

# 2021 round of TSE EURL EQAs: Results feed-back

Torino/Rome, Italy

PRNP sheep and goat genotyping EQA  
**PTGS21** and **PTGG21**

Barbara Chiappini



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# PT ORGANIZATION

- ❖ Blood samples collection and preparation of aliquots
- ❖ 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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# 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 **GS2101**, **GS2102** and **GG2151**, **GG2152**) :  
  
**GS**= Genotyping Sheep    **GG**= Genotyping Goat  
21= for the year 2021  
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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- ❖ The samples were shipped on 29<sup>th</sup> November 2021
- ❖ The deadline for submission of results was 28<sup>th</sup> January 2022
- ❖ The results had to be uploaded using an Excel file preset 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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**PTGS21 Submission file (excel format):**

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

• **Laboratory ID**

Note that 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 correct genotype)



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2	Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino	
3	Istituto Superiore di Sanità – Roma	
4	PROFICIENCY TESTING FOR VETERINARY LABORATORIES	
5	SCHEME:	PRNP sheep genotyping
6	ID	GS21
7	DISTRIBUTION DATE:	xx/11/2021
8		
9	Method used	
10		
11	Please, fill ONLY the grey cells in tables below	
12	Laboratory ID	Date of receipt
13		Date of testing
14		
15	Sample ID	Genotype
16	GS2101	
17	GS2102	ALRR/ALRR
18	GS2103	ALRR/ALRQ
19	GS2104	ALRR/ALRH
20	GS2105	ALRR/ALHQ
21	GS2106	ALRR/ALRQ
22	GS2107	ALRR/ALRH
23	GS2108	ALRR/VLRQ
24	GS2109	ALRQ/ALRQ
25	GS2110	ALRQ/ALRH
26		


**PTGG21 Submission file (excel format):**

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

• **Laboratory ID**

Note that 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 correct genotype)

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		PROFICIENCY TESTING FOR VETERINARY LABORATORIES	
SCHEME:		PRNP goat genotyping	
ID		GG21	
DISTRIBUTION DATE:		xx/11/2021	
Method used			
Please, fill ONLY the grey cells in tables below			
Laboratory ID	Date of receipt	Date of testing	
Sample ID	Codon 146 Genotype	Genotype	Comments
GG2151	N/N		
GG2152	N/N		
GG2153	N/S		
GG2154	S/S		
GG2155	N/D		
	D/D		
	S/D		



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# EQA - 2021

## PTGS21

21 Participants

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

## PTGG21

14 Participants

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



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## Test Method Used on the **PTGS21** and **PTGG21**

**SANGER SEQUENCING**

**REAL TIME PCR**

**REAL TIME PCR and MELTING CURVE ANALYSIS**

**REAL TIME PCR and PCR+RFLP+DGGE**

**PRIMER EXTENTION 136, 154, 171 and 141**

**DGGE and RFLP ANALYSIS**



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## Genotypes included in the **PTGS21**

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



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# GS - PTGS21

Sample ID	Expected result
GS2101	ALRR/ALRR
GS2102	ALRR/ALRQ
GS2103	VLRQ/VLRQ
GS2104	ALRR/AFRQ
GS2105	ALRR/ALRR
GS2106	ALRH/ALRH
GS2107	ALRQ/VLRQ
GS2108	ALRR/ALHQ
GS2109	ALRR/ALRH
GS2110	ALRR/ALRQ



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Results tabulation for PT GS21: PRNP sheep genotyping

Distribution date: 29/11/2021

Sample ID	GS2101	GS2102	GS2103	GS2104	GS2105	GS2106	GS2107	GS2108	GS2109	GS2110
Gold Standard	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
Laboratory ID	Genotype reported									
005	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
011	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
093	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
057	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
100	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
093	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
100	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
137	ALRR/ALRR	ALRR/ALRQ	ALRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
197	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
229	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
305	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
359	ALRR/ALRR	ALRQ/ALRR	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRQ/ALRR
381	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
417	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
604	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
619	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
722	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
769	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRQ/ALHQ	ALRR/ALRQ	ALRR/ALRQ
853	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
780	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
858	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
907	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
948	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ
981	ALRR/ALRR	ALRR/ALRQ	VLRQ/VLRQ	ALRR/AFRQ	ALRR/ALRR	ALRH/ALRH	ALRQ/VLRQ	ALRR/ALHQ	ALRR/ALRH	ALRR/ALRQ

