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Measurement of Serum IgG in Foals by Radial Immunodiffusion and Automated Turbidimetric Immunoassay

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Hypogammaglobulinemia as a result of failure of transfer of passive immunity (FTPI) is an important risk factor for infectious disease in neonatal foals. The current gold standard for determining serum immunoglobulin concentrations is radial immunodiffusion (RID). The purpose of this study was to compare immunoglobulin concentrations measured by RID with those determined by an automated turbidimetric immunoassay (TIA), which has a much shorter turnaround time. Immunoglobulin concentrations were measured by both RID and TIA in serum collected from 84 neonatal foals. Sixty-seven foals had results within the linear range for both assays. Sensitivity and specificity of TIA for diagnosis of FTPI with IgG ≤ 800 mg/dL were 0.81 (95% CI 0.70–0.88) and 0.86 (95% CI 0.76–0.93) and with IgG ≤ 400 mg/dL were 0.63 (95% CI 0.35–0.86) and 0.92 (95% CI 0.87–0.95), respectively. A significant linear relationship was found between IgG concentrations determined by TIA and RID (TIA = 0.9511RID + 8.4354; R^2 = .59, $P < .0001$). The coefficients of variation for between-run and within-run precision for the TIA were 2.5 and 3%, respectively. Storage of samples from 10 foals at −20°C for 10–12 months resulted in a reduction in TIA-measured serum IgG concentration of −17.6% (SD = 3.7%), indicating that long-term storage of samples at −20°C should be avoided. The results of this study indicate that measurement of serum IgG by TIA can be used to evaluate foals for FTPI.

Key words: Horse; IgG measurement; Immunoglobulin; Transfer of immunity.

Transfer of passive immunity is crucial to the health of a neonatal foal. In contrast to cats and dogs, no immunoglobulin transfer occurs across the equine placenta, and foals are born hypogammaglobulinemic. Therefore, immunoglobulin transfer in the initial postnatal period by ingestion of good-quality colostrum is critical for the establishment of passive immunity in the foal. The rate of uptake of colostral immunoglobulins by the foal's small intestinal epithelial cells is highest in the 1st 6–8 hours after birth and then begins to decline. Immunoglobulin is not absorbed after 24–36 hours of age. If a foal fails to suckle in the immediate postpartum period, there is a low to absent concentration of immunoglobulin, resulting in a high incidence of neonatal infectious disease.[1–4]

Failure of transfer of passive immunity (FTPI) can result from injury or illness in the neonate, inadequate immunoglobulin production by the dam, or failure of the neonate to absorb the ingested colostrum.[4,5] The incidence of FTPI in foals is estimated to be between 2.9 and 25%.[6] Foals that are immunodeficient because of FTPI are susceptible to many infections, especially septicemia, omphalophlebitis, septic arthritis, and respiratory infections.[1–4] Thus, a sensitive and specific diagnostic test to identify FTPI is valuable in the management of neonatal foals.

The current gold standard for determination of serum immunoglobulin concentration is the serial radial immunodiffusion (RID) assay.[7,8] In this test, serum is placed on a gel matrix that contains antibodies against each specific immunoglobulin isotype (IgA, IgG, IgM). The immunoglobulin in serum diffuses outward from the well and precipitates with the antibody at the zone of equivalence. The diameter of the precipitin ring is read manually and is proportional to the immunoglobulin concentration in the serum.[8–10] These measurements are compared with measurements from standards to estimate immunoglobulin concentrations. This method is both time and temperature dependent and relies on skilled technical personnel. Coefficients of variation reported for RID in humans and dogs range from 2.9 to 5.2% within runs and 4.4 to 9.9% between runs.[11–13] Equine RID most likely has similar variation, but a coefficient of variation for RID was not measured in this study. The major disadvantage with RID lies in the time it takes to generate and interpret the results. Most tests require between 18 and 24 hours before results can be provided to the clinician.[12] This delay is a major impediment to early diagnosis and treatment of FTPI in foals.

Turbidimetric immunoassay (TIA) is based on the principle of immunologic agglutination and light scattering of the agglutination products. TIA has been available for many years for measurement of IgG.[14,15] However, its use has been confined largely to research settings and originally required manual preparation of the reaction tubes. An automated TIA has been developed that can be performed on routine chemistry analyzers.[a] This procedure can be run on serum or plasma (sodium heparin or ethylenediaminetetraacetic acid [EDTA]) samples. The advantages of this TIA over RID include automation, a shorter turnaround time (<1 hour after submission to the laboratory), and elimination of human error in measurement of the precipitin ring diameters.

