Microbial Identification

From Basic to Advanced

Frank E. Matos, M.S.
QC Manager of Science and Technology
Sanofi Genzyme, Framingham, MA
Outline of Presentation

- Compendial Requirements
- Method Selection
- Basic Characterization Methods
- Methods based on Phenotypic technologies
- Automated Methods based on Genotypic Technologies
- Qualification and Implementation
No shortage of methods

• There are many techniques and technologies available for microbial characterization and identification.
• These range from century old techniques to state of the art automated systems.
• New technologies typically are highly accurate and reliable but old methods still serve microbiologists well.
• Each methodology has advantages and limitations.
• No single application will work for every identification situation.
• The microbiologists job is to match the best technique for each identification application.
Microbial Characterization and Identification

Genotypic Identification
(MicroSEQ)

Phenotypic Identification
(Vitek, Biolog, MIDI Sherlock)

Microbial Characterization
(Colony morphology, Gram stain, KOH string, Catalase, Coagulase)

Strain Typing
MALDI-TOF
The Need to Identify

• Identification is a crucial activity for routine microbial control of a manufacturing facility.
  ✓ As part of your environmental monitoring you set alert and action levels and you want to identify if you exceed or start to see a trend.

• Identifying a contaminating organism will provide useful information that will aid in its removal or destruction.
  ✓ Gram positive think of “human origin” contamination
  ✓ Molds and yeast; think your facility has been compromised by water damage

• The need for microbial identification is specifically cited in the compendia.
Microbial Identification

• Need for microbial identification is specifically mentioned in several USP chapters:

• USP Chapter <71> Sterility Tests
  ✓ OBSERVATION AND INTERPRETATION OF RESULTS- The test may be considered invalid after determination of the identity of the microorganisms isolated from the test, the growth of this species (or these species) may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test procedure.
Microbial Identification

• USP Chapter <62> Microbiological Examination of Non Sterile Products: Test for Specified Organisms
  ✔ Interpretation—The possible presence of S. aureus is indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests.
  ✔ The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.
Microbial Identification

• FDA Guidance for Industry Sterile Drugs Produced by Aseptic Processing- Current Good Manufacturing Practice
  ✓ “In Aseptic Processing, one of the most important laboratory controls is the establishment of an environmental monitoring program...”
  ✓ At minimum the program should require species (or, where appropriate, genus) identification of microorganisms in ancillary environments at frequent intervals to establish a valid, current database of contaminants present in the facility during processing (and to demonstrate that cleaning and sanitization procedures continue to be effective)...”
  ✓ Characterization of recovered microorganisms is an important aspect of the environmental monitoring program. Environmental isolates often correlate with the contaminants found in a media fill or testing failure...
Method Selection

• FDA Guidance for Industry Sterile Drugs Produced by Aseptic Processing- Current Good Manufacturing Practice
  ✓ “Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic technique. These methods are especially valuable for investigations into failures (e.g., sterility test; media fill contamination). However, appropriate biochemical and phenotypic methods can be used for the routine identification of isolates”
Method Selection

• Specimens for Microbial identifications originate from multiple areas and often have different levels of criticality.
  ✓ Air and surface monitoring
  ✓ Personnel monitoring
  ✓ Raw material and water testing
  ✓ In-Process and Finished product
  ✓ Biological Indicators (BIs) growth

**WHAT’S THE MOST APPROPRIATE METHOD TO USE ?**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>ID method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterility Test Failure</td>
<td>?</td>
</tr>
<tr>
<td>Water monitoring excursion</td>
<td>?</td>
</tr>
<tr>
<td>Media Fill Failure</td>
<td>?</td>
</tr>
<tr>
<td>Surface Sampling of ISO 8</td>
<td>?</td>
</tr>
</tbody>
</table>
Isolation of Pure Cultures

• The first step in identification is to isolate the microorganisms in pure culture.
  ✔ Typically accomplished by successive streaking of the colony of interest in a quadrant pattern on solid media with the objective of obtaining separate colonies.

• Technique allows phenotypic expression and growth of sufficient inoculum for use in identification procedure.
  ✔ Expression of phenotype may be affected by isolate origin, media selection and growth conditions. Therefore identification result using a phenotypic system may be affected by media and growth conditions.
The Quadrant Method

C. albicans in SDA agar
Basic Characterization Methods

• Phenotypic Characteristics useful for Characterization and Identification
  ✓ Characteristics of the microorganisms in pure culture: colony color, size and shape
  ✓ Microscopic: Gram reaction, cell size and shape, presence of spores, flagella.
  ✓ Biochemical: Fermentation, carbon utilization, enzyme patterns
  ✓ Physiological: Growth temperature, oxygen tolerance

\[ E. \textit{coli} \text{ in MacConkey agar} \]

\[ S. \textit{aureus in TSA} \]
Basic Characterization Methods

• Gram staining is a basic microbiological technique used to differentiate two large groups of bacteria based on their different cell wall constituents. It distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet.

