Development of Staphylococcal enterotoxin CRMs: 15 years of collaboration between ANSES and JRC

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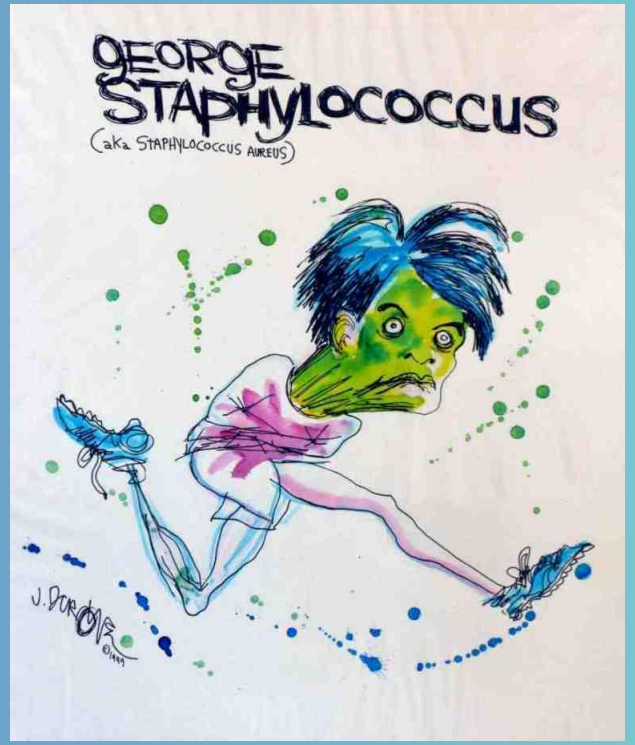
European Commission

Reflection and outlook

IRMM-359, SEA in cheese CRM	2011 - 2016
EQuATox, i.a. SEB PT	2012 - 2014
EuroBioTox	2017 - 2023
i.a. EURM-110, SEB calibrant CRM	2017 - 2021
ERM-BD359, SEA in cheese CRM	2025; ongoing
ERM-BD360, SEB in composite food CRM	will start 2026

IRMM 359 – SEA in cheese



Commission Regulation (EC) No 1441/2007 defines microbiological criteria for food stuffs. For SEs in cheeses, milk powders and whey powders: "not detected" in 5 sub-samples using the so-called ESM (European Screening Method ; ISO 19020 since 2017

European Union One Health Zoonoses Report: joint annual publication of EFSA and ECDC: notoriously high number of FPO cases with SEs involved

Insufficient hygiene practices during processing, cooking or distribution of food

Typical symptoms: nausea, vomiting, abdominal pain, diarrhoea, …

SEA is the SE type often involved in food-poisoning outbreaks related to SEs

How did the project evolve?

2010: Letter SANCO to IRMM to look into possibilities to collaborate with EURL CSP for the development of a *Staphylococcus aureus* enterotoxin CRM

Collection of technical information

Tripartite meeting SANCO – EURL CPS - IRMM

2011, visit of H. Schimmel and R. Zeleny at annual EURL CPS meeting, pre-meeting with EURL CPS staff to discuss technical and organisational issues towards a CRM

Feasibility study at IRMM for manufacturing of suitably homogeneous and stable materials (mixing, freeze-drying, upscaling issues); supported by ANSES

CRM project kick-off July 2012

Work distribution

JRC: coordination and lead of CRM project, processing, design of homogeneity, stability and characterisation studies, technical and statistical data evaluation, value assignment, reporting

