IRT neonatal screening ELISA

Enzyme immunoassay for the quantitative determination of Immunoreactive Trypsin (IRT) in human newborn blood spot samples. For neonatal screening of cystic fibrosis (CF).

REF RE53275/RE53279

480 / 2400

2-8 °C

For research use only. Not for use in diagnostic procedures.
1. **INTENDED USE**

Enzyme immunoassay for the quantitative determination of Immunoreactive Trypsin (IRT) in human newborn blood spot samples. For neonatal screening of cystic fibrosis (CF).

2. **SUMMARY AND EXPLANATION**

Cystic fibrosis (CF) is one of the most common autosomal recessive diseases caused by mutations in the CF transmembrane conductance regulator gene (CFTR). CF can result in death at an early age, primarily from progressive lung disease, although a number of organs are often involved. CF occurs at an incidence of approximately 1:2000 - 1:5000 live births in Europe and North America. The first European experiences in cystic fibrosis (CF) newborn screening (NBS) date back to the early nineteen seventies, with pioneering programmes examining the albumin content of meconium. The elevation of Immunoreactive Trypsin (IRT) in the blood of neonates with CF and its measurement in dried blood spots was first described in 1979. During the following decade, the determination of IRT levels in heel blood was introduced in several countries. Further improvement was possible after cloning of the CFTR gene in 1989 and subsequent identification of common population specific CFTR gene mutations allowed inclusion of DNA testing into screening protocols. Studies have shown that early diagnosis of CF through neonatal screening, combined with aggressive nutritional therapy, can significantly enhance long-term nutritional status. The two most common protocols for CF screening are (a) measuring IRT on one sample and then on a second sample, and (b) IRT testing followed by DNA mutation analysis on the same sample [1, 2, 3].

3. **TEST PRINCIPLE**

IRT neonatal screening ELISA is a “sandwich” type of solid-phase enzyme immunoassay, based on two biotinylated monoclonal antibodies specific to Trypsin 1 and Trypsin 2 and a third polyclonal antibody specific to Trypsin, which is immobilised on the inner surface of wells. Trypsin molecules from the sample bind to the immobilised antibody and anti-Trypsin-biotin conjugates. The wells are washed with wash buffer to remove any material not bound on the inner surface of the wells and enzyme conjugate is added to each well. The wells are washed again to remove any conjugate not bound to the conjugated antibody and substrate is added into each well. The intensity of the colour that develops after the substrate incubation is proportional to the amount of antigen in the sample. Results of samples can be determined directly using the standard curve.

4. **WARNINGS AND PRECAUTIONS**

1. For in-vitro diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.
10. Avoid contact with Stop solution. It may cause irritations and burns.
5. STORAGE AND STABILITY
The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.
The microtiter strips are stable up to the indicated expiry after the kit is broken. Make sure that the broken bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Blood Spots
Blood from the newborn’s heel should be collected only from the medial or lateral section of the plantar surface. Usual precautions for blood collection should be observed. After puncturing of the heel, the first drop of blood should be wiped away with a sterile gauze.
Touch the collection card against a large suspended drop of blood and allow a sufficient quantity of blood to soak into the filter paper in one go so that it fills the pre-printed circle completely. Repeat the procedure to fill the required number of pre-printed circles on the collection card.
Allow the blood spots to air-dry for 3 h at room temperature away from direct sunlight.

Because the standards are spotted on filter cards from Whatman 903® and the filter card material has a significant influence on the results (see LIMITATIONS OF THE PROCEDURE), Whatman 903® paper cards MUST be used for the patient samples. Don’t squeeze the puncture site during the collection since this will cause haemolysis or dilution of the blood with tissue fluid. Don’t apply successive drops of blood to the same pre-printed circles. Don’t touch or smear the blood spots.
Ensure the blood spot samples are visually okay (e.g. no blood smears, no coagulates, no finger-prints on the spots).

Cystic fibrosis screening does not require a change in current blood sampling practice. Most reported collection point of time is between 3rd and 5th day after birth.
National and country specific guidelines to sample collection point of time must be considered.

