DATA SHEET

User's Manual

Aeromonas salmonicida

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Aeromonas salmonicida

Antigenic ELISA diagnostic kit for Aeromonas salmonicida.

Introduction

Aeromonas salmonicida **is the bacterial agent causing furunculosis. It affects a large number of fresh- and saltwater fish species. The disease is called furunculosis because it causes furuncles to develop in various tissues of the body. The bacterium was discovered 100 years ago, but it is not known exactly how the disease is transmitted. The disease is seasonal, with acute cases occurring when the water temperature is above 20°C and chronic cases developing at temperatures below 13°C. In the acute form of the disease the fish's colour darkens and the fish stops eating. The viscera are haemorrhagic and the kidney tissue becomes very soft. The spleen dilates and the hepatic parenchyma becomes haemorrhagic and spotted with petechiae. In the chronic form of the disease the mortality rate is lower and more gradual. Furuncles are visible on the fish's skin. The disease can be treated by antibiotics. The diagnosis can be made directly in the field from diseased skin tissue extracts or after isolating the bacterium on selective medium. Of course, the latter method is more sensitive. It nevertheless calls for exact identification of the pathogen by means of a specific test such as ELISA or the agglutination of latex spheres sensitised with specific antibodies.**

Principle of the test

Rows A, C, E and G of the 96-well microtitration plates have been sensitised by specific monoclonal antibody against *Aeromonas salmonicida*. These antibodies capture the corresponding pathogens present in the samples. The other rows on the plate (B, D, F and H) have been sensitised with monoclonal antibody that is not specific for the bacterium. This provides a genuine negative control to determine specific binding on the microtitration plate. Using such a control reduces the number of false positives considerably. The samples are diluted in dilution buffer and incubated on the plate for 1 hour at room temperature. After this first incubation, the plate is washed and the conjugate, a peroxidase labelled anti-*Aeromonas salmonicida* specific monoclonal antibody, is added and the plate is incubated with the conjugate for 1 hour at room temperature. After this second incubation, the plate is washed again and the enzyme substrate (hydrogen peroxide) and chromogen tetramethylbenzidine (TMB)) are added. This chromogen has the two advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If *Aeromonas salmonicida* is present in the sample, the conjugate remains bound to the microwells containing the bacterial antigen and the enzyme catalyses the transformation of the colourless chromogen into a blue compound. The intensity of the resulting blue colour is proportionate to the titre of *Aeromonas salmonicida* in the sample. The signals recorded for the negative control microwells (sensitised with non-specific monoclonal antibody) are subtracted from the corresponding positive microwells (sensitised with specific monoclonal antibody against *Aeromonas salmonicida*). A control antigen is provided with the kit for the purpose of validating the test results. This control antigen is composed of a lyophilised *Aeromonas salmonicida* culture.

Composition of the kit

- **Microplates: Two 96-well microtitration plates (12 X 8 strips). Rows A, C, E and G are sensitised by anti-*Aeromonas salmonicida* specific antibody, while rows B, D, F and H are sensitised by the control antibody (non-specific monoclonal antibody).**
- **Washing solution: One 100-ml bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to room temperature until the disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water. Store the diluted solution at 4°C.**
- **Dilution buffer: One 50-ml bottle of 5x concentrated buffer for diluting samples and conjugate. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. Store the diluted solution at 4°C. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.**
- **Conjugate: One 500-µl vial of anti-*Aeromonas salmonicida* conjugate (horseradish peroxidase-labelled anti-*Aeromonas salmonicida* monoclonal antibody).**
- **Control antigen: 2 vials containing the *Aeromonas salmonicida* control antigen. Reconstitute this antigen with 0.5 ml of distilled or demineralised water. The reconstituted reagent is kept at -20°C. Divide the reconstituted antigen into several portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent can be kept for several months.**
- **Chromogen solution: One 2-ml drop-dispenser bottle of the chromogen tetramethylbenzidine (TMB). This reagent must be kept at 4°C.**
- **Substrate solution: One 30-ml bottle of the hydrogen peroxide substrate solution. Store this reagent at 4°C.**
- **Stop solution: One 15-ml bottle of 1 *M* phosphoric acid stop solution.**

Precautions for use

- **This test may be used for *in vitro* diagnosis only and is strictly for veterinary use.**
- **The reagents must be kept at between 4 and 8°C. The conjugate must be stored as a concentrated solution at 4°C. The positive reference must be stored at -20°C once it has been reconstituted. The reagents cannot be guaranteed if their shelf-life dates have expired or they have not been kept under the conditions described in this insert.**
- **Do not use reagents from other kits.**
- **The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.**
- **Discard all solutions contaminated with bacteria or fungi.**
- **The stop solution contains 1 *M* phosphoric acid. Handle it carefully.**

Procedure

1- **Bring all the reagents to room temperature at least 30 minutes before use.**
2- **Remove the microtitration plate from its wrapper.**
3- **Dilute the samples in the dilution buffer. The dilution must be adapted to the sample (bacterial culture or tissue sample).**
4- **Add 100-µl aliquots of the diluted samples to the wells as follows: sample 1 in wells A1 and B1, sample 2 in wells C1 and D1, etc. Proceed in the same manner for the positive reference (ex.: G1 and H1).**
5- **Incubate the plate at room temperature for 1 hour.**
6- **Rinse the plate with the washing solution, prepared as instructed in the section 'Composition of the Kit', as follows: Empty the microplate of its contents by flipping it over sharply over a sink. Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill all the used wells with the washing solution using a spray bottle or by plunging the plate in a vessel of the right dimensions, then empty the wells once more by turning the plate over above a sink. Repeat the entire operation two more times, taking care to avoid the**

formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.

7- **Dilute the conjugate 1:50 with the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.25 ml of diluent). Add 100 µl of the diluted conjugate solution to each well. Incubate at room temperature for 1 hour.**
8- **Wash the plate as described in Step 6 above.**
9- **Prepare the indicator solution extemporaneously as follows: Add 12 drops (500µl) of chromogen to 9.5 ml of the substrate solution (enough for 1 plate). Pipette onto the plate immediately in aliquots of 100 µl per microwell. The solution must be completely colourless. If a blue colour appears at this stage, this means that the solution has been contaminated with peroxidase. If this happens, the solution must be discarded and a new one made up using perfectly clean glassware and equipment.**
10- **Incubate for 10 minutes at room temperature and protected from the light. This time is given as a guideline only; in some circumstances it may be useful to lengthen or shorten the incubation time.**
11- **Add 50 µl of stop solution to each microwell.**
12- **Read the optical densities in the microwells using a plate reader and a 450 nm filter. The results must be read as soon as possible after the stop solution has been added since the chromogen may crystallise in wells with strong signals and distort the results accordingly.**

Interpreting the results

Calculate the net optical density of each sample by subtracting from the reading for each sample well the optical density of the corresponding negative control. Proceed in the same way for the positive control antigen. The test is validated only if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than the value given on the quality control data sheet attached to the package insert. The limit of positivity for the antigen is 0.150. Any sample that yields a difference in optical density that is greater than or equal to 0.150 is considered positive. Conversely, any sample that yields a difference in the optical density that is less than 0.150 is considered negative.