ABSTRACT

The objectives of this study were (1) to determine the differences in IgG and total protein (TP) content of serum and plasma samples collected from the same calves; (2) to evaluate the correlation between calf serum and plasma IgG levels, Brix scores, and TP concentrations; (3) to determine whether different cut-off values should be used for plasma and serum to assess failure of transfer of passive immunity (FTPI) in dairy calves; and (4) to evaluate the level of agreement between results obtained from using serum and plasma samples of the same calves to assess FTPI using optimal cut-off values. Blood samples (n = 217) were collected from Holstein calves at 3 to 10 d of age on 30 commercial dairy farms in Nova Scotia and Newfoundland, Canada. Paired serum and plasma samples were analyzed for IgG concentration by the reference radial immunodiffusion assay, transmission infrared (TIR) spectroscopy, digital and optical Brix refractometers, and optical TP refractometer. The IgG concentrations measured by RID and TIR spectroscopy in serum were similar to those in plasma. However, the Brix and TP refractometer readings were significantly higher in plasma than in serum. The prevalence of FTPI in serum and plasma samples based on a RID-IgG concentration <10 g/L was 43.3 and 46.5%, respectively. The RID-IgG concentration was correlated with TIR-IgG (r = 0.92 and 0.89), digital Brix (r = 0.80 and 0.80), optical Brix (r = 0.77 and 0.77), and optical TP (r = 0.75 and 0.77) refractometers in serum and plasma, respectively. The correlations between paired serum and plasma IgG content were 0.85 by TIR spectroscopy, 0.80 by digital Brix, 0.77 by optical Brix, and 0.79 by optical TP refractometer. The optimal cut-off values for TIR spectroscopy, digital Brix, optical Brix, and TP refractometers to assess FTPI using serum were 13.1 g/L, 8.7% Brix, 8.4% Brix and 5.1 g/dL, respectively; and the optimal cut-off values with plasma were 13.4 g/L, 9.4% Brix, 9.3% Brix and 5.8 g/dL, respectively. When using these optimal cut-off values, the level of agreement (88.1%) between results derived from testing serum and plasma by TIR spectroscopy was substantial, with a kappa (κ) value of 0.76. The results derived from testing serum and plasma by digital Brix refractometer showed substantial agreement (83.4%), with a κ value of 0.65, which is higher than the agreement and κ value (74.7% and 0.51) reported for the optical Brix refractometer. Substantial agreement (81.6%) between serum and plasma TP was also obtained when using the optical TP refractometer, with a κ value of 0.63. In conclusion, serum or plasma samples can be used interchangeably for measuring IgG concentrations and assessing FTPI in dairy calves. However, different cut-offs must be used to assess FTPI depending on the sample matrix. Furthermore, results obtained from serum samples showed higher agreement with the reference RID assay than those obtained from plasma samples.

Key words: calves, serum, plasma, immunoglobulin G, radial immunodiffusion

INTRODUCTION

Dairy calves are born without any acquired immunity because immunoglobulins cannot cross the placental structure of the cow during gestation (Borghesi et al., 2014). Calves rely on the passive transfer of IgG through consumption of maternal colostrum provided within the first hours of life (McGrath et al., 2016). Failure of calves to ingest or absorb sufficient amount of collostral IgG results in failure of transfer of passive immunity (FTPI) (Weaver et al., 2000; Godden, 2008; Beam et al., 2009). There is a recognized association between FTPI (IgG <10 g/L) and short- and long-term health and productivity in calves (Cuttance...
et al., 2018; Lora et al., 2018). Calves with FTPI are more susceptible to infectious diseases and have higher morbidity and mortality (Windeyer et al., 2014). Furthermore, FTPI is associated with decreased milk yield and increased culling rates in dairy heifers during the first lactation (Chuck et al., 2018). Thus, monitoring of calves for FTPI is essential to identify herd management deficiencies and ensure timely detection and implementation of interventional measures, which can reduce the morbidity, mortality, and production issues associated with FTPI in the herd (Furman-Fratczak et al., 2011).

