

# 2023 round of TSE EURL EQAs: Results feed-back

Rome, Italy

PRNP sheep and goat genotyping EQA  
**PTGS23** and **PTGG23**

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TSE EURL

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Istituto Superiore di Sanità - Rome

# 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 alphanumeric code (for example **GS2301**, **GS2302** and **GG2351**, **GG2352**) :  
  
**GS**= Genotyping Sheep    **GG**= Genotyping Goat  
23 = for the year 2023  
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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**PT list – Select PT\***

PRNP Sheep Genotyping



Date of expiration: 07/12/2023

**PT Code\***

GS23

**NRL ID Code\***

Insert your ID Code

**Test method used\***

Please indicate the title, identification code and details of the method used.

**Date of receipt\***

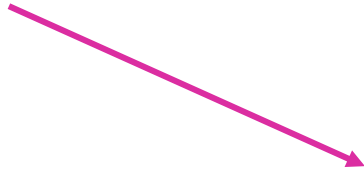
DD-MM-YYYY 

**Date of testing\***

DD-MM-YYYY 

**Note (optional)**

Your specific NRL ID code



Title and details of your method



Molecular technique used (RT-PCR, Sanger sequencing etc.)



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- ❖ The samples were shipped on 7<sup>th</sup> of November 2023
- ❖ The deadline for submission of results was 7<sup>th</sup> of December 2023
- ❖ The results had to be uploaded on the dedicated platform of the official website of EURL
- ❖ **PTGS23:** All participants were requested to report the genotypes at codons 136, 141, 154, 171 of the PrP in allelic format (e.g., ALRQ/AFRQ)
- ❖ **PTGG23:** 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 - 2023

## PTGS23

24 Participants

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

## PTGG23

15 Participants

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



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# PTGS23 and PTGG23

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



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# Genotypes included in the **PTGS23**

Genotypes of samples used for the PT were selected to include the major PRNP allele combination with an over-representation of those genotypes most frequently observed

GENOTYPES
ALRR/ALRR
ALRR/ALRQ
ALRQ/ALRQ
ALRQ/AFRQ
ALRQ/ALRH
ALRQ/VLRQ
ALRQ/ALHQ
ALHQ/ALHQ



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# Gold Standard - **PTGS23**

Sample ID	Expected result
GS2301	ALRQ/ALHQ
GS2302	ALRQ/AFRQ
GS2303	ALRQ/VLRQ
GS2304	ALRQ/VLRQ
GS2305	ALHQ/ALHQ
GS2306	ALRR/ALRR
GS2307	ALRR/ALRQ
GS2308	ALRQ/ALRH
GS2309	ALRQ/ALRQ
GS2310	ALRR/ALRQ



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# Gold Standard – PTGS23



## PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GS23: PRNP sheep genotyping

Distribution date: 07/11/2023

Sample ID	GS2301	GS2302	GS2303	GS2304	GS2305	GS2306	GS2307	GS2308	GS2209	GS2310
Gold Standard	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
Laboratory ID	Genotype reported									
11	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
93	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
100	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
137	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
197	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
229	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
305	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
359	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
422	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
548	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
566	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
591	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
604	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
619	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
691	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
731	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
780	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
791	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
853	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
857	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
858	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
907	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ
948	ALRQ/ALHQ	ALRQ/AFRQ	ALRQ/VLRQ	ALRQ/VLRQ	ALHQ/ALHQ	ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRR/ALRQ

### Comments :

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

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

### Conclusion:

All the laboratories have passed this PT round successfully.

Date: 12 December 2023

Giuseppe Ru, TSE EURL Director

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The information contained in this Report must be considered confidential.

All the laboratories have reported the samples as the intended results



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# Gold Standard - PTGG23

Sample ID	Expected result for codon 146	Expected result for codon 222
GG2351	N/N	Q/Q
GG2352	N/D	Q/Q
GG2353	N/N	K/K
GG2354	N/N	Q/K
GG2355	S/S	Q/Q



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# Gold Standard – PTGG23



## PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GG23: PRNP goat genotyping

Distribution date: 07/11/2023

Sample ID	GG2351		GG2352		GG2353		GG2354		GG2355	
Gold Standard	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
Laboratory ID	Genotype reported									
6	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
26	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
50	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
113	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
354	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
392	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
491	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
754	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
756										
757	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
768	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	K/K	S/S	Q/Q
774	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
835	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
891	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q
930	N/N	Q/Q	N/D	Q/Q	N/N	K/K	N/N	Q/K	S/S	Q/Q

### Comments :

Lab 756 didn't submit any result. For this reason the laboratory failed this PT round.  
 Lab 768 - Sample GG2354: The laboratory reported the codon 222 genotype K/K instead of Q/K. For this reason the laboratory failed this PT round.  
 All the other laboratories have reported all samples as the intended results.  
 Lab 113 also reported the following additional notes: GS2351-211 R/Q, GS2352-240 P/P; GS2354-240 S/P; GS2355-240 P/P. Also these data are correct.

