Evaluation of a point-of-care portable analyzer for measurement of plasma immunoglobulin G, total protein, and albumin concentrations in ill neonatal foals

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Objective—To compare the diagnostic performance of a point-of-care (POC) analyzer with that of established methods for the measurement of plasma IgG, total protein, and albumin concentrations in neonatal foals.

Design—Evaluation study.

Animals—100 neonatal foals < 7 days of age.

Procedures—Plasma IgG, total protein, and albumin concentrations were measured with a POC analyzer via an immunoturbidimetric method. Corresponding measurements of plasma IgG, total protein, and albumin concentrations were measured by means of automated biochemical analyzers via automated immunoturbidimetric, biuret, and bromocresol green dye–binding assays, respectively (standard laboratory methods).

Results—The sensitivity and specificity of the POC analyzer for detection of failure of passive transfer of immunity (FPTI) in foals were 80.7% and 100%, respectively, when FPTI was defined as a plasma IgG concentration < 400 mg/dL and were 75.9% and 100%, respectively, when FPTI was defined as a plasma IgG concentration < 800 mg/dL. The POC analyzer overestimated plasma albumin concentrations and, to a lesser extent, plasma total protein concentrations, compared with values determined with the standard laboratory methods.

Conclusions and Clinical Relevance—Results suggested the POC analyzer was acceptable for determination of plasma IgG and total protein concentrations in ill foals. The POC analyzer overestimated plasma albumin concentration such that its use was clinically unacceptable for the determination of that concentration. The POC analyzer provided timely measurements of plasma IgG concentrations, which is necessary information for the assessment of passive transfer of maternal antibodies to neonatal foals. (J Am Vet Med Assoc 2013;242:812–819)
mize complications associated with FPTI. As a result, numerous quantitative and semiquantitative diagnostic tests have been developed for the assessment of passive immunity in foals.16 The single RID method is a quantitative test for measurement of serum IgG concentration, and historically, its use has been considered the gold standard. However, the RID test is expensive, it is not readily available, it is susceptible to human error during measurement of the precipitin-ring diameter, and its results are temperature dependent and not available until 18 to 24 hours after the test is initiated.1,2 Additionally, the serum IgG concentration determined via RID kits from different manufacturers can vary substantially for the same sample.16 The immunoturbidimetric assay is an alternative method for the measurement of serum or plasma immunoglobulin concentrations in which an antibody directed against a specific analyte (ie, IgG) binds to that analyte and forms a complex that causes a change in the turbidity of the sample.17 The change in turbidity reduces the amount of light transmitted through the sample; the residual light transmitted through the sample is measured via spectrophotometry and provides a measure of the analyte concentration.17 Numerous immunoturbidimetric assays, including an IgG assay, have become automated so that the test can be performed on most automated biochemical analyzers, thereby minimizing potential human errors in measurement, improving precision, and shortening the turnaround time for results, compared with results obtained via an RID method. In fact, automated immunoturbidimetric assays have been recommended as an alternative to the RID method as the gold standard for measurement of serum or plasma IgG concentration.18–20

Hypoproteinemia and hypoalbuminemia are other common clinicopathologic abnormalities in foals with diseases such as enterocolitis, internal parasitism, and proliferative enteropathy (Lawsonia intracellularis infection).21–23 Although measurement of serum or plasma total protein and albumin concentrations is readily available, the ability to provide an accurate and affordable POC measurement of total protein and albumin concentrations may expedite the development of treatment plans, allow more frequent monitoring of total protein and albumin concentrations, and be beneficial for the care of critically ill foals.

A quantitative immunoturbidimetric assay for the measurement of serum or plasma IgG, total protein, and albumin concentrations has recently become commercially available by means of a POC portable analyzer. Although determination of serum IgG concentration in horses by the use of an immunoturbidimetric method via automated biochemical analyzers has been validated,16–20 the use of an immunoturbidimetric method via a POC portable analyzer has not been investigated. In veterinary medicine, it is important to evaluate the accuracy of a diagnostic test in the population for which it will be used before it is recommended for making decisions about or monitoring clinical response to treatment. The purpose of the study reported here was to investigate the performance of a POC portable analyzer that uses an immunoturbidimetric method to measure plasma IgG, total protein, and albumin concentrations in a group of ill foals and compare those results with results obtained via standard laboratory methods.

