

Multiplex detection using xMAP

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PLANT RESEARCH INTERNATIONAL
WAGENINGENUR

Available technologies

	IF	ELISA	MIA	FCM
Detection limit	10^3	10^4	10^4	10^4
Speed	low	high	very high	very high
Multiplexing	low	none/low	max. 100	max. 7
Wash steps	yes	yes	none/yes	none
Dilutions	yes	yes	no	yes
Vitality	no	no	no	yes



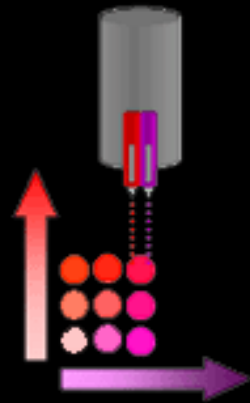
Outline

- Technique
- xSamples
 - Bacteria
 - Virus
- Summary
- Conclusion



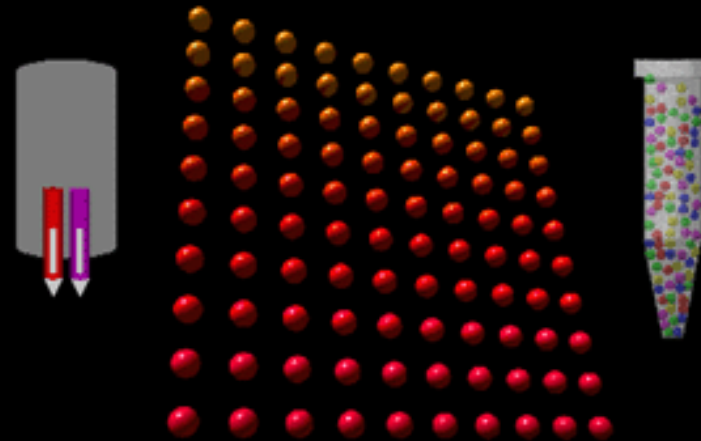
Luminex Technology

Color-coded Microspheres



Unique microsphere sets are color-coded using a blend of different fluorescent intensities of two dyes.

100 Color-codes = 100 Simultaneous Tests



Using this method, over 100 distinct microsphere sets can be created.



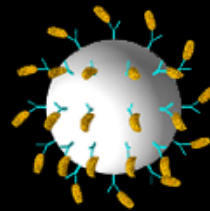
Luminex Technology

Microspheres as Molecular Carriers



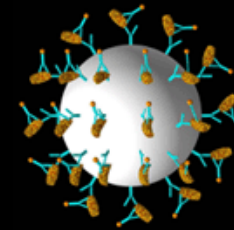
To perform a test, thousands of probes are bound to the microsphere.

Capturing the Sample Molecule



While suspended in a test sample, the bound probes collect molecules.

Tagging the Reaction

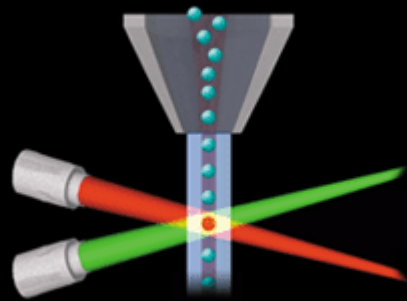


Fluorescently-labeled Reporter tags bind to the sample molecule.



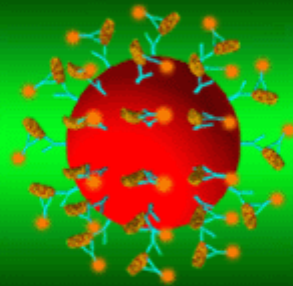
Microspheres/beads

Microspheres in a Fluid Stream



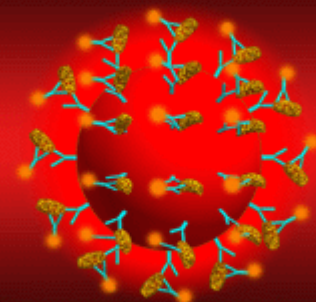
Precision fluidics align the microspheres in single file, and pass them through the lasers one at a time.

One Laser Excites Molecular Tags



Reactions are measured with fluorescent intensity and reported in real time.

Second Laser Excites Microsphere

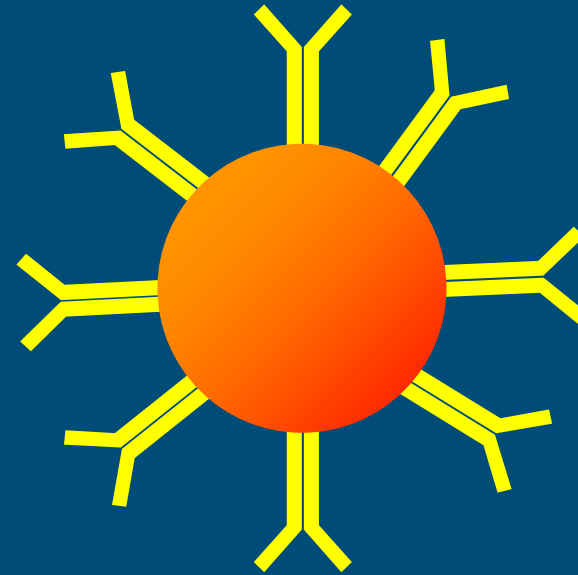


Fluorescent intensity of the microsphere identifies the reaction.



