تأثير بعض المنظفات الكيميائية وطرق غسيل بقع الدماء الجافة على البصمة الوراثية الجسدية

مجدى حسن وآخرون

الدليل المادى هو وسيلة فعالة فى ربط الجناة بمسرح الجريمة، والكشف عنه من الإجراءات اللازمة لاستكمال باقى التحقيقات. والدم هو أحد الأدلة المادية الأكثر شيوعا فى جرائم العنف ويعتبر مصدرًا مهمًا للمعلومات التى تكون حاسمة حين يعثر عليه فى مسرح الجريمة وبعد تحليل الطب الشرعى.

وقد أجريت هذه الدراسة للتعرف على تأثير بعض المنظفات الكيميائية على بقاء الحامض النووى الوراثى فى البقع الدموية المغسولة يدويا ومدى تأثير تلك المنظفات على ظهور السمات الوراثية الجسدية. وتضمنت الدراسة استخلاص الحامض النووى لعدد من العينات بعد غسلها بالماء فقط، والصابون (الأريال)، وهيبوكلورات الصوديوم (الكلوركس)، والصابون وهيبوكلورات الصوديوم معا وتم التحليل الكمى للعينات وعمل التكثير لمواقع السمات الوراثية، وبعد ذلك تم تحديد السمات الوراثية باستخدام جهاز التحليل الجينى.

وقد أظهرت الدراسة أن عينات البقع الدموية المغسولة بالماء العادى لم تتأثر فى محتواها، وعينات البقع الدموية المغسولة بالصابون يدويا تأثرت من حيث محتوى السمات الوراثية لبعض العينات، أما العينات التى تم غسلها بالكلوركس نتج عنها فقد كامل لبعض السمات الوراثية لبعض العينات أما العينات التى تم غسلها بالصابون والكلوركس نتج عنها الاختفاء التام للسمات الوراثية لبعض العينات. وتوصى الدراسة بعدم إهمال أى أثر دموى موجود على ملابس قد تعرضت للغسيل بقصد إخفاء معالم الجريمة.

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Table(2) STR genotyping of DNA isolated from studied blood stains

Samples		Alleles															
		D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	Amelogenin	D5S818	FGA
1	UW	11,14	29,29	10,11	10,11	14,19	6,9	2,121	1,131	19,20	13,14	14,15	7,8	13,13	XY	11,12	23,24
	WW	11,14	29,29	nd	nd	14,19	6,9	12,12	11,13	19,20	13,14	14,15	7,8	nd	XY	11,12	23,24
	AW	11,14	29,29	nd	nd	14,19	6,9	12,12	11,13	19,20	13,14	14,15	7,8	nd	XY	11,12	23,24
	CW	11,14	29,29	Nd	10,11	14,19	6,9	12,12	11,13	19,20	13,14	14,15	7,8	nd	XY	11,12	23,24
	ACW	11,14	29,29	10,11	10,11	14,19	6,9	12,12	11,13	19,20	13,14	14,15	7,8	nd	XY	11,12	23,24
2	UW	12,13	30,30	13,13	11,12	16,18	6,9,3	10,12	10,11	17,18	14,14,2	15,18	8,10	12,12	XY	10,11	22,24
	WW	12,13	30,30	13,13	11,12	16,18	6,9,3	10,12	10,11	17,18	14,14,2	15,18	8,10	12,12	XY	10,11	22,24
	AW	12,13	30,30	13,13	11,12	16,18	6,9,3	10,12	10,11	17,18	14,14,2	15,18	8,10	12,12	XY	10,11	22,24
	CW	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	XY	nd	nd
	ACW	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	XY	nd	nd
3	UW	12,14	30,32,2	13,13	12,12	16,17	7,9	9,14	12,13	17,22	12,2,13	16,17	8,9	15,15	XY	12,12	21,22
	WW	12,14	30,32,2	13,13	12,12	16,17	7,9	9,14	12,13	17,22	12,2,13	16,17	8,9	15,15	XY	12,12	21,22
	AW	12,14	30,32,2	nd	12,12	16,17	7,9	9,14	12,13	17,22	12,2,13	16,17	8,9	15,15	XY	12,12	21,22
	CW	12,14	30,32,2	nd	12,12	16,17	7,9	9,14	12,13	17,22	12,2,13	16,17	8,9	nd	XY	12,12	21,22
	ACW	12,14	nd	nd	nd	16,17	7,9	nd	nd	nd	12,2,13	16,17	8,9	nd	XY	12,12	nd
4	UW	12,13	29,30,2	10,12	9,12	15,16	7,9	11,12	11,13	20,25	13,14,2	16,17	8,8	16,17	XY	12,13	20,22
	WW	12,13	29,30,2	10,12	9,12	15,16	7,9	11,12	11,13	20,25	13,14,2	16,17	8,8	16,17	XY	12,13	12,13
	AW	12,13	29,30,2	nd	9,12	15,16	7,9	11,12	11,13	20,25	13,14,2	16,17	8,8	16,17	XY	12,13	12,13
	CW	12,13	29,30,2	nd	9,12	15,16	7,9	11,12	11,13	20,25	13,14,2	16,17	8,8	16,17	XY	12,13	12,13
	ACW	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	16,17	8,8	16,17	XY	12,13	nd
5	UW	14,15	29,30	8,11	11,12	16,18	7,8	12,12	11,11	17,17	10,13	16,16	8,8	10,29,2	XY	11,12	21,21
	WW	14,15	29,30	8,11	11,12	16,18	7,8	12,12	11,11	17,17	10,13	16,16	8,8	10,29,2	XY	11,12	21,21
	AW	14,15	29,30	8,11	11,12	16,18	7,8	12,12	11,11	17,17	10,13	16,16	8,8	10,29,2	XY	11,12	21,21
	CW	14,15	29,30	8,11	11,12	16,18	7,8	12,12	11,11	17,17	10,13	16,16	8,8	10,29,2	XY	11,12	21,21
	ACW	14,15	29,30	nd	11,12	16,18	7,8	12,12	11,11	17,17	10,13	16,16	8,8	nd	XY	11,12	21,21

