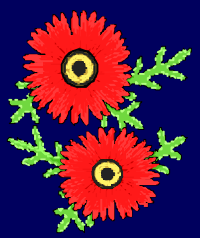
Developing a Technology Strategy

Chet Sutula

Agdia, Inc.

Elkhart, IN USA



While preparing this talk, I came to realize I have been chasing technology for quite a long time—

1st for nearly 20 years in human diagnostics

And more recently for 25 years developing plant pathogen diagnostics

It is also clear that other persons have positions in which the developing a technology strategy is their major responsibility—and I'm certain you will hear much more about this from them at this meeting.

So in this presentation, I will try to share some ideas that I believe can be important in developing a comprehensive, current strategy

no single technology--no matter how appealing--is able to serve all of our needs

Focus on the problem – Don't marry the technology

Many examples of this even in my own experience:

Companies that introduced and dominated the urine and blood glucose markets, e.g., Ames Co., Boehringer-Mannheim, Kodak were not the ones to introduce the modern blood glucose meter

DipStick Tests

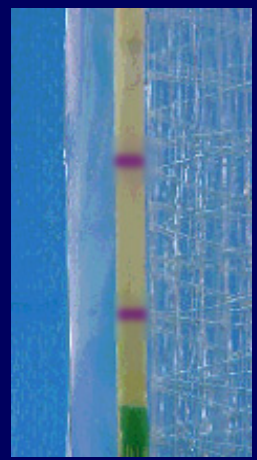
Electrochem Glucose Meter

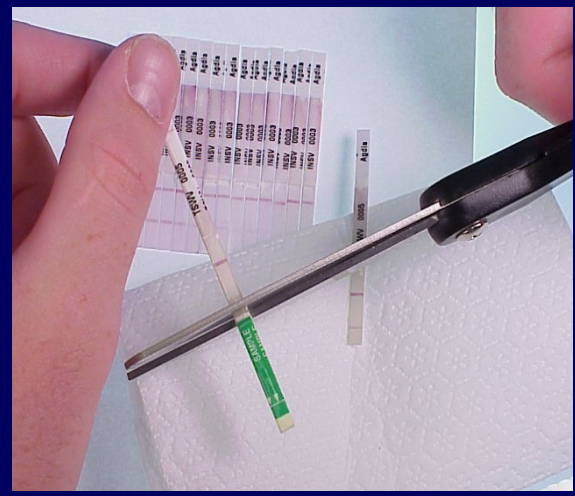
Agdia developed ELISA, at first, for use by growers

Spending too much time on ELISA delayed ImmunoStrips®

Insert strip

10-15 minutes





Record and store the result

Diverse Needs/Goals Require a Variety of Technologies

Field and on-site first detection

- Field/visual inspection
- Sentinel plots
- Tissue Print Assays
- ImmunoStrips
- Nucleic acid Strips
- Los Alamos Assay
- DARPA HISSS
- LLMIT Canary
- PNA-Dye Bleaching
- Others??

Confirm Field/on-site Results

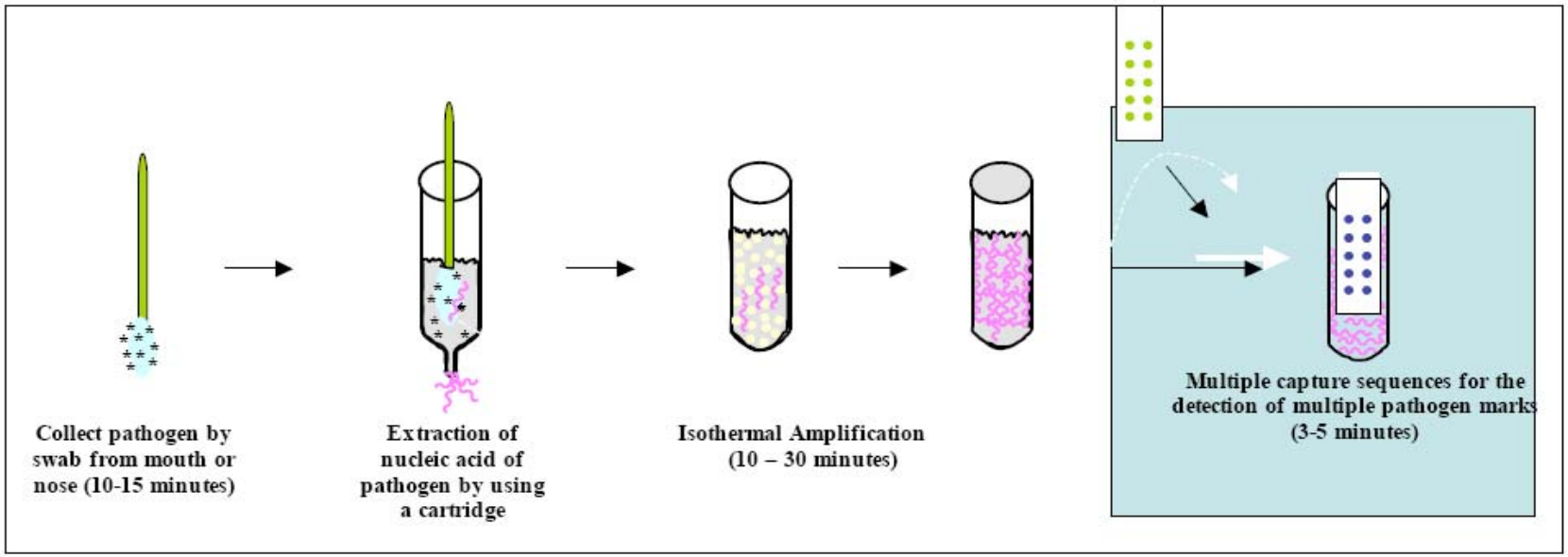
- Molecular and other analytical methods to confirm field result by independent approach, e.g.,
- Real Time PCR
- Rev-Dot Blot MacroArray

