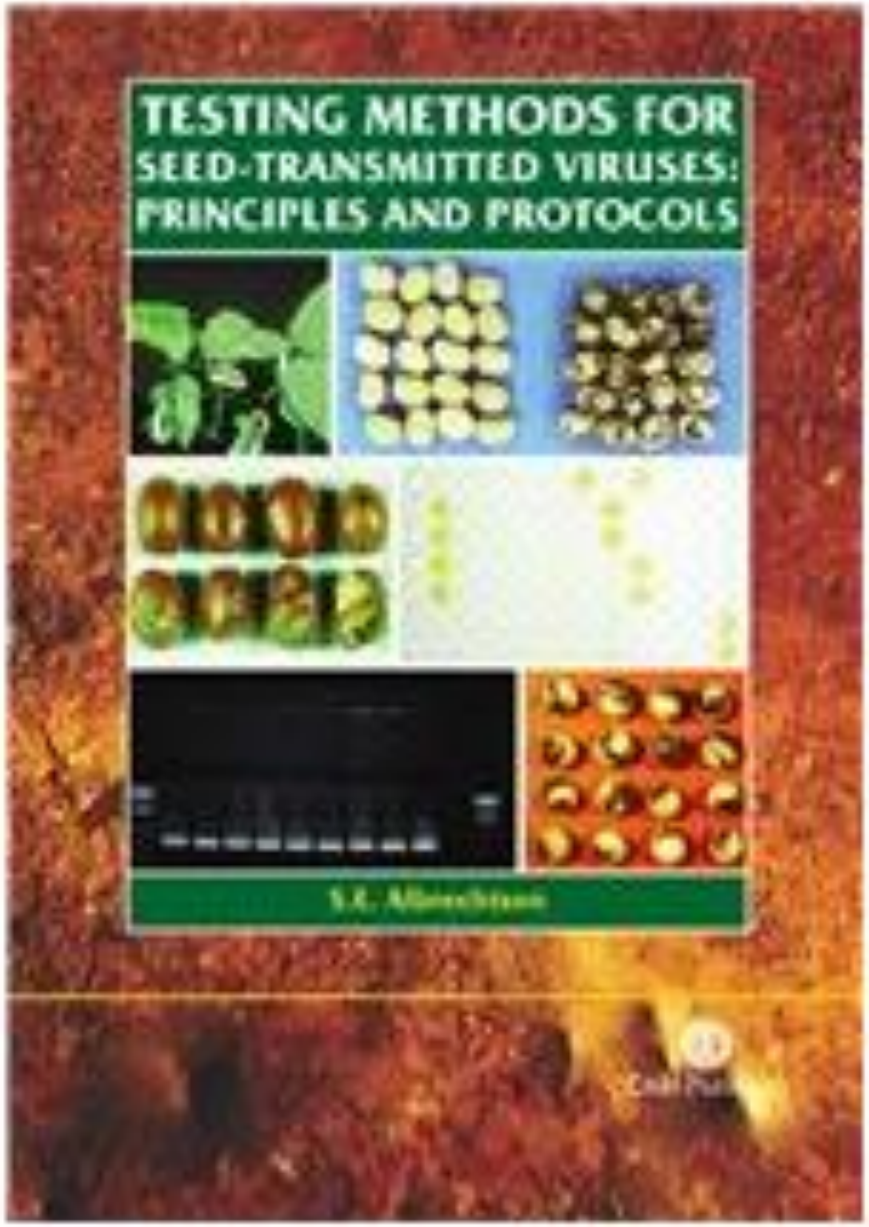
# SOROLOGIA PARA DETECÇÃO DE VÍRUS EM SEMENTES

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## TRANSMISSÃO DE VÍRUS POR SEMENTES

### A. Características

- 1/4 dos vírus conhecidos são transmitidos por sementes.
- Vírus crípticos
- Transmissão de 0 - 100%, maioria < 50%.
- Transmissão é função da hospedeira e do vírus.

EX: Vírus necrose branca do fumo

Não é transmitido por semente de fumo
É transmitido por semente de *Nicandra physaloides*

EX: Vírus do mosaico amarelo do feijoeiro

Não é transmitido por sem. de diversos *Phaseolus*
É transmitido por sem. de *Vigna sinensis*

- Época em que a planta foi infectada.
- Longevidade do vírus na semente: meses até anos.

