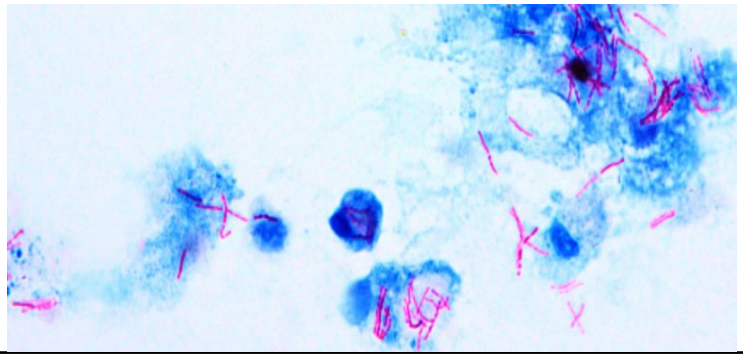


MYCOBACTERIOLOGY TESTING SERVICES GUIDE

DR. KATHERINE A. KELLEY
CONNECTICUT DEPARTMENT OF
PUBLIC HEALTH LABORATORY

Connecticut Department of Public Health
Mycobacteriology Laboratory

395 West Street, Rocky Hill, CT 06067
(860) 920-6649

Introduction

The Dr. Katherine A. Kelley Connecticut Department of Public Health Mycobacteriology Laboratory serves as a resource for hospitals and health clinics in confirming and identifying *Mycobacterium tuberculosis* complex (MTBc) and other mycobacteria of clinical interest. The Mycobacteriology Laboratory receives and processes several hundred specimens a year while providing accurate results in a short time. The quick turnaround of results ensures that submitters and clinicians are prepared to provide the most appropriate treatment for their patients. This guide will outline the process of how to submit a specimen for testing, as well as serve as an educational tool in the procedures conducted to confirm that a specimen harbors MTBc.

Who We Are

Bacteriology/Bioterrorism Response/Mycobacteriology Department

Rear, Left to Right : Dave Santoro, Hongli Dong, Mary Anne Banevicius, Beata Harvey, Doneisha Rumble, Rik Martinez
Front, Left to Right : Mark Harkins, Jocelyn Florano Boguszewski, Christina Nishimura, Barbara Wang

Not Pictured : Nick Brunetti

Connecticut Department of Public Health Laboratory Director
Jafar Razeq, PhD, HCLD (ABB)

Infectious Diseases Division Director
Anthony Muyombwe, PhD, HCLD (ABB)

Bacteriology/Bioterrorism Response/Mycobacteriology Supervisor
Christina Nishimura

Specimen Submission Procedures

Timely and accurate testing is contingent upon proper specimen submission procedures being exercised by the submitter or clinician. This includes appropriate **specimen collection** and **shipping**, in addition to a properly filled **clinical test requisition form**.

Specimen Collection

Correct specimen collection is a critical first step in the analytic process. Appropriate shipping conditions also ensure specimen quality is maintained throughout transport to the Laboratory.

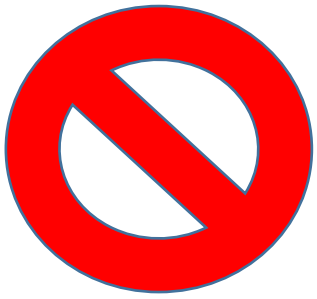
Acceptable Specimens and Shipping Conditions

Specimen Type	Shipping Container	Acceptable Testing Volume	Shipping Conditions
Pulmonary Specimens (sputum, bronchial wash, bronchial lavage)	TC OL40 or similar container	2-5 mL	Ice Pack
Extra-Pulmonary Body Fluids	TC OL40 or similar container	2-5 mL	Ice Pack
Tissues and CSF	TC OL40 or similar container	Tissue samples, biopsies; CSF recommended minimum volume 0.25 mL	Ambient temperature
Blood and Bone Marrow	Blood collection tubes containing heparin (**green top**) or sodium polyanethol sulfonate SPS (**yellow top**)	2-5 mL	Ambient temperature
Culture Isolates (Referred Cultures)	Lowenstein-Jensen agar or Middlebrook 7H10 and 7H11 agar; liquid media from automated test systems (i.e., BACTEC MGIT broth)	2-5 mL or solid media	Ambient temperature

PLEASE NOTE:

- Swabs are strongly discouraged as a clinical specimen source.
- The shipment of plate cultures is discouraged.
- Insufficient volume of specimen may not allow for complete testing.
- Extreme overgrowth of non-acid fast organisms may render a specimen unusable.

