



Dry Reagent Storage

Solving Key Challenges in
Dry Reagent Integration for
High-Performance Microfluidics



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ABSTRACT

Microfluidic cartridge applications are attracting a rapidly growing interest in a wide variety of diagnostic and research fields. This growth is driven by the advantages these cartridges provide, such as enabling use by non-trained or non-laboratory personnel, automating assay runs with minimal hands-on time, and delivering quick results when time matters.

However, many conditions must be met, and thorough requirement analysis is essential when developing a new microfluidic cartridge. Integrating reagents, particularly dried reagents,

is a critical factor that requires dedicated design work and consideration of many factors, not only for storage but also for ensuring precise assay performance.

While commonly used standard processes exist, they have disadvantages that can increase manufacturing time, overall costs, and cartridge production complexity. Fortunately, specific solutions, such as our Reagent Plug and our more specialized Elasto Plug, are available to address these issues, specifically for lyophilized pellets.

INTRODUCTION

Microfluidics-based cartridges play a vital role in the In Vitro Diagnostics (IVD) market, particularly in clinical chemistry, immunology, and hematology applications. In the past decade, however, microfluidics has gained traction in the molecular diagnostics and clinical microbiology sectors. The COVID-19 pandemic significantly accelerated the development of rapid Point-of-Need/Point-of-Care (PON/POC) systems for molecular diagnostics, while healthcare challenges such as global Antimicrobial Resistance (AMR), often referred to as the 'silent pandemic,' offer new opportunities for technological innovations in microfluidics. Although high levels of integration are essential for platforms serving these applications, even droplet-fluidics-based cartridges are advancing toward greater integration, particularly concerning reagents. Given the potential for future disease outbreaks or quality monitoring in biopharmaceutical production process, emerging opportunities like wastewater surveillance may create additional future prospects.

In the PON/POC market segment, microfluidic cartridges aim to provide ready-to-use solutions for complex assays while ensuring user-friendliness. These cartridges enable testing by non-laboratory or non-trained personnel and require minimal user interaction. In addition to application-specific functional and fluidic circuit control elements, all necessary reagents

are stored directly on the cartridge. The only user interactions needed are sample transfer and inserting the cartridge into a dedicated instrument to run the assay.

One of the main challenges in developing a microfluidic cartridge is the storage of reagents, which is particularly critical during high-volume production. When examining specifications for storing dry reagents, various factors and conditions must be considered, including drying processes, cartridge assembly requirements and processes, and environmental aspects related to the production infrastructure.

This White Paper focuses on integration concepts for dry reagents in microfluidic cartridges. It will outline the challenges and present our specific technical solutions for overcoming them.



PRODUCT DEVELOPMENT: THE DRY REAGENT INTEGRATION CHALLENGE

The integration of dried reagents into microfluidic cartridges presents a multi-faceted challenge, requiring solutions that address both reagent stability and consistent assay performance. As illustrated, these challenges span environmental sensitivity, reconstitution dynamics, and design considerations, all of which must be carefully balanced to achieve a scalable, ready-to-use cartridge solution.

01 USER-FRIENDLY DESIGN

Many cartridges are intended for use by non-trained or non-laboratory personnel, asking for a minimum of user interactions.

- › All reagents must be stored on the cartridge, making it ready-to-use, consistent, and safe.

02 REAGENT STORAGE

To maintain reagent stability within the cartridge (shelf life) and to avoid costs associated with temperature management during storage, reagent drying is a common practice.

03 DIVERSE DRYING NEEDS

One cartridge may hold a variety of different reagents — each with its specific requirements for drying processes (oven-/air-drying, lyophilization).

04 ENVIRONMENTAL FACTORS

Dried reagent handling and integration are challenging due to:

- › Sensitivity to environmental factors (humidity, temperature, and light).
- › Reconstitution performance in the microfluidic circuit: the process must be fast, homogeneous, and free from outgassing or de-bubbling.

05 SHELF-LIFE OPTIMIZATION

The cartridge design must reflect the storage conditions for reagent stability over the targeted shelf life.

06 PACKAGING SPECIFICATIONS

The packaging concept must meet the specifications for reagent stability throughout the shelf life.



