Milk and Milk Products

Annual Report 2008 Dutch National Reference Laboratory

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Summary

This annual report of the Dutch National Reference Laboratory (NRL) Milk and Milk Products describes the activities of the NRL in 2008. Nowadays the NRL reference tasks for milk and milk products are based on the specific rules for raw milk and milk products as described in Commission Regulation EC 853/2004. These tasks include the parameters: Total flora (TF), Somatic cells count (SCC), and Alkaline phosphatase activity (AP).

Currently, EC 1664/2006 states:
When checking against the criteria laid down in Annex III, Section IX, Chapter I, Part III to Regulation (EC) No 853/2004, the following standards must be applied as reference methods:
- EN/ISO 4833 for the plate count at 30°C;
- ISO 13366-1 for the somatic cell count.
- When determining alkaline phosphatase activity, ISO standard 11816-1 must be applied as reference method.

RIKILT has an accreditation for the SCC and TF reference methods and is working on the application for accreditation for the AP reference method.

Activities of the NRL Milk and Milk Products during 2008 included:
- **Participation in the annual CRL-NRL workshop.** The 2008 annual workshop took place on 9-10 October in Vienna, Austria. This workshop was dedicated to various aspects of Alkaline Phosphatase testing, like legal limits for ewe's and goat's milk, AP testing in milk from other species, and AP testing in various cheeses. In addition, the results from the 2007 proficiency test on AP and the work program for following years were discussed.
- **Participation in CRL-organised interlaboratory studies.** In November 2008, RIKILT participated in the interlaboratory trial for enumeration of somatic cells by the reference method.
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1 Introduction

In 1992 RIKILT was assigned as National Reference Laboratory (NRL) on Milk and Milk Products, according to directive 92/46/EEG laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. AFSSA-LEFHQA in Paris was assigned as the Community Reference Laboratory (CRL).


Generally, reference tasks on e.g. Listeria, Salmonella, E.coli, and S. aureus nowadays are horizontally (all foods) regulated instead of vertically (milk and milk products only), and specific CRL/NRLs have been designated accordingly. Formerly established CRL/NRLs were kept, but with revised tasks. Today the reference tasks for milk and milk products are based on the specific rules for raw milk and milk products as described in Annex III, section IX of EC 853/2004. These reference tasks include the parameters: Somatic cells count (SCC), Total flora (TF), and Alkaline phosphatase activity (ALP). This report describes the activities of the Dutch NRL Milk and Milk Products in 2008.
2 NRL tasks

The responsibilities of National Reference Laboratories are described in article 33 of EG 882/2004 (see Annex I) and this is implemented by RIKILT through the following activities:

- Development, validation and independent quality control of official reference methods and providing information to Routine Field Laboratories
- Participation in the annual CRL-NRL workshops
- Responding to CRL questionnaires
- Participation in CRL-organised interlaboratory studies
- Participation in CRL-training courses
- NRL ring trials
- Accreditation of the reference methods
- Communication on developments in related methods and legislation matters
- Annual report of activities (from 2006 onwards)
3 Results 2008

3.1 Annual CRL/NRLs workshop 2008

The annual workshop of 2008 took place on the 9th and 10th of October at AGES (Austrian Agency for Health and Food Safety) in Vienna, Austria. This workshop was dedicated to the various aspects of Alkaline Phosphatase testing, and topics as outlined below were discussed. The results of the proficiency tests of 2005/2006 and 2007 on AP were presented and proved to be satisfactory. In addition, the robustness of the AP method in milk, the outcome of the statistics sub-workgroup and the alternative statistical evaluation were presented.

In order to set legal limits for goat's and ewe's milk, results concerning these types of milk were gathered and discussed. The variability between the results lead to the decision to launch a second trial requesting participation of countries who did not participate in the first round. Furthermore, a circular letter will be launched gathering more information on the national market of pasteurised goat's milk. Limits for AP could be used as marker for proper pasteurisation for ass milk, however it was not suitable for mare and camel milk. To set up legal limits for pasteurised cheeses, more data from tests with soft and hard cheeses made from cow's milk are required. A circular letter on this subject will be launched.

Acceptance of alternative AP methods was discussed and will be further investigated by the CRL to assess equivalence between methods. Concerning the topic AP reactivation, it was decided that a project proposal will be written and submitted to DG-Research for financial support. Finally, the AP work program for the following years was also discussed. The minutes of this workshop are given in Annex II. Slides of the various presentations are available on request.

