

Quantitative marker of Immunoglobulin G (IgG) in foal

Vcheck Foal IgG

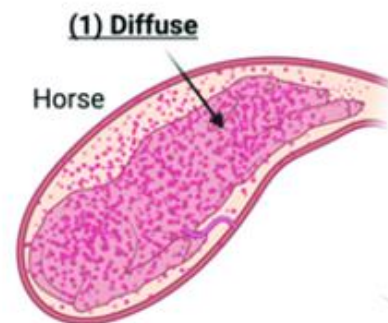
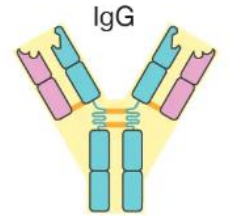
BIONOTE Marketing team

September 2023



Immunoglobulins in Foals

- **Immunoglobulins (Ig): Antibodies (IgM, IgD, IgA, IgE, and IgG)**
 - Protein used by the immune system to neutralize antigens (pathogenic bacteria and viruses)
 - **Immunoglobulin G (IgG): 75% of serum immunoglobulin**
- **Foals have low concentrations of circulating immunoglobulins at birth,** because the diffuse epitheliochorial placenta does not allow antibody transfer during pregnancy¹⁾.
- Foals begin to produce immunoglobulins immediately after they have been exposed to antigens, **but protective concentrations of immunoglobulins may not be reached until they are 2 months old.**



◀ The feature of equine placenta (diffuse epitheliochorial placenta)

Clinical utility of IgG in Foals

Immunoglobulin G in Foals

- **Transfer of maternal antibodies is critical for the well-being of foals.**
 - Foals are entirely dependent on maternal immunoglobulins (**primarily IgG**, lesser quantities of IgA and IgM) absorbed through ingestion of mare's colostrum **in the first 24 hours of life**¹).
 - Immunoglobulin is not absorbed after 24–36 hours of age ²).
- **Failure of passive transfer (FPT)** : failure to absorb colostrum antibodies sufficient to achieve a IgG
 - **Healthy foals** consuming good-quality colostrum: IgG concentrations > 800 mg/dL
 - **Complete FPT**: IgG concentrations < 400 mg/dL after 24 hours of age
 - **Partial FPT**: serum IgG concentrations between 400 and 800 mg/dL
- **Incidence:** FPT (ranges from 3–24% in foals), partial FPT (ranges from 14–31%)¹.

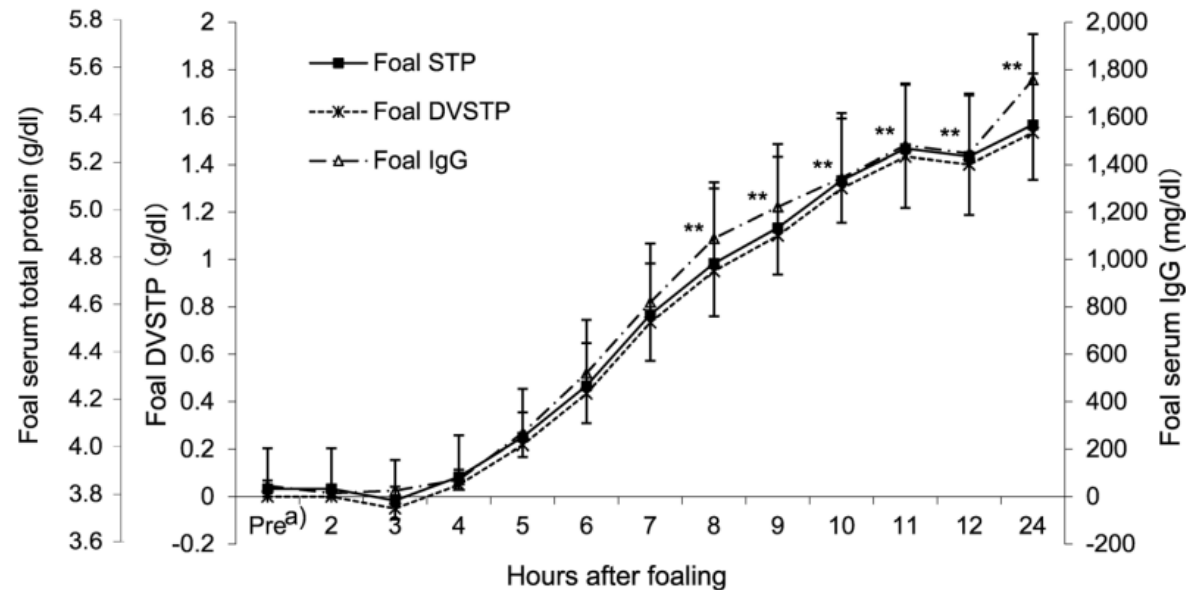
Clinical utility of IgG in Foals

Immunoglobulin G in Foals

- Foals with insufficient serum immunoglobulin (IgG) levels are **at increased risk of infectious diseases**, such as septicemia, arthritis or respiratory tract infections.
- **Several factors which can lead to FPT**
 - Poor colostrum quality (Not sufficient quantities IgG < 3,000 mg/dl)
 - Delayed suckling by the foal (mares reluctant to let foals suckle)
 - Ingestion of too little colostrum
 - Failure of the foal to absorb IgG from the colostrum

Clinical utility of IgG in Foals

IgG concentrations in Foals



▲ Changes in foal serum IgG from before suckling to 24 hr after birth

- **From 4 hr after birth,**
 - ✓ The foal serum IgG: tendency to increase
 - ✓ The colostrum/milk IgG: tendency to decrease
- **6-12 hours:** maximum antibody transfer

Result Interpretation

Interpretation of IgG results

- **Early testing for IgG concentration in newborn foals can identify potential cases of FPT.** (Treating foals with FPT may require hospitalization and costs can exceed \$1500 per foal¹.)
- Test the foal's IgG concentrations **after 12 hours after birth** to assess passive transfer status.
- If the foal has partial or complete FPT, you must administer antibodies via fresh or frozen oral colostrum (within the first 12-18 hours of life), commercial products, or intravenous plasma (if 24 hours have passed).

