TRIPLE J FARMS Product #628411
BELLINGHAM, WA

Radial Immunodiffusion Test
For Quantitation of Camelid IgG
In Serum or Plasma

ATTENTION

Plate must be refrigerated and stored upside down. Also avoid near freezing temperatures. Accuracy can only be assured with proper storage.

1. Summary

Single radial immunodiffusion tests have evolved from the work of Fahey and McKelvey[1] and Mancini et al[2]. They are specific for the various proteins in serum or other fluids and depend on the reaction of each protein with its specific antibody.

When the wells in antibody containing gels are completely filled with the antigen, the precipitin rings that develop after 10-20 hours at room temperature are measured. The diameter of the ring and the logarithm (base 10) of the protein concentration are related in a linear fashion. Using appropriate reference standards, the concentration of unknown samples may be measured.

Immunoglobulin G (IgG) is one of the first line of defenses against encapsulated bacteria and streptococci. The majority of the newborns IgG is obtained from the dam's colostrum in the first 16 hours after birth providing the cria nurses. This is called passive transfer. In passive transfer the IgG from colostrum provides antibodies to infectious agents that the dam has been exposed to or immunized against. The time it takes IgG to drop to half it's original titer in mammals ranges from 20 to 30 days. The cria can start producing its own IgG in sufficient quantities after 30 to 90 days.

2. Principle

Radial immunodiffusion is based on the diffusion of antigen from a circular well radial into a homogeneous gel containing specific antiserum for each particular antigen. A circle on precipitated antigen and antibody forms, and continues to grow until equilibrium is reached. The diameters of the rings are a function of antigen concentration. After overnight incubation, the zone diameters of reference sera are plotted against the logarithm (base 10) of the antigen concentration. If equilibrium is reached, the reference sera zone diameters are squared and plotted against their concentration (linear). At intervals in between, a linear relationship does not occur. Unknown concentrations are measured by reference to the standard curve.

3. Reagents

A. Radial immunodiffusion plates contain specific antiserum in agarose gel, 0.1M phosphate buffer pH 7.0, 0.1% sodium azide as bacteriostatic agent, l ug/ml amphotericin B as an antifungal agent. Plates also contain 0.002M ethylenediaminetetraacetic acid. Store at refrigerator temperatures (2 to 8 C).

B. Camelid Reference sera -- (Pooled llama serum at four levels*). Contains sodium azide (0.1%) as bacteriostatic agent. Store at refrigerator temperature.

4. Specimen Preparation And Handling

A. Collect whole blood without anticoagulent and allow to clot at room temperature.

B. Separate serum by centrifugation at about 200 rcf within 2-3 hours after collection.

C. Plasma may be used, but non-specific precipitation of fibrin may obscure precipitation rings. In addition, liquid anticoagulents such as ACD fluid will dilute the specimen.

5. Procedure

A. Materials Provided

1. One Radial Immunodiffusion plate.
2. Reference Sera: 3 x.2 mls.
3. Directions for use.

B. Materials Required

1. Blood collection tubes
2. Centrifuge (200rcf)
3. Microliter dispenser (5 microliters)
4. Normal control sera (optional)
5. Measuring device -- calibrated in 0.1mm increments
6. Two cycle semi-logarithmic graph paper and/or linear graph paper.

C. General

1. Do not overfill or underfill wells. An improperly filled well yields erroneous results and the same specimen should be placed in another well. Overfilling with a 5 microliter sample indicates that some gel shrinkage has occurred.
2. Reference serum zone diameters should be measured at the same time as test sera. If a delay in measurement is anticipated allow sufficient intervals between filling wells.
3. The time of filling each plate should be marked on the cover and if more than one plate is filled, they should be read in order of filling.
4. Excess moisture is required to prevent drying. Replace each plate in its plastic bag and reseal carefully before incubation.
5. Shrinkage of gel or oval shaped wells indicate drying and the plate should not be used.
6. If temperature fluctuations are anticipated, the plates in their bags may be incubated in an insulated container. Fluctuations in temperature may result in multiple precipitin ring formation.
7. Unused sections may be run at a later day if the plate has been stored at 2 to 8 C between incubations in its plastic bag. Check carefully for evidence of drying. Store upside down.
8. Rough granulation of the gel indicates freezing, plates should be discarded.