The workshop was attended by 37 participants from 27 NRL's from 25 EU Member States or countries from the European Free Trade Association (EFTA), as well as from EC/ DG SANCO "Health & Consumer Protection" (Thierry Chalus and Paolo Caricato), and the CRL MMP, Alkaline Phosphatase team, and the CRL coordination team. Roger Wood, from UK FSA (Food Standards Agency, i.e. UK competent authority) was also invited as an expert for his skills on statistics. Thierry Chalus took this opportunity to inform the NRLs that it was his last workshop with the milk and milk products network, and to introduce Paolo Caricato who is going to follow-up on this topic. The list of attendance is given in the minutes (Annex II).

3.2 CRL Questionnaires

The outcome of the questionnaire on reference materials for somatic cells counting of October 2007 became available in February 2008 and can be found in Annex III.
3.3 Participation in CRL interlaboratory studies

3.3.1 CRL interlaboratory study on alkaline phosphatase activity

The CRL organised an interlaboratory trial on alkaline phosphatase activity in cows milk in November 2007. RIKILT participated in this trial, using the reference method IDF 155/ISO 11816-1. The final report was received in September 2008 (available on request). There were 19 participants in this study. Repeatability and reproducibility figures calculated using the data of the network proved to be considerably better in comparison with those given in the ISO standard 11816. RIKILT z-scores for cow's whole milk, cow's semi-skimmed milk and cow's skimmed milk were all between -1.5 and +1.0, and thus satisfactory.

3.3.2 CRL interlaboratory study on enumeration of total flora at 30°C

RIKILT participated in October 2007 in the CRL interlaboratory study on enumeration of total flora at 30°C, using the reference method EN-ISO 4833. During the 2006 CRL Workshop in Kiel, it was decided that some additional participants needed to be invited to participate in this trial: the laboratories, to be identified by the NRLs, which are in their respective countries entrusted to estimate CC values (conversion characteristics) between the reference method (EN-ISO 4833) and the instrumental methods (flow cytometry). For the Dutch situation therefore Qlip, Zutphen (formerly: Milk Control Station) also participated in this interlaboratory study. The report on the results became available in April 2008. Results from 44 of the 48 participants were used for statistical calculations. Overall, for a microbiological method enumerating micro-organisms, the precision data were good, both in terms of repeatability (RSDr of 1 %) and in terms of reproducibility (RSDR of 4 %). Most of the participants (82 %) shown a satisfactory individual performance, in terms of precision (k-ratios) or trueness (z-scores). Therefore it was concluded that as a whole, the network of NRLs and additional laboratories is competent to enumerate total flora in raw milk, using the official reference method. RIKILT and Qlip Zutphen achieved good scores in this ring trial. In more detail, the individual k-ratio's and z-scores of RIKILT shown in Table 1.

Table 1 Individual k-ratio's and z-scores of RIKILT

<table>
<thead>
<tr>
<th>Lab code</th>
<th>k-ratio's</th>
<th>z-scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low level</td>
<td>Medium level</td>
</tr>
<tr>
<td>RIKILT</td>
<td>39</td>
<td>0.18</td>
</tr>
</tbody>
</table>

3.4 Participation to CRL training courses

No training courses were organised in 2008.
3.5 Analytical activities

3.5.1 Ring trials

Due to the limited number of Routine Field Laboratories (RFL), it is not appropriate to organize specific ring trials. However, the RFL labs as well as the NRL lab do participate in common ring trials in the dedicated topics. RIKILT participated with satisfactory results to the following ring trials in 2008:

- Total flora at 30°C in milk (Qlip, 4 x 4 samples)
- Somatic cells count, microscopy (Cecalait, 2 x 10 samples)

3.5.2 Reference material for calibration of automated somatic cells count

The RFL for somatic cells count, MCS/Qlip, uses an automated method (Fossomatic) for routine samples. The equipment for this needs to be calibrated every 3 months and RIKILT participates in this calibrations by testing designated samples by the microscopic reference method for somatic cells count (ISO 13366-1). Calibration tests were carried out in January, March, June, September, and December 2008.

3.5.3 Alkaline phosphatase

The reference method for alkaline phosphatase (ISO 11816-1) was elaborated to produce the validation data needed and the method (RSV N0335) will be submitted for accreditation as soon as possible. Since no official standard reference material is available, reference material was produced by spiking ALP-free milk with raw milk to an expected level of ca. 500 mU/l. Qlip Leusden also uses this type of in-house reference material. No common ring trials are available and therefore a secondary control system is used by always repeating one sample from the previous series of samples during the current one.

