Webinar Transcript

Best practices in optimizing sample processing for improved flow cytometry

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Host:
Cole Jones (CJ)
Miltenyi Biotec, Product Manager

Speakers:
Chris Spring, M.Sc., CCy (CS)
Flow Cytometry Core Specialist, Keenan Research Centre for Biomedical Science, St. Michael's Research Hospital, Toronto

Rebecca McHugh, PhD (RM)
Science Training Manager, Miltenyi Biotec

Transcript:
CJ- It's a pleasure to be here today. Before we begin, I'd like to thank everyone for joining the webinar. As Christina mentioned, we'll be discussing Miltenyi Biotec's best practices in sample processing for flow cytometry. Our speakers, Chris Spring and Rebecca McHugh, will share some examples and considerations with respect to implementing these best practices.

Chris Spring is a flow cytometry core specialist at the Keenan Research Center for Biomedical Sciences at St. Michael's research hospital in Toronto, Canada. Given his unique role within a flow cytometry core, Chris will be speaking about the key benefits of incorporating Miltenyi Biotec's techniques and technologies into a flow core that supports multiple labs and projects. Chris, thank you for joining us today.

Rebecca McHugh is Milentyi Biotec's science training manager in San Diego, California. She offers a number of hands-on training courses ranging from general topics such as flow cytometry basics and foundations for cellular experiments, to more specialized training topics such as MACSQuant® Analyzer training and antigen-specific T cell analysis training.

Given her role within science training, Rebecca will be highlighting some of the best practices in sample preparation and present some key considerations to think about when applying flow cytometry analysis to cellular experiments. Thank you for joining us today, Rebecca.

To begin addressing Miltenyi Biotec's best practices in sample preparation, first I would like to highlight a few of the general workflow items on which we will be focusing. The workflows include three main steps: tissue dissociation, sample preparation and flow cytometry. Traditionally, tissue dissociation consisted of extracting tissue samples followed by a manual disaggregation, as well as incubation of the tissue with a homemade enzyme cocktail. Typically, the investigator chooses an enzyme or set of enzymes based on a previous publication and then titrates that enzyme for optimal performance. This can take a considerable amount of time and once that single-cell suspension is produced, direct cell separation or isolation of the desired population

is performed and the selected cells are then analyzed on a flow cytometer. But how does this process work when analyzing less frequent or rare populations? The rest of this webinar will address this question and highlight how modern methods for incorporating Miltenyi Biotec's instrumentation such as our gentleMACS™ Dissociator, our autoMACS® Pro Separator, and our MACSQuant® Analyzer, and how these instruments will optimize and standardize sample processing for improved flow cytometry.

Now Chris, since you are working with these instruments in your flow core, can you tell us more about how these devices have augmented your services that you provide for a flow core facility?

