PRACTICAL GUIDANCE FOR CLINICAL MICROBIOLOGY

Practical Guidance for Clinical Microbiology Laboratories: Diagnosis of Ocular Infections
e00070-19
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Summary: The variety and complexity of ocular infections have increased significantly in the last decade since the publication of Cumitech 13B, Laboratory Diagnosis of Ocular Infections (L. D. Gray, P. H. Gilligan, and W. C. Fowler, Cumitech 13B, Laboratory Diagnosis of Ocular Infections, 2010). The purpose of this practical guidance document is to review, for individuals working in clinical microbiology laboratories, current tools used in the laboratory diagnosis of ocular infections. This document begins by describing the complex, delicate anatomy of the eye, which often leads to limitations in specimen quantity, requiring a close working bond between laboratorians and ophthalmologists to ensure high-quality diagnostic care. Descriptions are provided of common ocular infections in developed nations and neglected ocular infections seen in developing nations. Subsequently, preanalytic, analytic, and postanalytic aspects of laboratory diagnosis and antimicrobial susceptibility testing are explored in depth.

REVIEWS

Invasive Haemophilus influenzae Infections after 3 Decades of Hib Protein Conjugate Vaccine Use
e00028-21
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Summary: Haemophilus influenzae serotype b (Hib) was previously the most common cause of bacterial meningitis and an important etiologic agent of pneumonia in children aged <5 years. Its major virulence factor is the polyribosyl ribitol phosphate (PRP) polysaccharide capsule. In the 1980s, PRP-protein conjugate Hib vaccines were developed and are now included in almost all national immunization programs, achieving a sustained decline in invasive Hib infections. However, invasive Hib disease has not yet been eliminated in countries with low vaccine coverage, and sporadic outbreaks of Hib infection still occur occasionally in countries with high vaccine coverage. Over the past 2 decades, other capsulated serotypes have been recognized increasingly as causing invasive infections. H. influenzae serotype a (Hia) is now a major cause of invasive infection in Indigenous communities of North America, prompting a possible requirement for an Hia conjugate vaccine. H. influenzae serotypes e and f are now more common than serotype b in Europe. Significant year-to-year increases in nontypeable H. influenzae invasive infections have occurred in many regions of the world. Invasive H. influenzae infections are now seen predominantly in patients at the extremes of life and those with underlying comorbidities. This review provides a comprehensive and critical overview of the current global epidemiology of invasive H. influenzae infections in different geographic regions of the world. It discusses those now at risk of invasive Hib disease, describes
the emergence of other severe invasive H. influenzae infections, and emphasizes the importance of long-term, comprehensive, clinical and microbiologic surveillance to monitor a vaccine’s impact.

**Advances in the Microbiology of Stenotrophomonas maltophilia**  
*Joanna S. Brooke*

Summary: *Stenotrophomonas maltophilia* is an opportunistic pathogen of significant concern to susceptible patient populations. This pathogen can cause nosocomial and community-acquired respiratory and bloodstream infections and various other infections in humans. Sources include water, plant rhizospheres, animals, and foods. Studies of the genetic heterogeneity of *S. maltophilia* strains have identified several new genogroups and suggested adaptation of this pathogen to its habitats. The mechanisms used by *S. maltophilia* during pathogenesis continue to be uncovered and explored. *S. maltophilia* virulence factors include use of motility, biofilm formation, iron acquisition mechanisms, outer membrane components, protein secretion systems, extracellular enzymes, and antimicrobial resistance mechanisms. *S. maltophilia* is intrinsically drug resistant to an array of different antibiotics and uses a broad arsenal to protect itself against antimicrobials. Surveillance studies have recorded increases in drug resistance for *S. maltophilia*, prompting new strategies to be developed against this opportunist. The interactions of this environmental bacterium with other microorganisms are being elucidated. *S. maltophilia* and its products have applications in biotechnology, including agriculture, biocontrol, and bioremediation.

**Viral Respiratory Pathogens and Lung Injury**  
*Nicola Clementi, Sreya Ghosh, Maria De Santis, Matteo Castelli, Elena Criscuolo, Ivan Zanoni, Massimo Clementi, Nicasio Mancini*

Summary: Several viruses target the human respiratory tract, causing different clinical manifestations spanning from mild upper airway involvement to life-threatening acute respiratory distress syndrome (ARDS). As dramatically evident in the ongoing SARS-CoV-2 pandemic, the clinical picture is not always easily predictable due to the combined effect of direct viral and indirect patient-specific immune-mediated damage. In this review, we discuss the main RNA (orthomyxoviruses, paramyxoviruses, and coronaviruses) and DNA (adenoviruses, herpesviruses, and bocaviruses) viruses with respiratory tropism and their mechanisms of direct and indirect cell damage. We analyze the thin line existing between a protective immune response, capable of limiting viral replication, and an unbalanced, dysregulated immune activation often leading to the most severe complication. Our comprehension of the molecular mechanisms involved is increasing and this should pave the way for the development and clinical use of new tailored immune-based antiviral strategies.