Materials and Methods

Animals—One hundred foals < 7 days of age with various medical problems that were admitted to the Hofmann Equine Neonatal Intensive Care Unit at the University of Florida Veterinary Medical Center were enrolled into the study. All study procedures were approved by the University of Florida Institutional Animal Care and Use Committee, and owner consent was obtained for each foal prior to study enrollment.

Sample collection—From each foal at the time of hospital admission, a venous blood (5 mL) sample was collected into a blood collection tube that contained heparin. Blood samples were centrifuged for 15 minutes at 5,000 × g. The plasma was removed from each sample and stored at −80°C until analyzed.

Determination of plasma IgG, total protein, and albumin concentrations via standard laboratory methods—Each plasma sample was thawed, and plasma IgG, total protein, and albumin concentrations were measured with automated biochemical analyzers. Specifically, plasma IgG concentration was determined via an immunoturbidimetric assay that used goat anti-equine IgG antiserum,12–14 IgG concentration was determined via a biuret assay, and albumin concentration was determined via a bromocresol green dye–binding assay. Quality control for the automated biochemical analyzers was performed daily.

Determination of plasma IgG, total protein, and albumin concentration via a POC portable analyzer—Additionally, for each sample, plasma IgG, total protein, and albumin concentrations were measured via immunoturbidimetric spectrophotometry by use of a commercially available POC portable analyzer in accordance with the manufacturer’s instructions. Briefly, the POC analyzer was calibrated by use of the reagent vials while the plasma samples and reagent vials were warmed to room temperature (approx 22°C) for 20 minutes. To each reagent vial, 10 μL of plasma was added, then the vial was mixed gently and incubated at room temperature for 5 minutes. Subsequently, the reagent vial was placed in the POC analyzer and covered with a plastic shroud to inhibit ambient-light interference with the analyzer’s spectrophotometric function. At analysis completion, the concentration of plasma IgG, total protein, or albumin was recorded. Quality control was performed for the POC analyzer in accordance with the manufacturer’s instructions daily. For each analyte evaluated throughout the study, the reagent vials (IgG, total protein, and albumin) originated from the same lot number.

Statistical analysis—For the purpose of the present study, plasma IgG, total protein, and albumin concentrations determined via the standard laboratory methods were considered the gold-standard, or definitive, measurements for all samples. On the basis of plasma IgG concentration results obtained via the standard laboratory method, 2 different cutoff values were
used to define FPTI (plasma IgG concentration, < 400 mg/dL and < 800 mg/dL) in the study foals, and the sensitivity, specificity, accuracy, and likelihood ratios for plasma IgG concentration results obtained via the POC analyzer for diagnosing FPTI were determined at each of those 2 cutoffs. For results obtained via both the standard laboratory method and the POC analyzer, a positive test result for FPTI was defined as a plasma IgG concentration < 400 mg/dL or < 800 mg/dL, respectively, whereas a negative test result was defined as a plasma IgG concentration ≥ 400 mg/dL or ≥ 800 mg/dL, respectively. Although sensitivity and specificity are fixed characteristics of a diagnostic test, predictive values for positive and negative test results vary with the prevalence of the disease in the population. Therefore, after the sensitivity and specificity of the POC analyzer for diagnosis of FPTI were determined, the respective predictive values for positive and negative test results were calculated for prevalences of FPTI ranging from 0% to 100%.

