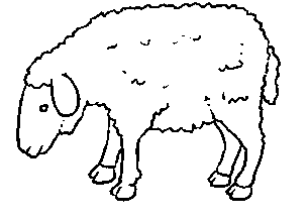
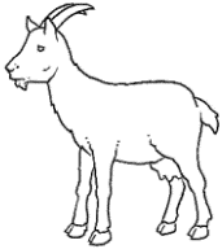


2022 round of TSE EURL EQAs: Results feed-back

20th TSE EURL/NRL Annual Meeting - Rome - Italy 2 – 3 October 2023



PRNP sheep and goat genotyping EQA GS22 and GG22

Gabriele Vaccari
ISS - Rome



EURL-TSE

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

Istituto Superiore di Sanità - Rome

PT ORGANIZATION

- Blood samples collection and preparation of aliquots (all year long, thanks to Cyprus and those Laboratory that will contribute to provide blood sample to EURL-TSE)
- Storage of samples at -20°C
- Quality and genotype of the sample (verified with an accredited method - EN ISO/IEC 17025)
- Stability assessment

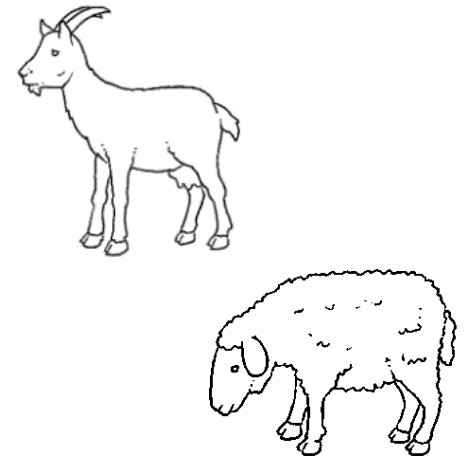


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Istituto Superiore di Sanità - Rome

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 GS2201, GS2202, etc. and GG2251, GG2252. etc) :
- GS= Genotyping Sheep GG= Genotyping Goat
- 22= for the year 2022
- number from 01 to 10 for sheep samples or from 51 to 55 for goat samples
- Each laboratory received its own individual “Laboratory identification number”
- A different code has been assigned to the laboratory for each different EQA

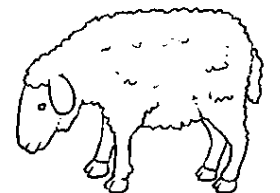
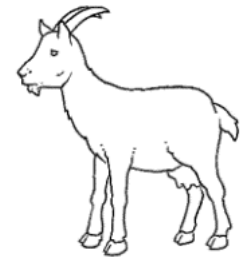


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PT ORGANIZATION

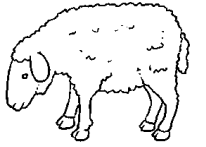
- The samples were shipped on 24th October 2022
- The deadline for submission of results was 5th December 2022
- The results had to be uploaded using an Excel file present for the insertion of the genotypes
- PTGS21: All participants were requested to report the genotypes at codons 136, 141, 154, 171 of the PrP in allelic format (e.g., ALRQ/AFRQ)
- PTGG21: 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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PT GS22 ORGANIZATION



- PTGS22 Submission file (excel format):
- Molecular technique used (RT-PCR, Sanger sequencing etc.).
- Laboratory ID
- A different code has been assigned to the laboratory for each different EQA
- Results and comments for each sample (drop-down menu to select the genotype)

	TSE EURL Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino Istituto Superiore di Sanità – Roma	
SCHEME:	PROFICIENCY TESTING FOR VETERINARY LABORATORIES	
ID	PRNP sheep genotyping	
DISTRIBUTION DATE:	GS22	
	24/10/2022	
Method used		
Please, fill ONLY the grey cells in tables below		
Laboratory ID	Date of receipt	Date of testing
Sample ID	Genotype	Comments
GS2201		
GS2202	ALRR/ALRR	
GS2203	ALRR/ALRQ	
GS2204	ALRR/ALRH	
GS2205	ALRR/ALHQ	
GS2206	ALRR/AFRQ	
GS2207	ALRR/VLRQ	
GS2208	ALRQ/ALRQ	
GS2209	ALRQ/ALRH	
GS2210		



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Test Method Used on the PTGS21