In 2007 was started with a system to compare results on blind samples that were already tested by Qlip Leusden before. Four series of 5 samples each were tested this way in 2008 and this system will be continued in the future to reach the number of data that will be sufficient to evaluate the performance of the labs statistically.

3.6 Communication

3.6.1 Contacts with Qlip Leusden and Zutphen

Qlip in Zutphen (former MCS) was visited on 11 December 2008. Experiences and methods were exchanged and laboratory facilities were visited. Qlip in Leusden (former COKZ) would have been visited in combination with the RIKILT project 71.313.51, "Government supervision COKZ", but due to several circumstances this visit in 2008 was cancelled.

New visits are planned for 2009, especially because of the upcoming change in Project leadership of the NRL task.
3.7 Legislation

During the 2006 Workshop in Kiel already some Regulation amendments were announced and these became official by the publication on 6 November 2006 of COMMISSION REGULATION (EC) No 1664/2006, amending Regulation (EC) No 2074/2005 as regards implementing measures for certain products of animal origin intended for human consumption and repealing certain implementing measures.

No new amendments or developments in legislation were received from the CRL in 2008. Consequently, current legislation (2008) is given below per topic.

3.7.1 Official method Total Flora count

1. When checking against the criteria laid down in Annex III, Section IX, Chapter I, Part III to Regulation (EC) No 853/2004, the following standards must be applied as reference methods:
   (a) EN/ISO 4833 for the plate count at 30°C.

2. The use of alternative analytical methods is acceptable:
   (a) For the plate count at 30°C, when the methods are validated against the reference method mentioned in point 1(a) in accordance with the protocol set out in EN/ISO standard 16140 or other similar internationally accepted protocols.
   In particular the conversion relationship between an alternative method and the reference method mentioned in point 1(a) is established according to ISO standard 21187.

RIKILT is accredited (www.rva.nl L014) for this reference method, based on RIKILT standard operating procedure RSV A0016.

3.7.2 Official method Somatic Cells Count

1. When checking against the criteria laid down in Annex III, Section IX, Chapter I, Part III to Regulation (EC) No 853/2004, the following standards must be applied as reference methods:
   (b) ISO 13366-1 for the somatic cell count.

2. The use of alternative analytical methods is acceptable:
   (b) For the somatic cell count, when the methods are validated against the reference method mentioned in point 1(b) in accordance with the protocol set out in ISO 8196 and when operated in accordance with ISO standard 13366-2 or other similar internationally accepted protocols.

RIKILT is accredited (www.rva.nl L014) for this reference method, based on RIKILT standard operating procedure RSV A0716. The final version of the revised ISO 13366-1/IDF 148-1 was published in 2008 and implemented as such.

3.7.3 Official method Alkaline Phosphatase activity

1. When determining alkaline phosphatase activity, ISO standard 11816-1 must be applied as reference method.
2. The alkaline phosphatase activity is expressed as milli units of enzyme activity per liter (mU/l). A unit of alkaline phosphatase activity is the amount of alkaline phosphatase enzyme that catalyses the transformation of 1 micromole of substrate per minute.

3. An alkaline phosphatase test is considered to give a negative result if the measured activity in cow’s milk is not higher than 350 mU/l.

4. The use of alternative analytical methods is acceptable when the methods are validated against the reference method mentioned in point 1 in accordance with internationally accepted protocols.

RIKILT has validated the reference method and is pursuing accreditation, based on RIKILT standard operating procedure RSV N0335.
4 Conclusions and recommendations

In 2008, RIKILT has been involved in various NRL activities, such as participation in the annual CRL-NRL workshop, participation in the interlaboratory trial for enumeration of somatic cells by the reference method, and several other analytical activities.

The two main laboratories in the dairy area with statutory tasks merged in 2008, i.e. MCS (Zutphen) and parts of COKZ (Leusden), and were renamed Qlip (a commercial laboratory). At the same time other parts of COKZ became fully public (ZBO). At present the division of tasks in control and legislative matters is not always fully transparent. This is a prerequisite for NRLs to operate as demanded by legislation. In 2009, meetings are planned to have the situation and procedures clarified and updated.

At the moment, Alkaline Phosphatase NRL work is limited to the matrix milk only. In view of the European developments at CRL/NRL level, it is recommended to cover a larger range of matrices which differ considerably from milk from an analytical perspective, such as cheese, milk powder, infant food, in 2009/2010.
5 References

EU legislation:
general principles and requirements of food law, establishing the European Food Safety Authority
and laying down procedures in matters of food safety.

