Case Study 2: House Fire

Microbiology Laboratory Summary

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Case Study Scenario

* "Rescued from a house fire Marian is hospitalized with second degree burns extending deep into the dermis layers in her upper limbs. After three days in hospital during which time she has regular changes of her wound dressings, she is released home to the care of her sister who expresses confidence in her ability to maintain the needed dressing changes. With a keen interest in natural remedies Marian and her sister treat her burn wounds with honey. A few days later Marian begins to experience pain associated with her burns and notices that they seem to be producing more fluid than before. After a bad nights sleep her sister takes her back to the hospital where the doctor notes that one of the wounds is expressing pus. A swab of the pus is sent to the laboratory and Marian is again hospitalized. The laboratory grows Pseudomonas aeruginosa from the wound and Marian is started on antibiotics."
(from PATH417A UBC wiki page).

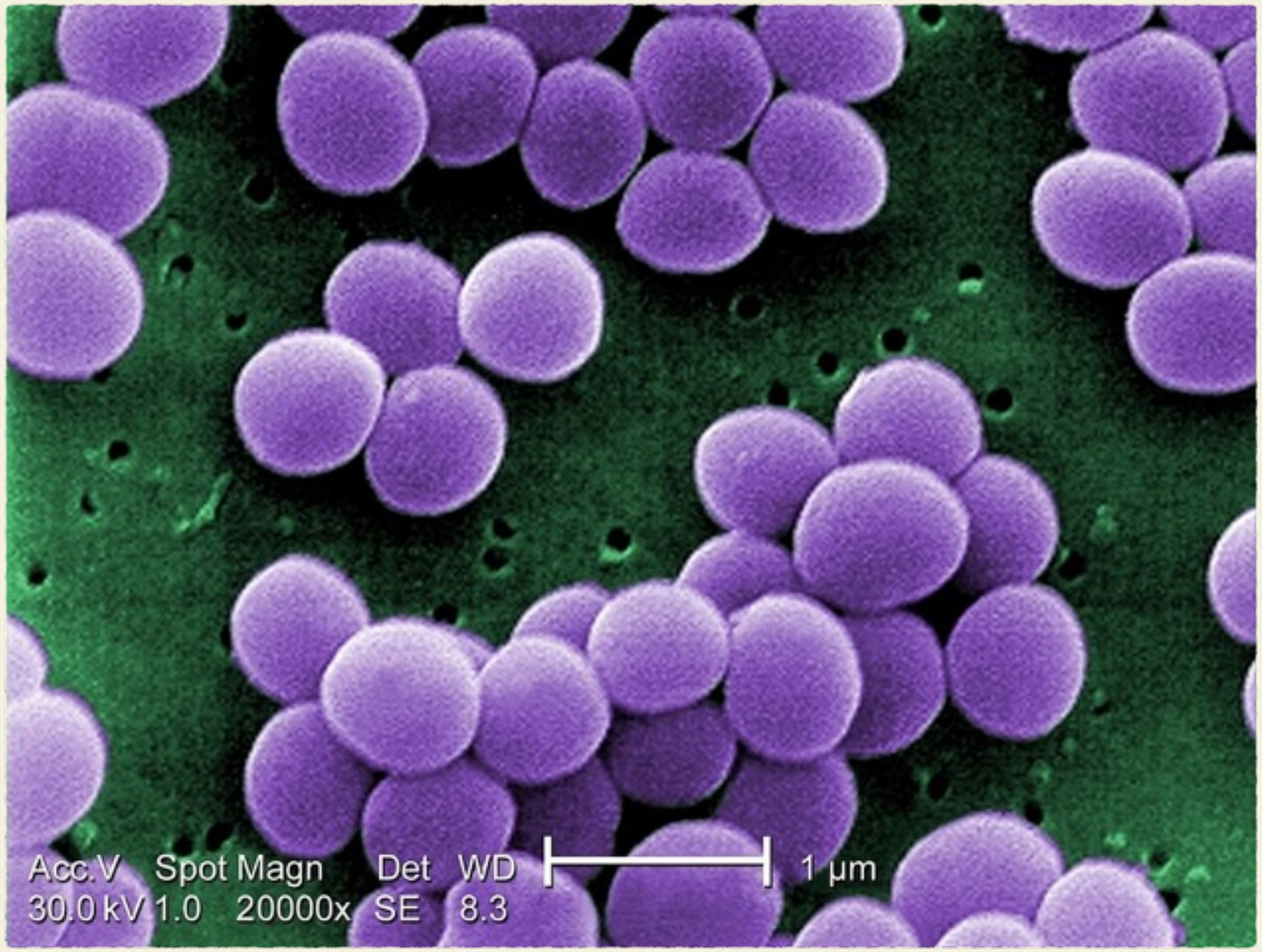
Question 1: What are the most common bacterial pathogens associated with the scenario?

Pathogens associated with the scenario

Staphylococcus aureus

Klebsiella pneumoniae

Acinetobacter baumannii



Staphylococcus aureus

Most common opportunistic pathogen to infect burn patients

Staphylococcus aureus

* Can be normal flora in human body (ie. skin, upper respiratory tract)
* Facultative anaerobic
* Non-motile bacterium
* Grape-like under a microscope
* Cause of skin infections; can induce illnesses that range from minor (impetigo and pimples) to severe (pneumonia and sepsis).

Staphylococcus aureus: Transmission

* Direct skin contact
* Contact through contaminated objects or equipment
* Contact with pus from infected wound

