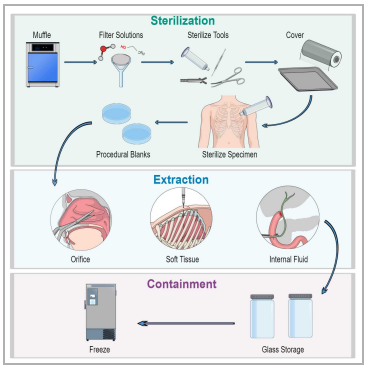

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A protocol to prevent plastic contamination during sample collection of cadaveric tissues

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ABSTRACT

Research on microplastic pollution and its effects on atmospheric, aquatic, and terrestrial environments has grown rapidly as potential risks for human health are recognized. Recently, microplastic particles have been detected in a variety of human tissue samples (e.g., blood, placenta, lung tissue), but studies use different methods of collection, preparation, and analysis, making results difficult to compare. In particular, the risk of microplastic contamination during sample collection is high and can potentially produce misleading results. Here we present a set of guidelines to mitigate all sources of potential plastic contamination while collecting cadaveric tissue samples. We discuss specific protocols for the collection of three tissue types: (1) internal body fluids; (2) soft tissue; and (3) tissue lining body orifices. Guidelines for each tissue type include sterilization of equipment and specimen, extraction of the sample, and containment of the cadaveric specimen after removal. Each methodology has the potential to be modified to collect samples from any anatomical region within the human body. Representatives from each tissue type (intestinal contents, lung tissue, and contents from the nasal cavity) are presented.

Keywords: Cadaveric tissue, Contamination prevention, Guidelines, Microplastics, Sample collection

GUIDELINES

Guidelines

We recommend storing filtered ethanol and water in BOD bottles as screw cap bottles have been shown to shed MPs from the plastic cap (1). We also recommend allowing dissection tools to dry in a laminar flow hood or clean bench as it has been shown to reduce the risk of exposure to airborne particulates (2).

References

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2. Wesch C, Elert AM, Wörner M, Braun U, Klein R, Paulus M. Assuring quality in microplastic monitoring: About the value of clean-air devices as essentials for verified data. Scientific Reports. 2017;7(1):5424.

MATERIALS

Materials and equipment

- Ethanol (50 – 70%)
- Purified water
- Membrane filters
- BOD bottle (1 L)
- Dissection tray
- Aluminum foil
- Metal scalpel (10 blade)
- Glass syringe with 16 G needles
- Glass beakers (250 - 500 mL)
- Razor
- Linear surgical stapler
- Ring forceps (7 inch)
- Straight hemostat (9 inch)
- Stainless steel biopsy punch
- Glass specimen jars
- Laminar flow hood or clean bench

Safety warnings

Working with cadavers can pose the risk of exposure to infectious disease. Cadavers should be screened for the following infectious pathogens prior to any sample collection: Tuberculosis, hepatitis B and C, AIDS and HIV, MRSA, VRSA, or VRE, SARS-CoV-2, meningitis, septicemia, and prions that cause transmissible spongiform encephalopathies such as Creutzfeldt-Jakob disease (CJD).

Proper protective clothing, gloves, and good hygiene measures can reduce the risks of working with a cadaveric specimen to a minimum.

Scalpel blades are sharp, thin, and can easily be broken. Lacerations from a scalpel blade can cause serious injury and increase the exposure risk to blood borne pathogens. Never try to insert, adjust, or remove a scalpel blade with your hands. Properly dispose of blades in a sharps container immediately after use.

BEFORE START INSTRUCTIONS

Before start

1. Wear a 100% cotton lab coat throughout the entire procedure.
2. Wear 100% cotton under lab coats.
3. Nitrile gloves should be worn during tissue collection.
4. The color of lab coats, clothing, and gloves should be documented.
5. No plastic tools or products should be used throughout the entirety of the procedure.
6. Cover pre-cleaned glassware with aluminum foil and muffle all at 450° overnight.
7. Dissection tools used per sample should either be new or pre-cleaned, disinfected, and autoclaved.
8. Filter all ethanol and water that will be used throughout the procedure.

Note

The filter used should have a pore diameter of at least half the size of the smallest particle targeted.
Common options are GF-C (1.2 µm), and GF-F (0.7 µm).

Note

Filtered ethanol and water should be stored in a BOD bottle when not in use.

Sterilization of tools

1 Rinse tools with particle-free water 3 times.

1.1 Rinse dissection trays, scalpel handles, forceps, hemostats, stainless steel biopsy punch, and glass syringes with particle-free water. (1/3)

1.2 Rinse dissection trays, scalpel handles, forceps, hemostats, stainless steel biopsy punch, and glass syringes with particle-free water. (2/3)

1.3 Rinse dissection trays, scalpel handles, forceps, hemostats, stainless steel biopsy punch, and glass syringes with particle-free water. (3/3)

Note

Be sure forceps and hemostats remain under running, particle-free water for at least 60 seconds to remove any contamination.

Note

Point the tools in a downward direction when rinsing them to ensure the particulate matter is removed. For forceps and hemostats, be sure the water is moving in the same orientation as the metal teeth on the tissue-gripping end of the tool.