SETTING THE RIGHT BASIS IN PRODUCT DEVELOPMENT – REQUIREMENT ANALYSIS

When developing a new microfluidic cartridge, the primary goal is to design a high-quality, cost-competitive product to ensure marketability. Alongside quality and economic considerations, the assay itself — with its specifications, requirements, and related production infrastructure — serves as the main design driver.

In general, all these factors will influence the design and vice versa. Therefore, conducting a thorough assay requirement analysis to establish the foundation for product design and developing a sustainable production concept is crucial for achieving repeatable cartridge-to-cartridge assay performance in the final product. When dry reagents are involved, handling and storage requirements can become quite demanding. The most common requirements for cartridges containing dried reagents, based on the aforementioned categories, are outlined as follows.

QUALITY

- ▶ **Storage:** Storage (up to 2 years) may not impact the product/reagent quality
- ▶ **Safety:** The product must not be a potential safety risk for users
- ▶ **Packaging & Transportation:** The design must ensure that neither the reagent nor the product itself will be damaged

ECONOMICS

- ▶ **Low-cost:** Low-cost product while maintaining high quality
- ▶ **Scalability:** Scalability of the product design to enable high-volume production
- ▶ **Standardization:** Apply generic design elements and processes to limit the need for dedicated, specialized equipment

PRODUCTION

- › **Light:** Some reagents are sensitive to light exposure (e.g., fluorophores); providing a shield is important
- › **Humidity:** Sensitivity to humidity, controlled conditions for handling dried reagents needed
- › **Temperature:** Temperature sensitivity, controlled temperature in production and storage area needed
- › **Particles:** Avoid particle contamination; a cleanroom environment is needed
- › **Cleanliness:** No contamination of the product allowed (e.g., Nucleic Acids, DNases/RNases, cross-contamination of cartridge reagents, bioburden, etc.)

ASSAY PERFORMANCE

- › **Consistency:** No intra-operator variations in performance and results
- › **Repeatability:** Test and retest performance is essential.
- › **Reconstitution:** No residues/full reconstitution, no loss of dried reagents before or during the reconstitution process
- › **Reagent Concentration:** Repeatable reconstitution performance in each cartridge
- › **Reagent Volume:** The reagent concentration may not vary from cartridge to cartridge, asking for a steady and comparable liquid volume
- › **Reagent cross-contamination:** Reagents used in the cartridge may impact the reagents for downstream assay steps due to carry-over, which must be considered in the fluidic circuit layout



ON-CHIP STORAGE OF DRIED REAGENTS

While widely used solutions like blisters are available for integrating wet reagents, the integration of dried reagents remains limited in terms of technical concepts, and there is no standardized solution. When trading off common integration concepts for reagents used in microfluidic products against specific requirements, scalability, and cost implications, several aspects still need to be tackled with innovative technical solutions.

COMMON METHODS TODAY

The most common practices for integrating dried reagents into microfluidic cartridges include:

ON-CARTRIDGE DRYING

Reagent Drying Directly within the Cartridge

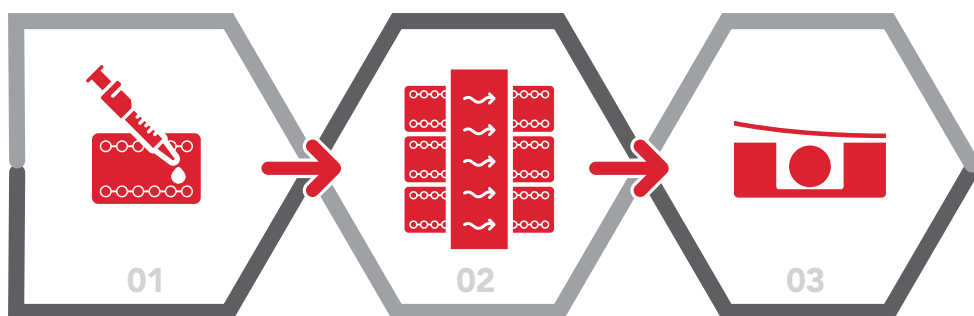
PICK & PLACE PELLETS

Pick and Place of Dried Reagent Pellets (Mostly Lyophilized)



ON-CHIP REAGENT DRYING

To dry reagents directly within the cartridge, the liquid reagent must be applied to a designated area. This can be accomplished using automated pipetting, spotting robots, or spraying techniques. Following this step, the reagent and the entire microfluidic cartridge undergo a drying process, which may include lyophilization, air drying, or oven drying at elevated temperatures.



