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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Malaria screening

REF: RT-66

Detection of the P.falciparum, P.malariae, P.vivax, P.ovale and P. knowlesi genome with *Real Time PCR*

INTRODUCTION AND PURPOSE OF USE

The system *Malaria screening* is a qualitative test that allows the DNA amplification and detection, by means of *Real Time PCR*, of P.falciparum, P.malariae, P.vivax, P.ovale and P. knowlesi genome correctly extracted by biological samples, without species discrimination.
The Procedure allows the detection of the DNA target by means a genomic amplification reaction.
The analysis of the results is made using a Real Time PCR analyzer (thermal cycler integrated with a system for fluorescence detection and a dedicated software).

CONTENT

The kit contains reagents enough to perform 48 amplification tests

	Quantity	Description
R1	3 x 220 µl	**Amplification mMix** dNTPs, Tris-HCl, KCl, $MgCl_2$, Taq Polymerase, *AmpErase* Uracil N-Glycosylase (*UNG)* Nuclease-free water, ROX (Pink Cap)
R2	3 x 130 µl	**Plasmodium probes Mix** Upstream primer, downstream primer, Target probe (FAM), Internal control (β-globin) Probe (VIC) Nuclease-free water (White cap)
R3	3 x 35 µl	**Plasmodium High concentration**
R4	3 x 35 µl	**Plasmodium Low concentration**
R5	1 x 30 µl	**Negative Control**

Instruction for use: **ST.RT66-ENG.9**

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED

Disposable latex powder-free gloves or similar material;
Bench microcentrifuge (12,000 - 14,000 rpm);
Micropipettes and Sterile tips with aerosol filter;
Vortex;
Plastic materials (microplate and optica adhesive cover);
Heat block (only for extraction)
Dry block shacker for 1.5ml conical tubes
Magnetic rack for 1.5ml conical tubes
EZ1 Advanced XL DSP Virus Card. - Ref. 9018703 - QIAGEN.
ATL Buffer - Ref. 939016 - QIAGEN.

Reagents

The Malaria screening kit was developed and validated to be used with the following extraction method:

Manual Extraction
Ref. 51304/51306
QIAmp DNA mini kit.
The kit allows the DNA extraction from tested samples. The kit contains reagents for 50/250 samples.(QIAGEN).

Automatic Extraction
Ref. 62724. EZ1 XL DSP Virus Kit
The kit allows the automatic viral DNA from Human samples.
The kit contains reagents for 48 samples. (QIAGEN)

Ref. ZP02001. MagPurix Blood DNA Extraction Kit 200
The kit allows the automatic viral DNA from Human samples.
The kit contains reagents for 48 samples. (ZINEXTS LIFE SCIENCE CORP)

Manual/Automatic extraction (Siemens)
10629800 - VERSANT® Sample Preparation 1.2 Reagents kit box 1. The kit allows the manual DNA extraction from Human samples. The kit contains reagents enough to perform the DNA extraction for 96 samples.

10629801 - VERSANT® Sample Preparation 1.2 Reagents kit box 2. The kit allows the manual DNA extraction from Human samples. The kit contains reagents enough to perform the DNA extraction for 96 samples.

Instruments

The Malaria screening kit was developed and validated to be used with the following instruments:

Extraction System
Ref. 9001492. EZ1 Advanced XL.
Robotic Workstation for the automatic purification of the nucleic acids until 14 samples simultaneously (QIAGEN)

Ref. ZP01005. MagPurix 12A.
Robotic Workstation for the automatic purification of the nucleic acids until 12 samples simultaneously (ZINEXTS LIFE SCIENCE CORP)

Real Time PCR
The Malaria screening kit was developed and validated to be used with the following real time PCR instruments:

- *7500 Fast* from Lifetechnologies
- *StepOne Plus* from Lifetechnologies
- *Rotor-Gene Q* MDx from QIAGEN
- *Versant kPCR AD* from *Siemens or Stratagene MX3005P/MX3000P*
- *CFX96 Real Time PCR System* from *BioRad*
- *mic Real Time PCR cycler from Bio Molecular System*

Please ensure that the instruments have been installed, calibrated, checked and maintained according to the manufacturer's instruction and raccommendations

SAMPLES AND STORAGE

The *Malaria screening*. system must be used with extracted DNA from the following biological samples: **whole Blood EDTA**. Collected samples must be shipped and stored at +2 - +8°C and used within 3 days from the collected data.
Store the sample at -20°C if it is used after 3 days.

