
Histologic Validation of Vacuum Sealed, Formalin-Free Tissue Preservation, and Transport System

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Abstract

We describe five validation trials of new vacuum sealing technologies that change the approach to the preanalytic “front end” of specimen transport, handling, and processing and illustrate their adaptation and integration into existing Lean laboratory operations with reduction in formalin use and personnel exposure to this toxic and potentially carcinogenic fixative. These trials provide histologic assessment by numerous pathologists of tissues processed in this new paradigm and define the financial advantages of applying this technology to the postanalytic or “back end” process of tissue storage. We conclude that the TissuSAFE and SealSAFE vacuum sealing systems are both promising technologies for preserving fresh human specimens that can promote a safer environment by markedly reducing formalin use in operating room theaters and can minimize formalin use by laboratories.

Keywords

Formalin • Vacuum sealing • Tissue specimens • Histology

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With the advent of personalized medicine to individualize the profiling of patients for targeted therapies, the historical role of pathology to make an accurate tissue-based histologic diagnosis is now being challenged with new requirements. These include more timely diagnoses with triage and preservation of fresh tissues and documented quality control of the numerous currently uncontrolled preanalytic specimen variables of fresh and formalin fixed tissues that could affect the sensitivity of molecular based testing. These preanalytic variables will be important for assessment of biologic molecules at all levels of DNA, RNA, protein, and small signaling molecules. The new challenge for pathologists is to close the preanalytic “gaps” and identify molecular friendly techniques and technologies to be able to assure these requirements while relying on the advantages of formalin whose role as a fixative for morphologic based diagnoses is well over 100 years old [1].

In this chapter we describe five validation trials of new vacuum sealing technologies that change the approach to the preanalytic “front end” of specimen transport, handling, and processing and illustrate their adaptation and integration into existing Lean laboratory operations with reduction in formalin use and personnel exposure to this toxic and potentially carcinogenic fixative. These trials provide histologic assessment by numerous pathologists of tissues processed in this new paradigm and define the financial advantages of applying this technology to the postanalytic or “back end” process of tissue storage.

The technologies tested here are the TissueSAFE high vacuum biospecimen transfer system and the SealSAFE system, the latter capable of resealing specimens post-dissection and dispensing formalin into the vacuum-sealed bags based on a preset ratio related to specimen weight (Milestone Medical Srl, Bergamo, Italy). Experiences of others with the TissueSAFE system in Europe at a university based laboratory have been published previously [2, 3].

1 Validation Trial #1—Defining the Parameters of Temperature and Time

We initially tested the TissueSAFE device with human specimens from at the Main Hospital Operating Rooms (Ors) that arrived fresh in the adjacent Frozen Section Laboratory. We manipulated variables of temperature and time with vacuum sealed preservation of fresh specimens at 4, 7, 25 °C and held for variable times (24, 48, 72 h) at those temperatures before dissection and formalin fixation (Fig. 1). This was compared to samples from the same specimens that were immediately formalin fixed. Pathologists were blinded to the pairs being compared. Specimens were designated to any of 15 specialists and general surgical pathologists who evaluated the histologic features from hematoxylin and eosin stained glass slides based on a three part scheme of 1 = acceptable for diagnosis; 2 = inferior quality for diagnosis;

