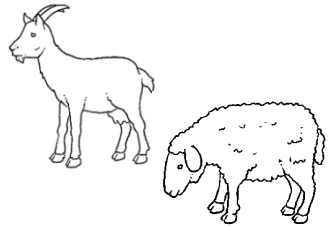


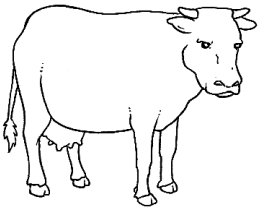
2025 round of TSE EURL EQAs: Results feed-back

23rd Annual Meeting of the TSE EURL Rome, Italy – 7-8 May 2026

1. DISCRIMINATORY WESTERN BLOT IN SMALL RUMINANTS



2. DISCRIMINATORY WESTERN BLOT IN BOVINE



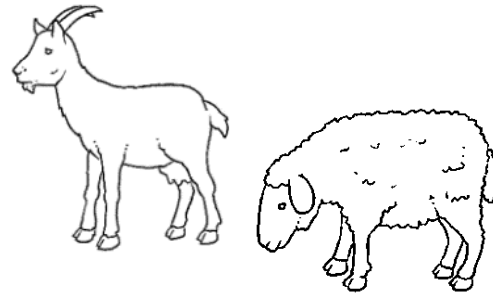
EURL-TSE

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

Istituto Superiore di Sanità - Rome

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DISCRIMINATORY WESTERN BLOT IN SMALL RUMINANTS



EURL-TSE

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

Istituto Superiore di Sanità - Rome



DISCRIMINATORY WB IN SMALL RUMINANTS (DS25)

N. Of participating NRLs in the EAQ round 2025

☐ 16 Labs

DISTRIBUTION

27 October 2025



DEADLINE

30 November 2025

Samples dispatched

Sample ID	Expected results	Genotype	Sample origin/characteristics
DS2501	Classical scrapie	ARQ/VRQ	Pool of classical scrapie samples from sheep
DS2502	BSE not excluded	ARQ/ARQ	Pool of samples from sheep with experimental BSE
DS2503	Classical scrapie	ARQ/ARQ	Pool of classical scrapie samples from sheep
DS2504	Classical scrapie	AHQ/AHQ	Pool of classical scrapie samples from sheep
DS2505	Classical scrapie	ARQ/ARQ	Pool of classical scrapie samples from sheep*

* Kindly provided by Alba Moreno



DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS25)

Results – Methods

N° of methods used per Lab	Total
one test method	14
two test methods	2

Test method used	N° of Labs
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	12
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 *	/

