Microarray Detection and Typing of Livestock and Poultry Viruses

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Assay Development

- detection of multiple pathogens
  - Livestock/Poultry HC diseases
- simultaneous detection and typing
  - FMDV serotyping
  - AIV H and N-typing
  - NDV pathotyping
- species differentiation of viral vectors
  - Chiroptera

Work Flow

Samples

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DNA/RNA Extraction

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Amplification

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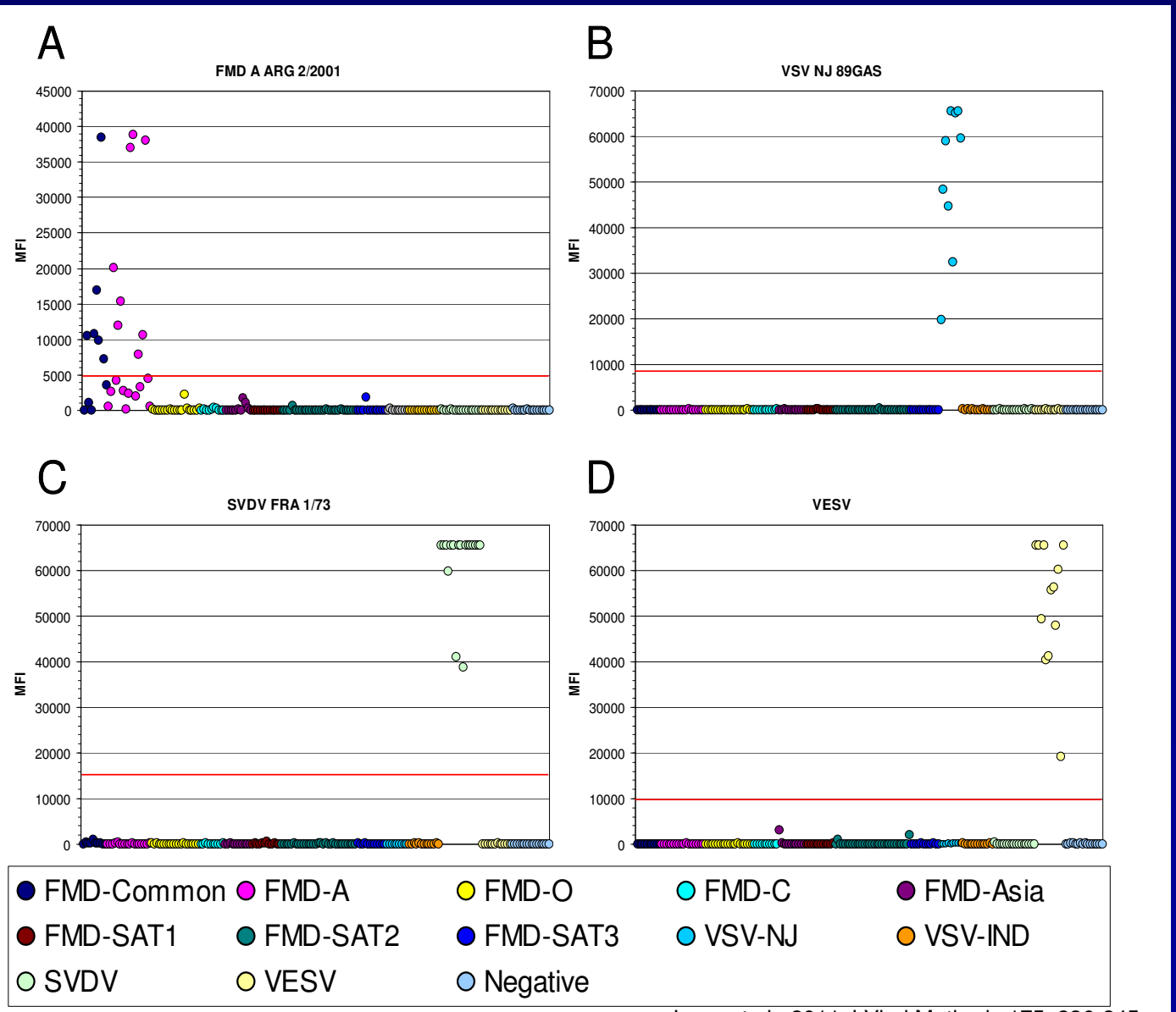
Microarray

