Comparison of Two ELISA Screening Tests and a Non-Commercial Glutaraldehyde Coagulation Screening Test for the Detection of Failure of Passive Transfer in Neonatal Foals (21-Nov-2003)

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Abstract
CITE enzyme-linked immunosorbent assay (ELISA) was an unacceptable screening test for failure of passive transfer because of poor sensitivity. SNAP ELISA and glutaraldehyde coagulation tests are more appropriate screening tests. Specificity of the SNAP ELISA is better than the glutaraldehyde coagulation test. A second confirmatory test should be considered when using the glutaraldehyde coagulation test.

1. Introduction
Foals are born with a naïve immune system. They require the ingestion and absorption of maternal colostrum to provide their protective antibodies. The primary colostral immunoglobulin is IgG, and it can be absorbed for the first 18 - 24 h of life. Most foals have IgG concentrations of > 1000 mg/dl after colostral immunoglobulin absorption [1]. At 24 h of age, foal IgG levels > 800 mg/dl are accepted as normal [2]. Foals with IgG levels < 800 mg/dl are said to have failure or partial failure of passive transfer (FPT). Studies indicate that FPT occurs in 12 - 44% of foals [3-7]. A high correlation has been shown between foals with FPT and the development of life-threatening septicemia [4,8-10]. Foals with a serum IgG concentration of < 400 mg/dl have the highest risk of developing sepsis or other infections in the first 6 wk of life [11]. Early detection of FPT in foals is essential so that an exogenous source of immunoglobulins, such as plasma, can be administered early in life.

Commercial test kits to screen for FPT are routinely used by equine practitioners. Certain IgG screening tests such as enzyme-linked immunosorbent assay (ELISA) immunoglobulin tests have been considered potentially superior screening tests, because these tests measure the foal's immunoglobulin concentration with a specific equine anti-IgG antibody [12]. In contrast, tests such as the glutaraldehyde coagulation test is a non-specific test, because it detects other proteins in addition to immunoglobulins, such as fibrinogen, acute phase proteins, albumin, and hemoglobin [13]. Previous studies have reported the accuracy of a screening test compared with the gold standard, radial immunodiffusion (RID) [13-16]. Accuracy measures the frequency of agreement or correlation between the two tests. However, what happens when the screening test does not agree with the RID? Either it is reporting a foal to have FPT when its serum IgG concentrations are truly adequate (a false positive), or it is reporting that the foal has adequate serum IgG concentrations when in fact it does have FPT (a false negative). A test that produces few false positive results has a high specificity, whereas a test that produces few false negative results has a high sensitivity. Ideally, a test for FPT should be both highly sensitive and highly specific such as the equine RID IgG test. Unfortunately, this test requires 18 - 24 h before a result can be obtained. The test also requires more technical skill and is best performed in a laboratory setting [14]. In contrast, most screening tests for FPT in foals are relatively simple to perform, and the results are available in minutes. It is essential that screening tests for FPT have a high sensitivity to minimize the number of foals with FPT misclassified as having adequate passive transfer (false negative).

To make rational decisions about an individual foal's IgG status, one needs to know the sensitivity and specificity of the test
being used to detect FPT as well as the prevalence of FPT in the neonatal foal population. With this information, the positive predictive value (PPV) and negative predictive value (NPV) of the screening test can be determined. The PPV and NPV is a measure of confidence a practitioner will have in a positive or negative test result, respectively [14]. For example, let us assume a test for FPT has a PPV of 90% and a NPV of 70%. If a foal tests positive for FPT, a practitioner could be confident 9 of 10 times that the test result is correct (PPV). In contrast, if the foal tests negative for FPT, the practitioner is only confident that result is correct 7 of 10 times (NPV). It is essential for a FPT screening test to have a high NPV so that a foal with FPT is not misclassified by the test as having adequate passive transfer.

