

Evaluation of Digital and Optical Refractometers for Assessing Failure of Transfer of Passive Immunity in Dairy Calves

I. Elsohaby, J.T. McClure, and G.P. Keefe

Background: Failure of transfer of passive immunity (FTPI) is the underlying predisposing risk factor for most early losses in dairy calves. Refractometers, either optical or digital, can be used to assess FTPI as a part of calf health monitoring program on dairy operations.

Objectives: To evaluate the performance of and differences between digital Brix and optical refractometers for assessing FTPI in dairy calves.

Animals: Two hundred Holstein calves from 1 to 11 days of age.

Methods: A cross-sectional study was designed to measure serum IgG concentration by radial immunodiffusion (RID) assay, digital Brix and optical refractometers. The correlation coefficients (r) between the 2 refractometers were plotted against each other and against the measured IgG concentration from RID. The Se, Sp, and accuracy of digital Brix and optical refractometers for assessing FTPI using previously recommended cut-offs were calculated. A receiver operating characteristic curve was created and used to identify the optimal cut-off for this dataset.

Results: The RID IgG concentration was positively correlated with digital Brix ($r = 0.79$) and optical ($r = 0.74$) refractometers. The best combination of Se (85.5%), Sp (82.8%), and accuracy (83.5%) of digital Brix refractometer was at 8.3% Brix. For optical refractometer, the best combination of Se (80%), Sp (80.7%), and accuracy (80.5%) was at 5.5 g/dL.

Conclusions and Clinical Importance: Both refractometers exhibited utility in assessing FTPI in dairy calves.

Key words: Immunoglobulin G; Radial immunodiffusion assay; Serum total protein.

Failure of neonatal calves to absorb colostral immunoglobulin G (IgG) is termed failure of transfer of passive immunity (FTPI).^{1,2} Calves have FTPI if the serum IgG concentration is <1,000 mg/dL.^{3,4} FTPI has been associated with reduced growth rates, increased morbidity and mortality, increased risk of being culled, and decreased first-lactation milk production.^{5–8} Consequently, FTPI has effects on the survival, health, and productivity of dairy heifers.⁹ Prevalence of FTPI among US dairy calves in 2007 was 19–40%.¹⁰

Several assays are available for evaluating the adequacy of transfer of passive immunity in neonatal calves on dairy farms and monitoring FTPI is an important part of preventive herd health protocols.^{3,11,12} Radial

Abbreviations:

AUC	area under the curve
AVC	Atlantic Veterinary College
IgG	immunoglobulin G
FTPI	failure of transfer of passive immunity
RID	radial immunodiffusion
Se	sensitivity
Sp	specificity
STP	serum total protein
ROC	receiver operating characteristic curve

immunodiffusion (RID) is a direct measurement of IgG concentration in calves and, although this test is the industry gold standard,¹³ it is laboratory-based, relative expensive, requires a minimum of 18–24 hours to obtain the results and, thus, is not practical for routine, on-farm monitoring of adequacy of transfer of passive immunity. Refractometry has been used for many years,¹³ and other assays, such as the zinc sulfite turbidity test, sodium sulfate turbidity test, serum γ -glutamyl transferase activity, whole blood glutaraldehyde coagulation test, and ELISA have been described as having varying degrees of accuracy for predicting IgG concentrations in dairy calves.^{3,11,12}

Refractometers, either digital Brix or optical refractometer, are an effective on-farm tool to evaluate colostrum quality^{14,15} or determine FTPI in calves,^{16,17} because immunoglobulins are the greatest constituent of total protein in neonatal calf blood besides albumin.^{11,13,18} The relationship between IgG and serum total protein (STP) has been confirmed in many studies.^{3,4} Using an STP concentration of ≤ 5.0 or ≤ 5.2 g/dL most accurately predicted FTPI by optical refractometer,^{16,19} while for digital Brix refractometer, a Brix percentage of <7.8% Brix may be used to identify FTPI in dairy calves.²⁰ The objectives of this study were to

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This study was performed at Maritime Quality Milk Laboratory, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Canada.

