

Effect of formaldehyde in liquid-based cytology preservation solutions on molecular testing for lung cancer specimens

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Objective

Liquid-based cytology (LBC) specimens of lung adenocarcinoma may be used for genetic analysis in the near future. However, formaldehyde contained in LBC preservation solutions is well-known to cause DNA fragmentation, and the progress of fragmentation fails us to accomplish molecular testing. In this study, we determined whether LBC specimens can be used for epidermal growth factor receptor (EGFR) mutation analysis in human lung adenocarcinoma (HLAC) cell lines.

Materials

- Human lung adenocarcinoma cell lines
 - A549 (EGFR_{wild-type})
 - H1975 (EGFR_{exon 20 T790M and exon 21 L858R})
 - PC-9 (EGFR_{exon 19 del})
- Preservation solution
 - CytoRich™ Red preservative (BD) (23.3% isopropanol, 10% methanol, 6.7% ethylene glycol, and 0.4% formaldehyde, pH7.5)
- DNA extraction kit
 - QIAamp DNA Mini Kit (QIAGEN)

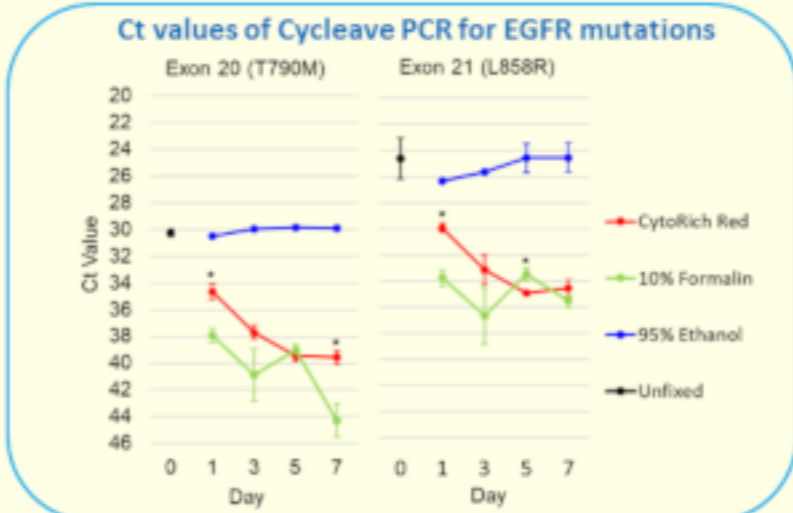
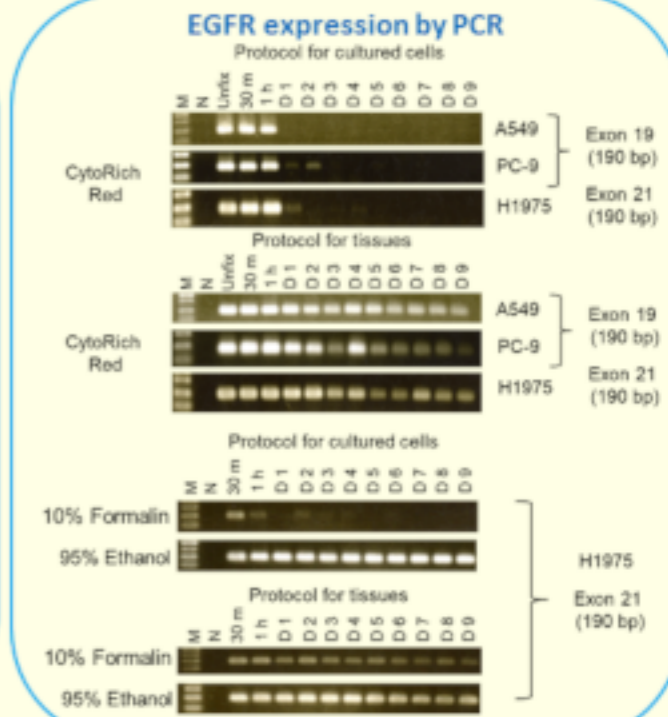