Staphylococcus aureus: Virulence Factors

Toxins

Adhesion proteins

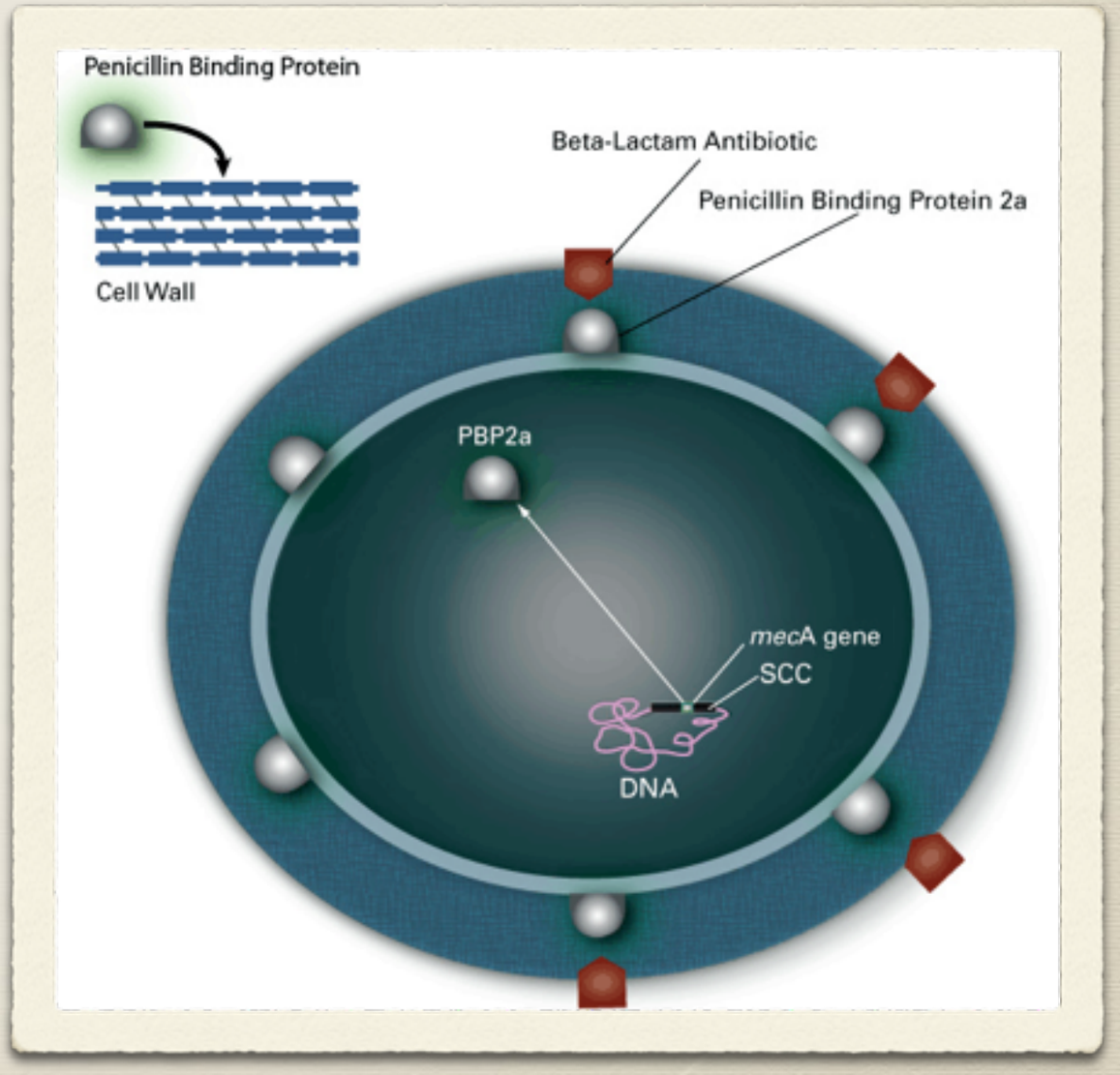
Colonizing proteins

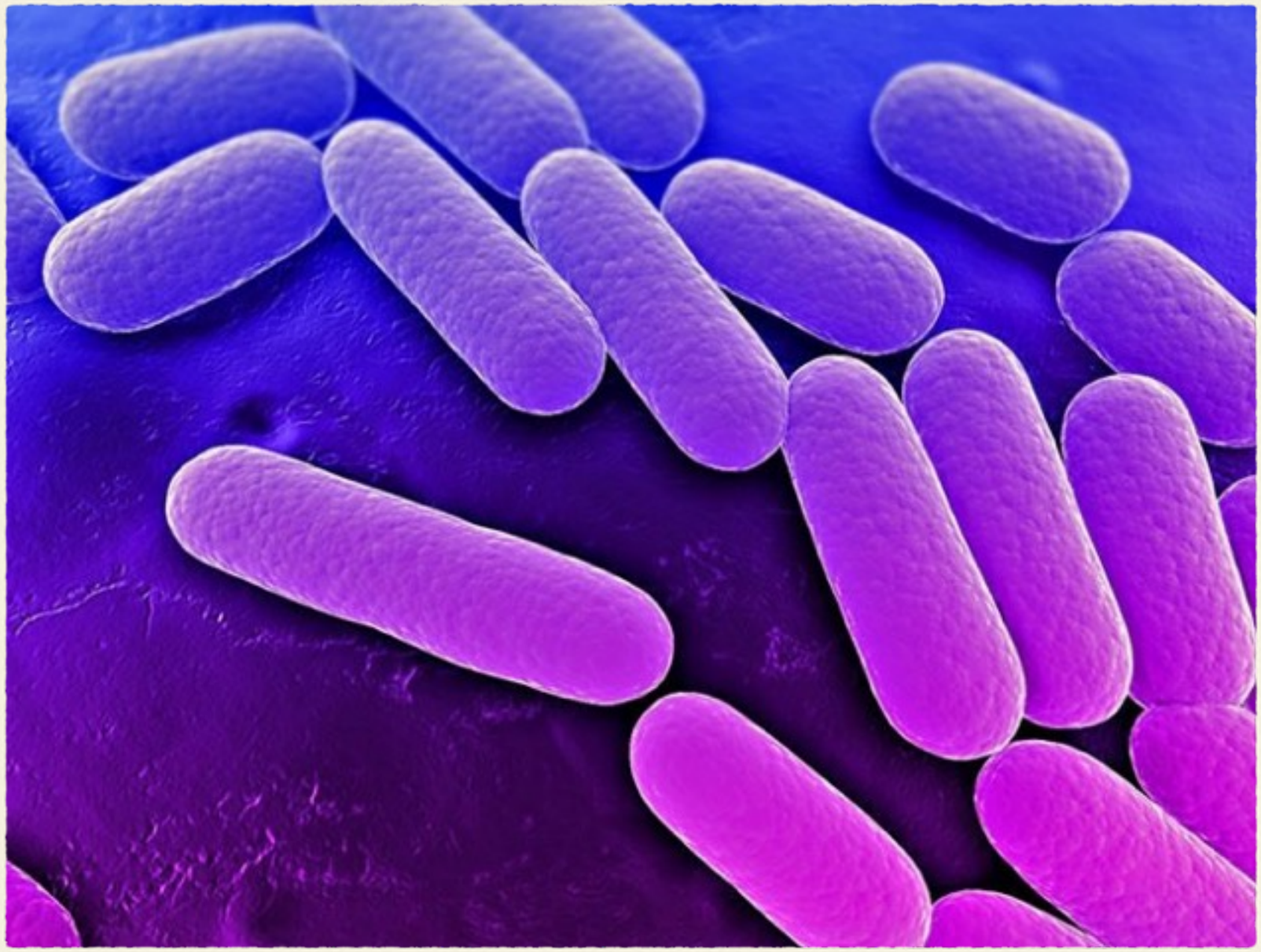
Enzymes

Superantigens

Staphylococcus aureus: mecA gene

* Can gain a mutation that encodes a modified penicillin-binding protein
* Grants resistance to methicillin and other beta-lactam antibiotics
* Bacteria complicated to treat because of this





Klebsiella pneumoniae

A nosocomial, opportunistic pathogen

Klebsiella pneumoniae

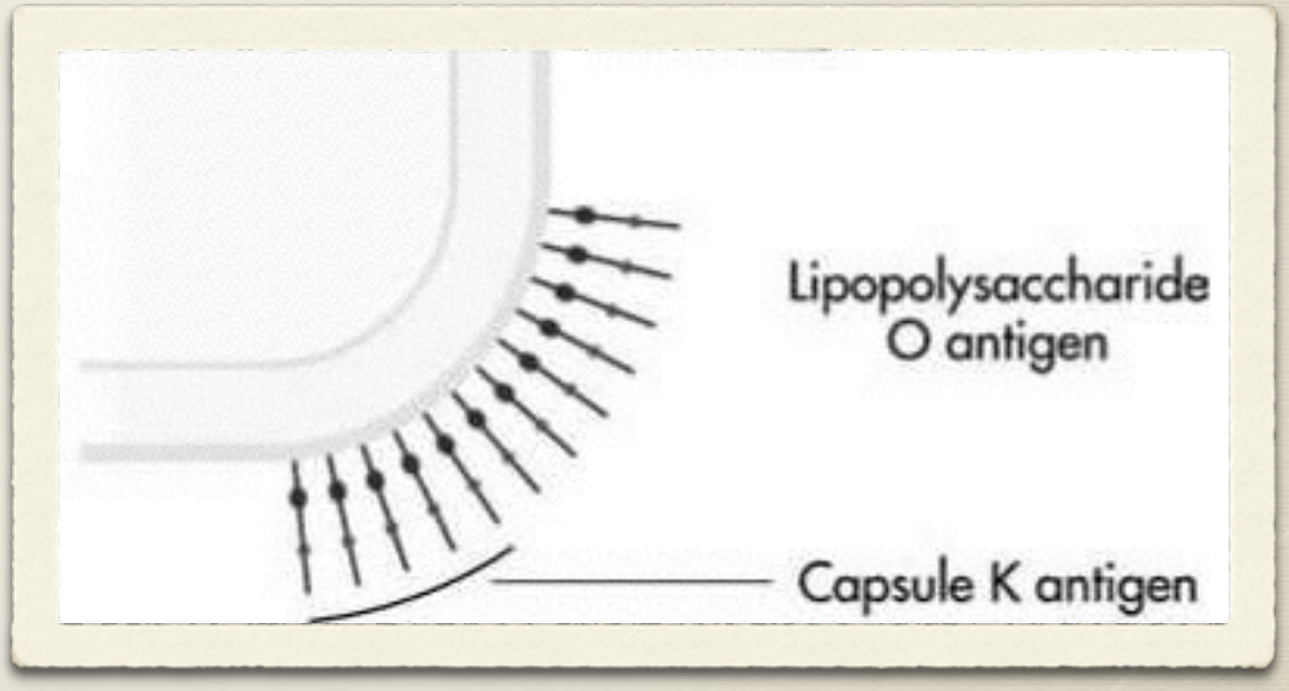
* Non-motile, facultative anaerobic
* Rod-shaped, gram-negative bacterium
* Found in normal flora of skin, mouth, intestines
* Infection usually in lungs
    * Destruction through inflammation, necrosis, and hemorrhage of lung tissue
* Nosocomial infections: urinary tract, respiratory tract, skin

