TECHNICAL NOTE

Lyophilisation (freeze drying) process for external INR and D-dimer quality assurance programme

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For almost 30 years, Waikato DHB has offered a quality assurance (QA) programme across numerous disciplines. Originally lyophilisation of internally sourced coagulation and microbiology QA samples was performed twice a year for the current year, at an approved external laboratory. However, it was observed that the integrity of the lyophilised product deteriorated over extended periods. As a result, our laboratory made the decision to purchase their own freeze dryer in 2014 to enable lyophilisation to be performed internally throughout the year.

Lyophilisation (freeze drying) is the removal of ice or other frozen solvents from a material through the process of sublimation and the elimination of bound water molecules through the process of desorption (1) Controlled freeze drying keeps the product temperature low enough during the process to avoid alterations in the dried product appearance and characteristics. It is an ideal method for preserving an extensive selection of heat-sensitive materials such as plasma, proteins, microbes, pharmaceuticals, and tissues.

The lyophilisation process is an ongoing process that takes several days to complete. The journey begins with our patient samples. Samples are categorised based on their INR and D-dimer results. We aim to get a variety of results. Pathlab Hamilton assists us by sending samples with high INR results. Samples with similar results are pooled together and frozen at -20°C. The serology results for each pool must be negative for HIV, hepatitis B and hepatitis C. Pools with positive immunology serology are discarded.

The lyophilisation process takes four days. Each day has a crucial process required to yield an optimal final product for the participants to receive. On day 1, individual pools are thawed and combined to make a 40 ml pool. Glycine, Hepes, and sucrose are added to the pool to help support the coagulation proteins during the lyophilisation process. This allows consistent coagulation results to be obtained post-lyophilisation (a pre-frozen sample is tested as a baseline). Vials are labelled with the appropriate survey number and 1 ml is pipetted into each vial. A rubber stopper is placed halfway on to each vial. This is purposely done to allow the air to escape. The samples will explode if the air is not released. The vials are placed in a polystyrene box for 24 hours at -70 degrees (Figure 1.)

Figure 1.

On Day 2 the freeze dryer is turned on and warmed up. Samples are transferred quickly to the freeze dryer. It is important that the samples do not thaw, as this will ultimately result in a failed run due to 'caramelisation' of the plasma. The pressure dropping should cause the icicles on the vials to flake off, this indicates a successful run. This process takes 24 hours (Figure 2).

Figure 2.

On Day 3 the pressure is further lowered for the final drying step. This gives adequate final vacuum pressure to eliminate all capillary or molecular bound water (1). Finally, on Day 4 the lyophilisation process is complete. The rubber stopper is pushed down to seal the vial. A vial from the run is selected and reconstituted to make sure the pre- and post-lyophilisation results are comparable. The vials are stored at -20°C until the dispatch date (Figure 3).

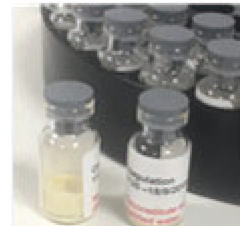

Figure 3.

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