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The Microbiopsy™ User Guide

Minimally Invasive Skin Sampling for Comfort, Efficiency and Precision

Introduction

Thank you for choosing the Harpera™ Microbiopsy™ Punch (IUO) based on a patented Microbiopsy technology for your clinical research studies! The Harpera Microbiopsy Punch is intended to offer a minimally invasive, suture-free, scar-free and virtually painless option for skin Microbiopsy.

This user guide will help you get started with the handling and processing of Microbiopsy specimens. Our goal is to provide you with the current common practices from the dermatology community utilizing the device for storing and analyzing skin Microbiopsy specimens with modern molecular techniques. This guide focuses on the current considerations for:

1. Collecting a skin Microbiopsy specimen
2. Retrieving a Microbiopsy collector from the Harpera Microbiopsy Punch
3. Storing a Microbiopsy specimen
4. Processing a Microbiopsy specimen for molecular analysis

Please note that this document provides guidance to facilitate the adoption of Microbiopsy specimen handling in the laboratory. We always recommend the reader review and adapt the proposed procedures as per local guidance.

What is a Microbiopsy and What Are the Benefits of Utilizing the Harpera Microbiopsy Punch?

The patented Microbiopsy technology is at the heart of the Harpera Microbiopsy Punch, featuring a Microbiopsy collector crafted from high-precision laser-cut stainless steel. The Microbiopsy collector is designed for virtually pain-free skin penetration, collecting thousands of skin cells (or about 0.29-115 µg of skin tissues) in a single punch. The handle allows safe and easy manipulation during the sampling procedure and retrieval of the Microbiopsy specimen.

The Benefits of Microbiopsy:

Minimally Invasive

The Microbiopsy punch design offers a virtually painless, suture-free experience, ideal for collecting specimens in sensitive areas, such as the face.

Precision Sampling

Collects specimens within and at vicinity of targeted site. Suitable for all topical sampling procedures.

Ease of Use

Designed with a controlled punch for easy sampling and safe handling before and after the procedure. The Harpera Punch facilitates specimen collection in the clinic or out in the field.

Versatility

Offering an advanced method for collecting specimens in various environments. Suitable for a range of clinical and research dermatology applications.

Additional technical details about the Harpera Microbiopsy Punch can be found in the Harpera Product Brochure, available on our Harpera Resources web page.

Considerations for Collecting Your First Microbiopsy Specimen

Prior to collecting your first Microbiopsy specimen, please ensure you have read the Harpera Microbiopsy Punch's instructions for use thoroughly and that you are familiar with its procedures. Before you begin, do not hesitate to seek help from our microsampling specialists at Trajan Scientific and Medical to discuss the most adequate protocol that suits your needs.

Dermatology professionals utilizing the Harpera Microbiopsy Punch have considered the following procedures prior to using the Harpera on participants:

- The use of mild skin cleansing methods (e.g. water, mild cleanser, RNase-free water, antiseptic, alcohol wipe). Feedback showed that the use of these cleaning methods does not impact on the expected analytical performance.
- Stretching the skin. Dermatologists typically stretch the skin prior to and during a biopsy procedure, to relax skin tension lines. This procedure can also be applied with the Harpera Microbiopsy Punch.

Tip: Sampling on hairless skin seems to yield a larger Microbiopsy specimen.

