From Swab to Requisition: Interpreting Microbiology Reports

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Disclosure

- Jim has consulted with Arjo, and Crede Technologies during the past two years.
- Neither of these companies have had input into this presentation from a commercial interest
- Any images are used for support of presentation and do not imply a recommendation or endorsement

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Braveheart – Slayer of Germs

Fundraiser for IFIC: International Federation of Infection Control!
Top fundraiser every year I have taken part like this!
If interested in donating to support IFIC, please send me a note via my email (last slide).

3

Objectives

- At the end of the session, the learner will:
  - Be able to list at least two pieces of information required on a requisition to ensure identification and clinically relevant results
  - Understand the proper collection of urine and stool
  - Understand issues with swab and fluid specimens
  - Recognize how poor specimens can lead to overuse of antibiotics

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Diagnostic Stewardship (Morgan 2023)

- Diagnostic stewardship is a collection of practices focused on changing the use of diagnostic tests, at the steps of test ordering, laboratory processing, and reporting of results
  - 2 key underlying premises of diagnostic stewardship:

    (1) that misdiagnosis often results from excessive and unnecessary culturing and

    (2) holding back treatment of positive cultures is very difficult for clinicians.

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Diagnostic Stewardship (Curran 2022)

- …ordering the right tests for the right patient at the right time to inform optimal clinical care.
- Diagnostic stewardship is an integral part of antibiotic stewardship efforts to optimize antibiotic use and improve patient outcomes, including reductions in antibiotic resistance and treatment of sepsis.

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Jim as a Medical Laboratory Technologist

- In Microbiology, I have minimal control over specimens I receive
- No healthcare provider learns how to take a specimen
- This presentation was first put together in ~1988

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The Laboratory Rules

- Provide as much information to the lab as possible:
  - site, appearance, time of collection, antibiotics in use
    - Bad: leg swab
    - Better: Left leg lesion swab
    - Best: Aspiration Left leg post op draining abscess

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The Laboratory Rules

- Get to lab as soon as possible
  - delays will allow susceptible bugs to die
  - delays will allow hardier bugs to grow
- Check with lab on refrigeration if there is a delay
  - Urines for sure!

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Swabs

- Nylon/cotton
- Flocked
  - Electrostatic
  - Release organisms better into a broth

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SWABBING

- Moisten swab if swabbing dry body parts
  - Use transport media or sterile saline
- Different swabs for different specimens
  - Will vary by hospital/clinic
  - C&S, chlamydia, anaerobic, charcoal

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In the Court of the Crimson King – Wikipedia

https://www.mantacc.com/96000d--foam-sampling-nasopharyngeal-swab-with-78mm-breakpoint358525-16602880932341834.html

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Mid Stream Urines

- Like it says:
  - First urine flow washes distal urethral flora away
  - Very difficult with demented; elderly; physically challenged
  - Full void is not appropriate
    - Nor is sterile bedpan
  - In/Out catheter

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Mid Stream Urines

- Catheters: use alcohol cleaned collection port
- If catheter is indwelling > 7days and patient appears symptomatic, change catheter and collect specimen (Hooton 2010, Garcia 2017, Septimus 2026)
  - ?discard first 15 mL?

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URINES

- Get to laboratory (within 48 hours) or refrigerate as soon as possible
  - *E. coli* will reproduce every 20 minutes in urine at room temperature
- Explain collection to patient – never assume!
- Use of "cleanser" controversial
  - Water and gauze works well

https://www.solocup.com/products#category=Cups
www.Grainger.ca

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URINES

- Other collection devices
  - Agar paddle
    - Dip or pour completely over both sides
  - Vacutainer
    - Maintain asepsis

https://www.medischevakhandel.nl/public/data/image/article/5099/11160/large/dipslides-uricult-10-stuks.jpg
https://www.bd.com/en-ca/products-and-solutions/products/product-families/bd-vacutainer-complete-urine-collection-kits

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URINES

- Colony count: a standardized amount of urine is plated onto culture plate, and number of colonies growing are counted.
- Significant levels were determined in symptomatic women volunteers.
  - >100,000 organisms per mL of urine
  - >100 x 10^8 / L

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URINES

- >100
- One organism with sensitivity
- Two organisms with or without sensitivity (usually with)
- Three organisms:
  - "Mixed with Urogenital flora", "mixed flora", "probable contamination"
    - Indicates collection issue

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URINES

- 10-100
  - One organism – with or without sensitivity
  - Two or more organisms: mixed
- <10
  - No significant growth

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URINES AND ANTIBIOTIC STEWARDSHIP (ASP)

