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BIOLOGICAL INDICATOR OF: exposure to *M. tuberculosis*

SYNONYMS: TB, tubercle bacilli

SAMPLING		MEASUREMENT	
SAMPLER:	FILTER (PFTE filter, 37-mm)	**TECHNIQUE:**	POLYMERASE CHAIN REACTION (PCR)/MICROPLATE READER [2]
FLOW RATE-RANGE:	4 L/min or higher [1]	**ANALYTE:**	*M. tuberculosis*
RECOMMENDATION:	if the airborne particle concentration is low, sample for at least 8 hours and/or use high volume sampling; in the laboratory studies, sampling times were 10 min	**WAVELENGTH:**	450 nm
PRESERVATIVE:	none	**QUALITY CONTROL:**	3 laboratory negative PCR controls, all should read less than 0.25 absorbance units; 2 laboratory positive controls, both should read 2.0 absorbance units or greater
SHIPMENT:	follow CDC guidelines for interstate shipment of human pathogen (42 CFR Part 72); may ship at ambient temperature	**RANGE:**	purified *M. tuberculosis* H37Ra DNA, 1–300 copies; H37Ra mycobacteria, approximately 4 to 1950 particles (all higher ranges will test positive)
SAMPLE STABILITY:	1 week or more at ambient temperature; indefinitely at –20 °C	**ESTIMATED LOD:**	approximately 20 mycobacteria particles (from air samples)
CONTROLS:	2 or more field blanks		

APPLICABILITY: This is a qualitative method which permits the detection of airborne *M. tuberculosis* particles. It will detect approximately 20 or greater *M. tuberculosis* particles. This method does not indicate how many particles were detected.

INTERFERENCES: Positive interferences, *M. bovis*, *M. bovis BCG*; negative interferences, metals, and other unknown airborne particulate matter. (Note: to detect suspected negative interferences, spike field samples which resulted in negative readings with *M. tuberculosis* H37Ra DNA or H37Ra particles and rerun assay. Alternately, the Roche positive control may be used instead of H37Ra.)

OTHER METHODS: The measurement technique was originally developed by Roche Diagnostic Systems for the analysis of clinical samples [2]. Various other *M. tuberculosis* detection methods are now available, such as Gen-Probe [3,4] and Digene [5].

REAGENTS:

1. Filter stripping solution containing 1% Triton X-100 in 10 mM Tris-HCl, pH 8.0. Alternately, the Sputum Wash solution (Roche) may be used.
2. Roche reagent kit, the AMPLICOR *Mycobacterium tuberculosis* test, containing various solutions and controls.
3. Bleach (5.25% sodium hypochlorite).

See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: polytetrafluoroethylene (PTFE) filter, 37-mm, 1.0-µm pore size, with cellulose support pad in plastic, three-piece filter cassette (Costar #130810).
2. Sampling pump, 4 L/min or greater, with flexible connecting tubing.
3. Petri dishes, polystyrene, 50-mm (Gelman #7242, or equivalent).
4. Forceps and/or tweezers.
5. Disposable gloves.
6. Platform clinical rotator.
7. Serological pipets, sterile, disposable, 2.0-mL.
8. Microcentrifuge tubes, 2.0-mL.
9. Microcentrifuge.
10. Heating block or water bath.
11. PCR thermocycler and accessories.
12. Microplate reader.
13. Microplate washer.
14. Microplate incubator.
15. Aerosol barrier pipettor tips or positive displacement micropipettors.
16. Sample bags, size 10.2 × 15.2 cm.

SPECIAL PRECAUTIONS: Entering rooms contaminated with airborne TB is a health risk. Appropriate respiratory protection should be worn [6]. Filter samples should be opened in a biosafety cabinet. Once the samples are heated for the specified time period, there is little risk to the laboratory worker conducting the analysis. The TB PCR method is extremely sensitive. Therefore, segregation of the various laboratory activities is essential in order to prevent the generation of false positives.

SAMPLER ASSEMBLY:

1. Assemble each filter cassette in a clean environment wearing disposable, preferably powder free or low powder, gloves.
   a. With forceps, insert support pad and PTFE filter into lower section of cassette.
   b. Attach middle and top cassette sections and insert plugs.
   c. Place shrinkable sealing band around cassette and air dry.
   d. Place each filter cassette into a sample bag for transport (one sampler/bag).

