DIRECT 16S
BACTERIAL DETECTION & IDENTIFICATION WITH 16S rRNA GENE DIRECTLY FROM CLINICAL SPECIMENS

Direct 16S is broad-range amplification of the bacterial 16S rRNA gene followed by DNA sequencing, and serves as a valuable, culture-independent method for bacterial detection and identification directly from clinical specimens. Using the RipSeq® sequence analysis software from Isentio also makes Direct 16S a solution for patients with mixed infections.

WHY DIRECT 16S?
Diagnostic methods to detect and identify bacteria in a specimen are normally dependent on the presence of viable bacteria that can be grown on standard media in the laboratory. Culture-dependent methods can be less reliable than culture-independent methods, especially when a patient has received one or more doses of antibiotics prior to specimen collection, when specimen transportation is suboptimal or when the suspected bacterial pathogens cannot grow on standard media.

Bacterial DNA are stable and can remain in clinical material after cell death caused by antibacterial therapy or inappropriate specimen transport. Important pathogens like S. pneumoniae, HACEK-group bacteria, anaerobes, and Haemophilus influenza can be dead upon arrival to the lab. Pathogens like Chlamydia, Mycoplasma, Tropheryma and Ehrlichia will not grow or grow extremely slowly on standard culture media. Delayed results can also occur in mixed infections, where repeated isolation and subcultivations to isolate bacteria can take up to a week or more.

The 16S rRNA gene is found in all bacteria on the small ribosomal subunit and can detect all bacterial pathogens. Unlike species-specific molecular tests, the clinician does not need to know the putative pathogen prior to submitting a clinical specimen for Direct 16S. By using a culture independent method like Direct 16S, concerns about specimen integrity, prior antibacterial therapy, or identifying bacteria from mixed infections are minimized.

The latest advances in polymerase chain reaction (PCR) and sequencing technology can offer a more accurate, rapid method for detection and identification. Direct 16S can detect and identify pathogens from a clinical specimen (e.g., heart valves, abscesses, pleural fluids) within the course of a day.

WHEN IS DIRECT 16S ADVANTAGEOUS
The advantage of Direct 16S is that the method is independent of culture and can provide answers within a shorter time compared to conventional methods. Direct 16S is optimally applied for clinical situations when:

» A patient has received antibiotics prior to specimen collection
» A clinician has a high index of suspicion for an infection caused by atypical or anaerobic bacteria
» The infection may be caused by multiple bacteria (mixed infections often occur in abscesses)

Direct 16S will not provide bacteria susceptibility data. However, prompt identification of putative pathogen(s) will provide guidance for targeted antibacterial therapy.

OPTIMAL CLINICAL SPECIMENS
EXAMPLE OF RELEVANT SPECIMEN TYPES

» Abscesses in internal organs (brain, lung, spleen, liver, pancreas, kidney, ovaries, aorta)
» Retroperitoneal abscesses
» Deep soft-tissue or muscular abscesses
» Aspirate/biopsies from spondylodiscitis and other bone related infections
» Sterile body fluids (pleural, synovial, bile, CSF, pericardial)
» Heart valves
USEFUL REFERENCES

The following is a list of some articles that will give you some background and show the clinical relevance of Direct 16S sequencing.

Comprehensive Diagnostic Strategy for Blood Culture–Negative Endocarditis: A Prospective Study of 819 New Cases
Clinical Infectious Diseases 2010; 51(2): 131–140

Direct 16S rRNA Gene Sequencing from Clinical Specimens, with Special Focus on Polybacterial Samples and Interpretation of Mixed DNA Chromatograms
Kommedal Ø, Kvello K, Skjåstad R, Langeland N, Wiker H G

Comparison of broad-range bacterial PCR and culture of cerebrospinal fluid for diagnosis of community-acquired bacterial meningitis
Clinical Microbiology and Infection 2007; 13: 879–886

NON-RELEVANT SPECIMEN TYPES

- Samples from areas in direct contact with mucus membranes or skin (BAL, pus from perianal abscesses, vaginal swabs, superficial wounds etc.)
- Very small biopsies or heavily diluted aspirates
- Abscesses/samples from locations in direct connection to intestine

HOW TO COLLECT AND SEND A SPECIMEN FOR DIRECT 16S

Specimens should be transported in sterile containers without additives. Sterile water might be contaminated with DNA from *Pseudomonas* sp. and similar bacteria. In general we recommend ≥200 µl of liquid material and a “finger nail” size for solid specimens to achieve optimal test sensitivity.

The lab form should contain information about why you request a Direct 16S sequence analysis (e.g. antibiotic treatment, anaerobe infection, slow-growing bacteria, atypical bacteria or potential multi-bacterial infection). This information will help the lab determine what is the most appropriate method of analysis.

FOR MORE INFORMATION

Optimized lab protocols (DNA extractions procedures, primers, enzymes) and a web-based software for sequence analysis of single- and multi-bacterial infections are available from Isentio AS (www.isentio.com). We can provide you with more information about the RipSeq software and Direct 16S and also tell you if there is a lab nearby you that uses RipSeq. Note that guidelines on sample collection may differ between laboratories. For more information please contact your lab or Isentio:

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RipSeq – SEQUENCE ANALYSIS SOFTWARE FOR MIXED INFECTIONS

Software is now available for sequence analysis of mixed infections and thereby improves the capabilities of sequencing directly from the primary, clinical specimen. The data file that is generated from sequencing is simply uploaded into the software, and analyzed within seconds. This software analysis tool, RipSeq, is currently in use by many laboratories around the world. Ask us about laboratories using RipSeq in your area.

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