REFERENCE RANGE

< 400 mg/dL	400 ~ 800 mg/dL	> 800 mg/dL
Failure of passive transfer in foal	Partial failure of passive transfer in foal	Successful passive transfer in foal

* Each laboratory should establish its own reference interval, as reference values may vary depending on the test population.

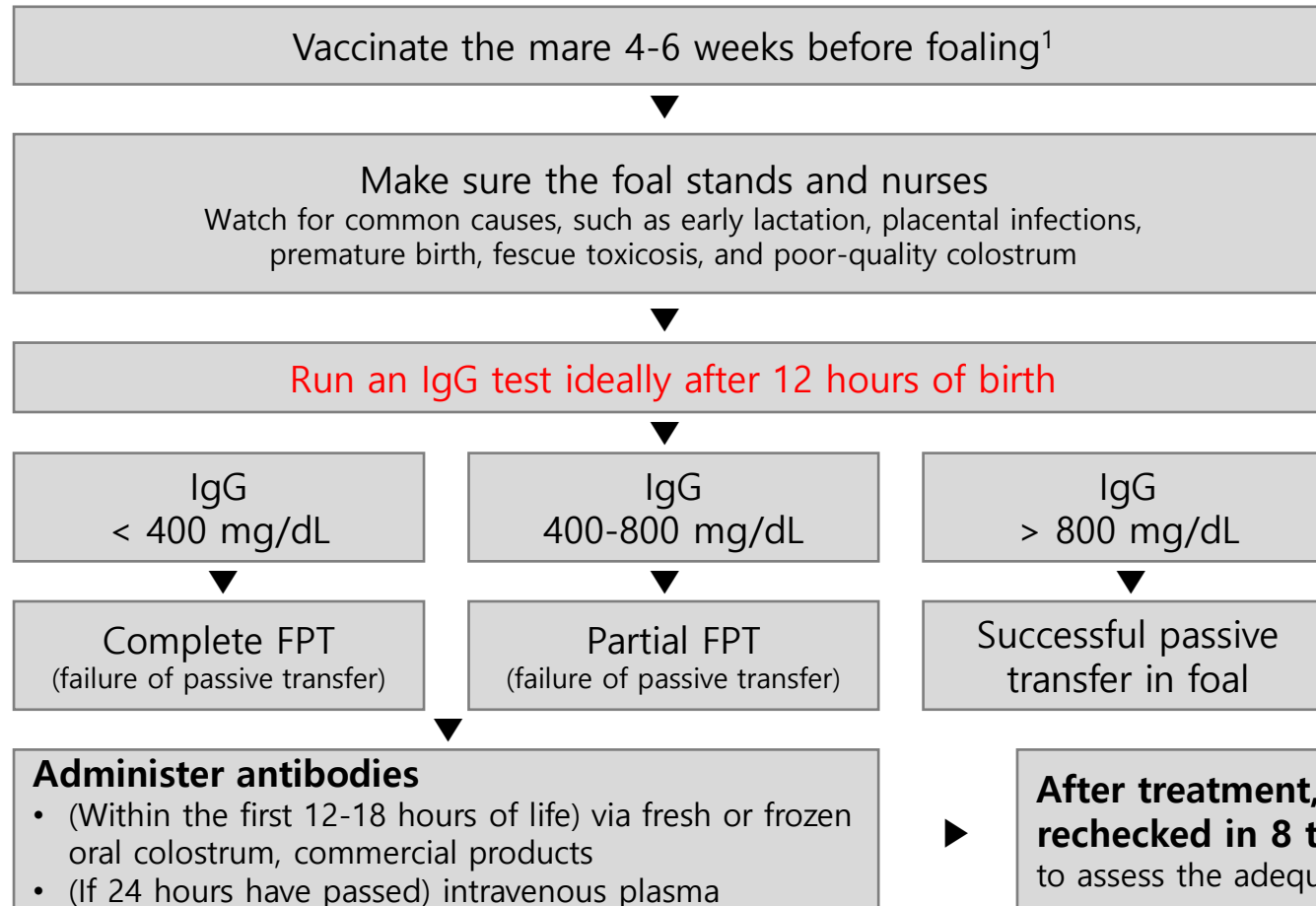
* The veterinarian in charge must conduct a clinical diagnosis along with the measured results of this reagent, clinical symptoms, and other test results.

◀ Reference range of Vcheck Foal IgG



Diagnostic Algorithm

Algorithm to prevent Failure of Passive Transfer



Case Study

- A foal born in September, 2021
- The foal was nursing from the mare within two to three hours after birth.
- When monitoring the mare carefully in the days prior to foaling, the mare drip milk for several days to weeks.
- IgG measurements were performed 12 hours after the foal was born. **The IgG result was 520 mg/dl.**
→ It was interpreted as "partial FPT".
- Oral immunoglobulin supplement (Seramune, Sera, Inc.) was immediately administered at the manufacturer's recommended dosage.

