TestLine®

## Instruction for use

# EIA COVID-19 RBD IgM

REF CoRM96

Σ 96

**Kit for professional use**

IVD CE

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# CONTENT

# 1 Document Records

| Revision No. | Version No. | Revision Description |
|---|---|---|
| ZM01566 | 8 | Revision according to IVDR requirements |
| ZM01577 | | Revision of analytical performance values |
| ZM01591 | | Addition of the Dried Blood Spot testing |

# 2 Intended Purpose

The immunoenzymatic assay is intended for the diagnosis, monitoring and screening SARS-CoV-2 (COVID-19) infection using IgM antibodies in human serum, plasma or dry blood spot in the general population. The semi-quantitative and quantitative manual assay is designed for professional use in a laboratory.

# 3 Introduction

Coronaviruses, which were discovered in the 1960s, belong to the family of enveloped RNA viruses. They fall in the group of zoonotic infections that cause diseases of the respiratory and digestive tracts in humans and animals (birds, mammals). Coronaviruses cause diverse clinical pictures, from common cold to severe respiratory syndromes (MERS, SARS and COVID-19). The majority of known coronaviruses circulate among animals. Alpha- and Beta-coronaviruses can infect only mammals whereas Gamma- and Delta-coronaviruses infect both birds and mammals. Alpha- and Beta-coronaviruses occur in humans. A total of 7 types of human coronaviruses are known so far – 229E, NL63, OC43, HKU1, MERS, SARS, SARS – 2.

Transmission: The infection can be transmitted from an infected person 1–3 days before the onset of the disease. The new coronavirus is a respiratory virus. It is primarily transmitted to an individual through a close contact with an infected person, during which infectious droplets spread to the environment, especially when the infected person talks, coughs and/or sneezes. Things freshly contaminated with secretions of an infected person can also contribute to the transmission. The virus has been successfully isolated from samples taken from the lower respiratory tract (bronchoalveolar lavage). Viral RNA has been detected in nasopharyngeal and throat swabs, serum, blood, rectal swabs, saliva, urine and faeces.

The virus has been found in airway samples 1–2 days before the onset of symptoms and up to 8 days after the onset in case of a mild disease, longer in case of a more severe disease development. Susceptibility seems to be general. Existing experience suggests that the infection is as likely in children as in adults but with milder clinical manifestations. Immunity to COVID-19, if any, has not been established so far. Reported mortality ranges from 2% to 3%. Due to the several-day-long interval between the first symptoms and the onset of the antibody response (the "window period"), serological tests play only a supporting role and, as stressed by the WHO, the results of such tests should always be verified by direct detection of the virus to diagnose an acute COVID 19 disease. Determination of the level of antibodies present after the disease is also an option.

SARS-CoV-2 virus (COVID-19) contains four structural proteins: spike (S), nucleocapsid (N), envelope (E) and membrane (M) protein. The most commonly used antigens in diagnosis include:

Nucleocapsid protein (NP) encapsulates viral genomic RNA and forms a major component of the viral structure. NP is a highly antigenic epitope and is associated with several virus-host interactions.

The receptor-binding domain (RBD), a subunit of the Spike S1 protein, specifically binds to the angiotensin-converting enzyme 2 (ACE2) of the host cell. The binding of RBD to ACE2 is highly associated with the formation of neutralizing antibodies.

# 4 Test Principle

The kit is intended for detection of specific IgM antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgM conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgM antibodies in the sample.

**Antigen Used**

Recombinant Receptor-binding domain (RBD) antigen and combination of S1 Spike relevant SARS-CoV-2 mutations

