RCL2: A Potential Formalin Substitute for Tissue Fixation in Routine Pathological Specimens

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Abstract

The present study investigated the suitability of RCL2, an alcohol-based formalin-free fixative, for tissue fixation in routine histopathological examination and assessed tissue suitability for ancillary investigations.

A variety of tissue types was tested. The suitability for microtomy and quality of histomorphology, histochemistry, immunohistochemistry, fluorescent and silver in-situ hybridization analysis as well as extracted genomic DNA were assessed on RCL2-fixed paraffin embedded tissue sections and compared with the same tissue types fixed in formalin.

Microtomy was straightforward in most paraffin blocks. There were no significant difference in quality of tissue morphology between RCL2 and formalin-fixed tissues. The quality of histochemical staining, immunohistochemistry and in-situ hybridization were comparable to that of formalin-fixed tissues. Genomic DNA concentration was higher in RCL2-fixed tissues.

In conclusion, this study has shown that RCL2 is a potential formalin substitute suitable as a fixative for use in routine histopathological examination without requiring major adaptations to the methodology.

Keywords: Histopathological examination; RCL2; Tissue fixation

Introduction

Formalin has long been the most popular fixative in many routine pathology laboratories because of its ease of use, low cost and fast fixation. Furthermore, a wide range of histological techniques can be performed easily on formalin-fixed tissues [1]. However, formalin based fixation, while it preserves the cellular and architectural morphology, impairs DNA and RNA quality, hampering molecular analyses of routinely fixed tissues [2-7]. In addition, formalin is highly toxic and may pose health hazards to laboratory personnel [3,8-11].

To overcome these limitations, several aldehyde-free fixatives have been tested. Thus far, the most promising results have been obtained with alcohol-based fixatives [3]. RCL2 is a new commercially available alcohol based non-cross-linking formalin-free fixative that could potentially replace formalin.

The suitability of RCL2 as a fixative for routine histopathological examination was tested in this study, in a wide range of tissue types focusing on the quality of the tissue for its morphology and architecture. In addition, the quality of immunohistochemical labeling using a large panel of antibodies, molecular techniques including fluorescent and silver in-situ hybridization (FISH and SISH) and genomic DNA extraction were assessed on the RCL2-fixed tissues. The possibility of using RCL2 diluted in 95% alcohol (instead of the recommended 100% solution) without compromising the quality of the fixed tissues was also explored, as this may further reduce the cost of tissue fixation.

Material and Methods

Forty-nine tissue sections were taken from 36 benign fresh surgical specimens; namely, an ovarian mucinous cystadenoma, an ovarian mature cystic teratoma, an ovarian endometriotic cyst, three hysterecctomy specimens for uterine fibroid, a myomectomy specimen for uterine fibroid, five breast fibroadenomas, two normal placentas, a benign thyroid and 18 tonsillectomies. Representative tissue samples from each specimen were fixed overnight [12] in either 5 volumes of RCL2 diluted in 100% ethanol (RCL2-100) or 5 volumes of RCL2 diluted in 95% ethanol (RCL2-95) or 10 volumes of neutral buffered formalin. The same protocol used for formalin fixed tissues were applied to RCL2 fixed tissues in all techniques (including processing, embedding, H&E staining, histochemical staining, immunohistochemistry and molecular studies) without major modification.

Histopathological evaluation was performed by two observers (N.M., M.G.). Qualitative assessments of hematoxylin and eosin (H&E), histochemical and immunohistochemical staining of formalin-fixed paraffin-embedded (FFPE) and RCL2-fixed paraffin-embedded (RCLPE) sections were performed in a blinded fashion and the results were scored on a three-point scale. Correlation of data from formalin, RCL2-100 and RCL2-95 fixed tissues for morphological and immunohistochemical assessment was analysed using Pearson’s Chi-square test, 2 sided P.
The quality of fluorescence in-situ hybridization (FISH), silver in-situ hybridization (SISH) and extraction of genomic DNA was also compared on FFPE and RCLPE tissue sections on limited number of tissues.

Results

The study showed that the majority of RCLPE tissues were suitable for microtomy. The RCL2-fixed tissues also produced good-quality H&E sections (Figure 1), with more than 90% of cases receiving a good score (i.e. score of 3) for morphological features. In fact, RCLPE tissues received a slightly better score for cytoplasmic assessment. Some discrepancies observed for a few antibodies in different tissue types. However, there was no statistical difference between formalin- and RCL2-fixed tissues (formalin versus RCL2-100, P=0.066; formalin versus RCL2-95, P=0.120; χ² test). Modification of the routine immunohistochemical protocols may be required for certain antibodies when tissues are fixed in RCL2.

Figure 1: Assessment of tissue morphology and ancillary tests in RCL2-fixed tissues. A, B, C pictures show good preservation of cellular structures in RCL2-fixed tissues. These include cilia (left), muscle striations (center) and keratohyaline granules (right). D, E, F, G: left to right: Perl's stain on RCL2-fixed section highlights the blue haemosiderin pigment. Strong, diffuse expression of smooth muscle actin (SMA) on a section of RCL2-fixed myometrium. Strong Ki67 labelling in germinal center B cells of RCL2-fixed tonsil and strong CD34 expression in capillary endothelial cells on section of RCL2-fixed placenta. H, I: Fluorescence in-situ hybridization (FISH) analysis using break-apart probes for BCL2 gene on section from RCL2-fixed tonsil shows two fused normal signals of good intensity in each nucleus (left). Silver in-situ hybridization (SISH) for HER2 gene on section from RCL2-fixed breast tissue shows clear 1-2 silver-impregnated black signals in the nuclei indicating presence of normal copy of HER2 gene (right).

The results of FISH showed that this technique worked on RCPLE tissues, producing signals of comparable intensity to those observed on formalin-fixed tissues (Figure 1). In addition, RCL2-100 fixed tissues contained more signals than the RCL2-95 fixed tissues. Of interest are the results of SISH for the HER2 gene, which showed stronger signals in RCLPE tissues than in formalin-fixed sections (Figure 1). Extraction of genomic DNA also showed higher DNA concentration from specimens fixed in RCL2 (six to seven times higher in RCL2-95-fixed specimens and four to seven times higher in RCL2-100) than that in formalin-fixed tissues.

Conclusion

In conclusion, this study has highlighted that RCL2 is potentially a good substitute for formalin. We found that the quality of tissue fixation is not affected when RCL2 is diluted in 95% ethanol, which improves the cost-effectiveness of the histopathological examination of routine specimens. One main advantage of RCL2 is that it does not require major changes in the protocols that are normally used by laboratories. However, more sensitive handling is required for some tissue blocks and further optimization of immunohistochemical methods may be needed with certain antibodies to obtain consistent results.

References