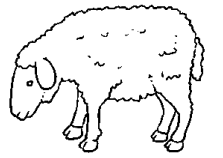
- SANGER SEQUENCING
- REAL TIME PCR MGB
- REAL TIME PCR and MELTING CURVE ANALYSIS
- MELTING-CURVE ANALYSIS, LightCycler 480
- PRIMER EXTENSION FOR POLYMORPHISMS AT 146 AND 222 CODONS
- TAQMAN AND SANGER SEQUENCING
- PYROSEQUENCING



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Genotypes included in the PTGS22



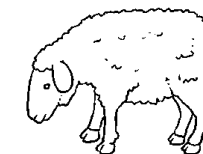
GENOTYPES
ALRR/ALRH
ALHQ/ALHQ
ALRR/ALRQ
ALRQ/AFRQ
ALRR/ALHQ
VLRQ/VLRQ
ALRR/ALRR
ALRQ/ALRQ
ALRR/VLRQ
ALRR/ALRR



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Tabulation of the PTGS22



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GS22: PRNP sheep genotyping

Distribution date: 24/11/2022

Sample ID	GS2201	GS2202	GS2203	GS2204	GS2205	GS2206	GS2207	GS2208	GS2209	GS2210
Gold Standard	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
Laboratory ID	Genotype reported									
011	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
023	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
035	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRQ/ALRQ	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
057	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
093	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
100	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
137	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
197	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
229	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
305	ALRQ/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/ALRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRQ/VLRQ	ALRR/ALRR
359	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
422	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
566	ALRH /ALRR	ALHQ/ALHQ	ALRQ /ALRR	AFRQ/ALRQ	ALHQ /ALRR	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	VLRQ /ALRR	ALRR/ALRR
591	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
604	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
619	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
742	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
780	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
791	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
858	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR
907	ALRR/ALRH	ALHQ/ALHQ	ALRR/ALRQ	ALRQ/AFRQ	ALRR/ALHQ	VLRQ/VLRQ	ALRR/ALRR	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR

Lab 035: The laboratory reported the wrong genotype for the sample GS2207

Lab 305: The laboratory reported the wrong genotype for Sample GS2201, GS2204 and GS2209.

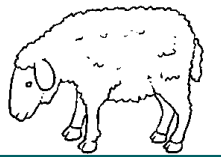
All the other laboratories have reported all samples as the intended results



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Contact with the Laboratories PTGS22



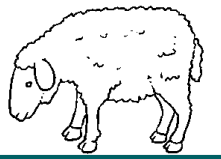
- Request to the laboratory to identify what was the cause of this deviation
- EURL offered technical assistance and support to resolve any problems
- EURL asked for a report of this analysis to evaluate the correct identification of the critical points.
- Sending an additional set of samples to verify if the corrective actions have been effective to identify a solution the non-compliance.



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Response from the Laboratory



Lab 305: The laboratory reported the wrong genotype for samples GS2201, GS2204 and GS2209.

ALRQ/ALRH instead of ALRR/ALRH
ALRQ/ALRQ instead of ALRQ/AFRQ
ALRQ/VLRQ instead of ALRR/VLRQ

... “Bloods which arrived had different consistency as usual, black colour and were completely thawed.

They proceed nucleic acid extraction with Biorobot based magnetic beads, and eluates had not clear colour.

Sequencing curves were low signal and heterozygote positions were unclear due to low signal.”

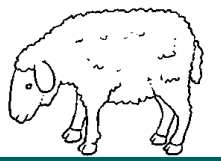
... “Could you be so kind to send me amplification and sequencing primer’s sequences which are used in other lab.”



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Response from the Laboratory



Lab 035: The laboratory reported the wrong genotype for sample GS2207
ALRQ/ALRQ instead of ALRR/ALRR

... “The root cause was a technical error during the extraction of DNA from the original samples, which lead to low-grade DNA yield in the samples. Further, during sequencing there was cross-sample contamination, resulting in the incorrect results in our first submission. We noticed this contamination and re-run our sequencing with better results, unfortunately however one sample had very low sequence quality, which resulted in the incorrect genotype observed in our final results. We have identified the points where the contamination and extraction failures occurred and consider the process corrected.”