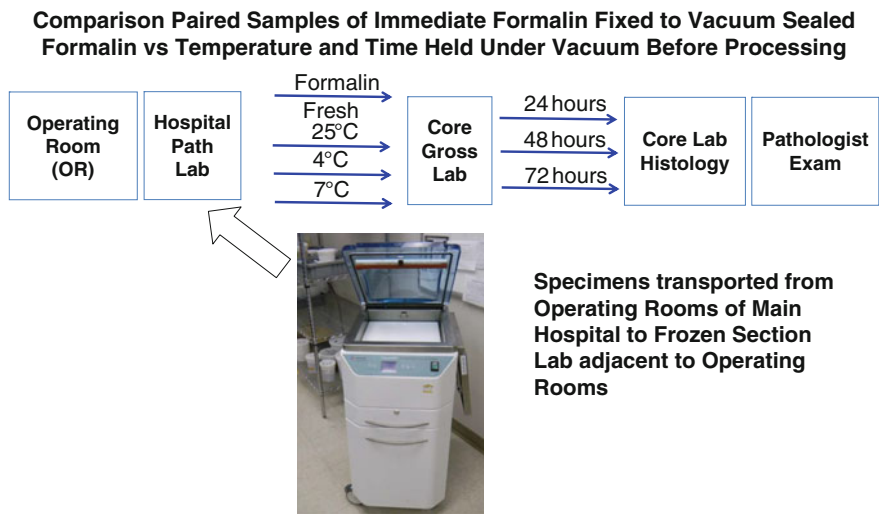


Fig. 1 Validation trial #1 main hospital specimens

3 = unacceptable for diagnosis. Of 50 blocks of tissue processed after sealing at 4 and 7 °C and held in simulated transport times of 24 and 48 h, 46 were found acceptable for diagnosis (Fig. 2). Four blocks of liver sealed at 4 °C were judged of inferior staining quality. These were considered under processed on review by the manager of the histology section. At a simulated transport time of 72 h, additional inferior quality stained slides were noted and therefore this prolonged time was no longer evaluated.

Variables Time and Temperature Under Vacuum

50 blocks evaluated-Formalin vs vacuum sealed
46 blocks acceptable after 24-48 hours at 4°C and 7°C

Large Specimens	1 acceptable	2 Inferior	3 unacceptable	HEMATOXYLIN AND EOSIN Scoring Scale
Leg	1	0	0	
Liver	1	1*	0	<ul style="list-style-type: none">• 1 = ACCEPTABLE FOR DIAGNOSIS• 2 = INFERIOR QUALITY• 3 = UNACCEPTABLE FOR DIAGNOSIS
Lung	1	0	0	
Lymph node	1	0	0	
Ovary	1	0	0	
Pannus	1	0	0	<ul style="list-style-type: none">* •4 liver blocks under-processed•Processed at 48 + 72 hrs at 4°C
Uterus	2	0	0	
Total	8	1	0	

Fig. 2 TissueSAFE histologic assessment of morphologic preservation

2 Validation Trial #2—Defining the Transport System from Community Hospital

One of our chief goals was to transport human tissues in the fresh state from a community hospital 25 miles away to a continuous flow core laboratory for dissection and processing (Fig. 3). Because we had to satisfy the requirement of five courier runs per day from the community hospital to the core laboratory, we innovated a means of transporting the vacuum sealed specimens in an insulated cooler between layers of plastic grids with conventional ice cubes in Ziploc bags place above and below the specimens (Fig. 4). An RFID card was included with each specimen run to record a temperature and time log for the transport run (Fig. 5). The optimal temperature achieved for transport with this mechanism was a stable 4 °C. In this pilot we evaluated 11 medium and large size tissue specimens assessed at transport delay times of 24 and 48 h on ice at 4 °C. All were judged to be acceptable for diagnosis by pathologists. The histologic assessment showed no differences in quality for specimens held in the chilled, airless state for 24 and 48 h (Fig. 6).

The TissueSAFE device has been subsequently located in the OR area and OR circulator nurses have been trained to triage and seal the specimens. We have now transported hundreds of specimens (roughly 56 % of OR based surgical volumes) from this community hospital in this manner for the past year without incident or pathologist complaint. The process receives very high marks from OR leadership and nurses. Large cubes of formalin are no longer stored and used in this OR suite to fill large specimen containers. Only small biopsy containers prefilled with small

- *Nurses Seal Specimens in Operating Room*
- *Transport Container Modifications*
- *Temperature Control*



Specimen transport 25 miles from Community hospital to Core Lab

Under vacuum at 4°C, 24-48 hours before fixation

Fig. 3 Validation trial #2 transport system from community hospital



Fig. 4 Transport by insulated cooler with conventional ice cubes in Ziploc bags



Fig. 5 RFID card records the run temperature

amounts of formalin are used in these ORs for small specimens and needle biopsies. The enhanced safety from reduced exposure of OR personnel to formalin is considered priceless by OR leadership.

Based on this TissueSAFE process, this community hospital laboratory has used 135 fewer gallons of formalin for an annual cost savings of \$1,688. The cost of consumables, plastic sealing bags replacing plastic bucket, was neutral. Additional savings not calculated are the courier fuel costs of transporting heavier specimen containers that would have been filled with formalin.

