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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Malaria panel

REF: RT-61 or RT-61S

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

INTRODUCTION AND PURPOSE OF USE

The system Malaria panel is a qualitative test that allows the DNA amplification, by means of *Real Time PCR*, of P.falciparum, P.ovale P.malariae and P.vivax genome extracted from biological samples.
The procedure allows the detection of the DNA target (*18s Ribosomal RNA gene*) through a genomic amplification reaction.
The analysis of the results is made using a Real Time PCR analyser (thermal cycler integrated with a system for fluorescence detection and a dedicated software).

CONTENT

The kit contains reagents enough to perform 24 amplification tests:

	Quantity	Description
R1	2 x 360 µl	**Amplification mMix** dNTPs, Tris-HCl, KCl, $MgCl_2$, Taq Polymerase, *AmpErase* Uracil N-Glycosylase (*UNG),* Nuclease-free water, ROX (Pink Cap)
R2	2 x 110 µl	**falciparum/ovale probes Mix** Upstream primer, downstream primer, P.falciparum probe (FAM), P.ovale Probe (VIC), Internal control (β-globina) Probe (CY5) Nuclease-free water (White cap)
R3	2 x 110 µl	**malariae/vivax probes Mix** Upstream primer, downstream primer, P.malariae probe (FAM), P.vivax Probe (VIC), Internal control (β-globina) Probe (CY5) Nuclease-free water (Yellow cap)
R4	2 x 35 µl	**P. falciparum positive control**
R5	2 x 35 µl	**P. ovale positive control**
R6	2 x 35 µl	**P. malariae positive control**
R7	2 x 35 µl	**P. vivax positive control**
R8	1 x 30 µl	**Negative Control**

Istruction for use: **ST.RT61-ENG.10**

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 optical adhesive cover);
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

RT-66 – Plasmodium Screening
The system allows the P.falciparum, P.vivax, P.malariae and P.ovale genomes amplification without give the identification of the species. The kit contains reagents for 48 tests.

Reagents

The **Malaria Panel 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 panel 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 panel kit was developed and validated to be used with the following real time PCR instruments

- *7500 Fast* 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 recommendations

SAMPLES AND STORAGE

The Malaria panel system must be used with DNA extracted from the following biological samples: **Whole Blood EDTA**. Collected samples must be shipped and stored at +2 - +8°C.
Store the sample at -20°C if not used within 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 (12 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**
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 the four Plasmodium was not detected in DNA extracted from the sample, but it may also contain Plasmodium 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 Panel*** at –20°C..
The ***Malaria Panel*** kit is shipped on dry ice. The kit components should be frozen. 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 Extraction

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 SETTING:

Lifetechnologies 7500 fast

Turn the instrument and the computer on and open the control software. Click on "**Advance Setup**": by default the software will shows the page "**experiment properties**". Write in the "**experiment name**" the file name, choose the type of instrument (**7500 or 7500fast),** 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	Quencher
P.falciparum probe:	FAM	NFQ
P.ovale probe:	VIC	NFQ
P.malariae probe:	FAM	NFQ
P.vivax probe:	VIC	NFQ
IC (β-globin) **probe:**	CY5	NONE

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: P. falciparum and P.ovale controls: select wells of the plate and set the targets (falciparum probe, ovale probe and βglobin probe). Select "**Assign target to selected wells**" in the blank, the "**task Standard (S)**" for falciparum and ovale targets.
Select an area of the plate where the controls will be placed: P. malariae and P.vivax controls: select wells of the plate and set the targets (malariae probe, vivax probe and βglobin probe). Select "**Assign target to selected wells**" in the blank, the "**task Standard (S)**" for malariae and vivax targets.
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 both mixes (falciparum/ovale and malariae/vivax).
Select an area of the plate where samples will be placed: select for every sample two wells of the plate:
Well 1: falciparum probe, ovale probe and βglobin probe
Well 2: malariae probe, vivax probe and βglobin probe
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 targets.
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 25 µl.
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 25 µl, 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** and **red** 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 the "Edit Gain" and set the Gain for the three channels:

Reporter	Gain
Green	6
Yellow	4
Red	2

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 stroke can be saved in the desired position by the user.
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 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**"
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 or 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. To turn 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	8
HEX	4
CY5	2
ROX	1

Click on button **"setup"** in the toolbar and choose "**Plate Setup".**
Sign the wells correspondent to positive controls P.falciparum and P.ovale. Define the control positions in the menu by setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
Pos. Control	FAM/HEX/CY5/ROX	ROX	None

Clicking on every single well it will appear the window "**well information**".
Choose the name of the positive control (*P.falciparum positive control and P.ovale positive control).*
It's possible to set the name of the dye near the name of the analysed target:

