Clinical Microbiology for the General Pathologist

A Crash Course in 120 minutes

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- I have no financial relationships to disclose.
- I will not discuss any off-label therapeutics..

Outline

- Outline
- Management of the clinical microbiology laboratory (30 minutes)
  - Components of clinical microbiology – From stains to susceptibility
  - QC/QA/QM
- Pathogens in the clinical microbiology laboratory – Relevance, identification and susceptibility testing. (60 minutes)
  - Routine bacteriology
  - *C. difficile*
  - Mycobacteriology
  - Virology
  - Serology
- Slides provided for self-study: Mycology and Parasitology
- Other important areas not covered here: Anaerobic bacteriology, Enteric bacteriology, prions, next-gen sequencing, whole genome sequencing…

Key components of clinical microbiology

- Major components of microbiology include:
  - Stains
  - Culture methods
  - Identification methods
  - Susceptibility testing methods
  - Molecular diagnostics
  - Quality control, quality assurance
    - Proficiency testing
  - Laboratory management, accreditation, workload, staffing…

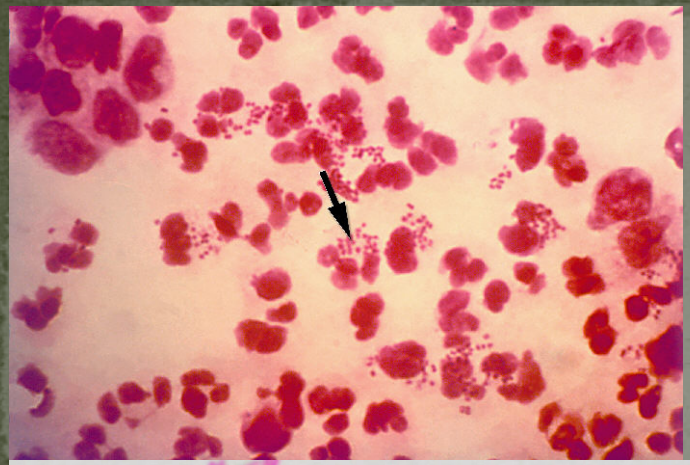
Stains

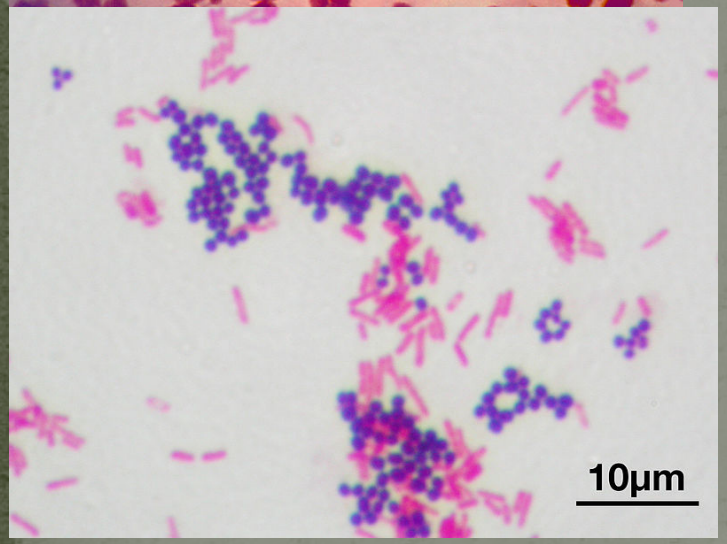
Yes, we still use some!

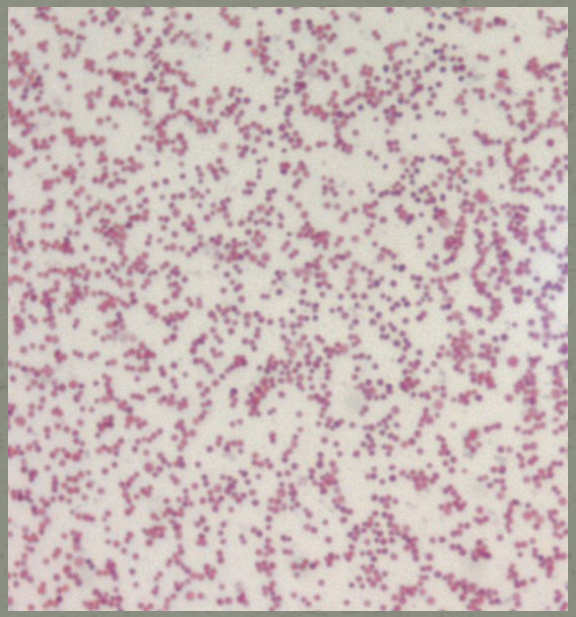
Stains in microbiology

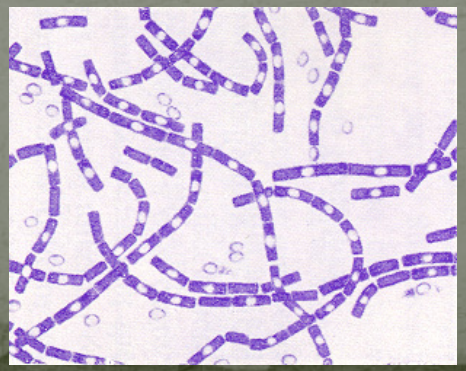
- Several stains are commonly used in microbiology.
  - Extent of use and selection of stain depends on the level of microbiology performed.
- Two major categories of stains used in micro:
  - Chromogen stains (Gives visible colour to structures)
  - Fluorescent stains (allows structures to fluoresce under UV light –Antibodies, selective fluorochromes.

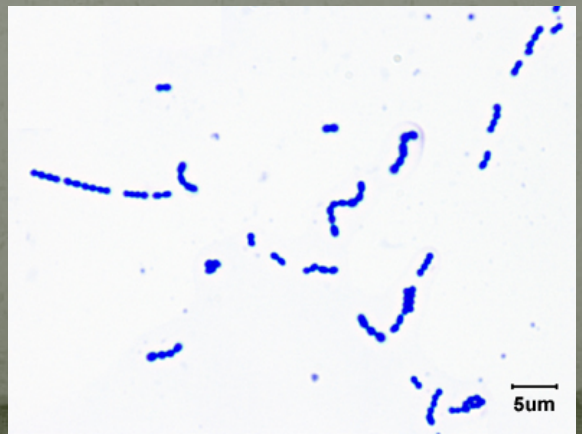
Gram Stain











Organism morphology

- Gram positive
  - Cocci in chains: *Streptococcus*, *Enterococcus*, *S. pneumoniae*.
  - Cocci in clumps: *Staphylococcus*, *Micrococcus*, *Aerococcus*.
  - Small rods: *Corynebacterium*, *Cutibacterium*, *Listeria*, some *Actinomyces* and *Lactobacillus*.
  - Large rods: *Bacillus*, *Lactobacillus*, *Clostridium*
- Gram negative
  - Rods: *Enterobacterales*, *Pseudomonas*, many "non-fermenters", some anaerobes.
  - Coccobacilli: *Haemophilus*, *Francisella*, *Brucella*, *Bordetella*, some anaerobes, *Acinetobacter*.
  - Diplococci: *Neisseria*, *Moraxella*, *Veillonella*

Other common bacterial stains

- Kinyoun (Carbol Fuchsin)
  - Phenol-acid based stain for acid fast organisms. Workhorse AFB stain.
- Uncommon use in microbiology:
  - Zeil-Neilson (Carbol Fuchsin)
    - Heat-acid based stain for acid fast organisms.
  - Warthin Starry stain
    - Used to stain poorly staining bacteria, *Bartonella,* spirochetes.
  - Wright's and Giemsa's
    - Useful for cell structure and intracellular bacteria (e.g. *Anaplasma*)
    - Used for intracellular parasites (*Leishmania, Plasmodium*)

Stains for fungi

- Most clinical specimens for fungal culture should be clarified with KOH before fungal staining in microbiology labs.
- Most commonly used in Micro are:
  - Lactophenol cotton blue: Simple stain binds to fungi, algae.
    - Simple "one drop and let it sit" preparation. Used on cultured moulds.
  - Calcofluor white: Fluorescent dye that binds to chitin.
    - Most sensitive of fungal dyes, but prone to artefact. usually used with KOH on specimens that aren't tissue sections.

Fluorescent stains in microbiology

- Auramine-rhodamine
  - Binds to mycolic acids of AFB. More sensitive than carbol fucshin stains.
  - Less specific – *Pseudomonas aeruginosa*, some *Corynebacterium* sp., aerobic actinomycetes will stain positive.
- Acridine orange
  - Binds to DNA. Useful for faintly staining or non-staining bacteria.
  - Prokaryotic and eukaryotic DNA stain differently.
- Immunofluorescent stains

Culture

Tried and true?

Principles of culture

- Need suitable medium
  - Agar/broth (most common)
    - Nutrients/supplement (eg. Heme, NAD, cysteine)
    - Selective agents
    - Growth or metabolic indicators
  - Cell culture and animals
- Suitable temperature
- Suitable atmosphere.

Routine media

- Sheep blood agar
  - Basal medium enriched with sheep blood.
  - Shows characteristic haemolysis reactions of organisms.
- Chocolate agar
  - Basal medium enriched with isovitalX (mixture of nutrients for the growth of fastidious organisms)
  - Allows growth of *Haemophilus* and other fastidious organisms.
- Maconkey agar
  - Selective and differential medium for the growth of enteric gram negative organisms.
  - Allows differentiation of lactose fermenters.
- Brucella Agar w/vit. K,
  - Enriched medium with blood and vitamin K for the recovery of fastidious and anaerobic organisms.
- PEA
  - Selective medium, enriched with blood usually used for anaerobes.
  - Inhibits gram negative organisms, allows visualization of haemolysis reactions.
- KVL
  - Selective medium for isolation and identification of gram-negative anaerobes.
- BBE
  - Medium for the selective isolation of *Bacteroides*.

Routine media (fungi)

- Saboureau
- Brain-Heart-Infusion
- Potato-dextrose agar
- Mycosel – inhibits most commensal non-pathogens
- Inhibitory mould agar: Acidic and contains chloramphenicol.

Other media you may want to know about

- Mycobacterial media
  - Middlebrook, LJ, Dubos
- Enteric media (HE, SS, Camply, Skirrows, TCBS, XLD, SMAC…)
- Media for susceptibility testing
  - Mueller-Hinton, Mueller-Hinton with blood and Haemophilus Test Medium.
- BCYE, Tinsdale, Theyer-Martin, Reagan Lowe, GC base…
- Chromogenic media
  - Multiple permutations: For yeasts, enteric pathogens, VRE, MRSA, uropathogens

Identification of bacteria

- Definitive identification of cultured organisms in clinical labs takes several forms:
  - Stains (Gram primarily)
  - Biochemical metabolism/Products of metabolism
  - Antigenic expression
  - Molecular
  - Proteomic composition (MALDI-TOF)

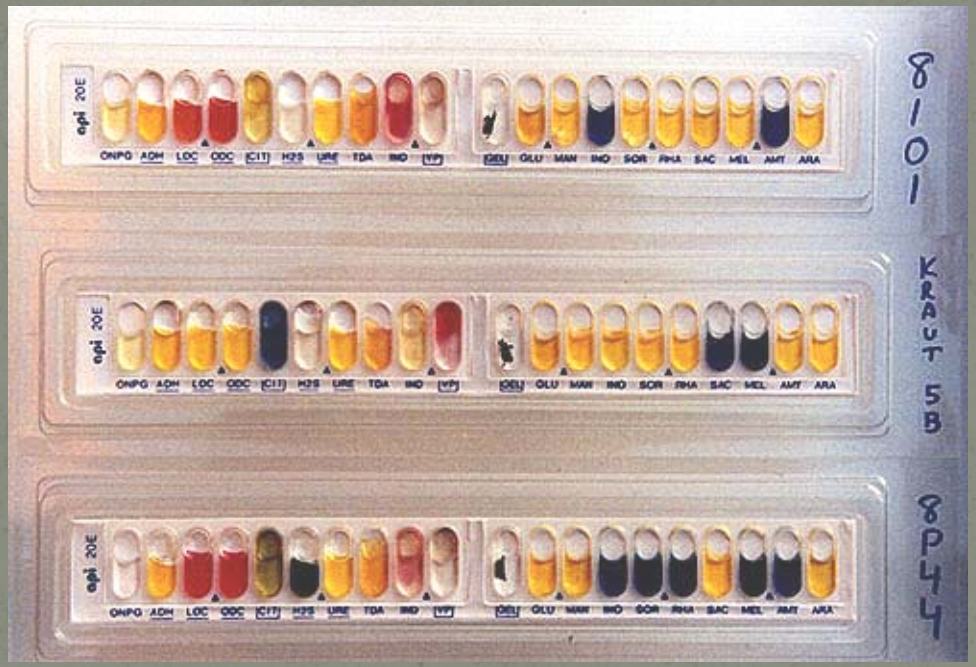
Biochemical metabolism and products

- Traditional method of identification
  - Commonly used, but being replaced.
- Metabolism of specific substrates varies enormously by organism and can be used to identify organisms by pattern of metabolism.
- Principles are:
  - Substrates and indicators included in media.
  - Substrate and indicator in a solution.

Rapid biochemical tests in common use

- Catalase
  - Primalrily *Staphylococcus* (+), *Streptococcus* (-), *Enterococcus* (-), *Corynebacterium* (+), *Listeria* (+), *Clostridium* (-), *Bacillus* sp (+) *Cutibacterium acnes* (+).
- Coagulase
  - *Staphylococcus* spp.
- Indole
  - *E. coli* (+), *Aeromonas* (+), *Pseudomonas* (-), (H)ACEK, *Proteus, Pasteurella*.
- Oxidase
  - *Pseudomonas* (+), (H)ACEK, Pasteurella
- Pyrilidonylarylamidase (PYR)
  - GAS, *S. lugdunensis*, and *Enterococcus* (+)
- Leucine aminopeptidase
  - Viridans *Streptococcus* group (+)

API strips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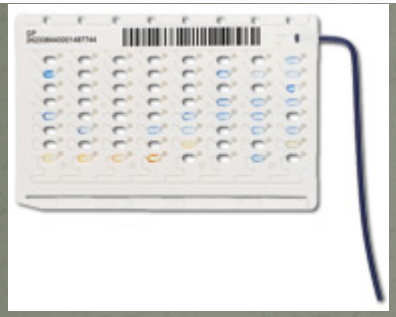
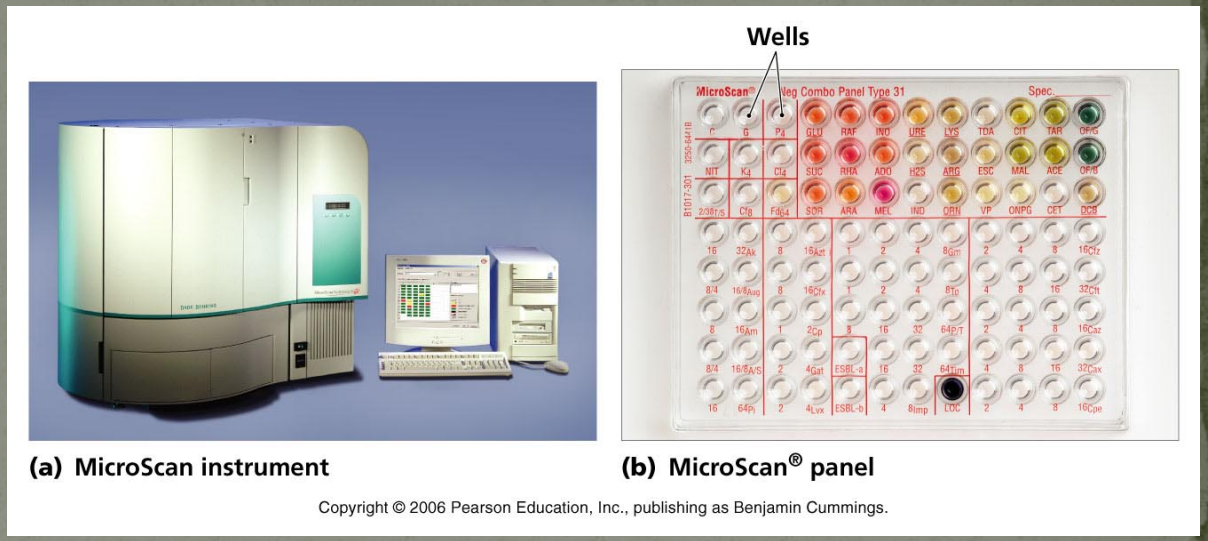
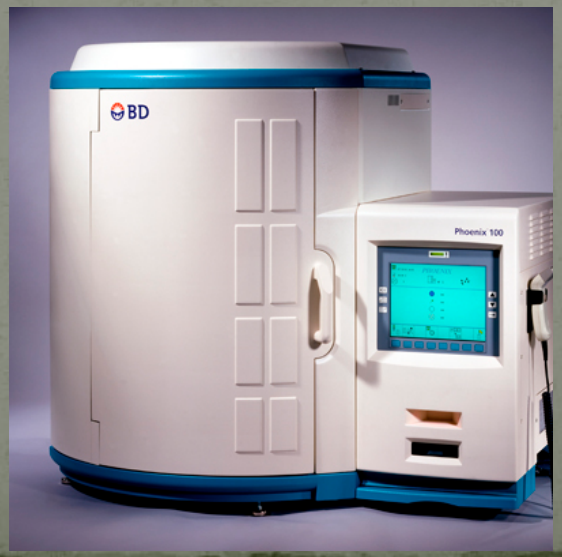
Fully automated systems



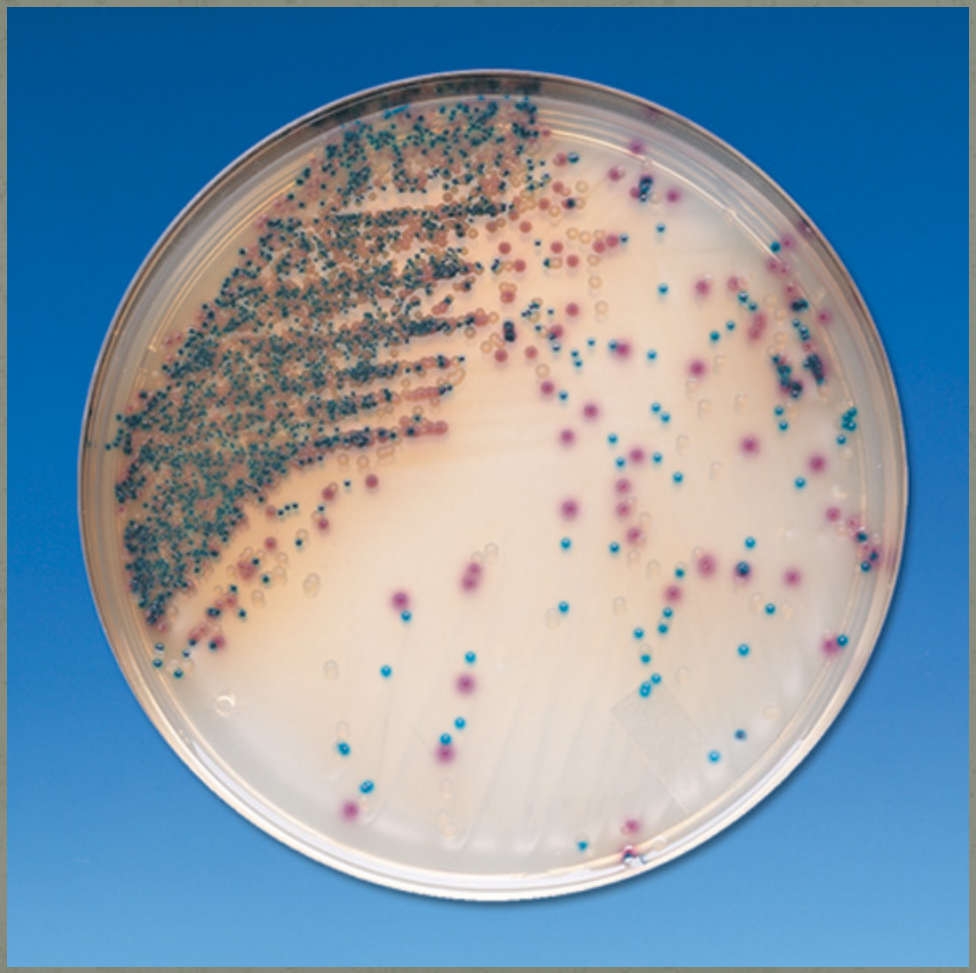


(a) MicroScan instrument

(b) MicroScan® panel



Chromogenic media

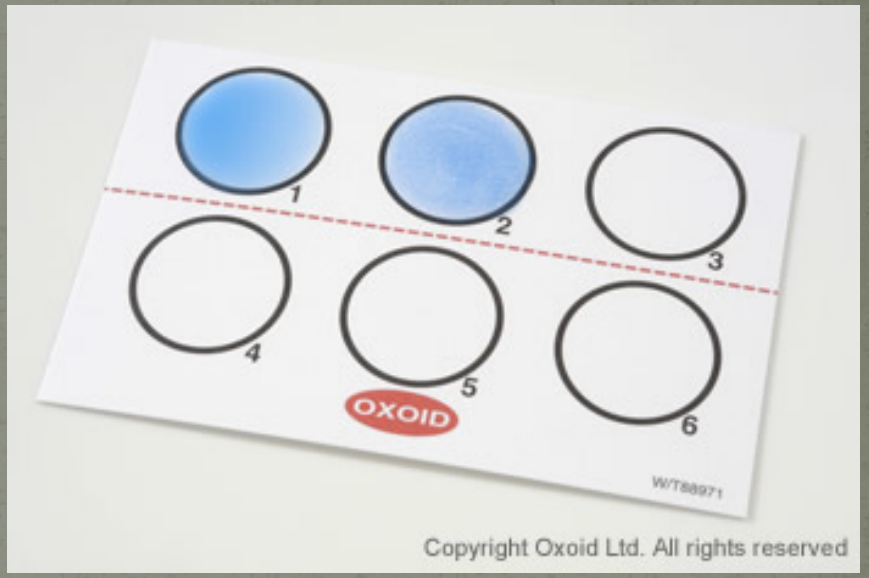


Antigenic expression

- Commonly used for the identification of relatively inert organisms or where rapid identification from culture is desired.
- Antibodies bind to bacterial antigens (usually surface, capsule or membranes.
  - Agglutination, enzyme and DFA are common methods.
- Cross-reactions may occur

