

# PrioCHECK<sup>®</sup> FMDV NS ELISA: Summary of Performance

## Introduction

This Summary of Performance shall give an overview of the performance of the PrioCHECK<sup>®</sup> FMDV NS ELISA.

## Detection of carrier animals with the PrioCHECK<sup>®</sup> FMDV-NS

Calfs were experimentally infected with different doses of a FMDV strain A/Tur 14/98 vaccine (see below). Samples were taken at different days post infection (d.p.i.). All carrier animals were detected positive after 6 d.p.i., resulting in a sensitivity of 100% to detect carrier cattle (with this limited amount of animals).

### Samples:

- Calf 1-5: vaccinated with the full dose of FMDV strain A/Tur 14/98 vaccine
- Calf 6-10: vaccinated with a quarter dose of FMDV strain A/Tur 14/98 vaccine
- Calf 11-15: vaccinated with one-sixteenth dose of FMDV strain A/Tur 14/98 vaccine
- Calf 16-17: control group, non-vaccinated

**Table 1: Detection of carrier animals**

Calf nr	Days post infection										
	0	6	8	10	13	27	55	111	223	441	665
1	31	<b>89</b>	<b>91</b>	<b>92</b>	<b>93</b>	<b>93</b>	<b>91</b>	<b>91</b>	<b>81</b>	<b>68</b>	
2	22	<b>51</b>	<b>83</b>	<b>84</b>	<b>86</b>	<b>86</b>	<b>89</b>	<b>93</b>	<b>92</b>	<b>94</b>	<b>86</b>
3	40	<b>57</b>	<b>81</b>	<b>84</b>	<b>87</b>	<b>90</b>	<b>87</b>	<b>81</b>	<b>91</b>	<b>92</b>	<b>93</b>
4	<b>63</b>	<b>69</b>	<b>88</b>	<b>91</b>	<b>93</b>	<b>91</b>	<b>95</b>	<b>96</b>	<b>95</b>	<b>95</b>	<b>96</b>
5	39	<b>60</b>	<b>85</b>	<b>89</b>	<b>91</b>	<b>93</b>	<b>94</b>	<b>95</b>	<b>93</b>	<b>94</b>	<b>93</b>
6	24	<b>59</b>	<b>87</b>	<b>89</b>	<b>89</b>	<b>90</b>	<b>91</b>	<b>91</b>	<b>90</b>	<b>90</b>	<b>90</b>
7*	33	<b>56</b>	<b>91</b>	<b>94</b>	<b>93</b>	x	<b>94</b>	<b>94</b>	x	<b>X</b>	<b>X</b>
8	16	<b>52</b>	<b>92</b>	<b>94</b>	<b>94</b>	<b>94</b>	<b>95</b>	<b>95</b>	<b>96</b>	<b>96</b>	<b>96</b>
9	38	<b>56</b>	<b>83</b>	<b>86</b>	<b>86</b>	<b>92</b>	<b>95</b>	<b>94</b>	<b>96</b>	<b>96</b>	<b>95</b>
10	30	<b>54</b>	<b>80</b>	<b>84</b>	<b>86</b>	<b>87</b>	<b>85</b>	<b>87</b>	<b>94</b>	<b>95</b>	<b>94</b>
11	22	<b>58</b>	<b>59</b>	<b>80</b>	<b>80</b>	<b>91</b>	<b>94</b>	<b>95</b>	<b>93</b>	<b>94</b>	<b>95</b>
12	41	<b>52</b>	<b>86</b>	<b>93</b>	<b>93</b>	<b>93</b>	<b>94</b>	<b>96</b>	<b>96</b>	<b>95</b>	<b>95</b>
13	32	<b>48</b>	<b>86</b>	<b>89</b>	<b>90</b>	<b>91</b>	<b>90</b>	<b>93</b>	<b>92</b>	<b>95</b>	<b>95</b>
14	25	<b>43</b>	<b>70</b>	<b>82</b>	<b>88</b>	<b>93</b>	<b>93</b>	<b>92</b>	<b>93</b>	<b>89</b>	<b>78</b>
15	45	<b>69</b>	<b>92</b>	<b>94</b>	<b>94</b>	<b>91</b>	<b>93</b>	<b>94</b>	<b>95</b>	<b>94</b>	<b>94</b>
16	33	<b>53</b>	<b>62</b>	<b>74</b>	<b>78</b>	<b>89</b>	<b>89</b>	<b>79</b>	<b>72</b>	<b>68</b>	<b>60</b>
17	45	<b>49</b>	<b>82</b>	<b>91</b>	<b>92</b>	<b>91</b>	<b>92</b>	<b>93</b>	<b>95</b>	<b>95</b>	<b>95</b>