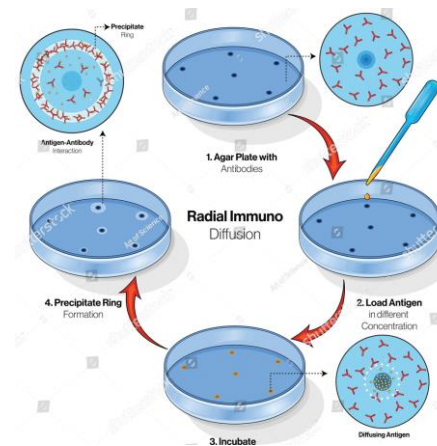


example photos

Gold standard method

Gold standard method for the IgG test

- Several screening tests have been developed over the years for evaluating the IgG concentration in foals.
- **The single radial immunodiffusion (SRID) test**
: considered to be the most accurate test for quantitative measurement of IgG concentration in foals
- **However, results of SRID test are generally not available for 24 hr.**
The 24-hr delay in obtaining test results decreases the usefulness of this test for routine use on a breeding farm, in a field situation or at a veterinary clinic because rapid results are required for therapeutic intervention in FPT cases in foals.



▲ Radial immunodiffusion (RID) test

Performance

Strong correlation with a gold standard method

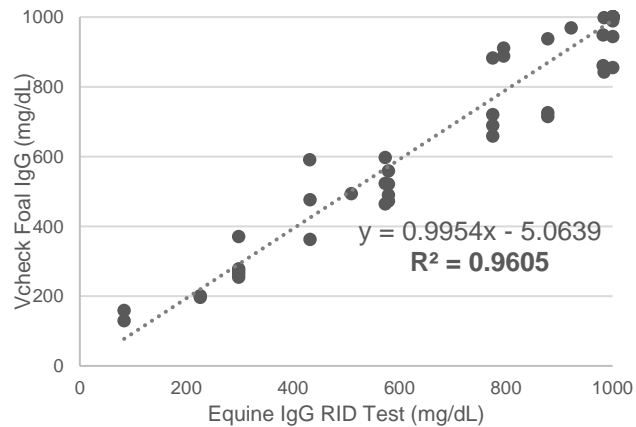


▲ Triple J Farms IgG

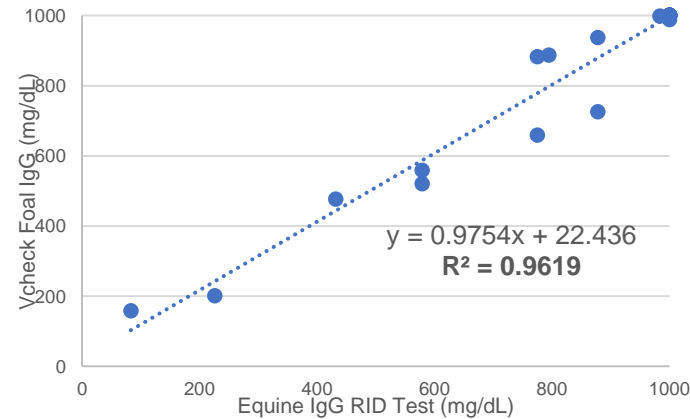
- The reference method of Vcheck Foal IgG: Triple J Farms IgG* (RID assay)

*Validated assay¹

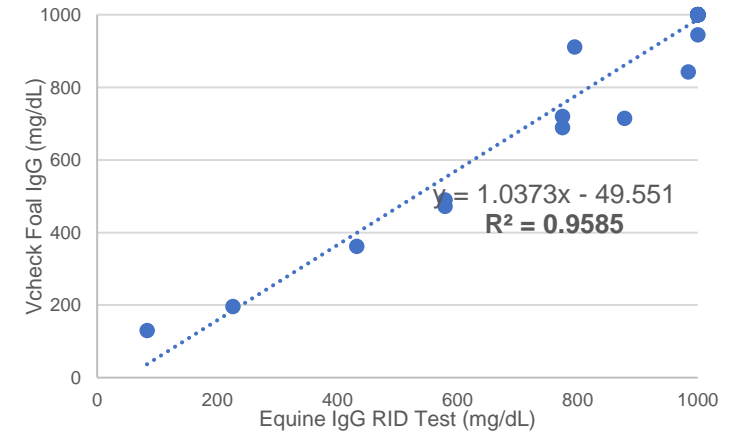
- There is a **strong correlation (slope 0.995, R²=0.96)** was found between the two test methods when analyzing 102 whole blood, plasma, and serum samples².



All sample types (n=102)



Serum (n=42)



Whole blood (n=40)

Performance

Better performance than IDEXX SNAP KIT



▲ IDEXX SNAP Foal IgG
Results are interpreted as '<400 mg/dl', '400 mg/dl', '400-800 mg/dl', '800 mg/dl', or '>800 mg/dl'.

- When compared to the RID test (cut-off 800 mg/dl)
 - ✓ **Vcheck:** sensitivity of 97% (64/66), specificity of 81.3% (13/16)
 - ✓ **IDEXX SNAP:** sensitivity of 92.4% (61/66), specificity of 75% (12/16)
- 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.

Vcheck (vs. RID)

IgG		RID			Total
		<400	400-800	>800	
Vcheck (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)

IDEXX SNAP (vs. RID)

IgG		RID (mg/dl)			Total
		<400	400-800	>800	
SNAP (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)

Is the 'colostrum IgG test' useful?

- Serum IgG concentration in a newborn foal correlates well with the IgG concentration in its dam's colostrum¹.
- However, one report has indicated that **foals that fail to nurse due to neonatal weakness or delayed development of the nursing reflex have low IgG concentrations** despite adequate equine colostrum quality¹.

Is the 'total protein (TP) test' useful?

- One of the most rapid and inexpensive tests (by refractometry)
- However, several reports have indicated that this test is an **unreliable indicator of FPT** in foals because unlike in calves, **there is a wide range of total protein concentrations in newborn foals**¹.

Thank you for your time!

