EQUINE

Evaluation of five commercially available assays and measurement of serum total protein concentration via refractometry for the diagnosis of failure of passive transfer of immunity in foals

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Objective—To determine and compare sensitivity, specificity, accuracy, and predictive values of measurement of serum total protein concentration by refractometry as well as 5 commercially available kits for the diagnosis of failure of passive transfer (FPT) of immunity in foals.

Design—Prospective study.

Animals—65 foals with various medical problems and 35 clinically normal foals.

Procedure—IgG concentration in serum was assessed by use of zinc sulfate turbidity (assay C), glutaraldehyde coagulation (assay D), 2 semiquantitative immunoassays (assays F and G), and a quantitative immunoassay (assay H). Serum total protein concentration was assessed by refractometry. Radial immunodiffusion (assays A and B) was used as the reference method.

Results—For detection of IgG < 400 mg/dL, sensitivity of assay H (100%) was not significantly different from that of assays C, E, and G (88.9%). Specificity of assays H (96.0%) and G (95.8%) was significantly higher than that of assays C (79.4%) and E (78.1%). For detection of IgG < 800 mg/dL, sensitivities of assays H (97.6%), D (92.9%), C (81.0%), and G (81.0%) were significantly higher than that of assay F (52.4%). Specificity of assays F (100%), G (94.7%), and H (82.8%) was significantly higher than that of assays C (56.9%) and D (58.6%). Serum total protein concentration ≤ 4.5 g/dL was suggestive of FPT, whereas values ≥ 6.0 g/dL indicated adequate IgG concentrations.

Conclusions and Clinical Relevance—Most assays were adequate as initial screening tests. However, their use as a definitive test would result in unnecessary treatment of foals with adequate IgG concentrations. (*J Am Vet Med Assoc* 2005;227:1640–1645)

Although newborn foals are immunocompetent at birth, they are born with negligible concentrations of circulating antibodies. Immunoglobulins acquired from the dam's colostrum are essential for preventing infection during the lag time between exposure to pathogens and development of a protective immune response. The predominant immunoglobulin in equine colostrum is IgG with lesser quantities of IgA and IgM.[1] Healthy foals consuming good-quality colostrum have IgG concentrations > 800 mg/dL. **Failure of passive transfer (FPT)** of immunity is typically defined as serum IgG concentrations < 400 mg/dL after 24 hours of age, whereas partial FPT is defined as serum IgG concentrations between 400 and 800 mg/dL. The incidence of FPT in foals has ranged between 3% and 20%.[1–9]

Sepsis is the leading cause of disease and death in newborn foals.[10] Results of several studies[4–6,8,10] indicate a positive correlation between FPT and bacterial sepsis. In a prospective study,[11] 7 of 8 colostrum-deprived foals developed clinical signs of infection, and bacterial sepsis was confirmed via bacteriologic culture in 5 foals. Results of these studies emphasize the importance of early diagnosis and treatment of FPT.

Radial immunodiffusion (RID) has long been recognized as the gold standard for determination of IgG concentrations in equine serum.[12] Radial immunodiffusion assays for measurement of equine IgG are commercially available from various manufacturers. To the authors' knowledge, the agreement between results of RID assays from various manufacturers has never been determined. The major disadvantage of RID is that it takes 18 to 24 hours to obtain test results. This prolonged incubation results in a considerable delay in treatment of foals with FPT. In addition, RID relies on the availability of laboratory material and skilled technical personnel.

Estimation of total protein concentrations in serum by refractometry represents a rapid, inexpensive, and accurate test for the diagnosis of FPT in calves.[13] However, because of the wide range of serum total protein concentrations in newborn foals, refractometry was an unreliable indicator of FPT in foals in 1 study.[14] Several rapid stall-side screening tests have been used to estimate the concentration of IgG in neonatal foals. These tests use such mechanisms as zinc sulfate turbidity, glutaraldehyde coagulation, latex agglutination, turbidimetric immunoassays, and enzyme immunoassays.[4,15–19] These methodologies have been explained in detail elsewhere.[12,19,20] Many of these assays are now commercially available as kits, which are commonly used by horse owners and veterinarians. However, their diagnostic performance has not been evaluated and compared in a single independent study.