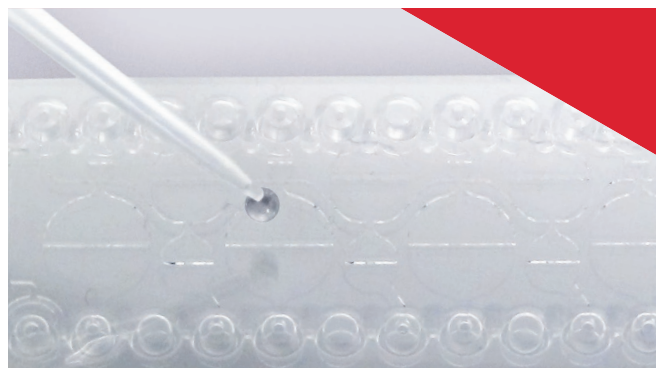
Example sequence of direct reagent application and drying.

SCHEME REAGENT DRYING

- 01** Liquid reagent is pipetted into the cartridge via backside (no cover film applied)
- 02** A few cartridges undergo a drying process in the oven, or similar
- 03** After drying, a cover film is applied

While this method allows for precise positioning of the reagent, it has significant disadvantages. First, it requires special equipment for localized pipetting, spotting, or spraying, and the production steps can be time-consuming. Additionally, drying the reagents directly within the cartridge presents several drawbacks, which can lead to increased time and cost. To begin with, the entire cartridge must be handled during the drying process, affecting spacing

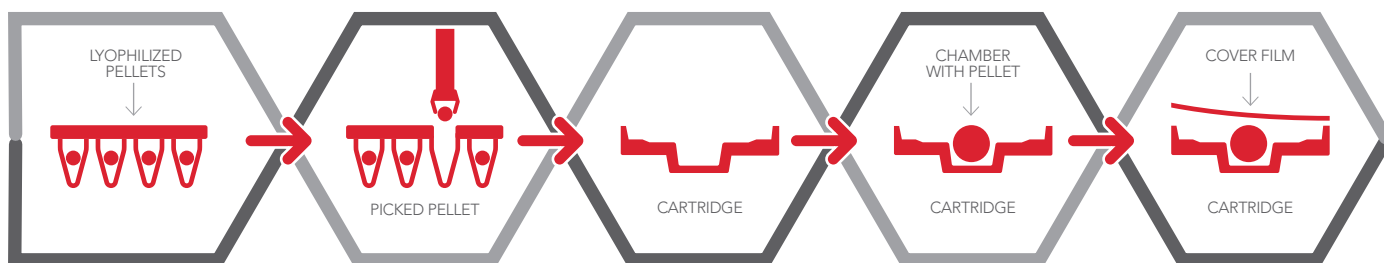
and throughput. Furthermore, drying conditions must be uniform for all reagents on the cartridge. Also, integrated reagents must pass through all subsequent manufacturing steps, posing a risk to their functionality. Finally, the production infrastructure must satisfy essential environmental conditions such as controlled humidity, light exposure, and temperature, further impacting production infrastructure complexity and ultimately increasing the cost of goods.



Reagent being pipetted into a cartridge.

PICK & PLACE OF LYOPHILIZED REAGENT PELLETS

LyoBeads/Spheres are typically produced by dispensing a defined volume of liquid reagent into liquid nitrogen, followed by a lyophilization step. Lyophilization is a low-temperature process beneficial for temperature-sensitive reagents, such as enzymes or antibodies. The industrial standard primarily uses spherical LyoBeads in microfluidic applications, but the limited spacing and dimensions in cartridges have prompted contract manufacturers to explore flexible shapes for Lyocakes (hemispherical, disk, etc.).



Example sequence of a pick and place process for lyophilized reagents.

Generally, lyophilization as an industrial batch process, separates the drying process from cartridge manufacturing, improving manufacturing throughput and reducing the time spent on reagent drying. This process also enables the use of industrial pick-and-place mechanisms to transfer LyoBeads to the cartridge.

