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Evaluation of laboratory and on-farm tests to estimate colostrum quality for dairy cows

M. Röder,[1] S. Borchardt,[1] W. Heuwieser,[1]* E. Rauch,[2] R. Sargent,[3] and F. Sutter[1]
[1]Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Königsweg 65, 14163 Berlin, Germany
[2]Unit of Animal Welfare, Behavioral Science, Animal Hygiene and Husbandry, University of Veterinary Medicine, Veterinärstr 13/R, 80539 Munich, Germany
[3]Saskatoon Colostrum Company Ltd., Saskatoon, SK, S7K 6A2, Canada

ABSTRACT

The objectives of this study were to evaluate different analytical methods to determine colostrum quality in dairy cattle, including one laboratory-based method (ELISA) and 4 on-farm tests. We hypothesized that the colostral IgG concentration using different analytical methods, such as ELISA (mg/mL), digital Brix refractometer (% Brix), colostrometer (specific gravity and mg/mL), an outflow funnel (seconds), and a lateral flow assay (mg/mL), were highly correlated with the reference method, radial immunodiffusion (RID; mg/mL) and would generate comparable results. Colostrum samples were collected from 209 Holstein Friesian cows on 2 commercial dairy farms in Germany. Colostrum weight and colostrum temperature were measured. Test characteristics, such as optimum thresholds, sensitivity, specificity, and area under the curve (AUC) were determined using a receiver operating characteristic curve analyses for each test. Out of 209 colostrum samples assessed by RID, 186 (89%) samples had high quality (≥50 mg IgG/mL), while 23 colostrum samples (11%) showed poor quality with IgG concentrations less than 50 mg/mL. The mean IgG concentration (±SD) was 101.3 ± 45.9 mg/mL and the range was 6.0 to 244.3 mg/mL. The Pearson correlation coefficient (r) between RID and ELISA was r = 0.78. In comparison to RID, Pearson correlation coefficients for the on-farm tests were: r = 0.79 (digital Brix refractometry), r = 0.58 (colostrometer: specific gravity), r = 0.61 (colostrometer: temperature corrected), r = 0.26 (outflow funnel) and r = 0.43 (lateral flow assay), respectively. The optimal threshold to identify high-quality colostrum using ELISA was 50.8 mg/mL with sensitivity 91.3%, specificity 92.3%, and AUC of 0.94. For the on-farm tests sensitivity ranged from 95.7% (Brix refractometry) to 60.9% (lateral flow assay). Specificity ranged from 88.6% (lateral flow assay) to 75.9% (colostrometer: temperature corrected). The AUC ranged from 0.93 (Brix refractometry) to 0.73 (outflow funnel). Based on the AUC, ELISA (0.94) and Brix refractometry (0.93) can be considered highly accurate. In conclusion, the ELISA is accurate to assess colostrum quality. Regarding the on-farm tests only the digital Brix refractometer and the colostrometer were adequate to determine colostrum quality.

Key words: colostrum quality, on-farm test, colostrum management

INTRODUCTION

Due to the impermeability of the bovine placenta for maternal antibodies calves are born immunonaive (Weaver et al., 2000; Barrington and Parish, 2001). To acquire passive immunity it is essential for neonatal calves to ingest adequate volumes of high-quality colostrum during their first hours of life (Weaver et al., 2000; Baumrucker et al., 2010; Godden et al., 2019; Fischer-Tlustos et al., 2021). For the utmost transfer of passive immunity (**TPI**), colostrum should be ingested within the first 2 h postnatum. The absorption of maternal IgG from colostrum across the small intestinal epithelial cells is greatest in the first hours of life and progressively decreases after the first day of life (Weaver et al., 2000; Barrington and Parish, 2001). The concentration of antibodies in calf serum allows health monitoring of the calf population. Failed transfer of passive immunity (**FTPI**) was defined as serum IgG concentrations <10 mg/mL in calves 24 to 48 h old (Weaver et al., 2000; McGuirk and Collins, 2004; Godden et al., 2019) and is associated with an increased risk for mortality and morbidity. Lombard et al. (2020) recently proposed new standards including 4 serum IgG categories (excellent, good, fair, and poor) with serum IgG levels of ≥25.0, 18.0–24.9, 10.0–17.9, and <10 mg/mL, to reduce the risk of mortality and morbidity in dairy calves. Raboisson et al. (2016) detected greater hazard ratios for bovine respiratory disease (1.75), di-

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*Corresponding author: w.heuwieser@fu-berlin.de

arrhea (1.51), overall morbidity (1.91) and mortality (2.12) for calves suffering from FTPI. Therefore, it is essential to provide high-quality colostrum for the first feeding to ensure a sufficient maternal IgG supply. In bovine colostrum 85% to 90% of the total immunoglobulins are represented by IgG (Larson et al., 1980). The concentration of colostral IgG is considered the reference method to assess colostrum quality (Godden et al., 2019). Bovine colostrum of high-quality is defined as colostrum with an IgG concentration ≥50 mg/mL (McGuirk and Collins, 2004).

