The reagents in one package are sufficient for 20 tests.

b) There are control bands at the upper end of the test strips:

3. Specific antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

There are control bands at the upper end of the test strips:

a) The reaction control band under the strip number.

b) The conjugate control band (IgG) is used to check the detected specific antigens.

c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of each antigen band (see 9.2. Evaluation).

4.2 Materials required but not supplied

- Incubation trays (can be purchased as needed from MIKROGEN)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker

4.2 Materials required but not supplied

- Vortex mixer or other rotators
- Vacuum pump or similar device
- Volumetric cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for bio-hazardous materials

5 Shelf life and handling

Store reagents at +2 °C to 8 °C before and after use, do not freeze.

Subject all ingredients for at least 30 minutes to room temperature (+18 °C to 25 °C) before beginning the test. The test procedure is carried out at room temperature.

Washing Buffer, Milk Powder, Dilution Buffer, Conjugate and TMB can be interchanged between the different recomLine and recomBlot test systems. If these components carry the same symbols, consider the shelf life of these components.

Mix the concentrated reagents and samples thoroughly before use.

Avoid a build up of foam.

Open the tube containing the test strip immediately before use to avoid condensation. Leave unused strips in the tube and continue to store at +2 °C to +8 °C (reseal tube tightly, test strips must not become moist before the test!)

The strips are marked with the serial number, as well as the test code.

The packages bear an expiration date. After this has been reached no guarantee of quality can be offered.

Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.

The test should only be carried out by trained and authorised personnel.

In case of significant changes by the user to the product and/or the instructions for use, application may be beyond the purpose specified by MIKROGEN.

Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Add patient samples, test strips and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells. Carefully drain liquids.

The strips must be completely wetted and immersed throughout the entire procedure. Automation is possible; you will receive further information from MIKROGEN.

6 Warnings and precautions

For In vitro diagnostic use only.

All blood products must be treated as potentially infectious.

The test strips were manufactured with inactivated whole cells of viruses and other pathogens. All reagents and samples must be considered infectious and treated as such.

Suitable disposable gloves must be worn throughout the entire test procedure.

The reagents contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion and chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive mixture with copper or lead azide.

All siphoned liquids must be collected. All containers must include appropriate disinfectants for the inactivation of pathogenic human viruses and other pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods of the manufacturer must be observed.

Use incubation trays only once.
Instructions for use (English)

7. Sampling and preparation of reagents

7.1 Samples

The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling, so as to avoid haemolysis. Avoid Microbial contamination of the samples. Insoluble substances must be removed from the sample prior to incubation. The use of heat-inactivated, icteric, haemolyzed, lipemic or turbid samples is not recommended.

Caution!

If the tests are not carried out immediately, the samples can be stored for up to 2 weeks at 2°C to 8°C. Prolonged storage of the samples is possible at -20°C or below. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results. Avoid more than 3 cycles of freezing and thawing.

7.2 Preparation of solutions

7.2.1 Preparation of ready-to-use wash buffer A

This buffer is required for serum and conjugate dilution as well as washing stages. Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.

First, the skimmed milk powder is dissolved in wash buffer A concentrate, and then deionised water is added to bring the solution up to the final volume (dilution: 1 + 9). The quantities required for a defined number of test strips are to be mathematically determined according to the following formula (device-specific dead volume is not considered):

\[
\text{Formula} = \frac{\text{number of strips}}{10} \times 20 \\
\text{Example:} = \text{number of strips} \times 20
\]

Ready to use wash buffer A can be stored at 2°C – 8°C for up to four weeks. The ready to use wash buffer A is odourless and easily marred.

7.2.2 Preparation of conjugate solutions

The conjugate solution must be prepared immediately before use. It is not possible to store the ready for use conjugate solution. One part of the conjugate concentrate is diluted with 100 parts of the ready to use wash buffer A (1 + 100). The quantities required for a defined number of test strips are to be calculated according to the following formula:

\[
\text{Formula} = \frac{\text{number of strips}}{10} \times 20 \\
\text{Example:} = \text{number of strips} \times 20
\]

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate for 1 to 3 strips.

