



MEDICINE
KING SAUD UNIVERSITY

Microbiology

team 436



OSPE

"لا حول ولا قوة إلا بالله العلي العظيم" وتقال هذه الجملة إذا دهم الإنسان أمر عظيم لا يستطيعه ، أو يصعب عليه القيام به .

أول ثلاث سلايدات لمراجعة أساسيات المايكروبايولوجي

Introduction : Bacteria

How to differentiate between **GRAM POSITIVE** and **GRAM NEGATIVE** by gram stain ?

By color:

Blue \ purple

Red \ pink

It could be **coccus** or **bacillus**

It could be **coccus** or **bacillus**

eg.

- **Staphylococcus**
- **streptococcus**

→ Let's talk about them in the next slide

Introduction :

Gram positive cocci :

staphylococcus In clusters

streptococcus In chain or pairs

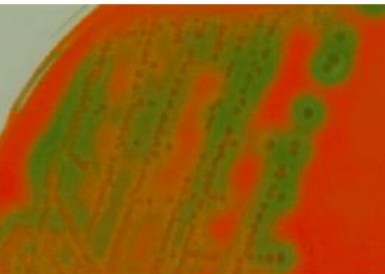

To differentiate between them we use catalase test


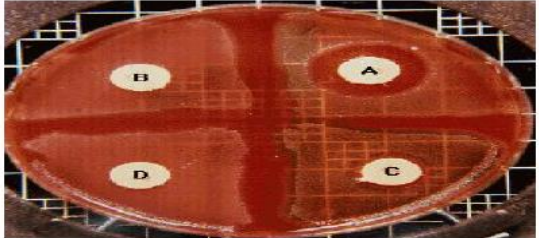
Bubbles :
(+ve) = staph



NO Bubbles:
(-ve) = strept





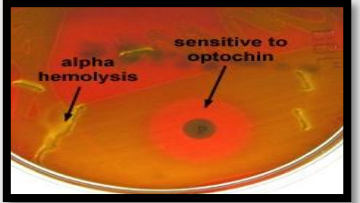

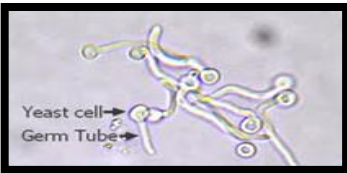
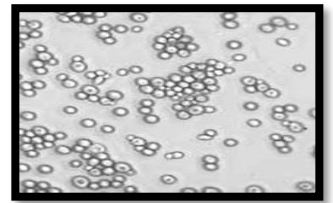
Streptococcus are divided into 2 :
(when we culture them in blood agar)

Alpha Hemolytic (α) → تُكون لون أخضر من حولها في الطبق		
Species	Further test	Result
<i>Streptococcus viridans</i> <i>Streptococcus pneumoniae</i>	We differentiate between them by using the "Optochin Test"	<i>Pneumoniae</i> = Sensitive <i>Viridans</i> = Resistant
		"المنسيف أو البوزيتيف دائماً هو المتفاعل"

Beta Hemolytic (β) → تُحلل الدم من حولها في الطبق وينتج عن ذلك ظل باللون الأبيض		
Species	Further test	Result
Group A (<i>Pneumonia</i>) Group B (<i>Agalactiae</i>) Group C	We differentiate between them by using the "Bacitracin Test"	Group A = Sensitive Group B and C = Resistant
		"لاحظ/ي تفاعل الـ A دون سواها من الأنواع"

Introduction :

Different Test Used in Lab .

Test	Use	Positive	negative
CATALASE TEST	To differentiate between <u>Staphylococcus</u> & <u>Streptococcus</u>	 Staphylococcus	 Streptococcus
BACITRACIN SUSCEPTIBILITY	To differentiate between <i>Streptococcus gp.A</i> & any other group in beta haemolysis Streptococcus spec.	 Group A Streptococcus Beta-hemolytic Sensitive to Bacitracin	 Beta hemolytic Bacitracin resistant Group B Streptococcus
OPTOCHIN SUSCEPTIBILITY (optochin disk)	To differentiate between <i>Streptococcus pneumoniae</i> & other alpha haemolysis Streptococcus spec.	 alpha hemolysis sensitive to optochin S. pneumoniae	
GERM TUBE TEST *for fungus	FOR IDENTIFICATION OF CANDIDA ALBICANS	 Yeast cell Germ Tube	