There are different on-farm tests commercially available to estimate the colostrum quality. Nevertheless, the measurement of colostrum quality with on-farm tests should be easy to perform and it has to be accurate (Bartier et al., 2015). The measurement of dissolved solids in colostrum determined by refractometry is a user-friendly way to indirectly assess the colostral IgG concentration (Bartier et al., 2015). Digital Brix refractometry uses the refraction of a light beam to assess how much dissolved solids are present in a fluid, detecting its relative density in % Brix. It has been shown that it is a reliable tool for determining colostral IgG concentration (Chigerwe et al., 2008; Bielmann et al., 2010; Morrill et al., 2012; Quigley et al., 2013). Correlation coefficients (**r**) to identify high-quality colostrum range from r = 0.64 to r = 0.87 comparing Brix refractometry and radial immunodiffusion (**RID**; Bielmann et al., 2010; Vandeputte et al., 2014; Bartier et al., 2015; Coleman et al., 2015). According to Bielmann et al. (2010) high-quality colostrum in Holstein Friesian cows can be identified using a threshold of ≥22% Brix.

The determination of specific gravity of colostrum with a colostrometer is a conventional method that has been established a long time ago (Fleenor and Stott, 1980). It measures the specific gravity and determines colostral IgG concentration indirectly. It has been shown to have good correlation with the IgG concentration assessed by RID (r = 0.77; Bartier et al., 2015) or measured by ELISA (r = 0.79; Lemberskiy-Kuzin et al., 2019). The specific gravity is influenced by the dissolved solids in colostrum. The greater the IgG concentration of colostrum, the higher its specific gravity and the greater the uplift of the colostrometer. In a less dense colostrum sample, the colostrometer sinks deeper. Colostrum of good quality shows a specific gravity ≥ 1,047 in Holstein Friesian cows (Fleenor and Stott, 1980). Though, this method has some limitations as the specific gravity depends, for instance, on breed of the dam (Morin et al., 2001) and the temperature of the colostrum (Mechor et al., 1991, 1992). Colostrum should be evaluated at 22°C to obtain reliable results (Conneely et al., 2013). Furthermore, the colostrometer is made of glass and therefore fragile.

Another indirect analytical method is the use of an outflow funnel. The outflow funnel measures the time in seconds a defined volume of the fluid flows through it. This type of measurement is based on the viscosity of a fluid and on the assumption that an increased viscosity is associated with greater concentrations of immunoglobulins in colostrum. Similar to the specific gravity, the viscosity depends on the temperature of colostrum. The outflow funnel requires a temperature of 30°C to perform the measurement.

A direct analytical method is the lateral flow assay which determines the IgG concentration (mg/mL) in colostrum based on an antigen-antibody reaction. Bovine IgG in colostrum reacts with anti-bovine IgG of the test strip. To perform the analysis, the test strip is immersed in a diluted colostrum sample. It is a semi-quantitative immunochromatographic test, which uses color-labeled antibodies that bind colostral IgG. The resulting antigen-antibody complexes migrate through the test strip until they encounter the reading area (antibodies for fixation of the antigen-antibody complexes). The fixation results in a visible color change due to the accumulation of the color-labeled antigen-antibody complexes. In addition, the test contains a control line to ensure that the sample migrated completely through the test strip. The concentration of IgG in the colostrum sample is proportional to the intensity of the test line. By taking a picture of the test line with the smartphone camera using the SmartStrips App the line intensity is interpreted and compared with a stored standard curve. The measurement range of the test is from between 2 and 120 mg/mL. Values lower than 2 mg/mL are displayed as <2 and values higher than 120 are displayed as >120 mg/mL.

A laboratory-based direct analytical method is the ELISA which is based on an antigen-antibody reaction. Immune complex enzyme reactions result in a color change that can be measured photometrically to determine the colostral IgG concentration.

Up to now, RID is still considered the reference method for measuring IgG concentration in colostrum (Ahmann et al., 2021). Disadvantages of RID are the limited test range, low reproducibility, and long incubation times. Furthermore, this method is time-consuming and expensive and therefore not feasible for calf health monitoring and management (Fleenor and Stott, 1980). Therefore, ELISA could be a useful alternative analytical method.

The objectives of this study were to evaluate 4 on-farm tests (digital Brix refractometer, colostrometer, outflow funnel, lateral flow assay) and an ELISA with the reference method to determine colostrum quality. We hypothesized that the colostral IgG concentration using ELISA, digital Brix refractometer, colostrometer,

outflow funnel, and lateral flow assay were highly correlated with the reference method RID.

