

### Summary

The newly updated horse-side Foal IgG test from VMRD is a lateral flow assay that offers objective quantitation of equine IgG values on the VMRD Reader, also used for VMRD SAA. It does not require refrigeration and is calibrated against the gold standard VMRD radial immunodiffusion (RID) test. Numerical results are reported between 50-2500 mg/dL using whole blood.

The specific sample volume needed is collected using a capillary device and then diluted twice in buffer. Three drops of the double diluted sample are applied to the test cartridge, and the test is run for 10 minutes either using the self-time function on the reader or timed by the user. Results are then interpreted and displayed by the reader as a numerical value.

The VMRD RID has been used historically in numerous publications as the reference test for other assays and for evaluating passive transfer in foals.<sup>1-10</sup> Although no longer commercially available, VMRD applied this extensive background in IgG quantitation to development of the VMRD Foal IgG lateral flow test, using this gold standard RID internally for validation and calibration. The lateral flow calibration system keeps results consistent over time using RFID cards that are programmed with specific details for each lot and attached to the side of the reader.

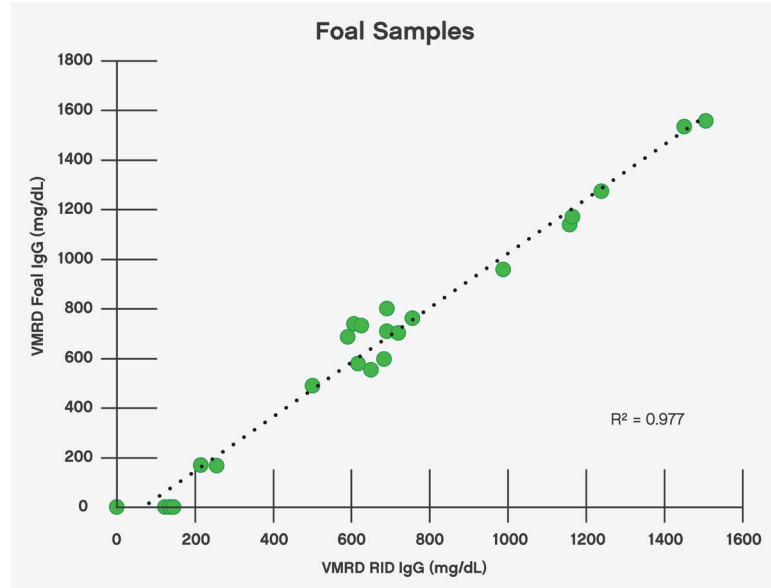
With quantitative results from the VMRD Foal IgG test, veterinarians can effectively tailor their treatment decisions with specific values and better monitor response to plasma therapy. For most accurate results, it is best to acclimate the test and reader to a temperature between 60-80°F prior to running. Although temperatures outside that range are often encountered when working with foals, results will be most reliable if these guidelines can be followed, particularly when tracking values for an individual foal before and after treatment.

### Comparison to RID reference standard

Excellent correlation was observed between the VMRD RID and lateral flow tests for both foal field samples and a dilution curve.

#### Foal Samples

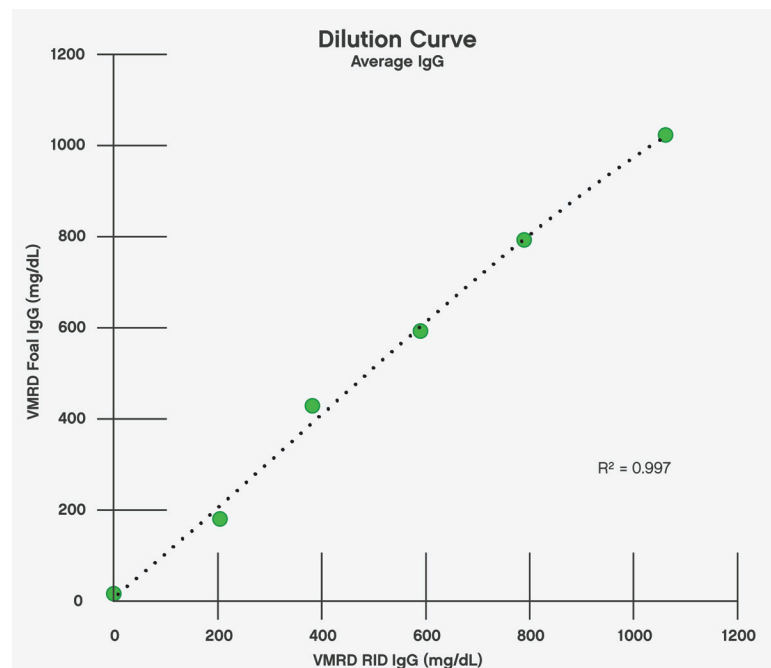
The IgG concentrations of 23 field samples from foals were determined with the VMRD RID, then compared with the results from the VMRD Foal IgG lateral flow test. This sample set included 11 foals with partial failure of passive transfer (400-800 mg/dL) and six with complete failure (<400 mg/dL). Correlation of lateral flow with RID for these foal samples was excellent with an  $R^2$  value of 0.977.



