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The promise of microsampling, delivered

TECHNICAL GUIDANCE

short and long-term storage instructions for dried Mitra® specimens

Abstract

Dried bio-fluid specimen samples have several advantages over wet matrices like plasma/serum, urine, and liquid whole blood. The major benefits being simplified sample collection, shipment, and storage. This technical note presents the various storage solutions for dried specimens collected using Mitra® devices based on volumetric absorptive microsampling technology. Mitra devices feature the original, patented absorbent VAMS® tip, which simplifies the collection of a fixed-volume sample for analysis. Many studies on dried matrices require samples to be transported to various locations for testing, stored for short-term routine use or for long-term biobanking. While some laboratories may store the sampled VAMS tips from Mitra devices at room temperature (RT) for short term, some may prefer storing them inside the freezer for a longer period. Here we discuss the various storage options, stability of analytes and how to effectively store samples depending on study requirements.

Introduction

Storage plays an important role in eliminating waste, reducing contamination, and reusing samples for further studies at a later time. Proper storage controls moisture, maintains stability of the analyte and prevents microbial growth.

Our storage solutions are designed to keep samples dry and have a small footprint, making it convenient to store samples on a benchtop or stack them inside a freezer. Drying desiccant sachets placed inside the storage specimen bags absorb moisture and assist in rapid drying of the sampled tips, thus contributing to the stability of the analyte.

Sample processing and analytics are analyte specific, however, several published research papers demonstrated that temperature did not seem to negatively impact the results at specific durations. Elevated temperature studies were performed to mimic the 'journey of a sample' from the point when remotely collected Mitra microsamples were posted through the mail to the point when they arrived at a laboratory for processing and analysis. The results indicated that when microsamples were dried in the presence of desiccant, temperature did not seem to have a deleterious effect.

Stability

A recent study from Quanterix using Mitra devices with VAMS for dried blood sampling shows stability of the SARS-CoV-2 protein IgG. The results are very promising, showing SARS-CoV-2 protein IgG to be stable for up to a month at RT and up to a week during transportation under extreme environmental conditions at 37°C.

Figure 1 illustrates the [IgG VAMS] % bias from Day 1 for up to one month (RT). [IgG VAMS] refers to extract from spiked venous whole blood tested for SARS-CoV-2 protein IgG.

Less than 20% change of SARS-CoV-2 protein IgG activity was observed at room temperature for 4 weeks. Donor 4 was slightly beyond the acceptable range but with a consistent trend. This result might be explained by a high CV for the readings on Day 1 skewing the expected value for subsequent time points. This suggests that the dried blood VAMS tip samples are stable for up to 1 month at room temperature.

Room Temperature Stability

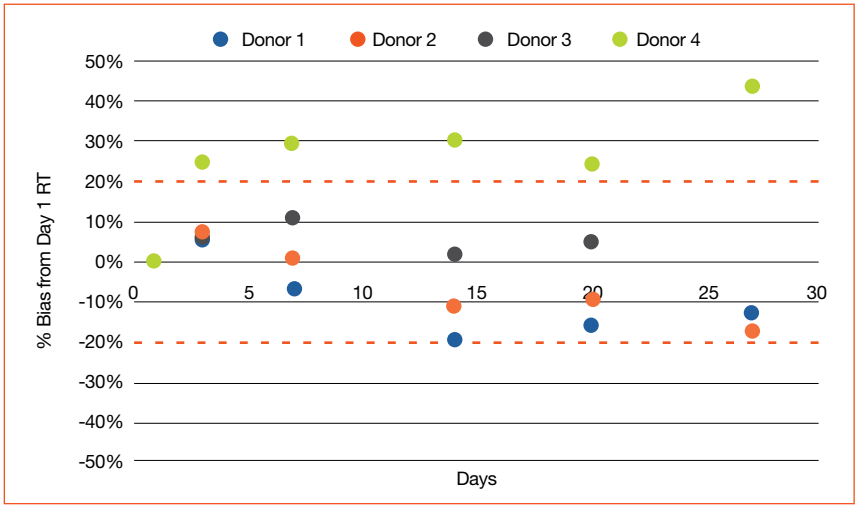


Figure 1. One-month stability at room temperature. SARS-CoV-2 protein IgG reading on Day 1 was normalized as 100%, the rest of the time points were calculated by dividing [IgG VAMS] of each time point by [IgG VAMS] of Day 1.

