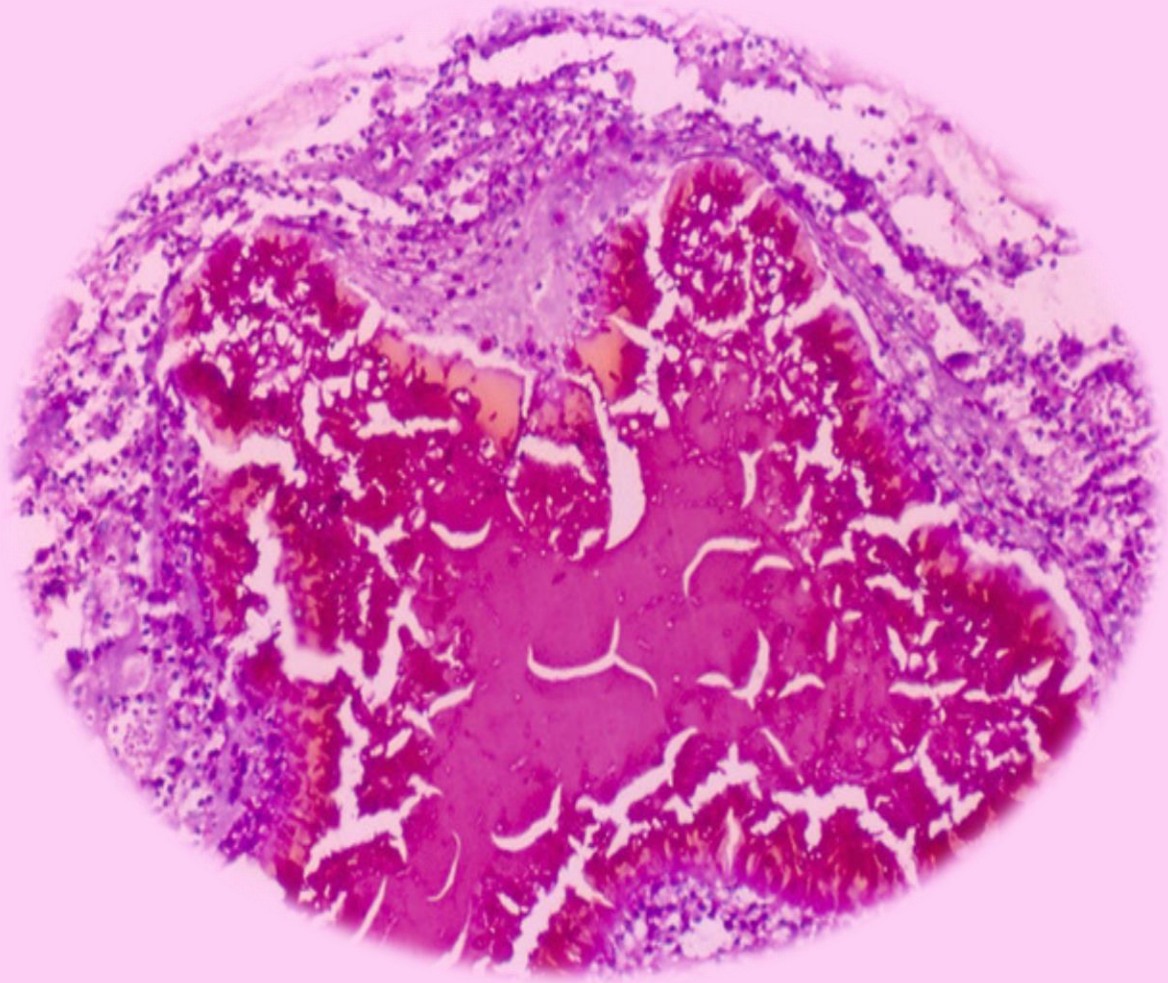
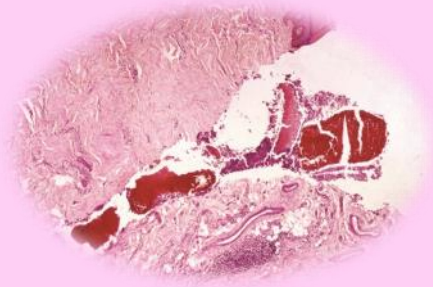
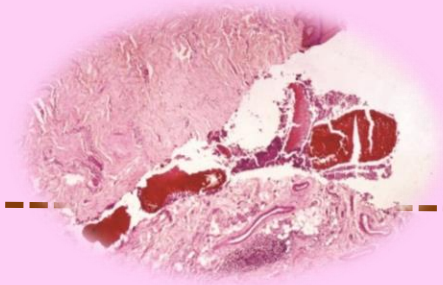


