D5.1 Organisation and conduct of inter-laboratory comparison tests under Tox-Detect

WP5 Inter-laboratory ring trial scheme

Responsible Partner: Anses

Contributing partners: Anses, INRAe, Pasteur Institue, BFR, NWI, Sciensano

GENERAL INFORMATION

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	Other international stakeholder(s): …… Social Media: ..... **Other recipient(s):** .....

Table of Contents

1 List of Abbreviations

ILC	Inter-laboratory comparison
WP	Work Package
TL	Task Leader
SOP	Standard Operating Process
ELISA	Enzyme-linked Immunosorbent Assay
MALDI ToF	Matrix Assisted Laser Desorption Ionization - Time of Flight
LC-MS	Liquid Chromatography-Mass Spectrometry/mass Spectrometry
LOD	Limit of Detection
RSD	Relative Standard deviation
ISO	International Organization for Standardization

2 Definitions

(ILC) material	Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in proficiency testing.
Replicate	Complete repetition of a sample analysis including sample preparation.
ILC item, sample	Defined volume or mass of ILC material filled into a suitable container (e.g. vial) for dispatch and use by the ILC participants.
Test portion	The amount of the unit or subsample used for analysis.
Method developper	In charge of the development of the method, and the transfer of the method to participants.
Task Leader	In charge of the management and coordination of tasks dedicated to Maldi-TOF (T. Skjerdal), LC-MS (J. Masquelier) and ELISA (M. Michaut).
ILC organizer	In charge of the organisation of the Inter Lab test (sample preparation and characterisation, invitation, sample dispatch, results assessment, report of ILC…).

3 Background and objectives

The inter-laboratory comparison (ILC) tests will enable the transfer of the methods developed in this project to different partners within the consortium and will allow the identification of success factors critical for bacterial strains characterization and detection of their toxins. These ring tests are prerequisite for the use of the developed methods in routine analysis in the longer term.

The purposes for these ILC include:

- Evaluation of the performance characteristics of a method (qualitative and/or quantitative),
- Help in identification of "best practices" for the analysis of these toxins,
- Establishment of the effectiveness and comparability of test or measurement methods,
- Identification of critical gaps in the detection technology, both under qualitative and quantitative aspects ,
- Identification of interlaboratory differences,
- Education of participating laboratories based on the outcomes of such comparison.

An overview of the WP5 actions is presented in Figure 1 below:

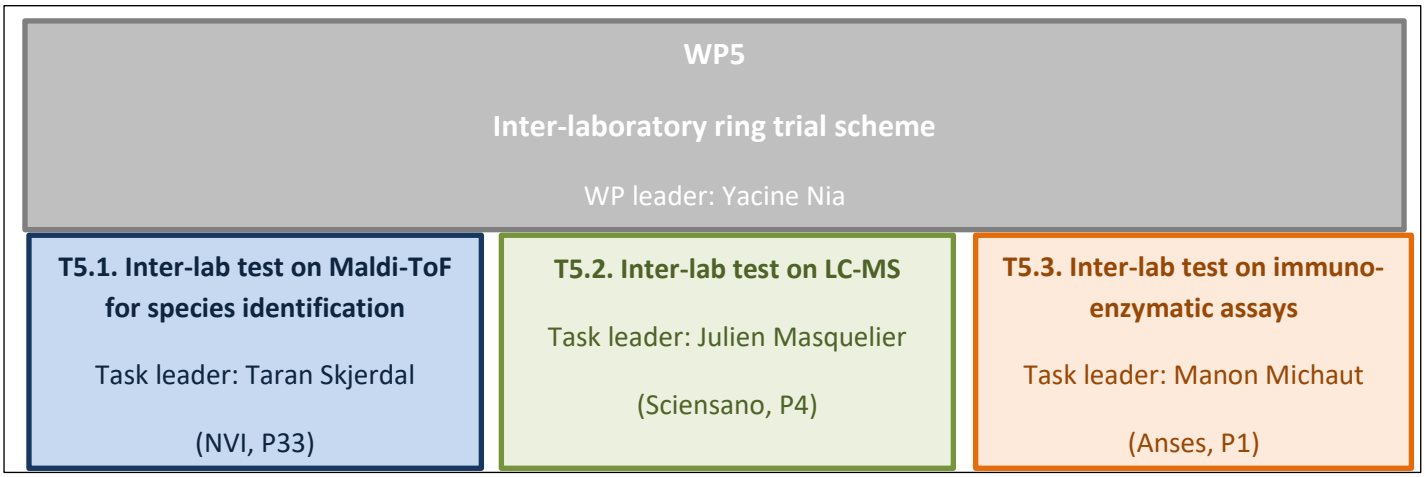


Figure 1: WP5 actions overview

Eight inter-laboratory comparison tests will be organized in order to check performance of:

(i) MALDI-ToF based analysis with a selection of reference bacterial strains as established within the framework of the WP1 (*Staphylococcus aureus, Bacillus cereus, Clostridium perfringens),*

(ii) Mass spectrometry methods using LC-MS (*Staphylococcus aureus, Bacillus cereus, Clostridium perfringens),* developed within the framework of WP3,

(iii) Immuno-enzymatic assays (*Staphylococcus aureus, Bacillus cereus),* developed within the framework of WP4.

