Journal of Forensic and Legal Medicine 117 (2026) 103023

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/yjflm

Research Paper

# Validation of forensic cleaning processes undertaken within Sexual Assault Referral Centres

Michelle Gaskell [a,b,*], Lesley Clifford [c], Aaron Jones [c], Guylaine. O Hanford [a], Kevin Sullivan [a]

[a] Forensic Capability Network, Dorset Police, Dorset, UK
[b] University of Central Lancashire, UK
[c] Cellmark Forensic Services, Abingdon, UK

ARTICLE INFO

*Keywords:*
Validation
DNA decontamination
Sexual assault referral centre (SARC)
Forensic Cleaning

ABSTRACT

This paper describes the retrospective validation of long-established cleaning processes used within Sexual Assault Referral Centres (SARCs), for which there are variations between facilities in the detail of the cleaning approach that is applied, including whether bleach/hypochlorite cleaning reagents are permitted depending on local health and safety requirements. Six cleaning reagents commonly used within UK SARCs and Forensic Science Providers were assessed in this validation study: Chemgene $HLD_4H$, Virkon, Microsol, Selgiene, Virusolve were tested along with Presept which was the only reagent containing bleach. Additional comparison testing was also conducted on Chemgene Medlab. These were evaluated for their DNA decontamination capability by cleaning dried-on body fluid stains deposited on typical examination room surfaces and then assessing the level of DNA remaining (percentage yield). Impact of changing different cleaning parameters were assessed against an environmental indicator guide that provides insight into SARC facility cleanliness. Differences in effectiveness of decontamination varied according to body fluid type with DNA in blood being most readily removed followed by saliva, and semen was the hardest to decontaminate. Likewise, different surfaces varied in their resilience to decontamination with Formica being the easiest to clean and vinyl the hardest. Bleach-based reagent Presept gave the best decontamination test results overall, whilst non-bleach cleaners Virkon and Selgiene were also very effective. However, as a general rule, provided double spray/wipe cycles are performed using manufacturers' recommended concentrations and a 30 s contact time, the cleaning effectiveness of all reagents were assessed to be generally acceptable in most circumstances. The exception to this rule was cleaning dried semen on vinyl, which was the most challenging body fluid/surface combination to decontaminate. It is recommended that extra care is taken in cleaning vinyl surfaces such as the examination couch and consider additional measures if necessary.

## 1. Introduction

An ever-present challenge faced by forensic medical examination facilities within SARCs and police custody suites is managing the risk of DNA contamination in order to prevent evidence from being potentially compromised during the examination process. This challenge is amplified by the advancements in genetic technologies significantly increasing the sensitivity of forensic DNA analysis.[1–4] To address this, a raft of measures have been developed to minimise the risk of DNA contamination including personnel training, cleaning protocols, plus use of appropriate DNA-free consumables and personal protective equipment (PPE). Scientific studies on the effectiveness of DNA decontamination procedures are often undertaken primarily from a laboratory perspective i.e., considering removal of cell-free DNA/Polymerase Chain Reaction (PCR) product rather than cellular contamination within a clinical environment. In one such study, various laboratory cleaning regimes were evaluated on dried cell-free DNA, from which it was concluded that the mechanical action of cleaning has an impact on DNA removal and that hypochlorite solution is the most effective for removal of traces of amplifiable DNA.[5] This confirmed outcomes of an earlier study in which hypochlorite solution proved most effective for removing DNA, blood, semen and skin cells.[6] A more recent study relevant to clinical environments assessed the effectiveness of cleaning reagents on cell-free DNA and cellular DNA within blood stains.[7] This

* Corresponding author. 138 Croesonen Parc, Abergavenny, Monmouthshire, NP7 6PG, UK.
*E-mail address:* michelle_gaskell@hotmail.co.uk (M. Gaskell).

https://doi.org/10.1016/j.jflm.2025.103023
Received 22 October 2024; Received in revised form 27 October 2025; Accepted 9 November 2025
Available online 17 November 2025

latter study undertook an informal survey of the decontamination methodologies being used by European Forensic Service Providers (FSP) and assessed ten of these including use of various chemicals and direct UV light treatment. This identified a significant reduction in effectiveness when cleaning cellular DNA stains compared with cell-free DNA. Bleach/hypochlorite was again determined to be the most effective against cell-free DNA but treatment with Virkon was the best for removal of DNA within blood stains. This greater resilience of cellular DNA highlights a challenge for decontamination within SARCs where significant levels of body fluids are frequently encountered. Of these body fluids, semen contamination can be more problematic to remove effectively and has been attributed as the likely source in an Office of the Forensic Science Regulator (OFSR) investigation into a SARC serious contamination incident.[8] This and other quality issues identified within SARCs have culminated in the UK Forensic Science Regulator (FSR) requiring all SARCs in England and Wales to be accredited to the international quality standard ISO/IEC 15189 *Medical Laboratories-Requirements for Quality & Competence*[9] and compliant with relevant elements of the FSR Code of Practice.[10–12]

To date most SARCs in England and Wales have yet to assess their cleaning processes from the perspective of a DNA anti-contamination measure. The aim of this exercise was to centrally validate the cleaning approaches typically utilised by SARCs, including an assessment of the efficacy of the most commonly used cleaning reagents, as part of their overall DNA anti-contamination approach. By doing so the intention was to provide assurance that existing processes are fit for purpose or identify improvements that are needed. Thereby reducing the work required by individual SARCs to just conducting local verification exercises to provide the required levels of assurance that their individual cleaning regimes are fit for purpose, and compliant with the requirements of ISO/IES 15189[9] and relevant sections of the FSR Code of Practice.[10] By extension, this exercise is also intended to validate the related cleaning processes undertaken within custody suites: whilst there is less pressure from a regulatory perspective to accredit these facilities, they present analogous risks and challenges to the Criminal Justice System (CJS) regarding contamination.

## 2. Materials and methods

### 2.1. General

All testing and analysis were undertaken within a dedicated test facility at Cellmark Forensic Services, Abingdon, UK. Consumables and equipment used are listed in Table 11.

Formica, vinyl, metal and plastic were included in this study as identified by forensic healthcare practitioners and SARC managers to be the most commonly encountered surfaces within SARCs.

Cleaning of spiked test surfaces and subsequent swabbing for DNA recovery were conducted by individuals trained and competent in cleaning procedures and environmental swabbing employed. Two experimental operators conducted the 10 experiments to maximise consistency of the cleaning actions throughout the experiments and minimise the contribution of operator variability to any differences observed.

The body fluids, shown in Table 1, were used with each diluted and vortexed to improve homogeneity of the samples. They were not all from the same source, but each individual body fluid type was from a single source donor who provided written consent prior to involvement.

### 2.2. Test methods: overview

Samples of 10 μl body fluids were deposited on different surfaces and then subjected to different cleaning regimes before sampling from the deposition area and assessing how much DNA had been removed by the cleaning process. Each individual test combination of body fluid/substrate/cleaning variation was replicated 5 times to enable statistical significance of the observed results to be assessed.

**Table 1**
List of body fluids, volumes and dilutions used throughout the experimentations and the storage conditions used for body fluids.

| Body fluid | Volume | Dilution | Storage | Additional information |
|---|---|---|---|---|
| Blood | 0.65 ml | 1 in 2 dilution in an isotonic saline | Fridge storage (i.e. 4 °C) | In sealed container with recommended anticoagulant and preservative at 4 °C |
| Semen (from non-vasectomised male) | 3.6 ml | | Stored frozen until required | Thawed out and vortexed immediately prior to use |
| Saliva | 0.65 ml | | | |

Body fluid samples were pipetted on to 3 cm wide circles which were indelibly marked areas of the substrate and spaced 50 cm apart. Each sample deposit was spread out with the pipette tip and left at room temperature for at least 2 h to fully dry. The decontamination methodologies subsequently applied to clean the surfaces were based on current practice within SARCs with direct comparison of the most commonly used cleaning reagents. Following cleaning, the deposition area was re-swabbed with forensic DNA grade moist and dry cotton swabs to recover biological material that may have remained. Sampling by swabbing was chosen for this exercise as it targets the exact point of deposition of the treated contaminant and is a routinely undertaken process for environmental monitoring (EM). Work surfaces were re-used as required, using a bleach followed by water clean conducted in-between the cleaning experiments, and with relevant EM samples also taken, which is a previously validated Cellmark Forensic Services cleaning process accredited to ISO/IEC 17025 *Testing and Calibration Laboratories* standard.[11] Appropriate control deposits of the body fluids were also made and swabbed directly as described above but without any cleaning undertaken so that reduction due to cleaning could be ascertained.

Studies have shown that semen stains can be extremely resilient, and DNA can be recovered from them even after being washed and aged for decades.[14] Semen samples can have higher overall human DNA concentrations than blood samples. Notably, a 2024 study[15] found significantly higher DNA concentrations in semen compared to blood when assessing detection and collection on various fabrics. Based on these studies and feedback from forensic practitioners and the OFSR, semen was identified as the most problematic body fluid to decontaminate, therefore this was used for much of the evaluation in this study. Formica and Chemgene $HLD_4H$ were typically the default cleaning reagent and surface used in this study where other variables were being evaluated, as these were most commonly encountered/used in SARCs at the time of this study.