The purpose of the study reported here was to determine and compare sensitivity, specificity, accuracy, and predictive values of measurement of serum total protein concentration by refractometry as well as 5 commercially available kits for the diagnosis of FPT. An additional objective was to determine the agreement between 2 commercially available RID assays for the quantitation of equine IgG.

Materials and Methods

Samples and assays—Blood was collected at the time of admission from 65 foals evaluated at the Hofmann Equine

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Neonatal Intensive Care Unit of the University of Florida Veterinary Medical Center for various medical problems. In addition, blood was collected 2 to 24 hours after parturition from 35 clinically normal foals. Blood samples were permitted to clot at 22°C for 15 minutes. Serum was separated and frozen at –80°C until assayed. Each sample was thawed and tested for IgG content by use of RID plates from 2 manufacturers[a,b] (assays A and B), zinc sulfate turbidity[c] (assay C), glutaraldehyde coagulation[d] (assay D), 3 semiquantitative enzyme immunoassays[e–g] (assays E, F, and G), and a handheld quantitative colorimetric immunoassay[h] (assay H; **Table 1**). Each assay was performed exactly as described by the manufacturer. The serum total protein concentration was estimated by refractometry.[i] All assays were performed by the same experienced individual. Samples were coded in such a way that the individual performing the assays was unaware of the source of the serum sample (ill vs healthy foal) and RID assay results.

Data analysis—For each sample, the average of IgG concentrations obtained from the 2 brands of RID plates (assays A and B) was used as the reference method to which the other assay results were compared. Sensitivity, specificity, and accuracy of assays C, E, F, G, and H were calculated for the detection of serum IgG concentrations at breakpoints of < 400 mg/dL and < 800 mg/dL. Assay D only permits evaluation of IgG concentrations at a cutoff value of < 800 mg/dL. Sensitivity, specificity, and accuracy among assays were compared by use of the McNemar test for paired proportions.

The diagnostic performance of measurement of serum total protein concentration by refractometry was assessed by use of **receiver operating characteristic (ROC)** curve analysis.[21] The true-positive rate (sensitivity) was plotted as a function of the false-positive rate (100 – specificity) for each possible serum total protein concentration cutoff value. Each value on a ROC curve represents a sensitivity-specificity pair corresponding to a particular decision threshold. The **area under the curve (AUC)** of a ROC curve is a summary statistic of overall diagnostic performance of a test. Receiver operating characteristic curves for diagnostic tests with perfect discrimination between negative and positive reference samples have an AUC equal to 1.00 (100% sensitivity and 100% specificity). Assays can be distinguished as noninformative (AUC, 0.50), less accurate (AUC, 0.50 to 0.70), moderately accurate (AUC, 0.71 to 0.90), and perfect (AUC, 1.00).[21] The 95% **confidence interval (CI)** for the AUC was calculated. Confidence intervals that do not include the 0.5 value indicate that a given test has an ability to distinguish between foals with and without FPT.

Although sensitivity and specificity are fixed characteristics of a test, predictive values of positive and negative tests will vary with the prevalence of the disease. To simulate the diagnostic performance of each assay, predictive values of positive and negative test results were estimated on the basis of a prevalence of FPT of immunity of 15%. This value was selected on the basis of the median value of the prevalence of FPT in several studies.[1–9]

Agreement between assay H and the reference method and agreement between assays A and B were determined by use of the method reported by Bland and Altman.[22] For each cutoff value (< 400 mg/dL, 400 to 800 mg/dL, and > 800 mg/dL), bias was calculated as the mean difference between the reference method and assay H or as the mean difference

Table 1—Comparison of various assays for detection of IgG in equine serum.