Several methods have been developed to measure IgG concentration in dairy calves either directly or indirectly. Direct methods include radial immunodiffusion (RID) assay (McBeath et al., 1971), ELISA (Gelsinger et al., 2015), infrared spectroscopy (Elsohaby et al., 2016), and an automated turbidimetric immunoassay (Alley et al., 2012). Indirect methods include refractometry (Deelen et al., 2014), zinc sulfate (ZnSO4) turbidity test, glutaraldehyde coagulation test, and sodium sulfate turbidity test (Tyler et al., 1996; Parish et al., 1997; Weaver et al., 2000). Although the RID assay is the historical reference standard method for direct quantification of IgG concentration in serum, plasma, colostrum, and milk (Pritchett et al., 1994; Tyler et al., 1996; Weaver et al., 2000; Bielmann et al., 2010), it is a laboratory-based assay, takes 18 to 24 h to obtain a result, requires skills to read the plates, has a high cost, and uses reagents with a short shelf life (Liu et al., 2007; Riley et al., 2007). Thus, ELISA and infrared spectroscopy have been used recently for direct quantification of serum and colostrum IgG concentration (Baumrucker et al., 2014; Gelsinger et al., 2015; Elsohaby et al., 2016, 2017). Results from direct methods have been used in the development of the indirect methods by adjusting the refractive index, Brix scores, specific gravity, or total protein (TP) levels to the IgG concentrations (Deelen et al., 2014; MacFarlane et al., 2014; Villarroel et al., 2014; Hernandez et al., 2016).

Earlier research studies have used serum or plasma IgG and TP concentrations to assess FTPI in dairy calves (MacFarlane et al., 2014; Villarroel et al., 2014). However, to our knowledge, no direct comparisons have been made between the performance of RID, TIR spectroscopy, Brix, and TP refractometers using serum and plasma samples from the same calves. Thus, the objectives of our study were (1) to determine whether IgG concentration, Brix scores, and TP values differ in serum and plasma samples of the same calves; (2) to evaluate the correlation between calf serum and plasma IgG levels, Brix scores, and TP concentrations; (3) to determine the optimal cut-off values and the diagnostic test characteristics of the TIR spectroscopy, Brix, and TP refractometers to assess FTPI using serum and plasma of the same calves; and (4) to evaluate the level of agreement between results obtained using serum and plasma samples.

**MATERIALS AND METHODS**

**Calf Enrollment and Sampling**

Holstein calves (n = 217) from 30 commercial dairy farms in Nova Scotia (n = 24) and Newfoundland (n = 6), Canada, were sampled between April and September 2015. Herds were selected by veterinary clinics in the study area, and each farm delivered between 5 and 11 samples. At each farm, whole blood samples were collected from 3- to 10-d-old calves by jugular venipuncture using a 20-gauge, 1-inch hypodermic needle (BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ), into a sterile, plastic Vacutainer tube with and without lithium heparin (BD Vacutainer, Becton Dickinson Co.) to obtain plasma and serum samples, respectively. Samples were labeled with the farm name, calf identification number, and collection date, and then stored on ice for transportation to the Maritime Quality Milk Laboratory, University of Prince Edward Island (UPEI). Serum and plasma samples were separated by centrifugation at 1,500 × g for 15 min at ~20°C. Three aliquots of serum and plasma were collected and stored at ~−80°C for later analysis. In the end, 217 paired serum and plasma samples were available for analysis. This study was conducted in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009) under a protocol (#15-001) approved by the Animal Care Committee at UPEI. The sample size calculation for the study was based on finding a difference of 0.2 g/L between serum and plasma IgG concentrations, considering that the standard deviation of the IgG concentration 1.0 g/L (Deelen et al., 2014; Elsohaby et al., 2015), and α and β errors of 0.05 and 0.20, respectively. A sample size of 198 calves was targeted for enrollment in this study. The sample size calculation was performed using Java Applets for Power and Sample Size (www.stat.uiowa.edu/~rlenth/Power; Lenth, 2006–2009).

