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ARTICLE

EVALUATION OF RAPID TESTS FOR THE DETECTION OF β-LACTAM AND TETRACYCLINE ANTIBIOTICS IN MILK

The objective of this study was to evaluate two kits for the detection of β-lactam and tetracycline antibiotics in milk (kit 1: rapid detection of β-lactam and kit 2: rapid detection of β-lactam and tetracycline).
These kits, manufactured by Bioeasy, are commercialised in France by Humeau.
The tested method is based on a strip immuno-chromatographic technique and is applicable to two different matrixes: raw milk or reconstituted dried milk.
The study was performed in 2 steps:

1. **- Determination of the threshold detection of the method for 14 antibiotics (kit 1) or 18 antibiotics (kit2)**
2. **- Determination of the threshold detection and robustness of the method for 1 antibiotic.**

PRINCIPLE OF THE METHOD

This method is based on a strip immuno-chromatographic technique.

Inoculation and incubation:

200µl of sample are introduced, after homogenisation, in a micro-cell containing the reagent. This micro-cell is incubated during 3 minutes at 40 °C ±3 °C. This first step enables the interaction between the potential antibiotics contained in milk and the reagents contained in the micro-cell.
After this first incubation, the strip is introduced in the micro-cell. A second incubation during 3 minutes for the kit 1 and of during 5 minutes for the kit 2 enables the migration of the reactional mix in the strip.

Reading and interpretation of the results:

The reading was visually realised. An automatic reader exists but was not used within this evaluation.

You will find below the graph and the interpretation grid of the test results for the detection of a molecule:

Figure 1: Interpretation of the results

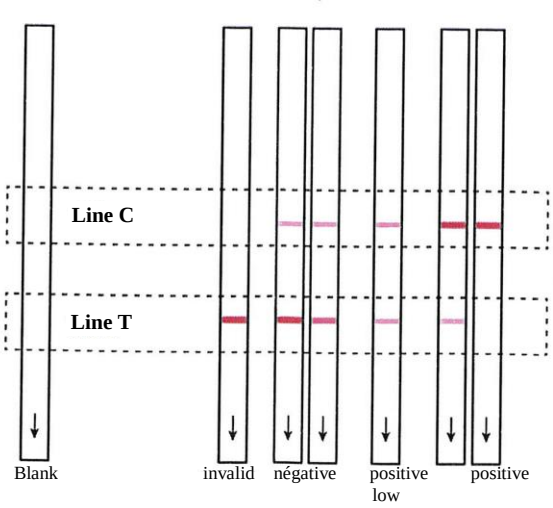

Tableau 1: Interpretation of the results

Comparison of the colour intensity between the T test line and the C control line	Results of analysis	Interpretation of the results
T>C	**NEGATIVE**	The milk sample do not contain antibiotic or contains a quantity of antibiotic lower than the limit value of detection (indicated in the notice).
T=C	**POSITIVE LOW**	The milk sample contains a quantity of antibiotic equal to the detection value (indicated in the notice).
T<C or absence of T line	**POSITIVE**	The milk sample contains a quantity of antibiotic higher than the detection value (indicated in the notice).

MATERIAL AND METHODS

1- Material

For this study, we used:
- metrologically verified 200 µl micropipette
- 3 incubators provided by Humeau for the incubation at 40 °C ±3 °C.

2- Basic raw milk

Each implemented basic raw milk was analysed by the method to evaluate and by the kit of an other provider (Kit C), based on the same strip immuno-chromatographic principle, in order to verify the absence of antibiotic in the basic milk.

2.1 Determination of the threshold detection for 14 antibiotics

For the both evaluated kits, the tests were performed with mix cow raw milk from PDO Comté cheese cooperative. The raw milk implemented was different for each tested antibiotic.

2.2 Determination of the detection threshold and robustness for 1 antibiotic

Two PDO mix raw milks and a non PDO mix raw milk were used to study the variability due to the origin of the milks.

3 - Preparation of the samples

The stock solutions were prepared at 1 mg/ml according to the annex 2 of the AFNOR certification N° NF102[1] requirements, which defines the solvent used for the stock solution, and the temperature and its maximum storage time. The standards were prepared by successive dilutions of the stock solution. Les solutions filles ont été préparées par des dilutions successives de la solution mère. A little volume of the final dilution was added in the basic raw milk to constitute the sample to test.

4 - Protocol

The limits of detection (LOD) forecasted by the provider are in the Humeau notice.
The kit C was used to verify the concentrations of the tested samples.

