Mycobacterium & Aerobic Actinomycetes

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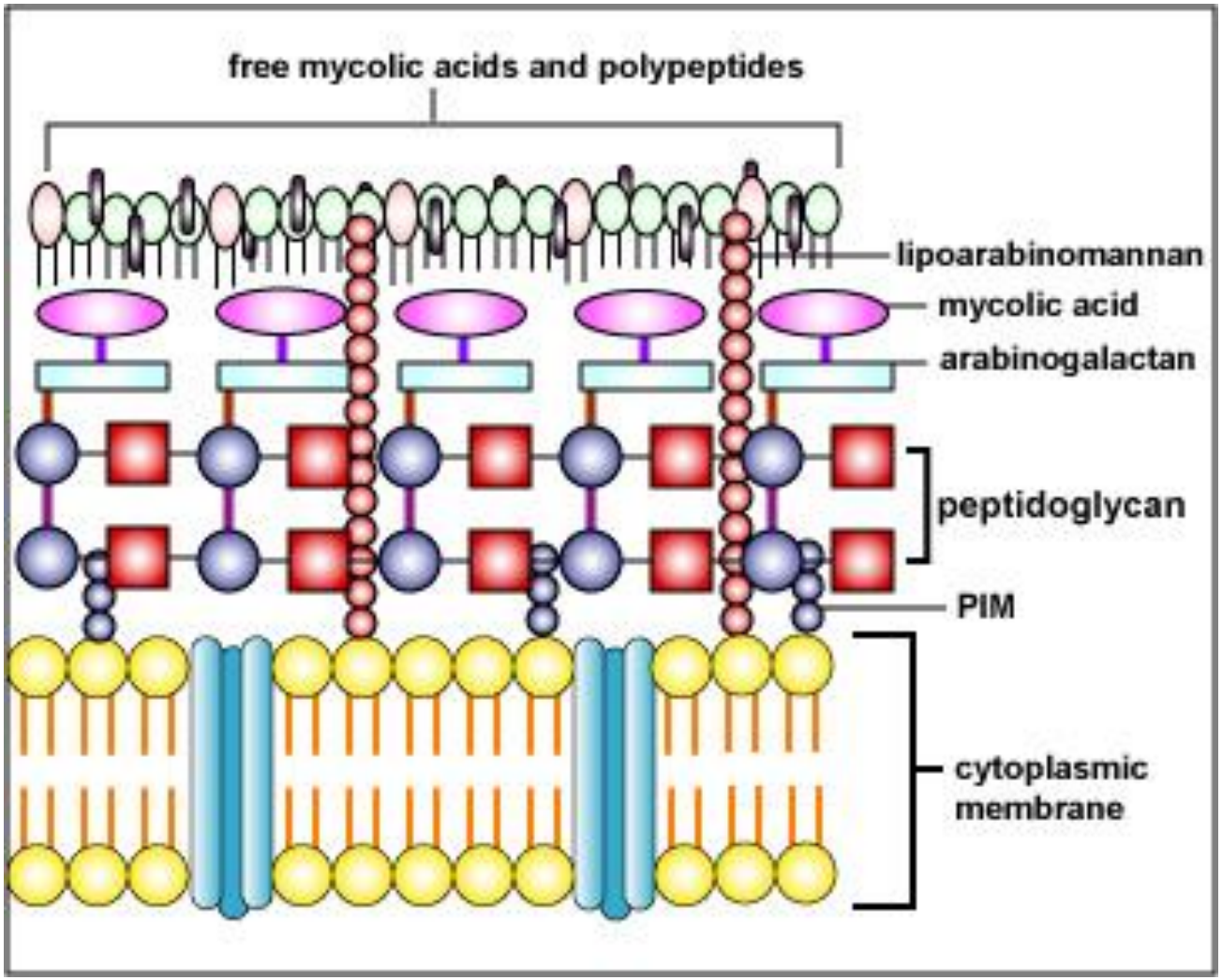
Mycobacteria: The acid fast bacilli

- Aerobic, non-sporeforming, non-motile rods
- Gram positive cell wall; but stain poorly
- 0.2–0.6 µm x 1-10 µm, may branch
- Mycolic acids in cell wall with long chains (60-90 carbons)
- 61-71% GC
- Not nutritionally fastidious, but grow slowly
- Generation time 20 hours
- 5-10% CO_2 is optimal

Mycobacterium spp. Cell Wall



Microscopy and/or NAAT for respiratory specimens

Fluorochrome - 24 hr of receipt

NAAT ≤ 48hr of specimen
Collection (respiratory specimens)

Culture – incubate 6 - 8 weeks

Broth based; automated detection: ≤ 14 d of receipt

ID of MTBC ≤ 21 d of specimen receipt Growth on solid media

Antibiotic Susceptibilities

1st line drugs: < 30 d specimen receipt
2nd line drug testing if resistance

Basic Considerations for Isolation

- Slow growth of mycobacteria may mean overgrowth with flora may be problematic.
- Slow growth may mean processing of more material might improve yield.
- Optimize growth
- Use biosafety - *M. tuberculosis* complex may grow

Specimens

- Respiratory
  - Sputum, expectorated or induced
    - 5-10 ml
    - Guideline for MTB & Infection Control:
      - 3 specimens, ≥8 hrs apart
      - Max of 3 in 24 hrs
      - At least 1 first morning.

        CAP MIC.31100 & MMWR 54:RR-17 (2005)
    - ATS Guideline for NTM:
      - 3 specimens useful for establishing clinical significance

        Am J Respir Crit Care Med 175:367 (2007)
  - Do not pool sputum
  - Refrigerate if transport is delayed >1 hr

Specimens

- Respiratory
  - BAL; bronchial aspirates, or washes (>5 ml)
    - Process max amount possible w/pre-centrifugation step
  - Bronchial brush
  - Fine needle aspirates (FNA) & biopsies
  - Gastric aspirate or lavage
    - 3 first morning specimens (5-10ml, more if lavage)
    - Used for pediatrics
    - Neutralize stomach acid with sodium bicarbonate (to pH =7) if not processed in 4 hours.

Specimens

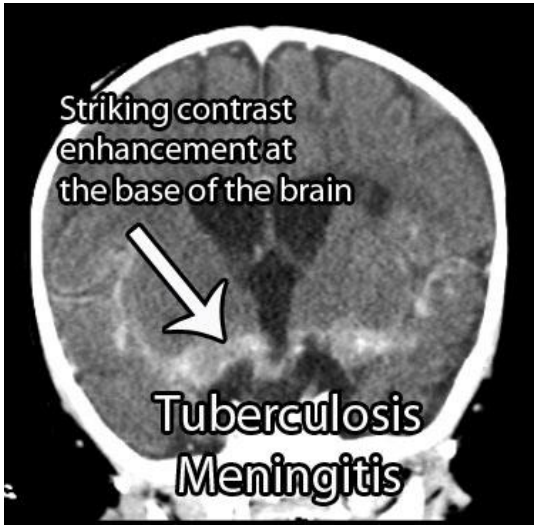
- Tissue, abscess, wounds

  (fluid or tissue preferred over swab)

  - Tissue
    - Request ~1 gram, if possible
    - Collect from advancing margin of cutaneous ulcers
    - Do not moisten tissue with non-sterile water or saline
    - Grind tissue prior to AFB processing
    - Tissue in formalin is unacceptable
  - Pus, aspirate material (as much as possible)
  - Swabs are discouraged
    - Poor yield
    - Hydrophobic mycobacteria not released

Specimens

- Body fluids
  - 5 to 15 mls, if possible
  - ≥5 mls CSF
- Urine
  - First morning 3X/week
  - 20 -40 mls preferred
  - Do not accept pooled or 24 hr collections due to contamination.
- Stool
  - AIDS patients for MAC
  - ≥1 gram in clean container



BIOHAZARD

Safety considerations for specimen processing

"BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. "

Biosafety in Microbiological and Biomedical Laboratories (BMBL)
5th Edition

Process in Biosafety Cabinet

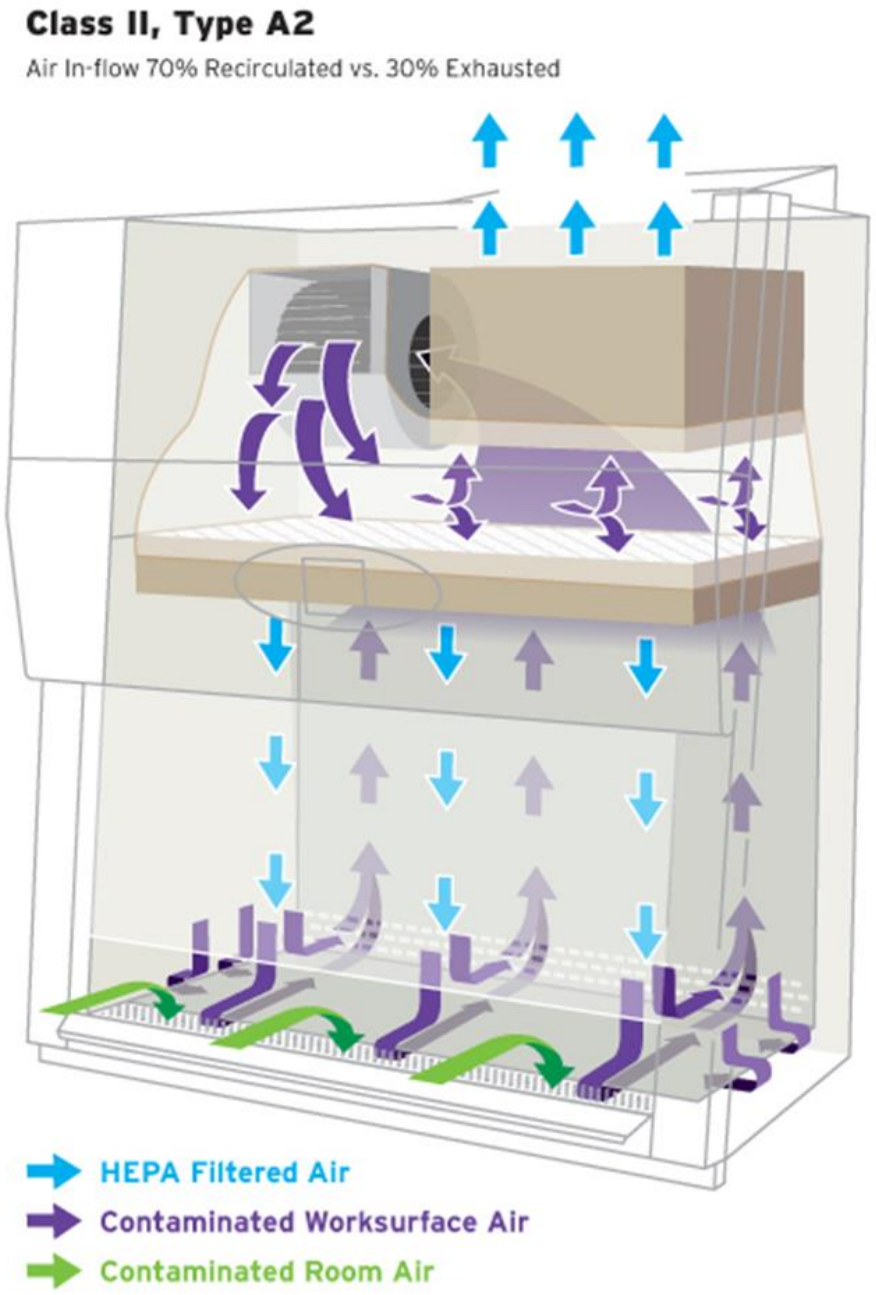


Image 1 of 3

Expectorated Sputum

What is the challenge of 6 week incubation?

Digestion & decontamination

Digestion:

- Liquifies mucin to release bacteria
- Allows contact of non-mycobacterial species and decontaminating agents

Decontamination:

- Decreases burden of non-mycobacterial species that may over-grow mycobacteria

Specimens:

Sputum, gastric washing, BAL, bronchial washing, tracheal aspirate, voided urine, stool, abdominal fluid, other contaminated specimens.

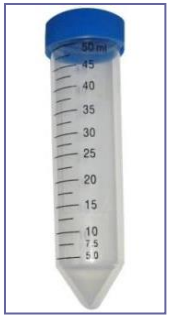
All specimens will be concentrated.

Specimen Processing: Decontamination Methods

- NaOH and n-acetylcysteine (NALC)
  - Most common
  - Commercially available
  - 2-4% NaOH to retain viability of AFB
  - Neutralize w/PO_4 buffer 0.067M, pH 6.8
- Oxalic Acid (5%)
  - For specimens contaminated with *P. aeruginosa, esp.* for Cystic Fibrosis patients
  - Follows NaOH-NALC
  - May decrease recovery of MAI

Gilligan P. Clin Lab Med (2014) 34:197-217

Specimen Processing



Digestion Reagent

Liquefaction

15 min

Neutralize

Centrifuge at 3000g
(contained)

Decant

Media, slides

Important Steps in Processing:

- Centrifuge to concentrate specimens with >10 ml volume prior to processing. 3000 g for 15 min.
- Adhere strictly to timing of digestion to prevent killing AFB & allow liquefaction.
- Limit batch size.
- To prevent aerosolization:
  - Do not open tubes prior to 15 min digestion time. Only open tubes in BSC.
  - Centrifuge in contained carriers. Open these in BSC.

Important Steps in Processing:

- To prevent cross-contamination:
  - Decant gently, avoiding splashing. Open 1 tube at a time.
  - Add reconstitution reagent with separate pipette for each specimen. Work on 1 tube at a time.

Culture Media – General Guidelines

- Use both liquid & solid media.
- Add media with antimicrobials to specimens from contaminated sites.

MIC.32250 **Specimen Inoculation** **Phase I**

Specimens (other than blood) are routinely inoculated on media that support optimal growth of the majority of clinically relevant mycobacterial species.

NOTE: The use of two types of media (for specimens other than blood), including one liquid medium (when possible) or a comparable culture method, is recommended for optimal isolation of mycobacteria.

