PCR*opsis*™ Reagent RVD with RVD Enhancer facilitates extraction-free amplification of human and bacterial targets from blood dried on filter paper

Francis Buan Hong Lim[1], Abhignyan Nagesetti[1], Kevin Moreno[1], Agustin Galecio[1], Ian Cheong[1,2,3,4], Obdulio Piloto[1]

Affiliations:
[1] Entopsis, Inc., USA
[2] Temasek Life Sciences Laboratory, Singapore
[3] Department of Biological Sciences, National University of Singapore, Singapore
[4] Pathnova Laboratories Pte Ltd, Singapore

Correspondence to: info@entopsis.com

Abstract:
Diagnostic and forensic samples come in various forms; most often on swabs. Samples dried on filter paper offer a low-cost alternative with advantages for both wealthy and less wealthy countries. Paper samples facilitate easy, large-scale transport of samples to centralized testing laboratories. Moreover, they tend to be less bio-hazardous and concentrate analytes given their dehydrated nature. Hole punches of dried samples can be easily fed into automated processes. Such samples can facilitate blood-borne disease testing of people and animals, environmental testing, and forensics. Here we demonstrate that PCR*opsis*™ Elution Buffer can elute human and bacterial material from blood dried onto Whatman filter paper. Furthermore, PCR*opsis*™ Reagent RVD with RVD Enhancer mediates extraction-free PCR from the eluted sample, resulting in cycle threshold (Ct) values comparable to traditional DNA extraction. This report demonstrates the capability of PCR*opsis*™ Next Generation Direct PCR™ technology to process blood samples dried onto filter paper.

Results:

Cells Detected	Eluted Sample - Unprocessed	Isolated DNA - Qiagen QIAamp	Reagent RVD with RVD Enhancer
Human	0	31.08	29.45
Bacteria	0	21.34	22.36

Figure 1. Reagent RVD with RVD Enhancer facilitates amplification of human and bacterial gene targets from dried blood samples. Human DNA from dried blood and residual bacteria DNA is amplified to similar degrees when the sample is processed using traditional DNA extraction or Reagent RVD with RVD Enhancer before PCR amplification. The use of neither approach results in no DNA amplification from the same sample. Samples were tested in triplicates and the average is shown.

Key Conclusions:

- PCR*opsis*™ Reagent RVD with RVD Enhancer can process dried blood samples with residual bacterial contamination comparable to traditional DNA extraction.
    - The unprocessed eluted sample did not show any DNA amplification, demonstrating that Reagent RVD with RVD Enhancer is capable of resolving PCR inhibition found in dried blood samples.

- PCR*opsis*™ Elution buffer is capable of eluting human and bacterial test samples dried onto Whatman filter paper.

Methods:

Materials:

- Entopsis:
    - PCR*opsis*™ Elution Buffer
    - PCR*opsis*™ Reagent RVD with RVD Enhancer
    - PCR*opsis*™ 5x PCR Master Mix
- IDT: hGAPDH and degenerate bacterial (16S rRNA) primers
- Milllipore Sigma: Whatman Filter Paper
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler
- Qiagen: QIAamp DNA Kit

Prepare dried blood samples:

1. Add drops of fresh human blood onto Whatman filter paper
2. Place filter paper outdoors, in the shade, for 12 hours to accumulate microbial contaminants
3. Use a hole puncher to create evenly sized filter paper samples

PCR*opsis*™ Reagent RVD with RVD Enhancer:

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

DNA extraction:

Isolate DNA from eluted sample according to the manufacturer's protocol using Qiagen QIAamp DNA Kit.