Assay	Sample type	Sample volume (mL)	Time	Tests per kit	Cost per kit ($)	Cost per test ($)
A[a]	S	0.003	18–24 h	30	85.00	2.83*
				60	150.00	2.50*
B[b]	S, P	0.005	5–18 h	24	68.40	2.84*
H[h]†	S, P	0.005	20 min	10	120.00	12.00
				20	200.00	10.00
C[c]	S	0.1	1 h	10	50.00	5.00
				24	95.00	3.96
E[e] and F[f]	S, P	0.2	20 min	3	22.50	7.50
				6	36.00	6.00
				12	69.00	5.75
				24	132.00	5.50
G[g]	S, P, B	unspecified	7 min	10	136.50	13.65
D[d]	S, B	1.5	5 min	10	27.50	2.75
				25	50.00	2.00

*Price for each test will be higher if only a few samples are evaluated at a time because standards must be evaluated each time. †The cost of the analyzer required for this assay is $695.00.
S = Serum. P = Plasma. B = Blood.

Table 2—Accuracy and diagnostic performance (95% confidence intervals [CIs]) of 4 commercially available assays for the detection of serum IgG at concentrations < 400 mg/dL in neonatal foals.

				Predictive values (prevalence of 15%)	
Assay	Accuracy	Sensitivity	Specificity	Positive test result	Negative test result
H[h]	97.0 (91.5–99.4)[a]	100 (89.5–100)[a]	96.0 (88.5–99.1)[a]	81.5	100
C[c]	82.0 (73.1–89.0)[b]	88.9 (70.8–97.7)[a]	79.4 (68.4–88.0)[b]	43.2	97.6
E[e]	81.0 (71.9–88.2)[b]	88.9 (70.8–97.7)[a]	78.1 (66.9–86.9)[b]	41.7	97.5
G[g]	93.4 (87.3–97.7)[a]	88.9 (70.8–97.7)[a]	95.8 (88.3–99.1)[a]	78.9	95.8

Data given as percentages. To simulate the diagnostic performance of each assay, predictive values of positive and negative test results were estimated on the basis of a prevalence of failure of passive transfer (FPT) of immunity of 15%.
[a,b]Within a column, values with different superscript letters are significantly ($P < 0.05$) different.

between RID assays A and B, respectively. The limits of agreement were reported as bias ± (1.96 × SD of the bias). A Kruskal-Wallis 1-way ANOVA on ranks was used to compare bias at IgG concentrations < 400 mg/dL, 400 to 800 mg/dL, and > 800 mg/dL. When appropriate, pairwise multiple comparisons were performed by use of the Dunn test. A value of $P < 0.05$ was considered significant.

Results

The prevalence of FPT at serum IgG concentrations < 400 mg/dL and < 800 mg/dL was 27% and 42%, respectively. Serum IgG concentrations determined by the reference method (RID) were < 400 mg/dL in 27 samples, 400 to 800 mg/dL in 15 samples, and > 800 mg/dL in 58 samples. For detection of IgG concentrations < 400 mg/dL, sensitivity of assay H was not significantly different from that of assays E, C, and G. Specificity and accuracy of assays G and H were significantly higher than that of assays C and E (**Table 2**). For detection of IgG concentrations < 800 mg/dL, sensitivity of assays C, D, G, and H was significantly higher than that of assay F. Specificity of assays F, G, and H was significantly higher than that of assays C and D (**Table 3**). Accuracy of assays G and H was significantly higher than that of assays C and D.

Serum total protein concentration as estimated by refractometry ranged between 3.3 and 8.2 g/dL (mean ± SD, 5.3 ± 1.0 g/dL). The ROC area of serum total protein concentration for detection of serum IgG concentrations < 400 mg/dL was 0.82 (95% CI, 0.73 to 0.89). By use of a cutoff value of ≤ 4.5 g/dL, the sensitivity and specificity of the serum total protein concentration were 51.9% and 95.9%, respectively (**Table 4**). The ROC area of serum total protein concentration for detection of serum IgG concentrations < 800 mg/dL was 0.80 (95% CI, 0.71 to 0.87). By use of a cutoff value of ≤ 4.5 g/dL, the sensitivity and specificity of the serum total protein concentration were 42.9% and 96.6%, respectively (**Table 5**).

