

**21st Annual Meeting of the TSE EURL
Rome 13th – 15th May 2024**



**2023 round of TSE EURL EQAs:
Results feedback**

**BSE AND SCRAPIE CONFIRMATORY
IMMUNOBLOTTING**

Maria Mazza
IZSPLVA - Turin



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BSE AND SCRAPIE CONFIRMATORY IMMUNOBLOTTING

PLANNING

Each NRL has been identified with a new code, different for the two PTs



Samples were stored at -80 °C until shipment by the courier on dry ice

Each round included 5 samples of frozen brainstem homogenates of cattle and sheep

Set of samples included information on their preparation, manipulation and transmission results



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BSE AND SCRAPIE CONFIRMATORY IMMUNOBLOTTING

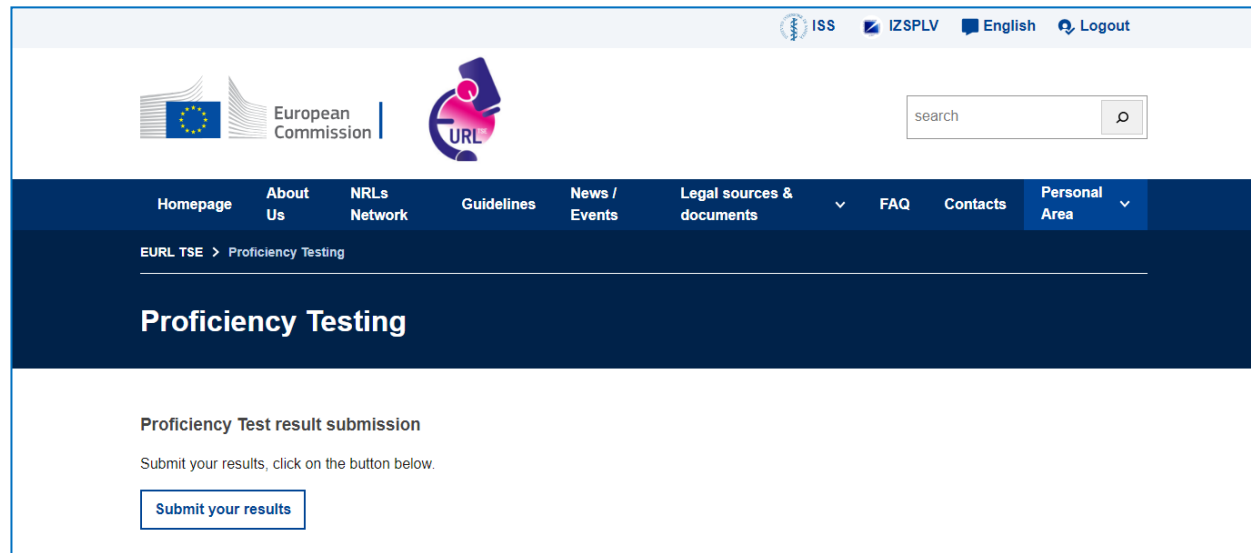
Shipping and Transmission results

Samples were shipped on 17th and 18th July 2023

Deadline for submission of results was on 31st August 2023

(extended 18th September 2023)

<https://www.eurl-tse.eu/proficiency-testing/>



The screenshot shows the website interface for EURL TSE Proficiency Testing. At the top, there are logos for the European Commission and the EURL TSE network, along with a search bar. The navigation menu includes links for Homepage, About Us, NRLs Network, Guidelines, News / Events, Legal sources & documents, FAQ, Contacts, and Personal Area. The main content area is titled "Proficiency Testing" and contains the text "Proficiency Test result submission" and "Submit your results, click on the button below." Below this text is a button labeled "Submit your results".



