PCR*opsis* by Entopsis

PROTOCOL: Extraction-free Processing of Dried Swabs

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Sample:

- Sample dried onto a synthetic swab

Materials:

- Entopsis Inc.:
  - PCR*opsis*™ Reagent RVD-E (see product IFU)
- Test sample

Methods:

1. Thoroughly mix Reagent RVD-E to ensure homogeneity, but avoid creating bubbles unnecessarily
   1. Reagent RVD-E has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed

2. Elute material from swab:
   1. Add ~100 µL of Reagent RVD-E to transport tube with swab
   2. Make sure the swab is at least partially submerged into Reagent RVD-E
   3. Vortex for ~30 seconds, 3 times, to elute sample

3. Specimen lysis & nucleic acid stabilization:
   1. Transfer ~50 µL of eluted sample into a thin-walled PCR tube / plate and then cap tube or apply plate sealer to plate to prevent evaporation
   2. Heat at 95˚C
      1. Mammalian: 5 minutes
      2. Viruses: 10~15 minutes
      3. Bacteria: 15~20 minutes
      4. NOTE: heating for a longer period of time does not negatively affect results
   3. Let cool at room temperature for ~10 seconds before continuing

4. Pipette up & down to ensure complete mixing

5. Use lysed / stabilized sample in your desired RT-qPCR / qPCR procedure
   1. Lysed / stabilized sample should represent 15%~30% of your final RT-qPCR mixture (i.e., 3~6 µL sample into a total volume of 20 µL)
   2. You might observe increasing PCR inhibition when your PCR mixture consist of >35% processed sample