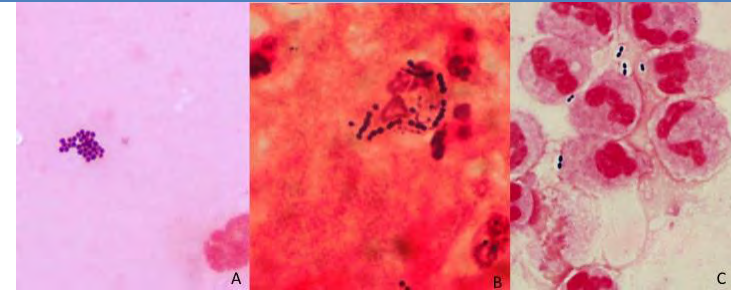


# Bacterial Gram Stain morphology

- Gram positive cocci
  - Clusters: *Staphylococcus*
  - Pairs and chains: *Streptococcus*, *Enterococcus*
- Gram positive bacilli
  - **Large:** *Clostridium*, *Bacillus*
  - **Small regular:** *Listeria*, *Erysipelothrix*, *Lactobacillus*
  - **Small irregular:** *Corynebacterium*, *Turicella*, *Dermabacter*, *Brevibacterium*, *Actinomyces*, *Propionibacterium*, *Gardnerella*, *Arcanobacterium*, *Actinobaculum*, *Bifidobacterium*, Aerobic actinomycetes
  - **Beaded:** aerobic actinomycetes: *Nocardia*, mycobacteria
- Gram negative cocci: *Neisseria*, *Moraxella*, *Acinetobacter*, *Kingella*, *Veillonella*, *Acidaminococcus*, *Megasphaera*, *Haemophilus*, *Pasteurella*, *Brucella*, *Bordetella*, *Actinobacillus*, *Francisella*, *Eikenella*, *Cardiobacterium*
- Gram negative bacilli: curved, safety pin



**NB: Shape, arrangement and abundance**

**: Tissue inflammation**

**Culture should be interpreted in conjunction with Gram stain**

# Gram negative bacilli


- Straight, medium-long, thin
  - *Pseudomonas*, Glucose-nonfermenters
- Straight, Short-medium, thick with rounded ends
  - *Enterobacteriaceae*
- Straight, Tapered ends
  - *Capnocytophaga*, *F. nucleatum*, *Leptotrichia*
- Curved
  - *Vibrio*, *Campylobacter*, *Helicobacter*, *Arcobacter*
- Filamentous
  - Antimicrobial-affected, some *Proteus* spp.
- Faint-staining, variable length
  - *Legionella*, *Bacteroides*
- Safety pin, Bipolar staining
  - *Burkholderia pseudomallei*, *Klebsiella*, *Pseudomonas*, *Yersinia*, *Francisella*, *Pasteurella*



