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Development and validation of a nephelometric immunoassay for IgG_1 in milk

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Summary. A nephelometric immunoassay, with a detection range of 0·3 to 5 g IgG_1/l, was developed for the determination of immunoglobulin in bovine milk. The assay exhibited no significant cross-reactivity with α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein or β-lactoglobulin and 39 % cross-reactivity with IgG_2. The nephelometric assay was compared with ELISA and RID (24 h and 48 h incubations) assays using 105 duplicate milk samples covering IgG_1 values ranging from 0·45 to 1·8 g_1. The results obtained from all assays showed good agreement with the exception of those obtained by the RID assay (24 h incubation) which gave lower results in samples containing more than 1·2 g IgG_1/l. It was concluded that the nephelometric assay is a reliable, rapid and convenient method suitable for the quantification of IgG_1 in milk. The assay can be configured for routine high-throughput milk quality assurance for IgG_1 in dairy laboratories.

Keywords: Bovine, colostrum, immunoassay, nephelometric, RID

The detection of colostrum contamination is important for determining the quality of bovine milk. Milk contaminated with colostrum contains high concentrations of serum proteins making it unsuitable for the preparation of many high quality products (Auldist *et al*. 1993). Colostrum in milk causes 'burn on' on hot surfaces in dairy process lines such as heat exchangers and evaporators (Newstead, 1976) and increases manufacturing plant down-time as more frequent cleaning is required (Auldist *et al*. 1993). The functional properties and colour of many dairy products are affected by contamination with colostrum. These problems are most serious in countries such as New Zealand, Australia and Ireland, in which the majority of cows calve during a short period in spring. In New Zealand a penalty system (for milk suppliers) has been established to promote the withholding of colostrum from the milk supply (NZCPI, 1998). Since the introduction of this system there has been a marked improvement in the quality of early season milk and of the resultant dairy products. This has resulted in 'extremely significant' financial gains to dairy companies (Franks, 1988). Dairy industries in other countries practising non-seasonal milking would also gain from the introduction of colostrum penalties to ensure supply of high quality milk for processing.

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IgG_1 concentrations are higher in colostrum than in milk, and may exceed 30 g/l in the first day of lactation in both Jerseys and Friesians (Auldist *et al.* 1993). By the fifth day of milking, concentrations fall to 1–2 g/l with concentrations of < 1 g/l in succeeding days (Auldist *et al.* 1993). IgG_1 concentration therefore makes an ideal marker for the presence of colostrum in milk and may be used to define a penalty level for colostrum contamination.

IgG_1 measurement is not only used to determine milk quality, it is also employed to determine the quality of colostrum. Some farms supply raw colostrum to dairy factories where it is dried for use as a functional food ingredient in sports nutrition products and snack foods and as an ingredient in skin creams. The quality of the supply is determined by measuring the concentration of IgG_1 in the raw colostrum and a penalty is applied if this fails to meet a minimum standard.

IgG_1 concentrations in milk or colostrum are normally measured by radial immunodiffusion (RID) or enzyme linked immuno-sorbant assay (ELISA) (Li-Chan & Kummer, 1997). Both methods however are labour intensive and time consuming. Nephelometric immunoassays (NIA) provide an alternative method for quantifying IgG_1 in milk. This methodology combines speed, automation, assay robustness and precision (Price *et al.* 1983; Svendsen & Blirup-Jensen, 1991). A particle-enhanced nephelometric assay for the quantification of IgG_1 in milk has been developed by Montagne *et al.* (1991). This is a competitive immunoassay that uses agglutination by polyacrylic microsphere-bovine IgG conjugates with anti-bovine IgG antibodies to produce light scattering complexes. Formation of these complexes is inhibited by free IgG in solution. Large milk dilutions (up to 25000-fold) are required with this assay and it is not readily automated. Nephelometric immunoassays have also been used to quantify a range of other proteins in milk and dairy products (Humbert *et al.* 1991) and are suitable for characterising raw milk for the optimisation of dairy product manufacturing.

This paper describes the development of a nephelometric immunoassay for quantifying IgG_1 in milk. This assay is compared with conventional IgG_1 assays and the advantages and disadvantages of each are discussed.