Slide Microarray – Vesicular Disease Chip



Lung et al., 2011 J Virol Methods 175: 236-245

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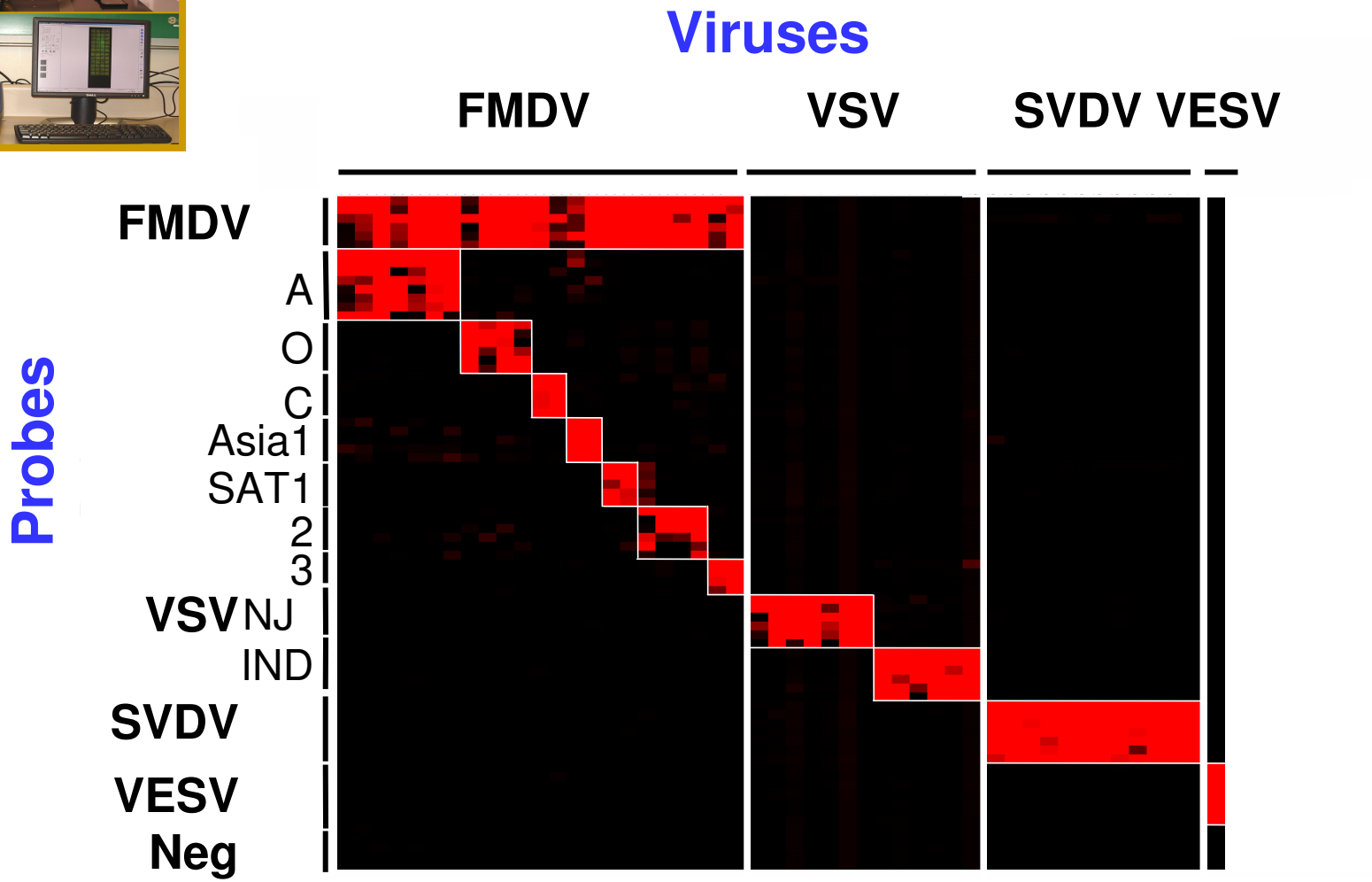
Vesicular Disease Chip



Lung et al., 2011 J Virol Methods 175: 236-245

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Electronic Microarray (NanoChip-400)

- Automated probe spotting, hybridization, washing, reporting
- All 400 test sites are independently controlled

