APPLICATION NOTE

Revolutionizing umbilical cord sample preparation with the Omni LH 96 automated homogenizer workstation for total protein quantification.

Summary

Automation has emerged as a groundbreaking innovation in laboratories, revolutionizing scientific research and experimentation processes by delivering high-throughput results. By integrating advanced technologies, laboratories have transitioned from manual to automated procedures, leading to unparalleled efficiency and precision while saving the cost of manual labor. Unlike traditional manual methods, automated systems can perform tasks with unwavering accuracy, minimizing the margin of error in experiments. This consistency is pivotal in scientific research, where even minor variations can significantly impact the outcomes of sensitive assays. Automated workstations can also accelerate the pace of experimentation allowing researchers and technicians to shift focus to more intellectually stimulating tasks like data analysis and new experimental planning.

These automated processes provide exceptional precision and accuracy; however, most automated workstations are tailored to cater to only one part of sample preparation process such as liquid handlers, exclusively managing pipetting duties, or plate hotels managing storage of reagents and prepared samples. If a sample requires homogenization before pipetting, these liquid handlers are deemed a semi-automatic process, necessitating some degree of manual handling and supervision, thus lacking a truly “walk-away” solution. Similarly, many homogenizers, while efficient in homogenizing samples, have their limitations in capabilities when it comes to liquid addition and transfer of homogenate to more high throughput friendly vessels like well plates.

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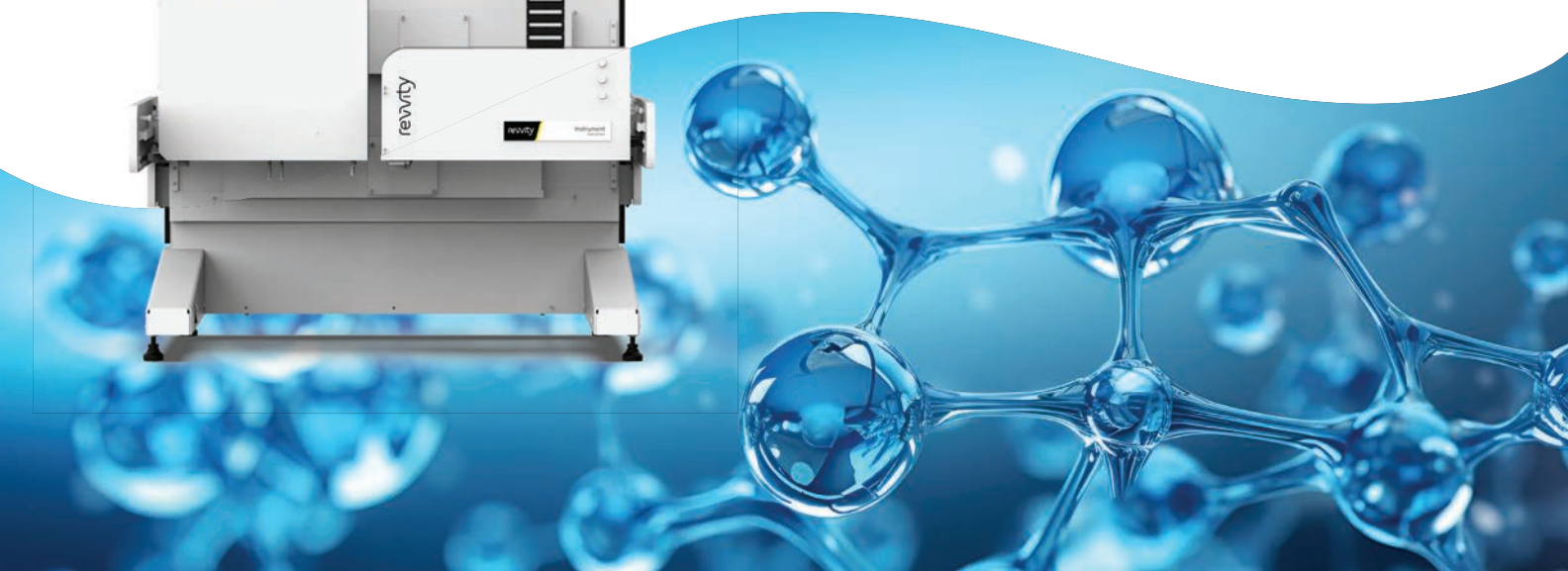