4 General requirements

Each ILC organizer should:

- Be trained in the method to transfer it in his/her laboratory and test method repeatability and reliability according to the method developer prescriptions in his/her laboratory
- Check ILC item (strain, culture supernatant) availability based on WP1.
- Announce the ILC and nominate the participants (at least) **3 months** before sample dispatch
- Announce sample dispatch by providing a short document giving a general overview of the purpose of the ILC and its conditions (e.g. Materials, (minimum) number of samples) including an overview of the timeline. Note: spiking levels and number of replicates should not be indicated before the reception of results from participants

5 Test plan

The ILC organizers shall document a detailed plan (Appendix 1) before the beginning of the proficiency testing scheme that adresses the objectives, purpose and basic design of the proficiency testing scheme including the following information:

- Name, type and purposes of the ILC,
- Identification of the coordinator and participants,
- ILC schedule,
- Previsional number of samples and detailed sample preparation,
- Packaging, labelling and distribution of ILC items,
- Information on the performing conditions of the analyses,
- Results report information,
- Statistical information.

A detailed planning (ILC schedule) should be drafted by the ILC organizer (and presented to the WP5 leader for approval).

6 ILC participants invitation

The ILC invitation (Appendix 2) letter must be sent to the participants at least **3 months** before the test is to begin. This invitation shall be sent with the ILC presentation document (Appendix 3) detailing the ILC purposes, performance conditions and statistical information.

7 ILC items

7.1 Sample composition

The ILC organizers shall:

- Discuss the appropriate ILC items selection (strains, culture supernatant) with WP5 partners and methods developers,
- Define samples composition (pure bacterial culture or mixed cultures with two or three pathogens/ supernatant containing one or more toxin) (see suggested composition),
- It is highly recommanded to include high, medium and low concentration samples of the individual toxin if possible. For exemple, low concentration level could correspond to the LOD of the developped method whereas medium and high could correspond to 5X and 10X the LOD,
- Select the replicate number to be analysed for each ILC,
- Evaluate the stability and homogeneity of the ILC, as well as expected values (see paragraph 8.1) and associated uncertainties if relevant,
- Ensure the stability of samples by performing stability tests and informing participants concerning the samples stability period,
- Use well characterized bacterial strains from the reference collection established in WP1 should be used in the ILC as well as strains from partner's collection. They should be sufficiently characterized to ensure that they can be detected by ILC methods (MALDI-ToF),
- Include both typical and atypical strains of targeted bacteria in the scheme program to challenge the MALDI-ToF method,
- Ensure that the matrices used in the trials are bacterial culture for MALDI-ToF ILC and culture supernatants for ELISA and LC-MS ILC.

7.2 Suggested sample composition for each type of ILC, MALDI-TOF, LC-MS, ELISA

Examples of samples selection and spiking levels are listed bellow, they can be adapted according to the particularity of each developed method

7.2.1 MALDI-ToF ILC

Samples dedicated to the ILC are a pure bacterial culture containing:

Table 1: Suggested sample composition ILC MALDI-TOF

Staphylococcus aureus	***Bacillus cereus***	***Clostridium perfringens***
6 strains from reference collection (WP1)	6 strains from reference collection (WP1)	6 strains from reference collection (WP1)
3 sufficiently characterized typical strains from partner's collection*	3 sufficiently characterized typical strains from partner's collection*	3 sufficiently characterized typical strains from partner's collection*
Either** - 3 or more strains from the partner's collection obtained during recent surveillance, outbreak investigation or routine analysis, or - 1 or more untypical CPS strain from the partners collection	Either** - 3 or more strains from the partner's collection obtained during recent surveillance, outbreak investigation or routine analysis, or - 1 or more untypical Bc strain from the partners collection	Either** - 3 or more strains from the partner's collection obtained during recent surveillance, outbreak investigation or routine analysis, or - 1 or more untypical Cp strain from the partners collection
1 species mix → with 2 different bacterial species***	1 species mix → with 2 different bacterial species***	1 species mix → with 2 different bacterial species***
Blank: bacterial strains other than CPS, Bc and Cp	Blank: bacterial strains other than CPS, Bc and Cp	Blank: bacterial strains other than CPS, Bc and Cp