4.1 Determination of the detection threshold for 14 antibiotics or 18 antibiotics

14 molecules of β-lactam and 4 molecules of tetracycline were tested.
At least 3 concentrations per kit were evaluated for each molecule (announced, a little higher and a little lower LOD). According to the first results obtained, the following concentrations were re-adjusted.
For each antibiotic molecule and for 3 concentrations, 5 samples were analysed in duplicate, many other concentrations were tested on 1 sample in duplicate.
The evaluation was only performed on a batch. It was then not possible to evaluate the inter-batches variability.

4.2 Determination of the detection threshold and robustness for 1 antibiotic

To confirm the results obtained, a second study was performed on a molecule already tested in the first study by varying the batches of the kits and the origin of the implemented milk.
This second study was performed on dicloxacillin, which presented, in the first study, a limit of detection 10 times lower than the announced LOD. Three milk from different origins and two different batches per kit were evaluated.
The results obtained on 10 samples analysed in duplicate by concentration, type of milk and kit enable to determine the capacity of detection (Ccβ) according to the AFNOR certification N° NF102[1] recommendations. Ccβ is the most low content of substance that can be detected, identified and/or measured in a sample, with a β error probability of 5 %.

RESULTS

During the evaluation, any strip has presented invalid tests, the control line was always present.

1 Determination of the detection threshold for 14 antibiotics

The tables below present the totality of the results obtained during the evaluation after the analysis of the 5 samples in duplicate by concentration. The LOD announced by the provider and the maximum regulatory limits (MRL) according to the European Regulation No37/2010 are joined.
The observed LOD column summarises the limits of detection noted in this evaluation. A colour code enables to situate them according the LOD announced by the supplier.

Table 2: Limits of detection obtained for Kit No 1 (detection of β-lactamin)

Family	Antibiotic	LOD announced by the supplier (ppb)*	Observed limit of detection (ppb)*	MRL (ppb)*
ß-lactamin	Penicillin G	**1-1.5**	0.5-1	4
	Ampicillin	**2-4**	0.5-1	4
	Amoxicillin	**2-4**	0.5-1	4
	Oxacillin	**5-7**	2-3	30
	Cloxacillin	**5-7**	2-3	30
	Dicloxacillin	**10-20**	1-2.5	30
	Nafcillin	**20-30**	2.5-5	30
	Cefapirine	**8-10**	6-8	60
	Cefalonium	**6-8**	1-2	20
	Cefazoline	**40-50**	20-30	50
	Cefoperazone	**4-6**	1-2	50
	Cefquinome	**12-18**	2.5-5	20
	Ceftiofur	**80-100**	40-60	100
	Cefacetrile	**25-30**	15-20	125

Table 3: Limits of detection obtained for Kit N° 2 (detection of β-lactamin and tetracycline)

Family	Antibiotic	LOD announced by the supplier (ppb)*	Observed limit of detection (ppb)*	MRL (ppb)*
ß-lactamin	Penicillin G	**1.5-2**	1.5-2	4
	Ampicillin	**3-5**	1-3	4
	Amoxicillin	**3-5**	1-2	4
	Oxacillin	**5-7**	2-3	30
	Cloxacillin	**6-8**	2-3	30
	Dicloxacillin	**10-20**	1-2,5	30
	Nafcillin	**20-30**	2.5-5	30
	Cefapirin	**15-18**	6-8	60
	Cefalonium	**6-8**	2-4	20
	Cefazoline	**40-50**	30-40	50
	Cefoperazone	**4-6**	1-2	50
	Cefquinome	**12-18**	2.5-5	20
	Ceftiofur	**80-100**	40-60	100
	Céfacétrile	**25-30**	15-20	125
Tétracycline	Doxycycline	**30-50**	5-10	ND
	Chloretetracycline	**30-50**	10-20	100
	Oxytetracycline	**30-50**	5-10	100
	Tétracycline	**30-50**	10-20	100

Observed limit = announced LOD
Announced limit/2 < Observed limit < announced limit
Observed limit < announced limit/2

Key for reading the colour code for the interpretation of the differences between the announced LOD and the observed limits of detection

The limit of detection of kit 1 for Penicillin G, Cefapirin and Cefacetrile is between the LOD/2 and the announced LOD. For all the other molecules, the limit of detection observed is very below the announced LOD.

For kit 2, the limit of detection observed for Penicillin G corresponds to the announced LOD. For Ampicillin, Cefazolin and Cefacetrile, the limit of detection observed is between the LOD/2 and the announced LOD. For all the other molecules, the limit of detection observed is very below the announced LOD.