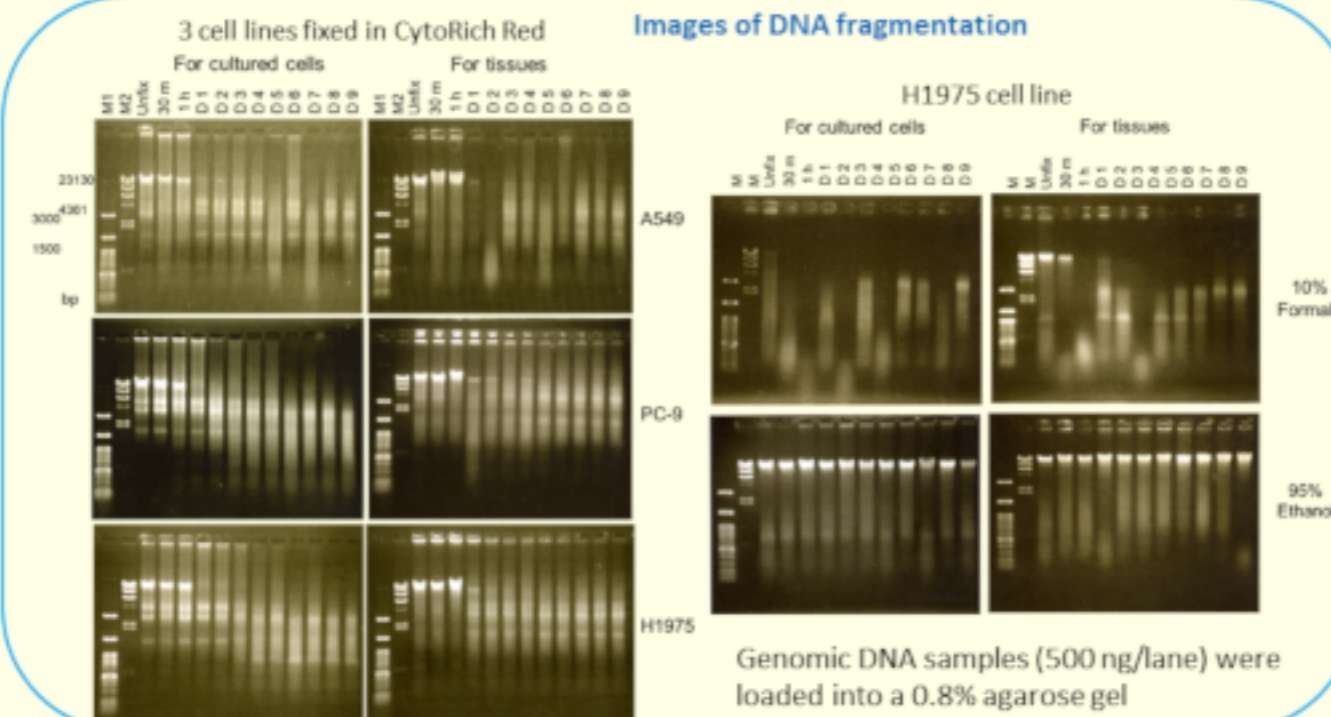
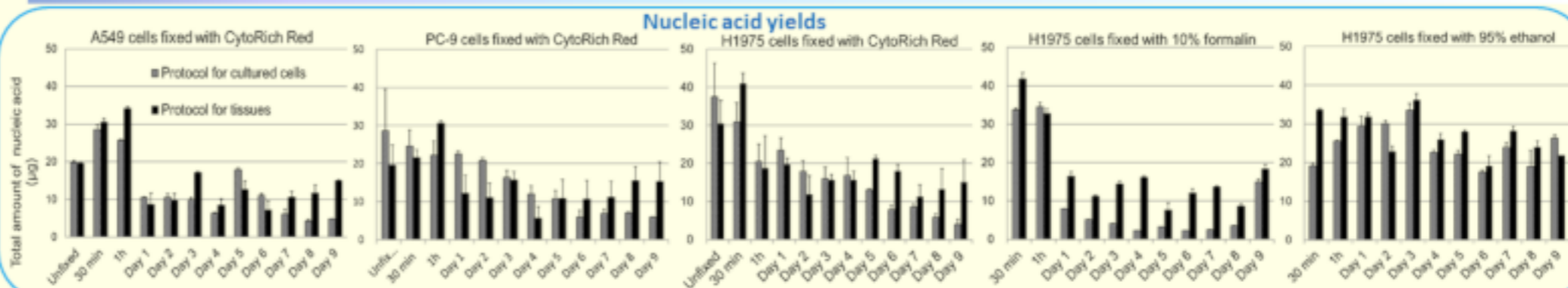
Methods

- Each cells (1x10⁶) was fixed with 1mL of CytoRich™ Red. As controls, cells were also fixed in 10% formalin or 95% ethanol.
- Genomic DNA was extracted using 2 protocols (one for cultured cells and the other for tissues)

	Protocol for cultured cells	Protocol for tissues
Lysis with proteinase K at 56°C	Buffer AL	Buffer ATL
Subsequent lysis at 70°C	No treatment	Buffer AL

- Different fixation times were tested for each protocol: 30 min, 1 h, and 1–9 d.
- We evaluated the effect of fixation time on DNA fragmentation, polymerase chain reaction (PCR) amplification, and EGFR mutation detection.

Results



Conclusion

DNA extracted from HLAC cell lines immersed in CytoRich Red for several days at room temperature can be utilized for molecular testing. When LBC specimens are used for targeted molecular testing, the appropriate preservative solution and extraction protocol first should be determined.

COI: We provided CytoRich™ Red preservative from Becton, Dickinson and Company.