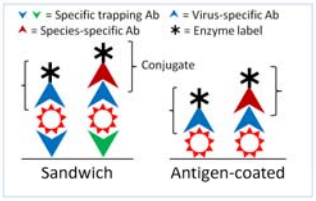
National Plant Diagnostic Network
Virus Diagnostics workshop

Serology-based assays



What are Viruses?

-- Viruses are very small (submicroscopic) infectious particles (virions) composed (minimally) of a protein coat and a nucleic acid core. Some viruses may also be enveloped by a membrane (with the addition of glycoproteins or lipids).

-- They carry genetic information encoded in their nucleic acid, which typically specifies two or more proteins. Translation of the genome (to produce proteins) or transcription and replication (to produce more nucleic acid) takes place within the host cell and uses some of the host's biochemical "machinery".

-- Viruses do not capture or store free energy and are not functionally active outside their host. They are therefore parasites (and usually pathogens) but are not usually regarded as genuine microorganisms.

-- Most viruses are restricted to a particular type of host. Some infect bacteria, [are called bacteriophages], whereas others infect algae, protozoa, fungi [mycoviruses], invertebrates, insects, vertebrates or vascular plants.

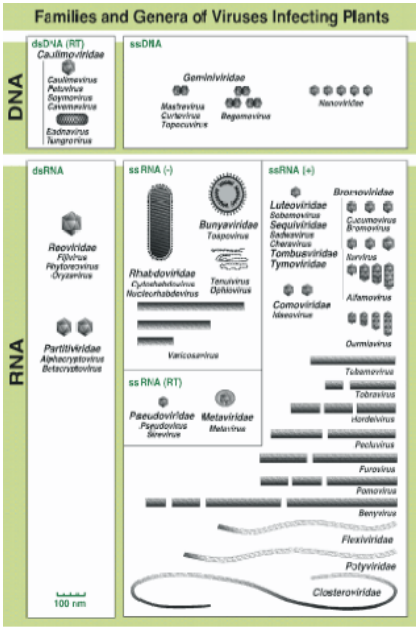
How are Viruses Classified?

Particle morphology:
- Isometric, spherical
- Flexuous or rigid rods
- Bullet-shaped
- Enveloped or non-enveloped

Genome:
- ssRNA, dsRNA
- ssDNA, dsDNA
- Plus- or minus-sense
- Mono- or polycistronic

Host:
- Vertebrates (Animals), Invertebrates, Insects, Plants, Algae, Protoza, Fungi, Bacteria

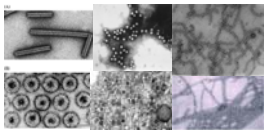
Transmission:
- Airborne, Fluids, Cells, Vector (such as insects)



Virus Detection Methods

An early step in any approach to treat and manage viral diseases involves detection and identification of the pathogen.

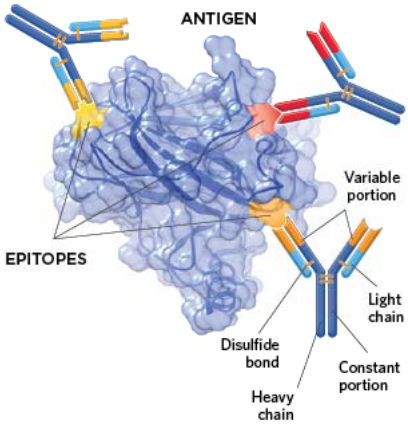
Positive identification of plant viruses can be accomplished by variations or combinations of at least four different technologies:



- Plant host range & transmission (a biological assay)
- Electron microscopy (a biophysical assay)
- Serology-based assays
  [e.g., Enzyme-linked immunosorbent assays]
- Nucleic acid-based analysis (a molecular approach)
  - o dsRNA analysis
  - o Polymerase chain reaction [PCR] assays
  - o Macroarray and Microarray
  - o "Next Generation" sequencing

Serological assays = antibody-based

- Antibodies are large proteins (~150 kDa).
- Four polypeptides—two heavy chains and two light chains—are linked by disulfide bonds to form a Y-shape molecule.
- The amino acid sequences at tips of the short ends of the Y vary greatly between antibodies produced by different B cells, while the rest of the molecule is relatively consistent.
- The variable portion of the antibody binds in a specific region (epitope) on a foreign protein (antigen) and signals the immune system to the presence of an invader.



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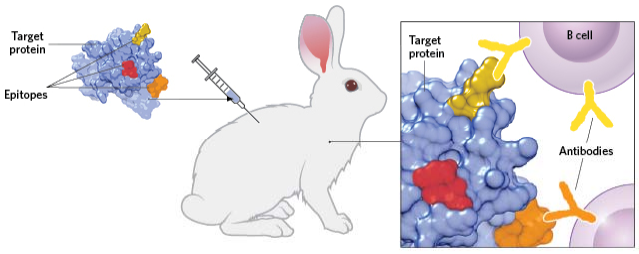
Serological assays = antibody-based - 2



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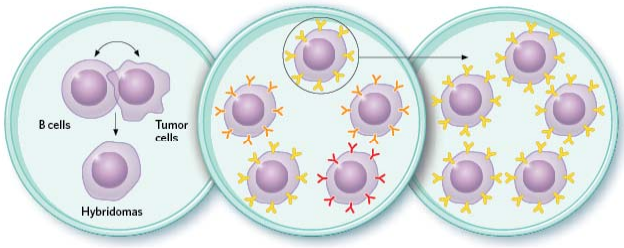
- To produce antibodies, researchers immunize lab animal with protein of interest.
- The animal's B cells then generate antibodies that bind to different regions, epitopes, on the protein.
- The diverse antibodies that bind to the target protein circulate in blood stream of animal.
- The antisera can be isolated and antibodies purified for use.
- Because they're produced by many B cells, and the sera has antibodies that bind numerous epitopes, these are called polyclonal antibodies.

Serological assays = antibody-based - 3



- Alternatively, the immunized animals' B cells can be isolated from the spleen or lymph nodes and fused with a tumor cell to generate immortal hybridoma lines.
- The secreted antibodies from single-cell derived hybridoma clones are monoclonal antibodies.
- Each monoclonal antibody recognizes only a single epitope of an antigen and is extremely specific.
- Those cell lines that produce the desired antibody against a specific or unique epitope of the target protein can then be grown as mouse ascities fluids or in large bioreactors to scale up production of the antibody.