Culture Media - Liquid

- Improve sensitivity
- Decreased time to detection
- Choices:
  - Classical 7H9 or Dubos
  - Commercial media & automated systems; 3 FDA-cleared

Supplements

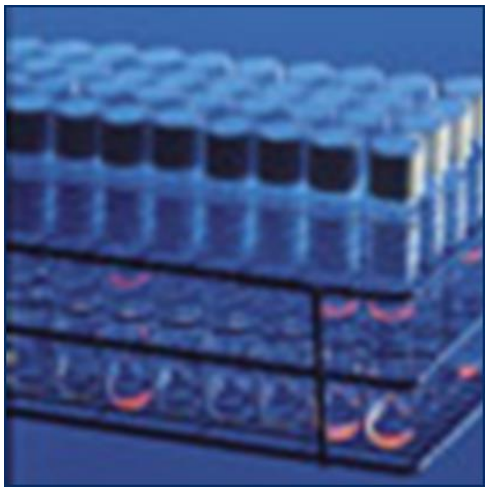
- OADC = oleic acid, bovine serum albumin, D-glucose, catalase
- PANTA = polymixin, amphotericin B, nalidixic acid, trimethoprim, azlocillin

- Redigest if bacterial overgrowth

Becton Dickinson: MGIT Mycobacterial Growth Indicator Tube

Automated 960 Instrument

Manual



bioMerieux BacT/Alert System

MP Bottle

Thermo-Fisher VersaTREK Myco

Culture Media: solid agar, chemically-defined

- Middlebrook 7H10 & 7H11
  - Base w/salts, vitamins, cofactors
  - 2% glycerol; enhances MAC
  - Enrichment: OADC, casein hydrolysate
    - OADC = oleic acid, bovine serum albumin, D-glucose, catalase
  - Easily-visualized colonies in 10-12 days

Selection:

- Mycobactosel 7H11 sel: cyclohexamide, lincomycin, nalidixic acid
- Mitchison 7H11 sel: carbenicillin, polymixin B, trimethoprim, and amphotericin B

Culture Media: Egg-based

Lowenstein Jensen; others

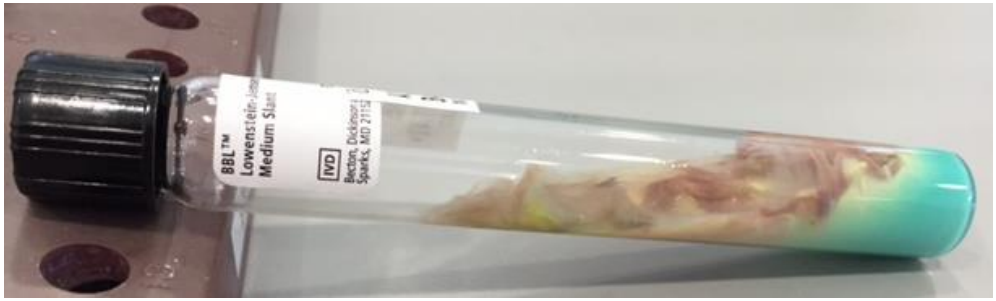
- Egg yolk or whole egg
- Glycerol
- Potato flour
- Malachite green - inhibits contaminating flora
- Ingredients neutralizes toxic substances
- Long shelf-life
- Good recovery of MTB

Selection:

- Mycobactosel LJ: cycloheximide, lincomycin, nalidixic acid
- Gruft LJ: penicillin & nalidixic acid

Disadvantages of LJ:

- Variability of ingredients effects growth of some species
- Slower growth than 7H11
- Media liquefies if contaminated



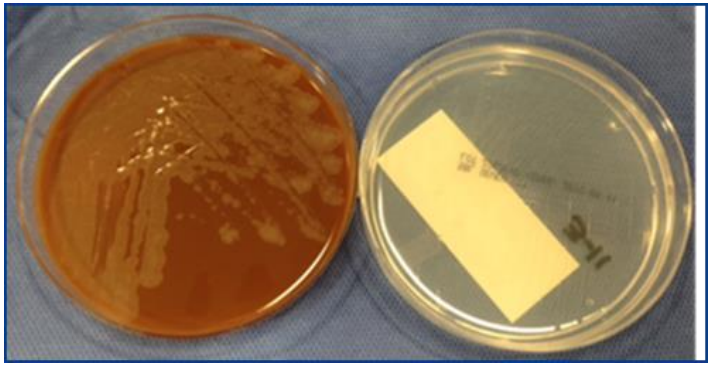
Disadvantages of 7H11:

- Excessive heat or light causes deterioration & release of formaldehyde

Special media & incubation considerations:

- Incubate skin, soft tissue & specimens from extremeties at 35°C and ~30°C.

- Skin & soft tissue: Add chocolate or media supplemented with hemin, hemoglobin or ferric ammonium citrate (*M. hemophilum*)



- *M. genavense*:
  - Supplement 7H11 with mycobactin J.
  - Bactec 7H12 PZA test medium
  - Acidified Middlebrook agar supplemented w/blood and charcoal

Blood & Bone Marrow Culture

- Ordered for AIDS & immunocompromised
- Major bone marrow pathogens: MTB, MAI
  - Osteomyelitis has more etiologies
- Blood pathogens:
  - MAI & MTB
  - *M. haemophilum*
  - rapid growers causing line infections
    - grow in routine blood culture systems within 5 days
  - Rarely other NTM

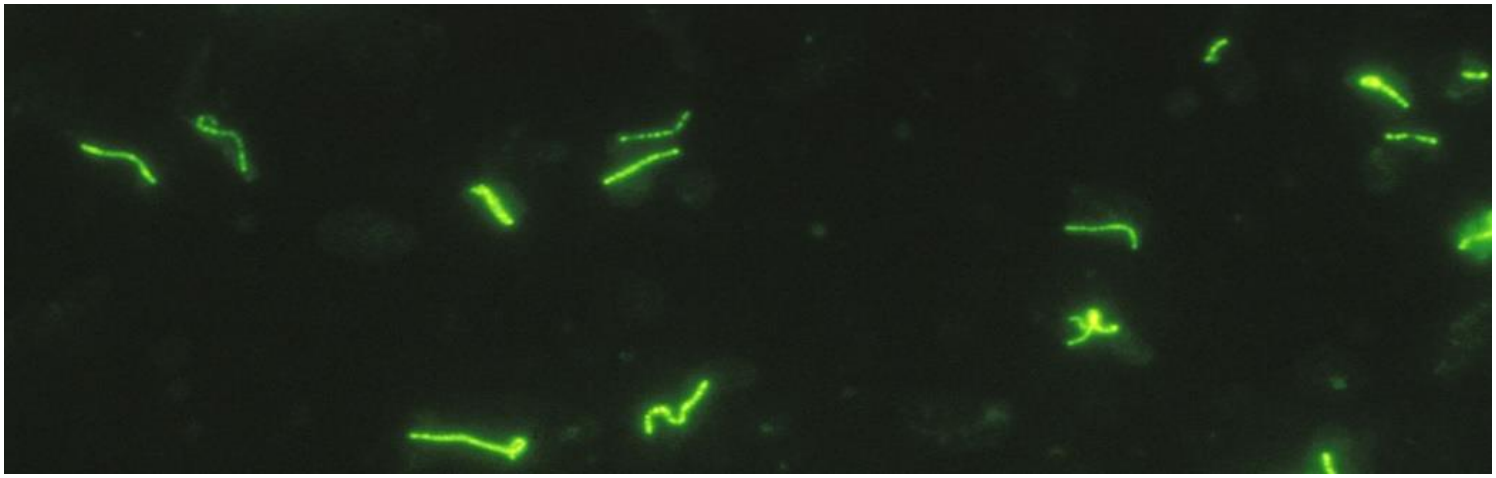
Blood & Bone Marrow Cultures

- Release adherent/intracellular mycobacteria
- Lytic bottles in automated systems
- Lysis centrifugation system i.e Isolator
- 1 culture usually sufficient; 5 mls
- No digestion/decontamination

Bactec 9000 & FX40

Auramine Rhodamine (Fluorochrome)

- Increased sensitivity over ZN or Kinyoun
- Faster – read on 40X instead of 100X oil
- Auramine & rhodamine are non-specific fluorochromes that bind mycolic acids and resist decolorization
- Decolorize - 0.5% HCl in 70% ETOH
- Counterstain - potassium permanganate



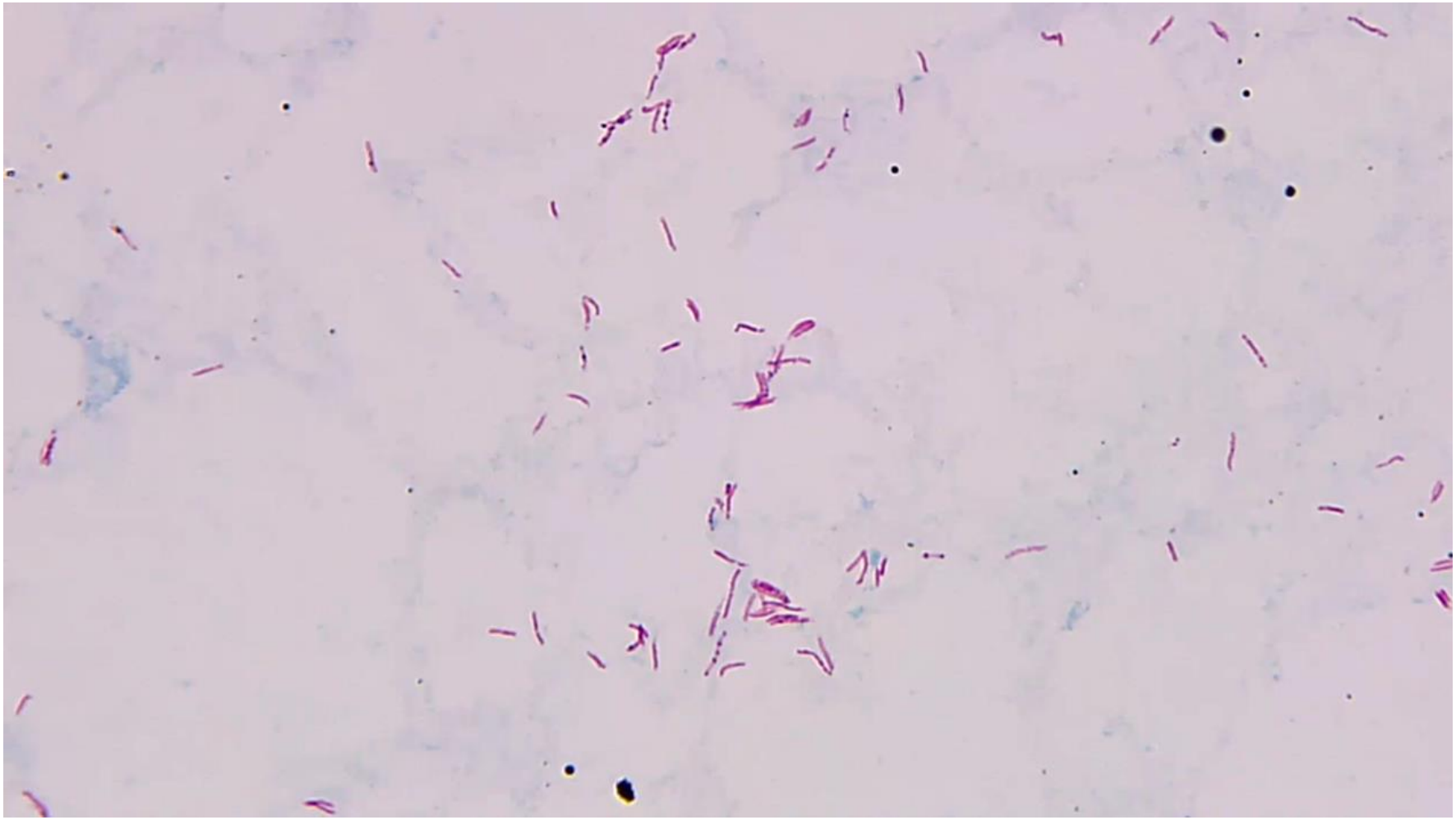
Stains - Traditional

Confirm fluorochrome

Confirm liquid media or colony acid-fastness

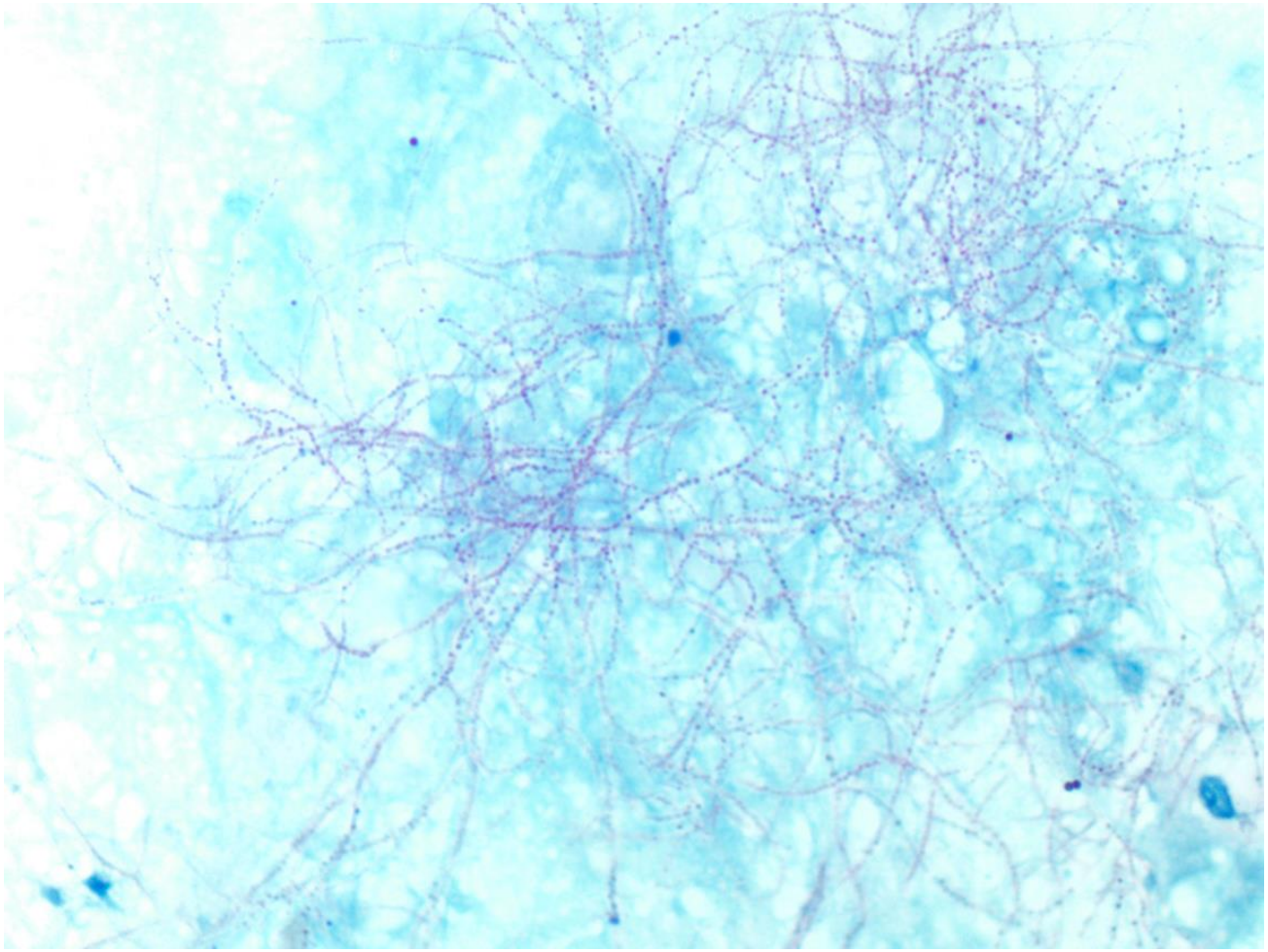
- Ziehl Neelson
  - Primary stain – carbol fuchsin in ethanol & phenol
    - Heat slide to steaming & maintain for 3-5 min at 60ºC
    - Cool slide, then wash with water
  - Decolorize – acid-alcohol (3 mls HCl in 97mls 95% ETOH)
  - Counterstain – methylene blue 20-30 sec
- Kinyoun
  - Steaming is replaced by a cold stain that uses more phenol in primary staining step

