# *Progetto Trieste – Veterinary Drug Residues*

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# *FINAL REPORT*

## *Laboratory Proficiency Testing for Food Analysis*

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and of ILAC MRA for the accreditation schemes TL, ML, CL and INSP

## 2017

## *Veterinary Drug Residues*

## *Round of September*

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22/11/2017 rev.0

## INTRODUCTION

Test Veritas S.r.l has been accredited EN ISO/IEC 17043:2010 "Conformity assessment - General requirements for proficiency testing" by Accredia.

Also, the quality management system has been assessed and certified by SGS Italia as meeting the requirements of ISO 9001 / UNI EN ISO 9001:2008 for development and production of materials for agri-food analyses and proficiency supply.

117 laboratories participated to *"Progetto Trieste 2017, round of September - Laboratory Proficiency Testing – Analytical field: veterinary drug residues"*.

The test materials were dispatched on September 4th and 5th, 2017. The deadline for sending the results was October 6th 2017.

112 laboratories returned test results.

Unless 10 out of 112 laboratories (participants n. 07, 21, 61, 82, 88, 109, 106, 115, 116 and 117) sent back the test results after the deadline.

Participants were from 41 countries (Argentina, Austria, Bangladesh, Belgium, Benin, Bolivia, Bosnia and Herzegowina, Botswana, Brazil, Chile, Colombia, Cyprus, Denmark, France, Ghana, Germany, Honduras, India, Ireland, Israel, Italy, Montenegro, Morocco, Netherlands, New Zealand, Nigeria, Paraguay, Portugal, Romania, Russian Federation, Slovenia, South Africa, Spain, Sudan, Sweden, Thailand, Tunisia, United Kingdom, United Republic of Tanzania, United States, Uruguay).

This Proficiency Testing Scheme offered 7 different sets of test materials (biological matrices, see Table a).

Test materials "A" were provided for performance evaluation of confirmatory techniques while test materials "B" were provided for performance evaluation of screening methods. Each material is composed by two different subsamples.

**Table a**: test materials.

| Test material Code | matrices | |
|---|---|---|
| T1730 | lyophilized tuna muscle | 50g A x 2 - 50g B x 2 |
| FI1731 | lyophilized fish muscle | 20g A x 2 - 10g B x 2 |
| M1732 | lyophilized bovine muscle | 30g A x 2 - 15g B x 2 |
| M1733 | bovine muscle | 20g A x 2 - 10g B x 2 |
| M1734 | lyophilized swine muscle | 20g A x 2 - 10g B x 2 |
| FI+SH1735 | lyophilized fish and shrimps | 20g A x 2 - 10g B x 2 |
| FI1736 | lyophilized fish muscle | 20g A x 2 - 10g B x 2 |

The molecules that could be present in the test materials of *Progetto Trieste 2017, round of September - Veterinary Drug Residues* are shown in Tables b and c. The analytes that were actually present are in **bold**.

**Table b**: Class of substances and molecules that had to be examined for screening and confirmatory methods.

| Test Material | Class | Molecules | Incurred |
|---|---|---|---|
| T1730-1 | histamine | **histamine** | yes |
| FI1731-1 | tetracyclines | **oxytetracycline (OTC), epioxytetracycline (eOTC)*,** chlortetracycline (CTC), epichlortetracycline (eCTC)*, doxycycline (DC), epidoxycycline (eDC)*, tetracycline (TC), epitetracycline (eTC)* | no |
| M1732-1 | quinolones and fluoroquinolones | enrofloxacin (ENRO), ciprofloxacin (CIPRO), **flumequine (FLUM),** danofloxacin (DAN), oxolinic acid (OXO), norfloxacin (NORF), marbofloxacin (MRB)*, sarafloxacin (SAR)*, nalidixic acid (NAL)* | yes |
| M1733-1 | tetracyclines | oxytetracycline (OTC), epioxytetracycline (eOTC)*, chlortetracycline (CTC), epichlortetracycline (eCTC)*, **doxycycline (DC)**, epidoxycycline (eDC)*, tetracycline (TC), epitetracycline (eTC)* | yes |
| M1734-1 | sulphonamides | sulfamethazine (SMZ), sulfadimethoxine (SDM), sulfamerazine (SMR), sulfadiazine (SDZ), sulfamonometoxine (SMM), sulfathiazol (STZ), sulfamethoxypyridazine (SMP)*, sulfaquinoxaline (SQX)* | blank |
| FI+SH1735-1 | nitrofurans metabolites | AOZ, **AMOZ,** SEM**,** AHD* | no |
| FI1736-1 | chloramphenicol | **chloramphenicol (CAP)** | no |

*test / measurand not ISO/IEC 17043:2010 accredited

**Table c:** Class of substances and molecules that had to be examined for screening and confirmatory methods.

| Test Material | Class | Molecules | Incurred |
|---|---|---|---|
| T1730-2 | histamine | histamine | blank |
| FI1731-2 | tetracyclines | oxytetracycline (OTC), epioxytetracycline (eOTC)*, **chlortetracycline (CTC), epichlortetracycline (eCTC)***, doxycycline (DC), epidoxycycline (eDC)*, **tetracycline (TC), epitetracycline (eTC)*** | no |
| M1732-2 | quinolones and fluoroquinolones | enrofloxacin (ENRO), ciprofloxacin (CIPRO), flumequine (FLUM), danofloxacin (DAN), oxolinic acid (OXO), norfloxacin (NORF), marbofloxacin (MRB)*, sarafloxacin (SAR)*, nalidixic acid (NAL)* | blank |
| M1733-2 | tetracyclines | **oxytetracycline (OTC), epioxytetracycline (eOTC)***, chlortetracycline (CTC), epichlortetracycline (eCTC)*, doxycycline (DC), epidoxycycline (eDC)*, tetracycline (TC), epitetracycline (eTC)* | no |
| M1734-2 | sulphonamides | sulfamethazine (SMZ), sulfadimethoxine (SDM), sulfamerazine (SMR), sulfadiazine (SDZ), **sulfamonometoxine (SMM),** sulfathiazol (STZ), sulfamethoxypyridazine (SMP)*, sulfaquinoxaline (SQX)* | no |
| FI+SH1735-2 | nitrofurans metabolites | AOZ, AMOZ, **SEM,** AHD* | no |
| FI1736-2 | chloramphenicol | **chloramphenicol (CAP)** | no |

*test / measurand not ISO/IEC 17043:2010 accredited

## PREPARATION OF TEST MATERIALS, HOMOGENEITY, DISTRIBUTION

### Preparation

**Muscle (incurred)**

A number of incurred matrices were provided by suppliers. Incurred matrices were mixed with blank matrices. The obtained samples were homogenized with a cutter mixer and dispensed into vials. Homogeneity was assessed, then test materials were kept at controlled temperature until dispatch.

