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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2018;7(1):173-7

Investigation of the microorganisms decaying blood evidences

Murat Ogdur[1], Huseyin Cakan[2], Filiz Ekim Cevik[2]

[1]*Istanbul Provincial Police Department, Istanbul, Turkey*
[2]*Istanbul University, Institute of Forensic Sciences, Istanbul, Turkey*

Received 12 April 2017; Accepted 07 November 2017
Available online 01.02.2018. with doi: 10.5455/medscience.2017.06.8734

Abstract

Many biological evidences that submitted to forensic laboratories are not convenient for DNA analysis. Because of they are not collected and packaged correctly and not stored in the appropriate environment. For this reason, DNA test and many other non-DNA tests cannot be performed [1]. In order to protect blood evidence, should be known the reason of the deterioration. For this intention, we investigated the kind of microorganisms which decay the evidences, the effect of temperature, effect of package types and resistance of biological evidence to putrefaction. We aimed to determine the best condition for evidence safety. In our study, evidence modules were prepared using five different grounds, three different temperatures, two different time durations and three different package types. After the waiting periods, packages were opened and microbiological analyzed. At the end of microbiological analyzes, it determined that, as the waiting time of packages increases, microbial reproduction increases. The most decaying was seen on blood drips on the wall. After wall, respectively wood, sponge, fabric and knife are decaying by microorganism. The most putrefaction is seen at 4^0C, the least putrefaction is seen in the room conditions. Paper envelopes and cloth bags have been found to be better for protecting evidences. However, evidences that were completely dry were found to be better preserved in nylon bags.

Keywords: Evidence, microorganism, blood, putrefaction, crime scene

Introduction

Forensic investigations are starts at the crime scene. The most important stage of the forensic inquiry is the investigation of the crime scene. When this stage is done correctly, the correct result is reached in solving judicial events [2]. The purpose of the crime scene investigation is to recognize the evidence, to collect them and bring evidences to the laboratory for analysis [3].

Every evidence incoming to a forensic laboratory is judged by courts only if it is evaluated and interpreted with accurate and accepted scientific techniques [4, 5]. Biological evidence is often gathered from the scene because it contains DNA and is used in DNA analysis and in the identification of crime and criminality. Mistakes were made during the collection, packaging, waiting and transportation of evidences, not only affect to DNA analysis in the negatively, but also cause delays in justice [6].

Blood is one of the most important biological evidences. We encounter in many cases frequently, especially in the case of murder and wounding [7]. Many bloody biologic findings are damaged by bacteria, fungi and other microorganisms and their enzymatic reactions since the content of the blood is suitable for microbial reproduction. Therefore, the criminal tests are inconclusive [1]. Microorganisms digest cell membranes of blood cells, assists with exoenzymes, and these exoenzymes disrupt the DNA structure [8].

In our study, it was aimed to learn the effect of temperature, environment condition and package types on the decaying of blood samples at different times. Our other aim is to learn to what kinds of diseases could be caused to the staff whom working in the forensic sciences.

Material and Methods

Material supplying and modules

The blood samples used in this study was taken from Blood Center of Cerrahpaşa Faculty of Medicine. The blood samples analyzed microbiologically to determine if there have any producers. Modulation was prepared from sterile blood. We used three different types of packages. These are paper envelopes, nylon bags and cloth bags. We transfer some blood drops to wall, wood, fabric, sponge and knife. When we select these surfaces, we thought the most encountered surfaces in various forensic events. Blood modules created in laboratory conditions.

Preparing and packing the modules

One milliliter of blood was infected with a sterile injector onto specially prepared sterile surfaces. The packaging was done after

***Coresponding Author:** Murat Ogdur, Istanbul Provincial Police Department, Istanbul, Turkey
E mail: murat__ogdur@hotmail.com

three hours, considering the average time is nearly three hours between the occurrence of the crime and the collection of evidence in crime scene.