### Conclusion:

All the laboratories have passed this PT round except Laboratories 756 and 768.

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

Date: 12 December 2023

Giuseppe Ru, TSE EURL Director

**Lab 756:** The laboratory didn't submit any result and failed this PT round.

**Lab 768:** The laboratory reported the genotype K/K instead of Q/K at codon 222 . For this reason the laboratory failed this PT round.

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

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# PTGG23 - Laboratory 768

Reported Genotype 222	Expected Genotype
K/K	Q/K

- ❖ The laboratory immediately collaborated with us to identify the causes of this mistake
- ❖ The mistake was a consequence of the misinterpretation of the data by the operator even if the raw data associated with the analytical method was correct
- ❖ A second set of samples was submitted to repeat the PT, included the genotype wrongly identified
- ❖ Resolution of the problem: laboratory provided correct results on the new set of unknown samples



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# Gold Standard – PTGG23 (Second round)



## PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GG23R: PRNP goat genotyping - Second shipment

Distribution date: 15/01/2024

Sample ID	GG23R1		GG23R2		GG23R3		GG23R4		GG23R5	
Gold Standard	N/N	K/K	N/N	Q/Q	N/N	Q/K	S/S	Q/Q	N/D	Q/Q
Laboratory ID	Genotype reported									
768	N/N	K/K	N/N	Q/Q	N/N	Q/K	S/S	Q/Q	N/D	Q/Q

**Comments :**

The laboratory 768 reported all samples as the intended results.

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

**Conclusion:**

Laboratory 768 passed this PT round.

Date: 1st February 2024

Giuseppe Ru, TSE EURL Director



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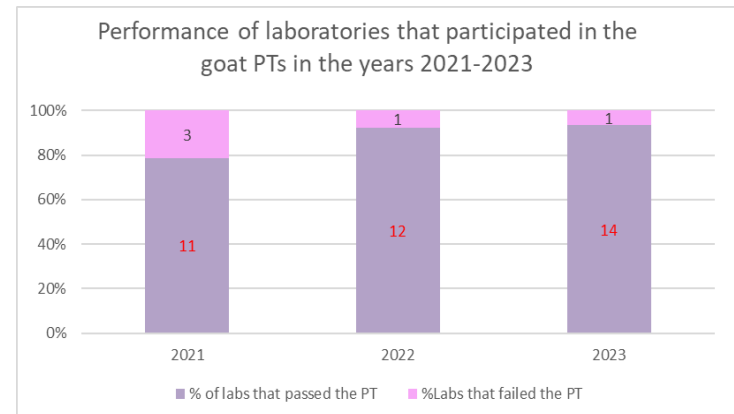
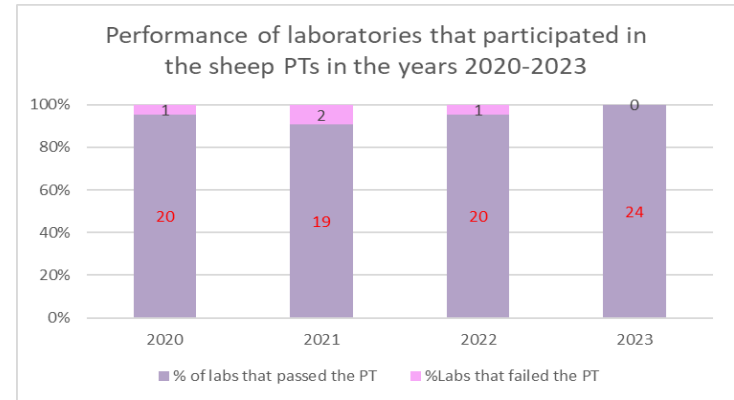
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# PERFORMANCE OF THE LABORATORIES

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

❖ There was an improvement

❖ The participation in the PTs has helped to highlight critical issues in order to improve laboratory performance



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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

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Michele Di Bari  
Jessica Del Bravo  
Michela Conte  
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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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