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SUPPLEMENT ARTICLE

# Pathology and Telepathology Methods in the Child Health and Mortality Prevention Surveillance Network

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This manuscript describes the Child Health and Mortality Prevention Surveillance (CHAMPS) network approach to pathologic evaluation of minimally invasive tissue sampling (MITS) specimens, including guidelines for histopathologic examination and further diagnostics with special stains, immunohistochemistry, and molecular testing, as performed at the CHAMPS Central Pathology Laboratory (CPL) at the Centers for Disease Control and Prevention, as well as techniques for virtual discussion of these cases (telepathology) with CHAMPS surveillance locations. Based on review of MITS from the early phase of CHAMPS, the CPL has developed standardized histopathology-based algorithms for achieving diagnoses from MITS and telepathology procedures in conjunction with the CHAMPS sites, with the use of whole slide scanners and digital image archives, for maximizing concurrence and knowledge sharing between site and CPL pathologists. These algorithms and procedures, along with lessons learned from initial implementation of these approaches, guide pathologists at the CPL and CHAMPS sites through standardized diagnostics of MITS cases, and allow for productive, real-time case discussions and consultations.

**Keywords.** CHAMPS; pathology; telepathology MITS; child mortality.

Though the under-5 mortality rate has decreased globally, the gap in child mortality rates between high-income and low-income countries remains large. In 2017, the under-5 mortality rate in low-income countries was 69 deaths per 1000 live births, and neonatal deaths accounted for 46% of under-5 deaths in 2016 [1]. Laboratory testing is critical in diagnosing infectious disease during investigation of an infant or child death. Traditionally, etiologic agents of fatal infectious diseases were diagnosed by autopsy in conjunction with microbial culture of tissues, when performed. However, hospital autopsy rates are in decline [2], and the sensitivity and specificity of microbiological methods such as culture may be affected by antemortem treatments or postmortem contamination. As a result, the ability to detect and correctly identify the cause(s) of fatal pediatric infections has been reduced; clinical diagnostic errors might be missed, opportunities to improve medical treatment might be lost, and healthcare statistics might be biased. Deaths associated with nonspecific signs and symptoms, and those occurring in the community, are the most challenging and are common among pediatric deaths. Knowledge about the cause of death and etiology-specific contributions is important for selecting strategies to further reduce infant mortality [3].

The continuing development of innovative diagnostic techniques has been essential to infectious disease diagnoses and for determining causes of death; however, there are substantial disparities between antemortem and postmortem diagnoses [4]. At the same time, clinical autopsy rates, including perinatal autopsies, have declined for multiple reasons, including low number and time constraints of pathologists, less promotion by physicians, and refusal by bereaved families [5]. Nonetheless, pathologic evaluation is critical to accurately determine the cause of an individual's death, and also for detection, surveillance, and research of emerging and reemerging pathogens. To help bridge this gap, minimally invasive tissue sampling (MITS) methods are being developed as a potential substitute for conventional autopsies [4, 6, 7]. The MITS technique is implemented by the Child Health and Mortality Prevention Surveillance (CHAMPS) project, and represents the first use of MITS for the purpose of longitudinal mortality surveillance in a large number of case patients without comparison of MITS with traditional autopsy specimens [4, 8–11]. CHAMPS is also unique in that it employs the use of many different laboratories and pathologists from vastly different

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locations, and with different backgrounds and infrastructure, with the goals of determining causes of death and building pathology capacity in each country associated with the project. Given these complexities, it was essential to develop standardized methods for histopathologic evaluation and further testing of MITS, and for achieving concordance and knowledge sharing between sites through telepathology.

## HISTOPATHOLOGICAL EVALUATION OF MITS: DEVELOPMENT OF STANDARDIZED CHECKLISTS

Given the multicentric nature of the CHAMPS study and the variability in the associated pathologists' backgrounds, along with the potential variety of pathogenic processes to be encountered, it was critical to develop a standardized checklist of potential histopathologic findings for each tissue type to ensure thorough, systematic, and comparable review of all cases. These checklists were developed jointly by the CHAMPS Central Pathology Laboratory (CPL) and partners in conjunction with CHAMPS sites, and incorporate information obtained from histopathologic features commonly observed in a pilot study. Sample checklists for liver, lung, and brain are provided in Supplementary Figures 1A–C. For each, there is a list of detailed tissue-specific findings and a separate list of tissue-specific overall diagnoses to be evaluated by the pathologist. Importantly, checklists also include measures of overall sample quality, including technical adequacy of target tissue collection, indicated by number of cores or core fragments of target tissue present (Figure 1A and 1B). Other measures of sample quality include tissue autolysis, postmortem bacterial overgrowth, presence of pertinent tissue components (eg, meninges in brain specimens), and contamination (Figure 1C–F). Spaces are also available to describe incidental tissues present and to document testing to be completed in each case.

## ALGORITHMS FOR INFECTIOUS DISEASE TESTING OF MITS

Different infectious agents can cause characteristic but nonspecific inflammatory responses in tissues, which depend on (1)

