CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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DNA Sequence Analysis for Identification of Microorganisms

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Effective Date:
May 1, 2011

Performed:
Typically set up Monday through Friday and resulted when assay is complete.
Expected Turn-Around-Time (TAT) is 1-3 days from receipt of specimens at CPAL.

Mnemonic:
MICROID

Method:
PCR amplification and Di-deoxy chain terminating DNA sequencing chemistry (Sanger Method). DNA sequence data is compared to DNA sequence database data to identify the "Best Match" sequence.

Specimen (IMPORTANT):
Specimen transport tubes are provided by CPAL. Call the CPAL laboratory to obtain transport tubes. These tubes contain a solution (*PrepMan® Ultra*) that is required for the inactivation of the microorganisms and denaturation of their genomic DNA.
The 2 mL screw cap specimen transport tubes (labeled MICRO ID) contain about 200 uL of PrepMan® Ultra. To submit a specimen for sequence identification, place a small amount of a pure culture into the solution in the specimen transport tube. Typical quantities of sample are, for example, a small bacterial colony, or a portion of a large bacterial colony. More is not better! A similar amount of eukaryotic samples are required, in cases in which identification is being requested for those organisms. Include any relevant information about the submitted specimen (ie nature of sample bacterial, mold, fungi, Mycobacteria, etc). Place specimen transport tube into an individual labeled contained (ie biohazard bag, conical tube, etc) and send to CPAL.

Reference Ranges:

No specific reference ranges are associated with this type of assay.

Summary:

The laboratory identification of bacterial and certain eukaryotic species can, on occasion be challenging. Many sophisticated and complex methods exist and are implemented in the modern clinical microbiology laboratory to ascertain a timely and accurate identification of clinically relevant microorganisms. In certain instances there is a need for additional laboratory methods that can provide information about the molecular character of a microorganism as an adjunct to the identification and characterization process.

The molecular characterization of the 16S or 18S rDNA sequence of a microorganism can aid in the rapid and specific identification of otherwise difficult to identify organisms. Ribosomal RNAs (rRNA) are present as major macromolecular structures in the ribosome of eukaryotic and prokaryotic organisms. The rRNAs serve both structural and functional purposes and have been highly conserved throughout the course of evolution. The largest of these rRNA molecules are designated the 16S rRNA and 23S rRNA in prokaryotic organisms and 18S rRNA and 28S rRNA in eukaryotic organisms (The "S" refers to the Svedburg unit, a measure of molecular size).

Molecular phylogenies are based on the sequence similarities and differences in many potential molecules. The ribosomal RNAs represent good molecules for the studies of molecular phylogeny (or systematics) and have been extensively studied. Molecular sequence databases (such as GenBank/NCBI, or the Ribosomal Database Project, Michigan State University) contain thousands of representative DNA (RNA) sequences from organisms across the spectrum of evolutionary lineages.

The 16S and 18S rRNA molecules represent an appropriate analyte for the determination of microorganisms' identities and similarities via DNA (RNA) sequence comparisons because;

1) The 16S and 18S rRNA molecules are greater than 1500 bases in length. This provides a significant amount of sequence information yet is small enough to represent a manageable amount of sequence information. Sequence analysis of the entire molecule is usually not necessary, as certain regions contain enough variability to accurately assign an appropriate identification, based on the sequence information obtained. This depends on the phylogenetic position of the organism being analyzed.
2) The 16S and 18S rRNA molecule consists of highly conserved sequences (useful for primer design and sequence stability) and regions of hyper-variable primary and secondary sequence structure (provide specificity in sequences comparisons).
3) Public domain databases (GenBank) are easily searchable. This provides a mechanism in which sequence data can be used to identify other, previously characterized organisms with identical or similar 16S and 18S rRNA sequences.

The ability of DNA sequence analysis of the hsp65 gene to differentiate between several of the very closely related Mycobacterium species that have virtually identical 16S rDNA gene sequences may be exploited to aid in the identification of organisms known or suspected to be Mycobacterium that may be identifiable via hsp65 sequence analysis. This will be used in those instances in which the 16S rDNA determinations alone cannot provide "species level" information and is accomplished using the sequencing protocol.

Result Reporting

Results are reported as "Best Match". This means that the results are based on comparison to database entries of numerous genes from numerous organisms. The best match result that is produced in laboratories that offer this assay is simply a reporting of likely microorganism identification, based on this sequence data match. In cases in which more than one organism is listed as a Best Match, all listed microorganisms should be considered in the final identification since this is an indication that there were multiple matches in the database queries. The Best Match result should be considered in the context of the entire clinical presentation of the patient and should be correlated with other laboratory findings. This result is based on sequence analysis of a representative region of the rDNA gene of the submitted organism. DNA sequence data is used to query appropriate DNA sequence databases, including NCBI GenBank. While the data contained in this, and other, database collections are capable of assisting in the correct identification of many microorganisms, the results should only be used as a guide for clinical identifications. This assay has been shown to reliably assist in the identification of microorganisms at various levels (ie genus, species) but the possibility of misidentification can not be entirely eliminated due to a variety of laboratory and biologic variables.

Note:

This test was developed and its performance characteristics determined by The Central Pennsylvania Alliance Laboratory, LLC. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.