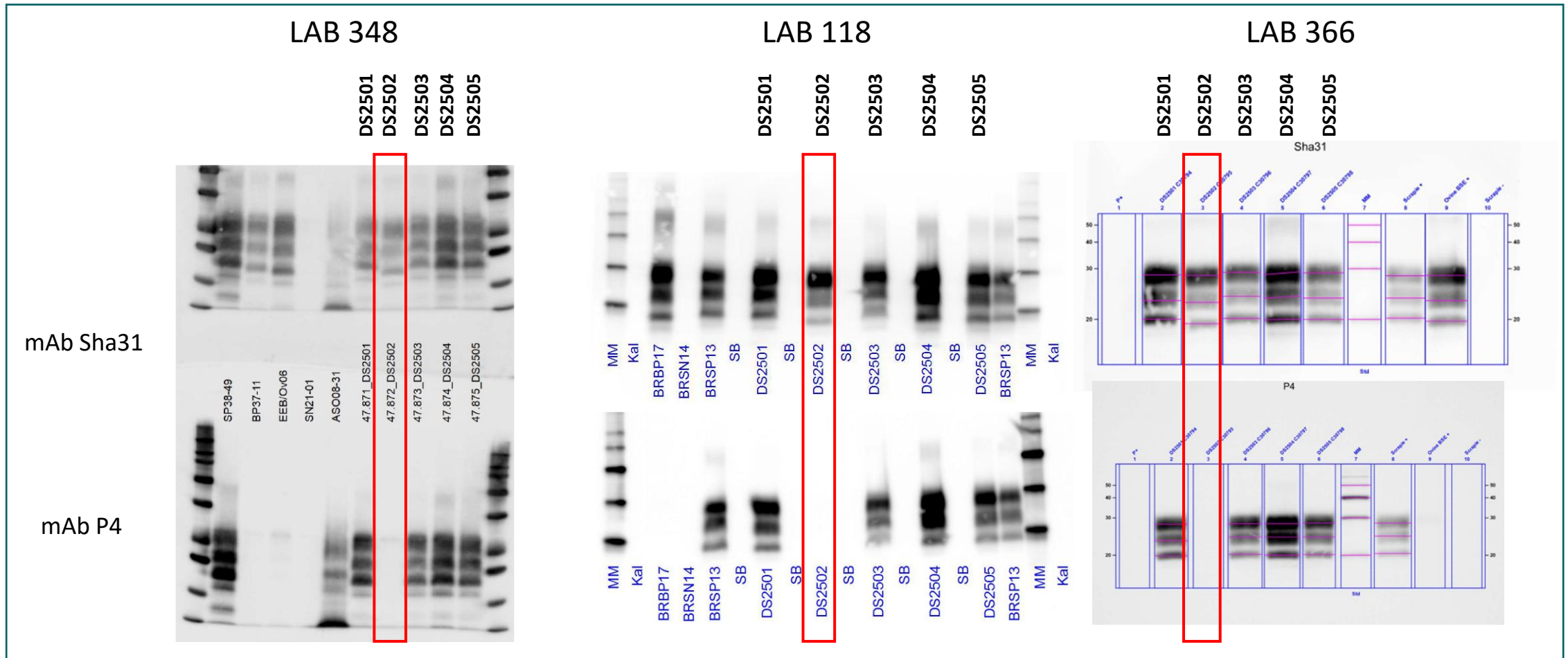
* Prionics kit discontinued

RESULTS

All the laboratories have reported the samples as the intended results

Some examples

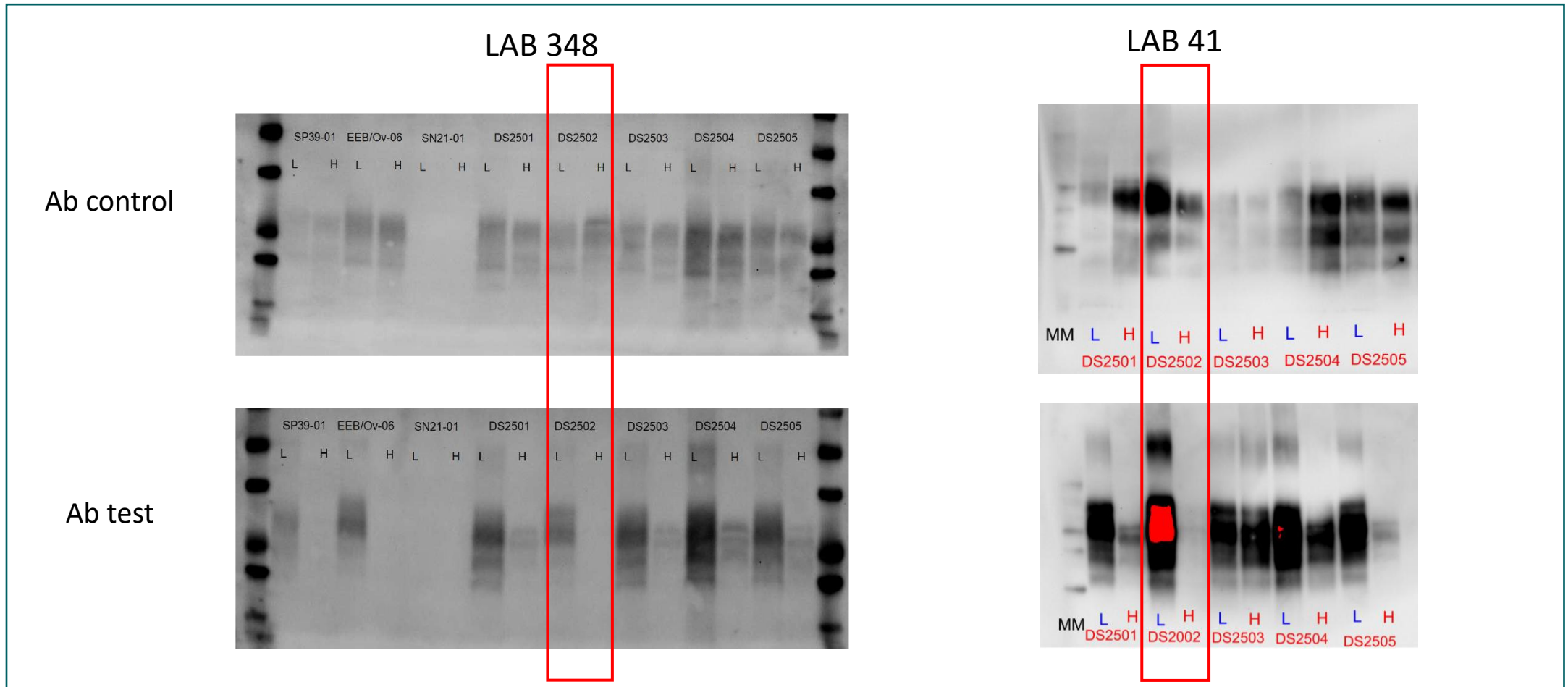
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method