Out line:

- Case 1: Pharyngitis – group A streptococcus
- Case 2 : Pneumonia - Streptococcus Pneumoniae.
- Case 3: otitis media - Haemophilus influenzae
- Case 4: Diphtheria - Corynebacterium diphtheriae
- Case 5: Pulmonary TB
- Case 6: Candida albicans
- Case 7: Aspergillus niger

Case 1: A 5 year boy was brought to KKUH, outpatient department complaining of **fever** and **sore throat**. He had regular vaccination history. On examination his temp. was **38.5°C**, the tonsil area and pharynx were obviously inflamed with some **foci of pus**.

• What is the differential diagnosis?*

✓ Acute Pharyngitis and/or Tonsillitis – virus infection

• What investigation should be done?

✓ Throat swab.

✓ Catalase test.

✓ Culture of the throat swab on blood agar. هو الأدق لكن يحتاج وقت

✓ Rapid Antigen Detection Test (RADT). ** (false negative) الأسرع لكن مو دائما يعطي النتيجة الصحيحة.

✓ Bacitracin susceptibility test.

✓ Gram stain of the throat swab.(microscope). في حالة الثروت سواب غير مفيد بسبب وجود النورمل فلورا



* (differential) دامه قال *
فنذكر كل الممكن بسبب هذه
الاعراض

إذا سوينا الاختبار وطلع بوزيتف فهو **
قروب أي ستربت ونعالج وخلص
أما إذا سويناها وطلعت النتيجة فولز
فنظل مو متأكدين ونسوي الكلتشر عشان
نتأكد هو قروب اي سترب او لا

Case 1:

The tests showed :

TEST	RESULT
• CULTURE ON BLOOD AGAR	Beta haemolysis (colonies surrounded with clear zone of haemolysis)
• CATALASE TEST	No bubbles → catalase negative *
• GRAM STAIN FROM CULTURE	gram positive cocci in chains *
• BACITRACIN SUSCEPTIBILITY TEST	Bacitracin Susceptible colonies



CULTURE ON BLOOD AGAR



CATALASE TEST



GRAM STAIN FROM CULTURE



BACITRACIN

- What is the likely identity of the organism?
 - ✓ Beta haemolytic Group A Streptococcus. (streptococcus pyogenes)
- What is the best antibiotic therapy for this child?
 - ✓ Penicillin for 10 days. If allergic, use Erythromycin.
- what complication may this child have after 6 weeks period **If not treated ?**
 - Rheumatic fever.
 - Acute glomerulonephritis.

كلا الاختبارين يعطوني نفس المعلومة اللي هي انه ستريبت لكن الكاتليز أدق*

Case 2: A 28 Year Old Female presented to the accident and emergency of KKUH with a sudden onset of **fever, right sided chest pain and productive cough** of purulent sputum. On examination her temperature was 39 °C. There **were Rhonci and dullness** on the right side of the chest X-ray showed massive **consolidation** on the right side of the chest.

- **What is the differential diagnosis?**
 - ✓ Streptococcus Pneumoniae, S.aureus, Haemophilus influenzae All cause Chest infection (Lobar pneumonia).
- **What investigation should be done?**
 - ✓ CBC.
 - ✓ Gram stain from sputum .
 - ✓ Culture of The sputum on blood agar.
 - ✓ catalase test.
 - ✓ Optochin susceptibility test.
 - ✓ Antibiotic susceptibility test

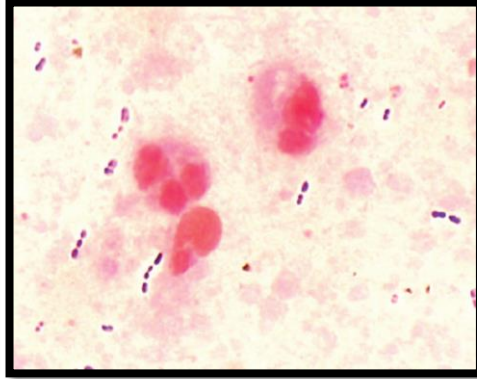
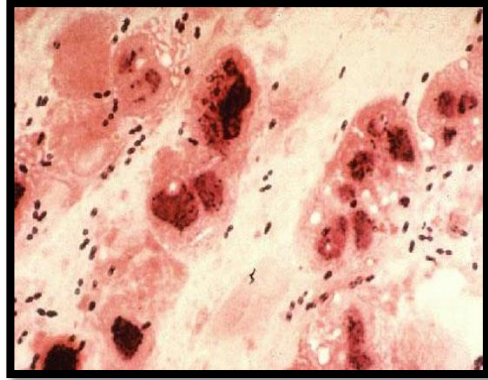
The chest X- ray done showed massive consolidation on the right side of the chest. →

