



DIAGNOSTIC PERFORMANCE OF NSP TESTS



NRL for FMD

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**IZSLER, Brescia, Italy
PANAFTOSA, Rio, Brasil
CODA-VAR, Ukkel, Belgium
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IAH, Pirbright, UK
KVI, Bet Dagan, Israel
FLI, Riems, Germany
SAP, Ankara, Turkey
DFVF, Lindholm, Denmark
FAO, Rome, Italy**



Objective:

Comparative evaluation of tests available in Europe for the detection of Ab to NSP

A consortium of laboratories within the research group, was established to answer these questions

In Europe:

- Which NSP tests are available in Europe?
- What is their validation status?
- How their performances compare with those of the OIE index test?

Approach: collection of well defined experimental and field sera and testing in parallel with the tests under evaluation

Brescia workshop; 3 – 15 May 2004

**IZSLER
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Analysis of results

Volume of data is adequate to validate the tests for use in cattle

Analyses performed for cattle

- ✓ Diagnostic Specificity
- ✓ Diagnostic Sensitivity
- ✓ Analysis of correlation between tests (specificity & sensitivity covariances)
- ✓ Analytical Sensitivity
- ✓ Rate of NSP antibody seroprevalence detected in post outbreaks surveys
- ✓ Analysis of concordance/discrepancy (field samples)

More samples are needed to establish sensitivity in sheep and pigs - Preliminary data on comparative Sp and Se based on limited sets of sera available

Collection of well defined cattle sera

≈ 2500 sera, field and experimental animals, contributed by 9 countries (Belgium, Denmark, Germany, Israel, Italy, The Netherlands, Turkey, United Kingdom, Zimbabwe) and South America

CATTLE condition	NON INFECTED		INFECTED				Post Outbreak ISRAEL ZIMBABWE	Total
	Non Vacc	Vacc	Non Vacc		Vaccinated			
			C+	C-/?	C+	C-/?		
Total sera	675 ^F	425	21	41	225	325	867 ^F	2579
Total cattle	675	425	13	41	67	218	867	2306

Diagnostic Specificity - RESULTS

1100 cattle sera → 675 naïve, from 4 EU countries
→ 425 vaccinated with European vaccines

Test	UBI	SVANOVA	CEDI	BOMMELI	BRESCIA	PANAFTO
screening	98.5%	98.5%	98.1%	97.6%	97.4%	97.2%
Retest pos	99%	99%	99.2%	98.8%	99.7%	98.1%

- No significant differences between vaccinated and non-vaccinated populations were found with any of the 6 NSP-ELISA: **Vaccination with European vaccines does not affect specificity**
- Specificity ranges from **97.2% to 98.5%** in the different ELISA, at the first screening test
- Specificity **improves significantly after retesting** false-positives, **overcoming 99%** with the more specific tests

Diagnostic Specificity – RESULTS (2)

Two further observations

- **False-positives exhibit usually borderline values in all NSP-ELISA**
- **Specificity covariance: false-positive results are usually not correlated (a serum reacting as false-positive in one test is usually negative in all other tests – none coincident positive result in more than é tests)**

These observations are “strategic” and can be considered when interpreting results of NSP-assays and when designing a testing system

NSP tests performance Diagnostic Sensitivity

- Evaluated using experimentally infected cattle
- 612 sera from 322 cattle (some sequential bleedings)
- Animals categorised according:
 - time after infection (7 dpi → > 100 dpi)
 - vaccinated or not vaccinated
 - evidence of infection (VI, PCR,
 - persistent infection (carriers)

Diagnostic Sensitivity - RESULTS

NON VACCINATED CATTLE EXPOSED TO INFECTION (n° 54)

Days after exposure	N° cattle	NCPanaf-screening	IZS-Brescia	Ceditest	Svanovir	Chekit	UBI	p value
7 - 14	5	100%	100%	100%	100%	100%	100%	1,000
15 - 27	27	100%	100%	100%	100%	100%	100%	1,000
28 - 100	26	100%	100%	100%	96.2%	92.3%	100%	0,301
> 100	2	100%	100%	50%	50%	50%	50%	0,486

