Enzygnost® Syphilis

**Intended Use**

Enzyme immunoassay for the qualitative detection of specific antibodies to *Treponema pallidum* in human serum or plasma.

The enzyme immunoassay is processed using the ELISA processors, BEP® III System and BEP® 2000 System or BEP 2000 Advance® System. A non-automated processing of the test is also possible.

**Summary and Explanation**

Syphilis (synonym: lues) is caused by infection with the pathogen *T. pallidum* and is transmitted predominantly by sexual intercourse. In cases of maternal infection during pregnancy there is a risk of transmission to the fetus (congenital syphilis). Furthermore, a risk of transmission via blood transfusion exists.

In patients with florid syphilis the pathogen can be detected only in the early phase of the disease and normally also only in the aqueous exudate of the primary lesion. Syphilis antibodies on the other hand can be detected already within a few weeks after the infection.

Serological diagnosis of lues is complicated by the frequent incidence of rheumatoid factors, other autoantibodies and cross-reactions with other related bacteria, especially with spirochaetes such as *T. phagedenis* and *Borrelia burgdorferi*. The specificity of Enzygnost® Syphilis is not impaired by these factors.

Enzygnost® Syphilis detects all the relevant classes of antibodies and exhibits a high degree of sensitivity at all stages of the disease.

A negative result in the Enzygnost® Syphilis test excludes a *Treponema* infection with a high degree of certainty.

As the test procedure can be automated and has a high sensitivity and specificity, Enzygnost® Syphilis is suitable for testing large series of samples, e.g. for donor screening in blood banks, in prenatal care programs and for testing at-risk groups.

Enzygnost® Syphilis exhibits good correlation with the Treponema-pallidum-Hemagglutination Assay (TPHA), e.g. Cellognost® Syphilis H from Siemens Healthcare Diagnostics.

Enzygnost® Syphilis is also suitable as a confirmatory test for samples that are initially reactive in a different screening test (e.g. TPHA), as the test likewise exhibits a good correlation with the Fluorescent Treponemal Antibody Absorption test (FTA-ABS).

For assessing the need for treatment and for therapeutic monitoring, reactive samples should be retested in the VDRL test (Venereal Disease Research Laboratory test) from Siemens or in an IgM-specific test such as the FTA-ABS/IgM test.

**Principle of the Method**

Enzygnost® Syphilis is a competitive one-step enzyme immunoassay. *T. pallidum*-specific antibodies (IgG and/or IgM) contained in the sample and the POD-labelled antibodies (Anti-*T. pallidum*/POD Conjugate) compete for binding to the *T. pallidum* antigens coated onto the wells of the microtitration plate.
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 intensity of the yellow color produced is inversely proportional to the activity of T. pallidum-specific antibodies contained in the sample.

Reagents

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Materials provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYPH</td>
<td>Enzygnost® Syphilis</td>
</tr>
<tr>
<td>MTP</td>
<td>Enzygnost® Syphilis test plate</td>
</tr>
<tr>
<td>CONJUGATE</td>
<td>Anti-T. pallidum/POD Conjugate</td>
</tr>
<tr>
<td>REFER P</td>
<td>Anti-T. pallidum Reference P</td>
</tr>
<tr>
<td>REFER N</td>
<td>Anti-T. pallidum Reference N</td>
</tr>
<tr>
<td>WASH POD</td>
<td>Washing Solution POD*</td>
</tr>
<tr>
<td>SUBSTRATE</td>
<td>Buffer/Substrate TMB*</td>
</tr>
<tr>
<td>CHROMOGEN</td>
<td>Chromogen TMB*</td>
</tr>
<tr>
<td>STOP POD</td>
<td>Stopping Solution POD*</td>
</tr>
<tr>
<td>EMPTY VIAL</td>
<td>Empty bottle for Chromogen Working Solution*</td>
</tr>
<tr>
<td>CHROM SOL</td>
<td>Polyethylene bag</td>
</tr>
<tr>
<td></td>
<td>Adhesive foils</td>
</tr>
<tr>
<td></td>
<td>Barcode table of values</td>
</tr>
<tr>
<td></td>
<td>Instructions for Use</td>
</tr>
</tbody>
</table>

* These components are also included in the kit Supplementary Reagents for Enzygnost®/TMB (REF OUVP).