Materials and methods

Milk samples

Milk samples ($n = 160$) were collected from farms and submitted to the SAITL Dairy Laboratory during August 1999. At this time of year, most cows in New Zealand are in early lactation. Whole milk samples were stored at 4 °C and analysed within 4 d of receipt. Alternatively, milk samples were defatted, diluted, and then frozen at –18 °C. Frozen samples were assayed within 1 month of freezing and immediately after thawing.

Nephelometric immunoassay for IgG_1

Antibody production. Antibodies against IgG_1 (Chemicon, No. AG130, Chemicon International Inc., Temecula, USA) were raised in hens and extracted from eggs using caprylic acid and ammonium sulphate as described by McLaren *et al.* (1994). The soluble antibody was stored at 4 °C in 0·4 g/l sodium azide.

Preparation of milk samples. Milk was defatted by centrifugation at 2200 ***g*** for 5 min at room temperature. A 10-μl aliquot of the defatted milk sample was diluted to 1000 μl with 0·05 M-phosphate buffer–0·15 M-NaCl–5 mM-EDTA–0·5 g Triton X-100/l–0·5 g sodium azide/l, pH 7·3 (PBSet) using a Gilson 402 dilutor dispenser (Gilson, Villers-le-Bel, France).

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Nephelometric immunoassay of bovine IgG_1. The NIA for IgG_1 was developed on a Behring Nephelometric Analyser from Behringwerke Diagnostica, Marburg, Germany. Assay parameters were; 100 μl sample, 60 μl antibody (10 mg/ml), and 100 μl reaction buffer (10% PEG 6000 in PBSet). The assay was performed at room temperature ($\sim$ 22 °C). After mixing of the reaction components, the intensity of scattered light was determined at 10 s and 6 min, the change in intensity between these times being used as the signal output. A 1·8 g IgG_1/l standard (ColTest IgG_1 standards, Immuno-Chemical Products Ltd (ICP Ltd), Auckland, New Zealand) was automatically diluted in PBSet by the nephelometer to give standards from 50 to 3·1 mg IgG_1/l. These standards were used to produce a five-point calibration curve, fitted using logit-log function analysis. The calibration curve had a detection range for undiluted milk from 5·0 to 0·31 g IgG_1/l. The same calibration curve was used to calculate the IgG_1 concentrations for all 160 milk samples. A quality control milk sample of known IgG_1 concentration (1·3 g/l) was run with each batch of 74 samples.

Limit of detection. The limit of detection (LOD) was assessed by assaying diluent samples (PBSet) 20 times. The LOD was determined from the standard curve using the mean plus three-times the standard deviation of the signal from the diluent samples.

Specificity. Assay specificity was determined by comparing the signal produced by purified α_{S1}-casein, α_{S2}-casein, κ-casein, β-casein, β-lactoglobulin or IgG_2 with the IgG_1 standard curve. Each protein was individually assayed at a concentration of 10 and 100 g/l for α_{S1}-casein, β-casein or κ-casein, 50 and 100 g/l for α_{S2}-casein, 25 and 100 g/l for β-lg, and 1 and 10 g/l for bovine IgG_2.

Assay recovery. Assay recovery was determined by supplementing a milk sample with bovine IgG_1 at concentrations of 0·31, 0·63, 1·25, 2·5 and 5 g/l. The samples were analysed and the percent recovery of added IgG_1 calculated.

Linearity. Linearity was assessed using a serial dilution of a freeze-dried sample of colostrum, which was collected within 24 h of calving. This was reconstituted to 160·9 g/l in PBSet and then diluted 1/650, 1/1300, 1/2600 and 1/5200 in PBSet. The dilutions were analysed by NIA and linearity of the dilution series determined.

Precision. Within-run, between-run and total precision were analysed using three milk samples containing low (0·45 g/l), medium (1·10 g/l) and high (1·75 g/l) values of IgG_1. Precision was calculated according to AOAC guidelines by one-way analysis of variance using data from five replicate analyses assayed on each of 5 d (Mishalanie, 1996). Precision was also determined for the RID and ELISA IgG_1 assays using the above method.