Serological assays = antibody-based - 4

POLYCLONAL ANTIBODIES	MONOCLONAL ANTIBODIES
• **Immunogen:** Must be pure. Antibodies are generated to 'all' proteins and analytes in the immunogen. • **Binds specific epitope?:** Typically no. • **Advantages:** Diverse antibodies against different epitopes. More tolerant to changes to the antigen (denature, polymorphism, heterogeneity). • **Production:** Low cost. 2–4 months. Entirely in animal models (rabbit, goat, horse). • **Disadvantages** - **Lot-to-lot heterogeneity:** High - **Shelf life:** Limited - **Affinity and specificity:** Vary for a given target.	• **Immunogen:** 'Crude' or semi-purified antigens are acceptable. Antibodies generated to non-target antigens (epitopes) are not selected in hybridoma screening assays. • **Binds specific epitope?:** Yes. • **Advantages:** Highly specific recognition of only one epitope of a target antigen. Minimum 'noise'. - **Lot-to-lot heterogeneity:** Low - **Shelf life:** Unlimited • **Production:** Higher cost. 6-8 months. Requires animal models with tumor parent cell line (mouse, rat). • **Disadvantages** - More vulnerable to loss of epitope through mutation .

Serological assays = antibody-based - 5

Virus-specific vs. Broad-spectrum (highly cross-reactive) antibodies

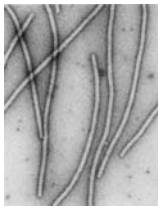
Journal of General Virology (1991), **72**, 25–36. *Printed in Great Britain*

Comparison and differentiation of potyvirus isolates and identification of strain-, virus-, subgroup-specific and potyvirus group-common epitopes using monoclonal antibodies

Ramon Jordan* and John Hammond

United States Department of Agriculture, Agricultural Research Service, Florist and Nursery Crops Laboratory, Building 004, Room 208, BARC West, Beltsville Agricultural Research Center, Beltsville, Maryland 20705, U.S.A.

- Immunogen:
  - Mixture of 12 diverse potyviruses
  - Native/whole virions, free subunits, denatured subunits
- Selection: Same (vs. "healthy plant proteins")



Serological assays = antibody-based - 6

Virus-specific vs. Broad-spectrum (highly cross-reactive) antibodies

Table 1. *Potyvirus isolates used in this study*

Virus	Acronym/isolate	Source/donor*
Bean yellow mosaic virus	BYMV GDD	Gladiolus, Utah, U.S.A.
Bean yellow mosaic virus	BYMV G82-18	Gladiolus, Ore., U.S.A.
Bean yellow mosaic virus	BYMV G82-29	Gladiolus, Ore., U.S.A.
Bean yellow mosaic virus	BYMV 49er	Gladiolus, Ca., U.S.A.
Bean yellow mosaic virus	BYMV Sierra Snow	Gladiolus, Ca., U.S.A.
Bean yellow mosaic virus	BYMV Goldfield	Gladiolus, Ca., U.S.A.
Bean yellow mosaic virus	BYMV Ice Cap	Gladiolus, Fla., U.S.A.
Bean yellow mosaic virus	BYMV Ideal A	Iris, Wash., U.S.A.
Bean yellow mosaic virus	BYMV Maud 2	Orchid, Pa., U.S.A.
Bean yellow mosaic virus	BYMV Wa Tulip	Tulip, Wash., U.S.A.
Bean yellow mosaic virus	BYMV G	R. H. Lawson, Md., U.S.A.
Asparagus virus 1	AV-1 N	G. Mink, Wash., U.S.A.
Bean common mosaic virus	BCMV PV25	ATCC, Md., U.S.A.
Carnation vein mottle virus	CVMV	Dianthus, Md., U.S.A.
Celery mosaic virus	CelMV PV374	ATCC, Md., U.S.A.
Cowpea aphid-borne mosaic virus	CpAbMV	G. Thottappilly, Nigeria
Clover yellow vein virus	CYVV Pratt	R. H. Lawson, Md., U.S.A.
Iris mild mosaic virus	IMMV	Iris, Wash., U.S.A.
Iris severe mosaic virus	ISMV	Iris, Wash., U.S.A.
Lettuce mosaic virus	LMV PV63	ATCC, Md., U.S.A.
Maize dwarf mosaic virus	MDMV A	A. G. Gillaspie, Md., U.S.A.
Maize dwarf mosaic virus	MDMV B	A. G. Gillaspie, Md., U.S.A.
Papaya ringspot virus	PRSV-W1 PV380	ATCC, Md., U.S.A.
Pea mosaic virus	PMV 204-1	R. H. Lawson, Md., U.S.A.
Pea mosaic virus	PMV PV89	ATCC, Md., U.S.A.
Pea seed-borne mosaic virus	PSbMV PV184	ATCC, Md., U.S.A.
Pepper mottle virus	PeMV NC165	J. W. Moyer, N.C., U.S.A.
Plum pox virus	PPV PV286	ATCC, Md., U.S.A.
Plum pox virus	PPV	S. Hartt, Md., U.S.A.
Pokeweed mosaic virus	PoMV	Pokeweed, Md., U.S.A.
Potato virus A	PVA	R. W. Goth, Md., U.S.A.
Potato virus Y	PVY-3	R. W. Goth, Md., U.S.A.
Potato virus Y	PVY-C	V. D. Damsteegt, Md., U.S.A.
Soybean mosaic virus	SMV PV94	ATCC, Md., U.S.A.
Statice virus Y	StaVY	R. H. Lawson, Md., U.S.A.
Sugarcane mosaic virus	SCMV D	A. G. Gillaspie, Md., U.S.A.
Sweet potato feathery mottle virus	SPFMV Common	J. W. Moyer, N.C., U.S.A.
Sweet potato feathery mottle virus	SPFMV Ibadan	G. Thottappilly, Nigeria
Sweet potato latent virus	SPLV	J. W. Moyer, N.C., U.S.A.
Tobacco etch virus	TEV NAT	W. G. Dougherty, Fla., U.S.A.
Tobacco etch virus	TEV PV69	ATCC, Md., U.S.A.
Tobacco vein mottle virus	TVMV	K. M. Franklin, Ken., U.S.A.
Tulip breaking virus	TBV	A. F. L. M. Derks, Netherlands
Tulip chlorotic blotch virus	TCBV	W. P. Mowat, United Kingdom
Turnip mosaic virus	TuMV PV134	ATCC, Md., U.S.A.
Turnip mosaic virus	TuMV Line 2	Tulip, Wash., U.S.A.
Vallota mosaic virus	VaMV	Vallota, Md., U.S.A.
Watermelon mosaic virus II	WMV II	J. Poulston, Ca., U.S.A.
Zucchini yellow mosaic virus	ZYMV	J. A. Dodds, Ca., U.S.A.

