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ACTILAIT

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ARTICLE

QUALITY PROVISIONS FOR THE PRODUCTION OF Cecalait® SECONDARY REFERENCE MATERIALS (SRMs)

For many years, Actilait (technical institute for milk and dairy products) has been proposing external reference materials (SRMs) for the calibration or control of methods in chemistry and microbiology, under the trademark Cecalait®.
Within the context of the quality control of analytical methods, secondary reference materials are complementary tools to proficiency tests to ensure the quality of the laboratories' determinations.
These secondary reference materials must therefore be produced according to a series of very strict quality provisions:
- From the outset, for milk SRMs, the milk must be selected and undergo treatments to avoid any physical alteration in order to ensure its quality and good preservation.
- The determination of the assigned values is the key point of the process. It is carried out by "expert" laboratories, rigorously monitored by the quality surveillance committee (CSQ). Moreover, a series of complementary technical dispositions ensure the quality of the assigned values sent to user laboratories.
- Finally, the express transport in well-defined conditions concludes this process.

A reference material is defined as a material (sample) one or more of whose properties are sufficiently established and defined.

Many types of reference materials exist:
- external reference materials (MRE), whose assigned value per analyte is determined by an interlaboratory test,

- internal reference materials (MRI), whose assigned value is established by the user (comparison with the MRE value, pure solutions...).

The Cecalait® reference materials proposed by Actilait are external reference materials (MRE). Two types of samples are proposed according to their purpose:

① **Calibration of a measuring instrument:** In this case, the samples are often multi-level, covering the totality of the range of application of the method. They are used to establish the calibration function (most often according to a linear model) between the instrument and the reference. Within the context of quality control associated with the adjustment, the laboratory will then proceed with a verification step (with or without specific samples) and an examination of the statistical parameters of the calibration (slope, standard deviation of deviations, residual standard deviation of regression...).

Concerning Cecalait® SRMs, this is the case for amido black, somatic cells, lipolysis (copper soap), urea, cryoscopy and infrared (median and high range (pre-calibration)) samples.

② **Quality control of measurements*:*** In this case, the samples are mainly mono-level. Following the analysis of the material by the laboratory, the results obtained are compared with the reference value and the laboratory interprets the deviation observed in relation to the tolerance (Shewhart-type control charts, comparison with a tolerance fixed beforehand, calculated according to the principle of the critical difference at 95 %).

Concerning Cecalait® SRMs, are included according to the matrix or the domain:

- *Milk: chemical method (Gerber, Kjeldahl, dry matter…),*
- *Butter and cheese: composition parameters (dry matter / moisture, fat, nitrogen, chloride…),*
- *Microbiology: microorganisms at 30 °C, coagulase positive Staphylococcus and E. coli.*

The production and determination of these samples are subjected to a series of measures throughout the various phases of the process.

The process can be split up into 3 main stages:

- Sample preparation,
- Determination of assigned values,
- Dispatch to clients.

1- Sample preparation:

In general, the physico-chemical samples are prepared in-house either from milk (preserved with bronopol) or dairy products, or externally in pilot industrial plants. The microbiological samples are prepared with milk and then freeze-dried to ensure their stability.

♦ *Chemistry:*

• Concerning the samples prepared with raw milk, the bacteriological quality of the milk has to be good and it must not have undergone any physical treatment that may deteriorate it, such as pumping, cooling or reheating... In practice, it will always be from small quantities of milk, from a single milking without any cooling and directly taken from the cheese dairy when the milk is delivered.

Moreover, all the pre-treatment operations must not deteriorate the milk or its components. With suitable equipment it is therefore possible to carry out the following operations: siphoning, separation of compounds by micro- or ultra-filtration.
Supplementary stabilisation treatments can, if necessary, be applied for certain criteria (for example heat treatment for lipolysis samples).

• For the other matrixes (butter, cheese), the samples supplied by pilot plants must be received in their final format. The pilots have been chosen to ensure the homogeneity of the batches sent to the laboratories (test performed in the sample feasibility phase).

• The principle of the distribution in vials is to ensure the quality of the milk (in particular fat) and guarantee the homogeneity of the batch produced. For milk samples, the initial mixtures are distributed in vials under constant magnetic agitation and without any air being incorporated by siphoning. The vials used in this case are provided with screw-caps (triple seal) to ensure air tightness and are filled to the brim to avoid churning.