**Clinical Laboratory Biosafety Gaps: Lessons Learned from Past Outbreaks Reveal a Path to a Safer Future**  

Summary: Patient care and public health require timely, reliable laboratory testing. However, clinical laboratory professionals rarely know whether patient specimens contain infectious agents, making ensuring biosafety while performing testing procedures challenging. The importance of biosafety in clinical laboratories was highlighted during the 2014 Ebola outbreak, where concerns about biosafety resulted in delayed diagnoses and contributed to patient deaths. This review is a collaboration
between subject matter experts from large and small laboratories and the federal government to evaluate the capability of clinical laboratories to manage biosafety risks and safely test patient specimens. We discuss the complexity of clinical laboratories, including anatomic pathology, and describe how applying current biosafety guidance may be difficult as these guidelines, largely based on practices in research laboratories, do not always correspond to the unique clinical laboratory environments and their specialized equipment and processes. We retrospectively describe the biosafety gaps and opportunities for improvement in the areas of risk assessment and management; automated and manual laboratory disciplines; specimen collection, processing, and storage; test utilization; equipment and instrumentation safety; disinfection practices; personal protective equipment; waste management; laboratory personnel training and competency assessment; accreditation processes; and ethical guidance. Also addressed are the unique biosafety challenges successfully handled by a Texas community hospital clinical laboratory that performed testing for patients with Ebola without a formal biocontainment unit. The gaps in knowledge and practices identified in previous and ongoing outbreaks demonstrate the need for collaborative, comprehensive solutions to improve clinical laboratory biosafety and to better combat future emerging infectious disease outbreaks.

**How To Prepare for the Unexpected: a Public Health Laboratory Response**

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Summary: Public health laboratories (PHLs) continue to face internal and external challenges to their abilities to provide successful, timely responses to public health crises and emerging threats. These laboratories are mandated to maintain the health of their communities by identifying, diagnosing, and warning constituents of potential and real health emergencies. Due to the changing characteristics of public health threats and their cross-jurisdictional nature, laboratories are facing increased pressure to ensure that they respond in a consistent and coordinated manner. Here, the Association of Public Health Laboratories (APHL) Emerging Leaders Program Cohort 11 members have compiled stories from subject matter experts (SMEs) at PHLs with direct involvement in crises to determine the characteristics of a successful response. Experts examined a diverse selection of emerging threats from across PHLs, including infectious diseases, opioids, natural disasters, and government shutdowns. While no public health crisis will be identical to another, overarching themes were consistent across subjects. Experiences from SMEs that could improve future responses to emerging threats are highlighted.

**Mobile Oxazolidinone Resistance Genes in Gram-Positive and Gram-Negative Bacteria**

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Summary: Seven mobile oxazolidinone resistance genes, including cfr, cfr(\(B\)), cfr(\(C\)), cfr(\(D\)), cfr(\(E\)), optrA, and poxtA, have been identified to date. The cfr genes code for 23S rRNA methylases, which confer a multiresistance phenotype that includes resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A compounds. The optrA and poxtA genes code for ABC-F proteins that protect the bacterial ribosomes from the inhibitory effects of oxazolidinones. The optrA gene confers resistance to oxazolidinones and phenicols, while the poxtA gene confers elevated MICs or resistance to oxazolidinones, phenicols, and tetracycline. These oxazolidinone resistance genes are most frequently found on plasmids, but they are...
also located on transposons, integrative and conjugative elements (ICEs), genomic islands, and prophages. In these mobile genetic elements (MGEs), insertion sequences (IS) most often flanked the cfr, optrA, and poxtA genes and were able to generate translocatable units (TUs) that comprise the oxazolidinone resistance genes and occasionally also other genes. MGEs and TUs play an important role in the dissemination of oxazolidinone resistance genes across strain, species, and genus boundaries. Most frequently, these MGEs also harbor genes that mediate resistance not only to antimicrobial agents of other classes, but also to metals and biocides. Direct selection pressure by the use of antimicrobial agents to which the oxazolidinone resistance genes confer resistance, but also indirect selection pressure by the use of antimicrobial agents, metals, or biocides (the respective resistance genes against which are colocated on cfr-, optrA-, or poxtA-carrying MGEs) may play a role in the coselection and persistence of oxazolidinone resistance genes.

