Mycobacterium species

Training Reference

Specimens

- Mycobacterial infections can occur in almost any anatomical site, due to either MTB or Nontuberculous Mycobacteria (NTM).
- A variety of clinical specimens other than sputum (e.g., urine, feces, CSF, pleural fluid, aspirates, or biopsy specimens) may be submitted for examination.

Processing Specimens

- Decontamination of a specimen should be attempted only if it is thought to be contaminated.
- Tissues or body fluids collected aseptically usually do not require pretreatment.

Digestion and Decontamination

- 1-2% Sodium Hydroxide (NaOH)
  - digestant & decontaminant
- *N*-acetyl-L-cysteine
  - liquefying agent
- Phosphate buffer
  - stops digestion/decontamination process

Smear Microscopy

- Performed on pretreated and concentrated specimens or on culture isolates.
- The overall sensitivity of the smear for clinical specimens has been reported to range from 22 to 80%.
- Even though the smear lacks sensitivity, it allows rapid identification of the most contagious patients.

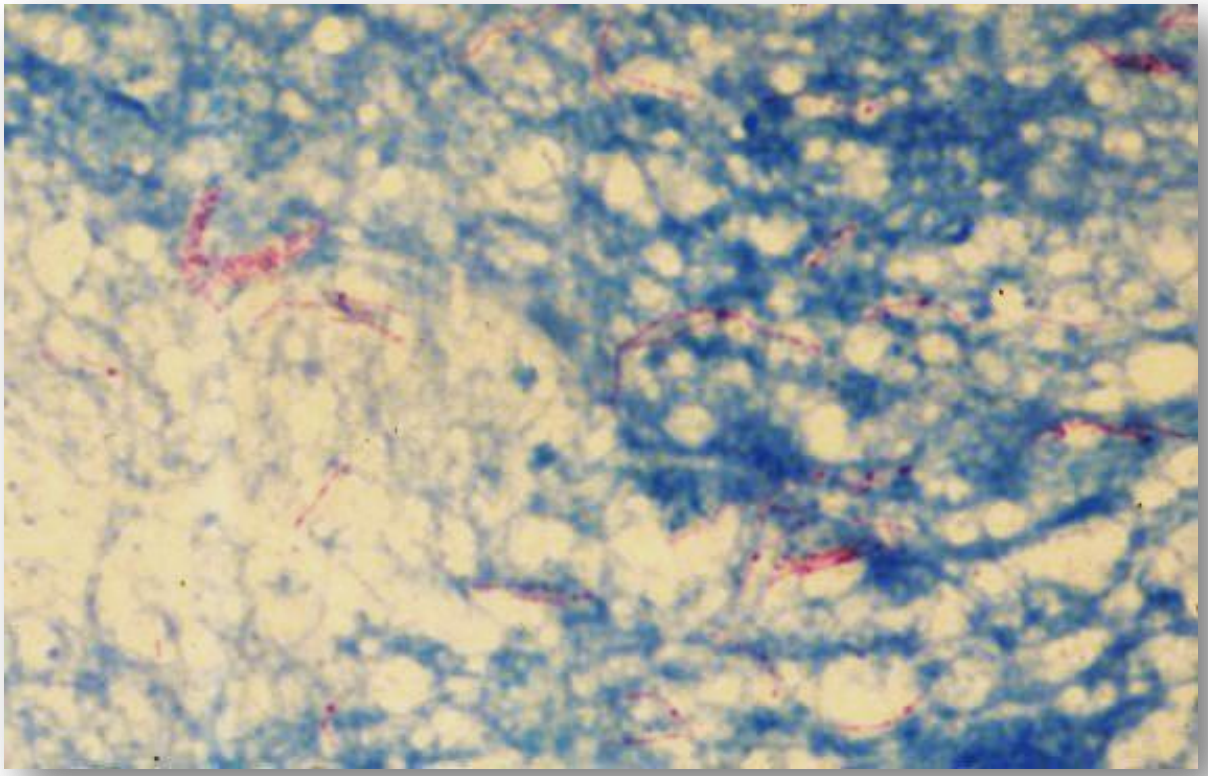
Stain Procedures

- Gram stain is not suitable for mycobacteria. They may appear as clear zones or as beaded gram-positive rods, particularly rapidly growing mycobacteria.
- Special acid-fast staining procedures are necessary to promote the uptake of dyes. Phenol allows penetration of the stain. Mycobacteria are able to form stable complexes with dyes such as fuchsin.
- The cell wall mycolic acid residues retain the primary stain even after exposure to acid-alcohol.
- AFB are approximately 1 to 10 µm long and typically are slender rods that may appear curved or bent.

Kinyoun Stain Procedure

- Flood slide with carbol fuchsin 5 min
- Rinse slide with water
- Flood with 3% acid-alcohol 2 min and until no more color drains from the slide
- Rinse slide with water
- Methylene blue 2 min
- Rinse with water, drain and dry
- Examine with 100X oil immersion with light microscope

Kinyoun 1000x



Positive: red-stained rods against blue background

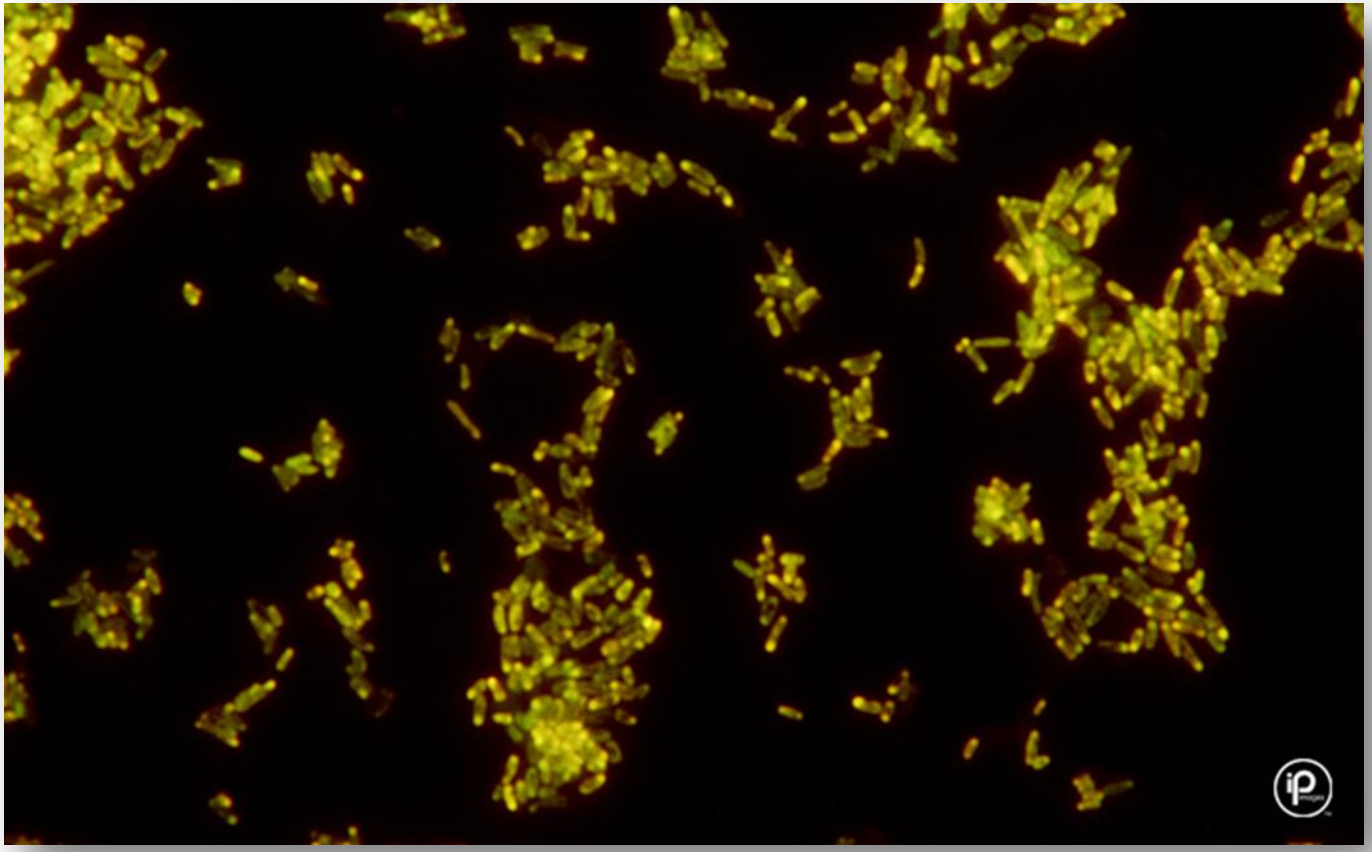
Auramine-Rhodamine

- Fluorescent dyes
- Acid-fast bacilli are more readily seen read at a lower power (X250) than the carbol fuchsin stain; therefore, more material can be examined in a given period. At the lower magnification, a minimum of 30 fields of view should be examined.
- Must distinguish bacilli from acid-fast artifacts

Auramine-Rhodamine Procedure

- Flood the slide with fluorochrome stain 15 min
- Rinse slide with water
- Flood with 0.5% acid-alcohol decolorizer 2 min
- Rinse slide with water
- Flood with potassium permanganate 2 min
- Rinse slide with water, drain and dry
- Examine with a fluorescent microscope with a 25X or 40X objective

Auramine-rhodamine 1000x



Positive: yellow to orange fluorescence against a black background

Smear Reporting

- All negative should be reported as negative.
- Positive fluorochrome-stained smears should be confirmed by carbol fuchsin staining. Confirm positive smears by having them reviewed by another experienced reader.
- Quantity of AFB observed on the smear should be reported (refer to procedure).
- If only one or two organisms are seen on an entire smear, this should be noted but not reported.

Culture Methods

- More sensitive than smear, detecting as few as 10 to 100 organisms/mL.
- Selective agents are used to prevent overgrowth by contaminating bacteria or fungi.
- Broth media are preferred for a rapid initial isolation of mycobacteria.