Klebsiella pneumoniae: Transmission

* Person-to-person contact
* Contamination from environment

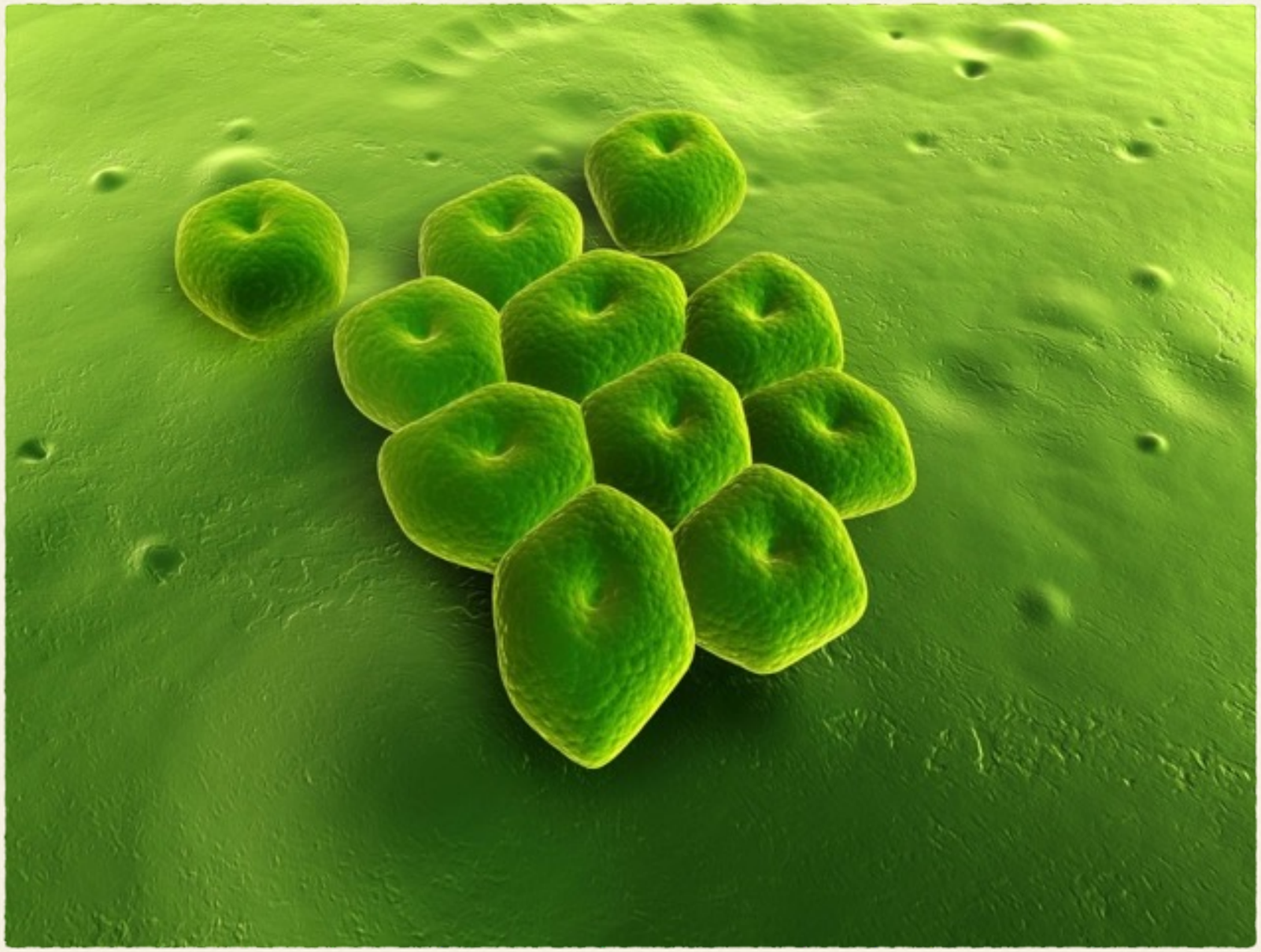
Klebsiella pneumoniae: Pathogenic Antigens

* K-antigen: capsular polysaccharide
* O-antigen: component of lipopolysaccharide



Klebsiella pneumoniae: Tests and Treatments?

* Resistant to multiple antibiotics
    * Tests performed by microbiology labs can be useful in determining which antibiotics are effective against the infection
* Potential treatment methods:
    * Third or fourth generation quinolones and carbapenems
    * Monotherapy and combination treatment



Acinetobacter baumannii

The opportunistic water organism

Acinetobacter baumannii

* Rod-shaped, gram-negative bacteria
* Preferentially colonizes in water environments
* Infections typically occur in organ systems with high fluid content
    * Urinary tract, respiratory tract, and cerebral spinal fluid.

Acinetobacter baumannii: Who's at risk?

Targets compromised immune systems. Burn wound patients at risk.

Acinetobacter baumannii: Virulence Factors

Protective capsule around each cell

Adhesion protein A

Multiple antibiotic resistance mechanisms: small RNA, biofilm formation, and efflux pump

Acinetobacter baumannii: Tests and Treatments?

* Symptoms indistinguishable from other opportunistic pathogens
* Laboratory tests needed in proper diagnosis
* Evolved resistance to multiple drugs
    * Treatment can be difficult and complicated

Question 2: What samples are taken for laboratory testing and how important is the Microbiology Laboratory in the diagnosis of this particular infectious disease?

Techniques for Taking Blood Wound Specimens

* Sample during biopsy
* New technique involving dermabrasion to enable deeper tissue (less invasive)
* Wound fluid sample (if sufficient volume is being produced to allow for aspiration)
* Wound swabbing: performed using cotton-tipped swabs to sample superficial wound fluid and tissue debris
* Also: contact plates and capillarity gauze sampling

Best sampling for this case scenario…

* Sampling of the burn wound and pus by surface swabs
    * Convenient and nominally invasive method
* Also possible: Tissue biopsy
    * May give a better prediction of septic infection and extent and depth of infection
    * BUT: Less practical

If systemic infection…

Blood and urine samples may also be needed.

Wound Tissue Samples

* Obtained aseptically to avoid contamination
* Multiple surface swabs or biopsies of different areas
* Taken from the leading edge of the burn wound may be more likely to include specimens with clinical significance
* Swab viable tissue and avoid contamination with necrotic tissue or pus

Transport of sample and other important details

* Transport immediately for inoculation onto culture media within 2 hours after collection
* Sample in sterile container or swab transport system
* Inoculation of culture media must occur within 24 hours
* Transport and storage occur at room temperature

