RECOVERING DNA FROM WATER TRAPS AS A BEHAVIOUR OF POST-HOMICIDE CLEAN-UP, LEARNT FROM FORENSIC AWARENESS STRATEGIES.

By

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Declaration

I declare that this thesis does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due reference has been made in the text. I declare that all reported experimentations performed in this research were carried out by myself, except where any other contributions are acknowledged.

Signed: Jamie-lee Webb

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LITERATURE REVIEW

FORENSIC AWARENESS STRATEGIES CULTIVATED THROUGH MEDIA AND ITS EFFECT ON CRIME SCENE EXAMINATIONS.

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ABBREVIATIONS

Crime Scene Investigation	CSI
Detection Avoidance	DA
Detection Avoidance Strategies	DAS
Degradation Index	DI
Deoxyribonucleic Acid	DNA
Forensic Awareness	FA
Forensic Awareness Strategies	FAS
Kastle Meyer	KM
Millilitres	ML

Modus Operandi	MO
Natural Born Killers	NBK
Red Blood Cells	RBC
Short Tandem Repeats	STR
Television	TV
Triclosan	TSC

ABSTRACT

Television is one of the largest sources of messages and images in history (2) and is watched by billions, including those that would, or will, become perpetrators of serious crimes. (3). So, what messages or ideas are future perpetrators seeing? And what perceptions are they taking from the programs they tune into almost every day? Media demonstrates behaviour, and these common behaviours are what viewers cannot discriminate between reality and entertainment. (5) There are scant studies on what knowledge viewers gain from programmes with violent behaviours and their impact on their perception of reality. Could a potential perpetrator gain such information from cultivating media programmes that showcase the glamourous and fictional renderings of scientific investigations (6), such as Crime Scene Investigations

(*CSI*) and *Law and Order*? The theory behind the cultivation of such media messages is that Forensic Awareness (FA) and Detection Avoidance (DA) form a cultural zeitgeist known as the *CSI Effect* (9). While the existence and impact of the *CSI Effect* continue to be studied and debated on its effects in the courtroom, a literature search exposes little published information about the potential effect on criminal activity. (15) Various forms of literature are compared and discussed on their involvement in criminal behaviour, from the judicial system, sexual homicides and prisoner studies, cultivation from the media, violence in films and current known forensic awareness and detection avoidance behaviours.

A research gap inspired the project's experimental aims from evaluating the minimal literature. Aiming to determine if potential criminals know they are performing detection avoidance behaviours as knowledgeable forensic awareness strategies. Criminals may manipulate or degrade evidence left behind through chemical agents and "washing" of the crime scene to adopt these forensic awareness strategies. The proposed project will determine if chemical manipulation prevents biological evidence, such as Deoxyribonucleic acid (DNA) through blood evidence, from being recovered at scenes. Or if degrative techniques also prevent blood evidence examination through presumptive and confirmatory testing. This project also aims to determine if a standard DNA recovery volume can be created, given the amount of blood recovered from the aqueous sink environment.

Keywords:

Forensic Awareness, Detection Avoidance, DNA, Bleach, HemaTrace, CSI, STR.

1.0 INTRODUCTION

Television (TV) is one of the most influential media platforms globally. Since its market debut in the 1930s, homes worldwide now have a recorded 2.5 sets in each household (1). Television is one of the largest sources of messages and images in history (2) and is watched by billions, including those that would or will become perpetrators of serious crimes (3). So, what messages or ideas are future perpetrators seeing? What perceptions are they taking from the programs they tune into almost every day? Studies by Morgan and Shanahan 2012 (4) show that those who spend large amounts of time watching television are more likely to distort their perceptions of reality and reflect the most common and recurrent fictional messages. While other advances in technology such as the internet and increased accessible ways to watch media, TV still dominates the flow of information and words that pass our eyes and ears each day (4). So, which cultural norms does media hold responsibility for maintaining? (5) The most significant shows convey action, sexual promiscuity, and lastly, violence. (5) Media demonstrates behaviour, and these common behaviours are

what viewers cannot discriminate between reality and entertainment. (5) There are inadequate studies on what knowledge viewers gain from programmes with violent behaviours and their impact on their perception of reality. Perpetrators who would not usually commit such acts may be emboldened due to false information or belief they have gleaned from media. Or have unfounded confidence in their ability to perpetrate such crimes and get away with it, utilising clumsy and often exaggerated methods of evidence destruction from certain fictional TV shows.

Could a potential perpetrator gain such information from cultivating media programmes that showcase the glamourous and fictional renderings of scientific investigations (6) such as Crime Scene Investigations (*CSI*) and *Law and Order*? Subsequent studies have argued that specific programming genres can exert differential cultivation effects on viewers' perceptions (7). According to this cultivation theory, our actions are based on the social reality partly created and influenced by TV (8).

TV shows such as *CSI* and *Law and Order* have become some of the most popular TV programs globally, with over 63 million viewers recorded for *CSI* in 2011 alone. What knowledge or significance do these shows place on specific technologies or techniques used by forensic examiners, methods that permit evidence destruction, and even the possible limitations of forensic investigators? The technologies and methods depicted on shows such as *CSI* are not factual, and it is estimated that 40% of the portrayed science is fiction (8) allowing the representation of forensic science and its examiners to be distorted and exaggerated (8).

It can be argued that perpetrators have become aware of what aids will help or condemn them in crimes. The theory behind the cultivation of such media messages is Forensic Awareness (FA) and Detection Avoidance (DA) form a cultural zeitgeist known as the *CSI Effect* (9). The *CSI Effect* can be best described as the impact of certain TV shows that depict the significance of the evidence and the technologies used to analyse it (10). However, a small demographic has taken these depictions as a sure reality and has raised evidence expectations. (11). These depictions have influenced the general public's attitudes and prospects (12) towards using forensic evidence in trials, with these attitudes becoming a predisposition towards a conviction or acquittal. (12). Literature research has established that high frequencies of crime television viewing are associated with the inflated value of evidence in trials (13). It, therefore, creates a Perceived realism (14).

While the existence and impact of the *CSI Effect* continue to be studied and debated on its effects in the courtroom, a literature search exposes little to limited published information about the potential effect on criminal activity. (15)

While the *CSI Effect* tends to be treated with more amusement than concern (16), suggested perhaps are the more severe aspects of the *CSI Effect,* the knowledge potential, and what information convicted criminals can gain from watching these shows (3). Shows such as *CSI* can drastically increase a criminal's insights of what not to do at crime scenes (3) and even a possibility of manipulating, destroying, degrading, or eliminating evidence (17). As criminals become more and more educated about investigators and their examination techniques, they leave less evidence (3).

"Numerous law enforcement officials have openly stated that they believe shows, such as *CSI* have educated potential perpetrators even more" (3). Because of this, forensic examiners are now expected to find DNA and other forensic evidence at every scene, which not only is impossible but absurd to expect (3). This same expectation is also found in the jurors of criminal trials, and justice decisions are based on it (18).

Labelled "*The Police Chief Effect*" by Strom and Hickman 2014 (8), it claims that criminals are now adopting countermeasures to prevent detection from forensic evidence. For exmaple using bleach to clean up or degrade biological evidence or wearing gloves to avoid leaving fingerprints (19). Taking these additional steps and even adapting a *Modus Operandi* (MO) in a crime scene to hide or manipulate evidence to ultimately avoid police apprehension is now being recognised as Forensic Awareness Strategies (FAS) (20). Through the amount of media publicity given to the importance of certain evidence types used in criminal apprehension, this *CSI Effect* could increase a perpetrator's recognition of the need to become greatly forensically aware (21). Although influenced by TV shows such as *CSI*, some offenders have shown their capacity to adapt and develop new and innovative ways to commit crimes, with or without FAS (21). Substantial evidence has suggested that the use of DA and FA behaviours has had a real impact on homicides being correctly classified as suspicious deaths and, therefore, adequately investigated (22).

It is possible that perpetrators who successfully use specific DA and FAS are not identified by law enforcement (19). The lack of attention to how convicted criminals

may have used any DA or FA while committing crimes, either through interviews or studies on the crime scene evidence, bridges a gap in the literature (17). Establishing any DA or FA that perpetrators might have used may allow an indicator and, therefore, is an essential step towards counteracting the ill effects of DA and FAS (23).

2.0 DISCUSSION

The following sections aim to address the current literature on violence and criminal acts in media as educating and influencing the public and future criminals. Literature from various backgrounds is compared and discussed on their involvement in criminal behaviour leading to forensic manipulative strategies

2.1 CSI EFFECT IN THE JUDICIAL SYSTEM

Research into any literature on the *CSI Effect* showed extensive studies on its disturbance in the judicial system. From re-educating judges on distinguishing between science and media-influenced pseudo-science (11) to the prosecution counsel negatively shining a light on missing evidence to the fictional technologies used on evidence. Jurors have become influenced to the evidence they expect to be presented

in court. One of the first studies by Podlas in 2006 (24) showed that presenting prosecutors used what juror viewers had seen on TV shows like *CSI* to cast reasonable doubt in their decision making, so much that the author believes that the entertaining media contributes to the public misunderstanding of the law (11). Although it seems to work both ways, defence counsels use the absence of evidence as capitalisation in the necessity to find their client guilty. Regardless if there is no apparent reason to present said absent evidence; E.g., there were no fingerprints left on the crime scene after a break-in; because the offender wore gloves (11). Jurors have gained unrealistic expectations about the quality, quantity, and availability of scientific evidence (9). Baskins and Sommers in 2010 (12) focused on crime show viewing habits and the public's attitude towards how they see the evidence. They believed that the attitudes of the public influenced by these shows have resulted in a preposition towards conviction or acquittal in a criminal trial (12). Their telephone survey of 1201 registered voters had a pretrial attitude towards evidence and testimonies but not their willingness to convict or acquit based on the presence or absence of evidence (24).

A 2008 study done by Shelton focused on surveying 1000 enrolled jurors for evidence of a *CSI Effect* based on their viewing habits. The survey found that 46% of the sample pool expected to see evidence in every criminal case, while 22% expected to see DNA evidence in every case (25). However, the study did have limitations; it is unsure whether the expected evidence was related to the crime? E.g., did the jurors expect to see DNA evidence on a fraudulent crime? Other research by Cole and Villa in 2007 (16) searched into the influence TV shows had on the judicial systems to what evidence is

to be expected and its reliability. They reviewed over 416 news items that contained keywords such as *CSI Effect*. They found that crime shows taint the juror's pool with impossibly high expectations of the evidence collected from a scene. The portrayed expectations for forensic examiners to collect every type of evidence from a scene are absurd (3). So, do the public and potential jurors understand the use and role of DNA evidence? Brewer and Ley 2010 (26) studied the publics' perceptions of DNA evidence, and after telephone surveying 908 people, their key finding showed that only 54% of the pool understood the word DNA. 55% of the pool found DNA to be very reliable, and 53% would convict on the grounds of DNA evidence. However, this study also has limitations. It was never disclosed the age or education status of their subject pool. They also cross-referenced this to the DNA evidence portrayal in the show *CSI* with another study by Jankowski, Brewer, and Ley in 2012 (27). The analysis of 6 seasons with random episode assortments displays that DNA evidence was seen in 86% of episodes, analysed in 77%, and used 39% of the time to solve a case. Is this because DNA is often epistemologically stronger than other forms of evidence? (27) Or have viewers and the public been conditioned that it's the most famous evidence found in TV shows. Therefore, it must be of the highest importance to viewers, which has become their self-perceived understanding of DNA (27).