**Muscle (spiked)**

Spiking solutions were prepared. Blank matrices were spiked. The obtained samples were homogenized with with a cutter mixer and dispensed into vials. Homogeneity was assessed, then test materials were kept at controlled temperature until dispatch.

**Lyophilized muscle (incurred)**

A number of incurred matrices were provided by suppliers. Incurred matrices were mixed with blank matrices. The obtained samples were homogenized with a cutter mixer and lyophilized. After mixing thoroughly, samples were dispensed into vials. Homogeneity was assessed, then test materials were kept at controlled temperature until dispatch.

**Lyophilized muscle (spiked)**

Spiking solutions were prepared. Blank matrices were spiked. The obtained samples were homogenized with with a cutter mixer and lyophilized. After mixing thoroughly, samples were dispensed into vials. Homogeneity was assessed, then test materials were kept at controlled temperature until dispatch.

**Lyophilized fish (spiked)**

Spiking solutions were prepared. Blank matrices were spiked. The obtained samples were homogenized with with a cutter mixer and and dispensed into amber bottles and lyophilized. Homogeneity was assessed, then test materials were kept at controlled temperature until dispatch.

**Lyophilized shrimps (spiked)**

Spiking solutions were prepared. Blank matrices were spiked. The obtained samples were homogenized with with a cutter mixer and and dispensed into amber bottles and lyophilized. Homogeneity was assessed, then test materials were kept at controlled temperature until dispatch.

**Lyophilized tuna (incurred)**

A number of incurred matrices were prepared in our laboratories. Contaminated matrices were mixed with blank matrices. The obtained samples were homogenized with a cutter mixer and lyophilized. After mixing thoroughly, samples were dispensed into foil-laminated pouches. Homogeneity was assessed, then test materials were kept at controlled temperature until dispatch.

### Subcontractor

No activity was outsurced.

### Homogeneity and stability

Appropriate homogeneity studies assessed that test materials are sufficiently homogeneous for the measurands under study.

Appropriate stability studies assessed that test materials are stable for the time that elapsed between the dispatch of the samples and the deadline for submission of results.
Information about homogeneity and stability studies are available under request.

### Labels

To avoid labelling errors, the Test Material "-1" and "-2" are always labelled on different days.

### Distribution

In accordance with the stability established, the test materials were shipped in proper containers and conditions.
Dispatch was followed by the courier's parcel tracking system on the web site.
Shipments outside the EU can follow different procedures.
The parcel contains:

- A number of units of test material.
- A covering letter with the necessary instructions for use and storage of the materials, together with indications for reporting results in the on-line form.
- A letter with recommendations on how to use lyophilized samples.

An e-mail informed participants that materials were shipped. The e-mail contained an attached copy of the covering letter and MSDS.

## PERFORMANCE EVALUATION CRITERIA

### Laboratory code and confidentiality

Confidentiality is guaranteed. Participants are identified in the Final Report by a randomly assigned code.
The laboratory codes were communicated to participants trough the web restricted area.

### Confirmatory result and concentration value

In confirmatory analysis, the compounds are separated by chromatographic techniques (GC, HPLC, UPLC, LC...); afterwards they are detected by MS, FLD, DAD, etc...
Participants were asked to report results corrected for recovery. If correction is not applied, the value is not included in the assigned value statistics.

- "< of…" means that the analyte was not detected.
- "> of…" means that the analyte was detected but not quantified.
- "NS" (Not Searched) means that the laboratory did not perform the analysis.

### Screening results and concentration value

In screening analysis, participants use techniques as ELISA, RIA, lateral flow, microbiological assay, etc...
Participants have to indicate their qualitative results as "analyte detected" or "analyte not detected".
Also screening results can be quantitative (concentration value corrected for recovery).
For quantitative results:

- "=…" means that the analyte was detected and quantified
- "< of…" means that the analyte was not detected
- "> of…" means that the analyte was detected but not quantified
- "NS" (Not Searched) means that the laboratory did not perform the analysis.

In reference to the method used, results can be expressed for a single analyte or for an analyte group.

## Decision Limit (CCα) and Detection Capability (CCβ)*

The decision limits and detection capability for the relative compounds were claimed by the laboratories.

"-" means "Not Reported".

*EU Decision 657/2002, *Official Journal of the European Communities 2002*. L221: 8-36.

## False Positive Results

The false positive results obtained by the laboratories are shown.

- "None" means that no false positive results were reported. This corresponds to a good performance for both screening and confirmatory methods.
- "-" means that evaluation was not applicable (e.g. the laboratory search only compound that was present).

It is not possible to classify as false positives concentrations lower than the assigned value of an intended blank sample. In this case concentrations are reported just for information.

## False negative results

In case of false negative confirmatory results, no evaluation is given.

In case of false negative screening results, evaluation is given as described (see screening assessment).

## Elaboration of laboratory data

In the statistical data processing, all the data submitted by the participants are elaborated considering two decimal places. In case there are not declared decimal places, they are considered as corresponding to "zero" (E.g. 25=25,00 - 25,3=25,30 - 25,32=25,32).

# CALCULATION OF THE ASSIGNED VALUE

## The Assigned Value $x_{pt}$

The Assigned Value $x_{pt,}$ is the value attributed to a particular property of proficiency test items (definition from ISO13528:2015).

In the routine, the results from the confirmatory analysis (chromatographic techniques) are considered a reference; they are used with legal purpose (as regulatory requirement). Instead, screening methods have the purpose to analyze in a short time a wide quantity of samples; in case of "positive" results, the data will be verified through the use of chromatographic techniques.

Because of above described, the Assigned Value $x_{pt}$ derives just from participants' quantitative results obtained with confirmatory analysis. The screening results are compared to the Assigned Value $x_{pt}$ obtained from the confirmatory data.

The procedure for determining the Assigned Value $x_{pt}$ is described below.

After excluding results that are identified as invalid the data population was checked for normality and for the presence of outliers by applying appropriate statistics and visual presentations. For both spiked and incurred test materials, $x_{pt}$ represents the value of concentration obtained from Algorithm A (ISO 13528:2015) or from the median. The chosen value will be reported under the assigned value table.

In case of "blank" test materials, the threshold above which the analyte should not be present is based on the capability of participants to determine the analyte. The statistical "mode" is chosen as the estimator.

Sometimes very low concentrations are quantified. When it occurs, the concentration value is assigned only if proper statistics are applicable.

