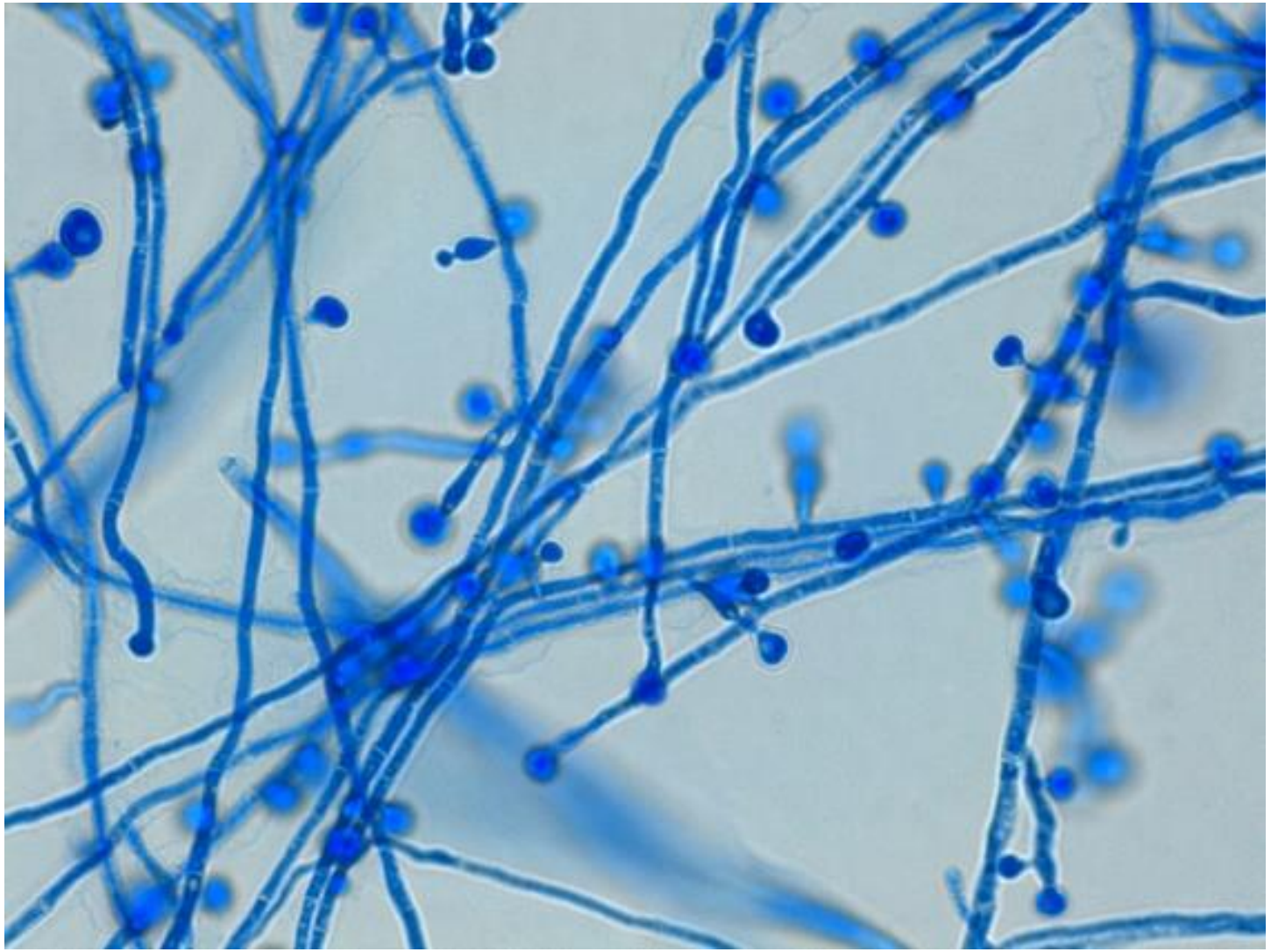


CASE OF THE MONTH

Microbiology

Resident Leads:
Teslin Sandstrom, PGY4 MM
Chinelo Iwegim, PGY2 MM

Faculty Lead:
Dr. Susan Poutanen, Medical Microbiologist and Infectious Disease Physician at UHN/Sinai

January 13, 2026

CASE INTRODUCTION

- Ms. B is a previously healthy 26-year-old female, who has presented to the Royal Victoria Regional Health Center in Barrie with a 5 day history of worsening fever, productive cough, and dyspnea.
- Triage vitals are as follows: BP 85/50, HR 135, RR 25, SpO2 86% on room air, Tmax 38.5°C

GOOD or BAD

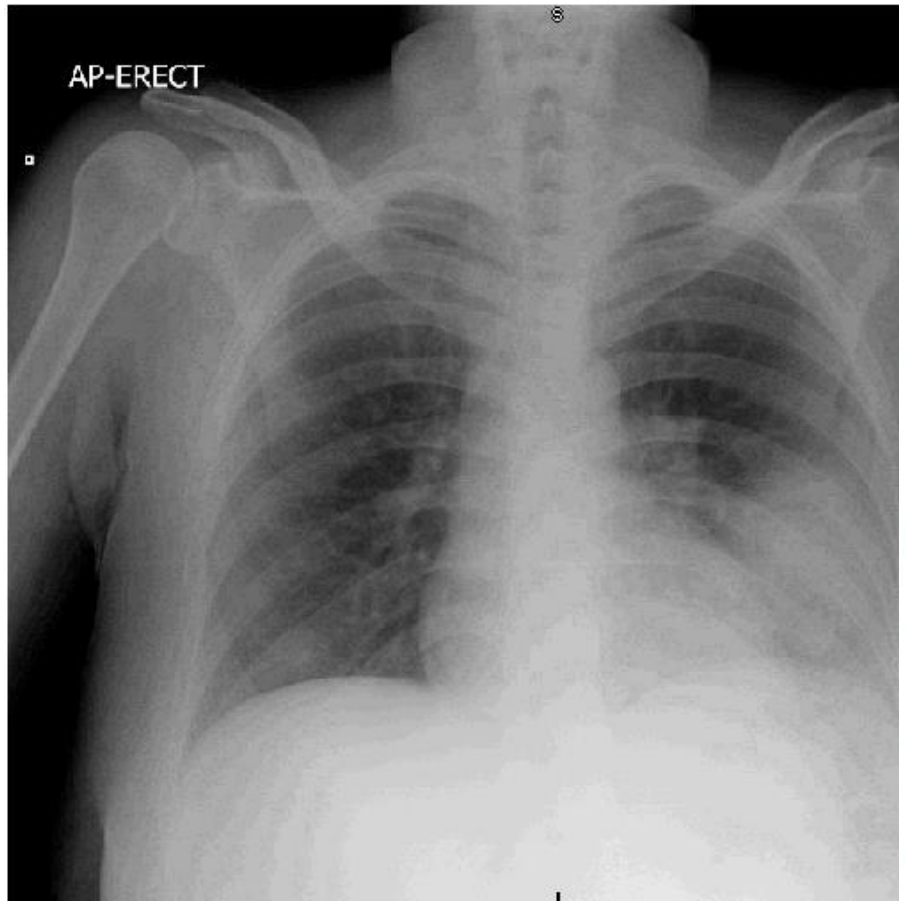
- On exam:
  - **Gen:** Alert, oriented x1 (person)
  - **Cardiac:** Pulses present and equal bilaterally. Extremities cool. JVP flat. NS1/S2 with no extra heart sounds. No stigmata of endocarditis on palms/soles.
  - **Resp:** Increased WOB. Actively coughing. Crackles to the left lung base on auscultation. You perform tactile fremitus. The ED staff laughs at you.
  - **Abdo:** Soft, non-tender. No masses or organomegaly.
  - **Derm:** No mucosal or skin lesions. No rashes.

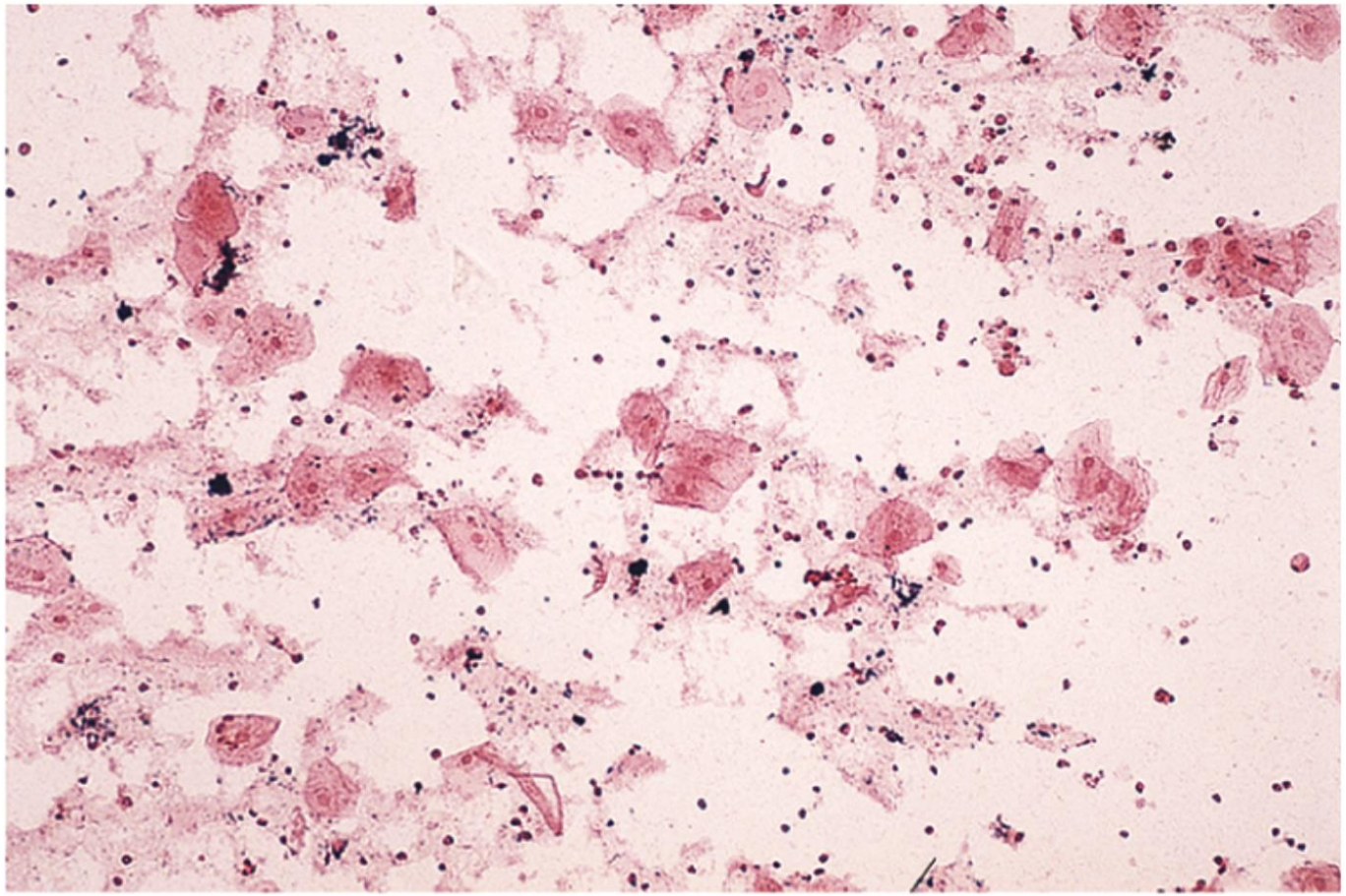
CASE INTRODUCTION[1]

DIAGNOSIS?	RELEVANT HISTORY?	FIRST STEPS?

[1]Although Medical Microbiologists don't routinely see patients, clinical teams will rely on our expertise to help guide investigations (and to a certain degree, management), so a strong clinical foundation is important!

CASE INTRODUCTION





Disclaimer: This is chest x-ray is from Google and not from this specific patient's case.

Gram stain from Manual of Clinical Microbiology 13th Ed. Ch19. Specimen Collection, Transport, and Processing

CASE INTRODUCTION

- Over the next 48h, Ms. B's clinical status worsens:
  - Antibiotics are broadened to meropenem and vancomycin
  - Norepi and vasopressin are started for hemodynamic support
  - Hypoxic respiratory failure resulting in intubation and transfer to TGH

- 12 hours after Ms. B's admission to TGH, you receive a page from the ICU…
  - Everything that was sent from RVH was negative, but the sputum culture was rejected! Can we please ask them to process it?
  - We are about to bronch this patient, what should we order?