Immunological detection (MIA)

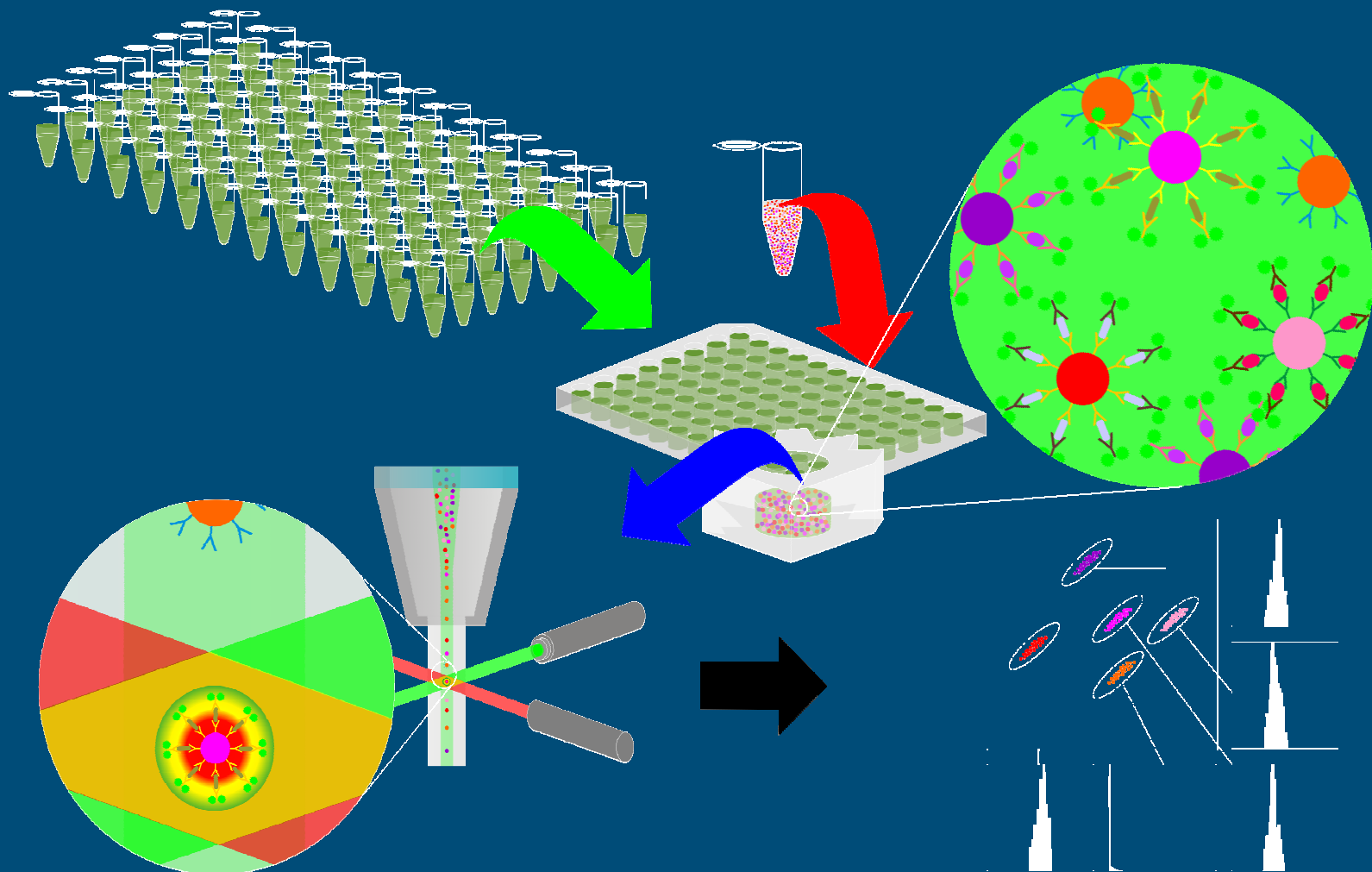
1st antibody



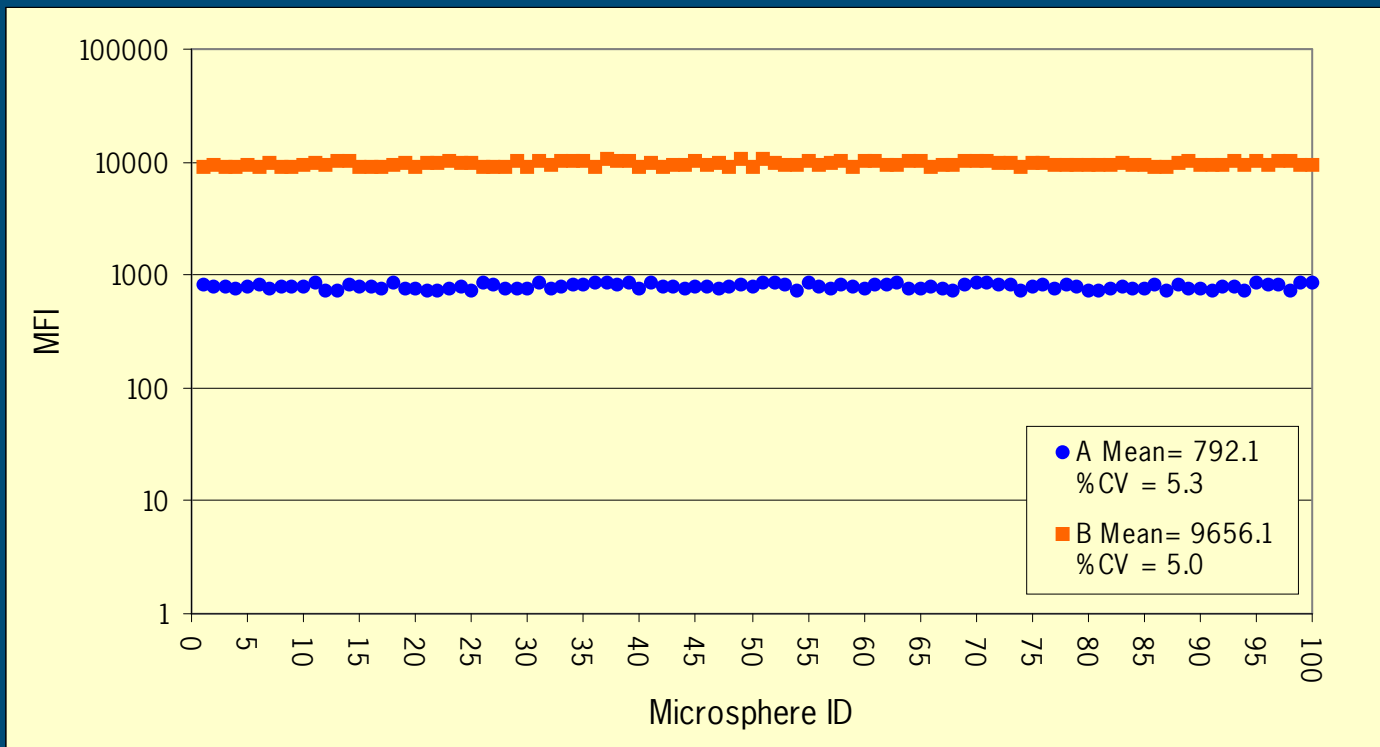
Bead has a specific address



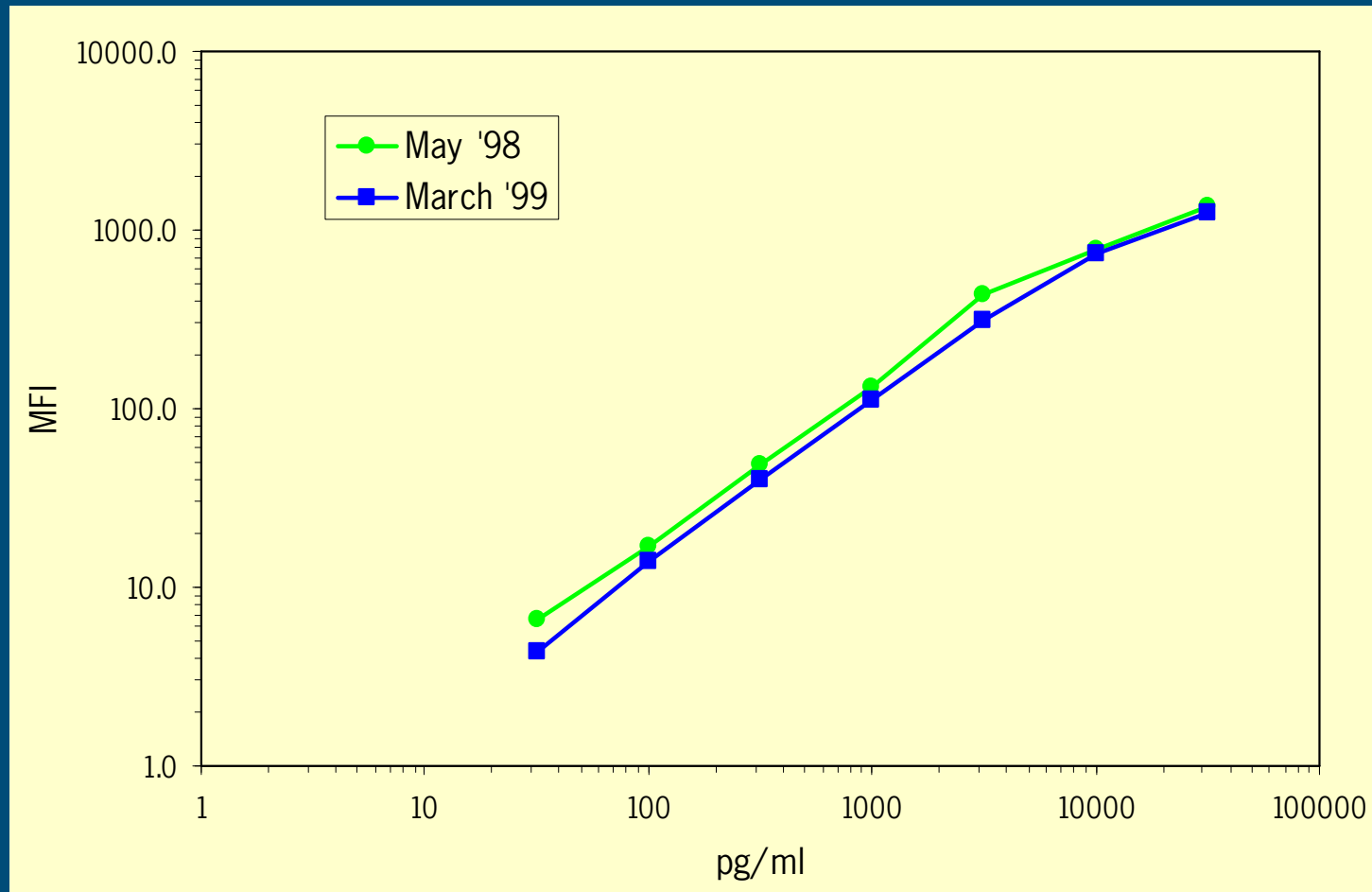