**TABLE 12.9** Examples of Relative Importance of Seed Transmission for Viruses of Various Virus Genera[a]

| Virus Genus/ Group | No. Members | | Type of Potential Injury[b] | | | | | | % Seed Transmission |
|---|---|---|---|---|---|---|---|---|---|
| | In Group | Seed-Borne | A | B | C | D | E | F | |
| *Alfamovirus* | 1 | 1 | + | + | + | | | | 1–23 |
| *Bromovirus* | 6 | 1 | + | + | + | | | | +[c] |
| *Capillovirus* | 4 | 1 | | | | | | | 1–60 |
| *Carlavirus* | 60 | 2 | | | | | | | 2–90 |
| *Carmovirus* | 18 | 2 | | | | | | | 10–40 |
| *Caulimovirus* | 34 | 1[d] | | | | | | | [d] |
| *Closterovirus* | 28 | 1 | | | | | | | + |
| *Comovirus* | 15 | 6 | + | + | + | | | | 1–90 |
| *Cryptovirus* | 31 | 31 | | | | | | | 100 |
| *Cucumovirus* | 3 | 3 | + | + | + | | | | <1–1 |
| *Dianthovirus* | 5 | 0 | | | | | | | |
| *Enamovirus* | 1 | 1 | | | | | | | 1–2 |
| *Fabavirus* | 4 | 0 | | | | | | | |
| *Geminivirus* | 102 | 1 | | | | | | | + |
| *Hordeivirus* | 4 | 1 | + | + | + | | + | | + |
| *Ilarvirus* | 17 | 8 | | | | + | | | 1–90 |
| *Luteovirus* | 7 | 0 | | | | | | | |
| *Marafivirus* | 3 | 0 | | | | | | | |
| *Nepovirus* | 40 | 17 | + | + | + | | | | 3–100 |
| *Plant reovirus* | 14 | 0 | | | | | | | |
| *Potexvirus* | 36 | 4 | | | | | | | 1–6 |
| *Potyvirus* | 179 | 16 | + | + | + | + | + | + | <1–80 |
| *Rhabdovirus* | 15 | 1 | | | | | | | + |
| *Sobemovirus* | 14 | 4 | | | | | | | 1–80 |
| *Tenuivirus* | 11 | 0 | | | | | | | |
| *Tobamovirus* | 17 | 7 | + | + | + | | | | 1–20[e] |
| *Tobravirus* | 3 | 3 | + | + | + | | | | 1–35 |
| *Tospovirus* | 13 | 1 | | | | | | | Up to 95 |
| *Tombusvirus* | 13 | 1 | | | | | | | + |
| *Tymovirus* | 23 | 3 | | | | | | | + |
| Viroids | 15 | 5 | | | + | + | | | + |

*Data from Stace-Smith and Hamilton (1988) and from AAB Descriptions of Plant Viruses.*

[a] *Note that not all members of genus were tested for seed transmissibility.*

[b] *A, survival of inoculum; B, dispersal of inoculum; C, primary inoculum source; D, contamination of germplasm lines; E, contamination of virus-free planting material; F, direct crop losses due to plants arising from infected seed.*

[c] *+indicates that no % values were given.*

[d] *BSV is apparently seed transmitted in Musa but probably by activation of integrated viral sequences.*

[e] *Seed-transmission of TMV probably due to contamination.*

Hull, 2012

Legenda Tabela 21.1

A: sobrevivência do inóculo
B: disseminação do inóculo
C: fonte primária do inóculo
D: contaminação de germoplasmas
E: contaminação de material propagativo livre de vírus
F: dano direto na produção originário de sementes infectadas

## B. Valor epidemiológico

- Perpetua o vírus sob condições adversas.

- Foco inicial de inóculo na cultura.

- Introdução e estabelecimento do vírus em novas áreas, países.

- Presença em bancos de germoplasma: efeito no melhoramento.

