Technical note: Evaluation of digital refractometers to estimate serum immunoglobulin G concentration and passive transfer in Jersey calves

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ABSTRACT

Previous data have demonstrated that refractometers can be used to estimate serum IgG, and that a cut-point of 7.8% Brix should be used to identify failure of passive transfer (FPT) in 1-d-old Holstein calves. The objective of the present study was to validate the use of refractometry to estimate serum IgG concentrations and evaluate FPT in Jersey calves. Blood samples ($n = 97$) were obtained from 1- to 3-d-old Jersey calves and centrifuged at $3,300 \times g$ for 20 min at 25°C. Serum was analyzed for % Brix, total protein (TP), and refractive index (nD) using a Sper Scientific Digital Refractometer (model #300036, Sper Scientific, Scottsdale, AZ) within 12 h of sampling. Samples were then frozen and later analyzed in the laboratory for IgG by radial immunodiffusion. The mean serum IgG concentration for all calves was 23.7 mg/mL (SD = 12.5), with a range of 2.3 to 65.5 mg/mL. Mean serum % Brix was 8.9 (SD = 1.1; range 6.5 to 12.0). Serum % Brix was moderately correlated with IgG concentration ($r = 0.77$). Total protein and IgG were moderately correlated ($r = 0.790$). Regression was used to determine cut-points for approximately 10, 12, and 14 mg of IgG/mL and to determine the sensitivity and specificity of refractometry to identify FPT (serum IgG <10 mg/mL at 24 h of life). Brix cut-points analyzed were 7.1, 7.3, and 7.6%; TP cut-points were 4.6, 5.0, and 5.5 g/dL; and nD cut-points were 1.34332, 1.34271, and 1.3448, respectively, for 10, 12, and 14 mg of IgG/mL. The 7.3% Brix and 4.6 g/dL TP cut-points resulted in the greatest percentage of samples being correctly classified. These data suggest that digital refractometry is an acceptable and rapid method to estimate immunoglobulin G in Jersey calf serum.

Key words: serum, Jersey, refractometry, passive transfer

Technical Note

Newborn calves require maternal colostrum (MC) as soon after birth as possible to benefit from passively transferred immunity. Calves are born agammaglobulinemic and rely on absorption of immunoglobulin from high-quality colostrum (Godden, 2008). Colostrum contains many nutrients, including vitamins, minerals, fat, and proteins; however, the most important factors in colostrum are immunoglobulins to prevent infection from disease pathogens (Smith et al., 1964). The period during which a calf can acquire macromolecules through intestinal absorption is limited after birth; their ability to absorb macromolecules decreases rapidly after 12 h with mean gut closure time of 24 h after birth (Stott et al., 1979; Bush and Staley, 1980). This is largely due to the replacement of fetal-type enterocytes with adult-type enterocytes and results in gut closure and cessation of absorption of antibodies into the blood (Smith, 1985). Neonates rely on the transfer of these maternal antibodies to provide passive immunity until the calf is able to synthesize their own (Smith, 1948). Calves that do not achieve adequate passive transfer are classified as having failure of passive transfer (FPT) with serum IgG concentration <10 mg/mL after 24 h of age (Weaver et al., 2000; Godden, 2008). Calves classified as FPT have increased risk of morbidity and mortality (McEwan et al., 1970; Boyd, 1972; McGuire et al., 1976; Tyler et al., 1999). Many factors affect the success of passive transfer, including the volume of MC fed, the immunoglobulin concentration, and the time elapsed postpartum before feeding (Stott and Fellah, 1983; Nocek et al., 1984; Jaster, 2005).

Radial immunodiffusion (RID) is the industry gold standard to determine a direct measurement of IgG concentration in bovine serum. However, RID assays require a long incubation time of approximately 24 h, as well as training to perform the assay properly. In addition, RID assays have the potential to be inaccurate due to inconsistencies in internal standards (Ameri and Wilkerson, 2008). Because newborn calves have a relatively short period before gut closure, there is a clear need for a rapid on-farm test to assess immune status.

