

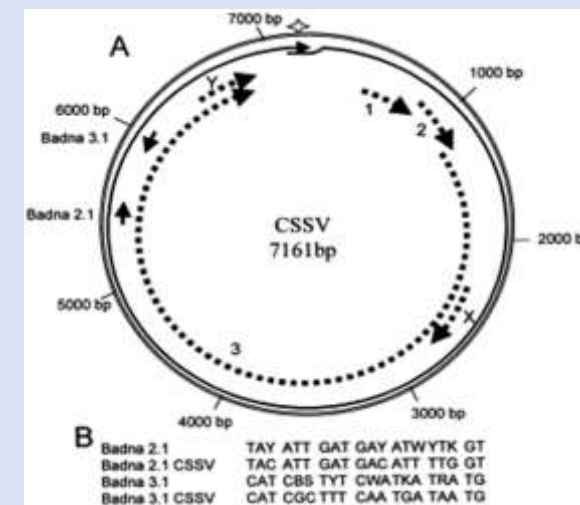
Development of immunoassays to detect Cocoa Swollen Shoot Virus

Jacqueline M Barnett^{1,2}, Sue Tyler^{1,2}, Janice Kiely², Richard Luxton², Jean-Philippe Marelli Joel Allainguillaume¹

1. Faculty of Applied Science, University of the West of England, Bristol, UK. BS161QY
2. Institute of Biosensing Technology, University of the West of England, Bristol, UK. BS161Q
3. MARS Wrigley, Davis Plant Science Laboratory, California, US. Davis 95616

Cacao Swollen Shoot Virus Disease(CSSVD)

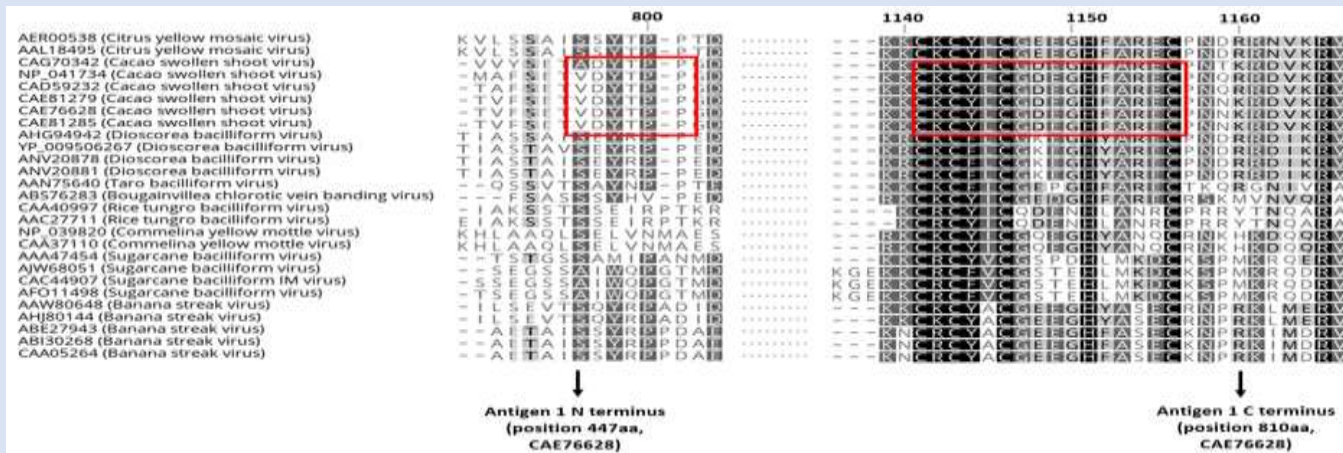
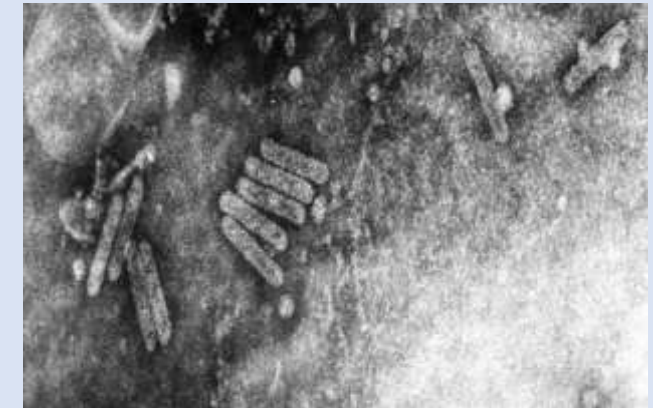
- The global chocolate market is predicted to be worth USD 200.4 billion by 2028
- Cacao Swollen Shoot Virus disease (CSSVD) has been identified as the main disease threat to productivity of chocolate.
- There is no rapid test available to detect CSSVD



