

Detecting viruses using microarrays

Neil Boonham

Central Science Laboratory



Update on virus detection work

- (1) EU project DiagChip – Finished 2005
- (2) Defra virus chip – Started 2006



(i) Potato pathogen array

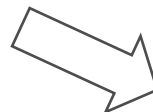
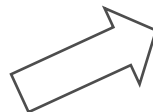
- EU FP5 funded project (www.diagchip.co.uk)
- Investigate the feasibility of detecting the EU quarantine potato pathogens using array techniques
 - 12 Viruses
 - 2 Bacteria
 - 1 Fungi
 - 6 Invertebrates
 - 1 viroid
 - 1 phytoplasma



1 Sample



2 Total Nucleic Acid Extraction



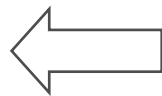
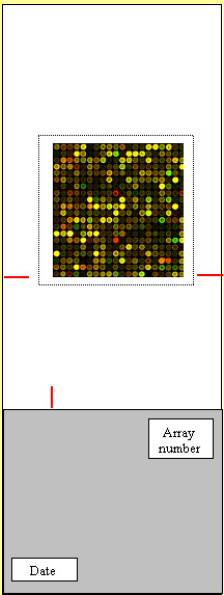
3 RNA labelling



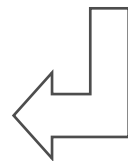
PCR labelling

Total time = 1.2 days
(hands on \approx 2 hours)

