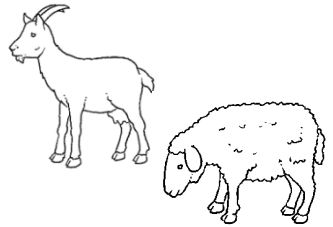


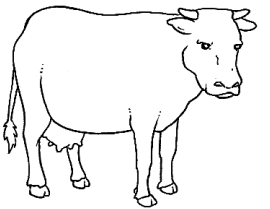
2023 round of TSE EURL EQAs: Results feed-back

Joint Workshop of the European Reference Laboratories for Transmissible Spongiform Encephalopathies and Animal Proteins
Rome, Italy – May 13-15, 2024

1. DISCRIMINATORY WESTERN BLOT IN SMALL RUMINANTS



2. DISCRIMINATORY WESTERN BLOT IN BOVINE



Laura Pirisinu
ISS - Rome



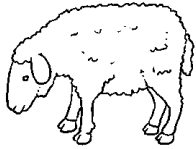
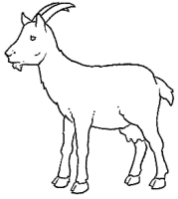
EURL-TSE

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 EQA (DS23)



EURL-TSE

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

Istituto Superiore di Sanità - Rome



DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS23)

Planning

- 17 NRLs** expressed interest to participate in EAQ round 2023 for Discriminatory WB in small ruminants
- Each NRL has been identified with a code (different for each EQA)
- The samples were stored at -80°C until shipment on dry ice
- All NRLs received an email informing the imminent dispatch of samples for 13 November 2023
- Inside the box were the cover letters with instructions for performing the proficiency tests, safety information, execution times and contacts.
- Submission of the results was carried out online via the new website <https://www.eurl-tse.eu/>
 - Login > Personal Area > Proficiency Testing > Submit your results > PT list – Select PT > Discriminatory Western Blot in Small Ruminants
- The deadline was 15 December 2023.



DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS23)

Samples dispatched

- 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
DS2301	Classical scrapie	ARQ/VRQ	Pool of classical scrapie samples from sheep
DS2302	Classical scrapie	VRQ/VRQ	Classical scrapie sample from sheep
DS2303	BSE not excluded	ARQ/ARQ	Pool of samples from sheep with experimental BSE
DS2304	Classical scrapie	ARQ/ARQ	Classical scrapie sample from sheep
DS2305	Classical scrapie	ARQ/ARQ	Classical scrapie sample from sheep



DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS23)

Results tabulation (sent 17/01/2024)

N° of methods used per Lab	Total
one test method	15
two test methods	2
Total participating Labs	17

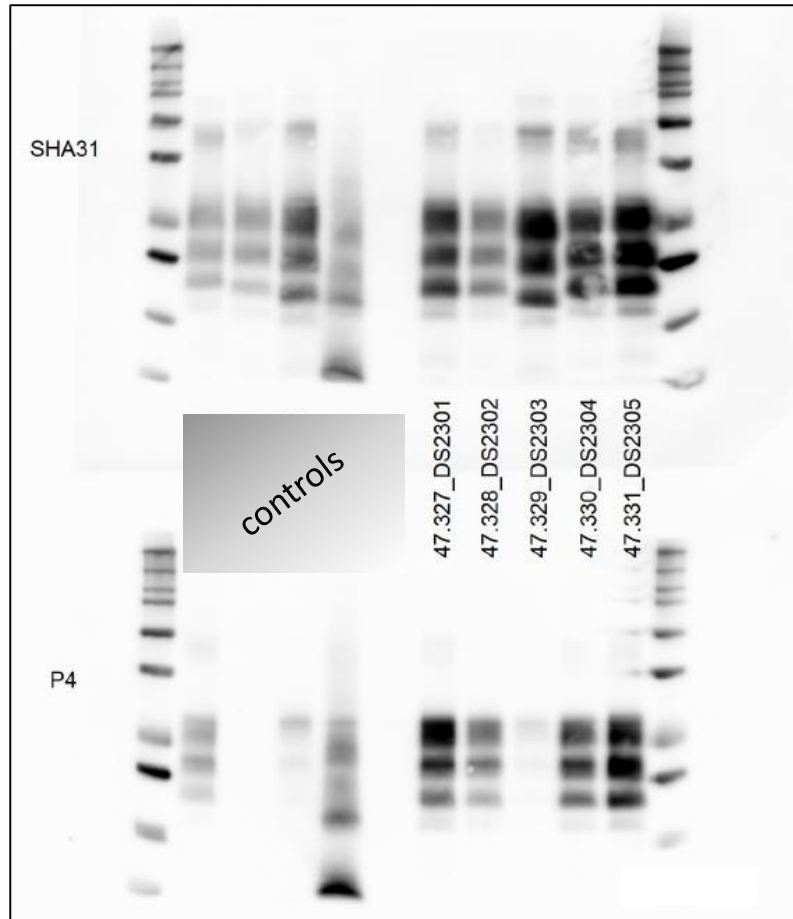
Test method used	N° of Labs
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	13
Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)	3
FLI Discriminatory Western blot Method	1
ANSES Discriminatory Western blot Method	1
ISS Discriminatory Western blot Method	1
APHA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

