alethia™

Malaria and Malaria PLUS DNA Amplification Assays RESEARCH USE ONLY

DNA Amplification Assays for the Detection of *Plasmodium sp.*

REF **480925RUO, 481125RUO** RUO

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTENDED USE
The Alethia Malaria and Alethia Malaria PLUS DNA amplification assays, performed on theAlethia Incubator/Reader™, are qualitative research use only tests for the direct detection of *Plasmodium sp.* DNA in human venous EDTA whole blood specimens.

Alethia Malaria assays utilize loop-mediated isothermal DNA amplification (LAMP) technology to detect *Plasmodium sp.* DNA by targeting segments of the *Plasmodium* genome. Alethia Malaria assays do not distinguish between *Plasmodium* species.

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REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.
For Alethia Malaria, Catalog Number 480925RUO
1. **Alethia Malaria Test Device:** Two-chambered device containing lyophilized amplification reagents (DNA polymerase, deoxynucleotide triphosphates) and either *Plasmodium sp.*-specific primers (TEST Chamber) or human mitochondrial DNA-specific primers (CONTROL Chamber).
2. **Alethia Buffer I**: Lysis solution containing 0.2N sodium hydroxide.
3. **Alethia Sample Preparation Apparatus IV (SMP PREP IV):** Tris-buffer solution containing 0.09% azide as a preservative.
4. **Alethia Tube I:** 1.5 mL tubes

For Alethia Malaria PLUS, Catalog Number 481125RUO
1. **Alethia Malaria Test Device:** Two-chambered device containing lyophilized amplification reagents (DNA polymerase, deoxynucleotide triphosphates) and either *Plasmodium sp.*-specific primers (TEST Chamber) or human mitochondrial DNA-specific primers (CONTROL Chamber).
2. **Alethia Buffer I:** Lysis solution containing 0.2N sodium hydroxide.
3. ***M-prep* Buffer II:** Solution containing phenol red and 0.09% azide as a preservative.
4. ***M-prep* Buffer III:** Tris-buffered solution with 0.09% azide as a preservative.
5. ***M-prep* Column:** 5.0 cm long column with twist-off tip and plug seal cap containing chromatography resin in a tris-buffered solution containing sodium azide (0.09%) as a preservative.
6. **ST Tubes:** 2.0 mL screw-top microcentrifuge tubes.

MATERIALS NOT PROVIDED
1. Disposable latex gloves, powder free
2. DNase/RNase-free, aerosol resistant pipette tips
3. Blood collection tubes with EDTA anticoagulant

EQUIPMENT NOT PROVIDED
1. Interval Timer
2. Vortex mixer (optional)
3. Micropipette capable of dispensing 50 µL
4. Micropipette capable of dispensing 250 µL (Catalog Number 481125RUO only)
5. Alethia Incubator/Reader ™, Meridian Bioscience, Inc. Catalog Number: 610189

PRECAUTIONS
1. **All reagents are For Research Use Only. Not for use in diagnostic procedures.**
2. Do not interchange kit reagents and Test Devices between lots. Individual lots of Tube I and ST Tubes are interchangeable within each kit type provided they are within the assigned expiration date when used.
3. The Alethia Incubator/Reader may produce incorrect results if the Malaria assay program is not used for testing.
4. Follow Biosafety Level 2 and Good Laboratory practices during testing.[1] Treat all specimens and used Test Devices as capable of transmitting infectious agents. Do not eat, drink or smoke in areas where specimens or kit reagents are handled.
5. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
6. Quality Control Programs for Molecular Testing Laboratories, including proper use and care of equipment, should be employed.[2]
7. The Alethia Malaria Test Devices contain lyophilized reagents. The protective pouches should not be opened until ready to perform the assay.
8. The Alethia Malaria Test Devices include a latch feature that is designed to prevent contamination of the test area with amplification product. Do NOT use Test Devices with broken latches.
9. Dispose of used Alethia Malaria Test Devices, ***M-prep*** Columns, and tubes immediately after processing. Leave the Test Device latch securely in place. Do NOT open the Test Device after processing. Opening the device after amplification may result in contamination of the test area with amplification product.

