100% concordance for the positive and negative samples. The PCR results compared with the results of culture for *M. tuberculosis* are as follows. The results are for a total of 230 specimens. Because the MWP and CA PCR systems gave identical PCR results, the classification of PCR positive or negative applies to both the MWP and the CA tests. Of the 31 PCR-positive samples, 20 were culture positive and 11 were culture negative. All 199 PCR-negative samples were culture negative.

The results of the MWP and CA tests for HCV are as follows. Sixty samples were positive by both tests, and 154 samples were negative by both tests. No sample testing positive by one test was negative by the other test. Therefore, there was 100% concordance between the two PCR systems.

In the carryover studies with the CA system, a strong positive result was simulated by using a sample containing 3×10^{14} copies of the *C. trachomatis* amplicon per ml. The strongly positive sample was placed in tubes on the thermal cycler and held at 20, 71, or 91°C. One pattern used to load the rings was to alternate the strongly positive samples with negative samples in adjacent wells. Another pattern studied was to start with four strongly positive samples in a row and then insert a negative sample. After temperature equilibration, the detection process was initiated and detection proceeded in the usual manner. There was no carryover at any temperature, whether from a single positive sample or by a gradual buildup of possible contamination with four positive samples and then a negative sample.

For the CA system, work flow studies based on the time study methods of the former CAP workload recording method were used to determine total hands-on time to produce and report a final result. Efforts were made to make these time studies equivalent to those used in the CAP workload system. The equivalent workload times were as follows: for *C. trachomatis*, *N. gonorrhoeae*, and the IC MultiPlex results, 3.3 min total; for *M. tuberculosis*, 4.4 min; and for HCV, 7.9 min. The IC results, if desired, could be obtained with no additional increase in the workload because of the automated detection process in the CA system.

One of the significant findings was the importance of organization of the work flow process in order to maximize throughput with a single CA instrument. For example, Fig. 2 shows the approximate times to the completion of test results in our laboratory when we ran 24 samples for *C. trachomatis-N. gonorrhoeae*, 12 samples for HCV, and 12 samples for *M. tuberculosis* (including controls) in a single day, in the order specified. The first effort was strictly sequential and produced a slower throughput. By proper organization, we were able to achieve a faster throughput, as shown in the time line for a quicker work flow in Fig. 2. We found that it was important to have two people working together for 30 to 60 min at the start of each workday. One person worked with the instrument to load reagents and prepared it for start-up, while the other person performed specimen preparation for the first run of the day. Once the instrument was loaded and started at the earliest time possible, both people could then continue with sample preparation for subsequent assays. Later, a single person could

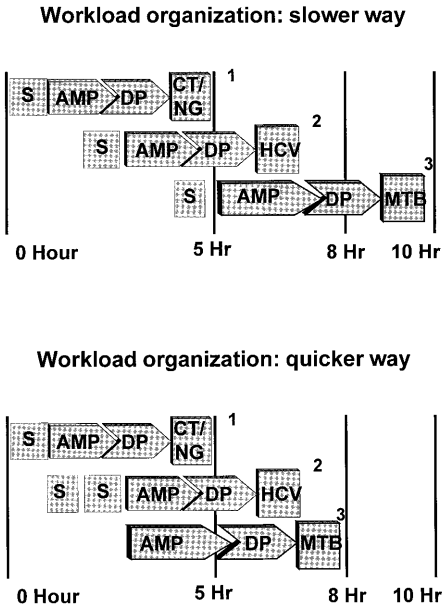

FIG. 2. Organization of workload by using the CA PCR system to produce completed results for 24 samples containing *C. trachomatis-N. gonorrhoeae*, 12 samples containing HCV, and 12 samples containing *M. tuberculosis* within 8 h. 1, Time line to process the *C. trachomatis-N. gonorrhoeae* analytes. S, sample preparation for the assay; AMP, the amplification step; DP, detection process; CT/NG, the time that results are completed for the *C. trachomatis-N. gonorrhoeae* analyte runs. 2, HCV designates the time that results are available for the HCV analyte run. 3, MTB, the time that results are available for the *M. tuberculosis* analyte run. The initial samples for the *M. tuberculosis* PCR were concentrated sputum samples prepared previously. The entire process for all three runs involved 92 detections which were produced in 7.3 h, with 3.8 h of total hands-on time. ICs were run with 20 samples.