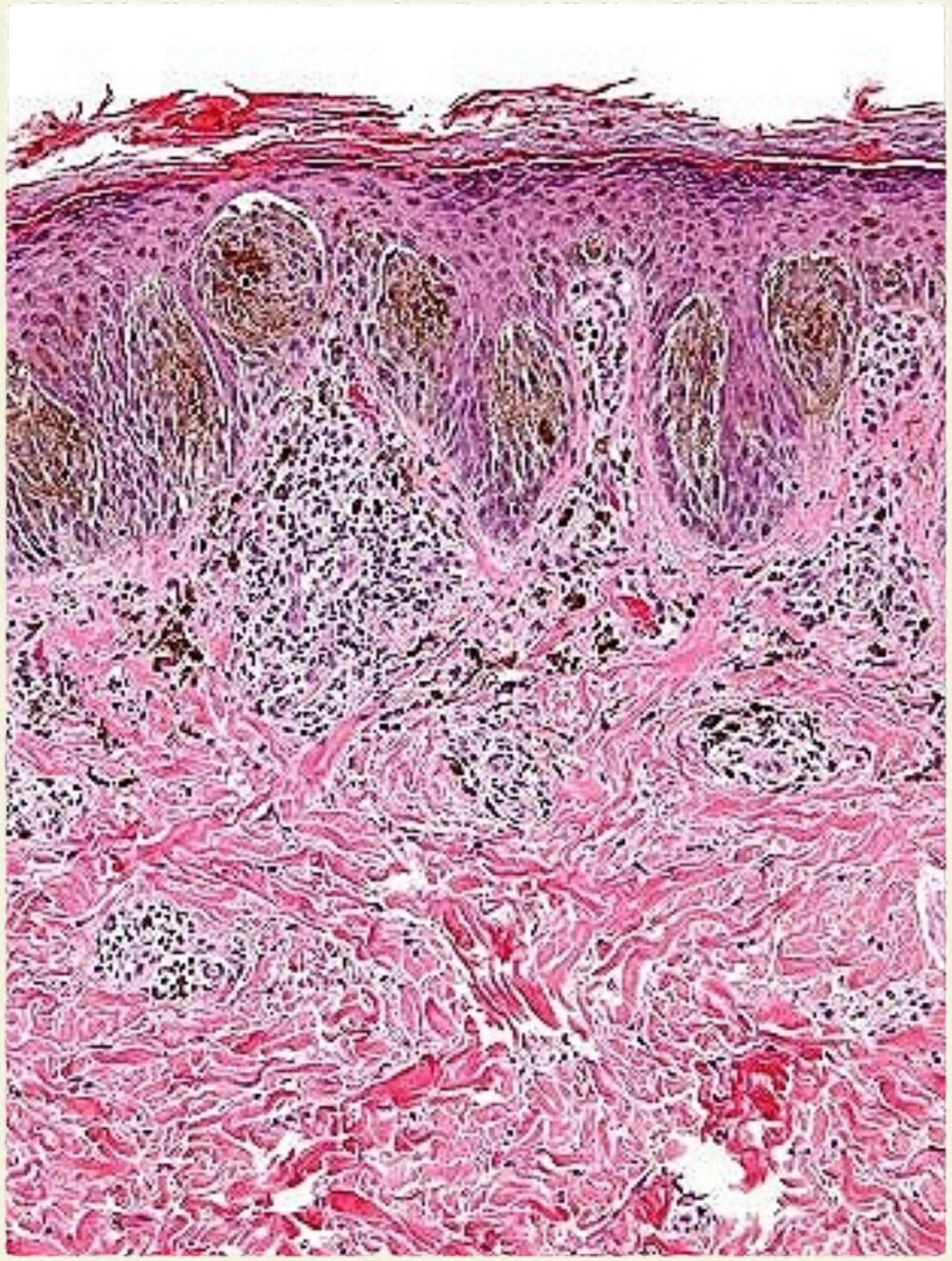
Importance of microbiology laboratory for diagnosis

* Confirming that a tissue is actually infected
* Organisms in Wound =/= Infection
* Determining antimicrobial susceptibility of the organisms colonizing the burn wound
* Determining appropriate treatment options (includes antibiotic treatment)

Question 3: Explain the tests that will be performed on the samples in order to detect any of the potential bacterial pathogens causing this disease.

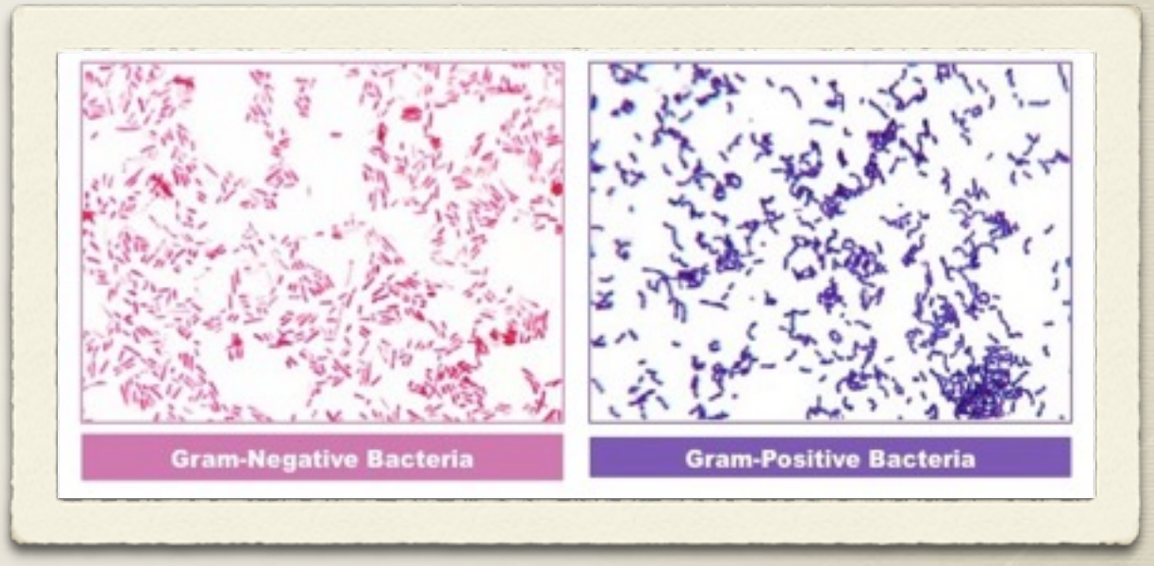
Histologies

Biposy histology can be used to visualize different types of microorganisms.



Gram-staining

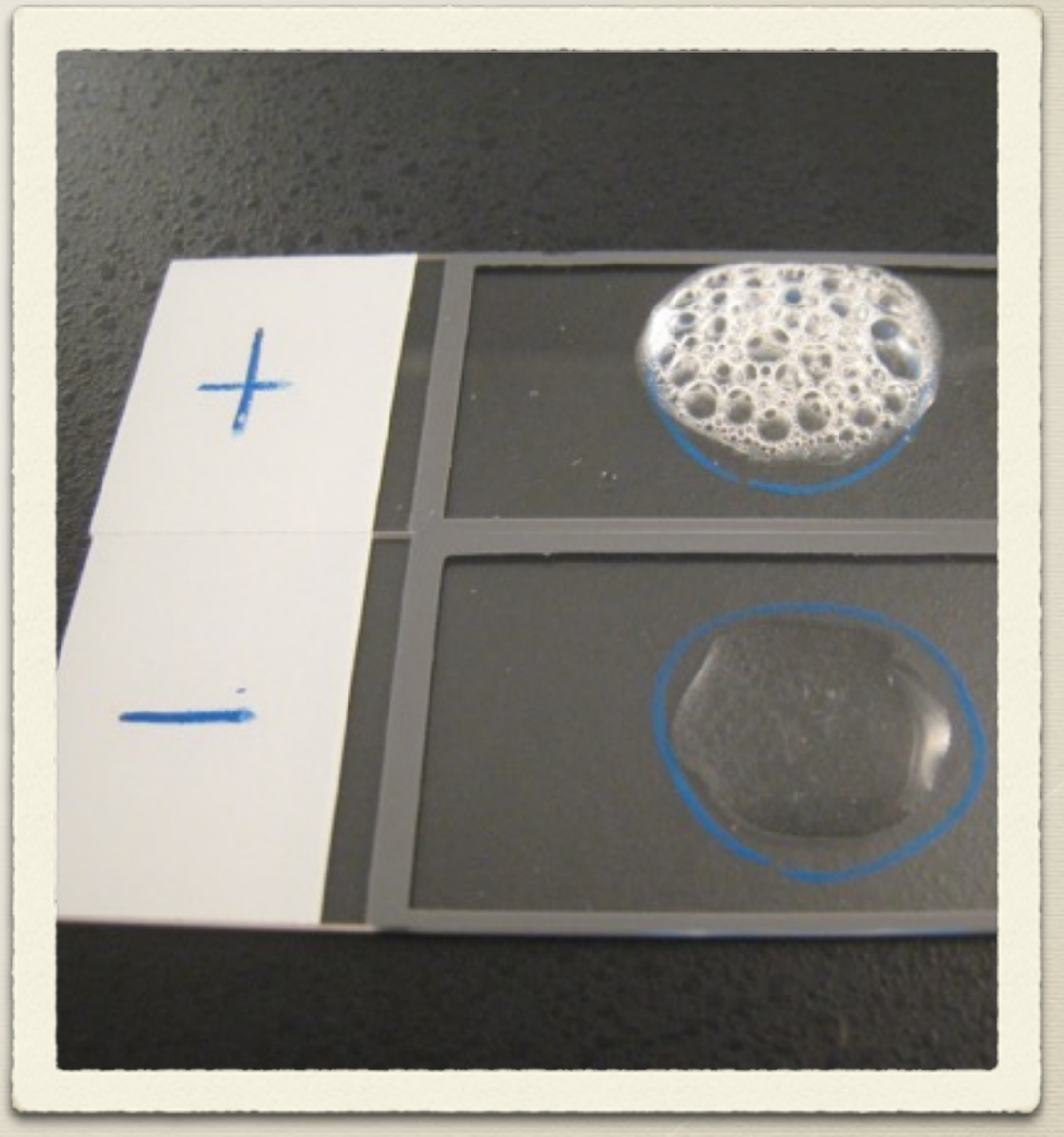
* Determines cell wall composition of the bacteria
* Gram-positive: violet (crystal violet dye)
* Gram-negative: pink (safranin)