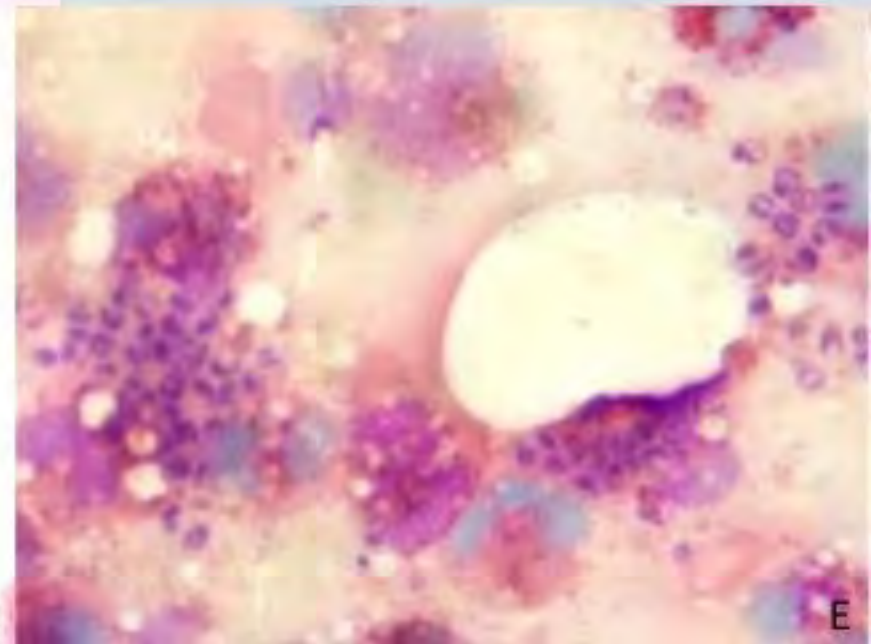
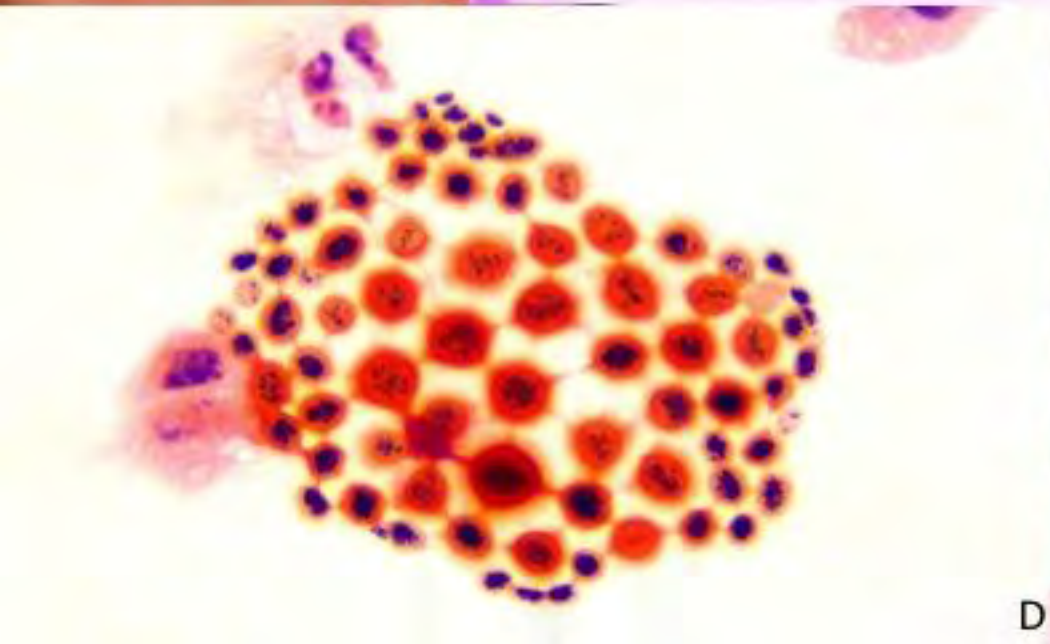
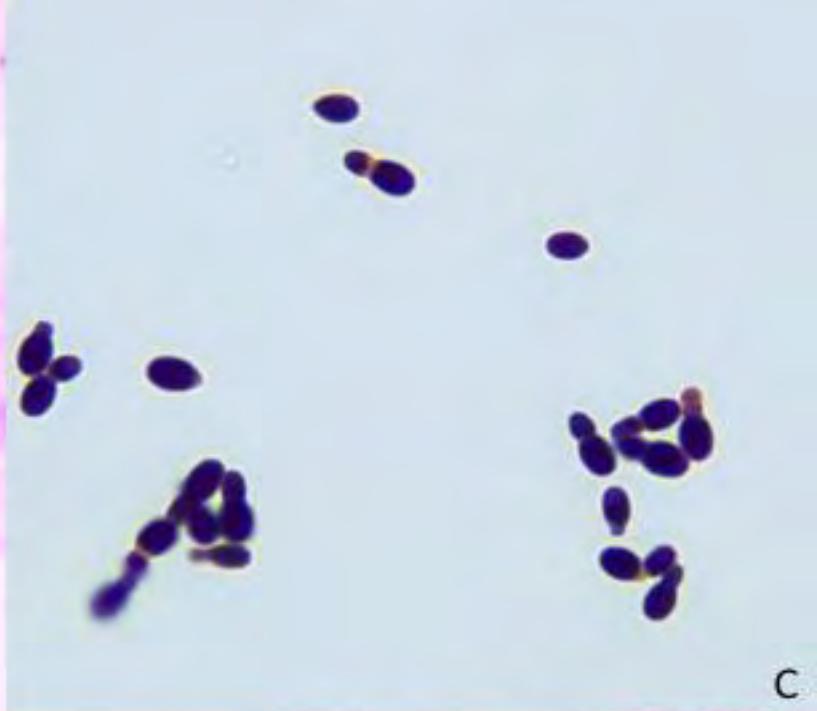
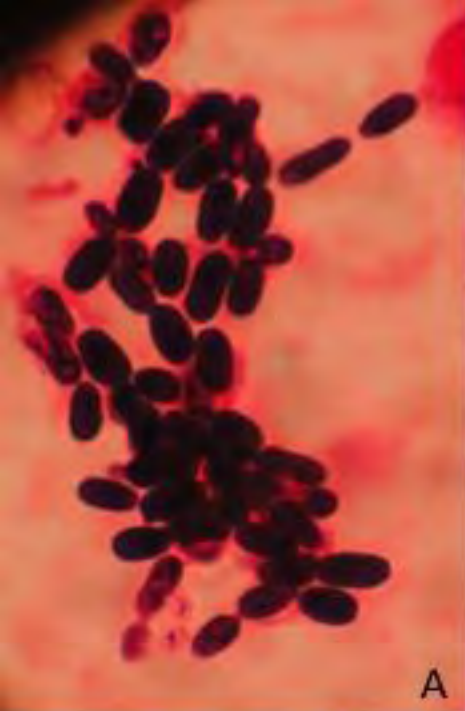
"Average" GNR  
Think enterics

