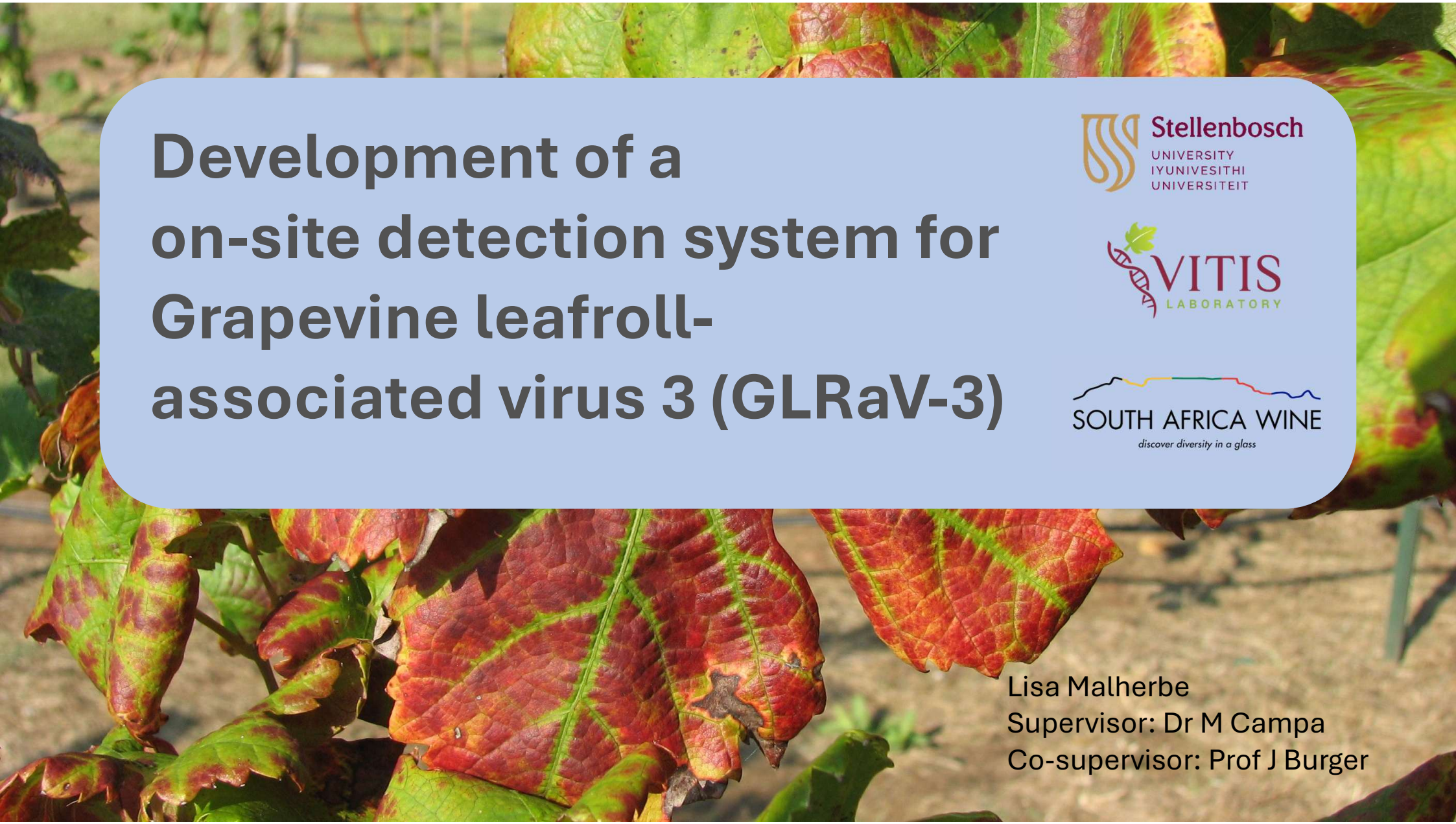


Development of a on-site detection system for Grapevine leafroll-associated virus 3 (GLRaV-3)

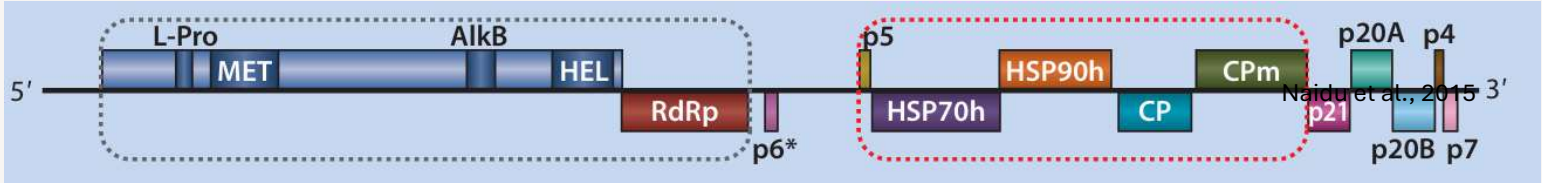
Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT

VITIS LABORATORY

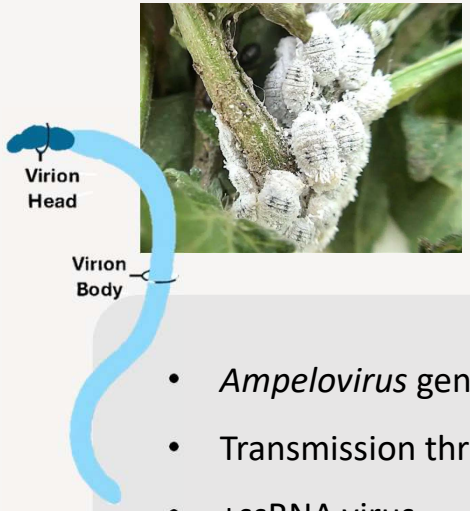
SOUTH AFRICA WINE
discover diversity in a glass

Lisa Malherbe
Supervisor: Dr M Campa
Co-supervisor: Prof J Burger

1 Background



Naiduret al., 2015



- *Ampelovirus* genus (*Closteroviridae* family)
- Transmission through mealybugs
- +ssRNA virus



1 Background

Red cultivars



Maree et al., 2013

White cultivars



Maree et al., 2013

- Once infected, always infected
- Complete plant removal

Current detection methods include:

- PCR and qPCR
- ELISA testing



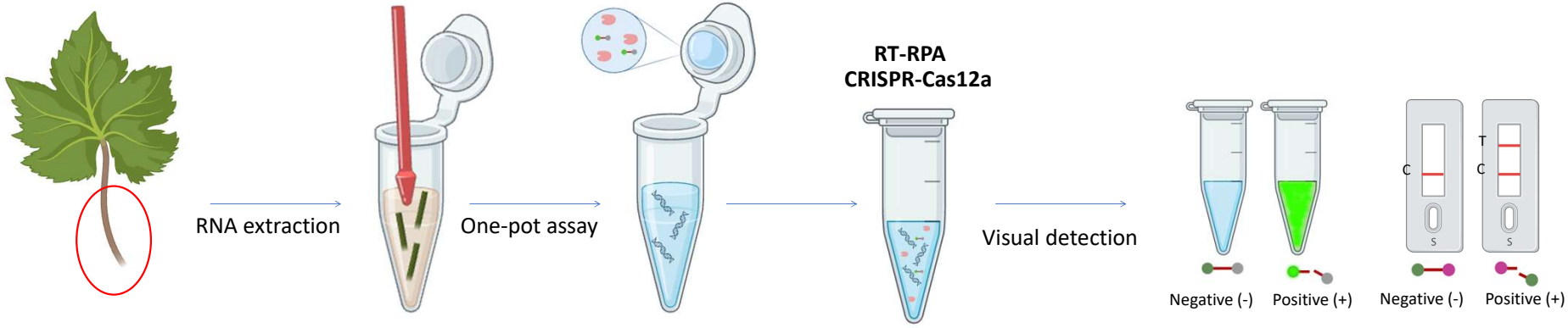
Problem statement

Can we make a GLRaV-3 detection system which is, quick, reliable, and user friendly?