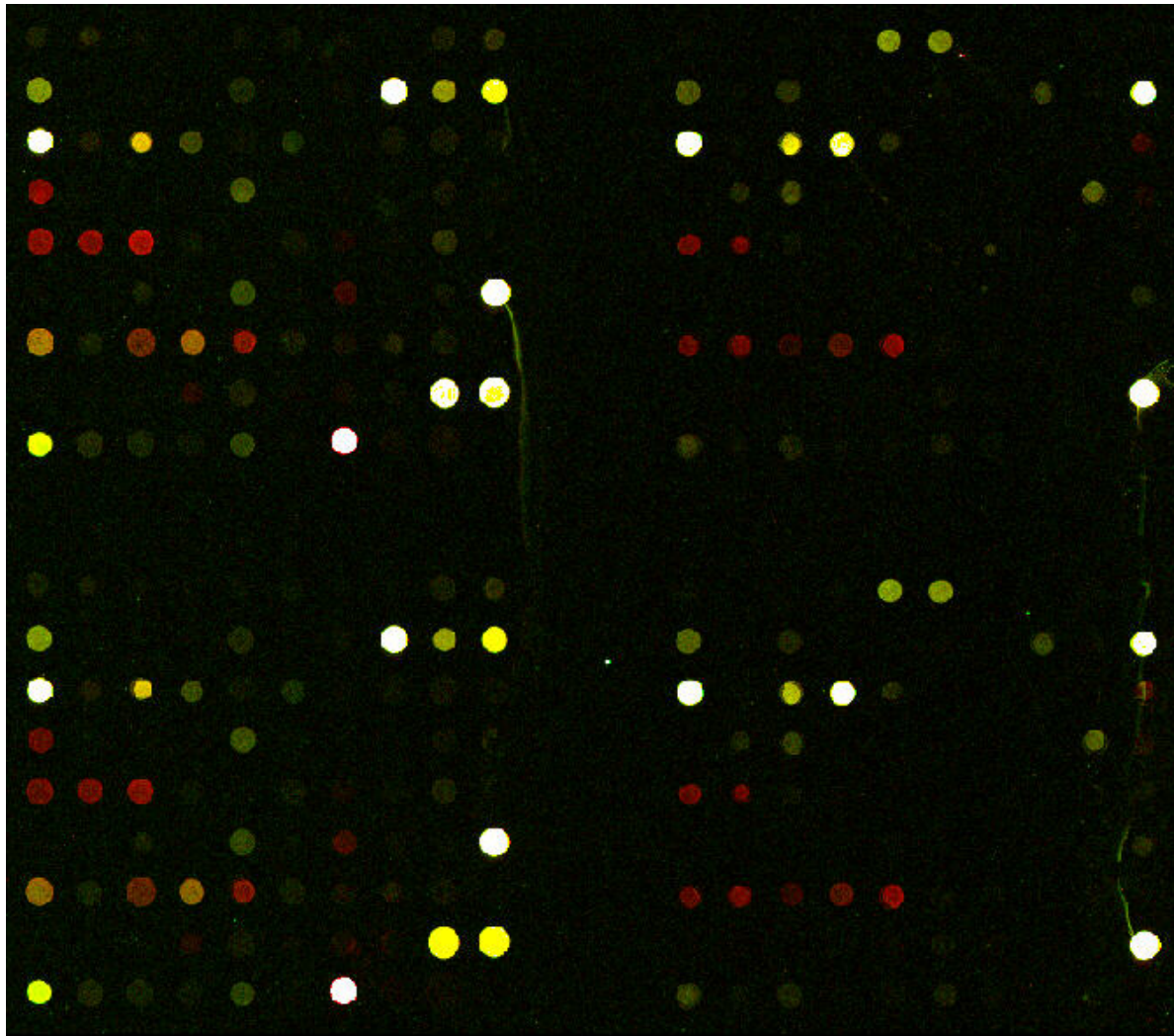
5 Scan Arrays



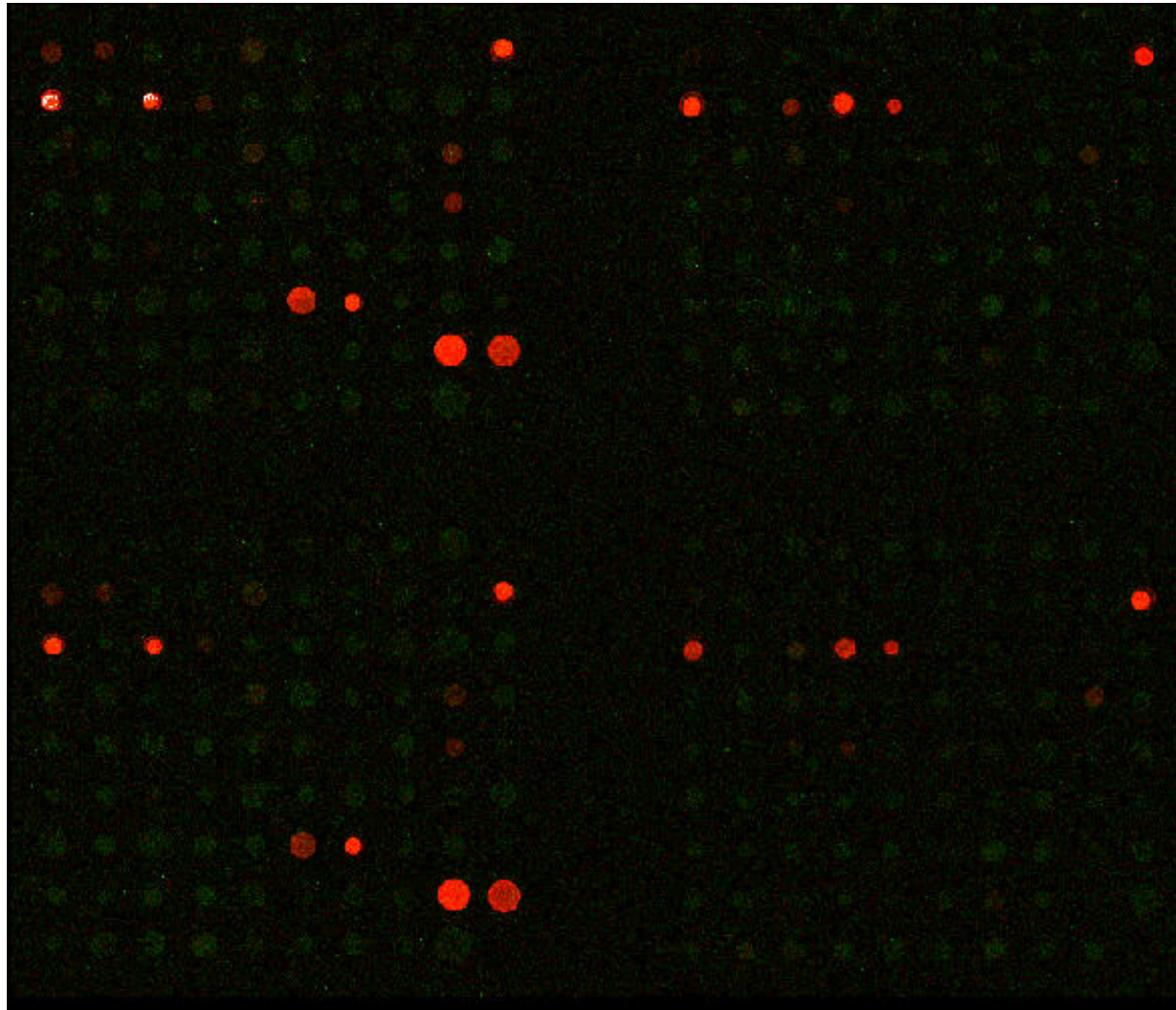