**The strains can be the reference strains used in the lab, or another strain that fulfils the criteria for well characterised strains. If the lab does not have its own, sufficiently characterised strains, a panel of strains will be offered by the organiser. The minimum criteria for a well characterised, typical strains is that it gains the typical characteristics as given in the ISO standard for detection. Strains with different toxin gene profiles are desired. WGS data or other typing data are desired, but not required.*

***The purpose of inclusion of such strains is to obtain a larger variety of strains and include recently isolated strains in the test, in order to challenge the methodology. Only strains for which data can be shared within the ToxDetect team should be included.*

****The mix of species will serve as an example of a poorly prepared sample, and lead to false negatives or positives*.

7.2.2 LC-MS

An ILC dedicated to LC-MS developed methods will be performed on culture supernatant naturally contaminated or spiked by targeted toxins. Naturally, contaminated supernatant can be obtained using toxigenic strains selected within the frame of WP1 (D1.1). A protocol for toxin production in culture medium will be provided by WP1 or WP2.

Samples dedicated to the ILC for LC-MS must contain at least one blank sample, and one spiking (contamination) level for each targeted toxin. As LC-MS is a highly specific method, it is possible to prepare single samples spiked with a mix of toxin.

Table 2: Suggested sample composition ILC LC-MS

<table>
<tr><th colspan="2">Staphylococcus aureus</th><th colspan="2">Bacillus cereus</th><th colspan="2">Clostridium perfringens</th></tr>
<tr><td rowspan="3">Toxin SEO</td><td>1 low concentration</td><td rowspan="2">Nhea</td><td>1 low concentration</td><td rowspan="7">Cpe</td><td>2 Low concentrations</td></tr>
<tr><td>1 medium concentration</td><td>1 high concentration</td><td>2 Medium concentrations</td></tr>
<tr><td>1 high concentration</td><td rowspan="2">Cytk2</td><td>1 low concentration</td><td rowspan="3">2 High concentrations</td></tr>
<tr><td rowspan="3">Toxin SEN</td><td>1 low concentration</td><td>1 high concentration</td></tr>
<tr><td>1 medium concentration</td><td rowspan="2">Smase</td><td>1 low concentration</td></tr>
<tr><td>1 high concentration</td><td>1 high concentration</td></tr>
<tr><td>Supernatant from atoxin S. Aureus strain</td><td>1 supernatant from atoxin S. Aureus strain</td><td rowspan="2">Supernatant from atoxin B. Cereus strain</td><td rowspan="2">1 supernatant from atoxin B.cereus strain</td><td rowspan="3">Supernatant from atoxin C. Perfringens strain</td><td rowspan="3">1 Supernatant from atoxin C. Perfringens strain</td></tr>
<tr><td>Other SE producing strain</td><td>1 other SE producing S. Aureus strain supernatant</td></tr>
<tr><td>Mix containing SEN and SEO toxins</td><td>1 Mix containing SEN and SEO toxins</td><td>1 mix → with 2/3 different toxins</td><td>1 mix at medium concentration</td></tr>
<tr><td>Blank</td><td>1 Blank (culture medium)</td><td>Blank</td><td>1 Blank (culture medium)</td><td>Blank</td><td>1 Blank (culture medium)</td></tr>
</table>

7.2.3 Immuno enzymatic assays

Table 3: Suggested sample composition ILC immune-enzymatic assays

<table>
<tr><th colspan="2">Staphylococcus aureus</th><th colspan="2">Bacillus cereus</th><th colspan="2">Clostridium perfringens</th></tr>
<tr><td rowspan="3">Toxin SEO</td><td>1 low concentration</td><td rowspan="2">Nhea</td><td>1 low concentration</td><td rowspan="8">Cpe</td><td>2 Low concentrations</td></tr>
<tr><td>1 medium concentration</td><td>1 high concentration</td><td>2 Medium concentrations</td></tr>
<tr><td>1 high concentration</td><td rowspan="2">Cytk2</td><td>1 low concentration</td><td rowspan="4">2 High concentrations</td></tr>
<tr><td rowspan="3">Toxin SEN</td><td>1 low concentration</td><td>1 high concentration</td></tr>
<tr><td>1 medium concentration</td><td rowspan="2">Smase</td><td>1 low concentration</td></tr>
<tr><td>1 high concentration</td><td>1 high concentration</td></tr>
<tr><td>Supernatant from atoxin S. Aureus strain</td><td>1 supernatant from atoxin S. Aureus strain</td><td rowspan="2">Supernatant from atoxin B. Cereus strain</td><td rowspan="2">1 supernatant from atoxin B.cereus strain</td><td rowspan="3">Supernatant from atoxin C. Perfringens strain</td><td rowspan="3">1 Supernatant from atoxin C. Perfringens strain</td></tr>
<tr><td>Other SE producing strain</td><td>1 other SE producing S. Aureus strain supernatant</td></tr>
<tr><td>Mix containing SEN and SEO toxins</td><td>1 Mix containing SEN and SEO toxins</td><td>1 mix → with 2/3 different toxins</td><td>1 mix at medium concentration</td></tr>
<tr><td>Blank</td><td>1 Blank (culture medium)</td><td>Blank</td><td>1 Blank (culture medium)</td><td>Blank</td><td>1 Blank (culture medium)</td></tr>
</table>