FAM	HEX	CY5
P.falciparum	P.ovale	β-Globin

Click on button **"setup"** in the toolbar and choose "**Plate Setup".**
Sign the wells correspondent to positive controls P.malariae and P.vivax. Define the control positions in the menu by setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
Pos. Control	FAM/HEX/CY5/ROX	ROX	None

Clicking on every single well it will appear the window "**well information**".
Choose the name of the positive control (*P.malariae positive control and P.vivax positive control).*
It's possible to set the name of the dye near the name of the analysed target:

FAM	HEX	CY5
P.malariae	P.vivax	β-Globin

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/CY5/ROX	ROX	None

Clicking on every single well will appear the window "**well information**", and you can set NTC as the name.

Set near the name of the fluorescent reporters, the name of the analysed targets:

Negative control falciparum/ovale mix

FAM	HEX	CY5
P.falciparum	P.ovale	β-Globin

Negative control malariae/vivax mix

FAM	HEX	CY5
P.malariae	P.vivax	β-Globin

Sign the wells correspondent to samples. Define the sample positions in the right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
Unknown	FAM/HEX/CY5/ROX	ROX	None

Clicking on every single well it will appear the window "**well information**"; it is possible to write in the name or the code of the sample.

Set near the name of the fluorescent reporters, the name of the analysed targets:

FAM	HEX	CY5
P.falciparum	P.ovale	β-Globin

Clicking on a second well for the same sample, it will appear the window "**well information**"; it is possible to write in the name or the code of the sample.
Set near the name of the fluorescent reporters, the name of the analysed targets:

FAM	HEX	CY5
P.malariae	P.vivax	β-Globin

In the toolbar choose the sheet **"Thermal Profile Setup"** and set thermal cycle 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

After preparing the plate and inserting it in the instrument, press the button "**Run**", selecting the sheet Thermal profile status and check the accuracy of the thermal profile.
Select the box **Turn Lamp Off** at the end of execution. Push the button Start: the software will ask to indicate the name of saved 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, VIC and CY5). 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.
In the windows Assay Setup 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
Falciparum/Malarie	FAM	BHQ®-1
Ovale/Vivax	VIC	BHQ®-1
B-Globine	CY5	BHQ®-2

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 1 min)** from the profile and click on (Acquire data)

In the window acquire on, check that are present the correct acquire channels **(Green,Yellow** and **red**)
In the **Temperature Control** choose **standard Taq polymerase** and in the **Reaction Volume** set a volume of **25 µl.**
Save as the **Malaria Panel 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 panel 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 **falciparum/ovale probes Mix;**
Defrost a tube of **malariae/vivax probes Mix;**
Mix carefully by vortex **175 µl** of **Amplification mMix** and **105µl** of **falciparum/ovale probes Mix** (the prepared master mix will allow to perform **12 reactions** of amplification: **2 positive controls, 1 negative control and 9 samples**.
Mix carefully by vortex **175 µl** of **Amplification mMix** and **105µl** of **malariae/vivax probes Mix** (the prepared master mix will allow to perform **12 reactions** of amplification: **2 positive controls, 1 negative control and 9 samples**.

Distribute, in the amplification plate, **20 µl of just reconstituted falciparum/ovale mix** in chosen positions, as already setted on the instrument software.
Distribute, in the amplification plate, **20 µl of just reconstituted malariae/vivax mix** in chosen positions, as already setted on the instrument software.
Distribute, in the negative control position, **5 µl** of the negative control.
Distribute, in chosen position for each sample, **5 µl** of corresponding sample.

Distribute, in chosen positions for the positive controls, **5 µl** of P.falciparum and P.ovale, P.malariae and P.vivax.
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 caps; the presence of air bubbles is not influential on the test, because the centrifugal force of the rotor will allow automatic deletion.
Transfer the plate in the instrument and push the button "**Start Run**".

QUALITATIVE ANALYSIS

Lifetechnologies 7500 Fast
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 **P.falciparum target**. Check the correct setting of the threshold
Select in the "**Option**" window inside the "**Target**" pop-up menu the **P.ovale target**. Check the correct setting of the threshold.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **P.malariae target**. Check the correct setting of the threshold.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **P.vivax 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 an 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)".**
Setting the graphic in **"Linear scale"**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**. Clic on "**Outlier removal**" e set up the "**Percentage of largest FI change**" at 5%.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.
Select the "**Quantification**" sheet and click on "**cycling A (yellow)".**
Setting the graphic in **"Linear scale"**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**. Clic on "**Outlier removal**" e set up the "**Percentage of largest FI change**" at 5%.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.
Select the "**Quantification**" sheet and click on "**cycling A (red)".**
Setting the graphic in **"Linear scale"**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**. Clic on "**Outlier removal**" e set up the "**Percentage of largest FI change**" at 5%.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.

