Validation of a Point-of-Care Quantitative Equine IgG Turbidimetric Immunoassay and Comparison of IgG Concentrations Measured with Radial Immunodiffusion and a Point-of-Care IgG ELISA

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Background: Point-of-care (POC) diagnostic tests with good sensitivity and specificity are needed for diagnosing failure of transfer of passive immunity (FTPI) in foals. Turbidimetric immunoassays (TIA) have these characteristics and provide quantitative results. A commercially available TIA-based POC test (POC-TIA) has not been validated in horses.

Objective: To validate a POC-TIA and compare results of POC-TIA, a POC-ELISA, and radial immunodiffusion (RID).

Animals: Heparinized blood samples (n = 127) from 48 hospitalized foals (<12 hour to 48 days).

Methods: Prospective validation study. IgG concentrations were measured using RID (gold standard), POC-TIA, and POC-ELISA. Agreement between assays was assessed using Bland–Altman analysis. Sensitivity and specificity were calculated using ROC curves. Inter- and intra-assay coefficients of variation (CVs) and linearity were evaluated for POC-TIA.

Results: The mean bias (95% limits of agreement) between RID and POC-TIA was −4 (−185 to 176), 27 (−201 to 255), and 308 (−377 to 993) mg/dL for samples with IgG concentrations of <400, 400–800, and >800 mg/dL, respectively. Sensitivity and specificity at optimal cutoff were 94 and 100% for the POC-TIA and 94 and 100% for the POC-ELISA to detect IgG <400 mg/dL, and 85 and 87% (POC-TIA) and 69 and 79% (POC-ELISA) to detect IgG >800 mg/dL. Intra- and interassay CVs for POC-TIA ranged between 1.6–3.8 and 11.9–18.8%, respectively. Linearity of the dilution series was preserved (R² > 0.96).

Conclusions and Clinical Importance: The POC-TIA provided unambiguous results and had sufficient sensitivity, specificity, accuracy, and precision to be used as an alternative to other POC tests to assess FTPI in foals.

Key words: Failure of transfer of passive immunity; Foal; Horse; Radial immunodiffusion.

Failure of transfer of passive immunity (FTPI) is common in neonatal foals and predisposes them to severe infections. Early detection of FTPI is therefore important to ensure adequate and timely treatment. Immunoglobulin G (IgG) concentrations of >800 mg/dL measured 12–48 hour postnatum are generally considered adequate, and FTPI is commonly defined as partial FTPI at IgG concentrations of 400–800 mg/dL or total FTPI at IgG concentrations <400 mg/dL.

A variety of quantitative and semiquantitative methods have been used to determine IgG concentrations in neonatal foals, but radial immunodiffusion (RID) is considered the gold standard for the diagnosis of FTPI.

However, RID has a long turnaround time, requires expertise in result interpretation, and is costly. Serum electrophoresis has been suggested as an alternative gold standard to RID and may be more accurate and reliable in interpretation, but electrophoresis also requires specialized laboratory equipment and has a relatively long turnaround time.

Several rapid, inexpensive point-of-care tests for clinical use, including an ELISA, zinc sulfate turbidity test, glutaraldehyde coagulation tests, and latex agglutination tests, have been shown to have acceptable sensitivity and specificity. However, these tests provide only semiquantitative results and are subject to interpretation error. Estimates of IgG concentrations based on serum total protein concentrations are considered unreliable, and serum total globulins have limited sensitivity and specificity.

An automated turbidimetric immunoassay developed for quantitative IgG measurements in horses has adequate sensitivity and specificity, but testing requires a laboratory chemistry analyzer and is not convenient for...
use in daily practice. More recently, point-of-care analyzers that use the turbidimetric immunosassay technique have been developed. One of these analyzers has good sensitivity and specificity, but is no longer available. Another point-of-care turbidimetric analyzer (POC-TIA) is currently marketed, but there are thus far no published validation studies.

The objective of this study was to validate the POC-TIA and compare the performance of the POC-TIA and of a widely used point-of-care ELISA (POC-ELISA) to the gold standard RID assay.

