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IDAHO DEPARTMENT OF  
HEALTH & WELFARE

## Special Edition!

- *M. tuberculosis* – CDC-recommended turn-around-times
- A TB Controller's Perspective
- Clinical Forum interviews Steve Gregoire
- TB or not TB? word puzzle
- Upcoming Teleconferences

## Rapid delivery of TB samples to the laboratory

Steve Gregoire

Rapid identification of *M. tuberculosis* infection expedites patient therapy, enables prompt epidemiological investigation, and allows confirmation of treatment efficacy. If a laboratory cannot identify *M. tuberculosis* in a timely manner, patient care may be compromised and the chain of transmission may continue. A delay between the collection of the sample and receipt in the laboratory will slow the confirmation of TB and the drug-susceptibility results. It may also delay the initiation of therapy, prolong infectiousness, allow inappropriate therapy, and present missed opportunities to prevent transmission. These problems are believed to have led to the resurgence of TB in the early 1990's and contributed to the emergence of multidrug-resistant TB in the United States<sup>1</sup>.

*M. tuberculosis* is a very slow growing organism that can take weeks to identify and characterize using conventional methods. Even with the increased use of molecular diagnostics, the importance of rapid delivery of samples to the lab cannot be overlooked. The rate of successful cultivation and identification of *M. tuberculosis* in a clinical sample increases substantially when the time between collection and laboratory analysis is minimized. Lengthy transport times may compromise samples by increasing overgrowth of commensal flora and reducing the viability of any mycobacteria present (see Table 1 for the effect of time on the recovery of *M. tuberculosis* from sputum with +AFB smears).

Table 1 Sputum cultures up to 28 days after Sputum collection. Reprinted with permission from Banda et al.<sup>2</sup> Copyright 2000 by copyright holder.

Initial AFB Smear result	Storage of Sputum	No. of specimens	Positive sputum cultures for <i>M. tuberculosis</i>				
			Day 0	Day 7	Day 14	Day 21	Day 28
3+	room	10	10	9	4	2	3
	refrigerator	10	10	10	8	8	9
2+	room	10	10	9	5	7	5
	refrigerator	10	10	10	9	8	7
1+	room	10	10	8	6	3	3
	refrigerator	10	9	9	7	4	4

For these reasons, Tenover and colleagues recommend that samples ideally be delivered to the laboratory within 30 minutes of collection, but at least within 24 hours<sup>3</sup>. The delivery of samples to the Idaho Bureau of Laboratories (IBL) within 24 hours from collection can be logistically challenging. These challenges include the lack of a state courier system, the cost of using commercial shipping vendors, and the remoteness of some locations. To help facilitate sample transport, IBL supplies TB collection kits, instructions and guidance on sample submission. However, it was recently discovered during an TB services outreach survey conducted by the IBL, that 56% of clients who use IBL TB Lab services were unfamiliar with the importance of delivering samples to the lab <24 hours<sup>4</sup>.

The importance of rapid delivery is also incorporated into the CDC's Division of Tuberculosis Elimination (DTBE) program recommendations, which are intended to expedite diagnosis of *M. tuberculosis* infection and to facilitate the timely completion of drug-sensitivity testing. Specifically, the DTBE has established turn-around-time (TAT) goals for the rapid receipt of samples to the laboratory, detailing that samples should be received at the laboratory within 24 hours of collection<sup>5</sup>.

Table 2 below shows the time between date of collection and receipt of samples at the Idaho Bureau of Laboratories over a period of four years, showing little variance<sup>6</sup>.

**Table 2** Rapid delivery of specimens to the Laboratory.

Year	% specimens received within 1 calendar day	% specimens received within 2 calendar days	% specimens received within 3 calendar days
2006	56%	67%	89%
2007	51%	Not Available	Not Available
2008	42%	62%	76%
2009	51%	72%	86%

IBL continues to monitor TAT of samples between collection and receipt, and will continue to respond to feedback about shipping challenges from our clients. IBL will regularly evaluate this information and address any transit issues that are discovered. The importance of rapid delivery and the integrity of specimens sent to IBL are a critical component to ensure timely identification of *M. tuberculosis* infection. The rapid delivery of samples has room for improvement and IBL will continue to provide outreach in an effort to reduce transit time.