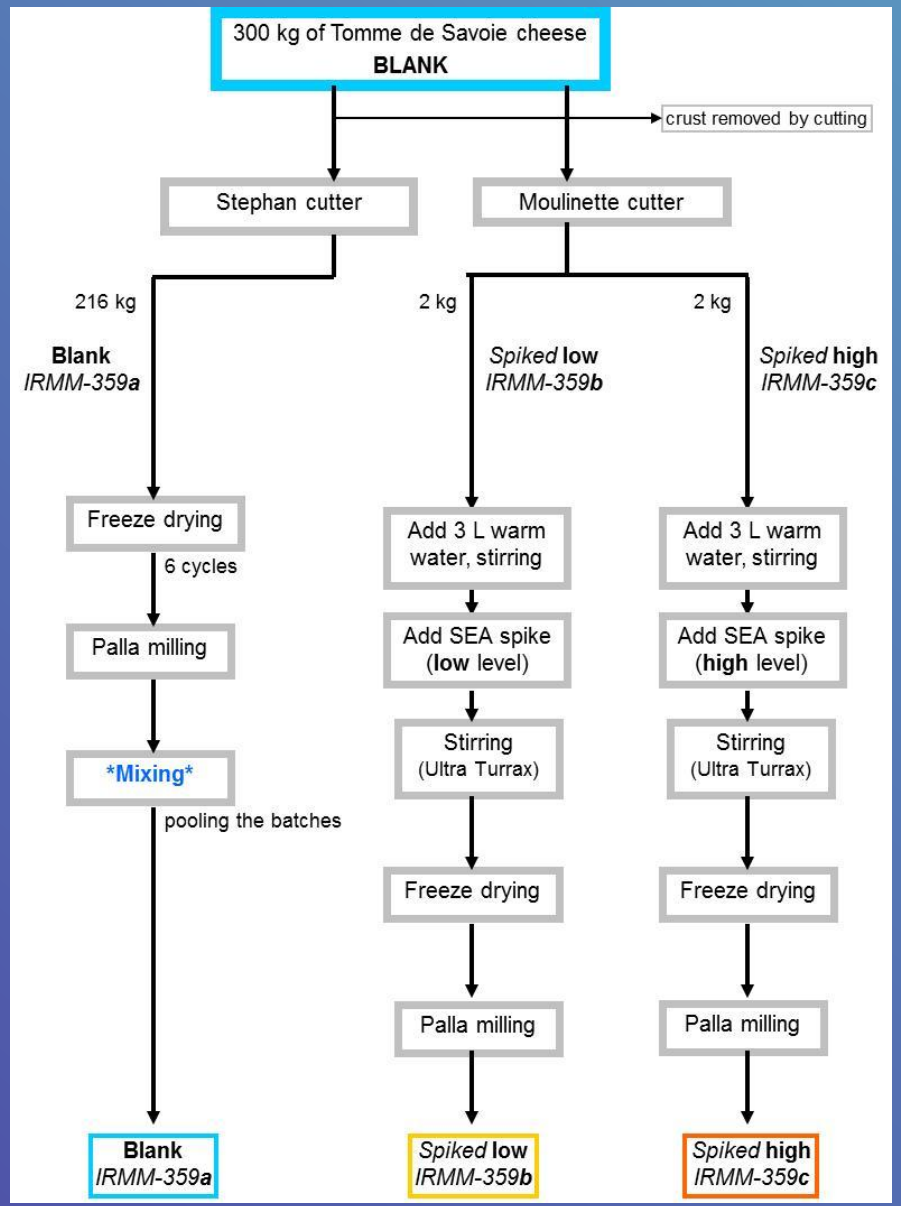
ANSES: conceptual contribution and measurement contributions:

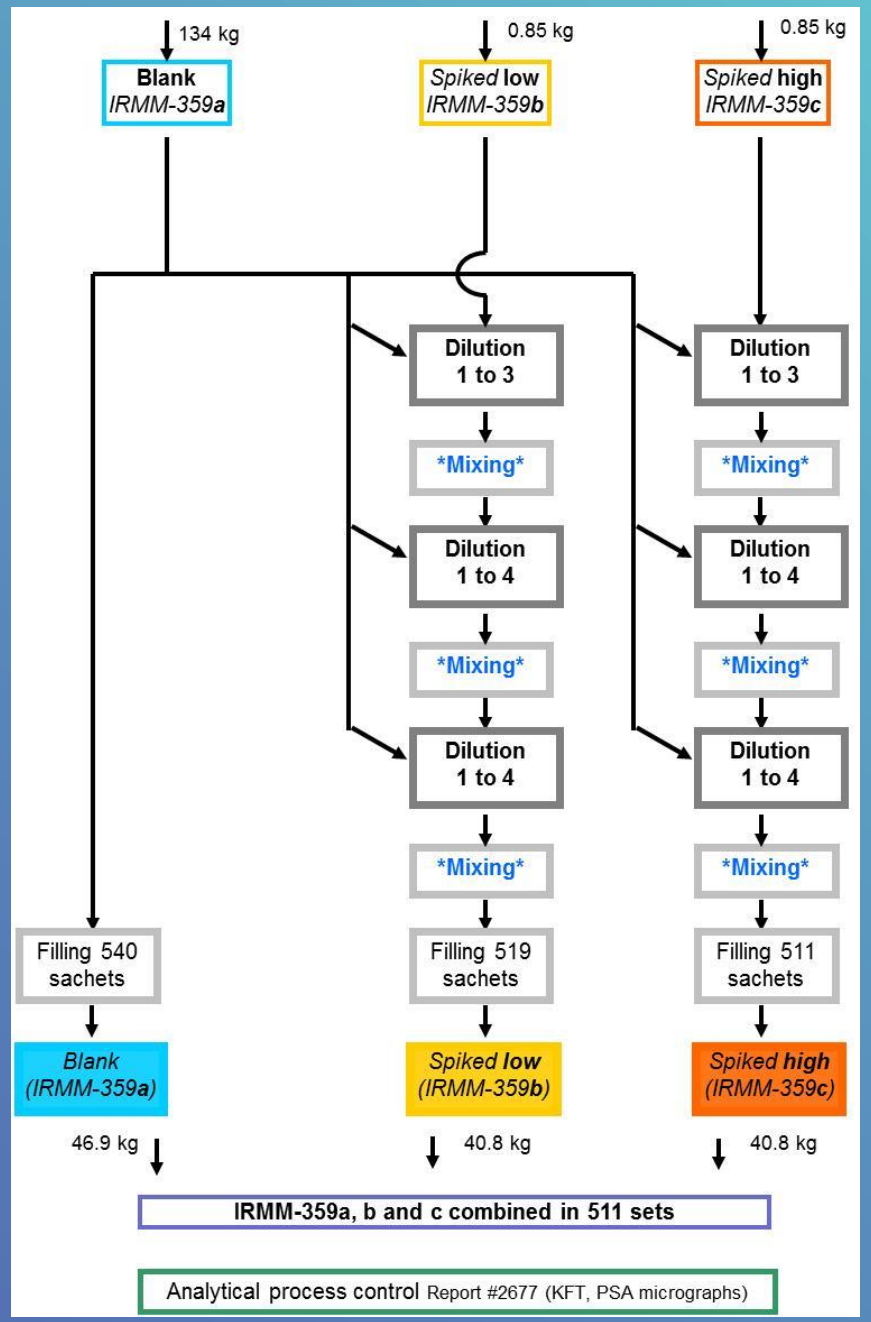
homogeneity, stability, characterisation, stability monitoring