Table 2. *Properties and reactivity of MAbs and mouse polyclonal antibodies with virus, virus subunit and SDS-denatured virus preparations from BYMV GDD in TAS- and ACP-ELISA tests, using either rabbit polyclonal antibody precoated plates or antigen-coated plates*

MAb		TAS-ELISA*		ACP-ELISA		
No.	Ig isotype	Virus†	Subunit	Virus	Subunit	SDS-protein
PTY 1‡	G2a	2§	9	7	9	9
PTY 2	A	2	4	4	5	7
PTY 3	G1	1	3	3	3	6
PTY 4	G1	1	3	3	3	3
PTY 5	G1	1	1	3	3	7
PTY 7	G1	1	1	2	2	6
PTY 8	G1	1	1	4	4	8
PTY 9	G1	1	1	2	2	5
PTY 10	G1	0	0	1	1	2
PTY 11	G1	1	1	2	2	3
PTY 12	G1	2	2	2	2	3
PTY 13	G2a	5	6	7	5	9
PTY 14	G2a	3	6	7	5	9
PTY 17	G1	2	2	3	2	5
PTY 18	G2a	2	2	2	2	5
PTY 20	G2a	2	1	2	1	2
PTY 21	G2a	9	9	9	9	9
PTY 22	G2a	6	4	5	4	9
PTY 24	G2a	7	8	8	7	9
PTY 25	G1	6	6	6	5	8
PTY 28	G1	3	4	8	6	8
PTY 30	G1	5	5	5	5	8
PTY 31	G1	6	5	6	5	9
PTY 32	G2b	6	5	8	6	9
PTY 33	G2a	8	8	8	7	9
PTY 34	G2a	8	7	8	7	9
PTY 35	G1	5	5	5	5	8
PTY 36	G1	6	6	6	6	9
PTY 37	G1	6	5	5	5	8
PTY 43	G1	6	6	5	6	7
NS1 myeloma	–	0	0	0	0	0
Mouse polyclonal antibody	–	8	6	5	5	7

Serological assays = antibody-based - 7

Virus-specific vs. Broad-spectrum (highly cross-reactive) antibodies

Table 4. *Differentiation of diverse potyviruses in ACP-ELISA* using potyviral cross-reactive PTY MAbs*

	PTY MAb																
Potyvirus†	1	2	3	4	5	7	8	9	10	11	12	13	20	21	25	33	MS‡
BYMV§	+++	+++	++	++	+++	+++	++−	++	(+)	+	+	+++	(+)	+++	+++	+++	+++
CYVV	+++	+++	++	++	++	++	++	++	+	++	(+)	+++	++	−	−	−	++
PMV‖	+++	+++	+++	+++	+++	+++	++−	+++	−	++	(+)	+++	++	−	−	−	++
IMMV	+++	+++	++	+++	+	+	+	+	−	−	+	−	−	−	−	−	++
PVY-3	+++	+	−	++	(+)	(+)	++	(+)	++	(+)	−	−	−	−	−	−	++
PeMV	+++	−	++	−	−	−	−	+	+++	−	++	−	−	+++	+	+++	+
TVMV	+++	−	+++	−	−	−	−	−	+++	+++	+	+++	−	−	−	−	+
TEV¶	+++	+++	−	−	++	+	++	++	++	+	+	−	−	−	−	−	+
TCBV	+++	+++	−	−	++	++	++	++	++	+	+	+	−	−	−	−	++
TuMV**	+++	++	−	−	++	++	++	++	++	+	(+)	−	−	−	−	−	++
TuMV Line 2	+++	++	−	−	++	++	++	++	−	−	(+)	−	−	−	−	−	++
PSbMV	+++	+++	+++	−	−	−	−	−	−	++	−	−	−	−	−	−	+++
TBV	+++	+++	−	++	−	−	−	−	+	−	+	−	(+)	−	−	−	+
SMV	+++	−	−	(+)	−	−	−	−	+	−	−	−	(+)	−	−	−	+
PaMV	+++	+++	+	−	−	−	+	−	−	−	+	−	(+)	−	−	−	+++
MDMV B	+++	+	++	++	+	−	++	(+)	(+)	−	−	−	−	−	−	−	+
MDMV A	+++	−	+	(+)	−	−	−	−	−	−	−	−	−	−	−	−	(+)
SCMV D	+++	++	+++	+	−	−	−	−	+	−	+	−	−	−	−	−	+
SPFMV††	+++	++	(+)	+	−	−	−	−	−	−	−	−	−	−	−	−	+
SaVY‡‡	+++	+++	++	−	−	−	−	−	−	−	−	−	−	−	−	−	+
CelMV	+++	++	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−
AV-1	+++	−	(+)	+	−	−	−	−	+	−	−	−	−	−	−	−	+
CpAbMV	+++	−	++	−	−	−	−	−	+	−	−	−	−	−	−	−	(+)
ZYMV	+++	−	−	++	−	−	−	−	(+)	−	−	−	−	−	−	−	++

Serological assays = antibody-based - 8

Virus-specific vs. Broad-spectrum (highly cross-reactive) antibodies

Table. Summary of an example of ELISA results of various healthy and potyvirus-infected ornamental plant samples using the broad-spectrum reacting genus potyvirus monoclonal antibody PTY-1 in an antigen-coated plate assay.