PRECAUTIONS USE

This kit is for in *vitro* diagnostic (IVD), for professional use only and not for in vivo use.
After reconstitution, the amplification master mix must be used in one time (16 reactions). Repeat thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently. At all times follow Good Laboratory Practice (GLP) guidelines.
Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
Avoid any contact between hands and eyes or nose during specimens collection and testing.
Handle and dispose all used materials into appropriate bio-hazard waste containers. It should be discarded according to local law.
Keep separated the extraction and the reagents preparation.
Never pipette solutions by mouth.
Avoid the air bubbles during the master mix dispensing. Eliminate them before starting amplification.
Wash hands carefully after handling samples and reagents.
Do not mix reagents from different lots.
It is not infectious and hazardous for the health (see Material Safety data Sheet – MSDS).
Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
Read carefully the instructions notice before using this test.
Do not use beyond the expiration date which appears on the package label.
Do not use a test from a damaged protective wrapper

LIMIT OF THE METHOD

Use only extracted DNA from **Whole blood collected in EDTA.**
Do not use contaminated DNA with extracted mucoproteins or hemoglobin: the latter inhibit the amplification reaction of nucleic acids and may cause invalid results .
The extreme sensitivity of gene amplification may cause false positives due to cross-contamination between samples and controls. Therefore, you should:

- physically separate all the products and reagents used for amplification reactions from those used for other reactions, as well as from post-amplification products;
- use tips with filters to prevent cross-contamination between samples;
- use disposable gloves and change them frequently;
- carefully open test tubes to prevent aerosol formation;
- close every test tube before opening another one.

The proper functioning of the amplification mix depends on the correct collection, correct transportation, correct storage and correct preparation of a biological sample.
As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and other laboratory tests done on the patient.
A negative result obtained with this product suggests that the DNA of Malaria was not detected in DNA extracted from the sample, but it may also contain Malaria DNA at a lower titre than the detection limit for the product (detection limit for the product, see paragraph on Performance Characteristics); in this case the result would be a false negative.
As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results.

STORAGE AND STABILITY

Store the product ***Malaria screening*** at –20°C..
The ***Malaria screening*** kit is shipped on dry ice. The kit components should be frozen.
If one or more components are not frozen upon receipt or if the tubes have been compromised during transport, contact Clonit srl for assistance.
An intact and well stored product has a stability of 12 months from the date of production. Do not use beyond the expiration date which appears on the package label.
Repeat thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently

ANALYTICAL PROCEDURE

Manual Exraction
Ref. 51304/51306 - QIAmp DNA mini kit. (QIAGEN).

Procedure to Whole blood
Follow the instructions inside the kit *QIAmp DNA mini kit*.
Elute the sample in 50 µl of buffer AE

Samples are now ready for amplification or storage at -20°C

Automatic extraction (QIAGEN)
Ref. 62724 - EZ1 XL DSP Virus Kit on *EZ1 Advanced XL*

Procedure to Whole Blood
Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.
Volume of sample to be used:

Whole blood [µl]	*ATL [µl]*	*Final volume Samples [µl]*
200	*200*	*400*

Preparation of the Carrier
Solve completely the lyophilize RNA carrier in elution buffer (AVE), from 310 µl, split in aliquots and store to –20 ± 5°C. Not thawing and freezing the aliquots more than 2 times.
For each analyzed sample, dilute 3,6 µl of a original solution include the RNA Carrier in total volume of 60 µl using elution buffer (AVE)
Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.
Select the protocol starting from 400 µl of samples with the elution of 60 µl.