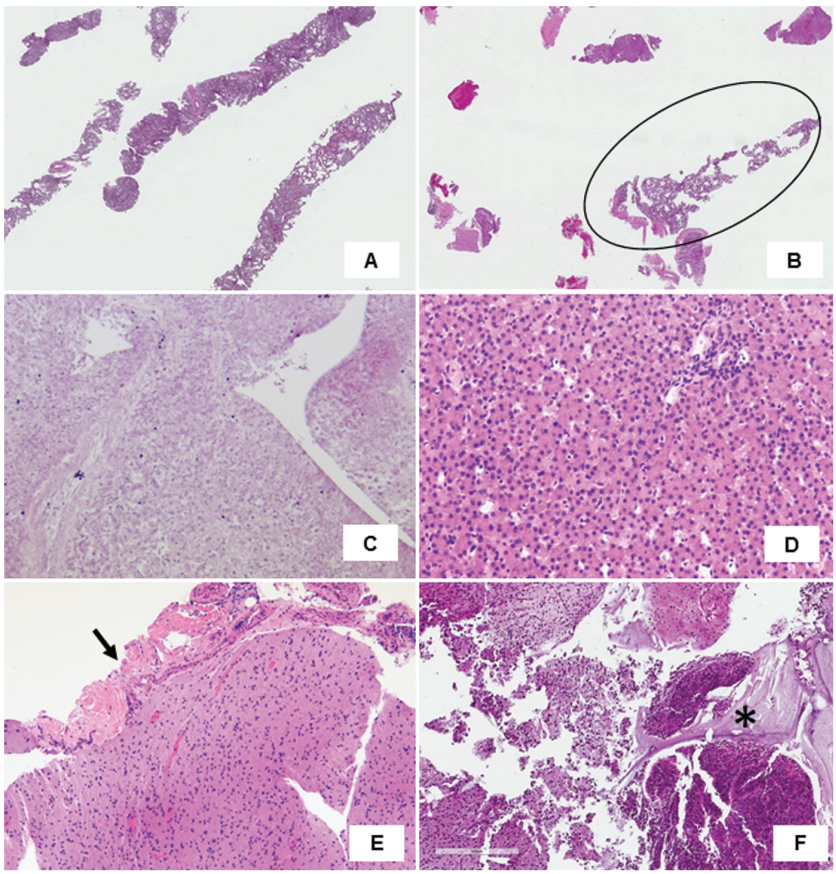


**Figure 1.** Examples of technical challenges often encountered in the microscopic examination of minimally invasive tissue sampling (MITS) cases. *A*, Lung sample showing 5 core fragments, which is considered adequate for evaluation. An "adequate" sample is defined as when at least 2 full cores worth of target tissue are present. *B*, Lung sample showing 1 small fragment of lung (circle) with no significant findings and fragments of skeletal muscle, which is an inadequate sample (<1 core fragment). An "inadequate" sample is defined as either no target tissue present, or <2 cores of target tissue present with no significant findings. *C* and *D*, Liver histopathology shows autolysis (*C*) in macerated fetus and normal liver (*D*) in early neonate for comparison. *E*, Brain tissue from central nervous system posterior sampling showing meninges (arrow), which are important for evaluating brain MITS for infectious processes; *F*, Brain tissue with nasal content (*) introduced during sampling. Nasal content includes mucous, neutrophilic inflammation, and yeasts.

the anatomy of the affected organ and route of pathogen entry; (2) virulence factors associated with the pathogen (ability to gain access to host, biologic products that damage host cells); and (3) host response (intrinsic host defenses and innate immunity, adaptive immunity and immunocompetence, genetic predisposing factors). Conversely, a single microorganism can elicit a variety of different patterns of inflammation (eg, pneumococcal pneumonia vs pneumococcal sepsis). Furthermore, other laboratory methods, such as culture and multiplex polymerase chain reaction (PCR) (TaqMan Array Card [TAC] in the case of CHAMPS; see Diaz et al in this supplement]) performed on autopsy specimens, can yield multiple possible causative agents for consideration or exclusion as a cause of the observed histopathologic changes seen. To clarify laboratory testing results, demonstration of a microorganism by special stains, immunohistochemistry (IHC), and/or PCR within lesions observed in the tissues provides support or confirmation of causation, while lack of detection by these methods may suggest absence of the agent or low sensitivity of the assay in formalin-fixed, paraffin-embedded (FFPE) tissue, detection of commensals or contaminants, or sampling discrepancy. Given the potential wide variety of pathogens on the list of differential diagnoses in CHAMPS MITS specimens, algorithms for infectious disease testing were developed using a syndromic approach, in an attempt to incorporate and standardize the use of all the various testing modalities employed in this project. These algorithms were based on histopathologic patterns of disease most commonly encountered in a CHAMPS pilot study: bronchopneumonia, interstitial pneumonia, meningitis, sepsis, and aspiration in stillbirth or early neonate deaths (Figures 2–6). More than 1 algorithm may be applicable in CHAMPS cases (eg, pneumonia and sepsis). In general, algorithms start with histopathologic

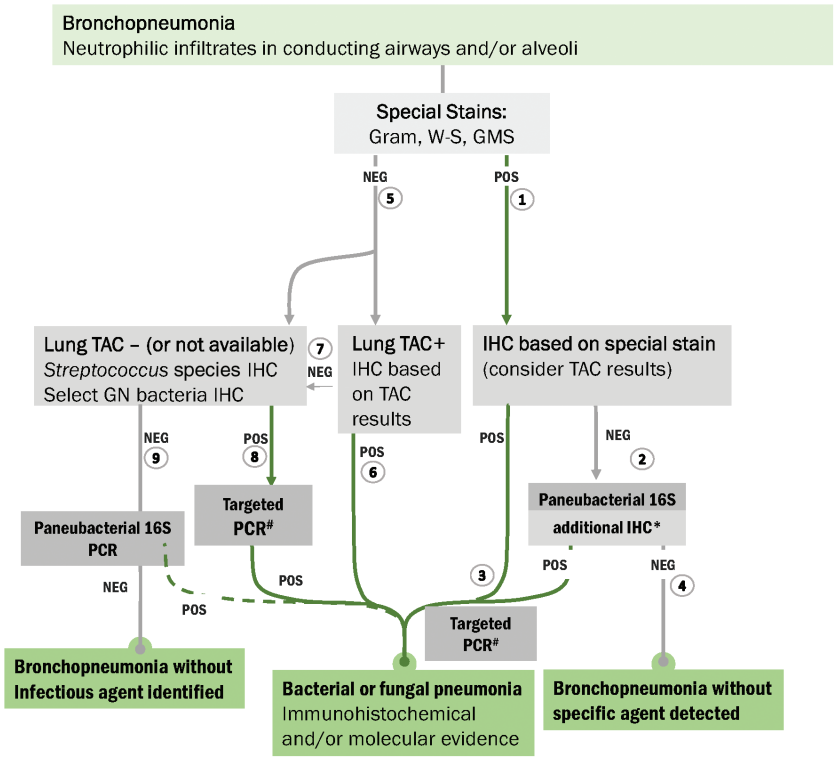


**Figure 2.** Algorithm for evaluation of bronchopneumonia in minimally invasive tissue samples from children aged <5 years. Bronchopneumonia is characterized by neutrophilic infiltrates within air spaces and prompts performing special stains (Gram, Warthin-Starry [W-S], Grocott methenamine silver [GMS]) to evaluate for bacteria or, less commonly, other agents such as fungi. If special stains are positive (POS) (right side of algorithm) (1), immunohistochemistry (IHC) is performed for relevant agents with compatible morphology and special stain characteristics seen. (Agents detected on lung TaqMan Array Card [TAC] with compatible features seen by special stains should be included in IHC testing.) If these special stains and TAC-guided IHC are negative (2), paneubacterial 16S polymerase chain reaction (PCR) with sequencing and additional IHC testing (*) to include *Streptococcus* species and select gram-negative (GN) bacteria are performed on the formalin-fixed, paraffin-embedded (FFPE) tissue. (Select GN bacterial IHC assay uses an antibody raised against *Klebsiella pneumoniae* but is also known to detect *Escherichia coli*, *Haemophilus influenzae*, and *Pseudomonas* species, among others. Immunostaining with this antibody is interpreted in conjunction with results of other tests [ie, TAC, FFPE-based PCR, cultures] to identify a specific agent.) At either point when positive IHC results are obtained (3), targeted PCR is performed for further identification when available, and a final diagnosis of agent-specific pneumonia is made when possible. If an etiologic agent is not identified by IHC or PCR testing (4), a diagnosis of "bronchopneumonia with no agent detected" is made. If special stains are negative (NEG) (left side of algorithm) (5), IHC is performed for any potentially relevant agents detected in lung TAC; positive IHC results (6) confirm agent-specific pneumonia. If no agents are detected on lung TAC or TAC results are not available, or if IHC for agents detected in lung TAC is negative (7), then screening by IHC for *Streptococcus* species and select GN bacteria is performed. If positive (8), targeted PCR is performed for further identification and a diagnosis of agent-specific pneumonia is made. If *Streptococcus* species and select gram-negative bacterial IHC is negative (9), then paneubacterial 16S PCR is performed as needed to attempt bacterial identification. #Targeted PCR is performed when available.

