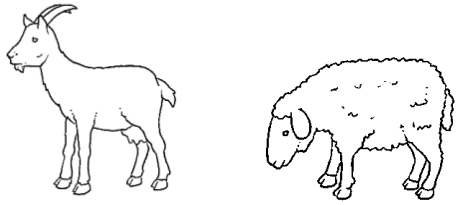
2024 round of TSE EURL EQAs: Results feed-back

22nd Annual Meeting of the TSE EURL Torino, Italy – 12-13 May 2025



Discriminatory Western Blot in Small Ruminants EQA (DS): results of the 2024 round

EURL-TSE
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin

Istituto Superiore di Sanità - Rome

DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS24)

Planning and samples dispatched

- **16 NRLs** expressed interest to partecipate in EAQ round 2024 for Discriminatory WB in small ruminants
- The NRLs received the samples on October 21, 2024
- The deadline had been set for November 30 (extended to December 6)

- 5 samples of frozen brainstem homogenates (50/50 mix of tissue and distilled water)
- Tested for homogeneity and stability

Sample ID	Expected results	Genotype	Sample origin/characteristics
DS2401	**Classical scrapie**	ARQ/VRQ	Pool of classical scrapie samples from sheep
DS2402	**Classical scrapie**	ARQ/ARQ	Pool of classical scrapie samples from sheep
DS2403	**Classical scrapie**	ARQ/ARQ	Dilution 1:2 of sample DS2402
DS2404	**Classical scrapie**	ARQ/VRQ	Dilution 1:2 of sample DS2401
DS2405	**BSE not excluded**	ARQ/ARQ	Pool of samples from sheep with experimental BSE

DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS24)

Results tabulation

N° of methods used per Lab	Total
one test method	13
two test methods	3
Total partecipating Labs	**16**

Test method used	N° of Labs
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	13
Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)	3
FLI Discriminatory Western blot Method	1
ANSES Discriminatory Western blot Method	1
ISS Discriminatory Western blot Method	1
APHA Prionics-based Hybrid Western blot Method *	/

* Prionics kit discontinued

RESULTS

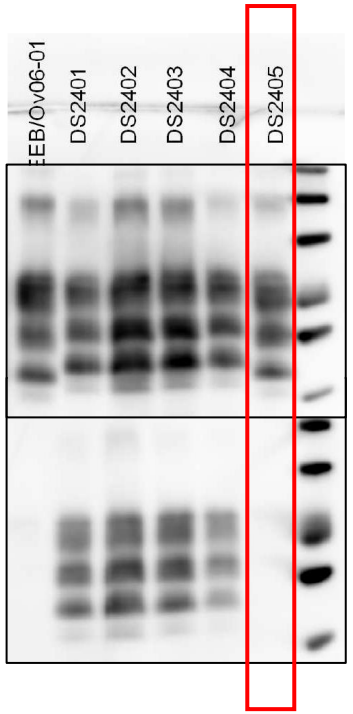
All the laboratories have reported the samples as the intended results

