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Quantification of Immunoglobulin G in Bovine and Caprine Milk Using a Surface Plasmon Resonance-Based Immunosensor

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Detection of colostrum in bovine and caprine milks is essential for dairy industries to avoid negative economical and technological consequences. One of the best markers of the presence of colostrum is immunoglobulin G (IgG). Two quantification methods have been evaluated for IgG in bovine or caprine milk, based on the real-time immunodetection of IgG by surface plasmon resonance (SPR) spectroscopy. Calibration curves were established by extracting affinity data from the sensorgrams, either using the residual bound IgG level after the association and dissociation phases or using the IgG binding rate during the association phase. The binding rate method allows for substantially reduced analysis times of below 4 min, which make it compatible with the milking time of small ruminants. Moreover, the binding rate method showed a better analytical performance, with lower detection limit and higher precision and accuracy than the residual binding method.

KEYWORDS: Optical biosensor; immunodetection; milk; immunoglobulin G; surface plasmon resonance; binding rate

INTRODUCTION

The formation of colostrum occurs during the first 5−7 days after calving in cows and goats. Its composition differs significantly from lactation milk by the presence at high level of biologically active components such as the immunoglobulin (Ig) antibodies, which ensure protection against infections to the newborn (*1*−*4*). According to legislation in many countries, the milk intended for human consumption may not contain colostrum. The reason of this limitation lies in the economical, technological, and sensorial consequences of colostrum addition on the quality of dairy products and their preparation (*5*−*7*). The presence of colostrum reduces the functional properties of milk such as heat stability and impairs fermentation processes. The increase of the amount of soluble proteins by colostrum addition leads to off-flavors in bovine pasteurized milk and to higher cleaning frequency of the hot surfaces in the dairy process lines (*6*−*9*). Nevertheless, the addition of colostrum to milk to increase its soluble protein content is one of the most common frauds encountered with milk.

The detection of colostrum in milk requires the selection of a marker. The presence of colostrum leads to a high level of soluble proteins, in particular of immunoglobulin G (IgG), which represent > 50% of the total amount of proteins. The ratio between the IgG concentration in colostrum and bovine milk can reach from 100 to 150 (*1*, *10*). Similarly, in caprine milk, this ratio is around 70 between the 1st milking and the 14th milking (*7*, *11*). The lower variations of the other soluble proteins make IgG the best marker of colostrum presence in milk (*12*). Furthermore, IgG quantification can also be useful to detect other milk abnormalities that make it unsuitable for human consumption. For example, previous studies revealed that the IgG level in cow's milk increases and can double during mastitis caused by major pathogen infections (*13*, *14*).

To date, legislation about milk selling is based on the number of days after calving. As an example, in France, milk cannot be sold within 7 days postpartum, but there is no unified worldwide regulation for this delay (reviewed in ref *1*). Using the IgG content as a criterion to authorize or not the commercialization of milk would be a great improvement. Furthemore, this would be particularly helpful for detecting frauds. The maximum IgG content cannot be calculated from the number of days after calving. The IgG concentrations in milk and colostrum were found to vary considerably, depending not only on the race of the animal, the time of year (*15*−*18*) but also on the methodology applied for quantification (*19*). IgG_1 concentrations in the bovine and caprine first-milking colostrums were reported to vary from 18 to 92 mg/mL and from 19.9 to 94.5 mg/mL, respectively (*11*, *20*, *21*). The base level of IgG in bovine milk was found to be around 0.5 mg/mL (*14*, *22*, *23*). Some studies on caprine milk also report high variations of the IgG base level, which is typically below 1 mg/mL after 7 days of lactation postpartum (*7*, *11*, *24*, *25*). Although disturbance of downstream processes was the main reason for forbidding colostrum in milk, the IgG threshold needs to be defined with respect to the impact of the IgG concentration on the functional properties of milk. This is highly dependent on the composition of the added colostrum. The addition of 5% of first-milking colostrum, corresponding to an IgG concentration of 3.4 mg/mL, according to Levieux et al., was shown to affect the properties of bovine milk (*1*, *6*, *16*).

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Conversely, 10% of colostrum from the fifth or sixth day postpartum (~0.9 mg/mL) had no influence (*11*, *16*, *26*). Few studies have been published regarding goat's milk. When the effects of the addition of bovine and caprine colostrums at different times after calving were compared, the disturbance of the functional properties was stronger for goat's milk (*6*). Clearly, more correlating studies are required to precisely determine an IgG concentration threshold.