CS: Sure, thanks Cole. So just to give you a little bit of context, the flow facility that I run has a series of pieces of infrastructure. We largely have some flow cytometers as well as a cell sorter, but we also have a series of pieces of infrastructure for upstream and downstream cell biological applications. We also have a whole slew of different users looking at very different things. One of the things that we have alluded to is the gentleMACS, and that's allowed us to do a better methodology in terms of tissue dissociations. We typically have lung and spleen samples coming through the facility. As you'd mentioned, lots of folks will go out there and do a very old-style manual preparation of these cells to dissociate them and bring them into the facility. That usually involves surgical isolation of the tissue in question, crude homogenizing by mincing or pushing spleens through mesh, and/or a dissolution of the tissue into a single-cell suspension through a cockatil of enzymes such as DNASe and/or EDTA. What I find, is that most of the time people do this based on publications that are older. They're questionable and they're limited in depth in terms of what they describe. The user then goes through and filters these samples to remove un-dissociated pieces and then they proceed to stain and perform flow cytometry or other cellular analyses. Now, what we've found, is that by including the gentleMACS Dissociator in the core facility and the use of the C tubes which generate single-cell suspensions, we have greatly improved the workflow. In my sister-core upstairs, we have folks using M tubes to generate molecular homogenate for genomic analysis, sort of a high-level population analysis as opposed to sort of the single-cell transcriptomic analysis that you'd get out of, say, a Fluidigm C1. Those systems go off to the genomics core, I typically see single-cell suspensions coming to the flow core. What we've found is that this really enhances the workflow that folks have. I've had people do old, manual-style dissociation and by comparison when they transition to an optimized, cocktail-based kit solution, that they typically purchase from Miltenyi, and then use the gentleMACS for organ dissociation, they get much better yield of their cell populations of interest. Viability from batch to batch is also very reproducible and their basal cell populations are also extremely reproducible, which we find is not the case with old, manual-style tissue dissociations. People will sometimes go with old, manual-style dissociation because they think it's going to be cheap and quick, but the reality is that they often spend a lot more time on optimization. They take a protocol straight out of the materials and methods, rather than titrate these things themselves and end up wasting a lot of sample, time and money on antibodies instead of using a kit-based solution. Typically, once the users of our facility have a single-cell suspension from an organ of interest, they will go do flow cytometry. Sometimes those cell populations are not particularly abundant and there is a requirement to enrich for a particular cell population or sub-population. This is something that we, as I mentioned, have a FACS-based cell sorting system and electro-static droplet system in the facility. However, not all sorts are made equal and they're not all accomplishable by taking your raw cell suspension and doing a cytometric sort. What this has looked like in the past is, isolation of cells, however you so choose to do so, stain, and then either wash or don't wash those cells, followed by a FACS-based cell sort. However, we do have sorts where that's not accomplishable. You can then use the autoMACS Pro Separator, which is a magnetic-based system, to either enrich for a cell population of interest or deplete an undesirable cell population. You can take billions of cells and process them by enrichment or depletion in a very very short period of time, on the order of 10 minutes, and it also effectively washes away any unbound antibody should that be something that's actually required for your specific sort. And then you can move on to do cell sorting which we'll talk about in detail a little bit later. You get very viable cells, they're very pure, they aren't sitting around for long periods of time, and you get short sorts, which means that if you are doing genomic analysis on these things that your RNA species are actually quite well preserved. And another side effect of this is that if you're purifying for cell population A, and

you need a negative control that has no cell population A in it, you actually get both fractions back so you can use the cell depleted fraction as the negative control. Which a lot of our users, which have adoptive mouse transfer experiments, actually find quite helpful. In the end, again, in the context of my particular facility, folks are often looking to then do flow cytometry after they have the cell population that they would like to analyze. And with most of our instruments, this looks like me coming in the morning and doing QC on the instrument, the users isolate the cells that they need, they then stain them. Hopefully, they've brought the appropriate comp controls for compensation and FMOs, etc., but as we all know, that that's not always the case. They then do their flow cytometry and are supposed to wash the machine in a multi-user environment, but again, that's not always the case. And then they take their data off and re-gate it and analyze it with a 3rd party software as needed. In the context of the MACSQuant that we have in the facility, there are several steps where we've found this to be simplified or enhanced in terms of functionality. The QC of the MACSQuant is incredibly easy, you just barcode scan the bottle, put a drop in a tube, anyone can do it. My users do do this. It's not something I allow them to do on other instruments, because they can mess with laser delays, and other things that are relatively catastrophic. There's also a pre-enrichment possibility within the MACSQuant itself. For an example, you could have a magnetic CD34 antibody if you wanted to analyze CD34 positive cells in the plasma. The magnet turns on, purifies those cells, and then you basically elute those cells by turning the magnet off and then running your actual flow analysis on that pre-enriched population. I have a lot of basic users who rely on software to compensate their samples, and this thing about other instruments, is that software often tells you that its' done a good job, but there's no proof of principle. The MACSQuant will show you that the compensation is correct on your samples by giving youa N-by-N matrix, so you don't end up running these things under bad comp settings and find out 6 months later that you've been doing it all wrong. From a multi-user environment, I particularly like that you literally can't walk away and not wash the cytometer, because we do have lazy users that do that. The instrument, when you shut it off, will wash itself with its own buffers. It's very resistant to clogging, so in terms of a cleanliness perspective in a multi-user environment, it is very, very useful. If you run lots of plates, 96-well plates with the mini-sampler on the MACSQuant, it is completely walk-away. You can put your stuff on there, leave, it will clean itself and shut itself down when you're done and you can come back to analyze data. You can get away with very small sample volumes. There's no way I'd ever put 20 microliters on, say, a Fortessa, but you can easily do that on a MACSQuant because it's a syringe-based system as opposed to a constant-pressure system. Simple users that don't need to, say, mathematically model their data, often have the data analyzed as they like when they acquire it. The mqd file format on the MACSQuant allows them to take it off, open it up in another program, right click, say analyze it for me, and it comes up exactly as they've seen it. They can take that and plop that data into their publication or their lab meeting presentation. Overall, what we've found in our facility is that the systems are very automated, they're very foolproof, they're user-friendly, they have lots of advantages in a multi-user environment, and that's had an impact on both the core facility as well as the quality of the data that folks are taking away from the core.