Samples are now ready for amplification or storage at -20°C

Automatic Extraction (ZINEXTS)
Ref. ZP02001 - MagPurix Blood DNA Extraction Kit 200 on *MagPurix 12A*

Procedure to Whole Blood
Follow the instructions inside the kit *MagPurix Blood DNA Extraction Kit 200.*
Select the protocol starting from 200 µl of samples with the elution of 50 µl.

Samples are now ready for amplification or storage at -20°C.

Manual extraction (SIEMENS)
Ref. 10629800 - VERSANT® Sample Preparation 1.2 Reagents kit box 1.
Ref. 10629801 - VERSANT® Sample Preparation 1.2 Reagents kit box 2.
Follow the instructions supplied by Siemens and elute it in 70 µl of Elution buffer. Transfer 55 µl of eluted sample to an appropriately size tube.

Samples are now ready for amplification or storage at -20°C

SOFTWARE SETTINGS:

Lifetechnologies 7500 fast/StepOne plus

Turn the instrument and the computer on and open the control software. Click on "**Advance Setup**": by default the sofware will shows the page "**experiment properties**". Write in the "**experiment name**" the file name, choose the type of instrument (**7500 or 7500fast / StepOne or StepOne Plus),** the type of reaction (**quantitation standard curve),** the type of reagents used (**Taqman®Reagents**) and the analysis reaction time (**Standard ≈ 2 hours to complete a run**).
Open the page named "**page setup"** (sheet **Define Target and Samples**).
In the window **Define Targets** set:

Target	Reporter	Quencer
Plasmodium probe:	FAM	TAMRA
IC (β-globin) **probe:**	VIC	TAMRA

Set the samples' name in the window "**Define Samples**".
In the same page "**plate setup**" select the sheet "**Assign Target and Samples**". On the screen you will see the microplate draft.
Select an area of the plate where the controls will be placed: select wells of the plate and set both targets (Plasmodium and β-globin). Select "**Assign target to selected wells**" in the blank, the "**task Standard (S)**" for Plasmodium target and set the controls' concentration.
Choose an area in the plate where negative control will be placed: select "**Assign target to selected wells**" in the blank, the "**task Negative (N)**" for the Plasmodium target.
Select an area of the plate where samples will be placed: select the wells and set both targets (Plasmodium and β-globin). Link every well to a sample, through the window "**Assign samples to selected wells**".
For each sample, select in the blank "**Assign targets to selected wells**" the "**task UnKnown (U)**" for the Plasmodium target.
Set ROX as passive reference, using it as normalizer of detected fluorescence.
Open "**Run Method**" (sheet **Graphic View**) and set the thermal cycling as follows, with the "collect data" in annealing/extension phase:

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

In the window "**Reaction volume plate per well**" set a volume of 25µl.

After having prepared the plate, and correctly inserted in the instrument, press the button "**Start Run**".

Rotor-Gene Q MDx

The experiments can be set using the **Quick Start Wizard** or the **Advanced Wizard**, which appears when the software is started.
Select the wizard "**Advanced**". As a first step, select the model "**Two Step Reaction**" with a double click in the "**New Run**".
In the next window, select the type of rotor installed on the instrument from the list that appears. Check the "**Locking Ring Attached**", check the checkbox and then click "**Next**".
Enter the name of the operator and the reaction volume of 25µl, and then click "**Next**".
In the next window click on "**edit profile**". Set the following thermal cycle:

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

Select the annealing / extension from the thermal profile and click on "**Acquiring A to cycling**."

