Comparison of M4 and M4RT Media for Transporting Cervical Swab Samples for PCR Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Jaber Aslanzadeh and Mathew Jones
Department of Laboratory Medicine, University of Connecticut Health Center, Farmington, Connecticut

Abstract. In a prospective study, M4RT medium was compared to the traditional M4 medium to transport cervical swab specimens for *Neisseria gonorrhoeae/Chlamydia trachomatis* (NG/CT) PCR testing using the Roche COBAS Amplicor. Two cervical swab samples were collected from 270 consecutive patients screened for NG/CT in a satellite facility. The swabs were placed individually in M4RT and M4 medium and were immediately refrigerated, transported to the laboratory on wet ice, and stored at 2 to 8°C until the PCR testing was performed within 7 da of collection. Seven of the cervical swab samples transported in M4 or M4RT were PCR positive for CT. Two additional samples transported in M4RT and a third swab transported in M4 were CT PCR positive. These samples were PCR negative in the alternative medium. Similarly, 12 of the cervical swabs transported in M4 or M4RT were NG PCR positive. Three additional swabs transported in M4 media were NG PCR positive. Initially, 2 of these samples when transported in M4RT were NG PCR equivocal and were considered NG PCR positive on repeat testing. Similarly, 2 additional swab samples transported in M4 RT media were NG PCR positive. These samples, when transported in M4 media, were NG PCR equivocal or negative. However, on repeat testing the equivocal sample was considered NG PCR positive. We conclude M4 and M4RT transport media are equally reliable for transporting cervical swab samples for NG/CT PCR testing. M4RT medium is more convenient to use, as it did not require refrigeration until it was inoculated with the clinical sample.

Keywords: microbiological media; sexually transmitted diseases, gonorrhea, chlamydiasis, cervical culture

Introduction

*Chlamydia trachomatis* is a gram-negative, non-motile bacterium that exists as an obligate intracellular parasite of eukaryotic cells due to their inability to synthesize ATP [9]. *Neisseria gonorrhoeae* is a Gram-negative diplococcus, cytochrome oxidase positive, non-motile and non-sporeforming bacterium [3,5]. Both *C. trachomatis* and *N. gonorrhoeae* are exclusively human pathogens. In fact, *N. gonorrhoeae* and *C. trachomatis* are the leading causes of sexually transmitted disease (STD) in the United States [9,11]. These infections are highest in high-density urban areas among persons under 24 yr of age who have multiple sexual partners and engage in unprotected sexual intercourse. An important factor that may contribute to the persistence of these infections is the occurrence of asymptomatic carriers, particularly in women [11]. While the majority of these infections are uncomplicated, if untreated they may lead to severe complications such as endometritis, pelvic peritonitis, and tubo-ovarian abscesses. To prevent the consequences of these infections in high-risk populations, it is imperative to use an accurate laboratory diagnostic test with utmost sensitivity to identify the infected individuals.

In recent years, several highly sensitive molecular based techniques that rely on enzymatic
amplification of unique sequences of bacterial chromosomes or their plasmids have been developed.

The most common assays utilized in clinical laboratories are based on the Polymerase Chain Reaction (PCR), Ligase Chain Reaction (LCR), or Transcription Mediated Amplification (TMA) assay kits that are manufactured by Roche Diagnostics (Indianapolis, IN), Abbott Laboratories (Abbott Park, IL), and Gen-Probe (San Diego, CA), respectively. These assays have been evaluated extensively by previous investigators [1-3,6,10,12].

The Roche COBAS Amplicor CT/NG procedure is a multiplex PCR test that simultaneously amplifies and detects *C. trachomatis* and *N. gonorrhoeae* in a clinical sample. Given the multiplicity of the target sites for amplification in each of these organisms, the theoretical sensitivity of the test is less than one organism. However, the clinical sensitivity of Amplicor CT/NG, similar to clinical sensitivity of most other PCR assays, is diminished by the presence of PCR inhibitors, technical problems at the time of sampling, improper transport, or storage.