One tough sample matrix, umbilical cord, has emerged as a compatible matrix for detecting illicit drug exposure to infants during pregnancy. It can also identify potential biomarkers indicative of various diseases through proteomics applications.[1] The National Institute on Drug Abuse (NIDA) highlighted a rise in illicit drug use during gestation, underscoring the urgency for high throughput homogenization of these samples.[2] Nevertheless, obtaining an umbilical cord homogenate that can be pipetted is challenging due to the cord's cartilaginous and fibrous tissue structure.

Herein, we address the challenge presented in working with a tough sample matrix by showcasing the effectiveness of the Omni LH 96 automated homogenizer workstation and 12 mm Hard Tissue Omni tip in an automated tube to plate solution
for preparation of umbilical cord samples for total protein analysis.

Materials & Methods

Equipment

- Omni LH 96 automated homogenizer workstation (Cat # 23-010)
- 12 mm Hard Tissue Omni Tip™ (Cat # 321250)
- 50 mL x 24 Tubes Standard Sample Rack (Cat # 23-HWR-12)
- 50 mL Tubes with Screw Caps (Cat # 19-6650)
- Disposal Rack for Dirty Probes/Tips (Cat # 23-HWA-13B)
- Rack for 12 mm Clean Probes/Tips (Cat # 23-HWA-13D)

Procedure

Two methods for extracting soluble protein from umbilical cord homogenate were created on the Omni LH 96 automated homogenizer workstation to examine the platform's ability to uniformly homogenize samples and deliver consistent downstream analysis. Using on-board functionality of the Omni LH 96 automated homogenizer workstation, the following was performed: the first method utilized the on-board function to weigh tubes and samples to quantify sample starting masses. After quantifying each sample and prior to dissociation, the instrument dispensed liquid buffer using a predetermined ratio of buffer volume to sample weight. The second method used a fixed volume of processing buffer, dispensed by the automated homogenizer workstation, to prepare samples in ascending starting weights.

Sample acquisition

De-identified paraformaldehyde-fixed human umbilical cord was procured by Emory University Hospital (Atlanta, Georgia) Tissue Procurement Lab under internal IRB .

Samples prepared using weight by volume addition

Human umbilical cord samples were sorted into mass tiers to create 5 groups of samples. Each weight group consisted of 4 replicates weighing 250 mg, 300 mg, 400 mg, 500 mg, and 750 mg each with a 1 % tolerance. Samples were then placed into 50 mL conical tubes that had been pre-weighed by the Omni LH 96 using the weighing function. Phosphate buffered saline (PBS) was then added to each sample tube using a predetermined weight by volume ratio of 40 µL of PBS to 1 mg of tissue sample. The homogenization parameters were set in the Omni LH 96 software as shown in Table 1. Samples were serially homogenized on the Omni LH 96 automated workstation utilizing a 12 mm Hard Tissue Omni Tip according to parameters in Table 1.

Samples prepared using fixed volume addition

Human umbilical cord samples were sorted into mass tiers to create 5 groups of samples. Each group consisted of 4 replicates weighing 125 mg, 250 mg, 300 mg, 500 mg, and 750 mg each with 1% tolerance. All samples were transferred to 50 mL conical tubes on the processing deck and were diluted with a fixed volume of 10 mL of PBS pipetted by the Omni LH 96. Samples were serially homogenized on the Omni LH 96 automated workstation utilizing a 12 mm Hard Tissue Omni Tip according to parameters in Table 1.