# 5 Materials Provided

| | | |
|---|---|---|
| MICROPLATE | **Microtitre Plate**<br>coated with antigen, 12 x 8 wells in bag with desiccant | 1 pc |
| CONTROL - CAL1 | **Negative Control (Calibrator 1) 5 U/ml**<br>Solution containing no specific human antibodies, ready to use | 1 × 2 ml |
| CUTOFF CAL2 | **CUT-OFF (Calibrator 2) 20 U/ml**<br>Solution containing specific human antibodies in cut-off concentration, ready to use | 1 × 3 ml |
| CONTROL + CAL3 | **Positive Control (Calibrator 3) 80 U/ml**<br>Solution containing specific human antibodies, ready to use | 1 × 2 ml |
| CAL4 | **Calibrator 4 (320 U/ml)**<br>Solution containing specific human antibodies, ready to use | 1 × 2 ml |
| CONJUGATE | **Conjugate**<br>Solution containing peroxidase labelled animal immunoglobulin to human IgM, ready to use | 1 × 15 ml |
| DILUENT 2 | **Sample Diluent 2**<br>Buffer with protein stabilisers, ready to use | 1 × 105 ml |
| SUBSTRATE 2 | **TMB-Complete 2**<br>Chromogenic substrate solution containing TMB/$H_2O_2$, ready to use | 1 × 15 ml |
| WASH 20x | **Wash Solution**<br>20× concentrated buffer | 1 × 75 ml |
| STOP | **Stop Solution**<br>Acid solution, ready to use | 1 × 15 ml |
| | **Instructions for use** | 1 pc |

# 6 Other Material Required for Test Performance

Single- and multichannel pipettes
Disposable tips
Microplate washer
Timer
Incubator (37°C)
Microplate reader

# 7 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

### Sample Preparation and Storage

Samples listed in the intended use may be used for the examination. The following human body liquids can be used for testing: serum, citrate plasma or dry blood spot. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Dry blood spot samples must be taken in the prescribed amount and fill in all fields on the card, insufficient blood may affect the test result.

A coagulating blood collection tube is recommended for serum collection. A citrated plasma collection bag is recommended for plasma collection. Other types of plasma (EDTA, heparin) may be used but they are not recommended since anticoagulants may affect the test result. A collection card is used to collect the dry blood spot.

Follow the manufacturer's instructions when using commercial or other specially modified samples.

Clinical samples collected within standard medical procedures into standardized tubes are ready for immediate use. Centrifugation or other separations are not required.

The examined samples can be stored at +2 °C to +8 °C for a maximum of 1 week.

# 8 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2 °C to +8 °C for one week.

The Controls and the Calibrators are supplied ready to use, do not dilute further!

The Conjugate is supplied ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

### Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

# 9 Preparation of Samples

Mix gently the Sample Diluent prior to use.

### Dilution of sera and plasma samples

Dilute well mixed samples 1:101 with the Sample Diluent:

E.g.: 10 µl of sample + 1 ml of the Sample Diluent

Mix well.

### Processing of dry blood spots

Remove the elution field target from the card with a 6 mm punch and place in a tube with 600 µl of Sample Dilution Solution, shake gently by hand, place on a shaker and shake for 30 min so that there is slight movement of the solution in the tube, then let stand for another 30 min at laboratory temperature, then shake the solution gently and use in the test without further dilution. For testing in analysers, the target must be removed from the tube.

# 10 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls (calibrators) and the diluted samples according to the working schedule.

**Semiquantitative evaluation in Index of Positivity (IP)**

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

**Quantitative evaluation in Units U/ml**

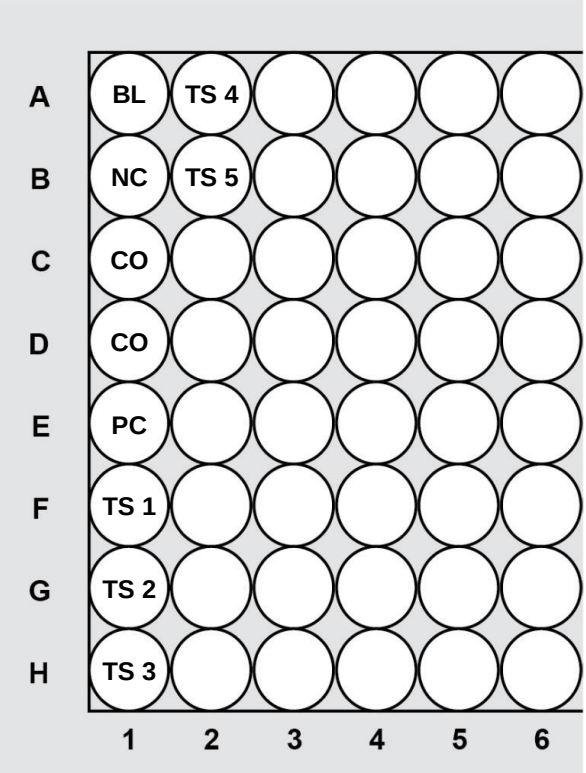
- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 2 wells.
- Pipette 100 µl of the Calibrator 4 into 2 wells.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