The last three reviewed research articles were primarily focused on the evidence behind a *CSI Effect*. First was Smith, Stinson, and Patry in 2011(9). Their study was based on the assessment of the *CSI Effect* in a court review. They interviewed 127 death investigators and 15 forensic experts and surveyed over 320 jury-eligible adults and 148 self-proclaimed *CSI*-heavy viewers. The interviewed prisoners were surveyed

on their reliability while analysing 250 newspapers and viewing various CSI TV seasons for the correct portrayal of police procedures, evidence collection, errors, and incrimination frequencies.

The key findings were short and straightforward; the *CSI Effect* does exist, with the more prominent belief coming from lawyers. However, lawyers were not a part of the survey. Next was Strom and Hickman in 2014 (8). This review bridges its critical findings to become common phrases used in articles years after. They assessed whether or not the *CSI Effect* is real and what effect forensic-based television programs had on the judicial system. They broke down their findings into "effects," such as the Strong or Weak Prosecutor Effect with the exploitation of absence evidence; The Defendant Effect where the need for evidence to say their client is guilty.

The Police Chief Effect is the recognition by criminals to what gets them apprehended and using DA strategies.; and, the Victim Effect, where the victim is conditioned to have all types of evidence collected from a scene to have the best possible chance of apprehension. These findings were focused on minor group effects instead of the courtroom as a whole. They found no evidence of an effect due to the small focus, but instead believe that the juries are affected by what they watch on television rather than a blanket TV controls a person's belief. Lastly, Maeder and Corbett of 2015 (14) looked beyond the frequency to a perceived realism of the *CSI Effect*. They focused on the effects of television crime shows on the realism they convey. The study had mock jurors participate in a trial with DNA evidence. After the jurors were surveyed on their

TV viewing habits and attitudes, they found a direct effect and perceived realism (14) on what they saw. This perceived realism then influences information processing and decisions.

2.2 SEXUAL HOMICIDES AND PRISONER STUDIES

When most people think of Detection Avoidance Strategies (DAS), it's usually hiding their identity, manipulating aspects of the scene, or removing evidence from a scene that can connect the offender. All these strategies strongly relate to sexual assault and sexual homicides, where most DA literature is found. The thought of linking certain aspects of DAS from offenders to their MO was by Davies in 1992 (20). He studied the behaviours of offenders helping identify and link like crimes. However, this concept heavily relied on victim statements being very detailed about the events or from prisoner interviews which Machado in 2012 (28) carried out. She interviewed 31 male prisoners convicted of a wide range of crimes on their attitudes towards what DNA evidence can mean for their apprehension. Their exposure was from images in media, other prisoners, and viewing habits before conviction. She found that the prisoners were sceptical of the fictionalised portrayal of DNA technology yet still saw it as infallible and one of the most powerful tools (28). Machado also revealed that prisoners used conversations with other inmates to learn how to commit a crime and decrease detection and conviction. Spending time in prison was a vital skill to distinguish reality in crime shows (28). A prisoner stated, "*CSI* is fiction, but it teaches

you how to commit a crime. They're teaching us how we should kill a person" (28). Although the study never disclosed the education of these prisoners or if they are repeat offenders with exposure to the judicial system.

Further research into DA in sexual homicides led to author Beauregard and associates producing numerous studies. In a 2013 paper with Martineau (19), the author assessed 350 cases of sexual homicide, with 250 of those solved to assess the use of FAS to increase the chances of avoiding the police. Their study showed not DAS but simular adaptive behaviours, especially sexual homicide indicators, semen evidence, and identity. In a further study with Balemba in 2013 (29), the author looked at the same 350 cases to determine the type of sexual offence concerning how offenders would approach a scene and what DAS they take. They classified the sexual homicides into sloppy/reckless, violent/sadistic, and forensically aware. They found that the sexual homicides of those offenders classed as forensically aware used more strategies to avoid apprehension, such as body removal, semen prevention, and hiding their identity (29). Again, this study has limitations; they didn't disclose whether the forensically aware criminals were first-time offenders or not, knowing that experienced sex offenders are more proficient in eluding apprehensions, utilising their experience, and even planning crimes (30). Lastly, the 2020 paper by Beauregard and Chopin (30) reviewed all previously mentioned, Davies (20), Hickman (8), Cole and Dioso-Villa (16), and Martineau (19), to research the amount of current literature on the commission process from police on FAS. They found that collection and analysis of specific evidence considered a DA or FAS was entirely upon the rational choice of the investigating officer and if their training included recognising such indicators. They

found that the research became a paradox; investigators un-trained to find FA or DA indicators wouldn't then be trained to recognise them again in the future.

2.3 CULTIVATION FROM MEDIA

Cultivation is a long-termed cumulative effect from heavy media viewing and its flooding of consistent messages (24). These messages can become a significant source of general perceptions, ideologies, beliefs, and specific assumptions that we have learned from repetitive television, most likely beginning in infancy (2). These TV viewing rituals show no signs of weakening as their consequences are increasingly felt worldwide, especially those images of violence (2). The proliferation of channels has not led to substantially greater content diversity (2). Cultivation studies focus primarily on the impact of violence, sex, and drugs (32,33) on viewers, but also the viewer's ability to differentiate between the conceptions of reality and fiction (2). Although media can emulate cultural norms, sexual promiscuity or violent behaviour may be learned from film or music (33). Violence on TV may also be learned and repeated; situations can be evident in acts such as mass shootings, influenced by media, seen in the copycat shootings from Columbine Colorado 1999 to Jonesboro, and Arkansas 2000 (32).

Small studies such as those by Sparks and Sparks 2007 (, 34) found that from 2000 people surveyed, many were conscious they had directly imitated acts of physical harm that they had witnessed from media such as movies. Their research into other

studies found experiments by Libert and Baron 1971, Stein and Fredich 1972, and Berkowitz and Alloto 1973 (34). They showed that children and college students acted aggressively towards each other after viewing violent behaviour on TV. This research came down to four hypotheses; the catharsis hypothesis by Feshbach 1955 (34), were watching violent media vents the violent behaviour; the priming hypothesis by Berkowitz 1994 (34), where aggression cues could trigger subsequent aggression; the arousal hypothesis from Zillmann 1991 (34), where arousal inducing properties of media violence, cause emotional reactions to occur immediately; and lastly the desensitisation hypothesis also by Zillmann, where sensitivity to violence becomes increasingly dull, and violent behaviour may become more aggressive as it is no longer recognised as over-violent.

One of the first studies by Grebner, Gross et al. in 2002 (2) explores how TV viewing contributes to audience conceptions and actions of violent behaviour through programme analysis of shows ideal for adults, adolescents, and children. They found exaggerated perceptions of violence in the real world, leading viewers to believe people are involved in violence any given week (2). A literature review by Morgan and Shanahan in 2012 (4) analysed papers by Grebner and Gross and Grabe and Drew. They investigated the extent to which TV contributes to the viewers' conceptions of social reality. Their key findings included a programme genre-specific culture, those who watch crime shows have a high fear of real-world crime (4). Grabe and Drew examined the extent of varying TV channels' potential cultivation of fears and behavioural perceptions related to viewers' exposures and attention to portrayed violence (31). From random telephone surveys of adults, there was a significant

variation from the varying genre channels and their influence on a viewer's orientation to crime (31). They also found that from a study by Oliver and Armstrong in 1995 (31), higher perceived realism for crime came from shows such as *Cops* than crime drama like *CSI*. If shows like *CSI* don't express and instil criminal fear, what do they instil in their viewers?

Role models? Horror? Instructions? Autoptic pornography? Many researchers believe that TV shows like *CSI* affect viewers, but of course, they're highly opinionated. Authors such as Tait published an article in 2006 (5) claiming shows such as *CSI* and *Bones* have a novel portrayal of a necrophiliac gaze. These gothic horror shows create desensitisation of the imagery of violence within or upon the human body. Any gasping or revulsion is slowly phased out through prolonged viewing habits. (5) However, those who commit homicides involving sexual acts may find these shows erotic.

Other studies found that certain viewing groups may find these shows inspirational or have potential role models. A paper by Weaver, Salamonson et al. in 2012 (13) interviewed 135 Australian first-year forensic science university students on their depictions of TV shows such as *CSI*, *Dexter*, and *Bones*. They were asked about the overall impressions of accuracy, ethics, professionalism, and any role models they saw. The majority of the sample pool disparaged the programmes' realism and their portrayal of unrealistic forensic representation. (13) However, some students saw role models within the shows and say they contributed to their motivation for a forensic degree. However, others found role models in the protagonist of the TV show *Dexter*, the character responsible for multiple murders (13).

2.4 VIOLENCE IN FILMS

Finding role models in films isn't uncommon; most viewers have a favourite character or protagonist. Although most viewers know that these actions and behaviours of their favourite characters are fictional, other viewers become encouraged or inspired to act as their role models do, because of certain films' violence and sexual behaviours, Acts and Regulations are created to moderate film and TV content. Examples of these moderations are the Videos Recording Act 1984 (35) and the Australian Classification Board (36). The slogan "Video Nasties" was a colloquial term created by Mary Whitehouse in 1982 (37). This term became the idea behind the Video Recordings Act in 1982 of moderating video works that depict violence, sex, or incite a criminal offence to a high classification (38). The Acts have banned many films from public viewing or purchase. However, Directors and companies find loopholes through cutting and re-editing the films to show less explicit content. Such Films that were banned until re-made or re-edited were the *Texas Chainsaw Massacre* of 1974 (39)*, I Spit On Your Grave* of 1998 (40), and *A Serbian Film* of 2011 (41). While most have been re-released by new producers, the last two films mentioned are still banned worldwide. However, different countries have varying reasons for banning certain films. What seems acceptable in Australia may be religious blasphemy in Israel; for example, *The Hunger Games* (42), released in Australia in

2012, is still banned in Vietnam because it depicts young children using violence on each other (43).

The ongoing debate remains on whether regulations should be harsher, given some released films' effects. The most infamous example of a film that has influenced over three brutal murders and four known mass school shootings is “Natural Born Killers” (NBK) of 1994 (44-48).

Journalist Owen Gleiberman once wrote that NBK “deposits the audience directly into the souls of sociopaths and that the film dares to ask what we are made of” (49). Although the film has never been banned under any Acts, it has been the primary influence behind many deaths in countless articles and headlines. The most famous was the Columbine High School Massacre in 1999 by Harris and Klebold (50).

Eric Harris and Dylan Klebold murdered twelve students and one teacher in Colorado, United States. The journals of both boys were later seized and deciphered to show idealisation towards the NBK film (50). Both boys even discussed which famous Hollywood director would adapt their story; Spielberg or Tarantino? (50). However, two years prior, in Kentucky, high school student Michael Carneal killed three students and injured five others in the Heath High School shooting of 1997 (51). Interestingly, one deceased student’s father filed a lawsuit against Warner Bro’s film for its violent influence on Carneal. It was, however, dismissed (51). Another Lawsuit was filed against the production companies’ film after the Savage and Byers murders of 1995 (52). Edmondson and lover Darras killed and injured Savage and Byres in Mississippi and Louisiana, respectively, after watching NBK the night before (52). Byers surviving

her injury, filed a lawsuit against Oliver Stone, director of NBK (44), was also dismissed. John Grisham, an American author and friend of deceased Savage, was quoted saying

"Filmmakers should be held accountable for their work when it incites viewers to commit violent crimes" (53).

Lastly, the Richardson Family Murders in 2006 (54) brought the NBK film back into the spotlight. 23-year-old Jeremy Steinke and his 12-year-old girlfriend Jasmine Richardson murdered Richardson's parents and 8-year-old brother in Alberta, Canada. Steinke was interviewed and quoted "Going Natural Born Killer on her family" (55).