Figure 2 illustrates the [IgG VAMS] % bias from Day 1 for 11 days (37°C). Less than 20% decrease of SARS-CoV-2 protein IgG activity was observed at 37°C for 1 week. After storing VAMS tip samples for 11 days, the SARS-CoV-2 protein IgG activity for 3 out of 4 samples had a significant decrease. This suggests that the dried blood VAMS tip samples are stable under extreme environmental condition (37°C) during transportation for up to 1 week.

37°C Stability

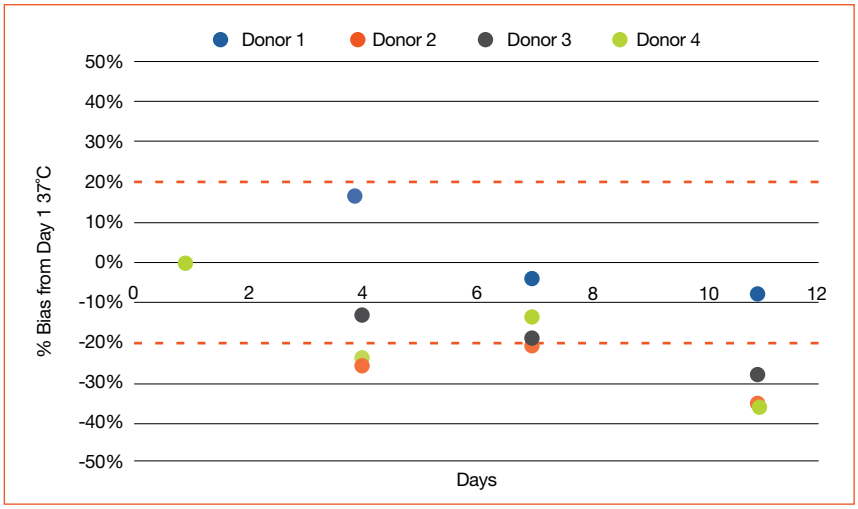


Figure 2. 11 days stability at 37°C. SARS-CoV-2 protein IgG reading on Day 1 was normalized as 100%, the rest of the time points were calculated by dividing [IgG VAMS] of each time point by [IgG VAMS] of Day 1.

Figure 3 illustrates the Freeze-Thaw (F-T) stability of dried blood VAMS tip samples for up to three freeze-thaw cycles. No significant [IgG VAMS] decrease was observed with fresh extractions, one F-T, two F-T and three F-T cycles. The average [IgG VAMS] % bias from fresh extracts (no F-T) was within 20%. This suggests that the samples are stable for up to three freeze-thaw cycles.

Freeze-Thaw Stability (Day 1 RT)

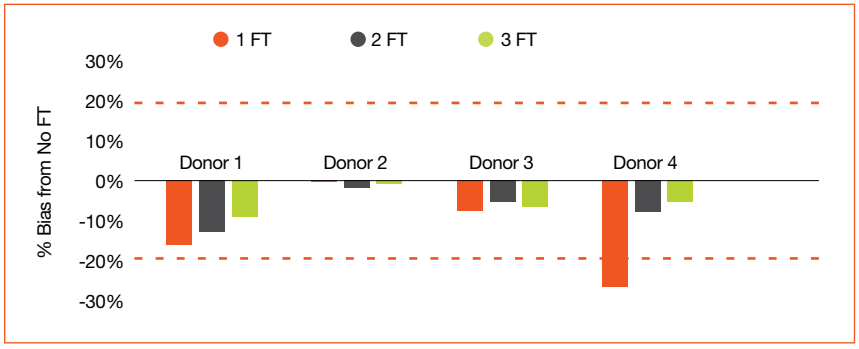


Figure 3. Freeze-thaw stability. [IgG VAMS] % bias from fresh extracts was plotted as a function of the donors over 3 freeze-thaw cycles.

