

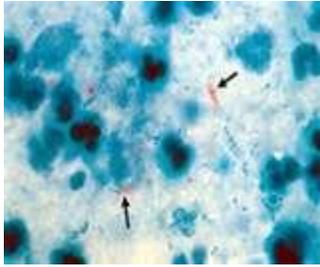
## SPECIAL MICROBIOLOGY SECTION



[Mycobacteriology](#)

[Mycology](#)

[Parasitology](#)



## MYCOBACTERIOLOGY

*Mycobacterium, M. tuberculosis, TB*

### INTRODUCTION

The Tennessee Department of Health (TDH) Mycobacteriology Laboratory provides isolation and identification testing of all *Mycobacterium* species (including *M. tuberculosis* complex and non-tuberculosis Mycobacteria). Public and private health care providers may submit sputum and other clinical specimens and reference specimens.

Sputum and specimens from other sources are concentrated and stained with fluorochrome acid fast stain and are cultured for isolation and identification. Solid and liquid media are used for isolation. Species identification is accomplished by routine biochemical characterization, pigment characterization, Mass spectrometry (MALDI-TOF), Line Probe, DNA probe or any combination of these methods.

Drug susceptibility tests are routinely performed on first time isolates of *M. tuberculosis complex*. Isoniazid (INH), Rifampin, Ethambutol and Pyrazinamide (PZA) are the antibiotics to which susceptibility is determined. Specimens for sensitivity testing on pathogenic Mycobacterium other than *M. tuberculosis* (MOTT) are referred to another laboratory upon special request.

**The Tennessee Medical Laboratory Act requires laboratory directors to send isolates of any *Mycobacterium* species to the TDH Laboratory for surveillance purposes.**

## SPECIMEN COLLECTION

### Clinical specimens

- **Collect clinical specimens in a plastic 50 ml conical sputum tube.**
- **Sputum:** Collect a series of 3 to 5 single samples, collected at least 8 hours apart, but preferably all early morning. A volume of 5 to 10 ml is adequate for each sample.
- **Induced (or nebulized) sputum:** These specimens are usually very watery and should be labeled as "induced" so that they will not be mistaken for saliva. Saliva is an unsatisfactory specimen.
- **Bronchial washings:** Collect up to 40 ml.
- **Gastric lavage specimens:** Collect early in the morning or 8 hours after eating or drug therapy. Buffer immediately with 100 mg of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) or other alkaline buffer. Deliver to the laboratory as soon as possible.
- **Tissue:** Aseptically collect and transport to the laboratory at once without preservative.
- **Urine:** A series of single, mid-stream specimens, voided in the early morning, should be submitted (rather than a 24-hour pooled specimen) without preservative and on ice packs.
- **Feces:** Only fecal specimens from confirmed or suspected AIDS or other immunocompromised patients will be accepted. Collect a minimum of 1 gram of feces.
- **Blood:** Collect 10 ml of blood in a sterile tube containing heparin, sodium polyanethole sodium (SPS), or citrate.
- **Other specimens:** Collect aseptically following the proper procedure for the type of specimen. Other specimens may include pleural fluid, pus, joint fluid, laryngeal or wound swab and spinal fluid. DO NOT use any transport medium. Phone the TDH Mycobacteriology Laboratory before submitting if any questions arise.

## **Reference Specimens**

Only pure cultures will be accepted. You can submit either the isolate on the original (primary) medium or a subculture on an appropriate medium after growth is visible. Laboratories electing to submit original cultures should make sure visible growth is evident before mailing and should hold a subculture in their laboratory. Subcultures are to have visible growth upon submission.

## **SPECIMEN IDENTIFICATION**

1. Complete **all** the provider and patient information areas on the requisition Form PH-4182. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name or other acceptable unique identifiers. Two unique identifiers must be present on the specimen and requisition. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

## **SPECIMEN SHIPMENT**

1. Pack the specimen following the shipping guidelines currently in use by your preferred carrier. Refer to the following packaging and shipping Quick Guides for Category A or B shipments and remember to make sure the individual who packs is currently certified.
2. If the State Lab Courier Service will be used, affix the MYCO label, the RED NASHVILLE courier label for clinical specimens, return address and appropriate infectious substance label (Category A or Category B) to container.
3. Ship the specimen to the Tennessee Department of Health Laboratory in Nashville as soon after collection as possible.

## REPORTING AND INTERPRETATION OF RESULTS

Clinical specimens are tested for the presence or absence of *Mycobacterium* species by smear and culture methods. Initial specimens that are smear positive are tested by GeneXpert MTB/RIF Assay and reported. Negative cultures are incubated for 6 weeks before the specimen is reported as No Growth of *Mycobacterium tuberculosis*. Isolates from clinical specimens and reference cultures are identified to the genus and species level.

