PACT Web Seminar 11: July 31, 2008 "Validation Processes"
Question & Answer Session Transcription
Speaker: Dr. Carolyn Keever-Taylor

Question 1: Do you have a validation procedure or policy in your facility? I believe that if your lab is AA BB or CAP certified you are required to have one.

Answer: Yes we do. What I showed you was our template that comes from that SOP. I essentially outlined our SOP for you in this talk, but it is a written document.

Question 2: How do you validate or verify procedures that do not have number values that you can analyze? For example, procedures for shipping cells.

Answer: Well, for that specific example there are numbers that you can analyze. You would monitor the external temperatures as your control for your shipping and you can monitor the temperature inside your shipping container. If you want to know whether or not the product is functional at the end of the shipping, you may do cell counts and viability. I don't think there are very many procedures where there isn't something that you can quantify. I appreciate that not every validation study that you are going to do is going to fit all of the elements that I've described, but you can't validate something if you don't have something to measure. And it could be as simple as the viability of your product.

Question 3: What do you do if your validation fails?

Answer: Well then you don't use that process. You start over. You can't use a procedure or a piece of equipment or a process that you can't validate. That's just not acceptable. Maybe that's too simple an answer. I'm not sure what the questioner really wanted to know. If you can identify why your validation fails, the first thing I would try to do is correct whatever it is that caused it to fail. You change your conditions until you can achieve the outcome that you want, or at some point you may just conclude that you can't validate this procedure, and you can't use it.

Question 4: How do you decide the number of tests to use for a validation?

Answer: So that's probably the hardest question to answer when designing a study. In doing my own research before I ever started doing validations, and listening to various presentations such as this one, you hear people throw out numbers. I can say that one is probably almost never enough. Three, for some studies may be enough. For others you may need six, and for some you may need twenty. It really depends on the nature of the results and how variable they are. If you do three experiments and the results are absolutely the same and you've tested over a range you feel is suitable then three may be enough. If you've done three measurements and they vary, say more than 10 % then you need to do more measurements. For some studies you can probably get help with the numbers you need to use from a statistician. I don't think there are very many institutions that don't have some sort of statistical support, and there are times when it may be appropriate to get some guidance. And of course there are practical issues and it may just not be feasible or even possible to do too many replicates. Your statistics certainly need to reach a defined level of significance, and if they don't you probably haven't done enough samples.

Question 5: How do you determine critical parameters for testing as well as accuracy? An example would be 10% variation.

Answer: You decide how much variation you are willing to accept in your system. For our example study comparing the phenotype of the product at receipt versus after overnight storage, we were willing to accept a 10% difference in the results? Part of what I look at, and maybe I didn't explain very well why we are looking at the intra assay-variation. So part of what I do is first I try to look at how much difference did I get just repeating the same tests on the same products. So, any of you who do flow know that your results can be affected by a number of things. The staining that you did; but probably more so how it is acquired; how it's analyzed. If you have two techs doing the same sample there's always going to be some difference. So first figure out what your intra-assay variation is and then decide how much difference you are willing to accept for your study. It's really something the laboratory itself has to decide. If I've got a coefficient of variation within my assay of 5% and I'm not willing to accept any bigger difference then I would set a 5% difference as my limit, but if I feel that a 10% difference really biologically doesn't make that much of a difference in the process, then I'll accept the bigger difference. So it really is somewhat subjective. I think it has to be based on your experience and your knowledge of what the end product needs to be. And I often say to my techs this is not a chemistry lab. In a chemistry lab, if you're off by a couple of drops, your pH could zoom way up. A small change can make a big difference. For a lot of what we do you can really make a big change that might not make such a difference. That's what you have to identify. You have to identify where the differences really do matter. And that's part of the validation process. That's identifying what are those critical points.

Question 6: Do you have a recommendation of a mock product to use to validate a new controlled rate freezer?

