Reagent Storage on Microfluidic Cartridges

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Abbreviations

FACS = Fluorescence-activated cell-sorting

HRP = Horseradish peroxydase

PCR = Polymerase chain reaction

PoCT= Point-of-Care-Testing

PoNT = Point-of-Need-Testing

Summary

In this paper, we present solutions for on-chip reagent storage which are scalable and thus allowing mass manufacturing of microfluidic consumables.

Reagent storage (both liquid and dry) is one of the most critical aspects in volume manufacturing of microfluidic consumables for point-of-care-testing. Providing storage concepts which allow utilization of standardized handling platforms is a big advantage.

Separating the raw card manufacturing processes from the reagent preparation and storage steps reduces both cost and risk of dry reagent storage.

Simple test platforms allow us to evaluate and optimize the dry and/or liquid reagent storage concept for any new assay.

1. Introduction

In this white paper on reagent storage, we will show you how to make use of the outstanding growth rates in the Point-of-Care-/Point-of-Need-Microfluidic-Test (PoCT/PoNT) markets for your company. We will describe the central challenges in the way of successful development of PoN-/PoC-Applications. In the main of this white paper, we will describe solutions to these central challenges.

2. Definitions

In this white paper, we will elaborate on "Microfluidic Devices," "Point-of-Need-Testing" (PoNT), and "Point-of-Care-Testing" (PoCT).

2.1. Microfluidic Devices

There is no commonly accepted definition of the term "Microfluidics." In different contexts, it describes both the science and the technology of dealing with Fluids in spaces which are constrained to the sub-millimeter-scale.[1]

Hence, Microfluidic Devices are devices which control fluids in the range of microliters to picoliters, and manipulate them to flow through channels with dimensions from tens to hundreds of micrometers. That is exactly what happens in both PoNT and PoCT.

2.2 Point-of-Need-Testing (PoNT)

In this text, the term Point-of-Need-Testing (PoNT) refers to all kinds of diagnostic tests performed outside of reference laboratories[2].

In the last two decades PoN-Testing was increasingly applicated in at least the following areas:

1. Food Testing[3]

2. Environmental and Industrial Testing[4,5]

3. Human and Veterinary Medicine[6,7]

5. Forensics[8]

2.3 Point-of-Care-Testing (PoCT)

The term Point-of-Care-Testing (PoC-Testing) is commonly used wherever out-of-labtests are used for human diagnostics[2]. The International standard ISO 22870, Point-of-Care-Testing (PoCT) - Requirements for quality and competence, defines PoCT as: "[…] testing that is performed near or at the site of a patient with the result leading to possible change in the care of the patient[9]" Alternative Terms for Point-of-Care-Testing are "[…] near patient testing (NPT), bed side testing, Physician Office Lab (POL) testing, off site testing, alternative site testing […]." Although the term Point-of-Need-Testing is broader, there is much more scientific literature on PoCT.

To a certain extent, the two terms PoNT and PoCT can be used interchangeably in the technical context of this white paper. Therefore, we will refer here mostly to literature on the subject of PoCT.

In this paper we focus on Microfluidic Devices. Other and fluidically simpler PoCTs like lateral flow tests and test strip (dip sticks) – for example Drug of Abuse (DoA) tests, pregnancy tests, etc.) are not within the scope of this white paper.

3. Requirements for Market Access

The requirements for access to the rapidly growing market of PoNT-/PoCT-Devices can be distinguished into quality and economic requirements.

3.1 General Quality Requirements

Typically, PoNT- and PoCT-Systems comprises an instrument and a (disposable) cartridge. In addition to being performed outside the laboratory, the testing is often done by non-laboratory/non-trained personnel[10]. That is the reason, why user-friendliness is so important.

These requirements imposed some constraints on both the instrument and the cartridge design. As a result, a trend towards simpler instruments and more complex, self-contained cartridges can be observed.

Hence, the underlying idea when designing a PoC consumable is to provide a cartridge which runs without liquid interfaces to the instrument. This is achieved by storing the required reagents and corresponding buffers on the consumable.

Depending on the assay complexity, a significant number of (different) buffers and/or reagents must be stored on the cartridge. In typical PoCT-applications, the number varies between one and four per card (but can be as large as 20). The reagent type and volume varies with the assay.