Sample	ELISA A_{405} OD	Sample	ELISA A_{405} OD
Euphorbia milii (EuRSV)	0.9	*Osteospermum* (LMV)	1.1
Omphalodes (OmVY)	1.6	*Schizostylis* 'Kafir Lily' (BYMV)	1.8
Spiranthes Orchids (SpiMV-2, -3)	2.2	*Tricyrtis* 'Toad Lily' (TrVY)	0.7
Spiranthes cernua (DsMV)	0.6	*Verbena* (BYMV-PMV)	0.8
Lily (LMoV)	1.2	*Ornithogalum* (OrMV)	2.3
Brugmansia (CDV)	1.9	New Guinea Impatiens (IFBV)	0.8
Potato virus Y (in tobacco)	2.8	*Bean yellow mosaic virus* (purified)	2.3
Healthy *Nicotiana tobacum*	<0.1		

Serological assays = antibody-based - 9

Virus-specific vs. Broad-spectrum (highly cross-reactive) antibodies

- U.S. Letters Patent Broad-Spectrum Potyvirus Monoclonal Antibody:
Jordan, R.L. and Hammond, J. *Monoclonal antibodies against potyvirus-associated antigens, hybrid cell lines producing these antibodies, and use therefor*. U.S. Letters Patent 5,043,263; issued August 27, 1991.

- Able to detect most all aphid-transmitted potyviruses
- PTY-1 Licensed to Agdia, Inc. as the "POTY Group Test"
- Recently released Potyvirus Group ImmunoStrips
- Advantage: One test able to detect >150 of virus species
- Disadvantages:
  - Requires other tests to ID the potyvirus.
  - Not "universal" (does miss a few potyviruses).

Serological assays = antibody-based - 10

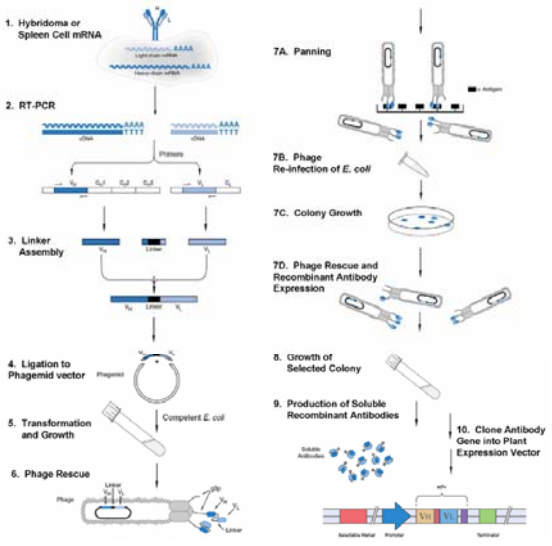
Antigen-binding single-chain variable antibody fragment , ScFV, antibodies

- ScFvs are a recombinant DNA engineered fusion protein of the variable regions of an antibody's heavy (VH) and light chains (VL), connected with a short linker peptide.

- They can be created using phage display, where the antigen-binding domain is expressed as a single peptide on surface of phage particles.

- ScFvs can also be created directly from sub-cloned heavy and light chains derived from a hybridoma.

- Unlike McAbs, scFvs are produced in bacteria cell cultures such as E. coli.



Kamo, Jordan, Hsu, Hu (2008) Antibodies in plants. Floriculture, Ornamental and Plant Biotechnology, Vol 5, p311-318

Serological assays = antibody-based - 11

Antigen-binding single-chain variable antibody fragment , ScFV, antibodies

Cloning and Construction of Single-chain Variable Fragments (scFv) to *Cucumber Mosaic Virus* and Production of Transgenic Plants

J.A. Aebig[1,4], H.H. Albert[2], B.L Zhu[1,3], J.S. Hu[3] and H.T. Hsu[1]
[1]FNPRU, Agricultural Research Service, U.S. Dept. Agr. Beltsville, MD 20705
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[3]Dept. Plant and Environ. Protection Sciences, University of Hawaii, Honolulu, HI 96822
[4]Present address: NIH/NIAID/MVDU, 5640 Fishers Lane, Rockville, MD 20850, USA
[5]Present address: FFA Sciences, 3550 General Atomics Court, San Diego, CA 92121, USA

Proc. XI[th] IS on Virus Diseases in Ornamentals
Ed. C.A. Chang
Acta Hort. 722, ISHS 2006

Transgenic Plants Expressing a Single-chain Fv Antibody to *Tomato spotted wilt virus* (TSWV) are Resistant to TSWV Systemic Infection

M.Q. Xu[1], H.P. Li[1], M. Wang[1], Z.C. Wu[1], W.B. Borth[1], H.T. Hsu[2] and J.S. Hu[1]
[1] Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI 96822 USA
[2] USDA-ARS, Floral and Nursery Plants Research Unit, Beltsville, MD 20705 USA

Development of single chain variable fragment (scFv) antibodies against *Xylella fastidiosa* subsp. *pauca* by phage display

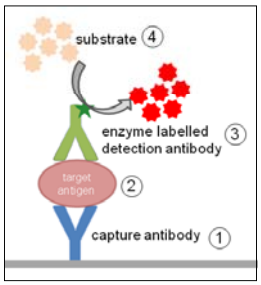
Qing Yuan[a,1], Ramon Jordan[b], Ronald H. Brlansky[c], Olga Istomina[d], John Hartung[a,*]

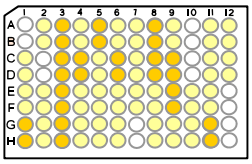
Development of single chain variable fragment (scFv) antibodies against surface proteins of '*Ca. Liberibacter asiaticus*'

Qing Yuan[a,1], Ramon Jordan[b], Ronald H. Brlansky[c], Olga Minenkova[d], John Hartung[a,*]

Enzyme-linked immunosorbent assay [ELISA] (a serological test)

- ELISA is an immunological assay commonly used to measure/detect antigens, proteins, or antibodies in biological samples.
- ELISA assays are generally carried out in 96 well plates, allowing multiple samples to be measured in a single experiment.
- Each ELISA measures a specific antigen, and kits for a variety of antigens, including plant viruses, are widely available.
- The most common ELISA is a "sandwich" ELISA: consisting of an anti-target capture antibody (1), the target antigen (2), an enzyme-labeled anti-target detection antibody (3), and a substrate (4).