The dairy industries are asking for an IgG quantification method with as short as possible analysis times, usable for the systematic control of large numbers of samples. Several quantification techniques of IgG in milk or in colostrum have been already developed. The majority of these are based on immunoassays such as nephelometric immunoassay (NIA) (*12*, *27*, *28*), particle-enhanced nephelometric assay (*29*), enzyme-linked immunosorbent assay (ELISA) (*30*, *31*), or radial immunodiffusion assay (RID) (*22*, *32*). ELISA and RID methods require incubation times, which make them time-consuming (24 h for ELISA and 24−48 h for the RID) and as a consequence are hardly used in systematic screening. The nephelometric technique allows quantification of IgG in a concentration range of 0.3−5 mg of IgG_1/mL with an analysis time of 30 min. However, before this assay can be performed, the samples need to be clarified and filtered. The sample preparation step limits the use of this method for routine assays (*12*, *27*).

In this study, we used surface plasmon resonance (SPR) for the real-time and label-free immunodetection of IgG. The SPR biosensor immunoassay is known to be a fast and powerful technique for the quantitative analysis of components in complex food matrices (*33*, *34*) and more precisely of proteins in milk (*35*−*39*). Here, the immunodetection is based on the specific binding of bovine or caprine IgG to anti-bovine or anti-caprine IgG immobilized onto carboxymethylated dextran surfaces. Usually SPR assays are end point methods, based on the measurement of the residual bound IgG value at the end of the dissociation phase. Indyk and Filonzi proposed an IgG quantification based on this measurement principle that required 8 min per milk sample (*36*). We evaluated the possibility of reducing the analysis time by recording the calibration curves through the measurement of the binding rate in the first seconds of the IgG/anti-IgG association (*40*). The detection limit, the precision, and the accuracy of the former and latter SPR methods were evaluated and compared. The assay used to obtain reference values for the IgG concentrations was radial immunodiffusion, which is considered to be the industry standard (*5*).

MATERIALS AND METHODS

Reagents. Amine coupling 1-ethyl-3-(3-dimethylaminopropyl) carbodiimine−HCl (EDC, 0.4 M), *N*-hydroxysuccinimide (NHS, 0.1 M), ethanolamine−HCl (1 M, pH 8.5), sodium acetate buffer (10 mM, pH 4,5), glycine−HCl (10 mM, pH 1.5), Sensor Chip CM5, and HBS-EP running buffer (10 mM Hepes, 150 mM NaCl, 3.4 mM EDTA, 0.005% surfactant P20, pH 7.4) were all obtained from Biacore (GE Healthcare, Aulnay-sous-bois, France).

Antibodies. Rabbit anti-bovine IgG (whole molecule; B5645) and rabbit anti-caprine IgG (whole molecule; G4018) were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Immunoglobulin G. Commercially available bovine IgG (IG 01-2) and caprine IgG (IG 03-2) were from P.A.R.I.S. (Compiègne, France). IgG standard solutions (25−10000 ng/mL) were prepared daily in HBS-EP or in HBS-EP containing NaCl at a final concentration of 0.5 M.

Milk Samples. Raw bovine milk was collected from Prim Holsteins in early and middle lactation. Raw caprine milk was collected from Alpines in early and middle lactation. Milk samples were stored at −18 °C. Milk samples were diluted to 1/1000 prior to SPR analyses.

Biosensor Measurements. *Apparatus*. The Biacore X optical biosensor was from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Instrument operations and data processing were performed with Biacore X control software 4 and Biaevaluation software 4.1, respectively.

Antibody Immobilization on the Sensor Surface. Rabbit anti-bovine IgG or rabbit anti-caprine IgG was immobilized covalently on CM5 sensor chips by amine coupling at 25 °C. Briefly, the carboxymethyl dextran matrix was activated by injection of a solution of EDC and NHS (1:1 v/v) (10 μL/min, 10 min). Then, 100 μL of a solution of anti-bovine or anti-caprine IgG (50 μg/mL in 10 mM sodium acetate, pH 5.0) was injected at 10 μL/min. Finally, unreacted NHS-ester groups were deactivated with ethanolamine (1 M, pH 8.5, 10 μL/min, 10 min). Final immobilization levels were determined in resonance units (RU, where 1 RU = 1 pg/mm^2) from the sensorgram. Following immobilization and between analyses, the chip was stored at 4 °C in a desiccator. The ligand-free CM5 chip, used as the control surface, was prepared with the same immobilization procedure, without the antibody solution injection step.

