

2024 round of TSE EURL EQAs: Results feed-back

Turin, Italy

PRNP sheep and goat genotyping EQA
PTGS24 and **PTGG24**

Barbara Chiappini



TSE EURL

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PT ORGANIZATION

- ❖ Blood samples were collected and aliquoted in criotubes of 0,5 ml 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 each time 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 collected in EDTA and shipped on dry ice.
- ❖ Each sample was identified with a unique alphanumerical code (for example **GS2401**, **GS2402** and **GG2451**, **GG2452**) :

GS= Genotyping Sheep **GG**= Genotyping Goat
24 = for the year 2024
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
- ❖ **PTGS24:** All participants were requested to report the genotypes at codons 136, 141, 154, 171 of the PrP in allelic format (e.g., ALRQ/AFRQ)
- ❖ **PTGG24:** All participants were requested 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 - 2024

PTGS24

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	

PTGG24

15 Participants

Austria	Spain
Belgium	UK
Germany	Czech Republic
Greece	
Italy	
Latvia	
Netherlands	
Poland	
Portugal	
Romania	
Slovakia	
Slovenia	



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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 - **PTGS24**

Sample ID	Expected result
GS2401	ALRR/ALRQ
GS2402	ALRQ/AFRQ
GS2403	ALHQ/ALRH
GS2404	ALRH/ALRH
GS2405	ALRR/VLRQ
GS2406	ALRQ/ALRQ
GS2407	ALRR/ALRQ
GS2408	ALRR/ALRR
GS2409	ALRR/ALRH
GS2410	ALHQ/VLRQ



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Gold Standard – PTGS24



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GS24: PRNP sheep genotyping

Distribution date: 23/09/2024

Sample ID	GS2401	GS2402	GS2403	GS2404	GS2405	GS2406	GS2407	GS2408	GS2409	GS2410
Gold Standard	ALRR/ALRQ	ALRQ/AFRQ	ALHQ/ALRH	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
Laboratory ID	Genotype reported									
011	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
081	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
093	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
100	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
197	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
229	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
305	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
359	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
548	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
566	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
584	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
591	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
604	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
619	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
722	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
731	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
780	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
853	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
857	-	-	-	-	-	-	-	-	-	-
858	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
907	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
948	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ
961	ALRR/ALRQ	ALRQ/AFRQ	ALRH/ALHQ	ALRH/ALRH	ALRR/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRH	ALHQ/VLRQ

Comments :

Lab 857 didn't submit any result. For this reason the laboratory failed this PT round.

Lab 548 - Sample GS2405: The laboratory reported the genotype ALRQ/VLRQ instead of ALRR/VLRQ. For this reason the laboratory failed this PT round.

Lab 566 - Sample GS2410: The laboratory reported the genotype ALRQ/VLRQ instead of ALHQ/VLRQ. For this reason the laboratory failed this PT round.

Lab 619 - Sample GS2404: The laboratory reported the genotype ALRQ/ALRH instead of ALRH/ALRH. For this reason the laboratory failed this PT round.

All the other laboratories have reported the samples as the intended results.

Lab 584 also submitted the results using the wrong NRL ID code.

Comments provided by Clarissa Ferreri, Istituto Superiore di Sanità - Rome

Conclusion:

All the laboratories have passed this PT round except Laboratories 057, 566, 619 and 857.

The informations contained in this Report must be considered confidential.

Firmato digitalmente da:

Ru Giuseppe

Date: 4 November 2024

Firmato il 04/11/2024 16:49

Seriale Certificato: 3441766

Valido dal 02/04/2024 al 02/04/2027

Giuseppe Ru, TSE EURL Director

PTGS24

LAB ID	EXPECTED RESULT	REPORTED GENOTYPE
Lab 548	ALRR/VLRQ	ALR ^Q /VLRQ
Lab 619	ALRH/ALRH	ALR ^Q /ALRH
Lab 566	ALHQ/VLRQ	AL ^R Q/VLRQ

- ❖ Laboratory 857 didn't submit any result and failed this PT round
- ❖ Laboratory 584 submitted the results using the wrong ID code



