Lecture 10 Part 1
Advantages and Limitations of Laboratory Assays

- Advantages and Limitations of:
  - Staining (and wet prep and fixation)
  - Antimicrobial Sensitivity Testing
  - Immunodiagnostic tests
  - Molecular diagnostic tests
  - Environmental testing
Advantages and limitations of Staining
ADVANTAGES OF STAINING

- Microscopic examination of stained preparations enables the relative sizing, morphology & arrangement of microorganisms to be seen clearly.
- Assists in the detection of cells, especially pus cells.
ADVANTAGES OF STAINING

- Refer to advantages of Gram Stain
- In addition, histological or Cytological examination is useful for diagnosing infections with agents that are difficult or impossible to culture
  - Chlamydia
  - Cytomegalovirus
  - Genital herpes
  - Histoplasma
- Stain can be used to identify some organisms to the species level e.g. *Plasmodium*.
LIMITATIONS OF STAINING

- Cannot be used to identify fresh specimens or motile organisms
- If staining is done from uncultured specimens collected from unsterile sites, the specimens cannot be used in differentiating pathogenic organisms from normal flora
ADVANTAGES OF WET PREPARATION

- Identification of motility in parasite & bacteria e.g. *Trichomonas vaginalis*; *Vibrio cholerae*
- Identification of morphology of the organisms like parasite & fungi e.g. cysts, eggs, fungal spores / vegetative forms
LIMITATIONS OF WET PREPARATION

- Only useful in the identification of large size organisms like parasites & fungi
- Cannot be used to identify viruses & most bacteria
- Cannot be used to identify organisms to the species level, except in fungal identification
HEAT FIXATION

- Organisms like *M. tuberculosis* are not killed by the usual heat techniques used to fix sputum smears
- Heat fixation is recommended for *M. leprae* smears
ALCOHOL FIXATION

- It is far less damaging to organisms than heat
- Cells, especially pus cells, are also preserved
- It is recommended for fixing smears which contain intracellular organisms
ALCOHOL FIXATION

- It is more bactericidal than heat
- For example, *M. tuberculosis* is rapidly killed in sputum smears after applying 70% v/v alcohol
Use of other chemicals is sometimes necessary to fix smears which contain particularly dangerous organisms to ensure all the organisms are killed.
ADVANTAGES OF STAINING

- Bacteria can be divided into groups & differentiated by their staining reaction (e.g. **Gram positive** & **Gram negative** organisms & **Acid fast** bacilli).
ADVANTAGES OF GRAM STAINING

- Assists clinicians in determining
  - the initial direction of therapy (empiric)
  - the need for isolation precautions

- For example, Gram negative diplococci in CSF suggest meningococci

<table>
<thead>
<tr>
<th>Step</th>
<th>Microscopic Appearance of Cell</th>
<th>Chemical Reaction in Cell Wall (very magnified view)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Crystal violet</td>
<td>Gram (+)</td>
<td>Gram (+)</td>
</tr>
<tr>
<td></td>
<td>Gram (-)</td>
<td>Both cell walls affix the dye</td>
</tr>
<tr>
<td>2. Gram's iodine</td>
<td>Dye complex trapped in wall</td>
<td>No effect of iodine</td>
</tr>
<tr>
<td>3. Alcohol</td>
<td>Crystals remain in cell wall</td>
<td>Outer membrane weakened; wall loses dye</td>
</tr>
<tr>
<td>4. Safranin (red dye)</td>
<td>Red dye masked by violet</td>
<td>Red dye stains the colorless cell</td>
</tr>
</tbody>
</table>

Insight 4.2
Advantages and Limitations of Antimicrobial Sensitivity Testing
ADVANTAGES OF MICROBIAL CULTURE & SENSITIVITY

Culture techniques are used to isolate pathogens in pure culture so that they can be identified & if indicated, tested for sensitivity.

ADVANTAGES OF MICROBIAL CULTURE & SENSITIVITY

- Sensitivity testing is used to select effective antimicrobial drugs against susceptible & resistant pathogens
- Results of antimicrobial sensitivity tests may also help identify pathogenic bacteria
FACTORS THAT INFLUENCE INTERPRETATION OF RESULT

- A zone of inhibition can be formed by penicillin-resistant *Staphylococci* if the amount of beta-lactamase (Penicillinase) is insufficient to inactivate the penicillin close to the disc.

http://fig.cox.miami.edu/~cmallery/255/255enz/penicillin.gif
FACTORS WHICH INFLUENCE DISK DIFFUSION TESTS

- This may result in sensitive strains being reported as resistant.
- If growth is too light, the inhibition zones will be larger which may result in relatively resistant strains being reported as sensitive.

