



# Molecular Validation Study of Nucleic Acids Extraction from Vacuum Sealed Surgical Pathology Specimens

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## Abstract

**Background:** Formalin is the universal fixative in surgical pathology laboratories world over, however, it is a highly toxic irritant and potentially carcinogenic. It requires diligent care in its usage. To eliminate formalin use for specimen transport to the laboratory, we placed the surgical specimens in plastic bags under-vacuum sealing. Our aim was to assess if this method of transport could be used routinely without compromising integrity of the nucleic acids in this molecular era.

**Design:** 15 surgically resected specimens from 3 anatomic sites (5 colon, 5 lung, 5 uterus) were selected for this pilot study. Specimens were placed in plastic bags under-vacuum sealing and transported at 4°C. 4 normal tissue samples using a 5 mm skin biopsy punch were collected at 0, 1, 2, 3 hour in duplicate; one snap frozen at -80°C & the other processed as formalin fixed paraffin embedded tissues (FFPE) [60 fresh, 60 FFPE]. Manual DNA column extraction protocol for genomic DNA isolation & total RNA isolation protocol to isolate total RNA were run. DNA/RNA quantities were evaluated by spectrophotometry. DNA integrity was assessed by amplification of commercial Control Size Ladder mix generating a series of amplicons of 100, 200, 300, 400 base pairs (bp). RNA integrity was assessed by real-time quantitative reverse transcription PCR by measurement of expression of  $\beta 2$  microglobulin (B2M) transcripts. Cycle threshold (Ct) values of 30 fresh & 30 for FFPE tissue samples were used with a cut-off of > 37.0 Ct for acceptable RNA quality.

**Results:** H & E stained sections from all the FFPE samples showed optimal histologic preservation. The yield of extracted DNA & RNA was adequate with good purity for both fresh & FFPE tissues [A260/A280 OD ratios >1.10 (range DNA:1.10-2.11, RNA:1.32-2.73)]. Fresh tissues: Total recovery between 6.46ug to 238.51 ug, the amplified DNA yielded product sizes of 300 bp for 54/60 samples, 400 bp for 53/60 samples. RNA from all the samples was of good quality, with acceptable B2M amplification in 28/30 samples. FFPE tissues: Total recovery between 3.12 ug to 81.09ug, the amplified DNA yielded product sizes of 300 bp for 60/60 samples, 400 bp for 60/60 samples. RNA from all the samples was of good quality, with acceptable B2M amplification in 29/30 samples.

**Conclusions:** Our data suggests that the DNA and RNA integrity is well preserved after 3 hours of vacuum sealing of the surgical specimens without formalin fixation. Vacuum-based preservation of surgical specimens is a viable, molecular friendly and environmentally-safe option for tissue transport to laboratory.

## Background

Formalin is a universal fixative in surgical pathology laboratories and used world over, however, it is a highly toxic irritant and potentially carcinogenic. Upmost care is necessary with its usage.