Time (seconds)
Electronic
Hybridization

Time (hours)
Passive
Hybridization

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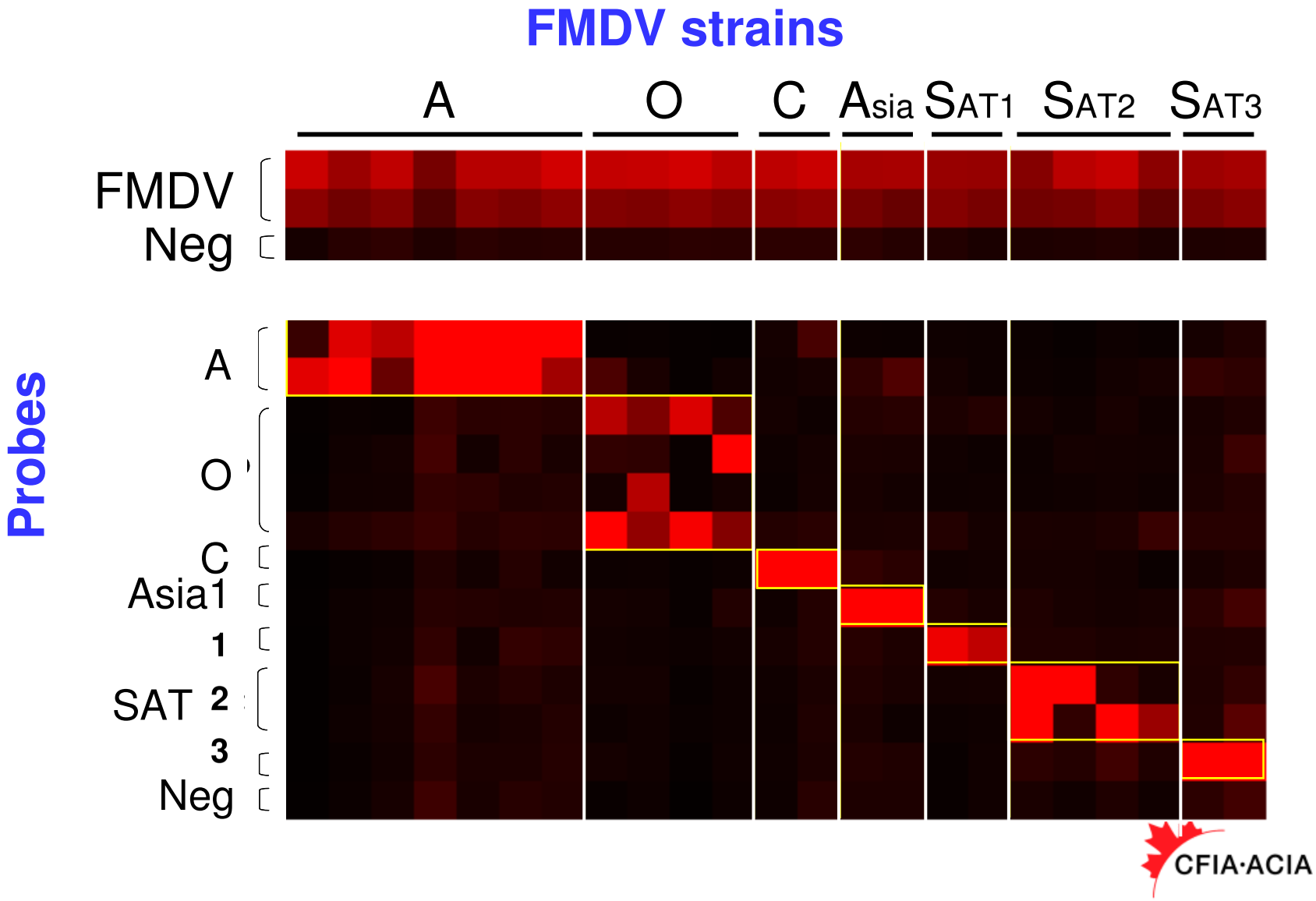
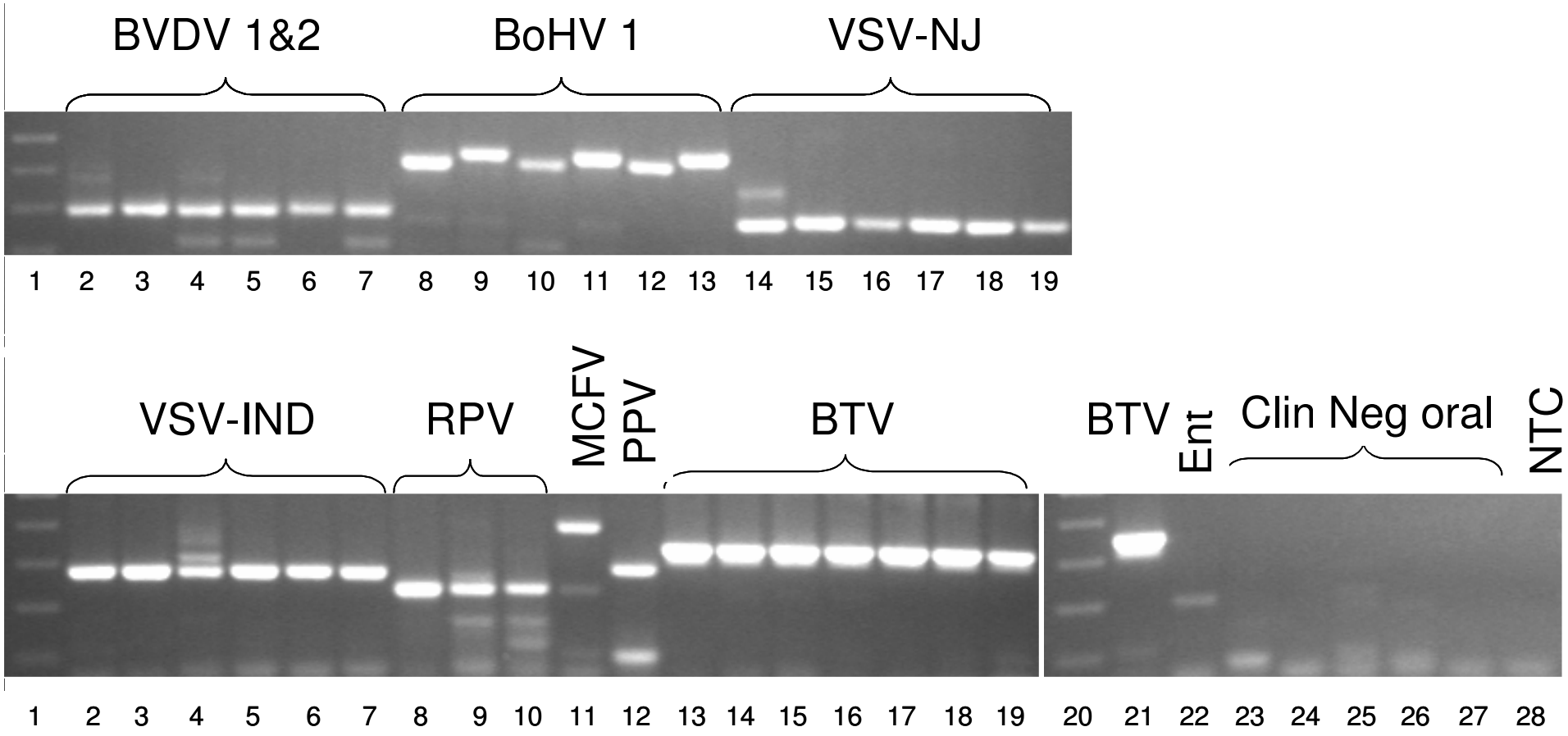
FMD Detection and Serotyping (NanoChip400)



Bovine HC Assay multiplex PCR



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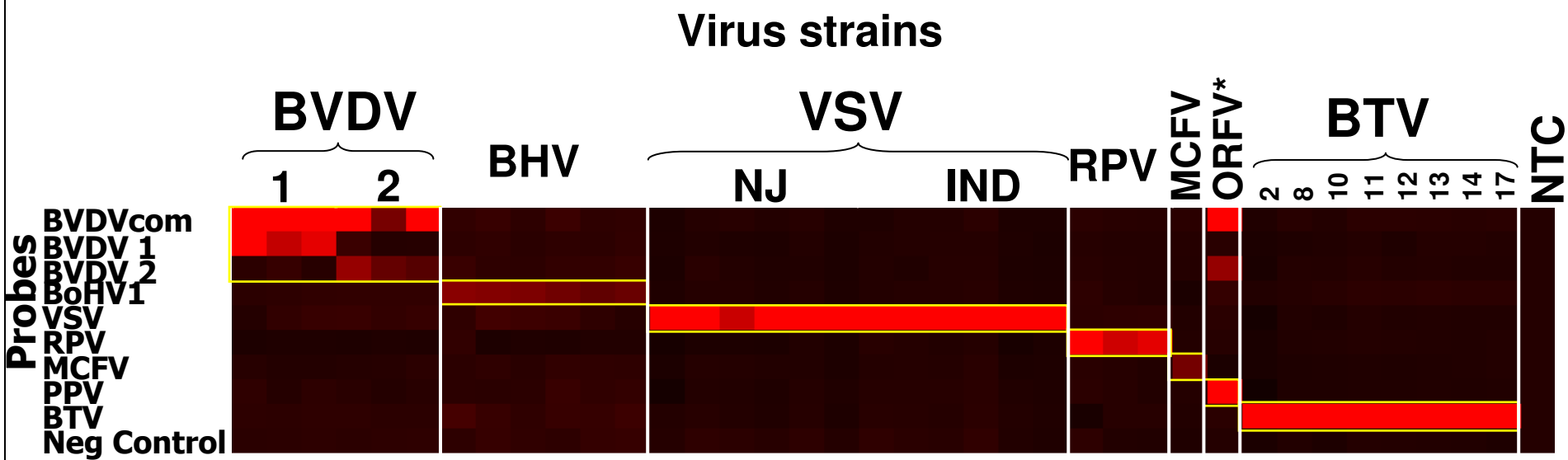
Bovine HC Assay (NanoChip400)