The purpose of this study was to compare serum immunoglobulin concentrations of neonatal foals determined by RID and automated TIA. We hypothesized that the TIA would provide IgG results comparable to the standard RID.

Materials and Methods

Serum was collected from 84 foals <3 weeks of age. Forty-eight foals were presented to The Ohio State University (OSU) Veterinary

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Table 1. Between-run precision study for turbidimetric measurement of lgG for individual foals.

	Coefficient of Variation									
	3446	8771	3417	0417	7986	8362	8322	0486	8042	8205
Day 1	518	910	740	430	505	761	899	419	644	675
Day 2	516	885	753	419	516	782	867	420	638	662
Day 3	507	896	745	417	486	761	824	411	639	627
Day 4	517	910	768	421	495	718	826	407	646	649
Day 5	510	919	736	420	507	721	828	407		
Day 6	536	944	758	406	507	767				
Day 7	545	933	784	421	515					
Day 8	517	954	758	414	501					
Day 9	536	904	758	418	506					
%CV	3	2	2	2	2	3	4	2	1	3

Teaching Hospital and 36 were seen by the OSU Ambulatory Service. A mixture of healthy foals and foals presented for various medical problems, including fever, pneumonia, swollen joints, and sepsis, were evaluated. Blood was collected into a tube with no additives and allowed to remain at room temperature until clotted (~10–20 minutes). The sample then was centrifuged, and the serum was transferred to a clean plastic tube.

The IgG concentrations of the samples were analyzed within 4 hours of sample collection by both RID[b] and an automated TIA.[a] The sample for analysis by TIA was mixed with a buffered solution containing a high concentration of antibody (goat anti-equine IgG). Antigen-antibody complexes form large aggregates that scatter light. This results in turbidity proportional to the immunoglobulin concentration of the sample. The nonscattered light is measured by spectrophotometry at 700 nm, a wavelength that is not absorbed by either the sample or the immunoprecipitate.[14,15] The assay used in horses is performed by comparing the nonscattered light from the horse sample with that of a standard curve prepared from the instrument with 6 different dilutions of equine calibrator.[c] An equine control is run with each sample or group of samples.[c] Under these assay conditions, the manufacturer claims there is no cross-reaction among IgG, IgA, and IgM.[d]

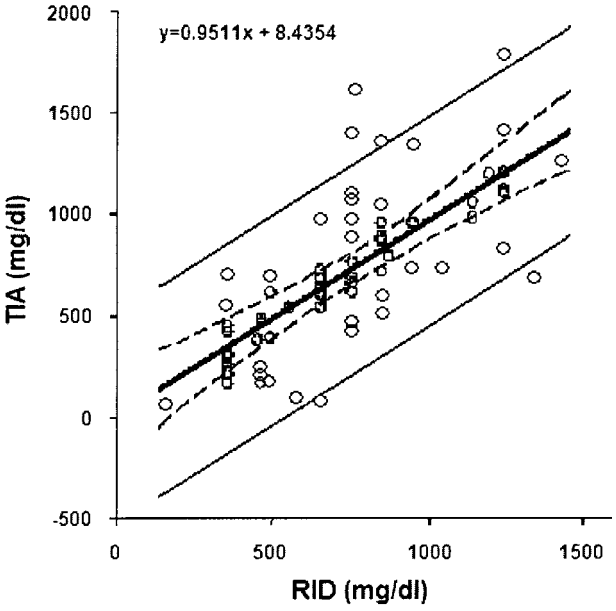

Fig 1. Serum IgG concentrations determined by radial immunodiffusion (RID) and turbidimetric immunoassay (TIA). The thick solid line represents the line of best fit, and the dashed line represents the 95% confidence interval for the line of best fit. The thin solid lines signify the prediction interval (the 95% confidence interval for new observations).

After analysis, samples were stored at −20°C. They then were thawed and reanalyzed 10–12 months later to determine the effect of long-term freezing. The percent difference between the initial measurement and the postfreezing measurement was calculated for each sample.