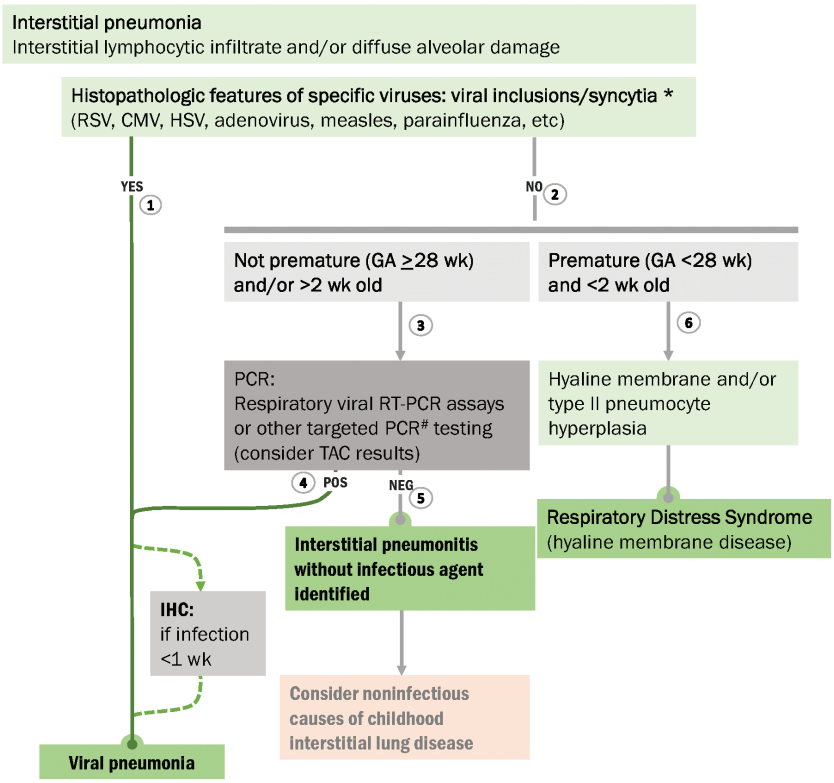


**Figure 3.** Algorithm for evaluation of infectious etiology in lungs with interstitial pneumonia in minimally invasive tissue samples from children aged <5 years. Interstitial pneumonia is characterized by lymphocytic interstitial lymphocytes and/or diffuse alveolar damage (hyaline membranes, type II pneumocyte hyperplasia) and prompts evaluation for primarily viral etiologies in infants >2 weeks. (*Some causes of bacterial sepsis, particularly *Neisseria meningitidis*, should also be considered in cases of lymphocytic interstitial pneumonitis.) If histopathologic features of specific viruses (eg, characteristic viral inclusions or syncytia) are seen (1), then a diagnosis of agent-specific viral pneumonia is made. If clinical history indicates duration of illness <1 week, immunohistochemistry (IHC) may be performed for further confirmation. If features of a specific virus are not seen (2), then age at death and gestational age (GA) at birth are critical to further interpretation of lung findings. If the infant was >2 weeks old at death, and GA at birth was >28 weeks (3), polymerase chain reaction (PCR) testing for respiratory viruses (influenza A/B, parainfluenza viruses 1–4, and respiratory syncytial virus) is performed on the formalin-fixed, paraffin-embedded tissue. Detection of other respiratory viruses in lung TaqMan Array Cards should prompt testing for those agents. Positive PCR leads to a diagnosis of agent-specific viral pneumonia (4), whereas negative PCR (5) results in a diagnosis of interstitial pneumonitis without infectious agent identified and consideration of noninfectious causes of childhood interstitial lung disease. If the infant was <2 weeks old at the time of death, and GA at birth was <28 weeks (6), the presence of hyaline membranes, with or without type II pneumocyte hyperplasia, is compatible with a diagnosis of infant respiratory distress syndrome (hyaline membrane disease). #Targeted PCR is performed when available. Abbreviations: CMV, cytomegalovirus; GA, gestational age; HSV, herpes simplex virus; IHC, immunohistochemistry; NEG, negative; PCR, polymerase chain reaction; POS, positive; RSV, respiratory syncytial virus; RT-PCR, reverse-transcription polymerase chain reaction; TAC, TaqMan Array Card.

findings compatible with a particular syndrome and testing proceeds from less specific methods (eg, special stains to identify presence of bacteria, fungi) to more agent-specific methods (ie, IHC for specific agents or groups of agents). Targeted (group- or pathogen-specific), conventional (with amplicon sequencing), or real-time PCR/reverse-transcription (RT) PCR assays are utilized for confirmation of specific microorganisms detected by other less specific methods or when sensitivity of IHC is known to be lower than PCR (eg, respiratory viral infections without viral cytopathic effect observed on hematoxylin and eosin [H&E]–stained slides), and paneubacterial 16S ribosomal RNA gene PCR, followed by Sanger sequencing, is utilized for tissues where a bacterial agent is highly suspected but not detected by other methods. In such cases, if an agent is detected by broad-range PCR, targeted IHC and/or PCR may then be performed; thus, there may be potential for multiple "feedback loops" within algorithms. Site-specific modifications to algorithms are also made when geographic trends in infectious disease are identified. For example, detection of a large number of nosocomial *Acinetobacter baumannii* infections at 1 CHAMPS site by tissue-based PCR led to addition of routine IHC screening for this agent in all cases of acute bronchopneumonia from that site.