* Prionics kit discontinued

Some examples of better WBs

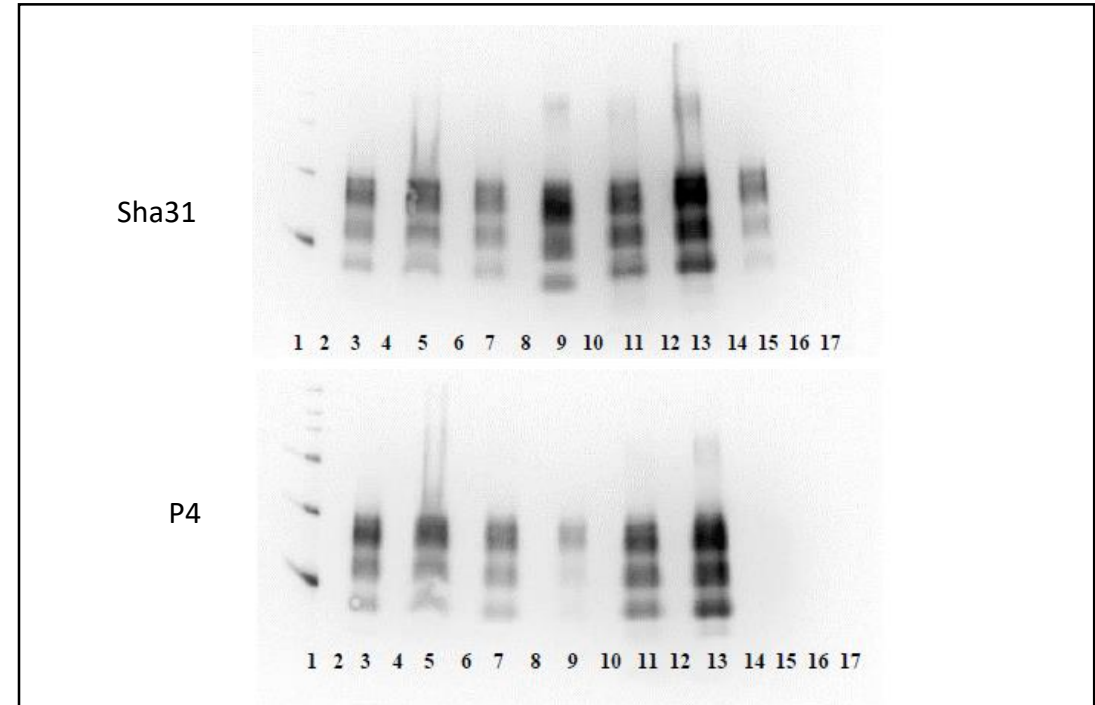
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method

