PCR*opsis* by Entopsis

SUGGESTED PROTOCOL:
Direct PCR from Urine Samples

v. 20230206

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 123 (see product IFU)
  - PCR*opsis*™ UrineA (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*™ UrineA with 1 mL PCR*opsis*™ Reagent 123 (called **123-UrineA solution** from here on)
   1. This mixture is stable for at least 24 hours at room temperature

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*™ 123-UrineA 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 downstream applications