Standard Operating Procedure For Hematoxylin and Eosin Staining

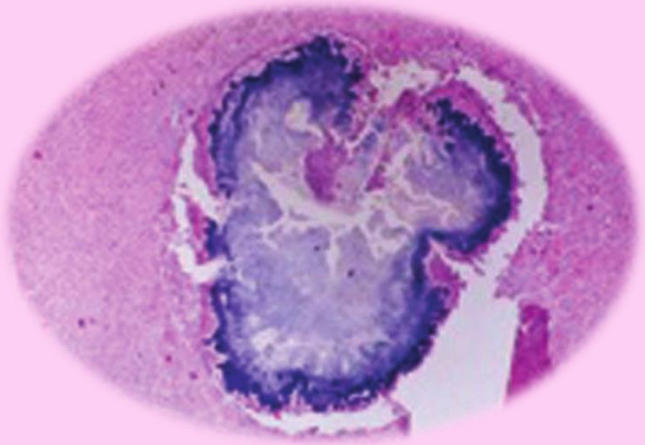
The Mycetoma Research Center
University of Khartoum
WHO Collaborating Center on
Mycetoma & Skin NTDs





Standard Operating Procedure

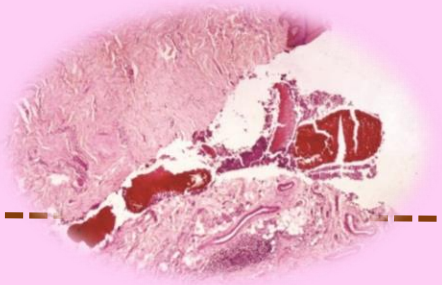
for Hematoxylin and Eosin (H&E) Staining



Number: 004/SP/MRC/2024

Date: April 03 2024

Purpose & Scope



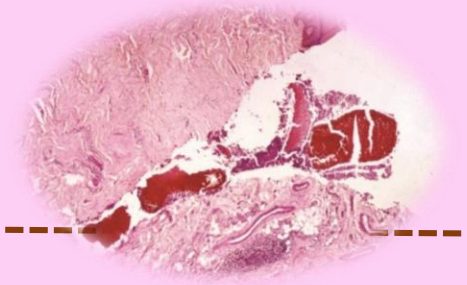
Purpose

This SOP provides a standardised method for performing Hematoxylin and Eosin (H&E) staining of paraffin-embedded tissue sections to ensure consistent and high-quality staining results.

Scope

This SOP applies to all personnel in the histopathology laboratory involved in staining tissue sections using the H&E technique.

Responsibilities



Laboratory Technicians

Responsible for performing the staining procedure according to this SOP.

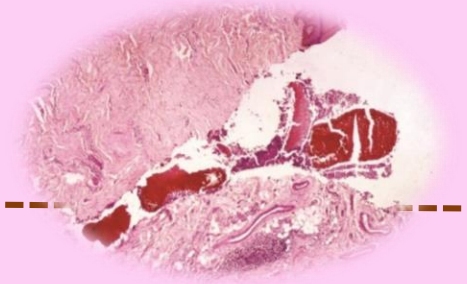
Laboratory Supervisor

Ensures that the SOP is followed and that the equipment and reagents are available and maintained.

Quality Control Personnel

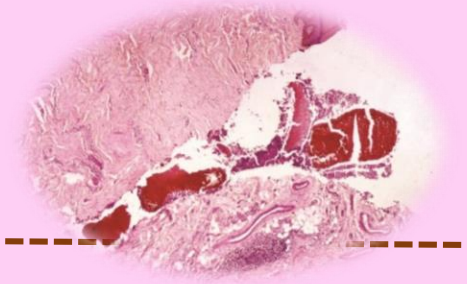
Responsible for verifying the accuracy and consistency of the staining results.