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Bovine-HC Assay: clinical and spiked samples

Virus	No. of Samples	No. of Animals	Sample Type	Microarray Detection
VSV (NJ/IND)	24	4	oral & nasal	• 2 dpi for both serotypes
RPV	16	4	oral & nasal	• 6 dpi
BTV	7	1	whole blood	• 8 dpi
BVDV-1 & 2	6 (spiked)	3	oral & nasal	• all samples
BoHV-1	4 (spiked)	2	oral & nasal	• all samples
PPV	6 (spiked)	3	oral & nasal	• all samples
Negative	42	34	oral & nasal	• negative

Total Samples n=105

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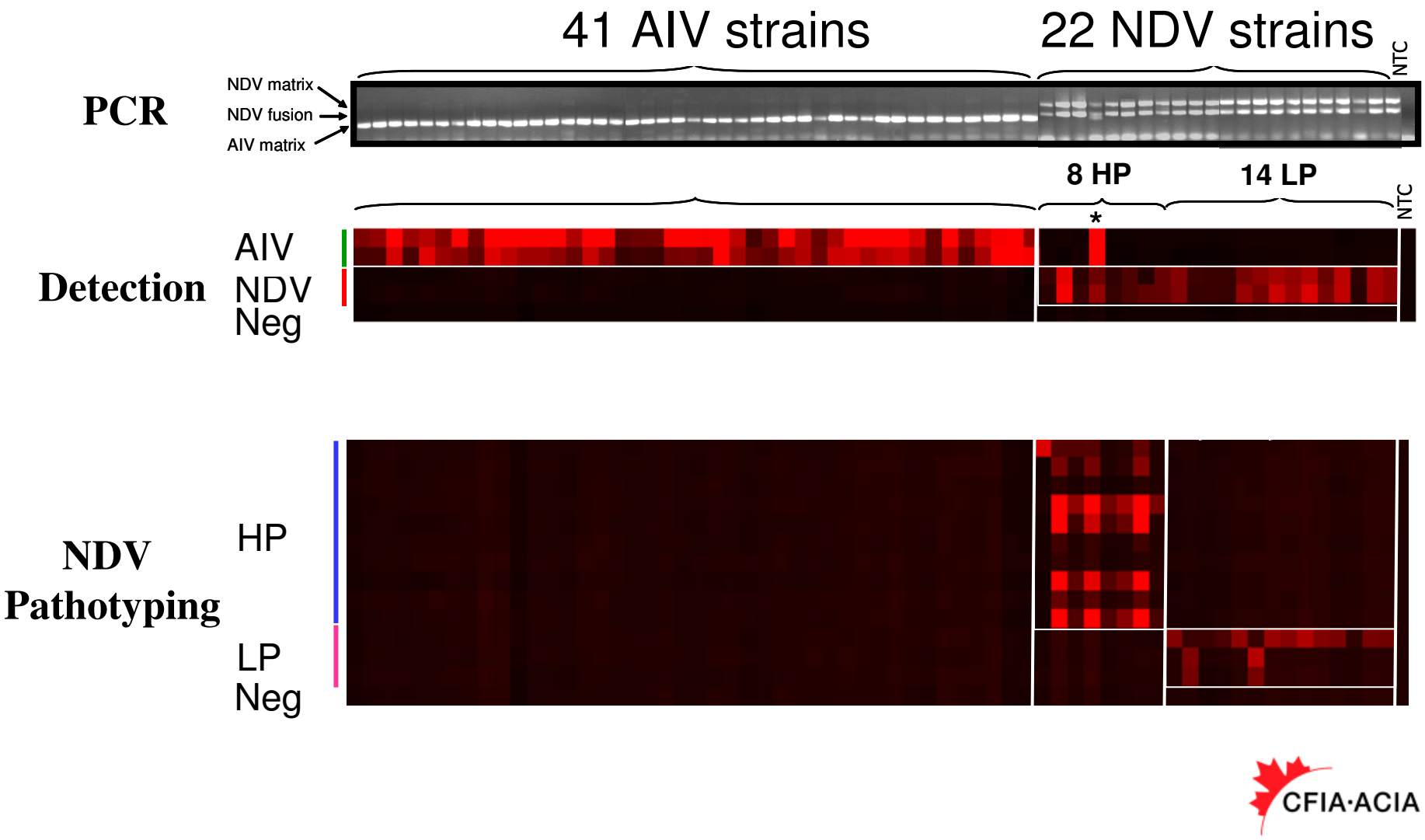
AIV/NDV Detection & NDV Pathotyping

(NanoChip400)



