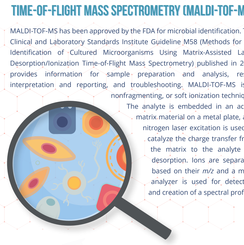
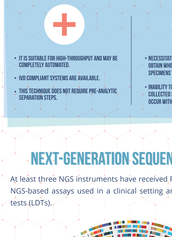
EMERGING TRENDS IN MICROBIOLOGY DIAGNOSTIC METHODS

Standard procedure for confirming an infectious disease requires specimen collection and test selection. Routine diagnostic tests include microscopic examination, culture and biochemical tests, serological tests including agglutination and ELISA, and genetic testing. More advanced diagnostic methods are emerging, focused on speed, accuracy, and cost-effectiveness.

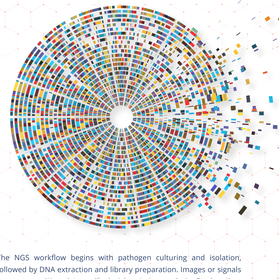


MATRIX ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF-MS)

MALDI-TOF-MS has been approved by the FDA for microbial identification. The Clinical and Laboratory Standards Institute Guideline M58 (Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) published in 2017 provides information for sample preparation and analysis, result interpretation and reporting, and troubleshooting. MALDI-TOF-MS is a nonfragmenting, or soft-ionization technique.



The analyte is embedded in an acidic matrix material on a metal plate, and nitrogen laser excitation is used to catalyze the charge transfer from the matrix to the analyte for desorption. Ions are separated based on their *m/z* and a mass analyzer is used for detection and creation of a spectral profile.



ADVANTAGES & LIMITATIONS OF MALDI-TOF-MS

+

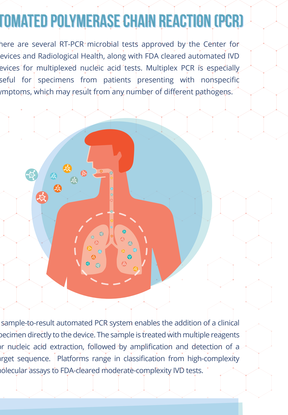
- IT IS SUITABLE FOR HIGH-THROUGHPUT AND MAY BE COMPLETELY AUTOMATED.
- IVD-COMPLIANT SYSTEMS ARE AVAILABLE.
- THIS TECHNIQUE DOES NOT REQUIRE PRE-ANALYTIC SEPARATION STEPS.

−

- NECESSITATES MICROORGANISM CULTURE TO OBTAIN WHOLE-CELL OR EXTRACTED PROTEIN SPECIMENS WITH A MINIMUM NUMBER OF CFUs.
- INABILITY TO SEPARATE MULTIPLE SPECTRA COLLECTED SIMULTANEOUSLY, WHICH MAY OCCUR WITH POLYMICROBIAL CULTURES.

NEXT-GENERATION SEQUENCING (NGS)

At least three NGS instruments have received FDA approval, and many NGS-based assays used in a clinical setting are laboratory-developed tests (LDTs).



The NGS workflow begins with pathogen culturing and isolation, followed by DNA extraction and library preparation. Images or signals are converted into base calls during primary analysis. Further data processing in secondary analysis includes trimming and filtering, and sequence reads are assigned to a reference sequence or assembled with do novo assembly. The identification of clinically significant findings in tertiary analysis is used to generate a final report.

ADVANTAGES & LIMITATIONS OF NGS

+

- NGS MAY BE USED FOR WHOLE GENOME SEQUENCING (WGS) ON BACTERIAL ISOLATES FROM A SINGLE, OR MULTIPLE PATIENTS.
- OFFERS RAPID BACTERIAL IDENTIFICATION, AND HAS THE CAPACITY TO DIFFERENTIATE BETWEEN CLONES (IT IS ESPECIALLY USEFUL FOR OUTBREAK CONTROL).
- THE TECHNIQUE IS BECOMING INCREASINGLY AUTOMATED AND COSTS CONTINUE TO DECREASE.

−

- NGS GENERATES COMPLEX DATA THAT NECESSITATES INTERPRETATION BY A CLINICAL MICROBIOLOGIST TO ENSURE THE REPORT IS DESIGNED TO ASSIST THE PHYSICIAN IN SELECTING AN APPROPRIATE TREATMENT.
- SENSITIVITY AND SPECIFICITY OF INDIVIDUAL PLATFORMS CANNOT BE COMPARED DIRECTLY.

AUTOMATED POLYMERASE CHAIN REACTION (PCR)

There are several RT-PCR microbial tests approved by the Center for Devices and Radiological Health, along with FDA cleared automated IVD devices for multiplexed nucleic acid tests. Multiplex PCR is especially useful for specimens from patients presenting with nonspecific symptoms, which may result from any number of different pathogens.

A sample-to-result automated PCR system enables the addition of a clinical specimen directly to the device. The sample is treated with multiple reagents for nucleic acid extraction, followed by amplification and detection of a target sequence. Platforms range in classification from high-complexity molecular assays to FDA-cleared moderate-complexity IVD tests.

ADVANTAGES AND LMITATIONS OF AUTOMATED PCR

+

- AUTOMATING PCR SIMPLIFIES WORKFLOWS AND LIMITS SPECIMEN HANDLING TO REDUCE THE RISK OF CONTAMINATION.
- AUTOMATED MULTIPLEX INSTRUMENTS ARE SUITABLE FOR RAPID DETECTION OF A GREATER NUMBER OF TARGETS THAN TRADITIONAL PCR.
- REDUCED COST PER TEST WHEN MULTIPLE SPECIMENS ARE PROCESSED TOGETHER.

−

- PATIENT CARE DECISIONS SOMETIMES REQUIRE RAPID TESTING, AND AUTOMATED ON-DEMAND TESTING IS MORE COSTLY THAN BATCH TESTING.