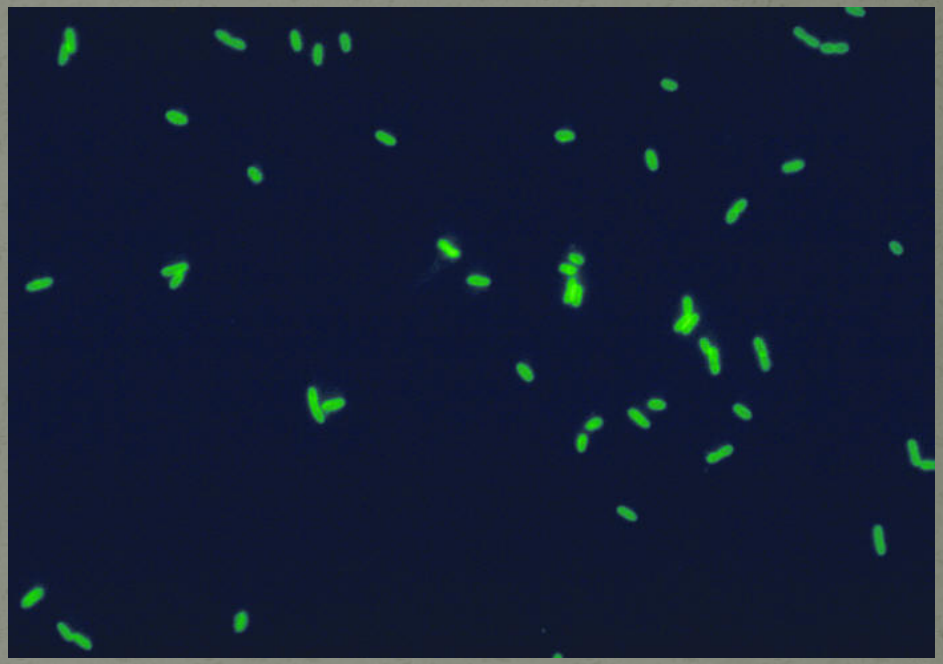
Agglutination

- Commonly used for:
  - β-haemolytic stretococci
  - *Salmonella* sp.
  - *Shigella sp.*
  - *S. aureus*
  - *H. influenzae*



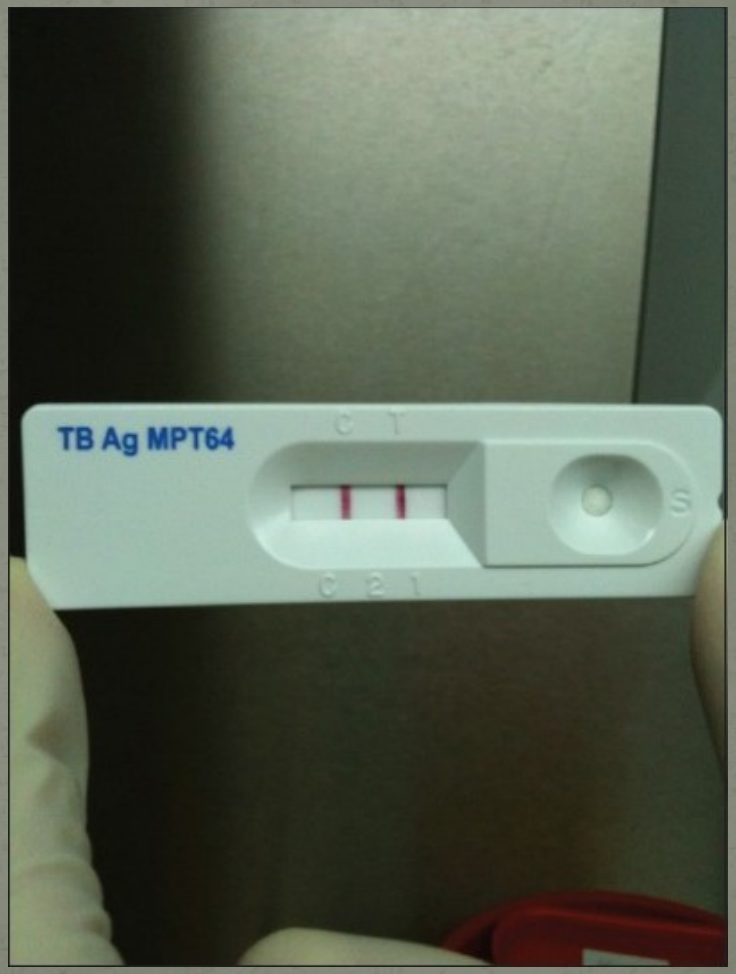
DFA

- Commonly used for:
  - *N. gonorrheae*
  - *Legionella* sp.
  - *Yersina pestis*
  - *Francisella* sp.



Immunoassays for identification

- MPT64 antigen for culture confirmation of TB cultures.



Molecular tools for identification

- Identification using molecular techniques is powerful, specific and can be used when all other methods fail.
- Directed PCR most cost effective if ID is suspected.
  - *Mycoplasma, S. aureus, Enterococcus, Legionella, TB, Brucella, Bacillus anthracis, Leptospira, Coxiella, Bartonella*…many others!
- 16S RNA gene PCR commonly used where identification is completely unknown.
  - Works well from pure cultured bacteria.
  - Can be done directly from samples.
- Specific molecular probes
  - Used for TB most frequently, also Gonococcus, GBS.

Proteomic analysis

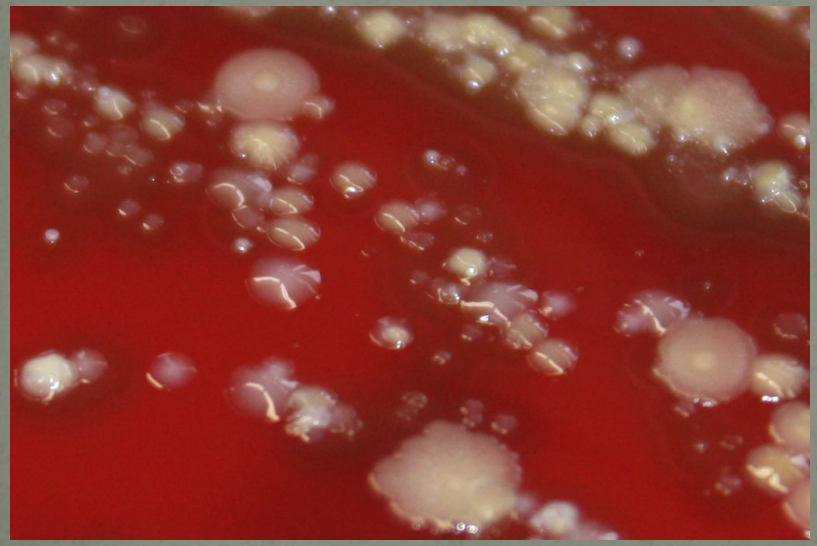
- Recent addition to identification.
- Uses the mass spectrum of the bacterial proteome and compares spectra to known library.
- Closely matching proteome spectra likely represent closely related or identical organisms.

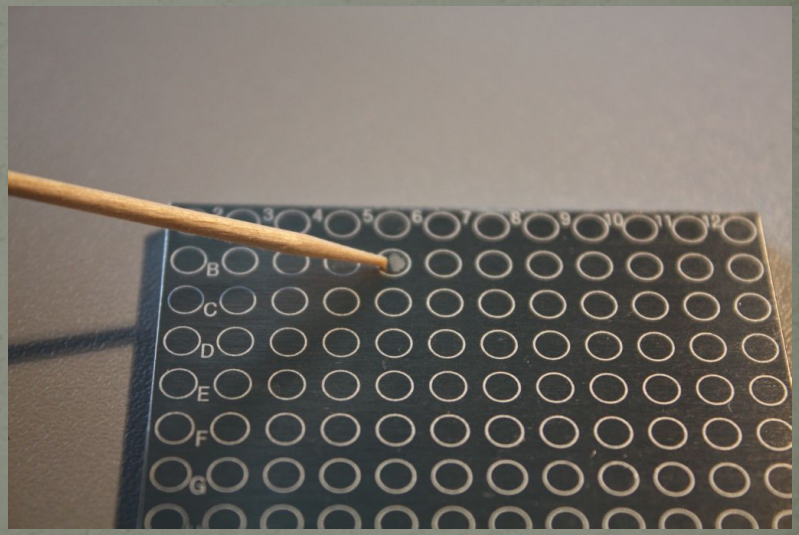
MALDI-TOF

- Simple procedure, RAPID (minutes) identification, extremely affordable (marginal cost ~$0.20 per identification).
- Can be used directly on positive blood cultures using washing procedures or after brief incubation on solid media.
- Can be used after very brief incubation (~3-5h)
- Might also be used directly on patient specimens (e.g. urine) – in development.
- Possible applications for susceptibility testing in the future.

MALDI-TOF

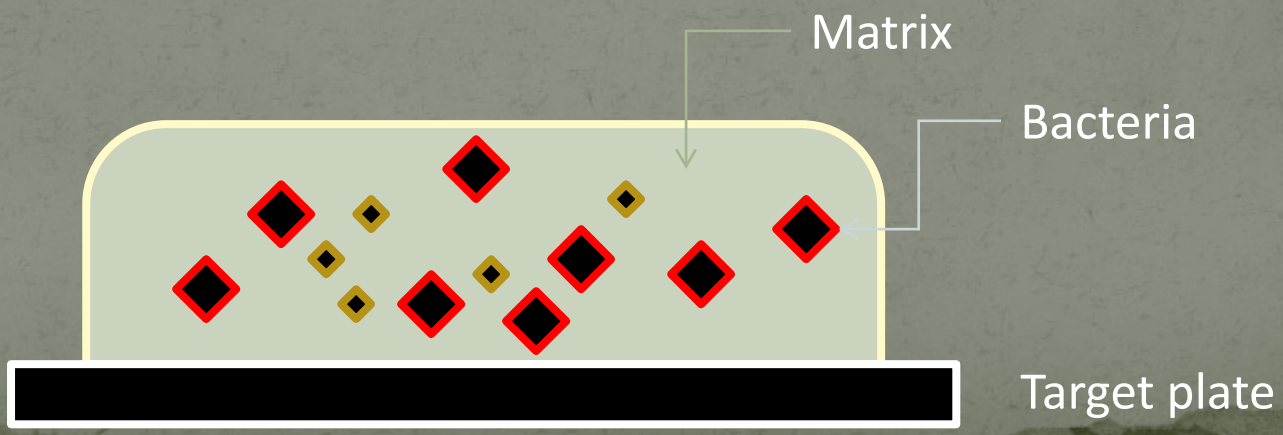
- Pick one colony (isolated but only need one colony)
- Inoculate to plate





Specimen analysis

Matrix Assisted Laser Desorption/Ionization



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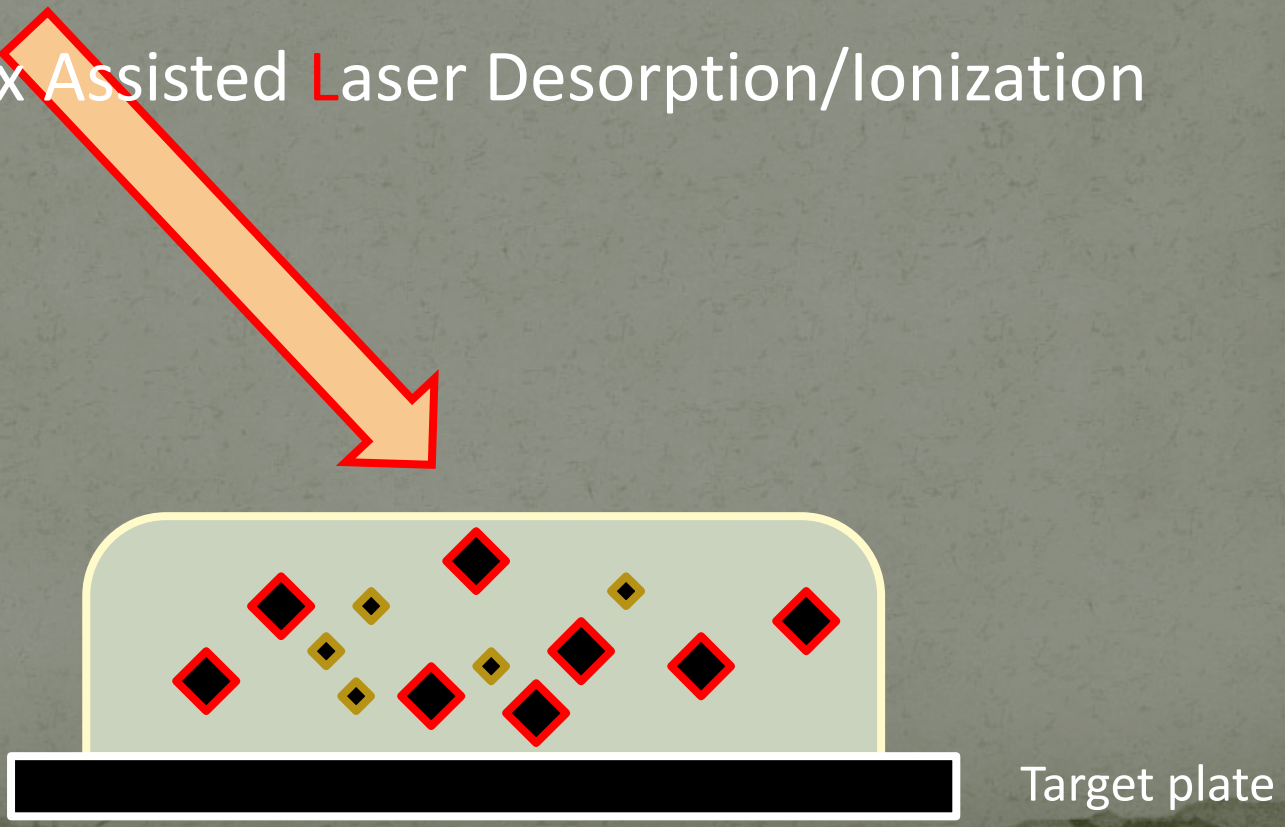

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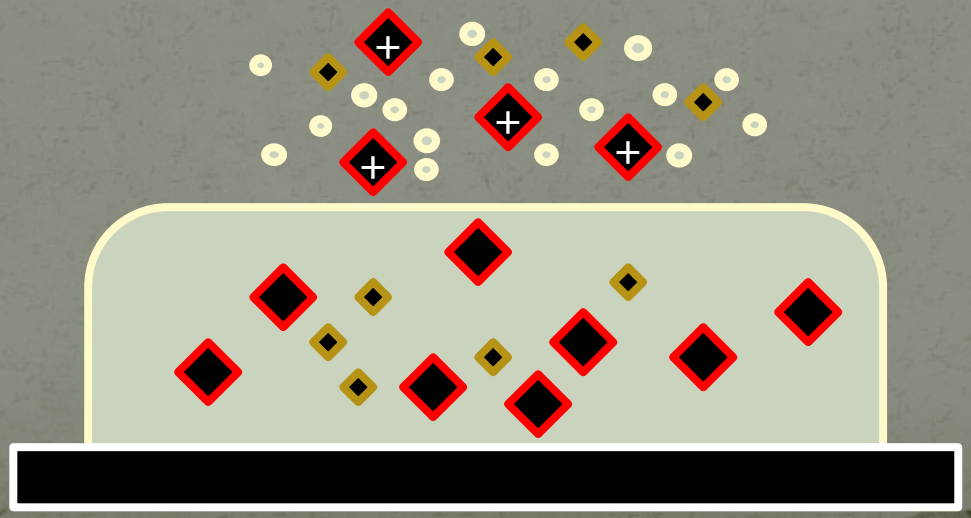

