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Technical note: Capillary electrophoresis as a rapid test for the quantification of immunoglobulin G in serum of newborn lambs

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ABSTRACT

Finding a rapid and simple method of serum IgG determination in lambs is essential for monitoring failure of passive transfer of immunity. The aim of this study was to assess the ability of capillary electrophoresis (CE), an instrument mainly used in blood serum protein analysis, to estimate IgG content in serum of newborn lambs through determination of only total Ig percentage by comparing the results with those obtained with radial immunodiffusion (RID), the reference method for serum IgG quantification. Serum samples were collected at 24 h after birth from 40 Sarda lambs. The IgG concentration measured by RID and serum total Ig concentration measured by CE were (mean ± standard deviation) 29.8 ± 16.1 g/L and 37.8 ± 15.0%, respectively. Data provided by RID and CE analysis showed a polynomial trend (RID = $0.02CE^2 - 0.04CE + 4.13$; coefficient of determination, $R^2 = 0.96$), displaying a strong relationship between these 2 methods. Applying the polynomial equation, the IgG values were predicted. Predicted IgG values were highly correlated (r = 0.98) and related ($R^2 = 0.96$) to IgG values obtained by RID assay. These data were subjected to Bland–Altman analysis, revealing a high level of agreement between CE and RID methods with a bias that was not different from 0 (−0.04 g/L) and agreement limits of −6.38 g/L (low) and +6.30 g/L (high). In addition, the linear regression analysis between differences (dependent variable) and average of IgG concentration by CE and RID (independent variable) did not show proportional bias ($R^2 = 0.01$). In conclusion, CE is a reliable instrument for a lamb health monitoring program, where Bland–Altman analysis also confirmed that the CE method can be a suitable alternative to RID.

Key words: lamb, immunoglobulin, capillary electrophoresis, method comparison

Technical Note

Lambs are born without passive immunity. Thus, the transfer of an adequate quantity of IgG from colostrum is crucial for their survival and well-being (McGuirk and Collins, 2004). Failure of passive transfer of immunity (**FPTI**) occurs when lambs do not acquire an adequate blood IgG concentration within 24 to 36 h of life (Castro-Alonso et al., 2008). The occurrence of FPTI is closely associated with a reduced growth rate, increased morbidity, and mortality (Stelwagen et al., 2009). A herd health prevention protocol should be established for the rapid assessment and monitoring of the adequacy of passive immunity transfer in lambs. Thus, finding a fast and reliable method for estimating IgG content in serum could offer great benefits to sheep farmers.

Radial immunodiffusion (**RID**) is the gold standard method for serum IgG quantification, but it is time consuming (18–24 h to determine the results), laborious, expensive, and has limited use for routine analysis of IgG concentration. Other assays were developed as alternatives to RID for the determination of serum Ig concentration, including ELISA (Alves et al., 2015; Hogan et al., 2015; Zakian et al., 2018) and capillary electrophoresis (**CE**; Regeniter and Siede, 2018). Capillary electrophoresis offers many advantages, such as high separation efficiency, short run time, simple instrumentation, minimum operation cost, and compatibility with small sample volumes. Furthermore, CE is a well-established technique that is routinely used in human and animal clinical laboratories for screening abnormal serum protein profiles (Regeniter and Siede, 2018).

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Serum proteins are separated into 6 main fractions, with each zone containing 1 or more proteins: gamma globulins, β-2 globulins, β-1 globulins, α-2 globulins, α-1 globulins, and albumin (Jellum et al., 1997). The most common application of CE is the detection of the monoclonal component (i.e., Ig) present in the gamma globulin fraction (Henskens et al., 1998; Miyazaki and Suzuki, 2016). Moreover, CE can be used for colostrum quality assessment (Lopreiato et al., 2017) and milk adulteration for species substitution determination (Trimboli et al., 2017, 2019). These features make CE an attractive tool that is useful in livestock, particularly in sheep breeding, and monitoring FPTI in lambs. In lamb serum, most Ig are IgG, the cut-off value of which for FPTI diagnosis is still being debated; results of observational studies indicate serum concentrations of IgG <0.8 or <15 g/L (Hunter et al., 1977; Sawyer et al., 1977). Capillary electrophoresis provides Ig concentration in grams per liter, but it requires measuring serum total protein concentration with other methods. The resulting increase in time and cost of analysis is the main limitation for large-scale application of CE in FPTI screening. During the first 24 h of life, the relative composition of the main serum protein fractions of lambs does not change markedly except for the Ig fraction, which tends to increase with time due to colostrum ingestion (Nagyová et al., 2017). This represents a physiological mechanism of lambs (and newborn ruminants in general) after the acquisition of passive immunity. Monitoring the total Ig percentage (**Ig%**) in serum using the CE method could be a fast and reliable approach to detecting the rate of passive immunity acquisition in a timely manner. Hence, the objective of this study was to evaluate the use of Ig% determined by CE to estimate IgG content in serum of newborn lambs and to compare the results with RID assay as a reference.