• The Importance of the Gram Stain
  ✓ Divides most bacteria into two main groups: Gram positive and Gram negative
  ✓ First step in many identification schemes
  ✓ Aids in the selection of the appropriate ID card in some automated systems
Gram Stain

• A widely used microbiological staining technique that is very useful for bacterial identification.

• Developed by Danish bacteriologist Hans Christian Gram in 1884 while working in the morgue of the city hospital in Berlin.

• Assign most bacteria to one of two large groups: Gam positives stain purple and Gram negatives stain pink.

• A very basic but not fool proof procedure. Not all organisms can be stained using the Gram technique and there are Gram variable organisms.
Method and Principle of the Gram Stain

1. Application of crystal violet (purple dye)
2. Application of iodine (mordant)
3. Alcohol wash (decolorization)
4. Application of safranin (counterstain)

**KEY**
- Crystal violet
- Iodine
- Alcohol
- Safranin

**GRAM-POSITIVE**
- peptidoglycan
- membrane

**GRAM-NEGATIVE**
- peptidoglycan
- outer membrane
- membrane
Spore Staining

• Performed by using malachite green stain for bacterial spores.
  • Heat or steam is required to get Malachite Green into the endospores (and the vegetative cells as well).
  • The use of a positive control is recommended to verify proper technique.
# Useful Biochemical Screening Techniques

<table>
<thead>
<tr>
<th>Test</th>
<th>Principle</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidase Test</strong></td>
<td>When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) purple color end product</td>
<td>Differentiate <em>Neisseria</em> and <em>Moraxella</em> from <em>Acinetobacter</em>; pseudomonas and <em>Vibrionaceae</em> from <em>Enterobacteriaceae</em>. Aid in identification of <em>Aeromonas</em>.</td>
</tr>
<tr>
<td><strong>Catalase Test</strong></td>
<td>Hydrogen peroxide, H2O2 is decomposed to O2 by catalase</td>
<td>Differentiate Bacillus from Clostridium. Streptococcus from micrococcus-staphylococcus</td>
</tr>
<tr>
<td><strong>Coagulase Test</strong></td>
<td>Enzyme cause clotting of plasma.</td>
<td>Differentiate Staphylococcus aureus (positive) from Staphylococcus epidermidis (negative)</td>
</tr>
<tr>
<td><strong>Indole</strong></td>
<td>Tryptophan from proteins is converted to indole</td>
<td>Differentiate <em>Escherichia</em> (positive) from most species of <em>Klebsiella</em> (usually negative)</td>
</tr>
<tr>
<td><strong>Methyl Red</strong></td>
<td>Mixed-acid fermentation results in acid production detected by pH indicator; and color change</td>
<td>Differentiate <em>Escherichia</em> (pos, culture red) from <em>Enterobacter</em> and <em>Klebsiella</em>(usually negative, culture yellow)</td>
</tr>
</tbody>
</table>
Identification using Phenotypic Methods

• Phenotypic methods rely on expression of phenotypic characteristics to identify a microorganism.
• Biochemical reactions, carbon utilization and enzymatic patterns are useful phenotypic characteristics for identification.
• Accuracy of the identification is affected by factors such as media and temperature that affect phenotypic characteristics of the organism.
• If executed properly phenotypic methods are accurate and reliable.
## Examples of Commercial Phenotypic Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Technology</th>
<th>Application</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>API® Strips</td>
<td>Biochemical Reactions</td>
<td>Gram positives, Gram negatives, anaerobes, yeasts</td>
<td>bioMérieux</td>
</tr>
<tr>
<td>BBL™ Crystal</td>
<td>Biochemical Reactions</td>
<td>Gram positives, Gram negatives, anaerobes</td>
<td>BD</td>
</tr>
<tr>
<td>Vitek® 2</td>
<td>Biochemical Reactions and enzyme patterns</td>
<td>Gram positives, Gram negatives, yeasts</td>
<td>bioMérieux</td>
</tr>
<tr>
<td>Biolog</td>
<td>Carbon Utilization</td>
<td>Gram positives, Gram negatives, yeasts and molds</td>
<td>Biolog</td>
</tr>
<tr>
<td>MIDI Sherlock™</td>
<td>Fatty Acid Analysis by Gas Chromatography (GC)</td>
<td>Gram positives, Gram negatives, yeasts</td>
<td>MIDI</td>
</tr>
<tr>
<td>MALDI TOF</td>
<td>Mass Spectrometry</td>
<td>Gram positives, Gram negatives, anaerobes, yeasts and molds</td>
<td>Bruker, bioMérieux</td>
</tr>
</tbody>
</table>
Advantages and Limitations of Phenotypic Methods

