

scenarios put forth. We would certainly welcome any additional probable scenarios to explain the trauma observed.

The focus of this article was to examine the extensive injuries the individual sustained in order to ascertain the most probable manner of death. As we are aware that cause of death is a medical determination, there is no attempt in this article to ascertain cause of death. We are simply stating that the severe injuries sustained by this individual most likely seriously incapacitated him.

Finally, several of the injuries to the scapula and vertebrae have been attributed to contraction of particular muscles. However, our understanding of avulsion fractures as a result of forcible tearing or pulling suggested that these injuries could also be classified as avulsion fractures. For instance, fractures of the inferior and superior scapular angle, where there is muscle attachment, are often classified as avulsion fractures.

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**Commentary on** Introna F, Di Vella G, Campobasso CP. Determination of postmortem interval from old skeletal remains by image analysis of luminol test results. *J Forensic Sci* 1999; 44(3):535-8.

Sir:

I have a few questions for the authors followed by some comments on luminol. What was the history of the bones examined in the study? Were the bones from burials or were they from non-buried, relatively pristine bodies? Did the bones undergo any cleaning procedures prior to luminol treatment?

A forensic scientist must always be very careful when interpreting luminol results. In this study, the authors took appropriate steps to eliminate false positives that could result from plant peroxidases; however, other sources of contamination can cause false luminol positive reactions. Copper, copper salts, ferricyanide, iron ions, cobalt ions, and sodium hypochlorite (bleach) can cause luminol to fluoresce (1-3). Any of these substances could come in contact with bones, particularly bones that have been buried in mineral rich soil and bones that have been cleaned with tap water and/or bleach. I have seen luminol react with copper salts that have leached into the fabric surrounding the copper rivets of blue jeans. I have also seen luminol react with black fingerprint powder. When using the suggested method for aging bones, the scientist must be aware of other substances that can cause variation in the fluorescent intensity of luminol. Standards, such as known bone samples of varying PMI, and controls, such as a soil sample collected from the area surrounding the bone, clothing associated with the remains, and bone cleaning materials, should be used in conjunction with this type of analysis.

## References

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## Authors' Response

Sir:

Thank you very much for the comments regarding our article: "Determination of postmortem interval from old skeletal remains by image analysis of luminol test results." We do really appreciate them and certainly agree that forensic scientists must always be very careful when interpreting luminol results.

The goal of our study is testing a simple and easy distinction method between two broad groups of skeletal remains frequently examined during forensic investigations: "modern" (less than 50 years) and "ancient" (more than 50 years) bones. The paper is a preliminary effort to the evaluation of correlating the time since death with blood remnants in bone tissue. Luminol is very sensitive, reacting rapidly to the most minute traces of blood, but it is a presumptive test, capable of delivering both false positives and false negatives. For example it does not differentiate between human and animal blood (1).

Major sources of false positives are chemical oxidants, catalysts, and salts of heavy metals such as copper and nickel. To avoid the possible influence of the most common substances (such as iodine, rust, household bleach, formalin and plant peroxidases such as are found in horseradish, citrus fruits, bananas, watermelon and numerous vegetables), we washed in distilled water all the bone samples and heated them to 100°C for a period of 5 min prior to testing with luminol solution. This temperature does not appreciably affect the heme portion of the hemoglobin responsible for the luminescence reaction and destroys the plant peroxidases.

However, as you stated in your comments, metal surfaces such as copper, copper salts, ferricyanide, iron ions, cobalt ions and sodium hypochlorite (bleach) are particularly likely to yield false positives. To avoid the possible influence of these substances we followed procedures as reported in a previous paper on this topic (2) collecting bone powder from the inner compact tissue of the mid-shaft of each femur. Compact bone is, in fact, far less susceptible to physical and/or surface contamination than trabecular bone with its large surface area to volume ratio and multiple cavities that easily become filled with contaminating soil and clay particles. After removing the periosteal (outer) and endosteal (inner) surfaces and pulverizing the compact tissue samples into a fine bone powder using a grinder no other particular cleaning procedures were used except a second washing in distilled water.

Regarding the history of the bone samples examined, the femora belonging to the "ancient" group examined (fourth and fifth group with PMI ranging between 50 and over 80 years) were from human remains found in different ossuaries (crypts) of old Roman Catholic churches. For these latter bones the original burial conditions are still not well defined and for some skeletons completely unknown. However, based on the negative results of image analysis of luminol tests for this latter "ancient" group we can exclude manifest false positives since only one femur (PMI ranging between 50 and 60 years) revealed a very faint light-reaction (see the weaker luminance recorded from the powdered bone than the other groups). The most of femora (33 out of 60) belonging to the "modern" group (first, second and third group with PMI ranging between 1 month and 35 years) were from skeletal remains found outdoors, in open fields, during forensic investigations. The rest of femora belonging to "modern" group (27 out of 60) came from cemetery exhumations. These bodies were buried in wooden coffins embedded both beneath the soil and in cement niches for urns; actually, we do not know exactly which coffins were lined with metal (zinc) plate or which kind of clothing was associated with the remains. Consequently, it was not possible to standardize the variations caused by burial environments, since the examined material came from different sites such as