Some examples
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method

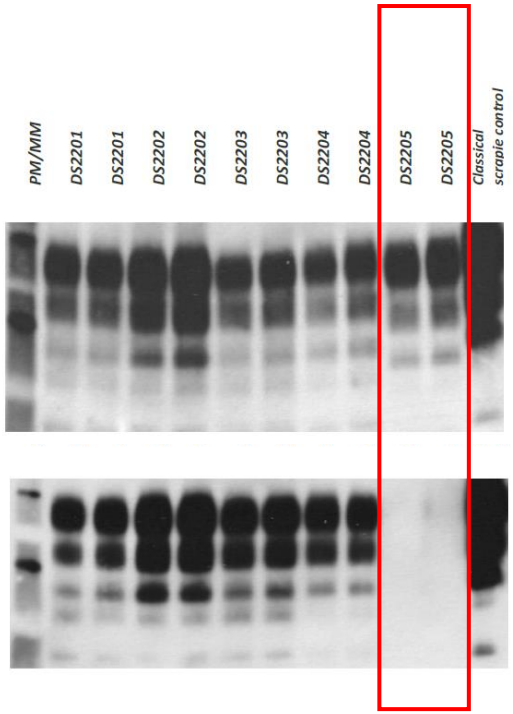
mAb Sha31

mAb P4

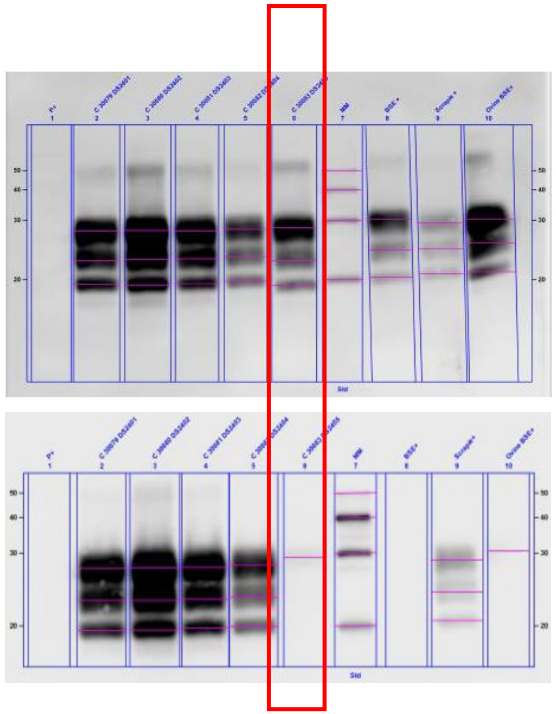
LAB 348



LAB 993



LAB 366



* Original submitted images modified

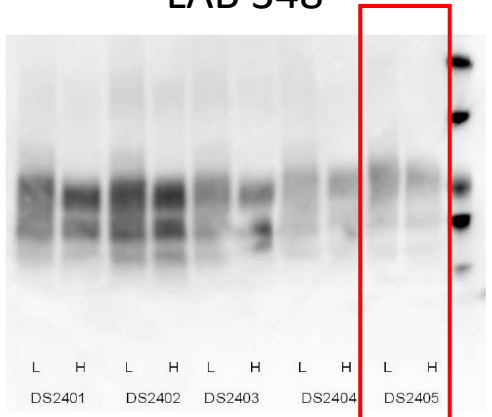
RESULTS

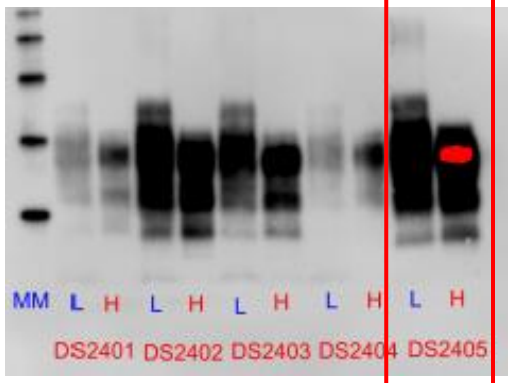
Some examples
Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)

