PAS

Cytochemical staining on blood or bone marrow smears for the differential diagnosis of acute leukemias

10 x 4 test **REF 3081**

PREFACE

The kit has been designed to reduce the reagents volume and minimize the exposure of the operator to the chemicals contact between the laboratory personnel and toxic reagents, to simplify the procedure and the disposal of the reagents. Based on current knowledge, the least toxic and polluting reagents were used in the kit**.**

REACTION PRINCIPLE

The Periodic Acid-Schiff (PAS) reaction is an important method for identifying lymphatic cellular elements. Along with the peroxidase and esterase reactions, it is one of the three cytochemical stains crucial for the differential diagnosis of acute leukemias. The method involves incubating blood or bone marrow smears with periodic acid and Schiff's reagent. The reaction detects glycogen present exclusively in the cytoplasm: its oxidation by periodic acid results in the formation of free aldehyde groups which produce an intense red-purple staining particularly evident in granulocytes, in the presence of Schiff's reagent. The intensity and frequency of stained granules in cells are evaluated under an optical microscope. The kit can also be used to differentiate lymphoblastic from myeloblastic leukemia since some non-Hodgkin lymphomas and, especially, chronic lymphocytic leukemia present an increase in cellular glycogen.

REAGENTS AND MATERIALS

Kit components:	**REF 3081**
REAGENT 1 Periodic acid	**1 x 45 mL**
*** REAGENT 2** Schiff's reagent	**1 x 45 mL**
PLATES Disposable multi well (4 wells for each plate)	**10**
COVER in black color for the plates	**1**

(*) Dangerous reagents are market by an asterisk. Refer to MSDS.

STABILITY: sealed and stored at 2-8°C, reagents are stable up to the expiration date on the label.

REAGENTS NOT PROVIDED

FIXATIVE:

Preparation:	formaldehyde 37%	1 volume
	absolute ethanol	9 volumes

COUNTERSTAINING: Harris hematoxylin.

MATERIALS REQUIRED BUT NOT PROVIDED

Optical microscope 400x or 1000x for slide reading.
Pipettes with disposable tips or Pasteur pipettes for sampling and dispensing the reagents.
Timer.
Deionized water

SAMPLE

Peripheral blood smears (preferably capillary) or bone marrow smears. Blood samples can be collected with EDTA or heparin. Blood or bone marrow smears can be stored at room temperature (18-26°C) and protected from dust for several days without any significant variation in activity. Fixed slides can be stored for many weeks.

PROCEDURE

A) FIXATION OF THE SLIDES (see notes)

1. Fix the air-dried smears for 1 minute in the fixing solution.
2. Wash both sides of the slide in plenty of deionized water, drain it, and wait till it is dry. The recommended fixative contains formaldehyde. Even a small amount of formaldehyde on the slides can inhibit the enzyme. Therefore, it is necessary to completely remove the fixative.

B) PAS REACTION

Bring the reagents at room temperature before use.

1. Put the needed multi-well plates on a flat surface.
2. Put the slides on the plate with the smear facing downwards, towards the bottom of the well, to ensure that the working solution come into contact with the smear.
3. Put the slide against one of the two long edges of the well. Between the longer side of the slide and that of the well, there will be a long groove into which the reagents will be injected.
4. Take 1 mL of Reagent 1 using a pipette or a Pasteur pipette. Insert the tip of the pipette or Pasteur pipette into the central area of the groove and slowly inject the reagent. It will spread in the well, coming into contact with the smear. Less than 1 mL is enough to fill the well. Proceed the same way with the other slides.
5. Incubate for 10 minutes at room temperature (18-26°C).
6. Remove the slides with tweezers or fingers (wearing disposable gloves) and rinse them in running water for 10 minutes. To facilitate this step, gently press one end of the slide so that the other one lifts.
7. Place the slides on new multi-well plates.
8. Take 1 mL of Reagent 2 and inject it into the groove.
9. Incubate for 30 minutes at room temperature (18-26°C).
10. Remove the slides and rinse them in running tap water for 5 minutes.

C) COUNTERSTAINING (see notes)

1. Counterstain with Harris hematoxylin for 5 minutes.
2. Rinse in running tap water, dry, and read under the optical microscope.

RESULTS

Glycogen appears as red-purple granulations in the cellular cytoplasm, while the nucleus is stained in a pale green.

Are PAS-positive:
1) The cells of granulocytic origin, except for the myeloblast.
2) The cells of megakaryocytic origin, characterized by strong positivity.

Are PAS-negative:
1) The cells derived from plasma cells.
2) The cells of the normal erythroid lineage.
3) Normal lymphocytes.

PATHOLOGY

The erythroblasts of erythremia and erythroleukemia are characterized by a marked granular PAS positivity in proerythroblasts and basophilic erythroblasts, with diffuse positivity in further maturation phases. Lymphoblasts in acute lymphoblastic leukemia (ALL) are frequently positive (LLA-common) with granular positivity (some granules in the cytoplasm), and this aspect is used in diagnostics. Megakaryoblasts may have peripheral positivity. Plasma cells are usually positive. In chronic lymphocytic leukemia (CLL), lymphosarcoma, Hodgkin's lymphoma, and some infectious diseases (e.g., mononucleosis), an increased number of lymphocytes containing small PAS-positive granules are present.

NOTES

Plates can be used for fixing and counterstaining the smear. In this case, arrange the slides as described in section B) and inject the fixing solution or dye into the groove instead of the reagents. Follow the fixation and counterstaining times and the corresponding washes as described in sections A) and C).

DISPOSAL OF WASTE

Dispose of reagents and used materials according to the regulations of your country.

BIBLIOGRAPHY

Available upon request.

KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	read instructions for use

IVD CE Ed. 03- Dec. 2023 RR

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