RESPIRATORY BENCH

An Introduction

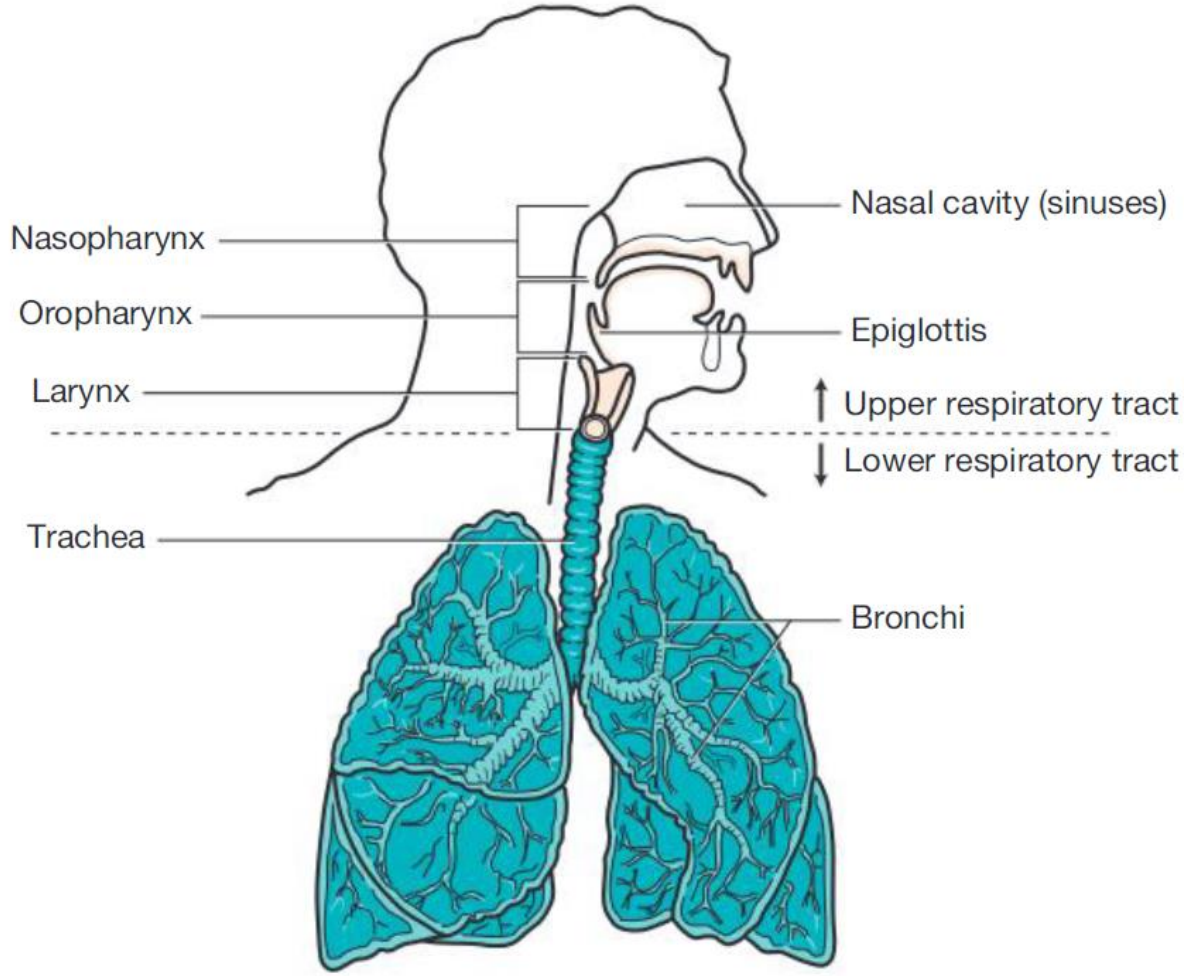
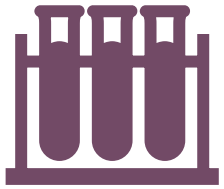


Fig. 32.1 Anatomy of the respiratory tract.

LABORATORY WORKFLOW



Pre-Analytical

Examination ordering

Specimen collection

Specimen transport

Specimen receipt, accessioning, and processing

Analytical

Examination method selection

Examination performance

Results review and follow-up

Laboratory results interpretation

Post- Analytical

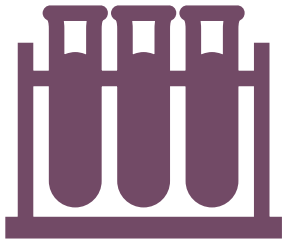
Communication of alert values and issuance of preliminary reports

Release of final reports

Specimen management

CLSI QMS01, 5th Ed.

LABORATORY WORKFLOW



Pre-Analytical

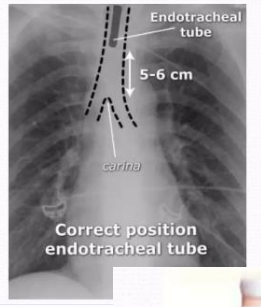
- Examination ordering
- Specimen collection
- Specimen transport
- Specimen receipt, accessioning, and processing

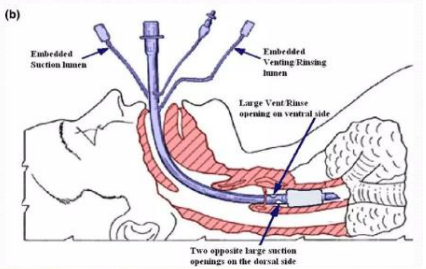
Impacts diagnostic accuracy
Prevents false negatives/positives
Reduces need for recollection
Supports antimicrobial stewardship
Protects laboratory staff

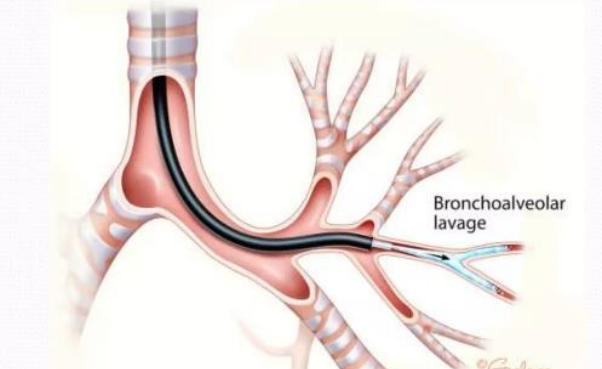
CLSI QMS01, 5th Ed.

UPPER RESPIRATORY TRACT	CONDITIONS	SPECIMEN TYPES
EYES	Conjuctivitis, keratitis, retinitis, orbital cellulitis, endopthalmitis	Conjuctival swab, corneal scraping, vitreous fluid,
EARS	Otitis media Otitis externa (malignant) Mastoiditis	Tissue biopsy, fluid aspirate
NOSE/THROAT	Laryngitis Pharyngitis Rhinosinusitis Epiglotittis Pertussis	Nasopharyngeal Swabs/aspirates
MOUTH	Dental Abscess, Gingivitis/Periodontal Disease, Oral thrush	Oropharyngeal swabs/aspirates

LOWER RESPIRATORY TRACT	CONDITIONS	SPECIMEN TYPES
Lungs	Pneumonia: Viral: SARS-CoV-2, Influenza, RSV; Bacterial: common and uncommon organisms, Fungal – Pneumocystis, aspergillus, histoplasma, blastomycosis etc Tuberculosis & Non-tuberculous infection Pleural empyema	Sputum/ Induced sputum Gastric aspirate (Tb in children) Endotracheal aspirates (ET) Bronchoalveolar lavage (BAL) Thoracocentesis aspirate







PRE-ANALYTICAL CONSIDERATIONS

Timing

Within 72hrs of symptom onset if possible

Consider incubation period and organism load is highest.

Collection Method: Use appropriate swabs (e.g., flocked nylon swabs are recommended for their efficient release of cells and secretions) and avoid cotton or calcium alginate swabs, which can contain PCR inhibitors.

Label with the right patient ID (at least 2 identifiers) & sample type

Transport Media

Appropriate viral or universal transport media (VTM or UTM) to prevent inactivation of organisms

Storage & Storage
Store at 2-8°C for up to 72 hours after collection. If shipping delayed > 72hrs, store at -70°C or below.

Prolonged stay at room temperature can cause loss of some viral RNA/DNA for some viruses and media types, increasing the risk of false-negative results, especially in samples with low viral loads.

Handling
Minimize freeze-thaw cycles, as this can degrade nucleic acids.

Protect samples from unnecessary light exposure. Adhere to strict biosafety guidelines, especially for high-risk pathogens, to prevent contamination and ensure personnel safety.

Attaway CC and Wang H. Laboratory testing for respiratory viruses for the clinician. Cleveland Clinic Journal of Medicine September 2024, 91 (9 suppl 1) S42-S49; DOI: https://doi.org/10.3949/ccjm.91.s1.07

PRE-ANALYTICAL CONSIDERATIONS

- For bacterial and fungal culture:
  - Sputum
  - ETT aspirate
  - BAL
  - *Tissue biopsy can be considered for ?invasive fungal infection
  - **NP swab for PCR (*Mycoplasma pneumoniae/Chlamydia pneumoniae*)

Collected and sent in sterile container

- For *Mycobacterium tuberculosis*:
  - Sputum
  - BAL

Processed by PHOL

- Microscopy (acid-fast staining)
- *M. tuberculosis* complex PCR (if AFB+)
- Culture

ANALYTICAL CONSIDERATIONS

Sample Quality Criteria

Sputum

1. Bartlett score
2. Murray and Washington grading system
3. Gecklet et al
4. Van Scoy
5. Barry
6. Heineman and Radano

CLSI QMS01, 5th Ed.

Bartlett Score

Based on microscopic examination

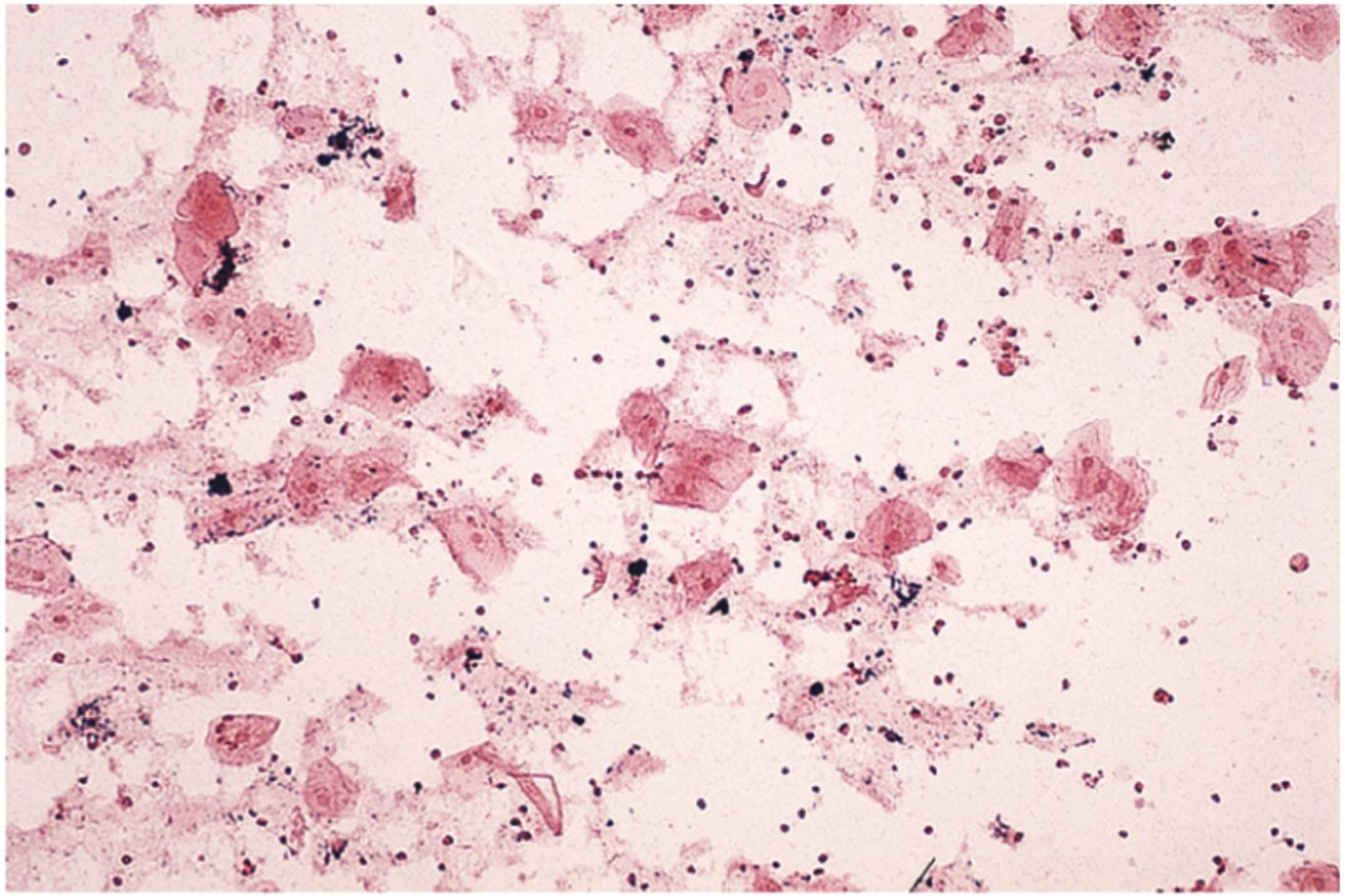
1. Number of Neutrophils / field
2. Number of squamous epithelial cells / field

(+) Score = acceptable sample

(-) Score = Non acceptable sample

Number of Neutrophils /10 X LPF	GRADE
<10	0
10-25	+1
>25	+2
Presence of mucus	+1
Number of Epithelial Cells /10 X LPF	
10-25	-1
>25	-2
TOTAL SCORE	

BACK TO THE CASE:



"The sputum culture was rejected! Can we please ask them to process it?"

