Received: 27 October 2025 | Accepted: 4 April 2026

DOI: 10.1002/evj.70172

ORIGINAL ARTICLE

Equine Veterinary Journal WILEY

Validation of a stall-side immunoglobulin assay for use in equine reproductive management

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Funding information
Future Medicines Institute, UKRI SIP, Ulster University; The Health Frontiers TIC Project supported by PEACEPLUS Programme, Special EU Programme Body (SEUPB)

Abstract

Background: Equine foals receive IgG from mare colostrum through passive transfer. Failure of passive transfer (FPT) is a significant risk to the foal's life, leaving them vulnerable to infection and sepsis. Radial Immunodiffusion (RID) and immunoturbidimetric assays quantify IgG present in a foal sample but require a laboratory to complete. Accurate, reproducible, stall-side testing to rapidly quantify IgG would allow for expedited clinical decisions, with potential to improve equine foal care and survival.

Objectives: To evaluate the analytical and clinical performance of a stall-side IgG lateral-flow test and reader (Sidekick), assessing its use as a point-of-care (POC) test to provide reliable results and agreement of IgG quantification with gold-standard methods.

Study Design: Retrospective cohort.

Methods: Stall-side LFD IgG test (Sidekick) was assessed with serial diluted foal sample (0.8–20 g/L, three replicates) and Stall-side LFD was compared to RID and Immunoturbidimetric assay. Serum/plasma samples from 10 foals created two cohorts, one representing successful passive transfer and one representing failed passive transfer. Agreement in IgG quantification between matched blood and plasma samples by the stall-side LFD was tested in three foals.

Results: The Stall-side LFD IgG test showed excellent recovery and high repeatability, 96.3%–109.5% across the clinically relevant range of 4–8 g/L (CV% 3.7–10). Overall, there was strong agreement with the reference method and strong agreement between matched whole-blood and plasma samples.

Main Limitations: Small cohort (10 foals).

Conclusions: The stall-side IgG assay (Sidekick) delivers repeatable results enabling on-the-spot decisions and reducing delays from sample processing, transport and result reporting, with potential to enable informed decision-making on whether to initiate or continue treatment for a foal with FPT.

L. Moore and A. McLain shared first authorship.

KEYWORDS
horse, IgG, lateral flow, reproduction, stall-side testing

1 | INTRODUCTION

Immunoglobulin G (IgG) is the most abundant antibody class and acts as a key mediator of the immune response in equines.[1,2] Equine foals are born with a low level of immunoglobulins, and through passive transfer (PT), foals will absorb IgG from the mare's colostrum during the first 6–12 h of life.[3] The estimated incidence of failure of passive transfer (FPT) ranges from 10% to 20%,[4,5] with causes including poor quality colostrum[6] and lack of suckling by the foal.[5] FPT is a common cause of death in equine foals,[7] leaving them exposed to infections such as pneumonia caused by *Rhodococcus equi*[8] or *Escherichia coli* which can cause serious illnesses like sepsis.[9] When FPT is diagnosed, a veterinarian may prescribe a transfusion of donor equine plasma to boost IgG levels,[10] which has been shown to improve outcomes and survival of newborn foals.[4] Failure of passive transfer is diagnosed using a blood test that measures IgG concentration. The normal levels of IgG in a foal with successful PT can vary by age, breed, and setting, but are typically in the range of 10–50 g/L. While several cut-offs for FPT were originally proposed, 4 g/L became established as the minimum IgG concentration required for foal survival based on empirical evidence. Since the 1970s, <4 g/L has been described as FPT, 4–8 g/L as partial FPT and >8 g/L successful PT.[11] In 2015, a large population study validated these traditional cut-offs, showing that foals with <8 g/L of IgG have proportionally decreased odds of survival.[12]

The gold standard for measuring equine IgG in serum has traditionally been the radial immunodiffusion assay (RID).[13–15] RID is used less frequently in clinical laboratories due to the long incubation times required. This has led to immunoassays, such as the immunoturbidimetric assay, being more favoured due to their quicker turnaround time for results.[13,15] While these assays are suited to regional laboratories, their requirement for specialist automated equipment makes them less suited to local or ambulatory clinics. Point-of-care, stall-side testing is increasingly used for many applications which require a timely decision on intervention, such as the detection of acute phase proteins that could be indicative of infection.[16] Stall-side testing for FPT can employ different methodologies, such as refractometry, which is an inexpensive method but has the limitation that it relies on measurement of total proteins rather than IgG specifically.[17–19] Additionally, a semi-quantitative IgG lateral flow device (LFD) for foals, the SNAP Foal IgG test, has shown utility for stall-side measurement of IgG; however, interpretation of the coloured dot output and the semi-quantitative nature of this readout can be a limitation.[20,21]

In this study, a fully quantitative, stall-side lateral flow test for IgG concentration in newborn foals was evaluated (Sidekick IgG) to determine the accuracy, precision, and reproducibility of it as a diagnostic assay and compare it to the current gold standard methodology.