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of use</td>
<td>Results influenced by culture conditions</td>
</tr>
<tr>
<td>Low Cost</td>
<td>Variability due to factors such as isolates not exhibiting their typical biochemical characteristics</td>
</tr>
<tr>
<td>Established technologies with long history of use</td>
<td>Small library of known organisms</td>
</tr>
<tr>
<td>Accurate</td>
<td>High re-test rate when compared to genotypic methods</td>
</tr>
<tr>
<td>Provide meaningful information</td>
<td></td>
</tr>
</tbody>
</table>
API® Strips

• A simple manual microbial identification method
• Consists of a plastic strip that holds multiple mini-test chambers
• Each compartment represents a biochemical test.
• Useful for identification of Gram positives and Gram negatives
• Established technology that has been available to microbiology labs for many years.
• Low cost and easy to use.
API® Strips

The seven digit numerical profile is compared with the numerical profile in the API 20 E analytical profile index book (or apiweb) to obtain the organism identification.
BD BBL™ Crystals

• Similar to API strips but organism suspension is inoculated into wells of a plastic tray followed by placing a second tray containing the reagents for the biochemical tests.

• After incubation and reading of results, a numerical profile is created and compared to a database of known organisms.

• Low cost and easy to use.
BioMérieux Vitek®

- An automated microbial identification system based growth based technology.
- Developed by NASA in the 1970s
- Available in three formats
  - Vitek 2 compact
  - Vitek 2
  - Vitek 2 XL
- System is found in industrial and clinical laboratories.
- System is CFR part 11 capable
BioMérieux Vitek®

• Method employs 64-well reagent cards that each contain and individual test substrate
• Must perform a Gram stain to select the appropriate card for your isolate.
• Can be used with several card types:
  ✓ GN
  ✓ GPC & GP
  ✓ YST
  ✓ BCL
BioMérieux Vitek® - Procedure

• A vacuum pulls the organism suspension into the card wells
• The cards are sealed and loaded into the unit where these are incubated at 35.5 ± 1 C.
• The operator walks away and the system automatically reads the reaction in the cards and compares the results to a database of known organisms.
• Vitek is a highly capable system and its limitations have to do with the size of the database and the preparation of the isolate.
### BioMérieux Vitek® - Advantages and Limitations

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of use</td>
<td>Need accurate Gram stain</td>
</tr>
<tr>
<td>Automated</td>
<td>Results influenced by culture conditions</td>
</tr>
<tr>
<td>Low Cost</td>
<td>Small library size</td>
</tr>
<tr>
<td>Established technologies with long history of use</td>
<td></td>
</tr>
<tr>
<td>Provide meaningful information about organism</td>
<td></td>
</tr>
</tbody>
</table>
Biolog

• An automated microbial identification system based growth based technology.
• Designed for industrial and environmental microbiology.
• Identification to the species level obtained by patterns of carbon utilization.
• System is based on the use of a 96-well plate that contains different carbohydrate sources in each well with tetrazolium redox dye.
• If the microorganism is capable of utilizing the carbohydrate the well turns purple indicating a reduction of the dye.
• Based on the results of the carbon utilization results a metabolic fingerprint is created that allows the user to identify the unknown microorganism.
Early adopters of the system had to choose a specific type of microplate for either Gram positive or Gram negative.