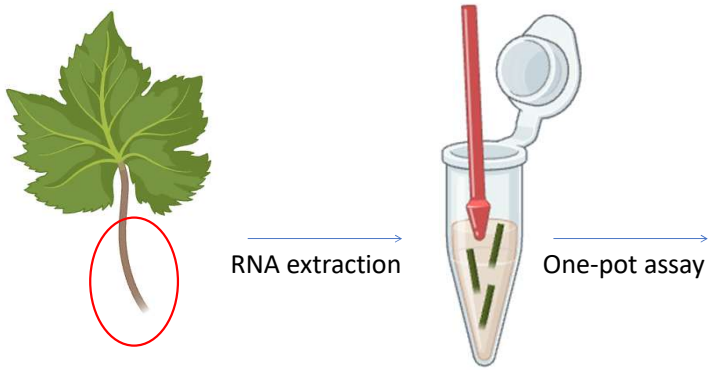
2 Approach

Development of a **crude sap-based RT-RPA-CRISPR/Cas12a one-pot system**



2 Approach

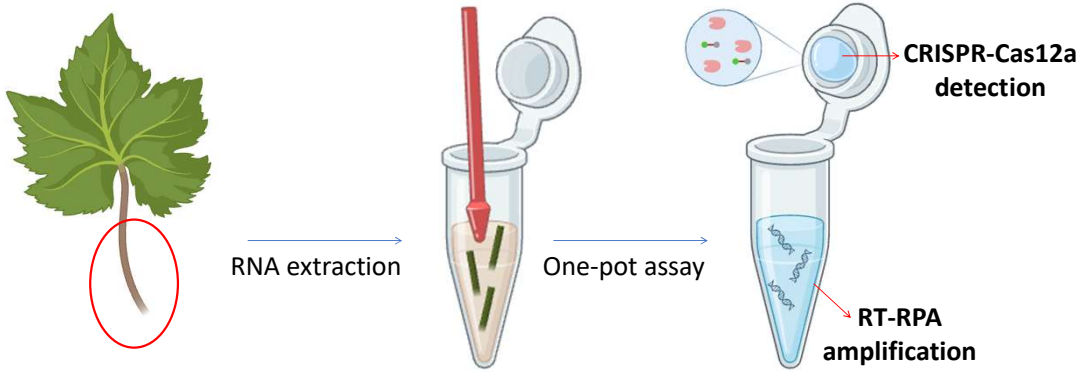
Development of a **crude sap-based RT-RPA-CRISPR/Cas12a one-pot system**



Buffer = 20 mM NaOH, 6% PEG200
Leave undisturbed for 4 min at room temp

2 Approach

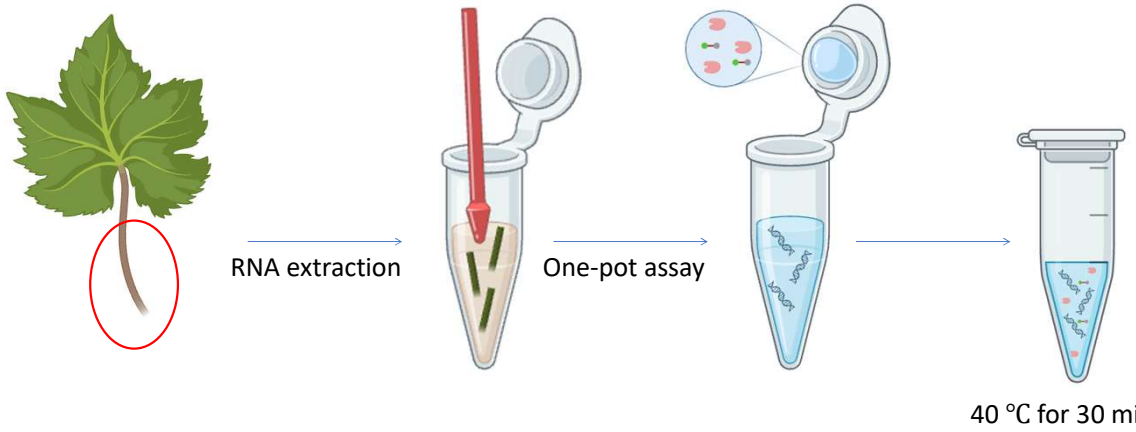
Development of a **crude sap-based RT-RPA-CRISPR/Cas12a one-pot system**



40 ℃ for 20 min

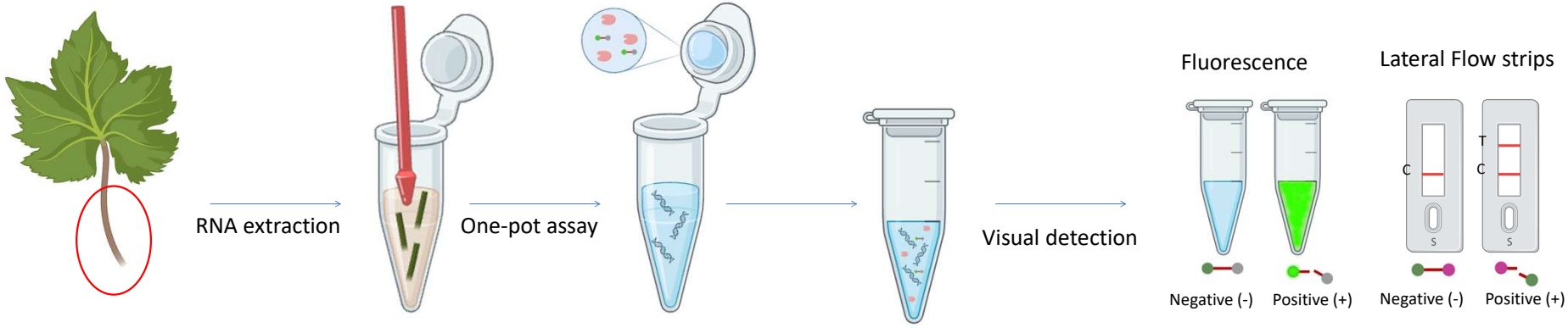
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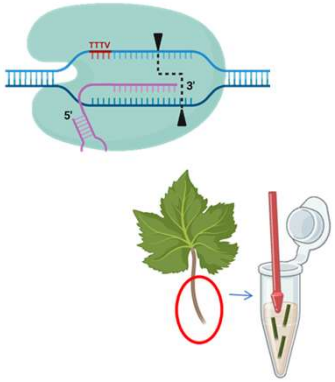
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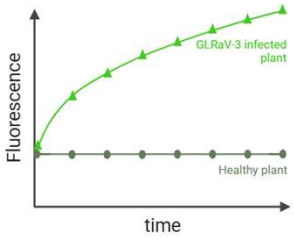