4 Hybridisation



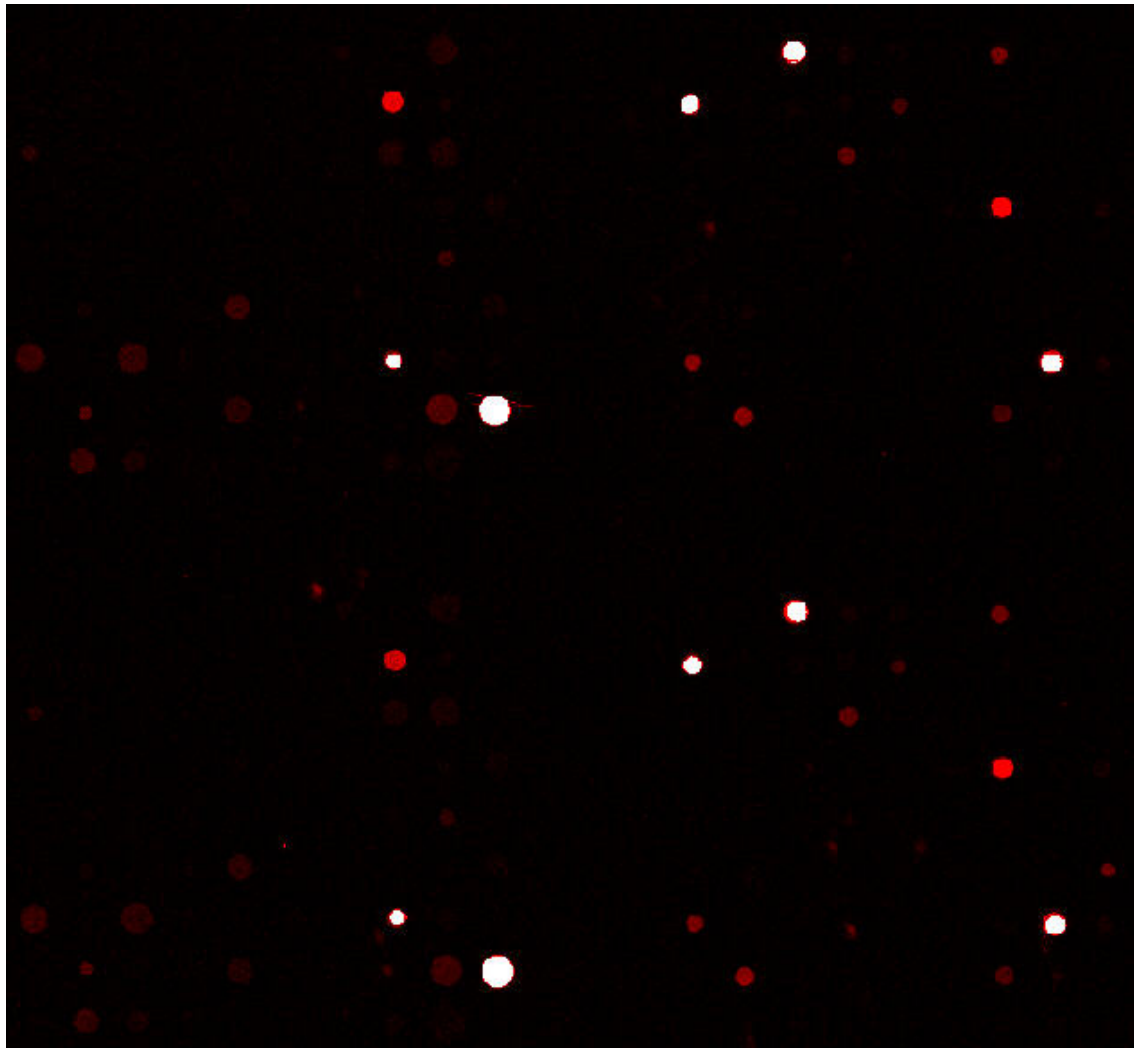
Results: Mixed infection of potato viruses



