Quantifying cross-contamination from tissue homogenization with a rotor-stator homogenizer equipped with a stainless steel generator probe.

In research labs, rotor-stator homogenizers are critical pieces of equipment as they are routinely used as the first step in many experimental workflows. Rotor-stator homogenizers are used to break down solid samples to create a homogenous mixture and release internal analytes such as proteins and nucleic acids prior to downstream analyses. The rotor-stator homogenizer functions by rapidly spinning the rotor with high speed, creating a vacuum that pulls the media between the rotor and outer stator. The media is pushed axially until they reach the gaps in the stator at which point the media is subjected to rapid deceleration forces that lead to sample disruption; a process known as shearing (Figure 1).

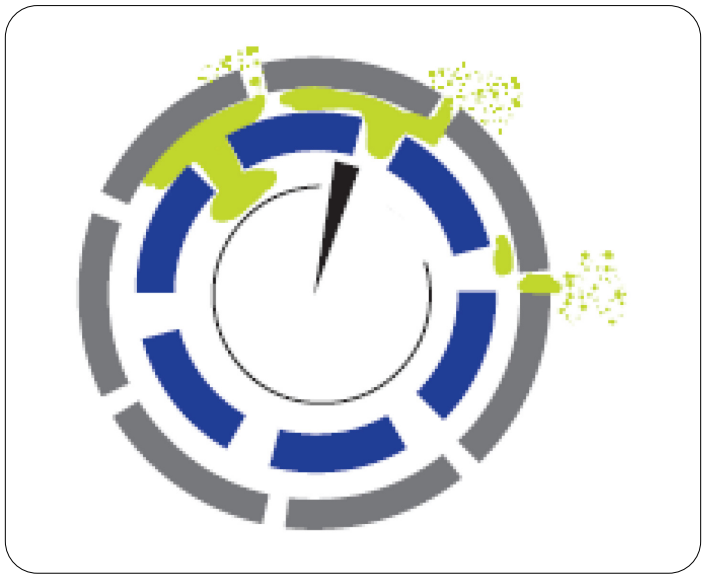
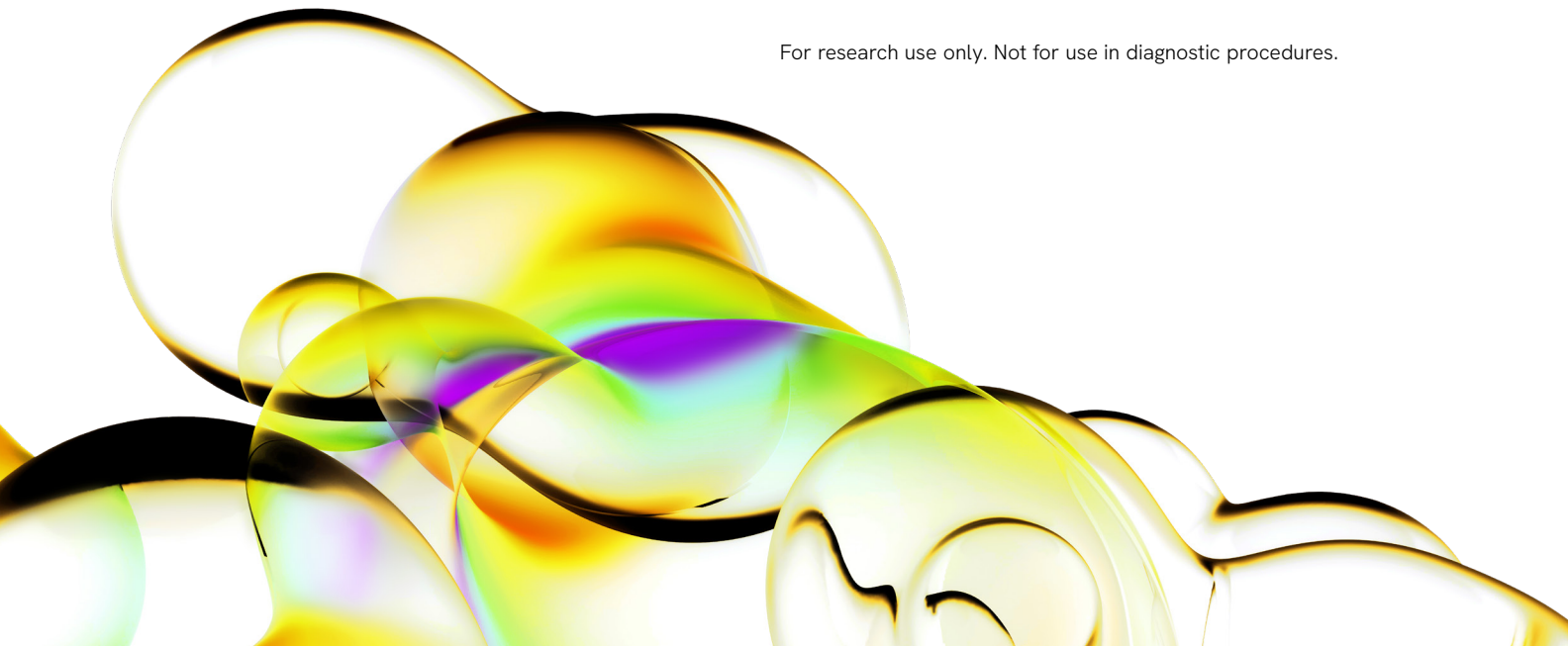


Figure 1. Principle of rotor-stator design.

