BIOSYNEX AMPLIQUICK® Helminths

Faecal parasite detection kit using gene amplification.

For professional *in vitro* diagnostic use only.

EN

CE

REF 3150066_SEC01 / 3150066_SEC02 / 3150066_TR02

1 I OBJECTIVES

BIOSYNEX AMPLIQUICK® Helminths is an *in vitro* molecular diagnostic kit for the qualitative detection of ten intestinal helminths in DNA extracts obtained from stool samples. It is presented in the form of ready-to-use pre-filled microplates of master mix. The test specifically detects *Schistosoma mansoni, Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura, Diphyllobothrium latum, Taenia spp, Hymenolepis nana, Enterobius vermicularis, Ancylostoma duodenale, Necator americanus*. A second kit, BIOSYNEX AMPLIQUICK® Protozoans (Ref. 3150067) that allows the qualitative detection of ten intestinal protozoans from the same DNA extracts, is available. The kit is intended to be used by laboratory staff for *in vitro* molecular diagnostics only.

2 I INTRODUCTION

Intestinal parasite infections constitute one of the biggest public health issues in the world. As well as affecting millions of people in developing countries, these diseases are also observed in industrialised nations. They are usually caused by two types of intestinal parasites: helminths and protozoans.

Helminths are parasitic worms that can live in humans and some animals, and can be divided into two main families: nematodes (round worms – *Ascaris, Trichuris, Ancylostoma*, etc.) and platyhelminths (flat worms – *Taenia, Diphyllobothrium, Hymenolepis*, etc.). The infections caused by these parasites (helminthiases) cause abdominal pain, nausea and vomiting, heartburn, diarrhoea and ulcerative gastric lesions. Infestations of high intensity can cause malnutrition as well as growth and physical development problems.

Compared with conventional methods (microscopy), molecular detection methods present a number of advantages for the detection of intestinal parasites in stool samples, including higher sensitivity and specificity, the ability to target multiple parasites (multiplex), a reduced turnaround time and faster results.

3 I PRINCIPLE

The BIOSYNEX AMPLIQUICK® Helminths kit is an *in vitro* diagnostic test based on real-time polymerase chain reaction (PCR) technology. Kits with reference numbers 3150066_SEC01, 3150066_SEC02 and 3150066_TR02 consist of microplates with 12 strips of 8 wells. The first 5 wells of each strip are pre-filled with master mixes and the last 3 are empty. Each of these strips can be used to test one patient sample. The master mixes are ready to use and contain dNTPs, MgCl2, fluorescent primers and probes, Taq polymerase and reaction buffer. The test kit is presented in the form of 96-well plates that can be divided into strips of 8 wells each.

The test consists of a PCR step enabling specific amplification and simultaneous detection of sequences of interest on:

- the cytochrome C oxidase subunit I gene of *Schistosoma mansoni*
- the small subunit 18s ribosomal RNA gene of *Strongyloides stercoralis*
- the small subunit 18s ribosomal RNA gene of *Ascaris lumbricoides*
- the small subunit 18s ribosomal RNA gene of *Trichuris trichiura*
- the cytochrome C oxidase subunit I gene of *Diphyllobothrium latum*
- the intergenic spacer (IGS) of *Taenia spp*
- the internal transcribed spacer 2 (ITS2) of *Hymenolepis nana*
- the 5s ribosomal RNA gene of *Enterobius vermicularis*
- the internal transcribed spacer 1 (ITS1) of *Ancylostoma duodenale*
- the internal transcribed spacer 1 (ITS1) of *Necator americanus*

This method uses probes labelled with the fluorophores FAM, HEX and Cy5. The master mixes present in the plates enable the simultaneous amplification of two parasite parameters with the FAM and HEX probes, and internal amplification control with a third probe labelled with fluorophore Cy5. This internal control (CIEZ sequence) identifies any PCR inhibition, thus excluding false negative results. A procedural or in-process control (IPC), amplified in the fifth well of the strip (also with a Cy5-labelled probe) validates the DNA extraction and purification steps.

Increased fluorescence signal is only detected if the complementary target sequence of the amplified probe is present in the sample. The fluorescence signal is therefore directly proportional to the amplification of the target during the amplification phase. The Cq (quantification cycle) value corresponds to the cycle at which fluorescence starts to increase exponentially compared with the background noise.

This amplification kit can be used with purified DNA extracts obtained from stool samples treated with the BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit (ref 3150065).