#### Pneumonia: Bronchopneumonia and Interstitial Pneumonia

Pneumonia due to viral and/or bacterial agents is the single most common cause of death in children worldwide, is most prevalent in South Asia and sub-Saharan Africa [12], and is the most frequent histopathologic finding in CHAMPS cases to date (data not published). Identification of the specific agents associated with childhood pneumonias is important for public health surveillance and disease control, and for development of effective diagnostic, therapeutic, and prophylactic protocols

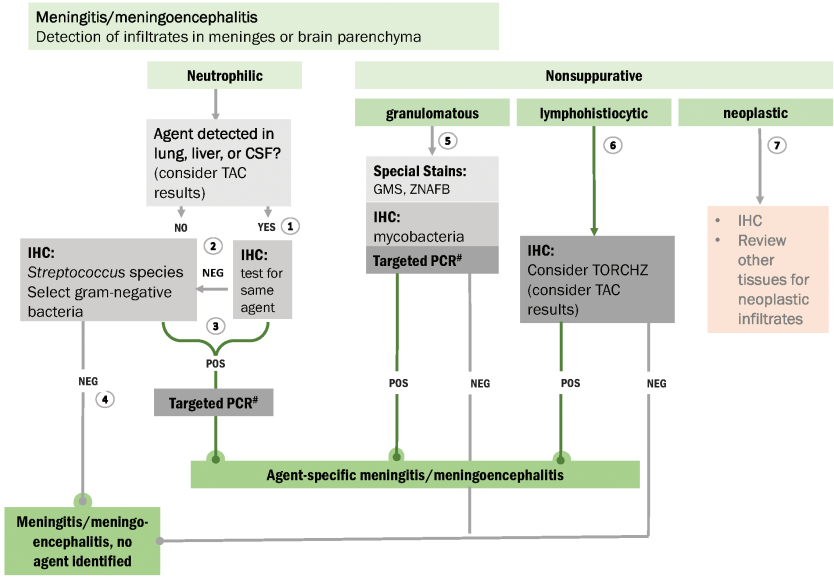


**Figure 4.** Algorithm for evaluation of brain samples with histopathologic diagnosis of meningitis or meningoencephalitis in minimally invasive tissue samples from children aged <5 years. Meningitis and meningoencephalitis are characterized by detection of cellular infiltrates in the meninges and brain parenchyma, and the character of the infiltrate directs diagnostic testing. Neutrophilic infiltrates prompt consideration of primarily bacterial etiologies, and initial immunohistochemical (IHC) testing should be performed for agents detected in other tissues (lung, liver, cerebrospinal fluid [CSF], blood) by IHC or TaqMan Array Card (TAC) (1). Strong consideration should be given to agents detected in CSF by TAC. If agents are not detected in other tissues or CSF TAC is negative or not available, or if agents detected in CSF TAC are negative by IHC (2), then screening by IHC for *Streptococcus* species and select gram-negative bacteria is performed. If at any point positive IHC results are obtained (3), targeted polymerase chain reaction (PCR) assays are performed on the formalin-fixed, paraffin-embedded tissue for further identification and a diagnosis of agent-specific meningitis/meningoencephalitis is made when possible. If an agent is not detected by IHC (4), the diagnosis is meningitis/meningoencephalitis, no agent identified. Nonsuppurative infiltrates warrant testing for other bacteria and nonbacterial agents, or consideration of neoplasia. Granulomatous infiltrates (5) direct to testing by special stains, IHC, and/or PCR for mycobacteria and fungi, whereas lymphoplasmacytic infiltrates (6) prompt more consideration of viral and protozoal agents (especially *Toxoplasma*, rubella, cytomegalovirus, herpesviruses, *Treponema*, and Zika). Cellular infiltrates with features of neoplasia (7) should be characterized by appropriate IHC if warranted and available, and other tissues reviewed for evidence of neoplasia. #Targeted PCR is performed when available. Abbreviations: CSF, cerebrospinal fluid; GMS, Grocott methenamine silver; IHC, immunohistochemistry; NEG, negative; PCR, polymerase chain reaction; POS, positive; TAC, TaqMan Array Card; TORCHZ, *Toxoplasma*, rubella, cytomegalovirus, herpesviruses, *Treponema*, and Zika; ZNAFB, Ziehl-Neelsen acid-fast bacilli stain.

[13–16]. Evaluation of postmortem lung tissue might confirm antemortem microbiological diagnoses, but more importantly, through demonstration of agents within lesions in the tissue, can help to distinguish causative agents from contaminants, false positives, or postmortem bacterial overgrowth, and can assess the relative contribution of different agents in cases of polymicrobial infection [17].

Bronchopneumonia is most commonly associated with bacterial etiologies, and the algorithm begins with identification of neutrophilic infiltrates in conducting airways (bronchi and bronchioles) and/or alveoli (Figure 2). If agents are not detected on TAC or Gram stain, IHC testing for *Streptococcus* species and select gram-negative bacteria (most pertinently *Klebsiella pneumoniae*) is performed to rule out these common causes of childhood pneumonia. The select gram-negative bacterial IHC assay uses an antibody raised against *K. pneumoniae* but is known to be broadly cross-reactive with other gram-negative bacteria of relevance to CHAMPS cases, including but not limited to *Escherichia coli*, *Pseudomonas* species, and *Haemophilus influenzae*. Of note, IHC may be more sensitive than Gram stain for bacterial detection in patients who received antibiotic treatment. If these agents are not detected by IHC, broad-range paneubacterial 16S PCR assays may be performed. IHC is performed in cases of noncontributory Gram stain results because previous antibiotic therapy can diminish or alter the Gram staining characteristics of bacteria.

The interstitial pneumonia algorithm begins with identification of a pulmonary interstitial lymphocytic infiltrate (Figure 3) and/or diffuse alveolar damage (evidenced by hyaline membranes and/or type II pneumocyte hyperplasia). Consideration of the child's age and gestational age at birth are critical when navigating this algorithm, as the noninfectious condition infant respiratory distress syndrome (IRDS; hyaline membrane disease), which is common in the first few weeks of life in premature infants with surfactant deficiency, can histomorphologically resemble an infectious pneumonia seen in older infants. After exclusion of IRDS, the interstitial pneumonia algorithm targets primarily viral agents. Histopathologic features of specific viruses (eg, syncytia and inclusions) direct to testing for these agents (Figure 7C); otherwise, histopathologic findings are nonspecific and so RT-PCR assays for some of the childhood respiratory viruses