All procedures of this study followed the common good clinical practices (EMA, 2000; VICH GL9: Guidelines on Good Clinical Practice EMA/CVMP/VICH463202/2009) and received approval from the Ethical Animal Care and Use Committee of the Magna Græcia University of Catanzaro (protocol no. 458/09-01-2019). Blood samples were collected at 24 h after birth from 40 Sarda lambs between April and July 2019 on a commercial dairy farm located in the Calabria region of Italy. Blood samples were collected via jugular venipuncture using a 21-gauge, 1-inch hypodermic needle into sterile plastic blood collection tubes (Vacuette, Greiner Bio-One GmbH, Kremsmunster, Austria) containing a clotting activator. Samples were transported in a cooler to the veterinary laboratory at the University of Magna Græcia (Catanzaro, Italy). The serum was separated by centrifugation at 1,700 × *g* for 10 min at room temperature within 6 h of collection and was stored at −20°C until use.

Capillary electrophoresis was performed using a Minicap CE system (Sebia, Lisses, France) that automatically performs a whole analysis process. All lamb serum samples and a serum control were analyzed in a single analytical session. Serum proteins were analyzed using a Capillarys Protein(E)6 kit (Sebia) according to the manufacturer's instructions. Capillary electrophoresis was equipped with two 17-cm (16 cm to detection point) × 25 μm i.d. coated fused-silica capillaries. Capillaries were prepared with sample buffer (150 m*M* borate solution; pH 9.9 ± 0.1) and washed after each analysis with wash solution (containing an alkaline solution at pH ~12). The instrument requires a dead volume of 300 μL of serum; the sample is diluted 1:10 with sample buffer, and the volume injected is 1 nL. The injection was carried out at the anode using a pressure (hydrodynamic injection) of 2,000 Pa for 1 s. Electrophoresis was operated under positive polarity at 7.8 kV. The total time of migration was 215 s, and electropherograms were recorded with a delay of 88 s. The software program (Phoresis Core All-in-One, Sebia) recorded in real time the variation of absorbance at 200 nm and produced typical electrophoretic peaks. After manual selection of the total Ig fraction area on the electrophoretogram, the instrument software automatically provided the percentage of total serum Ig as follows:

$$\text{total Ig\%} = (\text{total Ig fraction area}/\text{total curve area}) \times 100.$$

A commercial RID assay (Sheep and Goat IgG ID-Ring test, IDBiotech, ImmunoDiffusion Biotechnologies SARL, Issoire, France) was used as the reference method for determining IgG serum concentrations (g/L). Serum samples were thawed at room temperature (20–24°C) and then vortexed for 10 s to ensure good homogeneity. Subsequently, the RID assay was performed according to the manufacturer's instructions using 15 μL of 1:300 diluted 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 20 h of incubation at 37°C using an IDRing Viewer system (IDBiotech, ImmunoDiffusion Biotechnologies SARL).

Statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC). Results obtained using RID (IgG, g/L) and CE (Ig, %) methods were tested for normality using the Kolmogorov–Smirnov normality test and were used to calculate descriptive statistics (mean, standard deviation, median, mini-

Table 1. Descriptive statistics for CE (capillary electrophoresis) and RID (radial immunodiffusion) assays

Item	Mean	SD	Median	Minimum	Maximum
Serum Ig by CE, %	37.8	15.0	41.5	3.2	57.8
Serum IgG by RID, g/L	29.8	16.1	26.5	1.4	60.2

mum, and maximum values). A second-order polynomial regression analysis with total percentage of Ig concentration measured by CE (Ig), as predictor, and IgG concentration measured by RID (IgG), as outcome of interest (RID = $aCE^2 - bCE + c$), was performed, where a and b are the coefficients of equation and c is the y-intercept. Moreover, a Pearson correlation coefficient was carried out to measure the correlation between the proposed method (CE) and the gold standard method (RID). Statistical significance was declared at $P < 0.05$. The polynomial regression equation was applied to predict IgG values from total percentage of Ig concentration measured by CE. The predicted IgG values were first tested to evaluate the correlation of CE against the RID method by Pearson correlation and linear regression, and then they were used to estimate the agreement and the bias between the CE and RID methods by Bland–Altman analysis (Bland and Altman, 2003). Furthermore, the regression analysis was performed between the differences in measurements (CE − RID) and the average of IgG using the CE and RID methods (Lopreiato et al., 2017).

To evaluate the use of the Ig% parameter to estimate serum IgG levels in lambs, it was necessary to perform CE analysis on serum samples covering the range of values that this parameter can assume. Serum IgG concentrations measured by RID fell in a range of values between 1.4 and 60.2 g/L (IgG mean value: 29.8 ± 16.1 g/L; Table 1), covering a broad interval of values suitable for the purpose of this study. Serum Ig% concentrations obtained by CE ranged from 3.2 to 57.8% (Ig% mean value: 37.8 ± 15.0%; Table 1). The relationship between serum IgG concentration measured by RID and serum Ig% concentration measured by CE showed a polynomial trend (RID = $0.02CE^2 - 0.04CE + 4.13$); Ig% was highly related with IgG concentration measured by RID, explaining 96% ($R^2 = 0.96$; $P < 0.05$) of the variation (Figure 1). To evaluate the agreement and bias between the CE and RID methods, IgG values were predicted from Ig% measured by CE, applying a polynomial equation; it was verified that they were highly correlated (r = 0.98; $P < 0.05$) and related to IgG by RID (RID = 1.00CE + 0.01; $R^2 = 0.96$; $P < 0.05$; Figure 2). These values were used in Bland–Altman analysis, showing good agreement with a bias not different from zero (−0.04 g/L; $P > 0.05$), an upper agreement limit of 6.30 g/L, and a lower agreement limit of −6.38 g/L (Figure 3). In addition, the regression analysis between differences of predicted IgG values and IgG measured by RID (dependent variables)

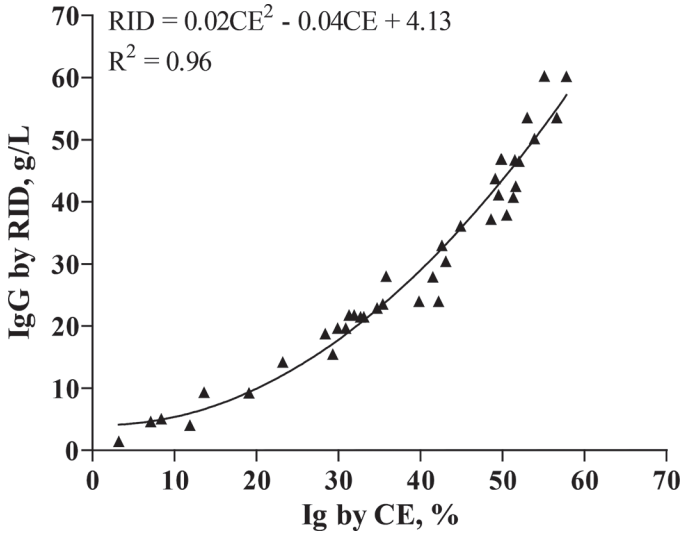

Figure 1. Relationship between Ig (%) measured by capillary electrophoresis (CE) and IgG (g/L) measured by radial immunodiffusion (RID) in serum of 24-h-old lambs.