Table 1: Typical Reagents Already Stored on PoC Cards for Various Applications

Application	Reagent Type
Sample Preparation / Conditioning	Enzymes (e.g. Proteinase K), Lysis / Binding Buffer, Magnetic Beads, Wash-, Dilution-, Elution Buffer
Molecular Assay	Amplification Mastermixes
Molecular Assay / Immunoassay	Substrates / Conjugates (e.g. HRP-Conjugates, Luminol…) Antibodies
µFACS	Antibodies , Beads

Any reagent storage concept must fulfill the following quality-related requirements:

- Protection and preservation of reagent stability during manufacturing and assembly as well as during long-term storage
- Chemical and/or biological compatibility with the reagent(s) to be stored; no leaching of substances which could affect the assay

More quality-related requirements become visible when we start to differentiate between liquid and dry reagent storage.

3.2 Quality Requirements for Liquid Reagent Storage

While pierceable aluminum pouches have been used in the past for fluid packaging, newer developments include the storage in blister packs and/or storage tanks on the chip; both options equipped with so called frangible seals.

Which concept is the right one for a given application depends on a number of aspects. In addition to the shelf-life and compatibility related aspects mentioned above, the manufacturing process chain is a key consideration. Typical requirements include:

- Shelf life (typically 6 – 24 months)
- Storage of both water and alcohol based liquids
- Controllable opening mechanism (manually or with the help of a simple mechanics)
- Controllable fluid release
- Volume capabilities µL to mL range

3.3 Quality Requirements for Dry Reagent Storage

Most integrated PoC cartridges require storage of dry reagents. Storing reagents in a dry (or lyophilized state) can be required to ensure the desired shelf life. Again, depending on the assay complexity, the number of (different) reagents can be significant. Some cartridges require storage of up to 15 different dry reagents.

The time needed for reagent preparation (drying/lyophilization) protocols depends on the reagent and can vary between minutes and days (!). As one can imagine, this has a significant impact on the cartridge manufacturing workflow.

State of the art procedures for bringing dry reagents into a cartridge still includes dispensing and successive drying of the liquid reagent within selected microfluidic chambers and channels directly onto the cartridge. Other concepts rely on the sensitive handling of reagent pellets.

Three more quality requirements for dry reagent storage handling occur, when:

- The drying/lyophilization protocols and/or environmental conditions differ for each reagent and if
- The cartridge manufacturing procedures are not compatible with delicate reagents

3.4 Economical Requirements

From an economic perspective, for any new assay a proof of concept is needed and — after that milestone is passed — any new PoNT/PoCT device needs to have scalability and mass producibility. Hence, the economic requirements are:

- Scalable, industrial manufacturing platform available
- No significant increase of the overall cartridge cost due to the storage concept
- Filling of reagents can be performed as a final step in the manufacturing process chain (thus the reagent is not affected by any of the cartridge manufacturing and/or assembly steps)

4. Ways to Meet These Requirements

Meeting both quality and economic requirements may seem a challenging task. In the following chapter we will describe new solutions for on-chip reagent storage which can help to achieve these goals.

4.1 Blister Packs with Frangible Seal for Liquid Reagents

The logical advancement of stand-alone blisters for liquid reagent storage (both from a functional and a manufacturing perspective) is the integration of the blister as an integral part of the cartridge.

For this blister storage concept, the blister dome is formed of a composite-layer film, assembled to the cartridge backbone and then filled with the liquid reagent.

A small channel connects the blister to the rest of the fluidic network of the cartridge. This channel provides a frangible seal. Once the blister is pressed by the instrument (or manually), this seal opens.

With such a concept, a controlled release of the liquid reagent is achieved. The materials used offer protection against environmental conditions and the reagent is stored without residual air in the blister.

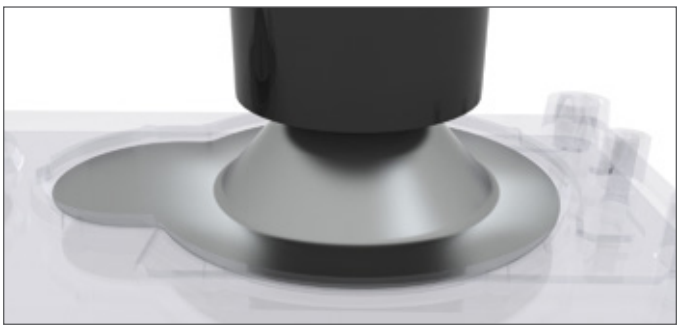


Figure 1 — Blister Storage

The picture shows a plunger pressing down on a on-chip blister pack. The pressure increase opens the frangible seal and the liquid is "squeezed out" into the microfluidic channel network. Typical opening forces are in the range of 20 – 50 N, depending on design. Note that the blister is not a stand-alone part. Instead, it is integral part of the cartridge. The picture shows a blister with a volume of approx. 0.5 mL.

