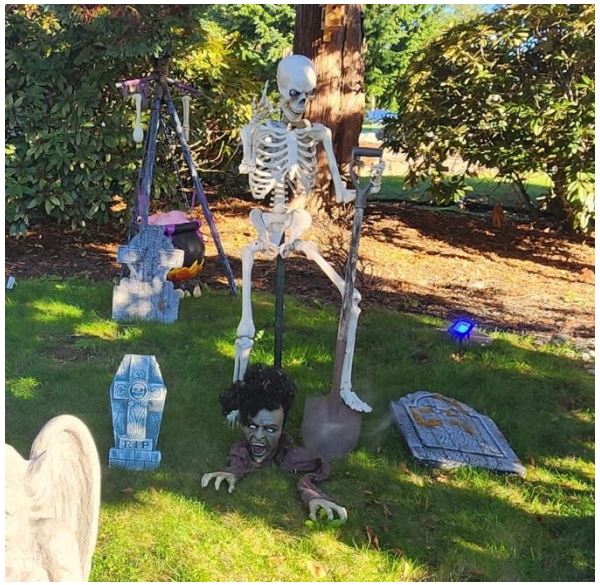
BEBPA'S HALLOWEEN HORRORS

Assays that have scared and scarred you!

The bleachening

Who: Mel Davis-Pickett, Director of CMC Technical Development at Immix BioPharma

Assay Type: ☐ Binding ☐ Cell-Based ☐ Animal-Assay ☒ Flow Cytometry



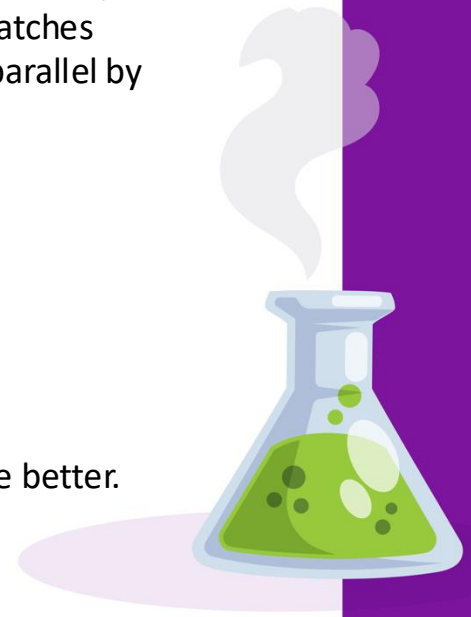
We had recently instituted new cleaning procedures for our flow cytometer.

The assay we were running was a relatively tame surface staining panel: activation markers, viability dye, nothing intracellular. Low risk. We sent our samples in, prepped from the same donor batches we'd used for months. The viability looked solid under the microscope. Stains were done in parallel by a trusted analyst. QC controls passed on the bench.

Then the data came back:
Fluor signals were distorted.
Viability was wrecked.
And FSC/SSC? Warped beyond recognition.

First thought: bad sample thaw. Second: staining error. Third: Fluidics?
We dug in and noticed something curious. The early wells were the worst. Later ones? A little better. Last few? Almost normal.

That's when we remembered the bleach flush.



The bleachening

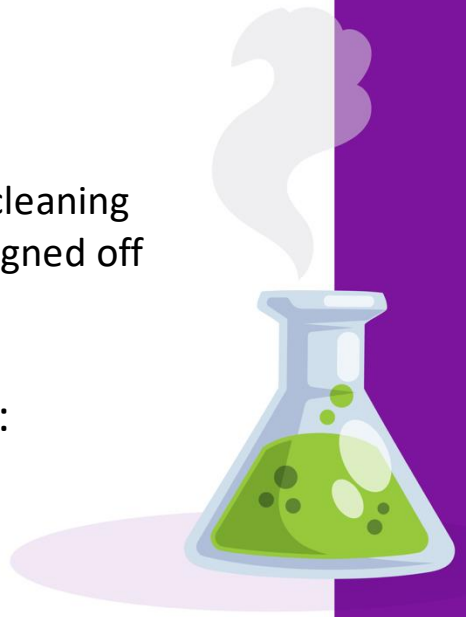
Turns out the system had been decontaminated the night before with the full 10% sodium hypochlorite flush. But instead of running three full tanks of clean sheath post-bleach, the operator only ran one. That left a whisper of bleach in the lines—just enough to cause low-level cell damage, particularly for the early wells.

