Procedure

Specimens should be collected by approved veterinary techniques. Allow the blood to clot, and centrifuge to collect serum. Use serum within 72 hours, or freeze until ready to assay. Avoid repeated freezing and thawing of samples.

Add reference standards by carefully pipetting 3µl of each into the first wells. Be sure to lift the pipette off of the well bottom once the filling process has begun.

Pipette 3µl of the specimen(s) to be tested into the remaining well(s).

Replace the cover firmly on the plate and leave undisturbed, rightside up at room temperature for 18-24 hours outside of the mylar pouch.

After 18-24 hours, the diameters of the rings may be read and a standard curve established. Measure each diameter either directly off the plate or by using a comparator. Read each diameter in mm. If all wells are not used, replace the lid securely and store at 4-8 °C inverted in the mylar pouch provided.

Plot the diameters of the reference standards versus the concentration of the reference standards as indicated on the supplied graph paper. Draw a standard curve. Alternatively, a calculator with the capability of establishing a regression line may be used to determine the concentration of the unknowns. Specimens with diameters beyond the range of the standard curve may be retested either by redilution if the rings are too large or by concentration if the rings are too small.

Interpretation

Information sheets regarding immunodeficiencies and immunoproliferative disorders of horses, cows and dogs are available through VMRD, Inc.

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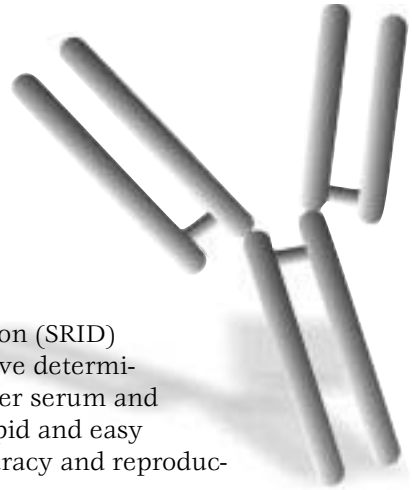
IMMUNOCHECK



SRID Kit

Single Radial Immunodifusion

For Veterinary Research Use Only

Principle

The technique of single radial immunodiffusion (SRID) is the most widely used method for quantitative determination of classes of immunoglobulins and other serum and plasma proteins. This technique combines rapid and easy sample application with a high degree of accuracy and reproducibility.

The method is derived primarily from the works of Fahey, and of Mancini. Antiserum specific for the protein to be measured is incorporated into agarose gel. The sample antigen diffuses into the gel containing the antibody, and a ring of precipitation forms that is proportional in size to the concentration of the antigen. A linear relationship exists between the diameter of the ring and the concentration of the antigen when plotted on semi-log graph paper. This method is time and temperature dependent.

Limitations

Results are limited by the assay range of the kit, although dilution of the specimen to be tested will extend the range. When diameters greater than the highest reference standard occur, dilute the sample and rerun the assay. If the diameter of the ring is too small, the sample may be concentrated. VMRD supplies differeent ranges of test kits to accomodate your testing needs. Please see immunocheck at www.vmrd.com

Kit Contents

1. Ready-to-use single radial immunodiffusion (SRID) plates containing monospecific antisera in buffered agarose
2. Four reference standards (preserved with 0.09% sodium azide)
3. 3 µl precision pipette
4. Instruction sheet with graph paper.

