Product Validation Guide for ZooOpsis™

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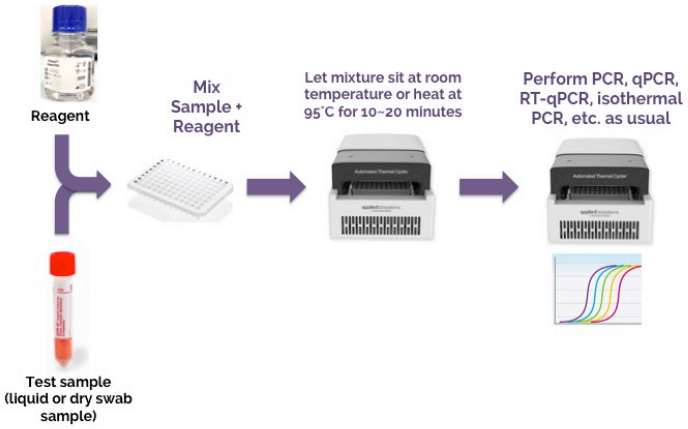
Introduction:
The following guide provides suggestions for people inexperienced with Next Generation Direct PCR, would like to optimize their testing procedure, and/or validate a direct PCR product. The recommendations below provide a good starting point from which to optimize and/or miniaturize sample processing. It's highly recommended to follow the suggested conditions and advice below before altering protocols stated in the product's Instructions for Use (IFU) documents.

One should first read and understand the product IFU before reading this supplementary document.



Why may testing conditions change when transitioning PCR testing from traditional nucleic acid extraction to extraction-free direct PCR?

- Extraction-free direct PCR does not produce isolated DNA or RNA, instead it produces a mixture where PCR inhibitors are sequested and nucleic acids available to bind primers and enzymes
- Some PCR kits may need to be optimized to handle non-purified nucleic acids; for example, change the amount of sample added to the PCR mixture, add more magnesium, or change the volume of the PCR mixture
- There may be less absolute or available DNA or RNA in the PCR mixture when using direct PCR, as such, one may benefit from allowing the reverse transcriptase extra time to convert RNA into cDNA and program extra PCR cycles to ensure detection of true positive samples

General Recommendations:

1. Invert the reagents before use to ensure complete mixing of the components

2. Use fresh samples since sub-optimal results are often observed with frozen samples
    a. Some fake urine products can be used for preliminary studies

3. Run samples on qPCR Thermocycler for ~5 extra cycles than when using traditionally extracted nucleic acid samples
    a. Direct PCR often results in ~3 Ct greater than traditional PCR

4. General recommended RT-qPCR conditions:
    a. Reverse Transcription:
        i. 45~50˚C for **30 minutes** (allows extra time to produce cDNA)
        ii. 95˚C for 2 minutes
    b. DNA Amplification: 3-step PCR often results in better Ct values than 2-step
        i. 95˚C 5 seconds
        ii. 55˚C 15 seconds
        iii. 72˚C 15 seconds
    c. Extension: 72˚C 60 seconds
    d. Hold: 4˚C

5. Process ~20 µL reagent + ~20 µL sample before miniaturizing this step to ensure satisfactory signal is observed
    a. The reagent should always be first added to the PCR tube before adding the sample

6. Initial tests should employ a ~20 uL PCR mixture before miniaturizing the assay to 10 ~ 15 µL to ensure satisfactory signal is observed
    a. 3 µL, 4 µL, and 5 µL of processed sample into a final PCR mixture of 20 µL should be tested to determine the optimal ratio for your PCR kit

Specific recommendations for urine samples:

1. Pre-process urine samples with Lysis Beads when examining bacteria, yeast, and /or fungi to ensure complete lysis
    a. Vortexing samples for ~5 minutes on high with the Lysis Beads is sufficient for most applications

2. When possible, centrifuge >10 mL of urine to collect a decent amount of target microorganisms / cells
    a. Resuspending the urine pellet in 200 ~ 250 µL of residual urine is usually optimal for most applications

3. Urine samples potentially containing high concentrations of drugs, toxins, or insoluble material should be diluted with saline buffer before centrifugation
    a. For example, 15~20 mL of urine can be mixed with 30~35 mL of saline buffer and then centrifuged. The residual urine, used to resuspend the cell pellet, will contain a lower concentration of potential PCR inhibitors.

Specific recommendations for swab samples in transport medium:

1. Pre-process samples with Lysis Beads when examining bacteria, yeast, and /or fungi to ensure complete lysis
    a. Vortexing samples for ~5 minutes on high with the Lysis Beads is sufficient for most applications

2. Ensure the transport medium does not contain known PCR inhibitors, such as alcohols, guanidinium thiocyanate, and other enzyme inhibitors
    a. Most non-inactivating transport mediums should be compatible with direct PCR applications

3. When analyzing cells from swab samples, the transport medium can be centrifuged and most of the residual liquid removed to concentrate target cells
    a. This is rarely a necessity, but can improve the detection of low abundant microorganisms

Specific recommendations for saliva samples:

1. Saliva samples should be free of visible debris
    a. Most debris will settle by letting the sample sit for ~5 minutes

2. Saliva (100%) or saliva in transport mediums can be used with direct PCR products, however the transport medium cannot inhibit enzymes
    a. Most non-inactivating transport mediums should be compatible with direct PCR applications

Specific recommendations for plasma, serum, and whole blood:

1. Avoid samples with lysed red blood cells, since they may result in sub-optimal results

2. Start by testing a 1:5 and 1:10 ratio of Zoo*Opsis*™ WBC to fresh whole blood

3. Zoo*Opsis*™ WBC is ideal for amplifying gene targets from white blood cells, and may not be the best choice for amplifying gene targets from viruses or bacteria found in blood

4. The Zoo*Opsis*™ Blood Kit is best suited for amplifying gene targets from viruses and bacteria found in plasma or serum
    a. The Zoo*Opsis*™ Blood Kit may be suitable for amplifying circulating DNA, but this has not been thoroughly tested

5. When using the Zoo*Opsis*™ Blood Kit, your processed sample should consist of 10~15% of the PCR mixture. Adding too much processed sample to the PCR mixture will result in poor amplification due to PCR inhibition.