- **What is the most likely organism?**
 - ✓ Streptococcus Pneumoniae.
- **What should have been the empirical therapy for this case and why?**
 - ✓ Ceftriaxone+ Vancomycin.
 - Because the organism may be Penicillin resistant.

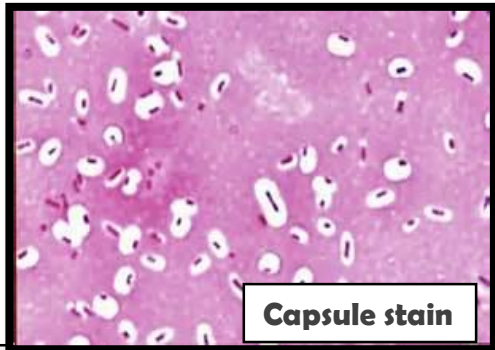


▶▶ MICROSCOPIC APEARANCE

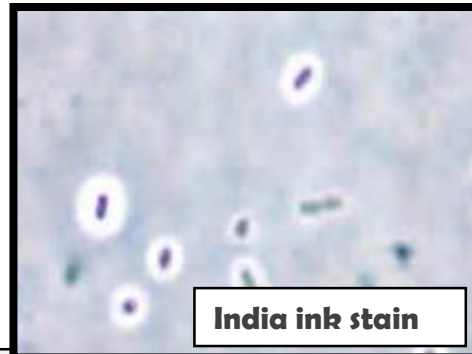
Gram stain From sputum showed :
Gram positive diplococci (arranged in piers) +
pus cells



Negative Stains showing capsule:



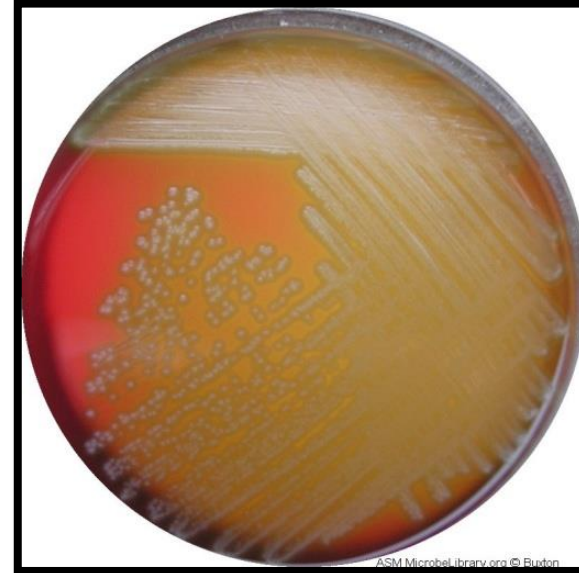
Capsule stain



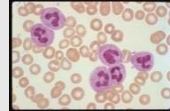


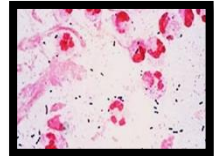
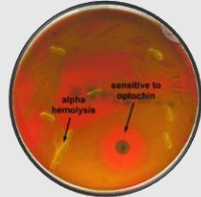
India ink stain

▶▶ culture

Sputum culture showed:
Alpha haemolysis on blood agar
(colonies surrounded by partial
haemolysis with greenish color).