Definitive confirmation and Regulatory Action

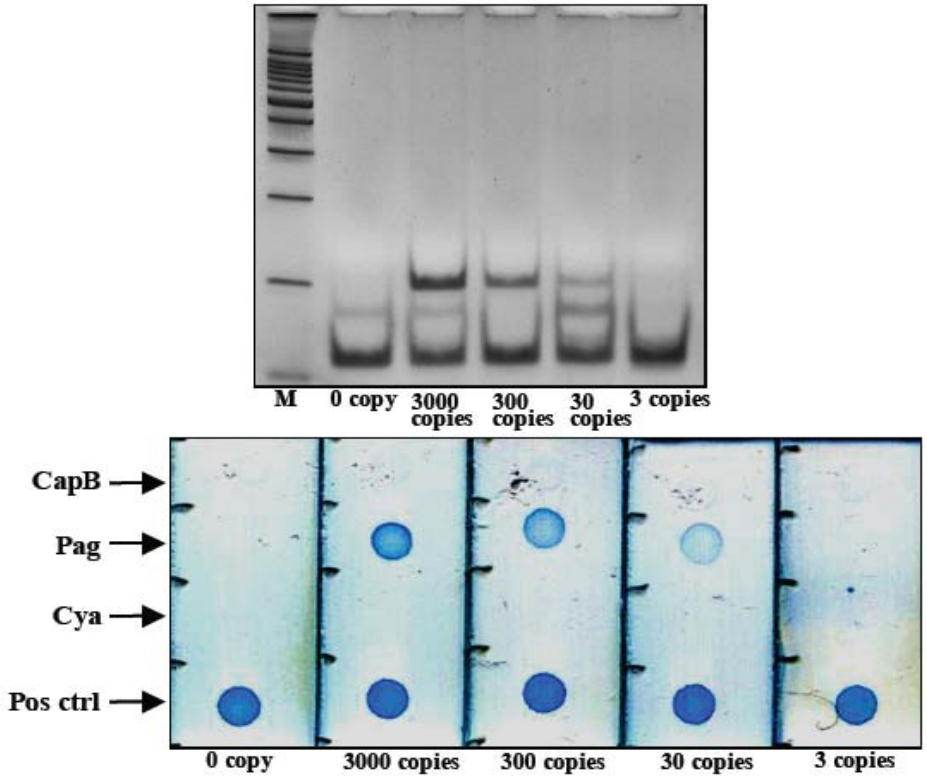
- Whatever method that provides certainty of ID and supports regulatory action
- HR PCR Seegene
- NA Sequencing

Nucleic Acid-based Dipstick Assays

1. Extraction of nucleic acid
2. Isothermal amplification of pathogen sequence
3. *Amplification product detection*



Dipstick Detection Limit: 30 Ba Genomic DNA Copies



Electrophoresis and dipstick detection of amplification products from genomic Ba DNA
M: 100 bp DNA ladder (Promega)

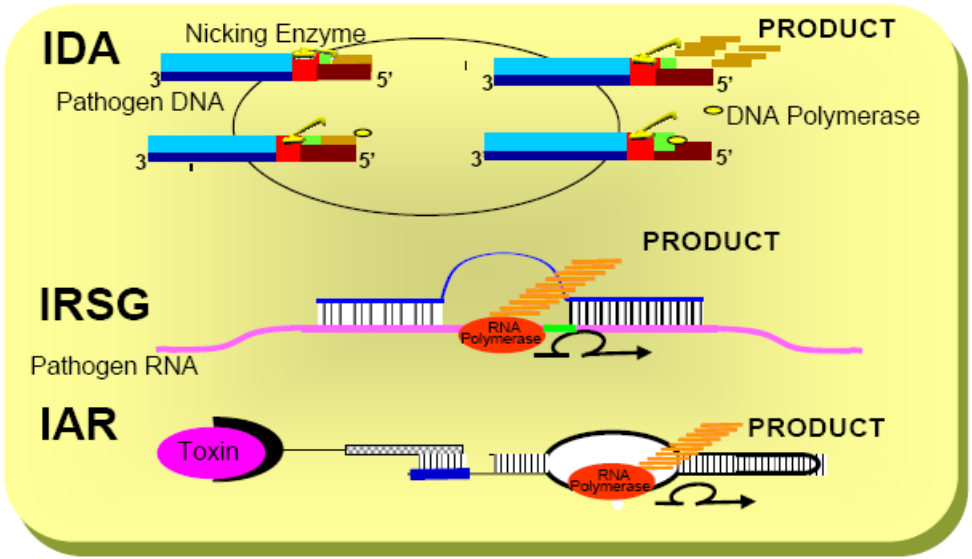
Los Alamos
NATIONAL LABORATORY

Handheld Isothermal Silver Standard Sensor (HISSS)

HISSS Goal

The goal of the Handheld Isothermal Silver Standard Sensor (HISSS) program is to develop a handheld sensor that is capable of identifying biological weapon threats across the entire threat spectrum including bacteria, viruses and toxins. The HISSS sensor will be based on novel high-speed isothermal detection methods for DNA, RNA and protein toxins.