2 Determination of the detection threshold and robustness for 1 antibiotic

Table 4: Results by type of milk and by batch (in % of positive results for n analyses)

KITS	BATCH	Concentration in dicloxacillin (ppb)	Results	PDO milk No 1 n=20	Non-PDO milk n=20	PDO milk No 2 n=20
KIT No1	BATCH A	2.5	% pos	100%	100%	100%
		2	% pos	100%	100%	100%
		1.5	% pos	95%	30%	85%
	BATCH B	2.5	% pos	100%	100%	100%
		2	% pos	100%	100%	100%
		1.5	% pos	90%	30%	85%
KIT No 2	BATCH C	2.5	% pos	100%	100%	100%
		2	% pos	100%	95%	100%
		1.5	% pos	30%	0%	0%
	BATCH D	2.5	% pos	100%	100%	100%
		2	% pos	100%	95%	100%
		1.5	% pos	90%	0%	0%

Ccβ: capacity of detection

The capacity of detection for dicloxacillin is of 2 ppb for all the kits and milk tested, except for the kit No 1 on a milk and a batch with a Ccβ of 1.5 ppb, but the difference is not significant: 100% of positive results / 20 at 2 ppb, against 95% at 1,5 ppb.

Table 5: Results by kit (in % of positive results for n analyses)

Concentration in Dicloxacillin n=120	1.5 ppb	2 ppb	2.5 ppb
KIT No 1 Detection of β-lactam	69%	100%	100%
KIT No 2 Detection of β-lactam and tetracycline	20%	98%	100%

Ccβ: capacity of detection

The capacity of detection for dicloxacilline was globally evaluated at 2 ppb, and is identical for the both kits.

GENERAL CONCLUSION

This detection test for β-lactam and tetracycline antibiotics is quick and convenient to implement, and negative and positive control confirming the performance of the batch are from now on provided with the kit. Its implementation is simple and no particular technical qualification is necessary. The interpretation of the results by visual reading is not difficult thanks the 3 possibilities of results: negative, low positive or positive. Moreover, the lines, after air drying of the strips during 3 minutes, are more marked and it is easier to view the intensity, especially in limit of detection.

For the both kits, detection thresholds lower or equal to the LOD announced by the provider were observed in the first study performed on 10 molecules of β-lactam and 4 molecules of tetracycline.

These results with a determination of Ccβ at 2 ppb for the both kits were confirmed and refined with the second study. The robustness of the method was also demonstrated by this study performed with many batches, different origin milk and different technicians, during several days.

[1]*AFNOR certification N° NF102 : « Protocole de validation des méthodes de détection et de quantification des résidus de médicaments vétérinaires dans les produits agro-alimentaire - Exigences relatives aux études préliminaire et interlaboratores menées par un Laboratoire expert" révision n° 1 : 1er juin 2017*

ARTICLE

DETERMINATION OF TRUE PROTEIN CONTENT BY THE AMIDO BLACK METHOD Description and critical points

The amido black method is a practical method allowing the determination of the true protein content of milk [(NT-NPN) x 6.38]. This method is standardised at the AFNOR level as No NF V 04-216.
This method is applicable to cow, goat and ewe raw milk, and also processed milk if the protein composition (caseins-seric proteins report) has not been modified.

***Note**: NF V 04-216 standard contains also information annexes to determine the true protein content in products with contents different from milk and also for products with different protein composition.*

- **Analytical principle:**

The general principle of this method consists to add a amido black solution to a test sample of milk. This operation will create an insoluble complex between the proteins of the samples and the amido black. The complex is then eliminated by centrifugation (or filtration) and the optical density of the supernatant amido black solution is measured using a colorimeter (at λ of 578 or 620 nm), and transforms into true proteins concentration thanks a calibration equation.

- **The reagent: the amido black solution**

The amido black solution is constituted of:

- Amido black 10 B dye
- Sodium hydrogen orthophosphate (di-hydrated)
- Citric acid (monohydrated)

This solution can be bought in ready to use solution or realised by the laboratory according to the protocol described in the § 5.2.1 of the standard.
In the both cases, this solution has to be verified by the laboratory:

- pH must be of 2.4 ± 0.1. This characteristic is important for the formation of the insoluble complex when the solution is added to the milk test sample. To realise this verification, the pH-meter must be adjusted on the measurement range (from pH 2 to pH 7 for example).
- The optical density at 620 nm (c = 10 mm) of the solution at 1 % must be between 0.695 and 0.735. The accuracy of the dilution must be controlled to have a correct measurement (the distributor used for the milk sample test should be used to exactly deliver 1 ml of the amido black solution in a 100 ml graduated flask). The measurement should be performed with an optical path of 10 mm (± 0.1 mm), only a SOG (special optical glass) enables this dimension. A single-use plastic flask does not allow to guarantee this type of specification (its measurement is also very difficult due to the characteristics of the plastic used).

