Evaluating Cell Sorter Cleaning Procedures Across ABRF-FCRG Institutions by Testing for Common Contaminants (Poster # 22)

Roxana del Rio, University of Vermont; Kathleen Brundage, West Virginia University; Alan Bergeron, Dartmouth College; Andrew Box, Stowers Institute for Medical Research; Matt Cochran, University of Rochester Medical Center; Monica DeLay, Cincinnati Children's Hospital; Maris Handley, Massachusetts General Hospital; E. Michael Meyer, University of Pittsburgh Cancer Institute; Alan Saluk, The Scripps Research Institute; Peter Lopez, New York University Langone Medical Center.

The Association of Biomolecular Resource Facilities

FLOW CYTOMETRY RESEARCH GROUP

BACKGROUND

- Cell sorting plays an important role in many *in vitro* and *in vivo* studies, including genomic studies in which single cell isolation is required.
- Then, it is critical that during the passage of the cell through the sorter that there is minimal contact with eukaryotic and prokaryotic cells and debris.
- Any cell product that come together with sorted cells has the potential to affect their functional properties (i.e. activation, proliferation), or unwanted nucleic acids may be amplified during downstream assays.

JUSTIFICATION

- As ABRF-Flow Cytometry Research Group, we are interested in developing best practices for maintaining a "clean" sorter.
- The short term goal for this study is to determine how "clean" sorters are using regular cleaning procedures. The long term goal is to provide recommendations on how to improve (if necessary) aseptic sorting procedures.

METHODS

1) Participants: 8 FC Shared-Resource Labs (SRL); 19 instruments tested (5 BD Aria I, 7 BD AriaII, 2 BC MoFlo, 2 BC Astrios, 1 BD Influx, 1 BioRad S3, 1 PL Avalon).
2) Pre-sorted sample (from sheath tank and/or stock bottle) and post-sorted stream were collected on aseptic conditions and distributed to 2 labs to perform tests.
3) The first test-lab performed endotoxin (ThermoFisher Sci, Cat. 88282; colorimetric), and RNase (ThermoFisher Sci, Cat. AM1964; fluorometric) assays; the second test-lab evaluated bacteria and fungus contamination assays (ThermoFisher Sci/Molecular Probes, Cat. 7028; fluorometric). Additionally, we surveyed the standard cleaning regimen that each supplier FC-SRL does in a regular basis.

RESULTS

Figure 1.- Detection of Bacteria and Fungus by Flow Cytometry: Syto9 (nuclei staining); Calcofluor (fungal cell walls); WGA-TR (bact)

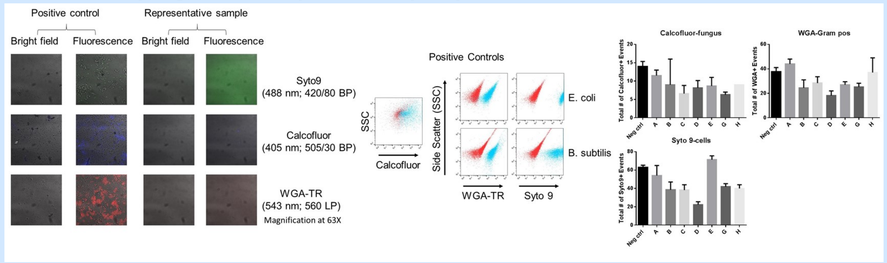

Figure 2.- Detection of RNase: at 5 and 20 minutes after addition of substrate

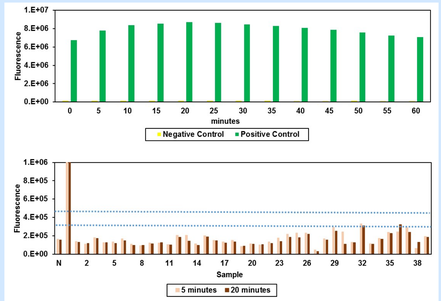

Table I.- Detection of Endotoxin

Site	Number of Instruments	Bacteria/Yeast	Pre-instruments	Post-instruments
A	1	B/Y	+	+
B	1	B	-	-
C	4	Y	+	+ (A&S) – (M&I)
D	3	No	+	+ (all)
E	2	B	-	-
F	2	No	-	-
G	5	B/Y	+ 1x PBS - Water	(+) all
H	1	No	-	+

Table II.- Shared-steps on cleaning procedures between participating labs

Common cleaning procedures:
1) Autoclave sheath tank (and ethanol tank) at least every other month. Rinse tanks with 10% bleach may be recommended.
2) Depending upon the system, every week run through sorter bleach and sterile water (in some cases ethanol as well).
3) Every other month replace filters and sample lines.
4) Before and after sorting, flush sample line with Contrad → bleach → sterile water

CONCLUSIONS

• In general, there is not a common procedure to keep sorters clean of contaminants. Instead, we have shown that different aseptic practices used among participating labs keep sorters clean.

•The sheath fluids used were either hand-made or by different manufacturers (ThermoFisher, Leinco, Sigma, Hospira, and BioSource). No difference on sterility/cleanliness was detected.

•Regardless of the cleaning procedure utilized, instruments are consistently free of RNases, fungus and bacteria (cells).

•Our results showed that endotoxin (a component of the membrane of Gram-negative bacteria), it is a common contaminant found on sheath tank and/or PBS (general) reservoir. However, it is most likely to be detected in instruments that sort microorganisms (bacteria) than in instruments that do not sort bacteria.

•The presence of endotoxin on stream/sorted fluid is regardless of the cleaning procedure utilized.

FUTURE DIRECTIONS

- Instruments that were positive for endotoxin will be re-tested in-house (second test).
- Instruments tested positive for second time will follow a protocol of decontamination suggested by McIntyre, C et al (Application Note, BD Biosciences, Nov 2009), followed by a third test.
- We expect to test for mycoplasma as wells, since mycoplasma is a common contaminant on cultures and can be easily pass into a sorter instruments.

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