4 I KIT CONTENTS

Equipment provided

5 ready-to-use microplates divisible into 8-well strips
1 positive control (CONTROL +, red cap, 200µL q.s. 15 reactions)
1 negative control (CONTROL -, green cap, 200µL q.s. 15 reactions)
1 procedural control (CONTROL IP, white cap, 620µL q.s. 60 reactions)
1 control master mix (CONTROL Mmix, blue cap, 420µL q.s. 30 réactions)
5 pouches of strips of transparent caps
1 instruction leaflet

Equipment required but not supplied

BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit (Ref: 3150065)
DNA extraction kit
Powder-free disposable gloves
Micropipettes & filter tips
Real-time PCR thermal cycler
Centrifuge for microplate or PCR strips

The real-time PCR device used for the test must be an “open” system with at least the following key features:

- Real-time PCR quantitative tests.
- Programmable thermal cycler block (0.1mL low-profile for REF 3150066_SEC01 or 0.2mL high profile for REF 3150066_SEC02 and 3150066_TR02).
- Excitation source: LEDs, lamp or laser.
- Set of filters (Excitation/Emission wavelengths) for detecting the “reporter” fluorophores of the FAM, HEX and Cy5 probes.
- Connection to a computer using specific analysis software to retrieve fluorescence data and interpret the results.

REF 3150066_SEC01

The kit has been validated for use with the following thermal cyclers: CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad), CFX96 Opus™ Real-Time PCR Detection System (Bio-Rad), QuantGene 9600 (BIOER), QuantStudio 5 System (Applied Biosystems), Dt Lite 48/96 (DNA Technologies), LightCycler480 (Roche).

REF 3150066_SEC02 and 3150066_TR02

The kit has been validated for use with the following thermal cyclers: CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad), CFX96 Opus™ Real-Time PCR Detection System (Bio-Rad), QuantGene 9600 (BIOER), QuantStudio 5 System (Applied Biosystems), Dt Lite 48/96 (DNA Technologies).

If another thermal cycler is used, please perform a validation of the BIOSYNEX AMPLIQUICK® Protozoans kit before using the test.

5 I PRECAUTIONS

- For *in vitro* diagnostic use. For professional laboratory use only.
- Use the kit and its components by the expiry date only.
- - Wells and caps are for single use only. Do not reuse.
- Do not expose the Master Mixes (plates and control master mix tubes) to direct light for extended periods of time.
- For best results, carefully follow the storage procedure and conditions.
- In the event of damage to the packaging only (no breakage or leakage), the kit remains usable. If the aluminium sealing film is punctured or has detached, do not use the plate or strip in question. If a tube is damaged or leaks, do not use it.
- Follow good laboratory practice. Use disposable powder-free laboratory gloves throughout the test procedure. Consider the samples potentially infectious and handle them with care, as per laboratory guidelines.
- Centrifuge the plates before opening and carefully peel back the aluminium film to avoid spillage of the master mix.
- Centrifuge the tubes before opening; open them one at a time, making sure to close them properly between each pipetting to avoid any contamination. Preferably open and add the negative control before the positive control.
- The positive control contains significant amounts of the target sequences. It can therefore potentially contaminate the other components of the kit if good molecular biology practices are not followed. To limit this risk of contamination, it is recommended to store this component outside the kit as soon as the kit is opened.
- The daily processing of a large number of samples and the high sensitivity of the PCR process may generate false positives through contamination, if care is not taken. Pre-PCR, post-PCR and DNA extraction handling operations should therefore be done in different rooms. The work flow in the laboratory must follow a one-way system.
- Wear disposable gloves in each zone and change them before passing from one zone to another.
- Clean up any sample splashes using appropriate disinfectant.
- Dispose of contaminated or empty kit components in a biological waste bin. Comply with local regulations on biowaste disposal.
- If using the BIOSYNEX AMPLIQUICK® Helminths kit leads to death or serious deterioration of health, the manufacturer and the local competent authority must be notified. If in doubt, report it.
- Safety data sheet available on request.

6 I STORAGE AND STABILITY

The kit is dispatched frozen and the components should arrive frozen. Upon arrival, they should be stored at -20°C. Under such conditions, the reagents remain stable until the expiry date specified.

The positive and negative controls and the procedural control can tolerate up to 30 thaw/freeze cycles..

The control Master mix can undergo up to 15 thaw/freeze cycles without affecting reagent performance.

Only remove and defrost the number of well strips or plates of master mix needed. As the plates and strips are ready to use, there is no need for repeated thaw/freeze cycles.

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It is recommended that plates or strips containing master mixes are placed in a cooling rack or on ice once they have been removed from the freezer and when samples are being added. Once the aluminium film has been removed, use the plate or wells immediately.

7 I SAMPLE COLLECTION AND STORAGE

Collect and store samples in accordance with the instructions included in the BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit (Ref. 3150065).

8 I EXTRACTION OF NUCLEIC ACIDS

You can use your own DNA extraction systems or commercial kits. We recommend respecting a 2:1 ratio of test volume to elution volume for the DNA extraction step (e.g. if 200µL of pretreatment supernatant is used for extraction, the DNA is eluted in 100µL). The kit has been validated for use with the following extraction kits:

- Macherey-Nagel NucleoMag Pathogen
- Macherey-Nagel NucleoMag Microbiome
- Macherey-Nagel Nucleomag Dx Pathogen
- QIAGEN QIAamp® Fast DNA Stool Mini kit
- Amplix Bacterial DNA extraction kit
- MagNa Pure Roche
- Singuway Nucleic acid extraction reagents MTQM036

Comment: The Macherey-Nagel NucleoSpin DNA Stool kit is not compatible with the use of the BIOSYNEX AMPLIQUICK® Helminths amplification kit.

