TSE EURL
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 DB19: Discriminatory western blot in bovine

Distribution date: 12/11/2019

Lab. ID	Date of receipt	Date of testing	Test method used	Kit Manufacturer	Batch	Expiry date	Method details	Antibodies used	Batch	Expiry date
127	06/11/2019	03/12/2019	APHA Hybrid WB	BioRad	8M0043	20/04/2021		Sha31 and P4	2620317	feb-21
277	13/11/2019	02/12/2019	ANSES WB	TeSeE-WB for PrPres extraction	9F044	20/11/2020		TeSeE-WB Blocking/AB/ABII	9F044	20/11/2020
								SAF84	0115/reconstitution2	02/03/2021
441	13/11/2019	21/11/2019	TeSeE TM Western Blot	Bio-Rad	8M0043	27/04/2020		Anti-Prp Mab	8M0043	27/04/2020
								Sheep Anti-Mouse IgG-HRP	8M0043	27/04/2020
472	14/11/2019	02/12/2019	FLI BSE Discriminatory Immunoblot	In house	NA	NA	PTA Immunoblot	mab L42	NA	NA
								mab P4	NA	NA
514	13/11/2019	22/11/2019	VLA Hybrid method	BioRad	8M0043	24/04/2020		P4	2620137	feb-19
								Sha31	8M0043	24/04/2020
532	14/11/2019	17/12/2019	in-house WB	Bio-Rad			Samples digestion according to Bio-Rad TeSeE sheep and goat ELISA with 5 x PK concentration	Sha31	A0111	31/12/2020
								12B2	01/01/2019	31/12/2020
539	13/11/2019	19/11/2019	hybrid WB	Prionics	W190101G	13/05/2020		9A2	N.A.	N.A.
								12B2	N.A.	N.A.
567	13/11/2019	23/01/2020	TeSeE Western Blot Hybrid method with specific mAb	BioRad	9F0044	20/11/2020	PESIG/EET-12	SHA31	9É0044	24/11/2020
								12B2	1142008	
567*			Discriminatory Western Blot pK Treatment Protocol	Prionics	W190101G	13/05/2020		6H4	W190101G45	13/05/2020
								L42	741012	
572	13/11/2019	04/12/2019	APHA Biorad Hybrid WB	BioRad	8M0043	27/04/2020	SOP. SE198 Ed7	Sha31	Biorad Kit	27/04/2020
								P4	R-Biopharm (C12705)	01/08/2021
								SAF84	Cayman Chemicals (R4754)	29/10/2020
688	13/11/2019		The APHA BioRad TeSeE-based Hybrid Western Blotting Method	TeSeE Western Blot BioRad - CEA	8M0043	27/04/2020	Double immunolabelling with Sha31 from the kit and P4	AbI / AbII	8M0043	15 and 19/05/2020 respectively
								P4	2620317	01/02/2019
766	13/11/2019	23/01/2020	AHVLA Bio-Rad TeSeE-based Hybrid WB Bio-Rad 3551169, R-Biopharm AG R8008	BIO-RAD	8M0043	27.04.2020		SHA31	8M0043	27.04.2020
								P4	2620317A	31/08/2021
766*				R-Biopharm	8M0043	27.04.2020		L42	7750818	31/07/2020
								P4	2620317A	31/08/2021
803	12/11/2019	21/11/2019	ISS BSE Dicriminatory WB	in-house	/	/	/	L42	7750818	31jul2020
								12B2	260313	03/04/2021
959	14/11/2019	12/12/2019	Discriminatory Western blot	In house	n.a.	n.a.	SOP 00-14-1647	6H4	W190101G	13/05/2020
								L42, SAF 84 & 12B2	n.a.	n.a.

***1st alternative test**

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Lab. ID	Sample No.	Result Core Antibody	Result Terminal Antibody	Interpretation	Comments	Sample No.	Result Core Antibody	Result Terminal Antibody	Interpretation	Comments
127	DB1901	3 bands, lower than C type	Reduced staining	L type BSE		DB1902	3 bands, strong di and mono bands	Reduced staining	Classical BSE	
277	DB1901	Low MW, bi&monoGlyco	idem core AB	L-type		DB1902	Low MW, biGlyco	idem core AB	C-type	
441	DB1901			BSE		DB1902			Scrapie	
472	DB1901	positive	negative	L-Type		DB1902	positive	negative	C.-Type	
514	DB1901	visible signal	no signal	potential L-type		DB1902	visible signal	no signal	classical bovine BSE	
532	DB1901	positive	negative	L-BSE	monoglycosylated band most prominent	DB1902	positive	negative	C-BSE	diglycosylated band most prominent
539	DB1901	positive	negative	L-type	comparable amount on di- and mono-glycosylated bands, no difference in migration distance of non-glycosylated band compared with classical BSE with core antibody, no reactivity with 12B2	DB1902	positive	negative	C-type	predominance of di-glycosylated band, no reactivity with 12B2
567	DB1901	Positive	Negative	L-Type BSE		DB1902	Positive	Negative	C-Type BSE	
567*	DB1901	Medium positive (pK mild condition)	Negative (pK Stringent condition)	L-Type BSE		DB1902	Strong positive (pK mild condition)	Strong positive (pK Stringent condition)	C-Type BSE	
572	DB1901	Positive. Di- and mono- intensities similar. Particularly highlighted with SAF84 antibody	No signal.	L-type BSE profile		DB1902	Positive. Di-glycosylated band most intense	No signal.	Classical BSE profile	
688	DB1901	Positive(similarity between di- and mono-glycosylated bands and non-glycosylated band lower when compared with BSE-C control)	Negative	BSE-L	1,721	DB1902	Positive(predominance of di-glycosylated band with classical profile)	Negative	BSE-C	over 3,500 / 1/10-3,062
766	DB1901	three bands	no signal	Classical BSE		DB1902	three bands	no signal	Classical BSE	Atyp.BSE L type ?
766*	DB1901	three bands	no signal	Classical BSE		DB1902	three bands	no signal	Classical BSE	Atyp.BSE L type ?
803	DB1901	positive	negative	L-type BSE	low glycotype	DB1902	positive	negative	C-type BSE	high glycotype
959	DB1901	Positive; Mono and Di band equally prominent.	Negative	L type BSE		DB1902	Positive; Di band most prominent	Negative	C type BSE	