Bartlett Score = -1 (???

Number of Neutrophils /10 X LPF	GRADE
<10	0
10-25	+1
>25	+2
Presence of mucus	+1
Number of Epithelial Cells /10 X LPF	
10-25	-1
>25	-2
TOTAL SCORE	

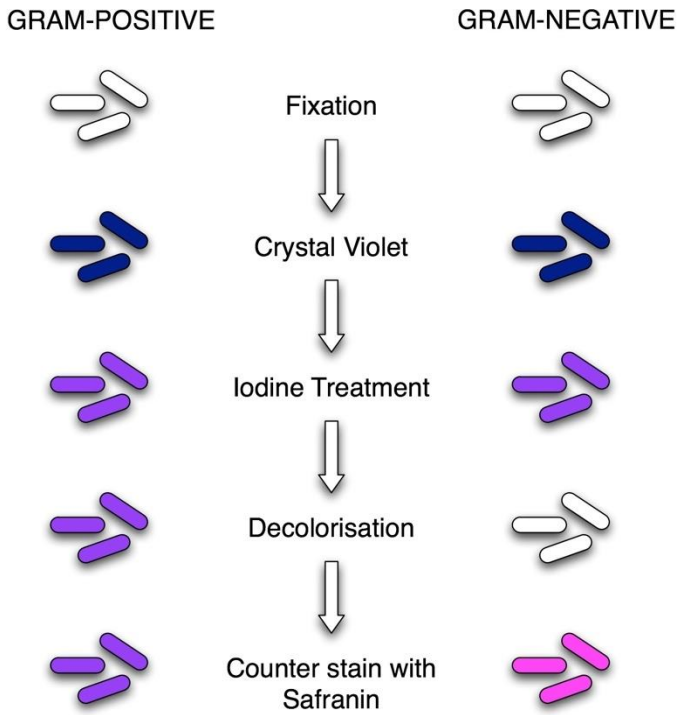
BACK TO THE CASE:

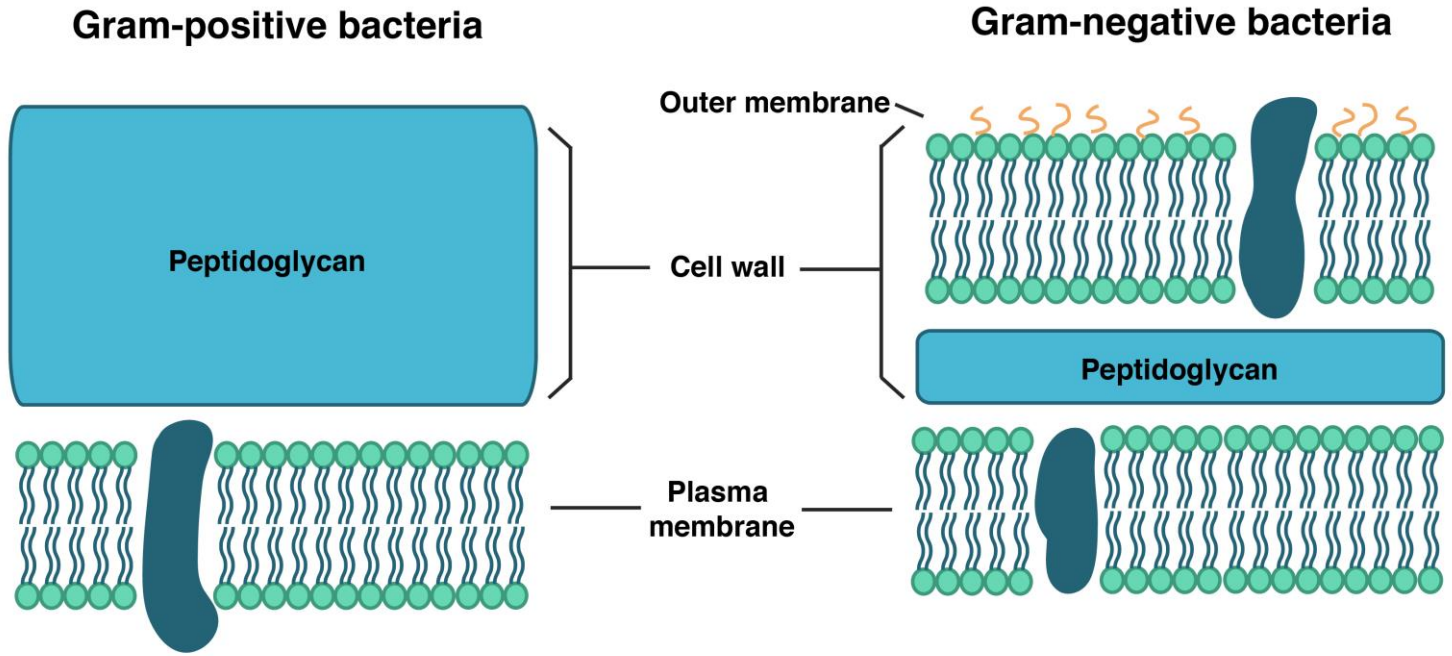
Ms. B's BAL fluid arrives in the lab.

45 minutes later, you are pulled aside by the Respiratory bench technologist who says "Myself and another MLT reviewed that gram stain, but we aren't sure what we are looking at. Can you take a look?"

TIME TO REVIEW:

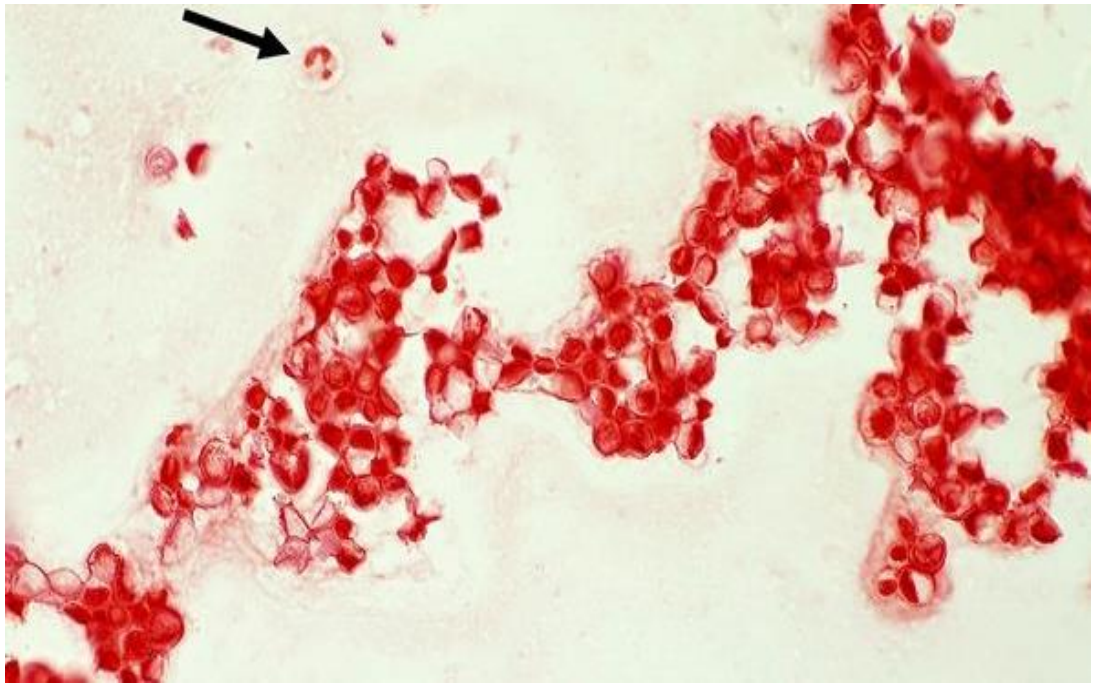
The Gram Stain[1]

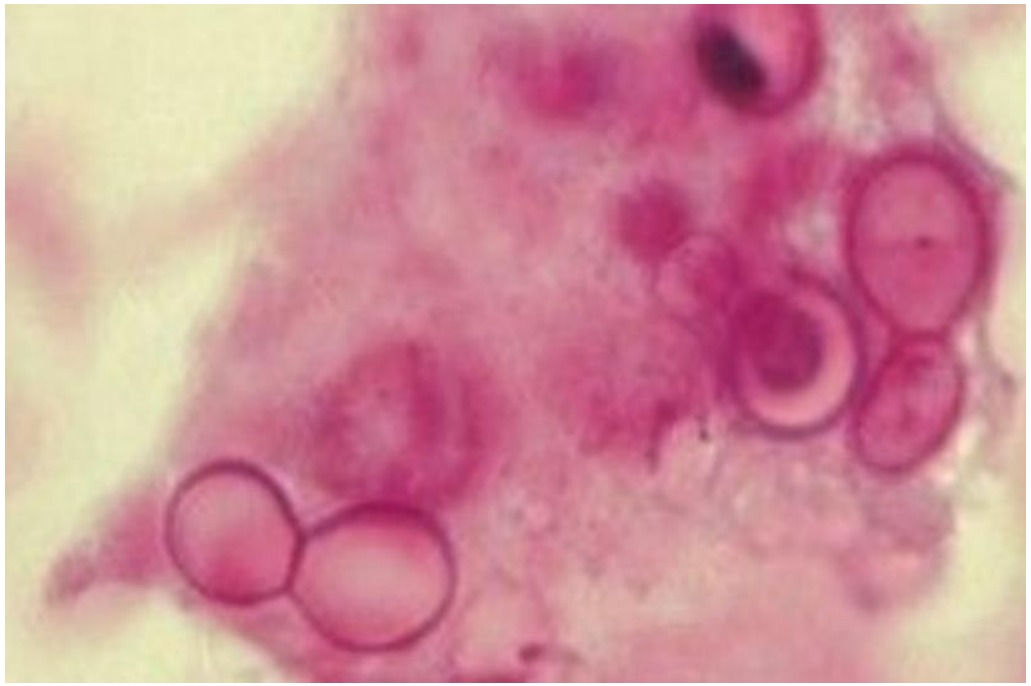




[1] Other non-bacterial organisms and human cells will pick up the stains used for Gram staining, but we reserve the categorization of "gram positive" and "gram negative" to bacteria when reporting.

BACK TO THE CASE:





The left panel shows the gram stain of the BAL fluid under 400X, with a small arrow pointing to a neutrophil. You then examine the same gram stain under 1000X with oil immersion (right panel).