SPR-Biosensor Assays. The instrument system was equilibrated with the HBS-EP running buffer at 25 °C. For generation of the calibration curves and for determination of the IgG concentrations in milk samples, the reference IgG solutions and the milk samples diluted in HBS-EP, NaCl 0.5 M, were injected for 120 s onto the antibody-modified sensor surface at a flow rate of 25 μL/min at 25 °C. Residual binding responses were acquired 20 s after the end of the IgG or milk sample injection and were measured relative to the baseline. Binding rates were collected over the period of 5−30 s after the start of the injection. The surface was finally regenerated by injection of 50 μL of 10 mM glycine−HCl, pH 1.5, at a flow rate of 50 μL/min.

Radial Immunodiffusion Assays. A commercial kit (RL 200.3) (The Binding Site, Saint-Egrève, France) was used to determine the total IgG concentration in bovine milk and to verify the concentration of commercially available bovine IgG standards. IgG concentrations in goat's milk and goat's IgG standard concentration were determined using a semiautomated single-radial immunodiffusion technique as described by Levieux (*22*). Caprine or bovine IgG measurements were performed in triplicate.

Statistics. The method accuracy was estimated using the radial immunodiffusion assay as the reference method. The degree of accuracy was represented by the normalized accuracy ratio (A_N). The assay results were considered to be significantly accurate if $A_N \leq 2$ (*41*).

$$A_N = \left| \frac{x_i - x_{ref}}{\sqrt{u_i^2 + u_{ref}^2}} \right|$$

x_i is the mean concentration (mean of three measurements) determined by the method used, x_{ref} is the mean concentration (mean of three measurements) obtained by the reference method, and u_i and u_{ref} are the standard deviations related to x_i and u_{ref}, respectively.

RESULTS AND DISCUSSION

Immobilization of the IgG Antibodies. Rabbit polyclonal anti-bovine IgG and anti-caprine IgG, both purified by affinity chromatography, were successfully immobilized by amine coupling onto a CM5 surface. **Figure 1** illustrates a rabbit anti-bovine IgG immobilization onto a carboxylmethyl dextran matrix. The immobilization levels yielded ca. 14500 ± 1100 RU for the anti-bovine IgG and 24750 ± 750 RU for the anti-caprine IgG. These values correspond to high ligand density: 14.5 ± 1 and 24.7 ± 0.7 ng/mm^2 for anti-bovine and anti-caprine IgG, respectively, according to the calibration made by Stenberg with radioactive monoclonal antibodies (*42*). The levels of antibodies immobilized onto the CM5 surface correspond to the values obtained by Indyk and Filonzi, that is, 12000 RU for rabbit anti-bovine IgG (*36*).

Calibration Curves. Calibration curves were generated for bovine IgG and also for caprine IgG. Dilutions of standard bovine or caprine IgG solutions were injected onto the corresponding sensor surfaces with immobilized anti-bovine IgG or

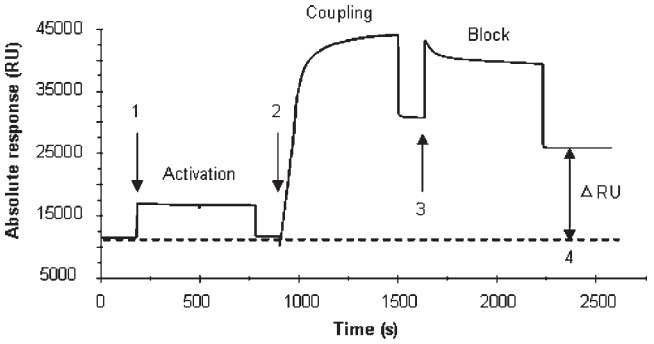

Figure 1. Immobilization of rabbit anti-bovine IgG on CM5 sensor surface at 10 μL/min. Surface activation was performed with 100 μL of EDC/NHS mixture (arrow 1), the antibody coupling by injection of 100 μL of antibody at 25 μg/mL in 10 mM sodium acetate, pH 5 (arrow 2), and the blocking with 100 μL of ethanolamine 1 M (arrow 3). The ligand immobilization level is indicated by the difference to an initial baseline (ΔRU).