The high-throughput sampler (HTS, i.e plate loader) fed the rows one by one. Early samples were exposed to just enough bleach carryover in the fluidics to nuke viability and strip off CD markers. Our panel collapsed, our plots were chaos, and our controls looked like cell death art projects.

The worst part? Everything ran “normally.” Only the samples were screaming.

From then on, we asked for: (1) A minimum two-tank rinse post-bleach (2) Post-cleaning validation runs with a known “canary” sample and (3) a timestamped flush log, signed off before every critical campaign.

Because sometimes what kills your assay isn’t contamination:
it’s the cure...



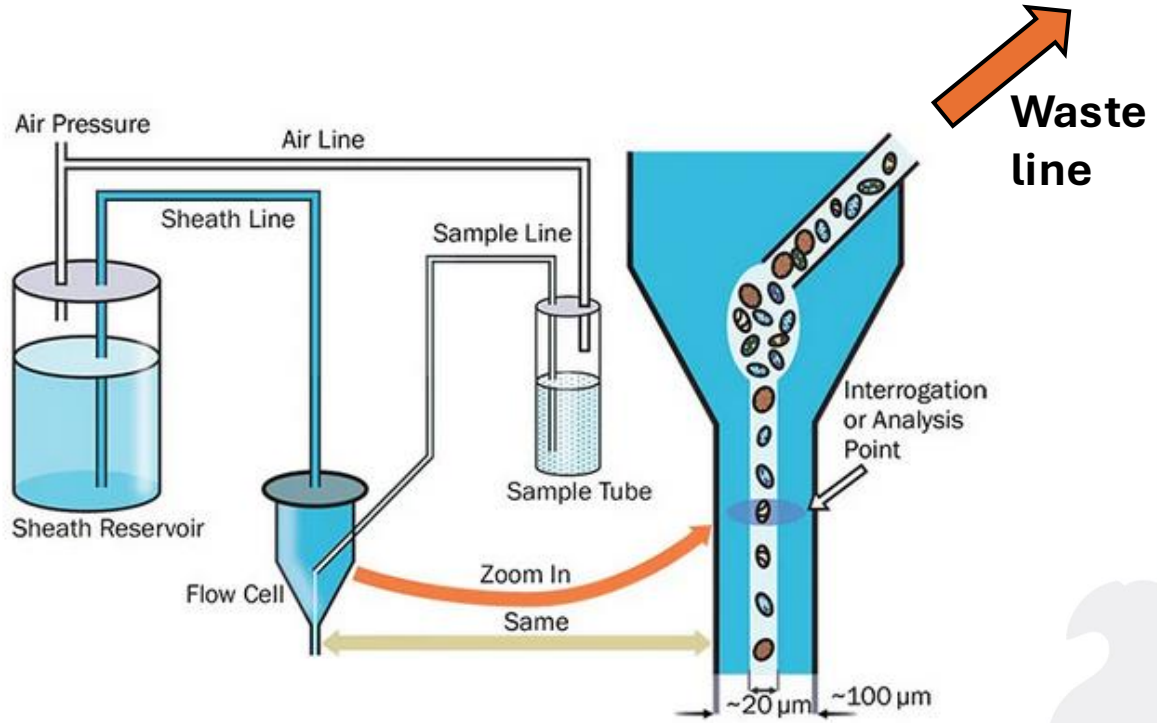
The bleachening

Under Normal Conditions:

Core Stream Formation: The sample is injected into a central stream of sheath fluid under pressure; this hydrodynamically focuses cells into a single-file line as they pass through the laser interrogation point

Pressure Regulation: Sheath and sample pressures are controlled to maintain a stable stream width and consistent event rate.

Collection and waste flow: After interrogation, fluid carrying analyzed cells exits into the waste tank under vacuum; the system continuously recirculates sheath from the supply tank to maintain flow stability.



What happened:

- A 10% bleach clean was run, but the post-clean was too short; The first wells saw the highest residual concentration. Some active chlorine lingered in the sheath, flow cell, and sample lines, as time went by the oxidant diluted, so the samples looked less impacted
- Oxidative injury + altered sheath chemistry changed the cell morphology so FSC/SSCplots and oxidized membranes looked warped even though voltages and pressure were normal

HAPPY HALLOWEEN!