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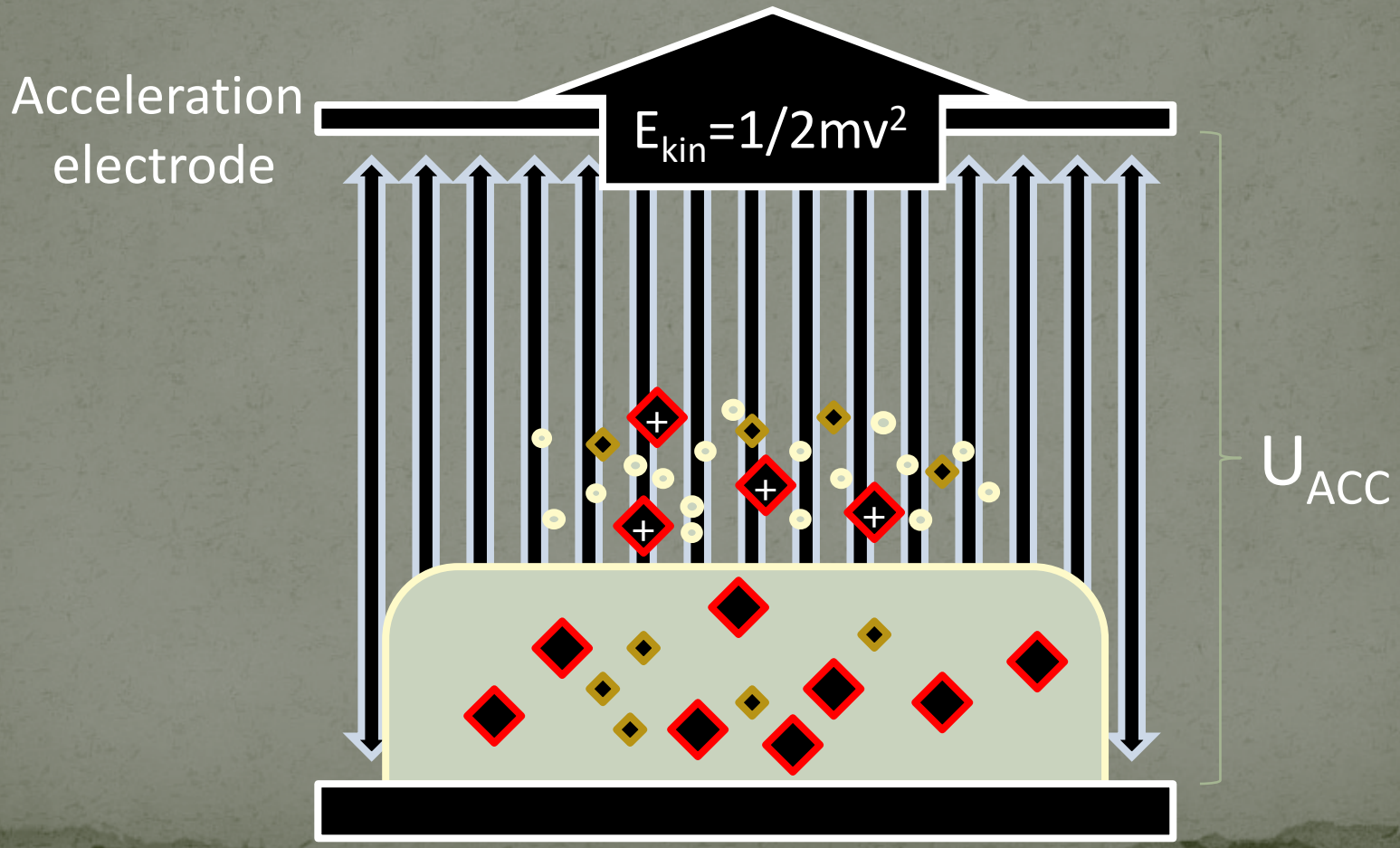
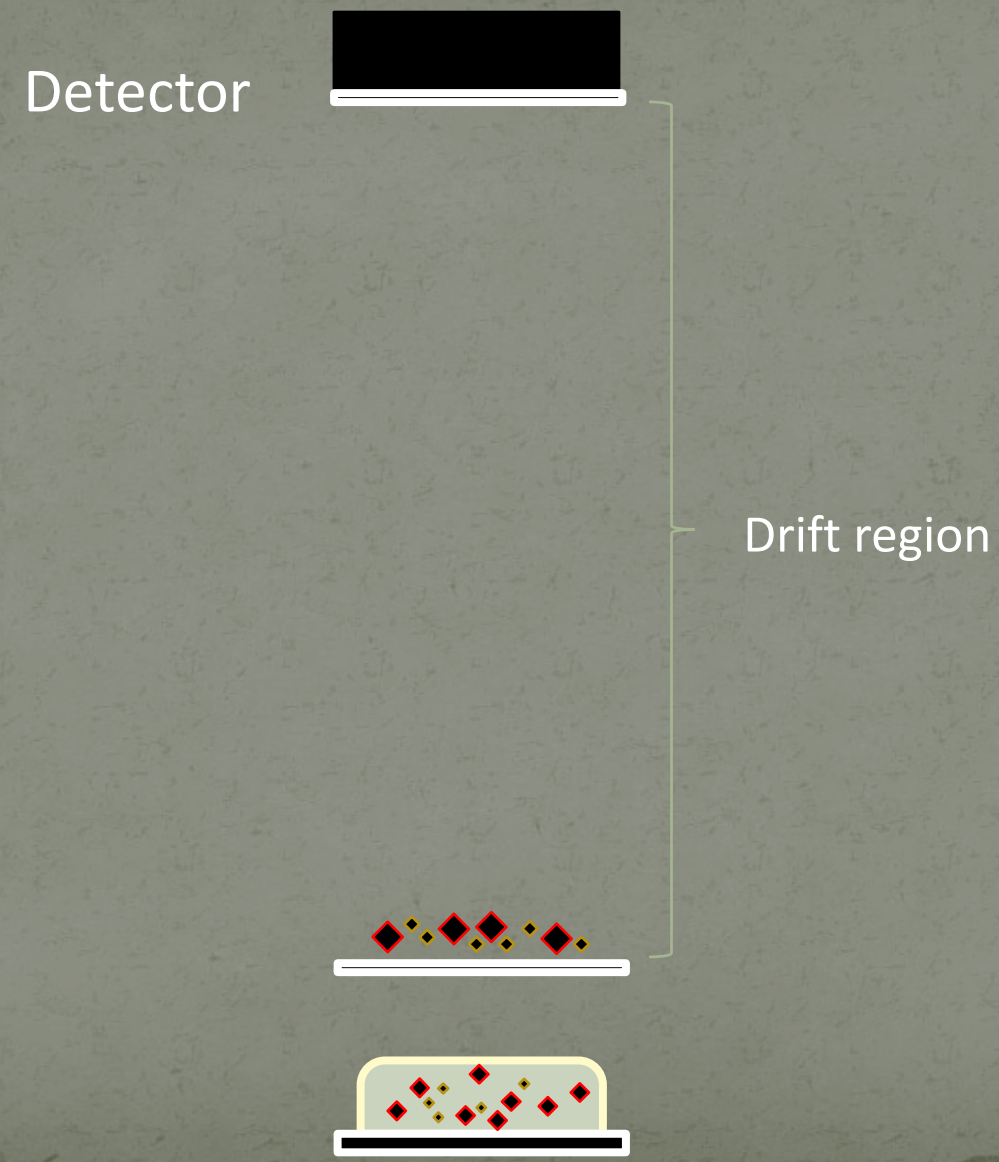
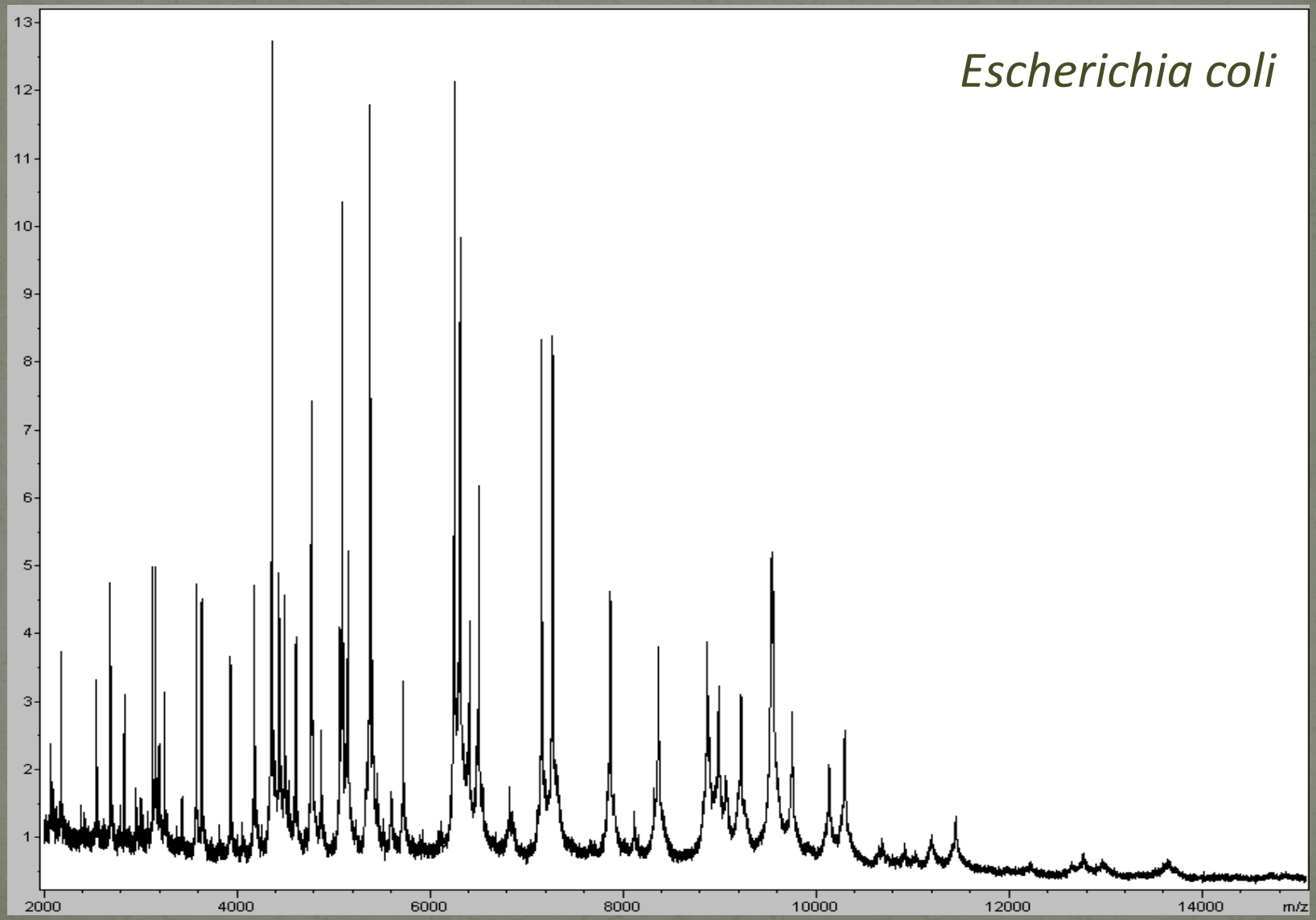



Detector
Drift region

Production of spectra (reading) = < 1 Minute



Non-culture methods

Demanding a bigger slice of the pie

Non-culture methods

- Microbiology laboratories are increasingly using non-culture methods for the identification of pathogens in clinical specimens.
  - Molecular detection
  - Antigen detection

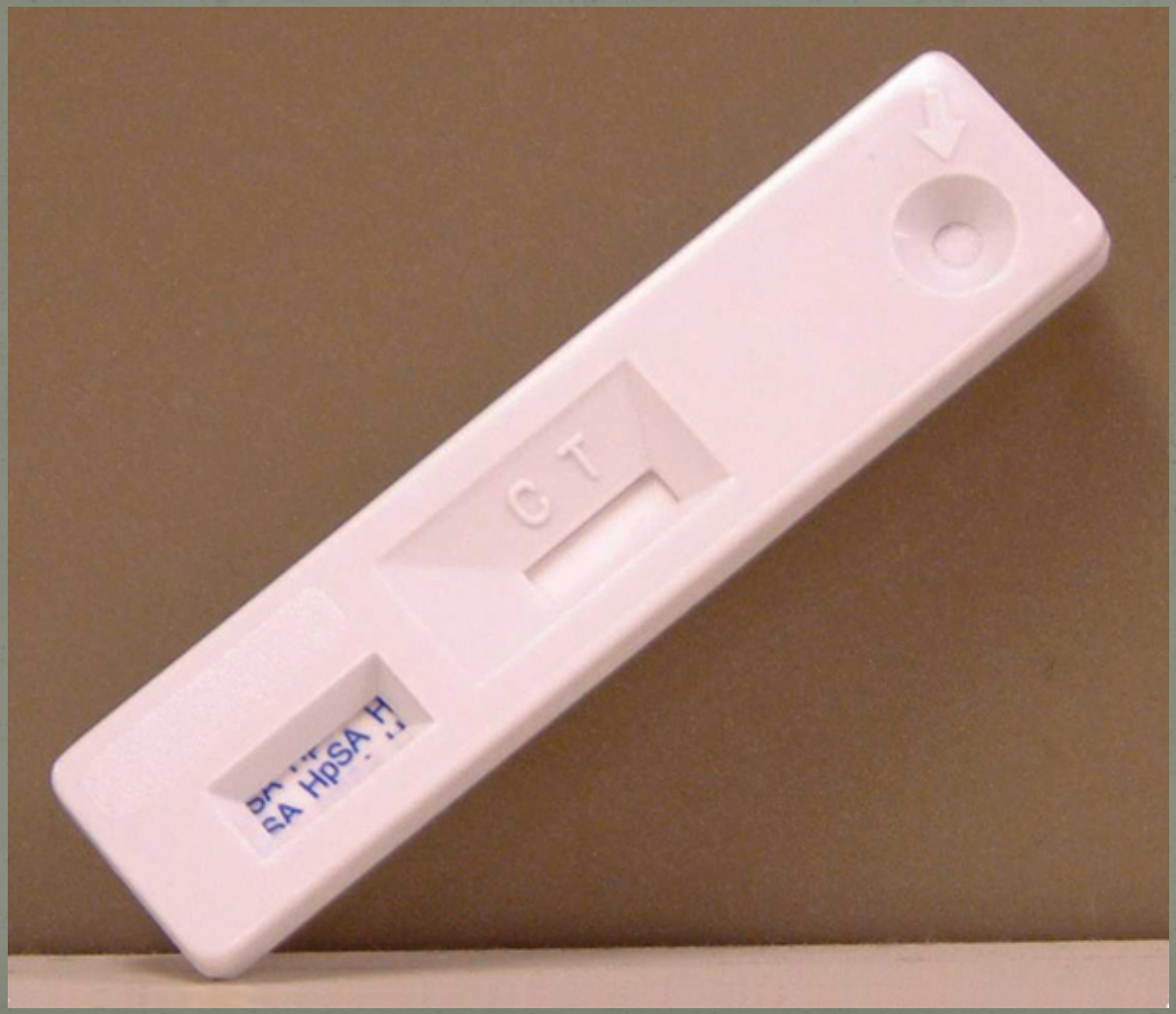
Antigen detection

- Antigen detection can be used to identify organisms directly from patient samples.
- Principle is a specific antibody that binds to a specific antigen of a known pathogen and creates a visible reaction.
  - Membrane IA, agglutination, ELISA.
- Caution with interpretation of results.
  - Cross reactivity
  - Sensitivity

Common use examples

- Group A Streptococci (throat)
- *Helicobacter pylori* (stool)
- *Legionella* (urine)
- *S. pneumoniae* (urine)
- *Clostridioides difficile* (toxin in stool)
- Cryptococcal antigen (CRAG)
- *Trichomonas*

Membrane IA



Molecular detection of pathogens

- Very versatile: Nearly all pathogens can be detected and can be applied to most specimen types.
- Very sensitive.
- Main disadvantages are inability do differentiate viability, lack of organism recovery, lack of susceptibility testing (usually), potential for cross contamination/cross reactions and cost.
- 16S PCR and sequencing can be used to identify bacteria from monomicrobial specimens with high accuracy, even if they are dead. 18S/ITS also possible for fungi, but less experience.

Methods in common use

- PCR
- RT-PCR
- Real-time PCR
- TMA, HDA and others
- Increasingly, amplification (NAAT) methods have been simplified and massively multiplexed.
- Sequencing
- Various typing methods

Simple multiplex/massive multiples assays

Susceptibility testing

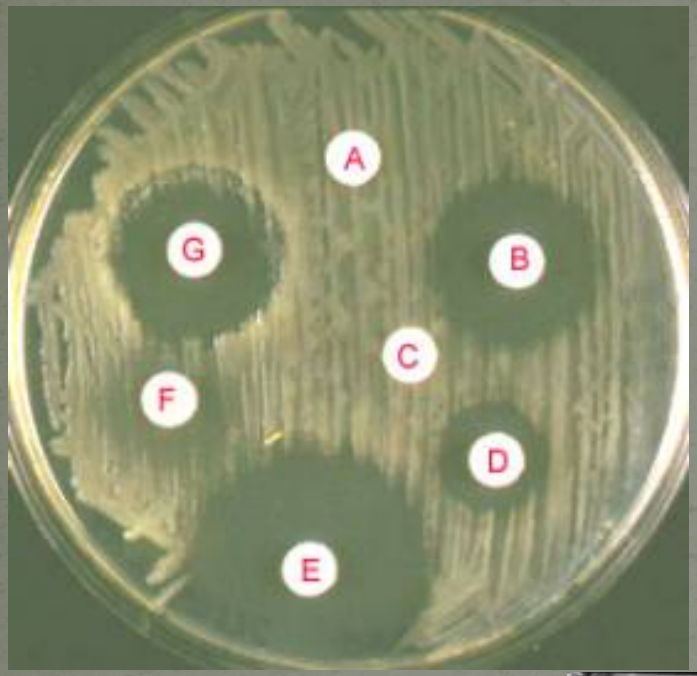
Yes, that's what they are all waiting for.

Susceptibility testing

- Routine susceptibility testing of bacteria is done using one or several methods:
  - Broth dilution (most automated systems use modifications of this method)
  - Agar dilution
  - Diffusion (disk or gradient strip)
  - Rapid enzymatic methods (eg. β-lactamase methods, ESBL, CPE)
  - Molecular detection of resistance determinants – Emerging field
- Method is organism specific.
  - Medium used, incubation times, temperature and atmosphere are strictly controlled.
- Interpretation (S,R,I) relates to many factors.
  - Interpreted according to recognized national standards (e.g. CLSI, EUCAST).
  - New SDD category in CLSI complicates reporting.
- New AST methods
  - MALDI-TOF, microscopically observed growth, detection of genetic resistance determinants may revolutionize AST methods and TAT.
  - Direct AST from blood cultures

New antimicrobial agents you should know about

- Linezolid, Tedizolid
- Ceftaroline, ceftobiprole
- Daptomycin
- Avibactam (with ceftazidime, azteronam)
- Meropenem-vaborbactam
- Imipenem-relebactam
- Ceftolozane-tazobactam
- Fidaxomicin
- Ertapenem, doripenem
- Oritavancin, dalbavancin, telavancin
- Cefidericol
- Bedaquidine
- Plazomicin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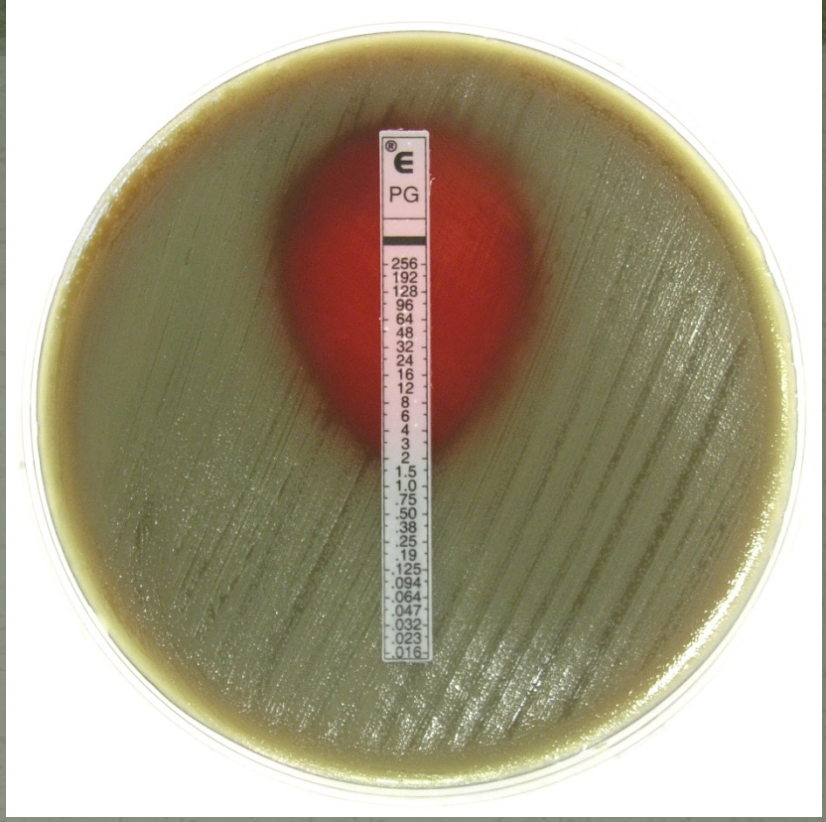
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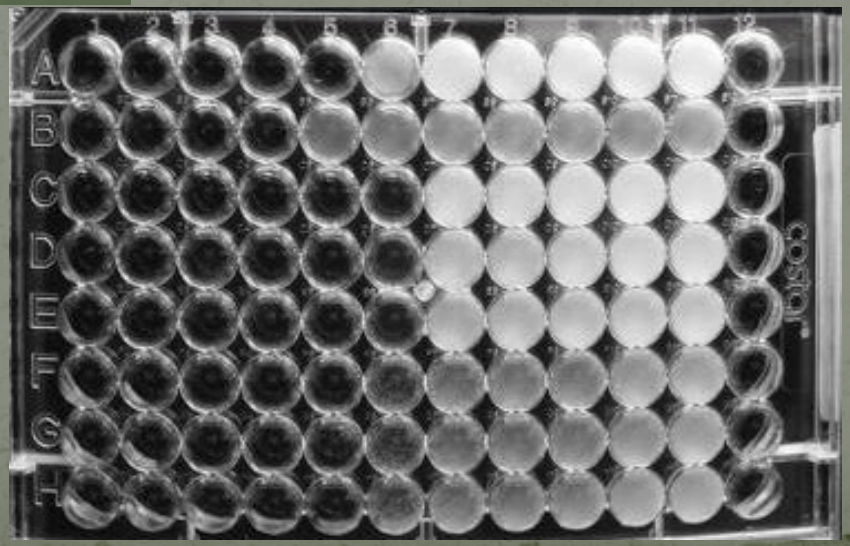
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Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINS											
A	Ampicillin	10 µg	≥17	–	14–16	≤13	≤8	–	16	≥32	**(4)** Results of ampicillin testing can be used to predict results for amoxicillin. See general comment (2).
O	Piperacillin	100 µg	≥21	–	18–20	≤17	≤16	–	32–64	≥128	
O	Mecillinam	10 µg	≥15	–	12–14	≤11	≤8	–	16	≥32	**(5)** For testing and reporting of *E. coli* urinary tract isolates only.
β-LACTAM COMBINATION AGENTS											
B	Amoxicillin-clavulanate	20/10 µg	≥18	–	14–17	≤13	≤8/4	–	16/8	≥32/16	
B	Ampicillin-sulbactam	10/10 µg	≥15	–	12–14	≤11	≤8/4	–	16/8	≥32/16	
B	Ceftolozane-tazobactam	30/10 µg	≥21	–	18–20	≤17	≤2/4	–	4/4	≥8/4	**(6)** Breakpoints are based on a dosage regimen of 1.5 g **administered** every 8 h.
B	Ceftazidime-avibactam	30/20 µg	≥21	–	–	≤20	≤8/4	–	–	≥16/4	**(7)** Breakpoints are based on a dosage regimen of 2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h **administered** over 2 h. **(8) Disk diffusion may overcall resistance for isolates with zones of 18–20 mm; confirmatory MIC testing is indicated.**
B	**Meropenem-vaborbactam**	**20/10 µg**	**≥18**	**–**	**15–17**	**≤14**	**≤4/8**	**–**	**8/8**	**≥16/8**	**(9) Breakpoints are based on a dosage regimen of 4 g (2 g meropenem + 2 g vaborbactam) every 8 h administered over 3 h.**
B	Piperacillin-tazobactam	100/10 µg	≥21	–	18–20	≤17	≤16/4	–	32/4–64/4	≥128/4	
O	Ticarcillin-clavulanate	75/10 µg	≥20	–	15–19	≤14	≤16/2	–	32/2–64/2	≥128/2	

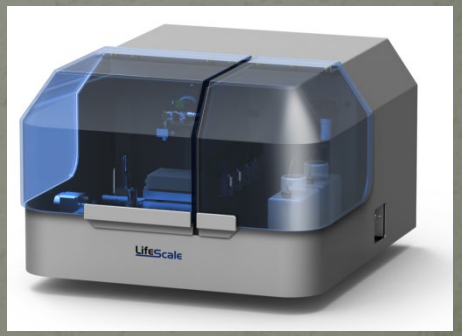
Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)											
(10) WARNING: For *Salmonella* spp. and *Shigella* spp., 1st- and 2nd-generation cephalosporins and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.											
(11) Following evaluation of PK-PD properties, limited clinical data, and MIC distributions, revised breakpoints for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in this table. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary for the dosage indicated below. When using the current breakpoints, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant). However, ESBL testing may still be useful for epidemiological or infection control purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in Table 3A.										
Breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli*, *Klebsiella* spp., or *Proteus* spp., ESBL testing should be performed (see Table 3A). If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.											
(12) *Enterobacter*, ***Klebsiella* (formerly *Enterobacter*) *aerogenes***, *Citrobacter*, and *Serratia* may develop resistance during prolonged therapy with 3rd-generation cephalosporins as a result of derepression of AmpC β-lactamase. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing repeat isolates may be warranted.											
A	Cefazolin	30 µg	≥23	–	20–22	≤19	≤2	–	4	≥8	**(13)** Breakpoints when cefazolin is used for therapy of infections other than uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Breakpoints are based on a dosage regimen of 2 g **administered** every 8 h. See comment (11).
U	Cefazolin	30 µg	≥15	–	–	≤14	≤16	–	–	≥32	**(14)** Breakpoints when cefazolin is used for therapy of uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Breakpoints are based on a dosage regimen of 1 g **administered** every 12 h. See additional information in CEPHEMS (ORAL).
C	Ceftaroline	30 µg	≥23	–	20–22	≤19	≤0.5	–	1	≥2	**(15)** Breakpoints are based on a dosage regimen of 600 mg **administered** every 12 h.

