CURRENT TRENDS IN HAPTOGLOBIN PHENOTYPE DETERMINATION IN FORENSIC INVESTIGATION OF BLOOD EVIDENCE

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Abstract. *The determination of haptoglobin phenotypes in bloodstains is widely applied in forensic examinations, particularly in cases of disputed parentage, possible child substitution, and limited or inconclusive ABO blood group results. This study aimed to evaluate the influence of bloodstain age and environmental factors on the stability and reliability of haptoglobin phenotype detection. Blood samples were obtained from healthy volunteers (18–60 years) and analyzed in native blood and in dried bloodstains deposited on paper, cotton, wool, and blended fabrics. Samples were exposed to direct sunlight, humid conditions, and soil burial for defined time intervals. Results demonstrated that haptoglobin phenotype detectability is strongly dependent on both environmental exposure and substrate type. Under direct sunlight (+25 to +30°C), reliable detection was maintained for up to 3–4 days. In humid conditions (+15 to +20°C), phenotypes remained detectable for up to one month. Soil storage (+10 to +15°C) allowed identification for up to five months, after which diagnostic reliability was lost. Thus, the forensic applicability of haptoglobin phenotyping in bloodstains is determined by the nature of the substrate and the duration and conditions of environmental exposure.*

Keywords: *forensic examination, blood analysis, haptoglobin, physical evidence, bloodstains.*

Introduction. In modern forensic medical practice, immunological and serological methods remain essential for the examination of biological evidence. Although the ABO blood group system is widely used for blood classification, its discriminatory power is often limited in complex forensic cases, such as mixed biological traces, disputed biological relationships, and cases involving individuals with identical ABO profiles. In such contexts, supplementary genetic marker systems, particularly the haptoglobin (Hp) polymorphic system, significantly enhance the evidential value of expert conclusions.

Haptoglobin is a serum glycoprotein of the α_2-globulin fraction with genetically determined polymorphism (Hp 1-1, Hp 2-1, Hp 2-2). Its forensic application is well established; however, the reliability of phenotype determination in bloodstains may be influenced by environmental conditions and substrate characteristics.

Objective. To evaluate the impact of environmental exposure and storage conditions on the stability and diagnostic reliability of haptoglobin phenotype identification in forensic bloodstains.

Materials and Methods. Blood samples were collected from healthy adult volunteers (18-60 years) after initial phenotyping of haptoglobin in native blood. Based on phenotype distribution, participants were grouped into Hp 2-1, Hp 2-2 (n=20 each), and Hp 1-1 (n=10).

Venous blood was applied to different substrates (paper, cotton, wool, and blended fabrics) without anticoagulants, air-dried, and subsequently exposed to controlled environmental conditions:

- direct sunlight exposure (up to 4 days),
- humid storage conditions (up to 4 weeks),
- soil burial conditions (up to 5 months).

Haptoglobin phenotyping was performed using vertical polyacrylamide gel electrophoresis (PAGE) with benzidine-based visualization of protein

fractions.

Results. After 2 days of exposure to direct sunlight, a general decrease in the intensity of haptoglobin fractions was observed in all bloodstains. Nevertheless, successful phenotype determination remained possible in all samples deposited on paper and wool, and in nearly all cotton samples, with the exception of 5 cases (10%). The greatest diagnostic difficulties were observed in blended fabric, where absence of fraction separation was recorded in 30% of cases, predominantly corresponding to the Hp 2-2 phenotype. Hp 2-1 samples generally demonstrated clear fraction separation across paper, cotton, and wool substrates, except for a single cotton stain, whereas Hp 1-1 samples showed the highest stability with reliable visualization across all substrates except one blended fabric sample.

After 3 days of sunlight exposure, a pronounced degradation of electrophoretic patterns was noted, with reliable phenotyping possible only in 28% of paper samples and 10% of cotton samples. Among positive results, Hp 1-1 predominated (60% in paper; 20% in cotton), while Hp 2-2 was absent in cotton samples. No detectable fraction separation was observed in blended or wool fabrics.

After 4 days of exposure under open sunlight conditions (+25 to +30°C), complete loss of haptoglobin fraction separation occurred in all examined samples, accompanied by a visible color change of bloodstains from red-brown to brownish-gray. In all control samples, haptoglobin phenotyping remained unaffected.