Storage: 2-8 °C Keep away from heat or direct sunlight.
Stability: up to 4 months [1]

7. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Quantity RE53275</th>
<th>Quantity RE53279</th>
<th>Symbol</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 12 x 8</td>
<td>25 x 12 x 8</td>
<td>MTP</td>
<td>Microtiter Plate Transparent. Coated with anti-human Trypsin antibodies (rabbit).</td>
</tr>
<tr>
<td>1 x 6 x 8</td>
<td>2 x 6 x 8</td>
<td>CAL A-F</td>
<td>Standard A-F Contains: human blood+Trypsin spotted on Whatman 903® paper. 8 Blood Spots / card. Concentrations see labels or QC certificate.</td>
</tr>
<tr>
<td>1 x 3 x 8</td>
<td>4 x 3 x 8</td>
<td>CONTROL 1-3 Control 1: low Control 2: middle Control 3: high Contains: human blood+Trypsin spotted on Whatman 903® paper. 8 Blood Spots / card. Concentrations / acceptable ranges see labels or QC certificate.</td>
<td></td>
</tr>
<tr>
<td>1 x 10 mL</td>
<td>5 x 10 mL</td>
<td>ASSAYBUF CONC</td>
<td>Assay Buffer Concentrate (20x) Contains: PBS Buffer, Tween, Protease Inhibitor, casein, preservatives.</td>
</tr>
<tr>
<td>1 x 1 mL</td>
<td>5 x 1 mL</td>
<td>Biotin-AB CONC</td>
<td>Biotin anti-IRT Ab Concentrate (100 x) anti-human Trypsin antibodies (mouse), conjugated to biotin.</td>
</tr>
<tr>
<td>1 x 0.6 mL</td>
<td>5 x 0.6 mL</td>
<td>ENZCONJ CONC</td>
<td>Enzyme Conjugate Concentrate (100x) Contains: streptavidin-Poly-HRP, preservatives.</td>
</tr>
<tr>
<td>5 x 15 mL</td>
<td>4 x 90 mL</td>
<td>TMB SUBS</td>
<td>TMB Substrate Solution Ready to use. Contains: TMB (Tetramethylbenzidine).</td>
</tr>
<tr>
<td>5 x 15 mL</td>
<td>4 x 90 mL</td>
<td>TMB STOP</td>
<td>TMB Stop Solution Ready to use. Contains: 1 M H₂SO₄.</td>
</tr>
<tr>
<td>2 x 100 mL</td>
<td>10 x 100 mL</td>
<td>WASHBUF CONC</td>
<td>Wash Buffer Concentrate (10x) Contains: PBS, Tween.</td>
</tr>
<tr>
<td>10 x</td>
<td>50 x</td>
<td>FOIL</td>
<td>Adhesive Foil black</td>
</tr>
</tbody>
</table>
8. MATERIALS REQUIRED BUT NOT SUPPLIED
1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 50; 100-500; 1000 µL
2. Blood collection cards (Whatman 903®)
3. Blood spot puncher, 3 mm (e.g. Sauer, Hannover, Germany)
4. Orbital shaker (400-600 rpm)
5. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
6. Empty vials for preparation of Biotin and Enzyme Conjugate
7. Vortex mixer
8. 8-Channel Micropipettor with reagent reservoirs
9. Wash bottle, automated or semi-automated microtiter plate washing system
10. Bidistilled or deionised water
11. Paper towels, pipette tips and timer

9. PROCEDURE NOTES
1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps must be followed strictly and in line with the instructions. Use calibrated pipettes and devices only.
2. Once the test has been initiated, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap vials that are not used. Do not reuse wells/tubes or reagents. Unused wells should be returned immediately to the resealed pouch including the desiccant.
4. It is advised to determine standards, controls and samples in duplicate in order to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. The following precautions should be taken when dried blood spots for the determination are punched:
   1. Punch the discs only from uniformly-covered dried blood spots.
   2. For double determinations, use only two nearby discs from one blood spot only, in order to avoid a chromatographic effect as much as possible.
   3. Note: Do not punch too close to the edge of the blood spot (1 mm away from the edge)!
9. Ensure all dried blood spots, without exception, are removed from the microtiter plate before starting washing.
10. Check the blood spot samples are visually okay (e.g. no blood smears, no coagulates, no finger-prints on the spots).
11. Unused wells/tubes and dried blood spots should be returned immediately to the resealed pouch, including the desiccant.
10. **PRE-TEST SETUP INSTRUCTIONS**

### Preparation of concentrated components (1 Microtiter Plate)

<table>
<thead>
<tr>
<th>Dilute / dissolve</th>
<th>Component</th>
<th>with</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mL</td>
<td><strong>WASHBUF CONC</strong></td>
<td>900 mL</td>
<td>bidist. water</td>
<td>1:10</td>
<td>Mix vigorously.</td>
<td>2-8°C</td>
<td>4 weeks</td>
</tr>
<tr>
<td>2 mL</td>
<td><strong>ASSAYBUF CONC</strong></td>
<td>38 mL</td>
<td>bidist. water</td>
<td>1:20</td>
<td>Resolve crystals at 18-25°C.</td>
<td>2-8°C</td>
<td>24 h</td>
</tr>
<tr>
<td>160 µL</td>
<td><strong>BIOTIN-AB CONC</strong></td>
<td>16 mL</td>
<td>diluted Assay Buffer</td>
<td>1:101</td>
<td>Mix &gt; 10 min without foaming.</td>
<td>28-25°C</td>
<td>up to 1h</td>
</tr>
</tbody>
</table>