The value is not assigned when $p < 8$, where "*p*" is the number of data after invalid results rejection.

In case of $8 \leq p < 15$ the uncertainty attributable to Assigned Value is not negligible.

## *z*-score and $\sigma_{pt}$ (standard deviation for proficiency assessment):

For quantitative data (confirmatory and screening), the participant's result is converted into a z-score according to the equation:

z-score = $(x_i - x_{pt}) / \sigma_{pt}$

where:

- $x_i$ is the analyte concentration value reported by the laboratory;
- $x_{pt}$ is the assigned value (obtained with confirmatory methods);
- $\sigma_{pt}$ is the standard deviation for proficiency assessment calculated from $b\, x_{pt}$.
- $b$ = %RSD / 100, (RSD = Relative Standard Deviation)

the %RSD value comes from the Horwitz equation (Horwitz, W., 1988, *Pure Appl. Chem*. 60, 855-864)

$$\%RSD = 2^{(1-0.5 \log X_{pt})}$$

where $x_{pt}$ is expressed as a dimensionless concentration.

$\sigma_{pt}$ is related to the concentration of the analyte: it comes from Horwitz equation (unless otherwise specified); in case of contamination less than 10 ppb the Thompson equation modified Horwitz equation (Thompson, M., 2000, *Analyst* 125, 385-386). In particular circumstance $\sigma_{pt}$ is chosen from Proficiency Test provider's (PTp) experience, derived from previous rounds. The adopted criteria is reported in the specific test material table.

The laboratory performance evaluation was established taking into account the following criteria for z-score:

| | | |
|---|---|---|
| *acceptable (satisfactory)* | when | $\lvert z \rvert \leq 2$ |
| *warning signal (questionable)* | when | $2 < \lvert z \rvert \leq 3$ |
| *action signal (unsatisfactory)* | when | $\lvert z \rvert > 3$ |

## The standard uncertainty of the assigned value $u(x_{pt})$

The standard uncertainty $u(x_{pt})$ is calculated as:

$u(x_{pt}) = s^* / \sqrt{p}$

where:

- $s^*$ is the robust estimate of the participant standard deviation;
- $p$ is the number of participants.

In case of median as estimator, the standard deviation is calculated as $s^* = MADe$ (where MADe is the Median Absolute Deviation).

When the standard uncertainty is too high, the assigned value could be inaccurate.

Therefore:

- In case $[u(x_{pt})]^2/\sigma_{pt}^2 > 0.5$, the consensus value is not determined and individual laboratory performance scores are not reported. Summary statistics are provided only for information.
- In case $0.1 < [u(x_{pt})]^2/\sigma_{pt}^2 \leq 0.5$, the assigned value and z-score will be given in *italic* (provided only for information). In this case the uncertainty is not negligible, and evaluation could be affected.

It may happen that only few participants confirm the presence of some analytes in the test materials. In such cases, the presence of an analyte is considered:

- “unconfirmed”, when less then 25 % of participants detect the compound;
- “confirmed”, when 25% of participants, or more, detect the compound (the minimum number of positive results is anyway three).

## Screening assessment

Participants who use screening methods, have to provide qualitative answer (detected/not detected). If they are able, they have to indicate in addition "less than..." or "greater than...".

The results are classified as "satisfactory" in the following cases:

- The laboratory detects the analyte or the group of analytes that are effectively present in the test material.
- The laboratory does not detect the analyte or the group of analytes that are not effectively present in the sample.

The results are classified as "unsatisfactory" in the following case:

- The laboratory does not detect the analyte or the group of analytes that is/are effectively present in the sample, but according to the method specifications the analyte/analytes is/are detectable. It means that a false negative has been reported.

The results are classified as "questionable" in the following case:

- The laboratory detects an analyte or a group of analytes that were not effectively present in the sample. It means that false positive has been detected. The false positive results are not considered unsuitable, because routine screening positive results should be confirmed by chromatographic methods.

The results are classified as "congruent" in the following case:

- The laboratory does not detect the analyte that is effectively present, because his method does not allow it. This is an information concerning the capability of the method. The participant should take in consideration if his method has the appropriate capability in respect his requirement.

The results are not classified, therefore "not applicable" in the following case:

- The laboratory detects the analyte or a group of analytes that were not effectively present in the sample, but the level detected is lower than declared value. In this case it is not possible to evaluate its results.

Because of the different country legislations, the regulatory limits are not considered in the results evaluation.

## Evaluation screening table

Example of the performance criteria for the screening are reported in the table below.

**Table d:** example of evaluation.

| TEST MATERIAL CONTAMINATION | RESULT (PROVIDED BY PARTICIPANT) | EVALUATION | Z-SCORE |
|---|---|---|---|
| Contamined material (the analyte is present above or below the regulatory limit) Assigned Value $X_{pt}$ = 6 ppb (from confirmatory methods) | detected | satisfactory | not provided |
| | detected = 5 ppb | satisfactory | provided |
| | detected > 8 ppb | satisfactory | not provided |
| | not detected | unsatisfactory | not provided |
| | not detected < 1 ppb | unsatisfactory | not provided |
| | not detected < 10 ppb | congruent | not provided |
| Blank material Value < 6 ppb (from confirmatory methods) | not detected | satisfactory | not provided |
| | not detected < 10ppb | satisfactory | not provided |
| | detected | questionable | not provided |
| | detected = 5 ppb | questionable | not provided |
| | detected > 8 ppb | questionable | not provided |
| | detected = 1 ppb | not applicable | not provided |

## Graphical presentation

Confirmatory and screening results are shown in different tables. This approach takes into account the different purpose of the methods.

When screening participants receive a z-score, it will be shown in the “bar-chart of z-score” (chart reported under the table “assigned value and standard deviations”).

