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جامعة المستقبل
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Advanced Laboratory Techniques

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Lecture / 7

Staining used in diagnosis microorganisms (Bacteria, parasites and fungi) /part 2

Staining used in diagnosis microorganisms // part 2

Staining used in diagnosis of parasite

- In the diagnosis of parasites, various staining techniques are employed to visualize and identify different types of parasites and their life stages.
- There are some common staining methods used in the diagnosis of parasitic infections:

❖ **Giemza Stain:**

- Giemza stain is a versatile stain that is commonly used for the detection of blood and tissue parasites, such as Plasmodium species (malaria), Trypanosoma species (Chagas disease and African sleeping sickness), and Leishmania species.
- It provides good contrast and allows for the observation of intracellular parasites within host cells.

Procedure:

- Prepare a thin smear of the specimen on a clean microscope slide. This can be blood, bone marrow, or other cellular samples.
- Flood the smear with methanol for fixation. Allow it to air dry. Fixation is essential to preserve the cellular morphology.
- Prepare a working solution of Giemsa stain by diluting the stock solution with distilled water. The dilution ratio may vary depending on the specific protocol or stain concentration.
- Flood the fixed smear with the Giemsa stain solution, ensuring that the entire smear is covered. Allow it to stain for the specified time (usually 10-30 minutes).
- After staining, rinse the slide with running tap water to remove excess stain.
- Allow the slide to air dry. It can use bibulous paper or blotting paper to speed up the drying process.
- Once the slide is dry, mount it with a coverslip using a mounting medium, such as Canada balsam or a water-based mounting medium.
- Examine the stained slide under a microscope. Giemsa stain is known for providing good contrast between different cell components and structures.

❖ **Wet Mount Preparation:**

- While not a traditional staining method, the wet mount involves placing a fresh specimen in a drop of liquid on a slide and covering it with a coverslip.
- This method is often used for the rapid detection of motile parasites, such as protozoa, in their fresh, unstained state.

Procedure:

- The microscope slides and coverslips are clean and free of any contaminants. If needed, clean them with a suitable cleaning agent and allow them to air dry.
- Using a microbiological loop or inoculating needle, collect a small sample of the microorganism or specimen the want to observe. This can be a drop of liquid containing the microorganisms or a portion of a solid specimen.
- Place a drop of sterile saline solution or water on the center of a clean microscope slide.
- Gently transfer the microorganism sample onto the drop of liquid on the slide. Be careful not to introduce air bubbles.
- Carefully place a coverslip over the liquid and microorganism sample. Lower the coverslip at an angle to avoid trapping air bubbles. The coverslip is in contact with the liquid and that it covers the specimen adequately.
- Place the prepared wet mount slide on the stage of the microscope.
- Use the lowest magnification objective lens to locate the specimen. Once located, switch to higher magnifications for a more detailed observation.
- Adjust the focus and lighting as needed to obtain a clear and well-illuminated view of the specimen.
- Observe the microorganisms in their natural, hydrated state. Note any movement, shape, or other characteristics.
- After observation, dispose of the wet mount slide appropriately.
- Wet mounts are temporary preparations, and observations should be made promptly as the specimen may dry out.
- The choice of mounting medium (saline solution, water, etc.) depends on the nature of the microorganisms being observed.
- Be cautious not to use too much liquid, as excessive depth can make it challenging to focus on the specimen.

❖ **Trichrome Stain:**

- Trichrome staining is commonly used for the identification of intestinal parasites, including Giardia lamblia and Entamoeba histolytica, in stool samples.
- The stain highlights the morphology of the parasites and their internal structures.

Procedure:

- Prepare the parasite samples, either in the form of tissue sections or smears, depending on the type of parasite.
- If the samples are embedded in paraffin, deparaffinize them by immersing the slides in xylene or a xylene substitute. Follow this with a series of descending concentrations of alcohol (e.g., 100%, 95%, 70%, and 50% ethanol) to rehydrate the tissue.
- Follow the specific instructions provided with the trichrome staining kit for each staining step. The kit usually includes solutions for the following steps:

- Hematoxylin staining for nuclei.
- Differentiation step to highlight cellular structures.
- Biebrich Scarlet-Acid Fuchsin staining for cytoplasmic components.
- Aniline blue staining for connective tissues and collagen fibers.

- Rinse the slides thoroughly with running tap water or distilled water between each staining step to remove excess stain.
- If necessary, dehydrate the slides through a series of ascending concentrations of alcohol (e.g., 95%, 100% ethanol).
- Clear the slides by immersing them in xylene or a xylene substitute.
- Mount the slides with a mounting medium suitable for permanent preservation and cover them with coverslips.
- Allow the slides to air dry before examining them under a microscope.
- Examine the stained parasite samples under a microscope to observe the stained structures and components. Different parasites may show specific staining patterns.

