

INFECTION PREVENTION AND THE MICROBIOLOGY LAB

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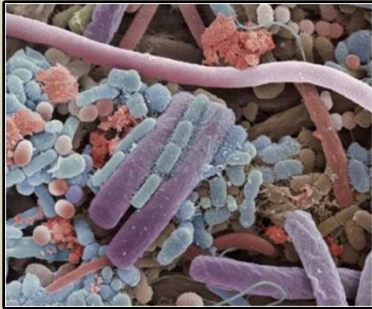


Illustration: Don Smith

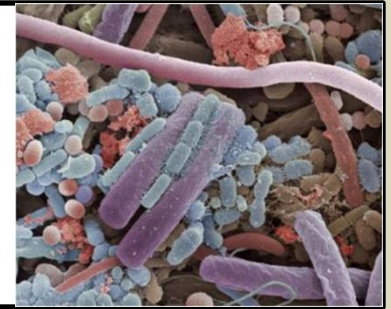


Overview

- Terminology/definitions
- Preanalytic: Specimen collection/submission
- Analytic: What happens in the Micro lab
- Postanalytic:
 - Reporting/susceptibilities
 - Interpreting the reports



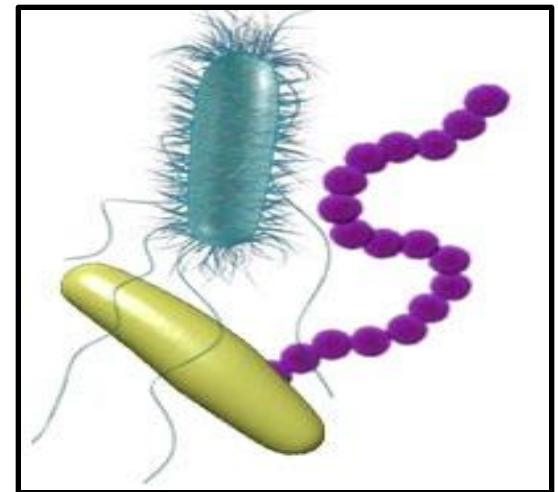
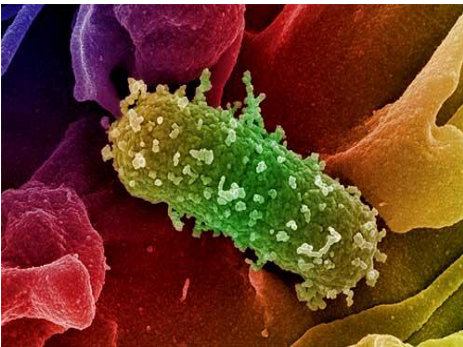
Terminology



- Normal flora:
 - Bacteria and some yeasts present at a variety of sites
 - Skin, mucosal surfaces
 - Do not cause disease under normal circumstances
 - Participate in maintaining health
- Colonizer: present on mucous membranes, noninvasive, no host response
 - VRE in stool, MRSA in nares
- Pathogen: causing infection, invasive with host response
- Normal flora and colonizers can become pathogens

Functions of Normal Flora

- Provide some nutrients (vit. K)
- Help develop mucosal immunity: stimulate immune system with cross reactivity against some pathogens
- Prevent colonization by potential pathogens
- Aid digestion



Factors Influencing Normal Flora

- Local environment
 - pH, temperature, oxygen levels, nutrients
- Diet
- Age
- Health/Immune status
- Antibiotics
- Flora changes with eruption of teeth, weaning, onset/cessation of ovarian function

Normal Flora by Site

- Most normal flora is anaerobic
- Skin
 - Coagulase negative staphylococci
 - Diphtheroids/*Corynebacterium* sp.
 - *Propionibacterium*
 - *Staphylococcus aureus*
 - Viridans group Streptococci
 - *Bacillus* sp.
 - *Malassezia furfur*
 - *Candida*
- Nares
 - Coagulase negative staphylococci
 - Viridans group streptococci
 - *Staphylococcus aureus*
 - *Neisseria/Moraxella*
 - *Haemophilus*
 - *Streptococcus pneumoniae*

- Mouth/oropharynx
 - Viridans group streptococci
 - *Veillonella* sp
 - *Fusobacterium* sp.
 - *Treponema* sp.
 - *Prevotella/Porphyromonas*
 - *Neisseria/ Moraxella*
 - *Streptococcus pneumoniae*
 - Beta hemolytic strep (*Strep mlleri/anginosus*)
 - *Candida*
 - *Haemophilus*
 - *Corynebacterium/diphtheroids*
 - *Actinomyces*
 - HACEK
 - *Staphylococcus aureus*
 - *Lactobacillus*

Normal Flora by Site

- Colon

- *Bacteroides*
- *Fusobacterium*
- *Clostridium*
- *Peptostreptococcus*
- Enteric GNRs
- *Enterococcus*
- *Lactobacillus*
- Viridans streptococci
- *Candida*



- Stomach

- *Lactobacillus*
- Viridans streptococci
- Staphylococci
- *Peptostreptococcus*

- Small Intestine

- *Lactobacillus*
- *Bacteroides*
- *Clostridium*
- *Enterococci*
- Enteric GNRs



Normal Flora by Site

- Urethra

- Coagulase negative staphylococci
- Diphtheroids/
Corynebacterium sp.
- Viridans streptococci
- *Bacteroides*
- *Fusobacterium*
- *Peptostreptococcus*

- Vagina

- *Lactobacillus*
- *Peptostreptococcus*
- Diphtheroids/
Corynebacterium sp.
- Viridans streptococci
- *Candida*
- *Gardnerella vaginalis*

Specimen Collection

- Avoid contamination from indigenous flora, to ensure a sample representative of the infectious process
- Select the correct anatomic site from which to obtain the specimen
- Submit tissue or needle aspirates when possible
- Collect adequate volumes; insufficient material may yield false negative results



Specimen Collection

- Try to collect specimens before administering antimicrobials
- Request direct smears when appropriate
- Label each specimen container with the patient's name, MR, source, specific site, date, time of collection, and initials of collector
- Designations of wound or abscess are acceptable as long as the exact anatomic location is also stated
- Transport specimen to lab ASAP



Swabs

- Limited volume
- Should only be used for specimens from mucous membranes
- Have no place in the OR
- Organisms get caught in fibers and die
- Anaerobes die upon exposure to air but survive in fluids and tissues



Blood Cultures

- Quality of collection affects microbial recovery, contamination rates, and the ability of physicians to interpret test results.
- Even with good collection technique, 1%-3% of blood cultures are found to be contaminated (rates are higher in teaching hospitals and EDs)
- Meticulous attention to skin antisepsis is necessary to prevent contamination



Blood Cultures

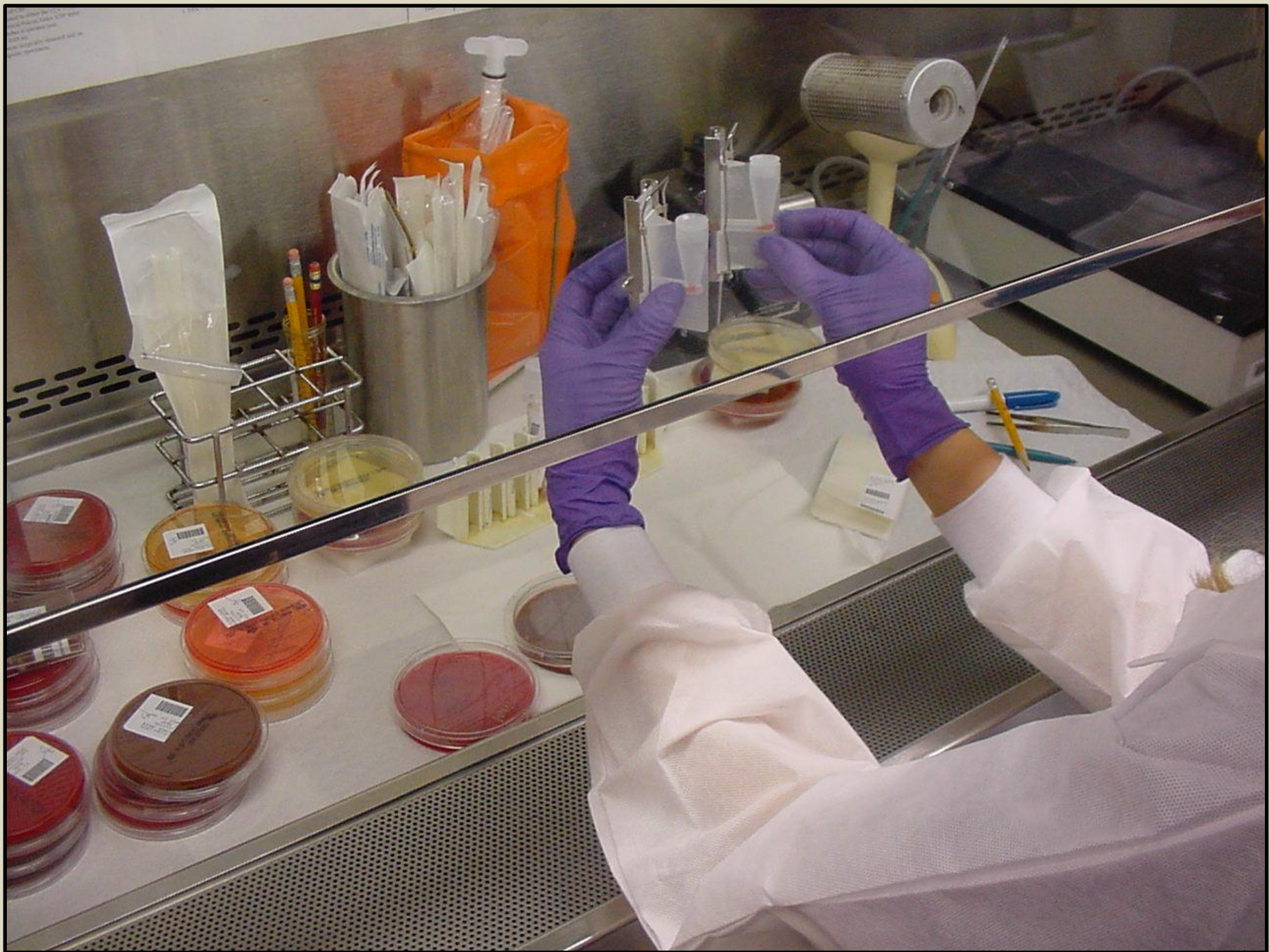
- 2-3 cultures from different venipuncture sites are recommended
- A single culture is inappropriate
- A single draw for multiple sets is inappropriate
- Volume of blood cultured is the most important variable in recovering a pathogen
- 20 cc should be drawn from each venipuncture site with 10cc added to each bottle (aerobic and anaerobic)