CJ: Well, thank you Chris. So having discussed several of the benefits of this new workflow, I'd like to take a look at a real-world example.

We recently performed a demonstration of this methodology for the flow core at the Ontario Institute for Cancer Research. Here, their ultimate goal was to isolate $CD31^+$ lung endothelial cells from whole lung tissue. To do this, they needed to dissociate mouse lung, then deplete $CD45^+$ leukocytes, followed by enriching for the $CD31^+$ endothelial cells from the $CD45^-$ population, and then analyze the isolated endothelial cells for purity and viability.

They wanted to evaluate our instruments as well, so we brought in the gentleMACS Octo Dissociator with Heaters, the autoMACS Pro Separator and the MACSQuant Analyzer. They used the gentleMACS and our Lung Tissue Dissociation Enzyme Kit to disaggregate the mouse lung tissue, the autoMACS Pro Separator for both the $CD45^+$ leukocyte depletion and the $CD31^+$ endothelial cell enrichment. And the MACSQuant Analyzer was used for flow cytometric analysis to then assess the viability and purity of the isolated endothelial cells.

Here are the results from this demonstration. The far left graph shows all cells from the dissociated

mouse lung in a forward and side scatter plot. The center graph shows the sample after depleting the $CD45^+$ leukocytes, here, showing side scatter over CD45. And the far right graph shows the sample after the $CD31^+$ endothelial cells enrichment from the $CD45^-$ population. And here, looking at side scatter over CD31. Chris, since your core supports labs doing similar work, I was wondering how does this data compare to what you've observed?

CS: We actually had a group that did do a similar kind of experiment. And, what they started with was doing a manual dissociation of lung and in the end they were looking for B cells and then an activation marker of B cells. First of all, what we found with manual dissociation was that the forward and side scatter plot that you see on the far left, for the lung, looked absolutely atrocious. I mean it was just all over the place. You could barely find the cell population and discriminate it from anything else. In addition to that, they had serious changes in their baseline B cells from control tube 1 to control tube 2, so they should be really quite identical and that was not at all the case. If you're looking for changes from your control group to your disease group to your treatment group and you have massive variation in your controls alone, you're just never going to be able to find that. These folks did change to using the gentleMACS for dissociation of their lung. Because they had very consistent viability and yield of their basal B cell population, looking at the activation states within their model system of disease was actually very successful. They were able to go on to publish their results and it was all from simply changing their dissociation protocol.

CJ: Thank you Chris. So it sounds like reproducibility and the cleaner data is definitely advantageous here. Having looked at an example of where the cell type of interest is more abundant, Rebecca will now review some considerations for flow analysis of rare cell populations.

RM: Thank you Cole and thank you Chris as well as LabRoots for having me join you today on this webinar.