In the next window, select **yellow** from the **available channels** and add it to **acquiring channel** along with the green channel and click "**OK**". In the next window click on "**OK**" and then click "**Next**".
Click on "Edit Gain" button and set the following values for each channel:

Reporter	Gain
Green	6
Yellow	4

To begin the course, click on the button "**Start Run**". You can save the model before you begin your run by clicking on "**Save Template**".
After clicking on the button "**Start Run**" window appears "**Save As**". The user can save the stroke in the desired position.
Once the run started, the window "**Edit Samples**" allows you to set the name of samples and controls in the positions in which they were loaded on the instrument.
Select the locations where you placed the controls. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**Positive control**".
Select the location where you placed the Negative Control and name it as Negative Control. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**Negative Controls**"
Select the location of each sample and enter the name or code of the patient. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**UnKnown**"
At the end of the operation, click "**OK**" in the "**edit samples**" and wait until the end of the race for the analysis (see "**Interpretation of Results**").

Versant kPCR AD O Stratagene MX3005P/MX3000P

Turn the instrument on and wait until both green lamps have fixed light, turn on the computer and start the control software. In the principal screen will appear the window "**New Experiment Options**": select "**Experiment type": quantitative PCR (Multiple Standard).**
Turn the lamp on 20 minutes before doing a new experiment. For turning the lamp on, click on the icon of the lamp in the tool bar or select "**Lamp On**" from the menu **"Instruments".**
Verify the right setting of the gain of the fluorescent reporters: in the menu of settings, choose: **"Instrument"** and then **"Filter set gain setting"**.

Reporter	Gain
FAM	4
HEX	4
ROX	1

Click on button **"setup"** in the toolbar and choose "**Plate Setup".**
Sign the wells correspondent to calibrators. Define the calibrator's positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
Pos. Control FAM	FAM/HEX/ROX	ROX	None

Clicking on every single well will appear the window "**well information**", and you can choose the name of the positive control (*Plasmodium High Control* and *Plasmodium Low Control*).
Identify the wells correspondent to Negative control. Define the Negative control positions in the menu by setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
NTC	FAM/HEX/ROX	ROX	None

Clicking on every single well will appear the window "**well information**", and you can set NTC as the name.
Sign the wells correspondent to samples (2 wells for samples). Define the sample positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
Unknown	FAM/HEX/ROX	ROX	None

Clicking on every single well will appear the window "**well information**", and you can insert the name or the code of the sample.
It's possible to set the name of the dye near the name of the analyzed target:

FAM	HEX
Plasmodium	β-Globin

In the tool bar choose the sheet **"Thermal Profile Setup"** and set the correct thermal cycle and reading the fluorescence in the annealing/extension step.

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

After making the plate and inserting it in the instrument, press the button "**Run**", selecting the sheet Thermal profile status and check the correctness of thermic profile.
Select the box **Turn Lamp Off** at the end of execution. Push the button Start: the software will ask you to indicate the name of save the file and will begin the analysis.

CFX 96 Real Time PCR

Turn the instrument and the computer on and start the control software. In the principal screen will appear the window "**Startup wizard**": select "**CFX96"** and press **"ok".** In the next window push "**create new**" and set the thermal protocol and the reaction volume (25µl), with the "collect data" in annealing/extension phase:

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

Save the protocol and click the next button. The software will open in default the sheet "**plate**". Click "**create new**", select **"Fluorophores button"** to choose fluorophores (FAM and VIC). Select the locations where they were positioned the controls of known concentration and choose the **"Sample Type" Standards.** Click "**Load**" check boxes to load fluorophores and Type or select Target Name. Select the location where you placed the Negative Control. Choose the **"Sample Type" NTC.** Click "**Load**" check boxes to load fluorophores and Type or select Target Name
Select the location of each sample and enter the name or code of the patient. Choose the **"Sample Type" Unknown.** Click "**Load**" check boxes to load fluorophores and Type or select Target Name
Save the plate clicking the next button and start the experiment

mic Real Time PCR cycler

Creating a New Assay

The *Assay* contains information regarding the target amplicons and the qPCR conditions and the analysis type required for the assay along with various analysis parameters.
Select **New** from the tool bar menu and then **Assay** from the drop-down list.
Select **Assay Setup** and click on **Information**.
In the windows Information, select the **Chemistry Type** and choose **Hydrolysis Probes**
Enter the name of the amplicon target and select the **add button** to setup another Target.

