Freeze-Drying COVID-19 Diagnostics: Formulation & Process Development and Lyophilizer Selection

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Lyophilization may be considered a relatively gentle drying process, but there are still risks and pitfalls when applying it to biomolecular reagents in diagnostic tests that are different to the freeze-drying of general pharmaceuticals.

Reagents typically used in ELISA and PCR based diagnostic kits for diseases, such as COVID-19 tend to contain labile components, such as enzymes that need preserving for longevity in the supply chain or antibodies to test for a patients response to the virus, which can be challenging to stabilize for commercial use.

Recently, Dr Kevin Ward, Director of Research and Development at Biopharma Group, UK presented a webinar exploring various aspects of formulation and cycle development in the lyophilization of diagnostic reagents, including those required for COVID-19 testing that may be different to regular diagnostics. This tech note summarizes the webinar and includes a selection of questions from the Q&A session.

Lyophilization of Biomolecular Diagnostic Reagents

Different molecules exhibit different sensitivities to processing and storage conditions and, in the case of biomolecules, not only does the molecule need to remain chemically intact during the freeze-drying process, but it is also necessary for the 3D structure to be maintained. The goal is to minimize the degradation of activity that can be seen due to factors such as aggregation and denaturation.

There are additional challenges with biomolecule-based diagnostics, in particular for COVID-19 that are less obvious than when freeze-drying other molecules; these are discussed in the webinar and summarized below:

Low-volume Diagnostics

Diagnostic kits tend to use small volumes of reagents that can evaporate quickly. While a few microliters of these reagents in tubes may evaporate less, samples on plates or in microfluidic channels that are exposed throughout loading are more susceptible to evaporation. With the urgency of testing for COVID-19, high throughput is essential and therefore many COVID-19 diagnostic kits take place in 96-well plates, chips or microfluidic channels. This problem can be partly overcome by loading into a cooled freeze dryer.

Stabilization of Biomolecules

Although most biomolecular components (e.g. antibodies and enzymes) are not sensitive to conditions of freeze-drying there are some that are, and it is worth noting what effects these may cause. A sensitivity to cold denaturation can cause aggregation or precipitation of proteins but this may be solved by cooling slowly or adding a surfactant. It is also possible that some components are damaged by freeze concentration effects that can also be

SP VirTis Ultra Pilot and Small Production Freeze Dryer

The low moisture load of diagnostic kits allows the use of compact and efficient 'pilot' freeze-drying equipment with large product shelf capacity to floor ratio. The Ultra capacity can easily handle up to 5,000ea 2mL vials or 144ea 96-well plates.

SP VirTis Benchmark Production Freeze Dryer

VirTis Benchmark production freeze dryers are designed for larger batch sizes of diagnostic products. Round product chambers are typical and internal ice condenser designs can help vapor flow for low-Tc diagnostic products being dried at very low shelf temperatures. Shelf capacity ranges from 2m^2 up to 20m^2.

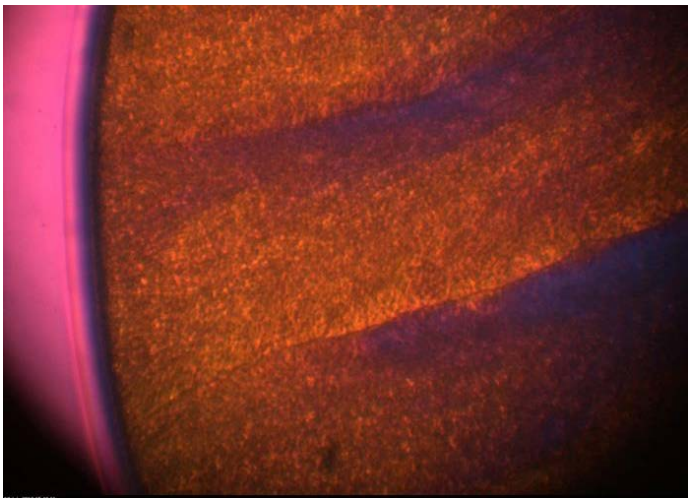
accompanied by a pH shift. Phosphate buffer can be the main culprit of this and a change of buffer or cooling quickly can often remedy this problem. Proteins can also be susceptible to interfacial effects that may be proportional to the surface area of ice crystals, but these effects can often be combated by cooling slowly or using controlled nucleation to increase the size of the crystals and reduce the surface area. Dehydration stresses can destabilize a protein. In some cases, structural water is required for the structure to be maintained; the addition of cryo- or lyo- protectants can alleviate this and avoid dehydration stresses by immobilizing proteins and/or mimicking the action of structural water.

