Bacterial Identification by 16S rRNA Gene Sequence Analysis

Background

All bacteria contain 16S ribosomal RNA (rRNA) genes of approximately 1500 base pairs (bp) in length. rRNA genes contain regions of variable DNA sequence that are unique to the species carrying the gene. The species identity of an unknown bacterium may therefore be deduced from its unique rRNA gene sequence.

Applications

16S based identification of bacteria may be used in one of two ways:

1. Direct Specimen Analysis. May be performed directly on the primary specimen after routine culture fails to exhibit growth. Consider ordering when prior cultures are negative, or where there is a high likelihood of uncultivable bacteria (e.g. due to intensive antimicrobial therapy) being present.

2. Isolate Identification. May be used when an organism is recovered in culture, but conventional microbiology methods fail to identify the isolate. This will usually be noted by the comment “Unable to further identify by routine biochemical methods. If clinical considerations warrant further testing, please contact the Microbiology Laboratory immediately for information regarding methods and fees.” Additionally, the physician/referring lab will be consulted on isolates from high priority specimens (e.g. blood) where 16S ID is indicated.

3. Hold for 16S Analysis. Order SEQHOLD when submitting a fluid, tissue, or other high value specimen for culture and/or histology where there is a concern that uncultivable microorganisms (e.g. fastidious microorganisms or following intensive antimicrobial therapy) may be present. Specimens ordered with SEQHOLD will be available for two weeks for subsequent 16S analysis.

Clinical Examples (drawn from the Marshfield Laboratories case files)

Patient presents with spinal meningitis, CSF specimen collected:
- Gram stain = Moderate WBCs, No microorganisms observed
- Aerobic Culture = No growth at 48 hrs
- Direct rRNA sequence analysis of CSF yielded *Neisseria meningitidis*

Diabetic patient whose chronic foot wound infection is cultured:
- Gram stain = Rare WBCs, Few Gram positive cocci
- Anaerobe culture = Tiny, fastidious cocci, unable to ID by conventional microbiology
- Isolate identified by rRNA sequence analysis as *Finegoldia magna*

Patient presents with fever of unknown origin:
- Routine blood cultures are negative. Direct rRNA sequence analysis of EDTA whole blood = *Anaplasma phagocytophila* (an organism not normally cultivable)

Principle

rRNA genes are first amplified using PCR technology. After amplification, PCR cycle sequencing is performed, and the rRNA sequence determined using a capillary sequence analyzer. The resulting sequence is then matched to known rRNA sequences in GenBank® (NIH, U.S. DHHS) and Ridom (Ridom GmbH, Wurzburg), and validated using a rigorous review process.
Limitations

1. While the use of PCR amplification increases the sensitivity of rRNA sequence analysis of patient specimens, very low numbers of organisms may nevertheless be below the lower limit of detection.

2. As is true of any PCR based method, naturally occurring inhibitors may lead to a false negative result. Acid formalin used in fixed tissues may also inhibit the PCR reaction.

3. Small amounts of naturally or artificially introduced bacteria or bacterial DNA amplified by the PCR reaction may lead to false positive results. Submit specimens collected using sterile technique in sterile container for best results.

4. While each result produced by nucleic acid sequence analysis is carefully reviewed at multiple levels, nucleic acid databases may contain errors having the potential to result in an incorrect identification.

Contact Information

Marshfield Labs Customer Service 800-222-5835.

Questions or Comments

Please refer any questions or comments to:

Interpretation

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Technical

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