**Remarks:**

- 1:10 dilution of WASHBUF with bidist. water
- 1:20 dilution of ASSAYBUF with bidist. water
- 1:101 dilution of BIOTIN-AB with diluted Assay Buffer

**Stability:**

- 2-8°C: up to 4 weeks
- 2-8°C: up to 24 h
- 28-25°C: up to 1 h
- 28-25°C: up to 3 h

**Do not use polypropylene bottles for the preparation of Enzyme Conjugate.**

If you are using several vials of the concentrated Enzyme Conjugate, it is highly recommended to pool the solutions and to establish the working solution from this pool.

11. **TEST PROCEDURE**

1. **Punch out one disc (3 mm Ø) of each blood spot Standard (A - F), Control 1-3 and sample** with a blood spot puncher and put the discs into the assigned well of the coated MTP.
   - **Note:** Do not punch too close to the edge of the blood spot!

2. **Pipette 150 µL of diluted Biotin anti-IRT Antibody** into each well. Make sure that all discs are immersed in the liquid. Cover plate with black adhesive foil.

3. **Incubate 2 hours at RT (18-25°C) on a plate shaker (400-600 rpm).**

4. **Remove adhesive foil. Discard incubation solution and all discs by tapping the inverted plate on a paper towel. Wash plate 4x with 300 µL of diluted Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.

5. **Pipette 100 µL of diluted Enzyme Conjugate** into each well. Cover plate with black adhesive foil.

6. **Incubate 60 min at RT (18-25°C) on a plate shaker (400-600 rpm).**

7. **Remove adhesive foil. Discard incubation solution. Wash plate 4x with 300 µL of diluted Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.

8. **If available, use an 8-Channel Micropipettor for adding Substrate and Stop Solution. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.**

9. **Pipette 100 µL of TMB Substrate Solution** into each well.

10. **Incubate 30 min at RT (18-25°C) on a plate shaker (400-600 rpm). Avoid direct sun light.**

11. **Stop the substrate reaction by adding 100 µL of TMB Stop Solution into each well. Color changes from blue to yellow.**

12. **Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 15 min after pipetting of the Stop Solution.**
12. QUALITY CONTROL
The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.
In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS
The obtained OD of the standards (y-axis, linear) is plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with Cubic Spline or 4 Parameter Logistics.
To calculate the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).
The concentration of the samples can be read directly from the standard curve.

Typical Calibration Curve
(Example. Do not use for calculation!)

<table>
<thead>
<tr>
<th>Standard</th>
<th>Trypsin (ng/mL)</th>
<th>OD\text{mean}</th>
<th>OD/OD_{\text{max}} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>0.144</td>
<td>6%</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>0.425</td>
<td>17%</td>
</tr>
<tr>
<td>C</td>
<td>45</td>
<td>0.646</td>
<td>26%</td>
</tr>
<tr>
<td>D</td>
<td>79</td>
<td>0.946</td>
<td>38%</td>
</tr>
<tr>
<td>E</td>
<td>157</td>
<td>1.514</td>
<td>60%</td>
</tr>
<tr>
<td>F</td>
<td>362</td>
<td>2.504</td>
<td>100%</td>
</tr>
</tbody>
</table>

![Typical Calibration Curve](image)
14. EXPECTED VALUES
Based on the assumption that the expected values of immunoreactive trypsin (IRT) follow a normal distribution, it is common to use floating cut-off because of the variations in IRT levels (e.g. ethnic or seasonal variations). There are several options for an initial threshold based on percentile values of the normal distribution measured in single determinations (95\textsuperscript{th}, 97.5\textsuperscript{th}, 99\textsuperscript{th} or 99.5\textsuperscript{th} percentile values). In general, a policy of replication in duplicate should be adopted for all samples with IRT above a preliminary threshold. As an example for normal distribution a small study on 474 newborns with the IBL IRT neonatal screening ELISA can be shown as follows:

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>31.3 ng/mL</td>
</tr>
<tr>
<td>Median</td>
<td>28.4 ng/mL</td>
</tr>
<tr>
<td>75\textsuperscript{th} percentile</td>
<td>40.8 ng/mL</td>
</tr>
<tr>
<td>90\textsuperscript{th} percentile</td>
<td>52.4 ng/mL</td>
</tr>
<tr>
<td>95\textsuperscript{th} percentile</td>
<td>63.8 ng/mL</td>
</tr>
<tr>
<td>99\textsuperscript{th} percentile</td>
<td>84.2 ng/mL</td>
</tr>
</tbody>
</table>

Depending on the application of samples of different populations of newborns it is highly recommended that each laboratory establishes its own range of normal values and that this distribution of values is coordinated with the recommendations of the responsible society of this geographic region.

