JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2006, p. 612–613 Vol. 44, No. 2
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.2.612–613.2006

False-Positive Amplified Mycobacterium Tuberculosis Direct Test Results for Samples Containing *Mycobacterium leprae*

Pam Chedore,[1]* George Broukhanski,[1] Zev Shainhouse,[2] and Frances Jamieson[1]

Ministry of Health and Long-Term Care, Public Health Laboratories Branch, Etobicoke, Ontario,[1] and The Scarborough Hospital-General Division, Toronto, Ontario,[2] Canada

Received 29 September 2005/Returned for modification 7 November 2005/Accepted 20 November 2005

Nucleic acid amplification tests are widely used in mycobacteriology laboratories to rapidly detect *Mycobacterium tuberculosis* complex directly in clinical specimens. A positive result provides an early diagnosis of tuberculosis, allowing initiation of appropriate therapy and public health measures.

The Amplified Mycobacterium Tuberculosis Direct (MTD) test uses isothermal transcription-mediated amplification and a hybridization protection assay to detect rRNA from *Mycobacterium tuberculosis* complex (MTBC) in clinical specimens. The test is approved by the U.S. Food and Drug Administration for use with respiratory specimens that are acid-fast bacillus (AFB) smear positive and smear negative. Numerous published studies have demonstrated that the MTD test is also sensitive and specific when it is used to test nonrespiratory samples, including lymph nodes. Studies report that the specificity of the MTD test for nonrespiratory specimens ranges from 97.7 to 100% (3, 5).

This report describes a false-positive MTD test result from a smear-positive (and, ultimately, culture-negative) lymph node specimen from a patient later clinically diagnosed with lepromatous leprosy. The diagnosis was confirmed in the laboratory by a positive in-house PCR assay result for *Mycobacterium leprae* (6) and a negative PCR test result for *M. tuberculosis* (1).

A 27-year-old male patient from India presented with a 4-month history of fever, anorexia, malaise, weight loss, and erythema nodosum-like lesions on his legs and forearms. A biopsy of an enlarged inguinal lymph node revealed caseating granulomata and numerous acid-fast positive bacilli on Ziehl-Neelsen staining. A portion of the node was sent to the Central Public Health Laboratory (CPHL) for mycobacterial culture. Due to thenar muscle wasting, a biopsy of the skin on a forearm nodule was performed and confirmed a clinical diagnosis of leprosy. Fite staining of the initial lymph node demonstrated organisms which were morphologically typical of *M. leprae*. The formalin-fixed, paraffin-embedded skin biopsy specimen was submitted to CPHL for PCR testing for the detection of MTBC and *M. leprae.* The patient was initially treated for tuberculosis and leprosy. Treatment for tuberculosis was discontinued when cultures and PCR for *M. tuberculosis* were reported to be negative. The response to leprosy therapy was excellent.

At the laboratory, the lymph node biopsy specimen was ground in a small amount of sterile buffer. The ground tissue was then processed by the standard NaOH-*N*-acetyl-L-cysteine method for 5 min. A fluorescence-stained smear of the processed tissue showed 3+ AFB. The MTD test was performed according to the laboratory protocol for new smear-positive specimens. Following the manufacturer's instructions a 450-μl aliquot of the processed tissue was tested. Other aliquots were inoculated into two cultures, a Mycobacterium Growth Indicator Tube (Becton Dickinson, Sparks, MD) and a Lowenstein-Jensen solid medium slant. The cultures were incubated at 37°C for 7 weeks.

The MTD test result was positive, at 1,875,376 relative light units (RLUs). The positive control was 2,862,128 RLUs, and the negative control was 1,894 RLUs (see Table 1 for the cutoff values). All cultures were negative for growth at 7 weeks.

Due to the clinical suspicion of leprosy, approximately 200-μl aliquots of the processed lymph node material were tested by in-house PCR assays for both *M. leprae* and MTBC (1, 6). The primers for *M. leprae* PCR target the 18-kDa protein gene, and the primers for *M. tuberculosis* PCR target the IS*6110* insertion sequence. DNA was extracted from the skin biopsy specimen by using a DEXPAT (TaKaRa Bio Inc. Shiga, Japan) extraction kit and was tested as described above. Both the lymph node biopsy specimen and the skin biopsy specimen were positive for *M. leprae* and negative for MTBC by the in-house PCR assays.