## PARTICIPANTS

Argentina
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Austria
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- Analytec - Labor für Lebensmitteluntersuchung und Umweltanalytik, Salzburg

Belgium
- FLVVG - Federaal Laboratorium voor Voedselveiligheid, Gentbrugge

Benin
- LCSSA, Laboratoire Central de Sécurité Sanitaire des Aliments, Cotonou

Bolivia
- Laboratorio LIDIVET- SENASAG, Santa Cruz de la Sierra

Bosnia and Herzegovina
- Veterinary Institute Bihac

Botswana
- Botswana National Veterinary Laboratory, Gaborone

Brazil
- LANAGRO/RS Laboratorio Nacional Agropecuario, Porto Alegre
- LANAGRO-SP, National Laboratory of the Ministry of Agriculture, Livenstock and Food Supply of Brasil, Campinas
- LANAGRO - GRO Ministério da Agricultura Pecuária e Abastecimento, Laboratório Nacional Agropecuário em Goiás, Goiânia

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- Bureau Veritas Chile - AC Andes Control, Colina, Santiago
- Ceimic Chile, Santiago
- Corthorn Qualità Chile s.a., Santiago
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- Gestión de Calidad y Laboratorio s.a., Santiago
- Laboratorio Labser Ltda., Rancagua
- SAG, Servicio Agricola y Ganadero, Punta Arenas
- SAG, Servicio Agricola y Ganadero, Laboratorio Quimica Ambiental y Alimentaria, Santiago
- SGS Chile Ltda., Laboratorio Agri-Concepción Santiago

Colombia
- INVIMA, Istituto Nacional de Vigilancia de Medicamentos y Alimentos, Bogotà

Cyprus
- LCFAO, Laboratory for the control of Foods of Animal Origin, Ministry of Agriculture, Natural Resources and Environment, Nicosia

Denmark
- Danish Crown, Horsens
- Fødevarestyrelsen, Ringsted

France
- Laboratoire Cereco Sud, Garons
- LABEO, Laboratoire Departamental de l'Orne, Alencon Cedex
- LEAV, la Roche sur Yon
- Nestlé France s.a.s., Cergy Saint Christophe

Ghana
- Ministry of Health/Food and Drugs Authority, Accra

Germany
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- Deutsches Institut für Lebensmitteltechnik, Quakenbrück
- Eurofins WEJ Contaminants GmbH, Hamburg
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- Export Inspection Agency, Kochi
- Export Inspection Agency, Chennai
- Intertek India pvt. Ltd., Delhi
- Intertek India pvt. Ltd., Hyderabad

Ireland
- Irish Equine Centre, Naas, Co. Kildare
- Marine Institute, Galway
- Public Analyst's Laboratory, Dublin
- Veterinary Public Health Regulatory Laboratory, Laboratories Department Food and Marine, Co. Kildare

Israel
- Kimron Veterinary Institute, National Residue Control Laboratory, Bet-Dagan

Italy
- Agricola Tre Valli, San Michele Extra, Verona
- Alpha ecologia s.r.l., Firenze
- Chelab s.r.l., Resana
- Fiorital s.p.a., Venezia
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- Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia
- Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffi", Sassari
- Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo
- Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro
- Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Umbertini", Bologna
- Istituto Zooprofilattico Sperimentale del Mezzogiorno, della Campania e Della Calabria, Portici
- Laboratori Vailati s.r.l., San Paolo
- R&C Lab S.r.l., Altaviila Vicentina
- Tecnocibus s.r.l., Ponte San Giovanni
- Voltan s.p.a., Marcon

Montenegro
- DOO Centar za Ekotoksikološka ispitivanja Crne Gore, Podgorica

Morocco
- Chef du Service du Contrôle et des Expertises /DPIV/ONSSA, Akkari, Rabat

Netherlands
- Food and Consumer Product Safety Authority, Wageningen
- NutriControl B.V. Analytical Service, Veghel
- Rikilt, Wageningen

New Zealand
- AsureQuality Ltd, Lower Hutt

Nigeria
- NAFDAC,National Agency for Food and Drug Administration and Control Central Laboratory Complex - NIGERIA, Lagos

Paraguay
- Grupo Multilab S. A., Area Econatura, Asunción

Portugal
- LABIAGRO, Laboratorio Quimico, Agroalimentar e Microbiologico, Lda., Oeiras

Romania
- DSVSA, Directia Sanitara Veterinara si Pentru Siguranta Alimentelor, Bacau

Russian Federation
- The All-Russian State Center for Quality and Standardization of Veterinary Drugs and Feeds (VGNKI), Moscow

Slovenia
- UL Veterinary faculty, National veterinary institute, Institute for food hygiene and bromatology, Ljubljana

South Africa
- ARC-OVI, Agricultural Research council, Onderstepoort Veterinary Institute Residue Lab, Pretoria

Spain
- Ainia, Parque tecnológico de Valencia, Paterna
- Alkemi s.a., Coslada (Madrid)
- Anfaco-Cecopesca, Vigo
- Aquimisa s.l., Salamanca
- Calitec s.c.p., Barcelona
- Centro Salud Publica de Valencia, Laboratorio de salud publica, Valencia
- Laboratorio de Salud Pública de Murcia, Consejeria de sanidad, Murcia
- Dyglab, Madrid
- Instituto de Salud Pública de Navarra, Seccion de Laboratorio de Salud Pùblica, Pamplona
- Laboratorio de Salud Pública de Burgos, Burgos

- Laboratorio de Salud Pública de Jaén, Jaén
- Laboratorio Agrario Regional de la Junta de Castilla y León, Burgos
- Laboratorio de Salud Pública de Galicia, Lugo
- Laboratorio de Salud Pública del Principado de Asturias, Oviedo
- Laboratorio de Salud Publica de Palma, Palma De Mallorca
- Laboratorio de Salud Publica, Departamento de salud y consumo, Gobierno de Aragón, Zaragoza
- Laboratorio Municipal de Bilbao, Bilbao
- Laboratorio Regional de Salud Publica de la Comunidad de Madrid, Madrid
- Laboratorio de Salud Pública de Toledo, Toledo
- Eurofins Analisis Alimentario SLU, Coslada, Madrid
- Servicio Laboratorio y Control (SeLyC), Santander

Sudan
- CVRL, Central Veterinary Research Centre - Khartoum

Sweden
- National Food Agency, LENC - Livsmedelsverket, Uppsala

Thailand
- Betagro Sciente Center Company Limited, Pathumthani
- AMARC, Asia Medical and Agricultural Laboratory, Bacau

Tunisia
- CNSTN, Centre National des Sciences et Technologies Nucleaires Ariana

United Kingdom
- AFBI, Agri-Food & Biosciences Institute, Belfast, Northern Ireland

United Republic of Tanzania
- TFDA, Tanzania Food and Drug Authority, Dar es Salaam - United Republic of Tanzania

United States
- Certified Laboratories, Inc., Plainview, Melville
- Trilogy Analytical Laboratory Inc., Washington

