



## ORIGINAL RESEARCH

## An Accuracy-Based Approach to the Microbiologic Diagnosis of Pulmonary Infection: Part III

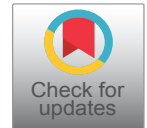
John Ferguson, MD, MPH<sup>1</sup>, Cyprien Jungels, DO<sup>2</sup> and Michael Gailey, DO<sup>3</sup>

<sup>1</sup>Rocky Mountain Pulmonary and Critical Care, 3555 Lutheran Pkwy, Suite 150, Wheat Ridge, CO, USA

<sup>2</sup>Rocky Vista University, 8401 S Chambers Rd, Parker, CO, USA

<sup>3</sup>Vista Pathology Laboratory, 1032 E Jackson St, Medford, OR, USA

\*Corresponding author: John Ferguson, MD, Rocky Mountain Pulmonary and Critical Care, 3555 Lutheran Pkwy, Suite 150, Wheat Ridge, CO 80033, USA



### Abstract

**Introduction:** Similar to that of bacterial infection as described in Part 1 and fungal infection described in Part 2, the performance of diagnostic tests for viral pneumonia and parasitic pneumonia are not well described.

**Methods:** We undertook a literature search to assess the accuracy of diagnostic tests for pneumonia, identified through a search of MEDLINE-indexed journals. Sensitivity and specificity of diagnostic tests for pneumonia were calculated with respect to various reference standards.

**Results:** A battery of diagnostic testing is adequate to rule out most pathogens leading to viral pneumonia, lymphatic filariasis, and *Toxoplasma*. Testing is inadequate to exclude, and empiric treatment should be considered, for clinical suspicion of *Hantavirus*, *Herpes Simplex Virus*, *Strongyloides*, Roundworm, Hookworm, *Paragonimus*, and *Toxocara*.

**Conclusion:** Most viral pathogens may be excluded using a combination of viral PCR and serology. In contrast, the presence of parasitic pathogens is difficult to exclude by current diagnostic testing. Clinical judgment is necessary in ruling out these causes of pneumonia.

### Keywords

Pneumonia, Diagnosis, Accuracy

### Introduction

Increased use of immunosuppressive medications, along with improved longevity of patients with oncologic diseases have led to increased complexity in diagnosing opportunistic infection of the lung. The

use of multiplex PCR has increased viral detection, but gaps in the diagnosis of viral infection remain. In regions that are not endemic to parasitic infection, the yield of diagnostic testing is likewise uncertain.

In Part 3 of this review, we will assess the literature and discuss the accuracy of diagnostic tests for infectious pneumonia caused by both viral and parasitic pathogens.

### Viral Pneumonia

#### Influenza

*Influenza* virus is a single-stranded negative-sense RNA virus in the family *Orthomyxoviridae* consisting of two common subtypes, A and B.

The sensitivity of cell culture for *Influenza A* (Flu A) and *Influenza B* (Flu B) among PCR-positive cases has been reported at 69% [1] and 89% [2], and 81% [1], respectively, and reduced to 73% [3] in cases diagnosed by either serology or PCR. Culture is not ordinarily recommended, however, as it provides only 1% [3] additional sensitivity to the combination of PCR and serology, and requires over 72 hours, limiting its utility.

Rapid antigen diagnostic tests (RIDTs) give results in minutes and are generally less expensive than PCR, although they are significantly less sensitive. The pooled sensitivity of traditional RIDTs has been reported at 62% [4] of cases positive by either PCR or culture, with a specificity of 98% [4]. The pooled sensitivities of the more

recently developed digital immunoassay (DIA) rapid testing for FluA and FluB have been reported slightly higher at 80% [5] and 77% [5], respectively. Although rapid antigen tests are commercially available, with only moderate sensitivity they are not recommended when PCR is available.

A serologic diagnosis of *Influenza* can be made, but like culture, is less accurate than PCR. ELISA IgM sensitivity has been reported in 84% [3] of cases detected by either PCR or culture. A fourfold titer rise of EIA has been demonstrated in only 47% [6] of PCR-positive cases, but has been reported to provide an additional sensitivity of up to 34% [6] to that of PCR, and may be complementary. Similarly, among PCR-positive cases, IF has been reported positive in 71% [7], with a specificity of 94% [7], but IF requires serial evaluation to demonstrate a diagnostic fourfold titer rise. The time required to make a serologic diagnosis limits its utility, although the reduced specificity of serology illustrates the many PCR-negative cases. Both ELISA and CF are commercially available, but are not generally recommended for diagnosis.

PCR can be used to rapidly detect the *Influenza* virus. The pooled sensitivity of rapid NAAT for FluA and FluB have been reported 92% [5] and 95% [5], respectively, with a specificity of 98% [5]. As previously noted, there are cases that PCR fails to detect, but the sensitivity of PCR in cases detected by either culture or serology has been reported at 92% [3] and 98% [8], with a specificity of 84% [3] and 98% [8]. Viral mPCR PPA with Flu PCR has been reported at 90% [9], 93% [10], 95% [11], and 100% [12,13] with a NPA of 100% [9,11-13]. PCR remains the diagnostic test of choice for Flu due to its excellent diagnostic accuracy, but either empiric treatment or further diagnosis with culture and serology should be considered if there is ongoing clinical suspicion and the need for a diagnosis.

### Parainfluenza virus

*Parainfluenza virus* (PIV) is a single-stranded negative-sense RNA virus in the family *Paramyxoviridae* made up of subtypes 1-4.

Cell culture sensitivity has been reported at 11% [14], 36% [15], 63% [16], and 67% [5] of PCR-positive cases, with a specificity of 100% [16]. However, as with Flu, cell culture takes time while adding very little sensitivity to PCR alone [14-16], and is therefore generally not recommended.

The sensitivity of PIV antigen detection by IF has been reported at 50% [17] and 53% [16] of PCR-positive cases, but as is true with culture, adds minimal additional sensitivity to PCR alone [16,17]. Likewise, conventional fluorescent antibody (FA) fails to add cases to those detected by PCR, with a sensitivity reported at 33% [18] and 66% [19], a rate which remains consistently

low when separated into subtypes PIV1, PIV2, and PIV3 reported at 11% [20], 36% [20], and 69% [20], respectively. Although commercially available, antigen tests are not recommended due to inferior sensitivity.