Molecular detection of resistance

- NAAT and sequencing can be used to detect resistance mechanisms in bacteria.
  - Used for beta-lactamases, mecA, vanA/B in blood cultures, isolates and direct specimens: e.g. Biofire assay, Luminex Verigene, in-house assays.
  - Used for rapid identification of INH, ETB, RIF resistance in TB.
- Some mutations and genes have good predictive value for resistance, others less ideal.
- Some resistant isolates have no obvious (known) genetic marker for resistance.
- Requires a robust database of resistance determinants.
- WGS may eventually provide comprehensive information of the resistome of bacteria.
  - Emerging field that requires improved databases and information technology infrastructure.

New methods in the horizon – phenotypic AST in hours



Reports and post-analytical issues

Now makes sense of it…

Reporting issues

- Reporting results can be complicated in microbiology and interpretation is important.
- Special issues in microbiology include:
  - Relevance
  - Selective reporting of antimicrobial susceptibility
  - Critical reporting
  - Infection control reporting
  - Diseases of public health importance
  - Comments

Relevance of reports

- Stool culture
  - 4+ Acinetobacter baumanii
- Vaginal-rectal swab (36 weeks pregnant)
  - 3+ Group C Streptococcus
- Throat culture
  - 3+ *S. aureus*
- Throat culture
  - 3+ *N. meningitidis*
- Sputum culture
  - 1+ *S. pneumoniae*

Key points - relevancy

- Humans are covered with bacteria.
  - Few are pathogenic.
- Relevance depends on the site, the relative amount and type of resident flora and relative quantitation of putative pathogen.
- CRITICAL to establish criteria for specimen relevance!!
- In most instances, any report with identification from the micro lab will be seen as valid and representative of infection unless clarified.
- Always report absolute pathogens and culture positive sterile sites.

Choosing wisely

1. Don't collect urine specimens for culture from adults who lack symptoms localizing to the urinary tract or fever unless they are pregnant or undergoing genitourinary instrumentation where mucosal bleeding is expected.
2. Don't routinely collect or process specimens for Clostridium difficile testing when stool is non-liquid (i.e., does not take the shape of the specimen container) or when the patient has had a prior nucleic acid amplification test result within the past 7 days.
3. Don't obtain swabs from superficial ulcers for culture as they are prone to both false positive and false negative results with respect to the cause of the infection.
4. Don't routinely order nucleic acid amplification testing on cerebrospinal fluid (e.g., herpes simplex virus, varicella zoster virus, enteroviruses) in patients without a compatible clinical
5. Don't routinely obtain swabs during surgical procedures when fluid and/or tissue samples can be collected.

Selective reporting

- Microbiology laboratories play a large role in stewardship.
- For any given pathogen, many antimicrobials are tested but only a limited amount should be reported.
  - Varies by organism and sample.
  - Allows reasonable choice of antibiotics but limits use of broad spectrum agents.
  - Should be decided with clinicians, but guidelines exist (CLSI M100 document)
  - Common examples include reporting broad spectrum agents only on MDR organisms, cephalosporin restriction, pharmacokinetic variables.

QC and QA

- Considered one of the largest components of laboratory workload.
- Overall, assures that the quality of lab results is high and that the results are impacting patient care.
- Must be done regularly.
- Must be very well documented.
  - Bulk of work is actually documentation.

QC

- QC is the verification and documentation that individual tests, media, atmospheres in the lab work as they should.
  - Some done daily (eg. most spot tests, temperatures, MALDI-TOF, susceptibility testing)
  - Some weekly (eg. susceptibility testing after appropriate justification)
  - Some done by batch/lot number (eg. most commercial tests, media).
  - Some done only when test is performed
- No lab should provide results if an appropriate QC system is in place, tests are working and documentation can prove it.
- Requirements vary according to accrediting bodies.
- Antibiotic QC is a particularly complex field. GPs should know what to do if values are out on weekly (or daily) QC, how many values need to be in before resuming weekly QC and what to do in the interim.
- Generally, antibiotic QC should be done each day of testing unless the laboratory can prove that QC values are consistently in control, in which case frequency may be decreased (e.g. qweek)

Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; **iron-depleted CAMHB for cefiderocol (see Appendix I)[1]**
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)
Staphylococcus aureus **ATCC® 25923 (for *Salmonella* enterica ser. Typhi azithromycin disk diffusion testing only; see Table 4A-1)**

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

Refer to Tables 3A, 3B, and 3C for additional testing, reporting, and QC for **Enterobacterales**.

QA

- Measurement and documentation of the overall lab performance in laboratory based tasks.
  - Evaluates lab process, result consistency, protocol adherence, accuracy of results from receipt to report.
  - Common examples include proficiency samples, TAT audits, correlation between expected results and final results, deviations from statistical norms, general audits.

Quality Management

- Measures (and documents) the performance of laboratory systems in the overall health care system and impact of corrective interventions.
  - Common examples are audits of antimicrobial use, impact of tests of treatment, hospital stay, infection control workload, outbreak control, interventions to improve compliance with sample requirements…
  - Usually accomplished through periodic audits of lab results in relation to patient or hospital outcomes.

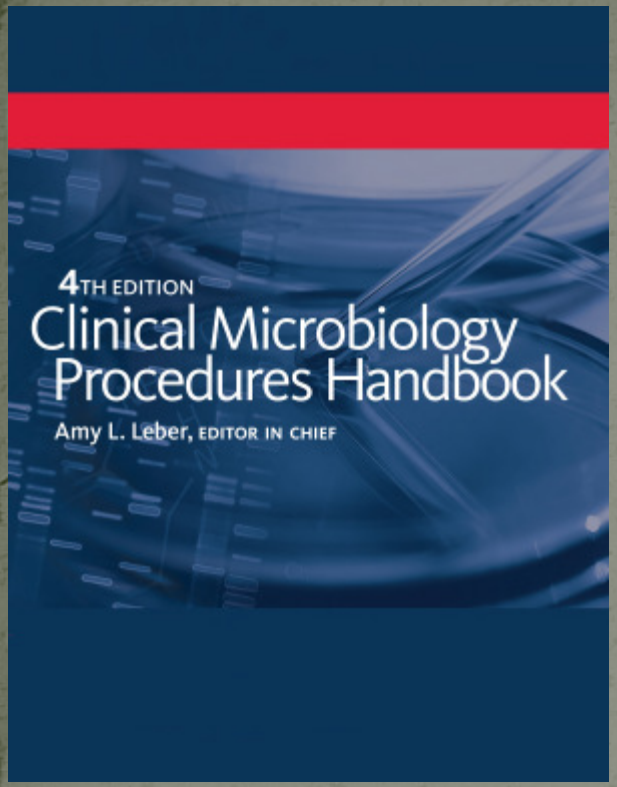
Part II

Pathogens

Relevance, Identification and Susceptibility

Key points

- There's more than one way to skin a bug
  - One or a few methods are described here – many others are acceptable.
  - In particular, MALDI-TOF is replacing conventional identification methods in many laboratories.
- Identification, susceptibility testing and relevance must be determined with evidence based approaches.
- Excellent references exist.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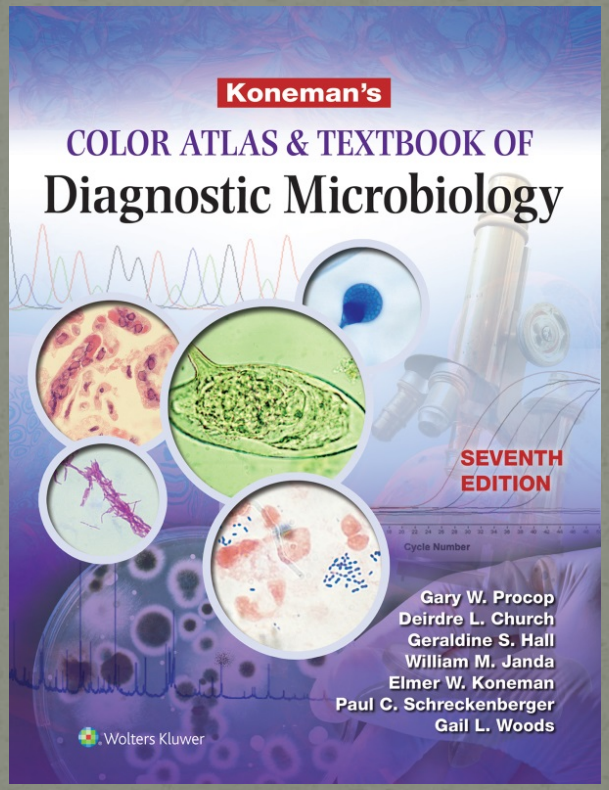
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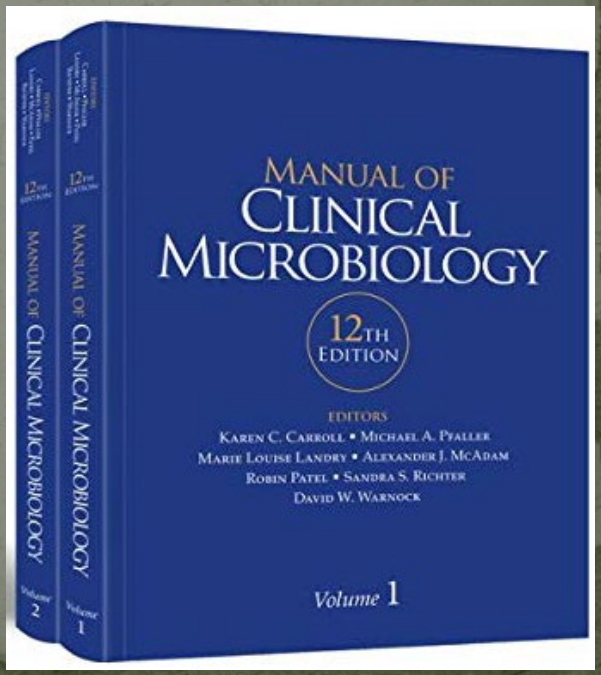
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EDITORS
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DAVID W. WARNOCK
Volume 1

Bacterial pathogens

- Bacteria are generally the only pathogens sought in smaller microbiology laboratories managed by GPs.
- However, as molecular procedures are simplified, virus detection may become more “mainstream”.
- Generally, ~90% of hospital microbiology laboratory workload is related to bacteriology.
- Note that virtually all of these can be identified by MALDI-TOF.

Top bacterial pathogens – a selected list

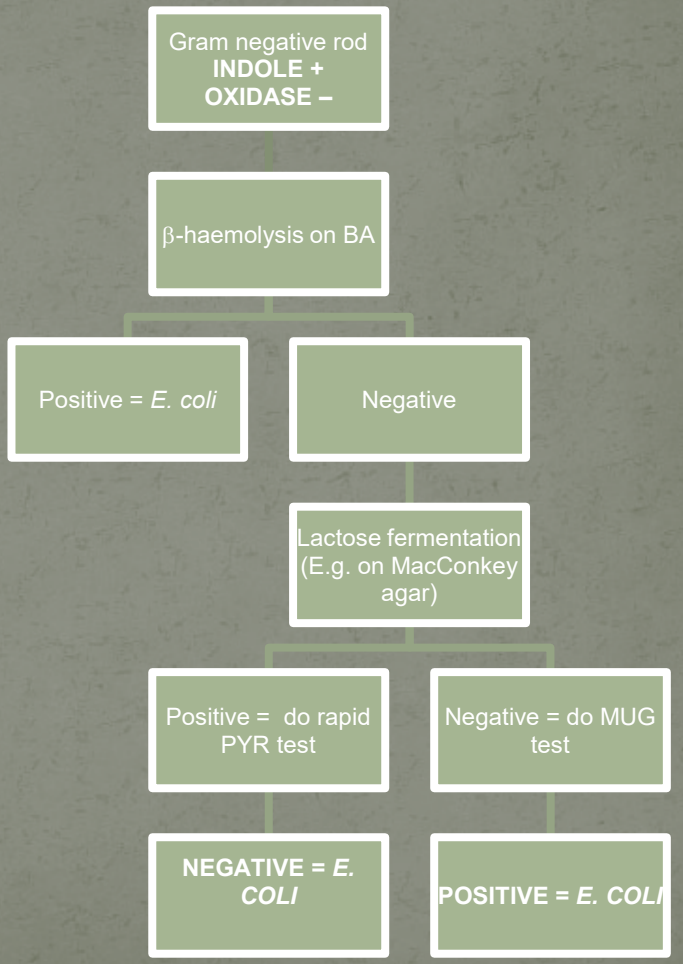
1. *E. coli*
2. *S. aureus*
3. *P. aeruginosa*
4. *S. pneumoniae*
5. *K. pneumoniae*
6. *Enterococcus spp.*
7. *H. influenzae*
8. β-haemolytic streptococci (A & B)

Escherichia coli - Relevance

- #1 cause of UTI in the hospital and community.
- #1 cause of bacteraemia in most hospitals.
- Common cause of VAP and HAP
- Common cause of wound infection in DFU.
- Most common facultative gram-negative associated with intra-abodominal infections.

Escherichia coli - identification

- Readily identified using commercial identification systems: Expensive, long turnaround and mostly unnecessary!
- Rapid identification methods are reliable, faster and often preferred.
- Chromogenic media useful for urine specimens.

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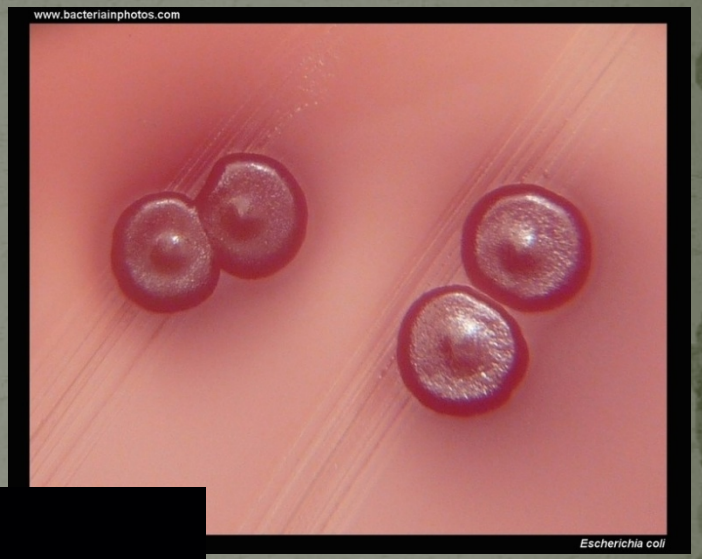
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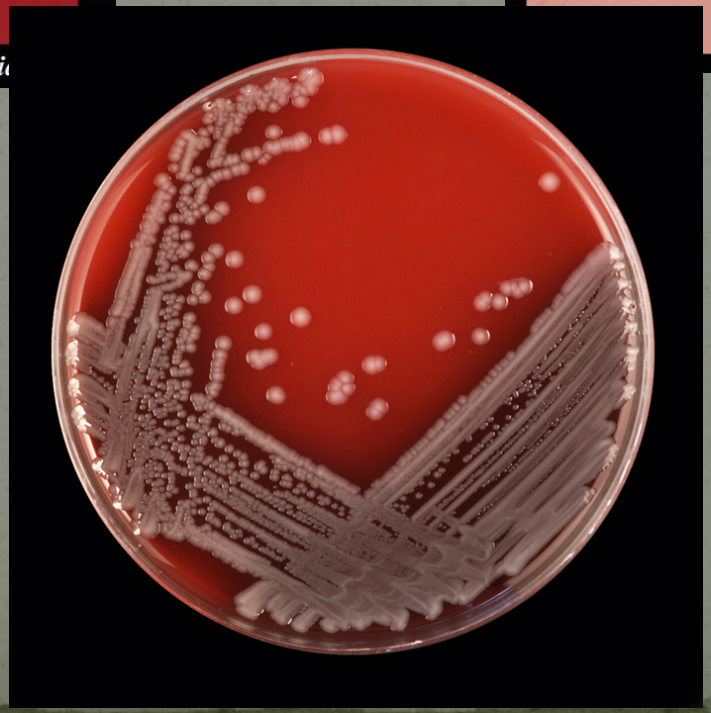
Escherichia coli



Escherichia coli – Identification pitfalls

- *Shigella* sp. are virtually identical to *E. coli* and rapid methods should not be used to ID *E. coli* from stool. Always use conventional methods and serotyping if *Shigella* sp. is suspected.
  - *Shigella* cannot be differentiated from *E. coli* (and vice versa) by MALDI-TOF.
- *E. coli* O157:H7 is metabolically different that most *E. coli*. Use serotyping and conventional identification if it is suspected.
  - Rapid biochemical methods shouldn't be used for stool samples.
  - Serotypes cannot be identified by MALDI-TOF.
- *Aeromonas* sp. is usually haemolytic, indole positive and looks like *E. coli*. Always do oxidase (+ for *Aeromonas* sp.) when performing identification.