  The optical path directly affects the measurement of the optical density and this verification is necessary to ensure that the measurements will be made in the linearity field of the colorimeter.

- **The material:**

The amido black method uses many types of materials, which, must meet standardised or not requirements:

- **Tubes** allowing the distribution of milk and amido black solution. There is no real specification for these tubes. However, after distribution of milk and amido black solution, a significant "empty volume" must be available for a good stirring of the mix.
- **System(s) of distribution**
  The system(s) of distribution must meet NF V 04-216 requirements, that is:
  - 1 ml ± 0.02 with CV % < 0.15 % for the milk system of distribution
  - 20 ml ± 0.05 with CV % < 0.20 % for the amido black system of distribution

This verification must be performed according to the ISO 8655-6 standard requirements: Piston-operated volumetric apparatus: gravimetric methods for the determination of measurement error (on 10 measurements).

A fault to these requirements could cause, either repeatability problems (CV %), or a measurement zone out the linearity zone of the colorimeter (mean volume).
Many systems exist and may be used to distribute milk and amido black solution (single or combined) if they fulfill recommendations described above: automatic distributor for distribution of milk and amido black, positive-displacement pipette for milk and piston distributor for amido black.

- **Stirring system**

The objective of the stirring system is to permit the mix of the tubes and then the fixation of amido black on the proteins, a turning system is appropriate (wheels, mechanical system,…).
The tubes should be stir 10 minutes to ensure the optimal fixation of the dye on the proteins.

NB: As the number of « places » available on the stirrer is limited, amido black (or the milk-amido black mix) should be only distributed in tubes, which will be immediately stirred after the distribution.

- **Centrifuge**

The objective of the centrifugation is the separation of the pellet (proteins and amido black) and the supernatant (residual amido black solution) to measure its absorbance (concentration) using the colorimeter. This instrument has to produce an acceleration of about 350 g to allow the separation of the precipitate and the supernatant obtained after 5 minutes. In practice, the majority of laboratories use the centrifuges used for the « Gerber » method.

- **Photometer**

It must be equipped with a measurement cell with an optical path from 0.2 to 1 mm (in practice, we exclusively meet cells of 1 mm). The wavelengths must be between 550 and 620 nm (in practice, the wavelengths used, corresponding to the maximum of the amido black sensibility, are 578 and 620 nm).
At the liquid-conductive level, the pipe length in the cell must be limited, because it generates tracing (marking by a value of the 1st tube different from the other tubes of a same sample)
The apparatus piloted by a computer may allow an adjustment of the instrument.

NB: 2 instruments are authorised for use in the milk payment: ATL33 and CECIL 2031/2041

- **Calibration (adjustment) and verification:**

To predict results in g of true proteins per liter or by kg of milk, the photometer has to be calibrated using milk standard (realised from dried milk) setting the measurements (3 standards per range: Rich (R), Medium (M) and Poor (P) are available).

The software of the instrument enables to realise a prediction model between the absorbance of the supernatant solution and the concentration of proteins of the standard

According to the instruments, many mathematical models are possible: the curvilinear model is the most adapted (it is the nearest to the instrumental response), if it cannot be done, a linear model must be suitable (it could be then possible to recalculate the model externally (Excel) from absorbance obtained for the standard and their respective concentrations in proteins).

Concerning the procedure: after the realisation of a blank sample from a dilution of the amido black solution (prediction in « equivalent proteins in the order to 40 to 42 g/liter for a cow milk type range), analysis of at least 3 samples per standard to calculate the calibration function.

A verification must be then realised analysing the control milk (cow whole raw milk), whose the value obtained in proteins (3 samples at least) must be in accordance to the reference value of the control milk (maximum limit ± 0.15 g/liter). If the value of the control milk is non-concordant inside this limit, the initial adjustment is not validated.

- **Stability monitoring:**

It is necessary to verify the stability of the measurement for each analytical set. Indeed, many factors can influence the prediction using the initial calibration function: temperature of the samples and amido black solution (affecting the distributed volume), evolution of the distributors, intrinsic value of zero, drift of the photometer…).