A serologic diagnosis by EIA has been reported at 13% [19] and 49% [21] of PCR-positive cases, with a specificity of 98% [21], but has been reported to add an additional 33% [21] and 53% [22] to the sensitivity of PCR alone, and should be considered in PCR-negative instances where there is a high clinical suspicion. EIA serology is not commercially available, however. The sensitivity of CF has been reported at only 64% [23] that of ELISA serology. Although CF is commercially available, it is not recommended due to the poor accuracy.

Nasopharyngeal PCR sensitivity has been reported at 100% [13] of DFA-positive cases and 98% [24] and 100% [16] of cases found positive by either PCR or IF, with a specificity of 98% [16]. Viral mPCR PPA with PIV PCR has been reported at 92% [9], 95% [16], 98% [12], and 100% [11,13], with a NPA approaching 100% [9,11-13,25]. PCR is the diagnostic test of choice for PIV due to its excellent accuracy, but a negative should still prompt consideration of serology or culture.

### Respiratory syncytial virus

*Respiratory Syncytial Virus* (RSV) is a single-stranded, negative-sense RNA virus in the family *Paramyxoviridae*, consisting of subtypes A and B.

Unlike Flu and PIV, cell culture is excellent for RSV having been reported positive in 88% [26], 92% [27], and 100% [5] of cases positive by antigen detection or PCR, with a specificity of 100% [26,28]. Like other viruses, however, the time and skill required to culture RSV and the excellent sensitivity of PCR limits its utility of cell culture for this purpose.

RSV DFA sensitivity has been reported pooled at 83% [29] and 87% [22] of culture-positive cases, 92% [28] of cases positive by either culture or antigen detection, and 70% [17], 90% [30], and 94% [20] of PCR-positive cases, with a specificity of 96% [28] and 98% [30]. Although DFA may be positive in culture-negative RSV infection, it adds less than an additional 1% sensitivity to PCR [17,20]. The pooled sensitivity of ELISA antigen detection has been reported at 83% [22] of culture-positive cases. The pooled sensitivity of the rapid RSV immunoassay antigen test for PCR-positive cases has been reported at 74% [29] and 75% [27], with a specificity of 99% [27]. Immunoassay and DFA antigen tests are commercially available, but add little value over PCR and are generally only recommended only when PCR is unavailable.

Serologic methods can be used to supplement culture and PCR. A fourfold EIA IgG titer rise has been reported in 12% [19] and 70% [21] of PCR-positive cases and 85% [31] of cases positive by either PCR or culture, with a specificity of 97% [31]. EIA has been reported to

provide an additional sensitivity of 11% [21], 26% [19], and 27% [32] to PCR and 26% [31] to the combination of culture and PCR, but is unfortunately not commercially available. CF has approximately half the sensitivity of EIA [32] and is not known to add sensitivity to the combination of EIA and PCR. Therefore, CF is not recommended [33].

PCR can be rapidly performed for a diagnosis of RSV infection. The sensitivity of nasopharyngeal PCR is excellent, having been reported at 91% [13] of DFA-positive cases, 94% [34] and 100% [35] of culture-positive cases, and 98% [36] of cases positive by either IF or culture, with a specificity of 100% [34]. Nasal swabs are less sensitive than nasopharyngeal aspirate, limiting their utility, and in the setting of a negative nasal swab, bronchoscopy should be considered. Viral mPCR PPA with RSV PCR has been reported at 93% [9] and 99% [11,12], with a NPA of 98% [12] and 100% [9,11]. PCR accuracy is excellent and remains the diagnostic test of choice, but as with Flu and PIV, PCR may not detect certain cases and if negative should be supplemented with serology or culture.

### Human metapneumovirus

*Human metapneumovirus* (HMPV) is a negative-sense RNA virus in the family *Paramyxoviridae*.

Culture is challenging and the cytopathologic effects can require more than two weeks to occur [37], making it of limited utility. Culture sensitivity among PCR-positive cases has been reported at 32% [38], 68% [39], and 100% [9], and adds minimal sensitivity to PCR alone. Similar to most other cases of respiratory viruses, cell culture is not recommended.

IF sensitivity has been reported at 73% [40] and 85% [38] of PCR-positive cases, with a specificity of 97% [40]. Similarly, among PCR-positive cases, DFA sensitivity has been reported at 50% [41], 63% [42], and 74% [43], with a specificity of 94% [43] and 100% [42]. DFA is commercially available, but generally not recommended due to the superior accuracy of PCR.

The sensitivity of serologic detection by EIA has been reported at 33% [9], 60% [21], and 78% [44] of PCR-positive cases, with a specificity of 98% [21], but EIA can provide an additional 20% [21] and 46% [9] sensitivity to PCR alone. Serology should be considered in PCR-negative cases, although not widely commercially available.

The pooled sensitivity of PCR has been reported at 91% [12] of cases detected by either DFA or culture, with a specificity of 100% [12]. Among DFA-positive cases only, the sensitivity has been reported at 67% [45] and 95% [42], and among culture-positive cases has been reported at 80% [46] and 100% [5,35], with a specificity of 97% [45] and 100% [35,46]. mPCR PPA with HMPV PCR has been reported at 93% [9] and 98% [11,12] with

a NPA of 98% [47] and 100% [9,11,12]. The reduced sensitivity against a DFA or culture reference standard indicates that multiple modalities may be necessary to confidently rule out infection.

### Human enterovirus

Human Enteroviruses (EVs) are positive-sense RNA viruses in the family *Picornaviridae* that can lead to either upper or lower respiratory tract infection.

The sensitivity of cell culture has been reported at 28% [48] of seropositive cases, at 33% [49], 35% [50], and 60% [51] of PCR-positive cases, and at 61% [52] of those positive by either PCR or NASBA, with a specificity of 95% [51] and 99% [49,52]. Culture may be positive in PCR-negative infection, but also requires several days to perform, limiting its utility.

A serological diagnosis can also be obtained for EV infection, but also with less sensitivity than culture or PCR. IgM neutralizing antibody sensitivity has been reported at 29% [48] of culture-positive cases, with a specificity of 78% [48]. The sensitivity of ELISA IgA, IgG, or IgM has been reported at 73% [53] of PCR-positive cases. The accuracy of CF is uncertain. Serology is not commonly utilized as it does not always provide additional sensitivity to PCR, but CF is nevertheless widely commercially available and may be considered in PCR-negative cases.

Nasopharyngeal PCR has been reported positive in 84% [49], 86% [51], and 100% [50] of culture-positive cases, and 88% [52] of cases positive by either culture, PCR, or NASBA, with a specificity of 93% [49] and 95% [52]. mPCR cannot distinguish Enterovirus from Rhinovirus, but does have a PPA with EV PCR of 95% [9] and 98% [11,12], with a NPA of 94% [12], 96% [9], and 100% [9]. PCR is the test of choice in the diagnosis of Enterovirus, but can be supplemented with serology in PCR-negative cases where clinical suspicion is high.

