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TITLE: GENETIC DIFFERENCES IN CONCENTRATION OF IMMUNOGLOBULINS M AND G_1 IN SERUM AND COLOSTRUM OF COWS AND IN SERUM OF NEONATAL CALVES

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Blood samples collected from 187 beef calves 24 and 36 hours post-parturition were analyzed for concentrations of IgG_1 and IgM. Analogous immunoglobulin levels were measured in serum and colostrum samples from their dams. Correlations between serum and colostrum levels of both immunoglobulins in the dam were low as was the correlation between colostrum and calf serum concentration of IgG_1. Variance in serum and colostral immunoglobulin concentrations was analyzed based on a model including sire breed, sire within breed, dam breed, age of dam and sex of calf. Of these, breed of sire, breed of dam and age of dam were the most important sources of variation. Calves of Simmental and Pinzgauer sires tended to have lower IgM and IgG_1 levels (P=.07) than calves sired by Hereford x Angus or Tarentaise bulls. Calves of Hereford x Angus dams had consistently higher values compared to calves of Hereford dams. Hereford x Angus cows tended to have higher colostrum and lower serum concentrations of both immunoglobulins than Hereford cows. A fetal sire effect was demonstrated for serum IgM values in that cows mated to Simmental

bulls had significantly lower serum IgM concentrations than cows bred to sires of other breeds. Except for serum IgM in the calf and the cow, calves of older cows tended to yield higher concentrations of immunoglobulins.

Heritability estimates for calf serum IgM at 24 and 36 hour postpartum were .30 ± .26 and .35 ± .26, respectively, while those for IgG_1 were .52 ± .28 and .69 ± .30. The influence of sires within breeds on immunoglobulin concentrations in serum or colostrum of their mates was not statistically significant. High genetic correlations for calf immunoglobulin concentration across time (24 versus 36 hours) and across class (IgG_1 versus IgM) suggest that ability to absorb immunoglobulins through the gut following parturition is non-selective for antibody type. Relatively high repeatabilities for these traits imply that observed differences among individuals were due in part to permanent genetic and(or) environmental differences.

GENETIC DIFFERENCES IN CONCENTRATION OF IMMUNOGLOBULINS M AND G_1 IN SERUM AND COLOSTRUM OF COWS AND IN SERUM OF NEONATAL CALVES

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INTRODUCTION

Ingestion of maternal antibodies via colostrum is essential for the well-being and survival of calves (McGuire et al., 1976; Brignole and Stott, 1980). The amount of passive immunity acquired by the newborn ruminant is dependent upon several factors, including age at initial feeding, the degree of selectivity exerted by the intestinal epithelium and the mass of immunoglobulins (Ig's) ingested in the colostrum (Kruse, 1970; Stott et al., 1979b; Bush and Staley, 1980). In addition, breed differences for immunoglobulin levels in both offspring and dam have been demonstrated (Baumwart et al., 1977; Halliday et al., 1978; Bradley et al., 1979).

As a part of a larger experiment, this study was designed to investigate possible differences in the ability of calves to acquire maternal immunoglobulins (IgG_1 and IgM) within the first 24 to 36 hours of life. Calves were sired by Pinzgauer, Simmental, Tarentaise, Hereford or Hereford x Angus bulls mated to Hereford or Hereford x Angus cows. Breed differences among dams in serum and colostrum IgG_1 and IgM concentration also were examined as were fetal effects on maternal immunoglobulins. Heritabilities, genetic correlations and repeatabilities of the various traits were computed.

MATERIALS AND METHODS

Population and Management. In June of both 1978 and 1979, Hereford (H) and Hereford x Angus (HxA) cows representing 17 birth years (1960 to

1977) were randomly divided within breed and age categories into four groups. Three groups were mated by artificial insemination with semen from Simmental (S), Pinzgauer (P) or Tarentaise (T) bulls. The fourth group was mated by natural service to H or HxA bulls. Cows were maintained throughout most of the year on perennial grass/legume pastures. Supplemental feed of grass/legume hay and(or) silage was provided in the winter months and prior to calving in March and April of 1979 and 1980.

