**Article**

# The Use of the Hexagon OBTI Test for Detection of Human Blood at Crime Scenes and on Items of Evidence

## Part I: Validation Studies and Implementation

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**Abstract**: The Hexagon OBTI kit was adapted for human blood detection at crime scenes as well as on items of evidence. The detection limit was increased while its specificity was kept unchanged. The implementation of the kit by the Israel National Police was accomplished with the introduction of a newly designed "blood testing" carrying case. The case contains all the items and reagents required for the Kastle-Meyer presumptive blood identification test and the Hexagon OBTI test.

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## Introduction

Currently, crime scene technicians of the Israel National Police (INP) use the Kastle-Meyer (KM) color blood test at crime scenes [1, 2]. This presumptive test gives a strong indication for blood but is not human specific [2]. Following the KM test, the stain is transferred to the laboratory for DNA analysis. In some cases, DNA profiling is not required; however, the investigator must determine whether the stain contains human blood. In these cases, the Precipitin test [2] is performed to determine the source of the stain. Transferring the sample from the crime scene to the laboratory and its analysis is time- and money-consuming.

The Hexagon OBTI test, a commercial kit designed for detection of fecal occult blood [3], was validated by Hochmeister et al. for forensic identification of human blood [4, 5]. The kit consists of a test device and a collection tube. The test procedure is simple and does not require a laboratory setting [3]. The OBTI test is therefore suitable for identification of human blood at crime scenes [4, 5].

This paper reports the results of a validation study carried out for the purpose of implementing the Hexagon OBTI test in the detection of human blood at crime scenes. The Hexagon OBTI kit was included as a component of a new "blood testing" carrying case for crime scene technicians.

## Materials and Methods

### *Body Fluids Stains Preparation*

Whole blood samples or blood diluted from human donors and animals were placed on sterile gauze pads and air-dried at room temperature. Diluted blood was prepared by serial dilution in water, and stains were prepared as above.

Animal species tested were orangutan (*Pongo pygmaeus*), donkey (*Equus asinus*), camel (*Camelus bactrianus*), pigeon (*Columba livia*), chicken (*Gallus gallus*), cat (*Felis catus*), dog (*Canis familiaris*), goat (*Capra hircus*), sheep (*Ovis aries*), cow (*Bos taurus*), turkey (*Meleagris gallopavo*), horse (*Equus caballus*), ostrich (*Struthio camelus*), rabbit (*Oryctolagus cunic-*

*ulus*), trout (*Oncorhynchus mykiss*), mountain gazelle (*Gazella gazella*), lemur (*Lemur sp.*) and squirrel monkey (*Saimiri sciureus*).

Thalasemic and newborn bloods were obtained from the Israel Blood Bank. Semen was obtained from a semen bank. All samples were deposited on sterile gauze pads.

Vaginal secretions, mucus, mother's milk, urine, and perspiration were directly deposited by donors on sterile gauze pads. All stains were air-dried at room temperature.

*Hexagon OBTI Test*

The test was performed essentially according to the manufacturer's instructions [3]. The dry stains were scraped using the applicator, which is an integral part of the cap of the collection tube supplied with the kit. In some experiments, a thread from a stained gauze pad was immersed directly into 2 ml of buffer in the collection tube. The collection tube containing the sample was manually shaken and incubated at room temperature for up to 30 minutes. Two drops were transferred to the test device. The results were recorded within 10 minutes from application time. The appearance of a blue test line indicated a positive result (Figure 1).

In some experiments, the time was extended up to one hour, and in some experiments, the volume of the sample buffer was reduced to 0.2 ml in order to increase the sensitivity of the test.

*Kastel-Meyer Test*

The test was performed as described by Cox [1] with a slight modification: phenolphtalin (Sigma P-89031) was employed instead of phenolphtalein and the reduction with zinc powder was omitted. The immediate development of a pink color was recorded as a positive result.


*Figure 1*

*Hexagon OBTI collection tube (upper) and test device (lower): the appearance of a blue test line indicates a positive result.*

## Results and Discussion

### *Validation Study*

The purpose of the present study was to adapt and validate the Hexagon OBTI kit for use at crime scenes. The sampling method used (scraping dry stains with the applicator provided with the kit and direct immersion in the collection tube) is simple and suitable for field application.

As expected, all blood samples, human and animals, reacted positively in the KM test. Human blood from thalasemic and newborns also reacted positively with the OBTI test. Blood obtained from animals other than primates reacted negatively even after a one-hour incubation in the testing device. These results are in agreement with the reports of Hochmeister et al., who found the Hexagon OBTI test to be human and primate specific [4, 5]. However, Rowely has shown that domestic ferret's blood reacted positively, probably because of a short sequence of amino acids that is common to domestic ferret (*Mustela puterius fero*) and human hemoglobin [6].

In an attempt to enhance the sensitivity of the test, the amount of the sampling buffer was reduced to 0.2 ml instead of 2 ml. In these conditions, two out of three samples of mucus gave positive results following a one-hour incubation in the test device (but not during the recommended 10-minute period). One of six semen samples reacted positively within 10 minutes from application. This is similar to the findings of Hochmeister et al. and Spear and Binkley, who detected hemoglobin in body fluids [4, 7]. However, all body fluids samples, including those that reacted positively with the Hexagon OBTI test, were negative when tested by the KM test. Various animal bloods (excluding primates) were also tested in 0.2 ml volume, and all produced negative results following up to a one-hour incubation after application to the test device.

Human blood diluted up to 1:250 was detected within the recommended 10-minute development time. Blood diluted to 1:1000 could also be detected when a stained thread was immersed in a smaller volume of 0.2 ml (Table 1). Immersing a 0.5 cm cotton thread removed from the stain directly into the collection tube which contained 2 ml buffer increased the detection limit to 1:500 dilution. Blood diluted to 1:1000 was detected by extending the detection time to 15 minutes. The KM presumptive blood test and the precipitin test failed to detect the 1:250 diluted bloodstains. The lower limit of detection reported by Hochmeister et al. was up to blood dilution of 1:1,000,000 [5]. This discrepancy is the consequence of different sampling and extraction procedures. The amount of blood sampled in our experiments is only a fraction of that sampled by the method of Hochmeister et al. [5], but the sampling and extraction protocols used here are more in line with crime scene applications.

The Hexagon kit was tested in the laboratory on bloodstains from forensic casework. Among 35 casework stains that reacted positively with the KM test, 34 yielded positive results within the recommended 10-minute development time. One stain, which was KM positive and Hexagon OBTI negative, turned out to contain dog blood as determined by a Precipitin test using antidog antiserum.

*Kit Implementation by Crime Scene Units*

Following the validation study at the laboratory, the Hexagon OBTI kit was distributed to five Serious Crime Unit districts to evaluate its performance in the field. For that purpose, a new

| | Time interval between application to results recording | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Immediately | | 5 min | | 10 min | | 15 min | | 30 min | | 60 min | |
| | Volume of incubation buffer | | | | | | | | | | | |
| Dilution | 2 ml | 0.2 ml | 2 ml | 0.2 ml | 2 ml | 0.2 ml | 2 ml | 0.2 ml | 2 ml | 0.2 ml | 2 ml | 0.2 ml |
| 1:250 | + | + | + | + | + | + | + | + | + | + | + | + |
| 1:500 | + | + | + | + | + | + | + | + | + | + | + | + |
| 1:1000 | - | + | - | + | - | + | + | + | + | + | + | + |
| 1:2000 | - | - | - | - | - | - | - | - | - | - | - | - |
| 1:4000 | - | - | - | - | - | - | - | - | - | - | - | - |
| 1:8000 | - | - | - | - | - | - | - | - | - | - | - | - |
| 1:16000 | - | - | - | - | - | - | - | - | - | - | - | - |