NASBA sensitivity has been reported at 91% [52] of PCR-positive cases, with a specificity of 92% [52], but is also infrequently used and is not commercially available.

### Human rhinovirus

Human Rhinovirus (RV) is a positive-sense RNA virus in the family *Picornaviridae* consisting of greater than 100 serotypes that presents with symptoms similar to EV.

Cell culture sensitivity has been reported at 11% [49], 30% [53,54], 31% [55], 56% [56], and 65% [57] of PCR-positive cases, with a specificity approaching 100% [49,53,55]. Culture may be positive in PCR-negative cases, however, and so may be considered when PCR is negative [58].

Serology for RV has been performed, but is infrequently used due to poor sensitivity. CF sensitivity has been reported at only 17% [59] of PCR-positive

cases. In addition to its limited diagnostic value, serology is not commercially available.

Nasopharyngeal PCR sensitivity has been reported at 80% [56], 95% [57], 97% [54,60], and 100% [49,53] of culture-positive cases, with a specificity of 59% [49], 64% [56], 84% [54], and 93% [55], lowered by both cross-reaction with Enterovirus [51] and asymptomatic shedding [48]. Additionally, nasal swabs may be inferior to nasopharyngeal aspirate, with a reported sensitivity of 74% [61] of cases detected by nasopharyngeal aspirate. As previously noted, mPCR does not distinguish Enterovirus from Rhinovirus, but has a PPA with RV reported at 95% [9] and 98% [11,12], with a NPA of 94% [12], 96% [9], and 100% [11]. PCR is highly accurate and is the test of choice in diagnosing Rhinovirus, however culture should be considered for PCR-negative cases.

### Adenovirus

*Adenovirus* (AdV) is a double-stranded DNA virus of the family *Adenoviridae* with the most commonly implicated serotypes 1 through 5, 7, 14, 19, and 37 [25].

Cell culture sensitivity has been reported at 26% [62] and 100% [63] of PCR-positive cases and 40% [62] of those detected by IF, with a specificity of 88% [62].

Fluorescent antigen detection sensitivity has been reported in only 24% [20] and 26% [62] of PCR-positive cases, and 36% [62], 68% [64], and 87% [65] of culture-positive cases, with a specificity of 80% [62]. Immunofluorescence adds only 2% sensitivity to PCR [20] and is generally not recommended. LFIA techniques, however, have improved sensitivity to a reported 55% [66], 92% to 97% [63], and 97% [67] of culture-positive cases, and 97% to 100% [63] of PCR-positive cases, with a specificity of 99% [66]. AdV DFA is commercially available, but is not recommended whenever PCR is available.

Serologic detection by EIA has been reported at 3% [9] and 23% [21] of PCR-positive cases and 44% [64] of culture-positive cases, but requires serial titers to demonstrate a fourfold rise, limiting its utility. Although serology fails to provide additional sensitivity to culture [68], the addition of serology to PCR has been reported to provide an additional 3% [9] and 24% [21] sensitivity and should be considered if culture is not obtained. EIA serology is not widely commercially available. CF is even less sensitive than EIA, and is not recommended [69].

The sensitivity of AdV PCR in nasopharyngeal specimens has been reported 61% [64], 76% [70], 87% [71], 91% [62], 97% [63], and 100% [71] of culture-positive cases, 100% [17] of antigen-positive cases, and 100% [72] of cases positive by either AdV antigen or culture, with a specificity of 20% [62], 78% [73], and 99% [17,53]. Nasal PCR has been reported positive in only 44% [61] of cases positive by a nasopharyngeal aspirate, and a negative nasal swab should prompt consideration

of bronchoscopy, nasal wash, or serology. PCR may be negative in culture-positive cases, and a negative PCR should also prompt consideration of cell culture or serology. mPCR PPA with AdV PCR has been reported positive at 89% [9], 95% [12] and 100% [11,73], with a NPA of 96% [73], 97% [12], and 99% [9,11]. As with many viruses, PCR is the first line diagnostic test but can be supplemented with culture if suspicion is high. In the absence of culture availability, serology should be considered.

### Coronavirus (CoV: 229E, HKU1, NL63, OC43, SARS-CoV, SARS-CoV-2)

*Coronaviruses* (CoVs) are single-stranded RNA viruses within the family *Coronaviridae*. The most commonly recognized strains include NL63, OC43, 229E, HK1, Severe Acute Respiratory Distress (SARS-CoV and SARS-CoV-2), and Middle Eastern Respiratory Syndrome (MERS).

Cell culture sensitivity has been reported at 41% [74] and 50% [75] of PCR-positive cases. Culture provides additional sensitivity to the use of PCR alone, but like culture for most viral infections, requires time and skill and should only be considered when PCR fails to detect the virus.

BALF DFA sensitivity has been reported at 80% [76] of PCR-positive cases, but is not recommended due to the superior sensitivity of PCR. Additionally, it is by no means certain that it provides additional sensitivity to PCR.

Among cases of SARS-CoV-2 PCR-positive infection, chemiluminescent immunoassay (CLIA) IgM or IgG pooled sensitivity has been reported at 98% [77], with a specificity of 97% to 98% [77]. ELISA IgM or IgG pooled sensitivity has been reported at 84% [77] of PCR-positive cases, with a specificity of 98% [77]. LFIA IgM or IgG pooled sensitivity has been reported at 66% [77] of PCR-positive cases, with a specificity of 97% [77]. Among PCR-positive cases, the pooled sensitivity of either CLIA or ELISA has been reported at 85% [78,79], with a specificity of 92% [79] and 99% [78]. CLIA serology can provide additional cases to PCR and should be considered when PCR is negative.

Among cases of SARS-CoV-1 infection, loop-mediated amplification (LAMP) sensitivity has been reported at 71% [80] of cases detected by either serology or PCR, but it is not widely commercially available.