- Beware
  - asymptomatic bacteriuria (definitions Septimus 2026)
    - Common in elderly
  - urogenital contamination
- Yeasts
  - Collection issue?
- Focused program in ICU reduced over-utilization (Al-Bizri 2022)

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URINES

- Catheter urines are not treated any differently than midstream urines
  - Rapidly colonized
- Cystoscopic urines usually have all flora reported
  - Try to limit cultures as usually done for pathological reasons

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URINES

- Suprapubic catheters are a catheter urine
- Suprapubic aspirates are considered sterile fluids
  - Pediatrics
- Gram stains can be requested in consultation with laboratory

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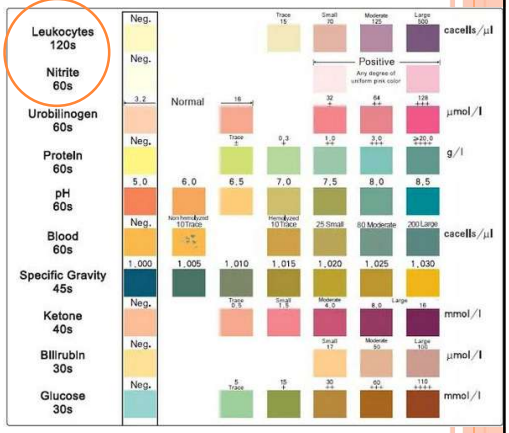
URINALYSIS

- Collection should also be midstream urine or in/out catheter
- A negative dipstick indicates patient does not have an infection
- A positive dipstick indicates nothing!



https://gavingiovannoni.substack.com/p/how-to-interpret-a-urine-dipstick

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STOOL SPECIMENS

- Feces Facts
  - 1 gram = $1x10^{12}$ organisms dry weight
- Should be collected free from urine and water
  - If possible!
  - Container, paper under seat, plastic wrap under seat, bathtub
  - Line diapers with plastic??

https://www.medline.ca/en/products/lab-equipment

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Stool Specimens: General

- Only add enough stool to bring fluid level up to line on container
- No preservative for *C. difficile*, Rotavirus, Norovirus
  - *C. difficile* specimen must take shape of container
- Parasite preservative is poisonous
  - Formaldehyde

https://www.albertahealthservices.ca/assets/wf/lab/if-lab-pc-frm-cal-stool-specimen-for-bacterial-pathogen-testing-mi6000.pdf

25

Stool Specimens

- Travel history very helpful
  - Under-resourced areas of world, floods, coastal waters, day care
- Virus cultures
  - Best to check with lab first

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Stool Specimens

- Most laboratories will not culture (C&S) stool from inpatients who have been in hospital greater than 3 days
  - *C. difficile* first
  - Then C&S if proper whining is done

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Stool Specimens (C&S)

- Listed pathogens
- Negative report usually indicates what is looked for routinely
  - Negative for Salmonella, Shigella, Yersinia and Campylobacter (*E. coli* 0157, Aeromonas, etc.)
- Parasites and *Clostridioides difficile* are a separate request

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Stool Specimens – PCR Panels

- Numerous organisms/toxins looked for
- Issue with viral pathogen detection along with possible colonization of *Clostridioides difficile* and what to do

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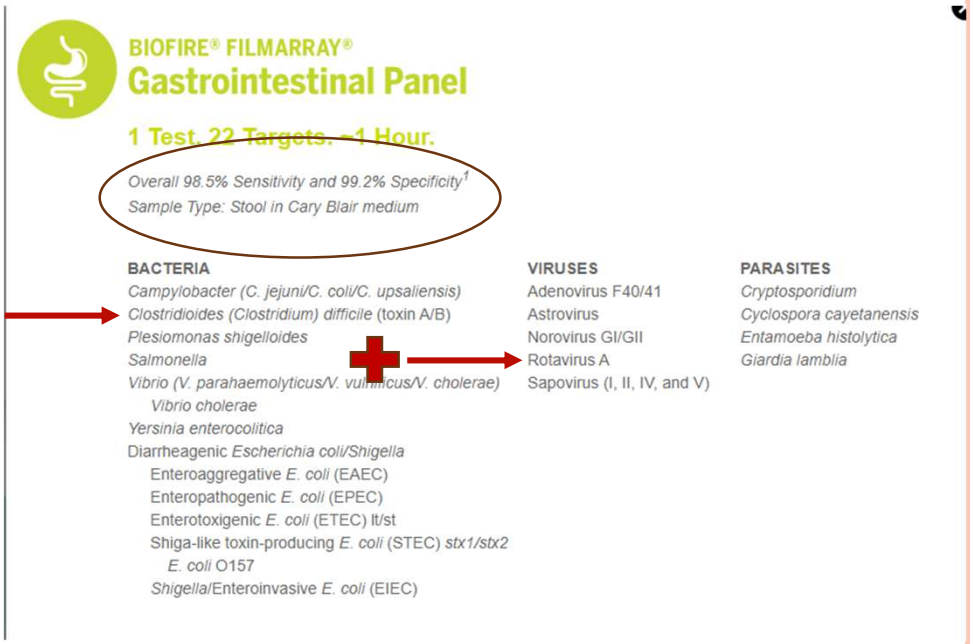
PCR

