

## Technical Note

## Histology safety: now and then

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Histology safety usually focuses on general laboratory issues, but this article concentrates on the hazards affecting the individual histotech and their evolution in the last half a century. Using the information from a survey especially designed for the occasion, the hazards were divided into 4 groups, and their prevalence was expressed as percentages for national and foreign laboratories. All the laboratories received a “safety index” (SI) with an average value of  $0.77 \pm 0.11$  for 63 national laboratories and  $0.69 \pm 0.13$  for 22 foreign laboratories, these 2 averages being statistically different ( $P < .02$ ). The historical evolution of the SI required answering the same questionnaire retrospectively, and so it was done for 17 laboratories with an SI average of  $0.27 \pm 0.12$  for 1955/1989 and  $0.77 \pm 0.13$ , almost 3 times larger for 1990/2007, with improvement of all safety issues. The technological, organizational, and regulatory advances before 1989 showed an unremarkable effect on the SI, and the only circumstance considered as the driving force behind the almost triple increment of the SI during 1990/2007 was the awareness that the AIDS epidemic instilled in the minds and consciences of the medical laboratory personnel in general. Even after almost tripling the average SI value in 2007, national histology laboratories obtained a grade average of “C+” only, leaving room for improvement.

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**1. Introduction**

All forms of human activity involve safety risks, some, such as fishermen, miners, or lumberjacks [1], are riskier, but even the most sedentary jobs involve health risks, such as the development of a potentially lethal leg blood clot after sitting for long periods of time.

Medical laboratory (ML) jobs in general and histology tasks in particular are not risks-free activities because of the wide range of chemical, mechanical, biologic, and environmental hazards the histotech (HT) is exposed to, all of which can pose immediate or long-term health consequences.

Although safety issues have been in the mind of almost everybody for more than 30 years, not many articles on the subject have been published. *Laboratory Medicine*, from the American Society for Clinical Pathology, has published since 1979 fifteen articles on histology safety, out of 50 on the general subject. The *Journal of Histotechnology*, official

publication of the National Society for Histotechnology, published 24 articles since 1977, and *Histologic*, first sponsored by Miles (1971) and later by Sakura (1995), has published only 9 short articles about safety.

Safety issues play such an important role today that each laboratory has a safety officer whose work is appreciated by the average HT from being a great help to an absolute hindrance, being the length the HT has been in the field inversely reflected in that scale. With exceptions, “survivors” of the nasty histology environment are the least appreciative of safety measures, and the rejection exists either if the safety officer comes from the laboratory ranks or from its bureaucracy, because many of their indications are seen as disruptive, having nothing to do with any sort of “suicidal attitude.”

This article deals with hazards the histology personnel has been exposed to in a historical context, comparing their evolution from the 1950s until present day regulations and safer environment. The changes have been dramatic, but there is still room for improvement, especially in personal awareness of the risks, mainly in small and specialized laboratories both in the United States and abroad.

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Histology is an art performed in very consistent ways around the world [2], so we HTs belong to a class not limited by country frontiers and are exposed to similar hazards all around the world.

## 2. Materials and methods

During the last week of March 2007, 82 colleagues from the United States and 11 foreign countries answered a questionnaire specifically designed to determine the HTs' personal safety conditions. The questionnaire was distributed and answered using the resources of *Histonet*, a free list server with more than 1600 members worldwide (<http://www.histonet@utsouthwestern.edu>).

The 72 questions, some self-excluding, focused on activities representing personal risks of chemical, mechanical, biologic, or environmental nature, each with a "correct" or "safe" answer. The total of "safe" answers, divided by the total number of questions applicable to each laboratory, permitted to calculate a "safety index" (SI) with theoretical limits between 0 and 1, similar to grading an academic test.

The answers, grouped into categories for national and foreign laboratories, and expressed as percentages of "unsafe" conditions, were tested for statistical significance of the observed differences using standard procedures, with *P* value less than .05 as the accepted limit and an  $\alpha$ -type error [3].