2 Approach

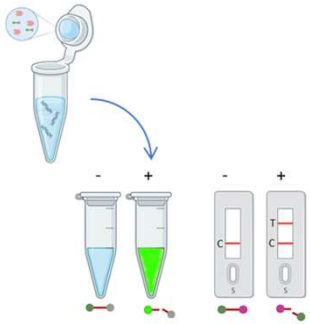
1. Identity suitable CRISPR/Cas12 targets & optimise a crude RT-RPA assay



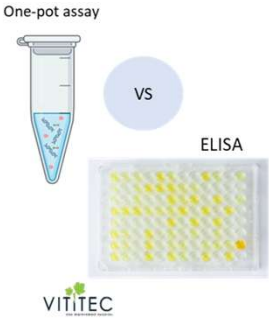
2. Optimitise the CRISPR/Cas12 detection assay



3. Develop a one-pot system and optimise the read-out systems

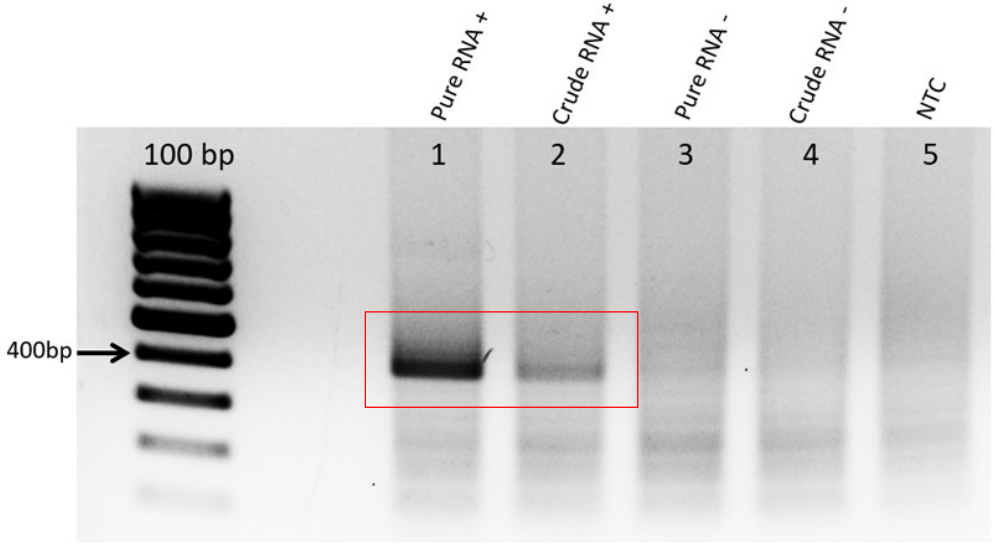


4. Blind validation of detection system



3 Key Findings

1. Crude extraction compatibly and effectiveness



Key:
+ = GLRaV-3 infection
- = No GLRaV-3 Infection

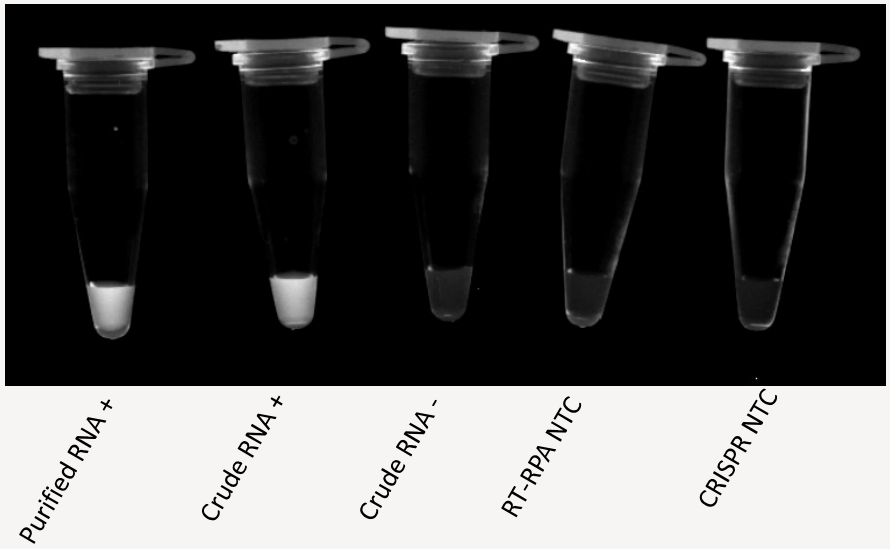
Results:

The crude RNA extraction can be used under RT-RPA conditions for GLRaV-3 detection