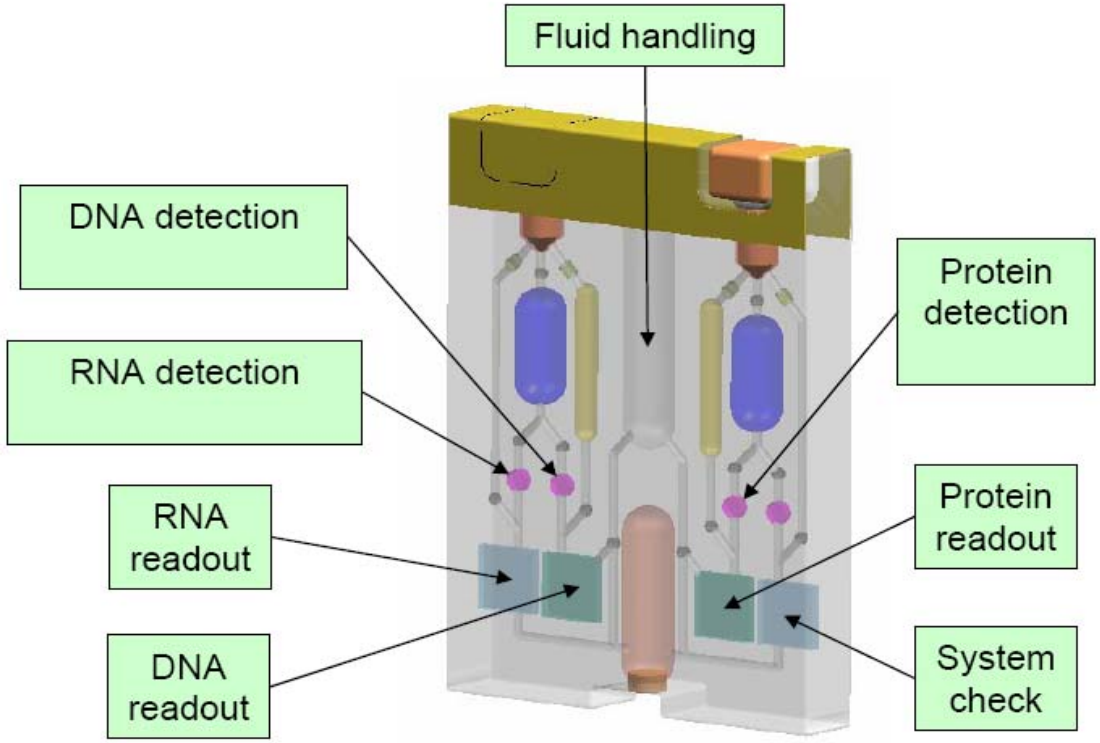
Detection Method	Today: Laboratory Testing	Tomorrow: Handheld Testing
DNA – based	PCR	Isothermal DNA Amplification (IDA)
RNA – based	RT-PCR	Isothermal RNA Signal Generation (IRSG)
Toxin – based	ELISA	Isothermal Antibody Recognition (IAR)



Conceptual Design of Handheld Instrument

Notional Handheld Instrument

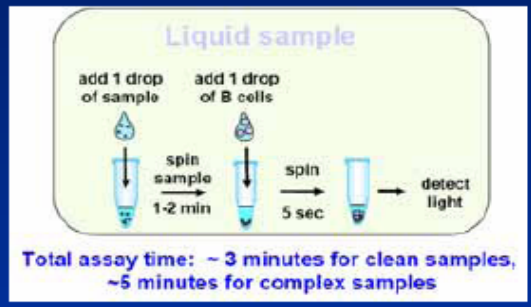
Notional Sample Cartridge



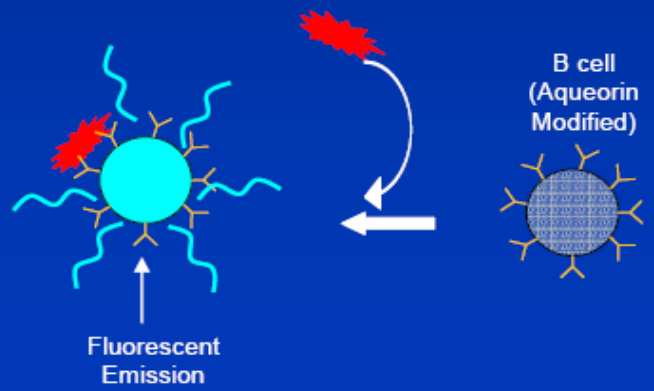
The handheld sensor will be capable of being operated by military personnel in the field environment

CANARY
(Cellular Analysis & Notification of Antigen Risk & Yields)

- ✓ Developed at MIT-LL
- ✓ Excellent for Biological Agents
  - ✓ *Bacillus anthracus* (anthrax)
  - ✓ *Yersinia pestis* (plague)
  - ✓ FMD (Foot and Mouth Disease) virus
  - ✓ *E. coli*
- ✓ Highly sensitive response in seconds
- ✓ Detection of Toxins – Developmental Stage

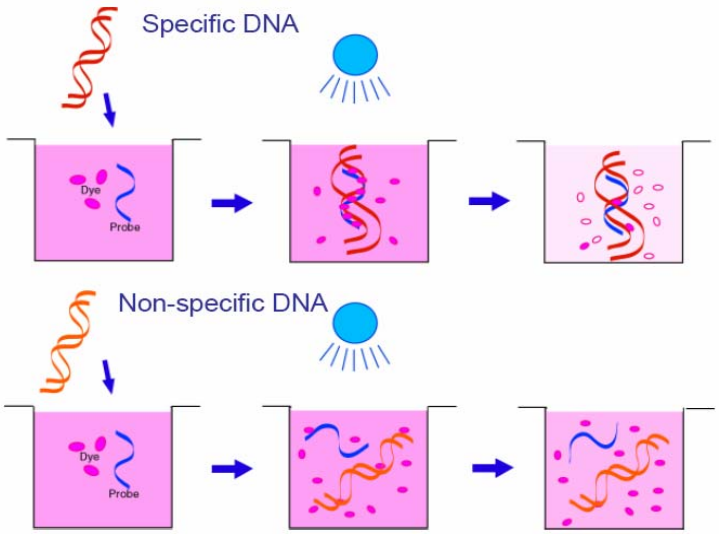


CANARY Bioassay



From: The Road to a Real-Time In Situ Monitoring System by Denise K. MacMillan, ERDCEL, Omaha, NE

