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Comparison of single radial immunodiffusion and ELISA for the quantification of immunoglobulin G in bovine colostrum, milk and calf sera

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ABSTRACT

The overall objective was to compare immunoglobulin G (IgG) concentrations measured by single radial immunodiffusion (sRID) and ELISA-based methods in samples of bovine colostrum and transition milk from contrasting breed types (Limousin × Friesian ($n = 10$) and Holstein ($n = 10$)). Jugular blood samples were collected at 48 h post-birth from beef ($n = 10$) and dairy ($n = 10$) calves and sera harvested subsequent to colostrum consumption. Absolute colostrum IgG values determined by ELISA showed poor agreement with mean (SD) IgG values measured using sRID, fixed bias (sRID – ELISA) was 31.89 (±9.84) mg/mL; having wide limits of agreement (12.61–51.17) and a low concordance coefficient (0.26). The agreement between ELISA and sRID when measuring serum IgG was greater than that of colostrum, fixed bias (sRID – ELISA) was 12.36 (±6.60) mg/mL; having narrower limits of agreement (−0.58 to 25.30) and serum IgG concentrations had a greater concordance coefficient (0.44) between samples. Calf sera IgG measured using the indirect zinc sulphate turbidity test showed a strong correlation with the sRID and ELISA methods ($P < .001$), $R^2 = 0.78$ and $R^2 = 0.77$ respectively. Overall, the ELISA and sRID methodologies had a strong positive association with almost a twofold (1.8) difference between values; therefore, they provide diverse absolute values of IgG concentration.

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ELISA; single radial immunodiffusion; zinc sulphate turbidity test; Brix refractometer; IgG; calf; colostrum

Introduction

Immunoglobulin G (IgG) is the most abundant isotype found in bovine colostrum and represents over 75% of the total immunoglobulin (Ig) concentration (Korhonen et al. 2000), and consequently, quality of colostrum is assessed with reference to the content of this specific Ig class. Newborn calves are dependent on the transfer of Ig from colostrum as a source of protection against infectious-disease-causing pathogens (Weaver et al. 2000; Godden, 2008). Failure of passive transfer (FPT) occurs when calves have inadequate (<10 mg/mL) Ig circulating in their blood at 24 h post-birth (Tyler et al. 1996; Beam et al. 2009). Several studies have reported calves with FPT to be at greater risk of neonatal morbidity, pre-weaning morbidity and mortality compared to calves which achieved adequate passive transfer (APT) at 24 h (Donovan et al. 1998; Tyler et al. 1999; Godden et al. 2009). FPT is a major problem in Ireland, with 56% of samples submitted by private veterinary practitioners and from dead calves submitted for post mortem returning values consistent with FPT (DAFM/AFBI 2015).

It has been established by several researchers (Kehoe et al. 2007; Morrill et al. 2012; Conneely et al. 2013) that colostrum quality is extremely variable between individual cows and herds. Beef cows generally produce colostrum of greater quality compared to dairy breeds (Guy et al. 1994; Lorenz et al. 2011); this may be a result of the beef cows producing crossbred calves (Vann et al. 1995). An Irish study looking at different genotypes of beef suckler cows have reported colostral IgG concentrations > 150 mg/mL from both Charolais and beef × dairy cross breeds; this is sufficient to provide APT to the calf providing the calf is supervised during the period after birth to ensure suckling occurs (McGee et al. 2005).

Assessment of the concentration of IgG in colostrum and calf serum, following the intake of colostrum is crucial for monitoring APT of immunity to dairy and beef calves; and evaluating colostrum feeding practices on different farms. Various laboratory techniques are currently available to measure the passive transfer of immunity from dam to calf, including refractometry (Bielmann et al. 2010; Quigley et al. 2013), sodium sulphite test (Pfeiffer and McGuire 1977), zinc sulphate turbidity (ZST) assay (McEwan et al. 1970), whole blood glutaraldehyde coagulation (Tyler et al. 1996), γ-glutamyl transferase (Braun et al. 1982), single radial immunodiffusion (sRID) (Fleenor and Stott 1981; Ameri and Wilkerson 2008; Fecteau et al. 2013) and enzyme-linked immunosorbent assay (ELISA) (Kummer et al. 1992; Hurley et al. 2004; Lee et al. 2008). Weaver et al. (2000) reported sRID and ELISA to be the only true and direct measures of serum IgG; the remainder of tests mentioned merely estimate the IgG concentration based on the level of total globulins or other proteins whose passive transfer are associated with IgG.

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Rumbaugh et al. (1978) compared four different assays to assess IgG concentration in foals' serum, findings showed ZST and serum electrophoresis to be satisfactory when compared with sRID; however sodium sulphite precipitations were very unpredictable and deemed inadequate to determine serum IgG concentration. sRID is considered to be the most commonly reported method used to detect FPT in calves as findings suggest that the immunoassay performs well in detecting calves with FPT (Dawes et al. 2002; Ameri and Wilkerson 2008; Fecteau et al. 2013). However, it is an expensive and time-consuming test, requiring an incubation period of 18–24 h. In contrast, ELISA method has a higher throughput, is less costly and analysis can be completed in a much shorter time (Lee et al. 2008). Consequently, recent research has used ELISA method to quantify IgG levels in colostrum, plasma and sera (Vetter et al. 2013; Baumrucker and Bruckmaier 2014; Gelsinger, Jones, et al. 2015; Gelsinger, Smith, et al. 2015).

The standards used in immunochemical assays can influence the quantification of Ig in bovine milk and serum. Although there are differences between milk and serum composition in terms of IgG subclasses, no IgG standards are commercially available for the detection of IgG in milk or dairy produce (Li-Chan and Kummer, 1997). A substitute to using IgG that has been specifically purified from a milk source is to use bovine IgG_1 as a standard in ELISA as it is commercially available, and reports from Li-Chan and Kummer (1997) have shown only slight differences between the quantification of IgG_1 and IgG in milk.