Natural Born Killers is said not to be the only infamous, influential violent film. Films such as *Scream (56)* and *A Nightmare on Elm Street (57)* have been catalysts in many murders' confessions. *Scream,* a 1996 film (56) based on the real-world murders of the Gainesville Ripper 1990 (58), soon became one of the most famous horror movies of the 1990s. It also inspired the violence of Daniel Gill and Robert Fuller in 1999 (59) and Thierry Jaradin in 2001 (60). Gill and Robert were charged with the attempted murder of classmate Ashley Murray when authorities found propaganda and idealisation drawings from the film in schoolbags of both boys (59). Jaradin murdered Allison Cambler by stabbing her 30 times in a premeditated attack after becoming inspired by the *Scream* film (60). *A Nightmare on Elm Street* was the catalyst behind Daniel Gonzales's murder spree in 2004 (61). Reporter Owen Bowcott stated Gonzales

"Emulated the slaughter inflicted by the evil figure of Freddy Krueger" (61).

Finally, an idea created for an online art competition developed into a video game and then a film in 2018 catalysed a violent altercation. *Slenderman* was an innocent project by artist Eric Knudsen (62) that led to the brutal stabbing of 12-year-old Payton Leutner (62). Although she suffered 19 stab wounds, Leutner survived to see her attackers convicted. Both Offenders, 12-years old at the attack and still serving sentences of 25 and 40 years in a mental health institution (62). Both attackers, Morgan Geyser and Anissa Weier were dedicated believers and followers of the Slenderman cult fiction. They believe killing Leutner would "Prove the existence of Slenderman" (62) and allow them to become his disciples. It is unknown how many video games can be held responsible for violent acts, to which the extent of research is far beyond the reach of this review.

Although any media viewer who cannot distinguish between fiction and reality amongst violent or criminal activities in films needs to access mental stability, it does raise questions on why films and other media don't all have warning messages displayed to say these criminal acts are fictionalised, and professionals perform violet altercations? We see warnings on film piracy and what age group is suitable for the film, but not what behaviours or actions in the movies may disturb or educate viewers.

2.5 FORENSIC AWARENESS AND DETECTION AVOIDANCE

Certain detection avoidance behaviours have been documented throughout police investigations. In literature, these include removing, degrading evidence,

offenders protecting their identity, manipulating bodies, or staging certain parts of a crime scene (23). Extant literature on DA behaviours shows that they are most likely in homicides or sexual homicides where the victims and offenders are accustomed (23). However, there is scarce research on perpetrators purposely using DA behaviours to skew police investigations rather than just avoiding apprehension. Research by Ferguson and McKinley (23) found numerous studies investigating the extent of offenders using any DA behaviours in solved serial or child homicides. Niemeyer, Pepper, and Salfasi in 20008 (23) examined 85 cases and found that 17 offenders, 20%, used adaptive cleaning acts (23). Brown and Keppel (23) of 2012 examined 347 child homicides. They found that 52.7% employed countermeasures (23). Lastly, Ferguson and McKinley examined 171 homicide cases in Victoria, Australia and found 68% removed or destroyed evidence (23).

DA behaviours heavily rely on the offender and their knowledge or control. An offender who kills for the first time may experience a flight or fight response and flee a scene before using any DA measures (22). In contrast, experienced offenders may decide whether or not to commit a crime by weighing the rewards, efforts, and costs (30). These offenders will then use the least amount of DA behaviours to avoid apprehension (29), given that they have planned a crime (17). Planning also allows offenders to choose which DA behaviours to use strategically. This FA can lead investigators in determining if evidence has been destroyed or removed on purpose and is crucial to case solvability (23). Offenders may become forensically aware of investigative practices and modify their behaviour or MO at scenes (19). A relatively new concept in Criminology, FAS are becoming evident in offenders taking additional

steps and behaviours to purposely conceal any previous known MOs used in crime (19). The research on the prevalence of FAS used by offenders and its impact on the investigative process is limited (19).

Yet, further study again heavily relies on what is observed by the investigator's office or revealed through offender interviews. Current FAS are not limited to crime scene staging, altering elements to disguise the type of crime, and elaborate staging, altering at least four or five elements of the scene (63). How offenders know which elements to manipulate or which trace evidence is the most important has been linked to the *CSI Effect*. According to Strom and Hickmann 2012 (8), the rise of the deception of forensic technologies and practices on fictional drama televisions has led to offender FA recognition and therefore manipulated their MOs (30). In Durnal's 2010 study (3), he interviewed one police officer who stated,

"It used to be virtually unheard of for a criminal to use bleach to clean up a bloody mess. Today, the use of bleach is not unusual in a planned homicide." (3).

Can we blame the *CSI Effect* for this learned FA, or are criminals becoming educated through other sources? In 2005 (64), Creamer conducted a study where he tested the effect of bleach on blood and if the blood was still visible after the treatment. He found that the bleaches' effect on blood made the interaction more chemiluminescent. Therefore, forensic examiners would be able to use this visible increase as an indicator of scene manipulation. However, it is questioned how long offenders become aware of this phenomenon.

3.0 EXPERIMENTAL DESIGN ELEMENTS

3.1 PRESUMPTIVE AND CONFIRMATORY TESTING FOR BLOOD AT CRIME SCENES

Blood is one of the most significant and frequently encountered types of DNA evidence associated with forensic investigations of death and violent crimes (65). Therefore, it makes perfect sense for forensic investigators to analyse possible stains for blood. Frontline field forensic examiners use presumptive and confirmatory testing kits to test (a) blood and (b) human blood. Presumptive and confirmatory testing in this research project will determine if blood is recovered from our washing attempts. Therefore, continuing with quantification to of find DNA. The presumptive blood kits to be used are the Kastle Meyer test (KM), a Phenolphthalein solution to show a presence of blood, based on a peroxidase reaction of haemoglobin with a pink colour change (66). The other is Hemastix™, a test that detects the peroxide-like activity of haemoglobin in a sample; levels are determined through a colour and index chart. These kits are presumptive as they detect the presence or level of blood but not the species of origin. The confirmatory test used is HemaTrace®, an antigen-antibody reaction. The antibodies present on the testing card react with the human haeme antigen found in haemoglobin, forming a lattice to give a visible pink dye band (67).

3.2 HUMAN-SPECIFIC HAEMOGLOBIN

As the proposed experiment involves human blood and various chemical effects on its degradation, first, a look into the structure of red blood cells (RBC) and what causes degradation.

The haemoglobin found inside human RBC is a protein synthesised to transport oxygen from the lung capillary gas exchange to the body. (68). Four monomers of haeme and globin units bonded together to create the complex functioning unit (Figure 1). The differential of the haemoglobin unit and its amino acid sequence varies between species; this allows confirmatory identification of human blood through forensic investigative techniques such as HemaTrace® (68). As unique and identifiable DNA is found within blood components, criminals avoid leaving such evidence at scenes. Therefore, blood has become advantageous evidence to find and confirm.

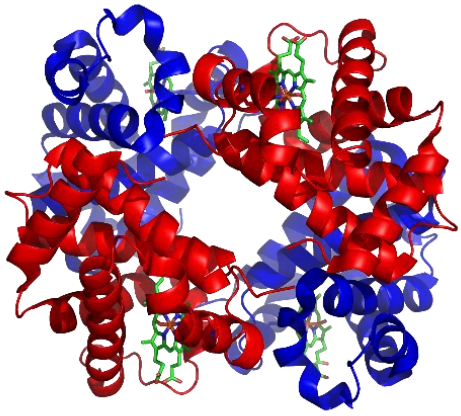

Figure 1- Structure of human haemoglobin, with four monomers of haeme and globin creating one unit (72).

3.3 DEGRADATION

Degradation of RBC is described as haemolysis or rupturing. This lysis results in the release of the haemoglobin while it undergoes further degradation by bodily enzymes until excretion. However, blood degradation at a crime scene would not undergo such bodily controlled process. Instead, environmental contributors cause rupture, such as exogenous reactive oxygen chemicals (73). Understanding the degradation process of haemoglobin outside the body, at crime scenes, has not been fully reached in literature (68). Nor has it been studied; the extent various chemicals have on the degradation index of RBC. Literature by Marrone and Ballantyne (2009) (74) and Wood *et al.* (2005) (75) do suggest the reasoning behind dried blood degradation of RBC, however not the degradation effects if the blood is suspended in an aqueous solution.

It is crucial to understand human haemoglobin's degradation process, given that haemoglobin is the primary detection molecule in many forensic examination blood kits (68), HemaTrace (Figure 2), Hemastix, and Kastle Meyer test. It's imperative to understand the degradation of DNA from RBC if a degradation index is to be established to measure the varying effects of different chemicals, such as bleach, hydrogen peroxide, or household hand soap, against a control.

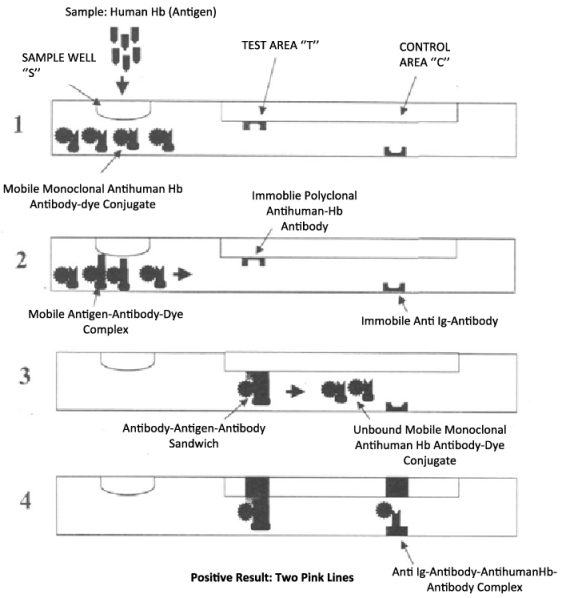

Figure 2- Hematrace process representation **(68).**

3.4 DEGRADATIVE AND INHIBITING AGENTS

Criminals have become more forensically aware of what cleaning agents and methods can manipulate or degrade evidence at a crime scene; deliberately removing or destroying biological evidence are problems forensic examiners routinely face (69). A wide variety of chemicals are now used to affect bloodstains, which, as imagined, poses many problems in analysing any bloodstains found at the scene (70). Because of chemical manipulation, it becomes unknown what condition any blood is found in at a scene. Therefore, any potential contamination or DNA degradation makes recovering a reliable profile difficult (71).

3.4.1 BLEACH (SODIUM HYPOCHLORITE)

Sodium Hypochlorite is the most common chemical component in various bleach cleaning agents. Bleach works by oxygen molecules being released in an oxidation process. The oxygen molecules break the chemical bonds found in chromophores, changing the molecules' ability to reflect light; the absence of light reflection is seen as white through human eyes. Bleach's effect on human haemoglobin reduces haematocrit levels and denatures the RBC structures (76). Bleach is also the most commonly used chemical to clean up blood evidence in a crime scene due to its destructive reactions (77). Offenders may not dilute the bleach available to them to destroy all evidence. Therefore, bleach will not be diluted in the research project; a base level to begin is a 1:1 Ratio.

The hypochlorite contained in household bleach also strongly reacts with forensic examinations luminol reaction techniques. However, it has been shown that substrates washed with bleach to eliminate blood create a higher positive reaction to luminol with a rapid appearance and higher brightness and duration (77-78). These differences in luminol tested bleached and unbleached substrates make it possible to distinguish between areas attempted to clean by an offender and those that haven't, indicating a FAS.

3.4.2 HYDROGEN PEROXIDE

Just like bleach, hydrogen peroxide is an oxidising agent. Hydrogen peroxide is naturally found in the body but is controlled by enzymes such as catalase (73). It's toxic due to the peroxide ions' oxidation of membrane lipids, proteins, and DNA (79). Therefore, any interaction with organic matter will cause degradation of the haeme molecules found in haemoglobin (80). As the Hydrogen peroxide is less aggressive than bleach, consumers use it frequently as a disinfectant.