Development of reagents for a CSSV immunoassay

CSSV is a Pararetrovirus

Polyprotein (211 kDa) produced from ORF3 comprising, proteolytic cleavage produces several proteins, a cell-to-cell movement protein, **coat protein**, an aspartyl proteinase, a reverse transcriptase and a ribonuclease H



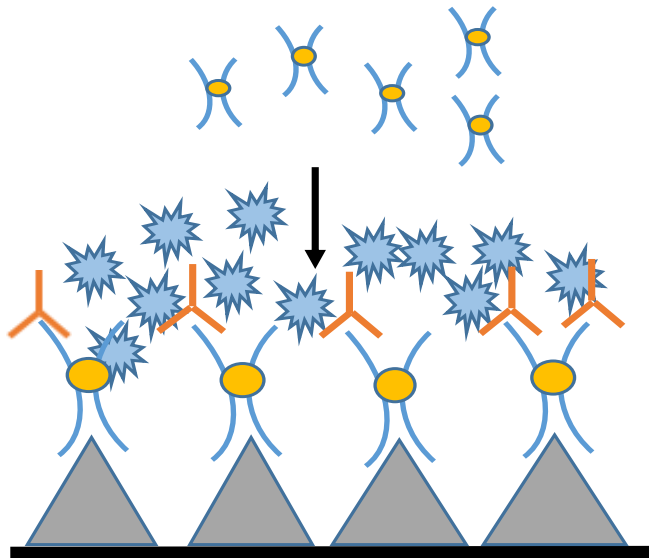
Recombinant Coat Protein Antigen (CSSV-CP-01)