MATERIALS AND METHODS

Colostrum was collected on 2 dairy farms in northern Germany from February 2020 to August 2020. Because all colostrum samples were obtained during routine farm management practices, the study was in accordance with the Institutional Animal Care and Use Committee of the Freie Universität Berlin. A sample size calculation was performed as described by Moinester and Gottfried (2014) for different half-widths (**w**) and Pearson correlation coefficients (r), expecting the desired 95% confidence interval (**CI**). For r = 0.80 and w = 0.05 a colostrum sample size requirement of n = 205 was needed.

A total of 213 colostrum samples were collected from clinically healthy primiparous (n = 86) and multiparous cows (n = 127). Colostrum samples were excluded if they were considered bloody or mastitic. The farms had a separate side-by-side milking parlor for 6 fresh cows near the calving area. Cows were milked 3 times daily at regular milking times (0430, 1230, and 1930 h) regardless of the individual calving time. Therefore, colostrum was tested mostly at the regular milking times. Otherwise, colostrum was stored in the milking bucket with a lid in the refrigerator at 8°C. Colostrum was milked into a separate milking bucket after the teats were predipped, forestripped, and dry wiped using a clean paper towel. Forestripping involved the manual removal of 2 streams of colostrum from each teat after thoroughly milking out the teat sealant. This manual stimulation lasted 30 s. The time interval between manual stimulation and attachment of the milk unit clusters was 60 s. The vacuum of the milking equipment (Flo-Star MAX, Boumatic Robotics GmbH, Kempten, Germany) was 45 kPa and the milk-to-rest ratio was at 60:40. After milking, the teats of the cows were dipped with iodine (Jod 5000, CID Lines N.V., Ieper, Belgium). Relevant information such as cow identification, parity, date, and time of parturition were obtained from the on-farm documentation.

Colostrum Sample Analysis

The assessment of colostrum quantity, temperature and quality was carried out in the milking parlor, immediately after milking until approximately 3 h after colostrum harvest. After each milking, the bucket with the colostrum was weighed with a digital hanging scale (LS 06 luggage scale, Beurer GmbH, Ulm, Germany) and the weight of the empty bucket subtracted. The temperature was measured by immersing a digital thermometer (digital probe thermometer 30.1018, TFA Dostmann GmbH & Co. KG, Wertheim-Reicholzheim, Germany) approximately 20 cm into the colostrum at the center of the bucket.

The quality of all colostrum samples was evaluated with 4 on-farm tests. Sampling was done directly from the milking bucket after mixing thoroughly the colostrum.

1) Digital Brix refractometer (Misco PA201, Misco, Solon, OH) with an automatic temperature calibration. Accurate measurements were possible between 0 and 50°C. Before each batch of samples, the device was calibrated with distilled water at room temperature (20°C). Two drops of colostrum were applied to the prism of the digital Brix refractometer using a disposable syringe (2 mL, Henry Schein, Langen, Germany) and the quality was measured in % Brix.
2) The specific gravity was assessed with a colostrometer (Colostrometer, Albert Kerbl GmbH, Buchbach, Germany) in a cylinder (diameter 3.5 cm, height 25 cm) filled with 500 mL colostrum. The device was immersed in the colostrum filled cylinder and the specific gravity determined by reading the scale. To control the influence of different colostrum temperatures on colostral IgG concentration, the equation according to Mechor et al. (1992) was used:

$$\text{IgG concentration (mg/mL)} = 853 \times (\text{specific gravity}) + 0.4 \times (\text{Celsius degrees}) - 866.$$

3) The viscosity of colostrum was assessed using an outflow funnel (ColostroCheck, Quidee GmbH, Homberg, Germany) with a volume of 100 mL. It was immersed into the milking bucket and raised. Thereafter, the time was stopped until the colostrum passed through the funnel. Colostrum with an outflow velocity of $\geq$24 s was classified as good quality colostrum according to the specifications of the manufacturer.
4) A lateral flow assay (SmartStrips IgG Colostrum, Bio-X Diagnostics, Rochefort, Belgium) was carried out according to the manufacturer's instructions. To obtain a first dilution, 20 μL of colostrum was transferred into the first dilution vial by using the pipette from the test kit. The vial was closed and swiveled. From this first vial, another 20 μL of the dilution was pipetted into a second vial in the same manner, using a new pipette. The test strip was placed into the second vial for 10 min and the test line

photographed with a smartphone by using the SmartStrips App.

Aliquots of colostrum were collected and transferred into sterile vials (Cryovial 2 mL, Simport, Bernard-Pilon, Canada). For further analysis, one aliquot each was shipped on dry ice to the Veterinary Science Department, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich for ELISA analysis and to The Saskatoon Colostrum Co. Ltd. (**SCCL**; Saskatoon, SK, Canada) for IgG analysis via RID.