Solid Media

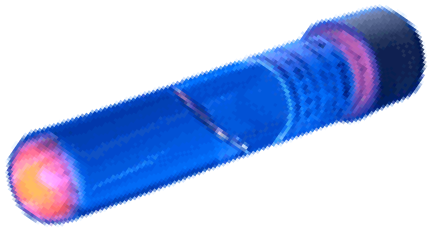
- Solid media allows for detection of mixed cultures
- Egg-Based Media (L-J)
  - Supports the growth of most mycobacteria
  - Contamination occurs more easily
- Agar-Based Media (Middlebrook 7H11)
  - Less contamination and earlier and easier visibility of colonial morphology

Liquid Media

- Broth media may be used for both primary isolation and subculturing of mycobacteria.
- Liquid media yield significantly more rapid results than solid medium-based cultures.
- Isolation rates for mycobacteria are higher.
- Middlebrook 7H9

MGIT (Becton Dickinson)

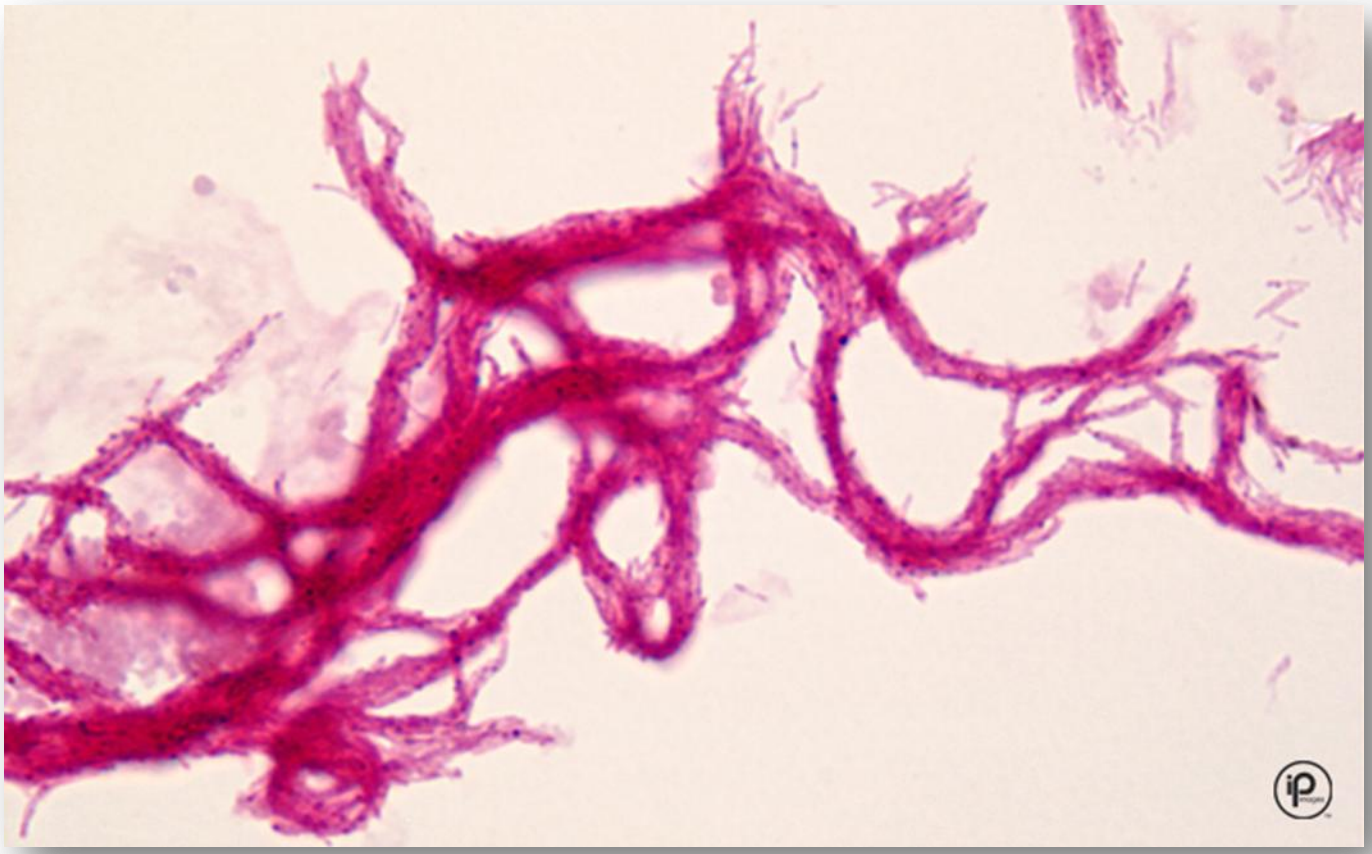
- Contains a modified Middlebrook 7H9 broth in conjunction with a fluorescence-quenching-based oxygen sensor to detect growth of mycobacteria.
- Growth of mycobacteria or other microorganisms in the broth depletes the oxygen, and the indicator fluoresces brightly when the tubes are illuminated with UV light at 365 nm.



Identification of Mycobacteria

- Confirm that organism isolated is acid-fast
- Growth rate
- Optimum growth temperature
- Colony morphology
- Photoreactivity
- Mycolic acid analysis by HPLC

Positive with cording (Kinyoun 1000x)



In liquid medium *M. tuberculosis* often exhibits serpentine cording, but cords are also seen with some NTM species such as MAC, *M. gordonae, M. chelonae,* and *M. marinum.*

Growth Rate

- Species dependent
- Influenced by media, temperature, and inoculum size
- Range of recovery time 3-60 days
- Rapid growers < 7 days

Temperature

- The optimum incubation temperature for most cultures is 35 ± 2°C.
- Some species have a lower optimum temperature (*M. marinum, M. ulcerans, M. chelonae, M. haemophilum*). These organisms typically infect skin and soft tissue. A second set of media must be inoculated and incubated at 30 ± 2°C.

Photoreactivity

- Photochromogenic
  - produce carotene pigment upon exposure to light (yellow to orange)
- Scotochromogenic
  - produce pigment in the dark or in the light
- Nonchromogenic
  - colonies remain buff-colored

Mycolic Acid Analysis

- HPLC analysis of the mycolic acid pattern for identification of *Mycobacterium* can provide identification more rapidly than time-consuming phenotypic tests.
- Mycolic acids are extracted from the cell walls and saponified. The mycolic acids are then derivatized to esters and separated by chromatography. The identification of species is based on the comparison of the test isolate's pattern of peaks with patterns from a library of known reference strains.
- **Results obtained by HPLC analysis should be confirmed by the presence of fundamental specific phenotypic properties, like growth rate, colony morphology, and pigmentation.**

Slowly Growing Mycobacteria *Mycobacterium tuberculosis* Complex

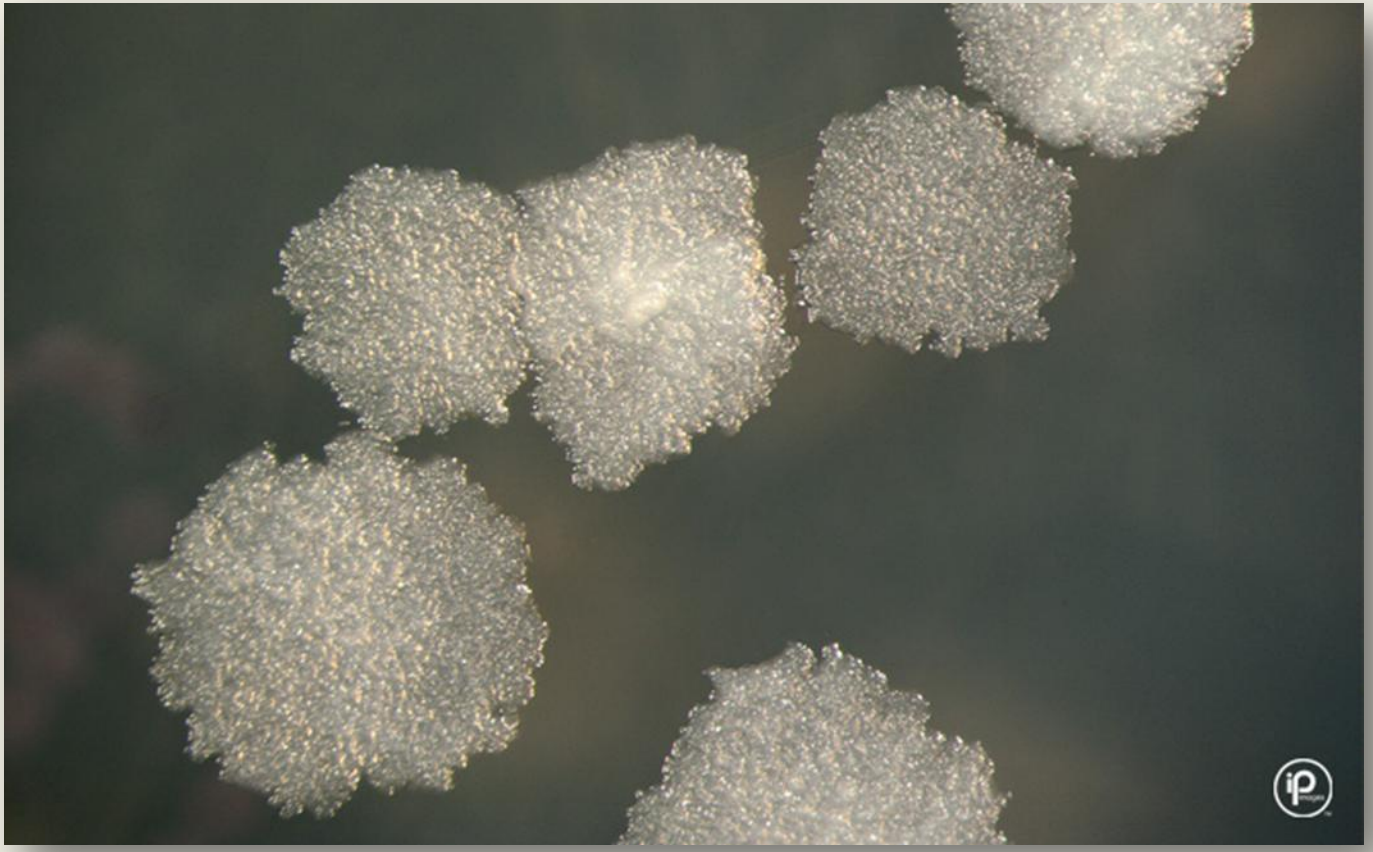
- The MTB complex includes *M. tuberculosis, M. bovis, M. bovis* BCG, *M. africanum, M. caprae, M. microti, "M. canettii,"* and *M. pinnipedii*.
- Tuberculosis in adults is a slowly progressive process characterized by chronic inflammation and caseation and formation of cavities.
- Extrapulmonary manifestations of *M. tuberculosis* infection include cervical lymphadenitis, pleuritis, pericarditis, synovitis, meningitis, and infections of the skin, joints, bones, and internal organs.

Nonchromogenic Slow Grower

Mycobacterium tuberculosis

- Growth rate: 12-28 days
- Colony morphology on 7H11: rough, buff, flat, spreading; irregular periphery

