Intended Use

Enzyme immunoassay for the qualitative detection of specific IgG and IgM antibodies to HCV (hepatitis C virus) in human serum or plasma.

The enzyme immunoassay can be processed using the ELISA processors BEP® III System, BEP® 2000 System or BEP 2000 Advance® System as well as the Quadriga® Systems. A non-automated processing of the test is also possible. The product is for in-vitro diagnostic use.

Summary and Explanation

HCV is the causative agent of most Non-A, Non-B hepatitis (NANBH) transmitted by blood or blood products\(^1\). Infection occurs predominantly by intravenous drug use (sharing used syringes). Transmission by blood or blood products was reduced significantly in countries with high quality standards by introducing a mandatory screening process of blood and plasma donations in blood banks and also by manufacturers of plasma-based products (in Germany one transmission per 2 to 20 million blood donations)\(^2\). HCV can lead to an acute hepatitis (approximately 25 %), but in most cases the course is asymptomatic. 60 to 80 % of HCV infections become chronic. A chronic hepatitis C may develop within 20 to 30 years into liver cirrhosis and proceed to liver carcinoma. Approximately 27 % of the diagnosed liver cirrhoses and 25 % of liver carcinomas are caused by an HCV infection\(^3,4\). A chronic HCV infection is the primary cause of liver transplantation. A prophylaxis by vaccination is currently not available\(^5,6\).

Principles of the Procedure

The specific IgG and IgM antibodies to HCV contained in the test sample bind to the antigens in the reaction wells of the Anti-HCV 4.0 test plate. The Anti-HCV 4.0 Conjugate binds to these specific antibodies. The enzyme portion of the conjugate causes the Chromogen Working Solution to turn blue. This reaction is stopped by the addition of Stopping Solution POD, which causes a color change to yellow. The color intensity is a measure of the immunochemical reactivity of the HCV-specific antibodies in the sample.
## Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Storage</th>
<th>Stability once opened</th>
<th>Stability once diluted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzygnost® Anti-HCV 4.0</strong></td>
<td>microtitration plate coated with an antigen mixture of recombinant protein c33 (Escherichia coli) for the NS3 region and synthetic peptides for the core respectively NS4 region of HCV</td>
<td>2–8 °C in the bag with desiccant</td>
<td>28 days</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>test plate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MTP</strong></td>
<td>96 wells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample Buffer</strong></td>
<td>TRIS/HCl buffer with TRITON X-100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2–8 °C</td>
<td>28 days</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>DILUENT</strong></td>
<td>45 mL or 85 mL</td>
<td>15–25 °C</td>
<td>6 x 8 hours&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Conjugate Buffer</strong></td>
<td>TRIS/HCl buffer with Tween 20 and BSA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.a.</td>
<td>use immediately</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>REAGENT DILUENT</strong></td>
<td>12.5 mL or 75 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Conjugate</strong></td>
<td>mixture of anti-human IgG/POD conjugate (rabbit) and anti-human IgM/POD conjugate (rabbit) in TRIS/HCl buffer, colored blue&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2–8 °C</td>
<td>n.a.</td>
<td>28 days</td>
</tr>
<tr>
<td><strong>0.7 mL or 3.2 mL</strong></td>
<td></td>
<td>15–25 °C</td>
<td>6 x 8 hours&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Control, negative</strong></td>
<td>stabilized anti-HCV negative human serum, colored green&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2–8 °C</td>
<td>28 days</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td>1 mL</td>
<td>15–25 °C</td>
<td>6 x 8 hours&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12 weeks</td>
</tr>
<tr>
<td><strong>Control, positive</strong></td>
<td>stabilized anti-HCV positive human serum, colored red&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2–8 °C</td>
<td>28 days</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td>1 mL</td>
<td>15–25 °C</td>
<td>6 x 8 hours&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12 weeks</td>
</tr>
</tbody>
</table>

<sup>a</sup> Use each component by the expiry date at the latest.

<sup>b</sup> Preservative: phenol (≤ 1 g/L)

<sup>c</sup> Number of cycles of standing time open in the laboratory (when used within 28 days after first opening and closed storage at 2–8 °C between cycles).