Susceptibility

- Generally a very susceptible organism.
  - Susceptibility done using broth, disk or automated methods.
- Aminopenicillins, fluoroquinolones, cephalosporins, furans, aminoglycosides, tetracyclines, sulphonamides, carbapenems all effective for wild-type.
  - Dramatic increase in fluoroquinolone resistance concerning.
- ESBL-producers increasingly common:
  - Resistance to all penicillins, ?cephalosporins.
    - Make note of cephalosporin breakpoints
  - Infection control issue?
  - ~5-20% of isolates depending on region.
  - Concerning cause of UTI associated bacteraemia requiring empiric use of carbapenems.
- *New fluoroquinolone breakpoints!*

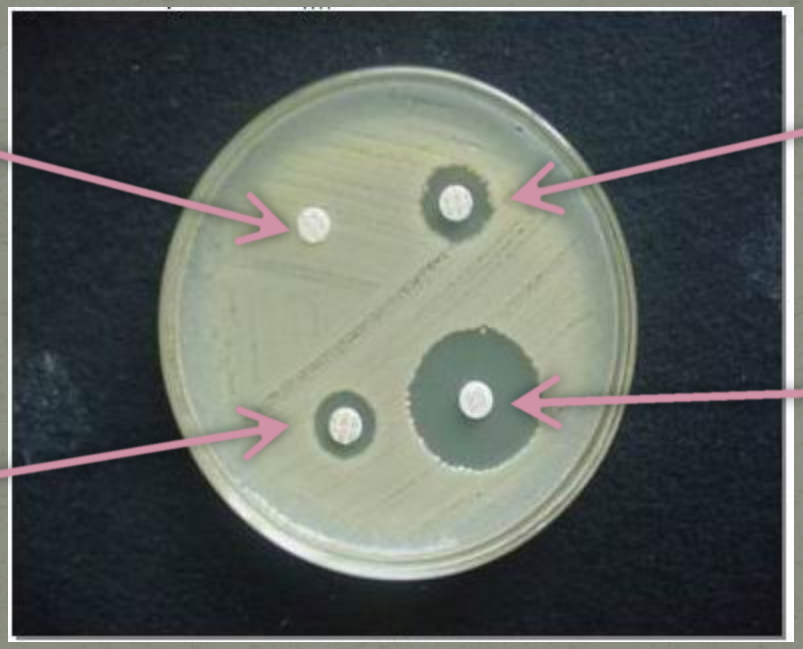
ESBL screening and confirmation

- All *E. coli* (or *Klebsiella* and *Proteus* sp.) with ceftriaxone or ceftazidime MIC > 1 μg/mL are suspect ESBL-producers.
- Confirmation can be achieved using beta-lactamase inhibition tests
  - Commonly called "double disk test".
  - Principle is to inhibit ESBL using a beta-lactamase inhibitor.
- No longer needed with new breakpoints
  - Unless it is an IP&C issue in your hospital

ESBL confirmation

Ceftazidime disk

Ceftriaxone disk



Ceftazidime disk with clavulanate

Ceftriaxone disk with clavulanate

> 5mm difference for either = ESBL

Carbapenemase production

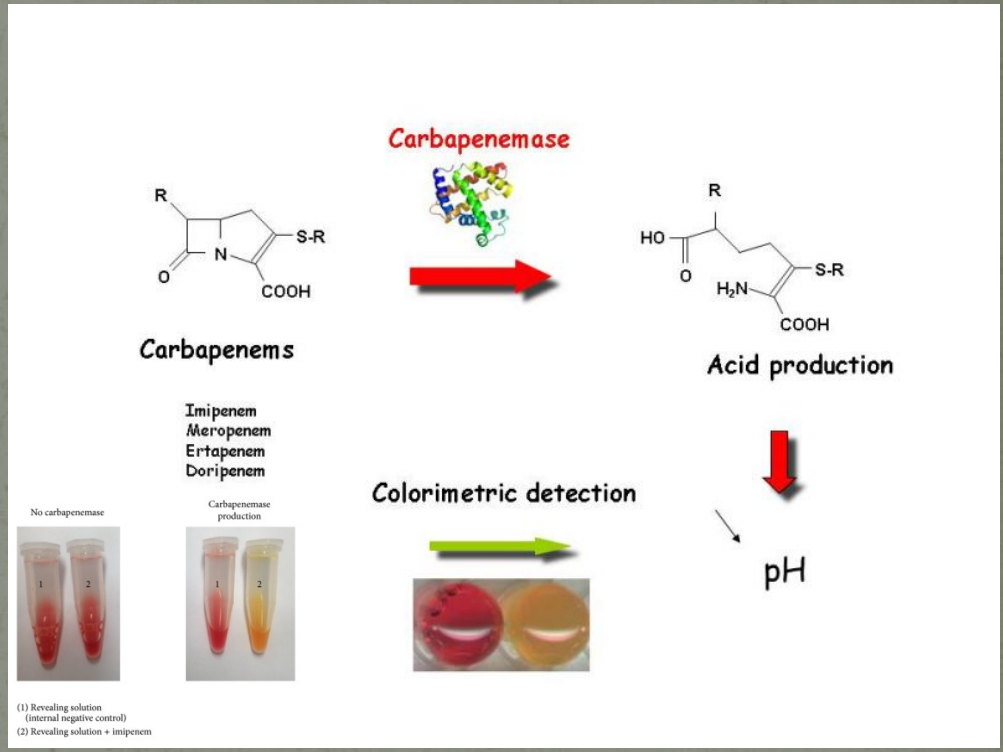
- Carbapenemases can occur in a variety of Gram-negative bacteria, but those in Enterobacteria are particularly significant.
- Resistance to carbapenems in Enterobacteria is more commonly caused by AmpC or ESBL production with impermeability rather than carbapenemase production.
- CLSI guidelines offer good screening tests, but confirmation is required.
- Multiple carbapenemase enzyme types exist: KPC, NDM, VIM, IMP, SME, OXA…

Carbapenem resistance - screening

- CLSI recommends either:
  - Ertapenem 10μg disk or 1μg/mL broth
  - Meropenem 10mg disk or 1μg/mL broth
- Ertapenem is more sensitive but less specific.
- Only screens for resistance, not carbapenemase production.
- CLSI recommends Carba NP test or modified carbapenem inactivation (mCIM) test for confirmation.
  - Carba NP
    - High sensitivity and specificity, rapid (2h) TAT.
    - Lower sensitivity for OXA enzymes.
    - Good for *Pseudomonas* and *Enterobacteriaceae*
  - mCIM
    - High sensitivity and specificity, including OXA enzymes.
    - Good for *Pseudomonas* and *Enterobacteriaceae*
    - Requires overnight incubation

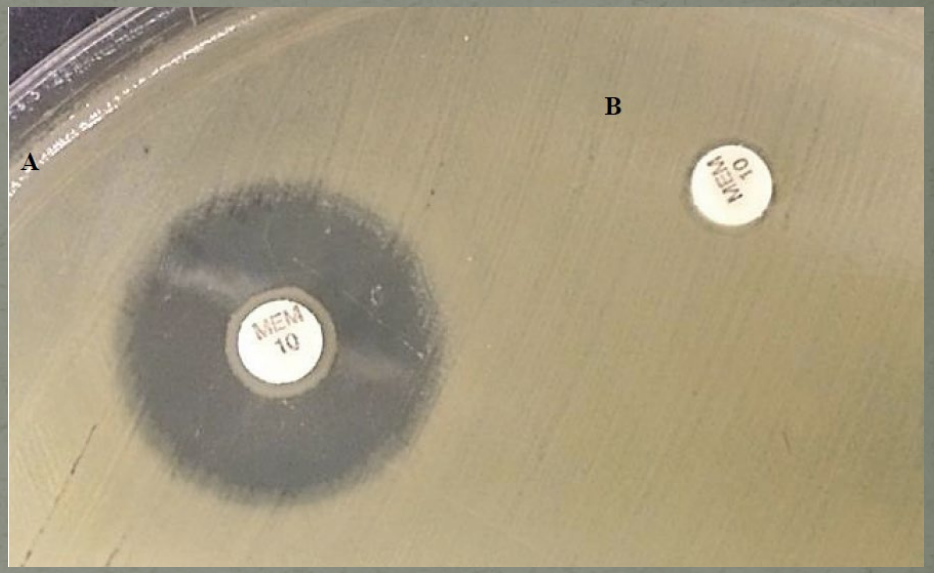
Phenotypic confirmatory tests

- CarbaNP Test:
  - Few reports of false negative results except for OXA enzymes.
  - Reliable for many types of carbapenemases.
  - No false positives reported.
  - Takes 2 hours.
  - Works for Enterobacteriaceae and *Pseudomonas*.



Phenotypic confirmatory tests

- Carbapenem inactivation test (mCIM)(possibly the best test)
  - >99% sensitivity and specificity.
  - Reliable for many types of carbapenemases.
  - 4 hour incubation of meropenem disk in broth with test strain.
  - Remove disk and place on lawn of susceptible *E. coli*.
  - Zone<15mm indicates carbapenemase production.
  - Works for Enterobacteriaceae *Pseudomonas* and likely *Acinetobacter*.



Immunoassays and PCR

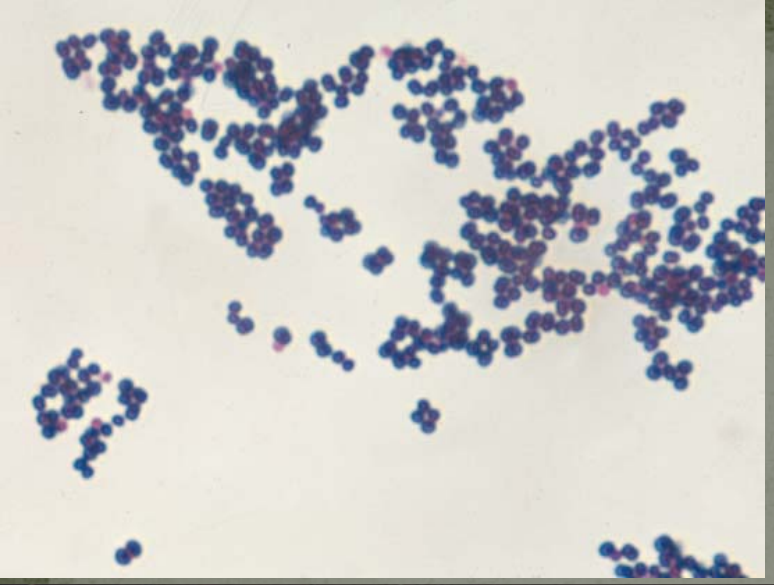
- Immunoassays and PCR can also be used to detect carbapenemases in suspect isolates.

S. aureus - Relevance

- #1 cause of all wound infections in community AND hospital.
- #2 cause of bacteraemia in most hospitals.
- #1 cause of septic joints.
- Common cause of pneumonia in hospitals.

S. aureus - Identification

- Rapid identification is possible for >95% of *S. aureus* isolates.
- Typical colony, gram-positive cocci in clumps, catalase positive and slide coagulase positive.
  - Identifies ~80-90% of *S. aureus*.
  - Some isolates require tube coagulase test.
  - Very few require identification panels or biochemical identification.
- Thermostable DNase is another method of identification.
  - Can be applied directly to blood culture bottles.

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S. aureus – identification pitfalls

- *Micrococcus* sp. are similar but coagulase negative, usually form GP tetrads and colonies are different.
- *S. lugdunensis*, *S. schleferi* are slide coagulase positive.
- *S. pseudintermedius, S. intermedius*, *S. hyicus, S. argentus* may be tube coagulase positive.
- *S. intermedius*, *S. hyicus* and *S. schleiferi* may be thermonuclease positive.
- About 5-10% of *S. aureus* are slide coagulase negative. Always do tube coagulase if suspicious.

S. aureus - Susceptibility testing

- Broth, diffusion and automated methods all acceptable for testing.
- Wild-type strains are susceptible to oxacillin (methicillin), cephalosporins, sulfonamides, macrolides, lincosamides, glycopeptides, fluoroquinolones.
- Methicillin resistance is common (~30%) and confers resistance to **all** beta-lactams and carbapenems.
  - Except ceftaroline and ceftobiprole

MR screening and confirmation

- 30µg cefoxitin disk or broth/agar with 6-8mg/L cefoxitin has become standard method for most MR detection.
- Chromogenic media popular for screening carriers, rapid detection.
- Definitive confirmation can be by mecA PCR or PBP2' by latex agglutination.
- Direct molecular detection of mecA is available.
- MecC gene is harder to detect with phenotypic methods and will not be detected by PCR.

MR detection – method is organism dependant!

Table 2C. *Staphylococcus* spp. (Continued)

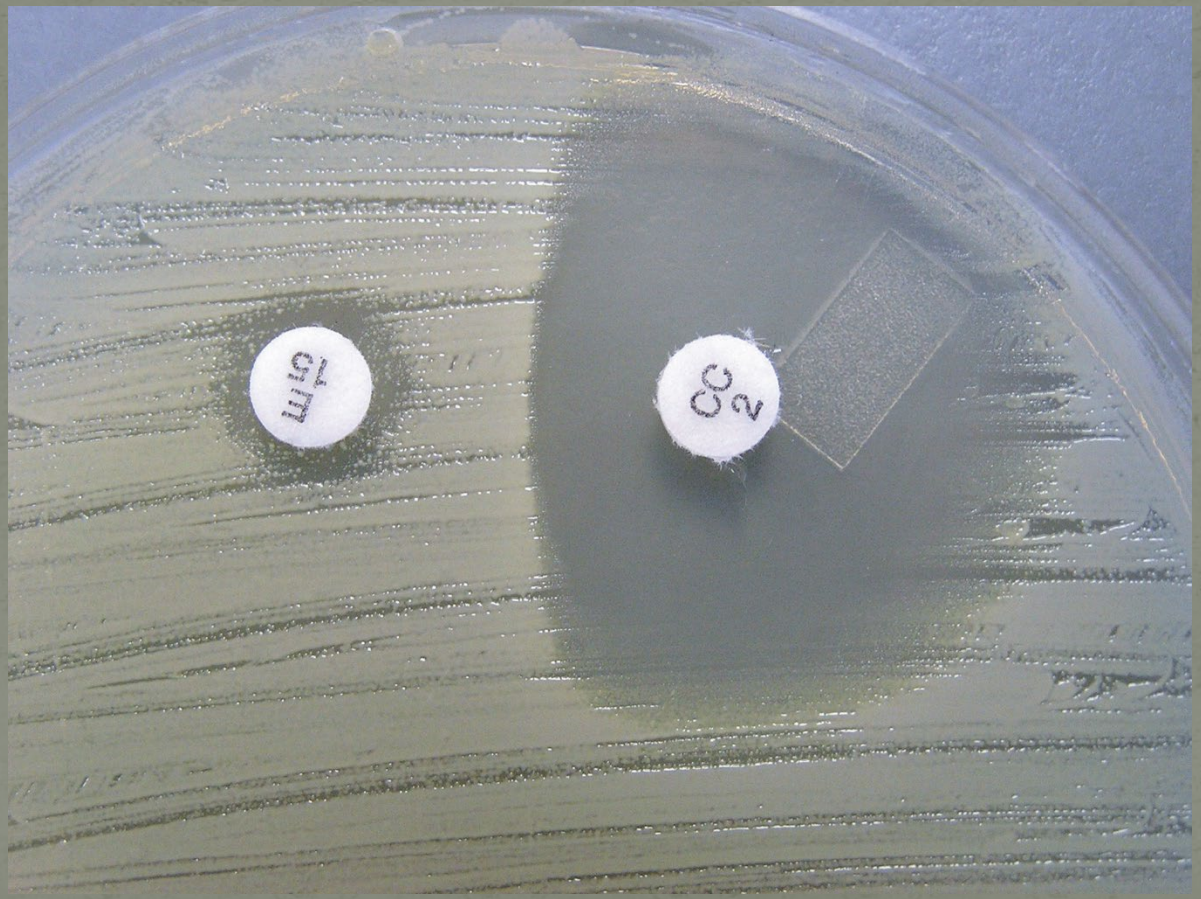
Test/Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
				S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)												
A	Oxacillin	*S. aureus* and *S. lugdunensis*	–	–	–	–	–	≤2 (oxacillin)	–	–	≥4 (oxacillin)	**(13)** Oxacillin disk testing is not reliable **for *S. aureus* and *S. lugdunensis*.** **(14)** For isolates of *S. aureus* that do not grow well on CAMHB or unsupplemented MHA (eg, small-colony variants), testing on other media (eg, BMHA) does not reliably detect *mecA*-mediated resistance. Testing for PBP2a using induced growth (ie, growth taken from the zone margin surrounding a cefoxitin disk on either BMHA or a blood agar plate after 24 hours incubation in 5% CO_2) or *mecA* should be done. See general comments (5) and (6) and comments (8), (11), and (12).
			30 µg cefoxitin (surrogate test for oxacillin)	≥ 22	–	–	≤21	≤4 (cefoxitin)	–	–	≥8 (cefoxitin)	
A	Oxacillin	***S. epidermidis***	1 µg oxacillin	**≥18 (oxacillin)**	**–**	**–**	**≤17 (oxacillin)**	**≤0.25 (oxacillin)**	**–**	**–**	**≥0.5 (oxacillin)**	**See general comments (5) and (6) and comments (8), (11), and (12).**
			30 µg cefoxitin (surrogate test for oxacillin)	**≥25 (cefoxitin)**	–	–	**≤24 (cefoxitin)**	–	–	–	–	**(15) Cefoxitin MIC testing is not reliable for detecting *mecA*-mediated resistance in *S. epidermidis*.**
		S. pseudintermedius and *S. schleiferi*	1 µg oxacillin	≥18	–	–	≤ 17	≤0.25	–	–	≥0.5	**(16)** Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius* and *S. schleiferi*. **See general comments (5) and (6) and comments (8), (11), and (12).**
A	Oxacillin	**Other *Staphylococcus* spp. (excluding** *S. aureus, S. lugdunensis, S. epidermidis, S. pseudintermedius, and S. schleiferi*)	30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 **(cefoxitin)**	–	–	≤ 24 **(cefoxitin)**	≤0.25 (oxacillin)	–	–	≥0.5 (oxacillin)	**(17)** For ***Staphylococcus* spp. other than *S. aureus, S. lugdunensis, S. epidermidis, S. pseudintermedius, and S. schleiferi***, oxacillin MIC breakpoints may overcall resistance. Isolates for which the oxacillin MICs are 0.5–2 µg/mL have been shown to be *mecA* positive and *mecA* negative. Isolates from serious infections with MICs in this range may be tested for *mecA* or for PBP2a. See general comments (5) and (6) and comments (8), (11), and (12).

Organism	Acceptable Methods				
	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
S. aureus	Yes	Yes	Yes	No	Yes
S. lugdunensis	Yes	Yes	Yes	No	No
S. epidermidis	**No**	**Yes**	**Yes**	**Yes**	**No**
S. pseudintermedius	No	No	Yes	Yes	No
S. schleiferi	No	No	Yes	Yes	No
Other *Staphylococcus* spp. (not listed above)	No	Yes	Yes*	No	No

Inducible clindamycin resistance

- Clindamycin resistance in *S. aureus* may be inducible and requires special testing.
- All erythromycin resistant, clindamycin susceptible strains should be tested for inducible resistance.
- Erythromycin is the inducer of choice
  - Broth and disk methods exist.

“D” test for inducible resistance

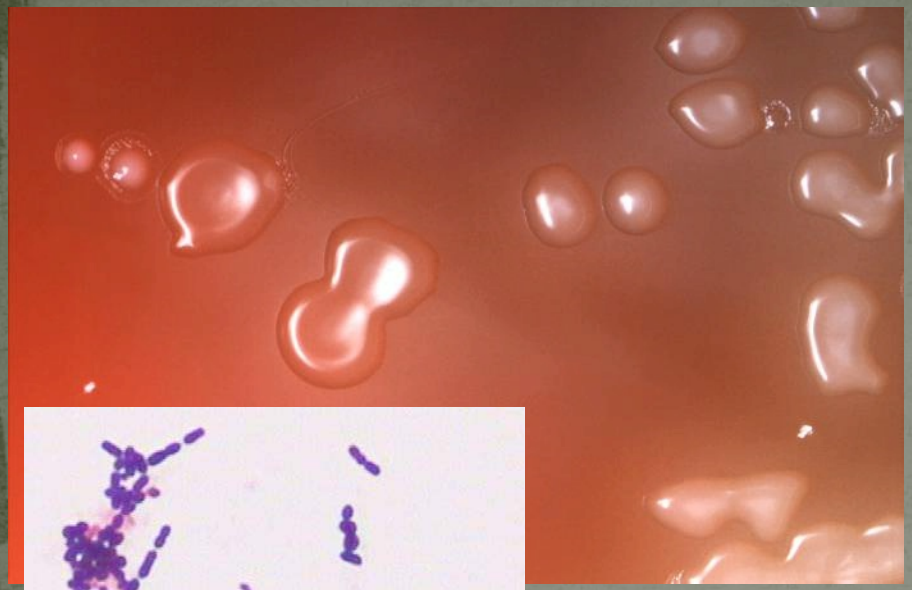


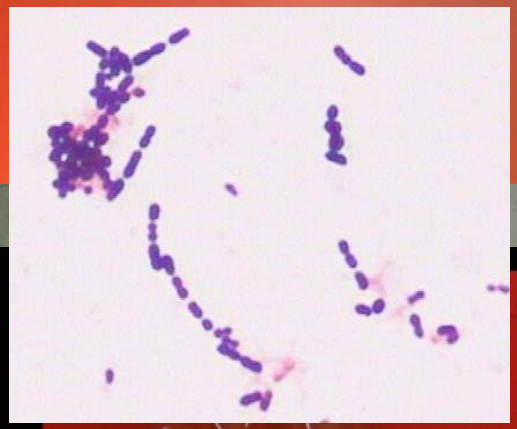
S. pneumoniae - relevance

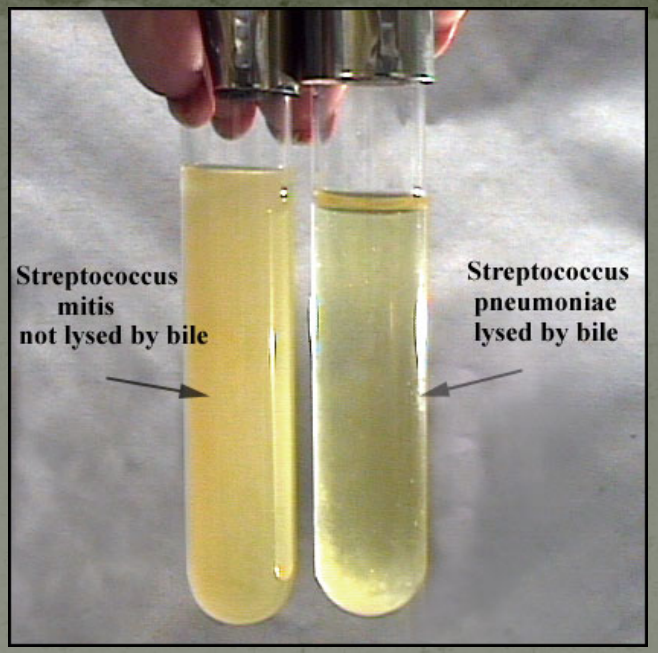
- #1 cause of pneumonia
- #1 cause of bacterial meningitis
- Common cause of bacteraemia
- Common cause of SBP.
- #1 cause of bacterial otitis media

S. pneumoniae - Identification

- Readily identified from blood agar plates grown in CO_2.
- Grey, α-heamolytic, translucent, mucoid or centrally depressed, Gram-positive cocci in pairs or short chains.
- Catalase negative.
- 2% bile (DCA) solubility confirms *S. pneumoniae*.
- Optochin ("P disk") less reliable due to low specificity and sensitivity.