This verification can be realised with transfer milks(1) (3 samples with contents near standards contents realised from raw milk and a control whole milk immediately defined after calibration) or standard milks and the control milk.

In practice, these milks are analysed at least in duplicate in each analytical set and the values observed are compared to the target values defined after the initial adjustment:

- If the values observed for the transfer milks remain in a limit ± 0.15 g/liter in relation to the target values, the stability of the measurement is confirmed
- If the deviations between the values obtained for one or many transfer milks and the target values are higher than ± 0.15 g/liter, calculate a mathematical correction of the results, either using a curvilinear correction equation, or using a mean factor of correction (on the basis of the values observed for the transfer milks). This mathematical correction is first applied to the « crude » value of the control milk: the mathematical correction calculated with the values of the transfer milks will be validated if the target is found with a tolerance of ± 0.15 g/liter.
- When the correction is validated, it is applied to the « crude » values of the other milks of the analytical set..

(1) *On prendra soin de vérifier la stabilité des laits de transferts sur la période d'utilisation*

Conclusion:

The amido black method is a practical and quick method enabling to predict results on milks equivalent to results obtained by the Kjeldahl method.
Its implementation is relatively easy but its procedure, particularly the adjustment and the verification operations of stability have to be control to ensure the quality of the results

STANDARDS, DRAFT STANDARDS

Classification in alphabetical order by theme

ISO standards under development

INFANT FORMULA AND ADULT NUTRITIONALS	
ISO/DIS 20636 December 2017	INFANT FORMULA AND ADULT NUTRITIONALS Determination of vitamin D by liquid chromatography-mass spectrometry
MICROBIOLOGY OF THE FOOD CHAIN	
ISO/DIS 17410 January 2018	MICROBIOLOGY OF THE FOOD CHAIN Horizontal method for the enumeration of psychrotrophic microorganisms
MILK, MILK PRODUCTS, INFANT FORMULA AND ADULT NUTRITIONALS	
ISO/DIS 15151 January 2018	MILK, MILK PRODUCTS, INFANT FORMULA AND ADULT NUTRITIONALS Determination of minerals and trace elements - Inductively coupled plasma atomic emission spectrometry (ICP-AES) method
ISO/DIS 21424 January 2018	MILK, MILK PRODUCTS, INFANT FORMULA AND ADULT NUTRITIONALS Determination of minerals and trace elements - Inductively coupled plasma mass spectrometry (ICP-MS) method
MILK AND MILK PRODUCTS	
ISO/DIS 17678 February 2018	MILK AND MILK PRODUCTS Determination of milk fat purity by gas chromatographic analysis of triglycerides (reference method)
SENSORY ANALYSIS	
ISO/DIS 13301 October 2017	SENSORY ANALYSIS Methodology - General guidance for measuring odour, flavour and taste detection thresholds by a three-alternative forced-choice (3-AFC) procedure

ISO published standards

MICROBIOLOGY OF THE FOOD CHAIN	
ISO 10272-1 June 2017	MICROBIOLOGY OF THE FOOD CHAIN Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 1: Detection method *Replace ISO 10272-1:2006*
ISO 10272-2 June 2017	MICROBIOLOGY OF THE FOOD CHAIN Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 1: Colony count technique *Replace ISO 10272-2:2006*
ISO 21528-1 June 2017	MICROBIOLOGY OF THE FOOD CHAIN Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection of *Enterobacteriaceae* *Replace ISO 21528-1:2004*
ISO 21528-2 June 2017	MICROBIOLOGY OF THE FOOD CHAIN Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count technique *Replace ISO 21528-2:2004*

STANDARDS - REGULATIONS

REFERENCE MATERIALS	
ISO/IEC 17025 November 2017	General requirements for the competence of testing and calibration laboratories *Replace ISO/IEC 17025:2005 + ISO/IEC 17025/AC1:2006*
REFERENCE MATERIALS	
ISO GUIDE 35 August 2017	REFERENCE MATERIALS Guidance for characterization and assessment of homogeneity and stability *Replace ISO GUIDE 35:2006*
SENSORY ANALYSIS	
ISO 6658 July 2017	SENSORY ANALYSIS Methodology - General guidance *Replace ISO 6658:2005*
ISO 8588 July 2017	SENSORY ANALYSIS Methodology - "A" - "not A" test *Replace ISO 8588:1987*
ISO 10399 December 2017	SENSORY ANALYSIS Sensory analysis - Methodology - Duo-trio test *Replace ISO 10399:2004*