RESULTS

Some examples

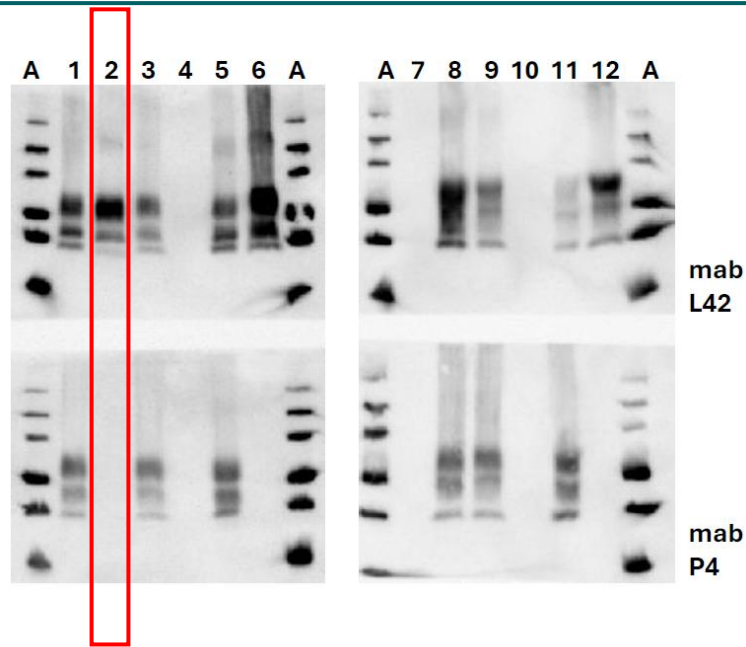
Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)



“home made” WBs

FLI, ANSES, ISS

FLI



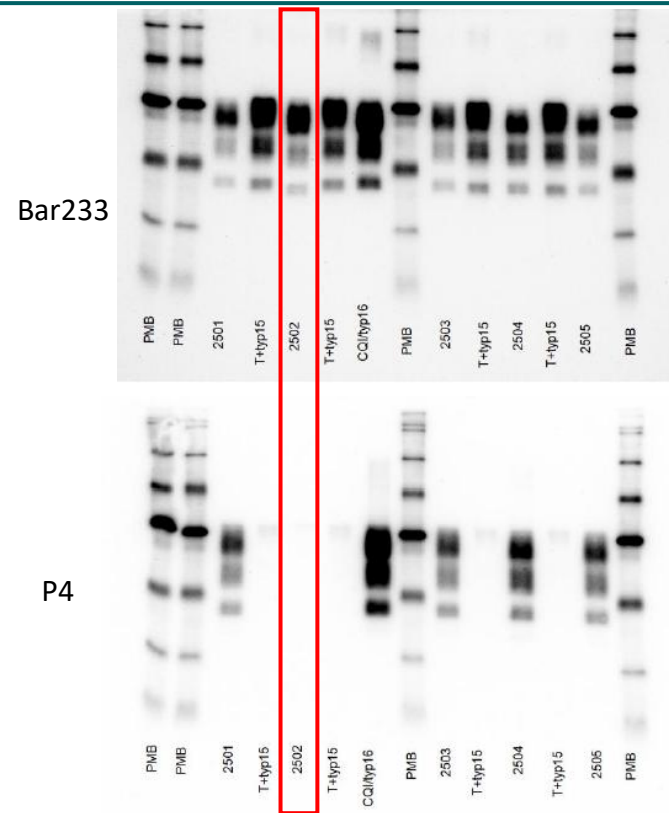
Dual Color Marker A
DS2501 1
DS2502 2
DS2503 3
Loading Buffer 4
Scrapie Control 5
BSE Control 6

Loading Buffer 7
DS2504 8
DS2505 9
Loading Buffer 10
Scrapie Control 11
BSE Control 12