**RID Analysis**

Serum and plasma samples were thawed at room temperature (20–24°C) and vortexed for 10 s. A commercial RID assay (Bovine IgG RID Kit; Triple J Farms, Bellingham, WA) was used as the reference method to measure serum and plasma IgG concentra-
tions. The RID assay was performed according to the manufacturer’s instructions, using 5 μL of undiluted sample in each well tested alongside the manufacturer’s standards. Diameters of precipitating rings were measured using a handheld caliper after 18 to 24 h of incubation at room temperature. Serum and plasma samples with IgG concentrations greater than the manufacturer’s stated performance range for the assay (>30 g/L) were diluted (1:1) with deionized sterile water and retested. All samples and assay standards were tested in duplicate. The average of the assay standards was used to build a calibration curve to determine the IgG concentration for each serum and plasma sample. The final IgG concentration for each sample was determined by calculating the average of the 2 replicates. The results were considered acceptable if the coefficient of determination of the calibration curve derived from the standards was ≥0.97.

**TIR Spectroscopy Analysis**

Infrared spectra were acquired for serum and plasma samples using a transmission infrared (TIR) spectrometer (Tensor 37, Bruker Optics, Milton, ON, Canada) equipped with a deuterium tryglycine sulfate detector and controlled by proprietary software (Opus ver. 6.5, Bruker Optics). Thawed serum and plasma samples were diluted (1:1) with deionized sterile water and tested in replicates of 6 by evenly spreading 10-μL aliquots into 5-mm-diameter wells within an adhesive-masked, 96-well silicon microplate (Riley et al., 2007). An empty well served as the background reference for each microplate. A total of 1,302 (217 serum or plasma samples × 6 replicate) spectra were collected, over a wavenumber range between 4,000 and 400 cm$^{-1}$ with a nominal resolution of 4 cm$^{-1}$, with 512 scans collected for data acquisition. Collected spectra were converted into printable (PRN) format (Grams/AI version 7.02, Thermo Fisher Scientific, Waltham, MA) and imported into MATLAB (MathWorks R2016b, Natick, MA). A previously developed partial least squares regression model built for prediction of serum IgG concentration from infrared spectra (Elsohaby et al., 2014) was used to predict serum and plasma IgG concentrations. The IgG concentration was predicted from each spectrum and, subsequently, the IgG concentration for each serum and plasma sample was calculated as the average of the 6 replicate IgG values.

**Refractometer Analyses**

Serum and plasma samples were allowed to thaw at room temperature (20 to 24°C), and analysis of Brix scores was performed using a digital Brix refractometer (PAL-1, Atago Co. Ltd., Bellevue, WA), with a scale from 0 to 52% Brix, and an optical Brix refractometer (model 300001; SPER Scientific, Scottsdale, AZ), with a scale from 0 to 32% Brix. Samples were also analyzed for TP using an optical handheld refractometer (RHC-00ATC handheld refractometer, Westover, Woodinville, WA). For the digital refractometer, approximately 250 μL of serum or plasma was used, and the Brix score was determined by transmitting light through the sample in the prism and recording the reading on a digital scale. For the optical refractometers, approximately 50 μL of serum or plasma was placed on the prism and held up to a light source; the result was read through the viewfinder at the interface between light and dark areas. Before each analysis, the refractometers were cleaned and calibrated with distilled water at room temperature. The reading of the optical refractometers was determined first, to avoid any bias in the results by the technician.