The CITE [a] ELISA test has been widely used among equine practitioners for over a decade. However, in 1999 the manufacturers discontinued the CITE ELISA test and replaced it with the SNAP [b] ELISA test. After the introduction of the SNAP ELISA test, practitioners reported that they were getting a larger percentage of foals testing positive for FPT than with the previous CITE ELISA test. This could have been caused by either the SNAP ELISA having a higher sensitivity and/or a lower specificity compared with the CITE ELISA test. Evidence that some of these positive SNAP ELISA test results for FPT were wrong (false positive) suggested that this new test had a lower specificity compared with the CITE ELISA test. This resulted in widespread concern over the reliability of the new SNAP ELISA test, specifically in its PPV. Many practitioners switched to other diagnostic screening tests such as the glutaraldehyde coagulation test or modified the method for performing the SNAP ELISA so that results were more consistent with the previous CITE ELISA test. In 2001, the manufacturer modified the SNAP ELISA and reported that the problems with the test had been resolved. During this time period, we were evaluating six FPT screening tests, including the CITE ELISA test, using serum from neonatal foals. At the end of the first foaling season, the CITE ELISA test was discontinued, and the SNAP ELISA was used in its place. Therefore, we had the opportunity to critically evaluate the sensitivity, specificity, and PPV/NPV of the CITE ELISA, the first version of the SNAP ELISA, and the revised version of the SNAP ELISA (SNAP ELISA 1 and SNAP ELISA 2, respectively). We also evaluated a non-commercial version of another popular screening test for FPT, the glutaraldehyde coagulation test.

2. Materials and Methods
Foals for this study came from the Maritime Provinces of Canada, primarily Prince Edward Island. Foals, 24 - 72 h of age, had blood collected for serum IgG quantitation as part of a routine post-partum foal examination. Blood was allowed to clot, and serum was separated and stored in a -80ºC freezer until all screening tests could be performed. Private practitioners submitted the majority of the foal samples. The two ELISA tests were run according to the manufacturer's instructions. The non-commercial glutaraldehyde coagulation test [14], was prepared and performed as described in previous studies. Equine RID IgG [c] test was the gold standard for determining foal's serum IgG concentrations and was performed according to the manufacturer's instructions. For the RID IgG testing, each serum sample was tested in duplicate, and the average of the results was used to determine IgG concentration. The results from the three screening tests were compared with the results of the equine RID IgG test. The sensitivity and specificity for each test was determined for the serum IgG concentration breakpoints, < 400 and < 800 mg/dl. Test results below the breakpoint (< 400 or < 800 mg/dl) were considered a positive test result, and those above the breakpoint were considered a negative test result. Because the methodology of the SNAP ELISA had changed between 2000 and 2001, the sensitivity and specificity of the first version (SNAP ELISA 1) and second version (SNAP ELISA 2) is reported.

Data Analysis - RID IgG concentrations were ascertained using a least squares linear regression equation, determined by four known standards and calculated using a statistical computer software program [d]. The sensitivity (Se), specificity (Sp), and prevalence (P) of FPT were calculated for each screening test by the formulas shown in Table 1, using a statistical software program [e]. Ninety-five percent confidence intervals were determined for the Se and Sp of all screening tests as well as the prevalence. PPV and NPV was calculated using the formulas:

PPV = Se x P/([Se x P] + [(1 - Sp) x (1 - P)])
NPV = Sp x (1 - P)/([Sp x (1 - P)] + [(1 - Se) x P])

Table 1 - Calculations for Sensitivity, Specificity, PPV, and NPV

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Positive for FPT</th>
<th>Negative for FPT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for FPT</td>
<td>a</td>
<td>b</td>
<td>(a + b)</td>
</tr>
<tr>
<td>Negative for FPT</td>
<td>c</td>
<td>d</td>
<td>(c + d)</td>
</tr>
<tr>
<td>Total</td>
<td>(a + c)</td>
<td>(b + d)</td>
<td>Prevalence (a + c)/(a + b + c + d)</td>
</tr>
</tbody>
</table>

RID IgG Results = < 800 mg/dl (or < 400 mg/dl).
FPT, failure of passive transfer.
3. Results
A total of 201 foals were tested for FPT. The prevalence of FPT, based on serum RID IgG test results, was 37.8% (95% CI = 31 - 45%) for IgG < 800 mg/dl and 21.9% (95% CI = 16 - 28%) for IgG < 400 mg/dl. Not all FPT screening tests were performed on all foals' serum samples. The discontinuation of CITE ELISA and SNAP ELISA 1 only allowed for testing of the serum samples collected during the time these assays were available. The SNAP ELISA 2 and glutaraldehyde coagulation test were tested on the majority of the foals. The sensitivity and specificity of each screening test when detecting foal serum IgG concentrations < 800 and < 400 mg/dl are presented in Table 2 and Table 3, respectively. The CITE ELISA had a poor sensitivity (54 - 55%) but the best specificity (100%) of any of the screening tests. The glutaraldehyde coagulation test had the best sensitivity (95 - 100%) but the worst specificity (58 - 80%) of any of the screening tests. The SNAP ELISA 1 test had a better sensitivity (87 - 93%) but poorer specificity (65 - 92%) compared with SNAP ELISA 2 (sensitivity 76 - 88%, specificity 90 - 95%). The PPV and NPV for foals with serum IgG concentrations < 800 and < 400 mg/dl are presented in Table 4. SNAP ELISA 1, SNAP ELISA 2, and glutaraldehyde coagulation tests had NPV > 90% but PPV < 85%. The CITE ELISA test had NPV of only 78 - 88% but 100% PPV. When using the glutaraldehyde coagulation and SNAP ELISA 2 in series testing (both tests must be positive for FPT to call the foal positive for FPT), the sensitivity and specificity for detecting serum IgG concentrations < 800 mg/dl was 92% and 93%, respectively. This resulted in a NPV of 95% and a PPV of 89% when using these two tests in series.