7.3 Sample preparation

The ILC organizer shall:

- Establish and implement procedures to ensure appropriate acquisition, collection, preparation, handling, storage and disposal of all ILC items in accordance with the plan described
- Select an homogenous and stable culture medium,
- Prepare ILC items (spiked culture medium or culture supernatant) in accordance with procedures (see plan test) or send a detailed procedure to participants who should prepare the samples in their laboratory,
- Prepare a sufficient amount of ILC items to cover ILC tests for all participants, homogeneity and stability tests, and supplementary items.

Table 4: Example of calculation of test unit number to prepare

Participant number : 3

2 contamination levels	**replicat / participant = 2**	**Test unit number for homogeneity**	**Test unit number for stability**
Blank	2	3 (in duplicate)	2 (in duplicate)
Contamination level 1	2	10 (in duplicate)	3 (in duplicate)
Total test unit number / participant	4	13 (in duplicate)	5 (in duplicate)
Total test unit number = (4*3) + 13 +5 = 30			

7.4 ILC items characterisation

Only homogeneity and stability studies will be conducted according to ISO 13528:2015 [2]

- Criteria for suitable homogeneity and stability shall be established and shall be based on the effect that inhomogeneity and instability will have on the evaluation of the method's performance,
- A preliminary stability and homogeneity study must be done before the ILC in order to determine ILC period and spiking levels. The ILC sample batch will be prepared taking into account preliminary study results,
- Homogeneity and stability studies must be done on the same batch of sample used for the ILC.

7.4.1 Homogeneity study

The organising laboratory has to show that the ILC material prepared is sufficiently homogenous [1, 2]. Therefore, at least 10 units per material (exept for blank) are to be randomly selected and analysed under repeatability conditions in (true) duplicates for homogeneity (20 data are expected).

7.4.2 Stability of ILC material

- The stability of ILC materials must be ensured for the duration of the ILC by the organising laboratory [1-3].
- Therefore, at least 3 units per material (exept for blank) are to be randomly selected and analysed under repeatability conditions in (true) duplicates for stability (6 data points are expected).
- The stability study must cover the whole time period of an ILC, from sample storage until the reception of the complete set of results,
- The study has to be done prior to the ILC using a sample set identical to the one planned to be used in the ILC (a procedure for checking stability during the course of a proficiency testing round is given in [1,2]). In the case of samples tested on several concentration levels only the lowest level of contamination must be tested. This will help to optimise the workload,
- The simplest approach is to take measurements on three sets of units at two points in time, in order to draw conclusions about future stability based on change over the elapsed time. For exemple, if the period of the ILC (from sample storage until data reception) is fixed at 30 days, the 1st stability point (3 samples analysed in duplicates) will be done on day 1 (storage day) and the 2nd stability point (3 samples analysed in duplicates) will be performed after day 30 (deadline for returning results by participants),
- The temperature selected for sample storage, transport and during the stability test must be the temperature that will be used in parcels during sample shipping.

7.5 Packaging, labelling and distribution of ILC items

- The actual sample dispatch has to be announced ~1 week prior to shipment as a reminder for all participants. This announcement contains information on the shipment/packging and storage conditions for the samples.
- The ILC organizer shall organise the ILC items transport
- The ILC organizer shall specify relevant environmental conditions for the transport of ILC items
- Where relevant the ILC organiser shall monitor the pertinent environmental conditions during transport and assess the impact of environmental influences on the ILC item
- The proficiency testing provider shall follow a procedure to enable the confirmation of delivery of the ILC items
- Selected methods are provided as "ready to use pack" including a detailed SOP and necessary tools and reagents to participants
- Each participating laboratory shall receive a clear set of instruction covering storage conditions (temperature), how to handle the samples (i.e: if reconstitution or dilution is necessary), safety data

sheets, latest dates for performing examinations, how to report the results with the samples (hard copy or electronically)

7.6 ILC instructions and result file

An instruction document is sent together with the samples and also provided by e-mail together with the Excel reporting form (Appendix 4). The design of the Excel result file has to be improved for each method with WPL to accommodate the needs of the individual toxins/methods.