VIDAS SET2, Ridascreen SET Total

~ 500 extractions and VIDAS analyses/Ridascreen analyses

Processing scheme

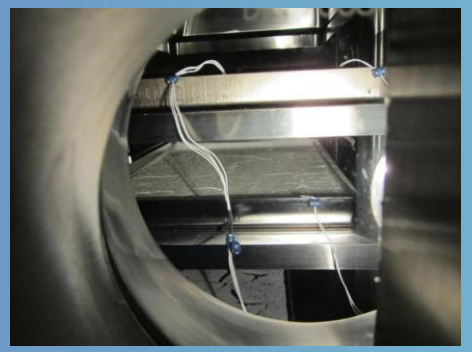




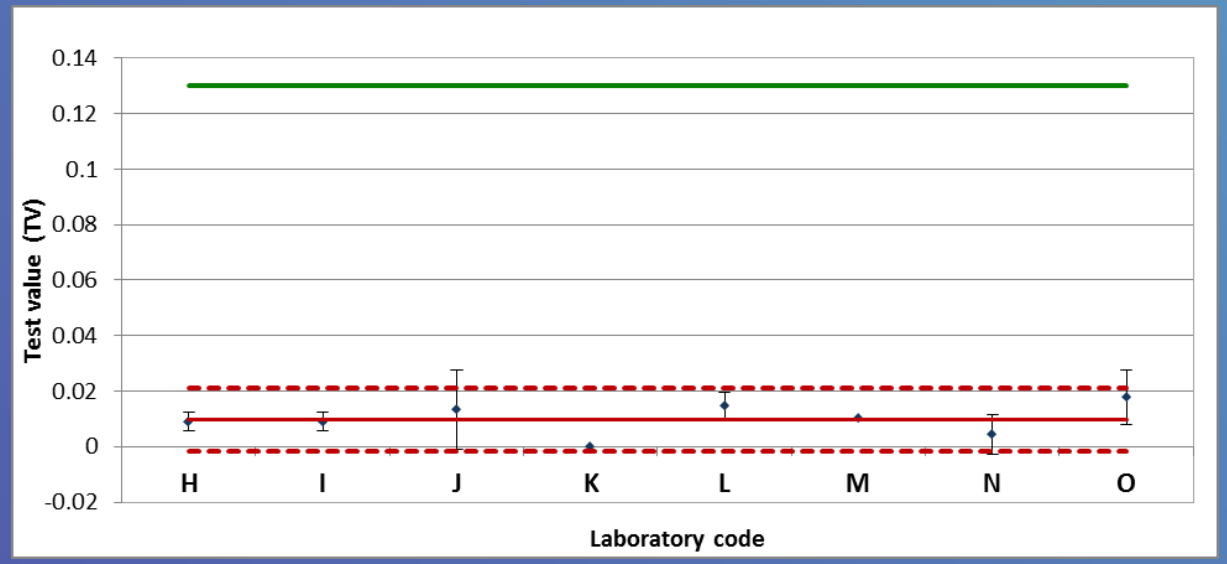
Impressions from processing



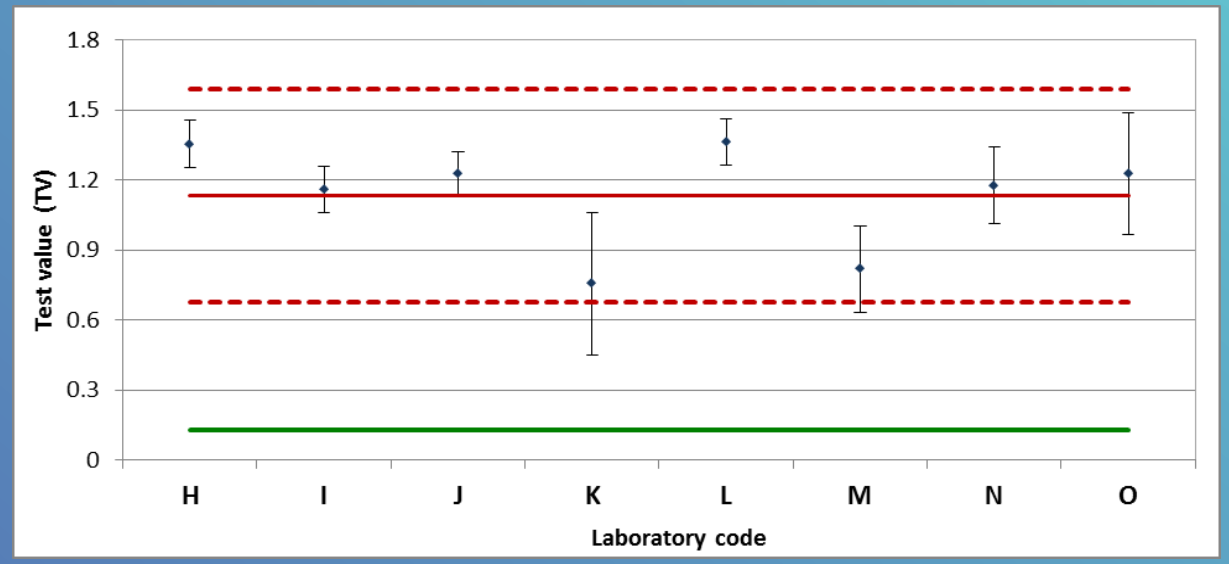



Characterisation - ESM with VIDAS SET2

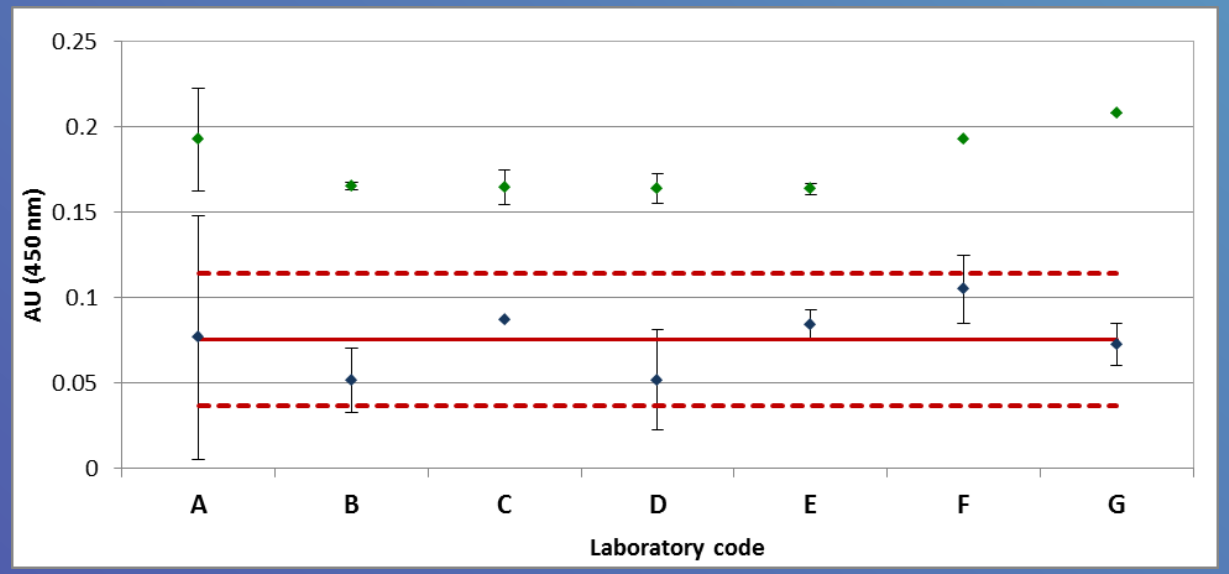


IRMM-359a

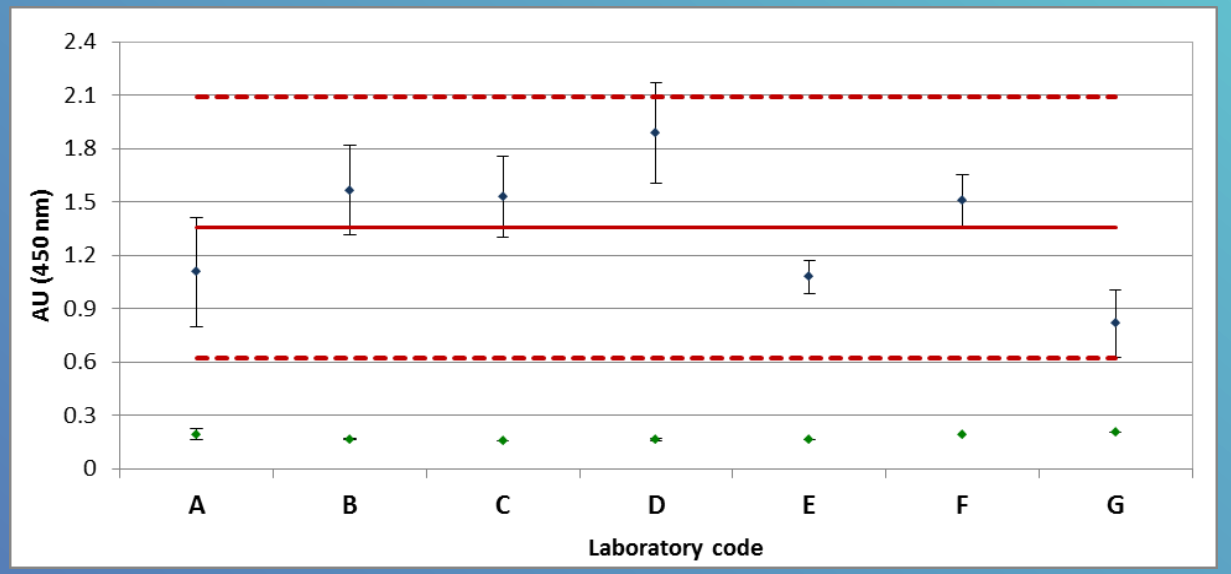


IRMM-359b

Characterisation - ESM with Ridascreen SET Total

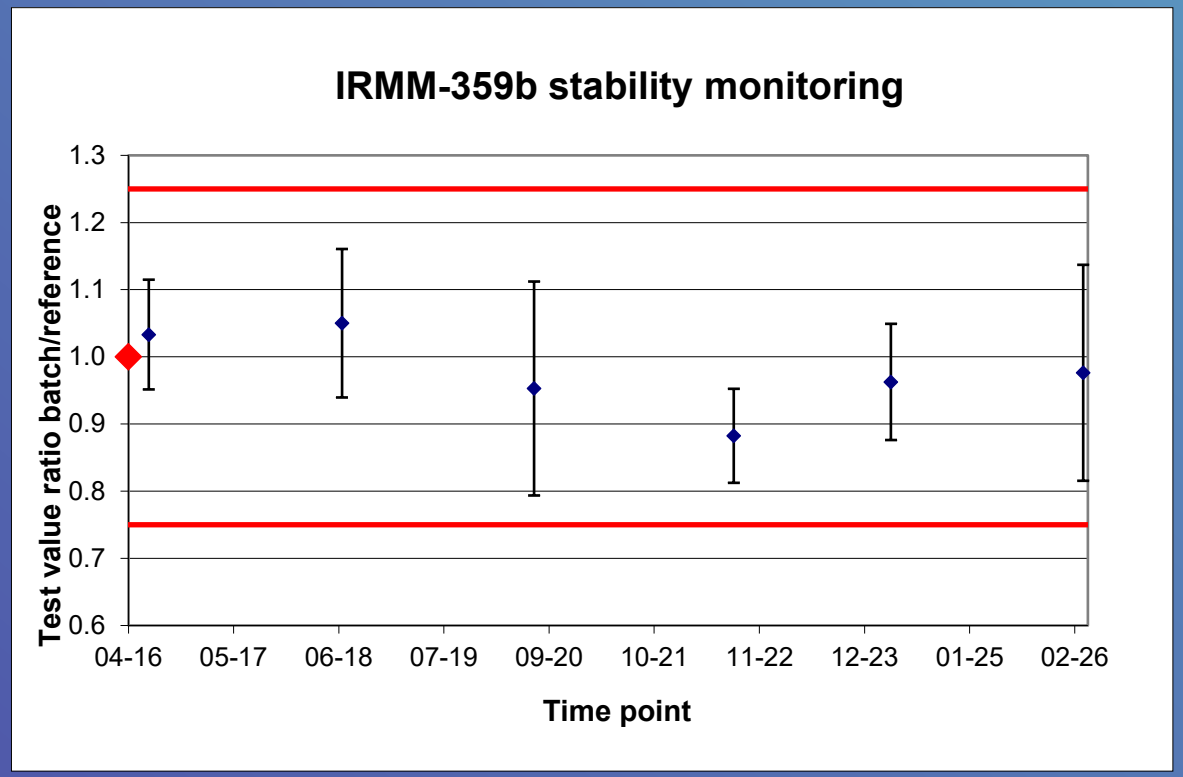


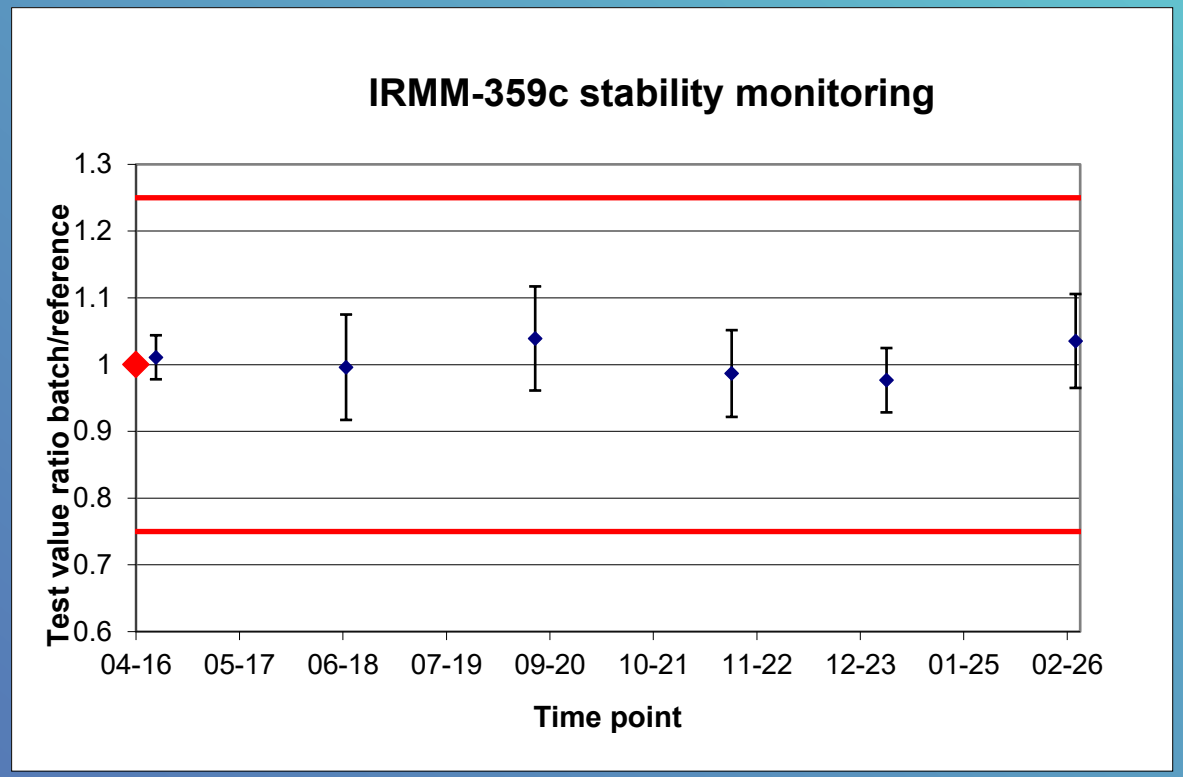
IRMM-359a



IRMM-359c

Stability monitoring





Certified values

Presence/absence approach; values have the meaning of a probability of detection, expressed as either diagnostic specificity (IRMM-359a) or diagnostic sensitivity (IRMM-359b/c)