**C. Tipos de transmissão de vírus por semente**

1. Infecção da plântula por vírus aderido à parte externa da semente
   Eliminação: calor seco 70-76°C, 1 a 3 dias
   Solução 10% de Fosfato trisódio, 30 minutos

2. Transmissão verdadeira ou embriogênica

**D. Rotas para infecção do embrião:**

Diretamente da planta mãe ou pólen

- Infecção do meristema floral (vírus crípticos)

- Infecção direta do embrião

Problema: isolamento do embrião dos tecidos maternos, ausência de ligações vasculares

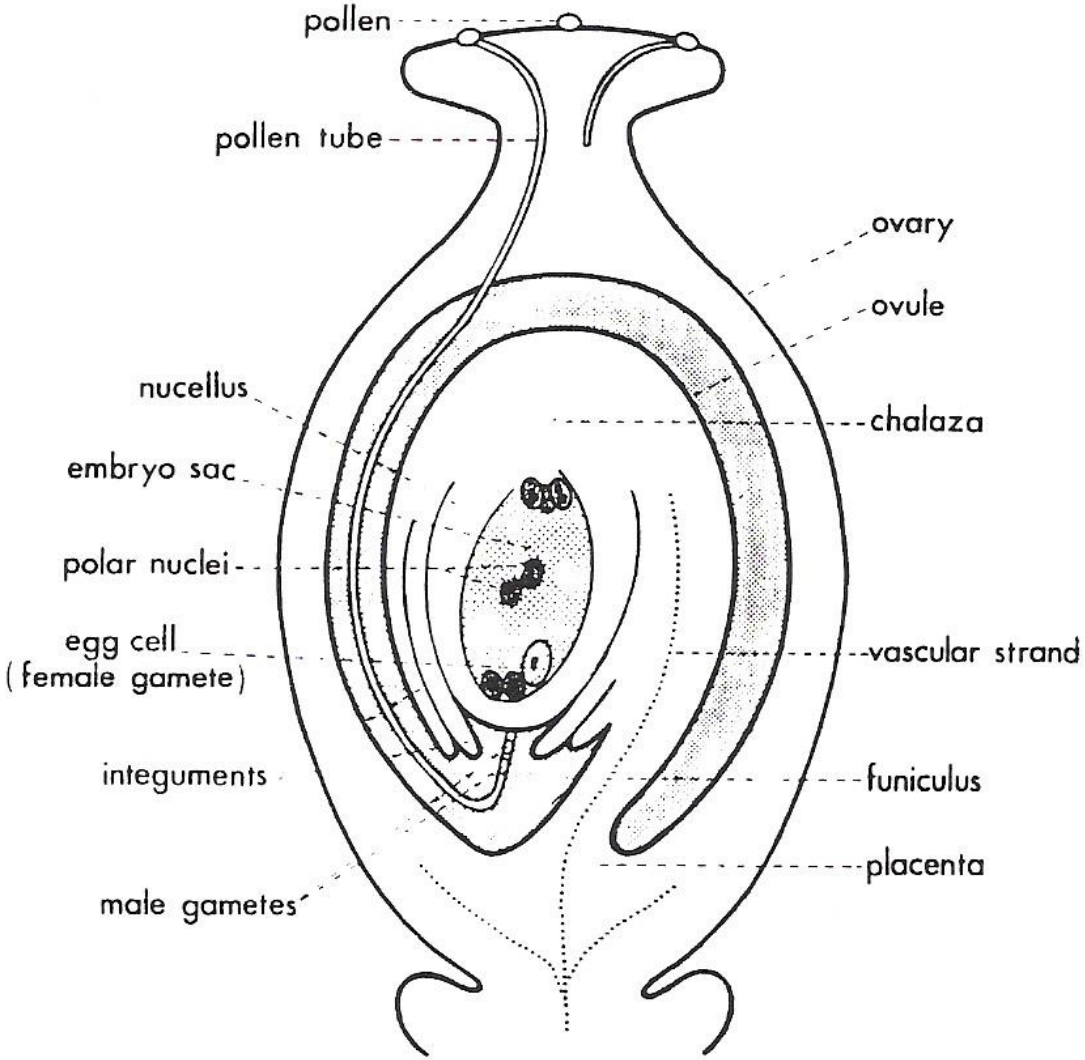



**Fig. 4-4.** Diagram representing the anatomy of the ovule (the megasporangium of the seed plant) within the ovary. (From Bos, 1977.)

# SOROLOGIA DE VÍRUS DE PLANTAS

## 1. Terminologia

**ANTÍGENO:** Qualquer substância capaz de induzir uma resposta de imunidade quando injetada em um animal.

**IMUNOGÊNICO:** capacidade que uma subst. tem de induzir uma resposta de imunidade.

- Não pode ser constituinte do animal.
- Peso molecular > $10^4$.
- Estrutura definida.

**ANTICORPO:** Proteínas pertencentes ao grupo das imunoglobulinas, produzidas nos linfócitos, e que aderem especificamente aos antígenos.

