# Rapid real-time detection of *Fusarium oxysporum* along with an endogenous control in cannabis and other hosts using isothermal AmplifyRP® technology

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## Introduction

*Fusarium oxysporum* (Fo) is a soilborne fungal species with more than 120 pathogenic formae speciales in the family of *Nectriaceae*. This pathogen infects a wide range of economically important crops such as soybean, barley, and cannabis, and causes destructive diseases like vascular wilt and root rot. Exclusion of Fo through rapid containment is the most effective management strategy, making early and accurate detection of Fusaria important for the management of diseases in cannabis and other host plants (1). Utilizing the leading isothermal amplification technology recombinase polymerase amplification (RPA) (2), Agdia Inc. has developed its AmplifyRP® platform for rapid detection of multiple plant pathogens (3-4). Here, we report the development of a rapid, real-time isothermal AmplifyRP® XRT test that detects the fungal pathogen *Fusarium oxysporum* in cannabis and additional host plants.

## Materials and methods

AmplifyRP® primers and probes were designed from sequence conserved regions of Fo and screened for their performance. A pair of primers and an internal probe were chosen from the EF1 gene for the final assay due to their specific and sensitive amplification. The selected EF1 primers and probe were balanced with primers and a probe for an endogenous plant gene to serve as an internal control. This is a duplex assay simultaneously amplifying both the EF1 target and endogenous control gene. The assay is optimized to run at 42°C for 20 minutes with the amplification and detection criteria held within a barcode for use in the AmpliFire®, a portable fluorescence reader as shown in **Figure 1**. The results are displayed in real time and the final data can be exported and further analyzed.

As outlined in **Fig. 1**, crude extracts from plant stem or root tissues were prepared by grinding them in a mesh bag with the 1:20 (tissue weight/buffer volume) GEB. 5ul of the extract were transferred to and mixed with 100 µl of the Pellet Diluent (PD1). Each reaction pellet was rehydrated with 25 µl of this mix and tested with barcode-guided AmpliFire®. The amplification process and results are displayed on the AmpliFire® screen for the target Fo EF1 gene and an endogenous plant gene as an internal control.

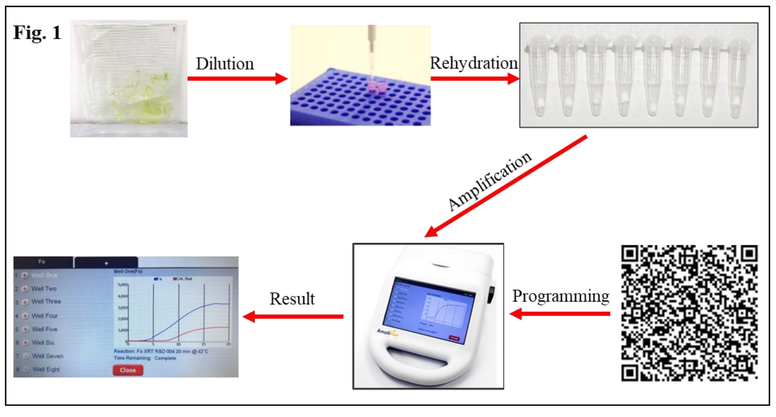


**Figure 1**. A workflow of the testing process for the Fo AmplifyRP® XRT assay.

## Results & discussion

### 1. Specific detection

Fungal cultures were obtained from USDA/ARS NRRL Culture Collections and other sources. Crude extracts from these fungal cultures were tested in the presence of hemp extract with Fo AmplifyRP® XRT. Similarly, crude extracts from healthy and infected plants were also tested for the presence of Fo. This assay detected all Fo formae speciales tested. **Figure 2** below illustrates the detection of several Fo formae speciales. Results from cross reactor testing against various healthy plants and pathogens suggest that this Fo AmplifyRP® XRT assay is specific to Fo.

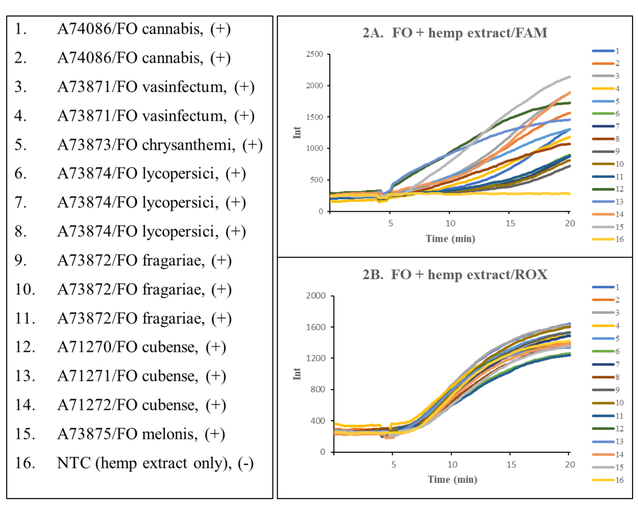


**Figure 2**. Detection of representative *Fusarium oxysporum* formae speciales (see the list on the left) with the AmplifyRP® XRT assay. The target is detected through FAM channel and shown on the top (**2A**) and the internal control is detected through ROX channel and shown on the bottom (**2B**).