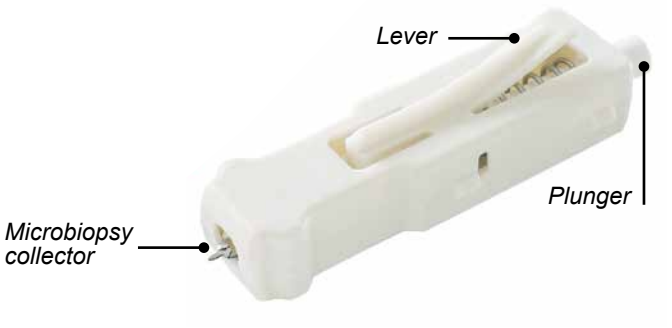
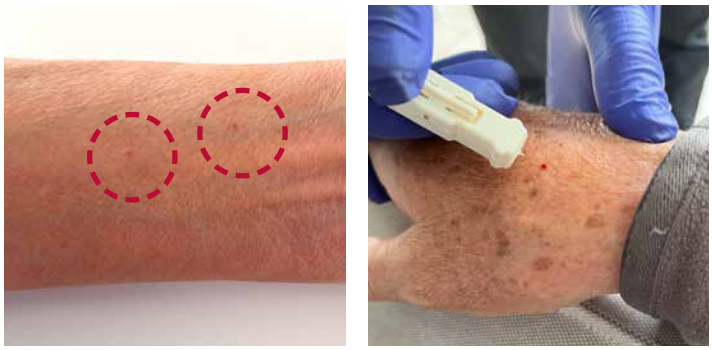


Figure 1: The Harpera Microbiopsy Punch and its key components

During the application of the Harpera Punch, make sure you press firmly (with the device perpendicular to the skin area). When pressing the lever ensure your fingers do not touch the plunger that will move during the activation step. You should hear a soft "CLICK" after you have activated the device.

After activation, the device can be immediately removed from the skin area. A small but visible mark on the skin indicates a successful sampling procedure (Figure 2a). A minimal bleeding at the sampling site may occur as well (Figure 2b). A Microbiopsy specimen is now collected onto the Microbiopsy collector.



(a) (b)

Figure 2: (a) Local erythema a few minutes after the sampling of two Microbiopsy specimens, (b) Minimal bleeding can occur after the collection of a Microbiopsy specimen using the Harpera Microbiopsy Punch.

Please refer to your local guidance prior to the use of the Harpera Punch on participants and again, please do not hesitate to seek help from our microsampling specialists at Trajan Scientific and Medical to discuss protocols that may better suit your needs.

Retrieving a Microbiopsy Collector From the Harpera Punch

After the specimen is collected, the technician or healthcare professional may process it and/or ship it to a designated laboratory for analysis.

This section explains the steps to retrieve the Microbiopsy collector from within the device.

There are several ways to retrieve the Microbiopsy collector containing the Microbiopsy specimen from the Harpera Punch. The most appropriate retrieval process will depend on the subsequent storage and/or analytical workflow.

This guide presents the two most common procedures for retrieving the Microbiopsy collector from the device for placement into a receptacle for subsequent storage and analysis of the specimen. The steps outlined below take place after the collection of a skin Microbiopsy specimen from a participant and are usually performed by the technician or healthcare professional at the sampling site, or in the designated laboratory.

WARNING:

The steps below may subject the end-user to exposure to the sharp element of the Microbiopsy collector and the collected specimen.

Handle the Microbiopsy collector with care.

Make sure the Harpera Microbiopsy Punch is not set - plunger pulled and locked - prior to opening its casing.

In the event of using tools (e.g. tweezers, pliers) during the manipulation of the Microbiopsy collector, please ensure their cleanliness (i.e. sterile) prior each use.

Option 1: By Opening the Harpera Punch

1. **OPEN** the Harpera Punch casing from the side using the notch and access the plunger (Figure 3a-b). The use of tweezers can facilitate its opening.
2. (optional) **REMOVE** the spring from the plunger
3. **UNLOCK** the Microbiopsy collector from the plunger (Figure 3c).
4. **RETRIEVE** the Microbiopsy collector from the plunger and transfer it into a receptacle (e.g., a microcentrifuge tube as seen in Figure 3d) with the tip facing down, either manually or using a pair of tweezers, or a disposable pipette tip. In such a case, the Microbiopsy collector can be either pushed into the tube or pulled from the back end.

   Alternatively, the Microbiopsy collector can be pulled and transferred directly into a tube after Step 1 using a pair of pliers or tweezers. Ensure you have a good grip on the Microbiopsy collector.

