2020 round of TSE EURL EQAs: Results feed-back

Torino/Rome, Italy

PRNP sheep genotyping EQA

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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 collected in EDTA and shipped on dry ice.
- Each sample was identified with a unique alphanumerical code (for example GS2001, GS2002) :

  GS= Genotyping Sheep
  20= for the year 2020
  number from 01 to 10 for each sample

- Each laboratory received in 2019 its own individual “laboratory code”
- The samples were shipped on 16th November 2020
- The deadline for submission of results was the 15th December 2020

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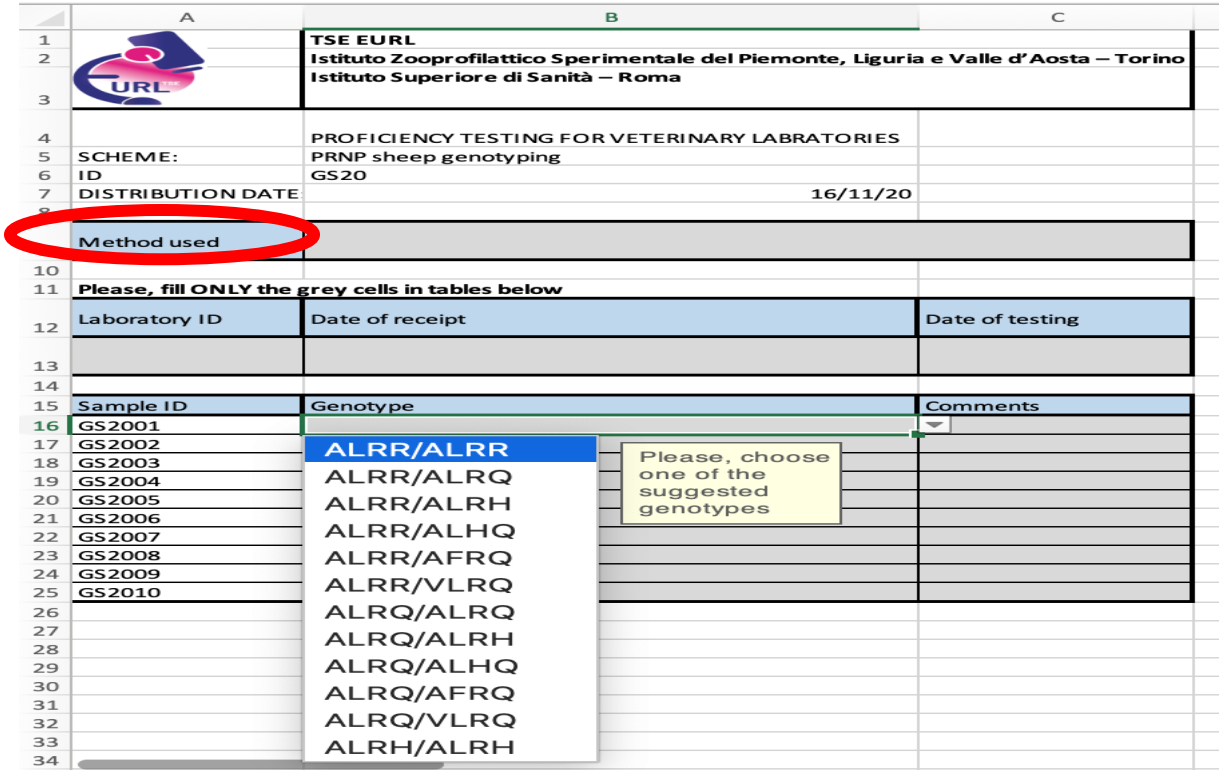
- The results had to be uploaded with the link: https://forms.gle/kEwaPF9b8DFqSiyd8 using an Excel file preset for the insertion of the genotypes

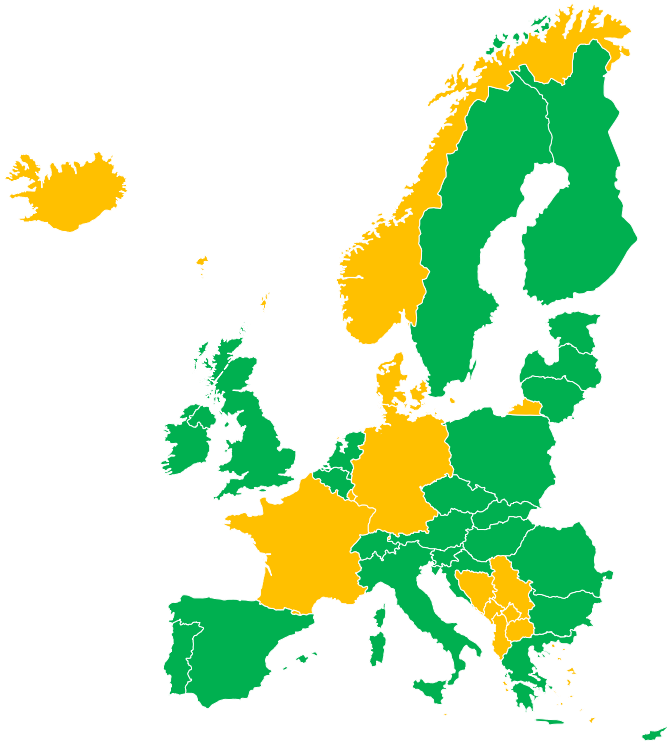
- All participants were requested to report the genotypes at codons 136, 141, 154, 171 of the PrP in allelic format (e.g., ALRQ/AFRQ).



