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Validation and Implementation Study for Identification of Human Blood from Different Forensic Evidence

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Abstract

Crimes aggression is a serious social and public health issue that requires an urgent valid and reliable forensic medical examination. identification of perpetrator is crucial in criminal investigation mainly in homicide, suicide, Robbery, and rapes. DNA amplification technology enables forensic scientists to profile a victim or suspect from small amounts of biological samples found on evidence or at crime scenes. Blood is one of the sample types utilized for profiling, however if these samples fail to provide a DNA profile it can be important to determine that the sample was in fact human blood or not. The ability to detect blood is vital in forensic cases involving sexual assaults and criminalistics. Could the stain be blood? Forensic serologists have been asked this question innumerable times. Hemoglobin is located in erythrocytes and predominantly serves to carry oxygen and carbon dioxide within the body. One of the most invaluable tools in a forensic science laboratory is the test used to screen for blood. In many cases involving suspected blood, there is an insufficient amount of stain to proceed beyond the screening test. Such situations place an increased importance on the sensitivity and specificity of the presumptive test employed. The chosen test must be sensitive enough to detect low concentrations of blood, and at the same time, it should possess a relatively high degree of specificity. In addition to these important features, the test should be safe, be simple to use, and provide rapid results.

Objectives: To verify and compare the validity of the one step chromatographic sandwich immunoassay as a new technique, evaluate the specificity and sensitivity for human blood origin detection and determine cross reaction with any of domestic animal's blood also old Kastel – Meyer test use as screening nonspecific screening test for blood detection.

Materials and Methods: The material and methods were used Hema Trace ABA card® kits for detection Human blood origin the principle of test is one step Chromatographic Sandwich Immunoassay. Total evidence (300) are inclusion in this study (95 outer clothing =, 50 knifes, 10 truncheon, 45 underwear, 40 light weapons, 5 hang rope) were investigated for ori-

gin of human blood also old Kastel – Meyer test use as screening nonspecific test for blood about 27 different animals blood were used for detect cross-reaction and specificity.

Results: Kastle-Meyer(KM) color blood is presumptive test gives a strong indication for blood but is not human specific. The new immunochromatographic sandwich method to be a highly sensitive, convenient and rapid test for the identification of human blood origin in forensic laboratory also the kit is highly specific for human blood without showing cross reaction with any of domestic animal's blood that study her except ferret (Mustela puterius). There is a high significant relation of evidences type with positive results mainly for outer clothing than other evidence (P = 0.0005).

Conclusion: The rapid membrane test (one step chromatographic sandwich immunoassay) easy to implement into routine casework protocols and provides identifying human blood origin from different evidence without showing cross reaction with any of domestic animals except ferret. Kastel-Meyer (KM) should be limited for screening blood detection its non-human specific. Using rapid Membrane assay leads to reduce the non-human stains sent to biology for DNA profiling reducing costs, time, and effect.

Keywords: Criminalistics, Biological Fluids, Validity and Reliability

Introduction

Forensic scientists are often asked to determine, both in the field and in the laboratory, whether a particular stain is blood or not. This is a surprisingly difficult question to answer with certainty. For many years, forensic science laboratories relied heavily upon the benzidine test to screen for blood. Because of the carcinogenic effects of benzidine, other suitable replacements have been sought by laboratories. The Kastle-Meyer test a forensic presumptive blood test, where the chemical indicator phenolphthalein is used Phenolphthalein, a clear dye immediately turns pink if oxidized by hemoglobin and hydrogen peroxide. A positive KM test indicated the presence of blood, Human blood was confirmed through the use of a lateral flow immunochromatographic device [1].

Hemoglobin has a molecular weight of 64.5 KD and is composed of 4 amino acid chains, two of which are identical to around 4% of the total weight of the molecule with concentration between 120 – 160 mg/ml and 140 – 180 mg/ml for women and men respectively, hemoglobin is one of the most abundant proteins in blood. Various confirmatory tests for the identification of blood are based on the detection of hemoglobin but do not allow differentiating between human and non-human blood. Wet blood has more value than dried blood because more tests can be run. For example, alcohol and drug content can be determined from wet blood only. Blood begins to dry after 3-5 minutes of exposure to air. As it dries, it changes color towards brown and black. Blood at the crime scene can be in the form of pools, drops, smears, or crusts. DNA can be extracted from blood (if WBCs which always contain a nucleus are present) also from, sperm, bone marrow, tooth pulp, urine and hair roots. Blood, however, is commonly used in DNA testing [2,3].

Since 2011 the laboratory of the MLD has switched to use **one-step chromatographic sandwich immunoassay** as efficient technique for Human blood origin investigation. In the past before 2011 Anti-human hemoglobin in precipitation technique was used, this method was time consuming and need high proficiency with long preparation. Many presumptive tests for Blood detection was used like Luminol test reaction with blood results in the production of light, Leucomalachite test, the leucomalachite green mixed with acetic acid and distilled water and Kastle-Meyer color test a mixture of phenolphthalein and hydrogen peroxide is tested on the unknown liquid or dry stain ,unfortunates all above methods have time consuming with many false negative and positive results, produces false positive when exposed to any oxidation material [3,4]

Diagnostic Test and The Gold Standard

Diagnostic tests are all the tests that physician can use in the process of making the diagnosis of a particular disease. It is a procedure performed to confirm or determine the presence of a disease in a person suspected of having the disease, usually following the report on signs and symptoms, or based on the results of other medical tests [5].