Changes in IgG_1 in mammary secretions of cattle following calving. Colostrum and milk samples were collected twice daily from eight Friesian × Jersey cattle, commencing at the afternoon milking immediately following calving and continuing for 10 d postpartum. Samples were held at 4 °C until processed, usually within 48 h of collection. Samples were defatted and diluted 1/100 in PBSet as previously described then stored frozen at −18 °C until analysed by NIA. Samples containing more than 5 g IgG_1/l were further diluted (1/5 or 1/10 in PBSet) before re-analysis.

RID for bovine IgG_1

The RID assay (Coltest™) was carried out according to the manufacturer's instructions (ICP Ltd). A positive displacement pipette was used to add 5 μl of standards containing 0·45, 0·9, 1·35 or 1·8 g IgG_1/l to four wells on a RID plate. Samples (5 μl) were then added to the remaining wells of the plate. The plates were placed on a level surface for either 24 or 48 h, after which the precipitin rings of the

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samples and standards were measured with a McSwiney ruler and the square of the zone diameter calculated. A standard curve was prepared by performing a linear regression of the zone size of the precipitin ring (mm^2) against the IgG_1 standard concentration. The concentration of IgG_1 in the samples was determined by interpolation from the standard curve.

ELISA for bovine IgG$_1$

ELISA (Rapid Coltest™) was performed according to the manufacturer's instructions (ICP Ltd). Solutions used with the ELISA were supplied by ICP Ltd. Samples and standards (0·45, 0·90, 1·35 and 1·80 g IgG_1/l) were diluted 20 000 × with diluent buffer in two stages using a Modumatic Auto-Diluter (Tri-Continent Scientific, Inc., Grass Valley, CA 95945 USA). Diluted samples and standard were transferred by adding 100 μl to appropriate wells on a Rapid Coltest™ ELISA plate, after which the plate was incubated for 60 min at room temperature in the dark. After washing with wash solution, 100 μl of enzyme conjugate was added to each well and the plate incubated as described above. After a further wash step, 100 μl of substrate was added and the plate incubated for a further 60 min at room temperature in the dark. Stop solution (100 μl) was added to each well and the optical density at 405 nm measured using a Dynatech MR7000 Microplate Reader (Dynatech Laboratories Inc., Middlesex, UK). A log-log or semi-log standard curve was prepared and sample concentrations determined from the standard curve.

Method comparison

Milk samples ($n = 160$) submitted to SAITL were used to compare the NIA, ELISA and RID (24 and 48 h incubation) assays. Of these samples, 86 were randomly selected while a further 59 were selected because they contained high levels of IgG_1 (> 1·35 g/l) as determined by ELISA and RID 24 h. The remaining 15 samples were selected because they contained moderate values of IgG_1 as determined by ELISA. Each sample was re-analysed in duplicate for IgG_1 content by ELISA, NIA and RID (24 and 48 h incubation) and duplicates compared using linear regression. Milk samples ($n = 105$) with average values within the RID measuring range (0·45 and 1·8 g IgG_1/l) as determined by RID (24 h incubation), were selected for method comparison using linear regression analysis. Difference plots (Hollis, 1996) were used to detect any systematic bias in the duplicate results from each assay method.

RESULTS

Optimisation of an assay for IgG_1 on a Behring Nephelometric Analyser was accomplished by varying antibody and reaction buffer volumes and concentrations to produce a Heidelberger and Kendall curve (Heidelberger & Kendall, 1935; Price *et al*. 1983). This curve displayed a signal varying from 31 to 775 bits giving a working range for IgG_1 from 0·3 to 5·0 g/l with a safety margin of 5–25 g/l (Fig. 1) before the assay output signal fell back into the output signal range of the assay standards.

Limit of detection. Using PBSet as a blank sample, the limit of detection of the assay was determined to be 0·13 g IgG_1/l.