Evaluating the evolution of the SI during the last 50 years required answering the same questionnaire in retrospect, remembering the working conditions years ago. Completing this fundamental phase required the contribution of 9 "seasoned" colleagues specially recruited for the task, to add to the author's retrospect, and included data from 17 laboratories.

## 3. Results

### 3.1. Chemical hazards

Even when some toxic chemicals with 8 hours of time-weighted averages (TWAs) ranging from 0.1 ppb (mercury oxide) to 1 ppm (benzene and dioxane) or 2 ppm (aniline oil and chloroform) have been almost completely eradicated from the histology laboratory, the average HT is still exposed to many other chemicals, some with similar or even higher toxic levels (Table 1).

Although with very different TWA levels, the 2 fundamental chemical hazards for the HT are formalin (TWA, 0.75 ppm) and xylene (TWA, 100 ppm), both known for their long-term effects [6,7].

The principal chemical hazards (Table 2) vary from risks when performing special stains manually to processing some tissues manually, and although the percentages are higher for half of the sources in foreign laboratories, the overall difference is not statistically significant ( $t_{18} = 0.43$ ,  $P > .70$ ,

Table 1

Eight hours of TWA for some chemicals frequently used in the histology laboratory [4]

Toxic level at	Chemical substance
0.01 ppb	Silver nitrate (silver metal dust/fumes)
0.02 ppb	Osmium tetroxide
0.05 ppb	Potassium dichromate; uranyl nitrate <sup>a</sup>
0.1 ppb	Iodine; picric acid (explosive)
0.2 ppb	Potassium permanganate
0.5 ppb	Chromium trioxide (chromic acid)
1 ppb	Ferric chloride; oxalic, phosphotungstic, and sulfuric acids
2 ppb	Hydroquinone; paraffin wax fumes; sodium hydroxide
10 ppb	Aluminum hydroxide; glycerin mist
0.1 ppm	Potassium iodide; sodium barbital
0.2 ppm	Glutaraldehyde (mutagenic agent)
0.5 ppm	Chlorine
0.75 ppm	Formalin; paraformaldehyde (both carcinogens)
1 ppm	Hydrogen peroxide
2 ppm	Nitric acid; sodium hydroxide
5 ppm	Formic and hydrochloric acids; phenol
10 ppm	Acetic acid
25 ppm	Ammonium hydroxide
100 ppm	Xylene

ppb = parts per billion (equivalent to mg/m<sup>3</sup>); ppm = parts per million (equivalent to g/m<sup>3</sup>, 1 ppm = 1000 ppb).

<sup>a</sup> One hundred milliliter of 1% aqueous solution of uranyl nitrate undergoes about 12 000 disintegrations/s (a specific activity of 123 Bq/mL) equivalent to 0.26  $\mu$ g of radium [5].

NS). Processing tissues manually is a risk now in the rise because of the increased use of nonautomated microwave ovens.

Performing special stains manually or preparing the staining solutions in the laboratory is cost effective [8] but involves handling toxic chemicals.

Again, in spite of the known chemical hazard they pose, formalin is still the fixative of choice and xylene the most used antemedium, but it is interesting to note that both are used less in foreign countries than in the United States in disregard for known alternatives [9–13].

Similarly, recycling xylene is costwise and environmentally advisable, but the practice imposes additional exposure to it, especially when using distilling recyclers, to be coped with not always followed additional precautions. The same concerns apply to recycling ethanol and especially formaldehyde, even with nondistilling recyclers.

### 3.2. Personal risks

Personal risks vary from injuries in the laboratory to long-term effects from not ergonomically designed work stations or repetitive motion injuries due to larger and heavier manual microtomes. These types of conditions are more evident now with a prevalently aging histology workforce [14–16].

All personal risks are higher in foreign countries (Table 3) for a significant difference ( $t_{12} = 2.67^*$ ,  $P < .05$ ).