D. Performance Of Test

1. Remove plates from refrigerator to room temperature approximately 30 minutes before filling wells. Do not open bag until ready for use.
2. If excess moisture is present, remove plate from bag and remove cover until evaporation has dried the surface and wells. Replace cover until used.
3. For best results, three wells should be filled with reference sera for each plate. Location of each should be noted. Mix each vial of reference serum thoroughly.
4. Deliver specimen to well by placing the pipette tip at the bottom of the well. Allow the well to fill to the top of the agar surface. Avoid bubbles to ensure proper volume and diffusion of sample. Visualization may be aided by placing the plate on dark background. If practice is required, a used plate may be utilized.
5. More consistent results are obtained when wells are filled with a 5 microliter pipette.
6. Mark time of completion on plate cover and replace cover.
7. Replace plate in bag and reseal carefully.
8. Incubate plates upright on a flat surface at room temperature (20 24 C) for 8-10 hours for Overnight readings and over 24 hours for End Point readings. See C6 above.

E. Calibration

1. Using the reference sera provided in kits determine their ring diameters to the nearest 0.1mm.
2. Using 2 or 3 cycle semi-logarithmic graph paper, plot the concentration on the Y axis and the zone diameters on the linear or X axis for each protein for Overnight readings.

3. Using regular graph paper, plot the concentration on the X axis and the zone diameters squared on the Y axis for each protein for End Point readings.

4. Draw a straight line of "best fit" between the three points. A curved line usually indicates that the incubation time and\or temperature should be reduced for overnight values. For valid results, a smooth curve should be fitted to the points and control sera included for additional verification.

F. Quality Control

For consistent results and a comparison of lot to lot, day to day, and week to week variations, a "normal" and abnormal serum should be included each day. The diameters and concentrations obtained can be charted to determine means and standard deviations. For the same specimen, an appropriate series of wells on the same plate should yield diameters within 0.2mm of one another. Control sera should be freshly thawed or reconstituted.

G. Reference Sera

All reference sera supplied have been calibrated from two Standard sera. The Standard Sera were calibrated against the appropriate purified proteins.

6. Results

Determine the concentration of each unknown of specimen protein by reading its zone diameter on the reference curve and the corresponding concentration from the X axis. Zone diameter must be squared for End Point calibration.

7. Interpretation Of Results And Limitation Of The Procedure

A. When an unknown diameter exceeds that of the top standard, the specimen should be diluted with saline and rerun.

B. When an unknown diameter is smaller than that of the lowest standard, its concentration should be reported as "less than" the concentration of the reference serum. If available, "low level" radial immunodiffusion plates may be utilized.

C. Lack of a precipitin ring may be due to :

1. sample not applied to well
2. a concentration too low to be detected by the method
3. a concentration too high, resulting in the formation of soluble complexes, which are not precipitated

D. ***These plates do not measure substitute colostrum sources of IgG from Goat, Sheep, or Cow.***

8. Expected Values

Determination of normal ranges has been established by analysis of serum from random blood donors in the Northwest USA. Variables such as sex was not studied. Other unknown variables may also affect each laboratory's results. The range is listed as comprising 10-90% of the ranked population.

Newborn Before Suckling 2-17 mg/dl (based on 20 samples)	Average[10, 11] 7.7 mg/dl
Newborn 24 Hours Old After Suckling 200-4500 mg/dl (based on 250 samples)	Average[10, 11] 1657 mg/dl
Weanlings 4 months to 6 months old 315 - 1351 mg/dl (based on 118 samples)	Average[10, 11] 780 mg/dl
Adult Llama Titers (2 years & older) 800-3000 mg/dl (based on 38 samples)	Average[10, 11] 1574 mg/dl
Colostrum Titers 2000-35000 mg/dl (based on 292 samples)	Average[10, 11] 16315 mg/dl

The incidence of failure of passive transfer (FPT) of immunoglobulins has been estimated to be between 2.9 and 25% [3,4,5] in horses. Partial passive transfer has been defined as immunoglobulin levels of 200 to 400 mg/dl. Total failure of passive transfer has less than 200 mg/dl.

The minimum level of IgG necessary to protect a cria from infection depends upon a number of factors, including the types of bacteria in the environment, management and stress factors and the colostral antibody titer against specific bacteria in the environment. Evidence suggests that crias should have IgG concentrations greater than 800 mg/dl.

The half-life of IgG from colostrum is 18 to 23 days [6,7] therefore serum immunoglobulin levels are lowest between 1 to 2 months of age [8,9].

These values are intended as a guideline -- each laboratory should establish its own "normal" range. Values vary with age and should be separately established.

9. Performance Characteristics

A. For investigational use only. The performance characteristics of this product have not been established.

10. References

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