M4 transport medium (MicroTest, Inc., Lilburn, GA) is required by the FDA to transport cervical or urethral swab samples for COBAS Amplicor CT/NG testing. To maintain the integrity of M4 medium, it is recommended that the medium be refrigerated at all times. Recently, a new M4 room temperature (M4RT), that does not require refrigeration during storage has been developed. Both media consist of modified Hank's balanced salt solution supplemented with bovine serum albumin, gelatin, sucrose, and glutamic acid. They are buffered to pH 7.3±0.2 with HEPES buffer and phenol red is used as the pH indicator.

M4 medium contains vancomycin, amphotericin B, and colisitin to inhibit growth of contaminating normal microbial flora. M4RT medium, however, contains gentamycin instead of vancomycin and amphotericin B.

The aim of the present study was to determine whether M4RT is an adequate substitute for M4 medium for transporting cervical samples for CT/NG PCR testing by the COBAS Amplicor.

**Materials and Methods**

In a prospective study, 2 cervical swab samples were collected from 270 consecutive patients who were screened for CT/NG in a satellite facility. The swabs were placed individually in M4 and M4RT medium and were immediately refrigerated until transported to the testing site on wet ice. All specimens were stored at 2-8°C and tested for CT/NG within 7 days of collection.

PCR amplification was performed in accordance with the manufacturer’s protocol. Briefly, all liquid was expressed from the swab by pressing it against the side of the tube. An aliquot (100 µl) of each sample was treated with 100 µl of CT/NG lysis solution (Tris-HCl buffer, <1% solublizer, and 0.09% sodium azide) and incubated at room temperature for 10 min. Following incubation, 200 µl of CT/NG specimen diluent (Tris-HCl solution containing 6 mM MgCl₂, <25% detergent, and 0.05% sodium azide) was added to each tube and incubated at room temperature for 10 min. An aliquot (50 µl) of the processed specimen was transferred into an amplification tube (A-tube) containing 50 µl of amplification mixture.

PCR components included biotinylated primers, the thermostable Taq DNA polymerase, deoxynucleoside triphosphates (dNTPs), including deoxyuridine (in place of thymidine) triphosphates, uracil N-glycosylase (UNG), and reaction buffer (Tris-HCl buffer, EDTA, 100 mM KCl, glycerol 0.01%). PCR was performed in a COBAS Amplicor analyzer for 35 cycles.

The PCR target for *N. gonorrhoeae* is a highly conserved DNA sequence (M. Ngo Pii) of ~1044 base-pairs that is not found in most other non-gonococcal Neisseria species [7]. The Amplicor CT/NG tests for *N. gonorrhoeae* use biotinylated primers SS01 and SS02 to amplify a sequence of ~201 nucleotides [7]. Similarly, *C. trachomatis* contains a ~7,500 base-pair cryptic plasmid that is common to all serovars of *C. trachomatis* [8]. The COBAS Amplicor CT/NG test for *C. trachomatis* uses the biotinylated primers CP24 and CP27 to amplify a sequence of ~207 nucleotides within the cryptic plasmid DNA of *C. trachomatis* [6]. PCR
amplification products were detected automatically by the COBAS analyzer.

PCR results were interpreted as recommended by the manufacturer. Specimens with equivocal findings for CT were considered inconclusive and repeat samples were collected. However, specimens with equivocal findings for NG were repeated in duplicate and interpreted using all three test results. Any repeat equivocal samples were followed-up with fresh specimens.

Results

As listed in Tables 1 and 2, of the 270 submitted specimens, 250 (93%) samples were PCR negative for NG and 261 samples (97%) were PCR negative for CT. Four samples transported in either media were PCR positive for both CT and NG. Seven cervical swab samples transported in M4 or M4RT were PCR positive for CT. Two additional samples transported in M4RT and a third sample transported in M4 were PCR positive for CT. These samples were PCR negative in the alternative transported media.