24 and 48 hours under vacuum at 4°C before fixation

Large Specimens	1 Acceptable 24 hours	1 Acceptable 48 hours	2 Inferior or 3 Unacceptable
Fallopian tube	1	n/a	0
Fistula soft tissue	1	1	0
Gall bladder	1	1	0
Placenta	1	1	0
Small bowel	2	2	0
Stomach	3	3	0
Thyroid	1	1	0
Uterus	1	1	0
Total	11	10	0

Fig. 6 TissueSAFE histologic assessment of morphologic preservation after transport in coolers on ice at 4 °C

3 Validation Trial #3—Evaluating the Histology of Wider Variety of Large Specimens

In this trial we obtained a wider variety of tissues from the ORs of the Main Hospital for histologic evaluation after vacuum sealing with the TissueSAFE device located in our Pathology specimen receipt adjacent to the large theater of 32 ORs. These sealed specimens were then transported at intervals throughout the day at 4 °C to the core gross lab within another building at transport times of 1–10 h.

Of 122 medium and large size specimens transported using the TissueSAFE, 97 % were assessed by 15 pathologists as histologically acceptable for diagnosis (Fig. 7). Only four specimens were considered inferior for histologic assessment and none were found to be unacceptable. The specimens considered inferior for diagnosis were one each of kidney, prostate transurethral resection chips, small bowel, and uterus. There was no root cause in the first two specimen types however in the latter two specimen types we have since created standards that require hollow organs to be opened before vacuum sealing.

Large Specimens	1 acceptable	2 Inferior	3 unacceptable
Appendix	4	0	0
Brain	1	0	0
Colon	11	0	0
Gall bladder	14	0	0
Heart	1	0	0
Kidney		1	0
Liver	4	0	0
Lung	3	0	0
Prostate TURP	1	1	0
Skin	3	0	0
Small bowel	3	1	0
Stomach	20	0	0
Soft tissue	2	0	0
Thyroid	7	0	0
Uterus	28	1	0
Tonsils	13	0	0
Miscellaneous*	7	0	0
Total	122	4	0

* Miscellaneous: hemorrhoid, hydrocele, heart valve, artery, endometrium

Fig. 7 Validation trial #3 TissueSAFE histologic assessment of large specimen morphologic preservation

4 Validation Trial #4—Evaluating the Histology of Needle Biopsies

We separately assessed the feasibility of transporting needle biopsies in the fresh state with the TissueSAFE device. We simulated clinical biopsies with a pathologist’s assistant using a Biopty gun to take needle biopsies from 35 freshly delivered resection specimens from the main ORs. The needle cores were placed in specimen