**Materials and Methods**

**Animals**

Foals presented to the Equine Hospitals of the Universities of Zurich and Bern between February and July 2015 were included in the study. Foals were included if testing for FTPI was considered indicated by the attending clinicians. Multiple samples from the same foal were included when IgG concentrations were measured on multiple occasions throughout the course of treatment. The study was performed in accordance with the animal use guidelines of the Swiss legislation and approved by Cantonal Veterinary offices in Zurich and Bern. The “Standards for the Reporting of Diagnostic Accuracy guidelines” were followed.

**Samples**

Blood samples were obtained from the jugular vein by direct venipuncture or from an indwelling intravenous catheter. 6 mL of native blood and 3 mL of heparinized blood were collected from each foal. Native blood was allowed to clot at room temperature for 60 minutes before centrifugation at 1341 g for 11 minutes. The serum was then transferred to cryotubes and frozen at –80°C. Heparinized blood was centrifuged at 1341 g for 11 minutes at room temperature, and plasma was used immediately to perform POC-TIA and POC-ELISA. The remainder of the plasma was transferred to a cryotube and frozen at –80°C.

**Laboratory Analyses**

The POC-TIA and POC-ELISA were run according to the manufacturers’ recommendations on heparinized plasma and evaluated by an experienced clinician. Results from POC-ELISA were read before the results from POC-TIA. Quality control of the POC-TIA analyzer was carried out as recommended by the manufacturer throughout the study period. Linearity of the POC-TIA dilution series was determined by linear regression using 2 plasma samples of high concentration measured undiluted and diluted with phosphate-buffered saline at concentrations of 1:2, 1:4, 1:8, and 1:16. Intra-assay variability of the POC-TIA was assessed by the coefficient of variation of ten replicate measurements of each of 3 samples, analyzed on the same setup of the assay. Interassay variability of the POC-TIA was assessed by the CV of 5 replicate measurements of each of 3 samples (low, medium, and high concentration), analyzed on different setups of the experiment. Coefficients of variation of <25% were considered acceptable. Samples for intra-assay and interassay variability were randomly selected based on low (<400 mg/dL), medium (400-800 mg/dL), and high concentration (>800 mg/dL).

The frozen serum samples were shipped as a single batch on dry ice to MAI P/S laboratory (Spring Valley, WI) for RID analyses that were conducted with a commercial RID test kit. The laboratory personnel were blinded to the results of the POC-TIA, and POC-ELISA tests. Standard curves were performed on each individual RID plate using the manufacturer’s suggested protocol with serial dilutions of a commercial USDA certified Equine IgG prepared with sterile goat serum. In addition to standards and unknowns, all RID plates included 3 control samples (high, 1900 mg/dL; mid, 950 mg/dL; and low, 475 mg/dL), supplied by the manufacturer of the RID plates and, on 3 plates, interassay (between plates) and intra-assay (within the same plate) replicates of 3 test samples chosen to represent high, mid, and low levels of IgG. The same 3 laboratory technicians processed all samples to reduce subjectivity. The average of the 3 reads was reported as the result of RID. The same USDA approved standard was used for all RID plates to reduce variability.

**Statistical Analyses**

Descriptive statistics were used to summarize data. Agreement of the POC-TIA with RID assay was evaluated using Bland–Altman analysis, whereby data were analyzed separately for samples with RID IgG concentrations of <400, 400–800, and >800 mg/dL, respectively. The mean bias, the 95% confidence interval (CI) of the mean bias, and the 95% limits of agreement (LOA) were calculated. Samples were categorized based on RID as ≤800 mg/dL (partial or total FTPI), and >400 mg/dL (total FTPI) to evaluate the performance of the POC-TIA and POC-ELISA. Receiver operator characteristics (ROC) were used to determine the accuracy of POC-TIA. The area under the ROC curve (AUC) was calculated, and the Youden index (maximum {sensitivity + specificity − 1}) was used to identify the optimal cutoff points for both sensitivity and specificity of the POC-TIA to detect IgG concentrations <400 mg/dL (total FTPI) and ≤800 mg/dL (partial or total FTPI), respectively. Other cutoffs were chosen to maximize either specificity or sensitivity of the assay. Test characteristics at the respective cutoff values were summarized by reporting sensitivity, specificity, and positive (≥LR) and negative (<LR) likelihood ratios. Similarly, the performance of the POC-ELISA was evaluated after generating ROC curves from the predicted probabilities from logistic regression. In addition, classification agreement of samples with IgG >800 mg/dL, 400–800 mg/dL, and <400 mg/dL between POC-TIA and RID assay and between POC-ELISA and RID assay was evaluated using the Kappa statistic with linear weights. The level of significance was set at P < 0.05. Analyses were performed by commercial software.

