Lyophilization: The Future of Molecular Diagnostics

It's no surprise that the need for molecular diagnostic assays is growing at an unprecedented rate. With new biomarkers being discovered and standard catalog reagents becoming more accessible, diagnostic developers and manufacturers can create molecular tests faster than ever before.

Still, there are challenges to overcome. Standard catalog reagents, most commonly formulated with glycerol and other additives for stability in wet chemistry format, require cold-chain handling and storage. Additionally, reaction components (e.g., master mix and oligonucleotides) are typically shipped and stored separately, requiring multiple mixing steps at the point of use in laboratory settings to achieve the desired functionality. If reagents are not handled, stored, or used properly, molecular diagnostic tests could result in false positive or false negative results. Minimizing storage and handling requirements of reagents can maximize the efficiency and accuracy of the test. The COVID-19 pandemic in particular has shown just how important it is to effectively diagnose and make decisions at the point-of-care.[1]

Diagnostic assay developers may design assays using standard catalog reagents available in wet chemistry format because they are widely available, easy to source, and simple to combine with other components in a laboratory setting for optimization. Although wet chemistry is the traditionally used format, some reagents are not stable at ambient temperatures long-term and therefore need cold-chain shipping and handling, adding risk and cost to logistics associated with sourcing the reagents for the test. In addition, laboratory capital equipment investments are needed in the form of freezers or refrigerators. Investing in freezer space may restrict not only the number of reagents that can be stored, but also limit the number of diagnostic tests clinical laboratories can run.[2]

To combat these hurdles, scientists are turning to lyophilized solutions, a centuries-old technique that is on the rise. The global freeze-drying/lyophilization equipment market is projected to reach USD 7.3 billion by 2031 from USD 4.9 billion in 2020, at a CAGR of 8.2% from 2022 to 2031. The rapid growth in contract manufacturing and lyophilization services across the world, rising demand for lyophilized products (especially for the manufacturing of new biologics and injectable formulations), and technological advancements in lyophilization methods are the major factors driving the growth of this market.[3]

In this white paper, we discuss the role lyophilization plays in aiding molecular diagnostic companies in their quest to provide products that remain stable and effective when stored in ambient conditions, and don't require the need for extensive freezer space or cold chain logistics. We also highlight the importance of working with a partner from the beginning of your development process through to commercialization to ensure that diagnostic tests are reliable and efficient for accurate patient diagnoses.

Lyophilization: A Promising Solution

Lyophilization is a process where enzymes or reagents are freeze-dried into solid, stable particles. This process is widely used to preserve a variety of materials, including food, pharmaceuticals, and biological samples. A major advantage in molecular diagnostic applications is that the enzymes can be lyophilized, and in many cases, in combination with the necessary buffer components and/or oligonucleotides (e.g., primers and probes) necessary to run the test, thereby completely eliminating any mixing or handling steps at the point-of-use. Samples and their associated target nucleic acids can simply be used to rehydrate the freeze-dried reagents and the test is ready to run.

There are four steps in the lyophilization cycle:[4]

1. **Freezing**: Creates the ice matrix that holds the substance and other excipients. The material to be freeze-dried is frozen to a temperature below its eutectic point. This helps preserve the integrity of the sample by ensuring that all molecules are in a stable state.

2. **Evacuation**: Reduces pressure below the vapor pressure of ice. A vacuum is used to reduce the pressure in the lyophilizer to below the triple point of water.

3. **Primary Drying**: Adds heat to drive the sublimation of water vapor from the ice onto the freeze dryer condenser coils. This step removes any moisture that is present in the sample, which can interfere with the testing process.

4. **Secondary Drying**: Adds heat to remove residual moisture.

Together, these four steps help to ensure that the lyophilized products are consistent and reliable.

The Many Benefits of Lyophilization

Unlike wet reagents, lyophilized products are solid and stable until resuspended. This provides a solution that addresses some of the challenges seen in molecular diagnostics.