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Twenty-four-hour serum total protein (TP) and serum IgG concentrations are positively correlated (McBeath et al., 1971; Calloway et al., 2002; Foster et al., 2006) and can be utilized to evaluate FPT. Previous data from our laboratory demonstrated that use of a digital refractometer is effective for use in rapid estimation of serum IgG concentrations in Holstein calves. Our laboratory recommended refractometer cut-points of 7.8% Brix and a refractive index of 1.34448 to evaluate failure of passive transfer in calves without regard to breed differences (Morrill et al., 2013). A more recent report by Deelen et al. (2014) evaluated the use of Brix refractometry compared with the measurement of serum TP by refractometry. They suggested a value of 8.4% Brix to accurately evaluate FPT in Holstein calves. Although the effectiveness of the refractometer in determining passive immunity has been evaluated for the Holstein breed in multiple reports (McBeath et al., 1971; Morrill et al., 2013; Deelen et al., 2014), there is still a need to evaluate the refractometer for use in the Jersey breed. The Jersey breed is becoming more prevalent among US dairy producers because of its greater heat stress tolerance (Smith et al., 2013) and improved efficiencies such as feed use with less environmental impact (Capper and Cady, 2012).

The most recent National Animal Health Monitoring System (NAHMS) report stated that 14% of heifer calves had FPT and 6.2% of US dairy operations routinely measure passive transfer status of calves (NAHMS, 2014). This is a small improvement for the dairy industry—the 2007 NAHMS report documented an FPT rate of 19.2% and just 2.1% of operations routinely measuring passive immunity status of calves (NAHMS, 2007). These data suggest that although there is a small increase in the use of refractometry as a rapid on-farm test, US dairy producers do not widely use this tool for the measurement of FPT, and FPT in calves remains a significant issue on many dairy farms.

To test the efficacy of refractometry in Jersey calves, serum samples (5 mL) were collected from 1- to 3-d-old Jersey calves located in New York, Vermont, and Iowa ($n = 97$). Blood was collected by jugular venipuncture into evacuated tubes and allowed to clot. Serum was separated by centrifugation at $3,300 \times g$ for 20 min at 25°C and frozen (−20°C). Samples were divided into 2

aliquots. One was analyzed by refractometer (digital refractometer, model #300036; Sper Scientific, Scottsdale, AZ) within 12 h, and the second was frozen for later analysis. Approximately 50 μ L of bovine Jersey serum was placed on the refractometer prism and refractive index (nD), TP, and % Brix (sugar content) were measured and recorded. The nD is the refractive index of a solution measured at the wavelength of the sodium D-line (589.3 nm) at 20°C. The % Brix value can be determined using the polynomial fit to the ICUMSA (2009) table: $\text{Brix} = \{[(11,758.74 \times \text{nD} - 88,885.21) \times \text{nD} + 270,177.93] \times \text{nD} - 413,145.80\} \times \text{nD} + 318,417.95 \times \text{nD} - 99,127.4536$. The refractometer was calibrated with distilled water before each set analyses.

Frozen samples were packed on ice and shipped to Iowa State University (Ames) and stored (−20°C) until further analyzed. Samples were allowed to thaw to room temperature and then analyzed for IgG using RID analysis. Five microliters of Jersey serum was added to each well of a bovine IgG RID test plate (Triple-J Farm, Bellingham, WA). Radial immunodiffusion plates were incubated for 24 h and the diameter of the precipitin ring was measured. The diameter of the ring was compared with a standard curve created by the internal test standards (1.96, 14.02, and 27.48 mg/mL) to determine IgG concentration. Samples that had an IgG concentration outside of the highest internal test standard were serially diluted with distilled water, mixed well, and reanalyzed.