An ROC curve was generated to determine the optimal plasma IgG concentration determined via the POC analyzer to use as the cutoff for diagnosis of FPTI in foals at each of the 2 cutoffs for the standard laboratory method used to define FPTI (plasma IgG concentration, < 400 mg/dL and < 800 mg/dL).24 The agreement between the plasma IgG, total protein, and albumin concentrations obtained via the POC analyzer and those obtained via the standard laboratory methods was evaluated via the Bland-Altman method.25 For each sample and analyte (IgG, total protein, and albumin), the bias was calculated as the analyte concentration determined via the standard laboratory method minus the corresponding analyte concentration determined via the POC analyzer. The mean bias and 95% limits of agreement (mean ± 1.96 SD) were calculated. A positive bias value indicated that the portable analyzer underestimated the concentration of an analyte, whereas a negative bias value indicated that the portable analyzer overestimated the concentration of an analyte, compared with the concentration of that analyte determined via the standard laboratory method.

For each sample and analyte (IgG, total protein, and albumin concentrations), measurements were classified as low (IgG concentration, ≤ 400 mg/dL; total protein concentration, ≤ 4.5 g/dL; and albumin concentration, ≤ 2.5 g/dL), intermediate (IgG concentration, > 400 to < 800 mg/dL; total protein concentration, > 4.5 to < 7.0 g/dL; and albumin concentration, > 2.5 to < 3.5 g/dL), or high (IgG concentration, ≥ 800 mg/dL; total protein concentration, ≥ 7.0 g/dL; and albumin concentration, ≥ 3.5 g/dL). Then a Kruskal-Wallis 1-way ANOVA by ranks was performed to evaluate the effect of the magnitude of the low, intermediate, or high) of a measurement on the bias of that measurement for each analyte. When appropriate, pairwise multiple comparisons were performed via a Dunn test. For plasma IgG concentration, bias was significantly associated with the magnitude of the measurement; therefore, the relative bias was calculated for each sample as follows: (IgG concentration determined via the standard laboratory method – IgG concentration determined via the POC analyzer)/((IgG concentration determined via the standard laboratory method + IgG concentration determined via the POC analyzer)/2) × 100. Similar to bias, a positive value for relative bias indicated that the POC analyzer underestimated the concentration of an analyte, whereas a negative value for relative bias indicated that the POC analyzer overestimated the concentration of an analyte, compared with the concentration of that analyte determined via the standard laboratory method.

The agreement between measurements obtained via both the standard laboratory methods and the POC analyzer was also assessed via the Lin concordance correlation coefficient, which compares 2 techniques that measure the same variable without the inherent bias of establishing a gold standard.26-27 The Lin concordance correlation coefficient (ρc) integrates a measurement of precision and accuracy: ρc = ρ × Cb, where ρ is the Pearson correlation coefficient, which measures how far each observation deviates from the best-fit line (ie, precision), and Cb is a bias correction factor that measures how far the best-fit line deviates from a 45° line through the origin (ie, accuracy); a ρc equal to 1 indicates perfect agreement between the 2 techniques.26-27 All analyses were performed with commercial statistical software,26 and values of P < 0.05 were considered significant.

Results

Plasma IgG concentration—Of the 100 plasma samples evaluated, the plasma IgG concentration determined via the standard laboratory method was < 400 mg/dL in 31, 400 to < 800 mg/dL in 23, and ≥ 800 mg/dL in 46. Thus, when FPTI was defined as a plasma IgG concentration < 400 mg/dL as determined via the standard laboratory method, the POC analyzer had a sensitivity of 80.7%, specificity of 100%, accuracy of 94%, and likelihood ratio for a negative test result of 0.19 (the likelihood ratio for a positive test result could not be determined because there were no false-positive results [ie, the denominator was 0]). When FPTI was defined as a plasma IgG concentration < 800 mg/dL as determined via the standard laboratory method, the portable analyzer had a sensitivity of 75.9%, specificity of 100%, accuracy of 87%, and likelihood ratio for a negative test result of 0.24 (the likelihood ratio for a positive test result could not be determined because there were no false-positive results [ie, denominator was 0]). The predictive values for positive (IgG concentration, < 400 mg/dL and < 800 mg/dL, respectively) and negative (IgG concentration, ≥ 400 mg/dL and ≥ 800 mg/dL, respectively) test results were determined for the entire range (0% to 100%) of FPTI prevalence and plotted (Figure 1) for the POC analyzer.