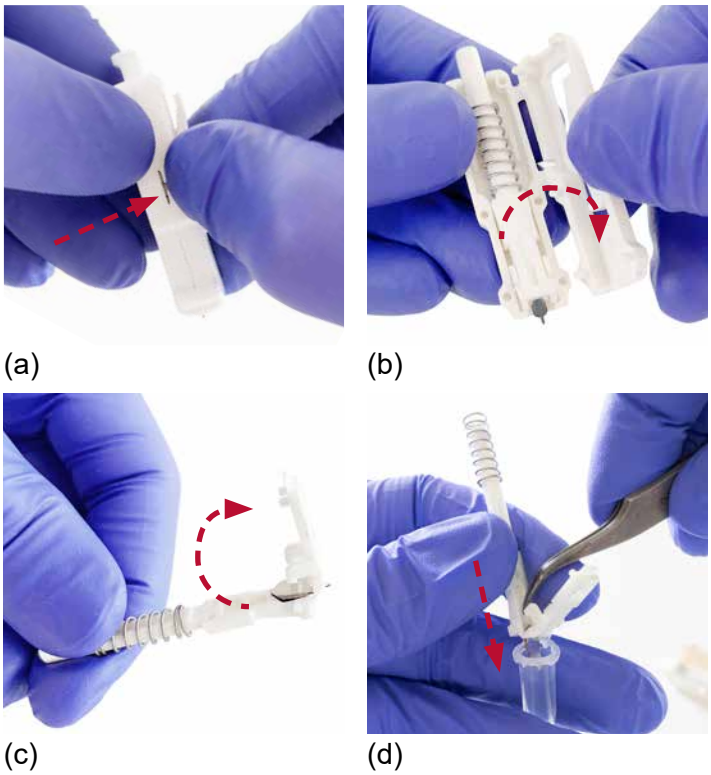


Figure 3: (a-b) OPEN the casing using the notch, (c) UNLOCK the plunger, (d) RETRIEVE the Microbiopsy collector & transfer into a tube.

Option 2: By Pulling the Microbiopsy Collector Directly

1. **PUSH** the plunger so that the Microbiopsy collector is visible at the front-end of the Harpera Punch.
2. **GRAB** the Microbiopsy collector below the folded plate, ideally using forceps (i.e. AF13-Forceps), pointed tip pliers or hemostat (Figure 4a). Ensure you have a good grip on the Microbiopsy collector.
3. **PULL** the Microbiopsy collector out of the plunger (Figure 4b). Several back and forth movements can facilitate its removal.
4. **RETRIEVE** the Microbiopsy collector & transfer it into a receptacle, (e.g., a microcentrifuge tube as seen in Figure 4c), with the tip facing down.

(a)

(b)

(c)

Figure 4: (a) PUSH the plunger and GRAB the Microbiopsy collector below the folded plates, (b) PULL the Microbiopsy collector out of the plunger, (c) PLACE the Microbiopsy collector into a tube.

For more information about the retrieval processes of the Microbiopsy collector, please visit the Harpera Resources web page to watch our user guide videos.

When transferring the Microbiopsy collector into a tube, we recommend placing it with the tip facing down toward the bottom of the tube to minimize the volume of extraction buffer. Typically, a minimum volume of 25-50 µL of reagent is recommended to ensure the tip of the Microbiopsy collector is fully submerged.

Pooling multiple Microbiopsy collectors into a 0.5 mL or bigger tube (2 mL) will enable the extraction of more Microbiopsy specimens, thus leading to a greater amount of material for further analysis. Some researchers have collected and pooled up to 5 Microbiopsy specimens from a single collection area and a single study participant.

Alternatively, the Microbiopsy collector can be placed in other containers or on a glass slides for microscope analysis.

Storing a Microbiopsy Specimen

The Microbiopsy specimen and consequent samples can be stored either in dry or wet formats prior to analysis. Below are some common storage methods used by the dermatology community for Microbiopsy specimens.

Dry Preservation:

- Snap frozen: The Microbiopsy collector containing the specimen can be placed into a tube and snap frozen at -20°C or -80°C.