Nasopharyngeal PCR sensitivity for 229E has been reported at 50% [81] of culture-positive cases and 62% [76] of cases positive by either culture or serology. PCR for OC43 has been reported at 40% [81] of culture-positive cases and 100% [76] of cases positive by either culture or serology. PCR for HK1 has been reported at 100% [76] of cases positive by either culture or serology. PCR for NL63 has also been reported at 100%

[76] of cases detected by either culture or serology. The sensitivity of PCR for these four strains combined has been reported at 87% [76] of cases positive by either culture or serology. The sensitivity of PCR for SARS-CoV-1 has been reported at 78% [80] of serologically-detected cases, while PCR sensitivity for MERS-CoV has been reported at 100% [82] of culture-positive cases, with a specificity of 100% [82], but evaluated in only a small sample. SARS-CoV-2 nasal PCR sensitivity has been reported at 73% [80] of cases positive by either culture or serology. The accuracy can vary by the source of collection with sputum, saliva, and nasopharyngeal aspirate exhibiting a pooled sensitivity of 97% [79], 62% [79], and 73% [79], respectively. mPCR PPA for 229E PCR has been reported at 92% [12] and 100% [11], with an NPA of 99% [11] and 100% [12]. OC43 PPA has been reported at 81% [12] and 97% [11], with an NPA approaching 100% [11,12]. NL63 PPA has been reported at 95% [11] and 100% [12] with a NPA of 99% [11,12]. HKU1 PPA has been reported at 93% [11] and 100% [12], with a NPA of 99% [12] and 100% [11]. PCR is the diagnostic test of choice, although cell culture and serology may detect additional cases.

### Human bocavirus

*Human Bocavirus* (HBoV) is a DNA virus in the *Parvoviridae* family, consisting of HBoV-1 and HBoV-2.

HBoV has not yet been isolated in cell culture and thus the sensitivity of viral culture is currently unknown.

EIA serology sensitivity has been reported at 59% [83], 70% [84], 77% [85], 82% [86] of nasopharyngeal PCR-positive cases, and 92% [84] of serum PCR-positive cases, with a specificity of 99% [84] and 100% [86]. Serology for HBoV is not yet widely commercially available.

HBoV-1 antigen sensitivity has been reported at 76% [87] of PCR-positive cases, with a specificity of 100% [87]. Antigen testing is also not commercially available, but also would not appear to add any increased sensitivity over PCR.

The sensitivity of nasopharyngeal PCR has been reported at 75% [88], 88% [83], and 100% [84,86,88] of serology-positive cases. The sensitivity of nasal swab PCR has been reported at only 50% [61] of those found positive by nasopharyngeal aspirate. Accordingly, a negative nasal swab should prompt consideration of bronchoscopy or nasal wash. Specificity has been reported at 78% [86] and 96% [88], and is higher for mRNA than DNA. Like serology, PCR is not yet commercially available.

### Cytomegalovirus

*Cytomegalovirus* (CMV), or *Human herpesvirus-5*, is a DNA virus in the family *Herpesviridae*. Risk factors for CMV pneumonia include immunocompromising

conditions such as AIDS, stem-cell transplantation, and the use of immunosuppressive medications [89].

BALF culture sensitivity has been reported at 60% [90] of clinically diagnosed cases, which may be misleadingly low as cases of CMV diagnosed clinically often have alternative diagnoses, but nevertheless has also been reported at 69% [90], 86% [91] and 89% [92] of those either clinically diagnosed or identified through immunohistochemical detection. BALF culture sensitivity has been reported positive in 96% [93] and 100% [94] of cases confirmed by tissue culture and histopathology, with a specificity of 45% [94], 95% [91], 97% [90], and 100% [93]. The specificity of the combination of culture and immunostaining has been reported up to 100% [91], making this virtually diagnostic of infection. Culture is of questionable utility however, as the virus may require up to four weeks to grow and has both limited sensitivity and specificity, is often detected in asymptomatic patients, and is not routinely utilized. CMV blood culture sensitivity has been reported at only 25% [95] of those with antigenemia, and 38% [96] of those with PCR-positive infection, severely limiting its yield.

BALF cytology sensitivity has been reported at 29% [93] of those positive by either histopathology or tissue culture and at 56% [92] of cases with immunohistochemical detection, with a specificity of 100% [93]. Among clinically diagnosed cases, transbronchial biopsies demonstrate typical features in 93% [90]. Biopsies may display cytopathic changes in asymptomatic patients, possibly indicating early infection, but should be performed when there is clinical suspicion of CMV disease.

BALF fluorescent antibody staining sensitivity has been reported at 59% [93] and 89% [97] of histopathology-proven cases, and 100% [91] of cases detected by immunohistochemistry, with a specificity of 95% [91], 99% [95], and 100% [93]. The combination of staining and culture has been reported to increase the sensitivity to 100% [92], although specificity is reduced. BALF DFA sensitivity has been reported at 78% [98] of culture-positive cases, with a specificity of 100% [98].

The sensitivity of the CMV pp65 antigen has been reported at 85% [99] of those with histopathologic confirmation, and pooled at 65% [100] of PCR-positive cases, with a specificity of 94% [100] and 100% [99]. The pp65 antigen has been reported to add up to an additional pooled 12% [100] sensitivity to the use of PCR alone. Therefore, PCR and pp65 antigen testing should ideally be used concurrently, although the pp65 antigen is not widely commercially available.

EIA IgG serology sensitivity has been reported at 30% [101] of PCR-positive cases, but has poor specificity due to cross-reaction with *Epstein-Barr Virus* and *Human herpesvirus-6*. IgM sensitivity has been reported as

low as 0% [101], but when positive, can persist for months. Passive acquisition of IgG to CMV can also occur in transplant recipients. With poor sensitivity and specificity, serology has no clear role in diagnosis of acute CMV infection and is not recommended.

BALF CMV PCR sensitivity has been reported at 91% [102,103] of clinically diagnosed cases, and 86% [71], 98% [104], and 100% [91,94,104] of culture-positive cases. The specificity has been reported at 77% [103,105], 87% [105], 94% [91], 95% [72,102], 99% [99], and 100% [94]. Serum PCR sensitivity has been reported at only 50% [106] and 60% [94] of histopathology-proven infection, and thus a negative serum PCR should prompt consideration of bronchoscopy. BALF PCR has adequate sensitivity to rule out CMV infection, but due to poor specificity, histopathology and cytology should also be obtained when there is clinical suspicion of infection.

### Herpes simplex virus

Herpes simplex viruses (HSV-1, HSV-2) are members of the family *Herpesviridae*. Risk factors for HSV pneumonia include hematopoietic stem cell transplantation or HIV.

The sensitivity of a surface swab culture has been reported at 83% [107] of clinically diagnosed cases, meaning cases which generally present with a characteristic vesicular rash. The sensitivity is lower, however, having been reported at only 33% [108] of lung specimens obtained from autopsy-confirmed cases, and in surface swabs at 50% [109] of PCR-positive cases, with a specificity of 100% [109]. The sensitivity of BALF culture for HSV is unknown.

