

An elution-independent collection device for rapid sampling of microorganisms and nucleic acids for PCR assays

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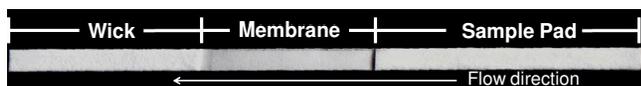
INTRODUCTION

- A novel elution-independent collection device (EICD), Oklahoma State University, patent pending reference number 2010.26, was designed to collect microorganisms and recovery of nucleic acids for PCR based assays without intermediate elution steps between sample collection and PCR processing.
- Dipstick format lateral flow devices are commonly used in the field of pathogen detection because they are inexpensive, easy to use, rapid, portable and disposable.
- EICD is an alternative to traditional lengthy nucleic acid (NA) extraction with commercial kits, which can take about 10-30 minutes to be completed.

METHODOLOGY

EICD PROTOTYPE DESIGN

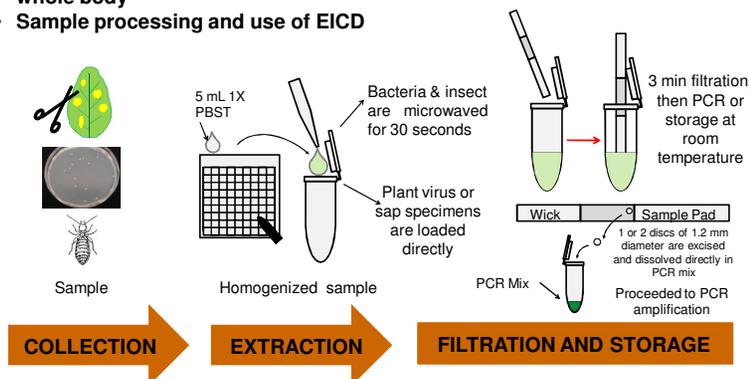
- EICD design follows a dipstick format of lateral flow devices (3, 4) but including a central soluble element.
- EICD design and composition:



- One sample pad out of 22 materials was selected based on fast flow rate. Testing included 10 replications each and used 1X PBS-T (Phosphate Buffered Saline Tween-20) with green dye in initial stages.

EICD PROTOTYPE TESTING

- Samples: Tobacco plants infected with *Tobacco Mosaic Virus (TMV)*, *Erwinia tracheiphila* (bacteria), and *Liposcelis brunnea* (Insect psocid) whole body
- Sample processing and use of EICD



- **Control treatments:** Samples extracted using commercial kits, for TMV (QIAGEN Rneasy® plant Mini Kit), for *L. brunnea* (ZYGEM prepGEM™) and freeze-thaw method for *E. tracheiphila*.

PCR Amplification:

RT-PCR (TMV): Two-step Reverse Transcriptions were carried out in 10 μ L volume following manufacturer's instructions (Invitrogen SuperScript® III RT, Cat. No. 18080093).

PCR amplifications were carried out in 20 μ L volume following manufacturer's instructions (Promega GoTaq® Green Master Mix, Cat. No. M7123).

RESULTS AND DISCUSSION

- TMV, *E. tracheiphila*, and *L. brunnea* diagnostic targets were amplified directly from EICD without elution intermediate steps (Figures 1 and 2).
- A monthly lapsed PCR based assay with plasmid DNA carrying a segment of (*Phymatotrichopsis omnivora*) showed storage and amplification of targeted plasmid DNA for a year (Figure 3).

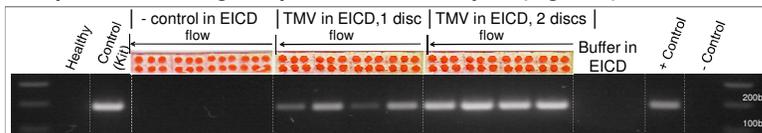


Figure 1. RT-PCR result for TMV Infected plant processed using EICD. One or two discs sample were taken from different locations within the membrane. Positive amplifications of TMV samples directly from EICD are shown here.

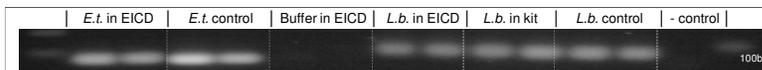


Figure 2. Positive PCR amplifications of *Erwinia tracheiphila* (*E.t.*) and *Liposcelis brunnea* (*L.b.*) from 1 disc sample.

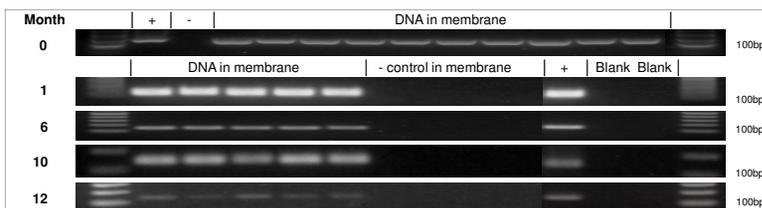


Figure 3. Positive PCR amplification of plasmid DNA stored in EICD for 1 year at room temperature.

CONCLUSIONS

- EICD facilitates a rapid sample collection by eliminating intermediate elution steps required by most extraction methods.
- EICD soluble element streamlines PCR processing.
- EICD can be used in a broad number of taxa.
- EICD allows sample storage for more than a 1 year.
- EICD is user-friendly and is transferable to non-highly trained personnel.
- EICD has application in health, animal, plant, forensics and biosecurity.
- Validation of EICD for a larger and more diverse types of samples is currently being conducted.

References:

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