The overall objective of this study were to compare and assess the accuracy and degree of agreement between ELISA and sRID measuring the IgG concentration in colostrum (first milking), milk (fifth milking), and serum from dairy and beef animals. A secondary objective was to use Brix refractometry and ZST to approximate IgG content in bovine colostrum and milk, and in calf sera, and correlate these estimates with values directly measured by sRID and ELISA. Several studies have reported IgG concentrations measured using ELISA and sRID method, but limited research has been carried out to appraise IgG concentrations quantified using both methods. Use of test method differs between researchers; for example, some report results using sRID and others use the ELISA method, and therefore standardization of these assays is essential to allow accurate comparisons between results within the literature.

Materials and methods

Animal feeding management

The birth of each calf was carefully supervised. Dairy calves were removed approximately 15–20 min post-parturition before suckling could occur, while beef bred calves remained with their dams. Dairy calves were weighed and placed in a straw-bedded pen where they were then blood sampled and received colostrum (~3 L) from their own dam via oesophageal tube within 2.5 h post-birth. Post-birth, all beef calves were weighed and closely supervised to ensure suckling of their own dam and any calf not suckling within 1 h of birth or showing signs of weakness was assisted to suckle the cow.

Sample collection

Dairy (Holstein ($n = 10$)) and beef (Limousin × Friesian ($n = 10$)) cows and their calves were included in the study. Colostrum (first milking) samples were collected from individual dairy and beef cows immediately post-parturition. A further milk sample (fifth milking) was collected from all cows post-parturition. Samples were stored at −20°C until IgG analysis. Blood samples were collected from the dairy (Holstein ($n = 10$)) and the beef (Limousin × Friesian ($n = 10$)) calves via the jugular vein using a 10 mL vacutainer tube containing no anticoagulant (BD, Oxford, UK), subsequent to colostrum intake from their dams at 48 h post-birth. This specific time point after birth was chosen to represent optimum serum IgG concentrations. Serum was obtained from these blood samples following centrifugation (1500 × g at 7°C) and frozen at −20°C until IgG analysis. Pooled colostrum and milk from both dairy and beef breeds, and serum (48 h post-birth) from calves were included in the analysis.

Quantification of commercial IgG_1

Using a Bicinchoninic Acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL, USA), a commercial IgG_1 antibody (1 mg/mL; Bethyl labs, UK) was quantified against a standard curve of bovine gamma globulin (BGG fraction II, 2 mg/mL in a 0.9% aqueous NaCl solution containing sodium azide; Pierce). The micro-plate assay procedure was performed in accordance with the manufacturer's instructions.

ELISA

The ELISA method used to quantify colostral and calf serum IgG concentration was performed according to manufacturer's instructions (Bio-X Diagnostics, Jemelle, Belgium).

Preparation of colostrum IgG standards and test samples

Colostrum standards ranging between 250,000 and 14,632 ng/mL IgG were prepared. It was recommended by the manufacturer that colostrum samples should be diluted 1:1000 in phosphate buffer saline (PBS); however, to obtain a range throughout the standard curve, three different dilutions of each sample were prepared (1:500, 1:1000 and 1:2000). Aliquots of dairy/beef colostrum (0.5 mL) were pooled from each cow ($n = 10$ cows) from two separate milkings: (1) colostrum from milking 1 (high IgG level) and (2) colostrum from milking 5 (low IgG level). Batches were divided and labelled into 0.2 mL aliquots for freezing (to avoid freeze/thawing). These were included as an inter-plate control. All colostrum samples and standards were added to a 96-well dilution plate (100 µL/well), in duplicate. Duplicates were acceptable when the coefficient of variation was <15%. The coefficients of variation for colostrum (milking 1) at a dilution of 1/500, 1/1000 and 1/2000 were 11.7%, 10.9% and 12.9%, respectively.

A commercial IgG_1 antibody (1 mg/mL; Bethyl laboratory, Cambridge, UK) was included on the plate as a positive control. This was added to the plate at a concentration of 50 and 100 µg/mL IgG. A colostrum sample with an IgG

concentration within the mid-range of the standard curve was included on each plate as an inter-plate control.

Serum IgG concentration

Sera dilutions were selected based on recommendations by the manufacturer that serum samples should be diluted 1:225 in PBS; however, to obtain a range of values within the standard curve, three dilution rates were applied to the plate in most cases. Therefore, samples were diluted accordingly; the range of dilutions used were the following: 1:225, 1:450 and 1:900. A commercially available reference serum (24 mg/mL IgG, 10 mg/mL IgG_1, 14 mg/mL IgG_2, 0.11 mg/mL IgA and 1.8 mg/mL IgM; Bethyl laboratory, Cambridge, UK) was diluted to an IgG concentration of 48,000 ng/mL for total IgG. A serum sample with an IgG concentration within the mid-range of the assay range was included on each plate as an inter-plate control. Aliqouts of dairy/beef calf sera (0.5 mL) were pooled from each calf ($n = 10$ calves) from two separate time periods: (1) calf sera from time before colostrum feeding (very low IgG level) and (2) calf sera from time period after colostrum feeding (48 h) (high IgG level). Batches were divided and labelled into 0.2 mL aliquots for freezing (to avoid freeze/thawing). These were included as an inter-plate control. All samples and standards were added to a 96-well dilution plate (100 µL/well), in duplicate.

The horse radish peroxidase conjugate was diluted 50 fold in the dilution buffer i.e. 250 µL conjugate in 12.5 mL of dilution buffer and 100 µL was added to each well of the dilution plate and then 100 µL of this was transferred to the test plate. Each test plate was incubated at room temperature for 1 h. The plate was washed four times using wash solution. Chromogen solution (tetramethylbenzidine) was added to each well (100 µL/well) and the plate was protected from light and incubated for 10 min at room temperature. Stop solution (containing 1 M phosphoric acid) was carefully added to the plate (50 µL/well) and the absorbance at 450 nm was read using a plate reader (Tecan Magellan, Männedorf, Switzerland). Negative controls (PBS) were included on the test plate; these values were averaged and then subtracted from the sample and standard absorbance values. A standard curve was constructed using a four parameter curve fit using the average values from duplicate standard wells; this illustrated the relationship between absorbance and IgG concentration. The coefficient of determination value of each standard curve had to exceed 0.99 on the standard curve to be included within these results.