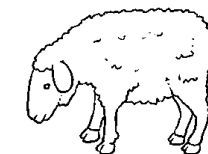
... “Another factor we considered is that we have only a very limited amount of technicians who are familiar this technique, since genotyping sheep and goats is rarely done in our lab. Because of this, sickness and other personnel absences can cause major delays in processing the samples. This was an issue during the 2022 PT and subsequently our results were late. We are currently training another technician for this technique, and will hopefully have less delays in the future.”



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Tabulation of the PTGS22R



Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino
Istituto Superiore di Sanità – Roma

PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GS22R: PRNP sheep genotyping - Second shipment

Distribution date: 17/07/2023

Sample ID	GS22R01	GS22R02	GS22R03	GS22R04	GS22R05	GS22R06	GS22R07	GS22R08	GS22R09	GS22R10
Gold Standard	ALRQ/AFRQ	ALRR/ALRH	VLRQ/VLRQ	ALHQ/ALHQ	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALHQ	ALRR/ALRR
Laboratory ID	Genotype reported									
035	ALRQ/AFRQ	ALRR/ALRH	VLRQ/VLRQ	ALHQ/ALHQ	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRR	ALRR/ALRR	ALRR/ALHQ	ALRR/ALRR
305	ALRQ/AFRQ	ALRR/ALRH	VLRQ/VLRQ	ALHQ/ALHQ	ALRQ/ALRQ	ALRR/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALHQ	ALRR/ALRR

Comments :

Lab 035: The laboratory reported the wrong genotype for the sample GS22R07 (ALRR/ALRR instead of ALRR/ALRQ). For this reason the laboratory failed this PT round.

Lab 305: The laboratory reported all samples as the intended results.

Lab 035: The laboratory reported the wrong genotype for the sample GS22R07
ALRR/ALRR instead of ALRR/ALRQ

Lab 305: passed the PT

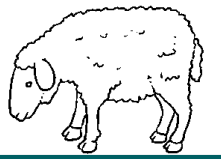
Due to the short time the laboratory



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Contact with the Laboratories PTGS22



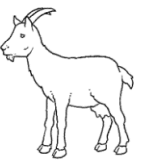
- Request to the laboratory to identify what was the cause of this deviation
- EURL offered technical assistance and support to resolve any problems
- EURL asked for a report of this analysis to evaluate the correct identification of the critical points.
- As the date of the PTGS23 genotyping is approaching, the Laboratory will verify if the corrective actions have been effective to identify a solution the non-compliance with the PTGS23.



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Test Method Used on the PTGG21



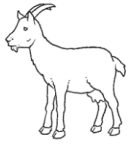
- SANGER SEQUENCING
- REAL TIME PCR MGB
- REAL TIME PCR and MELTING CURVE ANALYSIS
- PRIMER EXTENSION FOR POLYMORPHISMS AT 146 AND 222 CODONS
- TAQMAN AND SANGER SEQUENCING
- PYROSEQUENCING




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PTGG22 ORGANIZATION



- PTGG21 Submission file (excel format):
- Molecular technique used (RT-PCR, Sanger sequencing etc.).
- Laboratory ID
- A different code has been assigned to the laboratory for each different EQA
- Results and comments for each sample (drop-down menu to select the genotype)

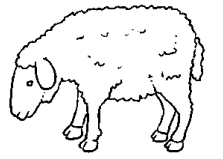
A	B	C	D
		TSE EURL Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino Istituto Superiore di Sanità – Roma	
		PROFICIENCY TESTING FOR VETERINARY LABORATORIES	
	SCHEME:	PRNP goat genotyping	
	ID	GG22	
	DISTRIBUTION DATE:	24/10/2022	
Method used			
Please, fill ONLY the grey cells in tables below			
Laboratory ID	Date of receipt		Date of testing
Sample ID	Codon 146 Genotype	Codon 222 Genotype	Comments
GG2251			
GG2252	N/N	of the suggested	
GG2253	N/S		
GG2254	S/S		
GG2255	N/D		
	D/D		
	S/D		



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Genotypes included in the PTGG22



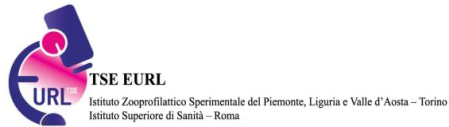
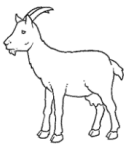
GENOTYPES	
146	222
N/N	K/K
N/N	Q/K
N/N	Q/Q
N/N	Q/K
N/N	Q/Q



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Tabulation of the PTGG22



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GG22: PRNP goat genotyping

Distribution date: 24/11/2022

Sample ID	GG2251		GG2252		GG2253		GG2254		GG2255	
Gold Standard	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
Laboratory ID	Genotype reported									
026	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
104	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
113	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
354	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
392	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
702	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
754	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
757	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
774	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
835	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
891	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	S/S	Q/Q
911	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q
930	N/N	K/K	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q

Lab 891 - Sample GG2255: The laboratory reported the codon 146 genotype S/S instead of N/N. For this reasons the laboratory failed this PT round.

