## Comparative performance of in-clinic tests and the reference laboratory test for foal immunoglobulin G

Key Words: Vcheck, Equine, Foal, IgG, RID, POC-ELISA, Comparison

**BIONOTE study** 

#### Introduction

Failure of transfer of passive immunity (FTPI) in foals is associated with a risk of infection and death. The current diagnostic gold standard is the quantification of immunoglobulins using radial immunodiffusion (RID).<sup>1)</sup> However, RID has several drawbacks, including lengthy processing time, the need for skilled interpretation of results, and high costs.<sup>2)</sup> Several rapid, inexpensive point-of-care tests for clinical use, including an enzyme-linked immunosorbent assay (ELISA), have been shown to have acceptable sensitivity and specificity. However, these tests provide only semi-quantitative results and are subject to interpretation error.<sup>2)</sup>

More recently, a point-of-care (POC) analyzer that utilizes the fluorescent immunoassay (FIA) technique has been developed. A commercially available Vcheck Foal IgG test kit provides quantitative results.

### Purpose

The aim of this study was to validate a fluorescent immunoassay and compare the results of a POC-ELISA, and radial immunodiffusion (RID), which has previously validated for measuring immunoglobulin G (IgG) concentrations.<sup>2)</sup>

## Materials and Methods

A total of 82 fresh equine whole blood and serum samples with varying IgG concentrations were received and used for the purpose of this study conducted by the BIONOTE laboratory. No samples exhibiting heavy hemolysis, lipemia, or other serum clots were included. The samples were analyzed using a Vcheck Foal IgG test kit (BIONOTE) and a SNAP Foal IgG test kit (IDEXX) according to the manufacturer's instructions, respectively. The remaining samples were measured using a RID test (Triple J Farms Equine IgG) at the BIONOTE laboratory by laboratory technicians.

## Results

The test results for the correlation of equine IgG measurements between Vcheck and SNAP kit with the RID test are shown in

Figures 1-2. Samples outside the measurement range (100-1,000 mg/dl) of the Vcheck Foal IgG test kit were excluded from the analysis. The SNAP test kit yields semi-quantitative results based on the color intensity of the sample spot, and values were assigned arbitrarily by the evaluator. When the color intensity of the sample spot is the same as the 400 mg/dl or 800 mg/dl calibrator spot, it was assigned values of 400 mg/dl or 800 mg/ dl, respectively. If the color intensity was lighter than the 400 mg/dl calibrator spot, it was assigned a value of 200 mg/dl, and if it was darker than the 800 mg/dl calibrator spot, it was assigned a value of 1000 mg/dl. If the color intensity was darker than the 400 mg/dl calibrator spot but lighter than the 800 mg/ dl calibrator spot, it was assigned a value of 600 mg/dl.

A very strong correlation (slope 1.01,  $\mathbf{R}^2 = 0.96$ ) was found between the Vcheck and the RID test when analyzing 82 whole blood and serum samples (Figure 1). However, when comparing the SNAP kit and RID test, a relatively low correlation (slope 0.84,  $\mathbf{R}^2$ =0.68) was observed (Figure 2).

When classifying the results of the Vcheck and SNAP tests based on the reference range, which serves as the interpretation criterion for assessing FTPI in foals, the Vcheck demonstrated a concordance rate of 92.7% (76/82) compared to the RID test. In contrast, the SNAP kit exhibited a relatively lower concordance rate of 87.8% (72/82) compared to the reference method. Furthermore, when using a cut-off of 800 mg/dl, the Vcheck exhibited a sensitivity of 97% (64/66) and specificity of 81.3% (13/16) compared to the RID test. On the other hand, the SNAP kit showed a sensitivity of 92.4% (61/66) and specificity of 75% (12/16).

### Conclusion

Based on our comparative analysis, the in-clinic Vcheck test demonstrated superior performance compared to the SNAP kit when evaluated against the reference RID test for measuring foal IgG levels. The higher sensitivity and specificity of the Vcheck test highlight its potential as a valuable tool for assessing foal health and detecting cases of FTPI. However, further research and validation studies are necessary to confirm these findings and establish the clinical utility of in-clinic tests for foal care.

# Comparative performance of in-clinic tests and the reference laboratory test for foal immunoglobulin G



Equine IgG RID Test (mg/dL)



| IgG               |           | RID   |           |       | Total |
|-------------------|-----------|-------|-----------|-------|-------|
|                   |           | < 400 | 400 - 800 | > 800 | IULdi |
| Vcheck<br>(mg/dl) | < 400     | 4     | 1         | 0     | 5     |
|                   | 400 - 800 | 0     | 8         | 2     | 10    |
|                   | > 800     | 0     | 3         | 64    | 67    |
| Total             |           | 4     | 12        | 66    | 82    |

**Concordance rate 92.7% (76/82)** Sensitivity 97.0% (64/66, cut-off 800 mg/dl) Specificity 81.3% (13/16, cut-off 800 mg/dl)



Equine IgG RID Test (mg/dL)

**Fig. 2**. Comparison between the SNAP kit and the RID test for IgG concentration using 82 serum and plasma samples

| IgG             |           | RID   |           |       | Total |
|-----------------|-----------|-------|-----------|-------|-------|
|                 |           | < 400 | 400 - 800 | > 800 | IUldi |
| SNAP<br>(mg/dl) | < 400     | 4     | 1         | 0     | 5     |
|                 | 400 - 800 | 0     | 7         | 5     | 12    |
|                 | > 800     | 0     | 4         | 61    | 65    |
| Total           |           | 4     | 12        | 66    | 82    |

**Concordance rate 87.8% (72/82)** Sensitivity 92.4% (61/66, cut-off 800 mg/dl) Specificity 75.0% (12/16, cut-off 800 mg/dl)

Table 1-2. Classification of the results of the Vcheck and SNAP kits based on the reference range

Reference

1) L. Tscheschlok, et al. Howard. Comparison of IgG concentrations by radial immunodiffusion, electrophoretic gamma globulin concentrations and total globulins in neonatal foals. Equine Veterinary Journal 0 (2016) 1–6

2) Ujvari S, et al. 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. J Vet Intern Med. 2017 Jul;31(4):1170-1177.

