

# Product information

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## Riemerella vet ELISA

RUO

REF

DERIEMVT0880



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**CONTENTS**

|  |    |
|--|----|
| 1. INTRODUCTION                          | 3  |
| 2. INTENDED USE                          | 3  |
| 3. PRINCIPLE OF THE ASSAY                | 3  |
| 4. MATERIALS                             | 4  |
| 5. STABILITY AND STORAGE                 | 4  |
| 6. REAGENT PREPARATION                   | 4  |
| 7. SAMPLE COLLECTION AND PREPARATION     | 5  |
| 8. ASSAY PROCEDURE                       | 5  |
| 9. RESULTS                               | 6  |
| 10. SPECIFIC PERFORMANCE CHARACTERISTICS | 7  |
| 11. LIMITATIONS OF THE PROCEDURE         | 7  |
| 12. PRECAUTIONS AND WARNINGS             | 8  |
| <br>                                     |    |
| BIBLIOGRAPHY                             | 9  |
| ABBREVIATIONS                            | 9  |
| SUMMARY OF TEST PROCEDURE                | 10 |
| SYMBOLS USED WITH DEMEDITEC ASSAYS       | 12 |

## 1. INTRODUCTION

The pathogen of infectious serositis (riemerellosis) is a 0.2-0.5 x 1.0-2.5 µm large, immobile, gram-negative rod-shaped bacterium of the genus Riemerella. Riemerella can be found worldwide and it is particularly important in the breeding of ducks and geese, since it leads to considerable economic losses. In addition to ducks (main host) also geese and turkeys, and more rarely chicken and wild birds are infected. Infection sources are latently infected adult animals. Here the pathogens persist in the mucous membranes of the upper respiratory tract. Infection occurs from animal to animal or indirectly by drinking water, interspersing etc. The acute disease usually affects young animals and is often fatal.

Symptoms of riemerellosis are:

- Catarrhal to fibrinous rhinitis, conjunctivitis and sinusitis
- Central nervous system disorders ("crooked")
- Lameness
- Heartbeat- and liver infections
- Catarrhal Enteritis
- Greenish diarrhea
- In the chronic course, brain inflammation and joint inflammation occur

The infection can be diagnosed by the following methods:

- Cultivation of riemerella in the embryonated egg or in cell culture
- Serologically by ELISA

## 2. INTENDED USE

The Riemerella vet ELISA is intended for the qualitative determination of antibodies against Riemerella in veterinary serum.

## 3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

## 4. MATERIALS

### 4.1. Reagents supplied

1. **SORB** **MT** **Microtiterplate**: 12 break-apart 8-well snap-off strips coated with Riemerella antigens; in resealable aluminium foil.
2. **SAM** **DIL** **Sample Dilution Buffer**: 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH  $7.2 \pm 0.2$ ; coloured yellow; ready to use; white cap;  $\leq 0.0015\%$  (v/v) CMIT/MIT (3:1).
3. **STOP** **SOLN** **Stop Solution**: 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
4. **WASH** **SOLN** **20x** **Washing Buffer (20x conc.)**: 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH  $7.2 \pm 0.2$ , for washing the wells; white cap.
5. **ENZ** **CONJ** **Conjugate**: 1 bottle containing 20 mL of peroxidase labelled antibody to avian IgY in phosphate buffer (10 mM); coloured blue; ready to use; white cap.
6. **SUB** **TMB** **TMB Substrate Solution**: 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB),  $< 0.1\%$ ; ready to use; yellow cap.
7. **CAL** **C** **Positive Control**: 1 vial containing 2 mL; coloured yellow; ready to use; red cap;  $\leq 0.02\%$  (v/v) MIT.
8. **CAL** **B** **Cut-off Control**: 1 vial containing 3 mL; coloured yellow; ready to use; green cap;  $\leq 0.02\%$  (v/v) MIT.
9. **CAL** **A** **Negative Control**: 1 vial containing 2 mL; coloured yellow; ready to use; blue cap;  $\leq 0.0015\%$  (v/v) CMIT/MIT (3:1).

For hazard and precautionary statements see 12.1

For potential hazardous substances please check the safety data sheet.