SCHEME PICK & PLACE PELLET:

- ▶ Liquid reagents droplets are freeze-dried
- ▶ The pellets are picked and placed into the cartridge
- ▶ A cover film is then applied

However, this process still requires dedicated drying equipment and a precise pick-and-place

mechanism tailored to the shape and texture of the LyoBeads. Environmental conditions such as temperature, humidity, and light exposure may also be critical. Since lyophilized reagents can be fragile and porous, careful handling during the pick-and-place transfer is essential. Additionally, securely storing the pellets within the cartridge requires special design features to prevent them from dislocating or getting damaged during transportation and handling.



Cartridge chamber with and without pellet.

DRYING ON A CARTRIDGE

- › Reagent applied at a dedicated position
- › No separate interface for reagent integration is required
- › Drying is an integral part of the manufacturing process; the production line needs to implement related processes and equipment
- › Complete cartridge experiences drying process; materials and components need to comply with drying conditions
- › Impact on timing and costs (cycle time per cartridge, spacing)
- › Physical environment and operation conditions must be respected downstream of reagent integration (a risk that downstream processes negatively impact the reagents)

LYOBEADS/SPHERES/CAKES

- › Drying process can be done off-line, with no need for integral processes and equipment in the production line
- › Benefits from industrial standards for reagent drying as well as integration via pick and place
- › Throughput in the drying process
- › Reconstitution properties may benefit from the porosity of LyoBeads
- › Need for a dedicated interface for reagent integration to fully exploit advantages of off-line reagent drying
- › Lyophilized reagents may be more challenging with respect of handling (texture, shape, stability), trade-off between stability and reconstitution
- › Compliance of LyoBead size with microfluidic circuit dimensions, risk of dead volume zones
- › Physical environment and operation conditions must be respected downstream of reagent integration

Although established methods for integrating dried reagents are widely used, they have significant disadvantages, as outlined in the table above. While LyoBeads or reagent pellets generated using alternative drying techniques offer clear advantages over on-chip reagent drying, these benefits can only be fully achieved if the cartridge is designed with the appropriate interface for reagent integration. This design must allow for the integration of reagents at a stage in the manufacturing process where subsequent procedures do not affect the LyoBeads, ensuring their functionality — both biologically and fluidically — is maintained during reconstitution. Ideally, this interface would facilitate the transfer of LyoBeads at the very end of the assembly process.

OUR SOLUTION: THINXXS REAGENT PLUG PLATFORM

To address the challenges associated with efficient, high-throughput drying of reagents and handling of the dried reagent pellets during manufacturing, thinXXS has developed our **Reagent Plug** (RP) Platform. This platform serves as a carrier for reagents being dried offline, simplifying and improving processes and cycle times, particularly in high-volume production.

We also introduce a new enhancement to the platform, the **Elasto Plug**. This new plug type represents an advanced design variant that extends the range of RP applications and ensures compatibility with the industrial LyoBead standard. Both our Reagent Plug and Elasto Plug are presented below.

Our RP concept was developed to tackle the most critical challenges of implementing dry reagent integration in volume production, which include:

- ▶ Throughput and cycle times
- ▶ Material and process compatibility
- ▶ Manufacturing line complexity
- ▶ Operating conditions & production infrastructure
- ▶ Maintenance of reagent functionality
- ▶ Reagent multiplexing capabilities (handling different reagents and drying procedures)
- ▶ Costs of goods sold COGS impact

Additionally, we aimed to develop a concept with maximum flexibility to enable robust cartridge platform capabilities, allowing a single cartridge design to support multiple assay panels. This initiative led to further requirements, including:

- ▶ A generic reagent integration interface to establish standard interface designs and processes
- ▶ The ability to integrate reagents at the very end of the assembly process chain
- ▶ The enablement of 'raw card' manufacturing, where dedicated cartridges for each panel can be created from the stock of raw cards by integrating panel-specific reagents