**Results**

**Animals**

A total of 127 samples were collected from 48 foals (87 samples from 20 foals presented to the Equine Hospital of University of Zurich and 40 samples from 28 foals presented to the Equine Hospital of the University of Bern). The sex of the foals was female in 18/48 (37.5%) and male in 30/48 (62.5%) of cases. The age at sampling ranged from <12 hours to 38 days, with a median of 4 days. Breeds included 19 Warmblood horses, 10 Freiberger, 8 ponies, 2 donkeys, 2 draft horses, 2 Lusitanos and one each of Standardbred, Thorobred, Friesian, Quarter Horse, and Paso Fino.

Presenting complaints recorded were FTPI (7/48), colic (7/48), sepsis (5/48), noninfectious orthopedic disease (5/48), healthy foals accompanying their sick mare (5/48), omphalitis (3/48), pneumonia (3/48), weakness (2/48), uroabdomen (2/48) and one each of prematurity, white muscle disease, inguinal hernia, soft tissue
trauma, wry nose, fever, and esophageal obstruction. The presenting complaint was not recorded for 2/48 foals. Samples

A single blood sample was collected from 29 foals. Foals from which more than one sample was collected were as follows: 2 samples (n = 7), 3 samples (n = 3), 4 samples (n = 1), 5 samples (n = 1), 6 samples (n = 2), 7 samples (n = 1), 9 samples (n = 1), eleven samples (n = 1), thirteen samples (n = 1), and 14 samples (n = 1). A total of 67/127 samples were taken after plasma transfusion. Based on RID analyses, IgG concentrations were >800 mg/dL in 75/127 (59%) samples, 400–800 mg/dL (partial FTPI) in 36/127 (28%) samples, and <400 mg/dL (total FTPI) in 16/127 (13%) samples. Any FTPI (partial or total) was present in 52/127 (41%) of samples.

Evaluation of POC-TIA

Linearity of the dilution series was preserved for the POC-TIA assay (Fig 1, Table 1). Intra-assay variability was within acceptable limits with CVs between 1.6 and 3.8% (Table 2). Interassay variability was higher (CVs between 11.9 and 18.8%) than intra-assay variability for all IgG concentrations (Table 2), but still within acceptable limits. Agreement between POC-TIA and RID using the Bland–Altman analyses revealed a mean bias of ~4 mg/dL (95% CI, −53 to 45), 27 mg/dL (95% CI, −13 to 66), and 308 mg/dL (95% CI, −515 to −239) for samples with RID IgG concentrations of <400, 400–800, and >800, respectively (Fig 2). For samples with RID IgG concentrations of >800 mg/dL, a proportional error was evident with POC-TIA showing a greater tendency to underestimate IgG concentrations as measured by RID assay at higher concentrations (Fig 2).

The AUC of the ROC curves for the POC-TIA was 0.99 and 0.92 to detect IgG concentrations of <400 and ≤800 mg/dL, respectively, when compared to the RID assay as the gold standard (Fig 3). The Youden index for detecting RID IgG concentrations of <400 and ≤800 mg/dL was associated with POC-TIA cutoff concentrations of 299 and 687 mg/dL, respectively. Cutoff concentrations for detecting RID IgG concentrations with highest sensitivity and specificity are given in Tables 3 and 4.

Table 1. Analytic accuracy of POC-TIA assessed by linear regression of dilution series in spiked plasma samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Starting concentration (mg/dL)</th>
<th>Slope (95% CI)</th>
<th>Y-intercept (95% CI)</th>
<th>P</th>
<th>R²</th>
<th>P (deviation from line of equality)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.379</td>
<td>1.013 (0.74–1.28)</td>
<td>−183.5 (−649.7 to 282.8)</td>
<td>0.0012</td>
<td>0.97</td>
<td>1.000</td>
</tr>
<tr>
<td>B</td>
<td>2.906</td>
<td>0.9948 (0.706–1.283)</td>
<td>−110.6 (−543.6 to 322.5)</td>
<td>0.0016</td>
<td>0.96</td>
<td>1.000</td>
</tr>
</tbody>
</table>