Overview of multiplex detection



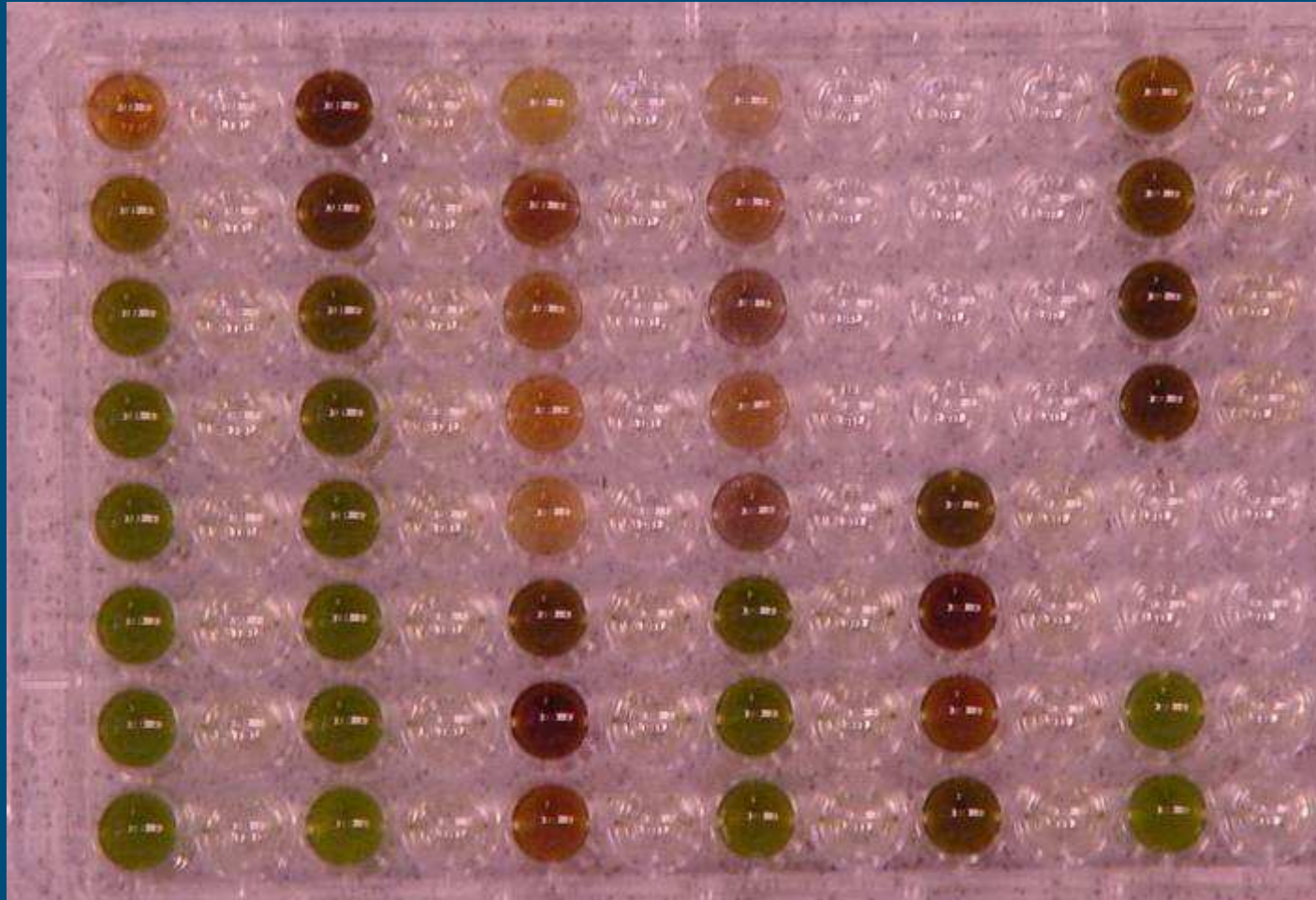
Reproducibility



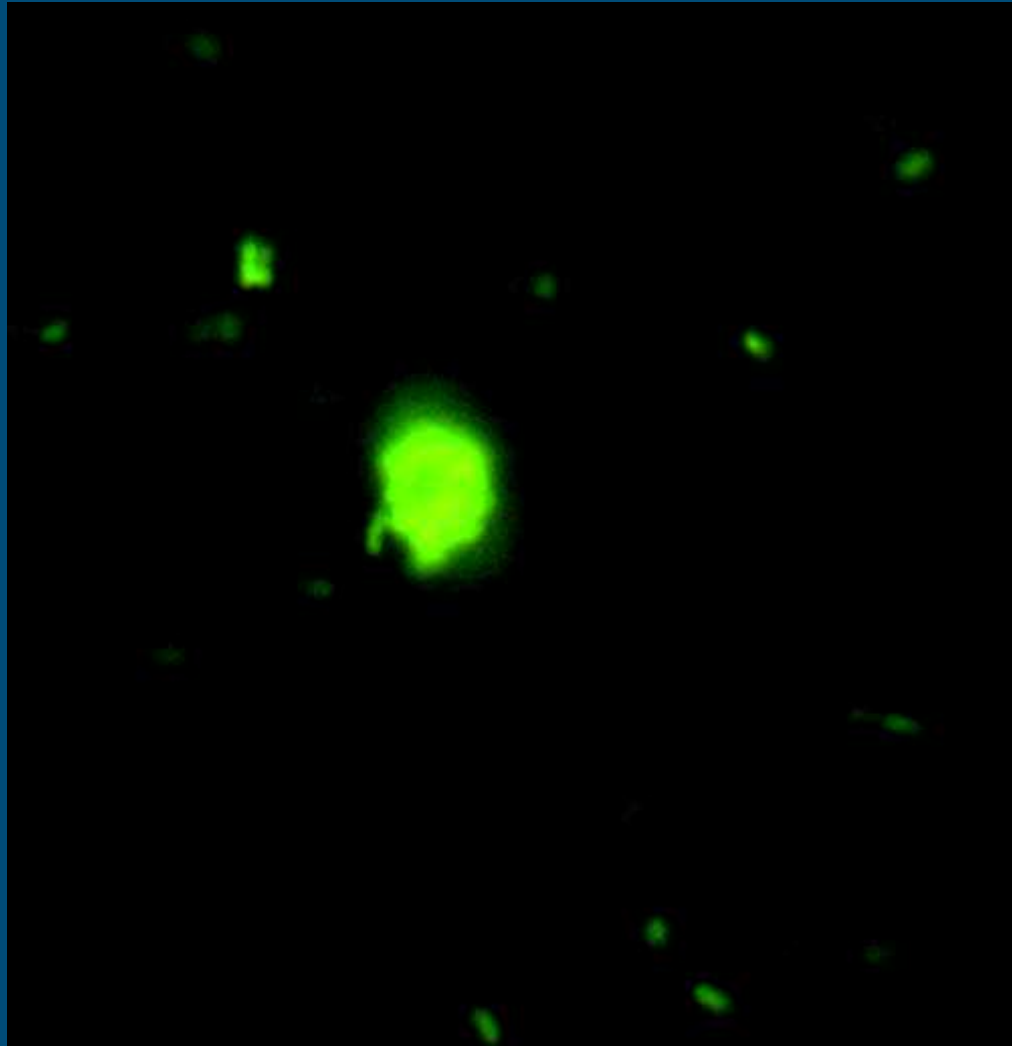
Stability



Potato tuber and potato leaf extract



MIA Bead + *Rsol*



Potato Viruses

- Potato Virus X & Y, Potato Leafroll Virus
- Transmitted by aphids and humans
- Controlled by planting certified seed
- Destroy infected plants
- Severe yield losses



healthy



PVX



PVY



PLRV



No Wash Procedure

- 50 μ l of extract + 50 μ l buffer/beads
- Incubate 15 minutes at room temperature
- Add reporter antibody
- Incubate 30 minutes at room temperature
- Run and analyze