Among the most notable improvements are the almost total substitution of the dangerous-to-handle large steel

Table 2  
Chemical hazards

Source of the chemical hazard <sup>a</sup>	% of laboratories	
	United States	Foreign States
Special stains are performed manually	87	91
Formalin as fixative	81	64
Xylene as antemedium	59	41
Xylene or xylene substitutes are recycled	54	27
Most staining solutions are prepared in the laboratory	41	82
Coverslipping is carried out manually	38	45
Alcohol is recycled	38	18
Not all known carcinogens have been eliminated	34	64
Both alcohol and xylene or substitutes are recycled	27	14
Not all mercury-containing reagents have been eliminated	23	68
Routine staining (hematoxylin and eosin) is manual	21	23
Manual coverslipping is not carried out in a fume hood	18	18
The chemical hygiene plan is not mandatory	16	73
There have been no protocol changes to safer procedures	16	9
Formalin is recycled	15	0
Some tissues are processed manually <sup>b</sup>	10	10
Cassetting is not carried out in a fume hood	6	14
Grossing is carried out in a poorly ventilated area	5	9
Formalin, alcohol, and xylene or substitutes are all recycled	3	0
All tissues are processed manually	2	14

<sup>a</sup> US average vs foreign average:  $t_{18} = 0.43$ ,  $P > .70$ , NS.

<sup>b</sup> Include manually processing some small/special biopsies, transmitted electron microscopy specimens, and tissue processing with nonautomated microwave ovens.

microtome knives by the sharper and less dangerous disposable blades, the introduction of motorized microtomes, and the elimination of the use of mercury-containing thermometers.

Table 3  
Personal risks

Personal risk source <sup>a</sup>	% of laboratories	
	United States	Foreign States
Not all working stations are ergonomically designed	51	59
There are no motorized microtomes	44	50
There is no special antifrost bite training <sup>b</sup>	36	45
There are no anti slip/fall surfaces in exposed areas	30	55
Electrical outlets are not tested for “earth” connection	27	36
Back injuries prevention is not included in the training	23	55
Mercury-containing thermometers are in use	16	73
Sandals and open-toed shoes are permitted	13	23
Portable hoods do not have activated charcoal	13	18
Some instruments are not “earthed”	10	23
Steel (large) microtome knives are used	6	9
Disposable blades are not prevalent	3	9
There are no fume hoods	2	9
There are no special containers for sharp objects	0	5

<sup>a</sup> US average vs foreign average:  $t_{12} = 2.67^*$ ,  $P < .05$ .

<sup>b</sup> For deep-frozen specimens and techniques using liquid nitrogen.

### 3.3. Safety standards risks

They include risks from not being trained in first aid to not having standard operating procedures available to all employees (Table 4). Even when the risks are higher in foreign laboratories for 71% of the standards, the differences are not significant ( $t_{15} = 2.11$ ,  $P > .90$ , NS).

It is interesting to note that 38% of the laboratories do not decontaminate the working stations at the end of each shift, that 31% pregnant employees are not assigned low-risk tasks, and that there are no uncontaminated working areas in 15% of the laboratories.

Even 10% of the laboratories do not enforce the prohibition of smoking, drinking, or eating in the laboratory, in defiance of the 1992 prohibition by Joint Commission on Accreditation of Health Care Organizations (JCAHO).

### 3.4. Environmental hazards

Three quarters of 16 sources (Table 5) have higher percentages in foreign laboratories for a significant difference ( $t_{14} = 2.49^*$ ,  $P < .05$ ) and range from refrigerators and freezers not explosion safe to improper flammable storage and waste chemicals disposed into the public sewer system.

Table 4  
Personal safety standards risks

Not followed safety standard <sup>a</sup>	% of laboratories	
	United States	Foreign States
The safety program does not include first aid instructions	49	45
Prohibitions of wearing contact lenses/applying makeup are not enforced	43	64
Personnel are not tested annually for effects of exposure to chemicals	41	82
Working stations are not decontaminated at the end of each shift	38	41
There are no lockers for employees	31	32
Pregnant employees are not assigned low-risk tasks	31	27
Protective attire is not mandatory	29	14
There is no lounge-designated area for employees	23	41
There is no monitoring program for formalin and/or xylene exposure	15	55
There are no uncontaminated working areas	15	9
Prohibition of eating, drinking, and smoking are not enforced	10	18
There are no special regulations/precautions for autopsies	6	5
Protective equipment is not required <sup>b</sup>	5	9
There is no written chemical hygiene plan	5	18
The chemical hygiene plan does not include blood-borne pathogens	5	18
The standard operating procedures are not available to all employees	3	23
Material Safety Data Sheets (MSDS) are not available to all employees	0	9

<sup>a</sup> US average vs foreign average:  $t_{15} = 2.11$ ,  $P > .90$ , NS.