SAMPLING:

2. Calibrate each pump with a representative filter cassette in line.
3. Sample closed-face at a flow rate of 4 L/min or greater. The sampling time period depends on the suspected concentration. If the concentration is unknown, sample for long time periods (hours).
   NOTE: If the airborne particle concentration is unknown, assume the particles are present at a low concentration.
4. Reinsert cassette plugs, place each filter cassette in a sample bag (one cassette/bag), and pack securely for shipment. Ship at ambient temperatures, according to CDC guidelines for shipment of human pathogens (42 CFR Part 72).

SAMPLE PREPARATION:

5. Place 2.0 mL of filter stripping solution in a 50-mm Petri dish.
6. Remove the PTFE filter with a forceps or tweezers.
7. Wet both sides of the filter by touching each side of the filter to the stripping solution and then place the filter (one filter/dish), sample side up, in the dish. Cover tightly.
8. Place the dishes on the platform clinical rotator and strip the filters for 30 min. The stripping solution should move back and forth across the surface of the filter.
9. Transfer the stripping solution from each filter to a 2.0-mL microcentrifuge tube.
10. Centrifuge at 12500 × g for 10 min and decant the supernatant into a beaker containing bleach. (Residual stripping solution should be removed from the microcentrifuge tubes).
11. Add 100 µL Roche lysis reagent to each microcentrifuge tube, close lids tightly, and heat at 60 °C for 45 min.
12. Follow steps described in the AMPLICOR *Mycobacterium tuberculosis* test booklet contained in the Roche reagent kit.

CALIBRATION AND QUALITY CONTROL:

13. Calibrate the PCR thermocycler and microplate reader according to the manufacturer's instructions.
14. Prepare positive and negative controls as described in the Roche reagent booklet.
    NOTE: Negative and positive controls are included in the Roche reagent kit. Include 3 negative controls and 2 positive controls each time the test is performed, randomizing the positions of these samples in the test.
    Discard the run:
    a. If one or more of the negative control values exceeds 0.25 absorbance units.
    b. If either of the positive control values falls below 2.0 absorbance units.

MEASUREMENT:

15. *Mycobacterium tuberculosis* is considered present in the sample if the absorbance of the unknown sample is equal to or greater than 0.35 absorbance units. A sample yielding an absorbance value less than 0.35 absorbance units is considered negative for *Mycobacterium tuberculosis*.

CALCULATIONS:

16. Since this is a qualitative method (positive/negative), no special calculations are required.

REFERENCES:

[1] Schafer MP, Fernback JE, Jensen PA [in press]. Sampling and analytical method development for qualitative assessment of airborne mycobacterial species of the *Mycobacterium tuberculosis* complex. Am Ind Hyg Assoc J (submitted).
[2] Devallois A, Legrand E, Rastogi N [1996]. Evaluation of Amplicor MTB test as adjunct to smears and culture for direct detection of *Mycobacterium tuberculosis* in the French Caribbean. J Clin Microbiol *34*:1065–1068.
[3] Pfyffer GE, Kissling P, Wirth R, Weber R [1994]. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J Clin Microbiol *32*:918–923.
[4] Bodmer T, Gurtner A, Schopfer K, Matter L [1994]. Screening of respiratory tract specimens for the presence of *Mycobacterium tuberculosis* by using the Gen-Probe amplified *Mycobacterium tuberculosis* direct test. J Clin Microbiol *32*:1483–1487.

[5] Huang T-S, Liu Y-C, Lin H-H, Huang W-K, Cheng D-L [1996]. Comparison of the Roche AMPLICOR MYCOBACTERIUM Assay and Digene SHARP signal system with in-house PCR and culture for detection of *Mycobacterium tuberculosis* in respiratory specimens. J Clin Microbiol *34*:3092–3096.
[6] NIOSH [1996]. Protect yourself against tuberculosis—a respiratory protection guide for health care workers. DHHS (NIOSH) Publication No. 96-102.

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