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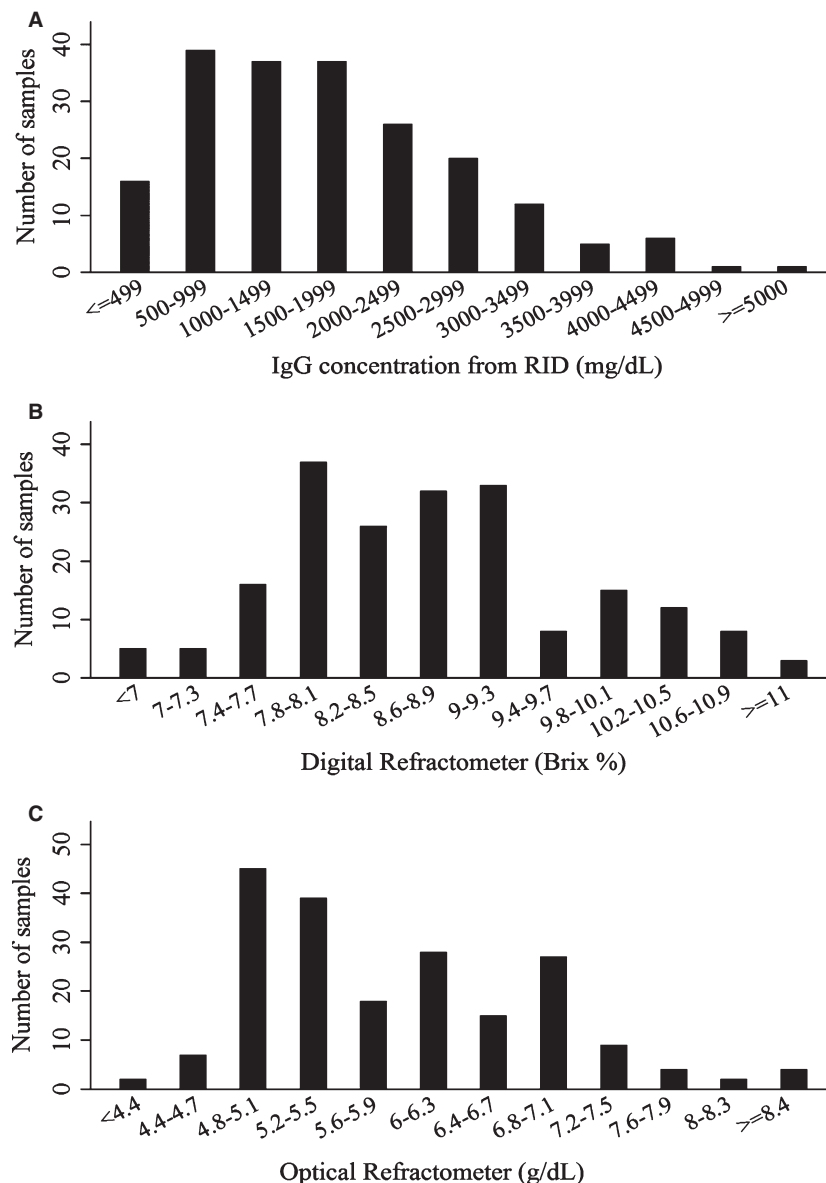


Fig 1. Frequency distributions of (A) serum immunoglobulin G (IgG) concentration measured by radial immunodiffusion (RID) assay, (B) percentage Brix (%Brix) determined by digital Brix refractometer, and (C) serum total protein determined by optical refractometer for 200 Holstein dairy calves.

determine the sensitivity, specificity, and accuracy of both digital and optical refractometers for assessing success of transfer of passive immunity in dairy calves, and to evaluate the agreement between 2 types of refractometers.