Negative Reaction
(No Detectable Antigen)

Positive Reaction
(Sample Has Detectable Antigen)

Strongly Positive Reaction
(Sample Has More Antigen)

Enzyme-linked immunosorbent assay [ELISA] - 2

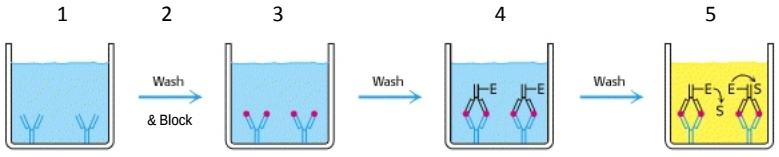
- Low tech, low volume and can be high throughput using automated systems.
- Requires that a good quality virus-specific antibody (polyclonal or monoclonal) is available (prepared in the lab, or purchased).
- Assay can be qualitative or quantitative, but they all need highly specific and sensitive antibodies.
- Relatively inexpensive, good for experimental identification and viral titer assay, and for large scale virus surveys and certification programs.
- Many variations and formats for different purposes.

➢ A good overall ELISA reference discussing formats, protocols, 'blocking', background issues and troubleshooting can be found in the "Technical Guide for ELISA" published by KPL [http://www.kpl.com/docs/techdocs/KPL ELISA Technical Guide.pdf].

Enzyme-linked immunosorbent assay [ELISA] - 3

The basic sandwich ELISA method is stepwise in the order shown:

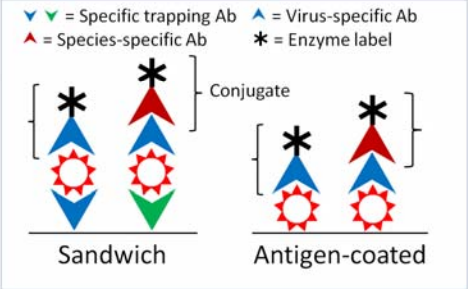
(1) The 1st step is to coat the ELISA plate wells with the anti-antigen capture antibody. (Any excess, unbound antibody is then washed from the plate).
(2) The wells are then washed and unbound plastic sites are "blocked" with heterologous proteins.
(3) Next, the sample (e.g., plant tissue extract or purified virus) is added. Any target antigen (virus) found in the sample will bind to the capture antibody already coating the plate.
(4) The enzyme-labeled detection antibody is then added. Detection antibody binds to any target antigen (virus) already bound to the plate. [If the target is not present, the detecting antibody will be washed away in the subsequent wash step].
(5) Finally, a substrate is added to the plate. ELISA assays are usually chromogenic using a reaction that converts the substrate into a colored product, which can be measured using an automated plate reader.



Enzyme-linked immunosorbent assay [ELISA] - 4

Formats

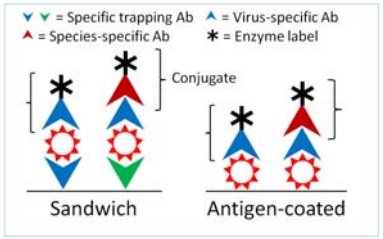
- Many variations and formats for different purposes
- Antibody trapping of virus 'sandwich'
  - Double-antibody sandwich (DAS-ELISA)
  - Triple-antibody sandwich (TAS-ELISA) (requires 2 different species and an anti-species conjugate)
- Antigen-coated plate (ACP-ELISA)
- Direct detection: labeled virus-specific antibody
- Indirect detection: virus-specific antibody recognized by labeled species-specific antibody
- Antibodies can be virus-specific or cross-reactive (broad-spectrum) polyclonal antisera or monoclonal antibodies (e.g. Poty Group test)



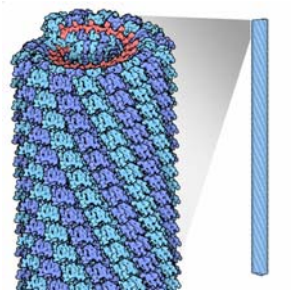
Enzyme-linked immunosorbent assay [ELISA] - 5

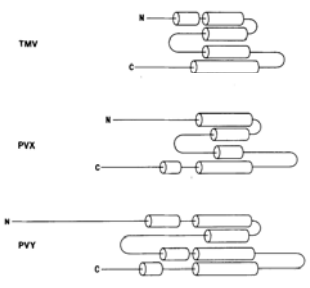
Which variation or format is best?

- How many different antibodies are available to your target?
- Polyclonal or Monoclonal?
- Same or different animal species?
- Are the antibodies virus-specific or cross-reactive (broad-spectrum)?
- Antibodies react to repeating (virion exposed) epitope(s) or not? [Cryptotope vs. Mematope]
- Epitope(s) linear or conformational?



Example:





Enzyme-linked immunosorbent assay [ELISA] - 6

Table 2. *Properties and reactivity of MAbs and mouse polyclonal antibodies with virus, virus subunit and SDS-denatured virus preparations from BYMV GDD in TAS- and ACP-ELISA tests, using either rabbit polyclonal antibody precoated plates or antigen-coated plates*

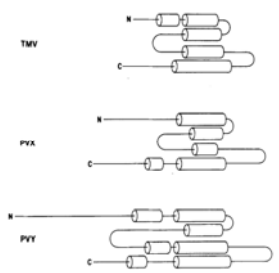
MAb		TAS-ELISA*		ACP-ELISA		
No.	Ig isotype	Virus†	Subunit	Virus	Subunit	SDS-protein
PTY 1‡	G2a	28	9	7	9	9
PTY 2	A	2	4	4	5	7
PTY 3	G1	1	3	3	3	6
PTY 4	G1	1	3	3	3	3
PTY 5	G1	1	1	3	3	7
PTY 7	G1	1	1	2	2	6
PTY 8	G1	1	1	4	4	8
PTY 9	G1	1	1	2	2	5
PTY 10	G1	0	0	1	1	2
PTY 11	G1	1	1	2	2	3
PTY 12	G1	2	2	2	2	3
PTY 13	G2a	5	6	7	5	9
PTY 14	G2a	3	6	7	5	9
PTY 17	G1	2	2	3	2	5
PTY 18	G2a	2	2	2	2	5
PTY 20	G2a	2	1	2	1	2
PTY 21	G2a	9	9	9	9	9
PTY 22	G2a	6	4	5	4	9
PTY 24	G2a	7	8	8	7	9
PTY 25	G1	6	6	6	5	8
PTY 28	G1	3	4	8	6	8
PTY 30	G1	5	5	5	5	8
PTY 31	G1	6	5	6	5	9
PTY 32	G2b	6	5	8	6	9
PTY 33	G2a	8	8	8	7	9
PTY 34	G2a	8	7	8	7	9
PTY 35	G1	5	5	5	5	8
PTY 36	G1	6	6	6	6	9
PTY 37	G1	6	5	5	5	8
PTY 43	G1	6	6	5	6	7
NS1 myeloma	–	0	0	0	0	0
Mouse polyclonal antibody	–	8	6	5	5	7