**Statistical Analysis**

Statistical analysis was performed using Stata statistical software (version 15.0; StataCorp, 2017), with results considered significant at $P < 0.05$. Descriptive statistics for the serum and plasma IgG, Brix scores, and TP concentrations were calculated, and normality was assessed by application of the Shapiro-Wilk test. A paired Student’s $t$-test was performed to evaluate differences between serum and plasma IgG, Brix scores, and TP concentrations. Serum and plasma TIR-IgG values, Brix scores, and TP concentrations were plotted against each other and the reference RID-IgG values. From these plots, correlation coefficients ($r$-values) were determined. The receiver operating characteristic (ROC) curve was created to plot the true positive rate against the false positive rate for TIR spectroscopy, Brix and TP refractometers. The area under the curve in the ROC plot for each assay with serum and plasma samples was calculated. The optimal cut-points for each assay were determined ($cutpt$ command in Stata; Clayton, 2013). The diagnostic test characteristics—sensitivity ($Se$), specificity ($Sp$), positive predictive value ($PPV$), negative predictive value ($NPV$), and accuracy—of TIR spectroscopy and Brix and TP refractometers were calculated to assess FTPI in dairy calves, using a serum and plasma RID-IgG concentration of 10 g/L as the cut-off value. Sensitivity was defined as the proportion of calves with FTPI (<10 g/L) that were correctly identified as such, and Sp was defined as the proportion of calves without FTPI (>10 g/L) that were correctly identified as such. The PPV was defined as...
the probability that a calf with a positive test result has FTPI, whereas NPV describes the probability that a calf with a negative test result has adequate transfer of passive immunity. Accuracy was defined as the proportion of calves that were correctly classified. Bland-Altman plots were used to examine the difference and interchangeability between serum and plasma TIR-IgG, Brix scores, and TP concentrations (Bland and Altman, 1995). The level of agreement between TIR, Brix, and TP refractometers for detecting FTPI was assessed for paired serum and plasma samples, followed by calculation of Cohen’s kappa statistic ($\kappa$).

**RESULTS AND DISCUSSION**

**Descriptive Statistics**

The distribution of serum and plasma IgG concentrations measured by RID, TIR spectroscopy, and Brix and TP refractometers was normal ($P > 0.05$). Descriptive statistics (mean, SD, minimum, and maximum) of the IgG concentration in calf serum and plasma obtained by RID, TIR spectroscopy, and Brix and TP refractometers are presented in Table 1. In this study, we detected no significant differences between serum and plasma IgG concentrations measured by RID ($P = 0.073$) and TIR spectroscopy ($P = 0.131$), which is similar to previous reports in the literature (McEwan et al., 1970). Conversely, the differences between serum and plasma Brix scores and TP concentrations measured by refractometers were significant ($P = 0.001$), which is consistent with the results reported in other studies (Naylor and Kronfeld, 1977; MacFarlane et al., 2014; Villarroel et al., 2014).

Approximately half of the serum (94/217) and plasma (101/217) samples had RID-IgG concentrations <10 g/L, generating an FTPI prevalence of 43.3 and 46.5%, respectively. The prevalence of calves with FTPI in this study is consistent with what has been previously reported in Atlantic Canada by Elsohaby et al. (2014; 51%) and higher than the prevalence reported in other Canadian provinces by Deelen et al. (2014; 4.75%) and Atkinson et al. (2017; 16%). The difference in the prevalence of FTPI in the present and previous studies may be related to the age of calves at sampling and number of farms enrolled in each study, as well as the management practices on the farm (Godden, 2008). In the present study, only 34 out of 217 calves were sampled between 6 and 10 d of age. These samples may not represent a valid prevalence estimate for FTPI in these herds. However, the 30 farms used in this study had previously reported a high proportion of poor-quality colostrum (48% of colostrum had IgG <50 g/L; Elsohaby et al., 2017), so FTPI estimates are likely higher than would be expected, compared with farms feeding high-quality colostrum to calves.