**ANTISSORO:** soro sanguíneo contendo anticorpos.

**DETERMINANTE ANTIGÊNICO OU EPITOPO:** é a sequência de aminoácidos na molécula protéica do antígeno que é complementar à molécula do anticorpo.

Determinante sequencial: 5 - 7 a.a.

Determinante conformacional: sequência de a.a. sob certa conformação dentro da molécula.

## 2. Classes de imunoglobulinas (Ig)

IgG, IgA, IgM, IgD e IgE
IgG = 75% da Ig do soro.
Estrutura da IgG

# MOLÉCULA DE MIOGLOBINA: EPITOPOS

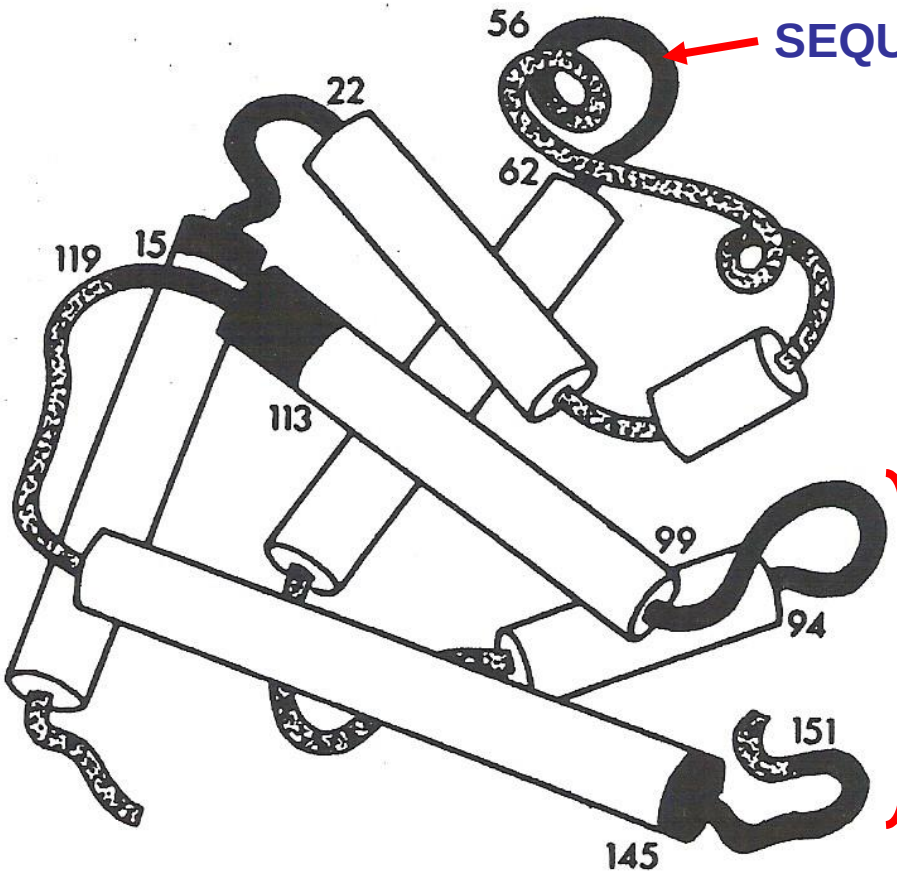



**Fig. 1.1.** Three-dimensional structure of myoglobin. The helices are represented as cylinders and the antigenic sites studied by Atassi (1975) are shown as black portions. The residue numbers correspond to the extremities of the different epitopes.