mab
L42

mab
P4

ANSES

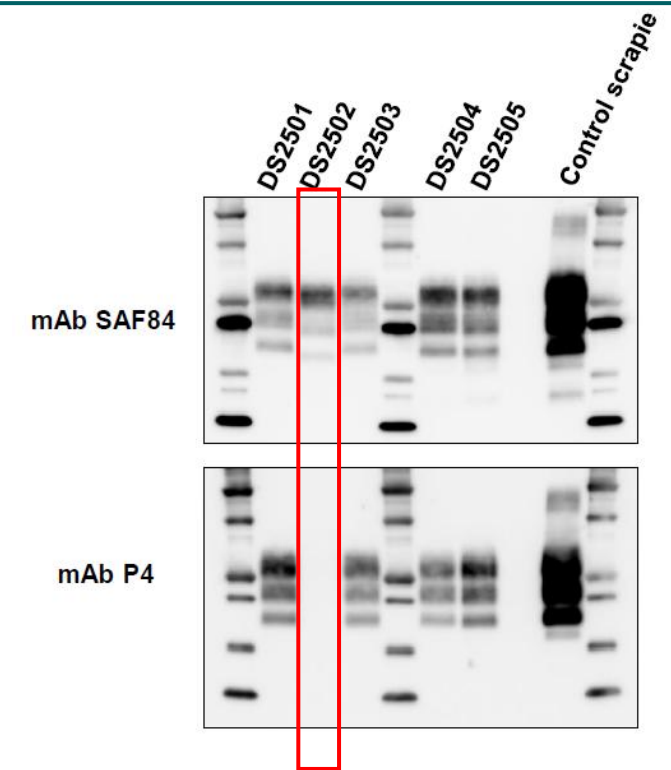


Bar233

P4

- Differential Molecular Weights (dmw) from cattle BSE
 - Dmw > 0,2 → not suspect
 - Dmw < 0,2 → re-tested
- Differential Immuno-reactivity (score as ++, +, +/-, -)
 - P4 signal ≥ Bar 233 → not suspect
 - P4 signal << Bar 233 → suspect

ISS



mAb SAF84

mAb P4

- Differential Molecular Weight (dmw) from scrapie
- Differential Immuno-reactivity (as SAF84/P4 ratio)
 - Dmw < 0,5 → suspect
 - SAF84/P4 > 2
 - Dmw ≥ 0,5 → not suspect
 - SAF84/P4 ≤ 2



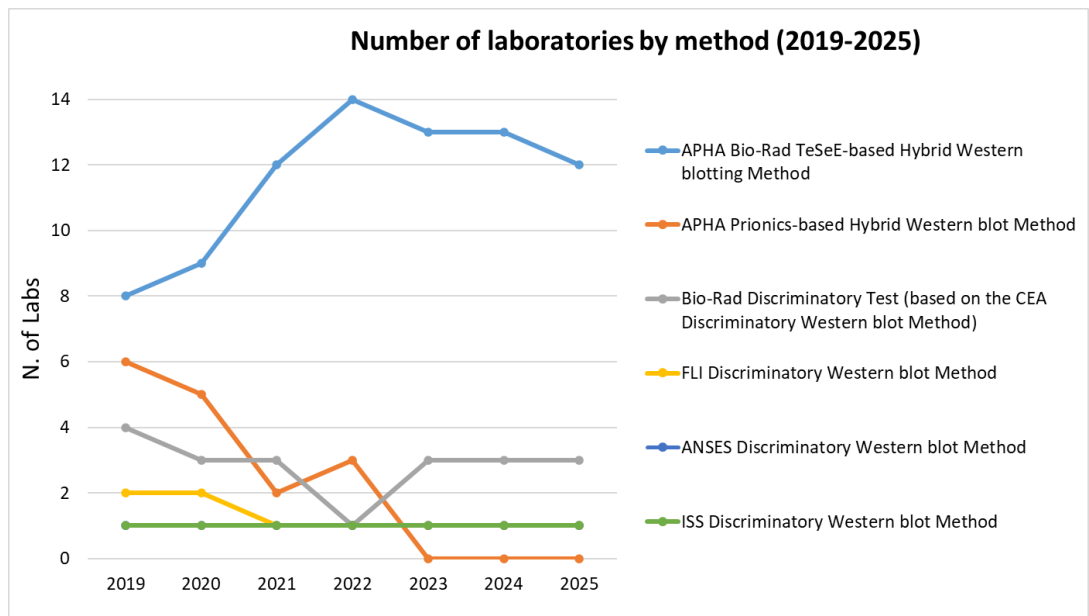
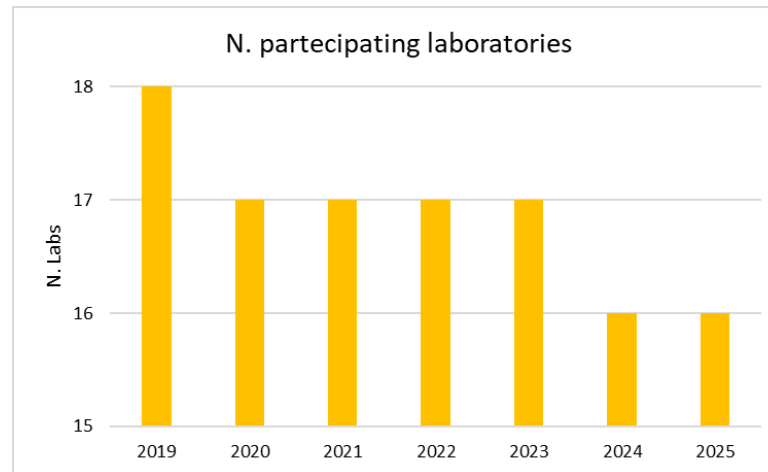
Discriminatory testing trend (2019-2025) – Small ruminants

