

2025 round of TSE EURL EQAs: Results feed-back

Rome, Italy

PRNP sheep and goat genotyping EQA
PTGS25 and **PTGG25**

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PT ORGANIZATION

- ❖ Blood samples were collected and aliquoted in EDTA criotubes and stored at -20°C before the shipment
- ❖ Quality and genotype of the samples an aliquot of each of them was verified with an accredited SOP - EN ISO/IEC 17025 based on Sanger sequencing method
- ❖ Stability assessment: an aliquot of each blood sample was analyzed after two cycle of freezing and defrosting to verify the good signal of amplification



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PT ORGANIZATION

- ❖ Laboratories received a set of 10 sheep blood samples and/or 5 goat blood samples shipped on dry ice.
- ❖ Each sample was identified with a unique alphanumeric code (for example **GS2501**, **GS2502** and **GG2551**, **GG2552**) :

GS= Genotyping Sheep **GG**= Genotyping Goat
25 = for the year 2025
number from 01 to 10 for sheep samples or from 51 to 55 for goat samples
- ❖ In order to guarantee the anonymity, each laboratory received its own “Identification code”
- ❖ A different code was assigned to the laboratory for each different EQA



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- ❖ The results had to be uploaded on the dedicated platform of the official website of EURL
- ❖ **PTGS25:** All participants were required to report the PRNP genotypes at codons 136, 141, 154, 171 in allelic format (e.g., ALRQ/AFRQ)
- ❖ **PTGG25:** All participants were required to report the genotypes at codons 146 (N/N, N/S, S/D, N/D, S/S and D/D) and 222 (Q/Q, Q/K, K/K) of the PrP



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EQA - 2025

PTGS25

23 Participants

Austria	Lithuania
Belgium	Netherlands
Croatia	Norway
Cyprus	Poland
Czech Republic	Portugal
Estonia	Romania
Germany	Slovakia
Greece	Slovenia
Hungary	Spain
Iceland	Sweden
Italy	UK
Latvia	

PTGG25

13 Participants

Austria	UK
Belgium	
Germany	
Greece	
Italy	
Latvia	
Netherlands	
Poland	
Portugal	
Slovenia	
Spain	
Sweden	



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PTGS24 and PTGG24

Method Used for PRNP genotyping in sheep and goat

SANGER SEQUENCING

REAL TIME PCR

REAL TIME PCR and MELTING CURVE ANALYSIS

PRIMER EXTENTION 136, 154, 171 and 141

DGGE

PYROSEQUENCING



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Gold Standard - PTGS25

- ❖ Samples sent to participating laboratories were selected to represent the major PRNP allele combinations

Sample ID	Expected result
GS2501	ALRR/ALHQ
GS2502	VLRQ/VLRQ
GS2503	ALRQ/VLRQ
GS2504	ALRR/ALRQ
GS2505	ALRR/ALRR
GS2506	ALRQ/ALRH
GS2507	ALRQ/ALRQ
GS2508	ALRR/ALRQ
GS2509	ALRQ/ALRQ
GS2510	ALRQ/AFRQ



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Gold Standard – PTGS25

Sample ID	GS2501	GS2502	GS2503	GS2504	GS2505	GS2506	GS2507	GS2508	GS2509	GS2510
Gold Standard	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
Laboratory ID	Genotype reported									
011	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
023	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
081	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
093	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
100	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
137	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
197	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
229	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
305	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
359	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
548	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
566	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
591	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
604	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
619	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
722	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
731	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
780	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
853	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
857	-	-	-	-	-	-	-	-	-	-
858	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
907	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ
961	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ	ALRQ/AFRQ

- ❖ **Lab 857** didn't submit any result and failed this PT round, moreover it was not able to participate in a second round of PT due to internal issues
- ❖ All the other laboratories have reported the samples as the intended results

Gold Standard - PTGG25

Sample ID	Expected result for codon 146	Expected result for codon 222
GG2551	N/D	Q/Q
GG2552	N/N	Q/Q
GG2553	N/N	Q/K
GG2554	N/N	K/K
GG2555	N/N	Q/Q