Overall, direct sunlight exposure exceeding 2 days significantly compromises the diagnostic reliability of haptoglobin phenotyping, with blended fabrics demonstrating the highest susceptibility to degradation.

Under humid storage conditions, all samples allowed reliable phenotyping during the first 2 weeks despite early putrefactive changes. By the end of the second week, absence of fraction separation was observed in 10% of

blended fabric and 14% of wool fabric samples, predominantly corresponding to Hp 2-2, while Hp 2-1 accounted for the remaining cases. At day 19, non-diagnostic results increased to 54% in wool and 42% in blended fabrics, while paper and cotton samples generally retained acceptable resolution.

At the beginning of the fourth week, haptoglobin fractions remained detectable in most paper samples, although 8% (Hp 2-1 and Hp 2-2) were non-diagnostic. In contrast, no fraction separation was obtained in cotton, wool, or blended fabrics. By the end of week four, phenotyping became impossible in all substrates, whereas control samples remained fully interpretable.

Thus, prolonged humid storage (>2 weeks) significantly reduces diagnostic reliability, and after 4 weeks, haptoglobin phenotyping becomes impractical.

Soil storage demonstrated a slower degradation pattern. Up to 8 weeks, phenotyping remained stable. After 9 weeks, reduced fraction intensity was observed, with non-diagnostic results in 10% of cotton and 20% of blended fabric samples, predominantly Hp 2-2. At 3 months, only minor changes were noted; however, an additional 14% of blended fabric samples became non-diagnostic, mainly involving Hp 2-2, with occasional Hp 1-1 and Hp 2-1 cases.

At the middle of the fourth month of the study, a marked reduction in the intensity of haptoglobin fractions was observed on electropherograms, with absence of fraction separation in approximately 50% of examined samples. The inability to determine haptoglobin phenotype was recorded in 34% of paper samples, 38% of cotton samples, and in 54% and 56% of samples deposited on other substrate types, respectively. Across all groups, negative results were most frequently associated with the Hp 2-2 phenotype, while the Hp 1-1 phenotype demonstrated the highest stability and lowest rate of non-detection.

By the end of the fourth month, the proportion of positive findings decreased significantly. The highest rate of successful phenotyping was observed in bloodstains on paper (28%), with positive identification in 40% of

Hp 1-1, 30% of Hp 2-1, and 20% of Hp 2-2 samples. In cotton-based stains, phenotyping was successful in 14% of cases, with the highest detection rates for Hp 1-1 and Hp 2-1 (20% each) and the lowest for Hp 2-2 (5%). In blended and wool fabrics, detectable haptoglobin fractions were observed in only 10% and 8% of cases, respectively; no Hp 2-2-positive samples were identified on these substrates, whereas Hp 2-1 and Hp 1-1 phenotypes accounted for 10–20% of positive results. By the 20th week of observation, haptoglobin phenotyping was no longer achievable in any of the examined samples.

In the control group at week 20, a general reduction in fraction intensity was also noted; however, haptoglobin profiles remained largely interpretable. On paper substrates, only 4% of samples (all Hp 2-2) yielded negative results, while cotton substrates showed 6% non-detection (Hp 2-2 in two cases and Hp 1-1 in one case). In blended fabric, positive results were obtained in 28% of samples, with complete absence of fraction separation in Hp 2-2 cases; positive identification rates reached 35% for Hp 2-1 and 70% for Hp 1-1. On wool fabric, positive results were observed in 88% of cases, although partial reduction in fraction intensity was noted in Hp 2-1 samples, while Hp 2-2 and Hp 1-1 phenotypes showed non-detection in 25% and 10% of cases, respectively.

Conclusion. Environmental conditions and substrate type significantly influence the stability and forensic detectability of haptoglobin phenotypes in bloodstains. Direct sunlight exposure leads to complete loss of diagnostic value within 4 days, while humid conditions allow reliable analysis for up to 2 weeks. Soil storage permits longer preservation of detectable fractions, although diagnostic reliability declines substantially after 3-4 months. The Hp 1-1 phenotype exhibits the highest resistance to environmental degradation, whereas Hp 2-2 is the most unstable. Paper substrates provide the most favorable conditions for long-term preservation of haptoglobin fractions. These findings emphasize the importance of considering environmental exposure and

evidence handling conditions when applying haptoglobin phenotyping in forensic biological investigations.

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