### Smears

The provider is notified by telephone of a positive smear on a new patient on the day of receipt of the specimen. The turn around time of a positive smear on a patient who has a previous positive smear or on a negative smear is 1 working day after receipt. A hard copy is mailed to the provider.

<b>SMEAR REPORTING</b>	
<b>Smear Negative</b>	AFB Not Found
<b>Smear Positive</b>	Less than 1 per high power field <b>or</b> 1-10 per high power field <b>or</b> 10+ per high power field (depending on the quantitation)

## GeneXpert/MTB/RIF Assay

This test is performed on initial (new positive) smear positive specimens. Smear negatives are not routinely tested. However, a test can be ordered by contacting the Special Bacteriology Section at 615-262-6369. Positive results are phoned to the provider. Hard copies of both positive and negative results are forwarded to the provider by mail.

RESULT	INTERPRETATION
<b>MTB DETECTED; Rif Resistance DETECTED</b>	<p>The MTB target is detected within the sample</p> <p>~ A mutation in the rpoB gene has been detected            ~SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control.            ~ Probe Check (QC1 and QC2): PASS. All probe check results pass.</p>
<b>MTB DETECTED; Rif Resistance NOT DETECTED</b>	<p>The MTB target is detected within the sample</p> <p>~ A mutation in the rpoB gene has not been detected            ~SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control.            ~ Probe Check (QC1 and QC2): PASS. All probe check results pass.</p>
<b>MTB DETECTED; Rif Resistance INDETERMINATE</b>	<p>The MTB target is detected within the sample</p> <p>~ A mutation in the rpoB gene could not be determined due to insufficient signal detection.            ~SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control.            ~ Probe Check (QC1 and QC2): PASS. All probe check results pass.</p>
<b>MTB NOT DETECTED;</b>	<p>The MTB target is not detected within the sample</p> <p>~SPC: PASS. The SPC met the acceptance criteria.            ~ Probe Check (QC1 and QC2): PASS. All probe check results pass.</p>

**Cultures**

If growth occurs at any time during the 6-week incubation period, identification procedures begin. Turn around time is 1 to 2 weeks after growth for identification by MALDI-TOF or Line Probe and 3 to 4 weeks after growth for identification by biochemical methods. Another 2 weeks is required for drug susceptibility tests to be completed.

<b>CULTURE REPORTING</b>	
Negative	No <i>Mycobacterium</i> found.
<b>Positive for <i>M. tuberculosis complex</i></b>	<i>M. tuberculosis complex</i>  If a method is used that is non-FDA approved it will be noted on the report.
<b>Positive for other <i>Mycobacterium species</i></b>	<i>Mycobacterium</i> _____ (species) If a method is used that is non-FDA approved it will be noted on the report.

**Susceptibility Testing**

Drug susceptibility testing is performed only on *M. tuberculosis-complex* (M.tbc) isolates. Results are usually available within 2 weeks after the organism has been isolated and identified. Each new isolate automatically receives susceptibility testing. Susceptibility testing is also performed if M.tbc is still being cultured from the patient after 3 months. If resistance is shown to any of the drugs listed below, the organism is sent to the Centers for Disease Control and Prevention (CDC) for confirmation and additional drugs testing.

<b>SUSCEPTIBILITY REPORTING</b>		
Drug (mcg/ml)	Result	*S = Susceptible or R = Resistant
INH (0.1)	*	
Rifampin (1.0)	*	
Ethambutol (5.0)	*	
Pyrazinamide (100)	*	

Drug Susceptibility testing for organisms other than M.tbc must be requested (615-262-6369) and in many cases payment for testing must be arranged.

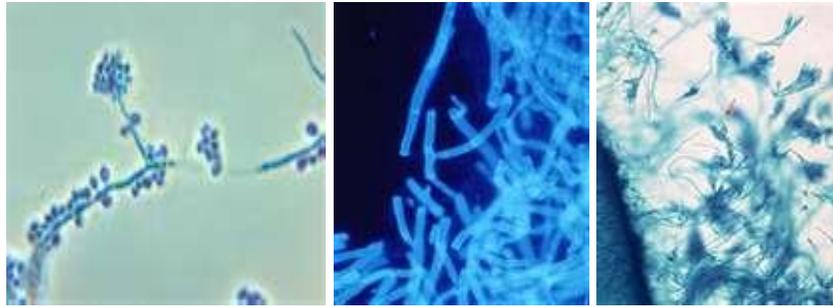
## **TEST REPORTS**

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the regional office and to the health department in the county where the patient lives.