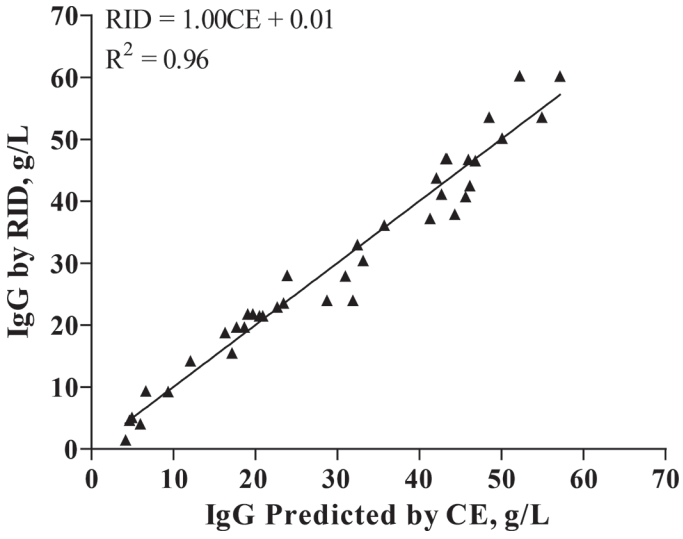

Figure 2. Relationship between values of IgG predicted by applying polynomial equation measured by capillary electrophoresis (CE) and IgG values measured by radial immunodiffusion (RID).

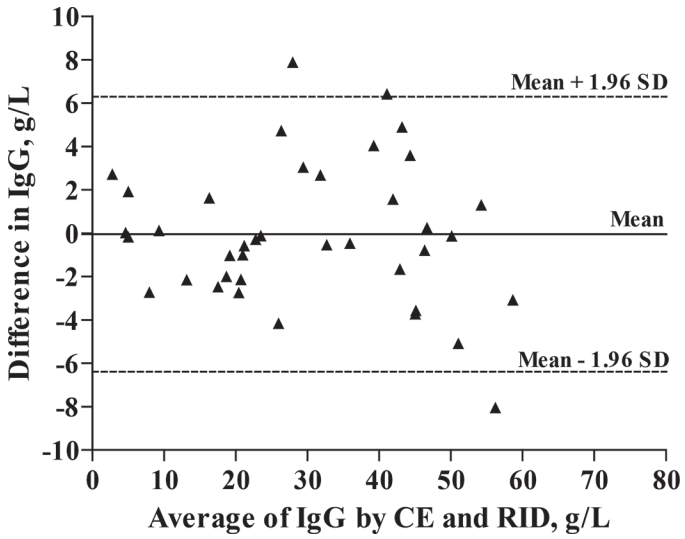

Figure 3. The difference between capillary electrophoresis (CE) and radial immunodiffusion (RID) concentration (g/L) plotted against the average of CE and RID values. The difference was calculated as CE − RID. The solid line represents the mean of differences (mean = −0.04 g/L), and the dashed lines represent the limits of agreement (upper agreement limit = 6.30 g/L; lower agreement limit = −6.38 g/L).

and their mean (independent variables) confirmed the absence of proportional bias ($y = -0.02x + 0.60$; $R^2 = 0.01$; $P = 0.52$; Figure 4).

The relationship between IgG, measured by RID, and Ig%, measured by CE, showed a polynomial trend

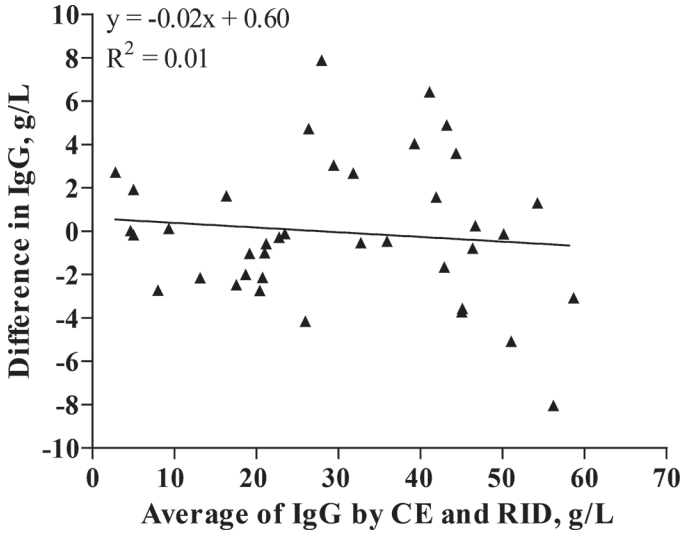

Figure 4. Relationship between the difference and average of IgG concentration measured by the capillary electrophoresis (CE) and radial immunodiffusion (RID) methods. The difference was calculated as CE − RID. The solid line represents the linear regression curve ($y = -0.02x + 0.60$; $R^2 = 0.01$; $P = 0.52$).