(CE) No 852/2004 on the hygiene of foodstuffs.


(CE) No 854/2004 laying down specific rules for the organisation of official controls on products of
animal origin intended for human consumption.

(CE) No 882/2004 on official controls performed to ensure the verification of compliance with feed
and food law, animal health and animal welfare rules.

(CE) No 2073/2005 on microbiological criteria for foodstuffs.

(CE) No 2074/2005 etc.

(CE) No 1664/2006 amending Regulation (EC) No 2074/2005 as regards implementing measures for
certain products of animal origin intended for human consumption and repealing certain
implementing measures.

ISO documents:
NEN-EN-ISO 4833:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the
enumeration of microorganisms - Colony-count technique at 30 degrees C.

NEN-ISO 8196-1:2000 Milk - Definition and evaluation of the overall accuracy of indirect methods of
milk analysis - Part 1: Analytical attributes of indirect methods.

NEN-ISO 8196-2:2000 Milk - Definition and evaluation of the overall accuracy of indirect methods of
milk analysis - Part 2: Calibration and quality control in the dairy laboratory.

NEN-EN-ISO 11816-1:2006 Milk and milk products - Determination of alkaline phosphatase activity

NEN-EN-ISO 11816-2: Milk and milk products - Determination of alkaline phosphatase activity - Part
2: Fluorimetric method for cheese.

(Reference method).


**RIKILT standard operating procedures (in Dutch):**

RSV A0016 Voedingsmiddelen en diervoeders - Bepalen van het aantal aeroob kweekbare micro-organismen bij 30°C; plaatmethode.

RSV A0716 Melk - Bepaling van het aantal somatische cellen; fluorescentie microscopie (telling).

RSV N0335 Milk and milk products-Determination of alkaline phosphatase activity-Fluorimetric method for milk and milk-based drinks.

**Other references:**

Annex I    Responsibilities of NRLs as described in EC 882/2004

1. Member States shall arrange for the designation of one or more national reference laboratories for each Community reference laboratory referred to in Article 32. A Member State may designate a laboratory situated in another Member State or European Free Trade Association (EFTA) Member and a single laboratory may be the national reference laboratory for more than one Member State.
2. These national reference laboratories shall:
   (a) collaborate with the Community reference laboratory in their area of competence;
   (b) coordinate, for their area of competence, the activities of official laboratories responsible for the analysis of samples in accordance with Article 11;
   (c) where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing;
   (d) ensure the dissemination to the competent authority and official national laboratories of information that the Community reference laboratory supplies;
   (e) provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans adopted in accordance with Article 53;
   (f) be responsible for carrying out other specific duties provided for in accordance with the procedure referred to in Article 62(3), without prejudice to existing additional national duties.
3. Article 12(2) and (3) shall apply to national reference laboratories.
4. Member States shall communicate the name and address of each national reference laboratory to the Commission, the relevant Community reference laboratory and other Member States.
5. Member States that have more than one national reference laboratory for a Community reference laboratory must ensure that these laboratories work closely together, so as to ensure efficient coordination between them, with other national laboratories and with the Community reference laboratory.
6. Additional responsibilities and tasks for national reference laboratories may be laid down in accordance with the procedure referred to in Article 62(3).
7. Paragraphs 1 to 5 shall apply without prejudice to more specific rules and in particular Chapter VI of Regulation (EC) No 999/2001 and Article 14 of Directive 96/23/EC.
Annex II  Minutes of the CRL/NRLs Workshop 2008

Report of the 11th Workshop of the National Reference Laboratories for Milk and Milk Products

(Version 19th December 2008)

9 & 10 October 2008
AGES, Vienna, Austria
1. Opening: Thursday 9th October, 9.30 am

Franz VOJIR, representative of the Austrian competent authority (Ministry of Health), opened the meeting insisting on the importance of National Reference Laboratories with their role to ensure through proficiency testing trials that the official control laboratories produce accurate values performing and to assist the competent authorities.

Rochus NEPF, Head of the Food Control Division of the Austrian Agency for Health and Food Safety (AGES), welcomed the attendees and presented briefly AGES, an institute in charge of risk assessment along the food chain from agriculture to food safety, composed of 59 laboratories.

Bertrand LOMBARD, Co-ordinator of the Community Reference Laboratory Milk & Milk Products (CRL MMP), introduced the meeting with a special thank to Claudia KRALIK and AGES for hosting this workshop, and also the Austrian Ministry of Health for supporting the social event organised on Thursday evening. He also recalled that the first workshop dedicated to alkaline phosphatase (AP) activity had taken place in Maisons-Alfort on 24 & 25 October 2002.