FACTORS WHICH INFLUENCE DISK DIFFUSION TESTS

Other factors which influence disc diffusion tests include the:
- volume
- moisture content
- pH
- constituents of the agar medium
- concentration, storage & application of the discs
An organism should be reported as sensitive to cotrimoxazole only when some sensitivity is shown to both sulphamethoxazole & trimethoprim.
FACTORS THAT INFLUENCE INTERPRETATION OF RESULT

- Some zones of inhibition, however, have a heaped up clearly defined edge as seen in sensitive strains.
- All strains showing a heaped up edge are reported as resistant!
  - This is the most important finding in the detection of penicillin-resistant Staphylococci especially if the organism is a urinary pathogen.
FACTORS WHICH INFLUENCE DISK DIFFUSION TESTS

- A large zone of inhibition is produced by an antimicrobial that diffuses rapidly & a smaller zone by one that diffuses more slowly.
Smaller size zones are produced by polymyxins (colistin & polymyxin) because they diffuse slowly in agar due to their large molecular size.

http://www.biomedcentral.com/content/figures/1471-2334-6-100-1.jpg
FACTORS THAT INFLUENCE INTERPRETATION OF RESULT

- Zone size will be markedly reduced if bacterial growth is too heavy.
- *Proteus* strain often swarm into the area of inhibition; however, the actual zone of inhibition is usually clearly outlined.

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LIMITATION OF ANTIMICROBIAL SENSITIVITY TESTS

Sensitivity tests measure antimicrobial activity against bacteria under laboratory conditions (i.e. in-vitro activity), rather than in the patient (i.e., in-vivo activity).
LIMITATION OF ANTIMICROBIAL SENSITIVITY TESTS

It cannot be assured, therefore, that an antimicrobial which kills or prevents an organism from growing in-vitro will be a successful treatment.
LIMITATION OF ANTIMICROBIAL SENSITIVITY TESTS

Selecting appropriate antimicrobial treatment also involves considering:

- the patient’s clinical condition,
- the type & site of the infection,
- any history of drug hypersensitivity.
LIMITATION OF ANTIMICROBIAL SENSITIVITY TESTS

◆ It is also necessary to know the activity of the different drugs, including their
  ◆ rates of absorption,
  ◆ diffusion in the tissues.

http://epswww.unm.edu/coursinf/eps462/graphics/diffusion.gif
LIMITATION OF ANTIMICROBIAL SENSITIVITY TESTS

- metabolism
- excretion
- possible toxicity
- effects on the patient’s normal microbial flora
DISADVANTAGES OF MICROBIAL CULTURE & SENSITIVITY

- It takes time for organisms to grow hence results are delayed.
- Sensitivity testing is limited to bacteria.
- Further testing might still be required to identify the isolates e.g. Biochemical test, Gram stain
Hence, it is relatively non-specific & has limited usefulness in determining true relatedness.
Advantages and limitations of Immunodiagnostic Tests
ADVANTAGES OF IMMUNODIAGNOSTIC TEST

- Used to diagnose a microbial disease when the pathogen is not present in routine specimens or if present, is not easily isolated & identified by other available techniques.
ADVANTAGES OF IMMUNODIAGNOSTIC TEST

- Provides an early diagnosis or presumptive diagnosis of diseases such as meningitis or cholera
ADVANTAGES OF IMMUNODIAGNOSTIC TEST

Used to identify & if indicated, serotype a pathogen that has been isolated by culture e.g. V. cholerae, Salmonella sp.

http://www.intergenomics.de/new/pics/host_pathogen_interaction_symbol.gif
ADVANTAGES OF IMMUNODIAGNOSTIC TEST

- Used to measure antibody levels to determine the prevalence, spread & control of infectious diseases
DISADVANTAGES OF IMMUNODIAGNOSTIC TEST