* Using enrichment, selective, or differential media, one can study different enzymatic capabilities and metabolic pathways of different species.
    * ie. MacConkey n°3 media: selects Gram-negative bacteria, differentiates between Lac + bacteria (red) and Lac - bacteria (yellow or colorless)

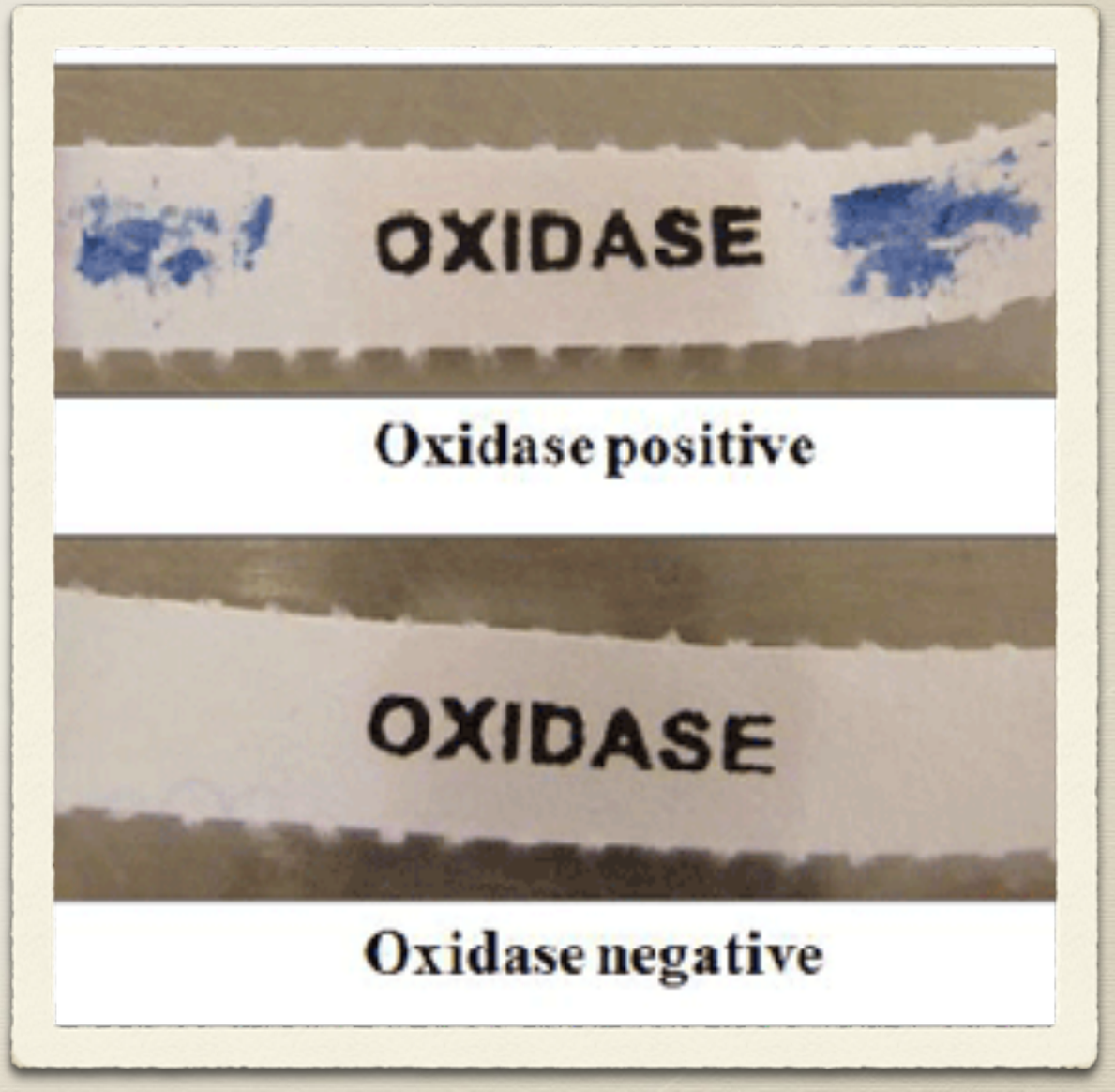
Catalase Test

* Subjects bacteria to H_2O_2 solution
* Bubbles indicates a catalase+ bacteria (release of oxygen molecules)



Oxidase Test

* Looks for cytochrome c oxidase
* Blue color = oxidase+
* Colorless = oxidase bacteria

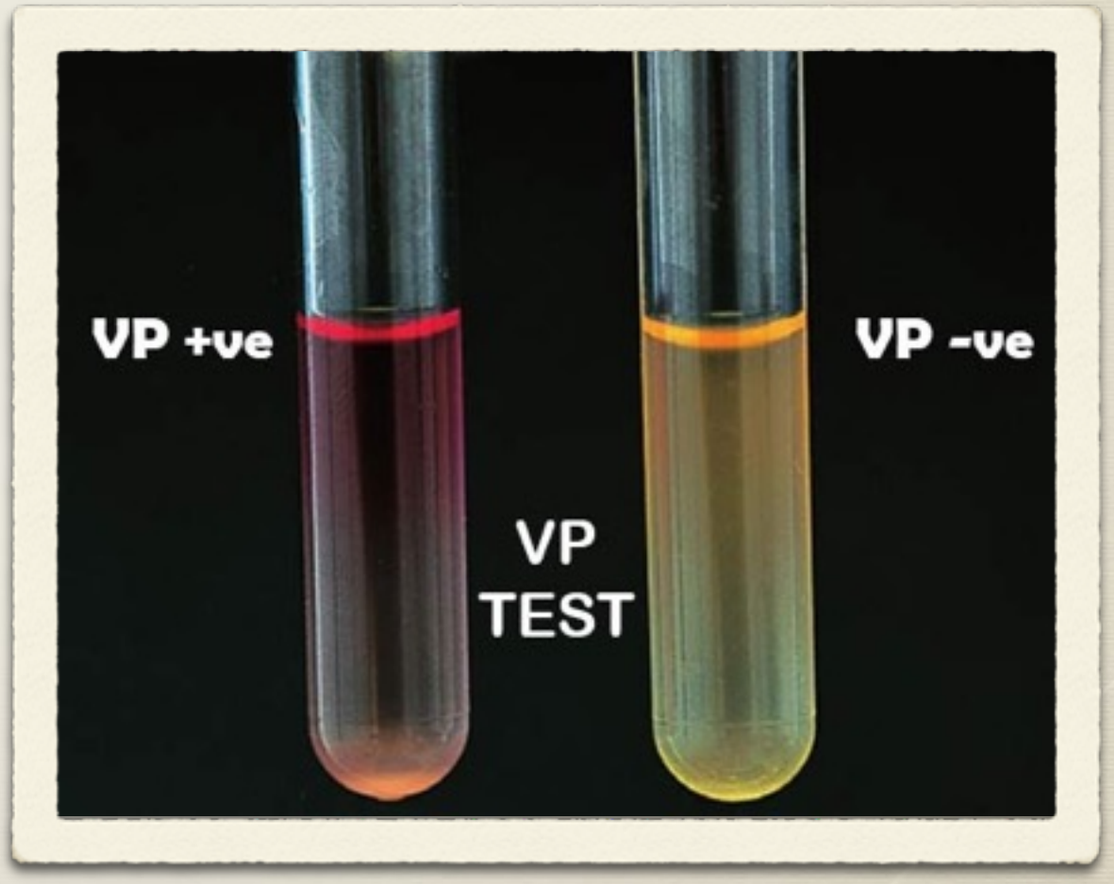


Triple Sugar Iron Test

* Analyzes the following:
    * Colony morphology, pyocyanin pigment production
    * Survival at high temperatures around 44°C
    * Ability to ferment carbohydrates and reduce sulfur
* Provides detailed information to narrow down potential pathogens in the infection