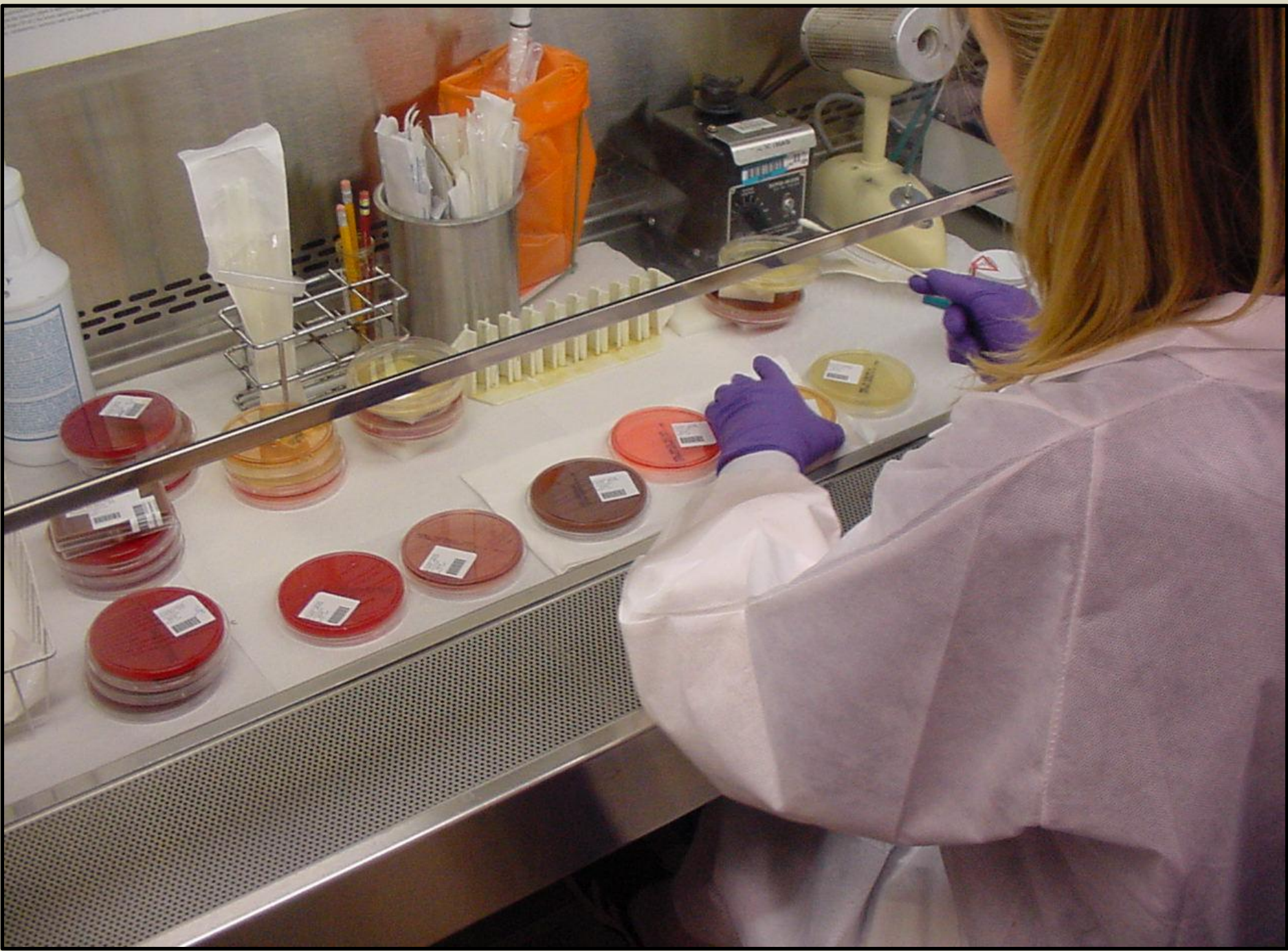
Specimen Rejection

- No label or requisition does not match specimen
- Prolonged transport
- Improper or leaking container
- Specimen unsuitable for request
- Duplicate specimens on the same day for the same request (except blood and tissue)
- Sputum specimens consisting of oropharyngeal secretions
- Routine bacterial stool cultures on patients in-house >3 days

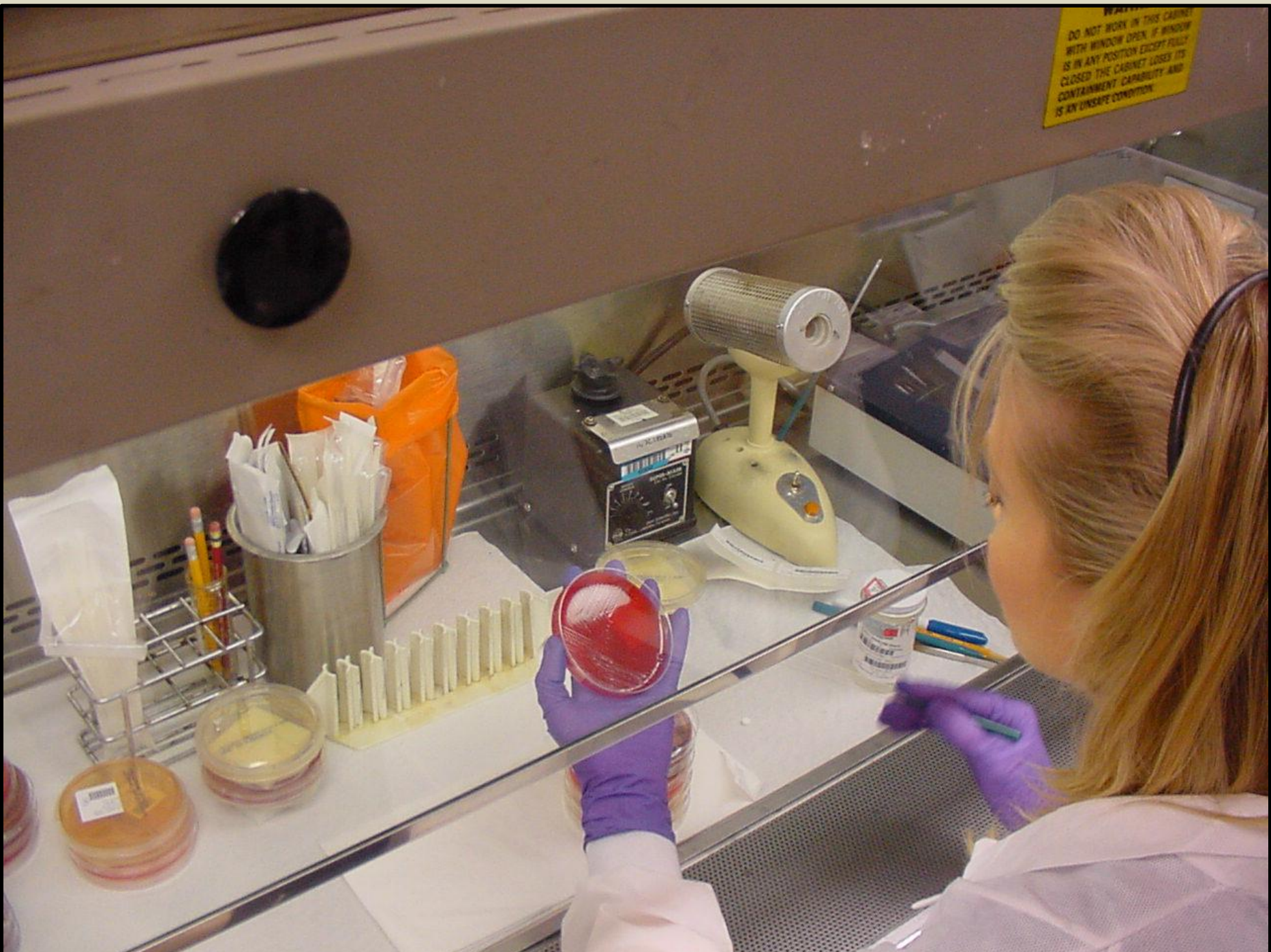




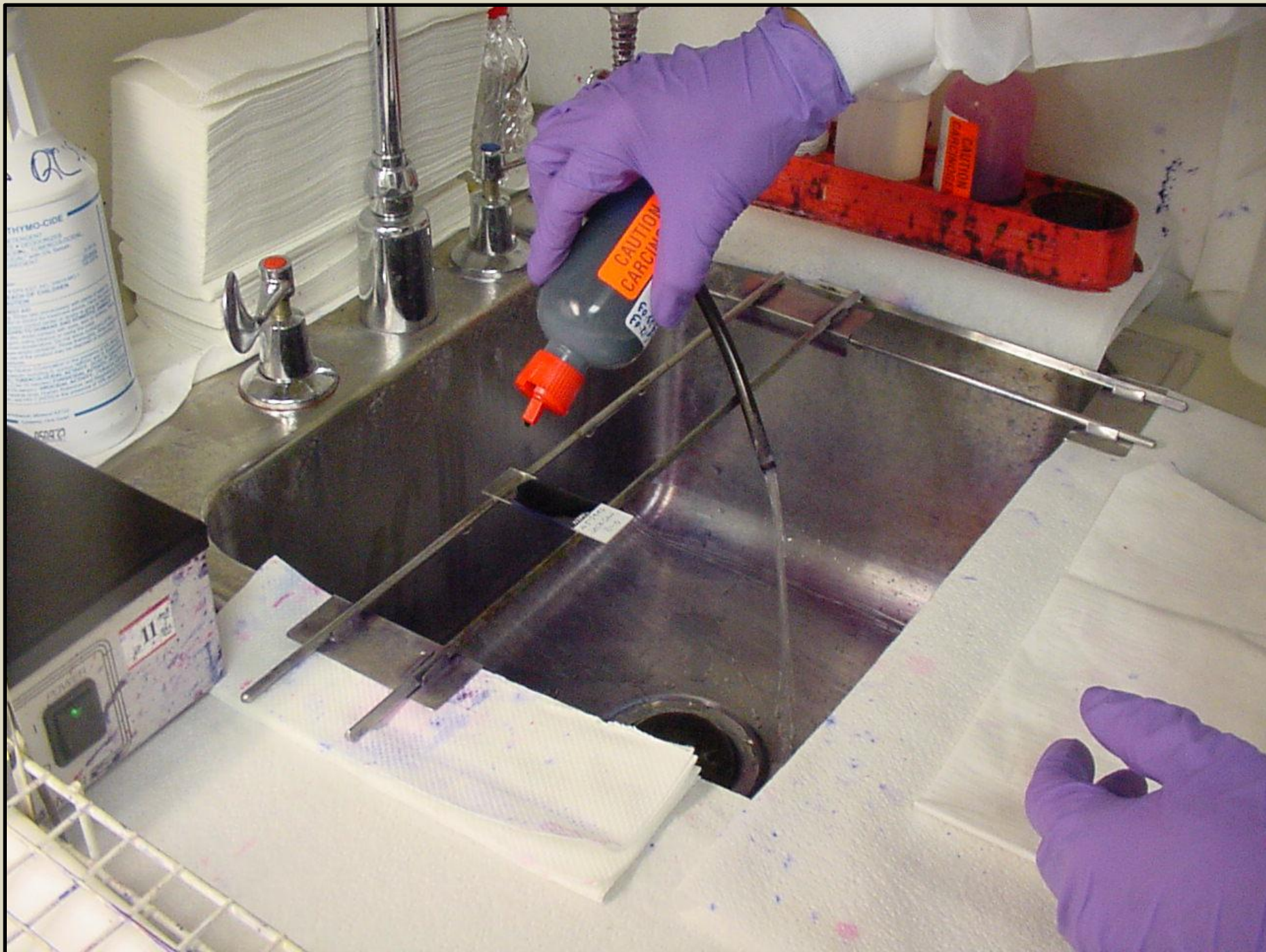




WARNING
DO NOT WORK IN THIS CABINET
WITH WINDOW OPEN. IF WINDOW
IS IN ANY POSITION EXCEPT FULLY
CLOSED THE CABINET LOSES ITS
CONTAINMENT CAPABILITY AND
IS AN UNSAFE CONDITION.

