

Histopathology of Fungal Infections

Diagnosis of Fungal Infections

- Diagnosis of a mycotic disease ideally includes:
 - Observation of typical symptoms
 - Demonstration of fungus in lesion with accompanying host reaction
 - Isolation of causative agent
- Not all the above can be accomplished in every type of fungal disease
- Other methods that can be used to aid in the diagnosis of a fungal infection include the detection of fungal:
 - Antigens
 - Antibodies
 - Metabolites
 - Cell wall markers
- More modern molecular-based methods are now available
- Some of the above methods are not yet available for many pathogenic fungi, particularly those that are somewhat unusual
- Remaining method for diagnosis includes the histopathological examination of biopsy material in order to observe:
 - Characteristic features of specific etiological agent
 - Host response to infection

Histological Stains for Fungi

- Hematoxylin and eosin (H&E)
 - Color of fungi: pink to pinkish blue
 - Applications:
 - Demonstrates inflammatory response
 - Stains some fungi
 - Allows determination of innate pigmentation by invading fungus
 - Demonstrates Splendore-Hoeppli material
 - Stains most nuclei of yeast-like fungi

- Limitations:
 - Does not stain many fungi
 - Does not stain filamentous bacteria
 - Is not adequate for screening tissue with sparse number of fungal elements
- Gomori's methenamine silver (GMS) [often referred to as 'silver stain']
 - Color of fungi: black brown on a light green background
 - Applications:
 - Stains most fungi, viable or not
 - Can stain filamentous bacteria
 - Limitations:
 - May overstain fungi and obscure internal details
 - Cannot detect host response
- Periodic acid-Shiff (PAS)
 - Color of fungi: red pink on a green background
 - Application: stains most fungi, viable or not
 - Limitations:
 - Masks innate color and internal details
 - Many tissue elements take up the stain
 - Cannot detect host response
 - Does not stain filamentous bacteria
- Gridley fungus (GF)
 - Color of fungi: purplish red on a yellow background
 - Application: stains most fungi
 - Limitations:
 - Masks innate color
 - Non-viable cells do not stain
 - Cannot demonstrate host response
 - Does not stain filamentous bacteria

- GMS with H&E counterstain
 - Stain of choice if only one slide available for histopathological examination
 - Color of fungi: black brown fungi on a red-pink background
 - Applications:
 - Permits study of host response
 - Excellent for detecting fungi and filamentous bacteria
 - Limitation: cannot determine innate fungal color
- Mucin (mucicarmine) stains
 - Mayer's or Southgate's preparations
 - Application: stains of mucopolysaccharide capsular material of fungi, e.g., *Cryptococcus*
 - Limitation: Not specific for *Cryptococcus*
- Modified Gram's stains
 - Brown-Hopps' and MacCallum-Goodpasture preparations
 - Application: stains Gram-positive filamentous bacteria
 - Limitation: does not selectively stain fungi
- Modified acid-fast stains
 - Ziehl-Neelsen's and Kinyoun's preparations
 - Application: stains Gram-positive filamentous bacteria
 - Limitation: does not stain fungi
- Modified Fontana-Masson
 - Applications:
 - Stains cell walls of *Cryptococcus* and other melanin producing fungi
 - Accentuates weakly pigmented agents of phaeohyphomycosis
 - Limitation: may stain fungal elements that are immature or innately not pigmented
- Whitening agents
 - Calcofluor White, Uvitex, and others
 - Application: stains cell walls of fungi
 - Limitation: need a fluorescent microscope

Histopathological Identification

- Tissue sections can be used to observe fungal elements and particular attributes that may be characteristic of certain species
- Fungi can appear as
 - Hyaline or pigmented (phaeoid)
 - One of four broad morphological categories
 - Yeast-like
 - Hyphae
 - Endosporulating spherules
 - Granules
- Other defining features of fungal forms in vivo include
 - Size and shape of cells
 - Cell wall thickness
 - Number and shape of blastoconidia (buds)
 - Presence or absence of septations
 - Capsules
 - Number of nuclei
 - Presence of pseudohyphae, hyphae, or arthroconidia
- Immunohistological staining is also used to detect and identify fungi in tissue
 - Can be direct or indirect staining, i.e., one step or multi-step process
 - Often fluorescent-tagged antibodies are used
 - Other 'tags' include
 - Gold-silver complexes
 - Enzyme complexes (e.g., peroxidases)