The cleaning reagents tested were Virkon, Chemgene $HLD_4H$, Microsol, Selgiene, Virusolve and Presept. This ensured that most of the cleaning reagents used by SARCs as identified in a survey (shown in Table 2) were included in this study. For Presept, an additional water wipe was deployed after its use as an additional health and safety and anti-corrosion precaution.

The experimental work was conducted by Cellmark Forensic Services at their facilities in Abingdon, UK. A list of all consumables and instruments used throughout are provided in Table 11. The experimental work comprised of a setup of experiments 1–10 (as detailed in 2.5 Experimental Method section) followed by DNA recovery, extraction, quantification and analysis using an ISO/IEC 17025[11] accredited processes. The swabs from each of the experiments were extracted and purified using the EZ1® and EZ2® Investigator kit (Qiagen) and the BioRobot® EZ1 workstation (Qiagen), using the 200 μl lysis procedure. Each extract was eluted into a 50 μl volume of Tris-EDTA buffer. An extraction negative control sample was included to monitor any contamination events.

**Table 2**
Results from a cleaning reagent survey to identify which products are used by SARCs and FSPs in England and Wales.

| Group | Organisation/Survey | Cleaning Reagent | Surface | Additional Notes |
|---|---|---|---|---|
| FSPs | **FSP A** | 1 % Rely + On™ **Virkon®** followed by cleaning with water | Bench | Cleaning processes validated and accredited to ISO/IEC 17025.[11] |
| | | bleach | Floor | |
| | | **Chlorhexidine** wipe | Pens etc | |
| | **FSP B** | **Virkon** (currently) Previously **Microsol, Presept** | All | |
| | **FSP C** | **Presept** then water | All | |
| SARCs England and Wales | **SARC Survey: 25 Responses** | **Chemgene HLD$_4$H** 11 sites (8 sole use) | All surfaces | 1 in 10 to 1 in 100 dilution depending on application |
| | | **Microsol** 6 sites (4 sole use) | | |
| | | **Selgiene** 3 sites (3 sole use) | | |
| | | **Virusolve** 3 sites (3 sole use) | | |
| | | **Distel** 3 sites (1 sole use) | | |
| | | **Clinelle** 1 (1 sole use) | | |
| | | **Actichlor** 1 (1 sole use) | | |
| | | **Presept** 3 sites | Floor | Floor cleaning only |
| | | **Selgiene** 1 site | | |
| | | **Chemgene HLD$_4$H** 2 sites | | |

Quantification was performed using a 7500 Real-time PCR system with Qiagen's Quantiplex PRO RT PCR kit. The DNA quantification results were used to determine the volume of DNA placed in the PCR reaction. The samples were amplified using Thermo Fisher's AmpFLSTR™ NGM Select™ PCR Amplification kit.

Following amplification, all samples were run on a 3500 Genetic Analyser. After electrophoresis, the samples were interpreted using Genemapper® IDX v1.5 software. The DNA profiling results for the samples were compared and statistically evaluated (as per the equations described in Table 12) where appropriate. Results were assessed using a number of measures to evaluate and express the effectiveness of decontamination: % allele count reduction measured by comparing number of alleles recovered from swabs taken post cleaning versus no cleaning is a very simple measure, as is quantifying recovered DNA post clean and the latter can also be expressed as % yield which compares recovery of DNA from swabbing a seeded area before and after cleaning.

A Cellmark EM Indicator Guide was used to assess the suitability of the cleaning throughout the experiments, this provided the criteria for the number of alleles above set thresholds which uses a Red/Amber/Green (RAG) scoring system, summarised in Table 3. These guides are intended to provide SARCs with an easy assessment of how well cleaning is working within their facility and give guidance for actions to take to investigate inefficiencies such as checking if a returned profile matches one on the staff elimination database (SED). A SED is a UK DNA database that holds DNA profiles from individuals who work with or come into contact with crime scene evidence, such as crime scene investigators, laboratory staff, SARC staff, and manufacturers. Its purpose is to allow for the elimination of these individuals' DNA when staff DNA profiles are identified on crime samples preventing them from being mistakenly linked to a crime. An SED is used for purposes of investigating potential contamination identified from EM samples.

**Table 4**
Summary of the number of tests conducted within each experiment using each of the 3 body fluids and number of control samples taken.

| Experiments | Body Fluids Seeded | | | Controls |
|---|---|---|---|---|
| | Semen | Blood | Saliva | |
| 1: Mechanical action | 25 | 0 | 0 | 18 |
| 2: Cleaning reagent on different body fluids | 35 | 35 | 35 | 9 |
| 3: Spray v wipe | 35 | 0 | 0 | 3 |
| 4: Duplicate spray/wipe cycles | 45 | 0 | 0 | 6 |
| 5: Different cleaning reagent concentrations | 30 | 0 | 0 | 3 |
| 6: Cleaning reagent contact times | 90 | 0 | 0 | 9 |
| 7: Different substrates | 66 | 0 | 0 | 6 |
| 8: Wiping instrument controls | 70 | 0 | 0 | 3 |
| 9. Double spray/wipe on vinyl all cleaning reagents | 30 | 0 | 0 | 8 |
| 10. Additional mechanical action | 15 | 0 | 0 | 3 |
| **Total 579** | **441** | **35** | **35** | **68** |

Following research conducted into the risk of DNA contamination in the SARC environment[16] a new criteria was developed and published into regulatory guidance[13] by the FSR, shown in Table 3.

For clarity and simplicity of presentation, the results in this report are shown primarily as allele counts scored as RAG in accord with Cellmark's EM Indicator Guide, unless otherwise stated.

**Table 3**
Table of two RAG rating criteria's for SARC EM results, one developed by Cellmark Forensic Service and the second set by FSR guidance.[13]

| Risk Level | Cellmark Forensic Services Environmental Indicator Guide | | FSR Environmental Monitoring Guide[13, 16] | | |
|---|---|---|---|---|---|
| | Alleles | Response | Alleles | DNA Quantification | Response |
| Green | (0-10 alleles) | Pass: No response required | (0-10 alleles) | <0.0002 | No action required |
| Amber | (11-24 alleles) | Pass: action may be required | (11-34 alleles) | 0.0002 < 0.004 | Re-clean and check EM result against SED |
| Red | (>24 alleles) | Fail: immediate action required | (>34 alleles) | 0.004 or more | Re-clean and re-sample: check EM result against SED |

### 2.3. Experimental setup

In total 579 tests as shown in Table 4 were conducted in the 10 experiments described below in order to assess the impact of different aspects of cleaning and variables within the cleaning process.

### 2.4. Statistical analysis

All equations used in the statistical analysis of the experimental results are provided in Table 12.

### 2.5. Experimental Method

#### 2.5.1. Experiment 1: impact of mechanical action

The efficacy of cleaning regimes has been identified to be a combination of both cleaning reagent and physical actions. This limited study assessed the impact of different mechanical actions, i.e., directional circular wiping versus directional linear wiping, in order to settle on one approach for the rest of the experiments, thereby removing a variable from the experimental design. This was assessed by testing five replicate semen spots dried onto Formica for each of the wiping actions shown in Fig. 1.

Assessment of the impact that cleaning a contaminant has on surrounding areas of substrate was also made, i.e., whether the body fluid is simply being spread out by the cleaning action rather than being removed, by taking additional swabs 10 cm from the seeded area. The experimental set-up has been provided in Table 13.

On completion of these experiments the work surface was thoroughly cleaned using a validated bleach and water method and environmental monitoring conducted of the selected areas previously spotted with semen. The results from this experiment determined the mechanical cleaning action used for the subsequent experiments, and the outcomes dictated the level of additional monitoring for the following experiments.

#### 2.5.2. Experiment 2: impact of cleaning reagents on different body fluids

Effectiveness of the six chosen cleaning reagents was assessed for the removal of DNA within dried stains of body fluids, namely blood, semen and saliva. 10 µl of each body fluid was dried onto Formica. The surface was sprayed with the cleaning reagent using the manufacturers'/suppliers' recommended concentrations for spray applications. After 30 s, the substrate was wiped clean with paper towel using circular wiping and left to dry before sampling the spotted areas. Controls of body fluids not subject to cleaning were included, plus substrate negative controls. Table 15 details the experimental set-up for Experiment 2.

#### 2.5.3. Experiment 3: comparison of the effectiveness of spraying and then wiping versus wiping with a pre-impregnated wipe

The effectiveness of spraying with a cleaning reagent and then wiping it clean was compared with wiping using a pre-impregnated wipes to identify the most effective cleaning approach, 10 µl of blood, semen and saliva were spotted on to Formica, as per Experiment 2. The surface was cleaned using commercially available pre-prepared wipes. For Presept, solution was sprayed onto disposable cloth, as no pre-impregnated wipes were available. The substrate was wiped clean with paper towel using circular wiping and allowed to dry before sampling. For Chemgene $HLD_4H$, both pre-impregnated wipes plus paper towel sprayed with the reagent were included, to determine whether there was a difference in cleaning performance. Details of the experimental setup are shown in Table 16.

