Significant Developments, Trends and Approaches in Next Generation Flow Cytometry

September 2018 – *As interest in personalized medicine deepens and the efficacy of immuno-therapeutics improves, the capability of flow cytometry has expanded through the availability of new higher complexity flow cytometry instrument designs, reagents, and analysis algorithms that allow more complete profiling of the immune response. These flow cytometry capabilities aid in the discovery and the development of new therapeutics targeted at manipulating the tumor micro-environment and immune response.* Applied Clinical Trials *recently sat down with Mark Edinger, the scientific affairs director of flow cytometry at Q² Solutions, to talk about current flow cytometry practices, the value of next-generation flow cytometry (NGFC), and the most recent significant technological developments and applications of this technology. The information he shared is that of Q² Solutions.*

Applied Clinical Trials: Could you give us a brief overview of your experience in flow cytometry?
Edinger: I started in flow cytometry in late 1978 while at the Cleveland Clinic where I worked for 21 years building clinical and research flow cytometry labs and defining flow cytometry practice. During part of that time, I also worked as a consultant for Becton Dickinson and joined BD in 1998. I worked there for 14 years in marketing and R&D and supported global and national accounts. In 2012, I joined what was then Quintiles and what is now Q^2 Solutions. That's about 40 years of flow cytometry experience.

ACT: Could you provide an overview of past and current flow cytometry practice?
ME: Flow cytometry has gone through a long and accelerating development track starting with digitization. While its demise has often been predicted, its seems only to have gained greater utility over time. At the beginning, flow cytometry was very primitive and cost prohibitive. We used pulse height analyzers left over from the nuclear weapons programs and, with no monoclonal antibodies, we generated our own or bought polyclonal antibodies that we conjugated in our laboratories. Now, we have lots of bright new fluorochromes, can readily buy a wide variety of monoclonal antibody conjugates, lasers are inexpensive and much more reliable, and computing power has become very inexpensive. Today, we can build a flow cytometer that is exquisitely powerful for one-tenth of the cost of the original flow cytometers. The technology has matured, come into more and more hands, and has become more powerful, which is an exquisite combination of outcomes.
In the late 1970s and early 1980s flow cytometry was a method in search of an application. With the HIV epidemic, CD4 T Cell enumeration was the first overwhelming important use of flow

cytometry, along with leukemia and lymphoma diagnosis and cell sorting for research purposes. Flow cytometry has now become central to the development of new anticancer and autoimmune immunotherapies by the pharmaceutical and biotech industries.

ACT: Why are drug developers choosing NGFC, and what are the most common applications that they choose?

ME: Flow cytometry is really the best way to profile the immune response and monitor the tumor microenvironment in immunotherapy studies. At the beginning, we only had one-color linear analog readouts. Then, we progressed to two-, three- and then four-color logarithmic readouts. This was followed by digitization of data acquisition in the late 1990s that allowed more reliable instrument setup, standardization, and the addition of many more analytes from a single tube or well. In the last few years, there have been two major developments. One is mass flow cytometry, called CyTOF, which allows the investigation of 40+ multiple markers in a single sample. The other is Coarse Wavelength Division Multiplexing adopted from the telecommunication industry that has the capability of simultaneously detecting 40+ analytes from a single tube or well rather than the current 28 color limitation of older fluorescence based designs.

ACT: Are there any common misconceptions about NGFC?

ME: Yes. Several new data analysis algorithms are being used. There's t-SNE, viSNE, SPADE, CITRUS and others, which provide visually simplistic displays of complex data sets that allow discovery of new phenotypes, and functional cellular subsets. But, if you look at the underlying parameter by parameter data, sometimes the resolution is not what it needs to be, and we should not get too far away from looking at the actual stain index for of individual markers in flow cytometry to see how they perform in terms of resolution. When we're looking at these complex algorithms during sophisticated data analysis, we must not lose sight of the underlying quality of the raw data in these analyses.

The other weakness is that although the instruments so easily produce complex results, operators should not lose track of the fundamentals of good practice. The flow cytometer can be a black box that produces data, but everything up to that point—instrument setup and standardization, the processing and handling of the samples, panel design, and assay validation and deployment—become even more critical. We continue to pay meticulous attention to the complete process as we have operationalized flow cytometry at Q² Solutions. We make sure that the entire process is clearly defined and that we've developed an assay for optimal individual

analyte resolution so that when we go to the more complex algorithms for analysis, the data underpinning them is solid.