Single radial immunodiffusion (sRID)

The bovine IgG test kit (200–3000 mg/dL) was performed in accordance with manufacturer instructions (Triple J Farms, Bellingham, WA, USA). Each kit consisted of 3 sRID plates, 24 wells per plate containing goat antiserum raised against bovine IgG within an agarose gel (0.1 M phosphate buffer, 0.1% sodium azide, 1 µg/mL of amphotericin B, 0.002 M EDTA; pH 7.0), requiring 5 µL of sample applied to each well and 3 standard solutions of bovine serum (1.96, 14.02 and 27.48 mg/mL of IgG). Standard solutions and samples were applied directly to the plate (5 µL/well) using 5 µL wiretrol micropipettes (Drummond Scientific, Broomall, PA, USA). Reference serum of known IgG concentration (24 mg/mL IgG) was included on each plate as a positive control (Bethyl laboratory, Cambridge, UK).

Defatted colostrum (fat was removed through centrifuging before freezing) were diluted appropriately in PBS at 1/2, 1/3 and 1/4 to fall within the range of the standard curve. All sera and defatted colostrum were analysed in triplicate (inter-day assays). Plates were incubated for 24 h at room temperature to obtain an end point reading. The diameters of the precipitin rings were measured using a 'Peak' Viewer 7X Lupe Magnifier (Triple J Farms, Bellingham, WA, USA). The diameters of the precipitin rings were plotted against the IgG concentrations of the standards using linear regression, and the concentrations of the samples were interpolated from this. Results from the three replicates were used to calculate the mean IgG concentration, the standard deviation and the coefficient of variation.

Zinc sulphate turbidity (ZST) test

Serum samples were analysed for ZST units, a control (100 µL of serum and 6 mL of distilled water) and test samples (100 µL of serum and 6 mL of zinc sulphate (0.208 g/L) distilled water) were prepared and incubated at room temperature for 1 h; samples were transferred into cuvettes and read using a spectrophotometer with a 623 nm filter, as described by McEwan et al. (1970).

Refractometry

A handheld digital refractometer was used to determine the brix score of colostrum (Bellingham and Stanley, Kent, UK). Before use, the refractometer was calibrated to zero using distilled water. The digital Brix determined the Brix score of the colostrum by shining a light through the sample in the well, measuring the index of refraction, and presenting the reading in Brix units on a digital scale, as described by Bielmann et al. (2010).

Statistical analysis

Statistical analyses were performed using GenStat, 16th edition (VSN International 2015). Statistical differences were considered significant at $P < .05$. Linear mixed model (LMM) methodology using the REML commands available in GenStat was used to analyse the difference between IgG results for individual colostrum and sera samples using the different test kits. Cow, batch of kit and plate were fitted as random effects in the model and a factorial arrangement of kit type, breed, milking number as fixed effects in the colostrum samples. Calf, batch of kit and plate were fitted as random effects in the model and a factorial arrangement of kit type, breed and time point after calving as fixed effects in the serum samples. The same analysis was carried out for pooled colostrum and sera samples; however, individual cow or calf was not included as a random effect. LMM using REML was also used to analyse the linearity of IgG results produced between kits at different dilutions for

colostrum and serum. Plate was included as a random effect and dilution factor as a fixed effect. A repeatability coefficient (Bland and Altman 1999) was used to measure the repeatability of test kits. Lin's concordance correlation coefficient was conducted to measure the agreement between IgG results obtained from test kits. This agreement between the IgG concentrations measured by ELISA and sRID kits was further assessed using the Bland–Altman method. The percentage recovery for each commercial standard of known concentration was calculated as the measured value for the standard divided by the expected value and converted to a percentage.

Results

Scatter plots and regression analysis for sRID and ELISA are represented in Figure 1. There was a positive correlation between the ELISA and sRID methods; IgG concentration in first milking colostrum ($R^2 = 0.83$; $P < .001$) and calf serum at 48 h post-birth ($R^2 = 0.97$; $P < .001$). The type of assay significantly affected colostrum and serum IgG concentration, with values determined using the ELISA kit being consistently lower than sRID, as shown in Table 1. However, while the absolute IgG concentration was less, there was a strong correlation between the values determined for the same samples using both methods. On average, the IgG values determined by sRID were approximately 1.8 times greater than the values produced using the ELISA method when assessing colostrum and calf sera IgG concentration. Breed of animal had a significant effect on calf sera IgG concentration, beef calves had a much greater mean serum IgG concentration at 48 h post-birth when quantified using sRID and ELISA, 34.39 and 19.26 mg/mL respectively compared to dairy calves, 20.76 and 11.30 mg/mL respectively. Breed had no effect ($P > .05$) on IgG concentration at milking 1 or milking 5, post-parturition (Table 1).

sRID and ELISA method showed a poor level of agreement between colostral IgG concentrations at the first milking post-partum; the Bland–Altman method showed a fixed bias (sRID – ELISA) of 31.89 ± 9.84 mg/mL and a wide limit of agreement (12.61–51.17), as shown in Figure 2; the concordance co-efficient between ELISA and sRID was also poor within colostral IgG values (0.26). The agreement between ELISA and sRID when measuring serum IgG was greater than that of colostrum, fixed bias was (sRID – ELISA) 12.36 ± 6.60 mg/mL, with a narrower limit of agreement (−0.58 to 25.30), and there was also a greater concordance coefficient (0.44) within calf sera IgG values.

Table 1. Mean IgG (mg/mL) concentration in first milking (colostrum), fifth milking and calf serum measured by ELISA or sRID method.