Wet Preservation:

- RNAlater Fixative: RNAlater™ Stabilization Solution from Invitrogen™ (e.g. P/N: AM7020) or RNAprotect® Tissue Reagent from Qiagen (e.g. P/N: 76106) are some examples of stabilizers that can be used to preserve the Microbiopsy specimen on the Microbiopsy collector at room temperature for several days (e.g., 1 week at 25°C) or frozen for longer periods. Please refer to the manufacturer's instructions for more details about the procedure of use.
- Extraction Buffer: The specimen can be directly extracted from the Microbiopsy collector using extraction buffers. After extraction of the sample and removal of the Microbiopsy collector, the extracted material can be stored following the kit protocol – typically a few hours after extraction. The specimen may also be snap frozen for a longer storage period. Please refer to the manufacturer's instructions for more details about the procedure of use.

Please refer to your local guidance prior to storing a Microbiopsy specimen and do not hesitate to seek help from our microsampling specialists to implement the most suitable storage workflow that meets your analytical and institutional needs.

Extraction Kit Examples Used in The Literature for Processing Microbiopsy Specimens

Because of the low quantity of specimen collected – a Microbiopsy – it is recommended to select an extraction kit that is specifically designed for single cell analysis (1 to <1000 cells), such as Laser Capture Microdissection (LCM) kits.

Below is a non-exhaustive list of reagents, methods, technologies and end-point instrumentations that Key Opinion Leaders (KOLs) have used over the years to process and analyze Microbiopsy specimens.

Stabilizers:

- RNAlater™ Stabilization Solution (Invitrogen™, AM7020)
- RNAprotect® Tissue Reagent (Qiagen, 76106)

Molecular Kits:

- RNA/DNA extraction kits:
  - RNeasy® Micro Kit (Qiagen, 74004)
  - Arcturus® PicoPure® RNA Isolation Kit (Thermo Fisher, KIT0204)
  - QIAamp® DNA Micro Kit (Qiagen, 56304)
  - Maxwell® 16 LEV Blood DNA Kit (Promega, AS1290)
  - REPLI-g® Single Cell Kit (Qiagen, 150343)
  - QuantiTect® Whole Transcriptome Kit (Qiagen, 207045)
  - SMARTer® Stranded Total RNA-Seq Kit (Takara, 634485)
  - Agilent DNA 12000 Kit (Agilent, 5067-1508)
  - Agilent RNA 6000 Pico Kit (Agilent, 5067-1513)
  - TruSeq™ RNA Library Prep Kit v2 (Illumina®)
- cDNA Synthesis (SensiFAST™, BIO65053)
- Proteins/Proteomics
  - Proximity Extension Assay (PEA) technology (Olink® Proteomics)
  - Pierce Micro BCA™ Protein Assay Kit (Thermo Fisher, 23235)

Quantification and Gene Detection:

- Qubit™ 1X dsDNA High Sensitivity (HS) (Invitrogen™, Q33230)
- Qubit™ RNA HS Assay Kit (Invitrogen™, Q32852)
- TaqMan® Assays, Human, RNase P (Applied Biosystems, 4403326)

End-point Instruments:

- Hyphenated-MS platforms
- Sequencing systems via HiSeq® 2000 (Illumina®), Sanger sequencing method
- Real time qPCR systems via:
  - QuantStudio® 6 Flex, QuantStudio® 5, ViiA™ 7, Applied Biosystems 7500 (Thermo Fisher)
  - Biomark™ HD (Fluidigm®)
  - CFX Connect™ (Bio-Rad Laboratories)
- Quantitative fluorometer-based assay via Qubit® fluorometers (Thermo Fisher)
- Microscopy via Zeiss 510 META confocal microscope, Stemi 2000C stereo microscope (Carl Zeiss Microscopy GmbH)

Tip: We recommend pre-cooling all the sterile processing tools, such as the 2 mL microcentrifuge tubes, on dry ice to ensure the integrity of RNA and prevent sample loss.

Tip: Because of the initial low amount of tissue, it is important to minimize the number of analytical steps to ensure the integrity of RNA and prevent sample loss. KOLs have found that starting the extraction with a dried Microbiopsy will yield more genetic material than with a Microbiopsy stored in RNALater™ at -80°C.