**Correlation Coefficients**

The correlation coefficient between serum IgG levels was 0.92, as estimated by TIR spectroscopy and RID assay (Figure 1A), similar to the correlation ($r = 0.94$) reported between RID and TIR spectroscopy for dairy calves (Elsohaby et al., 2016). The correlations between serum RID-IgG concentrations and Brix scores measured by digital and optical Brix refractometers were 0.80 (Figure 1B) and 0.77 (Figure 1C), respectively. The serum TP measured by optical TP refractometer was also correlated with serum RID-IgG concentration ($r = 0.75$; Figure 1D). Although similar correlations between serum Brix scores or serum TP and RID-IgG concentration have been reported (Elsohaby et al., 2015; Hernandez et al., 2016), others have found numerically higher correlation coefficients from 0.87 to 0.93 between refractometry and RID assay (Morrill et al., 2013; Deelen et al., 2014). Plasma IgG concentration measured by RID assay was positively correlated with IgG concentration obtained by TIR spectroscopy ($r = 0.89$; Figure 2A), Brix scores obtained by digital Brix ($r = 0.80$; Figure 2B) and optical Brix ($r = 0.77$; Figure 2C) refractometers, and TP concentration measured by optical TP refractometer ($r = 0.77$; Figure

### Table 1. Descriptive statistics of serum and plasma IgG concentrations, Brix scores (%Brix), and total protein (g/dL) in 217 dairy calves

<table>
<thead>
<tr>
<th>Method</th>
<th>Serum</th>
<th></th>
<th></th>
<th></th>
<th>Plasma</th>
<th></th>
<th></th>
<th></th>
<th>P-value: t-test²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
<td>SD</td>
<td>Minimum</td>
<td>Maximum</td>
<td>P-value: t-test²</td>
</tr>
<tr>
<td>RID (g/L)</td>
<td>13.34</td>
<td>9.25</td>
<td>1.64</td>
<td>51.42</td>
<td>12.56</td>
<td>9.18</td>
<td>1.77</td>
<td>59.36</td>
<td>0.073</td>
</tr>
<tr>
<td>TIR spectroscopy (g/L)</td>
<td>14.00</td>
<td>8.57</td>
<td>-4.80</td>
<td>41.37</td>
<td>13.54</td>
<td>7.87</td>
<td>-1.3</td>
<td>41.38</td>
<td>0.131</td>
</tr>
<tr>
<td>Digital Brix (% Brix)</td>
<td>8.64</td>
<td>0.91</td>
<td>6.8</td>
<td>12.3</td>
<td>9.15</td>
<td>0.92</td>
<td>7.1</td>
<td>13.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Optical Brix (% Brix)</td>
<td>8.75</td>
<td>0.99</td>
<td>6.3</td>
<td>12.2</td>
<td>9.25</td>
<td>0.97</td>
<td>7.1</td>
<td>13.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Optical total protein (g/dL)</td>
<td>5.24</td>
<td>0.83</td>
<td>3.6</td>
<td>8.6</td>
<td>5.64</td>
<td>0.85</td>
<td>3.8</td>
<td>9.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

¹RID = radial immunoassay; TIR = transmission infrared spectroscopy.
²Paired t-test differed significantly if P-value <0.05.
These findings are higher than the correlation previously reported between refractometry and RID (McBeath et al., 1971; r = 0.72) and between ELISA and RID (Gelsinger et al., 2015; r = 0.59) using plasma samples.

The wide range of reported correlation coefficients could be explained by variations in the source of serum and plasma samples and the instrument variation between refractometers used in these different studies (Vandeputte et al., 2011). In the current study, 217 samples were collected from 30 farms, whereas Deelen et al. (2014) and MacFarlane et al. (2014) collected 400 serum samples from 5 farms and 61 plasma samples from 7 farms, respectively. Furthermore, variations in the correlation coefficients in these studies could be due to the differences in non-IgG contents in serum and plasma samples (Pfeiffer et al., 1977). Refractometry measures IgG content indirectly by measuring serum TP and plasma TP, which are affected by a calf’s health status (dehydration; Tyler et al., 1999). Consequently, a number of studies have reported a poor correlation between plasma TP and IgG concentration because of variable fibrinogen content in plasma samples (Naylor and Kronfeld, 1977). Others have reported that the nonimmune components of serum proteins might lead to poor correlation with IgG concentration (Pfeiffer et al., 1977). Thus, the use of caprylic acid to separate IgG from other serum proteins has been investigated (Morrill et al., 2013).