LAB 243



*modified

LAB 933

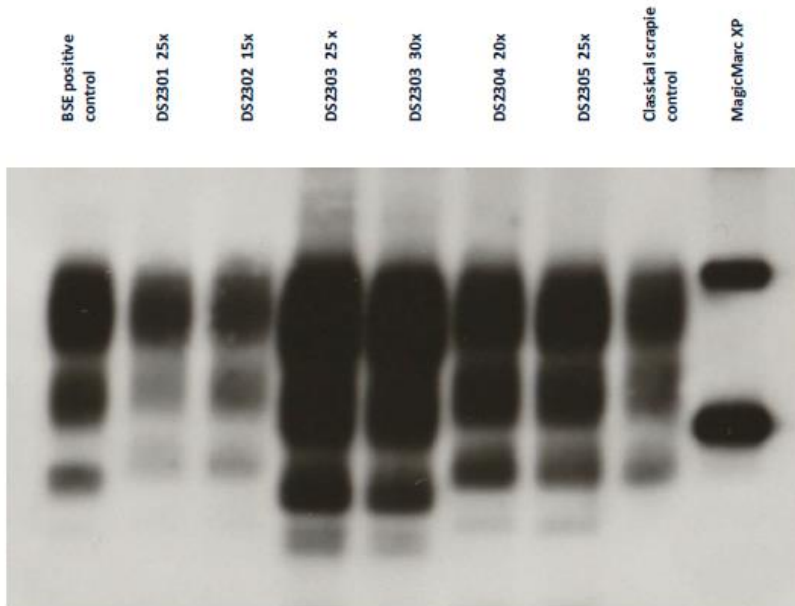


- | | |
|--|---|
| 1. Magic Mark Standard | 10. Negative control – Proteinase K+ |
| 2. Negative control – Proteinase K+ | 11. Sample No. DS2304 |
| 3. Positive – scrapie (mAb SHA31) / (mAb P4) | 12. Negative control – Proteinase K+ |
| 4. Negative control – Proteinase K+ | 13. Sample No. DS2305 |
| 5. Sample No. DS2301 | 14. Negative control – Proteinase K+ |
| 6. Negative control – Proteinase K+ | 15. Positive - BSE (mAb SHA31) / (mAb P4) |
| 7. Sample No. DS2302 | 16. Negative control – Proteinase K+ |
| 8. Negative control – Proteinase K+ | 17. Kaleidoskop Prestained Standard |
| 9. Sample No. DS2303 | |

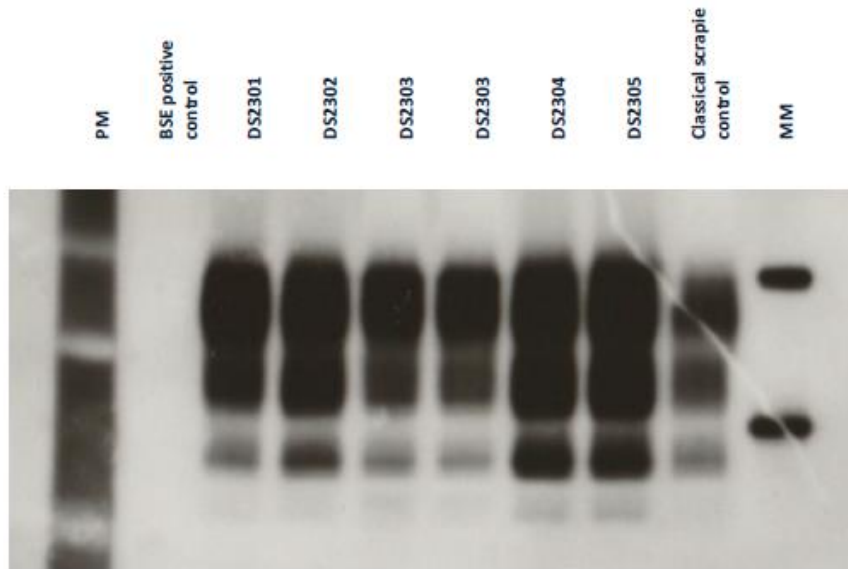
Some examples

APHA Bio-Rad TeSeE-based Hybrid Western blotting Method

mAb I.: SHA31



mAb I.: P4



ATTENTION

very nice blots qualitatively but you cannot dilute the sample with one antibody. You need to observe how the samples behave and react with mAbs under the same conditions