Between-run precision for the TIA was evaluated by measuring samples from 10 different horses once daily for 4–9 days and calculating the coefficient of variation (CV; Table 1). Each sample was divided into 4–9 aliquots (1 for each day) and stored at −20°C to avoid the effects of repeated freeze-thaw. Within-run precision of the TIA was evaluated by measuring IgG from the same foal 11 times on the same day.

All results were compiled and analyzed by Analyze-It software for Microsoft Excel. Sensitivity and specificity were calculated with standard formulas, with the use of the RID as the gold standard and ≤400 mg/dL and ≤800 mg/dL as clinically relevant cutoff concentrations. The 95% confidence intervals were calculated by a chi square contour method.[16–18] The relationship between serum IgG concentrations measured by RID and TIA was analyzed by linear regression.

Results

Of the 84 samples analyzed, 11 were ≤400 mg/dL and 42 were ≤800 mg/dL when measured by RID. With the TIA, 13 foals were ≤400 mg/dL and 40 foals were ≤800 mg/dL. Sixty-seven foals had results within the linear range for both methods (<1,600 mg/dL) and were included in the statistical analysis of linearity. There was a linear relationship between serum IgG concentrations determined by RID and TIA such that TIA = 0.9511RID + 8.4354 ($P < .0001$, $R^2 = .59$; Fig 1). Standardized residuals were normally distributed (Fig 2). The CV between runs was 2.3%, with a range of 1–4%; within runs, it was 3%.

Eighty-four samples were used to calculate sensitivity and specificity. Sensitivity was 0.63 (95% CI, 0.35–0.86) and specificity was 0.92 (95% CI, 0.87–0.95) for ≤400 mg/dL. Sensitivity was 0.81 (95% CI, 0.70–0.88) and specificity was 0.86 (95% CI, 0.76–0.93) for ≤800 mg/dL. There was a decrease of 17.6% (range 21.2–10.9%; SD = 3.7%) in serum IgG concentration determined by TIA in samples before and after storage for 10–12 months at −20°C.

Discussion

This study demonstrates the utility of TIA measurement of IgG in neonatal foals. Sensitivity and specificity are sim-

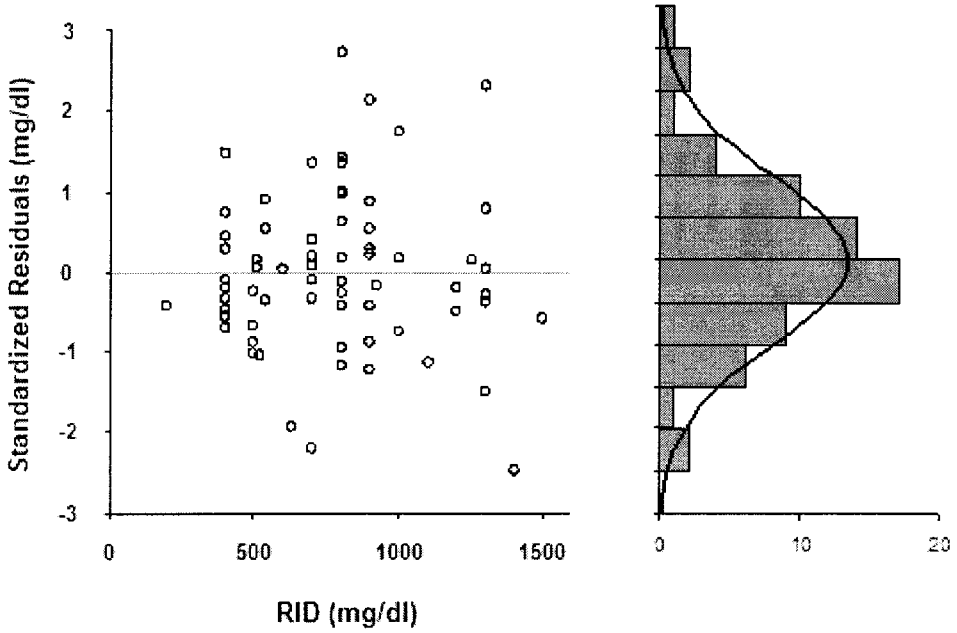

Fig 2. Standardized residuals for the relationship between IgG concentrations determined by RID and TIA.

ilar to currently used tests,[19] and the assay had acceptable interrun and intrarun variability. The method is suitable for use in veterinary laboratories for the measurement of serum IgG concentrations in foals for the diagnosis of FTPI.

