The Mitra® or hemaPEN® device – how to choose?

The need for microsampling

Since the first clinical publication on dried blood spot (DBS) in 1963 for use in neonatal screening, microsampling of blood and other biological fluids has gained a lot of interest in many bioanalytical fields. However, over the years there have been many challenges associated with the original dried blood spots. This led to a desire for better sample quality and quantitation, which can be solved by next generation microsampling devices, such as the Mitra® and hemaPEN®, which take a volumetric sampling approach. Previous application notes elaborated on the limitations of current DBS technology and how volumetric devices can solve many of those limitations while offering additional benefits.[1,2]

Covid-19 pandemic – an urgent need for remote sampling

It wasn't until the Covid-19 Pandemic that there was an acute need for institutions to turn to volumetric microsampling tools for their research projects. The need for remote sampling was primarily due to social distancing and government lockdowns, where people could not (or did not want to) travel to the clinic or lab to have venous blood collected. There was an urgency for ongoing studies to continue and new studies to be initiated, especially research conducted to understand the SARS-CoV-2 virus that causes Covid-19 disease.

As the number of people attending clinics declined, researchers needed alternative ways to obtain blood samples remotely, so they turned to remote devices based on volumetric absorptive microsampling. The pandemic validated volumetric microsampling as a key tool for obtaining quality samples and enabling many remote clinical trials. One such trial was conducted by the National Institutes of Health (NIH), for which around 11,000 self-sampling kits containing Mitra microsampling devices were sent out to study participants at home. The purpose of the NIH study was to help understand the degree of undiagnosed Covid-19 cases in the United States.[3] The study was a great success, leading to the discovery that there were 4.8 times more Covid-19 cases than had been previously diagnosed or recorded.[4] This study, and others like it, confirmed the utility of remote self-sampling as a reliable and convenient alternative to venipuncture.

Prior to the Covid-19 Pandemic, running research trials remotely was a 'nice to have' option for many organizations. Since the onset of the Pandemic, however, increasing numbers of institutions have started using remote microsampling devices to run remote research trials. They have reported that remote sampling tools like the hemaPEN and Mitra offer many benefits compared to standard venipuncture or conventional DBS sampling methods.

A short history of Mitra and hemaPEN

Figure 1 - hemaPEN (left) and Mitra (right) microsampling devices

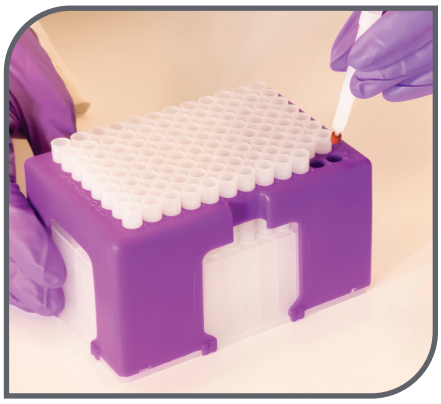

Figure 2 - Mitra 96-Autorack

The Mitra microsampling device was first released at the annual conference of the American Society for Mass Spectrometry (ASMS) in 2014, five years after its conception, as a quantitative device that accurately collected 10 µL capillary blood samples. Like DBS, the microsamples collected on Mitra devices could be dried and mailed to a central laboratory for rapid extraction analysis. Early on it was clear that Mitra devices and volumetric microsampling overcame many of the issues observed with the original dried blood spot technique.

Since the Mitra device was initially launched by microsampling device company Neoteryx, it has evolved to feature options for larger tip sizes (20 and 30 µL) in addition to the original 10 µL volumetric absorbent microsampling tips. These VAMS® tips allow for assays that require larger sample input volumes (such as whole genomic sequencing). Neoteryx later released complementary tools that enhanced sample processes with Mitra, including the Mitra 96-Autorack™ (Figure 2), which simplifies lab processing of dried matrix samples and enables automation workflows. After raising its profile as a world leader in volumetric microsampling, Neoteryx was acquired by Australian biomedical company Trajan Scientific and Medical (Trajan), becoming its microsampling product brand and supplying Mitra devices and other microsampling solutions to all four corners of the world.