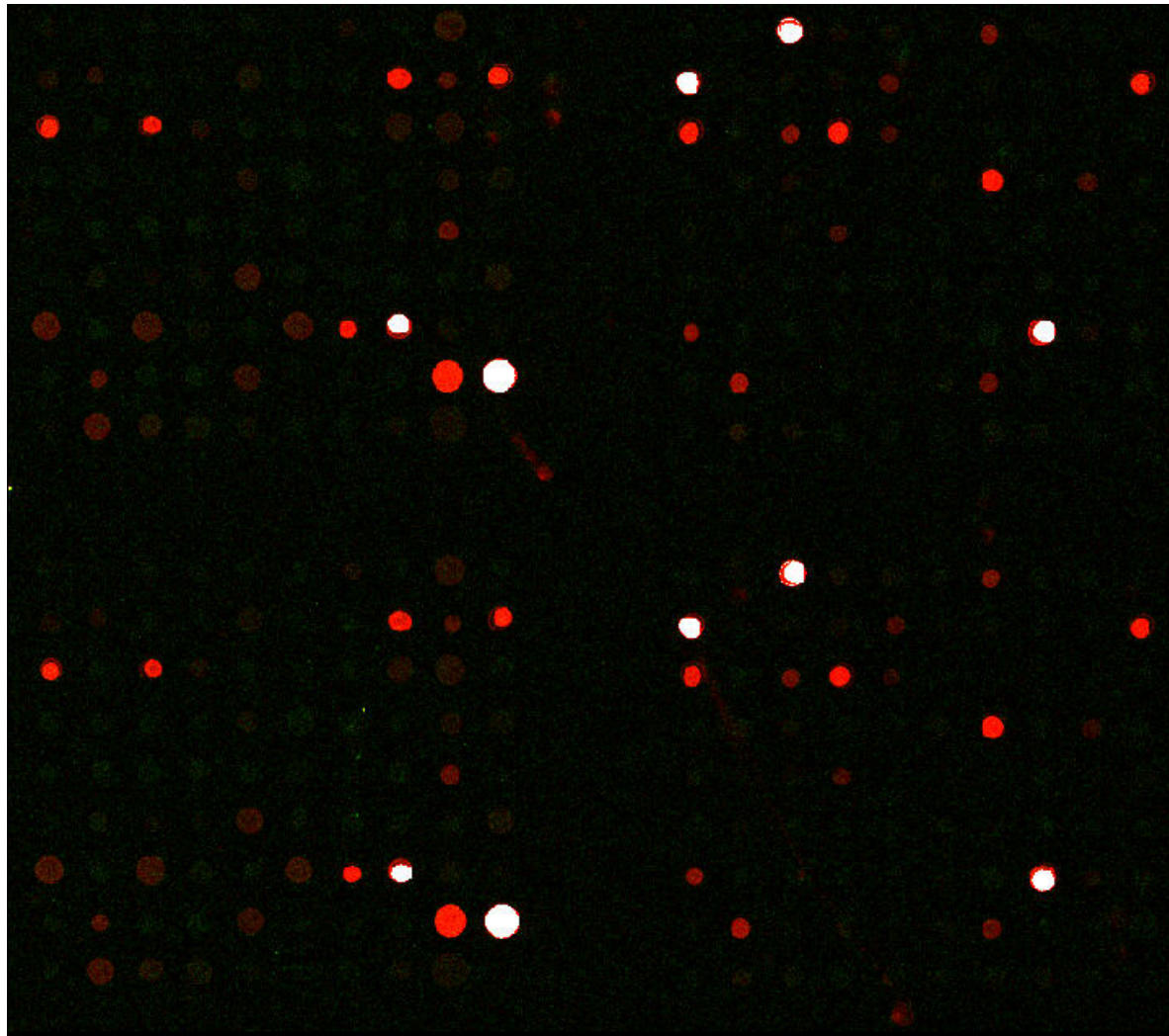
Results: Bacteria



Results: Nematode

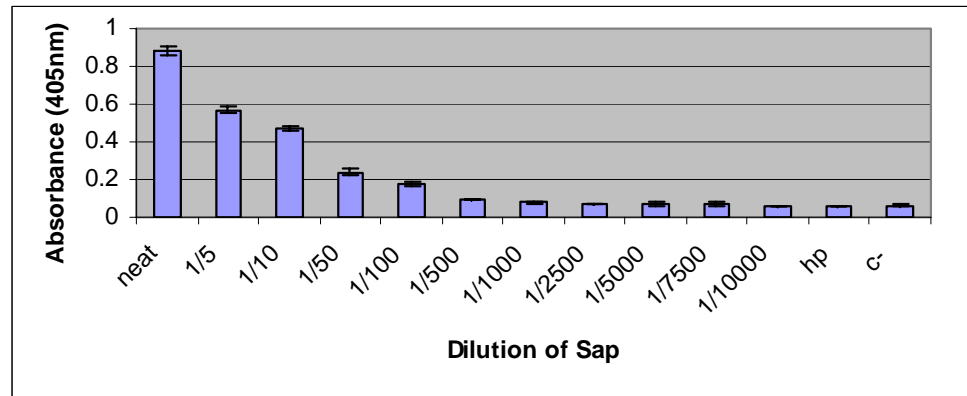


Results: Nematode and Bacteria



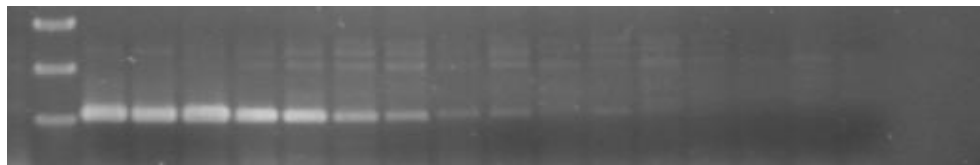
Sensitivity

ELISA



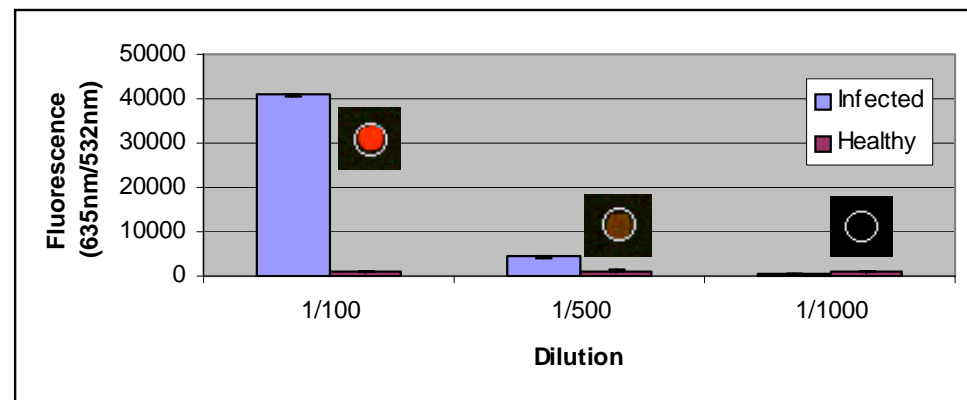
1/100

PCR



1/5000

Array



1/500

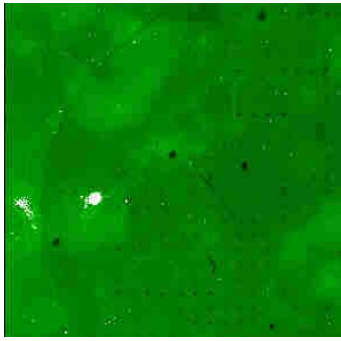


Ringtesting

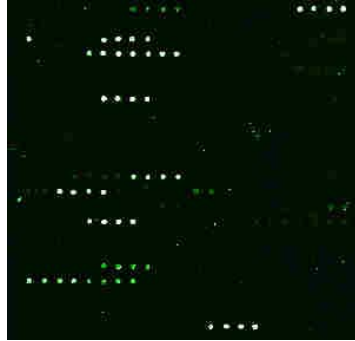
- 7 Laboratories
 - Mixed expertise from array experts to non-molecular
 - Identical 3 day training
- 6 samples
 - Samples 1-3 through the whole protocol
 - Samples 4-5 through the DNA labeling protocol
 - Sample 6 through RNA labeling protocol



