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Carey-Ann D. Burnham

Laboratory Detection of Sepsis: Biomarkers and Molecular Approaches 413

Stefan Riedel and Karen C. Carroll

Sepsis, severe sepsis, and septic shock cause significant morbidity and mortality worldwide. Rapid diagnosis and therapeutic interventions are desirable to improve the overall mortality in patients with sepsis. However, gold standard laboratory diagnostic methods for sepsis, pose a significant challenge to rapid diagnosis of sepsis by physicians and laboratories. This article discusses the usefulness and potential of biomarkers and molecular test methods for a more rapid clinical and laboratory diagnosis of sepsis. Because new technologies are quickly emerging, physicians and laboratories must appreciate the key factors and characteristics that affect the clinical usefulness and diagnostic accuracy of these test methodologies.

Molecular Detection of Respiratory Viruses 439

Richard S. Buller

Over the past several years a wide variety of molecular assays for the detection of respiratory viruses has reached the market. The tests described herein range from kits containing primers and probes detecting specific groups of viruses, to self-contained systems requiring specialized instruments that extract nucleic acids and perform the polymerase chain reaction with little operator input. Some of the tests target just the viruses involved in large yearly epidemics such as influenza, or specific groups of viruses such as the adenoviruses or parainfluenza viruses; others can detect most of the known respiratory viruses and some bacterial agents.

Molecular Diagnostics and Parasitic Disease 461

Shawn Vasoo and Bobbi S. Pritt

Molecular parasitology represents an emerging field in microbiology diagnostics. Although most assays use nonstandardized, laboratory-developed methods, a few commercial systems have recently become available and are slowly being introduced into larger laboratories. In addition, a few methodologies show promise for use in field settings in which parasitic infections are endemic. This article reviews the available techniques and their applications to major parasitic diseases such as malaria, leishmaniasis, and trichomoniasis.

Invasive Fungal Infections: Biomarkers and Molecular Approaches to Diagnosis 505

Audrey N. Schuetz

The biomarkers galactomannan and 1,3-β-D-glucan have been well studied over the past years and are gaining a role in the diagnosis of invasive

fungal infections. Although not as well studied until recently, molecular methods for the diagnosis of invasive fungal infection are also being evaluated. Outcomes data for molecular testing are expanding, but have not yet provided enough evidence for inclusion of molecular diagnostics in formal clinical guidelines. Lack of standardization and validation of the various molecular assays and platforms has hindered their widespread acceptance in the evaluation of invasive fungal infections, although the future is promising.

Advanced Techniques for Detection and Identification of Microbial Agents of Gastroenteritis **527**

Sherry A. Dunbar, Hongwei Zhang, and Yi-Wei Tang

Gastroenteritis persists as a worldwide problem, responsible for approximately 2 million deaths annually. Traditional diagnostic methods used in the clinical microbiology laboratory include a myriad of tests, such as culture, microscopy, and immunodiagnostics, which can be labor intensive and suffer from long turnaround times and, in some cases, poor and Yi-Wei Tang sensitivity. This article reviews recent advances in genomic and proteomic technologies that have been applied to the detection and identification of gastrointestinal pathogens. These methods simplify and speed up the detection of pathogenic microorganisms, and their implementation in the clinical microbiology laboratory has potential to revolutionize the diagnosis of gastroenteritis.

Molecular Approaches and Biomarkers for Detection of *Mycobacterium tuberculosis* **553**

Robert F. Luo and Niaz Banaei

Tuberculosis (TB) continues to be a public health emergency, compounded by the lack of adequate diagnostic testing in many regions of the world. New advances in the molecular detection of *Mycobacterium tuberculosis*, including faster and simpler nucleic acid amplification tests, have resulted in rapid and cost-effective methods to diagnose TB and test for drug resistance. Ongoing research on biomarkers for TB infection may lead to new tests for blood, urine, breath, and sputum. Sustained investment in the development and dissemination of diagnostic tests for TB is critical for increasing TB case finding, placing patients on appropriate treatment, and reducing transmission.