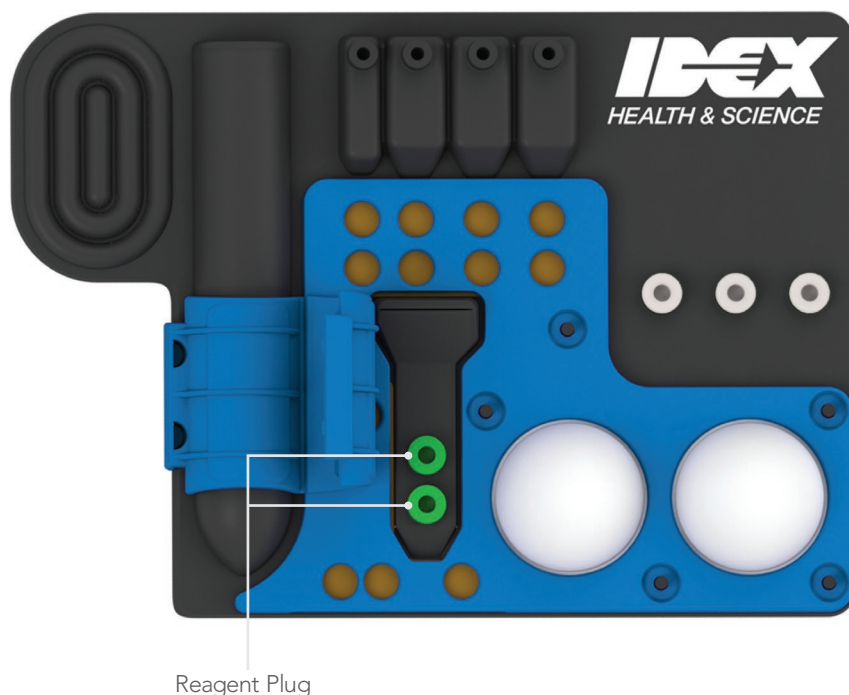


REAGENT PLUG

Our Reagent Plug is made from a rigid polymer that serves as both a platform for drying liquid reagents and a carrier for integrating the dried reagent into the microfluidic cartridge through a dedicated interface known as the RP port. The process begins with pipetting the liquid reagent onto the RP for drying. After it's dried, the reagent is inserted into the microfluidic cartridge. For more information, please refer to our white paper on Reagent Storage on Microfluidic Cartridges at <https://www.thinxxs.com/en/downloads>

Our RP platform effectively addresses various challenges and basic requirements, including:

- Precise, leak-tight integration of reagents
- Material and design flexibility for adapting the RP to assay-specific reagent needs and properties
- The option to stock dried reagents for on-demand cartridge production based on panel forecasts
- Compatibility with standard pick and place workflows





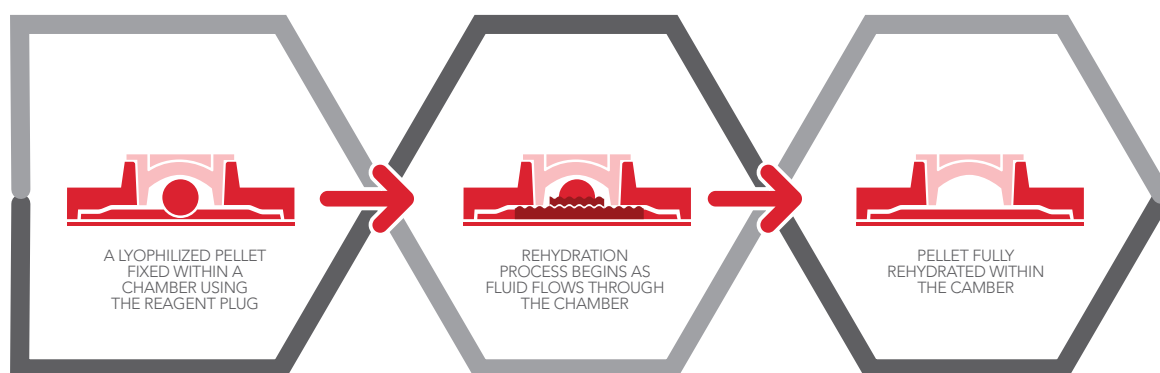
With our Reagent Plug, customized drying processes can be achieved, and off-the-shelf dried reagent pellets can be integrated. In this scenario, the generic cartridge interface serves as a loading chamber, while the RP secures the pellet during cartridge transport and handling, ensuring a fluidic seal for the interface.

Additionally, our system allows for quick and easy standardized integration. The pellet can be inserted into low-volume chambers, enabling rehydration with only a few microliters — a feature often limited in most existing design solutions.