The stability of lyophilized products translates to increased shelf life at ambient conditions and cost savings as diagnostic manufacturers and laboratories no longer have to pay for cold chain handling, shipping, delivery, and freezer space.[2] It also helps improve lab workflow efficiency by having reagents preserved indefinitely after receipt, minimizing wait periods before use (e.g., needing to thaw a frozen set of reagents prior to use).[2]

Another hidden benefit of lyophilization is its positive impact on the environment. With reduced contamination from toxic refrigerants and coolants, elimination of dry ice minimized packaging, and decreased energy use, environmental footprint can be reduced.[2]

And when it comes to cost, lyophilization can help reduce expenses. Given that lyophilized products are stable at room temperature; electrical, space and equipment costs can be reduced by eliminating the need for freezers and refrigerators. According to the Healthcare Distribution Alliance (HDA), 49% of warehouse volume is used to store refrigerated or frozen products[1], so removing cold storage needs significantly reduces warehouse requirements.[5]

While the cost savings from room temperature storage are significant, those attributed to cutting cold chain shipping logistics may be even greater. In 2018, the pharmaceutical cold chain logistics industry was valued at over $15 billion and is predicted to expand to $18 billion by 2022.[1] Of that $15 billion, $10.6 billion is transportation costs and $4.4 billion is packaging related. Consider how much money is wasted by cold chain packaging. Over-packing wastes resources and fuel, and adds to transportation costs, while under-packing leads to a damaged or spoiled product that must be replaced.[2] Using lyophilization to replace your cold chain with products that can ship at room temperature can dramatically bring down costs across multiple categories.[5]

Lyophilization May Help Make Point-of-Care Diagnostics More Accessible

For point-of-care diagnostics to be successful, tests need to be portable, simple to use, accurate, and durable, allowing the test to be performed in a variety of point-of-use locations: a local pharmacy, a rural hospital, or a remote village, far from the clinic. Historically, machines and reagents used in most molecular diagnostics meet few if any of those criteria.[1]

Furthermore, the COVID-19 pandemic has strained the infrastructure for manufacturing and delivering molecular diagnostics around the globe. Material shortages, limited amount of testing facilities, lengthy times to provide results to patients, and both cost and logistics associated with rapid testing scale-up all pose challenges to the success of established clinical diagnostic methods for detecting viral infections. For most molecular diagnostic tests, especially in a high-complexity and high-

throughput setting, polymerase-chain-reaction (PCR), or in the case of COVID-19: reverse-transcription (RT)-PCR, is the basic technology responsible for the test. However, to run such diagnostics reliably, clinical labs need a PCR machine for thermocycling and analysis, along with a stock of enzymes and reagents. While PCR machines have become more compact and simplified over time, most PCR enzymes and reagents remain in glycerol, making storage and handling conditions difficult.[1]

To alleviate these challenges, diagnostic tests need to be easier to use and inexpensive to send to any type of location. This is where lyophilization can play a major role. The ability to distribute RT-qPCR tests without relying on cold-chain storage during shipping is through freeze drying the reagents and enzymes.[6]

Fortis Life Sciences formulates enzymes, master mixes, and other reagents required for nucleic acid amplification to be lyo-compatible, ensuring functionality of lyo-ready products are equivalent or better to the performance of wet chemistry format.

Key Considerations for Successful Lyophilization

Most developers of molecular diagnostics products are experts at identifying the functional requirements of their assay or the performance of their platform but manufacturing the assay components in a lyophilized format may not be as trivial of a process. Our experience shows that there is no magical "one size fits all" approach for creating a reliable and robust lyophilized assay for a particular application. You need knowledge and expertise at every step.

For successful lyophilization, it's critical to have an understanding of the biology and chemistry of the reagents, the physics underlying the process, and how these can impact assay performance.[2,7]

When developing a new lyophilization product, several key parameters need to be considered to achieve a successful outcome. The product to be lyophilized, its formulation, and the container closure system and device design, and assay workflow all need to be taken into account. It is also important to consider the desired shelf life of the product and degradation that may occur during storage. The freeze-drying process is also a critical parameter that needs to be optimized in order to achieve the desired outcome. By taking all of these factors into consideration, it is possible to develop a final product that meets the specific needs of the product being processed.