Mycobacterium tuberculosis 7H11



Mycobacterium tuberculosis L-J

Mycobacterium bovis

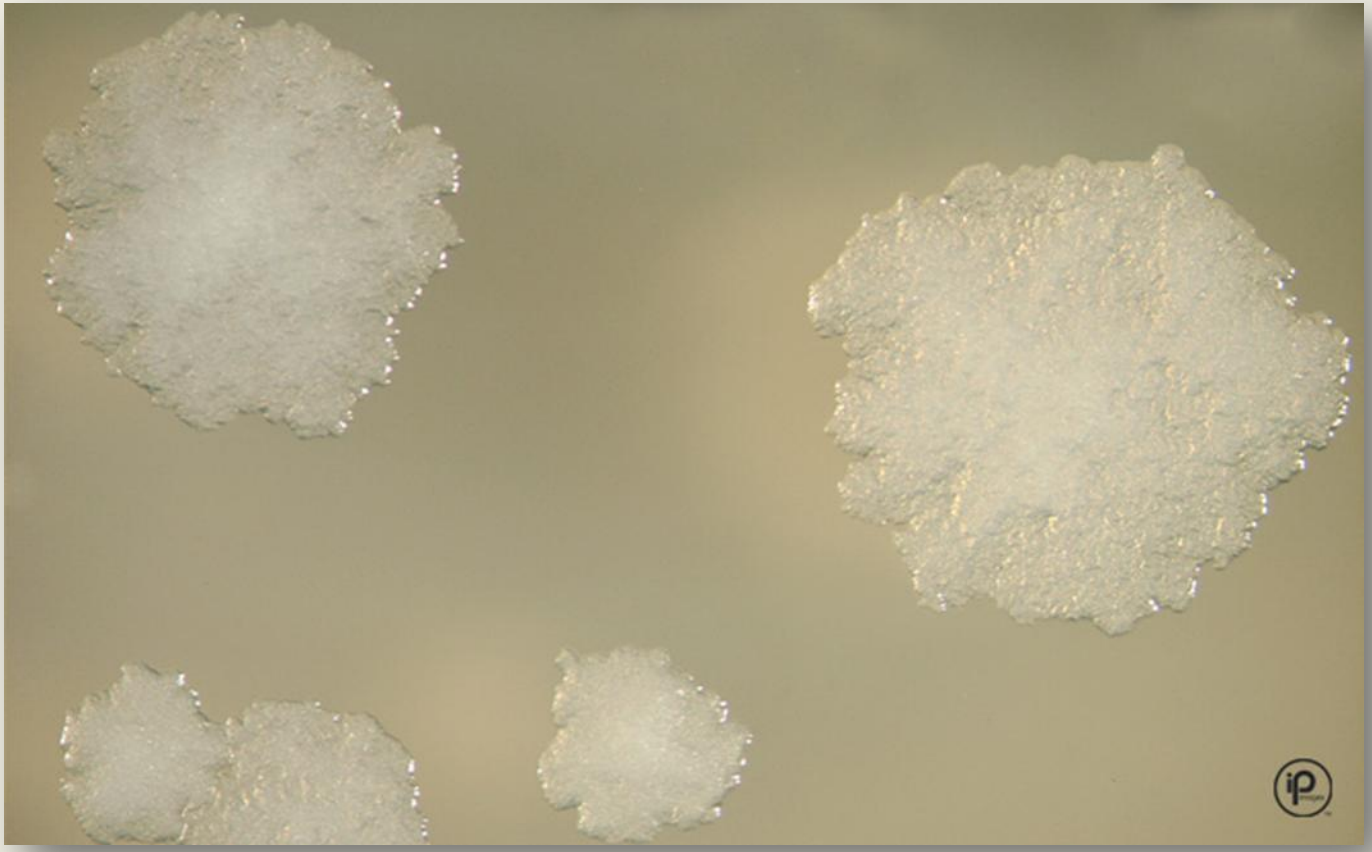
- *M. bovis* causes tuberculosis in warm-blooded animals, such as cattle, dogs, cats, pigs, parrots, and also in primates and humans.
- Human disease is very similar to that caused by *M. tuberculosis* and treated accordingly, except that pyrazinamide is ineffective due to inherent resistance of *M. bovis*.
- In many parts of the world, bacillus Calmette-Guérin (BCG) is still used for vaccine purposes.
- In rare instances, BCG may disseminate as a complication of intravesical BCG immunostimulation against bladder cancer.

Nonchromogenic Slow Grower

Mycobacterium bovis

- Growth rate: 25-90 days
- Colony morphology on 7H11: colorless to buff, small, thin, irregular edges and granular surface, raised, rough, later wrinkled and dry

Mycobacterium bovis 7H11



Mycobacterium bovis L-J

Slowly Growing Mycobacteria
Nontuberculous Mycobacteria (NTM)

- NTM are widely distributed in the environment.
- In culture, they may represent a contaminant or an opportunistic pathogen.
- Determination of the clinical significance based upon multiple factors, including clinical setting, host-specific factors, species, the pathogenic potential of the organism, the number of positive cultures, the source of the culture isolate, and quantification of the organisms detected (by smear and culture). For example, although the incidence of a specific NTM such as *M. gordonae* in cultures is high, the pathogenicity of this species is very low, in contrast to that of species such as MAC and *M. kansasii.*

Slowly Growing Mycobacteria
Nontuberculous Mycobacteria (NTM)

- The most common infections with NTM currently are pulmonary diseases.
- MAC is the most commonly isolated pathogenic slowly growing NTM, but other species of NTM also cause disease. In the US, *M. kansasii* infection is the second most frequently recovered pathogenic species.

Slowly Growing Mycobacteria
Nontuberculous Mycobacteria (NTM)

The American Thoracic Society clinical criteria for non TB mycobacterial lung disease requires the presence of pulmonary symptoms and lung nodules or cavities, with the exclusion of other diagnoses. Microbiological diagnosis requires at least:

1) two sputa or one bronchial wash or lavage with a positive culture or
2) a lung biopsy showing granulomatous inflammation or AFB, with one or more sputum or bronchial washings that are culture positive

(Am J Respir Crit Care Med. 175:367 to 416, 2007)

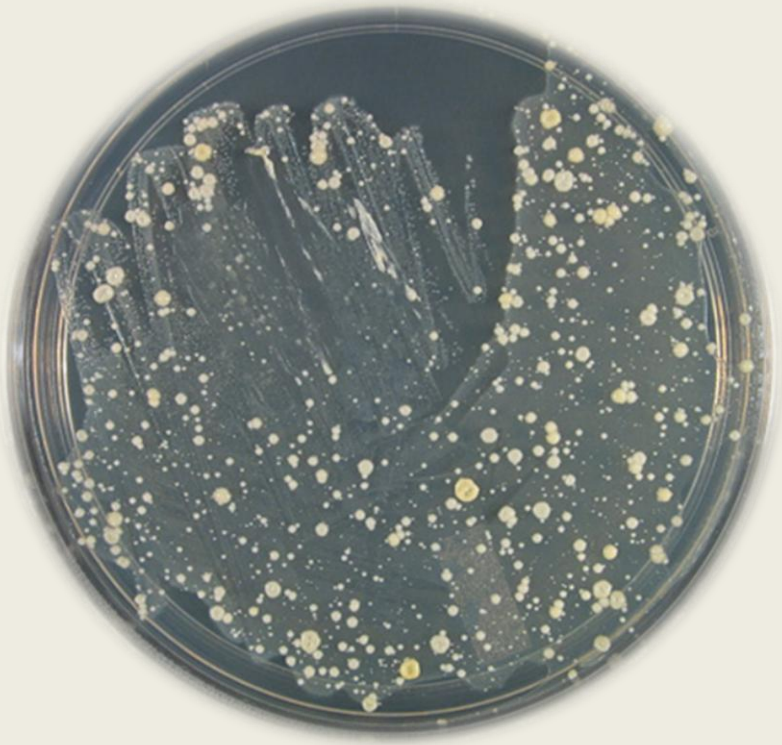
Slowly Growing Mycobacteria
Nontuberculous Mycobacteria (NTM)

- Other infections include skin and soft tissue, lymphatic, and disseminated infections.
- Disseminated infections most often occur in patients with advanced HIV disease, but non-HIV-infected patients can also be affected.

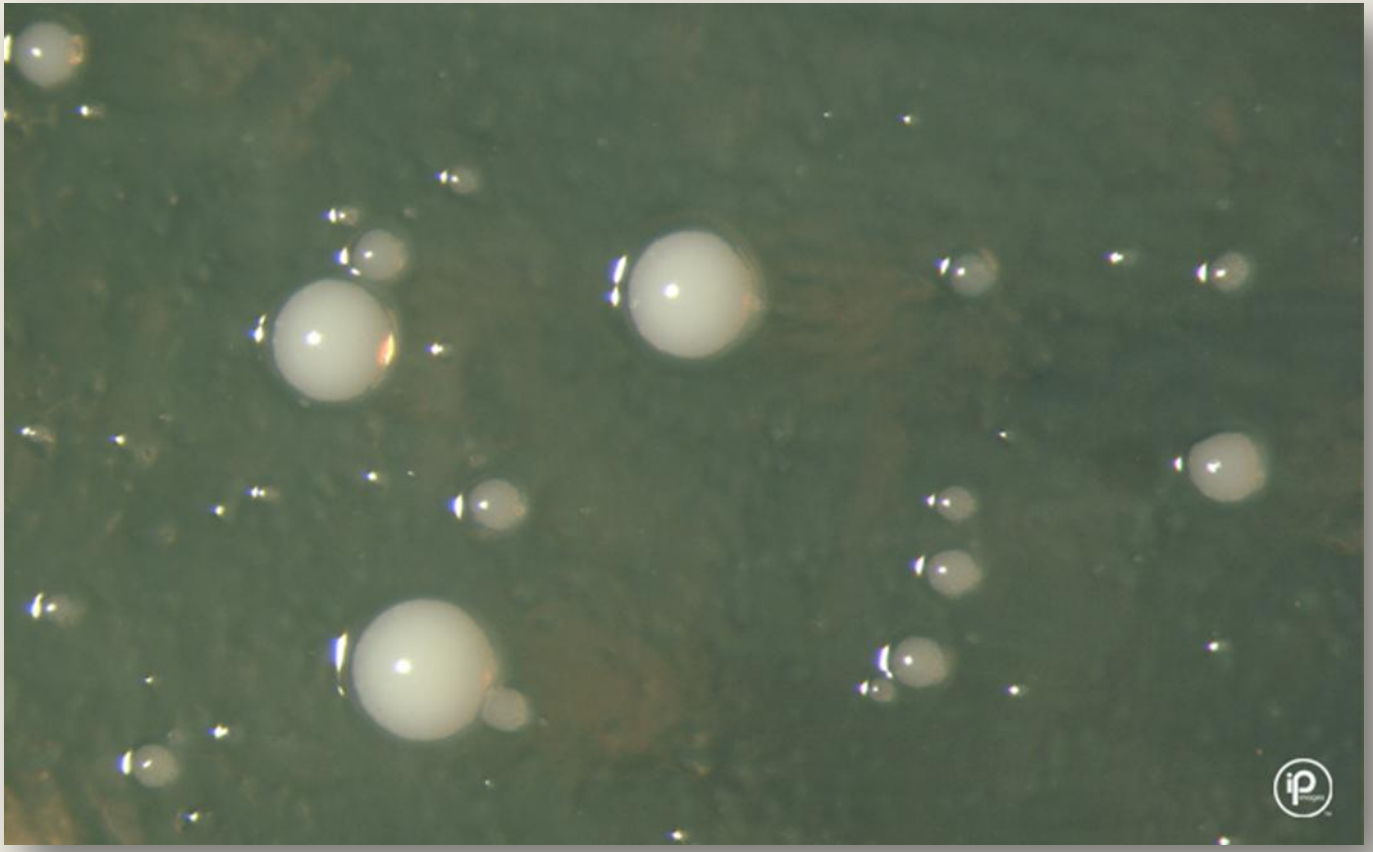
Nonchromogenic Slow Grower

Mycobacterium avium complex

- Growth rate: 10-21 days
- Colony morphology on 7H11: buff to yellow, thin, transparent, glistening or matte, smooth, entire, rounded; some rough and wrinkled