NEW EU REGULATIONS

Classification is established in alphabetical order of the first keyword

FOOD INGREDIENT

O.J.E.U. L 295, 14th November, 2017 – Commission Implementing Decision (EU) 2017/2078 of 10 November 2017 authorising an extension of use 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/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.295.01.0077.01.ENG

O.J.E.U. L 313, 29th November, 2017 – Commission Implementing Decision (EU) 2017/2201 of 27 November 2017 authorising the placing on the market of 2'-fucosyllactose produced with *Escherichia coli* strain BL21 as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council
http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.313.01.0005.01.ENG

O.J.E.U. L 336, 16th December, 2017 – Commission Implementing Decision (EU) 2017/2354 of 14 December 2017 authorising an extension of use of Chia seeds (*Salvia hispanica*) as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council
http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.336.01.0049.01.ENG

O.J.E.U. L 337, 19th December, 2017 – Commission Implementing Decision (EU) 2017/2375 of 15 December 2017 authorising the placing on the market of *N*-acetyl-D-neuraminic acid as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council
http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.337.01.0063.01.ENG

P.D.O. / P.G.I.

O.J.E.U. C 247, 21st October, 2017 – Application for approval of a minor amendment made public in accordance with the fifth subparagraph of Article 6(2) of Commission Delegated Regulation (EU) No 664/2014 supplementing Regulation (EU) No 1151/2012 of the European Parliament and of the Council with regard to the establishment of the Union symbols for protected designations of origin, protected geographical indications and traditional specialities guaranteed and with regard to certain rules on sourcing, certain procedural rules and certain additional transitional rules [Queso de la Serena (cheese) (PDO)]
http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.247.01.0012.01.ENG

STANDARDS - REGULATIONS

O.J.E.U. C 251, 2nd August, 2017 – Publication of an application for approval of a minor amendment in accordance with the second subparagraph of Article 53(2) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Lietuviskas varskes suris (cheese) (PGI)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.251.01.0022.01.ENG

O.J.E.U. C 286, 30th August, 2017 – Publication of an application for approval of a minor amendment in accordance with the second subparagraph of Article 53(2) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Montasio (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.286.01.0011.01.ENG

O.J.E.U. C 297, 8th September, 2017 – Publication of an application for approval of a minor amendment in accordance with the second subparagraph of Article 53(2) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Idiazabal (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.297.01.0010.01.ENG

O.J.E.U. C 299, 9th September, 2017 – Publication of an amendment application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Saint-Nectaire (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.299.01.0007.01.ENG

O.J.E.U. L 244, 22nd September, 2017 – Commission Implementing Regulation (EU) 2017/1595 of 21 September 2017 approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Gorgonzola (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.244.01.0001.01.ENG

O.J.E.U. C 317, 23rd September, 2017 – Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Traditional Welsh Caerphilly/Traditional Welsh Caerffili (cheese) (PGI)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.317.01.0010.01.ENG

O.J.E.U. L 256, 4th October, 2017 – Commission Implementing Regulation (EU) 2017/1788 of 22 September 2017 entering a name in the register of protected designations of origin and protected geographical indications [Ossolano (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.256.01.0004.01.ENG

O.J.E.U. L 269, 19th October, 2017 – Commission Implementing Regulation (EU) 2017/1788 of 22 September 2017 entering a name in the register of protected designations of origin and protected geographical indications [Danbo (cheese) (PGI)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.269.01.0010.01.ENG

O.J.E.U. L 273, 24th October, 2017 – Commission Implementing Regulation (EU) 2017/1931 of 17 October 2017 approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Bleu d'Auvergne (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.273.01.0002.01.ENG

O.J.E.U. C 358, 24th October, 2017 – Publication of an application for approval of a minor amendment in accordance with the second subparagraph of Article 53(2) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Grana Padano (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.358.01.0009.01.ENG

O.J.E.U. C 361, 25th October, 2017 – Publication of an amendment application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Laguiole (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.361.01.0042.01.ENG

O.J.E.U. C 368, 28th October, 2017 – Publication of an amendment application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Squacquerone di Romagna (cheese) (PDO)]

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.C_.2017.368.01.0016.01.ENG

STANDARDS - REGULATIONS

OFFICIAL CONTROLS

O.J.E.U. L 195, 27th July, 2017 – Commission Regulation (EU) 2017/1389 of 26 July 2017 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards the designation of the EU reference laboratory for foodborne viruses

http://eur-lex.europa.eu/legal-content/FR/TXT/?uri=uriserv:OJ.L_.2017.195.01.0007.01.ENG