Specificity. No signal was detected with α_{S1}-casein, β-casein or κ-casein for concentrations up to 100 g/l. α_{S2}-Casein at 50 g/l gave no signal, but at 100 g/l a signal equivalent to 1 g IgG_1/l was recorded. β-Lg, at 25 and 100 g/l, gave a signal

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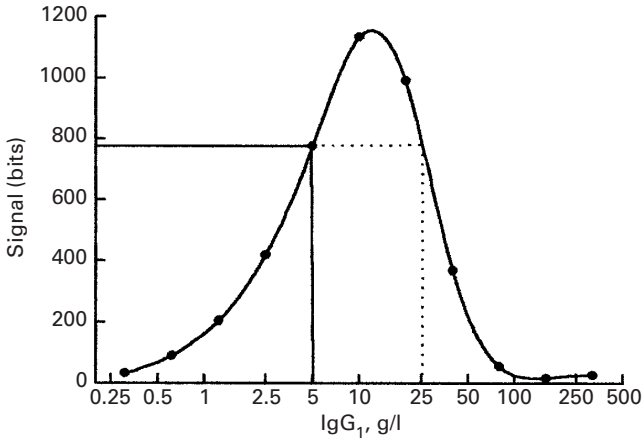


Fig. 1. Heidelberger & Kendall (1935) curve of the IgG_1 nephelometric immunoassay. The working curve (0·3 to 5 g IgG_1/l) is shown within the solid lines while the safety margin (see text) to 25 g/l, is shown by the dotted lines.

Table 1. *Within-run and between-run variability for different IgG_1 assays. Three milk samples were prepared containing low (0·45 g/l), medium (1·10 g/l) and high (1·75 g/l) levels of IgG_1. Milk samples analysed five times on each of five separate days*†

(Values are means and standard deviations, for $n = 25$†)

	IgG_1 concentration, g/l				
	Mean	Within-run SD	Between-run SD	Total SD	Total % CV
NIA					
Low	0·42	0·03	0·01	0·03	6·5
Medium	0·99	0·02	0·03	0·04	3·7
High	1·59	0·04	0·05	0·06	4·0
ELISA					
Low	0·39	0·04	0·05	0·07	17·4
Medium	0·96	0·09	0·13	0·16	16·7
High	1·42	0·19	0·04	0·19	13·3
RID 24 h					
Low	0·48	0·03	0·02	0·04	7·8
Medium	1·00	0·07	0·05	0·08	8·1
High	1·37	0·13	0·00	0·13	9·8
RID 48 h					
Low	0·47	0·03	0·03	0·05	9·4
Medium	0·98	0·06	0·04	0·07	7·1
High	1·43	0·15	0·00	0·15	10·4

equivalent to 2·3% of that obtained from an equal concentration of IgG_1. IgG_2, at 10 g/l, exhibited a signal equivalent to 39% of that obtained from an equal concentration of IgG_1.

Analytical recovery. Average analytical recovery for triplicate milk samples fortified with bovine IgG_1 at 0·31, 0·63, 1·25, 2·5 and 5·0 g/l was 101% ± 1·7%, 96% ± 1·0%, 95% ± 1·4%, 90% ± 0·2% and 93% ± 0·3% (mean ± SD), respectively. A linear regression with $r^2 = 0·9996$ was obtained when the added IgG_1 concentrations were compared to the values obtained by the assay.