	Method			Significance		
	ELISA	sRID	SED[a]	Breed[b]	Kit[c]	K × B
Pooled calf serum[d] (beef and dairy)	15.28[e]	27.58[e]	1.17	0.01	<0.001	<0.001
Calf serum[d]						
Beef ($n = 10$)	19.26	34.39	2.74	–	–	–
Dairy ($n = 10$)	11.30	20.76	2.74	–	–	–
Pooled first milking (colostrum) (beef and dairy)	45.04[e]	78.51[e]	3.26	0.01	<0.001	0.03
First milking (colostrum)						
Beef ($n = 10$)	51.55	88.35	5.17	–	–	–
Dairy ($n = 10$)	38.52	68.67	5.17	–	–	–
Pooled fifth milking (beef and dairy)	8.80[e]	17.75[e]	7.65	0.11	0.23	0.30
Fifth milking						
Beef ($n = 10$)	9.97	19.65	5.79	–	–	–
Dairy ($n = 10$)	7.62	15.84	5.79	–	–	–

[a] Standard error of the difference (SED).
[b] Breed = Limousin × Friesian (beef); Holstein (dairy).
[c] Kit = ELISA (Bio-X diagnostics, Jemelle, Belgium) and sRID (Triple J farms, Bellingham, WA, USA).
[d] Calf serum at 48 h post-birth.
[e] Pooled sample (beef and dairy).

Both ELISA and sRID showed good reproducibility when quantifying colostrum and calf sera, ELISA method showed an inter-day coefficient of variation of <6% when measuring colostral IgG. The average inter-day coefficient of variation for calf sera was ≤15%. Using sRID method, colostrum plates showed an inter-day variation of 7.6% and serum plates showed an inter-day variation of 14.6%.

Recovery of commercial IgG_1 antibody and reference serum having known amounts of IgG concentration are shown in Table 2. Recovery of commercial IgG_1 antibody was poor within the ELISA, having a recovery of only 34% and it was beyond the limit of detection for sRID method. Recoveries of >100% IgG were obtained using reference serum of known IgG concentration in both the sRID and ELISA.

A linear relationship was found when dairy and beef colostrum ($R^2 = 0.94$, $R^2 = 0.98$) and dairy and beef sera samples ($R^2 = 0.95$, $R^2 = 0.90$) were analysed at a range of different dilutions by ELISA. There was no difference between values

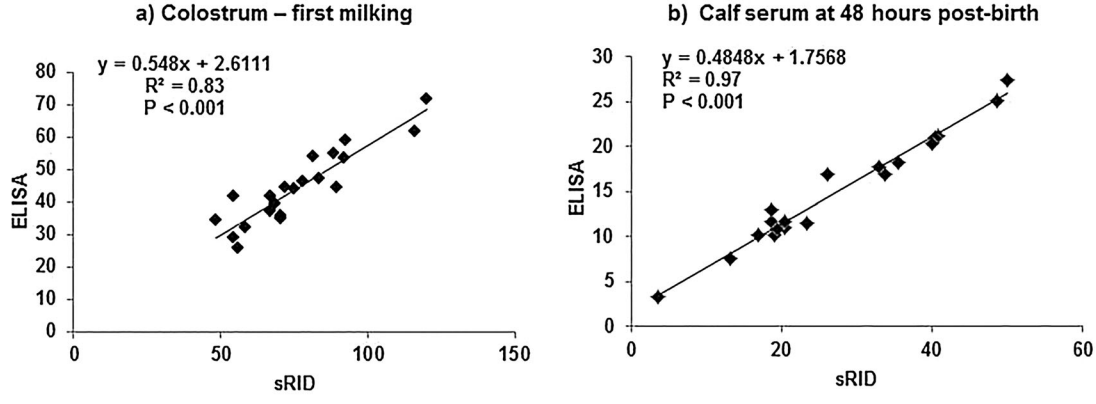

Figure 1. Correlation of IgG concentration measured by sRID and ELISA method in first milking colostrum and calf serum samples at 48 h post-birth.