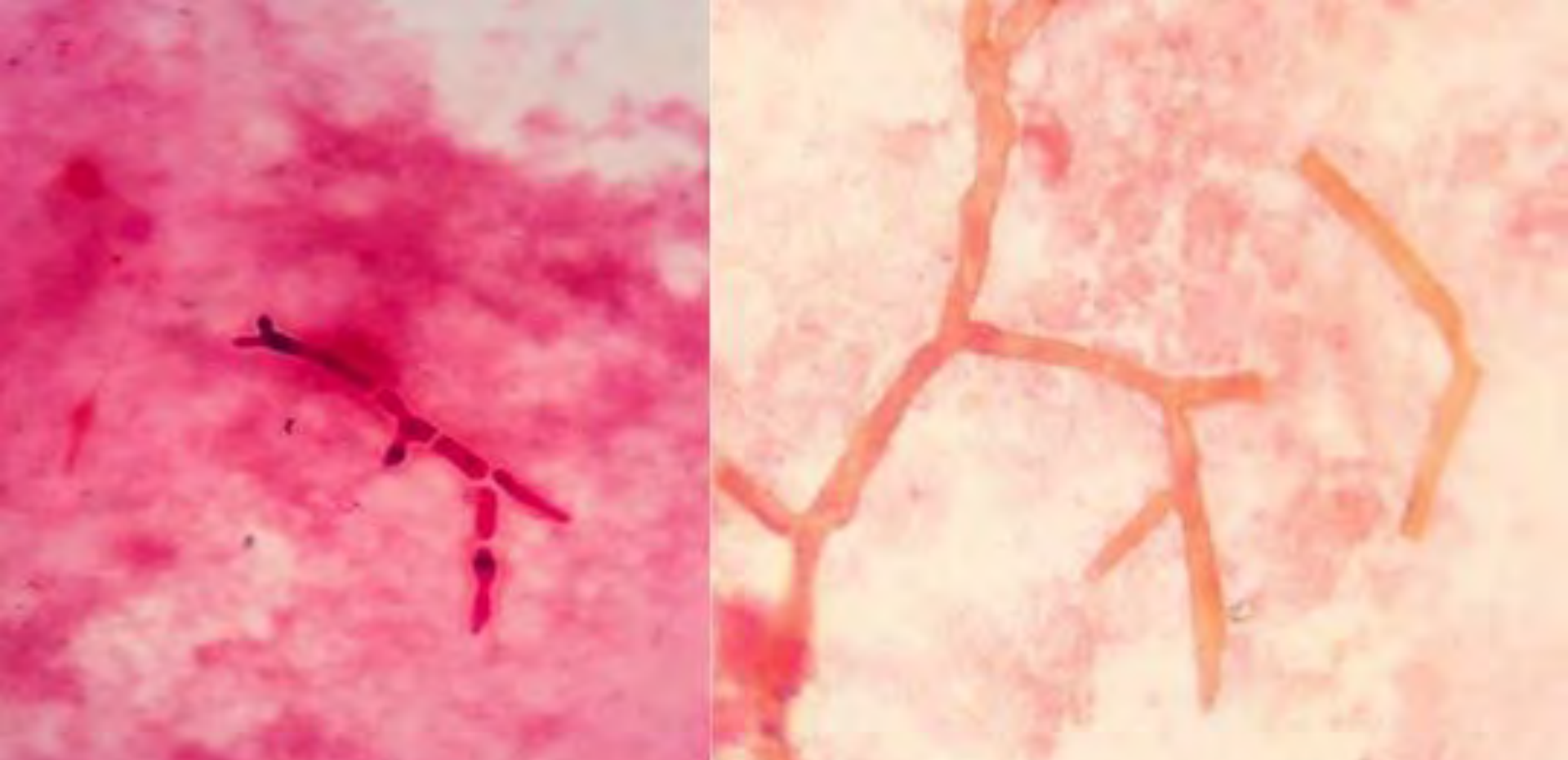


Short pleomorphic GNR  
Think *Haemophilus*



Long, thin GNR  
Think *Fusobacterium*





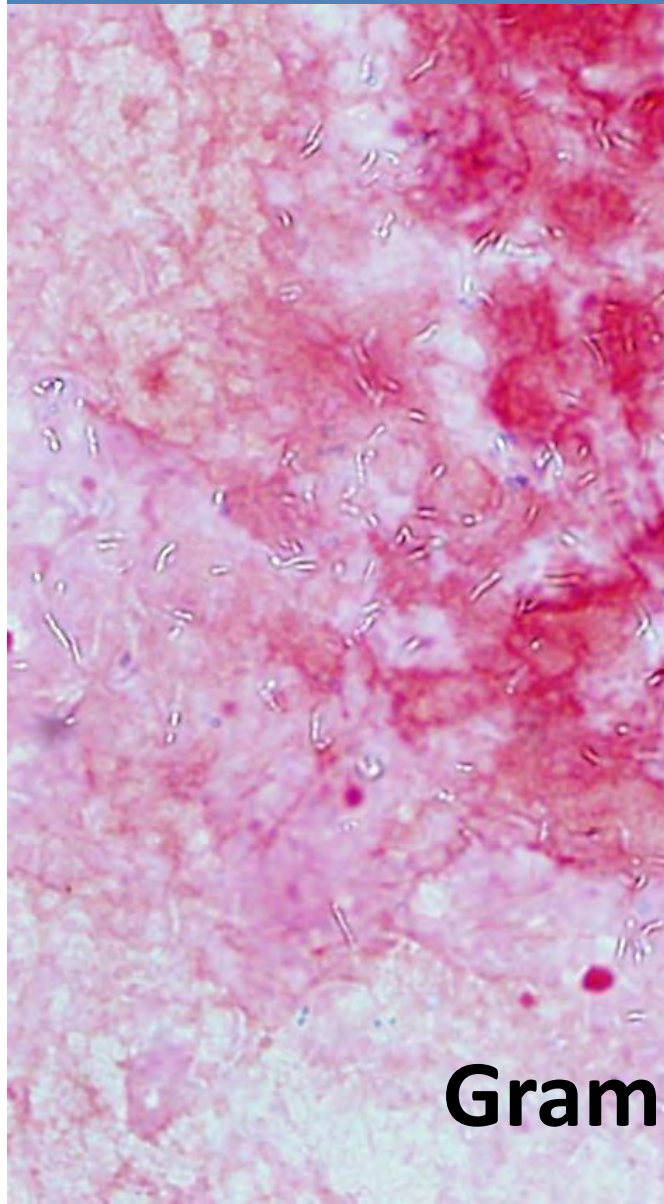
## **The sensitivity of fungal culture compared with the morphological identification of fungal elements seen in tissue sections**