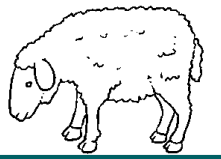
All the other laboratories have reported all samples as the intended results



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Contact with the Laboratories PTGG22



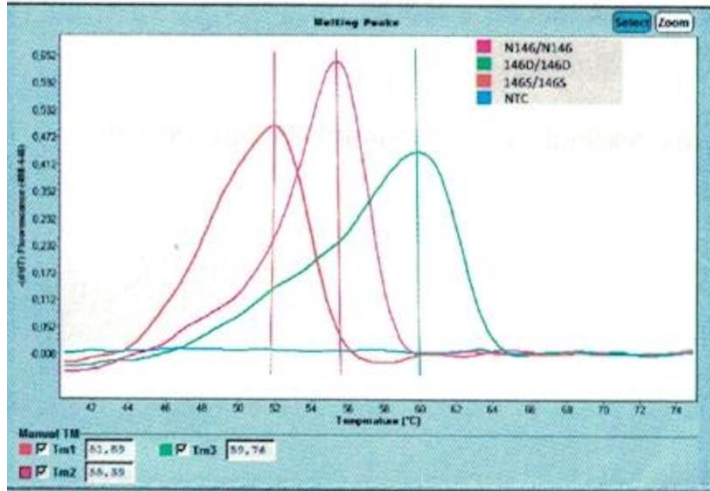
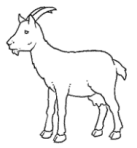
- Request to the laboratory to identify what was the cause of this deviation
- EURL offered technical assistance and support to resolve any problems
- EURL asked for a report of this analysis to evaluate the correct identification of the critical points.
- Sending an additional set of samples to verify if the corrective actions have been effective to identify a solution the non-compliance.



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Response from the Laboratory



LC Red 640

Sample data for PRNP 146

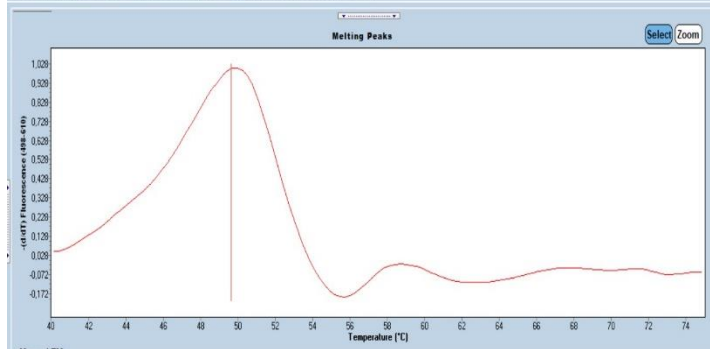
Position	Amino Acid	wt/mut	Melting temperature
			96 FS
146	N	Wildtype	55.4°C
146	D	Mutation	59.8°C
146	S	Mutation	51,9°C

Sample GG2255 is a

146 N/N 222 Q/Q

And it was reported as

S/S 146 Q/Q 222



Sample GG2255 is a 146 N/N 222 Q/Q genotype it holds additional mutation at two additional codons 142 and 138.

So we believe that the Melting temperature of the amplicon is influenced by additional polymorphic site.

Specific controls should be included in each amplification session

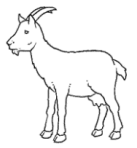


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Tabulation of the PTGS22R



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GG22R: PRNP goat genotyping - Second shipment

Distribution date: 24/07/2023

Sample ID	GG22R51		GG22R52		GG22R53		GG22R54		GG22R55	
Gold Standard	N/N	Q/K	N/N	Q/Q	N/N	K/K	N/N	Q/Q	N/N	Q/K
Laboratory ID	Genotype reported									
891	N/N	Q/K	N/N	Q/Q	N/N	Q/K	N/N	Q/Q	N/N	Q/K

Comments :

Lab 891 - Sample GG22R53: The laboratory reported the codon 222 genotype Q/K instead of K/K. For this reasons the laboratory failed this PT round.

