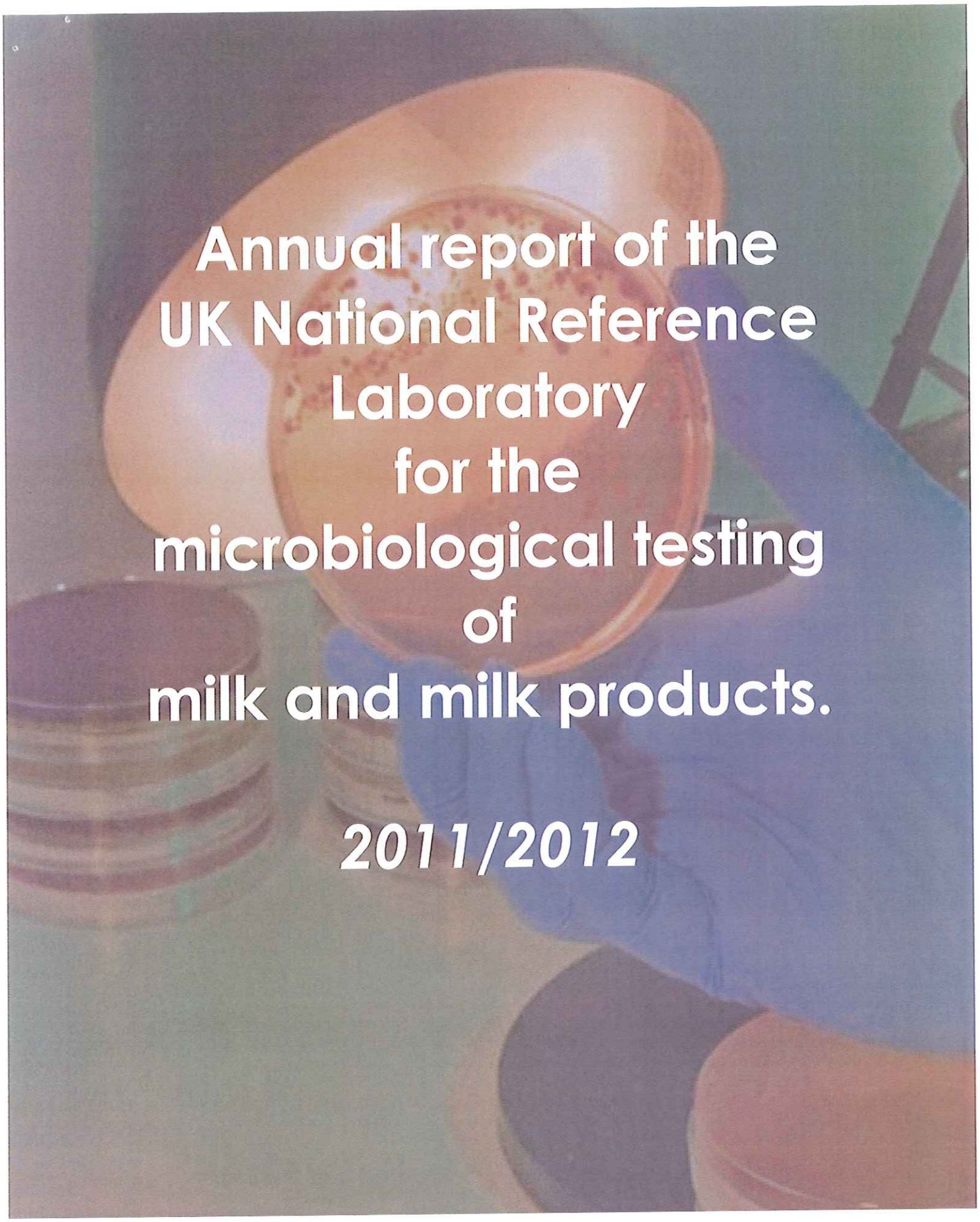

Annual report of the UK National Reference Laboratory for the microbiological testing of milk and milk products.

2011/2012

afbi Agri-Food and Biosciences Institute

Contents

Annual report of the UK National Reference Laboratory for the microbiological testing of milk and milk products.

The National Reference Laboratory (NRL) for the microbiological testing of milk and milk products for the UK is based at the Agri-Food and Biosciences Institute (AFBI) in Belfast, Northern Ireland.