Mycobacterium avium complex 7H11



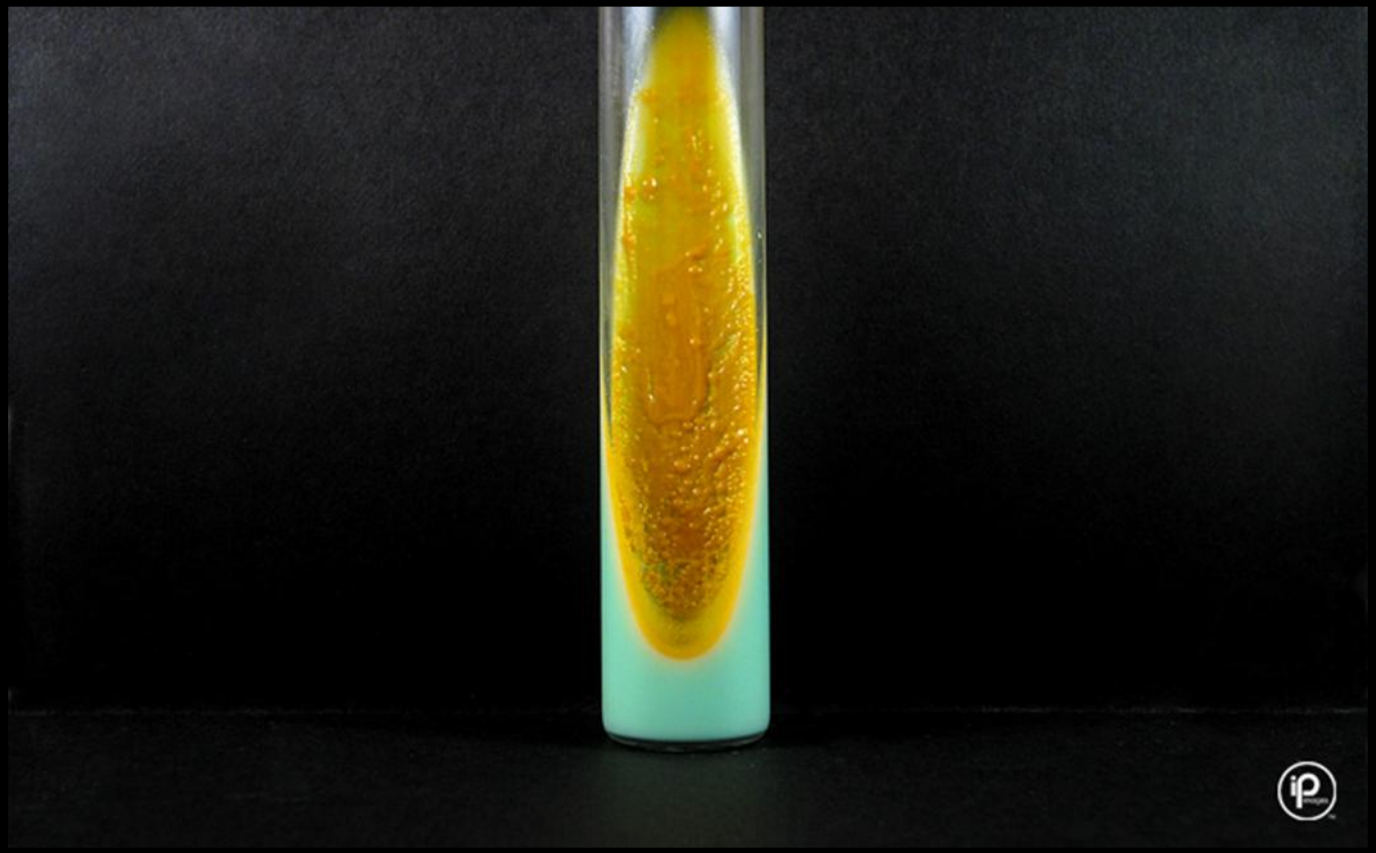
Mycobacterium avium complex L-J

Scotochromogenic Slow Grower
Mycobacterium gordonae

- Growth rate: 10-25 days
- Colony morphology on 7H11: yellow to orange, round, smooth, convex, glistening

Mycobacterium gordonae 7H11

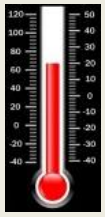
Mycobacterium gordonae L-J



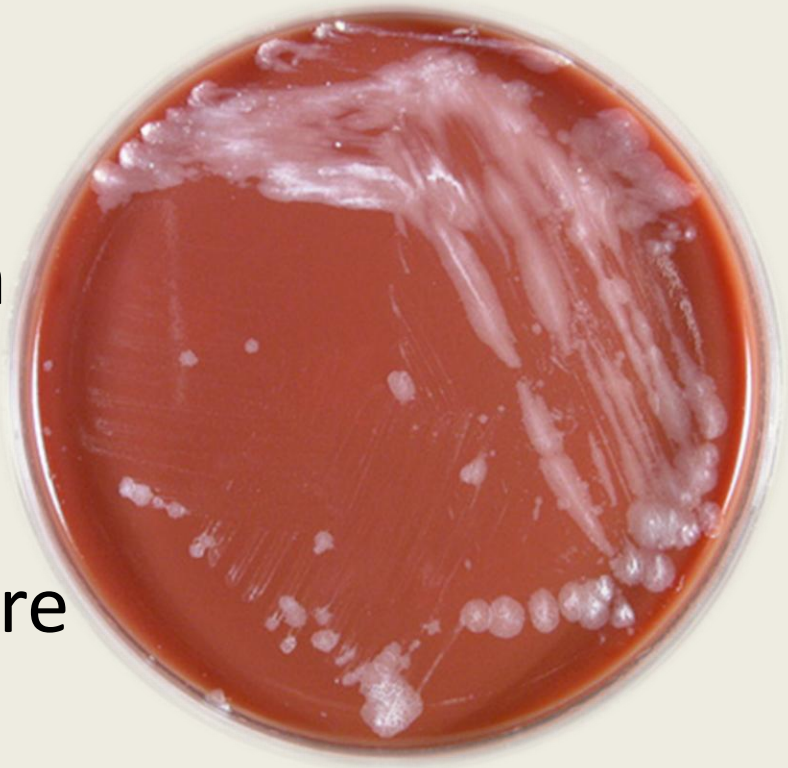
Nonchromogenic Slow Grower

Mycobacterium haemophilum

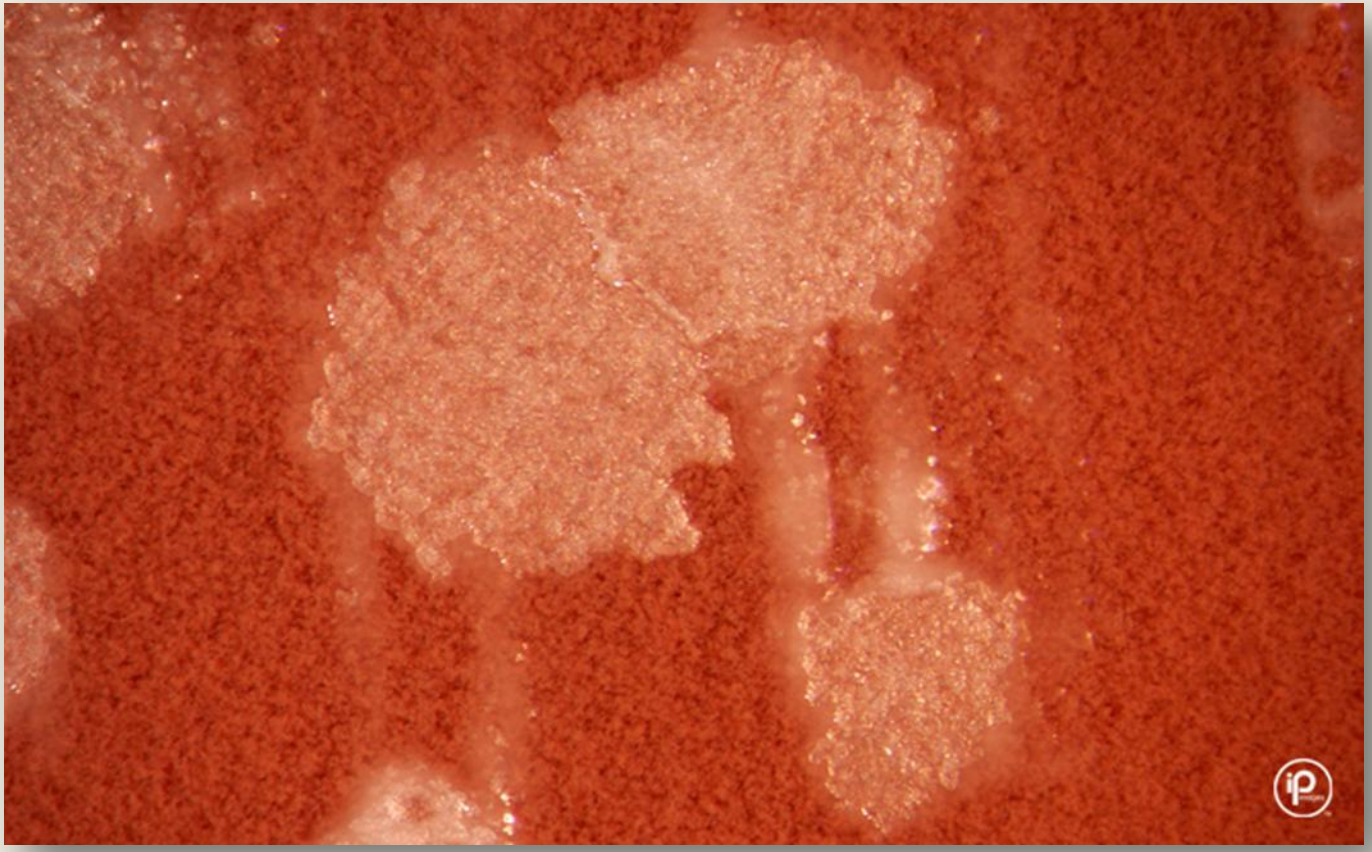
- Growth rate: 14-28 days
- Colony morphology on CHOC: buff to gray, smooth to rough; requires hemin for growth