Materials and Methods

Serum Samples

Calves ($n = 203$) from 5 commercial dairy farms in Prince Edward Island and 1 farm in Nova Scotia were enrolled in the study between June and October, 2013. Animals eligible for inclusion in this study were Holstein calves from 1 to 11 days of age that appeared physically healthy and were not visibly dehydrated. Whole blood was collected from calves by jugular venipuncture using a 20-gauge, 1-inch hypodermic needle, into a sterile, plastic

Vacutainer tube without anticoagulant.^a Samples were transported in a cooler to Maritime Quality Milk Laboratory, University of Prince Edward Island (UPEI). Serum was separated by centrifugation at $1,500 \times g$ for 10 minutes at $20\text{--}24^\circ\text{C}$ within 5 hours of collection. Three aliquots of serum were collected and stored at -80°C . Serum from Nova Scotia samples was separated by centrifugation at $1,500 \times g$ for 10 minutes at a local veterinary clinic and then frozen at -20°C until transported to UPEI. In the end, 3 samples were missing and thus not available for analysis ($n = 200$). This study was approved by the University of Prince Edward Island Animal Care Committee and performed according to the guidelines of the Canadian Council on Animal Care.²¹

RID Analysis

A commercial RID assay^b was used as the reference method for determining IgG serum concentrations. Serum samples were

allowed to thaw at room temperature (20–24°C) and then vortexed for 10 seconds. Subsequently, the RID assay was performed according to manufacturer's instructions, using 5 μ L of undiluted serum sample in each well. The same manufacturer's standards

(the same lot) were used on all RID assays. Diameters of precipitated rings were measured after 18–24 hours of incubation at room temperature, using a hand-held caliper. Each of the samples and assay standards were tested in replicates of 5. The averages of the 5 replicates of the assay standards were used to build a calibration curve that was subsequently used to determine IgG concentrations for the serum samples. The final IgG concentration for each sample was determined by calculating the average of the 5 replicates. Serum samples with IgG concentrations greater than the manufacturer's stated performance range for the assay (>3,000 mg/dL) were diluted (50:50) with deionized sterile water and retested.

Digital Brix and Optical Refractometers Analysis

Serum samples were thawed at room temperature and vortexed for 10 seconds, then tested by the digital Brix refractometer^c and the optical hand-held refractometer^d to assess FTPI. For the digital Brix refractometer, approximate 250 μ L of serum were used, and then the Brix score of the liquid was determined by shining a light through the sample in the prism, measuring the index of refraction, and representing the reading (%Brix) on a digital scale. For the optical refractometer, approximate 100 μ L of serum were placed on the prism and the sample cover was lowered. The refractometer was then held up to a light source, and the STP (g/dL) value was read at the line between the light and dark areas that appeared on the scale.

Statistical Analysis

Descriptive statistics for the results of RID, digital Brix, and optical refractometers were calculated. Results from the digital (% Brix) and optical (g/dL) refractometers were plotted against each other and against the measured IgG concentration from RID in mg/dL. From these plots, correlation coefficients (r) were determined. Epidemiological diagnostic test characteristics (sensitivity, specificity, predictive values, and accuracy) were calculated to evaluate clinical applicability of digital Brix and optical refractometers for the diagnosis of FTPI, using previously recommended cut-off values^{4,20,22} and IgG concentrations of <1,000 mg/dL (measured by RID test) as FTPI positive cases.^{4,11} Sensitivity was defined as the proportion of calves with serum IgG concentration <1,000 mg/dL that were test-positive. Specificity was defined as the proportion of calves with serum IgG concentration \geq 1,000 mg/dL that were test-negative. A receiver operating characteristic curve (ROC) was created to plot the true positive rate against the false positive rate for both the digital and optical refractometers using (roctab command in stata).⁶ The computed Se, Sp, and accuracy for each of the possible cut-off values were tabulated and the best cut-off value was defined as the one give optimum combination of Se, Sp, and accuracy. The area under the curve (AUC) in the ROC plot, with a 95% confidence interval (CI), was calculated. AUC, a commonly used index of the overall ability of a test to discriminate a target condition, was used to compare the performances of each of the refractometers.²³ The level of agreement between results of digital Brix and optical refractometers were assessed using McNemar's test²⁴ for paired data to check for bias, followed by calculation of the kappa statistic.