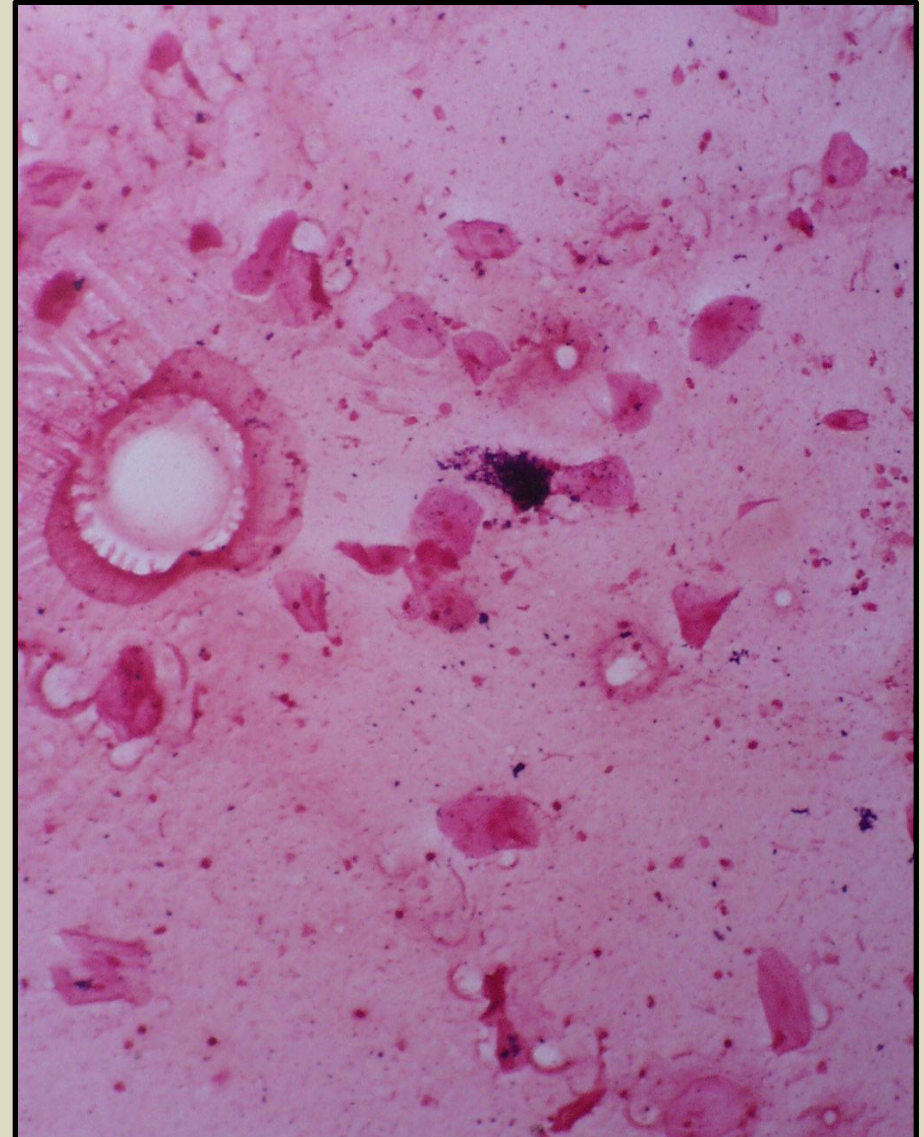
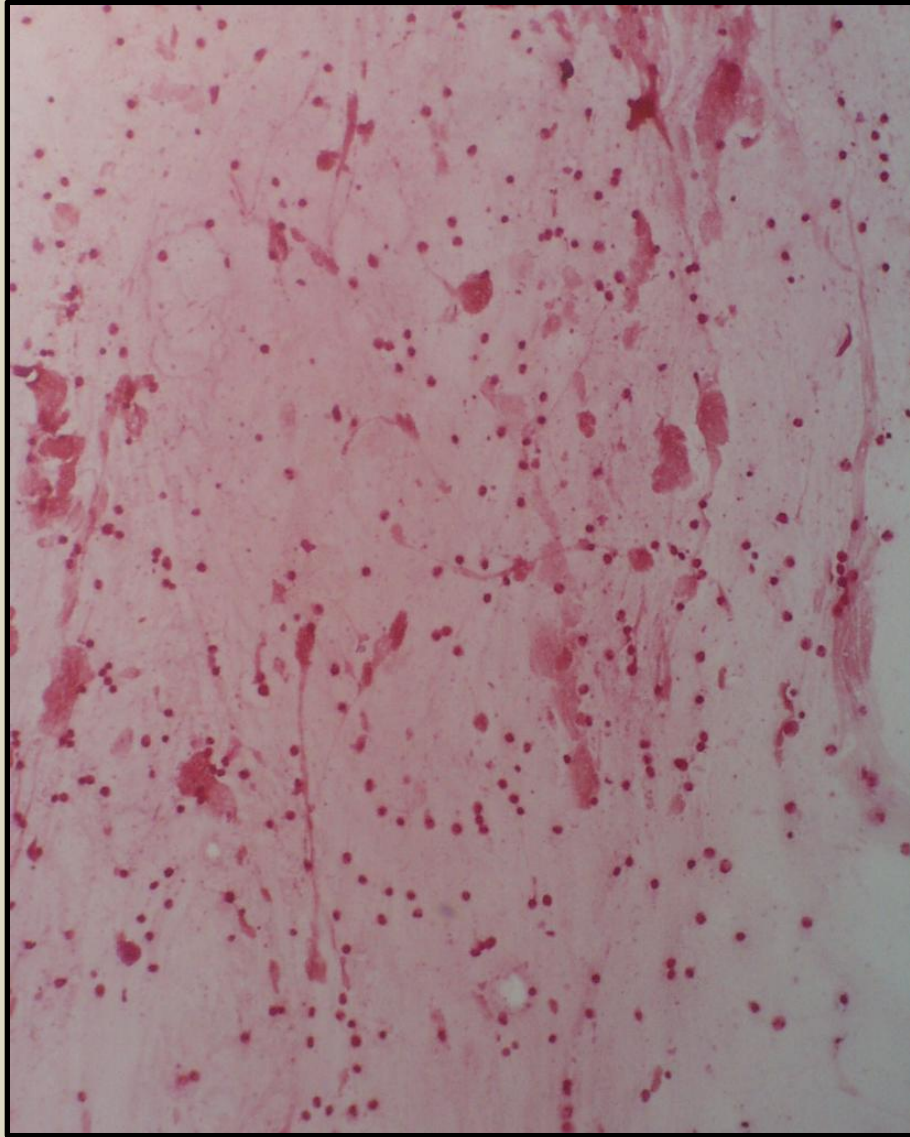