To further investigate the discrepant MTD test result obtained with the node specimen, we obtained *M. leprae* culture material from the National Hansen's Disease Programs at Louisiana State University. The culture material received consisted of *M. leprae* bacteria that were grown in the footpads of nude mice, homogenized, and suspended in 1 ml of 7H12 broth at a concentration of 10^9 bacteria per ml. At CPHL, the material was serially diluted to concentrations of 10^6 to 10^4. These dilutions were tested by the MTD test, and corresponding smears were made and stained with auramine-rhodamine. AFB smears were positive to a concentration of 5×10^4 per ml (Table 1). The results obtained by the MTD test were positive to a concentration of 10^5 AFB per ml, with an indeterminate result obtained with 5×10^4 bacteria per ml. Both the smear and the MTD test gave negative results at 10^4 bacteria per ml (Table 1).

While leprosy is rarely encountered in developed countries, the potential to miss the diagnosis exists when a tissue speci-

* Corresponding author. Mailing address: Ministry of Health and Long-Term Care, Public Health Laboratories Branch, TB and Mycobacteriology, 81 Resources Road, Etobicoke, Ontario M9P 3T1, Canada. Phone: (416) 235-5993. Fax: (416) 235-6013. E-mail: pam.chedore@moh.gov.on.ca.

TABLE 1. Results of MTD testing and AFB smears for samples containing *M. leprae*

Sample	RLUs		MTD interpretation[b]	AFB smear result
	MTD test	MTD test positive control		
Lymph node	1,875,376	2,862,128	Positive	3+
ML[a] (10^6)	1,479,934	2,344,839	Positive	2+
ML (10^5)	1,096,960	2,344,839	Positive	1+
ML (5×10^4)	119,779	2,344,839	Indeterminate	±
ML (10^4)	3,164	2,344,839	Negative	Negative

[a] ML, *M. leprae* culture material.
[b] MTD test RLU cutoff and interpretation: >500,000 RLUs, positive; 30,000 to 499,000 RLUs, indeterminate; <30,000 RLUs, negative.

men demonstrating caseating granulomata and AFB from *M. leprae* yields a false-positive MTD test result, leading to a misdiagnosis of tuberculosis. Nasal carriage and shedding of *M. leprae* occur in leprosy patients and their contacts (4). In countries where both tuberculosis and leprosy are endemic, testing of a respiratory specimen by the MTD test could yield a false-positive result, which would also lead to a misdiagnosis of tuberculosis.

When a clinical suspicion of leprosy exists and tissue specimens are AFB smear positive, mycobacterial culture negative, but MTD test positive, further testing for *M. leprae* by alternate methods such as PCR is highly recommended.

We thank T. Gillis, National Hansen's Disease Programs at Louisiana State University, for provision of *M. leprae* culture material. We also thank Joanne Blair, Mahaswaran Chithamparapillai, and Daria Pyskir for technical assistance and Suzanne Lombardi for secretarial assistance.

REFERENCES

1. **Eisenach, K. D., M. D. Cave, J. H. Bates, and J. T. Crawford.** 1990. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J. Infect. Dis. **161:**977–981.
2. Reference deleted.
3. **Kerleguer, A., M. Fabre, J. J. Bernatas, Gerome, P. Nicand, E. V. Herve, and J. L. Koeck.** 2004. Clinical evaluation of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct test for rapid diagnosis of tuberculosis lymphadenitis. J. Clin. Microbiol. **42:**5921–5922.
4. **Klatser, P. R., S. van Beers, B. Madjid, R. Day, and M. Y. L. de Wit.** 1993. Detection of *Mycobacterium leprae* nasal carriers in populations for which leprosy is endemic. J. Clin. Microbiol. **31:**2947–2951.
5. **Pfyffer, G. E., P. Kissling, E. M. I. Jahn, H.-M. Welscher, M. Salfinger, and R. Weber.** 1996. Diagnostic performance of Amplified Mycobacterium Tuberculosis Direct test with cerebrospinal fluid, other nonrespiratory, and respiratory specimens. J. Clin. Microbiol. **34:**834–841.
6. **Williams, D. L., T. P. Gillis, R. J. Booth, D. Looker, and J. D. Watson.** 1990. The use of a specific DNA probe and polymerase chain reaction for the detection of *Mycobacterium leprae*. J. Infect. Dis. **162:**193–200.