EQA for ovine PRNP genotyping

21 Participants

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



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Test Method Used on the PT

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 PTGS20

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.

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

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Genotypes included in the PTGS20

Sample ID	Expected result
GS2001	ALRH/ALHQ
GS2002	ALRR/ALRR
GS2003	ALRR/VLRQ
GS2004	ALRR/ALRR
GS2005	ALRQ/ALHQ
GS2006	ALHQ/ALHQ
GS2007	AFRQ/VLRQ
GS2008	ALRQ/ALRQ
GS2009	ALRR/ALRQ
GS2010	ALRQ/VLRQ

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Results tabulation for PT GS20: PRNP sheep genotyping

Distribution date: 16/11/2020

Sample ID	GS2001	GS2002	GS2003	GS2004	GS2005	GS2006	GS2007	GS2008	GS2009	GS2010
Gold Standard	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
Laboratory ID	**Genotype reported**									
005	ALHQ/ALRH	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALHQ/ALRQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
011	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
015	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
093	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
100	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
130	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
197	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
229	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
305	ALRQ/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/AFRQ	ALRQ/VLRQ
359	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
381	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
417	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
604	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
619	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
722	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
769	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
780	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
858	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
907	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
948	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ
981	ALRH/ALHQ	ALRR/ALRR	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALHQ/ALHQ	AFRQ/VLRQ	ALRQ/ALRQ	ALRR/ALRQ	ALRQ/VLRQ

Comments :

provided by Gabriele Vaccari, Istituto Superiore di Sanità - Rome

Lab 305 - Sample GS2001: The laboratory reported the genotype ALRQ/ALHQ instead of ALRH/ALHQ and Sample GS2009 reported ALRR/AFRQ instead ALRR/ALRQ.
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.

04 February 2021

Giuseppe Ru, director of TSE EURL

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Laboratory 305

136	141	154	171	Expected Genotype	Reported Genotype
A/A	L/L	R/H	H/Q	ALRH/ALHQ	ALRQ/ALHQ
A/A	L/L	R/R	R/Q	ALRR/ALRQ	ALRR/AFRQ

- 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
GS2011	AFRQ/VLRQ
GS2012	ALRR/ALRQ
GS2013	ALRH/ALHQ
GS2014	ALRR/VLRQ
GS2015	ALRR/ALRR
GS2016	ALRQ/ALHQ
GS2017	ALRQ/VLRQ
GS2018	ALRQ/ALRQ
GS2019	ALHQ/ALHQ
GS2020	ALRR/ALRR

- 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.

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Results tabulation for PT GS20R: PRNP sheep genotyping

Distribution date: 16/03/2021

Sample ID	GS2011	GS2012	GS2013	GS2014	GS2015	GS2016	GS2017	GS2018	GS2019	GS2020
Gold Standard	AFRQ/VLRQ	ALRR/ALRQ	ALRH/ALHQ	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALRQ/VLRQ	ALRQ/ALRQ	ALHQ/ALHQ	ALRR/ALRR
Laboratory ID	**Genotype reported**									
305	AFRQ/VLRQ	ALRR/ALRQ	ALRH/ALHQ	ALRR/VLRQ	ALRR/ALRR	ALRQ/ALHQ	ALRQ/VLRQ	ALRQ/ALRQ	ALHQ/ALHQ	ALRR/ALRR

Comments :
provided by Gabriele Vaccari, Istituto Superiore di Sanità - Rome

Laboratory 305 reported all samples as the intended results

Conclusion:

Laboratory 305 has passed this PT round.

29/03/2021
Giuseppe Ru, director of TSE EURL

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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 have developed and validated two Real-Time PCR method that allows the identification of PRNP genotype at codons 222 and 146 respectively

- For codon 222 the genotyping assay consisted of a mix of sequence-specific forward and reverse primers and two TaqMan MGB (minor grove binder) probes labelled with FAM and VIC

- discrimination between the three possible genotypes (Q/Q, Q/K and K/K)

PRNP Goat Genotyping

- For codon 146 we have developed two different assay: one for N/S polymorphism and one for N/D polymorphism
- The assay consisted of a mix of sequence-specific forward and reverse primers and three TaqMan MGB (minor grove binder) probes labelled with FAM, VIC and NED

- The methods developed are 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

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EQA rounds

EQA for PRNP Sheep Genotyping and EQA for PRNP Goat Genotyping

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