



RADIAL IMMUNODIFFUSION TEST FOR EQUINE IgG

The **RID** test is a means of quantifying the immunoglobulin G (IgG) in the equine species, and is especially useful in newborn foals for diagnosis of Failure of Passive Transfer and Partial FPT. The test is specific for various proteins in serum or other body fluids, and it depends on the reaction of each protein with its specific antibody, which is impregnated in the agar gel on the plate.

There are 24 test wells in each plate, into each of which is placed 5 microlitres of test solution.

RID is based on the diffusion of antigen (IgG) from a circular well, radially into a homogeneous gel containing specific antiserum for Equine IgG. Where the IgG and its antibody combine in the gel, an area of precipitation, which shows as a white opacity, forms around the well, and continues to grow until equilibrium (end point) is reached. The diameters of the rings are therefore a function of concentration of IgG. If equilibrium is reached (after 24 hours), the precipitation zone diameters are squared and plotted against their concentration on the straight line (best fit) plot, using the control solutions of known concentrations of IgG. If a result is required more urgently, say after 8 hours, the precipitation zone diameters of the test sera/plasma are plotted against the logarithm (base 10) of the line established using the known concentrations of the control solutions. At intervals in between, a linear relationship does not occur.

Serum or plasma may be used in this test. If plasma is used, a non-specific precipitation of fibrin may obscure the precipitation rings. In addition, the anticoagulant used in the production of that plasma will dilute the specimen to some degree.

RESULTS:

- Insurance companies insist on levels of greater than 8g/L (800mg/dL) before they will insure foals at 24 hours. Such levels are statistically associated with minimal incidence of disease within the first 2 months of life
- Blood levels of 4-8g/L are statistically associated with increased incidence of disease in the first 2 months of life – **transfusion with one bag of EQUIPLAS® will be of assistance**
- Blood levels of 2-4g/L are classified as Partial FPT – **transfusion with 1-2 bags of EQUIPLAS®Plus may be necessary to achieve blood IgG levels of 8g/L**
- Blood levels of 0-2g/L are classified as FPT – **transfusion with 2 bags of EQUIPLAS®PLUS may be necessary to achieve blood IgG levels of 8g/L**

The RID test is the most accurate estimation of immunoglobulin G (IgG).



HANDY HINTS:

1. Use all of the control solutions (there are usually 3) when running tests on a new plate for the first time.
2. Use only ONE control solution when running subsequent tests. If its diffusion ring is the same diameter as the one on the first test, read the concentration/s of the test solution/s from the graph established on that first test. In this way, up to 11 individual tests can be run on the one plate. However 21 tests can be run if there are enough test sera to test, at that time.
3. Run standard tests for 24 hours minimum. Read this result from plotting the control solution diffusion ring diameters (squared) onto standard graph paper. Plot the (known) concentrations of the control solutions on the X axis, and the diameter of diffusion squared on the Y axis.
4. For urgent tests, run them for as little as 8 hours, and read using logarithmic graph paper; drawing the line of "best fit" between the control solution plots, using the curved rulers that mathematicians use (Shipp's curves) to draw this line.
5. Double or even triple layers of diffusion can appear, and are quite normal. They simply mean that the IgG's have separated into one or more of their three subclasses – IgGa, IgGb, and IgGc. Measure to the outermost ring to achieve the most accurate results. These subclasses are closely related and difficult to analyze separately.
6. If FPT is suspected, use the low IgG RID plates, which measure IgG levels of 80-800mg/dL.
7. A gammaglobulin aemic foals may need 2 bags of EQUIPLAS®PLUS to achieve blood levels of 8g/L IgG.
8. Never freeze the plates (the gel takes on a granular appearance).
9. Never use them if they have dried out (the gel shrinks away from the edges of the dish).
10. Always fill the wells with the control solutions at the same time as the test solutions.
11. Always perform the test when the plate and reagents have been allowed to warm to room temperature (approximately 20 degrees C).
12. Where the room temperature is colder:
 - allow the test to run for a longer period; or
 - run the test in an insulated container or incubator at 20 to 25 degrees C.
13. Always store the plate in the refrigerator, upside down, so the ridges in the plate can be felt on the top.
14. Remove the lid from the plate and allow to dry, if moisture droplets can be seen inside the lid, before performing a test.
15. Use a 5 microlitre pipette to fill the wells, rather than merely filling the wells to the top, which is less reliable.
16. Always place leftover control solutions into your sharps container for disposal, as they are biological fluids and should be destroyed.

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