**Direct detection of fungus in tissues was significantly more sensitive than culture**

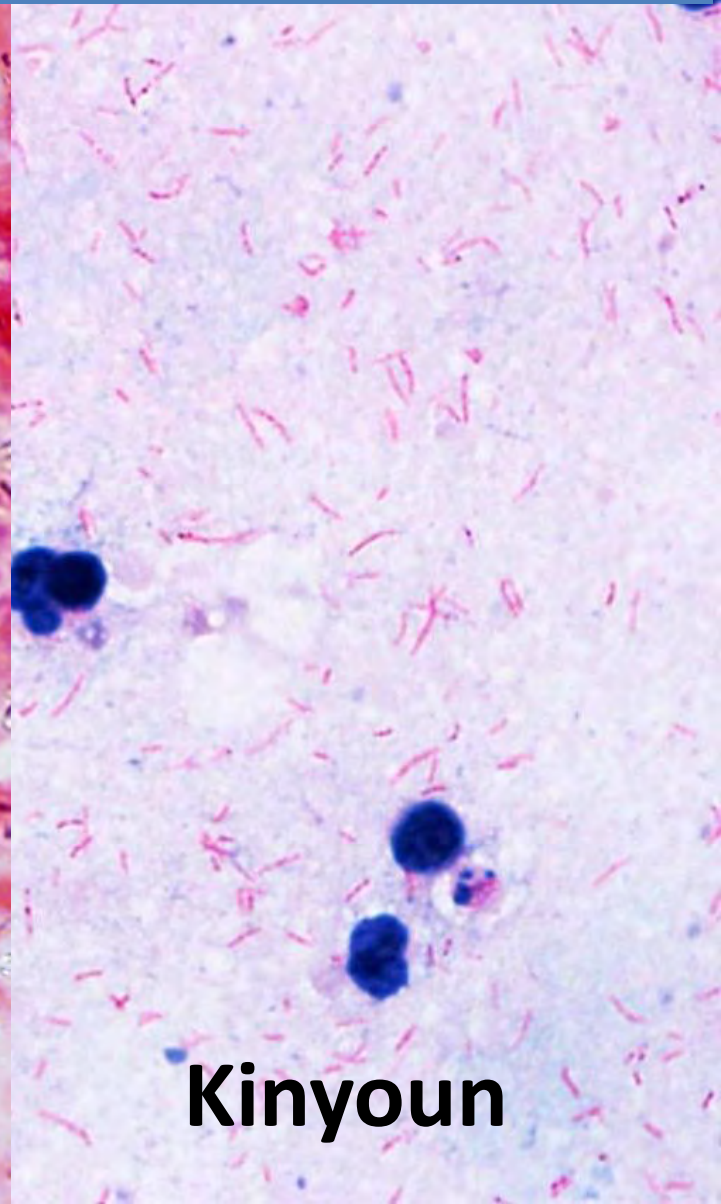
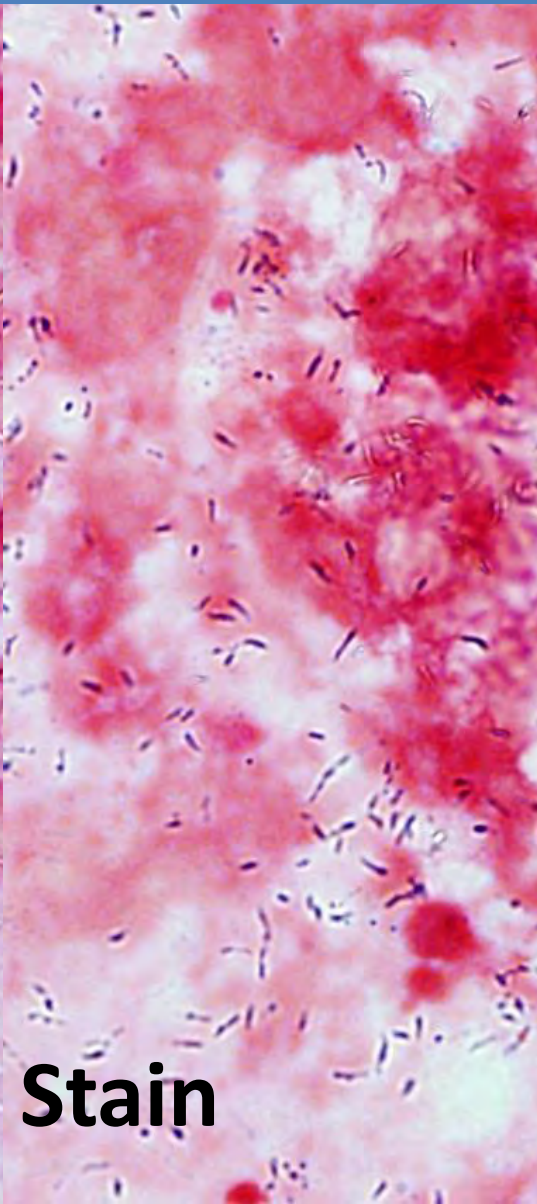
**Culture was positive in only 52% of the autopsy cases found to have fungal infections and only 30% of surgical specimens in which fungi were identified on direct examination.**