complete the runs by occasionally going back to add and remove samples and reagents as needed. The addition of A rings took less than a minute, and switching reagents was also a quick process because of bar coding and the use of reagents from the manufacturer-supplied kit. By these techniques, the throughput for the workload example stated above could be improved so that all results were available in approximately 7.3 h (Fig. 2). While the total time to complete all runs was 7.3 h, because of proper overlapping of the runs, the results for certain analytes were available sooner. For example, for *C. trachomatis-N. gonorrhoeae* results were ready in 5.5 h, for HCV the time to results was 6.6 h, and for *M. tuberculosis* the time to results was 7.3 h. During the 7.3 h needed to produce results for all 92 tests, the total hands-on time to produce those results was approximately 3.8 h. Producing all detection results (including IC results) during a single 8-h shift could be important for smaller laboratories.

If higher throughput is desired and if personnel are available on a second shift for the minor duties of switching rings and reagents, then all specimen preparation can be done during the first 8-h period. Any remaining samples for testing can be placed on the instrument during the next 8-h period. For optimum efficiency, the samples and reagents can be mixed in the A rings in advance so that they are ready to be placed on the instrument. These prepared A rings must be refrigerated and held for a time that does not exceed the manufacturer's recommendations. For HCV, the holding time once a specimen was mixed with the amplification reagents was up to 1 h. For the other two tests, the holding times could be up to 8 h. Periodic exchange of A rings takes less than a minute of hands-on time. This exchange increases the throughput for a single instrument to 208 detections when testing 72 samples for *C. trachomatis-N. gonorrhoeae*, 12 samples for HCV, and 24 samples for *M. tuberculosis* (including controls) in 16 h. The 208 detections included 180 microbe-specific assays and 28 assays for the internal control monitor. For that amount of work, 7.3 h of hands-on time was required. Depending on the analyte, approximately 40 to 80% of the hands-on time is for specimen preparation. For *C. trachomatis-N. gonorrhoeae* the time to final results was 7.8 h. For HCV the time to final results was 11.7 h, and for *M. tuberculosis* the time to final results was 14.9 h. Additional ways to improve throughput would be to prepare the HCV specimens the day before and freeze the samples to have them ready when they were needed the next day. It takes only 9 s, on average, to add a sample to the master mixture.

DISCUSSION

When using either PCR system, sample preparation was performed manually; however, amplification was fully automated in the MWP system, and both amplification and detection were fully automated in the CA system. With the CA system, the laboratory technician was able to walk away after loading the detection reagents and placing the specimens in the thermal cycler module. At the start of each workday, the instrument could be loaded with up to 24 samples for simultaneous amplification. When amplification was finished, the 24 samples could be moved to positions for analyte detection, while 24 additional samples could be placed in the amplification positions. This brought the total to 48 samples in various stages of processing. Aliquots of the amplification products were robotically transferred to the detection module, which performed all reagent additions, incubations, and washes and measured the absorbance of each reaction mixture. Level sensors ensured that sufficient quantities of each reagent were available.

After learning the importance of checking specimens for precipitation on the CA system, we were able to obtain complete correlation between the two PCR systems for MultiPlex testing for *C. trachomatis* and *N. gonorrhoeae* using 199 prospectively obtained samples. We also compared the PCR results for *C. trachomatis* and *N. gonorrhoeae* with those of culture. For *C. trachomatis*, there were 19 culture-positive samples, 18 of which were PCR positive by both PCR systems. There were 179 culture-negative specimens that were negative by both PCR systems. One additional culture-negative specimen was PCR positive when it was independently tested in both PCR systems. False-negative culture results with positive PCR results have been reported, and it is possible that this specimen could represent a false-negative culture result (2, 10). Conversely, one sample was culture positive but tested negative by both PCR systems. There was no attempt to fully arbitrate the results for these samples, but overall sensitivity appeared to be equivalent for each PCR system compared with that for culture. Analysis of the internal control results indicated that there was PCR inhibition in the PCR-negative, culture-positive sample. Diluting the sample 1:20 and repeating the assay produced a positive PCR result for both *C. trachomatis* and the IC. This demonstrated the usefulness of the IC. It could be used to determine which samples yield invalid results and therefore need to be retested.