# IMUNOGLOBULINA G - IgG

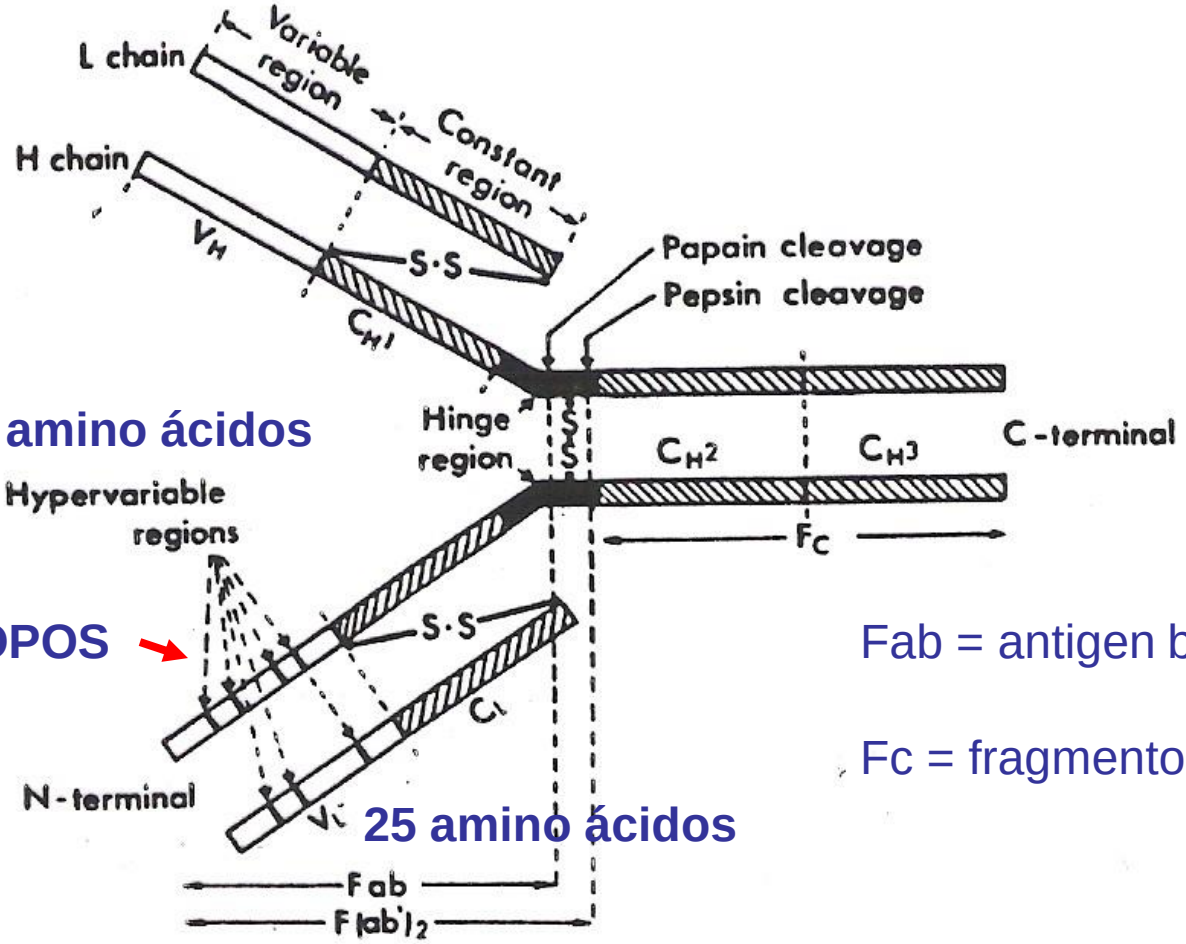



Fig. 2.1. Rabbit IgG molecule showing the interchain disulfide bridges, the location of the different domains, and the fragments obtained by papain and pepsin cleavage.