### **Examples of Unacceptable Specimens**

1. The specimen was not properly identified.
2. The patient identifier on the specimen does not exactly match the identifier on the form.
3. The specimen was broken or leaked in transit.
4. The specimen was submitted in a non-regulation container.
5. The specimen was submitted in 5% formalin, Cary-Blair or other preservative.
6. There was no specimen in the bottle.
7. The patient information was not complete.
8. The specimen was received more than 10 days after collection.

## MYCOLOGY



### INTRODUCTION

Until relatively recent times mycology cultures were performed only in a few cases. It was sufficient to determine that a person had a pathogenic fungus and to identify that pathogen. Most other mycology cultures were reported as "No pathogenic fungi isolated." Other fungi, even if identified, were designated as saprophytes.

Today many patients are immunocompromised or immunosuppressed. Patients with diabetes who have become ketonic, cancer patients who are receiving chemotherapy, transplant recipients who must take immunosuppressive drugs and persons who have developed AIDS are likely at some time to develop a fungal disease.

With any of these patients, mycoses can develop rapidly. Along with diseases caused by the common pathogens, it has become increasingly evident that many organisms formerly considered to be saprophytes are causing serious and in some cases life-threatening disease processes in these immunocompromised individuals.

Specimens to be tested for agents of superficial or cutaneous mycoses are accepted from public and private health care providers. Other clinical material is not routinely accepted (contact the Mycology Section if testing of other sources is desired). Reference cultures of medically important isolates are also accepted for the identification of yeast and cutaneous, subcutaneous and systemic fungi.

Yeasts are identified on characteristic microscopic morphology on selected media, MALDI-TOF or by their assimilation of carbohydrates in the API 20 C kit. Fungi are identified by their growth rate, the size and color of the hyphae

and by the arrangement and origin of the conidia they produce. MALDI-TOF, sequencing, or biochemical tests are used if appropriate.

The Mycology Section identifies aerobic actinomycetes and fungus-like bacteria. They are identified primarily by biochemical tests and MALDI-TOF.

*Histoplasma capsulatum* and *Blastomyces dermatitidis* are identified by microscopic morphology and DNA probe testing.

Antimicrobial testing of fungi is not performed in this laboratory.

## **SPECIMEN COLLECTION**

### **Clinical specimens**

The following specimens can be submitted for isolation of dermatophyte fungi:

- **Hair:** Clip hair from infected area. Pluck hairs in ringworm infections. Place the hair in a sterile screw-capped container.
- **Nail:** Swab the nail with gauze soaked in 70% alcohol. Allow the nail to dry and scrape the infected nail with a sterile scalpel. Place the scrapings in a sterile screw-capped container.
- **Skin:** Swab the infected skin with gauze soaked in 70% alcohol. Allow the skin to dry and scrape the lesion with a sterile scalpel. In ringworm infections of smooth skin, collect the specimen from the active border of the lesion. Place in a sterile screw-capped container.

### **Reference cultures**

Only pure cultures are accepted. An isolate can be submitted on the original culture media or a subculture with visible growth is also acceptable. Fungal and yeast cultures may be shipped in screw-capped tubes of Sabouraud's agar or other appropriate media. Seal tightly with parafilm or waterproof tape. Plates are acceptable only if they are sealed, placed in a plastic bag and hand-delivered to the laboratory (never mail plates).

## **SPECIMEN IDENTIFICATION**

1. Complete **all** the provider and patient information areas of Requisition Form PH-4182 and mark test requested. Include pertinent clinical information with each specimen and testing requested.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

## **SPECIMEN SHIPMENT**

1. Pack the specimen following the shipping guidelines currently in use by your preferred carrier. Refer to the following packaging and shipping Quick Guides for Category A or B shipments and remember to make sure the individual who packs is currently certified.
2. If the State Lab Courier Service will be used, affix the MYCO label, the RED NASHVILLE courier label for clinical specimens, return address and appropriate infectious substance label (Category A or Category B) to container.
3. Ship the specimen to the Tennessee Department of Health Laboratory in Nashville as soon after collection as possible.

## **REPORTING AND INTERPRETATION OF RESULTS**

- Clinical specimens are reported within 4 to 6 weeks.
- Molds are reported within 4 weeks of receipt.
- Yeasts are reported within 1 week of receipt.
- Aerobic actinomycetes and fungus-like bacteria are reported within 6 weeks.
- *Histoplasma capsulatum* and *Blastomyces dermatitidis* are reported within 2 weeks.
- Organisms are reported using the genus and species designations consistent with descriptions in the American Society for Microbiology's *Manual of Clinical Microbiology* and Davise Larone's *Medically Important Fungi: A Guide to Identification*. If the organism is identified by Gen-Probe, this will be indicated on the report. For molds, if no conidia are formed after 4 weeks and sporulation cannot be induced, the culture is reported as "non-sporulating fungus, unable to identify."