Table 3—Accuracy and diagnostic performance (95% CIs) of 5 commercially available assays for the detection of serum IgG at concentrations < 800 mg/dL in neonatal foals.

				Predictive values (prevalence of 15%)	
Assay	**Accuracy**	**Sensitivity**	**Specificity**	**Positive test result**	**Negative test result**
H[h]	89.0 (81.2–94.4)[a]	97.6 (87.4–99.9)[a]	82.8 (70.6–91.4)[a]	50.0	99.5
C[c]	67.0 (56.9–76.1)[b]	81.0 (65.9–91.4)[b]	56.9 (43.2–69.8)[c]	24.9	94.4
F[f]	80.0 (70.8–87.3)[a,b]	52.4 (36.4–68.0)[c]	100 (95.0–100)[b]	100	92.3
G[g]	88.9 (81.0–94.3)[a]	81.0 (65.9–91.4)[b]	94.7 (85.4–98.9)[a,b]	73.0	96.6
D[d]	73.0 (63.2–81.4)[b]	92.9 (80.5–98.5)[a,b]	58.6 (44.9–71.4)[c]	22.4	97.9

Data are given as percentages. To simulate the diagnostic performance of each assay, predictive values of positive and negative test results were estimated on the basis of a prevalence of FPT of immunity of 15%.
[a–c]Within a column, values with different superscript letters are significantly ($P < 0.05$) different.

Table 4—Diagnostic performance (95% CIs) of measurement of serum total protein concentrations via refractometry at various cutoff values for detection of serum IgG concentrations < 400 mg/dL in neonatal foals.

			Predictive values (prevalence of 15%)	
Cutoff value (g/dL)	**Sensitivity**	**Specificity**	**Positive test result**	**Negative test result**
≤ 4.5	51.9 (32.0–71.3)	95.9 (88.4–99.1)	69.0	91.9
≤ 5.0	74.1 (53.7–88.8)	69.9 (58.0–80.1)	30.3	93.9
≤ 5.5	92.6 (75.7–98.9)	41.1 (29.7–53.2)	21.7	96.1
≤ 6.0	96.3 (81.0–99.4)	17.8 (9.8–28.5)	17.1	96.9

Data are given as percentages. To simulate the diagnostic performance of each assay, predictive values of positive and negative test results were estimated on the basis of a prevalence of FPT of immunity of 15%.

Table 5—Diagnostic performance (95% CIs) of measurement of serum total protein concentrations via refractometry at various cutoff values for detection of serum IgG concentrations < 800 mg/dL in neonatal foals.

			Predictive values (prevalence of 15%)	
Cutoff value (g/dL)	**Sensitivity**	**Specificity**	**Positive test result**	**Negative test result**
≤ 4.5	42.9 (27.7–59.0)	96.6 (88.1–99.5)	68.7	90.5
≤ 5.0	66.7 (50.5–80.4)	75.9 (62.8–86.1)	32.8	92.8
≤ 5.5	85.7 (71.4–94.5)	44.8 (31.7–58.5)	21.5	94.7
≤ 6.0	95.2 (83.8–99.3)	20.7 (11.2–33.4)	17.5	96.1

Data are given as percentages. To simulate the diagnostic performance of each assay, predictive values of positive and negative test results were estimated on the basis of a prevalence of FPT of immunity of 15%.

Table 6—Summary statistics (mg/dL) of the differences between the reference method* and a handheld quantitative colorimetric immunoassay[h] (assay H) or the difference between 2 radial immunodiffusion[a,b] assays (assays A and B) at serum IgG concentrations < 400 mg/dL, 400 to 800 mg/dL, and > 800 mg/dL.