ID LAB	2019	2020	2021	2022	2023	2024	2025
41							
118							
176						ND	ND
182							
188							
341							
366							
565							
601							
933							
954							
983							
985							
143-51		ND	*	*	ND	ND	ND
287-66							
352-993							
357-910							
469-243							

	PASSED
	Passed on 1st repeat round
	Failed

ND: the NRL did not participate

*Results not submitted



* Prionics kit discontinued

- Lab 188 provided the expected results
- Raw data analysis revealed critical limitations:
 - Low sensitivity
 - Poor image quality (observed in both methods)

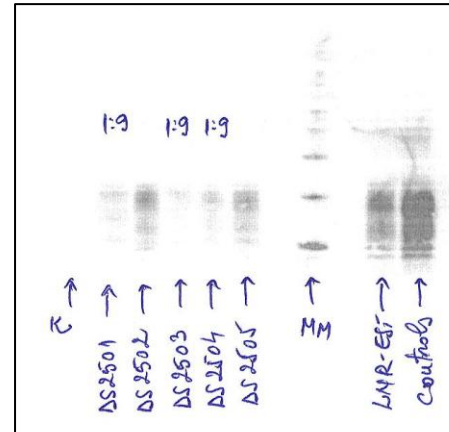
Recommendation

Review and optimize:

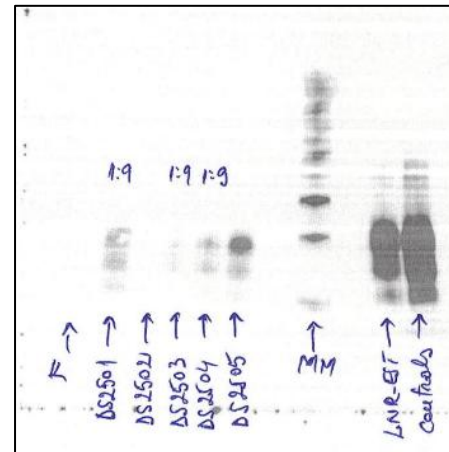
- Protocols
- Procedures
- Reagents used
- Training of personnel responsible for the test

APHA Bio-Rad TeSeE-based Hybrid Western blotting Method

Sha31

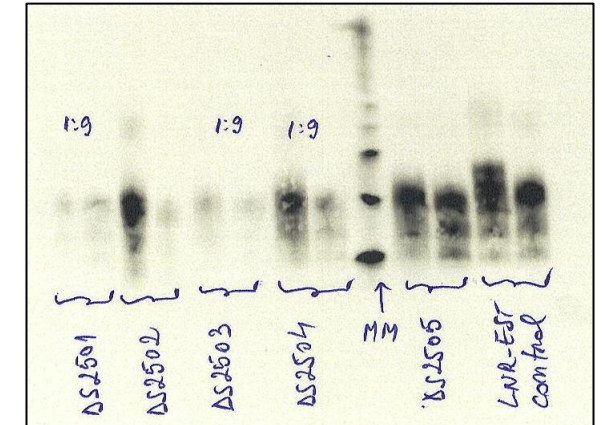


P4

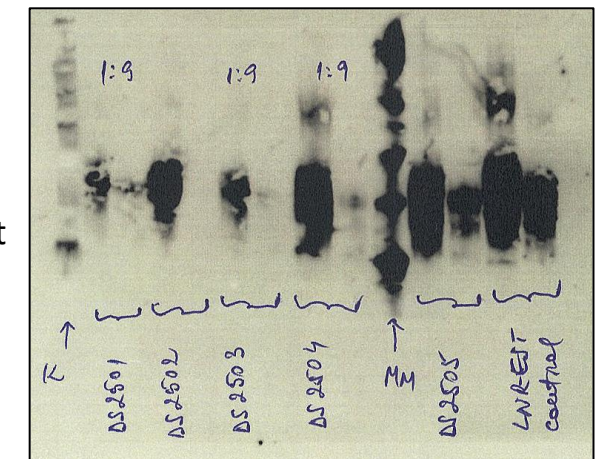


Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)

Control



Test





CONCLUSION/SUGGESTIONS

- Overall results are consistent with previous years:
 - Number of laboratories passing the PT
 - Methods applied
 - Participant distribution
- However, a gradual qualitative decline is observed:
 - Reduced attention to reagents and protocols
 - Decreased technical performance in test execution
 - Some laboratories should improve method quality and sensitivity
- Potential contributing factors:
 - Staff turnover over time
 - Reduced focus or engagement

Support:

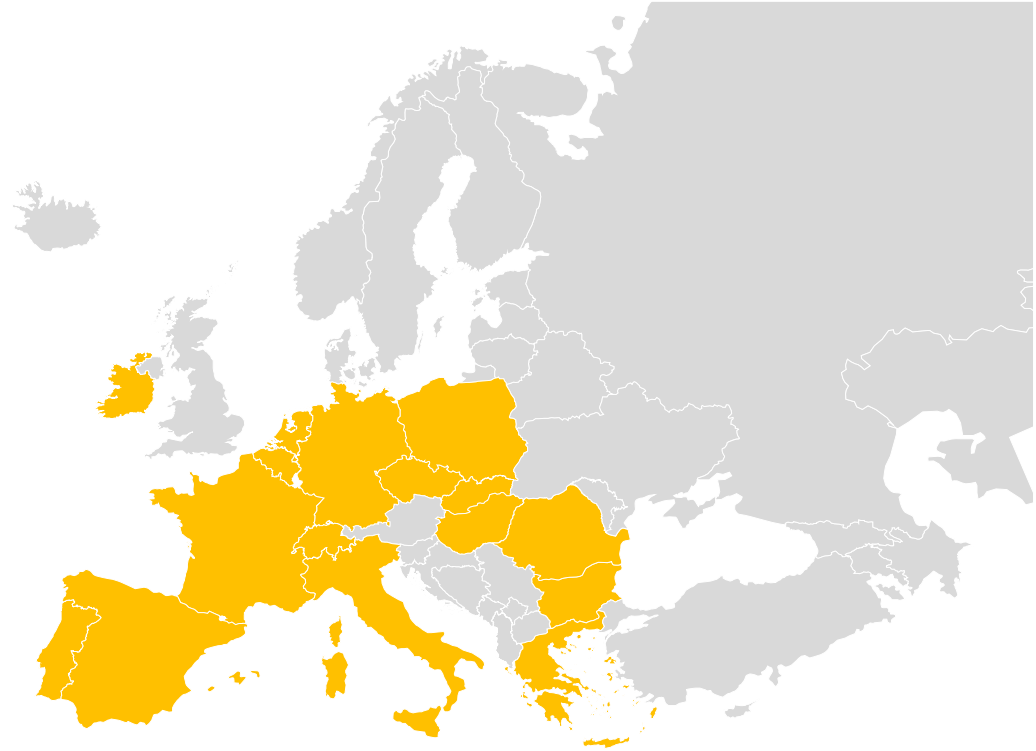
- Technical assistance, discussion and training are available upon request



DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS25)

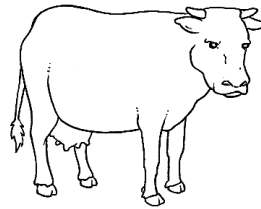
Laboratories passed this PT round successfully

- | | |
|-------------|-----------------|
| 1. Belgium | 10. Netherlands |
| 2. Bulgaria | 11. Poland |
| 3. Czech R. | 12. Portugal |
| 4. France | 13. Romania |
| 5. Germany | 14. Slovakia |
| 6. Greece | 15. Spain |
| 7. Hungary | 16. Switzerland |
| 8. Ireland | |
| 9. Italy | |



2025 round of TSE EURL EQAs: Results feed-back

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DISCRIMINATORY WESTERN BLOT IN BOVINE



EURL-TSE

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

Istituto Superiore di Sanità - Rome



DISCRIMINATORY WB IN SMALL RUMINANTS (DS25)

N. Of participating NRLs in the EAQ round 2025

☐ **11 Labs**

DISTRIBUTION

27 October 2025



DEADLINE

30 November 2025

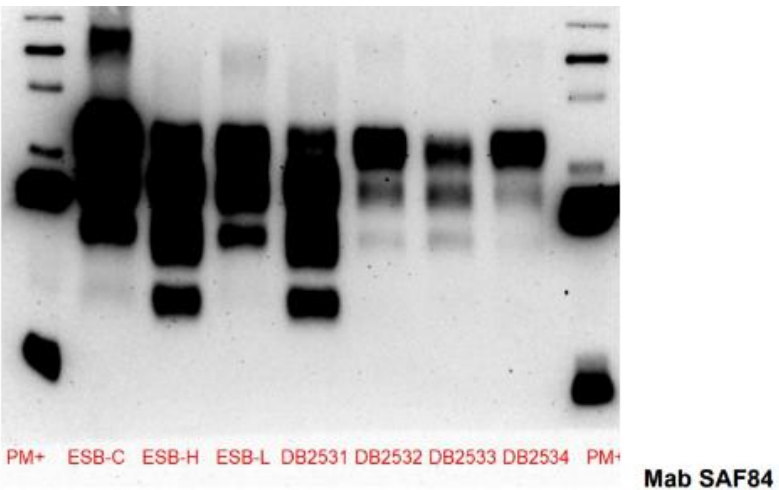
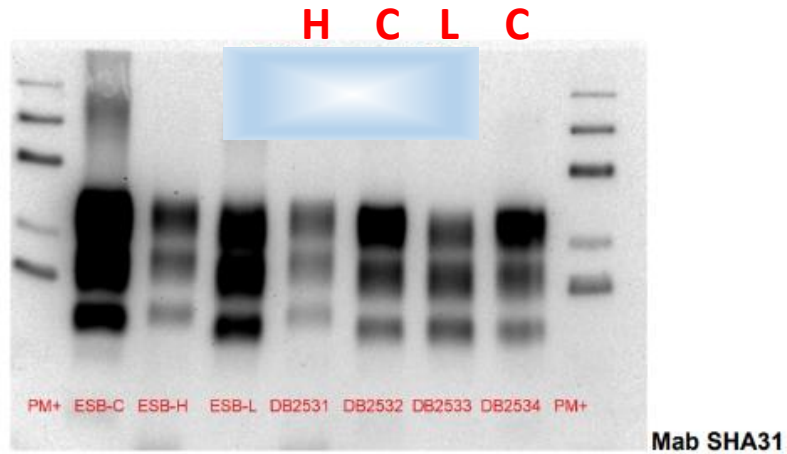
Samples dispatched