At the Department of Veterinary Science in Munich the colostral IgG testing was performed via sandwich ELISA according to Erhard et al. (1999). With PBS-Tween (20%), the colostrum samples were diluted in a ratio of 1:50,000. The assessment of the IgG concentration via ELISA was performed as described in Sutter et al. (2019). The ELISA was based on coating and conjugating the IgG with anti-bovine IgG coupled to a peroxidase enzyme. The catalyzed color change was measured photometrically. The mean value of the IgG concentration in each well of one column resulted in the final colostrum concentration (mg IgG/mL). Colostrum containing ≥50 mg/mL IgG was regarded as high-quality colostrum (McGuirk and Collins, 2004).

The assessment via RID (reference method) was performed in SCCL as described in Shivley et al. (2018). The RID was based on the measurement of antibodies and antigens by their precipitation, which involves diffusion through an in-house prepared 24-mL agarose plate, using commercially available ingredients and reagents. The diffusion denotes precipitation in gel. A plate reader (digital RID reader AD400, The Binding Site Inc., San Diego, CA) was used to measure the diameters of the precipitin rings surrounding the wells. A regression line was generated for each plate for the variable R (ring diameter) versus log 10 (concentration) by using the results (ring diameters) obtained for each of the 2-fold dilutions and a spreadsheet (Excel, Microsoft Corp., Redmond, WA). By using the regression line of the bovine IgG standard obtained for each plate the IgG concentration for the test sample was determined. The diameters were entered into a template where IgG concentration (mg/mL) and the regression line was calculated.

Statistical Analysis

Pearson correlation coefficients were determined using distribution plots. The results of ELISA and the on-farm tests were plotted against the reference method obtained by RID. Correlation coefficients and Bland-Altman plots were generated using Excel (Office 2010, Microsoft Deutschland Ltd., Munich, Germany). Bland-Altman plots were used to quantify the agreement between 2 quantitative measurements by using statistical limits of agreement, which were calculated by using the mean and the standard deviation (**SD**) of the differences between these 2 measurements (Bland and Altman, 1999, 2003). The quantitative difference of the 2 measurements were plotted against the average of the 2 measurements (RID and ELISA). The limits of agreement were expressed as the mean difference ± 1.96 SD (Bland and Altman, 1999, 2003). By definition 95% of the data points lie within ± 1.96 SD of the mean difference.

For all test methods, the mean IgG concentration (means ± SD) and sensitivity (**Se**), specificity (**Sp**), positive predictive value (**PPV**) and negative predictive value (**NPV**) and area under the curve (**AUC**) were calculated using MedCalc software (version 15.6.1, MedCalc, Mariakerke, Belgium) with RID as reference method. Sensitivity was defined as the probability of a test result correctly indicating poor colostrum quality (i.e., IgG < 50 mg/mL). Specificity was defined as the probability of a test result correctly indicating good colostrum quality with IgG ≥50 mg/mL. The PPV was defined as a predictive probability of a test result correctly indicating poor colostrum (IgG < 50 mg/mL). The NPV was defined as a predictive probability of a test result correctly indicating good colostrum quality (IgG ≥ 50 mg/mL).

By plotting the true positive rate against the false positive rate, a receiver operating characteristic (**ROC**) curve was generated, and the optimal thresholds were assessed. The optimal threshold was defined as the point on the curve with the highest combined sensitivity and specificity and its deduction was based on the AUC according to Swets (1988) as perfect (AUC = 1), highly accurate (0.9 < AUC <1), very accurate (0.7 < AUC <0.9), accurate (0.5 < AUC < 0.7), and as noninformative (AUC = 0.5). Accuracy describes the variance of a measurement from its true value while precision refers to the dispersion of the measurements (Ranstam, 2008). A significant statistical difference was defined for variables when $P < 0.05$; a statistical tendency was specified as differences between $P \geq 0.05$ and $P \leq 0.10$. The ROC curve analyses using RID as the reference method generated the test characteristics (optimal thresholds, Se, Sp, PPV, NPV, and AUC) for the on-farm tests and ELISA to identify high-quality colostrum (≥50 mg/mL).

RESULTS

Four samples could not be analyzed by RID due to high viscosity (out of 213). Due to missing data some colostrum samples were excluded from the analysis with

Table 1. Descriptive statistics of 4 on-farm and 2 laboratory tests to determine colostrum quality

Test	Unit	n	Mean ± SD	Minimum	Maximum
Brix refractometry[1]	% Brix	213	24.3 ± 4.7	11.9	38.0
Colostrometer[2]	Specific gravity	200	1,054.6 ± 12.9	1,025.0	1,077.0
Colostrometer	mg/mL	200	49.7 ± 11.2	23.2	70.0
Outflow funnel[3]	s	211	32.1 ± 36.9	15.6	240.8
Lateral flow assay[4]	mg/mL	212	96.4 ± 33.5	20.0	120.0
ELISA	mg/mL	205	78.9 ± 29.2	3.5	179.5
Radial immunodiffusion	mg/mL	209	101.3 ± 45.9	6.0	244.3

[1]Misco PA201 Brix refractometer.
[2]Colostrometer (Albert Kerbl GmbH).
[3]ColostroCheck (QUIDEE GmbH).
[4]SmartStrips IgG Colostrum (Bio-X Diagnostics).

the lateral flow assay (n = 1), the outflow funnel (n = 2), ELISA (n = 5), and colostrometer (n = 13). For the final analyses, 209 colostrum samples were considered.