Comments :

provided by Gabriele Vaccari, Istituto Superiore di Sanità - Rome

Lab. 137 - Sample GS2103: The laboratory reported the genotype ALRQ/VLRQ instead of VLRQ/VLRQ. For this reason the laboratory failed this PT

Lab. 769 - Sample GS2104: The laboratory reported the genotype ALRQ/ALRQ instead of ALRQ/AFRQ. Sample GS2108: The laboratory reported the genotype ALRQ/ALHQ

instead of ALRR/ALHQ. Sample GS2109: The laboratory reported the genotype ALRR/ALRQ instead of ALRR/ALRH. For these reasons the laboratory failed this PT round.

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

Conclusion:

Laboratories 137 and 769 have failed this PT round.  
All the other laboratories have passed this PT round.

02 March 2022

Giuseppe Ru, director of TSE EURL

## PTGS21- Laboratory 137

136	141	154	171	Expected Genotype	Reported Genotype
A/V	L/L	R/R	Q/Q	VLRQ/VLRQ	<u>A</u> LRQ/VLRQ

- ❖ The laboratory made a careful analysis of the causes and concluded that there was a problem in the equipment used for the analyzes. **Comparison with the manufacturer is ongoing.**
- ❖ From the analysis of the raw data that the laboratory has sent us, it is possible that the cause is a contamination in one of the phases of the process. We suggested to include a negative extraction control (water instead of blood) and a negative amplification control (water instead of DNA) in each analytical session.
- ❖ **Laboratory still resetting the SOP**



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## PTGS21 - Laboratory 769

136	141	154	171	Expected Genotype	Reported Genotype
A/A	L/L	R/R	Q/Q	ALRR/AFRQ	ALRR/AL <u>R</u> Q
A/A	L/L	R/H	Q/Q	ALRR/ALHQ	ALR <u>Q</u> /ALHQ
A/A	L/L	R/R	R/Q	ALRR/ALRH	ALRR/ALR <u>Q</u>

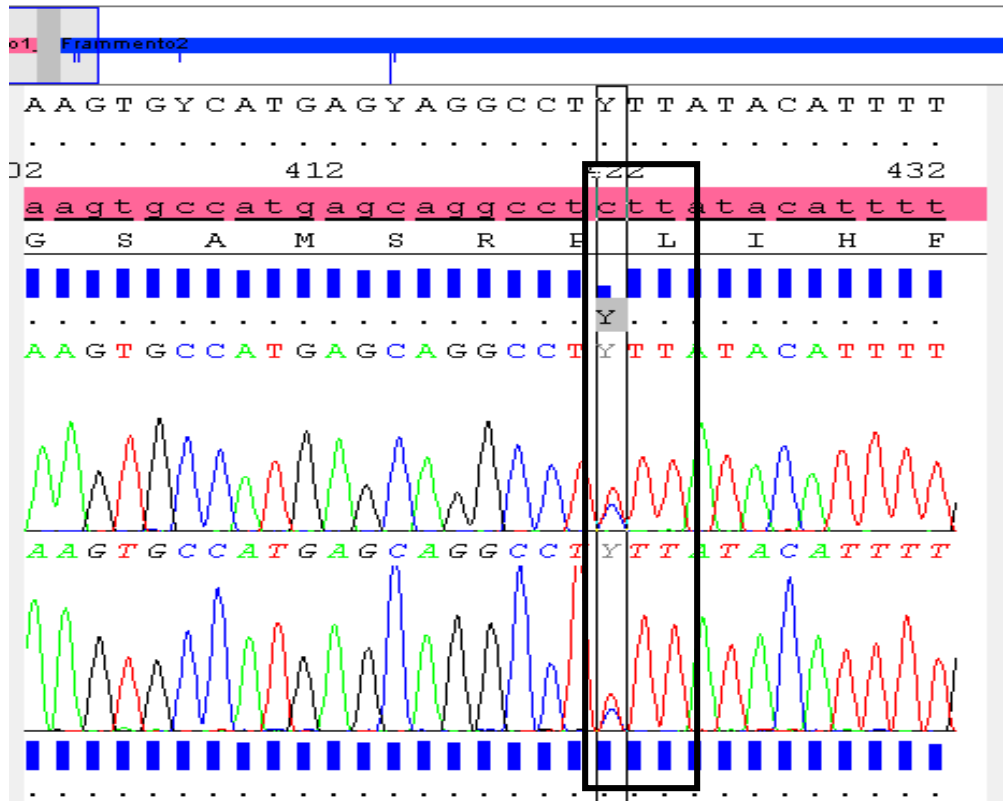
- ❖ The Laboratory has immediately identified the critical point and the causes of the mistake
- ❖ The error was a consequence of the incorrect interpretation of the data by the operator but the raw data associated with the analytical method was correct.