Roll-call of delegates

Each delegate introduced himself (see the list of attendance, in annex 1). 27 NRLs from 25 EU Member States (MSs) or countries from the European Free Trade Association (EFTA) were represented, as well as EC/ DG SANCO “Health & Consumer Protection” (Thierry CHALUS and Paolo CARICATO), and the CRL MMP, Alkaline Phosphatase team, and the CRL coordination team. Apologies were received from Celcídina PIRES-GOMES (PT-NRL).

Roger WOOD, from UK FSA (Food Standards Agency, i.e. UK competent authority) was also invited as an expert and particularly for his worldwide known skills on statistics.

Thierry CHALUS took this opportunity to inform the NRLs that it was his last workshop with the milk and milk products network, and to introduce Paolo CARICATO who was going to follow-up on this topic. Bertrand LOMBARD, in the name of the CRL, thanked him warmly for his support to the work of the CRL and NRL network during all these years.

The agenda and presentations are available on the following URL:

http://crl.ergap.free.fr/espacc/?key=ce49a9ed59c8a880437bca4a0596a3da

2. General information

T. CHALUS shortly reminded the roles and duties of the CRL and NRLs. Further to a request at the 2007 workshop, Thierry CHALUS promised that he would prepare after the present meeting a flow-chart on the EU regulations for milk hygiene.
3. Proficiency tests on alkaline phosphatase

3.1. Outcome of the meeting of the statistics working sub-group

Marina NICOLAS (CRL MMP, Unit CALAS) presented the conclusions of the meeting organised on 24/01/2008 in Brussels.

- To take for the assigned value the robust mean.
- To take for the standard deviation for proficiency assessment (op) the standard deviation obtained from the participants results.
- To calculate z-scores for each replicate and for all results even those not respecting the repeatability limit.

3.2. PTs 2006 and 2007

Anne-Cécile BOITELLE (CRL MMP, Unit CALAS) presented the results of the 2 last PTs.

A reminder was made on the 2005/2006 PT: the outcome had been already presented at the 2007 Workshop. The 2007 PT was dedicated to cow’s milk. 42 samples of whole, semi-skimmed and skimmed milk were dispatched to each NRL. The results were satisfactory, the repeatability and reproducibility values of the LNRs network were better that the values given in the EN ISO Standard. Two laboratories did not send back results, which was regarded as a non satisfactory situation: the CRL will send a letter to DG SANCO on that. One laboratory was rejected because of late submission of results and because the method specifications were not respected and another one was also eliminated from the statistical evaluation because of an important bias compared to all other results. As for the individual performance of laboratories, only one laboratory showed a high frequency of unsatisfactory z-scores. A new version of the report will be dispatched after the workshop, including the outcome of the exchanges with the participants having had unsatisfactory results.

⇒ The PT scheduled in 2008 had to be postponed to 2009, with the agreement of DG-Sanco, due to financial constraints, it would be dedicated to goat’s milk.

3.3. Alternative statistical evaluations

Miguel Ángel RODRÍGUEZ LOPERENA (Laboratorio Agroalimentario de Santander, SPNRL) presented the different alternative statistical evaluations using in particular robust statistics.

3.4. Determination of AP in milk: robustness of the method

Birgitt ROSSMANN (AGES, AT-NRL) presented the work done in her laboratory on these aspects.
4. Goat’s and ewe’s milk: legal limits

Marina NICOLAS introduced the topic, stressing that the case of goat’s milk is different from that of cow’s milk, given the lack of industrially pasteurised goat’s milk. Thus the study could only be conducted under laboratory pasteurisation conditions.

Experimental studies had been previously undertaken and had shown that a limit of 356 mU/l for a properly pasteurised milk could be acceptable for France, Portugal, Spain and Switzerland. But at the 2005 workshop, Theofanis SAGRIS (Veterinary Laboratory of Larissa, GR-NRL) had reported concerns that milk from some Greek goat species may give higher AP levels.

4.1. Results on goat’s milk by NRLs

Thomas BERGER (Agroscope Liebefeld-Posieux, CH-NRL), Constantinos ECONOMIDES (Laboratory for the Control of Foods of Animal Origin, CY-NRL) presented the studies performed in their laboratories. Marina NICOLAS presented results on Greek, Slovak and Romanian goat milk samples.

The outcome was that the 350 mU/l limit was acceptable for Switzerland but not acceptable for Cyprus, Greece and Romania (limit not respected for properly pasteurised milk).