1. BSE positive control

2. DS2301 dilution: 1:25 Classical scrapie

3. DS2302 dilution: 1:15 Classical scrapie

4. DS2303 dilution: 1:25 BSE not excluded

5. DS2303 dilution: 1:30 BSE not excluded

6. DS2304 dilution: 1:20 Classical scrapie

7. DS2305 dilution: 1:25 Classical scrapie

8. Classical scrapie control

9. MagicMarc XP

1. Page Ruler, Prestained Protein Ladder

2. BSE positive control

3. DS2301 dilution: 1:30 Classical scrapie

4. DS2302 dilution: 1:25 Classical scrapie

5. DS2303 dilution: - BSE not excluded

6. DS2303 dilution: 1: 2 BSE not excluded

7. DS2304 dilution: 1:25 Classical scrapie

8. DS2305 dilution: 1:30 Classical scrapie

9. Classical scrapie control

10. MagicMarc XP



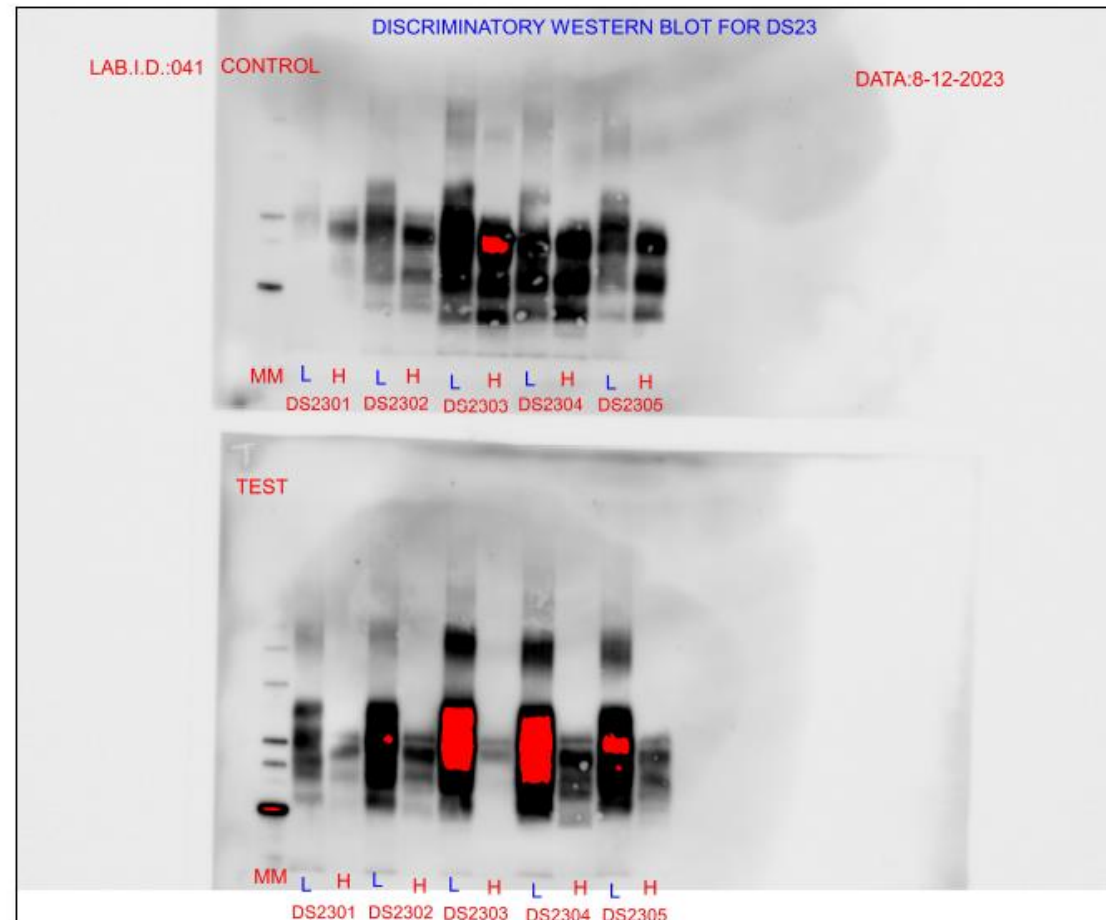
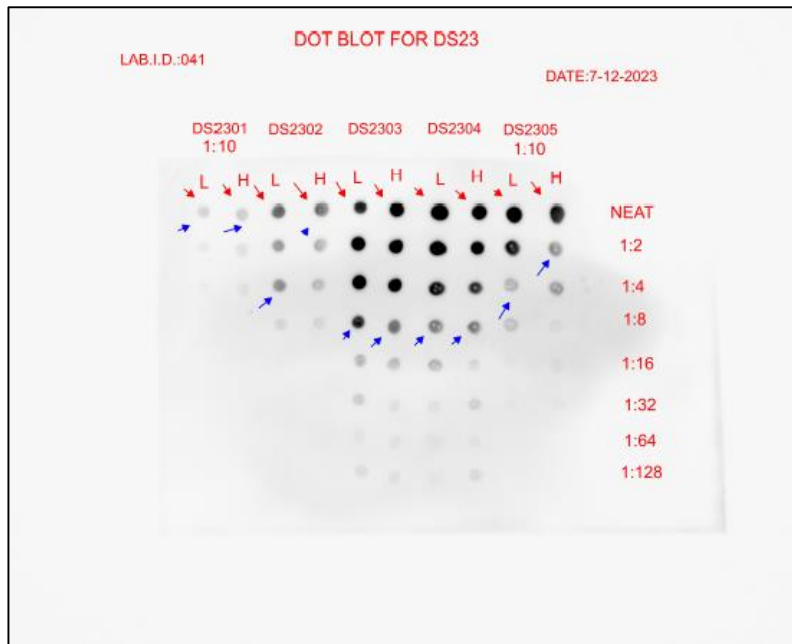
Some examples of better WBs

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

Lab 041.

