



# A LATERAL FLOW DEVICE FOR SIX HONEYBEE VIRUSES

production and evaluation of the antisera



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Buenos Aires, 19-26 september 2011

[www.bee-doc.eu](http://www.bee-doc.eu)





# BEE DOC



- WP1 pathogen-parasite-pesticide interactions - individual bees
- WP2 pathogen-parasite-pesticide interactions - colonies
- WP3 genomic responses to infections & pesticides
- WP4 resistance genes to *N. apis* & *N. ceranae*
- WP5 diagnostic tools**
- WP6 pathogen surveillance
- WP7 treatment & control
- WP8 information & outreach
- WP9 project coordination





## LATERAL FLOW DEVICE

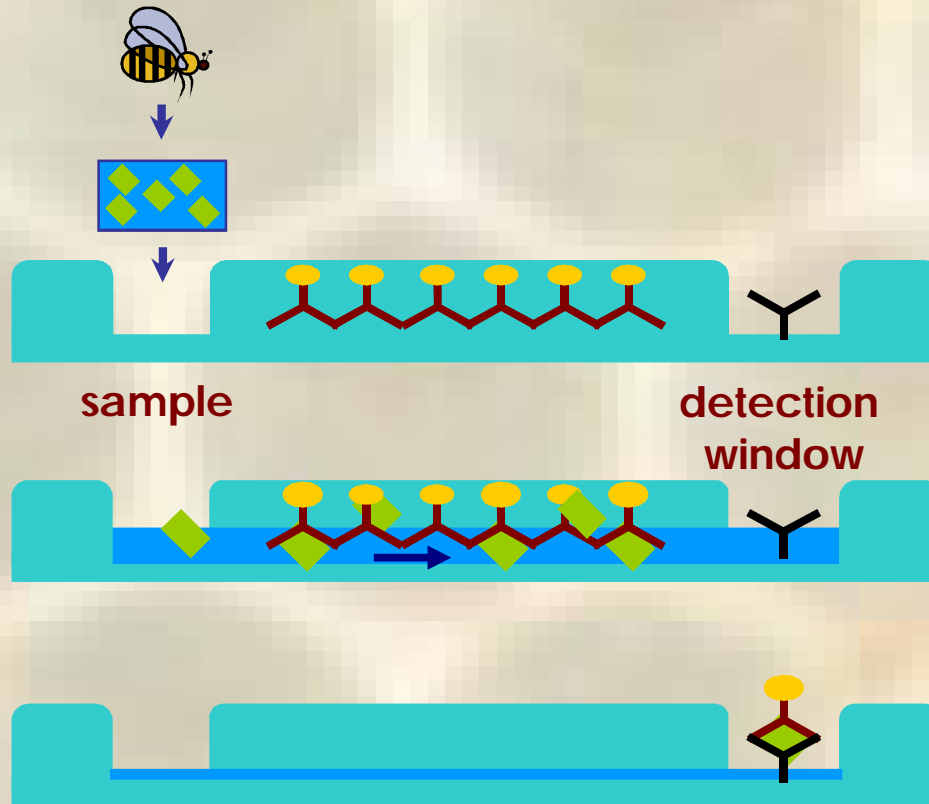


ABPV  
DWV  
CBPV  
BQCV  
SBPV  
SBV

ACCURATE  
SENSITIVE  
ROBUST  
SIMPLE  
FAST  
CHEAP



# Lateral Flow Device





# STRATEGY



## 1. Synthetic peptide antigen

- pure
- no virus production/cell culture
- reproducible
- commercial scale antigen synthesis
- intact virion capture with sandwich approach