Unacceptable Specimens:



- **Unlabeled or illegibly labeled specimens**
- **Broken or leaking specimens**
- **Blood and bone marrow collected in tubes other than heparin or SPS**
- **Specimens without two distinct patient identifiers**

Clinical Test Requisition

A **clinical test requisition** (OL-9B) must accompany every specimen (Appendix A) and is also available electronically – copy and paste this link into your browser:

https://portal.ct.gov/-/media/Departments-and-Agencies/DPH/laboratory/labhome/lab-forms/ClinTestReq_OL9B_FILL-Up1-10-24.pdf

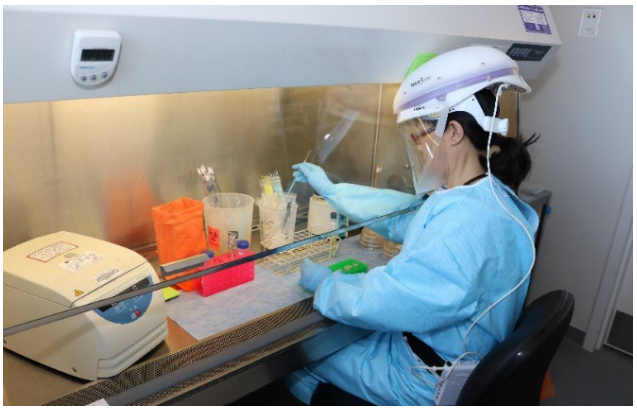
The following information is required on the clinical test requisition (as indicated in Appendix A):

- Name and address of submitter (and/or profile number)
- Patient name or unique identifier, date of birth and address
- Specimen type or source of collection
- Date of specimen collection
- Testing requested
- This information **must** match the labeling on the specimen itself. **Two distinct patient identifiers** that match between the specimen labeling and the requisition information are required.
- Any missing or incorrect information may delay the release of results until the issue is resolved.
- Any specimens that are unlabeled or illegibly labeled will be rejected for testing.

Cepheid GeneXpert Nucleic Acid Amplification Testing (NAAT)

If **nucleic acid amplification (NAA) testing** is requested (please see special specimen requirements regarding this request below), **an additional form** must be filled out by the submitter or clinician (Appendix B). This form is available electronically – copy and paste this link into your browser:

https://portal.ct.gov/-/media/Departments-and-Agencies/DPH/laboratory/labhome/lab-forms/MTB-NAAT-REQUEST-FORM-CTDPH-LAB-0418.pdf



WHAT IS NAA TESTING AND ARE THERE SPECIAL SPECIMEN REQUIREMENTS?

Nucleic acid amplification (NAA) testing (using the Cepheid GeneXpert) is used to identify the presence of MTBc and potential resistance to rifampin, an important first-line drug. This test is offered for **unprocessed** sputum, bronchial wash and bronchial lavage **clinical specimens only**. This testing requires that the **patient has received less than three days of antituberculous therapy,** and that the **specimen is collected less than ten days before receipt in the laboratory**.

Collection Kits and Shipping Materials

Specific **specimen collection kits** for mycobacterial clinical specimens (TC OL40) and shipping materials are available free-of-charge by the Department of Public Health Laboratory Outfit Room.

For collection kits and shipping supplies, contact the DPH Laboratory Outfit Room at: (860) 920-6674 or (860) 920-6675, or by email at dph.outfitroom@ct.gov

ADDITIONAL SHIPPING INFORMATION

Shipping of cultures known or suspected of containing *Mycobacterium tuberculosis* complex must be packaged and shipped in compliance with "Category A Infectious Substances" guidelines by appropriately trained personnel. Please refer to the following for additional guidance:

https://www.phmsa.dot.gov/sites/phmsa.dot.gov/files/2020-04/Transporting-Infectious-Substances-Safely.pdf

https://shop.saftpak.inmarkinc.com/products/shipping-category-a-biological-substance-and-related-materials-training-course