Another study from Ghent University demonstrates Iohexol could be accurately quantified for renal function assessment in the pediatric population within a hematocrit range of 20–60%. Long-term stability of Iohexol in sampled Mitra devices was demonstrated up to 245 days under different storage temperatures.[2]

For more information on stability please visit https://www.neoteryx.com/mitra-vams-online-publication-library and filter under “Key Topics” for “Stability Studies”.

Anatomy of a Mitra Device

Mitra devices are available in several formats: clamshell, cartridge and 96-Autorack. The device consists of an absorptive VAMS® tip attached to the Mitra sampler body. These are housed inside a clamshell or cartridge case. The VAMS tip is the functional part of the device that rapidly and precisely wicks 10, 20, or 30 µL volumes per tip. The sampler bodies below the tip are designed to mimic a pipette tip so they can be arranged in a 96-well format for multiplexing and are compatible with liquid handling instrumentation. The outer housing of the clamshells and cartridges are designed to protect the samples during drying and transport. Both the formats are available packaged in individual specimen bags with desiccant inside to keep the sample dry and prevent contamination.

The clamshell and cartridge devices can be stored individually in their respective specimen bag (short-term) or in the recommended storage bag with desiccants (long-term). The 96-Autoracks can be stored long-term inside the storage bag with desiccants. Please refer to: short- and long-term storage below.

Clamshell
Economical remote-sampling

Absorptive tips
Collects 10, 20, or 30 µL of liquid specimens (< 4% RSD).

Sampler Body
One end holds the absorptive tip, while the other facilitates high-throughput processing. The body of device has area for labeling

Cartridge
User-friendly at-home sampling

96-Autorack
Designed for high-throughput processing

Short- and Long-Term Storage

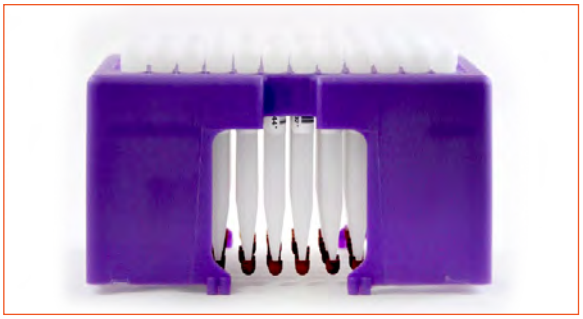
Neoteryx offers both short-term and long-term storage solutions of sampled Mitra clamshell and cartridge devices that have dried in transit to the laboratory, each catered for specific study needs.

Short-term Solutions

Empty 96-Autorack™

Typical Use:	Accessioning individual sampler bodies with VAMS tips out of a Mitra clamshell or cartridge device into a 96-well footprint for near term (within 1-2 days) benchtop processing & storage at room temperature
Part Number:	AC-DR01
Description:	Mitra 96-Autorack | Empty

AC-DR01: 96-Autorack (Empty)
Fits on standard 1.0mL & 2.0mL 96-well plates. It's used to accession samples collected using either the Cartridge or Clamshell format & build plates for high throughput sample processing



96-Autorack (Fully Loaded)
Mitra samples for illustration only, not included with part number AC-DR01

Empty 96-Autorack™ Paired with a Deep Well, 2mL Collection Plate

Typical Use:	Same as the empty 96-Autorack, but the collection plate adds an extra layer of protection against sample cross-contamination
Part Number:	AC-DR02
Description:	Mitra 96-Autorack | Empty with Well Plate

AC-DR02: 96-Autorack (Empty) with a well plate
It prevents cross contamination of samples

96-Autorack (Fully Loaded) with a well plate
Mitra samples for illustration only, not included with part number AC-DR02