Stored unopened at 2 to 8 °C, all components of the test kit may be used up to the expiry dates given on the labels.

Table 1 contains information on the on-board stability of reagents on the various systems.

### Warnings and Precautions

For *in-vitro* diagnostic use.

The test was developed for testing individual samples, not for pooled samples.

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**CAUTION! POTENTIAL BIOHAZARD**

Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and (with the exception of Anti-HCV 4.0 Control, positive) hepatitis C virus (HCV) using either tests found to be in conformance with the *In Vitro* Diagnostic Directive in the EU or FDA approved tests. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.
Caution: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

It is advisable to wear protective gloves throughout the entire test procedure. Please follow the recommendations of the manufacturer concerning the compatibility between gloves and exposed materials.

Buffer/Substrate TMB, Chromogen Working Solution and Stopping Solution POD must not be allowed to come into contact with heavy metal ions or oxidizing substances (do not use pipettes with metal parts which are in direct contact with the liquid). The substrate reaction steps must not be performed in the vicinity of disinfectants containing hypochlorite. If the Chromogen Working Solution has spontaneously developed a blue color before being transferred into the test plate, this indicates that the solution is contaminated; in such cases, prepare a fresh solution in a clean container. Skin contact with the above mentioned solutions is to be avoided.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements. It is recommended that solid infectious materials should be autoclaved for at least 1 hour at 121 °C. All aspirated liquids should be collected in two receptacles connected in series. Both should contain a disinfectant suitable for inactivating human pathogens. The concentrations and times specified by the manufacturer must be observed.

Safety Data Sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Preparing Reagents

Bring all reagents and test samples to 15 to 25 °C before starting with the test. Do not remove the foil pouch from the test plates during this step. If reagents or reagent working solutions need to be mixed, avoid foam formation.

To avoid a frequent change of syringes when processing large series of samples on the BEP® III System, the kit 10 x 96 (Q) is recommended.

Anti-HCV 4.0 test plate: Before starting the test processing, remove not required strips from the holder and store these in the enclosed polyethylene bag for later use.

Anti-HCV 4.0 Sample Buffer: ready to use
Anti-HCV 4.0 Conjugate Buffer: ready to use
Anti-HCV 4.0 Conjugate: To obtain the conjugate working solution dilute Anti-HCV 4.0 Conjugate 1+25 with Anti-HCV 4.0 Conjugate Buffer, e.g., for one test plate, add 0.5 mL Anti-HCV 4.0 Conjugate into a vial with 12.5 mL Anti-HCV 4.0 Conjugate Buffer. Shake gently to mix. Accordingly, when using the kit 10 x 96 (Q) 3 mL of Anti-HCV 4.0 Conjugate are added to a bottle with 75 mL Anti-HCV 4.0 Conjugate Buffer for seven test plates. Document this step by using the check box on the label. Mix thoroughly, e.g., by repeatedly inverting the bottle.

Anti-HCV 4.0 Control, negative: ready to use
Anti-HCV 4.0 Control, positive: ready to use

Specimen Collection and Handling

Collecting the Specimen

Suitable specimens are individual samples (human sera or CPDA/EDTA/heparinized/citrated plasma) obtained by standard laboratory techniques.

Storing the Specimen

The samples should be stored for no more than 8 days at 2 to 8 °C. If samples are frozen within this period, they can be stored at below −20 °C for at least 3 years if repeated freeze-thaw cycles are avoided.
**Procedure**

**Materials Provided**

<table>
<thead>
<tr>
<th>Component</th>
<th>2 x 96</th>
<th>10 x 96</th>
<th>10 x 96 (Q)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPIH</strong></td>
<td>2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1 x 45 mL</td>
<td>5 x 45 mL</td>
<td>3 x 85 mL</td>
<td></td>
</tr>
<tr>
<td>2 x 12.5 mL</td>
<td>10 x 12.5 mL</td>
<td>2 x 75 mL</td>
<td></td>
</tr>
<tr>
<td>2 x 0.7 mL</td>
<td>10 x 0.7 mL</td>
<td>2 x 3.2 mL</td>
<td></td>
</tr>
<tr>
<td>1 x 1 mL</td>
<td>3 x 1 mL</td>
<td>3 x 1 mL</td>
<td></td>
</tr>
<tr>
<td>1 x 1 mL</td>
<td>3 x 1 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>polyethylen bag</td>
</tr>
</tbody>
</table>