## 2. Egg-derived antisera

- affinity purification
- reproducible
- commercial scale antibody synthesis





# PEPTIDE DESIGN

## 1. Bioinformatics

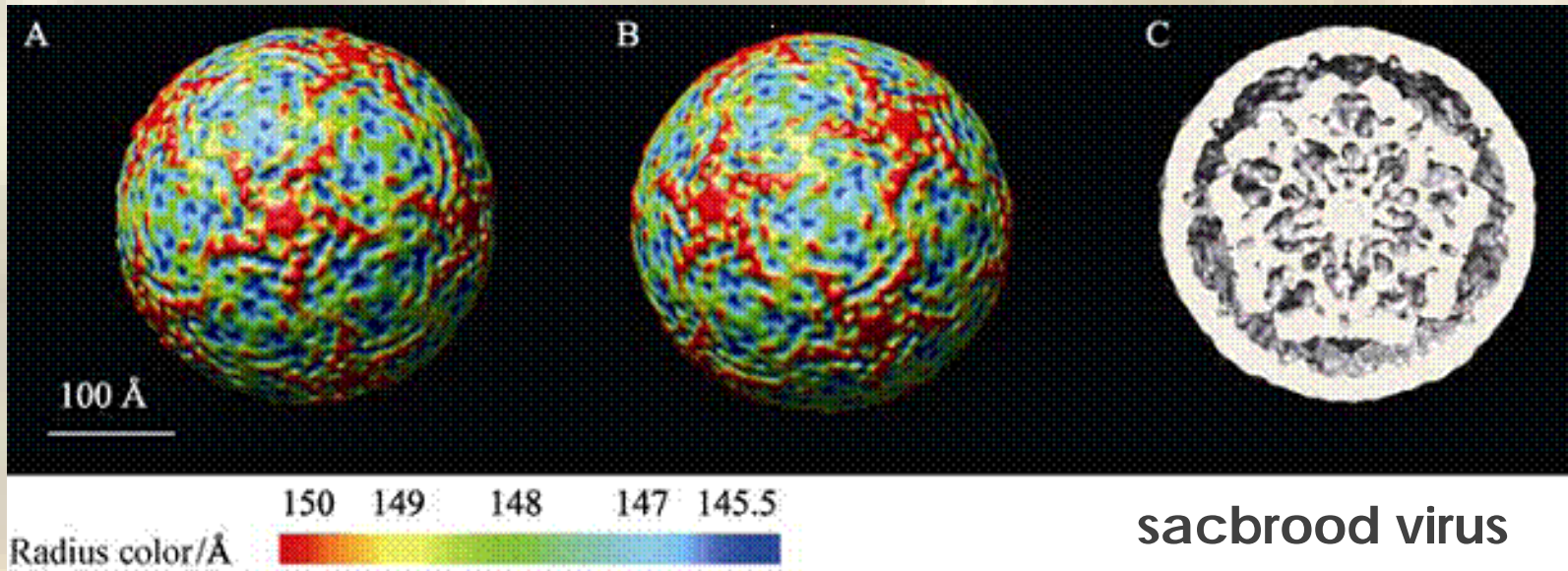
- virus sequence
- virion structure
- Western Blots – natural antisera
- diagnostic antisera vs denatured purified capsid proteins