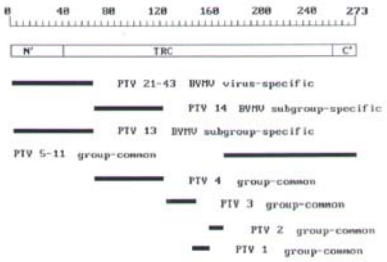
Table 6. *Characterization of the coat protein antigenic determinants recognized by the PTY MAbs*

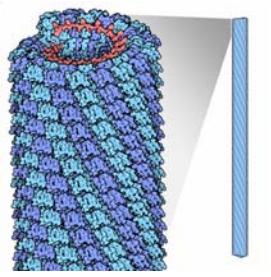
Epitope			PTY MAb	Distribution		
Site	Type*	Characteristic†		BYMV‡	BYMV-subgroup§	Other potyviruses‖
A	C	Con	1	9/9	12/12	30/30
B	C	C	2	9/9	12/12	19/30
C	C	C	3	9/9	12/12	15/30
D	C	C	4	9/9	12/12	19/30
E	C	C	5	9/9	12/12	7/30
F	C	C	7	9/9	12/12	6/30
G	C	C	8	9/9	12/12	8/30
H	C	C	9	9/9	12/12	8/30
I	C	C	10	9/9	9/12	18/30
J	C	C	11	9/9	12/12	7/30
K	C	C	12	9/9	11/12	13/30
L	M	BS	13	9/9	12/12	2/30
M	C	BS	14	9/9	12/12	0/30
N	M	BS	17,18	9/9	11/12	0/30
O	M	C	20	8/9	10/12	5/30
P	M	BV	21	8/9	8/12	1/30
Q	M	BV	22,24	8/9	8/12	0/30
R	M	BV	25	8/9	8/12	1/30
S	M	BV	28	8/9	8/12	0/30
T	M	BV	30	7/9	7/12	0/30
U	M	BV	31,32	7/9	7/12	0/30
V	M	BV	33	6/9	6/12	1/30
W	M	BV	34 to 36	6/9	6/12	0/30
X	M	BV	37	5/9	5/12	0/30
Y	M	BSt	43	1/9	1/12	0/30

* C, Cryptotope; M, metatope.
† Con, Conserved; C, common; BS, BYMV-subgroup; BV, BYMV-virus; BSt, BYMV-strain.

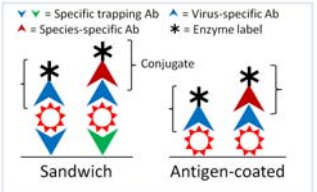
Enzyme-linked immunosorbent assay [ELISA] - 7







- Virus-specific sites – Virion external; "repeated"
  - DAS-, TAS-, or ACP-ELISA
- Broad-spectrum sites – Internal; Subunit only
  - ACP-ELISA



Enzyme-linked immunosorbent assay [ELISA] - 8

Need More Sensitivity?

To get the most sensitivity from an assay, the following factors must be addressed:

- Background noise can usually be minimized by optimizing the blocking and washing steps. The lower the signal, the lower the background must be in order to detect a positive result.
- Low signal due to low level attachment of the bound molecule can often be overcome by testing different plates or by switching to covalent linkage to the plate.
- Low signal can be amplified by incorporating indirect labeling techniques or by switching from colorimetric to chemiluminescent substrates.
- Low signal can sometimes be amplified by increasing the incubation times, allowing the binding steps to come to equilibrium.

Enzyme-linked immunosorbent assay [ELISA] - 9

Ideal blocking agents have the following characteristics:

- Effectively block nonspecific binding of assay reactants to the surface of the well
- Do not disrupt the binding of assay components that have been adsorbed to the well.
- Act as a stabilizer (prevent denaturation) of assay reactants on a solid surface
- Are not cross-reactive with other assay reactants
- Possess no enzymatic activity that might contribute to signal generation of the substrate or degradation of the reactants
- Perform all of the above reproducibly from lot-to-lot

The most typical protein blocking agents are:

- Bovine serum albumin – BSA
- Non-fat dry milk – NFDM
- Normal serum
- Casein or caseinate
- Fish gelatin
- Proprietary vendor reagents (e.g., Roche Diagnostics; DIG Wash and Block Buffer Kit)
- NOT Tween or other detergents

Enzyme-linked immunosorbent assay [ELISA] - 10

Difficulty differentiating between a low-positive and negative?
Background signal (noise!) is a common ELISA problem.

The good news is that there are many recommendations out there for how to resolve the issue. The only problem is...your assay is unique just like everyone else's assay. The best place to start is to rule out the classic culprits.

Top 5 Key Causes

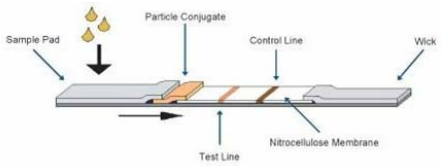
1. Insufficient blocking.
2. Insufficient plate washing.
3. Cross reactivity between antibodies and samples on the plate surface.
4. Primary (capture), secondary (detecting), or conjugate antibody concentration is too high.
5. Incubation time is excessive for your sample antibodies and/or substrate.

Recommended Solutions

1. Consider an alternative blocker, the same blocker may not work for all ELISAs.
2. Wash 3 times with 3 minute soak periods between each wash.
3. A. Dilute your antibodies and samples in your block solution to avoid cross-reaction.
   B. Remove non-target specific antibodies [cross-absorb polyclonal antisera against "healthy sap" using membrane-bound extract].
4. Titrate your antibodies to a concentration that provides the right sensitivity.
5. Try shortening your incubation times.