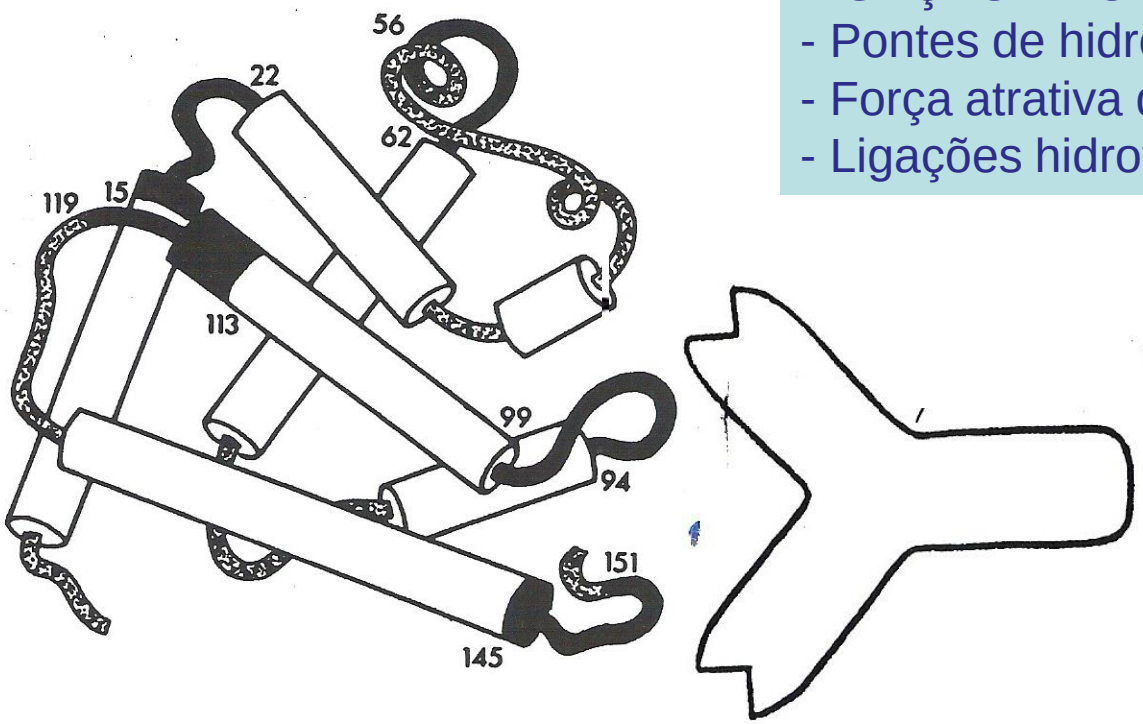
Van Regenmortel (1982)

Fab = antigen binding fragment

Fc = fragmento cristalizável

FC: alta afinidade pela proteína A de *Staphylococcus aureus*

# REAÇÃO SOROLÓGICA = ANTÍGENO + ANTICORPO




LIGAÇÃO NÃO COVALENTE:
- Pontes de hidrogênio
- Força atrativa de van der Waals
- Ligações hidrofóbicas

# ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay)

**Vantagens:** Sensível (1 ng/ml), rápido, econômico,

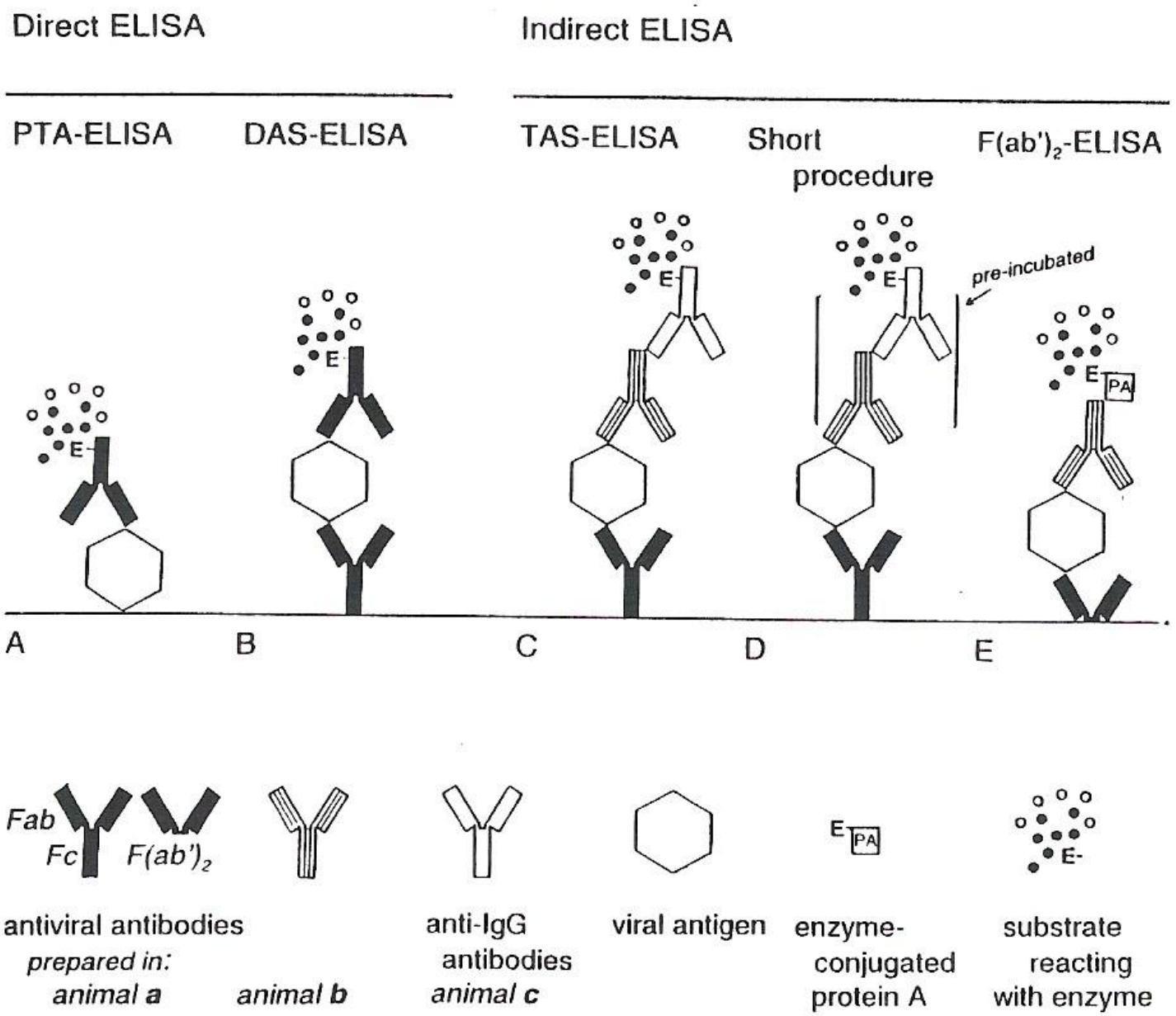
baixo custo, seguro e teste de várias amostras.