Mycobacteriology Lab Services Available

METHOD	PURPOSE	TIMEFRAME FOR RESULTS (From Date of Receipt)
Acid-fast bacilli (AFB) Smear	Determine the presence or absence of mycobacteria in a clinical specimen. Monitor response to drug treatment. Characterize acid fast organisms in a culture isolate.	Within 24 hours (except for specimens received Friday after noon) Positive clinical smears from a new patient are communicated to the submitter as ***critical values*.***
Nucleic Acid Amplification Testing (NAAT)	Determine the presence of MTBc DNA in a clinical pulmonary specimen, and the presence or absence of genetic markers for rifampin resistance, a potential indicator of multi-drug resistant (MDR) MTBc.	Within 24-48 hours (except for specimens received Friday after noon) All results, positive or negative, are communicated to submitters as ***critical values*.***
Culture and Identification	Recover viable mycobacterial specimens and monitor drug treatment. Identification and characterization of mycobacterial species. Isolation of MTBc for susceptibility testing.	More than 80% of MTBc and non-tubercular mycobacteria (NTM) are identified within 14 days of receipt. The first MTBc isolate from a new patient is communicated to submitter as *a* ***critical value*.*** Clinical specimens are allowed to incubate for 42 days (6 weeks) before being reported as culture negative.
Antimycobacterial Susceptibility Testing (AST) for MTBc	Determination of resistance or susceptibility of MTBc isolates to a variety of first line antimycobacterial drugs.	AST profiles are reported for more than 80% of isolates within 21 days of identification as MTBc. Resistances are communicated to submitter as ***critical values*.***
Submission to Collaborating Laboratories	Specimens may be referred to collaborating labs for additional or confirmatory testing. In the case of rifampin resistance detected by NAAT, molecular detection of drug resistance (MDDR) analysis at the Centers for Disease Control and Prevention (CDC) can provide very rapid and broad initial susceptibility profiles.	Turnaround for results may vary depending on the collaborating lab. MDDR results are typically returned within seven days of package receipt at the CDC.

Times to result will vary depending on quality of specimen collection, storage, transport, and media.

****Critical Values are time-sensitive results that must be communicated to the submitter by phone***

Mycobacteriology Lab Testing Sequence

Specimen Receipt at the Laboratory

(Turnaround times refer to specimens received Monday through before noon on Friday)

24 Hours

- Fluorescent smear (clinical specimen) or Kinyoun smear (referred culture) are reported as acid-fast bacilli seen or not; clinical specimens are processed and subcultured; incubation begins.
- New patient clinical specimens with acid-fast positive fluorescent smears are reported as *critical values*.

24 - 48 Hours

- NAA test performed, if requested, or if a new patient has an acid-fast positive smear from a clinical specimen. If rifampin resistance is detected, specimen will be forwarded to CDC for molecular detection of drug resistance (MDDR). Results are typically returned within seven days after package receipt at the CDC.
- All NAAT results, positive or negative, are reported as *critical values*.

~ 14 Days

- If mycobacteria are detected, species identification of MTBc or non-tuberculous mycobacteria (NTM) will be finished in about 80% of all specimens. Methods include both molecular techniques (PCR) and mass spectroscopy (MALDI-TOF).
- First isolates of MTBc for a new patient are reported as *critical values*.

