

Using less than 10% formalin fixed tissue samples in vimentin immunohistochemical detection: possible impact on Oncobiological analysis?

Context

There are very few circumstances where the diagnosis of malignancy is made in the absence of histopathological confirmation¹. For prognosis and therapeutic guidance, it is also essential to support all decisions with knowledge of the oncobiology provided by immunohistochemistry and other molecular methodologies²⁻³. Regarding this goal, it is essential to obtain/preserve adequate diagnostic material⁴. In case of tissue samples, the most widely used fixative is 10% neutral buffered formalin⁴. However, this concentration is not supported by consistent scientific evidence.

Aim

To compare immunohistochemical results considering less than 10% formalin concentrations, in order to support the proper use of this reagent and also to explore the possibility of decreasing the formalin use, since its toxicity is known⁵⁻⁶.

Methods

Three formalin concentrations (10%; 7,5%; 2,5%) and 70% ethanol (representing non-formalin fixation) were used to fix, for 48 hours at room temperature, similar sized human placenta samples, with a cold ischemia time of 15 minutes. For each fixative, 30 paraffin blocks were prepared, which, after sectioning, were subjected to vimentin (NCL-L-VIM-V9) immunohistochemical detection (Ventana Benchmark GX - OptiView DAB), has this structural protein is commonly used as a fixation quality biomarker⁷. Immunohistochemical results were assessed by two experts using the Global Immunohistochemistry Score (GIS) providing a 0-100 points score⁸. The GIS results were analyzed using descriptive statistics and Kruskal-Wallis test with pairwise comparison.

Results

The results were (mean-standard deviation): 2,5% formalin (90,10-0,89); 7,5% formalin (86,77-0,93); 10% formalin (82,94-1,24); 70% ethanol (84,66-1,22). The Kruskal-Wallis test revealed differences between 2,5% formalin and all the other samples (Kruskal-Wallis with multiple comparisons without bonferroni correction - Kruskal-Wallis $H=19,54$; $p<0,05$). The internal consistency between the two observers was considered satisfactory ($\alpha=0,72$).

Conclusions

Results sustain that 2.5% formalin provided best immunohistochemical results in this context. Even considering the exploratory type of this research, it is possible to consider the reduction of formalin concentration used in fixation.

References

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Diagram 1

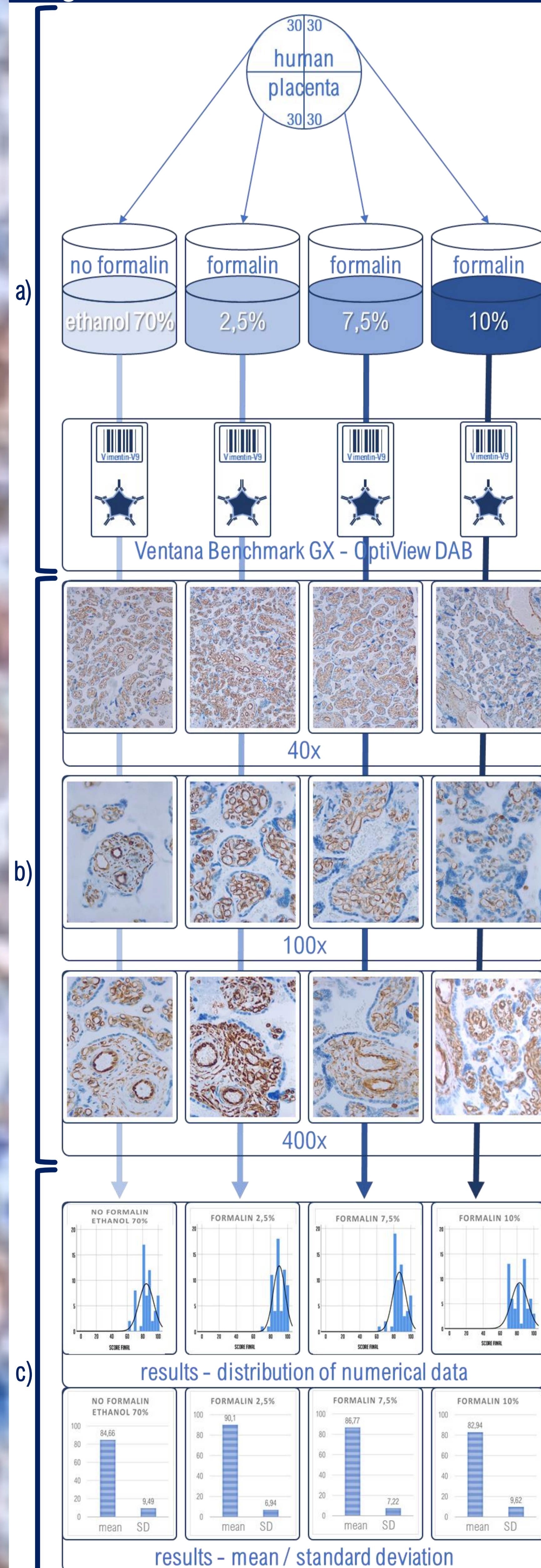


Diagram 1:

- a) experimental procedure;
- b) immunohistochemistry results;
- c) statistical results.