TIME TO REVIEW:

Respiratory specimen acceptance/rejection

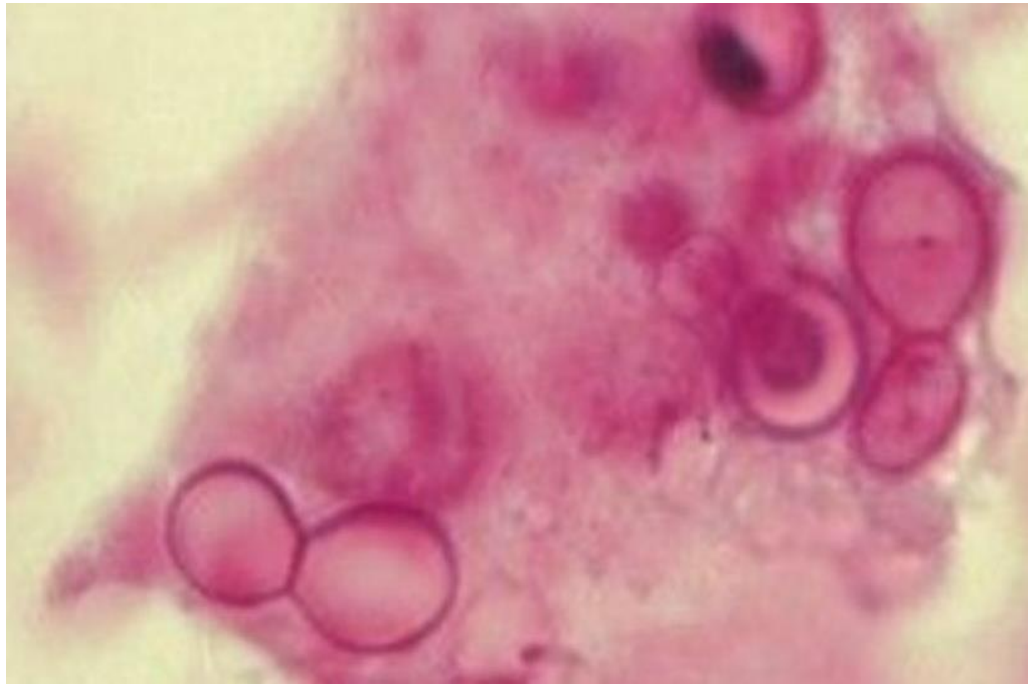
Do we see ≥10 squamous epithelial cells per LPF? **NO**

Do we see PMNs? **YES** This specimen is appropriate for culture.

Do we see bacteria?

Do we see something else…?

BACK TO THE CASE:



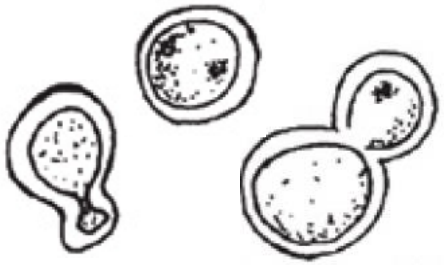
Sputum gram stain examined under 1000X with oil immersion.

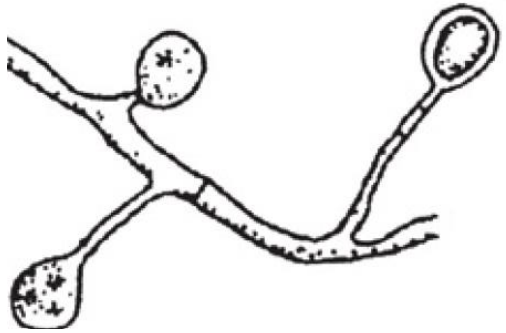
What are some words that we can use to describe what we are seeing on gram stain?

- Large (5-20um)
- Yeast form with broad-based budding
- Thick, doubly-refractile cell wall

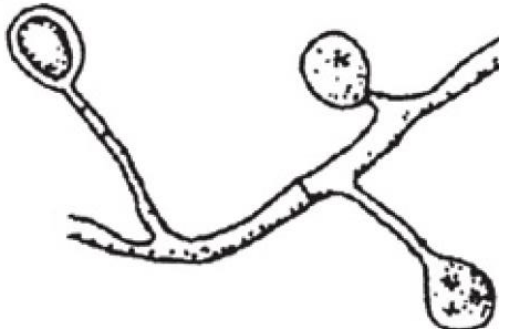
Presumptive ID: *Blastomyces* spp

LET'S "DEEPICT"





Blastomyces



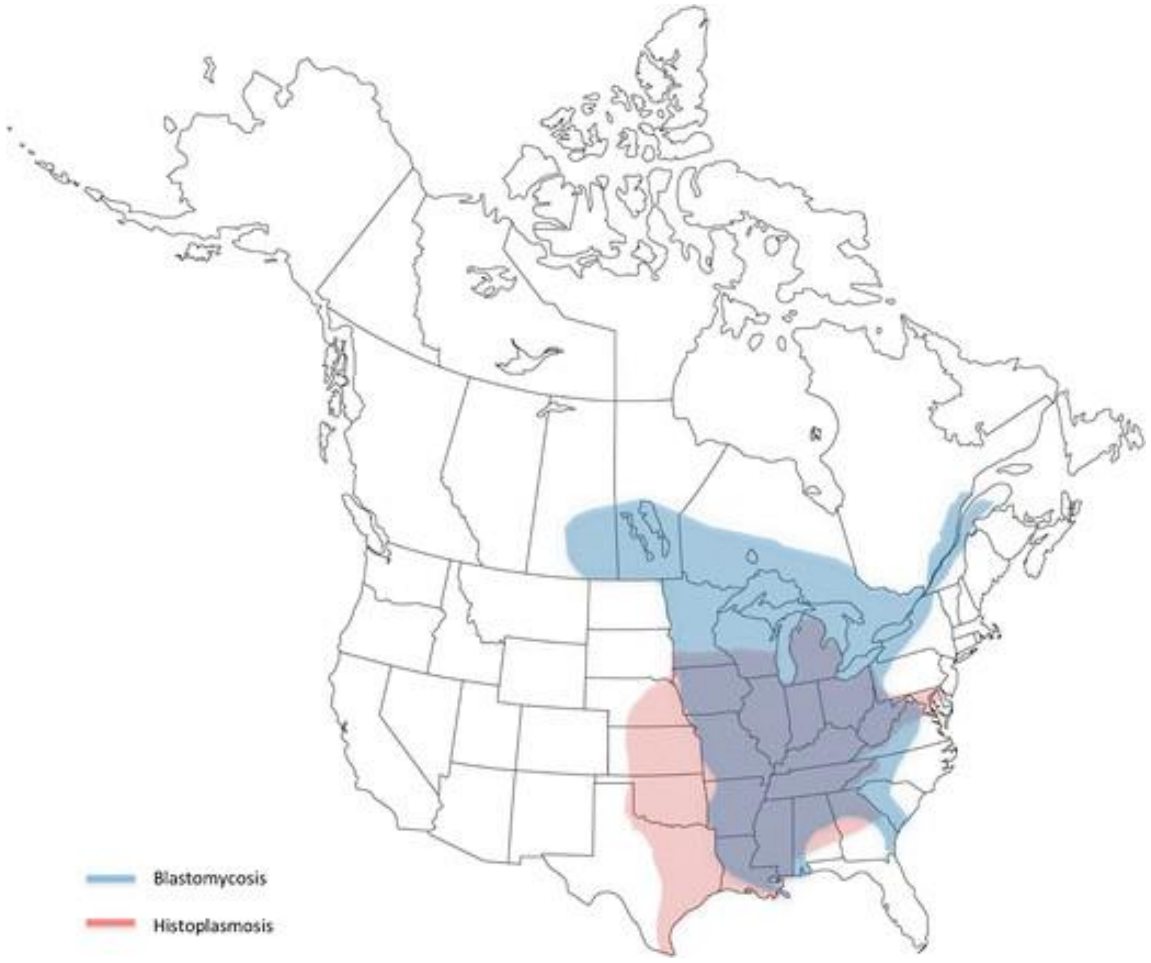
BLASTOMYCES:
Definition & Etiology

- *Blastomyces* spp. is a thermally dimorphic fungi
  - The causative agent of Blastomycosis
  - Most commonly: *B. dermatiditis* and *B. gilchristii*
  - Others: *B. helices, B. percursus, B. emzantsi,* and *B. parvus*

- Key feature: Mold in the cold (25ºC); Yeast in the heat (37ºC)
  - Other dimorphic genera of clinical relevance include *Histoplasma, Coccidioides,* and *Paracoccidioides*

BLASTOMYCES:
Epidemiology

- *B. dermatiditis/gilchristii* are endemic to North America, with predilection for areas that are warm, moist, acidic and rich in organic debris[1,2]
  - Mississippi River Valley
  - Great Lakes Region (USA and CAN)
  - St. Lawrence Seaway
  - Northwestern Ontario and Manitoba



DOI:10.1007/s12281-016-0260-7

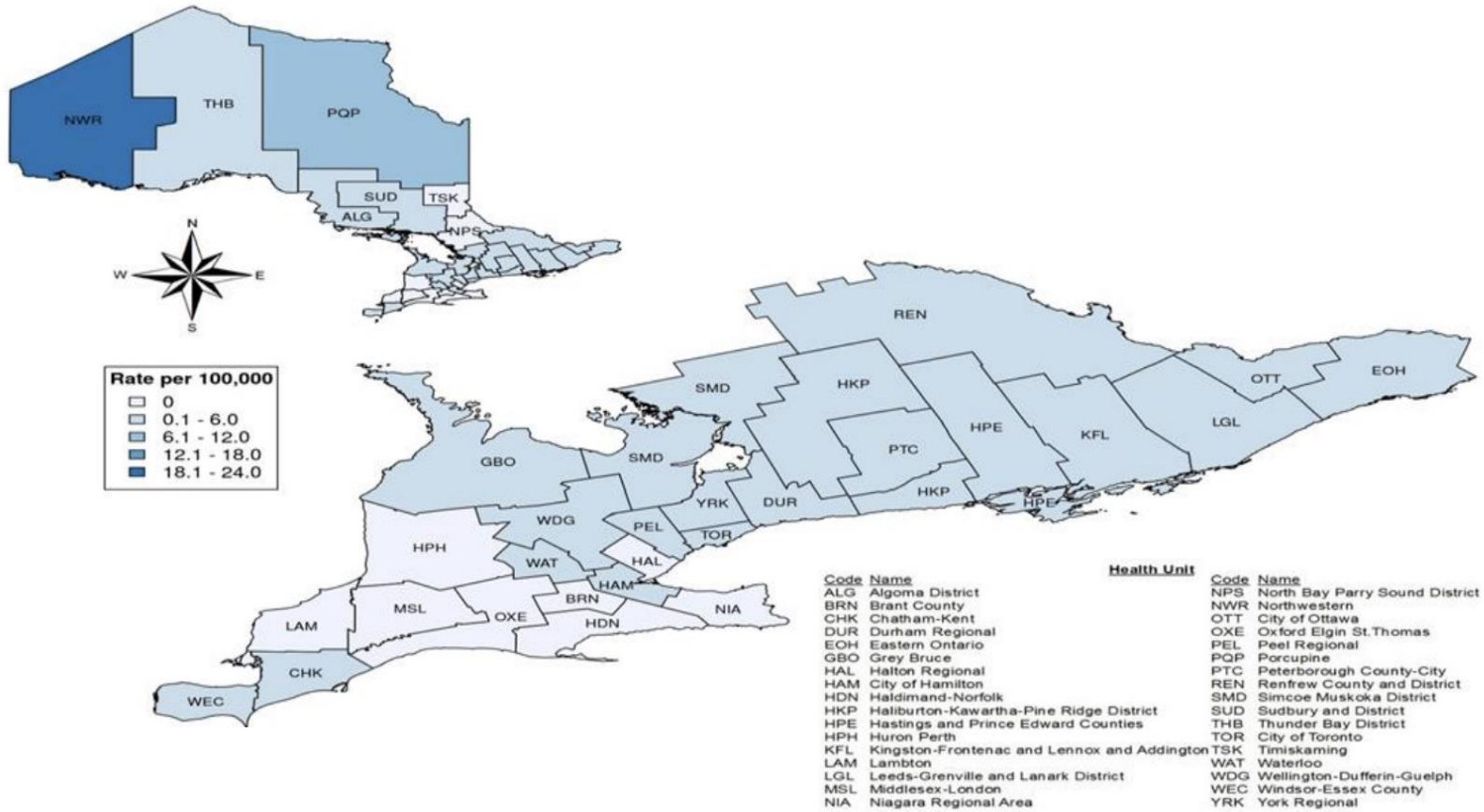
[1]We call these types of organisms "saprophytic"

[2]*Blastomyces* has also been identified in BC as well as in the Maritime provinces, and range is likely expanding due to climate change.