The SAS PROC CORR (SAS 9.2; SAS Institute Inc., Cary, NC) was used to determine the relationship between IgG, TP, nD, and % Brix. PROC MEANS of SAS was used to determine the variable means, and PROC GLM was used to determine differences in variable means by sex and age. Each refractometer measurement—TP, nD and % Brix—was regressed (PROC REG of SAS) on IgG measured by RID and cut-points of 10, 12, and 14 mg of IgG/mL of serum were calculated. Epidemiological diagnostic test characteristics (sensitivity, specificity, and predictive values) for each cut point were subsequently calculated using PROC FREQ of SAS. Sensitivity was defined as the probability of a test result (nD, TP, or % Brix) indicative of FPT for a sample with IgG <10 mg/mL as determined

Table 1. Descriptive statistics of serum samples from Jersey calves

Variable	n	Mean	SD	Minimum	Maximum
IgG (mg/mL)	97	23.7	12.5	2.3	65.5
Brix (%)	97	8.9	1.1	6.5	12
Total protein (g/dL)	97	5.8	1.0	3.7	8.4
Refractive index (nD)	97	1.34608	0.00176	1.34280	1.35110

Table 2. Diagnostic test characteristics for digital refractometry (% Brix) of serum samples compared with radial immunodiffusion assay for determination of IgG

Cut-point	Test characteristic ¹ (%)					Test high/low ³
	Sensitivity	Specificity	PPV	NPV	Correctly classified ²	
7.1	97.7	22.2	92.5	50.0	90.7	93/4
7.3	97.7	44.4	94.5	66.7	92.8	91/6
7.6	94.3	66.7	96.5	54.6	91.8	86/11
7.8	86.4	96.2	96.2	33.3	84.5	79/18

¹PPV = positive predictive value; NPV = negative predictive value.

²Percentage of samples correctly classified as adequate or failure of passive transfer.

³Number of samples declared high (>10 mg of IgG/mL) or low (<10 mg of IgG/mL) by Brix refractometry. Analysis by radial immunodiffusion indicated that 88 and 9 samples were high and low, respectively.

by RID. Specificity was defined as the probability of a test result (nD, TP, or % Brix) indicative of adequate passive transfer for a sample with IgG >10 mg/mL as determined by RID. Positive predictive values (**PPV**) and negative predictive values (**NPV**) were calculated using the prevalence of samples with an RID IgG of greater than or less than 10 mg/mL and a refractometer reading above or below the cut-point, respectively. McNemar's test was used to determine differences in test characteristics between cut-points. Significance was determined at $P \leq 0.05$.

A total of 108 samples of Jersey serum were collected; only 97 samples remained in the final data set due to missing data points. Descriptive data of serum sample measurements are presented in Table 1. Mean serum IgG concentration, as determined by RID, was 23.7 mg/mL (SD = 12.2), with a range from 2.2 to 65.0 mg/mL. In total, 88 samples had IgG concentrations ≥ 10 mg/mL and 9 samples had IgG <10 mg/mL, indicating FPT; thus, 10% of calves had FPT. This is similar to the 8.4% reported by Trotz-Williams et al. (2008) in a calf-level study, and much less than the 19.2% estimated by Beam et al. (2009) from the NAHMS (2007) report and less than the 37.1% reported by Trotz-Williams et al. (2008) in a herd-level study.

Mean % Brix (8.9; SD = 1.1) and nD (1.34608; SD = 0.00176) were similar to values reported by Morrill et al. (2013) in Holstein calves. Our laboratory previously indicated cut-points of 7.8% Brix and 1.34448 nD to estimate adequate passive transfer for the Holstein breed (Morrill et al., 2013). These values, along with other cut-points, were evaluated using Jersey serum. Cut-points of 7.1, 7.3, and 7.6% Brix resulted in similar percentages of samples being correctly classified as adequate passive transfer or failure of passive transfer (Table 2). No differences between cut-points were detected ($P > 0.05$).