### 4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)

### 4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate wells
- Pipettes to deliver volumes between 10 and 1000  $\mu\text{L}$
- Vortex tube mixer
- Distilled water
- Disposable tubes

## 5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

## 6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them before starting the test run!

### 6.1. Microtiterplate

The break-apart snap-off strips are coated with Riemerella antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

### 6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20...25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

### 6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

## 7. SAMPLE COLLECTION AND PREPARATION

Use turkey serum samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

### 7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with Sample Dilution Buffer. Dispense 10 µL sample and 1 mL Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

## 8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to  $37 \pm 1$  °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour  $\pm$  5 min at  $37 \pm 1$  °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!  
Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

### 8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

**Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the-plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

## 9. RESULTS

### 9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank:** Absorbance value < **0.100**
- **Negative Control:** Absorbance value < **0.200** and < **Cut-off**
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value > **Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

### 9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43  
Cut-off = 0.43

#### 9.2.1. Results in Units [U]

$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Units} = \text{U}]$

Example:  $\frac{1.591 \times 10}{0.43} = 37 \text{ U}$

### 9.3. Interpretation of Results

Normal value ranges for this ELISA should be established by each laboratory based on its own sample populations in the geographical areas serviced.

The following values should be considered as a guideline:

|           |          |  |
|-----------|----------|--|
| Cut-off   | 10 U     | -  |
| Positive  | > 11 U   | Antibodies against the pathogen are present.<br>There has been a contact with the antigen (pathogen resp. vaccine).  |
| Equivocal | 9 – 11 U | Antibodies against the pathogen could not be detected clearly.<br>It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as <b>negative</b> . |
| Negative  | < 9 U    | The sample contains no antibodies against the pathogen.<br>A previous contact with the antigen (pathogen resp. vaccine) is unlikely.   |

## 10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications. The performance data have been established with samples of turkey. Due to the nature of the anti avian conjugate this ELISA should react with other avian species also. More detailed information is available on request.

### 10.1. Precision

| <b>Intraassay</b> | <b>n</b> | <b>Mean (E)</b> | <b>CV (%)</b> |
|-------------------|----------|-----------------|---------------|
| #1                | 24       | 1.705           | 1.50          |
| #2                | 24       | 0.047           | 8.08          |
| #3                | 24       | 0.996           | 2.76          |

| <b>Interassay</b> | <b>n</b> | <b>Mean (U)</b> | <b>CV (%)</b> |
|-------------------|----------|-----------------|---------------|
| #1                | 12       | 41.56           | 3.96          |
| #2                | 12       | 2.07            | 4.09          |
| #3                | 12       | 2.05            | 3.39          |
| #4                | 12       | 15.58           | 2.45          |
| #5                | 12       | 15.47           | 2.55          |

### 10.2. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

Specificity turkey: > 98 % (95 % confidence interval: 79.41 % - 100.0 %).

### 10.3. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

Sensitivity turkey: > 98 % (95 % confidence interval: 69.15 % - 100.0 %).

### 10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

### 10.5. Cross Reactivity

Cross-reactions can not be excluded.

## 11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

## 12. PRECAUTIONS AND WARNINGS

- Only for research use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

### 12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)

Therefore, the following hazard and precautionary statements apply.



|                |           |  |
|----------------|-----------|--|
| <b>Warning</b> | H317      | May cause an allergic skin reaction.                             |
|                | P261      | Avoid breathing spray  |
|                | P280      | Wear protective gloves/protective clothing.                      |
|                | P302+P352 | IF ON SKIN: Wash with plenty of soap and water.                  |
|                | P333+P313 | If skin irritation or rash occurs: Get medical advice/attention. |
|                | P362+P364 | Take off contaminated and Wash it before reuse.                  |

Further information can be found in the safety data sheet.

### 12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.



**BIBLIOGRAPHY**

Sandhu TS. 2008. *Riemerella anatipestifer* infection, p 758–764 In Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE, editors. (ed), *Diseases of poultry*, 12th ed. Blackwell Publishing, Hoboken, NJ

Subramaniam S, Huang B, Loh H, Tan H-M, Chua K-L, Frey J, Kwang J. 2000. Characterization of a predominant immunogenic outer membrane protein of *Riemerella anatipestifer*. *Clin. Diagn. Lab. Immunol.* 7: 168–174

Sandhu T. 1979. Immunization of white Pekin ducklings against *Pasteurella anatipestifer* infection. *Avian Dis.* 23: 662–669

Higgins DA, Henry RR, Kounev ZV. 2000. Duck immune responses to *Riemerella anatipestifer* vaccines. *Dev. Comp. Immunol.* 24: 153–167

Hu Q, Ding C, Tu J, Wang X, Han X, Duan Y, Yu S. 2012. Immunoproteomics analysis of whole cell bacterial proteins of *Riemerella anatipestifer*. *Vet. Microbiol.* 157: 428–438