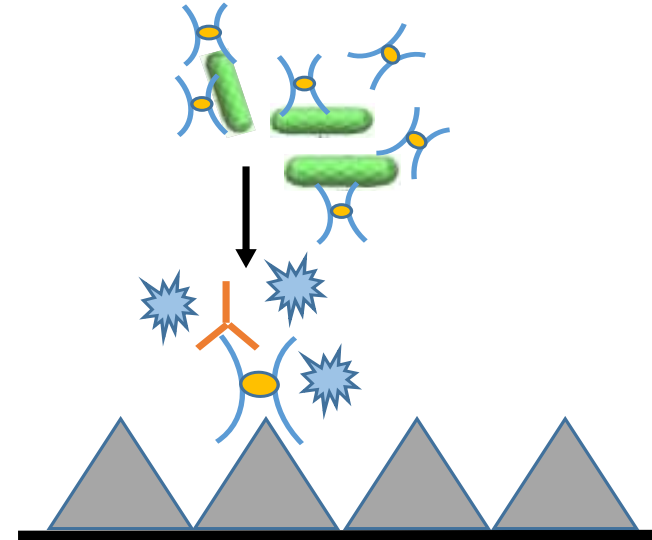
Recombinant antibodies

CSSV Competitive ELISA

Healthy leaf extract



CSSV infected leaf extract



Microtitre plate well



Anti-CSSV coat protein antibody F(ab)₂



Antigen – CSSV-CP-01



Cocoa Swollen Shoot Virus Particle

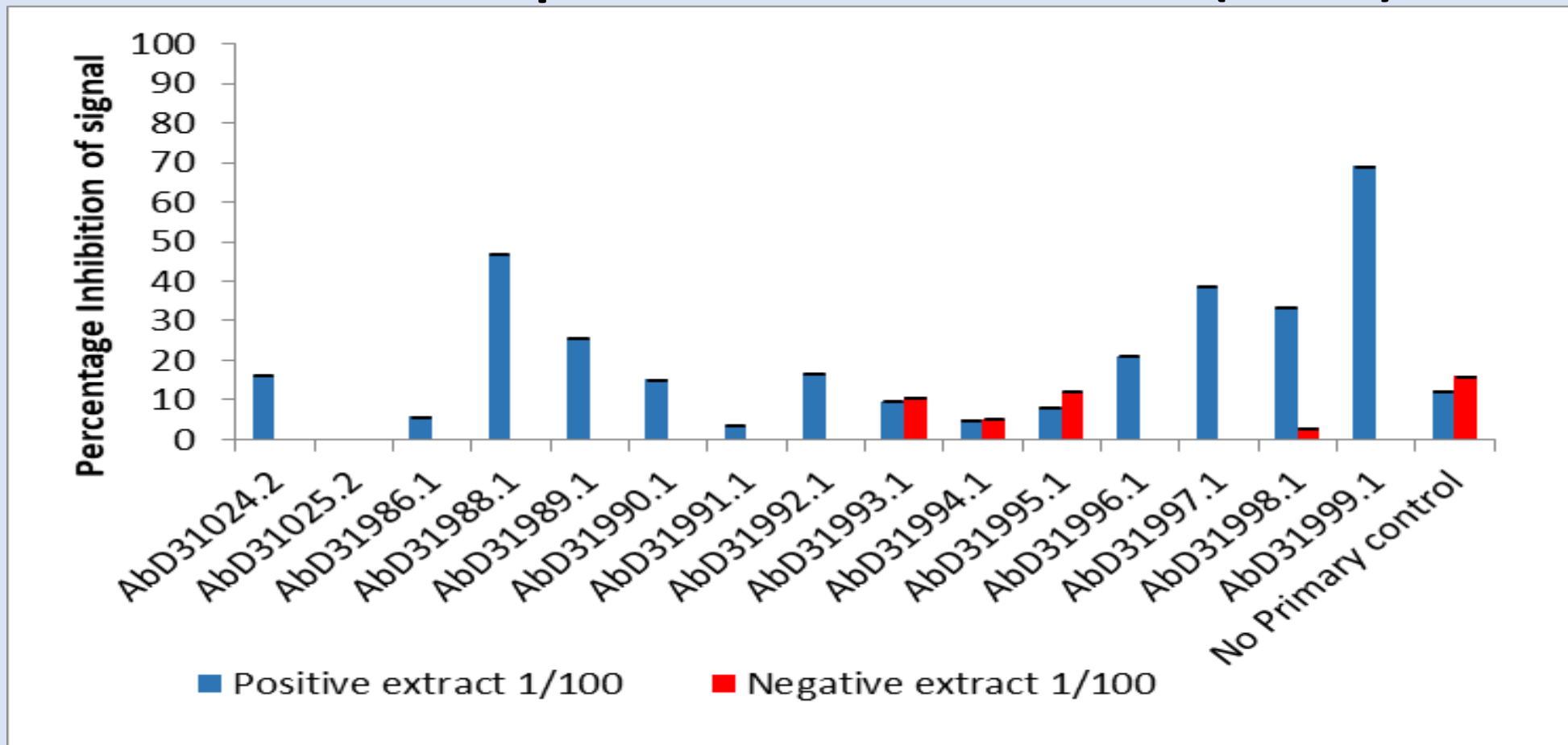


Goat anti-human IgG F(ab')₂: (HRP)