SCHEME FOR LYOBEAD WITH REAGENT PLUG (RP):

- ▶ A lyophilized pellet fixed within a chamber using the Reagent Plug
- ▶ Rehydration process begins as fluid flows through the chamber
- ▶ Pellet fully rehydrated within the chamber

The Reagent Plug has limitations when it comes to larger diameter reagent pellets, particularly LyoBeads, which usually measure 2–3 mm in diameter. While it is possible to incorporate LyoBeads by adapting the RP geometry — the design of the front end that interfaces with the pellet — there is an increased risk of creating dead volume zones once the pellet is resuspended. Design considerations must always account for pellet size and the corresponding plug geometry, especially in low-volume applications. To address this issue, we developed a new variant of the Reagent Plug: the Elasto Plug (page 15).



Rehydration of a pellet with the Reagent Plug.

REAGENT PLUG (FOR REAGENTS DRIED ON RP SURFACE)

- › Standardized carrier and interface for integrating dried reagents
- › Drying process is separate from cartridge manufacturing, allowing for reagent stock options
- › The size of the RP can be scaled according to the reagent volumes during drying
- › Standard pick and place process for insertion
- › Standardized MTP format is used for handling, independent of RP design
- › Reagent remains in place
- › Supports platform capabilities (one cartridge – multiple panels)

HOWEVER, THERE ARE SOME CONSIDERATIONS:

- › The geometry of the RP may limit the volume of reagents that can be dried after the cartridge and RP interface design is fixed
- › While the RP size can be scaled to handle higher reagent volumes or dried pellet sizes, this may impact fluidic performance due to increased dead volume



REAGENT PLUG (USED WITH LYOBeads/ DRIED PELLETS)

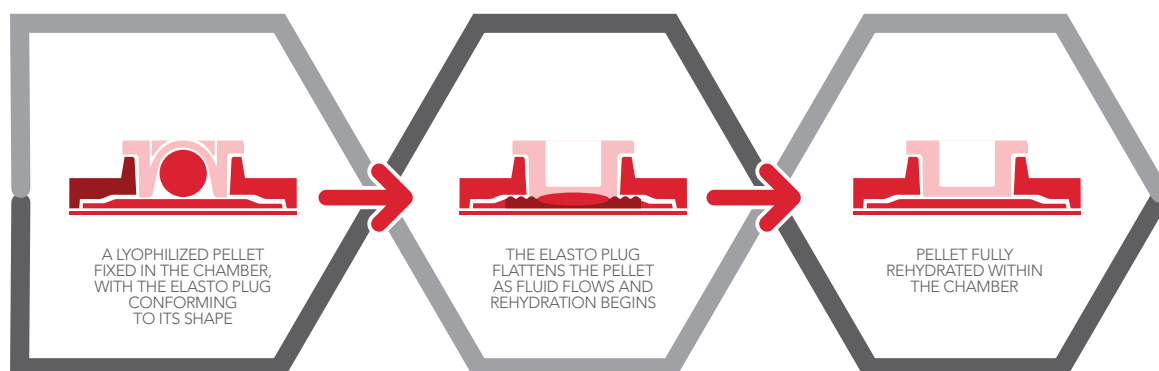
- › Standardized interface component for integrating dried reagents (Reagent Plug + port on cartridge)
- › Drying process is separate from cartridge manufacturing, allowing for the option of reagent stock
- › Option to scale the size of the Reagent Plug according to LyoBead/dried pellet size
- › Standard pick and place process for insertion (LyoBead/pellet + Reagent Plug)
- › Reagent remains securely in place
- › Standardized MTP format approach for handling, independent of RP design
- › Supports platform capabilities (one cartridge – multiple panels)

CONSIDERATIONS:

- › The geometry of the Reagent Plug may restrict adjustments to the dimensions of the LyoBead/dried pellets once the design of the cartridge and Reagent Plug interface is finalized
- › While the size can be adjusted to fit various LyoBead/dried pellet sizes, the resulting Reagent Plug geometry may affect fluidic performance, such as dead volume

ELASTO PLUG

While the Reagent Plug works as a reagent carrier, the Elasto Plug is designed specifically for securing and handling lyophilized reagent pellets in microfluidic cartridges.



Rehydration of a pellet with the Elasto Plug.