ACT: How is Q² Solutions using NGFC to advance customer study needs?

ME: We first started by installing matched high-performance filter sets in our instruments and quantitatively standardizing all our instruments globally. Most laboratories use a bead or beads for characterization and standardization. Beads are inexpensive and are easy to use, but they have some limitations that must be taken in to account when they are used. Using cells for instrument characterization, standardization, and setup would be very difficult, and in some cases impossible.

It is better to standardize instruments with fluorochrome-specific beads rather than beads that contains multiple surrogate fluorescent molecules. At Q² Solutions, we have developed fluorescence control beads that are custom manufactured for us that allow us to set global setup and standardization targets for all our global instruments. We have 40 instruments globally in our eight global laboratories. Our goal was to make sure we can produce the exact same result from any one of those machines.

We accomplished this by optimizing the dynamic range of all the detectors using a composite instrument constructed from characterization data from every instrument, setting targets with fluorochrome specific beads, and using these bead targets to standardize each instrument globally. This is extremely important when you're working in clinical trials with multiple instruments across multiple sites. We also use daily characterization data to monitor all global instruments at a central location in Edinburgh, Scotland. This constant monitoring allows us to make sure all instruments are performing correctly and to identify trends that will help avoid an impending instrument failure or identify an instrument in need of service to stay within Q² Solutions specific predefined performance limits. The transparency we get from using this characterization data—both to maintain the operation of the machines and to standardize them—has been critical to the success of our global operations.

We also have a team of dedicated analysts to limit the variability and subjectivity of the data analysis, and a team of senior data analysts to handle more complex assays and assays that demonstrate greater variability sample to sample, such as L&L MRD. The number of analysts for a given panel is limited to a small highly trained group to eliminate as much subjectivity in manual data analysis as is possible.

In addition, we generate QC material and establish a reference range for the reportable populations in every assay. This QC material is run with every assay set acquired, with the assay results reported only when the QC material results are within the established QC ranges. The assay is repeated until the

QC ranges are met. We're very tightly standardized and controlled globally and that's really been the key to our success.

ACT: What are best practices to keep in mind?

ME: Instrument characterization and standardization, with a dedicated assay development laboratory, are key. The Q^2 Solutions Translational Science Laboratory performs all the assay development. We engage in regular discovery activities and we record clone and conjugate comparisons, along with all our learning from validating upward of 500 different assays in the last six plus years. We use that experience to get better at what we do. We also have a long-term plan against which we execute and track our progress.

Flow cytometry is divided into three different areas. The Translational Science Laboratory schedules and conducts assay development and deployment; Science develops the overall flow cytometry and workflow strategies, designs assays, provides consultation and technical oversight, and drafts operational SOPs; Global Cytometry conducts and oversees day-to-day production laboratory operations on a global basis. We have implemented centralized operational oversight to limit variability, enhance reliability, and have best practices in place through our SOPs. Our SOPs are written in such a way that the last instructions in each are identical, with the variable and difficult parts of instrument and assay setup handled by the proficient senior staff. Critical setup steps are prioritized to a few technologists in each of the laboratories, who execute set up, compensation, and standardization. We're also building in automation to remove variability in individual operator performance.

ACT: How do you see NGFC changing in the coming 5–10 years, and how might those changes affect the future of clinical trials and drug development?

ME: We will shortly be able globally to obtain much more and better correlative information from a single sample with 40+ color instruments; there is much more correlative data when everything is run in a single tube or a single plate well. This also helps limit the amount of blood required for a given assay, which is a central focus of ours and the clinical trials community. This will ultimately benefit the patient and facilitate clinical trials that may not have been possible up until now because of the blood volume previously needed. Also, new algorithms are laying the groundwork for a whole new realm of discovery: we can see markers or discover lineages, populations, or functionality that have not been recognized before, because we did not previously have the capabilities for analyzing them more rapidly and thoroughly. This will drive a whole new realm of discovery and accelerate discovery overall. The next 10 years will be amazing in NGFC.