1 year old



https://go.biomerieux-usa.com/

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Clostridioides difficile

- Many labs use PCR
  - Positive predictive value (PPV) depends on normal expectation of a positive
  - In low prevalence populations, low PPV
  - In high prevalence populations, high PPV
- Negative is negative (no *C. difficile* present)
- Some labs perform screening tests GDH

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GDH

- Glutamate Dehydrogenase Test – Considered an antigen of *C. difficile*
- Produced in high amounts by both toxigenic and non-toxigenic CD, and some other Gram neg organisms
- Indicates organism is present or not present (negative predictive value is high)

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Table. Characteristics of Commonly Used Stool Tests for *Clostridioides difficile* Infection

Test	Sensitivity (95% CI), %[a]	Specificity (95% CI), %[a]	Mechanism of action	Test detection target
Nucleic acid amplification test (NAAT)	96 (93-98)	94 (93-95)	Nucleic acid amplification testing (such as polymerase chain reaction)	Genes that encode toxins produced by *C difficile*
Glutamate dehydrogenase (GDH) antigen test	94 (89-97)	90 (88-92)	Enzyme immunoassay	GDH, a protein present in toxigenic and nontoxigenic *C difficile*
C difficile toxin A/B	83 (76-88)	99 (98-99)	Enzyme immunoassay	*C difficile* toxins A and B
NAAT and toxin A/B	82.9 (80.0-85.6)	99.6 (99.4-99.7)		
GDH antigen test and toxin A/B	81.8 (78.8-84.5)	99.5 (99.4-99.6)		
GDH antigen test positive/toxin A/B negative and NAAT	81	100		
GDH positive antigen test/toxin A/B negative and CCNA	81	100		

[a] Sensitivity and specificity values are in comparison with cell cytotoxicity neutralization assay (CCNA) and derived from Planche et al[1] and Crobach et al.[3]

Nicholson 2023

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CD Screens

- GDH screen + then PCR, Toxin Elisa
- PCR + and Toxin + ➡ treat (?symptoms?)
- PCR + and Toxin - ➡ colonization

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CD ASP

- Don't want to treat colonized patients who test positive for *C. difficile*.
- Algorithm for patient treatment as possible source of diarrhea
  - Laxatives
  - Tube feeds

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C. DIFFICILE

- Do not test for cure!
  - Test can be positive for weeks after resolution of symptoms
  - If symptoms recur within 8 weeks, not a new case (Ontario Guidelines)
  - Check State guidelines
- Asymptomatic carrier state ranges from 4 – 20%

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C. DIFFICILE AND HAI

- Did we give the patient the spore?
  - Healthcare Associated
  - Possibly only 10-20% (Teska 2021)
- Did we precipitate the infection by giving antibiotics to a colonized patient?
  - Antibiotic Stewardship
  - Diagnostic Stewardship

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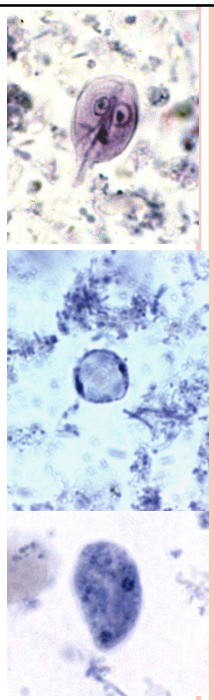
STOOL PARASITES