Any questions?

Key points

- ✓ Transfer of maternal antibodies is critical for the well-being of foals to achieve a IgG.
- ✓ Early testing for IgG concentration in newborn foals can identify potential cases of FPT.
- ✓ The Vcheck had superior performance compared to the SNAP kit when evaluated against the reference RID test for measuring foal IgG levels

Quantitative marker of Foal IgG

Vcheck Foal IgG

Vchck Foal IgG

Vcheck Foal IgG

Quantitative marker of Immunoglobulin G in foal

✓ Simple and useful method of assessment of neonatal foals' IgG levels.



- **Species** : Horse
- **Sample** : Whole blood (EDTA or heparin) 10 μ l
or Serum, Plasma (EDTA or heparin) 5 μ l
- **Testing time** : 5 minutes
- **Measurement Range** : 100 ~ 1,000 mg/dl
- **Storage Condition** : 2 ~ 30 $^{\circ}$ C

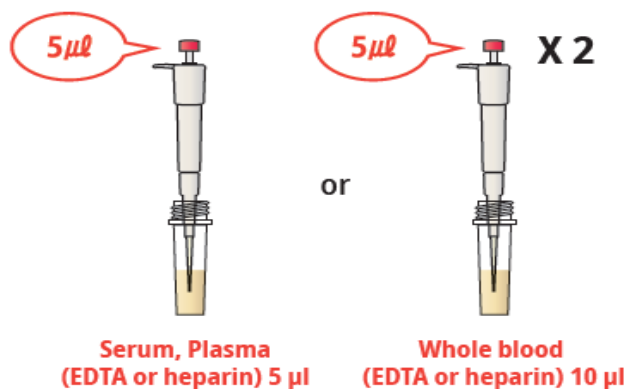
Vchck Foal IgG

Vcheck Foal IgG

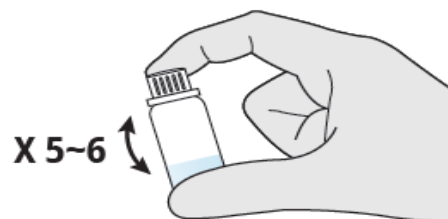
Quantitative marker of Immunoglobulin G in foal

- ✓ Simple and useful method of assessment of neonatal foals' IgG levels.

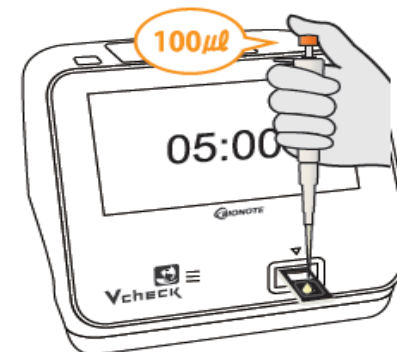
Add 5 μ l of serum, plasma (EDTA or heparin) or 10 μ l of whole blood to the assay diluent bottle



Close the bottle cap and shake for 5-6 times to mix thoroughly



Add 100 μ l of the mixed sample into the test device



< 400 mg/dL

Failure of passive transfer in foal

400 ~ 800 mg/dL

Partial failure of passive transfer in foal

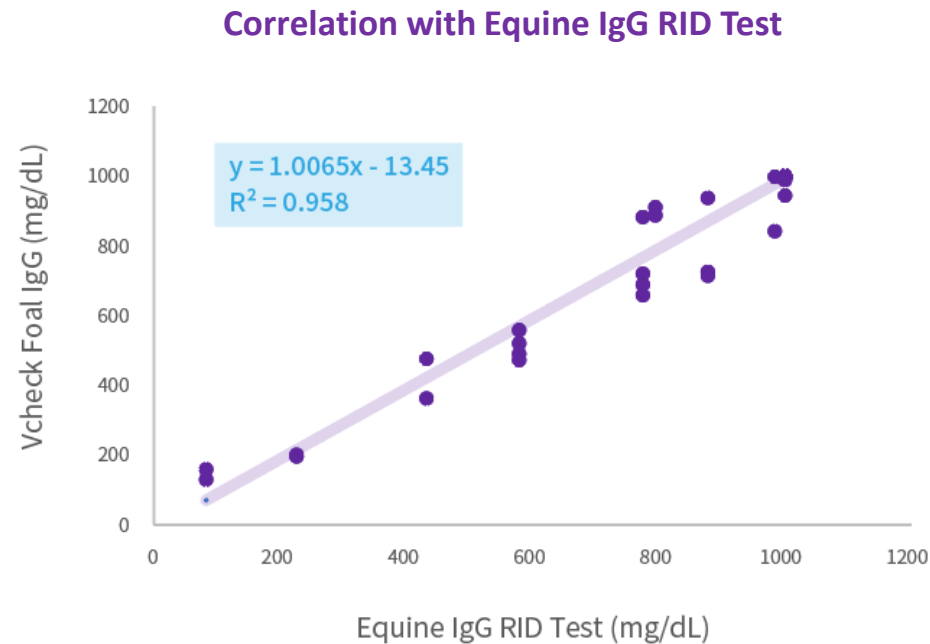
> 800 mg/dL

Successful passive transfer in foal

Performance

Correlation

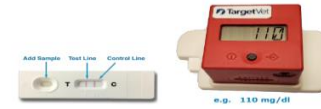
Vcheck Foal IgG has a **strong correlation** ($y=1.0065x - 13.45$, **R²=0.958**) with the reference method (Equine IgG RID), which has been used in reference laboratories