Clear discrimination under high condition

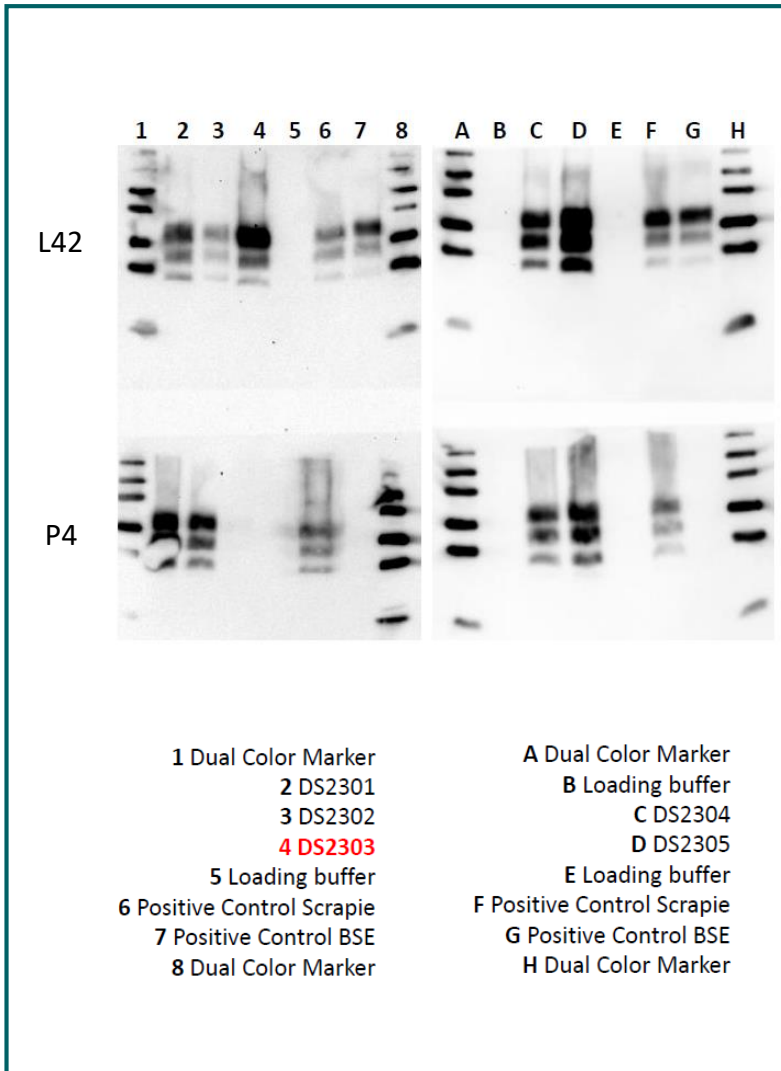
Put attention to analyse images/exposures with non-saturating signal



