

## **Sample Preservation - bloods**

About this time last year I wrote an article on Biobanking and the legislation that surrounds it. This is obviously only one aspect of the whole process. Equally important is ensuring that samples are collected and maintained in a high quality environment. How you do this probably depends on what you wish to do with them downstream and is somewhat unclear at present. What we as researchers need to ensure is that we give ourselves and others as much chance as possible to be able to use our precious stored archives of samples in the future. However and whatever we do needs to be stringently documented to enable this. The rest will be determined over time. Below I have listed some food for thought and some (hopefully) useful information that may help you decide on processing and storage of blood derivatives. Most of the information has been derived for proteomics work but we can use this to inform collection for other types of work.

### *Blood to serum / plasma*

One thing that has been known to be a problem in clinical testing for many years is the patient/subject themselves. How often do we think about patient posture, time of day, fasting vs. non-fasting, previous phlebotomy, concurrent other diseases or draw order? Probably not as often as we should. What are the actual effects of the disease we are studying (if we are) on the sample and is it due to some other parameter that we aren't interested in such as organ function or reduced numbers of circulating cells?

Our next source of variation comes from the make and composition of the tube that we take the blood into. It is known that polymers derived from different brands of plastics may cause ion suppression effects in some systems.

Next we need to address what we wish to store, whole blood, serum or plasma. These will all have their different collection criteria i.e. anti-coagulant vs. clot accelerator. Using a proteomics approach 80-90% of proteins/peptides found were common to both serum and plasma. However there are obvious notable differences in molecules involved in the coagulation cascade. It is the substances that show a difference between the collection methods that are of most interest. For example citrate and EDTA both chelate calcium but have marked differences in their proteomic profiles in certain systems. Heparin not only binds its target antithrombin III but also a variety of other proteins too. The type of clot activator used does not appear to have a big influence on the sample.

Time between sampling and processing can have large and variable effects on samples. Serum appears to be the most sensitive to this factor followed by EDTA-plasma>heparin-plasma>citrate-plasma. Virtually all of the changes appear in serum samples within the first 30min of venipuncture and peak by 60min whereas all of the changes in plasma happen after 4h. If samples are left for 24h before processing substantial changes can be observed. Citrated samples change very little but EDTA changes significantly. This is probably due to the greater stability of platelets in citrate. The question needs to be asked – do you want consistency between samples or capture the moment in time that the sample is taken?

Processing is also likely to have an effect on the composition of the samples i.e. spin speed, time and temperature. If samples are not spun fast enough then contamination with cellular material is likely. Additionally spinning of samples in cold conditions can lead to the activation of platelets.

Storage is another area of variability. There appears to be much more of a trend towards storing samples at -80°C currently, which is probably appropriate, but should we snap freeze? What about freeze thaw cycles, what truly is permissible and in terms of aliquoting what is practical? And how long should we be storing samples for?

The observation that the patterns in sera or plasma depend on how the sample has been collected, stored and assayed leads to a practical consideration in the coming worldwide effort to characterize the proteome of human blood. It has been suggested that one of the most important standards set by the Human Proteome Organization (HUPO) should be the standardisation of sample collection procedures. In terms of sera profiling for disease, standards should be set for time and temperature at which blood is clotted, the conditions of centrifugation, the time sera remains unfrozen, and how it is aliquoted, frozen, thawed, and used. Without such standards, it is apparent that it will be impossible to meaningfully compare the results obtained in one laboratory with those of another. It matters little which reasonable sample standard is adopted, as long as one is. What needs to be determined is whether as a community we decide to implement what is practical at all sites or limit our collection to only those sites that can provide high quality specimens.

Next month – Urine, DNA and Tissue

Best Wishes  
Dr Julie Williams