Analysis Summary of the MTB/NTM DST Performance Evaluation Results (example)

Analyses of the (month, date and year) Performance Evaluation Results for *M. tuberculosis* complex and Non-tuberculous Mycobacteria Drug Susceptibility Testing Reported to the Centers for Disease Control and Prevention by Participating Laboratories

This report is an analysis of laboratory test results reported to the Centers for Disease Control and Prevention (CDC) by participant laboratories for the four *Mycobacterium tuberculosis* complex and one *M. xenopi* strains shipped in (month, date,year). Participant laboratories received either four *M. tuberculosis* strains only or four *M. tuberculosis* and one NTM strain. Testing results were received and analyzed from 140 of 153 (92%) laboratories participating in this shipment. Of the laboratories that did not provide results, three had import permit or license issues; six laboratories had staff, laboratory construction, or culture media problems; and one laboratory no longer participates in the program.

Descriptive Information on Participant laboratories

Figure 1 shows the laboratory classification reported by 140 of the participants. Participants consisted of 76 health departments, 46 hospitals, 12 independents, and 6 "other" type of laboratories.

Figure 2 provides the distribution of the annual volume of *M. tuberculosis* isolates tested for drug susceptibilities by participating laboratories in calendar year xxxx.

Figure 3 lists the biosafety levels reported by participant laboratories for *M. tuberculosis*. All laboratories are strongly encouraged to consult the CDC/NIH manual, Biosafety in Microbiological and Biomedical Laboratories (4th edition) for recommendations and to determine their correct biosafety level.

Figure 4 provides a breakdown of the test procedures used by the participating laboratories for *M. tuberculosis* drug susceptibility testing. Participants were asked to check test methods used. Some methods, such as the proportion method with Lowenstein-Jensen (LJ) media, may reflect procedures used by international participants. The 'other' methods listed were BACTEC 960 (MGIT), microtiter, and LJ resistance ratio method.

Figure 5 provides information on the test procedures used by the participating laboratories testing *M. xenopi.*

M. tuberculosis test results:

The aggregate test results are provided in separate tables, representing strains F, G, H, I and J to facilitate comparison among laboratories. Table 1 for the *M. tuberculosis* complex strains F, G, H, and I is constructed to include the results for the radiometric (BACTEC), agar proportion (AP), Lowenstein Jensen (LJ) proportion, and other methods at each concentration of drug. The test results are listed in the appropriate (susceptible or resistant) columns with a corresponding total number of tests (Sum) column provided as a denominator for determining the level of consensus. This report contains all results reported by participating laboratories, including many drug concentrations with only one result.

In Table 1 the concentrations recommended by CDC and the NCCLS for the primary (isoniazid, rifampin, pyrazinamide, and ethambutol) and secondary (streptomycin, ethionamide, kanamycin, capreomycin, and p-amino-salicylic acid) antituberculosis drugs are highlighted for

the conventional and radiometric methods. Participants should note that the new NCCLS tentative standard (Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic

Actinomycetes; Tentative Standard-Second Edition, NCCLS document M24-T2 [ISBN 1-56238-423-6] NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2000) recommends testing streptomycin as a secondary drug and also adds ofloxacin and rifabutin to the list of recommended secondary drugs. Participants should note that these recommended combinations reflect the critical concentrations of antituberculosis drugs in 7H10 agar and those concentrations for the BACTEC method that directly correlate with the critical concentrations in the conventional method (1-4). When two concentrations are highlighted, such as for isoniazid and ethambutol, the lower concentration is the critical concentration that should always be included to determine whether the *M. tuberculosis* isolate is resistant.

Three strains of *M. tuberculosis* which have been previously reported as resistant to the low-level of INH were tested by participants in this shipment. **For Strain F**, resistance to the low-level concentration (0.2 µg/ml) of isoniazid (INH) by agar proportion (AP) was reported by 70% (23/33) of laboratories, while 73% (81/111) of laboratories reported resistance to the 0.1 µg/ml concentration of INH by the BACTEC method. Ninety-four percent (30/32) of laboratories reported the culture susceptible to the higher concentration of INH by BACTEC (0.4 µg/ml), and 97% (35/36) of laboratories performing AP at the higher concentration reported susceptible for this culture. Two percent of laboratories reported the culture resistant to pyrazinamide.