Ziehl Neelson: Positive Sputum (100X oil)



Modified Kinyoun or ZN

Uses weaker decolorizer; 3% HCl is replaced with 0.5 to 1% sulfuric acid



AFB Smear Evaluation & Reporting (300 fields)

Pfyffer MCM, 11th Ed. (2015) Chp 30

	Quantification of AFB		
	Carbol Fuchsin Stain	Fluorochrome stain	
	x1000	X250	X450
No AFB seen	0	0	0
Indeterminate	1-2/300 fields(3)	1-2/ 30 fields (1)	1-2/70 fields (1.5)
1+	1-9/100 fields(1)	1-9/10 fields	2-18/50 fields
2+	1-9/10 fields	1-9/field	4-36/10 fields
3+	1-9/field	10-90/field	4-36/field
4+	>9/field	>90	>36/field

Notifications:

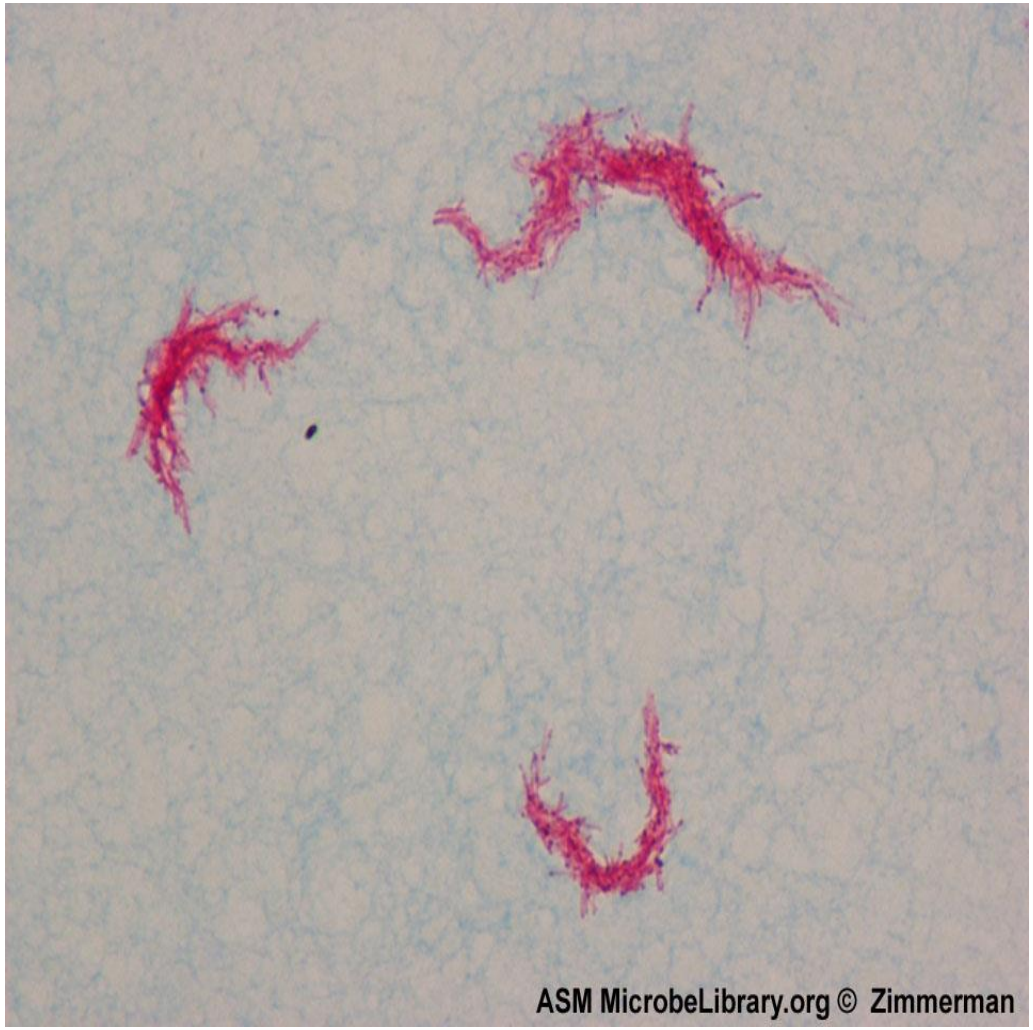
- Positive direct smear (or initial culture) results, notify:
  - Care-provider
  - Infection Prevention

Also notify provider and IP:

- If PCR positive for MTB
- If culture positive for MTB

Cording of Acid Fast Bacilli

- Usually indicates *M. tuberculosis*
- Few other species will display cording



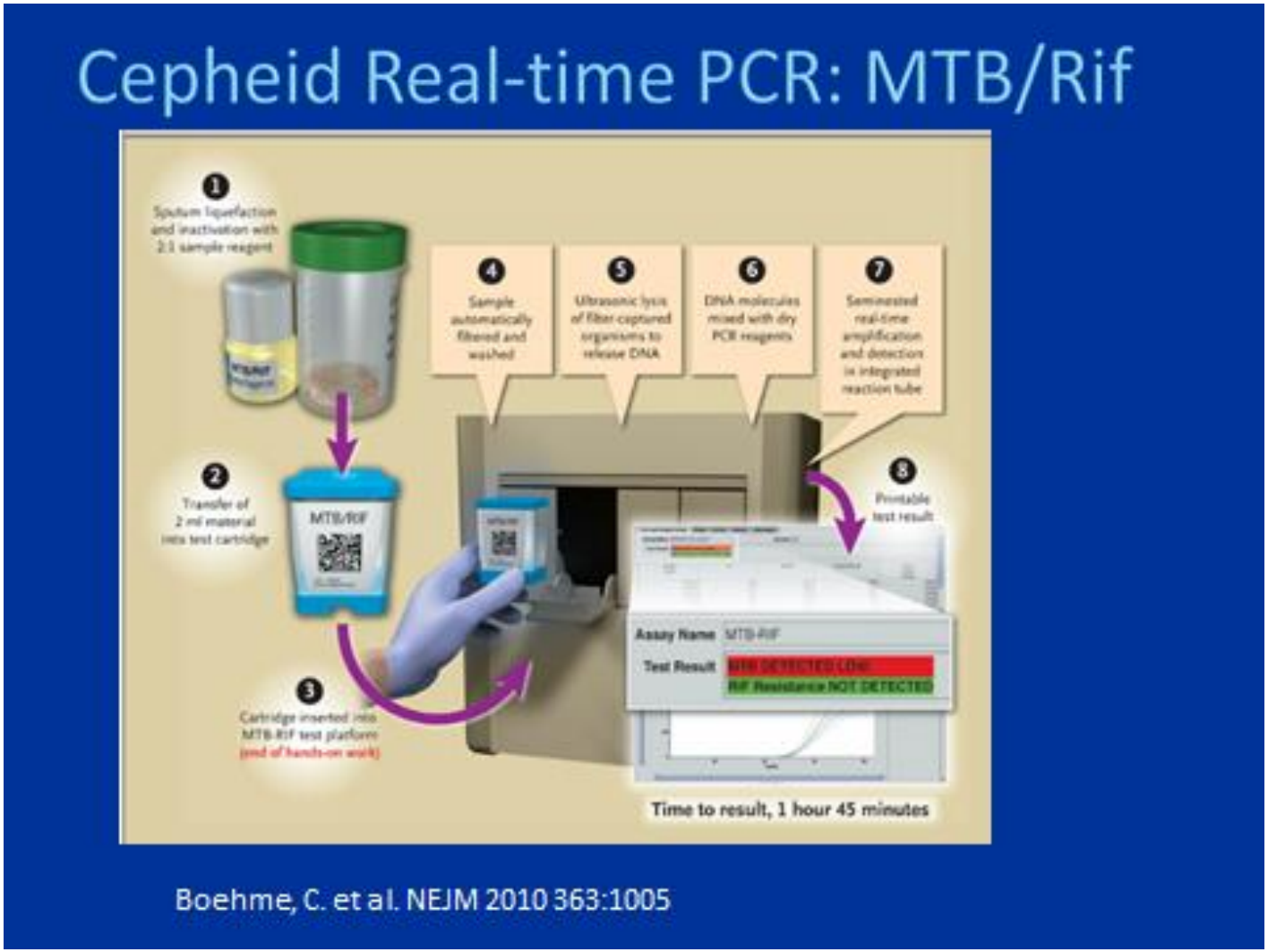
Nucleic Acid Amplification Testing from Clinical Specimens

"CDC recommends that NAAT be performed on at least 1 respiratory specimen from each pt with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered…, and for whom the result would alter case management or TB control activities, such as contact investigations."

- Shorten time to diagnosis from 1 to 2 weeks to 1 to 2 days!
- Can be very sensitivity, depending on body site and organism burden

Always perform routine smear and culture in addition to NAAT!

Cepheid Real-time PCR: MTB/Rif



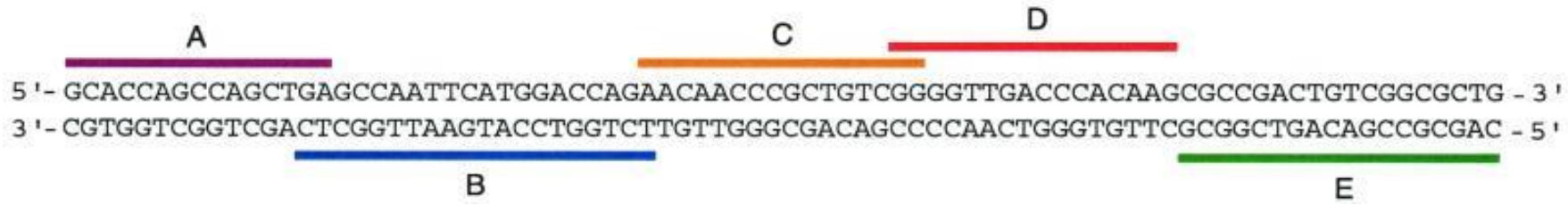
Boehme, C. et al. NEJM 2010 363:1005

Cepheid Real-time PCR

- Limit of detection ~100 CFU/ml
- Specificity: 100% for cx neg samples
- Sensitivity:
  - Smear positive 98%
  - Smear neg/cx pos: 68%

(Seingart 2013 Cochran Rev)

Nested PCR for *rpo*B w/molecular beacon probes



Interpretation

NAAT Result	Smear Result	Interpretation
Positive	Positive	Presumptive MTB
Positive	Negative	Consider testing 2nd specimen; Use clinical judgment
Negative	Positive	Rule-out inhibitors; May test an additional specimen, & if negative, assume NTM; Use clinical judgment;
Negative	Negative	Use clinical judgment and await cx results

Traditional Classification of Mycobacteria

- *M. tuberculosis* complex
- *M. leprae:* Hansen's bacillus
- Runyon classification-MOTT (NTM)
  - **Group I-----Photochromogens**
    Pigmented (yellow) only after being exposed to light; remain non-pigmented without light exposure.
  - **Group II ---Scotochromogens**
    - Pigmented (Yellow-orange) in dark and after light exposure
  - **Group III --Nonchromogens/slowly growing**
    - Remain buff or non-pigmented and require >_7 days to grow on sold media
  - **Group IV---Rapid growers**
    - Grow on solid media in ≤ 7 days
    - Usually non-pigmented

Identification of Mycobacteria from Culture

- Identify to the species level, if possible
- Clues to species:
  - growth rate and temperature tolerance
  - pigmentation
  - staining characteristics
- Common biochemicals – out-moded
  - thiophene-2-carboxylic hydrazide (T_2H), niacin, nitrate, NaCl tolerance, catalase (semi-quant & heat tolerance), urease, tween hydrolysis, arylsulfatase, pyrazinamidase

Non-biochemical Identification Methods

- Probes for rRNA (Accuprobe)
- Pyrosequencing (hypervariable region rRNA)
- Full-length Sequencing (rRNA, *rpoB*, *hsp65*, others)
- MALDI TOF