3.4.3 HOUSEHOLD HAND SOAP

Soap a product used to kill microorganisms by disrupting their membrane lipid bilayer and denaturing proteins (80). Soap products now come in a large variety, including liquid, bar, organic and specialised. The hand soap used in the research project will be household antibacterial hand soap. Due to the influx of consumer wants on antibacterial in the world pandemic of COVID-19, antibacterial soaps are the most common in households.

Other components of Antibacterial soaps are Quinolones, a group of commonly used antibiotics to inhibit the growth of bacteria (81). However, commonly used Quinolones such as Triclosan (TCS) are known to rapidly inhibit DNA synthesis by promoting cell cleavage or bacterial DNA (81). It is unknown how the soap ingredient will affect the recovery of DNA for possible synthesis. However, a study by Nakanishi et al. (2020) (82) found that handwashing bloodstain clothes with handwashing soap,

dishwashing liquid, and even laundry detergent allowed DNA to be recovered and extracted from all garments, with fewer amounts coming from polyester clothes (82).

3.4.4 DEGRADATION INDEX

DNA can degrade from various environmental influences including extreme heat, sunlight, and chemical manipulation (83). DNA degradation is seen within Forensic examiner software when a DNA sample is quantified in the hope to generate a Short tandem repeat (STR) profile. Quantification kits such as Quantifier TM Trio Quantification are used in this research project. Providing not only a DNA recovery concentration but in addition an assessment for degradation and any inhibition (83).

The software used shows an STR electropherogram, an image of person-specific STRs. A skew in this electropherogram indicates sample degradation (83) (Figure 3).

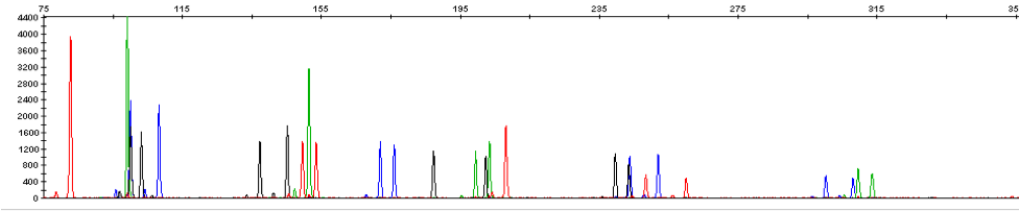

Figure 3- An electropherogram showing a ski-slope pattern of DNA degradation in a sample.

A Degradation Index (DI) has been included in the kit's software to evaluate degradation in forensic samples. This skew pattern manifests due to smaller DNA fragments remaining intact and amplifying well instead of larger DNA fragments being damaged and do not (83).

DI is automatically calculated within the software as equation (83),

$$DI = \frac{Concentration\ of\ Small\ DNA\ Target}{Concentration\ of\ Large\ DNA\ Target}$$

Any DNA fragments of approximately equal size will give a DI of one; therefore, a DI over one could indicate degradation (83).

3.4.5 EXPOSURE TIME

The degradative index and impact of chemical agents are dependent on the volume, dilution, and time spent under the conditions. While literature shows that degradative agents have an increased decomposition with extended exposure, little literature comments on the blood degradation while being in an aqueous dilution. The project's base-level time is twenty-four hours and one week. Therefore, it can be studied whether chemical degradative agents had higher DIs after twenty-fours in dilution or one week in dilution.

4.0 EXPERIMENTAL AIMS AND HYPOTHESIS

4.1 AIMS

The research presented in the literature review shows that chemical cleaning agents' exposures to blood in an aqueous environment may have a degradative effect on DNA recovery quantities. At the same time, the denaturing and manipulation of the blood molecule's structure may also result in the ability of blood to bind to any presumptive or confirmatory tests. A control treatment is, therefore, part of the treatment solutions.

As depicted in the literature review, the experiment aims to determine if common household cleaning agents cause a DI on DNA and if allowing the chemical/blood/water to sit a prolonged time will directly affect the DI of DNA. Lastly, if there is a standard blood recovery volume in aqueous solutions. Subsequently, there are three hypotheses to be tested.

4.1.1 EXPERIMENTAL HYPOTHESIS 1:

H_0: Exposure of 2ml of blood to either 25mls of Sodium Hypochlorite, Hydrogen Peroxide, Hand Soap, or No-Treatment diluted in 2500ml of water **will not** have a Degradative Index greater than 1.

H_1: Exposure of 2ml of blood to either 25mls of Sodium Hypochlorite, Hydrogen Peroxide, or Hand Soap diluted in 2500ml of water **will** have a Degradative Index greater than 1.

4.1.2 EXPERIMENTAL HYPOTHESIS 2:

$H_{0:}$ Prolonged blood exposure in a ~300ml chemical/water solution **is in**sufficient to cause a higher Degradative index.

$H_{1:}$ Prolonged blood exposure in a ~300ml chemical/water solution **is** sufficient to cause a higher Degradative index.

4.1.3 EXPERIMENTAL HYPOTHESIS 3:

$H_{0:}$ Blood recovery quantities from a ~300ml water dilution **will not** vary over replicates.

$H_{1:}$ Blood recovery quantities from a ~300ml water dilution **will** vary over replicates.

5.0 CONCLUSION STATEMENTS

From the literature reviewed, one can conclude that certain media depictions affect some viewers more than others since media still dominates the flow of information that passes our eyes each day (4). However, the intensity of the influence depends on a range of variables, such as viewing habits, attraction to violence, and even social upbringing (2). While the most common influence of media cultivation from crime-fighting drama shows such as *CSI* is extensively evident in the literature about its effect on the judicial system (11). Its impact is positively and negatively felt in

all parts of a criminal investigation, from the body discovery to the suspect sentence (3). As *CSI* started to gain popularity in 2006 (7), films in earlier years had a more significant impact on viewers, snowballing school mass shootings (47,48,59,51) and spree killings (45,46,49,55).

Media is still a significant demonstrator for behaviour (5). Unfortunately, some TV fictional behaviours are learnt from convicted criminals and aspiring offenders. It's these behaviours that some viewers cannot discriminate between reality and entertainment (5), such as exaggerated forensic techniques and even the importance and need for particular evidence samples. However, scant prisoner studies show that media may not be the only influence on criminals' behaviour. With others learning from other prisoners (28) and even from their own mistakes, many researchers still believe that future and current criminals learn adaptive behaviours from the deemed *CSI effect* of TV viewing habits.

The majority of FA and DAS crimes are sexual homicides (20). Most offenders want to hide their identity, wear gloves to prevent fingerprints (8) and remove any biological evidence from their victim (29), thus requiring DAS. However, it has not been thoroughly researched if offenders of just homicides show similar behaviours.

It has also not been extensively researched if criminals know that they purposely use FAS to avoid apprehension or do such behaviours because they heard or saw them on TV (17). Nor is there much research on what manipulation techniques offenders might use to pursue DA. These techniques range from destroying evidence through manipulation, degradation, or removal to stage aspects of a scene (23)

purposely. Such techniques most infamously known involve liquid bleach to manipulate or destroy biological fluids (64).

The proposed research project will see if chemical manipulation prevents biological evidence from being recovered at scenes; or if degrative techniques will also prevent forensic examiners from analysing presumptive and confirmatory blood testing. The project will also determine if a standard blood recovery volume can be created, given the amount of blood recovered from a diluted aqueous solution. Determining the dilution recovery limit of blood in the water will benefit forensic investigators in complex blood evidence samples and laboratory examiners in their efforts to retrieve evidence.

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Part Two

RESEARCH MANUSCRIPT

FORENSIC AWARENESS: DERIVING DNA FROM WATER TRAPS AS AN INDICATION OF HOMICIDE CLEAN-UP.

ABSTRACT

Blood is one of the most significant and frequently encountered types of Deoxyribonucleic acid (DNA) trace evidence associated with forensic investigations of death and violent crimes (1), and offenders are now learning which destructive aids will prevent, destroy or manipulate any biological evidence left at a scene (2). Extensive media cultivations of crime drama programmes and publicity were given to certain evidence types in criminal apprehension have said to directly articulate Forensic Awareness Strategies (FAS) used by criminals.

Research into possible DNA degrative agents showed that certain chemicals are the usual go-to for criminals because of their destructive effects (3). The objective of the present study was to address the effect of common household cleaning agents on the Degrative Index (DI) intensity of DNA derived from human blood. Furthermore, to investigate if, allowing the chemical/blood/water solution to sit a prolonged time will directly affect the DI intensity and if a standard blood recovery volume from an aqueous environment can be determined. As haemoglobin is the primary detection molecule in multiple forensic presumptive and confirmatory blood testing kits (9), understand what chemicals may impact or prevent results. To establish if a DI of DNA through various chemical manipulation can be created, this project was designed to test the varying effects of Bleach, Hydrogen peroxide and household hand Soap against control to evaluate their destructive tendencies.

From all treatments applied to the blood samples in the aqueous environments, it's evident that the most inhibitory chemical agent was Hand soap. The treatment with the most significant effect on DNA fragmentation is Hydrogen peroxide. Lastly, the treatment that allowed the least amount of DNA recovery was bleach.

From this data, DNA recovery was still viable after every chemical agent treatment, although some DNA was affected by degradation. Given this data collection and analysis, if a criminal had used any FAS involving chemical manipulation of blood in an aqueous environment such as a sink, there is a high chance the chemical will cause degradative effects after both 24 hours or one week.

Keywords:

DNA, Forensic Awareness, Homicide, Human Blood, Hema Trace, degradation, Forensic Science, KM.

1.0 INTRODUCTION

1.1 Forensic awareness by chemical agent manipulation

Blood is one of the most significant and frequently encountered DNA trace evidence associated with forensic investigations of death and violent crimes (1). Red Blood Cells (RBC) do not contain a nucleus but , White Blood Cells (WBC) specifically leukocytes do (4). DNA information used by forensic investigators and examiners is

quantified and profiled on the depositor of the bloodstain. To leave this exclusive information at a crime scene is therefore non-desirable. Criminals are becoming increasingly aware of techniques and tests that forensic investigators can use to take biological fluids left at crime scenes, such as blood, and use them to place an offender at the scene (5). Offenders are now also learning which destructive aids will prevent, destroy or manipulate any biological evidence left at a scene (2). There is a literature debate to determine if criminals are influenced by violent media depictions on Television (T.V.) or if they have other means of education. The forensic Awareness (F.A.) and Detection Avoidance (DA) of offenders have been linked to the adverse effects of a *CSI Effect*(6), rising from media shows as "*Crime scene investigation* (CSI)" and "*Law And Order."* Extensive media cultivations of crime drama show and publicity were given to certain evidence types in criminal apprehension have said to directly articulate Forensic Awareness Strategies (FAS) used by criminals. Literature research also shows that criminals purposely using FAS or DA behaviours significantly impacts homicides being correctly classified and investigated as such (7). However, scant research has been conducted on what particular FAS and DA behaviours are known to police and if using them at a crime scene can indicate criminal interference.

Current "clean-up indicators" at a scene are that of Bleach use. Investigators can tell when a substrate has been cleaned explicitly by Bleach, opposed to those that haven't through chemical enhancement methods such as Luminol testing. Creamer et al. (8) showed that a surface would show a higher chemiluminescence value when cleaned with Bleach than if the surface was stained with blood (8). Therefore, investigators can conclude an event that happened on this surface that Bleach then

manipulated. Are there other chemical agents that cause a destructive or degrative effect on human blood that investigators can use as an F.A. indicator within crime scenes?