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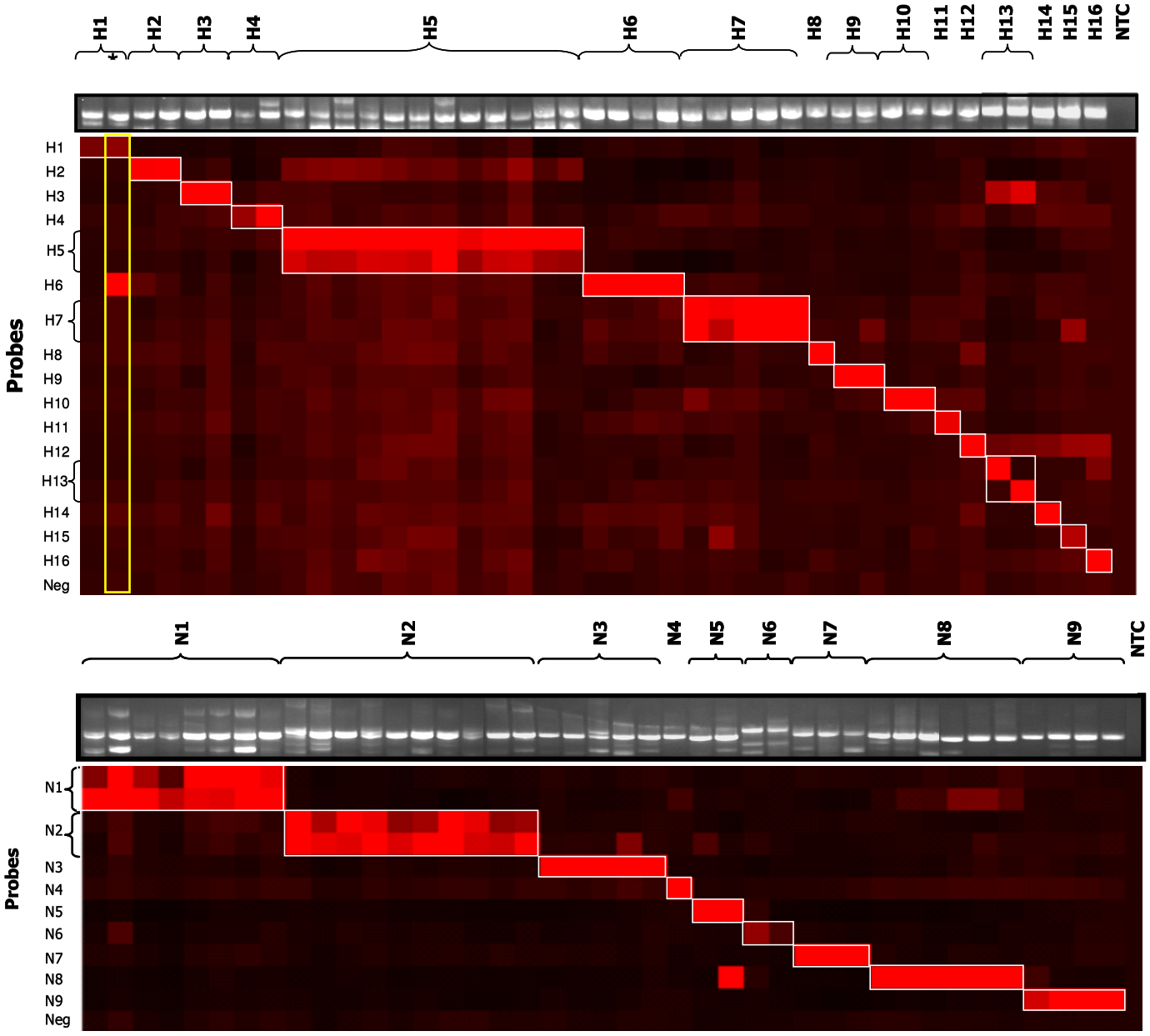
AIV H and N-typing (NanoChip 400)



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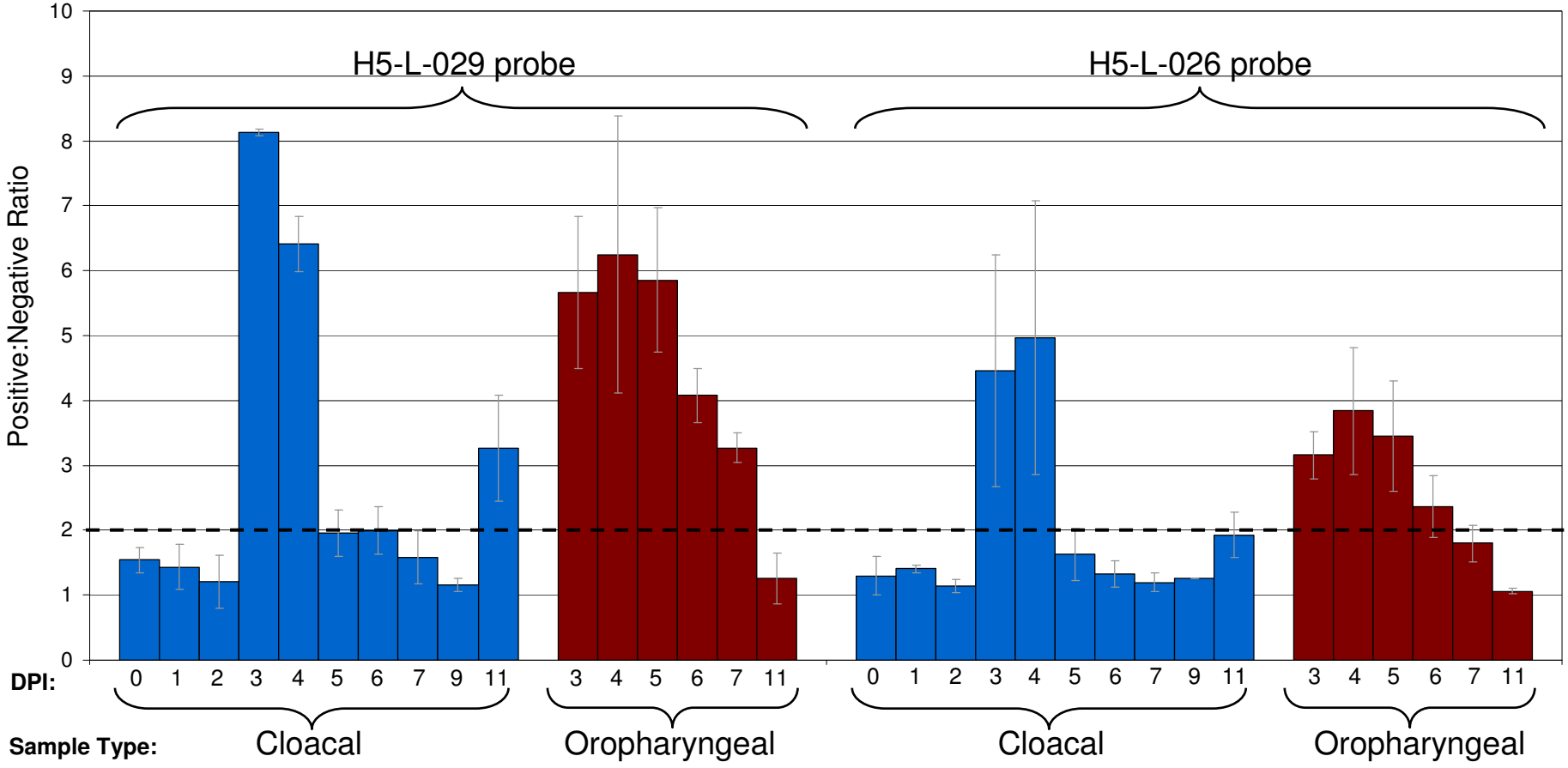
Detection of Early Infection (H5N2 HP)