I would like to take time right now to just talk about flow cytometry as an application for detecting and characterizing a very rare cell. When you do this, there are several things that you have to take into consideration. One of which is you should use some bright staining reagents, fluorochromes, as well as antibodies, to help pull away that population as well as you can from the background. In addition to that, having high-quality reagents, so reagents that have high-specificity and low background-binding, will aid in your ability to detect that rare event from the background population. Of course, using a flow cytometer that gives you a very low carryover between samples will aid with the detection of your rare event. Of course there's many different controls to choose from when you do flow cytometry. Not all of them are going to be helpful in your assay, but of course you do need to choose the right controls for your rare cell analysis. But what I really want to focus on today is the necessity of acquiring such a large number of events from your total sample just to give you some statistical significance in your characterization of the rare event. So with that, there's always the age–old question, "how many cells must I analyze?" And in all actuality, it depends. It really depends on the target cell that you're interested in characterizing. So, there's going to be a lot of stuff on this slide and mainly it is there just for your reference. I just want to talk about a few statistical considerations when looking at a rare event. One of which is if you are just trying to enumerate that rare event, there is an inherent error just to counting. The error in counting is the square root of the number of events that you've counted. So if you take a look at these examples I've written here, if you're counting 100 events, the error in that count is the square root of 100 or 10. Then if you translate that into precision of that count, you have a 10% CV. So as you increase the number of events you count, your error actually does increase, but your precision of your count improves. So the more events you count, the more precise your count will be. Beyond that, you can look at perhaps how reproducible your count or your method is. With this, you think about the statistical consideration of a confidence interval. So, if you would like to be 95% confident at what you're looking at, in general, that means that your measurement is going to fall within 2 standard deviations of your average of that measurement. Again, the detail on the slide is mainly just for your reference. I would like to combine these two statistical considerations on the next slide, here. And detail for you this little chart that I've put together. On this chart, it is detailing for you the number of events that you should count if you would like to be 95% confident that you're within a certain percentage of what's truly there in your tube or in your sample of interest. So if you would like to be 95% confident that you're within 10% of the measured value, then that

means that you have to acquire about 1500 of your TARGET events. Now, what if your target event was something so incredibly rare, such as maybe 1 in 100,000 events. That means to acquire 1500 of your target, you would now need to acquire over 153,000,000 of your total cell sample, and that can be quite unreasonable. All of these values that are detailed here in the red boxes, are generally going to be not capable of a flow cytometer to acquire in 1 data file. And even if it could, it would be very difficult to analyze a sample size that large. So why not consider enriching for your target event and therefore decreasing the number of events you absolutely have to acquire on the flow cytometer. So with this, I would like to talk about using a pre-enrichment technique to analyze a rare event such as an antigen specific T-cell. The frequency of these cells can be in a large range and if you look at trying to detect an antigen specific T-cell that maybe has never seen its antigen before, so a naïve cell, this can be incredibly rare in the peripheral blood. And even cells that might be reactive to auto-antigens would be quite rare. So we are going to use the technique of positive selection with our magnetic MicroBeads and in this technique we're actually going to be looking at antigen specific T-cells by their functional response, upregulating an activation marker, CD154, or better known as CD40-Ligand. Upon activation, seeing their antigen, the cells will upregulate CD154. We can target that with a magnetic particle, pass that cell sample over our column and magnet system, and those CD154$^+$ cells that have reacted to that antigen will be retained on the column. You can then wash-away or de-bulk, getting rid of non-reactive cells, and then basically concentrate your flow cytometry on your antigen-specific T cells. In this next slide, we are starting here with an un-stimulated population. It's already gated on CD4$^+$ lymphocytes, and we are looking at, on the X-axis, the CD154. And here, before stimulation, not much expression of CD154. After a 6 hour incubation, with, in this instance, BKV viral peptide, you can see that CD154 gets up-regulated and it's a very small percentage here, but pretty reasonable to look at by a flow cytometer. And if we look within this activated population, you can see that they are functionally responding by producing here IFN-γ. We also looked for a functional response of IL-10, but it doesn't appear that maybe IL-10 is a functional response to this antigen. Now, as mentioned, we were going to pre-enrich our sample targeting that CD154 and so here is an example of that enrichment, and so we now have a majority of the CD4 cells that we're looking at, are the ones expressing CD154. And now if we look at the subsequent cytokine production, of course you can still see and confirm that we have the IFN-g, but now you can start to see that there are a few cells within this antigen-reactive species that perhaps are making IL-10. In another example, also using another viral peptide, here, EBV, you can see after the stimulation, there is actually not much CD154 expression. So perhaps this donor is not responding to the EBV peptides. But we did do the enrichment as well for CD154 expression and after the enrichment you can see that there are some CD154$^+$ cells and these are also producing IFN-γ.