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# PTGS21 – 141 L/F



CTT → L  
(Leucine)

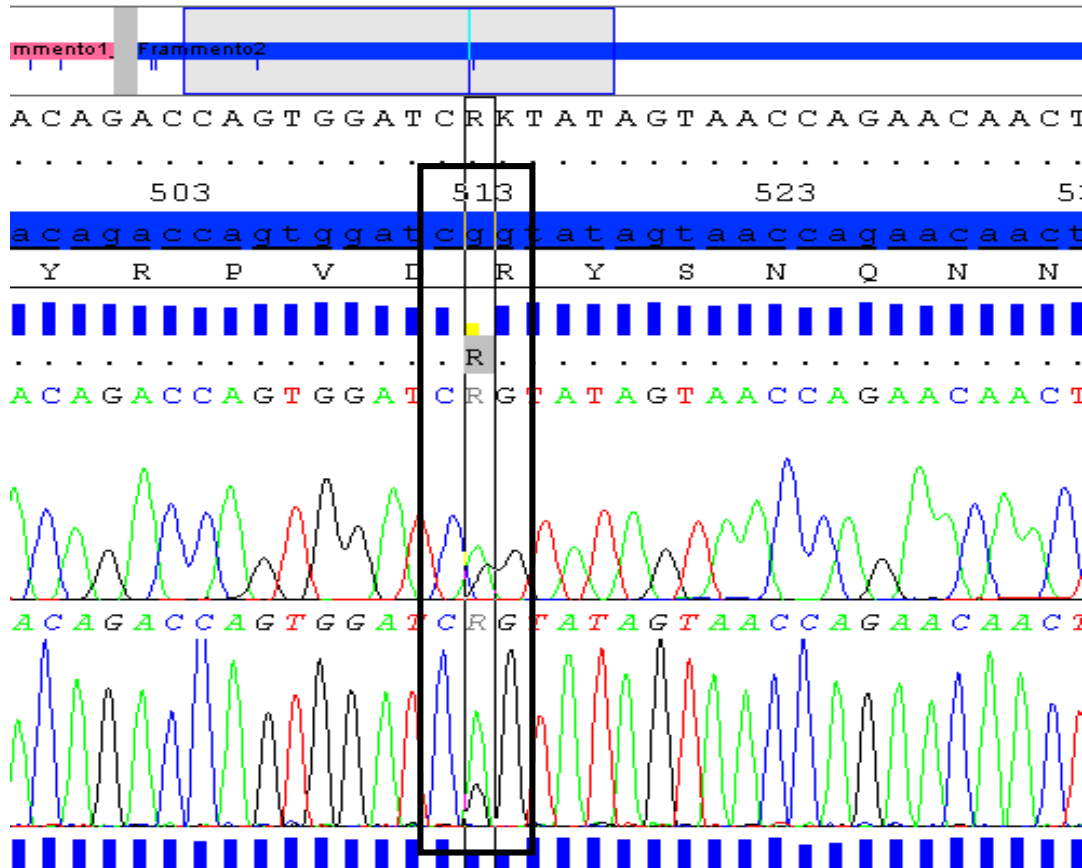
TTT → F  
(Phenylalanine)



