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

Diagnosis of Fungal Infections (cont.)

- 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

Diagnosis of Fungal Infections (cont.)

- 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

Haematoxylin and eosin (H&E) stained sections of lung tissue showing the broad, infrequently septate, thin-walled hyphae of Absidia corymbifera (left) and darkly-pigmented sclerotic cells of Fonsecaea pedrosoi. Source: www.doctorfungus.com
Histological Stains for Fungi (cont.)
- Limitations:
  - Does not stain many fungi
  - Does not stain filamentous bacteria
  - Is not adequate for screening tissue with sparse number of fungal elements

Histological Stains for Fungi (cont.)
- 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

Histological Stains for Fungi (cont.)
- 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

Splendore-Hoeppli material from a case of sporotrichosis.
Source: www.histopathology-india.net/ Soportri.htm

Histological Stains for Fungi (cont.)
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Methenamine silver (GMS) stained tissue section from a lung showing typical zygomycete hyphae and by chance a sporangium of Absidia corymbifera.
Source: www.doctorfungus.com

Periodic acid-Shiff (PAS) stain of Rhinosporidium seeberi depicting sporangia at different stages of sporangiospore development in the large sporangium as observed in a human nasal polyp.
Source: www.doctorfungus.com
Histological Stains for Fungi (cont.)

- **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

Histological Stains for Fungi (cont.)

- **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

Histological Stains for Fungi (cont.)

- **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*
Histological Stains for Fungi (cont.)

- 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

Histological Stains for Fungi (cont.)

- 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

Histological Stains for Fungi (cont.)

- 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

Histopathological Identification (cont.)

- 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

Histopathological Identification (cont.)

- 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)