- The ILC providers shall establish and implement procedures to ensure that test items are prepared in accordance with the plan described (according to ISO/IEC 17043:2010 (E))
- Dedicated results forms (Appendix 4) will be developed by each method's TL in accordance with methods developers and ILC organisers

8 Analysis of results

8.1 Expected values

ILC organizers shall determine expected values for each sample tested in quantitative analysis depending on its composition and contamination level. This expected value could be the mean value obtained during the homogeneity test on 20 units, plus/minus the relative standard deviation (RSD). The RSD shall be determined by methods developer for each method depending on SOP characteristics (RSD is indicated in each method SOP).

8.2 Qualitative results

The ILC organizer shall determine conformity of results (Obtained vs expected).

8.3 Quantitative results

Values obtained by participants will be compared to the expected values (obtained from homogeneity data) taking into account the uncertainty of the method (mean ± RSD%).

9 Report

In order to ensure the final ILC report, a common template will be proposed by the WP5 leader. These reports will be compiled in D5.2

10 References

1. *ISO/IEC 17043:2010 Conformity assessment – General requirements for proficiency testing*. 2010, International Organization for Standardization: Geneva, Switzerland.
2. *ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison*. 2015, International Organization for Standardization: Geneva, Switzerland.
    - B.1 General procedure for homogeneity check
    - B.4 Procedures for checking stability

11 Appendix

Annex 1: inter-lab test comparison detailed plan

INTER LAB TEST COMPARISON DETAILED PLAN			
ILC		**Version**	**01**

ILC detailed plan is an intern document of EJP TOX-Detect enabling to plan and organise the ILC. Whole agents engaged in ILC organisation have to be aware of this plan, related

ILC	
ILC name	
Type	
Purpose	This PT is organised within the WP5 ToxDetect EJP project This test plan reflects in particular the needs and expectations of the prescriber defined in the laboratory work program. It aims to measure the ability of participating laboratories to perform the test described below and to provide results in line with those expected. As part of its reference missions, ILPT also aims to guide laboratories that have obtained an unsatisfactory result in the implementation of corrective actions and to monitor their effectiveness. PT is organised according to the requirements of standard ISO 17043.
Identification of the organiser	
Name	
Adress	
Identification of the coordinator	
Name	Unit/team
☎	Email
Identification of the coordinator (link final participant list)	
Estimated participant number	
Conflict of interest, collusion and confidentiality	
■ **Conflicts of interest** (in case of ILC participation by the ILC organiser unit/team)	
No conflicts of interest - To avoid collusion in ILC organiser team/unit following measures have been established: -random codification of ILC matérial -paticipating agents are not involved in ILC organisation. Moreover agents involved in ILC organisation commits to: -respect ILC confidentiality -to not interact with ILC agents involved in ILC organisation -to secure ILC organisation documents	
■ **Collusion and confidentiality**	
To avoid collusion and/or confidentiality problems following measures have been established: -random codification of ILC material -in the participation form, the participating lab commits to don't cause any colllusion between participants or falsify results -Every mailing of documents is made on carbon copy to protect anonymity -decoys are add in test panel -assigned values are not communicated until all participants results is sent to the organiser	
ILC material preparation	
Analyte/matrix	
Levels of contamination	
Matrix/sample choice justification	see preliminary study
Quantity to prepare for a test unit	

Previsional test unit number

Levels of contamination	Test unit number per participant per level	Test unit number for homogeneity	Test unit number for stability			Supplementary test unit number	Total test unit number	Total amount
		Test unit number for homogeneity per tested	Stability points tested	Test unit number for	Total test unit number			
					0		0	0
					0		0	
					0		0	
					0		0	
					0		0	
					0		0	
					0		0	
					0		0	
					0		0	
					0		0	

■ **Raw material acquisition specification for test unit preparation:** matrix and blank type and specificity...

■ **Test unit preparation risks (contamination, blank preparation, environmental conditions…):** Risk analysis and precaution description (control, monitoring…)

■ **Test unit preparation modalities: method of operating, repartition (spiking, contamination), material list, consumables…**

■ **Codification of test unit**

■ **Packaging and shipment of test unit**	
Type de conditionnement	
Conservation of test unit before	
Shipment conditions of test units	
Shipment delay	
Modality in case of test unit reception problem (damaged package, non reception…)	In case of problem when sending / receiving parcels (conservation criteria not respected, damaged parcel, absence of samples, etc.), the following precriptions are taken: - return of a complete panel - return only damaged samples - Request for the return by the recipient laboratory of the damaged samples - cancellation of the participation of the laboratory because the samples can not be returned. The final report will mention the impossibility of involving the laboratory by specifying the precise cause. The participation fee is in this case canceled.