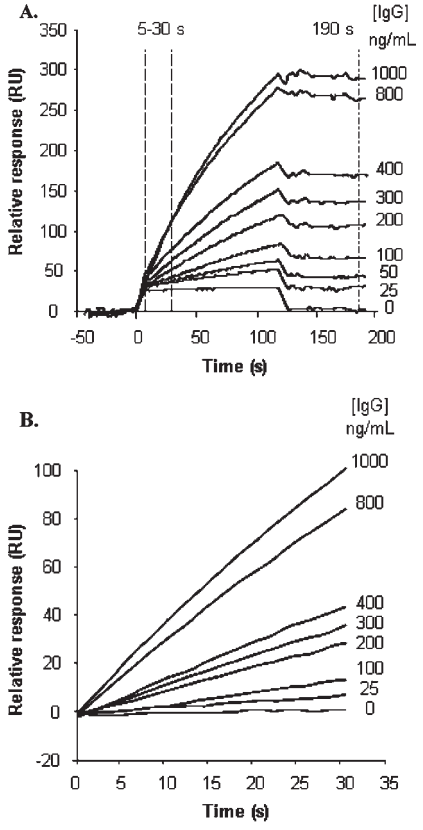

Figure 2. (**A**) Overlay sensorgrams illustrating the binding of bovine IgG upon injection of 50 μL of bovine IgG calibration solutions (0−1000 ng/mL) over anti-bovine IgG immobilized surface at flow rate of 25 μL/min. The two vertical lines identify the region of the association curve that was used for calculation of binding rates. The dashed line shows the time when the residual response was measured. (**B**) Zoom of the initial association curve parts until 30 s after injection.

anti-caprine IgG antibodies, respectively. **Figure 2** illustrates the SPR response due to the adsorption of bovine IgG onto the immobilized anti-IgG surface. After each injection cycle, a regeneration step was performed. A regeneration cycle takes 60 s. Four calibration curves were established by extracting two different affinity data from the sensorgram of bovine and caprine IgG.

Method 1. The calibration curves of the residual binding response against caprine and bovine IgG are shown in **Figure 3**.

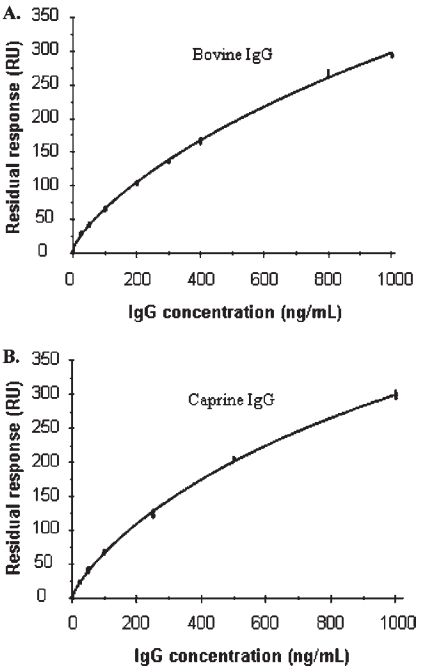

Figure 3. Calibration curve of residual SPR response versus bovine IgG concentration (**A**) and caprine IgG concentration (**B**). IgG in HBS-EP NaCl 0.5 M was injected at a flow rate of 25 μL/min for 120 s. For each concentration, the three data points of the reproduced measurements were plotted.

The value of residual binding was measured precisely 20 s after the end of the injection of the IgG samples. All measurements were reproduced three times with a relative standard deviation (rSD) of 2.2% for the bovine IgG calibration curve and of 2.5% for the caprine IgG calibration curve. The measurements were made with IgG concentrations varying from 0 to 1000 ng/mL. Above 1000 ng/mL, saturation of the binding sites was reached, limiting the dynamic range of the method. This led to a strong decrease in the analytical sensitivity (slope of the curve). The ranges of the residual binding response (0−300 RU) are similar for both bovine IgG and caprine IgG. The detection limits were determined from the mean measurements of representative blank samples (n = 8; mean + 3 × standard deviation) and were established at 4.1 ng/mL for bovine IgG and at 6.3 ng/mL for caprine IgG. The time required for a complete analysis cycle including regeneration and injection loop flushing was 5 min and 40 s.