Lab. Tests Results(Summary)

TEST	Result	
CBC	45,000/ ml 90% of the cells were neutrophils	
CULTURE ON BLOOD AGAR	Alpha haemolysis	
CATALASE TEST	No bubbles → catalase negative	
GRAM STAIN	gram positive diplococci in pairs	
Optochin SUSCEPTIBILITY TEST	Optochin Susceptible colonies	

Streptococcus pneumoniae (Pneumococcus)

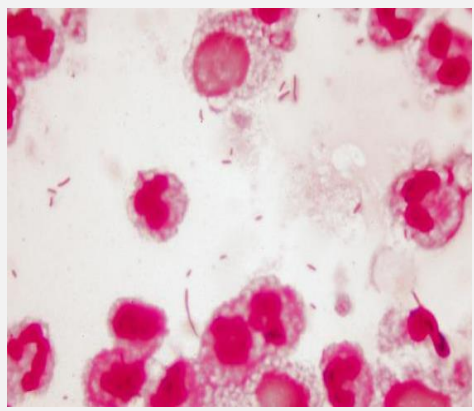
Case 3: A 3-year-old girl is brought to your office by her mother because she has a **fever** and complains that her **ear hurts**. She has no significant medical history. Her temperature is **38.8°C**, **injected tympanic membranes**.

- What is the differential diagnosis?
 - ✓ Haemophilus influenzae , S. arouse, Streptococcus Pneumoniae....(all cause otitis media)
- What investigation should be done?
 - ✓ Gram stain from ear discharge .
 - ✓ Culture of The specimen on blood and mackonckey agar.
 - ✓ Biochemical tests
 - ✓ Antibiotic susceptibility test

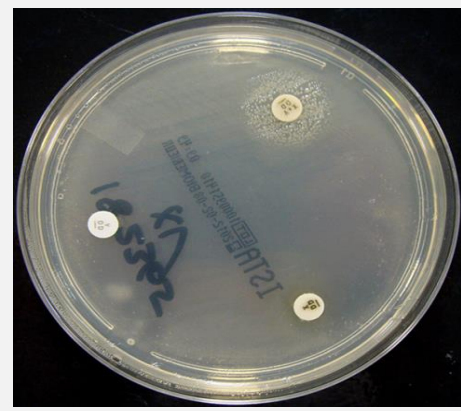
* دامه قال
(differential)
فنذكر كل الممكن يسبب
هذه الاعراض

The tests showed :

TEST	RESULT
• GRAM STAIN FROM EAR DISCHARGE	gram negative coccobacilli
• Nutrient agar with X and V factors:	Haemophilus influenzae grow around the disc containing X and V factors



GRAM STAIN



Nutrient agar with X and V factors

تنمو فقط في التشوكلت آغار, لأنها تحتاج مكونات موجودة فيه فقط(اكس وفي) Haemophilus influenzae
هذا اغار لا يحتوي شيء فقط نضع فيه الديسكز Nutrient agar

Case 4: A 5 year-old boy attended to the emergency department complaining of **sore throat** , **fever (38.5°C)**, and a noticed **pharyngeal pseudomembrane**

• What is the differential diagnosis?

✓ Diphtheria, 😊 اذكروا أي شيء آخر

What investigation should be done?

✓ Gram stain From culture.

✓ Throat swab culture on blood tellurite.

✓ ELEK's test

• What is the likely identity of the organism?

✓ *Corynebacterium diphtheriae*

• What is the best antibiotic therapy for this child?

✓ Anti-toxin

✓ Penicillin , If allergic, use Erythromycin.

• What complication may this child develop?

✓ Local complication (descent of pseudomembrane)

✓ Cardiac failure

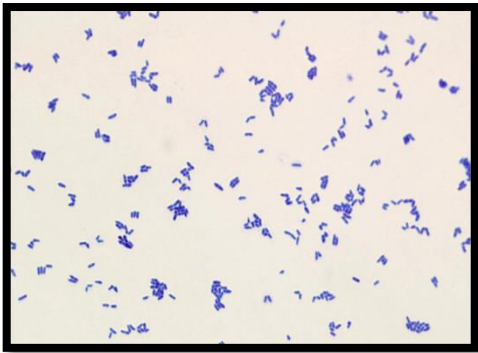
✓ adrenal infraction



▶▶ MICROSCOPIC APPEARANCE

Gram stain From culture showed :

Gram positive bacilli (chains' litter appearance)