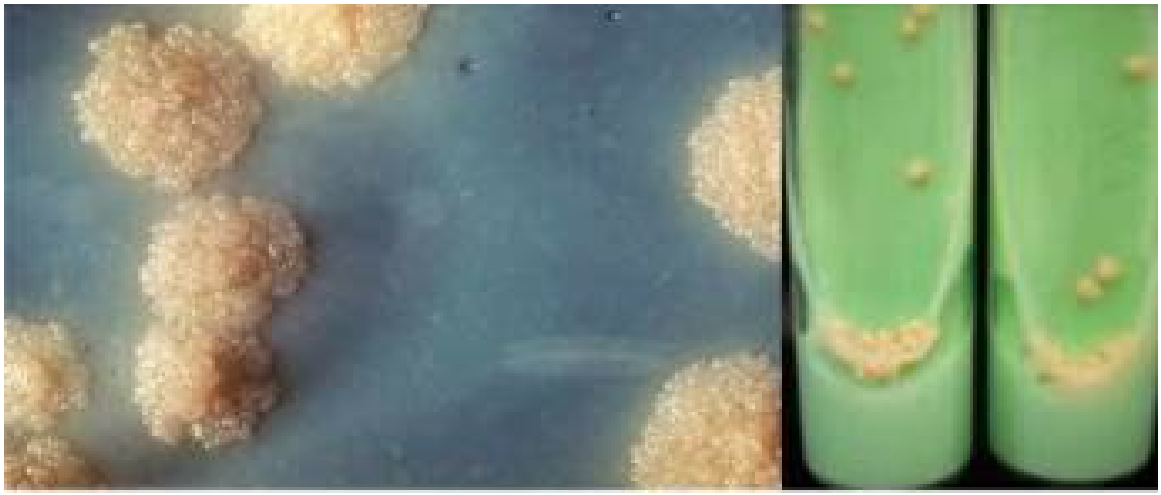
~ 21 Days

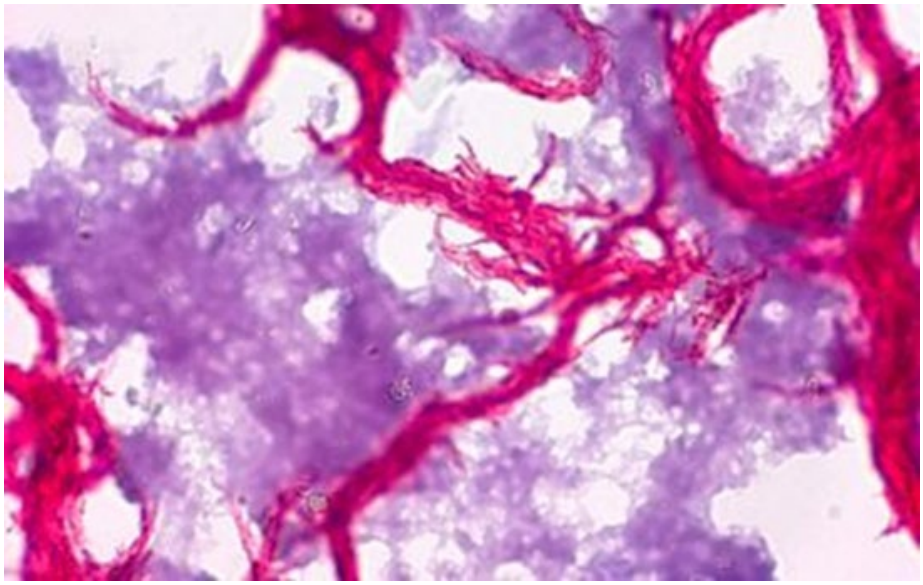
- AST result for a new patient's first MTBc isolate completed in more than 80% of specimens.
- Any resistances are reported as *critical values*.

AT 42 DAYS – CLINICAL CULTURES COMPLETE INCUBATION. IF NO GROWTH IS SEEN IN ANY SUBCULTURE, SPECIMEN IS REPORTED AS "MYCOBACTERIA NOT FOUND"

If you still have additional questions regarding submitting mycobacterial specimens or testing, please contact us:

- Mycobacteriology Laboratory - phone (860) 920-6649 or fax (860) 920-6721.
- TB Epidemiology Program - phone (860) 509-7698 for courier information and other questions
- The Mycobacteriology Laboratory operates Monday through Friday, 7:30 am to 4:00 pm. The laboratory is closed weekends and on various federal and state holidays.





Appendix A

♦ **Submitter (REQUIRED)**

♦ **LAB PROFILE Number:**

CLINICAL TEST REQUISITION
STATE OF CONNECTICUT
Dr. Katherine A. Kelley State Public Health Laboratory
395 West Street, Rocky Hill, CT 06067
CLIA ID 07D0644555
Phone 860-920-6500
Form OL-9B Rev. 01/10/2024

ACCESSION LABEL FOR CTDPH LABORATORY USE ONLY

♦ **DENOTES REQUIRED INFORMATION**

Section 1: Patient Information (***Please Print Clearly***)

♦ **Name (Last, First, M.I.) or Identifier:**

♦ **Street Address:** | ♦ **City, State, Zip:**

♦ **Date of Birth:** | Gender: ☐ Female ☐ Male ☐ Unknown | Home Phone:

Race (check all that apply): (♦ **Race/Ethnicity Information is Required for Blood Lead**)
☐ White ☐ Black/African Amer. ☐ Asian ☐ Amer. Indian/Alaska Nat. ☐ Nat. Hawaiian/Other Pacific Islander ☐ Other ☐ Unknown
Ethnicity: ☐ Hispanic ☐ Non-Hispanic ☐ Unknown