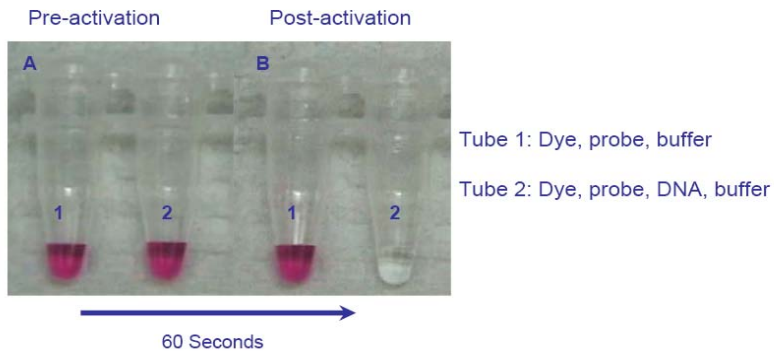
SmartDNA



SmartDNA: Investigen, Hercules, CA

Copied from:
Portfolio of new diagnostic technologies
36th IUATLD World Congress of Lung Health
21 October 2005
Mark Perkins, MD

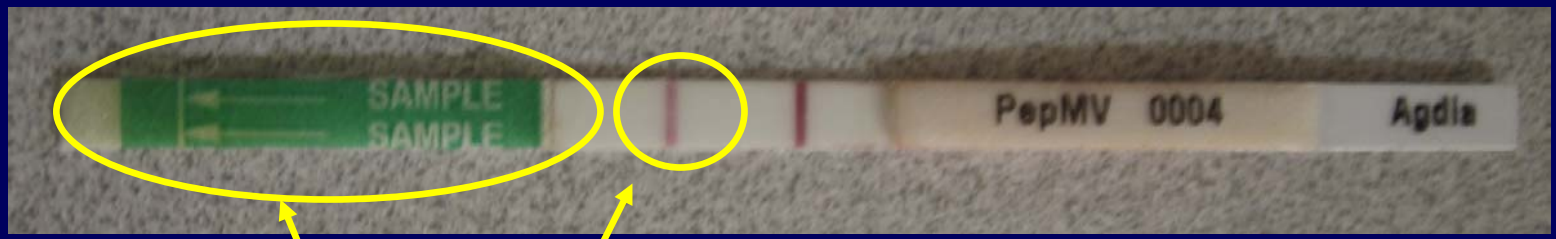
smartDNA™ assay



Methods used to confirm should be

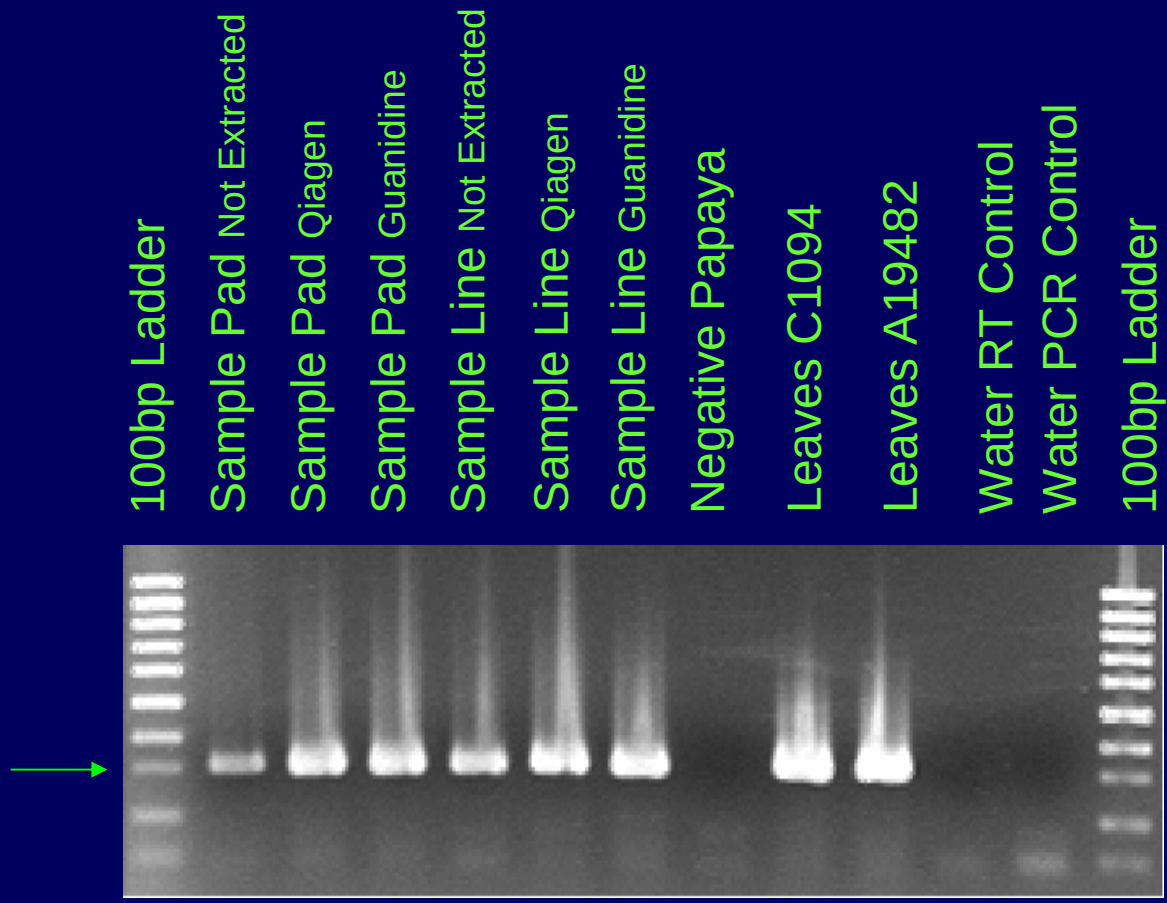
- ✓ independent of the detection technology
- ✓ able to use the identical sample / device which provided the original result