Uruguay
- División de Laboratorios Veterinarios - MGAP, Montevideo - Uruguay

## ABSTRACT

**Table e**: Overall evaluation.

| Test Material Code | analyte | number of evaluation | Z-score < 2 or satisfactory (n) | Z -score < 2 or satisfactory (%) |
|---|---|---|---|---|
| T1730A-1 | histamine | 21 | 16 | 76 |
| T1730B-1 | histamine | 11 | 11 | 100 |
| T1730B-2 | blank | 11 | 8 | 73 |
| FI1731A-1 | oxytetracycline | 13 | 12 | 92 |
| FI1731A-2 | chlortetracycline | 12 | 10 | 83 |
| FI1731B-1 | oxytetracycline | 8 | 5 | 73 |
| FI1731B-2 | chlortetracycline | 8 | 6 | 75 |
| M1732A-1 | flumequine | 19 | 18 | 95 |
| M1732B-1 | flumequine | 7 | 6 | 86 |
| M1732B-2 | blank | 10 | 10 | 100 |
| M1733A-1 | doxycycline | 26 | 24 | 92 |
| M1733A-2 | oxytetracycline | 29 | 25 | 86 |
| | oxytetracycline-epioxytetracycline | 13 | 11 | 85 |
| M1733B-1 | doxycycline | 22 | 18 | 82 |
| M1733B-2 | oxytetracycline | 23 | 21 | 91 |
| M1734A-2 | sulfamonometoxine | 21 | 18 | 90 |
| M1734B-1 | blank | 20 | 17 | 85 |
| M1734B-2 | sulfamonometoxine | 16 | 13 | 81 |
| FI+SH1735A-1 | AMOZ | 12 | 10 | 83 |
| FI+SH1735A-2 | SEM | 11 | 9 | 82 |
| FI+SH1735B-1 | AMOZ | 2 | 2 | 100 |
| FI+SH1735B-2 | SEM | 1 | 1 | 100 |
| FI1736A-1 | chloramphenicol | 17 | 15 | 88 |
| FI1736A-2 | chloramphenicol | 16 | 15 | 94 |
| FI1736B-1 | chloramphenicol | 6 | 6 | 100 |
| FI1736B-2 | chloramphenicol | 6 | 6 | 100 |

## REFERENCES

*"Progetto Trieste"* is managed in agreement to:


- UNI CEI EN ISO/IEC 17043:2010 Conformity assessment – General requirements for proficiency testing
- EURACHEM Selection, Use and Interpretation of Proficiency Testing (PT) Schemes, 2nd edition, 2011
- ISO 13528:2015 Statistical method for use in proficiency testing by interlaboratory comparisons
- ISO GUIDE 35 Reference materials – General and statistical principles for certification, 2006
- IUPAC Technical Report The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, 2006
- EURACHEM/CITAC Guide CG4 Quantifying Uncertainty in Analytical Measurement, 3rd edition, 2012
- ILAC-P10:2013 ILAC Policy on Traceability of Measurement Results
- ILAC-G8:03/2009 Guidelines on the Reporting of Compliance with Specification

# RESULTS

## Proficiency test T1730, result contents

**histamine in lyophilized tuna muscle, result of confirmatory "A";**

- **Test material T1730-1**
- **Test material T1730-2**

**histamine in lyophilized tuna muscle, result of screening "B";**

- **Test material T1730-1**
- **Test material T1730-2**

# Test material T1730, lyophilized tuna muscle, confirmatory

**Table 1: results of confirmatory analyses performed on test material T1730-1 (incurred sample): histamine detection.**

| T1730-1<br>histamine, assigned value: 94,00 mg/kg<br>satisfactory range: 78,82 - 109,18 mg/kg | | | | | | |
|---|---|---|---|---|---|---|
| **Lab.code** | **Result (mg/kg)** | **Recovery (%)** | **z-score** | **Decision limit CCα (mg/kg)** | **Detection capability CCβ (mg/kg)** | **ISO 17025** |
| 02 | 100 | 82 | **0,79** | - | 50 | YES |
| 03 | 100,2 | 106 | **0,82** | 5,0 | 20,0 | YES |
| 04 | 101,37 | 100 | **0,97** | 10 | 12 | YES |
| 06 | 95 | 87,1 | **0,13** | - | <10 | YES |
| 08 | 86,03 | 98 | **-1,05** | - | 25 | YES |
| 09 | 95,5 | 98 | **0,20** | 6 | 20 | YES |
| 14 | 109,89 | 100 | **2,09** | - | 5,00 | NO |
| 18 | 77 | 100 | **-2,24** | - | 40 | YES |
| 28 | 97,9 | 74,3 | **0,51** | - | - | - |
| 32 | 89,3 | 100 | **-0,62** | <50 | 50 | YES |
| 34 | 94 | - | **0,00** | 15 | 30 | NO |
| 39 | 89,98 | 97,38 | **-0,53** | 3,0 | 10,0 | YES |
| 42 | 97 | IS | **0,40** | - | 2 | YES |
| 43 | 104,1 | 100 | **1,33** | - | 50 | - |
| 54 | 97,20 | - | **0,42** | 9 | 25 | YES |
| 62 | 93 | 95 | **-0,13** | - | 20 | YES |
| 84 | 69,96 | 97,10 | **-3,17** | <0,20 | <2,00 | YES |
| 96 | 93,04 | 90,0 | **-0,13** | 15 | 25 | YES |
| 100 | 60,7 | 97,0 | **-4,39** | 5,0 | 5,0 | YES |
| 103 | 92,74 | 91,78 | **-0,17** | 25,00 | 50,00 | - |
| 115 | 69 | 90 | **-3,29** | 10 | 10 | - |

