RIDA®QUICK Malaria

Product code: N7006
Product code: N7007
1. Intended use
For in vitro diagnostic use. RIDA®QUICK Malaria is an immunochromatographic rapid test to qualitatively detect Plasmodium falciparum (P.f.), Plasmodium vivax (P.v.), Plasmodium ovale (P.o.) and Plasmodium malariae (P.m.) antigens in whole blood. It is intended for the quick diagnosis of malaria as soon as the first clinical symptoms appear.

2. Summary and explanation of the test
In many tropical countries, malaria is still a serious problem and in a few regions it is even a growing problem. A distinction is made between three different types of malaria. The most dangerous is the form caused by the Plasmodium falciparum pathogen. Known as malaria tropica, this form is almost exclusively responsible for the deaths and accounts for the majority of the malaria diseases imported to the industrial countries. The pathogens of the other types of malaria are Plasmodium vivax and Plasmodium ovale (malaria tertiana) as well as Plasmodium malariae (malaria quartana). In recent years, Plasmodium knowlesi has been included as the fifth malaria pathogen. It has a similar potential for disease as P. falciparum and cannot be differentiated from P. malariae microscopically and is often confused with it.
All malaria pathogens are transmitted through the bite of a female anopheles mosquito. The incubation period can range from a few days (malaria tropica) to several years (malaria quartana). The symptoms of malaria are relatively uncharacteristic. They often include flu-like symptoms without the signs of a common cold. These typically include bouts of fever with chills, headaches, back aches and pains in the limb. In case of atypical developments, symptoms can mostly be gastrointestinal symptoms. Subfebrility or afebrility can also occur. In case of malaria tropica, life-threatening complications can occur after the sixth day of illness, and they can be prevented through effective and early therapy. This is why an early diagnosis is indispensable.
The malaria prophylaxis is composed of the avoidance of mosquito bites and, if necessary, the intake of malaria medication in preventative or therapeutic dosages. However, resistances have already developed against each available malaria medication, particularly in the case of Plasmodium falciparum. Nevertheless, chemoprophylaxis can significantly reduce the risk of malaria in regions with multiple drug resistances. Taking malaria medication for self-treatment as needed (standby therapy) is only considered for short stays in malaria prone regions or on trips in regions with a very low malaria risk, as well as in cases of known intolerances against prophylactic medication. In such cases, it is recommended to take an easy to use malaria rapid test that is suitable for the trip. Particularly in regions where there is no practising doctor who is specialised in tropical diseases, a malaria rapid test is very helpful in early diagnosis, which can prevent life-threatening delays. Thus, if the rapid test is positive, the medication taken on the trips can be used in a targeted manner.
The common standard method for the minimal diagnosis of malaria in a laboratory is the detection of plasmodia in peripheral blood with the help of the “thick blood film” after Giemsa staining. The RIDA®QUICK Malaria rapid test is a quick alternative that can also be performed by persons without special training. The test can also be used as a screening method for serial
examinations in blood banks. In the event of microscopically uncertain parasite morphology or parasite morphology that can be difficult to assess, the test can also be used as a confirmatory test for malaria. The RIDA®QUICK Malaria rapid test is also very suited for treatment monitoring because it can detect the reduction of plasmodia antigens still circulating in the blood, even if the plasmodia morphology of the different blood stages in the blood smear is no longer recognisable under the microscope. Nevertheless, the definitive diagnosis of malaria is based on the microscopic examination of the blood, supported by further additional laboratory parameters in conjunction with the clinical diagnosis of the patient.