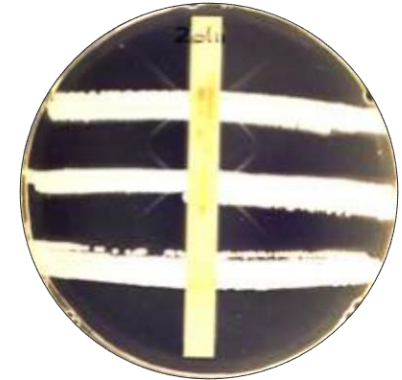
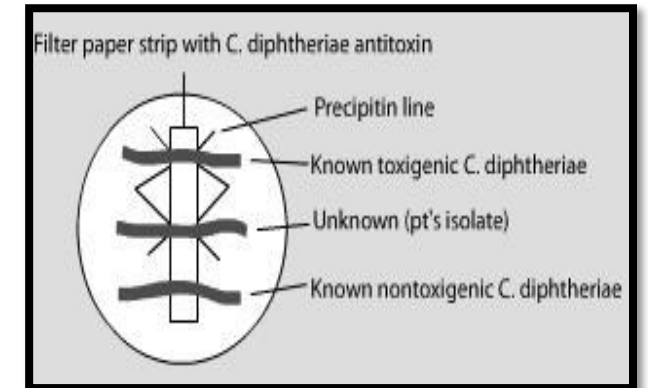


▶▶ culture

Throat swab culture on blood tellurite showed: Black color colonies



▶▶ ELEK TEST



Toxin from culture of *C. diphtheriae* diffused and react with the diphtheria antitoxin defused from strip and produce precipitation lines → which demonstrate positive test (Diphtheria exotoxin production)

Corynebacterium diphtheriae

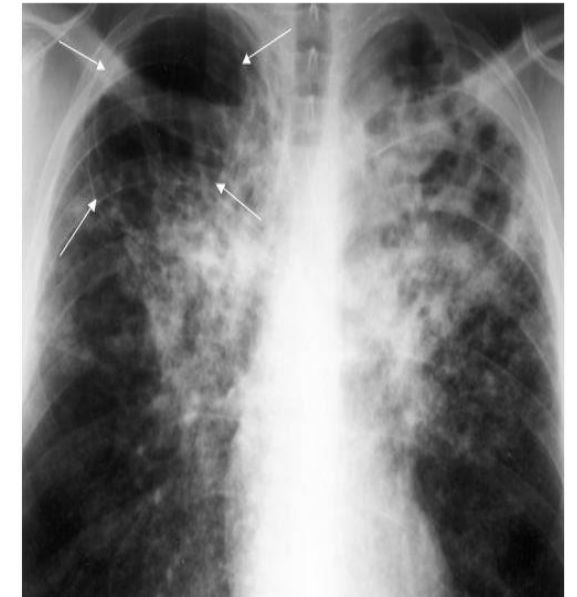
Case 5:

Abdul Karim is a 45 year old Saudi man who was admitted to KKUH because of 2-3 month history of **loss of appetite, weight loss**, and on and off **fever** with attacks of **cough**. On examination Abdul Karim looked weak with a temperature 38.6 °C, CVS and Respiratory system examination was unremarkable. two days before admission .he **coughed blood (haemoptysis)**, Abdul karim is diabetic for the last 5 years. His father died of tuberculosis at the age of 45 yr

- What is the differential diagnosis?
 - ✓ Chronic Pulmonary infection (TB, virus, fungal)
- What investigation- tests should be done?
 - ✓ X-ray
 - ✓ Sputum Microscopy: Ziehl-Neelsen stain (Shows Acid Fast Bacilli)
 - ✓ Culture: Growth on L.J medium (Selective for Mycobacteria)

The chest X- ray done showed multiple opacities and cavities.
The ESR was increased (85 m /hour).

- What is the probable diagnosis?
 - ✓ Pulmonary TB
- How can the diagnosis be confirmed?
 - ✓ Measurement of Interferon–Gamma (IFN γ).
 - ✓ If the morphology on LJ media showed buff rough and tough colonies.
 - ✓ If the growth occurred at 37°C and produced 5-10%CO₂.