The test plate, the conjugate as well as Anti-T. pallidum Reference P and Anti-T. pallidum Reference N 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. The same applies to the reagents Chromogen TMB and Buffer/Substrate TMB.

Materials required but not provided, for the kit 10 x 96 (Q)

Supplementary Reagents for Enzygnost®/TMB (REF OUVP)

The reagents Chromogen TMB and Buffer/Substrate TMB must be used only in the combination of lots stated for the Supplementary Reagents kit. The applicable lot numbers are the 6-digit lot numbers listed on the package.

Composition

Enzygnost® Syphilis test plate: microtitration plate coated with inactivated T. pallidum antigen (detergent extract from Nichols strain)

Anti-T. pallidum/POD Conjugate: IgG fraction from human serum conjugated with peroxidase in TRIS/HCl buffer, ready for use

Preservative: phenol (≤ 1 g/L)

Anti-T. pallidum Reference P: human serum containing specific antibodies to T. pallidum, nominal absorbance: ≤ 0.2

Preservatives: amphotericin (~ 5 mg/L)
gentamicin (~ 100 mg/L)

Anti-T. pallidum Reference N: human serum without antibodies to T. pallidum, nominal absorbance: ≥ 0.7

Preservatives: amphotericin (~ 5 mg/L)
gentamicin (~ 100 mg/L)
**Washing Solution POD:** phosphate buffer containing Tween
Preservative: phenol (≤ 1 g/L)

**Buffer/Substrate TMB:** hydrogen peroxide in acetate buffer
Preservative: n-butanol ( < 1 %)

**Chromogen TMB:** tetramethylbenzidine dihydrochloride

**Stopping Solution POD:** sulfuric acid (0.25 mol/L)

**Warnings and Precautions**

For *in-vitro* diagnostic use.

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

**Warning!** STOP POD

H290, H314: May be corrosive to metals. Causes severe skin burns and eye damage.
P280, P310, P301, P330, P331, P303, P361, P353, P305, P351, P338, P390, P501:
Wear protective gloves/protective clothing/eye protection/face protection.

Immediately call a POISON CENTER or doctor/physician. IF SWALLOWED: Rinse mouth.
Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Absorb spillage to prevent material damage. Dispose of contents and container in accordance with all local, regional, and national regulations.

**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 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.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

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.

For disposal, it is recommended that solid infectious materials should be autoclaved for at least one 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.

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 transferal 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 aforementioned solutions is to be avoided.

**Preparation of the reagents**

Bring all the reagents and test samples to 18 to 25 °C before starting with the test. Do not remove the foil pouch from the test plates during this step. Before starting the test processing, remove not required strips from the holder and store these in the enclosed polyethylene bag for later use (see Table 1). 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.

The Anti-T. pallidum/POD-Conjugate is ready for use. A slight flocculation which may occur due to bovine serum added as stabilizer is unproblematic.

For each test plate, dilute 20 mL of Washing Solution POD with distilled or deionized water to 400 mL.

For each test plate, dilute 1 mL of Chromogen TMB with 10 mL of Buffer/Substrate TMB using the supplied empty plastic bottle (Chromogen Working Solution). Store protected from light. After use, carefully rinse the bottle with distilled or deionized water.

It is also permissible to pour together the full contents of the Chromogen TMB vial and the Buffer/Substrate TMB vial into the empty bottle.

When using Supplementary Reagents for Enzygnost®/TMB, REF OUVP 27, the complete contents of the Chromogen TMB vial have to be transferred into the barcode labeled Buffer/Substrate TMB vial.

Storage and Stability
Stored unopened at the stated temperature, all components of the Enzygnost® Syphilis kit may be used up to the expiry dates given on the labels.

For complete stability and storage data of the opened, respectively diluted reagents, see Table 1.

Equipment Required

- **BEP® III:** for automated processing of the test after dispensing the samples as well as for evaluation
- **BEP® 2000/BEP 2000 Advance®:** for fully automated processing and evaluation of the test
- **Pipettes:** piston-type pipettes with fixed or variable volumes, or single- and multi-channel pipettes with adjustable volumes
- **Incubator:** incubator (37 ±1 °C) with homogeneous heat distribution or comparable incubation method
- **Washing device:** microtitration plate washer
- **Photometer:** photometer suitable for microtitration plates, measuring wavelength of 450 nm, reference wavelength of 650 nm (between 615 nm and 690 nm as appropriate)

All equipment used in the test must have been validated.

