PERLS

Cytochemical staining on blood, bone marrow or urinary sediment smears for the diagnosis of iron-deficiency anemia

10 x 4 tests **REF 3098**

PREFACE

The kit has been designed to reduce the reagents volume and minimize the exposure of the operator to the chemicals, 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.

PRINCIPLE

In an acid solution the organic iron present in the cellular cytoplasm reacts with potassium ferricyanide forming a blue-green complex (Prussian blue). The staining reveals the eventual presence of hemosiderin in erythrocytes (siderocytes), erythroblasts (sideroblasts), and phagocytic reticular cells. The presence of colored granules in cells is evaluated under an optical microscope. The kit is used in the diagnosis of all cases of hypo- or hyperferremia, in blood and also in urine.

REAGENTS AND MATERIALS

Kit components:	REF 3098
REAGENT 1 Potassium ferrocyanide (freeze-dried)	**10 x 6 mL**
REAGENT 2 Hydrochloric acid 0.2 N	**1 x 50 mL**
REAGENT 3 Harris Hematoxyline	**1 x 50 mL**
PLATES Disposable multi well (4 wells in each plate)	**10**
COVER in black color for the plates	**1**

Refer to MSDS.

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

REAGENTS REQUIRED BUT NOT SUPPLIED

FIXATIVE SOLUTION: absolute methanol.

MATERIALS REQUIRED BUT NOT SUPPLIED

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

SAMPLE

Blood (preferably from capillary), bone marrow or urinary sediment smears (or cytocentrifuged).
Blood samples may be collected in EDTA or heparin. Samples can be stored at room temperature (18-26°C) and protected from dust, without any significant variation in activity.
Fixed slides can be stored for many weeks.

PREPARATION OF REAGENT 1

Add 2.5 ml of distilled water to one vial of Reagent 1.
Gently shake until complete dissolution.
STABILITY: 30 days at 4°C, protected from light.

MANUAL ASSAY PROCEDURE

A) FIXATION OF THE SLIDES (see notes)

1. Fix the air-dried slides for 5 minutes in the fixing solution.
2. Wash both sides of the slide thoroughly in deionized water, drain, and wait till it is dry.

B) PREPARATION OF THE WORKING SOLUTION

Bring the reagents at room temperature before use. Unscrew the screw cap and carefully remove the rubber cap from a vial of Reagent 1.

1. Take 2.5 mL of Reagent 2 using a pipette or Pasteur pipette and add it to a vial of Reagent 1. Replace the rubber cap and shake by inversion until the lyophilized reagent is completely dissolved.

STABILITY: the working solution must be used right after it is prepared.

C) PERLS STAINING

1. Put the needed multi-well plates on a flat surface. Each plate and each bottle of working solution allows to run 4 determinations.
2. Place 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 the well, there will be a long groove into which the working solution will be injected will be injected.
4. Take 1 mL of working solution using a pipette or Pasteur pipette. Insert the tip into the central area of the groove and slowly inject the working solution. The solution 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. Cover the well with the black cover. If more plates are used, put them one over the other before covering he plate with the black cover to protect it from light. Incubate for 30 minutes at room temperature (18-26°C).
6. Remove the slides with tweezers or fingers (wearing disposable gloves) and rinse them thoroughly in distilled water. To facilitate this step, gently press one end of the slide so that the other one lifts up.

D) COUNTERSTAINING (see notes)

1. Counterstain with Reagent 3 for 5 minutes.
2. Rinse in running tap water, dry and read under a microscope. Experience in cytochemical techniques allows for the evaluation of slides without counterstaining.

RESULTS

In normal conditions, 20 to 50% of erythroblasts show small iron blue granules (sideroblasts) in the cytoplasm. When those inclusions encircle the nucleus, the cell is called ring-shaped sideroblast. Inclusions containing iron in erythrocytes are generally found only in splenectomized individuals. Macrophages which accumulate iron (sideromacrophages) can be observed in bone marrow.

PATHOLOGY

PERLS staining is particularly useful in the diagnosis of sideropenic anaemia: in this disease sideroblasts are considerably reduced or even absent. Instead, in sideroblastic anaemia the iron granules quantity in sideroblasts is highly increased.
The hemosiderine is present in the urinary sediment of patients affected by intravascular hemolysis and it is shown as free granules or granules inside epithelial cells (e.g. paroxysmal nocturnal hemoglobinuria).

NOTES

Plates can be used for fixing and counterstaining the smear. In this case, put the slides as described in section C) and inject the fixing solution or the dye into the groove instead of the working solution. Proceed as described in sections A) and D) for fixation, counterstaining, and washes.

WASTE DISPOSAL

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

BIBLIOGRAPHY

Available upon request.

MANUFACTURER

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KEY SYMBOLS

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

IVD CE

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