At the end of the waiting period, the knives were packed directly. The blood which in the fabric and sponge modules was cut off, the wood modules were scraped, and the wall modules were solved with saline water and collected by cotton swap. The blood modules were packed in paper envelopes, nylon bags and cloth bags. The packets were kept at 4°C, 25°C and 37°C. It was aimed to determine whether it would be better to leave the evidence in the refrigerator, at room temperature or in a hot environment such as a radiators or air conditioning for drying purposes.

Prepared modules were stored at three different temperatures for one and four weeks (Table-1). Waiting periods were determined, it was considered that underestimation of the two days would not be microbiologically significant. The evidence collected from crime scene under normal conditions would be studied in laboratories within an average of one week, and this time no more than four weeks.

Table 1. Blood materials kept in different ground, temperature and time

Nu.	Board	Temperature	Waiting Time
1	Wall	4°C, 25°C, 37°C	1 and 4 weeks
2	Wood	4°C, 25°C, 37°C	1 and 4 weeks
3	Cloth	4°C, 25°C, 37°C	1 and 4 weeks
4	Sponge	4°C, 25°C, 37°C	1 and 4 weeks
5	Knife	4°C, 25°C, 37°C	1 and 4 weeks

The Opening of the Packets and Analyzing

The prepared packages were stored in the refrigerator about 4°C, in the room conditions about 25°C and at 37°C. At the end of the waiting periods, the evidences packets were opened. The general situation of the packages investigated in terms of smell, humidity, color and coagulation status. The modules removed from the packages for microbiological analysis. The modules were dissolved in the Tryptic Soy Broth liquid medium at 37°C for 2 hours. The contaminant liquid solution was planted in the Endo and Mac Conkey medium for Gram negative bacteria; and planted in Chocolate, Bloody Gel and Tryptic Soy Agar (TSA) medium for Gram positive bacteria; and planted in Malt and Sabouraud medium agar for fungal culture. Endo, Mac Conkey, Chocolate, Bloody Gelatin and Tryptic Soy Agar media were incubated for 24-48 hours at 37°C; Malt and Sabouraud medium were waited at room temperature for 5-7 days.

Firstly, breeding plaques were examined macroscopically with naked eye, magnifying glass and stereo microscope to evaluate color, odor, colonization type and hemolytic status. At the end of the macroscopic examination, the samples were taken from the colonies were painted with simple or complex staining methods. Microscopic examination was performed after the staining. Bacteria and fungi seen on the microscope are photographed.

At the end of the microscopic examination, species identification was performed with API 20C and 20E, biochemical methods and commercial identification kits.

Results

90 different evidence packets that prepared in laboratory conditions were opened after waiting periods and analyzed microbiologically.

The relationship between the type of package and microbiological breeding is shown in table 2.

In our study, five different surface types which could be contaminated at the scene were used.

The distribution of microorganisms on these grounds is shown in table 3.

The effect of waiting temperatures of the packets on the putrefaction of the bloody evidence was investigated and the number of plaques observed reproduction was presented in Table- 4.

According to the length of time, the distribution of the microbiological reproduction in the sample evidence packets is shown in table 5.

The kind of microorganism obtained in the evidence package modules are shown in table-6.

Table 2. Reproducing scale according package type

Time	Kind of microorganism	Breeding status by package type			Total
		Paper envelope	Nylon bag	Cloth bag	
1 and 4 week	Gram positive cocci	18	39	33	90
	Gram positive rods	33	45	42	120
	Gram negative rods	6	8	6	20
	Mold and Fungi	20	28	24	72
Total		**77**	**120**	**105**	**302**
Rate (%)		**% 25,5**	**% 39,7**	**% 34,8**	**100,0**

Table 3. Total microbial growth in evidence packets kept for one and four weeks

Time	Kind of microorganism	Bloody ground type					Total
		Wall	Wood	Cloth	Sponge	Knife	
1 and 4 week	Gram positive cocci	24	15	18	6	27	90
	Gram positive rods	39	30	6	36	9	120
	Gram negative rods	8	6	2	4	0	20
	Mold and Fungi	24	14	18	16	0	72
Total		**95**	**65**	**44**	**62**	**36**	**302**
Rate (%)		**31,5**	**21,5**	**14,6**	**20,5**	**11,9**	**100,0**