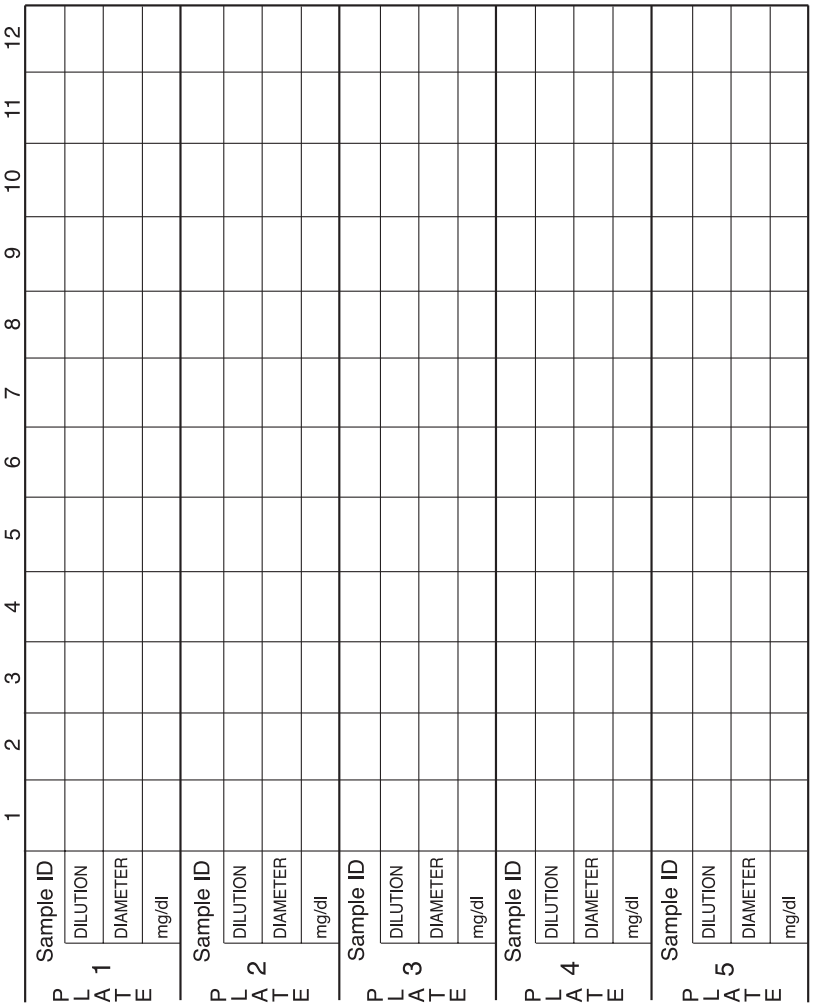
Materials Needed

All the materials required for this test are included in the kit. If dilutions of sample are necessary use glass or plastic tubes.