Although the concept of using a MultiPlexed PCR assay for the diagnosis of sexually transmitted diseases has been reported, this is the first commercially available automated MultiPlexed assay (11). Our study demonstrated that both *C. tra-*

chomatis-N. gonorrhoeae MultiPlexed PCR tests optimally coamplified two unique target nucleic acid sequences, each from two different organisms. The results of the second-generation *C. trachomatis* MWP test and the CA test (see Results) were comparable to those of the first-generation *C. trachomatis* AMPLICOR MWP test which was previously reported to have a sensitivity and a specificity of 97 and 99.7%, respectively, compared with those for culture (2). While some data on the usefulness of PCR for *N. gonorrhoeae* detection are available, few data are available on the usefulness of AMPLICOR assays (9).

For *M. tuberculosis* there was complete correlation between the results obtained with the two PCR systems. In most cases, the culture result and the PCR results also agreed. For the 11 samples that were PCR positive and *M. tuberculosis* culture negative, we attempted to review the clinical data to determine whether these could be false-positive results for PCR or false-negative results for culture. The arbitration was done by using previously described criteria (3). We were able to obtain clinical data for 10 of the 11 discrepant samples. Of those 10 samples, 9 were determined to be from patients diagnosed as having *M. tuberculosis* infection. Although we were unable to obtain a case history for the 11th patient, it is notable that there was also a positive acid-fast bacillus smear for the culture-negative sample. However, without access to the clinical information it was impossible to state conclusively whether this was a true-positive result for *M. tuberculosis*, so the result for that sample must remain inconclusive. The specimens with discrepant results were obtained primarily from patients taking therapy for *M. tuberculosis* infection, resulting in a PCR-positive, but culture-negative result. In conclusion, the results for the CA system for *M. tuberculosis* matched the MWP results. Moreover, the correlation with the culture result was also good, with results being similar to those obtained in our earlier studies by the AMPLICOR test for *M. tuberculosis* (3).

For HCV testing, there was again a complete correlation between the results for the two PCR systems. Of the 60 PCR-positive samples, 59 were ELISA positive for antibody to HCV and 1 tested ELISA negative. Of the 154 PCR-negative samples, 137 were ELISA negative. For the 17 ELISA-positive, PCR-negative samples, no attempt was made to arbitrate the results for these samples with additional testing and examination of clinical findings. Because immunological methods for detecting HCV infection are not reliable at all stages of the disease, the availability of an automated HCV PCR method should prove to be a useful addition to our diagnostic capability (7, 8).

For new molecular diagnostics laboratories, there is little information regarding standardized workload measurements of hands-on time for the various methods and the impact of instrumentation. Goto et al. (5) reported that the AMPLICOR MWP PCR assay took about 5 h to complete, but they did not perform a detailed workload analysis. CAP has been active in laboratory workload assessment for a number of years. The workload evaluation system currently supported by CAP is the Laboratory Management Index Program (LMIP) system. LMIP is a peer-group comparison of productivity, utilization, and financial indicators. LMIP is good for overall analysis of clinical laboratories and even well-established major subsections of the laboratory. That system uses indices to compare one laboratory with another and to help a laboratory compare current performance with past and expected performance. Prior to the use of LMIP, the CAP workload recording system was used (4). That system has the ability to focus on the impacts of specific instruments and procedures. The CAP workload recording system has been useful to laboratory managers and supervisors, especially for evaluating the change in personnel hours required to add new tests, operate new instrumentation, or switch to automation. In 1992, CAP decided that it could no longer afford the time and expense to support a laboratory management system giving that much detail. CAP stopped the development of new CAP workload numbers for new methodologies. The 1992 edition of the *Workload Recording Method and Personnel Management Manual* was the last complete edition. There were some minor updates to workload numbers in the 1993 edition of the CAP newsletter *CAP Today*. After CAP discontinued support for generating new CAP workload values, time studies determining CAP workload numbers for each new test method had to be made by interested individual laboratories. One of the authors of the present report (D.J.) had served on the CAP workload recording committee and had observed the process for determining approved CAP workload values for the CAP *Workload Recording Method and Personnel Management Manual*. The workload times for the CA system were determined by accounting for all categories included in the original CAP workload manual values. This included specimen preparation, periodic and routine maintenance, testing times, supervisor times, and reporting of results, plus other categories normally used to determine a CAP workload result. We also used a workload determination consultant who had experience producing CAP workload values. The consultant helped categorize the two PCR test processes and then independently performed timings of two technologists performing the assays with several different run sizes and on different days. Our workload values differed from the process originally used by CAP in that only one site rather than several sites was studied. Also, because of the newness of the assay, the time to do minor repair and routine maintenance of the instrument was based on our limited experience. Therefore, other sites should report their own workload value determinations before a national average can be obtained, and we consider our values to be only tentative until more studies are done. However, we did attempt to induce a range into the timing process by selecting technologists with different experience levels. Of the two technologists, one had more than 1 year of experience with the CA system and the other had only a few weeks of experience doing tests with the CA system. Despite the differences in levels of experience, their work times were quite close.