Nd = Alleles not found UW = unwashed WW =water washed AW= soap washed CW = Clorox washed ACW = soap and Clorox washed togther

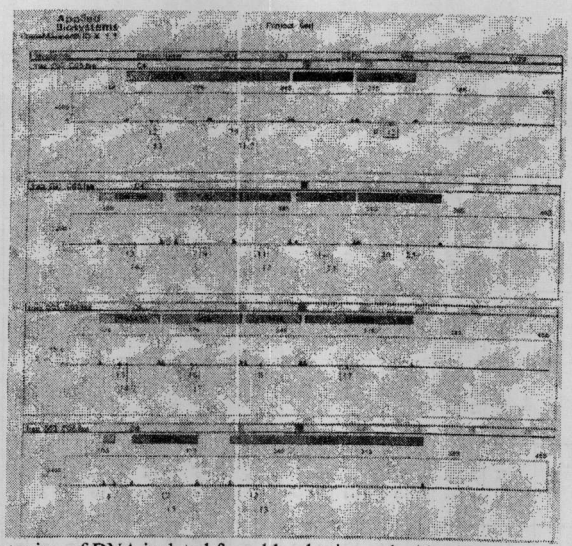


Fig (7) STR genotyping of DNA isolated from bloodstain washed by Cloroxshowingdrop alleles in (D7S820, and D18S51).

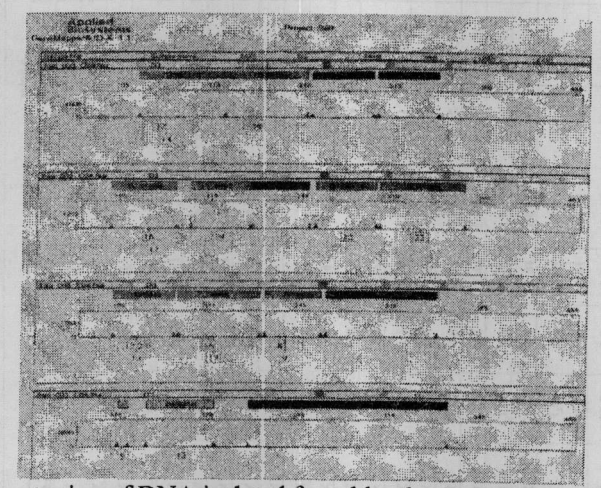


Fig (8) STR genotyping of DNA isolated from bloodstain washed by soap and Clorox together showingdrop alleles in: (D21S11,D7S820,CSFIPO,D13S317, D16S539, D2S1338, D18S51 and