3 Key Findings

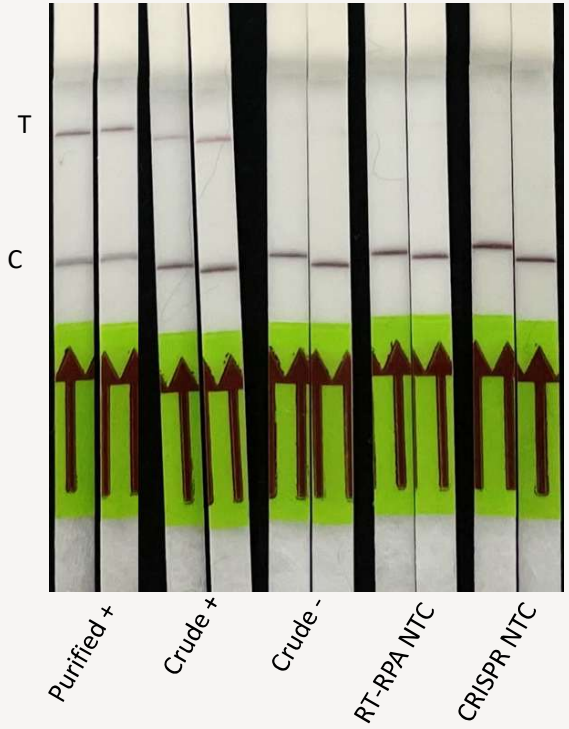
2. One-pot visual detection readout results

Fluorescence



VS

Lateral flow strips

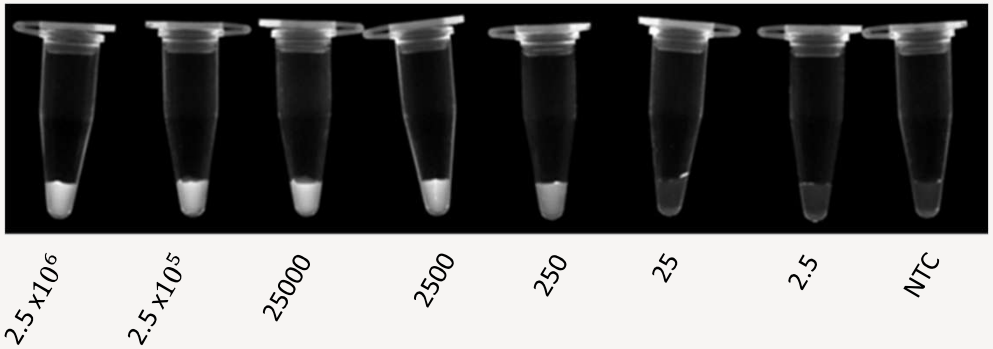


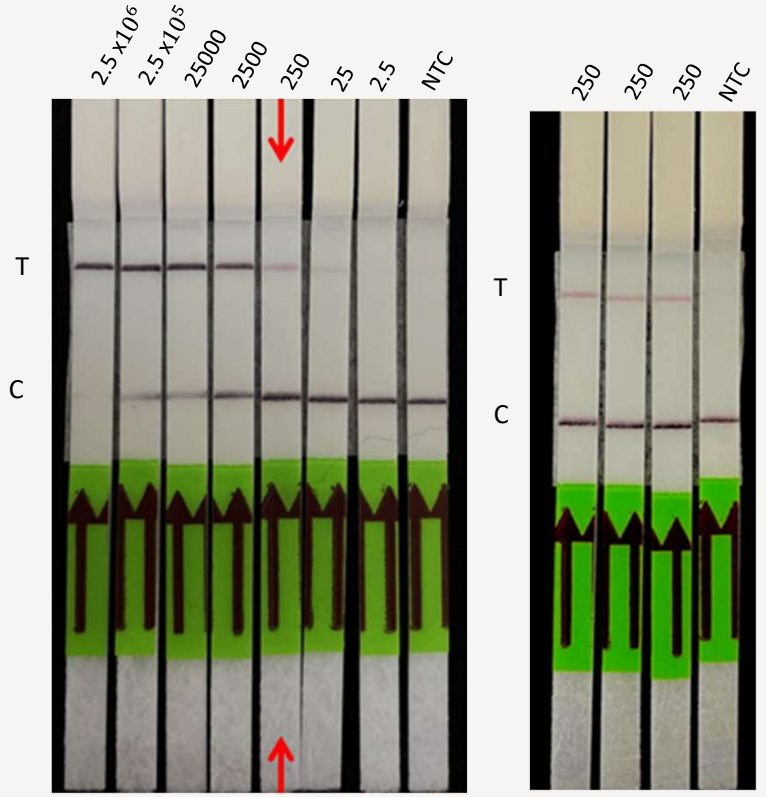
Key:
+ = GLRaV-3 infection
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3 Key Findings