- Giardia, Cryptosporidium, etc.
- *Blastocystis hominis*
  - In high numbers and absence of another pathogen
    - >5/HPF
- *Dientamoeba fragilis*
  - May hide in pinworm (*Enterobius vermicularis*)
- Special orders: Microsporidia, helminths



https://www.k-state.edu/parasitology/625tutorials/index.html

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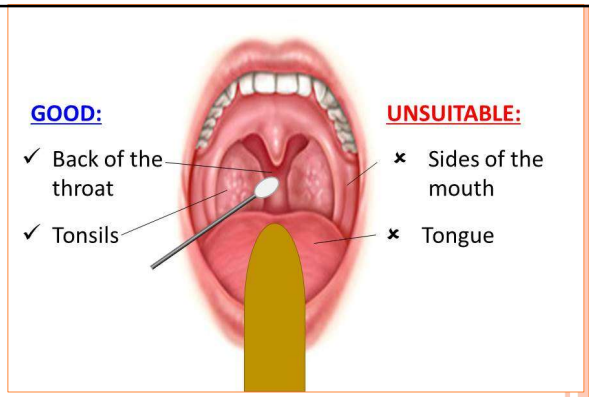
THROATS

- Swab:
  - tonsillar crypt
  - use tongue depressor
    - don't want roof of mouth
    - careful of "gag" reflex
  - say :"ahhhhhh"
    - removes uvula from field of view
- 1 mL saliva contains $1x10^8$ bacteria



https://www.sgh.com.sg/patient-care/specialties-services/pathology/pages/virology.aspx

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THROATS

- Group A Streptococci are always considered pathogens
  - Can be carried asymptomatically
- Groups C and G can be pathogens if present in predominating numbers.
- Rest of flora is viridans (alpha hemolytic) streptococci, anaerobes

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THROATS

- Rapid antigen testing
  - PPV 81%, NPV 93%
- Generally, in symptomatic patients
  - if positive, good
  - if negative, are you still going to culture?

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THROATS AND ASP

- Is it viral?
- Clinical picture is very important
- Carrier state of Group A strept is low (rapid tests)

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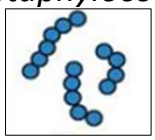
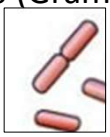
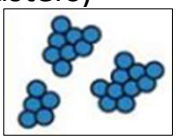
NOSE

- Useless for detecting sinusitis
  - We don't care how green the discharge is!
- Many normal flora organisms which are potential pathogens (in other body parts!)
  - *Streptococcus pneumoniae* (Gram positive cocci - chains)
  - *Haemophilus influenzae* (Gram negative cocco-bacilli)
  - *Staphylococcus aureus* (Gram positive cocci - clusters)

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NOSE

- MRSA Screening
  - Moisten swab
  - Bilateral anterior nares
  - Just hide the tip of the swab
  - Only need one swab for both nares

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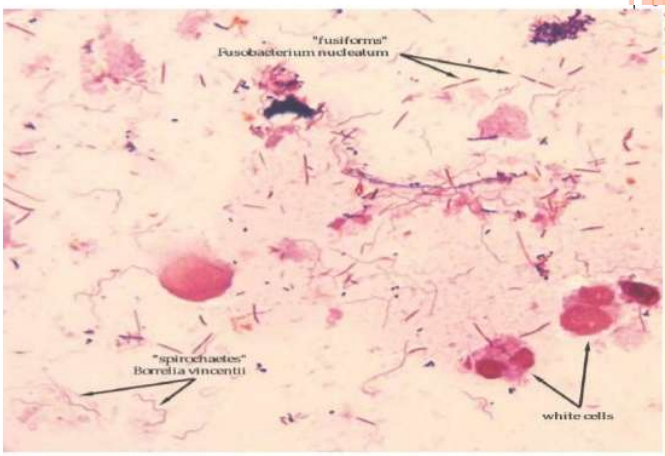
Mouth

- Lesion swab
  - Mention this on requisition
  - Vincent's angina
  - Spirochetes and fusiform bacteria
- Oral candidiasis (thrush)
  - Mention this on requisition



Oral specimen - Gram stain

https://image.slidesharecdn.com/examinationofthroat-131210153117-phpapp01/95/examination-of-throat-22-638.jpg?cb=1386689530

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Eye Swab

- Do not want "gunk" in inner canthus
- Pull out lower lid and rub swab gently along conjunctiva and lid junction
- Presence of epithelial cells indicate "surface"
- Gram stain should have very little in it if a good specimen has been collected

https://www.babycenter.com/ims/2013/04/bacterial-conjunctivitis_4x3.jpg.pagespeed.ce.-Da7W11_Tq.jpg

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Eyes – Non - Culture Method

- Eye lid glued together – bacterial
- Eye lid crusty but can open lid - viral
- Chlamydia needs a separate swab
- Need to interpret significance of organisms, as can be colonized with potential pathogens (*Str. pneumonia, St. aureus)*

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Sputum

- Collection is very important as is interpretation of results
- First morning specimen, good deep cough
  - No indication for rinsing mouth with sterile water first
- Q or Quality Score
  - based on number of polymorphonuclear cells and epithelial cells