CJ: Thank you Rebecca. Now, having reviewed some of the important considerations when doing flow analysis of rare cell populations, Chris, you've done similar work involving analysis of rare cells in your flow core, particularly with regard to pre-enrichment before rare cell analysis or even sorting. Would you mind elaborating on those experiences?

CS: Sure. What we've done is look at sorting cell populations that would otherwise not be particularly feasible in the context of FACS-based cell sorting. I'll take you through the model that the lab was trying to do before enrichment and also post-enrichment. The client lab was isolating mouse speloncytes, dissociating that tissue manually, and then taking that and enriching it properly on an autoMACS for the subset of the population that they were interested in. This enriched for their cells of interest upon which they performed further selection on a FACS-based cell sort system, which gave them large numbers of T-cells that they adoptively transferred into mice to accomplish an immunological model of disease. They transferred various populations, made their comparisons, and were able to publish their results. Now, the problem is that when they first attempted to do this, if you look at this, so here we have a CD3$^+$ population on the far left graph and in the middle graph, we have a red, basically CD8 T-cell population. Above that on the Y-axis in grey, we have a FITC$^+$, CD4$^+$, T-cell fraction, and then a CD25$^+$ fraction that spreads out to the right. Now, if you look at this from a cell-sort perspective, this population is only about 2% of your total events. And if we are going to sort this and try to get 2,3,4 million cells in a reasonably short period of time for adoptive transfer experiments, it was something that was really, quite frankly, unfeasible. The cell sort times were far too long, and the cells following the sort, they didn't look particularly good. Their

CD25 profile started to become altered and it was problematic. So, what I had them do, was use the autoMACS to pre-enrich their CD4 cells, and, this is something, like I mentioned earlier, which is very, very quick. They can put a massive, bulk amount of cells, billions of cells, through the instrument in 10 minutes, which you cannot do in a droplet-based sorting system. They were then now left with basically, just CD4 positive T cells going through the cell sorter. And now what you can see, is that 2% population of $CD25^+$ cells is now almost 9% of their population. And this makes a dramatic difference in their total cell-sort time and the quality of the cells that they got out the other end. Essentially, it took it from something that was un-accomplishable to something that was really quite manageable. We took these cells after the fact and did a purity check to made sure they were quite pure. As you can see there, there's a little bit of photo-bleaching of the CD25, but, basically they had very good populations that came out the other end and they were able to do their adoptive transfer experiments. This was probably about a 2-hour sort instead of a 8 or 9 hour sort, that just wasn't feasible. And, another thing that I don't have data on the slide for, but we've found a lot of use for the autoMACS, is in depletion as well. So we've had brain that we've depleted of myelin to do successful sorts for endothelial cells, which is essentially not doable when you do have all the myelin debris in your cell suspension.

CJ: Speaking of this pre-enrichment strategy for both flow analysis and even sorting in general, there are even some experiments that may REQUIRE pre-enrichment before cell sorting on a particular population of interest. We have some data from a lab up at the Cleveland Clinic, the lab of Dr. Jeremy Rich, in which the pre-enrichment of bulk tumor cells was required before isolation of rare cancer stem cells from a PDX tumor model. Rebecca, I was wondering if you could elaborate on the experiment, here, and the data, in terms of how that pre-enrichment strategy really did improve those downstream cell separations to ultimately be able to analyze and work with those rare cancer stem cells?