Cows were observed closely during calving. Because an objective of the study was to evaluate possible differences among individuals in their ability to acquire and absorb immunoglobulins, the exact time of first suckling was not standardized, nor was the frequency or duration of nursing following first ingestion. However, to prevent the likelihood of agammaglobulinemia, calves which had not suckled within 3 hours of postpartum were aided in the acquisition of colostrum. Calves were weighed at birth, and blood samples were obtained by venipuncture of the external jugular at 24 and 36 hours of age. With few exceptions, blood and colostrum samples were taken from each cow prior to suckling.

Individual blood samples were centrifuged at 1100 x g for 30 minutes; serum was removed and stored at -20°C. Whey was obtained from colostrum by ultracentrifugation (177,000 x g) for 90 minutes (Podrazky et al., 1975) and stored at -20°C. To retard spoilage, sodium azide (.02%) was added to both serum and whey samples before storage.

Preparation of Antisera. In order to quantify immunoglobulins in individual serum and whey samples, it was necessary to produce monospecific antisera to bovine IgG_1 and IgM. This was accomplished by

injecting two adult goats (I.M. and S.C.) with 4.8 mg each of bovine IgG_1 (Miles Research Product Division) emulsified with Freund's complete adjuvant. Goats were boosted at approximately 2 and 4 weeks with 2.5 mg bovine IgG_1 in Freund's incomplete adjuvant. Blood samples were collected into heparinized bottles and prepared as previously described. Goat and anti-bovine IgG_1 was purified by adsorption with soluble bovine IgG_2 (Miles Research Product Division). Following 3 hours incubation at $37^{o}C$ and 18 hours at $4^{o}C$, the adsorbed antiserum was centrifuged (10,000 x g), and the pellet was discarded.

Anti-IgM serum was prepared in a similar manner as that used for anti-IgG_1. Goats were immunized initially with 3.0 mg IgM (kindly provided by Dr. David Olson, Veterinary Science Department, University of Idaho) in Freund's complete adjuvant and boosted 2 weeks and 2 months later with 1.0 mg IgM in Freund's incomplete adjuvant. Anti-IgM was adsorbed with soluble bovine gammaglobulin (Calbiochem-Behring Corp.) and fetal bovine serum (Grand Island Biological Co.), concentrated to one-fourth its original volume.

The monospecificity of the antisera was confirmed by Ouchterlony double gel diffusion, immunoelectrophoresis, single radial immunodiffusion (SRID) recovery of known varying concentrations of standard immunoglobulins (Miles Research Product Division) in a pre-colostral calf sample and by the capability of monospecific antibiody to detect IgG_1 or IgM in known dilutions of serum samples.

Laboratory and Statistical Analysis. Individual serum and colostrum samples were analyzed by SRID (modification of Fahey and McKelvey, 1965,

as described by Blecha and Kelley, 1981). All samples were assayed in duplicate with immunoglobulin standards (Miles Research Product Division). In-house standard serum samples of known immunoglobulin concentration were assayed on each plate to determine intra- and interassay coefficients of variation. Plates were incubated at 21°C and immunoprecipitate ring diameters were measured with a calibrated SRID viewer (Transidyne General Corp.). The concentration of immunoglobulins was estimated by logarithmic linear regression analysis.

Data were analyzed by least-squares analysis of variance. Dependent variables were IgM and IgG_1 concentrations in cow colostrum and cow serum and in calf serum at 24 and 36 hours post-parturition. Independent variables initially included breed of sire, breed of dam, age of dam, calf sex, sire within breed of sire, year, all possible two factor interactions among fixed effects and the linear regression of dependent variables on birth weight. None of the interactions was statistically significant or important nor were the regressions on birth weight, so they were eliminated from subsequent analyses. Also, data were adjusted for year effects by expressing each observation as a deviation from its birth year mean, then adding the overall least-squares mean back to the deviation. From a second series of analyses, relationships among immunoglobulins in cow serum, colostrum and calf serum were determined. For example, the analysis of IgM in calf serum at 24 hours included the above listed main effects plus the regression on IgM in colostrum, and the analysis of IgG_1 in colostrum included these main effects plus the regression on IgG_1 in cow serum. In a third

series of analyses, breed of sire, sire within breed, breed of dam, age of same and calf sex were sources of variation. Heritabilities and genetic correlations were computed from results of these analyses. A fourth series of analyses with sources of variation for breed of sire, breed of dam, cow within breed, age of dam and calf sex allowed computation of repeatabilities. Standard errors of repeatabilities were computed according to the formula of Swiger et al. (1964).