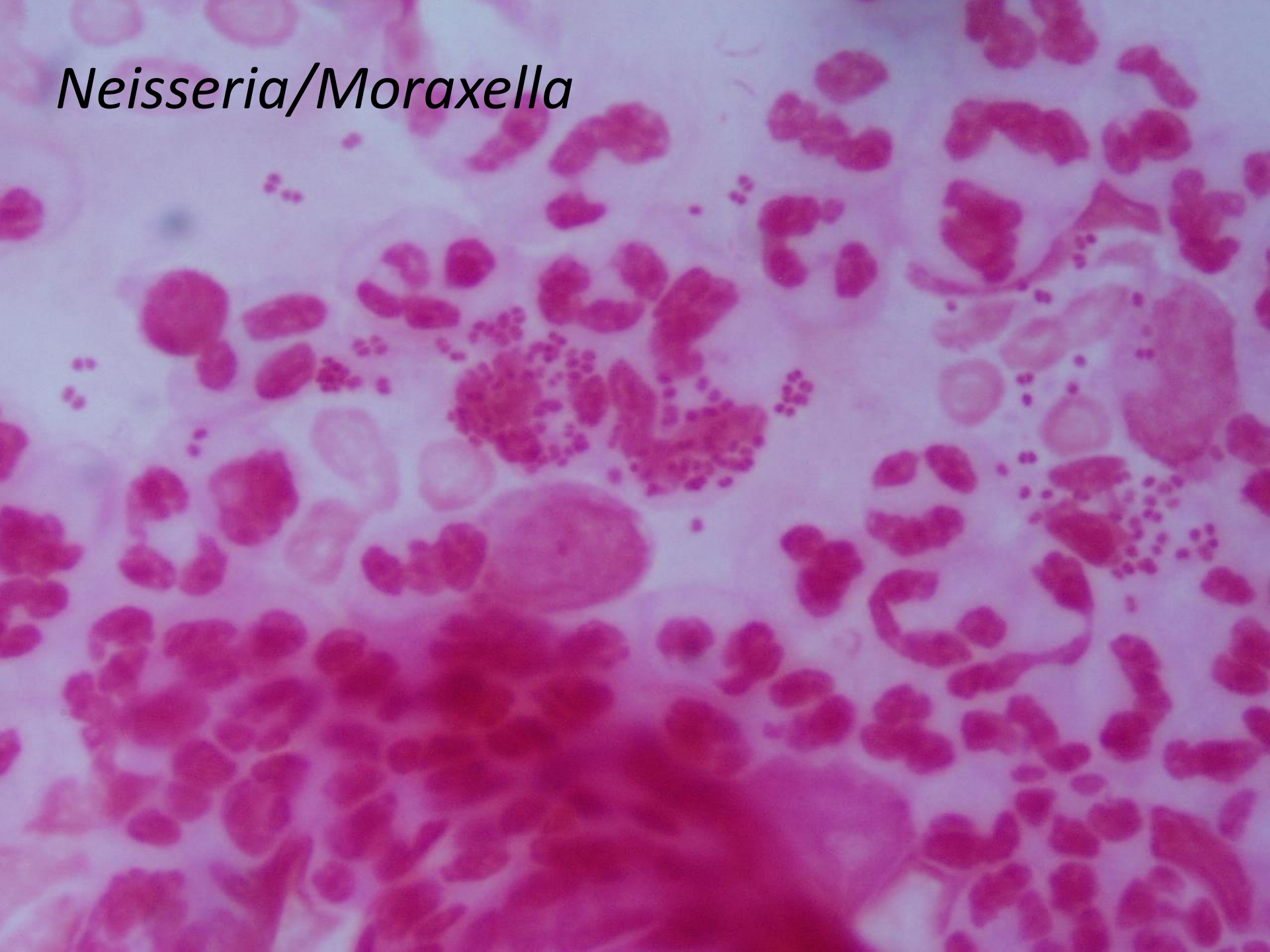




Sputum

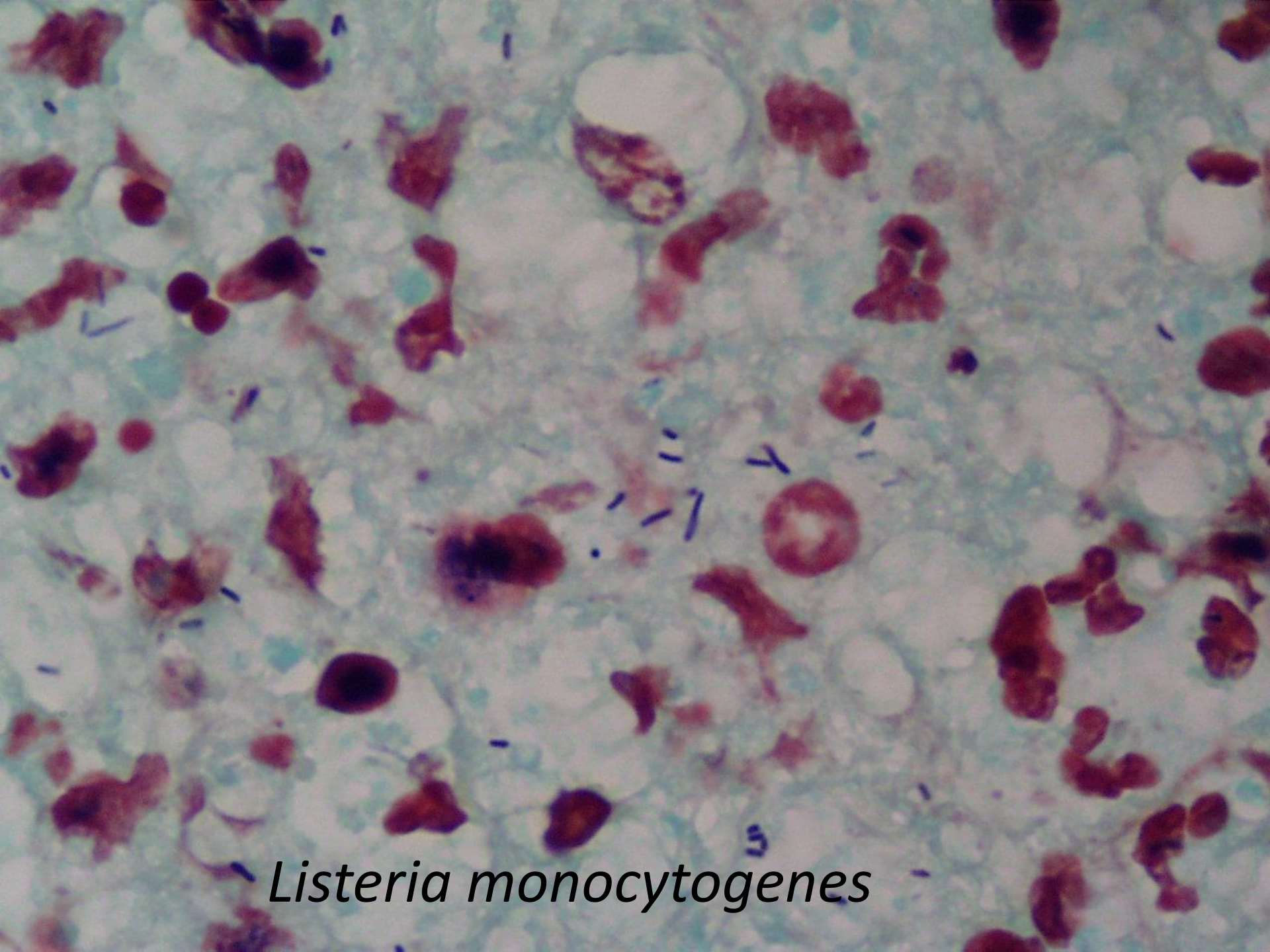


Neisseria/Moraxella



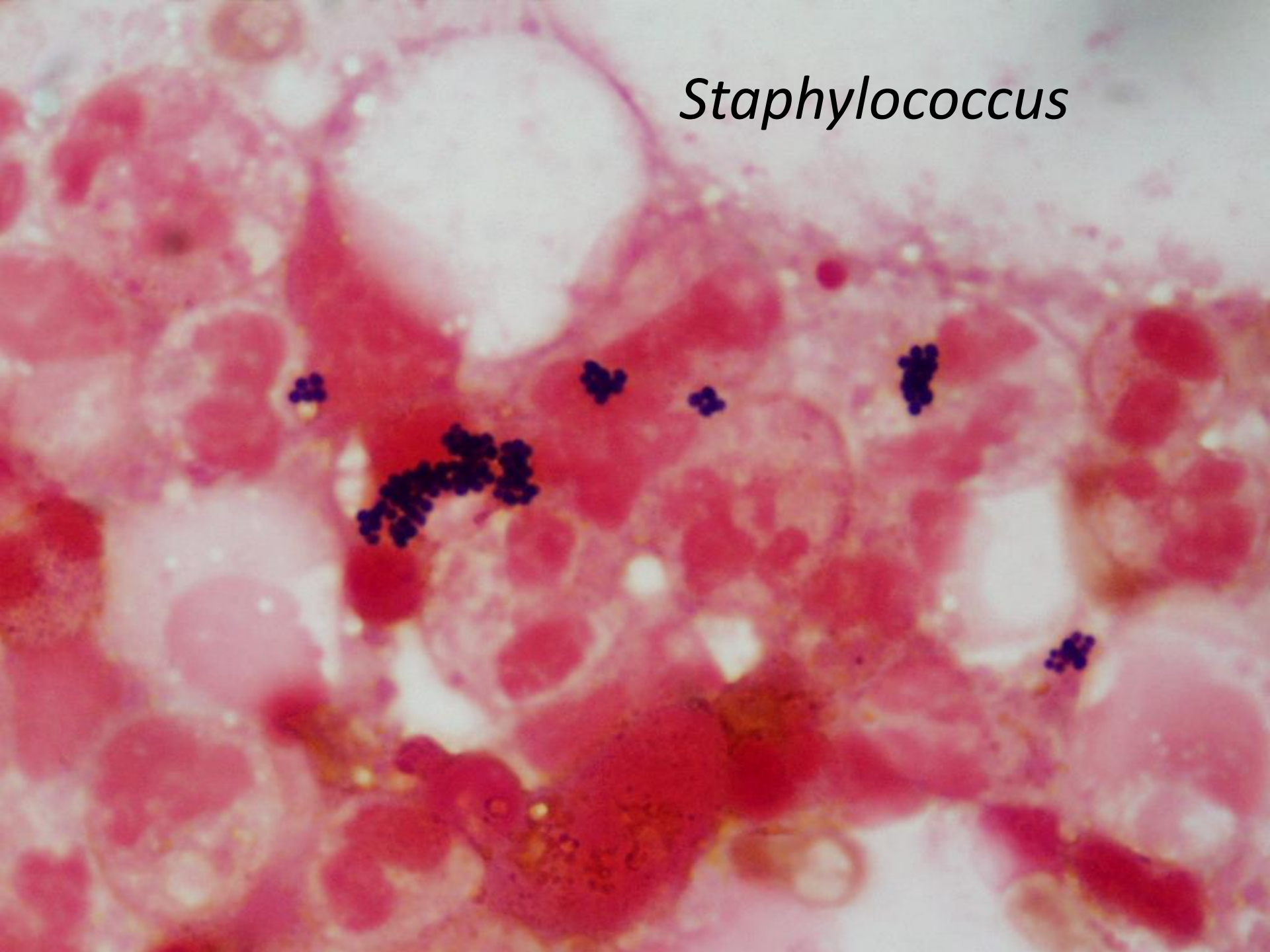


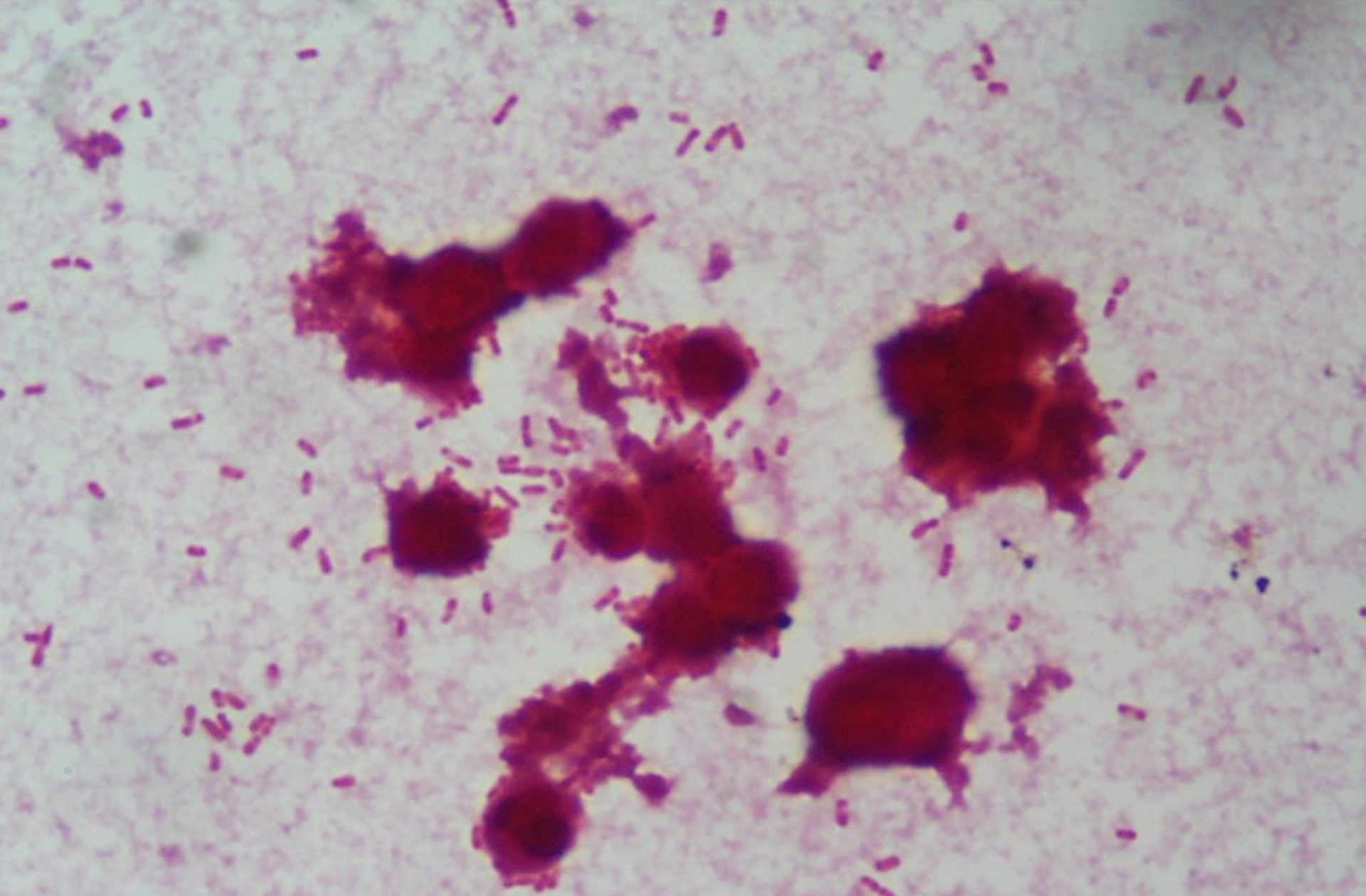
Haemophilus influenzae