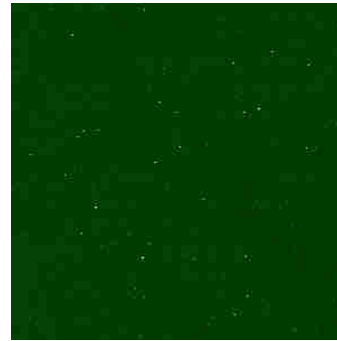
Healthy plants (sample 1)



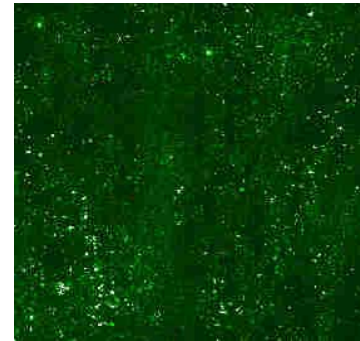
X



✓



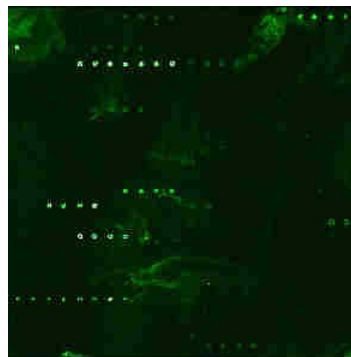
X



X



✓



✓

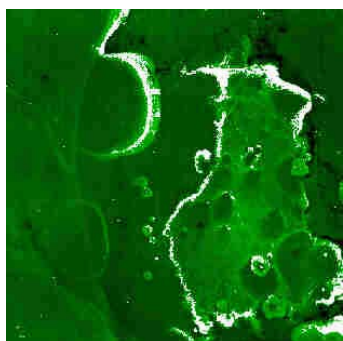


✓

4/7 correct



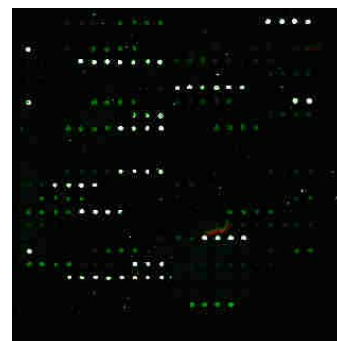
Potato virus Y (PVY) (sample 2)



X



✓



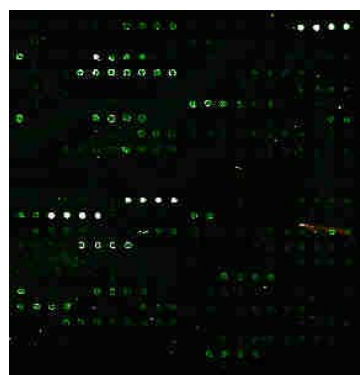
✓



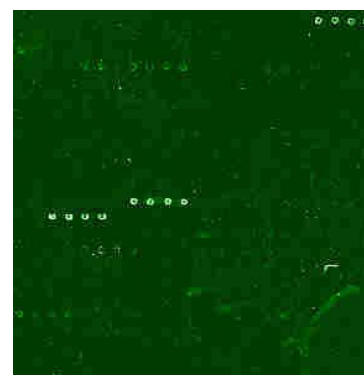
X



✓



✓

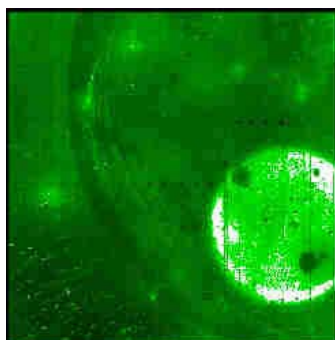


✓/X

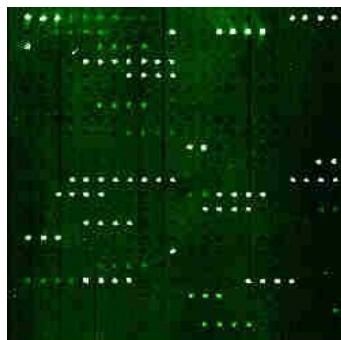
4/7 correct



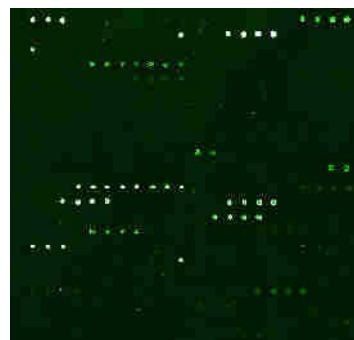
Potato virus X (PVX) (sample 3)



