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Equine IgG Test Kit FOALCHEK® A diagnostic test for determination of IgG levels in foals

General Information: In equine species, young are born without significant amounts of circulating protective antibody. Normally, maternal antibodies are transmitted to the foal in the colostrum ingested during the first 24 hours after birth. These maternal antibodies provide protection against a variety of pathogens common to the animals' environment until the foal matures sufficiently to produce its own immunoglobulins. It has been reported that 10-12 percent of foals fail to acquire even partially protective antibody levels from their dam's colostrum, and an additional 12% acquire only **partially** protective levels.¹⁻³ These foals are prone to infection which may result in serious illness or death. The survival rate of these animals, if diagnosed, may be improved by various treatments, particularly serum transfusions.^{2, 4-6}

McGuire and co-workers have suggested the following immunoglobulin levels as meaningful indicators of passive transfer ³.

- <200mg/dl failure of passive transfer
- 200mg-400mg/dl partial failure of passive transfer
- >400 mg/dl adequate transfer

Current trends indicate a number of veterinarians prefer to measure an IgG level >800 mg/dl.

Either of these IgG levels can be measured with the Foalchek® test kit. (See Test Procedures)

Description: Foalchek® is a highly sensitive latex agglutination test for IgG in foals. The test can be conducted on either blood or serum with results obtainable within minutes of collecting a blood sample.

Indications: The Foalchek® test is recommended as a routine part of neonatal health care to screen foals for passive transfer of colostral immunoglobulins from mares to foals. Immunoglobulin levels should be determined after allowing time for absorption. Although sampling between two and three days of age is optimal, twelve to fifteen hours after the first nursing is usually adequate.^{2,6} If a low test result is obtained before 24 hrs. of age, the test should be repeated 12-24 hrs. later.

Kit Contents:

<u>24 PACK</u> 24 glass bottles of diluent, 3 plastic squeeze bottles of latex, 1 vial containing fifty 5-microliter heparinized capillary pipettes, 1 plastic mixing stick, 24 disposable plastic Pasteur pipettes, 1 agglutination slide (black with 3 test rings).

<u>10 PACK</u> 10 glass bottles of diluent, 1 plastic squeeze bottle of latex, 1 vial containing twenty 5-microliter heparinized capillary pipettes, 1 plastic mixing stick, 10 disposable plastic Pasteur pipettes, 1 agglutination slide (black with 3 test rings.)

Specimen Requirements: Either whole blood or serum may be used for the test.

- Serum- collect blood specimen without anticoagulant and allow to clot. After retraction of the clot draw off 5 microliters of serum from the layer above the clot using a single 5 microliter capillary pipette. Touch the pipette to the serum, holding the pipette almost horizontal and allow it to fill. The capillary pipette must be completely filled from tip to tip. Care must be taken to assure that the capillary pipette does not contain air bubbles. Wipe excess serum from the outside of the pipette being careful not to draw any serum out of the pipette (fingers work best for this). Hemolysis does not interfere with the test.
- Blood- whole blood may be collected directly into the capillary pipettes or first placed into an anticoagulant tube (heparin of EDTA may be used). Either one or two capillary pipettes filled with whole blood are required for the test. (See Test Procedures) The capillary pipettes are filled in the same manner as with serum. The same care must be taken to insure the pipettes are completely filled and excess blood is removed from the exterior.

Test Procedure:

<u>A: TO TEST >400 mg/dl</u>:

Drop the filled 5 microliter pipette(s) into the diluent vial (one pipette if serum is used <u>or</u> two pipettes if whole blood is used).

<u>B: TO TEST >800 mg/dl</u>:

Drop one 5 microliter pipette of whole blood or alternatively, 2.5 microliters of serum into the diluent vial.

- 1. Allow <u>5 minutes</u> for the specimen to disperse throughout the diluent. Shake well to insure mixing before removing a sample for testing.
- 2. Use a plastic Pasteur pipette to draw a small amount of the specimen-diluent mixture, making sure that excess is removed from outside of the pipette. Deliver 2 drops inside the first slide ring. 3 drops inside the second ring and 4 drops inside the third ring of the glass slide. Take care to hold the pipette vertically to insure accurate dispensing of proper size drops.
- 3. Shake latex bottle to insure mixing. Remove cap and add 2 drops of latex to each slide ring. Hold dropper bottle vertically to insure accurate dispensing of proper size drops.
- 4. Use the provided mixing stick to stir the drops and spread them over the entire area inside the rings.
- 5. Gently tilt and rotate the slide to maintain suspension of the reactants
- 6. Observe for agglutination after rotating for approximately 15 seconds. If agglutination does not occur in all 3 rings, continue to rotate for an additional 45 seconds. Read slide and record finding at this time.

Some samples that are truly negative will appear to have glossy type patches floating on top. This is not to be considered a positive agglutination. The glossy sheen and patches on top are almost always linked with a negative sample.

Interpretation: Timing is important. Specimens with high IgG levels will agglutinate in all three rings within 15 seconds. When agglutination has not occurred in all 3 rings by 15 seconds, the slide should be rotated an additional 45 seconds, then read. The following table should be used to interpret the results at this time.

AGGLUTINATION	Procedure A	Procedure B
	IgG mg/dl	IgG mg/dl
all 3 rings+	>400	>800
- + +	200-400	400-800
+	<200	200-400
	<100	<200

Important: If one or more rings have a questionable or very weak agglutination reaction, these rings should be considered negative.

Precautions: The accuracy of this test is dependent upon delivery of consistent-size drops of both latex and serum diluents. It is, therefore, extremely important that care be taken to hold the dropper bottle and Pasteur pipette vertically while dispensing drops. Also, care must be taken to avoid delivering air bubbles with the drops of liquid. The agglutination slide should be rinsed with water and dried between tests.

Latex: If latex is difficult to squeeze from vial, or if it has a stringy appearance, <u>DO NOT USE</u>. Contact technical support.

Diluent: The diluent can become contaminated if opened prior to use. If diluent has a cloudy appearance, <u>DO</u> NOT USE.

STORAGE: Store latex between <u>35°F and 45°F</u>. Latex can degrade at extremes of low and/or high temperatures.

References:

- 1. Perryman.L.E. and McGuire, T.C. (1980) Evaluation for Immune System Failures in Horses and Ponies. JAVMA 176 (12), 1374.
- Crawford, T.B., McGuire, T.C., Hallowell, A.L., and Macomber, L.E., (1977) Failure of Colostral Antibody Transfer in Foals: Its Effect, Diagnosis and Treatment. <u>Proceedings of 23rd Annual Conv. AM. Assoc. Equine Pract. 265</u>.
- 3. McGuire, T.C., Crawford, T.B., Hallowell, A.L., and Macomber, L.E., (1977) Failure of Colostral Immunoglobulin Transfer as an Explanation for Most Infections and Deaths of Neonatal Foals. <u>JAVMA 170</u> (11), 1302.
- 4. Rumbaugh, G.E., Ardans, A.A., Ginno, D., and Trommershausen-Smith, A., (1979) Identification and Treatment of Colostrum-Deficient Foals. JAVMA 174 (3), 273.
- 5. Burton, S.C., Hintz, H.F., Kemen, M.J., and Holmes, D.F., (1981) Lyophilized Hyperimmune Equine Serum as a Source for Antibodies for Neonatal Foals. <u>Am. J. Vet. Res. 42</u> (2), 308.
- 6. Crawford, T.B. (1981) Practical Evaluation of the Immune Status of Foals. Proceedings of Iowa Vet. Med. Assoc. Ann. Mtg.