Descriptive Statistics

The RID analysis identified 186 (89%) high-quality colostrum samples (≥50 mg IgG/mL) and 23 poor-quality colostrum samples (<50 mg IgG/mL). Furthermore, 50.2% contained ≥100 mg IgG/mL, respectively. The mean IgG concentration (± SD) was 101.3 ± 45.9 mg/mL, and the range was 6.0 to 244.3 mg IgG/mL (Table 1). The mean temperature of colostrum (± SD) was 27.3°C ± 9.4 with a range from 2.6 to 38.2°C. The mean weight of colostrum (± SD) was 6.4 kg ± 3.8 with a range from 0.7 to 25.3 kg. The descriptive statistics for all tests are listed in Table 1.

Correlation Coefficients and Bland-Altman Plots

The Pearson correlation coefficients for the on-farm test compared with the reference method (RID) were between r = 0.71 (Brix refractometer) and r = 0.26 (outflow funnel; Figure 1A–E). The ELISA showed a moderate correlation to RID (r = 0.78; $P < 0.01$; n = 204; Figure 1F).

On average, the IgG concentration measured by ELISA was 21.6 mg/mL lower compared with RID (Figure 2). The limits of agreement were −37.9 mg/mL and 81.1 mg/mL (±1.96 SD; 95% CI).

Test Characteristics

Test characteristics to identify high-quality colostrum determined by the different on-farm tests, as well as by the laboratory method ELISA are summarized in Table 2. The analysis for ELISA identified an optimal threshold at 50.8 mg/mL with sensitivity 91.3%, specificity 92.3%, and AUC of 0.94. Sensitivity ranged from 95.7% (Brix refratometry) to 60.9% (lateral flow assay). Specificity ranged from 88.6% (lateral flow assay) to 75.9% (colostrometer after temperature correction). The AUC ranged from 0.93 (Brix refractometry) to 0.73 (outflow funnel).

DISCUSSION

As on-farm test, the digital Brix refractometer and colostrometer were adequate to determine colostrum quality but not the lateral flow assay and the outflow funnel. As laboratory method ELISA was accurate to assess colostrum quality compared with RID.

Colostrum Quality

In the present study, the mean and range of IgG concentration measured with RID was slightly higher than in previous studies (Kehoe et al., 2007; Chigerwe et al., 2008; Bielmann et al., 2010; Shivley et al., 2018), but in agreement with Morrill et al. (2012). For a comparison of IgG concentration across studies, it is important to consider the analytical details of the implementation of RID (Kehoe et al., 2011; Rivero et al., 2012) and ELISA (Baumrucker et al., 2010; Nowak et al., 2012) as removal of fat or heat treatment. The RID analysis detected 89% of the samples as high-quality colostrum, whereas 11% of the samples contained less than 50 mg IgG/mL. Chigerwe et al. (2008) and Bartier et al. (2015) reported 32% and 29.1% of poor-quality colostrum samples, respectively. One possible explanation is that the current study was conducted on 2 commercial farms. A wider range of environmental conditions and farm management practices is likely to be represented by a larger number of farms. Factors affecting colostrum quality can be divided into animal-related and environmental-related factors. It is known that dietary practices and trace mineral supplementation are associated with colostrum quality (Godden et al., 2019; Kincaid and Socha, 2004), as well as colostrum harvesting times more than 2 h after parturition negatively

