PROTOCOL: Direct PCR from Blood Dried onto Filter Paper

v. 20210810

Sample:

- Blood dried onto Whatman Filter paper
- Other samples may be suitable (must be verified by user)

Materials:

- Entopsis:
  - PCR*opsis*™ Elution Buffer
  - PCR*opsis*™ Reagent RVD with RVD Enhancer
- PCR master mix
- Primers
- Test sample
- Hole puncher
- Thin-walled PCR tubes
- qPCR Thermocycler

Methods:

1. Create hole punches of your test sample using a standard, sterile hole puncher
2. Place 1 hole punched filter paper sample into a 1.5 mL tube
3. Add ~100 µL of PCR*opsis*™ Elution Buffer to the tube
4. Vortex thoroughly for 1~3 minutes to elute material off the filter paper
5. Mix 20 µL PCR*opsis*™ Reagent RVD with RVD Enhancer with 20 µL of eluted sample in a thin-walled tube (0.2 ~ 0.6 mL) and cap tubes
6. Heat mixture of reagent + blood sample for 10 minutes at 95˚C and let cool at room temperature for ~10 seconds before continuing
7. Mix thoroughly
8. Add 5 µL of heated sample (top, clear phase of heated sample) to 15 µL of qPCR mixture.
   a. qPCR mixture:
      i. 5x PCR Master Mix: 4 µl
      ii. Forward primer (10 pmol/ µl): 1 µl
      iii. Reverse primer (10 pmol/ µl): 1 µl
      iv. Probe (10 pmol/ µl): 0.5 µl
      v. Nuclease-Free Water: 8.5 µl
9. Run samples on qPCR Thermocycler for 45 cycles.
   a. DNA Amplification:
      i. 95˚C 5 minutes (initial denaturation)
         1. 95˚C 30 seconds
         2. 55˚C 30 seconds
         3. 72˚C 30 seconds
      ii. 72˚C 60 seconds (final extension)
      iii. 4˚C hold