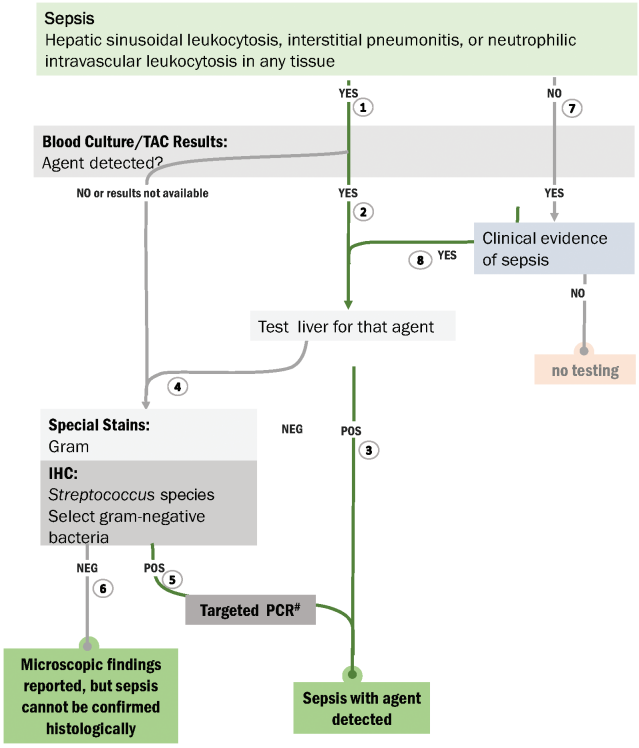


**Figure 5.** Algorithm for evaluation of tissue samples with histopathologic diagnosis of sepsis or clinical and laboratory suspicion of sepsis in minimally invasive tissue sampling (MITS) specimens from children aged <5 years. Histopathologic features of sepsis in MITS samples include hepatic sinusoidal leukocytosis, acute interstitial pneumonitis, or neutrophilic intravascular leukocytosis in any tissue. If these features are seen (left side of algorithm) (1), then immunohistochemical (IHC) testing should be performed on liver and other tissues with histopathologic features of sepsis for agent(s) detected on blood culture and/or TaqMan Array Card (TAC), if available (2). Positive (POS) IHC results confirm sepsis due to that agent (3). If blood culture and TAC results are negative (NEG) or not provided, or if IHC testing for agents detected by blood culture/TAC are negative (4), screening by Gram stain and IHC for *Streptococcus* species and select gram-negative bacteria are performed. If IHC is positive (5), targeted polymerase chain reaction (PCR) assays for identification of agent and/or further speciation are performed. If an agent is not confirmed by IHC (6), the diagnosis is limited to the histopathologic features observed (eg, hepatic sinusoidal leukocytosis, intravascular leukocytosis); a diagnosis of sepsis is not made unless bacteria are demonstrated by IHC. If histopathologic features of sepsis are not present in any tissue, but a relevant agent is detected on blood culture and/or TAC (7), correlation with clinical features is recommended and then testing of liver by IHC for that agent is performed only if the clinical history is compatible with known or suspected sepsis (8). #Targeted PCR is performed when available.

(influenza A/B, parainfluenza 1–4, and respiratory syncytial virus) are performed on RNA extracts from the FFPE tissues. Noninfectious causes of childhood interstitial lung disease must also be considered, though these diagnoses must take into account genetic, clinical, and other diagnostic information [18], which is often not available for CHAMPS cases.

#### Meningitis/Meningoencephalitis

Acute bacterial meningitis remains a significant cause of pediatric illness and death in low- and middle-income countries [19]. Detection of neutrophilic infiltrates in meninges or brain parenchyma is the most common histologic finding and directs testing toward primarily bacterial agents (Figure 4). Detection of bacteria in other tissues may help point to specific agents early in the workup. Nonsuppurative infiltrates warrant consideration of other bacteria and fungi, viral and protozoal agents, or neoplastic disease, depending on the character of the infiltrate.

#### Sepsis

Despite major advances in neonatal care and increasing research into neonatal sepsis, in developed countries, 40% of infants with sepsis die or experience major disability including significant permanent neurodevelopmental impairment [20, 21]. Prematurely born neonates experience the highest incidence of, and mortality from, sepsis among all age groups [20, 22, 23]. Histopathologic identification of hepatic sinusoidal leukocytosis, pulmonary interstitial pneumonitis, or neutrophilic intravascular leukocytosis in any tissue warrants testing for sepsis (Figure 5). Even in the absence of histopathologic features of sepsis, testing for agents detected by blood culture or TAC performed on fresh MITS specimens should be considered, especially in cases where another significant bacterial process (eg, pneumonia, meningitis) is detected or when clinical information strongly suggests sepsis. Demonstration of bacteria in sinusoids and/or Kupffer cells in the liver, or within vessels in any tissue, is sufficient for the histopathologic diagnosis of sepsis (Figure 7G), and testing may also be performed in other tissues with relevant histomorphologic features described above. When TAC, culture, or other tissue pathology does not aid in guiding testing for specific agents, liver is tested by IHC for *Streptococcus* species and select gram-negative bacteria in cases of highly suspected sepsis. Targeted PCR testing follows if positive results are obtained.

#### Aspiration in Stillbirths and Early Neonate Deaths

Stillbirth and neonatal death can be difficult to understand and prevent due to the complex relationship between biological, social, and healthcare factors. Currently, two-thirds of deaths among infants occur within the first month of life (neonatal mortality), and around 50% of all deaths in the first year happen during the first week of life (early neonatal mortality) [24]. Aspiration of amniotic fluid indicates intrauterine fetal distress (IUFD) and can be a major contributing factor to stillbirth and neonatal mortality (Figure 6). It is evidenced histopathologically by detection of squames and/or meconium in the fetal or neonatal lung, and can be associated with infectious (eg, chorioamnionitis) or noninfectious (eg, placental insufficiency) causes. While rare individual squames in the lung are considered an insignificant finding, the presence of at least moderate squames, or any amount of meconium, is pathologic and warrants a diagnosis of IUFD (Figure 7H). IUFD alone is often not associated with inflammation; therefore, if alveolar inflammation is seen, the algorithm directs