Confirmation of PepMV by PCR



Sample for PCR

Confirmation of the PepMV ImmunoStrip test data by PCR



Isolation and PCR amplification of plant viral nucleic acid from immunochromatographic devices.

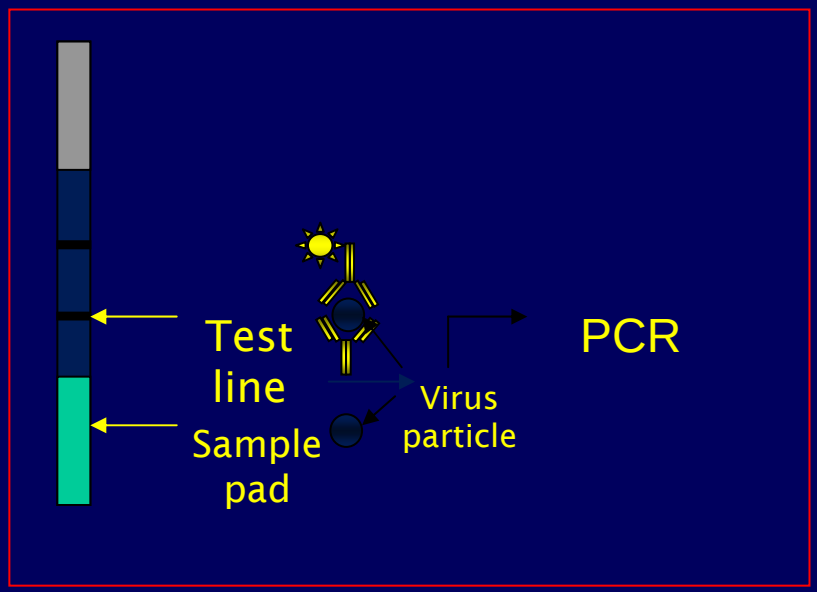
A.M. HARNESS (1), B.P. Kulemeka (1), J.A. Abad (2), and M.D. Bandla (1).
(1) Agdia, Inc., Elkhart, IN; (2) North Carolina State University, Raleigh, NC.