The Elasto Plug is crafted from a thermoplastic material, providing flexibility while ensuring stability for handling during production processes. Thanks to its material properties, the surface of the plug interfaces effectively with the reagent pellet. This allows the microfluidic chamber to be deflected mechanically or pneumatically, enabling a wide range of applications.

The implementation of lyophilized pellets is now more straightforward. The Elasto plug encloses the pellet and conforms to its shape. During rehydration, the surface of the plug returns to its original, less domed shape or can be deflected to create a completely flat surface. This significantly reduces the risk of dead volume. Additionally, entirely flat, non-domed surfaces can now be utilized with the Elasto Plug.

SCHEME FOR LYOPELLET WITH ELASTO PLUG:

- ▶ A lyophilized pellet fixed in the chamber, with the Elasto Plug conforming to its shape
- ▶ The Elasto Plug flattens the pellet as fluid flows and rehydration begins
- ▶ The pellet fully rehydrated within the chamber

Additionally, this concept provides more options regarding pellet sizes and introduces a flexibility for pellet design variants and volumes, even when the cartridge design is already established.

ELASTO PLUG

- › Standardized interface component for dried reagent integration (Reagent Plug + port on cartridge)
- › Drying process is separate from cartridge manufacturing
- › RP material properties allow compensation of dead volume zones after resuspension of dried reagents
- › RP material properties enable flexibility in LyoBead/dried pellet sizes without adopting RP geometry
- › Lyophilized pellets can be easily integrated into the cartridge
- › Standard pick and place process for insertion (LyoBead/dried pellet + Reagent Plug)
- › Reagent / pellet remains securely in place
- › Provides more options for altering lyophilized pellet size, even when cartridge design is fixed
- › Material properties support active mixing



MTP format carrier for Plugs (top), Elasto Plug with Pellet (right).

APPLICATION EXAMPLE WITH LYOPHILIZED PCR MIX



To demonstrate the functionality of the new Plug concept, qPCR runs were conducted using dedicated lyophilized Mastermix Beads.

First, an investigation of possible effects of material and mechanical stress on functionality resulting from fluid pumping was conducted. For these tests, rehydrated Mastermix Bead solution was pumped through a channel and plug chamber with either the Elasto Plug (silicone) or Reagent Plug (polypropylene) inserted.

Test groups were as follows:

► **Positive Control:**

Rehydration in a tube with a pipette (standard procedure, primers, probe, and DNA added afterward)

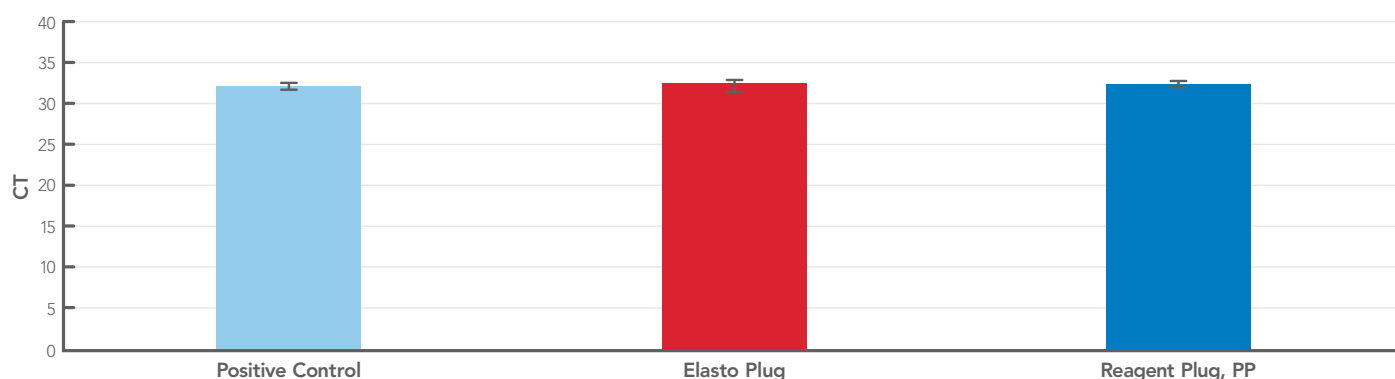
► **Elasto Plug:**

Rehydrated Mastermix pumped through microfluidic cartridge with inserted Elasto Plug (primers, probe, and DNA added afterward)

