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Evaluation of the New MB Redox System for Detection of Growth of Mycobacteria

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We evaluated a new mycobacterial culture system, MB Redox, for recovery rate and time to detection of mycobacteria from 742 consecutive respiratory specimens and compared the results to those found with Löwenstein-Jensen (LJ) medium. Twenty specimens (2.7%) were positive for *M. tuberculosis*: 17 on LJ medium and 19 in MB Redox, with 16 specimens positive in both media. In addition, 24 specimens (3.2%) were positive for nontuberculous mycobacteria (NTM), 20 on LJ medium, 18 in MB Redox, and 14 in both media. For *M. tuberculosis*, the mean times to detection were 28.9 days on LJ medium and 23.6 days in MB Redox, and for NTM, the mean times to detection were 40.6 days on LJ medium and 32.3 days in MB Redox.

Tuberculosis has reemerged as a significant public health problem in developed countries (11). In addition, infections caused by nontuberculous mycobacteria (NTM) are nowadays more frequent and involve more various agents (4, 14). Rapid identification of patients with active pulmonary tuberculosis is needed to speed up the implementation of drug therapy and contact investigation. For the diagnosis of NTM infections, sensitive culture systems are needed to isolate the various causative agents. These systems must also allow subsequent rapid identification and susceptibility testing, since adequate therapy depends on the NTM involved (14). Conventional diagnostic methods for tuberculosis and other mycobacterial infections include smear staining and culture of respiratory specimens (7). Smear staining is rapid and inexpensive, but is only 30 to 40% sensitive. Culture is more sensitive but slow. New culture systems allow early detection of mycobacterial growth by combining a liquid medium and a sensor based on oxygen consumption or metabolite production, such as in the radiometric BACTEC 460 and the more recent BACTEC 9000, as well as in the MGIT (Becton Dickinson), MB/BacT (Organon), and ESPII (Difco) systems (1, 8, 9, 12, 13).

MB Redox (Biotest, Dreiech, Germany) is a new culture system combining a liquid medium and a redox indicator which enables an easy macroscopic vision of growth (6). MB Redox tubes contain 4 ml of a Kirchner medium enriched with glycerol, horse serum, vitamins, and antibiotics, i.e., polymyxin B (50,000 U), amphotericin B (5 mg), carbenicillin (25 mg), and trimethoprim (2.5 mg). The tubes also contain an invisible tetrazolium salt which appears as red-to-violet particles when reduced by the growth of mycobacteria. This study was undertaken to evaluate this new system for the detection of growth of mycobacteria from specimens of the respiratory tract. The objectives were, first, to estimate the performance of the MB Redox system in comparison to that of conventional Löwenstein-Jensen (LJ) egg-based solid medium for recovery rate and time to detection of mycobacteria from respiratory specimens and, second, to evaluate the workflow, workload, and ease of use of MB Redox.

For 6 weeks, starting in November 1997, all of the consecutive respiratory specimens for detection of mycobacteria received at the Laboratory of Bacteriology in Pitie-Salpetriere Hospital, a 2,300-bed university hospital in Paris, were included in the study. Specimens were digested and decontaminated under biosafety conditions by the *N*-acetyl-L-cysteine–NaOH procedure (7). After centrifugation, the sediment was diluted in 1.5 ml of 0.2% bovine serum albumin (2) and distributed into two tubes of LJ medium (one with 0.4% sodium pyruvate and one without [0.25 ml in each tube]), one MB Redox tube (0.5 ml), and one glass slide for preparation of smears. LJ medium and MB Redox tubes were examined twice weekly during the first month and then once weekly for a total of 3 months for LJ medium and 2 months for MB Redox. Cultures were designated as positive when at least one colony of acid-fast bacilli (AFB) was observed on LJ medium and when red-to-violet sediment with AFB was observed in MB Redox. Time to detection was defined as the interval in days between the day of positive culture and the day of inoculation. We have compared statistically the culture results by using MB Redox and solid media, with a Yates test used to determine the recovery rate and a Fisher test used to determine the time to detection. Smears were stained with auramine fluorochrome. Mycobacteria grown on LJ medium and in MB Redox were identified by the Accuprobe identification test (GenProbe, San Diego, Calif.) for *M. tuberculosis* complex, *M. avium* complex, *M. kansasii*, and *M. gordonae* and by conventional methods for other species (7). Accuprobe identification tests were performed as recommended by the manufacturer, with one colony grown on LJ medium or with the pellet obtained after centrifugation of 1 ml of the MB Redox liquid culture when this medium was first or solely positive. Susceptibility testing was performed by the proportion method on LJ medium by using either colonies grown on LJ medium or the pellet obtained after centrifugation of 1 ml of MB Redox culture. Clinical data were collected for tuberculosis patients and for patients meeting the criteria for NTM infection (14).

