

Practical-11

PERIODIC ACID SCHIFFS (PAS) METHOD



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□ Solutions:

1) 1% Periodic acid

2) Schiff's reagent

3) Mayer's hematoxylin

- **Periodic acid-Schiff (PAS)** is a staining method used to detect glycogen and other polysaccharides in tissues.
- The reaction of periodic acid oxidizes the functional groups in glucose and other sugars, creating aldehydes that react with the Schiff reagent to give a **purple-magenta color**.
- A suitable basic stain is often used as a counterstain.
- The Periodic Acid Schiff (PAS) is used to demonstrate many other normal and pathological tissue constituents.

Solutions:

1% Periodic acid:

Preparation: Periodic acid-----1.0 gm
Distilled water-----100ml

Schiff's reagent:

Preparation:

1. Boil 200 ml of distilled water and add **1gm of basic fuchsin**.
2. Shake for **5min**. Cool exactly **50°C**.
3. Filter and add **20ml of N/1 HCL** of the filter.
4. Cool further to **25°C** and add **1gm of sodium or potassium metabisulphate**.
5. Store for 18-24 hours in the dark area and add **2gm activated charcoal** and shake the mixture **1min**.
6. Remove the charcoal by filtration and store at **0-4 °C**.

Do not over stain in Schiff's reagent - it is irreversible.

Wash for several minutes at step 5 to bring out the color of the Schiff's

PERIODIC ACID SCHIFFS (PAS)

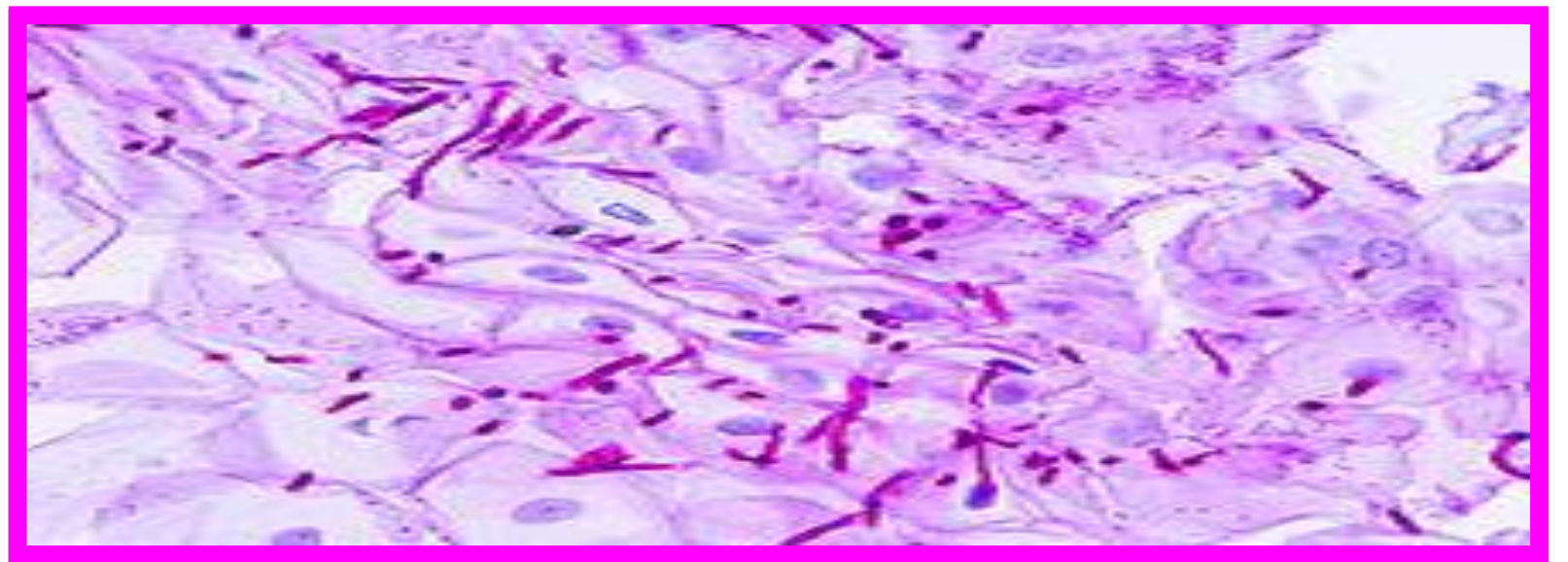
STAINING PROCEDURE:		
1. Remove paraffin wax	Xylene	
2. Remove xylene with	100 % absolute alcohol 2min	
3. Apply	95% alcohol	1min
4. Apply	70% alcohol	1min
5. Rinse in	Distilled water-	
6. Apply	1% periodic acid	5mins
7. Wash in	Tap water	2mins
8. Rinse in	Distilled water	
9. Treat with	Schiff`s reagent	15 to 30mins
10. Wash in	running water	10mins
11. Counter stain with	Mayers haematoxylin	5mins
12. Wash in	running water	1min
13. Blue in	Ammonia water	30secs
14. Wash in	running water	2mins
15. Deep in	70% alcohol	1min
16. Deep in	95% alcohol	1min
17. Dehydrate in 2 changes	100% alcohol	1min
18. Clear in 2 changes	Xylene	2mins.

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RESULT:

Polysaccharides—Red or Magenta color)

Nuclei---- Blue



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SHARPPENING OF MICRTOME KNIVES



Three stages are followed to prepare the microtome knives:

1. Grinding:

This is when setting is mould. It is done in the factory.2.

2. Honing:

Is done manually or by machine designed for this purpose. It is a rectangular block of nature or synthetic stone graded course. When using these types of stones, you have to apply lubricant.

3. Stropping:

It is to polish the knife edge after honing is important. This is done in a soft surface on a strop.

➤ **MICROTOME KNIFE:**

- Are very dangerous instruments. It should always be kept in its box with the lid fastened.
- Do not leave the knife attached to the microtome when not in use. All knives have edges.
- They are called cutting edge or knife.
- After prolonged use or after cutting very hard tissue, the cutting edge maybe destroyed (damage: presence of knifes, or blunt knife) so a new edge has to be created by a process called honing.