EIA serology sensitivity has been reported at 58% [110] among culture-positive cases and 94% to 100% [111] of those with a positive Western Blot, with a specificity of 68% to 85% [111]. Serology is not recommended in diagnosing acute HSV pneumonia due to a high prevalence of antibodies in the community.

DFA can be evaluated from body fluid, and is most commonly obtained from swabs from open vesicles. The sensitivity of DFA for HSV1 has been reported at 54% to 76% [112] and 76% for HSV1 [113] and 93% for HSV2 [112,113] of culture-positive cases, with a specificity of 99% [113]. DFA fails to add a meaningful number of additional positive cases to culture and is thus not recommended. The accuracy of DFA in BALF specimens is uncertain and so is also not recommended.

Cytologic identification of inclusion bodies has been reported in 55% [114] of PCR-positive cases. Cytology and tissue histopathology have been reported to identify CMV in 68% [110] and 80% [108] of culture-positive cases, respectively.

PCR sensitivity in vesicular lesions has been reported in 86% [109] of patients with viremia, 97% [112] of patients positive by antigen detection or viral culture,

and 83% [108] of clinically diagnosed cases, with a specificity of 93% [112], 96% [72], and 100% [109]. HSV PCR is commercially available and can supplement culture, although has not been extensively studied in BALF.

### Varicella-zoster virus (VZV)

*Varicella-Zoster Virus (VZV)* is a member of the family *Herpesviridae*, which may lead to pneumonia in immunocompromised patients. Very little data is available regarding the diagnosis of Varicella pneumonia, and the diagnosis is generally made from the characteristic skin rash.

The sensitivity of culture for vesicular lesions has been reported at only 44% [107] of clinically diagnosed cases and 56% [115] of DFA-positive cases, with a specificity of 97% [115]. Culture may be performed on BALF, although like HSV it has been poorly studied and the sensitivity of respiratory secretion culture is unknown. Presumably its sensitivity will be less than that of vesicular lesion culture.

DFA sensitivity for vesicular lesions has been reported at 92% [115] of culture-positive cases, with a specificity of 80% [115]. DFA can therefore detect additional cases to the poorly sensitive culture but has not yet been well studied in BALF and so has uncertain utility.

Serological methods may be utilized, but only with limited accuracy. ELISA IgM sensitivity has been reported at 25% [116] of PCR-positive cases. Like other diagnostic tests for VZV, serology has not been well-studied in cases of pneumonia. Both EIA and CLIA serology are commercially available, but there as yet is no clear role for serology in the diagnosis of VZV pneumonia.

VZV PCR sensitivity of vesicular lesions has been reported at 98% [115] of cases positive by either culture or DFA and at 97% [107] of clinically diagnosed cases, with a specificity of 91% [115]. BALF PCR sensitivity similarly has been reported at 96% [117] of clinically diagnosed cases. Despite limited data on BALF, PCR appears to be the diagnostic test of choice.

### Hantavirus

Hantaviruses are single-stranded, negative-sense RNA viruses of the family *Bunyaviridae*, consisting of over 20 different strains [118].

Culture is not generally performed due to the risk it presents to laboratory personnel, and moreover the sensitivity is uncertain.

ELISA IgM sensitivity has been reported at 73% [118] and 97% [119] of clinically diagnosed cases, and 100% [120] of PCR-positive cases, with a specificity of 100% [119]. PCR is more likely than ELISA to be positive in the first seven days, whereas ELISA is more likely to be positive thereafter. IF serology sensitivity has been reported at 40% [121] and 93% [122] of serologic cases

by ELISA, with a specificity approaching 100% [122]. ELISA serology is commercially available and should be used for the initial diagnosis of *Hantavirus*.

Serum PCR sensitivity has been reported at 33% [123], 50% [124], 93% [125], and 100% [120] of serology-positive cases by ELISA, and 85% [118] of clinically diagnosed cases, with specificity approaching 100% [125]. BALF PCR sensitivity has been reported at 88% [126] of seropositive cases. PCR is not commercially available, however, and is not certain to provide additional yield to serology.

## Measles

*Measles virus* is a negative-sense RNA virus in the family *Paramyxoviridae* that can lead to pneumonia, bronchiolitis, or bronchitis.

Among clinically diagnosed cases, the sensitivity of cell culture has been reported at 45% [127] and 54% [128,129], and among cases diagnosed by IF serology reported at 29% [130], 64% [129], 67% [127] and 89% [131]. Culture however, may require up to 10 days, severely limiting its utility [131].

Various serologic techniques can be used to diagnose *Measles*, including commercially available IF, ELISA, and CLIA. The sensitivity of IF has been reported at 34% [132] of PCR-positive cases, 46% [127] and 83% [130] of clinically diagnosed cases, and at 68% [127] and 89% [129] of culture-positive cases. IF and culture can be complementary as either may be individually positive. ELISA IgM sensitivity has been reported at 56% [133] of PCR-positive cases, and at 63% [134], 74% [135,136] 85% [137], and 98% to 100% [138] of clinically diagnosed cases. In the acute phase, IgM has been reported at 58% to 85% [138] of CF-positive cases, but in the convalescent phase has been reported to increase to 88% [136], 93% to 98% [138] and 97% [135], and may remain positive for over one month from the

onset of rash [136]. The specificity has been reported at 82% [136], and 87% to 100% [138]. CF sensitivity has been reported at 89% [135] of those found positive by ELISA IgM and similarly at 89% [130] of those positive by IF. CF is not commercially available, however, and also requires a longer processing time than ELISA, limiting its utility. Lastly, CLIA sensitivity has been reported at 97% [139] of cases diagnosed by ELISA IgM with a specificity of 93% [139]. Any one of the three available techniques (IF, ELISA, or CLIA) may be positive when used alone, and therefore either combining methods or using them sequentially can increase sensitivity.