RESULTS AND DISCUSSION

A summary of the distribution of records and the least-squares means for IgG, and IgM concentrations is given in table 1. Except for cow serum IgG_1, average values for immunoglobulins measured in this study were within the range of values reported in the literature (Bush et al., 1971; Logan et al., 1972; Butler, 1973; McGuire et al., 1976; Sasaki et al., 1977; Janota-Bassalik et al., 1978; Straub and Matthaeus, 1978; Halliday et al., 1978; Cabello and Levieux, 1978; Stott et al., 1979b) Our intra- and interassay coefficients of variation for IgM were 8.2% and 11%, respectively, and for IgG_1 were 9.1% and 7.4%, respectively.

IgG_1 in Cow Serum and Colostrum. While IgG_1 is the major component of bovine colostrum, it exists in about equal concentration with IgG_2 in serum. Mammary secretions of this antibody originate from blood serum and are transferred to colostrum prior to parturition via a specific transport mechanism (Larson et al., 1980). In analysis of these data, the regression of colostral IgG_1 on serum IgG_1 was not significant

(b=-.12, P=.85), and the residual correlation between the two variables from the third analysis was only -.05.

Hereford cows had higher levels of serum IgG_1 (P-.01) than did HxA cows, but for IgG_1 in colostrum, the breed ranking was reversed (P=.02). Neither sex of offspring nor breed of sire to which a cow was mated affected IgG_1 level in either serum or colostrum. Our study indicated that older cows (five or more lactations) had higher serum and colostrum IgG_1 values than younger cows, in general agreement with other research (Sasaki et al., 1977; Oyeniyi and Hunter, 1978; Frerking and Aeikens, 1978).

IgG_1 in Calf Serum. Unlike the highly selective transport mechanism at the blood-mammary barrier, the intestinal phase of absorption in the calf is relatively nonspecific for particular classes of immunoglobulins (Klaus et al., 1969; Halliday et al., 1978). Our findings support this in that calves absorbing high levels of IgG_1 tended concomitantly to have high levels of IgM (r=.66 and .71 at 24 and 36 hours following parturition, respectively). As expected, a high correlation existed between 24 and 36 hour IgG_1 concentrations (r=.92). The slightly higher IgG_1 level at 24 hours compared to 36 hours (48.2 mg/ml vs. 47.4 mg/ml) was not unexpected, since the capacity of the gut to absorb IgG_1 ceases at about 26 hours post-parturition (Stott et al. 1979a), and by 36 hours, some existing immunoglobulins may be undergoing metabolism in various biochemical and physiological processes of the calf (Logan et al., 1972; Baumwart et al., 1977) or may be equilibrating in extravascular spaces.

Regressions, from preliminary analyses, of IgG_1 in calf serum at 24 and 36 hours following parturition on IgG_1 in colostrum were b=.05 (P=.98) and b=-.68(P=.63), respectively; the residual correlations of 24 and 36 hour calf serum IgG_1 with colostral IgG_1 were .25 and .08, respectively. A similar lack of significant correlation between the concentration of immunoglobulins in the dam's colostrum and the corresponding concentrations in postsuckle calf serum at 48 hours has been demonstrated by McGuire et al. (1976).

Breed of sire influenced 24 hour IgG_1 level (P=.07). H and T sired calves had higher levels than did S and P sired calves, and calves with HxA sires were intermediate. A similar ranking was noted at 36 hours. Calves with HxA dams had higher IgG_1 values at both 24 and 36 hours (P=.09 and P=.04) than calves with H dams, corresponding to higher concentrations of the immunoglobulin noted earlier in HxA cow colostrum.