Tip: Ensure you use a minimal elution volume of about 10 µL in your analytical protocol.

For more information about RNA extraction procedures and Gene expression analysis, we recommend the reader to follow the protocol described in Lei et al., Absorbent Microbiopsy Sampling and RNA Extraction for Minimally Invasive, Simultaneous Blood and Skin Analysis. J. Vis. Exp. (144), e58614, doi:10.3791/58614 (2019).

Literature Referencing the Use of Harpera Microbiopsy Punch

Skin Cancer

Jain, M., *et al.* (2022). "Minimally invasive Microbiopsy for genetic profiling of melanocytic lesions: A case series." J Am Acad Dermatol 87(4): 903-904.

Sobarun, P., *et al.* (2017). "Microbiopsy Biomarker Profiling in a Superficial Melanoma Resembling a Pigmented Basal Cell Carcinoma." JAMA Dermatol 153(4): 334-336.

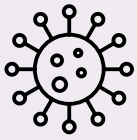
Dermatology Research Centre, School of Medicine The University of Queensland (2016), "Natural history and properties of naevi in advanced melanoma patients receiving treatment", CTRN 12616000272493 (ANZCTR)

Tan, J.-M., *et al.* (2015). "BRAFWild-Type Melanoma in Situ Arising In a BRAFV600E Mutant Dysplastic Nevus." JAMA Dermatology 151(4).

Skin Disorders

Preis, S., *et al.* (2022). "Munich atopy prediction study (MAPS): protocol for a prospective birth cohort addressing clinical and molecular risk factors for atopic dermatitis in early childhood." BMJ Open 12(9): e059256.

Yamada, M., *et al.* (2020). "Microbiopsy-based minimally invasive skin sampling for molecular analysis is acceptable to Epidermolysis Bullosa Simplex patients where conventional diagnostic biopsy was refused." Skin Res Technol.



Infectious Skin Disease

Van Henten, S., *et al.* (2024). "Evaluation of Less Invasive Sampling Tools for the Diagnosis of Cutaneous Leishmaniasis." Open Forum Infect Dis 11(4): ofae113.

Carter, E., et al. (2023). "A feasibility study of controlled human infection with intradermal Bacillus Calmette-Guerin (BCG) injection: Pilot BCG controlled human infection model." Wellcome Open Res 8: 424.

Liverpool School of Tropical Medicine (2023). "Using BCG Vaccine to Understand Tuberculosis Infection", NCT05820594 (ClinicalTrials.gov)

Cloots, K., *et al.* (2021). "Assessing L. donovani Skin Parasite Load: A Proof of Concept Study of a Microbiopsy Device in an Indian Setting." Front Cell Infect Microbiol 11: 645121.

Owen, S. I., *et al.* (2021). "Evaluation of qPCR on blood and skin microbiopsies, peripheral blood buffy coat smear, and urine antigen ELISA for diagnosis and test of cure for visceral leishmaniasis in HIV- coinfected patients in India: a prospective cohort study." BMJ Open 11(4): e042519.

Churiso, G., *et al.* (2020). "Minimally Invasive Microbiopsies as an Improved Sampling Method for the Diagnosis of Cutaneous Leishmaniasis." Open Forum Infect Dis 7(9): ofaa364.

Kirstein, O. D., *et al.* (2017). "Minimally invasive microbiopsies: a novel sampling method for identifying asymptomatic, potentially infectious carriers of Leishmania donovani." Int J Parasitol 47(10-11): 609- 616.

General Dermatology

Primiero, C. A., et al. (2024). "Skin 2.0: How Cutaneous Digital Twins Could Reshape Dermatology." J Invest Dermatol.

Hadeler E (2021). Innovations in translational research in dermatology: minimally invasive methods for biosample acquisition. Dermatol Online J. 2021 Oct 15;27(10).

Michele Fimiani, P. R., Elisa Cinotti (2020). Technology in Practical Dermatology, Springer Cham.