Comparisons	IgG (mg/dL)	Mean bias (± SD)	Limits of agreements
Reference method and assay H	< 400	46.7 ± 97[a]	–143 to 237
	400–800	256 ± 233[b]	–199 to 712
	> 800	385 ± 378[b]	–354 to 1,126
Assays A and B	< 400	72.7 ± 91.2[a]	–101 to 251
	400–800	–136.7 ± 239[b]	–605 to 332
	> 800	–61.5 ± 748[b]	–1,528 to 1,405

*For each sample, the average of IgG concentrations obtained from the 2 radial immunodiffusion assays[a,b] (assays A and B) was used as the reference method to which the other assay results were compared.

[a,b]Within a comparison, mean bias values with different superscript letters are significantly ($P \leq 0.01$) different.

The bias of assay H was significantly ($P < 0.001$) lower at serum IgG concentrations < 400 mg/dL than at higher concentrations (**Table 6**). Eleven of 21 (52%) samples with IgG concentrations between 400 and 800 mg/dL with assay H also had IgG concentrations between 400 and 800 mg/dL with the reference method. All 7 samples with IgG concentrations between 600 and 800 mg/dL with assay H gave IgG concentrations > 800 mg/dL with the reference method. There was perfect agreement between RID assays A and B for all 100 samples (100%) at a cutoff value of < 400 mg/dL. There was perfect agreement between RID assays A and B for 92 samples (92%) at a cutoff value of < 800 mg/dL. The bias between RID assays A and B was significantly ($P = 0.003$) lower at serum IgG concentrations < 400 mg/dL than at higher concentrations.

Discussion

Results of several studies[4–6,8,10] indicate a positive correlation between FPT and bacterial sepsis. However, many factors such as environmental and management conditions, stress, concurrent disease, and virulence of a given pathogen may also contribute to the development of sepsis.[3,23] Intravenous administration of plasma is typically indicated in foals with IgG concentrations < 400 mg/dL at 18 to 24 hours after birth.[23] Clinically normal foals with no apparent risk factors for sepsis and IgG concentrations between 400 and 800 mg/dL may not require administration of plasma if they are housed in a clean environment. However, it is generally recommended that foals at high risk for developing sepsis with IgG concentrations between 400 and 800 mg/dL at 18 to 24 hours after birth be administered IV plasma.[23] The definitions of test performance in the study reported here were calculated on the basis of threshold values of 400 and 800 mg/dL, to reflect these clinically relevant cutoff concentrations.

Although RID has universally been accepted as the gold standard for measurement of IgG concentrations in horses, to the authors' knowledge, the agreement between RID assays from various manufacturers has never been investigated. Agreement between RID assays A and B was good at low concentrations (< 400 mg/dL) with small mean bias and narrow limits of agreement. The limits of agreement considerably increased with increasing IgG concentrations (–1,528 to 1,405 mg/dL at concentrations > 800 mg/dL). One factor that may have contributed to the large bias was the difference in the high-concentration reference standards provided in the RID kits. The high-concentration standard for assay A has an IgG concentration of 1,600 mg/dL, whereas the high-concentration standard for assay B was 2,582 mg/dL. Nevertheless, the difference in the range of standards provided between manufacturers does not explain the wide limits of agreement obtained at concentrations between 400 and 800 mg/dL. Results of our study emphasize the need for universal reference standards among manufacturers of RID assays. Because there is no published information suggesting that a given RID assay is more accurate than the other, we chose to use the average value of the 2 RID assays as the reference method to which other tests were compared. Comparison of the 2 RID assays to a validated turbidimetric assay may have helped in determining which of the 2 RID assays was most accurate. Despite large bias between assays at high concentrations, there was perfect agreement between the 2 RID assays for the detection of IgG concentrations < 400 mg/dL and good agreement (agreement in 92% of samples) for the detection of IgG concentrations < 800 mg/dL.

When used for the purpose of screening, a test with a high sensitivity must be chosen. This ensures a high predictive value of a negative test, hence identifying of most foals with FPT. To confirm FPT, foals with positive results in the screening test should be tested with a different confirmatory test. In this second test, a high specificity and positive predictive value are required. At a cutoff value of 400 mg/dL, sensitivity was similar for each assay evaluated, resulting in negative predictive values > 95%. As a result, all assays evaluated would be suitable screening tests. However, specificity of assays C or E was significantly lower than that of assays G and H, resulting in much lower positive predictive values. Positive results with assays C or E should not be interpreted as an indication for treatment, and results should be confirmed with a more specific assay.