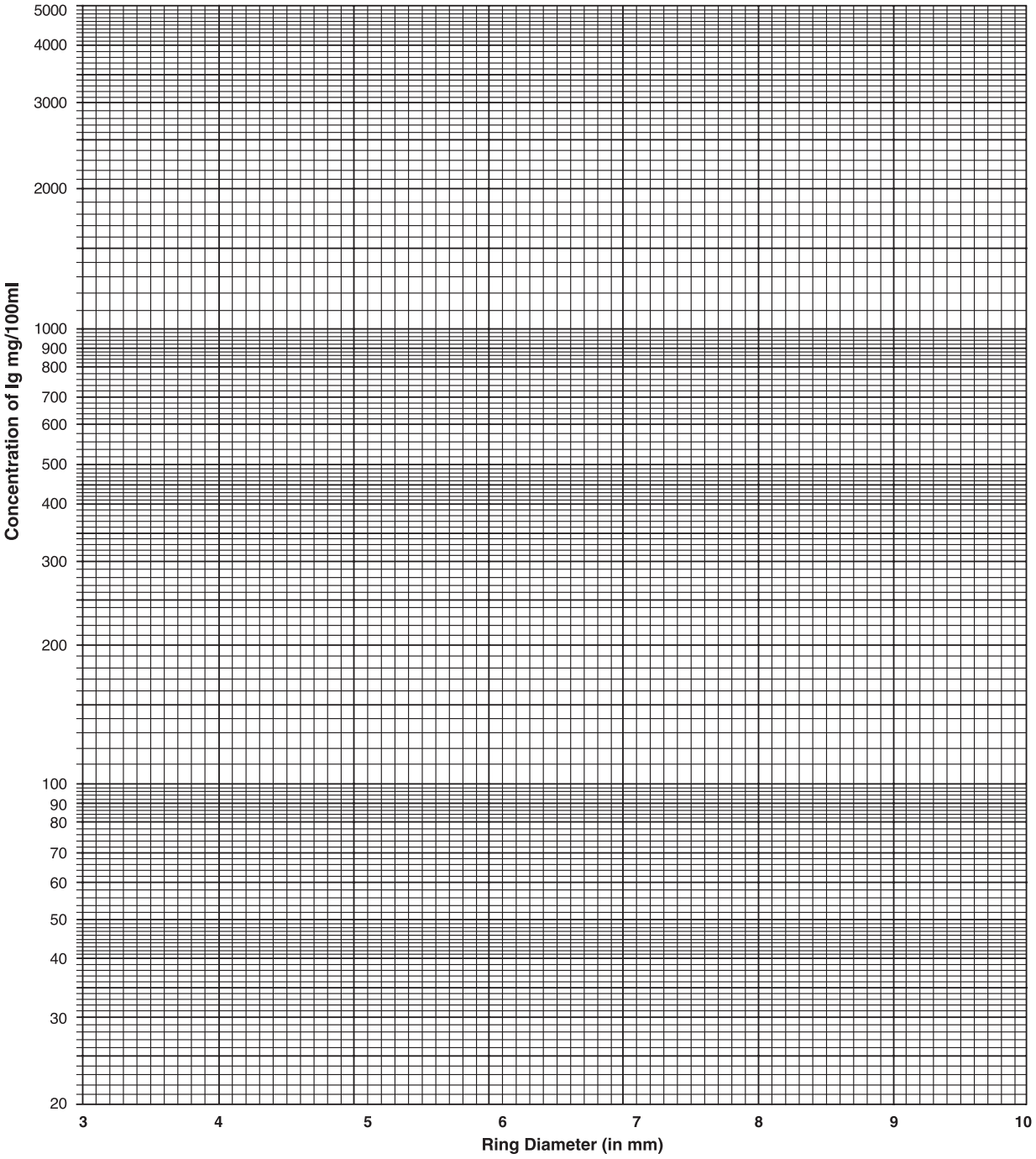
Storage and Precautions

Store plates in an inverted position at 4-8°C. **DO NOT FREEZE.** Keep plate tightly closed at all times. Between uses, plates may be stored inverted in their mylar pouches. The expiration date is listed on the box label.

VMRD, Inc. Radial Plates



Notes:



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FACTS ABOUT FPT IN CALVES

Failure of Passive Transfer (FPT) of Colostral Immunoglobulins in Calves

A calf is born essentially agammaglobulinemic and normally absorbs immunoglobulins from its dam's colostrum during the first 24 hours of life. These absorbed immunoglobulins protect the calf for the first 1-3 weeks of life from the onslaught of pathogens that are invariably present. Since the immunoglobulins in colostrum are concentrated from the cow's serum, they contain antibody activity to the pathogens to which the cow has been exposed or immunized. They afford no protection against the introduction of new pathogens.

The main point to consider about passive transfer in calves is that it fails to occur normally in a remarkably high number of cases. The prevalence of FPT varies with management and type of animal (beef or dairy), but is usually greater than 10% and may be as high as 40% in some dairy herds. Calves with FPT have a high risk of gastrointestinal and respiratory infections. The risk of death for calves with FPT is from 3 to 10 times greater than for calves that absorb adequate amounts of immunoglobulins. When calves dying of infections are considered, 90% have FPT.

The primary reasons for inadequate colostral immunoglobulin transfer are: 1) failure to obtain colostrum at an early enough age, and 2) ingestion of an insufficient volume of colostrum. Other factors affect the amount of colostrum absorbed, but to a much lesser degree. To prevent FPT, calves should be fed 3 liters of first milking colostrum during the first 6 hours after birth. In situations where hand feeding of colostrum is impossible, anything done to ensure nursing during the first 6 hours of life will be beneficial.

FPT can be detected in calves 24 hours after birth by measuring serum immunoglobulin. In situations where treatment is economically feasible, plasma can be given intravenously at a dosage of 20 ml/kg. If plasma is not available, 20 ml/kg of serum can be given intraperitoneally. Since antibody has a local protective effect in the intestine, colostrum should be fed as much as possible without causing scours. Administration of immunoglobulins should be done before any signs of infection, because their prophylactic effect is much greater than their treatment effect.

Suggested Criteria for FPT Diagnosis

Classification	Serum IgG_1 (mg/100 ml)	Prognosis
FPT	<800	Poor
Partial FPT	800-1600	Moderate
Normal	>1600	Good

REFERENCES

1. Gay, C.C. *Escherichia coli* and neonatal disease of calves. *Bacteriological Reviews* 29:75-101, 1965.

2. Selman, I.E., A.D. McEwan, and E.W. Fisher. Absorption of immune lactoglobulin by newborn calves. Attempts to produce consistent immune lactoglobulin absorptions in newborn dairy calves using standardized methods of colostrum feeding and management. *Res. Vet. Sci.* 12:205-210, 1971.

3. McGuire, T.C., *et al.* Failure of colostral immunoglobulin transfer in calves dying from infectious disease. *JAVMA* 169:713-718, 1976.

4. Pfeiffer, N.E., and T.C. McGuire. A sodium sulfite precipitation test for assessment of colostral immunoglobulin transfer in calves. *JAVMA* 170:809-811, 1977.