CI, confidence interval.
The weighted Kappa value characterizing agreement between POC-TIA and RID assay for the classification of samples as IgG < 400, 400–800, and > 800 mg/dL, respectively, was 0.64 (95% CI, 0.53–0.75). Total FTPI (<400 mg/dL) was falsely diagnosed by POC-TIA in 5/20 (25%) samples, and 1/16 (6%) samples with total FTPI based on RID was missed by POC-TIA. IgG concentrations ≤ 800 mg/dL (partial or total FTPI) were falsely diagnosed by POC-TIA in 2/50 (4%) samples, and 27/75 (36%) samples with IgG concentrations ≤ 800 mg/dL (partial or total FTPI) based on RID were missed by POC-TIA.

**Evaluation of POC-ELISA**

Based on the POC-ELISA results, 15/127 (12%) samples were classified as <400 mg/dL (total FTPI) and 52/127 (41%) as ≤ 800 mg/dL (partial or total FTPI). The AUC of the ROC curves for the POC-ELISA was 0.97 and 0.74 to detect IgG concentrations of <400 mg/dL (total FTPI) and ≤ 800 mg/dL (partial or total FTPI), respectively.
POC-TIA for IgG Measurement in Foals

Table 3. Sensitivities, specificities, and likelihood ratios of a point-of-care turbidimetric assay (POC-TIA) and point-of-care ELISA (POC-ELISA) to detect IgG concentrations <400 mg/dL (total FTPI, n = 16/127) as determined by the RID assay as the gold standard.

<table>
<thead>
<tr>
<th>Assay</th>
<th>AUC (95% CI)</th>
<th>Associated cutoff (mg/dL)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>+LR (95% CI)</th>
<th>−LR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POC-TIA</td>
<td>0.99 (0.96–1.00)</td>
<td>≤399*</td>
<td>93.8 (69.8–99.8)</td>
<td>100.0 (96.7–100.0)</td>
<td>0.06 (0.01–0.40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤595</td>
<td>93.8 (69.8–99.8)</td>
<td>95.5 (89.8–98.5)</td>
<td>20.81 (8.8–48.5)</td>
<td>0.07 (0.01–0.40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤488</td>
<td>100.0 (79.4–100.0)</td>
<td>90.1 (83.0–94.9)</td>
<td>10.09 (5.8–17.7)</td>
<td></td>
</tr>
<tr>
<td>POC-ELISA</td>
<td>0.97 (0.92–0.99)</td>
<td>≤400</td>
<td>93.8 (69.8–99.8)</td>
<td>100.0 (96.7–100.0)</td>
<td>0.06 (0.01–0.42)</td>
<td></td>
</tr>
</tbody>
</table>

AUC, area under the curve of ROC curve analysis; CI, confidence intervals; LR, likelihood ratio; RID, radial immunodiffusion; FTPI, failure of transfer of passive immunity.

*Optimal cutoff as determined by the Youden index.

Table 4. Sensitivities, specificities, and likelihood ratios of a point-of-care turbidimetric assay (POC-TIA) and point-of-care ELISA (POC-ELISA) to detect RID IgG concentrations ≤800 mg/dL (partial or total FTPI, n = 52/127) as determined by the RID assay as the gold standard.

<table>
<thead>
<tr>
<th>Assay</th>
<th>AUC (95% CI)</th>
<th>Associated cutoff (mg/dL)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>+LR (95% CI)</th>
<th>−LR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POC-TIA</td>
<td>0.92 (0.86–0.96)</td>
<td>≤595</td>
<td>36.4 (23.6–51)</td>
<td>100.0 (95.2–100)</td>
<td>0.63 (0.5–0.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤687*</td>
<td>84.6 (71.9–93.1)</td>
<td>96 (88.8–99.2)</td>
<td>17.31 (5.6–53.2)</td>
<td>0.32 (0.2–0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤804</td>
<td>98.1 (89.7–100.0)</td>
<td>62.7 (50.7–73.6)</td>
<td>2.63 (2.0–3.5)</td>
<td>0.03 (0.00–0.20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤864</td>
<td>100 (93.2–100.0)</td>
<td>49.3 (37.6–61.1)</td>
<td>1.97 (1.6–2.5)</td>
<td></td>
</tr>
<tr>
<td>POC-ELISA</td>
<td>0.74 (0.65–0.81)</td>
<td>≤800</td>
<td>69.2 (54.9–81.3)</td>
<td>78.7 (67.7–87.3)</td>
<td>3.25 (2.0–5.2)</td>
<td>0.39 (0.26–0.60)</td>
</tr>
</tbody>
</table>

AUC, area under the curve of ROC curve analysis; CI, confidence intervals; LR, likelihood ratio; RID, radial immunodiffusion; FTPI, failure of transfer of passive immunity.