Protein quantification

Post homogenization, 400 µL of each sample was transferred from the 50 mL conical processing tubes into a single 96-well plate utilizing the reformatting function of the Omni LH 96 automated homogenizer workstation. The well plate was then centrifuged at 2,700 x g for 5 minutes to pellet insoluble proteins and cellular debris. 25 µL of the supernatant from each well was transferred to an optically clear 96-well plate in triplicate wells. Pierce™ Rapid Gold BCA Protein Assay Kit (Fisher Scientific, Cat. # A53225) reagents were mixed according to manufactures instructions

to prepare a standard curve and prepare umbilical cord lysates for total protein extraction. Steps were followed in the detailed manufactures protocol to quantify both the standard curve and the unknown samples using an ELX808 plate reader. Data quantified from this assay is displayed below.

Data analysis

All BCA assay data (including the standard curve) was tabulated and plotted using Microsoft Excel 365. The BSA standard curves were plotted using the function y=mx+b. Protein concentrations (µg/mL) were obtained by interpolating sample signals to the BSA standard curve. These concentrations were plotted against the umbilical cord weight. Calculated protein concentration data from both fixed volume and weight by volume groups were analyzed to determine statistical significance using SPSS (version 29). In addition, an ANOVA (2-way) and Welch's Robust Tests of Equality were performed to determine significance between weight sets for protein concentration data from fixed volume group, as well as weight by volume group.

Table 1: Parameters used on the Omni LH 96 to homogenize umbilical cord samples.

Homogenizing Parameters			
Motor speed	20,000 rpm	Up down velocity	10 mm/s
Homogenizing duration	60 sec	Left right distance	3 mm
Height from bottom of tube	2 mm	Left right velocity	10 mm/s
Up down distance	2 mm	Delay for each movement	500 msec
Touch off Probes Parameters			
Distance from top of tube	25 mm	Motor speed	12,000 rpm
Distance from center of tube	3.2 mm	Touch off Time	2 sec

Results

The Omni LH 96 automated homogenizer workstation completely dissociated the samples and allowed for automated pipetting to reformat lysates for downstream total protein analysis. Average protein concentrations between replicates from each fixed volume weight group are listed in Table 2. Protein concentration from fixed volume group showed a linear relationship (R^2=0.98) indicating that protein concentration is affected by starting tissue weight when keeping buffer volume fixed. It was shown via ANOVA that protein concentrations between weight groups in fixed volume set are statistically significant from one another ($p \leq 0.001$).

Additionally, average protein concentrations between replicates from each weight group in weight by volume set are listed in Table 3 along with a graph showing distribution of weights in each weight group (Figure 2). Here we expected a non-significant difference between samples because the weight to volume increases are equal throughout samples. It was shown via ANOVA that protein concentrations between weight-to-volume groups are statistically insignificant from one another ($p \geq 0.05$).

Table 2: Average protein concentration and standard deviation between replicates in each weight group for fixed volume (n=12).

Starting Umbilical Cord Mass (mg)	125	250	300	500	750
Average protein Concentration (µg/mL)	2.8	29.9	77.4	142.4	217.6
Standard Deviation (µg/mL)	±10.2	±9.0	±31.3	±53.8	±76.7