### 2. Sensitive detection

DNA fragments containing the target region of Fo f. sp. *cannabis* or Fo f. sp. *vasinfectum* were synthesized and serially diluted 10-folds in the crude hemp leaf extract. This Fo AmplifyRP® XRT assay can detect as few as 10 ag of the target DNA per reaction from either Fo f. sp. *cannabis* (**Fig. 3A**) or Fo f. sp. *vasinfectum*. The limit of detection (LOD) was determined to be approximately 50 ag (32-72 copies) of the target DNA per reaction. As this is a duplex assay, it also simultaneously detects the endogenous plant gene as an internal control (**Fig. 3B**).

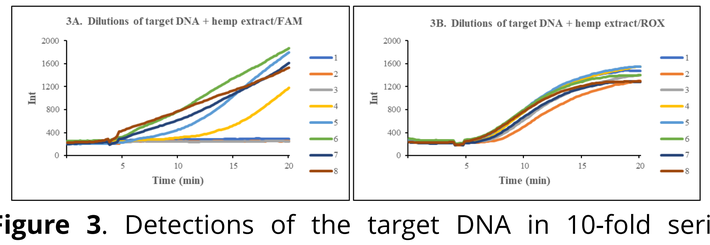


**Figure 3**. Detections of the target DNA in 10-fold serial dilutions in the presence of hemp leaf extract. **3A**, detection of the target DNA from 1.0 ag to 1.0 pg per reaction for reactions #2-8, respectively. The reaction #1 was no template control (NTC, hemp extract). **3B**, detection of the endogenous hemp control gene from the same set of samples.

### 3. Comparative detection

Ten-fold serial dilutions of a synthetic target DNA were spiked into crude hemp extracts and subsequently tested with the Fo AmplifyRP® XRT assay. Total DNA of the spiked extracts were also further purified and tested with conventional PCR and qPCR. The results from the XRT, PCR, and qPCR assays are shown in **Figure 4**. Both the AmplifyRP® XRT (**Fig. 4A & 4B**) and the qPCR (**Fig. 4D**) detected Fo down to 10 ag of the target DNA while the conventional PCR detected down to 1.0 fg of the DNA (**Fig. 4C**). This suggests that the AmplifyRP® XRT assay is as sensitive as qPCR and more sensitive than conventional PCR.

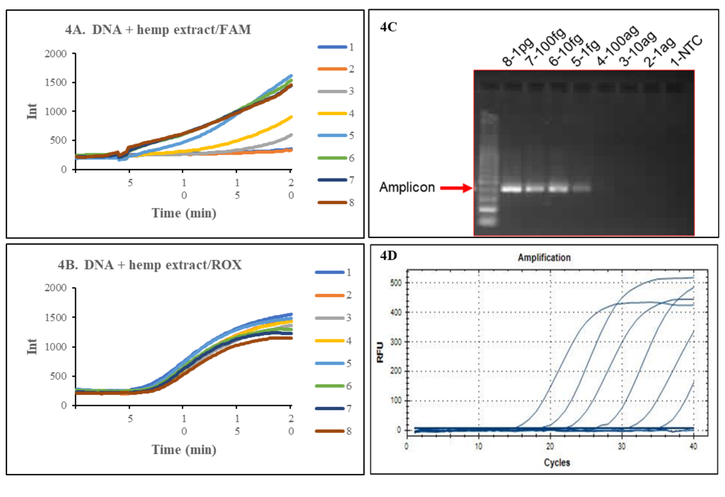


**Figure 4**. Detections of a target DNA with the Fo XRT (**4A & 4B**), PCR (**4C**), and qPCR (**4D**). Both the XRT and qPCR detected as low as 10 ag (reaction #3) of the DNA fragment while the PCR detected down to 1.0 fg (reaction #5) of the DNA. The DNA dilutions were 1.0 ag to 1.0 pg for reactions #2 to #8, respectively. The no-template-control (NTC) was healthy hemp leaf extract only.

## Conclusions

A real-time isothermal AmplifyRP® XRT assay for *Fusarium oxysporum* has been developed and commercialized utilizing RPA and AmpliFire®. The assay specifically detects *Fusarium oxysporum* formae speciales and does not cross react with hemp or other host plants. It is more sensitive than conventional PCR and as sensitive as qPCR. Crude extracts of host plants including cannabis are directly tested, and no DNA purification is needed. The protocol can be completed from sample to result within 30 minutes and the test is deployable in the laboratory and the field. This is an isothermal amplification duplex assay detecting both the target Fo and the endogenous plant control gene.

## Acknowledgments

Authors thank NRRL Culture Collection of USDA/ARS for providing us the fungal cultures used in this study.

## References

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