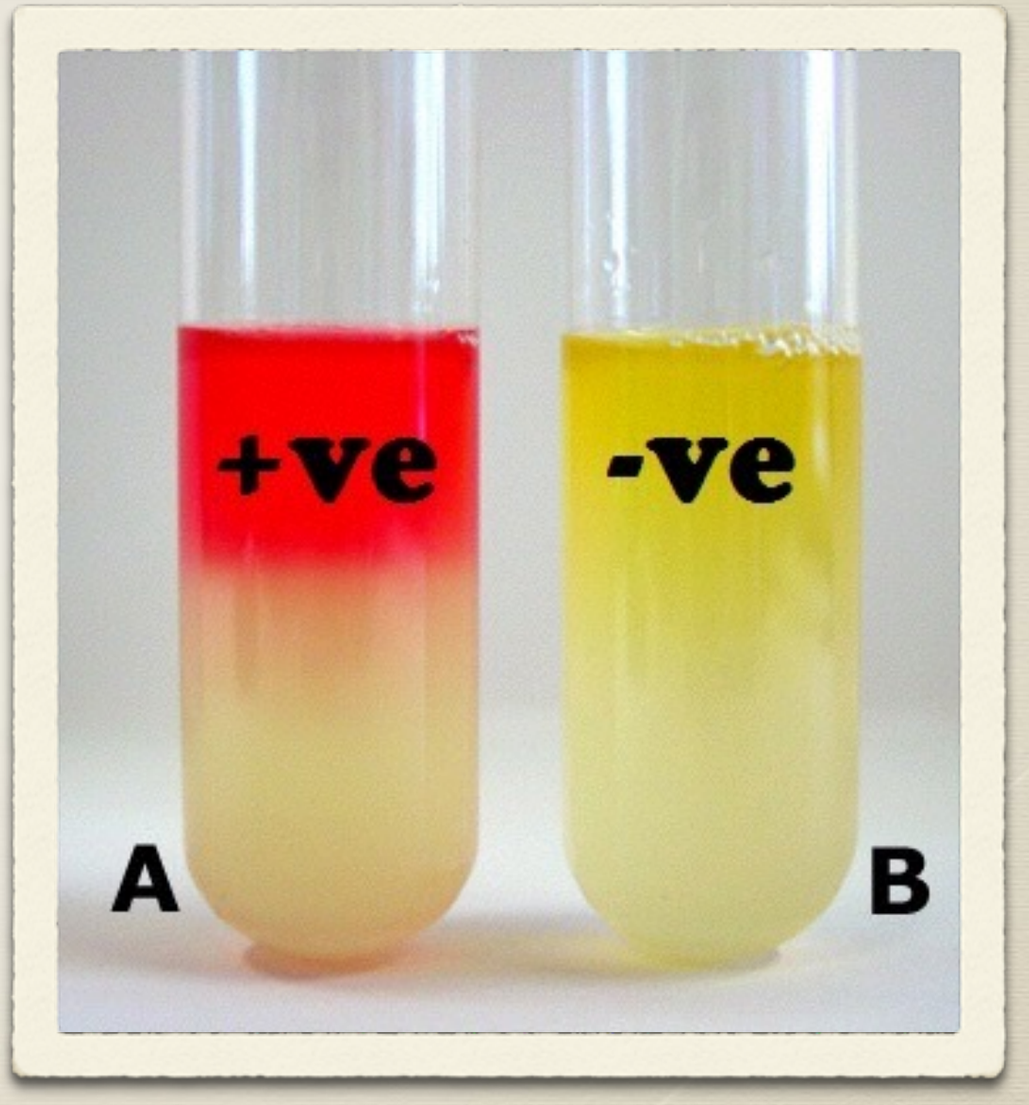
Voges-Proskauer (VP) Test

* Identify bacteria that ferment glucose to pyruvic acid and metabolize it to acetoin
* Separates Klebsiella-Enterobacter groups from E. coli (VP-negative) bacteria



Methyl-Red (MR) Test

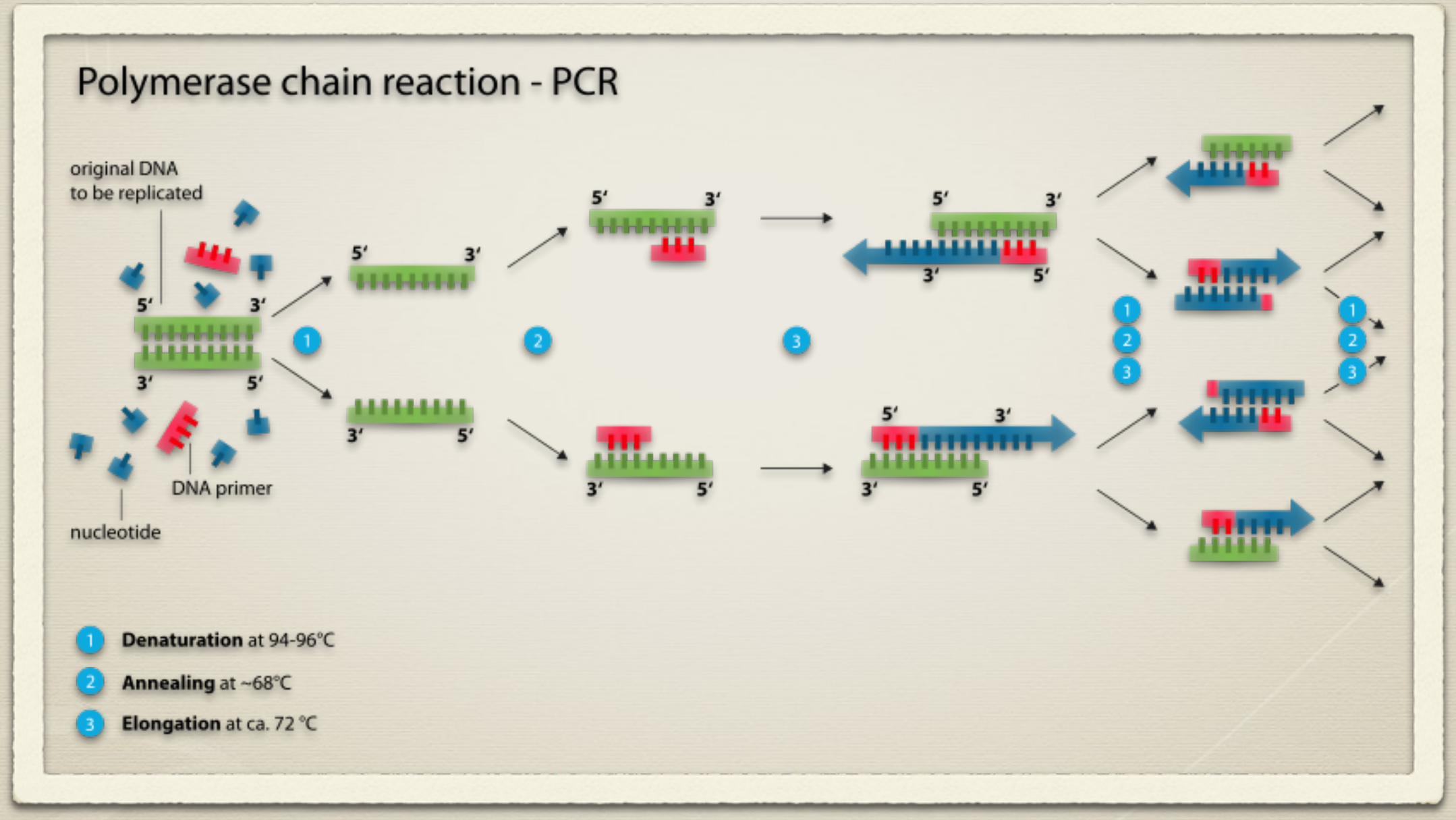
* Separates Enterobacteriaceae and Enterobacter aerogenes that decarboxylate their byproducts
* Create an alkaline environment and negative MR test