+ indicates a positive result
- indicates a negative result

*Table 1*

*Sensitivity of the Hexagon OBTI test.*

"blood-testing" carrying case was designed (Figure 2). The case components are contained in a hardened polyurethane internal structure. The carrying case includes two Hexagon OBTI kits (test devices and collection tubes) and bottles containing reagents for the KM test. The case also contains filter papers for sampling suspected stains prior to testing by KM reagents and plastic discs with filter paper soaked with diluted blood (1:100 in saline) for positive control tests (Figure 3). Instruction sheets for the KM and Hexagon OBTI tests are also included.

During the first month of assessment, two reported cases demonstrated the benefit of the Hexagon kit in crime scene use. In the first case, a burnt out car suspected to belong to a missing person was found. Searching the area, police found a body that was later identified as that of the missing person. The body was partially buried with only the upper back exposed. The exposed shoulders were very hairy and splashed with white lime powder, causing the officers first to believe that it was a dog. While waiting for the arrival of a pathologist, a crime scene technician sampled material from a small scratch on the exposed part of the body and, using the Hexagon OBTI kit, found it to be positive for human blood.

In the second case, a large puddle of suspected blood with a fragment of bone was found near a bank entrance. The police had no previous report or information that a violent crime had been committed in this area. A technician was alerted to the scene and performed KM testing, which provided a positive indication for blood. Human origin of the blood was further identified with the Hexagon OBTI kit, and a police investigation was initiated.

Both cases describe situations in which direct identification of human blood at the crime scenes assisted in the early stages of the investigations.

## Conclusions

The sensitivity of the Hexagon OBTI kit was enhanced by reducing the volume of the sample buffer to 0.2 ml (4 drops). However, the use of reduced sample buffer volume is recommended only under special circumstances, such as testing bloody fingerprints (see accompanying paper), and the 10-minute time interval from application to the test device and results reading should be kept. It is recommended that KM test be carried out

*Figure 2*
*The "blood testing" carrying case.*

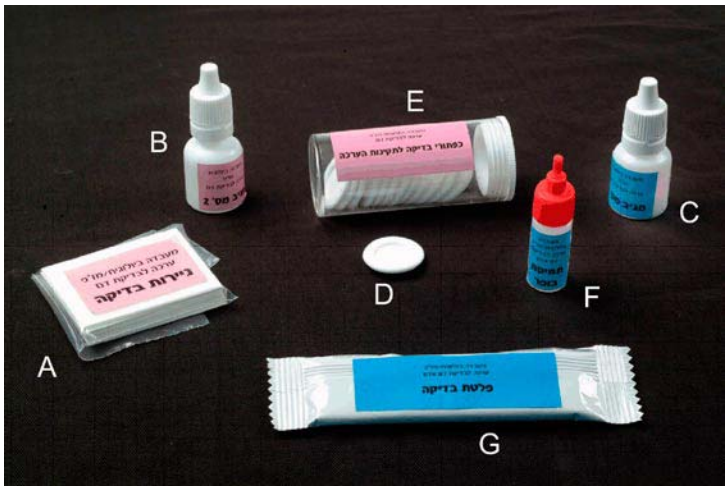


*Figure 3*
*Components of the blood testing carrying case.*
*A: Filter papers for sampling presumed blood stains.*
*B and C: Kastel-Meyer (KM) reagents 1 and 2.*
*D: Testing button: plastic discs with filter paper containing diluted blood (1:100) for KM positive control testing.*
*E: A cylinder containing testing buttons (as in D).*
*F: Collection tube*
*G: Hexagon OBTI test device.*

before the Hexagon OBTI test, especially when the latter is performed in a reduced volume.

The Hexagon OBTI kit was introduced as a component of a new "blood testing" carrying case for use by crime scene units.

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