\* Calf 7 died 111 days post infection

PI> 50% is considered positive in the PrioCHECK<sup>®</sup> FMDV-NS (bold)

## Sensitivity and specificity in carrier animals

Vaccine strain	% Sensitivity (+/n)	% Specificity (-/n)
A Turkey	100 % (140/140)	93 % (14/15)

**Results in the PrioCHECK® FMDV-NS ELISA (PI values) obtained with sera from vaccination challenge experiments in cattle sera (n=257).**

Sera were collected from cattle vaccinated and challenged with different FMDV strains, FMDV strain Asia-1, FMDV strain A22 Iraq, FMDV strain O1 Manissa (indicated in the tables). Samples were taken 4 weeks after vaccination and 7, 8 or 9 days after challenge infection. Latest after 9 d.p.i. all animals were detected positive.

**Table 2: Challenge with FMDV strain Asia-1**

Vaccination and challenge with FMDV strain Asia-1											
Nr.	vacc	8 dpi	Nr.	vacc	8 dpi	Nr.	Vac	8 dpi	Nr.	vacc	9 dpi
1	33	<b>60</b>	18	<b>54</b>	<b>85</b>	35	39	<b>76</b>	52	27	<b>80</b>
2	40	<b>89</b>	19	45	<b>81</b>	36	20	<b>82</b>	53	22	<b>87</b>
3	30	<b>56</b>	20	<b>58</b>	<b>78</b>	37	34	<b>87</b>	54	22	<b>82</b>
4	17	41	21	32	<b>88</b>	38	43	<b>81</b>	55	36	<b>69</b>
5	27	<b>54</b>	22	48	<b>64</b>	39	36	<b>84</b>	56	36	<b>68</b>
6	<b>51</b>	<b>50</b>	23	29	<b>77</b>	40	35	<b>88</b>	57	16	<b>84</b>
7	39	<b>50</b>	24	<b>54</b>	<b>82</b>	41	15	<b>66</b>	58	13	<b>82</b>
8	39	<b>76</b>	25	<b>59</b>	<b>78</b>	42	20	<b>66</b>	59	33	<b>74</b>
9	<b>50</b>	<b>55</b>	26	31	<b>78</b>	43	25	<b>80</b>	60	19	<b>86</b>
10	33	41	27	44	<b>71</b>	44	40	<b>90</b>	61	37	<b>74</b>
11	31	<b>78</b>	28	35	<b>70</b>	45	39	<b>81</b>	62	25	<b>82</b>
12	<b>62</b>	<b>65</b>	29	<b>50</b>	<b>79</b>	46	15	<b>85</b>	63	18	<b>80</b>
13	44	<b>82</b>	30	47	<b>86</b>	47	33	<b>85</b>	64	26	<b>87</b>
14	39	<b>80</b>	31	34	<b>76</b>	48	29	X	65	39	<b>86</b>
15	<b>53</b>	<b>82</b>	32	48	<b>52</b>	49	31	X	66	29	<b>83</b>
16	<b>55</b>	X	33	34	<b>82</b>	50	X	X	67	X	X
17	49	<b>84</b>	34	18	<b>75</b>	51	X	X	68	X	<b>84</b>

X: indicating that animal died during experiments or no sample was taken

**Table 3: Challenge with FMDV strain A22 Iraq**

Vaccination and challenge with FMDV strain A22 Iraq					
Nr.	Vacc	7 dpi	Nr.	vacc	9 dpi
69	44	<b>88</b>	86	28	<b>88</b>
70	41	<b>86</b>	87	45	<b>90</b>
71	36	<b>82</b>	88	10	<b>79</b>
72	21	<b>86</b>	89	32	<b>78</b>
73	41	<b>87</b>	90	27	<b>81</b>
74	46	<b>86</b>	91	11	<b>76</b>
75	47	<b>87</b>	92	3	<b>81</b>
76	27	<b>86</b>	93	13	<b>86</b>
77	40	<b>86</b>	94	42	<b>83</b>
78	<b>53</b>	<b>87</b>	95	19	<b>80</b>
79	29	<b>83</b>	96	19	<b>87</b>
80	22	<b>81</b>	97	19	<b>81</b>
81	29	<b>84</b>	98	14	<b>75</b>
82	41	<b>83</b>	99	47	<b>82</b>
83	36	<b>84</b>	100	28	<b>71</b>
84	48	X	101	31	<b>84</b>
85	35	X	102	33	<b>88</b>