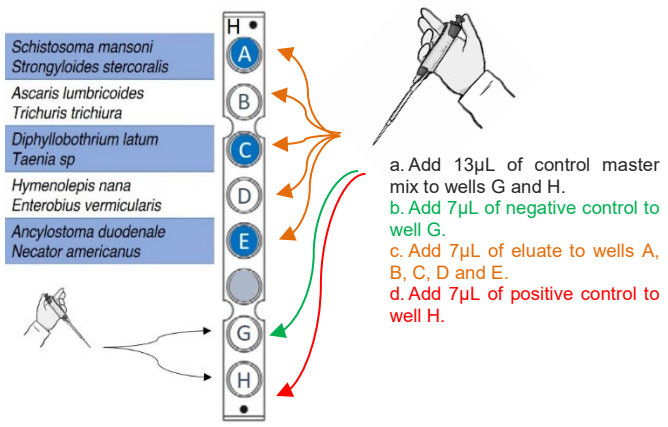
It is not necessary to extract the positive and negative controls using the nucleic acids extraction kit.
The procedural control provided in the kit should be added to the stool sample during pretreatment, before DNA extraction is performed (see the BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit instructions).
If the use of purified DNA is delayed, before adding it to the master mix, store it at 4°C or on ice if the test is to be done that day, otherwise store it frozen, at a temperature of at least -20°C.

9 I AMPLIFICATION PROTOCOL

Preparation of the divisible microplate- Option 1

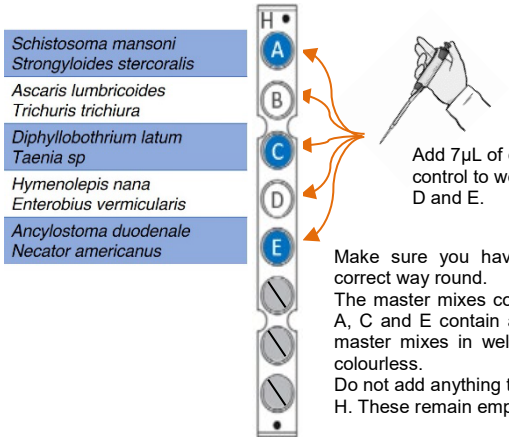
1. Take out one microplate and breakaway the necessary number of strips (remember: 1 strip corresponds to 1 patient).
2. Centrifuge the strips for a few seconds in order to recover any droplets present on the edges of the well or on the sealing film.
3. Gently remove and discard the aluminum foil.
4. Add 13µL of control master mix to wells G and H of a single strip. If you are using multiple strips at the same time, it is not necessary to deposit control master mix into each strip.
5. Add 7 µL of negative control to well G containing the control mix added in the previous step.
6. Add 7 µL of sample or to the first 5 wells of strips A to E; do not add to well F.
7. Finally, place 7µL of positive control in well H containing the Master mix control added in step 4.
8. Seal the wells with the transparent caps provided. . Do not use the aluminium film.
9. Centrifuge the strips for a few seconds.

Make sure you have the strip the correct way round.
The master mixes in wells A, C and E contain a blue dye. The master mixes in wells B and D are colorless.
Do not place anything in the well F, it should remain empty.



Preparation of the divisible microplate – Option 2

1. Take out the microplate and remove the number of 8-well strips you need (remember: 1 strip for 1 patient or 1 positive control or 1 negative control).
2. Centrifuge the strips for a few seconds to collect any drops that may be on the sides of the well or on the sealing film.
3. Carefully remove the aluminium film and discard. Place 7 µL of sample or control into the first 5 wells on the strip, wells A to E; do not place anything into wells F, G or H.



Make sure you have the strip the correct way round.
The master mixes contained in wells A, C and E contain a blue dye. The master mixes in wells B and D are colourless.
Do not add anything to wells F, G or H. These remain empty.

4. Seal the wells with the transparent caps provided. Do not use the aluminium film.
5. Centrifuge the strips for a few seconds.

Place the strips in the thermal cycler and launch the following amplification programme:

PCR programme:

Stage	Repetition	Temperature	Duration	Acquisition
Activation	1x	95°C	3 min	-
Denaturation	50x	95°C	05 sec	-
Hybridisation/elongation		58°C	20 sec	yes

Enter a reaction volume of **20 µL** in the thermal cycler programme.
Please refer to the thermal cycler user instructions for necessary information about programming.
Detection channel settings:

Targets	Fluorochrome
Schistosoma mansoni (mix A) *Ascaris lumbricoides (mix B)* *Diphyllobothrium latum (mix C)* *Hymenolepis nana (mix D)* *Ancylostoma duodenale (mix E)*	FAM
Strongyloides stercoralis (mix A) *Trichuris trichiura* (mix B) *Taenia spp* (mix C) *Enterobius vermicularis* (mix D) *Necator americanus* (mix E)	HEX
Internal control – CIEZ (mix A, B, C, D) or IPC (mix E)	Cy5

Comment: For the DNA Technology DT LITE 48/96 thermal cycler, the exposure settings should be as follows:
- FAM channel: 300
- HEX channel: 300
- Cy5 channel: 500

Before using this device, it is also important to set the height of the tubes. To do this, place the plate on the thermal cycler block, then close the lid; go to the "Preferences Device diagnostics" menu, select "Measure height of the tube" and click on "OK".

