APPENDIX A: Definitions of infection and suggested treatments

I. Definitions of Infections

Table 1: Proven invasive fungal disease (if any of the criteria apply)

	Molds*	**Yeasts**	**Agents of endemic fungal disease§**
Microscopy – sterile material	Histopathologic, cytopathologic, or direct microscopic examination† of a needle aspiration or biopsy specimen showing hyphal or melanized yeast-like forms with evidence of associated tissue damage (either microscopically or as an infiltrate or lesion by imaging)	Histopathologic or cytopathologic examination† of a needle aspiration or biopsy specimen from a normally sterile site excluding mucous membranes showing yeast cells e.g. *Cryptococcus* species indicated by encapsulated budding yeasts, *Candida* species showing pseudohyphae or true hyphae ‡	if culture is sterile or not obtained, histopathologic or direct microscopic demonstration of appropriate morphologic forms is considered adequate for dimorphic fungi having a truly distinctive appearance
Culture – sterile material	Recovery of a Mold or 'black yeast' by culture from a sample obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL, cranial sinus cavity, and urine.	Recovery of a yeast by culture from a sample obtained by a sterile procedure (including a freshly (<24h) placed drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process	Must be proven by recovery in culture from a specimen obtained from the affected site, and the host must at the same time have an illness consistent with a fungal infectious disease
Culture – blood	Blood culture that yields a Mold, e.g. *Fusarium* spp. in the context of a compatible infectious disease process	Blood culture that yields yeast (e.g. *Cryptococcus* species, *Candida* species), or yeast-like fungi (e.g. *Trichosporon* spp.)	Blood culture that yields an agent of endemic mycosis
Serology	Not applicable	**Disseminated cryptococcosis** cryptococcal antigen in CSF	**Histoplasmosis** diagnosis of disseminated disease can be established by means of a positive *Histoplasma* antigen EIA test on CSF, urine or serum, or by showing the presence of characteristic intracellular yeast forms in a peripheral blood smear or in bone marrow. **Coccidioidomycosis** demonstration of coccidioidal antibody in CSF, or a 2-dilution rise measured in two consecutive blood samples tested concurrently in the setting of an ongoing infectious disease process.

* if culture is available, append identification at genus or species level from culture.

† tissue and cells submitted for histopathologic or cytopathologic studies should be stained by Grocott-Gomori methenamine silver stain or by periodic acid Schiff stain to facilitate inspection of fungal structures. Where possible, wet mounts of specimens from foci related to invasive fungal infectious disease should be stained with a fluorescent dye (e.g., calcofluor or Blankophor ™)

‡ *Candida*, *Trichosporon* and yeast-like *Geotrichum* species and *Blastoschizomyces capitatus* may also form pseudohyphae or true hyphae

§ Histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, sporotrichosis and infection due to *Penicillium marneffei.* Onset within 3 months defines a primary pulmonary infection

Table 2 Criteria for defining probable invasive fungal disease (At lease one of each of the 3 elements must b present). Cases for which microbiologic criteria are absent are considered "Possible" and are not eligible for the RING Study.

Host factors

Host factors are not synonymous with risk factors and are characteristics by which individuals predisposed to invasive fungal diseases can be recognized. They are intended primarily to apply to patients treated for malignant disease and to recipients of allogeneic hematopoietic stem cell and solid organ transplant. These host factors are also applicable to those receiving corticosteroids and other T-cell suppressants as well as those with primary immune deficiencies

1) Recent history of neutropenia (< 0.5 x 10^9/L {<500 neutrophils/mm^3} for >10 days) temporally related to the onset of fungal disease
2) Receipt of an allogeneic stem cell transplant
3) Prolonged use of corticosteroids (excluding patients with allergic bronchopulmonary aspergillosis) at an average minimum dose of 0.3 mg/kg/day prednisone equivalent for > 3 weeks
4) Treatment with other recognized T-cell immune suppressants such as cyclosporine, TNF-α blockers, specific monoclonal antibodies such as alemtuzumab, nucleoside analogues during the past 90 days
5) Inherited severe immunodeficiency (e.g., chronic granulomatous disease, severe combined immunodeficiency)