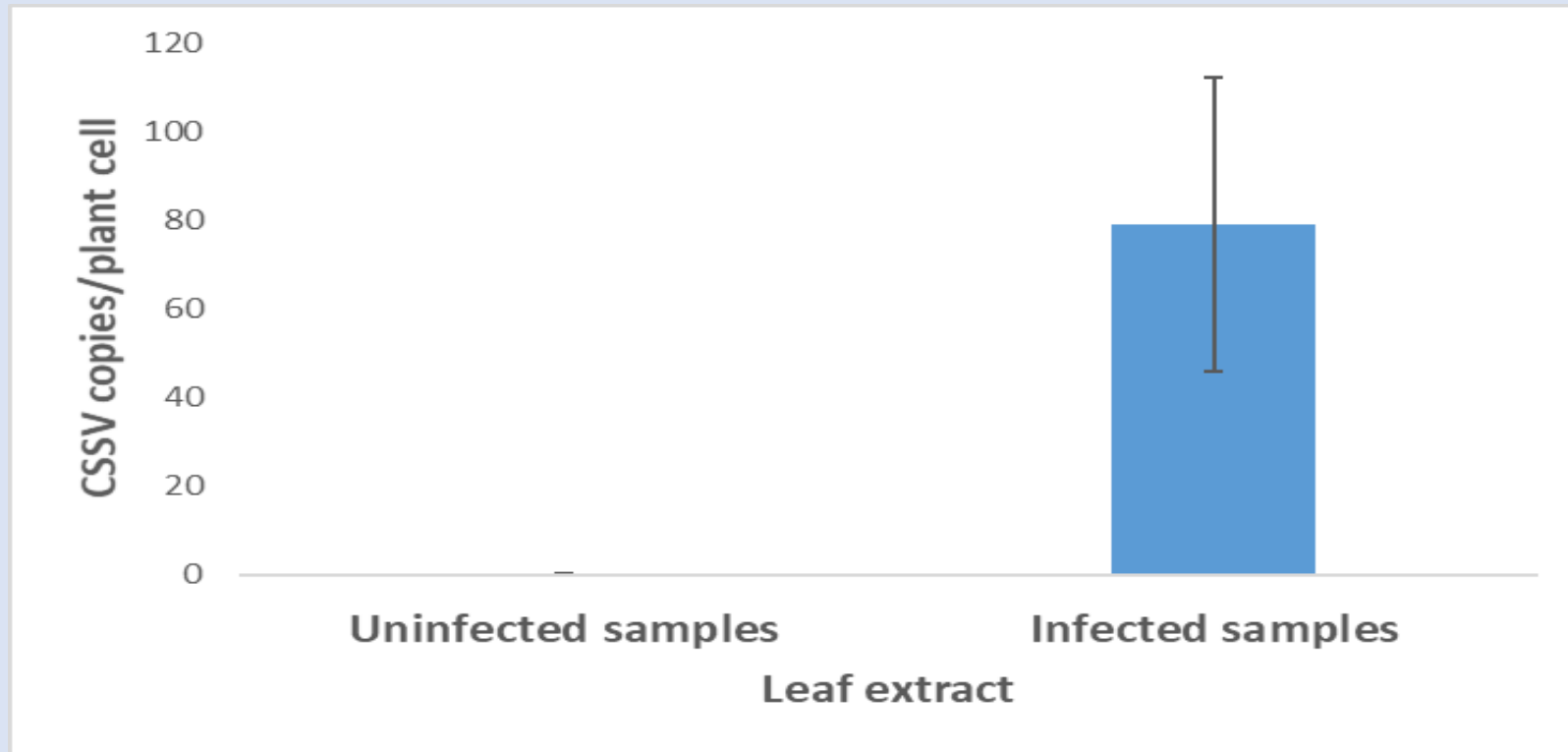


TMB substrate producing a coloured product.