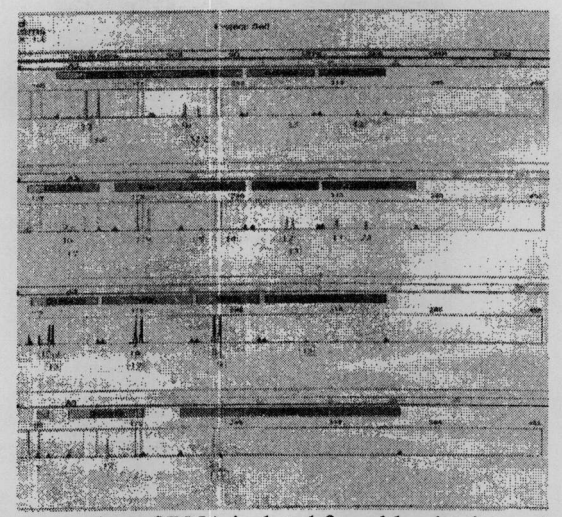


Fig (5)STR genotyping of DNA isolated from bloodstain washed by water.

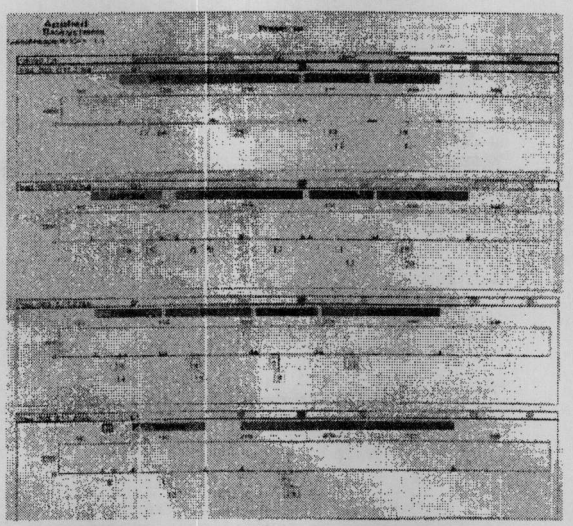


Fig (6)STR genotyping of DNA isolated from blood stain washed by soap showing drop alleles in cells (D7S820, CSFIPO and D18S51).