X: indicating that animal died during experiments or no sample was taken

**Table 4: Challenge with FMDV strain O1 Manissa**

Vaccination and challenge with FMDV strain O1 Manissa					
Nr.	Vacc	7 dpi	Nr.	vacc	9 dpi
120	32	<b>75</b>	137	18	<b>83</b>
121	<b>60</b>	<b>73</b>	138	35	<b>83</b>
122	41	47	139	33	<b>74</b>
123	<b>63</b>	<b>80</b>	140	33	<b>76</b>
124	45	<b>69</b>	141	49	<b>84</b>
125	<b>57</b>	34	142	<b>77</b>	<b>77</b>
126	<b>53</b>	32	143	28	<b>70</b>
127	<b>50</b>	X	144	<b>51</b>	<b>84</b>
128	<b>57</b>	<b>71</b>	145	44	<b>70</b>
129	<b>70</b>	<b>79</b>	146	23	<b>66</b>
130	36	<b>67</b>	147	<b>50</b>	<b>86</b>
131	5	<b>63</b>	148	36	<b>60</b>
132	<b>55</b>	<b>50</b>	149	43	<b>81</b>
133	37	<b>64</b>	150	22	<b>69</b>
134	44	<b>70</b>	151	35	<b>83</b>
135	44	<b>83</b>	152	X	<b>76</b>
136	45	<b>68</b>	153	X	<b>85</b>

X: indicating that animal died during experiments or no sample was taken

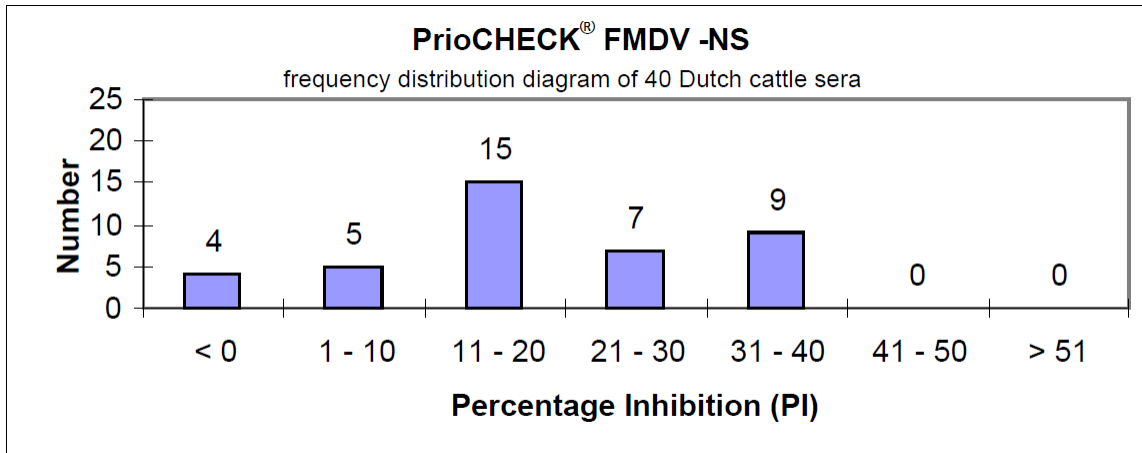
#### Sensitivity and specificity using different vaccine and challenge strains

Vaccine strain	% Sensitivity (+/n)	% Specificity (-/n)
O1 Manissa	91 % (31/34)	73 % (25/34)
A22 Iraq	100 % (34/34)	97 % (33/34)
Asia 1	97 % (66/68)	88 % (60/68)
<b>Average</b>	<b>96 %</b>	<b>87 %</b>

**Results obtained in the PrioCHECK<sup>®</sup> FMDV-NS (PI values) with negative field samples of sheep, goats, cattle and pigs.**