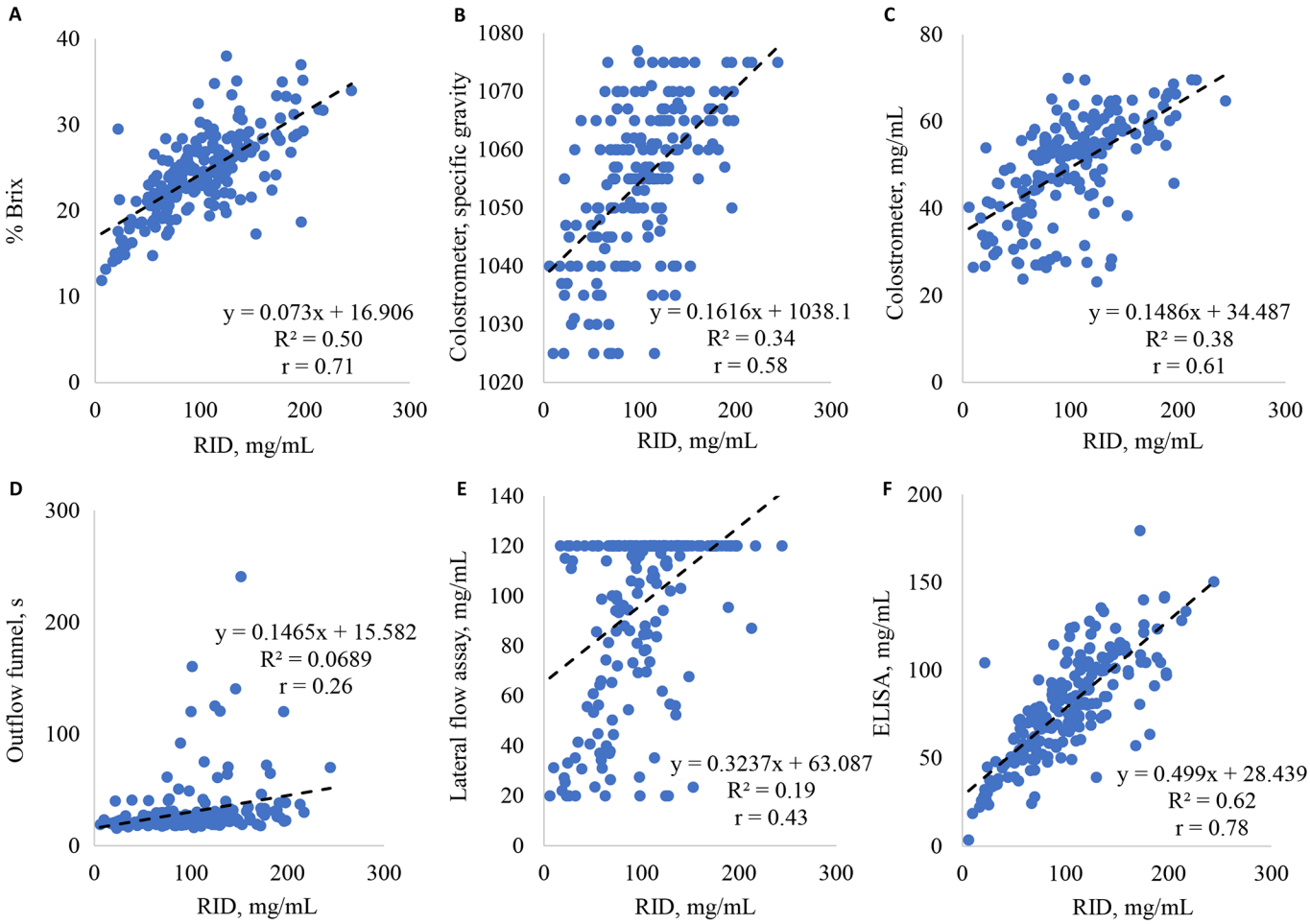

Figure 1. Comparison of 4 on-farm tests and 1 laboratory test to determine colostrum quality using the colostral IgG concentration (mg/mL) determined by radial immunodiffusion (RID) as the reference method. (A) Colostral IgG concentration assessed by RID compared with % Brix (n = 209; $R^2 = 0.50$; r = 0.71); (B) colostral IgG concentration assessed by RID compared with specific gravity using the colostrometer (n = 196; $R^2 = 0.34$; r = 0.58); (C) colostral IgG concentration assessed by RID compared with the IgG concentration (mg/mL) measured by the colostrometer after correction analysis (Mechor et al., 1992; n = 196; $R^2 = 0.38$; r = 0.61); (D) colostral IgG concentration assessed by RID compared with the outflow velocity using the outflow funnel (n = 207; $R^2 = 0.07$; r = 0.26); (E) colostral IgG concentration assessed by RID compared with the IgG concentration (mg/mL) measured by the lateral flow assay (n = 208; $R^2 = 0.19$; r = 0.43); (F) colostral IgG concentration assessed by RID compared with the IgG concentration (mg/mL) determined by ELISA (n = 204; $R^2 = 0.62$; r = 0.78).

affect the IgG concentration in colostrum (Moore et al., 2005; Chigerwe et al., 2008). Higher colostrum yield can have a dilution effect on colostrum as well as dry period length (Cabral et al., 2016). According to Conneely et al., (2013) a genetic standard deviation for IgG concentration is given (16.0 g/L) as well as a positive influence of parity on colostrum quality.