Before acquiring Neoteryx and its Mitra products, Trajan had initiated a program with the University of Tasmania to address the known limitations of DBS and develop its own volumetric dried matrix microsampler. This microsampling device, which was named the hemaPEN, could sample even smaller volumes (2.74 µL) of blood than the Mitra device. From one drop of blood from a single finger-prick, the hemaPEN could collect four capillary samples simultaneously and transfer them into a cartridge of 4 pre-punched DBS papers housed inside the device. The hemaPEN was designed as a tamper-proof device with integrated drying desiccant. In less than four years, hemaPEN was registered as a medical device and commercially released, showing comparable levels of performance to Mitra in terms of volumetric accuracy and precision (<5% RSD). Over the last few years, Trajan has continued to innovate and develop its microsampling product line, introducing hemaPEN-QC® (Figure 3) for rapid method development and processing, as well as tools for laboratory integration.

Figure 3 - hemaPEN QC – for method development and validation

With a growing product range from sample collection and processing through automation and analysis, Trajan now offers more microsampling options to our valued customers and build on our vision to provide scientific tools and end-to-end solutions that help enrich personal health.

Why two dried capillary blood microsampling devices?

Both hemaPEN and Mitra devices are capillary microsampling tools that can be used by virtually anyone, anywhere, at any time. However, they have distinct differences that offer the end-user a wider range of applications for microsampling.

Sampling technique

Both devices are designed to sample capillary blood and other biological fluids like plasma and urine. When it comes to blood, both devices are also self-indicating. In other words, the device users can see when a sample has been collected correctly. However, the two devices do differ in the sampling technique.

Figure 4 - Capillary sampling using Mitra - always sample from a positive angle

- The Mitra utilizes a one-step process to collect and store the sample. With Mitra, after lancing a fingertip or other sampling site, you must lower the microsampler onto the blood drop from above, or a positive position, to lightly touch the surface of the blood drop until it absorbs into the white VAMS tip (See Figure 4). The end-user must wait two seconds for the device tip to fully turn red with blood. After two additional seconds, the tip is fully sampled, and you can move to the next microsampler tip and repeat the process until all the sampler tips in the device cartridge have been correctly filled (sampled). Once each VAMS tip is fully red, it will not oversample - even if held in contact with the blood for longer than the recommended sampling time. The sampled device is closed in its plastic cartridge and mailed to the lab. Upon inspection in the lab, most sampled Mitra devices are found to be of high-quality, yielding data that often aligns with samples from standard venous collection. This is true as long as the method validation has demonstrated that both sampling events are correlative.
- The hemaPEN utilizes a two-step process to collect and store the sample. With hemaPEN, you can touch the hemaPEN tip to the surface of the blood drop from any angle. The blood drop fills 4 integrated EDTA coated glass capillary tubes simultaneously in about 10 seconds via capillary action (Figure 5).

  Once filled, the capillaries cannot oversample. Next, the hemaPEN is pushed back down upon its base (Figure 6) and the accurate volumes of blood contained within the capillaries are transferred onto 4 individual pre-punched DBS disks securely held in an internal cartridge. It must be noted that if insufficient blood is collected in any of the 4 capillaries (inaccurate volume) during the collection step, then the insufficiently filled capillary will not transfer the inaccurate volume of blood onto the DBS surface.

Figure 5 - Sampling using hemaPEN - sample from any angle

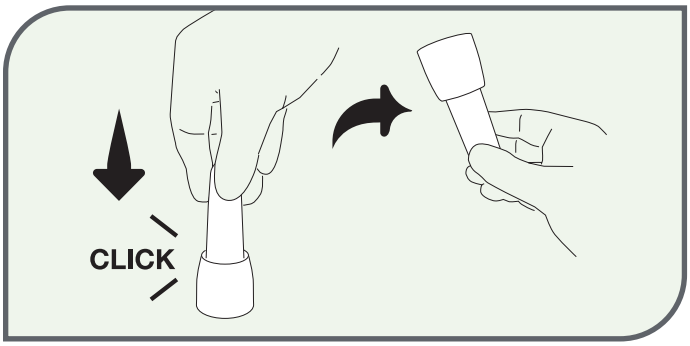

Figure 6 - Post sampling procedure to lock the samples inside

This feature prevents under- and over-sampling, while also guaranteeing that all of the DBS disks will contain an accurate and precise sample. In other words, the DBS disks are either volumetrically filled or blank. Lastly, once the end-user has finished sampling with hemaPEN, and pushes the tip of the device down onto its base until they hear a “click,” they will know they have locked the device, minimizing the risk of sample tampering or contamination. Sample drying occurs automatically, offering both convenience for the end-user and sample integrity for the laboratory.

Volume collection

Mitra and hemaPEN devices provide end-users the choice of various sampling volumes to fit their needs.