Lei, B. U. W., *et al.* (2019). "Absorbent Microbiopsy Sampling and RNA Extraction for Minimally Invasive, Simultaneous Blood and Skin Analysis." JoVE(144): e58614.

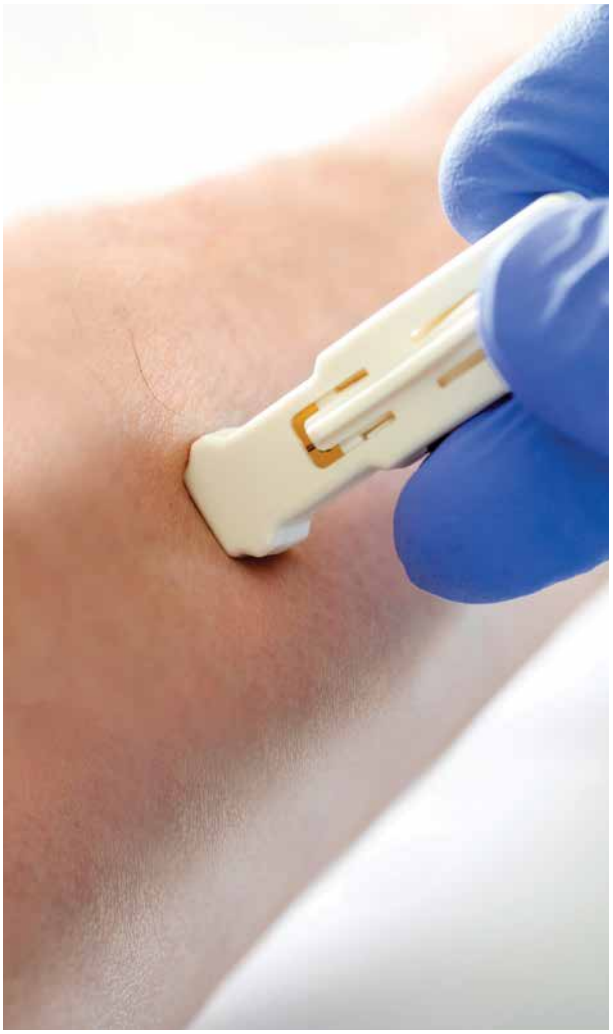
Lin, L. L., *et al.* (2013). "Microbiopsy engineered for minimally invasive and suture-free sub-millimetre skin sampling." F1000Res 2: 120.

Want more?

Access the full list of journal articles at www.neoteryx.com/harpera-micro-skin-biopsy

Microsampling Specialist Support

You can seek support from our microsampling specialists to ensure you implement the most suitable workflow that meets your analytical and institutional needs.



Thanks for Being a Skin Microsampling Innovator!

We hope you find this user guide helpful for your skin research projects.

For further information on how to develop robust methods using skin Microbiopsy specimens, we'd be happy to recommend several excellent resources in the literature that are worthwhile reading.

Should you have questions about skin Microbiopsy with the Harpera Punch, please feel free to contact a microsampling specialist at Neoteryx®, the microsampling product brand of Trajan Scientific and Medical.
For assistance, please send your questions to:
neo.info@trajanscimed.com.

Resources

Neoteryx offers microsampling content for further guidance and resources on its website. Please use the links below to explore our other Harpera Punch content.

1. Harpera product
2. Harpera resources
3. Microsampling resource library
4. Skin microsampling blogs

For information on Trajan's Neoteryx range of microsampling solutions, visit www.neoteryx.com.

About Trajan Scientific and Medical

Trajan is a global developer and manufacturer of analytical and life sciences products and devices founded to enrich personal health through scientific tools and solutions. We aim to support science that benefits people. Trajan's products and solutions are used in the analysis of biological, food, and environmental samples. Trajan has a portfolio and pipeline of new technologies which support the move towards decentralized personalized data-based healthcare. Trajan comprises more than 680 people with seven manufacturing sites across the US, Australia, Europe, and Malaysia, with operations in Australia, the US, Asia, and Europe. Trajan's products and solutions are marketed under multiple product brands and services, including Neoteryx. For more information visit www.trajanscimed.com.

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