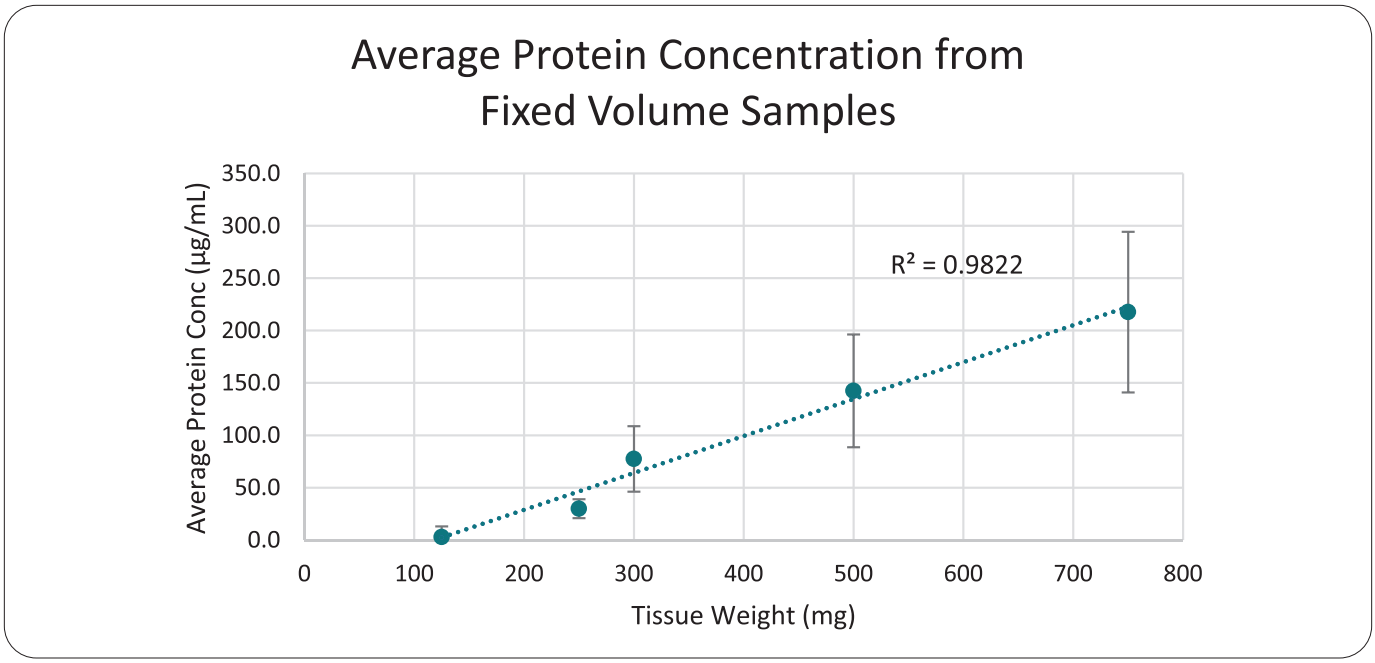

Figure 1. Linear regression distribution of average protein concentrations grouped by starting mass of samples prepared using fixed volume addition homogenization method.

Table 3. Results from BCA assay. Average absorbance and calculated protein concentrations for umbilical cord samples prepared using weight by volume addition (n=12).

Weights (mg)	250	300	400	500	750
Average protein concentration (µg/mL)	40.1	39.3	60.8	33.1	53.9
Concentration Standard deviation	±23.1	±13.8	±47.8	±12.8	±31.9