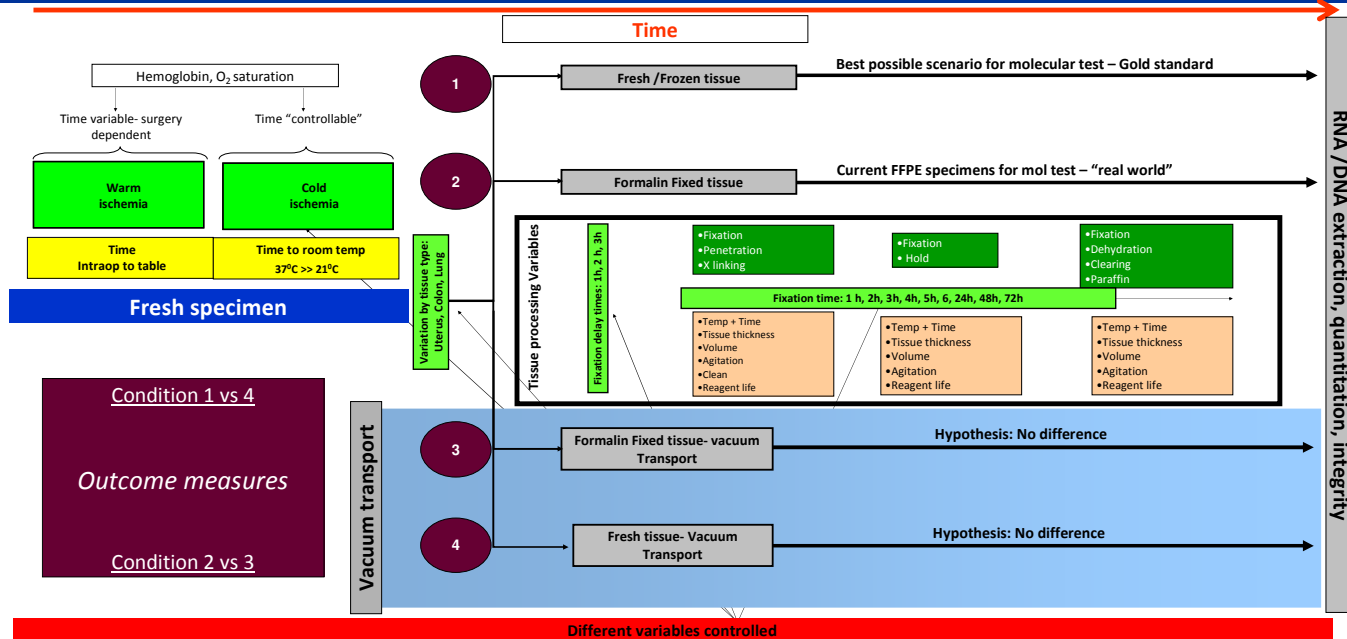
Our aim was to assess feasibility of formalin free transport of surgical specimens:

- to eliminate formalin use for specimen transport to the laboratory by using vacuum sealing and transporting the tissues at 4 °C.
- to assess if this method of transport is molecular friendly
- to assess morphologic changes affected by this method

## Materials and Methods

- 15 surgically resected specimens from 3 anatomic sites (5 colon, 5 lung, 5 uterus)
- Surgical specimens were placed in plastic bags under-vacuum sealing and transported at 4°C
- 4 tissue samples using a 5 mm skin punch biopsy were collected at 0, 1, 2, 3 hour in duplicate
  - one snap frozen at -80°C
  - one routinely processed as formalin fixed paraffin embedded tissues (FFPE).
- Manual DNA column extraction protocol was run for genomic DNA isolation
  - 60 fresh and 60 FFPE tissues
- Manual RNA isolation protocol was used to isolate total RNA from fresh and FFPE tissues.
- DNA/RNA quantity were evaluated by spectrophotometry.
- DNA integrity was assessed by amplification of commercial Control Size Ladder mix generating a series of amplicons of 100, 200, 300, 400, and 600 base pairs (bp).
- RNA integrity was assessed by real-time quantitative reverse transcription PCR (QRT-PCR) by measurement of expression of  $\beta 2$  microglobulin (B2M) transcripts.
  - Cycle threshold (Ct) values of 30 fresh frozen samples and 30 for FFPE tissue samples were used as a cut-off for acceptable RNA quality.

