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Forensic Identification of Human Blood: comparison of two one-step presumptive tests for blood screening of crime scene samples

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Resumo

O sangue é o fluido corpóreo mais comumente encontrado em cenas de crime. Testes presuntivos de passo único foram concebidos como testes imunológicos rápidos para a detecção qualitativa de hemoglobina humana em amostras de fezes (sangue oculto em fezes). A utilidade desses testes para fins forenses já foi demonstrada anteriormente. Neste artigo, apresentamos um estudo de comparação entre o kit Hexagon OBTI e kit FOB Passo Único Bioeasy nas análises de amostras de sangue diluído. Com o Hexagon OBTI kit, são alcançados resultados positivos em diluições de sangue total de até 1:1.000. A sensibilidade do teste diminui com amostras envelhecidas, se estas não foram armazenadas em baixas temperaturas, independentemente de qual teste presuntivo é usado. Testes de sangue total devem ser analisados com cautela tendo em vista que o efeito prozona pode interferir nos resultados. Comparando ambos os testes, OBTI Hexagon Kit é mais sensível na detecção de sangue diluído, mostrando uma janela de detecção mais ampla em todas as condições estudadas. Esse achado é interessante quando se analisa amostras forenses já que geralmente não estão disponíveis informações prévias relativas às amostras analisadas.

Palavras-Chave: **S**angue; Teste Presuntivo; Hexagon OBTI.

Abstract

Blood is one of the most common body fluid found at crime scenes. One-step presumptive tests have been designed as a rapid immunological test for the qualitative detection of human hemoglobin in stool samples (faecal occult blood) their usefulness for forensic purposes has been demonstrated before. In this study, we compare Hexagon OBTI kit and FOB One-step Bioeasy kit sensitivity in the analysis of diluted blood samples. With Hexagon OBTI, positive test results are achieved in whole blood dilutions up to 1:1,000. Sensitivity decreased with aged samples, if samples were not stored under low temperatures regardless of which presumptive test is used. Whole blood tests must take into consideration that "hook" effect may interfere. Comparing both tests, OBTI Hexagon Kit is more sensible to detect diluted blood, showing a wider detection window in all conditions. This is interesting when analyzing forensic samples as forensic analysts usually do not know about the history of the analyzed sample before its collection.

Keywords: **B**lood; Presumptive Test; Hexagon OBTI.

1. INTRODUÇÃO

Blood is the most common body fluid found at crime scenes. Several presumptive tests and confirmatory tests to identify blood have already been described [1].

One-step presumptive tests have been designed as a rapid immunological test for the qualitative detection of human hemoglobin in stool samples (faecal occult blood). Additionally, these tests are suited for forensic purposes to determine whether blood traces are of human/primate origin or not [2].

Some validation studies were carried out in order to determine efficacy and reliability of one-step presumptive blood tests for forensic casework [3-7]. Hochmeister et al. [3] tested primate blood, diluted blood and others humans secretions samples in order to measure specificity and

sensibility. Dilutions up to 1/1,000,000 tested positive with fresh blood. Hexagon OBTI kit is not specific solely for blood. Body fluids other than blood as extracts of saliva, urine, stool, vaginal secretion and semen, may contain trace amounts of hemoglobin and yield a positive result. Degradation and contamination studies were also carried out showing that the antigen is insensitive to a variety of environmental insults, except for exposure to certain detergents and household bleaches and prolonged exposure to certain preparations of luminol.

Hemon et al. [4] also determine specificity and sensibility testing blood samples beyond human, other human secretions as well as diluted human blood, finding that Hexagon OBTI test is primate specific and demonstrating positive results to dilutions up to 1:1,000 when a reduced buffer volume is used.

Johnston et al. [5] compared four presumptive blood tests. Dilution tests were performed showing positive results for Hexagon OBTI test in dilutions up to 1x103. Recently [7] compared faecal occult blood test with Kastel-Mayer/Coombs tests results in order to determine if there is consistency between the results obtained, finding concordance in 95.4 % of the cases and suggested that FOB tests are more efficient as it showed positive results in samples even more diluted than Kastel-Mayer/Coombs tests.

All these studies demonstrate the usefulness of the Hexagon OBTI kit for forensic analysis. To the best of our knowledge no study were conducted with FOB One-step Bioeasy kit.

This paper shows a comparison study between Hexagon OBTI kit and FOB One-step Bioeasy kit in the analyses of diluted blood samples and their suitableness for forensic casework.

2. MATERIAL AND METHODS

2.1. Blood dilution and stain preparation

Whole blood samples were obtained from one volunteer and diluted up to 1:50,000 (1:10, 1:50, 1:100, 1:250, 1:1,000, 1:5,000, 1:10,000, 1:50,000) using sterile water. Fifty microliters of each dilution were placed on filter paper. All samples were air dried for 24 h.

Filter paper samples were separated in three groups and maintained at specific temperatures (4ºC, 25ºC and 37ºC). Tests were performed weekly for the first month and then monthly until 4 months.

Negative controls (sterile water and Tris buffer pH 7.5) were also prepared and analyzed. All experiments were performed in triplicate and the results reported were the least sensitive observed.

2.2. Determination of sensitivity

Tests were performed according to manufacturer's instructions (product literature). Dried stains were cut out and immersed directly into 2 mL buffer in the collection tube. The collection tube was then manually shaken for 30 seconds and two drops were immediately dispensed onto the sample slot of the test bar. Results were recorded 10 minutes after application time. The development of a control test line indicated human blood was detected.

In some experiments, in order to increase the sensitivity of the test, sample buffer volume was reduced to 0.2 mL as in [4].

3. RESULTS AND DISCUSSION

Two one-step presumptive blood kits, Hexagon OBTI and FOB One-step Bioeasy, were compared for their sensitivity in the identification of dried bloodstains of varying dilutions.

With Hexagon OBTI kit, all bloods samples tested positive for human hemoglobin up to 1:1,000 dilution until week two. After that, blood samples diluted 1:1,000 only tested positive when maintained under low temperature (4ºC). Blood samples diluted 1:250 tested positive throughout the experiment under all conditions. Dilutions above 1:1,000 tested negative even with the reduced buffer volume of 0.2 mL

With Bioeasy FOB kit, samples maintained under low temperature (4oC) tested positive for dilutions up to 1:500, decreasing sensibility only on month four. Room temperature samples tested positive for dilutions up to 1:500 until week two decreasing during the experiment to positive results in samples diluted up to 1:100 in month four. Tests showed a faster decrease of sensibility for samples stored under 37ºC and after four months positive results were only observed in samples diluted up to 1:10. The results of diluted bloodstains are presented on Table 1.

The Hexagon OBTI kit results presented here are similar to those described by [4]. Although, data in literature are controversial; Johnston et al. [5] observed a higher sensitivity but only with reduced buffer volume; Human Diagnostic Worldwide [2] described positive tests results to blood dilution up to 1:1,000,000 but buffer incubation time was extended up to 2 hours. The observed differences may be a consequence of different sampling, extraction procedures, blood amount used and/or incubation time.

Whole blood tests, although positive, always present a faint blue test line. These are thought to be due to an excess of analyte, which prevents the simultaneous binding of solid-phase and liquid-phase monoclonal antibodies known as "hook" effect [6].

Comparing both tests, Hexagon OBTI demonstrates a wider detection window than FOB One-step Bioeasy tests

regardless which temperature the sample is stored. Sensibility always decreases with aged samples, unless if samples are stored in 4ºC and Hexagon OBTI test is used. Both tests showed more sensibility when samples are stored at lower temperatures. For samples maintained at high temperature sensibility decrease faster and positive results are achieve only until month 4 when FOB One-step Bioeasy is used.