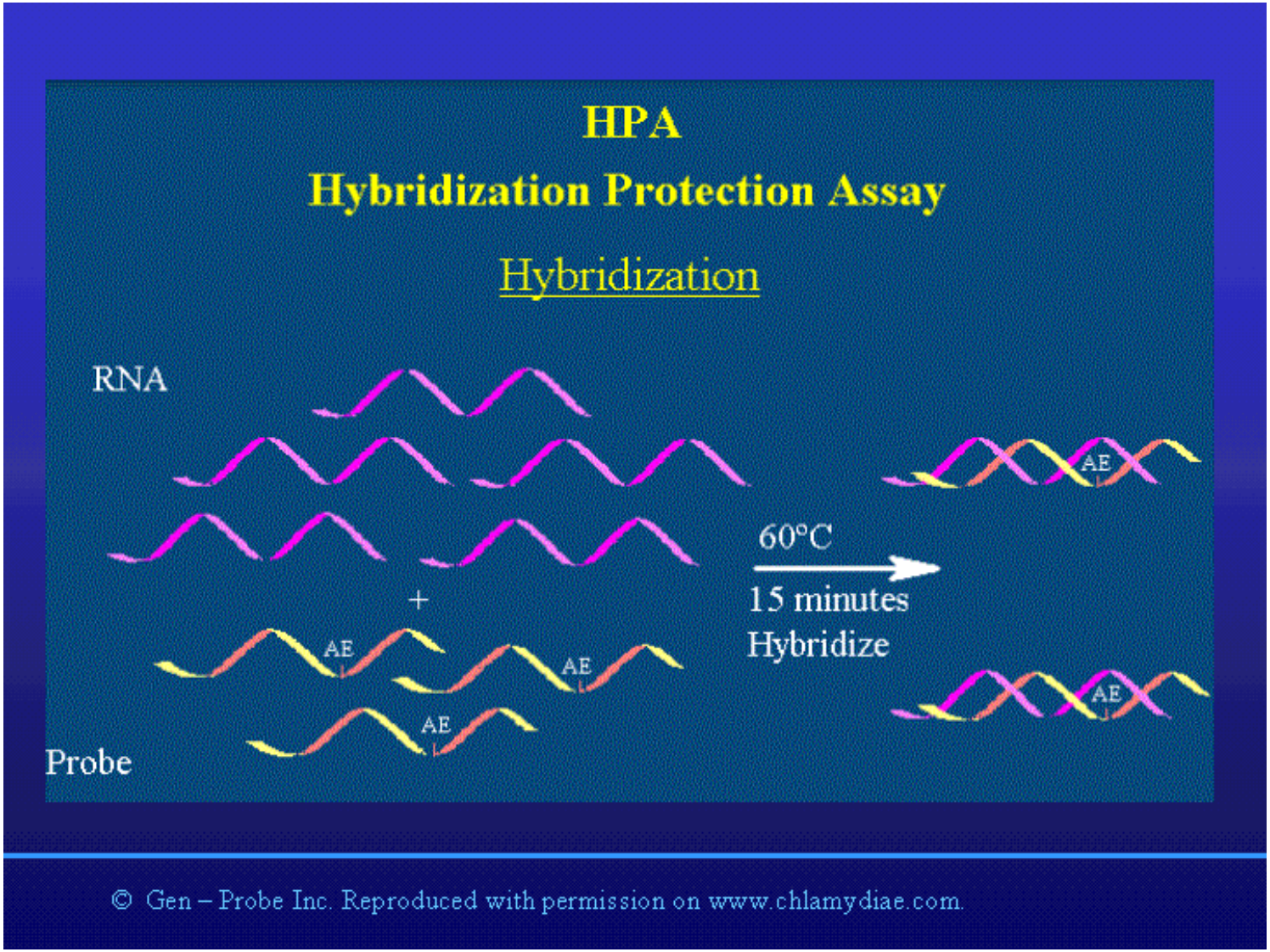
Others:

- Solid Format Reverse Hybridization Assays (not in U.S.)
- MPT64 immunochromatographic tests - MTBC
- HPLC of fatty acids
- PCR-RFLP
- Home brew: RT-PCR, High Resolution Melt, etc

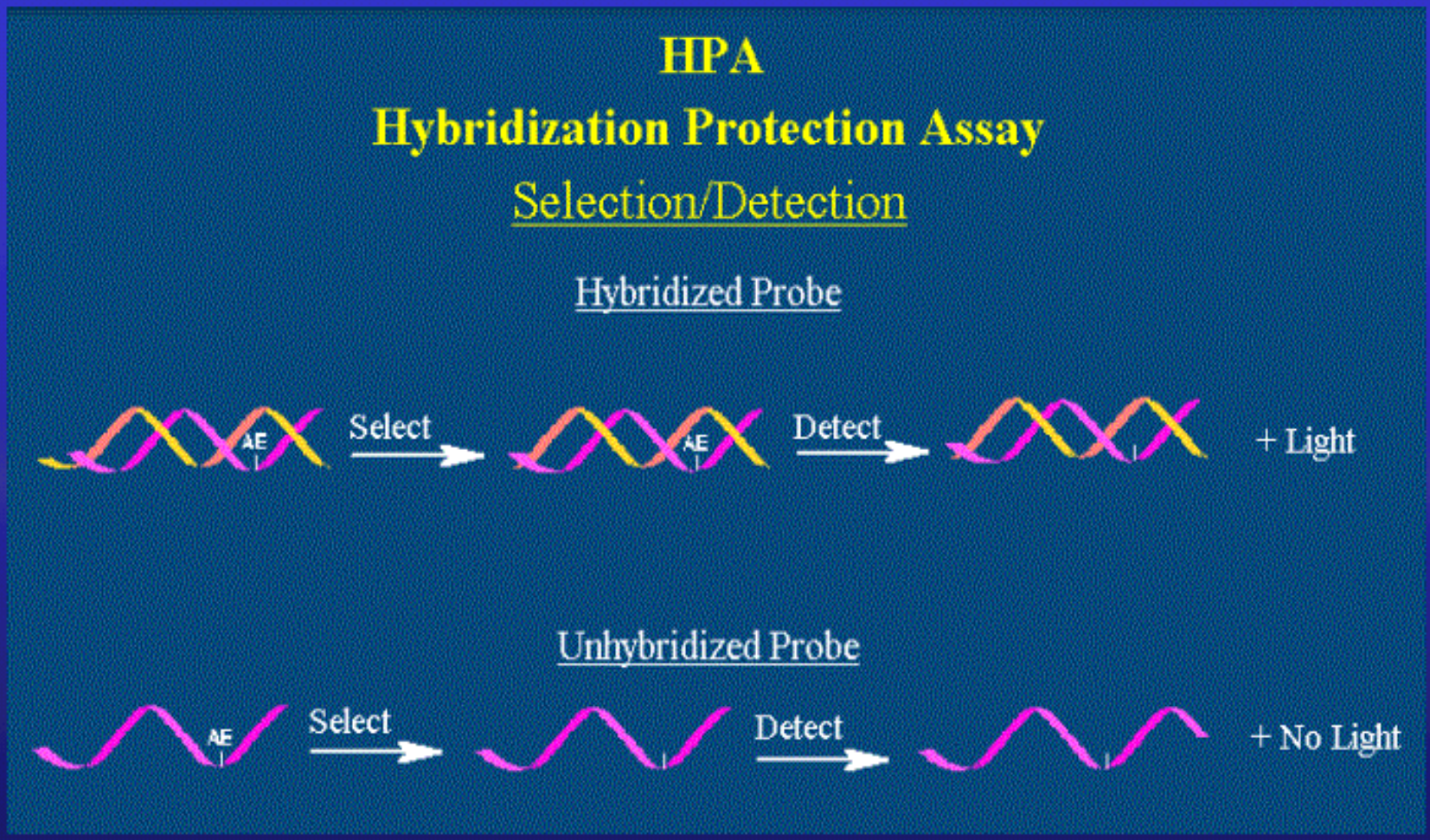
Identification from Positive Cultures
Available AccuProbe Assays – FDA cleared

- *M. tuberculosis* complex:
  - *M. tuberculosis*
  - *M. bovis*
  - *M. africanum*
  - *M. microtii*
- *M. avium-intracellulare* complex includes:
  - *M. avium, M. intracellulare, other species in MAI Complex*
- *M. kansasii*
- *M. gordonae*

Accu-Probe Methodology



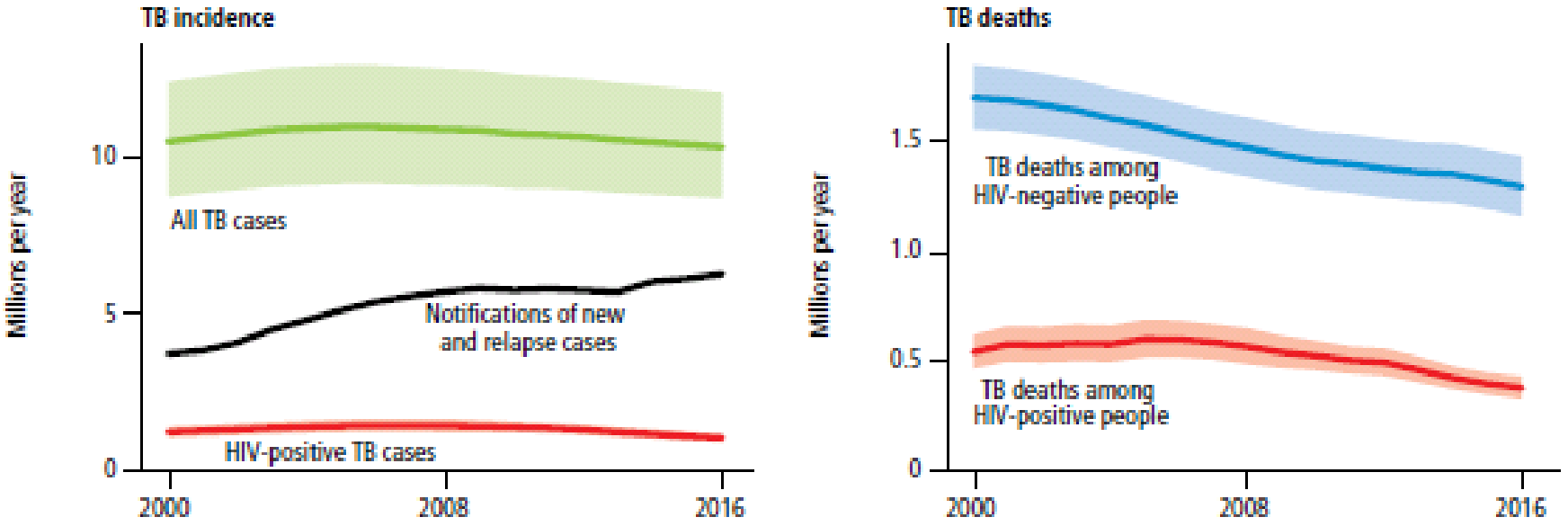
HPA
Hybridization Protection Assay
Selection/Detection



Estimated Global Burden of TB,
WHO 2017

FIG. 3.6

Global trends in the estimated number of incident TB cases and the number of TB deaths (in millions), 2000–2016. Shaded areas represent uncertainty intervals.

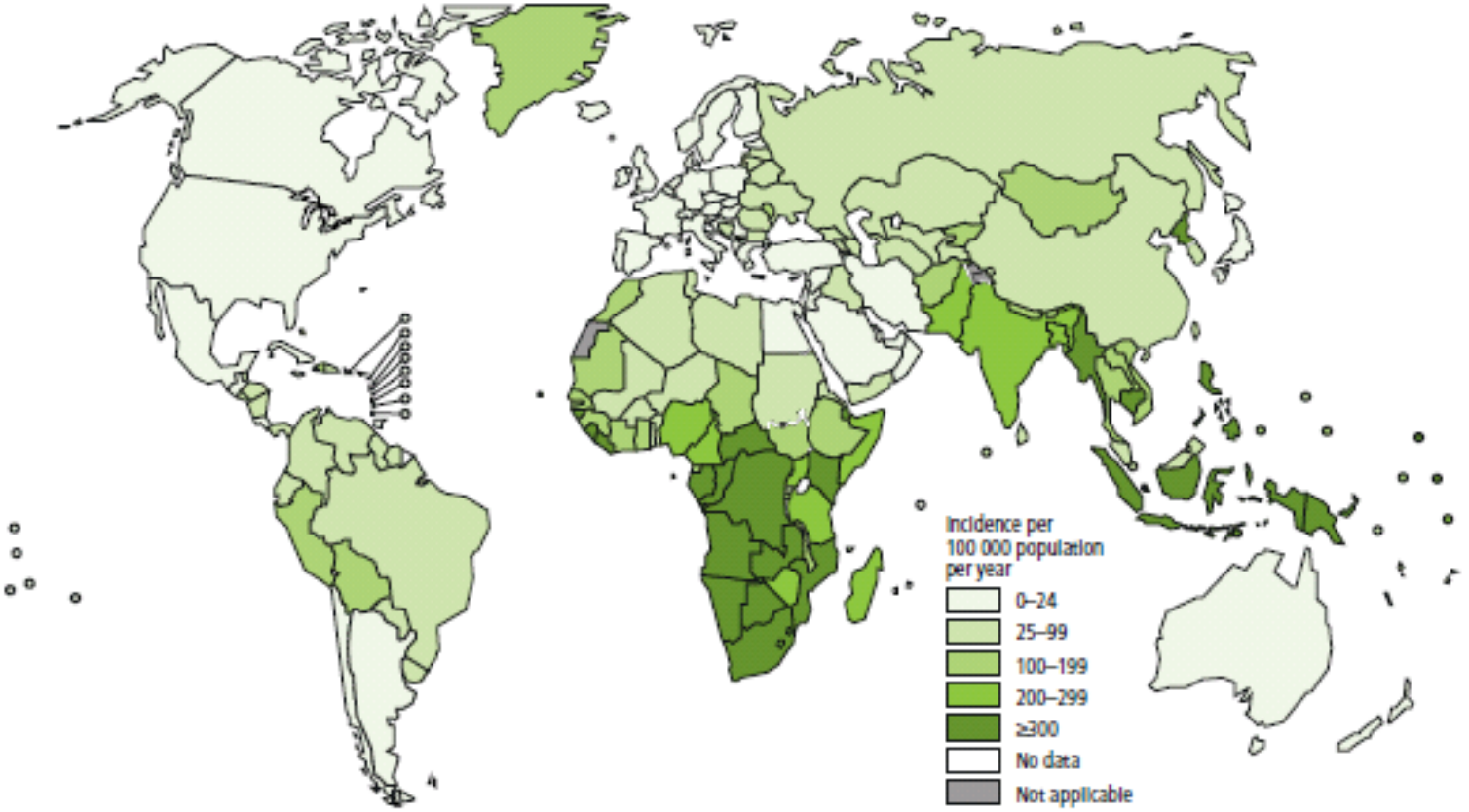


About 1/3 of world's population is infected

WHO Global tuberculosis report 2017

FIG. 3.4

Estimated TB incidence rates, 2016



Pathogenesis of *M. tuberculosis*

- Method of transmission
  - Pulmonary; droplet particles expelled by another infected human
  - Not found in the environment
  - Highly infectious