# Test material T1730, lyophilized tuna muscle, confirmatory

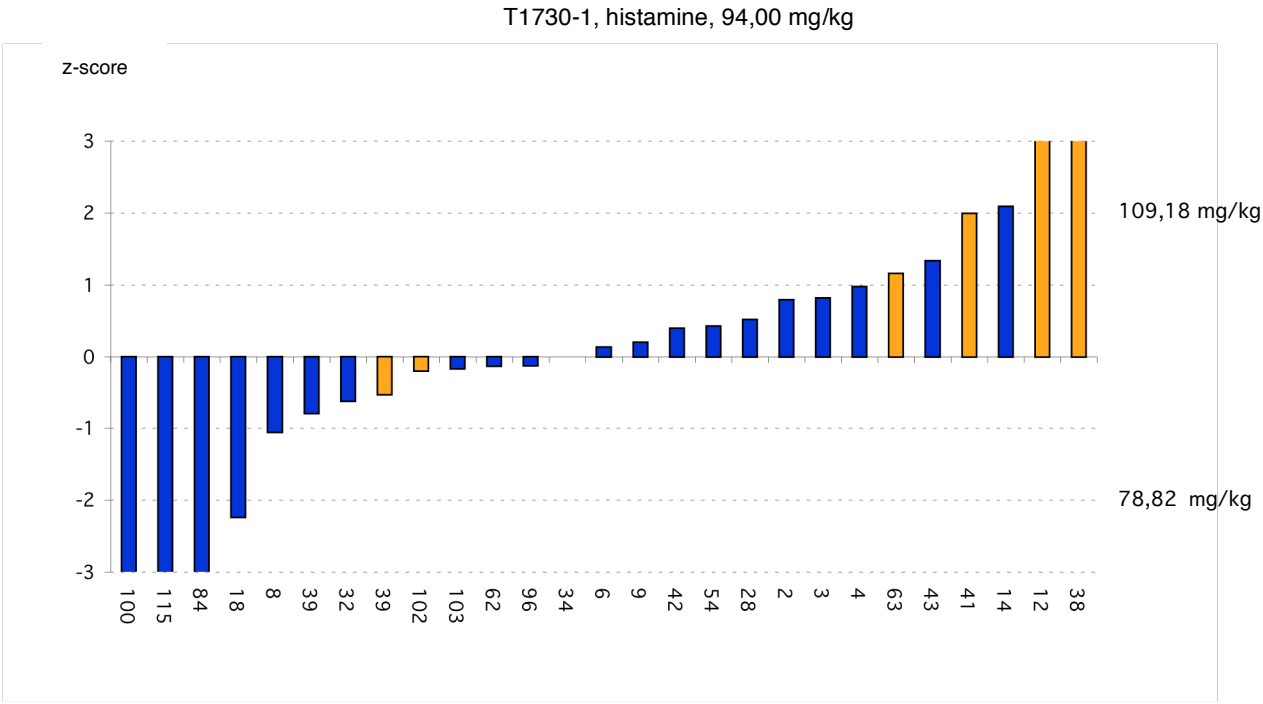
| | |
|---|---|
| Concentration, arithmetic mean (n = 21) | 91,09 mg/kg |
| Standard deviation (n = 21) | 12,35 mg/kg |
| Coefficient of variation (n = 21) | 13,56 % |

**Table 2: assigned value and target standard deviations.**

| Analyte | Assigned value (mg/kg) | | | | Standard deviation for proficiency assessment | | |
|---|---|---|---|---|---|---|---|
| | Data points, n | Median | MADe | CV | Standard uncertainty $u(x_{pt})$ | b | $\sigma_{pt}$ =b $x_{pt}$ |
| histamine | 21 | 94,00 | 6,97 | 7,41 | 1,52 | 0,08 | 7,59 |

*The median has been considered as the best possible estimate of the True Value.*

$[u(x_{pt})]^2 / \sigma_{pt}^2 \leq 0{,}1$ %




The assigned value has been calculated by confirmatory methods only

|z|≤2=acceptable  2<|z|≤3=warning signal
|z|>3=action signal  *=outliers

confirmatory *z*-score
screening *z*-score

# Test material T1730, lyophilized tuna muscle, confirmatory

**Table 3: results of confirmatory analyses performed on test material T1730-2 (blank sample): histamine detection.**

| T1730-2<br>histamine: <50,00 mg/kg | | |
|---|---|---|
| **Lab. code** | **histamine** | **False Positive mg/kg** |
| 02 | <50 | none |
| 03 | <20,0 | none |
| 04 | <10 | none |
| 06 | <10 | none |
| 08 | <25 | none |
| 09 | <20 | none |
| 14 | NS | none |
| 18 | <40 | none |
| 28 | <1 | none |
| 32 | <50 | none |
| 34 | 38 | histamine (38) |
| 39 | 1,39 | histamine (1,39) |
| 42 | <2 | none |
| 43 | <50 | none |
| 54 | <25 | none |
| 62 | <20 | none |
| 84 | <2,00 | none |
| 96 | <15 | none |
| 100 | <5,0 | none |
| 103 | <25,00 | none |
| 115 | >1 | histamine (>1) |

*This blank Test Material has been characterized during the proficiency test: the concentrations below the provided value can not be classified as false positive.*

# Test material T1730, lyophilized tuna muscle, screening

**Table 4: results of screening analyses performed on test material T1730-1 (incurred sample): histamine detection.**

| T1730-1<br>histamine, assigned value: 94,00 mg/kg | | | | | |
|---|---|---|---|---|---|
| **Lab. code** | **histamine** | **Evaluation** | **z-score** | **CCβ (mg/kg)** | **ISO 17025** |
| 02 | DETECTED | **satisfactory** | | - | YES |
| 08 | DETECTED >50 | **satisfactory** | | - | YES |
| 10 | DETECTED | **satisfactory** | | - | NO |
| 12 | DETECTED 129 | **satisfactory** | **4,61** | 2,5 | NO |
| 18 | DETECTED >70 | **satisfactory** | | - | YES |
| 38 | DETECTED 137 | **satisfactory** | **5,67** | 50 | YES |
| 39 | DETECTED 88,00 | **satisfactory** | **-0,79** | 10,0 | YES |
| 41 | DETECTED 109,12 | **satisfactory** | **1,99** | - | NO |
| 63 | DETECTED 102,8 | **satisfactory** | **1,16** | 10 | YES |
| 78 | DETECTED >300 | **satisfactory** | | - | YES |
| 102 | DETECTED 92,5 | **satisfactory** | **-0,20** | >1,00 | YES |

CRITERIA: see page 7. table d

# Test material T1730, lyophilized tuna muscle, screening

**Table 5: results of screening analyses performed on test material T1730-2 (blank sample): histamine detection.**

| | T1730-2<br>histamine: <50,00 mg/kg | | | |
|---|---|---|---|---|
| **Lab. code** | **histamine** | **Evaluation** | **False positive mg/kg** | **ISO 17025** |
| 02 | NOT DETECTED | **satisfactory** | none | YES |
| 08 | NOT DETECTED <50 | **satisfactory** | none | YES |
| 10 | NOT DETECTED | **satisfactory** | none | NO |
| 12 | NOT DETECTED <2,5 | **satisfactory** | none | NO |
| 18 | NOT DETECTED <70 | **satisfactory** | none | YES |
| 38 | NOT DETECTED <50 | **satisfactory** | none | YES |
| 39 | DETECTED 1,43 | **questionable** | histamine (1,43) | YES |
| 41 | NOT DETECTED <0,07 | **satisfactory** | none | NO |
| 63 | NOT DETECTED <10,00 | **satisfactory** | none | YES |
| 78 | DETECTED 20 | **questionable** | histamine (20) | YES |
| 102 | DETECTED 1,40 | **questionable** | histamine (1,40) | YES |