Materials and Reagents



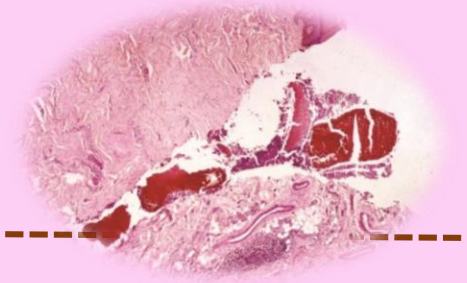
- Paraffin-embedded tissue sections (4-5 µm thick)
- Xylene
- Absolute ethanol (100%)
- 95% ethanol
- 70% ethanol
- Distilled water
- Hematoxylin solution (Mayer’s or Harris)
- Eosin Y solution

Materials and Reagents



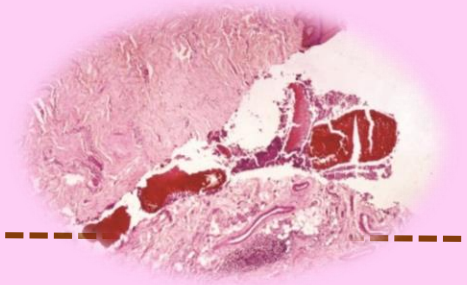
- Acid alcohol (0.3% hydrochloric acid in 70% ethanol)
- Ammonia water (0.1% ammonium hydroxide)
- Mounting medium (e.g., DPX)
- Glass slides and coverslips
- Slide rack and staining dishes
- Timer
- Fume hood
- Micropipettes and tips

Equipment



- Microscope
- Microtome
- Water bath
- Slide dryer or incubator
- Automated staining machine

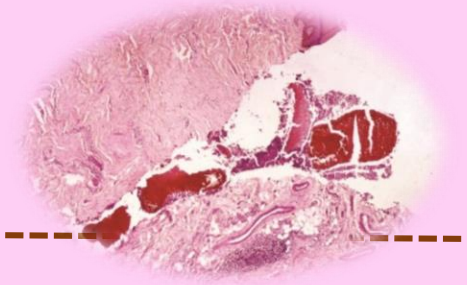
Procedure



1. Dewaxing and Rehydration

- Place the paraffin-embedded tissue sections on glass slides.
- Place the slides in xylene for 5 minutes (two changes).
- Transfer the slides to absolute ethanol for 2 minutes (two changes).
- Sequentially rehydrate the slides by placing them in 95% ethanol for 2 minutes, 70% ethanol for 2 minutes, and then rinse in distilled water for 2 minutes.

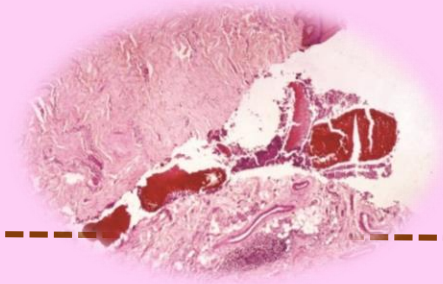
Procedure



2. Hematoxylin Staining

- Submerge the slides in hematoxylin solution for 5-10 minutes (time may vary depending on the hematoxylin type used).
- Rinse the slides in tap water for 1-2 minutes.
- Differentiate in acid alcohol for 1-2 seconds (optional, depending on desired intensity).
- Rinse quickly in tap water.
- Blue the slides by dipping them in ammonia water for 30 seconds or until the blue colour is apparent.
- Rinse the slides in tap water for 5 minutes to remove excess bluing agent.