10 I DATA ANALYSIS AND INTERPRETATION OF THE RESULTS

A. Test validation criteria

The threshold of the real-time PCR reaction corresponds to the level of signal that reflects a statistically significant increase compared with the baseline signal calculated; the value is set to distinguish a significant amplification signal from background noise. We recommend automatic setting of the threshold by the real-time PCR instrument software, rather than manual setting.

Negative control:
The fluorescence emitted must be below threshold. This is an indicator of non-specific amplification. If the fluorescence exceeds this threshold, check for the presence of an atypical curve.
In the event of an amplification curve, contamination or microtube distribution error is likely. Only the internal control signal should be amplified.

Positive control:
The positive control value should ideally be detected within 30 cycles (Cq ≤ 30). If there is no amplification of the positive control, an amplification issue or fluorescence detection problem (defective thermal cycler) is likely.

Internal amplification control and procedural control:
The internal control (wells A to D) ensure that the enzymes in the master mix are working. An amplification curve for the internal control should be observed in the Cy5 channel. The internal control value should ideally be detected within 35 cycles (Cq ≤ 35). However, two situations may occur in which there is no internal control amplification:

- If the target genes are initially present in the sample in a large number of copies, the internal control provided may not be amplified. This result is expected and does not invalidate the test. It should be interpreted as a positive result despite the absence of the internal control signal. This phenomenon is the result of amplification competition between the internal control and the targets present in a large number of copies.
- If the target genes in the FAM and HEX channels are not amplified and there is no amplification of the internal control in the Cy5 channel, no result can be returned. This situation points to the presence of PCR inhibitors or a technical problem with the performance of the test. The PCR should be repeated starting from the primary sample and preferably on DNA extract.

The procedural control (well E) ensures that, following pretreatment, the nucleic acids were correctly extracted during the extraction/purification step. A Cq value of equal to or more than 35 cycles (Cq ≥ 39) for a target-negative sample may indicate poor extraction of nucleic acids. We advise repeating the extraction.

B. Qualitative interpretation (positive or negative)

Signals above the threshold and **visually consistent with a typical PCR amplification curve** are considered positive results.
Some samples may present atypical curves that are not characteristic of amplification curves. In this event, the result cannot be interpreted and the sample should be reanalysed with controls.

Detection channels			Interpretation
FAM	HEX	Cy5	
-	-	+	Negative control
+	+	+	Positive control
-	-	+	Wells A to E: Negative sample presenting no DNA from any of the 10 target helminths.
+	-	+/-*	Well A: Sample positive for *Schistosoma mansoni* Well B: Sample positive for *Ascaris lumbricoides* Well C: Sample positive for *Diphyllobothrium latum* Well D: Sample positive for *Hymenolepis nana* Wells E: Sample positive for *Ancylostoma duodenale*
-	+	+/-*	Well A: Sample positive for *Strongyloides stercoralis* Well B: Sample positive for *Trichuris trichiura* Well C: Sample positive for *Taenia spp* Well D: Sample positive for *Enterobius vermicularis* Wells E: Sample positive for *Necator americanus*
+	+	+/-*	Well A: Sample positive for *Schistosoma mansoni* and *Strongyloides stercoralis* Well B: Sample positive for *Ascaris lumbricoides* and *Trichuris trichiura* Well C: Sample positive for *Diphyllobothrium latum* and *Taenia spp* Well D: Sample positive for *Hymenolepis nana* and *Enterobius vermicularis* Wells E: Sample positive for *Ancylostoma duodenale* and *Necator americanus*
-	-	-	Invalid sample, inhibition of PCR reaction or testing issue - Perform another test.

*In the event of positive signals in the target pathogen detection channels (FAM and HEX), the internal control signal is not required to validate the result. A high pathogen load may lead to the internal control signal being reduced or absent due to competition.

11 I TEST LIMITS

1. To obtain reliable results, the precautions for use and instructions should be carefully followed. Refer to the section on the interpretation of the results.
2. The BIOSYNEX AMPLIQUICK® Helminths kit is a diagnostic aid. The result of the PCR test must be compared with the clinical picture.
3. Test results should be interpreted within the epidemiological, clinical and therapeutic context.

12 I PERFORMANCE

- **Analytical sensitivity**

Target sequence detection limits:
The detection limit of the BIOSYNEX AMPLIQUICK® Helminths kit is defined as the concentration, in number of copies/µL, that can be 100% detected in a DNA sample specific to each target. It has been determined by performing serial dilution of reference samples with a known number of copies.