♦ Ordering Healthcare Provider: | ♦ Phone

Section 2: Specimen Information

Specimen Storage (Prior to Delivery):
☐ Refrigerated (2-8˚C) ☐ Frozen (<-20˚C) ☐ Room Temperature
Specimen Transport/Delivery:
☐ Cold (Ice pack) ☐ Frozen (Dry Ice) ☐ Room Temperature

Specimen Receipt (*CTDPH internal use only*)
˚C ☐ Room Temperature ☐ Refrigerated ☐ Frozen

Submitter Sample ID: | ♦ **Date Collected:** | Time Collected: ☐ AM ☐ PM

♦ **Specimen Source/Type:**
☐ Axilla/groin ☐ Blood (whole) ☐ Bronchial Wash ☐ Buccal cavity ☐ Cervix ☐ CSF ☐ Lesion ☐ Nasopharynx
☐ Oropharynx ☐ Plasma ☐ Rectum ☐ Serum ☐ Sputum ☐ Stool ☐ Urethra ☐ Urine ☐ Vagina
☐ Body Fluid, specify _____ ☐ Tissue, specify _____
☐ Other, specify _____

♦ **Section 3: Select Testing Requested**

Bacteriology

☐ AFB Clinical Specimen (Mycobacteria Smear & Culture)
☐ AFB Referred Culture (Mycobacteria for Identification)
Bacterial Isolate for Identification (**Check one**)
☐ Group A Streptococcus ☐ Group B Streptococcus ☐ *H. influenzae*
☐ *L. monocytogenes* ☐ Legionella ☐ *N. meningitidis* ☐ *S. pneumoniae*
☐ Campylobacter ☐ *E. coli* O157 ☐ Salmonella ☐ Shigella
☐ Shiga-toxin producing *E. coli* ☐ Vibrio ☐ Yersinia
☐ Other: _____
☐ Bioterrorism Agent Identification
Specify agent: _____
Bordetella pertussis ☐ Culture ☐ DNA amplification
☐ Carbapenemase colonization screening (Rectal swab)
Carbapenem resistant organism (**Please attach susceptibility results**)
☐ Fast Track (**[1]Epidemiology approval required**)
☐ CRE (Enterobacterales, specify organism) _____
☐ CRAB (*Acinetobacter baumannii*) ☐ CRPA (*Pseudomonas aeruginosa*)
☐ Enteric (Stool) Culture Suspect Organism: _____
☐ Shiga-toxin (+) Broth Culture

Blood Lead (**Uninsured Patients ONLY**) ♦ **Race/Ethnicity Required**
☐ Child Lead Screen (Capillary) ☐ Confirmation (Venous)

Mycology
☐ *Candida auris* identification (culture isolate)
☐ *Candida auris* screen
☐ Yeast ID/susceptibility testing (**Blood *Candida* spp. Isolates ONLY**)

Parasitology
☐ *Blood Parasite – Smear

Comments

Test, Agent, or Disease Not Listed (Specify):

Serology/Virology/Sexually Transmitted Infections

Arbovirus (**Please select all that apply**)
☐ *Eastern Equine Encephalitis Virus IgM Antibody
☐ *Powassan Virus IgM Antibody
☐ *West Nile/St. Louis Virus IgM Antibody
☐ Chlamydia/ Gonorrhea Nucleic Acid Amplification Test
☐ Hepatitis A Virus PCR (**[1]Epidemiology approval required**)
☐ Hepatitis B Surface Antibody
☐ Hepatitis B Surface Antigen
☐ Hepatitis C Testing
☐ Herpes Simplex IgG Antibody
☐ Herpes Simplex DNA amplification
☐ HIV-1/HIV-2 Antigen/Antibody
☐ HIV Viral Load
☐ Influenza/SARS-CoV-2 multiplex PCR
☐ Measles PCR
☐ MERS CoV PCR (Novel Coronavirus) (**[1]Epi Approval Required**)
☐ Mumps PCR
☐ *Non-Variola Orthopoxvirus PCR (**R/O Monkeypox Virus**)
☐ Norovirus PCR (**[1]Epidemiology approval required**)
☐ QuantiFeron-TB Test (**Specify ♦ Date AND Time Collected Above**)
☐ Syphilis Screen (Serum)
☐ Syphilis VDRL (CSF)
☐ Respiratory Panel
☐ *Trichomonas vaginalis* NAAT (urine/vaginal Only)

***Please provide:**
Symptoms _____
Symptom onset date _____
Travel history _____

[1]DPH Epidemiology and Emerging Infections: (860)509-7994

Appendix B

CONNECTICUT
Public Health

Mycobacterium tuberculosis complex
Nucleic Acid Amplification (NAA) Test Requisition

Katherine A. Kelley State Public Health Laboratory
395 West Street, Rocky Hill, CT 06067

For each clinical respiratory specimen where NAA testing is requested, complete this form, along with a Clinical Test Requisition, when submitting the specimen to the laboratory. Routine mycobacteria smear & culture will also be performed.