### **Examples of Unacceptable Specimens**

1. The specimen was not properly identified.
2. The patient identifier on the specimen does not exactly match the identifier on the form.
3. The specimen was broken in transit.
4. The specimen was submitted in formalin, Cary-Blair or other preservative.
5. Clinical specimen was received greater than 3 days after collection.
6. Reference specimen was non-viable.
7. Mixed reference specimen was submitted or received.

## **PARASITOLOGY**



### **INTRODUCTION**

Accurate clinical diagnosis of blood and intestinal parasitic diseases is difficult and requires laboratory confirmation. Demonstration of the diagnostic stages of invading parasites by direct microscopic examination of specimens is the most reliable method of establishing a diagnosis of most parasitic infections.

Diagnostic specimens for examination of the presence of human parasites and reference specimens are accepted from public and private health care providers. Routine screening of asymptomatic individuals is not recommended.

**The Tennessee Medical Laboratory Act requires laboratory directors to submit specimens of suspected or confirmed *Plasmodium species* (malaria) to the Tennessee Department of Health Laboratory for confirmation and surveillance.**

## SOURCES EXAMINED

- **Fecal material** is examined for amoebic cysts, intestinal flagellates and the ova of round worms, tapeworms, hookworms and flukes. Stained and unstained slides are prepared and examined after a concentration procedure has been performed.
- **Fecal specimens for the identification of *Cryptosporidium*** are tested by the direct immunofluorescent test and by microscopic examination.
- **Gross specimens (whole worms or proglottids)** such as tapeworms and roundworms that cause disease in man are identified by macroscopic and microscopic examination.
- **Blood slides** are examined for the presence of blood parasites. Submit Giemsa-stained thick and thin film slides and a vial of EDTA whole blood. *Specimens positive for blood parasites are forwarded to CDC for speciation and confirmation.*
- **EDTA Whole Blood** is tested by Malaria PCR for the presences of *Plasmodium falciparum, ovale, vivax or malarie.*
- **Perianal slides** are examined for *Enterobius vermicularis* (pinworm) infection.
- **Sputum and stool specimens** are examined in suspected cases of paragonimiasis (*Paragonimus*).
- **Duodenal drainage** is used for the diagnosis of *Strongyloides stercoralis* and *Giardia lamblia*.
- **Urine** is examined for *Schistosoma haematobium*.

Indirect (serologic) methods are performed at the Centers for Disease Control and Prevention (CDC) for a few diseases in which the organism is not readily demonstrated. These include trichinosis, echinococcosis, extra-intestinal amebiasis, Chagas' disease, African trypanosomiasis, filariasis and chronic schistosomiasis. Other methods are also utilized for identification of Leishmaniasis. Contact the Parasitology Section for information about this testing.

## **SPECIMEN COLLECTION**

### **Fecal material**

1. Patients infected with parasites pass the parasites intermittently. Multiple specimens should be submitted, collected from three separate bowel movements, preferably at three-day intervals.
2. Collect stool specimen in a clean, dry container. Do not mix with urine, water, dirt or paper.
3. Immediately place a nickel-sized amount of feces (approximately equal to the volume of Totalfix bottle provided by TDH. Do not allow feces to contaminate the outside of the container.
4. Place the cap on securely. Mix the feces well with the Totalfix by shaking the bottle for 1 minute.

**Cellulose tape for pinworms:** Performed via the cellulose tape method. Refer to Figure III-1. Directions also provided in each kit.

### **Blood parasites:**

1. Utilize proper technique in collection of whole blood into EDTA tube.
2. Make at least 1 thick and 1 thin smear and stain.
3. Fix and stain with Geimsa stain.

**Gross Specimens (whole worms or proglottids):** Preserve whole worms in 5 to 10% formalin. Proglottids may be preserved in formalin or placed in saline.

### **Sputum specimens**

1. Collect in 50ml conical tube.
2. Add equal amount of Totalfix.
3. Tightly seal container and mix well.

## Duodenal drainage

1. Collect according to facility's established procedure.
2. Place equal amounts of Totalfix and specimen in 50ml conical tube.
3. Tightly seal container and mix well.

**Urine:** Mid-day, sterile collection without preservative requested. 3 specimens suggested. Ship cold on ice packs.

## SPECIMEN IDENTIFICATION

1. Complete **all** the provider and patient information areas on the Requisition Form PH-4182 and mark the appropriate test. Include pertinent clinical information with each specimen and testing requested.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be examined**.