#### Dilution Curve

Normal horse serum with an IgG concentration of 1062 mg/dL was serially diluted in IgG-depleted horse serum to achieve a range of IgG concentrations spanning the most clinically relevant area of 0-1000 mg/dL. Specific IgG concentrations of all samples were characterized with the VMRD RID, and five replicates were then run for each sample using the VMRD Foal IgG lateral flow test.

Across the clinically important range of 0-1000 mg/dL IgG, the lateral flow test had excellent correlation with the reference RID, with an  $R^2$  value of 0.997.



## NVSL Reference Sample

A serum-based reference standard was obtained from the USDA National Veterinary Services Laboratory to use as an externally validated control. This reference standard, with a reported equine IgG concentration range of 411-523 mg/dL, was run in triplicate on the VMRD Foal IgG test as well as a quantitative competitor test. The VMRD result was an average concentration of 445 mg/dL with a range of 410-501 mg/dL, while the competitor delivered an average result of 643 mg/dL, with a range of 547 – 732 mg/dL.

USDA-NVSL Range (mg/dL)	VMRD RID (mg/dL)	VMRD Lateral Flow (mg/dL)	Competitor (mg/dL)
411-523	403	445	643

## Comparison to SNAP Foal IgG test

To compare the VMRD Foal IgG test to the commonly used SNAP Foal IgG test, 13 foal samples were run on both assays. Results from SNAP were reported using the manufacturer's designated categories, then compared to the quantitative VMRD Foal IgG test as well as the RID reference standard. Due to the subjective nature of the results, SNAP tests were read by three different technicians, with the consensus reported as the final result.

	RID mg/dL	VMRD mg/dL	SNAP mg/dL
Sample 1	<200	<50	<400
Sample 2	145	<50	<400
Sample 3	215	170	<400
Sample 4	255	167	<400
Sample 5	617	579	400-800
Sample 6	684	597	400
Sample 7	590	687	>800
Sample 8	719	710	400-800
Sample 9	690	733	<400
Sample 10	607	738	400-800
Sample 11	756	761	400
Sample 12	1158	1138	400-800
Sample 13	1239	1273	>800

Unnecessary treatment  
Failure to treat

The RID and VMRD Foal IgG test had excellent agreement. Despite the semi-quantitative nature of the SNAP test, five samples were incorrectly categorized. Four of these results were underestimated by SNAP (Samples 6, 9, 11, and 12) while Sample 6 was reported as normal (>800 mg/dL) with a RID value of 590 mg/dL.

## Intra-assay variation

Intra-assay variation for VMRD Foal IgG was evaluated by two technicians using three control samples, each representing a range of clinical significance. Each technician ran five replicates, for a total of 10 replicates for each sample. Variation ranged from 9-13% with the average variation at 11.3%.

	Approx. [IgG]	Average mg/dL	CV	Range (mg/dL)
FPT	200	170	12%	150-190
Partial FPT	600	625	13%	544-706
Normal	1000	991	9%	901-1080
Overall Average CV: <b>11.3%</b>				

## Conclusion

The VMRD Foal IgG lateral flow test correlates very well with the gold standard RID test, providing accurate, quantitative results using the VMRD Reader. When compared to the commonly used SNAP Foal IgG test, most samples were clinically consistent between tests, however the VMRD Foal IgG test matched more closely with the reference RID. With a total of 10 replicates per sample run by two different technicians, results from VMRD Foal IgG were very consistent for each tested concentration, averaging 11.3% variation.

This new option for horse-side quantitation of IgG in foals offers the convenience and accuracy of objective numerical results backed by VMRD's history as the gold standard in equine IgG quantitation by RID.

## References

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