

2019 round of TSE EURL EQAs: Results feed-back

Torino/Rome, Italy

PRNP sheep genotyping EQA

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 (in accordance with standard EN ISO/IEC 17025)
- ❖ Stability assessment



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PT ORGANIZATION

- ❖ Laboratories received a set of 10 sheep blood samples collected in EDTA and stored at -20 °C.
- ❖ Each sample was identified with a unique alphanumeric code:

GS= Genotyping Sheep

19= for the year 2019

number from 01 to 10 for each sample


- ❖ Each laboratory received its own individual “laboratory code”
- ❖ The samples were shipped on 21st October 2019
- ❖ The deadline for submission of results was the 16 December 2019



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- ❖ The results had to be sent by e-mail using an Excel file preset for the insertion of the genotypes
- ❖ All participants were requested to report the genotypes at codons 136, 141, 154, 171 of the PrP in allelic format (e.g., ALRQ/AFRQ).

1			TSE EURL
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	GS19	
7	DISTRIBUTION DATE:	22/10/19	
8			
9			
10	Please, fill ONLY the grey cells in tables below		
11	Laboratory ID	Date of receipt	Date of testing
12			
13			
14	Sample ID	Genotype	Comments
15	GS1901		
16	GS1902	ALRR/ALRR	
17	GS1903	ALRR/ALRQ	
18	GS1904	ALRR/ALRH	
19	GS1905	ALRR/ALHQ	
20	GS1906	ALRR/AFRQ	
21	GS1907	ALRR/VLRQ	
22	GS1908	ALRQ/ALRQ	
23	GS1909	ALRQ/ALRH	
24	GS1910	ALRQ/ALHQ	
25		ALRQ/AFRQ	
26		ALRQ/ALHQ	
27		ALRQ/AFRQ	
28		ALRQ/VLRQ	
29		ALRH/ALRH	



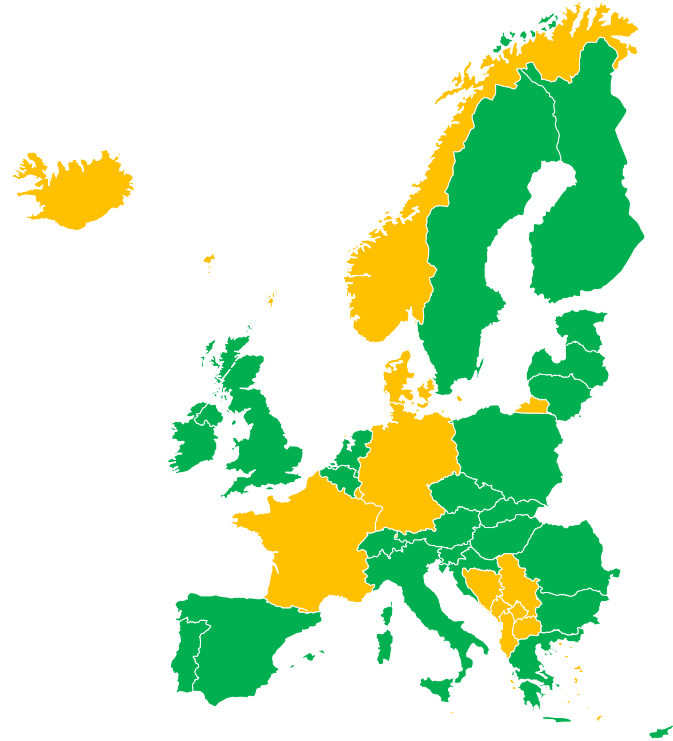
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EQA for ovine PRNP genotyping

21 Participants

2nd week of October delivery

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



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Test Method Used on the PT

Test method used	Number of laboratories
SANGER SEQUENCING	10
REAL TIME PCR	5
REAL TIME PCR and MELTING CURVE ANALYSIS	2
REAL TIME PCR and PCR+RFLP+DGGE	1
PRIMER EXTENTION 136, 154 e 171 + SEQUENCING 141	1
DGGE and RFLP ANALYSIS	1



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Genotypes included in the PTGS19

Samples shipped to the participant laboratories belonged to various PrP genotypes. They were selected to include all the *PRNP* allele combinations with an over-representation of those genotypes most frequently observed (e.g., ALRR/ALRR, ALRR/ALRQ and ALRQ/ALRQ).



