PCR*opsis*™ BCS Nano Produces Blood Cell Distributions Comparable to Buffy Coats without Centrifugation and Facilitates Next Gen Direct PCR™

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Abstract:
Blood is a highly valuable bio-fluid for diagnostic testing. However it contains red blood cells (RBC) and other components that inhibit polymerase chain reaction (PCR) – based analysis. As such, whole blood normally needs to be processed before PCR testing. This often involves centrifugations, sample transfers, and multiple steps for nucleic acid extraction. In this report, we show how PCR*opsis*™ BCS Nano mediates Next Generation Direct PCR™ from whole blood, without centrifugations, in a matter of ~15 minutes, and just a few simple steps. The observed cell profiles and cellular viability of whole blood processed with PCR*opsis*™ BCS Nano resembles that of buffy coats. Enriched white blood cells (WBC) can be directly added to downstream PCR applications without the need for nucleic acid extraction. PCR*opsis*™ BCS Nano saves time, money, and offers a highly compatible means of direct PCR from whole blood with automated systems.

Results:

	Volume [ml]	Lym [10^6/mL]	Mono [10^6/mL]	Gran [10^6/mL]	PBMC [10^6/mL]	Recovered PBMC [10^6 Cells]	PBMC Recovery [%]	Viability - Live [%]
Whole Blood	2.5	1.7	0.6	5.7	2.3	5.8	100%	95.52
Buffy Coat	0.2	2.5	0.9	7	3.4	0.68	11.83	94.11
BCS Nano	0.2	2.2	0.7	6.8	2.9	0.58	10.09	92.87

Table 1. BCS Nano and buffy coats results in comparable blood cell profiles and cellular viability. Both methods of enriching white blood cells result in comparable cellular profiles and cellular viabilities when examined on a Medonic M-Series Hematology Analyzer and a Beckman Coulter flow cytometer. Samples were tested in triplicates and averages shown.

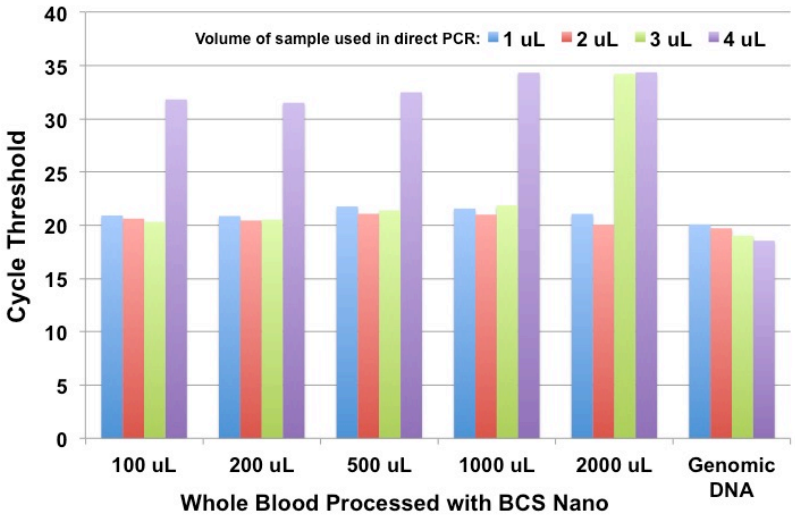

Figure 1. BCS Nano facilitates Next Generation Direct PCR™ from enriched blood cells without the need for nucleic acid extraction. BCS Nano sequesters PCR inhibitors normally found in whole blood and mediates nucleic acid extraction-free PCR. The use of tubes with diameters wider than 10 mm, as that used for the 2 mL sample, leads to sub-optimal cellular separation and sequestration of PCR inhibitors. Genomic DNA extracted from the whole blood samples was used as a positive control. Samples were tested in duplicates and averages shown.

Key Conclusions:

- Samples processed with PCR*opsis*™ BCS Nano result in concentrations of lymphocytes, monocytes, and granulocytes that parallel those observed in buffy coats

- PCR*opsis*™ BCS Nano enriches for white blood cells in ~15 minutes without the need for centrifugation, and is thereby highly compatible with automated approaches

- Samples processed with PCR*opsis*™ BCS Nano exhibit comparable cellular viability as whole blood and buffy coat samples, when determined by Flowcytometry (FC500, Beckman Coulter)

- Next Generation Direct PCR™ components in PCR*opsis*™ BCS Nano facilitate nucleic acid extraction-free PCR
    - These components are similar in nature to those found in PCR*opsis*™ Reagent RVD with RVD Enhancer, but tailored for blood samples

- The diameter of tubes used to process whole blood samples with PCR*opsis*™ BCS Nano should not be much larger than 10 mm to obtain optimal cellular separation and sequestration of PCR inhibitors

Methods:

Materials:

- Phosphate buffered saline (PBS)
- Fresh, whole blood
- Entopsis:
    - PCR*opsis*™ BCS Nano
    - PCR*opsis*™ 5x PCR Master Mix
- DuraClone IM Count (Cat# C00162, Beckman Coulter)
- Stellar Scientific: tubes
- IDT: human GAPDH primers / probe
- Qiagen: QIAamp DNA Blood Kit
- Medonic: M-series Hematology Analyzer (Boule Diagnostics)
- Flowcytometry (FC500, Beckman Coulter)
- Chai: Open qPCR Thermocycler

Processing Samples with PCRopsis™ BCS Nano:

1. Add 1 volume of homogenized BCS Nano to a 2 mL plastic tube tube using a 1 mL pipette tip
2. Add 5 volumes of whole blood sample to the tube with BCS Nano and mix thoroughly
3. Let the mixture incubate at room temperature for 15 minutes

4. Carefully collect the RBC-depleted supernatant and dilute cells in PBS

Processing Buffy Coat Samples:

1. Mix whole blood 1:1 with PBS
2. Centrifuge at 800xg for 10 minutes at room temperature with the brake off
3. Carefully remove the tube from the centrifuge
4. Carefully collect the buffy coat layer without collecting red blood cells and dilute cells in PBS

DuraClone IM Count:

1. Pipette 100 µL of whole blood or cell sample into DuraClone IM Count Tube
2. Vortex at high speed for 6-8 seconds and incubate for 15 minutes at laboratory conditions (18-25˚C)
3. Add 2 mL of VersaLyse, vortex on high for 1-3 seconds and incubate for 15 minutes at laboratory conditions (18-25˚C)
4. Analyze by flow cytometry

Samples tested on Medonic Hematology Analyzer:

1. Cells were washed with PBS
2. 200 µl of cells were analyzed

DNA extraction:

Isolate DNA from whole blood samples according to the manufacturer's protocol using Qiagen QIAamp DNA Blood Kit.

Quantitative PCR Analysis:

1. qPCR mixture:
    a. Test Sample: 1-4 µl (balance with water)
    b. PCR*opsis*™ 5x PCR Master Mix: 4 µl
    c. Forward primer (10 pmol/ µl): 1 µl
    d. Reverse primer (10 pmol/ µl): 1 µl
    e. Probe (10 pmol/ µl): 0.5 µl
    f. Nuclease-Free Water: 9.5 µl
2. Run samples on qPCR Thermocycler for 40 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