A total of 742 consecutive specimens were included in the study: 320 (43%) expectorated sputa, 265 (36%) gastric aspirates, 77 (10%) bronchoscopic aspirates, and 80 (11%) bron-

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TABLE 1. Recovery of *M. tuberculosis* and NTM from 742 respiratory specimens cultured on LJ medium and in MB Redox

Mycobacterial species (*n*)	No. (%) of specimens with positive culture in[a]:		
	LJ medium	MB Redox	Both
M. tuberculosis (20)	17 (85)*	19 (95)*	16 (80)
AFB positive (8)	8	8	8
AFB negative (12)	9	11	8
NTM (24 [100%])	20 (83)**	18 (75)**	14 (58)
M. kansasii (7)	5	5	3
M. avium complex (4)	4	2	2
M. xenopi (3)	2	3	2
M. gordonae (5)	4	3	3
M. fortuitum (1)	1	1	1
M. chelonae/abscessus (3)	2	3	2
Undetermined (1)	1	1	1
Total (44)	37 (84)	37 (84)	30 (68)

[a] *, $P = 0.59$; **, $P = 0.48$.

choalveolar lavage fluids. The contamination rates were 3.1% (23 specimens) for MB Redox and 2% (15 specimens) for LJ medium. The results for the rate of recovery of *M. tuberculosis* complex are presented in Table 1. Twenty (2.7%) specimens were positive for *M. tuberculosis* complex (20 *M. tuberculosis* isolates). Seventeen (85%) of them were positive on LJ medium, and 19 (95%) were positive in MB Redox, with 16 specimens being positive in both media. Eight specimens (seven sputa and one gastric aspirate) were smear-positive, and all of them were cultured in both media. The 12 other specimens (7 sputa, 3 bronchoscopic aspirates, 1 gastric aspirate, and 1 bronchoalveolar lavage fluid) were smear negative; out of them, 9 were cultured on LJ medium and 11 were cultured in MB Redox. The overall mean times to detection for the 16 specimens positive in both media were 28.9 days on LJ medium and 23.6 days in MB Redox. The mean time to detection observed for LJ medium was in accordance with those observed in studies by van Griefthuysen et al. (13), Rohner et al. (9), and Tortoli et al. (12), but longer than that in studies by Badak et al. (1), Cornfield et al. (3), and Pfyffer et al. (8), who reported a higher contamination rate. When restricted to smear-positive specimens, the mean times to detection were 20.5 days on LJ medium and 17 days in MB Redox, and when restricted to smear-negative specimens, the times to detection were 37 days on LJ medium and 30.1 days in MB Redox. Detailed results are presented in Table 2. Half of the positive cultures were observed after 18 days of incubation (i.e., after the fifth observation) in MB Redox and after 25 days (i.e., after the seventh observation) on LJ medium. In conclusion, the rate of recovery for *M. tuberculosis* seems slightly, but not significantly ($P = 0.48$), higher with MB Redox than with LJ medium, and the time to detection was slightly, but not significantly ($P = 0.29$), shorter. The 20 specimens positive for *M. tuberculosis* belong to nine patients with pulmonary tuberculosis (mean number of positive specimens per patient, 2.2). For four patients, the smear was positive for at least one specimen, and for the other five, all specimens were smear negative. This proportion is consistent with what is usually observed in our hospital and in other places for pulmonary tuberculosis (5).