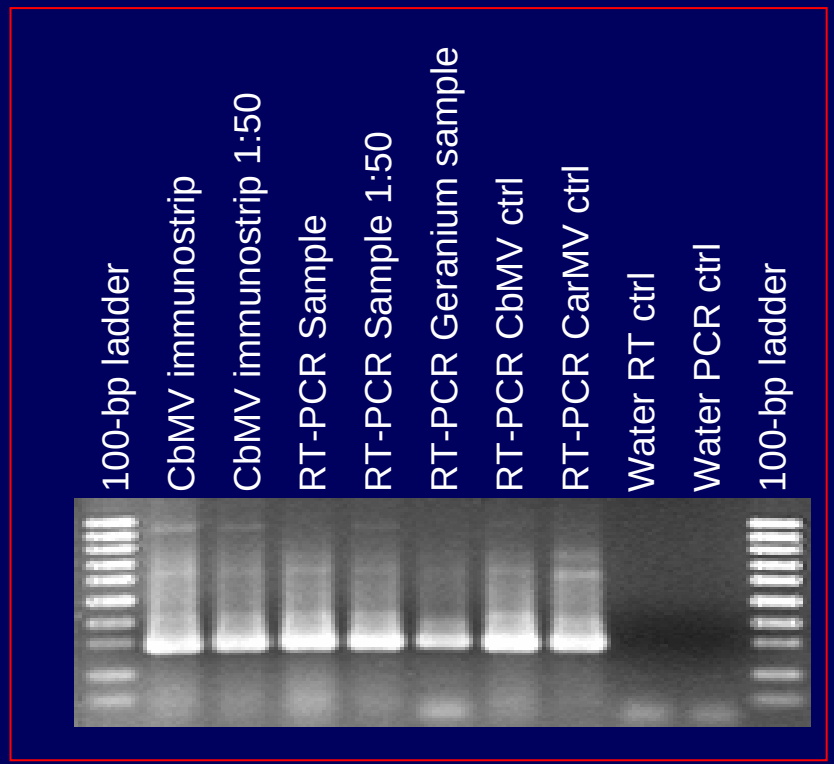
Confirming CbMV in Petunia

(2) Confirmation by Carmo Group PCR

RT-PCR of sample

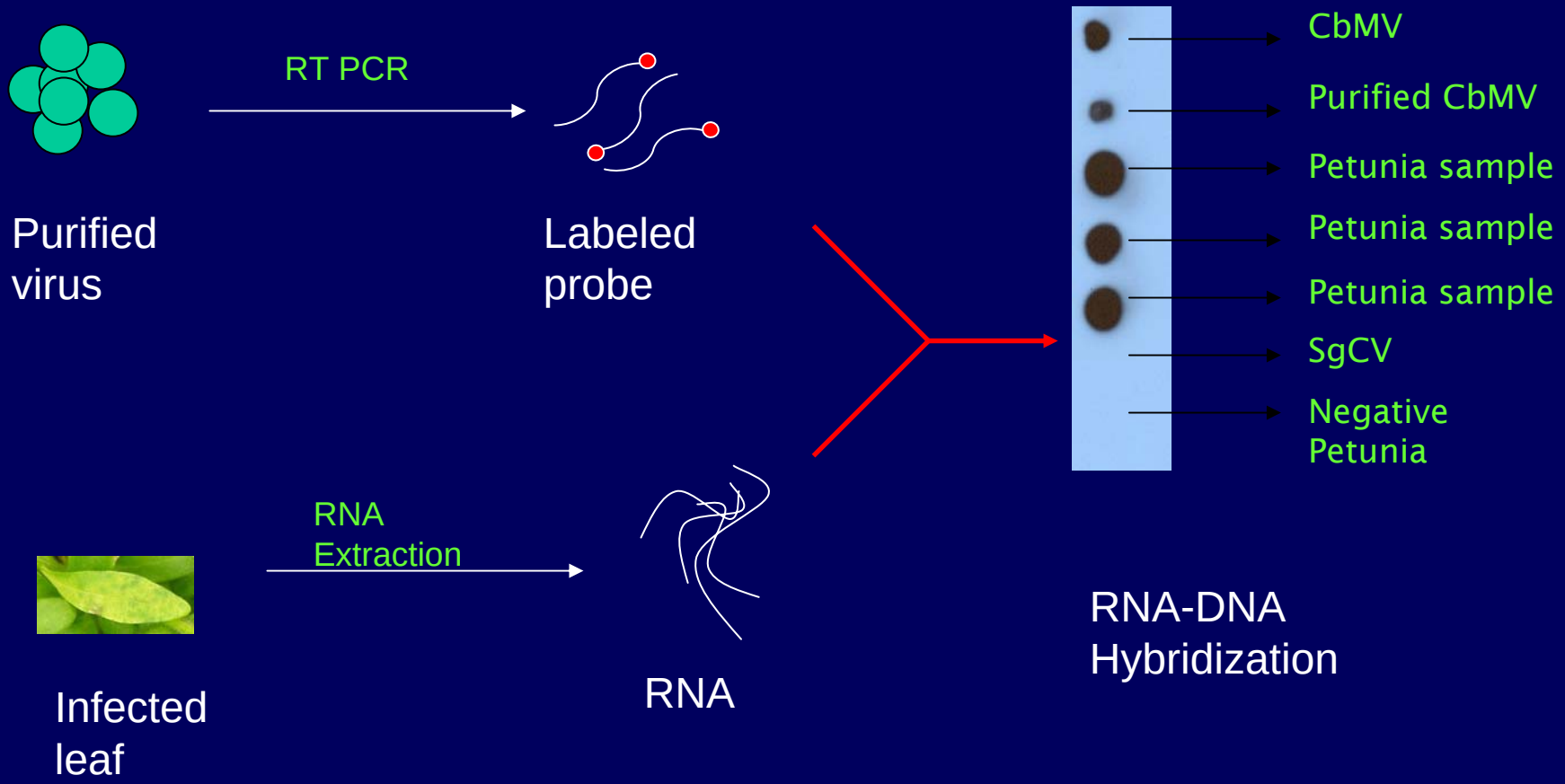
RT-PCR of ImmunoStrip



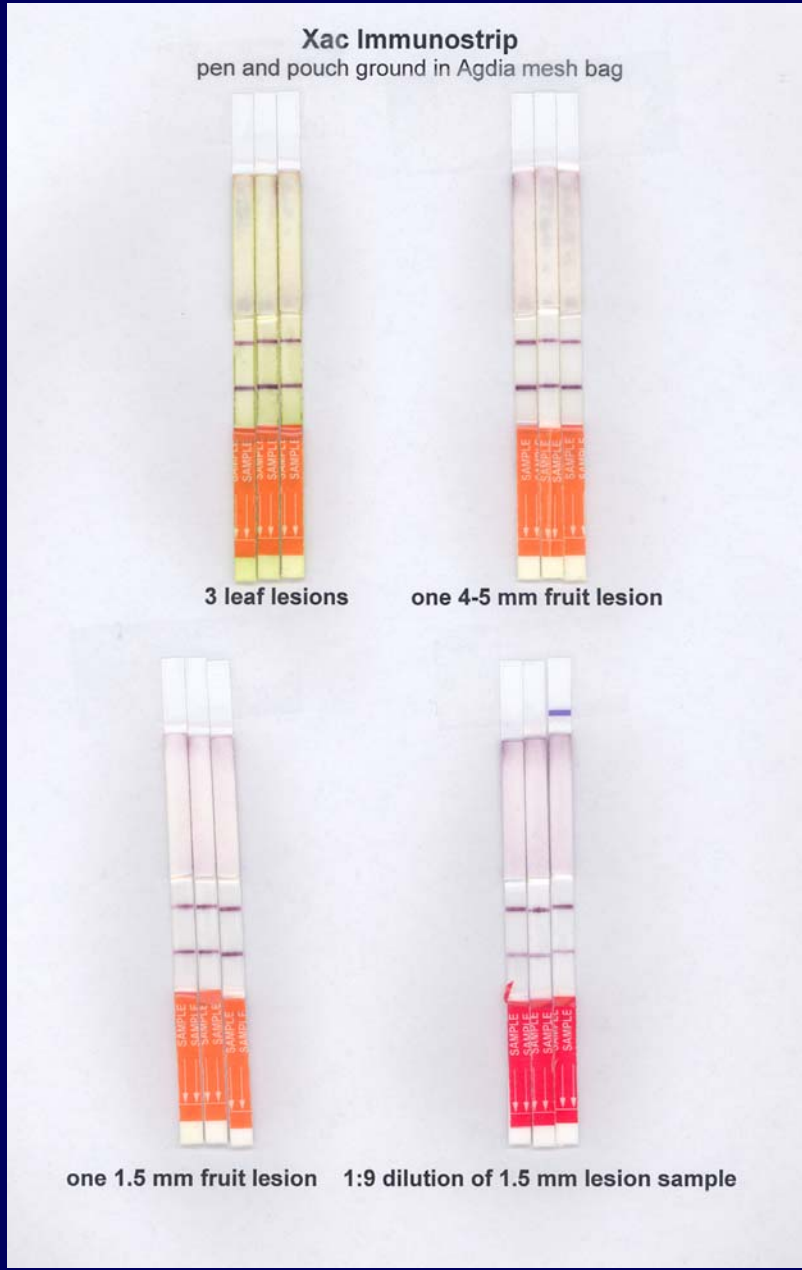


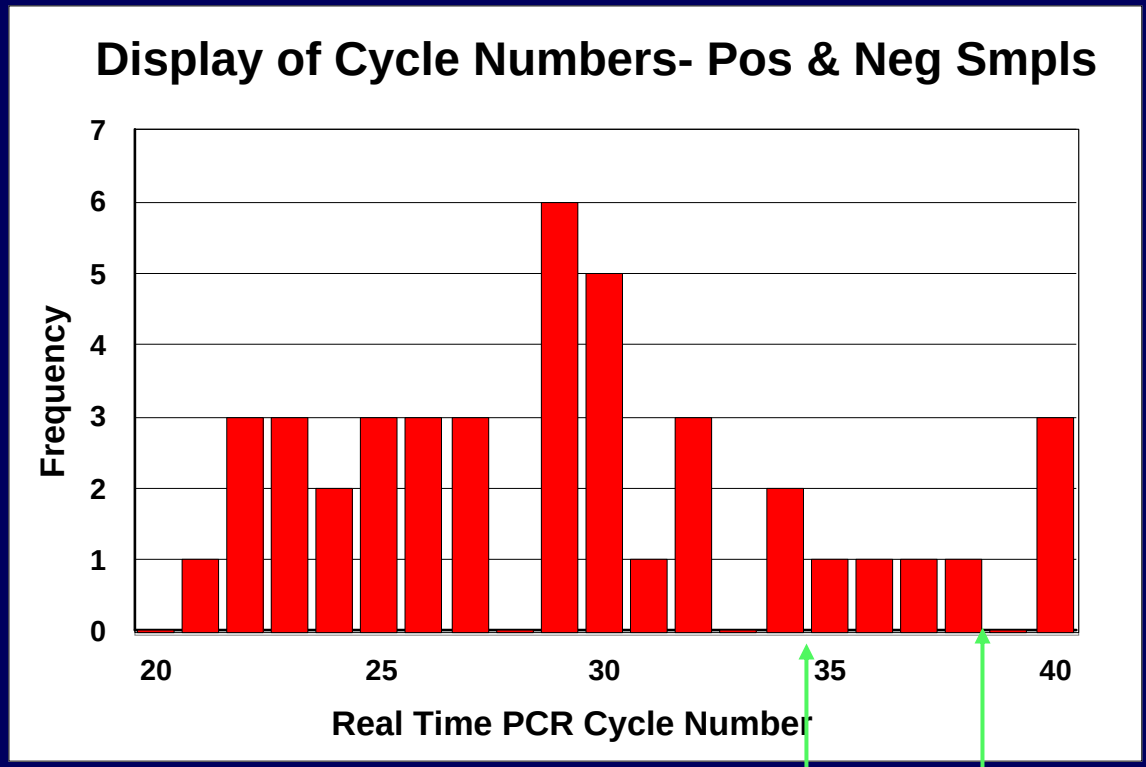
Confirming CbMV in Petunia

(3) Confirmation by DNA probe hybridization



Citrus Canker ImmunoStrip®





		Strip pos	Strip neg
PCR pos	39	35	4
PCR neg	3	0	3

Strip +, PCR +

Strip -, PCR +

Strip -, PCR -

Citrus Canker ImmunoStrip® Validation 2006

The Processes:

Confirming first detection, responding, test flow and lab management

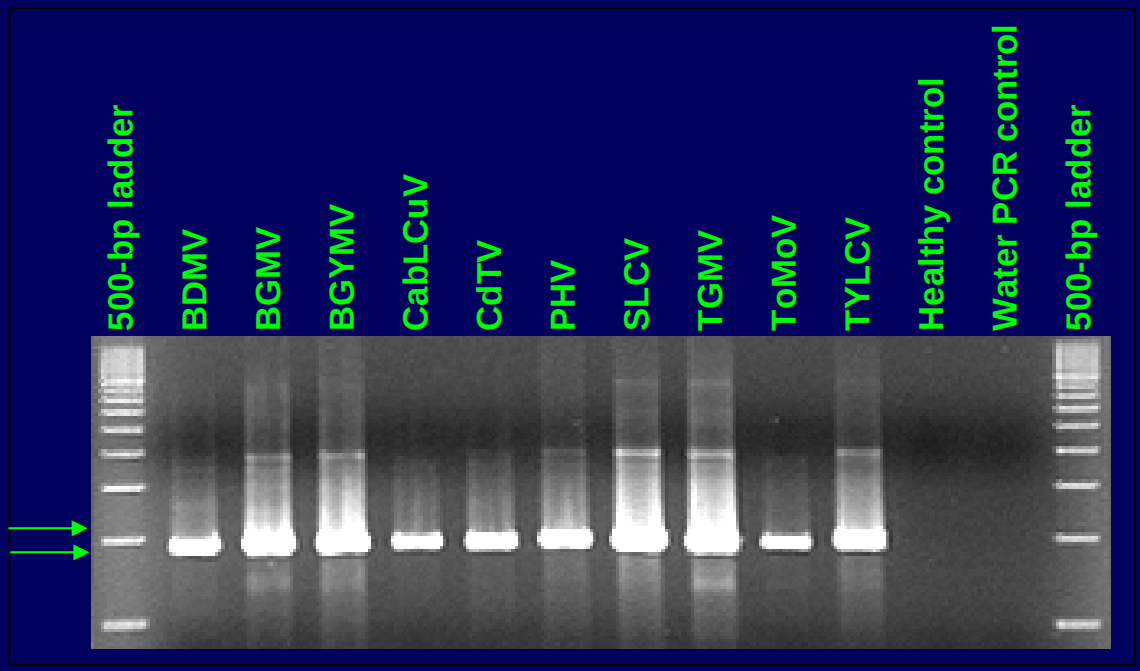
are simpler if the methods can be developed in a common/identical format and protocol

BEGOMOVIRUS GROUP

Viruses detected:

African cassava mosaic virus ***
Bean dwarf mosaic virus
Bean golden mosaic virus
Bean golden yellow mosaic virus
Cabbage leaf curl virus
Chino del tomato virus
Pepper huasteco virus
Squash leaf curl virus
Tomato golden mosaic virus
Tomato mottle virus
Tomato yellow leaf curl virus

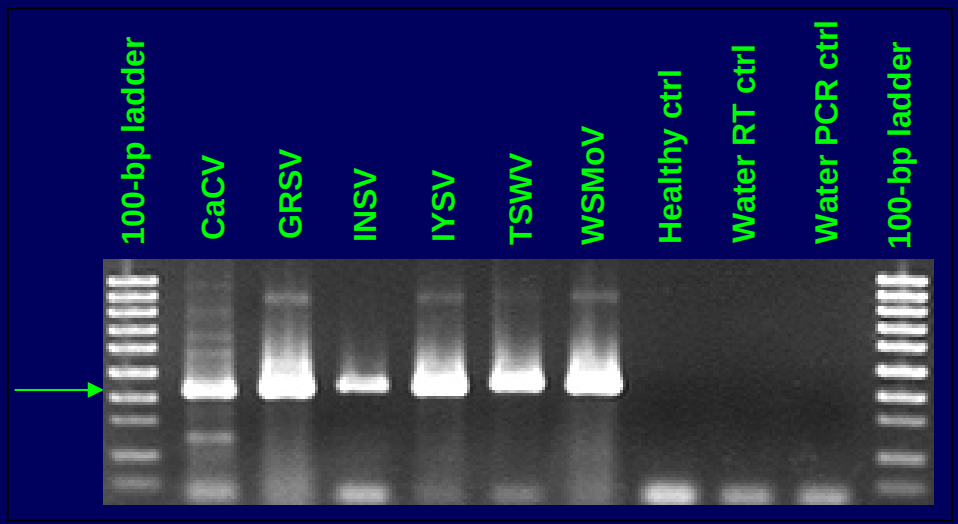
***Not shown



TOSPOVIRUS GROUP

Viruses detected:

Capsicum chlorosis virus
Groundnut ringspot virus
Impatiens necrotic spot virus
Iris yellow spot virus
Tomato spotted wilt virus
Watermelon silver mottle virus



Virus Group PCR tests developed at Agdia: 1996 to 2006

- Allexivirus
- Badnavirus
- Begomovirus
- Bromoviridae
- Bymovirus
- Capillovirus
- Carlavirus
- Carmovirus
- Caulimovirus
- Closteroviridae
- Comovirus
- Curtovirus
- Dianthovirus
- Fabavirus
- Foveavirus
- Furovirus
- Hordeivirus
- Ilarvirus
- Ipomovirus
- Luteoviridae
- Marafivirus
- Mastrevirus
- Nepovirus
- Potexvirus
- Potyviridae (Potyvirus)
- Rymovirus/Tritimovirus
- Sobemovirus
- Tenuivirus
- Tobamovirus
- Tobravirus
- Tombusvirus
- Tospovirus
- Trichovirus
- Tymovirus

Central Science Laboratory [UK]

Pests	Number of Tests
Viruses	32
Bacteria	7
Fungi	33
Invertebrates	7
Honey Bee Pathogens	9

CSL Real-Time PCR tests (all TaqMan™ assays)

Agdia's test development strategy

Sequence Product

Specific Virus [Pathogen] PCR

Group Virus PCR

ImmunoStrip®

ELISA

Summary

- Focus on the problem – Don't marry the technology
- Diverse Needs / Goals Require a Variety of Technologies
- Choose / Develop confirm methods that are:
  - independent of the first detection technology
  - able to use the identical sample / device which provided the original result
- Try to develop the methods in a common / identical format and protocol

Thank You