Vchck Foal IgG

Vcheck Foal IgG

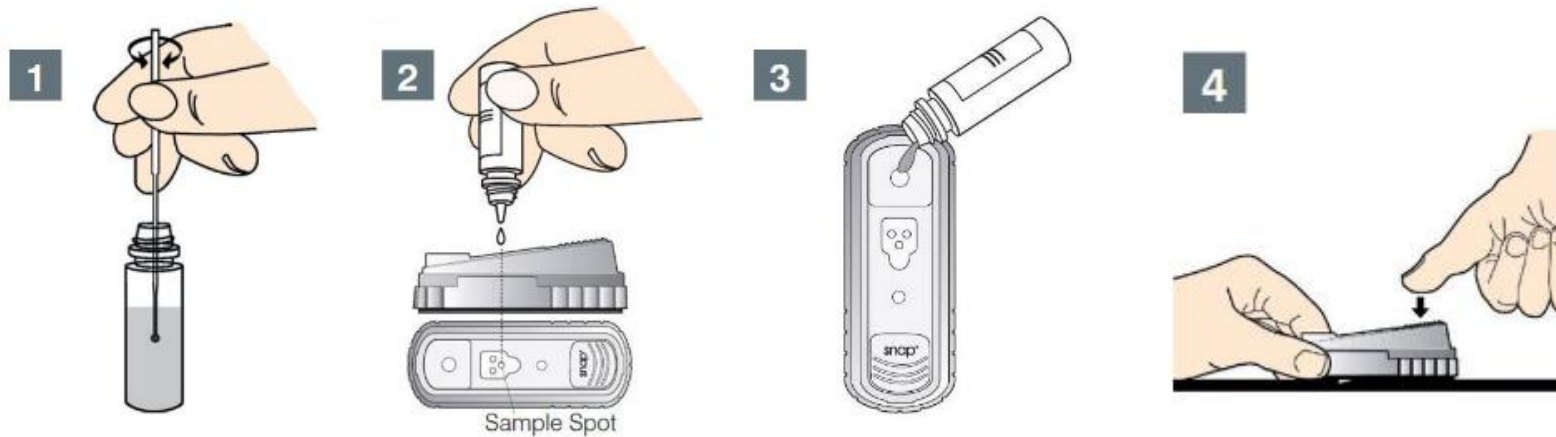
Quantitative marker of Immunoglobulin G in foal



Equine IgG	Vcheck F	IDEXX (USA)	TargetVet (USA)	VMRD (USA)
Product Name	Equine Foal IgG	SNAP Foal IgG	Immuno-Chek G	Foal IgG
Analyzer	V200, V2400	x	x	VMRD reader
Assay	Fluorescent immunoassay (FIA)	ELISA	Lateral Flow Tech	Lateral Flow Tech
Specimen	Serum, Plasma(EDTA, heparin), whole blood (EDTA, heparin)	Whole boold, Serum, Plasma(heparin, EDTA)	Serum, whole blood (Heparin, EDTA)	Serum, whole blood (EDTA)
Measurement Range	100 mg/dL~1,000 mg/dL	100-800 mg/dL	10-2700 mg/dL	200-1000 mg/dL
Test Time	5mins	7mins	10mins	10 mins
Storage	2-30°C	2~8°C	Room temperature	Room temperature
Reference Range	< 400 mg/dL Failure of passive transfer in foal 400 ~ 800 mg/dL Partial failure of passive transfer in foal > 800 mg/dL Successful passive transfer in foal	IgG > 800 mg/dL → adequate passive transfer (good/normal immune protection) IgG between 400 – 800 mg/dL → partial failure of passive transfer (partial protection) IgG < 400 mg/dL → complete failure of passive transfer (very susceptible to infection)	>800 Very Good Transfer 400 Partial Transfer 200 Total Failure	0-400 mg/dL: Very low IgG 400-800 mg/dL: Low IgG >800 mg/dL: Normal IgG
Package unit	5 Tests/Kit	5 or 10 Tests/Kit	5 or 10 Tests/Kit	15Tests/Kit
Note	•27 Items available •3 equine panel	semi-quantitative test	semi-quantitative test	Quantitative

Foal IgG

IDEXX –Snap Foal IgG



- Sample: Plasma(heparin), Serum, Whole blood
- Testint time: 7 mins
- Assay: ELISA
- Video: <https://www.youtube.com/watch?v=BuJMPCsSqNg>
- Sensitivity: 94.7%
- Result: : 100-800 mg/dL
- Package unit: 5T/kit