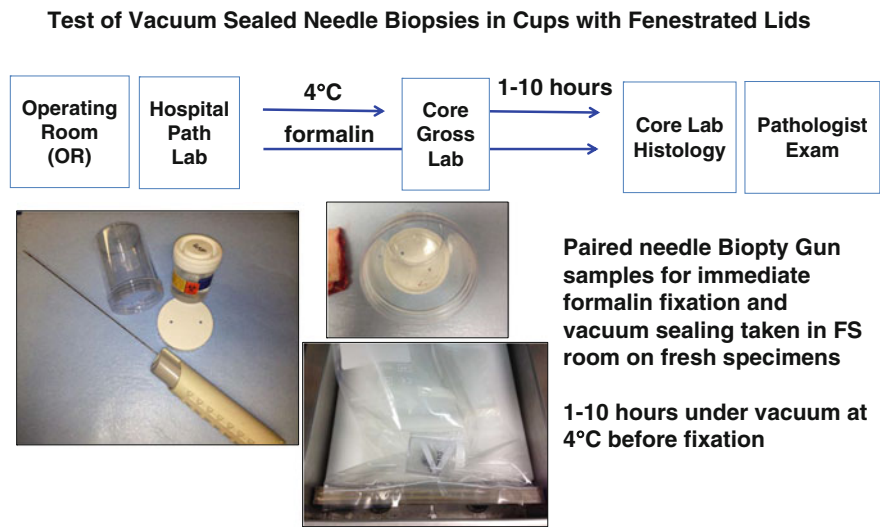


Fig. 8 Validation scheme #4 needle biopsy evaluation

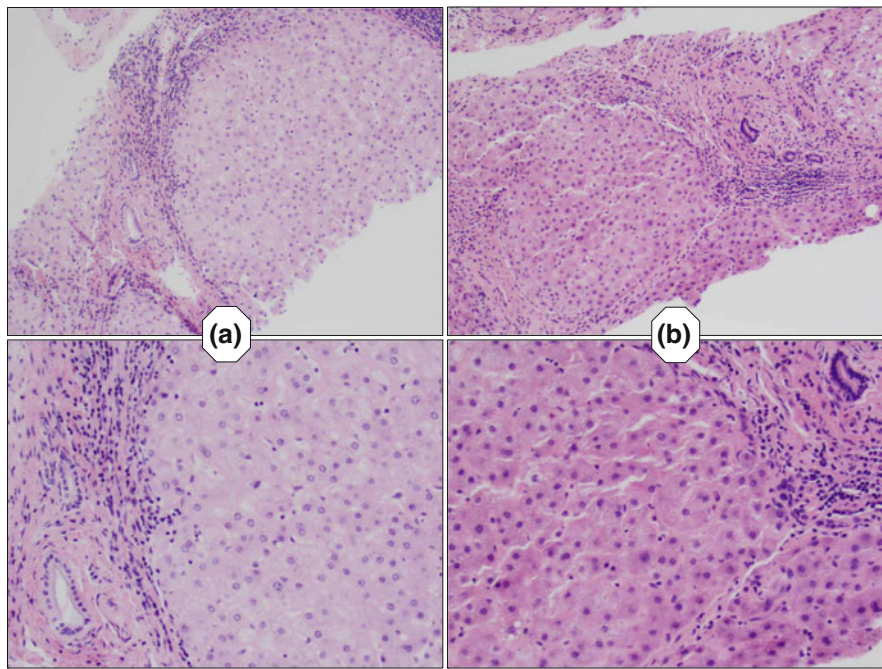


Fig. 9 Liver needle biopsies-**a** immediate formalin fixed, **b** vacuum sealed

Paired Needle Biopsy Comparison	Number cases	Vacuum Sealed Score Avg.	Immediate Formalin Score Avg.	HEMATOXYLIN AND EOSIN Scoring Scale <ul style="list-style-type: none">• 1 = ACCEPTABLE FOR DIAGNOSIS, OPTIMAL HISTOLOGY• 2 = ACCEPTABLE FOR DIAGNOSIS, LESS THAN OPTIMAL HISTOLOGY• 3 = UNACCEPTABLE FOR DIAGNOSIS
Colon cancer and adenomas	6	1.7	1.7	
Liver cancer and cirrhosis	4	1.4	1.0	
Kidney cancer				
Stomach cancer and gastritis	2	1.9	1.5	
Thyroid-cancer and goiter	6	2.0	1.5	
Uterus cancer and myomas	14	1.6	1.3	
Total	35	1.7	1.4	

Fig. 10 TissueSAFE histologic assessment of needle biopsy morphologic preservation

collection cups with fenestrated lids that were sealed under vacuum at 4 °C and transported to the core gross lab over 1–10 h. These test cases were assessed histologically in comparison to Biopty gun needle cores that were taken at the same time and fixed immediately in formalin (Fig. 8). Histologic assessment of suitability for diagnosis was more variable for the same test biopsies between the 15 pathologists. This was ascribed to inconsistent differences in deeper nuclear staining intensity and cytoplasmic eosinophilia seen in the sealed needle biopsy specimens (Fig. 9b) compared to the immediately formalin-fixed pairs (Fig. 9a). This is illustrated from liver core biopsies in Fig. 9. Because of the wider range of pathologist assessment, the scores were averaged and showed a small preference for the immediate formalin fixed biopsies (average score 1.4) compared to the vacuum sealed biopsies (average score 1.7) among the group of 15 pathologists who evaluated cancers and non-neoplastic diseases of colon, liver, kidney, thyroid, and uterus (Fig. 10). None were judged to be unacceptable for diagnosis.

5 Validation Trial #5—Evaluating Reduced Formalin for Tissue Storage

The SealSAFE device differs from TissueSAFE system in that this vacuum sealing device can dispense formalin into the sealing bags based on a preset ratio related to specimen weight determined on its platform scale (Fig. 11). This device is therefore capable of resealing specimens in formalin post-dissection.