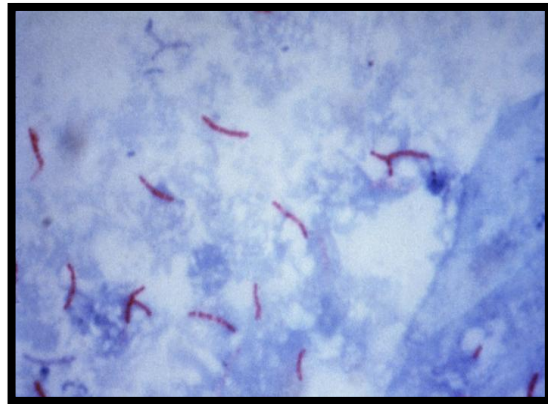
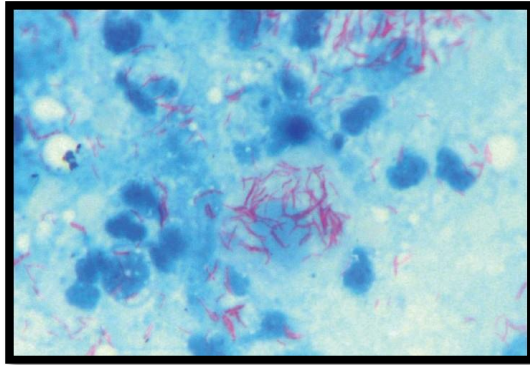


The chest X- ray done showed multiple opacities and cavities

Figure 8. Chest x-ray with bilateral upper lobe opacities (white areas) with multiple cavities including a very large cavity in the right upper lobe (arrows).

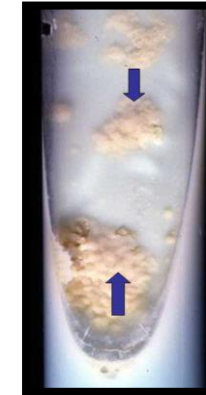
» MICROSCOPIC APPEARANCE

Ziel – Neelsen Stained Smear
From Sputum Showing:
Acid – Fast Bacilli (AFB)



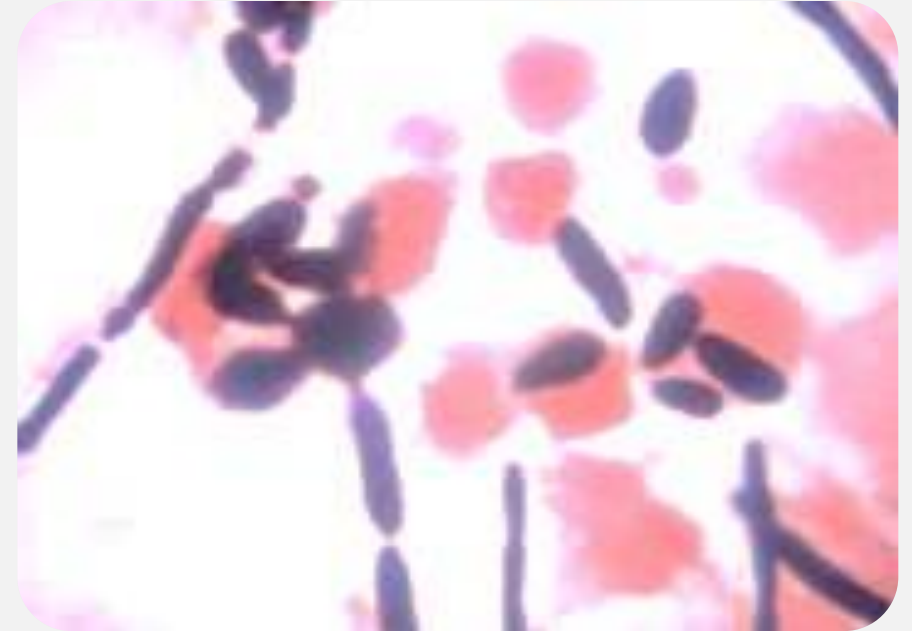
» culture

Sputum culture on Lowenstein–Jensen medium (selective for mycobacteria) showed:
showing growth of Rough, Tough and Buff colonies



Case 6: A 45-year old women who underwent bilateral **lung transplant** developed **fever** and **respiratory failure** 4 days post-operatively. She received immunosuppressive therapy. Gram stain of lung tissue biopsy shown below figure:

1. What is the differential diagnosis?
 - ✓ *Candida albicans*
2. What investigation should be done?
 - ✓ Gram stained of lung tissue.
 - ✓ Culture from sputum on SDA.
 - ✓ Germ tube test.
 - ✓ chlamydospore test.