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PTGS24 – second round



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GS24R: PRNP sheep genotyping - Second shipment

Distribution date: 02/12/2024

Sample ID	GS24R1	GS24R2	GS24R3	GS24R4	GS24R5	GS24R6	GS24R7	GS24R8	GS24R9	GS24R10
Gold Standard	ALRQ/ALRQ	ALRQ/AFRQ	ALRR/ALRR	ALHQ/VLRQ	ALRR/VLRQ	ALRH/ALRH	ALRR/ALRH	ALRR/ALRQ	ALRR/ALRQ	ALHQ/ALRH
Laboratory ID	Genotype reported									
548	-	-	-	-	-	-	-	-	-	-
566	-	-	-	-	-	-	-	-	-	-
619	ALRQ/ALRQ	ALRQ/AFRQ	ALRR/ALRR	ALHQ/VLRQ	ALRR/VLRQ	ALRH/ALRH	Not Determined	ALRR/ALRQ	ALRR/ALRQ	ALRH/ALHQ
857	ALRQ/ALRQ	ALRQ/AFRQ	ALRR/ALRR	ALRR/ALHQ	ALRR/VLRQ	ALRH/ALRH	ALRR/ALRH	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALHQ

Comments :

Lab 548 did not take part in this PT round.

Lab 566 didn't submit any result. For this reason the laboratory failed this PT round.

Lab 619 - Sample GS2407: The laboratory reported "Not Determined". For this reason the laboratory failed this PT round.

Lab 857 - Sample GS24R4: The laboratory reported the genotype ALRR/ALHQ instead of ALHQ/VLRQ. Sample GS24R8: The laboratory reported the genotype ALRQ/ALRQ instead of ALRR/ALRQ. Sample GS24R10: The laboratory reported the genotype ALRR/ALHQ instead of ALHQ/ALRH. For this reason the laboratory failed this PT round.

Comments provided by Clarissa Ferreri, Istituto Superiore di Sanità - Rome

Conclusion:

All the laboratories failed this PT round.

Date: January 30, 2025

Giuseppe Ru, TSE EURL Director

The informations contained in this Report must be considered confidential.

Firmato digitalmente da:

Ru Giuseppe

Firmato il 03/02/2025 17:35

Seriale Certificato: 3441766

Valido dal 02/04/2024 al 02/04/2027

InfoCamere Qualified Electronic Signature CA

Giuseppe Ru



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PTGS24 – second round

LAB ID	EXPECTED RESULT	REPORTED GENOTYPE
Lab 619	ALRR/ALRH	NOT DETERMINED
	ALHQ/VLRQ	ALRR/ALHQ
Lab 857	ALRR/ALRQ	ALRQ/ALRQ
	ALHQ/ALRH	ALRR/ALHQ

- ❖ Laboratory 566 didn't submit any result and failed PTGS24
- ❖ Laboratory 548 did not take part in this second PT round and failed PTGS24



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Gold Standard - PTGG24

Sample ID	Expected result for codon 146	Expected result for codon 222
GG2451	N/N	Q/K
GG2452	N/S	Q/Q
GG2453	S/D	Q/Q
GG2454	N/N	Q/Q
GG2455	N/N	Q/K



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Gold Standard – PTGG24



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GG24: PRNP goat genotyping

Distribution date: 23/09/2024

Sample ID	GG2451		GG2452		GG2453		GG2454		GG2455	
Gold Standard	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
Laboratory ID	Genotype reported									
006	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
050	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
113	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
392	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
476	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
652	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	K/K
751	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
754	N/N	Q/Q	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
757	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
768	-	-	-	-	-	-	-	-	-	-
774	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
835	N/N	Q/K	N/N	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
891	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
920	N/N	Q/K	N/S	Q/Q	N/D	Q/Q	N/N	Q/Q	N/N	Q/K
920	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/N	Q/Q	N/N	Q/K
998	-	-	-	-	-	-	-	-	-	-

Comments :

Lab 768 and Lab 988 didn't submit any result. For this reason they failed this PT round.