8 Test procedure

<table>
<thead>
<tr>
<th>No.</th>
<th>Execution</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temper all reagents for at least 30 minutes at 18°C - 25°C (room temperature) before beginning the test.</td>
<td>The test procedure is carried out at room temperature.</td>
</tr>
<tr>
<td>2</td>
<td>Prepare test strips</td>
<td>Do not touch the strips with bare hands - use the forceps. The strip number points upward. A well is required in the incubation tray (see 4.2) for each strip. The strips must be completely immersed.</td>
</tr>
<tr>
<td>3</td>
<td>Incubation of samples</td>
<td>Pipette the sample/control at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray. Cover the incubation tray with plastic cover and place in the shaker.</td>
</tr>
</tbody>
</table>

4. Washing

a) Carefully remove the plastic cover from the incubation trays.

b) Gently siphon serum dilution from the individual wells.

c) Pipette 2 ml of ready to use wash buffer A into each well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.

5. Incubation with conjugate

Add 2 ml of ready-to-use conjugate solution and incubate for 45 minutes with gentle shaking.

6. Washing

See under 8.4.

7. Substrate reaction

Add 1.5 ml of ready-to-use substrate solution and incubate for 8 minutes while shaking gently.

8. Stopping the reaction

Wash at least three times briefly with deionised water.

9. Drying the strips

Dry the strips between 2 layers of absorbent paper for 2 hours prior to analysis.

Carefully remove strips from water using plastic forceps. Store strip away from light.

9 Results

Caution:

Please do not use automated interpretation without consideration of the information on interpretation given below.

9.1 Validation – Quality Control

An analysis of the test can be carried out if the following criteria have been fulfilled:

1. Reaction control band (top line) with clearly visible stain, dark band

2. Antibody class (second band): the IgG conjugate control band must show a clear staining.

3. Cut-off control (third band): weak, but visible staining

Negative and positive controls are not required for evaluating the test. They may be carried out for internal quality control purposes where necessary.

The controls must have the following reactive antigen bands:

Positive control: gp120, gp41, p51, p31, p24, p17

gp105 and gp36 may react, but need not react.

Negative control: no

9.2 Evaluation

The analysis of the test strips can be visual or computer-assisted - using the test strip analysis software recomScan. The recomScan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted analysis is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 Assessment of band intensity

1. Note the date and batch number, as well as the detected antibody class, on the attached evaluation form.

2. Enter the sample identification numbers to the evaluation sheet.

3. Now stick the corresponding test strip onto the appropriate fields on the evaluation form using a glue stick. Align the test strip with the reaction control bands along the marked lines. Then use a transparent adhesive tape to attach the test strip to the left of the marked lines (do not tape over the reaction control band!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.

4. Now identify the bands of the developed test strips using the printed control strip on the evaluation sheet and enter it into the log sheet. For each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

GARLH006EN_2014-03
**9.3 Interpretation of test results**

The criteria for interpreting the test are to be taken from Table 2.

<table>
<thead>
<tr>
<th>Test result</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No bands ≥ cut-off or gp120 and/or gp105 ≥ cut-off</td>
</tr>
<tr>
<td>Borderline</td>
<td>Any band constellation that does not fulfill the criteria for negative or positive</td>
</tr>
<tr>
<td>Positive</td>
<td>Two ENV bands of the same HIV type (gp120 + gp41 or gp105 + gp36) ≥ cut-off or one ENV band (only gp41 or gp36) and at least one GAG band (p17, p24) or POL band (p31, p51) ≥ cut-off</td>
</tr>
</tbody>
</table>

**Table 3: Differentiation**

- **HIV 1**
  - The test result is positive and gp41 reacts ≥ cut-off and gp41 reacts significantly stronger than gp36
- **HIV 2**
  - The test result is positive and gp36 reacts ≥ cut-off and gp36 reacts significantly stronger than gp41
- **non-typeable**
  - The test result is positive and neither the criteria for HIV-1 nor for HIV-2 apply.

