INSTRUCTIONS FOR USE

MINI-SYSTEM PARAGREEN

1 - INTENDED USE

In vitro diagnostic device. The Mini-System Paragreen is a disposable plastic device for the collection, fixation, filtration and concentration by sedimentation of intestinal parasites from faecal specimens.

2 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The collection vials of Mini-System contain a fixative for the morphological preservation of the parasites. The built-in filters eliminate the food debris providing a clean sediment after centrifugation. The parasites are identified by the examination of the concentrated sediment by low magnification microscopy.
The concentration procedure is a modification of the Formalin Ether (Ritchie method), as recommended by WHO[1].
The Paragreen patented fixative contains a heterocyclic compound which releases small amounts of formaldehyde after the reagent preparation and when it comes in contact with the biological sample.
Based on the amount of formaldehyde generated, the product is NOT classified as hazardous under Regulation (EC) 1272/2008 (CLP) and subsequent amendments and adjustments. In addition, Paragreen does not contain alcohol and heavy metals.
The filtration is performed by two filters of 400 and 250 microns, the optimal size for obtaining a clean sediment and a good recovery of helminths eggs and protozoa. The filters are included in the collection vial which contains glass beads for facilitating the homogenous suspension of the specimen.
After fixation, a sedimentation tube is screwed to the bottom of the collection vial, forming a closed system, which is then centrifuged. The addition of ether or ethyl acetate, normally needed to allow the filtration, is not required as the filtration happens during the centrifugation. After discarding the supernatant, an aliquot of the sediment is examined microscopically. The concentration technique can be combined with a permanent staining to improve de detection of protozoa.

3A – MATERIAL PROVIDED - KIT CONTENT

Product	Type	REF	Pack
Mini-System Paragreen Kit content ▪ 13 mm collection vials with 4 mL Paragreen fixative, two filters, glass beads and collection spoon ▪ Sedimentation tubes ▪ Sticks	Kit	25RPP7000	150 tests 150 pcs 150 pcs 150 pcs

Note:
Midi System Paragreen is also available with 98 collection vials with 10 mL fixative (REF 25RPP8000).

3B – MATERIAL AVAILABLE – ACCESSORIES NOT INCLUDED IN THE KIT

Product	Type	REF	Pack
Mini-System Caps	Perforated caps for sedimentation tubes	25RPP01	500 pcs
Mini-System Screw Caps	Screw caps for sedimentation tubes	25RPP04AB	500 pcs

4 - MATERIALS REQUIRED BUT NOT PROVIDED

1. Transfer pipettes
2. Microscope slides and coverslips
3. Centrifuge
4. Lugol's iodine (optional)
5. Microscope

5 – SPECIMEN COLLECTION

Mini-System Paragreen is intended for the detection of parasites in faecal specimens.

1. Faeces should be presumably collected before antimicrobial or anti-diarrhoeal therapy where possible and between 10 pm and midnight, or early in the morning, before defecation or bathing.[2]
2. Ideally, collect three stool samples over no more than a 10-day period. It is usually recommended that specimens are collected every other day.[2]
3. Faecal concentrations are carried out on all specimens where examination of parasites is specifically requested and where there are definite clinical indications. All faecal samples from symptomatic individuals should be tested for *Cryptosporidium* oocysts.[2]
4. Avoid the use of antidiarrheal or laxative medications before collecting the sample.
5. Collect the faecal specimen in a clean dry recipient.
6. Avoid contamination of the specimen with urine or water.
7. Mix the specimen thoroughly with the wooden stick and using the spoon under the cap, transfer one spoonful of the specimen into the collection vial. With deep recipients, the wooden stick can be used to fill the spoon. For liquid stool transfer approximately 1 mL with a disposable pipette. Do not open the bottom cap of the tube in any moment. Mix to homogenize the diluted specimen.
8. Close the tube tightly and send it to the laboratory for processing.

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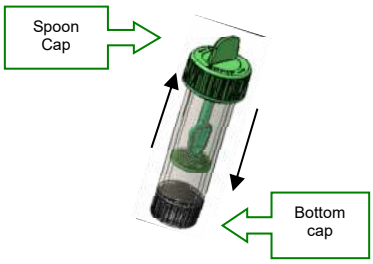
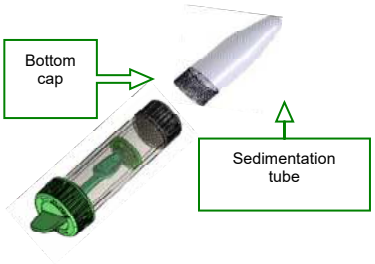
6 - TEST PROCEDURE

1- Shake vigorously the collection tube with the sample for 30 seconds

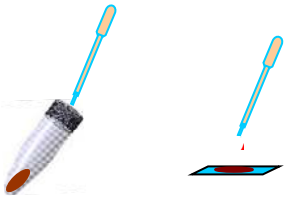


2- Keeping the vial upside down, replace the bottom cap with the sedimentation tube



3- Centrifuge for 3 minutes at 700 g (2000 rpm).