Sample ID	Expected results	Sample origin/characteristics
DB2531	H-BSE	Field isolate provided by Lucien Van Keulen
DB2532	C-BSE	APHA Reference Material
DB2533	L-BSE	Field isolate provided by Pawel Polak
DB2534	C-BSE	Dilution 1:2 of sample DB2532

DISCRIMINATORY WESTERN BLOT IN BOVINE EQA (DB25)

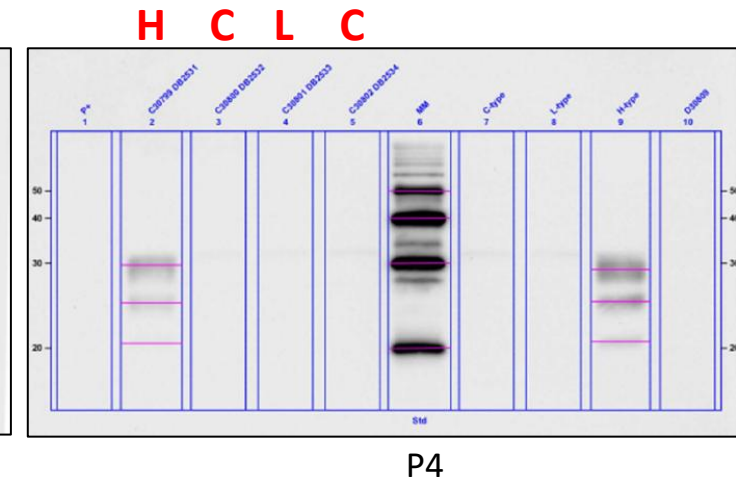
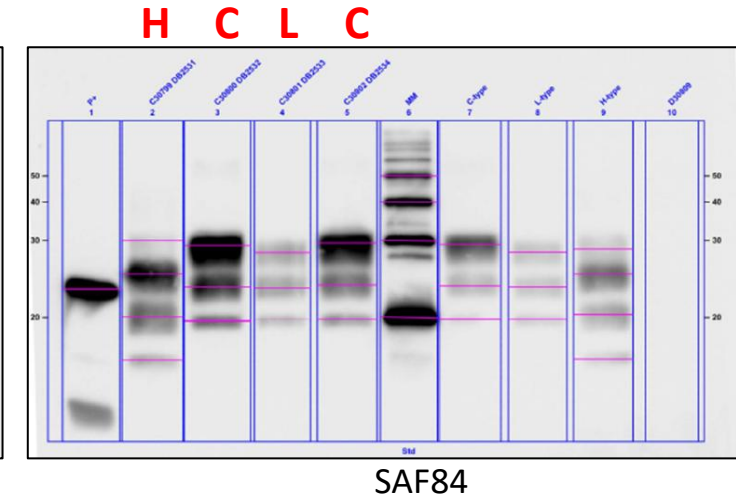
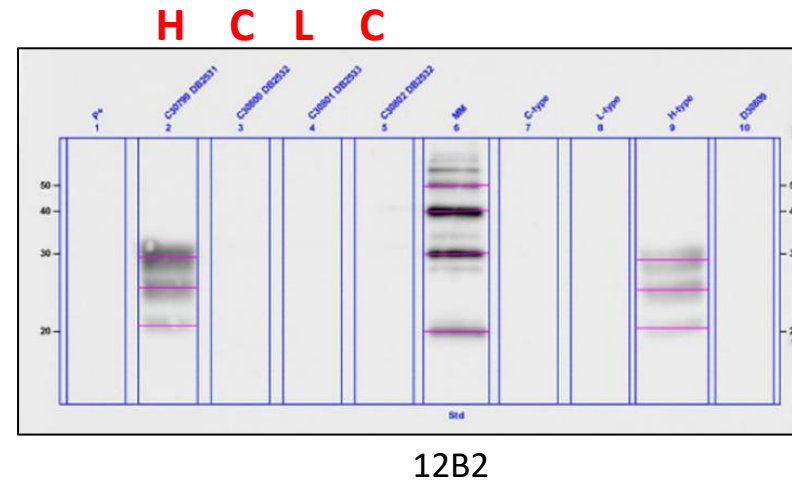
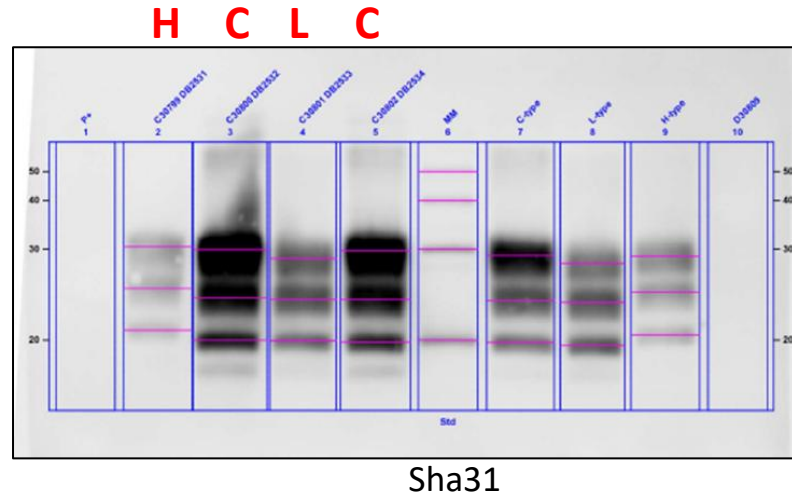
Some representative WBs