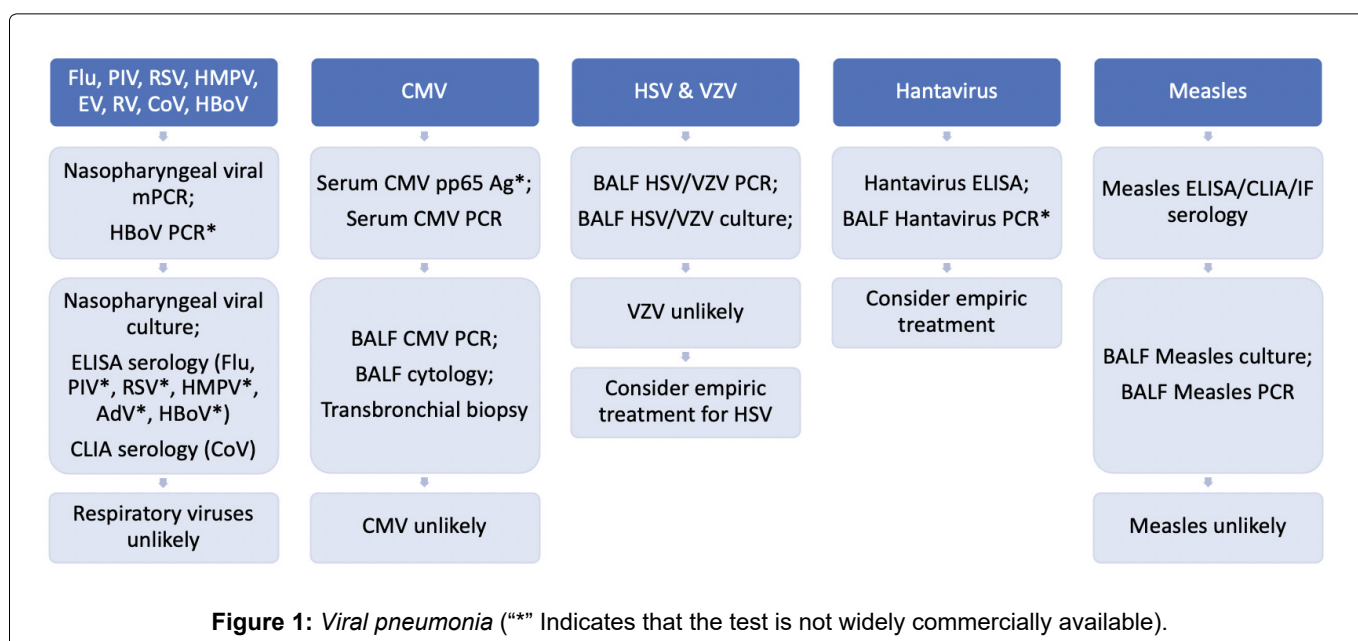
The sensitivity of PCR from respiratory secretions has been reported at 53% [140], 90% [133], and 98% [141] of seropositive cases by ELISA, 68% [133], 81% [142], and 96% [137] of clinically diagnosed cases, 85% [143] of culture-positive cases, and at 90% [143] of LAMP-positive cases. The sensitivity of PCR has been reported to be more than 50% higher [140] in the first two weeks of the rash than in the following two weeks and is thus the preferred test in the early stage of infection. PCR appears to have a higher sensitivity than serology in the acute phase, while serology sensitivity is superior in the convalescent phase. PCR has not been well evaluated in BALF, but can be performed in cases in which there is clinical suspicion.

LAMP sensitivity of respiratory secretions has been reported at 100% [143] of PCR-positive cases, and 95% [143] of culture-positive cases, but is not commercially available and also has uncertain utility (Figure 1).

## Parasitic Pneumonia

### Lymphatic filariasis (*Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, and others)

Lymphatic filariasis is a parasitic infection caused by nematodes such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* which are transmitted to



humans through a mosquito vector, leading to tropical pulmonary eosinophilia.

Successful detection of microfilaria (mf) by stool microscopy has been reported in 7% [144], 46% [145], 80% [146], and 85% [146] of cases with a positive serum circulating filarial antigen (CFA), but only 16% [147] of stool PCR-positive cases. The sensitivity of microscopic methods may vary, as thick smear detects 94% [146] of cases found by the micropore chamber method.

CFA cards can be used to detect the *Onchocerca gibsoni* circulating antigen (Og4C3) of *Wuchereria*. ELISA antigen sensitivity for *Wuchereria* has been reported at 95% [148-150], 98% [151], 99% [152], and 100% [145,146,153,154] of cases detected by either mf micropore or thick smear, with a specificity of 94% [153], 98% [151], and 100% [149,150], indicating the technique's ability to identify mf-negative cases. ELISA CFA sensitivity for *Brugia* is slightly less, having been reported at 75% [144] and 83% [149] of cases positive by microscopic blood smear. The CFA immunochromatographic test (ICT) sensitivity among serology-positive cases for *Wuchereria* has been reported at 90% [155], with a specificity approaching 100% [155]. The sensitivity, however, decreases to 60% [155] following the administration of drugs which are commonly used in endemic regions. In low-prevalence areas with cases which have been detected by the ELISA CFA, ICT sensitivity has been reported at only 36% [156]. Although it is not widely commercially available, ELISA CFA assay is recommended in conjunction with microscopy.

Serologic methods have been used to detect recombinant antigens such as Wb14 and WbT. ELISA sensitivity for *Wuchereria* has been reported at 75% [140] of clinically diagnosed cases, 90% [157] and 93% [157,158] of microscopically detected cases, and at 95% [159] of CFA-positive cases, with a specificity of 51% [159], 70% [159], 96% [158], 97% [157], and 100% [157]. Luciferase Immunoprecipitation System (LIPS) sensitivity for *Wuchereria* has been reported at 98% [160] of mf-detected cases, with a specificity of 100% [160]. ELISA IgG4 serology is commercially available, and may be complementary to microscopy and CFA. Positive serology may however, indicate past rather than active infection and should be interpreted with caution.

Serum PCR sensitivity for *Wuchereria* has been reported at 94% [143] of clinically diagnosed cases and 100% [147,161] of mf-positive cases, with a specificity of 100% [161]. The sensitivity of serum PCR for *Brugia* is uncertain, however the specificity approaches 100% [161] of microscopically detected cases. Neither *Wuchereria* nor *Brugia* PCR is commercially available and both are uncertain to add any additional sensitivity over CFA. Furthermore, the accuracy of PCR on BALF has not yet been thoroughly evaluated.

## ***Strongyloides stercoralis***

*Strongyloides stercoralis* is a nematode transmitted directly to humans through soil, leading to pneumonia with eosinophilia.