5. Davidson, J.N., *et al.* Relationship between serum immunoglobulin values and incidence of respiratory disease in calves. *JAVMA* 179:708-710, 1981.

6. McGuire, T.C., and D.S. Adams. Failure of colostral immunoglobulin transfer to calves: Prevalence and diagnosis. *Comp. Cont. Ed.* 4:S35-S40, 1982.

7. Naylor, J.M., *et al.* Plasma total protein measurement for prediction of disease and mortality in calves. *JAVMA* 171:635-638, 1977.

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BOVINE INFORMATION SHEET

VMRD, Inc.'s radial immunodiffusion plates are ready-to-use, with standards calibrated to determine samples within the normal range. Custom plates can be prepared to meet your specific needs.

FAILURE OF PASSIVE TRANSFER

The major immunoglobulins of bovine serum are IgG_1, IgM, and IgA. Clearly, the most important reason to measure immunoglobulin concentrations is to determine the adequacy of passive transfer in newborn calves. In a large number of studies, it has been shown that failure of passive transfer (FPT) occurs in 10 to 28% of calves and that these calves are more susceptible to infections. FPT accounts for most of the deaths in neonatal calves and many of the infections.

There is no placental transfer of immunoglobulin in cattle, and calves are born almost agammaglobulinemic (small amounts of IgG_1 and IgM are present). Prior to parturition, immunoglobulins, primarily IgG_1 with some IgM and IgA,

are concentrated in the colostrum. When calves obtain colostrum, they absorb whatever immunoglobulins are present across the intestinal epithelium and into the bloodstream via the lymphatics. Calves that have a normal transfer have post-suckle serum IgG_1 concentrations equal to or greater than the dam's serum.

Normal post-suckle immunoglobulin values are displayed in the table. A survey of several papers that have correlated the percentage of calves sick and dying with their IgG_1 concentrations indicate amounts below 8 mg/ml are failure of passive transfer (FPT) and amounts 8-16 mg/ml are partial failures (PFPT).[6]

IMMUNODEFICIENCY DISORDERS

Primary deficiencies of serum immunoglobulins are rare in cattle, although several have been reported. Those reported include agammaglobulinemia and selective IgG_2 deficiency.

NORMAL VALUES

The mean and standard deviation from several studies will be given, especially since normal post-suckle IgG_1 values in calves vary with time after birth and with different management practices among herds.

Bovine Serum Normal Values

(mg/100 ml ± S.D.)

Type	No. Tested	Age	IgG_1	IgG_2	IgM
Beef[1]	18	2-7 days	3700 ± 1300	50 ± 30	300 ± 200
	19	8-15 days	2500 ± 1000	40 ± 20	80 ± 20
	19	30 days	2200 ± 700	-	60 ± 20
	19	60 days	1100 ± 300	100 ± 100	120 ± 50
	19	90 days	1300 ± 300	110 ± 100	250 ± 70
Beef[2]	187	1 day	4800	-	320
Dairy[3]	983*	1 day	1130	-	290
Dairy[4]	7	1 day	3100 ± 200		
	7	7 days	2300 ± 900		

*Note: 42% of these failed to nurse or failed to absorb. The 42% FPT rate caused the mean to be too low.

Overview

(range)

	IgA	IgG	IgG_1	IgG_2	IgM
Serum	10-50	1700-2700	1050	790	250-400
Colostrum	390-490	3950-5050	3750-4760	190-290	420-490
Milk[5]	10-50	50-750	-	-	10-20

References

1. McGuire, T.C., *et al*. Failure of colostral immunoglobulin transfer in calves dying from infectious disease. *JAVMA* 169:713-718, 1976.
2. Norman, L.M., and W.D. Hohenboken. Genetic differences in concentration of immunoglobulins G(1) and M in serum and colostrum of cows and in serum of neonatal calves. *J. Animal Science* 53:1465, 1981.
3. Brignole, T.J., and G.H. Stott. Effect of suckling followed by bottle feeding colostrum on immunoglobulin absorption and calf survival. *J. Dairy Science* 63:451, 1980.
4. Klaus, G.B., *et al*. A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. *Immunology* 16:293, 1969.
5. Tizard, I. *Veterinary Immunology, An Introduction.* Philadelphia: W.B. Saunders Co., 1987. 401 pp.
6. McGuire, T.C., and D.S. Adams. Failure of colostral immunoglobulin transfer to calves: Prevalence and diagnosis. *Comp. Cont. Ed.* 4:S35-S40, 1982.

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