

PT-01: “Artificial digestion to detect *Trichinella* larvae in meat samples”

Instructions

PT scheme is managed through a dedicated web page (<https://pt-crlp.iss.it/>).

Each participant is registered in the system and has login credentials to access the web page.

To obtain credentials send a request, via e-mail, to the PT coordinator, once received the participant has to complete the identification form. STEP 1 OF THE WORKFLOW BELOW REPORTED

PT ITEMS

Description. Each PT item consists of a meatball made of 35 or 100 grams of minced meat (swine or horse), spiked or not with viable *Trichinella spiralis* larvae.

Production. Matrix consists of swine or horse meatball, without fat and fascia, weighing 35 or 100 grams (according to participant request). Analyte consists of *Trichinella spiralis* viable larvae obtained through artificial peptic digestion of experimentally infected mice carcass. Artificial peptic digestion is performed according to the method described in ISO standard 18743:2015/Amd.1:2023.

Note: PT items intended for non-EU countries are produced with encapsulated larvae because, due to customs controls, the package may be delivered more than five days after dispatch.

Distribution. Collected larvae are placed in a Petri dish, observed under a stereomicroscope, counted, transferred to a watch glass and afterwards to the meatball, where a hollow has been produced, by rinsing the watch glass with 150µL of PBS (phosphate buffered saline). The watch glass is then checked under the stereomicroscope to confirm that all larvae have been transferred. The hollow of the meatball is closed. In case of negative PT items, the meatball is not spiked with larvae.

Labeling. Each PT item is vacuum sealed to preserve meat freshness and ensure larvae survival. Each plastic bag is labeled with a unique code with no indication of contamination.

Homogeneity check. Since PT items are prepared individually, homogeneity is ensured by an accurate control of the number of larvae spiked into each PT item by two operators.

Packaging. Each PT panel (consisting of three PT items) is vacuum sealed in a bigger plastic bag and placed inside a polystyrene carton. Several ice packs are arranged in the package to maintain internal temperature between +4°C and +15 °C during shipping.

Stability and quality control. The stability of PT items in the package has been evaluated by *ad hoc* experiments made by PT provider (EURL-P) on vacuum sealed samples stored between +4°C and +15°C. Naked larvae remain viable up to five days from the date of preparation, while the encapsulated larvae remain viable up to 20 days from the date of preparation. Quality control requires that PT provider's staff analyse PT items twice a year. These PT items are produced at the same time as those sent to participant and analysed on the deadline date for submitting results.

SUBMIT A PARTICIPATION REQUEST

The dedicated website displays the dates of the PT scheme, typically five-six dates per year. Registered participant submits independently the request of participation selecting the meat type (swine or horse) and weight (35g or 100g). STEP 2 OF THE WORKFLOW BELOW REPORTED

PT ITEMS STORAGE

- Store PT items refrigerated at a temperature between +4°C and +15°C until the analysis is performed
- Check the packaging and its contents for correctness and completeness: three PT items properly labeled, vacuum sealed, internal temperature of the package between +4°C and +15°C
- Fill in the shipping form available at the dedicated web page. STEP 3 OF THE WORKFLOW BELOW REPORTED
- In case of discrepancy, fill in the claim form available at EURL-P website: <https://www.iss.it/documents/20126/6678302/Claim-Appeal-rev-1.pdf/f9bba72e-3032-88fd-9d05-2b04ad45d1f1?t=1716802008339> or contact via e-mail the person in charge of the PT

PT ITEMS ANALYSIS

1. The code assigned to participant is reported on a paper sheet contained in the package.

2. PT items treatment:

- treat PT items as routine samples, except for the mechanical homogenization process, to be replaced by manual homogenization (using a rod or spatula)
- record the codes reported on the plastic bag of each PT item (PT item identification code)
- handle PT items according to safety procedures required for infectious biological material, i.e. wearing personal protective equipment (coat, mask and gloves).

3. Analysis: perform the test method within five days after the date of production (same as the shipping date). Apply the ISO standard 18743:2015/Amd.1:2023 or one of the equivalent methods based on peptic digestion and direct observation of the larvae. It is recommended to perform the analysis when the PT item has reached the room temperature to prevent a drop in the temperature of digestive solution when adding the PT item. Warning: handle hydrochloric acid under a fume hood.