Answer: Well, we actually use just normal peripheral blood. Our collection center is a blood center which is fortunate for us. So it's fairly easy for us to get buffy coat products that would otherwise be discarded. So we often use those for validation studies. We may turn them into mononuclear cells by doing density gradients so they are more like a HPC product. This is an example of where you may want to do part of your study with a mock product, but you might want to follow up with confirming your process with the real product. This could be a partial product. You may take 10% of a patient product, with approval of course, to do this, to confirm that there is no effect on the CD34 content, if you did the initial studies with products that did not contain CD34. That's a very broad question, but we often use buffy coat products for our validation studies and for follow up we confirm with real products.

Question 7: How do you document the risks and benefit decisions made and associated with validation? For example, expense prohibits sufficient runs for statistical assurance.

Answer: Again, this isn't an easy question to answer. You have to do enough so that you are confident that the system works because when you are doing a real product, it's a patient's life that is in the balance. The validation of large scale clinical selection and depletion procedures is a tough one. At MCW we are getting ready to start doing a CD3 depletion protocol using an unnamed device. We actually got 50 buffy coats, and this took a long time, but we then did a density gradient separation to prepare mononuclear cells and ran them through the device so we would have a number of cells that at least came close to something we might do clinically. We were able to validate that we could indeed reduce the CD3 content to the level that we wanted, but there were no CD34+ cells in the product so we couldn't confirm that CD34 was going to be

recovered. This was a verification study, not a validation. This procedure is established in other laboratories, and in fact in the laboratory at the hospital we are going to be doing the study with, have done a number of these procedures. So by doing the buffy coats, we felt good that we could do the whole process and see good CD3 depletion. However, for the first product that we do we will do the flow analysis after the first selection to confirm the CD34 content and its preservation before we do any other part of the product. The products are almost always going to be split in this procedure, so we will not do the whole product at least for the first selection that we do. So it's difficult. Getting 50 buffy coats was tough so we did one study, but again it was a verification study, not a validation study. We certainly will hold off the second selection on the first clinical case to get flow results before we do another selection to complete the validation. So this is an example of one sample being tested, but we feel this is adequate because it is verification. If we were validating this device, say for a new antibody we wanted to use, then I would never take this into the clinic unless I had done a full-scale procedure. My own internal limit for brand new true validation studies is three. I would not ever put anything into place unless I had done it three times, and there are commercial places for a price that will sell you mobilized apheresis products, and if you are doing a true validation study then that is probably what you do need to do. You certainly do not want to put a procedure into place if you aren't sure that it is going to work.

Question 8: Is it sufficient for verification of a new test method to run samples simultaneously and then compare the data of an established method to the new method?

Answer: Yes it is. It is probably also a good idea. One of the nice things for some assays is that there are controls available, such as for flow assays, where there are cells with a known content of the subsets. And for a lot of other analytical assays you can get controls that have known content. Normally, I would make sure my controls have also been run in the test so there isn't any question that my current procedure truly is giving me correct answers and if I wanted to compare my new procedure against it I think that is acceptable.

Question 9: Is the testing of patient samples in real time using both methods sufficient to qualify the new method?

Answer: Whether it's an assay or whether it's a processing method that is what you do. You compare the new method to the method you are replacing it with. Most often this is what you do when you qualify something. For example, a new reagent source where you are more verifying than you are validating it. So frequently, that's what we do. We compare our new method against our old method. In some cases, the new method may be better than the old method, it maybe more sensitive or more reproducible. As long as it's as good as or better than the current method, then documenting that would be acceptable.

Question 10: Can you talk about the validation of the actual physical facility? For example, the laboratory rooms, the air filtration, temperature, and humidity? I am specifically thinking in terms of a new facility.

Answer: I am the lab director of a cell processing lab. I don't have a GMP facility. I could try to give you feedback on things I've heard at other talks, but facility validation is certainly not my area of expertise. I know some of the things they do, but I don't think I am really qualified to fully answer that. I know it's a complex process.