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Veterinary Medical Research & Development

BLUETONGUE VIRUS ANTIBODY TEST KIT

VLN/PCN 332/5010.00

Assay Instructions for Catalog Number: 288-100

General Information

This bluetongue virus immunodiffusion (ID) test detects precipitating antibodies to bluetongue virus in sera of ruminants. Antibodies to epizootic hemorrhagic disease virus are also detected. Test sera, if positive, will form a line that fuses with positive reference lines or that deviates the positive reference lines inward near the test serum well without formation of a visible line. Negative sera will neither form a line nor cause deviation of the positive reference lines.

Kit Contents

1 of bottle A . . . . . Bluetongue Virus Antigen, 0.75 ml
1 of bottle R . . . . . Positive Reference Serum, 2.15 ml
These reagents are sufficient to test up to 100 samples.

Materials Required but not Included in the Test Kit

Hot plate or microwave oven, balance, 250 ml flask, laboratory pipettes, refrigerator, standard gel cutter, vacuum pump or water-driven filter pump, micropipettor, plastic food container, high-intensity narrow-beam light source, 45°C waterbath (desirable, but not required), agarose (Sigma 05066 or equivalent), NaCl, distilled or deionized water, 60 x 15 mm or 100 x 15 mm plastic petri plates or 45 x 90 mm plastic tray.

Storage and Stability

Do not use components from other kits. Store all reagents at 2-8°C. **Do not freeze.** Reagents will remain stable until the expiration date when stored as instructed. **Do not use test kit past the expiration date printed on the box.** Use clean equipment to avoid contamination of reagents.

Preparation of Agar Gel

1. Make a 0.9% suspension of agarose in a solution of 0.85% NaCl in distilled or deionized water.
2. Dissolve the agarose completely by microwaving or heating on a hotplate.
3. Cool solution to 45°C and then transfer 6 ml of solution into 60 x 15 mm plastic petri plates, or 15 ml into 100 x 15 mm plastic petri plates, or 11 ml into 45 x 90 mm plastic trays.
4. Cool the agar at room temperature in a relatively dust-free environment. Remove the lids during cooling to permit the escape of water vapor.

Cutting Wells in the Agar Gel

A seven-well pattern is used with one center well and six wells in a circle around it. The wells are 4 mm in diameter and 1.8 mm apart. Agar is cut the same day it is poured, but should be sufficiently hardened so that the cut edges of the wells do not break down when agar plugs are removed. [Hardness of the agar may be increased by placing the plates (with the lids replaced) in a refrigerator upside down for an hour or two before cutting.] The agar plugs are suctioned from the wells using a metal or glass cannula drawn to a small opening (1-2 mm in diameter) and connected to a vacuum line. Care should be taken to avoid separating the agar from the plastic when removing the agar plugs. If moisture is observed in the wells prior to introduction of reagents or samples, it should be removed by suction. Agar plates should be used the same day they are cut.

Filling the Wells and Incubation of the Agar Plates

Place 20 µl of Bluetongue Virus Antigen (A) in the center well and 20 µl of Positive Reference Serum (R) in alternating peripheral wells, leaving three empty wells for samples (Figure 1). Place 20 µl of each sample into empty alternating wells. This arrangement provides a positive control line on each side of the test serum, thus facilitating accurate determination of lines of identity. Fill wells level with the agar surface, leaving no meniscus. Serum or antigen must not run on top of the agar. Allow the plates to set a few minutes before moving to reduce the possibility of spillage. Place the plates in a closed humid chamber and incubate at 23 ± 2°C.

Test Validation

For a test to be valid, the reference lines (between Positive Reference Serum wells and the center Bluetongue Virus Antigen well) must be easily visualized. These lines should be clearly visible right to the edge of negative sample wells. If not, the assay cannot be accurately interpreted.

Interpreting the Results

The test may be read at 24 hours of incubation and can be reread at 48 hours to confirm the results, especially when weak positive reactions are observed. The precipitin lines are most easily visualized using an intense narrow beam of light. It is also very helpful to read the plates against a dark background in reduced lighting conditions.

The following types of reactions are observed:

1. **Negative:** The reference lines continue straight into the test sample well without bending (Figure 1, S1, negative sample).
2. **Positive:** Control lines join with and form a continuous line with the line between the test serum and antigen (A) (Figure 1, S2, positive sample).
3. **Weak Positive:** The reference positive control lines bend slightly toward the antigen well (A) and away from the Positive Reference Serum wells (R) but do not form a complete line between antigen (A) and test serum (Figure 1, S3, weak positive sample). These reactions require careful observation and can be easily overlooked. All weak positive samples should be retested before reporting the results.
4. **Very Strong Positive:** The reference positive control lines turn toward the antigen well before they reach the well containing the test serum and there is a broad, hazy line between the test serum and antigen (A) (Figure 2, S1, strong positive sample). This line is situated very near the antigen well (A), especially if the plate is observed at 24 hours.
5. **Non-Specific Lines:** These lines are observed between the antigen and test serum well. However, the reference positive control lines will pass through the non-specific line and continue on into the test serum well of negative sera (not shown). The non-specific line does not form a continuous line with the reference positive control lines. The reference positive control lines will form more acute angles with a non-specific line than with a BTV-specific line of identity. The non-specific lines are formed by sample-antibody reactions with antigens other than BTV. A sample serum may produce a specific BTV line as well as a non-specific line (Figure 2, S2, positive sample with non-specific line). Care must be taken to be certain a specific reaction is not obscured by a non-BTV line. Retesting such samples and observing the reactions at frequent intervals may facilitate making determinations if the samples are positive or negative.
6. **Haze Around Well:** Occasionally a haze, due to lipids or other material in the serum, will form around the test serum well that may obscure the reference positive control lines near the sample well (Figure 2, S3). If the test is read at 24 and 48 hours, sometimes the results can be determined before the haze obscures the reaction. However, in some cases a determination cannot be made and another sample should be requested.

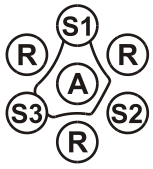

Figure 1.

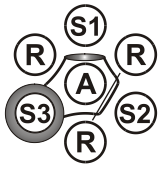

Figure 2.

Antibody-positive results indicate previous or current infection and/or immunization with bluetongue virus and/or infection with epizootic hemorrhagic disease virus. A negative result may indicate no previous or current infection with bluetongue virus but, because it takes several weeks from initial infection for precipitating antibodies to be detectable, retesting may be desirable to confirm negative results. Persistence of detectable precipitating antibodies is usually of long duration. However, cattle have been known to become seronegative in the AGID test after a few weeks.

Precautions

Kit components should be handled and disposed of as potentially hazardous. Do not eat, drink, or smoke where serum samples and kit reagents are handled. Do not pipette by mouth. Some reagents may be harmful if ingested. If ingested, seek medical attention. Do not use expired or contaminated reagents, or reagents from other kits or serials. Do not mix reagents from different serials of this same product.

Component A, Bluetongue Virus Antigen, contains sodium azide as a preservative.
Component R, Positive Reference Serum, contains sodium azide as a preservative.

Version 211202

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