4. Results: according to ISO standard 18743:2015/Amd.1:2023, results of artificial digestion method are expressed qualitatively, i.e. as “positive” if *Trichinella* larvae are recovered in the sample, or “negative” if no larvae are present. Participants are also requested to indicate the number of larvae recovered in each PT item. To report the results, participant has to complete the dedicated page within the restricted area of the website. STEP 4 OF THE WORKFLOW BELOW REPORTED

5. Deadlines:

- production and shipping are usually performed on Monday
- PT items should be delivered within 48-72 hours
- the deadline date for results submission is indicated in the “shipment form list” section of the website, generally one week after the production date. It is not allowed to submit results after the due date
- the final PT report is published on the EURL-P website according to the annual timetable available online (<https://www.iss.it/en/eurlp-prove-valutative-interlaboratorio>)

6. Perform evaluation: The results are considered “correct” if the participant properly identifies PT items spiked or not with larvae and “incorrect” in case of false positive or false negative identification. Results have to be expressed indicating the number of larvae detected in each PT item, allowing the PT provider a more accurate evaluation of participant performance. Due to the low number of larvae in PT items, no statistical parameters are applied for result evaluation. Each result is evaluated against the value assigned by the PT provider. The final evaluation is “positive” if all PT items are correctly identified otherwise, it is “negative”.

7. Report: PT provider drafts the Final PT Report including, for each participant, information on: i) the number of larvae spiked per PT item; ii) the number of larvae recovered by the participant in each PT item; iii) the final evaluation and iv) recommendation based on the participants performance and an updated summary of participants performance over successive PT rounds. To guarantee confidentiality each participant is identified by an alphanumeric code changed annually. The final PT report is published on the EURL-P website (<https://www.iss.it/en/rapporti-finali-prove-valutative-interlaboratorio>) and presented to the National Reference Laboratories during the annual workshop. The report is also sent to the European Commission (DG SANTE) within the time frame established by the latter.

8. Follow up: in case of negative result, the PT provider contacts the participant who fails the PT and sends a link to a form where the participant is asked to analyze the causes of failure and indicate the corrective actions planned or implemented.

9. Confidentiality: EURL-P retains the PT Reports for 10 years. PT provider staff sign a confidential agreement regarding acquired data. In the final PT report, participant is identified by alphanumeric code. The PT provider reserves the right to provide participant PT results to competent authority and accreditation body, upon request. In such cases, the PT provider informs the participant in writing.

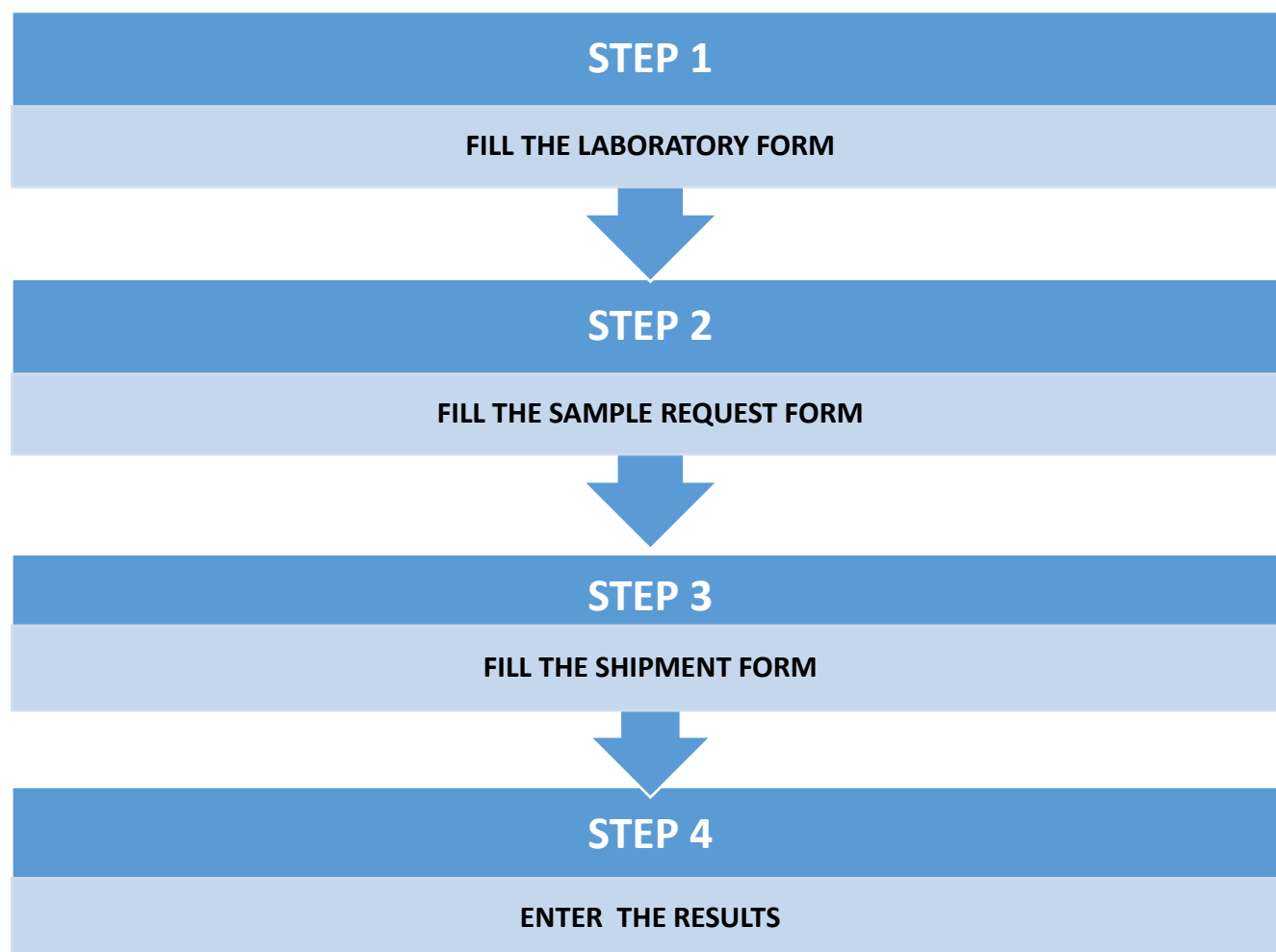
For any information or problem related to the web site access, please address:

Dr. Gianluca Marucci

e-mail: gianluca.marucci@iss.it

Workflow

Carefully follow the instructions below and **fill in** the check boxes included in the forms. Each participant and all data referring to (results, reports, etc.) is identified by a unique code.



Step 1. Laboratory form

The participant admitted in the PT have to fill in the laboratory identification form.

Proficiency Testing

Restricted Area - Laboratory : trichi-iss

◀ Start page : Laboratory form

Institute/company (*)	<input type="text" value="Istituto Superiore di Sanità"/>
Laboratory (*)	<input type="text" value="EURLP"/>
Address (*)	<input type="text" value="Viale Regina Elena, 299"/>
Zip code (*)	<input type="text" value="00161"/>
Country (*)	<input type="text" value="Italy"/>
City (*)	<input type="text" value="Roma"/>
Telephone (*)	<input type="text" value="+3949902304"/>
Fax (*)	<input type="text" value="+3949903561"/>
E-mail (*)	<input type="text" value="simone.caccio@iss.it"/>
URL	<input type="text"/>
Person in charge name	<input type="text" value="Simone"/>
Person in charge surname	<input type="text" value="Cacciò"/>
Notes	<input type="text"/>

Fiscal data

Address (^)	<input type="text"/>
Zip code (^)	<input type="text"/>
Country (^)	<input type="text" value=""/>
City (^)	<input type="text"/>
Telephone (^)	<input type="text"/>
Fax (^)	<input type="text"/>
E-mail (^)	<input type="text"/>
VAT (\$)	<input type="text"/>

In the section below the participant can insert data about the person/s to contact in case of problems.

	Name	Surname	Telephone	Email	Contact type
Edit Delete	<input type="text" value="Gianluca"/>	<input type="text" value="Marucci"/>	<input type="text" value="+3949902310"/>	<input type="text" value="marucci@iss.it"/>	<input type="text" value="Contact pers"/>

Insert new contact and then edit...

Step 2. Application for PT or request for sample submission for other purposes

From the main page, users can access the sections dedicated to application for PT and to requesting samples for various purposes (validation, training, inspections accrediting body audit).

These forms summarize the samples required by the laboratory/participant for each shipment.

To apply for PT dedicated to the National Reference Laboratories, which date is fixed by the PT provider every year, use the section **PT request list**.

To request a new shipment, click on **New PT request for NRLs**, the request list will be available, and the participant can select type and weight of items (pork or horse meat; 35g or 100g). By clicking on **Insert**, the participant sends the request to PTP (EURL-P). The participant can cancel the request by clicking on **Delete** up to 7 days before the shipment date. After that time, the items will be sent.