Clinical criteria*

Lower respiratory tract fungal disease	***Tracheobronchitis***	***Sinonasal infection***	***CNS infection***	***Disseminated candidiasis†***
The presence of one of the following "specific" imaging signs on CT: 1. Well defined nodule(s) with or without a halo sign 2. Wedge-shaped infiltrate 3. Air crescent sign 4. Cavity ***OR*** the presence of a new non-specific focal infiltrate ***PLUS*** at least one of the following: 1. Pleural rub 2. Pleural pain 3. Hemoptysis	Tracheobronchial ulceration, nodule, pseudomembrane, plaque or eschar seen on bronchoscopy	Imaging showing sinusitis ***PLUS*** at least one of the following: 1. Acute localized pain (including pain radiating to eye) 2. Nasal ulcer, black eschar 3. Extension from the paranasal sinus across bony barriers, including into the orbit	Focal lesions on imaging ***OR*** Meningeal enhancement on MRI or CT	At least one of the following: 1. Small, target-like abscesses (new nodular filling defects, bull's-eye lesions) in liver or spleen of a patient who has had candidemia within the previous 2 weeks 2. Progressive "cotton wool" exudates on ophthalmologic examination

* Must be consistent with the microbiological findings, if any, and must be temporally related to current episode.

† the presence of signs and symptoms consistent with sepsis syndrome indicates acute disseminated disease whereas their absence denotes chronic disseminated disease.

Table 2 continued

Microbiological Criteria	
Direct test - Cytology, direct microscopy or culture	**Mold**: sputum, BAL fluid, bronchial brush or sinus aspirate samples: the presence of fungal elements indicating a Mold **OR** recovery by culture of a Mold (e.g. *Aspergillus* spp., *Fusarium* spp., Zygomycetes, *Scedosporium* spp.) from sputum, BAL fluid or bronchial brush samples
	Candida*:* biopsy of skin ulcers or a swab of draining soft tissue lesions or fistulas : Detection of yeast by microscopy **AND** recovery of *Candida* species by culture of the lesion
Indirect tests (Detection of antigen, cell wall constituents or nucleic acid)	**Aspergillosis**: Galactomannan antigen detected in plasma, serum, BAL fluid or CSF, unless the subject has been treated with pipercillin/tazobactam within the last 48 hours (these drugs are associated with false positive galactomannan results).
	Mycosis other than cryptococcosis and zygomycoses Beta-D-glucan detected in serum Note: These tests are primarily applicable for aspergillosis and candidiasis and do not detect *Cryptococcus* species or Zygomycetes (e.g. *Rhizopus* spp., *Mucor* spp. *Absidia* spp.)

* Must be consistent with the microbiological findings, if any, and must be temporally related to current episode.
† the presence of signs and symptoms consistent with sepsis syndrome indicates acute disseminated disease whereas their absence denotes chronic disseminated disease.

II. **Definitions of Bacteremia**

Subjects with any positive isolate from bacterial blood cultures indicative of serious infection (e.g., gram negative bacteremia or S. aureus bacteremia; coagulase-negative staphylococcal bacteremias are excluded). Subjects must also be unresponsive to appropriate clinical and antimicrobial management for > 24 hours (i.e., ongoing pressor requirements or hemodynamic instability). Bacteremia in the absence of hemodynamic instability will not be grounds for inclusion in the study unless it has persisted for 72 hours in spite of appropriate antimicrobial therapy.

III. **Definition of Invasive Bacterial Tissue Infection**

Clinical signs and symptoms compatible with disease (sinusitis, pneumonia, intra-abdominal abscess) and radiographic evidence of disease and pure or predominant culture from sterile site biopsy or bronchoalveolar lavage (BAL). The positive culture is not required if the subject has a positive blood culture with an organism that is a plausible cause of the infection (e.g. isolation of Streptococcus pneumoniae from blood in a subject with pneumonia). Typhlitis (neutropenic enterocolitis) is defined as clinical signs and symptoms compatible with disease and typical radiographic evidence of disease with or without culture confirmation.

IV. **Suggested therapy for specific infections**

A. Invasive mold infections due to:
   1. Aspergillus species: voriconazole +/- caspofungin
   2. Zygomycetes (agents of "mucormycosis"): lipid formulation of amphotericin B (AmBisome or ABLC; 5mg/kg/day) is preferred first-line therapy. Posaconazole may be considered as salvage therapy.
   3. Fusarium species: voriconazole or lipid formulation of amphotericin B (AmBisome or ABLC; 5mg/kg/day)
   4. Scedosporium species: voriconazole

B. Candidemia or deep tissue invasive candidiasis: echinocandin (caspofungin, micafungin or anidulafungin), conventional amphotericin B ($\geq$ 0.6 mg/kg/day); lipid formation of amphotericin B (3 to 5 mg/kg/day). Removal of central venous catheter is advised.