2. Cover the microplate with the lid and incubate at 37 °C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate it at 37 °C for 30 minutes.
6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37 °C for 15 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

# 11 Working Schedule

Semiquantitative evaluation

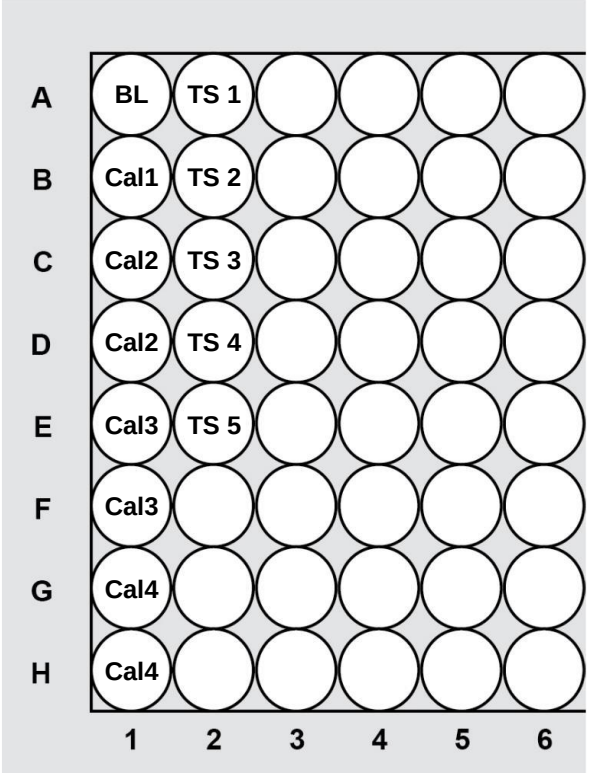
Index of Positivity (IP)




| | | |
|---|---|---|
| BL | Blank (empty well) | |
| NC | 100 µl | CONTROL - CAL1 |
| CO | 100 µl | CUTOFF CAL2 |
| PC | 100 µl | CONTROL + CAL3 |
| TS 1-x | 100 µl | diluted tested sample |

Quantitative evaluation

Units U/ml




| | | |
|---|---|---|
| BL | Blank (empty well) | |
| Cal1 | 100 µl | CONTROL - CAL1 |
| Cal2 | 100 µl | CUTOFF CAL2 |
| Cal3 | 100 µl | CONTROL + CAL3 |
| Cal4 | 100 µl | CAL4 |
| TS 1-x | 100 µl | diluted tested sample |

# 12 Quality Control

The test is valid if:

The absorbance of blank is lower than 0.150.

BLANK < 0.150

The absorbance of the Negative Control (Calibrator 1) is lower than half of the mean absorbance of CUT–OFF (Calibrator 2).

| CONTROL | - | CAL1 | < 0.5 × | CUTOFF | CAL2 |
|---|---|---|---|---|---|

The mean absorbance of CUT-OFF (Calibrator 2) is within a range of 0.200 – 1.000.

| 0.200 < | CUTOFF | CAL2 | < 1.000 |
|---|---|---|---|

The absorbance of the Positive Control (Calibrator 3) is 1.5-fold higher than the mean absorbance of CUT–OFF (Calibrator 2).

| CONTROL | + | CAL3 | > 1.5 × | CUTOFF | CAL2 |
|---|---|---|---|---|---|

The absorbance of the Calibrator 4 is higher than the absorbance of the Positive Control (Calibrator 3).

| CAL4 | > | CONTROL | + | CAL3 |
|---|---|---|---|---|

# 13 Results Interpretation

**Calculation of Index of Positivity (IP)**

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$IP = \frac{\text{Absorbance of serum, plasma}}{\text{Mean absorbance of CUT-OFF}}$$

Interpretation of the test results is described in the table (Table 1).