## SHIPMENT OF SPECIMENS

1. Pack the specimen following the shipping guidelines currently in use by your preferred carrier. Refer to the following packaging and shipping Quick Guides for Category A or B shipments and remember to make sure the individual who packs is currently certified.
2. If the State Lab Courier Service will be used, affix the MYCO label, the RED NASHVILLE courier label for clinical specimens, return address and appropriate infectious substance label (Category A or Category B) to container.
3. Ship the specimen to the Tennessee Department of Health Laboratory in Nashville as soon after collection as possible.

## REPORTING AND INTERPRETATION OF RESULTS

Specimens are reported within 1 to 3 working days of receipt. If large numbers of specimens are submitted, reporting may be delayed. If the specimen is sent to the Centers for Disease Control and Prevention, final identification may take more than 1 to 3 days.

Most parasites found, both pathogenic and non-pathogenic species, will be reported by their scientific names. Hookworm eggs are reported as "hookworm eggs." The diagnostic stage seen, cyst or ova, will be included in the report. Genus and species designations are consistent with the American Society for Microbiology's *Manual of Clinical Microbiology* and the *Atlas of Human Parasitology*.

### FECAL SPECIMEN REPORTING

**Parasites found:** All parasites found, pathogenic and non-pathogenic, are reported. The diagnostic stage seen, cyst or ova, is reported.

**No parasites found:** No ova and parasites identified.

### PINWORM SPECIMEN REPORTING

**Positive:** Positive for *Enterobius vermicularis* eggs.

**Negative:** No pinworms found by the cellulose-tape technique.

<b>BLOOD PARASITE SPECIMENS REPORTING</b>
<b>Positive Smear:</b> The specimen is reported as "Blood Parasite Seen" <b>Positive PCR:</b> Plasmodium _____(vivax, falciparum, or malariae) detected by real time PCR.
All positive are sent to CDC for speciation confirmation. Preliminary results are phoned to the provider.
<b>Smear Negative:</b> No blood parasites seen. <b>PCR Negative:</b> No Plasmodium species detected by real time PCR.

<b>CRYPTOSPORIDIUM REPORTING</b>
<b>Positive:</b> Positive for <i>Cryptosporidium</i> oocysts using the direct immunofluorescent test.
<b>Negative:</b> Negative for <i>Cryptosporidium</i> oocysts using the direct immunofluorescent test.

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the health department in the county where the patient lives.

## **Examples of Unacceptable Specimens**

### **All specimens**

1. The specimen was not properly identified.
2. The patient identifier on the specimen does not exactly match the identifier on the form.
3. The specimen or slide was broken in transit.
4. The specimen leaked in transit.

### **Fecal specimens including *Cryptosporidium***

1. The specimen was not submitted in Totalfix.
2. The specimen was submitted in a non-regulation container or other non-regulation specimen kit.
3. The specimen contains interfering substances such as barium, bismuth, gall-bladder dye, urine, or water.

### **Pinworm cellulose tape**

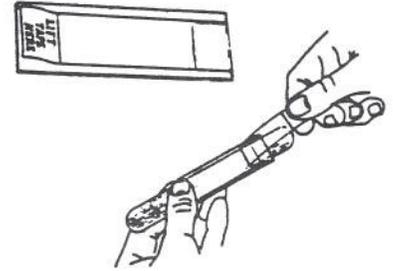
1. The cellulose tape was contaminated with fecal material or talcum powder.
2. The collection procedure was not followed.

### **Blood parasites**

1. The blood smear was too thick (blood flaking off slide).
2. The blood smear was too thin and not feathered at the end.

### Figure III - 1 Use of Cellulose Tape Slide Preparation for Diagnosis of Pin Worm Infections

1. Clear cellulose tape about 2 inches long and  $\frac{3}{4}$  inch wide is used. (Do not use frosted or "magic" tape as it is not transparent and cannot be examined readily with the microscope.) Hold the slide against a tongue depressor one-inch from the end and lift the long portion of the tape from the slide.



2. Place the tape over the end of the depressor to expose the sticky surface. Hold the tape and slide against the tongue depressor.



3. Spread the patient's buttocks to expose the anus. (Preferably take the specimen a few hours after the person has retired or immediately after waking. Do not clean the anal area before taking the specimen.) Press the sticky side of tape gently to anus two or three times.



4. Lay the tape smoothly on a clean glass slide, sticky side down. Press gently to the slide with a piece of tissue or gauze.



Pinworm eggs