**Cattle sera**

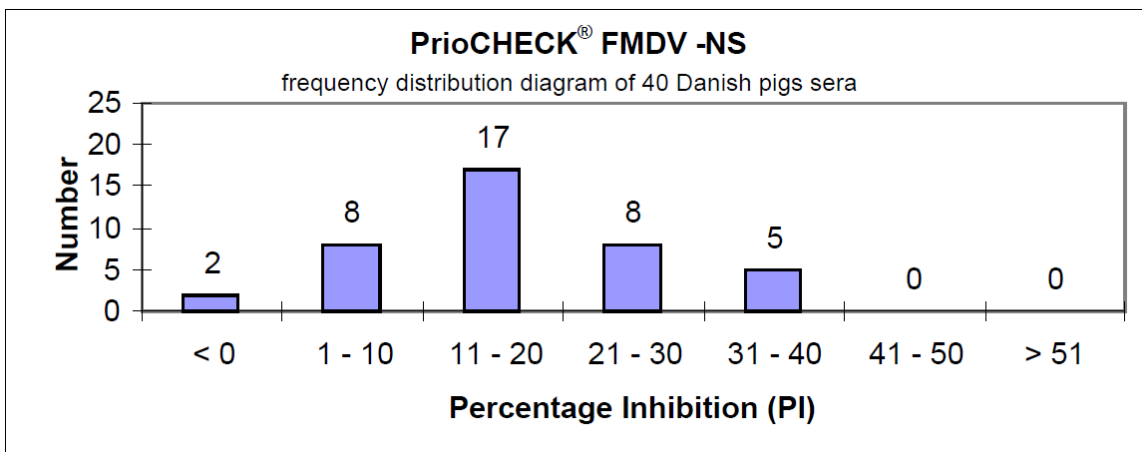
Serum samples from 40 Dutch cattle were tested. All Samples resulted negative.



PI values below 50% are considered as negative.

**Pig sera**

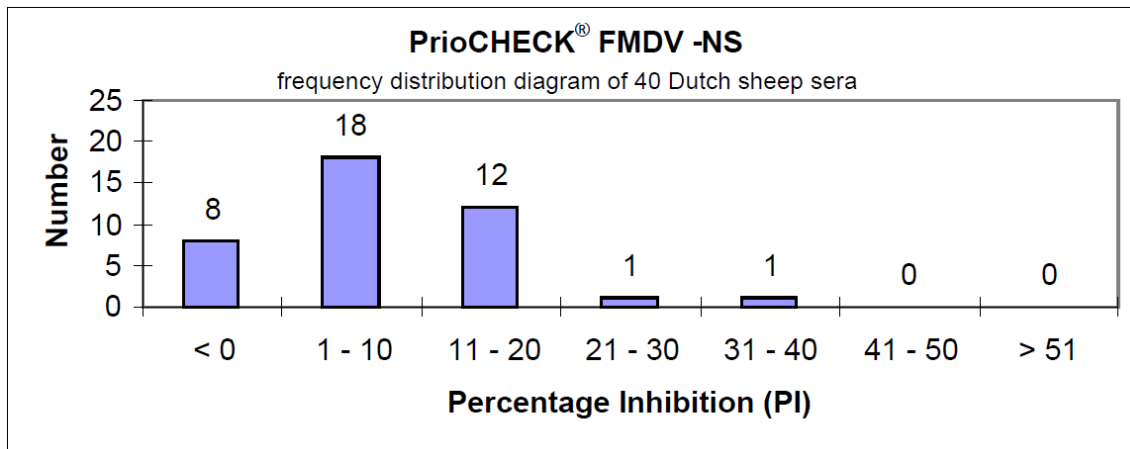
Serum samples from 40 Danish pigs were tested. All Samples resulted negative.



PI values below 50% are considered as negative.

### Sheep sera

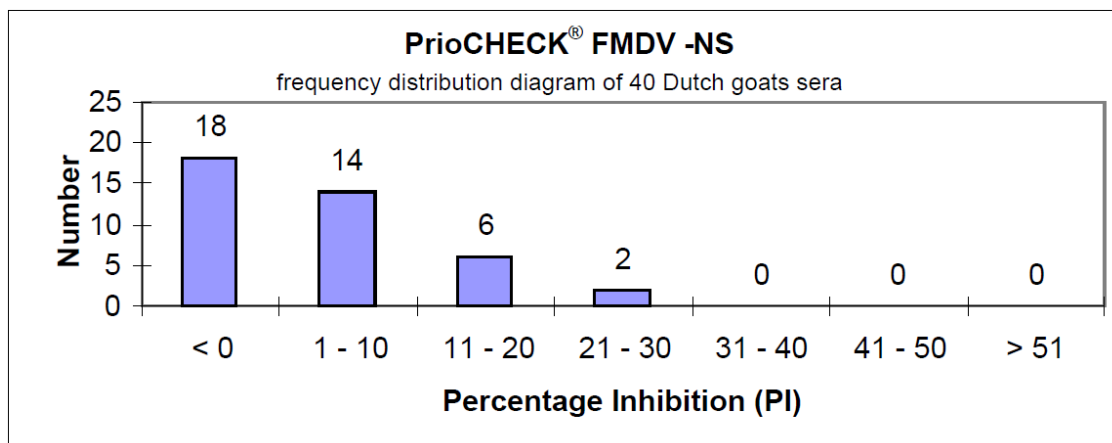
Serum samples from 40 Dutch sheep were tested. All Samples resulted negative.



