Dr. Paul Harrison Discusses Platelet Counting with the BD Accuri™ C6

Paul Harrison, PhD, is a scientist at the Churchill Hospital at Oxford University in the UK, president of the British Society of Haemostasis & Thrombosis, and a fellow of the Royal Society of Pathologists. His scientific research focuses on hemostasis and thrombosis with an emphasis on platelet biology and platelet function testing. Dr. Harrison spoke to us about his recent evaluation of the BD Accuri™ C6 flow cytometer for counting platelets.

Q: Can you tell us about the research in which you have used the BD Accuri C6?

Dr. Harrison: As a platelet biologist, I’m interested in many different aspects of hemostasis and thrombosis. In the Oxford Haemophilia and Thrombosis Center, we see patients with inherited and acquired platelet defects. We study their platelet function and elucidate the mechanism of their defects. Platelet counting plays an important part.

The problem in the field is that the majority of automated hematology analyzers are inaccurate at very low levels (thrombocytopenia). So, twelve years ago at University College London, we developed a new flow cytometry reference method. Because conventional flow cytometers don’t measure a given volume of liquid, we used the red blood cell/platelet ratio to accurately determine the platelet count. The ratio method was much more precise and accurate and became the new international reference method (IRM). The only problem is that the ratio method requires an [independent] RBC count. When I heard about the BD Accuri C6, I was interested in its ability to measure cell [concentrations] by direct volume. So we brought in the instrument for a student project to see if it could count platelets accurately.

Q: What were the findings?

Dr. Harrison: We initially showed that the ratio (reference) method on the BD Accuri C6 was equivalent to another flow cytometer I’ve had for a long time. And that’s exactly what you would expect. Providing you mix the samples properly, follow the protocol, and gate accurately on both platelets and red cells, you can determine a very accurate and precise platelet count.

When we compared the volume method with the reference method on the BD Accuri C6, it compared pretty well, although it wasn’t absolutely equivalent. In the samples with thrombocytopenia, the data suggested a slight bias.

But that is to be expected because of the nature of the test. The direct volume method is never going to be theoretically better than the ratio method. A ratio is always going to give the same ratio, irrespective of pipetting error, whereas with the direct volume method, you have to get the dilution and the amount of blood dead-on. My conclusion is that we can perform both the ratio method and the volume method on the BD Accuri C6.

Q: In what kinds of research would you feel confident using the direct volume method vs the reference method?

Dr. Harrison: In reality, we don’t use the IRM routinely in hematology blood samples. A reference method isn’t a routine method. We use it for research, for studying the accuracy of counters, and to calibrate our counters. The direct volume method isn’t quite as good as that, but it’s still reasonably accurate. It provides a rapid way of counting platelets in the physiological range right down to severe thrombocytopenic levels and probably beyond. So I would suggest that the direct volume method could be used in any platelet laboratory where researchers are interested in obtaining a reasonably accurate count quickly and easily, without the need for other equipment.

Q: What was your experience in using the BD Accuri C6?

Dr. Harrison: Nobody who walked into the lab could believe the size of the thing. They would say, “What’s that?” And I said, “It’s a flow cytometer.” And they said, “What?!” Its footprint is very, very small.
What’s really nice is that you can train somebody to use it—even if they’re totally inexperienced—very quickly. With a conventional cytometer, there are more variables to worry about—voltages, gains, and detectors. The immediate advantage of the BD Accuri C6 is that you don’t have to adjust any of those.

The student was an experienced hematologist, but he’d never done flow cytometry. So I showed him how to use the instrument, and he was away within about half an hour. We worked together evolving the method and establishing the threshold to eliminate instrument noise. Threshold and flow rate were really the only variables we had to worry about.

Q: Have you used the BD Accuri C6 as a teaching tool with others?

Dr. Harrison: It’s a wonderful training instrument. At the recent European platelet meeting in Maastricht, I ran the flow cytometry workshop with BD. We demonstrated the BD Accuri C6 to four different classes. In about 40 minutes, we were able to show them how to gate on platelets, how to count with the ratio and direct volume methods, and how to run other platelet assays.

We were very pleased with the instrument. As I say, when you first see it, you can’t believe it’s so compact. And its ease of use is fantastic, especially for inexperienced people. If I was running a flow cytometry lab and wanted a new instrument, this would be my instrument of choice.