#### 2.5.4. Experiment 4: single versus double spray/wipe cycles

The impact was assessed of undertaking different spraying and wiping cycles compared with a single round, reflecting the different approaches that were being used by different SARCs, including replication of a previously published SOP for SARC cleaning,[17] as well as a "wipe followed by spray" alternative. All cleaning reagents were assessed, and results compared against Experiment 2. The experimental design and results for Experiment 4 are shown in Table 18.

#### 2.5.5. Experiment 5: impact of different reagent concentrations

Alternative concentrations to those recommended by the manufacturers were assessed to determine the impact on the effectiveness of DNA decontamination. This used the cleaning process in Experiment 2 applied to semen dosed on Formica utilising different concentrations of the cleaning reagents, details of experimental set up are shown in Table 21.

The concentrations of the cleaning reagents used were determined based on the information shown in Table 5.

**Table 5**
Experiment 5. The manufacture recommended concentrations for all of the cleaning reagents used throughout the experiments and the alternative concentrations assessed in Experiment 5, with rational for these concentrations.

| Cleaning reagent | Manufacturer's recommended concentrate | Alternative concentration and reason for choice, tested in Experiment 5. |
|---|---|---|
| Virkon | 1 % | Antec international literature used concentration of Virkon at 3 % as an antibacterial measure |
| Chemgene HDL4H | 10 % for blood and body fluid spills | Manufacturer also suggested 1 % for general bactericidal use |
| Microsol | 5 % spray | Faculty of Forensic & Legal Medicine guidance[18] suggest 10 % is used |
| Selgiene | 10 % as maximum antimicrobial | Manufacturer suggest using 2.5 % as a general cleaner |
| Virusolve | 5 % for heavy contamination | Manufacturer suggest 0.5 % for intermediate contamination |
| Presept | 1250 ppm | Manufacturer suggests 10,000 ppm for occasional use on body fluid spillages. Please note an additional water wash was completed after this concentration as a health and safety and anticorrosion precaution. |

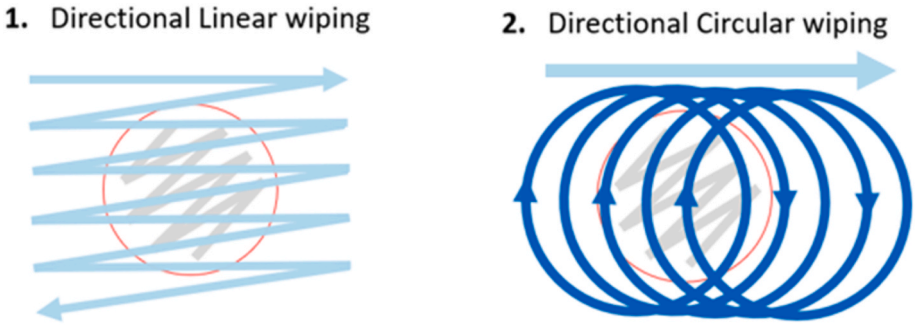


**Fig. 1.** Depiction of the two cleaning mechanical actions assessed in experiment 1.

#### 2.5.6. Experiment 6: impact of different reagent contact times

The impact of varying the time the cleaning reagent was in contact with the body fluid stain was assessed. A contact time of 30 s was utilised in Experiment 2 and this was expanded by repeating for 15 s, 1 min, and 5 min contact times using semen samples only. The experimental design for Experiment 6 is shown in Table 24.

#### 2.5.7. Experiment 7: effectiveness on different substrates

Effectiveness of cleaning different surfaces typically encountered within SARCs was assessed. The cleaning process used in Experiment 2 on Formica was repeated with semen samples spotted on to vinyl and metal surfaces. This required semen controls to be set up on the vinyl and metal, together with substrate negative controls taken from both surfaces. Results were compared directly against Experiment 2 from which the negative control results also applied. The experimental set up for Experiment 7 is shown in Table 25.

#### 2.5.8. Experiment 8: wiping instrumentation/small items

The effectiveness of cleaning instrumentation/small items with different cleaning reagents was assessed, for which wiping rather than spraying is typically utilised within SARCs. 1000 μl of a 1 in 100 dilution of liquid semen in isotonic buffer was applied to gloves and spread over them using the same action that is used to apply a cleaning reagent. The operator then handled a control knob on a colposcope or similar for 5 min. Half the knob surface was swabbed with wet and moist swabs to collect material present. The whole knob was then wiped down and the second half of the knob was then sampled with fresh wet and moist swabs. Chlorhexidine pre-impregnated wipes were included in this experimentation as they are used by one of the FSPs for cleaning small items. The experimental set up for Experiment 8 is shown in Table 26.

#### 2.5.9. Experiment 9: performance of reagents on most challenging body fluid/substrate combination

Experiment 9 assessed the efficacy of all cleaning reagents on the body fluid/substrate combination identified to be the most challenging (semen/vinyl), using the cleaning process identified in Experiment 4 to be the very effective i.e. a double spray/wipe, Table 27 details setup. This experiment included a small assessment of the new Chemgene Medlab on the most difficult combination, which entered the market as the replacement for Chemgene $HLD_4H$ during the study.

#### 2.5.10. Experiment 10: additional mechanical action assessment

This compared the cleaning process applied in Experiment 9 of a double spray/wipe using a circular action against variations of the wiping action. The former process had been identified in Experiment 4 to be very effective and Virkon was used throughout for the same reason. The experimental design is shown in Table 29, where Experiment 10.1 used "multiple unidirectional linear wiping" (Fig. 2) i.e. spray followed by five wipes repeated in one direction only, then this was repeated in a second spray/wipe cycle using a fresh cloth. 10.2 was the same as 10.1 but with turning of the cloth face between each of the five wipes per cycle. 10.3 was the same as 9.4 but with an additional final drying-off of the surface with fresh paper towel after the two spray/wipe cycles.

**Fig. 2.** Depiction of multiple unidirectional linear wiping.

## 3. Results

### 3.1. Experiment 1 results: impact of mechanical action

The full set of results for Experiment 1 are shown in Table 13 and Fig. 3, which include the % yield of semen that remained on the Formica surface post cleaning for both circular and linear cleaning actions using Chemgene $HLD_4H$ or a saline solution.

Average quantification detected was 0.0163 ng/μl after cleaning with Chemgene $HLD_4H$ using a circular action and a higher level of 0.0894 ng/μl using a zig zag linear action, corresponding to yields compared with the untreated control semen of 0.53 % and 2.90 %, respectively. These values contrast with control results obtained after bleach and water control decontamination which averaged 0.0003 ng/μl and a yield of 0.01 %. Conversely, the near spot controls detected low levels of DNA 0.0049 ng/μl after circular action cleaning but zero after linear action and no action controls. In the absence of a definitive difference from this limited assessment, due to the circular action providing a significantly greater reduction of DNA (99.5 %) when compared to linear action (97.1 %) determined by an unpaired *t*-test shown in Table 14, circular action was used for the subsequent Experiments 2–9 and mechanical action was re-visited in Experiment 10.

### 3.2. Experiment 2 results: impact of cleaning reagents on different body fluids

The results from Experiment 2 are shown in Table 15 and displayed in Fig. 4.

The % efficiency of the cleaning treatment (% allele count reduction compared to the control sample) is summarised in Table 6 applying the risk level criteria from the Cellmark EM Indicator Guide with green and amber levels denoting "pass" results, and red denoting a "fail".

Only Presept was efficient at removing DNA contaminant from all three body fluids (blood, semen and saliva) from the Formica work surface. Selgiene and Virkon gave the next best results overall. Presept was above a 95 % efficiency for the removal of DNA (based on allele count reduction) for all body fluids evaluated on the work surface, therefore less than 5 % DNA recovery following decontamination of the work surface with Presept.

#### 3.2.1. Blood contaminant

All of the cleaning reagents evaluated were shown to be effective at removing contaminant DNA from the dried blood staining seeded onto the work surface, based on the EM Indicator Guide the results would be classed as 'Pass'. The least efficient decontamination strategy was Chemgene $HLD_4H$, with recoveries of 0.38 % following cleaning. All other cleaning reagents evaluated were highly efficient in removing DNA with recoveries of 0.00 %.

#### 3.2.2. Semen contaminant

The cleaning of semen results suggest the least efficient decontamination strategies were Chemgene $HLD_4H$ and Virusolve for the removal of DNA from dried semen stains, with recoveries of 0.04 % and 0.05 % respectively, following cleaning. The other cleaning reagents evaluated (Virkon, Microsol, Selgiene, and Presept) were more efficient in removing DNA from dried semen stains with recoveries between 0.00 and 0.02 %.

