PROTOCOL: Direct PCR from Urine Samples

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Sample:

- Urine sample
  - cells pelleted at >1,400xg for ~10 minutes
  - most of the urine supernatant was removed following centrifugation, leaving only 100 ~ 250 µl of urine over the pellet

Materials:

- Entopsis Inc.:
  - PCR*opsis*™ Reagent RVD with RVD Enhancer (see product IFU)
  - PCR*opsis*™ Support (see product IFU)
  - PCR*opsis*™ Lysis Beads (see product IFU)
- PCR master mix
- Primers
- Test sample
- 2 mL round-bottom tubes
- Thin-walled PCR tubes
- qPCR Thermocycler

Methods:

PCR*opsis*™ Reagent Prep:

1. Thoroughly mix 2 µL of PCR*opsis*™ Support with 1 mL PCR*opsis*™ Reagent RVD with RVD Enhancer (called **RVD-Support solution** from here on)
   1. This mixture is stable for at least 24 hours at room temperature
   2. If poor results are initially observed, then add 10 ~ 20 µL of PCR*opsis*™ Support per 1 mL PCR*opsis*™ Reagent RVD with RVD Enhancer

After Reagent Prep:

1. Centrifuge 1.5~25 mL of urine at >1,400xg for 10 minutes in a 15 mL or 50 mL conical
   1. Higher volumes of centrifuged urine is expected to result in higher sensitivity
2. Remove supernatant and leave <250 µL of residual urine
3. Resuspend cell pellet using residual urine
4. Add roughly 0.25 grams of Lysis Beads to a 2 mL, round-bottom vial or well in a deep, 96-well plate
   1. One full PCR*opsis*™ Lysis Bead Scoop holds ~0.3 grams of beads
5. Add 200 µL of sample to the vial or well containing Lysis Beads
6. Cap tube or place a plate sealer on the deep well plate
7. Vortex on high for ~5 minutes to lyse microorganisms
8. Mix 20 µL PCR*opsis*™ RVD-Support solution with 20 µL of the sample pre-processed with PCR*opsis*™ Lysis Beads in a thin-walled tube or plate (0.2 ~ 0.6 mL)
   1. **For optimal results, the reagent needs to be added first to the tube/well before the sample is added.**

2. Ratio of sample to PCR*opsis*™ reagent will remain 1:1, but volume can be increased if needed (example: 30 µl : 30 µl and so forth)

9. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
10. Heat diluted sample at 95˚C for 15~20 minutes in a thermal cycler or controlled heat block with a lid, and let cool at room temperature for ~10 seconds before continuing
    1. Heating for a slightly longer period (extra 2~5 minutes) of time does not negatively affect results
    2. Make sure the heating device has reached the desired temperature before applying sample.
    3. You may need to increase the heating time if increasing the volume of sample and reagent past 100 µl of each
    4. Sample heating can be performed using a controlled heating block or thermal cycler; however a device lid is highly recommended to minimize popping of tube caps or unpeeling of the plate sealer
11. Mix heated sample and use lysed / stabilized sample in your desired PCR procedure
    1. Lysed / stabilized sample should represent 15% ~ 30% of your final PCR mixture (i.e., 3~6 µL sample into a total volume of 20 µL) depending on the polymerase used
    2. You might observe increasing PCR inhibition when your PCR mixture consist of >35% processed sample

Suggested PCR conditions:

1. Add ~5 µL 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
2. 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