2 | METHODS

Sample collection was carried out in Spring 2025 by registered veterinarians during routine phlebotomy of newborn foals (between 24 and 48 h old) located across two locations, in Ireland and Germany, with a total of 10 foals. Samples were collected during routine IgG testing as part of veterinary care of newborn foals, and the experimental testing was carried out on remnant material. Sample inclusion criteria included that foals were alive at the time of sampling, survived for a minimum of 4 h after sampling, and foals must not have been treated with a plasma infusion in the 24 h prior to sampling.

2.1 | Sample preparation and processing

2.1.1 | Serum and plasma sample preparation

Blood was drawn from the jugular vein using a 20-gauge needle and syringe by a veterinarian, during routine IgG testing as part of veterinary care of newborn foals, with this study using the remnant of the samples. Depending on the veterinarian's standard practice, foal blood samples were either collected in a plain blood collection tube, with no additives present, or in a K2 EDTA blood collection tube with K2 EDTA acting as an anticoagulant. Blood samples collected in plain collection tubes were allowed to clot for 1 h at RT. Samples were centrifuged at 1000×*g* for 10 min to separate serum or plasma from the red blood cells. Serum was collected from samples in plain collection tubes and plasma from samples in anticoagulant tubes; both were aliquoted and frozen at −20°C until analysis. It has been previously shown that serum and plasma contain the equivalent amount of IgG,[22] therefore, they were analysed together rather than as separate datasets.

For linearity and precision assessment of the Stall-side LFD (Sidekick) IgG assay, an equine foal serum sample of known IgG concentration was diluted serially in saline. For the comparison of the Stall-side LFD (Sidekick) IgG assay to other methods, two cohorts of samples were created to represent both PT and FPT. The first group represented successful PT and consisted of the 10 sera/plasma samples. The second group represented FPT and consisted of the same 10 sera/plasma samples diluted by 80% in pre-suckle equine foal serum (Biowest) that did not contain IgG. Creating this second group via dilutions was required due to the low incidence of FPT.

2.1.2 | Whole blood sample preparation

Blood was drawn from the jugular vein using a 20-gauge needle and syringe by a veterinarian into K2 EDTA plasma tubes for three foals. This collection was during routine IgG testing as part of veterinary

care of newborn foals, with this study using the remnant of the samples. Once the study commenced, the blood was diluted in pre-suckle blood from the same foal (to 50%, 33%, and 25%) to give a range of IgG values representative of different levels of passive transfer. Plasma was separated via centrifugation at 1000×*g* for 10 min, providing matched whole blood and plasma samples from the same three foals.

2.2 | Stall-side LFD (Sidekick) IgG assay

The Stall-side LFD (Sidekick) IgG assay (Sidekick Animal Health) was performed on serum/plasma samples as well as on matched whole blood samples, according to the manufacturer's instructions. Briefly, 2.5 μL of serum/plasma sample was added directly to the supplier buffer bottle, then 100 μL of sample-buffer mixture was applied to the test cartridge. For whole blood, the BloodCaptor™ device (supplied with Stall-side LFD (Sidekick) IgG kit) was used to draw a measured volume of blood into the bulb, before inserting it in the buffer bottle and mixing gently. Sample-buffer mixture was applied to the test cartridge and incubated for 5 min, and the result was read using the Stall-side LFD (Sidekick) reader.

2.3 | Radial immunodiffusion (RID)

IgG concentration was determined using the gold standard assay, radial immunodiffusion (RID) according to the manufacturer's instructions (Triple J Diagnostics). Briefly, 5 μL of serum/plasma was added to wells and allowed to form precipitin rings over 48 h at room temperature. The diameter of the rings was measured with callipers and IgG concentration was determined relative to reference samples included in the kit.

2.4 | Immunoturbidimetric assay

IgG concentration was tested by an immunoturbidimetric assay at Cornell University Animal Health Diagnostic Centre, USA, according to the manufacturer's instructions (Midlands Bioproducts).

3 | DATA ANALYSIS

3.1 | Linearity and precision

Replicate measurements at each nominal IgG concentration were summarised as mean, standard deviation (SD), and coefficient of variation (CV%). Linearity of observed versus expected concentrations was evaluated using ordinary least squares regression, with comparison against a categorical (factor-by-level) model for lack-of-fit testing. Recovery was calculated as 100 × (mean/expected). Acceptance criteria were CV ≤15% across the range, and recovery between 80% and 120%.