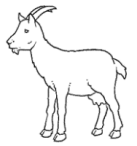
Sample GG22R53 is
N/N 146 K/K 222
however it was reported as
N/N 146 Q/K 222



EURL-TSE

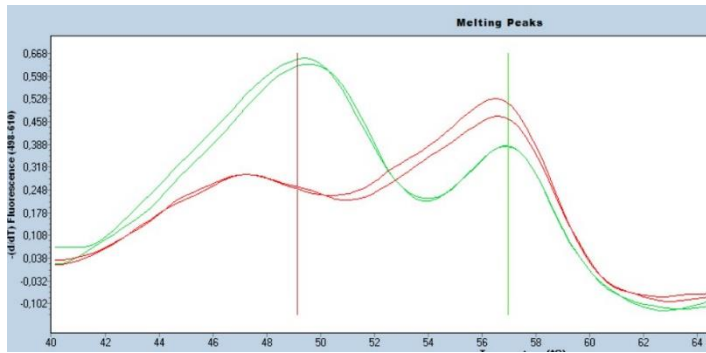
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Istituto Superiore di Sanità - Rome

Response from the Laboratory



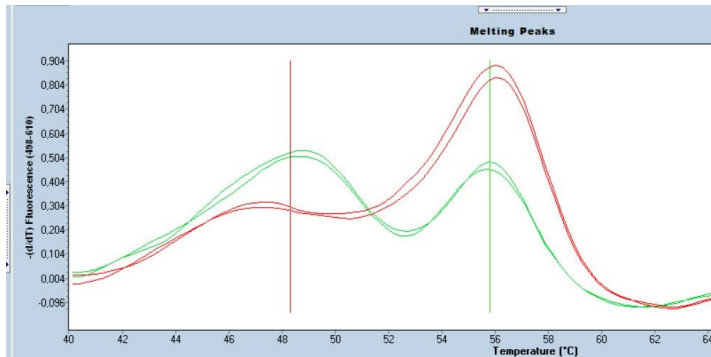
Sample GG22R53 is
N/N 146 K/K 222
however it was reported as
N/N 146 Q/K 222

DNA Extraction
from
Blood spotted
on cards

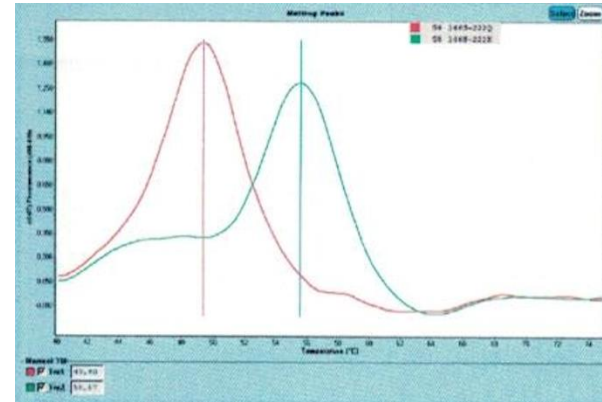


Q/K 222
K/K 222
(GG22R53)

DNA Extraction
directly from
Blood



Q/K 222
K/K 222
(GG22R53)



Sample data for PRNP 222

LC Red 610

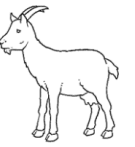
AA Position	Amino Acid	wt/mut	Melting temperature
			96 FS
222	Q	Wildtype	49.5°C
222	K	Mutation	55.5°C



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Contact with the Laboratories PTGG22



- Request to the laboratory to identify what was the cause of this deviation
- EURL offered technical assistance and support to resolve any problems
- EURL asked for a report of this analysis to evaluate the correct identification of the critical points.
- As the date of the PTGG23 genotyping is approaching, the Laboratory will verify if the corrective actions have been effective to identify a solution the non-compliance with the PTGG23.



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Technical aspect of Sheep and Goat PRNP Genotyping methods

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ISS

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IZPLVdA

Giuseppe Ru

Francesco Ingravalle

Cristiana Corci

- All participants to PTs
- Labs with non-compliant results for being responsive, collaborative and open to confrontation