Research into possible DNA degrative agents showed that certain chemicals are the usual go-to for criminals because of their destructive effects (3). The degradation of RBC is described as rupturing or haemolysis, resulting in the membranes and structure being broken down and releasing the haemoglobin molecules inside (8). This reaction is usually In Vivo, and any environmental contributors are from reactive oxygen chemicals, such as those found in Bleach and hydrogen peroxide (9). However, the literature has not fully understood exogenous influences on blood outside the body (10). The extant chemical agents have not been thoroughly studied on DNA degradation through RBC manipulation, especially in an aqueous solution.

As haemoglobin is the primary detection molecule in multiple forensic presumptive and confirmatory blood testing kits (9), understand what chemicals may inhibit results. To establish if a DI of DNA or haemoglobin through various chemical manipulation can be recorded, this project was designed to test the varying effects of Bleach, hydrogen peroxide and household hand Soap against control to evaluate their destructive tendencies. This DI will be measured through software designed to quantify STRs, give a DNA recovery assessment, and indicate degradation or inhibition (11).

1.2 Experimental aims

The objective of the present study was to address the effect of common household cleaning agents on the DI index of human blood. Furthermore if, allowing the chemical/blood/water solution to sit a prolonged time will directly affect the DI intensity and if a standard blood recovery volume from an aqueous environment can be determined.

2.0 METHODS AND MATERIALS

2.1 Blood collection

50 mL of male human blood was collected from a certified phlebotomist volunteer, Certification Number: AC74/1594. The blood was collected into EDTA tubes and refrigerated before use. Male human blood was used to minimise mixed DNA profiles, with all 50 mL being taken on one occasion to control RBC and WBC fluctuations.

2.2 Sink Apparatus set up

Using a 51mm, 2-inch BI-Metal drill bit (Craft-Right Engineering Works), a hole was drilled into a multi-purpose 9.3L bucket with sprout (Bunnings Warehouse). Using (Parfix) Bathroom and Kitchen Silicone Sealant, a 40mm PVC flange and 40mm male adaptor (Holman-Watermark) were glued together and then onto the bottom of the bucket to create a wastage pipe for the S-Trap pipe to fit onto. The 40mm S and P-Trap

pipe by Caroma (GWA Smarter Solution) was simply screwed and tightened into place on the bottom of the 40mm male adaptor. A 10L waste bucket was placed under the S and P-trap waste pipe to collect water drainage. A metal retort stand with two clamps holds the apparatus via clamping around the S and P trap first pipe bend. Placed close to a bench end, a 10L Adventure camper water carrier is placed on the end of the bench filled with tap water to allow water to flow into the bucket.

Twenty-four sink apparatus were made and used in total; buckets were individually labelled with Avery heavy-duty white labels with treatment to be used. The amount of time the S-trap contents will be held for (see appendix Figure 1.0).

2.3 Treatment Application

2ml of the Human male blood was pipetted onto Latex (Ansell Products) gloved hands placed briefly inside the multi-purpose bucket. For a timed 25 seconds, 2.5L of tap water was emptied from the 10L water camper carrier onto the gloved hands while mimicking a "washing" motion. (See Appendix Figure 2.0). Within the allocated time and water volume, 25mls of the bucket's assigned treatment was syringed using a NIPRO 25ml plastic syringe without needle from its corresponding 400mL Granulated glass stock bottle and ejected onto the gloved hands while being washed inside the bucket.

This was repeated six times for each of the four treatments: 3.83% Bleach (NaOCI) (Glitz), 0.03% Hydrogen Peroxide (H_2O_2) (Sanofi), Antibacterial Hand Soap (Dettol) and

No Treatment. Each treatment was done in triplicate for either a one week or 24Hour S-Trap content hold. In total, each treatment has six assigned buckets.

Once each triplicate had been completed, the S-Trap was immediately emptied into a labelled ~500mL PET plastic bottle container at room temperature stored closed out of direct sunlight for either a week or 24 hours. In Total, each treatment has six assigned PET plastic bottle containers.

2.4 Presumptive and Confirmatory Testing

After the allocated time on each stored container containing the S-Trap contents, the container was inverted three times to mix the solution. 1.0ml was pipetted out and aliquoted into a glass vile for presumptive and confirmatory blood testing. A fresh Cotton swab was saturated in the S-trap content solution; it was then tested using the Kastle Meyer (K.M) (Murdoch University) testing method. The first K.M. reagent, phenolphthalein, one was aliquoted onto the moistened swab, followed by the second K.M. reagent 2, H_2O_2 ; any visible colour change was recorded.

The same S-trap content solution underwent testing via the HemaStix (BLO) (SIEMENS) (12) testing method, as per the technical information sheet. A reaction strip was placed into the glass vile for absorption. A colour change was recorded against the reaction library on the HemaStix product bottle, and any colour development was recorded.

HemaStix Sensitivity for haemoglobin is 0.015-0.062mg/dL haemoglobin(12). Colour change given the amount of ca Cells/µl found are tabled below. A Strip result of Small has estimated 0.030-0.065 mg/dL amounts of blood (12).

Table 1.0- HemaStix reaction colour indicator for urinalysis involving blood quantities.

Non-Haemolyzed			Haemolyzed		Ca Cells/µl	
Neg	Trace(10)	Moderate(80)	Trace(10)	Small(25)	Moderate(80)	Large(200)

Lastly, the S-trap content solution was tested under the HemaTrace (Pathtech) method analysed per the ABACard® HemaTrace®(13) technical information sheet using the included buffer and small plastic dropper. Any line development was recorded.

All results would indicate if blood or a recordable haemoglobin level was present inside the S-trap content solution and if further testing is beneficial.

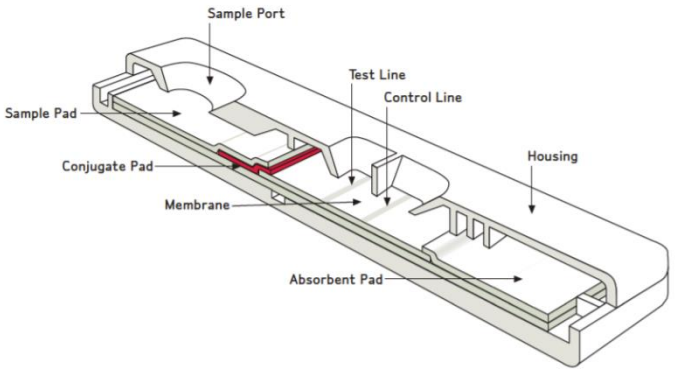

Figure 1.0 Schematic for Hema Trace® showing assays mechanics. (14)

2.5 Micro-Filter DNA Recovery

150mL of each 24 S-Trap content solution as filtered individually through the Micro-Funnel filter funnels across sterile 0.2µm Super Hydrophilic Polyethersulfone (PES) Membrane Disc Filters using a Laboratory Manifold (Pall Corporation, New York)(15). Due to the thicker viscosity of the Soap treated S-Trap samples, 0.45µm sterile PES Membrane Disc Filters were used for the 6 S-Trap content samples. It should also be noted that the Soap treated, and the H_2O_2 treated S-Trap content samples took an average of 1 hour to filter through the membranes, as opposed to the other treatments taking an average range of 3-30 minutes.

Each thin filter paper is placed inside a labelled Petri Dish until dry. Sterile scissors were also cleaned with 10% Bleach solution between each treatment; each Micro-Filter membrane was cut into eight pieces and placed into individual 2mL Eppendorf tubes, refrigerated until all samples could be DNA extracted

2.6 DNA Isolation

In a DNA free Murdoch University Laboratory, all 24 Microfiltered sample membranes previously cut were extracted using the QIAGEN QiAamp DNA Investigator kit (16). Following all isolation protocols for Total DNA from Paper and Similar Materials (16)**,** carrier RNA was prepared and added to the Buffer AL, and 50ul of Buffer ATE was added to MniElute spin Columns used in centrifugation. The twenty four MiniElute Columns were incubated at room temperature for 5 minutes to increase DNA yield(16). All Columns were then centrifuged as handbook instructed. Samples were then refrigerated until needed for qPCR.

2.7 qPCR Amplification

qPCR amplification was done using the Quantifier™ Trio DNA Quantification Kit (Applied Biosystems) (17). Recommended by the Quantifier™ Trio Kit User Guide, two ten-fold dilution series were prepared to make 50ng/µL, 5ng/µL, 0.5ng/µL, 0.05ng/µL and 0.005ng/µL standards. 2 unfiltered samples of PCR grade water were also prepared to act as a negative PCR control. All reactions were prepared and loaded into a 384-well plate and run in a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, United States) using TaqMan Reagents. The experiment was set up according to the Quantifier™ Trio Kit User Guide, and three targets were assigned: Male Y, Large Autosomal and Small Autosomal DNA.

3.0 RESULTS AND DISCUSSION

Two well-known forensic presumptive tests were tested with all samples after their allocated holding periods. K.M, a phenolphthalein chemical indicator test with a recorded sensitivity range of 1:100,000, 1:1 000,000 to 1:10, 000,000. (18-20) was used first and scored accordingly as a weak or visible solid chemical indication. With one '+' indicating a weak haemoglobin presence and '+++' indicating a strong haemoglobin presence. The samples were then tested using the (BLO) HemaStix test, using Tetramethylbenzidine (TMB) to detect the peroxide activity of haemoglobin by in a sample to a recorded sensitivity of 1:50,000 for an instant reaction, to 1:1,000,000 after 20 seconds development in the study by Cox et al. (20). The reaction strips are characterised by either Non-Haemolysed, Haemolysed or Ca Cells/ µl present in the

samples. These three reactions are then again divided into other haemoglobin levels found in the sample as g/dL units, e.g., a small Haemolysed sample has between 0.025 -0.065 mg/dL amounts of blood any colour change indication from the dry test strip is considered a positive result.

Table 2.0- Presumptive and Confirmatory test outcomes for each S-Trap sample contents for the four treatments in triplicates after a 24-hour hold at room temperature.

Presumptive and Confirmatory tests Outcomes of S-Trap contents

1a-1c All treatments 24 Hour hold

Treatment	**Kastle-Meyer**	**HemaStix**	Hema Trace®
Bleach 1a	+	Haemolyzed Small (25)	-
Bleach 1b	+	Ca cells Moderate (80)	-
Bleach 1c	++	Haemolyzed Small (25)	-
H_2O_2 1a	+	Non-Haemolyzed Trace (10)	-
H_2O_2 1b	+	Ca cells -Large (200)	+
H_2O_2 1c	++	Ca cells Moderate (80)	+

Soap 1a	+++	Ca cells Moderate (80)		Inconclusive -
Soap 1b	+++	Ca cells Moderate (80)		-
Soap 1c	+++	Ca cells Moderate (80)		-
Non-Treatment 1a	+++	Ca cells -Large (200)		+
Non-Treatment 1b	+++	Haemolyzed Small (25)		-
Non-Treatment 1c	+++	Ca cells -Large (200)		+

However, the K.M. test and HemaStix cannot determine the blood species' origin, and confirmatory tests are therefore required, such as the ABACard® Hema Trace®. The HemaTrace® test reaction works by human haemoglobin antibodies. It, therefore, is highly specific to human blood with a recorded sensitivity of 1:32,000 with a reduced buffer solution and a recorded 1:200,000 with no buffer solution by Johnston et al. (21). The reaction has three outcomes, Positive '+', Negative '- "or "Inconclusive", a failed control "C" line, or a positive T-test sample line without a positive C line is also an inconclusive result, See Figure 1.0.

Table 3.0- Presumptive and Confirmatory test outcomes for each S-Trap sample contents for the four treatments in triplicates after a one week hold at room temperature.

