# ELIstain Para-Color

**Differential staining process of parasitic elements in stools**

100 tests
(Ref. 66704)

**8000315-EN-2025-09_V3**

For *in vitro* diagnostic use only, for professionnal use only

## 1 - AIM

**ELIstain Para-Color** is a reagent for staining parasitic elements in stools during:

- A direct examination ;
- An examination of sediments obtained after concentration of parasitic elements by a two-phase method, with the exception of those which have already employed a staining agent.

Each kit allows 100 tests to be carried out.

## 2 - INTRODUCTION

Many parasites (protozoa - helminths) can cause intestinal or hepatic pathology. The presence of these parasites in intestines or bile ducts is confirmed by macroscopic and microscopic examination of stool. Clinical manifestations, patient examination including the notion of a stay in endemic areas, laboratory blood test results such as hyper-eosinophilia, guide parasitological diagnosis and the corresponding implementation technique (1,2).

## 3 - PRINCIPLE

**ELIstain Para-Color** is a differential staining process of parasitic elements using a mixture of staining agents including Lugol.
Its utilization facilitates parasitic elements detection which appear as a yellow, yellow-orange or brownish-yellow coloration on a more or less dark blue background (1,3).

## 4 - REAGENT

| Description | Quantity |
|---|---|
| R1: vial of 1 mL of Para-Color solution. | 1 |

## 5 - PRECAUTIONS FOR USE

- The reagent is intended for *in vitro* diagnosis only and should be handled by authorized and trained persons.
- Tests are for single use only.
- Patient samples are potentially infectious. They must be handled with the usual precautions in accordance with the hygiene rules and the regulations in force for this type of product in the country of use.
- Do not use reagent 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 any other source of ignition. No smoking.
P235: Keep cool.
P260: Do not breathe vapors.
P403 : Store in a well-ventilated place.

## 6 – SAMPLE COLLECTION AND TREATMENT

All stool should be collected without urine (which kills protozoa) and in a clean container that is dry and transparent but not necessarily sterile.

Given the fragility of some parasitic stages, such as protozoal vegetative forms, it is recommended to treat stool as soon as possible after collection, either within 6 hours if the sample is stored at room temperature or within 2-3 days if stored at 2-8°C.

## 7 – REAGENT PRESERVATION AND PREPARATION

The product is ready for use.
The reagent stored at 18-25°C, **protected from sunlight,** is stable until the expiry date indicated on the box.
It should not be frozen.

## 8 - EQUIPMENT REQUIRED BUT NOT PROVIDED

- Automatic pipette(s) with a pipetting volume adapted to the volume that will be measured;
- Haemolysis tubes;
- Physiological saline ;
- Taking stirrers;
- Vortex ;
- Pasteur pipettes ;
- Slides + cover glasses for microscopy;
- Microscope ;
- Contaminated waste containers.

## 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 (300 mg +/- 50 mg regardless of its consistency) and place it in a hemolysis tube containing 1 mL of solvent (physiological saline, distilled water or pH5 aceto-acetate buffer solution).
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 25 µL with a micropipette) of the stool suspension to the slide.
f. Mix thoroughly.
g. Place a cover glass over the stool suspension and examine under white light (blue filter) with a microscope.

Note: The preparation must be read extemporaneously to avoid lysis of certain fragile parasitic elements.

### Examination of sediment obtained after parasitic elements concentration by a two-phase method

a. Put in a suspension the sediment obtained after a two-phase concentration method (e.g. Bailenger's method) using 1 or 2 drops of physiological saline (do not allow the sediment to dry).
b. Using the micropipette, place 10 µL of **R1** reagent on a slide.
c. Using the Pasteur pipette, add 1 drop (or 25 µL with a micropipette) of the suspension to the slide.
d. Mix thoroughly.
e. Place a cover glass over the sediment suspension and examine under white light (blue filter) with a microscope.

## 10 – INTERPRETATION OF RESULTS

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

## 11 - CAUSES OF ERRORS AND TEST LIMITS

Some elements can lead to confusion in parasitic coprological diagnosis. These misleading aspects can lead to false-positive tests. The recognition of many residues of digestion is fundamental so as not to confuse them with parasitic elements (e.g. pollen grain, fungal spores, certain fruits hair, parasites in transit following the ingestion of contaminated animal liver, etc.) (4)

Some non-resorbable treatments may interfere with the recovery and collection of parasites (castor oil, mineral oil, bismuth, antibiotics, antimalarials, etc.).

A single examination of the parasitic elements of the negative stools is of no value. The examination must be repeated taking into account the existence of mutilation periods in the expression of certain parasites. In the event of a negative stools, it is necessary to take 2 additional samples at 4-5 day intervals between each collection.

The search for parasites must be carried out by personnel trained and qualified in parasitic coprology.