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Restricted Area - Laboratory :

	Request date	Preferential Date	Scheduled	Sample type	Weight
View Delete	9/20/2012	10/8/2012	No	Pork	100g

New PT request

New PT request NRLs (for national reference laboratories only)

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Restricted Area - Laboratory : trichi-iss

[Start page](#) : [Request list](#) : [Request](#)

PT sample request

Date	<input type="text"/>
Sample type	<input type="text"/>
Weight	<input type="text"/>
Notes	<input type="text"/>
<input type="button" value="Insert"/> <input type="button" value="Cancel"/>	

The **Request list** section allows users to request samples for purposes other than PT (validation, training, accreditation body audit). To request a shipment, click on **New request** and select:

- 1) the shipment date, among those scheduled;
- 2) the type of samples (pork or horse meat; 35g or 100g);

3) with or without *Trichinella* larvae;

4) the number of samples.

By clicking on **Insert**, the user sends the request. The user can cancel the request by clicking on **Delete** up to 7 days before the shipment date. After that time, the samples will be sent.

	Pref. Date	100g Sw.	100g Eq.	35g Sw.	35g Eq.	100g Sw.(*)	100g Eq.(*)	35g Sw.(*)	35g Eq.(*)
View Delete	9/10/2012	1				1			
View Delete	12/3/2012		1				1		
						(*) neg	(*) neg	(*) neg	(*) neg

New request...

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◀ Start page : Request list : Request

Request form

Service type

Date

Number of 100 g pork samples with *Trichinella* larvae

Number of 100 g horse meat samples with *Trichinella* larvae

Number of 35 g pork samples with *Trichinella* larvae

Number of 35 g horse meat samples with *Trichinella* larvae

Number of 100 g pork samples without *Trichinella* larvae

Number of 100 g horse meat samples without *Trichinella* larvae

Number of 35 g pork samples without *Trichinella* larvae

Number of 35 g horse meat samples without *Trichinella* larvae

Notes

Insert Cancel

Step 3. Entry of data related to sample receipt

The **List of shipments, results, and report printing** section summarizes the user's participation in PT and the different sample requests submitted, indicating the sample shipment date, the date of receipt at the laboratory, and, in the case of PT, the due date for submission of results to the EURL-P.

Restricted Area - Laboratory : iss_eurlp - L12

◀ Start page : Shipment form list

		Service	Shipment date	Arrival date	Due date
Print	View	Training	17/02/2011		24/02/2011
Print	View	Training	27/02/2011		07/03/2011
Print	View	Validation official method	14/02/2011		21/02/2011
Print	View	Proficiency Test	14/02/2011		21/02/2011

By clicking on **View**, the **Shipment form** is displayed. The participant/laboratory has to fill in the form by entering:

Shipment arrival date

Time (hour) of the package opening

Check of the package content (n. of samples)

Check of the package content conditions (sample integrity and refrigeration).

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◀ Start page : Shipment form list : Shipment form

Shipment date
 Arrival date
 Arrival time (es. 15.30)
 At what time did you open it (es. 15.30)
 Notes
 The package contains the number of meat ball samples requested ☒ Yes ☐ No Notes
 The samples are still under vacuum ☒ Yes ☐ No Notes
 The samples are still refrigerated in the package (temperature <10°C) ☒ Yes ☐ No

By clicking on **Update**, the participant/laboratory submits the requested data.

Step 4. Enter results

The table **Results** summarizes data of each item/sample received (code, type, weight), there are columns related to spiked larvae and recovered larvae, which are populated after the results submission by the laboratory/participant.

Results

	Code	Type	Grams	No. spiked larvae	No. recovered larvae
View	5604	Horse	100	2	0
View	4514	Horse	100	4	0
View	3134	Horse	100	5	0
View	2138	Horse	100	5	0
View	1127	Horse	100	10	0
View	3073	Horse	100	0	0

By clicking on **View**, the 'Sample results' window will open, and the participant/laboratory can enter the number of larvae recovered in the item/sample, and the method chosen to test the item/sample among the official methods which allow direct visualization of larvae.

Restricted Area - Laboratory : iss_eurlp - L12

◀ [Start page](#) : [Shipment form list](#) : [Shipment form](#) : [Sample results](#)

Sample : 9346

Sample type	<input type="text" value="Pork"/>
Grams	<input type="text" value="100"/>
Number of recovered larvae	<input type="text" value="4"/>
Method	<input type="text" value="Magnetic stirrer method for pooled sample digestion"/>
Notes	<input type="text"/>

By clicking on **Update**, the entered data is recorded. Data can be updated up to the deadline.

Print of the results evaluation

To print results evaluation, click on **Print** in the **Shipment form** (see Step 3).

In the case of PT, the generated document does not have the status of a PT Report.

The PT results are evaluated exclusively in the final PT Report available at the EURLP web site
<https://www.iss.it/-/pt-01-artificial-digestion-to-detect-trichinella-larvae-in-meat-samples>