Streptococcus pneumoniae

Dissolved colonies of *S.pneumoniae* in bile.

S. pneumoniae – identification pitfalls

- Normal colonizer of the pharynx – not always pathogenic!
- Some viridans group Streptococci (e.g. *S. mitis*, *S. salivarius, S. pseudopneumoniae*) may have similar morphology – bile insoluble.
- MALDI-TOF does not yet appear to be able to identify *S. pneumoniae* reliably (Claims from manufacturers would need to be rigorously evaluated!!).
  - Especially from *S. pseudopneumoniae*

Susceptibility testing

- Penicillin remains the drug of choice but resistance is increasing.
- **Different breakpoints exist for meningitis and non-meningitis isolates.**
  - **Oral penicillin ≤0.06 / 0.12-1 / ≥2**
  - **IV penicillin ≤2 / 4 / ≥8**
  - **IV penicillin meningitis ≤0.06 / ≥0.12**
  - **Ceftriaxone ≤1 / 2 / ≥4**
  - **Ceftriaxone meningitis ≤0.5 / 1 / ≥2**
- Testing done with MH agar/broth with blood.
- Oxacillin disk used to screen for penicillin resistance.
- No disk tests for penicillins, cephalosporins.
  - Automated methods are suspect.
  - Broth methods best.

Pseudomonas aeruginosa - Relevance

- Common cause of VAP.
- Common cause of diabetic foot infections.
- Common cause of hospital associated UTI.
- Common cause of otitis externa
- Most common gram-negative pathogen in CF patients.
- Several mechanisms for intrinsic antibiotic resistance.

P. aeruginosa - identification

- Identification is relatively simple and rarely requires biochemical panels or automated identification systems.
  - MALDI-TOF works, but not required for most strains.
- Typical colony (+/- metallic sheen, +/- haemolytic, +/-pigmented, +/- mucoid, “grape-like” odour).
- Oxidase positive, indole negative, gram-negative rods.


ASM MicrobeLibrary.org © Buxton

P. aeruginosa - Pitfalls

- Caution with identification from CF patients – many non-fermenting gram-negative rods may be present and some may look like *Pseudomonas*.
  - *Less of an issue in MALDI-TOF era.*

P. aeruginosa – Susceptibility testing

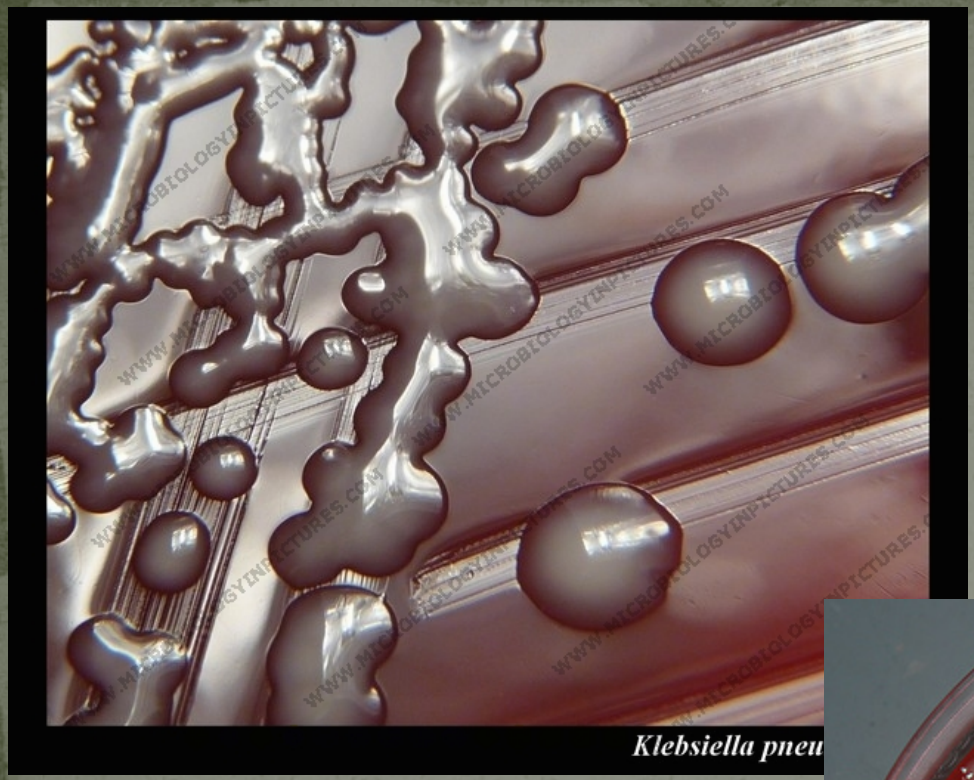
- Broth, disk and automated methods all available.
- *P. aeruginosa* is intrinsically resistant to many antimicrobials.
- Wild-type susceptible only to: Aminoglycosides, fluoroquinolones, ureidopenicillins (piperacillin), carbapenems (most), ceftazidime, ceftolozane, cefepime, colistin.
  - *New fluoroquinolone breakpoints have significantly reduced susceptibility rates!*
- Other antimicrobials may appear active *in-vitro* but should not be reported.
- Highly resistant isolates should be tested for susceptibility to colistin.

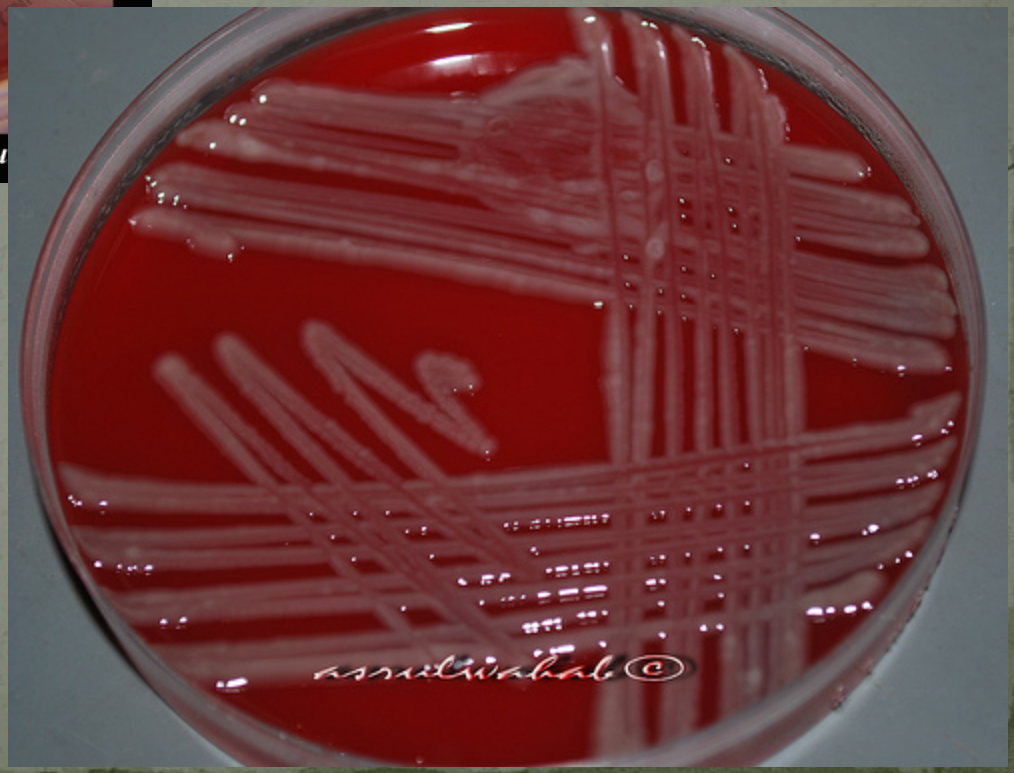
Klebsiella pneumoniae - relevance

- Overall, the most common relevant enterobacteria isolated from clinical specimens after *E. coli*.
- Common cause of HAP, UTI, complicated wound infections, surgical infections, liver abscesses…

K. pneumoniae – identification

- Colonies are gram-negative, typically mucoid, lactose fermenting and indole negative – but requires additional tests for identification.
- Most laboratories use automated ID systems for identification of this and most Enterobacteria.


Klebsiella pneu


asnulwahab©

K. pneumonaie – susceptibility testing

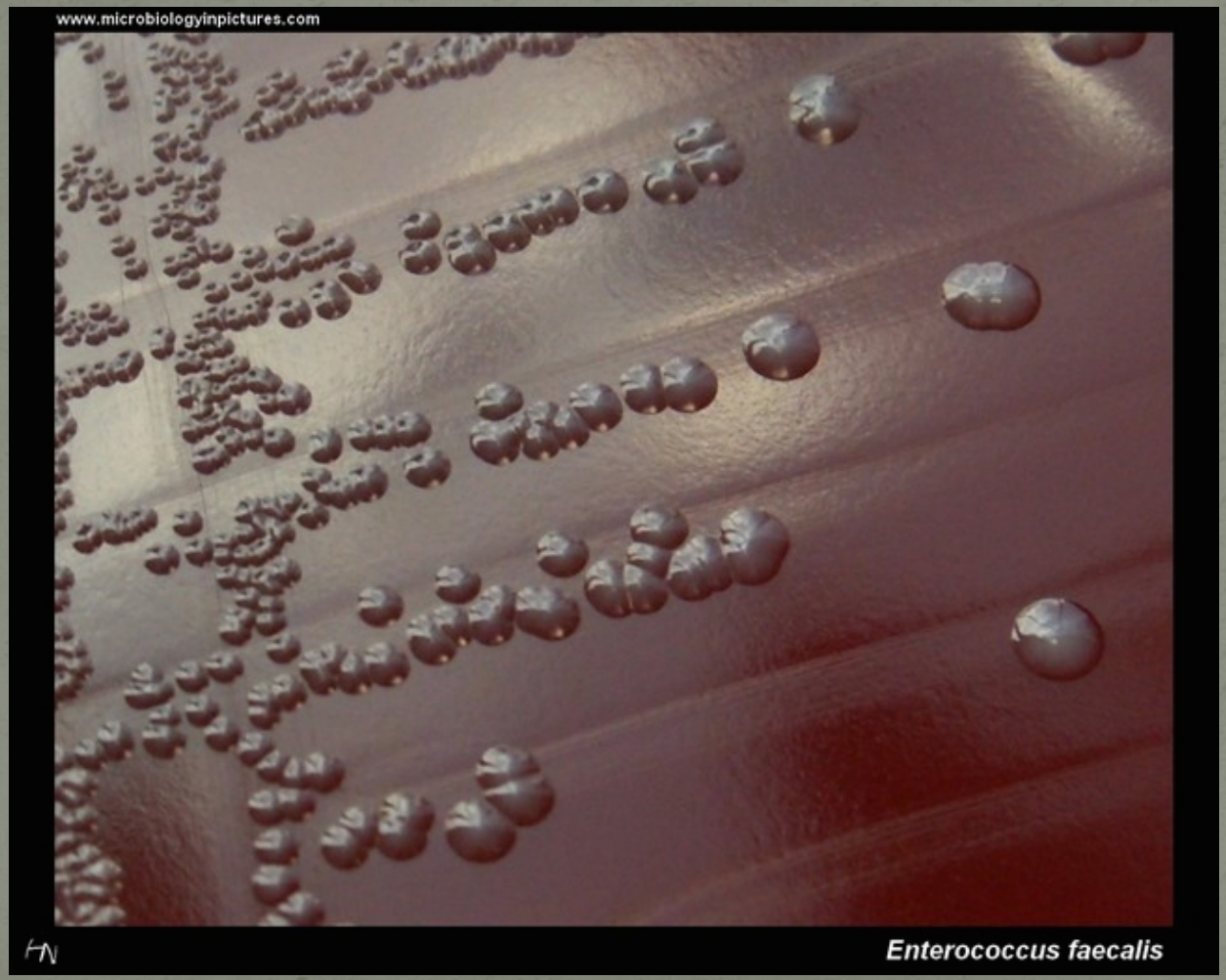
- ESBLs occur increasingly often and confer resistance to ALL penicillins and most cephalosporins.
  - Testing and screening same as for *E. coli.*
- CPE increasingly common (esp. KPC)
  - Testing and screening same as for *E. coli.*
- *New fluoroquinolone breakpoints!*

Enterococcus spp. - Relevance

- Normal resident of GI tract.
- Common cause of UTI in hospitalized:
  - Often no symptoms and catheter associated.
- Isolated from blood: Endocarditis, line infections, ascending UTI, abdominal source.
- Occasional pathogen in intra-abdominal infections.
- Rarely a pathogen elsewhere.
- Frequently resistant to many antimicrobials.

Enterococcus spp. - identification

- Simple algorithms will identify *Enterococcus* spp.
  - ~1-2 mm grey colonies
  - Gram positive cocci in chains/pairs
  - Catalase negative
  - non-haemolytic
  - PYR (pyrilidonylarylamidase) positive.
- Speciation requires additional testing.


www.microbiologyinpictures.com
HN
Enterococcus faecalis

Enterococcus – susceptibility testing

- Only vancomycin, ampicillin/penicillin and high level resistance to aminoglycosides should be generally be reported for blood isolates.
- Fluoroquinolones, furans, tetracyclines can be used for UTI.
- Only vancomycin and penicillin/ampicillin for other sites.
  - Additional agents are available for VRE
- Ceftriaxone now plays a role in the treatment (in synergy with ampicillin) of *E. faecalis* endocarditis. There are no standardized tests for susceptibility.

Vancomycin resistance

- Increasingly prevalent worldwide, occurs in outbreaks.
- Low virulence but may be difficult to treat.
- Usually in *E. faecium*, less commonly in *E. faecalis*.
- Only resistance conferred by VanA or VanB genes is relevant:
  - High level resistance, transmissible, epidemic.
- Treatment options include linezolid, tidezolid, daptomycin, tigecycline, tetracyclines, oritavancin, ampicillin (*E. faecalis*).

VRE

- Screening can be achieved using BHI medium with 6mg/L vancomycin.
  - Disk tests and broth tests can be used, but lower sensitivity
- VRE should be confirmed with identification tests (VRE are almost always *E. faecium* or *E. faecalis*)

VRE pitfalls

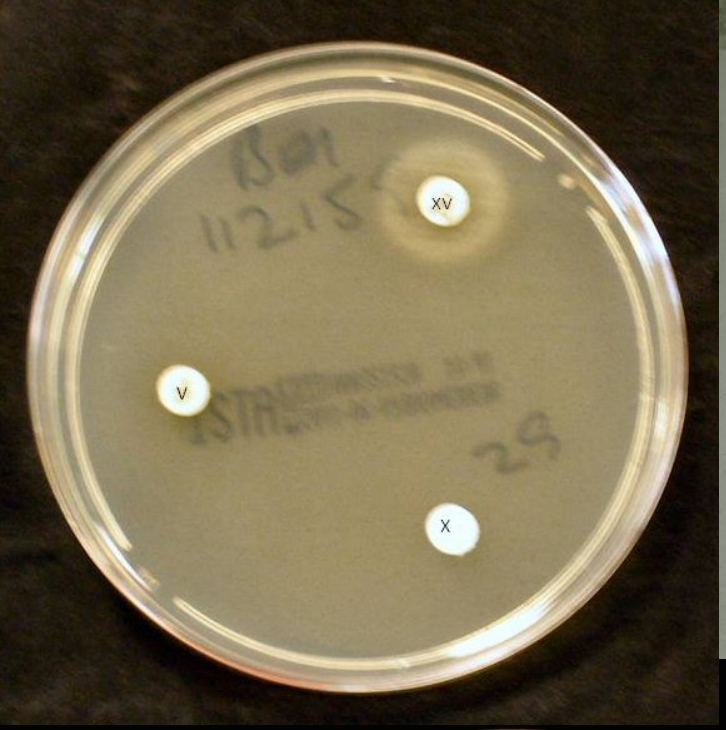
- *Enterococcus casseliflavus* and *E. gallinarum* are common gut commensals.
- Both have are have intrinsic low-level resistance to vancomycin.
  - MIC 8 – 32mg/L
  - Due to VanC gene
- They are susceptible to ampicillin, are not considered VRE and should not be reported in screening.

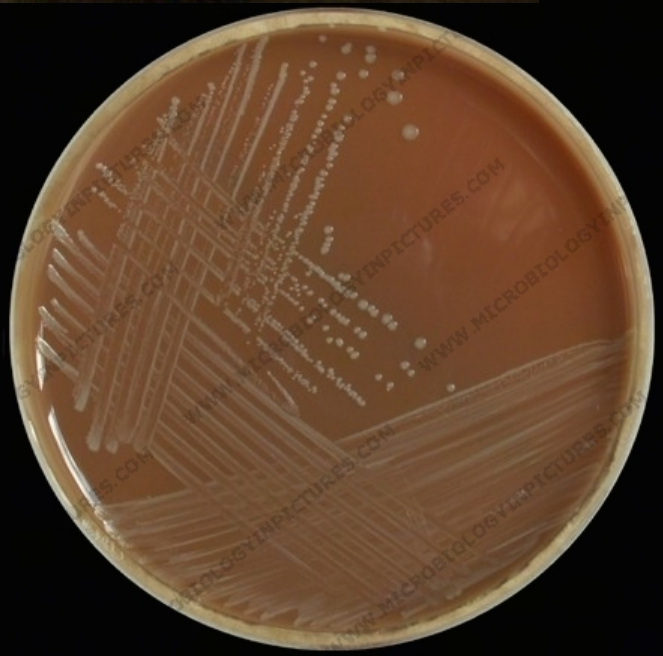
Haemophilus influenzae - relevance

- Common cause of pneumonia, bacteraemia, otitis media, meningitis.
- Disease most common at the extremes of age.
- Encapsulated typable strains tend to be more virulent (type a, b especially)

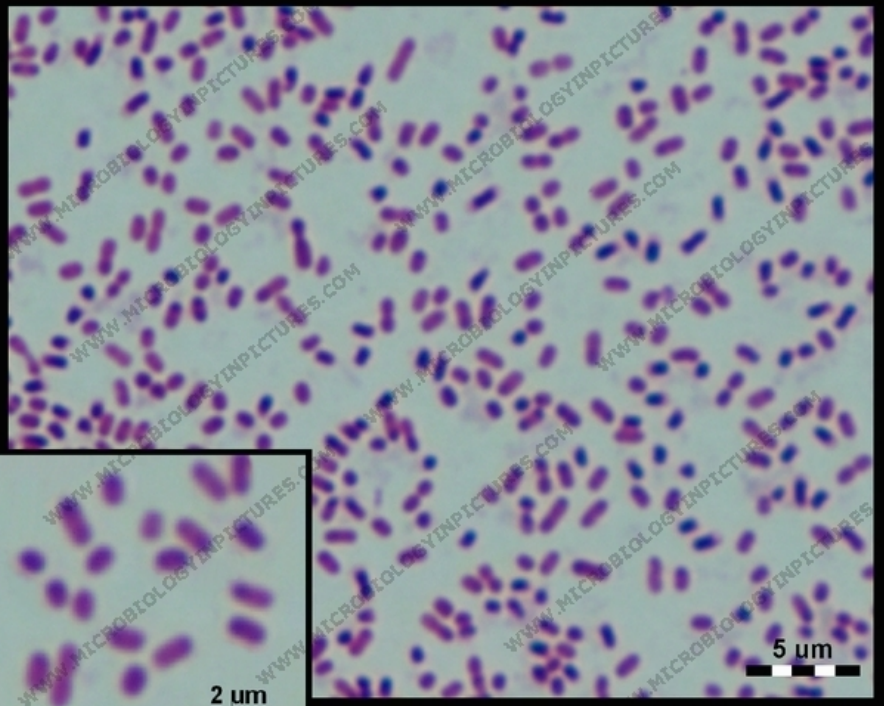
H. influenzae - identification

- Rapid identification methods readily available from respiratory or CSF cultures.
  - Caution when isolated from blood.
- Good growth of Gram-negative coccobacilli on chocolate agar at 24h, no growth on blood agar.
- Either negative for synthesis of porphyrin by the d-ALA test or requires both X and V factor for growth on basal media.





Haemophilus influenzae



Haemophilus influenzae

H. influenzae – identification pitfalls

- HACEK organisms, *Brucella* spp., *Francisella* spp., other *Haemophilus* spp. may be mistaken for *H. influenzae*
  - Avoid rapid algorithms from blood cultures if unsure.
  - Pure organism that grow on blood agar is not *Haemophilus influenzae*.
  - XV factors more specific.
  - MALDI-TOF works well and allows identification of uncommon *Haemophilus* sp.