3. Test principle

The RIDA®QUICK Malaria test is a single stage immunochromatographic rapid test for the combined detection of the histidine-rich protein (PfHRP-2) specific for \textit{Plasmodium falciparum} as well as plasmodia-specific lactate dehydrogenase, which occurs in all malaria pathogens. Capture antibodies against the HRP-2 antigen of \textit{Plasmodium falciparum} and against the plasmodia-specific LDH are immobilised in two cross-lines on the nitrocellulose membrane located in the cassette housing. Furthermore, there is a third line, the control line, to which the other specific capture antibodies are connected. In addition, antibodies fixed to the colloidal gold in appropriate specificity are located in the area for the application of blood sample. Lysis of the erythrocytes and the release of the plasmodia-antigens present in the specimen takes places after the blood sample is transferred to the provided funnel on the test cassette and the subsequent addition of reagents into the second funnel provided for this purpose. In this process, the existing antigen (PfHRP-2 and/or pLDH) binds to the gold-marked antibodies, flows through the cellulose membrane and crosses the three cross-lines with the immobilised antibodies in the process. In case of positive specimens, the antigen and gold-marked antibody complex is bound to these immobilised antibodies, and then condenses into one or two pink-coloured test lines (\(T_1\) and/or \(T_2\)) as well as the pink-coloured control line (\(C\)) that appears in all cases. Only the pink-coloured control line is visible in plasmodia-negative blood samples.

4. Package contents

The reagents in a kit are sufficient for 25 (N7006) or 5 (N7007) assays

<table>
<thead>
<tr>
<th>Cassettes</th>
<th>25 (5) assay</th>
<th>25 (5) individually packaged test cassettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>3 (1) mL</td>
<td>Vials with extraction reagent</td>
</tr>
<tr>
<td>Micro Pipet</td>
<td>25 (5) units</td>
<td>MICROSAFE® Tubes 5 µL</td>
</tr>
</tbody>
</table>

5. Reagents and their storage

The kit can be stored at 2 °C to 40 °C and can be used until the printed date of expiration. It must be protected against moisture and frost and stored in a dry location. After the expiry date, the quality guarantee is no longer valid. Similarly, the usability of the cassettes can no longer be guaranteed, if the cassette package is damaged.
6. Materials required but not provided
- Timer
- Waste container with a 0.5% sodium-hypochlorite solution

7. Precautions
For in vitro diagnostic only.
All test cassettes are intended only for one-time use and must not be reused.
Reagents and cassettes of different batches must not be mixed together.
This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always strictly adhere to the instructions for use for this test.
The reagents contain sodium azide as a preservative. This substance must not be allowed to come into contact with skin or mucous membranes.
Do not pipette samples or reagents with the mouth. Avoid contact with broken skin or mucous membranes. When handling the specimens, wear disposable gloves and when the test is finished, wash your hands. Do not smoke, eat, or drink in areas where samples are being processed.
All reagents and materials coming into contact with potentially infectious specimens must be treated with suitable disinfectants (e.g. sodium hypochlorite) or autoclaved at 121 °C for at least one (1) hour.

8. Collection and storage of samples
Blood specimens must be collected in clean standard tubes and may be stored for up to three (3) days at room temperature or at 2 °C to 8 °C before use. For longer storage, the blood samples can be stored frozen at -20 °C. To inhibit coagulation, the collection tubes for the blood samples can contain EDTA, citrate or heparin.

9. Test procedure
9.1. General information
The specimen, reagents and the test cassettes must be brought to room temperature (20 °C to 25 °C) before use. The test cassettes must be taken out of the additional package shortly before use. Once used, the test cassettes must not be re-used. The test must not be carried out in direct sunlight.

9.2. Sample preparation
The specimens collected and stored according to pt. 8, once they have reached room temperature (20 °C to 25 °C), can be used undiluted in the test.
Similarly, fresh blood after puncturing a fingertip previously disinfected with alcohol can be collected directly using the Micro Pipet provided with the 5-µL MICROSAFE® tube kit and used
according to the test procedure described below. For proper use of the MICROSAFE® Tubes, strictly observe the respective instructions.

9.3. Sample testing

Remove the test cassette [Cassette] from the foil and place on an even surface. Transfer 5 µL of blood by carefully touching the test membrane on the base of the specimen funnel (Blood) with the pipette tip of a disposable pipette, [Micro Pipet], contained in the MICROSAFE® Tubes kit or alternatively, a laboratory pipette that can be adjusted to 5 µL.