Quantitativo

Muito útil para testes de rotina.

**Desvantagens:** Não mede infectividade e reação inespecífica.

# TIPOS DE ELISA



# INTERPRETAÇÃO DO TESTE DE ELISA

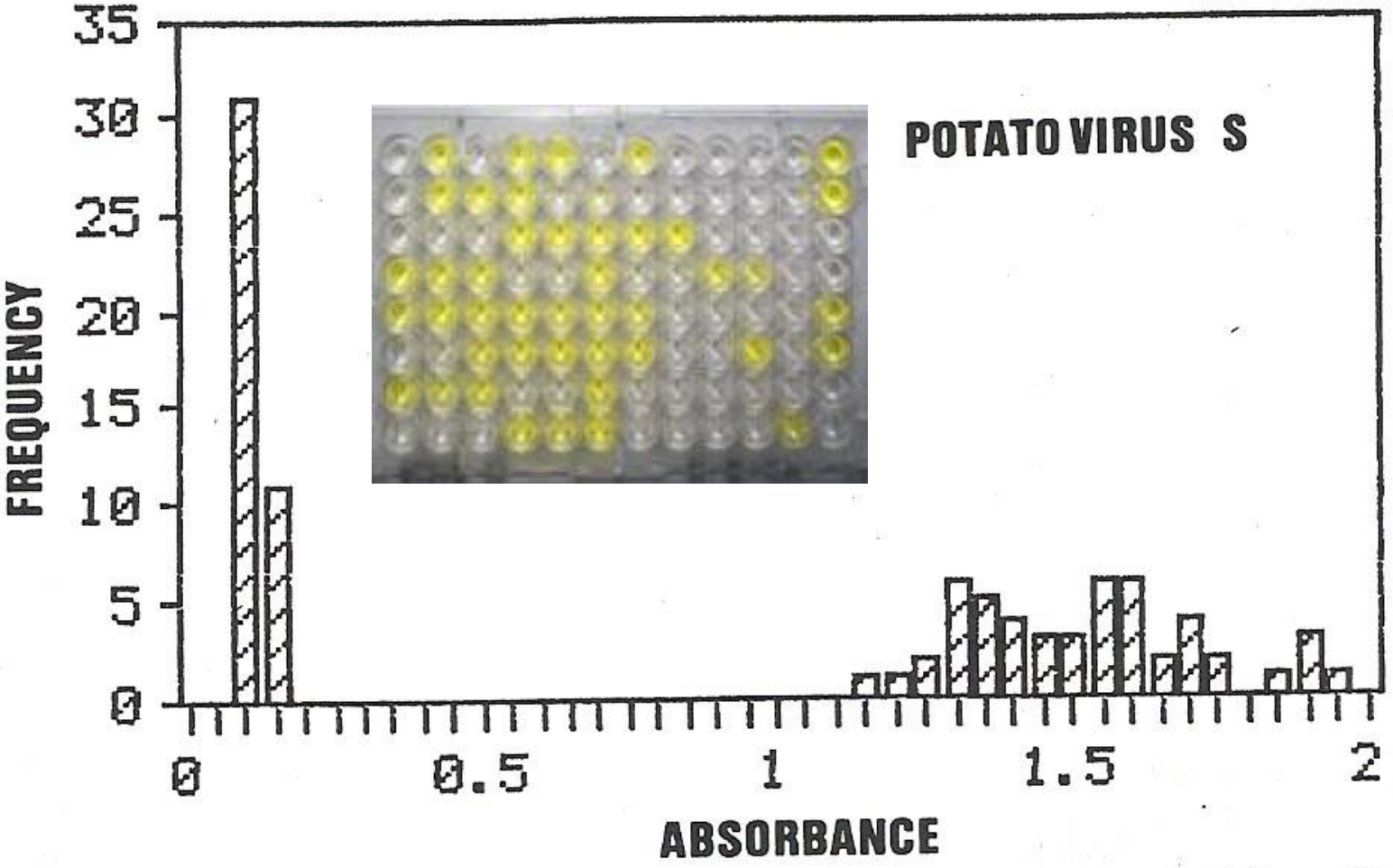



**Fig. 1. Histogram of ELISA results for potato virus S in plantlets derived from 90 characterized potato tissue cultures. The bimodal distribution of data is ideal, with a large interval of absorbance separating healthy (negative) plants on the left and diseased (positive) plants on the right.**

# Method for the Detection of *Lettuce mosaic virus* on Lettuce Seed and Seedlings

**Crop:** *Lactuca sativa*
**Pathogen**: *Lettuce mosaic virus* (LMV)

**Revision history:** Version 4.2, April 2015

## Sample and sub-sample size

The minimum sample size of the seedling assay is 2,000 seedlings and the maximum sub-sample size is 100 seedlings. For the seed assay the minimum sample size is 10,000 seeds and the maximum sub-sample is 500 seeds.

## Principle

Lettuce seeds or seedlings (according to the choice of the laboratory) are ground in a buffer solution to extract the virus. The extract is tested using DAS ELISA for the detection of LMV.

## Sensitivity and Restrictions on Use

- This test method is suitable for untreated seed.
- This test method is suitable for seed that has been treated using physical processes for disinfestation or seed that has been treated using chemicals for disinfestation provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- This test method has not been validated for seed treated with protective chemicals or biological substances. If a user chooses to test treated seed using this method, it is the responsibility of the user to determine empirically (through analysis, sample spiking, or experimental comparisons) whether the protective chemicals or biological substances have an effect on the method results.
