

Commentary on: Johnson E, Ames CE, Dagnall KE, Foster J, Daniel BE. Comparison of presumptive blood test kits including Hexagon OBTI. *J Forensic Sci* 2008;53(3):687–9

Sir,

After seeing this study in *JFS* we were expecting to read a more complete, comprehensive, and detailed study to compare the traditional presumptive blood tests with the Hexagon OBTI kit than was presented. Although the title claims that there is a comparison between presumptive tests for blood, the main study focuses solely on the Hexagon OBTI kit and there is virtually no new research presented.

Hochmeister et al. (1) performed validation tests for the Hexagon OBTI kit in 1999 and found a sensitivity of 1:100,000 when diluted in sterile water, a magnitude more sensitive than found in the current study under standard conditions. Hochmeister et al. (1) also conducted specificity, degradation, and contamination studies and determined that there was no effect on DNA recovery. This study was complimented by the work of Hermon et al. (2) who determined that sensitivity could be increased with a reduction of the buffer solution from 2 mL to 200 μ L and also with an increase in incubation time. The maximum sensitivity that Hermon et al. (2) achieved was 1:1,000, even with an increase in incubation times, which they attribute to different volumes of blood used for the initial sampling. They also tested the specificity of the assay using different species of animals as well as other human body fluids.

While the current study cites these previous works, it replicates the sensitivity and DNA recovery studies and other than the direct comparison to Kastle–Meyer (KM), leucomalachite green, and Hemastix[®] for sensitivity, and the 2 months' storage of the buffer solution prior to DNA extraction, there is no novel work presented.

Other major concerns with the paper include:

1. Only papers pertaining to the validation and use of Hexagon OBTI were cited. Previous research describing comparisons of other blood presumptive tests were not cited (3–5) and do not appear to have been consulted.
2. A minimal number of presumptive tests were tested for sensitivity at various dilutions, with only three repetitions for each. Three repetitions may not give a true reflection of the tests and does not allow any meaningful statistical comparisons to be made. No repeatability studies were reported to justify the choice of three repetitions. No previous studies were consulted for comparison. For instance, the described study found Hemastix[®] were more sensitive than the KM test, which is contrary to previous studies where the sensitivity of KM was found to be equal to that of Hemastix[®] (4). Only previous papers describing the sensitivity of the Hexagon OBTI kit were discussed.
3. Luminol, a highly sensitive and well-used presumptive blood test, was not included in the study. Other immunologic methods for the detection of primate blood were identified in the introduction, but none of these tests were included in the current study and no reason was given as to why they were excluded (i.e., previous studies indicating they were unsuitable). Previous research investigating HemeSelect[™] (6) and ABACard[®] HemaTrace[®] (7) are available but do not seem to have been referred to by the authors. In our view, results from those studies should have been included for comparison purposes.

4. The abstract describes the Hexagon OBTI test as primate specific, a point repeated throughout the study. In the concluding comments it is stated that the Hexagon OBTI test “would ascertain whether located stains are human blood.” We are concerned that the sentiments expressed in this statement have not been shown by the research carried out. In fact, the actual Hexagon OBTI user's manual states that the test will react with human, primate, ferret, and skunk hemoglobin (available http://www.bluestar-forensic.com/pdf/en/instructions_hexagon_obti.pdf). Although primates and skunk may not be encountered in the U.K. (where experimentation was undertaken and, presumably where the test would be used), ferrets are common household pets. The test may further react with other animals from the genus *Mustela* (ermine, ferrets, minks, and weasels).
5. Hexagon OBTI was demonstrated to be unable to detect undiluted blood or blood at a dilution of 1 in 10. Although a possible explanation of the high-dose hook effect was given for this false negative, no research was undertaken to prove this was responsible. No research was undertaken to provide a solution to this significant problem. This may significantly affect the operational usefulness of Hexagon OBTI as a blood presumptive test reagent.
6. The authors endorse the use of Hexagon OBTI even when there may be much better tests available, most recently the Rapid Stain Identification[™]-Blood test (8), which does not cross-react with ferret, skunk, or primate blood and exhibits no high-dose hook effect. Alternatively, there is a test for staining human blood *in situ* and identifying the DNA containing leukocytes (9).

References

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