Twenty-four (3.2%) specimens were positive for NTM, out of which 20 (83%) were positive on LJ medium and 18 (75%) were positive in MB Redox, with 14 (58%) positive in both media. The NTM species isolated are detailed in Table 1. The four species most frequently isolated in clinical laboratories (4), *M. avium* complex, *M. kansasii*, *M. xenopi*, and *M. gordonae*, were well represented. The rates of recovery were slightly superior on LJ medium than in MB Redox, with the difference not statistically significant ($P = 0.48$). For the 14 specimens which gave positive results in both media, the mean times to detection were 40.6 days on LJ medium and 32.3 days in MB Redox ($P = 0.44$), with large ranges of 11 to 90 days in MB Redox and 15 to 85 days on LJ medium (Table 2). Such wide ranges are usually observed in all studies (8, 12), since the time to growth widely differs with regard to NTM species (10). Three patients only were diagnosed for a NTM infection, making a total of 9 of the 24 specimens: 2 with a *M. kansasii* pulmonary infection and 1 with a disseminated *M. avium* complex infection with pulmonary localization. When considering only the specimens yielded by the three NTM infection patients, the rates of recovery were equal (78%) on LJ medium and in MB Redox (seven positive specimens in each medium and five in both media). The mean times to detection for these five specimens were 31.6 days on LJ medium and 39.8 days in MB Redox. Although there was a slight difference in favor of MB Redox for time to detection when considering all the specimens that were positive in both media, the difference was

TABLE 2. Time to detection of *M. tuberculosis* and NTM that grew both on LJ medium and in MB Redox

Specimen with positive culture	Mean days to detection (range)[a]	No. (%) of positive cultures detected by day:											
		7	11	14	18	21	25	28	35	42	49	56	>56
MB Redox													
M. tuberculosis	23.6* (11–49)	0 (0)	1 (6.3)	3 (18.8)	4 (25)	8 (50)	12 (75)	12 (75)	13 (81.2)	15 (93.8)	16 (100)		
AFB positive	17 (11–22)	0	1	3	3	7	8	8	8	8	8		
AFB negative	30.1 (17–49)	0	0	0	1	1	4	4	5	7	8		
NTM	32.3** (11–90)	0 (0)	2 (14.3)	4 (29.6)	4 (29.6)	6 (42.8)	7 (50)	9 (64.3)	9 (64.3)	10 (71.4)	11 (78.6)	13 (92.8)	14 (100)
LJ medium													
M. tuberculosis	28.9* (14–56)	0 (0)	0 (0)	1 (6.3)	3 (18.8)	6 (37.5)	8 (50)	8 (50)	13 (81.3)	14 (87.5)	15 (93.8)	16 (100)	
AFB positive	20.5 (14–33)	0	0	1	3	5	7	7	8	8	8	8	
AFB negative	37 (21–56)	0	0	0	0	1	1	1	5	6	7	8	
NTM	40.6** (15–85)	0 (0)	0 (0)	0 (0)	3 (21.4)	4 (28.6)	5 (35.7)	5 (35.7)	7 (50)	8 (57.1)	9 (64.3)	12 (85.7)	14 (100)

[a] *, $P = 0.29$; **, $P = 0.44$.

in favor of LJ medium when considering only the specimens yielded by the cases of NTM infection. These results need to be confirmed by a large study involving more isolates of each NTM species.

The workflow with the MB Redox system was similar to that with LJ medium. Accuprobe testing was performed with success with all of the MB Redox cultures, except for two which gave negative results on the day of the positive culture, but positive results after being subcultured on LJ medium. Some authors reported that genomic probes can be falsely negative with a culture obtained in liquid medium immediately at the time of the detection, and advocate performing identification after 2 or 3 days of additional incubation (9) or after subculture on solid medium (12). Indeed, when the culture was positive on LJ medium, identification was easily suspected on the basis of colony morphology, which was not possible when the culture was positive only in liquid medium. Susceptibility testing was directly performed by using the pellet from two MB Redox-positive tubes and led to reliable results.

The workload with the MB Redox system was identical to that with LJ medium. The use of MB Redox was secure, since inoculation did not require a needle. Because the tubes are ready to use (there are no growth complexes or antibiotics to add) and the detection system does not require any additional material, MB Redox was easy to use.