X



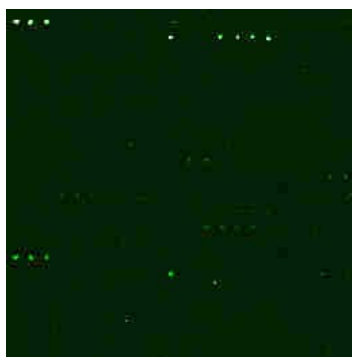
✓



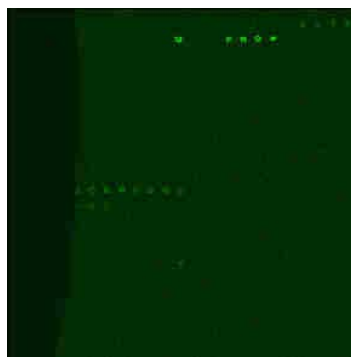
✓



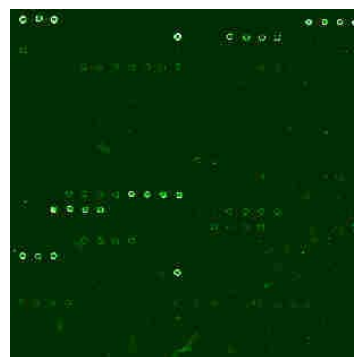
X



X / ✓



X / ✓



✓

5/7 correct



Clavibacter michiganense subsp. *sepedonicus* (sample 4)



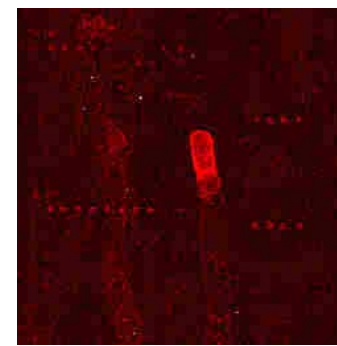
✓



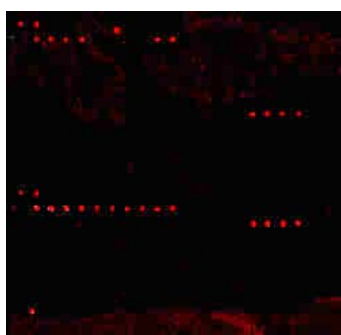
✓



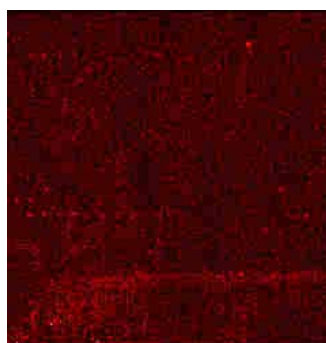
✓



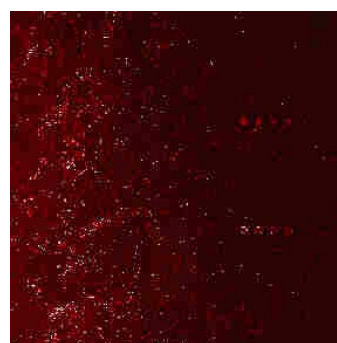
✓



✓



X

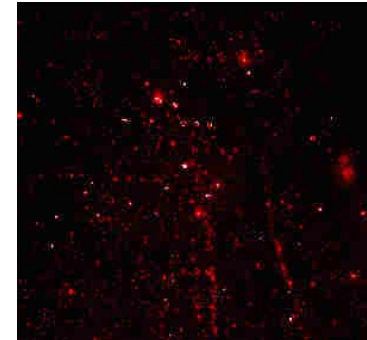
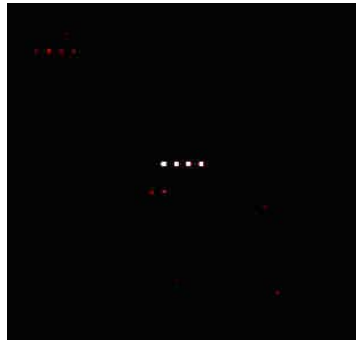
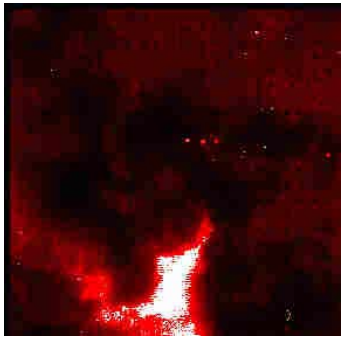


✓

6/7 correct



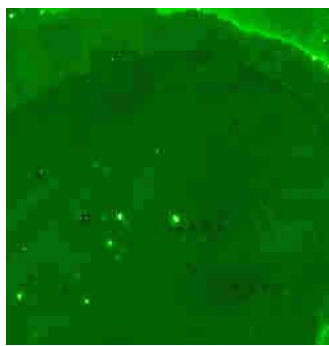
Ralstonia solanacearum (sample 5)



