

BOVINE LEUKOSIS AGID

SEROLOGICAL DIAGNOSIS OF ENZOOTIC BOVINE LEUKOSIS

1. GENERALITIES

Cattle affected by enzootic Bovine Leukosis (EBL) may be carriers of the virus without presenting any apparent clinical symptoms. Nevertheless, all infected animals, sick or not, develop virus-specific antibodies.

These antibodies generally appear 8 to 12 weeks after experimental infection. A negative serology test indicates that the animal has not been infected in the 12 weeks preceding the sampling.

The AGID bovine Leukosis immunodiffusion test reveals the presence of precipitating viral envelope glycoprotein (gp51) antibodies and to a lesser extent, the internal protein (p24) antibodies.

2. KIT PRESENTATION

One kit for 400 serodiagnosis contains :

- two vials of freeze-dried antigen to be reconstituted with **1.6 ml** of diluent,
- one 5 ml vial of diluent for antigen reconstitution.
- three vials of 'ready-for-use' positive reference serum (RS),
- five 100 ml vials of agar for immunodiffusion. This media has precise values for pH and ionic force, allowing optimal immunodiffusion and/or precipitation.

3. TECHNIQUE

3.1 Materials (not furnished)

- Plate support : Standard Petri plates (90 mm diameter) in glass or plastic;
- Agar-cutter, made of 7 cutting cylinders arranged in circle in order to cut in the agar :
 - . one 4 mm diameter central well,
 - . six 6 mm diameter peripheral wells,
 - . (3 mm distance between the edges of peripheral and central wells),
- Adjustable micropipette 20-100 μ l (with disposable tips).

3.2 Agar preparation

- The vial of agar is placed in a boiling water bath for 1.5 hrs.
Once melted, the agar should be placed in a $+56^{\circ}\text{C} \pm 3^{\circ}\text{C}$ water bath for at least 30 min.
- The glass or plastic support plate is placed on a flat horizontal surface.
- With the aid of a pre-heated pipette, distribute the agar in sufficient quantities in order to have a thickness of at least 2.6 mm. (19 ml of agar is needed for a standard 90 mm diameter Petri plate). Let the agar cool and solidify before cutting the wells. The solidification can be sped up by placing the plate at $+5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for one hour after distribution of the melted agar.
- Using the agar-cutter, cut the 7 wells in the agar as shown in diagram n°1. Remove the cut agar cylinders by suction.

3.3 Freeze-dried antigen reconstitution

- Reconstitute the freeze-dried antigen with 1.6 ml of diluent.

3.4 Reaction

Once the wells have been numbered as indicated in diagram n°2, using a micropipette with disposable tip, place in the following order :

- 73 µl of individual sera to be tested in wells 2, 3, 5 and 6 ;
- 73 µl of positive reference serum (RS) in wells 1 and 4 ;
- 32 µl of reconstituted BLV antigen in the central well.

Place the support plate in damp or humid atmosphere at room temperature ($+20^{\circ}\text{C} \pm 5^{\circ}\text{C}$) for at least 72 hours shielded from light.

3.5 Remarks

3.5.1 Use **one sterile pipette tip per reagent and per serum** in order to avoid contamination.

3.5.2 Freeze any unused reconstituted antigen at -20°C .

3.5.3 Avoid leaving the reagents at room temperature while performing the test.

3.5.4 Materials and reagents should be handled with the usual precautions, and all the used materials must be decontaminated.

3.5.5 It is recommended to prepare the agar plates either the day before or the actual test day.

3.6 Reading the Results

According to the Official Journal of the European Union (Directive 88/406, lines A-9) : The results can be read after 24 hours, then at 48 hours, but no final result can be obtained until after 72 hours.

a) A tested serum is positive if it forms a precipitation curve specific to the viral antigen for Bovine Leukosis and this curve coincides with the control serum curve ;

b) A tested serum is negative if it does not give a precipitation curve specific to the viral antigen for Bovine Leukosis and it does not bend the control serum curve ;

c) The reaction cannot be interpreted conclusively :

- (i) if the serum control curve is bent towards the well containing the Bovine Leukosis viral antigen without forming a visible precipitation curve with the antigen or,
- (ii) if it is not possible to interpret as either positive or negative. For non-conclusive reactions, repeat the test using concentrated serum."

The plates should be read on a black background with intense side-lighting. Test evaluation takes into account the presence of lines as well as the linking or non-linking to the precipitation lines obtained between the BLV antigen and the positive reference serum (RS) (See diagrams).