Method 2. The second method was proposed by Karlsson et al. and was applied here to reduce the analysis time (*40*). This method is based on the use of the diffusion-limited part of the binding curve for the analyte concentration determination. Binding rates were measured between 5 and 30 s after the start of the sample injection. Values collected during the beginning of the injection were excluded from the binding rate calculations to avoid sample dispersion effects (*40*). The calibration curves obtained by plotting the binding rates versus bovine and caprine standard IgG concentrations are shown in **Figure 4**. The calibration curve covered the IgG concentration range from 0 to 1000 ng/mL with measured binding rates from 0 to 3.4 RU/s for bovine IgG and from 0 to 5.7 RU/s for caprine IgG. The binding rates were proportional to the IgG concentration. In this case, they reflected the interaction kinetics and were below the mass transfer limiting value, determined by the limit up to which the binding rate is no more affected by the concentration and becomes constant. All measurements were reproduced three times with a relative standard deviation of 3.0% for the bovine IgG calibration curve

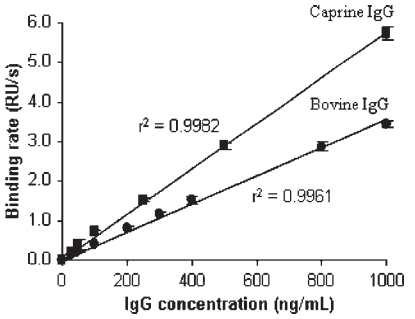

Figure 4. Calibration curves of binding rates versus bovine and caprine IgG concentration. Binding rates were obtained for IgG dilution injected onto immobilized anti-IgG at the flow rate of 25 μL/min, calculated from the data collected between 5 and 30 s after the start of the injection. Standard deviations were calculated from three independent measurements.

and of 3.1% for the caprine IgG calibration curve. For this method, the detection limit was established as 2.6 ng/mL for bovine IgG determination and as 3.1 ng/mL for caprine IgG. The required analysis time was shortened to 3 min and 55 s including the regeneration time, compared to the 5 min and 40 s for the method based on the residual binding measurement. This is a real improvement considering the large numbers of samples to be analyzed.

Baseline Stability. For both methods, the baseline was kept stable over 500 complete cycles of injection, followed by a regeneration. The stability of the binding capacity was evaluated by following the baseline value after series of 100 injection cycles of a bovine milk sample containing an IgG concentration of 500 μg/mL (diluted 1/1000 before injection). This procedure was then followed by regeneration steps. The standard deviation of the baseline value does not exceed 0.07% every 100 cycles.

Validation and Comparison of the Methods. The performance of the two methods was evaluated using different criteria.

Specificity. The first criterion was the method specificity, or the detection of a specific analyte in a complex matrix. In the course of this study, preliminary controls were performed to determine the analytical conditions that correspond to the absence of the milk component binding to the dextran support. The interaction of IgG standard solutions and an antibody-free sensor chip (control surface) were quantified. IgG residual binding levels and binding rates were negligible and did not exceed 2 RU and 0.1 RU/s for an IgG concentration of 10 μg/mL in HBS-EP NaCl 0.5 M. Binding responses of milk samples onto the control chip were also evaluated. The dilution of bovine or caprine milk samples and the HBS-EP buffer ionic strength were varied. The results are reported in **Table 1**. The residual bound IgG response and the binding rates reached negligible levels for an ionic strength of NaCl 0.5 M and a sample dilution up to 1/25 ($<$10 RU) and for starting dilutions of 1/100 and an ionic strength of NaCl 0.5 M ($<$0.1 RU/s), respectively. As expected, the increase of the buffer ionic strength prevented the electrostatic adsorption of milk components to the dextran. The concentration of sodium chloride was kept fixed at 0.5 M for establishing the calibration curves and for preparing the milk samples. Minimum sample dilutions of 1/100 were required for the method based on binding rate calculations.