The correlation coefficient between paired serum and plasma IgG concentrations was 0.85 for TIR spectroscopy (Figure 3A). The correlations between serum and plasma Brix scores estimated by the digital and optical Brix refractometers were 0.80 and 0.77, respectively.
However, the serum and plasma TP concentrations obtained by optical TP refractometer were positively correlated ($r = 0.79$; Figure 3C). Correlations reported in the present study are lower than those reported between serum and plasma TP by MacFarlane et al. (2014; $r = 0.98$) and Villarroel et al. (2014; $r = 0.91$). Pearson correlation coefficients between serum and plasma IgG, Brix scores, and TP concentrations are given in Supplemental Table S1 (https://doi.org/10.3168/jds.2018-15070).

**Diagnostics Test Characteristics**

Receiver operator characteristic curves were created to determine the optimal cut-off values for TIR spectroscopy and Brix and TP refractometers to detect FTPI (RID-IgG $<10$ g/L) using serum (Figure 4A) or plasma (Figure 4B) samples of the same calves. The test characteristics (Se, Sp, PPV, NPV, and accuracy) associated with these optimal cut-points are shown in Table 2 for each test with serum and plasma. These results showed that the optimal cut-off values for assessing FTPI using calf serum were different than those for plasma. Similar results were previously reported by MacFarlane et al. (2014), who used different cut-points for serum TP (5.2 g/dL) and plasma TP (5.6 g/dL) to assess FTPI in the same calves. The TIR spectroscopy in the present study showed higher Se and lower Sp than what has been previously reported for both transmission and attenuated total reflectance infrared spectroscopies (Elsohaby et al., 2015). Conversely, the Brix and TP refractometers showed similar Se, Sp, and accuracy to that reported by Elsohaby et al. (2015), lower Sp than that reported by Deelen et al. (2014), and...
lower Se than that reported in other studies (Thornhill et al., 2015; Hernandez et al., 2016). Furthermore, our TIR spectroscopy and Brix and TP refractometry results showed higher Sp and slightly lower Se in serum than in plasma of the same calves.

For each assay, using serum samples resulted in a higher number of calves being correctly classified as FTPI positive and negative than when plasma samples were used. Furthermore, when plasma was used, the assays, particularly the refractometers, showed relatively lower PPV compared with when serum was used. This means that when plasma is used with these assays, a substantial number of calves with adequate transfer of passive immunity will be classified as having FTPI (i.e., false positives). The cost of a false-positive FTPI diagnosis at calf level may be relatively low; however, the cost could be significantly higher if misdiagnosis results in changes in the management of calves and colostrum on the farm. The PPV and NPV of each assay with serum and plasma samples across populations with different FTPI prevalences are presented in Supplemental Figure S1 (https://doi.org/10.3168/jds.2018-15070).

Agreement Among Tests

The level of agreement among TIR spectroscopy, Brix and TP refractometry, and the reference RID assay for detection of FTPI was higher with serum samples than with plasma samples of the same calves, as assessed using the κ statistic (Table 2). A similar level of agreement with RID assays has been reported for TIR and attenuated total reflectance infrared spectroscopy (Elsohaby et al., 2016) and Brix and TP refractometers (Elsohaby et al., 2015).

Bland-Altman plots (Figure 5) revealed that the mean values of the difference between serum and plasma IgG concentrations provided by TIR spectroscopy was 0.46 g/L (Figure 5A). The level of agreement (88.1%) between results derived from testing serum and plasma by TIR spectroscopy was substantial, with a κ value of 0.76 (Table 3). This revealed that using either serum or plasma samples to measure IgG levels for the assessment of FTPI would result in similar classification of dairy calves with and without FTPI, which is consistent with the results previously published for calves (McEwan et al., 1970) and foals (Ujvari et al., 2017).