**Important:**
When using a MICROSAFE® Tubes [Micro Pipet], do not press the bulb while collecting blood. The filling automatically takes place up to the 5-µL marking once the pipette tip has been immersed into the blood sample. If the bulb is pressed, once the pipette tip has made contact with the test membrane on the base of the specimen funnel of the cassette, 5 µL of blood will be transferred to the test membrane. For this purpose, please observe the enclosed instructions on the micro-pipettes.

Inexact specimen volumes can result in incorrect results that cannot be analysed. Subsequently, following the same process, pipette four (4) drops of the reagent [Reagent] using the drop vial into the funnel marked “Reagent”. Please make sure that the reagent is completely absorbed by the membrane. This is the only way to guarantee that sufficient reagents combine with the drops of blood applied and flow through the membrane together in order to detect possible malaria pathogens. Through the inspection window of the cassette with the lateral markings T1, T2 and C, it is possible to observe how the mixture flows through the membrane. The C marking should appear no later than after five (5) minutes and a pink-coloured band should appear on the membrane. This means: the test was performed correctly and worked. Over the course of time, the membrane that starts out white only turns red due to the blood. The final result is first read after 30 minutes. Bands that appear later on have no influence on the evaluation of the test.

The band patterns are evaluated and interpreted according to the instructions enclosed with the kit.

10. Quality control, signs of reagent deterioration

The test should be evaluated only if the test cassette is intact prior to the pipetting of the specimen suspension and no changes in colour or bands can be seen on the test membrane. Furthermore, after an incubation period of five (5) minutes, at least the red-violet control bands must be visible. If they do not appear, check the following items prior to repeating the test:

- Storage life of the test cassettes and the reagents used
- Correct test procedure
- Contamination of the reagents

If, after a repetition of the test with a new test cassette, the control bands are still not visible, please contact the manufacturer or your local R-Biopharm agent.
11. Evaluation and interpretation

A maximum of three bands must appear, viewed from the blood application field, in the following sequence: A pink-coloured reaction band on the T$_2$ test line and another at the T$_1$ test line as well as the pink-coloured control band on control line C.

**If control band C is absent, the test cannot be assessed and is invalid!**

The following interpretations are possible:

- **Malaria positive:** all three bands (T$_2$, T$_1$, and C) or only one of the two T-bands are visible along with the C-band.
- **Malaria negative:** only control band C is visible.
- **Invalid:** no bands are visible or there is another constellation than the one described. Band discolourations that appear after more than 30 minutes, have no diagnostic value and must not be assessed. An overview of all constellation possibilities of the three specific bands T$_2$, T$_1$, and C can be found in the application instructions enclosed with the test kit.

12. Limitations of the method

The RIDA®QUICK Malaria test is a purely qualitative test for detection of specific soluble antigens of plasmodia in blood samples. It is not possible to associate between the intensity of the specific bands visible and the occurrence or severity of clinical symptoms.

The results obtained must always be interpreted in combination with the clinical signs and symptoms and other laboratory parameters.

A **positive** result does not rule out the presence of other infectious pathogens or causes.

A **negative** result does not rule out a possible infection with malaria pathogens. It can be caused by a distribution of plasmodia in the blood that was too low at the time of the collection of blood. If there are substantial anamnestic grounds for suspecting malaria, a second test with a new, fresh blood sample must be carried out around 12 hours later. A final diagnosis must always be verified or confirmed by further laboratory methods, such as the microscopic examination of the blood smear.

13. Performance characteristics

13.1. Clinical sensitivity and specificity

In a prospective study from January to December 2013, a total of 93 blood samples were examined in the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel with the RIDA®QUICK Malaria rapid test compared to the gold standard (microscopy of the thick blood film and the blood smear). The specimens came from patients with suspected malaria, who presented to TPH or were sent from hospitals, laboratories and established doctors from all around Switzerland. All specimens were tested in parallel in another commercially available rapid test. The two rapid tests detected the histidine-rich protein (HRP2) specific for *P. falciparum* and another different pan-malaria-specific protein on a second band for the detection of other plasmodia species. In the RIDA®QUICK Malaria rapid test, it is plasmodia-specific
lactate dehydrogenase and, in the NOW® Malaria, plasmodia-specific aldolase. The results are summarised in table 1. The results of both bands are summarised into an overall result. By means of microscopy, it was not possible to determine the species from one of the original 93 specimen. This specimen was not included in the evaluation.