The test plate, the conjugate, the conjugate buffer, the sample buffer, Anti-HCV 4.0 Control, positive as well as Anti-HCV 4.0 Control, negative must be used in the given combination of 6-digit lot numbers printed on the package, respectively stated in the enclosed barcode table of values.

**Materials Required but not Provided**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
</table>
| **Supplementary Reagents** for Enzygnost®/TMB, **OUVP** | Buffer/Substrate TMB  
Chromogen TMB  
Stopping Solution POD  
Washing Solution POD  
Adhesive foils  
empty bottle for the Chromogen Working Solution  
for details on kit size and components refer to the respective Instructions for Use. |
| **BEP® III System** | for automated processing and evaluation of the test after manual dispensing of samples and controls |
| **BEP® 2000 / BEP 2000 Advance® System** | for fully automated processing and evaluation of the test |
| **Quadriga® Systems** | for fully automated processing and evaluation of the test in combination with BEP® III |
| **Pipettes** | piston-type pipettes with fixed or variable volumes, or single- and multichannel pipettes with adjustable volumes |

The following items are required additionally if the test is not processed automatically:

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubator</strong></td>
<td>incubator (37 ±1 °C) with homogenous heat distribution, or similar incubation method</td>
</tr>
<tr>
<td><strong>Washing device</strong></td>
<td>microtitration plate washer</td>
</tr>
<tr>
<td><strong>Photometer</strong></td>
<td>photometer suitable for microtitration plates, measuring wavelength of 450 nm, reference wavelength of 650 nm (between 615 nm and 690 nm as appropriate). For SURE measurements, wavelength 405 nm is also required.</td>
</tr>
</tbody>
</table>

All the equipment used in the test must have been validated.
Test Procedure

Non-automated Test Procedure

1. **Preparation of Reagents**: Refer to “Reagents”.

2. **Assay scheme**: The necessary number of test plate wells is given by the number of test samples plus the number of determinations (n = 5) for Anti-HCV 4.0 Control, positive and negative.

3. **Pre-dispense buffer**: Dispense 200 µL of Anti-HCV 4.0 Sample Buffer into each required well of the test plate.

4. **Dispense samples**: Dispense 25 µL Anti-HCV 4.0 Control, negative into each of the first 3 wells (A1-C1), 25 µL Anti-HCV 4.0 Control, positive into the next well (D1) and 25 µL of undiluted sample into each of the subsequent wells. At the end of the series, respectively test plate, fill the last well with 25 µL Anti-HCV 4.0 Control, positive.

   **Alternative pipetting scheme**: Dispense 25 µL Anti-HCV 4.0 Control, negative into each of the first 3 wells (A1-C1), 25 µL Anti-HCV 4.0 Control, positive into each of the next 2 wells (D1-E1), and 25 µL of undiluted sample into each of the subsequent wells.

   **Important**:
   - Do not mix well contents!
   - It is not permitted to first pipette Anti-HCV 4.0 Control, positive into the wells at the start and end of the sample series, and then put the samples in-between.

   Each sample must be pipetted with its own pipette tip. The pipetting steps must be completed within 30 minutes per test plate. After completing the pipetting steps, seal the test plate with foil and place immediately into the incubator.

   **Pipetting control (optional)**:
   The correct pipetting of the controls and samples can easily be checked visually (Anti-HCV 4.0 Control, negative (green), Anti-HCV 4.0 Control, positive (red), sample and empty wells (clear)) or qualitatively by photometric measurement at 405 nm against 650 nm (the so-called SURE function). For details refer to the document "BEP® III System/ BEP® 2000 System/ BEP 2000 Advance® System SURE Specifications”.