Glycerol Effect

The presence of glycerol in PCR reagents (Fig 1) causes significant problems because it does not freeze under typical freezing conditions. This can be solved by diluting the glycerol out if a high enough stock solution is available or adding an excipient. However, there may still be glycerol in the dried cake which can impact stability and structure. In the case of the COVID-19 diagnostic kits, the shelf life may not be as essential as other diagnostic kits due to their requirement to be stored for only a few months at a time. They can also be stored at low temperatures so the presence of glycerol will have less effect on the stability of the active components.

Containers and Closures

The format of containers (e.g. vials, tubes, wells, chips, paper etc.) and closures has a significant long- or short- term effect of thermal transfer and stability. Even though there is a challenge to seal a container without letting any moisture in, it is worth noting some plates are more permeable than their seals to water (Fig 2). Dr Ward mentions the use of a controlled environment with reduced oxygen, low humidity conditions and using a flexible isolator to reduce the moisture levels in the product. For COVID-19 testing, initial performance and rapid throughput may be more important than long-term stability and therefore some of these measures may not be necessary.



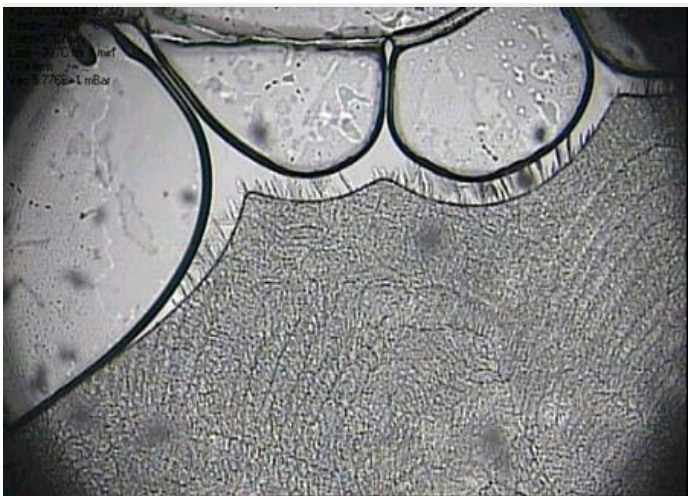


Figure 1 *Presence of Glycerol in PCR reagents*

Freeze-drying Equipment

As most companies are maximizing throughput over long-term stability for COVID-19 diagnostic kits, smaller pilot dryers may be adequate for freeze-drying the components of the diagnostic tests. A typical pilot freeze dryer, a SP VirTis Ultra (SP Scientific Products) can freeze dry up to 5,000 x 2 mL vials, which is high enough output for most diagnostics. Larger freeze dryers that are designed for the pharma industry have additional functions not required for diagnostics, for example steam sterilization.

Summary

It is important to adopt a freeze-drying strategy for diagnostic biomolecular components according to the particular sensitivity of the molecule in question, being mindful of the issues discussed above, but also taking into consideration that all the normal rules apply as for the freeze-drying of any other diagnostics. In the case of a COVID-19 diagnostic test, reagents will likely be processed in small fill volumes but high batch numbers and the priority is focused on throughput rather than long-term stability; therefore this needs to be considered when designing a lyophilization protocol specifically for a COVID-19 diagnostic test.

To view the full webinar and download the slides, please go to the archived webinars on our website https://www.spscientific.com/Webinars/Archives/.

Figure 2 *Sealing issues*