Results

Descriptive Analysis

The frequency distribution of the RID IgG concentrations was skewed to the right (Fig 1A) and ranged from 133 to 5,995 mg/dL. The mean and median of the

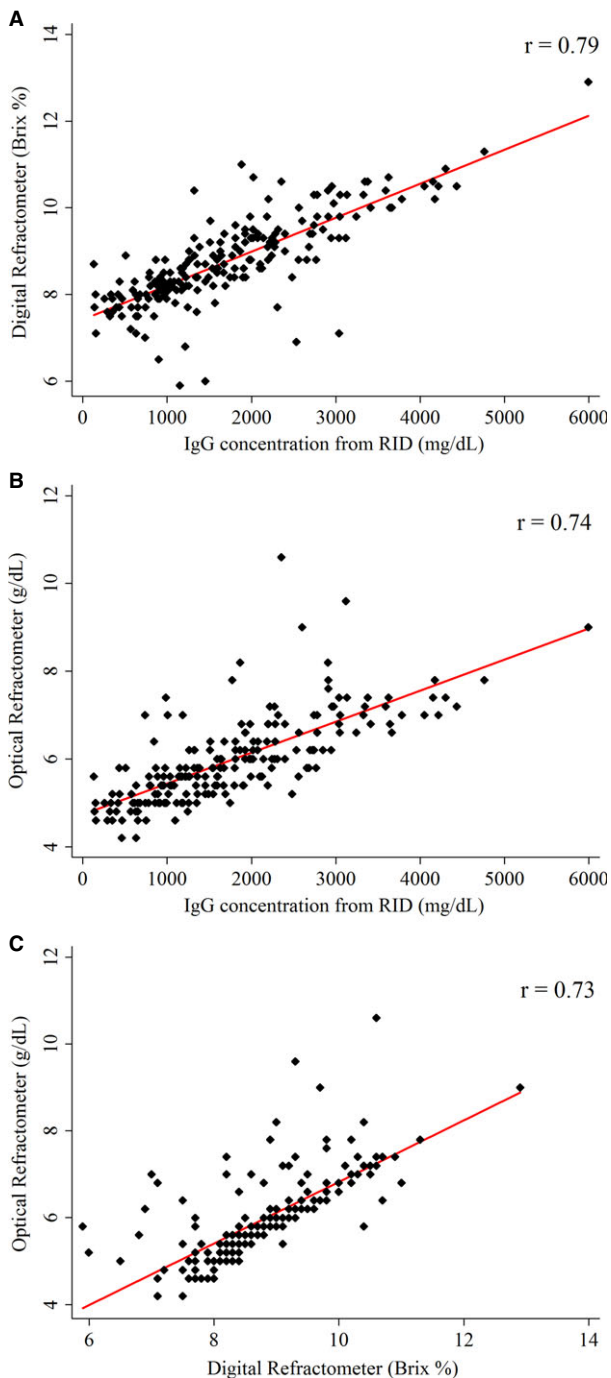


Fig 2. Scatter plots comparing (A) serum immunoglobulin G (IgG) concentration obtained by radial immunodiffusion (RID) assay with percentage Brix (%Brix), determined by digital refractometer ($r = 0.79$); (B) serum IgG concentration obtained by RID assay with serum total protein (STP), determined by optical refractometer ($r = 0.74$); (C) %Brix, determined by digital refractometer with STP obtained by optical refractometer ($r = 0.73$) for 200 Holstein dairy calves.

Table 1. Diagnostic test characteristics for the digital Brix and optical refractometers for assessing failure of transfer of passive immunity using previously recommended cut-off values compared with immunoglobulin G (<1,000 mg/dL) determined by radial immunodiffusion assay.

Refractometer	Test Characteristics					
	Cut-Off Value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Digital Brix values (%Brix)	7.8	32.7	94.5	69.2	78.7	77.5
	8.0	47.3	93.1	72.2	82.3	80.5
	8.2	76.4	85.5	66.7	90.5	83
	8.4	89.1	76.6	59	94.9	80
Optical values (g/dL)	5	25.5	98.6	87.5	77.7	78.5
	5.2	69.1	89	70.4	88.4	83.5
	5.5	80	80.7	61.1	91.4	80.5
	5.7	87.3	69	51.6	93.5	74

PPV, Positive predictive value; NPV, Negative predictive value.