5. **Incubate samples**: Incubate for 30 ±2 minutes at 37 ±1 °C, then proceed immediately to the wash step.

6. **Wash**: Remove foil and aspirate all wells. Fill each well with approximately 0.3 mL diluted Washing Solution POD, aspirate the plate, and repeat the wash cycle three times. After completing the wash cycles, proceed immediately to the next reagent dispensing step (otherwise the wells may dry out).

7. **Dispense conjugate**: Pipette 100 µL of Conjugate Working Solution into each well. Then seal the test plate with fresh foil and place immediately into the incubator.

8. **Incubate conjugate**: Incubate for 30 ±2 minutes at 37 ±1 °C, then proceed immediately to the wash step.

9. **Wash**: As described in step 6.

10. **Dispense substrate**: Pipette 75 µL of Chromogen Working Solution into each well, then seal the microtitration plate with fresh foil.

11. **Incubate substrate**: Immediately after the substrate dispensing step, incubate at 18 to 25 °C for 30 ±2 minutes, protected from light.

12. **Stop reaction**: Remove the foil. Add 75 µL Stopping Solution POD to each well, keeping to the same timing as during the substrate dispensing step.

13. **Measure**: Read the test plate at 450 nm within one hour. The recommended reference wavelength is 650 nm (or where appropriate between 615 and 690 nm).

Procedure for the BEP® III System

When using the BEP® III, the test plates must be prepared up to the sample dispensing step (steps 1 to 4 in the section "Non-automated Test Procedure"). Immediately afterwards place the uncovered test plates, i.e., not covered with foil, into the BEP® III. All subsequent
processing steps are performed fully automatically by the instrument (see BEP® III Instruction Manual).

The settings for the incubation times in the BEP® III software may differ from the times in the section “Non-automated Test Procedure” for technical reasons (system speed) but have been validated for Enzygnost® on the BEP® III.

Procedure for fully automated systems (BEP® 2000 and Quadriga®)

The sample dispensing steps and subsequent processing of the test are performed fully automatically by the analyzer (see respective Instruction Manual).

Sample processing with the BEP® 2000 and Quadriga® System may differ from the information given under “Non-automated Test Procedure”, but has been validated for Enzygnost® on the respective System.

Internal Quality Control

To evaluate the test the following criteria must be fulfilled:

1. Anti-HCV 4.0 Control, negative: 
   \[-0.010 \leq A \leq 0.150\]
2. Anti-HCV 4.0 Control, positive: 
   \[0.700 \leq A \leq 3.000\]

If one of the three absorbance values of Anti-HCV 4.0 Control, negative is outside the specification, this value can be neglected.

Both absorbance values for Anti-HCV 4.0 Control, positive must comply with the respective specification.

If these conditions are not met, the test is not valid for evaluation. In this case, the software of BEP® III, BEP® 2000 and Quadriga® will give the notice of an invalid test result. The measurements must be repeated after investigating the cause.

Results

The evaluations are performed automatically with the BEP® III, the BEP® 2000 and Quadriga® Systems. Please consult the relevant Instruction Manual. The following sections must be taken into account when performing measurements without software support.

Evaluation using the Cut-off

To calculate the cut-off, use the mean of the valid absorbance values of Anti-HCV 4.0 Control, negative and add a value of 0.320:

\[\bar{A}_{\text{neg}} + 0.320 = \text{cut-off}\]

Based on the criteria of the test, the samples are classified as follows:

Anti-HCV negative \( A < \text{cut-off} \)
Anti-HCV reactive \( A \geq \text{cut-off} \)

Evaluation using the Ratio

An interpretation of the test results is also possible by calculating the quotient of \(A_{\text{sample}}\) and cut-off:

\[\text{ratio} = \frac{A_{\text{sample}}}{\text{cut-off}}\]

The ratio is calculated automatically by the BEP® III, BEP® 2000 and Quadriga® Systems. With this method results from different runs can be standardized and made comparable with each other.