Table 4. Breeding plaque counts at different temperatures

Time	Reproduction condition	Waiting temperature of packets			Total
		4 °C	25 °C	37 °C	
1 week	Number of Plaques	45	39	55	139
	Rate (%)	32,4	28,1	39,6	100,0
4 week	Number of Plaques	62	56	45	163
	Rate (%)	38,0	34,4	27,6	100,0
Total	**Number of Plaques**	**107**	**95**	**100**	**302**
	Rate (%)	**35,4**	**31,5**	**33,1**	**100,0**

Table 5. The Distribution of microorganisms in evidence packets.

Microorganism	Waiting Time of Packets			
	1 Week		4 Week	
	Breeding Plaque Cont.	%	Breeding Plaque Cont.	%
Gram positive cocci	27	19,4	63	38,7
Gram positive rods	54	38,8	66	40,5
Gram negative rods	8	5,8	12	7,4
Mold and Fungi	50	36,0	22	13,5
Total	**139**	**46,0**	**163**	**54,0**

Table 6. Microorganisms are growth in evidence packages

Kind of microorganisms	Plate Number	Rate (%)
Aeromonas sp.	6	1,7
Aspergillus sp.	18	5,1
Bacillus sp.	84	23,9
Bacillus subtilis	36	10,2
Corynebacterium sp.	3	0,9
Esherichia coli	12	3,4
Micrococcus sp.	3	0,9
Micrococcus luteus	3	0,9
Mucor sp.	8	2,3
Penicillium notatum	4	1,1
Penicillium sp.	68	19,3
Koagulase Negative cocci	15	4,3
Sarcina sp.	9	2,6
Staphylococcus aureus	72	20,5
Streptobacillus sp.	2	0,6
Streptococcus agalactiae	6	1,7
Streptococcus sp.	3	0,9
Plaque number	352	100

Discussion

Usually, evidences are taken form crime scene. The crime scene investigation process includes maintaining scene security, preparing documentation, collecting and preserving physical evidence [9].

In the last decades, Biological evidences have a vital importance on enlighten the crimes, identification of the criminal and in defending the victim's right [10].

Blood and bloody evidences are the most common types of biological evidence worldwide [1].

Blood is very convenient for microbial reproduction because of the plenty of water and also the presence of the proteins in the blood and the pH value of the blood [10]. Blood is also added to some of the plate used in the production of microorganisms (Bloody Agar, Chocolate Agar etc.).

If the blood sample taken from the scene is dry, sterile physiological water is dripped onto the cotton swap and the sample is taken. If the blood stain is wet, the blood drip is taken without wetting. Portable bloody materials are taken directly, packed after drying. If a material will send to more than one laboratory after biological researching, a swap is taken from the material and then the evidence packaged. Besides, dried blood can scrape, but using physiological water is more practically. Where the blood is pumped, the blood is also taken to a sterile plastic container with a cold chain, but this method not convenient for evidence protection to against microorganism [11]. In our study, it is found that, the taking method of evidences from crime scene is not important for decaying. But, to dry the evidences is vital important for protection of evidences. None of the dry evidence was decay. Because, microorganisms are cannot reproduced without water and humidity [12].

The types of packages which used in packing are important in putrefaction. The maximum deterioration was seen in the nylon-specific airless bags, later in the cloth bags, at last, minimal deterioration was seen in the paper envelopes (Table 2). No reproduction was observed in entirely dried packs also nylon packages. Findings that packed entirely dry are less reproduced in nylon bags. Against the possible environmental humidity, nylon bags safety from moisture, especially in rainy winter months.