15. LIMITATIONS OF THE PROCEDURE
Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.
Any result with an elevated concentration has to be indicated as ‘presumptive positive’ and has to be confirmed with further sampling and testing. A false negative result of this assay cannot be excluded with absolute certainty.
Conditions which are known to cause anomalous analytical assay results include:
- sample spot not uniformly saturated with blood
- sample spots punched too close to the edge of the blood spot
- poorly collected and improperly dried specimens
- non-eluting blood spot due to deterioration of sample caused by exposure to heat and humidity
- contamination of blood spot filter paper with faecal material

For cross-reactivities, see PERFORMANCE.
The following blood components do not have a significant effect on the test results up to the below stated concentrations:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>5 mg/mL</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>91 mg/mL</td>
</tr>
</tbody>
</table>
16. PERFORMANCE

<table>
<thead>
<tr>
<th>Analytical Specificity (Cross Reactivity)</th>
<th>The following substances showed a cross reactivity lower than &lt;0.01%: Pepsinogen II PGC; alpha2-Macroglobulin; alpha-1-Antitrypsin; alpha-Chymotrypsin; gamma-Globulin, phospholipase A2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Sensitivity (Limit of Blank)</td>
<td>&lt; 6 ng/mL</td>
</tr>
<tr>
<td>Precision</td>
<td>Range (ng/mL)</td>
</tr>
<tr>
<td></td>
<td>Intra-Assay</td>
</tr>
<tr>
<td></td>
<td>Inter-Assay</td>
</tr>
<tr>
<td>Method Comparison versus Methods / Assays</td>
<td>IBL-Assay ELISA = 0.70 (IBL IRT LUM) + 6.91</td>
</tr>
<tr>
<td></td>
<td>IBL-Assay ELISA = 0.86 (IRT Colorimetric ELISA) - 7.46</td>
</tr>
<tr>
<td></td>
<td>IBL-Assay ELISA = 0.84 (IRT Fluorometric Assay) + 14</td>
</tr>
</tbody>
</table>

17. PRODUCT LITERATURE REFERENCES

**_symbols / Symbole / Symbôles / Símbolos / Σύµβολα**

<table>
<thead>
<tr>
<th>REF</th>
<th>Lot-No. / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote n.: / Αριθµός -Παραγωγή:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat.-No. / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / Αριθµός-Κατ.:</td>
<td></td>
</tr>
</tbody>
</table>

**Use by:** / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro: / Χρησιµοποιείται από: |

**No. of Tests:** / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / Αριθµός εξετάσεων: |

**CONC** / Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συµπύκνωµα |

**LYO** / Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασµένο |

**VD** / In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamiento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση. |

**Evaluation kit.** / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluación. / Kit de avaliação. / Kit di evaluatione. / Κιτ Αξιολόγησης |

**Keep away from heat or direct sunlight.** / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται µακριά από θερµότητα και άµεση επαφή µε το φως του ηλίου. |

**Manufacturer:** / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός: 

**Caution!** / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή! |

Symbols of the kit components see MATERIALS SUPPLIED. 
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. 
Voir MATERIEL FOURNI pour les symbôles des composants du kit. 
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. 
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. 
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. 
Για τα σύµβολα των συστατικών του κιτ συµβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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**IBL AFFILIATES WORLDWIDE**

<table>
<thead>
<tr>
<th>IBL International GmbH</th>
<th>IBL International Corp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flughafenstr. 52A, 22335 Hamburg, Germany</td>
<td>194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada</td>
</tr>
<tr>
<td>Tel.: + 49 (0) 40 532891 -0</td>
<td>Tel.: +1 (416) 645 -1703</td>
</tr>
<tr>
<td>Fax: -11</td>
<td>Fax: -1704</td>
</tr>
<tr>
<td>E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a></td>
<td>E-MAIL: <a href="mailto:Sales@IBL-International.com">Sales@IBL-International.com</a></td>
</tr>
</tbody>
</table>

**LIABILITY:** Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.