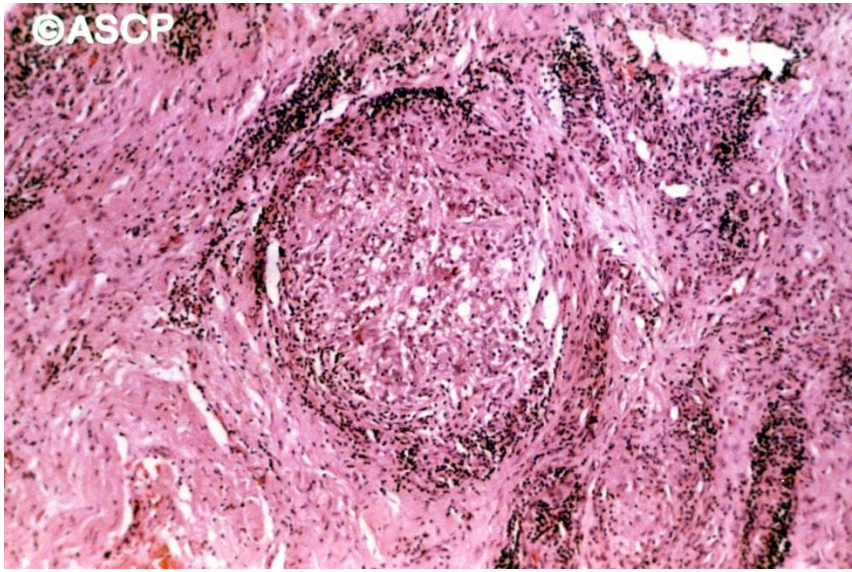
- Disease depends on:
  - Immune response
  - Infectious dose
  - Virulence of strains

Infection vs. Disease

- Once infected with MTB, bacilli are phagocytized & replicate intracellularly
- Host cellular immunity activated and replication usually ceases in 3-6 wks post-exposure;
- If intracellular organisms not eliminated: may remain latent in macrophages & immune response forms granuloma

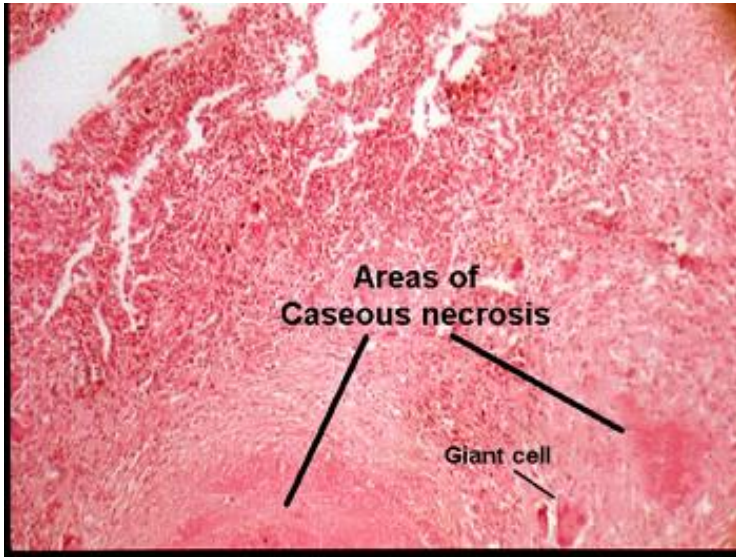
Host response to *M. tuberculosis*

- Localized collections of activated macrophages prevent further spread of bacteria.



- Granulomas – lymphocytes, multinucleated giant cells, fibrin, surrounding macrophages

- Larger necrotic or caseous granulomas become encapsulated with fibrin that protect the bacteria from macrophage killing.



- Reactivation disease: If immune system wanes, dormant MTB can be released

http://medsci.indiana.edu/c602web/602/c602web/pulmon/sl76_1.htm

Risk of Disease

- 5% of infected individuals may develop active disease within 2 yrs
- Immunocompetent individuals have about a 5-15% lifetime risk of developing tuberculosis disease after this
- In 10% of HIV+ patients, active disease develops in 1 yr after exposure
- Whether progression to disease occurs depends on immune status of host and the infectious dose

Clinical symptoms of Tuberculosis

- Primarily a lower respiratory tract (pulmonary) disease
  - Nonspecific complaints:
    - malaise
    - weight loss,
    - cough
    - night sweats
  - Sputum may be bloody or purulent or initially may be scanty in amount
  - X-ray will support the clinical diagnosis
  - 1 or more upper lobes can be involved; disease may include abscess formation and cavitation

- Extrapulmonary disease due to hematogenous spread during initial phases of multiplication
- Dissemination is also referred to as miliary disease with or without evidence of pulmonary disease
- Pott's disease – spread to spinal vertebrae and intervertebral disk spaces

Primary Therapy for MTB

- Rifampin
- Isoniazid (INH)
- Pyrazinamide
- Ethambutol

MDR TB: Resistance to INH and Rifampin

XDR TB: MDR TB plus resistance to quinolone and at least 1 of the 3 aminoglycosides (injectable anti-TB drugs)

Identification of *M. tuberculosis*

- *M. tuberculosis* – part of the MTB complex including:
  - *M. bovis* – warm blooded mammals; PZA resistant
  - *M. bovis BCG* – vaccine strain
  - *M. africanum*
  - *M. canetti*
  - *M. microti*
  - *M. caprae*
  - *M. pinnipedii*

- Colonies of MTB are rough and buff
- Generally reported as MTB Complex
- Specific molecular methods needed to distinguish within complex.

MTBC Biochemical Reactions (abbreviated table)

Species	Colony Morphology	Niacin Accum-ulation	T2H	Nitrate	Pyrazinamidase
√*M. tuberculosis*	**Rough**	**+**	**Resistant**	**+**	**Susceptible**
√*M. bovis*	**Rough**	**-**	**Susceptible**	**-**	**Resistant**
M. bovis BCG	Rough	-	Susceptible	-	Resistant
M. africanum	Rough	V	V	V	Susceptible
M. canettii	Smooth	-	Resistant	+	Susceptible
M. microti	Smooth	+	Susceptible	V	Susceptible
M. caprae	Smooth	-	Susceptible	-	ND
M. pinnipedii	Rough	V	ND	-	ND

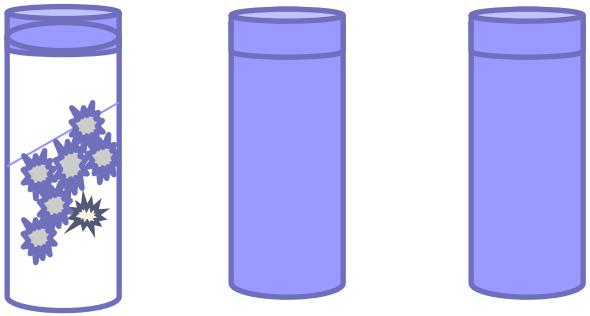
T2H = thiophene-2-carboxylic acid hydrazide

Non-tuberculous Mycobacteria

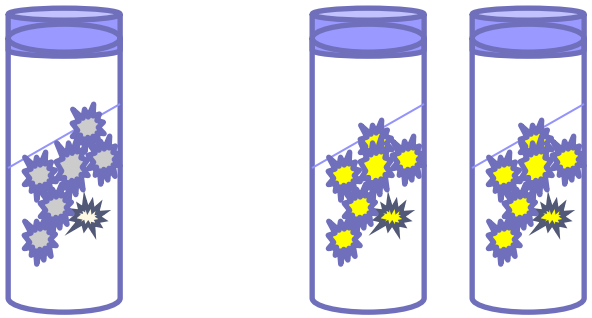
- Habitat
  - Free-living in soil and water
  - Not part of flora of humans or animals, but sometimes isolated from mucous membranes or skin of healthy individuals
- Human pathogenicity varies for different species
- Recovery may reflect contamination, saprophytic state or pathogenicity

Photoreactivity Test

Inoculate 3 tubes; shield 2 tubes from light.

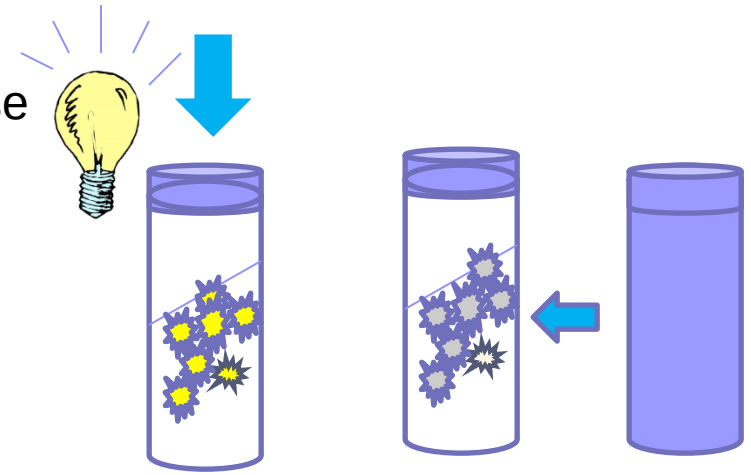


When growth occurs in unshielded tube, unwrap one.



If colonies pigmented in room light and dark, <u>scotocrhromogen</u>

If colonies not pigmented, expose to light.



Unwrap tube and compare to light exosed tube. If only pigment when exposed to light, <u>photochromogen</u>

Photochromogens

- *M. kansasii* – common pulmonary
- *M. marinum* – uncommon; causes skin infection
- *M. simiae* – uncommon; pulmonary infections
- *M. asiaticum* - uncommon
- *M. szulgai* – uncommon, usually pathogenic; orange scotochromogen at 37ºC; photochromogen at 25ºC.

Scotochromogens (Runyon Group II)

- *M. xenopi* –
  - Common in Canada & England, sometimes causes pulmon infection
  - (pigment intensifies with age of colony).
  - Temp optimum is 42ºC.
- *M. szulgai* – uncommon, usually pathogenic; orange scotochromogen at 37ºC; photochromogen at 25ºC.
- *M. gordonae* – common contaminant of water; orange
- *M. scrofulaceum* – uncommon, may cause lymphadenitis

Nonchromogens (Group III)

- *M. avium* complex – common, usually pathogenic (can be pigmented)
- *M. haemophilum* – uncommon, usually pathogenic; skin infection in HIV +
- *M. malmoense* – uncommon in U.S., usually pathogenic; pulmonary disease
- *M. ulcerans* – common in Africa, pathogenic ulcers

Rapid Growers

mostly common, sometimes pathogenic

- *M. fortuitum* group
- *M. chelonae*
- *M. abscessus* group
- *M. mucogenicum* – less uncommon; less pathogenic

M. avium-intracellulare complex
MAC or MAI

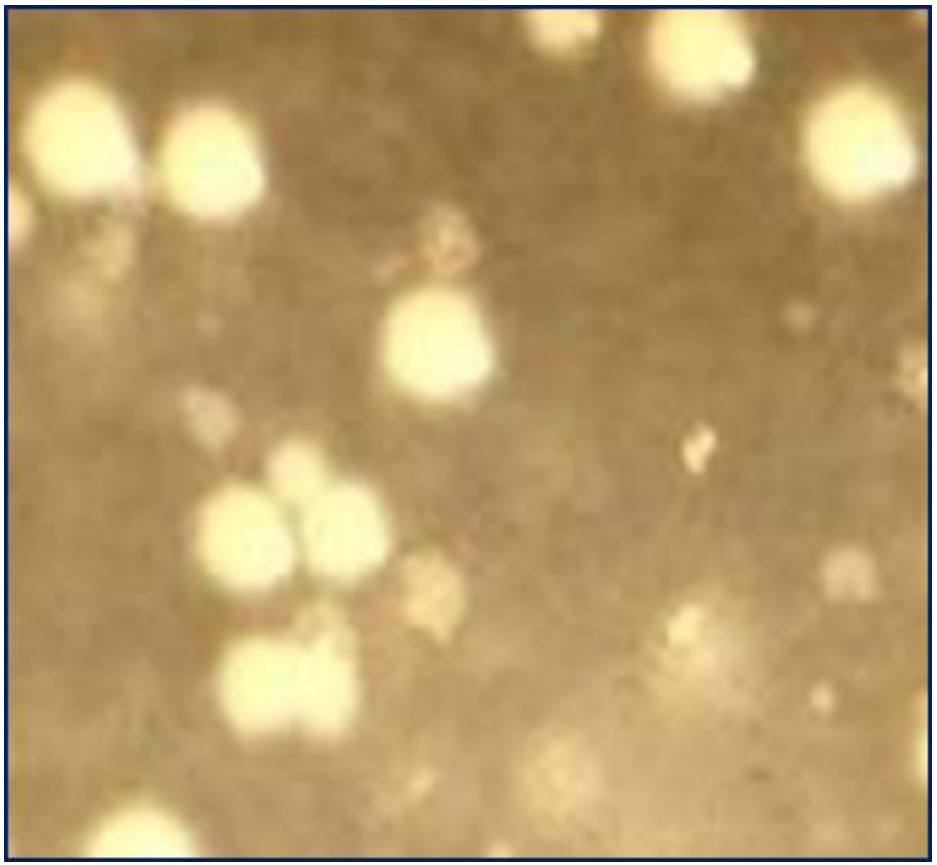
- Distinct Species; often not separated:
  - *M. avium* (90% of AIDS isolates)
  - *M. intracellularae* M. chimaera
  - Others
- Low pathogenicity in immunocompetent
- Common in cystic fibrosis and AIDS
- Mostly inert biochemically

M. avium complex Disease

- Pulmonary
  - Solitary pulmonary nodule
  - In elderly non-smoking women
    - patchy nodular infiltrates, associated with bronchiectasis
  - Older men with underlying pulmonary disease or hx smoking.
    - Slowly evolving cavitary disease like TB
- Lymphadenitis – children
- Disseminated – AIDS (CD4<100), replication in local lymph nodes, spread to many organs, esp lung & intestine, bacteremia.

MAI Complex: Colonies can be a mixture of smooth and rough; can be white or transluscent (more resistant). Usually relatively small colony size.