3.2 | Method comparison

For method comparison, analyses were restricted to a common measurable interval (≤20 g/L) to avoid extrapolating beyond the Stall-side LFD (Sidekick) reportable range and because comparator values such as '>30 g/L' are right-censored. Agreement between Stall-side LFD (Sidekick) and reference methods (RID and immunoturbidimetric) was assessed using Deming regression (error ratio = 1). Pearson correlation coefficients (*r*) and coefficients of determination (R^2) were also reported. Bland–Altman (BA) plots were generated using the convention test−reference, so that positive bias indicates higher Stall-side LFD (Sidekick) values relative to the comparator. Mean bias and 95% limits of agreement (LoA) were calculated as bias ±1.96 × SD of the differences.

3.3 | Threshold agreement

Due to clinical interpretation hinging on IgG cut-offs (<4 g/L = failure of passive transfer; 4–8 g/L = partial failed passive transfer; >8 g/L = successful passive transfer), agreement at these thresholds was evaluated using confusion matrices and weighted Cohen's κ, with observed (po) and expected (pe) agreement and 95% confidence intervals (CI). For this, all paired samples were included, as measurements above 20 g/L would still fall within the same band (>8 g/L).

3.4 | Sample matrix comparison

To assess the potential influence of sample type, we compared Stall-side LFD (Sidekick) results from whole blood versus plasma using Deming regression and Bland–Altman analysis of paired serial dilutions.

Statistical analysis was completed using R (v4.4.2) statistical programming language.

4 | RESULTS

4.1 | Linearity and precision assessment

To determine that the Stall-side LFD (Sidekick) IgG assay results were proportional across a clinically relevant range, an assessment of the assay linearity was carried out. A foal serum sample of known IgG concentration, determined by RID, was serially diluted in saline solution to provide reference IgG standards of 0.8, 2, 4, 6, 8, 14, 20 g/L to test with the Stall-side LFD (Sidekick) IgG assay, with three runs per concentration ($n = 24$).

The Stall-side LFD (Sidekick) IgG assay demonstrated strong linearity across the measurable range of foal IgG. Recovery ranged from 94% to 110% across the concentration range 2–20 g/L; recovery at 0.8 g/L was not measured due to this being below the limit of detection. Three replicates were compared for each concentration, with the exception of the 20 g/L where two replicates were compared as one

reading was above the limit of detection. The intra-assay variation was examined by coefficient of variation (CV), which ranged from 0% to 15.9%, confirming acceptable accuracy and reproducibility across the validated range (2–20 g/L) (Table 1).

Linear regression of observed versus expected concentrations produced a slope of 0.94 and intercept of 0.377 g/L ($R^2 = 0.98$), with no evidence of lack-of-fit ($p = 0.79$) or improvement from a quadratic model ($p = 0.95$). A scatter plot with fitted regression line further illustrated the linearity of the Stall-side LFD (Sidekick) IgG assay (Figure 1).

4.2 | Comparison with RID and immunoturbidimetric assay

Ten clinical foal samples (serum or plasma) of unknown IgG concentration and with successful PT were tested across three laboratory methods: Stall-side LFD (Sidekick) IgG Assay, RID, and Immunoturbidimetric assay. RID is the traditional gold standard laboratory method for testing IgG concentration, and the immunoturbidimetric assay is routinely used for IgG detection, both of which were used to compare against the Stall-side LFD (Sidekick) IgG assay. In order to test samples over IgG ranges relevant to PT and FPT, the 10 foal samples were diluted in pre-suckle serum (no IgG present) to concentrations representative of FPT. Samples were tested in parallel on the same three methods, creating one cohort for PT and one for FPT. Of the 20 samples tested, 3 of the neat samples were removed due to measuring above the common measurable interval (>20 g/L), resulting in an analysis sample of $n = 17$ for the Deming regression and Bland–Altman analysis; an $n = 20$ was preserved for ordinal agreement.

Stringent method comparison was conducted by quantifying the agreement between two methods with Deming regression and Bland–Altman analysis, first comparing the Stall-side LFD (Sidekick) IgG assay to RID and then to the immunoturbidimetric assay. Deming regression of Stall-side LFD (Sidekick) IgG assay versus RID showed close agreement, with a slope of 1.02 (95% CI: 0.87–1.16) and an intercept of −0.163 g/L (95% CI: −1.69 to 1.364), with Pearson correlation of 0.929 (Figure 2A). Bland–Altman analysis revealed a small positive bias (+0.319 g/L) with 95% limits of agreement from −3.466 to +4.105 g/L (Figure 2B, Table 2). Overall, the Stall-side LFD (Sidekick) IgG assay had good overall agreement to RID, with no significant differences between the methods detected.