Competitive ELISA results for fifteen recombinant antibodies and plant leaf extract (n=3)



CSSV qPCR results of uninfected (n=12) and CSSV New Juaben infected (n=23) leaf extracts from the Envirottron, UWE expressed as CSSV copies/cell

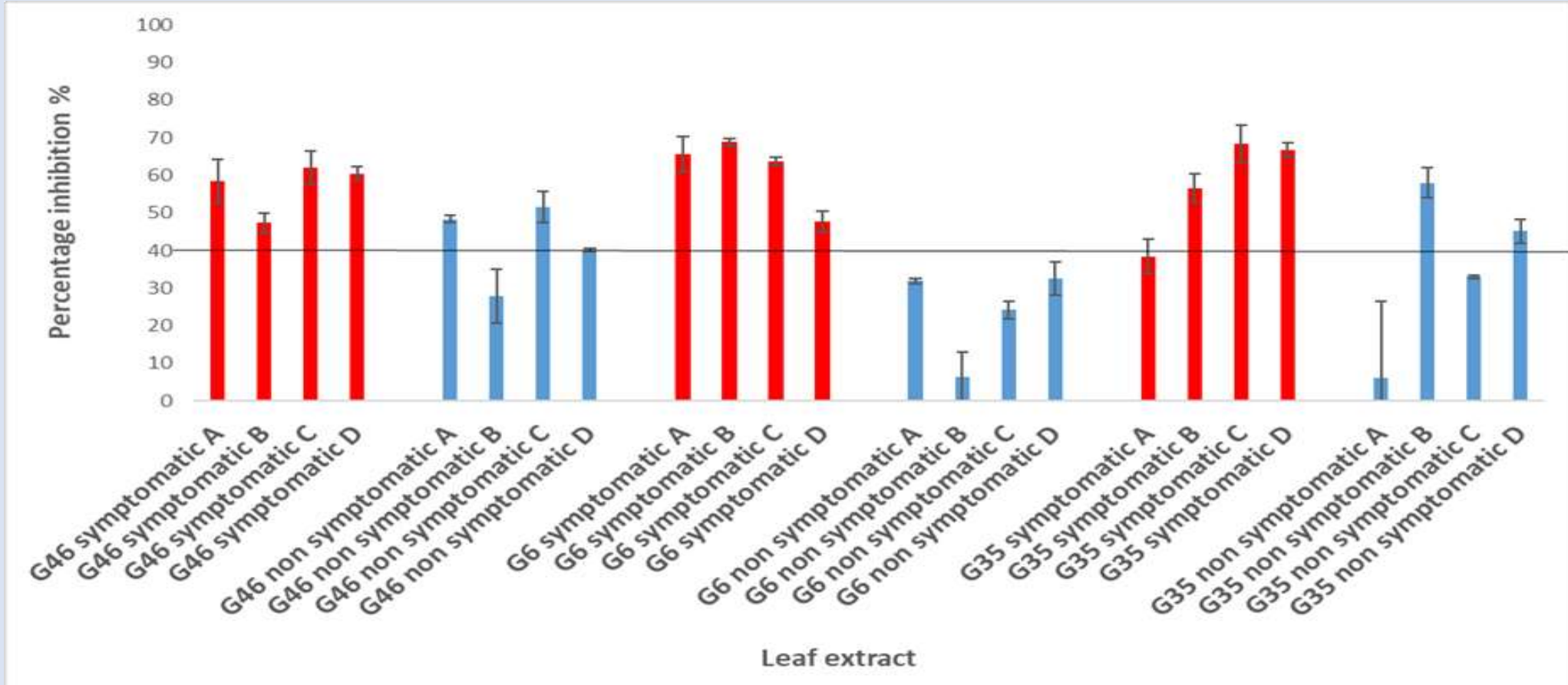


Envirotron – University of the West of England



Infected (New Juaben) and non-infected CSSV Theobroma Cacao plants are kept in the Envirotron

CSSV competitive ELISA results for **symptomatic** (RED), (n=3) and **non-symptomatic** (BLUE), (n=3)