As with serum and colostrum IgG_1 concentrations in the dam, age of dam was a significant source of variation in calf serum IgG_1 concentration. Calves born of younger cows had lower concentrations for this immunoglobulin (P=.07 and P=.08 at 24 and 36 hours, respectively) than did calves from older cows. Offspring sex did not influence IgG_1 concentration, in agreement with findings of Jensen and Christensen (1975) and Bradley et al. (1979).

IgM in Cow Serum and Colostrum. In contrast to IgG synthesis, IgM is produced within the mammary gland by plasmacytes (Lee and Lascelles, 1970; Brandon et al., 1971). Both blood serum and colostrum contain

less IgM than IgG_1. The residual correlation between serum IgM and colostrum IgM was low (.14). Since IgM is especially concentrated in bovine colostrum, it was not surprising to find colostrum concentrations approximately twice those of serum levels (8.99 mg/ml vs. 4.32 mg/ml), in agreement with results reported by Butler (1973).

HxA cows had higher levels of IgM in colostrum than did H cows, as was true for IgG_1. Cow age did not affect IgM in colostrum, nor was this immunoglobulin influenced by sex of offspring or by the breed of sire to which a cow was mated. However, there was an apparent effect of the breed of sire of calf on serum IgM (P=.05). Cows bred to Simmental sires had lower levels of circulating IgM. Stonaker and Knapp (1974) reported that sex of fetus influenced a dam's postpartum lactation yield. A fetal effect on milk production via the genetic contribution of its sire was established by Skjervold and Fimland (1975) and Adkinson et al. (1977). The present data demonstrates that this effect may exist for IgM concentration as well.

IgM in Calf Serum. As anticipated, we found a high correlation between 24 and 36 hour levels of serum IgM (r=.84) in calves. From preliminary analyses, the regressions of IgM at 24 and 36 hours in calf serum on IgM in colostrum were b=.05 (P=.03) and b=.06 (P=.01).

Both sire breed and dam breed influenced calf serum levels of IgM; significance probablities varied from .02 to .12. As with IgG_1, calves with S sires had the lowest immunoglobulin levels, and H sired calves were highest. Calves from HxA dams had higher IgM concentrations than calves from H dams, as was true for IgG_1 concentration.

Discussion of Breed Differences. Breed of calf's sire and(or) breed of calf's dam were important sources of variation in immunoglobulin level in all but one of the eight dependent variables in this study. Comparable findings for beef and dairy breeds have been reported. Baumwart et al. (1977) found that Holstein calves were more efficient than Ayrshire calves in absorbing total gammaglobulins, while Tennant et al. (1969) reported that immunoglobulin levels in Jersey calves were twice those in Holsteins. Selman et al. (1971) determined that Friesian x Ayrshire calves absorbed greater quantitites of immunoglobulins than did Ayrshires. Bradley et al. (1979) reported highly significant differences among beef breeds in immunoglobulin concentrations of calves, with Herefords ranking above Simmentals in total quantity of gammaglobulin. McGary et al. (1978) reported that Charolais x Brown Swiss crossbred cows had higher blood serum, colostrum and milk immunoglobulin and protein concentrations than purebred Charolais cows. Also crossbred calves (Simmental x Charolais-Brown Swiss) in their study had more total blood protein than Charolais calves. Halliday et al. (1978) demonstrated that Shorthorn x Galloway calves had significantly higher concentrations of IgG_1 and IgM than Hereford x Friesian calves and that their dams had higher IgG and IgM concentrations in the colostrum. In sheep, Halliday (1973) reported differences among breeds in ability of offspring to concentrate maternal immunoglobulins that were independent of the breed of dam rearing the lamb.