The Kruskal-Wallis 1-way ANOVA by ranks results indicated that the bias for the plasma IgG concentration determined via the POC analyzer was significantly (P < 0.001) associated with high (≥ 800 mg/dL) measurements of plasma IgG concentrations. However, relative bias was not significantly (P = 0.380) associated with the magnitude of the plasma IgG concentration determined via the POC analyzer, and the mean relative bias was −32.1% (95% limits of agreement, −52.2% to 18.1% [Figure 2]). The Lin concordance correlation coefficient was 0.86 (95% CI, 0.80 to 0.90), which suggested moderate agreement between the plasma IgG concentration...
measured via the standard laboratory method and that measured via the POC analyzer. When FPTI was defined as a plasma IgG concentration < 400 mg/dL, as determined via the standard laboratory method, the area under the ROC curve for the portable analyzer was 0.995 (95% CI, 0.946 to 1.00), which suggested that measurement of plasma IgG concentration via the POC analyzer was very accurate. The optimal cutoff value for plasma IgG concentration measured via the POC analyzer for diagnosis of FPTI was 620 mg/dL. At that cutoff, the sensitivity and specificity of the POC analyzer were 96.3% (95% CI, 87.3% to 99.5%) and 95.7% (95% CI, 85.2% to 99.5%), respectively, and the likelihood ratios for positive and negative test results were 22.2 and 0.04, respectively.

Plasma total protein concentration—The median plasma total protein concentration as measured via the standard laboratory method was 4.9 g/dL (range, 1.1 to 8.9 g/dL). Unlike plasma IgG concentration, the magnitude of plasma albumin concentration was not significantly (P = 0.528) associated with bias. The mean bias for plasma total protein concentration measured via the POC analyzer, compared with that measured via the standard laboratory method, was –0.33 g/dL (95% limits of agreement, –0.71 to 0.05 g/dL; Figure 3). The Lin concordance correlation coefficient was 0.92 (95% CI, 0.88 to 0.94), which suggested moderate agreement between the plasma total protein concentration measured via the standard laboratory method and that measured via the POC analyzer.

Plasma albumin concentration—The median plasma albumin concentration as measured via the standard laboratory method was 2.9 g/dL (range, 0.5 to 4.7 g/dL). Similar to plasma total protein concentration, the magnitude of plasma albumin concentration was not significantly (P = 0.528) associated with bias. The mean bias for plasma albumin concentration measured via the POC analyzer, compared with that measured via the
Results of the present study indicated that the use of a POC portable analyzer provided accurate measurement of plasma IgG and total protein concentrations within 5 minutes after initiation of the tests and may be beneficial for the rapid assessment of passive transfer of maternal antibodies in neonatal foals. During the critical care of ill neonatal foals, it is often necessary for rapid determination of various blood variables to identify clinicopathologic abnormalities (ie, electrolyte derangements), develop a list of differential diagnoses, and formulate a treatment plan. The increased availability of POC analyzers to veterinary clinicians has expedited and improved stall-side evaluation of ill horses and foals. Point-of-care analyzers that have been evaluated in horses include handheld devices that measure concentrations of glucose, lactate, cardiac troponin, and hemoglobin. Portable ultrasonography and POC blood gas and electrolyte analyzers have also become readily available, which allow immediate stall-side imaging and evaluation of blood acid-base and electrolyte status. Because foals with FPTI have a higher risk of developing an infection than do foals with adequate concentrations of maternal antibodies, rapid and accurate measurement of serum or plasma IgG concentrations is important for the management and care of neonatal foals. Treatment of FPTI in foals is time dependent; the capacity of the intestine to absorb colostral antibodies is greatest within the first 12 hours after birth and then begins to decrease.

