Commentary on Linch CA, Smith SL, Prahlow JA. Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. J Forensic Sci. 1998; 43(2):305–14.

Sir:

It was with some dismay that we read the above-cited article by Linch et al., who reported that, in their experience, the technique of Fluorescence In Situ Hybridization (FISH) was unsuitable for gender determination of hair.

Linch et al., reported that they failed to attain hybridization of commercially (VYSIS) available X- and Y-chromosome-specific alpha-satellite FISH probes to both archived and fresh hair samples. We had previously reported that FISH, using these probes, could correctly identify the gender of hair (1). In addition, we have reported using FISH successfully to identify the gender of cells in a number of different sample types as it could be applicable in forensic analysis (2–7). As a result of this discrepancy, we reviewed their methodology. The technique used was essentially that reported in our article (1) with one major exception. The cells were heat fixed to the slides. In our original report, cells from the hair bulb were attached using liquid nitrogen (2). The step of heating cells represents a critical error in their FISH methodology. In our experience with FISH, those of other colleagues, reports and recommendations in the literature (8,9) and "trouble shooting" recommendations by commercial companies (VYSIS, Venatana-Oncor), heating, baking, or flaming a slide prior to the hybridization step severely inhibits the efficient hybridization of DNA probes to the cells. Hence, probes do not hybridize well, if at all, and may result in inconclusive results and/or cause false hybridization signals. This appears to be the case with the observation made by Linch et al., wherein they report seeing either no signals (i.e., no hybridization), some hybridization or false hybridization. A simple change in the way they made slides would have solved their lack of FISH hybridization.

Linch et al. attempted to justify their negative results by stating that "FISH probes have inherent problems even when used with fresh viable cells. Loss of target DNA, poor penetration of probe, and incomplete or non-specific hybridization are problems associated with apoptotic, necrotic, and keratinizing cells. FISH requires examination of a large number of cells, the use of control cells on the same microscope slide as the evidence slide (due to critical temperature requirement) and sophisticated statistical analysis" (10). Those statements may have had validity some years ago, however they are no longer of critical concern with newer techniques and commercial probes. Techniques have been so well standardized that FISH is now used routinely for prenatal, postnatal and leukemia diagnosis (11–15). In fact, in microdeletion syndrome cases such as DiGeorge or William syndromes, FISH is the only truly confirmatory test. A number of the currently available probes have been FDA approved for clinical testing.

It is our recommendation that Linch et al. or any other investigator planning to use FISH, first thoroughly familiarize themselves with the technique and its potential pitfalls, before reporting conflicting information in the literature.

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Authors' Response

Sir:

Early reviewers and colleagues suggested we separate the paper into three articles: (1) Fluorescence in situ hybridization (FISH) gender typing of telogen hair club material, (2) Transmission electron microscopy (TEM) of telogen hair club material and anagen hair bulb material, and, (3) Polymerase Chain Reaction (PCR) nuclear DNA typing of all hair root stages. We protested however because we hoped the reader would appreciate the relationship between hair root morphology and expected DNA typing results if the three parts were taken as a whole. One of the main goals of the paper was to urge the reader to microscopically evaluate hair roots prior to attempting biotechnical methods.