Probe clogging

- Can be avoided by high speed centrifugation
- Plate centrifuge not sufficient
- Preferably no centrifuge step
- Filter plate to remove fibers
- Other procedure

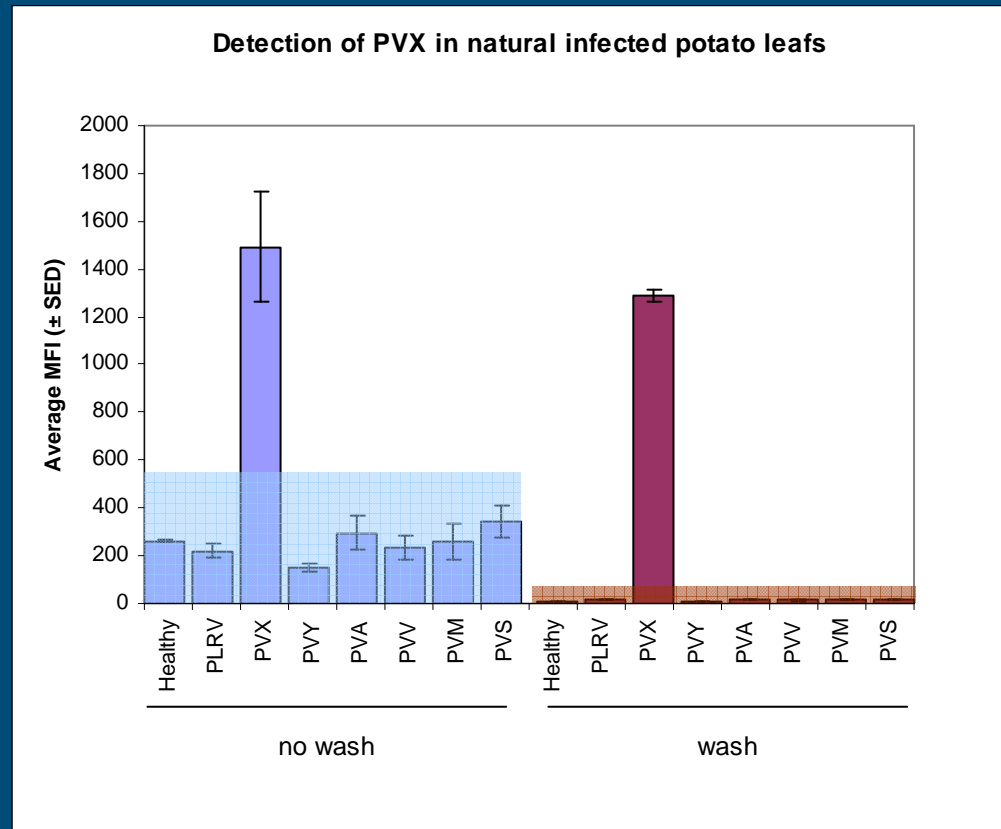


Wash Procedure

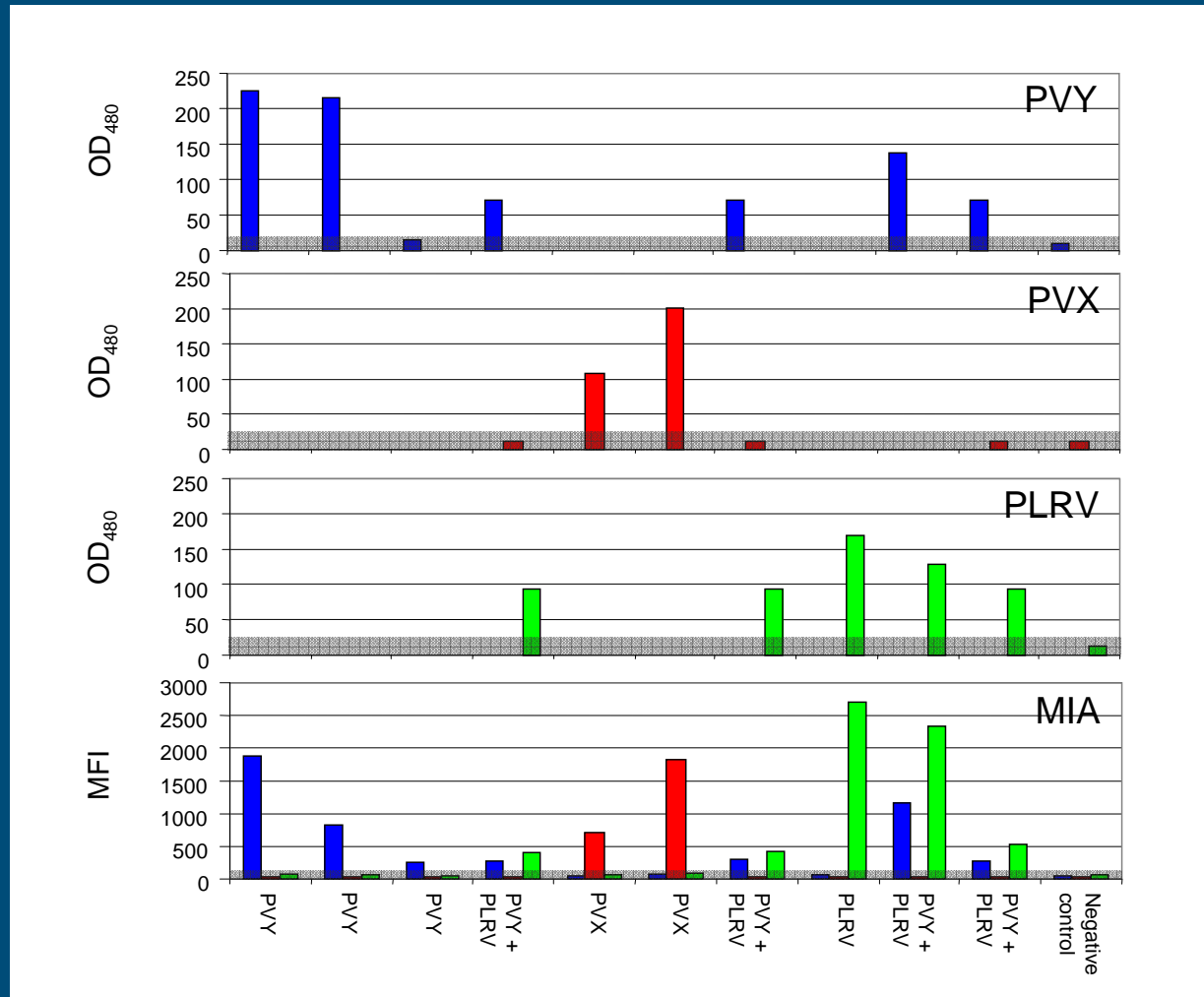
- 50 μ l of extract + 50 μ l buffer/beads
- Incubate 15 minutes at room temperature
- Remove sample
- Add buffer + secondary antibody
- Incubate 30 minutes at room temperature
- Wash and add SAPE for 15 minutes
- Run and analyze