4.2 Reagent Tanks with Frangible Seal

Depending on the volume to be stored, the concept of storing liquid reagents in cavities on the chip can be advantageous. Since most lab-on-a-chip platforms are made of polymers, it is essential to use polymers with sufficient barrier properties to allow storage of volatile reagents. Again, the cavities are closed with frangible seals or frangible seal film valves which are opened by the instrument.

The figure shows a test platform with four storage containers. Each container is closed with a frangible seal valve on either end which can be opened mechanically (e.g. using a simple push mechanism).

Figure 2 — On-Chip Storage Container

4.3 Solution for Liquid Reagent Shelf Life Enhancement

As described before, one of the key requirements for successful PoNT-/PoCT-Devices is long reagent shelf life. Both concepts described previously require that the cartridge backbone is made from material which provides a very good moisture barrier. For PoC applications, both Cycloolefins and Polypropylene have been used with good success. The figure to the right shows the measured weight loss of a blister filled with PBS buffer during storage.

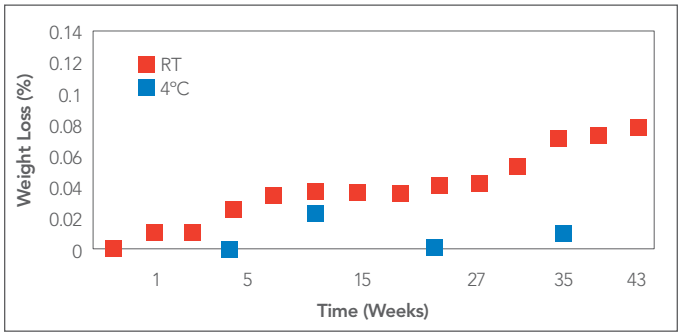


Figure 3 — Shelf Life During Storage

The figure shows the weight loss of a blister during storage (shown for a period of 1 year which corresponds to the desired minimum shelf life requirements of many PoC cartridges). No significant loss of buffer was measured in cases where the cartridge was stored at 4°C. The start volume was 500 µL PBS buffer stored in a on chip-blister mounted on a polypropylene cartridge.

4.4 Introducing the "Reagent Plug"

We now present an approach which is based on a simple, molded carrier — the "Reagent Plug." It can be used both for the proof-of-concept-phase and for mass production. Materials, surfaces, and surface coatings of such a reagent plug can be selected and optimized independently from the cartridge material(s).

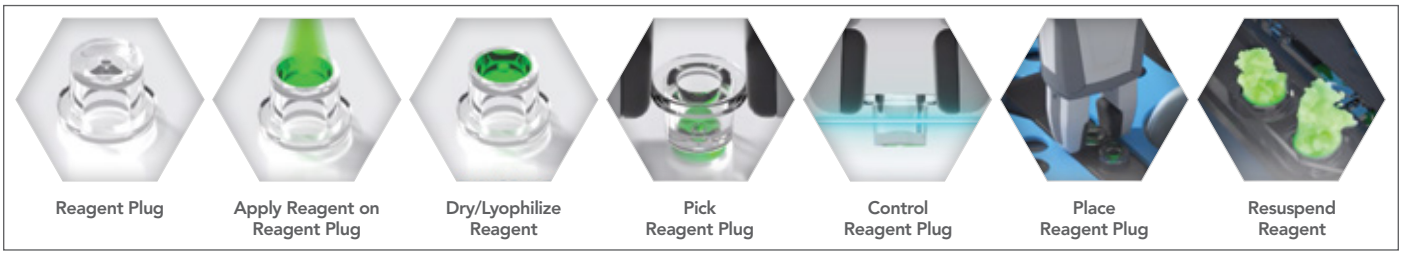


Figure 4 — Dry Reagent Preparation and Resuspension

Injection molded reagent plugs can be arrayed in a standard Titerplate format. All processes for dry reagent conditioning, reagent application, (freeze) drying, are then performed on this platform. The reagent preparation processes of different reagents do not interfere with each other and with the raw card manufacturing processes (e.g. welding, bonding, assembly)

Plugs are assembled into the fluidic body as the final assembly step. Industrial pick and place processes can be utilized for leak tight sealing of the plug via simple press fit or welding.