Instead of an uncertainty, a one-sided lower confidence limit was indicated

IRMM-359a: 100 % (97.3 %) IRMM-359b: 100 % (97.5 %) IRMM-359c: 100 % (97.6 %)

as obtained in several laboratories using ESM-VIDAS/Ridascreen SET Total;

traceable to the SI

Additional material information values:

Mean value of all VIDAS SET 2 results and lowest and highest individual value

Mean value of all Ridascreen SET Total results and lowest and highest individual value

EQuATox

Main objectives

Main funding: European Commission, DG ENTR

Establishment of an EU network focusing on quality assurance for the detection of high priority biological toxins

Overview and evaluation of existing methods and technologies

Define the status quo of toxin detection within EU-28

Generation and characterization of toxin reference materials (pure substances)

Four rounds of proficiency tests on different toxins to compare the analytical approaches

Set the basis for future development of certified reference materials and ISO-accredited PTs

Identification of best practices; identification of any critical gaps in detection technology

SEB Proficiency test

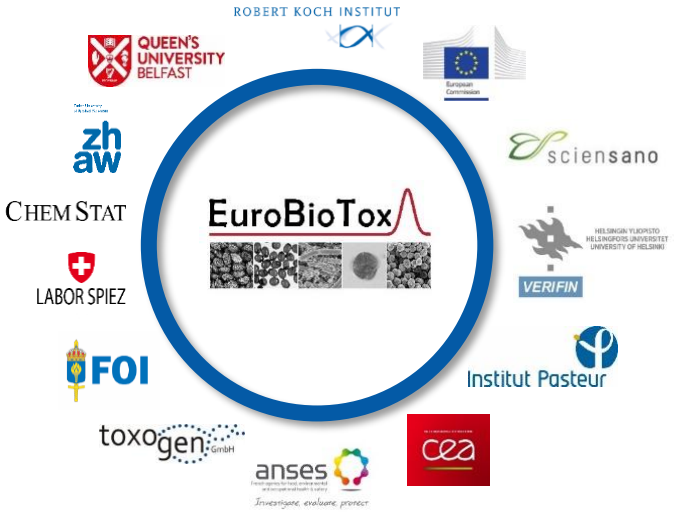
Matrices: buffer, milk

ANSES: PT organizer
JRC: quantification of SEB (Toxin Technology, US) by AAA using LC-MS/MS

Valuable information obtained for the successor project EuroBioTox

- PT matrices
- Spiking levels
- Methods
- Homogeneity and stability

Purity assessment of native SEB – recombinant SEB as more appropriate candidate for a SEB calibrant CRM



Main funding: European Commission, DG HOME

Main objectives

Certified reference materials: ricin, abrin, **SEB**, BoNT/A, BoNT/B
Repository of tools and reagents
Proficiency tests (2 per toxin + PT with forensic context)
Quality management and SOPs
Training courses (different methods; basic and advanced)
In-situ detection and forensics
Animal replacement methods

EuroBioTox and EQuATox

Experts from three different fields working together:

Food safety (e.g. ANSES, QUB) - Public health (e.g. RKI, Institut Pasteur) - Security/military/verification sector (e.g. FOI, CEA)

+ JRC (CRMs; standardisation; metrology) + Chemstat (statistics)

EURM-110 (SEB calibrant)

rSEB processed and aliquoted at Toxogen (A. Rummel and team)

JRC: coordination and lead of CRM project

ANSES: measurements contributions:

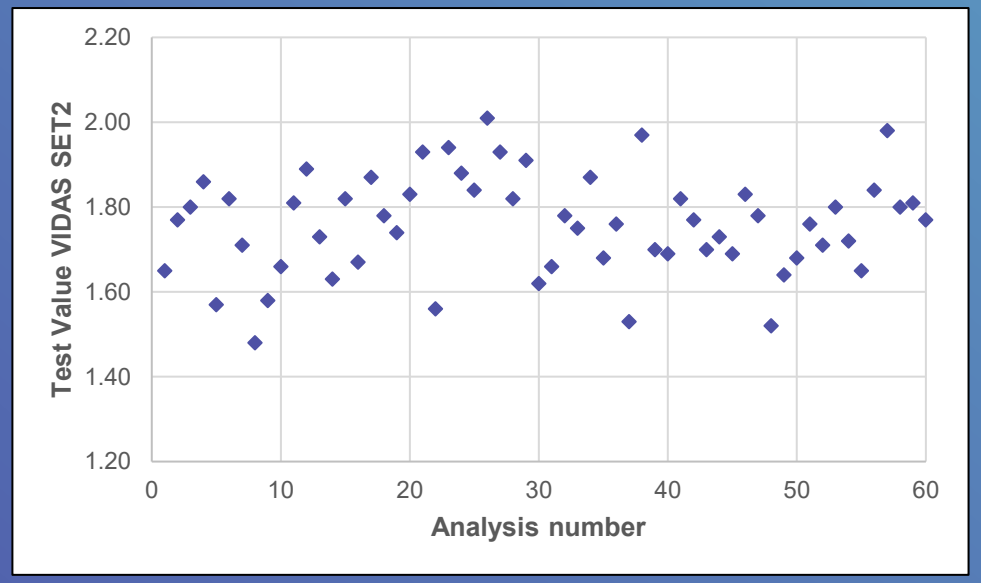
material purity and identity, homogeneity, stability, characterisation

in-house ELISAs (SEA-SEE); VIDAS SET2, Ridascreen SET Total, SDS-PAGE, LC-MS/MS

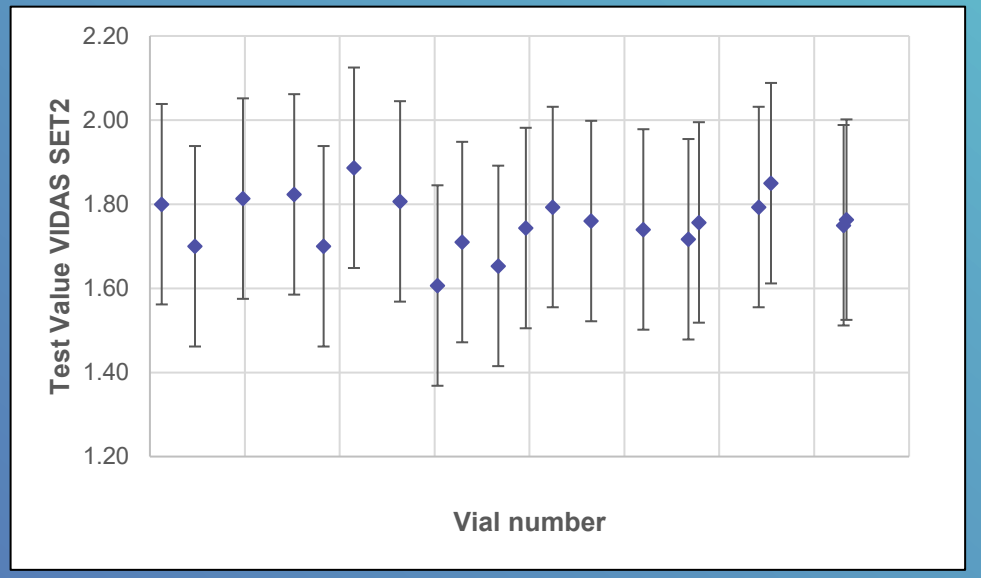
240 analyses VIDAS, 240 analyses in-house ELISA

