Introduction
Salmonella infections in cattle can cause serious economical and welfare losses in the cattle industry. Infection can be transmitted to humans by the consumption of infected meat or dairy products and cause severe health problems or even death. Infections caused by Salmonella strains belonging to serotypes B, C1 and D are the most frequent occurring and serious infectious. Salmonella Dublin (serotype D) is adapted to cattle and unlike most other types of Salmonella bacteria, has the tendency to persist in herds for decades. Additionally, Salmonella Dublin infections in humans are extremely invasive, and when compared to other Salmonella infection, mortality rate is high. In order to control the infection in infected herds it is necessary to cull the carriers and prevent production of new carriers. In Europe, Salmonella programs to control infections in swine, poultry and cattle have been successfully applied in the control program for Salmonella in Denmark since 2002. The PrioCHECK® Salmonella Ab bovine can be used to specifically detect infections caused by Salmonella serotypes belonging to the B and D group. The test is based on detection of antibodies directed against the O-antigens: 1, 4, 5, 12 and 1, 9, 12 respectively and can be used for large scale screening of (bulk) milk and serum.

Test Principle
The PrioCHECK® Salmonella Ab bovine is an indirect ELISA for the detection of Salmonella antibodies in milk of cattle directed against Salmonella in vitro detection of antibodies against Salmonella in milk of cattle. The PrioCHECK® Salmonella Ab bovine originates from the Danish Veterinary Institute and has been successfully applied in the control program for Salmonella in Denmark since 2002. The PrioCHECK® Salmonella Ab bovine can be used to specifically detect infections caused by Salmonella serotypes belonging to the B and D group. The test is based on detection of antibodies directed against the O-antigens: 1, 4, 5, 12 and 1, 9, 12 respectively and can be used for large scale screening of (bulk) milk and serum.

Kit Components
Store at 5±3°C until the expiry date. See kit label for actual expiry date. The shelf life of diluted, opened or reconstituted components is noted below, where appropriate. Chemical hazard data are available in section “Safety Regulations and R&S Statements” (Appendix II).

Component 1
Test Plate
Five Test Plates.

Component 2
Conjugate (30x)
(30x concentrate, dilute before use) One vial containing 2.2 ml of Conjugate.

Additional Material Required
- Demineralized or water of equal quality must be used for all steps.
- All reagents of the kit must be equilibrated to 22±3°C.
- Chemical hazard data are available in section “Safety Regulations and R&S Statements” (Appendix II).
**INCUBATION WITH CONJUGATE**

2.1 Empty the Test Plate and wash the plate 6 times with 200 to 300 µl of diluted washing fluid. Tap the plate firmly after the last wash cycle.

2.2 Disperse 100 µl of the working solution of the conjugate to all wells.

2.3 Seal the test plate with a plate sealer.

2.4 Incubate the plate(s) for 15 minutes at room temperature (22±3°C).

**INCUBATION WITH CHROMOGEN (TMB) SUBSTRATE**

3.1 Empty the Test Plate and wash the plate 6 times with 200 to 300 µl of diluted washing fluid. Tap the plate firmly after the last wash cycle.

3.2 Disperse 100 µl of the Chromogen (TMB) Substrate (Component 8) to all wells.

3.3 Incubate the plate(s) 15 minutes at room temperature (22±3°C).

3.4 Add 100 µl of the Stop Solution (Component 9) to all wells.

3.5 Mix the content of the wells of the plate(s).

**Note:** Start the addition of stop solution 15 minutes after the first well was filled with the Chromogen (TMB) Substrate. Add the Stop Solution in the same order and at the same pace as the Chromogen (TMB) Substrate was dispensed.

**READING OF THE TEST AND CALCULATING THE RESULTS**

4.1 Measure the optical density (OD) of the wells at 450 nm preferable within 15 minutes after color development has been stopped.

4.2 Calculate the mean OD<sub>450</sub> value of the Negative Control (wells A1 and B1).

4.3 Calculate the mean OD<sub>450</sub> value of the Positive Control (wells E1 and F1).

4.4 Calculate the corrected OD<sub>450</sub> value of the Positive Control, Validation Control and all controls and of the test samples according to the formula below.

The OD<sub>450</sub> of all samples is expressed as percent positivity (PP) of the OD<sub>450</sub> of Positive Control (PC) (wells E1 and F1) corrected with the mean OD<sub>450</sub> of the Negative Control (NC) (wells A1 and B1).

PP = \frac{\text{corrected OD}_{450} \text{ test sample}}{\text{corrected OD}_{450} \text{ Positive Control}} \times 100 - 10

**RESULT INTERPRETATION**

**Validation criteria**

5.1 The mean OD<sub>450</sub> of the Negative Control (wells A1 and B1) must be < 0.4.

5.2 The OD<sub>450</sub> of the Positive Control (not corrected) should be > 1.000.

5.3 The percent positivity of the Validation Control must be ≥ 30.

5.4 Not meeting these criteria is reason to discard the results of that specific test plate.

**Note:** If the OD<sub>450</sub> of the Positive Control (not corrected) is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case pre-warm the solution to 22±3°C or incubate up to 30 minutes.

**Interpretation of the percent positivity**

PP = < 35% (negative)

SALMONELLA-specific antibodies are absent in the test sample.

PP = ≥ 35% (positive)

SALMONELLA-specific antibodies are present in the test sample.

In well-advanced Salmonella control programs the test can be used with a different cut-off. It remains the responsibility of the respective authorities/users to implement such cut-offs.

**Appendix I**

**Notice**

This manual is believed to be complete and accurate at the time of publication. In no event shall PrioChins AG be liable for incidental or consequential damage in connection with or arising from the use of this manual.

**Liability**

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In the event of a breach of the foregoing warranty, PrioChins AG’s sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies PrioChins AG promptly of any such breach. If after exercising reasonable efforts, PrioChins AG is unable to repair or replace the product or part, then PrioChins AG shall refund to the customer all monies paid for such applicable product or part.

PrioChins AG shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by any customer from the use of its products.

PrioChins AG and PrioChins Lelystad B.V. are ISO 9001:2000 certified companies.

**Appendix II**

**Safety Regulations and R&S Statements**

1. **National Safety Regulations must be strictly followed.**

2. **R&S Statements**

   **Component 1**
   
   **Test Plate**
   
   Hazard Code: This product is not classified according to EU regulations.

   **Component 2**
   
   **Conjugate (30x)**
   
   Hazard Code: This product is not classified according to EU regulations.

   **Component 3**
   
   **Dilution Buffer (5x)**
   
   Hazard Code: This product is not classified according to EU regulations.

   **Component 4**
   
   **Washing Fluid (200x)**
   
   Hazard Code: This product is not classified according to EU regulations.

   **Component 5**
   
   **Negative Control**
   
   Hazard Code: This product is not classified according to EU regulations.

   **Component 6**
   
   **Validation Control**
   
   Hazard Code: This product is not classified according to EU regulations.