# Acid fast staining

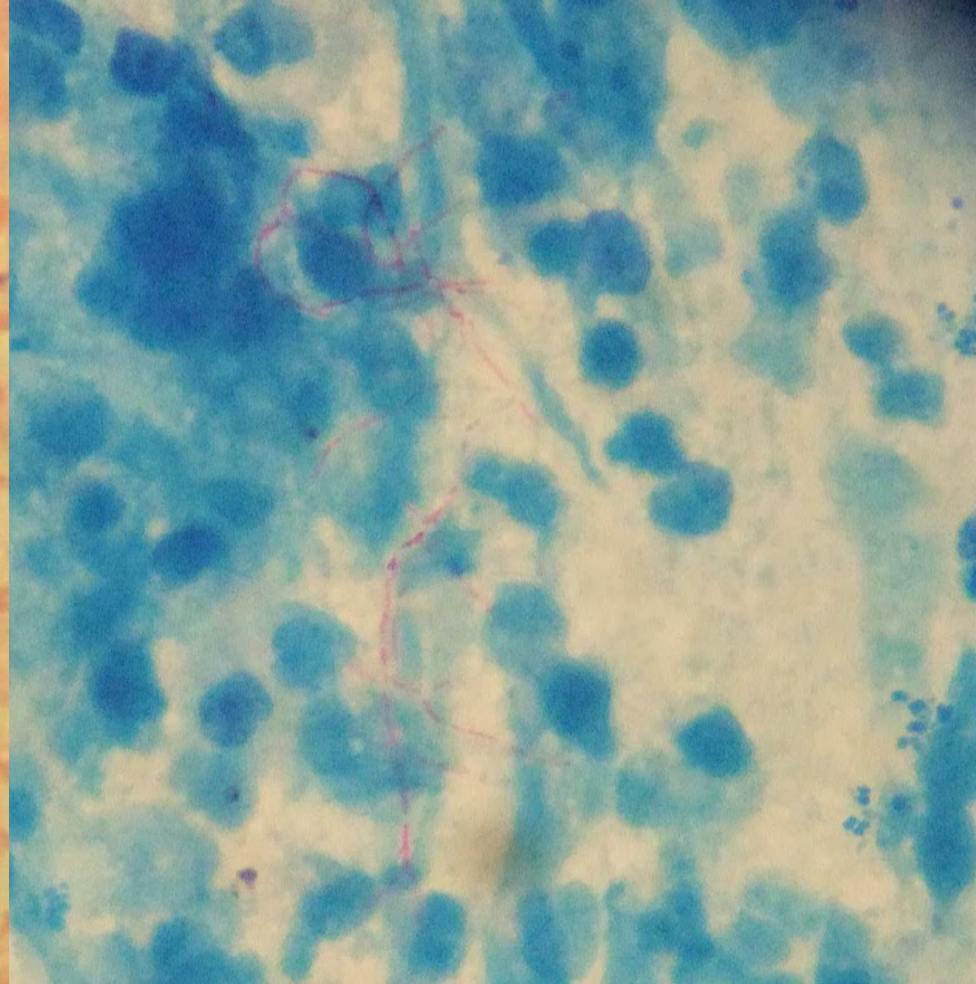
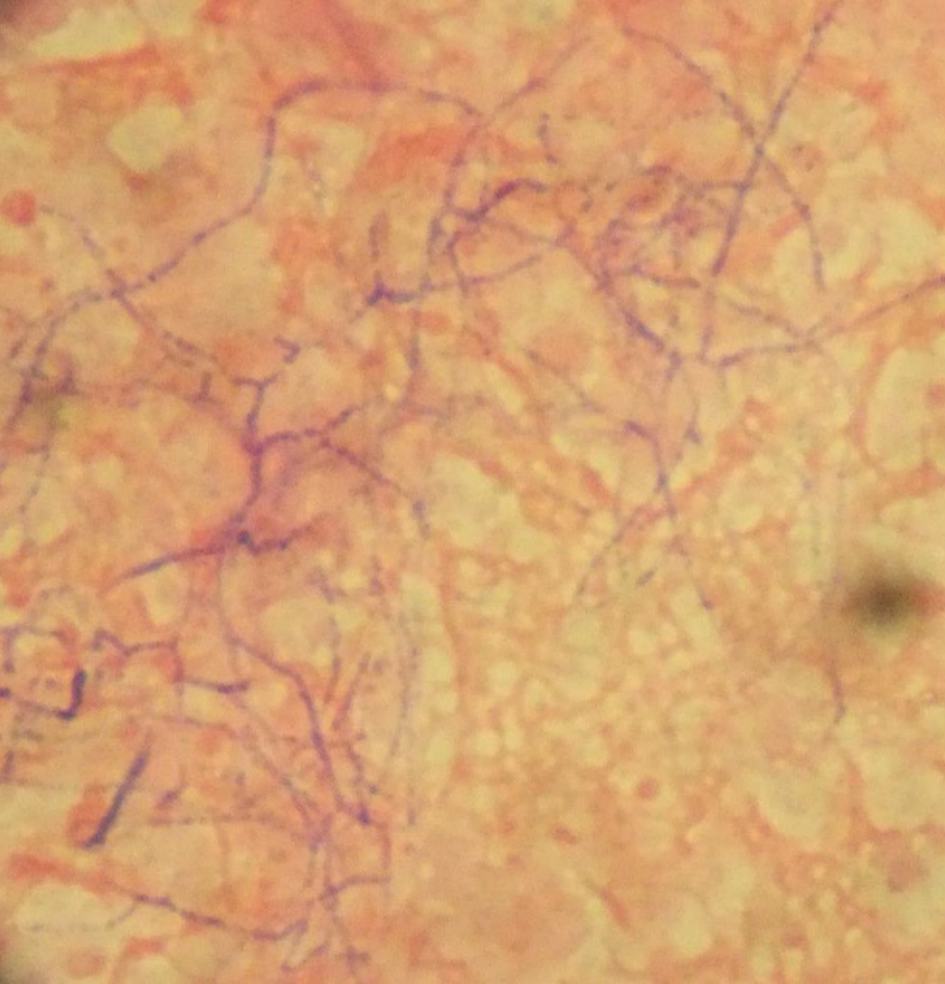


**Gram Stain**



**Kinyoun**



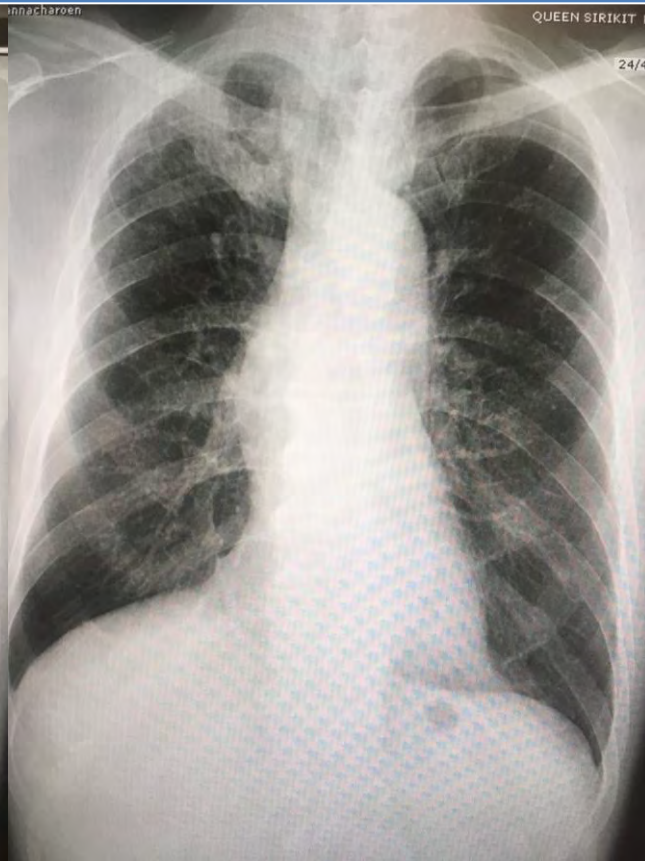


- **Modified acid fast organisms**
  - **Mycobacteria**
  - *Nocardia* (vs. Actinomyces)
  - Other aerobic actinomycetes: *Gordonia*, *Rhodococcus*, *Tsukamurella*, *Seigniliparus*
  - *Legionella micdadei*
  - Cysts of *Cryptosporidium*, *Cyclospora*, *Cystoisospora*; Microsporidia