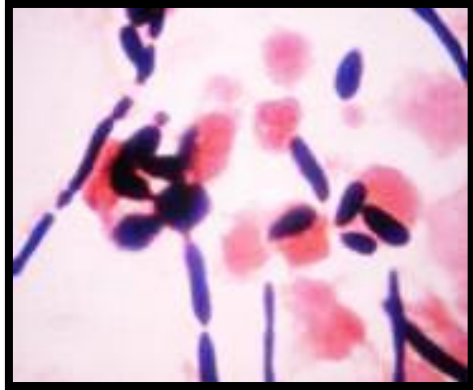


✓ *Candida albicans*

▶ MICROSCOPIC APPEARANCE

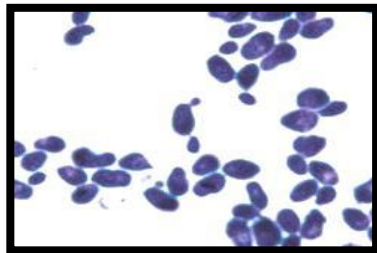
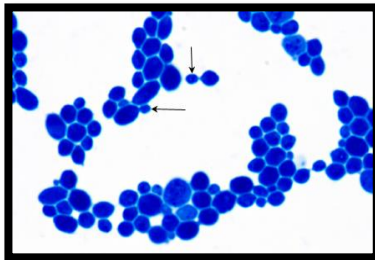
1- Gram stained of lung tissue showed :

Budding yeast cells & pseudohyphae.



2- Gram stain From culture showed :

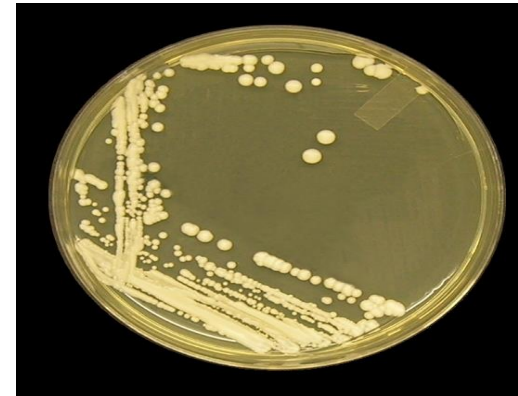
Gram positive oval budding yeast cells.



▶ culture

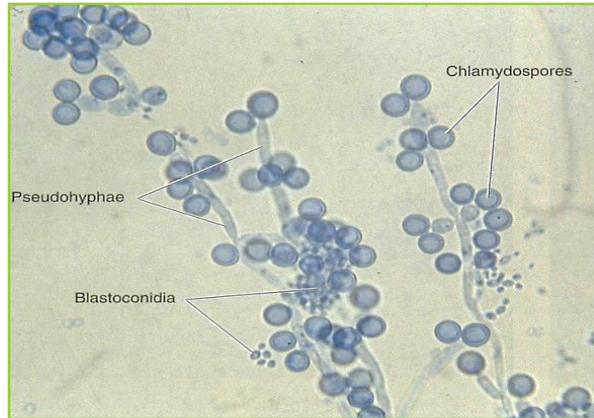
Culture from sputum on SDA (Sabouraud's Dextrose Agar) showed:

Cream color colonies.



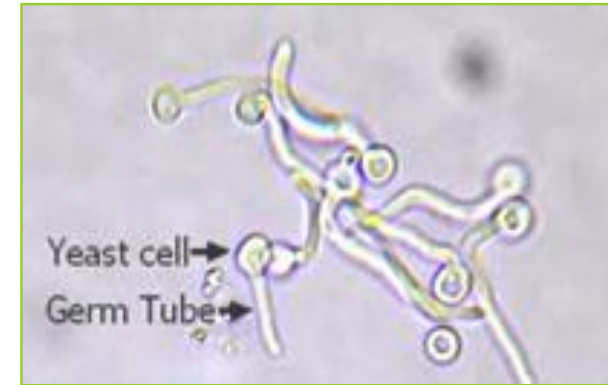
▶▶ CHLAMEDOSPORE TEST

CULTURE ON CORN MEAL AGAR



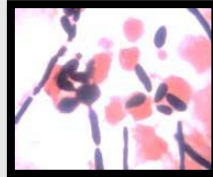
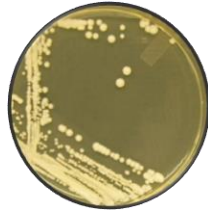