Comparison of the Stall-side LFD (Sidekick) IgG assay to immunoturbidimetric assay with Deming regression yielded a slope of 0.76 (95% CI: 0.67–0.85) and an intercept of 0.956 g/L (95% CI: −0.217 to 2.129), with Pearson correlation of 0.968 (Figure 2C). The Bland–Altman analysis confirmed a small negative mean bias (−0.475 g/L), with 95% limits of agreement from −3.253 to +2.304 g/L. This meant that Stall-side LFD (Sidekick) IgG assay gave a lower value than the

TABLE 1 Linearity and precision of Stall-side LFD (Sidekick) IgG assay for IgG reference samples.

IgG reference concentration (g/L)	Average reported concentration (g/L)	Standard deviation (g/L)	Intra-assay variation (% CV)	Recovery (%)
2	2.187	0.116	5.3	109.3
4	3.85	0.142	3.7	96.3
6	6.57	0.658	10.0	109.5
8	7.82	0.498	6.4	97.8
14	13.193	2.092	15.9	94.2
20	19.3	0.0	0.0	96.5
		Mean % CV	6.9	

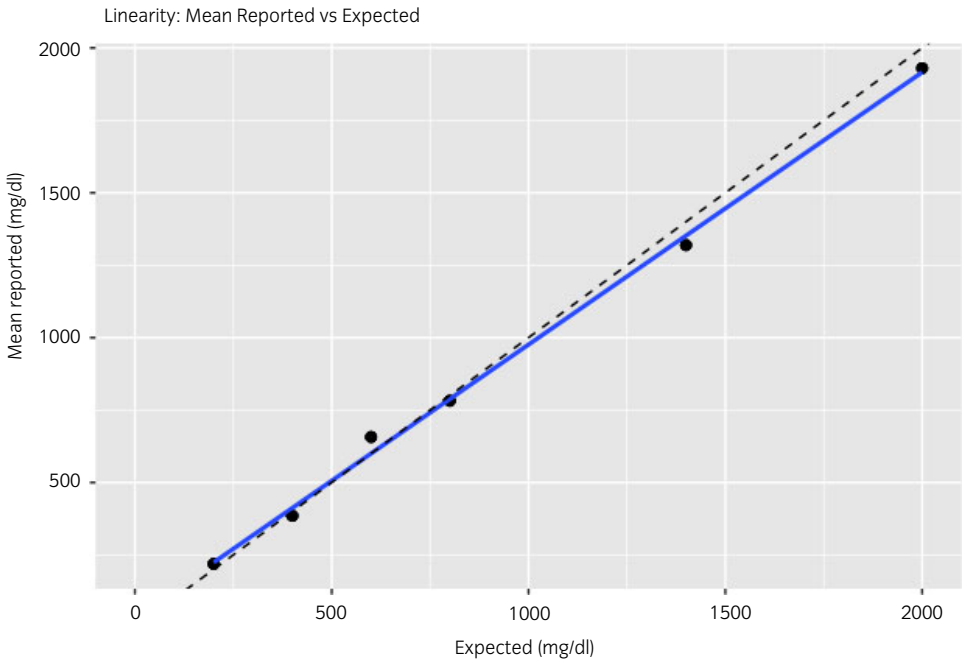

FIGURE 1 Measured concentration (Stall-side LFD (Sidekick) IgG assay) versus assigned levels (2–20 g/L). Point are individual runs (3 per concentration); the dashed line is the line of identify ($y = x$). Solid lines are ordinary least-squares fits for each lab, illustrating good overall linearity.