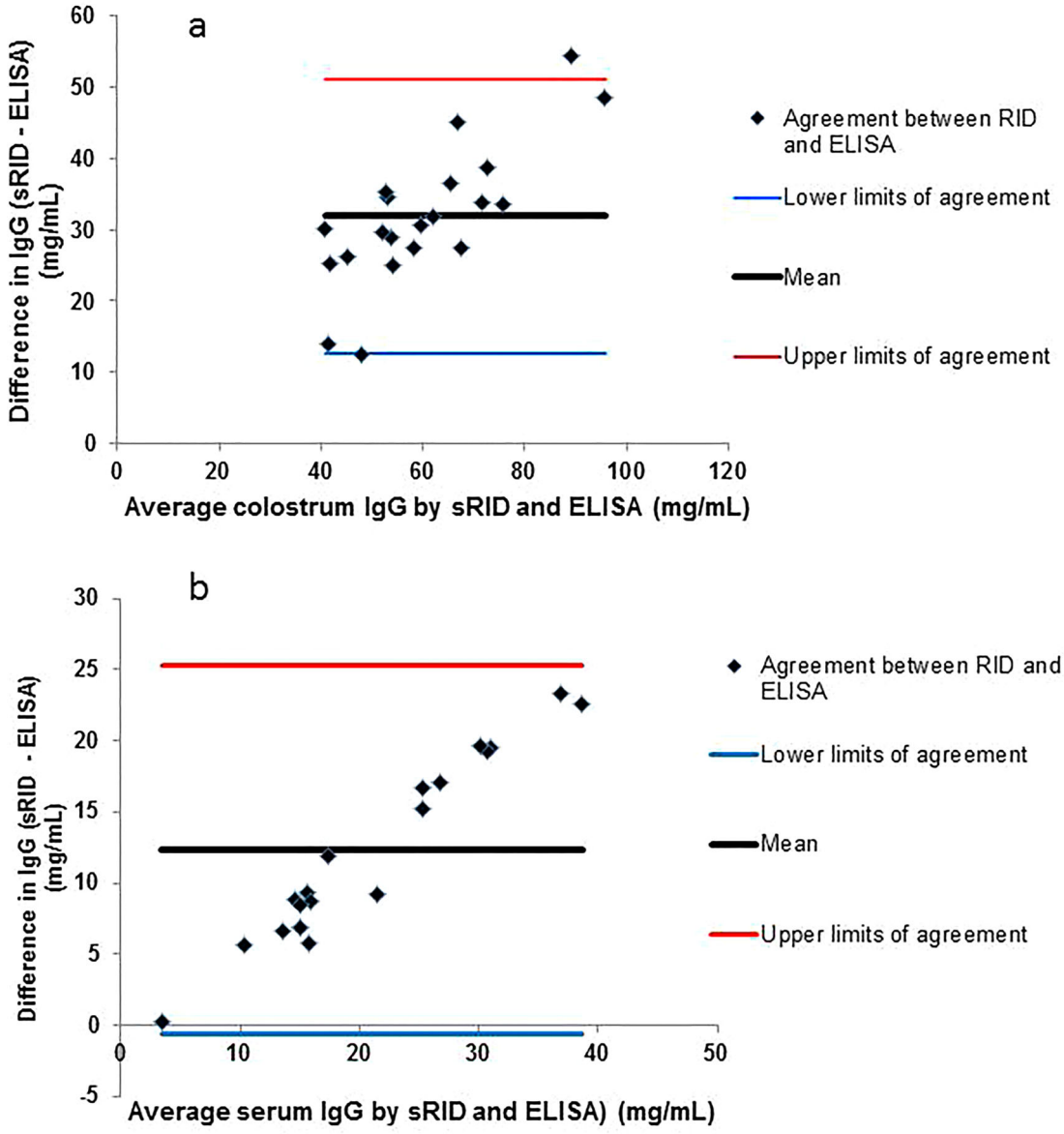

Figure 2. Bland–Altman plot with mean + 95% confidence limits of agreement showing level of agreement between (a), first milking colostrum and (b), 48 h calf sera IgG concentrations, using ELISA and sRID methods.

when beef calves sera were diluted; however, a difference ($P = .03$) was identified within dairy sera when diluted at a higher rate (Table 3), showing greater IgG levels in a more dilute sample.

The sRID and ELISA methods had a significant relationship with ZST ($P < .001$), $R^2 = 0.78$ and $R^2 = 0.77$ respectively, as shown in Figure 3. Brix scores were measured for colostrum collected at milking 1 post calving and we found a significant positive correlation between ELISA values and BRIX score ($R^2 = 0.58$; $P < .001$); there was a poorer relationship observed between sRID values and BRIX score ($R^2 = 0.36$; $P = .005$) (Figure 4). We found the average Brix score to be 27.3%, ranging from 22% to 35%.

Discussion

In an effort to elucidate the accuracy of sRID, ELISA, and indirect methods to quantify bovine IgG, we performed a validation study comparing colostrum and calf sera IgG concentration

Table 2. Recovery study using commercial IgG_1 and IgG standards within ELISA and sRID methodologies.

	ELISA				sRID		
Sample	Expected (ng/mL)	Actual (ng/mL)	Recovery (%)	Sample	Expected (ng/mL)	Actual (ng/mL)	Recovery (%)
Commercial IgG_1[a]	100,000	28,478	28.5	Reference serum	24,000,000	24,840,556	103.5
Commercial IgG_1[a]	50,000	17,205	34.4				
Reference serum[b]	48,000	54,844	114.3				

[a] Commercial IgG_1 standard (Bethyl, UK) – commercial bovine IgG_1 purified using salt fractionation and DEAD chromatography. Protein concentration was determined by A_{280nm} 1.4 equals 1.0 mg of IgG.
[b] Reference serum (Bethyl, UK; Cat # RSO-103). Assigned values: IgG: 24 mg/mL; IgG_1: 10 mg/mL; IgG_2: 14 mg/mL; IgA: 0.11 mg/mL; IgM: 1.8 mg/mL.

Table 3. Effect of dilution factor used within ELISA on the recovery of IgG concentration in colostrum and sera samples from beef and dairy animals.

Sample	Dilution factor	Observed value (mg/mL)	Expected value (mg/mL)	Recovery (%) when compared to that of recommended dilution rate	*P*-value
1st Milking (colostrum)					
Beef[1]	1/1000*	35.94[a]	35.94	100	0.99
	1/500	36.42[a]		101	
	1/2000	39.63[a]		110	
Dairy[2]	1/1000*	52.82[a]	52.82	100	0.46
	1/500	52.34[a]		99	
	1/2000	52.60[a]		99.6	
48 h Calf sera					
Beef[1]	1/225*	18.04[a]	18.04	100	0.55
	1/450	18.91[a]		105	
	1/900	19.43[a]		108	
Dairy[2]	1/225*	11.06[a]	11.06	100	0.03
	1/450	11.81[ab]		107	
	1/900	14.16[b]		128	

* As recommended by the manufacturer.
[1] Limousin × Friesian (beef).
[2] Holstein (dairy).

from two contrasting cow breeds. No validation was conducted on the ZST or Brix tests but rather the values were correlated to the two methods of direct measurement. Based on the variation observed in the literature, the minimum number of animals necessary to significantly differentiate two groups at a *P*-value of .05 and confidence interval of 90% was 10 animals per treatment (Dell et al. 2002). In the present study, a strong correlation was observed between the sRID and the ELISA method. In contrast to our findings, Gelsinger, Smith, et al. (2015) reported a weaker relationship between a modified ELISA (Bethyl Laboratories, Montgomery, TX, USA); and the protocol described by Vetter et al. (2013), and a commercially available sRID assay (Triple J Farms, Bellingham, WA, USA) when measuring colostrum ($R^2 = 0.36$; $P = .01$) and plasma IgG concentrations ($R^2 = 0.59$; $P < .01$), although these were still significant. The differences observed between these studies may be specific to the particular kits that were used. The strong correlation between both test kits in this present study indicates that there was an association between each set of IgG values; however, the absolute IgG values were dissimilar. Therefore, the application of a new cut-off value may enable comparison between kits; however, this would have to be empirically determined through further validation. This recommendation is in agreement with research by Gelsinger, Smith, et al. (2015), who reported that colostrum and plasma samples had significantly lower IgG concentration when measured by ELISA method compared to sRID. Colostrum IgG values determined by sRID were almost twofold that of ELISA; however, plasma values determined by sRID were only 1.3 times greater than values determined by ELISA method.

