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A complete read of the paper will show that we had FISH X-Y probe failure with telogen hair club material (trichilemmal keratin) and not with anagen hair bulb cells. Telogen hair clubs have no intact nuclei and anagen hair bulbs do, as revealed by the TEM part of the study. In 1997 FISH X-Y probes required interphase nuclei or metaphase chromosomes for success. We did not attempt FISH gender typing of the anagen hair bulb material because the practicing forensic community prefers the STR, amelogenin typing of such material for obvious reasons. FISH gender typing of trichilemmal keratin would be similar to FISH gender typing of fingernails absent soft tissue. There is a 1993 report of successful FISH gender typing in which the slides containing "sheath cells from the shaft of the hair roots" were heated to 80 degrees C for 20 minutes prior to the dehydration steps (1). It was refreshing to see investigators actually identify the material they were testing but, again, these types of hairs (anagen) are a waste of time for FISH X-Y forensic analysis since more informative methods exist for such cell rich materials (STR, amelogenin).

The commentators' use of the term "hair bulb" indicates their focus on anagen phase hairs which we did not use. Investigators not experienced with hair root microscopy do not know if they are testing clubs or bulbs, each of which may, or may not, also have follicular tissue present. In Prahlow et al., (2), Dr. Pettenati, Dr. Rao, and Dr. Prahlow reported successful FISH typing of "pulled" and "combed" hairs from autopsy patients without benefit of microscopic examination of the hair roots prior to typing. It is extremely difficult to comb the hair of an autopsy patient without obtaining some hairs that contain either sheath cells or bulb cells (not telogen clubs).

Forensic scientists do not have the luxury of testing clinical diagnostic material. Our brief touch of the micro slide to the hot plate to evaporate the acetic acid, as complained about, was a minor tissue insult compared to that suffered by hairs left at crime scenes. Forensic validation guidelines require that degradative environmental and matrix studies be performed on specimens prior to implementation of such biotechnologies for crime lab use (3–5). In other words, subject the telogen club (trichilemmal keratin) material to extreme temperatures, humidity, direct sunlight, dyes, soils, and foreign blood/semen/saliva contaminants; wash with an appropriate method (5), and then, attempt FISH gender typing if one expects to find interphase nuclei in keratin material. We did contact Vysis technical support about our results, March 1997, and they recommended purchase of their FISH apoptosis detection kit. (The telogen club is the final product of an apoptosis process that shrinks the hair root stem from the active (anagen) growth stage to the resting (telogen) stage). At that time the Vysis technical staff was not concerned about our brief specimen heat fixation method.

The focus of the FISH portion of the study was the telogen hair club since its exploitation for gender typing would be an addition to comparison microscopy and mitochondrial DNA D-loop sequence analysis, the only currently useful techniques for forensic comparison of such. Biomedical and forensic investigators should take the time to learn proper hair histiogenic micro structure and language. "Shed", "combed", "pulled", and "plucked" hair specimen categories only add to the confusing data that have been published using FISH, nuclear DNA PCR, and mitochondrial DNA PCR sequence methods. One must know the nature of the material actually being tested and account for the potential environmental insults the material may have had prior to arriving at the sterile laboratory.

We have no doubt that FISH is a useful methodology for clinical specimens. We have no doubt that FISH X-Y probes work on anagen hairs. FISH X-Y probes will not work on telogen hair clubs (absent attached follicular cells) no matter what methodology is used.

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Commentary on Willey P, Scott DD. Who's buried in Custer's grave? J Forensic Sci 1999;44(3):656–65.

Sir:

The excellent article, referenced above, was absolutely fascinating!

As a forensic dentist and a clinical dentist, I have the following comments. The suggestion that skull (Burial 8B) was a tobacco user and specifically a pipe smoker, due to "pipestem abrasion" on the left mandibular premolar teeth may not be perfectly accurate for the following reasons:

1. All of the left posterior teeth depict a degree of occlusal abrasion, but I believe that this abrasion was the result of bruxism. (I am sure that soldiers over 125 years ago had plenty of problems over which to clench and grind their teeth.)
2. I am not sure what pipestems were made of in the 1870's, but I cannot think of many materials suitable for pipestems harder than enamel, thus, I would expect the stem to yield before the enamel structure of the teeth.
3. If the individual were a pipe smoker, and clenched the stem in a chronic fashion, more than likely the stem would have caused a vertical downward movement of the involved tooth or teeth, much like an orthodontic appliance.

The bottom line: I would not think that one of the elements in eliminating Custer should be the fact that he was disdainful of smoking, simply because I don't believe there is ample evidence that the abrasion came from a pipestem in the first place! Eliminate him on other factors if you will, but not on that particular one.

Again, I thank the authors for a meticulous and interesting account of the events surrounding the death of Gen. Custer. The photographs, sketches and maps were very illustrative and engrossing.

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Authors' Response

Sir:

We appreciate Dr. Norman Sperber's comments and insights concerning our assessment of Burial 8B. We concur with many of his statements, particularly those concerning the exceptional service that the *Journal of Forensic Sciences's* editor and