#### References

<sup>1</sup> Shinnick, Thomas M, Iade, Michael, Ridderhof, John C. (2005). National Plan for Reliable Tuberculosis Services Using a Systems Approach. *MMWR*, 54(RR06), 1-12.

<sup>2</sup> Banda, H.T., Harries, A.D., Boeree, M.J., Nyirenda, T.E., Banerjee, A. Salaniponi, F.M. (2000). Viability of Stored Sputum Specimens for Smear Microscopy and Culture *International Journal of Tubercular Lung Disease*, 4(3), 272-274.

<sup>3</sup> Tenover, Fred C, Crawford, Jack T, Huebner, Robin E., Geiter, Larry J., Horsburgh Robert Jr., Good, Robert C. (1993). The Resurgence of Tuberculosis: Is Your Laboratory Ready? *Journal of Clinical Microbiology*, 31(4), 767-770. Retrieved from: <http://www.jcm.asm.org>

<sup>4</sup> IBL TB Needs assessment Survey Review and Summary 2010.

<sup>5</sup> Healthy People 2010, 14 Immunization and Infectious Diseases; Infectious Diseases and Emerging Antimicrobial Resistance, 14-11 Tuberculosis.

<sup>6</sup> Cooperative TB agreement IBL 2010

#### New IBL Bureau Chief



**Dr. Christopher Ball** recently accepted the position of Chief for the Bureau of Laboratories. Dr. Ball has provided exceptional leadership to the bureau in his acting capacity over the last 14 months. Welcome Dr. Ball!

## In the Mycobacteriology Lab

The Idaho Bureau of Laboratories (IBL) provides mycobacteriological services to health care facilities in Idaho. Services include smears and cultures for clinical specimens, isolation and identification of reference samples, nucleic acid amplification for detection of *M. tuberculosis* in clinical specimens, and drug susceptibility testing on first isolates from a TB confirmed patient.

Steve Gregoire is the primary mycobacteriologist for the State TB lab. Steve has been with IBL since February of 2004 and has had his hand in TB for the last 4 years before taking the position as the primary microbiologist approximately a year ago. The following is an interview with Steve about the TB lab.

**Clinical Forum:** Describe the work area for TB.

**Steve:** The TB lab is a laboratory suite located inside the BSL3 complex at IBL. TB lab houses two biosafety cabinets (BSCs), a VersaTREK liquid culture system, four CO<sub>2</sub> incubators, two centrifuges, and an exit-only autoclave. Use of personal protective equipment (PPE) varies from wearing a N100 respirator, closed front gown, gloves, and eye protection when performing work in a BSC, to a disposable lab coat when performing other duties.

**Clinical Forum:** How does a submitter send a specimen to IBL for TB testing?

**Steve:** Specimens are received at IBL from 8 am-5pm Monday through Friday via UPS, FedEx, USPS, and various couriers. In general, specimens should be received at IBL within 24 hours of collection. IBL provides TB collection kits to our clients free of charge.

**Clinical Forum:** Please describe the workflow through the TB lab.

**Steve:** Processing of raw sample occurs daily, Monday through Friday. Raw samples are digested/decontaminated and inoculated on both solid and liquid media. Also, a direct smear is prepared and typically read within 24 hours of receipt, as per the CDC-recommended turnaround time. We also perform QuantiFERON TB-Gold testing and DNA probes weekly.

**Clinical Forum:** What type of equipment do you use?

**Steve:** We use the VersaTREK liquid culture system for automated detection of all mycobacteria species and also for drug susceptibility testing (DST) on TB isolates. The VersaTREK detects mycobacterial growth by automatically monitoring (every 24 minutes) the rate of oxygen consumption within the headspace of the culture bottle. The Lowenstein-Jensen (LJ) slants and

Middlebrook 7H11 selective biplates are read weekly to check for suspicious growth. We also have DNA probes specific for *M. tuberculosis* complex, *M. avium* complex or *M. gordonae*, which are used on cultures that meet specific criteria. The other non-tubercular mycobacteria (NTM) are identified using the ABI 310 genetic analyzer for 16S ribosomal DNA sequence analysis.

**Clinical Forum:** Do you perform direct nucleic acid amplification?

**Steve:** An in-house assay for direct detection of *M. tuberculosis* by PCR is currently in development. Presently, the nucleic acid amplification test (NAAT) is performed at the Montana Public Health laboratory Monday through Friday. So, if all goes smoothly, we can forward samples overnight for a result by close of business the next working day.