◆ Some techniques are labor intensive e.g. CFT
◆ Older techniques lack sensitivity & specificity

http://www.rehab.research.va.gov/jour/06/43/1/hatzakisf02.gif
SEROTYPING

- Standardized reagents are lacking for most species & many isolates of certain species are not typeable.
- It is not as discriminatory as genotypic analysis & requires maintenance of large stocks of typing antisera.
Fig. 1 - Banding patterns of *A. baumannii* generated by *Eco*RI (1a) and by *Hind*III (1b). Size marker (*H. aegyptius* 3031 *Eco*RI DNA digest, fragment sizes in kilobases) in lanes 1 and 8 (Fig. 1a) and 1 and 9 (Fig. 1b). In Fig. 1a, ribotypes A, B, C, D, E, F, H, I, J, K, L and M are in lanes, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13 and 14, respectively. Ribotype G obtained after digestion with *Eco*RI is not shown in this blot. In Fig. 1b, ribotypes A, B, C, D, F, G, H, I, J, K, L, and M are shown in lanes 2, 3, 4, 5 and 6, 7, 8, 10, 11, 12, 13, 14, and 15, respectively. Ribotypes D and E obtained after digestion with *Eco*RI displayed the same profile when clived with *Hind*III (lanes 5 and 6 of Fig. 1b).
Advantages and limitations of Molecular Diagnostic Tests
ELECTROPHORESIS

- When the test gel is examined, related organisms may have the same pattern of movement.
- It is used with most common bacterial pathogens; but, it is labor intensive & time consuming.
POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

PAGE has helped to identify the species of some organisms that are difficult to evaluate by routine laboratory methods & can detect different strains within a species.

However, PAGE patterns are often complex & difficult to interpret.
IMMUNOBLOTTING

- It has been used to study *C. difficile* & *S. aureus*.
- It is inexpensive & relatively rapid.

http://www2.kobe-u.ac.jp/~katsuda/KImage_data/KRes_photo/immu_assayf1.gif
MULTILOCUS ENZYME ELECTROPHORESIS (MLEE)

- Mathematical analysis can be used to quantify the differences between two isolates with different ETs.
- For some species, MLEE may have less discriminatory power than other methods that are technically easier.
PLASMID PROFILE ANALYSIS

- Problems in profile interpretation caused by supercoiled or circular plasmid DNA
- REA produces consistent patterns by gel electrophoresis.
Plasmid REA gives better discrimination & reproducibility & is the method of plasmid analysis most widely used at present.
Chromosomal DNA PROFILE ANALYSIS

- Chromosomal REA patterns, unlike those obtained with plasmid REA, typically consist of hundreds of bands, hence interpretation of result may be difficult & time consuming.

Figure-1: REA profiles for MTB isolates representing identical patterns in standard and clinical strains after digestion with Hae III and Bst EII restriction enzymes. Lanes 1-6: Bst EII digestion; lane 7: DNA size marker; lanes 8-13: Hae III digestion. Marker positions are indicated on the left (base pairs).

http://www.pjms.com.pk/issues/aprjun107/fig_tab/technique_fig1.gif
One advantage of this type of testing is that some probes may allow simultaneous assessment of epidemiological interrelationships & definition of other clinically relevant characteristics.
For example, mechanism of antibiotic resistance, the presence of specific antibiotic resistance genes can be analyzed.

Unfortunately, this method is time consuming, labor intensive & technically difficult.

http://www.gbiosciences.com/Image/BE315.jpg
PULSE FIELD GEL ELECTROPHORESIS OF CHROMOSOMAL DNA (PFGE)

- This method is a powerful tool for typing numerous organisms.
- It is capable, in theory, of typing all bacterial isolates.
- PFGE is highly discriminatory & reproducible.
PULSE FIELD GEL ELECTROPHORESIS OF CHROMOSOMAL DNA (PFGE)

- PFGE can separate large DNA fragments that conventional agarose gel electrophoresis, which uses a constant electric field, cannot adequately separate.

http://www.cdc.gov/pulsenet/images/pfge_process.gif
The method, however, is not rapid & requires relatively expensive equipment.

It is the most widely used method for molecular epidemiology.
One of the most valuable attributes of PCR is its ability to detect DNA from organisms which cannot be cultivated.

http://users.ugent.be/~avierstr/principles/pcrsteps.gif
A major problem with PCR-based techniques is that they may also amplify minute amounts of contaminating DNA, thus producing erroneous results.
DISADVANTAGES / LIMITATIONS OF MOLECULAR DIAGNOSTIC TEST

- This is unlike culture systems, which are capable of detecting multiple pathogens simultaneously from the same specimen.
DISADVANTAGES / LIMITATIONS OF MOLECULAR DIAGNOSTIC TEST

- The cost of utilizing molecular technology far exceeds the cost incurred by conventional microbiologic methods


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Advantages and Limitations of Environmental Testing
There are some limitations with sampling time because most air sampling devices were originally developed to sample particles rather than fungi.

http://www.firstrays.com/Pictures/packed_particles.jpg