- The sample volume for Mitra includes 10 µL, 20 µL and 30 µL (Figure 7). We recommend using 10 µL microsamples for applications where analytical sensitivity is not often an issue, such as LC-MS/MS applications and some high-sensitivity immunoassays including some ELISA applications, and PCR applications.

  We recommend 20 µL tips for more sensitive LC-MS/MS assays as well as the majority of ligand binding applications. The 30 µL tips are recommended for applications where more volume is needed, such as whole genomic sequencing. The lowest volume that can be collected is 1 x 10 µL tip and the highest volume per unit is 4 x 30 µL tips - yielding 120 µL of collected blood which, in theory, could be used for a multitude of different assays from 1 collection event.

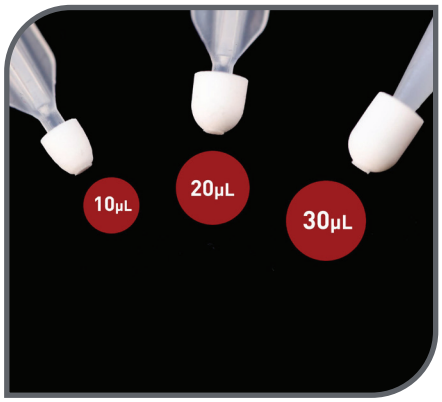

Figure 7 - Mitra tip sizes (10, 20 and 30 µL)

- In contrast to Mitra, hemaPEN collects 2.74 µL per DBS disk with 4 disks per hemaPEN (Figure 8). The disks can be pooled to produce up to 10.96 µL maximum sample volume per collection event. The DBS disks are ideal for assays where sensitivity is not an issue, including many LC-MS/MS applications, high sensitivity immunoassay (including certain ELISA applications), and certain PCR applications. Most DBS applications are compatible with hemaPEN samples because hemaPEN integrates industry-standard DBS substrates, which minimizes method modification. Refer to the application note that details this.[1]

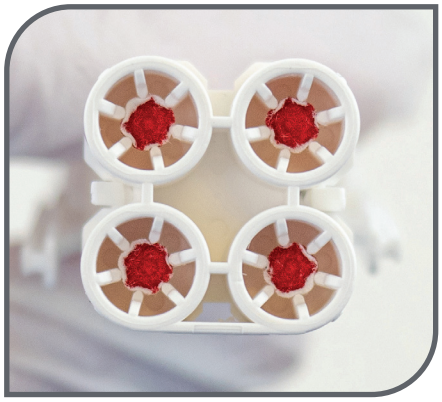
Figure 8 - A cartridge of 4 DBS disks found in the hemaPEN

The material

Both hemaPEN and Mitra produce dried matrix samples. The sampling material in both devices is hydrophilic and designed to rapidly absorb biological fluids. Furthermore, although the matrices found in hemaPEN and Mitra differ from one another, they are both designed to minimize analyte interactions and degradation and maximize extraction efficiencies (though this must be checked during method validation.)

- The sampling material on the VAMS tip of the Mitra device is made from a patented hydrophilic polymer and is designed to absorb biological fluids rapidly and accurately with a precision of <5% RSD. The VAMS tips are mounted on the plastic sampler body. They are dome shaped to aid rapid and complete volumetric absorption.
- The sampling material of the pre-punched DBS disks found inside the hemaPEN device is sourced from either Whatman 903 or PerkinElmer 226, two industry-standard DBS materials. Although there is no major difference in performance of these materials, when converting traditional DBS methods to hemaPEN it is good practice to choose a hemaPEN with the same matrix as used in the lab's previous DBS method. This will minimize any extraction biases that could occur if different matrices are used.[1]

Laboratory handling

Both the hemaPEN and Mitra devices are designed to work with standard laboratory workflows including extractions into 96-well plates or Eppendorf tubes. However, there are some differences between the hemaPEN and Mitra laboratory workflows, as follows.