■ **Particular modalities on the conditions of the implementation of the analyses to communicate to participants (preparation and storage of test units, environmentals conditions, safety rules…)**

Statistical plan - Performance evaluation	
■ **Homogeneity test**	
Homogeneity test description (tests, visual criteria…)	
Acceptence criteria of	
■ **Stability test**	
Stability test description (tests, tested points, tested levels…)	
Tested conditions (shipping conditions, storage, analysis	
Acceptence criteria of stability test	
■ **Data analysis- participant's perfromance évaluation**	
Method and data analysis informations	
Method	quantitative /qualitative
Informations about method performance and potential impact on statistical test choice	Potential bias method information Dispersion of the method (reliability (intra-lab), repetability, reproductibility (inter-lab)? -Others informations about the method (Detection and quantification limit, sensibility, specificity...) -Uncertainity of the result ?
Results distribution	Purposeless for qualitative method Normal distribution Poisson distribution Log Normal distribution Binomiale distribution
Data transformation before data analysis	No transformation LOG
Results pre treatment	
Criteria for refusing results (results not used when processing data or participant evaluation)	- Failure to meet the deadline for analysis - Non respect of the method defined in the plan test - Comparison support that does not comply with the specifications (transport, conservation, ...) - aberration value (préciser les critères d'exclusion) - Non-compliance of internal control, blank - Failure to respect the unit of measurement - Not respecting the number of significant figures - Unreadable results - Format of the result not respected etc.
Verification of the respect of the instructions (except criteria of refusal) - modalities of processing	- Non respect of forms To use - Non respect of the date of delivered results …
Procedures for processing missing results (partly or totally)	- No evaluation of participants in case of missing results whether in whole or in part - No evaluation on missing results
Performance of participants	
Assigned value definition	
Standard deviation definition	
Assessment of the performance of participants for accuracy (calculations and conformity criteria)	- z-score - z-score' - z score compounds (weighted, squares) - Specificity rate, sensitivity rate, accuracy rate
Evaluation of participants' proficiency for precision (type of calculations and conformity criteria)	Mandel k graph

Information on the conditions of realisation of the analyses	
Method of analysis	
Number of tests to be performed on each sample	
Period analysis	
Dely for presenting results	
Conditions for presenting results (unit of measurement, number of	
Other informations	
Report	
Deadline for sending the	
Deadline for sending final report	
Means of sending the report	Byleter Bye mail (a proof convention will be established at the time of registration) Bydownloading the report on a website (a proof convention will be established at the time of registration)
Confidentiality agreement regarding reports	The results were processed confidentially and were transmitted to all participants while preserving their anonymity.

Risk analysis (other than method application)	
Critical points ☐ ☐	Risk management tools
Test unit selection	Preliminary study
Package lost/ delivery error	Delivery service choice
Failure in homogeneity or stability study	Agents habilitation
Data collect	Two readings
Statistical plan choice	
Calculation failure	Result calculation sheet
Neutrality and confidentiality	cf § Conflict of interest, collusion and confidentiality
Other	
Other informations	