Target Name	5' Modifier	3' Modifier
Plasmodium	FAM	BHQ®-1
B-Globine	VIC	BHQ®-1

In the windows **Assay Setup** click on **Profile**

Activation	Cycling	Cycles
50° C 2 min	95° C 15 sec 60° C 1 min	45
Add Hold		
95°C 10 min		

Select the **Cycling (60°C 1min)** from the profile and click on (Acquire data)

In the window acquire on, check that are present the correct acquire channels **(Green** and **Yellow**)

In the **Temperature Control** choose **standard Taq polymerase** and in the **Reaction Volume** set a volume of **25 µl.**
Save as the **Malaria Screening Assay.**

Creating a New Run

Select **New** from the tool bar menu and then **Run** from the drop-down list
Click on **Assays** and choose the **Malaria Screening Assay.**
Click on **mic Idle** and choose **start a run.**
Save the Experiment and click **Start Run** in the dialogue box.
Once the run started, the window "**Samples**" allows you to set the name of samples and controls in the positions in which they were loaded on the instrument.
Select the locations where you placed the **controls**.
Clicking on the box next to "**Type"** correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analysed. Select "**Positive control**".
Select the location where you placed the **Negative Control** and name it as **Negative Control**.
Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analysed. Select "**Negative Controls**"
Select the location of each sample and enter the name or code of the **patient.**
Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analysed. Select "**UnKnown**"

PREPARATION OF THE REACTIONS:

Defrost a tube of **Amplification mMix**;
Defrost a tube of **Plasmodium probes Mix;**
Mix carefully by vortex **210µl** of **Amplification mMix** and **126µl** of **Plasmodium probes Mix** (the mix as produced is enough to prepare **16 reactions** of amplification: **2 positive controls, 1 negative control and 13 samples**).
Distribute, in the amplification plate, **20µl of just reconstituted mix** in chosen positions, as already set on the instrument software.
Distribute, in the negative control position, **5µl** of the negative control.
Distribute, in chosen position for each sample, **5µl** of corresponding sample.
Distribute, in chosen positions for the positive controls, **5µl** of **low control** and **high control**.
Seal up accurately the plate using an optical adhesive film and verify that there aren't air bubbles in the mix, to avoid interferences in the amplification.
For the Rotor-Gene Q MDx, seal each tube with the appropriate cap. The air bubbles presence is not influently; the rotor centrifugal force will allow automatic deletion.
Transfer the plate in the instrument and push the button "**Start Run**".

QUALITATIVE ANALYSIS

Lifetechnologies 7500 Fast, StepOne Plus.

At the end of the PCR run, the software automatically opens the "**Analysis**" window in the "**Amplification plot**" sheet on the menu on the left.
Select the wells corresponding to the positive control, negative control and samples for analysis.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **Plasmodium target**. Check the correct setting of the threshold.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **IC Control target**. Check the correct setting of the threshold.

The analysis of the results is made selecting from the menu in the left the page **"Analysis"**.

From the page "**Amplification Plot**" verify the amplification plot for every single sample.
Opening the sheet "**view well table**" in the right side of the software it is possible to verify the data obtained from experiments: Threshold Cycles, emitted fluorescences etc…
Clicking from the menu file and selecting the box export, the window "**export properties**" will open. Indicate the file name, select the position to save it (**Browse**) and click on button **"Start export".** In this way the software will permit to save a excel file with all the data corresponding to selected experiment.

Rotor-Gene Q MDx

At the end of the PCR run open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (green)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold**".
Open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (yellow)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Enter in the space provided "**CT calculation – Threshold"** the threshold value indicated in the table.