Based on the criteria of the test, the samples are classified as follows:

Anti-HCV negative Ratio < 1.0
Anti-HCV reactive Ratio \( \geq 1.0 \)
Assessment of the Results

Reactive test samples (absorbance ≥ cut-off, respectively a ratio ≥ 1) have to be tested again in duplicate. A sample is considered repeatedly reactive if at least one repeat measurement has an absorbance value ≥ cut-off, respectively a ratio ≥ 1.0.

If the absorbance value of both repeat measurement is < cut-off respectively ratio < 1.0, the sample is considered anti-HCV negative according to the test criteria.

According to current knowledge, a positive result cannot determine with certainty that there is infectious HCV in the blood, nor can a negative test result exclude for certain the presence of HCV. All reactive samples must therefore be clarified according to a recognized confirmation method.

Results should always be interpreted in conjunction with the patient’s medical history, clinical presentation, and other findings.

Limitations

1. Anticoagulants (citrate, CPDA, EDTA, heparin) do not interfere with the test result.
2. When testing icteric samples (up to 30 mg/L bilirubin), hemolytic samples (up to 18.5 g/L hemoglobin), samples containing rheumatoid factors (up to 1 800 IU/mL) and lipemic samples (up to 8 g/L triglycerides), no interference with the test has been observed.
3. Samples from pregnant women and samples containing the following potentially interfering substances were investigated: ANA, HBsAg, as well as antibodies to HAV, HBV, HIV, CMV, EBV, TBE virus und dengue virus. With these samples no interference with the test results have been observed.
4. Antibodies to *E. coli* do not interfere with the test result.
5. No interferences have been observed with heat-treated samples (30 minutes, 56 °C).
6. Incompletely coagulated sera and microbially contaminated samples should not be used. Any particulate components in the sample (e.g., fibrin clots, erythrocytes) should be removed before the test.
7. If thawed samples are used, ensure that the material is thoroughly homogenized.
8. Highly reactive samples may cause a precipitation of the dye during the stopping reaction. This does not interfere with the photometric evaluation.
9. The control sera were produced using native human sera. Therefore, turbidity may occur but does not impair the test result.
10. This product is not intended for use with samples drawn post mortem.
11. Siemens Healthcare Diagnostics has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these Instructions for Use.

Performance Characteristics

Specificity

For the determination of specificity, 17,701 anti-HCV-negative sera were investigated at 4 evaluation sites and a specificity of 99.92 % (initial testing) and 99.93 % after retesting was obtained. For the determination of specificity in plasma, 2886 EDTA plasmas were investigated at 2 sites and a specificity of 99.83 % (initial testing) respectively 99.90 % after retesting was obtained.

The results of the specificity studies are summarized in the following table.
In relation to sample population, test procedure, and other factors different values may be obtained, which however have to be in accordance with the Common Technical Specifications (CTS).

**Sensitivity**

The diagnostic sensitivity was determined using 808 anti-HCV positive samples. All samples were tested as reactive.

The reactivity of the test with seroconversion samples was investigated using 42 seroconversion panels. It was found that Enzygnost® Anti-HCV 4.0 exhibits a sensitivity in detecting seroconversions which is comparable to or better than similar tests. Nevertheless, it cannot be ruled out that individual samples may escape detection when the test is used on a large scale.

The results of the sensitivity studies are summarized in the following table.

<table>
<thead>
<tr>
<th>Sample population</th>
<th>Number of samples</th>
<th>Reactive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute infection</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Coinfected with HIV</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Chronic infection</td>
<td>216</td>
<td>216</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>134</td>
<td>134</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Genotype 5</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Genotype 6</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Status positive without known genotype</td>
<td>219</td>
<td>219</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>808</strong></td>
<td><strong>808</strong></td>
</tr>
</tbody>
</table>

**Precision**

4 test samples with different anti-HCV specific reactivities were tested to determine the repeatability and the within-device variation coefficients (CV) (8-fold replicates in 5 runs). The calculation was performed using analysis of variance.