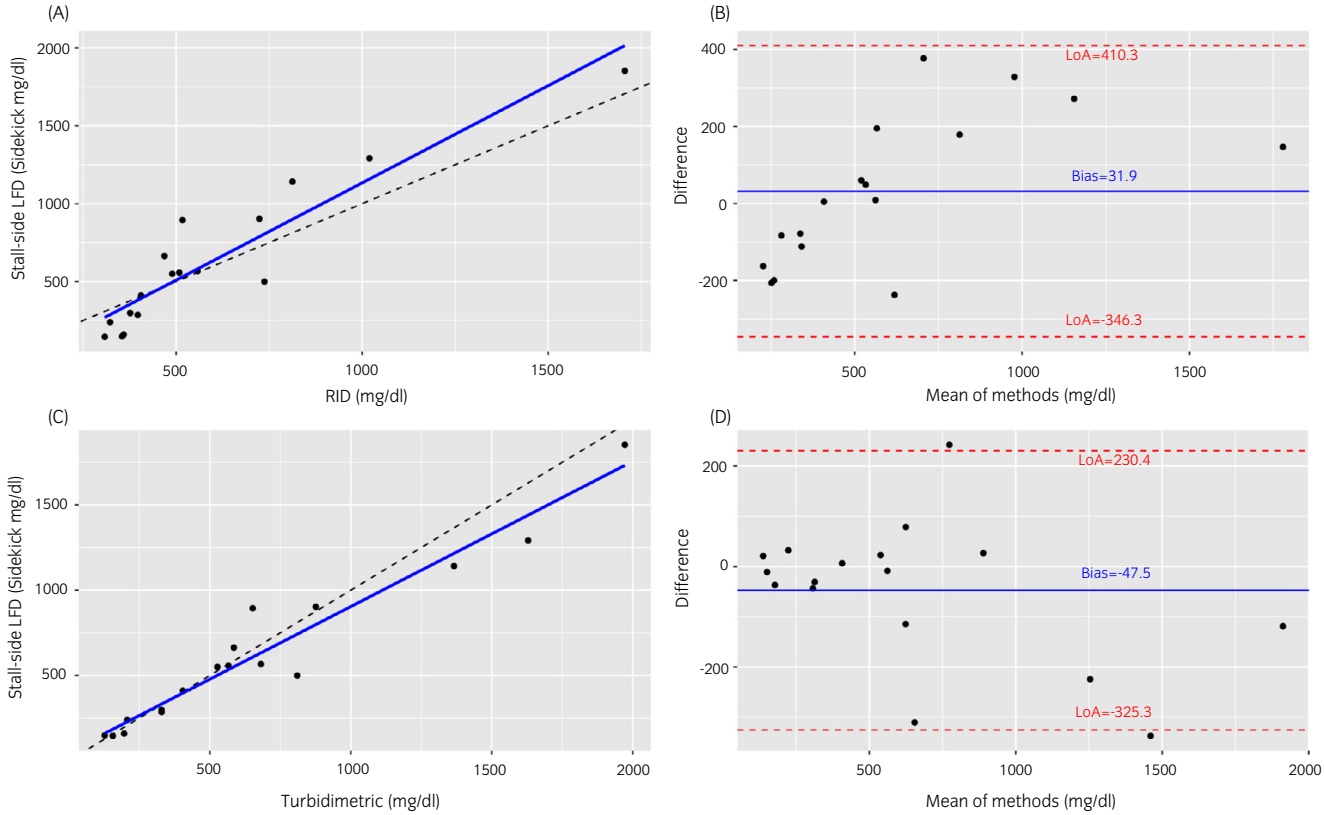

FIGURE 2 (A) Deming regression of Stall-side LFD (Sidekick) against RID. Solid line is the Deming fit (slope 1.02, 95% CI: 0.87–1.16; intercept −0.163 g/L, 95% CI: −1.69 to 1.364); dashed line is identity ($y = x$). $N = 20$. (B) Bland–Altman plot showing the difference (Stall-side LFD (Sidekick) − RID) versus the mean of methods. Solid line indicates mean bias (+0.319 g/L); dashed lines show 95% limits of agreement (−3.466 to +4.105410.5 g/L). (C) Deming regression of Stall-side LFD (Sidekick) against Immunoturbidimetric assay. Solid line is the Deming fit (slope 0.76, 95% CI: 0.67–0.85; intercept 0.956 g/L, 95% CI: −0.217 to 2.129); dashed line is identity ($y = x$). $N = 20$. (D) Bland–Altman plot showing the difference (Stall-side LFD (Sidekick) − Immunoturbidimetric) versus the mean of methods. Solid line indicates mean bias (−0.475 g/L); dashed lines show 95% limits of agreement (−3.253 to +2.304 g/L).

TABLE 2 Method comparison of laboratory comparators and sample matrices.

Comparison	Intercept (95% CI)	Slope (95% CI)	R^2	Bias (g/L)	Lower LoA	Upper LoA
Stall-side LFD (Sidekick) vs. RID	−16.3 (−169.0 to 136.4)	1.02 (0.87 to 1.16)	0.98	+0.319	−3.466	+4.105
Stall-side LFD (Sidekick) vs. Immunoturbidimetric	95.6 (−21.7 to 212.9)	0.76 (0.67 to 0.85)	0.98	−0.475	−3.253	+2.304
Whole Blood vs. Plasma	−23.7 (−443.4 to 135.7)	1.12 (0.94 to 1.87)	0.97	+0.682	−2.525	+3.889

immunoturbidimetric assay; however, this was more apparent at higher IgG concentrations, where this discrepancy would not impact clinical decisions (Figure 2C, Table 2).