The cross-reactivity of anti-bovine IgG and anti-caprine IgG with individual milk proteins was measured by injecting individual protein solutions onto antibodies coupled to CM5 chips. Solutions of α-casein (12.5 mg/mL), β-casein (9 mg/mL), κ-casein (3.2 mg/mL), α-lactalbumin (0.5 mg/mL), β-lactoglobulin (4 mg/mL), serum albumin (4 mg/mL), and lactoferrin (2 mg/mL) at concentrations corresponding to those expected in the milk were diluted at 1/100 in HBS-EP, NaCl 0.5 M, before injection. For all of the proteins, the responses measured 20 s after the end of the injection were below 10 RU and the binding rates below 0.1 RU/s. These results demonstrate the absence of nonspecific binding in the measured responses and, as a consequence, the IgG detection specificity in bovine and caprine milk diluted at or below 1/100 in HBS-EP, NaCl 0.5 M.

Table 1. Residual Binding and Binding Rates of Diluted Bovine and Caprine Milks at Different Ionic Strengths[a]

	0.15 M NaCl		0.5 M NaCl	
sample dilution	response (RU)	binding rate (RU/s)	response (RU)	binding rate (RU/s)
		Bovine Milk		
1/25	22.7 ± 0.6	0.42 ± 0.07	9.8 ± 0.3	0.38 ± 0.05
1/50	12.7 ± 1.0	0.39 ± 0.04	3.6 ± 2.8	0.29 ± 0.06
1/100	4.4 ± 1.2	0.10 ± 0.03	0.2 ± 0.6	0.06 ± 0.02
		Caprine Milk		
1/25	25.4 ± 1.0	0.45 ± 0.05	9.8 ± 1.3	0.37 ± 0.09
1/50	12.7 ± 0.3	0.43 ± 0.06	4.8 ± 1.1	0.30 ± 0.06
1/100	6.1 ± 0.6	0.16 ± 0.02	0.4 ± 1.0	0.07 ± 0.01

[a] Means and standard deviations were calculated from three independent measurements.

Precision. The second criterion for method validation was the SPR assay precision. It was determined considering intra-assay (within run) and interassay (between runs) variations. Intra-assay variations were determined by 12 successive analyses of the same sample on the same sensor surface. Two milk samples were tested each time, with two different IgG concentrations: 326 and 824 μg/mL for bovine milk and 328 and 997 μg/mL for caprine milk. In the end point assay, the intra-assay repeatability was 4.2% (n = 12) for the lowest IgG level in bovine raw milk and 8.8% (n = 12) for the highest IgG level. For the binding rate method, the intra-assay relative standard errors were 3.8% (n = 12) and 4.5% (n = 12), respectively. The intra-assay precisions for both IgG levels in goat's milk were 4.9% (n = 12) and 8.5% (n = 12), for the residual binding response method and 3.2% (n = 12) and 4.5% (n = 12) for the binding rate method, respectively. The interassay variation was evaluated by analyzing the sample in four different runs on three independent sensor surfaces. The IgG concentrations of the milk tested for the interassay repeatability were 500 μg/mL for bovine and caprine milks. The between-run assay precisions for the bovine or caprine raw milks were 6.1% (n = 12) and 7.9% (n = 12) for the end point response method and 4.7 and 5.0% for the binding rate assay, respectively. The intra- and inter-repeatabilities were as good as with the method reported by Indyk and Filonzi (*36*). The precision of the transient procedure was better than the one obtained with the steady state method, in relation with the graphic resolution. Starting from the same milk sample, its preparation and injection involve automatic introduction of errors, which can be responsible for a part of the RU value variability. This variability is enhanced by determining the IgG concentration from the steady state calibration curves compared to the binding rate curves. The low graphic resolution of the end point curves is due to the absence of linearity (**Figures 3** and **4**). The influence of the curve shape is confirmed by the strong increase of the intra-assay variation at high IgG concentrations for the end point method. This increase is related to the slope decrease. Conversely, the linear relationship between the IgG concentration and the binding rate allows the same standard deviation range to be maintained for all IgG concentrations.