differing from results reported in previous studies (Elsohaby et al., 2015; Renaud et al., 2018). Renaud et al. (2018) reported that IgG concentration determined by RID was linearly related to serum total protein concentration determined by a new semiquantitative antibody. Furthermore, Elsohaby et al. (2015) found a linear relationship between serum IgG determined by RID and percentage Brix determined by a digital refractometer (% of TS) and serum total protein (g/dL) determined by an optical refractometer. The polynomial relationship reported herein can be explained by the nature of serum Ig% determined by CE. The Ig% is calculated as the ratio of total Ig fraction area and total curve; therefore, it is a relative measure influenced by variation in other serum proteins that may occur during the first hours after birth. Nevertheless, in newborn lambs the Ig fraction is the main protein fraction and is subjected to strong variation after colostrum ingestion (Nagyová et al., 2017). These features make Ig% an appropriate marker of adequate acquisition of passive immunity. Moreover, the Bland–Altman analysis showed good agreement between serum-predicted IgG values and IgG using the RID method, confirming that CE is suitable for a reliable estimate of IgG in lamb serum. Compared with RID assay, CE has a shorter turnaround time, commonly defined as the time from when a test is ordered until the result is reported (Hawkins, 2007); the entire procedure from serum preparation to CE analysis requires approximately 20 min compared with almost 24 h with RID assay. This could be one of the most important considerations in the use of CE in serum IgG estimation, particularly because sheep farmers can successfully monitor the passive immunity acquisition after colostrum ingestion. However, CE is a laboratory method, but it is fully automated and does not require intensive training of technicians.

The results of this study highlight a close relationship between the CE method and RID assay, endorsing CE as a reliable method for determining IgG content in serum of lambs at a very early stage after birth. This allows farmers and veterinarians to directly and quickly optimize interventions on the farm if FPTI occurs. Further studies will be carried out to establish the optimal cut-off value of total Ig percentage that can discriminate between lambs with and without FPTI.

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REFERENCES

Alves, A. C. C., N. G. G. Alves, I. J. J. Ascari, F. B. B. Junqueira, A. S. S. Coutinho, R. R. R. Lima, J. R. O. R. O. Pérez, S. O. O. De Paula, I. F. F. Furusho-Garcia, and L. R. R. Abreu. 2015. Colostrum composition of Santa Inês sheep and passive transfer of immunity to lambs. J. Dairy Sci. 98:3706–3716. https://doi.org/10.3168/jds.2014-7992.

Bland, J. M., and D. G. Altman. 2003. Applying the right statistics: Analyses of measurement studies. Ultrasound Obstet. Gynecol. 22:85–93. https://doi.org/10.1002/uog.122.

Castro-Alonso, A., N. Castro, J. Capote, A. Morales-delaNuez, I. Moreno-Indias, D. Sánchez-Macias, P. Herraez, and A. Argüello. 2008. Short communication: Apoptosis regulates passive immune transfer in newborn kids. J. Dairy Sci. 91:2086–2088. https://doi.org/10.3168/jds.2007-0814.

Elsohaby, I., J. T. McClure, and G. P. Keefe. 2015. Evaluation of digital and optical refractometers for assessing failure of transfer of passive immunity in dairy calves. J. Vet. Intern. Med. 29:721–726. https://doi.org/10.1111/jvim.12560.

EMA. 2000. VICH GL9: Guidelines on Good Clinical Practice CVMP/VICH/595/98-FINAL. www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004343.pdf.

Hawkins, R. C. 2007. Laboratory turnaround time. Clin. Biochem. Rev. 28:179–194.

Henskens, Y., J. de Winter, M. Pekelharing, and G. Ponjee. 1998. Detection and identification of monoclonal gammopathies by capillary electrophoresis. Clin. Chem. 44:1184–1190. https://doi.org/10.1093/clinchem/44.6.1184.