< 400

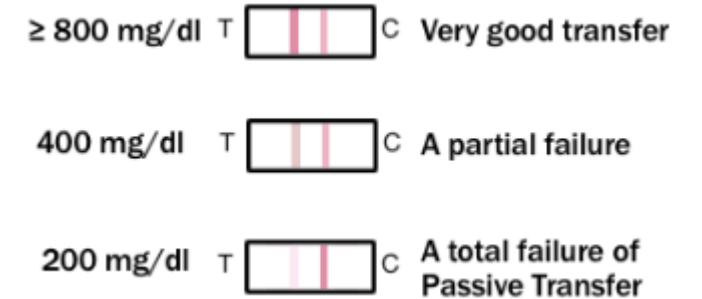
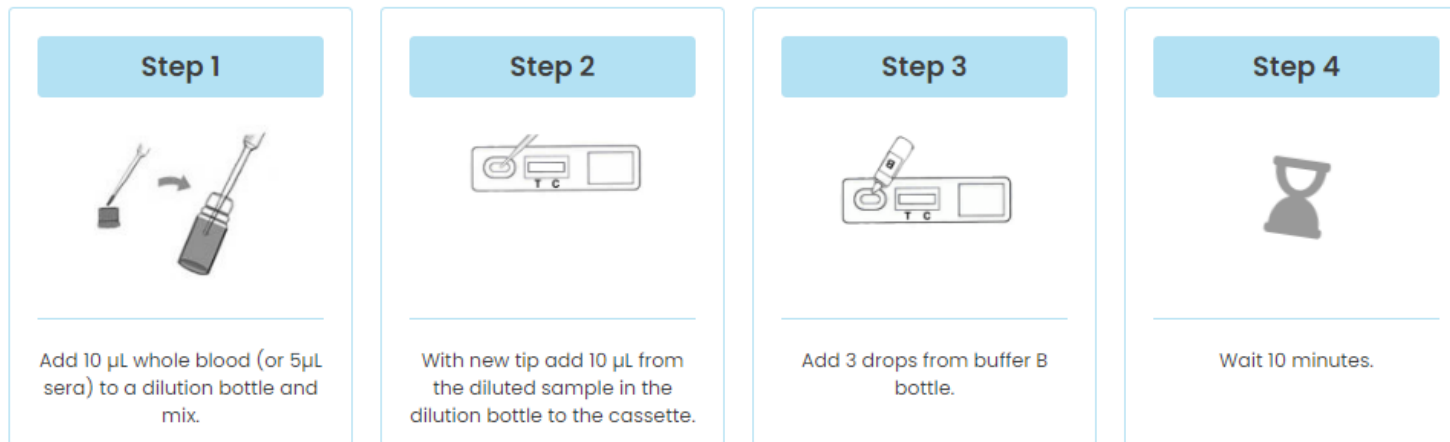


>800



Foal IgG

Target vet- Immuno Check G



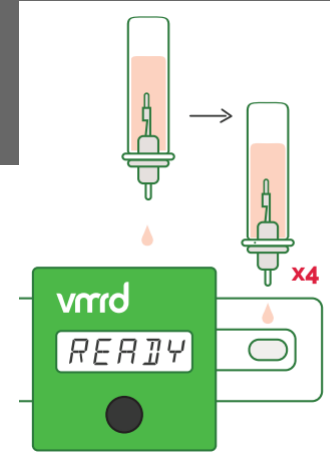
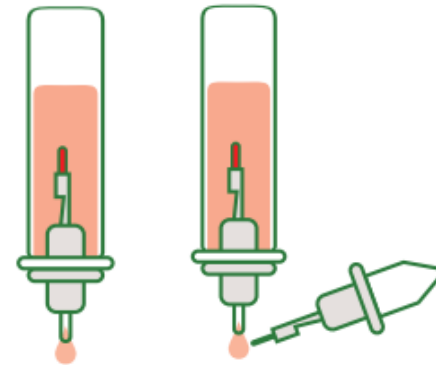
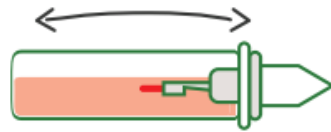
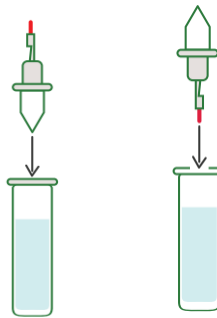
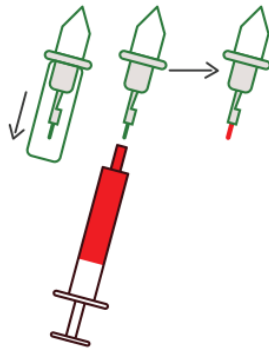
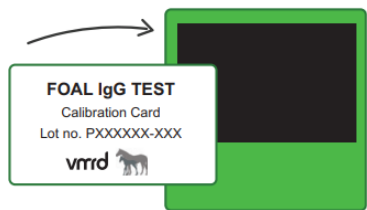
- Sample: Serum, Whole blood
- Testint time: 10 mins
- Assay: Lateral flow tech
- Video: <https://www.youtube.com/watch?v=2qySoaU9Xi4>
- Sensitivity: 100%
- Result: : 10-2700 mg/dL
- Package unit: 5T/kit



e.g. 110 mg/dl

Foal IgG

VMRD Foal IgG

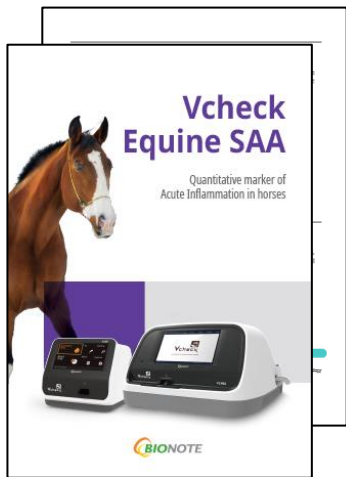


- Sample: Plasma(EDTA), Whole blood
- Testint time: 10 mins
- Assay: Lateral flow tech
- Video: <https://www.youtube.com/watch?v=Y9V6hIZ6jpM>
- Result: : 10-2700 mg/dL
- Package unit: 15T/kit

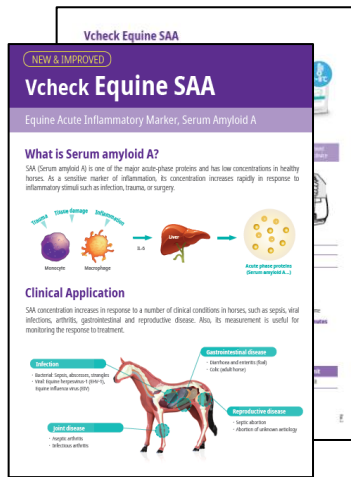
Marketing materials

✓ Brochures/leaflets

- Brochure: Detailed resource, showcases product values, used by vets to explain benefits and encourage tests.
- Leaflet/Pamphlet: Summarized content, highlights key product advantages, helps vets communicate benefits to patients.