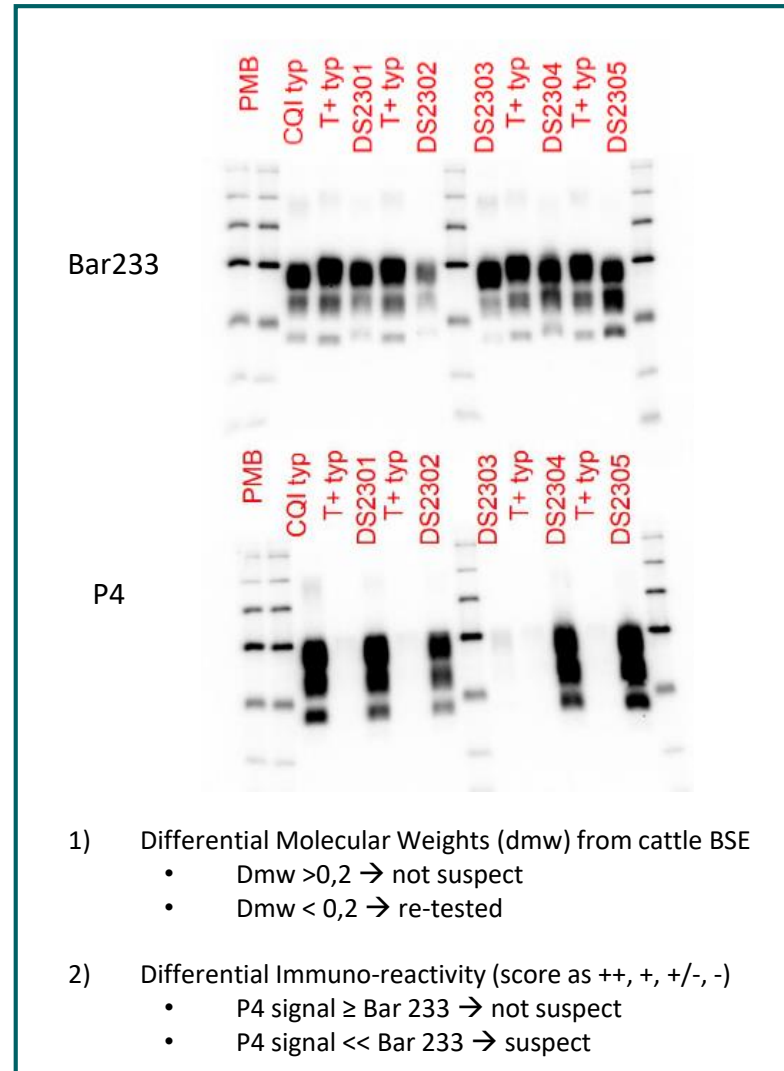
Some examples of “home made” WBs

FLI, ANSES, ISS

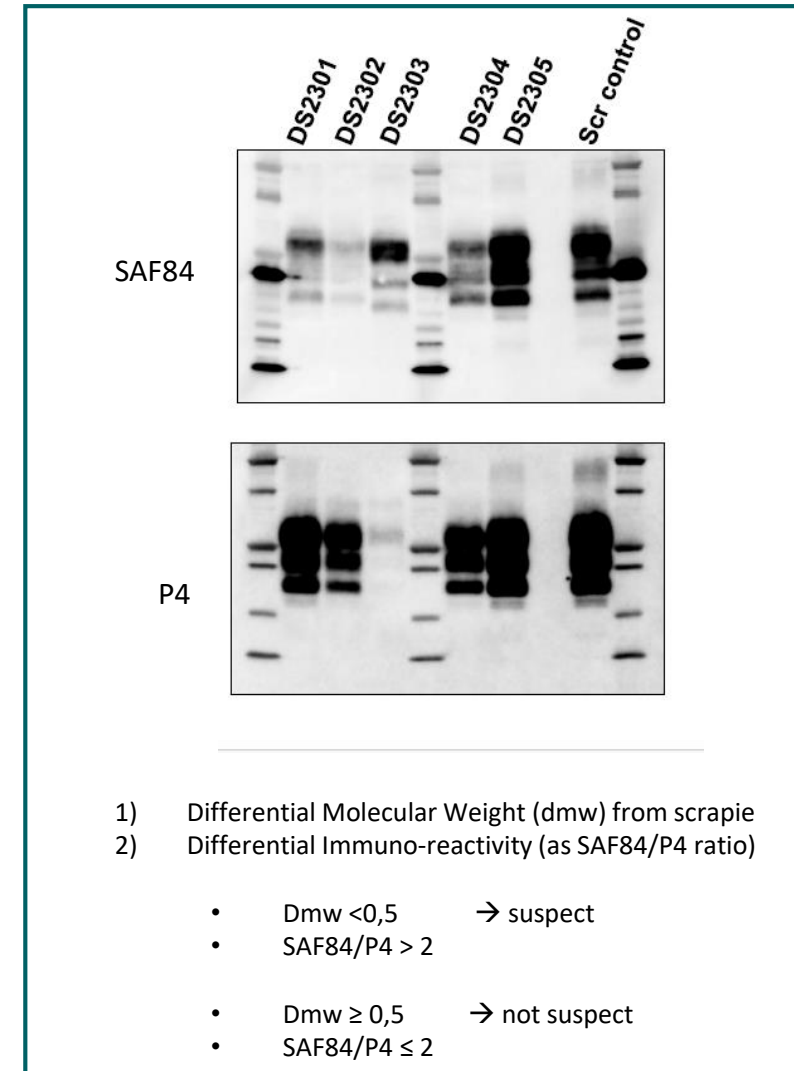
FLI



ANSES



ISS





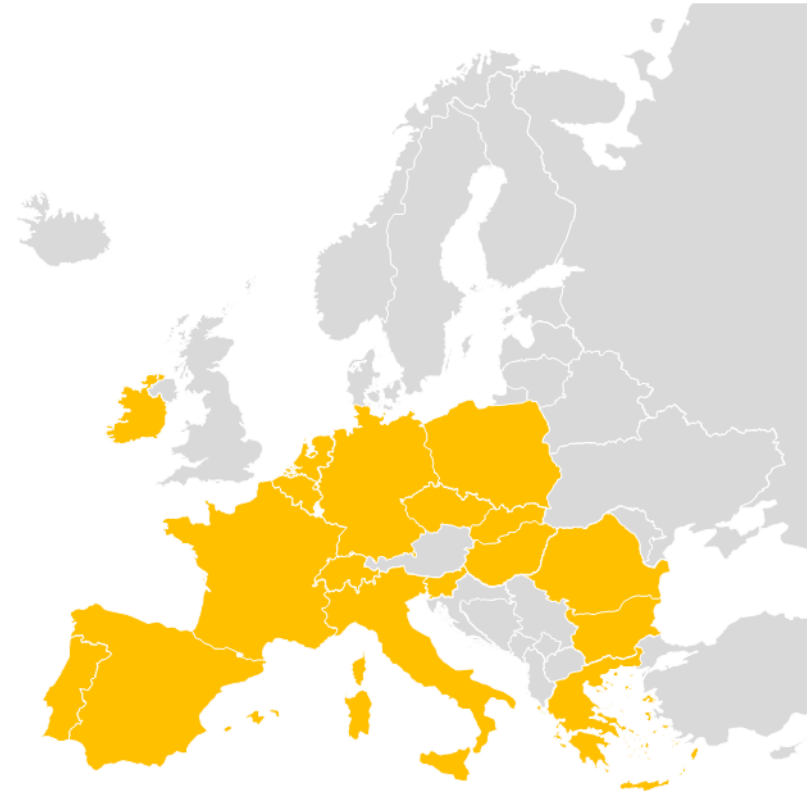
DISCRIMINATORY WB IN SMALL RUMINANTS EQA (DS23)