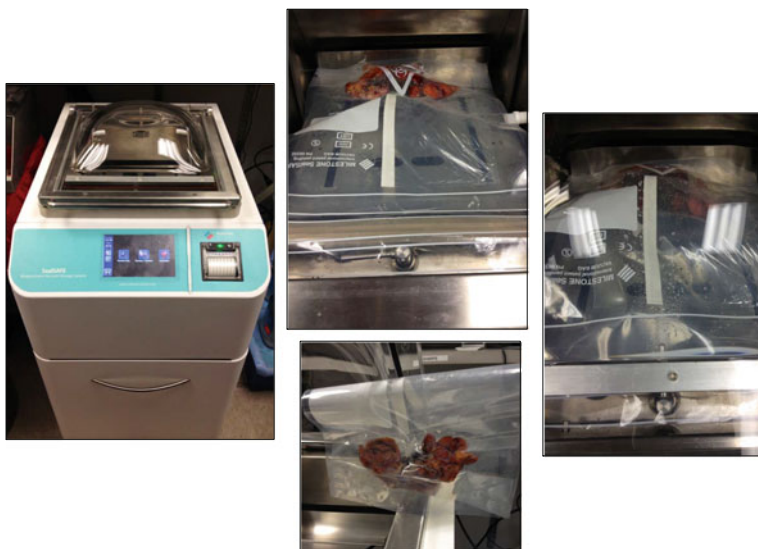


Fig. 11 SealSAFE

Dictums abound related to optimal tissue fixation and the importance of excess volume of fixative in relation to the total volume of tissue. Fixative to tissue ratios ranging from 10:1 to 50:1 can be found. The generally accepted but unscientific rule of thumb for formalin fixation is to immerse a specimen in a volume that is 10 times its weight. Unfortunately this results in large, heavy buckets of formalin that require expensive disposal. However, our paradigm for tissue handling is to receive specimens in the fresh state, to dissect specimens fresh and to fix in appropriate amounts of formalin only what tissue will be processed. Therefore we seek to use minimal formalin compared to the weight of the entire specimen in the initial processing and we subsequently desire to continue that miserly use of formalin into specimen storage.

In this trial of the SealSAFE device, we tested reduced formalin volumes related to specimen weight to preserve tissues in storage that may potentially require return dissection for morphologic assessment of the stored tissues by pathologists. We evaluated 10 specimen types (colon, gall bladder, small bowels, thyroid and uterus) at specimen: formalin weight ratios of 2:1 and 1:1. Specimens were sealed with formalin, held under vacuum at room temperature and sampled, with resealing, for histologic assessment after 24, 48, and 72 h with minimal formalin. Morphologic assessment demonstrated that all tissues at both formalin ratios were acceptable for diagnosis with no degradation in histology noted at 72 h (Fig. 12).

2:1 and 1:1 formalin to weight ratio 24, 48, 72 hours under vacuum at room temperature					HEMATOXYLIN AND EOSIN Scoring Scale
Specimens	1 Acceptable 24 hours	1 Acceptable 48 hours	1 Acceptable 72 hours	2 Inferior or 3 Unacceptable	
Colon	1	1	1	0	<ul style="list-style-type: none">• 1= ACCEPTABLE FOR DIAGNOSIS• 2= INFERIOR QUALITY• 3= UNACCEPTABLE FOR DIAGNOSIS
Gall bladder	2	2	2	0	
Small bowel	1	1	1	0	
Thyroid	1	1	1	0	
Uterus	5	5	5	0	
Total	10	10	10	0	

Fig. 12 SealSAFE histologic assessment of morphologic preservation in storage

6 Analysis of Upstream and Downstream Savings

A number of savings have been identified here, not the least of which is a safer formalin-free environment for all employees, especially in OR theaters where a spill clean up can close a room for many hours of hazardous waste containment and removal. Although it is a very good fixative and the basis of the histology artifact upon which pathologists define most microscopic diagnoses, formalin is well known to be a toxic and potentially carcinogenic substance. Restriction of its use should be strongly considered and is now possible.

7 Financial Savings

In our experience, using this combined process of vacuum sealing specimens at the “front and back end” processes of anatomic pathology resulted in reduced formalin usage at the community hospital of 135 gal/year, reduced formalin for tissue storage in the core laboratory of 468 gal/year, 43 % less storage shelf space used for specimens and a financial reduction to the institution of \$51,000 for routine rather than hazardous disposal of stored formalin fixed tissues because of the minimal formalin used.

8 Conclusions

We have successfully integrated these technologies into existing Lean operations to consistently obtain our goals of controlled preanalytic transport and fixation variables, rapid turnaround times, preservation of fresh tissues for biobanking, and reduction in use of formalin at the front end process of initial tissue fixation and the back end process of excess tissue storage before discard.

We conclude that the TissueSAFE and SealSAFE are both promising technologies employing vacuum sealing of human specimens that can promote a safer environment by markedly reducing formalin use in OR theaters and can minimize formalin use by laboratories. The investment in these technologies is offset by the perpetual savings associated with disposal expense of tissues that would ordinarily be stored in higher volumes of formalin.

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Pre-Analytics of Pathological Specimens in Oncology

Dietel, M.; Wittekind, C.; Bussolati, G.; von Winterfeld, M.
(Eds.)

2015, VI, 133 p. 65 illus., 61 illus. in color., Hardcover

ISBN: 978-3-319-13956-2