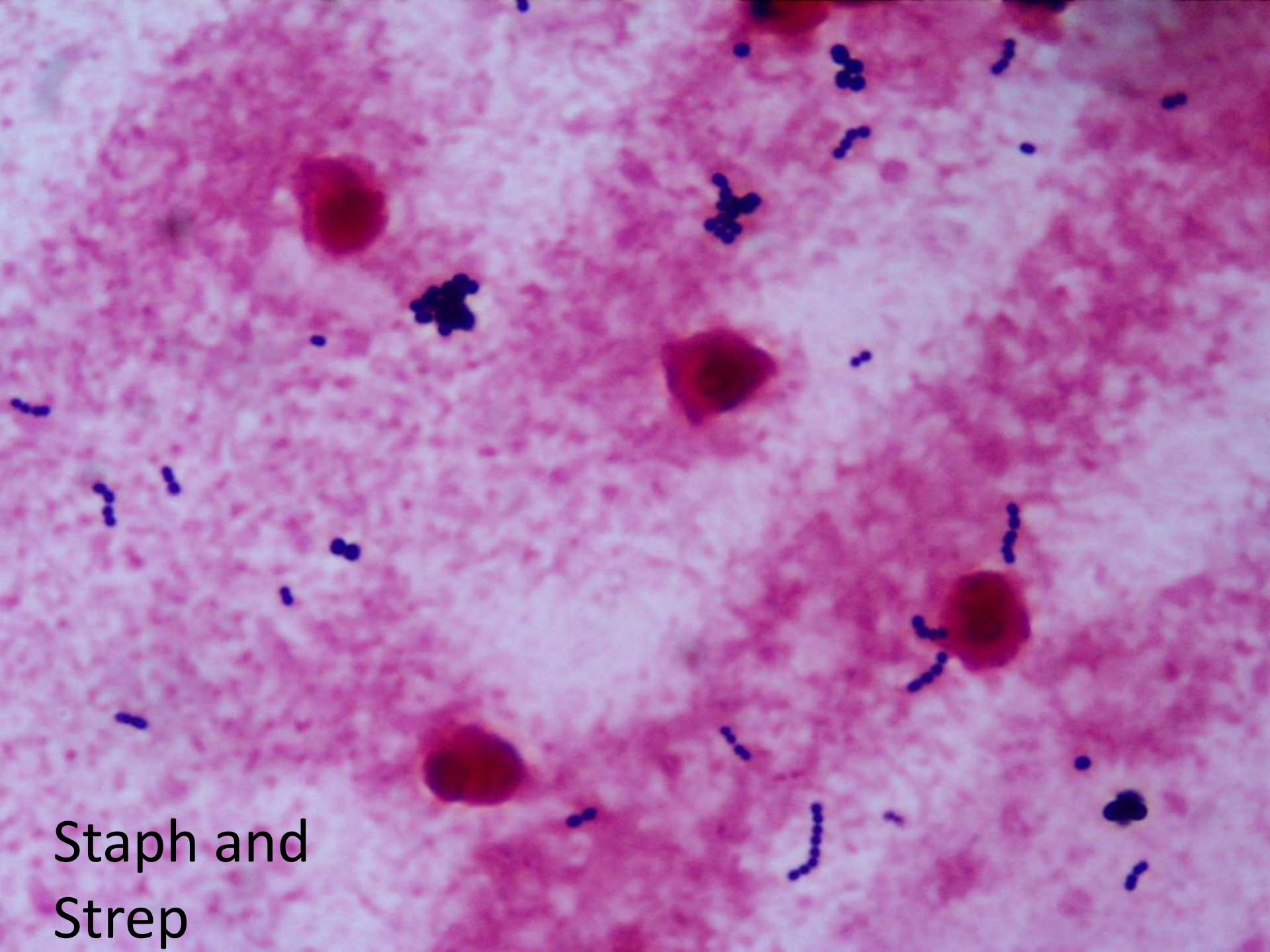
Listeria monocytogenes

Staphylococcus





Gram Negative Rods

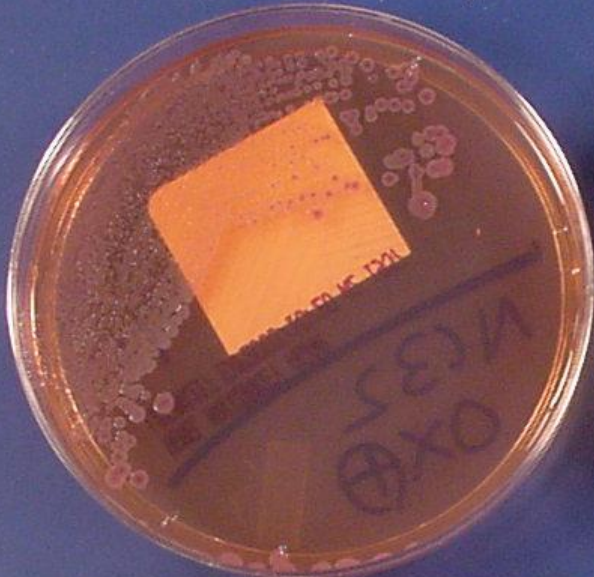


Staph and
Strep





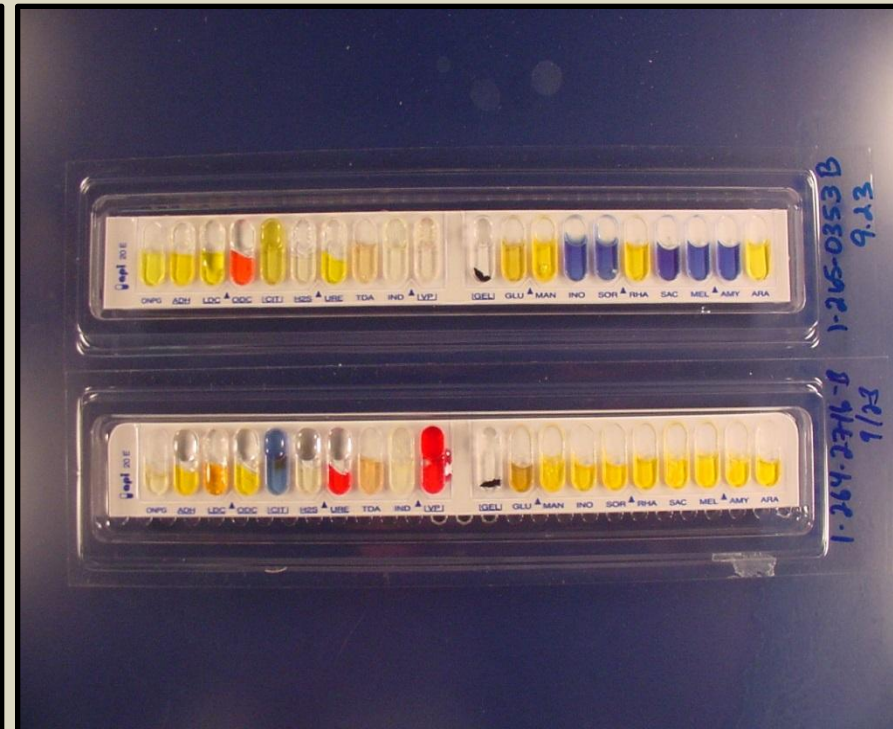
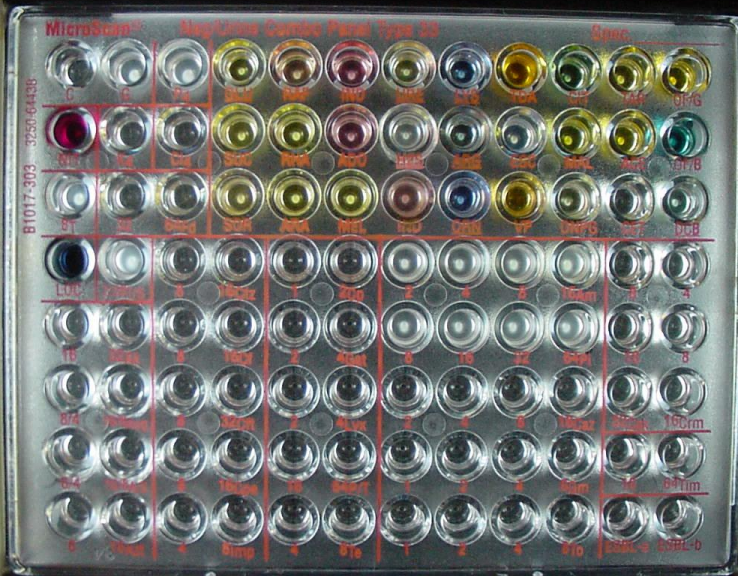