HAZARD AND PRECAUTIONARY STATEMENTS
Refer to the SDS, available at www.meridianbioscience.com, for Hazards and Precautionary Statements.

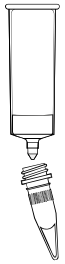
SHELF LIFE AND STORAGE
The expiration date is indicated on the kit label. Store the kit components at the temperature indicated on the label.

REAGENT PREPARATION
Ensure kit reagents are at room temperature (19-30 C) before use. Ensure Buffer I has been warmed to room temperature completely prior to use and no precipitate is visible. Incorrect results may be obtained if reagents are not brought to room temperature prior to use.

COLUMN PREPARATION (For Alethia Malaria PLUS Catalog Number 481125RUO only)
Visually inspect each ***M-Prep*** Column and ensure the column bed and filters were not disturbed during transportation. Columns with disturbed resin beds or improperly placed filters may not perform correctly.

Column Set-up:
1. Use 1 ***M-prep*** Column for each sample to be tested. Remove the top cap and the bottom twist-off tip.
2. Place the column tip into an ST Tube. The ST Tube should be held at a slight angle (approximately 15 degrees) from the ***M-prep*** Column.
   NOTE: Do not seat the column directly in the collection tube as this may create a seal and cause improper flow through the column. Refer to **Figure 1** for proper column and collection tube placement.
3. Let the ***M-prep*** Column drain into the ST Tube. Drained ***M-prep*** Columns should be used within 1 hour.
4. Proceed with Sample Preparation for **Alethia Malaria PLUS**.

FIGURE 1: *M-prep* Column and ST Tube placement



SPECIMEN COLLECTION AND PREPARATION
Sample type: Human venous whole blood samples with EDTA as a preservative.

Sample Collection: Venous whole blood samples should be collected into a specimen tube containing EDTA. Ensure the blood collection tube is inverted immediately after collection at least 8 times, or according to the manufacturer's instructions.

EDTA venous whole blood samples may be stored at 2-30 C after collection and during transportation to the laboratory. Samples should be tested as soon as possible, but may be stored for up to 7 days at room temperature (19-30 C) or up to 14 days refrigerated (2-8 C) prior to testing. Samples that will not be tested within this time frame should be frozen immediately at -20 C for up to 30 days until tested. Samples may be frozen and thawed 2 times after storage at -20 C prior to testing with the Alethia Malaria assays.

SPECIMEN PREPARATION
NOTE: Ensure that the Alethia Incubator/Reader is powered on and required performance verifications have been completed prior to initiation of SPECIMEN PREPARATION. Refer to the Alethia Incubator/Reader Operator's Manual for further information regarding instrument set-up and operation.

NOTE: Ensure specimens are at room temperature (19-30 C) before specimen preparation.

Specimens to be tested with the Alethia Malaria assay may be prepared using one of the following methods:

1. Alethia Malaria Specimen Preparation (Catalog Number 480925RUO)
   a. Invert EDTA whole blood specimen 2-3 times to mix.
   b. Add 50 µL of the collected venous whole blood sample (with EDTA) to one tube of Buffer I. Mix by inversion 5 times or by vortexing for approximately 10 seconds. Hold the sample for 2 minutes.
   c. Mix by inversion 5 times or by vortexing for approximately 10 seconds and immediately transfer 50 µL of lysate into SMP PREP IV. Mix by inversion 5 times or by vortexing for 10 seconds.
   d. Gently squeeze the SMP PREP IV and slowly collect 5 to 10 drops into a clean Tube I. Visually verify that the eluate is tinted red to reddish brown. Label the tube with the specimen identification and proceed to the Test Procedure.