## 2. Criteria

- antigenicity
- surface exposure
- hydrophobicity
- variation

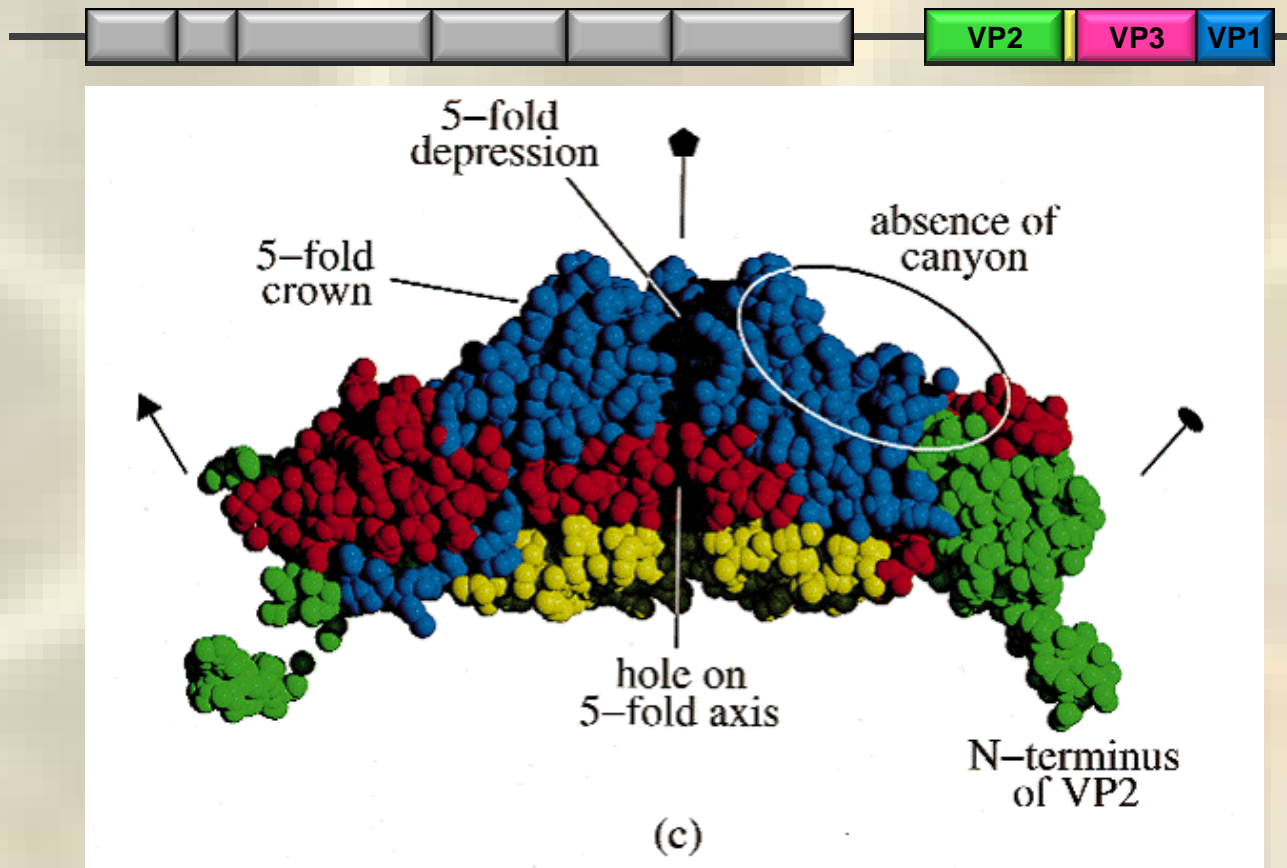


# VIRION STRUCTURE



Xie et al. (2009) Sci China Ser C-Life Sci

## PENTAMER CROSS-SECTION



Tate et al. (1999) Nat Struct Biol

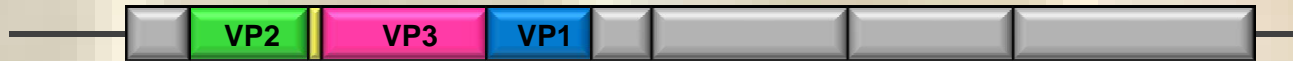


# VIRUS SEQUENCE

ABPV - BQCV



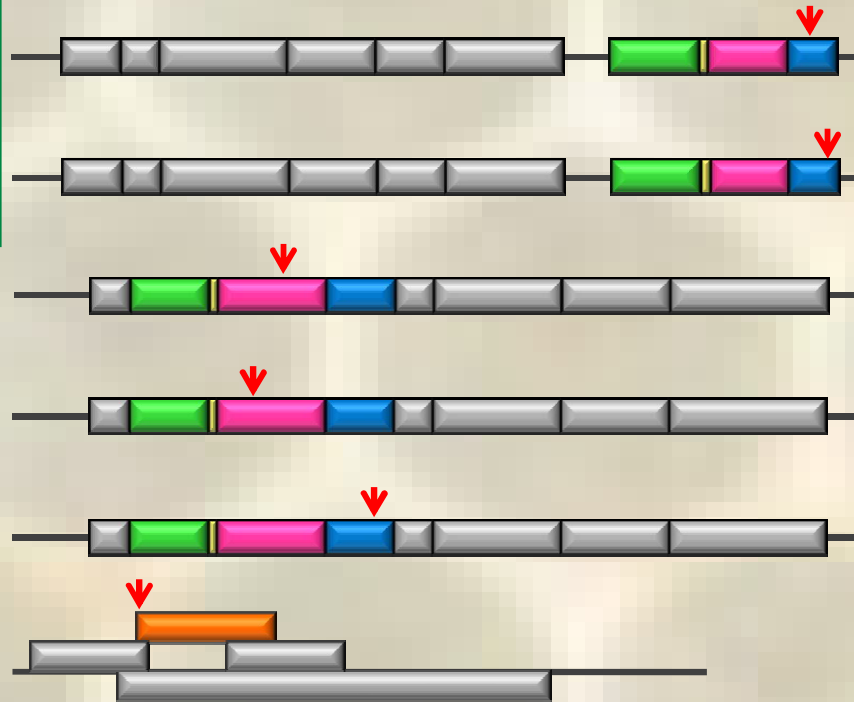
DWV - SBPV - SBV



CBPV



# PEPTIDE PREDICTIONS



VIRUS	Ag	Surface	HydPhob	ACGT
ABPV	PP	P	PP	P
BQCV	PP	~	~	PP
DWV	PP	P	P	P
SBPV	~	~	0	PP
SBV	PP	PP	~	PP
CBPV	PP	PP	PP	-



# ANTIBODY PRODUCTION



## 1. Carrier protein

- **Keyhole Limpet Hemocyanin (KLH)**
- high antigenicity
- strong immune response
- peptide conjugation