Specimens

Suitable specimens are individual samples (human serum or EDTA/heparinized/citrated plasma) obtained by standard laboratory techniques. Heparinized and citrated plasma specimens should be stored for no more than 3 days at 2 to 8 °C; whereas serum and EDTA plasma can be used up to 8 days under these storage conditions. If samples are to be stored for a longer period of time, they must be frozen.

Procedure

Non-automated Test Procedure

1. **Assay scheme:** The necessary number of test plate wells is given by the number of samples plus the number of determinations (n=6) for Anti-T. pallidum Reference P and N.

2. **Dispense samples:** Dispense 25 µL Reference N into each of 4 wells (A1-D1), 25 µL Reference P into one well (E1) and 25 µL of undiluted sample into each of the subsequent wells. At the end of the series respectively test plate, fill one further well with 25 µL Reference P.
As an alternative to the pipetting scheme above, it is also possible to pipette Reference P in duplicate after pipetting Reference N.

**Alternative pipetting scheme:** Dispense 25 µL Reference N into each of 4 wells (A1-D1), 25 µL Reference P into 2 wells (E1, F1), and 25 µL of undiluted sample into each of the subsequent wells.

The pipetting procedure must be finished within 30 minutes per test plate.

3. **Dispense conjugate:** Pipette 100 µL Anti-T. pallidum/POD Conjugate into each well. Then seal the plate with foil and place immediately into the incubator.

4. **Incubate:** Incubate for 90 ± 2 minutes at 37 ± 1 °C, then proceed immediately to the wash step.

5. **Wash:** Remove foil and aspirate all wells. Fill each well with approx. 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).

6. **Dispense substrate:** Pipette 100 µL of Chromogen Working Solution into each well, then seal the microtitration plate with fresh foil.

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

8. **Stop reaction:** Remove foil. Add 100 µL of Stopping Solution POD to each well, keeping to the same timing as during the substrate dispensing step.

9. **Read:** 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 and 2 in the section “Non-automated Test procedure”). Ensure that partially-loaded test plates are supplemented with water-filled strips to at least half plates (6 test strips). Immediately afterwards place the uncovered test plates, i.e. not covered with foil, into the BEP® III. All subsequent processing steps are then 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 System.

**Procedure for the BEP® 2000 System**

The sample dispensing steps and subsequent processing of the test are performed fully automatically by the analyzer (see BEP® 2000 Instruction Manual). When doing so, ensure that partially-loaded test plates are supplemented with water-filled strips to at least half plates (6 test strips).

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

**Internal Quality Control**

**Validation criteria**

To evaluate the test the following criteria must be fulfilled:

1. Anti-T. pallidum Reference N : $0.700 \leq A \leq 2.500$

2. Anti-T. pallidum Reference P: $-0.010 \leq A \leq 0.200$

If one of the absorbance values of the Anti-T. pallidum Reference N is outside the specification, this value can be neglected.

Both absorbance values of the Reference P must comply with the specification.

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

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

To calculate the cut-off, use the mean of the valid absorbance values of Reference N and multiply with 0.7:

\[ \overline{A}_{\text{Ref. N}} \times 0.7 = \text{cut-off} \]

The equivocal range is defined as:

\[ \overline{A}_{\text{Ref. N}} \times 0.6 \leq \text{equivocal range} \leq \overline{A}_{\text{Ref. N}} \times 0.7 \]

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

1. Anti-\( T. pallidum \) negative \( A_{\text{sample}} > \text{cut-off} \)
2. Anti-\( T. pallidum \) positive \( A_{\text{sample}} < \overline{A}_{\text{Ref. N}} \times 0.6 \)
3. Anti-\( T. pallidum \) equivocal \( \overline{A}_{\text{Ref. N}} \times 0.6 \leq A_{\text{sample}} \leq \text{cut off} \)

When a positive or equivocal result is obtained the sample is to be retested, but in duplicate. If, in the retest, both absorbance values are above or below the equivocal range, the initial equivocal result can be ignored and the sample can be classified as negative or positive, respectively.