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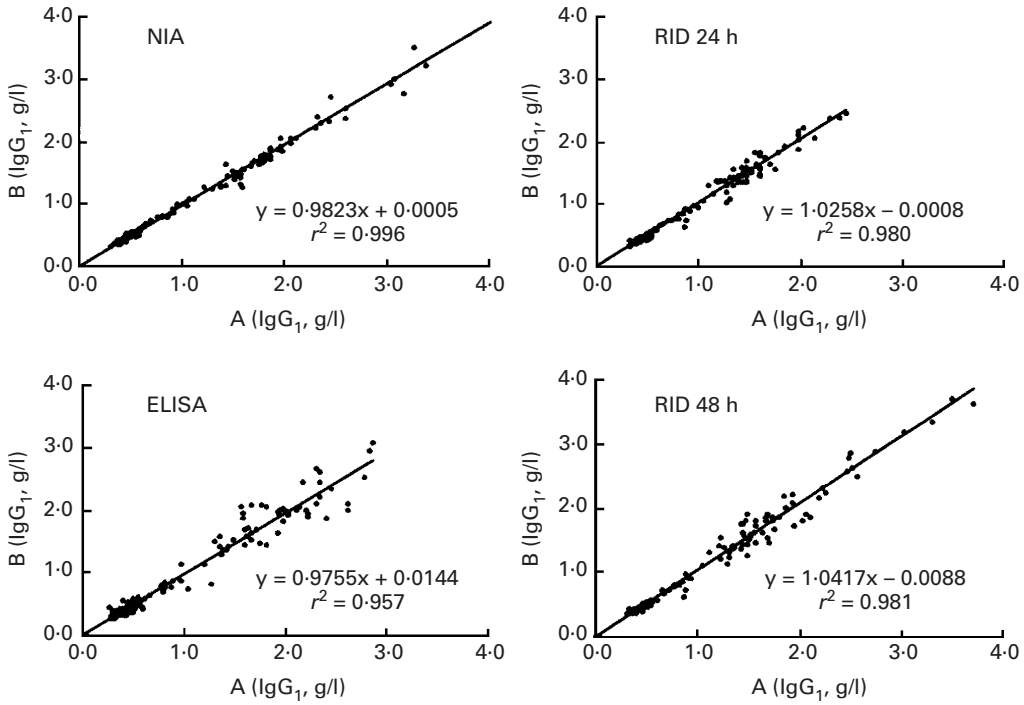


Fig. 2. Precision of each assay format, examined using regression analysis to compare duplicate samples (A & B) from milks and colostrums over a wide range of IgG_1 concentrations.

Linearity. Dilution of the colostrum sample from 4·4 to 0·6 g/l led to a linear reduction in IgG_1 concentration measured by the assay. The linear regression line had a slope of 1·10, a y intercept of 0·05 and a correlation coefficient of 0·9994.

Precision. Within-run, between-run and total variance for three milk samples containing high, medium and low levels of IgG_1 was calculated according to AOAC guidelines (Table 1). Sample duplicate regressions for each assay provided an additional indication of within sample precision. Fig. 2 shows that the NIA gave a higher correlation coefficient between duplicates than RID or ELISA.

Changes in IgG_1 in mammary secretions of cattle following calving. IgG_1 levels were determined for colostrum/milk from eight cows for 10 d after calving. IgG_1 levels rapidly decreased from day 1 until stable values of ~ 1 g/l were obtained by day 6–7 (Fig. 3). The average value for IgG_1 on day 1 was 30 g/l$\pm$3·5 SEM with the value declining rapidly thereafter to give a value of 3·5 g/l$\pm$0·45 SEM by day 3.

Method comparison

Only samples ($n = 105$) falling within the working range of the RID (24 h incubation; 0·45–1·8 g/l) were used in the comparison of the different assays (Fig. 4) as this assay is the current industry standard. NIA, ELISA and, to a lesser extent, RID (48 h incubation) gave similar results with a slope of approximately one and a y axis intercept of approximately zero. A comparison of the NIA with other assays showed that the slope of the regression line varied between 0·67 to 1·05 and the y intercept varied between $-0{\cdot}06$ to 0·20. Data analysis using difference plots showed a systematic divergence between IgG_1 values produced by the RID (24 h incubation) and those from the other assays when IgG_1 concentrations were above 1·2 g/l, with the RID consistently yielding lower levels.

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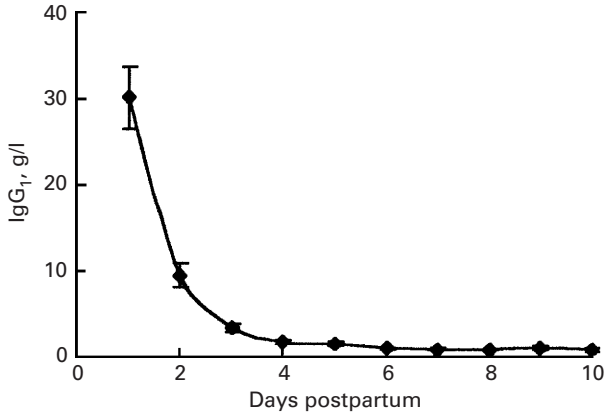


Fig. 3. Postpartum changes in the IgG_1 concentration (mean ± SEM) of colostrum/milk from eight cows for 10 d after calving. A rapid decline in values is seen from day 1 to 4, with values stabilizing after this time.