VACCINATED CATTLE EXPOSED TO INFECTION (n° 285)

ALL , WITH OR WITHOUT EVIDENCE OF INFECTION

7 - 14	176-177	49.7%	53.1%	49.7%	41.8%	51.1%	32.8%	0.002
15 - 27	131	60.3%	55.7%	52.7%	49.6%	52.7%	38.2%	0.012
28 - 100	108	69.4%	64.8%	63%	58.3%	50%	55.6%	0.052
> 100	47	72.3%	63.8%	74.5%	57.4%	38.3%	46.8%	0.001

Diagnostic Sensitivity - RESULTS

VACCINATED CATTLE EXPOSED TO INFECTION with evidence of infection (n° 164)

Days after exposure	N° cattle	NCPanaf-screening	IZS-Brescia	Ceditest	Svanovir	Chekit	UBI	p value
ONLY CARRIERS (CATTLE KNOWN TO BE PERSISTENTLY INFECTED AT 28 DAYS OR LATER, n° 67)								
7 - 14	29	58.6%	58.6%	58.6%	55.2%	58.6%	41.4%	0,531
15 - 27	36	72.2%	66.7%	63.9%	55.6%	58.3%	58.3%	0,483
28 - 100	66^{a)}	93.9%	86.4%	86.4%	71.2%	68.2%	77.3%	0.001
> 100	37 ^{a)}	89.2%	78.4%	89.2%	70.3%	48.6%	59.5%	0.000
NON CARRIERS OR UNDEFINED CARRIER STATUS (n° 97)								
7 - 14	56	58.9%	57.1%	55.4%	46.4%	68.7%	51.8%	Xx
15 - 27	61	68.9%	65.6%	60.7%	57.4%	63.9%	41%	Xx
28 - 100	26	26.9%	19.2%	23.1%	23.1%	15.4%	19.2%	Xx
> 100	10	10%	10%	20%	10%	0%	0%	Xx

a) including 4 cattle with questionable carrier status, and negative for all tests in both dpi class

Evidence that virus persistence maintains the level of Ab to NSP detectable for long time - In cattle that recover from infection Ab to NSP decay more rapidly

Diagnostic Sensitivity - RESULTS

VACCINATED CATTLE EXPOSED TO INFECTION without evidence of infection

Days after exposure	N° cattle	NCPanaf-screening	IZS-Brescia	Ceditest	Svanovir	Chekit	UBI	p value
ALL, NO EVIDENCE OF INFECTION OR UNKNOWN INFECTIOUS STATUS (n° 121)								
7 - 14	91-92	41.3%	48.9%	43.5%	34.8%	42.9%	18.5%	Xx
15 - 27	34	32.4%	26.5%	26.5%	29.4%	26.5%	11.8%	Xx
28 - 100	16	37.5%	50.0%	31.3%	62.5%	31.3%	25.0%	Xx
> 100	0	-	-	-	-	-	-	-
CATTLE WITH NO EVIDENCE OF INFECTION (VI neg/PCR neg) (n° 17)								
7 - 14	15	40%	45,7%	20%	53,3%	33,3%	26,7%	Xx
15 - 27	8	25%	12,5%	12,5%	12,5%	12,5%	0%	Xx
28 - 100	15	33,3%	46,7%	26,7%	66,7%	26,7%	20%	Xx
> 100	0	-	-	-	-	-	-	-

Finding of seroreactors to NSP; yet lacking any other evidence of infection: NSP serology may help to infer an infection condition that could be missed by any other test system

BRESCIA



Diagnostic Sensitivity Conclusions

Positive Sensitivity covariance: tests results are highly correlated among the six tests

The production of antibody to NSP is correlated to the extent of virus replication

Cattle non protected by vaccination and or carriers are more likely to be detected. *Sensitivity reaches 100% with any test in non vaccinated and infected cattle and approaches 90% with the better tests in vaccinated/carriers*