**Clinical Forum:** Do you perform drug susceptibility testing?

**Steve:** We test first line drugs on initial TB isolates, which includes Isoniazid, Rifampin, Ethambutol, and Pyrazinamide. MDR-TB isolates are shipped to the Centers for Disease Control and Prevention (CDC) for 2<sup>nd</sup> line drug testing. We also provided a forwarding service to National Jewish Health for DST requests on NTM isolates.



Steve Gregoire reading Ziehl-Neelsen smears

**Clinical Forum:** Has Idaho seen any MDR (multi drug resistant) or XDR (extremely drug resistant) TB cases?

**Steve:** We did see one MDR-TB case last year. We detected resistance to INH and Rifampin and subsequently forwarded the isolate to CDC to confirm our MDR diagnosis and to perform 2<sup>nd</sup> line drug testing. It turned out that the case was not XDR.

**Clinical Forum:** What is your reporting procedure?

**Steve:** We report all smear results, DNA probe results, and culture results via phone, fax or hard copy mailed to the submitting physician or laboratory the same day they are determined. The TB Controller’s office is also notified per Idaho Reportable Disease guidelines. NAAT results are received via phone from the Montana PHL and are promptly relayed to the submitter by phone.

**Clinical Forum:** How is the IBL TB program funded?

**Steve:** The CDC Tuberculosis Elimination and Laboratory Cooperative Agreement provides financial assistance that augments state contributions to TB program activities.

**Clinical Forum:** What final thoughts would you like to leave the submitting agencies with?

**Steve:** Please submit the very best sample you can, please make sure the cap on the sputum collection vessel is secure and the tube is labeled with the patient's name!

**TB or not TB?**

D A Y T N B T R D M I U V S X N P K Y G  
 W E B T E E J F T G B N C X E S H O Y N  
 K R C T I Z S F R Y R O N S Q Q O O E I  
 N O S O O L E L L K T F N X D M T R P D  
 K S I F N V I V E O Y E B N M K O B W R  
 S O T J W T Z B C E J A O H Y L C E V O  
 D L N O X U A H I N N R A N I Z H L F C  
 U I B N O U R M I T E L C N U X R D T E  
 W U G Y S O W E I F P S H O L W O D Q N  
 Q N B E M Y T I I N P E Q E K K M I U I  
 Z J R O S S V T T U A V C C I E O M C T  
 P M G E N T N U T W H T J S Q Z G E V N  
 P E U E Q A I U X E R F I R U P E O L E  
 N Q W C U G M O G A T Z D O K S N U S P  
 P O J Q B N R W N S I X G L N W G N A R  
 L A C I D F A S T B A C I L L I M U V E  
 S R I R A P I D G R O W E R I H P J R S  
 S U R B I F P Y Y U W K A L T J K R U D  
 E M I T D N U O R A N R U T E N V D C I  
 H O O U H M G T T H T U I W D H Z P H E

**Tuberculosis and the Laboratory  
 from the Perspective of Disease  
 Control and Prevention**

Idaho Office of Epidemiology, Food Protection,  
 and Immunization

ACID FAST BACILLI  
 DECONTAMINATION  
 DRUG SUSCEPTIBILITY  
 MDR-TB  
 PHOTOCHROMOGEN  
 RAPIDGROWER  
 SERPENTINE CORDING  
 TURN-AROUND-TIME

AEROSOL  
 DIGESTION  
 LOWENSTEIN-JENSEN  
 MIDDLEBROOK  
 QUANTIFERON  
 SCOTOCHROMOGEN  
 SPUTUM  
 ZIEHL-NEELSEN

Nationally, active tuberculosis (TB) is a disease<sup>1</sup> that is declining. In March, the Centers for Disease Control and Prevention (CDC) reported that 2009 had the lowest rate of new cases diagnosed (3.8 cases per 100,000 people) since national surveillance was started in 1953. Every year, about 10-15 Idaho residents are diagnosed with active TB disease; in 2009, 18 new cases of active TB disease were diagnosed—slightly higher than the average. Idaho data from 1987 onward can be found at: [http://healthandwelfare.idaho.gov/LinkClick.aspx?fileticket=XhTpep\\_g8b0%3d&tabid=378&mid=3844](http://healthandwelfare.idaho.gov/LinkClick.aspx?fileticket=XhTpep_g8b0%3d&tabid=378&mid=3844).