Symptomatic leaf extracts
11/12 leaf extracts - + ve

Non-symptomatic leaf extracts
4/12 leaf extracts - ve

Rapid assay development

ELISA

Antibody is detected through an enzyme tag where the substrate produces a coloured solution – lab based reader

Flow through assay

Colorimetric assay - antibody is detected through an enzyme tag where the substrate forms a coloured precipitate – lab based reader or hand held device

Florescent assay –antibody is detected through a biotin tag and this binds a fluorescent molecule (QDots) - lab based reader or hand held device

Flow through assay – colorimetric readout



Non-infected
leaf extract

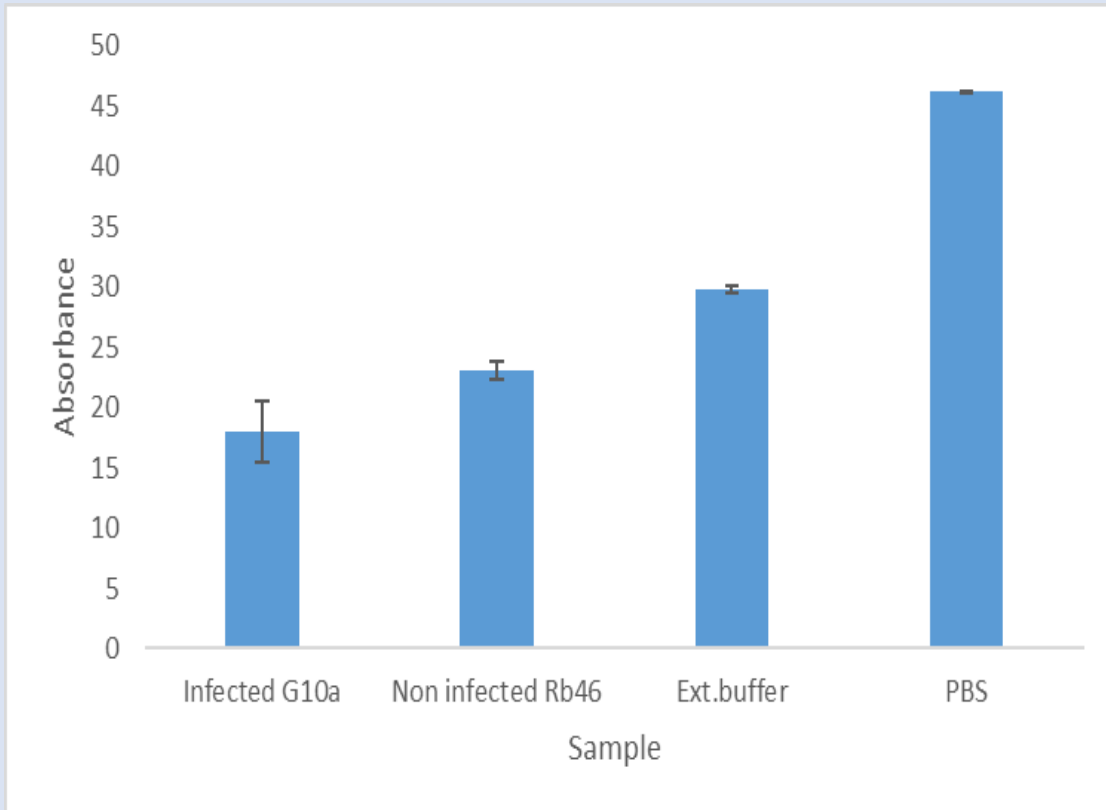


Infected
leaf extract

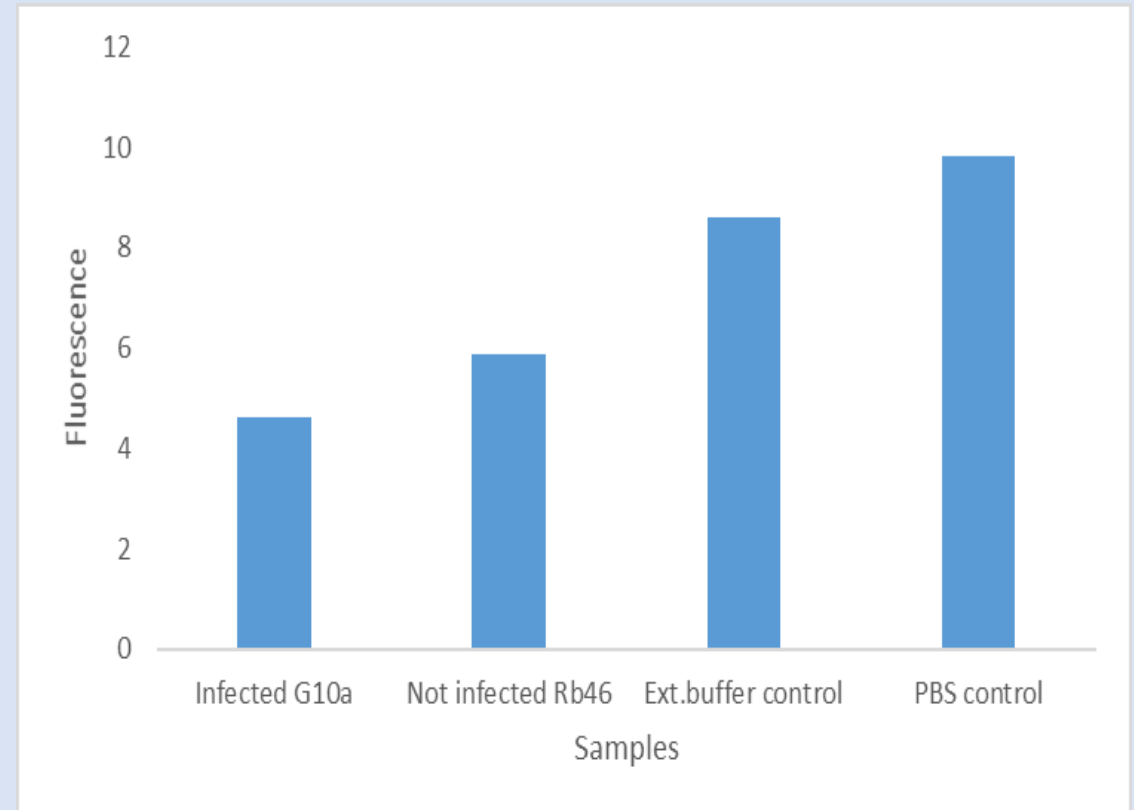
Competitive CSSV immunoassay