Test characteristics to evaluate adequate passive transfer based on TP readings are presented in Table 3. A cut-point of 5.5 TP is often used to evaluate FPT; however, the results from the current study indicate that a cut-point of 5.5 may be too high for Jersey calves, as a large number of calves would be inaccurately classified as having FPT. No differences between cut-points were detected ($P > 0.05$).

Although results based on nD are not as broadly used as TP and % Brix, characteristics to determine adequate passive transfer based on nD are given in Table 4. The 1.34448 cut-point (equivalent to 15.96 mg of IgG/mL) resulted in a greater percentage of samples

Table 3. Diagnostic test characteristics for digital refractometry (total protein) of serum samples compared with radial immunodiffusion assay for determination of IgG

Cut-point	Test characteristic ¹ (%)					Test high/low ³
	Sensitivity	Specificity	PPV	NPV	Correctly classified ²	
4.6	95.5	55.6	95.5	55.6	91.75	88/9
5.0	86.4	77.8	97.4	36.8	85.56	78/19
5.5	68.2	88.9	98.4	22.2	70.1	61/36

¹PPV = positive predictive value; NPV = negative predictive value.

²Percentage of samples correctly classified as adequate or failure of passive transfer.

³Number of samples declared high (>10 mg of IgG/mL) or low (<10 mg of IgG/mL) by total protein, using refractometry. Analysis by radial immunodiffusion indicated that 88 and 9 samples were high and low, respectively.

Table 4. Diagnostic test characteristics for digital refractometry (refractive index; nD) of serum samples compared with radial immunodiffusion assay for determination of IgG

Cut-point (nD)	Test characteristic ¹ (%)					Test high/low ³
	Sensitivity	Specificity	PPV	NPV	Correctly classified ²	
1.34332	93.5	60.0	97.0	33.3	91.8	92/5
1.34371	95.6	66.7	96.7	66.7	93.8	88/9
1.34410	66.7	50.0	12.0	88.0	90.7	95/13
1.34448	98.7	38.1	85.2	88.9	95.9	76/21

¹PPV = positive predictive value; NPV = negative predictive value.

²Percentage of samples correctly classified as adequate or failure of passive transfer.

³Number of samples declared high (>10 mg of IgG/mL) or low (<10 mg of IgG/mL) by refractive index refractometry. Analysis by radial immunodiffusion indicated that 88 and 9 samples were high and low, respectively.

being correctly classified as FPT or adequate passive transfer. This cut-point was determined based on the regression equation $y = 5,118.6x - 6,865.9$. No differences between cut-points were detected ($P > 0.05$).

Serum refractometer readings of % Brix, TP, and nD were moderately correlated with IgG concentration as determined by RID (Table 5). Correlations between IgG (as determined by RID), % Brix, and nD were slightly lower in this study compared with the results reported by Morrill et al. (2013; $r = 0.87$ and 0.86 , respectively) using Holstein calves.

In conclusion, digital refractometry is a rapid and accurate way to evaluate failure of passive transfer in 1- to 3-d-old Jersey calves by measuring serum TP or % Brix. Results from this study suggest that a cut-point of 7.3 or 7.6% Brix is acceptable and a maximum cut-point of 5.0 for TP should be used to evaluate adequate passive transfer in Jersey calves.

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Table 5. Correlation coefficients between serum IgG as determined by radial immunodiffusion (RID) and Brix, total protein (TP), and refractive index (nD)¹

Item	n	r		
		RID × Brix	RID × TP	RID × nD
All samples	96	0.78	0.79	0.78
Calf sex				
Male	37	0.64	0.74	0.63
Female	70	0.80	0.81	0.79
Calf age (h)				
1 d (0–24)	58	0.76	0.78	0.77
2 d (25–48)	33	0.82	0.81	0.81
3 d (49–72)	5	0.77	0.87	0.73

¹P-values for all samples, calf sex, and calves aged 1 and 2 d are <0.001; P-values for “calf age 3 d” are >0.05.

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