Similarly, 12 (4.4%) cervical swabs transported in M4 or M4RT were NG PCR positive. Three additional swabs transported in M4 medium were NG PCR positive. Two of these samples were NG PCR equivocal and one sample was NG PCR negative when transported in M4 RT. In addition, 2 additional swab samples transported in M4RT medium were NG PCR positive. These samples were either PCR negative or PCR equivocal when transported in M4 medium.

In subsequent testing of the discrepant results, irrespective of transport medium, all the NG PCR positive samples remained NG PCR positive and all the NG PCR negative samples remained NG PCR negative. The 2 equivocal samples transported in M4 medium were considered positive on repeat testing. Similarly, on repeat testing, the equivocal sample transported in M4RT was NG PCR positive.

The combination of 2 swabs resulted in 10 positive C. trachomatis and 17 positive N. gonorrhoeae. Overall, PCR performed on the M4-transported sample had a sensitivity of 80% and 94% for detecting C. trachomatis and N. gonorrhoeae, respectively. The sensitivity of PCR on M4RT transported swabs of these organisms was 90% and 88%, respectively.

Discussion

The proper transport of clinical samples to the testing site is one of the most important factors that influences the outcome of clinical testing. Thus, properly selected and validated transport medium for individual tests is essential for ideal patient care. Currently, there are at least three amplification techniques that are licensed by FDA for detecting CT/NG in clinical samples [1-3,6,10,12]. M4 medium is the recommended medium to transport cervical and urethral samples for Amplicor CT/NG testing. This medium, while reliable, requires refrigeration at all times.

Recently, MicroTest, Inc., developed a modified formulation of this M4 medium, M4RT, that does not require storage at 4°C prior to inoculation.

| Table 1. Comparison of M4 and M4RT media for transporting cervical swab samples for PCR detection of C. trachomatis by Roche COBAS Amplicor. |
|-----------------------------------|-----------------|-----------------|
|                                    | Resolved positives for C. trachomatis |
|                                    | M4 positive | M4 negative |
|___________________________________|-------------|-------------|
| M4RT positive                      | 7           | 2           |
| M4RT negative                      | 1           | 261         |

| Table 2. Comparison of M4 and M4RT media for transporting cervical swab samples for PCR detection of N. gonorrhoeae by Roche COBAS Amplicor. |
|-----------------------------------|-----------------|-----------------|
|                                    | Resolved positives for N. gonorrhoeae |
|                                    | M4 positive | M4 negative |
|___________________________________|-------------|-------------|
| M4RT positive                      | 15          | 1           |
| M4RT negative                      | 1           | 250         |
Because most PCR assays are sensitive to the presence of potentially inhibiting substances in the milieu, we evaluated the utility of M4RT for transporting cervical swabs. Two cervical swab samples were collected from 270 patients and placed randomly in M4 and in M4RT transport media. Swabs were transported to the testing site within 24 hr and PCR testing was performed by COBAS Amplicor for CT/NG within 5 da of collection.

PCR detected 8 CT and 15 NG among the 270 samples transported in M4 media. Similarly, PCR detected 9 CT and 14 NG among the samples transported in M4RT media. Results from our study clearly demonstrated that M4 and M4RT transport media are equally reliable for transporting cervical swab samples for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* PCR testing by COBAS Amplicor CT/NG. The overall sensitivities of PCR performed on the M4-transported sample were 80% and 94% for detecting *C. trachomatis* and *N. gonorrhoeae*, respectively. The sensitivity of PCR on M4RT-transported swabs of these organisms were 90% and 88%, respectively.

As presented in Tables 1 and 2, the combination of the 2 transport media, 2 swabs/patient, resulted in 2 additional NG positives and 1 additional CT positive findings by PCR testing. The minor difference in the performance of the two media was probably due to inherent sampling error. In our experience, M4RT medium was more convenient as it did not require refrigeration until it was inoculated with the clinical sample.

**Acknowledgement**

This work was supported by a grant from Roche Diagnostics Corporation.

**References**