The most accurate test for determining a disease is a "gold standard". Since it represents the best of the existing tests, we may consider the "gold standard" as a currently preferred method for diagnosing a specific disease [6]. It is often invasive or expensive; therefore, some other diagnostic test may be used instead. Hence, a newly designed test has to be initially validated by comparing its results with a gold standard due to establish the exact health status of a person.

Validity

Validity is the capability of a test to point out which people have a disease, and which do not. It is the test accuracy, or the extent to which a test can measure what should be measured [7]. Validity is estimated by two objective measures: sensitivity and specificity [8]

Study design

This is an observation and prospective study conducted in Iraqi Medical Legal Directorate / DNA Fingerprint department, Serology Division, which serves as the referral center for all Iraqi Provinces sexual assault victims and criminal crimes. From first of June 2014 to 31 of May 2015 about 300 evidence (120 outer clothing, 60 knifes, 15 truncheon, 55 underwear, 45 light weapon and 5 hang rope) were investigated for human blood origin also 27 different animals blood were used for cross reaction and specificity.

Ethical Consideration

This study was approved form scientific and ethical council in the Iraq medical legal directorate / Ministry of Health (MOH). This research was conducted based on Article 2 of the Iraqi Forensic Medicine Law of 2013.

Case Definition Inclusion and Exclusion Criteria

we receive evidence referred to us officially from police office and investigation bureau, evidence must be packaging correctly with paper envelops and sealed as standard requirement (Chain of Custody), evidence not get this criteria were exclusion, in this study contains 300 evidence (120 outer clothing, 60 knifes, 15 truncheon, 55 underwear, 45 light weapon and 5 hang rope) were investigated for detection human blood.

Sampling

During the one year prior to the implementation totally 300 forensic evidence (120 outer clothing, 60 knifes, 15 truncheon, 55 underwear, 45 light weapon and 5 hang rope) were evaluated for human blood origin identification and 27 different animals blood samples were used to evaluate cross reaction and Validity.

Outcome

An immunochromatographic one-step test kits was used to detect origin of blood, old method (Kastel – Meyer test) use as screening nonspecific test for blood.27 of different animal's blood were study cross reaction. Positive and Negative results are recorded for all forensic evidence and control.

Materials and Methods

An immunochromatographic one-step test for the human blood kits was used to detect origin of blood, old method (Kastel – Meyer test) use as screening test for blood non-human specific. From Feb 2015 to 31 of Jan 2016 about 300 criminals' evidence, crime scene tools and different clothing were referred to our directorate to detect blood origin.

Each new lot of kits must be validated using a positive and negative control before using in casework.

Known human blood (voluntary person with known blood group).

Blood stains from different evidence totally 300 referred to the medical legal directorate from all Iraqi province.

Known animals blood total 27 samples (from Baghdad markets animals Sheep (5), chicken (5), fish (5), Goats (5), calf (5) and ferret (2).

Thalassemia, Sickle cell anemia and newborn blood were obtained from the national Iraqi blood bank.

One Step ABA card® HemaTrace (manufactured by Abacus Diagnostics, West Hills, CA) Used according to manufacturer's directions. Diluted blood was prepared by serial dilution of dilution buffer.

Kastel-Meyer Test (KM) presumptive test for blood detection

The test was performed as described by Cox,1991.
1 - All evidence was rub with a dampened cotton swab.
2 - Swabs were add to test tube containing about 1 cm of sterilize distilled water, swish to release contents.
3 - Several drops (3) were add of Kastle -Meyer reagent.
4 - Several drops (2) were add of hydrogen peroxide.

A positive result will give a bright pink color

The test is based on the peroxide-mediated oxidation of reduced phenolphthalein in which the heme molecule acts as a catalyst. This test is nondestructive to the sample, which can be kept and used in further tests at the Laboratory. This test has the same reaction with human blood and animal blood so further testing would be required to determine which one it is.

Statistical analysis

The database was examined for errors using range and logical data cleaning methods, and inconsistencies were remedied. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 21 computer software (statistical package for social sciences) in association with Microsoft Excel 2013.