Several theories have been proposed to account for observed variation in calf absorption of immunoglobulins, such as differences among

breeds for calf vigor during the first hours following birth or differences in absorption efficiency of the calf's intestine due either to differences intrinsic to the gut or to differences in exogenous factors in colostrum that facilitate absorption (Tennant et al., 1969; Halliday, 1973). Offspring vigor is often associated with an optimum intermediate range of birth weights. Very small and excessively heavy animals may be less vigorous at birth (as discussed by Purser and Young, 1964, in sheep) and therefore may possibly be less capable of obtaining colostrum within the first critical hours following birth. This explanation does not seem likely in our experiment since all calves received colostrum within 3 hours postpartum. In addition, preliminary analyses indicated that birthweight was not a significant factor influencing variation in immunoglobulin concentration in either calves or dams. As mentioned earlier, low correlations between colostrum and calf serum immunoglobulins for IgG_1 and significant differences among breeds of calves for both immunoglobulins imply that differences within the intrinsic system of the calf do exist. These differences are no doubt enhanced by calf vigor and by what appear to be intrinsic differences in the dam for her ability to synthesize and, in the case of IgG_1, transport immunoglobulins.

Heritabilities, Genetic Correlations and Repeatabilities. Heritability estimates for calf serum immunoglobulins at 24 and 36 hours were .23 ± .26 and .35 ± .26 for IgG_1 and .52 ± .28 and .69 ± .30 for IgM (table 3). Roubicek and Ray (1972) and Jensen and Christensen (1975) reported h^2 of total serum protein in older calves of .06 to .30, and Lie (1979)

reported the heritability of total immunoglobulin in young bulls to be .54. These studies, however, examined genetic variation in active immunity. We are not aware of research to examine within breed genetic variation for ability of calves to acquire passive immunity following parturition. Our estimates suggest that it may be possible to select for changes in IgG_1 and IgM absorption in young calves. This in turn might influence resistance to calfhood diseases and increase calf survival.

In this experiment, however, calves that were either markedly above average or markedly below average for Ig concentrations did not differ from their contemporaries in survival or incidence of calfhood diseases. Cows markedly above or below average for Ig in colostrum and(or) serum also did not differ from their contemporaries for post-parturition illnesses. The limited amount of data for the low frequency of health problems in the herd at large may have been responsible for this lack of difference.

For immunoglobulins in cow serum and colostrum, "heritabilities" from paternal half-sib analysis of variance actually would reflect within breed genetic variation for fetal effects on dam traits, since dams of paternal half-sibs were no more closely related than average for the population. Our study did not provide evidence that such effects were important, since "h^2" of serum IgG_1 and colostral IgM were only .05 ± .23 and .15 ± .24, respectively, while sire components of variance for serum IgM and colostral IgG_1 were negative.

Genetic correlations were determined among 24 and 36 hour calf serum IgG_1 and IgM concentrations (table 3). High correlations existed between the two immunoglobulins within and across time. Genetic correlations between the two immunoglobulins also were high. Differences among calves for the ability to acquire and absorb various colostral immunoglobulins therefore apparently are largely the result of non-selective differences. Thus, calves genetically capable of acquiring and absorbing high levels of IgG_1 are also capable of concentrating IgM in large quantities.

Many cows had calves in both 1979 and 1980, so it was possible to estimate repeatabilities of cow and calf traits. For IgG_1 in cow serum and colostrum, repeatabilities were .52 ± .10 and .30 ± .14, while for IgM, these repeatabilities were .59 ± .09 and .23 ± .15, respectively. When calf serum immunoglobulins were considered as traits of the cow, repeatabilities were .52 ± .10, .38 ± .13, .47 ± .11 and .45 ± .16 for IgM at 24 and 36 hours and IgG_1 at 24 hours and 36 hours, respectively. A cow's inferiority or superiority for immunoglobulin concentration based on a single record for herself or from her offspring may therefore be a reasonably good estimate of future measurements of these immunoglobulins. Our results are in close agreement with Halliday et al. (1978) who found that cows with calves high in immunoglobulin concentrations one year tended to have calves with similar values a second year (r=.47).