RID IgG concentrations were 1,765 mg/dL (SD ± 1.039) and 1,594 mg/dL, respectively. The distribution of Brix and STP concentrations appeared approximately normally distributed (Fig 1B,C). The mean and median of Brix concentrations as measured by digital Brix refractometer were 8.8%Brix (SD ± 1.03) and 8.7%Brix, respectively, with a range of 5.9–12.9%Brix. The mean and median of STP concentrations as measured by optical refractometer were 6.0 g/dL (SD ± 1.0) and 5.8 g/dL, respectively, with a range of 4.2–10.6 g/dL. Fifty-five of 200 samples had IgG concentrations based on RID below a cut-off value of 1,000 mg/dL for FTPI positive cases, generating a true FTPI prevalence of 27.5%.

Correlation Coefficients

Correlation between each refractometer and the RID assay was determined using correlation plots of 200 serum samples. The RID IgG concentration was positively correlated with Brix concentration measured by digital refractometer ($r = 0.79$, Fig 2A) and with STP

measured by optical refractometer ($r = 0.74$, Fig 2B). Similarly, the correlation between digital Brix and optical refractometer results was 0.73 (Fig 2C).

Diagnostic Test Characteristics

The test characteristics of the digital Brix and optical STP refractometers were determined for the assessment of FTPI (serum IgG <1,000 mg/dL). The Se, Sp, PPV, NPV, and accuracy for digital Brix and optical refractometer at previously recommended cut-points are shown in (Table 1). A ROC was created to plot the true positive rate against the false positive rate for both digital Brix and optical refractometers (Fig 3). The cut-point of 8.3%Brix on digital refractometer yielded the best combination of Se (85.5%; 95% CI: 75.5–94.7%), Sp (82.8%; 95% CI: 78.7–90.8%), and accuracy (83.5%). Similarly, the best combination of Se (80%; 95% CI: 67–89.6%), Sp (80.7%; 95% CI: 73.3–86.8%), and accuracy (80.5%) for optical refractometer was achieved at 5.5 g/dL STP. The AUC of digital Brix and optical refractometers were 0.89 (95% CI: 0.85–0.94) and 0.88 (95% CI: 0.82–0.93), respectively.

Agreement between Tests

The agreement between results of the 2 refractometers and RID assay for the assessment of FTPI is presented in Table 2. The overall percent of agreement between results of digital Brix and optical

Table 2. Level of agreement between results of refractometers and radial immunodiffusion assay for assessing of failure of transfer of passive immunity in 200 dairy calves.

Refractometers	Cut-Off Value	Agreement	Kappa	P-Value
Digital Brix	8.3%Brix	0.83	0.59	.0001
Optical	5.5 g/dL	0.81	0.55	.0001
Digital versus Optical	—	0.85	0.65	.0001

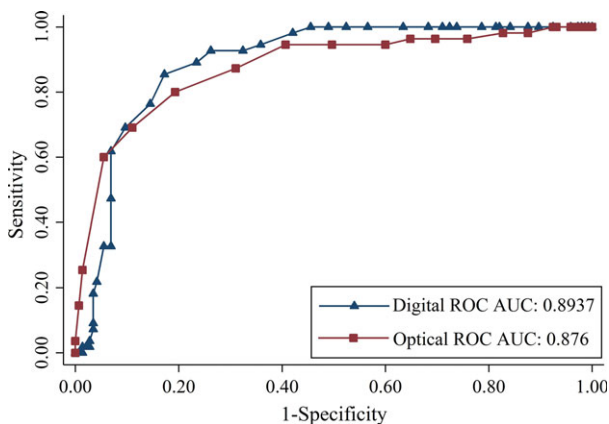


Fig 3. Receiver operating characteristic (ROC) curve, the best combination of sensitivity and specificity for digital and optical refractometers obtained at a cut-off value 8.3%Brix and 5.5 g/dL, respectively.

refractometers was 85%, with a corresponding kappa-value of 0.65. The McNemar's test for the sensitivity and specificity comparison, showed no significant difference ($P = .77$ and $P = .17$, respectively), between the proportion of calves classified as having FTPI by the 2 refractometers.