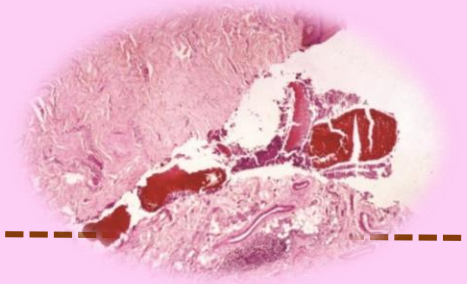
Procedure



3. Eosin Staining

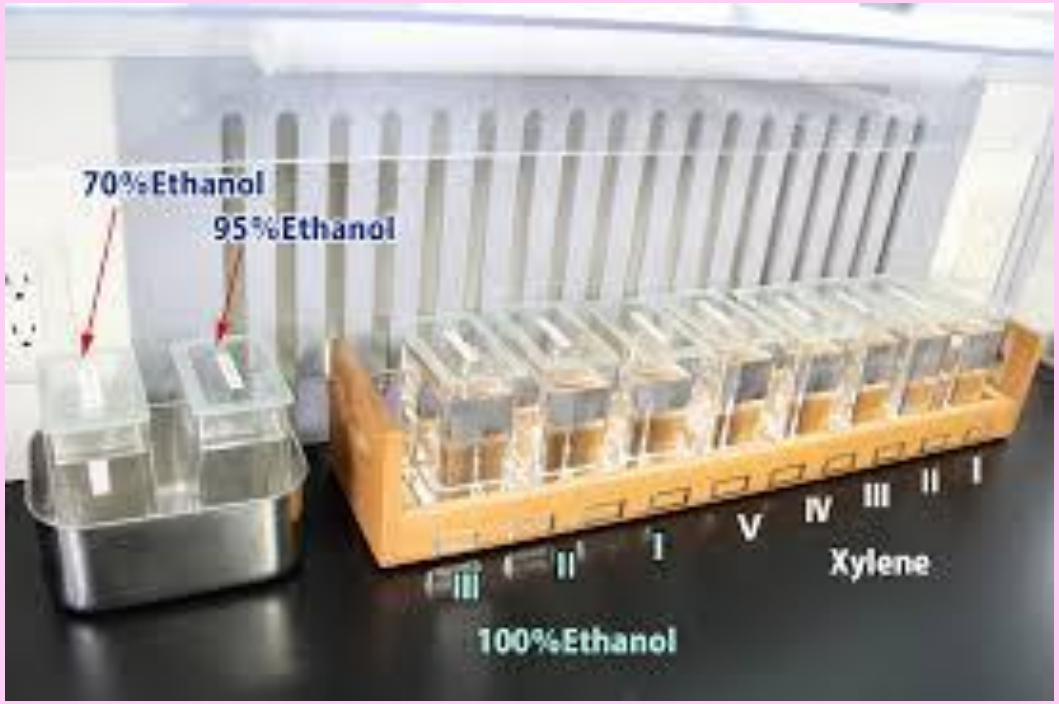
- Submerge the slides in eosin Y solution for 1-2 minutes.
- Rinse quickly in distilled water to remove excess eosin.

Procedure

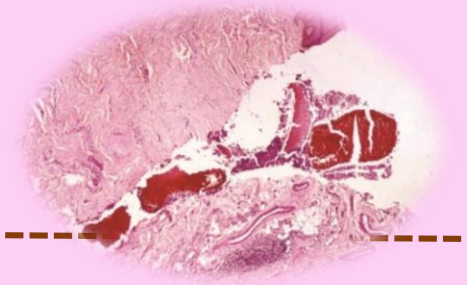


4. Dehydration and Clearing

- Dehydrate the slides by sequential immersion in 70% ethanol, 95% ethanol, and absolute ethanol (2 minutes each).
- Clear the slides in xylene for 5 minutes (two changes).



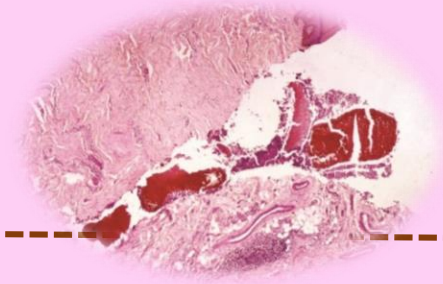
Procedure



5. Mounting

- Apply a drop of mounting medium to the tissue section and cover with a coverslip.
- Allow the slides to dry in a fume hood or an incubator.

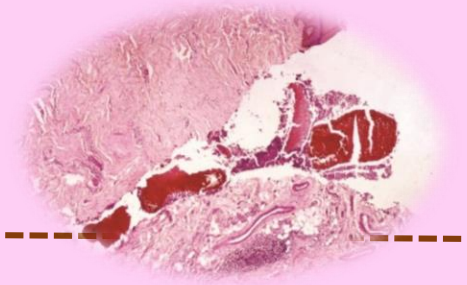
Procedure



6. Microscopic Examination

- Examine the slides under a microscope to ensure adequate staining and tissue morphology.