For research use only. Not for use in diagnostic procedures.



While rotor-stator homogenizers are extremely effective, with the exception of automated platforms like the Omni Prep 96 and LH96 workstations, they are one use tools in which samples must be processed on an individual bases. Between samples, researchers must clean the generator probe to remove sample particulate in order to prevent cross-contamination between samples. Typically the cleaning method involves running the homogenizer at low speed in a clean liquid to "spin out" particulate retained in the probe. In some cases solvents such as ethanol can expedite the cleaning procedure. To reduce cross-contamination risk significantly, especially for studies involving nucleic acids, the generator probe must be fully disassembled, cleaned and autoclaved prior to the next sample being processed.

As an alternative to stainless steel generator probes, disposable plastic Omni Tip™ generator probes can be disposed of after each use to significantly reduce cross-contamination risk (Figure 2).

Figure 2. 7 mm Hard Tissue Omni Tip™.

Herein, we evaluate the effectiveness of standard generator probe cleansing processes to quantify the degree of DNA and protein carryover from sample to sample using a standard stainless steel generator probe.

Materials & methods

Equipment

- **Omni Tissue Homogenizer (TH)** (Cat # TH115, TH220)
- **7 mm Stainless Steel Generator Probe** (Cat # B7-110ST)

Liver homogenization and carryover

1 g of Sprague-Dawley rat liver (Bioreclamation Inc.) was added to 3 mL of DDH_2O in a 15 mL conical centrifuge tube (Cat # 19-6615). The sample was homogenized with an Omni Tissue homogenizer (Cat # TH115) fitted with a 7 mm stainless steel probe (Cat # B7-110ST) for approximately 30-45 seconds until the sample was fully homogenized. The generator probe was then washed for 20 seconds in approximately 7 mL of DDH_2O, followed by a pulse for 5 seconds in a clean tube containing 3 mL of DDH_2O. The wash-pulse step was repeated 1 more time in clean tubes for a total of three 3 mL carryover samples, three 7 mL washes and one 3 mL liver sample.

DNA extraction and separation

300 µL was aliquoted from the original liver sample and each of the three carryover samples and added to 200 µL of commercially available tissue lysis buffer. 25 µL of Proteinase K was added to each buffered tube and incubate for 1 hour at 60 °C, while vortexing every 15 minutes. DNA was then extracted using a commercially available tissue DNA extraction kit per manufacturer's instructions. To avoid overloading the spin column during extraction, the sample was split in half and processed over two columns in volumes of 300 µL, then combined. 1 µL of each eluant was taken for DNA quantification on the NanoDrop Spectrophotometer (Thermo Fisher).

2 µL from each sample was then mixed with 10 µL TBE/UREA loading dye (BioRad) and separated on a 5 % TBE agarose gel (BioRad) in TBE running buffer (BioRad). Electrophoresis was carried out at 200 V for 45 minute. The gel was then stained in ethidium bromide for 15 minutes and visualized on a GelDoc EZ System after a single wash with DDH_2O.

Protein extraction and separation

500 µL of each homogenate was transferred to a clean 1.5 mL microcentrifuge tube and centrifuged at 10,000 x g for 10 min. The supernatant was removed and 1 µL was analyzed on a NanoDrop spectrophotometer at 280 nm to determine protein concentrations.

10 µL of the supernatant was added to Laemmli sample buffer (BioRad) and heated to 95 °C for 5 minutes. The proteins were separated by electrophoresis on a 4-20% Tris-Glycine SDS polyacrylamide gel at 200 V for 45 minutes in Tris-Glycine SDS running buffer (BioRad). The gel was then stained in InstantBlue (Expedeon) for 1 hour and visualized on a GelDoc EZ System (BioRad).

Results

Protein and DNA quantification

In this study we investigated the degree of cross-contamination generated through standard cleaning procedures following tissue homogenization with a stainless steel generator probe. Rat liver samples were homogenized and the probe was washed sequentially. Following each wash, both DNA and protein concentrations were quantified to determine the level of cross-contamination that would occur in a typical laboratory study. It's important to note that by fully disassembling the generator probe and washing the individual components, cross-contamination can be avoided. However, the focus of this study was to evaluate the utility of standard washing and "spin out" procedures as this is the most widely used method of generator probe cleaning. The protein and DNA yields were quantified on a NanoDrop spectrophotometer as shown in Table 1.

Table 1. Protein and DNA concentrations as a function of wash steps. LOD indicates limit of detection.