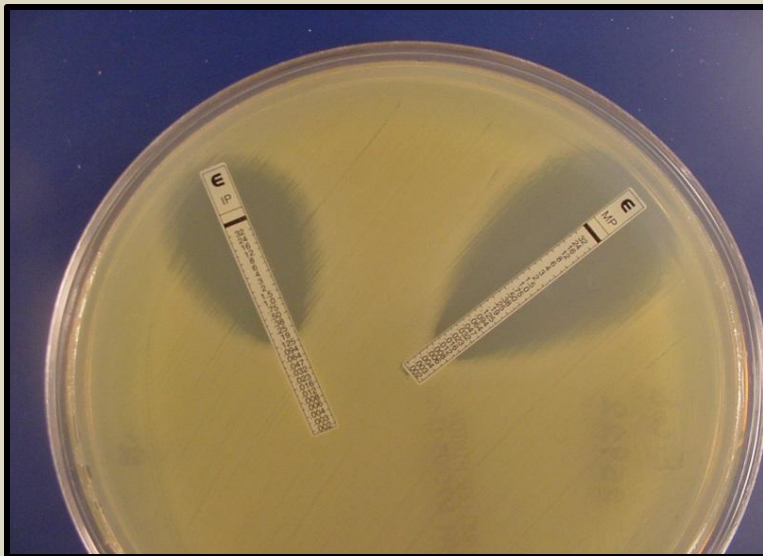


Identification

- Quick tests
- Automated systems
- MALDI-TOF



Susceptibility Testing

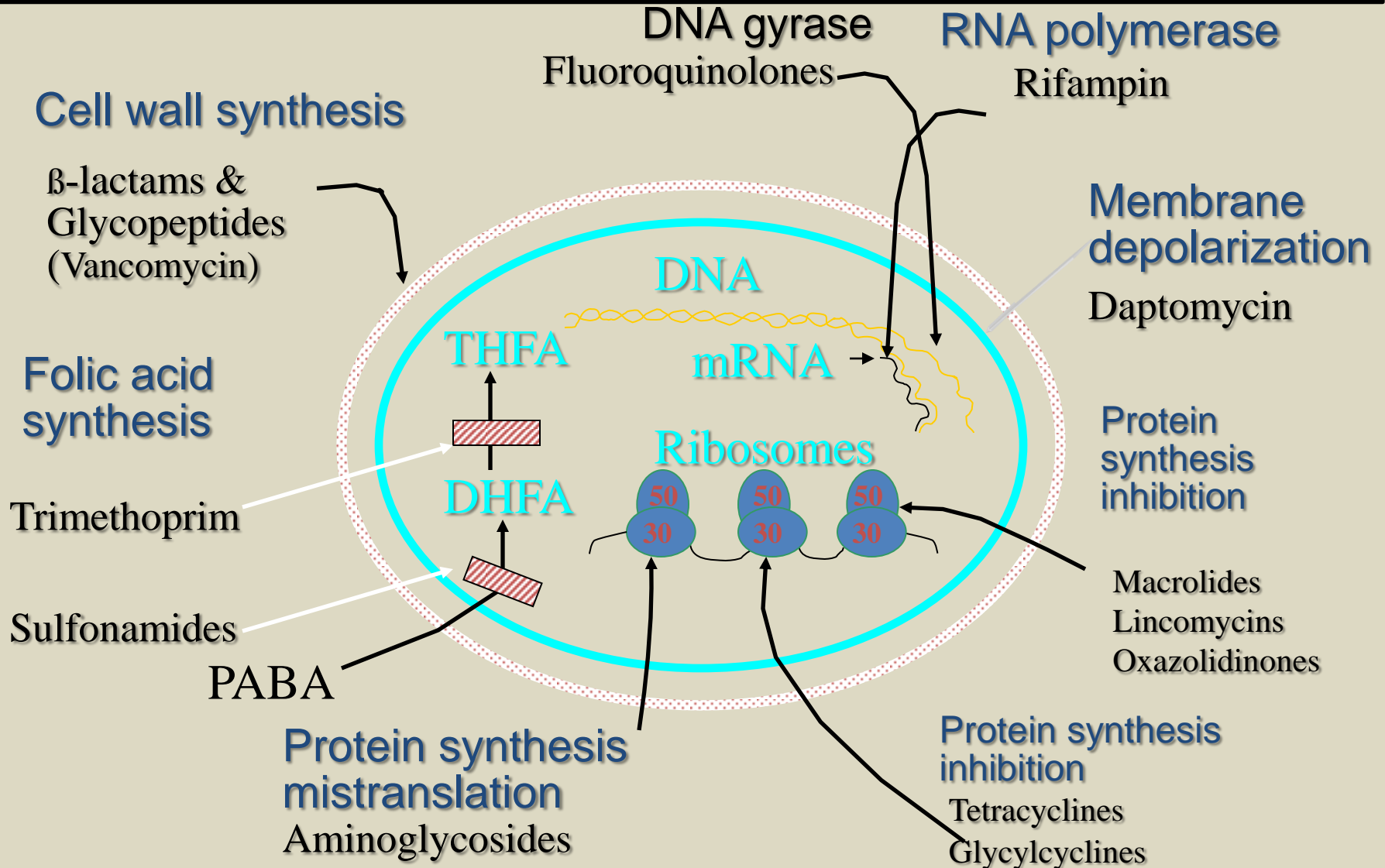


Susceptibility Report

- Moderate Growth *Escherichia coli*

| Antibiotic | M.I.C. | Interpretation |
|-------------------|--------|----------------|
| Ampicillin | >16 | R |
| Cefotaxime | <=8 | S |
| Ciprofloxacin | <=1 | S |
| Gentamicin | <=2 | S |
| Levofloxacin | <=1 | S |
| Piperacillin/Tazo | 64 | I |
| Trim/Sulfa | >2/38 | R |
| Ticarcillin/Clav | >64 | R |
| Tobramycin | <=4 | S |

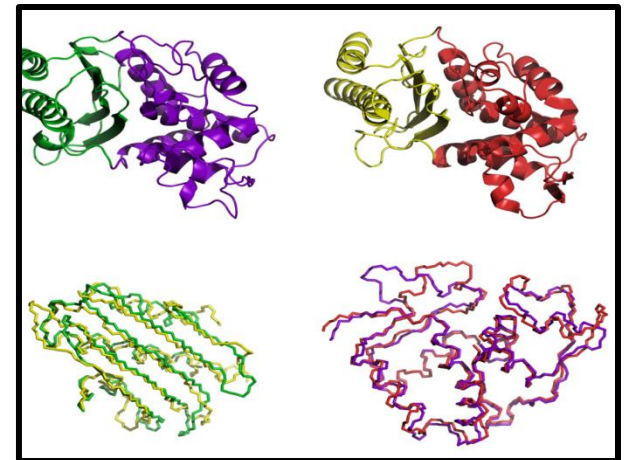
Antibiotic Mechanisms of Action



| Resistance Mechanism | Examples |
|--|--|
| Diminished Intracellular Drug Concentration | |
| Decreased Outer Membrane Permeability | β -Lactams (OmpF, OprD) |
| Decreased Cytoplasmic Membrane Transport | Quinolones (OmpF) Aminoglycosides (decreased energy) |
| Increased Efflux | Tetracyclines (tetA) Macrolides (mefA) |
| Drug Inactivation | β -Lactams (β -lactamases) Aminoglycosides (modifying enzymes) |
| Target Modification | Quinolones (gyrase modifications) β -Lactams (PBP changes) |
| Target Bypass | Glycopeptides (vanA, vanB) |

Mechanisms of Beta-Lactam Resistance

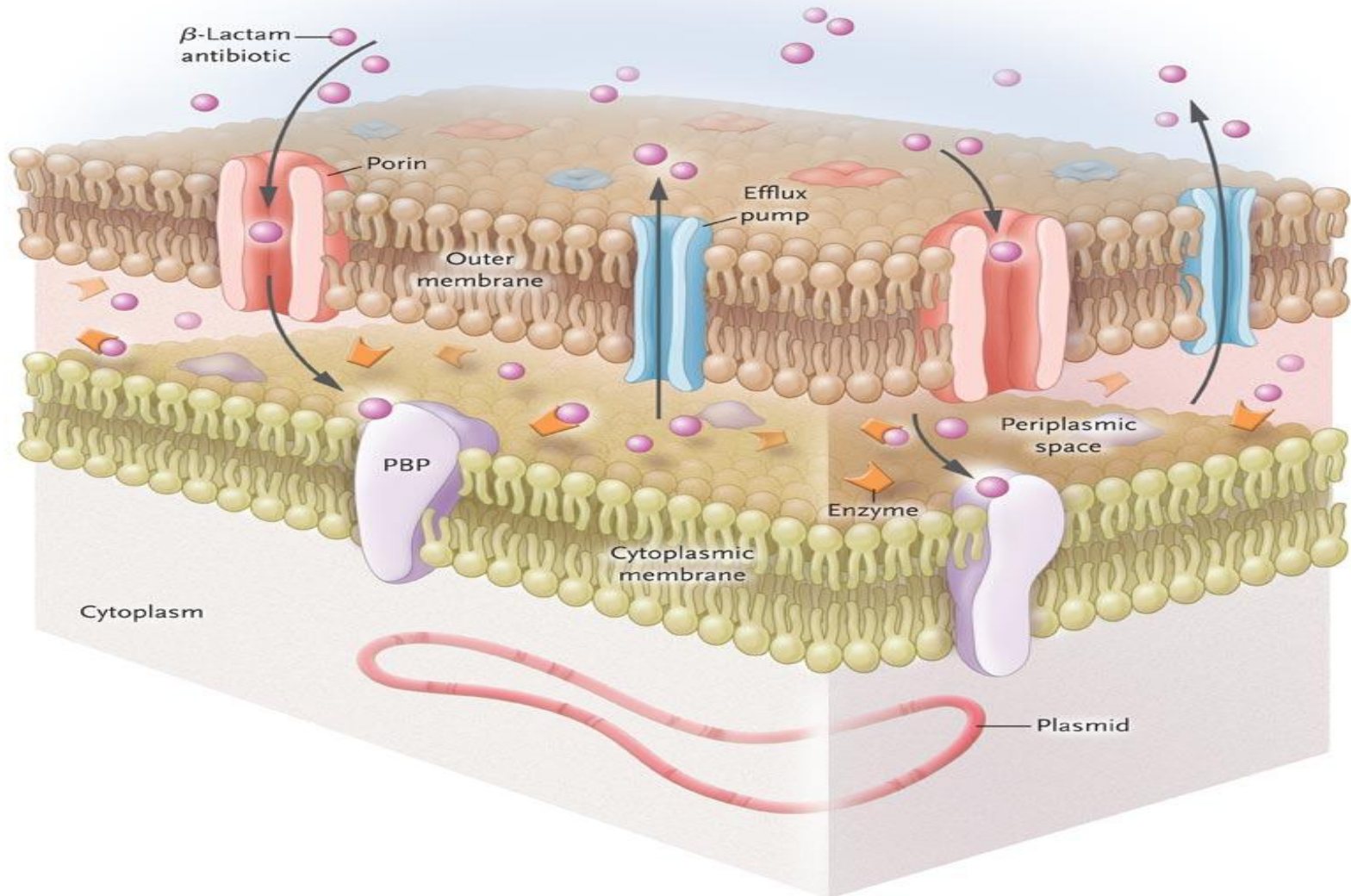
- β lactamases (Gm-/Gm+)
 - Hundreds of different types
 - We can only test for a few of them
- Altered/acquired PBPs (Gm-/Gm+)
- Decreased entry and/or active efflux (Gm-)