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Number of Foals</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde Coagulation</td>
<td>200</td>
<td>100% (100)*</td>
<td>58% (52 - 65)*</td>
</tr>
<tr>
<td>CITE ELISA</td>
<td>91</td>
<td>54% (44 - 65)</td>
<td>100% (100)</td>
</tr>
<tr>
<td>SNAP ELISA 1</td>
<td>63</td>
<td>93% (87 - 99)</td>
<td>65% (53 - 77)</td>
</tr>
<tr>
<td>SNAP ELISA 2</td>
<td>198</td>
<td>88% (84 - 93)</td>
<td>90% (86 - 94)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are the 95% confidence interval for sensitivity and specificity.
FPT, failure of passive transfer; RID, radial immunodiffusion tests.

<table>
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<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde Coagulation</td>
<td>200</td>
<td>95% (92 - 98)*</td>
<td>80% (74 - 85)*</td>
</tr>
<tr>
<td>CITE ELISA</td>
<td>91</td>
<td>55% (44 - 65)</td>
<td>100% (100)</td>
</tr>
<tr>
<td>SNAP ELISA 1</td>
<td>63</td>
<td>87% (78 - 95)</td>
<td>92% (85 - 98)</td>
</tr>
<tr>
<td>SNAP ELISA 2</td>
<td>198</td>
<td>76% (70 - 82)</td>
<td>95% (92 - 98)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are the 95% confidence interval for sensitivity and specificity.
FPT, failure of passive transfer; RID, radial immunodiffusion tests.

<table>
<thead>
<tr>
<th>Screening test</th>
<th>IgG &lt; 800 mg/dl*</th>
<th>IgG &lt; 400 mg/dl**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde Coagulation</td>
<td>59%</td>
<td>57%</td>
</tr>
<tr>
<td>CITE ELISA</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>SNAP ELISA 1</td>
<td>62%</td>
<td>75%</td>
</tr>
<tr>
<td>SNAP ELISA 2</td>
<td>84%</td>
<td>81%</td>
</tr>
</tbody>
</table>

*Prevalence for foals with serum IgG < 800 mg/dl was 37.8%.
**Prevalence for foals with serum IgG < 400 mg/dl was 21.9%.
PPV, positive predictive value; NPV, negative predictive value; FPT, failure of passive transfer.
4. Discussion

There was a large variability in sensitivity and specificity among the screening test. The CITE ELISA had a high specificity but a poor sensitivity. Other tests like the SNAP ELISA 1 and the glutaraldehyde coagulation test had good to excellent sensitivity but moderate to poor specificity. What is more important for a FPT screening test, high sensitivity or high specificity? By definition, screening tests should have high sensitivity so that very few foals with FPT go unidentified (false negative). The FPT screening tests with sensitivity > 90% were glutaraldehyde coagulation test and SNAP ELISA 1 test. The SNAP ELISA 2 test had fairly good sensitivity (88%) when detecting IgG concentrations < 800 mg/dl, but the sensitivity markedly decreased when detecting foals with serum IgG < 400 mg/dl (76%). The CITE ELISA test had poor sensitivity (53 - 55%), indicating that it was not an appropriate screening test for FPT. A previous study compared the sensitivity and specificity of multiple screening tests for FPT in foals, including CITE ELISA test and glutaraldehyde coagulation test [13]. The same study also found the glutaraldehyde coagulation test to have a high sensitivity and the CITE ELISA to have a poor sensitivity. Interestingly, the CITE ELISA, up to the time it was discontinued, was widely used as a screening test for FPT. Many considered it to be highly reliable when, in fact, the CITE ELISA test only detected about one-half of the foals with FPT. The increased number of FPT test positive foals identified by SNAP ELISA 1 test was caused by both its superior sensitivity and reduced specificity compared with the CITE ELISA, resulting in concerns from the field about its reliability. The manufacturer responded by modifying the SNAP ELISA. This resulted in a decrease in the sensitivity in the SNAP ELISA 2 compared with SNAP ELISA 1 but an improved specificity.