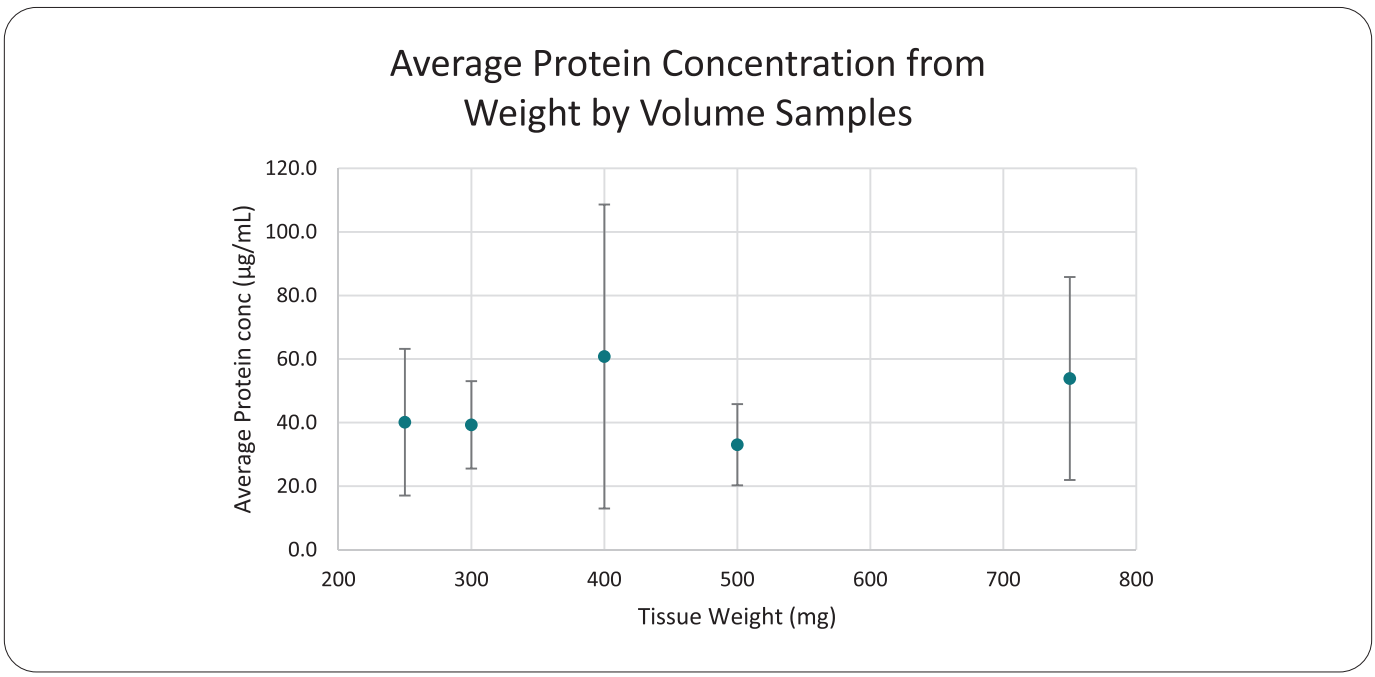

Figure 2. Distribution of average protein concentrations grouped by starting mass of samples prepared prepared using weight by volume addition homogenization method (n=12).

Conclusions

Walk away workflow automation and homogenization of fibrous and cartilaginous samples like umbilical cord is easily accomplished by the Omni LH 96 automated homogenizer workstation. A wide range of starting sample sizes from small, 125 mg, samples to larger, 750 mg, samples all readily homogenize in as little as 10 mL of processing buffer to as much as 35 mL of processing buffers using the 12 mm Hard Tissue Omni Tip prior to total protein determination. Robust statistical analysis of protein concentrations from both weight by volume and fixed volume groups, independently, reinforce the capabilities of the Omni LH 96 automated homogenizer workstation to weigh, dilute and homogenize samples precisely while returning data that aligns with statistical hypotheses. Furthermore, samples prepared using fixed volume dilution were found to have protein concentrations that are statistically significant which is expected as buffer volume was kept constant, and tissue weight increased. Samples prepared using weight by volume dilution were found to have protein concentrations that were not statistically significant from one another, resulting from the precision in sample prep when pre-diluting samples using a weight by volume addition scheme. Overall, the results indicate that homogenization, dilution, and reformatting of the samples on the automated workstation using weight by volume and fixed volume formats is achievable. The instrument's integrated weighing and liquid dispense platform can precisely determine sample and tube weights and prepare pre-determined weight by volume dilutions prior to sample homogenization with the user's buffer of choice.

To relate these results to the field of proteomics, total protein recovery is vitally important to downstream applications like enzyme linked immunosorbent assays (ELISAs) and Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) that depend on total protein recovery prior to targeted analyte detection. However, recovery of intracellular analytes is not limited to proteins. Other downstream analysis methods like HPLC, mass spectrometry, and DNA sequencing all benefit from repeatable sample preparation techniques. Ultimately, the Omni LH 96 automated homogenizer workstation and 12 mm Hard Tissue Omni Tip offers a robust and reliable solution that provides users with tube-to-plate automation prior to downstream assays.

References

1. Conrad, Matthew S., et al. "Proteomic analysis of the umbilical cord in fetal growth restriction and preeclampsia." *Plos* one 17.2 (2022): e0262041.

2. National Institute on Drug Abuse, National Pregnancy & Health Survey: Drug Use Among Women Delivering Live Births: 1992, National Institute on Drug Abuse, Rockville, 1996, pp. 1–F157.

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