Mitra

- Mitra devices are designed to work with 96-well plates and liquid handling robots, such as the Hamilton Star or Starlet.
  - 96-well tip attached workflow – Upon receipt, Mitra devices are removed from their foil specimen bags, the samplers are removed from their device cartridges, and the barcodes are scanned (optional, Figure 9). Note: All samplers in each cartridge are labeled with identical patient-centric barcodes specific to the cartridge.
  - After scanning, the samplers are manually placed into a Mitra 96-Autorack (Figure 10). The Mitra 96-Autorack is based on a standard 96-well footprint designed to hold Mitra samplers. They hang freely and don't touch the workbench. The 96-Autorack is also designed so that the samplers and their sampled VAMS tips are prevented from touching each other, avoiding any kind of cross contamination.
  - Once filled, the assembled racks are then placed over 2 ml, round well 96-well collection plates (containing pre-dispensed extractant), either manually, or using the 96-well handling tool of a liquid handling robot. The samplers are designed to fit certain pipette heads, allowing for individual sampler manipulation.
  - Once the 96-Autorack is assembled on the collection plate, extraction can commence (i.e., vortexing or sonication). Please refer to another resource, the Mitra Microsampling User Guide, to delve deeper into optimal extraction conditions for your assay.[5] After extraction, 96-Autoracks containing the extracted samplers are either safely discarded or the samplers themselves are discarded.
  - Leave the tip behind workflow – Occasionally, researchers prefer to remove the VAMS tip from the Mitra sampler body and drop the tip into a tube or 96-well plate. If this is the case, there are several ways to achieve this. The first is to push the VAMS tip off with a pipette tip. Another approach is to grip the VAMS tip with the lid of an Eppendorf tube and pull (Figure 11). Finally, if working with 96-well plates is preferable, then poke the tip through a single slitted Silicone/PTFE plate cover and pull back to detach it from the sampler body. It must be noted that this method is dependent on the size of the slit. Furthermore, this method may cause a buildup of dried blood dust on the sealing mat, especially if excess blood is found on the sampler bodies. If the slit is too large, the VAMS tip will not detach and will remain on the sampler body.

Figure 9 - Optional barcode scanning of a Mitra sampler

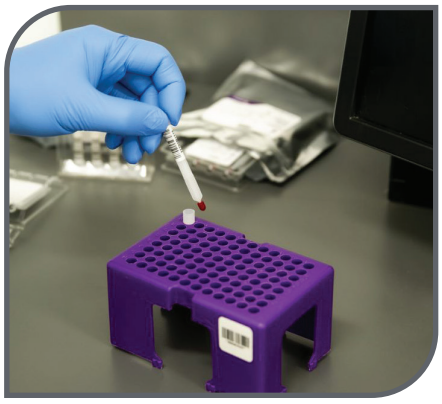

Figure 10 - Reformatting Mitra samplers into Mitra 96-Autoracks

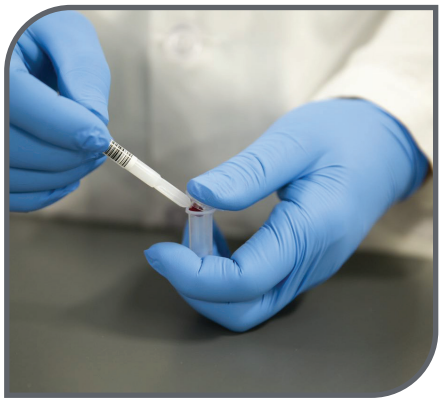

Figure 11 - Detaching a VAMS tip from its Mitra sampler body into an Eppendorf tube

hemaPEN

- hemaPEN devices are designed to work with both 96-well plate and Eppendorf tube workflows.
  - In the laboratory, hemaPEN devices are initially removed from their foil specimen bags and their individual 2D Data Matrix codes are scanned into a LIMS system.
  - Since hemaPEN microsampling devices are tamper resistant, an opening tool is used in the lab to cut open the sampled hemaPEN (Figure 13) and release the cartridge that contains the 4 DBS disks (Figure 14).
  - The cartridge is removed from its applicator automatically when added to a 96-well plate adaptor, where 24 cartridges (each with 4 DBS disks) can be assembled onto one 96-well plate (Figure 15). Alternatively, 4 cartridges can be placed over 16 pre-assembled Eppendorf tubes using an Eppendorf tube adaptor (Figure 16).
  - The DBS disks are then pushed out of the cartridges using a special disk accessioning tool or even a regular 10 µL pipette tip. Once the disks have dropped into the wells or tubes, they can be extracted using either vortexing and/or sonication.

Figure 12 - Scanning the 2D Data Matrix code of a hemaPEN

Figure 13 - Releasing DBS cartridge from hemaPEN

The 'extractions' section of the Mitra Microsampling User Guide provides guidance on how to extract from volumetrically collected samples.[6] Although the user guide focuses on Mitra, the extraction principles are common between both devices. Note: the only extraction method to watch out for when using the DBS disks from hemaPEN is 'impact assisted extraction' as this will homogenize the paper, requiring filtration or centrifugation steps.