Also in this case, you can print a report of the analysis by clicking on the **"Report"** window and selecting the file in the first **Quantification cycling A (green)** and then the file **cycling A (yellow).**

Versant kPCR AD o Stratagene MX3005P/MX3000P

Click on button "**Analysis**" in the toolbar. The software will open in default the sheet "**Analysis Term Setting**". Activate the buttons FAM and HEX in the lower part of the screen and select testing samples.
Click on sheet "**results**"; the software will open in default the page "**Amplification plot**". Check the correct setting of the threshold in the specific window "**Threshold fluorescence**", in the menu on the right of the screen.

Selecting the box **Text report**" from menu "**Area to Analyze**": in the right side of the screen it's possible to verify the data obtained from the experiments (Threshold Cycles, emitted Fluorescences etc..)
From the window **Text Report** it's possible to export the results obtained clicking **file, export** on main menu.

CFX96 Real Time PCR System

At the end of the PCR, select the "**quantitation**" sheet. On the top of the screen, select "**settings**" from the menu and choose "**Baseline Threshold…**" for both the parameters (Plasmodium and IC):
You can export the report pushing the paper block figure on the top of the screen.

mic Real Time PCR cycler

At the end of the PCR run, select **Cycling Analysis**, the software will, by default, plot baseline-corrected curves as fluorescence (y-axis) against cycle number (x-axis), in logarithmic scale, for the target that was chosen.

In the window Analysis, click on **Cycling** and add the target **Plasmodium**. In the window Parameters set the Method Dynamic, Exclusion Extensive, remove auto set Threshold and setting the correct Threshold.

In the window Analysis, click on **Cycling** and add the target **B-Globine**. In the window Parameters set the Method Dynamic, Exclusion Extensive, remove auto set Threshold and setting the correct Threshold.

INTERPRETATION OF RESULTS

In the Real Time PCR reaction the Ct values of specific probe for **Plasmodium** are used for detect the presence of the Target in analysis.
Fluorescence increase of the specific probe for **Plasmodium** (FAM) indicates the positivity of the sample for the target in exam.
The samples that were present Ct values> 40 should be subjected to further verification as close to the limit of sensitivity of the system.
As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and other laboratory tests done on the patient.
The use of positive and negative control in each amplification session allows to verify the correct functioning of the amplifcation mix and the absence of any contamination.
The instrument software is able to analyze the fluorescences that are emitted by the specific probe for **Plasmodium** (FAM) and by the specific probe for the positive internal control (β-globin VIC).
A proper functioning of the amplification mix can be verified analyzing these parameters:

Parameters	Ref.
Conc. High control (FAM)	Ct ≤ 27
Conc. Low control (FAM)	Ct ≤ 33

If the amplification reaction of each controls produces a Ct > 27 the session can't be considered valid and so it must be cancelled.
Be sure that there isn't any specific fluorescence increasing for examining target in negative control (FAM).
In the amplification reaction of each sample, the Ct values for the internal control (β-globin) specific probe are used for validating the analysis session. Beginning from extraction process until detection step. A good extraction performances presents internal control (β-globin) threshold cycle between 22 and 25.
Be sure that emitted fluorescence from internal control amplification has not a Ct > 28 or undetermined. If a sample presents an undetermined Plasmodium DNA and internal control Ct >28 means that some problems happened in the extraction step or in the amplification step; therefore the sample could be a false negative. **Repeat the sample**.

Detector FAM	Detector VIC/JOE	Assay	Sample
Ct undetermined	Ct > 28 o undetermined	Not valid	Repeat
Ct undetermined	Ct < 28	Valid	Negative
Ct positivo	Ct < 28	Valid	Positive
Ct basso	Ct > 28 undetermined	Valid	High Positive

PERFORMANCES

Analytical sensitivity:
Limit of sensitivity
For the purposes of this evaluation is considered as analytical sensitivity the highest serum dilution (title) to which a positive sample can be subjected without the system losing the ability to detect it as positive. The analytical sensitivity of the system was assessed by analyzing plasmid DNA, quantified by spectrophotometric analysis, containing the genomic region of interest (18S Ribosomal RNA gene) of the 4 plasmodium in serial dilutions from 100.000 copies to 1 copy of DNA in 5µl of extracted material added in the amplification reaction.