- Optimal growth temperature range: 25-30°C



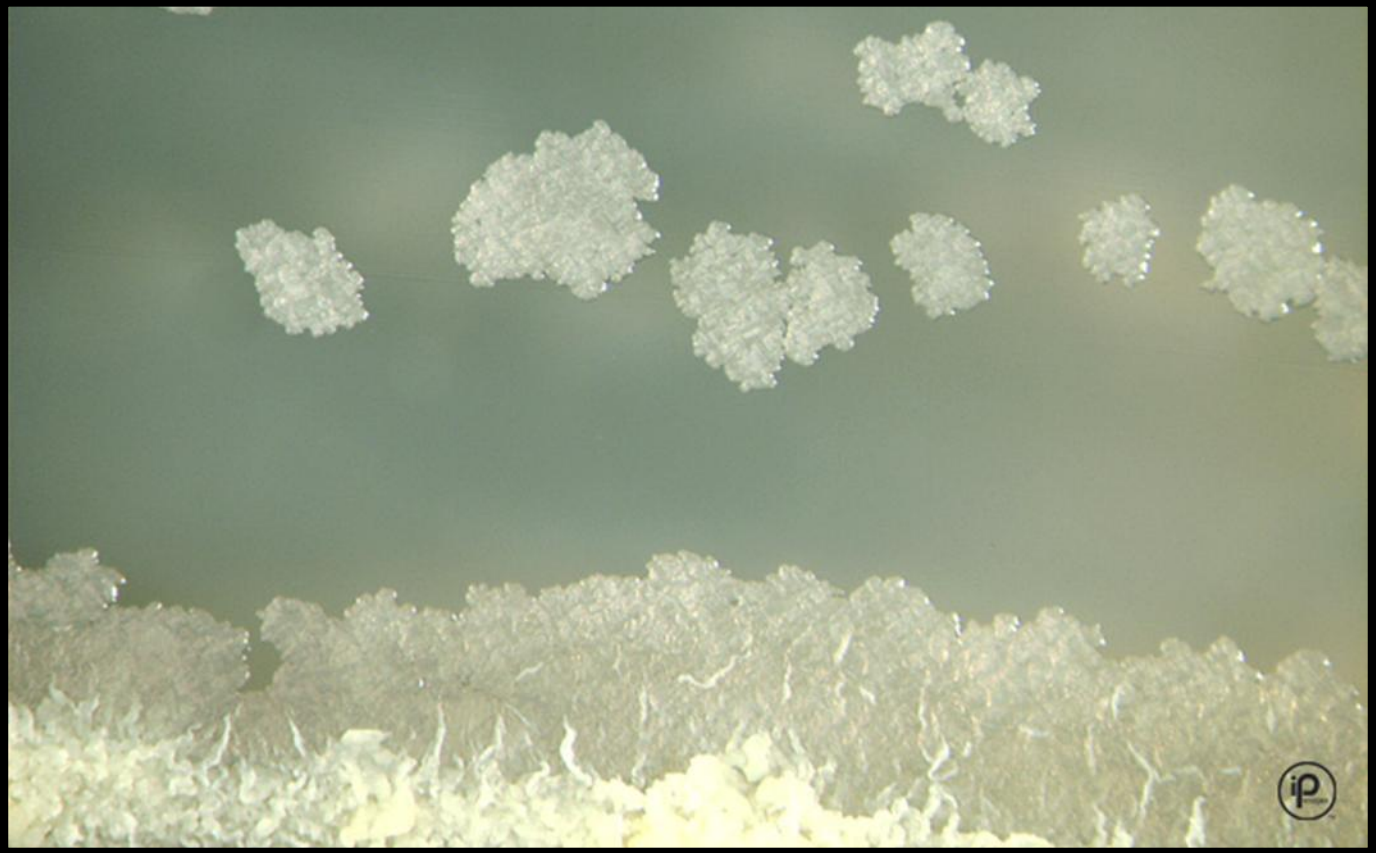
Mycobacterium haemophilum CHOC



Photochromogenic slow grower
Mycobacterium kansasii

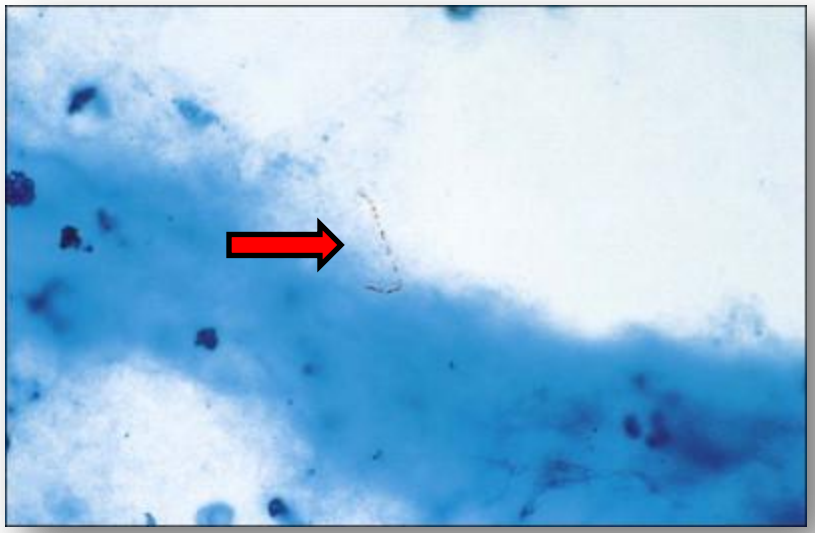
- Growth rate: 10-21 days
- Colony morphology on 7H11: buff, turns yellow after exposure to light, raised, smooth; some rough and wrinkled

Mycobacterium kansasii 7H11



Mycobacterium kansasii Microscopy

M. kansasii organisms can often be suspected in stained sputum smears by their large size and cross-banding appearance

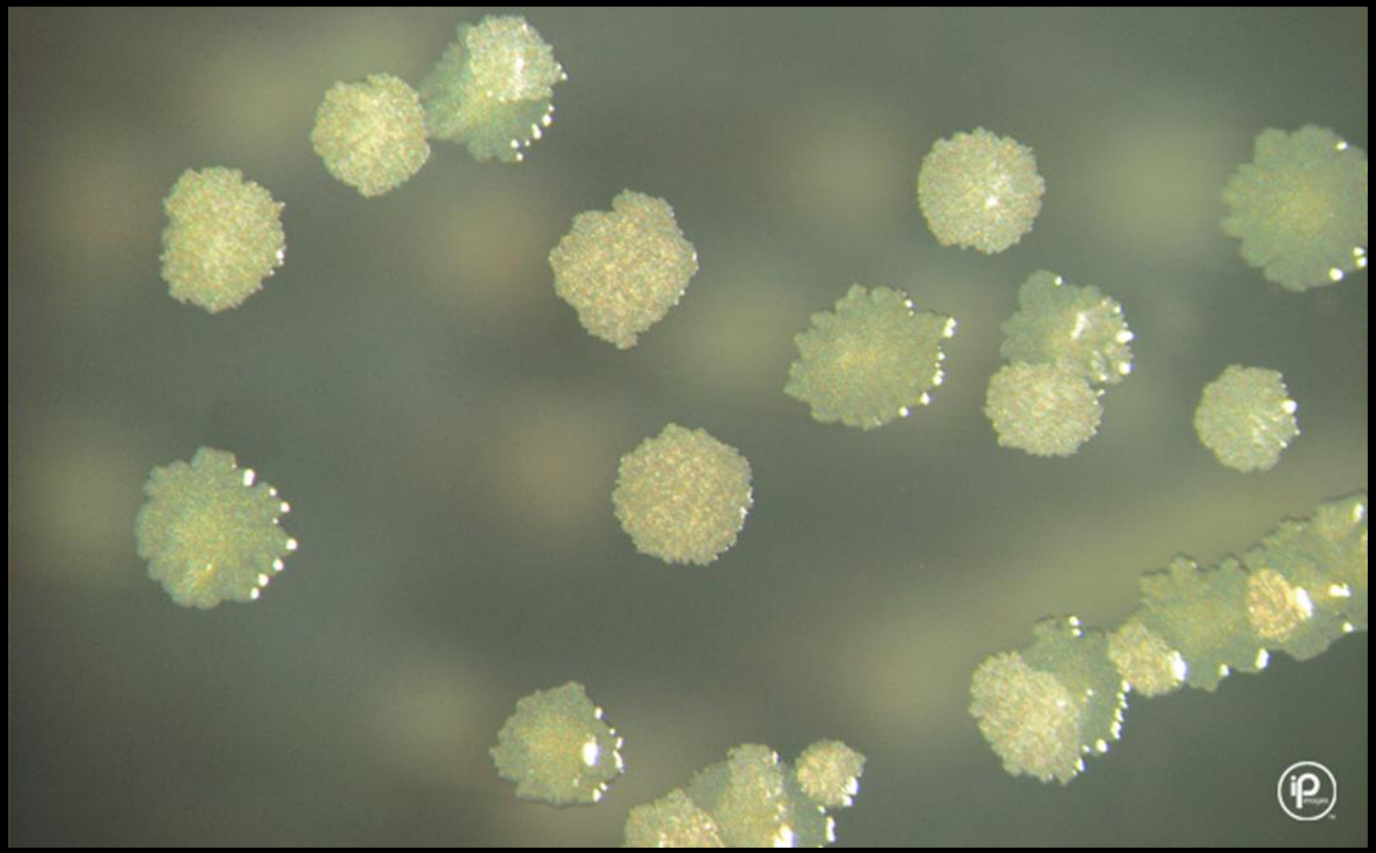


Mycobacterium kansasii
L-J Exposed to Light

Photochromogenic slow grower *Mycobacterium marinum*

- Growth rate: 5-14 days
- Colony morphology on 7H11: buff, turns yellow with exposure to light, round, smooth, some may be wrinkled
- Optimal growth temperature: 30°C

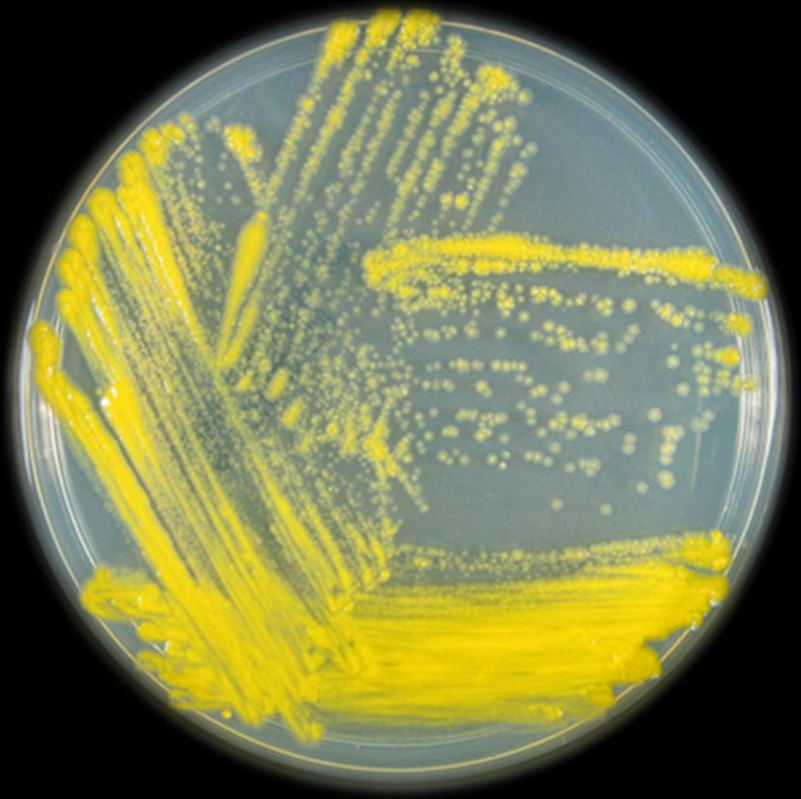
Mycobacterium marinum 7H11



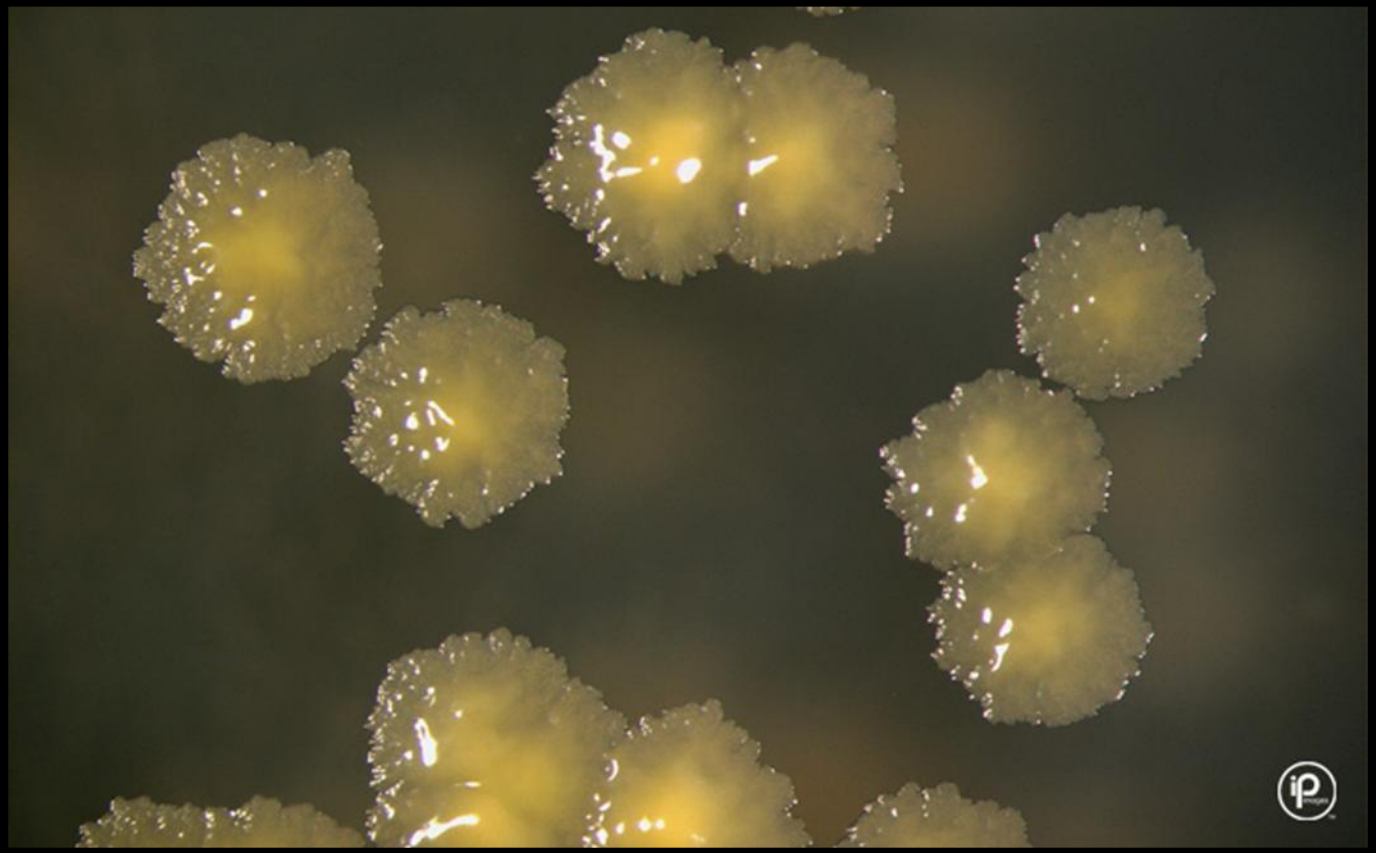
Mycobacterium marinum
L-J Exposed to Light