The variation of agreement shown between test kits in this current study may be due to the extensive dilution of colostrum samples within the ELISA assay compared to the minimal sample dilution required within sRID; this is in agreement with Gelsinger, Smith, et al. (2015). Contrastingly, Lee et al. (2008) demonstrated a modified ELISA (Bethyl Laboratories, Montgomery, TX, USA); and the protocol described by Vetter et al. (2013) to exhibit a good agreement (94%) with a commercially available sRID assay (Triple J Farms, Bellingham, WA, USA).

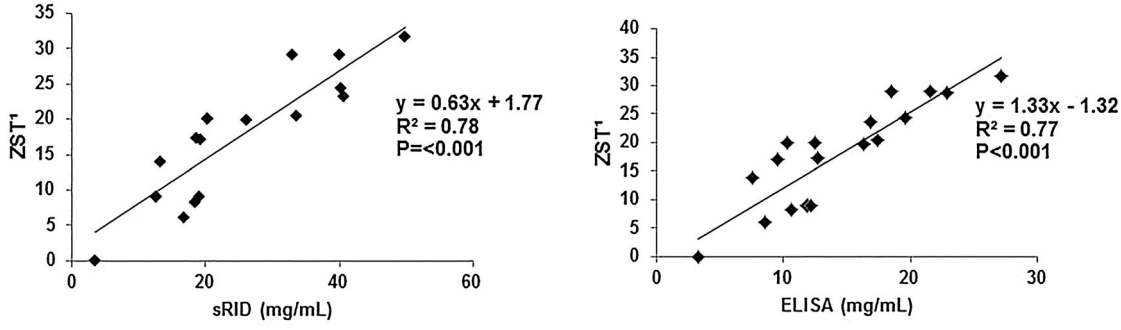

Figure 3. Correlation of IgG concentration measured by sRID, ELISA and ZST in calf serum samples at 48 h post-birth.

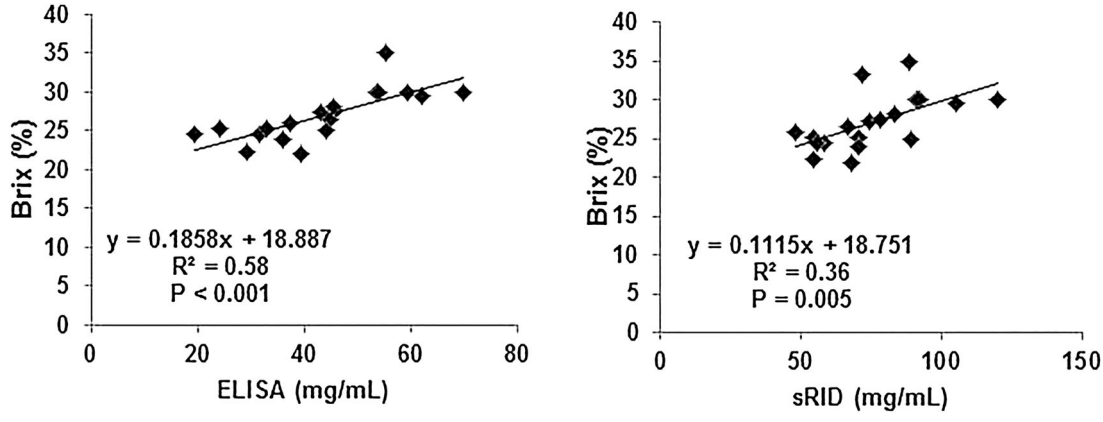

Figure 4. Correlation of IgG concentration measured by sRID, ELISA and BRIX score in first milking colostrum.

This may be a consequence of the specific test kits used, as there may be differences between IgG concentrations of standards supplied with kits depending on how they are quantified. A future development to these current findings is additional validation of these test kits to determine a new cut-off value which is suitable for application to ELISA values so that ELISA and sRID values can be compared accurately. It is evident that there is a strong correlation between the IgG concentrations determined using both test kits, although the absolute IgG concentrations from each test differ. Calves are reported to have successful passive transfer when their serum contains a minimum of 10 mg/mL IgG at 24 h post-birth (Weaver et al. 2000) and colostrum is deemed high quality at 50 mg/mL (McGuirk and Collins 2004); however, these cut-off values were established using sRID method (Tyler et al. 1996; Godden 2008), and therefore updated cut-off values determined using the ELISA method are required to prevent misdiagnosis of FPT in calves.

Breed of animal was significantly associated with the sera IgG concentration, our findings are in agreement with those reported by Suh et al. (2003) in which the mean IgG concentration in beef calves sera was approximately twofold that of dairy calves monitored for the first two weeks of life. Other studies have shown relatively similar serum IgG concentrations for beef (Waldner and Rosengren 2009) and dairy calves (Deelen et al. 2014). However, in the present study, there was no difference between colostrum IgG and breed of cow; this was in agreement with findings from Murphy et al. (2005), who observed cow breed to have no significant effect on colostral IgG1 concentration between five suckler cow breed types. Consequently this would suggest that the reason for higher concentrations of IgG circulating in the calves' blood is due to the increased intake of colostrum through natural suckling by the beef calves compared to manually feeding 3 L of colostrum to dairy calves at birth.