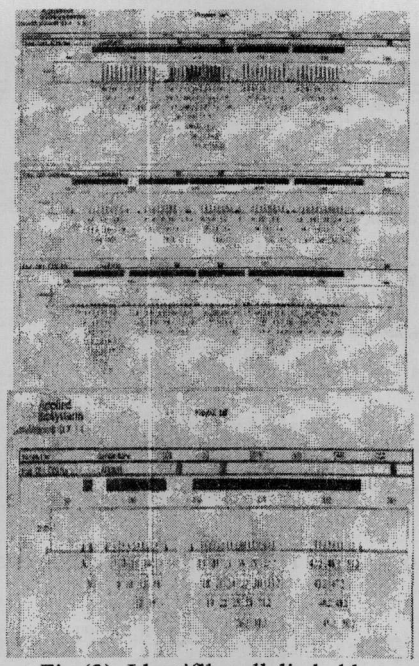


Fig (3) Identifiler allelic ladder

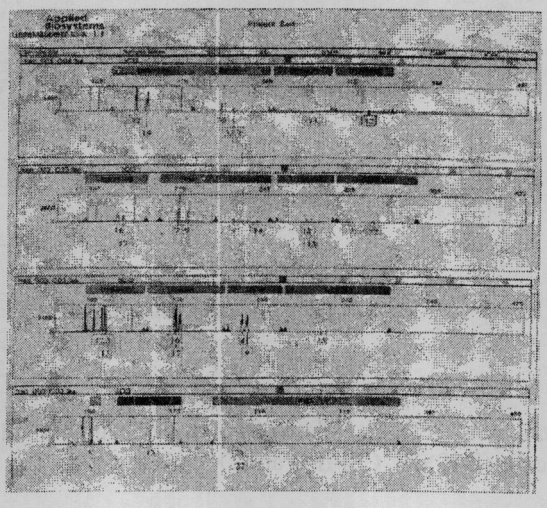
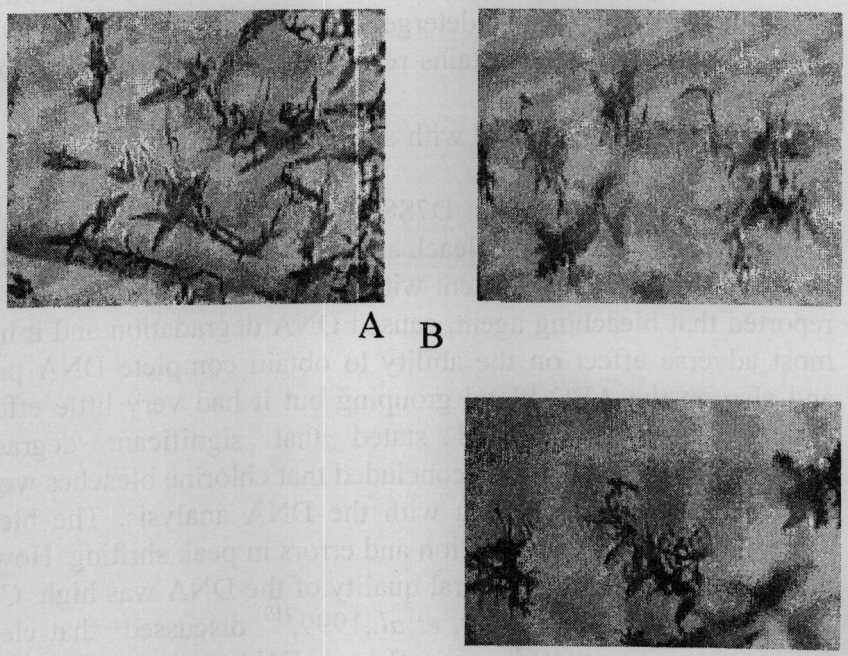
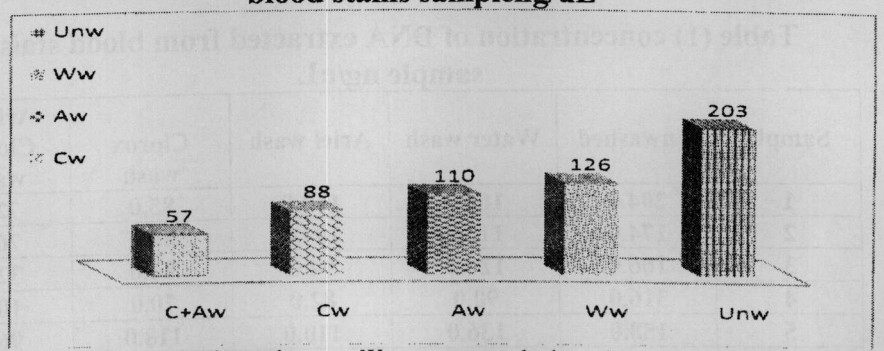


Fig (4)STR genotyping of DNA isolated from control blood sample



Fig(1) Showing blood crystal after (A) washing by water (B) washing by soap (C)washing by clorox

[redacted]) concentration of DNA extracted from different blood stains sampleng/uL



Unw = unwashed sample Ww = water washed Aw = soap washed
Cw = Clorox washed

In majority of the cases complete autosomal STR profile was obtained from water or detergent washed blood stains, whereas bleach-washing of blood stains resulted in the loss of 2-3 autosomal STR loci.

Blood stain washed with soap and Clorox together, produced partial human DNA profile.

Autosomal STR loci D7S820, CSFIPO and D18551 appeared to be the most affected by bleach and or detergent washing (Table 2). These results are in agreement with Passi,*et al.* (2012)[16]who reported that bleaching agent, caused DNA degradation and it has the most adverse effect on the ability to obtain complete DNA profiles and also on the ABO blood grouping but it had very little effect on species determination and stated that significant degradation occurs.Harris,*et al.*, 2006[17] concluded that chlorine bleaches were the most effective in interfering with the DNA analysis. The bleaches contributed to DNA degradation and errors in peak shifting. However, they also relate that the general quality of the DNA was high. Coy, *et al.*, 2005[18] andHochmeister, *et al.*,1999,[19] discussed that cleaning agents have aneffect on DNA analysis of blood stain.