Workload value determination was not done for the MWP system in the present study. However, in a previous study we determined the workload value for the MWP test for *C. trachomatis* to be as low as 3 min per sample for a large run of more than 120 samples (2). That time was for *C. trachomatis* only and did not include *N. gonorrhoeae* or IC testing. The workload value found during the present study was 3.3 min for the MultiPlex *C. trachomatis-N. gonorrhoeae* test with the IC. In conclusion, for approximately the same hands-on time as the MWP assay, the automated system was able to produce three test results: one for *C. trachomatis*, one for *N. gonorrhoeae*, and one for the IC. Additional studies are needed at multiple sites with various testing combinations before a completely accurate CAP-type average workload value can be obtained for each analyte.

For *C. trachomatis-N. gonorrhoeae* testing with the CA system, the elapsed time until the first PCR amplification station reload was 2.6 h. The first answers were produced after an additional hour, plus 0.02 h for each additional result. For *M. tuberculosis* testing with the CA system, the elapsed time until the first PCR amplification station reload was 2.9 h. The first answers were produced after an additional hour, plus 0.02 h for each additional result. For HCV testing with the CA system,

the elapsed time until the first PCR amplification station reload was 2.1 h. The first answers were produced after an additional hour, plus 0.02 h for each additional result. For maximum productivity with the CA system, it is important to move specimens from the 24 amplification and detection stations to the 24 detection-only positions as soon as possible. Since there are 12 specimens per ring, this involves moving two rings prior to the addition of two more rings to the instrument. This transfer takes less than 1 min and makes full use of the 48 detection stations on the instrument.

In our experience, the MWP format is suitable for high-volume tests (≥100 specimens per analyte per day), whereas the CA system is more efficient for testing of moderate volumes with multiple analytes and multiple runs. It is easily possible to analyze 72 samples per 8-h workday with multiple detections for each sample. It is possible to test 96 to 118 samples per day if the laboratory is staffed for 9.5 h per day and the instrument is allowed to operate overnight to finish the last testing run. The automated analyzer also offers the user the flexibility of MultiPlex testing because of the ease of coamplification and the detection of two or more targets. It can incorporate the use of internal controls to monitor PCR efficiency or inhibition. The CA system contains two onboard thermal cycler stations which can function independently and which thereby can allow two separate PCR runs to be performed at once. The CA can also support conditional ReFlex testing; for example, if test A is positive, tests B and C are performed, but if test A is negative, test D is performed.

Our automated CA PCR analyzer proved to be a user-friendly, low-maintenance instrument. In the course of our research spanning 1 year, the major problems were that the transfer arm mechanism failed once when a defective D cup could not be released. Another failure occurred once because of possible coring, and the probe needle had to be replaced. Coring occurred when plastic from a cap became embedded in the probe after it pierced the cap. Roche Molecular Systems corrected these initial problems with a modified probe and a new reagent cassette cap. No subsequent problems occurred.

These two AMPLICOR PCR systems provide the clinical laboratory with highly specific and sensitive PCR and DNA probe technology methods for the rapid and reliable detection of specific microorganisms. Our study demonstrated that the prototype *C. trachomatis-N. gonorrhoeae* combination PCR tests optimally coamplify two unique target nucleic acid sequences from a single endocervical swab specimen. The automated CA PCR system also had the ability to amplify and detect such diverse organisms as *M. tuberculosis* and an RNA virus such as HCV simultaneously by making use of the two separate amplification stations in the instrument. Overall, for 842 analytes tested, there was 100% concordance between the results of the manual AMPLICOR MWP PCR system and the automated CA PCR system.

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