**10 Limitations of the method - restrictions**

- When the positive findings are based entirely on an evaluation of two glycoprotein bands of the same HIV type (gp120+gp41 and/or gp105+gp36), a further sample should be tested two to four weeks later. As an additional precaution, an (RT) PCR test is recommended for the HIV genome.
- Patients with questionable results should be tested again after two to four weeks under all circumstances. As an additional precaution, an (RT) PCR test is recommended for the HIV genome. According to the literature, test results are often unclear in pregnant women.
- A negative test result cannot exclude an infection with the human immunodeficiency viruses. In the early phase of infection antibodies may not yet be present or not present in a detectable quantity. If infection with HIV is suspected, a further sampling and testing should be carried out after two weeks.
- Serological test results must always be considered in the context of other medical assessments of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- No correlation between positive antibody detection and infectiousness is possible.
- **Dark test strips:** Some patient samples can produce a dark, uniform or patterned staining across the entire nitrocellulose strip (e.g. on sera from patients with milk protein allergies). Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

**11 Test performance**

**11.1 Diagnostic specificity**

<table>
<thead>
<tr>
<th>recomLine HIV-1 &amp; HIV-2 IgG</th>
<th>HIV 1 (n=238)</th>
<th>HIV 2 (n=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Borderline</strong></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>238</td>
<td>103</td>
</tr>
</tbody>
</table>

sensitivity

238/238=100% 1+103/104=100%**

**including samples of subtypes A, B, C, D, F, G, CRF01, CRF02 from group M and group O.

**including one borderline result.

**11.2 Differentiation between HIV 1 and HIV 2**

<table>
<thead>
<tr>
<th>recomLine HIV-1 &amp; HIV-2 IgG</th>
<th>HIV 1 (n=238)</th>
<th>HIV 2 (n=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive to HIV-1</strong></td>
<td>233</td>
<td>0</td>
</tr>
<tr>
<td><strong>Positive to HIV-2</strong></td>
<td>0</td>
<td>101</td>
</tr>
<tr>
<td><strong>Differentiation not possible</strong></td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

**Correct differentiation**

233/238=98% 101/103=98%**

**11.3 Seroconversions**

Fifteen seroconversions panels with a total of 147 inspections were performed with the recomLine HIV 1 and HIV 2 in direct comparison with another commercially available confirmatory test. In two panels the recomLine HIV 1 & HIV 2 antibodies detected antibodies against HIV earlier than the comparative test. In one other panel the recomLine HIV 1 & HIV 2 were reactive one inspection later. In the remaining twelve seroconversion panels both confirmation tests detected the HIV antibodies at the same time.

**11.4 Diagnostic specificity**

<table>
<thead>
<tr>
<th>recomLine HIV 1 &amp; HIV 2 IgG</th>
<th>Blood donors (n=300)</th>
<th>Clinical samples* (n=340)</th>
<th>Potentially interfering samples** (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>298</td>
<td>336</td>
<td>54</td>
</tr>
<tr>
<td>Borderline</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Specifity 298/300=99,3% 336/340=98,8% 54/56=96,4%

* Samples from patients with acute hepatitis, recent EBV infection and various autoimmune diseases, pregnant women and samples from the laboratory routine.

** Lipemic, haemolytic and icteric samples, RF-positive samples, patients with hypergammaglobulinemia.

**11.5 Analytical specificity**

The analytical specificity is defined as the capacity of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.

a) **Interferences:** Control studies on potentially interfering factors have shown that anticoagulants (citrate, EDTA, heparin, CPD), haemolysis (up to 1000 mg/dl haemoglobin), lipoaemia, bilirubinaemia (up to 20 mg/dl bilirubin) or three cycles of freezing and thawing do not affect the performance of the test.
b) **Cross-reactions:** Potential interferences of antibodies against other organisms, as described in the literature regarding cross-reactivity in HIV confirmation tests (e.g. EBV, acute viral hepatitis) were examined in control studies. Additionally, conditions that are attributed to atypical activity of the immune system (antinuclear autoantibodies, rheumatoid factor) were tested. No cross-reactivities were detected (see 11.4).

**12 Literature**

13 Explanation of symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>∑</td>
<td>Content is sufficient for &lt;n&gt; applications</td>
</tr>
<tr>
<td>EVALFORM</td>
<td>Evaluation form</td>
</tr>
<tr>
<td>INSTRU</td>
<td>Instructions for use</td>
</tr>
<tr>
<td>CONT</td>
<td>Contents, includes</td>
</tr>
<tr>
<td>IVD</td>
<td>In vitro test</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch number</td>
</tr>
<tr>
<td>REF</td>
<td>Order number</td>
</tr>
<tr>
<td>Store at x°C to y°C</td>
<td></td>
</tr>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
</tbody>
</table>

14 Manufacturer and version information

recomLine HIV 1 & HIV 2 IgG

Instructions for use valid from: GARLHI006EN
2014-03

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