For Strain G, 79% (26/33) of laboratories reported resistance to INH 0.2 µg/ml with the AP method, and 75% (82/110) by BACTEC at 0.1µg/ml. One hundred percent of laboratories reported susceptible results by AP at 1.0 µg/ml, and 97% (31/32) of laboratories reported susceptible results at 0.4 µg/ml by BACTEC. The strain was reported as susceptible to other primary drugs by almost all laboratories.

Strain H was reported as resistant to 0.2 µg/ml of INH by 97% (33/34) of laboratories with the AP method. Ninety-nine percent (111/112) of laboratories reported resistance to the lower concentration (0.1 µg/ml) of INH by BACTEC. Ninety-seven percent (31/32) of laboratories found the isolate susceptible at the higher 0.4 µg/ml of INH by BACTEC. Of the laboratories testing the isolate by AP, 94% (34/36) found the isolate susceptible at the 1.0 µg/ml of INH. Twelve per cent of the laboratories detected resistance to pyrazinamide at 100 µg/ml with BACTEC. Forty percent (10/25) of laboratories detected resistance to ethionamide at 5.0 µg/ml by AP, while 75% (3/4) of laboratories detected resistance using BACTEC with the same concentration. Reports have associated ethionamide resistance with low-level INH resistance (1, 9).

Strain I was fully susceptible to the primary drugs by almost all laboratories (except 2% (2/90) detected resistance to pyrazinamide 100 µg/ml and 1% (1/110) detected resistance to rifampin at 2.0 µg/ml both with the BACTEC method).

Our providing test results for all drugs that are reported to CDC should not be construed as a recommendation or endorsement for testing particular drugs or concentrations with patient isolates of *M*. *tuberculosis*-complex. It is assumed that some of the drugs are being tested for research purposes or potential use in the few referral institutions that may treat patients with *M*. *tuberculosis* isolates resistant to almost all standard drugs. Laboratories should not add drugs to their testing regimen without the consultation of physicians having expertise in treating multi-drug resistant tuberculosis. Laboratories may contact their local TB control program for referrals of physicians with experience and expertise in treating multi-drug resistant tuberculosis.

Non-tuberculous Mycobacteria test results:

The aggregate test results are provided in Tables 2 and 3 for **Strain J**, *M. xenopi,* to facilitate comparison among laboratories. Table 2 represents either single or multiple drug concentrations with "breakpoint" susceptibility test results. Table 3 provides quantitative MIC test results. Fifty percent (5/10) of laboratories found this isolate resistant to INH at 0.2 µg/ml by the AP method, while 100% (8) of laboratories found it to be resistant at 0.1 µg/ml by BACTEC. For AP, 90% (9/10) of laboratories reported susceptible at 1.0 µg/ml concentration and 100% reported susceptible at 5.0 µg/ml. Results reported at higher concentrations of the drug were susceptible with the BACTEC method.

Of participants who tested **Strain J** (*M. xenopi)* 36% (5/14) reported the isolate resistant to rifampin 1.0 µg/ml with AP; however, 100% (8) reported it susceptible to rifampin 2.0 µg/ml by BACTEC. For *M. xenopi* isolates resistant to 1.0 µg/ml of rifampin, the recommended secondary drugs for susceptibility testing are ethambutol, isoniazid, streptomycin, clarithromycin, amikacin, ciprofloxacin, trimethoprim sulfamethoxazole or sulfamethoxazole (7).

One hundred percent (13) of participants found this isolate (*M. xenopi*) resistant to ethambutol 5.0 µg/ml in AP, and one of three laboratories testing at the higher 10 µg/ml of ethambutol found it to be susceptible. All laboratories testing with BACTEC at the 2.5 µg/ml detected resistance.

In Table 3, laboratories reported agar proportion MIC results for *M. xenopi*. The isolate was resistant to concentrations of ethambutol greater than 5.0 and 8.0 µg/ml but susceptible to 16.0 µg/ml. One laboratory reported resistance to rifampin at 4.0 µg/ml while another reported susceptible at the same concentration by AP.