3. Sensitivity limit of one-pot detection system

- Viral copy number
- Same detection limit for both fluorescence and LFS assays
- Early detection

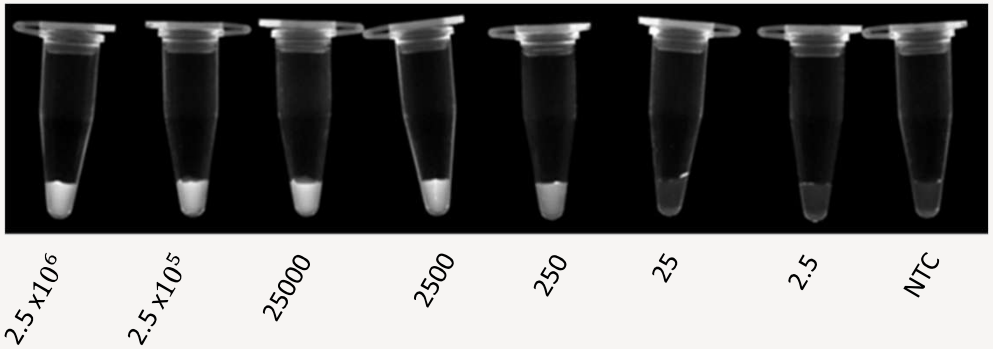




3 Key Findings

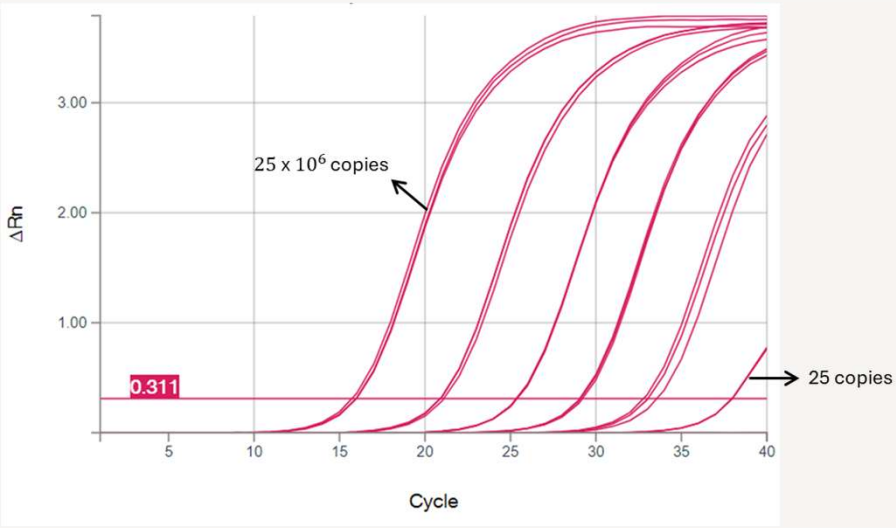
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VS

RT-qPCR results

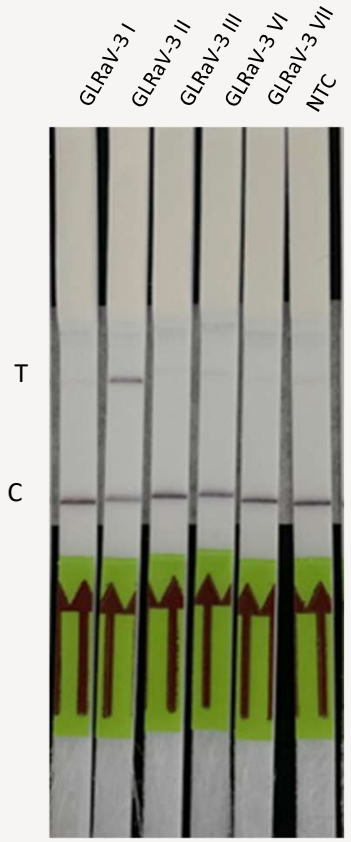


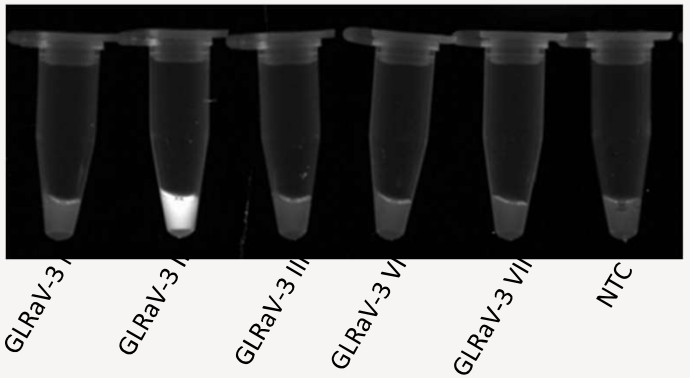
3 Key Findings

4. Specificity testing for one-pot detection assay

GLRaV-3 variant groups

- Tested the detection of other GLRaV-3 variant groups
- Test is specific to GLRaV-3 variant group II only





