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Title: Using a Chemical Biopsy for Graft Quality Assessment

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Introduction

REQUIRED:

1.1. **Natalia Warmuzińska:** Chemical biopsy is a new diagnostic solution in organ transplantation for quick and complex graft quality assessment. Currently there is no other low invasive method available that enables such analysis.

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

1.2. **Natalia Warmuzińska:** The main advantage of this technique is the possibility of monitoring graft tissue modifications over time of preservation due to the simplicity and low invasiveness of SPME probes.

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

OPTIONAL:

1.3. **Iga Stryjak:** The small diameter of the probe and big variety of biocompatible coatings make this method suitable for analysis of transplanted organs and applicable in medical research such as tumor analysis.

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

1.4. **Iga Stryjak:** When attempting this technique for the first time, pay attention to details and timing of particular steps, because small mistakes may heavily influence the results. The protocol is simple, but it is easier to understand and perform after visual demonstration.

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

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Animal Care Committee of the Toronto General Research Institute.
Procedures involving human subjects have been approved by the Bioethical Committee at Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Torun.

Protocol

2. Preparation of Probes and Extraction

2.1. Start by preparing a preconditioning mixture composed of 1 to 1 methanol and water **[1]**. Pipet 1 milliliter of the solution into each 2-milllilter glass vial **[2]** and place one probe in each vial **[3]**.

2.1.1. WIDE: Establishing shot of talent combining the water and methanol.

2.1.2. Talent adding 1 milliliter of the solution into a few vials.

2.1.3. Talent placing a probe into a few vials.

2.2. Agitate the vials on a vortex agitator at 1,200 rpm for 1 hour **[1]**, then rinse the probes with LC-MS grade water and sterilize them according to the standard surgical sterilization protocol or in sterile processing department **[2]**.

2.2.1. Talent putting the vials on the agitator and starting it.

2.2.2. Talent rinsing the probes with water.

2.3. When ready to extract the sample, open the sterile packaging **[1]** and insert 2 probes directly into the kidney cortex for 10 minutes per time point, making sure that the entire length of the coating is covered by the tissue matrix. Make sure to keep track of the time of sampling for each probe. **[2-TXT]**. *Videographer: This step is difficult and important!*

2.3.1. Talent opening the package with the probes.

2.3.2. LAB MEDIA: VID_20191018_152147_1.mp4 or IMG_20191015_074200.jpg, IMG_20191018_152139.jpg. Kidney with probes. **TEXT: See Text Manuscript for sampling time points** NOTE: May have to use lab media, not sure if this shot was filmed.

2.4. Retract the probe by pulling it out from the tissue **[1]** and immediately rinse the coating with LC-MS grade water to remove any remaining blood, making sure to rinse away from the surgical site **[2]**. *Videographer: This step is important!*

2.4.1. Talent removing the probe from tissue.

2.4.2. Talent properly rinsing the coating.

2.5. To transport the probes, place them in separate vials and close them, then place the vials in a box filled with dry ice or liquid nitrogen **[1]**. Store the samples at -80 degrees Celsius or immediately proceed with desorption **[2]**. *Videographer: This step is important!*

2.5.1. Talent placing a probe in a vial and closing it.

2.5.2. Talent putting the sample in the freezer and closing the door.

3. Desorption

3.1. Prepare a desorption solution composed of acetonitrile and water for metabolomic analysis **[1-TXT]** and another composed of isopropanol and methanol for lipidomic analysis **[2-TXT]**.

3.1.1. Talent making the desorption solution for metabolomic analysis, with the solution container clearly labeled. **TEXT: 80:20 acetonitrile : water (v/v)**

3.1.2. Talent making the desorption solution for lipidomic analysis, with the solution container clearly labeled. **TEXT: 1:1 isopropanol : methanol (v/v)**

3.2. Add 100 microliters of the solution to inserts in the 2-milliliter labeled vials **[1]** and place one probe in each vial **[2]**. Agitate the vials at 1,200 rpm for 2 hours **[3]**, then remove the probes from the vials **[4]** and proceed with LC-MS analysis **[5]**.

3.2.1. Talent adding solution to a vial.

3.2.2. Talent placing adding a probe to a few vials.

3.2.3. Vials on the agitator.

3.2.4. Talent removing the probe from the vial.

3.2.5. Talent operating the LC-MS system.

Results

4. Results: Metabolomic and Lipidomic Analyses

4.1. Liquid chromatography coupled with high resolution mass spectrometry **[1]** was used to carry out untargeted metabolomic **[2]** and lipidomic analyses **[3]**. The data was subjected to principal component analysis to assess quality and attain general insights regarding the results **[4]**.

4.1.1. LAB MEDIA: Figure 1.

4.1.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize A.*

4.1.3. LAB MEDIA: Figure 1. *Video Editor: Emphasize C.*

4.1.4. LAB MEDIA: Figure 1.

4.2. The quality control samples formed a tight cluster, confirming the quality of the analyses **[1]**. The studied groups exhibited relatively good separation, allowing for visualization of differences in metabolic and lipidomic profiles before and after transplantation, as well as during organ perfusion **[2]**.

4.2.1. LAB MEDIA: Figure 1. *Video Editor: Emphasize the quality control points in all 4 graphs (orange dots).*

4.2.2. LAB MEDIA: Figure 2.

4.3. A wide spectrum of extracted features was separated on both reversed phase and liquid chromatography columns **[1-TXT]**. Alterations in metabolite levels throughout the experiment were demonstrated with box-whisker plots of the selected metabolites **[2-TXT]**.

4.3.1. LAB MEDIA: Figure 4. *Video Editor: Label A "***Reversed Phase***" and B "***HILIC***".*

4.3.2. LAB MEDIA: Figure 3. *Video Editor: Label A and B "***Metabolites***" and C and D "***Lipids***".*

Conclusion

5. Conclusion Interview Statements

5.1. **Barbara Bojko:** When performing this procedure, keep in mind the organ's heterogeneity – place the probes in the desired location, keep the time of sampling identical at all points and secure the fiber properly after sampling.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.2, 2.5.1.*

5.2. **Barbara Bojko:** The biomarkers discovered with this method can be quantified using a sample extraction method and fast detection with analytical instrumentation or short LC-MS/MS method. Moreover, since no sample is consumed, routine analysis such as biopsy can be performed.

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

5.3. **Barbara Bojko:** The method can be adopted for other tissue metabolomics and lipidomics analysis without necessity for sample pretreatment and preparation. For specific metabolites, other extraction phases can be used to increase selectivity and sensitivity of the assay.

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