Lab 313



Lab 514

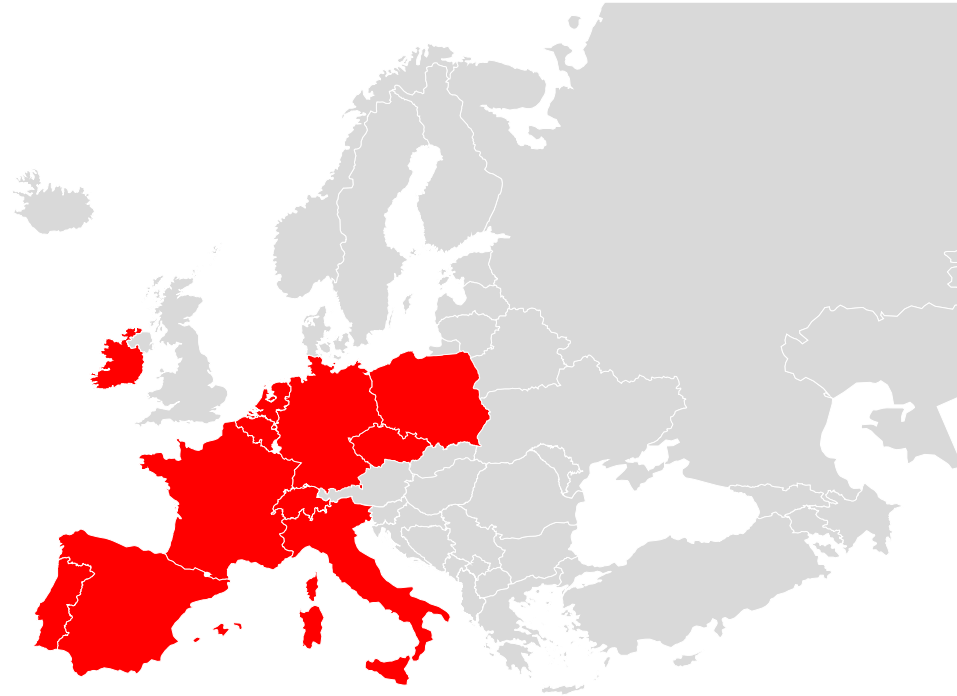
TeSeE WB kit BioRad



DISCRIMINATORY WESTERN BLOT IN BOVINE EQA (DB25)

Laboratories passed this PT round successfully

1. Belgium
2. Czech R.
3. France
4. Germany
5. Ireland
6. Italy
7. Netherlands
8. Poland
9. Portugal
10. Spain
11. Switzerland



Kind Reminder

Given the current difficulties in sourcing materials for bovine PT and the stock constraints, we kindly ask everyone not to register for the round unless there is a clear intention and readiness to participate.

This will help ensure that limited resources are used efficiently.

ACKNOWLEDGMENTS

- People who contributed to organize the PT rounds

ISS - Rome

Clarissa Ferreri
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Elena Esposito
Gabriele Vaccari
Geraldina Riccardi

IZPLVdA - Turin

Giuseppe Ru
Francesco Ingravalle
Elena Bozzetta
Maria Mazza
Cristiana Corci

- All participants to PTs

Discriminatory tests contact:

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