β-haemolytic streptococci - relevance

- Group A streptococci (*S. pyogenes*) is the nearly exclusive cause of bacterial pharyngitis, second most common cause of cellulitis, commonest cause of necrotizing fasciitis, cause of pneumonia, bacteraemia…
- Group B streptococci are a common cause of cellulitis in diabetics (esp. DFU), neonatal sepsis, puerperal sepsis and surgical infections.
- Group C, G and *S. anginosus* are causes of abscess, wound and surgical infections. (not discussed here)

β-haemolytic streptococci

- Generally easily identified.
- Presence of β-haemolysis on Sheep Blood agar in typical grey colonies >1mm at 24h,, Gram positive cocci in chains, catalase negative.
  - Large zone haemolysis = presumptive GAS
  - Narrow zone = presumptive GBS
- PYR test = positive for GAS.
- Latex agglutination is available for all common streptococci (but more expensive).
- MALDI-TOF frequently used (see pitfalls).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DIAGNOSTICS
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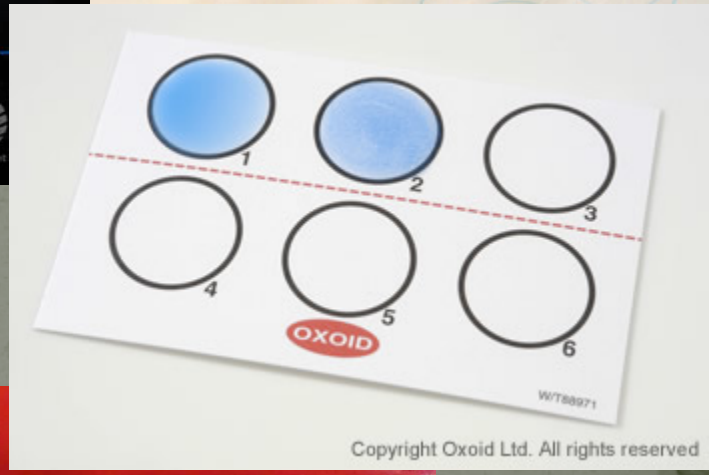
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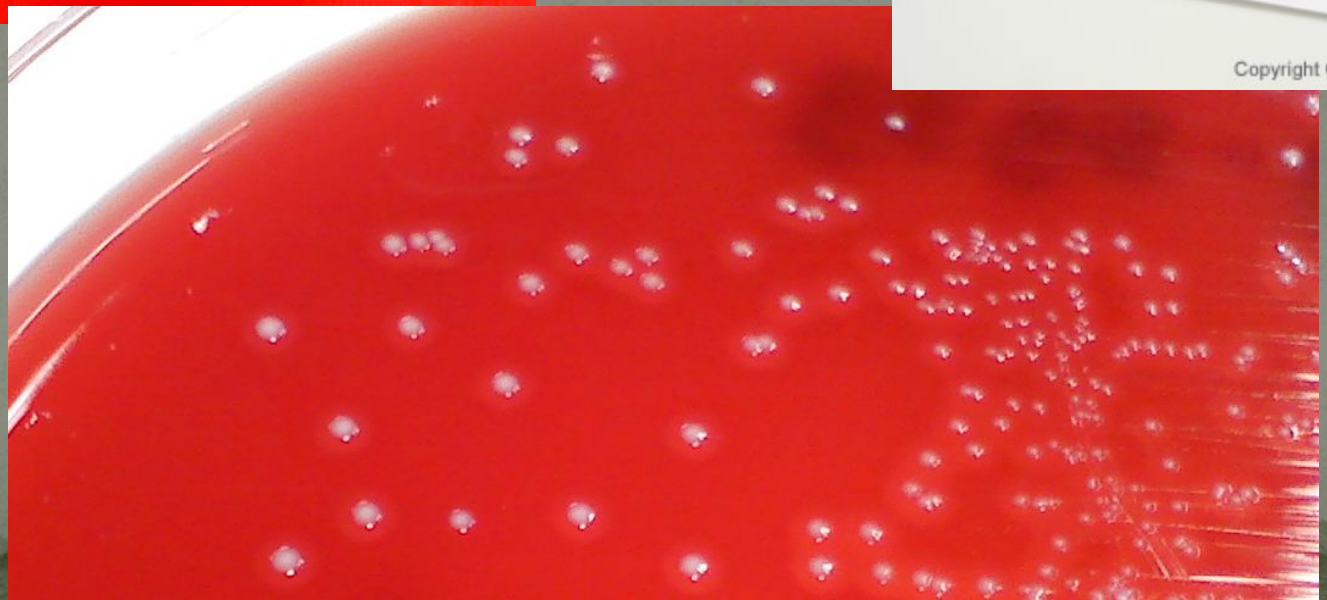


Pitfalls of identification

- GAS:
  - Occasional β-haemolytic Enterococci are also PYR positive.
  - May be very mucoid and look like gram-negative colonies
  - Difficulty in discrimination of *S. dysgalactiae*, *S. canis* and *S. pyogenes* by MALDI-TOF.
- GBS:
  - *Listeria* colonies look virtually identical, CAMP positive and carry Lancefield B antigen. Always do gram stain and catalase (+ for *Listeria*).
  - Non-haemolytic GBS occur. Look identical to *Enterococcus* spp. GBS is PYR negative.

Susceptibility testing

- Testing is not generally required.
  - All isolates are susceptible to penicillins and all β-lactams.
- Disk, broth methods are available. Automated methods are less common.
- Susceptibility testing should only be performed in very limited scenarios (e.g. anaphylactic penicillin allergy).
  - Clindamycin and erythromycin (not in GBS carriers!) can be considered.
  - D-test should be performed.

Clostridioides difficile

- Most common bacterial cause of diarrhoea in the hospital.
- Diarrhoea only caused by toxigenic strains.
- Diagnosis usually depends on demonstrating the toxin in a fecal specimen.

Clostridioides difficile toxin - detection

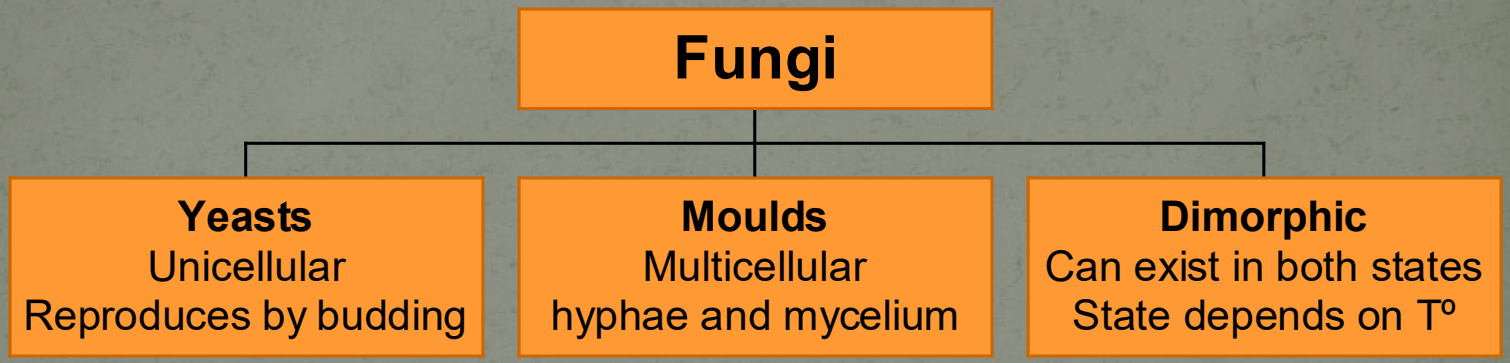
- Membrane Toxin A/B EIA.
  - Sensitivity generally low (~70%) – however Gold Standard may be suspect!
  - Rapid, easy to perform.
- Cytopathic effect.
  - Neutralizable CPE on HuFF cells is diagnostic and highly sensitive/specific.
  - Can be done directly on stool or from isolated organism
- PCR or NAAT for toxin gene
  - Highly sensitive and specific, but role of spores and low-level carrier states an issue that remains to be resolved.
- Culture
  - Highly sensitive, but role of spores and low-level carriers makes specificity suspect.

Toxin detection methods

Mycology

- Role of the general pathologist is two-fold:
  - Identification of fungal elements with LIMITED ability for organism identification from tissue sections.
  - More definitive identification of organisms from culture.
- Culture is always more definitive for identification but less specific for infection.
- MALDI-TOF is increasingly seen as a useful tool in mycology.
  - Already method of choice for yeast.
  - Used for moulds in some places.

Three main morphologies

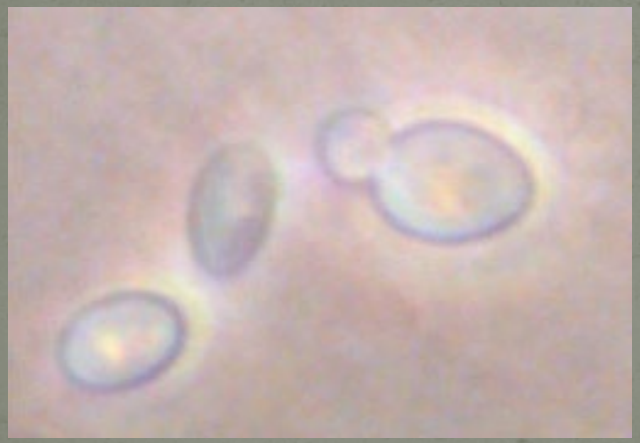


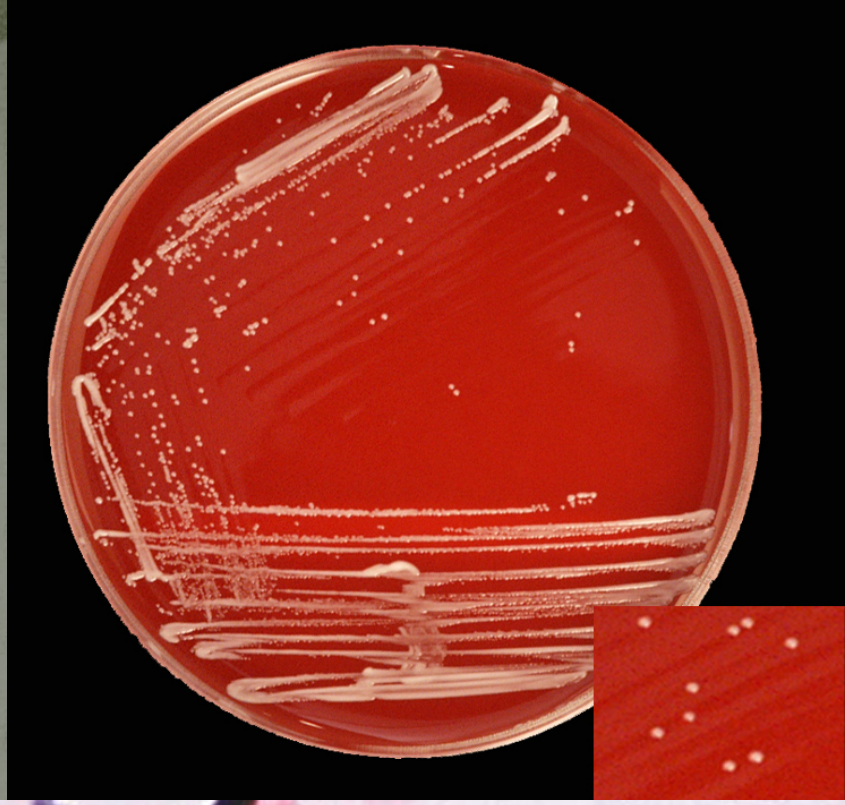
Candida

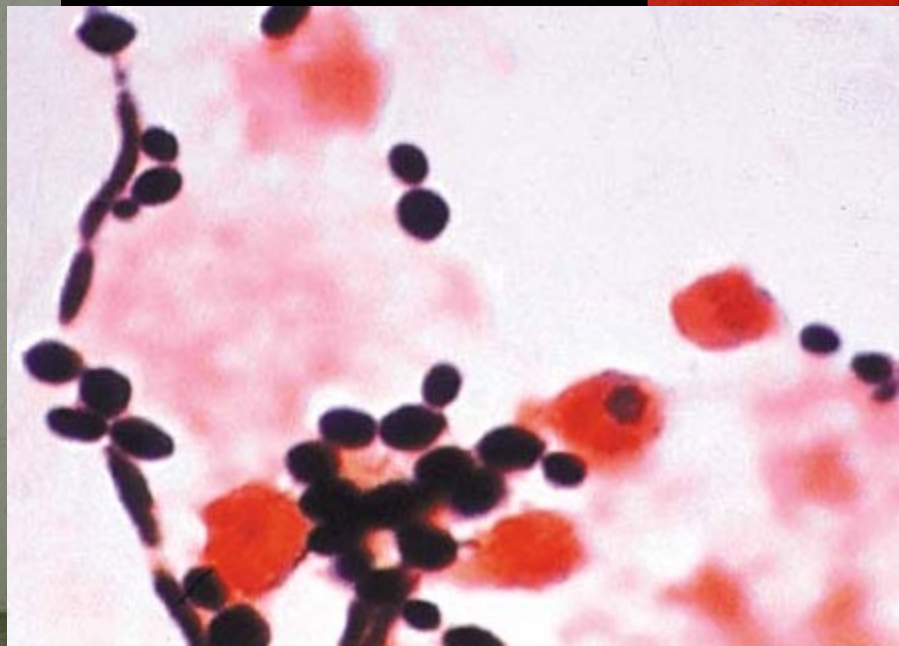
- Most common yeast isolated.
- All can form pseudohyphae and hyphae
- Generally unencapsulated
- Only *C. albicans* and *C. dublinensis* are germ-tube positive
- Urease negative
  - Differentiates from *Trichosporon*, *Cryptococcus* and *Rhodotorula*
- Identification using MALDI-TOF is typically very reliable.

Candida

- White colonies yeast on wet prep and gram stain dark purple.

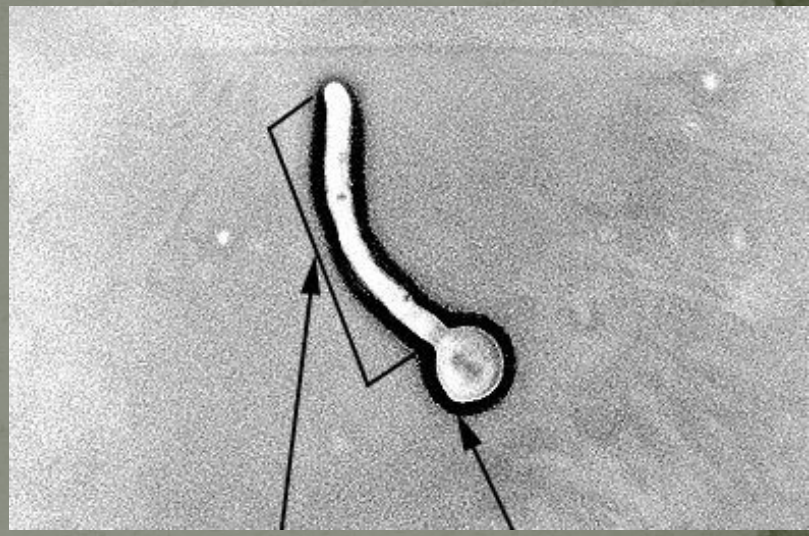


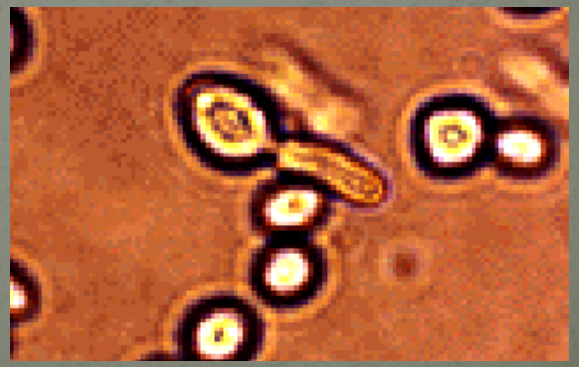




Germ tube

- Rapid test to diagnose *C. albicans* and *C. dublinensis* from other *Candida* spp.
- Incubate yeast in plasma for 3h.
- Observe for typical unconstricted germinating hyphae.





Other species of Candida

- *Candida albicans*
- *Candida tropicalis*
- ***Candida auris***
  - ***Multidrug-resistant, IPC issue.***
- ***Candida glabrata***
  - **Cystitis, azole resistance**
- ***Candida parapsilosis***
  - **Echinocandin resistance**
- ***Candida krusei***
  - **Azole resistance**
- *Candida lusitaniae*
  - Ampho resistance
- *Candida kefyr*
- *Candida guilliermondii*
  - Echinocandin resistance
- *Candida dubliniensis*
  - Azole resistance

- Differentiation is by MALDI-TOF, biochemical (esp. sugar assimilation), growth in cyclohexamide, @42ºC and cornmeal morphology.
- 18S/ITS gene PCR and sequencing another option for identification.

Cryptococcus neoformans

- Cause of meningitis in elderly or immunocomprimised
- Large capsule seen by india ink preparation on isolate or specimen.
- Cryptococcal antigen test is available – CSF or serum.
- Any capsulated, urease positive yeast is *Cryptococcus* sp. until otherwise proven.
  - There are a number of non-pathogenic species.
- *C. gattii* is an emerging pathogen in B.C, Western USA.
  - Pathogenic in immunocompetent
  - Level 3 pathogen!

Histopathology of fungi

- Speciation is generally not possible.
  - Except for few characteristic morphologies
- Significant overlap in size and morphology of most yeasts (and PCP).
  - Often "yeast-like elements" must be reported.
- Significant overlap in morphology and size of moulds.
  - Often "mycelial elements" can be reported.
  - May be possible to identify zygomycetes from hyalohyphomycetes from dematiaceous moulds but speciation usually impossible.
- Special stains (e.g Mucicarmine, Mason's), immunohistological stains and molecular diagnostics can be helpful.

Mycobacteriology

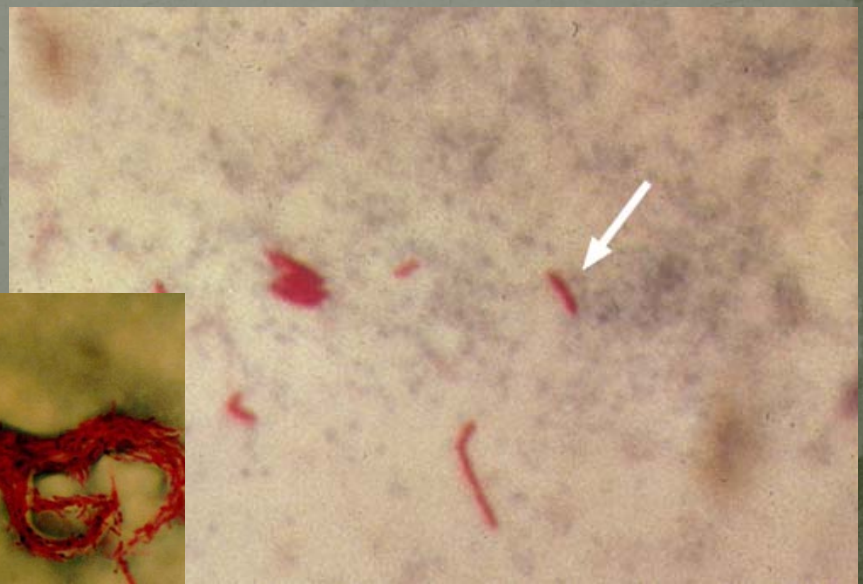
- Culture requires BSL3 containment and expertise in handling.
  - Generally the realm of reference labs.
  - Not discussed here…
- Smaller laboratories may be required to perform smears of concentrated specimens.
- Pathologists often called on for mycobacteria in tissue samples.

AFB smears in microbiology

- Specimens for culture are pre-treated and concentrated, then smeared and cultured.
- Labs without culture may pre-treat with hypochlorite and concentrate for smears.
  - Always send aliquots for culture!
- Smears are prepared using kinyoun (phenol-carbol-fuschin) stain and auramine-rhodamine.
- Speciation usually not possible with smears only.
  - New technologies (e.g. PCR) are changing this!