**Tools and Techniques for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)/COVID-19 Detection**

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Summary: The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory disease coronavirus 2 (SARS-CoV-2), has led to millions of confirmed cases and deaths worldwide. Efficient diagnostic tools are in high demand, as rapid and large-scale testing plays a pivotal role in patient management and decelerating disease spread. This paper reviews current technologies used to detect SARS-CoV-2 in clinical laboratories as well as advances made for molecular, antigen-based, and immunological point-of-care testing, including recent developments in sensor and biosensor devices. The importance of the timing and type of specimen collection is discussed, along with factors such as disease prevalence, setting, and methods. Details of the mechanisms of action of the various methodologies are presented, along with their application span and known performance characteristics. Diagnostic imaging techniques and biomarkers are also covered, with an emphasis on their use for assessing COVID-19 disease or monitoring disease severity or complications. While the SARS-CoV-2 literature is rapidly evolving, this review highlights topics of interest that have occurred during the pandemic and the lessons learned throughout. Exploring a broad armamentarium of techniques for detecting SARS-CoV-2 will ensure continued diagnostic support for clinicians, public health, and infection prevention and control for this pandemic and provide advice for future pandemic preparedness.

**Laboratory Detection of Malaria Antigens: a Strong Tool for Malaria Research, Diagnosis, and Epidemiology**

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Summary: The identification and characterization of proteins produced during human infection with *Plasmodium* spp. have guided the malaria community in research, diagnosis, epidemiology, and other efforts. Recently developed methods for the detection of these proteins (antigens) in the laboratory have provided new types of data that can inform the evaluation of malaria diagnostics, epidemiological investigations, and overall malaria control strategies. Here, the focus is primarily on antigens that are currently known to be detectable in human specimens and on their impact on the understanding of malaria in human populations. We highlight historical and contemporary laboratory assays for malaria antigen detection, the concept of an antigen profile for a biospecimen, and ways in which binary results for a panel of antigens could be interpreted and utilized for different analyses. Particular emphasis is
given to the direct comparison of field-level malaria diagnostics and laboratory antigen detection for the development of an external evaluation scheme. The current limitations of laboratory antigen detection are considered, and the future of this developing field is discussed.

**Echinococcoses in Iran, Turkey, and Pakistan: Old Diseases in the New Millennium**

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Summary: Echinococcosis is considered a cosmopolitan zoonosis caused by different species of small taeniid tapeworms of the genus *Echinococcus* and is regarded as a neglected zoonosis. Cystic and alveolar echinococcosis are endemic diseases of Tibetan, Pamir, and Iranian plateaus. All of the countries within the Iranian plateau are affected by echinococcosis. Pakistan, Turkey, and Iran are the three most populous countries of the region, highly endemic for echinococcosis. The three neighboring countries share strong cultural and socioeconomic ties. The present study aimed to provide a broad review of the status of cystic and alveolar echinococcosis, summarizing the current knowledge about geographical distribution, molecular epidemiology, and transmission dynamics of *Echinococcus granulosus sensu lato* and *Echinococcus multilocularis* in this region. Additionally, we aimed to understand disease burden and risk factors as basic requirements for establishing a surveillance system and planning prevention and control programs. A considerable body of information is available on different aspects of echinococcosis in this region; however, several information and research gaps need to be filled before planning control programs. None of the countries in the region have an elaborate echinococcosis control program. Effective control programs require multi/intersectoral coordination within a one-health approach with a long-term political and administrative commitment and enhanced international collaboration among the three countries.

**Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis**

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Summary: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), a rapidly evolving pandemic worldwide with at least 68 million COVID-19-positive cases and a mortality rate of about 2.2%, as of 10 December 2020. In about 20% of COVID-19 instances, the disease exhibits moderate to severe symptoms. Severe COVID-19 manifests as acute respiratory distress syndrome (ARDS) with elevated plasma proinflammatory cytokines, including interleukin 1β (IL-1β), IL-6, tumor necrosis factor α (TNF-α), C-X-C motif chemokine ligand 10 (CXCL10/IP10), macrophage inflammatory protein 1 alpha (MIP-1α), and chemokine (C-C motif) ligand 2 (CCL2), with low levels of interferon type I (IFN-I) in the early stage and elevated levels of IFN-I during the advanced stage of COVID-19. Most of the severe and critically ill COVID-19 patients have had preexisting comorbidities, including hypertension, diabetes, cardiovascular diseases, and respiratory diseases. These conditions are known to perturb the levels of cytokines, chemokines, and angiotensin-converting enzyme 2 (ACE2), an essential receptor involved in SARS-CoV-2 entry into the host cells. ACE2 downregulation during SARS-CoV-2 infection activates the angiotensin II/angiotensin receptor (AT1R)-mediated hypercytokinemia and hyperinflammatory syndrome. However, several SARS-CoV-2 proteins, including open reading frame 3b (ORF3b), ORF6, ORF7, ORF8, and the nucleocapsid (N) protein, can inhibit IFN type I and II (IFN-I and -II) production. Thus, hyperinflammation, in combination with the lack of IFN responses
against SARS-CoV-2 early on during infection, make the patients succumb rapidly to COVID-19. Therefore, therapeutic approaches involving anti-cytokine/anti-cytokine-signaling and IFN therapy would favor the disease prognosis in COVID-19. This review describes critical host and viral factors underpinning the inflammatory “cytokine storm” induction and IFN antagonism during COVID-19 pathogenesis. Therapeutic approaches to reduce hyperinflammation and their limitations are also discussed.