Results

The participants reported all samples as the intended results.

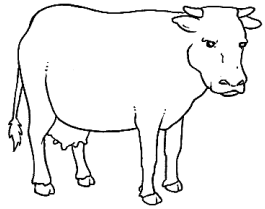
All Laboratories passed this PT round successfully!

- | | |
|-------------|-----------------|
| 1. Belgium | 10. Netherlands |
| 2. Bulgaria | 11. Poland |
| 3. Czechia | 12. Portugal |
| 4. France | 13. Romania |
| 5. Germany | 14. Slovakia |
| 6. Greece | 15. Slovenia |
| 7. Hungary | 16. Spain |
| 8. Ireland | 17. Switzerland |
| 9. Italy | |



2023 round of TSE EURL EQAs: Results feed-back

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DISCRIMINATORY WESTERN BLOT IN BOVINE EQA (DB23)



EURL-TSE

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

Istituto Superiore di Sanità - Rome



DISCRIMINATORY WESTERN BLOT IN BOVINE EQA (DB23)

Planning

- 11 Labs** expressed interest to participate in EAQ round 2023 for Discriminatory WB in bovine
- Each NRL has been identified with a code (different for each EQA)
- The samples were stored at -80°C until shipment on dry ice
- All NRLs received an email informing the imminent dispatch of samples for 13 November 2023
- Inside the box were the cover letters with instructions for performing the proficiency tests, safety information, execution times and contacts.
- Submission of the results was carried out online via the new website <https://www.eurl-tse.eu/>
 - Login > Personal Area > Proficiency Testing > Submit your results > PT list – Select PT > Discriminatory Western Blot in Small Ruminants
- The deadline was 15 December 2023.



DISCRIMINATORY WESTERN BLOT IN BOVINE EQA (DB23)

Samples dispatched

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

Sample ID	Expected results	Sample origin/characteristics
DB2331	C-BSE	Pool of UK Reference Material
DB2332	C-BSE	Pool of UK Reference Material
DB2333	H-BSE	Field isolate identified by Irish NRL (kindly provided by Ann Sharpe)
DB2334	L-BSE	Pool of field isolates from IT



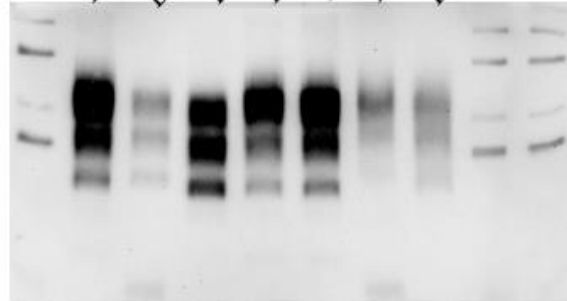
DISCRIMINATORY WESTERN BLOT IN BOVINE EQA (DB23)