The agreement between serum and plasma Brix scores and TP concentrations was assessed using Bland-Altman plots and showed no obvious systematic bias between serum and plasma results obtained by digital Brix (Figure 5B), optical Brix (Figure 5C), and TP (Figure 5D) refractometers. However, the results derived from testing serum and plasma by digital Brix refractometer showed a substantial agreement (83.4%).

Figure 3. Scatter plots comparing (A) serum and plasma IgG concentration obtained by transmission infrared (TIR) spectroscopy; (B) serum and plasma Brix scores measured by digital and optical Brix refractometers; and (C) serum and plasma total protein obtained by optical total protein refractometer for 217 paired serum and plasma samples.
with a $\kappa$ value of 0.65, which higher than the agreement and $\kappa$ value (74.7% and 0.51) reported for the optical Brix refractometer. The agreement (81.6%) between serum and plasma TP obtained by optical TP refractometer was slightly lower than that of the digital Brix refractometers (Table 3). The lower agreement between plasma and serum TP concentration might be attributed to the significant and consistent higher concentration of plasma TP than serum TP (Villarroel et al., 2014). Furthermore, plasma contains fibrinogen and other coagulation proteins, which are lost during serum processing (George, 2001). Plasma may also contain excessive disodium EDTA or lithium heparin due to underfilled blood collection tubes, which falsely

![Figure 4](image)

**Figure 4.** Receiver operating characteristic (ROC) curve analysis of IgG concentration measured by transmission infrared (TIR) spectroscopy, digital Brix refractometer (DBR), optical Brix refractometer (OBR), and optical total protein refractometer (OPR) compared with the reference radial immunodiffusion (RID) assay for (left) serum and (right) plasma samples collected from 217 dairy calves.

**Table 2.** Test characteristics of transmission infrared (TIR) spectroscopy and Brix and total protein (TP) refractometers for detecting failure of transfer of passive immunity in serum and plasma samples collected from 217 dairy calves using optimal cut-off values compared with IgG (<10 g/L) determined by radial immunodiffusion (RID) assay

<table>
<thead>
<tr>
<th>Method</th>
<th>Cut-off</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
<th>$\kappa^2$</th>
<th>RID high/low</th>
<th>Test high/low</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIR spectroscopy</td>
<td>13.1 g/L</td>
<td>93.6</td>
<td>86.2</td>
<td>83.8</td>
<td>94.6</td>
<td>89.4</td>
<td>0.79</td>
<td>123/94</td>
<td>112/105</td>
</tr>
<tr>
<td>Digital Brix</td>
<td>8.6% Brix</td>
<td>91.5</td>
<td>70.7</td>
<td>70.5</td>
<td>91.6</td>
<td>79.7</td>
<td>0.60</td>
<td>123/94</td>
<td>95/122</td>
</tr>
<tr>
<td>Optical Brix</td>
<td>8.4% Brix</td>
<td>72.3</td>
<td>86.2</td>
<td>80.0</td>
<td>80.3</td>
<td>80.2</td>
<td>0.59</td>
<td>123/94</td>
<td>132/85</td>
</tr>
<tr>
<td>Optical TP</td>
<td>5.1 g/dL</td>
<td>75.5</td>
<td>77.2</td>
<td>71.7</td>
<td>80.5</td>
<td>76.5</td>
<td>0.52</td>
<td>123/94</td>
<td>118/99</td>
</tr>
<tr>
<td><strong>Plasma samples</strong></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TIR spectroscopy</td>
<td>13.4 g/L</td>
<td>95.0</td>
<td>83.6</td>
<td>83.5</td>
<td>95.1</td>
<td>88.9</td>
<td>0.78</td>
<td>116/101</td>
<td>102/115</td>
</tr>
<tr>
<td>Digital Brix</td>
<td>9.4% Brix</td>
<td>93.1</td>
<td>58.6</td>
<td>66.2</td>
<td>90.7</td>
<td>74.7</td>
<td>0.50</td>
<td>116/101</td>
<td>75/142</td>
</tr>
<tr>
<td>Optical Brix</td>
<td>9.3% Brix</td>
<td>84.2</td>
<td>64.7</td>
<td>67.5</td>
<td>82.4</td>
<td>73.7</td>
<td>0.48</td>
<td>116/101</td>
<td>91/126</td>
</tr>
<tr>
<td>Optical TP</td>
<td>5.8 g/dL</td>
<td>81.2</td>
<td>68.1</td>
<td>68.9</td>
<td>80.6</td>
<td>74.2</td>
<td>0.49</td>
<td>116/101</td>
<td>98/119</td>
</tr>
</tbody>
</table>