In the present study, when FPTI was defined as a plasma IgG concentration < 400 mg/dL as determined via the standard laboratory method, the sensitivity and likelihood ratio for a negative test result for the POC analyzer were 80% and 0.19, respectively. When FPTI was defined as a plasma IgG concentration < 800 mg/dL as determined via the standard laboratory method, the sensitivity and likelihood ratio for a negative test result for the POC analyzer were 76% and 0.24, respectively. This indicated that the likelihood of a foal having FPTI decreased approximately 4- to 5-fold given a negative test result (plasma IgG concentration, ≥ 400 mg/dL or ≥ 800 mg/dL) has FPTI (ie, false-negative result) is 3.4% and 4.5%, respectively. The specificity of the POC analyzer for detecting FPTI was 100%; no foals with adequate passive transfer of immunity were misclassified as having FPTI (ie, had a false-positive result). Thus, use of the POC analyzer for measurement of plasma IgG concentration in neonatal foals is unlikely to result in overdiagnosis of FPTI and unnecessary administration of plasma to foals with adequate passive transfer of immunity, and only a small proportion of foals with FPTI will be misdiagnosed (ie, have false-negative results). A diagnostic method used to screen a population for any disease, including FPTI in foals, should have
high sensitivity because this will ensure a high predictive value for a negative test result and allow for identification of most diseased individuals. In the present study, the sensitivity of the POC analyzer was substantially improved if the plasma IgG concentration cutoff used to define FPTI in foals was increased. When FPTI was defined as a plasma IgG concentration < 400 mg/dL as determined via the standard laboratory method, increasing the cutoff for plasma IgG concentration measured via the POC analyzer from < 400 mg/dL to < 620 mg/dL improved the sensitivity of the POC analyzer for detection of FPTI from 80% to 97%. When FPTI was defined as a plasma IgG concentration < 800 mg/dL, as determined via the standard laboratory method, increasing the cutoff for plasma IgG concentration measured via the POC analyzer from < 800 mg/dL to < 1,150 mg/dL improved the sensitivity of the POC analyzer for detection of FPTI from 76% to 96%. However, in both instances, the increase in sensitivity was accompanied by a decrease in specificity.

Results of the present study suggested that measurement of plasma IgG concentration via the POC analyzer had a similar, if not slightly better, sensitivity and specificity, compared with that of other methods used to measure plasma or serum IgG concentration in foals.40–42 Semi quantitative laboratory methods used to measure serum IgG concentration in foals include zinc sulfate turbidity, latex agglutination, glutaraldehyde coagulation, and ELISA.16 Of those semiquantitative tests, the most commonly used test to detect FPTI in foals is the ELISA because it provides results within 10 minutes after test initiation and is commercially available, easy to use, and inexpensive. Although results of multiple studies19,20 indicate that the ELISA is fairly accurate, in 1 study40 this test had good sensitivity but poor specificity when used in a population of hospitalized foals. Moreover, because of the semiquantitative nature of the ELISA, the results are categorized into 1 of 3 classifications (ie, < 400 mg/dL, 400 to 800 mg/dL, or ≥ 800 mg/dL) rather than provided as the specific IgG concentration.

In a study16 in which 5 methods (zinc sulfate turbidity, glutaraldehyde coagulation, a quantitative colorimetric assay, and 2 semi quantitative immunoasays) for the measurement of serum IgG concentration in neonatal foals were compared, the sensitivity of the 5 assays for identifying foals with serum IgG concentrations < 400 mg/dL ranged from 89% to 100%, and the specificity ranged from 79% to 96%; the sensitivity of the 5 assays for identifying foals with serum IgG concentrations < 800 mg/dL ranged from 52% to 98%, and the specificity ranged from 57% to 100%. Thus, the performance of the POC analyzer evaluated in the present study was roughly equivalent, and in some instances superior, to those other 5 methods for identifying neonatal foals with plasma IgG concentrations < 400 mg/dL (sensitivity, 80%; specificity, 100%) and < 800 mg/dL (sensitivity, 76%; specificity, 100%) and has the additional benefit of the rapid provision of quantitative results.