RM: Sure, thanks Cole. So yes, I would like to just talk a little bit about the collaboration we had done with this group whose goal was to establish an orthotopic passaging system for their glioblastoma-patient derived samples. So their whole goal was to dissociate these primary tumors that they were receiving and orthotopically inject these into mouse, meaning actually grow them in the biologically relevant tissue that they are from. They were having many challenges trying to get this established. One of which was that implanting these into that relevant tissue really resulted in a lot of mouse cells infiltrating these tumors. So when they were harvesting these tumors out, they were having a really difficult time dissociating the human tumor cells from the infiltrating mouse cells. And that required trying to isolate them and general FACS sorting was taking several hours to do so. And it was also requiring them to genetically modify these primary tumors to express a fluorescent protein, just for their identification. Just to give you a little bit more detail on this, they were trying to establish a model system where they could grow the glioblastoma tumor in a mouse brain. In general, what happens, is as that tumor establishes within the brain tissue, it infiltrates a lot of the healthy brain tissue, and when you extract that tumor out, you are going to be harvesting a lot of the mouse cells as well. To help them with this, we suggested one of our kits, which is called a Mouse Cell Depletion Kit. The kit is a cocktail of magnetic MicroBeads that will target many of the types of mouse cells that can infiltrate a tumor: endothelial cells, stromal cells, and so forth. But with this, you really need to ensure that when you harvest and dissociate that tissue that you are not using any enzymes that can compromise the expression of the epitopes that we need to target. So, with that, we recommended to use the Tumor Dissociation Kit, human. What they had been using previously was a cocktail of enzymes that also affected some of the antigens that they wanted to sort for as well. This Tumor Dissociation Kit helped preserve all of those epitopes that they were wanting to target. So here is just an example of some of the data. If we look up here at the upper left-hand corner of the slide, this is the flow analysis prior to any depletion of mouse cells, so just after dissociation of the tumor. You can see that the actual cells themselves was less than 10% of the population of the dissociated tumor. Within those cells you can see the tumor cells, they are expressing the GFP. But there are also some cells that are not expressing the GFP and these are also just some lingering mouse cells that were being identified as well. After using the Mouse Cell Depletion Kit, you can see here that pretty much all of the cells are the GFP^+ tumor cells and you can see that this depletion also got rid of a majority of the debris that makes it really difficult to do flow sorting as well. And then on the right-hand side you can see

that their end result was really to sub-divide these tumor cells into the cancer stem cell population identified by the marker CD133. And so after doing this cleanup with the mouse cell depletion, they could easily sort for CD133 positive cells, and also use the CD133 negative cells as a control for their RNA-seq. You can see that they got quite a number of quality aligned reads in both of these sub-populations. So just to summarize a little bit about their overall goal, prior to incorporating some of the solutions that we could offer, they were really struggling with trying to do a 60-mice study in this orthotopic model. Utilizing the gentleMACS with the Tumor Dissociation Kit, they were able to dissociate the tumors efficiently, preserve all the antigens necessary, including that CD133, so they didn't have to do any post-dissociation culture for re-expression. So that lessened the amount of time to actually get the cells of interest for their study. By doing the mouse cell depletion, this enabled them to more easily sort these cells and, in all actuality, it enabled them not to even have to genetically modify them with GFP for tumor cell identification. Beyond that, by de-bulking the whole system using that mouse cell depletion, we went from several hours required for the sorting of these cancer stem cells to just 30 minutes per sample. And this then enabled them to really consider the ability to do the 60-mice cohort study.

CJ: Thank you Rebecca. Taking a look at the advantages at each step, it was mentioned that incorporating the gentleMACS technology into tissue dissociation allows for increased yield, consistent viability, reproducibility and even epitope preservation, which is clearly important when subsequent downstream antibody staining of key epitopes for analysis or further cell selection is required. Chris, earlier you mentioned that dissociation with the gentleMACS system is easier to optimize and troubleshoot. Can you discuss what that optimization and troubleshooting process might look like when more manual methods are used?