NAA testing will automatically be done on the first patient specimen submitted for routine mycobacteria smear & culture found to be **Acid-fast Bacilli (AFB) smear positive** by the CTDPH laboratory (the *M. tuberculosis* complex NAA Test Requisition is not required).

NAA Testing should **NOT** be ordered:

- When clinical suspicion is low (the positive predictive value of the test, the likelihood that the patient has tuberculosis when the test is positive, is low in such cases).
- To determine bacteriologic cure or to monitor response to antituberculous therapy

CTDPH TB Laboratory (Ph: 860-920-6649 / Fax: 860-920-6721)

CTDPH TB Control Program (860-509-7722)

Submission Requirements

- ☐ Clinical respiratory specimens (raw unprocessed): sputum, BAL, bronchial wash
- ☐ Patient has received no antituberculosis therapy, or less than three days of therapy at specimen collection.
- ☐ Specimens must be received by the laboratory within 10 days of collection.
- ☐ Test requests must be received within 7 calendar days of specimen receipt in the laboratory

Submitter Information

Authorized Submitter's Name: _____

Phone :_____ Fax: _____

Patient Information

Name:_____

Patient /Specimen ID #:_____ Date of Birth: _____

Specimen Information

Type / Source: ☐ Sputum ☐ Bronchoalveolar Lavage (BAL) ☐ Bronchial Wash

Date Collected:_____ Other Information_____

Rev. 05/02/2024

Acknowledgements:

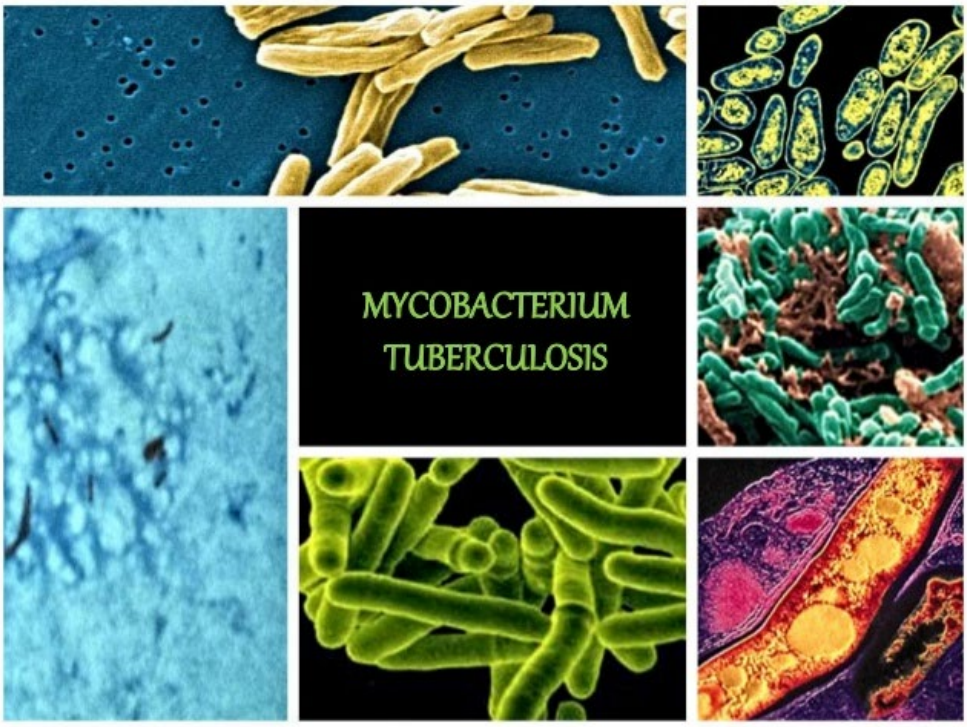
Rik Martinez

Christina Nishimura

Mark Harkins

Hongli Dong

Diane Charette



World TB Day **March 24**