Controlling the manufacturing processes to ensure the enzymes and reagents are compatible with lyophilization is also necessary for success. Specific components that are often present in off-the-shelf wet chemistry format, particularly glycerol, must be removed from the manufacturing process to make it compatible with lyophilization. Having an understanding of the following variables in the end application is critical to successfully making reagents lyo-compatible:

1. Source and format of the nucleic acid template (e.g. is it a crude lysate from saliva or an extracted/purified sample)

2. Compatibility and sensitivity of or to certain sales and buffer conditions

3. Device spatial/volume limitations

4. Concentration of and formulation of the enzymes

Designing the device and test with lyophilization compatibility in mind at the onset of the project is likely to lead to a more cost-effective and successful project. In many cases, initial optimization is performed with standard catalog wet reagents and then attempting to re-engineer the processes and components to be lyophilizable.[3] This can lead to a significant amount of wasted time and money trying to re-engineer the device or reagents; time that could have been saved by considering the lyophilized format from the beginning and working within those boundaries.

The Importance of Having a CDMO Partner

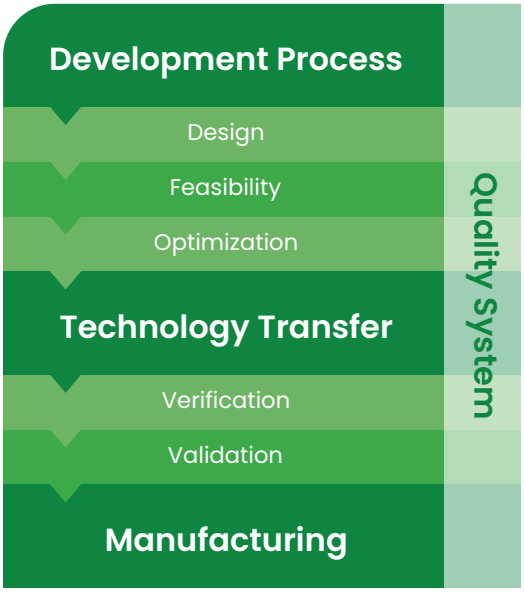
Despite the clear benefits of lyophilization, it can be challenging to build these capabilities in-house. You not only have to select the right excipients to stabilize active reagents, but also need to purchase specialized equipment and have the right expertise.[7] And even if a lab is successful, there may still be

robustness issues as well as the ability to scale and launch a product.

At Fortis, we recognize the advantages of having lyophilized reagents and assays. For this reason, lyophilization compatibility is a design input for all of our polymerases, enzymes and reagents from the start of each new project.

We work closely with our clients and their preferred lyophilization partner to ensure that we understand their device, constraints, and desired end results, so that we can manufacture a custom product to meet those requirements.

We strive to take a collaborative approach. This custom product development cycle begins with a consultation with our team of expert scientists; and one of the key questions we ask is whether the product will be needed in lyophilized format. Understanding the assay requirements early on is key for success.



Communication is also critical in any collaborative process, and we work hard to keep our customers informed. We are a data and systems driven organization, and we use these, along with collaborative input guided by our customers' needs, to establish the necessary protocols and quality control to ensure the product specifications are met.

At Fortis, we ensure that all our custom lyophilized enzymes or master mixes are:

- Capable of being stabilized in lyophilized or air-dried format
- Unaffected in terms of performance post rehydration and reactivation
- Developed and manufactured under our ISO:13485 quality management system from the start

Conclusion

As the access to molecular diagnostics increases, so too does the number of people who can benefit from them. Lyophilization is a groundbreaking technology that is opening up new molecular diagnostic possibilities for clinical laboratory scientists, point-of-care clinicians, and common people using at-home tests. . Lyophilization increases the number of tests that can be run in clinical labs previously restricted by capital equipment and logistics infrastructure or facilities. It provides clinicians a way to make fast bedside point-of-care decisions, and it enables people to know the status of their health and wellbeing in the comfort of their own home.

For more information, visit fortislife.com

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