When compared to Cellmarks EM indicator (Table 3) work surface decontamination using Virusolve, produced high-level DNA results (DNA profiles with allele counts above 25+ which would be classified as 'fail') indicating that significant DNA levels were detected following decontamination of the work surface with this cleaning reagent.

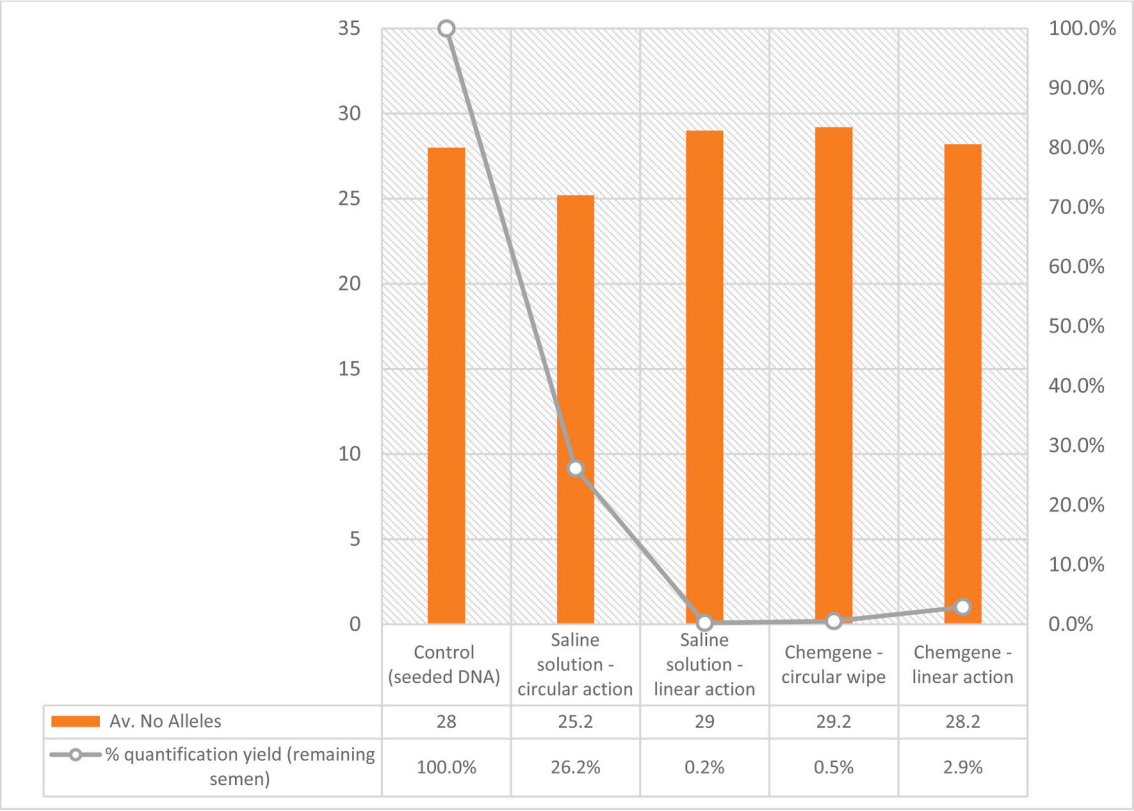


**Fig. 3.** Experiment 1 results for the assessment of different cleaning actions on a Formica surface dosed with semen using a saline solution or Chemgene $HLD_4H$ with a white paper cloth. Results are presented in allele count and % yield.

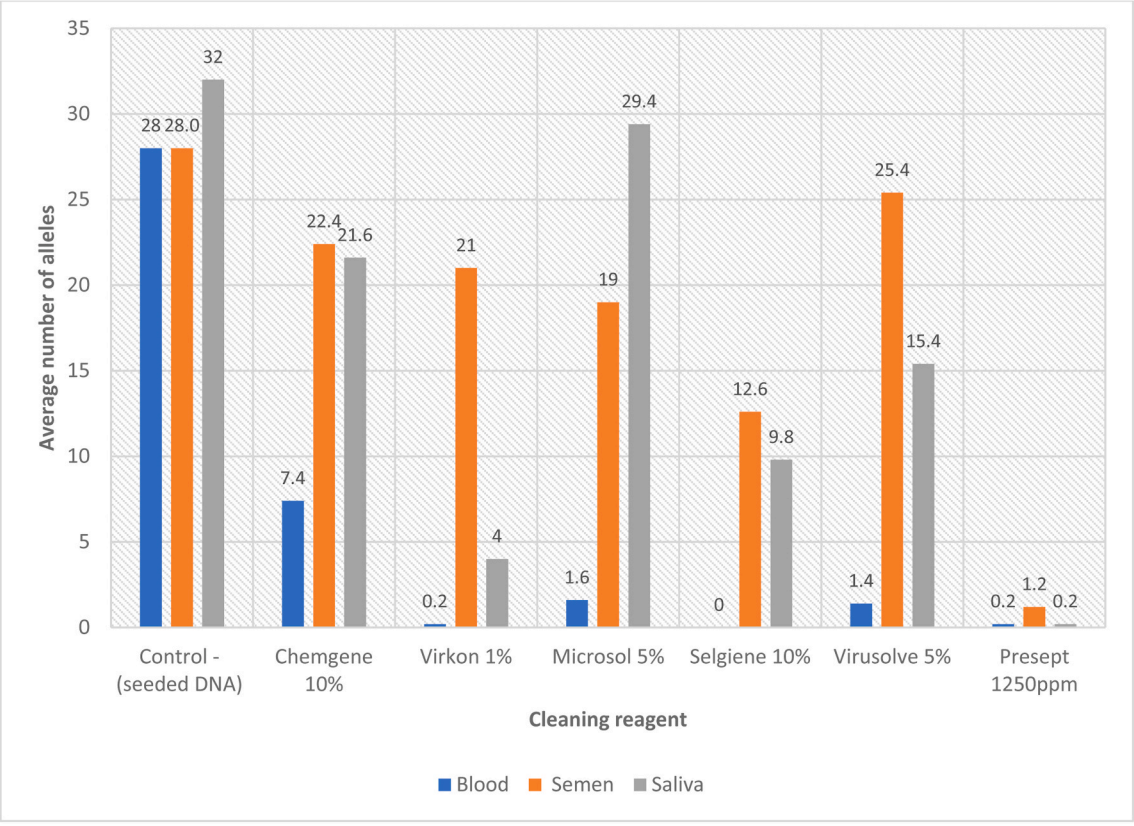


**Fig. 4.** Experiment 2. Graph of the average allele count of the remaining body fluids (blood, semen and saliva) following cleaning the seeded Formica surface using six different cleaning products, including control samples (without cleaning).

**Table 6**
Experiment 2. Summary of results for the assessment of the effectiveness of cleaning reagents on different body fluids: single spray/wipe cycle of semen on Formica. Results have been coloured as per their RAG rating using Cellmark's EM indicator Guide.

| | Blood | | Semen | | Saliva | | |
|---|---|---|---|---|---|---|---|
| | Average Allele count | % Allele reduction | Average Allele count | % Allele reduction | Average Allele count | % Allele reduction | |
| Presept 1250 ppm | 0.2 | 99% | 1.2 | 96% | 0.2 | 99% | Top performers |
| Selgiene 10% | 0.0 | 100% | 12.6 | 55% | 9.8 | 69% | |
| Virkon 1% | 0.2 | 99% | 21.0 | 25% | 4.0 | 88% | |
| Virusolve 5% | 1.4 | 95% | 25.4 | 9% | 15.4 | 52% | |
| Microsol 5% | 1.6 | 94% | 19.0 | 32% | 29.4 | 8% | |
| Chemgene $HLD_4H$ 10% | 7.4 | 74% | 22.4 | 20% | 21.6 | 33% | |

Chemgene $HLD_4H$, Virkon, Microsol and Selgiene DNA profiles generated were moderate-level results (DNA profiles with allele counts between 11 and 24), which would be classified as a 'Pass', but indicative that low levels of DNA were detected in the associated samples following cleaning. Presept was identified to be the most effective cleaning reagent for its ability to remove DNA contamination (from dried semen staining) from the work surface; based on the results from this study, an average of just 1.2 alleles were detected following cleaning. Based on the EM Indicator Guide the results would be classed as 'Pass', indicating that the cleaning/anti-contamination procedure had been effective.

### 3.2.3. *Saliva contaminant*

The cleaning of saliva results showed that the least effective reagents were Microsol and Chemgene $HLD_4H$ for the removal of DNA from dried saliva stains, with recoveries of 0.44 % and 0.17 % respectively. The other cleaning reagents evaluated (Virkon, Selgiene, Virusolve, and Presept) were more effective at removing DNA from dried saliva stains with recoveries between 0.00 and 0.05 %.

Following work surface decontamination with Chemgene $HLD_4H$ and Virusolve, the associated DNA profiles were moderate-level results (DNA profiles with allele counts between 11 and 24). Based on the EM Indicator Guide the results would be classified as a 'Pass', but indicative that low levels of DNA were detected in the associated samples following execution of the cleaning strategies.