⇒ Investigation on the influence of fat content and somatic cells should be conducted, along with the AP content.

4.2. Discussion on legal limits

One solution could have been to settle a limit with derogations in special cases for some MSs, but Thierry CHALUS explained that having a criterion for proper pasteurisation based on a limit with derogations may not be a good solution.

⇒ After a round table, it was decided to launch a second round of experiments to have a broader view of AP levels in goat’s milk. This study should involve in particular NRLs from countries which had not taken part to the first trial. In addition, NRLs would be asked to provide information on the national market of pasteurised goat’s milk. A circular letter would launch this second trial with results to be sent back to the CRL by 31 October 2009.

5. Milk from other species: pertinence of AP to control appropriate pasteurisation

5.1. Introduction

Thierry CHALUS introduced the topic in order to establish the frame of AP control. He mentioned that Regulation 853/2006, compared to the former Directive 92/46, has enlarged the scope of milk hygiene to all farm species, including mare, ass, camel. Moreover, he stressed that AP is tested not only to check if the pasteurisation is performed properly to guarantee consumers’ health, but also to guarantee animal health. The aim is to prevent importing diseases which may be carried by dairy products and which may be transmitted to European animals not infected before.
5.2. Mare milk

Jan DE BLOCK (ILVO - Eenheid Technologie en Voeding, BE-NRL) presented a study performed on mare milk. Mare milk is commercialised in Belgium, Germany, and Norway. This milk is of nutritional interest. As raw milk has low AP values (approx. 2 500 mU/l), AP cannot be a good marker of pasteurisation.

⇒ The CRL mandate would need to be enlarged to the investigation of other pertinent markers of pasteurisation than AP.

5.3. Ass milk

Luisa PELLEGRINO (Università degli Studi di Milano, Italy, for the IT-NRL) presented a study performed on ass milk. Ass milk is recognised to have a composition close to human milk, and is appropriate to prevent infant allergy. In this case AP was a good marker: AP levels for raw milk are at about 10 500 mU/l and decrease to about 200 mU/l when pasteurised.

5.4. Camel milk

Marina NICOLAS presented this topic.

United Arab Emirates had asked DG SANO an authorisation to export camel milk to Europe. This authorisation cannot be given because the AP content in camel pasteurised milk is too high and consequently doesn’t comply with the limit set for cow milk in the EU Hygiene rules. It was shown experimentally that phosphatase is not a pertinent marker to control correct pasteurisation in camel milk.

⇒ The CRL will collaborate with the Dubai Central Veterinary Laboratory on a project aiming to identify a pertinent marker for camel milk and validate analytical tools.

6. Alternative methods

6.1. Overview of a commercial PT scheme – The criteria approach

Roger WOOD presented the criteria approach, the overview of a commercial proficiency testing scheme. He presented in particular a comparison of the Fluorophos method and the Novalum method, concluding to a significant bias between the 2 methods and, in terms of precision, the Novalum being much more variable (2-3 times) than the Fluorophos. This finding can support the current choice of the Fluorophos as the reference method for official control in EU (see Regulation 1664/2006).

6.2. Equivalence of alternative methods versus the official reference method – use of conversion factors

Marina NICOLAS and Anne-Cécile DOITELLE presented that topic. Comparative studies were performed by the CRL between Novalum and Fluorophos methods. US FDA recognizes
both methods. But to fit the Fluorophos results, either the developer of the Novahum needs to change the calibration or a conversion factor needs to be defined.

Marina NICOLAS and Bertrand LOMBARD mentioned also a new approach for method validation, called accuracy profile, which enables interpretation of both the bias and precision, currently introduced in a French AFNOR Standard (NF V03-110) on in-house validation of quantitative chemical methods, as well in EN ISO 16140 Standard on method validation in food microbiology.

⇒ It was agreed that the CRL would investigate first requirements for method acceptance, then the possible use of the approach of accuracy profile (acceptability limits) to assess the equivalence between the Fluorophos and the Novahum methods.

7. Cheese, legal limits for pasteurised cheese

7.1. Analytical tools and revision of the ISO 11816-2/IDF 155-2 Standard

Marina NICOLAS presented this part. The CRL study, conducted preliminary to the revision of the IDF/ISO Standard, showed that the protocol for AP in cheese is much more efficient when using Turrax for sample preparation and the specific cheese buffer for extraction. The price of the buffer had been a limiting factor to its use but a reduction by half will apply from January 2009.