Schistosoma mansoni – COX1			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	35.66	12/12	100
5	35.74	12/12	100
2.5	37.07	12/12	100
1	38.43	10/12	83.33
0.5	38.21	3/12	25.0

The detection limit is 2.5 copies/µL.

Strongyloides stercoralis – RNA18s			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	33.94	12/12	100
5	34.86	12/12	100
2.50	36.01	12/12	100
1	36.03	12/12	100
0.50	38.03	11/12	91.67

The detection limit is 1 copy/µL.

Ascaris - RNA18s			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	33.32	12/12	100
1	36.49	12/12	100
0.5	37.74	11/12	91.67
0.25	38.81	11/12	91.67
0.01	38.94	6/12	50.00

The detection limit is 1 copy/µL.

Trichuris trichiura - RNA18s			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	34.08	12/12	100
1	37.73	12/12	100
0.5	39.25	11/12	91.67
0.25	39.61	11/12	91.67

The detection limit is 1 copy/µL.

Diphyllobothrium latum – COX1			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
5	27.82	12/12	100
2.5	28.49	12/12	100
1	29.90	12/12	100
0.5	30.66	12/12	100

The detection limit is 0.5 copy/µL.

Taenia sp – IGS			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	35.84	12/12	100
5	37.71	12/12	100
2.5	38.98	12/12	100
1	39.48	11/12	91.67

The detection limit is 2.5 copies/µL.

Hymenolepis nana – ITS2			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	34.53	12/12	100
1	38.75	12/12	100
0.5	38.10	11/12	91.67
0.25	38.87	8/12	66.67

The detection limit is 1 copy/µL.

Enterobius vermicularis – 5S rRNA			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	34.27	12/12	100
1	37.79	12/12	100
0.5	37.58	12/12	100
0.25	38.38	9/12	75

The detection limit is 0.5 copy/µL.

Ancylostoma duodenale – ITS1			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
5	34.20	12/12	100
2.5	34.90	12/12	100
1	37.46	12/12	100
0.5	37.81	10/12	83.33
0.25	39.09	8/12	66.67

The detection limit is 1 copy/µL.

Necator americanus – ITS1			
Number of copies/µL	**Mean Ct**	**Number of positives**	**Detection %**
10	34.2	12/12	100
5	34.6	12/12	100
2.5	35.7	12/12	100
1	38.1	11/12	91.7
0.5	37.6	11/12	91.7

The detection limit is 2.5 copies/µL.

- **Analytical specificity**

Cross reactivity
A panel of 79 DNA samples and 38 RNA samples from a biobank, listed in the following tables, were tested using the BIOSYNEX AMPLIQUICK® Helminths kit. For all these samples, no amplification of targets in the various master mixes was observed.

RNA	
Coronavirus Oc43	*Influenza A* H5
Coronavirus	*Influenza B*
Coronavirus SARS (2003)	*Measles*
Coxsackie A6	*MERS Coronavirus*
Coxsackie B1	*Mumps*
Coxsackie B5	*Norovirus*
Dengue 1 Virus	*Novel Influenza A* H1n1
Dengue 2 Virus	*Parainfluenza 1*
Dengue 3 Virus	*Parainfluenza 2*
Dengue 4 Virus	*Parainfluenza 3*
Echovirus 5	*Parainfluenza 4 A*
Enterovirus 68	*Respiratory Syncytial Virus* (Subtype A)
Rhinovirus	*Respiratory Syncytial Virus* (Subtype B)
Rotavirus	*West Nile Virus*
Rubella	*Yellow Fever Virus*
Tick-Borne Encephalitis Virus	*Zika Virus* (Asian Lineage)
Human Parainfluenza 1	*Zika Virus*
Influenza A H1	*Chikungunya Virus*
Influenza A H3	*SARS-CoV-2*