Poor recovery of IgG_1 antibody was observed within the ELISA; however, it has been previously reported that purified bovine IgG_1 is a suitable alternative for a colostrum-based IgG standard (Li-Chan and Kummer 1997). However, in contrast to this current study, Li-Chan and Kummer (1997) developed an in-house double sandwich ELISA for the quantification of IgG in bovine milk and this may have attributed to the difference between results. Recoveries of >100% IgG were obtained using reference serum of known IgG concentration in both the sRID and ELISA. This indicates that both kits are capable of quantifying IgG concentration. It is unknown as to why the reference serum IgG values are similar in both kits, yet the colostrum and sera samples when tested for IgG are very different.

Lee et al. (2008) reported ZST to be a useful screening device, showing high specificity but recommended confirmatory diagnosis of IgG concentration with a direct measure such as sRID; this is in agreement with the findings from this study. The Brix refractometer is a useful tool to indirectly assess the IgG concentration in colostrum; it is simple to use, rapid and inexpensive. Our findings correlated with findings from Bielmann et al. (2010), who reported the average Brix score to be 26.1% (range 13.6% and 37%). Quigley et al. (2013) determined a cut-off Brix score to be 21% to estimate colostrum IgG values above 50 mg/mL. In contrast to our findings, Bielmann et al. (2010) reported a stronger correlation ($R^2 = 0.73$; $P < .001$) between Brix score and sRID values when quantifying colostral IgG. Recent findings from Bartier et al. (2015) showed a correlation of $R^2 = 0.64$ between Brix and RID values. However, it has been suggested that the volume and proportion of protein in the colostrum may have an effect on the precision of the refractometer (Bielmann et al. 2010). Using foal sera, Davis and Giguere (2005) evaluated five commercially available assays, including ZST, glutaraldehyde coagulation assay, two semi-quantitative enzyme immunoassays (Quick test kits) and a quantitative immunoassay (handheld quantitative colorimetric immunoassay). Serum total protein concentration was assessed by refractometry, to quantify passive transfer of immunity. It was concluded from this investigation that the majority of these assays were sufficient for initial screening tests; however they were inadequate as a definitive test as it could result in unnecessary treatment of foals with adequate serum IgG concentrations (Davis and Giguere 2005).

In summary, the present study reports a strong correlation between IgG concentrations determined in bovine colostrum and sera using the commercially available ELISA and sRID assays, and suggests that values should not be directly compared in terms of absolute concentrations of IgG. We observed strong recovery of the known IgG within the reference serum using both test kits and the reproducibility from each kit was satisfactory. It may be the case that a new cut-off value can be applied to the ELISA assay to determine colostrum quality and FPT in newborn calves. Additional studies are required to produce a cut-off values for the ELISA to detect FPT in calves. Consequently, a detailed validation of test kits should be performed prior to any laboratory use for both colostrum and serum.

In conclusion, direct comparison of ELISA and sRID results from colostrum or serum IgG concentration is not recommended due to poor agreement between tests. This has implications for the interpretation of IgG values within the literature. Both assays provided consistent reproducible results over repeated analyses. Brix and ELISA showed the strongest correlation between indirect and direct measures of IgG concentration in colostrum. ZST showed strong correlation with both ELISA and sRID when quantifying IgG in calf sera; therefore, this quick and easy indirect test can be useful for the detection of FPT in newborn calves.

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References

Ameri M, Wilkerson MJ. 2008. Comparison of two commercial radial immunodiffusion assays for detection of bovine immunoglobulin G in newborn calves. J Vet Diag Invest. 20:333–336.

Bartier AL, Windeyer MC, Doepel L. 2015. Evaluation of on-farm tools for colostrum quality measurement. J Dairy Sci. 98:1878–1884.

Baumrucker CR, Bruckmaier RM. 2014. Colostrogenesis: IgG1 transcytosis mechanisms. J Mammary Gland Biol Neoplasia. 19:103–117.

Beam AL, Lombard JE, Kopral CA, Garber LP, Winter AL, Hicks JA, Schlater JL. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on us dairy operations. J Dairy Sci. 92:3973–3980.

Bielmann V, Gillan J, Perkins NR, Skidmore AL, Godden S, Leslie KE. 2010. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. J Dairy Sci. 93:3713–3721.

Bland JM, Altman DG. 1999. Measuring agreement in method comparison studies. Stat Methods Med Res. 8:135–160.

Braun J, Tainturier D, Laugier C, Benard P, Thouvenot J, Rico A. 1982. Early variations of blood plasma gamma-glutamyl transferase in newborn calves—a test of colostrum intake. J Dairy Sci. 65:2178–2181.

Conneely M, Berry DP, Sayers R, Murphy JP, Lorenz I, Doherty ML, Kennedy E. 2013. Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows. Animal. 7:1824–1832.

DAFM/AFBI. 2015. AFBI/DAFM all-island surveillance report (pdf 1,099Kb). https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/labservice/rvlreports/2016/2015AFBIDAFMAllIslandSurveillanceReport190117.pdf.

Davis R, Giguere S. 2005. Evaluation of five commercially available assays and measurement of serum total protein concentration via refractometry for the diagnosis of failure of passive transfer of immunity in foals. J Am Vet Med Assoc. 227:1640–1645.

Dawes ME, Tyler JW, Hostetler D, Lakritz J, Tessman R. 2002. Evaluation of a commercially available immunoassay for assessing adequacy of passive transfer in calves. J Am Vet Med Assoc. 220:791–793.

Deelen SM, Ollivett TL, Haines DM, Leslie KE. 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J Dairy Sci. 97:3838–3844.

Dell RB, Holleran S, Ramakrishnan R. 2002. Sample size determination. ILAR J. 43:207–213.

Donovan GA, Dohoo IR, Montgomery DM, Bennett FL. 1998. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. Prev Vet Med. 34:31–46.