- In the seed assay, one infected seed can be detected in a sub-sample of 500 seeds.
- A comparison of antibodies is recommended (1).
- In the seedling assay, one infected seedling can be detected in sub-sample of 100 seeds.

**Method for the Detection of *Squash mosaic virus* (SqMV), *Cucumber green mottle mosaic virus* (CGMMV) and *Melon necrotic spot virus* (MNSV) on Cucurbit seed**

| | |
|---|---|
| **Crop**: | Cucurbits (Watermelon (*Cirtullus lanatus* var. *lanatus*), Cantaloupe (*Cucumis melo* var. *catalupensis*) and melon (*Cucumis melo*)) |
| **Pathogens**: | *Squash mosaic virus* (SqMV), *Cucumber green mottle mosaic virus* (CGMMV) and *Melon necrotic spot virus* (MNSV) |
| **Date:** | August 2011 |

## Sample and sub sample size

The recommended minimum sample size is 2,000 seeds with a maximum sub-sample size of 100 seeds.

## Principle

The method, using ground seed in a DAS-ELISA, provides the option to simultaneously detect SqMV, CGMMV and MNSV in a single extract. The extract is tested in separate microtiter plates, one for each pathogen of interest; SqMV, CGMMV and/or MNSV. It can detect externally and internally located virions as well as infectious and non-infectious virions.

## Restrictions on Use

- This test method is suitable for untreated seed.
- This test method is suitable for seed that has been treated using physical processes for disinfestation or seed that has been treated using chemicals (such as hydrochloric acid, peroxyacetic acid, etc.) for disinfestation provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- Although ELISA is compatible with some seed treatment chemicals (Pataky et al., 2004), seed treatments may affect the performance of this test. It is the responsibility of the user to check for such antagonism and or inhibition by analysis, sample spiking, or experimental comparisons.