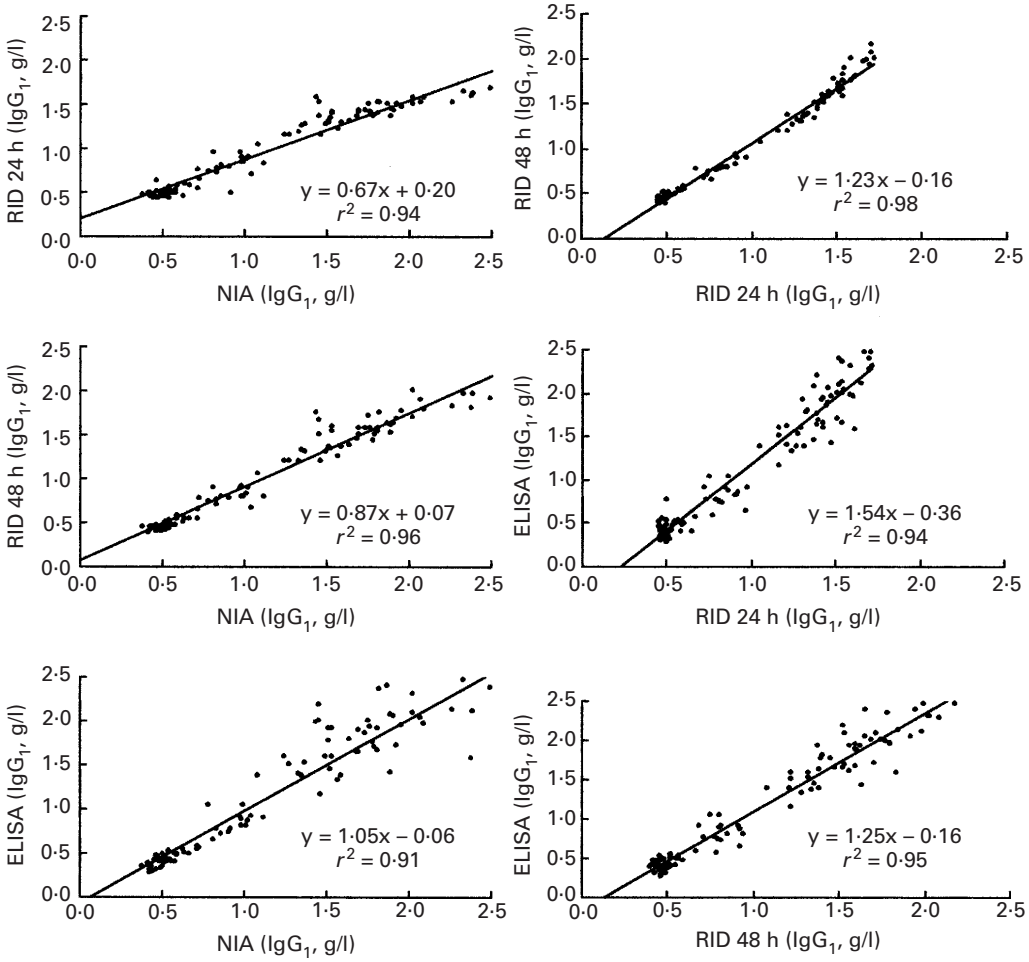


Fig. 4. Comparison, using linear regression, of 105 milk samples analysed by NIA, RID (24 and 48 hr) and ELISA. Data used for these graphs comprise those samples with values of between 0·45 and 1·8 g IgG_1/l as determined by RID 24 h.