$^a$Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; accuracy = percentage of correctly classified samples.

$^a$RID $\text{high/low} = \text{number of samples declared high (IgG} \geq 10 \text{ g/L) or low (IgG} < 10 \text{ g/L) by RID assay.}$

$^a$Test $\text{high/low} = \text{number of samples declared high (IgG} \geq 10 \text{ g/L) or low (IgG} < 10 \text{ g/L) by each assay.}$
increases plasma TP concentration (Dubin and Hunt, 1978).

**CONCLUSIONS**

Our results suggest that serum or plasma samples could be used interchangeably to measure IgG concentrations for assessing FTPI in dairy calves. However, different cut-off values should be used for serum and plasma to assess FTPI when using TIR or refractometers. Optimal cut-off values for TIR spectroscopy, digital Brix, optical Brix, and TP refractometers to assess FTPI using serum were 13.1 g/L, 8.7% Brix, 8.4% Brix, and 5.1 g/dL, whereas those for plasma were 13.4 g/L, 9.4% Brix, 9.3% Brix, and 5.8 g/dL.

### Table 3. Level of agreement between results obtained from using serum and plasma samples for assessing failure of transfer of passive immunity in the same 217 dairy calves

<table>
<thead>
<tr>
<th>Method</th>
<th>Serum cut-off</th>
<th>Plasma cut-off</th>
<th>Agreement (%)</th>
<th>$\kappa^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIR spectroscopy</td>
<td>13.1 g/L</td>
<td>13.4 g/L</td>
<td>88.1</td>
<td>0.76</td>
<td>0.0001</td>
</tr>
<tr>
<td>Digital Brix</td>
<td>8.7% Brix</td>
<td>9.4% Brix</td>
<td>83.4</td>
<td>0.65</td>
<td>0.0001</td>
</tr>
<tr>
<td>Optical Brix</td>
<td>8.4% Brix</td>
<td>9.3% Brix</td>
<td>74.7</td>
<td>0.51</td>
<td>0.0001</td>
</tr>
<tr>
<td>Optical total protein</td>
<td>5.1 g/dL</td>
<td>5.8 g/dL</td>
<td>81.6</td>
<td>0.63</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

$^1$TIR = transmission infrared spectroscopy.  
$^2\kappa = $ Cohen’s kappa value.
g/L, 9.4% Brix, 9.3% Brix, and 5.8 g/dL, respectively. Results obtained from using serum samples showed higher agreement with the reference RID assay than those obtained from using plasma samples.

ACKNOWLEDGMENTS

The authors thank summer program students, participating dairy farmers and veterinarians, as well as Theresa Andrews, Cynthia Mitchell, and Natasha Robinson (Maritime Quality Milk Laboratory, Charlottetown, PEI, Canada) for their technical assistance and data collection. This research was funded by Zoetis (Kirkland, QC, Canada) and Atlantic Canada Opportunities Agency (AIF: 195174). I. Elsohaby is supported by a Mitacs Elevate Postdoctoral Fellowship (IT09473).

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