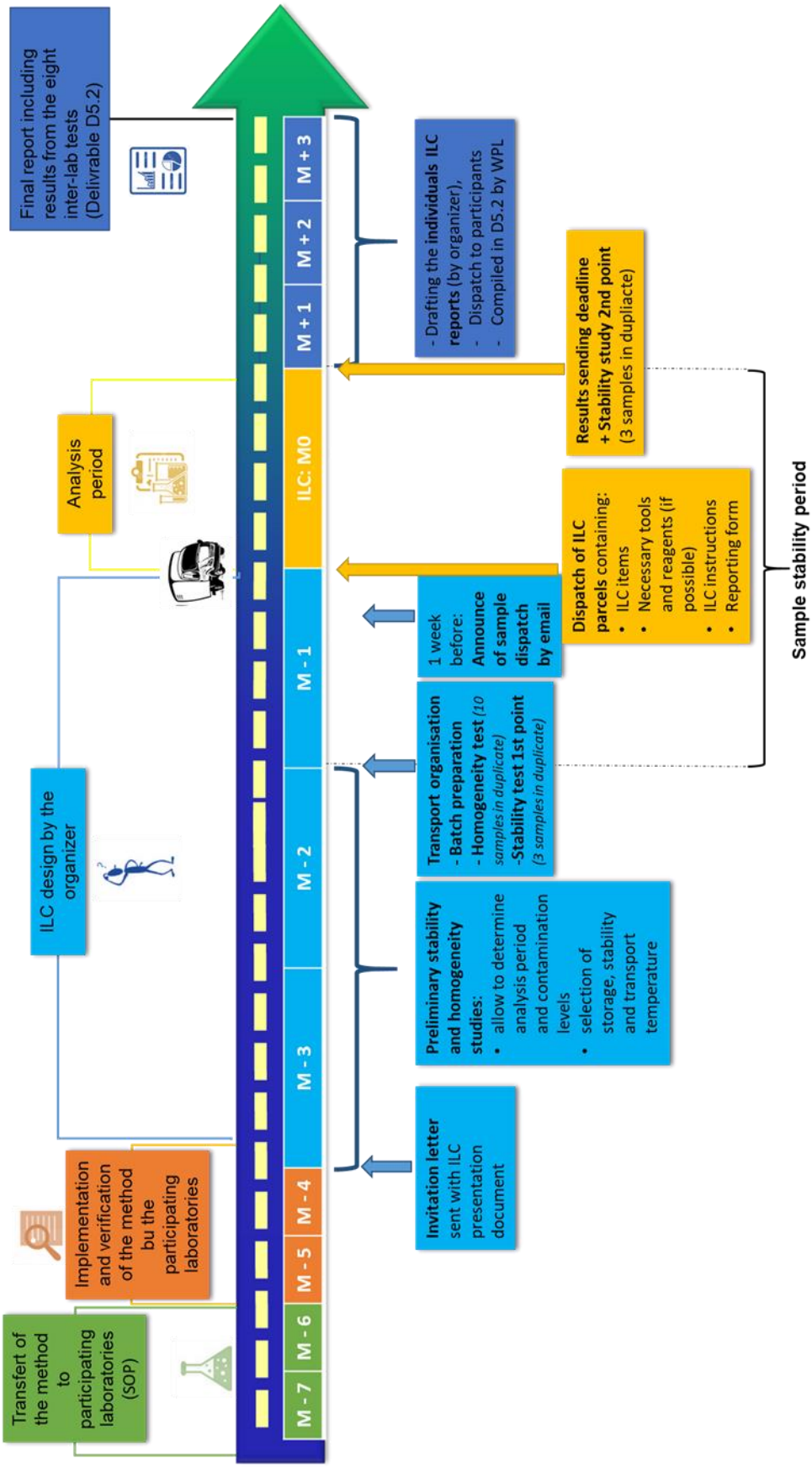
ILC planification validation

Involved agents signature attest : - they check their role and responsibilities -they are aware of risks and critical points of the procedure -they commit to inform the ILC coordinator of any problem or difficulty

Name	Date	Signature	Name	Date	Signature

GANTT for ILC

	Description	2020														2021										
		T1			T2			T3			T4					T1			T2			T3				
		25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45				
Development and transfer of the method																										
	Method developed (SOP available)									MS 3.2																
	Transfer of the method to ILC coordinator										MS3.3															
	Transfer of the method to ILC participants												D3.1+3.2+3.3													
	Implementation and verification of the method (SOP modifications if necessary)																									
	TC with method developer, ILC coordinator and ILC participants																									
	Report on the performance criteria for method harmonization															D3.4										
Preliminary work																										
	Selection of samples																									
	Proposal of draft of ILC schedule																									
	Preliminary tests on the selected matrices																									
	Drafting administrative documents (invitation...)																									
	Establishement of participating labs list																									
	Preparation of exportation documents																									
	Drafting of results form (Excel sheet)																									
	Contact for shipment																									
	Emailing of ILC annoucement													MS5.1												
	Dispatch of inter-lab tests documents																									
Sample preparation																										
	Homogeneity test																									
	Homogeneity assessment and conclusion																									
	Purchase, sampling																									
	Spiking, codification and storage																									
ILC																										
	Organisation of the pick-up																									
	Dispatch of samples and evaluation report to the participating labs																MS5.2									
	Analysis period																									
	Reception of ILC results																MS5.3									
	Assessment of results and exchange with labs if needed																									
Stability study																										
	Stability test (T° according to method developer prescription for sample conservation)																									
	Stability test (during ILC) (shipping, storage and analysis T°)																									
	Stability assessment and conlusion																									
Report on the WP5 ILC																										
	Dispatch of individual reports																									
	TC with WP5 coordination and T1s																									
	Drafting the final report																									
	Approval and dispatch f the final report																									


Final report including results from the eight inter-lab tests (Delivrable D5.2)
- Drafting the individuals ILC reports (by organizer),
- Dispatch to participants
- Compiled in D5.2 by WPL
Results sending deadline + Stability study 2nd point (3 samples in dupliacte)
Analysis period
ILC: M0
M + 1
M + 2
M + 3
Dispatch of ILC parcels containing:
• ILC items
• Necessary tools and reagents (if possible)
• ILC instructions
• Reporting form
1 week before: Announce of sample dispatch by email
Sample stability period
ILC design by the organizer
M - 1
M - 2
Transport organisation
- Batch preparation
- Homogeneity test (10 samples in duplicate)
-Stability test 1st point (3 samples in duplicate)
Preliminary stability and homogeneity studies:
• allow to determine analysis period and contamination levels
• selection of storage, stability and transport temperature
Implementation and verification of the method bu the participating laboratories
M - 3
M - 4
M - 5
M - 6
M - 7
Invitation letter sent with ILC presentation document
Transfert of the method to participating laboratories (SOP)