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discussion

The quality assurance parameters determined for the optimised NIA show that this assay is suitable for the quantification of IgG_1 in milk. Values of IgG_1 in milk normally range from 0·3 to 0·5 g/l (Newstead, 1976), while concentrations in early season milk may be greater than 2 g/l. NIA, with a detection range from 0·3 to 5·0 g/l, reliably quantified IgG_1 in milk samples covering this range. Analysis by NIA of mammary secretions from cows, 1–10 d postpartum, confirmed the rapid decline of IgG_1 with time as previously described by Lacy Hulbert *et al*. (1996) and Auldist *et al*. (1993) using RID.

The antibody used in the assay had no significant cross-reactivity to major milk proteins but had cross-reactivity with 10 g IgG_2/l of 39 % when compared to IgG_1 of the same concentration. This level of cross-reactivity to IgG_2 is expected for antibodies raised against IgG_1 (Li-Chan & Kummer, 1997) and a similar level of cross-reactivity to IgG_2 was determined for the RID Coltest™ (unpublished observations). The concentration of IgG_2 in milk is 0·05–0·1 gl (Eigel *et al*. 1984). Cross-reactivity of the antibody with IgG_2 would therefore contribute 0·02–0·04 g/l to assay readings. Both analytical recovery and linearity upon dilution showed that the assay was accurate across the full range of the standard curve and that the milk matrix did not affect the assay. Precision calculations show that within-run and between-run standard deviations were lowest for NIA followed by RID then ELISA. The poor precision of the ELISA may be due, in part, to the errors introduced by the extensive dilution of milk (1/20000) required for this assay. Measurement errors due to dilution would be less for RID (no milk dilution) and NIA (1/100 dilution).

RID assay (24 h incubation) is considered to be the industry standard for quantification of IgG_1 (Auldist *et al*. 1993) and was used to determine which samples were selected for method comparison by linear regression analysis. But early in this study it was noted that the 1·8-g IgG_1/l standard for the RID assay (24 h incubation) produced a zone size which fell below the linear line of best fit for the other standards. A comparison of this assay with NIA and ELISA showed that milk IgG_1 values were underestimated in the upper assay range (1·2–1·8 g/l), probably because the 24-h incubation time was insufficient for high values of IgG_1 to reach equilibrium in this assay. An improved linear relationship was obtained after 48 h incubation. All RID plates were therefore read after incubation for both 24 and 48 h. Later work (data not shown) demonstrated that a better relationship between RID results and those of other assays existed when the RID standard curve was constructed using a log-log regression rather than the linear regression recommended by the manufacturer of the RID Coltest™ (ICP Ltd).

The NIA assay has a number of advantages for the determination of IgG_1 in milk. These include high sample throughput, reduced operator input and increased precision of measurement over a wide concentration range. There are, however, two potential disadvantages of NIA. As a light scattering assay it is important that the sample is clear for accurate determination of IgG. This is achieved by separating milk fat by centrifugation followed by sampling the lower non-fat milk component by piercing a hole in the bottom of the centrifuge tube. This method of collecting non-fat milk is reliable and ensures that fat is not present in the sample. Dilution of non-fat milk in PBSet disrupts casein micelles resulting in a clear solution suitable for nephelometric analysis. The second disadvantage reflects the fact that the precipitin curve is bell shaped and that there are potentially two antigen concentrations for any light scattering value. The assay was developed to give a standard curve on the left,

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antigen excess, side of the precipitin curve. The safety zone of the assay was derived from the precipitin curve and encompassed all IgG_1 concentrations giving rise to a signal greater than that of the highest IgG_1 standard. Using this criteria the IgG_1 NIA assay had a safety zone of 5–25 g/l with a calibration range from 0·3 to 5 g IgG_1/l (Fig. 1). This safety zone reduces the possibility of incorrectly assaying milks containing very high concentrations of IgG_1. Milks would require IgG_1 levels of greater than 25 g/l to be falsely interpreted in the assay and this is highly unlikely to occur unless colostrum from 1–2 d postpartum was being analysed. To avoid this problem, any sample collected within the first 48 h of calving should be diluted 1/10 before analysis.