Long-term Storage Solutions

Storage Bag with Desiccant

Typical Use:	For long-term storage in a deep freezer of sampled Mitra devices (clamshells or cartridges): The desiccant contained within the storage bag keeps the samples dry, while the zip closure prevents moisture from entering the bag. A label on the outside of the bag enables samples to be easily marked and identified.
Part Number:	AC-SS01
Description:	Mitra Device Storage Solution

AC-SS01: 8"x10" Storage bag with 100g (20x 5g) of desiccant
Long term storage solution for clamshells (option 1) & cartrdige (option 2)

Option 1: Storage bag (A5-SS01) with Clamshells
Clamshells shown for illustration only, not included with the storage bag

Clamshells inside Storage bag
Multiple clamshells can be stored inside the storage bag as shown above

Option 2: Storage bag (AS-SS01) with Cartridge
Cartridges shown for illustration only, not included with the storage bag

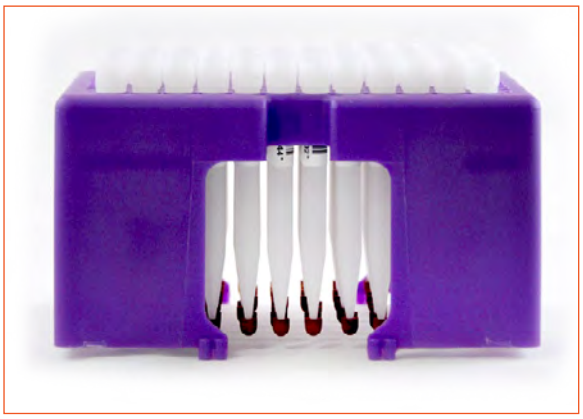
Cartridge inside Storage bag
Multiple cartridges can be stored inside the storage bag as shown above

Long-term Storage Solutions

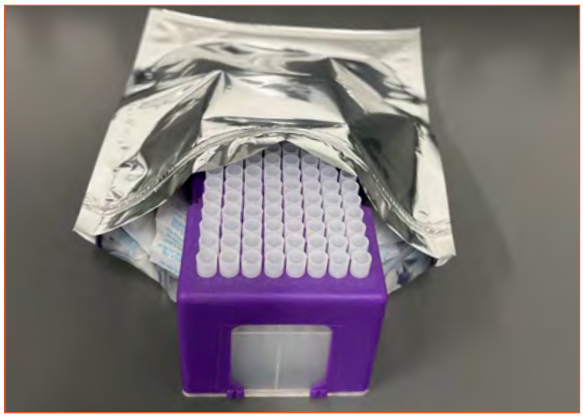
Storage Bag with Desiccant and 96-well Collection Plate

Typical Use:	For long-term storage in a deep freezer of sampled Mitra devices (clamshells or cartridges): The desiccant contained within the storage bag keeps the samples dry, while the zip closure prevents moisture from entering the bag. A label on the outside of the bag enables samples to be easily marked and identified.
Part Number:	AC-SS02
Description:	Mitra Device Storage Solution with 96-Well Collection Plate

8″x10″ Storage bag with 100g (20x 5g) of desiccant plus 96-well collection plate



96-Autorack (Fully Loaded)



Fully loaded 96-autorack with collection plate inside storage bag

Sealed 8″x10″ storage bags with fully loaded autorack and 96-well plate Filled storage bag dimensions are approximately 8″ x 10″ x 3.5″ (W x L x H).

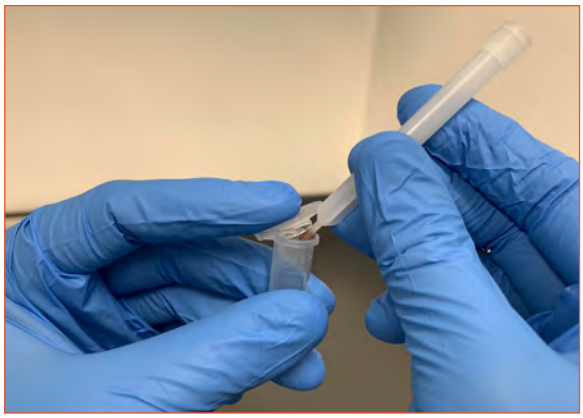
Deciding on the best storage solution for a study depends on how long the sample needs to be preserved, and the stability of the analyte at room temperature and inside the freezer. The application it's being used for and whether the samples need to be processed in batches or individually, also must be considered.