HA-NC	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-
qRT-PCR	-	-	-	S	+	+	S	-	-	-	+	+	+	S	S	-	-	-	-	S	+	+	S	-	-	-	+	+	+	S	S	-

Limits of Detection[a]

Virus	Titre	Microarray		Real-Time RT-PCR
		Multiplex Detection	Typing[b]	
AIV H5N2 A/Duck/British Columbia/26-6/2005	2×10^5	+	+/+	+
	2×10^4	+	+/+	+
	2×10^3	+	+/+	+
	2×10^2	+	+/+	+
	2×10^1	-	+/-	+
	2×10^0	-	-	-
AIV H7N3 A/Chicken/British Columbia/514/2004	4×10^5	+	+/+	+
	4×10^4	+	+/+	+
	4×10^3	+	+/+	+
	4×10^2	+	+/+	+
	4×10^1	-	+/+	-
	4×10^0	-	+/-	-
	4×10^{-1}	-	-	-
NDV DYHAMAZON ICE 99-10-25 (HP)	1×10^5	+	+	+
	1×10^4	+	+	+
	1×10^3	+	+	+
	1×10^2	-	-	+
	1×10^1	-	-	-
	1×10^0	-	-	-
NDV B1(LP)	1×10^5	+	+	+
	1×10^4	+	+	+
	1×10^3	+	+	+
	1×10^2	+	+	+
	1×10^1	+	-	+
	1×10^0	-	-	+
	1×10^{-1}	-	-	+
	1×10^{-2}	-	-	-

[a] The results for the RT-PCR developed in this study were identical to the microarray

[b] All AIV typing results were presented in the following order: H typing, N typing