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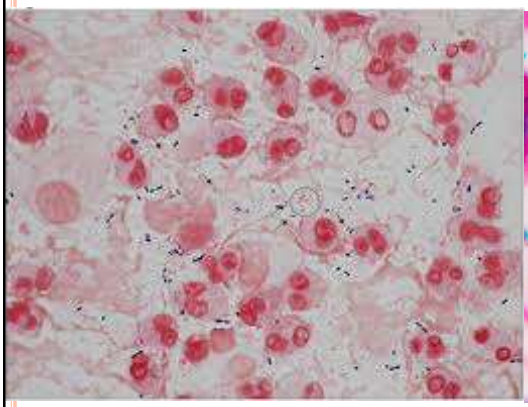
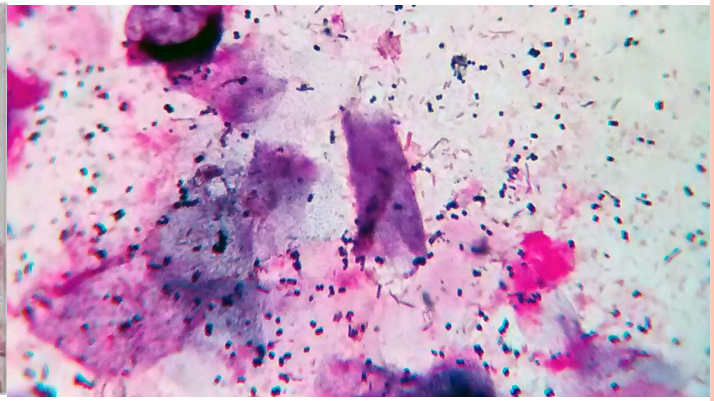
Q Score Pictures



Good Q Score



Bad Q Score

https://esmed.org/MRA/index.php/mra/article/view/4207
https://upload.wikimedia.org/wikipedia/commons/b/b8/Gram_stain_saliva.jpg

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Sputum

- If suctioning, use sterile suction catheter, into trap, do not use inline unless new
- Many potential pathogens are colonizers esp. if trach present. Overtreatment is prevalent (Prinzi 2022)
  - *Staph. aureus*
  - *H. influenza*
  - Pseudomonas (Gram negative rods)
  - Coliforms (Gram negative rods)

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Sputum

- Must look at signs and symptoms, X ray reports, other lab reports (Hematology)
- Gram stain can indicate predominating organism, but still could be colonizer
- Blood culture
- Comparison of potential pathogen to normal flora can also be helpful

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Sputum - ASP

- Many elderly patients will be colonized with Gram negative organisms in their oropharynx
  - *E. coli*, Klebsiella, Enterobacter, Proteus, Pseudomonas
- Many vented patients will have a colonized endotracheal tube
  - Pus may not be indicative of infection but of foreign body

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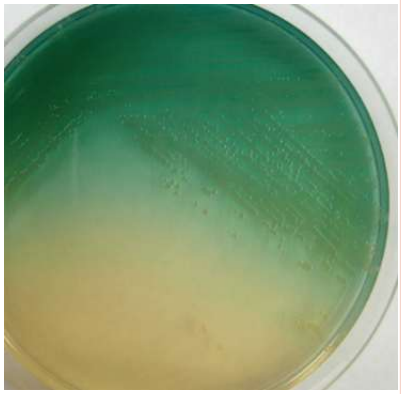
Ear

- Swab reddened area, no need for wax to be sent
- Not good specimen for diagnosis of otitis media, unless eardrum is ruptured.
- *Pseudomonas aeruginosa* most common cause of swimmer's ear (Otitis externa)

Pseudomonas aeruginosa



Jawetz et al, Chapter 16

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Vaginal Swabs

- Swab Vaginal vault
  - Use clear swab
- Remove excess mucous first
  - Grosses lab people out also!
- Will not culture for Gonorrhea or Chlamydia unless using nucleic acid methods