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Transmission of the results

The submission included the raw data (pictures of the blots, readings), the information about the kit batches used for the analysis plus any information considered important for the correct interpretation of the results



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Processing of results

Elaboration of a report with the PTs results for
BSE and Scrapie round



Correct results

laboratories suitable to
perform confirmatory tests

Incorrect results

analysis on a new
set of samples

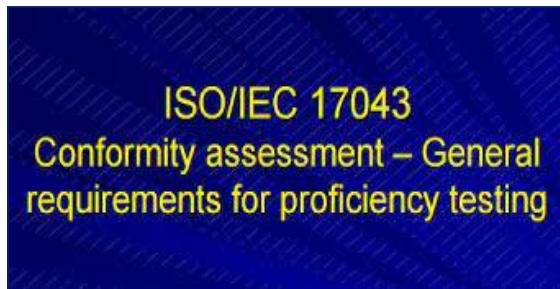
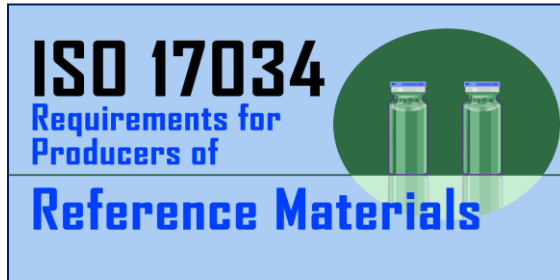


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EURL EQA round 2023: Quality Control

BSE and Scrapie Confirmatory Immunoblotting



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BSE Confirmatory Immunoblotting



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NRLs participating in Proficiency Testing 2023

BSE CONFIRMATORY IMMUNOBLOTTING

AUSTRIA

GREECE

ROMANIA

BELGIUM

HUNGARY

SLOVAKIA

BULGARIA

IRELAND

SLOVENIA

CROATIA

ITALY

SPAIN

CZECH REPUBLIC

THE NETHERLANDS

SWEDEN

FINLAND

NORWAY

SWITZERLAND

FRANCE

POLAND

GERMANY

PORTUGAL

22 NRLs and United Kingdom



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BSE CONFIRMATORY IMMUNOBLOTTING

The samples provided for the TSE quality assessment were homogenates of brainstem material from a panel of classical BSE-positive and BSE-negative animals

The tissue have been homogenised using a 50/50 mix of tissue and distilled water

Sample No	Result	Interpretation
WB2301	negative	////
WB2302	positive	strong
WB2303	positive	medium
WB2304	negative	////
WB2305	positive	medium

Sample WB2301 = sample WB2304

Sample WB2303 = sample WB2305



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Results Tabulation for BSE Confirmatory Immunoblotting Scheme: WB23

Primary Tests	No. NRLs
TeSeE Western Blot - Bio-Rad	19
In house method	2
§APHA Hybrid TeSeE WB	1
OIE SAF immunoblot	1

§APHA Hybrid TeSeE WB – used 2 mAbs: Sha31 and 12B2

Labs 092, 339, 605 used 1st alternative test

Lab 129 used 1st and 2nd alternative test



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Results Tabulation for BSE Confirmatory Immunoblotting Scheme: WB23

TeSeE Western Blot Bio-Rad used five batches



2H0050 (10 labs)

2K0051 (7 labs)

2K0047 (1 lab)

1K0051 (1 lab)

2K001 (1 lab)



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BSE Confirmatory Immunoblotting – Scheme WB23



ALL THE LABS PASSED THIS PROFICIENCY TESTING AT THE FIRST ROUND

EXCEPT THE LABS 717 AND 642 THAT PASSED AT THE SECOND ROUND



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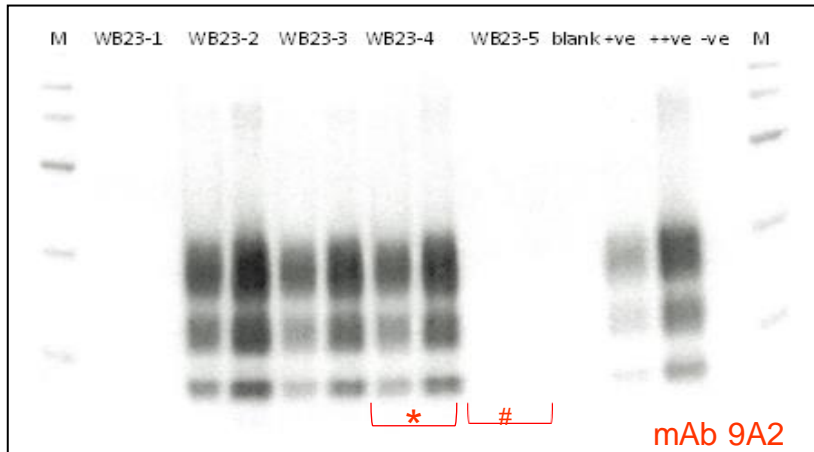
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BSE confirmatory Immunoblotting – Scheme WB23

