ELIstain Paratest

Staining and concentration of parasitic elements in stools

100 tests
(Ref. 66702)

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1 - AIM

ELIstain Paratest allows performing:
·the direct examination of parasitic elements in stools with **R1** reagent (Para-Color solution).
·the concentration of parasitic elements based on Bailenger's method with staining by **R1** reagent.
Each kit allows 100 tests to be carried

2 – INTRODUCTION

Many parasites (protozoa - helminths) can cause intestinal and hepatic events. The presence of these parasites in intestines or bile ducts was confirmed by macroscopic and microscopic examination of stool. Clinical manifestations, patient examination including the notion of living in endemic areas, laboratory tests results such as blood hypereosinophilia direct parasitological diagnosis and techniques to implement.

3 – PRINCIPLE

Direct examination after staining by R1 reagent (Para-Color solution)

Para-Color is a differential staining process of parasitic elements using a mixture of staining agents one of which is Lugol.
Its utilization facilitates the detection of parasitic elements which appear to be yellow, yellow-orange or brownish-yellow on a more or less dark blue background.

Concentration of parasitic elements based on Bailenger's method with staining by R1 reagent (Para-Color solution)

Two phase method of concentration using ether as an organic solvent and **R2** reagent (a pH5 aceto-acetate buffer solution) as the aqueous phase. The examination of sediment is performed after staining by **R1** reagent which facilitates the detection of parasitic elements which appear to be yellow, yellow-orange or brownish-yellow on a more or less dark blue background.

4 - REAGENTS AND MATERIAL

Description	Quantity
R1: vial of 4.5 mL of Para-Color solution.	**1**
R2: vial of 750 mL of pH5 aceto-acetate buffer solution.	**3**
TUBE - 30 mL : conical tubes of 30 mL.	**100**
TUBE - 10 mL : conical tubes of 10 mL.	**100**
SPATULA: spatulas.	**100**

5 – PRECAUTIONS

- The reagents are intended for *in vitro* diagnostic use only and must be handled by authorized personnel.
- Tests are for single use only.
- Patient samples are potentially infectious. They must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.
- Do not use reagents after the expiry date.

PARA-COLOR GHS02 – GHS08 – GHS07

H225 : Highly flammable liquid and vapour.

H315 : Causes skin irritation.
H319 : Causes serious eye irritation.

H373 : May cause damage to organs through prolonged or repeated exposure.

P210 : Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P235 : Keep cool.
P260 : Do not breathe vapours.
P403 : Store in a well-ventilated place.

6- SAMPLE COLLECTION AND TREATMENT

Due to the fragility of some of parasitic stages as protozoa vegetative forms, it is recommended to treat stools as soon as possible after their collection.

7 - STABILITY, STORAGE AND PREPARATION OF REAGENTS

The reagents are ready-to-use.
The reagents stored at 18-25°C, **sheltered from sunlight**, in their original packaging, are stable until the expiry date indicated on the box.
Do not freeze.

8 - MATERIAL REQUIRED BUT NOT SUPPLIED

- Automatic pipette(s) with a pipetting volume adapted to the volume that will be measured;
- Haemolysis tubes;
- Ether or ethyl acetate;
- Pasteur Pipettes;
- Slides + coverglasses for microscopy;
- Contaminated waste containers.
- Physiological saline;
- Vortex;
- Centrifuge;
- Microscope;

9 – METHOD

Direct examination after staining by R1 reagent (Para-Color solution)

a. Homogenize stools.
b. Take out a volume of stools equivalent to the size of a pea and place it in a haemolysis tube containing 1 mL of thinner (physiological saline, distilled water or **R2** reagent).
c. Triturate and shake it to obtain a homogeneous suspension (Vortex shaker).
d. Using the micropipette, place 10 µL of **R1** reagent on a slide.
e. Using the Pasteur pipette, add 1 drop (or, using the micropipette, add 25 µL) of the stools suspension to examine.
f. Mix well.
g. Place a coverglass over the stools suspension and examine by using a microscope having a white light (blue filter).