2. Alethia Malaria PLUS Specimen Preparation (Catalog Number 481125RUO)
NOTE: Sample elution steps with ***M-prep*** Columns should take no longer than 30 minutes. Samples that take longer than 30 minutes to elute should be discarded and re-tested with the original patient sample.
   a. Invert EDTA whole blood specimen 2-3 times to mix.
   b. Add 50 µL of the collected venous whole blood sample (with EDTA) to one tube of Buffer I. Mix by inversion 5 times or by vortexing for approximately 10 seconds. Hold the sample for 2 minutes.
   c. Mix by inversion 5 times or by vortexing for approximately 10 seconds and, using a micropipette, immediately transfer 250 µL of the prepared sample to the top of an appropriately labeled and prepared ***M-prep*** Column. Wait approximately 2 minutes, or until the sample has been absorbed by the column and flow stops. This step should take no longer than 30 minutes.
   d. Using a micropipette, add 250 µL of ***M-prep*** Buffer II to the top of the ***M-prep*** Column. Discard the pipette tip. The column will have a red appearance after the addition of ***M-prep*** Buffer II. Wait approximately 2 minutes, or until the red-colored buffer is absorbed by the column and flow stops. This step should take no longer than 30 minutes.
   e. Remove the last drop of liquid from the column tip with the ST Tube. Discard the tube.
   f. Place a clean ST Tube under the ***M-prep*** Column. Using a micropipette, add 250 µL of ***M-prep*** Buffer III to the top of the ***M-prep*** Column. Discard the pipette tip. Wait approximately 2 minutes or until flow stops. This step should take no longer than 30 minutes.
   g. Remove the last drop of liquid from the column tip with the ST Tube. Visually verify that the eluate is tinted red to reddish brown. Label the tube with sample identification information and proceed to the Test Procedure.

TEST PROCEDURE
NOTE: A maximum of 10 samples can be processed in a single Alethia Incubator/Reader run.
1. Remove 1 Alethia Malaria Test Device from its protective pouch per sample. Carefully open the device, holding the chambers such that the lyophilized reagents will not fall out upon opening. Place the device on a flat surface or in a rack that can accommodate the device.
2. Using a micropipette, transfer 50 µL of the sample to both the TEST (Left/White Bead) and CONTROL (Right/Yellow Bead) chambers of the Alethia Malaria Test. Take care to not introduce air to the reaction mixture. Do not mix reactions with pipette.
3. Close the Alethia Test Device and fasten the latches securely.
4. Tap device(s) on the bench top or mix to remove air bubbles. Carefully examine the Test Device(s) for rehydration of the Control/Test Bead, for air bubbles left in the chamber and liquid in the top of the device. If undissolved beads, air bubbles or liquid in the top of the device are noted, tap the device on the bench top and repeat visual inspection. Amplification and detection should be initiated within 15 minutes.
5. Repeat Test Procedure Steps for all samples to be tested.
6. Insert the Alethia Test Devices into the Alethia Incubator/Reader and initiate run **using the Malaria Program.** Results will be displayed at the conclusion of the run.

INTERPRETATION OF RESULTS

Sample ID	Reported Result	Interpretation
Specimen	POSITIVE	Sample contains *Plasmodium sp.* target DNA.
	NEGATIVE	No *Plasmodium sp.* DNA detected.
	INVALID	**No reportable result. Repeat the test using the original sample.** Inhibitory specimen, improper sample preparation, reagent failure, instrument failure or internal control failure.
Positive Control	POSITIVE	Valid positive control result. Reagents active at time of use, Alethia Incubator/Reader performing correctly.
	NEGATIVE	**Incorrect control result.** Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.
	INVALID	**No reportable result. Repeat entire assay run using original samples.** Improper sample preparation, reagent failure, instrument failure or internal control failure.
Negative Control	POSITIVE	**Incorrect control result.** Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.
	NEGATIVE	Valid negative control result. Reagents active at time of use, Alethia Incubator/Reader performing correctly.
	INVALID	**No reportable result. Repeat entire assay run using original samples.** Improper sample preparation, reagent failure, instrument failure or internal control failure.
EMPTY WELL	NONE	No Alethia Test Device in the Alethia Incubator/Reader Well. **OR** The Alethia Test Device present is compromised due to sample preparation failure, dirty device or improperly seated device. **Repeat the test using original sample.**

QUALITY CONTROL

1. Each device contains an internal control that controls for amplification inhibition, assay reagents, DNA preparation, and sample processing effectiveness. Human mitochondrial DNA, which serves as the internal control DNA, is isolated from the whole blood sample and processed through all steps of the procedure. Primers for amplification of internal control DNA are present in the Control Chamber of the Alethia Test Device.
2. Alternatively, previously characterized clinical or contrived *Plasmodium sp.* positive blood samples can be used as an external positive control. A qualified negative human whole blood sample with EDTA may be used as an external negative control.
3. A separate Test Device must be used for each external control reagent.