An automated immunoturbidimetric method was used as the standard laboratory method in the present study because investigators of other studies concluded that the automated immunoturbidimetric method yielded consistent (ie, repeatable) results when the same sample was tested multiple times during 1 day and when the same sample was tested once per day during a period of several days.19 Historically, the RID method has been used as the standard laboratory method for determination of serum IgG concentration against which other test methods are evaluated; however, results of another study40 suggest that there is substantial variation in agreement among commercially available RID assays, especially at serum IgG concentrations > 400 mg/dL. Moreover, results for the RID method are measured manually and reported in increments of 100 mg/dL, whereas the results of the immunoturbidimetric method are measured automatically and reported in increments of 1 mg/dL (ie, more precise). Other advantages of the immunoturbidimetric assay when compared with the RID assay include a shorter turnaround time (< 1 hour) for results and elimination of human error in measurement of the precipitin-ring diameter.20 In fact, investigators of 1 study concluded that the automated immunoturbidimetric method was as reliable as the RID assay for determination of plasma IgG concentration and was acceptable for use as a standard reference laboratory method.

In veterinary patients, measurement of serum or plasma total protein concentration is often used to estimate hydration status, monitor response to fluid therapy, and assess total protein content within the blood. Use of a handheld medical refractometer represents a readily available, low-cost, and rapid method for assessment of serum or plasma total protein concentration, and results obtained via that method are highly correlated with those obtained via standard laboratory methods.43–45 In the present study, the mean bias for measurement of plasma total protein concentration via the POC analyzer was −0.33 g/dL, which indicated that the POC analyzer overestimated total protein concentration slightly, compared with that measured via the standard laboratory method. The reference range for plasma total protein concentration in healthy neonatal foals is 4.3 to 8.1 g/dL45; thus, the magnitude of overestimation (0.33 g/dL) of plasma total protein concentration via the POC analyzer was small and likely to be clinically irrelevant. Moreover, the Lin concordance correlation coefficient (0.92) between the POC analyzer and the standard laboratory method for the measurement of plasma total protein concentration indicated good agreement, which suggested that the POC analyzer might be useful for the determination of plasma total protein concentration during the clinical evaluation of ill foals. However, the 95% limits of agreement for measurement of plasma total protein were wide (−1.2 to 0.54 g/dL), which indicated that the POC analyzer could overestimate plasma total protein concentration by as much as 1 g/dL, especially when the total protein concentration was high (ie, > 7 g/dL). Given that the serum or plasma total protein concentration can be accurately and affordably determined via a handheld refractometer, the purchase of a POC portable analyzer for the sole purpose of determination of plasma total protein concentration may not be justified.
In the present study, the POC analyzer overestimated plasma albumin concentration by a mean of 0.88 g/dL compared with the plasma albumin concentration determined via the standard laboratory method. The reference interval for plasma albumin concentration in healthy foals is 2.5 to 3.6 g/dL; thus, care must be taken in the interpretation of results obtained from the POC analyzer. For example, the POC analyzer could yield a plasma albumin concentration of 2.6 g/dL for a neonatal foal in which the actual plasma albumin concentration as determined via the standard laboratory method was 1.7 g/dL. This could result in the attending clinician making the assumption that the foal’s plasma albumin concentration was adequate and formulating an inappropriate treatment plan. Furthermore, the 95% limits of agreement for measurement of plasma albumin concentration were wide (−1.98 to 0.21 g/dL), compared with the reference interval for plasma albumin concentration in healthy foals. Therefore, we concluded that the POC analyzer was an unacceptable method for determination of plasma albumin concentration in neonatal foals.

Results of the present study suggested that use of the POC portable analyzer is an acceptable method for rapid, accurate, and quantitative determination of plasma IgG concentration in neonatal foals during the assessment of FPTI. The POC analyzer also provided acceptable results for determination of plasma total protein concentration; however, purchase of a POC portable analyzer for the sole purpose of measuring serum or plasma total protein may be unwarranted because use of a handheld refractometer for determination of total protein concentration is easier and more cost-effective. The POC portable analyzer was not an effective method for determination of plasma albumin concentration in neonatal foals.

References

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