

## Practical Approach to the Pathologic Diagnosis of Mixed Filamentous Fungal Infections in Burn Wounds: A Case Series

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**Introduction/Objective:** Opportunistic infections by fungi are a major source of morbidity and mortality in patients suffering from extensive burn wounds. Here we review a series of cases of infections by multiple fungi in burn wounds as diagnosed by histopathology and outline the key features for the pathologist to include in the report.

**Methods/Case Report:** Biopsies from patients with more than one fungal species identified in the laboratory in a concurrent culture or by PCR were included in this study. Three cases are presented with multiple fungi identified. Each case had yeast and at least one different hyaline mold species present on pathology; two cases additionally had mucormycetes present, with angioinvasion in one case. All organisms required microbiologic cultures and variably required molecular testing for full identification.

**Results (if a Case Study enter NA):** N/A

**Conclusion:** Pathologists should be aware of the possibility of infection by multiple fungal species in burn wounds. Fungal morphology in tissue sections should allow for detection and distinction of mucormycetes and other hyaline molds. Histopathologic correlation with culture and/or PCR results is essential to distinguish potential contaminants from true infection.

## Practical Risk Scoring System for Predicting Severity of COVID-19 Disease

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**Introduction/Objective:** SARS-CoV-2 has become an international pandemic with numerous casualties. The severity of the COVID-19 disease course depends on several clinical, laboratory, and radiological factors. This has led to risk scoring systems in various populations such as in China, but similar risk scoring systems developed based on the American veteran population are sparse. As a risk scoring system (RSS) would be very useful for future reference in similar pandemics, we share a simple Jhala Risk Scoring System (JRSS) developed early in the pandemic to assess the severity of disease risk.

**Methods/Case Report:** A retrospective review of all SARS-CoV-2 reverse transcriptase-polymerase chain

reaction (RT-PCR) tests collected and performed at the regional Veterans Administration Medical Center (VAMC) serving the Philadelphia and surrounding areas from March 17th, 2020 to May 20th, 2020. Data was collected and analyzed in the same year. These tests were reviewed within the computerized medical record system for demographic, medical history, laboratory test history, and clinical course. Information from the medical records were then scored based on the criteria of the JRSS.

**Results (if a Case Study enter NA):** The JRSS, based on age, ethnicity, presence of any lung disease, presence of cardiovascular disease, smoking history, and diabetes history with laboratory parameters correlated and predicted (with statistical significance) which patients would be hospitalized.

**Conclusion:** The JRSS reached statistical significance in its predictions on informing risk stratification for COVID-19 positive patients. Similar risk scoring systems may play a role in the rapid development of risk scoring in future pandemics of similar nature and thus provide a useful reference point. A simple RSS based on clinical parameters is a highly practical, cost effective and simple system to evaluate need for hospitalization, which is critical for operations in the intensive care unit and simultaneously the use of ventilators.

## Evaluation of indeterminate SARS-CoV-2 results with repeat testing on an alternative platform

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**Introduction/Objective:** Accurate SARS-CoV-2 results are crucial for patient management and infection prevention. Result confidence decreases with low viral load because near the assay's limit of detection (LOD), test results may alternate between positive and negative, as characterized by Poisson distribution for target analytes at low density. Low positive results may indicate past infection, early infection, a vaccinated individual with low level viral shed, or a false-positive result. EUA methods provide guidance on test interpretation, but laboratories should assess clinical accuracy. The purpose of this study was to assess clinical accuracy of specimens with low positive results.

**Methods/Case Report:** Respiratory specimens were tested by Cepheid Xpert® Xpress SARS-CoV-2 assay with positive results up to a Ct of 45. A low positive (defined as Ct  $\geq 35$ ), which could not be confirmed by Hologic Aptima® SARS-CoV-2 assay was reported as indeterminate and repeat testing recommended. Repeat testing occurred by Cepheid, Hologic, BioFire, Roche, or Quest assays. Retrospectively, final results were extracted from the LIS (Epic Beaker, Madison, WI, version May 2020) for 5-months (12/1/2020 to 5/31/2021), and chart review performed.