Yellow pigment (late)



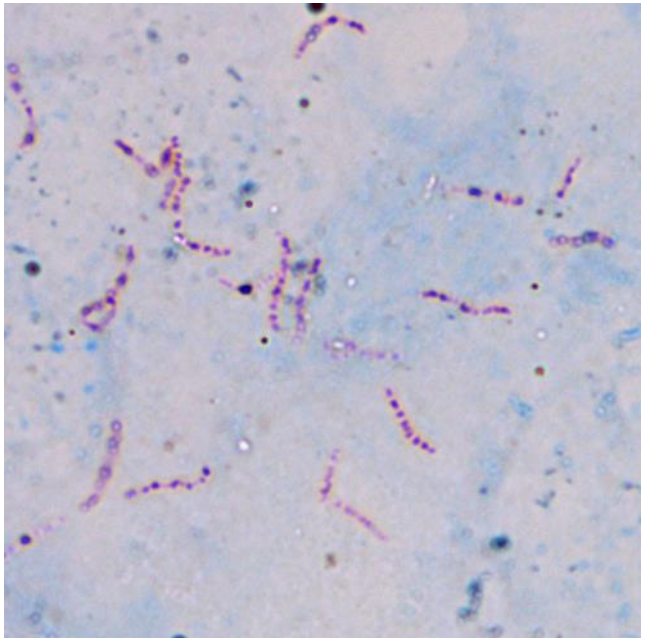
M. avium complex - Therapy

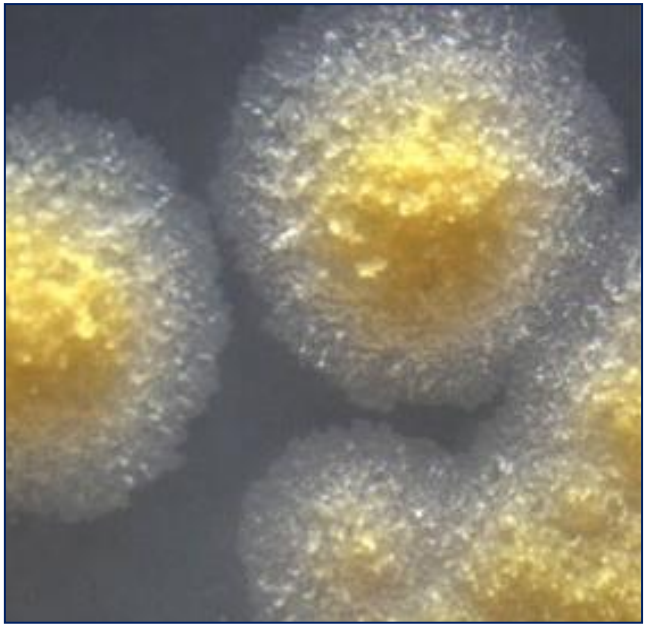
- Clarithromycin or Azithromycin (prophylaxis)
- Ethambutol
- Rifampin
  - Resistance may develop on monotherapy
- Pulmonary infection: Macrolide, rifampin/rifabutin, ethambutol combination.
- Intrinsically resistant to isoniazide and pyrazinamide

M. kansasii

- A photochromogen, golden color
- Reported in mine workers in UK and S. Africa
- Chronic pulmonary disease, usually upper lobe
- Can mimic TB
- Rare extrapulmonary infection

- Therapy: rifampin, INH, ethambutol, streptomycin

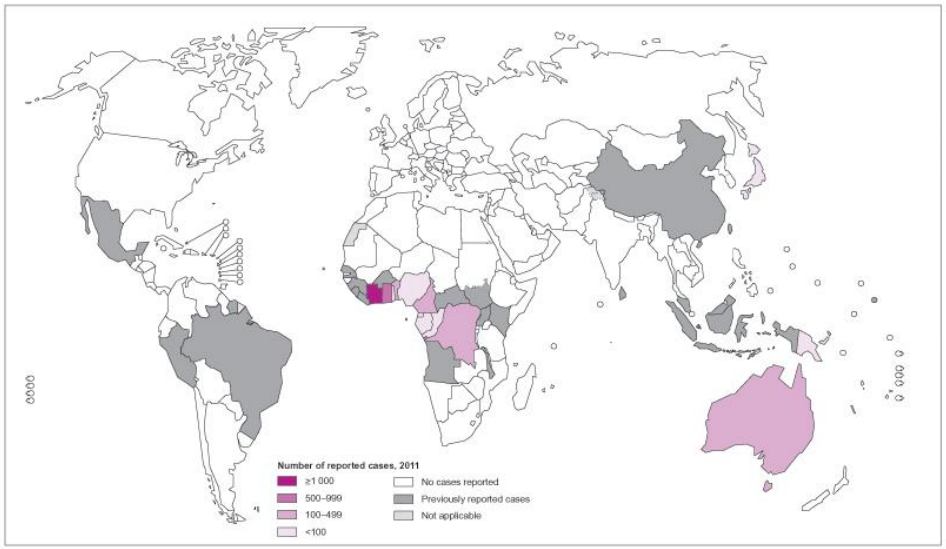




M. ulcerans

- 3rd most common mycobacterial infection worldwide
- Grows slowly at 30ºC. Sensitive to many decontamination protocols so often missed in culture.

Distribution of Buruli ulcer, worldwide, 2011



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

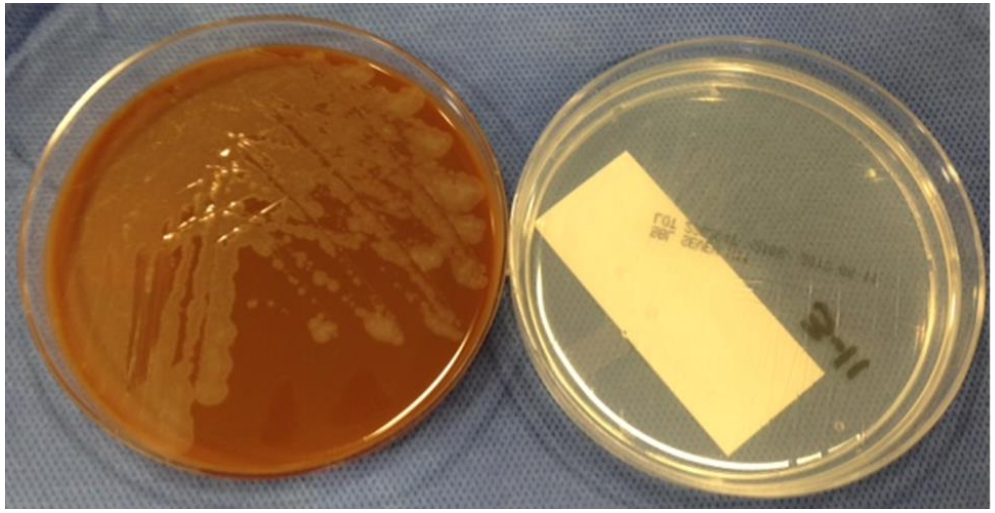
World Health Organization

Causes Buruli ulcer

A painless lump under skin at site of trauma (lower extremities). Shallow ulcer forms in a few weeks due to a toxin. Can disseminate widely & cause osteomyelitis

M. haemophilum

- Usually subcutaneous lesions in immunocompromised
- Nodules (may be multiple) on extremities
- May have cellulitis, abscesses, draining fistulas, osteomyelitis.
- **Grows at 30ºC. Requires source of hemin.**



M. marinum

- A photochromogen
- Cutaneous infections due to trauma associated with fish tanks, salt water, improperly chlorinated pools.
- Primary lesion may spread in sporotrichoid pattern
- Complications include tenosynovitis, bursitis, arthritis, osteomyelitis
- **Grows at 30ºC.**

M. leprae – Hansen's disease

- **Not cultivable on solid media or tissue culture; foot pad of armadillo or mouse**
- 2 Major forms
  - Tuberculoid – few organisms. Erythematous or hypopigmented plaques. Strong CMI, but weak humoral response. Many lymphocytes & granulomas.
  - Lepromatous – Many organisms. Highly infectious. Extensive tissue destruction & sensory loss. Involvement of nasal mucosa. Strong Ab response, weak CMI.
- Rx: dapson, clofazimine and rifampin

Rapid grower on BAP

- *Common:*
  - *M. fortuitum*, *M. chelonae, M. abscessus*
- *M. chelonae* often grows well at 30º C.
- Rapid growers positive for 3 day arylsulfatase

Rapid Growers

- *M. chelonae*
  - Localized post-traumatic wound infections, post-trauma or post-surgical corneal infections, catheter infections. Disseminated skin infections in immunosuppressed (corticosteroids).
  - Rx: clarithromycin, amikacin

- *M. fortuitum group*
  - Consists of *M. fortuitum*, *M. peregrinum* & other species
  - Causes localized, post-traumatic wound infections, catheter infections & surgical wound infections

Rapid Growers

- *M. abscessus*
  - Chronic lung infections, localized post-traumatic wound & surgical site infections, chronic otitis media, catheter infections. Disseminated skin infections in immunocomp.
  - Rough and smooth colony types in same culture
  - Often very resistant; Rx amikacin, imipenem, cefoxitin, & sometimes clarithromcyin
- *M. mucogenicum*
  - Sputum contaminant. Catheter infections
  - Wet, shiny colony
- *M. smegmatis*
  - Very rare infections.

MTB Susceptibility Testing is based on Critical concentration

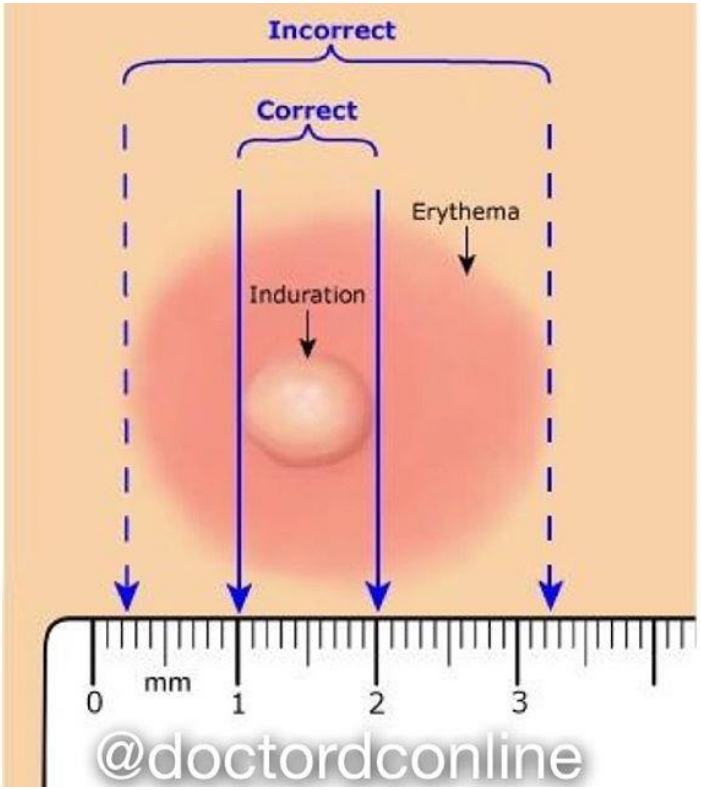
- The critical conc of a drug is the conc that inhibits growth of most bacteria (wild type) without affecting the growth of resistant mutants.
- The proportion of bacilli resistant to an anti-TB drug, above which a clinical response is unlikely, was set at 1%.
- Compared to growth control if >1% of bacteria grow in presence of drug, the drug will not be effective.

NTM Susceptibility Testing

- Rapid growers – microbroth dilution panels incubated 3 to 5 days
  - Test clinically significant isolates
- Slow growers – MAI, M. kansasii
  - Microbroth dilution panels
  - MAI
    - Test if clinically significant and patient previously treated
    - Test if patient relapse or acquires infection while on therapy

PPD testing

- Test for LTBI –latent TB infection
- *In vivo* delayed type hypersensitivity reaction
- Purified protein derivative (0.1 ml; 5 U) injected intradermally in forearm
- Read for induration at 48 to 72 hrs
- Positive:
  - ≥10 mm if risk factors;
  - ≥15 mm if no risk factors;
  - ≥ 5 mm if immunocomp. or exposed to TB.



Problems with PPD

- Difficult to interpret reaction
- False positives if BCG vaccinated (if >10mm new infection likely)
- Cross-reactions with NTM (<10mm induration)
- Patients must return at 48 hours
- False negatives due to anergy, very recent TB infection or very young age

Interferon gamma release assays (IGRA): QuantiFERON Test

- Designed to measure latent TB infection (LTBI)
- Measured release of γ-IFN from stimulated lymphocytes in heparinized blood (w/in 12 hrs).
- Cells in blood are stimulated overnight at 37° C with TB antigens (H), mitogen (M) or no antigen (N).
- Remove plasma and store for testing.
- γ-IFN quantified by ELISA. Standard curve is used to generate γ-IFN amounts.
- Optical densities are converted into I.U.s

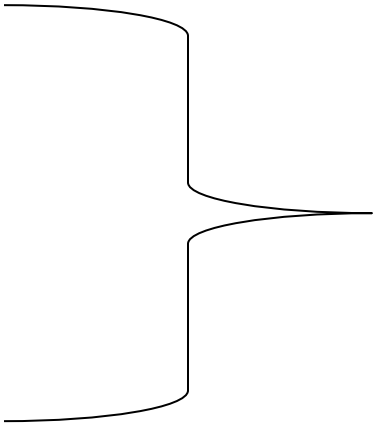
Interpretation of QuantiFERON

- *M. tuberculosis* infection likely
  - TB antigen ≥ 0.35
  - TB antigen >50% of Nil control
  - Mitogen-Nil in any amount
- Indeterminate
  - TB antigen <0.35 & any Nil result & Mitogen-Nil <0.5
  - Nil >0.7 and TB antigen <50% of Nil & any Mitogen-Nil result
- *M. tuberculosis* infection unlikely
  - TB antigen <0.35
  - Nil ≤ 0.7
  - Mitogen – Nil ≥ 0.5