Correlation Coefficients and Bland-Altman Plot

The results of the established methods including Brix refractometer (r = 0.71; $P < 0.01$) and colostrometer (specific gravity: r = 0.58, $P < 0.01$; after temperature correction: r = 0.61, $P < 0.01$), showed similar results to previously published studies. Correlation coefficients between Brix refractometry and RID ranged from 0.64 to 0.87 (Vandeputte et al., 2014; Bartier et al., 2015; Coleman et al., 2015; Morrill et al., 2015). Considering the correlation coefficients of the colostrometer and RID the published range of previous studies was 0.53 to 0.84 (Fleenor and Stott, 1980; Morin et al., 2001; Bartier et al., 2015; Løkke et al., 2016). This wide range of reported correlation coefficients might be caused due to variation of non-IgG protein content in the colostrum (Elsohaby et al., 2017). Morin et al. (2001) detected that specific gravity had higher correlation with colostral total protein (r = 0.76) than with IgG_1 (r = 0.53). Also, Fleenor and Stott (1980) reported a correlation of r = 0.84 ($R^2 = 0.699$) between specific gravity and the entire γ-globulin content. Furthermore, breed- and

Table 2. Test characteristics for 4 on-farm tests and one laboratory method for determining high-quality colostrum using radial immunodiffusion (RID) as the reference method (≥50 mg/mL)

Test method	n	Threshold[1]	AUC[2] (95% CI)	SE	*P*-value	Sensitivity	Specificity	PPV[2] (95% CI)	NPV[2] (95% CI)
Brix refractometry,[3] % Brix	209	21.3	0.93 (0.89–0.96)	0.04	<0.001	95.7	82.8	40.7 (27.4–55.1)	99.4 (96.5–100.0)
Colostrometer,[4] specific gravity	196	1,047	0.83 (0.77–0.88)	0.04	<0.001	81.8	78.2	32.1 (20.2–46.1)	97.1 (92.8–99.2)
Colostrometer, IgG, mg/mL	196	46.0	0.84 (0.78–0.88)	0.03	<0.001	86.4	75.9	31.1 (19.9–44.3)	97.8 (93.6–99.5)
Outflow funnel,[5] s	207	20.8	0.73 (0.67–0.79)	0.06	<0.001	65.2	76.1	25.4 (14.9–38.6)	94.6 (89.6–97.6)
Lateral flow assay,[6] mg/mL	208	55.7	0.74 (0.67–0.79)	0.06	<0.001	60.9	88.6	40.0 (23.6–58.2)	94.8 (90.4–97.6)
ELISA, mg/mL	204	50.8	0.94 (0.90–0.97)	0.03	<0.001	91.3	92.3	60.0 (42.1–76.1)	98.8 (95.8–99.9)

[1]Optimal threshold was determined by receiver operating characteristic curve analysis using the threshold with the highest sum of sensitivity and specificity to identify colostrum of good quality.
[2]AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value.
[3]Misco PA201 Brix refractometer.
[4]Colostrometer (Albert Kerbl GmbH).
[5]ColostroCheck (QUIDEE GmbH).
[6]SmartStrips IgG Colostrum (Bio-X Diagnostics).

Figure 2. Bland-Altman plot comparing the difference between total IgG concentration measured by radial immunodiffusion (RID; mg/mL) and ELISA (mg/mL; n = 204). On average, the IgG concentration measured by ELISA was 21.6 mg/mL lower compared with RID. The limits of agreement were 81.1 mg/mL and −37.9 mg/mL (±1.96 SD; 95% CI).

species-specific colostrum compositions affect the dissolved solids in colostrum (Kessler et al., 2021). Løkke et al. (2016) detected that fat content had a significant negative effect on specific gravity and a positive effect on % Brix results.

The outflow funnel and the lateral flow test showed poor correlations to the reference method RID. The Pearson correlation coefficient of the outflow funnel compared with RID was r = 0.26 ($P < 0.01$) clearly indicating no correlation between viscosity and IgG concentration. There is only limited research regarding the association between viscosity and IgG concentration of colostrum. Hallberg et al. (1995) and Maunsell et al. (1999) approached this topic in their study. Nevertheless, the viscosity determination was carried out by visual assessment and therefore it was rather subjective. Maunsell et al. (1999) concluded that there was no correlation between viscosity and IgG content, whereas Hassan et al. (2020) detected that the viscosity of colostrum from cows suffering from mastitis was lower than from healthy cows. Hassan et al. (2020) compared visually assessed colostral viscosity and dynamic colostral viscosity using a viscometer of 40 Holstein dairy cattle to colostral IgG concentration measured by colostrometer and % Brix refractometry. The correlation between the viscometer and colostrometer was moderate (r = 0.58; $P < 0.01$). To the best of our knowledge the outflow funnel has not been validated yet.