CRITERIA: see page 7. table d

# Test material T1730, lyophilized tuna muscle, screening

**Table 6: detection capabilities in screening analyses of histamine.**

**Detection capability- CCβ (mg/kg)**

| Lab. code | histamine |
|---|---|
| 02 | - |
| 08 | - |
| 10 | - |
| 12 | 2,5 |
| 18 | - |
| 38 | 50 |
| 39 | 10,0 |
| 41 | - |
| 63 | 10 |
| 78 | - |
| 102 | >1,00 |

# ANNEX

# ANNEX

## ANALYTICAL METHODS USED

## HISTAMINE IN LYOPHILIZED TUNA MUSCLE, T1730A

*Analytical methods used by partecipants*

| | histamine |
|---|---|
| **Methods** | **Laboratory Code** |
| HPLC-UV | 14-28-84 |
| HPLC/DAD | 02-03-04-06-09-54-96-103-115 |
| HPLC/FLD | 18-32-34-39-62-100 |
| HPLC/MS/MS | 08-42-43 |

| | histamine |
|---|---|
| **Is the analytical method a routine one ?** | **Laboratory Code** |
| YES | 02-03-04-06-08-09-14-18-32-34-39-42-43-54-62-84-96- 100-103-115 |

*Action level*

| | histamine |
|---|---|
| **Specify the concentration above which the material is considered not-compliant (mg/kg)** | **Laboratory Code** |
| 20 | 09 |
| 100 | 08-18-42 |
| 200 | 54 |
| 500 | 100 |
| 2000 | 84 |
| 100000 | 03-96-103 |
| 200000 | 39 |
| >112 | 02 |

*Number of replicates (1 replicate = 1 extraction)*

| | histamine |
|---|---|
| **Number of replicates** | **Laboratory Code** |
| 1 | 04-32-39-43-62-102 |
| 2 | 02-03-06-08-09-14-18-42-54-96-100-103 |
| 3 | 34-84 |

*Recovery*

| | histamine |
|---|---|
| **Recovery calculated by** | **Laboratory Code** |
| Certified Reference Material (CRM) | 08 |
| In-house Reference Material (RM) | 84 |
| Internal standard | 18-42 |
| Matrix matched calibration | 34-43-54-96 |
| Spiked sample | 02-03-04-06-09-14-32-39-62-100-103 |

### *Quality*

| histamine | |
|---|---|
| **Quality control implemented** | **Laboratory Code** |
| Control chart | 04-06-43-62 |
| Control chart with Certified Reference Material (CRM) | 08-14 |
| Control chart with in-house Reference Material (RM) | 100 |
| Duplicate analyses | 42 |
| Regular partecipation to proficency test (PT) | 02-03-09-84 |
| Spiked sample | 18-32-34-39-54-96-103 |

### *Reference*

| histamine | |
|---|---|
| **Reference** | **Laboratory Code** |
| ANSES Boulogne | 54 |
| Internal method | 02-03-04-06-08-09-14-18-34-42-62-84-96-103 |
| Journal Ag & Food Chemistry Vol 50 2002 | 100 |
| Torry Research Station, MAFF< Aberdeen, Scotland | 39 |

### *Source of standards for chromatographyc analysis*

| histamine | |
|---|---|
| | **Laboratory Code** |
| Sigma, Aldrich, Fluka | 02-03-04-06-08-09-14-18-32-39-42-54-63-84-96-100 |
| dr. Ehrenstorfer | 34-103 |

### *Sample preparation*
### *Amount of test sample*

| histamine | |
|---|---|
| **Sample volume (g)** | **Laboratory Code** |
| 1,1 | 103 |
| 2,5 | 42-62 |
| 5 | 02-03-06-08-09-18-54-96-100 |
| 10 | 04-32-34 |
| 25 | 39 |
| 50 | 84 |

### *Sample treatment*

| histamine | |
|---|---|
| | **Laboratory Code** |
| **SPE Extraction** | |
| trichloracetic acid 10% | 34 |
| **Other Extraction** | |
| formic acid | 43 |
| perchloridric acid | 03-04-06-09-54-84-96-100 |
| trichloracetic acid | 02-39-42-62 |
| water | 08 |
| water / methanol | 18-63 |
| **Digestion** | |
| trichloroacetic acid | 32 |
| **SPE clean up** | |
| OASIS® MCX | 18 |
| **Other clean up** | |
| anionic exchange column | 63 |
| centrifugation | 03 |
| diethyl ether | 100 |
| filtration | 08-09-39-62-63 |
| methanol | 18 |

### *HPLC methodology and conditions*

| histamine | |
|---|---|
| | **Laboratory Code** |
| Gradient | 02-08-14-39-54 |
| Isocratic | 03-04-06-09-18-32-34-42-43-62-84-96-100-103 |

| | |
|---|---|
| **Column phase** | |
| C18 | 02-03-04-06-09-14-32-34-42-43-54-62-84-96-100-103 |
| HILIC | 08 |
| $NH_2$ | 39 |
| Phenyl-Hexyl | 18 |
| **Column length (cm)** | |
| 0,05 | 43 |
| 5 | 06 |
| 10,0 | 54 |
| 12,5 | 62 |
| 15 | 02-42 |
| 15,0 | 39 |
| 20 | 34 |
| 25 | 09-14-18-84-96 |
| 25,0 | 103 |
| 50,0 | 32 |
| 150 | 04-08 |
| 250 | 03-100 |
| **Column diameter (mm)** | |
| 2,1 | 32 |
| 2 | 02-42 |
| 2,1 | 08 |
| 3,9 | 39 |
| 4 | 62 |
| 4,6 | 03-04-06-09-14-18-54-84-96-100-103 |
| 46 | 34 |
| **Particle size (µm)** | |
| 1,7 | 32 |
| 1,8 | 06 |
| 2,6 | 08-54 |
| 4,0 | 39-42 |
| 5 | 02-03-04-09-14-18-34-62-84-96-100-103 |
| **Chromatographyc column (type)** | |
| Agilent | 84 |
| Hypersil® | 34 |
| LiChroCART® Zorbax® | 96 |
| Luna® | 02-03-09 |
| Novapak® | 39 |
| Phenomenex Kinetex® | 08-54 |
| Prodigy® | 100 |
| Supelcosil™ | 04 |
| Synergi® | 42 |
| Waters BEH tecnology | 32 |
| Zorbax® | 06-18-84-103 |
| pursuit C18 | 62 |
| **Mobile phase** | |
| acetonitrile / methanol/phosphate buffer / sodium decano sulfonate | 103 |
| acetonitrile / phosphate buffer / 1-decane sulfonic acid | 96 |
| methanol / acetonitrile / water | 100 |
| acetonitrile / methanol / ammonium acetate | 32-39 |
| acetonitrile / phosphate buffer | 04-34-84 |
| acetonitrile / natrium acetate / 1-octansulfonic | 62 |
| ammonium formiate / water / acetonitrile | 08 |
| methanol / ammonium formiate | 42 |
| phosphate buffer | 18 |
| water / acetonitrile | 54 |
| water / methanol | 02-03-06-09 |
| **pH of mobile phase** | |
| 3 | 08 |
| 4,5 | 39-62 |
| 6,9 | 04-84-103 |
| 7,0 | 32 |