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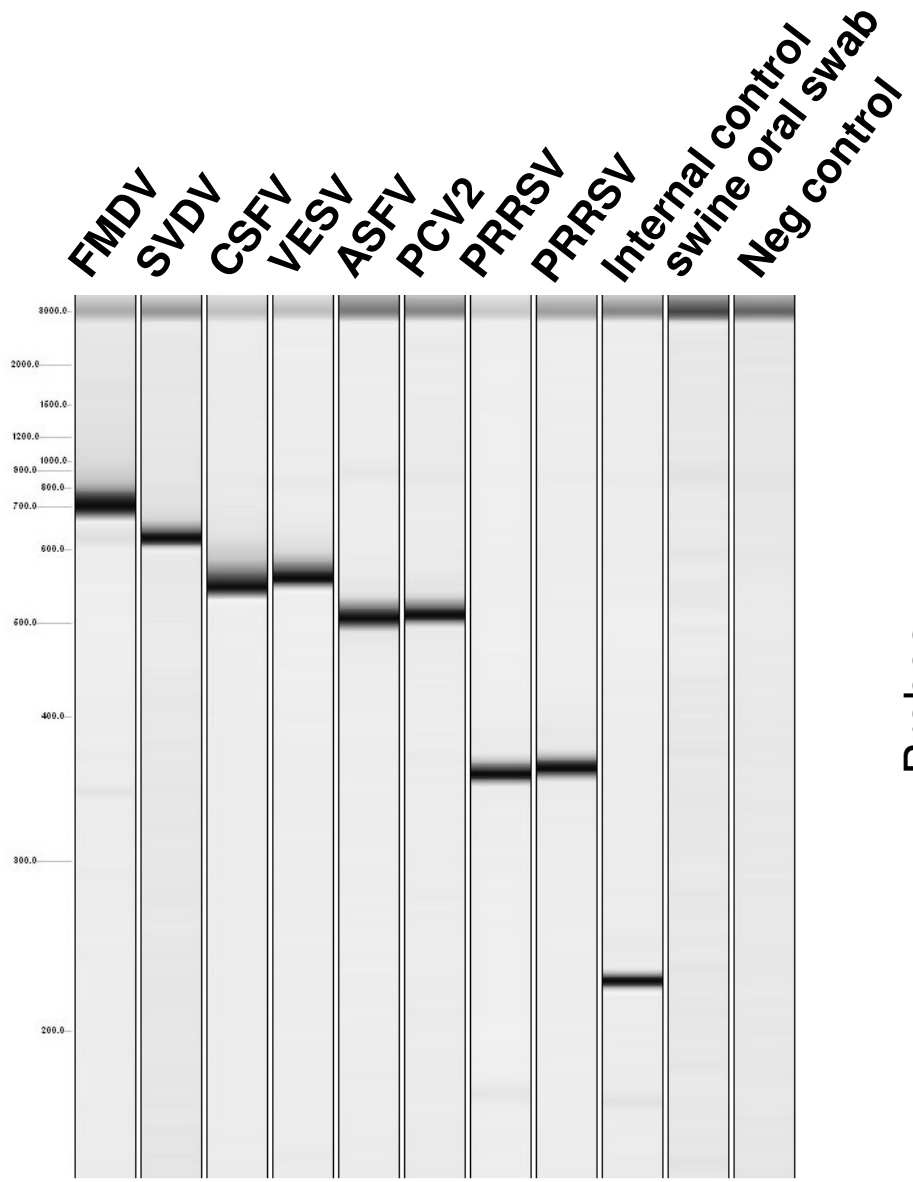
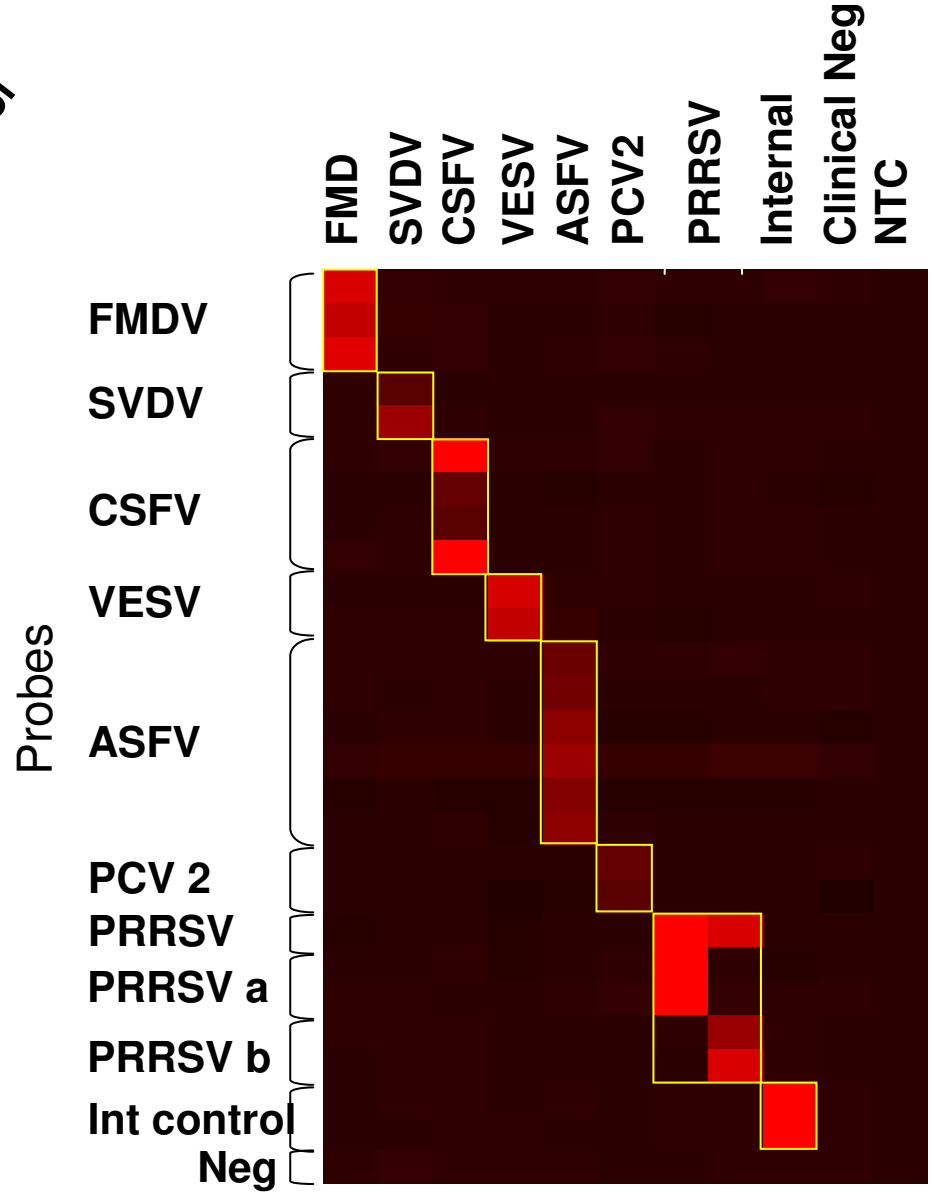
Swine HC Assay (NanoChip400)