Flow through assay using AbD31998.8 Bio

- Colorimetric detection



- Fluorescent detection



Portable CSSV Fluorometer – cover open

Top cover

Optics

Hinge to allow operator to load /remove cartridge

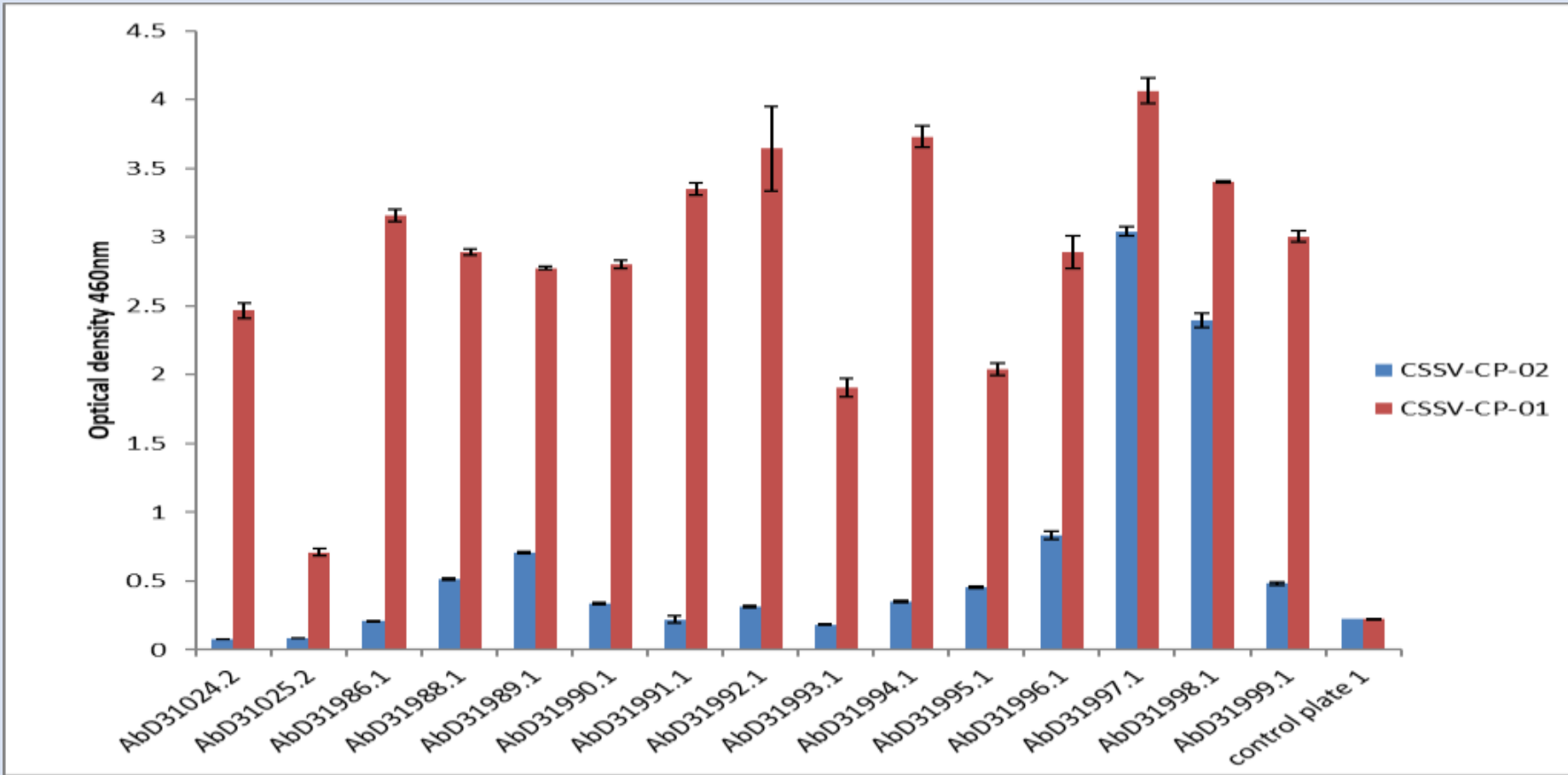
Cartridge and holder

Battery charger socket

Battery casing



Interaction of recombinant monoclonal antibodies with CSSV-CP-01 and CSSV-CP-02



Summary

- Recombinant reagents have been made to form an immunoassay to CSSV
- A competitive ELISA has been developed that can detect CSSV in symptomatic and non-symptomatic leaves of *Theobroma cacao* trees infected with the New Juaben strain of CSSV
- Rapid assays using a 'flow through' format have been developed with colorimetric and fluorescent readout that detect CSSV in symptomatic and non-symptomatic leaves
- The lead antibody binds to CSSV-CP-02 that has a sequence more representative of strains circulating in West Africa

Impact of the development of the CSSVD diagnostic

Impact

- The development of a portable tool to detect CSSD will allow for an early detection of the virus even in asymptomatic trees.
- The use of the CSSVD diagnostic will lead to improved surveillance and enable the screening of new planting material.
- This will represent a cost saving to the farmers in West Africa since the only control strategy available is cutting the infected tree, and knowing the presence of the disease at the beginning of an outbreak will result in less trees needing to be cut, therefore reducing the losses to the farmers.

Acknowledgements

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