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# PTGS21 – 171 R/Q



**CGG → R  
(Arginine)**

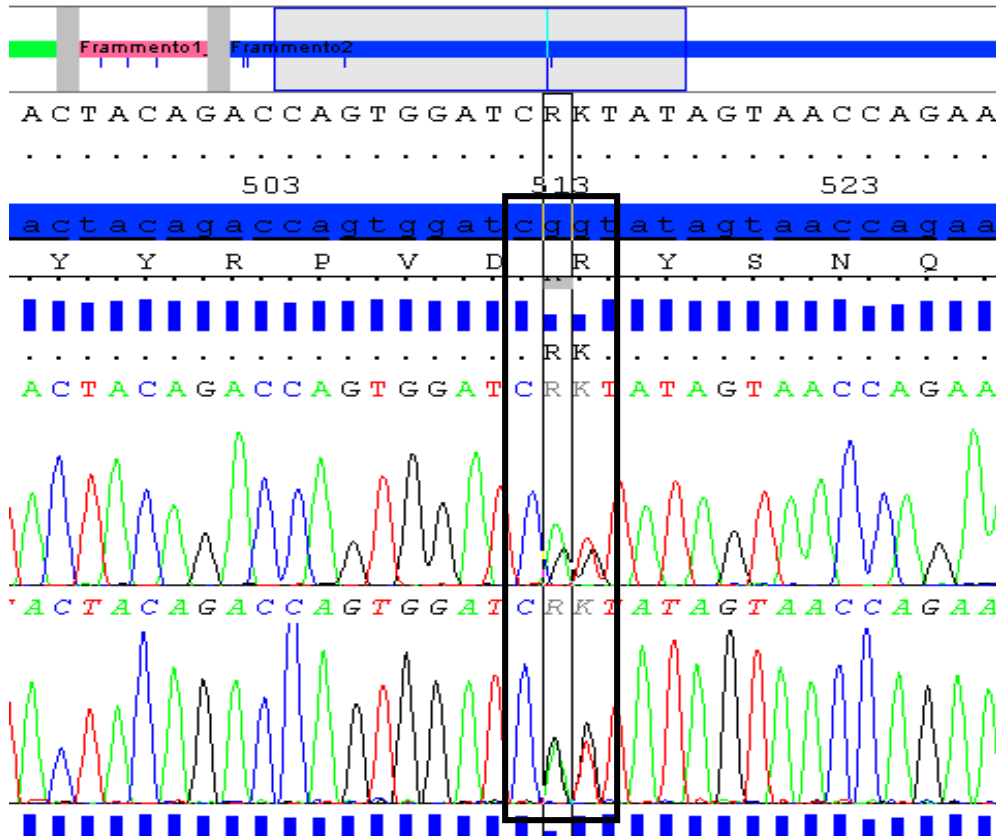
**CAG → Q  
(Glutamine)**



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# PTGS21 – 171 R/H



CGG → R (Arginine)	OK
CAT → H (Histidine)	OK
CAG → Q (Glutamine)	OK
CGT → R (Arginine)	?



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- ❖ For the laboratory 769 the analytical performance was considered unsatisfactory and a further set of samples was submitted to repeat the PT.
- ❖ Laboratory repeated the second round PT successfully

Sample ID	Expected result
GS2111	ALRQ/VLRQ
GS2112	ALRR/ALRH
GS2113	ALRR/ALRR
GS2114	VLRQ/VLRQ
GS2115	ALRR/ALRQ
GS2116	ALRR/ALRR
GS2117	ALRR/ALRQ
GS2118	ALRR/ALHQ
GS2119	ALRH/ALRH
GS2120	ALRR/AFRQ



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## GS - PTGG21

Sample ID	Expected result for codon 146	Expected result for codon 222
GG2151	N/N	K/K
GG2152	N/N	Q/K
GG2153	N/S	Q/Q
GG2154	S/D	Q/Q
GG2155	N/D	Q/Q



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Results tabulation for PT GG21: PRNP goat genotyping

Distribution date: 29/11/2021

Sample ID	GG2151		GG2152		GG2153		GG2154		GG2155	
	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
Gold Standard	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
Laboratory ID	Genotype reported									
113	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
222	N/N	Q/Q	N/N	Q/Q	N/S	Q/Q	*	Q/Q	N/D	Q/Q
268	N/N	K/K	N/N	Q/K	N/S	Q/Q	N/S	Q/Q	N/D	Q/Q
354	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
392	N/N	K/K	N/N	Q/K	N/S	Q/Q	N/S	Q/Q	N/D	Q/Q
476	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
754	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
757	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
774	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
835	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
881	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
891	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q
930	N/N	K/K	N/N	Q/K	N/S	Q/Q	S/D	Q/Q	N/D	Q/Q

