Assessment of different fixatives, fixation and tissue processing on morphology, antigenicity, and acid nucleic integrity from tumoral and non tumoral thyroid tissues

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Background and Aims

i) After a thorough discussion of the epidemiologic, experimental, and other relevant data, the working group of International Agency for Research on Cancer (IARC) concluded in 2004 that formaldehyde is carcinogenic to humans. In the epidemiologic studies, there was sufficient evidence that formaldehyde causes nasopharyngeal cancer, "strong but not sufficient" evidence of leukemia, and limited evidence of sinonasal cancer (1). *ii)* Molecular characterization of morphologic changes requires exquisite tissue morphology and DNA and RNA preservation; however, formaldehyde usually result in fragmented RNA.

Aims:To optimize molecular analyses on fixed tissues, we assessed morphologic, immunogenicity, DNA and RNA integrity in human thyroid from non tumoral and tumoral pathology when specimens were fixed for different time course in several non-formalin fixatives.

Materials and Methods

Fixatives: five fixatives were used in this study [10% neutral buffered formalin (NBF), RCL2, ExcellPlus, Finefix, and Glyofix). Fresh-frozen samples were used as controls.

Tissue preparation: same specimens of normal, hyperplasia, adenoma, and papillary carcinoma of thyroid (1 cm^3) were fixed in the different fixatives for 6 h, 12 h and 24 h and were processed overnight and paraffin embedded. After embedding, 3-µm paraffin sections from each block were stained with HE and PAS. Immunohistochemistry were performed using HBME1, CK19, Galectin 3, and TTF1 antibodies. Quantity and quality of DNA and RNA obtained from twenty 5 µm deparaffinized sections of tissue from each specimen were compared to those obtained from twenty 5 µm cryosections.

Results

Morphology: Example of typical morphology obtained from each fixatives are shown in figure 1. Tissues were adequate for histologic HE examination with all fixatives, while PAS observed with non formalin fixatives showed a darker staining.

Immunohistochemistry : Antibodies were used as purchased. Typical immunostaining were adequate for all fixatives, except TTF1. For this latter antibody, no nuclear staining were obtained when using a couple of non formalin fixatives (fig. 2).

Nucleic acids: Recovery of mRNA was reduced in both quantity and quality in the different alternative preservation methods comparatively to that obtained from snap-frozen tissues, but was always superior to that obtained from formalin-fixed samples and largely sufficient to perform reverse transcription polymerase chain reactions (fig. 3A).

Recovery of DNA from all the different alternative preservation methods was equally or slightly superior to formalin-fixed samples as determined by one-dimensional gel electrophoresis and polymerase chain reaction (fig. 3B). The DNA from the ethanol-fixed samples migrates on an agarose gel as a smear of fragments, ranging in size from several hundred base pairs to few kbp. The quality of the DNA was superior to that recovered from formalin-fixed tissue. Experiments using PCR showed that DNA from the ethanol-fixed tissue consistently amplified more robustly than DNA from formalin-fixed tissue

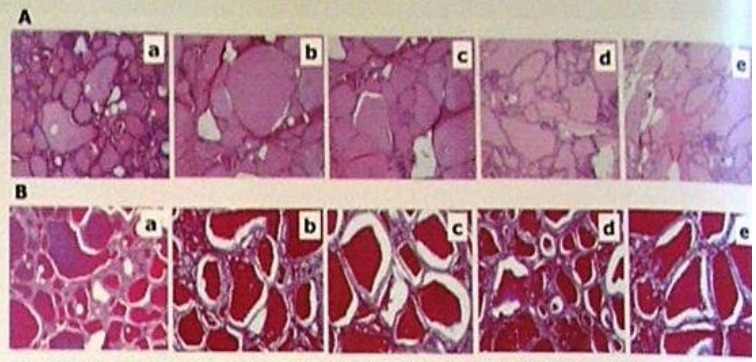

Figure 1. Normal specimens of thyroid fixed 24 h in formol (a), RCL2 (b), ExcellPlus (c) , Finefix (d) and Glyofix (e) [A: HES; B: PAS]

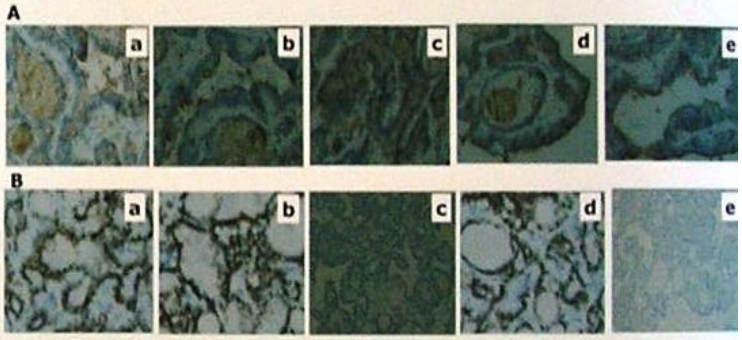

Figure 2. Papillary carcinoma specimens fixed 24 h in formol (a), RCL2 (b), ExcellPlus (c), Finefix (d), and Glyofix (e) [A: HBME1; B: TTF1]

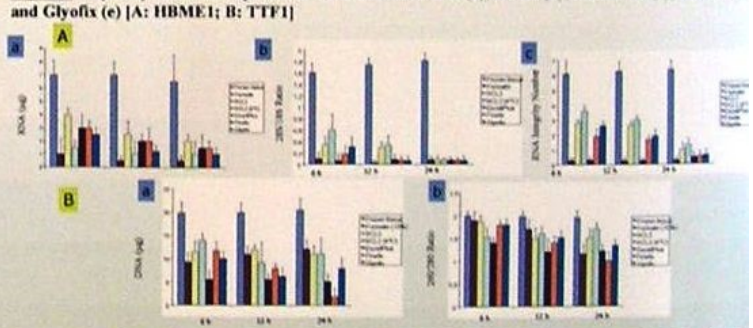

Figure 3. A. Quantity and quality of extracted RNA from thyroid adenomas at different time of fixation. a. Amount of RNA isolated based on different fixation and processing methods. b-c. Average quality of each fixative-derived RNA above the mean for 28S/18S ratio and RNA integrity number. B. Quantity (a) and quality (b) of extracted DNA from different specimens based on different fixatives and different time of fixation.

Conclusion

In conclusion, thyroid specimens fixed in the different alternative preservation methods used in this study, and embedded in paraffin, produce good histology, and immunohistochemistry and permit recovery of DNA, and RNA sufficient for several downstream molecular analyses. These results confirmed some of previous works concerning the evaluation of non-formalin fixatives and new fixatives and processing methods for morphological, immunohistochemical and molecular studies (2)

References

1. Cogliano VJ et al. Working Group for volume 88. Meeting report: summary of IARC monographs on formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2-propanol. *Environ Health Perspect* 2005; 113: 1205-8.
2. Gillespie JW et al. Evaluation of non-formalin tissue fixation for molecular profiling studies. *Am J Pathol* 2002; 160: 449-57.