Gen III Microplate is used to test for both Gram positives and Gram negatives.
Biolog

• Three configurations of the system are available:
  • Manual
  • Semi-automatic
  • Fully Automatic

• System software: Gen III & Retrospect
  • Is CFR part 11 capable
  • Includes module for tracking and trending
  • Database can be customized
## Biolog – Advantages and Limitations

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of use &amp; fast set up time</td>
<td>Results influenced by culture conditions</td>
</tr>
<tr>
<td>Different levels of automation available</td>
<td>Slow growing and fastidious organisms will extend the turn around time</td>
</tr>
<tr>
<td>No Gram stain required</td>
<td>Unreactive non-fermenters may be a challenge</td>
</tr>
<tr>
<td>Fast results (as little as 2 hr.)</td>
<td></td>
</tr>
<tr>
<td>Relatively large library size &amp; custom database building capable</td>
<td></td>
</tr>
<tr>
<td>Capable of molds identification</td>
<td></td>
</tr>
<tr>
<td>Trending and tracking software available</td>
<td></td>
</tr>
</tbody>
</table>
MIDI Sherlock™

- Amicrobial identification system that consists of extracting the fatty acids of the organism followed by gas chromatograph (GC).
- Automated system
- Capable of identifying Gram positives, Gram negatives and yeasts by analysis of fatty acids methyl esters (FAME)
MIDI Sherlock™

• The Sherlock identification system is comprised of the following:
  • Computer workstation loaded with the Sherlock and Agilent Chem Station software
  • Gas chromatography instrument, model 6850 or 7890 from Agilent Technologies

• The MIDI Sherlock identification procedure is straightforward and consists of:
  • Extraction of FA from a pure culture
  • Analysis of the extract using GC
  • Comparison of FA profile to a library of microbial species
MIDI Sherlock™

• System software:
  • Analysis software
  • Data Export software
  • Library generation software
  • Tracker/Cluster software
  • Electronic Records and Signatures software
  • Sherlock DNA software
MIDI Sherlock- Advantages and Limitations

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accurate</td>
<td>Results influenced by culture conditions</td>
</tr>
<tr>
<td>Simple set up</td>
<td>Slow growing organisms will extend the turn around time</td>
</tr>
<tr>
<td>No Gram stain or biochemical tests required</td>
<td></td>
</tr>
<tr>
<td>Low cost per test (~ $6)</td>
<td></td>
</tr>
<tr>
<td>Fast results (as little as 20 min)</td>
<td></td>
</tr>
<tr>
<td>Large library size (over 1,500 species)</td>
<td></td>
</tr>
</tbody>
</table>
MALDI TOF

• Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) is a proteotypic identification system

• Uses mass spectrometry to create a unique molecular fingerprint of highly abundant proteins from a microorganism

• The characteristic pattern (fingerprint) from these highly abundant proteins are matched with a database to determine the identity of the microorganism.

• Two versions of the system are available:
  • Vitek MS – bioMerieux (Marcy L’Etoile, France)
  • Bruker Microflex (Bruker Daltonics, Inc. Leipzig, Germany)
Organism identified as *Penicillium* species on MALDI-TOF system; confirmed as *Penicillium citrinum* on MicroSEQ system.
MALDI TOF

• Identifications may be obtained by:
  • Direct inoculation of the organism onto a target plate
  • Performing Formic Acid (FA) extraction of the sample prior to inoculating it onto the target plate.

• Capable of identifying Gram positives, Gam negatives, yeasts and molds.

• A highly accurate system with a low cost per test
MALDI TOF - Procedure

• Material from an isolated colony of microorganism is deposited or smeared on the MALDI target plate.
• After drying the smear on the target is covered with matrix solution (alpha-cyano-4-hydroxycinnamic acid)
• Target is placed in the source of the MALDI unit
• The instrument automatically measures the protein mass fingerprint for each sample spot
• Microorganisms are then identified by comparison of their individual peaks with those in the reference library.
### MALDI-TOF - Advantages and Limitations

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple set up</td>
<td>Cost of acquiring, running and maintaining the unit is high.</td>
</tr>
<tr>
<td>No Gram stain required</td>
<td>Direct inoculation method does not work well.</td>
</tr>
<tr>
<td>Low cost per test (&lt; $5)</td>
<td>Does not provide meaningful biological information about the isolate.</td>
</tr>
<tr>
<td>Fast results (as little as 20 min)</td>
<td></td>
</tr>
<tr>
<td>Highly accurate</td>
<td></td>
</tr>
<tr>
<td>Large library size (over 2,500 species)</td>
<td></td>
</tr>
</tbody>
</table>
Identification Using Genotypic Methods

• Identification of organisms based on analysis of the genetic properties of a microorganism

• Genotypic methods are more stable because nucleic acid sequences are highly conserved in most microbial species

• Genotypic methods are technically more challenging and time consuming

• Examples of genotypic methods are:
  • Nucleic acid hybridization
  • 16S and 23S RNA sequencing
  • Ribotyping
  • PCR

• Typically require investing in expensive equipment, reagents and supplies.
Identification Using Genotypic Methods

• Many labs opt for performing routine identifications using a phenotypic system and rely on genetic identifications for critical and investigational samples.