Presumptive and Confirmatory tests Outcomes of S-Trap contents **2a-2c All treatments One Week hold**				
Treatment	**Kastle-Meyer**	**HemaStix**		Hema Trace®
Bleach 2a	++	Ca cells -Large (200)		-
Bleach 2b	++	Ca cells -Large (200)		Inconclusive _
Bleach 2c	+	Haemolyzed Small (25)		-
H_2O_2 2a	+	Ca cells Moderate (80)		+
H_2O_2 2b	++	Ca cells Moderate (80)		_
H_2O_2 2c	++	Non-Haemolyzed Trace (10)		_
Soap 2a	+++	Ca cells -Large (200)		-
Soap 2b	+++	Ca cells		-

		Moderate (80)	
Soap 2c	+++	Ca cells -Large	-
		(200)	
Non-Treatment 2a	+++	Ca cells -Large	+
		(200)	
Non-Treatment 2b	+++	Ca cells -Large	+
		(200)	
Non-Treatment 2c	+++	Ca cells -Large	Inconclusive
		(200)	+

3.1 Phenolphthalein Testing

The K.M reactions for the four treatments in the 24-hour held S-Trap samples exhibited varying degrees in haemoglobin presence. Each treatment showed an average reaction in their triplicates, evident in Table 2.0. Bleach and H_2O_2 had the weakness reactions, indicated by '+ and ++", possibly explained as Bleach and Hydrogen Peroxide are known to degrade and denature the haemoglobin complex found in the blood. Studies by Passi et .al (22), Li et al. (23), Harris et al.(24) and Helmus and Pfeifer(3) found that cleaning Bleach agents gave DNA degradative and deleterious effects. Thus, no detectable DNA or the quality of DNA left wasn't high enough to produce a viable DNA profile. As Hypochlorite is a common component in household cleaning agents and is therefore often used to remove blood from crime scenes (22), it was chosen for this study. Although H_2O_2 is naturally found in the body

to remove accumulated unsaturated fatty acids (25), its volumes are controlled by Catalase and Glutathione Peroxidase (GSHDX) (9). Small amounts of uncontrolled H_2O_2 contribute to the degradation of heme (9) by affecting the proteins' membrane elasticity (25). With this knowledge, commercially available H_2O_2 was used in this study to see if the same heme degradation effects occur out of Vivo. Antibacterial hand Soap and the Non-Treatment samples showed the highest presence of haemoglobin, indicated by their "+++" in Table 2.0. As blood is not a bacterium and no other chemical treatments were tested on these samples, these samples are expected to have a large blood-water dilution volume still.

The K.M reactions for the four treatments in the one week held S-Trap samples exhibited almost the same haemoglobin presence as those held for only 24 hours, evident in Table 3.0. However, the K.M. test was able to pick up higher haemoglobin present in the Bleach and H_2O_2 treatments from the one-week hold treatments. However, the sensitivity of K.M. is said to be the highest in all presumptive blood tests alongside Luminol (18). A high K.M. reading was expected for both the hand Soap and the Non-Treatment samples, with a high "+++" reaction recorded for all triplicates seen in Table 3.0. It is interesting to see that after a week of sitting in a known large volume degrative solution, blood can still be detected via the K.M. test. These results give promising applications to forensic examiners currently researching DNA transfer in aqueous solutions and any DNA present on washed items (26).

3.2 HemaStix (BLO) Testing

The TMB presumptive test showed significant variance between samples held for only 24 hours and those held for one week. While there was a slight variance between the triplicates, except H_2O_2, overall blood was still detected in every sample, Table 2.0. The Bleach treatment 1a of the 24 hours held samples exhibited the lowest recorded haemoglobin levels with variation between small haemolysed cells (25) to Moderate Ca Cells µl (80). H_2O_2 showed a significant variance between the triplicates 1a to 1c, with the lowest levels recorded as Non-Haemolysed Trace (10) to Large Ca Cells µl (200). It is unknown why there is such variation between the triplicates; however, in a study by Cox et al., a positive interference result when stock solution H_2O_2 was added after 15-20 secs wait (20). Therefore, a variance in the haemoglobin levels may be indicated by adding the H_2O_2 treatment samples 1a-1c again after getting negative results.

Soap treatments 1a-1c all recorded moderate Ca Cells µl (80). In contrast, non-Treatment 1a-1c had the highest recorded haemoglobin levels of all treatments with two triplicates recording Large Ca Cells µl (200), evident in Table. 2.0. However, all recorded levels of haemoglobin in each sample from Trace (10) to Large Ca Cells µl (200) indicate a positive result, Table 1.0; only a non-colour change in the reaction strip is considered a negative haemoglobin reading, which was not seen in the 24 treatments from Tables 2.0-3.0

Surprisingly, the S-trap samples held for one week had the highest haemoglobin level readings out of all 24 samples. In Table 3.0, seven samples out of twelve recorded a level of Large Ca Cells µl (200), with non-Treatment 2a-2c all

recording the highest levels for all three samples. The treatments that showed the lowest haemoglobin levels were H_2O_2 2a-2c, recording from Trace (10) to moderate Ca Cells µl (80) levels. Also observed in treatments 1a-1c of H_2O_2 in Table 2.0. From these results only, it can be said that H2O2 has the most significant effect on haemoglobin in an aqueous environment regardless of time. It is unknown why the samples held for one week showed higher recorded levels of haemoglobin than those only held for a week. An answer could include that the complex haemoglobin structure has been degradative via the various treatments to give raw heme in the solution. The high sensitivity of the TMB test allowed a reaction; or regardless of the treatments, the TMB test would still be susceptible.

3.3 ABACard® Hema Trace® Testing

As the Hema Trace® test focused highly on its specificity to human haemoglobin, its sensitivity isn't on par with that of the K.M or HemaStix presumptive tests. Many exogenous variables impact the validation and reliability of the Hema Trace® test. These include pH, viscosity, Hema Trace® kit extraction Buffer, temperature, assay age, chosen assay antigen and even certain peroxidase-activity cleaners can disrupt or create false negatives and false-positive results (10, 21, 27, 28). Soap detergents have been shown to interfere with the protein interactions of the antigen-antibody complex given their viscosity to give inconclusive or negative assay results (28). While the exact Buffer contents and the type of antigen used in the kits are not commercially available knowledge, it is known that the antigen is insensitive to various environmental factors, except certain household Bleaches and detergents (28).

While such degradative factors like temperature can also denature proteins, affecting the Complementarity-Determining-Region (CDR) on the chosen assay's antigen (27). Unfortunately, the kits used in this study were stored in sun-exposed conditions with an expiration date surpassing the recommended shelf life of one year at a storage temperature of 28 degrees (27).

These factors mentioned above are a simple investigative query into the underwhelming outcomes of the 24 samples each undergone a Hema Trace® test. The 12 samples held for 24 hours had only four samples return a positive result, given the same samples were positive from both K.M and HemaStix tests. The only samples that gave positive results were those with a high HemaStix level of Large Ca Cells µl (200) and one Moderate Ca Cells µl (80) samples H_2O_2 1b, 1c and non-Treatment 1a and 1c, see Table 2.0. The Soap triplicate 1a is inconclusive due to a failed control line. Four Samples again were only positive in the 12 samples held for one week; surprisingly, it was again H_2O_2 and the Non-Treatment samples, H_2O_2 2a and non-Treatment 2a-2c. However, seven samples in this 12-sample pool had significant recorded haemoglobin levels but did not return a positive Hema Trace® result. The author believes it to be because of the "High Does Hook Effect" phenomenon. A significant limitation is presented in the Technical Information Sheet of the Hema Trace® kit (13). Having excessive quantitative amounts of human haemoglobin molecules in a sample, where the excess molecules bind to the immobile antibodies in the Test zone, preventing these antibodies from reacting with the mobile antigen complexes (28).

Given that Large Ca Cells of µl (200) of haemoglobin were recorded for these samples, yet negative Hema Trace® results have occurred, the author believes in this occurrence. Other explanations for those samples where no significant haemoglobin level recordings were evident can be the viscosity of the samples being too high to allow proper migration through the test membrane. As the Soap and the H_2O_2 treatments took the longest to filter through the micro-funnels (between 40 minutes to 2 hours.), it is believed this may again be the occurrence.

The census of this data was not a critique on presumptive testing sensitivity, specificity or validation of each test. Conversely, the data was to show that even after various chemical treatments, human haemoglobin can still be found in a large water dilution, with extended time holding periods as both the K.M. and HemaStix tests returned a positive result; Tables 2.0 and 3.0, for each of the 24 samples. It, therefore, can be concluded that regardless of 24 hours or one week, human blood at a dilution of 1% can be found in the aqueous environment of a plumbing S-Trap after a "washing" has been done. The following data will indicate how much DNA was recovered from the blood amounts found in each sample and if any degradation has impacted the ability to generate viable DNA profile information.

3.4 DNA Isolation and Amplification

DNA concentration analysis from the extraction and its amplification was crucial in determining if DNA could be recovered from aqueous environments, given it underwent various chemical manipulation. To determine any degradation, standard curves and Internal PCR Control (IPC) curves were established to provide comparative

analysis and additional details on quantitation techniques, efficiency and purity. The IPC found in quantitation kits like Quantifiler ™ Trio is a synthetic DNA strand that indicates that the assay worked as expected (17). Determined by its generated Cycle threshold (Ct) value, or the number of cycles the DNA took to be fully amplified (17). The IPC Ct values allow users to determine if any inhibitors impeded the DNA's amplification. All amplified DNA that gave a Ct value, once assessed for inhibition, was used to calculate the DNA concentration in each sample for its amplicon targets (large autosomal, male Y and small autosomal). By inputting the values into the generated standard curves (Figure 4.0-6.0) (Equation 1.0). The accuracy of each sample's concentration is limited to the pipetting accuracy when preparing the standard solutions. R^2 values are assigned to each standard curve to assess accuracy, the R^2 values for large and small autosomal and the Male Y amplicon targets were a strong positive. 0.9866, 0.9945 and 0.9982 respectively.

Table 4.0 – Cycle threshold values of the internal PCR controls (IPC) added to the five standard solutions samples for quantitation using the Quantifier ™ Trio.

IPC Standard	Ct Value
1	**20.093**
2	**22.788**
3	**24.966**
4	**23.867**
5	**24.616**

The DI for each chemical treatment on the samples were also calculated from the DNA concentrations of the large amplicon targets against the small targets (equation 2.0); any value >1 is indicated as a slight to moderate degradation (17). This value indicates which chemical agents significantly affected DNA recovery from aqueous environments after FAS.

3.4.1 Bleach

The total amount of DNA extracted from all twelve (12) bleach treated samples showed exciting results, given the time difference the blood sat in the bleach/water solution. While it was expected that the week-held samples would have less DNA, there were significantly higher amounts of recovered DNA than those held for 24 hours (see Table 5.0 (a)). However, the DNA recovered from the week samples were moderately degraded with a DI of 3.274 and had high amounts of DNA fragments (Small and large autosomal). It can be deduced that the bleach treatment primarily affected the quality of the DNA recovered and the recovery amount, suggesting that the bleach treatment slowly breaks apart the DNA fragments when held in solution for longer. Regarding sample inhibition, all samples were below the IPC Ct value range of the standards (Table 6.0 and Table 8.0 (a-b); therefore, the DI is accepted as actual degradation.

Table 5.0(a) - Data from twelve (12) duplicated biological samples processed using the Quantifier™ Trio system. Showing concentration (ng/µl) (Equation 1.0), Total amount of DNA extracted (ng) (Equation 3.0) and the Degradative index (DI) (Equation 2.0) after being treated with bleach.