AFNOR VALIDATIONS

During its October and November meetings, the Technical Committee of NF VALIDATION approved by vote:

Commercial name	Date	Certificate	Description
NEW VALIDATION			
3M™ PETRIFILM™ LACTIC ACID BACTERIA COUNT PLATE	Validation date: 23 Nov 2017 **End of validity: 23 Nov 2021**	**3M-01/19-11/17**	**Enumeration of mesophilic aerobic flora** All human food products (excluding yoghurts) and production environmental samples
RENEWALS OF VALIDATIONS			
3M™ PETRIFILM™ AEROBIC COUNT PLATE	Validation date: 29 Sep 1989 Renewal: 6 Sep 1993, 10 Sep 1997, 13 Dec 2001, 14 Jun 2005, 3 Jul 2009, 5 Jul 2013 and 2 Oct 2017 Extension: 27 Sep 2007 and 6 Feb 2015 **End of validity: 10 Sep 2021**	**3M-01/01-09/89**	**Enumeration of mesophilic aerobic flora** All human food products, pet food and industrial environment samples
3M™ PETRIFILM™ *ENTEROBACTERIACEAE* COUNT PLATE	Validation date: 10 Sep 1997 Renewal: 13 Dec 2001, 14 Jun 2005, 3 Jul 2009, 4 Jul 2013 and 2 Oct 2017 Extension: 1 Apr 2010 and 6 Feb 2015 **End of validity: 10 Sep 2021**	**3M-01/06-09/97**	**Enumeration of *Enterobacteriaceae*** All human food products, animal feeding and production environment samples
TEMPO AC	Validation date: 23 May 2013 Renewal: 2 Oct 2017 **End of validity: 23 May 2021**	**BIO-12/35-05/13**	**Enumeration of aerobic mesophilic flora** All human food, pet foods and production environment samples
THERMO SCIENTIFIC SURETECT *LISTERIA MONOCYTOGENES* PCR ASSAY	Validation date: 4 Nov 2013 Renewal: 22 Nov 2017 Extension: 21 Mar 2014, 17 Mar 2016 and 30 Jun 2016 **End of validity: 4 Nov 2021**	**UNI-03/08-11/13**	**Detection of *Listeria monocytogenes*** All human food products and production environmental samples
RAPID'*E. COLI* 2	Validation date: 19 Nov 1997 Renewal: 7 Mar 2002, 2 Dec 2004, 28 Nov 2008, 29 Nov 2012 and 22 Nov 2017 **End of validity: 2 Dec 2020**	**BRD-07/01-07/93**	**Enumeration at 44 °C of β-glucuronidase positive *E. coli*** All human food products
RAPID'*E. COLI* 2	Validation date: 2 Dec 2004 Renewal: 28 Nov 2008, 29 Nov 2012 and 22 Nov 2017 **End of validity: 2 Dec 2020**	**BRD-07/07-12/04**	**Enumeration at 37 °C of β-glucuronidase positive *E. coli*** All human food products
RAPID'*E. COLI* 2	Validation date: 2 Dec 2004 Renewal: 28 Nov 2008, 29 Nov 2012 and 22 Nov 2017 **End of validity: 2 Dec 2020**	**BRD-07/08-12/04**	**Enumeration at 37 °C of coliforms** All human food products