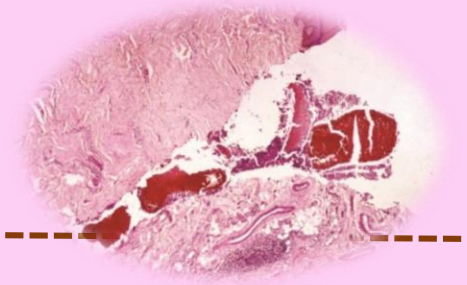
Procedure



7. Quality Control

- Include a control slide with each batch of staining.
- Record any deviations from the SOP and any issues with staining quality.

Procedure



8. Cleaning and Maintenance

- Clean all equipment and work surfaces after use.
- Dispose of used reagents according to laboratory safety guidelines.

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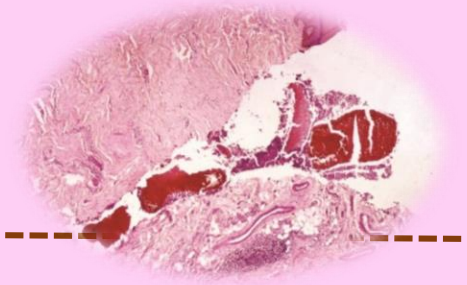
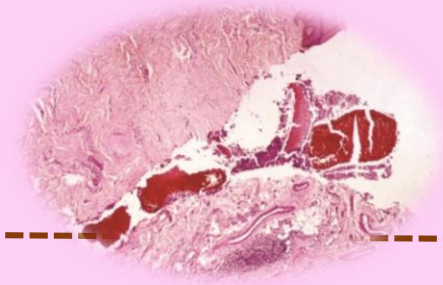
Procedure



Xylene	2 minutes
Xylene	2 minutes
100% ethanol	2 minutes
100% ethanol	2 minutes
95% ethanol	2 minutes
Water wash	2 minutes
Hematoxylin	3 minutes
Water wash	1 minute
Differentiator (mild acid)	1 minute

Water wash	1 minute
Bluing	1 minute
Water wash	1 minute
95% ethanol	1 minute
Eosin	45 seconds
95% ethanol	1 minute
100% ethanol	1 minute
100% ethanol	1 minute
Xylene	2 minutes
Xylene	2 minutes
Coverslip	

References



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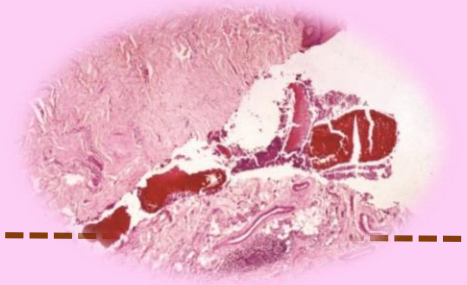


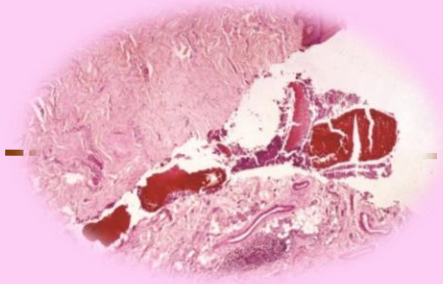
Attachments

Flowchart of the H&E staining process

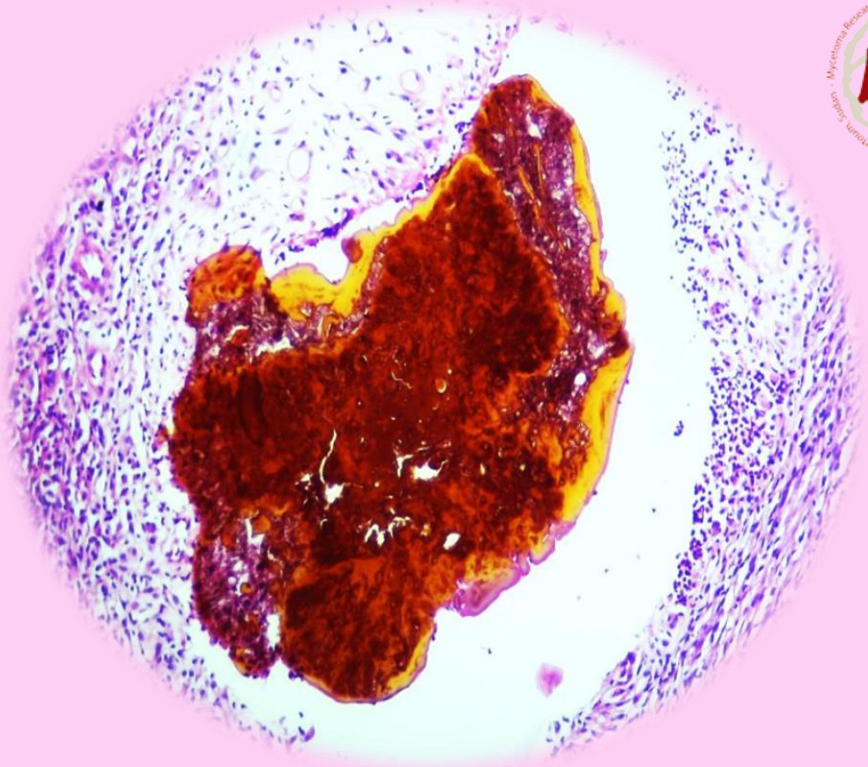
Troubleshooting guide for common staining issues