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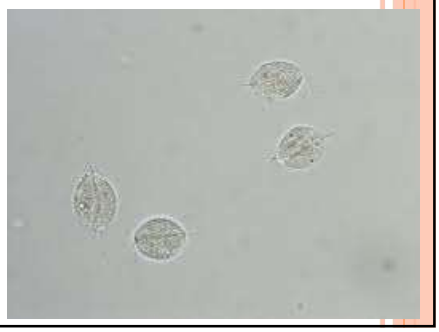
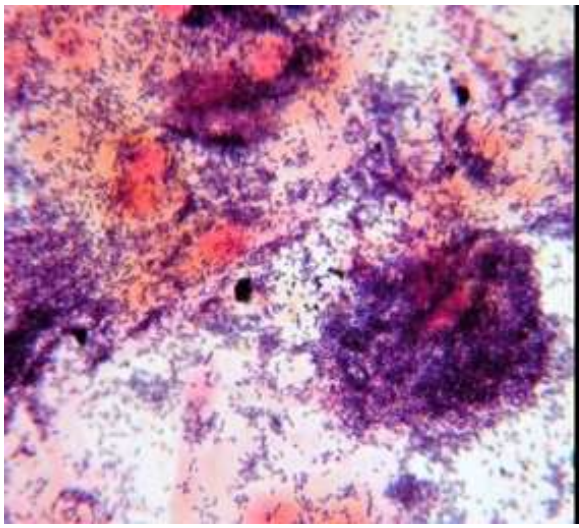
VAGINAL SWABS

- Presence or absence of normal flora
  - if replaced: bacterial vaginosis
  - "Results may not be reliable in post-menopausal women"*
- Yeast
- Trichomonas – needs to be looked at rapidly after collection
  - Some areas will have Trich culture medium



*https://cmpt.ca/wp-content/uploads/2016/06/Interpretation-of-Vaginal-Specimens.pdf
https://www.stepwards.com/?page_id=9101

55



Bacterial Vaginosis

Normal Vaginal Flora

http://thunderhouse4-yuri.blogspot.ca/2010/11/bacterial-vaginosis.html

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Yeast in Gram Stain

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Cervical

- May be able to test both gonorrhea and Chlamydia from one swab
- NAAT: Nucleic Acid Amplification Test
  - Sensitivity better with vaginal swab (Aaron 2023)

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Cervical

- Use charcoal (black) media
  - Easier on gonorrhea (GC)
- May need separate swab for Chlamydia
  - Cervix: insert until tip is not seen
  - Rotate and withdraw
  - Need columnar-epithelial cells

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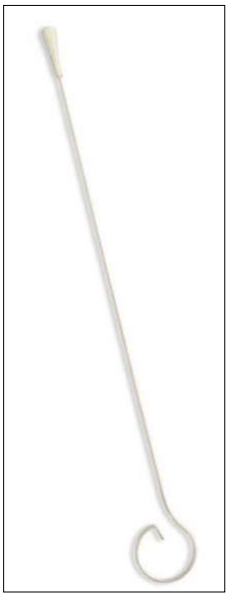
Urethral Swabs

Chlamydia

- Urethra: insert wire swab 2-3 cm
- Rotate 10 times and withdraw



https://www.dufortlavigne.com/en/produit/HAR25800A50

60

CHLAMYDIA

- Can also use first void urine
  - first AM specimen
  - No void for at least an hour
- Not as sensitive as NAAT testing with swab (Aaron 2023)

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CERVICAL/URETHRAL SWABS

- *N. gonorrhea* not isolated.
- *Chlamydia trachomatis* not found.
- NOTE: The lab can find GC in Vagina and yeast in cervix, but to really say it is not there with culture methodologies…
- Syphilis would be a separate request (check with lab)

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Wound Swabs

- Many potential pathogens can be normal colonizing flora
- Generally, treat on symptoms, unless patient septic
- Generally do NOT culture pressure injuries
- Irrigation of wound
  - 30 cc syringe with 18g needle

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Wound Swabs

- Clean wound first, then swab
  - Better to aspirate leading edge of erythema
- Debride before culturing?
- Do not want to see lots of epithelial cells

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Levine Quantitative Swab Technique

- Cleanse wound with normal saline.
- Pat dry wound bed with sterile gauze.
- Culture the healthiest looking tissue, excluding exudate, purulent, devitalized tissue.
- Spin the end of the sterile applicator over a 1cm x 1cm area for at least 5 seconds.
- Apply sufficient pressure to swab, causing tissue fluid to be expressed.