Treatments		Male Y DNA Concentration (ng/µl)	Large Autosomal DNA Concentration (ng/µl)	Small Autosomal DNA Concentration (ng/µl)	Total Amounts of DNA In Extraction (ng)	Degradative index (DI)
Bleach	24 Hour Hold	0.006	0.039	0.027	0.003	0.698
	1 One Week	0.634	0.132	0.433	0.038	3.274

3.4.2 Hydrogen Peroxide

The Hydrogen peroxide treatment showed higher total DNA extracted from the twelve (12) samples for those that were held for 24 hours, with 0.661 ng recovered (see Table 5.0 (b)). With a large amount coming from large autosomal amplicons, the DI on the 24-hour samples was <1, and therefore regarded as non-degraded. The DI for the one-week samples, however, was 2.331 and accepted as moderately degraded.

The H^2O^2 treatment is fast-acting and greatly affected the 24-hour samples' fragmentation; however, the treatment only affected the quality of the DNA in the one-week samples, suggesting that the longer the solution sat in its aqueous environment, the more degraded and less recoverable it became. No inhibition was noted in all H^2O^2 treated samples, as the recorded IPC Ct values were below the standards (Table 6.0 and Table 8.0 (a-b)); therefore, all degradative values are valid.

Table 5.0(b) - Data from twelve (12) duplicated biological samples processed using the Quantifier™ Trio system. Showing concentration (ng/µl) (Equation 1.0), Total amount of DNA extracted (ng) (Equation 3.0) and the Degradative index (DI) (Equation 2.0) after being treated with Hydrogen Peroxide.

Treatments		Male Y DNA Concentration (ng/µl)	Large Autosomal DNA Concentration (ng/µl)	Small Autosomal DNA Concentration (ng/µl)	Total Amounts of DNA In Extraction (ng)	Degradative index (DI)
Hydrogen Peroxide	24 Hour Hold	4.989	8.416	1.551	0.661	0.251
	1 One Week	0.549	0.027	0.048	0.034	2.331

3.4.3 Hand Soap

The hand soap treatment had the highest value of DI from all blood treatments. Surprisingly it was thought that an Anti-Bacterial hand soap would have non to little effect on DNA fragmentation and recovery volumes, given DNA is not a bacterium. However, the one-week held samples had the second-largest DNA recovery of all treatments at 7.803 (see Table 5.0 (c)). The one-week small autosomal concentration was the highest recorded value in all treatments at 105.1 (ng/µl). Though the IPC Ct values are within the acceptable range, table 6.0 and 8.0 (a-b), contamination cannot be ruled out. The data suggest that the soap treatment is highly effective for DNA fragmentation, and quality was given a long time held in solution. However, quality DNA can be recovered in large amounts after 24 hours in aqueous environments.

Table 5.0(c) - Data from twelve (12) duplicated biological samples processed using the Quantifier™ Trio system. Showing concentration (ng/µl) (Equation 1.0), Total amount of DNA extracted (ng) (Equation 3.0) and the Degradative index (DI) (Equation 2.0) after being treated with Hand Soap.

Treatments	Male Y	Large	Small Autosomal	Total Amounts of DNA In	Degradative index

		DNA Concentration (ng/µl)	Autosomal DNA Concentration (ng/µl)	DNA Concentration (ng/µl)	Extraction (ng)	(DI)
Hand Soap	24 Hour Hold	0.101	1.604	0.057	0.106	0.050
	1 One Week	18.175	6.710	105.1	7.803	5.786

3.4.4 non-Treatment

The non-Treatment was used as a negative control and a study to determine if a standard blood recovery volume from an aqueous environment can be determined. However, table 8.0 (a-b)(Appendix) shows that recovery concentrations for each triplicate had varying values and a total DNA recovery amount variation between 24 hours and one-week samples.

Neither the 24 hour nor the one-week samples had any degradation, with both values recorded below <1. Although a recovery standard couldn't be deducted from the data, it was concluded that blood can still be recovered from an aqueous environment; after being in a 0.1% solution for a week. As with the other treatments, no inhibition was

seen in the samples, as all IPC Ct values were within the standard curve range, Table 6.0.

Table 5.0 (d) - Data from twelve (12) duplicated biological samples processed using the Quantifier™ Trio system. Showing concentration (ng/µl) (Equation 1.0), Total amount of DNA extracted (ng) (Equation 3.0) and the Degradative index (DI) (Equation 2.0) after No Treatment.

	Treatments	Male Y DNA Concentration (ng/µl)	Large Autosomal DNA Concentration (ng/µl)	Small Autosomal DNA Concentration (ng/µl)	Total Amounts of DNA In Extraction (ng)	Degradative index (DI)
Non-Treatment	24 Hour Hold	3.885	1.309	0.358	2.277	0.274
	1 One Week	19.439	0.662	0.429	10.265	0.647

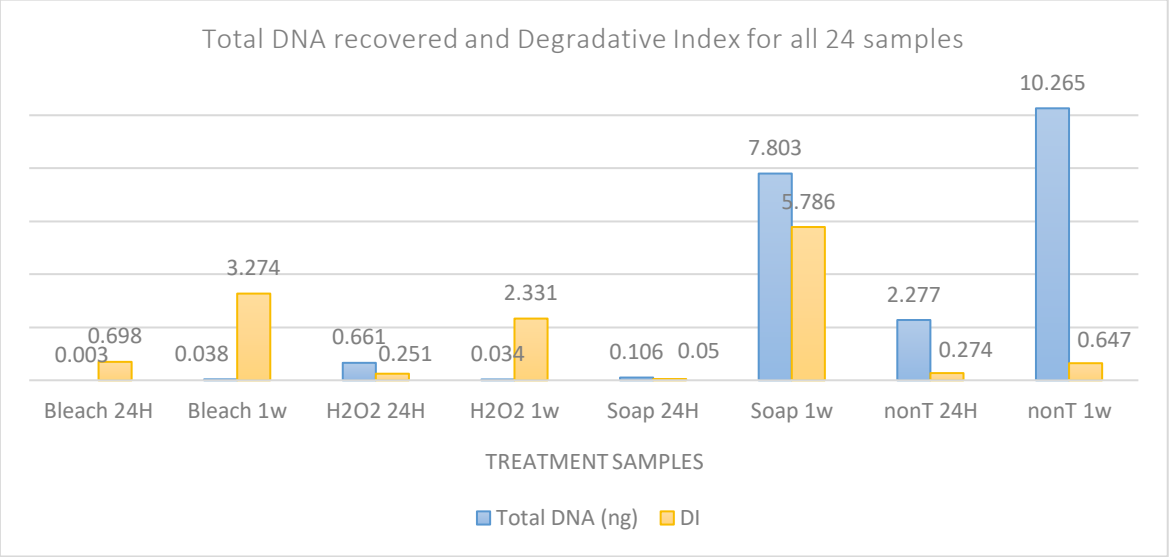

Figure 7.0 – Total DNA recovered and the corresponding DI for all 24 samples assayed by Quantifiler ™ Trio.

4.0 CONCLUDING STATEMENTS

From all treatments applied to the blood samples in the aqueous environments, it's evident that the most degrative chemical agent was hand soap with a DI recorded at 5.786 (Table 5.0 (c)). However, it was also the treatment with the highest DNA recovery, 7.909 ng (Table 5.0(c)), besides the non-treatment control. Although the most prominent DNA fragmentation was also soap at 105.1 small autosomal(ng/µl) (Table 5.0 (b)), this value was considered under contamination effects. Excluding this value, the treatment with the most significant effect on DNA fragmentation is Hydrogen peroxide with small autosomal fragments recorded at 1.551 (ng/µl) (Table 5.0 (b)), without a DI. The treatment that allowed the least

amount of DNA recovery was bleach at 0.041 ng (Table 5.0 (a)), a total sum recovery between 24 hours and one week see Figure 7.0.

From this data, DNA recovery was still viable after every chemical agent treatment, although some DNA was affected by degradation. From the Quantifiler™ Trio handbook (17), any DI between 1-10 is seen as only moderate. Therefore, a potential viable DNA profile could be created; however, only H^2O^2 after 24 hours has enough DNA recovered (0.661 ng, Table 5.0 (b)) without any DI (0.251, Table 5.0 (b)) to continue onto further testing, Excluding those of non-treatment.

Given this data collection and analysis, if a criminal had used any FAS involving chemical manipulation of blood in an aqueous environment such as a sink, there is a high chance the chemical will cause degradative effects after either 24 hours or a week, depending on the chemical. However, DNA recovery is still possible regardless of a prolonged time. Future research opportunities can explore how much of each chemical agent is needed in a dilution to prohibit DNA recovery and a DI higher than one completely.

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6.0 APPENDIX

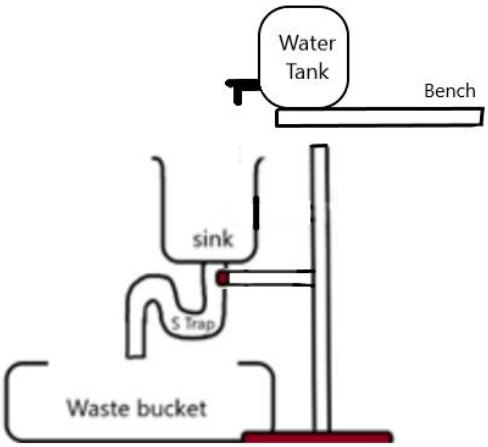

Figure 2.0 – Sink apparatus set up created by JL Webb 2021.

Figure 3.0 – Recommended hand washing steps, disregarding temperature and Soap application.

Table 6.0- Data from quantifying DNA from five (5) known standard solution concentrations. The cycle threshold (Ct) was calculated for each DNA component and internal positive control (IPC).

DNA component	Standard	Concentration (ng/μl)	Ct Value
Large Autosomal	1	50	15
	2	5	18.0
	3	0.5	21.9
	4	0.05	23.9
	5	0.005	11.9
Male Y	1	50	18.7
	2	5	22.5
	3	0.5	26.0
	4	0.05	29.1
	5	0.005	31.1
Small Autosomal	1	50	21.6
	2	5	24.6
	3	0.5	27.7
	4	0.05	30.6
	5	0.005	32.6

IPC	1	-	20.093
	2	-	22.788
	3	-	24.966
	4	-	23.867
	5	-	24.616

Table 7.0 (a)

Data from quantifying DNA from twelve (12) biological samples, including two (2) reagent blanks. The cycle threshold (Ct) values were calculated for each DNA component after averaged.

1a-1c All Treatments 24 Hour Hold			
Treatment	**Large Autosomal Ct Value**	**Male Y Ct Value**	**Small Autosomal Ct Value**
Bleach 1a	24.989	32.143	32.670
Bleach 1b	24.30	31.765	32.417
Bleach 1c	0.000	16.185	31.203
H_2O_2 1a	22.180	28.710	30.594
H_2O_2 1b	17.558	22.696	25.012
H_2O_2 1c	17.421	22.101	26.375
Soap 1a	22.531	29.889	30.807
Soap 1b	22.730	33.563	36.910
Soap 1c	20.344	29.193	29.819
Non-Treatment 1a	20.094	22.424	27.679
Non-Treatment 1b	22.553	30.686	14.655
Non-Treatment 1c	20.587	25.464	27.316
Reagent Blanks	18.517	28.992	-

Table 7.0 (b)

Data from quantifying DNA from twelve (12) biological samples. The cycle threshold (Ct) values were calculated for each DNA component after averaged.