It was observed that the surface on which the blood smears was found important in putrefaction. The largest number of reproduction was found on the wall and floor; the least reproduction was shown in knives (Table 3). The evidences maybe more exposed to microbial contamination on open surface areas [13, 14]. The reproduction of microorganism on the knives is shown on the handle part of the knives. There is no reproduction in the steel part of the knives. It is thought that some metal ions play an inhibitory role in microbial reproduction [15].

It was observed that the temperature of waiting place were important for microbial growth (Table 4). The least putrefaction was shown

at 25°C (in room conditions). The maximum putrefaction was observed at 4°C then second at 37°C. Especially, the most fungi growth was shown in the refrigerator. The reason of decaying in the refrigerator is humidity. Humidity in a refrigerator is more than external atmosphere. Any evidences packed in wet are decayed in every temperature and every condition. The highest putrefaction was shown in 37°C. This temperature is optimum temperature for most microorganisms' reproduction.

We see more putrefaction in four week waited packs of evidence than waited for a week. Microbial growth goes on by the time. According to the results of the experiment, it was determined that packages waited for four weeks contained 17,3% more microorganisms than for a week (Table 5). For this reason, findings collected from a crime scene should be sent to the laboratories as soon as in short time. Microbiological reproduction is very slightly into 24 hours. If possible, all evidence should be delivered to the laboratory within this time.

In our study, 90 pieces of evidence modules were prepared in order to evaluate the effect of waiting period on the microbial activity in bloody samples. These modules were opened after waiting time and cultivated in 630 agar plaques. Reproduction was observed in 302 (48%) of these plates. 139 (46%) plaque of these were obtained from the packs which were kept in one week and other 163 (54%) plaque from the packs which were kept for four weeks. At the end of our study, microbial reproduction in plaques was identified by various analysis methods. The most microorganisms which were fund can cause allergic bronchopulmonary aspergillosis, asthma, allergic sinusitis, and alveolitis. Some of these microorganisms produce aflatoxin B, which is known to be an inhibitory effect of the polymerase chain reaction (PCR) [16,17]. This toxin also caused to carcinogenicity, teratogenicity and mutagenicity. Metabolic effects of these toxins are inhibition of lipid synthesis and inhibition of clotting factor, including inhibition of DNA, RNA and protein synthesis, reduction in various enzyme activities, depression of glucose metabolism, phospholipids, free fatty acids, triglycerides and cholesterol and esters [18]. The risk of this microorganism which in a blood evidence collected for DNA analysis, is high.

Our study performed in Forensic Medicine Institute of Istanbul University, Microbiology and Parasitology Laboratory. We used sterile blood and sterile surface that not contaminating by any intervention. However, bloody dresses or other surfaces contaminated with blood in crime scenes may not sterile. In addition, the crime scene may be a closed area such as a hospital, a school, a workplace, or a house, as well as blood samples collected from open areas such as parks and streets. Therefore, it is estimated that the modules created in laboratory conditions have less microbiological diversity than the actual evidence. The owner of blood can vector nosocomial infections, HIV viral or other pathogen carriers, which can affect the microbial burden of the evidence [19].

Conclusions

The methods using in collection biological evidence is depends on the material. If possible, it is considered that the best method is a direct taking, and if it is not possible to obtain it directly, the method of scraping is considered to be better in terms of evidence security. Blood samples taken from the scene should be dry. Evidences must be packed in envelopes and not kept in refrigerators, and should not be transported in enclosed spaces such as transport boxes, in order to evaporate the material, even if the materials are installed, since moisture may be present.

Microorganisms that cause deterioration of biological evidence can growth in a cold environment such as a refrigerator, but they cannot reproduce when they lost their moisture. The refrigerator does not allow the evidence to dry because the humidity is higher than the outside and the refrigerator is a closed environment. For this reason, Evidences must be storage in the room conditions, instead of refrigerator and cold chain transport box, during waiting or transportation.

We cannot study DNA analysis, anaerobic microorganism and viruses. Doing new investigations will be beneficial in this area.

This study was supported by Scientific Research Projects Presidency of Istanbul University. Most welcome for this support. Project number is 29202.

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