Scotochromogenic slow grower
Mycobacterium scrofulaceum

- Growth rate: 10-14 days
- Colony morphology on 7H11: yellow, smooth, moist, yellow, round



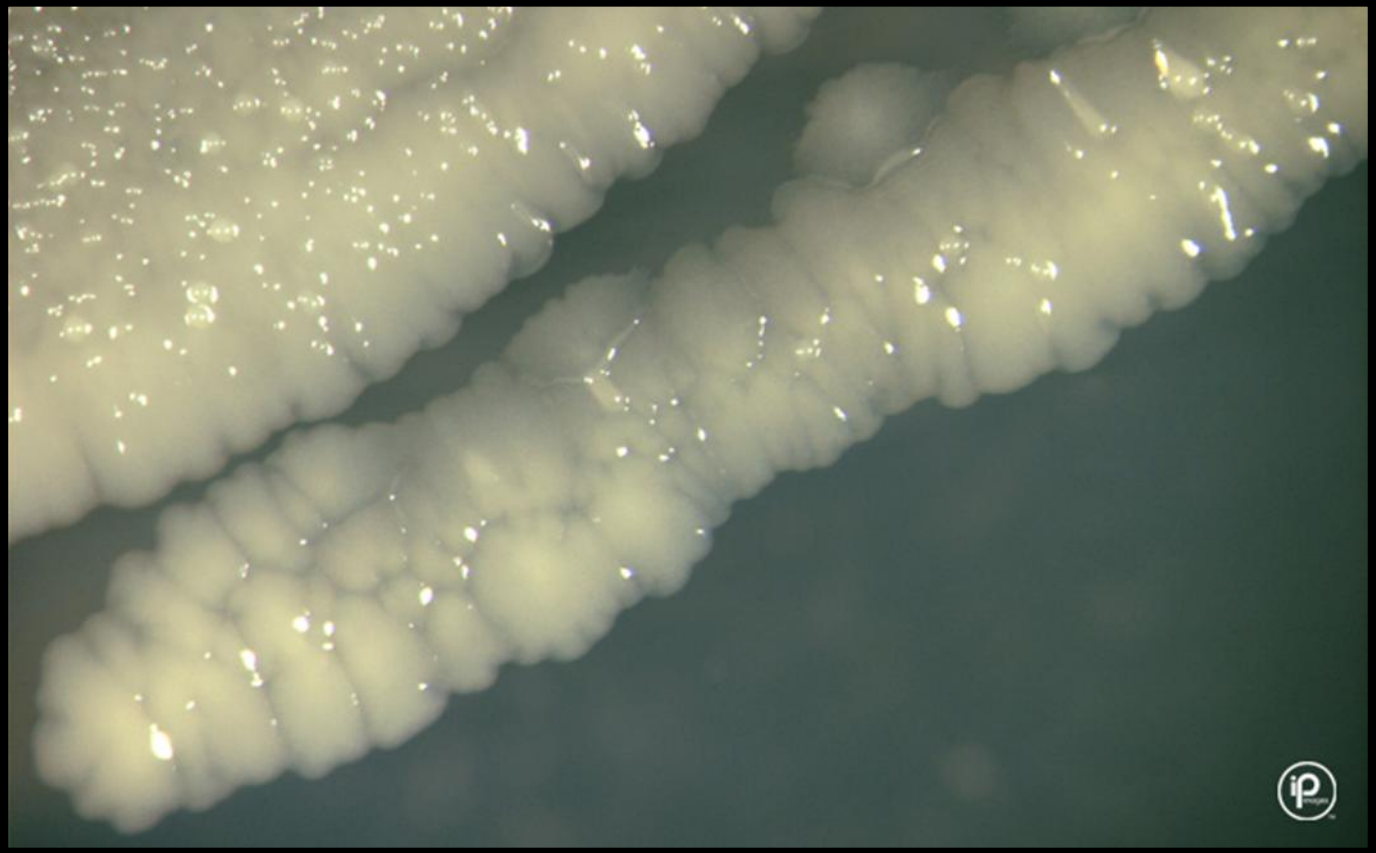
Mycobacterium scrofulaceum 7H11



Photochromogenic slow grower
Mycobacterium simiae

- Growth rate: 7-14 days
- Colony morphology: buff, turns yellow with exposure to light, smooth, domed

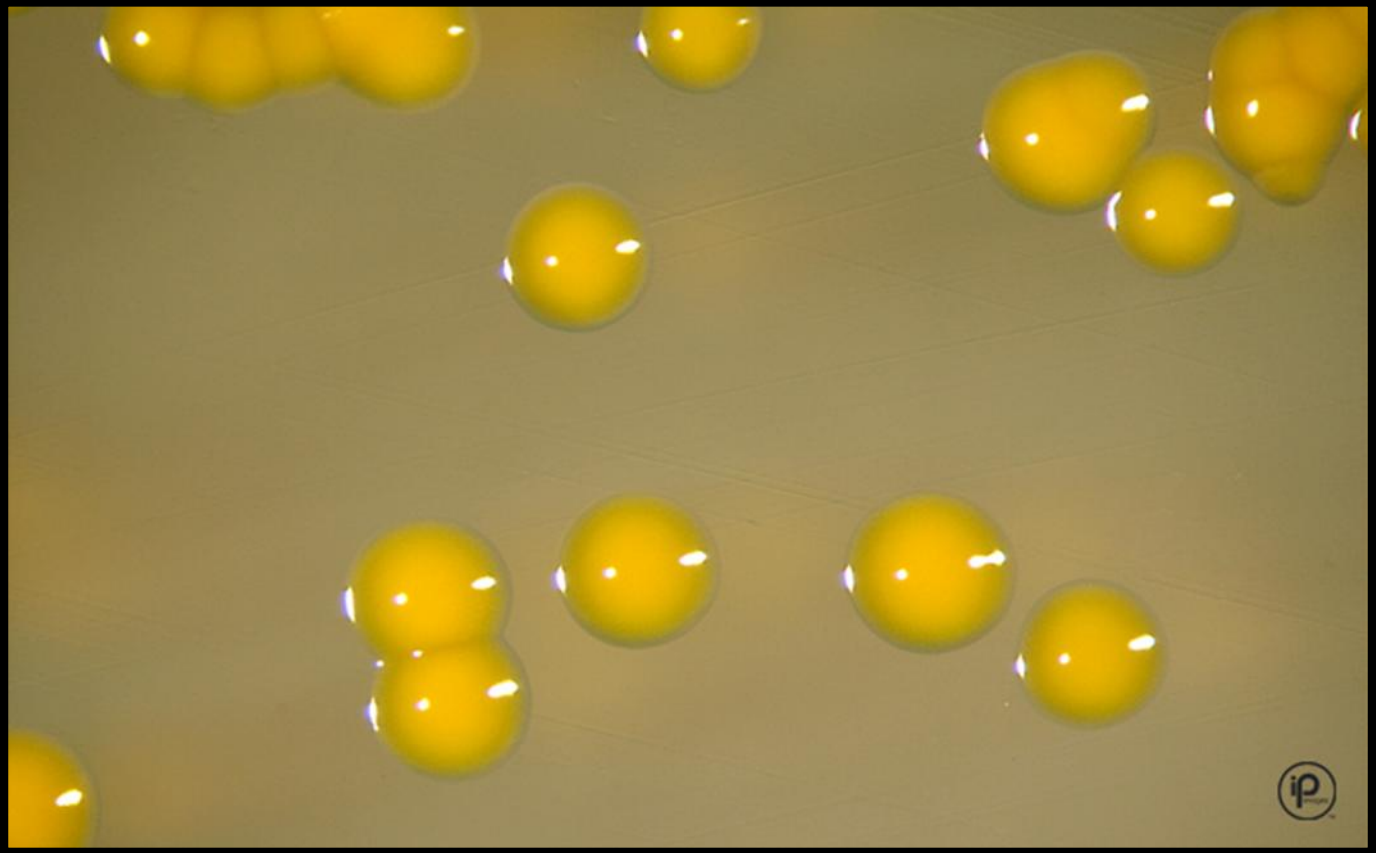
Mycobacterium simiae 7H11



Scotochromogenic slow grower
Mycobacterium szulgai

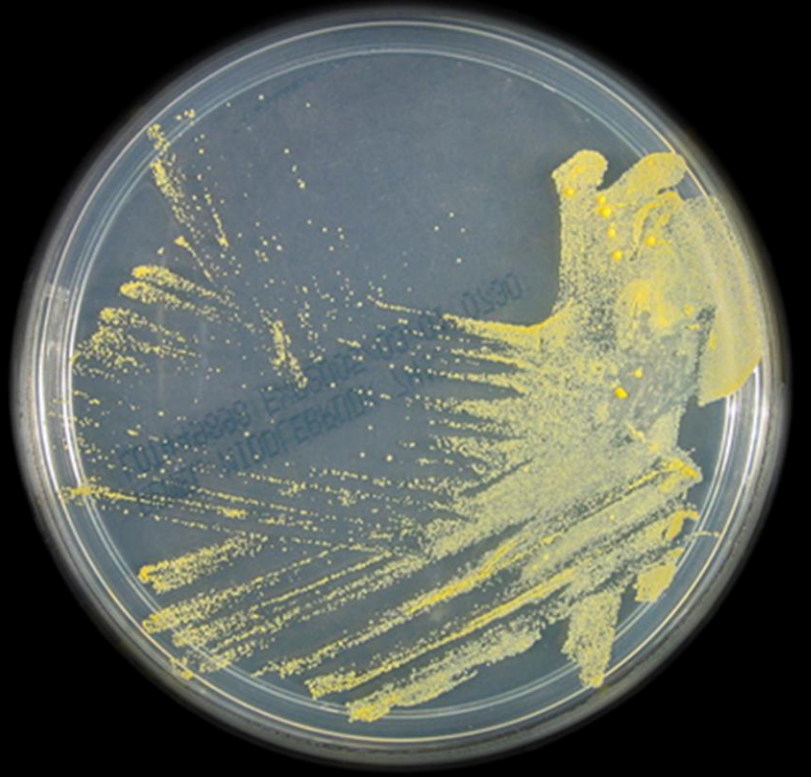
- Growth rate: 14-28 days
- Colony morphology: yellow to orange, smooth to rough; periphery somewhat irregular
- Scotochromogenic at 37°C
- Photochromogenic at 25°C