In all cases and before the establishment of the final diagnosis, the interpretation of the test must be carried out in the context of all the clinical, epidemiological and biological data, as well as other test results.

## 12 - QUALITY CONTROL

Quality control can be carried out from the controls sold by the companies KaliDiv and Eurobio SCIENTIFIC:

-Suspension of stool with *Giardia duodenalis* cysts (ref CQI KD-Col/PM/Susp-G.dK at KaliDiv): Subspherical (7-10 µm x 8-12 µm), contains two or four nuclei depending on its stage of maturity and residues of flagella and median bodies. Yellow to orange-yellow coloring on a blue background.

-Suspension of stool with vegetative forms of *Entamoeba histolytica* (ref CQI KD/Col/PM/Susp-Eh Fv from KaliDiv): Size from 15 to 40 µm with a slightly coloured ectoplasm and a granular endoplasm. The core is large, 6-8 µm. It has a regular and "beaded" fine peripheral chromatin with a small central or subcentral karyosome. Yellow to orange-yellow coloring on a blue background

-Suspension of stool with *Trichuris trichiura* eggs (ref 1809-CQI from Eurobio SCIENTIFIC): Lemon-shaped eggs with a clear lid on both ends. Yellow to orange-yellow coloring on a blue background.

## 13 PERFORMANCE

A first comparative study carried out between ELIstain Para-Color and examination without staining (direct or after concentration) showed that ELIstain Para-Color allowed the staining and detection of helminth eggs, vegetative forms and protozoan cysts.
The color difference between the parasitic elements (yellow or orange-yellow coloration) and background (blue) allowed a fast microscopic detection of these elements and facilitated their identification.

In a second study, the performance evaluation of the ELIstain Para-Color method was performed compared to conventional microscopic methods (wet mount on slide and lugol staining) (5).
The study involved the analysis of stool samples collected from 117 patients.

The detection performance of intestinal parasitic diseases, sensitivity, specificity, positive and negative predictive values of each method were calculated and expressed as percentages with 95% confidence intervals.
Note: In the absence of a standardized reference method, the results obtained after the combination of several techniques both on the direct examination and on the examination after concentration were considered as a reference.

The performances obtained during the direct examination by the ELIstain Para-Color method are described below:

| Species | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| *Endolimax nana* | 93.3 %<br>IC à 95 % [84.4 - 100%] | 95.2 %<br>IC à 95 % [86.1 - 100%] | 96.5 %<br>IC à 95 % [90.1 – 98.8%] | 90.9 %<br>IC à 95 % [83 – 95. 4%] |
| *Giardia intestinalis* | 92.9 %<br>IC à 95 % [85.1 - 100%] | 100 %<br>IC à 95 % [100 - 100%] | 100 %<br>IC à 95 % [100 - 100%] | 75 %<br>IC à 95 % [50.5 – 99.5%] |
| *Blastocystis hominis* | 86.7 %<br>IC à 95 % [69.5 - 100%] | 100 %<br>IC à 95 % [100 - 100%] | 100 %<br>IC à 95 % [100 - 100%] | 94.7 %<br>IC à 95 % [87.7 – 97.9%] |
| *Ascaris lumbricoides* | 100 %<br>IC à 95 % [100 - 100%] | 50 %<br>IC à 95 % [0 - 100%] | 98 %<br>IC à 95 % [94.1 - 100%] | 100 %<br>IC à 95 % [100 - 100%] |
| *Trichuris trichiura* | 100 %<br>IC à 95 % [100 - 100%] | 100 %<br>IC à 95 % [100 - 100%] | 100 %<br>IC à 95 % [100 - 100%] | 100 %<br>IC à 95 % [100 - 100%] |

*PPV : Positive Predictive Value*
*NPV : Negative Predictive Value*

### 13.1 – Repeatability

On Internal Quality Controls of *Trichuris trichiura* eggs, vegetative form *of Entamoeba histolytica* and cysts of *Giardia duodenalis,* the ELIstain Para-Color device has a 100% repeatability in terms of parasite detection on 10 staining tests carried out.

### 13.2 – Reproducibility

On Internal Quality Controls of *Trichuris trichiura* eggs, vegetative form of *Entamoeba histolytica* and cysts of *Giardia duodenalis*, the ELIstain Para-Color device has a reproducibility of 100% in terms of detection of the parasite on 10 staining tests carried out.

## 14 - WASTE ELIMINATION

Waste must be disposed of in accordance with the hygiene rules and current regulations in force for this kind of product in the country of use.
In case of accidental spillage of reagent, clean the work area with absorbent paper and rinse with water. In case of sample spillage, clean with bleach and absorbent paper.

## 15 BIBLIOGRAPHY


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Any serious incident related to the device shall be notified to the manufacturer and to the Competent Authority of the Member State in which the user is established.

Changes compared to the previous version are highlighted in gray.


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