• DNA sequencing of the first 500 base pairs of the 16S sequence is a popular method used in pharmaceutical identification labs and contract laboratories.

• Strain typing is useful for tracking the source of contamination.
### Examples of Commercial Genotypic Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Technology</th>
<th>Application</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MicroSEQ® 500</td>
<td>rRNA sequencing</td>
<td>Gram positives, Gram negatives, anaerobes, yeasts and molds</td>
<td>Applied Biosystems (AB)</td>
</tr>
<tr>
<td>RiboPrinter®</td>
<td>Ribotyping</td>
<td>Gram positives, Gram negatives, anaerobes, yeasts and molds</td>
<td>Hygena</td>
</tr>
<tr>
<td>Accuprobe</td>
<td>Nucleic Acid Hybridization</td>
<td>Mycobacterial, fungal and bacterial pathogens</td>
<td>Hologic®</td>
</tr>
</tbody>
</table>
## Advantages and Limitations of Genotypic Systems

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly accurate</td>
<td>Expensive equipment required</td>
</tr>
<tr>
<td>Reproducible</td>
<td>Cost per test is high</td>
</tr>
<tr>
<td>No gram stain required</td>
<td>Labor intensive</td>
</tr>
<tr>
<td>No specific culture media or growth conditions required</td>
<td>Require highly trained analysts</td>
</tr>
<tr>
<td>Extensive library of microorganisms</td>
<td>Extensive validation required</td>
</tr>
<tr>
<td>Preferred by regulators</td>
<td></td>
</tr>
</tbody>
</table>
MicroSEQ Microbial Identification System

- Fully automated identification system equipped with CFR part 11 capable software.
- Identifies bacteria to the species level by sequencing the universal 16 s rRNA gene.
- Fungi are identified by sequencing the D2 region of the 26S gene.
- After the sequencing step the MICROSEQ Analysis software compares the sample sequence results to sequences in the validated database library.
MicroSEQ Microbial Identification System

• System consists of
  • AB 3500 or 3500 XL Genetic Analyzer instrument (consisting of a capillary electrophoresis and detection unit).
  • Computer Workstation with Windows Operating System
  • Computer Monitor
  • MicroSEQ ID Analysis Software for control, data collection and sample analysis
  • Therma Cycler PCR unit
  • Printer
MicroSEQ Micrrial Identification System

5 step workflow for ID

1. Pick material from isolated colony
2. Extract DNA
3. Perform PCR
4. Sequence DNA
5. Identify the organism

MicroSEQ® 500 16S PCR Kit
MicroSEQ® 500 16S rDNA Sequencing Kit
MicroSEQ® D2 LSU fungal kit
## MicroSEQ Advantages and Limitations

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly accurate</td>
<td>Expensive to acquire and operate</td>
</tr>
<tr>
<td>No Gram stain required</td>
<td>Labor intensive</td>
</tr>
<tr>
<td>Growth requirement-independent</td>
<td>No strain differentiation capability</td>
</tr>
<tr>
<td>Fast results (in under 24 hr..)</td>
<td>Not practical for small volume labs</td>
</tr>
<tr>
<td>Large library size (over 2,000 species)</td>
<td></td>
</tr>
<tr>
<td>Customizable library</td>
<td></td>
</tr>
</tbody>
</table>
Riboprinter®

- Fully automated identification system equipped with CFR part 11 capable software.
- Identifies bacteria by analyzing DNA fragments produced by restriction enzymes.
- A ribotyping fingerprint is created for the organism of interest and compared to a fingerprint database of over 6,900 RiboPrint™ patterns.
- Can process up to 32 samples per day.
Riboprinter®

• System consists of
  • Characterization unit
  • Computer Workstation with Windows Operating System
  • Computer Monitor
  • Printer
  • Heat Treatment Station
  • Reagents, sample carriers, gel cassettes, membranes
Riboprinter®- Procedure

1. Pick material from isolated colony

2. Extract DNA

3. DNA cleaved at specific sites

4. Separate DNA fragments

5. Probe Hybridization

6. Label on Probe Visualized

7. Algorithms generates fingerprint

8. Fingerprint compared to database

**Lactobacillus buchneri**
**Lactobacillus delbrueckii ss. bulgaricus**
**Lactobacillus delbrueckii ss. delbrueckii**
**Lactobacillus fructivorans**
**Lactobacillus lincheni**
**Lactobacillus lincheni**
# Riboprinter Advantages and Limitations