LIMITATIONS OF THE PROCEDURE

1. This product can only be used with the Alethia Incubator/Reader instrument.
2. Alethia Malaria and Alethia Malaria PLUS do not distinguish between *Plasmodium* species.
3. Hematocrit (>57.5%) and hemoglobin (>30 g/dL) above normal physiological levels may produce invalid results with the Alethia Malaria assays.
4. Performance of the Alethia Malaria assays has not been established for packed red blood cell specimens.
5. Alethia Malaria and Alethia Malaria PLUS are qualitative assays and do not provide quantitative values or information about organism load.
6. The detection of nucleic acids is dependent upon proper specimen collection, handling, transportation, storage and preparation. Failure to observe proper procedure in any one of these steps can lead to incorrect results.
7. Organism nucleic acid may persist *in vivo*, independent of organism viability. Alethia Malaria and Alethia Malaria PLUS do not distinguish between viable and nonviable organisms.
8. As with all molecular-based tests, (A) False-negative results may occur from the presence of inhibitors, technical error, sample mix-up or low numbers of organisms in the clinical specimen; (B) False-positive results may occur from the presence of cross-contamination by target organisms, their nucleic acids or amplified product, and from non-specific signals.

REFERENCES

1. US Department of Health and Human Services PHS/CDC/NIH. Biosafety in microbiology and biomedical laboratories, Washington DC: US Government Printing Office, 2007.
2. CLSI: MM3-A2 Molecular diagnostic methods for infectious disease; approved guideline, 2nd ed. Wayne PA: Clinical Laboratory Standards Institute. 2006.

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Manufactured By

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SYMBOL USAGE

You may see one or more of these symbols on the labeling/packaging of this product:

Key guide to symbols

	Use By	CONTROL +	Positive control
LOT	Batch Code	CONTROL −	Negative control
IVD	In vitro diagnostic medical device	EC REP	Authorized Representative in the European Community
CE	This product fulfills the requirements of Directive 98/79/EC on in vitro diagnostic medical devices	SMP PREP DIL SPE	Sample Preparation Apparatus containing Sample Diluent
REF	Catalogue number		Do not freeze
	Consult Instructions for Use	RoHS	Restriction of Hazardous Substances
	Manufacturer		Caution, consult accompanying documents
	Single Use Only	STERILE R	Sterilization by gamma irradiation
	Female	STERILE EO	Sterilization by ethylene oxide
Σ	Contains sufficient for <n> tests	BUF RXN	Reaction Buffer
	Temperature limitation		ETL Registered Mark Certified
SN	Serial number		Recycle - do not dispose of as general waste
TEST	Test Device	HT TUBE	Heat Treatment Tube
	Date of manufacture		For IVD Performance Evaluation Only
	LASER RADIATION: Avoid Exposure to Beam		HOT SURFACE: Keep hands Away from Hot Surfaces
CAUTION	CAUTION: Laser Radiation	IPX-0	CAUTION: Protect from water
	CAUTION: Risk of Danger	CONTROL	Assay Control
BUF	Buffer	MIN OIL	Mineral Oil
MEDIA	Media		Warning
ST TUBE	Screw Top Tube	IUO	Investigational Use Only
RUO	Research Use Only	COL	Sample Preparation Column
BUF SMP	Sample Buffer	PRE REAG	Pretreatment Reagent
Rx Only	Prescription Use Only	SMP PREP	Sample Preparation
TUBE	Empty Tube		

For technical assistance, call Technical Support Services at 800-343-3858 between the hours of 8AM and 6PM, USA Eastern Standard Time. To place an order, call Customer Service Department at 800-543-1980.