► **Reagent Plug, PP:**

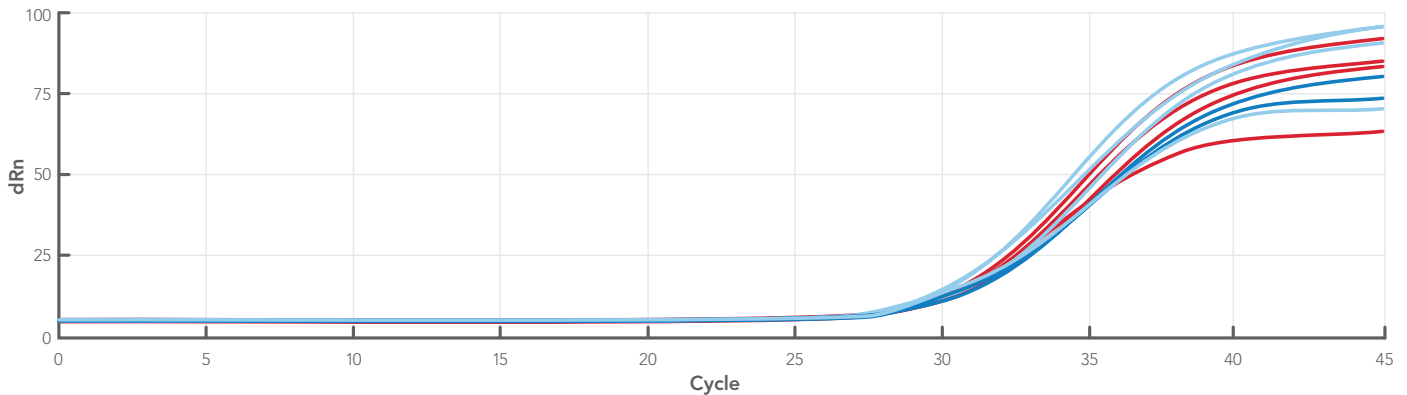
Rehydrated Mastermix pumped through the microfluidic cartridge with inserted Reagent Plug (primers, probe, and DNA added afterward)

MATERIAL AND PUMPING TEST



Material Test: Mean CT value, n=5.

MATERIAL TEST



As shown, no significant material and mechanical stress effect could be detected. Therefore, the process of the Mastermix Beads was transferred into the microfluidic cartridge, and tests were conducted to evaluate the consistency of the rehydration.

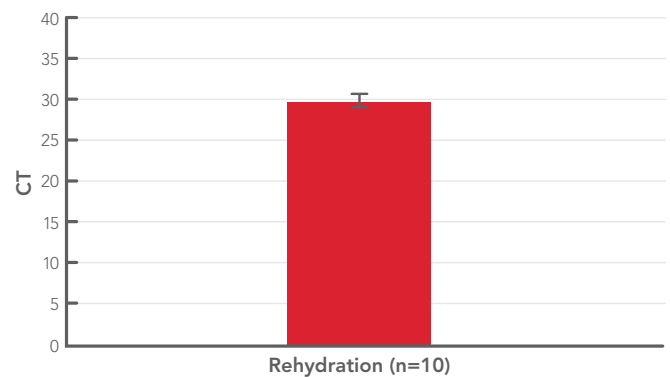
The following conditions were tested:

► Rehydration:

Rehydration with water in a microfluidic cartridge with inserted Elasto Plug (primers, probe, and DNA added afterwards)

Analysis of qPCR data showed comparable performance of rehydration within the cartridge and within a standard tube using a pipette.

REHYDRATION TEST WITH ELASTO PLUG



CONCLUSION

As summarized, there are several options for integrating and storing dried reagents on-chip in a microfluidic cartridge. However, the most common processes for direct application or implementation during production have disadvantages that call for more specific solutions to streamline production and enable mass manufacturing.

Our Reagent and Elasto Plug concepts address all the requirements for dry reagent storage while also offering an elegant solution for large-scale production settings. The proof of concept for our new Elasto Plug design successfully demonstrated its effectiveness.

For further information, including details on liquid reagent storage, visit www.thinXXS.com





For ordering, technical support,
and contact information
please visit www.thinxxs.com