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),** then the file **cycling A (yellow)** and finally the file **cycling A(red).**

Versant kPCR AD or 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 and CY5 in the lower part of the screen and select testing samples.
Select from the plate the wells corresponding to the positive control, negative control and samples in the analysis.
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…**"
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 **Falciparum/Malarie**. 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 **Vivax/Ovale**. 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 P.falciparum, P.ovale, P.malariae and P.vivax are used for detect the presence of the Target in analysis.
Increase of fluorescence of specific probe for **P.falciparum (FAM)** in the mix **falciparum/ovale** shows a positivity of the sample for **P.falciparum**.
Increase of fluorescence of specific probe for **P.ovale (VIC)** in the mix **falciparum/ovale** shows a positivity of the sample for **P.ovale**.
Increase of fluorescence of specific probe for **P.malariae (FAM)** in the mix **malariae/vivax** shows a positivity of the sample for **P.malariae**.
Increase of fluorescence of specific probe for **P.vivax (VIC)** in the mix **malariae/vivax** shows a positivity of the sample for **P.vivax**.
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 amplification mix and the absence of any contamination.
The instrument software is able to analyse the fluorescences that are emitted by the specific probe for P.falciparum (FAM), P.ovale (VIC/HEX), P.malariae (FAM), P.vivax (VIC/HEX) and by the positive internal control (β-globinCY5).
A proper functioning of the amplification mix can be verified analysing these parameters:

Parametro	Riferimento
P.falciparum control (FAM)	Ct ≤ 27
P.ovale control (VIC)	Ct ≤ 27
P.malariae control (FAM)	Ct ≤ 27
P.vivax control (VIC)	Ct ≤ 27

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/VIC/HEX).
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 stage.
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 Malaria DNA and internal control Ct >28 means that some problems happened in the extraction stage or in the amplification stage; therefore the sample could be a false negative. **Repeat the sample**.

Detector FAM	Detector CY5	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:
It is considered as analytical sensitivity the highest serum dilution (title) to which a positive sample can be diluted without that the system looses the ability to detect it as positive. The analytical sensitivity of the system was assessed by analysing plasmid DNA, quantified by spectrophotometric analysis, containing the genomic region of interest (18S Ribosomal RNA gene) of the protozoa in serial dilutions from 100.000 copies to 1 copy of DNA in 5µl of extracted material added in the amplification reaction.

	Conc.	1 cps	10 cps	100.000 cps
P.falc.	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.mal.	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 the DNA target added to the amplification reaction (2cps/µl), corrisponding to:

Extraction	Clonit	Siemens
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.ovale, P.malariae and P.vivax positive samples and the test is performed following the method advices. Positive samples are confirmed with another disposable method.

Sample	P.falciparum	P.vivax	P.ovale	P.malariae
n°	61	10	16	5
Positive	60	10	16	5
Negative	1	0	0	0
Clinic.Sens	98.36%	100%	100%	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 analysing human genomic samples tested and confirmed as negative with another disposable system.

Sample	P.falciparum	P.vivax	P.ovale	P.malariae
n°	30	30	30	30
Positive	0	1	0	0
Negative	30	29	30	30
Spec.diagn.	100%	97%	100%	100%

Traceability versus NIBSC controls material
The NIBSC standard (NIBSC code 04/176, Version 3.0, Dated 09/05/2012) was established as the 1st WHO International Standard for P.falciparum, with an assigned potency of $1x10^9$ International Units (IU) when reconstituted in 0.5 ml of nucelase-free water.

	7500 fast	Rotorgene Q	Kpcr Versant
P.falciparum	Positive	Positive	Positive
P.malariae	Negative	Negative	Negative
P.ovale	Negative	Negative	Negative
P.vivax	Negative	Negative	Negative

Ref: Padley DJ, Heath AB, Sutherlan C., Chiodini PL, Baylis SA; Collaborative Study Group Malaria. 2008 July 27:7:139. ***Establishment of the 1st WHO International Standard for P.falciparum DNA for nucleic acid amplification technique (NAT) based Assays***.

Analytical Specificity:
Test's specificity is guaranteed by the use of specific primers for determining P.falciparum, P.ovale, P.malariae and P.vivax.
The alignment of the choose regions for specific primers' hybridization for P.falciparum, P.ovale, P.malariae and P.vivax demonstrated: their conservation, the absence of significative mutations and the complete specificity for the analysed target.

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

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CE IVD
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