7.2. Study of French soft cheeses

Caroline DESBOURDES (having worked with the CRL MMP team on a one-year basis), presented a CRL study on French soft cheeses. According to this study, a limit of approximately 10 mU/g can be established for cow’s soft cheeses made from pasteurised milk. Further studies should be performed on pasteurised hard cheeses.

7.3. Study of Italian cheeses

Luisa PELLEGRINO presented an important study conducted on Italian hard cheeses. It appears that for Grana Padano, AP levels were very low due to the process of this raw milk’s cheese, except under the surface. AP controls should therefore be conducted only under the surface for big size cheeses.

7.4. Discussion

Only limits for pasteurised cheeses can be established in the frame of European legislation (Regulation 853/2006). Based on the present studies, a limit of about 10 mU/g could be envisaged for soft and hard cheeses.

⇒ Further experiments on soft and hard cheeses should be conducted by NRLs, especially others than the ones having already performed tests. For the time being, the experiments should concern soft and hard (not blue) cheeses from cow’s milk. The CRL would launch these experiments with an experimental design by a circular letter.
8. AP reactivation

8.1. AP, heat treatment and reactivation

Karl EKNER (Eurofins Norsk Matanalyse, NO-NRL) presented a project he had conducted in 1992 dealing with AP inactivation correlated to Salmonella and Listeria.

8.2. AP Reactivation, State of the art

Caroline VIGNAUD (CRL MMP, Unit CALAS) presented in a very complete way the state of the art regarding AP reactivation and recapitulated a number of questions still unanswered. CRL will continue investigations on this issue.

8.3. Discussion

Marina NICOLAS reported that a 6-months’ study on AP reactivation would be conducted in CRL in 2009. The CRL intends to investigate conditions that promote AP reactivation, the specificity of the phenomenon to matrices/processes, comprehension and, if possible, optimisation of the analytical method used to determine reactivated phosphatase.

Some NRLs reported cases of reactivation: Bernadette HICKEY (IRL-NRL) for cream, Miguel Angel RODRIGUEZ LOPERENA (SP-NRL) with butter (at 80°C) and UHT milk after 10 days. Several NRLs expressed the need to have a method to test reactivated AP, in particular in order to correctly interpret some positive results for AP.

Marina NICOLAS reported that the US were also very interested in this topic and particularly regarding reactivation in cheese. Thierry CHALUS considered that this issue called for some fundamental research. He therefore encouraged the CRL to prepare, in due time, with interested NRLs a research project, to submit to DG-Research. DG SANCO would support the project.

9. Setting up the work program for the following years

According to the needs identified during the workshop, and at the condition that the necessary resources will be made available to CRL MMP, a tentative work programme for 2009 was established (see Annex 2).

10. Closure of the workshop

B. LOMBARD closed the meeting on Friday at 1:30 pm, hoping that it was up to LNRs’ expectations. He thanked all the attendees for their participation and active contributions to the workshop.
11. Visit of the laboratory

Claudia Kralik and Julia Kathan (staff of the NRL for *L. monocytogenes*) guided two groups to a visit of the Austrian NRL activities including the NRL for *L. monocytogenes* and the microbiology department.
## 12. Annex 1 – List of attendance

### List of Attendance

**11th NRLs Workshop on Milk & Milk Products**

**October 9 & 10, 2008**

**AGES Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH**

**Vienna, Austria**

<table>
<thead>
<tr>
<th>Name and Surname</th>
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11th NRLs Workshop for Milk & Milk Products October 9 & 10, 2008.

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09/10 Oct 2008
**Annex II  Minutes of the CRL/NRLs Workshop 2008, continued**

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11th NRLs Workshop for MILK & Milk Products October 9 & 10, 2008.
Annex II  Minutes of the CRL/NRLs Workshop 2008, continued
Annex III  Outcome of the questionnaire on reference materials for somatic cells counting

Enquiry on the Reference Materials for Somatic Cells Counting in Raw Milk

Outcome of the enquiry to the European suppliers
13 February 2008

Bertrand LOMBARD, Coordinator
Community Reference Laboratory “Milk”, AFSSA-LERQAP
A. Background

The European Regulation 853/2004 lays down specific hygiene rules for food of animal origin. Its Annex III, Section IX is dedicated to raw milk and dairy products, and it defines in particular a criterion on somatic cells count (SCC) for raw cow's milk\(^1\).