**Table 1 Interpretation of test results**

| Index of Positivity (IP) | Evaluation |
|---|---|
| lower than 0.9 | negative |
| 0.9 to 1.1 | borderline |
| higher than 1.1 | positive |

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

**Quantitative evaluation in Units (U/ml)**

Construct a calibration curve by plotting the concentration (X) of the calibrators in U/ml against the corresponding absorbance (Y). Construct the calibration curve by single point cross connection. Read the values of antibody level (U/ml) in samples from the calibration curve. Interpretation of the quantitative test results is described in the table (Table 2).

**Table 2 Quantitative interpretation in Units (U/ml)**

| Antibody level (U/ml) | Evaluation |
|---|---|
| lower than 18 | negative |
| 18 to 22 | borderline |
| higher than 22 | positive |

Examination of borderline samples should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

# 14 Analytical performance

## 14.1 Specificity and Sensitivity

Specificity was determined in the panel of negative samples. Sensitivity was determined in the panel of positive samples. The number of samples tested and the results obtained are described in the table (Table 3).

## 14.2 Trueness (bias)

Trueness is the closeness of agreement between the average value obtained from a large number of measurement results and the reference value. Its measure is bias. The nature of the method does not allow quantitative determination of bias (and thus the trueness). The trueness of the method is ensured by clinical parameters such as sensitivity and specificity, comparison with the reference method and batch continuity. The obtained results are described in the table (Table 3).

## 14.3 Precision: Repeatability – Intra-assay (within run)

The precision is defined as the closeness of agreement between measured values obtained by replicate measurements on the same object under specified conditions. The Intra-assay repeatability is expressed as agreement level among sample replicates within a run of the assay (in one batch). The obtained results are described in the table (Table 3).

## 14.4 Precision: Reproducibility – Inter-assay (between-run)

Reproducibility is a measure of precision under a defined set of conditions which include the inter-assay, expressed as agreement level among sample replicates within runs of the assay in one batch. The obtained results are described in the table (Table 3).

## 14.5 Accuracy

Accuracy is defined as the closeness of agreement between the measured value and the reference value. It is expressed as an achievable measure of the combined uncertainty. The obtained results are described in the table (Table 3).

## 14.6 Analytical sensitivity – limits of detection and quantitation

The analytical sensitivity is the maximum binary dilution of CUT-OFF or international standard samples, respectively, giving absorbance significantly different from the background. The value is expressed as an index of positivity and/or a concentration in units. This value is a minimum limit of detection and quantification. The obtained results are described in the table (Table 3).

## 14.7 Measuring Range

The measuring range of the kit lies between values where the lower limit is determined by the analytical sensitivity value and the upper limit depends on the measuring capability of the equipment used.

## 14.8 Linearity

The linearity is the ability of the method to obtain final values proportional (directly or after mathematical transformation) to the concentration of analyte in the sample; it is expressed as the range in which the method provides linear results. The obtained results are described in the table (Table 3).

## 14.9 Hook effect

Hook effect is an immunological phenomenon that causes falsely low results in the presence of an excess amount of analyte. Its presence is detected by serial dilution of a highly positive sample (Table 3).

## 14.10 Comparison with the reference method

Comparison with the reference method was performed. The results of both methods are comparable, considering the differences of both methods and completely meeting the requirements if the agreement in the classification of the samples is at least 90% (Table 3).

**Table 3 Analytical Performance**

| Parameter | Value |
|---|---|
| Sensitivity – serum (n 805) | 99.88% |
| Sensitivity – plasma (n 58) | 99.99% |
| Sensitivity – dried blood spot (n 57) | 99.99% |
| Specificity – serum (n 160) | 99.37% |
| Specificity – plasma (n 588) | 97.57% |
| Specificity – dried blood spot (n 78) | 98.72% |
| Trueness (bias) | N/A |
| Precision: Repeatability | 6.45% |
| Precision: Reproducibility | 6.60% |
| Accuracy | 9.80% |
| Analytical sensitivity | IP 0.06 |
| | <5.00 U/ml |
| Linearity interval | IP 0.30–7.00 |
| | 6.82–320.00 U/ml |
| Hook effect | Not observed |
| Comparison with the reference method | at least 90% |

N/A – not applicable

## 14.11 Interference

Two samples (one negative plasma pool and one positive plasma pool) were spiked with potentially interfering substances. Results of interference testing are shown in the table (Table 4).