Several other methods are available to measure serum IgG concentration, but all are semiquantitative. The zinc sulfate turbidity test provides an estimate of immunoglobulin concentration and correlates with IgG as measured by RID.[7,20] A modification of the zinc sulfate test in foals has been described in which visual evaluation of turbidity correctly identified 84% of foals with <400 mg/dL of IgG as measured by RID. All of the FTPI foals were identified if a spectrophotometer was used to confirm borderline visual assessments.[21] This test has several limitations, including the precipitation of immunoglobulins other than IgG with the addition of zinc sulfate and a false increase in the estimated immunoglobulin concentration by hemolysis.[20,22] The glutaraldehyde coagulation test is based on cross-linking between glutaraldehyde and basic proteins in the blood, including immunoglobulins, fibrinogen, and other proteins.[12,23] It is reported to have a sensitivity of nearly 1.0 at either the 400 or 800 mg/dL cutoff, but specificity ranges from 0.58 to 0.88 for <800 mg/dL and from 0.80 to 0.90 for <400 mg/dL.[8,19] As with the zinc sulfate turbidity test, hemolysis will shorten the coagulation time, leading to overestimation of IgG concentration.[8,23] Finally, there is a SNAP test[e] that is a semiquantitative immunoassay. A recent paper reported sensitivity and specificity as 0.76 and 0.95, respectively, for IgG <400 mg/dL and 0.88 and 0.90, respectively, for IgG <800 mg/dL.[19] The sensitivities and specificities reported here for the TIA are similar to those of the SNAP test.

Regression analysis and precision studies demonstrate that the test described here is precise and yields a reasonable estimate of IgG concentrations as determined by RID. The linear relationship between IgG concentrations determined by RID and TIA has a slope close to 1 and a *y*-intercept that is not significantly different from zero, indicating reasonable correlation between the 2 methods. The coefficient of determination (R^2) was lower than ideal. This finding might reflect a ''binning'' phenomenon of the RID. The RID is measured manually and usually is reported in increments of 100 mg/dL, whereas the turbidimetric method is reported in increments of 1 mg/dL. This ''binning'' will result in a lower coefficient of determination. The standardized residuals (Fig 2) demonstrate that no bias occurred in TIA measurement of IgG. Precision studies for the TIA showed excellent precision both within and between runs, demonstrating that the methodological variation of the TIA is unlikely to introduce error into the clinical decision-making process.

The subjective method of RID measurement introduces variability into the measurement of IgG. The methodology leaves numerous places for technical error and variability in measurement. The RID test, although the current gold standard, might lack precision.[11–13]

The TIA has a linear range of 300–3,500 mg/dL, whereas the linear range of the RID is 400–1,600 mg/dL. Test results of the RID falling outside this range are interpreted as <400 mg/dL or >1,600 mg/dL. The extended analytical range of the TIA might not be clinically useful, but it might be important in monitoring immunoglobulin concentrations and response to treatment.

The TIA is reportedly not affected by hemolysis in calves[14] or by icterus or lipemia in humans,[d] but we did not evaluate the effect of these factors in foals. Antigen excess can occur (eg, gammopathy secondary to inflammation or immunoproliferative disease) and cause interference in the TIA assay by a high-dose hook effect.[14,d] This condition would be unusual in neonatal foals and is not likely to be a confounding problem.

Long-term freezing studies on these samples suggested

that care should be taken in interpreting measurements on samples that have been frozen at −20°C, as indicated by the relatively large decline in serum IgG concentration measured by TIA after freezing for 10–12 months.

IgG measurement by TIA is comparable to other methods currently used, including RID, zinc sulfate turbidity, glutaraldehyde coagulation, and SNAP ELISA. The precision of the TIA method is higher than that previously reported for RID and is likely to represent an analytical improvement.[11–13] The TIA has the added benefits of being completely automated and having a quick turnaround time. The TIA does require specific reagents but can be measured on standard chemistry analyzers that are commonly used in veterinary chemistry laboratories.

Footnotes

[a] Hitachi 911 Chemistry Analyzer, Roche/Hitachi, Indianapolis, IN
[b] IgG SRID kit, VMRD Inc, Pullman, WA
[c] TIA reagents, Midland Bioproducts Corporation, Boone, IA
[d] IgG [package insert, 2001], Roche Diagnostics Corporation, Indianapolis, IN
[e] SNAP Foal IgG Test Kit, IDEXX Laboratories Inc, Westbrook, ME

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