Discussion

Fifty-five of 200 samples had IgG concentrations <1,000 mg/dL based on RID, generating a true FTPI prevalence of 27.5%, which is consistent with the FTPI prevalence previously reported in the literature.^{10,11,25,26} However, the average values for serum IgG, %Brix, and STP concentrations determined in this study are slightly lower and the ranges wider than those found in a recently published article.²²

The correlation coefficients of the 2 refractometers compared to the RID determinations were similar. The Brix concentration measured by digital refractometer was positively correlated with serum IgG ($r = 0.79$; Fig 2A). However, higher correlation between digital refractometer and RID assay ($r = 0.87$) was reported, when Caprylic acid fractionation used to improve estimates of refractometer.²⁰ STP determined by optical refractometer was also positively correlated with serum IgG concentration ($r = 0.74$, Fig 2B). Although, similar correlation between STP and IgG as measured by RID ($r = 0.72$) have been reported,¹³ others have observed correlation coefficients from 0.67 to 0.93 between STP and IgG concentrations.^{22,27} This could be attributed to the use of digital refractometer to determine the STP in those studies. In the current study, STP determine by optical refractometer was positively correlated with % Brix ($r = 0.73$; Fig 2C). Perfect correlation ($r = 1.0$) between STP and %Brix was reported in a recent study.²² However, in that study they used of the same digital refractometer to determine both the STP and Brix concentrations, which might explain the perfect correlation.²²

For this study, the diagnostic test characteristics were established for both digital Brix and optical refractometers. These calculations provided an opportunity to identify utility and differences between both types of refractometers in the assessment of FTPI in dairy calves. The diagnostic test characteristics of both refractometers were determined at previously recommended cut-off values (Table 1). For example, the Se and Sp of digital refractometer at 8.4%Brix²² were 89.1 and 76.6%, respectively. The sensitivity and specificity of optical refractometer at 5.2 g/dL⁴ were 69.1 and 89%, respectively. The test characteristics determined were different at various cut-offs, which is related to a change of apparent prevalence of FTPI in the study population that altered the proportion of calves correctly identified.¹⁶ Se for detecting FTPI increased from 32.7% at 7.8%Brix to 89.1% at 8.4%Brix and from 25.5% at 5.0 g/dL to 87.3% at 5.7 g/dL (Table 1). In this study, the Se (85.5%) and Sp (82.8%) of digital refractometer at a cut-off value 8.3%Brix were very close to the Se (88.9%) and

Sp (88.9%) reported in a recent study, which used a cut-off value 8.4%Brix.²² Similarly, the Se (80.0%) and Sp (80.7%) of the optical refractometer at a cut-off value 5.5 g/dL were very similar to the Se (80%) and Sp (80%) previously reported with a cut-off value of 5.2 g/dL.⁴ The differences in the optimal refractometer cut-off values between studies might be attributed to instrument variation between refractometers used in these different studies.

The level of agreement between results of the 2 refractometers and RID IgG was high (Table 2). Similarly, the digital Brix refractometer showed high agreement (85%) with optical refractometer. This indicates that the 2 refractometers agree on the classification of calves with and without FTPI. Further, the McNemar's test showed a non-significant difference ($P > .05$) between proportions of calves classified as having FTPI by the 2 refractometers, indicating that the 2 refractometers performed similarly for FTPI assessment in calves.

In conclusion, both the digital Brix and optical STP refractometers show good potential for being useful management tools to be included in the calf health monitoring program on dairy operations. The results from this study suggest that the appropriate cut-off values for digital Brix and optical refractometers are 8.3% Brix and 5.5 g/dL, respectively, to assess FTPI in dairy calves. The 2 refractometers performed similarly in detection of FTPI.

Footnotes

- ^a BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ
 - ^b Bovine IgG RID Kit, Triple J Farms; Bellingham, WA
 - ^c PAL-1 digital Brix refractometer, Atago Co Ltd; Bellevue, WA
 - ^d Westover RHC-200ATC hand-held refractometer, Woodinville, WA
 - ^e Stata, version 13; StataCorp, LP, College Station, TX
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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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