<sup>b</sup> Include masks, respirators, goggles, face shields, gowns, fluid-resistant laboratory jackets, and gloves.

Table 5  
Environmental hazards

Source of the environmental hazard <sup>a</sup>	% of laboratories	
	United States	Foreign
Refrigerators and freezers are not explosion safe	68	55
There are no safety cans for flammables	41	41
Flammables are stored at a rate of more than 1 gal/24 sq ft	34	45
Nonexplosion safe refrigerators and freezers are not labeled as such	31	27
There is no hazard decontamination program or training in place	23	36
There are no sprinklers	21	41
Waste chemicals are disposed into the public sewer system	16	41
Lighting in working stations is inadequate	15	14
Personnel are not trained in the use of fire extinguishers	13	14
Airflow is not tested for compliance with recirculation rate regulations	13	36
There are no acid or alkali spills neutralizing substances	10	23
There are no contractors to dispose of waste/dangerous chemicals	6	27
There are no formalin spills neutralizing substances	6	18
There are no safety cabinets for flammables	5	18
There are no special containers for biohazardous material	3	9
Regulations are not followed when flushing chemicals	2	18

<sup>a</sup> US average vs foreign average:  $t_{14} = 2.49^*$ ,  $P < .05$ .

It is disturbing that some laboratories do not have a hazard decontamination program and training in place, and do not test the airflow for compliance with current regulations.

Seventy-two percent of the 67 tabulated hazard sources show higher-risk levels in foreign countries, but because of intrinsic variability, the differences are not statistically significant ( $t_{132} = 1.78$ ,  $P > .90$ , NS).

The tables indicate that some important hazard issues, such as using formalin or xylene substitutes, using protective attire and the existence of uncontaminated areas, less recycling of chemicals, and a greater percentage of protocol changes to eliminate noxious chemicals, provide some advantages to foreign over national laboratories. However, the advantages stop there, with better overall safety conditions for national laboratories.

### 3.5. Safety index

#### 3.5.1. General averages

Although not every tabulated hazard poses the same health risk, each constitutes a health concern for which each was assigned the same unitary weight when calculating the SI for individual or group of laboratories.

The SI for 63 national histology laboratories is  $0.77 \pm 0.11$  (from 0.37 to 0.94), and for 22 foreign laboratories,  $0.69 \pm 0.13$  (from 0.35 to 0.82). These 2 averages are statistically different ( $t_{83} = 2.58^{**}$ ,  $P < .02$ ), precluding the calculation of a general average index.

The SI average for 12 MLs from Canada [5], the UK [2], South Africa [3], Australia, and Argentina (1 each) is  $0.76 \pm$

Table 6  
Safety index averages from 1955 to 2007

Year or period	No. of laboratories	"Average year" (Fig. 1)	Average SI (Fig. 1)
1955	1	1955	0.11
1961–1968	3	1965	0.19
1970–1978	4	1975	0.33
1980–1989	4	1984	0.29
1990–1999	2	1995	0.67
2000–2004	1	2002	0.72
2003	1	2003	0.84
2007	<sup>a</sup>	2007	0.94
1955–1989	12		$0.27 \pm 0.12$
1990–2007	5		$0.77 \pm 0.13$

<sup>a</sup> Two laboratories with the maximum SI value of 0.94 in the 2007 survey, tabulated as "1" 1955/1989 vs 1990/2007:  $t_{15} = 7.35^{***}$ ,  $P < .001$ .