At a cutoff value of 800 mg/dL, sensitivity was greater with assay H (97.6%) and assay D (92.9%), followed by assay C (81.0%) and assay G (81.0%). Any of these tests would be adequate as a screening test. In contrast, the sensitivity of assay F was too low to justify its use as a screening test. The specificity of assay H (82.8%), assay C (56.9%), and assay D (58.6%) was significantly lower than that of assay G (94.7%) or assay F (100%). As a result, a positive test result with assays C, D, or H at a cutoff value of 800 mg/dL should not be interpreted as an indication for treatment, and results should be confirmed with a more specific test.

In the study reported here, the high sensitivity of assay D was consistent with results of other studies[24,j] evaluating various modifications of the glutaraldehyde coagulation test in foals. However, the specificity (59% to 94%) of glutaraldehyde coagulation–based assays in foals varies considerably between studies.[24,j] Similarly, the diagnostic performance of various modifications of the zinc sulfate turbidity test varies con-

siderably between studies.[25,26] In our study, use of assay C resulted in adequate sensitivity but poor specificity, especially at the cutoff value of 800 mg/dL. Assay C requires distinction between the amounts of precipitate in the test tube relative to a photograph provided by the manufacturer. The subjective nature of this test may have contributed to decreased performance observed in our study, compared with other less subjective screening tests. Results of 1 study[18] indicate that assay G was found to be 80% to 89% accurate for the detection of low (< 400 mg/dL) and high (> 800 mg/dL) IgG concentrations. However, IgG concentrations between 400 and 800 mg/dL with assay G were found to agree with RID in only 2 of 13 samples.[18] In our study, IgG concentrations between 400 and 800 mg/dL with assay G agreed with RID in 5 of 10 samples. Sensitivity and specificity of assay G in the study reported here were similar or slightly superior to those previously reported.[j]

Of the commercial assays evaluated in our study, only assay H had quantitative results. This assay resulted in the highest sensitivity, specificity, and accuracy at a cutoff value of 400 mg/dL. The agreement between assay H and RID was excellent at low (< 400 mg/dL) IgG concentrations with a small bias and narrow limits of agreement. In contrast, bias was significantly higher at concentrations > 400 mg/dL, with assay H generally underestimating RID results. Underestimation of RID results at high IgG concentrations resulted in lower specificity of the assay at a cutoff value of 800 mg/dL than at a value of 400 mg/dL. In our study, IgG concentrations between 400 and 800 mg/dL with assay H agreed with RID in only 11 of 21 samples. All 7 samples with IgG concentrations between 600 and 800 mg/dL with assay H gave IgG concentrations > 800 mg/dL by RID. Therefore, use of assay H will result in unnecessary treatment of some foals when a cutoff value of 800 mg/dL is used to decide if administration of plasma is necessary.

Refractometry only provides a crude estimate of serum total protein concentrations, and more accurate methods such as spectrophotometry are available. We choose to use refractometry because it can be performed on the farm without having to send the sample to a specialized laboratory. The choice of the most appropriate cutoff value for estimation of serum total protein concentrations by refractometry should include a consideration of the distribution of sensitivity and specificity in clinically normal foals and foals with FPT, the prevalence of disease in the population to be tested, and the consequences of false-positive and false-negative test results.[27] The consequence of missing FPT in a foal (false-negative result) may be, in the worst-case scenario, death from sepsis. On the other hand, the consequences of false-positive results would be the turmoil and financial losses associated with treatment of healthy foals and, rarely, adverse effects from administration of unnecessary plasma. Serum total protein concentration in foals prior to suckling varies widely.[14] In that study, the coefficient of correlation between measurement of serum total protein concentrations by refractometry and RID was 0.85. However, the wide dispersal of data points led to the conclusion that serious errors might result if serum total protein concentrations are used to estimate IgG concentrations in foals.[14] As a result, measurement of serum total protein concentration has not been recommended as a screening test for FPT in foals.