CS: Sure. The reality is that folks accessing a core facility should be taking their enzymes and optimizing these things, and titrating and all of that. But the reality is you get graduate students who come in, and they read a materials and methods that's missing half of the information, they try to employ that methodology and get a single cell suspension. And then when it doesn't work, they have no idea what to do. They really don't know where to begin with optimizing the protocol or what to titrate, and how. And they often ignore things like Rebecca's talked about, where, you don't just care about getting single cells reproducibly time and time again, but in preserving antigens that aren't as bright like CD133. So really that's when people kind of pull out their hair and say I have no idea what I'm going to do, I have no idea what to change to fix this. And they really do kind of switch over to using a time-saving technique like the gentleMACS.

CJ: OK, thank you. Then moving on to the autoMACS Pro Separator and with regards to cell separation. There seems to be a time-savings benefits here. Also, the effective wash of unbound antibodies is important when moving into those downstream assays that require subsequent antibody staining. And being able to use the depleted fraction as a control is also going to be advantageous for downstream analysis. Though, a lot of times, investigators are concerned about recovery and purity of selected cells. Rebecca, could you describe how the autoMACS Pro Separator can address this concern?

RM: Yea, sure. So one of the features that I really like about the autoMACS Pro Separator is the ability to choose different types of programs. A lot of these programs will differ in the controlled flow rate that goes over the column. And how our technology works is all a compromise between the ability to retain the cell on the column that's magnetically labeled and the ability to wash away your unwanted cells. And all of this is going to be a trade off with the flow rate. So you can choose a really fast flow rate if you want higher purity of the cells being retained on the column, so it's easier to wash away the cells you don't want. You can slow down the flow rate if you're really concerned about recovery, so that you can try and retain every single cell that has a magnetic antibody on the surface. So there's a lot of flexibility in using the autoMACS if your goal is, really to, let's say, flow sort for another sub-population. You can use the autoMACS Pro Separator to enhance the recovery of your one cell type of interest. And then you can go, then to flow sorting for the purity of those subsets in a more easy fashion.

CJ: And as for the MACSQuant Analyzer, Chris you previously mentioned several benefits such as automated startup and shut down, auto calibration, compensation, automatic clearing between samples

and even auto labeling. But could you discuss, in a little more detail, how these benefits or any other factors, are advantageous from a multi-user facility perspective?

CS: Sure. The reality is that these advantages are advantageous to everybody. They're advantageous to an individual lab, be it small or large. However, in a multi-user facility, they're absolutely critical. If you have things like clogs, or people not washing the machine, QC-ing it improperly, or getting compensation done improperly, in a multi-user environment where people often share templates, and have large cells or sticky cells, it's basically life or death. It makes huge differences in terms of the accessibility of the equipment and the results that come out of the facility. Which, then, of course turns into whether people are using your facility or not. Something else that I didn't particularly mention, that's not immediately obvious is that most cytometers have very large cases of buffer that you're required to use for sheath. They are expensive on shipping and they're cumbersome and then people don't fill up your instruments. Whereas the MACSQuant has these little 1.5L sheath boxes that last just as long as your standard 20L sheath box. Which again, is something very useful for a multi-user facility. Because people will bring down their 1.5L bottle, plug it into the side of the MACSQuant, and actually fill up the sheath. So it really comes down to the maintenance of the equipment, the use of the equipment, and the data that's coming out of the facility being improved by these types of things.

CJ: Well, thank you Chris and thank you Rebecca for reviewing some of these best practices and tips and tricks in optimizing sample processing for improved flow cytometry. I'd like to thank everyone for attending this webinar and if you're interested in more information, please feel free to use the contact information below. And I think now it's time for some questions and answers.

Q&A:

Q1 (for Chris): How is Q and C different between the MACSQuant and non-Miltenyi flow cytometers?