Exemplary results obtained from studies on the BEP® III are summarized in the following table.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Status</th>
<th>Mean absorbance (A)</th>
<th>Repeatability CV (%)</th>
<th>Within-device CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>positive</td>
<td>0.503</td>
<td>3.1</td>
<td>7.4</td>
</tr>
<tr>
<td>B</td>
<td>positive</td>
<td>0.861</td>
<td>4.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Sample</td>
<td>Status</td>
<td>Mean absorbance (A)</td>
<td>Repeatability CV (%)</td>
<td>Within-device CV (%)</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>C</td>
<td>positive</td>
<td>1.260</td>
<td>3.4</td>
<td>4.7</td>
</tr>
<tr>
<td>D</td>
<td>positive</td>
<td>1.515</td>
<td>4.3</td>
<td>4.6</td>
</tr>
</tbody>
</table>

**Note**

The values cited for specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for Enzygnost® Anti-HCV 4.0.

**References**


**Definition of Symbols**

- **Do not reuse**: YYYY-MM-DD
- **Use By**: 
- **Batch Code**: LOT
- **Catalogue Number**: REF
- **Manufacturer**: 
- **Contains sufficient for <n> tests**:  
- **In Vitro Diagnostic Medical Device**: IVD
- **Consult instruction for Use**: 
- **CE mark**: 
- **Reconstitution volume**: 
- **Keep away from sunlight and heat**: 
- **Contents**: CONTENTS
- **Level**: LEVEL
- **Caution, consult accompanying documents**: 
- **Authorized representative in the European Community**: EC REP
- **Biological Risks**: 
- **Temperature Limitation**: 
- **Non-sterile**: 
- **Temperature Limitation**: 
- **Consult instruction for Use**: 
- **CE mark**: 
- **Reconstitution volume**: 
- **Keep away from sunlight and heat**: 
- **Contents**: CONTENTS
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- **Temperature Limitation**: 
- **Consult instruction for Use**: 
- **CE mark**: 
- **Reconstitution volume**: 
- **Keep away from sunlight and heat**
Table 1  On-board Stability

<table>
<thead>
<tr>
<th>Material/reagent</th>
<th>System</th>
<th>Stability(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HCV 4.0 Sample Buffer</td>
<td>BEP® 2000, Quadriga®</td>
<td>6 x 8 hours(^e)</td>
</tr>
<tr>
<td>Conjugate Working Solution</td>
<td>BEP® 2000</td>
<td>24 hours or 6 x 8 hours(^e)</td>
</tr>
<tr>
<td></td>
<td>BEP® III, Quadriga®</td>
<td>6 x 8 hours(^e)</td>
</tr>
<tr>
<td>Anti-HCV 4.0 Control, negative</td>
<td>BEP® 2000, Quadriga®</td>
<td>6 x 8 hours(^e)</td>
</tr>
<tr>
<td>Anti-HCV 4.0 Control, positive</td>
<td>BEP® 2000, Quadriga®</td>
<td>6 x 8 hours(^e)</td>
</tr>
</tbody>
</table>

\(^d\) Use each component by the expiry date at the latest.

\(^e\) Number of cycles of standing time open in the systems (when used within 28 days after first opening and closed storage at 2–8 °C between cycles).
Table 2  Test Procedure

1. Preparation of the reagents

2. Dispense 200 µL Sample Buffer / well

3. Transfer:
   - 3 x 25 µL Control, negative
   - 1 x 25 µL Control, positive
   - 25 µL undiluted sample
   - 1 x 25 µL Control, positive
   - Do not mix!

4. Incubation:
   - 30 ±2 minutes
   - (37 ±1 °C)

5. Wash 4 x

6. 100 µL Conjugate Working Solution

7. Incubation:
   - 30 ±2 minutes
   - (37 ±1 °C)

8. Wash 4 x

9. 75 µL Chromogen Working Solution

10. Incubation (protected from light):
    - 30 ±2 minutes
    - (18 to 25 °C)

11. 75 µL Stopping Solution

12. (after max. 60 minutes)
    - Measure:
    - 450 nm vs. 650 nm

13. Test result