3 Key Findings

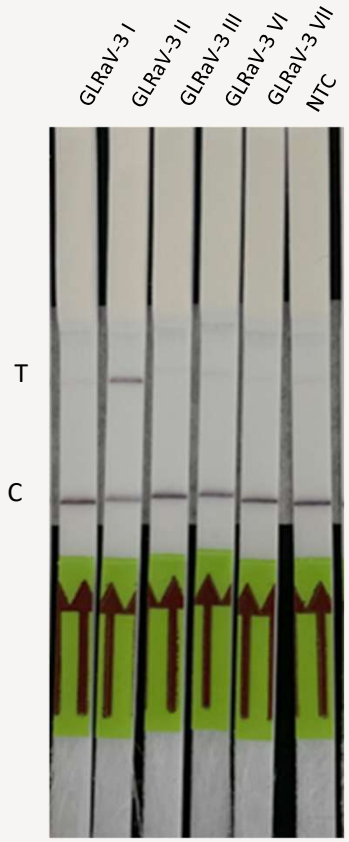
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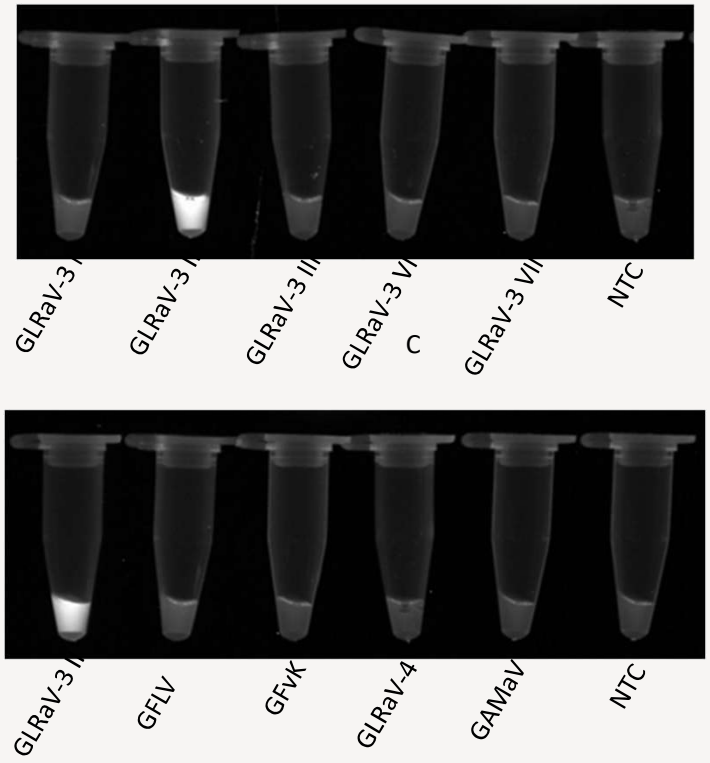
GLRaV-3 variant groups

- Tested the detection of other GLRaV-3 variant groups
- Test is specific to GLRaV-3 variant group II only

Grapevine viruses

- Tested the detection of other common grapevine viruses
- Fanleaf virus, fleck virus, GLRaV-4, asteroid mosaic-associated virus
- No cross-reactivity observed



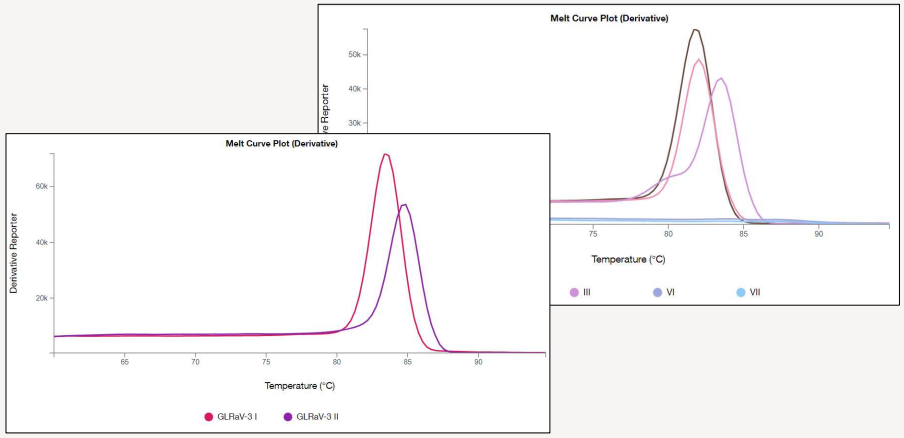


3 Key Findings

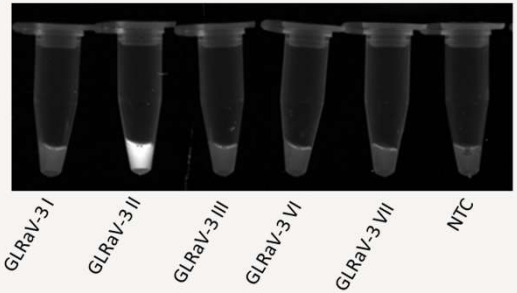
5. Blind Validation with commercial ELISA testing (Vititec)