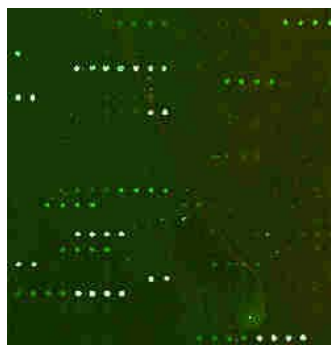
7/7 correct



Potato virus A (PVA) (sample 6)



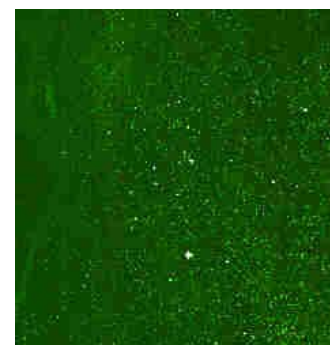
X



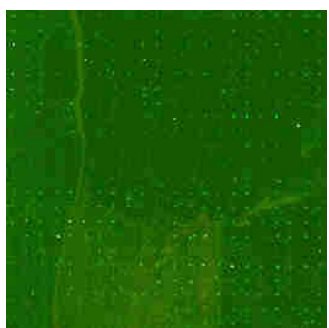
✓



✓



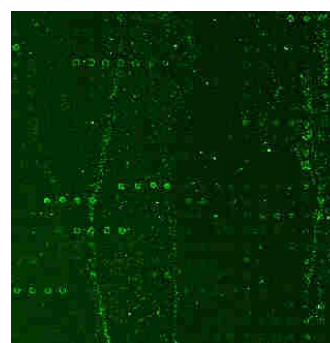
X



X



✓



✓

4/7 correct



Comparison of the laboratory performance

Testing Lab	Sample number					
	1	2	3	4	5	6
1	x	x	x	x	✓	x
2	✓	✓	✓	✓	✓	✓
3	x	✓	✓	✓	✓	✓
4	x	x	x	(✓)	(✓)	x
5	✓	✓	✓	✓	✓	x
6	✓	✓	✓	(✓)	✓	x
7	✓	x	✓	✓	✓	x



Comparison of the laboratory performance

Testing Lab	Sample number					
	1	2	3	4	5	6
1	x	x	x	x	✓	x
2	✓	✓	✓	✓	✓	✓
3	x	✓	✓	✓	✓	✓
4	x	x	x	(✓)	(✓)	x
5	✓	✓	✓	✓	✓	x
6	✓	✓	✓	(✓)	✓	x
7	✓	x	✓	✓	✓	x

Comparison of the laboratory performance

Testing Lab	Sample number					
	1	2	3	4	5	6
1	x	x	x	x	✓	x
2	✓	✓	✓	✓	✓	✓
3	x	✓	✓	✓	✓	✓
4	x	x	x	(✓)	(✓)	x
5	✓	✓	✓	✓	✓	x
6	✓	✓	✓	(✓)	✓	x
7	✓	x	✓	✓	✓	x

Comparison of the laboratory performance

Testing Lab	Sample number					
	1	2	3	4	5	6
1	x	x	x	x	✓	x
2	✓	✓	✓	✓	✓	✓
3	x	✓	✓	✓	✓	✓
4	x	x	x	(✓)	(✓)	x
5	✓	✓	✓	✓	✓	x
6	✓	✓	✓	(✓)	✓	x
7	✓	x	✓	✓	✓	x

Comparison of true positives and negatives

	True result					
		+			-	Total
Array result	+	24			0	24
			A	B		
	-	11*			7*	18
			C	D		
Total	35			7	42	

*13 samples would be repeated due to failed controls

Diagnostic sensitivity ($A/A+C$) = 68%

Diagnostic specificity ($D/D+C$) = 100%



Conclusions: ringtesting

- More training / experience needed by some labs
- 1 laboratory got perfect results
- PCR labeling more reliable than RT labeling
- 2 samples: either 'controls' or 'target' positive not both