## Study design



## Results

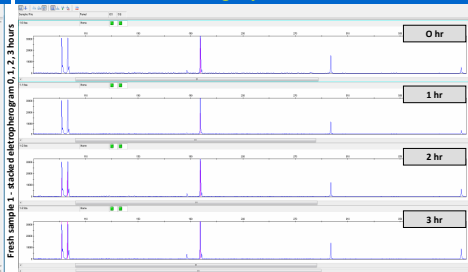
### Time points of specimen collection

Case Number	Tissues	Frozen Section Room		Surgical Pathology Room							
		Arrival Time to FS Room	Time Vacuum-Sealed	Zero Hour	1 HOUR		2 HOUR		3 HOUR		
1	Colon	11:30	11:40	FF	FF	FF	FF	FF	FF	FF	FF
2	Uterus	11:40	11:46	FF	FF	FF	FF	FF	FF	FF	FF
3	Uterus	11:50	11:56	FF	FF	FF	FF	FF	FF	FF	FF
4	Colon	14:20	14:36	FF	FF	FF	FF	FF	FF	FF	FF
5	Colon	15:09	15:17	FF	FF	FF	FF	FF	FF	FF	FF
6	Uterus	16:56	16:44	FF	FF	FF	FF	FF	FF	FF	FF
7	Uterus	12:50	12:56	FF	FF	FF	FF	FF	FF	FF	FF
8	Uterus	14:19	14:31	FF	FF	FF	FF	FF	FF	FF	FF
9	Uterus	10:34	10:43	FF	FF	FF	FF	FF	FF	FF	FF
10	Colon	15:35	15:43	FF	FF	FF	FF	FF	FF	FF	FF
11	Lung	13:50	14:08	FF	FF	FF	FF	FF	FF	FF	FF
12	Lung	13:35	13:42	FF	FF	FF	FF	FF	FF	FF	FF
13	Lung	11:55	12:05	FF	FF	FF	FF	FF	FF	FF	FF
14	Lung	10:20	10:21	FF	FF	FF	FF	FF	FF	FF	FF
15	Lung	9:56	10:05	FF	FF	FF	FF	FF	FF	FF	FF

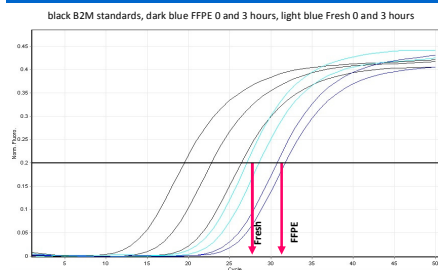
### FFPE DNA integrity- Size Ladder mix



### Fresh DNA integrity- Size Ladder mix



### RNA integrity- Beta 2 microglobulin transcripts



Sample ID	FFPE		Sample ID	Frozen	
	Ct Value	Frozen		Ct Value	Ct Value
1-0	26.60	22.99	9-0	26.77	22.73
1-3	27.36	29.40	9-3	27.10	24.40
2-0	26.68	23.77	10-0	27.52	24.30
2-3	27.14	26.54	10-3	24.89	24.53
3-0	27.16	23.08	11-0	26.79	25.70
3-3	28.15	24.44	11-3	25.07	33.85
4-0	fail	31.24	12-0	24.76	22.14
4-3	27.79	31.63	12-3	27.63	20.95
5-0	29.79	25.47	13-0	25.79	23.89
5-3	29.64	27.21	13-3	26.57	30.48
6-0	26.16	22.34	14-0	24.35	22.91
6-3	26.58	22.80	14-3	25.61	37.80
7-0	25.14	22.73	15-0	25.87	26.94
7-3	26.85	23.73	15-3	26.08	fail
8-0	29.67	22.81	Average	26.72	25.67
8-3	25.47	23.71	Range High	29.79	37.80
			Range Low	24.35	20.95

## Conclusion

- H & E stained sections from all the FFPE samples showed no significant morphologic artifacts with optimal histologic preservation.
- Our data suggests that the DNA integrity is very well preserved even after 3 hours of vacuum sealing of the surgical specimens without formalin fixation.
- RNA integrity was marginal in the FFPE with the delay in fixation while there was no significant decline in the fresh specimen cohort at any time point.
- Vacuum-based preservation of surgical specimens is a viable, molecular friendly and environmentally-safe option for tissue transport to laboratory.