LAB 348

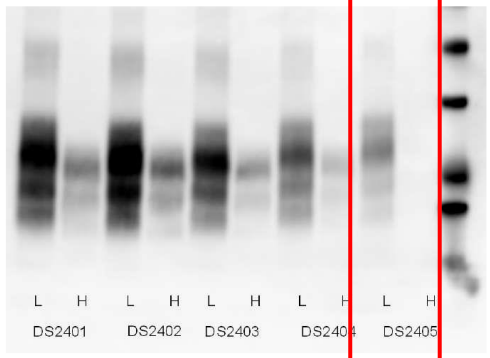
LAB 41

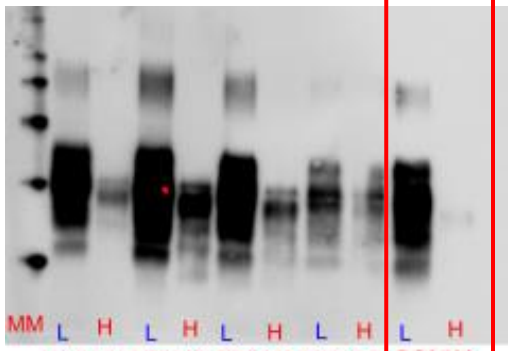
Ab control





Ab test





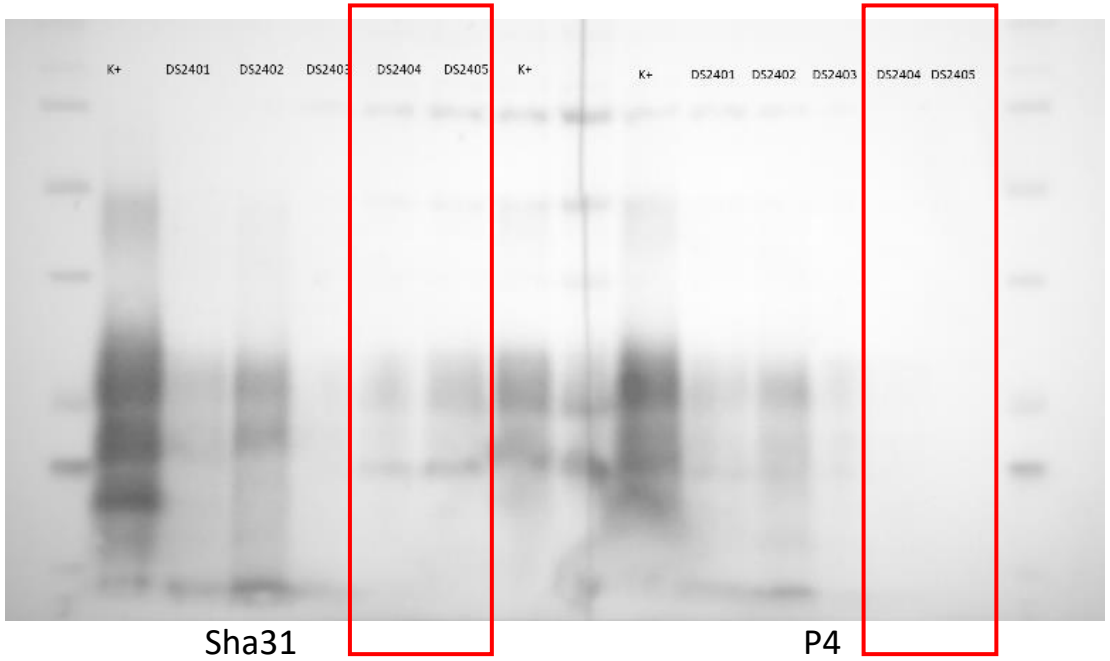
* Original submitted images modified

NON-COMPLIANT PERFORMANCE FOR LAB 066

Method used:
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method

The results reported were correct but did not align with the provided images:

- Low sensitivity
- Unclear electrophoretic pattern
- Non-glycosylated bands not well distinguishable in any of the samples
- Loss of signal for both sample DS2404 (scrapie) and DS2405 (sh-BSE) with P4



Sha31 P4

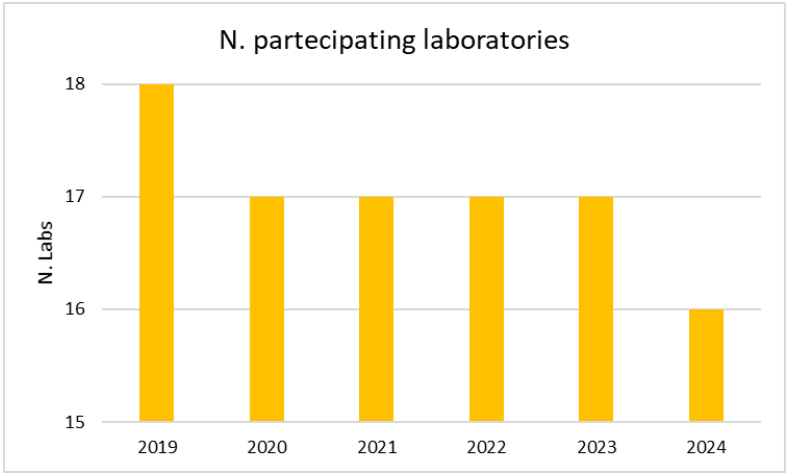
Actions taken:

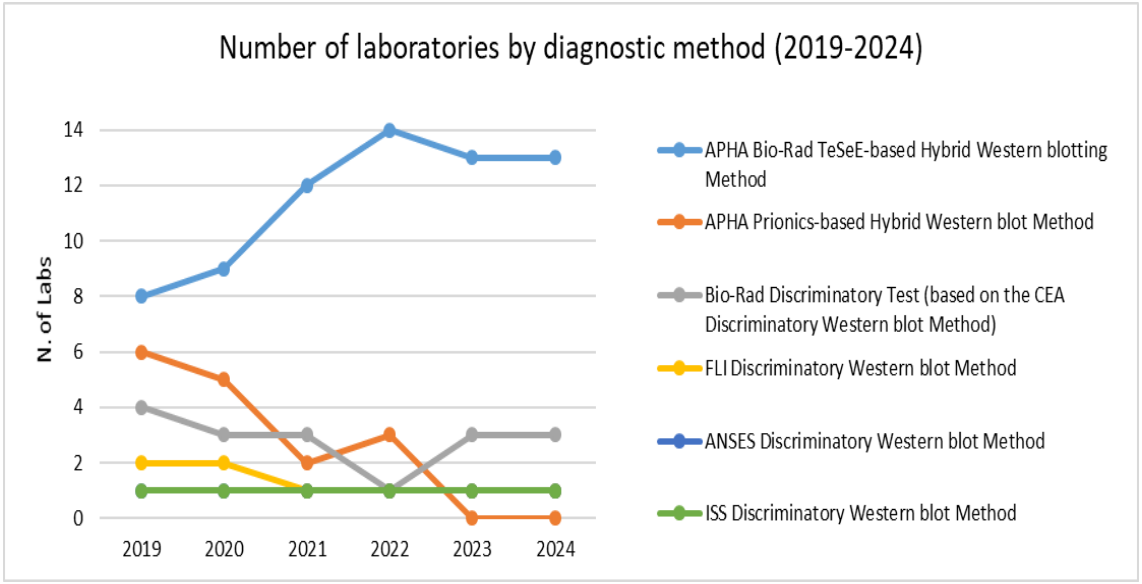
- The Lab was asked to provide additional images at different exposure times; however, these were not available.
- The Lab was requested to explain how the results were interpreted based on the Western blot.
- The explanations provided were not satisfactory.
- The Lab was invited to perform a new test using a new set of samples, and at the same time, was encouraged to revise the method to improve sensitivity and quality, enabling a clearer discrimination between classical scrapie and sh-BSE.
- The laboratory responded with full cooperation and confirmed that the test will be repeated.