DNA		
Adenovirus	*Escherichia coli (EAEC)*	*Mycoplasma hominis*
Adenovirus 41	*Escherichia coli (EIEC)*	*Neisseria gonorrhoeae*
Aspergillus fumigatus	*Escherichia coli (ETEC)*	*Neisseria meningitidis Sg A*
Bacillus cereus	*Escherichia coli (VTEC)*	*Neisseria meningitidis Sg B*
Bartonella henselae	*Francisella tularensis*	*Neisseria meningitidis Sg C*
Bartonella Quintana	*Gardnerella vaginalis*	*Papillomavirus type 16*
Bk Virus	*Giardia intestinalis*	*Papillomavirus type 18*
Bordetella holmesii	*Haemophilus ducreyi*	*Parvovirus B19 (Plasmid)*
Bordetella parapertussis	*Haemophilus influenzae*	*Rickettsia conorii*
Bordetella pertussis	*Helicobacter pylori*	*Salmonella enteritidis*
Borrelia afzelii	*Herpes simplex 1*	*Salmonella typhi*
Borrelia garinii	*Herpes simplex 2*	*Staphylococcus aureus (MecA-)*
Brucella abortus	*Hhv-6*	*Staphylococcus aureus (MecA+)*
Campylobacter jejuni	*Hhv-8*	*Streptococcus agalactiae*
Candida albicans	*Klebsiella pneumoniae (NDM-1)*	*Toxoplasma gondii*
Candida auris	*Legionella pneumophila*	*Treponema pallidum*
Chlamydia trachomatis	*Leishmania chagasi*	*Trichomonas vaginalis*
Chlamydophila pneumoniae	*Leishmania infantum*	*Trypanosoma cruzi*
Chlamydophila psittaci	*Listeria monocytogenes*	*Ureaplasma parvum*
Clostridium difficile	*Moraxella catarrhalis*	*Ureaplasma urealyticum*
Coccidioides immitis	*Mycobacterium avium*	*Varicella-Zoster Virus*
Coxiella burnetii	*Mycobacterium intracellulare*	*Vibrio cholerae*
Cryptosporidium parvum	*Mycobacterium kansasii*	*Yersinia enterocolitica*
Cytomegalovirus	*Mycobacterium tuberculosis*	*Acanthamoeba castellanii*
Enterococcus faecalis (VanB)	*Mycobacterium ulcerans*	*Borrelia burgdorferi*
Enterococcus faecium (VanA)	*Mycoplasma genitalium*	*Streptococcus pneumoniae*
Epstein-Barr Virus		

• Interference studies

Intra-well and inter-well interference and competition

To evaluate any potential competition effect between the targets of the BIOSYNEX AMPLIQUICK® Helminths kit, various samples containing a known number of DNA copies representing the 10 target helminths only, as well as combinations of these targets (intra-well competition) and the 10 target protozoans detected by the BIOSYNEX AMPLIQUICK® Protozoans kit (inter-well competition) were tested. No significant variation in the Cq values obtained in the various scenarios was observed. There is therefore no interference between the fluorescence channels or detection competition between these 20 parasitic targets. The BIOSYNEX AMPLIQUICK® Helminths kit detects the ten pathogens effectively in both single and multiple infections.

Chemical interference

Various substances that may be present in patients' stool samples may cause positive or negative interference on PCR results. Interference tests on the AMPLIQUICK® Helminths kit were performed using stool samples qualified as negative for the target intestinal parasites. The negative samples were spiked with specific purified DNA from the targets of interest at a known number of copies. Two dilutions were tested, one weak (+) and one strong (+++). These stool samples were loaded to 200 mg in the presence of the substances listed in the table below and were tested in the 5 master mixes of the kit.

Substance	Concentration	DNA dilution	Interference
Blood	40%	+	NO
		+++	
Haemoglobin	12.5%	+	
		+++	
Betamethasone (anti-inflammatory cream)	5%	+	
		+++	
	0.25%	+	
		+++	
Carrageenans, Zinc oxide, Titanium dioxide (haemorrhoid cream)	5%	+	
		+++	
	0.25%	+	
		+++	
Mucin	0.8%	+	
		+++	
Loperamide hydrochloride (Imodium)	5%	+	
		+++	
Ampicillin	152 µmol/L	+	
		+++	
Fatty acids	4.8%	+	
		+++	
Bismuth oxychloride + Salicylic acid (Pepto-Bismol)	5%	+	
		+++	
Starch	3%	+	
		+++	
Cellulose	4%	+	
		+++	
Pectin	3%	+	
		+++	

• Clinical performance

Clinical performance was determined using 131 stool samples from 6 different laboratories (1 Senegalese laboratory and 5 laboratories in mainland France) treated with the BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit.
As certain parasites are extremely rare in the available samples, we did not have access to positive samples for this study. Validation of this target was done using DNA extracts from a National Centre of Excellence (Centre National de Référence) and the parasitology laboratory at Hôpital Bichat (Paris).

The contingency table below shows the detection performance of the BIOSYNEX AMPLIQUICK® Helminths test and the BIOSYNEX AMPLIQUICK® Protozoans test, in comparison with microscopy. A breakdown of the results is given in the table in Appendix 1.

		Microscopy	
		Positives	Negatives
BIOSYNEX AMPLIQUICK®	Positives	120	72*
	Negatives	18**	16

*Samples found positive only by PCR with result confirmed using a CE marked commercially available kit
**Samples found negative only by PCR with result confirmed using a CE marked commercially available kit.

• Precision data

The precision data for the BIOSYNEX AMPLIQUICK® Helminths kit were determined based on 5 conditions:

- Intra-assay variation (within one test run)
- Inter-assay variation (between different test runs)
- Inter-laboratory variation
- Inter-operator variation
- Inter-batch variation

The precision data are expressed in terms of mean value, standard deviation and coefficient of variation, on the basis of the threshold quantification cycle (Cq) values for the DNA of the various targets. Three sample concentrations were tested: one high +++, one low + and one zero -.

Intra-assay variation:

	Master mix A								
	Schistosoma			Strongyloides			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	25.77	0.86	3.34
+++	22.93	0.08	0.34	21.01	0.06	0.27	25.88	1.49	5.77
+	34.33	0.30	0.88	35.40	0.35	1.00	25.78	0.70	2.71

	Master mix B								
	Ascaris			Trichuris			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.71	0.72	2.69
+++	20.83	0.10	0.46	21.88	0.06	0.25	27.55	1.18	4.30
+	34.74	0.36	1.05	33.59	0.18	0.55	26.83	0.38	1.42

	Master mix C								
	Diphyllobothrium			Taenia			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.55	0.72	2.72
+++	22.38	0.10	0.43	20.04	0.08	0.42	27.14	0.76	2.81
+	36.23	0.39	1.08	34.36	0.19	0.56	26.43	0.85	3.22

	Master mix D								
	Hymenolepis			Enterobius			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	28.59	0.70	2.45
+++	21.19	0.09	0.44	21.19	0.08	0.37	29.73	0.59	1.99
+	36.08	0.43	1.20	35.51	0.38	1.08	28.51	0.52	1.83

	Master mix E								
	Ancylostoma			Necator			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	29.86	0.11	0.36
+++	21.65	0.10	0.45	21.67	0.07	0.32	28.04	0.09	0.31
+	35.42	0.31	0.87	35.18	0.50	1.44	29.10	0.16	0.55

Inter-assay variation:

	Master mix A								
	Schistosoma			Strongyloides			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.93	0.35	1.31
+++	23.11	0.14	0.60	21.21	0.26	1.21	28.78	0.92	3.21
+	34.80	0.29	0.55	35.64	0.34	0.96	27.23	0.43	1.60

	Master mix B								
	Ascaris			Trichuris			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.01	0.25	0.97
+++	21.01	0.18	0.83	22.90	0.29	1.28	27.82	0.61	2.18
+	35.00	0.27	0.76	34.82	0.28	0.79	26.66	0.63	2.35

	Master mix C								
	Diphyllobothrium			Taenia			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	27.10	0.24	0.88
+++	22.63	0.29	1.28	20.63	0.53	2.59	27.62	1.12	4.06
+	36.70	0.19	0.51	35.38	0.24	0.68	27.34	0.32	1.15

	Master mix D								
	Hymenolepis			Enterobius			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	25.65	0.25	0.99
+++	20.76	0.39	1.86	20.80	0.34	1.63	28.66	0.26	0.90
+	34.74	0.60	1.69	35.20	0.51	1.45	27.47	0.66	2.42

	Master mix E								
	Ancylostoma			Necator			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	33.45	0.69	2.05
+++	21.43	0.25	1.19	22.45	0.40	1.80	29.93	0.27	0.90
+	35.09	0.24	0.68	36.03	0.41	1.15	30.27	0.28	0.93

Inter-laboratory variation:

	Master mix A								
	Schistosoma			Strongyloides			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	27.91	0.39	1.41
+++	22.56	0.14	0.60	20.77	0.04	0.17	28.93	2.05	7.10
+	34.12	0.20	0.59	34.87	0.21	0.61	27.80	0.37	1.35

	Master mix B								
	Ascaris			Trichuris			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.35	1.29	4.90
+++	20.50	0.06	0.31	22.36	0.16	0.70	27.93	0.49	1.76
+	34.48	0.26	0.76	34.24	0.05	0.13	26.77	0.36	1.36

	Master mix C								
	Diphyllobothrium			Taenia			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	28.44	1.15	4.05
+++	22.27	0.04	0.19	20.13	0.32	1.58	28.96	0.77	2.65
+	35.94	0.08	0.23	34.94	0.07	0.19	26.85	1.26	4.67

	Master mix D								
	Hymenolepis			Enterobius			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	25.93	0.31	1.21
+++	20.51	0.17	0.81	20.43	0.54	2.63	27.84	0.34	1.23
+	35.04	0.18	0.51	34.21	0.61	1.78	27.11	0.26	0.95

	Master mix E								
	Ancylostoma			Necator			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	32.95	0.29	0.89
+++	21.69	0.19	0.90	22.53	0.14	0.64	30.30	0.22	0.74
+	34.22	0.07	0.22	35.26	0.18	0.52	29.96	0.11	0.35

Inter-operator variation:

	Master mix A								
	Schistosoma			Strongyloides			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.78	0.59	2.19
+++	23.28	0.04	0.15	21.22	0.01	0.07	29.38	0.30	1.04
+	35.08	0.28	0.79	35.66	0.47	1.31	27.59	0.18	0.64

	Master mix B								
	Ascaris			Trichuris			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.28	0.33	1.24
+++	21.05	0.31	1.48	22.77	0.37	1.61	27.00	1.10	4.09
+	35.22	0.16	0.46	34.77	0.43	1.24	25.97	0.40	1.55

	Master mix C								
	Diphyllobothrium			Taenia			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	27.18	0.19	0.71
+++	22.69	0.34	1.50	20.56	0.32	1.55	27.99	0.91	3.26
+	36.46	0.25	0.70	35.30	0.12	0.34	27.06	0.83	3.06

