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Title: On-Site Sampling and Extraction of Brain Tumors for Metabolomics and Lipidomics Analysis

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **Y, 4 km**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Joanna Bogusiewicz**: This protocol can be used to perform an extraction from brain tumors in or next to a surgery room using solid phase microextraction fibers **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Paulina Zofia Gorynska**: This technique enables the extraction of small molecules directly from resected tumors and provides an opportunity for rapid intraoperative diagnostics by coupling extraction devices directly to analytical instrumentation **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Joanna Bogusiewicz**: This protocol focuses on brain tumors, but the strategy can be implemented for the diagnosis of many different cancers **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

OPTIONAL:

1.4. **Paulina Zofia Gorynska**: In addition, the method can be used for the characterization of other animal or plant tissues as well as for other types of samples, including biofluids, cells, and microorganisms **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

1.5. **Paulina Zofia Gorynska**: Take care not to touch the coating with bare hands. Also, inserting the entire coating into the sampled tumor and monitoring the timing of the subsequent steps are also important **[1]**.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

Protocol

2. Solid Phase Microextraction (SPME) Device Preparation and Sample Collection

2.1. To prepare the solid phase microextraction device, trim the coating of each solid phase microextraction device probe with mixed-mode or C18 (C-eighteen) coatings to an appropriate length according to the size of the tumor **[1]** and soak the probes in a 50:50 methanol:water solution for at least 1 hour before the sample collection **[2]**.

2.1.1. Talent trimming coating and soaking probes NOTE: Shot was split in 2 but also includes 2.1.2.

a) WIDE: the whole laboratory is presented

b) Focused on hands

~~2.1.2~~

2.2. As soon as possible after tumor removal **[0]**, immerse the probe fibers with LC-MS (L-C-M-S) grade water for 5 seconds **[1-TXT]** before inserting the fibers as far as possible into the brain tumor tissue sample to ensure that the entire extraction phase is located inside the tumor **[2-TXT]**

2.2.0 A Added shot 2.2.0 split in 3 parts: Surgery room presented (please use Take 1 from 2.2.0.A),

2.2.0 B The cut of dura presented

2.3.0 C Tumor removal presented(starting from **18sec.**) NOTE: Very important to show the tumor removal

2.2.1. Probe(s) being immersed *Videographer: Important/difficult step* **TEXT: LC/MS: liquid chromatography-mass spectrometry**

2.2.2. Fiber(s) being inserted *Videographer: Important/difficult step* **TEXT: Do not wash or pretreat tumor before SPME extraction**

2.3. Leave the probes within the tissue for precisely 30 minutes **[1]**. To eliminate sources of error due to the presence of artefacts from sources other than the sampled tumor, place conditioned probe fibers on the table for the same period of time **[2]**.

2.3.1. Talent setting timer, with tissue and probes visible in frame *Videographer: Important step*

2.3.2. Talent placing control probes *Videographer: Important step*

2.4. During the extraction, label HPLC (H-P-L-C) vials to be used for probe storage after the extraction **[1-TXT]**.

2.4.1. Talent labeling vial(s) **TEXT: HPLC: high-performance liquid chromatography**

2.5. At the end of the extraction, immerse the fibers in LC-MS grade water for 3 seconds to remove any blood or cell debris **[1]** and insert each probe through the bottom of the septa of a single HPLC vial cap to immobilize the fibers **[2]**.

2.5.1. Talent immersing fiber(s)

2.5.2. Septa being pierce

2.6. Then cap the vials with the immobilized fibers **[1]** and place the vials into an appropriate transportation container **[2]**.

2.6.1. Talent capping vial(s) NOTE: Shot combined with 2.5.2.

2.6.2. Talent placing vial(s) into container

2.7. Upon laboratory arrival, immediately place the vials into a minus-30 or minus 80-degree Celsius freezer for no more than 3 or 5 years, respectively **[1]**.

2.7.1. Talent placing vial(s) into freezer

3. Metabolomic Analysis Sample Preparation

3.1. Once all of the samples from a single experiment have been collected, place the vials containing the mixed-mode fibers at room temperature **[1]** and label 2-milliliters vials for desorption **[2]**.

3.1.1. WIDE: Talent placing vial(s) onto bench

3.1.2. Talent labeling vials

3.2. Place a glass insert into each vial **[1]** and add 300 microliters of freshly prepared 80:20 acetonitrile:water solution to each insert **[2]**.

3.2.1. Talent placing insert into vial(s)

3.2.2. Talent adding solution to insert(s), with solution container visible in frame

3.3. Fully immerse the coating of each probe in individual vials of the desorption solvent **[1]** and agitate the vials for 120 minutes at 1200 revolutions per minute on a vortex **[2]**.