Lab 717

INCORRECT RESULTS

TeSeE WB

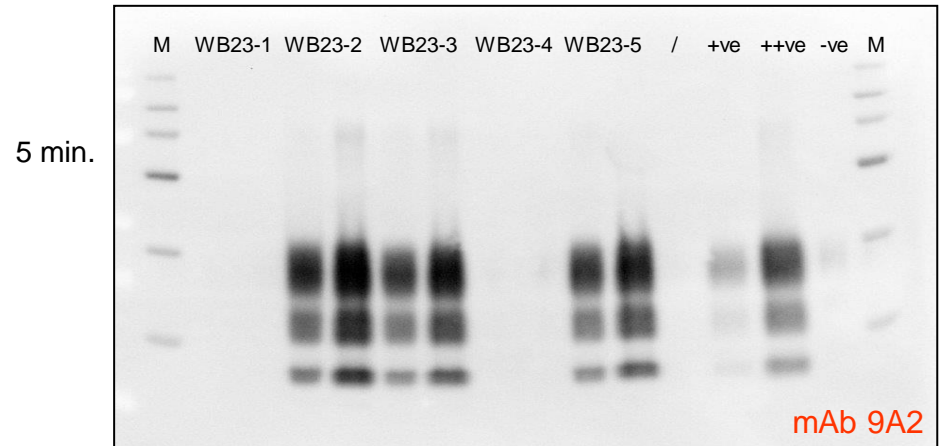


first round

- *WB2304 = positive (but negative)
- #WB2305 = negative (but positive)

The laboratory reported this mistake to a sample exchange

TeSeE WB



second round

All samples were identified correctly



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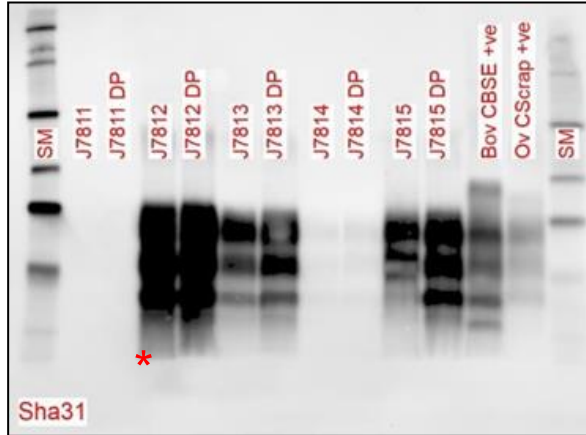
BSE confirmatory Immunoblotting– Scheme WB23

Lab 642

INCORRECT RESULTS

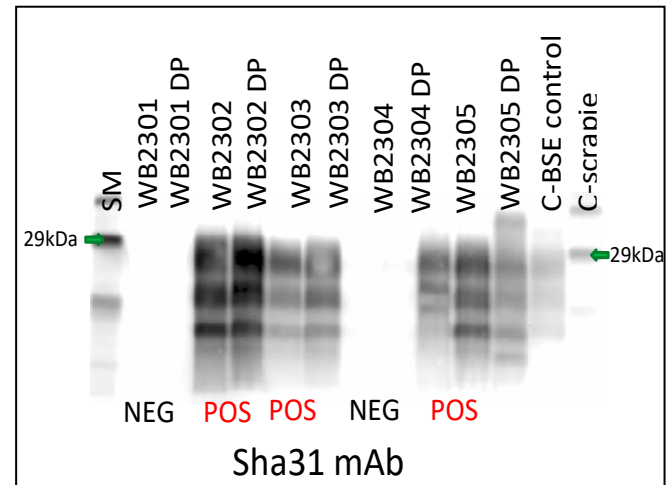
APHA Hybrid TeSeE WB

first round



*WB2304 = positive (but negative)