In conclusion, the rate of recovery, time to detection, workload, and workflow assessed with MB Redox compared well with those obtained with LJ medium. More precise appraisal of the MB Redox system would require a more extensive evaluation.

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REFERENCES

1. **Badak, F. Z., D. L. Kiska, S. Setterquist, C. Hartley, M. A. O'Connell, and R. L. Hopfer.** 1996. Comparison of Mycobacteria Growth Indicator Tube with BACTEC 460 for detection and recovery of mycobacteria from clinical specimens. J. Clin. Microbiol. **34:**2236–2239.
2. **Cernoch, P., R. Enns, M. Saubolle, and R. Wallace, Jr.** 1994. Cumitechs 16A. Laboratory diagnoses of the mycobacterioses. Coordinating ed., A. S. Weissfeld. American Society for Microbiology, Washington, D.C.
3. **Cornfield, D. B., K. G. Beavis, J. A. Greene, M. Bojak, and J. Bondi.** 1997. Mycobacterial growth and bacterial contamination in the Mycobacteria Growth Indicator Tube and BACTEC 460 culture systems. J. Clin. Microbiol. **35:**2068–2071.
4. **Falkinham, J. O., III.** 1996. Epidemiology of infection by nontuberculous mycobacteria. Clin. Microbiol. Rev. **9:**177–215.
5. **Jouveshomme, S., E. Cambau, D. Trystram, M. Spytzma, W. Sougakoff, J. P. Derenne, and J. Grosset.** 1998. Clinical utility of an amplification direct test based on ligase chain reaction in pulmonary tuberculosis. Am. J. Respir. Crit. Care Med. **158:**1096–1101.
6. **Naumann, L., N. Lehn, H. Wolf, and U. Reischl.** 1997. New rapid cultural nonradiometric method for the detection of mycobacteria, abstr. U-144, p. 568. *In* Abstracts of the 97th General Meeting of the American Society for Microbiology 1997. American Society for Microbiology, Washington, D.C.
7. **Nolte, F. S., and B. Metchock.** 1995. Mycobacterium, p. 400–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
8. **Pfyffer, G. E., H.-M. Welscher, P. Kissling, C. Cieslak, M. J. Casal, J. Gutierrez, and S. Rüsch-Gerdes.** 1997. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. J. Clin. Microbiol. **35:**364–368.
9. **Rohner, P., B. Ninet, C. Metral, S. Emler, and R. Auckenthaler.** 1997. Evaluation of the MB/BacT system and comparison to the BACTEC 460 system and solid media for isolation of mycobacteria from clinical specimens. J. Clin. Microbiol. **35:**3127–3131.
10. **Shinnick, T., and R. Good.** 1994. Mycobacterial taxonomy. Eur. J. Clin. Microbiol. Infect. Dis. **13:**884–901.
11. **Snider, D. E., Jr., M. Raviglione, and A. Kochi.** 1994. Global burden of tuberculosis, p. 3–11. *In* B. R. Bloom (ed.), Tuberculosis: pathogenesis, protection, and control. ASM Press, Washington, D.C.
12. **Tortoli, E., P. Cichero, M. G. Chirillo, M. R. Gismondo, L. Bono, G. Gesu, M. T. Simonetti, G. Volpe, G. Nardi, and P. Marone.** 1998. Multicenter comparison of ESP culture system II with BACTEC 460TB and with Lowenstein-Jensen medium for recovery of mycobacteria from different clinical specimens, including blood. J. Clin. Microbiol. **36:**1378–1381.
13. **van Griethuysen, A. J., A. R. Jansz, and A. G. M. Buiting.** 1996. Comparison of fluorescent BACTEC 9000 MB system, Septi-Chek AFB system, and Lowenstein-Jensen medium for detection of mycobacteria. J. Clin. Microbiol. **34:**2391–2394.
14. **Wallace, R. J., J. Glassroth, D. Griffith, R. Olivier, J. Cook, F. Gordin, and the American Thoracic Society.** 1997. Diagnosis and treatment of disease caused by non tuberculous mycobacteria. Am. J. Respir. Crit. Care Med. **156:** S1–S25.