❖ **Iron Hematoxylin Stain:**

- This stain is often used for the detection of tissue parasites, such as helminthes (worms).
- It enhances the visibility of the internal structures of parasites in tissue sections.

Procedure:

- Prepare the parasite samples, either in the form of tissue sections or smears, depending on the type of parasite.
- If the samples are embedded in paraffin, deparaffinize them by immersing the slides in xylene or a xylene substitute. Follow this with a series of descending concentrations of alcohol (e.g., 100%, 95%, 70%, and 50% ethanol) to rehydrate the tissue.
- Immerse the slides in Iron Hematoxylin staining solution for a specified period. The time may vary depending on the specific staining protocol and the stain's concentration.
- Rinse the slides thoroughly with running tap water or distilled water to remove excess stain.
- Differentiate the slides in acid alcohol or another differentiation solution. The differentiation step helps to enhance contrast by selectively removing excess stain.
- Rinse the slides again with running tap water or distilled water to stop the differentiation process.
- Immerse the slides in a bluing agent (e.g., Scott's tap water substitute) to counteract any residual acidity from the differentiation step.
- Dehydrate the slides through a series of ascending concentrations of alcohol (e.g., 95%, 100% ethanol).
- Clear the slides by immersing them in xylene or a xylene substitute.

- Mount the slides with a suitable mounting medium for permanent preservation and cover them with coverslips.
- Allow the slides to air dry before examining them under a microscope.
- Examine the stained parasite samples under a microscope to observe the nuclei stained by Iron Hematoxylin.

❖ **Calcofluor White Stain:**

- Calcofluor white is a fluorescent stain that binds to the chitin present in the cell walls of some fungi and parasites.
- It is commonly used for the detection of microsporidia and other fungal or parasitic structures.

❖ **Ziehl-Neelsen Stain (for Mycobacteria):**

- While primarily used for acid-fast bacteria, the Ziehl-Neelsen stain can also be employed for the detection of acid-fast parasites, such as Cryptosporidium species.

❖ **Silver Stains:**

- Silver stains, such as the Warthin-Starry stain, can be used to visualize certain parasitic structures, including the cysts and trophozoites of parasites like Giardia lamblia.

Staining used in diagnosis microorganisms // part 3

Staining used in diagnosis fungi

- Staining techniques are crucial in the diagnosis of fungal infections, aiding in the visualization and identification of fungal structures.
- There are some common staining methods used in the diagnosis of fungal infections:

❖ **Giemsa Stain:**

- Giemsa stain, which is commonly used for blood and tissue parasites, can also be employed for the staining of fungal elements.
- It provides good contrast and allows for the observation of fungal structures.

❖ **Calcofluor White Stain:**

- Calcofluor white is a fluorescent stain that binds to the chitin present in the fungal cell wall.
- It is particularly useful for the detection of fungi in clinical specimens, such as skin scrapings or nail clippings, under fluorescent microscopy.

❖ **Periodic Acid-Schiff (PAS) Stain:**

- The PAS stain is widely used in histology for the detection of carbohydrates and fungal elements.
- It stains fungal cell walls and other structures magenta, making them easily visible under a microscope.

❖ **Methenamine Silver Stain:**

- This silver stain is commonly used to visualize fungal structures in tissues.
- It stains fungal cell walls and some fungal structures, allowing for the identification of fungal elements.

❖ **India Ink Stain:**

- India ink staining is often used for the rapid detection of Cryptococcus neoformans, a yeast-like fungus commonly associated with meningitis.
- The capsule of Cryptococcus appears as a clear halo against the dark background of the India ink.

❖ **Lactophenol Cotton Blue Stain:**

- Lactophenol cotton blue is a stain used for the examination of fungal cultures.
- It helps in the visualization of fungal structures and aids in the identification of fungal species based on their morphological characteristics.

❖ **Gridley Fungus Stain:**

- This stain is specifically used for the detection of fungi in tissues.
- It involves the use of basic fuchsin and picric acid and is especially useful for identifying fungal elements in histopathology.

❖ **Wheatley Trichrome Stain:**

- Wheatley trichrome stain is often used to stain fungi in clinical specimens.
- It utilizes a combination of acidic and basic dyes to highlight fungal structures.

The choice of staining method depends on the type of specimen, the suspected fungal pathogen, and the information needed for accurate diagnosis. These staining techniques allow microbiologists and pathologists to observe the morphology of fungi, aiding in the identification and characterization of fungal infections.