There was probably diffusion of near samples



second round

All samples were identified correctly



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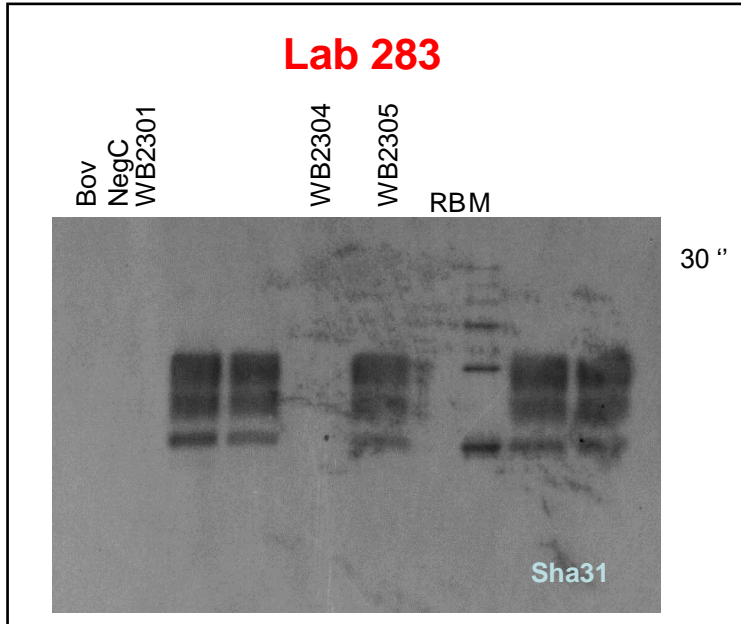
BSE confirmatory Immunoblot – Scheme WB23

TeSeE WB

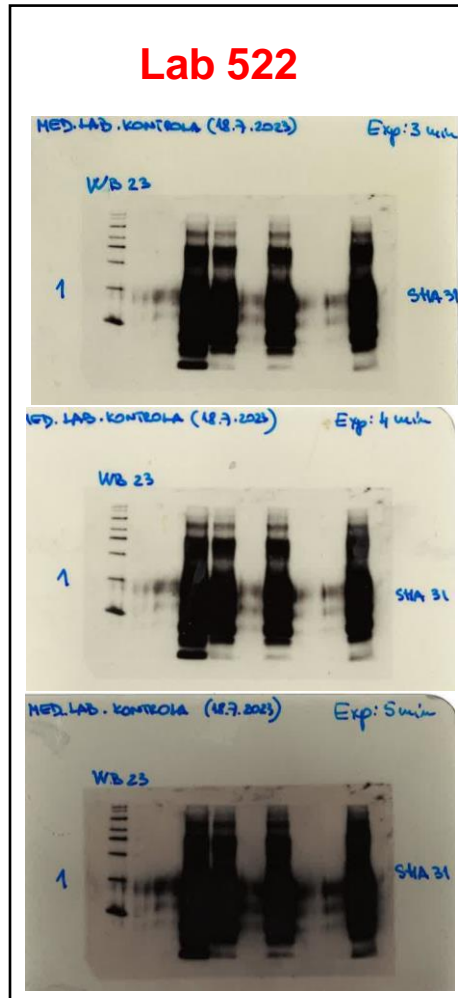


CRITICAL ISSUES

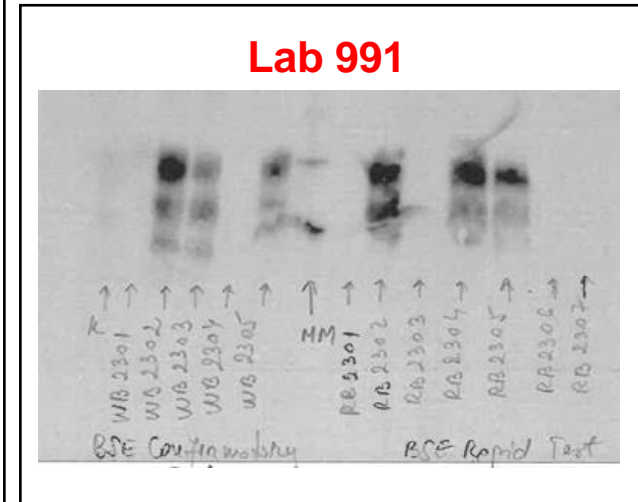
Lab 283



Lab 522



Lab 991



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Scrapie Confirmatory Immunoblotting