Discriminatory testing trend (2019-2024)

ID LAB	2019	2020	2021	2022	2023	2024
41						
118	2nd rnd					
176						ND
182						
188						
341						
366						
565						
601						
933						
954						
983						
985						
143-51		ND	*	*	ND	ND
287-66						ongoing
352-993						
357-910						
469-243						

ND: the NRL did not partecipate
*Results not submitted



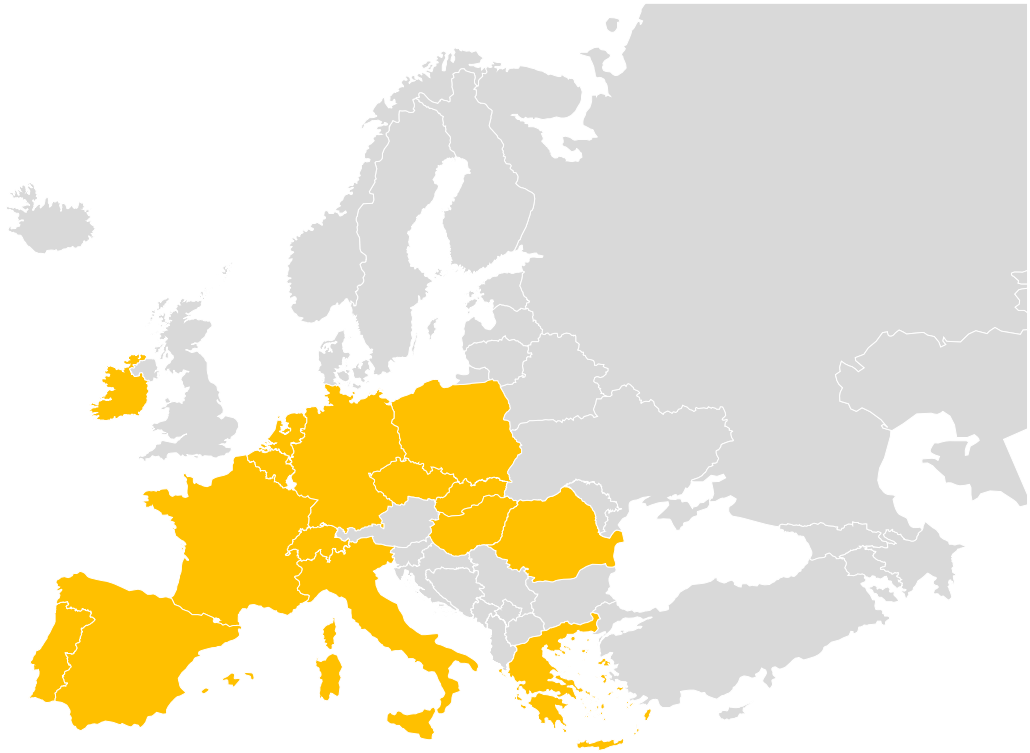


* Prionics kit discontinued

DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS24)

Laboratories passed this PT round successfully

1. Belgium
2. Czechia
3. France
4. Germany
5. Greece
6. Hungary
7. Ireland
8. Italy
9. Netherlands
10. Poland
11. Portugal
12. Romania
13. Slovakia
14. Spain
15. Switzerland



CONLCUSION/SUGGESTIONS

- Samples DS2403 and DS2404 were diluted versions of samples DS2402 and DS2401, respectively. In many cases, this difference was not clearly visible on the blots. Please consider evaluating the antibody concentrations used, the substrate incubation time for the chemiluminescence reaction and/or exposure time (acquisition of the images).

- Consider acquiring images at sequential exposure times to better visualize the differences between the samples.

- Some laboratories need to revise the quality and sensitivity of the methods currently in use. In many cases, it is difficult to distinguish the samples either due to a weak signal or an inadequate electrophoretic profile.

- We are always available to provide technical support, discussion, and training if needed. Please do not hesitate to contact us.

ACKNOWLEDGMENTS

- People who contributed to organize the PT rounds

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Cristiana Corci

- All partecipants to PTs

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