Automated Tissue Dissection Solution to Support Comprehensive Genomic and Immune Profiling

Jeffrey M. Conroy[1]; Guido du Pree[2]; Reinhold Wimberger-Friedl[2]; Joel Duckworth[2]; Roel Stroucken[2]; Erin A. DeBlasi[1]; Daniel Metzger[1]; Jie An[1]; Michael J. Collins[1]; Jonathan Andreas[1]; Nancy Kelly[1]; Linnea Menin[3]; Vanessa Process[3]; Thuy Dao[3]; Luca Beker[3]; Rachel Mittelman[3]; Martina Werner[3]; Kristopher Amirault[3]; Ulrich Thomann[3]; Eugenio Daviso[3]; Taylor Jensen; Shengle Zhang[1]; Durga P. Dash[1]

[1]Labcorp Oncology, Buffalo, NY, USA. [2]Xyall B.V. (https://xyall.com), Netherlands, [3]Covaris LLC (https://www.covaris.com), Woburn, MA, USA

Introduction

- Comprehensive genomic and immune profiling (CGIP) is a powerful precision medicine tool as it enables simultaneous identification of multiple biomarkers to guide cancer diagnosis, therapy selection and prognostication. For these reasons, CGIP is standard of care for many cancer types.
- CGIP requires formalin-fixed paraffin embedded (FFPE) tumor tissue collected as fine needle aspirations and biopsies (including core needle biopsy, resection and excision).
- To ensure successful CGIP testing, accurate enrichment of cancer cells within a specimen is critical (i.e., tumor content). Currently the manual process of scraping specified tissue regions of interest (ROI) from FFPE unstained slides (USS) is inefficient, subjective, labor-intensive and error prone.
- To address these issues, we evaluated the Xyall® Tissector High Throughput (HT) system, a fully automated tissue dissection solution for automated FFPE tissue scaping (Figure 1).

Figure 1. Xyall Tissector HT system is designed for continuous operation and can dissect over 80 tissue slides per hour using a disposable scraping head

Methods

- 10 FFPE tissue blocks representing real-world cases and different tumor types were sectioned as 5µm USS.
- Following H&E review, ROIs containing >20% tumor content were marked by an Anatomical Pathologist.
- The ROIs of the even-numbered slides for each case were dissected using the Xyall® Tissector HT automated system, while the same ROI for the odd-numbered slides were manually dissected by a trained Labcorp operator using a razor blade (Figure 2).
- Tissues scraped by both methods, including a set of replicates, were transferred to tubes for DNA/RNA co-extraction using the Covaris truXTRAC® FFPE SMART Solutions.
- ROI tissue scraping images and DNA/RNA quality and quantity (including yield, purity, fragment size, DNA amplifiability) were obtained and compared across both dissection methods (Figure 3).
- Additionally, a DNA ID test to assess sample contamination was performed.

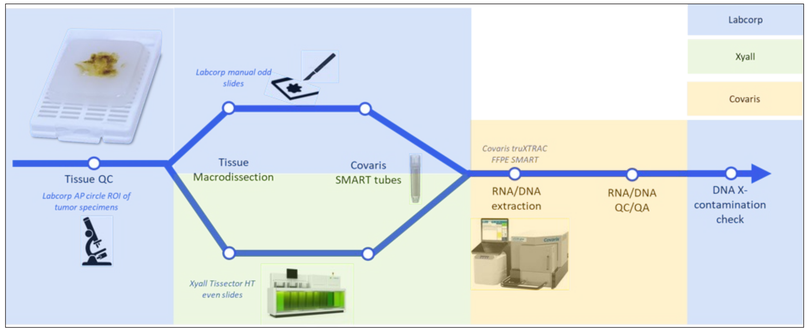

Figure 2. Tissector HT system study plan and stakeholders (Labcorp = blue, Xyall = green, Covaris = yellow)

The automated Tissector HT provides consistent and efficient scraping of cancer patient's FFPE tissue in specified regions of interest

Block ID	ROI Size #	Tumor type	Slide # Xyall	Slide # OS	Purpose	Total GEN3 tubes *	Total slides
RS-02271279	L	colon	1	2	replication	3 X, 3 L	8
			3	4			
			5	6			
RS-02270170	L	lung	1	2	replication	3 X, 3 L	8
			3	4			
			5	6			
CAL-02262549	L	colon	1, 3, 5, 7, 9, 11	2, 4, 6, 8, 10, 12	multi-slide	1 X, 1 L	14
CAL-02264169	S	colon	1, 3, 5, 7, 9, 11	2, 4, 6, 8, 10, 12	multi-slide	1 X, 1 L	14
RS-2273489	S	lung	1	2	replication	3 X, 3 L	8
			3	4			
			5	6			
RS-02299730	L	melanoma	1	2	multi-foci	1 X, 1 L	4
RS-02281621	L	lung	1, 3, 5, 7, 9	2, 4, 6, 8, 10	multi-foci, multi-slide	1 X, 1 L	12
RS-02696484	S	melanoma	1	2	replication	3 X, 3 L	8
			3	4			
			5	6			
SE-013299	L	melanoma	1	2	multi-foci	1 X, 1 L	4
SE-014066	M	melanoma	1	2	replication	3 X, 3 L	8
			3	4			
			5	6			