Microarray Automation

Samples

↓

DNA/RNA Extraction

↓

Amplification

↓

Microarray

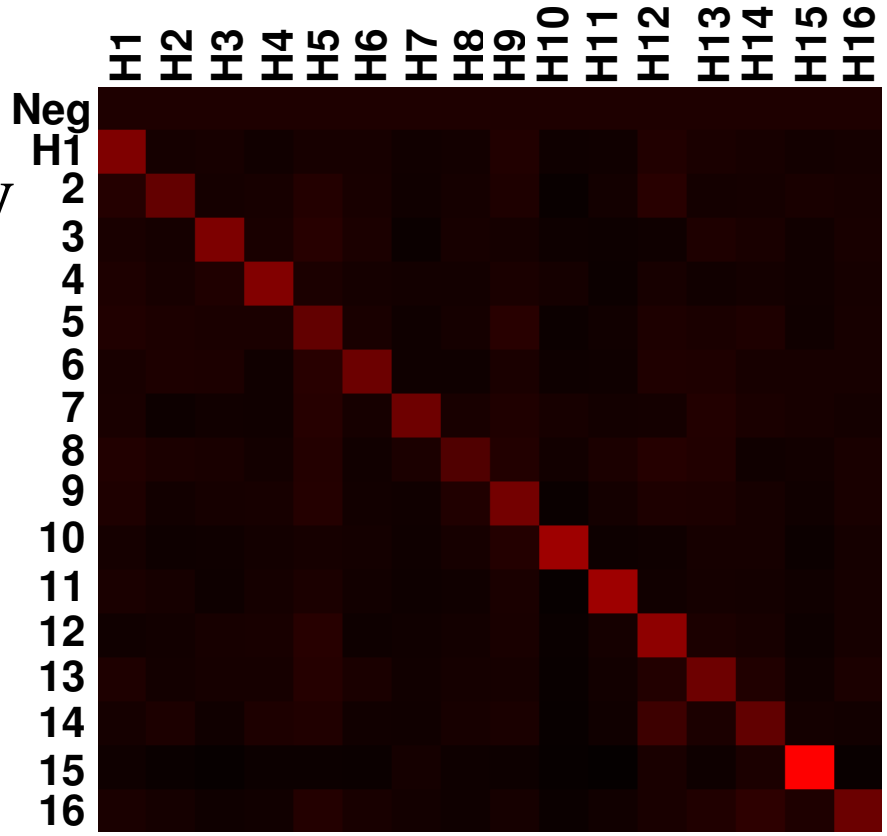
Fully Automated

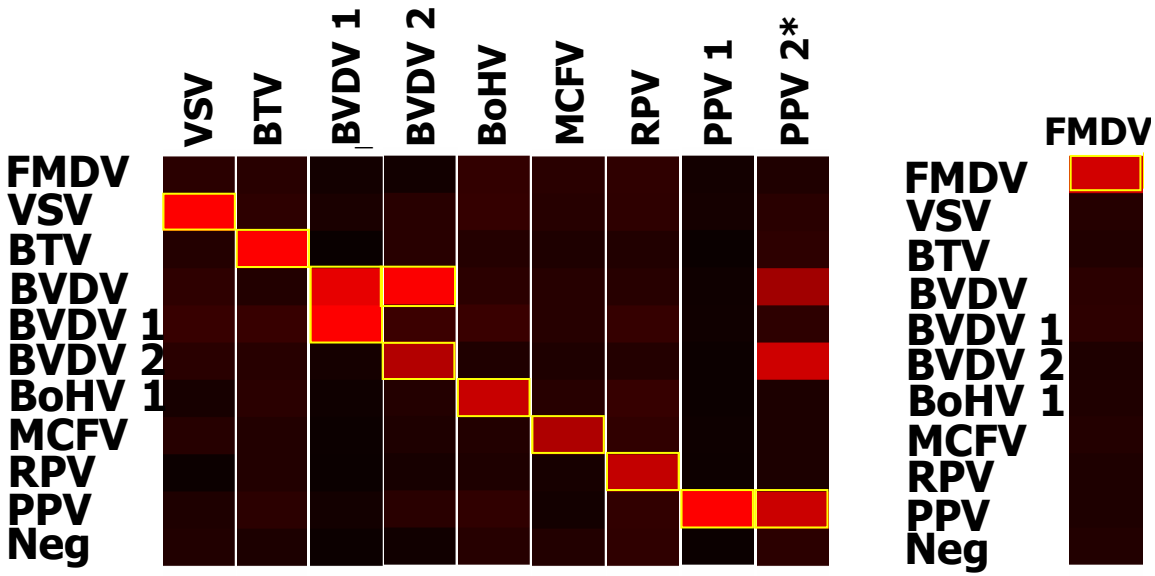
Fully Automated & Portable

Detection on new carbon electrode array

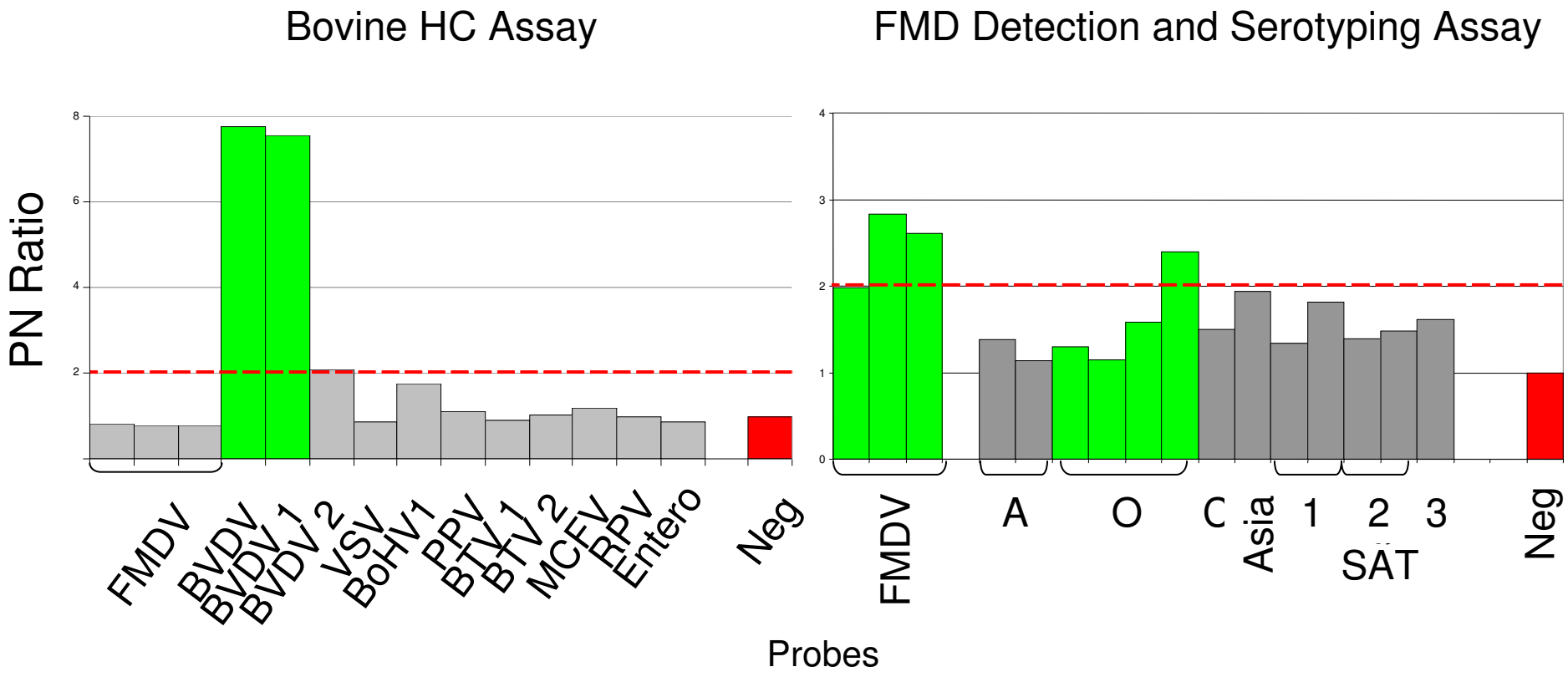
AIV H-typing

Bovine HC





Portable Integrated Instrument



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