Despite relatively wide LoA in absolute terms, threshold-based agreement between Stall-side LFD (Sidekick) IgG and the reference assays was excellent. Weighted κ was 0.923 for Stall-side LFD (Sidekick) IgG versus RID and 0.928 for Stall-side LFD (Sidekick) IgG versus immunoturbidimetric, with observed agreement 0.975 and expected agreement ~0.65 to 0.68 (Table 3). This indicates almost perfect agreement for classifying samples as <4, 4–8, >8 g/L and that the Stall-side LFD (Sidekick) IgG assay can be reliably used to detect IgG at these clinically relevant concentrations, detecting incidences of 400 mg/dL that would require a treatment intervention to supplement the low levels of IgG present as well as detecting incidences of >8 g/L where further action is not often required. It should be noted that the Stall-side LFD (Sidekick) should be used alongside the expertise of a veterinarian to decide upon treatment.

4.3 | Whole blood versus plasma

RID and immunoturbidimetric assays for IgG detection are typically tested with serum or plasma; therefore, this was the sample matrix used with the Stall-side LFD (Sidekick) IgG assay to compare the IgG detection to these reference methods. However, the Stall-side LFD (Sidekick) IgG assay is typically used with a whole blood sample, rather

TABLE 3 Ordinal agreement between Stall-side LFD (Sidekick) IgG assay and laboratory comparators.

Comparison	Weighted κ	Observed agreement (po)	Expected agreement (pe)	95% CI
Stall-side LFD (Sidekick) vs. RID	0.923	0.975	0.675	0.780–1.000
Stall-side LFD (Sidekick) vs. Immunoturbidimetric	0.928	0.975	0.655	0.783–1.000

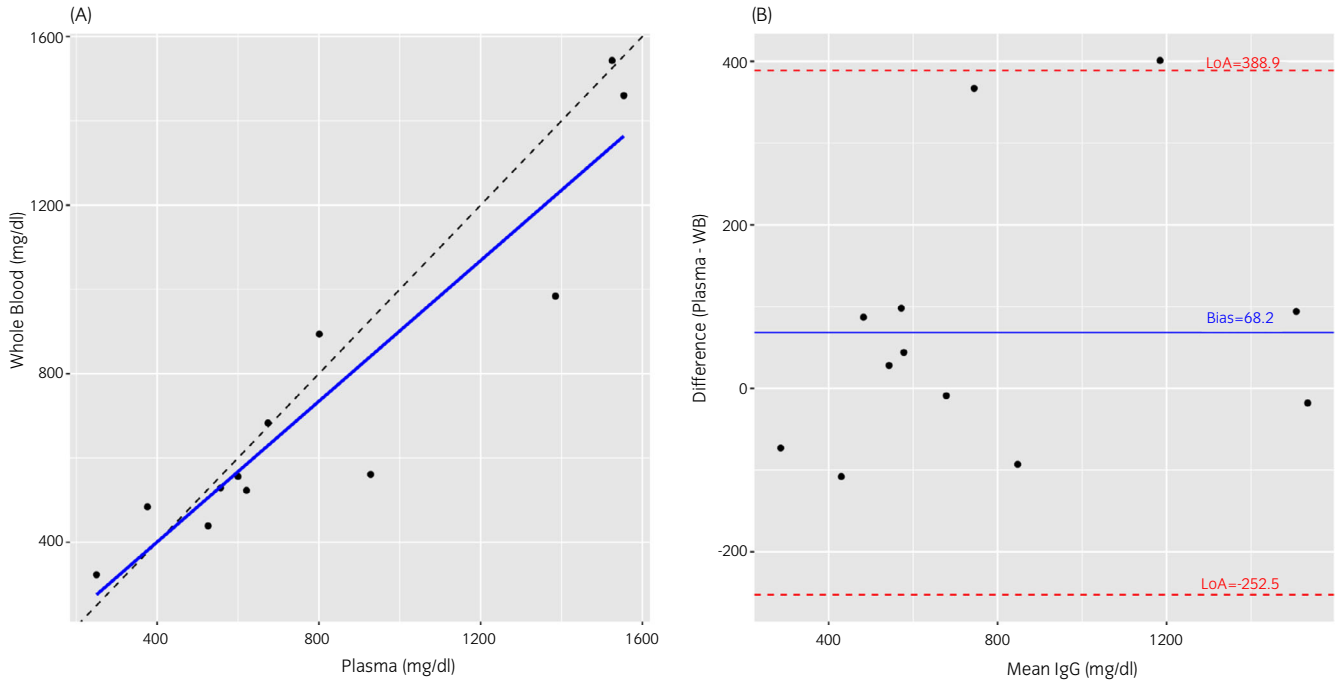

FIGURE 3 (A) Deming regression of Stall-side LFD (Sidekick) Whole-blood against Stall-side LFD (Sidekick) Plasma. Solid line is the Deming fit (slope 1.12, 95% CI: 0.94–1.87; intercept −0.237 g/L, 95% CI: −4.434 to 1.357); dashed line is identity ($y = x$). $N = 20$. (B) Bland–Altman plot showing the difference (Stall-side LFD (Sidekick) − RID) versus the mean of methods. Solid line indicates mean bias (+0.682 g/L); dashed lines show 95% limits of agreement (−2.525 to +3.889 g/L).

than a serum or plasma. An evaluation was carried out on IgG detection by the Stall-side LFD (Sidekick) IgG assay on matched whole blood and plasma samples from three foals. The whole blood samples were tested first, then processed to plasma for further testing with the Stall-side LFD (Sidekick) IgG assay.