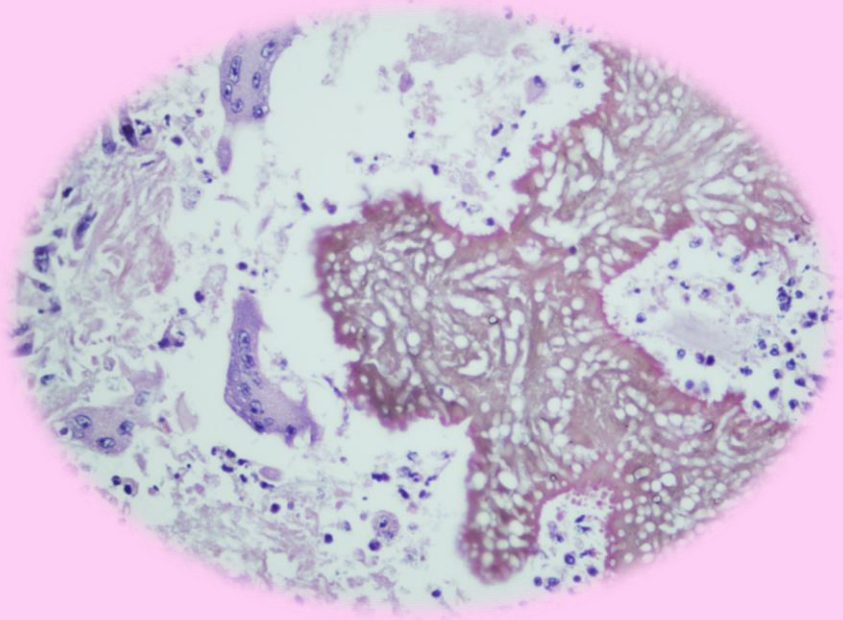
Document Control

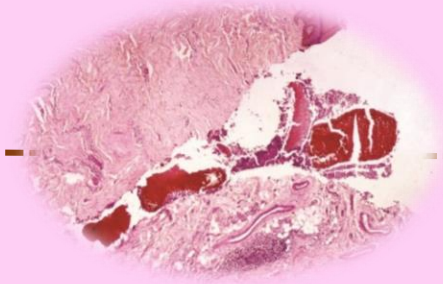
Version history and changes



Dark Brown to yellow grains, with fungal Hyphae at the center of the grains and yellow cement matrix was observed in the periphery of the grains surrounded by neutrophils, lymphocytes and macrophages. H&E stain X40

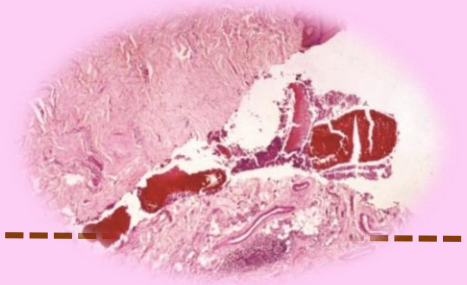






Madurella Mycetomatis grain with surrounding numerous neutrophils, macrophages, lymphocytes, and foreign body giant cells, H&E X20

Approval



This SOP was prepared, reviewed, and approved by

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Dr Abdullah Osman Ahmed	Senior Researcher	Abdullah
Prof Ahmed Fahal	Center Director	Fahal

April 03 2024

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