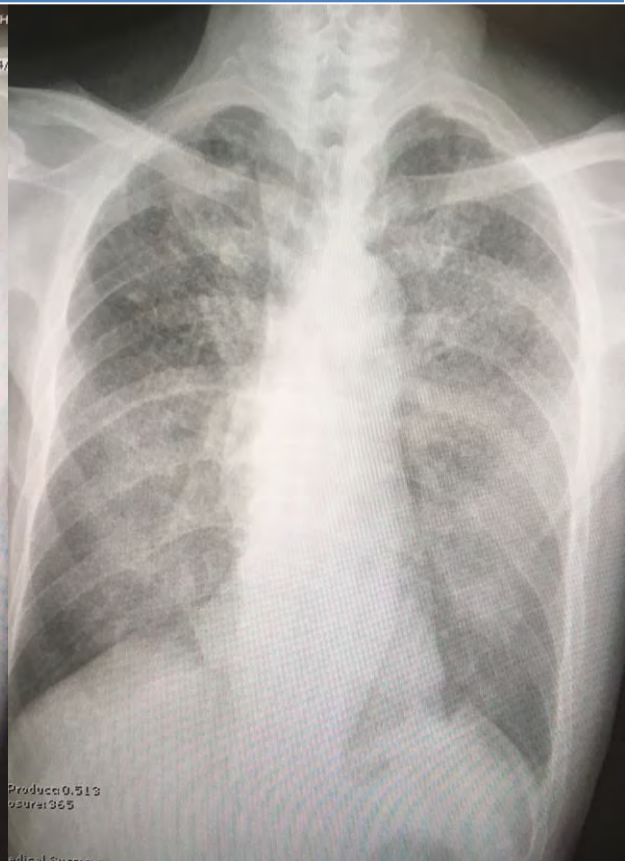
# A 60-year-old, previously healthy male with prolonged fever, productive cough and dyspnea for 4 months



4 months  
Negative AFB  
Positive GeneXpert  
No RIF resistance detected  
IRZE



2 months  
Feeling better  
IR



Fever, dyspnea





***Microscopic Examination:***

Section shows two pieces of tissue containing necrotic area surrounded by epithelioid histocytes, foamy macrophages, and some lymphocytes. Neither organism nor malignancy is definitely seen in H&E stain.

***Pathological Diagnosis:***

Right adrenal gland, Biopsy, Outside slide:

- Necrotizing granulomatous inflammation
- See HI-62-232 for special stains and MO-62-991 (PCR for TB)
- Positive result of real time PCR-based detection of *M. tuberculosis complex*.



# Kinyoun Stain

AFB stain

3.

Negative for AFB stain

Aerobic culture with AST\*

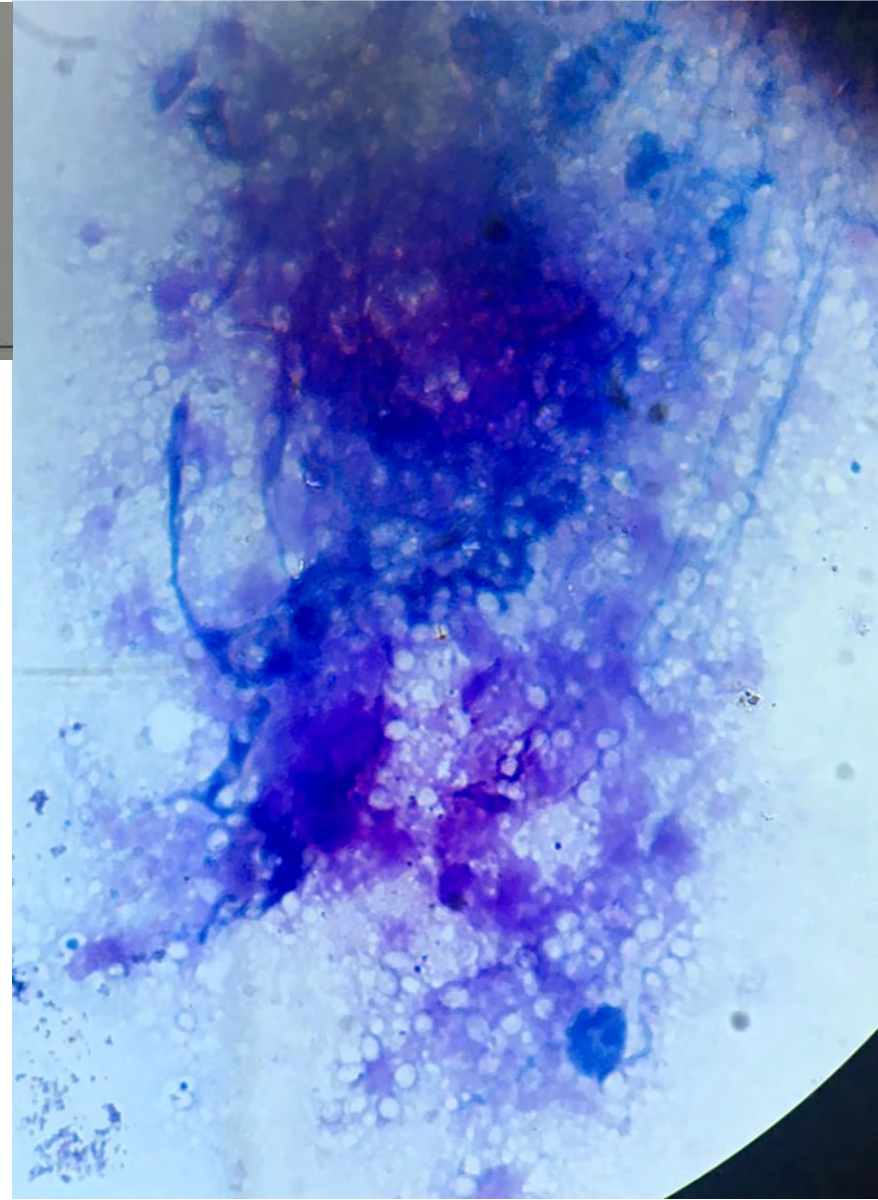
2.