Table 1: Results from 93 blood samples suspected of malaria in comparison with two commercial malaria rapid tests with the gold standard methods of the Swiss TPH Institute in Basel

<table>
<thead>
<tr>
<th>Microscopy of thick blood film/smear</th>
<th>RIDA®QUICK Malaria</th>
<th>NOW® Malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>-</td>
<td>6</td>
<td>5†</td>
</tr>
</tbody>
</table>

* P. ovale, parasitaemia < 0.1%

§ P. falciparum (1x), P. ovale (1x) parasitaemia < 0.1%, P. vivax (1x), P. malariae (1x)

# 5 x therapy control of previous microscopically positive specimen; 1 of 6 is PCR-confirmed positive

† 4 x therapy control of previous microscopically positive specimen, 1 of 5 is PCR-confirmed positive

Accordingly, the following test specifications are calculated for the two rapid test procedures:

Sensitivity: 97.7% 90.9%

Specificity: 87.8% 89.8%

13.2. Analytical sensitivity

The detection limit for the RIDA®QUICK Malaria rapid test was also determined in the Swiss TPH Institute through the serial dilution of two P. falciparum-positive blood samples and one P. vivax-positive blood sample. The dilution, in which a weak band was visible on the T1 or T2 line, was considered the detection limit. The detection limits determined in this manner were determined only for the two plasmodia species that were detected most frequently. They amounted to:

**P. falciparum**: 45 plasmodia/µL blood

**P. vivax**: 100 plasmodia/µL blood

13.3. Analytical specificity

In order to test possible cross reactions with potentially harmful components (rheumatoid factor (RF), anti-nuclear antibodies (ANA) and human anti-mouse antibodies (HAMA)) as well as various pathogens, different positive specimen for such components and pathogens were tested in RIDA®QUICK Malaria. Only one of the tested pathogens (HCV, HIV, HBV, dengue virus, Chikungunya virus, Schistosoma) showed a weak cross reaction. All specimens with ANA and HAMA as well as rheumatoid factor concentrations up to 300 IU/mL were negative. At
400 IU/mL RF, there was a weak discolouration on the \( T_2 \)-test band. Table 2 shows the results in an overview. Further possible cross reactions can generally not be excluded. Positive findings should always be verified prior to introducing a specific anti-malaria treatment through another laboratory standard method (see pt. 12 Limits of the Method).

Table 2: Results of tests on analytical specificity

<table>
<thead>
<tr>
<th>Pathogens/disruptive factors</th>
<th>Number of tested specimen</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chikungunya virus</td>
<td>10</td>
<td>Negative</td>
</tr>
<tr>
<td>Dengue virus</td>
<td>10</td>
<td>Negative</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>11</td>
<td>Negative</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>21</td>
<td>Negative *</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>14</td>
<td>Negative</td>
</tr>
<tr>
<td>Schistosoma</td>
<td>3</td>
<td>Negative</td>
</tr>
<tr>
<td>Rheumatoid factor 6 (200–400 IU/mL)</td>
<td></td>
<td>Negative #</td>
</tr>
<tr>
<td>HAMA 2 (400–600 ng/mL)</td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>ANA 5 (titre 160–1280)</td>
<td></td>
<td>Negative</td>
</tr>
</tbody>
</table>

* 3 of the 21 tested specimen resulted in a signal on the \( T_2 \) test band

# At 400 IU/mL, the result on the \( T_2 \) test band was weakly positive.
Appendix

Test-specific symbols:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Cassette</td>
<td>Test cassette</td>
</tr>
<tr>
<td>Reagent</td>
<td>Vials with extraction reagents</td>
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<tr>
<td>Micro Pipet</td>
<td>MICROSAFE®-Tubes 5 µL</td>
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References


