General Information

This agar gel immunodiffusion (AGID) test, also known as the Coggins-test, detects anti-EIAV p26 precipitating antibodies in equine serum and plasma.

Description and Principle

The agar gel immunodiffusion (AGID) test is a method whereby antigen and antibody diffuse toward each other in a semisolid medium to a point where the optimum concentration of each is reached. A band of precipitation occurs at this point.

In this test:

- Agar gel is cast and equidistant wells are cut out in agar.
- Recombinant EIAV p26 antigen is placed in the central well. The positive control and the samples to be tested are placed in the peripheral wells.
- After diffusion, EIAV p26 antigen–anti-p26 antibody complexes lead to the formation of precipitates (a whitish line visible to the naked eye).

After 24-48 hours, plates are examined:

- Samples are considered positive if a precipitation line forms with the antigen.
- Samples are considered negative if a precipitation line does not form with the antigen.

Digitally signed by Cucu Vasile
Date: 2024.03.29 11:30:49 EET
Reason: MoldSign Signature
Location: Moldova

Kit Components

Reagents for 200 tests	
Recombinant EIAV-p26 antigen	3.4 ml
Positive control	10 ml

This kit may be purchase with or without agar gel included (product codes: EIA-AGID and EIA-AGID-NOGEL, respectively).

The Antigen and the Positive control must be stored at 5°C (± 3°C) until the expiry date, or indefinitely at -20°C.

Materials required but not provided

1. Seven-well cutting tools. (Wells must be 2.4 mm apart and 5.3 mm in diameter.)
2. Mono micropipettors capable of delivering volumes of 50 µl, and 200 µl.
3. Disposable tips.
4. Hot plate, autoclave or microwave oven.
5. Balance and 250 ml flask.
6. High-intensity narrow-beam light source.
7. 100 mm diameter plastic petri plates.

Precautions

1. Do not pipette by mouth.
2. Do not use components from other kits.
3. Reagents contain sodium azide.
4. All single-use material used for the assays should be decontaminated by immersion in freshly prepared 5% sodium hypochlorite for minimum 1 hour before elimination, or by autoclaving at 120°C.

Agar gel

If not provided by IDvet (product code: EIA-AGID-NOGEL), prepare your own Agar gel as follows:

1. Borate buffer:

   Mix:
   - 2g NaOH
   - 9g Boric Acid (H_3BO_3)
   - 1L distilled water

   Adjust the resulting pH to 8.6 ± 0.2.

2. Prepare a 1% solution of Agar gel in the borate buffer and dissolved by either of two methods:
   - Boil the suspension to dissolve the agar and autoclave for seven minutes OR
   - Microwave agar solution for a total of 3 minutes at 30 second intervals or until agar dissolves.
3. Cool the agar to 45-50°C.
4. Add 15 ml of agar to a 100 mm diameter Petri dish.
5. Cool plates for 1 hour at room temperature 21°C (± 5°C). Store at 5°C (± 3°C). If uncut, plates can be stored for up to one week.

Testing Procedure

1. A seven-well pattern is used with one centre well encircled by 6 wells. The wells are 2.4 mm apart and 5.3 mm in diameter.
2. The day the agar is to be used, cut out wells when the agar is cold and hardened. Remove the agar plugs and leave lids ajar for 30 minutes before adding reagents and serum samples. Any remaining moisture in the wells should be suctioned out or allowed to evaporate.
3. Allow all reagents to come to room temperature 21°C (± 5°C) before use. Homogenize all reagents by inversion or Vortex.
4. Add:

- 50 µl of **recombinant EIAV-p26 antigen** to the centre well (see Figure 1).
- 50 µl of the positive control to wells 1, 3 and 5 (see Figure 1)
- 50 µl of each sample to be tested to the remaining wells 2, 4 and 6 (see Figure 1).

Note: testing of severely haemolysed or contaminated samples is not recommended. Empty wells must be filled with negative serum or isotonic NaCl solution (not PBS).

5. Incubate plates for **24 - 48 hours** at 21°C (± 5°C) in a moist chamber.

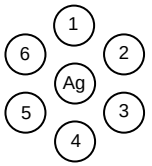


Figure 1

Reading the test

Use a narrow beam of intense, oblique light for reading. It should be adjustable for varying intensities and positions.

The reaction should be observed against a black background. A magnifying lens is helpful in some cases. Viewers made for observing stained immunodiffusion reactions are not suitable for reading this test.

Test Validation

The reference lines between the positive control and the antigen should be clearly visible up to the edge of negative serum wells.

Interpretation

Negative - Reference lines continue into the test sample well without bending, or with a slight bend away from the antigen well and toward the positive control (Figure 2, serum N°6, negative sample).

Positive: Reference lines join with and form a continuous line with the line between the test serum (serum N°2) and the antigen (Figure 2).

Weak positive – Reference lines bend slightly towards the antigen well and away from the positive control wells, but do not from a complete line between the antigen and the test serum (Figure 2, serum N°4).

Strong Positive: The lines turn toward the antigen well before they reach the well containing the test serum (serum N°10) and there is a broad, hazy line between the test serum (serum N°10) and antigen (Figure 3). This line is situated very near the antigen well (Ag).

Non-Specific Test Serum Lines: For a negative serum, the reference lines pass through the non-specific line and into the test serum well. For a positive serum, the non-specific line does not form a continuous line with the reference lines.

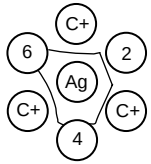


Figure 2

C+: Positive control

2: **Positive serum**

4: **Weak positive serum**

6: **Negative serum**

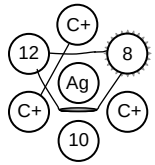


Figure 3

C+: Positive control

8: **Negative serum with halo**

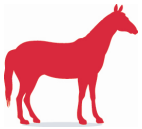
10: Strong positive serum

12: **Non-specific line**

IDvet
Innovative Diagnostics

Certified management system

IDvet EIA AGID



Agar gel immunodiffusion (AGID) test for the detection of EIA anti-p26 antibodies in equine serum and plasma

200 tests

February 2015:

- Change in the cooling temperature of the agar

September 2014:

- Empty wells should be filled with isotonic NaCl solution or negative serum (not PBS)

EIA-AGID / EIA-AGID-NOGEL ver 0215 GB

IDvet, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com