2 NSP tests show sensitivity performance comparable to the OIE index test (PANAFTOSA)

- CEDI Diagnostic (commercial kit)
- Brescia ELISA (in-house assay)



Seroprevalence rates detected in post outbreaks surveys

	PANAFTOSA	BRESCIA	CEDITEST	SVANOVA	CHEKIT	UBI
Israel	25.8 %	25.6 %	22.4 %	21.3 %	20.9 %	15.7 %
Zimbabwe	67.7 %	65.2 %	66.2 %	50.5 %	48.8 %	53.5 %

Origin	N°	Level of agreement		
		Positive in 6 tests	Negative in 6 tests	Discordant in 1 or > test
Israel	465	12.3 %	66 %	22.7 %
Zimbabwe	402	37.6 %	22.9 %	39.5 %

Analysis of discrepancies-concordance

PANAFTOSA; Ceditest and Brescia ELISAs contributed the higher number of positive-concordant results (higher relative sensitivity and concordance)

NSP tests diagnostic performance in post outbreak surveys - Conclusions

Higher detection rates and concordance shown by three tests

- PANAFTOSA
- Brescia
- Cedi

consistently with results on experimental models

Results from field samples provided estimates of NSP-antibody prevalence in different field situations and another possibility to compare tests

Due to circulation of FMDV SAT1 and SAT2 in Zimbabwe, the analysis enabled us to extend assays validation to these serotypes

Diagnostic performance of NSP ELISAs in small ruminants and pigs

	NON EXPOSED		EXPOSED		Total
	Non Vacc.	Vaccinated	Non Vacc.	Vaccinated	
SHEEP SERA	422 ^{a)}	12	172	100	706
PIG SERA	130 ^{a)}	54	15	70	269

SHEEP	NON INFECTED non vacc. & vacc.		EXPERIMENTALLY INFECTED *				
			Non-Vaccinated		Vaccinated		
			25-28 dpi		28 dpi		6-8 dpi
NSP-ELISAs	pos./total	Spec. %	pos./total	%	pos./total	%	pos./total
NCPanaftosa	9/434	97.92	9/9	100	3/6	50	4/16
IZS-Brescia	2/428	99.53	9/9	100	4/6	66.66	2/16
Ceditest	0/431	100	9/9	100	2/6	66.66	5/16
Chekit	0/431	100	9/9	100	2/6	33.33	1/16

PIGS	NON INFECTED non vacc. & vacc.		EXPERIMENTALLY INFECTED *				
			Non-Vaccinated		Vaccinated		
			> 20 dpi		> 20 dpi		≤14 dpi
NSP-ELISAs	pos./total	Spec. %	pos./total	%	pos./total	%	pos./total
Ceditest	0/152	100	12/12	100	10/18	55.5	5/42
IZS-Brescia	1/179	99.44	12/12	100	8/18	44.4	3/42
Chekit	6/181	96.69	12/12	100	3/18	16.6	2/40
UBI	10/184	94.56	12/12	100	4/18	22.2	2/42

* undefined infection/carrier status

Seroprevalence rates in sheep samples from affected areas

SHEEP	2004 outbreak non-vacc./pos to SP ISRAEL		2001 outbreak non-vacc./pos to SP UNITED KINGDOM		Serosurvey in vaccinated areas TURKEY ^{b)}	
	pos./total	Seroprev. %	pos./total	Seroprev. %	pos./total	Seroprev. %
NCPanaftosa	59/63	93.65	85/100	85	37/78	47.43
IZS-Brescia	59/63	93.65	77/100	77	33/78	42.30
Ceditest	56/63	88.88	60/100 ^{a)}	60	36/78	46.15
Chekit	55/63	87.30	40/100	40	38/78	48.72

a) test carried out in the laboratory of origin

b) possible bias due to pre-selection of samples on the basis of preliminary testing

- ✓ Homogeneous prevalence found by the 4 tests in the flock in Israel: uniform infectious status of animals within the flock
- ✓ Significant inter-assays variation in sheep from UK epidemic : bias?
- ✓ Lower prevalence of antibodies in sheep from Turkey: vaccinated regions?