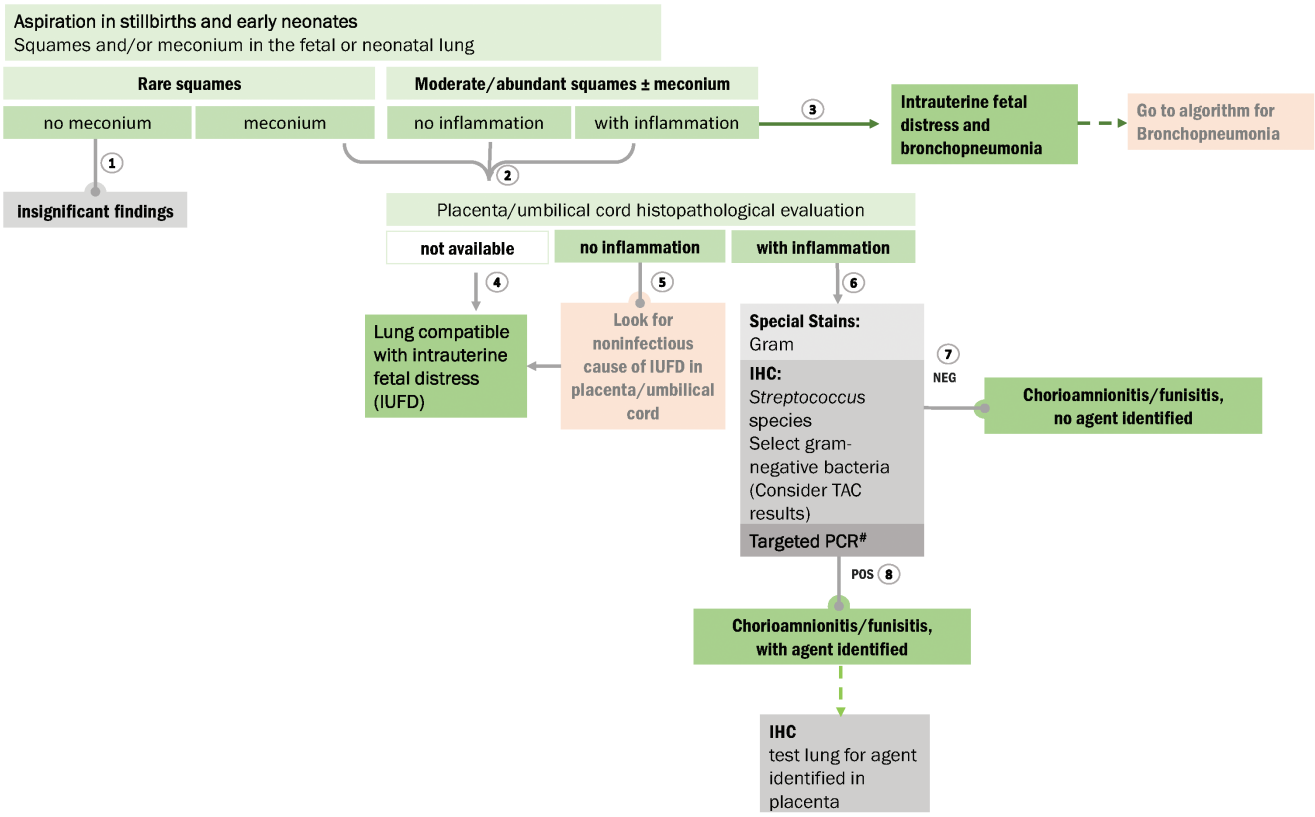


**Figure 6.** Algorithm for evaluation of aspiration in stillbirths or early neonates in minimally invasive tissue samples from the Child Health and Mortality Prevention Surveillance (CHAMPS) network from children aged <5 years. The presence of squames and/or meconium in the lungs of stillbirths or neonates indicates in utero amniotic fluid aspiration and warrants special consideration of potential causes, including placental evaluation for evidence of in utero infection or placental abnormalities. The presence of only rare squames without meconium is considered an insignificant finding (1), whereas the presence of moderate or abundant squames, and any amount of meconium, indicates intrauterine fetal distress (IUFD) and prompts evaluation of placental and umbilical cord tissues (2). If neutrophilic inflammation accompanies squames/meconium in the lung (3), the bronchopneumonia algorithm (Figure 2) should also be initiated to investigate for infectious etiologies. If placenta and umbilical cord are not available (4), then the diagnosis is limited to IUFD. If placenta and umbilical cord have no inflammation (5), they are evaluated for potential noninfectious causes of IUFD (eg, placental infarct). If these tissues do have inflammation (6), evaluation for infectious agents by special stains and immunohistochemistry including screening for *Streptococcus* species and select gram-negative bacteria is performed. Agents detected on lung, blood, or cerebrospinal fluid TaqMan Array Card should be prioritized for testing in the placenta. If agents are not identified (7), a diagnosis of chorioamnionitis/funisitis, no agent identified is made. If an agent is identified (8), the agent associated with chorioamnionitis/funisitis should also be tested for in the lung tissue. #Targeted PCR is performed when available. Abbreviations: IHC, immunohistochemistry; IUFD, intrauterine fetal distress; NEG, negative; PCR, polymerase chain reaction; POS, positive; TAC, TaqMan Array Card.

toward workup to exclude an infectious etiology (ie, bronchopneumonia algorithm). Also imperatively, evidence of IUFD or bronchopneumonia in the fetal or neonatal lung directs to examination of the placenta, fetal membranes, and umbilical cord for evaluation of chorioamnionitis or funisitis as the cause of ascending, in utero infection.

## ACHIEVING A DIAGNOSIS FROM MITS: CORRELATION OF HISTOPATHOLOGY, LABORATORY TESTING, AND CLINICAL HISTORY

The end product of the algorithmic histopathologic examination and laboratory testing of MITS cases is the CPL final pathology report, which incorporates macroscopic and microscopic descriptions of MITS specimens, results of special stains, IHC stains, molecular tests, final diagnoses, and a comment on clinicopathologic correlation to assist the Determining the Cause of Death (DeCoDe) panel in interpreting the significance of overall findings (see Blau et al in this supplement). In parallel, a separate final report is generated by the site pathologist detailing microscopic findings and limited ancillary testing (ie, special stains) performed on MITS specimens processed on site. The pathologists must consider the types and severity of histopathologic findings, together with the type(s) of agents detected, in order to speculate on their relative contributions to the death. The clinical history, including signs and symptoms, immunostatus (eg, human immunodeficiency virus infection), treatments provided, and maternal history and gestational age, is crucial to interpretation of pathologic findings.

## TELEPATHOLOGY: VIRTUAL DISCUSSIONS TO ACHIEVE CONSENSUS DIAGNOSES, TRAINING, AND QUALITY ASSURANCE

Telepathology is an emerging field utilizing digital communication technology and whole slide imaging (WSI) to transmit