The most effective cleaning reagents evaluated for the removal of DNA contaminant from dried saliva were found to be Selgiene, Virkon and Presept. Low-level DNA results were produced (DNA profiles with allele counts between just 0–10). Based on the EM Indicator Guide the results from all three of these cleaning reagents would be classified as a 'Pass,' indicating that all of the cleaning/anti-contamination procedures have been effective at removing contaminant DNA (from dried saliva stains) seeded onto a Formica work surface.

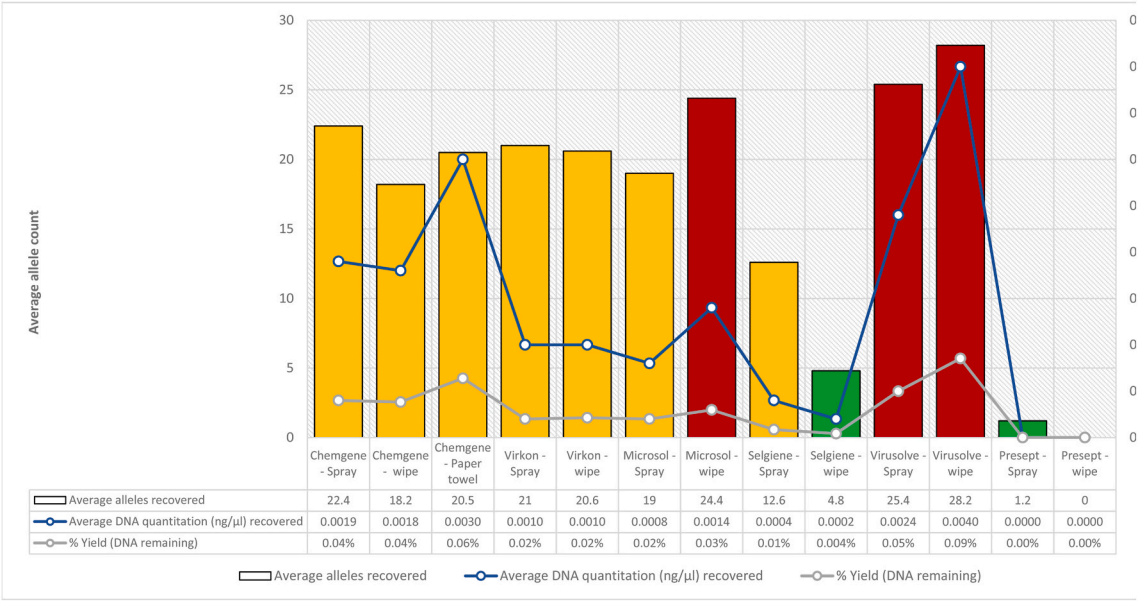


**Fig. 5.** Experiment 3. Graph of Experiment 3 results to show allele count and % reduction to determine the decontamination efficiencies of spraying/wiping versus use of pre-impregnated wipes using Cellmark EM Indicator Guide-RAG criteria.

**Table 7**
Experiment 4. Summary of results for the assessment of a single spray/wipe cycle versus double spray/wipe cycles. Results are shown as allele count and % allele reduction for the cleaning of semen stains on Formica (results coloured using Cellmark's EM Indicator Guide -RAG criteria).

| | | Presept 1250 ppm | Selgiene 10% | Virkon 1% | Virusolve 5% | Microsol 5% | Chemgene $HLD_4H$ 10% |
|---|---|---|---|---|---|---|---|
| Single spray/wipe | Average Allele count | 1.2 | 12.6 | 21 | 25.4 | 19 | 22.4 |
| | % allele reduction | 96% | 55% | 25% | 9% | 32% | 20% |
| Duplicate spray/wipe | Average Allele count | 1.2 | 9 | 0.4 | 7 | 11.2 | 7.2 |
| | % allele reduction | 96% | 68% | 99% | 75% | 60% | 74% |
| Chemgene $HLD_4H$ Spray/wipe + water/wipe dry | Average Allele count | N/A | | | | | 3.6 |
| | % allele reduction | N/A | | | | | 87% |
| Chemgene $HLD_4H$ Spray/wipe + chemical mist | Average Allele count | N/A | | | | | 6 |
| | % allele reduction | N/A | | | | | 79% |
| Virkon duplicate spray/wipe + wipe dry | Average Allele count | N/A | | 0 | N/A | | |
| | % allele reduction | N/A | | 100% | N/A | | |

### 3.3. *Experiment 3 results: comparison of the effectiveness of spraying and then wiping versus wiping with a pre-impregnated wipe*

When considering the most effective cleaning reagent overall for the removal of DNA (from dried semen) from Formica, the average allele count of the profiles generated after cleaning with each cleaning reagent and each strategy (spray vs wipes) was compared to the control samples (seeded surface not cleaned) to calculate the % efficiency (allele count reduction). This is summarised in Fig. 5, scored according to Cellmark EM Indicator Guide-RAG criteria, the full set of results for Experiment 3 are provided in Table 16.

The decontamination efficiencies (% allele count reduction) following cleaning was then compared. Only Presept obtained above a 95 % efficiency for reducing the allele count using both spray and wipe strategies. Selgiene was the next most efficient at reducing the allele count with 83 % efficiency using the reagent wipe and 55 % using the reagent spray. Again, there were overall differences observed in the efficiency of DNA removal between the different cleaning reagents and whether they were sprayed or wiped (using the pre-impregnated wipes) but the differences **were not** significantly different statistically as shown

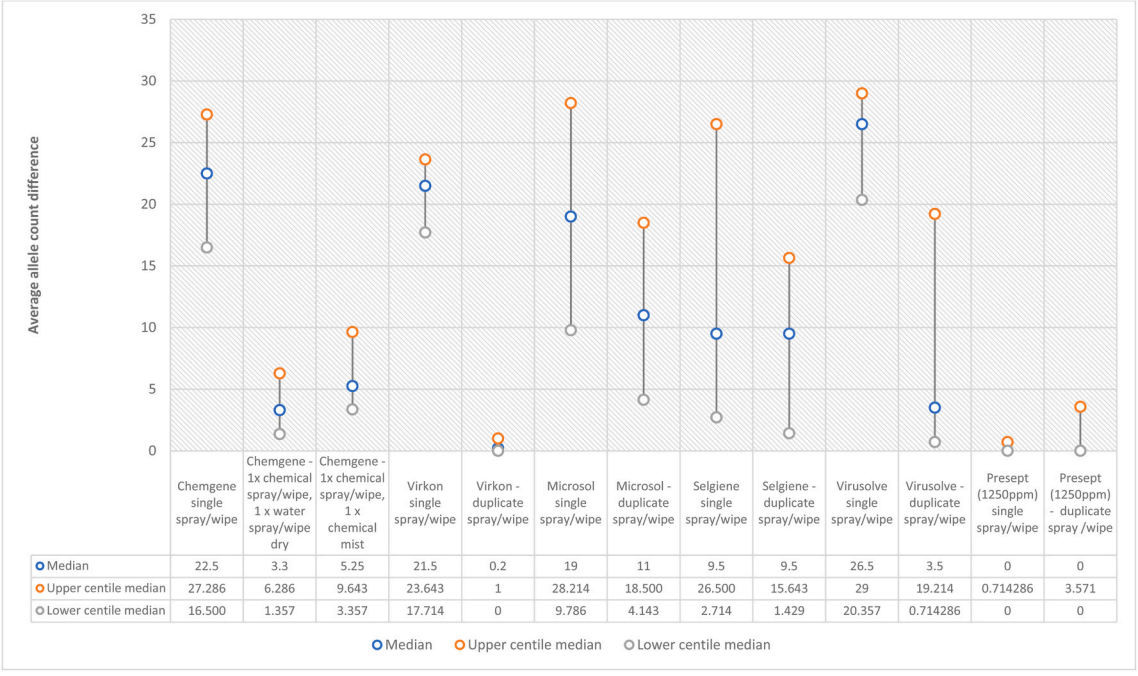


**Fig. 6.** Experiment 4. Kruskal-Wallis Nonparametric ANOVA statistical analysis for each of the six cleaning products for the assessment of a single spray/wipe strategy versus a duplicate spray/wipe.

**Table 8**
Experiment 5. Summary of results for the assessment of different cleaning reagent concentration presented as allele count and % Allele reduction. The reagent concentrations used are shown in Table 5 (using Cellmark's EM Indicator Guide- RAG criteria).

| | Lower concentration | | Higher concentration | |
|---|---|---|---|---|
| | Average Allele count | % allele reduction | Average Allele count | % allele reduction |
| Presept | 1.2 | 96% | 1.8 | 94% |
| Selgiene | 28.2 | 0% | 12.6 | 55% |
| Virkon | 21.0 | 25% | 17.0 | 39% |
| Virusolve | 15.6 | 44% | 25.4 | 9% |
| Microsol | 19.0 | 32% | 25.2 | 10% |
| Chemgene $HLD_4H$ | 9.8 | 65% | 22.4 | 20% |

in Table 17.