Regarding SCC, together with the network of National Reference Laboratories (NRLs), the importance of Reference Materials (RMs) was recognized. Indeed, for this determination, it was acknowledged that the reference method prescribed by Regulation 1664/2006 for controls performed in the frame of Regulation 853/2004, the microscopic method as in Standard ISO 13366-1, could not be used in routine and was difficult to implement correctly, being based on microscopy. Thus the use of RMs, in combination with the reference method or not, was regarded as necessary to determine reference values for SCC and to calibrate routine (instrumental) methods used to perform SCC controls.

The quality of RMs for SCC was therefore recognized of utmost importance. It was also noted that several RMs with different characteristics were provided in different European countries, thus leading to different reference values coexisting in Europe. In that frame, it was agreed that the CRL Milk would launch an enquiry to the suppliers of RMs for SCC in Europe. Based on its outcome, the CRL, in cooperation with the NRLs, may recommend certain RMs for use in the frame of Regulation 853/2004.

The enquiry has been launched by circular letter dated 03/10/07, replies were asked by 12/11/07.

Seven replies were received from:
- Mr Jim FLYNN, Teagasc, Cork, IRL;
- Mrs Petra FRANTLOVA, SVU, Prague, CZ;
- Mrs Suzana VACZIOVA, SVU, Nitra, SK;
- Mrs Silvia ORLANDINI, AIA, Roma, IT;
- Mr Harris VAN DEN BIDGAART, Qlip, Zutphen, NL;
- Mrs Karin KNAPPSTEIN, BIEL, Kiel, D;
- Mrs Jolanta ROLA, PIWET, Pabowy, PL.

A synthesis of the replies received is given thereafter. This synthesis will be discussed at the next general workshop of the NRLs Milk in 2009. The CRL, in cooperation with the NRLs, may recommend certain RMs for use in the frame of Regulation 853/2004.

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\(^1\) See Chapter I, clause III: ≤400 000 SCC/ml, rolling geometric average over a 3-month period, with at least one sample per month, unless the competent authority specifies another methodology to take account of seasonal variations in production level.
Annex III  Outcome of the questionnaire on reference materials for somatic cells counting, continued

Q.1 – Types of RMs for SCC provided
(UHT) whole milk: 3 RM providers\(^2\), half skimmed milk: 2
Raw milk: 4
Heat-treated milk: 4
Concentration range provided: 150 000 – 1 500 000 SCC/ml
Volume of each vial: 10 – 50 ml

Comments: UHT milk used to dilute raw milk to the targeted concentrations.

Q.2 - Production of RMs for SCC

Q.2.1 – Origin of the somatic cells (blood leucocytes, milk)?
Milk: 6
Bovine blood leucocytes in UHT milk: 1

Q.2.2 – Stabilization of the samples (thermal/chemical treatment-use of conservatives)
Chemical: 5 (Bronopol, Bronopol/potassium dichromate, Azidiol)
Heat treatment in association with a chemical conservative: 3

- Microscopic method only: 2
- CECALAIT standards (5: 0–800 cells, weekly): 1
- Microscopic method in combination with an instrumental method (Fossomatic): 4
  - use of the microscopic method by the SCC RM provider;
  - combination/comparison of the microscopic values with values obtained with 3 other laboratories (1 case) and with instrumental values in proficiency testing trials organized by the RM provider (8 – 70 laboratories);
  - uncertainty of the RM values: derived from the reproducibility standard deviation of the PT trials.

Q.4 – Quality Assurance measures to ensure the quality of RMs

Q.4.1 – Homogeneity studies
Performed: 5

Q.4.2 – Stability studies
Performed: 4 (at the expiry date)

Q.4.3 – Expiry date
All producers fix expiry dates: 5 days (1), 10 days (1), 1 month (2), 3 months (1), 4 months (2)

\(^2\) If not otherwise specified in the following, the figures given relate to the number of RM providers.
Annex III  Outcome of the questionnaire on reference materials for somatic cells counting, continued

Q.4.4 – Critical factors for shipment and use. SOP for the users

- Shipment and storage at refrigerated temperature (sometimes temperature to be recorded at arrival);
- RM to be tempered (40°C) and homogenized before use; heating/cooling process not to be repeated;
- Storage in lying position to be avoided;
- SOP for users: 4.

Q.4.5 – In the assurance quality system, systematic feed-back procedure for possible problems from customers

Yes : 3 (at least yearly feed-back questionnaire)

Q.4.6 – Link to other RM via inter-laboratory trials

All RM providers are related to at least an outside PT inter-laboratory trial: from 1 to 5 (national schemes or international ones, such as CECALAIT, BfEL-Kiel, ALP).