The sensitivity of the stool microscopic examination varies according to the method employed. Among clinically diagnosed cases, the pooled sensitivity of the Baermann method has been reported at 72% [162], agar plate culture (APC) at 89% [162], direct examination at 21% [162], and formaldehyde-ether (FEC) pooled at 48% [162]. Obtaining serial stool cultures can increase sensitivity. For example, among culture positive cases tested over seven consecutive days, only 53% [163] have been reported positive on day one. Stool APC appears to be the preferred coprologic test and among cases detected by either microscopy, FEC, Harada-Mori filter paper culture, or APC, the sensitivity has been reported at 96% [164]. Among cases detected by PCR, however, the sensitivity has been reported at only 18% [165]. Of these PCR-positive cases, the addition of the Baermann method to APC has been reported to increase sensitivity by 24% [165]. The reduced sensitivity when PCR is added to the reference standard indicates that coprologic techniques alone are insufficient to make a trustworthy diagnosis. Among cases serologically confirmed by ELISA or LIPS, the sensitivity of microscopy by either FEC, Baermann, or stool APC has been reported at 53% [166]. Lastly, of serologically diagnosed cases by IF, the sensitivity of microscopy by Lutz, Rugai, or APC has been reported at 95% [167]. Microscopic examination can be performed on BALF, although it is not certain to provide additional sensitivity to fecal microscopy [168].

Serologic methods may provide a useful supplement to direct examination. Luciferase Immunoprecipitation System (LIPS) sensitivity for *S. stercoralis* has been reported in 85% [166] of cases detected by stool microscopy, with a specificity approaching 100% [166]. ELISA IgG serology sensitivity has been reported at 75% to 91% [166], 79% [169], 90% [170] and 95% [171] of cases detected by stool microscopy, and at 69% [169] of PCR-positive cases, with a specificity of 29% [171], 76% [169], and 99% [166,170]. IF serology sensitivity has been reported at 94% [166] and 95% [167] of cases detected by stool microscopy, with a specificity of 92% [166] and 96% [167]. Only ELISA is widely commercially available and should be used in conjunction with stool detection.

Stool PCR can be used to detect *Strongyloides*. The pooled sensitivity of PCR has been reported at 71% [172] of cases detected by microscopy but reduced to 57% [172] of cases detected by either serology or microscopy, with a specificity of 95% [172]. Because serology may sometimes be positive due to remote infection, the true sensitivity is on the upper end of the range, but when used in isolation it lacks the sensitivity to rule out active infection. PCR on BALF is neither well studied nor commercially available.



## Roundworm (*Ascaris lumbricoides*, *Ascaris suum*)

Roundworms are a parasite from the nematode phylum transmitted to humans through the fecal-oral route. Pneumonia occurs as a result of migration through the lungs.

Stool microscopy can be performed with several methods including the Kato-Katz (KK), formaldehyde-ether (FEC), and spontaneous tube sedimentation (STS) technique. The sensitivity of KK thick smear method has been reported at 49% [173], 70% [174], and 90% [175] of PCR-positive cases, and at 50% [176] and 75% [177] of cases positive by any microscopic technique, with specificity at 67% [173] and 100% [174]. Therefore, utilization of multiple techniques may improve sensitivity. Among PCR-positive cases, sensitivity of microscopy by KK, FEC, or wet preparation has been reported at 67% [178].

PCR has been used to detect *Ascaris lumbricoides*. Stool PCR sensitivity has been reported at 31% [173] of cases detected by the KK method, 82% [179] of those detected by the FEC method, and at 80% [178], 95% [175], 97% [174], and 100% [180], of all microscopy-positive cases, with a specificity of 81% [173], and 83% [179], 93% [175], and 95% [174]. As is the case with other parasitic infections, the accuracy of PCR on BALF is unknown. Unfortunately, *Ascaris* PCR for either stool specimens or BALF is not widely commercially available.

LAMP sensitivity has been reported at 96% [181] of cases detected by the microscopic thick smear technique, with a specificity of 62% [181], but is also not widely commercially available.

## Hookworm (*Ancylostoma duodenale*, *Necator americanus*)

Hookworms are helminth nematode parasites transmitted through soil that following migration to the lungs, lead to eosinophilic pneumonia.

Identification of eggs by stool microscopy has been reported in 0% [182], 29% [178], 31% [174], 32% [173], 43% [179], and 88% [183] of PCR-positive cases, with a specificity of 99% [173] and 100% [174,183]. Utilizing multiple methods may increase the sensitivity of microscopy. For example, among cases detected by any of four methods, the sensitivities of the FEC, KK, STS, and APC have been reported at 64% [176], 56% [176], 78% [176], and 86% [176], respectively. Stool microscopy, although possessing limited sensitivity, is still the only widely commercially available test.

PCR is neither well studied in BALF nor commercially available, but so far, the accuracy of stool PCR has been promising. Among cases with egg detection by stool microscopy, the sensitivity of stool PCR has been reported at 0% [182], 43% [179], 79% [184], 89% [175], 90% [178], 92% [174], 93% [173], and 100% [183,185], with a specificity of 79% [186], 84% [173], 91% [174],

95% [179], 99% [173], and 100% [175,187].

## Paragonimus (*westermani*, *mexicanus*, *africanus*, and others)

*Paragonimus* is a lung fluke from the *Trematoda* phylum that infects the lungs of humans who ingest crustaceans which are eaten raw or undercooked.

*Paragonimus* eggs can be identified by microscopy in either sputum or stool samples, as patients may unknowingly swallow sputum. The sensitivity of sputum microscopy has been reported at 10% [185] and 62% [188] of cases serologically detected by ELISA, and 14% [185] and 46% [189], and 72% [190] of clinically diagnosed cases. Of these clinically diagnosed cases of pulmonary paragonimiasis, the sensitivity of stool detection has been reported to reduce to 65% [190]. Either sputum or stool can be independently positive, and of clinically diagnosed cases the sensitivity of combining stool and sputum can increase sensitivity to 85% [190]. Therefore, detection by both sputum and stool microscopy is recommended concurrently for initial testing. Empiric treatment should be considered for microscopy-negative cases in which there is still clinical suspicion, as alternative diagnostic tests are not commercially available.

Skin testing is infrequently utilized, but has been reported positive in 97% [191] of cases detected by either stool or sputum microscopy, but is not widely commercially available.

Serum antigen sensitivity has been reported at 42% [192] and 86% [193] of clinically diagnosed cases, and at 100% [193,194] of microscopy-positive cases, with a specificity of 94% [192]. The paragonimiasis antigen is not commercially available and is not currently recommended.

ELISA serology sensitivity has been reported at 89% [186], 90% [174], 96% [192], and 100% [188,195,196] of microscopy-positive cases, with a specificity of 96% [186], 97% [195,196], and 100% [197]. Of cases positive by microscopic egg detection, the sensitivity of IF exceeds CF, reported at 42% and 33%, respectively [198]. No serologic method is widely commercially available, but ELISA does show promise as a complement to microscopy.