### 3.4. Experiment 4 results: duplicate spray wipe cycles compared with single spray wipe cycles

The % efficiency (allele reduction) of each cleaning reagent strategy (duplicate spray/wipe) at reducing the allele count in the resulting DNA profiles is summarised Table 7.

The findings demonstrate that in general, when a duplicate spray/wipe cycle decontamination strategy was adopted, for all of the cleaning reagents evaluated **there was a significant reduction in recoverable DNA** from semen body fluid stains when compared to both i) the controls of body fluid stains not subject to cleaning (seeded DNA – no treatment controls) and ii) the results from Experiment 2 whereby a single spray/wipe cycle strategy was followed. The statistical analysis of this data is available in Table 19. In addition to Presept, Virkon obtained above a 95 % efficiency for reducing allele count using both cleaning strategies (duplicate spray/wipe, as well as duplicate spray/wipe and wipe dry).

The average allele count difference following cleaning using a single spray and wipe strategy versus using a duplicate spray and wipe strategy for each of the 6 cleaning reagents was statistically analysed using a variant of the ANOVA test, the Kruskal – Wallis Nonparametric ANOVA Table 20. Based on these results there was a statistically significant difference when comparing the average number of alleles remaining following cleaning using a duplicate spray/wipe strategy for the cleaning reagents Chemgene $HLD_4H$, Virkon and Virusolve when compared to a single spray/wipe strategy.

This analysis of the data illustrated in Fig. 6 shows that the mean allele count following cleaning with each of the cleaning products reduced following a second spray/wipe strategy, with the exception of Precept where the mean allele count was consistently 0 following a single spray/wipe and a duplicate spray/wipe strategy. There is a general trend that the level of uncertainly and consistency in the results increases with a second spray/wipe with the exception of Virusolve where the level of accuracy reduced by over half following a second spray/wipe.

### 3.5. Experiment 5 results: impact of different reagent concentrations

Results are shown in Table 21 and statistical analysis of the results provided in Tables 22 and 23, all six reagents demonstrated a reduction

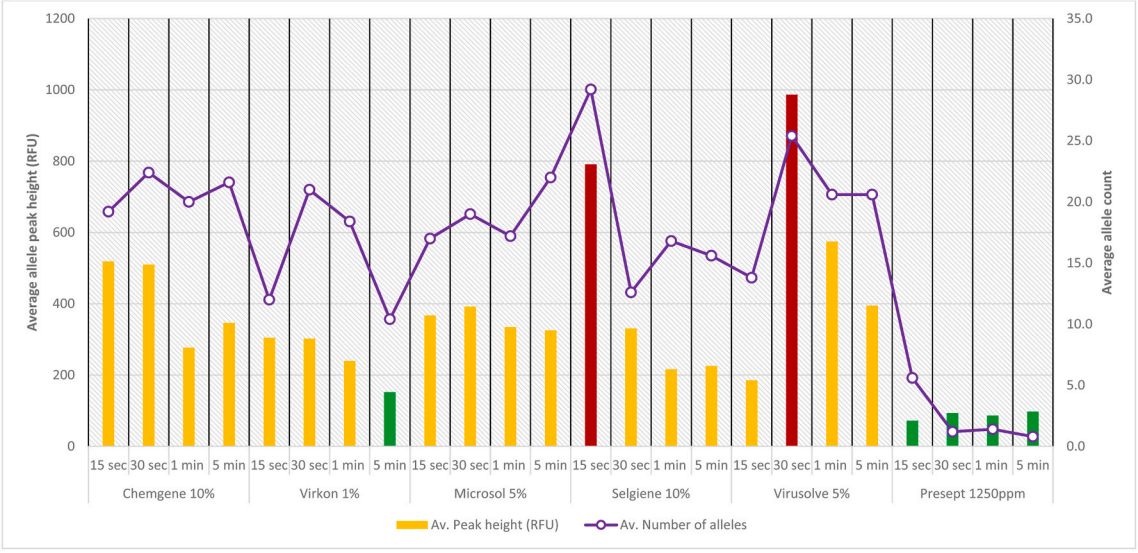


**Fig. 7.** Experiment 6. Graph of results to show a comparison of average allele count and peak heights (RFU) for different contact times (15 s, 30 s, 1 min and 5 min) of the cleaning reagent with semen seeded on to a Formica surface.

**Table 9**
Experiment 7. Summary of results on the effectiveness of cleaning on different substrates (Formica, Metal and Vinyl) as average allele count and % allele reduction, results coloured as per an assessment against the Cellmark EM Indicator Guide -RAG criteria.

| | Substrate - Formica | | Substrate - Metal | | Substrate - Vinyl | |
|---|---|---|---|---|---|---|
| | Average Allele count | % allele reduction | Average Allele count | % allele reduction | Average Allele count | % allele reduction |
| Presept 1250 ppm | 1.2 | 96% | 0.4 | 99% | 14.8 | 50% |
| Selgiene 10% | 12.6 | 55% | 28.4 | 3% | 29.4 | 0% |
| Virkon 1% | 21.0 | 25% | 15.8 | 46% | 25.6 | 13% |
| Virusolve 5% | 25.4 | 9% | 29.2 | 0% | 29.0 | 1% |
| Microsol 5% | 19.0 | 32% | 29.2 | 0% | 30.8 | -5% |
| Chemgene $HLD_4H$ 10% | 22.4 | 20% | 7.0 | 76% | 22.0 | 25% |

in recoverable DNA from semen following cleaning. These results were compared with those using default manufacturers' recommended concentrations (in Experiment 2) and are combined and summarised in Table 8.

Decontamination with Presept at 1 % and 3 % was identified as the most efficient, yielding a 96 % and 94 % respectively for the reduction in allele count. Selgiene, when tested at the lower concentration (of 2.5 %), was found to be the least efficient cleaning reagent tested for removing the DNA contaminant from the work surface. It obtained an overall efficiency calculation for the reduction in allele count of 0 % based on the results of this study but gave the second-best results when used at the higher concentration recommended by the manufacturer. Overall, no statistically significant improvements were observed in changing the concentrations of the reagents from the default manufacturers' instructions (Table 5) and given that the latter are based on optimisation of other factors that are also required of the cleaning reagents. i.e disinfection performance, it is therefore recommended to follow the manufacturer's instructions and ensure the concentration is suitable for the required use.

### 3.6. *Experiment 6 results: impact of different reagent contact times*

DNA levels were determined for 15 s, 30 s, 1 min and 5 min reagent contact times with the Formica surface for all six cleaning reagents. Plotted in Fig. 7 is a comparison of allele counts over time, scored using the Cellmark's EM Indicator Guide -RAG criteria, combining data from Experiment 6 and Experiment 2. Table 24 provides further results detail for comparison of DNA concentrations and % yield which demonstrate broadly the same pattern. The average peak height (RFU) was also calculated for each of the chemical reagents tested at all of the different

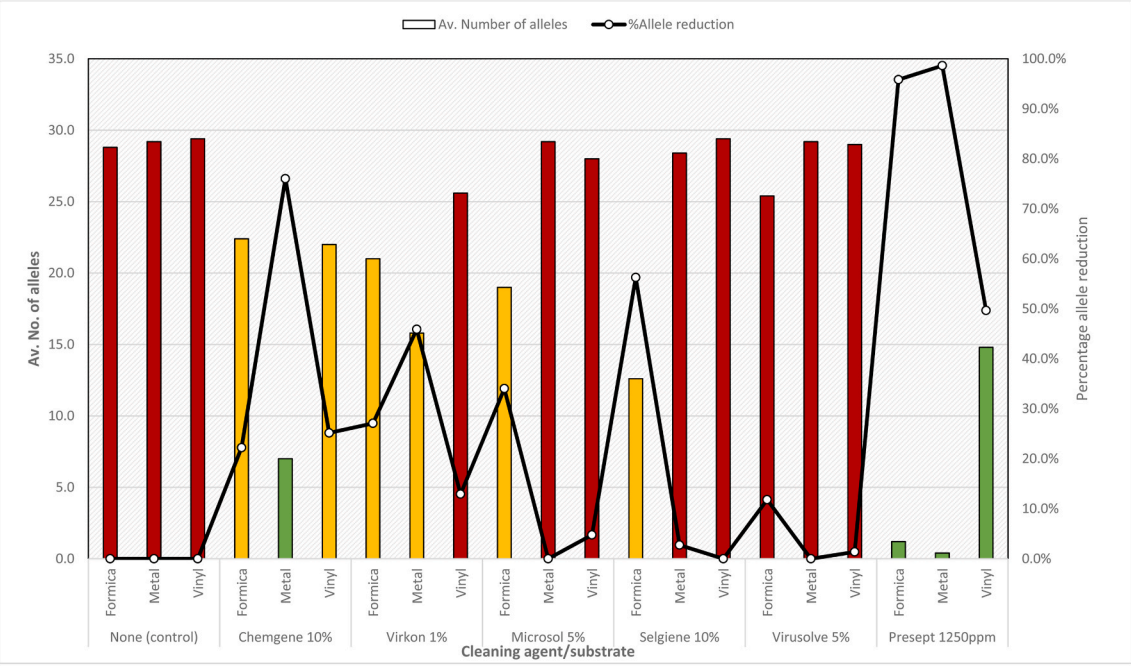


**Fig. 8.** Experiment 7. Graph of number of alleles and % allele reduction results for the assessment of different cleaning reagent/substrate combinations, all conducted using a single spray/wipe strategy.