As a conclusionwashed blood stain can be detected, byDNA - extraction and genotyping using standard laboratory procedure.STR Amplification kits comprising low number of loci, such as Minifiler, may yield reproducible and conclusive profiles resulting from detergent treated and bleached blood stains.

Table (1) concentration of DNA extracted from blood staisn sample ng/uL

Sample	Unwashed	Water wash	Ariel wash	Clorox wash	Ariel + Clorox wash
1	204.0	168.0	160.0	85.0	72.0
2	174.0	116.0	104.0	86.0	76.0
3	166.0	120.0	94.0	84.0	32.0
4	316.0	90.0	82.0	70.0	10,0
5	158.0	136.0	110.0	118.0	96.0
mean±SD	203±58.31	126±25.67	110±26.74	88±15.18	57±31.43

separated on 3130 Genetic Analyzer[14] and genotyped using Gene mapper ID-X software version 1.0/ 1.1. Duplicate extractions were carried out, PCR was performed usingSaiki, *et al.*, 1988[15]method.

Control samples

Profiles were obtained from blood samples prior to application and from stains prior tocleaning. These controls were used to help determine which contributory factor played themajor effect in compromising the quality of the resultant profile.

Results and discussion

The preliminary and confirmatory tests were performed toidentify the stains. The results are shown in Fig. 1. blood stain samples washed by water onlywere not affected and the crystals were observed.Blood stain samples washed by soap detergent were slightly affected and the crystals were observed in small amount, blood stain samples washed by Clorox were stronglyaffected and the crystals were observed in rare amount, where blood stain samples washed by soap and Clorox were strongly affected and the crystals were not observed after addition of Takayama reagent.

Out of all the cleaning agents bleach had the most deleteriouseffect on the quality of the DNA (Table 1 and Fig 2).

blood stains that were washed by water only were not affected in their content of DNAprofile or genetic feature (Fig 3, 4and 5) when compared to the treated blood stain.

blood stains of some samples that were washed by soap had been affected DNA in their content, profile and genetic feature. This is due to the fact that the soap compound contain chemical substances whichaffect DNA profile or the genetic feature (fig 6).

blood stains that were washed by Clorox had showncomplete loss in the genetic feature of some samples and had affect theDNA profile. These occur because of the presence of alkaloid chemicals in the clorox which effect or destroy autosomal DNA (Fig 7) .

The complete disappearance of the genetic features of blood stain that washed by Clorox and soap together occurred as a result of multipleeffects of Clorox and soap (Fig 8).

until the stain was invisible. Then they ran standard DNA PCR analysis on those washed stain samples.They used commercial forensic automated analyzers and compared results.Partial to full Profiler Plus™ DNA profiles of the victim'sblood (a non-visiblequantity) were obtained from areas of the suspect's vehicle and from swabbings of each of thesuspect's hands (after they had been washed with running tap water) in the mock "crime scenes[10].

Since very little information is available on the investigations of the effect of soap and bleachingagent (sodium hypochlorite)on serological markers and on qualitative and quantitative analysis of DNA from bloodstain.This study aimed to investigate the potential effect that these factors may have on DNA analysis.

Materials and methods

Samples, substrates and cleaning agents

Blood samples were obtained from 5 unrelated donors 2.5 ml from each one and the substrates used in this studywere 25 pieces of white Saudi uniforms cloth (30% cotton and 70% silk). All bloodstains were prepared on square pieces measuring about 25 cm^2. 0.5 ml of blood wereused to each one square ofthese pieces and these pieces divided into 5 groups.One group leaves as control without washing, and the other fourgroups wereexposed to different ways of hand washing (2-3 min) first group washed only by water (3 L), second group by soap compound (Arial, 42.5 g), third group bychlorinated bleach (Clorox, 60 ml), and fourth one soap and chlorinated bleach together.

All substrates were UV irradiated for 20 min prior to stain application. Standardvolume of blood was applied to each substrate and left to dry at room temperature (20-22 C°) for 48 hour. Then hand cleaning was doneand then left to dry again.