Options for method development and validation

Working with Mitra cartridges and hemaPEN devices offers very convenient methods for self-collection or assisted capillary collection. However, when using these devices in the laboratory for method development and validation, the patient centricity element of the device is no longer the focus. For example, the tamper resistance feature of the hemaPEN is not the priority once the sampled device arrives in the lab. Therefore, we supply ancillary tools that assist with sample access and processing, offering more convenience when developing and validating methods in the laboratory. Below are the best practices when developing and validating methods on hemaPEN and Mitra.

Figure 14 - DBS cartridge on its applicator after retrieval from hemaPEN

Figure 15 - Adding DBS cartridge to 96-well adaptor plate

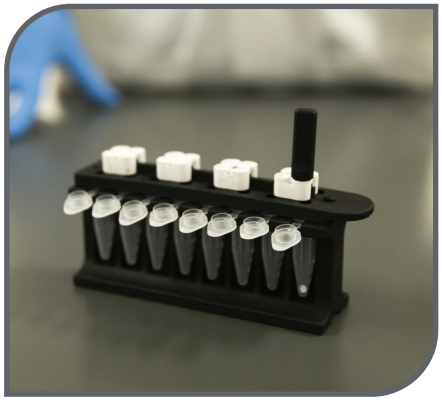

Figure 16 - DBS cartridges on Eppendorf tube adaptor (nearest cartridge shows disk accessioning tool above the cartridge)

Using Mitra

- When using Mitra, fully filled 96-Autoracks (Figure 2) are often the best option, especially if using the '96-well tip attached' workflow. The fully assembled 96-Autoracks contain 96 Mitra microsamplers individually barcoded in the rack placed over a collection plate.
- The first option is to separate the autorack from the 96-well plate for the extraction step.
- Next, take each individual microsampler and sample it with the fluid of interest, taking care not to fully immerse the sampler tip, as this will cause over-sampling. Once sampled, place the microsampler back in its rack and move on to the next microsampler. One drawback to this method is that it can be difficult to pick up microsamplers that are in the center of the plate, but a compatible pipettor can be used as a tip accessioning tool. However, an alternative method is to use a second empty autorack (rack B) where microsamplers can be picked up from rack 'A', sampled and reformatted into rack 'B' (Figure 10).
- After sampling is complete and the tips of all microsamplers have sufficiently dried (2-6 h minimum in low humidity or in a bag with desiccant), they are then ready for extraction.

Using hemaPEN

- When using hemaPEN for lab work, it is advisable to use the hemaPEN-QC tool (Figure 3). The hemaPEN-QC tool contains all the essential parts of the standard hemaPEN but without the tamper resistant "pen-like" housing and lockable base. The QC tool is an abbreviated version, consisting of just the hemaPEN tip with the glass capillaries for sample collection and the DBS cartridge on an applicator with desiccant. These sampling components are contained in an easy-open plastic storage tube (Figure 17).
- To use the hemaPEN-QC tool, open the storage tube to remove the contents and place them on the workbench.
- Take the pointed end of the hemaPEN-QC with the capillary holder, remove the plastic cap, and sample the biological fluid until the glass capillaries are full (Figure 18).
- Place this over the DBS-applicator assembly to transfer the fluid onto the DBS disks (Figure 19). You can rotate or twist the capillary holder to ensure you fully empty the capillaries.

Figure 17 - Contents of the hemaPEN-QC tool


Figure 18 - Sampling with hemaPEN-QC


Figure 19 - Transferring blood to the DBS cartridge assembly

- Once emptied, place the DBS-applicator assembly back in the storage tube container to allow for desiccant assisted drying.
- Once dry (2 h minimum), take the cartridge using the applicator and proceed with the extraction using the 96-well plate or Eppendorf methods (see previous section).

How to choose the right microsampling solution

We have created a decision tree to help you decide which microsampling solution is the best for your needs (Figure 20).

First, you will need to decide if your analytical method is sensitive enough to measure analytes from samples that are less than < 10 µL. If not, then Mitra would be a better option.

If the analytical workflow is adequate for samples less than < 10 µL and you need to collect less than 10 µL, then hemaPEN would be a better option.

If either works, then you will need to assess how important tamper resistance and sample protection are to your study. For example, if you are planning to analyze samples that could be subject to tampering or contamination from end-users (i.e., samples from addicts or from random drug screenings as part of a workplace testing scheme) or the environment, then hemaPEN would be a better option.