Analysis of paired serial dilutions from three foals showed consistent performance of the stall-side LFD (Sidekick) IgG assay across sample medium. Deming regression of plasma against whole blood measurements gave a slope of 1.12 (95% CI: 0.94–1.87) and an intercept of −0.237 g/L (95% CI: −4.434 to 1.357), with Pearson correlation of 0.930 (Figure 3A). Bland–Altman analysis indicated a small positive bias (0.682 g/L), with 95% limits of agreement from −2.525 to +3.889 g/L (Figure 3B, Table 2). There were no significant systematic differences detected between sample types, supporting the use of either whole blood or serum/plasma for stall-side LFD (Sidekick) IgG testing.

5 | DISCUSSION

The ability to accurately and rapidly test equine foals stall-side for IgG present in serum, plasma or whole blood has the potential to beneficially impact equine foal care by quickly identifying foals exhibiting FPT and expedite veterinary treatment decisions. Due to the time-sensitive nature of FPT, reducing a need for sample shipment and laboratory testing for IgG by implementing a reliable, stall-side test could enable earlier treatment interventions and has the potential to reduce incidences of serious complications such as sepsis.[7,9,10,12] A schematic of how RID, immunoturbidimetric assay and Stall-side LFD (Sidekick) IgG can be used for IgG detection is presented in Figure 4.

Linearity and precision analysis of the point of care Stall-side LFD (Sidekick) IgG assay strongly supports that it could be utilised for accurate IgG quantification across the 0.8–20 g/L range, which includes the clinically relevant threshold of 4–8 g/L needed for determining the success of passive transfer. Recoveries at 4, 6, and 8 g/L were 96.3%, 109.5%, and 97.8% respectively, with a mean % CV across all concentrations tested of 6.9%, indicating the assay's excellent repeatability. In addition to screening for cases of failure of passive transfer, the ability to accurately quantify results from 0.8 to 4 g/L and 8 to 20 g/L could enable monitoring of the foal, particularly after treatment with donor plasma.

Herein, the Stall-side LFD (Sidekick) IgG assay was rigorously compared to other established reference methods for determining IgG

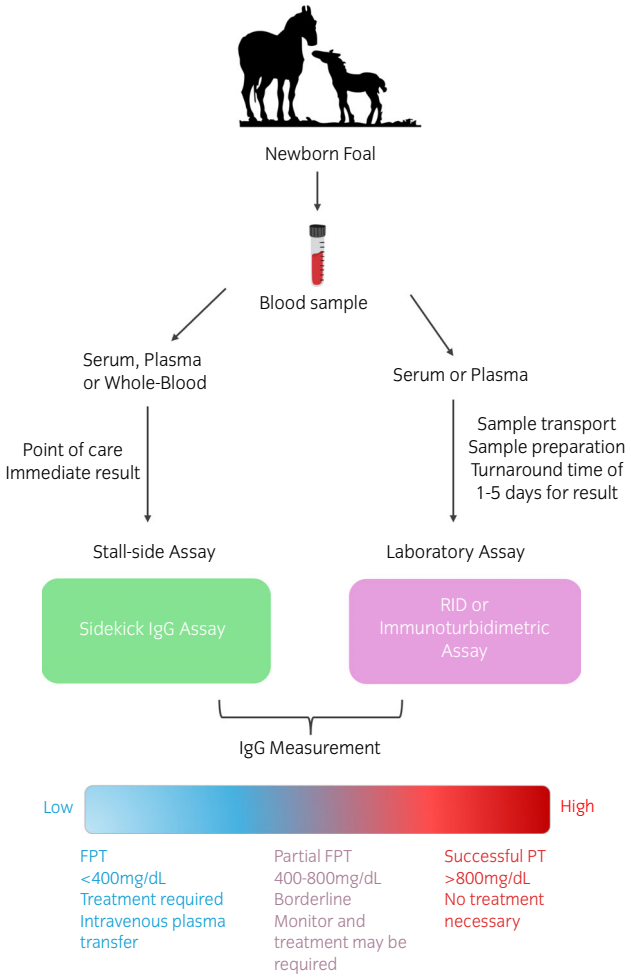

FIGURE 4 Schematic of how Stall-side LFD (Sidekick) IgG assay and laboratory comparators can be used for IgG testing of newborn foals for FPT detection. Figure created using free to use illustrations from pixabay and sketchify at Canva.com.