**Comments :**

provided by Gabriele Vaccari, Istituto Superiore di Sanità - Rome

- Lab 222 - Sample GG2151: The laboratory reported the genotype N/N Q/Q instead of N/N K/K.  
 Sample GG2152: The laboratory reported the genotype N/N Q/Q instead of N/N Q/K.  
 \* Sample GG2154: The laboratory reported no genotype for codon 146 noting that Codon 146 = RRT.  
 Several combinations possible, genotype could not be deduced.  
 For these reasons the laboratory failed this PT round.
  - Lab 268 - Sample GG2154 The laboratory reported the genotype N/S Q/Q instead of S/D Q/Q.  
 For this reasons the laboratory failed this PT round.
  - Lab 392 - Sample GG2154 The laboratory reported the genotype N/S Q/Q instead of S/D Q/Q.  
 For this reasons the laboratory failed this PT round.
- All the other laboratories have reported all samples as the intended results.

**Conclusion:**

Laboratories 222, 268 and 392 have failed this PT round.  
 All the other laboratories have passed this PT round.

02 March 2022  
 Giuseppe Ru, director of TSE EURL



## PTGG21 - Laboratory 222

Reported Genotype 222	Expected Genotype
Q/Q	K/K
Q/Q	Q/K

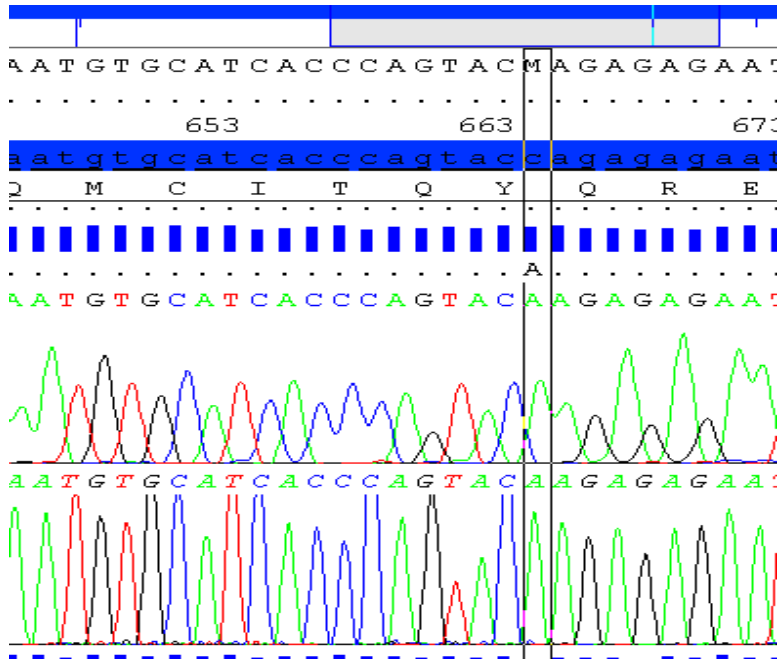
- ❖ The laboratory immediately collaborated with us to identify the causes of the error
- ❖ The error was a consequence of the difficult of interpretation of the data by the operator due to an incorrect setting of analysis parameter (dye set primer) but the raw data associated with the analytical method was correct (dye set primer KB\_3500\_POP7\_BDTv3.mob instead of KB\_3130\_POP7\_BDTv1.mob)
- ❖ **Laboratory still resetting the SOP**



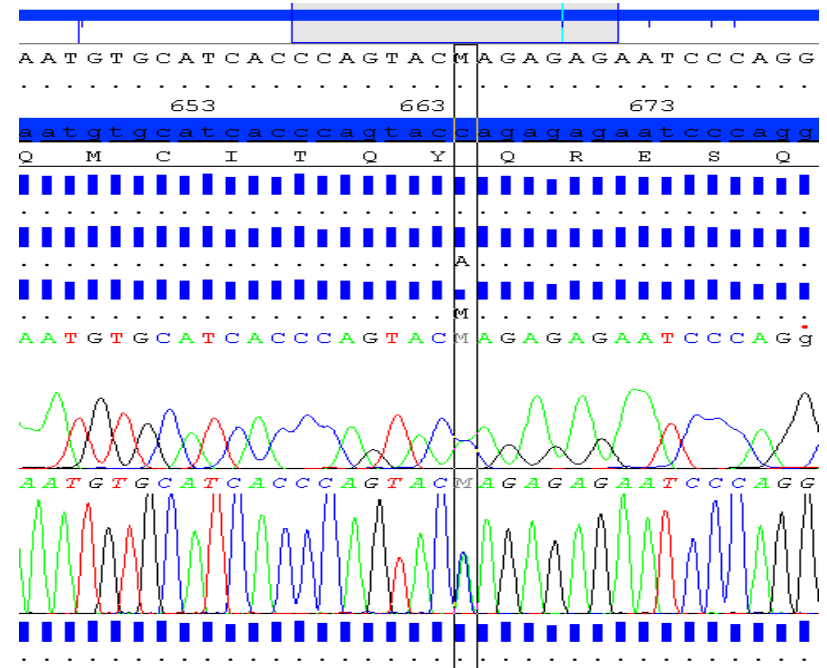
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# K/K