0.04, which is not statistically different to our national average ( $t_{73} = 0.71$ ,  $P > .50$ , NS) but is significantly higher than for the rest of the foreign laboratories with an average SI of  $0.61 \pm 0.17$  ( $t_{20} = 2.72^{**}$ ,  $P < .02$ ). It is necessary to point out that those 12 foreign MLs are in countries with strict laboratory regulations.

Veterinary, experimental, and very small laboratories in the UK [2], South Africa [1], and the United States [4] have an average SI of  $0.51 \pm 0.12$  (from 0.36 to 0.66) not statistically different ( $t_{15} = 1.43$ ,  $P > .80$ , NS) to the SI of the low-ranking foreign laboratories.

#### 3.5.2. Time evolution of the SI

So far, we have dealt with safety hazards and SI for several groups and types of laboratories, but that is the information from the 2007 survey. It is valid to ask, "Has it always been like that?"

Any HT working for more than 10 or 15 years in histology and reading the tabulated unsafe percentages can easily remember that, only a few years ago, things were worse.

*What has changed?* To answer that question, 9 colleagues joined the author in an experiment, each answering in

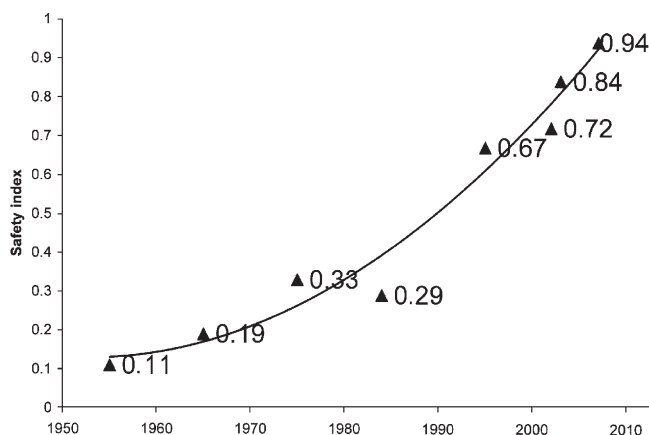


Fig. 1. Variation of the Safety Index from 1950 to 2007.



retrospect the same questionnaire used for the survey and trying to remember how things were from 1955 onward.

The information was tabulated (Table 6) and plotted (Fig. 1), with results that are both amazing and encouraging. The SI for the period 1955 to 1989 was  $0.27 \pm 0.12$ , with an unremarkable evolution during those 35 years, but for the period 1990 to 2007, only 8 years later, there is a surge of the SI value (average of  $0.77 \pm 0.13$ ), which is statistically different ( $t_{15} = 7.35^{***}$ ,  $P < .001$ ) and almost 3 (2.85) times larger.

The information from answered questionnaires between 1955 and 1983 was tabulated along with the national data for 2007 (Table 7), and it shows that all safety issues have improved notably, up to 17 times in the case of the use of steel knives. The general average improvement rate has quintupled, being more evident for risks that changed from 100% to lower values or safe practices that increased from an initial 0% upward.

Table 7  
Evolution of some safety issues from 1955/1983 to 2007

Safety issue	% of laboratories	
	1955-1983	2007
Tissue processors are used for all tissues <sup>a</sup>	64	98
Manual processing for all or some tissues	36 <sup>b</sup>	12 <sup>c</sup>
Manual routine staining (hematoxylin and eosin)	100	21
Manual coverslipping	100	38
Most staining solutions are prepared in the laboratory	100	41
Manual special staining	100	87
Formalin substitutes are used	0	19
Xylene substitutes are used	0	41
Airflow is tested for compliance with regulations	18	87
There is a chemical monitoring program for formalin and xylene	9	85
Personnel annually tested for effects of chemical exposure	9	59
Grossing is done in well-ventilated areas	36	95
Casseting is done under a hood	27	94
Steel blades are used for all or some sectioning	100	6
Disposable blades are used in most sectioning	0	97
There are motorized microtomes	0	56
Mercury-containing thermometers are prevalent	100	16
Most working stations are designed ergonomically	9	49
There are antislip/fall surfaces in exposed areas	9	70
Refrigerators and freezers are explosion safe	0	32
Prohibition on drinking, eating, and smoking are not enforced	45	10
There are safety cabinets for flammable and explosive chemicals	36	95
All working stations are decontaminated at the end of the shift	9	62
Waste chemicals are disposed into the public sewer system	82	16
Average SI	$0.26 \pm 0.13$	$0.77 \pm 0.13$

<sup>a</sup> Tissue processors before 1980 were neither self-enclosed nor had fume control.