In calves, use of refractometry with a cutoff value for serum total protein concentration of 5.5 g/dL results in a sensitivity of 94% and a specificity of 76% for the detection of serum IgG < 1,000 mg/dL.[13] In our study, a cutoff value of 5.5 g/dL in foals resulted in similar sensitivity but much lower specificity. Conversely, use of a lower cutoff value improved specificity to the detriment of sensitivity. Nevertheless, the use of serum total protein concentration may be of some clinical value in foals. On the basis of results of our study, a foal with a serum total protein concentration ≤ 4.5 g/dL is likely to have IgG concentrations < 400 mg/dL. The positive predictive value of this cutoff value (69.0%) was superior to that of half of the commercial kits evaluated (41.7% to 81.5%). In addition, a cutoff value of ≥ 5.5 g/dL for serum total protein concentration resulted in a sensitivity of 92.6%. The negative predictive value of such a cutoff value was > 96%, indicating that foals with serum total protein concentrations ≥ 5.5 g/dL were unlikely to have IgG concentrations < 400 mg/dL. Similarly, foals with serum total protein concentrations ≥ 6.0 g/dL were unlikely to have IgG concentrations < 800 mg/dL (negative predictive value of 96.1%). The negative predictive value of these cutoff values compares with those of the best screening tests evaluated. Foals with serum total protein concentrations between 4.5 and 5.5 g/dL had unpredictable IgG concentrations. This represented 42% of the foals in the study, therefore limiting the value of serum total protein concentration as a stand-alone screening test.

In the study reported here, many of the assays evaluated had good sensitivity and were acceptable as initial screening tests for IgG concentrations in foals. However, many of the assays evaluated lack specificity, and their use as definitive tests would result in unnecessary treatment of foals with adequate IgG concentrations.

a. Equine IgG RID Kit, courtesy of VMRD Inc, Pullman, Wash.
b. Equine IgG Test Kit, courtesy of Kent Laboratories/Triple J Farms, Bellingham, Wash.
c. Equi Z Equine FPT Test Kit, courtesy of VMRD Inc, Pullman, Wash.
d. Gamma-Check-E, courtesy of Plasvacc USA Inc, Templeton, Calif.
e. Midland Plasma Foal IgG Quick Test Kit (400 mg/dL), courtesy of Midland BioProducts Corp, Boone, Iowa.
f. Midland Plasma Foal IgG Quick Test Kit (800 mg/dL), courtesy of Midland BioProducts Corp, Boone, Iowa.
g. Snap Foal IgG, courtesy of IDEXX Laboratories, Westbrook, Me.
h. DVM Stat, courtesy of VDx Inc, Belgium, Wis.
i. Refractometer, American Optical Co, Buffalo, NY.
j. McClure JT, DeLuca JL, Miller J. Comparison of five screening tests for detection of failure of passive transfer in foals (abstr), in *Proceedings*. 20th Am Coll Vet Intern Med Forum 2002;770.

References

1. Kohn CW, Knight D, Hueston W, et al. Colostral and serum IgG, IgA, and IgM concentrations in Standardbred mares and their foals at parturition. *J Am Vet Med Assoc* 1989;195:64–68.

2. LeBlanc MM, Tran T, Baldwin JL, et al. Factors that influence passive transfer of immunoglobulins in foals. *J Am Vet Med Assoc* 1992;200:179–183.

3. Baldwin JL, Cooper WL, Vanderwall DK, et al. Prevalence

(treatment days) and severity of illness in hypogammaglobulinemic and normogammaglobulinemic foals. *J Am Vet Med Assoc* 1991;198: 423–428.

4. Clabough DL, Levine JF, Grant GL, et al. Factors associated with failure of passive transfer of colostral antibodies in Standardbred foals. *J Vet Intern Med* 1991;5:335–340.

5. Haas SD, Bristol F, Card CE. Risk factors associated with the incidence of foal mortality in an extensively managed mare herd. *Can Vet J* 1996;37:91–95.