Another Identifying Method: PCR Sequencing

* Creates copies of the bacterial genome
    * Can be sequenced and compared to existing genetic databases
* Used in cases where the bacteria cannot be cultured or identified through conventional means

Another Identifying Method: PCR Sequencing



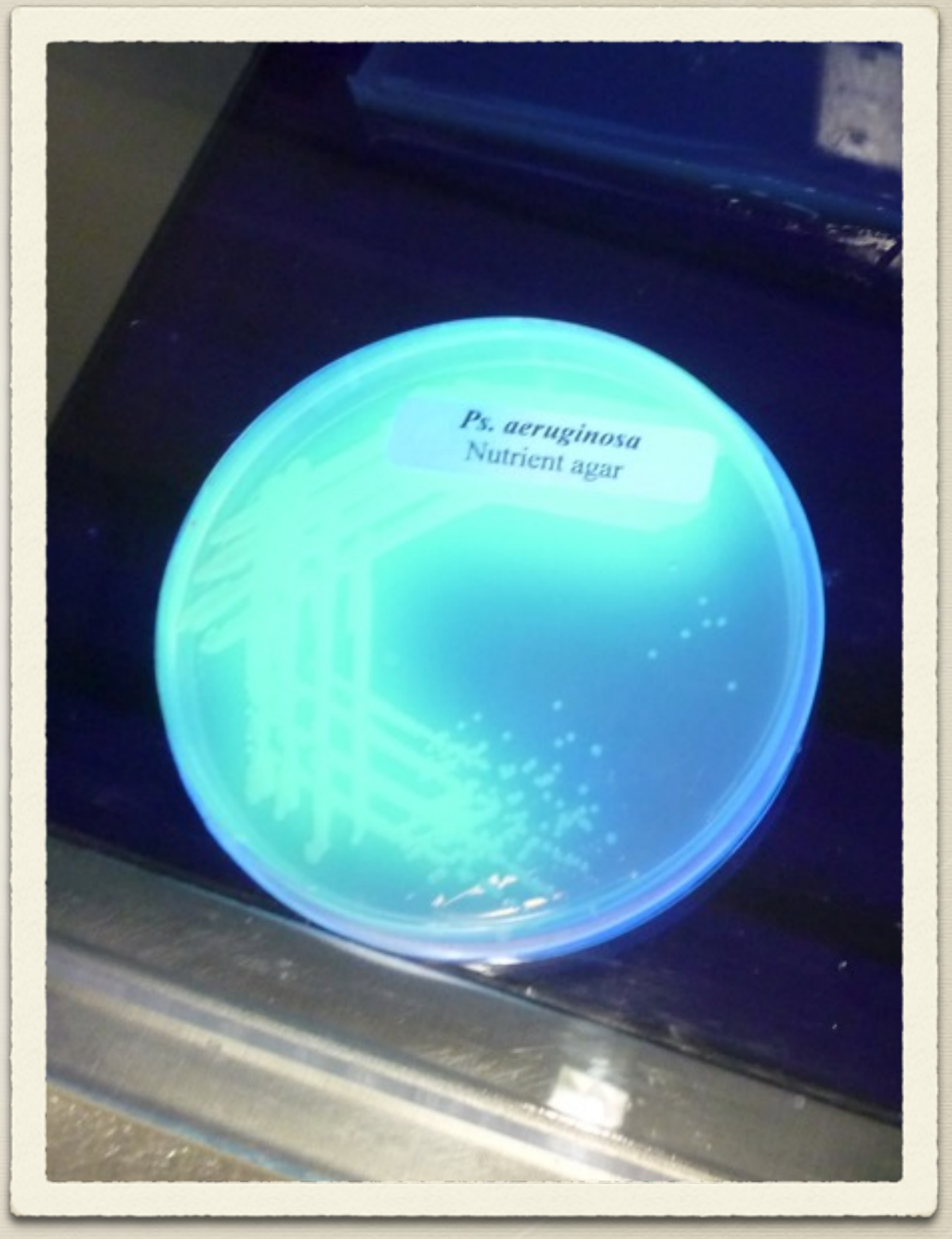
Question 4: What are the results expected from these tests allowing for the identification of the bacteria named in this case?

Oxidase and Catalase Test

* Positive test results for both tests
* Change to dark purple colour within 2min of oxidative reagent
* Distinct, grape-like odour
    * Production of aminoacetophenone
* Appear as pale yellow colonies on MacConkey agar plate
    * Non-lactose fermenting

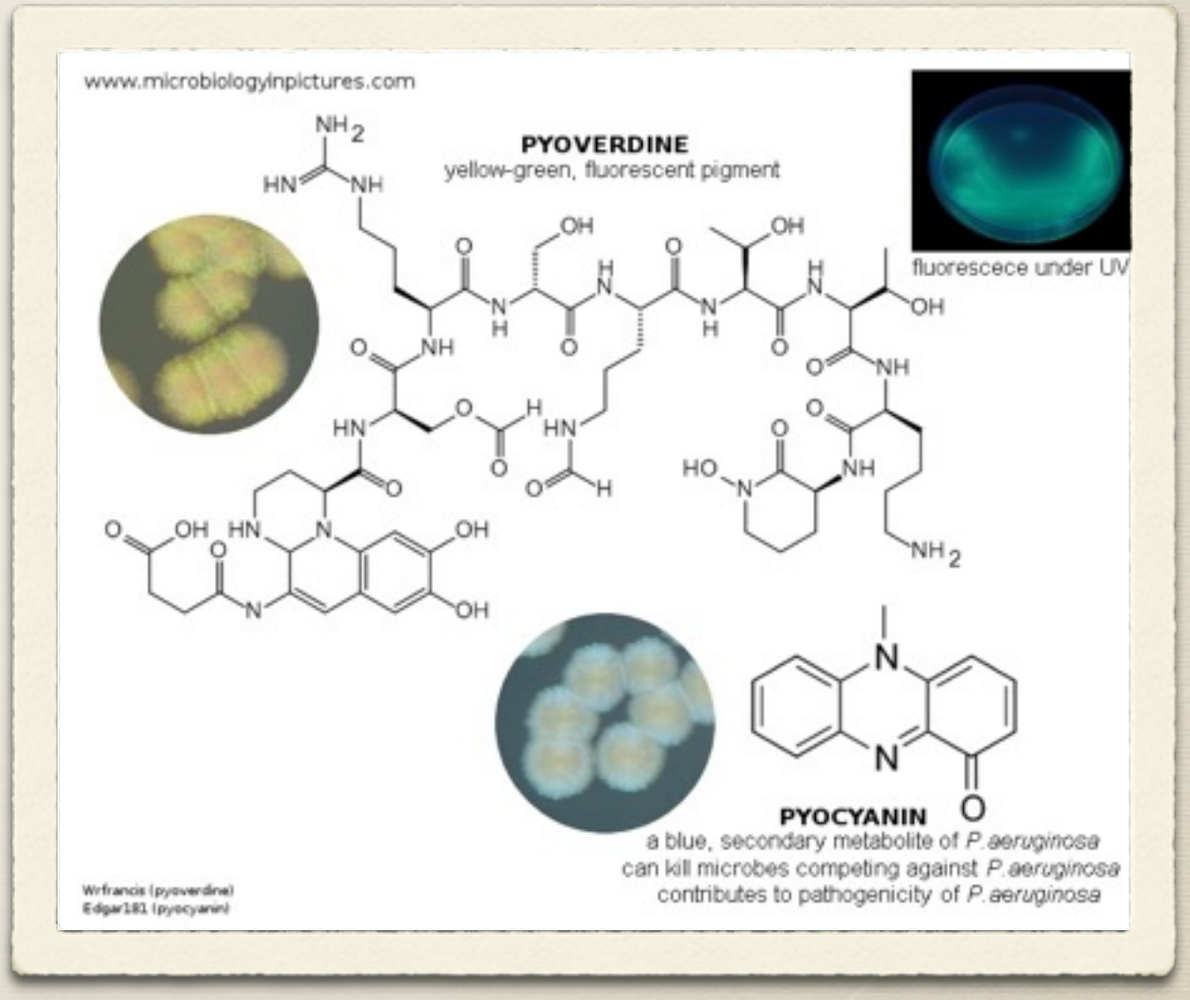
Oxidase and Catalase Test

Also: Detected by fluorescence of MacConkey agar plates under ultraviolet light.