(Misc 2004)

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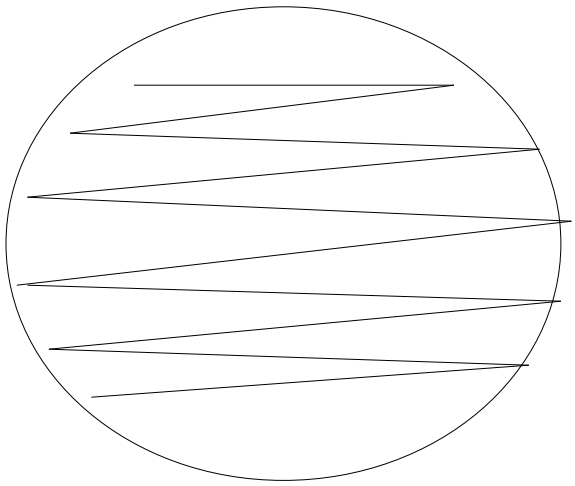
Wound Swabs – 10 Point



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Sterile Body Fluids

- In a properly collected fluid, any organism is a pathogen
  - Abscess fluid?? – NO!
  - If multiple organisms, treat based on underlying cause!
- Can use other tests to help interpret results
  - Cell counts
  - Chemistry analysis
  - Glucose, protein

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Blood Cultures

- Peripheral best, but lines can be used if poor venous access or if worried about line sepsis
- Use good antiseptic first, make sure to do palpating finger, even if gloved
- Disinfect top of bottle
  - Bacillus outbreaks
- Add appropriate volume of blood
  - Syringe and then add…

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Blood Cultures

- Check with lab on preferred timing
  - 2 sets – 95% sensitivity
  - 3 sets – 95.5%
  - 4 sets – 95.7%
- ~$5 per bottle…

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Blood Cultures - ASP

- Single bottle grows?
- Sepsis picture can direct treatment

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Viral Cultures

- Tricky!
  - Need special swabs and transport
- Not many labs are culturing anymore

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Viral Detection

- Respiratory Panel
  - Nasopharyngeal specimen
  - Uncomfortable – doing it right!
- Vesicle
  - Aseptically open vesicle, swab fluid, then rub base of lesion

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HTTPS://WWW.YOUTUBE.COM/WATCH?V=DVJNWEFMHJE

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VIRAL PANELS

- Multi Plex
- ePlex

Microbiology Flowsheet
Microbiology Miscl.
RVP Adenovirus
RVP Bordetella Pertussis
RVP Chlamydia Pneumonia
RVP Coronavirus 229E
RVP Coronavirus HKU1
RVP Coronarvirus NL63
RVP Coronavirus OC43
RVP Human Metapneumovirus
RVP Human Rhinovirus/Enterovirus
RVP Influenza A
RVP Influenza B
RVP Mycoplasma Pneumoniae
RVP Parainfluenza Virus 1
RVP Parainfluenza Virus 2
RVP Parainfluenza Virus 3
RVP Parainfluenza Virus 4
RVP Respiratory Syncytial Virus

Respiratory Panel →

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Viral Panels

- Influenza A, A (H1N1), B, C
- Parainfluenza viruses 1, 2, 3 and 4
- Coronaviruses NL63, 229E, OC43, HKU1
- Human metapneumoviruses A/B
- Rhinovirus
- Respiratory syncytial viruses A, B
- Adenovirus
- Enterovirus
- Parechovirus
- Bocavirus
- *Pneumocystis jirovecii*
- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*
- *Strept. pneumoniae*
- *H. influenzae* type B
- *Staph. aureus*
- *Moraxella catarrhalis*
- *Bordetella* spp.
- *Kleb. pneumoniae*
- *Legionella pneumophila / longbeachae*
- *Salmonella* species including internal control

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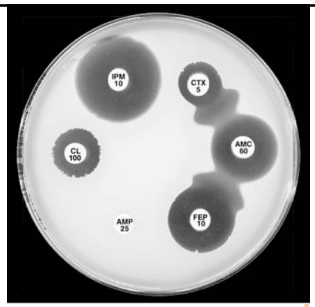
Antibiograms



- Will list common antibiotics to use
- May withhold some antibiotics
- Many reports are now listing the relative cost of the antibiotics
- Some antibiotics listed as resistant by class, based on another result or the genus isolated
  - *Enterobacter spp*. with extended spectrum beta lactamases

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ANTIBIOGRAMS

- Some organisms (*Haemophilus influenza*) will only have the Beta Lactamase reported
  - organism is sensitive or resistant to beta lactam antibiotics.
- Some organisms do not test well in vitro, and statements are used to cover problems
  - extended beta lactamases, synergy

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Presence of an antibiogram does not indicate significance!!!!

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Antibiotic Resistance and Disinfection

- Resistance to antibiotics does not change how an organism reacts to a disinfectant!
- Antibiotics are very 'lock and key'
- Disinfectants are like a sledgehammer!