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NRLs participating in Proficiency Testing 2023

SCRAPIE CONFIRMATORY IMMUNOBLOTTING

AUSTRIA

GREECE

PORTUGAL

BELGIUM

HUNGARY

ROMANIA

BULGARIA

ICELAND

SLOVAKIA

CROATIA

IRELAND

SLOVENIA

CZECH REPUBLIC

ITALY

SPAIN

FINLAND

THE NETHERLANDS

SWEDEN

FRANCE

NORWAY

SWITZERLAND

GERMANY

POLAND

23 NRLs and United Kingdom



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SCRAPIE CONFIRMATORY IMMUNOBLOTTING

The samples provided for the TSE quality assessment are homogenates of brainstem material from a panel of Scrapie-positive and Scrapie-negative animals

The tissue have been homogenised using a 50/50 mix of tissue and distilled water

Sample No	Result	Interpretation	Characterisation
WS2301	positive	strong	classical scrapie
WS2302	positive	medium	classical scrapie
WS2303	negative	////	///
WS2304	negative	////	///
WS2305	positive	medium	classical scrapie

Sample WS2301 = sample WS2305

Sample WS2303 = sample WS2304



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Results Tabulation for Scrapie Confirmatory Immunoblotting Scheme: WS23

Primary Tests	No. NRLs
TeSeE Western Blot Bio-Rad	18
In house method	3
§APHA Hybrid TeSeE WB	2
TeSeE Western Blot Bio-Rad *	1

§ APHA Hybrid TeSeE WB – used 2 mAbs: Sha31 and P4

* TeSeE Western Blot: detection also with P4

Labs 164 and 235 used 1st alternative test



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Results Tabulation for SCRAPIE Confirmatory Immunoblotting Scheme: WS23

TeSeE Western Blot Bio-Rad used six batches



2H0050 (9 labs)

2K0051 (8 lab)

2C0049 (1 lab)

2K0047 (1 lab)

1K0051 (1 lab)

2K001 (1 lab)



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Scrapie Confirmatory Immunoblotting– Scheme WS23

Congrats!

**ALL THE LABORATORIES PASSED THIS
PROFICIENCY TESTING**



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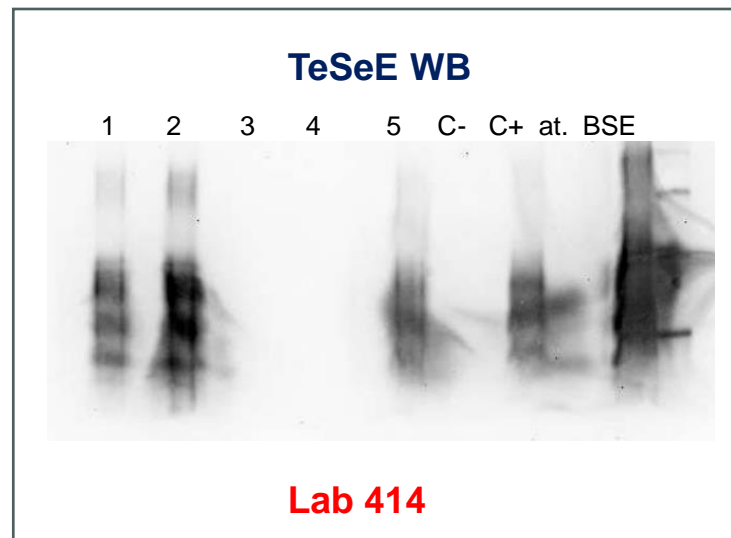
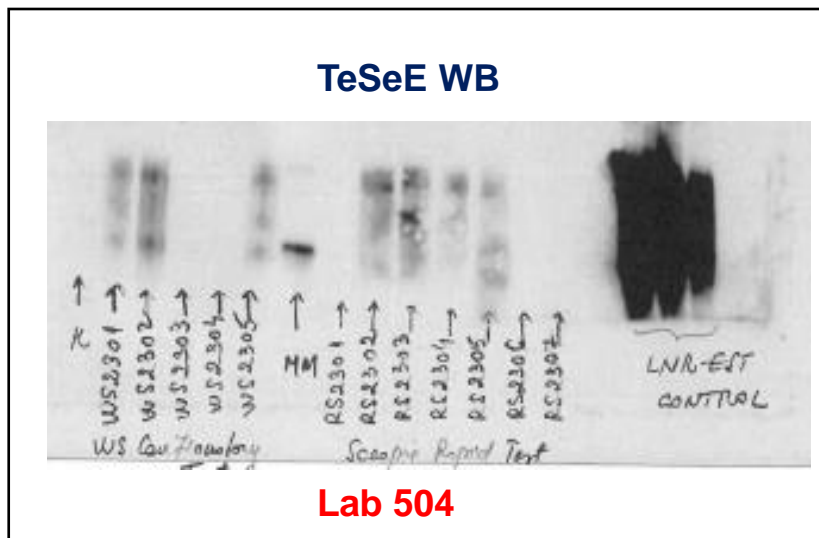
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Scrapie confirmatory Immunoblot – Scheme WS23

