

General Information

Agar gel immunodiffusion (AGID) test for the detection of Bovine Leukosis Enzootique (BLV) anti-gP51 antibodies in bovine serum and plasma.

Description and Principle

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

The antigen contains the BLV glycoprotein gP51.

Agar gel* is cast and equidistant wells are cut out in agar.

BLV antigen is placed in the central well, positive control and samples to be tested are placed in the peripheral wells.

After diffusion, the complex BLV antigen – anti-gP51 antibodies leads to the formation of precipitates presented in the shape of a visible whitish line by the naked eye.

Plates are examined:

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

* IDvet also offers a kit without agar gel, product code: BLV-AGID-NOGEL.

Kit Components

Reagents*	
BLV antigen	2 ml
Positive control	3 ml
Agar gel	200 ml

* Quantities supplied are indicated on the kit label.

- **Freeze-dried antigen:** store at +5°C (± 3°C) in the dark until expiry date.
- **The reconstituted antigen:** store for 3 days at +5°C (± 3°C) or until the expiry date at ≤ -16°C.
- **Freeze-dried serum:** store at + 5°C (± 3°C) in the dark until expiry date.
- **The reconstituted serum:** store for 3 days at +5°C (± 3°C) or until the expiry date at ≤ -16°C.
- **The agar gel** may be stored between +2°C / 26°C until expiry date.

For storage at ≤ -16°C, it is recommended to aliquot the antigen and the reconstituted serum in order to avoid multiple freezing thawing cycles. **An aliquot must not undergo more than 3 freezing-thawing cycles.**

It is recommended not to conserve the reconstituted serum more than four days at +5°C (± 3°C).

Material required but not provided

1. Mono micropipettors capable of delivering volumes of 10 µl and 100 µl.
2. Disposable tips.
3. Hot plate, autoclave or microwave oven.
4. Balance and 250 ml flask.
5. Standard gel cutter for BLV-AGID.
6. 85 mm-diameter Petri dish.

Precautions

1. Do not pipette by mouth.
2. Do not use components from other kits
3. Reagents contain sodium acid.
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.

Preparation of Agar gel provided by IDvet

1. Melt the agar gel in a water bath or a microwave oven until the gel becomes perfectly liquid and limp (approximately 30 minutes).
2. Let cool to approximately 45-50°C in order to avoid strong evaporation when the gel medium is poured in Petri dishes.
3. Pour 25 ml of the agar gel into an 85 mm-diameter Petri dish. The dish should be maintained perfectly horizontal. It is possible to adjust the volume of gel used per dish. However, ensure that enough gel is used to prevent overflowing of the wells containing the antigen or positive control. Do not move the dishes until the agar gel solidifies completely by a return to room temperature. Dishes containing the agar gel can be conserved for 2 days at +5°C (± 3°C) in a humid atmosphere and stocked upside-down. It is however preferable to prepare them the night before or the day of their use.

It is also possible to work with Petri dishes of different sizes. Adjust the volume of gel used in order to avoid overflowing of the antigen or serum wells.

Testing Procedure

Cut out wells in the Agar

Using a cutter for BLV-AGID, cut out 7 wells in the agar gel according to the diagram shown in Figure 1.

Remove the small cylinders of gel cut-out from the agar gel.

Reconstitution of freeze-dried components

1. **BLV antigen:** reconstitute in 2 ml of distilled water.
2. **Positive control:** reconstitute in 3 ml of distilled water.

The reaction

3. Add:
 - 32 µl (± 5%) of **BLV antigen** in the central well.
 - 73 µl (± 5%) of **positive control** in wells 1 and 4.
 - 73 µl (± 5%) of **each samples to be tested** in wells 2, 3, 5, 6.

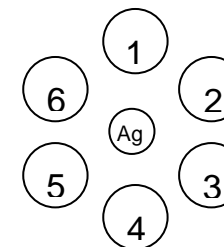


Figure 1: Arrangement of wells in the agar gel.

4. Place the dishes in a humid enclosure at +21°C (± 5°C).

Test interpretation

The test can be read after 24 hours or after 48 hours, but no final results can be obtained before 72 hours.

Validation

The positive control provided in the kit should react by forming a precipitation line with the BLV antigen (see Figure 2).

Interpretation (see Figure 2)

1. **Negative** – a serum is negative if it does not produce a specific precipitation line with the BLV antigen, and if it does not deflect upon the line of the positive control (Figure 2, serum N° 6).
2. **Positive** – a serum is positive if it forms a specific precipitation line with the BLV antigen, and if this line is situated in the prolongation of that of the positive control (Figure 2, sera N° 2 and N° 3).
3. **Weak positive** – control lines bend slightly towards the antigen well and away from the positive control well, but do not form a complete line between the antigen and test serum (Figure 2, serum N°5).
4. **Non-conclusive** – the reaction is only considered non-conclusive if:
 - a. a double line forms (Figure 3, serum N°6).
 - b. if the axes cut each other (Figure 3, serum N°2).
 - c. It is not possible to interpret as negative or positive.

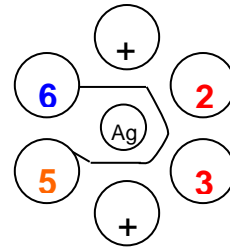


Figure 2: Result interpretation.

- +: Positive control
- 2: **Positive serum**
- 3: **Positive serum**
- 5: **Weak positive serum**
- 6: **Negative serum**

Example of non-conclusive reaction:

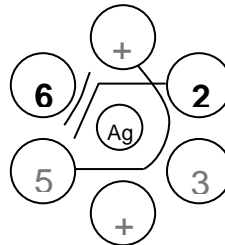


Figure 3: Non-conclusive results interpretation.

- +: Positive control
- 2: **Non-conclusive reaction**
- 3: Positive serum
- 5: Negative serum
- 6: **Non-conclusive reaction**

IDvet BLV AGID



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240 tests

February 2015:

🔔 Change in the cooling temperature of the agar

BLV-AGID ver 0215 GB