Anti-tuberculosis drugs to be tested for treatment of *M. xenopi* infections include isoniazid, rifampin and ethambutol. Similar to rifampin-resistant *M. kansasii, M. xenopi* infections may be very difficult to treat and all drugs should be tested (7, 10). Rifabutin is used in HIV-infected patients on treatment with protease inhibitors. Patient cultures which remain positive after 3 months of appropriate therapy should have susceptibility tests repeated (6, 7).

M. xenopi has been recovered from skin infections and bronchoscopy-associated pseudoinfections due to tap water contamination (6, 8). Isolates may grow in tap water at temperatures as high as 43-45 ºC (6). There were 31 results reported for the 36 laboratories performing susceptibility testing on *M. xenopi*. Although this isolate is reported to grow well at 37 ºC, it is not clear whether the “no growth” reported by some laboratories was related to incubation temperature or attempts to grow the isolate in broth microdilution. There have also been problems reported when attempting to grow *M. xenopi* in cation adjusted Mueller-Hinton broth (7).

The addition of NTM strains to this performance evaluation program should not be interpreted as recommendations for laboratories to adopt NTM drug susceptibility testing, especially if the laboratory has limited experience with these tests and methods. We encourage laboratories that perform NTM drug susceptibility testing to consult recommendations, references, and physicians with expertise in infectious diseases when selecting test methods, drugs, and test interpretations.

Special thanks to the following persons for reviewing this report: Nancy G. Warren, Ph.D., Laboratory Corporation of America; Richard Wallace, M.D., Ph.D., and Barbara Brown-Elliott, M.S., University of Texas at Tyler, TX; Gail L. Woods, M.D., Merck Company; Wendy Gross, M.S., TB Reference Laboratory, West Haven, CT.

REFERENCES

1. **Inderlied, C. B. and M. Salfinger**. 1999. "Antimicrobial Agents and Susceptibility Tests: Mycobacteria", p. 1601-1623. In Murray, Patrick R., Baron, Ellen Jo, Pfaller, Michael A., Tenover, Fred C., Yolken, Robert C. (ed.) Manual of Clinical Microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

2. **David, H. L.** 1971. Fundamentals of Drug Susceptibility Testing in Tuberculosis. DHEW Publication No. (CDC) 712165. Center for Disease Control, Atlanta.

3. **Kent, P.T and G.P. Kubica.** 1985. Public Health Mycobacteriology: A Guide for the Level III Laboratory. Centers for Disease Control, Atlanta.

4. **Siddiqi, S.H., J.E. Hawkins, and A. Laszlo**. 1985. Interlaboratory drug susceptibility testing of *Mycobacterium tuberculosis* by a radiometric procedure and two conventional methods. J. Clin. Microbiol. 22:919-923.

5. **Hawkins, Jean E., Wallace, Richard J. Jr., Brown, Barbara A**. 1991. Antibacterial Susceptibility Tests: Mycobacteria, p. 1138-1152. In Balows, Albert, Hausler, William J. Jr., Herrmann, Kenneth L., Isenberg, Henry D., Shadomy, H. Jean (ed.) Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

6. **American Thoracic Society**. 1997. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am. J. Respir. Crit. Care Med. 156:S1-S25.

7. **National Committee for Clinical Laboratory Standards Committee on Antimycobacterial Susceptibility Testing.** 2000. Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes; Tentative Standard-Second Edition. NCCLS. Wayne, PA.

8. **Gross, W.M., J.E. Hawkins, and D.B. Murphy.** 1976. Origin and significance of *Mycobacterium xenopi* in clinical specimens**.** Bull. Int. Union Tuberc. Lung Dis. 51:267-269.

9. **Banerjee, A., et al.** 1994. *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis.* Science 263:227-230.

10. **Wallace, R. J. Jr., Dunbar, D., Brown, B. A., Onyi, G., Dunlap, R., Ahn, C. H., Murphy, D. T.** 1994. Rifampin-resistant *Mycobacterium kansasii*. Clin. Infect. Dis. 18:736-743.