Genus & staining properties

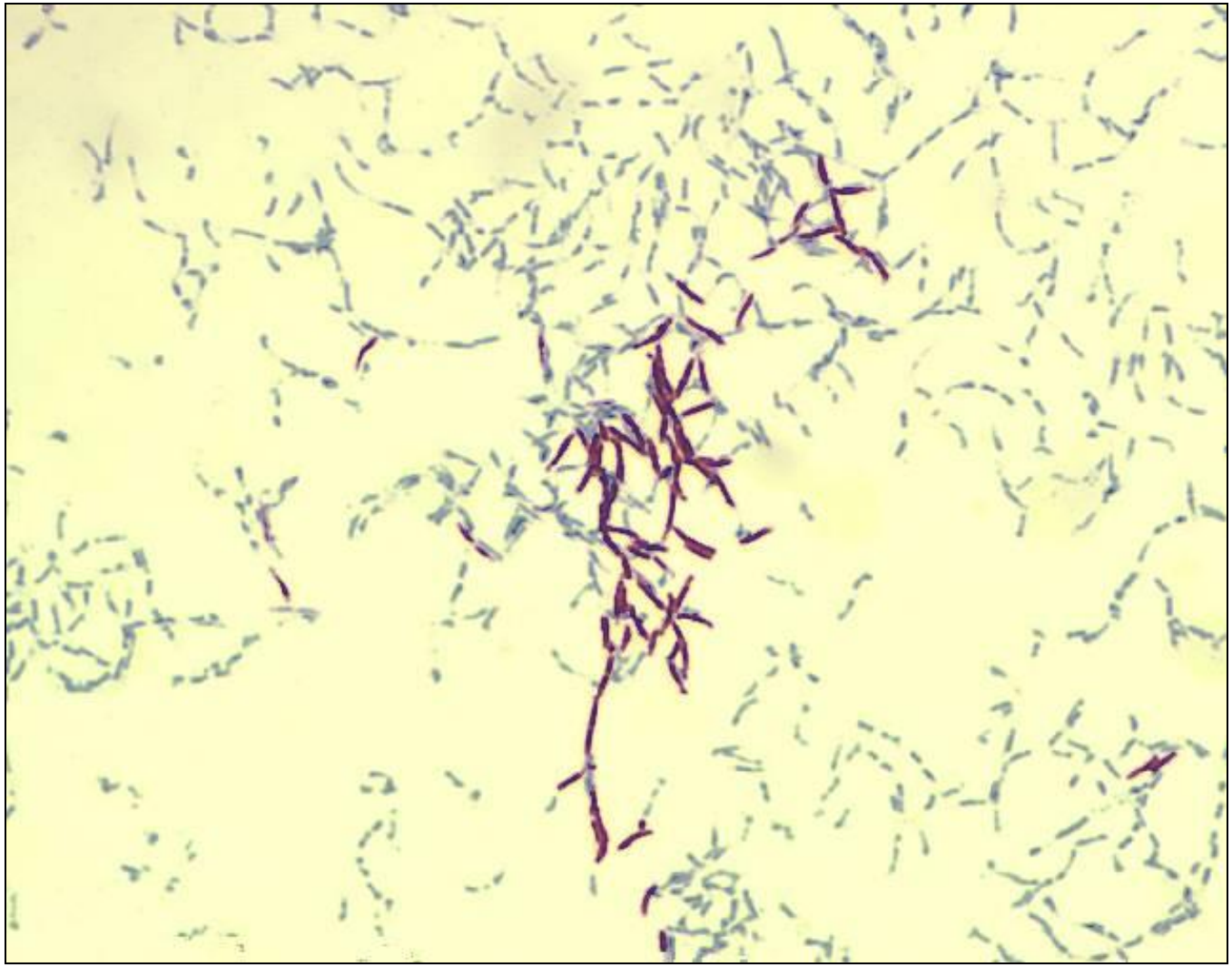
- *Mycobacterium* = acid fast

- *Nocardia*
- *Rhodococcus*
- *Gordonia*
- *Tsukamurella*



Weakly acid fast; stain with modified acid fast procedure

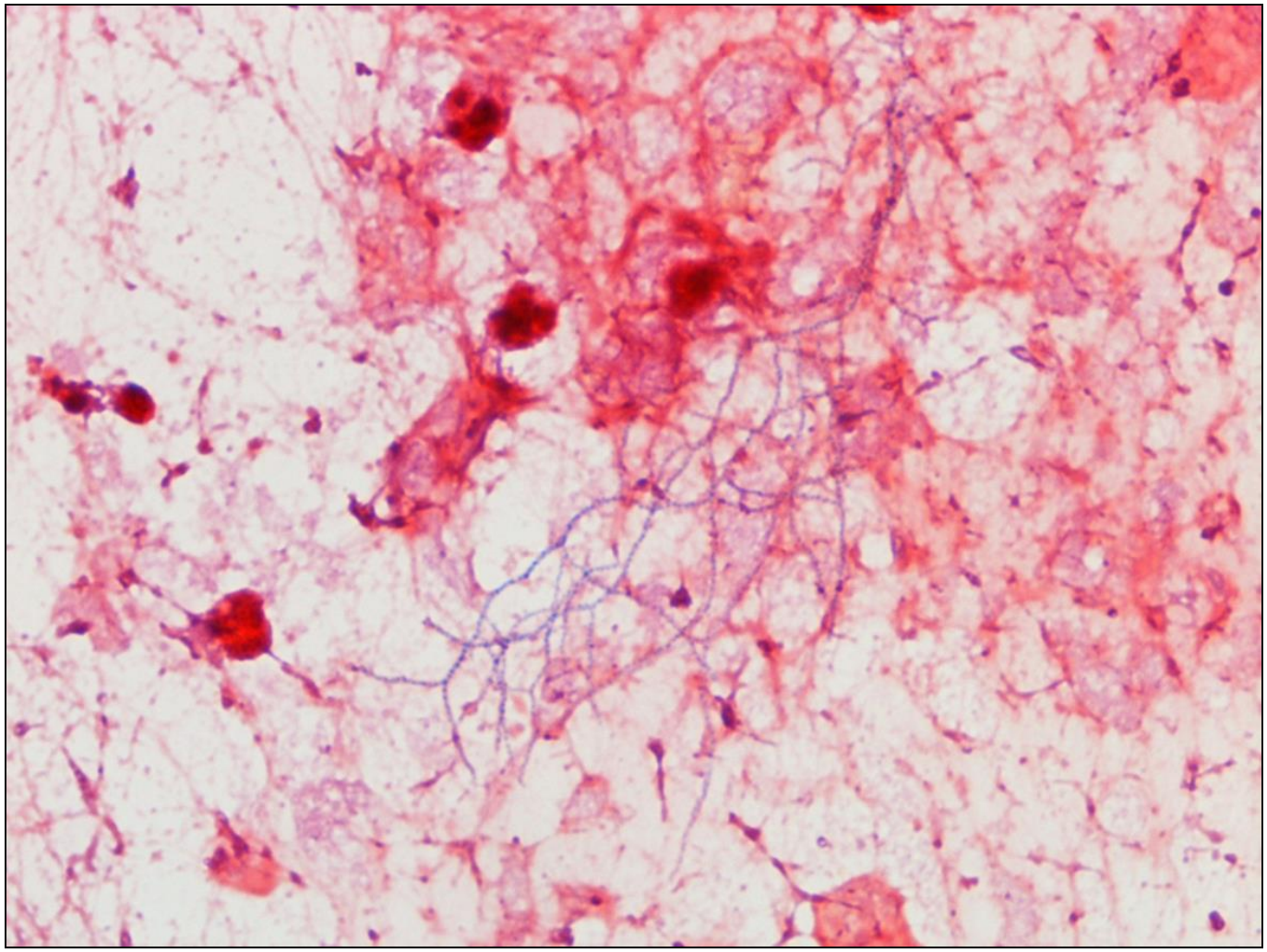
MAF - partial or weak acid fast staining



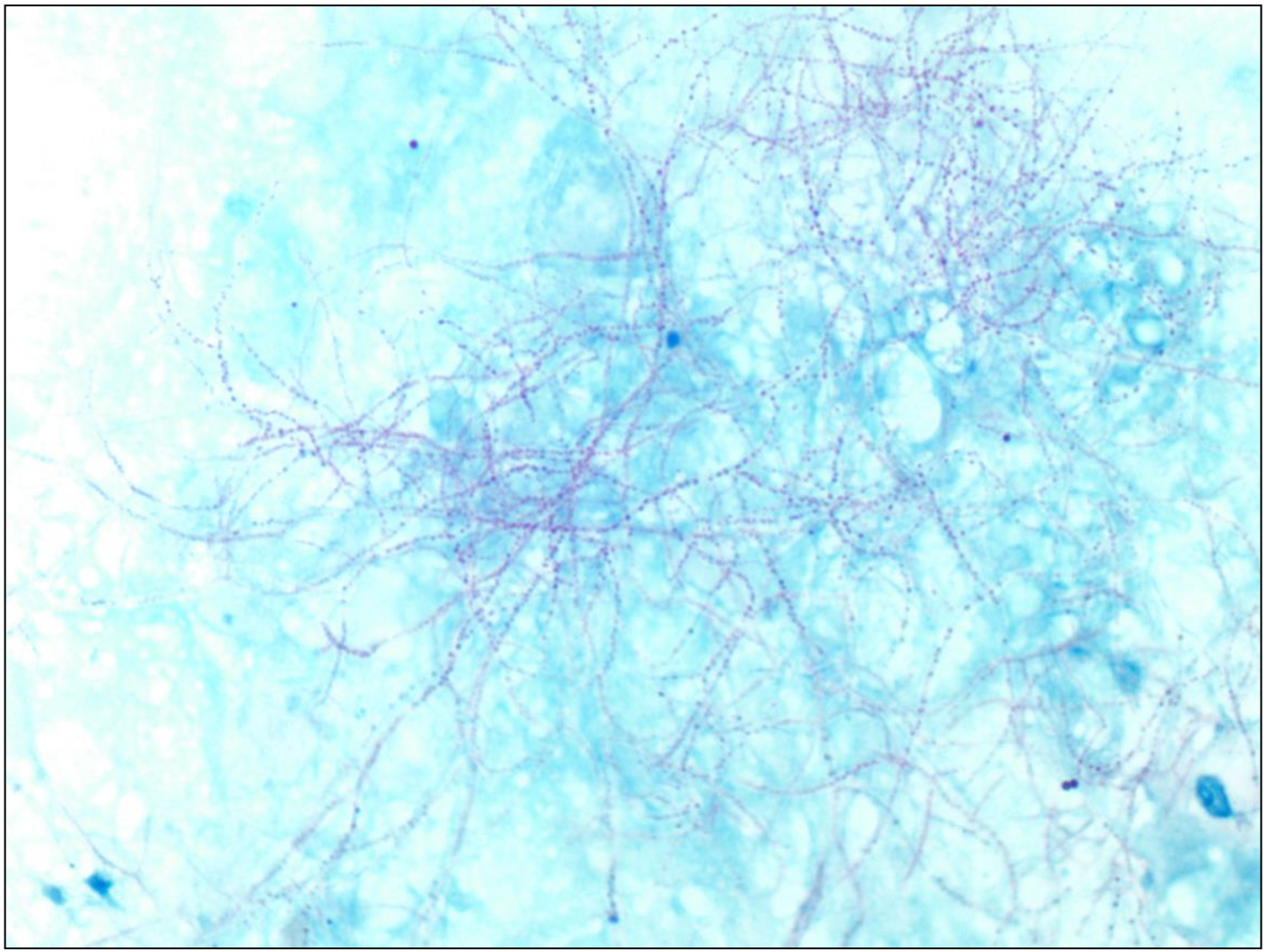
Modified Acid Fast Stain

- Used primarily for the direct detection of Nocardia in specimens
  - MAF+ branching rods:
    - Consider Nocardia species
  - MAF- branching rods:
    - Consider Streptomyces, Actinomyces
  - MAF+ non-branching rods:
    - Consider mycobacteria, Tsukamurella, Rhodococcus, Gordonia

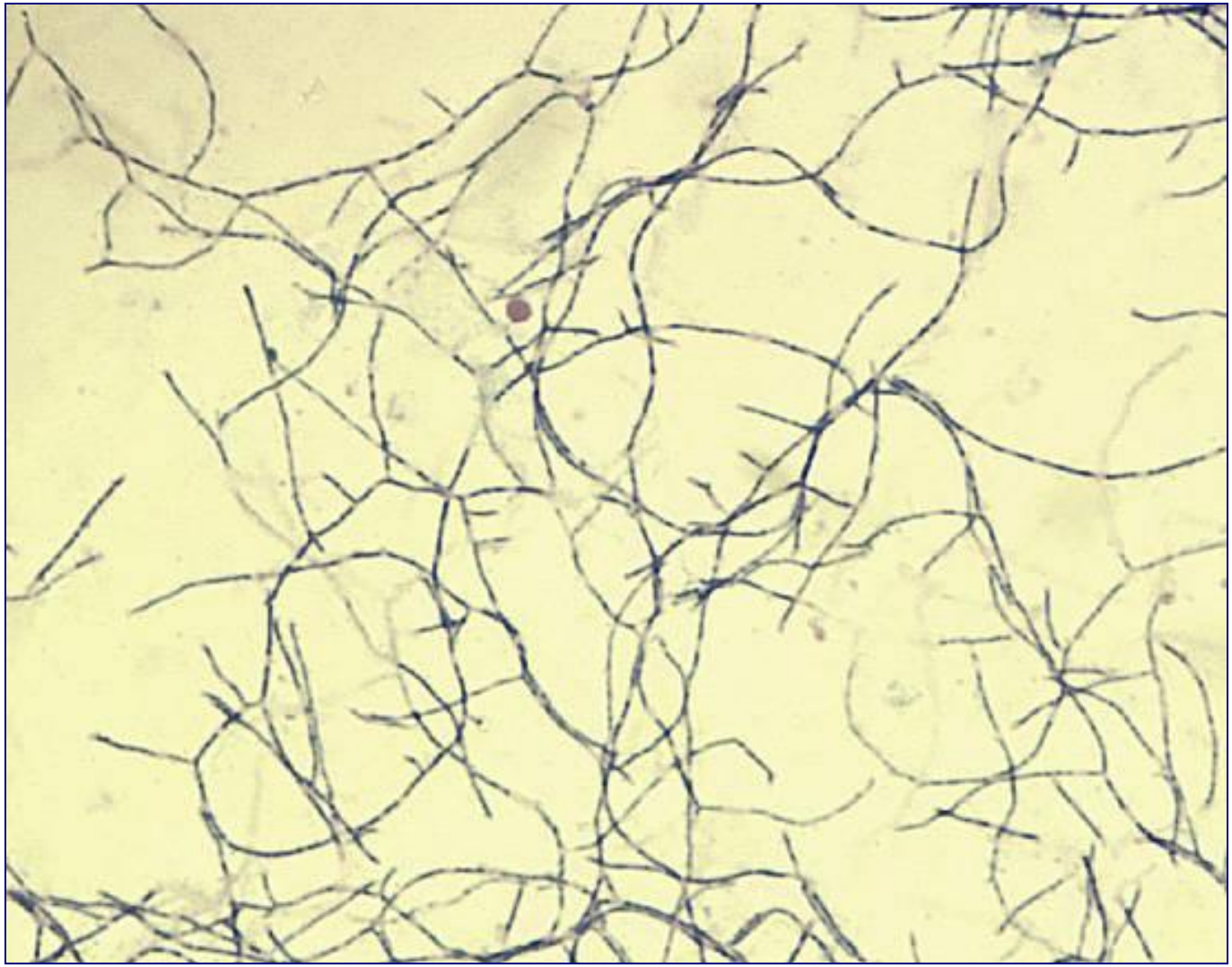
NOCARDIA GRAM STAIN – long, thin finely branching, beaded positive rods