The role of the NRL is to provide monitoring for the enforcement of EU Directive 882/2004- on official controls performed to ensure the verification of compliance with food and feed law, animal health and animal welfare rules.

Report Number:	AFBI/NRLREPORT/2011-2012
Authors:	S Cassidy, J Early, M Patterson, M Rowe
Date:	February 2012
Sponsor:	Food Standards Agency Aviation House 125 Kingsway London WC2B 6NH
Project Number:	PAU/222
NRL Auditor:	Dr Margaret Patterson
Lead Scientist:	Dr Michael Rowe
Senior Project Manager:	Mr John Early
Project Manager:	Miss Sharon Cassidy
Distribution:	1. Mrs Chelvi Leonard (FSA) 2. Dr Margaret Patterson

INTRODUCTION

It is important to recognise the hierarchy of enforcement authorities within the European Union. On the top tier is the relevant EU Reference Laboratory (EU-RL) which is based in Maisons-Alfort, Paris, France, and funded directly by the EU Commission. This Laboratory is responsible for monitoring the performance of the reference laboratories in Member States e.g. the UK National Reference Laboratory (UK-NRL) based in the Agri-Food and Biosciences Institute in Belfast, UK. Each of the NRLs are, in turn, responsible for the performance of Official Control Laboratories (OCLs) situated within their jurisdiction. Both the Member State NRLs and OCLs are funded by their respective Government departments for testing performed under the relevant legislation. An OCL is defined as a laboratory which generates results which can be used by the competent authority for enforcement purposes under the requisite EU directives. The object of the whole exercise is to facilitate free and fair trade between member states of the EU and ultimately to ensure the safety of the public and protect them from fraudulent purchases. It also acts as a 'touchstone' for those countries outside the EU who wish to export dairy products into the region.

In the proper pursuit of its function the UK-NRL has a number of responsibilities viz.

- Participate in ring trials organised by the EU-RL to ensure the satisfactory performance of the member state NRLs e.g. total viable counts .
- Participate in practical assessments to aid the formulation of EU legislation e.g. levels of alkaline phosphatase in pasteurised bovine milk cheeses.
- Monitor performance of national OCLs. Since the majority of these within the UK are United Kingdom Accreditation Service (UKAS) approved, which requires internal and external independent audit and satisfactory performance in internal and external quality assurance schemes, this is regarded as adequate. Hence, the expense which would be incurred by the UK-NRL directly auditing them, in addition, is deemed to be superfluous and an unnecessary draw on public resources.
- Disseminate relevant changes to British Standard (BSI) and International Standard Organisation (ISO) standards to the OCLs. The participation of the Lead Scientist of the UK-NRL on BSI Technical Committees AW/9 and AW/5 facilitates this function.
- Provide expert advice to the FSA or OCLs as required.
- Disseminate information coming from the EU-NRL. The circulation of a questionnaire to the national OCLs about their activities ensures that only relevant information is circulated to them and reduces any unnecessary burden of administration.
- Participate in workshops organised by the EU-RL on topics of concern.

It is recognised that the ultimate responsibility for the operation of the UK-NRL resides with the FSA and they provide the necessary finance. In pursuit of this responsibility the FSA can conduct an audit of the UK-NRL at any time.

Alternative methods for Total Viable Counts.

The testing of raw milk for total viable count is for one or both of two principal reasons viz enforcement purposes (EU Regulation 853/2004) or to enable the operation of quality payment schemes. In April 2011 a report was received from the EU Reference Laboratory (EU-RL) detailing an analysis of questionnaire responses concerning alternative methods, such as the use of Bactocount™ and Bactoscan™ systems for measuring the total viable microflora of raw milk in Member States.

This report was sent to all Member State National Reference Laboratories (NRLs). The UK NRL responded to the original questionnaire and collated the data from relevant UK laboratories so the UK data was taken into account in the overall analysis. This analysis was not restricted to bovine milk and milk from other animals was included e.g. buffaloes, mares and camels.

The main reason for this exercise was because many of the alternative methods measure all the viable bacteria in the sample, even those within clumps. Traditional viable plate counting methods cannot discriminate between cells within clumps and those in a planktonic state. A single viable cell will give rise, under the right conditions, to one colony and a clump of cells, perhaps containing hundreds of cells, will also only give rise to one colony. This means that the value obtained with most alternative methods will consistently be higher than the value obtained using a plate count. This necessitates the use of a conversion factor to transform the value obtained with alternate methods to its plate count equivalent. It was of concern to the EU-RL that different conversion factors may be being used even for the same models and this may adversely affect fair trade both within and amongst Member States.