TABLE 1. Distribution of Data and Least-Squares Means for Concentration of Immunoglobulins G_1 and M (mg/ml) for Calves and Their Dams

EFFECT	N	24 H Calf IgG_1	36 H Calf IgG_1	24 H Calf IgM	36 H Calf IgM	Cow Serum IgM	Cow Serum IgG_1	Colostrum IgM	Colostrum IgG_1
OVERALL	187	48.2	47.4	3.18	3.01	4.32	19.3	8.99	114.6
BREED OF SIRE									
Hereford (H)	49	55.4	54.1	3.77	3.51	4.52	19.6	9.43	115.8
Pinzgauer	41	42.0	41.8	3.03	2.72	4.52	18.0	9.13	113.8
Simmental	36	42.4	43.6	2.28	2.38	3.91	19.9	7.46	120.2
Tarentaise	41	52.7	50.4	3.32	3.03	4.18	19.3	10.06	123.8
H x Angus	20	48.5	47.3	3.50	3.42	4.48	19.8	8.86	99.2
BREED OF DAM									
Hereford (H)	118	45.5	44.2	2.97	2.78	4.35	21.0	7.87	112.6
H x Angus	69	50.9	50.7	3.39	3.24	4.29	17.7	10.10	116.6
AGE OF DAM									
3 yrs	27	44.9	42.3	3.57	3.39	4.56	17.4	8.00	93.4
4 yrs	26	41.1	41.6	3.00	2.90	4.94	18.5	6.92	87.3
5 yrs	38	48.3	47.6	3.22	3.13	4.34	15.2	9.52	115.2
6-10 yrs	78	53.3	52.1	3.15	2.95	4.29	19.5	9.68	130.5
11+ yrs	18	53.4	53.6	2.97	2.67	4.54	26.0	10.80	146.5
SEX OF CALF									
Female	91	49.5	48.1	3.14	3.03	4.21	19.6	8.63	113.6
Male	96	46.9	46.8	3.22	2.99	4.44	19.0	9.34	115.5

TABLE 2. Significance of Probabilities of Residual Mean Squares from Analyses of Variance of Calf and Cow Immunoglobulins

		TRAIT							
		24 H	36 H	24 H	36 H	SERUM		COLOSTRUM	
SOURCE OF VARIATION	df	IgM	IgM	IgG_1	IgG_1	IgM	IgG_1	IgM	IgG_1
Breed of Sire	4	.02[a]	.05	.07		.05			
Sire/Breed	28		.09	.03	.01				
Breed of Dam	1		.07	.09	.04		.01	.02	
Age of Cow	4			.07	.08		.01		.01
Sex of Calf	1								
Residual	148	2.69[b]	2.11	351.1	350.3	1.15	53.2	28.83	3804.8

[a]Significance probabilities greater than .10 are not given

[b]Residual mean square.

TABLE 3. Heritabilities of and Genetic and Phenotypic Correlations Among Calf Immunoglobulin Concentrations

	24 H IgM	36 H IgM	24 H IgG_1	36 H IgG_1
24 H IgM	.30[a] ±.26	1.29 ±.30	1.08 ±.26	1.22 ±.32
36 H IgM	.84	.35 ±.26	.88 ±.23	1.03 ±.19
24 H IgG_1	.66	.68	.52 ±.28	.95 ±.05
36 H IgG_1	.64	.71	.92	.69 ±.30

[a]The diagonal elements represent h^2 values. Above the diagonal are genetic correlations, and below the diagonal are phenotypic correlations.

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APPENDIX

SINGLE RADIAL IMMUNODIFFUSION (SRID) ASSAY

References:

Fahey, J.L. and E.M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol. 94:84.

Mancini, G., A.O. Carbonara and J.R. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem.

Materials:

Borate Buffer

	1 ℓ	0.5 ℓ
-Boric Acid	6.184 g	3.092 g.
-Sodium borate ($Na_2B_4O_7 \cdot 10H_2O$)	9.536 g	4.768 g.
-NaCl	4.384 g	2.192 g.
-NaN_3 0.1%	1.0 g	.500 g.

-Add to volumetric and bring to volume with distilled H_2O.

-Adjust to pH 8.4-8.5

Borate Saline Buffer

-5 part borate buffer
-95 parts saline (.85%)

Agar

1.2% Noble Agar (in borate-saline buffer)
-Autoclave agar 15-20 minutes to dissolve then place in a 54°C water bath and let cool to 54°C before use. You can dissolve agar by heating carefully over a bunsen burner.
Note: If antisera is in a fluid state, subtract the necessary amount of fluid from the buffer used in dissolving the agar. Final agar concentration must be 1.2%.