2a-2c All Treatments One Week Hold			
Treatment	**Large Autosomal Ct Value**	**Male Y Ct Value**	**Small Autosomal Ct Value**
Bleach 2a	24.876	31.236	30.839
Bleach 2b	22.894	19.110	29.152
Bleach 2c	11.246	16.552	32.619
H_2O_2 2a	24.971	24.983	29.758
H_2O_2 2b	24.795	32.106	33.241
H_2O_2 2c	12.583	29.755	30.140
Soap 2a	17.338	22.424	22.769
Soap 2b	25.914	31.790	30.360
Soap 2c	12.284	35.351	33.460
Non-Treatment 2a	22.812	22.585	28.227
Non-Treatment 2b	22.729	26.120	28.157
Non-Treatment 2c	20.378	25.156	27.263

Table 8.0 (a) – Twelve (12) biological samples including two (2) reagent blanks, Ct Values of target DNA amplicons and an Internal PCR Control (IPC) using Quantifier™ Trio system methods including Degradative index

1a-1c All Treatments 24 Hour Hold							
Treatment Samples	**Large Autosomal DNA Concentration (ng/µl)**	**Male Y DNA Concentration (ng/µl)**	**Small Autosomal DNA Concentration (ng/µl)**	**IPC Ct Value**	**The total concentration of DNA in the extract (ng/µl)**	**Degradative Index (DI)**	**Total Amount of DNA in Extraction (ng)**
Bleach 1a	0.03658	0.00562	0.01726				
Bleach 1b	0.081	0.009	0.028	22.554	0.072	0.698	0.003
Bleach 1c	0	0.00168	0.03704				
H_2O_2 1a	0.58763	0.05061	0.03929				
H_2O_2 1b	9.30816	4.16456	3.98819	22.529	15.216	0.215	0.661
H_2O_2 1c	15.3542	10.7538	1.40181				
Soap 1a	2.96830	0.02659	0.03948				
Soap 1b	0.40478	0.0017	0.00033	21.919	1.773	0.056	0.106
Soap 1c	1.43920	0.27674	0.16289				
Non-Treatment 1a	2.76299	11.02941	0.43288	23.104	5.553	0.274	2.776

Non-Treatment 1b	0.33498	0.08552	0.05638	
Non-Treatment 1c	0.83265	0.54046	0.58755	
Reagent Blanks	3673.198 31	-	-	-	3673.198 31

Table 8.0 (b) – Twelve (12) biological samples Ct Values of target DNA amplicons and an Internal PCR Control (IPC) using Quantifier™ Trio system methods including Degradative index

2a-2c All Treatments One Week Hold							
Treatment Sample	**Large Autosomal DNA Concentration (ng/µl)**	**Male Y DNA Concentration (ng/µl)**	**Small Autosomal DNA Concentration (ng/µl)**	**IPC Ct Value**	**Total concentration of DNA in extract(ng/µl)**	**Degradative Index (DI)**	**Total Amount of DNA in Extraction (ng)**
Bleach 2a	0.03446	0.18923	0.03690	23.322	6.287	3.274	0.038
Bleach 2b	0.26350	0.00002	1.24912				
Bleach 2c	0.09880	0.00098	0.01327				
H_2O_2 2a	0.03480	1.62051	0.07823	20.867	0.625	2.331	0.034
H_2O_2 2b	0.03527	0.00465	0.01034				
H_2O_2 2c	0.01325	0.02327	0.05712				
Soap 2a	54.49138	20.124668	315.44285	22.667	130.056	0.647	7.803
Soap 2b	0.01561	0.00527	0.05958				
Soap 2c	0.02074	0.00038	0.00907				

Non-Treatment 2a	0.18257	57.2553	0.28202				
Non-Treatment 2b	0.17614	0.38114	0.30760	21.640	20.531	5.786	10.265
Non-Treatment 2c	1.62976	0.68292	0.69760				

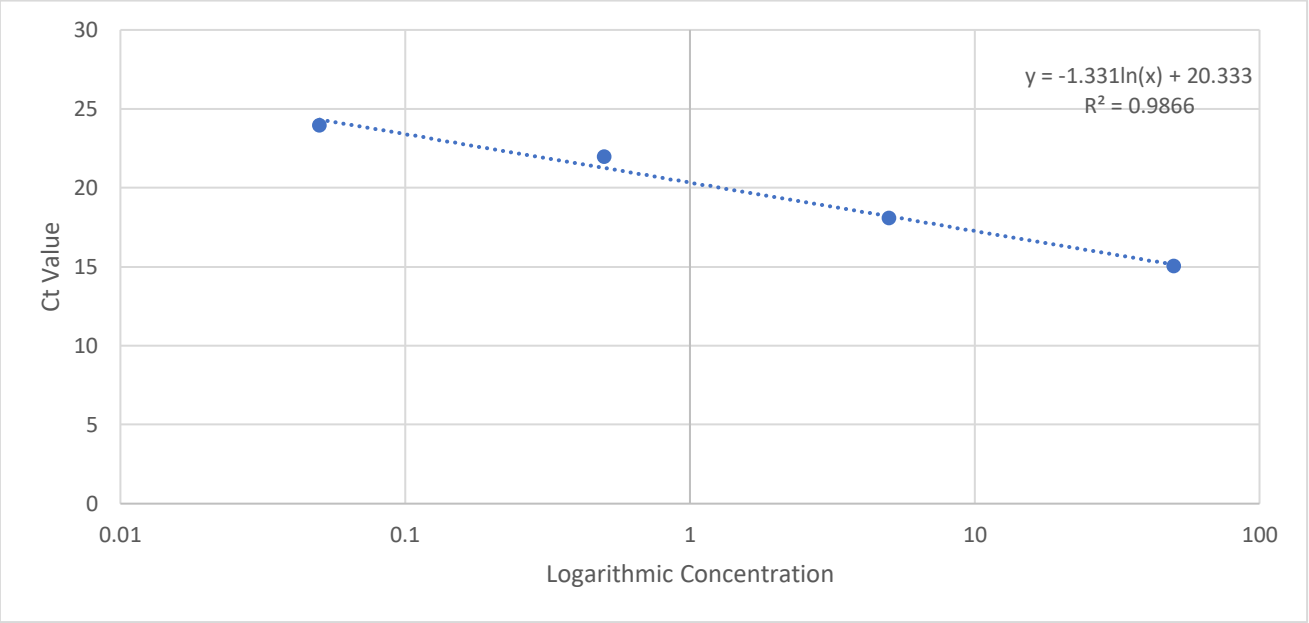

Figure 4.0 Standard curve of the large autosomal DNA component signifying the logarithmic concentration against the quantitated cycle threshold (Ct) value using the Quantifier™ Trio system

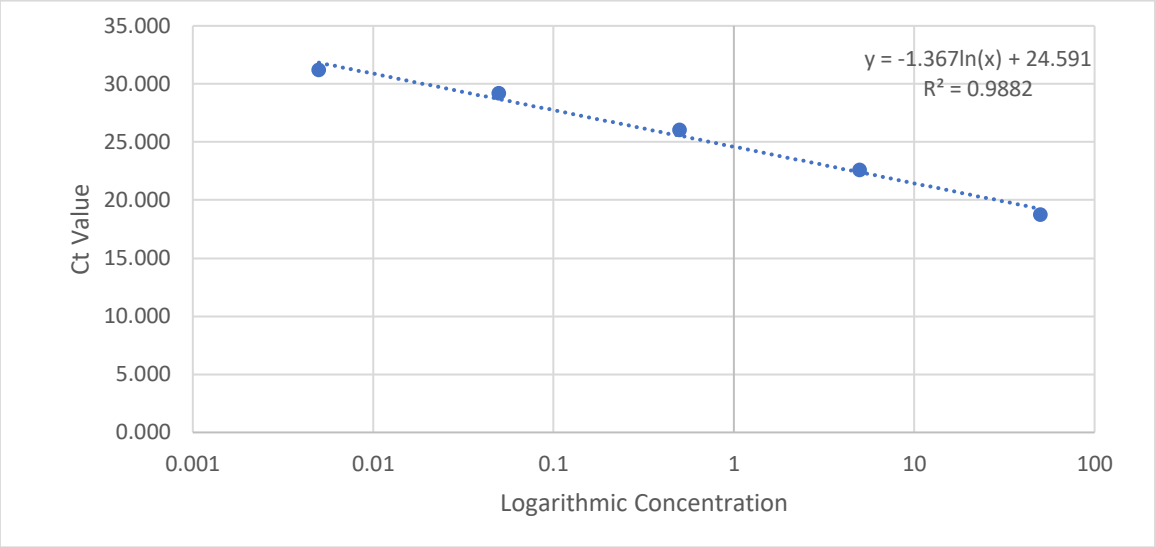

Figure 5.0: Standard curve of the male Y DNA component signifying the logarithmic concentration against the standard cycle threshold (Ct) value using the Quantifier™ Trio system.

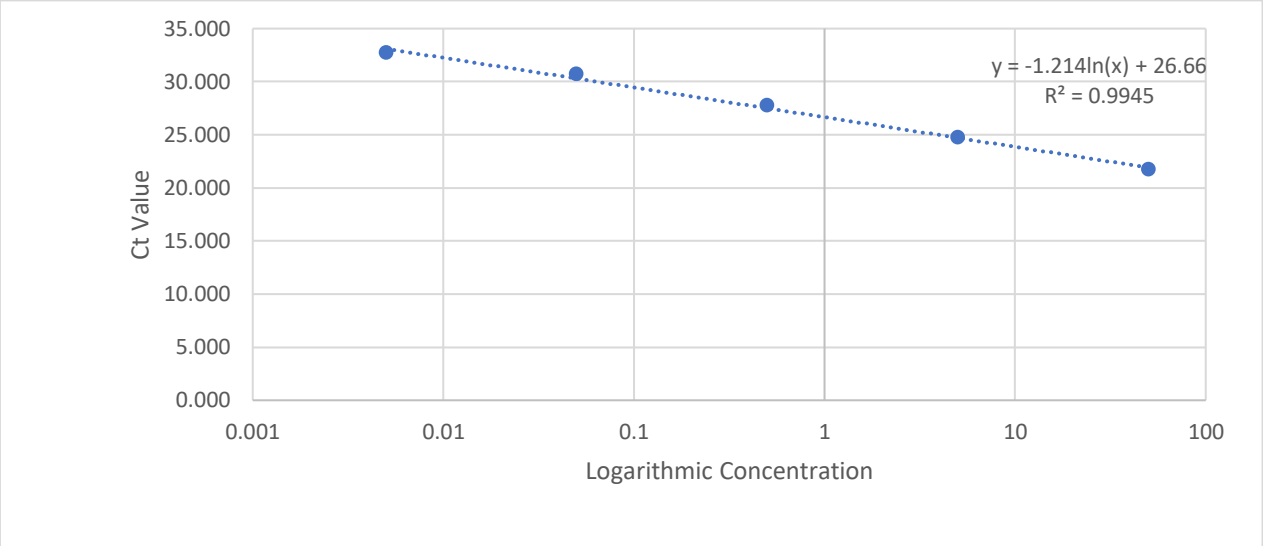

Figure 6.0 Standard curve of the small autosomal DNA component signifying the

logarithmic concentration against the quantitated cycle threshold (Ct) value using the Quantifier™ Trio system.

Equation 1.0 - Concentration (ng/µl) of DNA in the Quantifiler™ Trio based on the standard curve logarithmic equations.

$$Conc = EXP(\frac{(Ct\ Value - y\ intercept)}{gradient})$$

Equation 2.0 – Degradative Index (DI) of DNA concentrations from samples assayed by Quantifiler™ Trio

$$DI = \frac{Concentration\ of\ Small\ DNA\ Target}{Concentration\ of\ Large\ DNA\ Target}$$

Equation 3.0 -Total DNA (ng) in each sample assayed by Quantifiler™ Trio DNA.

$$\text{Total DNA (ng)} = \frac{Total\ Small\ autosomal + Male\ Y + large\ autosomal}{\text{extraction volume}}$$