Hogan, I., M. Doherty, J. Fagan, E. Kennedy, M. Conneely, P. Brady, C. Ryan, and I. Lorenz. 2015. Comparison of rapid laboratory tests for failure of passive transfer in the bovine. Ir. Vet. J. 68:18. https://doi.org/10.1186/s13620-015-0047-0.

Hunter, A. G., J. K. Reneau, and J. B. Williams. 1977. Factors affecting IgG concentration in day-old lambs. J. Anim. Sci. 45:1146–1151. https://doi.org/10.2527/jas1977.4551146x.

Jellum, E., H. Dollekamp, A. Brunsvig, and R. Gislefoss. 1997. Diagnostic applications of chromatography and capillary electrophoresis. J. Chromatogr. B Biomed. Sci. Appl. 689:155–164. https://doi.org/10.1016/S0378-4347(96)00333-7.

Lopreiato, V., C. Ceniti, F. Trimboli, E. Fratto, M. Marotta, D. Britti, and V. M. Morittu. 2017. Evaluation of the capillary electrophoresis method for measurement of immunoglobulin concentration in ewe colostrum. J. Dairy Sci. 100:6465–6469. https://doi.org/10.3168/jds.2016-12284.

McGuirk, S. M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. Vet. Clin. North Am. Food Anim. Pract. 20:593–603. https://doi.org/10.1016/j.cvfa.2004.06.005.

Miyazaki, K., and K. Suzuki. 2016. Capillary electrophoresis/immunosubtraction as a better alternative to immunofixation for detecting and immunotyping serum monoclonal proteins in patients with immunoglobulin light chain (AL) amyloidosis. Amyloid 23:221–224. https://doi.org/10.1080/13506129.2016.1232647.

Nagyová, V., C. Tóthová, and O. Nagy. 2017. The impact of colostrum intake on the serum protein electrophoretic pattern in newborn ruminants. J. Appl. Anim. Res. 45:498–504. https://doi.org/10.1080/09712119.2016.1218886.

Regeniter, A., and W. H. Siede. 2018. Peaks and tails: Evaluation of irregularities in capillary serum protein electrophoresis. Clin. Biochem. 51:48–55. https://doi.org/10.1016/j.clinbiochem.2017.09.017.

Renaud, D. L., T. F. Duffield, S. J. LeBlanc, and D. F. Kelton. 2018. Short communication: Validation of methods for practically evaluating failed passive transfer of immunity in calves arriving at a veal facility. J. Dairy Sci. 101:9516–9520. https://doi.org/10.3168/jds.2018-14723.

Sawyer, M., C. H. Willadsen, B. I. Osburn, and T. C. McGuire. 1977. Passive transfer of colostral immunoglobulins from ewe to lamb and its influence on neonatal lamb mortality. J. Am. Vet. Med. Assoc. 171:1255–1259.

Stelwagen, K., E. Carpenter, B. Haigh, A. Hodgkinson, and T. T. Wheeler. 2009. Immune components of bovine colostrum and milk. J. Anim. Sci. 87(Suppl. 13):3–9. https://doi.org/10.2527/jas.2008-1377.

Trimboli, F., N. Costanzo, V. Lopreiato, C. Ceniti, V. M. Morittu, A. Spina, and D. Britti. 2019. Detection of buffalo milk adulteration with cow milk by capillary electrophoresis analysis. J. Dairy Sci. 102:5962–5970. https://doi.org/10.3168/jds.2018-16194.

Trimboli, F., V. M. Morittu, C. Cicino, C. Palmieri, and D. Britti. 2017. Rapid capillary electrophoresis approach for the quantification of ewe milk adulteration with cow milk. J. Chromatogr. A 1519:131–136. https://doi.org/10.1016/j.chroma.2017.08.075.

Zakian, A., M. Nouri, A. Rasooli, M. Ghorbanpour, P. D. Constable, and M. Mohammad-Sadegh. 2018. Evaluation of 5 methods for diagnosing failure of passive transfer in 160 Holstein calves. Vet. Clin. Pathol. 47:275–283. https://doi.org/10.1111/vcp.12603.

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