**Table 4 Interference Results**

| Interfering substance | The result was not affected up to concentration: |
|---|---|
| Bilirubin | 0.4 mg/ml |
| Triacylglycerols | 20 mg/ml |
| Haemoglobin | 5 mg/ml |

## 14.12 Cross-reactivity

The assay was evaluated for potential cross-reactivity using samples positive for selected pathogens and factors. Results of testing are shown in the table (Table 5).

**Table 5 Results of Cross-Reacting Pathogens or Factors**

| Category | (n) | Positive result |
|---|---|---|
| Endemic HCoVs | 30 | 1 |
| EBV | 10 | 0 |
| Mumps virus | 10 | 0 |
| Measles virus | 9 | 0 |
| VZV | 17 | 0 |
| CMV | 20 | 0 |
| Influenza A, B virus | 10 | 0 |
| Chlamydia pneumoniae | 10 | 0 |
| Adenovirus | 6 | 0 |
| Bordetella pertussis | 5 | 0 |
| Toxoplasma gondii | 10 | 0 |
| Parvovirus B19 | 9 | 0 |
| HSV | 7 | 0 |
| Rubella virus | 5 | 0 |
| RF | 7 | 1 |
| Treponema pallidum | 10 | 0 |
| **Total** | **175** | **2** |

# 15 Clinical Performance

## 15.1 Diagnostic specificity and diagnostic sensitivity

Diagnostic specificity was determined in the panel of negative samples. Diagnostic sensitivity was determined in the panel of positive samples. The number of samples tested and the results obtained are shown in the table (Table 6, Table 7, Table 8).

## 15.2 Positive and negative predictive value

A positive predictive value is probability that a person is actually affected by infection if the result was positive. A negative predictive value is probability that a person is actually healthy if the result was negative. The results obtained are shown in the table (Table 6, Table 7, Table 8).

## 15.3 Likelihood ratio of the kit

The likelihood ratio of the kit for a positive test is the ratio of probability that an individual from affected population is diagnosed as positive by the test and probability that a healthy individual is misdiagnosed as positive.

The likelihood ratio of the kit for a negative test is the ratio of probability that an individual from affected population is misdiagnosed as negative by the test and probability that a healthy individual is diagnosed as negative. The results obtained are shown in the table (Table 6, Table 7, Table 8).

## 15.4 Expected values in population

Expected values in population are established based on the value results in a file of samples declared as negative and a file of samples declared as positive for the presence of specific antibodies. The results obtained are shown in the table (Table 6, Table 7, Table 8).

**Table 6 Clinical performance – serum**

| Parameter | Value | 95% Confidence Interval (CI) |
|---|---|---|
| Diagnostic sensitivity (n 805) | 99.88% | 99.31% – 100.00% |
| Diagnostic specificity (n 160) | 99.37% | 96.55% – 99.98% |
| Positive predictive value (n 805) | 99.88% | 99.31% – 100.00% |
| Negative predictive value (n 160) | 99.37% | 96.55% – 99.98% |
| Likelihood ratio of the kit for a positive test | >100 | - |
| Likelihood ratio of the kit for a negative test | 0.001 | - |
| Expected values in healthy population | 10.14 U/ml | 9.70 – 10.58 U/ml |
| Expected values in affected population | 278.14 U/ml | 272.18 – 284.10 U/ml |

**Table 7 Clinical performance – plasma**

| Parameter | Value | 95% Confidence Interval (CI) |
|---|---|---|
| Diagnostic sensitivity (n 58) | 99.99% | 93.84% – 100.00% |
| Diagnostic specificity (n 588) | 97.55% | 95.92% – 98.65% |
| Positive predictive value (n 58) | 80.56% | 69.53% – 88.94% |
| Negative predictive value (n 588) | 99.99% | 99.34% – 100.00% |
| Likelihood ratio of the kit for a positive test | 40.786 | - |
| Likelihood ratio of the kit for a negative test | <0.0001 | - |
| Expected values in healthy population | 9.57 U/ml | 9.33 – 9.81 U/ml |
| Expected values in affected population | 114.09 U/ml | 88.44 – 139.74 U/ml |