*Optimal cutoff as determined by the Youden index.

Discussion

A quantitative point-of-care test offers a great advantage compared to most currently available assays marketed for the assessment of FTPI in foals that give only semiquantitative results. In particular, semiquantitative tests giving a result between 400 and 800 mg/dL do not allow clinicians to distinguish animals with IgG concentrations bordering on total FTPI from those with concentrations close to adequate levels. Moreover, the effects of treatment cannot be as closely monitored by semiquantitative tests. The purpose of this study was to validate a quantitative POC-TIA and compare IgG concentrations measured with RID and both the POC-TIA and a widely used semiquantitative POC-ELISA. The POC-TIA showed both good linearity in dilution series and good precision, based on intra- and interassay CV. The test showed fair to good agreement with the gold standard RID and had better sensitivity and specificity than the POC-ELISA in this study.

The POC-TIA evaluated in this study showed good linearity in dilution series. Intra-assay variability (CVs, 1.6–3.8%) was similar to that reported in a previous study (CV, 3%). Evaluating an automated TIA performed on a routine laboratory chemistry analyzer showed good analytic performance of an assay. Small differences in manipulation can lead to variations. The more steps included in a protocol, the higher the chance for variation. This explains the slightly better coefficient of variation in the intra-assay experiment where measurements were carried out repeatedly compared to the interassay experiment, where the actual setup was repeated. The good analytic performance of the POC-TIA suggests that small differences in manipulation are not likely to lead to clinically relevant error in IgG measurement, and precise measurements of IgG at clinically relevant concentrations are possible.
Agreement between POC-TIA\textsuperscript{b} and the gold standard RID was good for samples with RID IgG concentrations of <400 and 400–800 mg/dL. For samples with RID IgG concentrations of >800 mg/dL, both considerable bias and proportional error were evident with the POC-TIA\textsuperscript{b}, measuring increasingly lower than RID assay with increasing IgG concentrations. However, as IgG concentrations above 800 mg/dL are considered to be adequate to protect foals against infections, this increasing bias is likely to be of little clinical importance.

A test with high sensitivity is desirable when assessing FTPI, as failure to diagnose (and treat) FTPI may have serious sequelae.\textsuperscript{14,20,21} Conversely, high specificity is necessary to avert unnecessary treatment of foals with plasma transfusions, which carries a risk of adverse effects and is associated with higher costs.\textsuperscript{22} The AUC of ROC curve analyses for POC-TIA\textsuperscript{b} to detect RID IgG concentrations <400 mg/dL (total FTPI) and \(\leq\)800 mg/dL (partial or total FTPI) was both very high and considerably higher than that of POC-ELISA\textsuperscript{a}. Moreover, optimal cutoff points based on the Youden index (i.e., 299 and 687 mg/dL) achieved a sensitivity and specificity similar or superior to those found with the POC-ELISA\textsuperscript{a}, and those previously reported using a different POC-TIA assay (80 and 100%, respectively)\textsuperscript{14} and an automated TIA (63 and 92%, respectively).\textsuperscript{8} Likewise, optimal cutoff points achieved sensitivities and specificities similar or superior to those of the POC-ELISA\textsuperscript{a}, and those previously reported using a different POC-TIA assay (76 and 100%, respectively)\textsuperscript{14} and using an automated TIA (81 and 86%, respectively).\textsuperscript{8} Although cutoff values determined by the Youden index resulted in some foals with FTPI being missed by POC-TIA\textsuperscript{b}, this can be averted by selecting higher cutoff values of 488 and 804 mg/dL to detect IgG concentrations <400 (total FTPI) and \(\leq\)800 mg/dL (total or partial FTPI, as measured by RID), respectively. These higher cutoff values result in sensitivities of 100 and 98.1%, respectively, decreasing the false-negative rate at the expense of lower specificities. Results of the present study therefore suggest that the POC-TIA\textsuperscript{b} provides sufficient diagnostic accuracy to assess FTPI in foals.