concentration: the gold-standard RID and immunoturbidimetric assays. Overall, the Stall-side LFD (Sidekick) IgG assay demonstrated very good continuity of results in comparison to the established reference methods across the clinically relevant range for identifying FPT. There was a good overall agreement observed in comparison to RID, but when measured against the immunoturbidimetric assay, the Stall-side LFD (Sidekick) assay tended to measure a lower concentration of IgG. However, across the clinically relevant range of 4–8 g/L there was confidence in the accuracy of concentrations reported by the Stall-side LFD (Sidekick) IgG assay within this range that impacts diagnosis of FPT. There is a small trade-off seen between the rapid, convenient nature of this point-of-care assay versus the sensitivity of the traditional laboratory methods for detecting these higher IgG concentrations, which is a known limitation of LFDs at higher antigen concentrations.[20] However, these higher IgG concentration samples are not relevant to the initial detection of FPT, since the assay is designed to detect a low level of IgG, indicating that FPT has occurred and highlighting that veterinary intervention is required. Higher IgG concentrations beyond 8 g/L are only relevant if FPT has been diagnosed, treated, and the veterinarian requires measuring of the treatment response. If measuring treatment response, using the same method as original testing, either Stall-side LFD or the same laboratory method, will ensure direct comparability of results.

It is recognised that the inability to run all the samples as whole blood on the stall-side LFD (Sidekick) assay is a limitation to the study, yet efforts were made to ensure there is no significant difference between the use of whole blood and plasma/serum with the stall-side LFD (Sidekick) IgG assay, with matched plasma/serum and whole-blood from three foals tested and compared. This analysis showed that there were no significant differences in IgG detection between these sample types and that the stall-side LFD (Sidekick) IgG assay could be used with both matrices equally and derive the same results. This result adds confidence to the use of stall-side fresh blood samples in the stall-side LFD (Sidekick) IgG assay, eliminating the need for any sample processing and direct use of drawn blood sample on site.

The sample size within this study is a limitation and the assessment of the Stall-side LFD (Sidekick) IgG assay performance across a larger sample cohort of foals of one collected sample type would be beneficial for further evaluating the clinical performance of this assay. However, this study provides evidence of the linearity, precision, and repeatability of the stall-side LFD (Sidekick) IgG assay over the cohort tested. While there is a slight reduction in precision in comparison to the traditional laboratory reference methods (RID and immunoturbidimetric assay), the use of a point-of-care test for assessment of FPT offers multiple benefits over standard laboratory testing. A POC assay would avoid delays in receiving results, reduce the need for sample transport, sample processing, specialised equipment, and training to test samples for IgG, enabling on-the-spot decision making and be particularly beneficial in ambulatory practices where access to laboratory may be more challenging. In conclusion, the Stall-side LFD (Sidekick) IgG assay could be implemented effectively to reliably test equine foals for FPT (<4 g/L IgG) in a rapid and repeatable manner, with the potential to impact real-time decision making, improving equine foal health outcomes.

FUNDING INFORMATION

Funding for this study was supported by Future Medicines Institute, UKRI SIP, Ulster University and The Health Frontiers TIC Project supported by PEACEPLUS Programme, Special EU Programme Body (SEUPB).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

L. Moore: Investigation; validation. **A. McLain:** Investigation; validation; writing – original draft. **S. H. McDowell:** Data curation; visualization; writing – review and editing. **C. J. Ledgerwood:** Formal analysis; data curation; visualization. **E. Bailie:** Writing – review and editing. **M. A. Nesbit:** Investigation; validation. **T. Moore:** Resources; project

administration; funding acquisition; conceptualization; methodology; writing – original draft; investigation.

DATA INTEGRITY STATEMENT

T. Moore had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis.

ETHICAL ANIMAL RESEARCH

Research ethics committee oversight not currently required by this journal: procedures were performed as part of clinical investigations.

INFORMED CONSENT

Owner consent was obtained for animals used in this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Ulster University's Research Portal at http://doi.org/10.21251/d6f9f476-5b6b-45a5-8e0c-d4ace7ebb725 [23].

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How to cite this article: Moore L, McLain A, McDowell SH, Ledgerwood CJ, Bailie E, Nesbit MA, et al. Validation of a stall-side immunoglobulin assay for use in equine reproductive management. Equine Vet J. 2026. https://doi.org/10.1002/evj.70172