Specificity testing: PVX



ELISA versus MIA



xMAP in AgBio

- Currently breakeven above 4 plex
- Only suited for HTP



xMAP emerging applications

- non amplified DNA/RNA detection
 - PRI



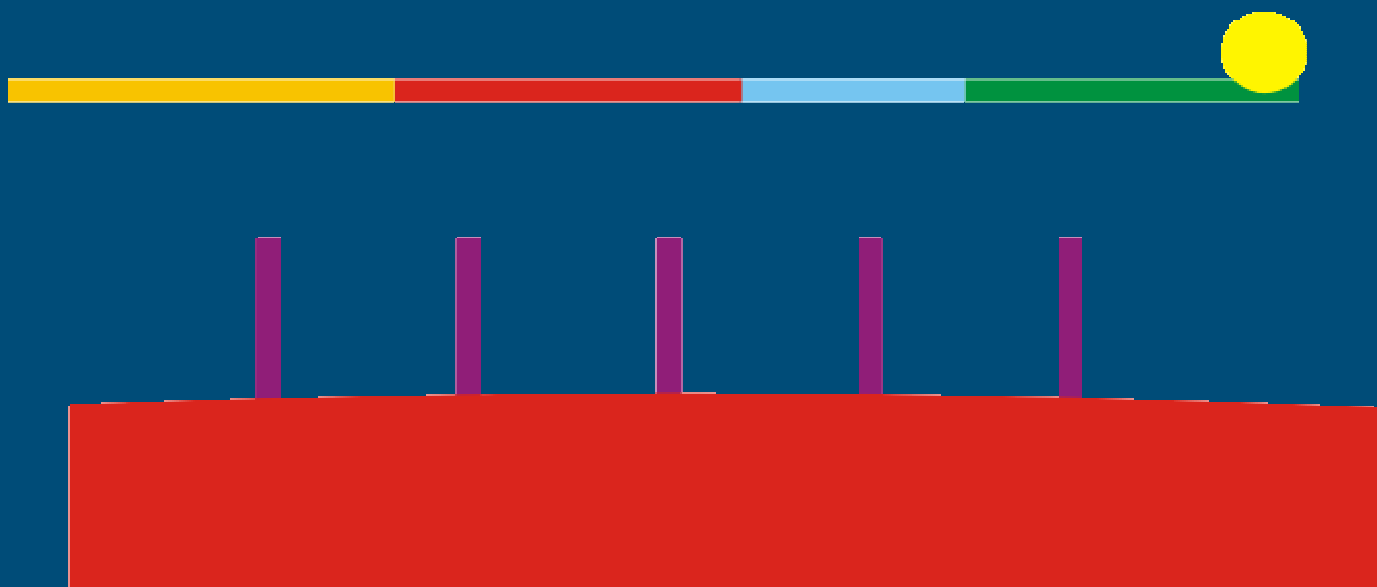
Padlock probes



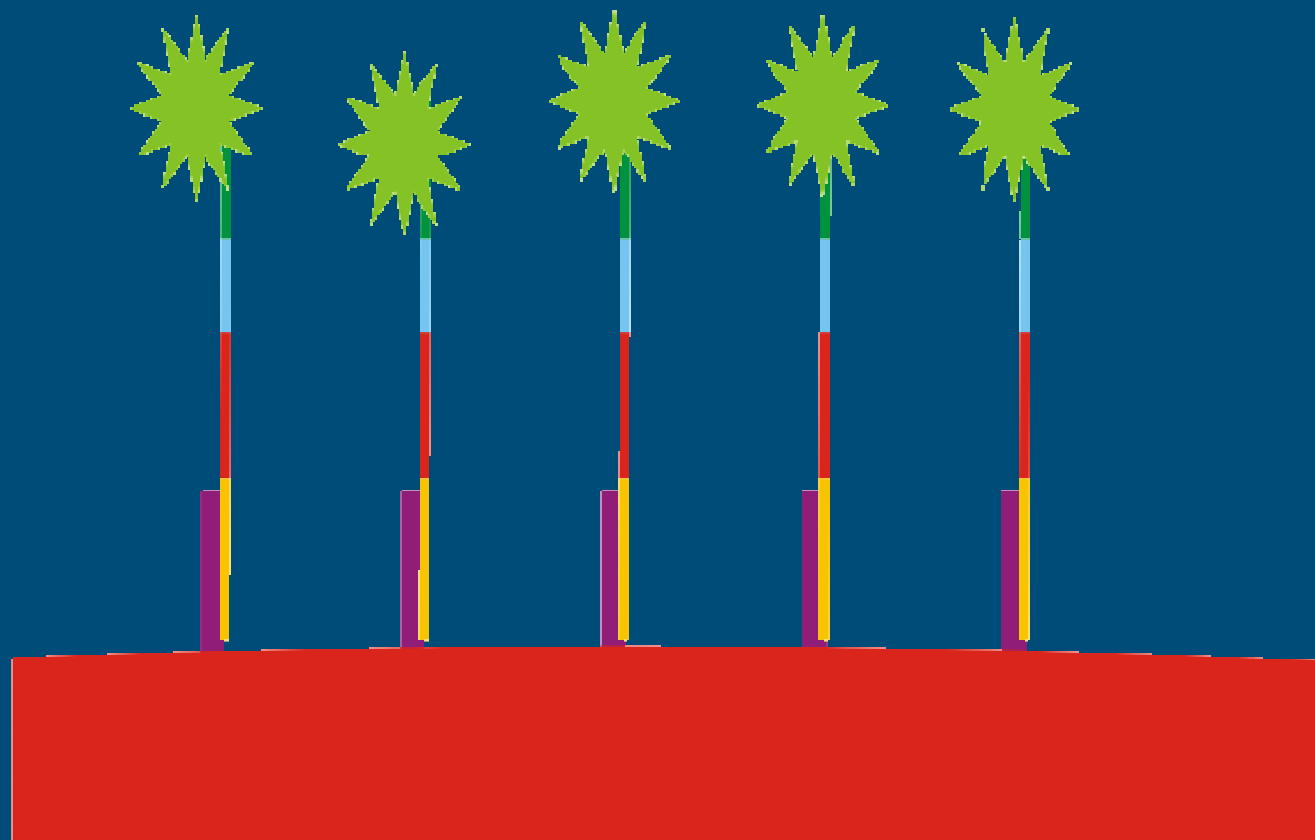
Padlock probes



Padlock probes



Padlock probes



Results

1	<i>Didymella bryoniae</i>	1 μ M	273
2	<i>D. bryoniae</i>	0.1 μ M	183
3	<i>D. bryoniae</i>	0.01 μ M	83
4	<i>D. bryoniae</i>	1nM	29
5	<i>D. bryoniae</i>	0.1nM	37
6	<i>D. bryoniae</i>	0.01nM	63
7	<i>Fusarium oxysporum</i>	0.1 μ M	39



Conclusions xMAP technology

■ Bacteria

- No wash assays possible
- Fast and sensitive
- Results comparable to ELISA
- Suitable for HTP routine analysis

■ Virus

- Wash procedure superior to no wash assay
- Procedure is robust and reliable
- Results comparable to ELISA
- Suitable for HTP routine analysis



Conclusions DNA/RNA

- xMAP technology is a versatile platform for DNA/RNA assessment
- Padlock; high specificity, sensitivity (low μM)



People

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Eisse de Haan