The lateral flow assay used in our study correlated weak with the reference method RID (r = 0.43; $P < 0.01$). In addition to the main components represented by immunoglobulins colostrum contains dif-

ferent components with immune enhancing properties which can influence the quality of colostrum as well as the test results (Puppel et al., 2019). Furthermore, the test performance may be susceptible to variations due to the individual performance steps (first and second dilution). On-farm tests that directly measure the IgG concentration in colostrum have only been evaluated in one study using an IgG assay measuring the optical density (Drikic et al., 2018). The correlation with RID for dairy and beef colostrum were r = 0.72 and r = 0.73, respectively. The weak correlation of the current lateral flow assay for colostrum contrasts a previous study in which the lateral flow assay for serum of the same manufacturer was evaluated in beef and dairy calves (Delhez et al., 2021). The correlation between lateral flow assay for serum and ELISA was r = 0.86 ($P < 0.01$), which indicates that it is an appropriate on-farm test for the assessment of TPI in calf serum. The results for the lateral flow assay obtained with colostrum do not provide accurate results in comparison to the reference method. To the best of our knowledge the lateral flow assay for colostrum has not been validated yet.

The highest correlation in the present study was assessed with the laboratory method ELISA (r = 0.78; $P < 0.01$) which is in accordance with Dunn et al. (2018), who observed a strong correlation (r = 0.91; $R^2 = 0.83$; $P < 0.01$). However, Gelsinger et al. (2015) reported a weaker correlation (r = 0.60; $R^2 = 0.36$; $P = 0.01$). The discrepancy is probably caused by different test kits both for ELISA and RID used. The Bland-Altman plot showed lower IgG concentrations measured by ELISA (21.6 mg/mL) compared with RID. The limits of agreement were wide (−37.9 to 81.1 mg/mL) because of a high mean difference and SD in both methods. The discrepancy between RID and ELISA increased with IgG concentration. Rising concentrations can cause increased variability of the measured values (Grouven et al., 2007). These results underline the findings of Gelsinger et al. (2015) and Dunn et al. (2018) that the additional validation of specific assay kits is needed to determine thresholds appropriate for application to ELISA, so that ELISA and RID values can be accurately compared. Further research is warranted to validate the established threshold with relevant clinical outcomes such as disease incidence rates and mortality.

Test Characteristics

The laboratory method ELISA, and the on-farm test digital Brix refractometer were highly accurate (0.9 < AUC <1) using the AUC as an indicator of overall test characteristics by Swets (1988). The 95% CI did overlap for the ELISA and the Brix refractometer and high Se and Sp was detected (ELISA: Se = 91.3%, Sp = 92.3%; Brix refractometer: Se = 95.7%, Sp = 82.8%). The colostrometer (specific gravity and temperature corrected) was very accurate, though, 95% CI of the AUC did not overlap with the CI of ELISA and Brix refractometry, indicating that test accuracy of the colostrometer was slightly less accurate than the test accuracy of ELISA and Brix refractometry. The 95% CI did not overlap for the outflow funnel and the lateral flow assay as well. Further, Se and Sp were moderate for the outflow funnel (Se = 65.2%, Sp = 76.1%) and the lateral flow assay Se = 60.9%, Sp = 88.6%, lateral flow assay), indicating that these tests cannot distinguish between high- and poor-quality colostrum without considerable numbers of false negatives and positives.

Based on our data the threshold for the digital Brix refractometer to identify high-quality colostrum is 21.3% Brix. This is consistent with previous studies in which varied between 20 and 23% Brix (Chigerwe et al., 2008; Bielmann et al., 2010; Quigley et al., 2013). Implementing an optimal threshold ensures that high- and poor-quality colostrum is correctly identified and not discarded or fed to calves, respectively.

The optimal threshold to identify high-quality colostrum with ELISA was 50.8 mg/mL. However, a laboratory specific threshold should be established based on relevant outcomes.

Specific gravity without and with temperature correction barely varied [specific gravity: Se = 81.8, Sp = 78.2, and AUC = 0.83; IgG (mg/mL): Se = 86.4, Sp = 75.9, and AUC = 0.84, mg IgG/mL]. The optimal threshold for specific gravity and IgG after temperature correction were 1,047 and 46.0 mg/mL, respectively. These findings are similar to previous research (Fleenor and Stott, 1980; Pritchett et al., 1994). More recent publications recommended higher thresholds (1,050 to 1,055) for specific gravity (Bartens et al., 2016; Løkke et al., 2016) and 60 to 90 mg/mL for IgG concentration (Chigerwe et al., 2008; Bartier et al., 2015). By increasing the thresholds, the risk of identifying poor colostrum (<50 mg/mL) falsely as good is decreased which ensures that more calves will be fed with colostrum of good quality (≥50 mg/mL). Alternatively, some high-quality colostrum would be falsely classified as not acceptable.

CONCLUSIONS

The laboratory method ELISA and the on-farm test digital Brix refractometer and colostrometer were suitable to assess colostrum quality. The predictive value of the colostrometer for colostral IgG concentration was lower than digital Brix refractometer and ELISA. Different threshold values must be considered. The

outflow funnel and the lateral flow assay cannot be recommended as on-farm tests to determine colostrum quality.

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