Concentration of parasitic elements based on Bailenger's (method with staining by R1 reagent (Para-Color solution)

a. Pour 20 mL of **R2** reagent in a 30 mL conical tube.
b. Homogenize stools.
c. Take out the equivalent of a knob of the stools (3 to 4 g or 3 to 4 mL if the stools are fluid) and place it in the **R2** reagent.
d. Triturate the stools by using a spatula and shake the mixture vigorously until a homogeneous suspension is obtained (Vortex shaker).
e. Permit the mixture to settle for 2 or 3 minutes for the sedimentation of the coarser particles.
f. Pour 5 mL of supernatant in a 10 mL conical tube.
g. Add 2.5 to 3 mL of ether.
h. Plug the tube and shake vigorously in order to obtain an emulsion (shake manually or with a Vortex shaker).
i. Take the plug out of the tube and centrifuge the mixture (150-200 g) for 5 minutes to "break" the emulsion.
j. In the case of gelling in the higher phase (due to lipophile residues), loosen the emulsion from the tube walls using the Pasteur pipette.
k. Eliminate the supernatant by turning the tube upside down.
l. Put the sediment in a suspension using 1 or 2 drops of physiological saline (do not allow the sediment to dry up).
m. Using the micropipette, place 10 µL of **R1** reagent on a slide.
n. Using the Pasteur pipette, add 1 drop (or, using the micropipette, add 25 µL) of the suspension to examine.
o. Mix well.
p. Place a coverglass over the sediment suspension and examine under a microscope using a white light (blue filter).

N.B.: POSSIBILITY OF USING ETHYL ACETATE AS ORGANIC SOLVENT INSTEAD OF ETHER

Ether (diethyl ether —C_2H_5-O-C_2H_5) is the reference solvent for **Bailenger** concentration method. It can be replaced by ethyl acetate in equal volume. It is however necessary to note that the solubilisation of certain faecal fragments is more difficult with ethyl acetate than with diethyl ether. **So, with some stools treated with ethyl acetate, the interface layer may be thicker and the more voluminous sediment may present numerous non-parasitic elements which may hamper microscopic observation.** It is then advisable to take back the sediment with more diluent, for example with 4 drops of physiological water instead of 2, and to make several assemblies for the microscopic examination.

10 - INTERPRETATION OF RESULTS

The parasitic elements appear to be yellow, yellow-orange or brownish-yellow on a more or less dark blue background.

11- CAUSES OF ERROR AND TEST LIMITS

In all cases, it is necessary that the clinical, epidemiologic and biological data are taken fully into consideration before establishing the final diagnosis.

12 – PERFORMANCE

A comparative study to search for parasitic elements was performed on 83 stools using conventional techniques (direct examination without staining - Bailenger without staining) and **ELIstain Paratest** kit.
For direct examination and examination after concentration, qualitatively, identical results were obtained with the different techniques in terms of identification of parasite and parasitical stage. It was noted that **R1** reagent (Para-Color solution) use permits easier reading.
Quantitatively, for a large number of stools, cysts or eggs number that were obtained with the different techniques was identical or similar. For a small number of stools, differences were observed but were considered not significant because less than 30% between the techniques used.
All the results concluded that techniques using reagents of **ELIstain Paratest** allowed detection at least equal to that of standard techniques.

13 - WASTE ELIMINATION

Waste should be disposed of in accordance with the hygiene rules and current regulations for this kind of product in the country of use.
If the reagent is spilled, clean the work area with absorbent paper and rinse with water. If a sample is spilled on the work area, clean using bleach and absorbent paper.

14 – BIBLIOGRAPHY

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Changes from the previous version are highlighted in gray

ELITech MICROBIO
Parc d'Activités du Plateau
19 Allée d'Athènes
83870 SIGNES
FRANCE
Tel : 04 94 88 55 00
Fax : 04 94 88 55 22
http://www.elitechgroup.com