**Results (if a Case Study enter NA):** A total of 19,969 tests were performed; 10.4% (n=2,083) were positive, 89% (n=17,728) negative, and 0.79% (n=158) indeterminate. Previous infection (up to 3 months prior) was documented in 18% (n=28) of indeterminate results and defined as true positive. Of remaining indeterminate results, 43% (n=68) had repeat testing as recommended by laboratory; 26% (n=18) were positive and 74% (n=50) were negative. The average number of days between indeterminate and negative result was 7.25 (range 1-38).

**Conclusion:** Result discordance occurred in < 1% of all samples, excellent agreement. For low positive samples, discordance was higher, as expected. It's impossible to determine if negative results from the 50 repeat samples were false-positive by Cepheid or false-negative by other methods. In summary, 32% (50/158) of indeterminate samples did not repeat as positive. Overall concordance was high and results fluctuate when low virus is present. In absence of symptoms, we conclude repeat testing is not routinely recommended. Laboratories must recognize that normal variability occurs near assay LOD and must critically assess performance against other methods with similar LODs to fully assess performance of EUA methods.

### **Necrotizing Fasciitis and Fatal Septic Shock Associated with *Streptococcus constellatus***

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**Introduction/Objective:** *Streptococcus anginosus* group consists of three species: *S. anginosus*, *S. intermedius*, and *S. constellatus* (SC), normal flora in the human oral cavity, gastrointestinal and urogenital tracts. What makes them unique is their ability to form abscesses.

**Methods/Case Report:** We report a case of a 43 year old morbidly obese bipolar male presenting with general malaise and diarrhea. He had a history of uncontrolled diabetes mellitus Type 2 and persistent right thigh wound. Admitting labs revealed diabetic ketoacidosis. Physical examination revealed a right medial thigh ulcer with necrotic scrotal skin, after which he underwent urgent surgical debridement. Wound culture grew SC. Debrided tissue measured 44 x 17 x 4 cm. Histology revealed abscesses next to zones of pale gray necrotic tissue having a granular appearance without associated neutrophils. Gram stain of these pale gray zones revealed sheets of Gram positive cocci, consistent with Stage III necrotizing fasciitis (NF), which has 47% mortality. He died shortly after surgery with death attributed to septic shock.

Toxic shock syndrome is most often associated with Group A *Streptococcus*. However, other rare pathogenic streptococci, including SC, may be associated with a similar syndrome. Gram stain is essential in confirming NF, characterized by pale gray necrosis with a granular appearance.

**Results (if a Case Study enter NA):** NA

**Conclusion:** Our case highlights the risk of fatal NF due to SC. Identification of SC in a deep wound warrants early surgical intervention for adequate source control and administration of appropriate antibiotic. Poor diabetic control and ulcerated chronic thigh wound predisposed our patient to fatal necrotizing fasciitis due to SC, confirmed by Gram stain.

### **Molecular Pathology/Diagnostics**

#### **Performance Comparison of Multiplexed Fluorescent Resonance Emission Transfer Hybridization Probes Across Roche LightCycler® Real-Time PCR Systems for the Detection of *Bartonella* species**

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**Introduction/Objective:** Molecular assays for *Bartonella* species are important in diagnosing infection and expediting patient treatment. Real time polymerase chain reaction (RT-PCR) using fluorescent resonance energy transfer (FRET) hybridization probes can be used to detect *Bartonella* species in blood and fresh/fixed tissue biopsies in RT-PCR instruments. Over time, new technologies and reagents are introduced and existing PCR primers and FRET probes must be re-validated on new platforms. This study aimed to compare the performance of a *Bartonella* RT-PCR assay using the sunseting Roche LightCycler® 2.0 (Roche Diagnostics, Indianapolis, IN) and newer LightCycler® 480 RT-PCR instruments.

**Methods/Case Report:** DNA was extracted from 132 historically positive, whole organism spiked, and historically negative whole blood and formalin fixed paraffin embedded (FFPE) samples. Samples were run on the LightCycler® 2.0 using instrument specific LightCycler® FastStart DNA Master HybProbe enzyme and compared to results generated using the LightCycler® 480 and its instrument specific LightCycler® 480 Genotyping Master enzyme.

During optimization, MgCl<sub>2</sub> concentrations and thermocycling profiles were adjusted. Accuracy, specificity, inclusivity, and limit of detection studies were performed. Crossing point (Cp), melting temperature (Tm), fluorescent peak and fluorescent background values were compared between the two instruments.