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Inhibition of PCR by Agar from Bacteriological Transport Media

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We observed inhibition of PCR in throat swabs submitted in routine bacteriological transport media. Experimental studies showed that agar, which was extracted with DNA by DNAzol (Gibco BRL, Gaithersburg, Md.), was the inhibitory agent. No inhibitory effect was observed with a transport medium which did not contain agar.

PCR is a powerful tool for detecting microorganisms. One of the barriers to the introduction of PCR in a diagnostic laboratory is the lack of data on the suitability of readily available specimen transport systems.

We wished to use PCR for the detection of *Mycoplasma pneumoniae*, using primers that had been validated by other workers (5, 6). We wanted to be able to have specimens collected by a simple and readily available method. Throat swabs have been used in published studies of the detection of *M. pneumoniae* by PCR (3, 4, 6) and have been found to have a lower rate of inhibition of PCR than nasopharyngeal aspirates in one study (4). However, the transport media used for throat swabs in these studies have been varied and included Trypticase broth with albumin and antibiotics (4), 2SP medium (3), or media for culture of *M. pneumoniae* (3, 6). We therefore set out to determine whether throat swabs in routine transport media available in clinics and doctors' offices in our area are suitable for PCR.

Throat swabs are usually sent to this laboratory in either Amies Clear transport medium or Chan Universal transport medium (NCS Diagnostics, Etobicoke, Ontario, Canada) and occasionally in Amies medium with charcoal (1). Chan Universal medium contains morpholinepropanesulfonic acid (MOPS), sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium thiosulfate, and agar at undisclosed concentrations. Amies Clear transport medium (modified Stuart's medium [1] without charcoal) is similar to Chan Universal medium but uses phosphate as a buffer and 0.1% sodium thioglycolate as a reducing agent. Specimens for isolation of *M. pneumoniae* are transferred on arrival at the laboratory to Mycoplasma Transport Medium (MTM). MTM consists of PPLO broth base (Difco, Detroit, Mich.) with 0.1% yeast extract, 0.002% Phenol Red, 20% horse serum, 0.5% glucose, and 1,000 U of penicillin per ml (pH 7.7). MTM can be supplied by the laboratory to clinicians but has to be stored frozen and brought to room temperature before use.

Published primer sequences were used to detect the normal human gene for β-globin (6). Swabs for PCR were expressed vigorously in 1 ml of phosphate-buffered saline (PBS). The PBS was centrifuged in a microcentrifuge, and the supernatant was discarded, leaving any pellet and approximately 0.1 ml of PBS. To this was added 0.5 ml of DNAzol reagent (Gibco BRL), and the sample was lysed by gentle pipetting. DNA was then precipitated with 0.25 ml of absolute ethanol, collected by centrifugation, and washed twice with 1 ml of 95% ethanol, and the pellet was dried thoroughly before being dissolved in water. The PCR was performed in a Perkin-Elmer PCR 2400. After an initial 4 min at 95°C, there were 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a period of further extension for 7 min at 72°C and cooling to 4°C. The PCR product was detected by looking for a band of the appropriate molecular weight in a 1.5% agarose gel stained with ethidium bromide.

Among routine throat swabs submitted to the laboratory, 6 of 44 in Amies Clear transport medium and 16 of 34 in Chan Universal transport medium were negative for β-globin by PCR. No positive PCR results were obtained from three swabs in Amies medium with charcoal. These results might be explained by poor collection technique or by inhibition of the PCR by the transport media.

We first investigated whether thioglycolate, thiosulfate, or charcoal is inhibitory to PCR. These substances were chosen because we thought they might interact chemically with the PCR and because our preliminary data obtained with routine throat swabs led us to focus on the differences between the various transport media. We used MTM as the diluent because we had been able to obtain positive PCR results for β-globin in all of 27 sputum and bronchoalveolar lavage samples that had been stored in MTM. We added various quantities of thioglycolate (0.05, 0.1, and 0.5%), thiosulfate (0.1, 0.5, and 1%), and charcoal (0.5, 1.0, and 2.0%) to MTM to mimic and exceed those expected in the transport media. Swabs of sputum samples were placed in the MTM with additives and kept at room temperature overnight. Each dilution of each substance was tested with swabs of four sputum samples. The PCR for β-globin was positive in all cases.

The next experiment was designed to test whether Amies Clear transport medium is truly inhibitory. We collected pairs of buccal swabs (Culturette EZ II; BBL, Cockeysville, Md.) from 24 volunteers. One of each pair was placed in Amies Clear transport medium, while the second was kept in its original container, which contained no transport medium. The PCR was performed after overnight storage at room temperature. All of 24 swabs in tubes without transport medium were

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positive for β-globin, while only 15 of the 24 swabs kept in Amies Clear transport medium were positive ($P < 0.01$).

A further experiment was carried out to determine whether the effect of Amies Clear transport medium might be due to a mechanical effect (removing the specimen from the swab) or due to inhibition by the transport medium itself. Pairs of buccal swabs were collected from 18 volunteers and expressed in PBS. A small fragment of Amies Clear transport medium (approximately 10 mg) was added to one of each pair of tubes before DNAzol treatment. Of the 18 samples with no added Amies Clear medium, 16 were PCR positive for β-globin while only 7 of 18 samples with added agar were PCR positive ($P < 0.05$). It was noted that the pellet obtained from specimens with added agar was bigger, presumably due to extraction of agar with DNA by the DNAzol.

We then tested whether agar itself, without the other constituents of the transport medium, could inhibit the PCR. Pairs of buccal swabs were collected as before, but this time a fragment of plain agar (1.35% in water; BBL) of approximately 10 mg was added to one of each pair of tubes of PBS after expression of the swabs. Among 20 samples without added agar, 17 were positive for β-globin while only 8 of 20 with added agar were positive ($P < 0.05$).

To determine the amount of agar required to inhibit the PCR, we collected and expressed 28 buccal swabs as before. The expressed material in PBS was then pooled and aliquoted into 1-ml volumes to provide a consistent source of β-globin genes. We then added 200, 100, 50, 25, 12.5, 6, or 0 μl of agar (1.35% in water) to each tube, giving four replicates for each volume of added agar. The PCR for β-globin was positive with all tubes containing ≤12.5 μl of agar but negative with all tubes containing ≥25 μl of agar.

We then investigated whether Multi-Microbe Medium (Micro Test Inc., Snellville, Ga.) would be suitable as a transport medium for PCR. Multi-Microbe Medium contains no agar and was designed as a transport medium for specimens for isolation of viruses, mycoplasmas, and chlamydiae. Pairs of buccal swabs were again collected. One of each pair was placed in Multi-Microbe Medium, while the second was kept in its original container without transport medium. PCR was performed after overnight storage. All of 24 pairs of swabs were PCR positive for β-globin.

We have found that placing swabs in Amies Clear medium causes inhibition of PCR and that the inhibitory component of the transport medium is agar that is dissolved by DNAzol and subsequently precipitated out with the DNA by ethanol. The experimental system used closely models throat swabs that may be routinely submitted to a diagnostic laboratory. It may be possible to avoid the inhibition problem for swabs in Amies Clear medium by using a different DNA extraction procedure. Other workers have noted inhibition of PCR by calcium alginate and aluminum swab shafts (7) and by mucolytic agents (2). Materials used for specimen collection, transport, and processing, as well as DNA extraction procedures, need to be carefully considered and optimized for PCR.

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