A recent study by Pusterla et al., [17] compared the SNAP ELISA to RID using the blood of 42 foals. The study found that the SNAP ELISA's accuracy was good when test results were < 400 and > 800 mg/dl but did poorly when SNAP ELISA's results were between 400 - 800 mg/dl. A similar trend was found in this study with the majority of misclassification occurring between 400 - 800 mg/dl. It is not stated when Pusterla et al., [17] performed the study. Therefore, it is unknown which version of the SNAP ELISA test was used; however, the sensitivity and specificity of the results of that study correlated best with the SNAP ELISA 1 results in our study. The use of whole blood in Pusterla's [17] study instead of serum used in this study may have also caused different results in sensitivity and specificity.

If an FPT screening test has poor specificity, some foals will be misdiagnosed as having FPT and be subjected to unnecessary and expensive therapy. The non-commercial glutaraldehyde coagulation test and SNAP ELISA 1 have marginal to poor specificity, especially at detecting serum IgG < 800 mg/dl (58 - 65%). The non-commercial glutaraldehyde coagulation test, with its high sensitivity, is an excellent screening test for FPT; however, because of its poor specificity, a confirmatory test is needed to verify a positive test result. RID IgG is the ideal confirmatory test but has a 18 - 24 h time delay and is more technically demanding. In contrast, the SNAP ELISA 2 test has a good specificity (90%) and could be used as a rapid confirmatory test. When the glutaraldehyde coagulation test is used as a screening test and SNAP ELISA 2 is used as a confirmatory test, the sensitivity and specificity of this diagnostic testing combination is 92% and 93%, respectively, for detecting serum IgG < 800 mg/dl. A critical assessment of the commercial glutaraldehyde coagulation test is needed to determine if the Se and Sp of that test is similar to the non-commercial glutaraldehyde coagulation test we used in this study.

The confidence that a practitioner has in an individual test result is described as PPV and NPV. A screening test for FPT should have a high NPV, indicating that a foal testing negative for FPT truly has adequate serum IgG concentrations. Based on the prevalence of FPT in this study, glutaraldehyde coagulation test and both SNAP ELISA tests had NPV of > 90%. A positive test result using a diagnostic test with a high PPV indicates a high likelihood that the patient tested is truly positive. The CITE ELISA had a PPV of 100%, whereas the SNAP ELISA 2 had a PPV of 81 - 84%. In contrast, the PPV of the non-commercial glutaraldehyde coagulation and the SNAP ELISA 1 tests were poor. This poor PPV of the SNAP ELISA 1 was partly responsible for the controversy over this test reliability compared with the CITE ELISA. The PPV of the test was markedly improved with the modification to SNAP ELISA 2. Practitioners using the glutaraldehyde coagulation test should consider verifying a positive FPT result with a RID IgG confirmatory test before pursuing a plasma transfusion because of its poor PPV. If rapid results are desired, the SNAP ELISA 2 test could be used in place of the RID IgG test as a confirmatory test. Using the glutaraldehyde coagulation tests as screening tests and the SNAP ELISA 2 as the confirmatory test resulted in NPV = 95% and PPV = 89%.

PPV and NPV change based on the disease prevalence within a population. The prevalence of FPT found in this study were within the range of previous reports [3-7]. The prevalence found in this study may have been biased, because practitioners could have preferentially submitted samples from foals they suspected to have FPT. In an effort to reduce that bias, we did
not charge a fee for the FPT diagnostic testing, hoping that they would be more likely to submit serum from all neonatal foals that they examined. If the prevalence of FPT was overestimated, the PPV would be overestimated and the NPV would be underestimated.

In conclusion, the CITE ELISA was unacceptable as a screening test for FPT because of its poor sensitivity and poor NPV. The SNAP ELISA 2 and glutaraldehyde coagulation tests are more appropriate screening tests, because they have a high sensitivity and NPV. The specificity and PPV of the SNAP ELISA 2 test is better than the glutaraldehyde coagulation test. A confirmatory test should be considered when using the glutaraldehyde coagulation test to screen for FPT in foals.

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Footnotes
[e] Stata 6.0, Stata Corp., College Station, TX, 77845.

References


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