Automation in the Clinical Microbiology Laboratory **567**

Susan M. Novak and Elizabeth M. Marlowe

Imagine a clinical microbiology laboratory where a patient's specimens are placed on a conveyor belt and sent on an automation line for processing and plating. Technologists need only log onto a computer to visualize the images of a culture and send to a mass spectrometer for identification. Once a pathogen is identified, the system knows to send the colony for susceptibility testing. This is the future of the clinical microbiology laboratory. This article outlines the operational and staffing challenges facing clinical microbiology laboratories and the evolution of automation that is shaping the way laboratory medicine will be practiced in the future.

MALDI-TOF Mass Spectrometry for Microorganism Identification 589

Tanis C. Dingle and Susan M. Butler-Wu

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid, reliable, and high-throughput diagnostic tool for the identification of microorganisms. The technology is unique in clinical microbiology, allowing laboratories to definitively identify bacterial and fungal isolates within minutes. The rapid turnaround time and minimal cost for consumables per specimen compared with conventional identification methods have resulted in MALDI-TOF MS being increasingly used in clinical laboratories worldwide. This article summarizes the current literature on MALDI-TOF MS for microbial identification and provides a preview of the method's potential future applications in clinical microbiology.

Beyond Identification: Emerging and Future Uses for MALDI-TOF Mass Spectrometry in the Clinical Microbiology Laboratory 611

Mari L. DeMarco and Bradley A. Ford

The routine use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized microorganism identification in the clinical microbiology laboratory. Building from these now common microorganism identification strategies, this review explores future clinical applications of MALDI-TOF MS. This includes practical approaches for laboratorians interested in implementing direct identification processing methods for MALDI-TOF detection of microbes in bloodstream infection (BSI) and urinary tract infection (UTI), as well as, post-analytical approaches for classifying MALDI-TOF spectral data to detect characteristics other and species-level identification (e.g. strain-level classification, typing, and resistance mechanisms).

Bacterial Strain Typing 629

Duncan MacCannell

Over the course of the past several decades, rapid advancements in molecular technologies have revolutionized the practice of public health microbiology, and have fundamentally changed the nature, accuracy, and timeliness of laboratory data for outbreak investigation and response. Whole-genome sequencing, in particular, is becoming an increasingly feasible and cost-effective approach for near real-time high-resolution strain typing, genomic characterization, and comparative analyses. This review discusses the current state of the art in bacterial strain typing for outbreak investigation and infectious disease surveillance, and the impact of emerging genomic technologies on the field of public health microbiology.

Diagnostic Assays for Identification of Microorganisms and Antimicrobial Resistance Determinants Directly from Positive Blood Culture Broth 651

Morgan A. Pence, Erin McElvania TeKippe, and Carey-Ann D. Burnham

The detection of blood stream infections is one of the most important functions of the clinical microbiology laboratory. Sepsis is a clinical emergency, and mortality increases if commencement of appropriate antimicrobial therapy is delayed. Automated blood culture systems are the most sensitive approach for detection of the causative agent of sepsis. Several

laboratory methods have been developed to expedite identification of organisms directly from positive blood culture broth. The principle and analytical performance characteristics of these methods are described in this review.

Future-Generation Sequencing and Clinical Microbiology **685**

Benjamin C. Kirkup, Steven Mahlen, and George Kallstrom

Sequencing technologies are changing the way both laboratory medicine and clinical practice impact patient care. This article focuses on the clinical microbiology laboratory and the potential benefits and limitations of coming generations of sequencing technology. Nucleic acid sequencing technology is rapidly outpacing the infrastructure needed to accurately educate, analyze, and interpret complex massive data sets that are rapidly becoming integrated into clinical practice.

Integration of Technology Into Clinical Practice **705**

Christopher D. Doern

It is an exciting time in clinical microbiology. New advances in technology are revolutionizing every aspect of the microbiology laboratory, from processing of specimens to bacterial identification; as a result, the microbiology laboratory is rapidly changing. With this change comes the challenge of selecting and implementing the technology that is most appropriate for each laboratory and clinical setting. This review focuses on issues surrounding implementation of new technology such that the improvements to clinical care are maximized.

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