Guidance on the Long-Term Storage of Mitra Samples

1. **Sampled Mitra devices can be stored long term in the formats below.**
    a. Multiple cartridges or clamshells within a Storage Bag with desiccant (P/N AC-SS01)
    b. Transfer fully dried individual samples out of the clamshell or cartridges into an empty 96-Autorack and then
    package that into a Storage Bag with desiccant and 96-well collection plate (P/N AC-SS02)
2. **Use an appropriate cryostorage system.**
    a. Analytes on Mitra have proven to be stable under a variety of temperatures and stability is dependent on the nature of the molecule, and the duration and temperature of storage. For example, some molecules have proven to be stable on Mitra devices at room temperature for many months, whereas some only a few days or hours. Care is needed to choose the best storage system. One rule of thumb is to use -80°C cryostorage where long-term stability is unknown. Although it can't be guaranteed that samples will remain stable under these conditions, such conditions have shown to give excellent stability to untargeted metabolomics markers.[3]
    b. Aim to store samples in the appropriate storage conditions as soon as possible to minimize any degradation.
3. **Avoid condensation.**
    a. Ensure samples remain sealed in bags with desiccant and that samples are fully dried (at least 2 hours) before storage.
    b. Avoid opening the bags while they are being stored in the freezer.
    c. When retrieved from storage, allow the samples to warm in ambient conditions before opening the bags. This minimizes any condensation forming on the samples, which may affect any analytical measurement.

Alternative to 96-well Format Solution

If space becomes a limitation when storing or biobanking Mitra in a 96-well format, then detaching the VAMS tip off the Mitra microsampler body into a tube, such as an Eppendorf® tube (see picture below), will allow for a reduced storage footprint. Furthermore, storing individual tips in this manner will allow for removal of individual tips, negating the need for freeze thawing of multiple samples in 96-well plates or individual devices. Before transferring the tip to the tube, make sure the tip is fully dry and the transfer from specimen bag with desiccant to the tube is done as quickly as possible. This will help to minimize any absorption of moisture from the air (especially in high humidity environments). Also ensure that the tubes are correctly labelled such that the chain of custody is maintained. Use of a



One method is to use the lid of an Eppendorf tube to remove the VAMS tip from the sampler body for an efficient storage solution

2D pre-barcoded tubes
*Needs to be sourced separately

Although there is presently no long-term data available using the “in tube” storage method, the Neoteryx R&D team is relatively confident that the analytes on the tip will remain stable without the use of desiccant. This is due to the low volume and sealed nature of the tube. Recent experiments have shown that placing a VAMS tip in an Eppendorf tube, stored in a -20°C freezer and allowed to warm up to room temperature yielded no observed condensation on the inside of the vial, as long as it was still sealed. However, condensation was observed on the outside of the tube, which was removed by wiping the tube with a tissue to prevent any build-up of ice on the tube’s exterior if it is to be put back into cryostorage.

Conclusion

Currently, dried specimen sampling has expanded into many arenas of infectious diseases, therapeutic drugs, and inherited metabolic disorders. Many epidemiological studies and clinical trials require samples to be stored for specific time points and over longer period. Storing dried liquid samples as shown above is a cost-effective option as it eliminates the need of cold shipping, utilizes less freezer space and are easy to process.

If you need assistance with your storage needs or would like to learn more about how other researchers have incorporated storage into their workflow, our Microsampling Specialists will be happy to help! We are also open to exploring other custom storage solutions with you.

Sources

1. Application of Volumetric Absorptive Microsampling Devices to the Simoa® SARS-CoV-2 IgG Antibody Test
2. Volumetric absorptive microsampling as alternative sampling technique for renal function assessment in the paediatric population using iohexol
3. Pre-analytic evaluation of volumetric absorptive microsampling and integration in a mass spectrometry-based metabolomics workflow

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