Some representative WBs

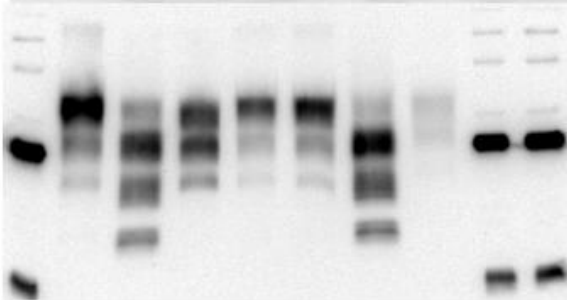
Lab 313

BSE-C control
BSE-H control
BSE-L control
DB-2331
DB-2332
DB-2333
DB-2334

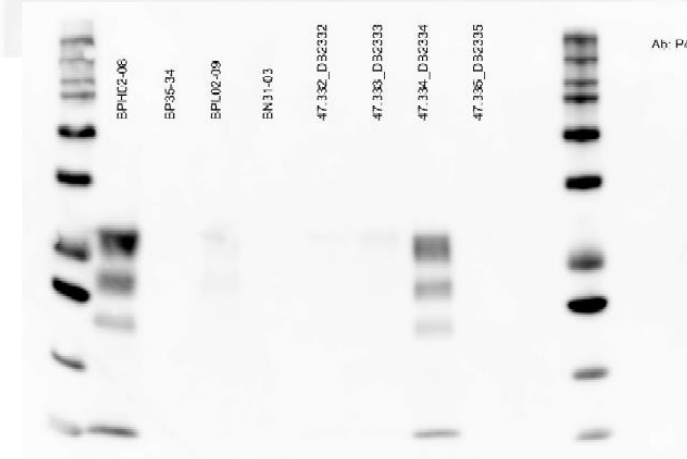
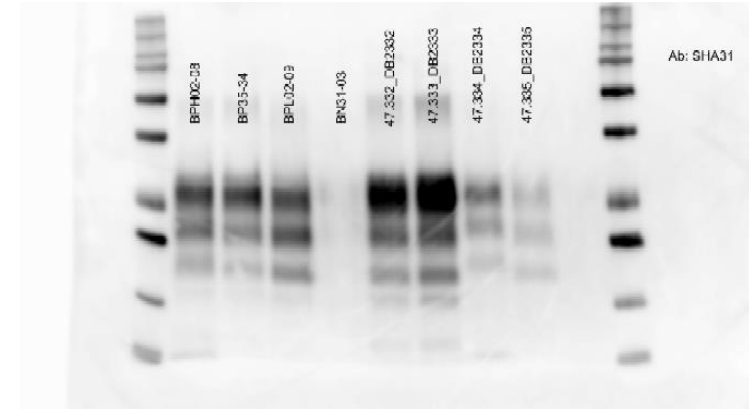
Detection with SHA31 antibody



Detection with SAF 84 antibody



Lab 811



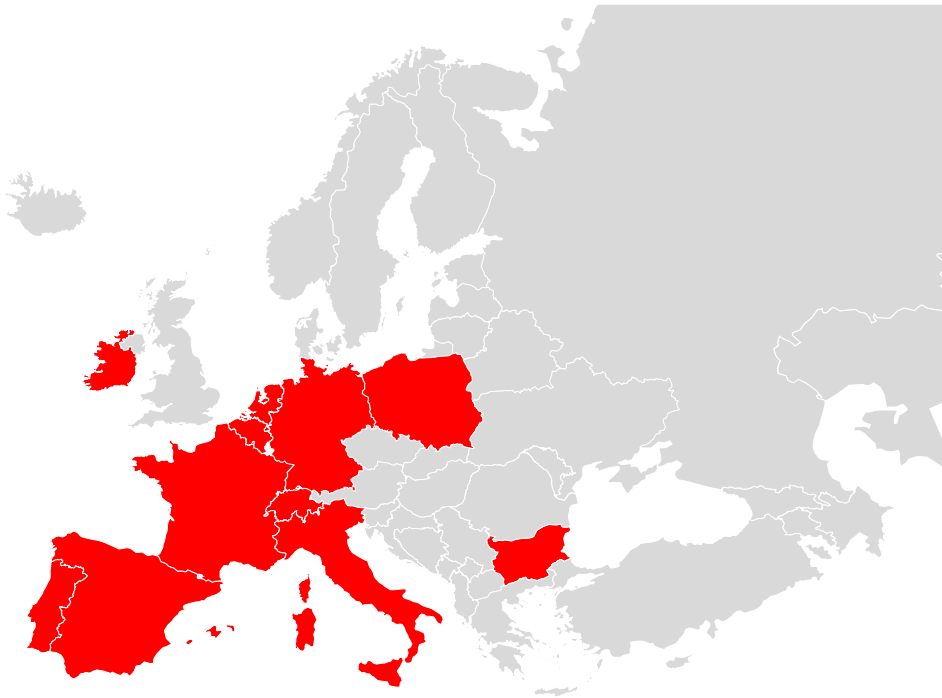


DISCRIMINATORY WESTERN BLOT IN BOVINE EQA (DB23)

The participants reported all samples as the intended results.

All Laboratories passed this PT round successfully!

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



ACKNOWLEDGMENTS

- People who contributed to organize the PT rounds

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Gabriele Vaccari
Geraldina Riccardi

IZPLVdA - Turin

Giuseppe Ru
Francesco Ingravalle
Elena Bozzetta
Maria Mazza

- All participants to PTs

Discriminatory tests contact:

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