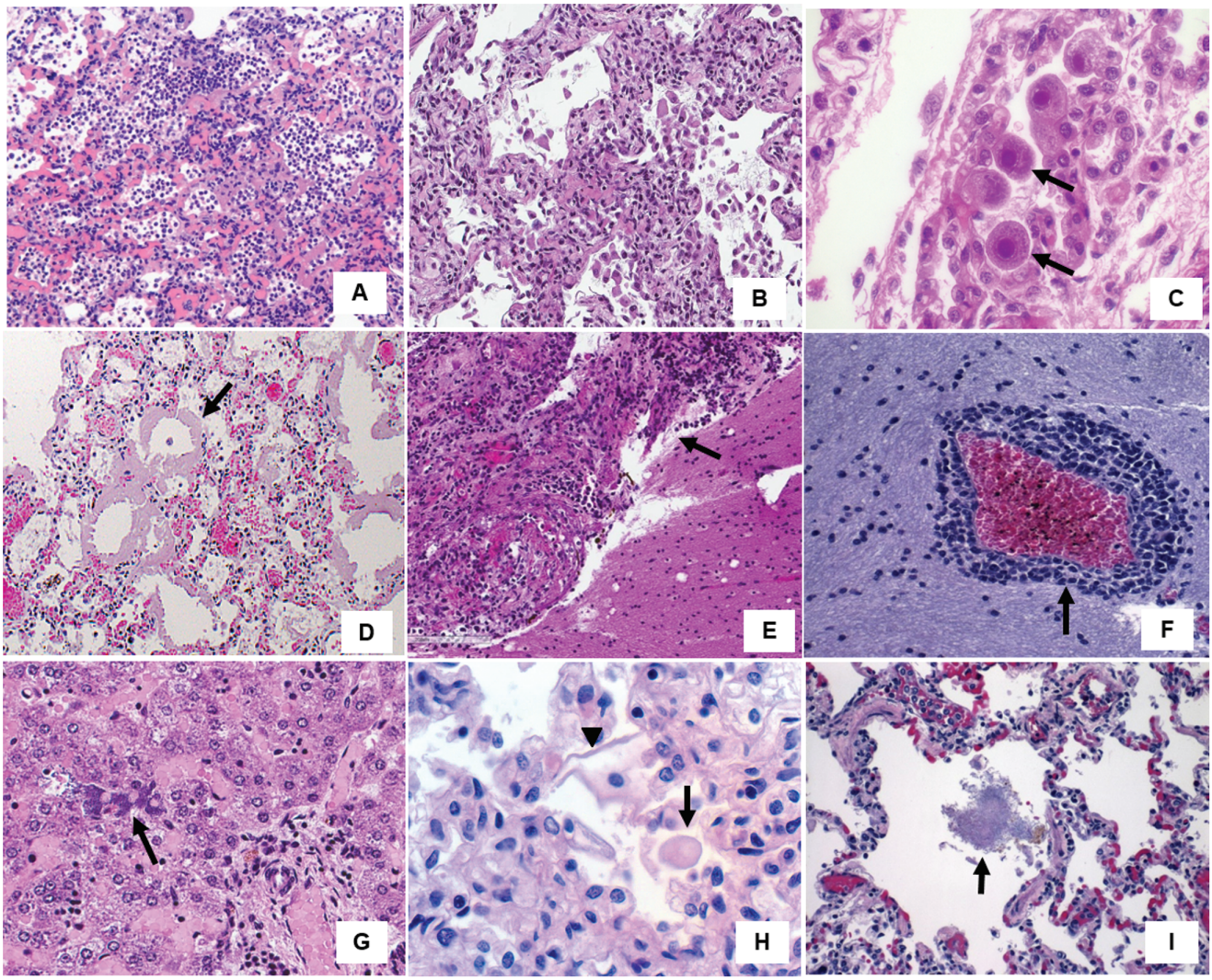


**Figure 7.** Common histologic patterns seen in minimally invasive tissue samples. *A*, Lung tissue shows alveolar spaces with neutrophilic inflammation (bronchopneumonia). *B*, Lung tissue shows expansion of alveolar septa by inflammatory infiltrates (interstitial pneumonitis). *C*, Lung tissue shows alveolar space with pneumocytes containing viral inclusions (cytomegalovirus infection; arrows indicate infected cells). *D*, Lung tissue shows hyaline membranes (arrow) lining alveoli in infant respiratory distress syndrome (hyaline membrane disease). *E*, Brain tissue shows neutrophilic meningitis (arrow). *F*, Brain tissue shows perivascular infiltrate of neoplastic lymphocytes in a child with lymphoma/leukemia (arrow). *G*, Liver tissue shows moderate extramedullary hematopoiesis and small clusters of bacteria in sinusoids (arrow). *H*, Lung tissue shows intra-alveolar squames (arrowhead) and meconium ball (arrow) in intrauterine fetal distress. *I*, Lung tissue shows intra-alveolar bacterial colonies (arrow) without inflammation compatible with aspiration.

digital versions of glass slides for long-distance, real-time discussions and consultations for diagnosis, second opinion, or educational purposes [25–27]. WSI, captured on slide scanners, can be stored, organized, annotated, and transmitted digitally, even to remote areas. The contribution of telepathology is especially relevant in remote locations that lack specialists and/or access to advanced testing methods (eg, special stains, IHC) [28], and provides opportunities for pathologists to participate in collaborative review of cases and research over distances. While several studies have demonstrated the utility of telepathology using WSI as an effective diagnostic modality in surgical pathology, mostly for neoplastic diseases, only rare studies have evaluated its utility for infectious disease diagnoses [27, 29–32]. CHAMPS is unique in that it is the largest prospective multicenter surveillance study utilizing MITS, and the first to implement telepathology primarily for identification of infectious causes of death.

The CHAMPS network is building an internet-based telepathology platform to connect pathologists from each of the sites and expert consultants from the CPL, to facilitate joint review of cases to reach consensus diagnoses. Within CPL, glass slides of MITS cases, including H&Es, special stains, and IHC, are scanned primarily using a large capacity scanner (Leica Aperio AT2) and the associated scanner acquisition software. The resulting WSI is transferred to the CHAMPS servers, where image viewing and annotation of significant findings occurs

**Table 1. Summary of Telepathology Case Selection and Consultation Process in the Child Health and Mortality Prevention Surveillance Network**

| Telepathology case selection and consultation process |
|---|
| • 1-hour session scheduled monthly between site and CPL. |
| • CPL identifies cases for which both site and CPL histopathology reports are available. |
| • CPL compares site and CPL reports for major discrepancies or important diagnoses and learning opportunities. Sites are also invited to suggest cases for discussion. Up to 10 cases are chosen for review at each session. |
| • CPL notifies sites of cases slated for telepathology session. |
| • Both CPL and sites upload scanned slides (hematoxylin and eosin, special stains, immunohistochemistry) into CHAMPS central server and annotate significant histopathologic findings. CPL and sites have access to both sets of slides and can review and annotate each other's slides to indicate areas for discussion. |
| • Telepathology session is conducted through online video conferencing with screen sharing for simultaneous viewing of whole slide images by sites and CPL. CPL provides clinical history and laboratory data for each case, then site and CPL findings are discussed in real time while viewing the slides. Discussion points and consensus diagnoses are documented in the telepathology report form during the session. |
| • CPL generates the final telepathology report and uploads to CHAMPS portal for inclusion in DeCoDe packet. |

Abbreviations: CHAMPS, Child Health and Mortality Prevention Surveillance; CPL, Central Pathology Laboratory; DeCoDe, Determination of Cause of Death panel.

(Supplementary Figure 2). All CHAMPS sites are equipped with a single slide scanner (NanoZoomer-SQ Digital slide scanner C131140-01- 40× mode-Hamamatsu) that generates WSI that can be viewed at magnifications up to 40×. At each site, a CHAMPS study technician is trained to scan the slides and transfer to the digital management system (Hamamatsu), where the program office information technology specialist uploads the WSI, provides the specific identification, and manages the image access for the project participants.

