Flow Cytometry Research Group 2016-2017 Study

Sheath Contamination Survey: An Examination of Common Laboratory Practices

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Background

Expanding upon our study from last year (*Evaluating cell sorter cleaning procedures across ABRF-FCRG institutions by testing for common contaminants*), the FCRG opened the study to non-FCRG members. To gather participants, a survey was distributed on the PUCL mailing list as well as the Google+ Flow Cytometry community. The survey collected information on respondents' instrumentation, practices, and contamination history.

Survey respondents were also asked to submit samples from their instrumentation for contamination testing. Samples from 47 sorters across 17 institutions were received for testing. Bacterial and RNase testing results are presented here while endotoxin testing is presented in our related poster (*Endotoxin Contamination of Cell Sorters: Evaluating Cleaning and Testing Procedures*).

Participants Profile

61 different facilities responded to our survey. Of those 57 respondents were from Shared Resource Laboratories. 106 cell sorters were described and were of the following types:

- Astrios – 4
- FACS Aria – 70
- Synergy – 7
- Influx – 5
- SH800 – 3
- ZXC - 2
- Jazz - 2
- MoFlo - 9
- S3 – 2
- Melody – 1
- Copas – 1

Protocol for Collecting and Testing Samples for Bacterial and RNAse Contamination

1. Sorters were started up as normally done following standard startup protocol for the lab
2. A sterile 10 ml pipet was used to collect samples from the sheath tank connected to the instrument
3. After turning on the sorter and fluidics following normal procedures, 10 ml of sheath fluid was collected by placing a sterile 15 ml conical tube under the fluid stream exiting the nozzle
4. Samples were shipped on wet ice or with freezer packs to the testing lab

Testing was done using the following kits:

Bacteria Contamination was detected using the BDBiosciences FACSMicroCount Reagents

RNAse levels were determined using the Applied Biosystems RNaseAlert Lab Test Kit (ThermoFisher catalog #AM1964)

Self-Reported Testing Practices

When asked about sheath fluid testing, 62% test only when contamination is reported by users or do not test at all. Total responses break down as follows (53 responses):

Testing Frequency	Responses	Percentage
Daily	2	4%
Weekly	11	21%
Monthly	7	13%
Never	8	15%
Only Upon Possible Contamination	25	47%

The most common location for sterility sample collection is directly from the flow cell with only half the respondents checking other areas such as the sheath tank. Self-reported sheath sampling locations (53 responses):

Collection Location	Responses	Percentage
Sheath Tank	24	53%
Flow Cell	40	89%
Sample Introduction Area	15	33%
Bulk Sheath container	9	20%

By far the majority of respondents are looking for bacterial or fungal contamination. Only four of the 45 responses indicated an interest in endotoxin, and only one indicated an interest in mycoplasma. Reported testing targets break down as follows:

Target	Responses	Percentage
Bacteria via Culture	45	100%
Fungus via Culture	33	73%
Endotoxin	4	9%
Mycoplasm	5	11%
RNAse	1	2%

36 of 44 (82%) indicated that they perform their own contamination testing of the sheath while the remainder hand their samples off to another institutional group for independent testing.

DID YOU KNOW…

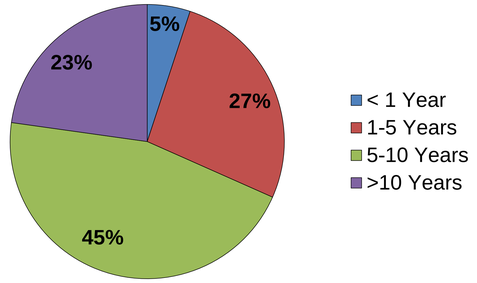
While once considered a common source of contamination, only 1 respondent indicated that they still test calibration beads for bacteria or fungus.

Instrumentation Quick Facts

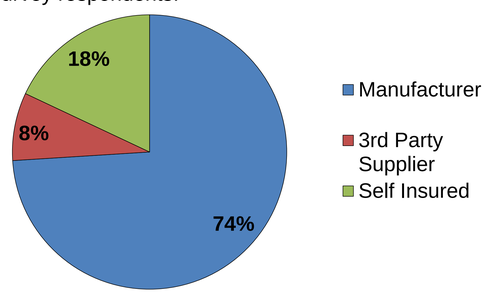
Of the 106 cell sorters described in the survey:

- 54 are housed within Biosafety Cabinets
- 55 are operated solely by laboratory staff while the remaining 51 allow trained users to operate the instrument
- Average reported instrument usage is reported at 76-100% though that number falls to 51-75% as the number of sorters within the facility increases.
- Sheath Sources break down as follows:
  - 51% of respondents use a commercial 1x saline product
  - 35% use a commercial concentrate saline product which is then diluted in-house
  - 14% manufacture their saline in-house

The average age of described cell sorters is 5-10 years and the results break down as follows:

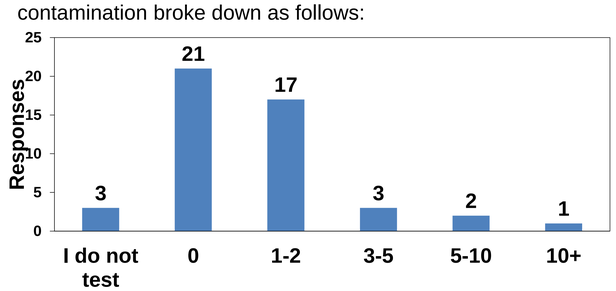


Manufacturer service contract is the preferred maintenance source for survey respondents:



Self-Reported Testing Results

21 of 47 respondents indicated no positive test results over the last 12 months. Those that did show instances of contamination broke down as follows:



Submitted Sample Testing Results

Bacterial Testing:

17 respondents submitted samples for bacterial testing. Each individual submitted a sample from the flow cell as well as the sheath tank. The sample was measured via flow cytometry, and the results were categorized as negative, low positive (+), positive (++), and highly positive (+++). Testing indicates less than half the samples are free of contamination :

	Negative	+	++	+++
Sheath Tank	2	6	4	5
Stream	12	2	0	3

RNase Testing:

47 samples were submitted for RNase testing. Two samples (1 from an Aria stream and another from an Aria sheath tank) were positive for RNase activity.

Instrumentation Decontamination Quick Facts

- 44% of respondents rely on the built-in instrument sterilization process once contamination is discovered
- Sterilization solutions used by those surveyed include:
  - 10% Bleach (78%)
  - 70% Ethanol (69%)
  - Hydrogen Peroxide (11%)
  - Sporicidin (4%)
- 59% of respondents do not autoclave their sheath tanks and rely entirely on chemical sterilization

Conclusions

1. Nearly half the survey respondents do not perform any proactive sheath testing and yet over half the received samples tested positive for bacterial/fungal contamination.
2. The prevalence of RNase in our testing pool was low, but it is worth noting that both positive tests were recorded in facilities that do no RNase testing.
3. Survey results at this point are limited and additional responses will present a better idea of current standard practices.

Follow the QR code to The current survey

Acknowledgements

We would like to thank all the individuals who answered our survey and provided samples for our study.