BLASTOMYCES:

Epidemiology

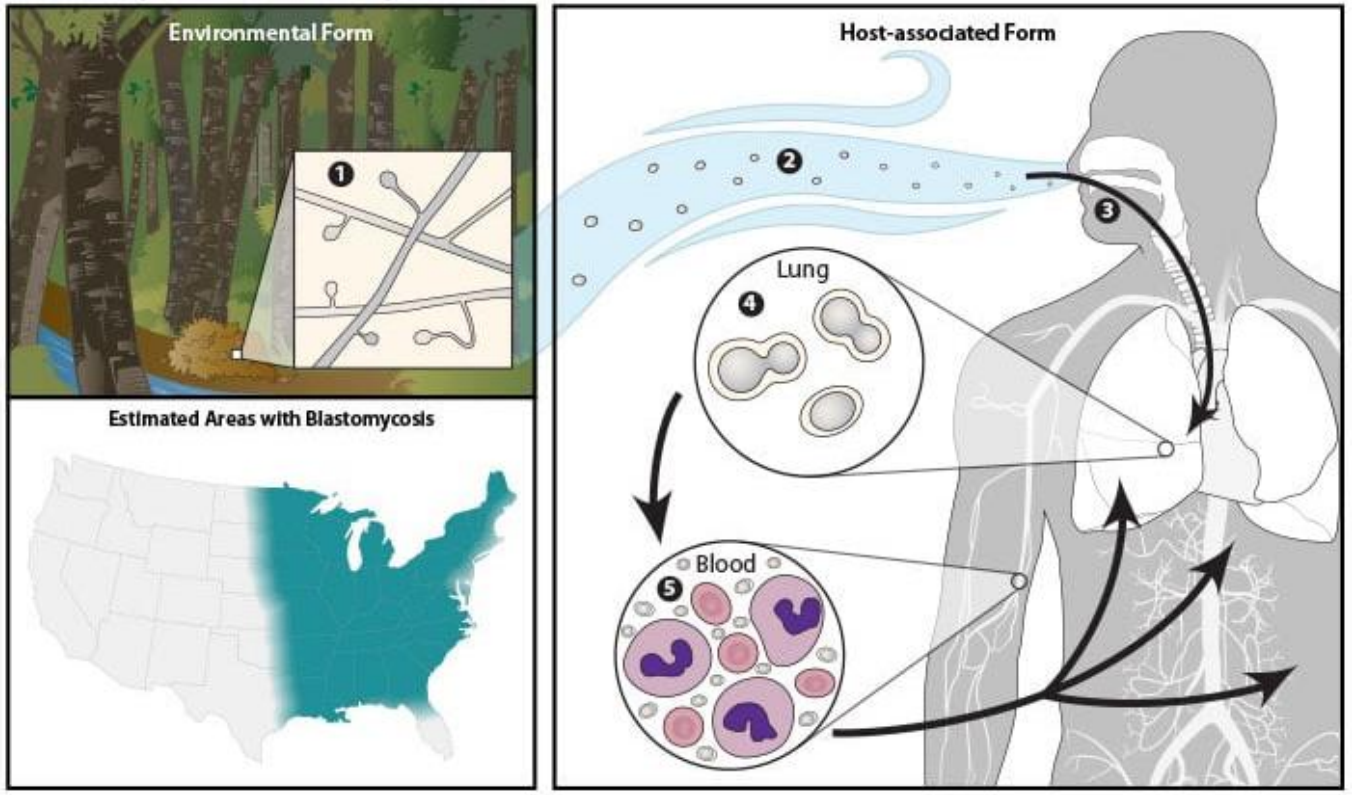
- Northwestern ON is hyperendemic, with rates exceeding 25 cases per 100,000 per year
- 0.4–1.3 cases per 100,000 per year elsewhere in North America[1]



[1]Khadilkar A, Waddell L, Acheson ES, Ogden NH. Perspectives on blastomycosis in Canada in the face of climate change. Can Commun Dis Rep 2024;50(11):400–11. https://doi.org/10.14745/ccdr.v50i11a04

BLASTOMYCES:

Pathogenesis



- Infection occurs via inhalation (and more rarely direct inoculation) of conidia from mold form
- Conversion to yeast form occurs once in body
- Dissemination can occur if immune response at primary site of infection is insufficient

Pathogen Safety Data Sheets: Infectious Substances – Blastomyces dermatitidis. Gov't of Canada. Available via: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/blastomyces-dermatitidis.html

BLASTOMYCES:

Pathogenesis

"My mom was diagnosed with pulmonary Blastomycosis. Does she need to self-isolate?"

Short answer – **NO**

- Human to human transmission of *Blastomyces* is exceedingly rare, but has been reported in the context of perinatal and sexual exposures.[1]
- The infectious form of this organism is the mold form via aerosolized conidia (more on this later)

[1]Pathogen Safety Data Sheets: Infectious Substances – Blastomyces dermatitidis. Gov't of Canada. Available via: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/blastomyces-dermatitidis.html

BLASTOMYCES:

Investigations and Lab Work-Up

- Fungal culture and direct examination (a.k.a. Microscopy)
- *Blastomyces* serology
- *Blastomyces* antigen enzyme-linked immunoassay (EIA)
- Molecular methods (e.g. PCR, sequencing)
- Histopathology

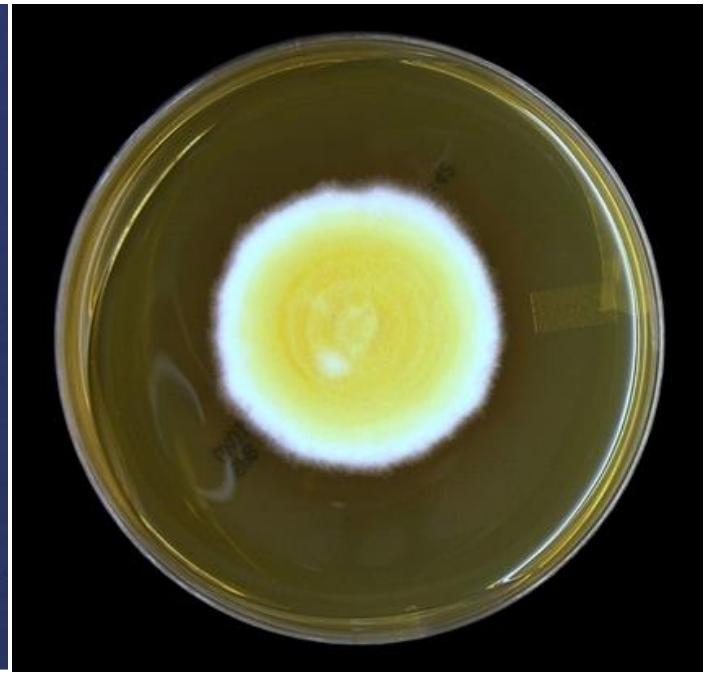
BLASTOMYCES:

Investigations and Lab Work-Up

- Fungal culture – some general principles:
  - **Temperature:** Optimal growth temperature for many clinically relevant fungi is 25-30°C, versus the 35-37°C for bacteria
  - **Time:** Growth on specialized agar may take up to 14-21 days, but many can be detected within 3-7 days.
  - **Media:** Often more acidic than bacterial isolation agars, with addition of sugars (e.g dextrose) and antibiotics to promote fungal growth while limiting bacterial overgrowth

BLASTOMYCES:
Investigations and Lab Work-Up

- *Blastomyces* spp.:
  - **Temperature:** Grown at 25–30ºC to main organism in mold form
  - **Time:** Slow; mycelial forms mature after 10 days
  - **Media:** Mold form can grow in the presence of cycloheximide, which can be added to common medias (e.g. Saboraud Dextrose agar, Brain-Heart Infusion agar)

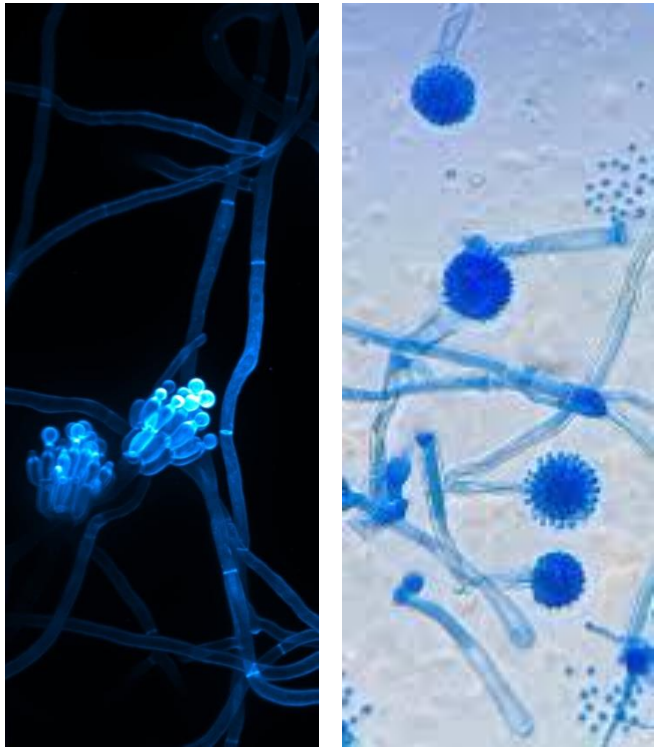


Images from Larone's Medically Important Fungi, 7th Ed. and the "Fun With Microbiology -What's Bugging You?" Blog.

BLASTOMYCES:

Investigations and Lab Work-Up

- Direct examination – some general principles:
  - **Size:** Much larger than bacteria; if you're not looking for it, you may miss it!
  - **Structure:** Aiming to identify the presence (or absence) and characteristics of specific structures (e.g. conidia, hyphae)
  - **Stains:** Visualization requires specific stains that enhance fungal cell features; different sample types may require different stains



BLASTOMYCES:
Investigations and Lab Work-Up

- *Blastomyces* spp. under the microscope:

	YEAST	MOLD
Stain	Primary specimen: • Gram stain • Calcofluor white fluorescent stain	Cultured organism[1]: • Lactophenol cotton blue
Size	Large, 8-15 µm in diameter	• Conidia are 2-10 µm in diameter • Hyphae are 1-2 µm in diameter
Key Ft's	• Oval to round • Broad-based budding (4-5 µm base) • Thick, "double-contoured," refractile cell wall	• Branched, septate hyphae • Single, small conidia with round or teardrop shape originating from short conidiophores (Lollipop appearance)

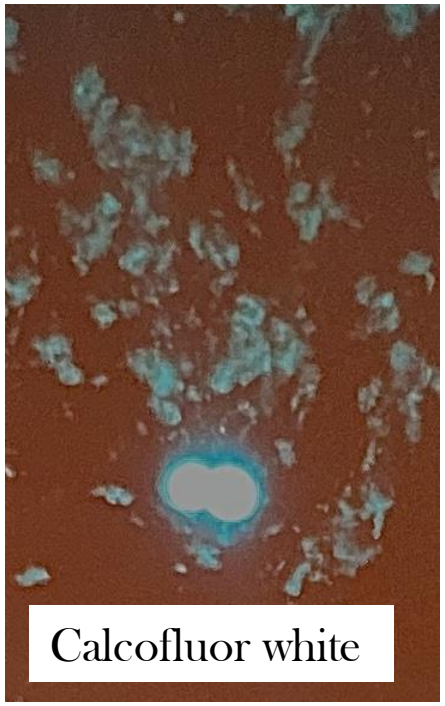
[1] Due to biosafety concerns for this organism, manipulation of cultures is never performed intentionally in a BSL-2 laboratory environment.

BLASTOMYCES:

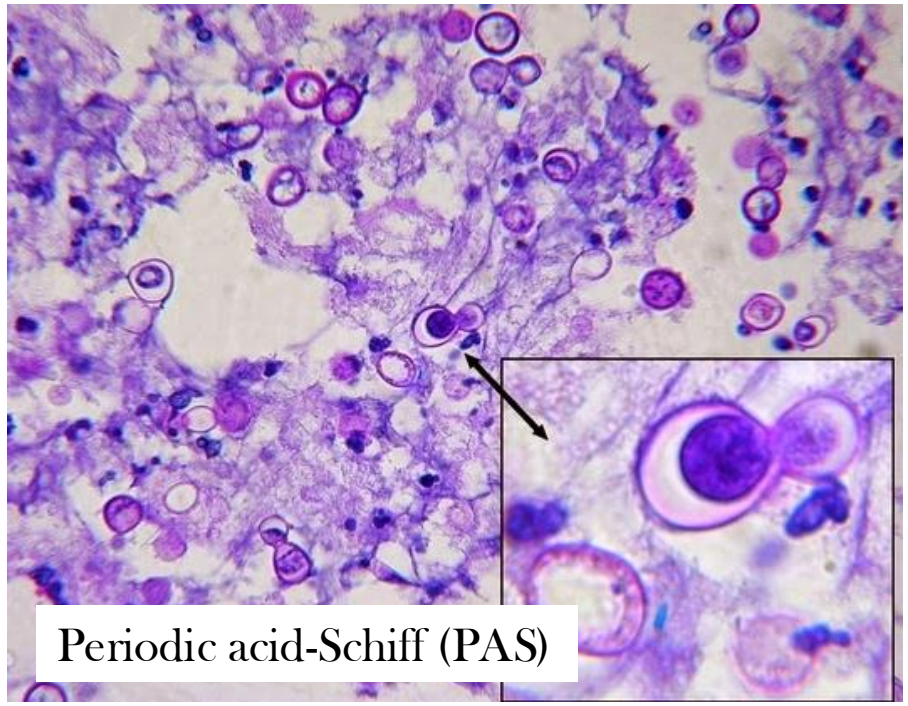
Investigations and Lab Work-Up

- *Blastomyces* spp. under the microscope:

Lactophenol cotton blue



Calcofluor white

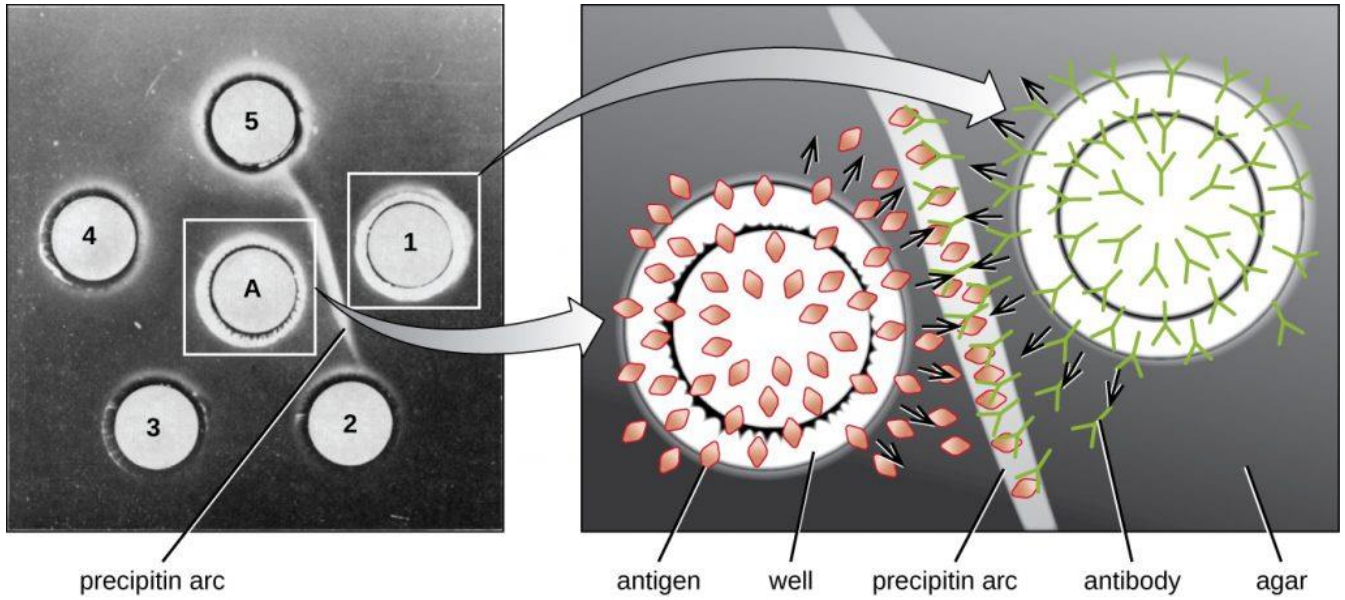


Periodic acid-Schiff (PAS)

Images from the "Fun With Microbiology -What's Bugging You?" Blog and my phone camera!

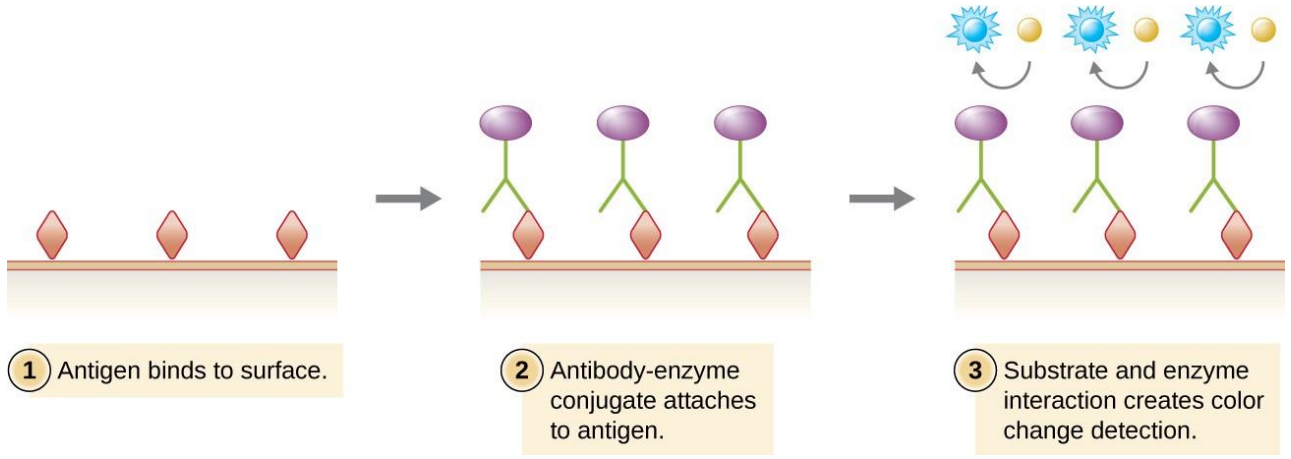
BLASTOMYCES:
Investigations and Lab Work-Up

- *Blastomyces* serology
  - Qualitative assay (Y/N) performed on serum only at our provincial reference lab
  - Immunodiffusion for detection of IgM and IgG
  - Specificity of 100% but Sensitivity of 32-80%



BLASTOMYCES:
Investigations and Lab Work-Up

- *Blastomyces* EIA
  - Quantitative assay (amount)
  - Performed by MiraVista Labs in the USA ($$$)
  - Requires Microbiologist approval
  - Specimen types include urine (most common), plasma, CSF, and BAL fluid



Target is the Blastomyces galactomannan antigen (cell wall component)
Complete cross reactivity with Histoplasma
Partial cross reactivity with Coccidioides and Aspergillus

BLASTOMYCES:

Clinical Presentation

Infection can be:

- Localized
- Disseminated
- Asymptomatic (50% of cases)

Sites of infection:

- Pulmonary
- Cutaneous
- Osteoarticular
- CNS (rare)

Symptoms:

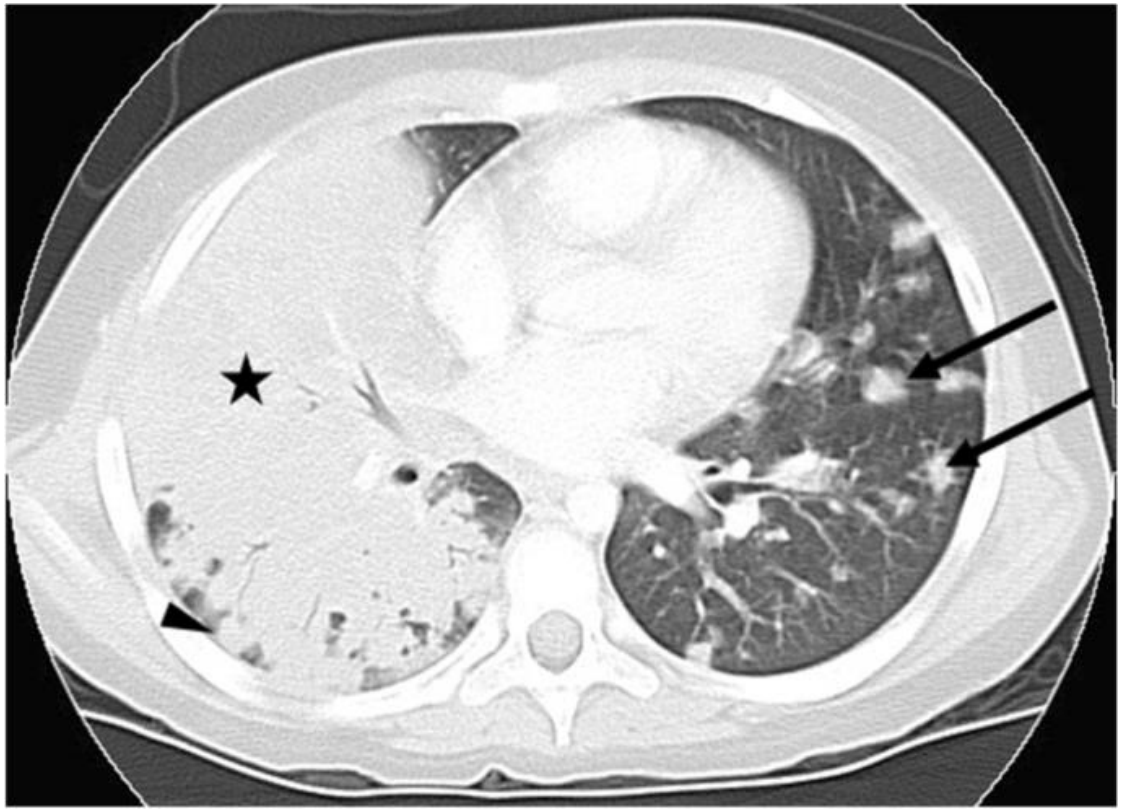
- **Resp:** Fever, cough (productive), dyspnea
- **Derm:** Nodules evolving into crusted ulcerations and verrucous lesions
- **Bone/Joint:** Pain, edema mimicking septic arthritis
- **CNS:** Focal neuro deficits, headache, confusion

IMPORTANT: Blastomycosis is often mistaken for malignancy! If in doubt, ask that fresh tissue is sent to the Microbiology lab for fungal culture.

Although these are the most common presentations, visceral involvement has also been reported.

BLASTOMYCES:

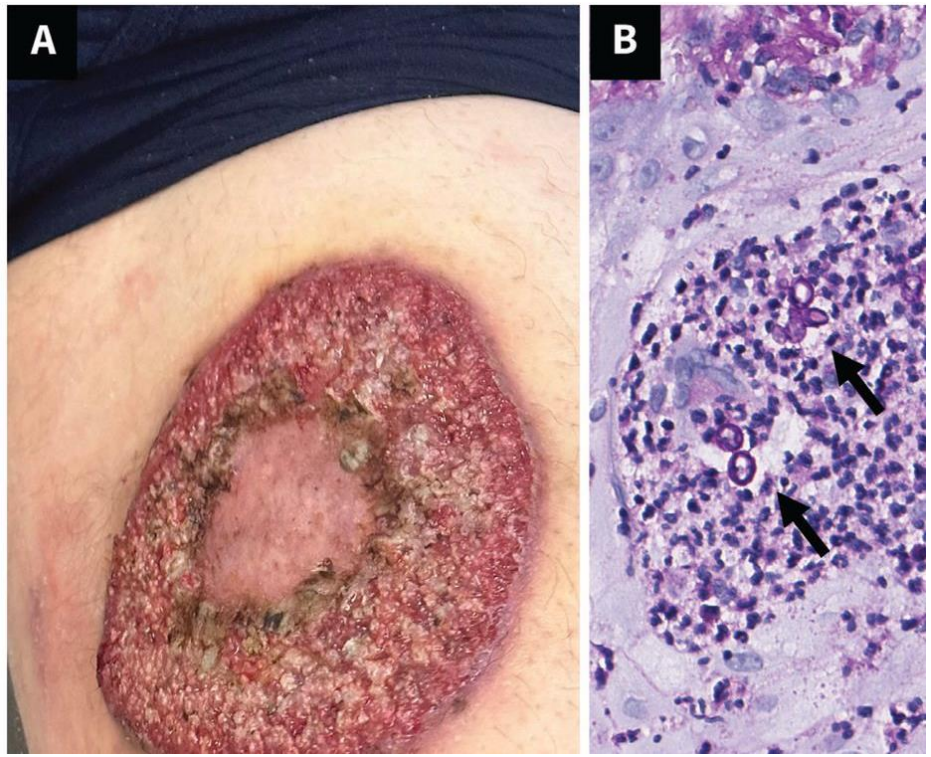
Clinical Presentation – Pulmonary

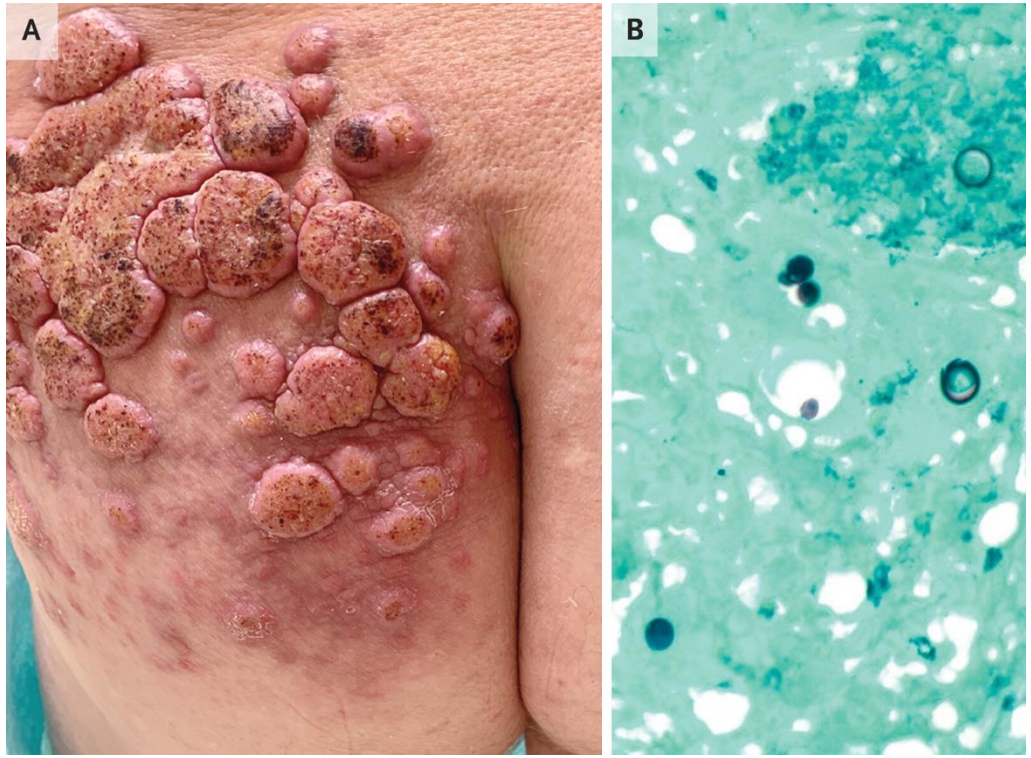


https://doi.org/10.1007/s00247-022-05402-x; https://doi.org/10.1016/j.mmcr.2024.100655