The development of the nephelometric immunoassay was facilitated by using hens to produce bovine anti IgG_1 antibodies. Hens provide an ideal vehicle for antibody production since they develop a greater immune response to bovine milk than mammals (Jensenius *et al.* 1981). Furthermore, a large and consistent supply of antibody can be obtained, with antibody extraction from eggs being fast and technically undemanding (McLaren *et al.* 1994).

In conclusion, the NIA for IgG_1 in milk or colostrum is a fast, convenient and reliable method that can be used for rapid analysis of samples. These features make the nephelometric assay ideal for the quantification of IgG_1 in the modern dairy laboratory.

REFERENCES

Auldist, M. J., Coats, S., Rogers, G. L. & McDowell, G. H. 1993 Characteristics of mammary secretion during early lactation from naturally-calving dairy cows and cows induced to calve prematurely. *The Australian Journal of Dairy Technology* **48** 15–19

Eigel, W. N., Butler, J. E., Ernstrom, C. A., Farrell, H. M., Jr., Harwalker, V. R., Jenness, R. & Whitney, R., McL. 1984 Nomenclature of proteins of cow's milk: fifth revision. *Journal of Dairy Science* **67** 1599–1631

Franks, R. 1988 Raw milk grading, present and future trends. *Proceedings of the 5th Seminar of the Dairy Cattle Society of the New Zealand Veterinary Association*, Auckland 55–62

Heidelberger, M. & Kendall, F. E. 1935 Quantitative theory of the precipitin reaction; study of azoprotein-antibody system. *Journal of Experimental Medicine* **62** 467–483

Hollis, S. 1996 Analysis of method comparison studies. *Annals of Clinical Biochemistry* **33** 1–4

Humbert, G., Collard-Bovy, C., Marchal, E., Linden, G., Montagne, P., Duheille, J. & Varcin, P. 1991 Microparticle-enhanced nephelometric immunoassay. 3. Application to milk and dairy products. *Journal of Dairy Science* **74** 3709–3715

Jensenius, J. C., Andersen, I., Hau, J., Crone, M. & Koch, C. 1981 Eggs: conveniently packaged antibodies. Methods for purification of yolk IgG. *Journal of Immunological Methods* **46** 63–68

Lacy-Hulbert, S. J., Malcolm, D. B., Copeman, P. J. A., Woolford, M. W. & Franks, R. 1996 Reduction in SCC and colostrum levels in milk after calving. *Proceedings of the New Zealand Society of Animal Production* **56** 263–265

Li-Chan, E. C. Y. & Kummer, A. 1997 Influence of standards and antibodies in immunochemical assays for quantitation of immunoglobulin G in bovine milk. *Journal of Dairy Science* **80** 1038–1046

McLaren, R. D., Prosser, C. G., Grieve, R. C. J. & Borissenko, M. 1994 The use of caprylic acid for the extraction of the immunoglobulin fraction from egg yolk of chickens immunised with ovine α-lactalbumin. *Journal of Immunological Methods* **177** 175–184

Mishalanie, E. A. 1996 *Intralaboratory Analytical Method Evaluation: Short course manual.* Gaithersberg: AOAC International

Montagne, P., Gavriloff, C., Humbert, G., Cuillière, M. L., Duheille, J. & Linden, G. 1991 Microparticle-enhanced nephelometric immunoassay for immunoglobulins G in cow's milk. *Lait* **71** 493–499

Newstead, D. F. 1976 Carotene and immunoglobulin concentrations in the colostrum and milk of pasture-feed cows. *Journal of Dairy Research* **43** 229–237

NZCPI 1998 *Farm Dairy Code of Practice.* Wellington: New Zealand Dairy Board

Price, C. P., Spencer, K. & Whicher, J. 1983 Light-scattering immunoassay of specific proteins: a review. *Annals of Clinical Biochemistry* **20** 1–14

Svendsen, P. J. & Blirup-Jensen, S. 1991 *Determination of human plasma proteins by turbidimetry and nephelometry.* Copenhagen: DAKO A/S

https://doi.org/10.1017/S0022029901005295 Published online by Cambridge University Press