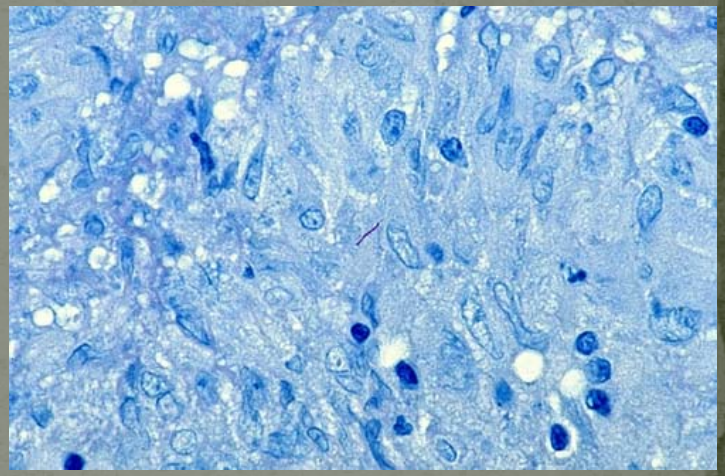
TB in sputum samples

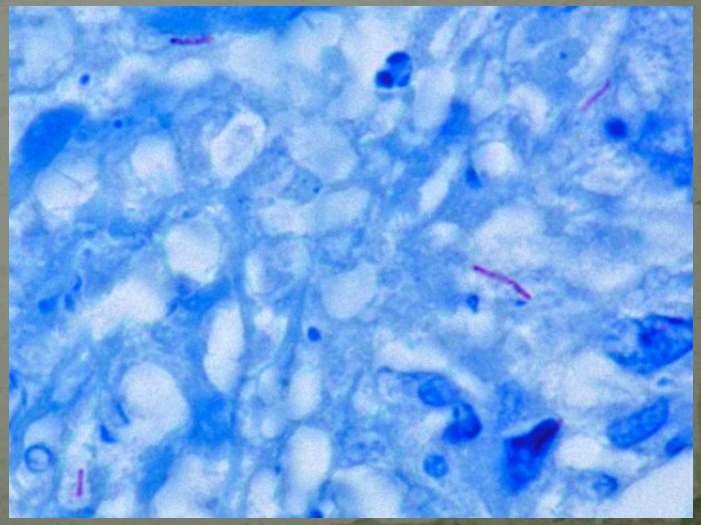
- In Canada, low AFB numbers is more common.
- >300 fields must be viewed.
- Auramine-rhodamine most sensitive.
  - Lower specificity – always confirm with Kinyoun.
- Cording may be present and points toward TB.
  - Uncommon *in vivo*



TB in tissues

- Paucity of AFB common.
- Cording very uncommon.
- Caseating granulomatous reaction is typical.
  - Tissue reactions may be atypical in immunocompromised.
- Diagnosis by PCR possible but generally requires fresh tissue!!





Virology

- Most virology is performed in reference laboratories.
- Antigen detection assays and PCR makes some assay accessible to smaller laboratories.
- Many viruses are considered infection control issues.

Influenza A & B & (C)

- Influenza A causes outbreak and pandemics of influenza.
- Influenza B causes limited outbreaks of influenza.
- Both are infection control issues.
- Diagnostics by EIA and NAAT are widely available.
  - EIA tend to have low sensitivity - exact number varies by strain and assay.
- Cell culture also possible but not commonly done anymore.


BinaxNOW®
Influenza A & B
Test
FLU B
FLU A
CONTROL
(+)
(-)

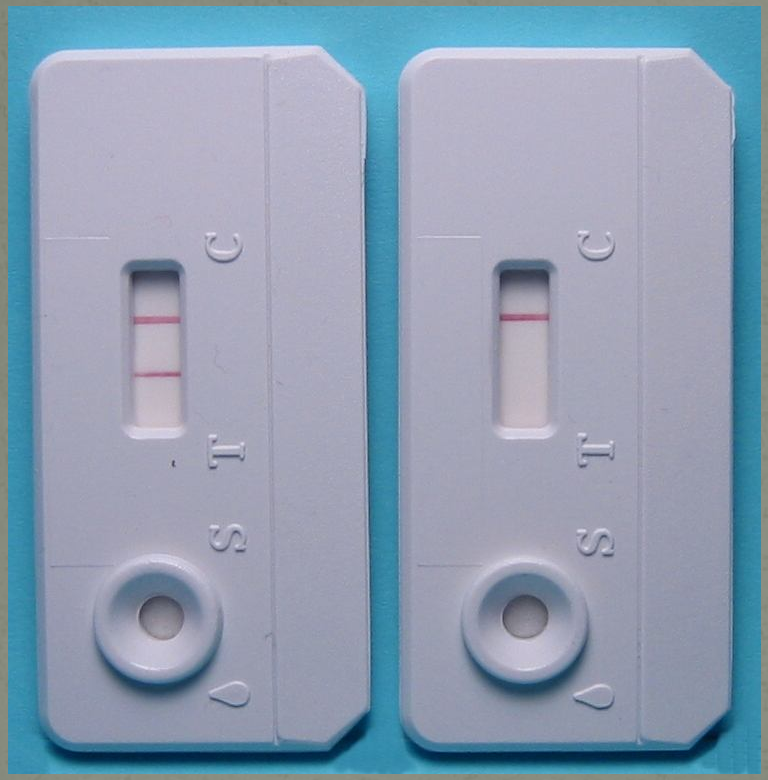

GeneXpert.

RSV

- Most common in children <5 years.
  - Causes bronchiolitis.
- Can be very serious and potentially deadly in neonates and premature infants.
- Diagnosis can be done by cell culture, but EIA methods and NAAT are popular.

HIV

- Diagnostics once the realm of reference labs.
  - Reference labs now generally do 4th generation ELISA, western blots and quantitative PCR.
- Rapid antibody tests using saliva, serum and whole blood now available and used in smaller laboratories – and POC. Simple RT-PCR assays also exist.
- All approved kits have high sensitivity and specificity but should be followed-up with reference tests.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OraQuick
ADVANCE
HIV-1/2

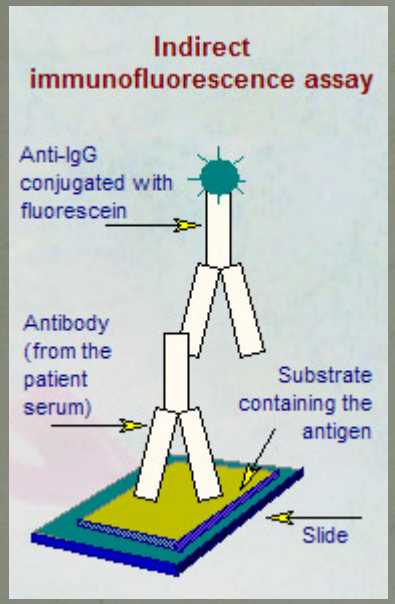
Serological detection of pathogens

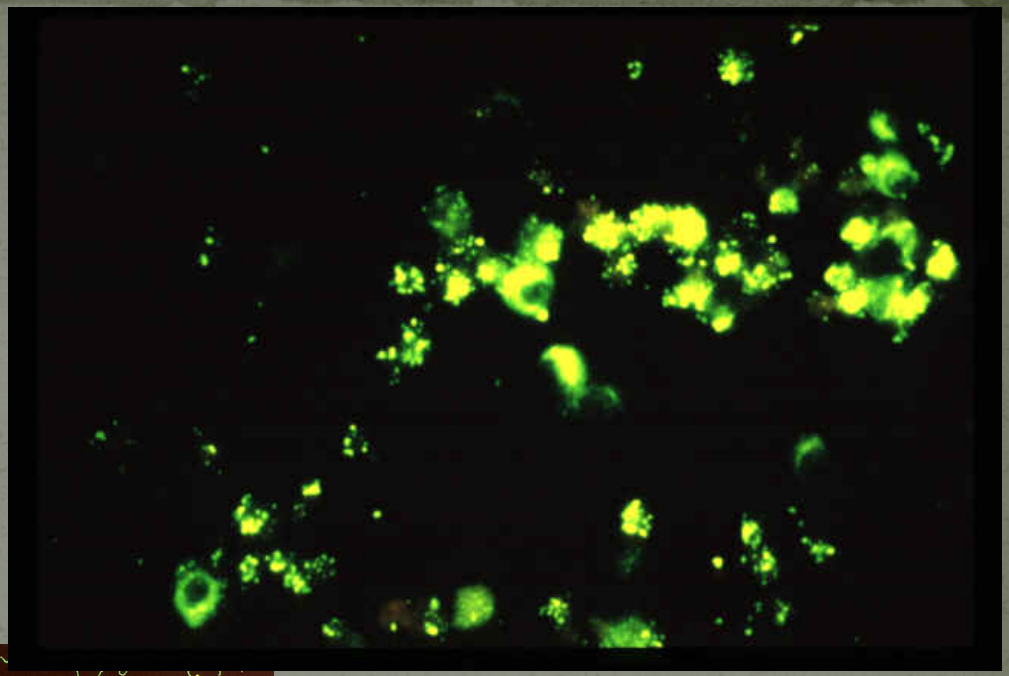
- Serological detection depends on the demonstration of targeted antibodies to a specific pathogen antigen.
- Several classes on antibody are produced by humans:
  - **IgG, IgM, IgA**, IgE, IgD
- Enormous variation in antibody response exists, but generally, presence of IgM or *rising* IgG level to a pathogen supports a recent infection and stable IgG levels implies a remote infection.

Methods

- Agglutination
  - Relatively simple method that observes an agglutination reaction between antibodies in the serum and an antigen. ASOT, Brucella and Syphilis (VDRL, RPR) are common examples.
- IFA
  - Antibodies from serum are bound to antigens on a slide (e.g. fixed organism).
  - A second anti human IgG (or IgM) bound to fluorophore is added and the fluorescence is observed by microscope.

IFA

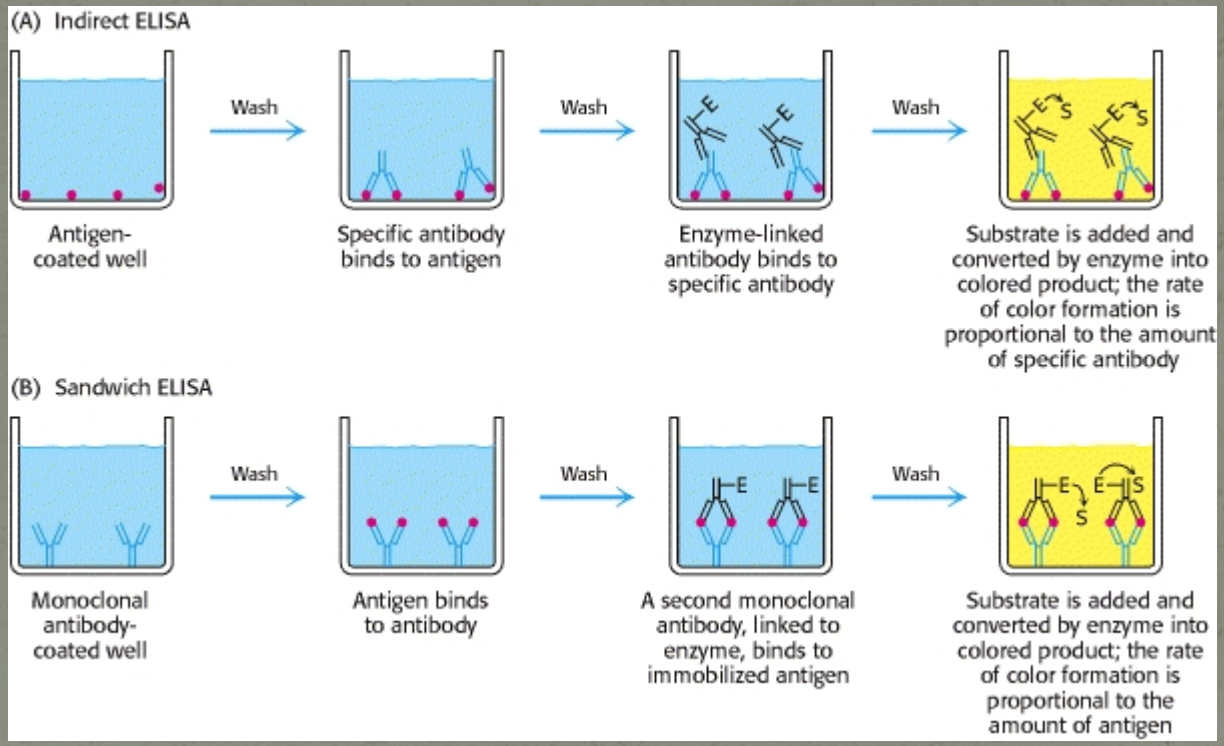

Indirect immunofluorescence assay
Anti-IgG conjugated with fluorescein
Antibody (from the patient serum)
Substrate containing the antigen
Slide



Methods (2)

- ELISA – enzyme linked immunosorbent assay.
  - Similar principle to IFA but a purified antigen is used in an plastic tray.
- Can be indirect ELISA or Sandwich ELISA
  - Depends on when patient antibodies are added to reaction.
- Targets IgG, IgM or others.
- Can be used to detect antigens or combinations of antigens and antibody (e.g. 4th generation HIV tests)

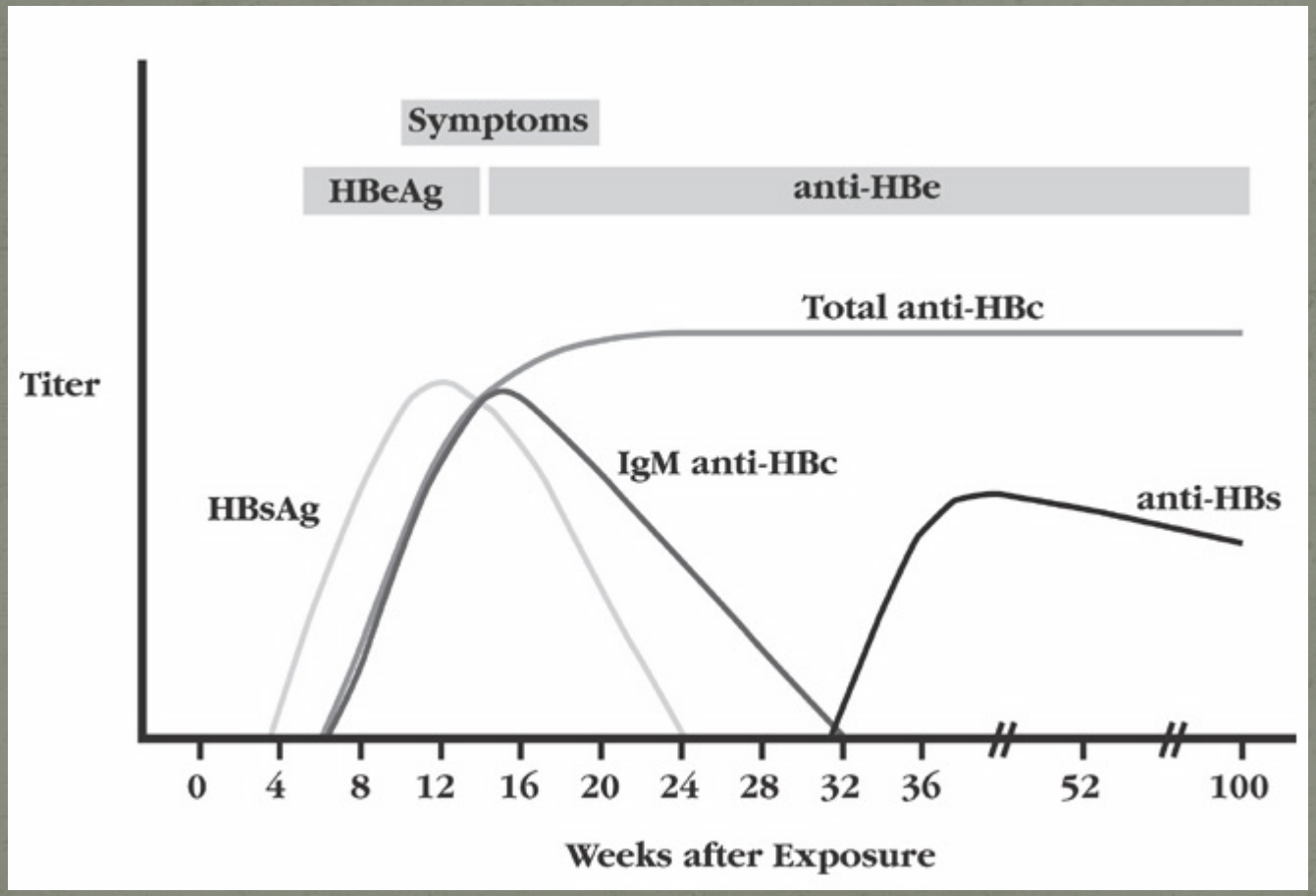
ELISA


(A) Indirect ELISA
Wash
Wash
Wash
E
E
E
S
E
S
Antigen-coated well
Specific antibody binds to antigen
Enzyme-linked antibody binds to specific antibody
Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of specific antibody
(B) Sandwich ELISA
Wash
Wash
Wash
E
E
E
E
S
S
Monoclonal antibody-coated well
Antigen binds to antibody
A second monoclonal antibody, linked to enzyme, binds to immobilized antigen
Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of antigen

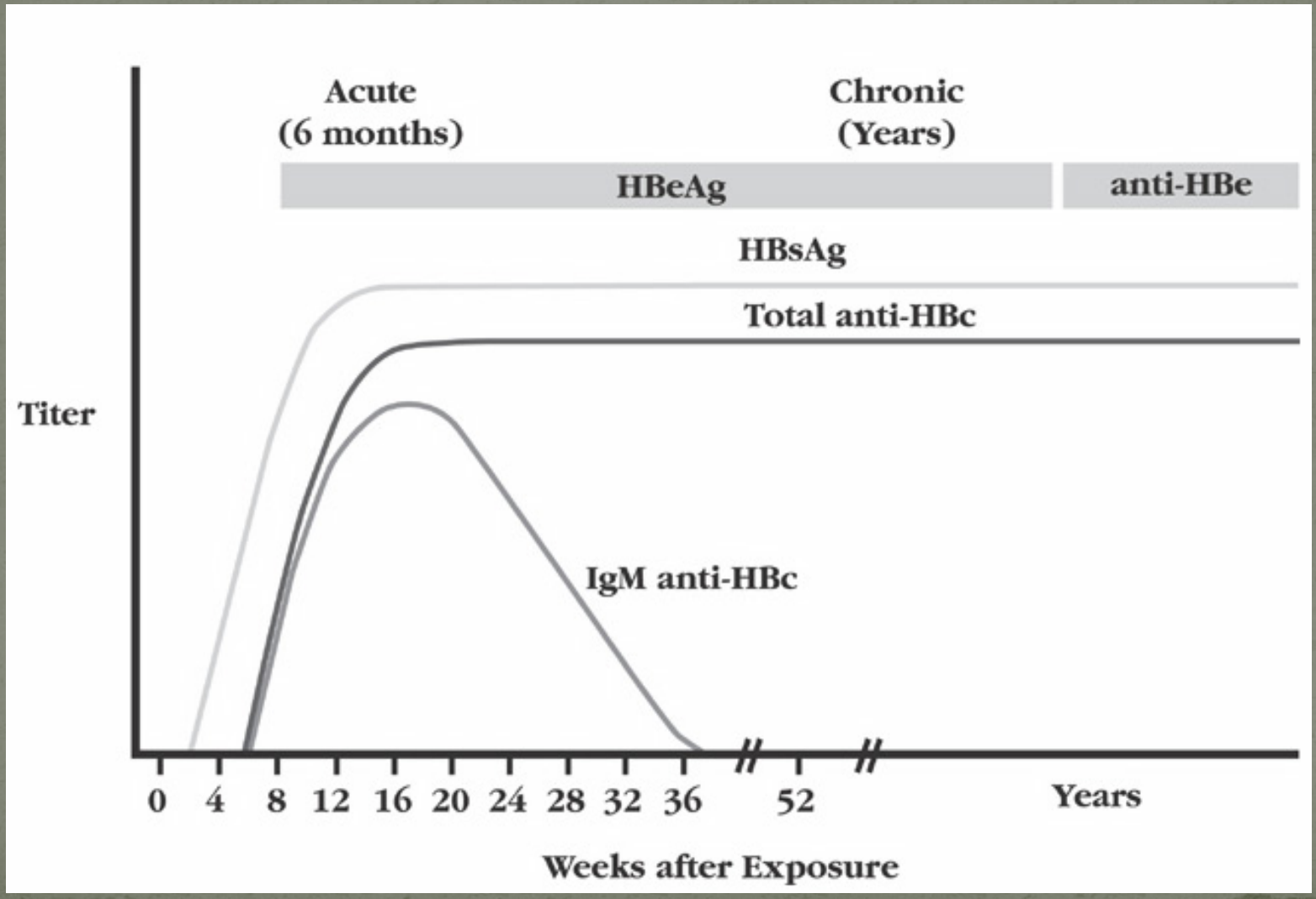
Serology – a single example HBV

- HBV has a number of relevant antigens and antibodies that are detected in serological assays:
  - HBsAg: Antigen expressed at the surface of virus. Present in acutely and chronically infected patients. Also the antigen in the vaccine.
  - HBsAb: Antibody to HBsAg. Created in response to resolved natural infection or vaccine. Confers immunity.
  - HBcAb: Antibody to core antigen of virus. Present only in individuals naturally infected.
  - HBeAg: Envelope antigen. Indicative of recent infection and generally more infectious when detectable. May persist.
  - HBeAb: Antibody to HBeAg. Present in natural infection (either resolved or late chronic).

Self resolving infection (~95%)



Chronic infection (~5%)



The end!