	Conc.	1cps	10cps	100.000cps
P.falc.	N° Test	15	15	15
	N° Positive	0	15	15
	N° Negative	15	0	0
P.mal.	N° Test	15	15	15
	N° Positive	0	15	15
	N° Negative	15	0	0
P.ovale	N° Test	15	15	15
	N° Positive	0	15	15
	N° Negative	15	0	0
P.vivax	N° Test	15	15	15
	N° Positive	0	15	15
	N° Negative	15	0	0

The analytical sensitivity allow to detect 10 copies of extracted Plasmodium DNA added to the amplification reaction, corrisponding to:

Extraction	Clonit	Siemens
A.Sensitivity	500 copies/ml	730 copies/ml

Clinical sensitivity:
For the purposes of this evaluation is considered as clinical sensitivity the skill of determining true positives in the totality of positive screened samples. The analysis is made on P.falciparum, P.malariae, P.vivax and P.ovale positive samples and the test is performed following the method advices. Positive samples are confirmed with another disposable method.

Samples		N	Positive	Negative
EDTA blood	P.falc.	61	61	0
EDTA blood	P.mal	5	5	0
EDTA blood	P.ovale	16	16	0
EDTA blood	P.vivax	10	10	0

Obtained results show a clinical sensitivity of 100%.

Diagnostic Specificity:
For the purposes of this evaluation is considered as diagnostic specificity the skill of the method of determining real negative samples. The diagnostic specificity of the system is valued analyzing human genomic samples tested and confirmed as negative with another disposable system.

Samples	N	Positive	Negative
Donors' EDTA blood	20	0	20

Diagnostic specificity is 100% for material extracted from EDTA blood.

Analytical Specificity:
Test's specificity is guaranteed by the use of specific primers for determining P.falciparum, P.malariae, P.vivax, P.ovale and P. knowlesi
The alignment of the choose regions for specific primers' hybridization for malaria plasmodium with available sequences of the 18S Ribosomal RNA gene region present in database, demonstrated: their conservation, the absence of significative mutations and the complete specificity for the analyzed target.

Traceability versus NIBSC controls material
The NIBSC standard (code 04/176, Version 3.0, Dated 09/05/2012) was established as the 1st WHO International Standard for Plasmodium falciparum DNA. It consists of a freeze-dried whole blood preparation collected from a partient transfusion.

	7500 Fast	StepOne	MX3000P	Rotor-Q	CFX96	micPCR	Expected
WHO standard	Positive	Positive	Positive	Positive	Positive	Positive	Positive

INTERFERENCES:

Verify that in the DNA extracted from the sample there is no contamination from mucoproteins and haemoglobin, to exclude possible inhibition of PCR reaction. The interference due to contaminants can be detected through a spectrophotometric analysis, verifying the ratio between the absorbance readings at 260 nm (maximum absorbtion of Nucleic Acids) and 280 nm (maximum absorbtion of Proteins). A pure DNA should have a ratio of approximately 1.8.

QUALITY CONTROL

It is recommended to include in each analytical run, as quality control of every extraction, amplification and detection step, an already tested negative and positive sample, or a reference material with known concentration.
In accordance with the Clonit srl ISO EN 13485 Certified quality Management System, each lot of Malaria screening is tested against predetermined specification to ensure consistent product quality.

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TECHNICAL ASSISTANCE

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IVD	*In vitro* diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	Code
	Manufacturer
Σ	Contains sufficient for <n> tests

EDMA code: 15051009
CND: W0105050299

The ***Malaria screening*** kit is CE marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/CE.

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CE IVD
for *in vitro* diagnostic use

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Revision 20th January 2021