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Title: Histology – Miller's Staining (modified)

Version: v1

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| SOP History | | |
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| Number | Date | Reason for Change |
| v1 | 28/04/20 | Original |
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1.0 Purpose –

This SOP describes the current procedure for carrying out Miller's Trichrome Staining on FFPE sections in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM carrying out Millers Trichrome Staining on FFPE sections in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing Millers Trichrome Staining on FFPE sections in this manner are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

***NB. Be extra careful when dealing with picric acid!
ALL USERS MUST SIGN THE RISK ASSESSMENT FOR PICRIC ACID
BEFORE CARRYING OUT THIS PROCEDURE.***



4.0 Procedure –

The Millers's Trichrome staining method is for visualising elastin (elastic connective fibres) and is typically used to identify blood vessels and pleural invasion in lung cancer. Miller's stain contains three active dyes, namely Victoria blue 4R (CI 42563), new fuchsin (CI 42520) and crystal violet (CI 42555). This is purchased ready to use from VWR.

Van Gieson's stain is a Picric acid and acid fuchsin mixture used in the differential staining of collagen and other connective tissue which can be used as a counterstain for other methods (such as Miller's, or Verhoeff Van Gieson elastic staining techniques).

Solutions and Reagents

Aqueous Picric Acid (1%):

Aqueous Picric Acid is purchased as a ready-to-use solution (VWR; #87897.180) supplied at 10g/Litre (1% concentration).

Miller's Elastic stain

This is supplied ready to use from VWR (#351154S) as it has excellent storage properties and may be reused for many years.

Make up 0.5% Acidified Potassium Permanganate (FILTER it before use)

[A] Make up 3% Sulfuric acid (3ml of sulfuric acid in 100ml DW).

[B] Dissolve 2.5g of potassium permanganate (#223468-500G) in 500ml of DW.

Remove 25ml from the 0.5% potassium permanganate [B] and replace with 25ml of 3% sulfuric acid [B]

- this will then become 0.475% potassium permanganate

NB. Be extra careful when dealing with sulfuric acid!

Make up 1% Acid Fuchsin as follows

Acid Fuchsin (#1052310025) 1g in 100mL DW

Make up 1% Oxalic acid solution as follows:

Oxalic acid (#75688-250G) 1g in 100mL DW.

Make up Van Gieson Solution as follows (make up fresh)

Saturated aqueous Picric Acid solution 14 parts

1% acid fuchsin 1 part

NB. Be extra careful when dealing with picric acid!
ALL USERS MUST SIGN THE RISK ASSESSMENT FOR PICRIC ACID
BEFORE CARRYING OUT THIS PROCEDURE.



De-wax & Re-hydrate

Always allow any excess fluid to drain from the slide rack before proceeding to the next solution.

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| 1. Dewax-Xylene | 1 | 5 minutes |
| 2. Dewax-Xylene | 2 | 5 minutes |
| 3. Dewax-Xylene | 3 | 5 minutes |
| 4. Rehydration-100% Alcohol | | 2 minutes |
| 5. Rehydration-100% Alcohol | | 2 minutes |
| 6. Rehydration-80% Alcohol | | 2 minutes |
| 7. Rehydration-50% Alcohol | | 2 minutes |
| 8. Wash in running water | | 2 minutes |

Staining

After hydration in water, place (oxidise) the slides in 0.5% acidified potassium permanganate for 5 mins.

Wash in water.

Decolourise with 1% oxalic acid for 2 mins.

Place in Miller's Solution (in a microwave) for 5mins.

Differentiate in 70% alcohol until only the elastic fibres are a deep blue colour.

Counterstain with Van Gieson solution for 5 mins.

Quickly dehydrate, clear and mount.

5.0 Personal protection –

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

6.0 Spillages –

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –

All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –

- 8.1 Risk assessments –
RA20232 (Histology – Miller's, Carstair's and Picro Sirius Red Staining)



9.0 Approval and sign off –

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