VIVEKANANDA COLLEGE THAKURPUKUR

KOLKATA-700063

NAAC ACCREDITED 'A' GRADE

Topic: Feulgen Reaction

Course Title: Cell Biology

Paper: CC-4

Unit: Laboratory Course

Semester: 2

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Name of the Department: Zoology

Preparation of permanent slides to demonstrate DNA by Feulgen reaction

Principle:

The Feulgen reaction is a highly specific method for DNA. When sections are treated with 1.0 M HCl at 60°C for 5-20 minutes, most of the RNA is broken down to soluble substances and lost from the tissue, but DNA is only partly hydrolysed. The purine and pyrimidine bases of the DNA are removed from the deoxyribose residues, which remain in their original positions and are capable of reacting as aldehydes. The aldehyde groups liberated by the Feulgen hydrolysis are then stained with Schiff's reagent imparting a deep pink to purple colour.

Reagents and their preparations:

a. 1M Hydrochloric acid

HCl (conc.)	8.5 ml
Distilled water	91.5 ml

b. Xylene
c. Graded series of alcohols: Absolute, 90%, 70%, 50%
d. Distilled water
e. Schiff's reagent

   1 g. Basic fuchsin was dissolved in 200 ml of boiling distilled water, removing the flask before adding the Basic fuchsin. The solution was allowed to cool to 50°C, and 2 g. Potassium metabisulfite added with mixing. The solution was allowed to cool to room temperature. Then 2 ml concentrated HCl was added, mixed, 2 g. Activated charcoal added and left overnight in the dark at room temperature. It was filtered through a No. 1 Whatman paper; the solution should be either clear or a pale yellow colour. It was stored in a dark container at 4°C.

f. Bisulfite solution

10% Potassium metabisulfite	5 ml
1M HCl	5 ml
Distilled water	90 ml

g. Counterstain (optional)

Light green	1%

h. DPX (or glycerine)

Method:

1. 1 M HCl was pre-heated to 60°C in a covered container.
2. Paraffin sections were de-waxed in xylene and hydrated through descending grades of alcohols.
3. Sections were placed in 1M HCl at 60°C for 8 minutes (for Carnoy or Formalin fixed sections).
4. Rinsed in water.
5. Immersed in Schiff's reagent, at room temperature for 30-45 min (until nuclei are stained pink).
6. Sections transferred directly to bisulfite water: 3 changes, each 10-15 seconds with agitation.
7. Washed in distilled water
8. Counterstained (if desired) in 1% light green, 2 min.
9. Washed briefly in water.
10. Dehydrated through ascending grades of alcohols, cleared in xylene and mounted in a resinous medium (or directly mounted in glycerine).

Results:

DNA:	Red-purple
Cytoplasm:	Green (if counterstain is used, otherwise colourless)

Notes:

1. If Schiff's reagent has been refrigerated, it must be allowed to warm to room temperature.
2. The purpose of the bisulfite rinse is to prevent the recolourization of Schiff's reagent, which occurs on dilution with water alone and can cause artifactual background staining.
3. Bouin's fluid and acid decalcifying agents can attack DNA and with prolonged contact may hydrolyse it excessively with consequent loss of stainability by the Feulgen method. So, Bouin's fixed tissue sections are not stained.
4. The ribosyl residues of any RNA that is not removed during the hydrolysis do not react as aldehydes. This property is due to the presence of a –OH group at position 2′ of ribose.

5. Schiff's reagent reacts with aldehydyes, but not with ketones, to from brightly coloured products. The two principal coloured compounds formed when Schiff's reagent reacts with tissue-bound aldehyde groups are alkylsulphonic acid derivatives of pararosaniline that exist in tautomeric equilibrium.

Applications:

The intensity of staining by the Feulgen method is proportional to the concentration of DNA. Thus it is possible to make microphotometric and morphometric observations and determine the quantities of DNA in the nuclei of cells. A valuable application of the Feulgen reaction in pathology is the quantitation of cells that contain abnormal quantities of DNA in malignant tumours.