

PROFICIENCY TESTING FOR VETERINARY LABORATORIES

Results tabulation for PT DB21: Discriminatory western blot in bovine

Distribution date: 06/12/2021

Lab. ID	Date of receipt	Date of testing	Test method used	Kit Manufacturer	Batch	Expiry date	Antibodies used	Batch	Expiry date
127	08/12/2021	03/02/2022	APHA BioRad TeSeE based Hybrid WB	Bio Rad	0L0046	07/04/2022	AB1 SHA & AB 2 P4	0L0046	07/04/2022
							P4	350315	giu-22
							HRP	64322730	30/12/2022
277	06/12/2021	08/02/2022	ANSES WB	TeSeE-WB for PrPres extraction	1C0047	17/08/2022	TeSeE WB Biorad Blocking buffer/AbI/AbII	1H0048	01/01/2023
							SAF84	SAF84/3	12/02/2023
472	09/12/2021		FLI-Test	in house	not relevant	not relevant	mab L42 (in-house)	not relevant	not relevant
							mab P4 (in-house)		
514	08/12/2021		APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	BioRad	0L0046	07/04/2022	P4	350315	giu-22
							Sha31	64403983	02/03/2024
532	08/12/2021		In-house WB Bio-Rad based	Bio-Rad TeSeE s/g, 5 x PK	1K0045	28-nov-22	Sha31	216	31/12/2022
							12B2		31/12/2022
539	09/12/2021	13-15/12/2021	TeSeE WB kit	Biorad	1C0047	17/08/2022	Sha31	1C0047	21/09/2022
							12B2	N.A.	N.A.
567	09/12/2021		TeSeE Western Blot Hybrid method with several mAb	Bio Rad TeSeE WB	1C0047	17/09/2022	Anti-PrP Mab (SHA31)	1C0047	21/09/2022
							P4 (R-Biopharm) and SAF84 (Bertin Bioreagent)	350315 and 0321	30/06/2022 and 03/08/2023
567*			Discriminatory Western Blot pK Treatment Protocol	pK was from Quiagen	166045008	14/02/2022	L42 (R-Biopharm)	7750818A	31/07/2022
572	07/12/2021		APHA Biorad Hybrid Western Blot	Biorad	1C0047	18/08/2022	Sha31	From kit above	18/08/2022
							P4	C13264	13/10/2023
688	10/12/2021		APHA Bio-Rad TeSeE- based Hybrid Method	Bio-Rad	8M0043	27/04/2020	AbII (SHA31)	1H0048	01/01/2023
							P4	2390320	lug-22
803	07/12/2021		ISS BSE Discriminatory WB				L42	7750818	31/07/2022
							12B2	nd	29/10/2022
959	09/12/2021	18/01/2021	Discriminatory Western blot	In house	n.a.	n.a.	6h4, L42, SAF84 & 12B2	N.a.	N.a.

*1st alternative test

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127	DB2131	C-BSE		DB2132	C-BSE		DB2133	H-BSE		DB2134	C-BSE	
277	DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE		DB2134	C-BSE	
472	DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE	very weak sample	DB2134	C-BSE	
514	DB2131	C-BSE		DB2132	L-BSE		DB2133	L-BSE	Weak sample. Tested twice with very similar result.	DB2134	C-BSE	
532	DB2131	C-BSE		DB2132	L-BSE		DB2133		negative	DB2134	C-BSE	
539	DB2131	C-BSE	dominance of diglycosyl band	DB2132	L-BSE	equal amount of di- and mono-glycosyl bands, lower migration of non-glycosyl band compared to C-type	DB2133	H-BSE	higher migration of non-glycosyl band compared to C-type, reactivity with N-terminal Ab, 12B2	DB2134	C-BSE	dominance of diglycosyl band
567	DB2131	C-BSE	The same deglycosylation profile as in the classical BSE positive control	DB2132	L-BSE	No signal with P4 mAb and the same signal distribution in the three fractions with SHA31 mAb	DB2133	H-BSE	Signal with P4 mAb and additional unglycosylated band with SAF84 mAb.	DB2134	C-BSE	The same deglycosylation profile as in the classical BSE positive control
567*	DB2131	C-BSE	No signal loss between mild (M) and strong (S) pK concentration	DB2132	L-BSE	Signal loss with the strong pK concentration and the same deglycoslation profile as in the L-type BSE positive control	DB2133	H-BSE	Signal loss with the strong pK concentration and the same deglycoslation profile as in the H-type BSE positive control	DB2134	C-BSE	No signal loss between mild (M) and strong (S) pK concentration
572	DB2131	C-BSE		DB2132	L-BSE		DB2133		Negative	DB2134	C-BSE	
688	DB2131	C-BSE	Predominance of diglycosylated band and no signal with mAb P4 (OD=0,280)	DB2132	L-BSE	Diglycosylated band similar to monoglycosylated band; lower molecular mass migration and no signal with mAb P4 (OD=0,896)	DB2133	H-BSE	High molecular mass migration and signal with mAb P4 (OD=1,272)	DB2134	C-BSE	Predominance of diglycosylated band and no signal with mAb P4 (OD=3,421; 1/10 dilution OD=0,811)
803	DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE		DB2134	C-BSE	
959	DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE		DB2134	C-BSE	

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Sample ID	Intended
DB2131	C-BSE
DB2132	L-BSE
DB2133	H-BSE
DB2134	C-BSE

Comments :

provided by Gabriele Vaccari, Istituto Superiore di Sanità - Rome

All laboratories reported the correct results, with the exception of laboratories **127, 514, 532** and **572**.

Laboratory **127** didn't report the correct result for sample DB2132.

Laboratory **514** didn't report the correct result for sample DB2133.

Laboratories **532** and **572** reported the sample DB2133 as negative.

Laboratories **127, 514, 532** and **572** were asked to re-test the samples, suggesting some modifications of the protocol used.

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Re-Testing

Lab. ID	Date of receipt	Date of testing	Test method used	Kit Manufacturer	Batch	Expiry date	Antibodies used	Batch	Expiry date	Sample No.	Result	Comments	Sample No.	Result	Comments	Sample No.	Result	Comments	Sample No.	Result	Comments
127	08/12/2021	26/04/2022	APHA BioRad TeSeE based Hybrid WB	Bio Rad	0L0046	07/04/2022	AB1 SHA & AB 2 P4	0L0046	07/04/2022	DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE		DB2134	C-BSE	
							P4	350315	giu-22												
							HRP	64322730	30/12/2022												
514	08/12/2021		APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	BioRad	1H0048	01/01/2023	12B2	260313		DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE	No signal with P4, weak signal with 12B2 (june 2022)	DB2134	C-BSE	
							Sha31	64403983	02/03/2024												
532	08/12/2021		Bio-Rad Hybrid Western blot	Bio-Rad	1H0048	01/01/2023	Sha31	1H0048	31/01/2023	DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE	very weak signal w/ 12B2 and Sha31	DB2134	C-BSE	
							12B2		31/12/2023												
572	07/12/2021		APHA Biorad Hybrid Western Blot	BioRad	IC0047	18/08/2022	Sha31	From kit above	ago-22	DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE	Re-test	DB2134	C-BSE	
							P4	C13264	13/10/2023												
							12B2														
							SAF84														

Comments :

provided by Gabriele Vaccari, Istituto Superiore di Sanità - Rome

After re-testing, laboratories 127, 514, 532 and 572 reported the correct results.

Conclusions:

In summary all the laboratories have passed this PT round.

Giuseppe Ru, director of TSE EURL