In conclusion the EU-RL considered this questionnaire analysis represented a good initial step towards harmonisation of conversion factors with the overall objective of establishing a single conversion factor per country for EU Member States.

14th Workshop for EU Member State NRLs

This was held on 2nd and 3rd of May 2011 at the EU-RL Headquaters at Maisons-Alfort, Paris and was attended by Miss Sharon Cassidy (Project Manager, UK NRL).

A report was delivered by Klaus Kostenzer (DG-SANCO) of an evaluation carried out between 2006 and 2010 of all 26 EU-RLs dealing with feed and food safety including the EU-RL for milk and milk products, the latter receiving a B^+ rating. This audit generated some minor specific recommendations which are currently being implemented by the EU-RL. The speaker also mentioned the replacement of ISO 21528-1:2004 (enumeration of Enterobacteriaceae by MPN method) with ISO 21528-2:2004 (enumeration of Enterobacteriaceae by colony count technique).

A session was devoted to discussion on the organisation and execution of proficiency trials which are used by Member State NRLs to monitor the performance of automated machines for total flora and somatic cell counts operating within their respective jurisdictions.

A report was given of the impact of various factors on the conversion factor for total microflora count using the Bactoscan machine on goat's milk. Factors considered included geographical location of the dairy and origin of the milk, breed of goat, feed used and season.

A presentation was delivered by Thomas Berger (NRL Switzerland) on progress towards formation of a working group tasked with development of a certified reference method for somatic cell counting. This was followed by a presentation on colostrum in cow's milk and the intention of DG Sanco to introduce hygiene requirements for this product in Regulation 853/2004.

Attention was finally focused on alkaline phosphatase. This is an intrinsic enzyme produced by ruminants and is inactivated by pasteurisation heat treatments. Detection of supposedly pasteurised milk of alkaline phosphatase above a threshold value is indicative of improper pasteurisation or post pasteurisation contamination. Although phosphatase activity in various products were discussed, along with detection methods, a lengthy discussion on alkaline phosphatase in cheese followed. This discussion concluded by agreement for the majority of Member States to determine the typical alkaline phosphatase levels in pasteurised bovine milk cheeses produced within their own national boundaries and the desirability of another proficiency trial for the NRLs on alkaline phosphatase in that product.

Meeting between UK-NRL representatives and directorate staff at FSA

This took place on 8th July 2011 at Aviation House, FSA Headquarters in London. Those participating for the NRL were Dr Michael Rowe (Lead Scientist, NRL) and Miss Sharon Cassidy (Project Manager, NRL). Those participating form the FSA directorate were Dr Andrew Damant and Chelvi Leonard (Scientific Methods and Laboratory Policy Branch; SMLP), David Alexander (Science and Knowledge Unit, Food Production Branch), and Karen Pratt and Chris Harvey (Hygiene Delivery Branch).

It was agreed that the SMLP Branch propose M. Rowe to be a member of BSI Technical Committee AW/5 which is concerned with chemical testing of food since this committee is concerned with somatic cell counting, an analysis which comes within the remit of the UK-NRL. The UK-NRL is already represented on BSI AW/9 which is the corresponding technical committee dealing with microbiological analyses of food.

Participation in a scoping exercise was requested by the EU-RL to determine typical levels of alkaline phosphatase in UK produced cheese made with bovine pasteurised milk. This was to be compared to an EU proposed threshold of less than 10 mU/g. It was considered important that UK produced cheeses tested should be representative both in terms of cheese variety and origin of production with the experimental design being validated by the Biometrics Branch of AFBI prior to commencement to ensure that the data generated would be statistically valid. It was also recognised that such an exercise was outwith the scope of the current contract and that a business case for the work should be prepared by AFBI and submitted to the FSA for consideration and approval. This has been approved by the FSA and is currently (January 2012) underway with the work scheduled to be completed within this financial year and the report prepared and submitted in the financial year 2012-2013.

Comparison for measurement of alkaline phosphatase using Charm and Fluorphos methods.

