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A comparative study of different laboratory storage conditions for enhanced DNA analysis of crime scene soil-blood mixed sample

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ABSTRACT

Crime scene investigation is an important step in the entire criminal investigation process because this is where evidence is gathered. Blood from the perpetrator or victim of a crime can be left at crime scenes or transferred to other materials such as clothing, knives and guns. Most often, this body fluid is contaminated with soil at outdoor crime scenes but this might be the only or the most important evidence in solving a crime. This work aimed at identifying the most appropriate method of storing crime scene soil-blood mixed sample prior to analysis at the laboratory. Human blood was mixed with soil and stored at three different storage conditions (i.e., Room temperature/25 °C, 4 °C and −20 °C). Samples stored at room temperature saw significant reduction in DNA concentration as storage time increased ($P = 0.001$). Samples stored at 4 °C saw a drastic decrease in DNA concentration just after two weeks of storage. By the eighth week of storage at 4 °C, there was no detectable DNA ($P = 0.000$). Samples stored at −20 °C recorded no specific pattern in decrease or increase in DNA concentration for the entire 12 week storage ($P = 0.324$). There were full STR Profiles generated for room temperature stored samples and −20 °C stored samples throughout the study. There were full, partial and null Profiles generated for 4 °C stored samples depending on the sample storage duration. In conclusion, −20 °C was identified as the best storage condition for soil-blood mixed sample followed by room temperature and 4 °C, respectively.

1. Introduction

1.1. Background

The world of crime is a complex place. A crime can be committed everywhere such as workplace, schools, places of residence, vehicles, on the streets, on water bodies and even on the Internet in modern times [1].

Every forensic process starts from the crime scene in order to obtain evidence for analysis. The aim of an investigation that follows the commission of a crime is to interpret correctly the facts, reconstruct the events and know what happened. Comparing all other forms of evidence available to an investigator (e.g. confessions, testimonies, and video recordings), physical evidence (body fluids, guns, knives, fingerprints, etc.) plays an important and exceptional role [2]. Blood is the most common body fluid found at crime scenes [3–5]. Blood provides a good ground for the growth of microbes and these microbes secrete biochemicals which degrade or destroy DNA in the blood [6]. At outdoor violent-related crime scenes, many situations may cause human biological evidence from the suspect or victim to be deposited in the soil [7]. This evidence can be analyzed using DNA to link the suspect or victim to the crime scene.

Microbial activities in soil can degrade blood DNA after deposition making the DNA unusable for Profiling. When tissue samples are exposed to harsh environmental conditions, DNA degradation occurs rapidly to the extent that DNA becomes unrecoverable. Various problems can occur from the analysis of degraded DNA samples and these include signal loss, peak imbalance and allele dropout [8], hence, crime scenes need to be processed as fast as possible and tissue samples properly stored.

1.2. QuantiFiler trio kit

The QuantiFiler Trio Kit quantifies total human DNA and total human male DNA at the same time. Results from real time PCR with QuantiFiler trio can assist the analyst to know:

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- If there is sufficient human DNA and/or human male DNA for subsequent STR analysis.
- The quantity of sample to use for PCR amplification.
- Ratio of male to female in mixed samples especially sexual assault samples.
- The quality of DNA (degradation and inhibition).

There are three targets of amplification in the QuantiFiler trio kit; small autosomal, large autosomal and Y target. The Small Autosomal and Y targets have short amplicons (75 to 80 bases) in order to maximize the chance of detecting degraded samples. The Large Autosomal has relatively longer amplicon (>200 bases) to aid an analyst know if the sample is degraded. The small autosomal quantity actually gives the concentration of the sample.

There is also an internal positive control which contains a synthetic template DNA. By assessing the internal positive control, an analyst can determine if a sample has zero concentration or contains inhibitors (Fig. 1).

ΔRn means Rn minus the baseline. Rn means the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye; thus, Rn is the reporter signal normalized to the fluorescence signal of Applied Biosystems ROX dye (Figs. 2 and 3).