## 2. Immunization

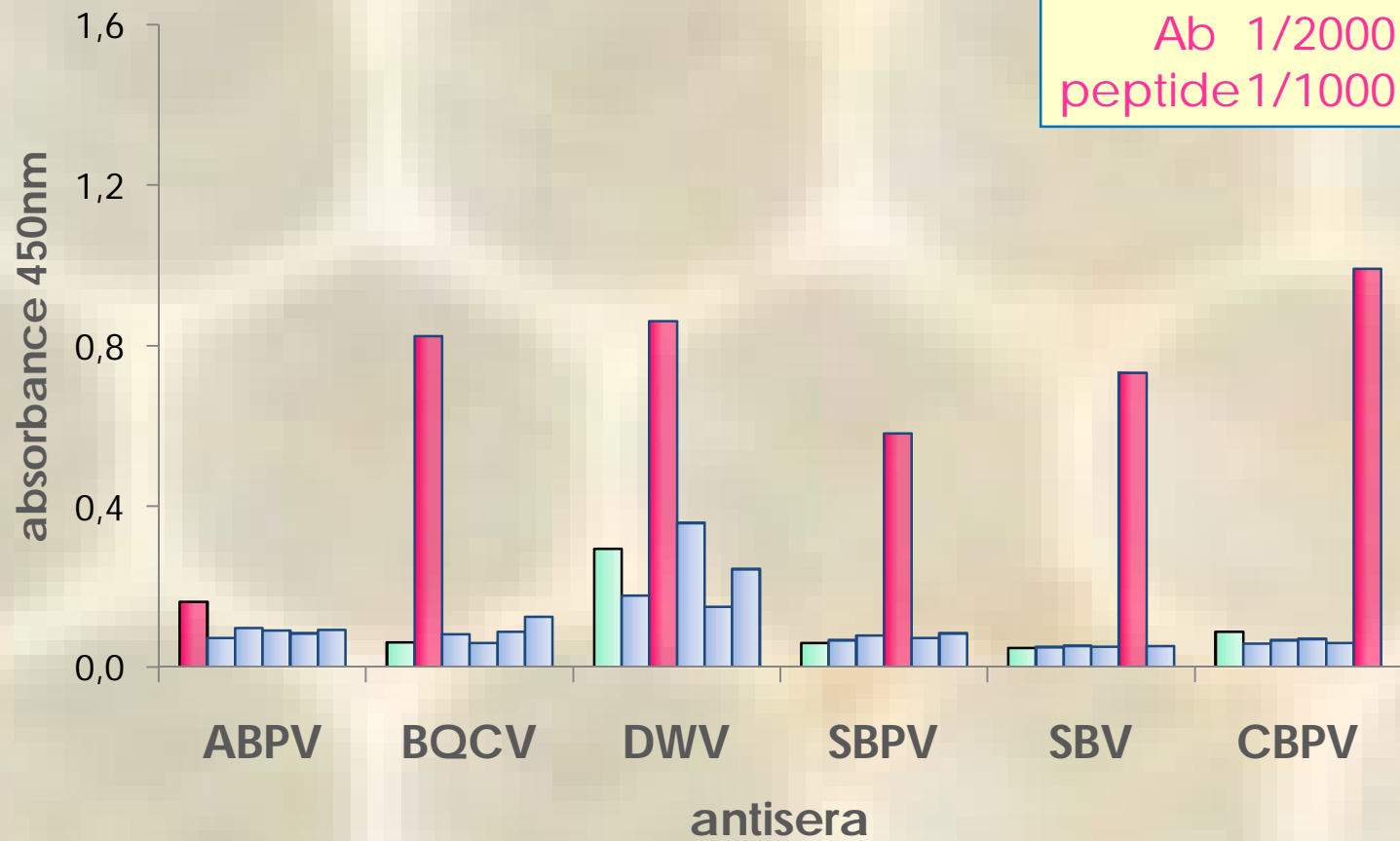
- chickens
- primary & booster injections
- antibodies purified from eggs

## 3. Purification

- yolk protein removal
- PEG precipitation
- Sepharose gel affinity purification vs peptides