PCR has been performed on BALF, but is neither well studied nor commercially available.

## *Toxocara* (*canis*, *cati*, and others)

*Toxocara* is a parasitic, zoonotic roundworm transmitted by the fecal-oral route which manifests with allergic symptoms, neurologic symptoms, and pneumonia. Hosts of the roundworm include cats, dogs, foxes, coyotes, and wolves.

The sensitivity of culture is not known and thus BALF culture is generally not recommended.

Serology is an effective means of diagnosis. EIA IgG sensitivity among clinically diagnosed cases has been reported uniformly at 87% [199], 91% [200], 92% [201], with a specificity of 86% [200], and 100% [199], although specificity can be limited due to cross-reactivity with *Strongyloides* and *Trichinella*. The sensitivity of ELISA IgE with a titer greater than 1 TU/liter has been reported at 81% [202] of cases positive by Western Blot, with a specificity of 54% [202]. Above a higher threshold of 50 TU/liter, the sensitivity lowers to 42% [202] while specificity increases to 96% [202]. Conversely, among cases positive by ELISA, the sensitivity of Western blotting has been reported at 100% [202]. Only *Toxocara* ELISA is commercially available but if negative, empiric treatment should still be considered [203].

While PCR has been performed on animals, information on its accuracy when used on human tissues is lacking. In addition, PCR is not commercially available.

### *Toxoplasma gondii*

*Toxoplasma gondii* is an obligate intracellular protozoan acquired through ingestion of uncooked meat or infected water. Risk factors include pregnancy, HIV, and solid organ transplantation.

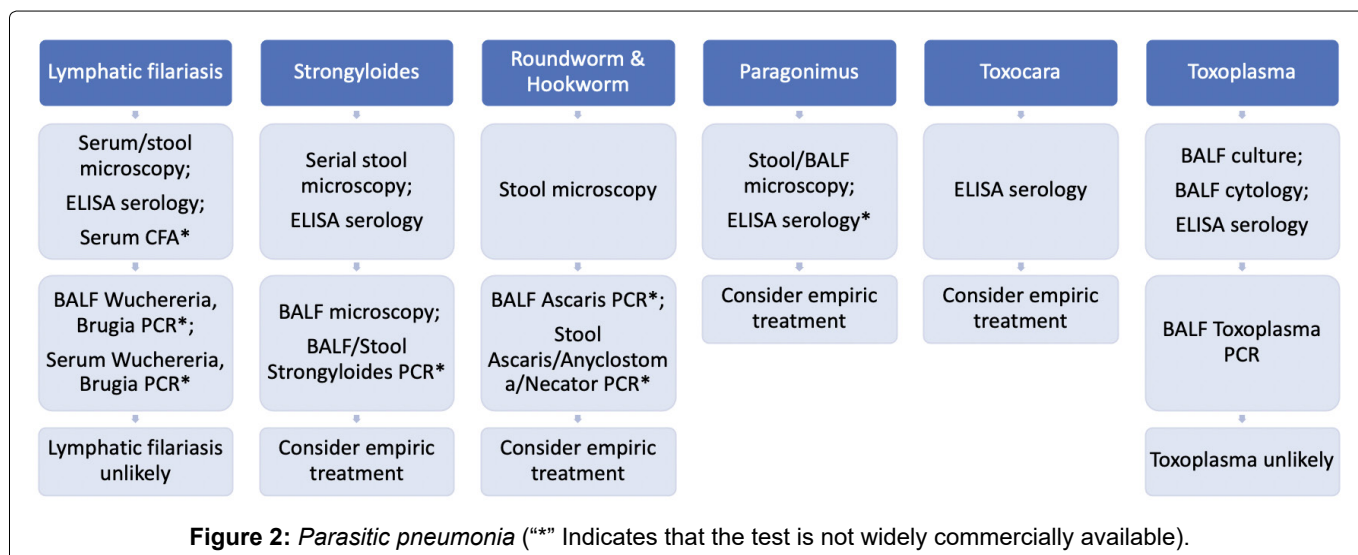
The sensitivity of BALF culture is unknown but culture of respiratory secretions can be performed [204]. Tissue culture sensitivity, however, has been reported at 100% [205] of microscopy-positive or serology-positive cases. The sensitivity of culture in fetal specimens with serologic evidence of infection, however, has been reported at only 80% [206]. Consistent with these findings, microscopic detection of BALF organisms by a Giemsa stain has been reported at 75% [204] of autopsy-confirmed cases, but increases to as high as 100% [204,207] of PCR-positive cases. BALF IF sensitivity has been reported at 33% [204] and 100% [205] of microscopy-positive cases, although as yet it has been evaluated only in small numbers. Because serology is not commercially available, microscopy-negative cases should be followed by either tissue sampling or empiric treatment.

Serologic methods are commonly used in the diagnosis of toxoplasmosis. EIA IgG has been present in 83% [208] of PCR-positive cases. ELISA IgM sensitivity has been reported at 100% [209] of clinically diagnosed cases, and at 100% [210] of those with positive BALF microscopy. The specificity has been reported at 78% to 99% [209], although IgM levels may sometimes remain elevated for months. Although not yet commercially available, serology does appear to have adequate sensitivity to be used in conjunction with PCR or microscopy.

BALF PCR sensitivity has been reported at 100% [204] of cases positive by microscopy [204] but only 50% [206] of those with a serologic titer rise, although as yet has been reported in only small numbers. As PCR is not known to provide additional sensitivity to the combination of microscopy and serology, its use is not currently recommended [207]. PCR is commercially available and may be considered, however, in cases of high clinical suspicion with either equivocal serology or negative BALF culture (Figure 1 and Figure 2).

### Conclusion

Although many pathogens still lack effective treatment, there may be value in identifying the presence of infection. Hospitalized patients with pneumonitis found to be PCR- and culture-negative are commonly diagnosed with drug-induced pneumonitis, radiation pneumonitis, organizing pneumonia, or nonspecific inflammation. In these instances, prolonged immunosuppressive therapy is commonly administered, which itself carries a risk of exacerbating the undiagnosed infection. Therefore, it is desirable to determine the causative organism to before treating with immunosuppressive therapies. Accurate diagnostic tests for several viral and parasitic pathogens are lacking. These instances require astute clinical judgement and a high index of suspicion is needed. Pathogen-targeted therapy should be considered even in the presence of non-diagnostic tests for most infectious agents.



## Contribution

All authors have contributed equally.

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## Conflicts of Interest

There are no conflicts of interest to report by any author.

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