Patients with active TB disease are diagnosed throughout Idaho, but most cases in the southern part of the state. Over the last five years the average age of TB cases in Idaho is 46 years of age; a majority of cases are male (55%). Anyone - including laboratorians - can become infected with *Mycobacterium tuberculosis* and develop active TB disease. However, people with certain risk factors have a higher likelihood of developing active TB disease. In Idaho common risk factors include being foreign-born, immuno-compromised (e.g., HIV positive, patients taking tumor necrosis factor inhibitors), and being contacts to a case of active TB disease. Additionally, cases of active TB

disease have been documented in laboratorians who have worked with samples from patients with active TB disease. Use of proper laboratory precautions prevents most transmission to laboratorians handling samples.

From the perspective of disease control and prevention of active TB disease, laboratories on all points of the time-continuum of patient care from diagnosis of disease to completion of treatment and testing of contacts are vitally important. Since 2005, over 80% of all TB cases have had pulmonary involvement and were therefore, potentially infectious. (TB can be diagnosed from non-pulmonary sites such as the pericardium and the skeletal system generally causing the patient to be non-infectious.) Timely and quick shipment of samples to a laboratory that can culture and perform drug susceptibility testing, such as the Idaho Bureau of Laboratories, is essential to clinicians treating the patient and initiating contact investigations to interrupt transmission of the disease.

## UPCOMING TELECONFERENCES

### "Clostridium Difficile Testing Algorithms"

July 20, 2010; 12:30 MNT

### Emerging Resistance Trends in Gram Negative Bacteria: Implications for Drug Therapy

August 17, 2010; 12:30 MNT

### Cytomegalovirus - The Underutilized Diagnostic Marker

September 21, 2010; 11:00 MNT

### TB Nucleic Acid Amplification Testing: Latest CDC Guidelines

September 28, 2010; 11:00 MNT

### Update in Anaerobic Bacteriology

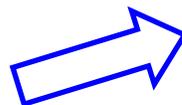
October 19, 2010; 12:30 MNT

### A Review of Medically Relevant *Corynebacterium* Species and Other Coryneforms

November 16, 2010; 12:30 MNT

### Case Histories and Surveillance for MDRO

January 18, 2011; 12:30 MNT



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## SOLUTION TO WORD PUZZLE

(Over, Down, Direction)

ACID FAST BACILLI (2,16,E)  
 AEROSOL (2,1,S)  
 DECONTAMINATION (1,1,SE)  
 DIGESTION (1,7,SE)  
 DRUG SUSCEPTIBILITY (20,18,NW)  
 LOWENSTEIN-JENSEN (1,16,NE)  
 MDR-TB (10,1,W)  
 MIDDLEBROOK (18,11,N)  
 PHOTOCROMOGEN (17,1,S)  
 QUANTIFERON (4,15,NE)  
 RAPIDGROWER (4,17,E)  
 SCOTOCHROMOGEN (14,1,SW)  
 SERPENTINECORDING (20,17,N)  
 SPUTUM (12,9,SW)  
 TURN-AROUND-TIME (14,19,W)  
 ZIEHL-NEELSEN (16,12,NW)

## Tuberculosis and the Laboratory from the Perspective of Disease Control and Prevention (continued)...

Rapid shipment is essential to the work of medical and public health personnel because of how slowly *M. tuberculosis* grows. New rapid PCR diagnostic tests for active TB disease are available and becoming more widely used (*e.g.*, nucleic acid amplification testing [NAAT]). **NAAT testing is now recommended for all suspected pulmonary TB patients since it can significantly shorten the time to diagnosis and onset of treatment.** However, these diagnostic tests do not eliminate the need to grow cultures to test for drug resistance. Rapid PCR tests to detect drug resistance to the two most important anti-tuberculosis drugs isoniazid (INH) and rifampin (RIF) are not currently approved by the Food and Drug Administration (FDA), but some laboratories have created reliable and validated "home-brew" PCR protocols to test for resistance to some drugs. Please contact the Idaho Bureau of Laboratories if you have any questions about TB testing.

<sup>1</sup>TB presents in 2 forms: latent TB infection (LTBI) and active TB disease. Only active TB disease is reportable in Idaho. LTBI is not infectious.