No growth

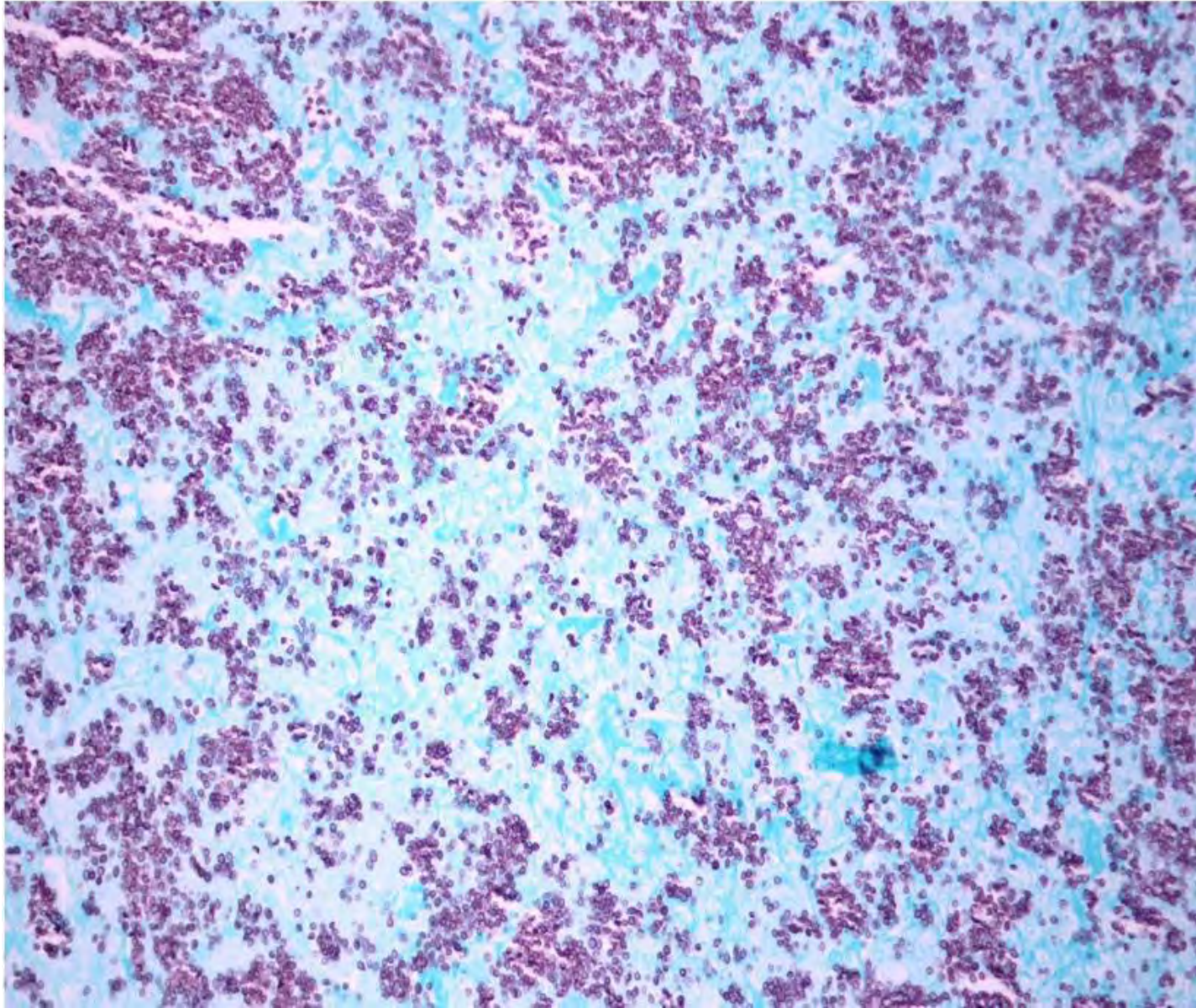
◇ Modified AFB stain

4.