Mycobacterium szulgai 7H11

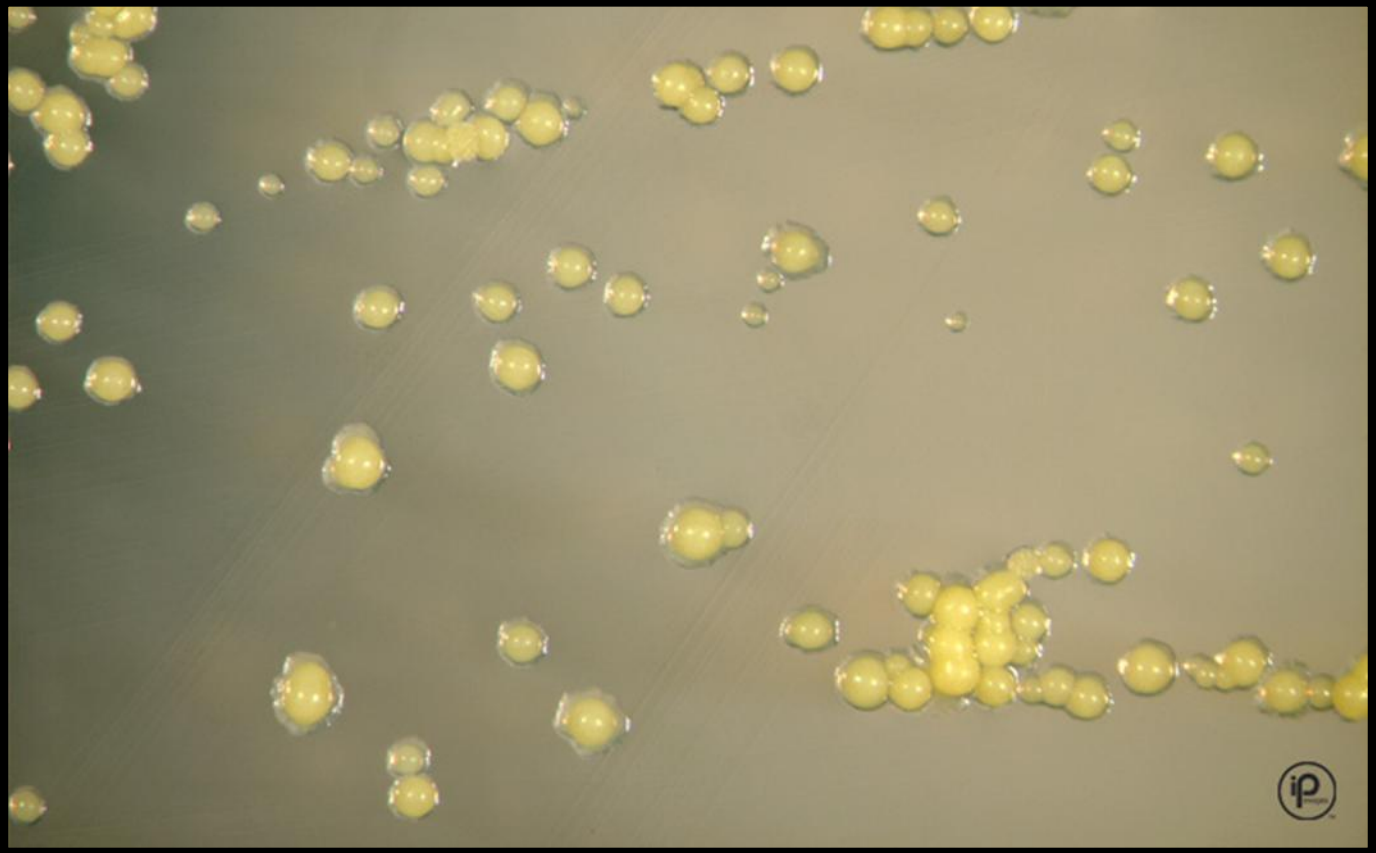


Scotochromogenic slow grower
Mycobacterium xenopi

- Growth rate: 28-42 days
- Colony morphology on 7H11: yellow, small, domed, smooth or rough, at 45°C, resemble miniature bird's nest
- Optimal growth temperature: 40-45°C



Mycobacterium xenopi 7H11



Mycobacterium xenopi L-J

Rapidly Growing Mycobacteria (RGM)

Currently 6 major groups	Species within each group
M. chelonae/M. abscessus group	*M. chelonae, M. immunogenum, M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, *M. salmoniphilum*
Mycobacterium fortuitum group	*M. fortuitum, M. peregrinum, M. senegalense, M. setense, M. septicum, M. porcinum, M. houstonense, M. boenickei, M. brisbanense, M. neworleansense*
M. mucogenicum group	*M. mucogenicum, M. aubagnense, M. Phocaicum*
M. smegmatis group	*M. smegmatis* and *M. goodii*
Early-pigmented RGM	*M. neoaurum, M. canariasense, M. cosmeticum, M. monacense*
M. mageritense/M. wolinskyi group	*M. mageritense, M. wolinskyi*

RGM Clinical Significance

- RGM are common in the environment.
- RGM are most frequently seen with post-traumatic wound infections.
- Sporadic cases of localized wound infections following medical or surgical procedures.
- Less commonly seen with disseminated cutaneous disease, bone & joint infections, CNS disease, corneal infections, otitis media, health-associated infections.
- Chronic lung infections can occur. *M. abscessus* is the causative agent in 80% of cases of pulmonary disease due to RGM.

RGM Clinical Significance

The American Thoracic Society clinical criteria for non TB mycobacterial lung disease requires the presence of pulmonary symptoms and lung nodules or cavities, with the exclusion of other diagnoses. Microbiological diagnosis requires at least:

1) two sputa or one bronchial wash or lavage with a positive culture or
2) a lung biopsy showing granulomatous inflammation or AFB, with one or more sputum or bronchial washings that are culture positive

(Am J Respir Crit Care Med. 175:367 to 416, 2007)

RGM Laboratory Observations

- Growth observed in culture within 7 days
- Cells may be < 10% acid fast and may not stain with the fluorochrome stain.

Nonchromogenic Rapid Grower

Mycobacterium abscessus

- Growth rate: 3-7 days
- Colony morphology: rough or smooth, buff

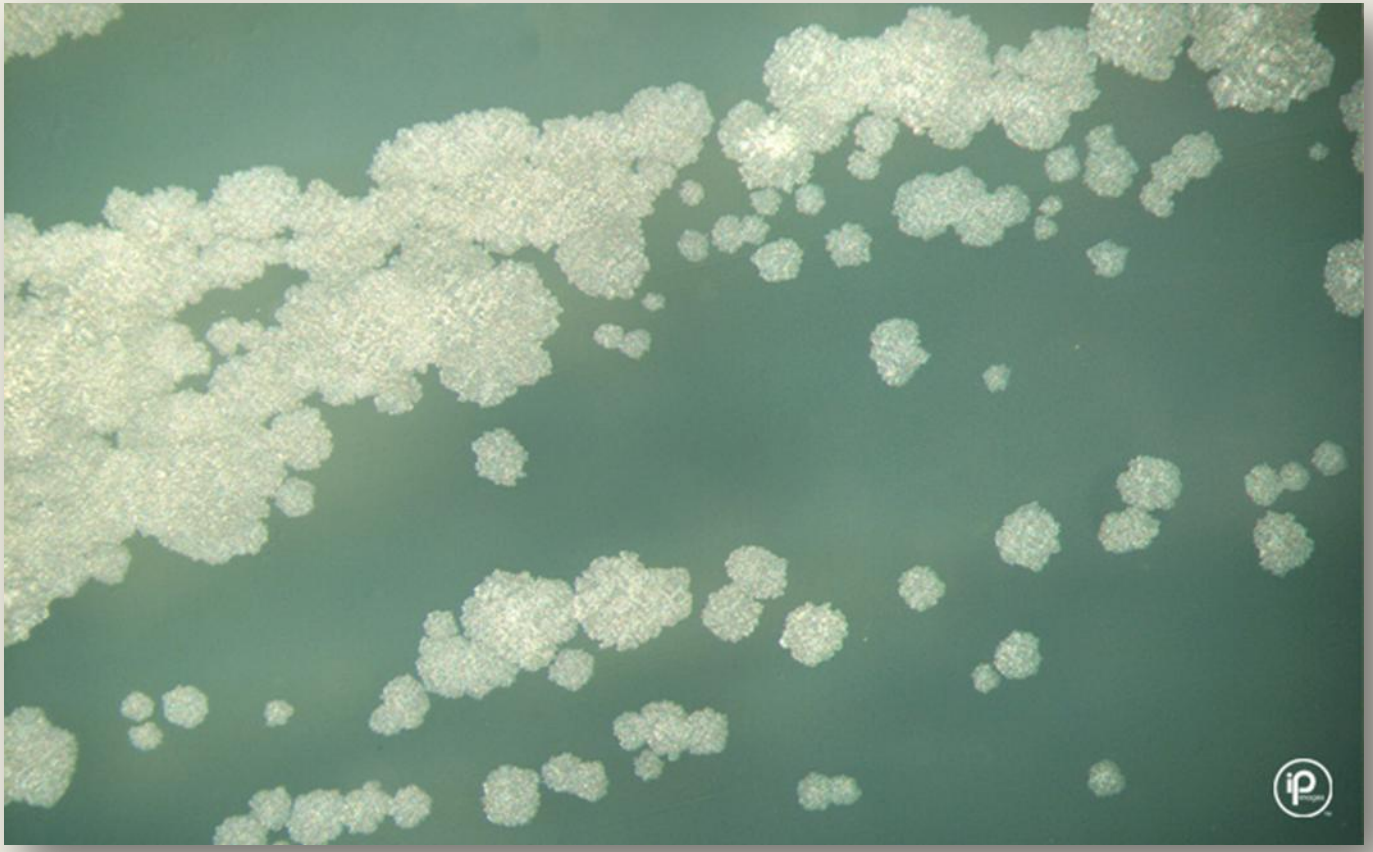
Mycobacterium abscessus 7H11

Nonchromogenic Rapid Grower

Mycobacterium chelonae

- Growth rate: 3-7 days
- Colony morphology: rough or smooth, buff
- Primary isolation may be more optimal at 30°C