DNA extraction and detection

DNA from hand- washed detergent and bleached blood stains was extracted[11] and quantifiedusing standard molecular biology techniques[12]. Autosomal STR loci were amplified using AmpFISTR ® Identifier™ Amplification kit[13]. Amplified STR loci were size

Introduction

Blood stain is the most common evidence at the crime scene in one form or another. It may be encountered in different types of crime cases like murder, rape and assaults. They are required to be properly analyzed properly for their nature, species origin and individual characteristics. In an attempt to hide evidence, perpetrators can wash and destroy blood stains, making them undetectable to the naked eye and investigatorscannot predict the conditions blood stainswere subjected to before analysis. A wide variety of chemicals are used to affect the nature of blood stains and poses lot of problems in their analysis of blood stains.

Potential evidential material is often adulterated and rarely uncontaminated or of high quality. Advances in techniques and increased levels of sensitivity have meant that this need not prohibit confirming or negating a relationship between evidential and suspect material.

It has been shown that complete DNA profiles can be obtained from non-visible quantities of blood and the sensitivity of the Kastle–Meyer (KM) presumptive blood test makes the sourcing of such trace evidence possible[1]. The nature of the support or substrate on which the blood is suspended can itself introduce contaminants to the evidential material[2]. Bloodstains have also been deliberatelyremoved from the crime scene by using a variety of bleaching agents[3].luminol had no destructive effect, on confirmatory tests, species test or elution methodfor the detection of blood group antigens[4] but again notedthat it could seriously affect the electrophoretic typing of enzymes[5].It has also been shown that following luminoltreatment, DNA can be extracted and subsequently analyzedusing PCR[6].

Hypochlorite is a common component in household bleaches and cleaners, which are often used to remove blood from crime scenes. Cleaning agents not only have the potential to contaminate the biological material but may also degrade DNA present thus making the production of a conclusive and reliable profile difficult[7],however, hypochlorite is volatile and comparablyfast evaporates from a surface[8]. Harris *et al.* (2006)[9] ran a multiphasic experiment. They washed various fabrics containing dried blood using various cleansers

SOMATIC SHORT TANDEM REPEAT (STR) DNA PROFILING OF HAND WASHED DETERGENT TREATED AND BLEACHED BLOOD STAINS

Abdul Aziz A. Bin Dukhyil[*], Magdy A. Hassan[**], Ahmed M. Refaat[***]

Abdul Rauf Choudhry[***], Saif A. Al Rashidy[***]

Abstract

DNA evidence, linking perpetrators to crime scenes, is crucial to many legal proceedings. Blood is one of the most common physical evidence in investigations of violent crimes. Forensic analysis of the blood found at a crime scene supply in many ways valuable information that can be decisive in solving of a crime. Blood stains can be found anywhere a violent crime was committed .

Blood stain patterns on the floor (from a dripping wound, for example) or spattered on the walls can be interpreted for crime scene reconstruction. DNA typing analysis can establish the genetic profile(s) of the participant (s) in a violent crime. Consequently, blood stains are among the most useful evidence for court. This fact is becoming well-established so that criminals now often attempt to clean up their crime scene.

Blood samples were obtained from 5 unrelated donors and the substrates used in this study were pieces of white Saudi uniforms cloth (30% cotton and 70% silk), these pieces of cloth impregnate with blood were treated by different ways of hand washing including only water, soap compound (Arial), chlorinated bleach (Clorox), in addition soap and chlorinated bleach together.

DNA from blood stains were extracted and quantified. Autosomal STR genotyping of DNA isolated from hand -washed, detergent- treated and bleached blood stains were size separated on 3130 Genetic Analyzer.

Blood stain washed by water only were not affected. In the majority of samples complete autosomal STR profile were obtained from water or and from detergent washed blood stains whereas bleach-washing of the blood stains resulted in the loss of 2-3 autosomal STR loci. Blood stain washed with detergent and Clorox together, produced partial human DNA profile. Autosomal STR loci D7S820, CSFIPO and D18551 appeared to the mostbe affected mostly by bleach and or detergent washing.

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*College of Applied Medical Sciences, Majmaah University.

** National Center for Social and Criminological Research.

*** College of Forensic Sciences, Naif Arab University for Security Sciences.