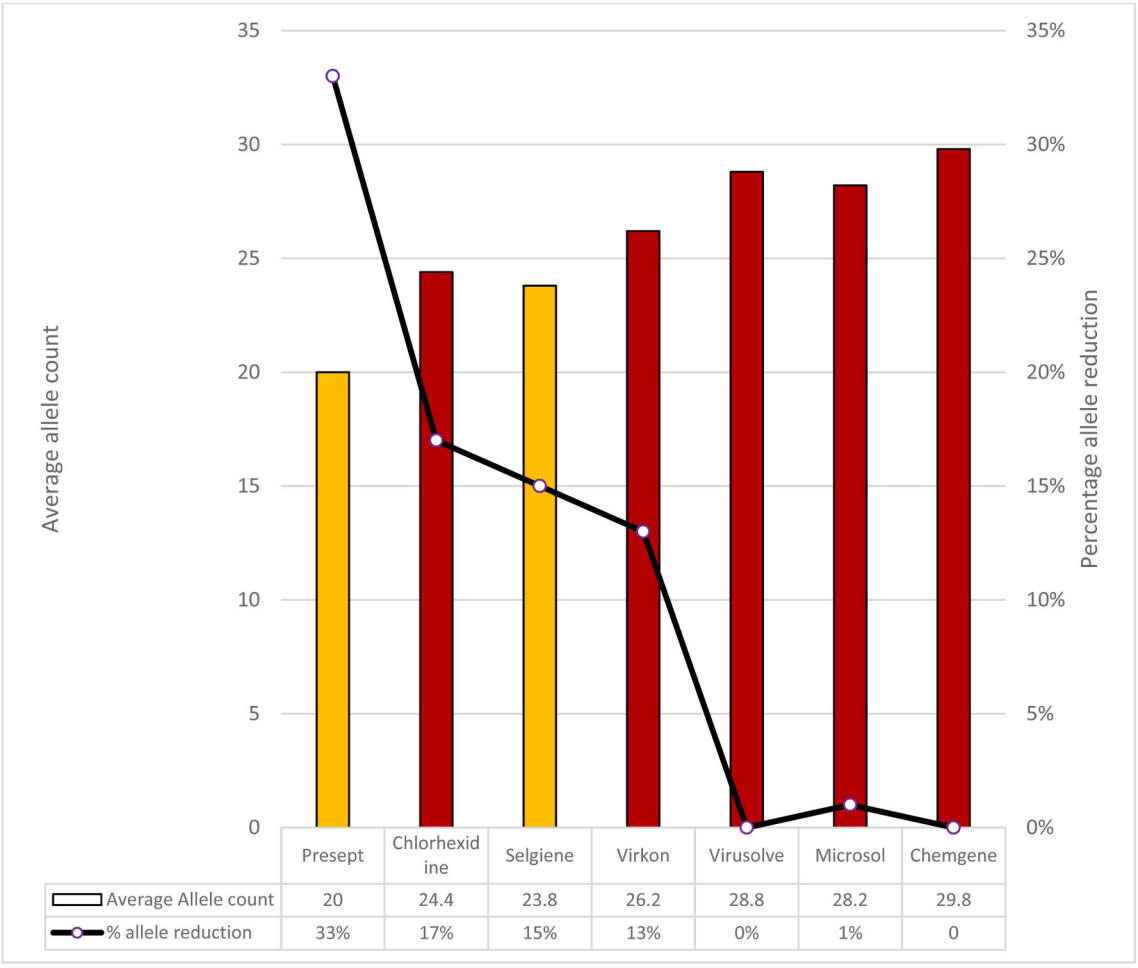


**Fig. 9.** Experiment 8. Average allele count results for cleaning sensitive instrumentation and % allele count reduction results following cleaning with the six different reagents. The colours of the bars indicate the relative risk category as per the Cellmark EM Indicator Guide-RAG criteria.

contact times with semen (shown in Fig. 7). The average peak heights were found to be representative of the DNA profiles generated.

Based on the results from this study only Presept was found to be highly efficient at removing the contaminant from the work surface at each of the contact times evaluated with recoveries of 0.01 % for 15 s and 0 % when the contact time was 30 s, 1 min and 5 min. Only 2/6 reagents had a greater % allele reduction at 5 min compared to the other times, which ranged from 1.4 % to 5.6 %. Overall, there was no clear

**Table 10**
Experiment 9: Summary of EM Guide Risk Levels for single vs double spray/wipe cleaning.

| | Single spray/wipe | | | | Duplicate spray/wipe | | | |
|---|---|---|---|---|---|---|---|---|
| | Total Av DNA ng/µl | Av. Allele Count | Cellmark EM Guide Risk Level | FSR EM Guide Risk Level | Total Av DNA ng/µl | Av. Allele Count | Cellmark EM Guide Risk Level | FSR EM Guide Risk Level |
| **Presept 1250 ppm** | 0.0005 | 14.8 | | | 0.0000 | 1.0 | | |
| **Virkon 1%** | 0.0036 | 25.6 | | | 0.0025 | 12.2 | | |
| **Selgiene 10%** | 0.0122 | 29.4 | | | 0.0042 | 24.0 | | |
| **Chemgene HDL$_4$L 10%** | 0.0096 | 22.0 | | | 0.0056 | 28.6 | | |
| **Microsol 5%** | 0.0415 | 30.6 | | | 0.0125 | 28.6 | | |
| **Virusolve 5%** | 0.0073 | 29.0 | | | 0.0140 | 30.2 | | |
| **Chemgene Medlab 10%** | 0.0463 | 28.6 | | | 0.024 | 28.6 | | |

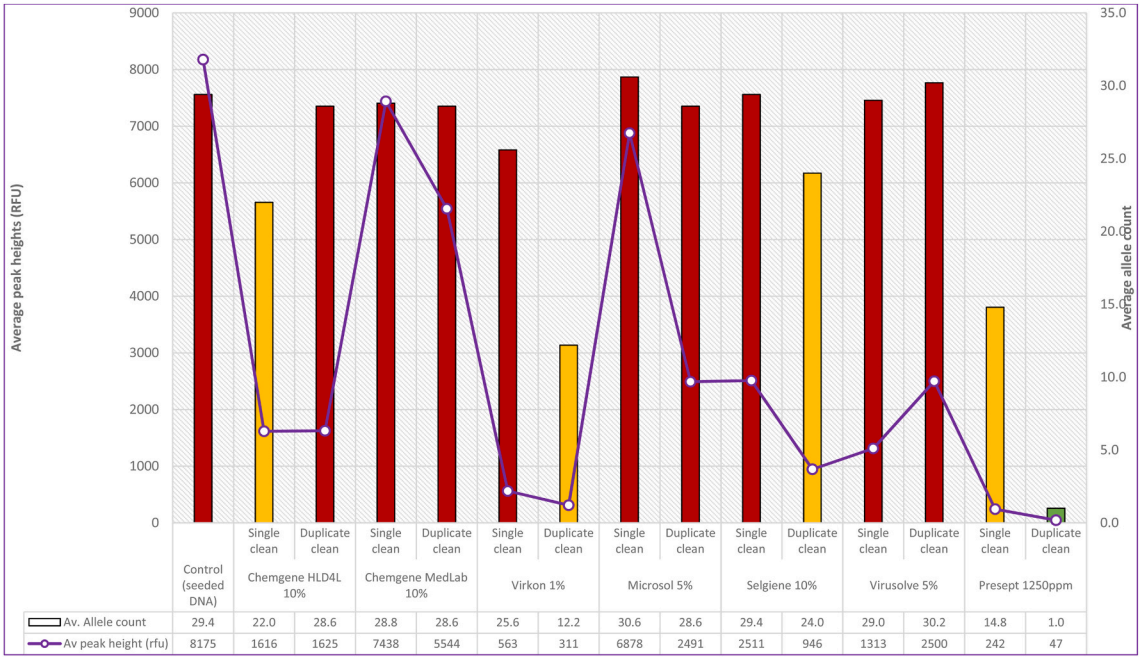


**Fig. 10.** Experiment 9: Graph of results shown as average residual DNA allele peak height after single and duplicate spray/wipe.