**ABBREVIATIONS**

|             |  |
|-------------|--|
| <b>CMIT</b> | 5-chloro-2-methyl-4-isothiazolin-3-one |
| <b>MIT</b>  | 2-methyl-2H-isothiazol-3-one           |

**SUMMARY OF TEST PROCEDURE****SCHEME OF THE ASSAY**  
Riemerella vet ELISA**Test Preparation**














|   |
|---|
| Prepare reagents and samples as described.<br>Establish the distribution and identification plan for all samples and standards/controls.<br>Select the required number of microtiter strips or wells and insert them into the holder. |
|---|

**Assay Procedure**

|   | Substrate Blank (A1) | Negative Control | Cut-off Control | Positive Control | Sample (diluted 1+100) |
|---|----------------------|------------------|-----------------|------------------|------------------------|
| Negative Control  | -                    | 100 µL           | -               | -                | -                      |
| Cut-off Control   | -                    | -                | 100 µL          | -                | -                      |
| Positive Control  | -                    | -                | -               | 100 µL           | -                      |
| Sample (diluted 1+100)  | -                    | -                | -               | -                | 100 µL                 |
| Cover wells with foil supplied in the kit<br><b>Incubate for 1 h at 37±1 °C</b><br>Wash each well three times with 300 µL of Washing Buffer                 |                      |                  |                 |                  |                        |
| Conjugate   | -                    | 100 µL           | 100 µL          | 100 µL           | 100 µL                 |
| <b>Incubate for 30 min at room temperature (20...25 °C)</b><br>Do not expose to direct sunlight<br>Wash each well three times with 300 µL of Washing Buffer |                      |                  |                 |                  |                        |
| TMB Substrate Solution  | 100 µL               | 100 µL           | 100 µL          | 100 µL           | 100 µL                 |
| <b>Incubate for exactly 15 min at room temperature (20...25 °C) in the dark</b>   |                      |                  |                 |                  |                        |
| Stop Solution   | 100 µL               | 100 µL           | 100 µL          | 100 µL           | 100 µL                 |
| Photometric measurement at 450 nm<br>(reference wavelength: 620 nm)   |                      |                  |                 |                  |                        |



## SYMBOLS USED WITH DEMEDITEC ASSAYS

| Symbol  | English                            | Deutsch                                      | Française  | Espanol                                     | Italiano                            |
|---|------------------------------------|--|--|---|-------------------------------------|
|  | European Conformity                | CE-Konformitätskennzeichnung                 | Conforme aux normes européennes                        | Conformidad europea                         | Conformità europea                  |
|  | Consult instructions for use       | Gebrauchsanweisung beachten                  | Consulter les instructions d'utilisation               | Consulte las Instrucciones                  | Consultare le istruzioni per l'uso  |
|  | In vitro diagnostic device         | In-vitro-Diagnostikum                        | utilisation Diagnostic in vitro                        | Diagnóstico in vitro                        | Per uso Diagnostica in vitro        |
|  | For research use only              | Nur für Forschungszwecke                     | Seulement dans le cadre de recherches                  | Sólo para uso en investigación              | Solo a scopo di ricerca             |
|  | Catalogue number                   | Katalog-Nr.                                  | Référence  | Número de catálogo                          | No. di catalogo                     |
|  | Lot. No. / Batch code              | Chargen-Nr.                                  | No. de lot   | Número de lote                              | Lotto no                            |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze                  | Contenu suffisant pour "n" tests                       | Contenido suficiente para <n> ensayos       | Contenuto sufficiente per "n" saggi |
|  | Note warnings and precautions      | Warnhinweise und Vorsichtsmaßnahmen beachten | Avertissements et mesures de précaution font attention | Tiene en cuenta advertencias y precauciones | Annoti avvisi e le precauzioni      |
|  | Storage Temperature                | Lagerungstemperatur                          | Température de conservation                            | Temperatura de conservación                 | Temperatura di conservazione        |
|  | Expiration Date                    | Mindesthaltbarkeitsdatum                     | Date limite d'utilisation                              | Fecha de caducidad                          | Data di scadenza                    |
|  | Legal Manufacturer                 | Hersteller                                   | Fabricant  | Fabricante                                  | Fabbricante                         |
| <i>Distributed by</i>   | Distributed by                     | Vertrieb durch                               | Distribution par                                       | Distribución por                            | Distribuzione da parte di           |
|  | Version                            | Version                                      | Version  | Versión                                     | Versione                            |
|  | Single-use                         | Einmalverwendung                             | À usage unique   | Uso único                                   | Uso una volta                       |