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Genotypes included in the PTGS19

ALRR/ALRR
ALRR/VLRQ
ALRQ/AFRQ
ALRR/ALRQ
ALRQ/ALRH
ALRQ/ALRQ
VLRQ/VLRQ

Sample ID	Expected result
GS1901	ALRR/ALRR
GS1902	ALRR/VLRQ
GS1903	ALRR/ALRR
GS1904	ALRQ/AFRQ
GS1905	ALRR/ALRQ
GS1906	ALRQ/ALRH
GS1907	ALRQ/ALRQ
GS1908	VLRQ/VLRQ
GS1909	ALRR/ALRQ
GS1910	ALRQ/ALRQ



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PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GS19: PRNP sheep genotyping

Distribution date: 22/10/2019

Sample ID	GS1901	GS1902	GS1903	GS1904	GS1905	GS1906	GS1907	GS1908	GS1909	GS1910
Gold Standard	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
Laboratory ID	Genotype reported									
005	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
011	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
093	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
100	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
130	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
137	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
197	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
229	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
305	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/ALRQ
359	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
381	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
417	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
604	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
619	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
722	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
769	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
780	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
858	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
907	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
948	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ
981	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/AFRQ	ALRR/ALRQ	ALRQ/ALRH	ALRQ/ALRQ	VLRQ/VLRQ	ALRR/ALRQ	ALRQ/ALRQ

Comments :

provided by Gabriele Vaccari, Istituto Superiore di Sanità - Rome

Lab 305 - Sample GS1908: The laboratory reported the genotype ALRQ/ALRQ instead of VLRQ/VLRQ. For this reason the laboratory failed this PT round.

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

Conclusion:

laboratory 305 has failed this PT round, all the other laboratories have passed this PT round.

13 February 2020

Giuseppe Ru, director of TSE EURL



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ERROR : Laboratory 305

136	141	154	171	Expected Genotype	Wrong Genotype
V/V	L/L	R/R	Q/Q	VLRQ/VLRQ	ALRQ/ALRQ

- ❖ 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 and the raw data associated with the analytical method was correct.



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- ❖ The analytical performance was considered unsatisfactory and a further set of samples was submitted to repeat the PT.

Sample ID	Expected result
GS1911	ALRQ/ALRQ
GS1912	ALRR/ALRQ
GS1913	ALRR/ALRR
GS1914	ALRR/ALRR
GS1915	ALRR/VLRQ
GS1916	ALRR/ALRQ
GS1917	ALRQ/ALRQ
GS1918	VLRQ/VLRQ
GS1919	ALRQ/ALRH
GS1920	ALRQ/AFRQ



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- ❖ Evidence of the resolution of the problems came to be the fact that the laboratory provided correct results on the new set of unknown samples.



PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT GS19R: PRNP sheep genotyping

Distribution date: 03/03/2020

Sample ID	GS1911	GS1912	GS1913	GS1914	GS1915	GS1916	GS1917	GS1918	GS1919	GS1920
Gold Standard	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRQ	ALRQ/ALRQ	VLRQ/VLRQ	ALRQ/ALRH	ALRQ/AFRQ
Laboratory ID	Genotype reported									
305	ALRQ/ALRQ	ALRR/ALRQ	ALRR/ALRR	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRQ	ALRQ/ALRQ	VLRQ/VLRQ	ALRQ/ALRH	ALRQ/AFRQ

Comments :

provided by Gabriele Vaccari, Istituto Superiore di Sanità - Roma

Laboratory 305 reported all samples as the intended results

Conclusion:

Laboratory 305 has passed this PT round.

19/03/2020
Giuseppe Ru, director of TSE EURL



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WORK IN PROGRESS

- ❖ The samples for the PTGS20 will be dispatched to the participating laboratories by November
- ❖ We have noted that some of the participant laboratories use more than one method for the determination of *PRNP* genotype. For this reason, in the next PT, the laboratories that deem it appropriate or necessary will be able to participate with more methods
- ❖ The submission of the results in the next PT will be by a web repository folders



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PRNP Goat Genotyping

- ❖ Since the changes made to the Regulation (EC) 999/2001 provide genetic based measure for the management of scrapie outbreaks also for goat, we are validating a Real-Time PCR method that allows the identification of PRNP genotype at codons 222 and 146
- ❖ The methods developed will be available to all laboratories and we can provide support for the validation of the methods
- ❖ We would like to thank Dr. Penelope Papasavva-Stylianou (Head of the State Veterinary Laboratories – Cyprus) for her collaboration in providing us the goat samples with polymorphisms 146 S / D



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Technical aspect of Sheep and Goat PRNP Genotyping methods

Contact details

Barbara Chiappini

+39 0649902392

barbara.chiappini@iss.it

EQA rounds

EQA for PRNP Sheep Genotyping and EQA for PRNP Goat Genotyping

Contact details

Gabriele Vaccari

+39 06 49902139

Gabriele.vaccari@iss.it



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Istituto Superiore di Sanità - Rome



Gabriele Vaccari
Michele Di Bari
Giuseppe Ru

Michela Conte
Jessica del Bravo
Geraldina Riccardi



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