♦ *Microbiology:*

• For the microbiological samples, the chosen treatment has to fulfil 2 objectives: guarantee the homogeneity between vials and obtain samples representative of a natural sample (diversity of the flora) to ensure the transferability of the results obtained to the routine samples.

• For the preparation of SRMs for the enumeration of microorganisms at 30°C, raw milk from several producers, with a natural, varied flora, is used. Concerning the SRMs for the enumeration of staphylococci and *E. coli*, the samples are prepared with sterilised milk contaminated with both of these strains and associated flora, isolated from dairy products.

• The freeze-drying diagram and the rehydration protocol have been studied so as not to deteriorate the bacteria and to enable their revivification before analysis.

- Homogeneity controls

Homogeneity tests are performed with routine analysers: infrared analyser for milk (for fat), flow cytometry germ counter for the microbiological parameters, cell counter for somatic cells. These controls are not systematically carried out on all the batches produced.

2- Determination of assigned values:

The assigned (reference) values are obtained by a specific interlaboratory test integrating 4 or 5 expert laboratories (according to the criteria).
These expert laboratories have been chosen because they have ISO 17025 accreditation, where possible, for the criteria under consideration, and are monitored yearly according to very strict specifications:

- Participation in at least two proficiency tests per year (of which at least 1 Cecalait®) with a minimum performance level of 75 % (3 tests out of 4 within the accepted tolerances).
- Inclusion of the laboratory's results in the calculation of the assigned values in at least 75 % of cases.

Each year the Cecalait® quality surveillance committee of Actilait decide on the renewal or not of the expert laboratories on the basis of their results over two rolling years in relation to the specifications.
In case of non-renewal the laboratory is informed and a new expert laboratory is sought for the criterion considered.

For the determination of the assigned value, the expert laboratories receive the samples for analysis. A series of quality provisions are integrated according to the criteria and the matrixes:

- Analysis in duplicate (Gerber, dry matter, nitrogen…),
- Analysis of month M-1 batch (with blind codification of month M and M-1 samples),
- Analysis of sample stability (fat by extraction),
- Analysis of pure solutions (Kjeldahl…),
- Instrumental verification of the results (lipolysis, somatic cells...).

The tolerances are accurately formalised for each procedure (maximum deviation between duplicates, tolerance according to M-1 samples, % recovery of pure solutions…).

Firstly, laboratories are selected on the basis of the quality controls above. The mean of their results is then calculated after elimination if necessary of any results "detrimental to the symmetry".
In the case of certain calibration samples (lipolysis, urea, somatic cells), a linearization of the different contents is realised according to the dilution factors used (mixture of a "rich" base and a "poor" base).

To be validated as the assigned value, the calculated mean has to fulfil two additional criteria: a minimum number of results taken into account and a maximum range (standard deviation of the series). These provisions, "minimum number of results" and "maximum standard deviation", are formalised for each SRM in the quality documentation.

If not, (failure to meet one or more of these provisions), the samples are sent to the expert laboratories again for analysis (2^{nd} analytical series).
The assigned value is then calculated as above, integrating the results of both analytical series.

Following this determination, a certificate indicating the assigned value(s), the associated uncertainty (in the majority of cases) and the use-by date is issued for this SRM.

3- Dispatch to clients:

Specific methods of packaging exist according to the matrixes and criteria of the SRMs:

- insulated boxes with ice or not.

These provisions are accurately established for each type of SRM (type of box, presence or not of ice and number of ice packs according to the box).

The samples are then sent by "express" for delivery the next day in the majority of European Union countries and within 2 to 3 days for other countries.

Only compliance with all these provisions guarantees the quality of the samples and the associated assigned values sent to the laboratories. Thus their use will effectively contribute to the process of controlling the quality of the results.