Mold for making Agar Gels

-Base plates and cover plates 107 mm x 82 mm x 1 mm
-U-shape metal frame, 1 mm thick
-Requires approximately 10 ml of agar-antibody solution to fill mold.

Pipette

-10 ml pipette for applying agar-antibody mixture

Pasteur Pipette

-For sealing outside of mold with agar to prevent leakage

Well Cutter

-For cutting wells (3 mm i.d.), from Miles Research Products, Miles Laboratories, Inc., Elkhard, Indiana 46515

5 µl Eppendorf and Tips

-For application of sample to wells

Incubator

-For incubation of plates in a humid atmosphere

-Incubator consists of a plastic airtight container, holding a 6 x 12 hole test tube rack on which the SRID plates are placed. The bottom of the container is covered with approximately 1/4 inch of H_2O.

Calibrating RID Viewer

-For measurement of immunoprecipitate rings, from Transidyne General Corporation, 903 Airport Dr., Ann Arbor, MI 48106

Antibody

-To be used at an appropriate dilution

Generally antibody dilution should cause immunoprecipitate ring diameters of $\leq$ 15.0 mm.

Standard Curve

-Prepare a standard curve of approximately 8 points of antigen that is to be assayed.

-A single standard curve should be run on the first and last plate of each assay.

In-House Standards (IHS)

-IHS A: undiluted serum sample
-IHS B: IHS A diluted 1:2
-IHS C: IHS B diluted 1:2
This varies from assay to assay
-Run each IHS on each plate in all assays

Samples

-If necessary, dilute in borate-saline buffer

-Run duplicates of each sample on separate plates

Procedure:

1. Make borate buffer and borate-saline buffer

2. Preheat water bath to 54°C

3. Make 1.2% Noble Agar solution

4. Add antibody to cooled Noble Agar solution
   -Use a dilution to obtain the most economical use of antiserum and still achieve clear distinct immunoprecipitate rings.

   -Anti-Bovine IgG_1 is in a fluid state and is used at a dilution of 1/8. For example, for 10 plates:

   1.32 g Noble agar
   96.3 ml 5% borate-saline
   13.7 ml Anti-Bovine IgG_1

5. Preparation of plates with plate mold
   -Place metal frame mold on base plate
   -Cover the frame with a glass plate
   -Tightly clamp the 3 pieces of the mold together
   -With Pasteur pipette apply a 5% agar solution onto mold edges to prevent leakage. Allow sufficient time for solidification.
   -Set the mold vertically on the lab bench and apply the heated agar-antibody mixture directly in the center of the mold with a preheated pipette (~10 ml/plate).
   -After the gel has solidified (15 min) loosen top edge of agar with a spatula and remove the clamps. Slide off the top plate carefully and remove the metal frame.

6. Application of antigen samples
   -Punch circular wells in agar using a German well-cutter
   -Remove gel plugs with a light suction
   -Apply 5 µl of standards or appropriately diluted samples with an Eppendorf pipette.

7. Incubate plates in a humid atmosphere for 48 hours at room temperature.

8. Measure and record the distance of migration (dia) with the Calibrating RID Viewer.

9. Transform mg/ml concentrations of std. curve to logarithmic values
   - -Put log mg/ml vs. diameter units on standard curve
   - -Compute linear regression for standard curve
   - -Calculate mg/ml for each sample using linear regression program
   - -Correct for dilution factor and report as mg/ml

10. General Comments
   - -Number of samples that can be assayed per plate will vary with the class of Immunoglobulin (35 for IgM and 25 for IgG_1)
   - -For concentrations of > 15 $mgml^{-1}$ discard duplicates if > 7 $mgml^{-1}$ difference and for concentrations of > 15 $mgml^{-1}$ discard duplicates if > 4 $mgml^{-1}$ difference
   - -This acceptance criteria typically results in CV's of 8% to 10% (all CV's are calculated on basis of $mgml^{-1}$; not ring diameter)