Results

known human blood with a hemoglobin concentration of approximately 13 g/dl was using, as expected all blood samples, human and animals, were reacted positively in the KM test human blood from thalassemia, newborn and Sickle cells anemia also reacted positively with the KM test, as shown in table 1. All animals blood gives Negative results in one step

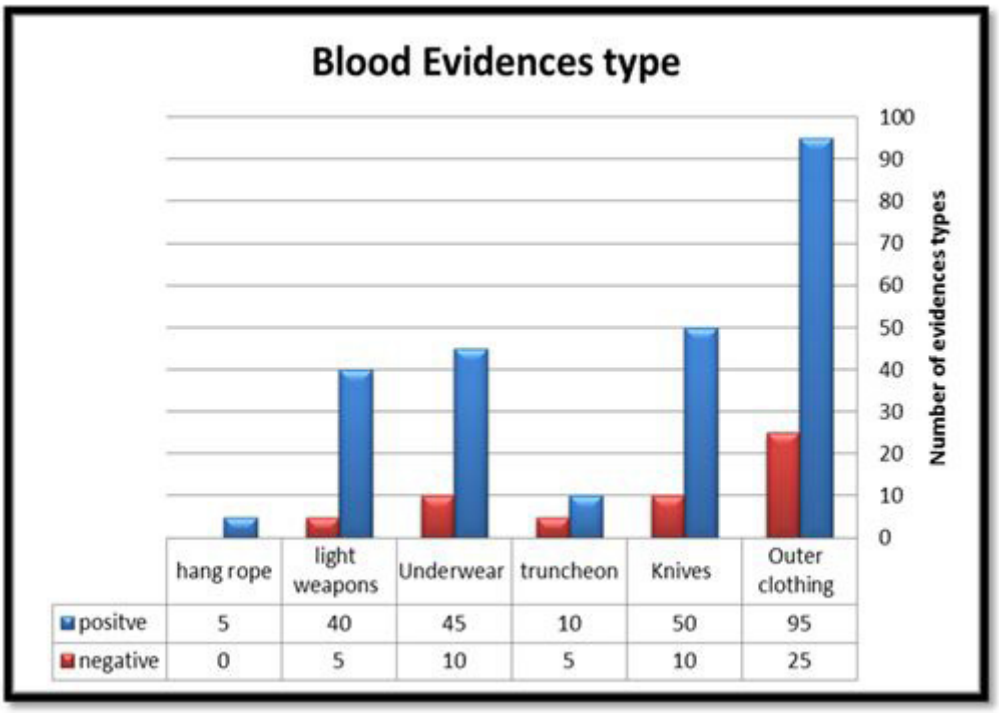

Figure:1 Represents different forensic evidence type

chromatographic sandwich immunoassay (ABA card® kit) except ferret blood, while 54 human blood control give positive results by this test. Domestic ferret blood reacted positively with the immunochromatographic sandwich method probably because of a similarity of short sequence of amino acids that is common on domestic ferret (Mustela puterius) and human hemoglobin [9,10].

The Kastle-Meyer test is a quick inexpensive test used to analyze evidence at a forensic laboratory and crime scene for the presence of blood. Phenolphthalein reacts with hydrogen peroxide in the presence of hemoglobin to turn from colorless to pink. These reagents provide a presumptive test for blood, as food samples which contain hemoglobin (meat) and certain vegetables will also generate a positive response. An additional benefit of this procedure is that the samples remain intact and can be used in further testing including DNA analysis [11]

Conclusion

The new immunochromatographic sandwich method is a convenient and rapid test for the identification of human blood. It is far superior to the other technique in terms of sensitivity and tolerance to different conditions. The correlation between the visual appearance of a blood stain and the ability to determine the presence of human blood makes the new immunochromatographic method an acceptable and highly useful tool for the forensic science community.

Positive Hema Trace result conclusively indicates the presence of human hemoglobin in the tested sample irrespective of the strength of the result, false positives will not be a serious consideration as ferret and higher primates will not be common consideration to the majority of crime scenes or evidences and will generally be referred to in case notes. Rowley has shown that domestic ferret blood reacted positively, probably because of a short sequence of amino acid that is common to domestic ferret and human hemoglobin [9].

Hematological genetic diseases caused by hemoglobin abnormality (hemoglobinopathies) in structure like sickle cell anemia, thalaseamia and blood from newborn were tested and give positive result in Kastel-Mayer and one-step chromatographic sandwich immunoassay this means abnormal Hb or Hb subtypes (HbA0, HbA2, HbS, HbF) reacted with monoclonal antibodies specific for human hemoglobin derivatives and Hb no loss their substrate activity to reducing agent.

In most important forensic cases it is sufficient to include or exclude samples based upon the results of the one-step chromatographic sandwich immunoassay. This test leads to re-

duce the non-human stains sent to biology for DNA profiling reducing costs, time, and effect. Use of the immunochromatographic method human hemoglobin (hHb) detection device was also evaluated in this study, the device can be utilized successfully as a combined hHb/DNA assay. This allows for the detection of hHb in a limited blood samples while preserving most of the DNA in that sample for DNA profiling.

Iraqi medical legal directorate is one of ministry of health directories, it's also depend on continental legal system, this system let us under supervisor of ministry of Justice and higher judges council , unfortunate the medical and biological information for judges and police officer are little ,we face sometimes problems with police station and courts in re-refer appeal cases (objections issues), refer biological evidences ,some packaging ,evidences storage ,evidences type collection , labeling and Sealing Evidence .

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