<sup>b</sup> Thirty-six percent process all tissues manually.

<sup>c</sup> Two percent process all tissues manually + 10% process manually some tissues.

Fig. 1 presents a very elegant exponential adjustment of the historical data, with a slope increment from the late 1980s up to our days.

#### 4. Discussion

So far, the fact is that the average SI almost tripled from 1955/1989 to 1990/2007, so the next valid question is, “Why?”

Before 1989, technological advances abounded, like the prevalent use of rotary instead of sledge microtomes (1960s), better quality and reliability of disposable blades (1970s), as well as the appearance in the early 1980s of tissue processors much better than those from 1940/1950s, along with embedding centers, automated stainers, and coverslippers [17].

All these new technologies increased safety by reducing the exposure of the HT to some hazards, but the SI value during the period did not change dramatically.

There were also organizational and regulatory advances before 1989. The American Society for Clinical Pathology, created in 1928, started certifying HTs in 1948 and histotechnologists in 1980. In 1961, the College of American Pathologists, created in 1947, started inspecting laboratories, and in 1970, Occupational Safety and Health Administration commenced its activities. In 1973, the National Society for Histotechnology was created, and in 1978, JCAHO and College of American Pathologists started evaluating hospital laboratories. For sure, those events had also an impact on the increment of the SI, but it was erratic and unremarkable.

*What other event or series of events could have turned the tide and improved the safety conditions in the histology laboratories almost 3-fold?*

It is the opinion of this author, shared with other colleagues, that the recognition by the Centers for Disease Control of the existence of the HIV/AIDS epidemic [18] and other viral infections [19] was the driving force behind the dramatic improvement of the safety conditions starting in the late 1980s, more exactly, in 1987, as shown by the inflection point of the curve in Fig. 1.

In 1983, the hazard communication or “right to know” was distributed to all laboratory personnel, followed in 1988 by Clinical Laboratory Improvement Amendments Act and in 1990 by Blood-Borne Pathogens from Occupational Safety and Health Administration, all becoming mandatory study materials and work operations guidelines, culminating in 1992 with the comprehensive JCAHO regulations.

This was a comprehensive body of safety regulations to act upon and, with available technologies, moneys allocated, and the knowledge and will to act, combined with personnel safety concerns, most histology laboratories, especially larger ones, enacted the regulations, made the investments, and assured a safer working environment for all personnel.

It is no coincidence that 7 national laboratories that reported not being under JCAHO regulations have an

average SI of  $0.56 \pm 0.10$ , statistically different ( $t_{61} = 6.00^{***}$ ,  $P < .001$ ) to the average SI of the laboratories following those regulations ( $0.80 \pm 0.08$ ).

It is wrong that safety measures of veterinary laboratories are less stringent because we have to remember that HIV probably transferred to humans via direct contact with primates during activities that involved the exposure to blood.

## 5. Conclusion

The AIDS epidemic, the most destructive in recorded history, with more than 25 million deaths so far, and the fear that it generates have been the driving force behind the histology safety improvements, but we cannot become complacent because, after all, national histology laboratories got an unimpressive grade average of just C+.

Many more improvements can take place, such as the substitution of formalin and xylene as prevalent chemical hazards, eliminating all carcinogens and mercury-containing solutions, rethinking the way recycling is carried out, completely automating routine staining and coverslipping, and changing to less hazardous procedures.

The histology laboratory should become more “ergonomically friendly,” redesigning all working stations and increasing the number of motorized microtomes. Injuries in the workplace, in the form of slip and falls, or back injuries should be reduced and the improvements continued until all the safety issues are addressed.

Are HTs any safer today? The answer is a resounding “Yes,” but we can do better, much better!

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