STANDARDS, DRAFT STANDARDS

Classification in alphabetical order by theme

ISO standards under development

MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS	
ISO/DIS 7218:2007/DAmd1 March 2012	MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS General requirements and guidance for microbiological examinations – Amendment 1

ISO published standards

CHEESE AND PROCESSED CHEESE	
ISO 27871:2011 (IDF 224) October 2011	CHEESE AND PROCESSED CHEESE Determination of the nitrogenous fractions
LACTOSE	
ISO 12779:2011 (IDF 227) November 2011	LACTOSE Determination of water content – Karl-Fischer method
LAIT	
ISO/TS 17193:2011 (IDF 208) December 2011	MILK Determination of the lactoperoxydase activity – Photometric method (Reference method)
MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS	
ISO/TS 10272-3:2010/Cor1:2011 December 2011	MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS Horizontal method for detection and enumeration of Campylobacter spp. – Part 3: Semi quantitative method – Technical corrigendum 1
MILK AND DAIRY PRODUCTS	
ISO 13082:2011 (IDF 218) November 2011	MILK AND MILK PRODUCTS Determination of the lipase activity of pregastric lipase preparation
SENSORY ANALYSIS	
ISO 3972:2011 October 2011	SENSORY ANALYSIS Methodology – Method of investigating sensitivity of taste

NEW EU REGULATIONS

Classification is established in alphabetical order of the first keyword

DIOXINS

O.J.E.U. L 320, 3rd December 2011 – Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:320:0018:0023:EN:PDF

FOOD ADDITIVES

O.J.E.U. L 295, 12th November 2011 – Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:295:0001:0177:EN:PDF

STANDARDS - REGULATIONS

O.J.E.U. L 295, 12th November 2011 – Commission Regulation (EU) No 1130/2011 of 11 November 2011 amending Annex III to regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives by establishing a Union list of food additives approved for use in food additives, food enzymes, food flavourings and nutrients

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:295:0178:0204:EN:PDF

O.J.E.U. L 295, 12th November 2011 – Commission Regulation (EU) No 1131/2011 of 11 November 2011 amending Annex II to regulation (EC) No 1333/2008 of the European Parliament and of the Council with regard to steviol glycosides

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:295:0205:0211:EN:PDF

FOOD INGREDIENT

O.J.E.U. L 313, 26th November 2011 – Commission Implementing Decision of 24 November 2011 authorising the placing on the market of flavonoids from *Glycyrrhiza glabra* L. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:313:0037:0040:EN:PDF

O.J.E.U. L 313, 26th November 2011 – Commission Implementing Decision of 24 November 2011 authorising the placing on the market of yeast beta-glucans as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:313:0041:0044:EN:PDF

HEALTH CLAIMS

O.J.E.U. L 299, 17th November 2011 – Commission Regulation (EU) No 1171/2011 of 16 November 2011 refusing to authorise certain health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:299:0004:0006:EN:PDF

HYGIENE

O.J.E.U. L 008, 12th January 2012 – Commission Regulation (EU) No 16/2012 of 11 January 2012 amending Annex II to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the requirements concerning frozen food of animal origin intended for human consumption

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:008:0029:0030:EN:PDF

INFORMATION TO CONSUMERS

O.J.E.U. L 304, 22nd November 2011 – Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation No 608/2004

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF

PESTICIDES / VETERINARY DRUGS

O.J.E.U. L 285, 1st November 2011 – Commission Implementing Decision of 27 October 2011 amending Decision 98/536/EC establishing the list of national reference laboratories for the detection of residues

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:285:0046:0052:EN:PDF

O.J.E.U. L 325, 8th December 2011 – Commission Implementing Regulation (EU) No 1274/2011 of 7 December 2011 concerning a coordinated multiannual control programme of the Union for 2012, 2013 and 2014 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:325:0024:0043:EN:PDF

PROTECTED DESIGNATIONS OF ORIGIN

O.J.E.U. L 260, 5th October 2011 – Commission Implementing Regulation (EU) No 987/2011 of 30 September 2011 entering a name in the register of protected designations of origin and protected geographical indications [Queso Casin (AOP) (cheese)]

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:260:0011:0012:EN:PDF

STANDARDS - REGULATIONS

O.J.E.U. L 260, 5th October 2011 – Commission Implementing Regulation (EU) No 987/2011 of 30 September 2011 entering a name in the register of protected designations of origin and protected geographical indications [Nanoski sir (AOP) (cheese)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:260:0013:0014:EN:PDF