CRITICAL ISSUES



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2023 round of TSE EURL EQAs: Results feedback

BSE AND SCRAPIE CONFIRMATORY IMMUNOBLOTTING

CONCLUSIONS

- ❑ All NRLs submitted the results of both PTs by the deadline
- ❑ Scheme WB23 – two labs not passed PT at the 1st round
- ❑ Scheme WS23 – all the labs passed PT at the 1st round
- ❑ Good wb image quality in most cases, but critical for one lab in both PTs (codes 991 and 504)



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2023 round of TSE EURL EQAs: Results feedback

BSE AND SCRAPIE CONFIRMATORY IMMUNOBLOTTING

KEEP IN MIND

- ❖ Scrapie surveillance - Always repeat negative samples detected with only mAbs mapping the N-terminus (i.e. P4) using mAbs mapping the PrP^{Res} core
- ❖ Take great care in identifying and management of samples
- ❖ Use disposable material when several samples are sampled in the same analysis session or apply cleaning procedures after each sample



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2023 round of TSE EURL EQAs: Results feedback

BSE AND SCRAPIE CONFIRMATORY IMMUNOBLOTTING

KEEP IN MIND

- ❖ Include negative and positive control samples in all analysis sessions
- ❖ Not use expired reagents and kits
- ❖ Dilute very strong samples to avoid diffusion between two neighbouring wells in the gel
- ❖ WB image with a low background to detect weak samples



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BSE and Scrapie Confirmatory Immunoblotting



The forthcoming 2024 round of EQAs BSE and Scrapie Confirmatory Immunoblotting



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About shipping

- The shipment of the samples is scheduled in November 2024.
- BSE and Scrapie confirmatory PTs will be sent along with sample sets for the rapid test.
- The samples will be packed with dry-ice and the shipment will be scheduled at the beginning of the week.
- E-mail to communicate the shipment.



**Please transmit the import permit as soon
as possible, if required and expired**



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BSE and Scrapie Confirmatory Immunoblotting

Submission PTs results

- **The results will be submitted by EURL web site**
- The submission of results is expected by the deadline
- The results submitted after the deadline will not be evaluated
- **Please include in the submission the raw data (pictures of the blots, readings, time exposure of pictures) and all the information about the kit batches used for the analysis**
- Report any information considered important for the correct interpretation of the results



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Confirmatory BSE and Scrapie Immunoblotting

SUBMISSION RESULTS ON WEB SITE

- Upload results for each applied test to the Eurl website
- Fill in all fields correctly
- Always indicate if the test used by the laboratory is the one routinely applied or as a first or second alternative test



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PTs results

The results of the PTs round 2024 will be reported at the next TSE EURL Meeting



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EURL Contact Immunolotting



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THANKS TO THE
WHOLE TEAM!!!

Maria Cavarretta

Daniela Loprevite



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THANKS FOR YOUR TIME!!!



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