M-PCR Test

* Pale and large translucent mucoid colonies of MacConkey agar plates.
* Most common seen pigments:
    * Pyroverdine (yellow-green).
    * Pycocyanin (blue-green).

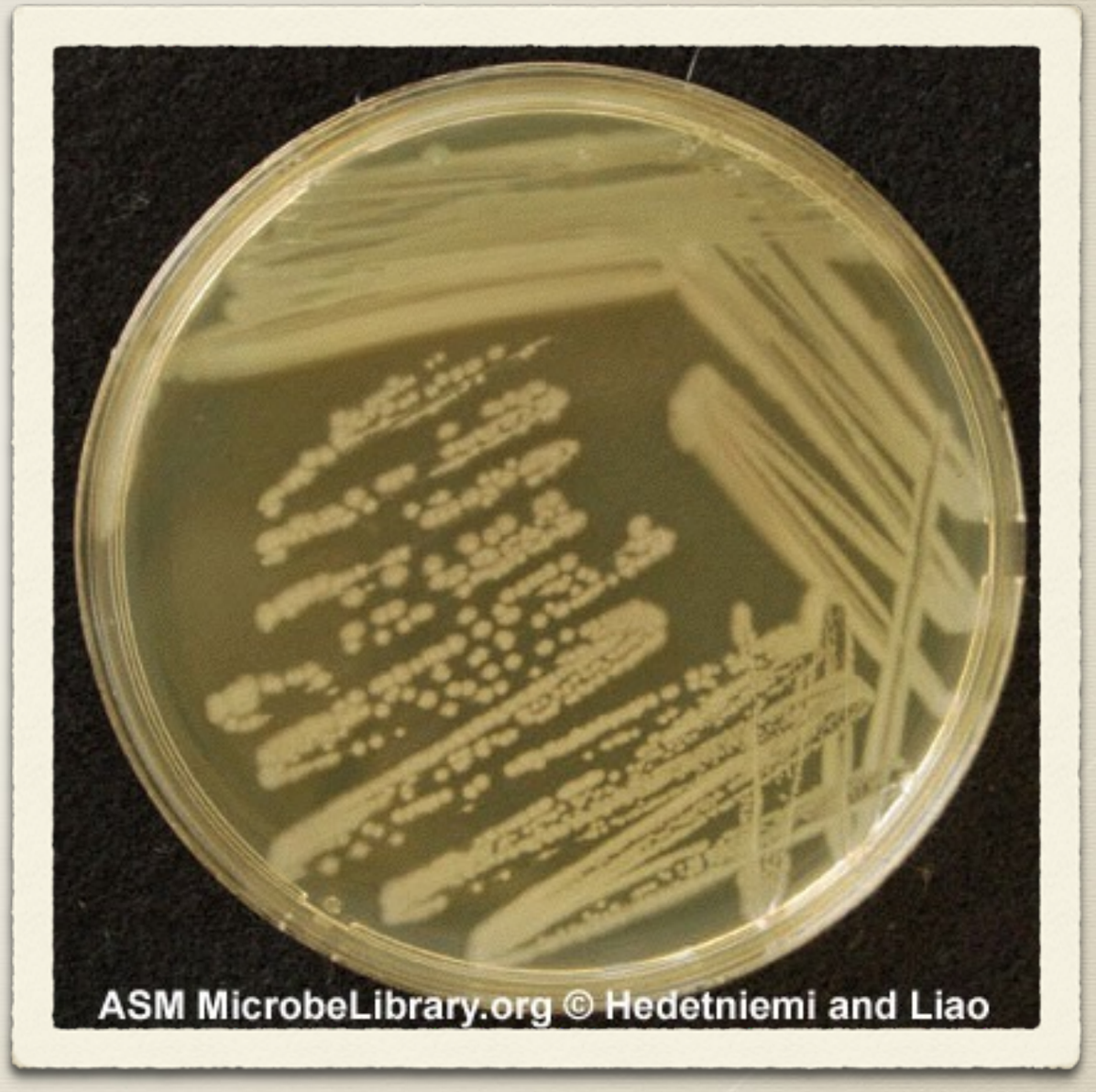


M-PCR Test

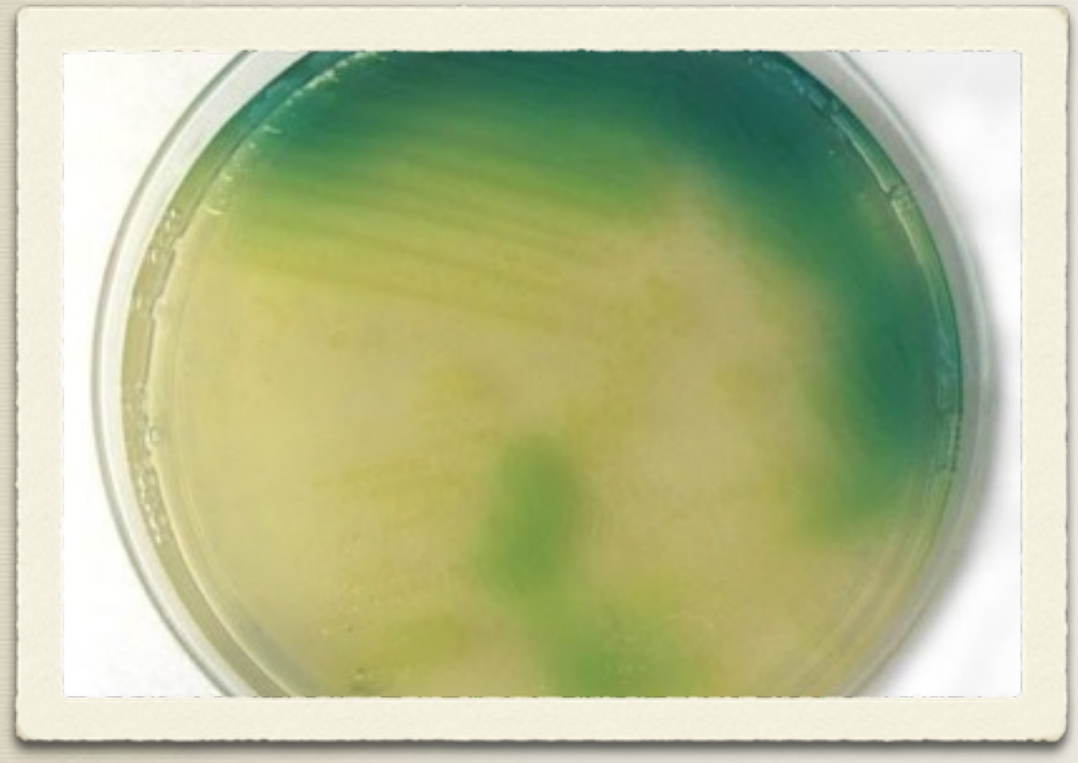
* Positive isolates display amplification of all four gene fragments:
    * gyrB
    * ETA
    * oprL
    * 16S rDNA
* Hight white blood cell count in skin biopsies

Agar Plate Test

P.aeruginosa appearance after being spread on the agar plate (sample from site of injury).



Clinical Sample Results: Two Smooth Colony Types



"Fried-egg".
Smooth, flat edges

Smooth mucoid colonies
(colonization/virulence)

Picture Sources (Question 1)

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* http://i.dailymail.co.uk/i/pix/2012/09/24/article-2207760-152DAAEF000005DC-950_468x313.jpg

Picture Sources (Question 2)

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Picture Sources (Question 3)

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* https://upload.wikimedia.org/wikipedia/commons/thumb/9/96/Polymerase_chain_reaction.svg/835px-Polymerase_chain_reaction.svg.png

Picture Sources (Question 4)

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