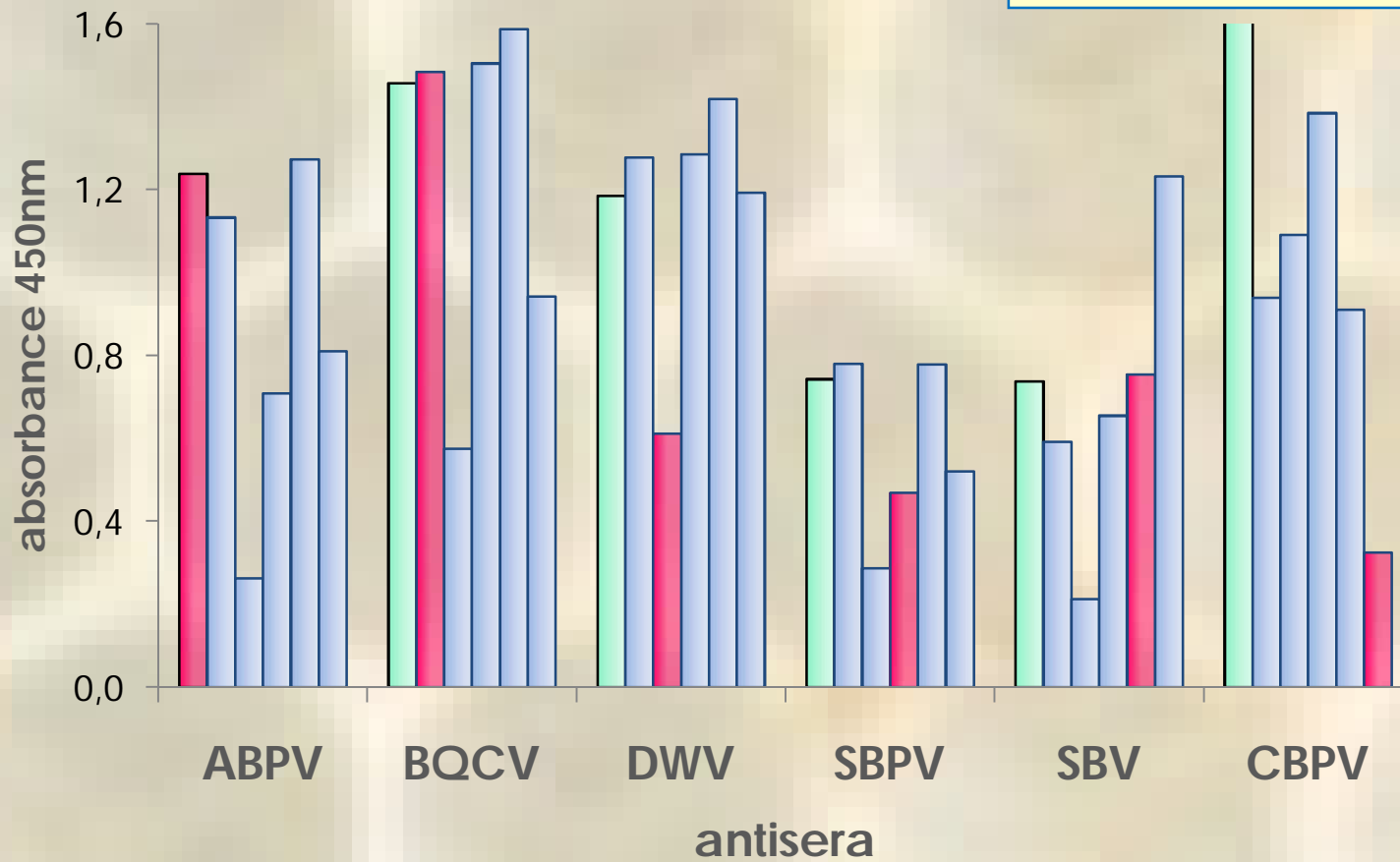
## CROSS-REACTIONS peptides





## CROSS-REACTIONS virus

Ab 1/2000  
virus 1/10,000





# BEE EXTRACT BACKGROUND



## 1. Virus extracts

- intervirus immunological cross-reaction?
- contaminated virus extracts?
- natural HRP activity?

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## 2. Keyhole Limpet Hemocyanin

- honeybee storage proteins
- massively produced
- strong immunogenic, high IgY titres
- strongly cross-reactions between invertebrates
- affinity purification

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P  
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## FUTURE EVALUATIONS

- Ab's vs pure KLH ELISA
- antiviral A/S vs peptides ELISA
- sandwich ELISA antiviral A/S & anti-peptide Ab's
- Western blot





# CONCLUSIONS

## 1. Strategy

- peptide design?
- antibody production?
- Ag specificity?
- natural virus extract specificity?

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## 2. Non-specific background

- research into cause of background
- further purification
- re-assess natural extract specificity
- alternative carriers (BSA)

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THANK YOU...

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