A recent comprehensive comparative study has been published (Albillos *et al.* 2011).
The conclusion of this study was as follows:
The alkaline phosphatase generated by an independent collaborative study reported in this paper using raw milk and fluid milk spiked with raw milk were commensurate with those samples prepared by the manufacturers' laboratories. Alkaline phosphatase values obtained with the Fluorophos method were similar to those obtained with the Charm method or were higher near the 350 mU/L legal limit. Values obtained with both methods were correlated linearly with the amount of raw milk used for spiking and limits of detection and quantification in milk and liquid fluid milk being similar. Determination by both methods was independent of the type of raw milk used (cow, goat or sheep), the type of matrix (whole milk, skim milk, cream or chocolate milk) being tested. Although the work was comprehensive the authors' indicated that more work may be required.

For more detailed information the reader is directed to:
Albillos, S.M., Reddy, R and Salter, R. (2011) Evaluation of alkaline phosphatase detection in dairy products using a modified chemiluminescent method and official methods. *Journal of Food Protection* **74:** 1144-1154.

Scoping exercise to determine typical alkaline phosphatase values for UK produced pasteurised bovine milk cheeses

It is the intention that the EU Commission will introduce a threshold value of less than 10 mU/L. In order to ensure that this will not disadvantage inter or intra community trade between Member States the EU-RL has requested the Member State NRLs to test representative samples of such cheeses and communicate the results to them for collation and analysis. The UK-NRL has prepared a representative list of UK produced cheeses (Appendix 1) taking account of cheese type and geographical origin. The testing programme is currently underway and practical aspects should be completed within this financial year.

Proficiency trial for the determination of total microflora of raw goat's milk.

The UK NRL as part of it's function perform proficiency trials organised by the European Union Reference Laboratory (EU-RL). The purpose of these trials is to monitor and assess the performance of all member state NRLs. This is to ensure that each laboratory is carrying out all the reference methods prescribed to analyse milk and milk products effectively.

The most recent proficiency trial took place in November 2011. This was to determine the total viable count in raw goat's milk using reference method ISO 4833 (Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of micro-organisms: Colony count technique at 30 degrees Celsius).

On 16 November 2011 the NRL received six samples of raw goat's milk to be analysed on 18 November 2011. To determine the total number of organisms the samples were processed as per ISO 4833 and the colony forming units per ml (CFU/ml) were recorded and sent to the EU-RL for analysis. The outcome of the trial is unknown but in due course a report will be issued by the EU-RL that will list the performance of each NRL in each Member State including the UK. This report will then be forwarded to the FSA and OCLs with a summary of the UK's performance and overall results.

Questionnaire to UK OCLs.

A follow up questionnaire (Appendix 2) was distributed to all UK OCLs within the NRL remit that test milk and milk products for enforcement purposes on behalf of a Competent Authority in November 2011.

The purpose of this exercise was to ensure that all the information held by the NRL, on the status of each OCL, was up-to-date including UKAS accreditation and the tests carried out on milk and milk products. All this information is considered important since the UK NRL can be subject to audit by the Commission and this provides evidence that the UK NRL is properly discharging its responsibility of monitoring the performance of each OCL within its jurisdiction.

To date the NRL is awaiting responses from the OCLs regarding the questionnaire. A final reminder letter was sent out to those laboratories who have not made a return on 20 January 2012. The NRL will then produce a report on the outcome of the follow up questionnaire to be distributed to the FSA and OCLs.

It should be recognised that the majority of OCLs are accredited by UKAS and participate in internal and external quality assurance schemes.

Miscellaneous activities

Throughout the year the UK-NRL has responded to queries form the OCLs on a number of topics and as a result of Dr Rowe's participation in the BSI Technical Committee AW/9, responsible for devising new standards for the microbiological testing of food, has alerted the OCLs to new standards as they come into force. Upon retirement of Dr Arthur Gilmour as FSA auditor of the UK-NRL Dr Margaret Patterson has been appointed. The role of the auditor is to monitor, at first hand, the performance of the NRL, to contribute to the annual report of the NRL and to verify the rectification of any non-conformances raised by the FSA on the operation of the NRL.