SMART

DNA

1. Quantitation
   - ng DNA (PicoGreen)
2. Qualitation
   - 260/280 (Nanodrop)
   - 260/230 (Nanodrop)
   - KAPA Q ratio
   - Fragment size (Fragment analyzer)

RNA

1. Quantitation
   - ng RNA (RiboGreen)
2. Qualitation
   - 260/280 (Nanodrop)
   - 260/230 (Nanodrop)
   - DV200 (TapeStation)

Evaluation (case and overall level)

1. Material scraped
   - Tissue ROI and paraffin (X/L ratio)
2. Quantitation
   - Xyall/Labcorp (X/L % change)
3. Qualitation
   - Xyall/Labcorp (X/L % change)
4. Replication
   - Standard Deviation (STD)
   - Average (mean)
   - Coefficient of Variation (CV)

Figure 3. FFPE specimen characteristics, slides sectioned, study purpose and downstream nucleic acid assessment. #S = small (3 x 3 mm²), M = medium (8 x 8 mm²), L = large (15 x 15 mm²); *X = Xyall, L = Labcorp

Results

- The Xyall® Tissector HT successfully scraped 19 of 20 ROIs with minimal deviation from the annotated boundary (Figure 4 as an example).
- Manual scraping resulted in more variability in scraped tissue mass and inclusion of peripheral paraffin and tissue in adjacent non-ROI regions.
- The average DNA quantity (Xyall = 92.3ng; Manual = 92.4ng), RNA quantity (Xyall = 956ng; Manual = 1014ng) and quality (260/280, 260/230) were very comparable between both methods when normalized to the ROI tissue scraped (Figure 5).
- For precision, the Xyall® Tissector HT replicates resulted in more consistent quality metric results (Figure 5) and less variability in DNA/RNA fragment size.
- The DNA ID test showed no evidence of sample cross-contamination in either method.

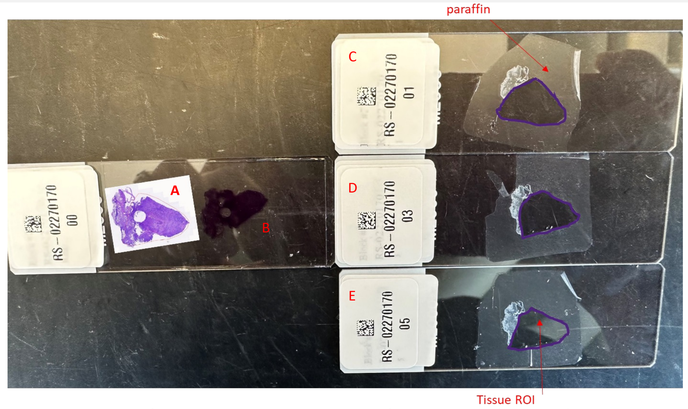

Figure 4. Case RS-02270170. A) H&E image with ROI marked red. B) H&E slide. C – E) Images of replicate unstained slides with tissue scraped and removed by Tissector HT in marked ROI.

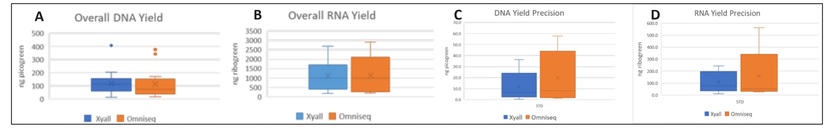

Figure 5. Comparison of overall DNA yield (A), RNA yield (B), DNA yield precision (C), and RNA yield precision (D) between dissection methods.

Conclusions

- The Xyall® Tissector HT is superior to manual methods in scraping FFPE tissue within a defined ROI, in minimizing excess paraffin, and in delivering consistent tissue sections for downstream DNA and RNA extraction and CGIP.
- The results show that the automated system can be considered for clinical implementation to support accurate and efficient dissection of patient's tumors.

Future Directions:

- Future study aims to validate and implement automated Tissector HT system for routine clinical use.

SLAS 2024 | Abstract ID:1684902

©2023 Laboratory Corporation of America® Holdings All rights reserved.