Threshold cycle is where an amplification curve and a threshold line meet. When the Threshold cycle (C_T) value of a target is less than 40, then positive amplification has occurred. The Internal positive control (IPC) C_T value is relatively the same in normal reactions but presence of inhibitors in the sample and/or DNA quantities can increase the IPC C_T value compared to the average IPC C_T value of the standards on the same reaction plate. When PCR inhibitors are in large concentration in a sample such that subsequent analysis could be affected, the IPC C_T flag is triggered for that particular sample. Large Autosomal target may be affected by the increasing inhibitor concentration before the Small Autosomal target and before the IPC C_T flag is triggered. Small rise in value of degradation index may be as a result of degradation and/or presence of inhibitors [9,10].

1.3. GlobalFiler PCR amplification kit

The GlobalFiler PCR Amplification Kit manufactured by Applied Biosystems uses a 6-dye, STR multiplex assay for amplifying human nuclear DNA. This kit amplifies: 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338), 1 Y-STR (DYS391), 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel) and Amelogenin (sex determining marker). This kit is very sensitive and can work in the midst of inhibitors [9,10].

1.4. Problem statement and justification

Bloodstains are often encountered on weapons, clothing and other materials and sometimes on the body of the victim. When these materials are discarded at outdoor locations, they may come into contact with soil [11]. Biological sample mixed with soil is often contaminated with materials that pose a threat to the DNA profiling process [7]. It is estimated that 10 billion microbes can be found in a gram of soil and they comprise thousands of different species [12,13]. These soil microbes destroy DNA in biological samples in a short period of time by fragmenting [6] and pose threat for successful DNA profiling.

Crime scenes containing biological evidence are usually processed early to prevent loss or destruction of evidence. Microbes in the soil, organic matter and storage temperature can all affect the evidence. Evidence submitted to the laboratory from crime scenes are usually not processed immediately due to large number of pending cases, unavailability of analysts or unavailability of reagents. There is thus the need to investigate the proper way of storing these evidence at the laboratory to prevent destruction prior to their processing.

1.5. Main objective

The objective of this study was to do a comparative evaluation of three laboratory storage conditions on soil-blood mixed samples found at crime scenes using DNA analysis.

2. Materials and methods

2.1. Ethical clearance

Ethical clearance for this study was obtained from the Committee of Human Research, Publications and Ethics of the Komfo Anokye Teaching Hospital and the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST).

2.2. Blood sample

Fresh adult male whole blood sample from a single person in a tube with Ethylenediaminetetraacetic acid (EDTA) anticoagulant

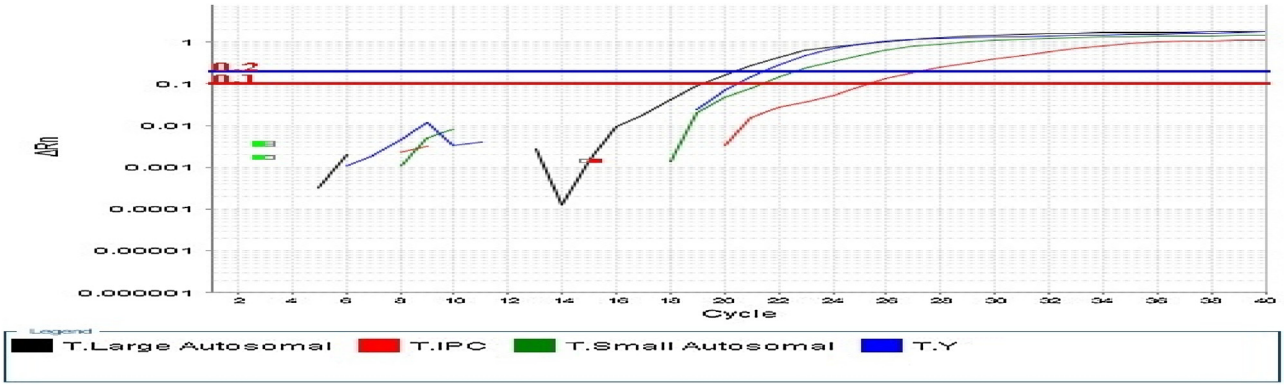

Fig. 1. Real-time PCR amplification plot for male sample using QuantiFiler trio kit.
Source: Ghana Police Forensic Science Lab.