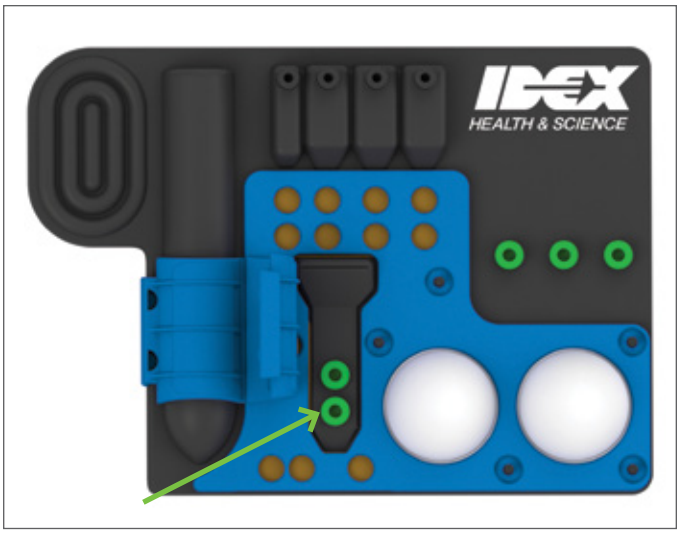


Figure 5 — PoC Cartridge with Reagent Plug(s)

Table 2: Typical Reagents Already Stored on PoC Cards for Various Applications

Application	Reagent Type
Sample Preparation / Conditioning	Lysis reagents, Binding Buffer, Magnetic Beads, Wash, and Elution Buffer
Molecular Assay	PCR Mastermix, Primer, Primer Mixes, Hybridization Buffer
Molecular Assay / Immunoassay	Misc. Reagents (e.g. Enzymes, Antibodies, Redox Reagents…) and Buffers
µFACS	Beads, Antibodies

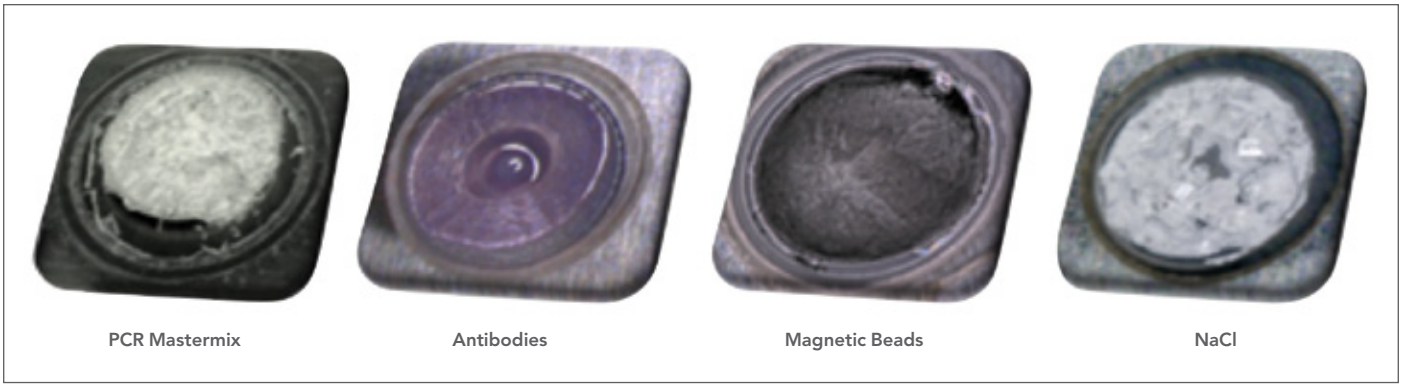


Figure 6 — Different Types of Dry Reagents on Plug

5. Test Platform for the Evaluation of Dry Reagent Storage in New Assays

For its proof-of-concept, any reagent storage concept must ensure protection and preservation of reagent stability during manufacturing, assembly, and long-term storage as well as chemical and/or biological compatibility with the reagent(s) to be stored. Further optimization of drying protocols and resuspension properties, e.g. by adding stabilizers, may be desirable for a specific assay.