Table 2. Comparison of IgG Content Determination in Cow's Milk by Radial Immunodiffusion, Residual Binding, and Binding Rate Methods[a]

	RID		SPR residual response			SPR binding rate		
days after calving	[IgG] (μg/mL)	rSD (%)	[IgG] (μg/mL)	rSD (%)	A_N	[IgG] (μg/mL)	rSD (%)	A_N
3	1055	4.0	1200	10.8	1.1	1025	2.4	0.6
7	711	5.6	617	7.1	1.6	694	6.2	0.3
7	824	6.2	891	5.9	0.9	811	2.1	0.2
7	757	1.8	828	6.3	1.3	770	3.5	0.4
10	600	5.5	578	5.4	0.5	590	4.3	0.3
10	638	4.7	656	3.6	0.5	634	2.2	0.1
10	642	7.0	603	6.6	0.6	640	4.4	0.1
20	308	8.1	282	3.9	1.0	314	3.5	0.2
20	326	2.8	309	4.2	1.1	314	3.5	0.8
20	509	1.8	502	4.4	0.3	510	2.3	0.1
20	535	2.4	550	8.4	0.3	526	5	0.2

[a] Milk samples were collected from different animals and at different times after calving. Relative standard deviations (rSD) were calculated from three independent measurements. Accuracy of both SPR assays was evaluated by using the normalized accuracy ratio (A_N) and RID assay as the reference.

Table 3. Comparison of IgG Content Determination in Goat's Milk by Radial Immunodiffusion, Residual Binding, and Binding Rate Methods[a]

	RID		SPR residual response			SPR binding rate		
days after calving	[IgG] (μg/mL)	rSD (%)	[IgG] (μg/mL)	rSD (%)	A_N	[IgG] (μg/mL)	rSD (%)	A_N
1	2000	1.0	2035	10.6	0.6	1995	1.5	0.1
2	1851	3.0	1760	10	0.5	1841	3.1	0.1
4	997	2.4	858	8.6	1.8	953	4.7	0.9
4	994	3.7	841	11.4	1.5	957	4.3	0.7
7	554	2.9	532	4.7	0.7	543	2.9	0.5
7	589	4.4	630	9.2	0.7	571	4.9	0.5
7	620	2.9	602	7.6	0.4	624	1.1	0.2
7	681	3.8	631	8.4	0.8	680	5	0
15	328	2.7	349	5.1	1.5	338	4.4	0.6
15	331	6.6	314	8	0.5	336	7.7	0.1
15	350	6.6	308	5.8	1.4	332	3.9	0.7
15	465	8.4	458	3.3	0.2	470	2.6	0.1

[a] Milks were provided from different animals and at different times after calving. Relative standard deviations (rSD) were calculated from three independent measurements. Accuracy of both SPR assays was evaluated by using the normalized accuracy ratio (A_N) and RID assay as the reference.

Accuracy. The last criterion used to evaluate the method's performance is the accuracy. To evaluate the accuracy of both methods, the SPR residual binding method and the SPR binding rate method, milk samples were collected at different milking times and from different animals and were analyzed. The accuracy was estimated by comparison of the analysis results with those obtained by the dairy reference method, the radial immunodiffusion technique (*5*). The accuracy was related to the normalized accuracy ratio (A_N), and the concentration result obtained was considered to be significantly accurate if $A_N \leq 2$. The results of the method comparisons are shown in **Table 2** for cow's milk samples and in **Table 3** for goat's milk samples. Both SPR assays present good accuracy, with A_N below 2. For the tested milk samples, the accuracy of the binding rate method is higher than the accuracy calculated for the residual binding method.

In conclusion, milk component quantification by label-free and real-time SPR immunodetection offers the advantages of stability, sensitivity, precision, and accuracy. Furthermore, these SPR assays provide fast analyses with automation possibilities, compared to the conventional techniques such as ELISA or RID. In this study, an analysis time improvement was obtained compared to the usual end point method that will be time-saving for the treatment of large numbers of samples. Calibration curves were established by calculating the IgG binding rate during the first 30 s of sample injection. Recently, Fonfria et al. used also successfully this approach for yessotoxin quantification by SPR (*43*). Furthermore, the method based on the binding rate allows an improvement of the limit of detection, the precision, and the accuracy compared to the end point method, in particular for high IgG concentrations. Furthermore, the total analysis time is below the threshold of 4 min, which is the milking time of small ruminants. This result is interesting for a possible integration of this assay in an online milking process. It is also conceivable to use this online detection for the monitoring of disease markers in milk, such as the IgG level for the detection of mastitis.

ABBREVIATIONS USED

A_N, normalized accuracy ratio; EDC, 1-ethyl-3-(3dimethylaminopropyl) carbodiimine; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; NHS, *N*-hydroxysuccinimide; RID, radial immunodiffusion; SD, standard deviation; SPR, surface plasmon resonance; rSD, relative standard deviation; RU, resonance unit.

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