# Q/K



AAG → K (Lysine)      K / K

CAG → Q (Glutamine)      Q / K  
AAG → K (Lysine)



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## PTGG21 - Laboratory 222

Reported Genotype 146	Expected Genotype
???	S/D

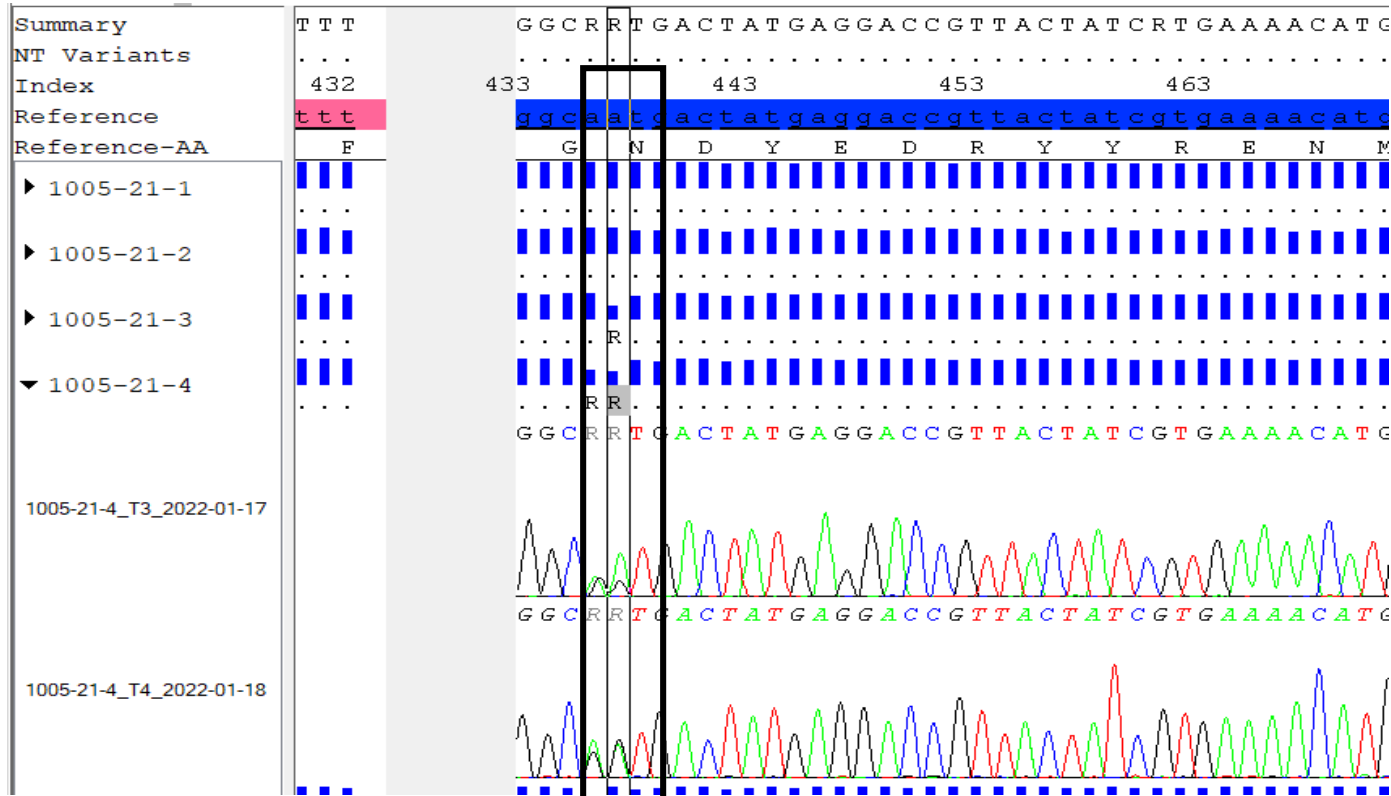
- ❖ The laboratory reported no genotype for codon 146 for one sample, noting that several combinations were possible and genotype could not be deduced
- ❖ The problem was a consequence of the difficulty of interpretation of the data by the operator but the raw data associated with the analytical method was correct
- ❖ **Laboratory still resetting the SOP**