Selected β -Lactamases of Gram Negatives

| β -Lactamase | Examples | Substrates |
|--------------------|-------------------------|---|
| Broad Spectrum | TEM-1, TEM-2, SHV-1 | Pen, amp, cefazolin, cefuroxime |
| ESBL | TEM, SHV | Cefotaxime, Ceftriaxone, ceftazidime, aztreonam, cefepime |
| | CTX-M | Cefotax>Ceftaz |
| AmpC | OXA | Ceftaz>Cefotax |
| | ACC, FOX, MOX | Same as ESBLs+ cephamycins, - CPE |
| Carbapenemase | KPC-1, 2, 3 | Same as ESBL+cephamycins and carbapenems |
| | IMP, VIM, NDM (metallo) | Same as KPC w/o aztreonam |

Potential Mechanisms of Antimicrobial Resistance



Antibiogram

Staphylococci

| Percent Susceptible by Broth Microdilution | No. Tested | Penicillin (a) | Nafcillin, Oxacillin (b,c) | 1st generation Cephe ms (c) | Vancomycin | Erythromycin | Clindamycin (d) | Gentamicin | Trimeth/Sulfa | Moxifloxacin | Tetracycline (Doxy) | Linezolid |
|--|------------|----------------|----------------------------|-----------------------------|------------|--------------|-----------------|------------|---------------|--------------|---------------------|-----------|
| Staphylococcus aureus, ALL(b) | 1827 | 14 | 62 | 62 | 100 | 50 | 75 | 98 | 99 | 60 | 95 | 100 |
| MRSA(ONLY) (c) | 692 | 0 | 0 | 0 | 100 | 5 | 45 | 96 | 98 | 12 | 96 | 100 |
| Staph. epidermidis | 62 | 11 | 23 | 23 | 100 | 33 | 62 | 63 | 63 | | | |
| Staph. lugdunensis | 15 | 57 | 93 | 93 | 100 | 93 | 100 | 86 | 93 | | | 100 |
| Staph. coagulase negative (other) | 424 | 13 | 33 | 33 | 100 | 41 | 62 | 61 | 57 | 38 | | 100 |
| Cost (\$) | | \$ | \$ | \$ | \$ | \$ | \$\$ | \$ | \$ | \$\$ | \$ | \$\$\$ |

(a) Penicillin-resistant staphylococci should be considered resistant to all penicillinase-sensitive penicillins, including ampicillin, amoxicillin, mezlocillin, piperacillin and ticarcillin.

(b) For empiric therapy where *S. aureus* is a potential pathogen, nafcillin and first generation cephalosporins are recommended drugs of choice for infections other than serious or systemic, for which vancomycin should be used until the susceptibility results are available.

(c) Oxacillin resistant staphylococci (MRSA & MRSE) should be considered resistant to all penicillins, cephalosporins, imipenem and beta-lactams including combinations with clavulanic acid, sulbactam and tazobactam. Oxacillin susceptibility predicts susceptibility to all other beta-lactams.

(d) Clindamycin induction test not performed on all staphylococcal isolates.

Determining relatedness

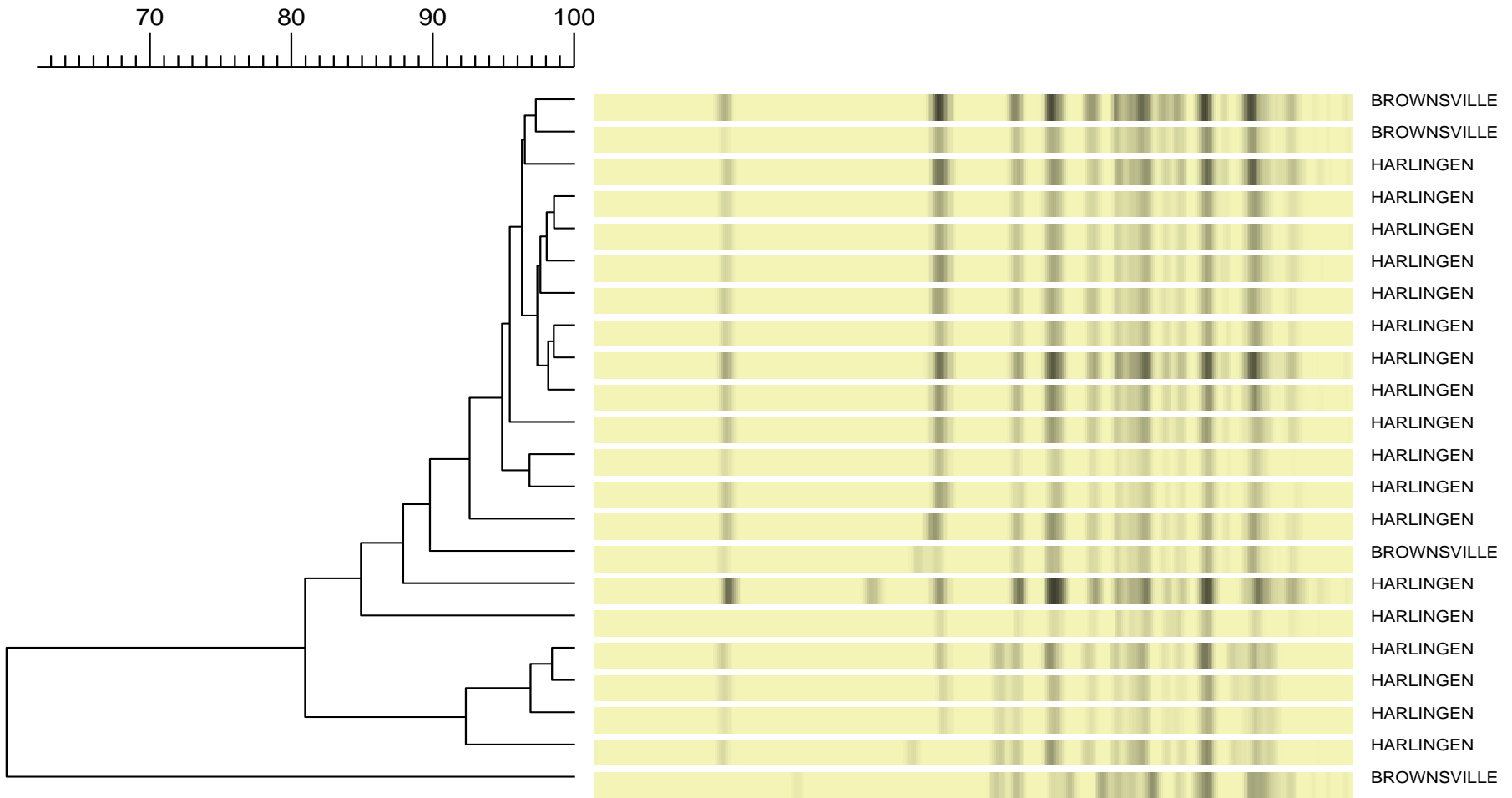
To identify and subtype pathogenic bacteria

- **Phenotype**
 - Antibiotic susceptibility
 - Unusual organism
- **Genotype**
 - Pulsed-Field Gel Electrophoresis (PFGE)
 - Restriction Fragment Polymorphisms (RFLP)
 - Polymerase Chain reaction (PCR)



WHY DO WE NEED TO SUBTYPE BACTERIA?

- Surveillance
- Epidemiology
 - DNA-based methods allow discrimination of strains that are indistinguishable based on biochemical or serological test
- Control of disease
 - Computerized data base at CDC-P for cross reference of isolates aids in:
 - Tracking of isolates
 - Emergency response
 - Assists in epidemiological studies
 - Develop control and education programs



DSHS Lab Dendrogram (image) of PFGE results
from HSR 11 January1- May 21 2008

Environmental Cultures

- Outbreak situations
- Educational purposes
- Soiled Equipment
- Routine monitoring



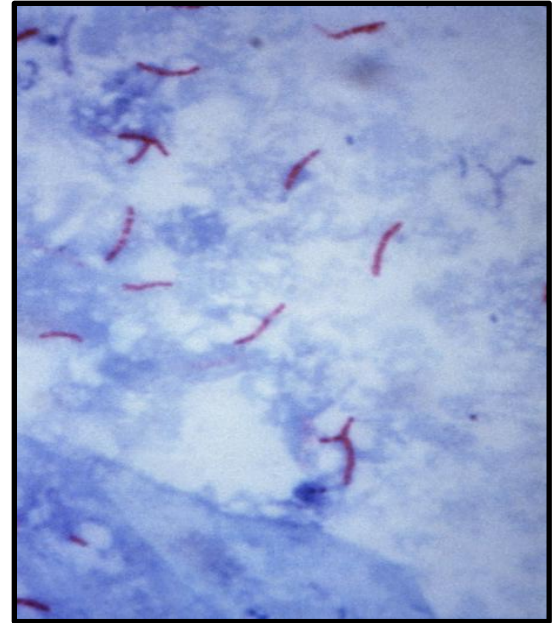
(C) 2007 Daniel Friedman - inspect-ny.com



Curiosity- Remember, you have to do something with the result

Acid Fast Bacilli (AFB)

- Mycobacteria
- High lipid content in cell wall
- Stain poorly with Gram stain
- Stain using carbol fuchsin
- Resist decolorization with acid-alcohol
- All specimens get a direct stain
- Sputum require decontamination and concentration procedure
- Require different media and longer incubation periods for isolation and identification



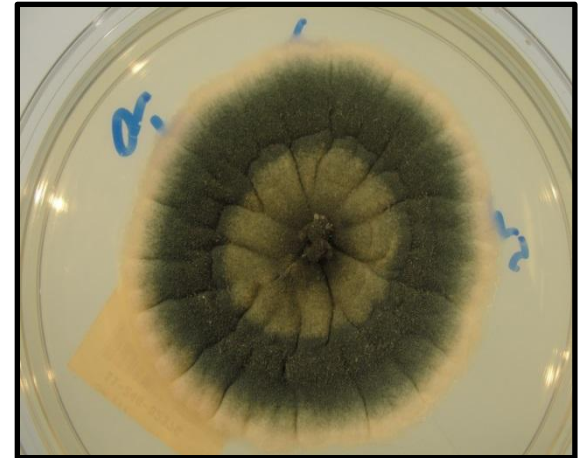
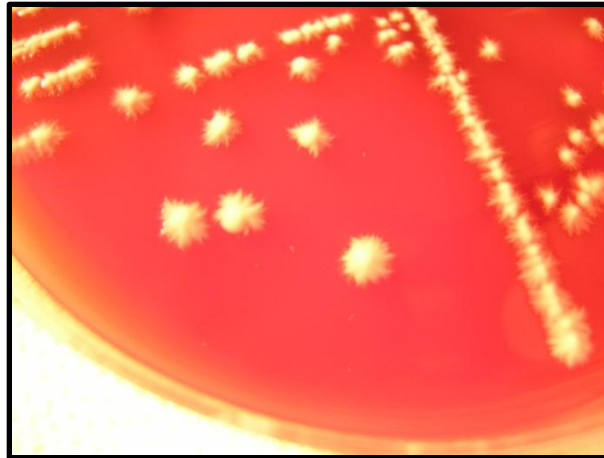
Mycobacteria