Lateral flow immunoassay (another serological test)

Lateral flow immunochromatographic assays
Lateral flow immunostrip assays
ImmunoStrip™ (Agdia, Inc)



- The immunochromatography strip test, or namely lateral flow test, operates similarly to the sandwich ELISA assay as a simple device intended to detect the presence or absence of the target antigen.
- It's an immunoassay in which the test sample flows along a supported membrane via capillary action.
- Probably the most well-known example of lateral flow tests are in-home pregnancy tests.
- Advantages of lateral flow tests compared to other immunoassays:
  - Quick – only takes a few minutes to obtain results.
  - Requires little samples or reagent(s) preparations.
  - Excellent for rapid single sample testing in field or lab
- Disadvantages:
  - Higher cost per sample
  - Less sensitive

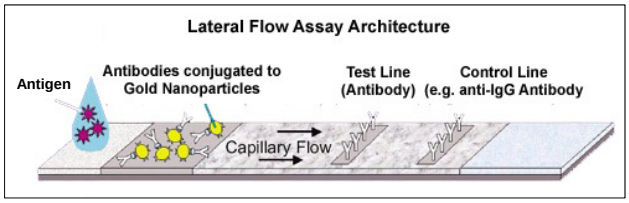
Demonstration: Agdia POTY ImmunoStrip Test

Lateral flow immunoassay - 2

The technology is based on a series of capillary beds, such as pieces of porous paper or sintered polymer. Each of these elements has the capacity to transport fluid (e.g., plant tissue extract) spontaneously.

1. The first element (the sample pad) acts as a sponge and holds an excess of sample fluid. This end of the "strip" is usually dipped into the sample extract.

2. Once soaked, the fluid migrates to the second element (conjugate pad) which contains bio-active particles [e.g., nanometer-sized Colloidal Gold] coated with target-specific antibodies that have been immobilized (conjugated) on the particle's surface.

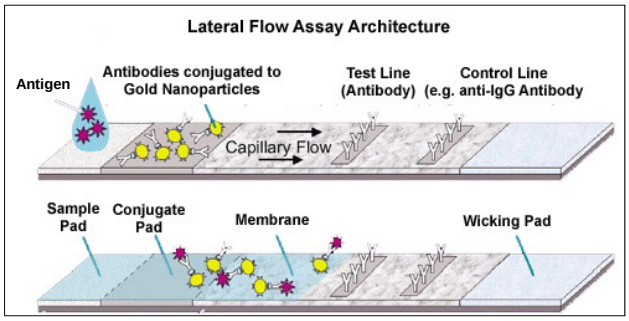


1 **2**

Lateral flow immunoassay - 3

3. While the sample fluid flows through the porous pad, the target antigen (virus protein) binds to the antibody-bound particles while migrating further through the third capillary bed (usually a nitrocellulose membrane).

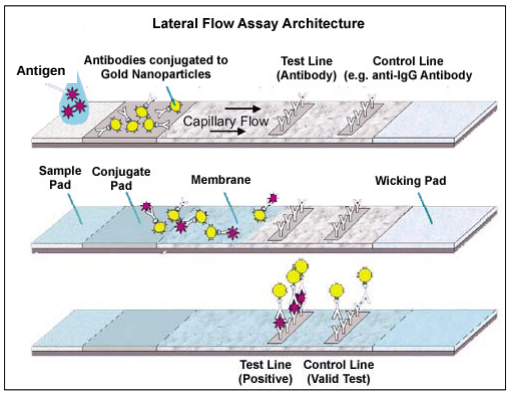
4. This material has two or more areas (often called stripes). The 'test' stripe contains another 'capture' molecule (usually another anti-virus antibody) which binds the virus-bound particle complex.



3 **4**

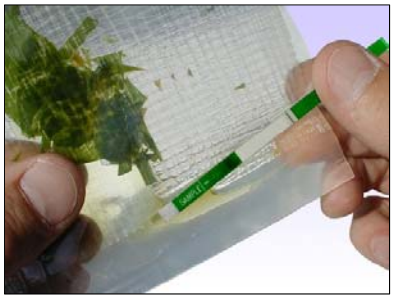
Lateral flow immunoassay - 4

5. After a while, when more and more fluid has passed the test stripe, particles accumulate and the stripe-area changes color. Accumulated Colloidal Gold has a red color.
6. The second stripe (the control; usually antibody anti-antibody) captures any antibody-bound particle and thereby shows that reaction conditions and technology worked fine.

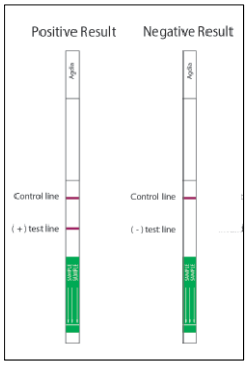


5 6

Lateral flow immunoassay - 5



- Tissue sample extracted in mesh bag
- ImmunoStrip placed in extract
- 5-10 min 'reaction'



- Graphic of expected results
- Positive vs. Negative

< Control

< Positive

- +

Example ImmunoStrip Test

Demonstration results almost done? - Agdia POTY ImmunoStrip Test

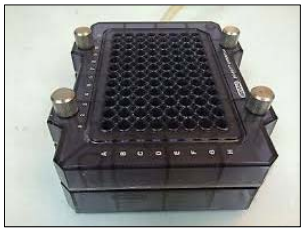
Other membrane-based serological assays

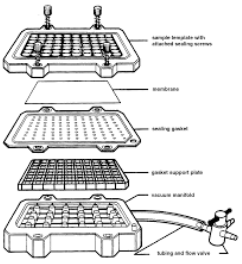
Dot-blots and Tissue prints

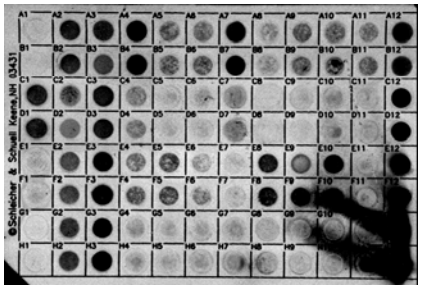
- Dot-blots
  - Antigen samples are “spotted” to a membrane, usually in a grid pattern [96-well format (w/, w/o device), or free-hand].
  - Variety of membrane compositions:
    - Nitrocellulose, nylon, PVDF
  - Proceed as per a direct or indirect ELISA; albeit the whole membrane is incubated in solutions in a tray and final substrate reaction product is insoluble (i.e., precipitates at reaction location).
  - E.g., as per a Western-blot after blotting of proteins from a gel.