2 Rinse dissection trays, scalpel handles, forceps, hemostats, stainless steel biopsy punch, and glass syringes with particle-free ethanol.

3 Place the tools in a laminar flow hood or clean bench to dry.

Note

To further protect the tools and dissection tray from particulates within the hood, place tools on top of aluminum foil within the hood, covering them with an upside-down dissection tray. Keep one edge of the dissection tray lifted by setting it on a forceps handle to ensure the tools can completely dry.

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4 Once all tools are dry, place them in the dissection tray(s) and cover the tray(s) with aluminum foil until needed.

Note

Be sure there are no tears or damage to the aluminum covering that will reduce its shielding function.

Sterilization of the cadaveric specimen

5 Locate area where tissue extraction will occur. Thoroughly scrub the area.

6 Using a razor, shave off external body hair from the area. Use the razor in multiple directions to ensure that all the hair is removed.

7 Using a glass syringe, flush the target area, in a downward direction, with particle-free water (3 times) followed by flushing it with particle-free ethanol. Repeat the flushing sequence 4 times.

7.1 Flush with particle-free water. (1/12)

7.2 Flush with particle-free water. (2/12)

7.3 Flush with particle-free water. (3/12)

7.4 Flush with particle-free ethanol. (1/4)

7.5 Flush with particle-free water. (4/12)

7.6 Flush with particle-free water. (5/12)

7.7 Flush with particle-free water. (6/12)

7.8 Flush with particle-free ethanol. (2/4)

7.9 Flush with particle-free water. (7/12)

7.10 Flush with particle-free water. (8/12)

7.11 Flush with particle-free water. (9/12)

7.12 Flush with particle-free ethanol. (3/4)

7.13 Flush with particle-free water. (10/12)

7.14 Flush with particle-free water. (11/12)

7.15 Flush with particle-free water. (12/12)

7.16 Flush with particle-free ethanol. (4/4)

Extraction of Tissue: Internal body fluids (representative bo.

8 Using a scalpel (size 10 blade), make a 7.5 cm vertical incision from the umbilicus running toward the xiphoid process.

Note

Be sure to only cut through the skin, adipose, and musculature. If you cannot visually see the depth of the incision, use your fingertips to feel that you have cut through the musculature but have not cut into the greater omentum covering the abdominal viscera or the abdominal viscera itself.

9 At both the superior and inferior points of the incision, use the scalpel to cut laterally in either direction. The lateral incision should not exceed 5 cm.

10 Using forceps and your fingers, locate the preferred section of the intestine and pull it out of the abdominal cavity without additional incisions, leaving any other viscera in place.

Note

A small pair of scissors can be used to cut through peritoneal structures that might be covering the intestine (e.g., greater omentum, posterior rectus sheath).

11 After the desired segment of the intestine is removed from the abdominal cavity, use the linear

stapler to secure contents within the intestine. Staple 2 sites, 3 cm from each other, on both the proximal and distal ends of the intestinal segment.

12 Use scalpel to cut between the staple sites on both ends.

13 Remove the segment of intestine so that contents can later be removed and analyzed.

Extraction of Tissue: Soft tissue (representative body regio..

14 Using a scalpel (10 blade), make a 5 cm incision in the intercostal space, cutting through skin and musculature.

Note

When possible, the body should be in a supine position. An incision between the 3^{rd} and 4^{th} or 4^{th} and 5^{th} rib on the superior aspect of the thoracic cage will often give more space to maneuver the tools and will also provide better, more visible access to the lung tissue.

15 Using your fingers, locate the lung tissue. Insert forceps or a hemostat into the incision, using the tool to grab and stabilize the lung segment.

16 Still holding the lung segment, push the biopsy punch through the tissue.

Note

If the biopsy punch does not transect the tissue segment, angle the punch in a superior direction while turning it 360 degrees, breaking the tissue within the biopsy punch free from the intact lung.

17 Remove the biopsy punch from the thoracic cavity and use forceps or a probe to push tissue through the biopsy punch and into a glass container.

18 Repeat until you have collected the desired amount of tissue.

Extraction of Tissue: Tissues lining body orifices (represent..

19

Insert the scalpel blade into one or both nostrils and gently scrape the lateral and medial walls, removing portions of the mucosal membrane.

20 Insert glass collection jar into nares.

> Note
>
> If the nostril diameter is larger than the glass collection jar, pinch the surrounding skin to seal the space.

21 Use a 16-gauge syringe needle to pierce the lateral nostril wall just superior to the inferior edge.

22 Using a new needle, draw particle-free water into a glass syringe and insert the needle into the nostril through the piercing. With the needle pointing in a superior direction, flush the nasal cavity. Allow the particle-free water and nasal contents to flow into the glass collection jar.

Containment of Tissue Samples

23 Seal the glass containment jar(s) with metal lid(s), aluminum foil, or glass lid(s).

24 Human tissues should be frozen at -20° C until they are ready for analysis preparation. Leave

additional space at the top of the collection jar to account for the expansion of water within the tissue.

Note

If tissues are to be stored in other liquids (e.g., formalin, formaldehyde) after extraction, be sure that these liquids are also filtered and added as a component of the blanks to later be analyzed for contamination.