- Tuberculosis (*M. tuberculosis*)
 - Direct specimen molecular tests
 - Culture isolates identified by DNA probe
 - Susceptibility testing routinely performed- RIPE (Rifampin, Isoniazid, Pyrazinamide, Ethambutol)
- MOTT (Mycobacterium Other Than Tb)/NTM (nontuberculous mycobacteria)
 - *M. kansasii*
 - *M. avium* Complex (MAC)
 - *M. abscessus/chelonae*
 - Criteria for determining significance of respiratory isolates
 - No direct specimen molecular tests readily available
 - Some DNA probes for culture isolate identification
 - Susceptibility testing available



Fungi

- Yeast
 - *Candida*
 - *Cryptococcus*
- Molds
 - *Aspergillus*
 - *Fusarium*
 - *Zygomycetes* (*Rhizopus*, *Mucor*)
- Dimorphic
 - *Histoplasma*
 - *Coccidioides*

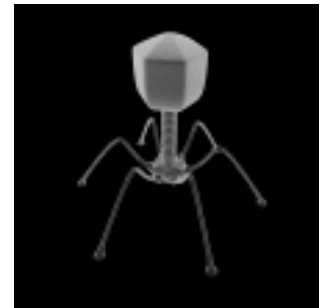


Diagnosis/Identification

- Direct specimen testing
 - Urine Histoplasma antigen
 - Galactomannan: *Aspergillus*
 - Beta-D-glucan (Fungitell): does not detect zygomycetes or *Cryptococcus*
 - Cryptococcal antigen
- Culture
 - Varying growth rates
 - Yeasts: morphology, biochemicals
 - Molds: morphology, newer technologies
 - Dimorphics: morphology, DNA probes

Viruses

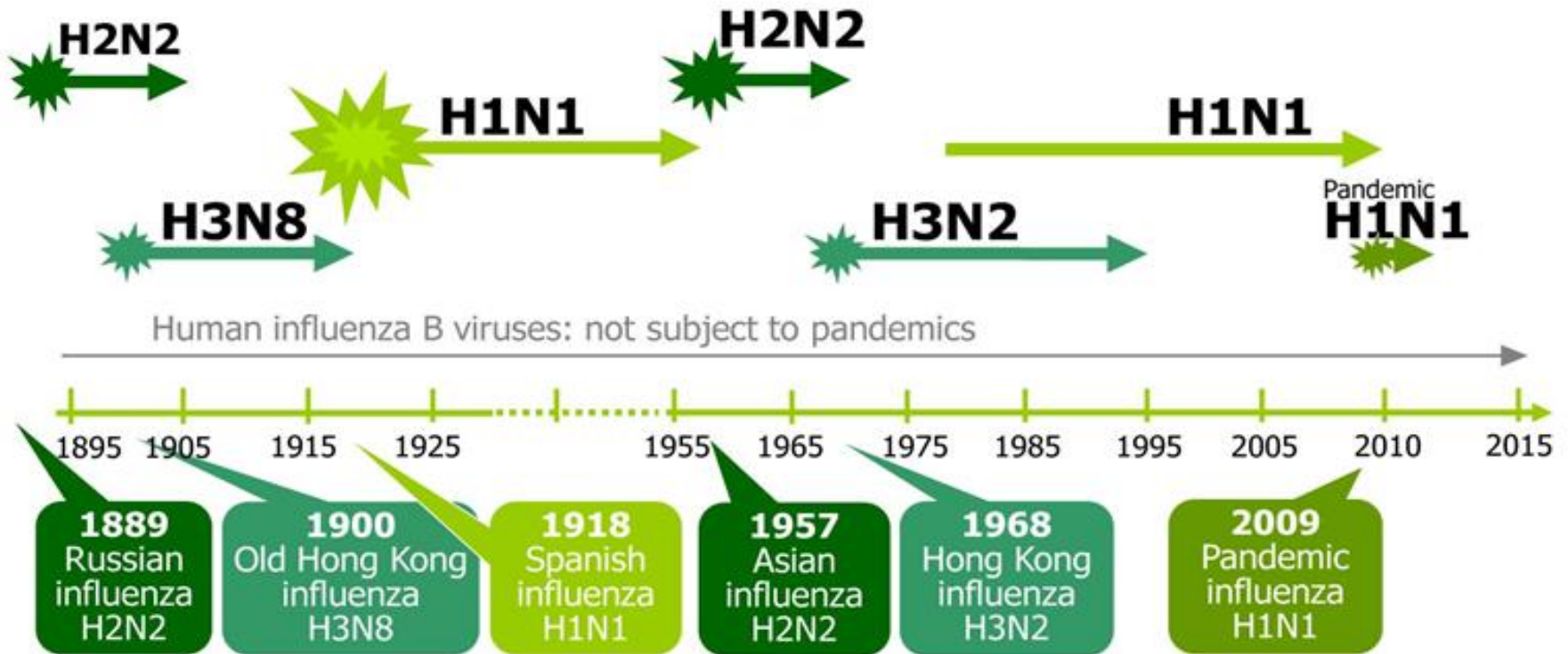
- Obligate intracellular pathogens
- Require living cells to grow
- Either RNA or DNA
- Only seen with an electron microscope
- Some have identifiable inclusions in tissues
- Not susceptible to routine antibiotics



Routes of Transmission

- Respiratory- Most common (flu, RSV, adeno)
- GI/oral fecal 2nd most frequent (HAV, Norwalk)
- Skin- bites (rabies) arthropod (dengue, WNV)
- Genital (HIV, HSV, HPV)
- Intrauterine/transplacental (HIV, CMV)
- Personal/Direct contact, Water and Food, urine, and nosocomial (RSV, Rotavirus)
- Blood borne (HIV, HBV, HCV)

Historical Pandemics



1918-19 Spanish Flu:
An estimated 20-40 percent of the worldwide population became ill.
DEATHS: 50 million

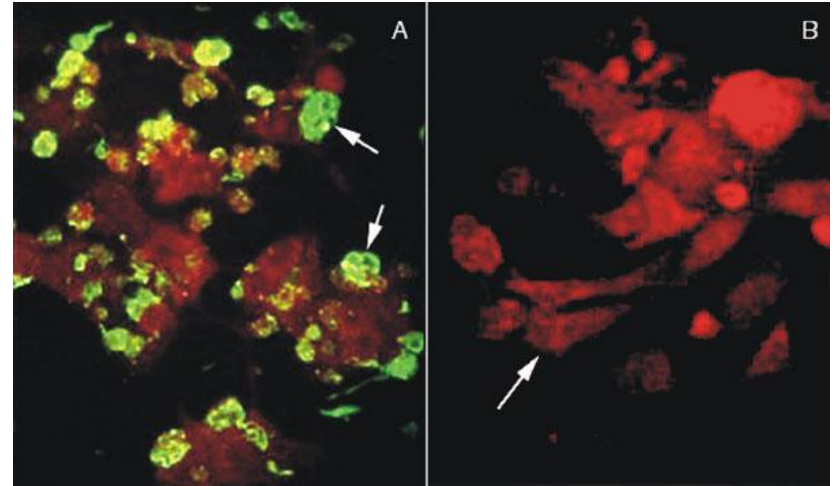
1957- 1958 Asian flu:
Virus was quickly identified due to new technology.
DEATHS: 2 million

1968-69 Hong Kong flu: Elderly were most likely to die.
DEATHS: 1 million

2009 Novel H1N1 flu: Young and pregnant were most likely to die.
CASES: estimate 89 million
CONFIRMED CASES: 482,300
DEATHS: 6071

Viral Testing

- DFA
 - Respiratory viruses
 - HSV/VZV
- Culture
 - Going away
 - HSV: skin/mucous membranes
 - CMV: urine in neonates
- Molecular
- Serology
- Susceptibility testing not routinely performed



Scenario 1

59 year old patient is admitted to your facility from an LTAC. Patient has a stage IV decubitus ulcer. Doctor requests wound culture. Final report states:

Moderate *E.coli*, pan sensitive

Moderate *S.aureus*, Methicillin Susceptible

Moderate *E.gallinarum*, Vancomycin Intermediate
(MIC = 8mcg/ml)



CASE SCENARIO 2

ACC # : X27592 ORD. LOC: BMT

Admit DATE: 01/03/2013

Source: BLOOD CULTURE

TRANSPORT: 30 MINUTES

COLL: 01/08/2013(1239)

REC: 01/08/2013(1300)

SPEC DESC: Blood cult, Peripheral

- ANC : 400 (01/04/2013 – 01/08/2013) - DIARRHEA SINCE ADMISSION

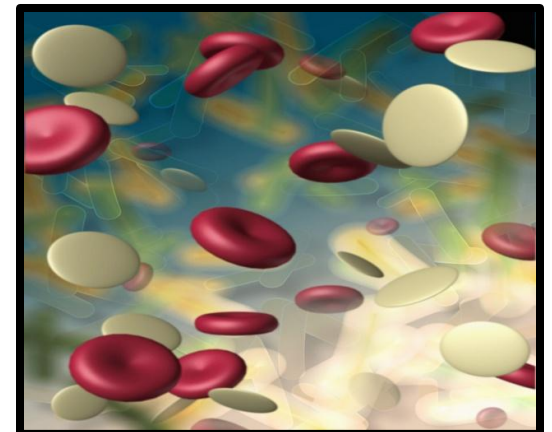
- BLOOD CULTURE: NO GROWTH ON ADMISSION

REPORT CULTURE :

1. *Enterococcus faecalis* IN BOTH BOTTLES AT <24 HOURS

Final Report: 01/12/13

Significant Pathogen: Yes/No



CASE SCENARIO 3

ACC # : X27378 ORD. LOC: 3N-318

Admit Date: 05/03/2013

ROUT CULT W/O GRAMS

TRANSPORT: 45 minutes

COLL: 05/12/2013 (1400)

REC: 05/12/2013 (1445)

SPEC DESC: LT KNEE Wound

Culture Report:

- ❖ HEAVY GROWTH *METHICILLIN RESISTANT STAPHYLOCOCCUS*
- ❖ Moderate Growth of Staphylococcus species (CNS)

Final Report: 05/15/13

Significant Pathogen: Yes/No



CASE SCENARIO 4

ACC # : F70269

LOC: 5W-515

ADMIT DATE:01/01/2013

STOOL CULT W/WBC SMR

TRANSPORT: 1.0 HOUR

COLL: 01/01/2013 (1200)

REC: 01/01/2013 (1300)

SPEC DESC: STOOL

STOOL WBC: 1. 10-15 WBCs / LPF Observed

CULTURE REPORT:

- ❖ LIGHT GROWTH OF *SHIGELLA SONNEI* ISOLATED
- ❖ MODERATE GROWTH OF VANCOMYCIN RESISTANT *ENTEROCOCCUS FAECIUM*

FINAL Report: 01/05/2010



QUESTIONS ?