PI values below 50% are considered as negative.

### Goat sera

Serum samples from 40 Dutch goats were tested. All Samples resulted negative.



PI values below 50% are considered

### Conclusion:

All samples from the different species were correctly tested negative.

## **Repeatability**

The repeatability was calculated based on the positive control and weak positive control sample from 22 different production lots.

## **Results**

Repeatability of the weak positive control: 7.64 %

Repeatability of the positive control: 3.51 %

## **Conclusion**

The repeatability of the test is for both control samples below 10 % and therefore it can be concluded that the PrioCHECK<sup>®</sup> FMDV-NS ELISA shows a good repeatability.

## External evaluations of the performance of the PrioCHECK® FMDV NS ELISA

The PrioCHECK® FMDV NS ELISA is broadly used in the field all over the world. There are numerous publications with the PrioCHECK® FMDV NS ELISA used.

In the following section some selected publication are referred which provide information on the performance of the PrioCHECK® FMDV NS ELISA.

### Comparison of commercial ELISAs for antibodies against FMDV non-structural proteins

*A. Dekker, P. Moonen, E.M. v.d. Linde*

- In total 1517 serum samples from cattle were tested. 953 negative samples, 564 positive samples from different experimental infection studies
- Different NS ELISA were compared
- The Cedi test (PrioCHECK® FMDV NS) showed the best performance from all assay in the study. The ELISA showed a very high sensitivity and specificity and detected antibodies of infected/vaccinated animals earlier than the other assays in the study. The ELISA showed further the lowest variation coefficient when testing dilutions of a positive control in each plate.
- **Conclusion:** The Ceditest ELISA (PrioCHECK® FMDV NS) is a very reliable and robust ELISA.

### Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus.

*Sørensen KJ1, Madsen KG, Madsen ES, Salt JS, Nqindi J, Mackay DK*

Arch Virol.1998;143(8):1461-76.

- Serum samples from vaccinated, with different monovalent vaccines, and naive cattle and sheep
- Different serum samples from experimental and field infected cattle and sheep
- The sensitivity for the 3ABC ELISA was 92% for sheep and 88% for cattle in this study.
- The specificity of the 3ABC ELISA was 100% for sheep and 99.8% for cattle in this study.
- **Conclusion:** The 3ABC ELISA was specific, sensitive and precise. The 3ABC ELISA is distinguishing between vaccinated and infected animals but not between carriers and non-carriers, and constituted a reliable means of detecting infected animals in a vaccinated population, irrespective of the serotype involved.

## **Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus**

*Brocchi E, Bergmann IE, Dekker A, Paton DJ, Sammin DJ, Greiner M, Grazioli S, De Simone F, Yadin H, Haas B, Bulut N, Malirat V, Neitzert E, Goris N, Parida S, Sørensen K, De Clercq K.*

Vaccine. 2006 Nov 17;24(47-48):6966-79. Epub 2006 May 6.

- 3551 serum samples were tested with six assays that detect antibodies to the non-structural proteins of FMD virus
- The sera came from naive, vaccinated, infected and vaccinated-and-infected animals; two-thirds from cattle, the remainder from sheep and pigs.
- **Conclusion:**
  - The commercial Ceditest kit (PrioCHECK<sup>®</sup> FMDV NS) and the in-house IZS-Brescia test perform comparably to the Index NCPanaftosascreeing ELISA and the highest sensitivities for both experimental and field sera were observed with these three tests.
  - In this trial, the highest specificities were provided by the IZS-Brescia and Ceditest ELISAs (PrioCHECK<sup>®</sup> FMDV NS) with 99.7% and 99.2%, respectively. For sheep and pigs, specificities approached 100%, even at the first screening.