For our "reagent plug," we use a simple test platform to evaluate and optimize the dry reagent storage concept for a new assay.

5.1 Testing for Rehydration

The first parameter to be verified is the amount of reagent that can be picked up by the buffer stream after having been dried on the reagent plug. Resuspension of reagents was tested using different flow rates and incubation times:

The following example illustrates the feasibility of this storage approach for PCR based assays:

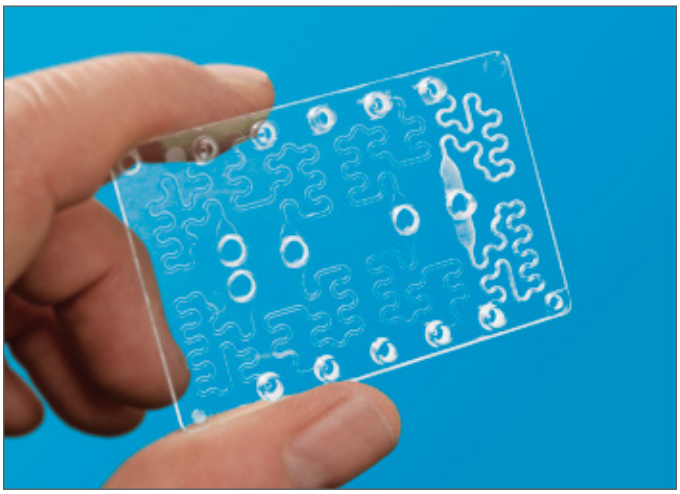
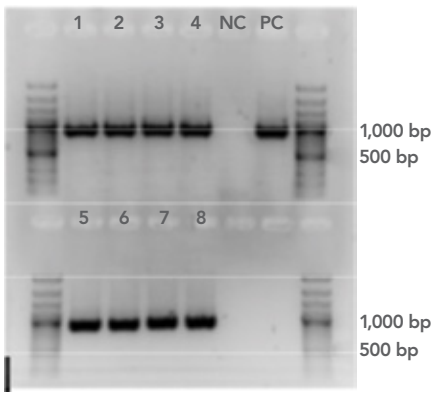


Figure 7— Test Chip for Rehydration Test of Dried Reagents
Microfluidic Test Platform with four chambers with Reagent Plug interfaces.

We have dispensed and dried primers (16sRNA-8F and -926R, universal primers for bacteria) and PCR Mastermix (Bioline, 2x MyTaq Mix) on Reagent Plugs.

5.2 Testing for Validity of Rehydrated Reagents

In a subsequent test, the reagents were rehydrated and PCR experiments were performed. Experiments with dried and resuspended primers showed results comparable to the positive control with fresh primers.



Lane Number	Description
1 – 2	Stored 21 Days
3 – 4	Stored 21 Days, Reference
NC	Negative Control
PC	Positive Control
5 – 6	Stored 24 Hours
7 – 8	Stored 24 Hours, Reference

Figure 8 — PCR Experiments with Dried and Reconstituted Primers

Functional testing on a model PCR for a 900 bp product from E. coli, E. coli genomic DNA was used as sample. As a reference, primers and PCR Mastermix were dried, stored, and resuspended in tubes.

5.3 Testing the Storage Approach for Liquid Reagents

On similar test platforms you can evaluate and optimize the liquid reagent storage-concept for your reagents. The first experiments to be done before settling on a concept, is to test the reagent stability during storage and, depending on the reagent and the assay, to test for leaching of substances which could affect the assay. This can be done on relatively simple test chips before the complete PoC card is manufactured. Furthermore, the effect of co-storage of dry and liquid reagents on the same card can be tested.

Figure 9 — Test Chips for Compatibility and Shelf Life Tests of Liquid Reagents
Left: Blister test Slide
Right: Test Slide with storage tanks and Reagent Plugs

6. Conclusion: The Advantages

Both the dry reagent storage and the liquid reagent storage concepts presented in this paper are designed to be compatible with a mass manufacturing environment for microfluidic consumables.

The reagent plug enables the drying process optimization parallel to the cartridge development.

The reagent plug meets all the qualitative requirements for successful PoN-/PoC-application described in this paper and:

- It helps to shorten the assay development phase
- It provides cost-effective dry reagent storage concept which is scalable and compatible with production settings

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