However, if tamper resistance is not of importance to the study, then the next thing to consider is if integrated desiccant is important for drying your samples. The hemaPEN integrates a desiccant within the device to avoid any misuse and to ensure all samples are dried and stored consistently, independent of user error. In contrast, Mitra provides the flexibility to include or not include a loose sachet of desiccant in the foil specimen bags in which Mitra devices are kept.

If integrated desiccant is not important, then the next question to ask is how important is rapid laboratory processing? The tamper resistant design of hemaPEN means that the cartridges need to be released from the pen using an opening tool. Although this is a straightforward procedure, it is not as quick as assembling a 96-well autorack with Mitra.

However, if you are not concerned about rapid lab processing, then either device option would potentially work. Your choice will then boil down to which sampling laboratory workflow works best for your project. If you need further assistance deciding, please contact our microsampling specialists, who can guide you to choosing the microsampling solution that would work best for you.

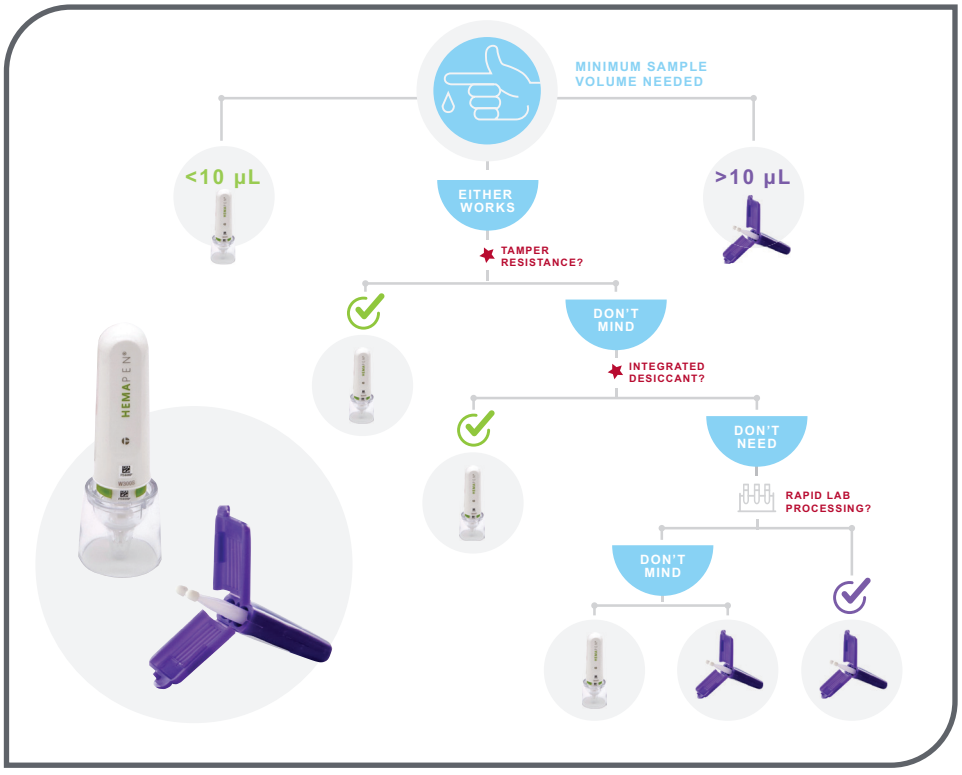

Figure 20 - How to choose your right microsampling format

[1] *Conversion of DBS to hemaPEN app note*
[2] *https://www.neoteryx.com/microsampling-blog/variances-in-dbs-observed-for-neonatal-screening*
[3] *https://www.niaid.nih.gov/news-events/nih-begins-study-quantify-undetected-cases-coronavirus-infection*
[4] *https://stm.sciencemag.org/content/13/601/eabh3826/tab-pdf*
[5] *https://hubs.ly/H0_RrQb0*

Mitra® devices are CE-IVD (IVDR) devices intended as specimen collectors and for the storage and transport of dried blood. They are available as registered IVD Devices in the European Union and United Kingdom, Australia, Brazil, China, and Canada, as well as multiple Health Ministries worldwide. In the USA, Mitra devices are supplied as a research use only (RUO) product to assist in method development, other research-related and non-diagnostic activities. Mitra has not been validated for use with any diagnostic testing.

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