Sample ID	Average protein concentration (mg/mL)	Average DNA concentration (mg/mL)
Liver Sample	53.01	241.67
Wash 1	3.63	177.97
Carryover 1	0.35	1.17
Wash 2	0.01	Below LOD
Carryover 2	Below LOD	Below LOD

As shown in table one, the initial extraction of the rat liver sample produced excellent protein and DNA yields. Following the initial homogenization, the probe was run in 7 mL of DDH_2O to clean out any tissue particulate remaining in the probe (Wash 1). Following the first wash, the probe was placed in a clean tube containing 3 mL of DDH_2O and run to mimic processing of a second sample (Carryover 1). This process was repeated with a second wash and carryover. Figure 3 shows the tube contents following one wash step. As expected, the first wash contained a large amount of tissue matter and small particulates were also observed in the first carryover sample following the second wash. Following protein/DNA extraction and quantification it was determined that the first carryover sample contained 0.35 mg/mL protein and 1.17 ng/µL of DNA. After the third wash no DNA was detected but proteins were detected at a concentration of 100 µg/mL. While no DNA was detected by spectrophotometric analysis it is possible that DNA would be present at concentrations below the limit of detection of our system. For studies performing PCR amplification, small quantities of nucleic acids below 1 ng/µL would certainly constitute contamination.

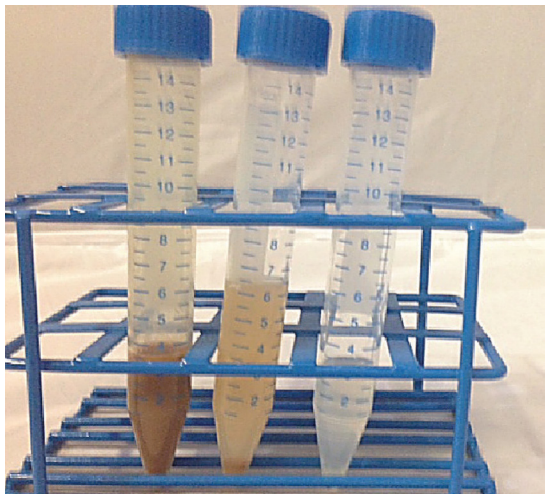


Figure 3. Tissue cross-contamination observed after one wash step.

Proteins and DNA were also visualized by gel electrophoresis and staining to evaluate the degree of cross-contamination following each wash step. Figures 4 and 5 are the protein gel and DNA gel respectively. The data presented in table 1 is further validated in the gel images. It is clear that even after a 20 second wash step that significant carryover is observed for both proteins and DNA. While protein was measured at 100 ng/mL in the second wash sample this concentration of protein is below the detectable limit for coomassie blue staining and thus no protein bands were observed in lane five.

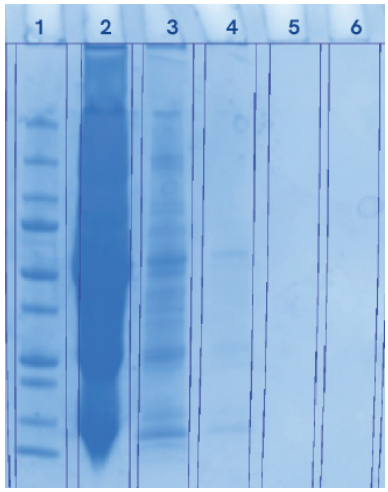


Figure 4. Protein polyacrylamide gel electrophoresis of liver homogenate and washes. Lane 1: Protein Ladder. Lane 2: Liver homogenate. Lane 3. Wash 1. Lane 4. Carryover 1. Lane 5. Wash 2. Lane 6. Carryover 2.

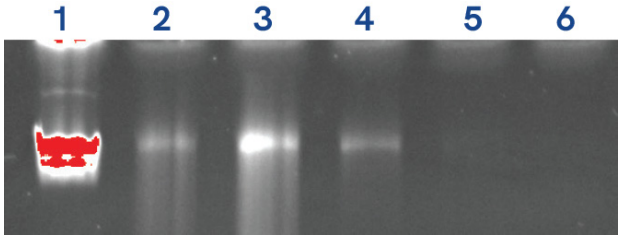


Figure 5. DNA agarose gel electrophoresis of liver homogenate and washes Lane 1: 2.5 kb DNA ladder. Lane 2: Liver homogenate. Lane 3: Wash 1. Lane 4: Carryover 1. Lane 5: Wash 2. Lane 6: Carryover 2.

Conclusion

Rotor-Stator homogenizers are capable of disrupting a wide range of samples and producing high protein and DNA yields. The extraction of both molecules from rat liver tissue was demonstrated here and washes were performed followed by quantification of cross-contamination following each step.

Despite the generator probe being washed multiple times, analyte carryover was observed. While cross-contamination risk can be significantly reduced by probe disassembly and washing, the majority of laboratories perform wash procedures as described here without disassembling the probe. In these cases, based on the carryover observed following two wash steps, it is recommended that at least two washes and probe "spin outs" be performed using clean tubes and buffer prior to the next sample being processed. For many studies this method should be sufficient to remove background contamination. However, significant care should be taken when performing PCR based studies as very little carryover is required to contribute to erroneous results. In these cases we would recommend disposable probes such as the Omni Tip™ plastic generator probes.

Omni Tissue Homogenizer (TH)

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