BLASTOMYCES:

Clinical Presentation – Dermatologic

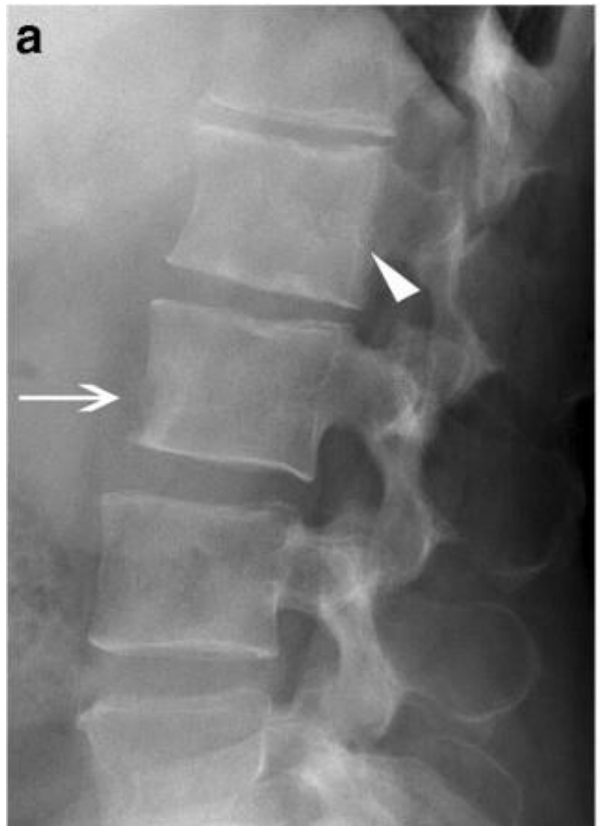


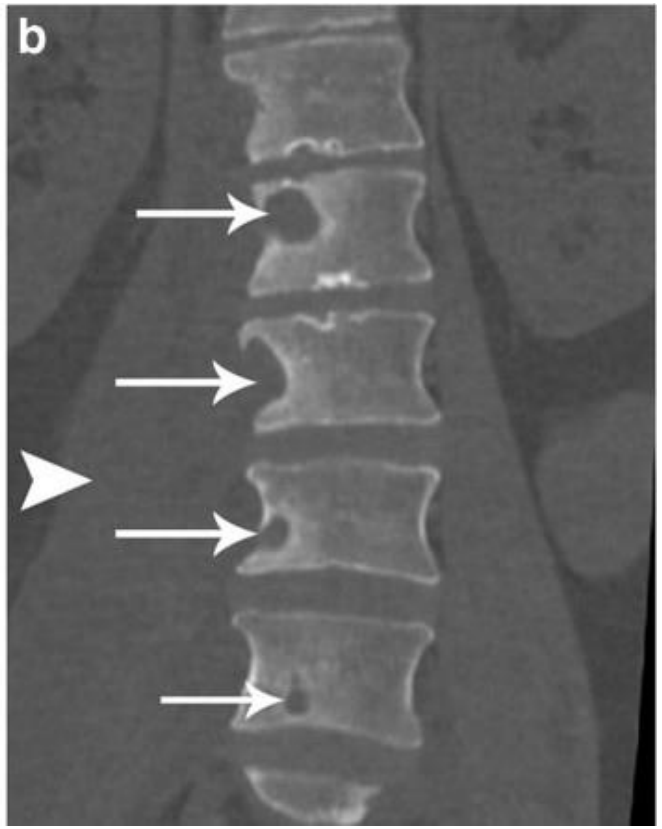


DOI: https://doi.org/10.1503/cmaj.241680; DOI: 10.1056/NEJMicm2116178

BLASTOMYCES:

Clinical Presentation – Osteoarticular

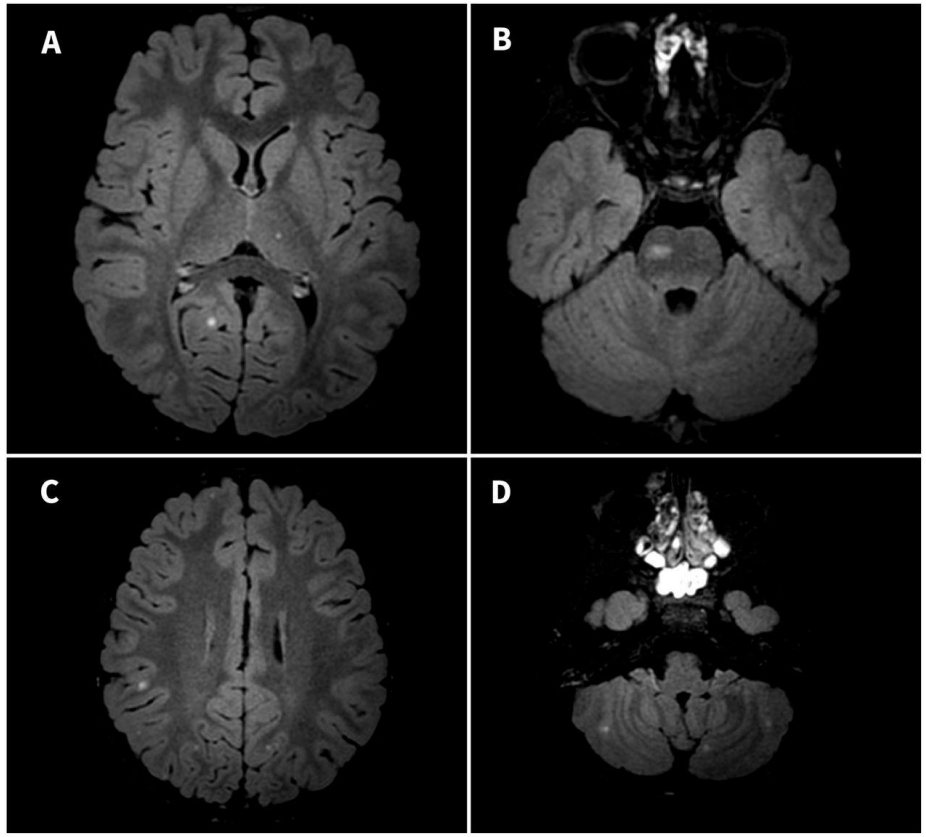


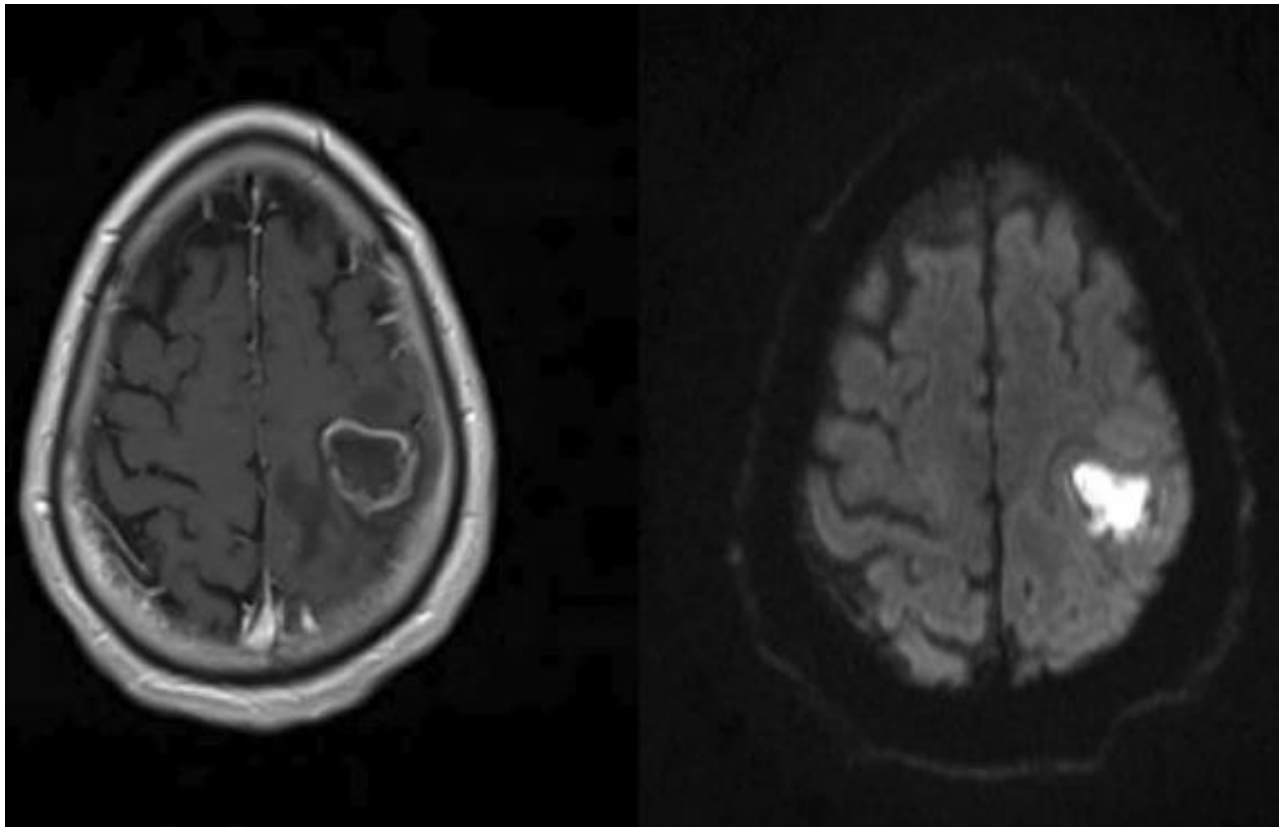


https://doi.org/10.1177/2473011423116666; https://doi.org/10.1007/s00256-019-03234-9

BLASTOMYCES:

Clinical Presentation – CNS



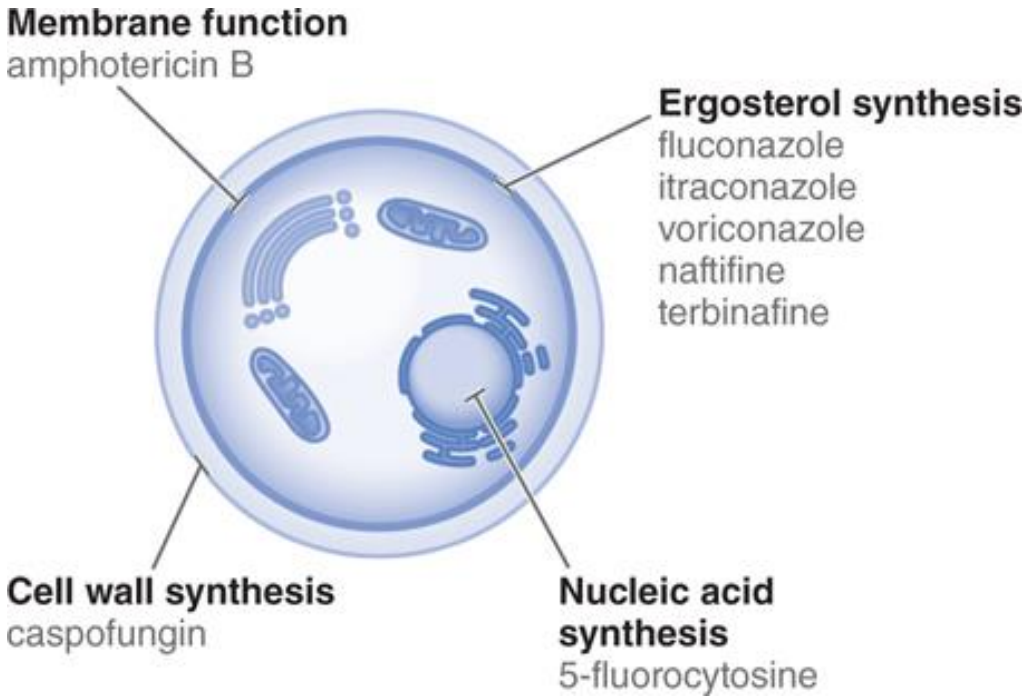


DOI: https://doi.org/10.1503/cmaj.241332; DOI: 10.1177/19418744419829276

BLASTOMYCES:

Treatment

- Dependent on severity of disease and anatomical site:
  - Oral itraconazole for 6-12 months for mild-to-moderate disease
  - Intravenous Liposomal Amphotericin B until clinical improvement for severe disease and/or CNS involvement , followed by step-down therapy with itraconazole for 6-12 months
  - Use of voriconazole as step-down therapy is only approved for treatment of CNS disease



Clinical Infectious Diseases, Volume 46, Issue 12, 15 June 2008, Pages 1801–1812, https://doi.org/10.1086/588300

BLASTOMYCES:

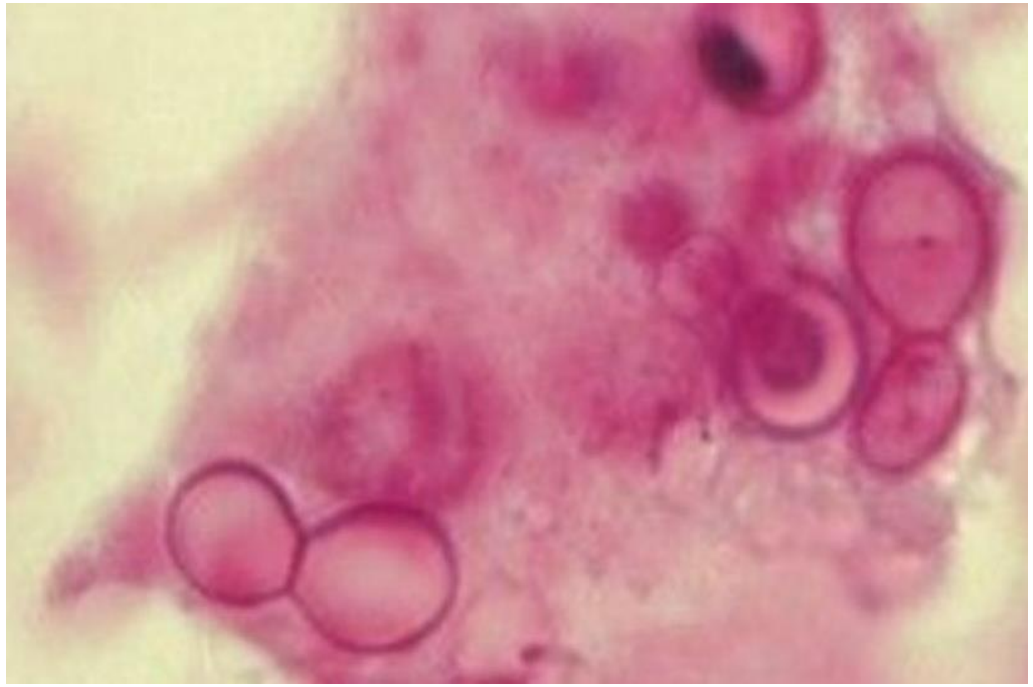
Treatment

"But how to we know it is susceptible?"

- Antifungal susceptibility testing is not routinely performed on *Blastomyces* spp. due to a lack of antifungal **breakpoints**
  - Breakpoints are minimum inhibitory concentration (MIC) values that have been demonstrated to correspond to clinical success or failure for a specific bug/drug combination and can therefore be used to infer "susceptible" or "resistant."

- The Microbiology lab and Infectious Disease physician may instead choose to use epidemiologic MIC data to hypothesize whether a certain agent may or may not be useful

BACK TO THE CASE:



Sputum gram stain examined under 1000X with oil immersion.

What actions should be taken in the Microbiology lab?

LABORATORY BIOSAFETY:

Some definitions

Biosafety: Containment principles, technologies, and practices that are implemented to prevent unintentional exposure to regulated materials, and their accidental release.

Containment: The physical enclosure within which specimens/pathogens are being worked on. Generally, the containment level and risk group of a pathogen are the same (e.g., RG2 pathogens are handled at CL2); with some exceptions.

Note: The only CL-4 lab in Canada is at the National Microbiology Lab in Winnipeg. It also has a clean CL-4 setup for training!

Canadian Biosafety Standard, Third Edition. Public Health Agency of Canada 2022-11.

LABORATORY BIOSAFETY:

Some definitions

Risk group (RG)
The classification of a biological agent (i.e., microorganism, protein, nucleic acid, or biological material containing parts thereof) based on its inherent characteristics, including pathogenicity, virulence, communicability, and the availability of effective prophylactic or therapeutic treatments.

The risk group describes the risk to the health of individuals and the public, as well as the health of animals and the animal population.

Canadian Biosafety Standard, Third Edition. Public Health Agency of Canada 2022-11.

LABORATORY BIOSAFETY:

Some definitions

Risk assessment (lab context): Site or procedure specific activity to identify and characterize hazards associated biological material, assess risk of each hazard to determine potential for exposure and develop and implement mitigation measures.

Risk assessment required prior to sample processing: patient symptoms, exposure history. Staff in lab reception flag high-risk samples upon receipt to minimize manipulation outside containment.

Other procedural opportunities for precaution during analytic phase

Canadian Biosafety Standard, Third Edition. Public Health Agency of Canada 2022-11.

RISK GROUP	INDIVIDUAL RISK	PUBLIC HEALTH RISK	INFECTION PREVENTION	EXAMPLES
1	Microorganism, nucleic acid, or protein that is not capable of or unlikely to cause human or animal disease	None	Not required	Non-pathogenic E. coli
2	Pathogen or toxin that poses moderate individual risk. are able to cause serious disease in a human or animal but unlikely to do so. and the risk of spread of diseases caused by these pathogens is low.	Low risk of spread of diseases caused Low public health/ community risk	Effective treatment and preventive measures are available	Influenza, RSV, S. aureus, Hepatitis A
3	High individual/ animal risk causing severe disease. Effective treatment and preventive measures are usually available	Low public health risk Low to high animal spread risk	Do not spread easily. Require strict precautions to prevent exposure. Effective treatment available.	Mycobacterium tuberculosis, HIV
4	High individual threat, cause severe disease and pose a high risk of transmission.	High risk of transmission.	Highest level of containment precautions necessary.	Ebola virus Marburg virus

Type of risk	Risk Group 1	Risk Group 2	Risk Group 3	Risk Group 4
Individual risk	Low	Moderate	High	High
Community risk	Low	Low	Low	High

BACTERIA

Bacillus anthracis
Brucella abortus, melitensis
Burkholderia mallei, pseudomallei
Chlamydophila psittaci
Coxiella burnetii
Francisella tularensis
Mycobacterium africanum, bovis, microti, tuberculosis
Orientia tsutsugamushi
Rickettsia japonica, prowazekii, rickettsia & typhi
Yersinia pestis

VIRUSES

Chikungunya virus
Eastern equine encephalitis virus
Hantaan orthohantavirus
HIV 1 & 2
Japanese encephalitis virus
Louping ill virus
Lymphocytic choriomeningitis mammarenavirus
Monkeypox virus
Mucambo virus
Murray Valley encephalitis virus
Powassan virus
Virus Powassan
Rabies lyssavirus
Severe acute respiratory syndrome-related coronavirus
St. Louis encephalitis virus
Venezuelan equine encephalitis virus
Western equine encephalitis virus
West Nile virus
Yellow fever virus

FUNGI

Blastomyces dermatitidis
Cladophialophora bantiana
Coccidioides immitis
Coccidioides posadasii
Cryptococcus gattii
Histoplasma capsulatum
Paracoccidioides brasiliensis

PARASITES

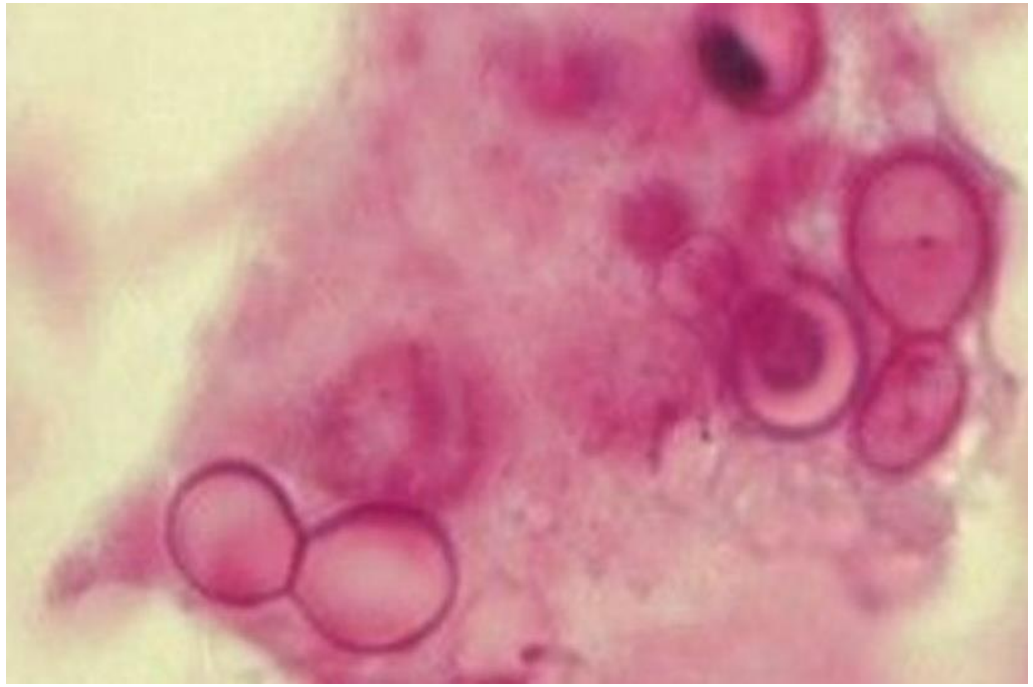
ONLY 1- Naegleria fowleri
AKA Brain eating amoeba

PRIONS

Bovine spongiform encephalopathy agent
Creutzfeldt-Jakob disease agent
Kuru agent
Variant Creutzfeldt-Jakob disease agent

Human Pathogens Act, Canada. Subsections 31(1) and (2) June 1, 2009. https://laws.justice.gc.ca/eng/acts/h-5.67/page-8.html. Last modified Nov 10, 2025

BACK TO THE CASE:



Sputum gram stain examined under 1000X with oil immersion.

What actions should be taken?

- Stop all open benchwork on specimens from this patient.
  - Wrap plates in parafilm and incubate offline
- Place RG3 flag on patient chart
- Assess whether a laboratory exposure has occurred
  - No PEP, but exposed individuals can self-monitor
- Fungal cultures should be sent to PHOL for definitive identification

CASE WRAP-UP:

The "Post-Analytical":

After reviewing the gram stain, you call the ICU staff and consulting Infectious Disease specialist:

"We are seeing fungal structures that are consistent with broad-based budding yeast. This will be reported out shortly, but this is most consistent with a *Blastomyces* spp. We will be sending these cultures to PHOL, and we expect to get a definitive ID in about 7-10 days."

CASE WRAP-UP:

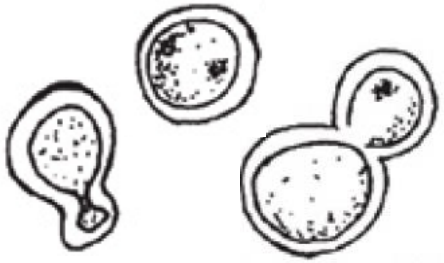
The "Post-Analytical":

8 days later, a fax arrives from PHOL:

"*Blastomyces dermatiditis* isolated."

You also receive an email from the ID staff, who tells you that Ms. B has stabilized on Amphotericin B and was extubated 2 days ago. She is awaiting a bed on the ward, and will be transitioned to oral itraconazole before she is repatriated to her community hospital.

"Thank you again for reviewing her gram stain so quickly! This made a huge difference in her receiving appropriate care."


The End