O.J.E.U. C 304, 15th October 2011 – Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Nostrano Valtrompia (AOP) (cheese)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2011:304:0015:0018:EN:PDF

O.J.E.U. C 304, 15th October 2011 – Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Squacquerone di Romagna (AOP) (cheese)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2011:304:0019:0022:EN:PDF

O.J.E.U. L 297, 16th November 2011 – Council Decision of 20 October 2011 on the conclusion of the Agreement between the European Union and the Swiss Confederation on the protection of designations of origin and geographical indications for agricultural products and foodstuffs, amending the Agreement between the European Community and the Swiss Confederation on trade in agricultural products
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:297:0001:0002:EN:PDF

O.J.E.U. L 297, 16th November 2011 – Agreement between the European Union and the Swiss Confederation on the protection of designations of origin and geographical indications for agricultural products and foodstuffs, amending the Agreement between the European Community and the Swiss Confederation on trade in agricultural products
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:297:0003:0047:EN:PDF

O.J.E.U. C 345, 25th November 2011 – Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Ser Korycinski Swojski (PGI) (cheese)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2011:345:0019:0023:EN:PDF

O.J.E.U. L 330, 14th December 2011 – Commission Implementing Regulation (EU) No 1298/2011 of 9 December 2011 approving a non-minor amendment to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Pélardon (PDO) (cheese)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:330:0007:0008:EN:PDF

O.J.E.U. C 364, 14th December 2011 – Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Bovski Sir (PDO) (cheese)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2011:364:0025:0028:EN:PDF

O.J.E.U. L 343, 23rd December 2011 – Commission Implementing Regulation (EU) No 1377/2011 of 20 December 2011 entering a name in the register of protected designations of origin and protected geographical indications [Salvo Cremasco (PDO) (cheese)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:343:0016:0017:EN:PDF

AFNOR VALIDATIONS

During its last meeting, the Technical Committee of NF VALIDATION approved by vote:

Commercial name	Date	Certificate	Description
NEW VALIDATIONS			
VIDAS UP *SALMONELLA*	Validation date: 6 Oct 2011 **End of validity: 6 Oct 2015**	**BIO-12/32-10/11**	**Detection of *Salmonella* spp.** All human food products (except raw milk cheese), animal feeding stuffs and production environment samples (except primary production stage)
IRIS *SALMONELLA*	Validation date: 7 Oct 2011 **End of validity: 7 Oct 2015**	**BKR-23/07-10/11**	**Detection of *Salmonella* spp.** All human food products, animal feeding stuffs and production environment samples (except primary production stage environment)
REVEAL *SALMONELLA* 2.0	Validation date: 7 Oct 2011 **End of validity: 7 Oct 2015**	**NEO-35/01-10/11**	**Detection of *Salmonella* spp.** All human food and animal feed
MICROSEQ® *LISTERIA* SPP.	Validation date: 1 Dec 2011 **End of validity: 1 Dec 2015**	**ABI-29/04-12/11**	**Detection of *Listeria* spp.** All human food and environment samples
MICROSEQ® *LISTERIA MONOCYTOGENES*	Validation date: 1 Dec 2011 **End of validity: 1 Dec 2015**	**ABI-29/05-12/11**	**Detection of *Listeria monocytogenes*** All human food and environment samples
RENEWAL OF VALIDATIONS			
TRANSIA PLATE *LISTERIA*	Validation date: 21 Nov 1995 Renewal: 11 Feb 2000, 11 Dec 2003, 4 Dec 2007 and 6 Oct 2011 **End of validity: 21 Nov 2015**	**TRA-02/06-11/95**	**Detection of *Listeria* spp.** All human food products and environmental samples
***SALMONELLA* PRECIS™**	Validation date: 4 Dec 2007 Renewal: 6 Oct 2011 **End of validity: 4 Dec 2015**	**UNI-03/06-12/07**	**Detection of *Salmonella*** All human and animal food products and production environment samples (except primary production stage environment)
SESAME *SALMONELLA* TEST	Validation date: 4 Dec 2007 Extension: 3 July 2009 Renewal: 7 Oct 2011 **End of validity: 4 Dec 2015**	**BKR-23/04-12/07**	**Detection of *Salmonella* spp.** All human food products and environmental samples
RAPID' *E. COLI* 0157:H7	Validation date: 27 Sep 2007 Renewal: 6 Oct 2011 **End of validity: 27 Sep 2015**	**BRD-07/14-01/08**	**Detection of *E. coli* 0157** All human food products and environmental samples
TAG 24 *SALMONELLA*	Validation date: 2 July 2007 Renewal: 1 Dec 2011 **End of validity: 2 July 2015**	**TRA-02/09-07/07**	**Detection of *Salmonella*** All human and animal food products
REBECCA™ BASE or REBECCA™ + EB	Validation date: 17 Jan 2008 Renewal: 1 Dec 2011 **End of validity: 17 Jan 2016**	**AES-10/06-01/08**	**Enumeration of *E. coli*** All human and animal food products