Fecteau GG, Arsenault J, Paré J, Van Metre DC, Holmberg CA, Smith BP. 2013. Prediction of serum igg concentration by indirect techniques with adjustment for age and clinical and laboratory covariates in critically ill newborn calves. Can J Vet Res. 77:89–94.

Fleenor WA, Stott GH. 1981. Single radial immunodiffusion analysis for quantitation of colostral immunoglobulin concentration. J Dairy Sci. 64:740–747.

Gelsinger SL, Jones CM, Heinrichs AJ. 2015. Effect of colostrum heat treatment and bacterial population on immunoglobulin G absorption and health of neonatal calves. J Dairy Sci. 98:4640–4645.

Gelsinger SL, Smith AM, Jones CM, Heinrichs AJ. 2015. Technical note: comparison of radial immunodiffusion and ELISA for quantification of bovine immunoglobulin G in colostrum and plasma. J Dairy Sci. 98:4084–4089.

Godden S. 2008. Colostrum management for dairy calves. Vet Clin North Am Food Anim Pract. 24:19–39.

Godden SM, Haines DM, Konkol K, Peterson J. 2009. Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. J Dairy Sci. 92:1758–1764.

Guy MA, McFadden TB, Cockrell DC, Besser TE. 1994. Regulation of colostrum formation in beef and dairy cows. J Dairy Sci. 77:3002–3007.

Hurley IP, Coleman RC, Ireland HE, Williams JHH. 2004. Measurement of bovine IgG by indirect competitive ELISA as a means of detecting milk adulteration. J Dairy Sci. 87:543–549.

Kehoe S, Jayarao B, Heinrichs A. 2007. A survey of bovine colostrum composition and colostrum management practices on Pennsylvania dairy farms. J Dairy Sci. 90:4108–4116.

Korhonen H, Marnila P, Gill HS. 2000. Milk immunoglobulins and complement factors. Br J Nutr. 84:S75–S80.

Kummer A, Kitts DD, Li-Chan E, Losso JN, Skura BJ, Nakai S. 1992. Quantification of bovine igg in milk using enzyme-linked immunosorbent assay. Food Agri Immunol. 4:93–102.

Lee SH, Jaekal J, Bae CS, Chung BH, Yun SC, Gwak MJ, Noh GJ, Lee DH. 2008. Enzyme-linked immunosorbent assay, single radial immunodiffusion, and indirect methods for the detection of failure of transfer of passive immunity in dairy calves. J Vet Inter Med. 22:212–218.

Li-Chan ECY, Kummer A. 1997. Influence of standards and antibodies in immunochemical assays for quantitation of immunoglobulin G in bovine milk. J Dairy Sci. 80:1038–1046.

Lorenz I, Mee JF, Earley B, More SJ. 2011. Calf health from birth to weaning. I. General aspects of disease prevention. Irish Vet J. 64:1–8.

McEwan AD, Fisher EW, Selman LE, Penhale WJ. 1970. A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clin Chim Act. 27:155–163.

McGee M, Drennan MJ, Caffrey PJ. 2005. Effect of suckler cow genotype on cow serum immunoglobulin (Ig) levels, colostrum yield, composition and Ig concentration and subsequent immune status of their progeny. Irish J Agr Food Res. 44:173–183.

McGuirk SM, Collins M. 2004. Managing the production, storage, and delivery of colostrum. Vet Clin North Am Food Anim Pract. 20:593–603.

Morrill KM, Conrad E, Lago A, Campbell J, Quigley J, Tyler H. 2012. Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. J Dairy Sci. 95:3997–4005.

Murphy BM, Drennan MJ, O'Mara FP, Earley B. 2005. Cow serum and colostrum immunoglobulin (IgG_1) concentration of five suckler cow breed types and subsequent immune status of their calves. Irish J Agri Food Res. 44: 205–213.

Pfeiffer NE, McGuire TC. 1977. A sodium sulfite-precipitation tests for assessment of colostral immunoglobulin transfer to calves. J Am Vet Med Assoc. 170:809–811.

Quigley JD, Lago A, Chapman C, Erickson P, Polo J. 2013. Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. J Dairy Sci. 96:1148–1155.

Rumbaugh GE, Ardans AA, Ginno D, Trommershausen-Smith A. 1978. Measurement of neonatal equine immunoglobulins for assessment of colostral immunoglobulin transfer: comparison of single radial immunodiffusion with the zinc sulfate turbidity test, serum electrophoresis, refractometry for total serum protein, and the sodium sulfite precipitation test. J Am Vet Med Assoc. 172:321–325.

Suh GH, Hur TY, Son DS, Choe CY, Jung YH, Ahn BS, Lee CY, Lee CG. 2003. Differences in the serum immunoglobulin concentrations between dairy and beef calves from birth to 14 days of age. J Vet Sci. 4:257–260.

Tyler JW, Hancock DD, Parish SM, Rea DE, Besser TE, Sanders G, Wilson LK. 1996. Evaluation of 3 assays for failure of passive transfer in calves. J Vet Inter Med. 10:304–307.

Tyler JW, Hancock DD, Thorne JG, Gay CC, Gay JM. 1999. Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves. J Vet Inter Med. 13:335–337.

Vann R, Holloway J, Carstens G, Boyd M, Randel R. 1995. Influence of calf genotype on colostral immunoglobulins in bos taurus and bos indicus cows and serum immunoglobulins in their calves. J Anim Sci. 73:3044–3050.

Vetter A, Argüello A, Baumrucker C, Bruckmaier RM. 2013. Short communication: fractional milking distribution of immunoglobulin G and other constituents in colostrum. J Dairy Sci. 96:5919–5922.

Waldner CL, Rosengren LB. 2009. Factors associated with serum immunoglobulin levels in beef calves from Alberta and Saskatchewan and association between passive transfer and health outcomes. Can Vet J. 50:275–281.

Weaver DM, Tyler JW, VanMetre DC, Hostetler DE, Barrington GM. 2000. Passive transfer of colostral immunoglobulins in calves. J Vet Inter Med. 14:569–577.