Although the overall Kappa agreement to classify samples as <400, 400–800, and >800 mg/dL was similar for the POC-TIA\textsuperscript{b} and POC-ELISA\textsuperscript{a}, results of ROC curve analyses suggest that POC-TIA\textsuperscript{b} is superior to POC-ELISA\textsuperscript{a}. However, as the POC-TIA\textsuperscript{b} measured lower than RID, assay-specific cutoffs are necessary to achieve optimal performance. Indeed, use of 400 and 800 mg/dL as cutoffs for the POC-TIA\textsuperscript{b} would result in lower specificities than those achieved with Youden index-based cutoffs.

Previous studies evaluating the POC-ELISA\textsuperscript{a} have reported sensitivities and specificities ranging of 89–90 and 79–96%, respectively, to detect RID <400 mg/dL, and 81–95 and 52–95%, respectively to detect RID \(\leq\)800 mg/dL.\textsuperscript{6,12} However, differences in prevalences of FTPI between studies and use of different RID assays make direct comparison with other studies difficult.

Multiple samples from foals were included in the study as samples were used to monitor the foal IgG concentrations over time after plasma transfusion. These samples were not considered repeated measures, as most of them received plasma for treatment of FTPI between sampling points.

As evaluation of the POC-ELISA\textsuperscript{a} is somewhat subjective and both the POC-TIA\textsuperscript{b} and POC-ELISA\textsuperscript{a} were run by the same operator at the same time, results of the POC-ELISA\textsuperscript{a} were read before performing the POC-TIA\textsuperscript{b}. Therefore, results of POC-TIA\textsuperscript{b} should not have influenced subjective reading of the POC-ELISA\textsuperscript{a}. The POC-TIA\textsuperscript{b} can be performed with both serum and

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**Fig 4.** Receiver operator characteristic (ROC) curves for the point-of-care ELISA (POC-ELISA) to detect IgG concentrations of <400 mg/dL (A) and \(\leq\)800 mg/dL (B) measured with radial immunodiffusion. ROC curves for were calculated from the predicted probabilities from logistic regression.
plasma. In this study, plasma was used for convenience although serum was used for RID assays. However, as a previous study showed no difference in IgG concentrations between serum and plasma using an automated TIA assay, no significant effect of matrix is expected.  

From a practical standpoint, the POC-TIA was found to be easy to perform, rapid (run time of approximately 15 minutes) and the measured concentrations are displayed digitally, giving unambiguous results that are not subject to individual interpretation bias.

In conclusion, the POC-TIA showed fair to good overall performance and its use in practice can be considered as an alternative to other point-of-care tests for the diagnosis of FTPI in foals. Assay-specific cutoffs are necessary to achieve optimal accuracy compared to RID.

**Footnotes**

* SNAP Foal IgG Test Kit, IDEXX Laboratories Inc, Westbrook, ME
* Rapid DVM test™ II, Value Diagnostics™, MAI Animal Health, Melksham, Wiltshire, UK
* RID Test for Equine IgG, product number 828411, Triple J Farms, Bellingham, WA
* Secondary Reference Serum (SRS), Product Number 2011, Midland BioProducts, Boone, IA
* Equitech-Bio, Kerrville, TX
* MedCalc, version 16.4.1 MedCalc Software bvba, Ostend, Belgium
* GraphPad Prism, version 5.03, GraphPad Software, San Diego, CA, USA, www.graphpad.com

**Acknowledgments**

The study was not supported by a grant.

**Conflict of Interest Declaration:** MAI Animal Health the company that produces and distributes the test we validated in this study (Rapid DVM) provided the test kits and reagents. The internal laboratory of the company also performed the radial immunodiffusion test we used as gold standard to compare the point-of-care test to. The company (and laboratory) had no influence on study design, data analysis, and writing of the manuscript. The laboratory of the company was blinded to the results of the point-of-care test we validated when the RID was run and afterward. Dr. Colin Schwarzwald serves as Associate Editor for the Journal of Veterinary Internal Medicine. He was not involved in the review of this manuscript.

**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

**References**