AFNOR VALIDATIONS

TEMPO TC	Validation date: 8 Dec 2005 Renewal: 4 Dec 2009, 4 Oct 2013 and 24 Nov 2017 Extension: 3 Feb 2011 **End of validity: 8 Dec 2021**	**BIO-12/17-12/05**	**Enumeration of total coliforms** All human food products and pet food
VIDAS EASY *SALMONELLA*	Validation date: 20 Sep 2005 Renewal: 2 Jul 2009, 4 Jul 2013 and 23 Nov 2017 Extension: 30 Jun 2011, 30 Jan 2014 and 14 Oct 2015 **End of validity: 20 Sep 2021**	**BIO-12/16-09/05**	**Detection of *Salmonella* spp** All human food products, feed products and production environmental samples (except primary production stage environment)
PATHATRIX® AUTO *SALMONELLA* SPP KIT LINKED TO SELECTIVE AGAR DETECTION	Validation date: 28 Nov 2013 Renewal: 23 Nov 2017 Extension: 30 Aug 2016 **End of validity: 28 Nov 2021**	**ABI-29/06-11/13**	**Detection of *Salmonella* spp** Heat processed milk and dairy products, raw beef meats, cocoa and cocoa based products
PATHATRIX® AUTO *SALMONELLA* SPP KIT LINKED TO SELECTIVE AGAR DETECTION	Validation date: 28 Nov 2013 Renewal: 23 Nov 2017 Extension: 30 Aug 2016 **End of validity: 28 Nov 2021**	**ABI-29/07-11/13**	**Detection of *Salmonella* spp** Heat processed milk and dairy products, raw beef meats, cocoa and cocoa based products
VIDAS UP *E.COLI* O157 INCLUDING H7 (ECPT)	Validation date: 18 May 2009 Renewal: 29 Mar 2013 and 23 Nov 2017 Extension: 3 Dec 2009, 30 Jun 2011 and 15 May 2014 **End of validity: 18 May 2021**	**BIO-12/25-05/09**	**Detection of *E. coli* O157** Raw meat products, raw milk and raw milk products, raw vegetables and production environmental samples
EXTENSIONS OF VALIDATIONS			
BAX SYSTEM PCR ASSAY *LISTERIA MONOCYTOGENES* 24E (AUTOMATISED)	Validation date: 1 Jul 2008 Renewal: 6 Jul 2012 and 18 Mar 2016 Extension: 26 Jan 2009, 12 May 2011 and 3 Oct 2017 **End of validity: 1 Jul 2020**	**QUA-18/05-07/08**	**Detection of *Listeria monocytogenes*** All human food products and production environment samples
BAX SYSTEM PCR ASSAY GENUS *LISTERIA* 24E (AUTOMATISED)	Validation date: 1 Jul 2008 Renewal: 6 Jul 2012 and 18 Mar 2016 Extension: 26 Jan 2009, 12 May 2011 and 3 Oct 2017 **End of validity: 1 Jul 2020**	**QUA-18/06-07/08**	**Detection of *Listeria* spp (except *Listeria* grayi)** All human food products and production environment samples
GENE-UP *SALMONELLA*	Validation date: 30 Jun 2016 Extension: 29 Sep 2016, 24 Mar 2017, 3 Jul 2017 and 23 Nov 2017 **End of validity: 30 Jun 2020**	**BIO-12/38-06/16**	**Detection of *Salmonella* spp** All human food products and production environment 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

FORTHCOMING EVENTS

4-6 April 2018 Rennes, France	10th Cheese Symposium	https://symposium.inra.fr/cheese2018/

IN THE PRESS – ON THE WEB

Classification in alphabetical order of keywords

ADDITIVES

Re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) as food additives

http://www.efsa.europa.eu/en/efsajournal/pub/4866

► Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient sources added to Food was asked to deliver a scientific opinion on the re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) as food additives. he Panel concluded that there is no safety concern for the use of pectin (E 440i) and amidated pectin (E 440ii) as food additives for the general population and that there is no need for a numerical ADI.

Re-evaluation of xanthan gum (E 415) as food additive

http://www.efsa.europa.eu/en/efsajournal/pub/4909

► The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of xanthan gum (E 415) as food additive. The Panel concluded that there is no need for a numerical ADI for xanthan gum (E 415), and that there is no safety concern for the general population at the refined exposure assessment of xanthan gum (E 415) as food additive.

Re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives

http://www.efsa.europa.eu/en/efsajournal/pub/4910

► The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of glutamic acid–glutamates (E 620–625) when used as food additives. The Panel noted that the exposure to glutamic acid and glutamates (E 620–625) exceeded not only the proposed ADI, but also doses associated with adverse effects in humans for some population groups.

Re-evaluation of alginic acid and its sodium, potassium, ammonium and calcium salts (E 400-E 404) as food additives

http://www.efsa.europa.eu/en/efsajournal/pub/5049

► LThis opinion deals with the re-evaluation of alginic acid and its sodium, ammonium and calcium salts (E 400-E 404) when used as food additives. The panel concluded that there was no need for a numerical Acceptable Daily Intake (ADI) for alginic acid and its salts (E 400, E 401, E 402, E 403 and E 404), and there was no safety concern at the level of the refined exposure assessment for the reported uses as food additives.

NOVEL FOOD

Safety of synthetic N-acetyl-d-neuraminic acid as a novel food pursuant to Regulation (EC) No 258/97

http://www.efsa.europa.eu/en/efsajournal/pub/4918

► Following a request from the European Commission, the EFSA delivered a scientific opinion on N-acétyl-d-neuraminic as a novel food. The panel concluded that it is safe when it is used as an ingredient in formulae and foods for infants and young children.

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