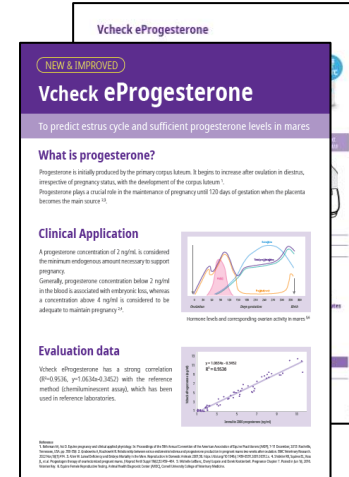
▲ E.SAA brochure



▲ E.SAA Leaflet



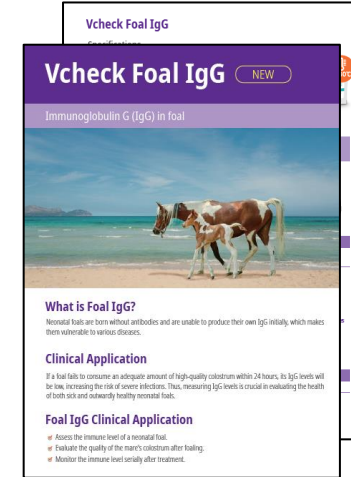
▲ ePRG brochure



▲ ePRG Leaflet



▲ Foal IgG brochure



▲ Foal IgG Leaflet

Marketing materials

✓ Evaluation

- **eProgesterone:** Comparative assessment with over 150 samples against the IMMULITE 2000 equipment demonstrated excellent correlation (R2=0.96), based on data from the BN Research Division.
- **E.SAA:** Using over 170 samples, a comparative assessment against the Eiken VET-SAA equipment demonstrated exceptional correlation (R2=0.98), based on data from the BN Research Division.
- **Foal IgG:** A strong correlation (slope 0.995, R2 = 0.96) was found between Vcheck and the RID test when analyzing 102 whole blood, plasma, and serum samples

Comparison of an in-clinic point-of-care assay to the reference method for the detection of equine progesterone

Key Words : Vcheck, Equine, Progesterone, Comparison, IMMULITE

BIONOTE study

Introduction
Progesterone is responsible for the suppression of behavioral estrus, closure of the cervix, alterations in endometrial glandular function, and other physiological events. It is also the most important hormone for the maintenance of early pregnancy to day 45 of the term.¹ Progesterone is required for early embryonic survival.²

Purpose
The aim of this study was to compare equine progesterone results obtained using the Vcheck assay with those obtained using the IMMULITE 2000 Progesterone, which has previously been validated for measurement of equine progesterone.

Materials and Methods
A total of 150 fresh equine serum and plasma samples with varying progesterone concentrations were received and used for the purpose of this study conducted by the BIONOTE laboratory. No samples were used that exhibited heavy hemolysis, lipemia, or other serum/ plasma abnormalities. Samples were analyzed using a Vcheck eProgesterone test kit (BIONOTE) according to the manufacturer's instructions. The remaining sample was measured with IMMULITE 2000 Progesterone run on an IMMULITE 2000 at the BIONOTE laboratory by laboratory technicians.

Results
The test results for the correlation of equine progesterone measurements between Vcheck and IMMULITE 2000 are shown in Figure 1-3. Samples outside the measurement range (1-30 ng/ml) of the Vcheck eProgesterone test kit were excluded from the analysis. A strong correlation (Slope 0.95, R² = 0.96) was found between the two test methods when analyzing 150 plasma and serum samples (Figure 1). When measuring plasma (heparin) samples (N = 33) and serum samples (N = 117) separately, a very high correlation of R² = 0.95 (Figure 2) and R² = 0.97 (Figure 3) was observed, respectively.

Conclusion
This paper presents a validation of point-of-care (POC) progesterone immunoassay in comparison to chemoluminescent immunoassay (CLIA), which has already been validated for the measurement of progesterone in equine samples. The performance of the Vcheck eProgesterone immunoassay was similar to the CLIA (IMMULITE 2000). Our study supports the conclusion that progesterone results generated by the POC immunoassay can be used interchangeably with CLIA results for clinical purposes.

BIONOTE

▲ ePRG

Comparison of an in-clinic point-of-care assay to the reference method for the detection of equine serum amyloid A

Key Words : Vcheck, Equine, SAA, Comparison, Eiken

BIONOTE study

Introduction
Serum amyloid A (SAA) is the major acute phase protein (APP) primarily produced by the liver during the acute phase response (inflammation process). It is a sensitive marker of inflammation. SAA concentration increases rapidly in response to inflammatory stimuli such as infection, trauma, or surgery. SAA measurements aid in the diagnosis, prognosis, and general assessment of health in horses.

Purpose
The aim of the study was to compare equine SAA results obtained using the Vcheck assay with those obtained using the Eiken VET-SAA assay, which had previously been validated for measurement of equine SAA.

Materials and Methods
A total of 170 fresh equine serum and plasma samples with varying SAA concentrations were received and used for the purpose of this study, conducted by the BIONOTE laboratory. No samples were used that exhibited heavy hemolysis, lipemia, or other serum/ plasma abnormalities. The samples were analyzed using a Vcheck Equine SAA test kit (BIONOTE) according to the manufacturer's instructions. The remaining samples were immediately frozen and shipped to the Animal Health Diagnostic Center (AHDC) at Cornell University on dry ice for the VET-SAA assay (Eiken Chemical Co., Tokyo, Japan).