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Antibiotics – Lock and Key

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Antibiotic Resistance

Antibiotic

Microorganism

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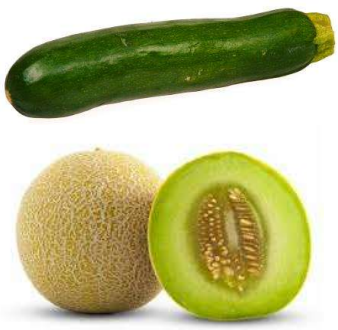
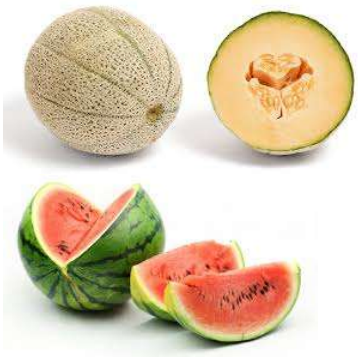
Analogy for Antibiotics





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The Family Tree

- **Family** • Cucurbitaceae
- **Genus** • Last Name
- **Species** • First Name

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Disinfectant and Microorganism

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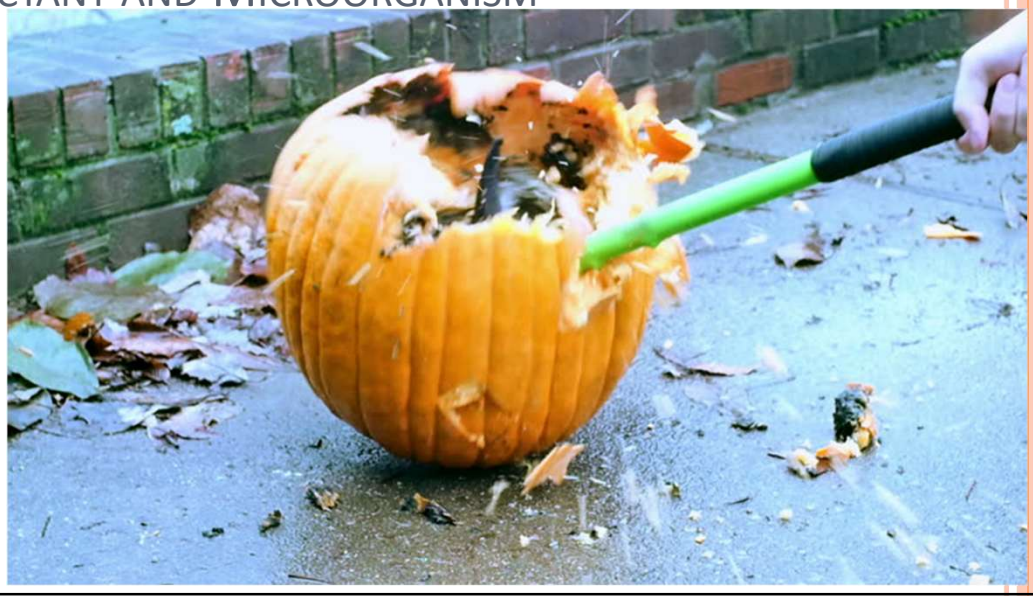
Disinfectant and Microorganism



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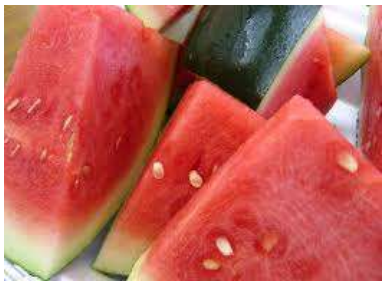
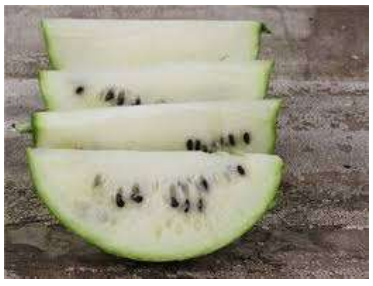
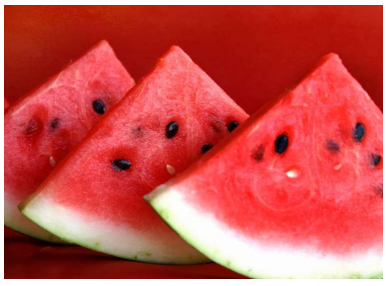
Genetic Mutations



Watermelon coli



Watermelon ESBL



Watermelon CRE

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SUMMARY

- If in doubt, check with the laboratory to find out what they mean!
- Never assume!
- Give the lab a good specimen and you will get a good result!
- Treat the patient's signs and symptoms, not the microbiology report

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SUMMARY - ASP

- Normal flora can be very misleading!!
- The lab has NOT seen the patient, we are just reporting the facts!
- Lab rounds should be considered
- Infectious disease are always available for consult!

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