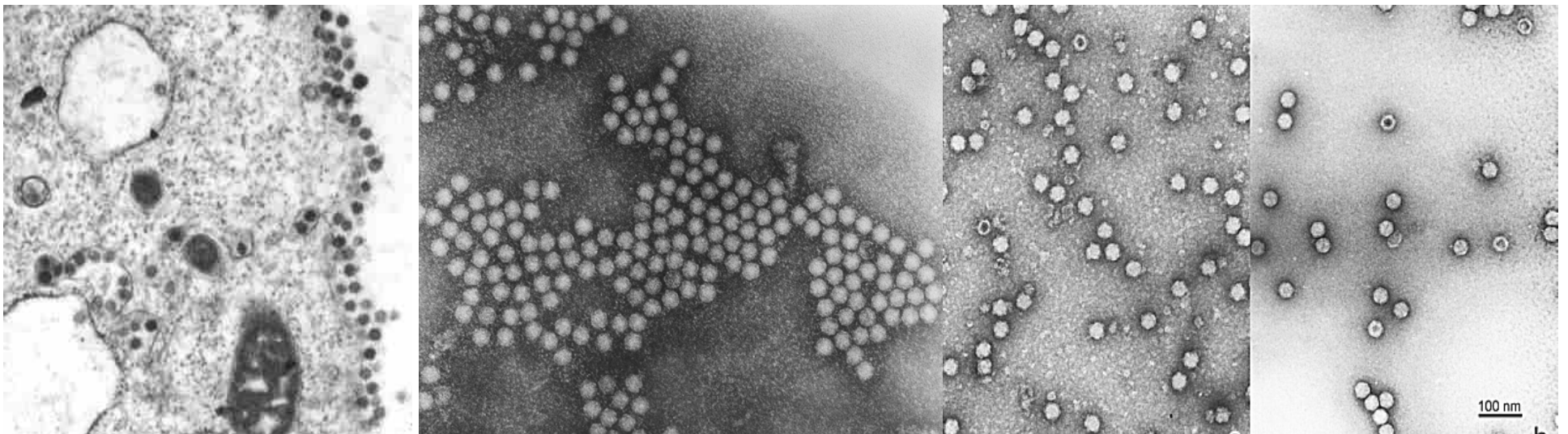
Conclusions: arrays so far

- Sensitivity similar to ELISA
- Suitable for 'diagnostic' testing, screening & identification
- Arrays being used in CSL diagnostic labs
- Most suitable for virus detection ?
- Sequencing more useful for fungi, bacteria and pests



(ii) Defra biosecurity chip

- Array detection of viruses important to Defra
- 500+ viruses of animals (including zoonotics), birds, plants, bees and fish
- Screening tool



Defra biosecurity chip

- Consortium of 7 laboratories
- Includes mandated testing laboratories



IAH Pirbright



THE ROYAL VETERINARY COLLEGE
UNIVERSITY OF LONDON



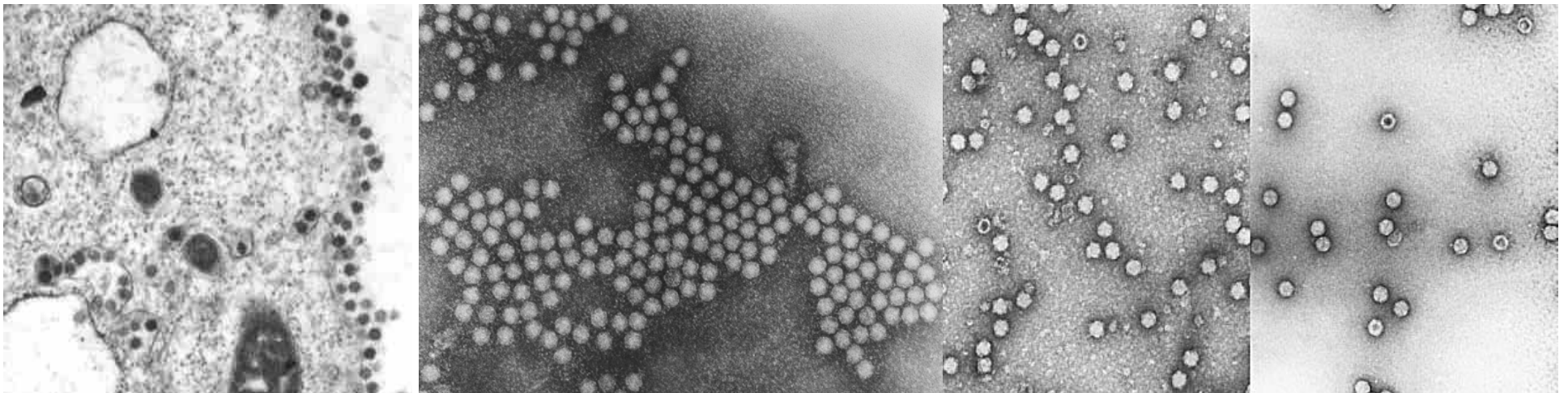
Cefas



IAH Compton

Main aims

- Investigate different array formats
- Amplification methods
- Bioinformatics – selecting oligos more effectively
- Effective detection at the genus level
- Redundancy to detect unknowns



Acknowledgements: CSL

- Jenny Tomlinson
- Anna Skelton
- Ian Barker



Acknowledgements: DiagChip participants

- DIAS, Slagelse Denmark
- PPS, Wageningen, The Netherlands
- PRI, Havlickuv Brod, Czech Republic
- SASA, East Craigs, Edinburgh, United Kingdom
- Universidade de Trás-os-Montes, Portugal
- NIB, Ljubljana, Slovenia



Acknowledgements: Biochip project

IAH-Compton

- Abu-Bakr Abu-Median
- Paul Britton
- Dave Cavanagh
- Mick Watson

VLA-Virology

- Rod Card
- Malcolm Banks
- Paul Golby
- Javier Nunez-Garcia

CEFAS

- David Stone

RVC

- Joe Brownlie
- Dirk Werling

HPA

- Nigel Silman
- Karen Kempsell
- Jane Burton
- James Oshota

CSL

- Ian Barker
- Neil Boonham
- Jenny Tomlinson

IAH-Pirbright

- Geoff Hutchings
- Andrew Shaw
- Scott Reid
- Don King
- Nick Knowles
- Juliet Dukes