- 25 samples were evaluated

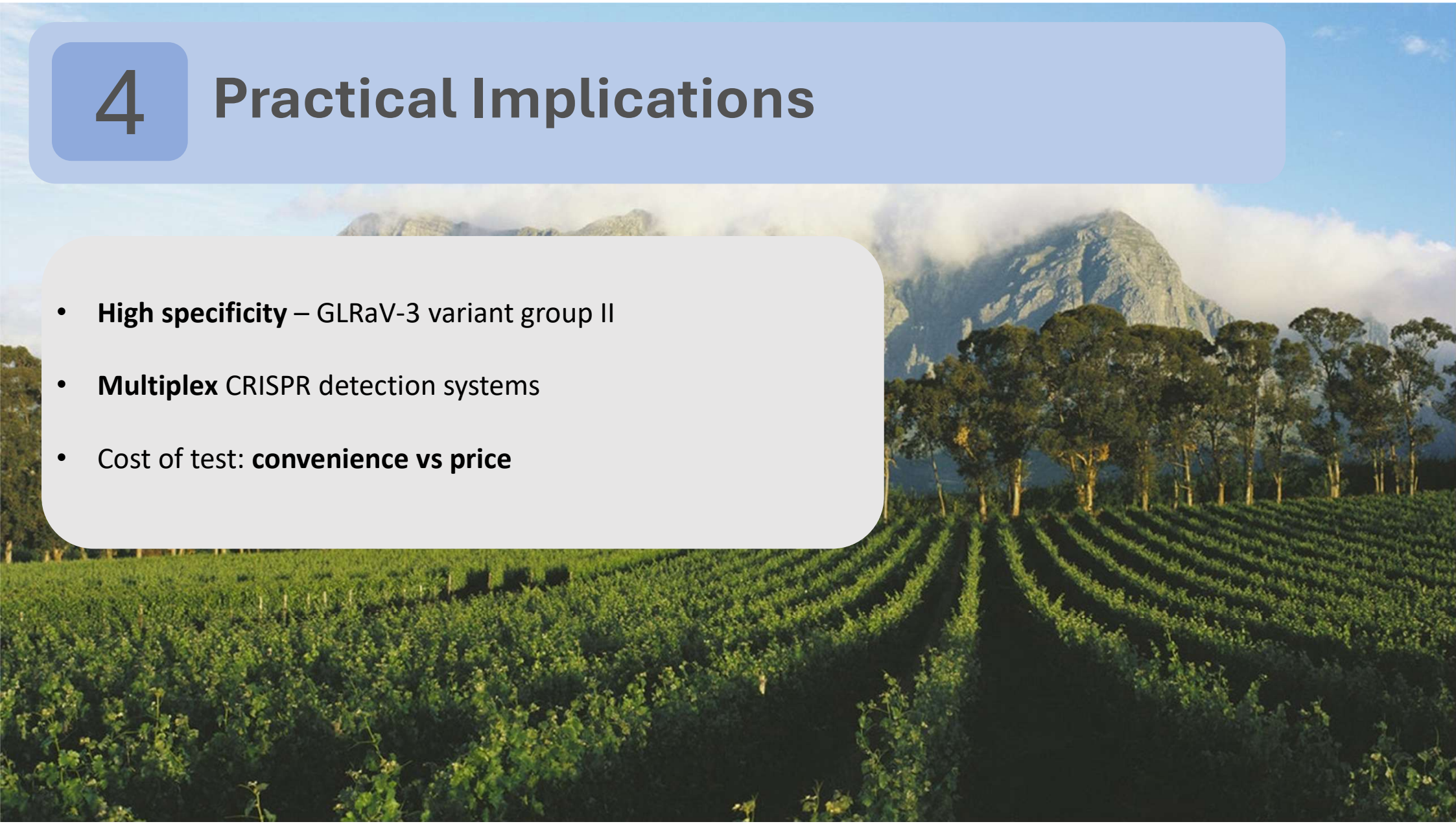
One-pot assay	ELISA
2/25	11/25



- Melt curve analysis and sequencing results indicated variant group specific infections:
- Only GLRaV-3 variant group II infections are detected by the one-pot assay (2/25 samples)



4 Practical Implications



- **High specificity** – GLRaV-3 variant group II
- **Multiplex** CRISPR detection systems
- Cost of test: **convenience vs price**

5 Take home message



- One step closer to a **on-site** GLRaV-3 detection method
- **Faster** and **easier** detection system
- **Adaptable** to many grapevine viruses


Thank you for listening!
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