	Master mix D								
	Hymenolepis			Enterobius			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	25.83	1.29	4.98
+++	21.13	0.37	1.77	21.04	0.35	1.65	28.73	0.21	0.74
+	36.14	0.80	2.21	35.41	0.54	1.52	27.78	0.38	1.37

	Master mix E								
	Ancylostoma			Necator			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	30.35	0.21	0.68
+++	21.59	0.37	1.70	22.45	0.32	1.42	30.15	0.24	0.80
+	35.02	0.45	1.27	35.96	0.25	0.71	30.35	0.21	0.68

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Inter-batch variation:

	Master mix A								
	Schistosoma			Strongyloides			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	28.39	2.00	7.05
+++	22.67	0.90	3.96	20.73	0.91	4.40	29.47	2.43	8.24
+	34.78	1.35	3.89	35.21	1.51	4.28	28.48	1.71	6.00

	Master mix B								
	Ascaris			Trichuris			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	26.93	2.45	9.11
+++	20.57	0.13	0.65	21.95	0.23	1.03	27.80	2.34	8.42
+	34.63	0.09	0.27	33.76	0.20	0.60	27.25	2.00	7.36

	Master mix C								
	Diphyllobothrium			Taenia			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	27.92	2.95	10.57
+++	21.69	0.95	4.40	20.01	0.39	1.94	29.55	1.50	5.08
+	36.16	0.83	2.30	34.62	1.24	3.58	28.73	1.68	5.85

	Master mix D								
	Hymenolepis			Enterobius			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	28.30	1.77	6.26
+++	20.21	0.85	4.22	20.45	0.61	2.99	28.91	1.99	6.90
+	35.15	0.46	1.32	34.56	0.75	2.18	27.41	2.48	9.04

	Master mix E								
	Ancylostoma			Necator			CIEZ		
	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)	Cq values mean	SD	CV (%)
-	N/A	N/A	N/A	N/A	N/A	N/A	32.60	0.89	2.72
+++	20.57	0.94	4.59	21.50	0.96	4.46	29.11	1.06	3.66
+	34.46	0.68	1.98	35.72	1.16	3.26	29.78	1.01	3.41

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SYMBOLS

	See the instructions leaflet		Contains enough for <n> tests	REF	Catalogue number
IVD	*In vitro* diagnostic medical device		Temperature limit		Do not re-use
	Manufacturer	LOT	Batch number		Expiry date
	Keep away from sunlight	Mmix	Master Mix	APQ	AMPLIQUICK®
CONTROL -	Negative control	CONTROL +	Positive control	CONTROL IP	Procedural control
CONTROL Mmix	Control Master mix		Pouches of strips of caps		Do not use if the packaging is damaged and see the instructions leaflet
CH REP	Representative in Switzerland		Importer		

IFU_3150066_EN_V01202205R02
Date of last revision: 11/2022
Latest modifications: Addition of precision for pre-analytical steps

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	Qualification							
	Positives				Negatives			
	Qualification	Inconsistent results	Negative results confirmed using another CE marked commercially available kit	Total number of inconsistent results	Qualification	Inconsistent results	Positive results confirmed using another CE marked commercially available kit	Total number of inconsistent results
	Positives with AMPLIQUICK® kits	Negatives with AMPLIQUICK® kits			Negatives with AMPLIQUICK® kits	Positives with AMPLIQUICK® kits		
Cryptosporidium spp	14	1	1	0	117	3	2	1
Dientamoeba fragilis	11	2	2	0	120	22	21	1
Enterocytozoon bieneusi	5	0	0	0	126	1	1	0
Encephalitozoon spp	3	0	0	0	128	0	0	0
Entamoeba histolytica	4	4	4	0	127	3	3	0
Entamoeba dispar	4	1	1	0	127	6	4	2
Blastocystis spp	32	2	2	0	99	30	30	0
Giardia intestinalis	15	3	2	1	116	4	4	0
Cystoisospora belli	3	0	0	0	131	0	0	0
Cyclospora cayentanensis	0	1	1	0	131	0	0	0
Schistosoma **(no CE marked kit commercially available to confirm result)**	6	0	0	0	125	4	0	4*
Strongyloides Stercoralis	5	1	1	0	126	0	0	0
Ascaris lumbricoides	15	1	1	0	116	1	1	0
Trichuris trichiura	7	1	1	0	124	3	3	0
Diphyllobothrium **(no CE marked kit commercially available to confirm result)**	2	0	0	0	129	1	0	1*
Taenia spp	4	0	0	0	127	0	0	0
Hymenolepis nana	4	1	1	0	127	0	0	0
Enterobius vermicularis	5	1	1	0	126	2	2	0
Ancylostoma duodenale	0	0	0	0	131	2	1	1
Necator americanus	0	0	0	0	131	0	0	0

Appendix 1: Table showing Clinical Study I performed on 131 microscopically qualified samples. Samples with inconsistent microscopy and PCR results were reanalysed using commercially available CE marked kits.

*Inconsistent results not confirmed using a rival CE marked kit as none available.