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# 146 S/D



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# PTGG21 - Laboratory 222

- ❖ CODON 146 RRT (A/G A/G T)
- ❖ There are 2 possible phase resolution

AAT → N (Asparagine)	OK
GGT → G (Glycine)	X
AGT → S (Serine)	OK
GAT → D (Aspartic Acid)	OK



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## PTGG21 - Laboratory 268

Reported Genotype 146	Expected Genotype
N/S	S/D

- ❖ The Laboratory has immediately identified the critical point and the causes of the mistake
- ❖ The error was a consequence of the incorrect interpretation of the data by the operator but the raw data associated with the analytical method was correct.
- ❖ A second set of samples was submitted to repeat the PT.
- ❖ Laboratory provided correct results on the new set of unknown samples.



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## PTGG21 - Laboratory 392

Reported Genotype 146	Expected Genotype
N/S	S/D

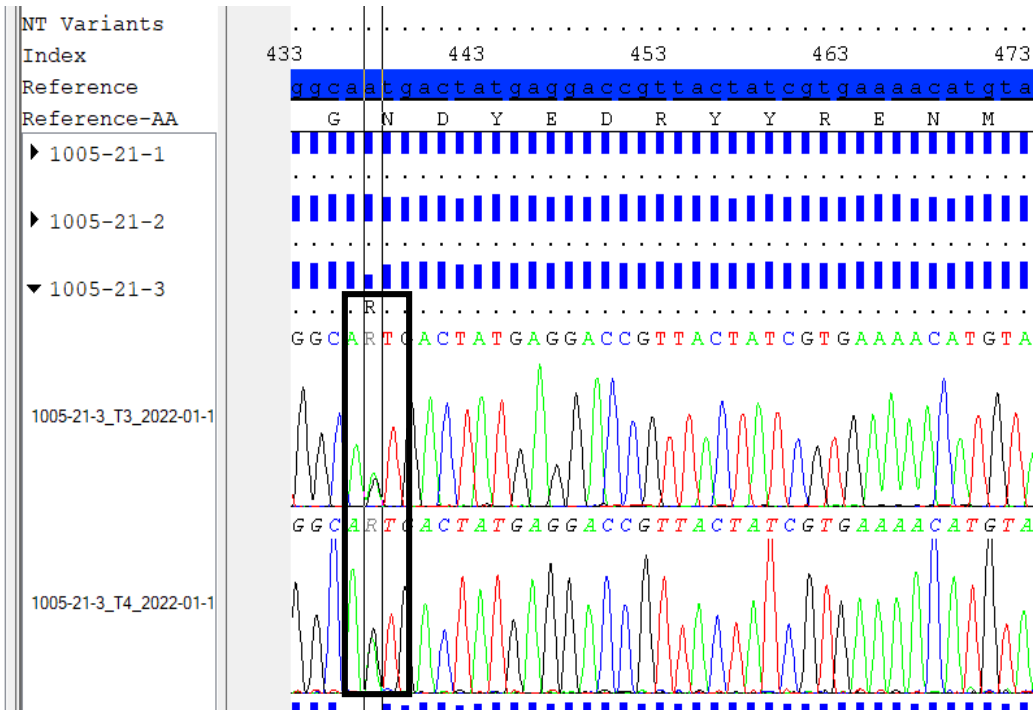
- ❖ The error was a consequence of the difficult of interpretation of the data by the operator due to an incorrect setting of analysis parameter.
- ❖ **Laboratory still resetting the SOP**



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# 146 N/S



AAT → N (Asparagine)	OK
AGT → S (Serine)	OK



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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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**Giuseppe Ru**  
**Bruno Rosalia**

**Michela Conte**  
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