Annex 2: invitation letter

Circular letter *addressed to: [method] ILC participant*

[place, date]

ICL Coordination:

- Name, function
- Mail
- Phone number

File followed by:

Unit:

Direct line:

E-mail address:

Ref.:

Subject: Invitation to the Tox-Detect inter-laboratory comparison trial dedicated to [method] challenge

Dear Colleagues,

We hereby announce that we are organising an inter-laboratory comparison trial dedicated to the detection of [toxin, bacteria] in [matrix]

Participating laboratories have to perform the [method] developed in Tox-Detect WP[3,4]

[ILC items number] samples will be sent out during Week [date]. All the necessary documents and useful information will be sent later on to the registered participants.

Please find appended 1 sheet "information to participants" which describes the organisation.

The results are to be transmitted by the [date]. The results are confidential and anonymously transmitted. An intermediate report will be sent to each participating laboratory and the final report will be distributed to all participants and Tox-Detect partners.

We remind you that as Tox-Detect partner, it is part of your duties to take part to ILC trials dedicated to challenge developed methods in the project. Therefore whether participating or not to the trial, please fill in the online participation form (link provided in the invitation email).

This participation form must be submitted no later than [date].

Do not hesitate to contact us in case of need.

With best regards,

[ILC coordinator]

[WP5 coordinator]

Appendix 3: ILC instructions and planification

INSTRUCTIONS TO PARTICIPANTS FOR CARRYING OUT THE INTERLABORATORY TEST

ILT code		**Version**	01

ILT	
Name of ILT	
Type	
Purpose	This PT is organised within the framework of the activities of the EURL "nom du LRUE" on prescription of Directorate-General for Health and Food Safety. This test plan reflects in particular the needs and expectations of the prescriber defined in the laboratory work program. It aims to measure the ability of participating laboratories to perform the test described below and to provide results in line with those expected. As part of its reference missions, ILPT also aims to guide laboratories that have obtained an unsatisfactory result in the implementation of corrective actions and to monitor their effectiveness. PT is organised according to the requirements of standard ISO 17043.
Identification of the Organiser	
Name	
Adress	

Identification of coordinator			
Name		**Unit/team**	
☎		**Email**	
Identification of the national external contact person (to be completed by the organiser)			
Name		☎	SO

Information on the conditions of realisation of the analyses	
Analyte/matrix pair or method	
Number of samples sent by the laboratory	0
Storage conditions of samples upon receipt	
Date of sample dispatch	
Method of analysis **Cliquez ici pour sélectionner le type de méthode d'analyse**	
Number of tests to be performed on each sample	
Conditions for presenting results (unit of measurement, number of significant figures …)	

Specific conditions to carry out the analyses	Samples should be treated in the same way as those you usually treat (sample preparation and handling, environmental conditions, safety rules, etc.) unless otherwise specified (see below).
Cliquer ici pour sélectionner le délai d'analyse	
Deadline for submitting results	
Statistical information	
Criteria for discarding results (results not used when processing data or participant evaluation)	- Failure to meet the deadline for analysis - Non respect of the method defined in the plan test - Comparison support that does not comply with the specifications (transport, conservation, ...) - aberration value (préciser les critères d'exclusion) - Non-compliance of internal control, blank - Failure to respect the unit of measurement - Not respecting the number of significant figures - Unreadable results - Format of the result not respected etc.
Other verification of proper implementation of the instructions (criteria of refusal) - modalities of assessment	- Non respect of forms to use - Non respect of the date of delivered results ... …
Procedures for processing missing results (in whole or in part)	- Justification of participant on Form LSA-FGE-0305 "List of deviations" - No evaluation of participants in case of missing results whether in whole or in part - No evaluation on missing results
Evaluation of participants' proficiency for accuracy (type of calculations and conformity criteria)	- z-score - z-score' - z score compounds (weighted, squares) - Specificity rate, sensitivity rate, accuracy rate
Evaluation of participants' proficiency for precision (type of calculations and conformity criteria)	Mandel k graph
Other information and comments	

Appendix 4: Results forms

Sample	Test portion used (mL or g)	Type of toxin	Result ng/mL or ng/g (precise)

Method

Item	Description
Method title	
Method description	
Sample preparation	
Used standards	
Is the method validated?	– Select answer –
References	
Comments	