Results
The test results for the correlation of equine SAA measurements between Vcheck and Eiken VET-SAA are shown in Figure 1-3. Samples outside the measurement range (0-1,000 ng/L) of the Vcheck Equine SAA test kit were excluded from the analysis. A strong correlation (Slope 1.06, R² = 0.98) was found between the two test methods when analyzing 170 plasma and serum samples (Figure 1). When measuring plasma (heparin) samples (N = 161) and serum samples (N = 20) separately, a very high correlation of R² = 0.98 (Figure 2) and R² = 0.97 (Figure 3) was observed, respectively.

Conclusion
This paper presents a validation of a point-of-care (POC) SAA immunoassay in comparison to a chemoluminescent immunoassay, which has already been validated for the measurement of SAA in equine samples. The performance of the Vcheck Equine SAA immunoassay was similar to that of the chemoluminescent immunoassay (Eiken VET-SAA). Our study supports the conclusion that SAA results generated by the POC immunoassay can be used interchangeably with chemoluminescent immunoassay results for clinical purposes.

BIONOTE

▲ E.SAA

Comparison between an in-clinic point-of-care assay and the reference method for detecting of equine immunoglobulin G

Key Words : Vcheck, Equine, Foal, IgG, RID, Comparison

BIONOTE study


Introduction
Failure of transfer of passive immunity (FTPI) in foals is associated with a risk of infection and death.¹ Healthy foals on well-managed farms may have sufficient serum immunoglobulin G (IgG) concentrations ranging from 400 to 800 mg/dL; however, these levels are inadequate for compromised or ill foals, which require a significant portion of hospitalized foals. For compromised or ill foals, an IgG concentration of 800 mg/dL or considered adequate. Detection of FTPI in sick or hospitalized foals, therefore, constitutes an important facet of these cases.²

Purpose
The aim of this study was to compare the results of equine IgG obtained using Vcheck assay with those obtained using the radial immunodiffusion (RID) test for Equine IgG (Eiken, Breda, The Netherlands), which has previously been validated for measurement of equine IgG.³

Materials and Methods
A total of 102 fresh equine whole blood, plasma, 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/ plasma abnormalities were included. The samples were analyzed using a Vcheck Foal IgG test kit (BIONOTE) according to the manufacturer's instructions. The remaining samples were measured using the Triple I Farms Equine IgG test kit at the BIONOTE laboratory by laboratory technicians.

Results
The test results for the correlation of equine IgG measurements between Vcheck and the RID test are shown in Figures 1-4. Samples outside the measurement range (100-1,000 mg/dL) of the Vcheck Foal IgG test kit were excluded from the analysis. A strong correlation (Slope 0.995, R² = 0.96) was found between the two test methods when analyzing 102 whole blood, plasma, and serum samples (Figure 1). When measuring whole blood samples (N=48), plasma (heparin) samples (N = 20), and serum samples (N = 42) separately, a very high correlation of R² = 0.96 (Figure 2), R² = 0.95 (Figure 3), and R² = 0.96 (Figure 4) was observed, respectively.

Conclusion
This paper presents a validation of point-of-care (POC) IgG immunoassay in comparison to the RID assay, which has already been validated for the measurement of IgG in equine samples. The performance of the Vcheck Foal IgG immunoassay was similar to the RID in whole blood, plasma, and serum samples. Our study supports the conclusion that IgG results obtained from the POC immunoassay can be used interchangeably with the RID results for clinical purposes.



BIONOTE

▲ Foal IgG

Marketing materials

✓ Fact sheet

- Quick product overview in one page.
- Showcases product range and benefits.
- Aids veterinarians in explaining options to customers.

VCHECK EQUINE 3 PANEL OVERVIEW

Veterinary equine diagnostic panel is used to assess the overall health and well-being of a horse. The panel identifies any underlying medical conditions that may require treatment or further clinical test. It is a useful tool to evaluate the health of horses and ensure that they are in optimal condition.

Equine 3 panel is provided on Vcheck platform, Vcheck is a Fluorescence Immunoassay Analyzer that utilizes Europlum for more accurate measurements. Vcheck provides in-house quantitative analysis with 29+ biomarkers available (cardiac, kidney, etc).

VCHECK PRODUCT PORTFOLIO

eProgesterone

TO PREDICT ESTRUS CYCLE AND SUFFICIENT PROGESTERONE LEVELS IN MARES

Progesterone is essential in sustaining pregnancy up until the placenta becomes the primary source, which happens after 120 days of gestation. Measuring progesterone levels also aids in determining a mare's reproductive cycle and optimizing pregnancy planning.

eProgesterone Clinical Application

- ✓ On-site Progesterone Analysis for Equines
- ✓ Evaluate corpus luteum (CL) during early pregnancy
- ✓ Monitoring the progesterone during pregnancy

Sample: Serum, Plasma (Heparin)
Testing Time: 15 minutes
Measurement Range: 1 - 30 ng/mL

≤ 2.0 ng/mL (≤ 20 weeks) > 2.0 ng/mL (> 20 weeks)

Low High (Gestation)

Equine SAA

TO ASSESS THE LEVEL OF INFLAMMATION IN HORSES

SAA (Serum amyloid A) is one of the major acute-phase proteins and has low concentrations in healthy horses. As a sensitive marker of inflammation, its concentration increases rapidly in response to inflammatory stimuli such as infection, trauma, or surgery.

Equine SAA Clinical Application

- ✓ Early detection of the presence of inflammation
- ✓ Monitoring the post-operative effects and recovery
- ✓ Serial monitoring of the response to treatment

Sample: Serum, Plasma (Heparin)
Testing Time: 5 minutes
Measurement Range: 10 - 1,000 mg/L

≤ 10 mg/L 10 - 20 mg/L > 20 mg/L

Normal Equivocal Abnormal

▲ Fact sheet

Thank you

BIONOTE Marketing team
September 2023