However, if the mean absorbance value of the sample is equivocal again, the sample must be reported as "equivocal". All samples with repeated equivocal or positive results should be investigated using a test for the detection of specific IgM in order to verify the presence of a recent infection requiring treatment.

If the confirmatory test initially provides a negative result but there are strong reasons for suspecting a recent infection, a second sample should be taken approximately 2 to 4 weeks after the first and this subsequent sample should then be tested in parallel with the earlier sample.

Assessment of the Results

A negative test result in Enzygnost® Syphilis excludes acute or past syphilis with a high degree of certainty.

"Prozone" phenomena, which in other test methods can lead to false negative results if the specific antibody titers are very high, have not been observed with Enzygnost® Syphilis.

A "positive" retest result signifies that \( T. pallidum \)-specific antibodies have been detected (see however the notes under "Limitations of the Procedure"). If no \( T. pallidum \)-specific antibodies were detected in an IgM-specific test performed at the same time it can be assumed that the patient had been infected with syphilis in the more remote past or had undergone successful treatment.

Limitations of the Procedure

1. Samples containing sodium azide must not be used!
2. It is probable that Enzygnost® Syphilis also detects infections with the \( T. pallidum \) subspecies \( T. carateum, T. pertenue \) and \( T. endemicum \), the causative agents of pinta, yaws, and endemic syphilis respectively. These treponematoses, which are endemic in parts of Africa and South America, cannot be differentiated serologically from syphilis\(^8,9\) but can however be distinguished based on the clinical picture.
3. Immunosuppressed patients and particularly HIV-infected persons often exhibit atypical serological constellations.
4. Anticoagulants (heparin, EDTA, citrate) and rheumatoid factors do not interfere with the test result.
5. Lipemic, icteric or hemolytic samples do not interfere with the test.
6. Samples containing ANA as well as \( Borrelia \) positive samples do not affect the test result.
7. No interferences have been observed with heat-treated samples (30 minutes, 56 °C).
8. Incompletely coagulated sera and microbially contaminated test samples should not be used. Any particles (e.g. fibrin clots, erythrocytes) contained in the sample should be removed prior to assay.

9. If thawed samples are used, ensure that the material is thoroughly homogenized.

10. If there is a strong color change after the stopping reaction, dye may precipitate. This does not interfere with the photometric evaluation.

11. The References were produced using native human sera. Therefore, turbidity may occur but does not impair the test result.

12. The test plate should be protected from vibration during the incubation phases. If using a water-bath as incubator, it should be non-circulating or a secured floatation aid has to be used. The wells of the plate must be in contact with the temperature-controlled water. If stabilizers are used to prevent microbial contamination of the water, care must be taken that neither the surface of the test plate nor the wells come into contact with the liquid since such contamination can lead to unspecific reactions.

13. Siemens 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.

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

Performance Characteristics

Sensitivity

In an initial clinical study 294 status-positive test samples were tested with Enzygnost® Syphilis. With this sample panel the test exhibited a sensitivity (after retesting initially equivocal results) of 97.3 %.

In a further study 369 status-positive samples were tested with Enzygnost® Syphilis. Here the sensitivity (after retest) was found to be 98.4 - 100 %.

Specificity

In several studies Enzygnost® Syphilis exhibited the following specificity values (after retesting initially equivocal/positive values):

<table>
<thead>
<tr>
<th>Samples</th>
<th>n</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient sera (syphilis status-negative)</td>
<td>755</td>
<td>100.0</td>
</tr>
<tr>
<td>Pregnancy sera</td>
<td>200</td>
<td>99.5</td>
</tr>
<tr>
<td>Serum samples of healthy blood donors</td>
<td>3455</td>
<td>100.0</td>
</tr>
<tr>
<td>Plasma samples of healthy blood donors</td>
<td>1094</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Precision