Nocardia – MAF Stain

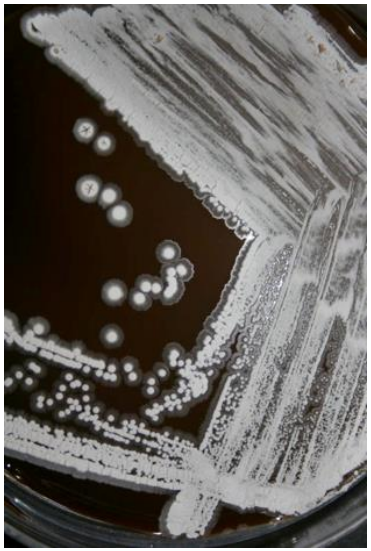


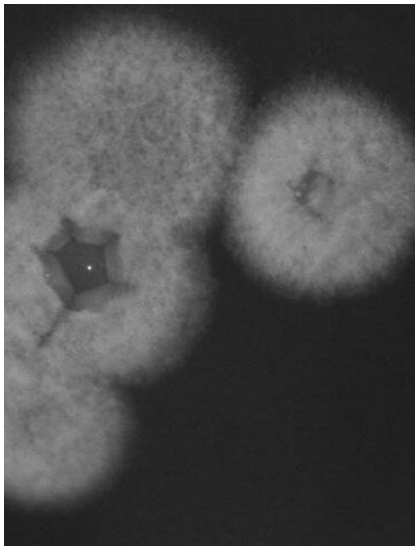
Streptomyces & Modified Acid Fast Stain



Nocardia spp.

- Gram + branching beaded bacilli; partial AFB
  - *N. cyriacigeorgica*
  - *N. farcinica*
  - *N. nova*
  - *N. brasiliensis*
  - *N. otitidiscaviarium*
  - Many others





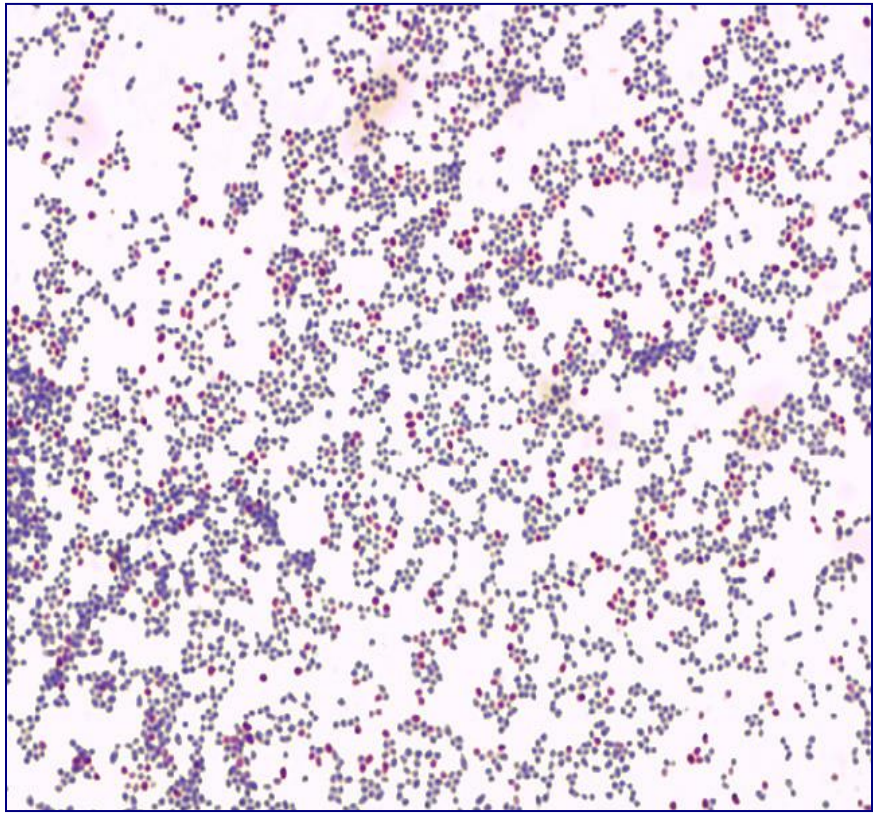
- Partially acid fast; aerial hyphae; resistant to lysozyme;
- Pulmonary and skin infections; dissemination to brain
- Susceptible to SXT and imipenem usually

Rhodococcus

Baroudi. Infectious Disease News, April 2013

Organism from pulmonary abscess from an AIDS patient.

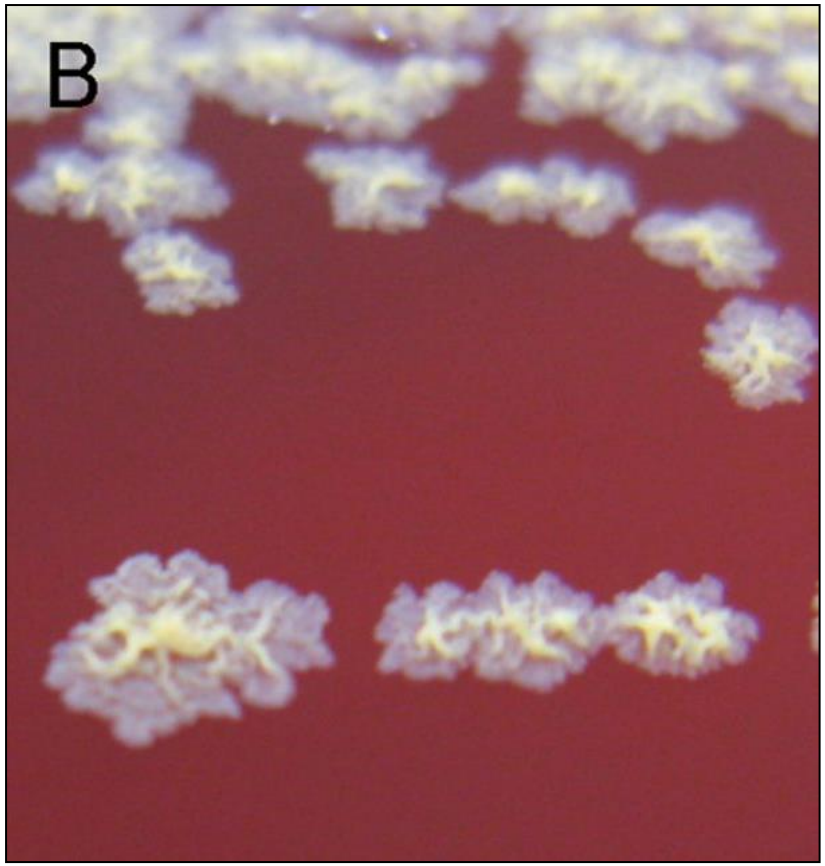
MAF from 2-day agar plate



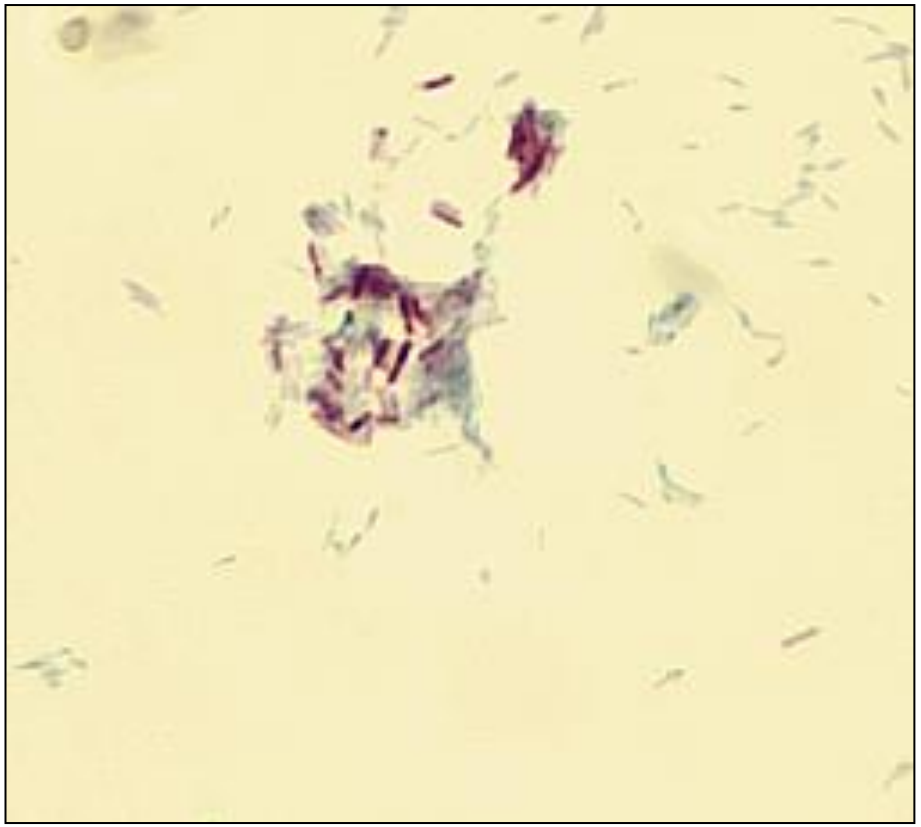
Gram stain from broth culture

Tsukamurella

Long, thin rods with no branching. Colony is small.
Can be smooth to rough and whitish to orange.



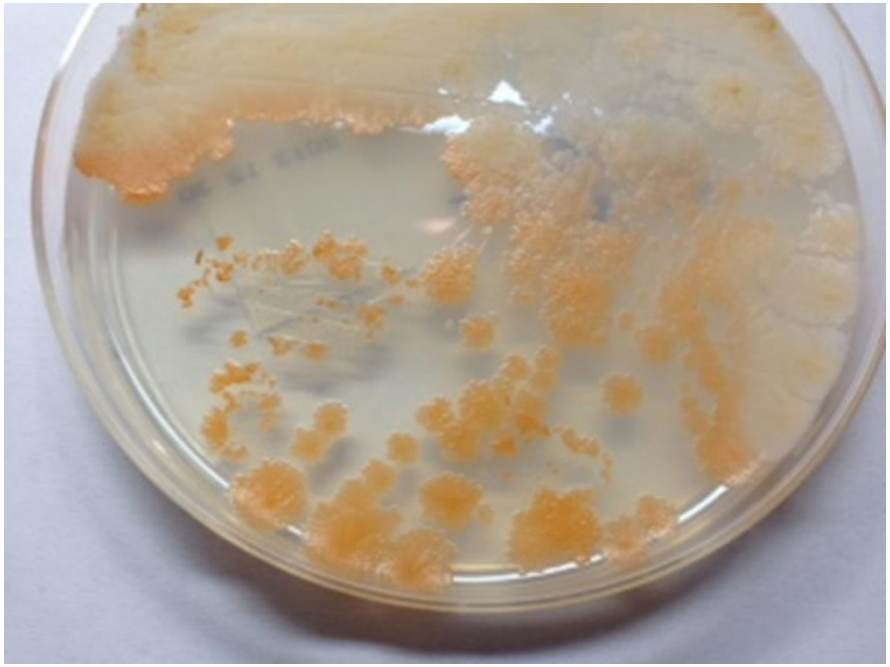
JCM 2014 52:2251



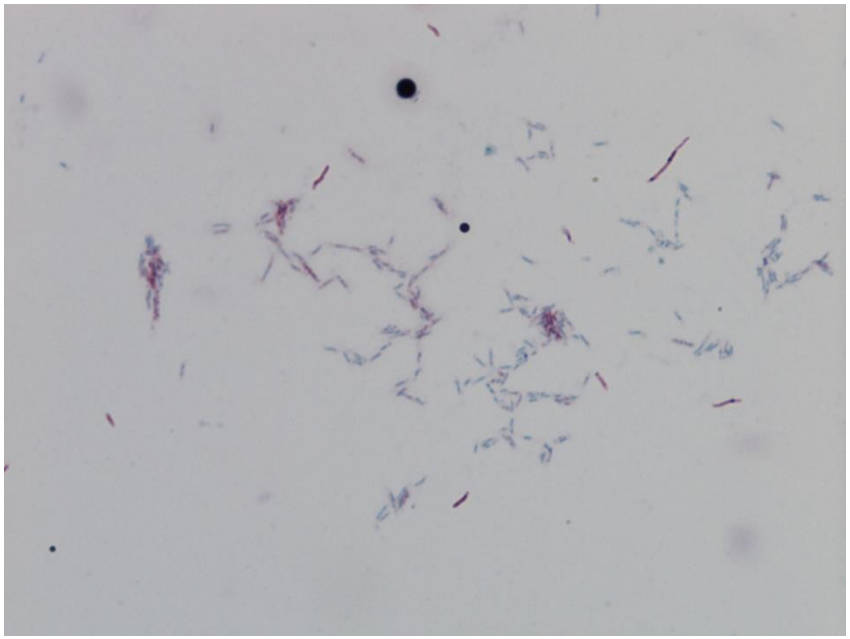
MAF – from Sheep blood agar

Gordonia

(genus split from *Rhodococcus*)



Middlebrook 7H11 agar



Modified acid fast stain from Middlebrook agar; 100X

Questions??