6. McGuire TC, Crawford TB, Hallowell AL, et al. Failure of colostral immunoglobulin transfer as an explanation for most infections and deaths of neonatal foals. *J Am Vet Med Assoc* 1977;170:1302–1304.

7. Morris DD, Meirs DA, Merryman GS. Passive transfer failure in horses: incidence and causative factors on a breeding farm. *Am J Vet Res* 1985;46:2294–2299.

8. Raidal SL. The incidence and consequences of failure of passive transfer of immunity on a Thoroughbred breeding farm. *Aust Vet J* 1996;73:201–206.

9. Perryman LE, McGuire TC. Evaluation for immune system failures in horses and ponies. *J Am Vet Med Assoc* 1980;176:1374–1377.

10. Cohen ND. Causes of and farm management factors associated with disease and death in foals. *J Am Vet Med Assoc* 1994;204: 1644–1651.

11. Robinson JA, Allen GK, Green EM, et al. A prospective study of septicaemia in colostrum-deprived foals. *Equine Vet J* 1993; 25:214–219.

12. Young KM, Lunn DP. Immunodiagnostic testing in horses. *Vet Clin North Am Equine Pract* 2000;16:79–103.

13. Tyler JW, Hancock DD, Parish SM, et al. Evaluation of 3 assays for failure of passive transfer in calves. *J Vet Intern Med* 1996; 10:304–307.

14. Rumbaugh GE, Ardans AA, Ginno D, et al. Measurement of neonatal equine immunoglobulins for assessment of colostral immunoglobulin transfer: comparison of single radial immunodiffusion with the zinc sulfate turbidity test, serum electrophoresis, refractometry for total serum protein, and the sodium sulfite precipitation test. *J Am Vet Med Assoc* 1978;172:321–325.

15. Bauer JE, Brooks TP. Immunoturbidimetric quantification of serum immunoglobulin G concentration in foals. *Am J Vet Res* 1990;51:1211–1214.

16. Beetson SA, Hilbert BJ, Mills JN. The use of the glutaraldehyde coagulation test for detection of hypogammaglobulinaemia in neonatal foals. *Aust Vet J* 1985;62:279–281.

17. Bertone JJ, Jones RL, Curtis CR. Evaluation of a test kit for determination of serum immunoglobulin G concentration in foals. *J Vet Intern Med* 1988;2:181–183.

18. Pusterla N, Pusterla JB, Spier SJ, et al. Evaluation of the SNAP foal IgG test for the semiquantitative measurement of immunoglobulin G in foals. *Vet Rec* 2002;151:258–260.

19. Davis DG, Schaefer DMW, Hinchcliff KW, et al. Measurement of serum IgG in foals by radial immunodiffusion and automated turbidimetric immunoassay. *J Vet Intern Med* 2005;19:93–96.

20. Weaver DM, Tyler JW, VanMetre DC, et al. Passive transfer of colostral immunoglobulins in calves. *J Vet Intern Med* 2000;14: 569–577.

21. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Prev Vet Med* 2000;45:23–41.

22. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–310.

23. Sellon DC. Secondary immunodeficiencies of horses. *Vet Clin North Am Equine Pract* 2000;16:117–130.

24. Clabough DL, Conboy HS, Roberts MC. Comparison of four screening techniques for the diagnosis of equine neonatal hypogammaglobulinemia. *J Am Vet Med Assoc* 1989;194:1717–1720.

25. Baird AN, Pugh DG, Rupp GP, et al. Detection of immunoglobulin G in the foal. *J Equine Vet Sci* 1987;7:124–129.

26. LeBlanc MM, Hurtgen JP, Lyle S. A modified zinc sulfate turbidity test for the detection of immune status in newly born foals. *J Equine Vet Sci* 1990;10:36–39.

27. Smith RD. Evaluation of diagnostic tests. In: *Veterinary clinical epidemiology: a problem-oriented approach*. 2nd ed. Boca Raton, Fla: CRC Press Inc, 1995;31–53.