AFNOR VALIDATIONS

REBECCA™ + EB	Validation date: 17 Jan 2008 Renewal: 1 Dec 2011 **End of validity: 17 Jan 2016**	**AES-10/07-01/08**	**Enumeration of *Enterobacteriaceae*** All human and animal food products
EXTENSION OF VALIDATION			
ALOA ONE DAY	Validation date: 27 Sep 2000 Renewal: 7 Apr 2005 and 30 June 2008 Extension: 10 Mar 2006, 15 Sep 2006, 1 Apr 2010 and 6 Oct 2011 **End of validity: 27 Sep 2012**	**AES-10/03-09/00**	**Detection of *Listeria monocytogenes* and *Listeria* spp.** All human food products and environmental samples
PROLONGATION OF VALIDATIONS			
COMPASS *LISTERIA* AGAR	Validation date: 4 Dec 2007 **End of validity: 4 Dec 2011** **Prolongation till: 4 June 2012**	**TEC-24/03-12/03**	**Detection of *Salmonella*** All human and animal food products
3M™ TECRA™ UNIQUE *SALMONELLA* TEST	Validation date: 12 Dec 2003 Renewal: 4 Dec 2007 **End of validity: 12 Dec 2011** **Prolongation till: 12 June 2012**	**BKR-23/05-12/07**	**Enumeration of *Listeria monocytogenes*** All human food products and environmental samples

The validation certificates and the recapitulative list are available at the following website address:
http://www.afnor-validation.com/afnor-validation-validated-methods/validated-methods.html

BOOKSHOP: LATEST PUBLICATIONS

The classification in alphabetic order of the first keyword allows you to consult the references according to your interests. The web site allows you to know more, or to order the book.

OTLES S. – **Methods of analysis of food components and additives, second edition** – CRCPress – November 2011 – ISBN: 9781439855526 – 534 pages

http://www.crcpress.com/product/isbn/9781439815526

This second edition contains new chapters on analytical quality assurance, the analysis of carbohydrates and natural toxins in foods. The analytical methods for chemical preservatives, pesticide residues, food allergens, and radioactive contaminants are also presented.

FORTHCOMING EVENTS

Classified in chronological order

CHEESE AND DAIRY PRODUCTS

26-29 February 2012 Paris, France	Cheese and Dairy Products Show	http://www.salon-fromage.com

IN THE PRESS – ON THE WEB

Classification in alphabetical order of keywords

ANTIBIOTICS

ELISA kits for screening compounds in milk

http://www.laboratorytalk.com/news/ran/ran234.html

► Randox Food Diagnostics is offering new ELISA screening kits for the rapid detection of antimicrobials in milk and milk powder.

BIOGENIC AMINE

Scientific opinion on risk based control of biogenic amine formation in fermented foods

http://www.efsa.europa.eu/en/efsajournal/pub/2393.htm

► EFSA has emitted a scientific opinion concerning the risk of the biogenic amine in fermented foods. This qualitative evaluation was conducted using data from the scientific literature, surveys, reports and consumption data. The present knowledge and data on toxicity of biogenic amines are limited, nevertheless, histamine and tyramine are considered as most toxic for the food safety.

ESCHERICHIA COLI

Broth made for presumptive enumeration of *E. coli*

http://www.laboratorytalk.com/news/lbm/lbm169.html

► The Lauryl Sulphate Tryptose broth modified with MUG and Tryptophan allows presumptive enumeration of *E. coli* in milk and milk products.

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