After training the pathologists on how to access and annotate digital slides in the CHAMPS central server, telepathology sessions between site and CPL pathologists were initiated. Since September 2017, the CPL has conducted approximately monthly sessions with each of the 4 sites. The primary goal at each session is to discuss discrepancies between site and CPL findings and reach a consensus diagnosis for each case by reviewing the digital images associated with cases of interest (Table 1). It is important to note that sites and CPL receive separate MITS specimens from each case, and discrepancies between tissue findings may therefore be real, due to variability in presence of lesions among the specimens. The goal of telepathology is therefore overall consensus diagnoses for the case as a whole, not necessarily consensus between site and CPL findings for each tissue type. The case discussions and consensus diagnoses are documented in a telepathology report that is made available to the DeCoDe panel to assist in interpreting overall pathologic findings. Through these case discussions, pathologists are simultaneously trained on assessment of tissue section adequacy and quality, interpretation of histopathologic findings, utilization of diagnostic algorithms, and standardization of microscopic diagnoses. Due to the high volume of CHAMPS cases, only a subset are chosen for telepathology review, with an emphasis on those with highly discrepant site/CPL results or those that showcase important diagnoses or learning opportunities. An overview of the telepathology case selection and consultation process is provided in Table 1, and a sample telepathology report is shown in Supplementary Figure 3.

The value of telepathology in CHAMPS is not limited to achieving consensus diagnoses; it is also vital to the CHAMPS goal of increasing local pathology capacity and expertise at sites. Scanned slides are evaluated for tissue adequacy (eg, amount of target tissue obtained, autolysis) and slide preparation quality (eg, thickness, tissue folds, staining quality, coverslipping) at the same time as evaluation for pathologic processes. While initial training activities incorporated targeted quality assessments of histology procedures by review of slides through telepathology (see Rakislova et al in this supplement), this ongoing quality assessment will be critical throughout the duration of CHAMPS, especially as increased capacity is transferred from the CPL to the sites.

In addition to technical quality assessment, telepathology facilitates training of pathologists in identification of pathologic processes in tissues. The telepathology platform allows for both real-time instruction during telepathology sessions, and for historical review of annotated case materials for future and repeated reference. To this end, the CPL is in the process of developing a more formal training module that will contain annotated slides with examples of characteristic histopathologic patterns of disease, along with explanation of ancillary testing and interpretation of results. This module will be available within CHAMPS and will be an invaluable tool for pathologists and trainees.

## DISCUSSION

While a full postmortem examination provides the greatest ability to determine the mechanism and/or cause of death, the use of MITS provides a significant opportunity to obtain similar information in cases where a complete diagnostic autopsy is not possible. Histopathologic evaluation of MITS and demonstration of infectious agents directly associated with tissue pathology, together with clinical information, is crucial to interpreting significance of findings. Testing (TAC, culture) of unfixed MITS specimens is helpful for guiding histopathologic testing, but can also result in extraneous results due to detection of commensals or contaminants that are noncontributory toward mortality. As such, the development of testing algorithms for common histologic findings in CHAMPS cases has been necessary for consistent, thorough evaluation of MITS specimens. It is important to note that these algorithms do not encompass every possible infectious etiology associated with

a particular histopathologic pattern; they represent general guidelines that have been found to be effective and efficient for identifying infections within the context of the CHAMPS study thus far. Furthermore, there are important histopathologic findings that are not incorporated into the algorithms, as they alone warrant consideration of specific infectious agents (eg, granulomas in any tissue warrants testing for mycobacterial and fungal agents). The algorithmic approach is modified/expanded on a case-by-case basis, taking into consideration factors such as clinical history, results of other laboratory testing, and geographic trends in infectious diseases. The algorithms have evolved as we have gained a greater understanding of the causes of childhood mortality and how to best diagnose them utilizing our available testing modalities, and they will no doubt continue to change as we further this endeavor.

While able to provide valuable information regarding causes of death, utilization of MITS specimens presents a number of technical challenges, the first of which is obtaining an adequate target tissue sample. For CHAMPS, MITS procedures are performed in low-resource settings and within limited time constraints, and are therefore done without imaging assistance to ensure targeted tissue/lesion collection. In some instances, especially stillbirths or small neonates, a sufficient amount of target tissue may not be obtained. Even if the target tissue is obtained, focal or small lesions may be missed (eg, focal lung abscess), and samples may therefore not be representative of pathologic processes present. Contamination of MITS by unintentionally collected tissues, such as gastrointestinal tissues in liver samples or nasal contents in transnasal brain samples, may introduce bacterial contaminants that complicate interpretation of ancillary tests. Furthermore, the MITS procedure does not target some organs that may be most relevant to determining cause of death (eg, structural cardiac abnormalities, gastrointestinal disease).

When target tissue is obtained, additional factors may contribute to a suboptimal sampling. Some examples include autolysis (especially in macerated fetuses), postmortem bacterial overgrowth, absence of pertinent anatomical components such as meninges in brain specimens or conducting airways in lung, and iatrogenic atelectasis in lung specimens. The small size of MITS specimens also sometimes results in tissue depletion when multiple IHC or PCR assays are needed. This can be especially challenging in cases where clinical history or fresh tissue test results are not available to help narrow testing early in the evaluation of a case.

Finally, achieving consistent diagnoses across pathologists and CHAMPS sites requires standardization of interpretation of histopathologic findings, with careful consideration of all test results available. TAC and IHC results are initially considered together to determine which of the identified pathogens, if any, are most likely contributing to the histopathologic changes seen, and ultimately to the cause of death. TAC may produce both pertinent and extraneous results and IHC assays are frequently broadly cross-reactive; therefore, considering both TAC and IHC together yields stronger confidence to diagnosis or exclusion of specific pathogens. Targeted PCR with sequencing on FFPE tissues can further confirm or help to exclude pathogens detected by TAC/IHC, and paneubacterial 16S PCR is valuable as a screening tool in cases with high suspicion for infection when other, less sensitive methods do not yield pathogens. Standardization not only of the diagnostic workup for cases, but also of the interpretation of potentially complex aggregate results, is challenging but crucial to accurately identifying infectious causes of death.

**Table 2. Telepathology Challenges**

| Challenges | Examples |
|---|---|
| Technical | Setup and maintenance of scanners and servers |
| | Digital file management |
| | Secure file sharing |
| | Internet connections |
| | Pathologist proficiency in viewing digital slides |
| | Quality of scanned slides/pixilation |
| Logistical | Time delay for scanning digital slides and annotation |
| | Coordination of schedules in multiple time zones for slide review and telepathology sessions |
| Interpersonal | Language barriers |
| | Working as a group to establish rapport between Central Pathology Laboratory and sites |

Telepathology facilitates standardization of diagnoses and training of pathologists and allows for continuous quality assessment during transfer of pathology capacity from CPL to sites. Though telepathology is an invaluable asset that enables virtual consultations, it is not without challenges, including those that are technical, logistical, and interpersonal, as briefly summarized in Table 2. Ultimately, CHAMPS and the telepathology platform will facilitate the development of a worldwide, collaborative network of pathologists with expertise in utilizing MITS for identifying causes of childhood mortality, and will lead to the implementation of mortality-reducing measures in CHAMPS, and potentially other, sites.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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