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Distribution date: 12/11/2019

Lab. ID	Sample No.	Result Core Antibody	Result Terminal Antibody	Interpretation	Comments	Sample No.	Result Core Antibody	Result Terminal Antibody	Interpretation	Comments
127	DB1903	3 bands	Strong staining of 3 bands	H type BSE		DB1904	3 bands, strong di and mono bands	Reduced staining	Classical BSE	
277	DB1903	High MW, biGlyco	+C-term	H-type		DB1904	Low MW, biGlyco	idem core AB	C-type	
441	DB1903			Atypical Scrapie		DB1904			BSE	
472	DB1903	positive	positive	H-Type		DB1904	positive	negative	C-Type	
514	DB1903	visible signal	visible signal	potential H-type		DB1904	visible signal	no signal	classical bovine BSE	
532	DB1903	positive	positive	H-BSE	reactivity with mAB 12B2	DB1904	positive	negative	C-BSE	diglycosylated band most prominent
539	DB1903	positive	positive	H-type	higher migration with core antibody compared with classical BSE, reactivity with 12B2	DB1904	positive	negative	C-type	predominance of di-glycosylated band, no reactivity with 12B2
567	DB1903	Positive	Positive	H-Type BSE		DB1904	Positive	Negative	C-Type BSE	
567*	DB1903	Medium positive (pK mild condition)	Negative (pK Stringent condition)	H-Type BSE		DB1904	Strong positive (pK mild condition)	Strong positive (pK Stringent condition)	C-Type BSE	
572	DB1903	Positive. Higher molelcular mass compared to classical BSE control	Positive. Lower molecular mass bands seen with SAF84 as expected with H-type BSE.	H-type BSE profile		DB1904	Positive. Di-glycosylated band most intense	No signal.	Classical BSE profile	
688	DB1903	Positive(predominance of di-glycosylated band and non-glycosylated band higher when compared with BSE-C control)	Positive	BSE-H	over 3,500 / 1/10-3,239	DB1904	Positive(predominance of di-glycosylated band with classical profile)	Negative	BSE-C	over 3,500 / 1/10-over 3,500
766	DB1903	three bands	weak signal	Atypical BSE	H type	DB1904	three bands	no signal	Classical BSE	
766*	DB1903	three bands	weak signal	Atypical BSE	H type	DB1904	three bands	no signal	Classical BSE	
803	DB1903	positive	positive	H-type BSE	MW higher than control scrapie, relative L42/12B2 ratio <2	DB1904	positive	negative	C-type BSE	high glycotype
959	DB1903	Positive; Di band most prominent; With SAF84 extra lower band.	Positive	H type BSE		DB1904	Positive; Di band most prominent	Negative	C type BSE	

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Distribution date: 12/11/2019

Comments :
provided by Gabriele Vaccari

Sample DB1901 L - type BSE positive brain homogenate.
All lab with the exception of lab 441 and 766 reported the correct result.
Lab 441 reported this sample as BSE and has therefore failed this PT
Lab 766 using both primary and first alternative method reported
this sample as Classical -BSE and has therefore failed this PT

Sample DB1902 Classical BSE positive brain homogenate
All lab with the exception of lab 441 reported the correct result.
Lab 441 reported this sample as Scrapie for this sample and has therefore failed this PT.
Lab 766 using both primary and first alternative method reported the correct results
however their supporting comments provide a doubt as to whether it is "Atyp. BSE L type"

Sample DB1903 H - type BSE positive brain homogenate
All lab with the exception of lab 441 reported the correct result.
Lab 441 reported this sample as Atypical Scrapie and has therefore failed this PT.

Sample DB1904 Classical BSE positive brain homogenate
All lab reported the correct result.

Conclusion:
In summary laboratories 441 and 766 have failed this PT round, all the other laboratories have passed it.
Laboratories 441 and 766 must stop Atypical BSE Discriminatory testing until the issues are resolved and
a further PT round is successfully repeated.

Please inform the EURL of the alternative Atypical BSE Discriminatory testing arrangements that will be in place
for the interim period.

Laboratories 441 and 766 have the following options:
1. Refer samples for Atypical BSE Discriminatory testing to the EURL.
2. Refer samples for Atypical BSE Discriminatory testing to an NRL within the EU who has passed this exercise.
3. If practicably possible, store samples for Atypical BSE Discriminatory testing until successful repeat PT has
been achieved.

The TSE EURL would like to receive confirmation regarding their chosen option, as listed above.
In the meanwhile Laboratories 441 and 766 are required to undertake an investigation into the root cause of the test anomalies and send this to the EURL.
The EURL will continue to provide any support or advice as required"

06 March 2020
Giuseppe Ru, director of TSE EURL