Mycobacterium chelonae 7H11

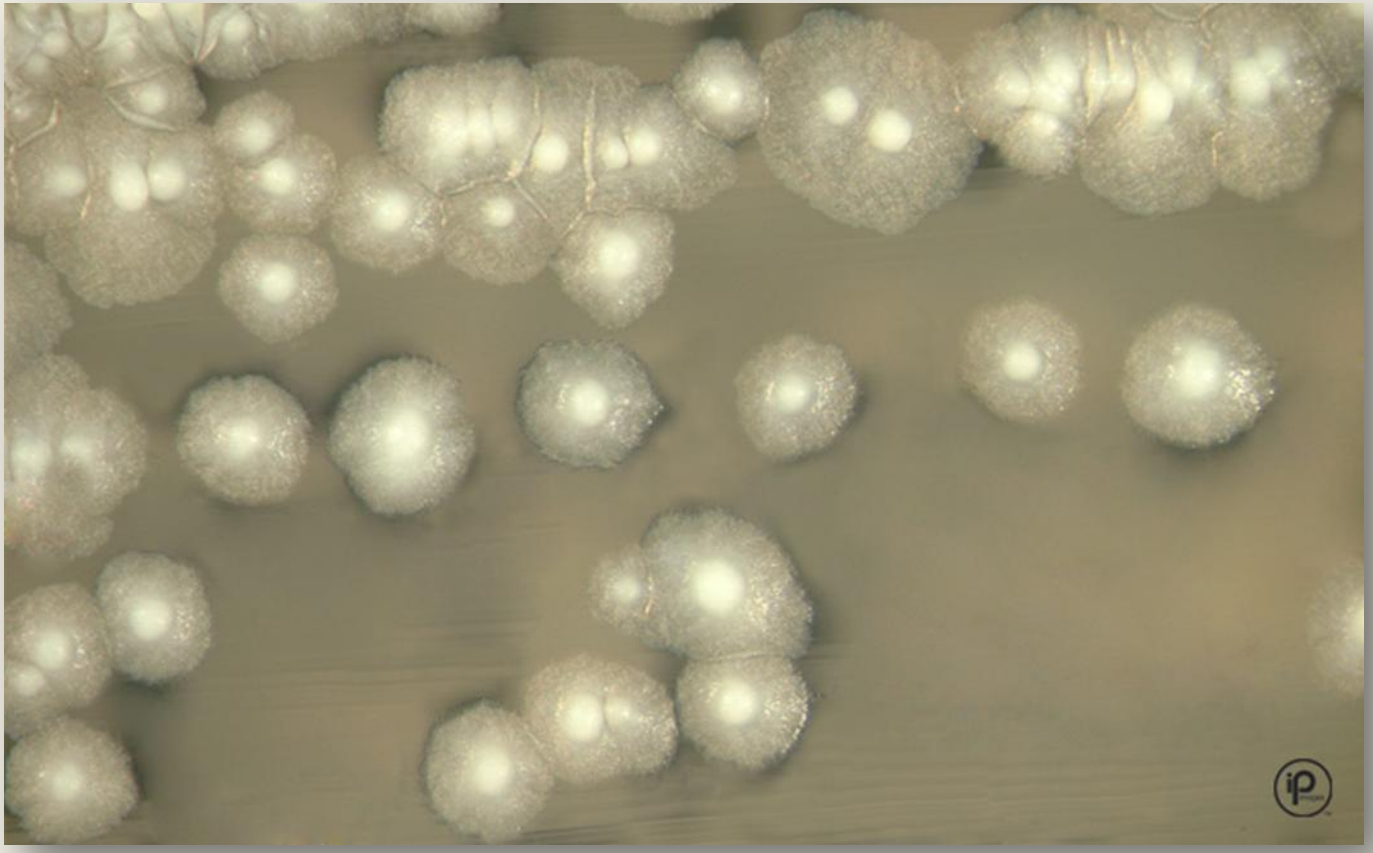


Nonchromogenic Rapid Grower

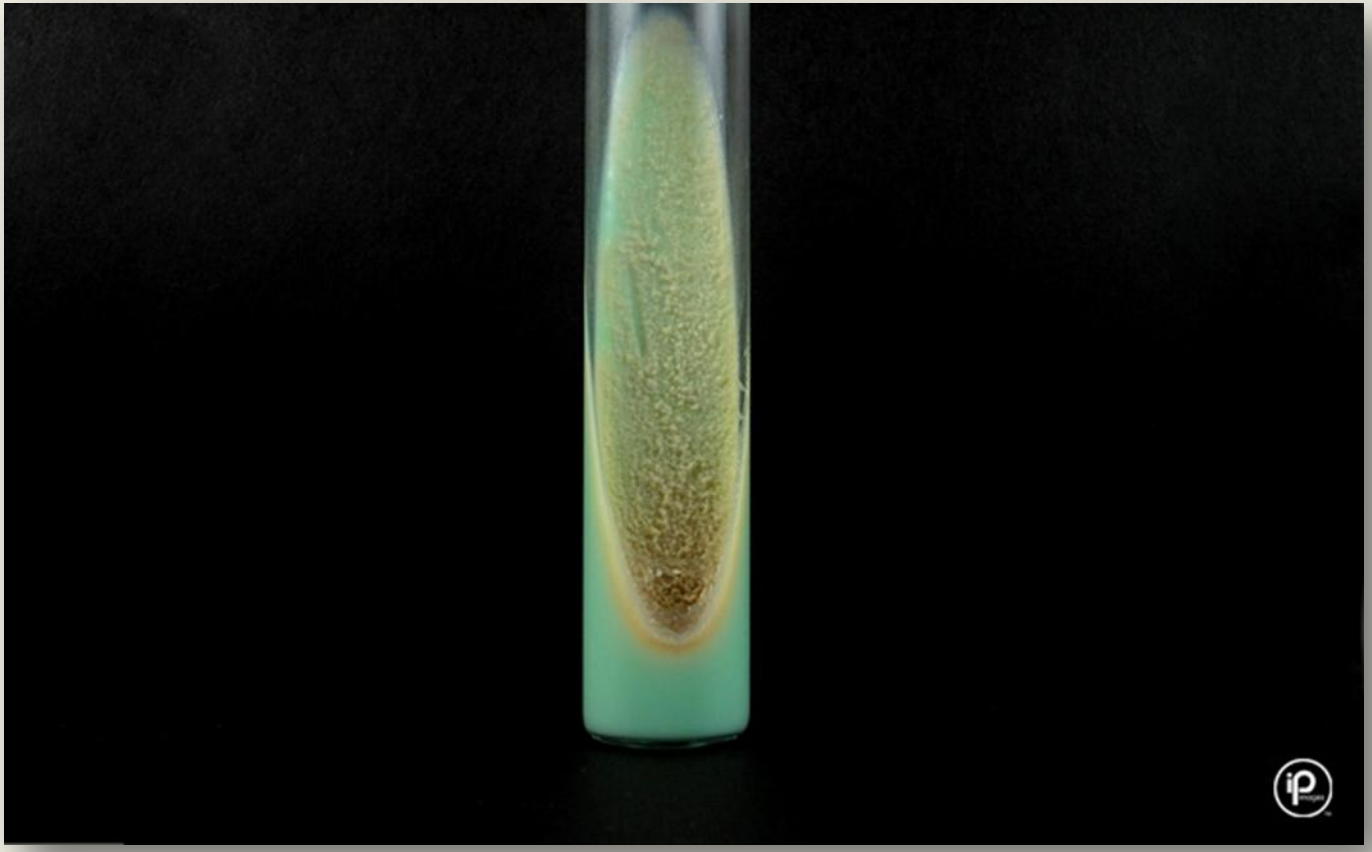
Mycobacterium fortuitum

- Growth rate: 3-7 days
- Colony morphology on 7H11: buff-colored, smooth, domed; sometimes rough branching filaments on periphery

Mycobacterium fortuitum 7H11



Mycobacterium fortuitum L-J

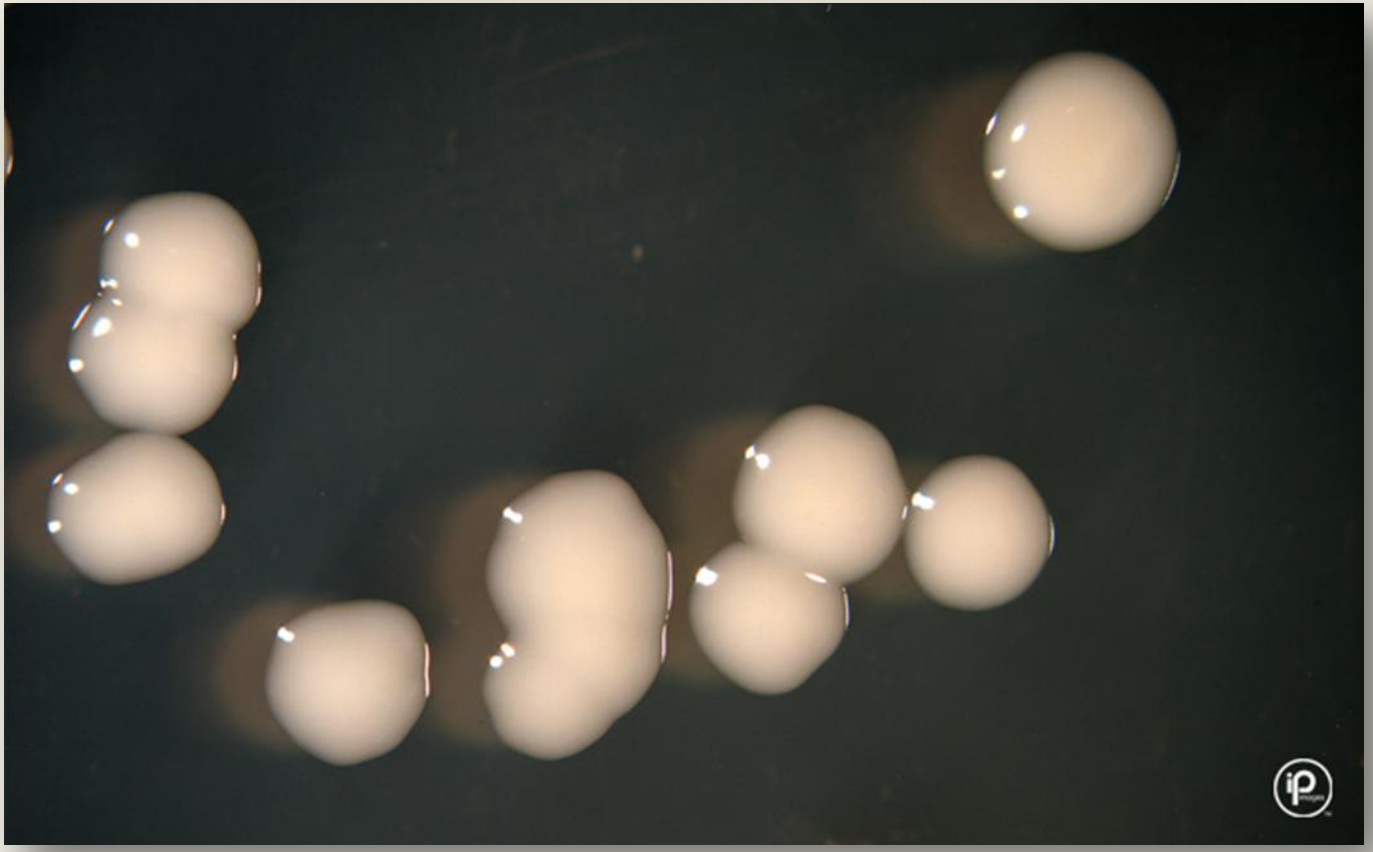


Nonchromogenic rapid grower

Mycobacterium mucogenicum

- Growth rate: 3-7 days
- Colony morphology: off-white, smooth, mucoid

Mycobacterium mucogenicum 7H11



Scotochromogenic Rapid Grower
Mycobacterium neoaurum

- Early-pigmented RGM
- Growth rate: 3-7 days
- Colony morphology: smooth, shiny, yellow-orange