**Table 8 Clinical performance – dried blood spot**

| Parameter | Value | 95% Confidence Interval (CI) |
|---|---|---|
| Diagnostic sensitivity (n 57) | 99.99% | 93.28% – 100.00% |
| Diagnostic specificity (n 78) | 98.72% | 93.06% – 99.97% |
| Positive predictive value (n 57) | 98.15% | 90.11% – 99.95% |
| Negative predictive value (n 78) | 99.99% | 95.32% – 100.00% |
| Likelihood ratio of the kit for a positive test | 78.000 | - |
| Likelihood ratio of the kit for a negative test | <0.0001 | - |
| Expected values in healthy population | 5.86 U/ml | 5.46 – 6.26 U/ml |
| Expected values in affected population | 122.20 U/ml | 90.57 – 153.83 U/ml |

# 16 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain the toxic component sodium azide or gentamicin, but in very low concentrations. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

### First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

### Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

### Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

# 17 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

**Microtitre Plate** – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

**Wash Solution** – use high quality distilled water for preparing the working strength Wash Solution.

**Washing procedure** – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

**TMB-Complete** – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

**Technical limitation of samples**

Materials of a human origin from donor population listed in the intended use were used for manufacture and development of the kit. Kits are intended for use in general population, unless otherwise stated.

When using samples from other specific populations (comorbid, immunocompromised, pregnant, paediatric population), the risk of a specific effect on the result of the applied test due to e.g. interference or cross-reactivity should be considered in the context of expert knowledge and current scientific knowledge.

**Other notes**

The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

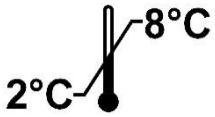
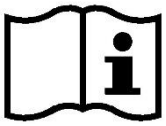
The kit might be used in all types of automatic EIA analysers. If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

**The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.**

# 18 References

1. Fu Y, Pan Y, Li Z, Li Y. The Utility of Specific Antibodies Against SARS-CoV-2 in Laboratory *Diagnosis. Front Microbiol*. 2021. 11 (603058): 1–10.
2. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends Immunol*. 2020. 41 (12): 1100–1115.
3. Liu Z, Xu W, Xia S et al. RBD-Fc-based COVID-19 vaccine candidate induces highly potent SARS-CoV-2 neutralizing antibody response. *Signal Transduct Target Ther.* 2020. 5 (282): 1–10.
4. Long QX, Liu BZ, Deng HJ et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med*. 2020. 26: 845–848.

## 19 IFU Symbols

| | |
|---|---|
|   | Temperature limitation |
|  | Keep dry |
|  | Expiry date |
|   | Lot number |
|  | Manufactured by |
|  | Consult instructions |
|   | Catalogue number |
|   | Number of tests |
|   | In vitro diagnostic medical device |

**Summary of EIA COVID-19 RBD IgM Protocol**

| Step No. | Symbol | Test steps |
|---|---|---|
| 1 |  | Dilute samples<br>serum/plasma 1:101 (10 µl + 1 ml)<br>processing of dry blood spots is described in chapter Preparation of Samples |
| 2 |  | Pipette Controls and diluted samples – 100 µl<br>Blank = empty well |
| 3 |  | Incubate at 37°C for 30 min |
| 4 |  | Aspirate and wash the wells 5× |
| 5 |  | Pipette Conjugate – 100 µl<br>Blank = empty well |
| 6 |  | Incubate at 37°C for 30 min |
| 7 |  | Aspirate and wash the wells 5× |
| 8 |  | Pipette Substrate (TMB-Complete) – 100 µl<br>Including blank |
| 9 |  | Incubate at 37°C for 15 min |
| 10 |  | Pipette Stop Solution – 100 µl<br>Including blank |
| 11 |  | Read colour intensity at 450 nm |