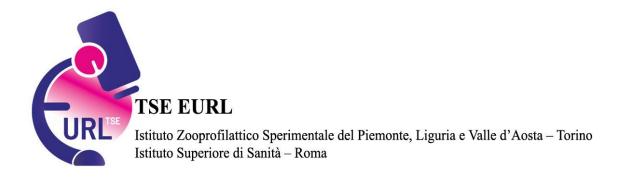


Results tabulation for PT DB21: Discriminatory western blot in bovine

Lab. ID	Date of receipt	Date of testing	Test method used	Kit Manufacturer	Batch	Expiry date	Antibodies used	Batch	Expiry date
							AB1 SHA & AB 2 P4	0L0046	07/04/2022
127	08/12/2021	03/02/2022	APHA BioRad TeSeE based Hybrid WB	Bio Rad	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
472	03/12/2021			in nouse	notrelevant	notrelevant	mab P4 (in-house)		
514	08/12/2021		APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	BioRad	0L0046	07/04/2022	Ρ4	350315	giu-22
514	08/12/2021		AFTIA BIO-Rau Tesel-based Hybrid Western biotting Method	BioRau	010040	0770472022	Sha31	64403983	02/03/2024
522	<b>532</b> 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
552					110045	20 1100 22	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
339	09/12/2021	13-13/12/2021		Biorad	100047	1770872022	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
507	03/12/2021		resel western blot nysna methoa with several mas		100047	1770372022	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
570	07/42/2024			5: 1	1000.17	10/00/0000	Sha31	From kit above	18/08/2022
572	07/12/2021		APHA Biorad Hybrid Western Blot	Biorad	IC0047	18/08/2022	Ρ4	C13264	13/10/2023
688	10/12/2021		ADUA Die Dad Teses, based Unbrid Method	Bio-Rad	8M0043	27/04/2020	Abii (SHA31)	1H0048	01/01/2023
000	10/12/2021	APHA Bio-Rad TeSeE- based Hybrid Method		bio-nau	01110045	27/04/2020	Ρ4	2390320	lug-22
803	07/12/2024		ISS DSE Digriminatory W/D				L42	7750818	31/07/2022
803	07/12/2021		ISS BSE Dicriminatory WB				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									

Distribution date: 06/12/2021

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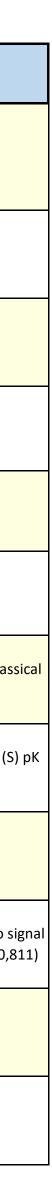


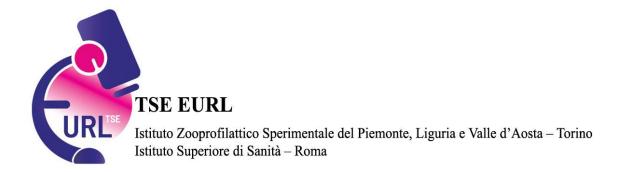
Results tabulation for PT DB21: Discriminatory western blot in bovine

	Lab. ID	Sample No.	Result	Comments	Sample No.	Result	Comments	Sample No.	Result	Comments	Sample No.	Result	Comments
	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 classi 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) 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 sig with mAb P4 (OD=3,421; 1/10 dilution OD=0,81
	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	
*1st	alternative test					-							·

\*1st alternative test

Distribution date: 06/12/2021





Results tabulation for PT DB21: Discriminatory western blot in bovine

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**.

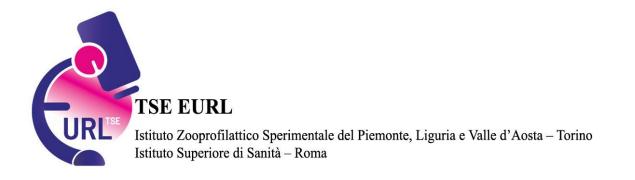
Laboratory **127** didn't report the correct result for sample DB2132.

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.

Distribution date: 06/12/2021



Results tabulation for PT DB21: Discriminatory western blot in bovine

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
			APHA BioRad TeSeE based Hybric WB				AB1 SHA & AB 2 P4	0L0046	07/04/2022		C-BSE		DB2132	L-BSE		DB2133	H-BSE		DB2134	C-BSE	
127	08/12/2021	26/04/2022		d Bio Rad	0L0046	07/04/2022	P4	350315	giu-22	DB2131											
							HRP	64322730	30/12/2022												
				BioRad			12B2	260313		 DB2131	C-BSE		DB2132	L-BSE		DB2133	H-BSE	No signal with P4, weak signal with 12B2 (june 2022)	DB2134	C-BSE	
514	<b>514</b> 08/12/2021		APHA Bio-Rad TeSeE-based Hybrid Western blotting Method		1H0048	01/01/2023	Sha31	64403983	02/03/2024												
500			Bio-Rad Hybrid Western blot	Bio-Rad	1H0048	01/01/2023	Sha31	1H0048	31/01/2023		C-BSE				3SE	DB2133	H-BSE	very weak signal w/ 12B2 and Sha31		C-BSE	
532	08/12/2021						12B2		31/12/2023	DB2131			DB2132	L-BSE							
							Sha31	From kit above	ago-22				DB2132	32 L-BSE					DB2134	C-BSE	
572	07/10/000		APHA Biorad Hybrid Western Blot	DioDod	100047	18/08/2022	P4	C13264	13/10/2023	002121						002122		Do tost			
572	07/12/2021			BioRad	IC0047		12B2			" DB2131	C-BSE					DB2133	H-BSE	Re-test			
							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

Distribution date: 06/12/2021

#### **Re-Testing**