3.3.1. Coating being immersed *Videographer: Important step*

3.3.2. Vial(s) being vortexed *Videographer: Important step*

3.4. Once the desorption is complete, remove the caps from the vials **[1]** and set aside 10-microliter aliquots from each vial containing extract from the tumor to prepare quality control sample and close the vials with new caps **[2]**.

3.4.1. Talent removing cap(s) *Videographer: Important step*

3.4.2. Talent adding 10-microliter aliquot to container *Videographer: Important step*

3.5. Then close the vials with new caps **[1]** and place the vials into the 4-degree Celsius autosampler of a liquid chromatography high resolution mass spectrometer **[2]**.

3.5.1. Talent capping vial(s)

3.5.2. Talent placing vial(s) into autosampler **TEXT: See text for metabolomic analysis details**

4. Lipidomic Analysis Sample Preparation

4.1. To prepare the samples for lipidomic analysis, place the vials containing the C18 fibers at room temperature **[1]** and label 2-milliliter HPLC vials for the desorption **[2]**.

4.1.1. WIDE: Talent placing vial(s) at RT

4.1.2. Talent labeling vial(s)

4.2. Place silanized glass inserts into the vials **[1]** and add 200 microliters of freshly prepared 50:50 isopropanol:methanol solution to each insert **[2]**.

4.2.1. Talent placing insert(s) into vial(s)

4.2.2. Talent adding solution to insert(s)

4.3. Fully immerse each probe coating into the solvent **[1]** and agitate the samples for 50 minutes at 1200 revolutions per minute on a vortex **[2]**.

4.3.1. Probe being immersed *Videographer: Important step*

4.3.2. Vial(s) being agitated *Videographer: Important step*

4.4. At the end of the time stop the desorption, by removing caps **[0]** and then set aside a 10-microliter aliquot from each vial containing extract from the tumor **[1]** to prepare the quality control sample and close the vials with new caps **[2]**.

4.4.0. Added shot: Talent removing caps

4.4.1. Talent adding aliquot to new container *Videographer: Important step*

4.4.2. Talent capping vial(s) *Videographer: Important step*

4.5. Then place the vials into the 4-degree Celsius autosampler of the liquid chromatography high resolution mass spectrometer **[1-TXT]**.

4.5.1. Talent placing vial(s) into autosampler **TEXT: See text for lipidomic analysis details**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.2., 2.3., 3.3., 3.4., 4.3., 4.4.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
2.2.

Results

5. Results: Representative Lipidomic and Metabolomic Tumor Analyses

5.1. The reproducibility of the instrumental analysis was determined to be very good based on the tight clustering of the quality control samples on the principal component analysis plot **[1]**.

5.1.1. LAB MEDIA: Figure 3A *Video Editor: please emphasize green QC data point*

5.2. These lipidomics data for samples **[1]** collected from patients with gliomas **[2]** and meningiomas **[3]** highlight the ability of the tumor samples to be distinguished according to their histological origin and malignancy **[4]**.

5.2.1. LAB MEDIA: Figure 3B
5.2.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize purple cloud/data points*
5.2.3. LAB MEDIA: Figure 3B *Video Editor: please emphasizes blue cloud/data points*
5.2.4. LAB MEDIA: Figure 3B

5.3. As illustrated with these metabolomics data **[1]**, gliomas can be further divided based on their degree of malignancy **[2]** and/or according to the presence **[3]** or absence of mutations of interest **[4]**.

5.3.1. LAB MEDIA: Figure 4
5.3.2. LAB MEDIA: Figure 4 *Video Editor: please sequentially emphasize Figure 4A then Figure 4B*
5.3.3. LAB MEDIA: Figure 5A *Video Editor: please emphasize blue data points*
5.3.4. LAB MEDIA: Figure 5A *Video Editor: please emphasize orange data points*

5.4. Statistical analysis also permits the selection of specific compounds within the studied groups **[1]**.

5.4.1. LAB MEDIA: Figure 5B *and 3C Video Editor: please sequentially emphasize data bars*

Conclusion

6. Conclusion Interview Statements

6.1. **Barbara Bojko**: It is essential to carefully control the extraction time and to maintain reproducible conditions for all of the samples **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.3.)

6.2. **Barbara Bojko**: Alternatively, samples can be desorbed from fiber directly to MS without chromatographic separation, allowing the targeted analysis of specific markers and shortening the entire procedure to several minutes **[1]**.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*