Negative for Modified AFB stain



# GMS Stain





**No test is 100% accurate**

# No test is 100% accurate

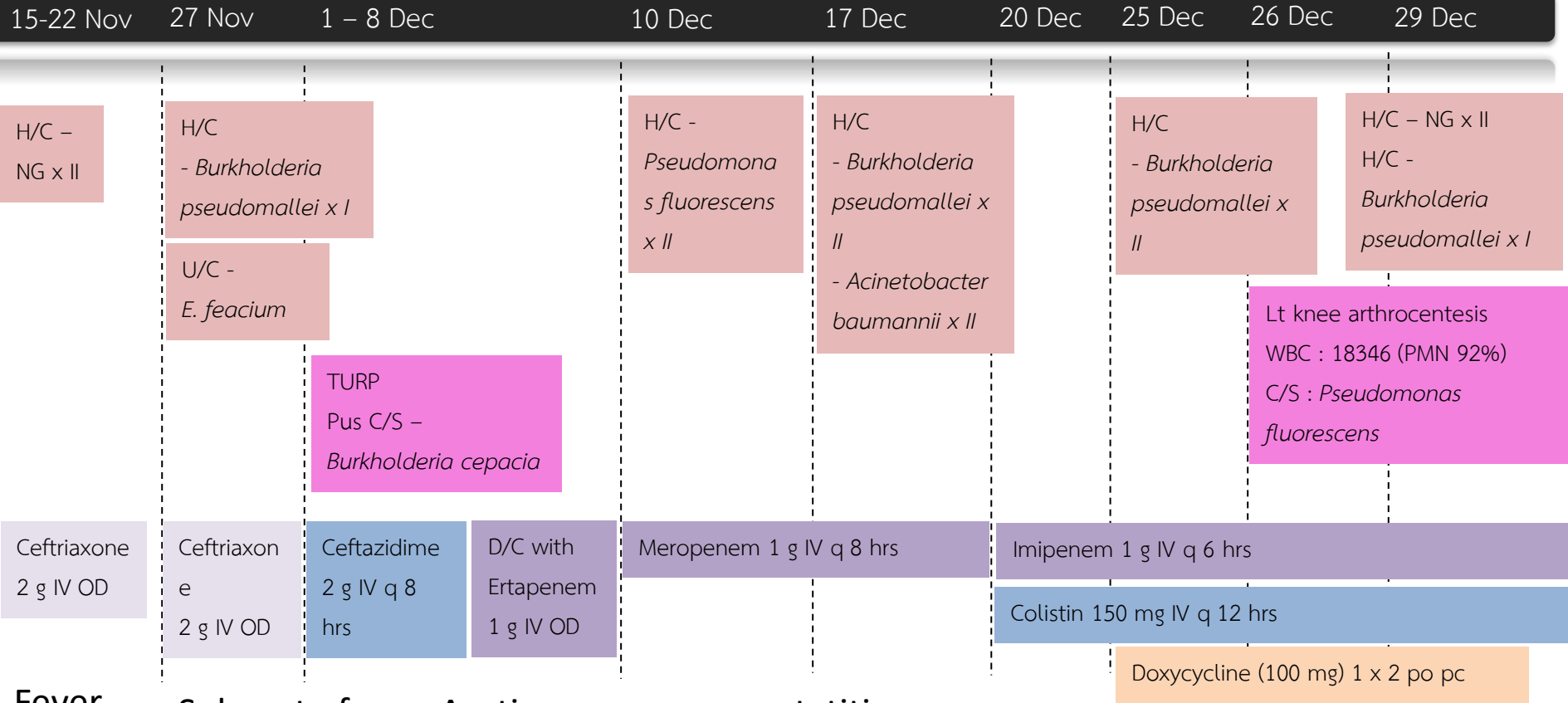
- All have some level of false positive or false negative results
- **FP/FN rates** dependent of sensitivity and specificity of specific test **and the prevalence/pretest probability of disease**

Positive test results are more accurate when pre-test probability is higher (clinically relevant)

- History, physical examination
- Prevalence and risk
- Tissue reaction

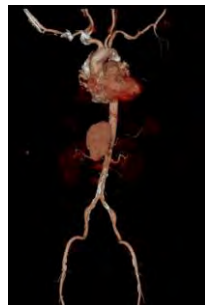
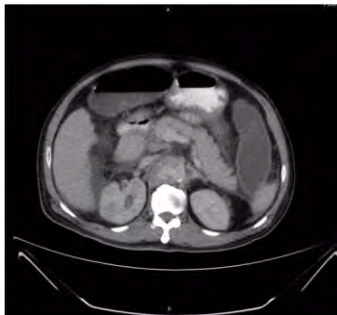


# A 65-year-old diabetic male presented with fever for 4 months



Fever  
Dysuria

Subacute fever: Aortic aneurysm, prostatitis



SPECIMEN: Blood-Hemoculture 1

\* Aerobic Culture \*

1. Burkholderia pseudomallei

	1-MIC	SIR	1-MIC	SIR	1-MIC	SIR
AMOXICIL/CLAV...	<=4	S				
CEFTAZIDIME....	4	S				
IMIPENEM.....	<=0.5	S				
TRIMETHO/SULFA.	<=1	S				

SPECIMEN: PUS From.....

\* Aerobic Culture \*

1. Numerous

Burkholderia cepacia

	1-MIC	SIR	1-MIC	SIR	1-MIC	SIR
CEFTAZIDIME....	4	S				
MEROPENEM.....	1	S				
TRIMETHO/SULFA.	<=1	S				

\* Aerobic Culture \*

1. Pseudomonas fluorescens.. (MDR)

	1-MIC	SIR	1-MIC	SIR	1-MIC	SIR
PIPERACI/TAZOB.	>64	R	LEVOFLOXACIN...	8	R	
CEFOTAXIME.....	>32	R	COLISTIN.....	>4	R	
CEFTAZIDIME....	4	(S)	TRIMETHO/SULFA.	<=1	(S)	
CEFTRIAXONE....	>32	R	TIGECYCLINE....	0.5	S	
CEFEPIME.....	32	R				
IMIPENEM.....	1	S				
MEROPENEM.....	4	S				
AMIKACIN.....	>32	R				
GENTAMICIN.....	>8	R				
CIPROFLOXACIN..	>2	R				



## Comparison of Automated and Nonautomated Systems for Identification of *Burkholderia pseudomallei*

Peter Lowe,<sup>1</sup> Catherine Engler,<sup>2</sup> and Robert Norton<sup>2\*</sup>

Central Queensland Pathology Laboratory, Mackay,<sup>1</sup> and Clinical Microbiology, Queensland Health Pathology Services, The Townsville Hospital, Townsville,<sup>2</sup> Queensland, Australia

System	Identification	% of strains
API 20E	<i>Burkholderia pseudomallei</i>	99
	<i>Chromobacterium violaceum</i>	1
API 20NE	<i>Burkholderia pseudomallei</i>	98
	<i>Chromobacterium violaceum</i>	2
Vitek 1	<i>Burkholderia pseudomallei</i>	99
	Unidentified organism	1
Vitek 2	Various nonfermenting gram-negative bacilli	37
	<i>Burkholderia cepacia</i>	24
	<i>Burkholderia pseudomallei</i>	19
	<i>Myroides</i> spp.	8
	Inconclusive identification	7
	<i>Chromobacterium violaceum</i>	2
	Unidentified organism	2
	<i>Pseudomonas aeruginosa</i>	1