| **Flow rate (ml/min)** | |
|---|---|
| 0,2 | 02 |
| 0,4 | 42 |
| 0,6 | 08-32 |
| 1 | 06-18-39-54-84-96 |
| 1,2 | 04-103 |
| 1,3 | 62 |
| 1,5 | 03-09 |
| 1,6 | 34 |
| 2 | 100 |
| **Post-column reagent** | |
| O-phthaldialdehyde (OPA) | 34-39-62 |
| **Injection volume (µl)** | |
| 5 | 02-08-32 |
| 10 | 39-42-54 |
| 20 | 04-06-18-34-62-84-103 |
| 25 | 100 |
| 30 | 03-09 |
| 50 | 96 |
| **UV em λ** | |
| 254 | 09-54 |
| 418 | 39 |
| 447 | 34 |
| 448 | 62 |
| 450 | 18 |
| 492 | 32 |
| 520 | 100 |
| **UV ex λ** | |
| 214 | 84 |
| 254 | 03-09 |
| 337 | 32 |
| 338 | 62 |
| 340 | 18 |
| 350 | 100 |
| 358 | 34 |
| 365 | 39 |

# HISTAMINE IN LYOPHILIZED TUNA MUSCLE, T1730B

### *Analytical methods used by partecipants*

| | **histamine** |
|---|---|
| **Methods** | **Laboratory Code** |
| ELISA | 02-08-10-12-18-41-78 |
| HPLC-FLD | 39 |
| HPTLC | 38 |
| Spectrofluorimetry | 63 |
| Fluorimetry | 102 |

| | **histamine** |
|---|---|
| **Is the analytical method a routine one ?** | **Laboratory Code** |
| YES | 02-08-10-12-18-38-39-41-63-78-102 |

### *Action level*

| | **histamine** |
|---|---|
| **Specify the concentration above which the material is considered not-compliant (mg/kg)** | **Laboratory Code** |
| 50 | 08 |
| 70 | 18 |
| 2500 | 12 |
| 50000 | 102 |
| 200000 | 39 |
| >100 | 02 |
| > 250 | 41 |

| histamine | |
|---|---|
| **In case of non-compliant results by screening, do you proceed with confirmatory analysis ?** | **Laboratory Code** |
| NO | 41 |
| YES | 02-08-18-38-39 |

***Number of replicates (1 replicate = 1 extraction)***

| histamine | |
|---|---|
| **Number of replicates** | **Laboratory Code** |
| 1 | 39-78 |
| 2 | 02-08-18-38-63 |
| 4 | 12 |
| 9 | 41 |

***Recovery***

| histamine | |
|---|---|
| **Recovery calculated by** | **Laboratory Code** |
| Control chart with Certified Reference Material (CRM) | 08 |
| Internal standard | 41-78 |
| Spiked sample | 39-63-102 |

***Quality***

| histamine | |
|---|---|
| **Quality control implemented** | **Laboratory Code** |
| Control chart with Certified Reference Material (CRM) | 08-41 |
| Control chart with in-house Reference Material (RM) | 63 |
| Duplicate analyses | 78 |
| Regular partecipation to proficency test (PT) | 02 |
| Spiked sample | 18-38-39-102 |
| Control chart | 02 |

***Reference***

| histamine | |
|---|---|
| **Reference** | **Laboratory Code** |
| Internal method | 02-38-63-78 |
| Kit insert | 08-12-18-41 |
| AOAC 977.13 | 102 |
| Torry Research Station, MAFF, Aberdeen, Scotland | 39 |

***Source of test kits***

| histamine | |
|---|---|
| **Manufacturer (Kit Code)** | **Laboratory Code** |
| Neogen | 10 |
| Neogen 9515 | 08 |
| BioSystems FCE3100 | 41 |
| Biooscientific 1032 | 78 |
| LDN Histamine Food ELISA | 18 |
| Tecna | 12 |
| r-Biopharm | 02 |

***Sample preparation***
***Amount of test sample***

| histamine | |
|---|---|
| **Sample volume (g)** | **Laboratory Code** |
| 1 | 02-78 |
| 5 | 08-18 |
| 10 | 38-41-63-102 |
| 25 | 39 |

*Sample treatment*

| | histamine |
|---|---|
| | **Laboratory Code** |
| **SPE Extraction** | |
| Dowex® | 102 |
| **Other Extraction** | |
| trichloracetic acid | 39 |
| water | 02-08-18-41 |
| water / methanol | 78-102 |
| **SPE clean up** | |
| Dowex® | 102 |
| **Other clean up** | |
| centrifugation | 41 |
| filtration | 08-39 |
| methanol | 78 |

*Calibration curve*

| | histamine |
|---|---|
| **Calibration Curve** | **Laboratory Code** |
| Linear regression | 39 |
| Point to point | 41-78 |
| No calibration curve, Definition of a B/B0 cut-off | 02 |

# TETRACYCLINES IN LYOPHILIZED FISH MUSCLE, FI1731A

*Analytical methods used by partecipants*

| | tetracyclines |
|---|---|
| **Methods** | **Laboratory Code** |
| HPLC-UV | 54 |
| HPLC/MS | 79 |
| HPLC/MS/MS | 21-40-81-82-87-92 |
| UPLC/MS/MS | 91-96-97-102-103-117 |

| | tetracyclines |
|---|---|
| **Is the analytical method a routine one ?** | **Laboratory Code** |
| NO | 102 |
| YES | 21-40-54-79-81-82-91-92-96-97-103-117 |

*Action level*

| | tetracyclines |
|---|---|
| **Specify the concentration above which the material is considered not-compliant (µg/kg)** | **Laboratory Code** |
| 20 | 102 |
| 25 | 40 |
| 100 | 82-96-103 |
| < 40 | 117 |
| CCα | 54-81 |

*Number of replicates (1 replicate = 1 extraction)*

| | tetracyclines |
|---|---|
| **Number of replicates** | **Laboratory Code** |
| 1 | 81-82 |
| 2 | 40-54-79-92-96-102-103-117 |