~ 540 analyses in total

Homogeneity

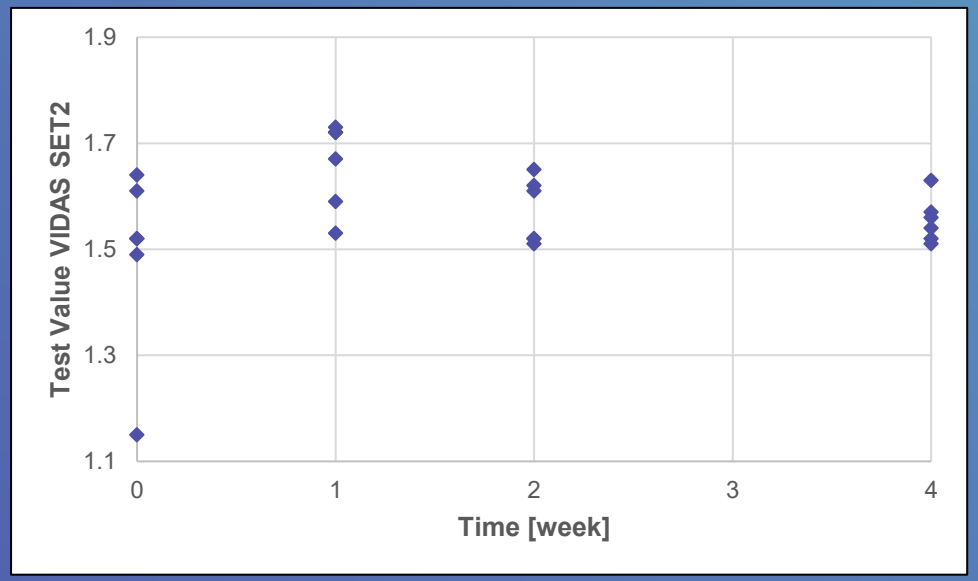


Analytical sequence

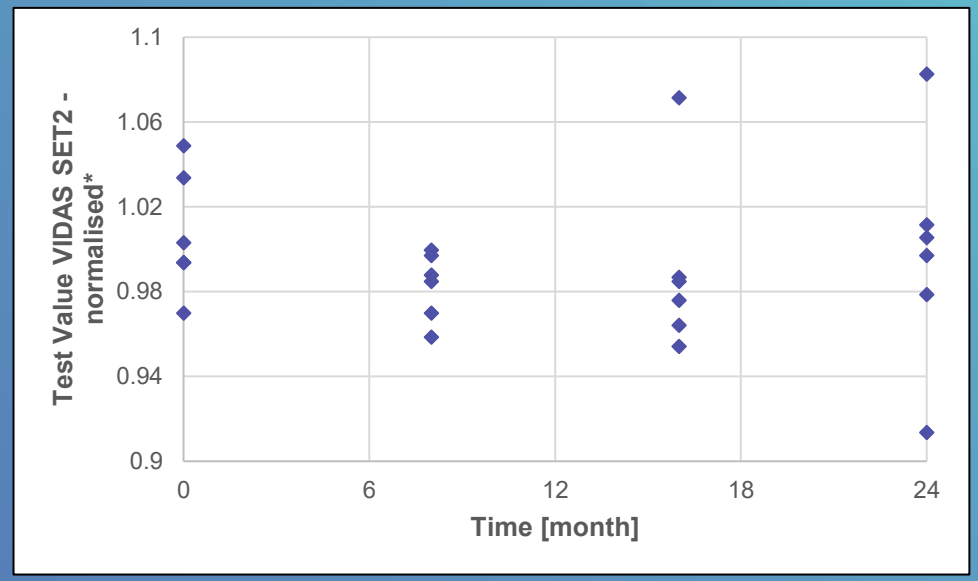


Filling sequence

Short- and long-term stability



+18 °C



-80 °C

Certified value: 1.39 ± 0.08 mg/mL and mg/g

as obtained by amino acid analysis in two laboratories

traceable to the SI

Additional material information values:

Mean value (n=36) VIDAS SET 2 measurements

Mean value (n=36) Ridascreen SET Total measurements

Mean value (n=6) determining the biological activity relative to two native SEB preparations using a cell-based assay

Mean value (n=16) determining the residual endotoxin content using a Limulus amoebocyte lysate (LAL) assay

Processing of ERM-BD359 - impressions

Processing of ERM-BD359 – impressions

ERM-BD360, SEB in composite food

Feasibility study: SEB in a food matrix

Rice salad (rice, ham, lettuce, tomato); material can be processed (freeze-dried powder), but very low SEB recovery due to the rice fraction

Quiche Lorraine (was one food matrix in the validation study for ISO 19020; SEA toxin)

Freeze-drying does not work (greasy pellet obtained)

QL slurry worked, 0.2 and 0.5 ng SEB/g QL are suitable concentration levels

Small scale homogeneity and short-term stability studies conducted

ANSES support in measurements: VIDAS SET2/Ridascreen SET Total; in-house ELISAs with pAbs and mAbs

CRM kick-off meeting June 2026

Poster/oral presentations related to the CRMs

IAFP Marseille 2013
SYMPOSTAPH Lyon 2014
IAFP Athens 2016
IAFP Brussels 2017
CBRN workshop* Helsinki 2017
IAFP Munich 2022
EUROTOX Ljubljana 2023
MBDC Munich 2023
FBTIS Maison-Alfort 2025

FEMS Leipzig 2013
SFM Paris 2015
ISSSI Seoul 2016
SFM Paris 2017
IMEKOFOODS online 2020
NBC CBRNE Lahti 2022
SIC CBRNE Rome 2023
Eurachem/CITAC online 2024

* marking the 20th anniversary of CWC

Peer-reviewed publications related to the CRMs

Zeleny, R., Emteborg, H., Charoud-Got, J., Schimmel, H., Nia, Y., Mutel, I., Ostyn, A., Herbin, S., Hennekinne, J.A. (2015) Food Chem. 168, 241-246

Zeleny, R., Nia, Y., Schimmel, H., Mutel, I., Hennekinne, J.A., Emteborg, H., Charoud-Got, J, Auvray, F. (2016) Anal. Bioanal. Chem.408. 5457-5465

Dorner, B.G., Zeleny, R., Harju, K., Hennekinne, J.-A., Vanninnen, P., Schimmel, H., Rummel, A. (2016) Trends Anal. Chem. 85, 89-102

Zeleny, R., Rummel, A., Jansson, D, Dorner, B.G. (2019) ACS Symposium Series. Vol. 1339, chapter 12, 185-202

… more planned …

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DG ENTR and DG HOME

Thank you