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Gold Standard – PTGG25

Sample ID	GG2551		GG2552		GG2553		GG2554		GG2555	
Gold Standard	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
Laboratory ID	Genotype reported									
006	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
113	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
222	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
392	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
476	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
757	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
768	-	-	-	-	-	-	-	-	-	-
774	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
835	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
891	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	Q/Q	N/N	Q/Q
911	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
920	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q
930	N/D	Q/Q	N/N	Q/Q	N/N	Q/K	N/N	K/K	N/N	Q/Q



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PTGG25

Lab 768 did not submit any results due to internal issues and was not able to participate in a second round of PT

Lab 891 - Sample GG2254: The laboratory reported for the codon 222 genotype Q/Q instead of K/K. The issue was due to an incorrect data entry in the EURL system and not to an analytical problem.

- ❖ This was identified only after the gold standard had been submitted. In accordance with the protocol, we then sent a second set of samples.
- ❖ Lab provided correct results on the new set of unknown samples



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LABORATORY PERFORMANCE

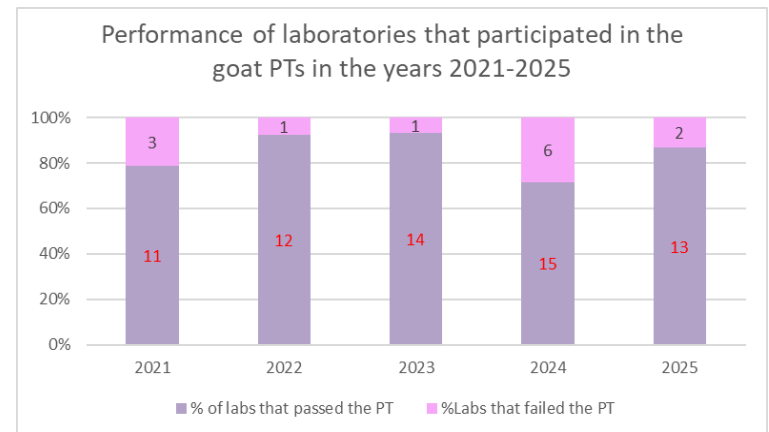
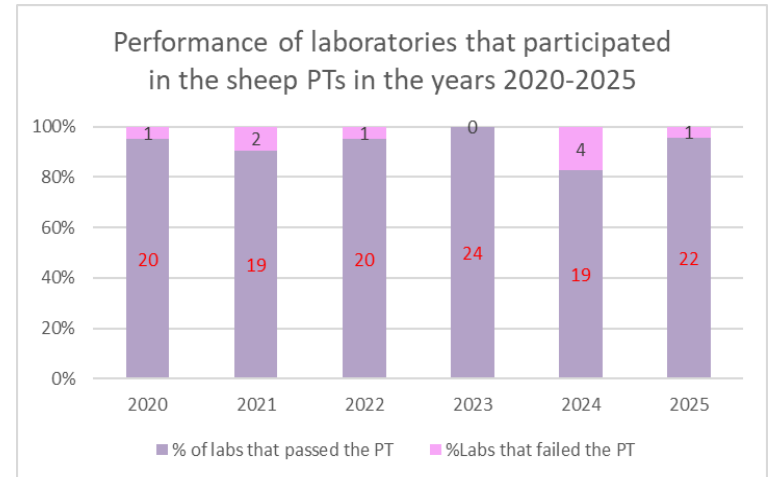
❖ Laboratory performances for each year, represented by the percentage of the labs that passed or failed the PTs

❖ Performances showed a slightly decline in 2024 for both PTs followed by an improvement in 2025



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CONCLUSION

- ❖ Participation in proficiency test scheme is an opportunity to identify and address laboratory weaknesses and helping to improve performance



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Technical aspect of Sheep and Goat PRNP Genotyping methods

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- All participants to PTs
- Cyprus and those Laboratory that will contribute to provide blood sample to EURL-TSE



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