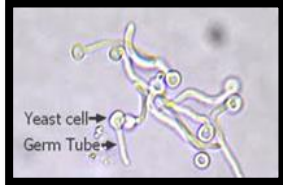
+Ve Test

▶▶ GERM TUBE TEST



+Ve Test

Lab. Tests Results(Summary)

TEST	Result	
Gram stained of lung tissue	Budding yeast cells & pseudohyphae.	
CULTURE ON SDA	Cream color colonies	
CHLAMEDOSPORE TEST	Chlamydospores, blastosconidia and pseudohyphae	
GERM TUBE TEST	Germination of tube	

Candida albicans

Case 7: A 15-year-old girl who was recently diagnosed with acute leukemia developed prolonged granulocytopenia (less than 100/ μ L) and refractory fever for 14 days and pulmonary signs or symptoms of pneumonia. Lung biopsy showed the following figure:

1. What is the differential diagnosis?

✓ *Aspergillus niger*

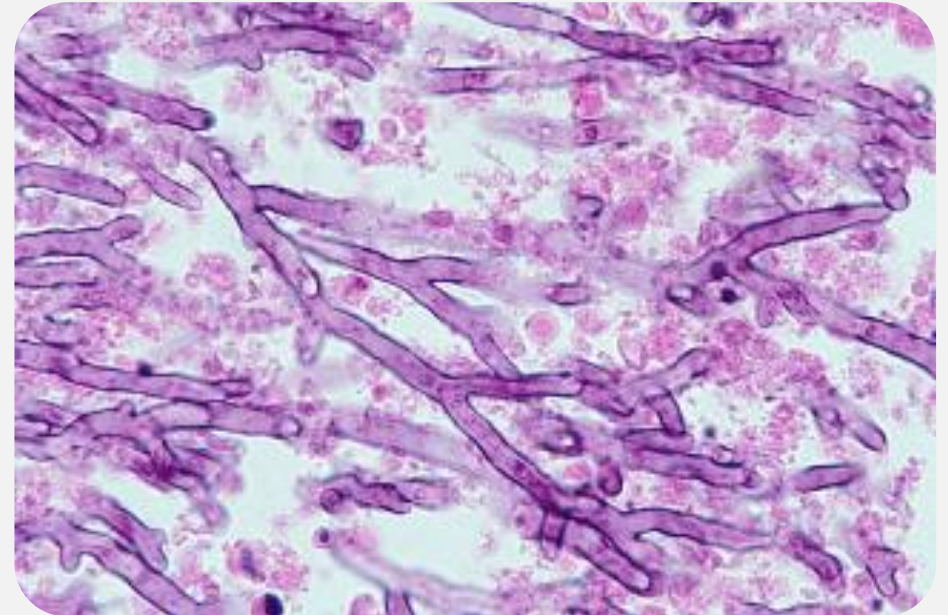
2. What investigation should be done?

✓ **Methenamine silver (GMS).**

✓ **H&E Stain.**

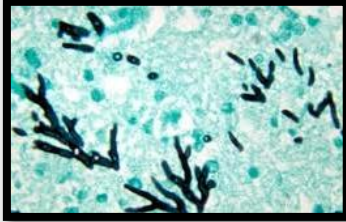
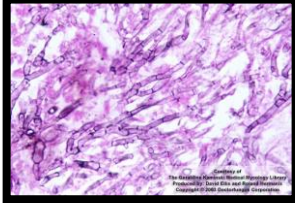
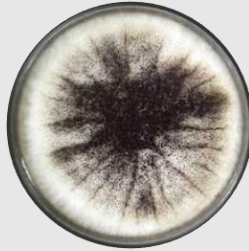

✓ **Culture from sputum on SDA.**

✓ **LPCB preparation from culture**



✓ *Aspergillus niger*

Lab. Tests Results(Summary)

TEST	Result	Images
Methenamine silver (GMS) tissue stain of lung:	Dichotomously branching fungal element	
H&E tissue Stain	Dichotomously branching fungal element	
Culture from sputem on SDA :	Salt and pepper appearance	
LPCB preparation from culture	Dark brown conidial heads and true hyphae	

Aspergillus niger