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly accurate system</td>
<td>Expensive to acquire and operate</td>
</tr>
<tr>
<td>Capable of identification at the genus, species and strain differentiation</td>
<td>Not for routine use</td>
</tr>
<tr>
<td>Analyses a much larger portion of the bacterial genome than other bacterial</td>
<td>Not practical for small volume labs</td>
</tr>
<tr>
<td>identification systems</td>
<td></td>
</tr>
<tr>
<td>Fast results in about 8 hr.</td>
<td></td>
</tr>
<tr>
<td>Strain-level analysis can be used to track a bacterial isolate in a facility</td>
<td></td>
</tr>
<tr>
<td>Place or point in time.</td>
<td></td>
</tr>
<tr>
<td>Established technology</td>
<td></td>
</tr>
<tr>
<td>User can create custom libraries</td>
<td></td>
</tr>
</tbody>
</table>
ACCUPROBE SYSTEM- Nucleic Acid Hybridization

• Identify specific microorganisms based on the ability of complementary nucleic acid strands to form double stranded complexes.

• Accuprobe uses a single stranded DNA probe with a chemiluminescent label that is complementary to the ribosomal RNA of the target organism.

• Most common application is screening for specific bacteria and fungal species in the clinical and research laboratories.

• Assay is rapid and highly sensitive.
ACCUPROBE SYSTEM - Nucleic Acid Hybridization

1. Pick growth material
2. Extract ribosomal RNA
3. Apply labeled DNA probe
4. DNA-RNA complex is formed
5. Luminometer measures DNA-RNA complex
6. Reading ≥ the cut off value is a + result
### ACCUPROBE SYSTEM - Nucleic Acid Hybridization

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>High specificity and accuracy</td>
<td>Require highly trained analysts</td>
</tr>
<tr>
<td>Capable of identification at the genus and species level</td>
<td>For confirmatory identification</td>
</tr>
<tr>
<td>Fast results</td>
<td></td>
</tr>
</tbody>
</table>
Implementing an Automated Microbial Identification System

• Project Deliverables
  ✓ User Requirement Specification (URS)
  ✓ Change Control
  ✓ Risk Assessment Document
  ✓ Validation Plan
  ✓ Electronic Records Electronic Signatures Document (ERES)
  ✓ Traceability Matrix
  ✓ IOQ and PQ Protocol
  ✓ Final Report
  ✓ SOP’s for Use and Maintenance of the Systems
  ✓ On the Job Training Documents
  ✓ Approved Change Control
User Requirements Specification (URS)

• Defines the user requirements for the identification system.
• Serves as a basis for procurement and qualification
• Must be prepared and approved prior to procuring the system
• Typically includes:
  ✓ Functional Requirements
  ✓ Functional Data
  ✓ Data Integrity
  ✓ Interface
  ✓ Utilities
  ✓ Environmental Requirements
Change Control

• Describes the scope of the project
• Contains the Implementation plan with detail of activities
• Contains assessment by Subject Matter Experts
• Lists the project deliverables
• Lists the system owner and approvers
Risk Assessment Document

- A formal document that documents the risks associated with implementing your ID system
- Follow your company’s RA procedure.
- May be part of change control document
Validation Plan

• Together with the change control defines the validation strategy for the new system
• Defines specific activities, deliverables and roles and responsibilities
• Includes overall acceptance criteria for the project
Project Planning – Microsoft Project
ERES / Traceability Matrix

• **ERES**
  - Document that establishes the parameters to be verified for computerized systems.

• **Traceability Matrix**
  - Ensures that the qualification addresses all user requirements and functions to be tested.
  - Provides traceability to related verification and validation activities along with pre-defined acceptance criteria.
Qualification Protocols

• Documents that guide the qualification of the system.
• May write separate protocols (IQ, OQ and PQ) or combine the challenges in a single protocol.
• Must execute in a sequential manner
• Must match the requirements of the URS
• May be a combination of vendor protocol and your own.
Operation and Maintenance SOP / OJT

• **SOP**
  • Contains the steps necessary to operate and maintain the system
  • Specifies the extent and frequency of routine maintenance

• **OJT**
  • A guide for training the users of the ID system
  • Focuses on the technique and skills required proper execution and documentation of the assay
Approved Change Control

• It’s a wrap! Deliverables have been completed and analysts are trained. Let’s use the system for routine ID.