Five test samples with different *T. pallidum*-specific antibody activities were evaluated in two laboratories with regard to the intra- and interassay coefficient of variation (CV) and yielded the following results:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean absorbance (A&lt;sub&gt;450&lt;/sub&gt;)</th>
<th>Intraassay CV (%)</th>
<th>Sample</th>
<th>Mean absorbance (A&lt;sub&gt;450&lt;/sub&gt;)</th>
<th>Interassay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F I</td>
<td>1.151</td>
<td>7.0</td>
<td>F I</td>
<td>1.410</td>
<td>5.3</td>
</tr>
<tr>
<td>F II</td>
<td>0.725</td>
<td>6.5</td>
<td>F II</td>
<td>0.830</td>
<td>4.5</td>
</tr>
</tbody>
</table>
### Sample Mean absorbance ($A_{450}$) Intraassay CV (%) Sample Mean absorbance ($A_{450}$) Interassay CV (%)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean absorbance ($A_{450}$)</th>
<th>Intraassay CV (%)</th>
<th>Sample</th>
<th>Mean absorbance ($A_{450}$)</th>
<th>Interassay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F III</td>
<td>0.661</td>
<td>7.3</td>
<td>F III</td>
<td>0.747</td>
<td>5.1</td>
</tr>
<tr>
<td>F IV</td>
<td>0.427</td>
<td>6.7</td>
<td>F IV</td>
<td>0.510</td>
<td>6.7</td>
</tr>
<tr>
<td>F V</td>
<td>0.060</td>
<td>7.8</td>
<td>F V</td>
<td>0.067</td>
<td>22.1</td>
</tr>
</tbody>
</table>

**Note**

The values cited for performance characteristics of the assay represent typical results and are not to be regarded as specifications for Enzygnost® Syphilis.

**Bibliography**

Definition of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>✗</td>
<td>Do not reuse</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch Code</td>
</tr>
<tr>
<td>🚨</td>
<td>Caution, consult accompanying documents</td>
</tr>
<tr>
<td>EC REP</td>
<td>Authorized representative in the European Community</td>
</tr>
<tr>
<td>🦠</td>
<td>Biological Risks</td>
</tr>
<tr>
<td>🔥</td>
<td>Temperature Limitation</td>
</tr>
<tr>
<td>⚠️</td>
<td>Non-sterile</td>
</tr>
<tr>
<td>📜</td>
<td>CONTENTS</td>
</tr>
<tr>
<td>🌞</td>
<td>Keep away from sunlight and heat</td>
</tr>
<tr>
<td>YYYY-MM-DD</td>
<td>Use By</td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue Number</td>
</tr>
<tr>
<td>🏗️</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>🛑</td>
<td>Contains sufficient for &lt;n&gt; tests</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td>📜</td>
<td>Consult instruction for Use</td>
</tr>
<tr>
<td>🏷️</td>
<td>CE mark</td>
</tr>
<tr>
<td>📜</td>
<td>Reconstitution volume</td>
</tr>
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</table>

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2015-04
Table 1  Storage and Stability

<table>
<thead>
<tr>
<th>Material/reagent</th>
<th>State</th>
<th>Storage</th>
<th>Stability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzygnost® Syphilis test plate, remaining strips</td>
<td>once opened</td>
<td>2–8 °C in the bag with the desiccant</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Anti-T. pallidum/POD Conjugate</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Anti-T. pallidum-Reference P</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
<tr>
<td>Anti-T. pallidum-Reference N</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
<tr>
<td>Chromogen TMB</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
<tr>
<td>Buffer/Substrate TMB</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
<tr>
<td>Chromogen Working Solution</td>
<td>diluted 1+10</td>
<td>2–8 °C 15–25 °C closed container, protected from light</td>
<td>5 days 8 hours</td>
</tr>
<tr>
<td>Washing Solution POD</td>
<td>once opened diluted 1+19</td>
<td>2–8 °C 18–25 °C</td>
<td>expiry date 1 week 1 day</td>
</tr>
<tr>
<td>Stopping Solution POD</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
</tbody>
</table>

- use each component by the expiry date at the latest
Table 2  Test Procedure

- Preparation of the reagents
  - 4 x 25 µL Reference N
  - 2 x 25 µL Reference P
  - 25 µL of each undiluted sample

- 100 µL conjugate
  - Incubation: 90 ±2 minutes (37 ±1 °C)
  - Wash 4x
  - 100 µL Chromogen Working Solution
    - Incubation (protected from light): 30 ±2 minutes (18 to 25 °C)
  - 100 µL Stopping Solution
    - (after max. 60 minutes)

- Measure: 450 nm vs. 650 nm

- in case of partially filled plates, add water-filled strips to half fill the plates

- BEP® 2000 fully automated test processing

- BEP® III automated test processing

- Test result