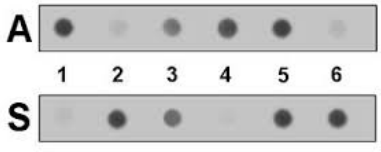
Other membrane-based serological assays - 2

Dot-blot - examples









Other membrane-based serological assays - 3

Tissue prints

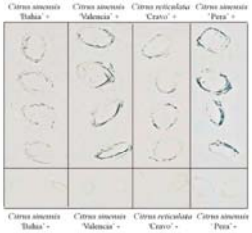
- Tissue printing
  - o Plant samples are “printed” (or “stamped” or ”smashed”) onto a membrane.
  - o Variety of membrane compositions:
    - Nitrocellulose, nylon, PVDF
  - o Proceed as per a direct or indirect ELISA; albeit the whole membrane is incubated in solutions in a tray and final substrate reaction product is insoluble (precipitates).
  - o E.g., as per a Western-blot after blotting of proteins from a gel.
  - o Advantages include:
    - Simple sample preparation: e.g., sliced edge from a rolled leaf
    - Can localize virus in specific tissues or organs

- Demonstration: tobacco leaf to nitrocellulose membrane

Other membrane-based serological assays - 4

Tissue print - examples

Anti-TSWV McAb (4A) or PcAb (4B) vs. TSWV-infected (A, B) or healthy (4C) *N. benthamiana* . Lin, Hsu, Hsu (1990) Phytopathology 80:824-828.



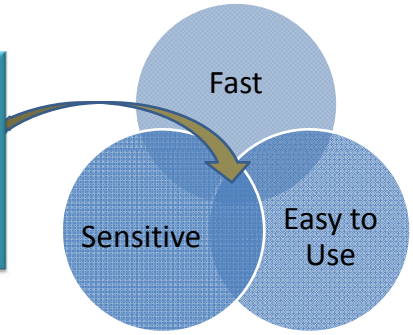
Example of Citrus leprosis virus-C p29 polyclonal antibody. (+ = infected; - = healthy.) Calegario et al (2013) Trop. Plt. Path. 38:188.

ScFv anti-HLB vs. citrus midribs; Hartung *et al* (unpublished).

CANARY® - Cellular Analysis and Notification of Antigen Risks and Yields

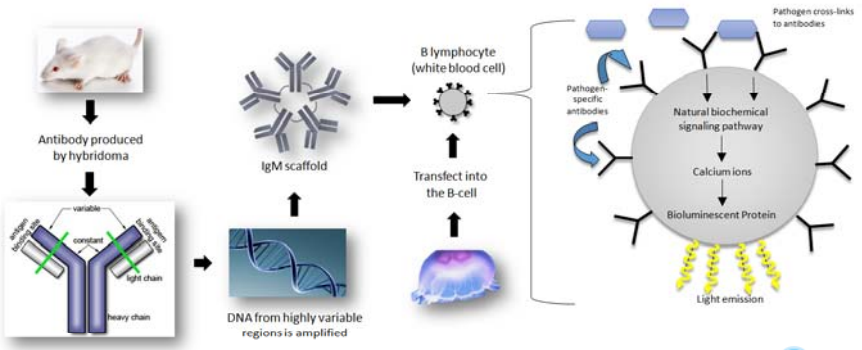
- Cell-based biosensor technology developed by MIT Lincoln Lab
- Published in Science: Vol. 301 11 July 2003 pg. 213-215
- Licensed to *PathSensors* (Baltimore, MD)
- Immuno-based system – more sensitive & faster than traditional methods
- Detects viruses, bacteria, fungi, proteins, and nucleic acids
- Highly specific
- Easy to use

CANARY® Technology
pg virus; 100-1000 cfu
5 minutes to result
Easy to use
Inexpensive



PATHSENSORS
IDENTIFYING PATHOGENS AT THE SPEED OF LIGHT

CANARY® BioSensor Technology - 2



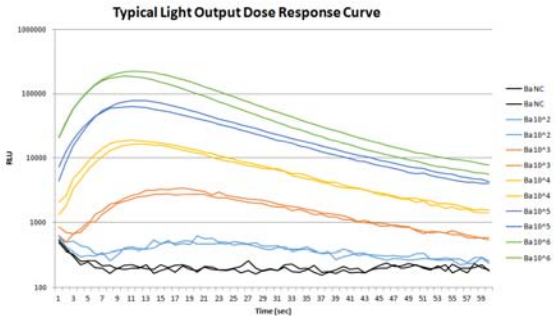
Proprietary information of PathSensors, Inc.

PATHSENSORS
IDENTIFYING PATHOGENS AT THE SPEED OF LIGHT

CANARY® BioSensor Technology - 3

PathSensors - Zephyr System:
- *Biosensor cell lines*
- *Centrifuge*
- *Touch-enabled PC*
- *Luminometer*
- *Barcode scanner*

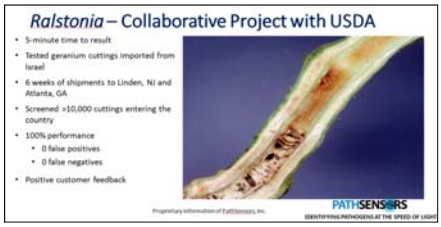
Light Output

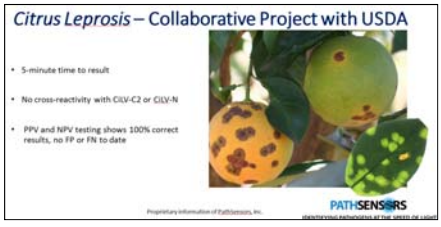


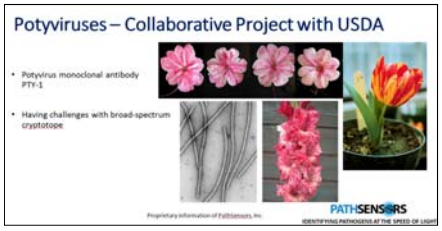
An algorithm is used to determine Positive or Negative call based on shape of light output over time

PATHSENSORS
IDENTIFYING PATHOGENS AT THE SPEED OF LIGHT

CANARY® BioSensor Technology - 4







PATHSENSORS
IDENTIFYING PATHOGENS AT THE SPEED OF LIGHT

Serology-based assays

Questions?

Thanks!

…Then break…