4- Discard the collection tube and all the supernatant. Re-suspend the sediment with 1-2 drops of water or saline, transfer few drops on a microscope slide and examine under the microscope at 100 x magnification



One drop of Lugol's Iodine can be added on the slide to enhance the visibility of parasites. A calibrated ocular micrometer is useful to measure helminths eggs for a correct identification. Examine the entire area of the coverslip in a systematic manner.
If needed, special caps can be ordered for the sedimentation tubes, useful for closing the tubes after centrifugation, to avoid spills and smell (REF 25RPP01). The cap is perforated so that a Pasteur pipette can be introduced to collect the sediment without the need of removing it. Screw caps are also available for permanent storage of positive samples (REF 25RPP04AB).

7 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory.

8- PERFORMANCES CHARACTERISTICS

- Mini System Paragreen was evaluated in a study on 121 stool specimens examined for parasites. Paragreen showed 93% correct results against 76% obtained by the classical in-house SAF concentration method.[3]
- Mini-System device was evaluated in a study on 100 stool samples for the detection of parasite structures, compared to the reference system consisting of the modified Ritchie technique. The concordance between the two methods was 94.6%, the sensitivity 87%, the specificity 100%, the predictive value of the positive result 96%, the predictive value of the negative result 100%.[4]
- The influence of the Paragreen Fixative on the performances of immunofluorescence and immunochromatographic assays for the detection of faecal parasitic antigens was evaluated. The results obtained show that Paragreen fixative does not interfere with the immunological tests since no false positive or negative results were seen.[5,6]

9 - LIMITATIONS OF THE METHOD

- Due to the intermittent shedding of the parasites, negative tests should be repeated from new specimens collected in different days.
- Fresh faeces specimens are essential for the examination of trophozoites.[2]
- Mini-System Paragreen is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

10- PRECAUTIONS AND WARNINGS

- Mini-System Paragreen is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- All samples must be treated as a potential source of infection. Ensure you follow correct laboratory guidelines at all time. Disposable gloves should be worn for all parasitology investigations.
- Sterilize all biohazard waste before disposal. Dispose the unused vials with the fixative and the sterilized vials inoculated with samples in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be

observed for the examination of samples collected from human and animal organic districts. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

11 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the products in the original package at 10-30°C. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Collection vials with Paragreen are a "closed system" for single use and must not be opened except when in use.

12 – REFERENCES

1- Bench aids for the diagnosis of intestinal parasites, second edition. Geneve: World Health Organisation; 2019. https://www.who.int/publications/i/item/9789241515344
2- Public Health England. (2017). Investigation of specimens other than blood for parasites. UK Standards for Microbiology Investigations. B 31 Issue 5.1. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-andconsistency-in-clinical-laboratories.
3- Tritten ML, Stauffer J, Siegrist HH, Lienhard R. Comparison of two commercial Concentration Devices for the Recovery of intestinal Parasites in Stools with the Reference Method (Poster) - Annual Swiss Society for Microbiology Meeting, Basel, 30 Aug – 1 Sep, 2017.
4- Lucrecia Acosta Soto, Luis Navarro Martínez, Fernando Jorge Bornay Llinares. Área de Parasitología, Departamento de Agroquímica y Medio Ambiente. Universidad Miguel Hernández de Elche. 04/08/2011- Unpublished data.
5- Maria Teresa Manfredi. Parasitology and Parasitic diseases Professor. Head of DIVET Lab Parasitology Laboratory, University of Milan. Evaluation of influence of the Paragreen Fixative on the performances of immunofluorescence assay for the detection of Giardia spp. cysts and Cryptosporidium spp. oocysts. September 2017. Unpublished report.
6- Maria Teresa Manfredi. Parasitology and Parasitic diseases Professor. Head of DIVET Lab Parasitology Laboratory, University of Milan. Evaluation of influence of the Paragreen Fixative on the performances of immunochromatographic assay for the detection of fecal antigens. September 2017 Unpublished report

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	For single use only	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 5	Updated layout and content	2023/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.