A1: (CS) Sure. So, the way that typically looks is of course a little bit instrument dependent. But most other instruments involve going into a portion of the software, running reference beads, and establishing settings and the performance of the instrument itself. Usually in, so, if we take the example of say, BD, because they are a large market of cytometers that people are familiar with. There is lots of information within those areas that is sensitive to folks that don't particularly know what they're doing if they haven't been well trained as flow cytometrists. So they can mess with instrument configurations unintentionally, they can change laser delays unintentionally, area scaling, things like that. More easily than they can do within the analytical portion of the program. So it's something that core facility people end up doing most of the work on and it's not a big deal, but when you have a large number of instruments, it can be a little bit time consuming. With the MACSQuant, really, you just scan a bottle's barcode, it tells you on the screen to put a droplet into the tube, you put a droplet into the tube, and it does everything else for you and then shows you the information. It also has a prompter on the screen that tells you this was the last time it was QC'd, maybe you should QC it or definitely you should QC it. It's nice to kind of take that and offset that on to users, as well as the fact that users are then able to see exactly what's happening and when.

Q2 (for Rebecca): What is the impact of the MicroBeads on the cells?

A2: (RM) Ok, so, in a way this is somewhat of a difficult question to answer. Our MicroBead and column technology is designed for the least amount of impact on the cell. The beads themselves are incredibly small, only 50 nanometers in size, about the size of a virus, and are really just a co-precipitate of dextran and iron. So basically sugar and iron and that's what cells use anyway. Now the column technology that we have enables such a high magnetic gradient that you only have to add such a small amount of this magnetism to the surface of the cells to be able to be retained in the column-magnet system. So, combining with the fact that the iron and sugar is so incredibly small as well as we are not adding a lot of that to the surface, it doesn't have a huge impact. So the surface area of these MicroBeads are not large enough for cross-linking of receptors and they are biodegradable. So, you know, depending on how the receptor is degraded, that is basically going to be the same pathway that that MicroBead will be degraded as well.

Q3 (For Chris): What criteria do you use to state that the RNA quality is better preserved?

A3: (CS) In that particular example, if I'm remembering correctly, we had folks actually take these samples to the genomics facility on site and they did a Bioanalyzer run and looked at degradation of the RNA to establish the fact that it was impaired.

Q4 (For Rebecca): Can you explain why the CD133 was not preserved prior to the gentleMACS dissociation system?

A4: (RM) So, what I believe, the enzyme that they were using prior to incorporating the human tumor dissociation kit was actually papain, I believe. That is a general enzyme required for brain dissociation and CD133 is actually sensitive to papain. So the tumor dissociation kit does not use papain as an enzyme, and therefore the CD133 was preserved post human tumor-dissociation and contact with the gentleMACS.

Q5 (for Cole): What tissues are compatible with the gentleMACS?

A5 (CJ): There's a broad array of tissue types that are compatible such as lung, liver, neonatal heart, adult brain, neonatal brain, postnatal brain, basically almost anything other than bone. Some other things would be spleen, skin, umbilical cord. There's a whole array of them.

Q6 (for Rebecca): How does the MACSQuant address concerns about inter-sample carry-over?

Q6 (RM): Sure. The fluidics of the MACSQuant are actually quite stringent in terms of washing through the flow cell. We have validated, if you use our standard inter-sample washing mode, a carry-over of less than .01%. That's pretty much at the reasonable determination of an event carry-over. In terms of comparison to other systems out there, it has one of the best stringencies in terms of inter-sample carry over.

Q7 (for Rebecca): What is the best practice to wash DMSO from thawed, frozen, cord-blood samples when performing a CD34 enumeration and viability testing?

A7 (RM): Personally, I do not have experience with this particular assay. However, I know we work with several researchers that are using cord blood and therefore CD34 isolation and enumeration. I can certainly look through some of our technical archives and see if we have any best practices for the post-thaw washing and perhaps send some of those recommendations to you.

-- END OF TRANSCRIPT --

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Miltenyi Biotec Inc. | 6125 Cornerstone Court East | San Diego | CA 92121 | USA
Phone +1 800 FOR MACS | Fax +1 530 745 2806 | macs@miltenyibiotec.com | **www.miltenyibiotec.com**
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