3.7 Specific cases

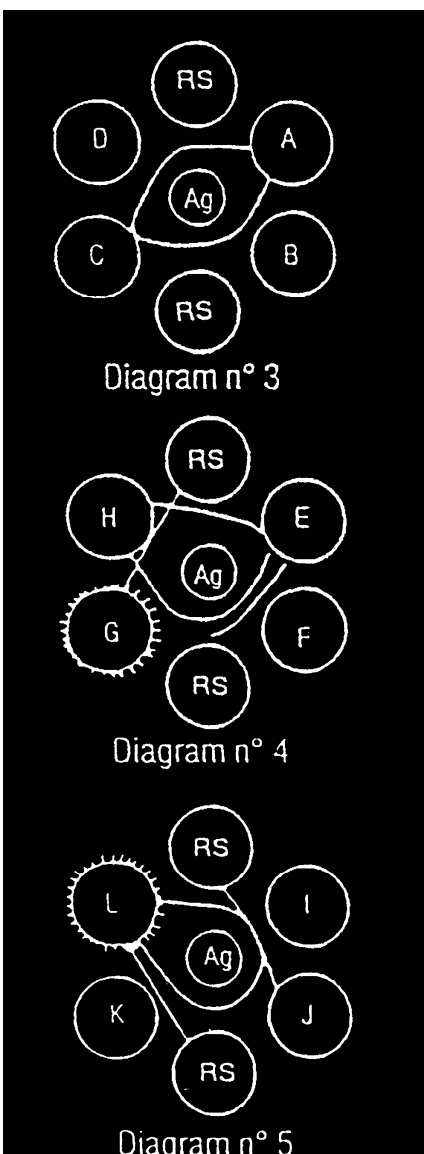
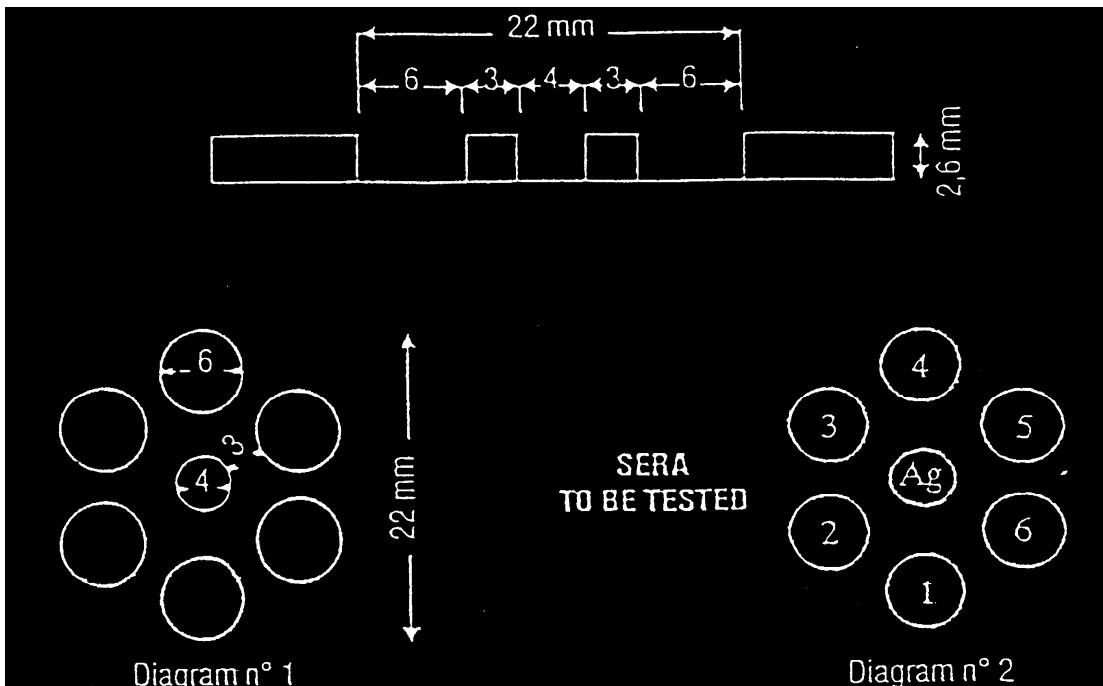
- 3.7.1 If there is no precipitation line visible between the antigen and the positive reference serum (RS), the test must be repeated.
- 3.7.2 When the reference line bends clearly towards the well containing the antigen and becomes tangent to the well(s) containing the serum to be tested, the latter is slightly positive (diagram 3, serum C). The detection of slightly positive serum is delicate : the bend of the reference line should be distinct. It is very often necessary to repeat the test before asserting that such serum is positive (diagram 4, serum E).
- 3.7.3 When the precipitation lines between the serum to be tested and the antigen appear and cut the reference line, this is a case of **non-specific precipitation lines**, which may hinder the reading of the precipitation line. Thus, despite the presence of this non-specific precipitation :
- serum K (diagram 5) is positive,
 - serum H (diagram 4) is negative.
- 3.7.4 Some positive sera may develop a second specific precipitation line corresponding to the p24 protein (diagram 4, serum F ; diagram 5, serum I).
- 3.7.5 A halo, caused by lipids and lipoprotein in the serum, may appear around a well and therefore hinder the reading of the precipitation lines. In this case :
- serum L (diagram 5) is negative,
 - serum G (diagram 4) is positive.
- If necessary, the test should be repeated.

NOTE : due to the presence of maternal antibodies absorbed from the colostrum, it is not advised to serologically test for Bovine Leukosis in calves of less than 6 months of age.

4. STORAGE

Store the kit between +5°C ± 3°C, shielded from light.

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INTERPRETATION OF THE RESULTS

RS: Positive reference serum with one precipitation line (gp51)
 A: Negative serum
 B & D: Positive sera
 C: Slightly positive serum

RS: Positive reference serum with one precipitation line (gp51)
 E: Doubtful serum
 F: Positive serum with two precipitation lines (p24 and gp51)
 G: Positive serum with halo
 H: Negative serum with non specific precipitation line (not related to the reference serum)

RS: Positive reference serum with one precipitation line (gp51)
 I: Positive serum with two non-disassociated precipitation lines (p24 and gp51)
 J: Positive serum
 K: Positive serum with one non-specific line
 L: Negative serum with halo