Contact Information

National Reference Laboratory for the microbiological testing of milk and milk products in the UK	Agri-Food and Biosciences Institute Food Microbiology Branch Newforge Lane Belfast BT9 5PX **Contact:** Sharon Cassidy **Telephone:** 02890 255321 **Email:** Sharon.cassidy@afbini.gov.uk
European Union Reference Laboratory (EU-RL) for milk and milk products	ANSES 23 Avenue du General de Gualle 94706 Maisons Alfort Cedex France **Contact:** Adrien Assere **Telephone:** +33 149 772 749 **Email:** Adrien.assere@anses.fr
Food Standards Agency	Aviation House 125 Kingsway London WC2B 6NH **Contact:** Mrs Chelvi Leonard **Telephone:** 44(0) 207 276 8969 **Email:** chelvi.leonard@foodstandards.gsi.gov.uk

Appendix 1

Cheese	Country of origin
Chesire Cheddar	England
Farm House Cheddar	England
Cornish Yarg	England
Chatel Cornish Brie	England
Cathedral City	England
Davidstow Cheddar	England
Eden Valley Brie	England
South Lakes Organic Cheddar	England
Bretlin Derby	England
Wensleydale	England
Swaledale Old Peculiar	England
Coverdale	England
Tremains Cheddar (Godminster Cheddar)	England
Ashdown Forresters	England
Double Gloucester	England
Hereford Hop	England
Stinking Bishop	England
Old Wincester	England
Lancashire	England
Red Leicester	England
Chevington Cheese	England
Coquetdale	England
Oxford Isis	England
Moorlander Oaksmoked Cheese	England
Cheddleton Cheese	England
The Staffordshire	England
Suffolk Gold	England
Somerset Camembert	England
Somerset Brie	England
St Eadburgha Cheese	England
Hawes Traditional Wensleydale	England
Hartington Bomber	England
Five Counties Cheddar	England
Caerphily	Wales
Golden Cenarth	Wales
Perl wen	Wales
Cadog	Wales
Snowdonia Black Bomber	Wales
Old Shire Cheddar	Wales
Y Fenni	Wales
Tintern Cheese	Scotland
Colliers Cheddar	Scotland
Drumloch	Scotland
Aitkit Cheese	Scotland
Aryshire Dunlop	Scotland
Black Crowdie (Gruth Dhu)	Scotland
Caboc	Scotland
Caithness Cheese	Scotland

Clava Brie	Scotland
Orkney Cheddar	Scotland
Mull of Kintyre	Scotland
Isle of Arran Dunlop	Scotland
Seriously Strong Cheddar	Scotland
Coleraine Cheddar	Northern Ireland
Drumkeel	Northern Ireland
Dromana Cheese	Northern Ireland
Fivemiletown Ballybrie	Northern Ireland

Appendix 2

National Reference Laboratory (milk/milk products) follow-up questionnaire for UK Official Control Laboratories.

1. Does your laboratory still undertake analysis of milk/milk products for enforcement purposes for a competent authority under EC Regulation 882/2004?

Yes ☐

No ☐

If you answered 'no' please give brief explanation below:

2. Do you find the information that the NRL sends you to be informative and of use? For example reports and BSI information.

Yes ☐

No ☐

If you answered 'no' please give brief explanation below:

3. Since November 2009 has there been the addition or removal of any tests involving milk/milk products from your UKAS schedule?

Yes ☐

No ☐

If you answered 'yes' please list these tests and whether they have been added or removed below:

4. As an OCL would you be interested in attending a future meeting that would be organised by the NRL and held at the FSA Headquarters in London?

Yes ☐

No ☐

5. What topics or subjects would you like to see discussed at the meeting?

6. Does your laboratory analyse pasteurised bovine cheese to determine alkaline phosphatase levels?

Yes ☐

No ☐

If you answered 'yes' what are the typical values you obtain?

7. Does your laboratory analyse milk for TVCs using an alternative method such as Bactoscan or Bentley etc?

Yes ☐

No ☐

If you answered 'yes' what alternative method do you use? Please if possible include a conversion factor.

8. Do you know of any other organisation or laboratory in your area that use instruments such as Bentley or Bactoscan?

Please list below:

9. As an OCL do you have any further questions, comments or suggestions you would like to bring to the NRL's attention?

Please state below:

10. If you have submitted a query to the NRL in the past, was it dealt with in a timely manner, and the answer sufficient to your needs?

Yes ☐

No ☐

If you answered 'no' please give brief explanation below:

Thank you for taking the time to complete this questionnaire

Name:

Organisation:

Address:

Telephone: