HAPTOGLOBIN TYPING IN BLOODSTAINS*

การตรวจหาแฮปโตโกลบินในคราบเลือด

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ABSTRACT

In crime scene, biological evidences which are usually found are bloodstains. Most forensic science laboratories can provide the answer for the detective, whether they are human or animal; and if human, to what kind of ABO group it belongs. Haptoglobin typing will also increase per centage accuracy of identification.

The detection of haptoglobin in bloodstains is carried out by extracted blood in tris-citrate buffer and run in starch gel electrophoresis in NaOH-boric and stained with benzidine-hydrogen peroxide. The persistence of haptoglobin varies according to the supporting medium and environment (heat, moisture) and also to its phenotypes. The duration for detection of haptoglobin in bloodstains is between 1 and 7 days.

บทคัดย่อ

แฮปโตโกลบิน เป็นสารประกอบพวกแอลฟากลัยโคโปรตีน ทำหน้าที่รวมตัวเป็นเฮโมโกลบินเกิดเป็นสารประกอบแฮปโตโกลบิน–เฮโมโกลบิน เพื่อจะป้องกันการสูญเสียเฮโมโกลบินจากร่างกายทางไต แฮปโตโกลบิน แบ่งออกเป็น 3 พวกใหญ่คือ Hp 1-1, Hp 2-1 และ Hp 2-2 การถ่ายทอดทางกรรมพันธุ์อาศัยยีนส์ 2 ตัวคือ Hp 1 และ Hp 2 ประโยชน์ที่นำมาใช้ในทางนิติวิทยาศาสตร์คือ ใช้ในการตรวจพิสูจน์ปฏิเสธความเป็นพ่อ ลูก และการตรวจพิสูจน์บุคคล

ในสถานที่เกิดเหตุคดีอาชญากรรมทั่ว ๆ ไป วัตถุพยานทางชีวะที่พบได้บ่อยที่สุดคือคราบเลือด การตรวจพิสูจน์คราบเลือดก็เพื่อจะได้ทราบว่า คราบเลือดนั้นเป็นของคนหรือสัตว์ ถ้าเป็นของคน เป็นหมู่ เอ บี โอ หรือ เอบี นอกจากการตรวจหาหมู่เลือดเอ บี โอแล้ว การตรวจหาหมู่ซีรัมพวกแฮปโตโกลบินยังจะสามารถช่วยในการตรวจพิสูจน์ยืนยันอีกด้วย

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การตรวจหาชนิดของแฮปโตโกลบินในคราบเลือดทำได้โดยสกัดคราบเลือดด้วย tris-citrate buffer โดยมี starch gel เป็นตัวกลาง นำไปแยกด้วยเครื่องอิเล็กโตรฟอเรซีส โดยมี NaOH-Boric acid เป็นบริดจ์บัฟเฟอร์ ย้อมด้วยสีเบนซิดีนและไฮโดรเจนเพอร์ออกไซด์ พบว่าความคงทนของแฮปโตโกลบินในคราบเลือดขึ้นอยู่กับขนาดของแฮปโตโกลบิน สภาพแวดล้อม (ความร้อน, ความชื้น) และสิ่งรองรับคราบเลือด ระยะเวลาที่สามารถตรวจพบแฮปโตโกลบินได้ คือ ระหว่าง 1 ถึง 7 วัน

INTRODUCTION

Bloodstains are one of the most frequent and important types of evidence encountered in criminal investigation. They are found at times in connection with almost every type of criminal activity and must not be overlooked. Bloodstain may be on the victim, the crime scene, the culprit and the weapon.

Haptoglobin is a α -glycoprotein which combines hemoglobin in serum to form haptoglobin-hemoglobin complex, to prevent the loss of iron via the kidney. Haptoglobin is divided into three main groups: Hp 1-1, Hp 2-1 and Hp 2-2. The inheritance of it is determined by two allele autosomal genes, Hp 1 and Hp 2. The haptoglobin is used in forensic science in case of disputed paternity and personal identification.

In bloodstain investigation, the following questions are liable to arise[4]:

Is it blood or not?

Is it human or animal blood?

Does the blood belong to male or female?

Does the blood come from a certain individual?

The most common chemical for presumptive test is benzidine. A solution, comprising of saturated solution of benzidine in glacial acetic acid and hydrogen peroxide, will turn blue with very small quantities of blood. This is a preliminary sorting test. A negative result excludes blood. A positive result should be followed by other confirmatory tests. Because this test is unspecific, since it is also positive with direct oxidising agents such as potassium permanganate, potassium dichromate, and copper sulphate.

These substances will give positive result only with the solution of benzidine in acetic acid.

Confirmatory test

a. Serological Test: This method is the most common used for various confirmatory procedures, having the advantages of speed and specificity. Precipitation caused by antihuman globulin, or other antisera, will confirm that the substance causing the stain is blood and will indicate whether the blood is human or animal.

b. Spectroscopic Test: A solution of reduced hemoglobin (obtained by adding reducing agents such as ammonium sulphide, 10% sodium dithionate, 0.2% hydrazine hydrate or sulphate to HbO_2) has a characteristic spectrum. The test is made more specific by adding various reagents

to the solution of the stain, to produce different products of hemoglobin, such as methemoglobin, which have their own characteristic spectrum.

c. Microscopic Test: In dried bloodstains, the various formed elements of the blood, red cell and white cell, etc. are usually destroyed. However, red cell can occasionally be identified if the stain is reasonably fresh, and their appearance may allow the source of the blood to be identified. Thus, red cells of avis are destroyed and those from a camel are oval[2].

For the question of individuality, the ABO grouping of dried bloodstains usually give satisfactory answers. Other blood group systems, MNS, Rh, Duffy, Kell etc. could be used but they are not popular as ABO.

Serum types could be used too. Detection of serum types in bloodstain has been described since the year 1961 by Durwald on haptoglobin and also in the year 1961, 1962 by Planques and Ducos respectively[3]. The studies on Gm typing in bloodstain were first described by Nielsen and Henningsen in 1963. Other serum types for example InV, Gc typing in bloodstain have also been studied too.

The possibility of detection of haptoglobin phenotype in bloodstains in different conditions and at various stages will be possible in these studies.

MATERIALS AND METHODS

Electrophoresis vessel

Plastic trays with dimension 12 × 12 × 6 cm are used. The upper halves of the side frames, 0.3 cm thick, are removable and screw to the lower halves.

Appropriate-sized buffer chamber was made from plastic plates, and small platinum wires were used as electrodes.

Chemical

1. Trizma Base No. T-1503 (Sigma Chemical Company, St. Louis, U.S.A)
2. Hydrolysed Starch for electrophoresis No. S-4501 (Sigma Chemical Company, St. Louis, U.S.A.)

Buffer system

1. Bridge buffer (0.3 M boric acid, 0.05 M sodium hydroxide)
2. Gel buffer (0.076 M Trizma base (tris-hydroxymethyl aminomethane) 0.005 M citric acid pH 8.6)

Preparation of the starch gel

The gel was prepared by boiling 14% hydrolysed starch in gel buffer in a suction flask. The suspension was continuously stirred with a magnetic stirrer, until it was ready to be used. Cease boiling and a suction pump was applied to degas the gel, when all air bubbles were completely removed, poured the solution into the tray. Allowed the gel to cool in room temperature for about 30 min and at 4°C for about 30 min.

Made slots in the gel by using single-edge razor blade and then put filter paper with equal width of the slots into the holes and withdrew. Serum sample was introduced into the slots with pasteurized pipettes.

Sample preparation

Packed red cells were washed in 0.9% normal saline three times. Hemolysed red cells were produced by adding distilled water with proportion 2 : 15.

Five drops serum sample were mixed with 2 drops of hemoglobin solution, incubated at 37°C for 30 min immediately before electrophoresis.

Bloodstains were prepared from known typing blood on clean white cotton cloth, kept in three conditions:

1. room temperature
2. direct sunlight
3. damp place

For the bloodstain analysis, a piece of bloodstained cloth (about 3 × 10 mm) was cut and soaked in tris-citrate buffer. It was put in a moist chamber at 4°C over night and inserted in the starch gel.

Electrical connection

Filter paper wads were soaked in the bridge buffer solution connecting the gel and the bridge compartments. Thick wads with a width corresponding to the entire tray were used. Similar filter paper bridge connected the electrode compartment and the bridge solution.

Electrophoresis time

Average voltage for electrophoresis was 4-6 V/cm for 4 h.

Slicing of the gel

At the end of electrophoresis released the screws on the rims of the tray to remove the upper side frames. With a very small wire, sliced the gel into two horizontal slabs along the edge of the new half-heighted sides.

Staining method

Saturated solution of the benzidine in glacial acetic acid was applied to the cut surface by using a soft brush and hydrogen peroxide, by means of a piece of filter paper.

RESULTS

The persistence of haptoglobin in bloodstains does not depend on sex but depends on phenotypes of them. Table 2 shows that the persistence of haptoglobin Hp 2-2 is least in every medium and environment. The nature of medium and environment has much effect on the persistence of haptoglobin (Table 1 & 2). It is found that :

For absorbent medium

a. The persistence of haptoglobin on absorbent medium in damp place are less stable than in condition of room temperature and direct sunlight (Table 1 & 2).

b. There is no significant difference of the persistence of haptoglobin between phenotypes of them in the same condition (Table 2).

c. The stable phenotype of haptoglobin is between 3 days (in damp place) and 5 days (at room temperature or under direct sunlight).

For non-absorbent medium

a. The persistence of haptoglobin direct sunlight is less stable than in room temperature and damp place (Table 1 & 2).

b. There is no significant difference of the persistence of haptoglobin between phenotype of them in condition of direct sunlight and damp place; but at room temperature, the difference of the persistence of haptoglobin between phenotypes are clearly seen, Hp 1-1 is the most stable and Hp 2-2 is the least (Table 2).

c. Hp 1-1 is the most stable (7 days) and Hp 2-2 is the least (1 day direct sunlight) (Table 2).

DISCUSSION

The most frequent laboratory examinations required for forensic purposes are the examination of articles for the presence of blood. The question "Does the blood come from a certain individual?" may be important in accusing a suspected person and of proving or disproving the truth of this statement. In general, most laboratories ABO grouping are used to solve this question by absorption-elution method. Serum grouping is also used too, to increase per centage accuracy of identification.

Starch gel electrophoresis divides haptoglobin into three main types (Figure 1). Detection of haptoglobin in bloodstains on absorbent material is available if the stains are not more than five days old in room temperature, but may be about three days in damp place. On non-absorbent material heat has much effect on the persistence of haptoglobin, so we cannot detect them if the stains are more than three days old. But under room temperature condition and in damp place, detection of them depends on the phenotypes for example, about 7 days for Hp 1-1, 5 days for Hp 2-1 and not more than three days for Hp 2-2.

Effect of sunlight on the persistence of haptoglobin in bloodstains is due to the process of "denaturation" caused by heat and ultraviolet light. Sunlight is not the only factor that produces denaturation but also moisture. Bacterial growth in damp place cause protein lysis from its digestion. So moisture as well as heat and ultraviolet light can cause denaturation.

The reason is that the difference of the persistence of haptoglobin on non-absorbent medium and absorbent medium which makes the difference between Hp 2-2 and Hp 1-1 by preserving Hp 2-2 bands rather than Hp 1-1 and Hp 2-1 bands in contrast to the persistence of haptoglobin on non-absorbent medium, is not yet known; further investigation should be done.

It is very difficult to interprete the result as well as to distinguish the difference between Hp 1-1 and Hp 2-1 if the 2-2 like components are absent, so the control serum should be done in every bloodstain typing to avoid the misinterpretation. The most suitable control serum is Hp 2-1.

SUMMARY

1. Haptoglobin grouping of bloodstains was performed on experimental stains on two kinds of material : absorbent (cloth) and non-absorbent material (wood, glass, cement floor). The stains were kept for 1, 3, 5 and 7 days. Each was subdivided into 3 groups. One group was placed in drying stage at room temperature, another in moist stage at room temperature and the last in drying stage under direct sunlight.

All the haptoglobin grouping was performed by starch gel electrophoresis.[1]

2. The result of typing in absorbent material at room temperature can be detected for 5 days in dry condition, but in damp place or moist chamber it is reduced to 3 days and shows no significant difference between their phenotypes.

3. The haptoglobin grouping, on non-absorbent material, at room temperature clearly shows that the persistence of haptoglobin is correlated to phenotypes of them, Hp 1-1 is the most stable and Hp 2-2 is the least. Hp 1-1 can be detected in bloodstains up to 7 days, but Hp 2-2 in only 3 days. The environment such as moisture showed no difference of the persistence.

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Table 1. The results of haptoglobin typing in bloodstain of different environment in room temperature, damp place, and direct sunlight.

Phenotype	Medium	No. of testing	Room temperature				Damp place				Direct sunlight			
			Positive result (days)				Positive result (days)				Positive result (days)			
			1		5	7	1	3	5	7	1	3	5	7
1-1	Wood	10	+ + +	+ +	+	+	+ + +	+	–	–	+ +	–	–	–
	Glass	10	+ + +	+ +	+	+	+ + +	+	–	–	+ +	–	–	–
	Cement floor	10	+ + +	+ +	+	+	+ + +	+	–	–	+ +	–	–	–
	Cotton	10	+ + +	+ +	+	–	+ +	+	–	–	+ + +	+ +	+	–
2-1	Wood	10	+ + +	+ +	+	–	+ + +	+	–	–	+ +	–	–	–
	Glass	10	+ + +	+ +	+	–	+ + +	+	–	–	+ +	–	–	–
	Cement floor	10	+ + +	+ +	+	–	+ + +	+	–	–	+ +	–	–	–
	Cotton	10	+ + +	+ +	+	–	+ +	+	–	–	+ + +	+ +	+	–
2-2	Wood	10	+ +	+	–	–	+ + +	+	–	–	+ +	–	–	–
	Glass	10	+ +	+	–	–	+ + +	+	–	–	+ +	–	–	–
	Cement floor	10	+ +	+	–	–	+ + +	+	–	–	+ +	–	–	–
	Cotton	10	+ + +	+ +	+	–	+ + +	+ +	+	–	+ + +	+ +	+	–

Table 2. The persistence of Haptoglobin in bloodstain of different environment in room temperature, damp place, and direct sunlight.

Phenotype	Medium	No. of testing	Positive result (% Finding)																						
			Room temperature								Damp place								Direct Sunlight							
			Male				Female				Male				Female				Male				Female			
			Day 1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7
1-1	Wood	5	100	20	20	20	100	20	20	20	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND	
	Glass	5	100	20	20	20	100	20	20	20	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND	
	Cement floor	5	100	20	20	20	100	20	20	20	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND	
	Cotton	5	100	20	20	0	100	20	20	0	100	20	0	ND	100	60	0	ND	100	20	20	0	100	80	40	0
2-1	Wood	5	100	20	20	0	100	20	20	0	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND	
	Glass	5	100	20	20	0	100	20	20	0	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND	
	Cement floor	5	100	20	20	0	100	20	20	0	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND	
	Cotton	5	100	80	20	0	100	20	0	ND	100	20	0	ND	100	60	0	ND	100	60	20	0	100	40	20	0
2-2	Wood	5	100	20	0	ND	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND		100	0	ND	
	Glass	5	100	20	0	ND	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND		100	0	ND	
	Cement floor	5	100	20	0	ND	100	20	0	ND	100	20	0	ND	100	0	ND		100	0	ND		100	0	ND	
	Cotton	5	100	60	60	0	100	20	0	ND	100	80	60	0	100	0	ND		100	60	40	0	100	20	0	ND