4. CONCLUSION

Those tests are rapids and simple to perform. With Hexagon OBTI positive test results are achieved in whole blood dilutions up to 1:1,000. Sensitivity decreased with aged samples, if they were not stored under low temperatures regardless of which presumptive test is used. Whole blood tests must be taken considering that "hook" effect may interfere. Comparing both tests, OBTI Hexagon Kit is more sensible to detect diluted blood, showing a wider detection window in all conditions. This is interesting when analyzing forensic samples as forensic analysts usually do not know about the history of the analyzed sample before its collection.

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REFERENCES

[1] J. H. An, K.-J. Shin, W. I. Yang, K. Y. Lee, "Body fluid identification in forensics", *BMB Reports* **45**(10): 545-553, 2012.

[2] Human Diagnostic Worldwide, "Design verification and product data for Hexagon OBTI". Rev. 012, 2012.

[3] M. N. Hochmeister, B. Budowle, R. Sparkes, O. Rudin, C. Gehrig, M. Thali, L. Schmidt, A. Cordier, R. Dirnhofer, "Validation studies of immunochromatographic 1-step test for the identification of human blood". *J. Forensic Sci.* **44**(3): 597-602, 1993.

[4] D. Hemon, M. Shpitzen, C. Oz, B. Glattstein, M. Aroury, R. Gafny, "The use of Hexagon OBTI test for detection of human blood at crime scene and on itens of evidence part I: Validation studies and implementation". *J. Forensic Identif.* **53**(5): 566-575, 2003.

[5] E. Johnston, C. E. Ames, K. E. Dagnall, J. Foster, B. E. Daniel, "Comparative of presumptive blood test kits including Hexagon OBTI". *J. Forensic Sci.* **53**(3): 687-689, 2008.

[6] S. A. Fernando, G. S. Wilson, "Studies of "hook" effect in one-step sandwich immunoassay". *J. Immunological Methods*, **151**: 47-66, 1992.

[7] P. Longo, C. R. Dias Filho, M. P. O. Valadares, E. C. Alonso, S. P. S. Gonçalves, E. Auler-Bittencourt. "Avaliação comparativa de teste imunocromatográfico para identificação forense de sangue humano". *R. Bras. Criminalística*, **1**:1-16-21, 2011.

Table 1. Results from one-step presumptive tests. + cells = positive results. +* = positive results only with reduced buffer volume. - = negative results.

Dilutions	Temp	Time	OBTI	FOB	Time	OBTI	FOB	Time	OBTI	FOB	Time	OBTI	FOB	Time	OBTI	FOB	Time	OBTI	FOB	Time	OBTI	FOB	Time	OBTI	FOB
1/1			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/10			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/50			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/100	25ºC		+	+		+	+		+	+		+	+		+	+		+	+		+	+*		+	+*
1/250			+	+		+	+		+	+*		+	+		+	+		+	-		+*	-		+*	-
1/500			+	+*		+	+*		+	-		+	-		+	-		-	-		-	-		-	-
1/1000			+	-		+	-		-	-		-	-		-	-		-	-		-	-		-	-
1/1			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/10			+	+		+	+		+	+		+	+		+	+		+	+		+	+*		+	+*
1/50			+	+		+	+		+	+		+	+		+	+		+	+*		+	-		+	-
1/100	37ºC		+	+		+	+		+	+*		+	-		+	-		+	-		+	-		+	-
1/250			+	+		+	+*		+	-		+	-		+	-		+*	-		+*	-		+*	-
1/500			+	+*		+	-		+	-		+	-		+	-		-	-		-	-		-	-
1/1000			+	-		+	-		-	-		-	-		-	-		-	-		-	-		-	-
1/1			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/10			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/50			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/100	4ºC		+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/250			+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
1/500			+	+		+	+		+	+		+	+*		+	+*		+	+*		+	+*		+	-
1/1000			+	-		+	-		+	-		+	-		+*	-		+*	-		+*	-		+*	-