Lab 652 - Sample GG2455: The laboratory reported the codon 222 genotype K/K instead of Q/K. For this reason the laboratory failed this PT round.

Lab 754 - Sample GG2451: The laboratory reported the codon 222 genotype Q/Q instead of Q/K. For this reason the laboratory failed this PT round.

Lab 835 - Sample GG2452: The laboratory reported the codon 146 genotype N/N instead of N/S. For this reason the laboratory failed this PT round.

Lab 920 - Sample GG2453: The laboratory reported the codon 146 genotype N/D instead of S/D. For this reason the laboratory failed this PT round.

All the other laboratories have reported all samples as the intended results.

Lab 652 and Lab 751 also submitted the results using the wrong NRL ID code.

Comments provided by Clarissa Ferreri, Istituto Superiore di Sanità - Rome

Conclusion:

All the laboratories have passed this PT round except Laboratories 652, 754, 768, 835, 920 and 998.

Date: 4 November 2024

Giuseppe Ru, TSE EURL Director

The informations contained in this Report must be considered confidential.

Formato digitalmente da:
Ru, Giuseppe
Firmato il 04/11/2024 16:47
Serializzato Certificato: 3441766
Valido dal 02/04/2024 al 02/04/2027
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PTGG24

LAB ID	EXPECTED RESULT	REPORTED GENOTYPE
Lab 652	222 Q/K	222 K/K
Lab 754	222 Q/K	222 Q/Q
Lab 835	146 N/S	146 N/N
Lab 920	146 S/D	146 N/D

- ❖ Laboratory 768 and 988 didn't submit any result and failed this PT round
- ❖ Laboratory 652 and 751 submitted the results using the wrong ID code



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Gold Standard – PTGG24 (Second round)



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GG24R: PRNP goat genotyping - Second shipment

Distribution date: 02/12/2024

Sample ID	GG24R1		GG24R2		GG24R3		GG24R4		GG24R5	
Gold Standard	N/N	Q/Q	N/N	Q/K	S/D	Q/Q	N/N	Q/K	N/S	Q/Q
Laboratory ID	Genotype reported									
652	N/N	Q/Q	N/N	Q/K	S/D	Q/Q	N/N	Q/K	N/S	Q/Q
754	N/N	Q/Q	N/N	Q/K	S/D	Q/Q	N/N	Q/K	N/S	Q/Q
768	-	-	-	-	-	-	-	-	-	-
835	N/N	Q/Q	N/N	Q/K	S/D	Q/Q	N/N	Q/K	N/S	Q/Q
920	N/N	Q/Q	N/N	Q/K	S/D	Q/Q	N/N	Q/K	N/S	Q/Q

Comments :

Lab 768 didn't submit any result. For this reason the laboratory failed this PT round.
All the other laboratories have reported all samples as the intended results.

Comments provided by Clarissa Ferreri, Istituto Superiore di Sanità - Rome

Conclusion:

All the laboratories have passed this PT round except Lab 768.

Date: January 30, 2025

Giuseppe Ru, TSE EURL Director

The informations contained in this Report must be considered confidential.

Firmato digitalmente da:

Ru Giuseppe

Firmato il 03/02/2025 17:35
Seriale Certificato: 3441766
Valido dal 02/04/2024 al 02/04/2027
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PTGG24 second round

- ❖ A second set of samples was submitted to repeat the PT, included the genotype wrongly identified
- ❖ Laboratory 768 did not submit any results and failed PTGG24
- ❖ All the other laboratories have reported all samples as intended results



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Technical aspect of Sheep and Goat PRNP Genotyping methods

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EQA rounds

EQA for PRNP Sheep Genotyping and EQA for PRNP Goat Genotyping

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ACKNOWLEDGMENTS

- Colleagues who contributed to organise the PT rounds

ISS

Clarissa Ferreri
Michele Di Bari
Jessica Del Bravo
Michela Conte
Geraldina Riccardi
Gabriele Vaccari

IZPLVdA

Giuseppe Ru
Francesco Ingravalle
Cristiana Corci

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



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