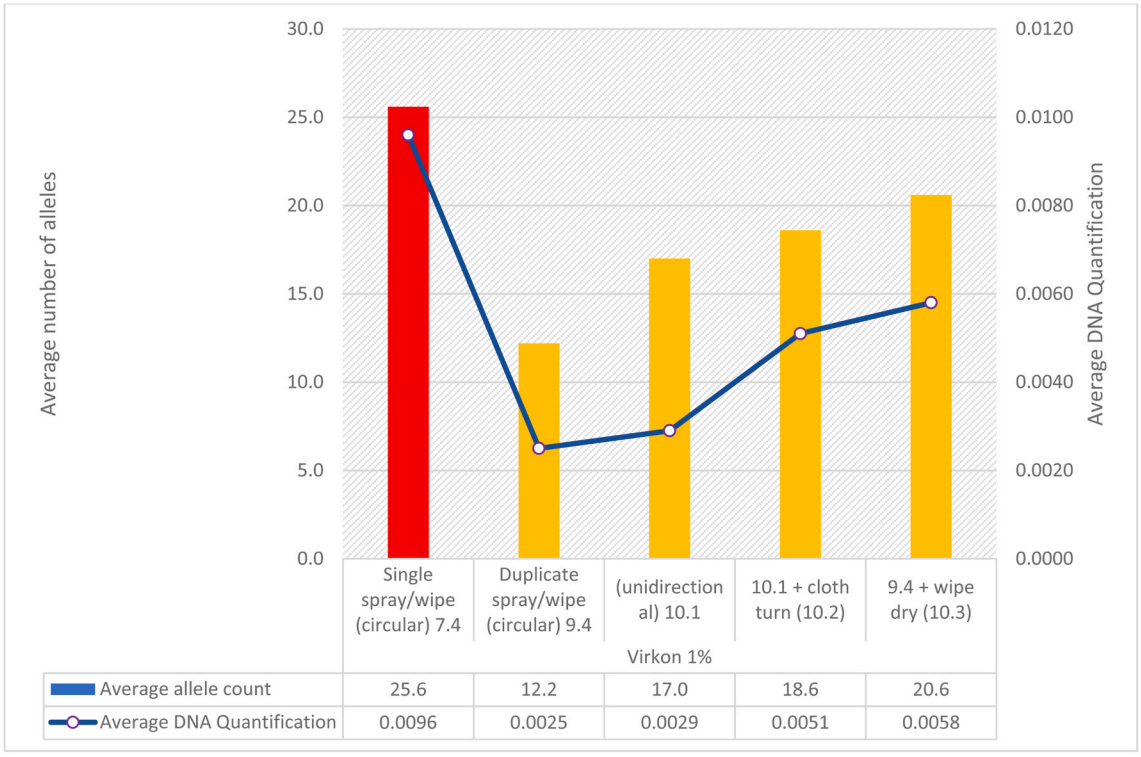


**Fig. 11.** Experiment 10. Average allele and average DNA quantification (ng/μl) results of different wiping actions.

indication that increasing the cleaning reagent contact time with the semen stain to 5 min with the cleaning reagents tested significantly improved the decontamination efficiency.

An assessment of the results against the Cellmark EM Indicator Guide found that at 15 s contact time 5/6 cleaning reagents had an average allele count within the amber range for a 'pass', with Selgiene providing a red 'fail' result. At 30 s 5secs 5/6 had an average allele count within the amber EM RAG range for a 'pass' with the exception of Virosolve providing a red 'fail' result and 2/5 cleaning reagents (Presept and Selgiene) had a greater % allele reduction at 30 s contact time opposed to 15 s.

Based on these results it seems reasonable to recommend a 'best practice' reagent contact time of approximately 30 s following spraying before wiping, for the best results. Although 15 s did provide equally effective results, a recommendation of approximately 30 s as a precautionary measure provides a more forgiving, pragmatic approach

allowing for natural deviations up to 15 s.

### 3.7. Experiment 7 results: effectiveness on different substrates

As before, the % efficiency (allele count reduction) was used to estimate the ability of each cleaning reagent to remove contaminant DNA from each of the tested work surfaces, results are shown in Table 25 and summarised in Table 9 and Fig. 8.

The decontamination efficiencies (% allele count reduction) following cleaning were compared. When considering the effectiveness of cleaning different surfaces typically encountered within SARCs only Presept was identified as consistently being above a **95 %** efficiency for the reduction in allele count from both the Formica and metal work surfaces. The decontamination efficiency reduced to **50 %** using Presept on the vinyl work surface, which suggests that it is more difficult to decontaminate this type of work surface, possibly due to the semi-porous nature and absorbency of the substrate.

Overall, the least effective cleaning reagents tested for reducing the allele count of the contaminant DNA from the metal and vinyl work surfaces were Microsol (metal 0 %, vinyl −5 %), Selgiene (metal 3 %, vinyl 0 %) and Virusolve (metal 0 %, vinyl 1 %).

A % allele reduction of −5 % from cleaning vinyl with Microsol is likely due to drop in, a term applied to a small numbers of low level short tandem repeats (STR) alleles, attributed to sporadic contamination. The difference between the control and Microsol on vinyl result is 1.4 alleles, not sufficient for any DNA comparisons.

### 3.8. Experiment 8 results: wiping instrumentation/small items

As previously, the % efficiency (allele count reduction) was used to estimate the ability of each cleaning reagent to remove contaminant DNA from each of the tested plastic handle surfaces. The full set of results are shown in Table 26 and the RAG status results are summarised in Fig. 9 (using Cellmark's EM Indicator Guide-RAG criteria).

The decontamination efficiencies (% allele count reduction) of cleaning with each of the cleaning reagents, using a wiping method to simulate the approach used for cleaning sensitive instrumentation at SARCs, were compared. Based on the results from this study, the decontamination efficiency was highest **(33 %)** using Presept, and ~ (15–17 %) using Selgiene and Chlorhexidine. The profiles were quite strong for all reagents which indicates it is more difficult to decontaminate contoured plastic surfaces than flat vinyl, metal or Formica surfaces, although it should be noted that the seeding levels used throughout these experiments is far greater than what would realistically be expected in a practical SARC forensic medical examination room setting and therefore challenging.

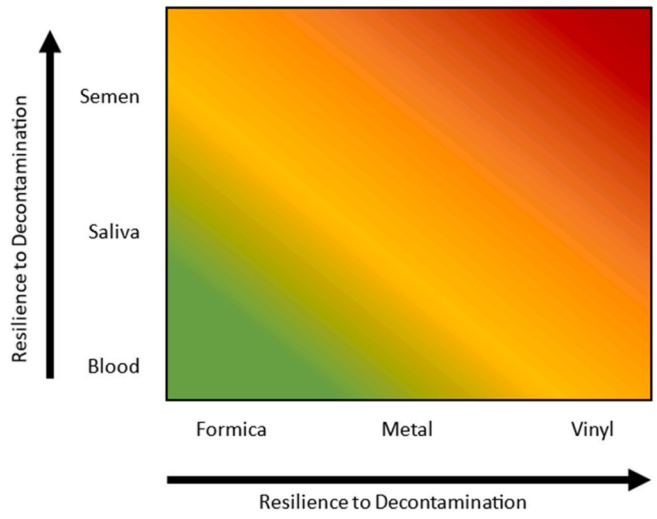


**Fig. 12.** – General schematic of resilience to DNA decontamination for different substrate and body fluid combinations.

### 3.9. Experiment 9 results: performance of reagents on most challenging body fluid/substrate combination

The remaining DNA sample results from single and double spray/wipes of semen stains on vinyl were scored using both the Cellmark's EM Indicator Guide and the criteria published in FSR SARC guidance[13] (Table 3), both of which utilise combined allele score values to assign green, amber and red risk levels. This is summarised in Table 10 and full results shown in Table 27, which demonstrates a slightly different categorisation of results between the two scoring systems.

As expected from Experiment 7 results, it is difficult to remove contaminant DNA (dried semen) from a vinyl work surface, possibly due to the semi-porous nature and absorbency of the substrate. All reagents demonstrated a reduction in average DNA recovered.

When Presept, Microsol and Selgiene cleaning reagents (duplicate spray/wipe cycle strategies) were compared to the results obtained from Experiment 7 (single spray/wipe cycle strategy) on the vinyl work surface the results were found to be statistically significant when tested using a student's *t*-Test (results shown in Table 28). The results were not statistically different for the other cleaning products, even following a duplicated spray/wipe cycle strategy work surface decontamination, the cleaning reagents (Chemgene $HLD_4H$, Microsol, and Virusolve) generated high-level DNA results (producing DNA profiles with allele counts above 25+).

Chemgene Medlab which will likely replace Chemgene $HDL_4L$ in the future was included in this experiment for comparison. A students t-test (Table 28) determined that the difference in quantification score between the two reagents in both the single spray/wipe and double spray wipes strategy assessments are statistically significantly different, despite similar allele count results. The average peak height (RFU) was also calculated for each of the cleaning strategies and were found to be representative of the DNA profiles generated (Fig. 10). There is a distinct reduction in average peak height for most of the cleaning reagents tested using the duplicate spray/wipe cycle strategy.

### 3.10. Experiment 10 results: additional mechanical action assessment

All tested duplicate spray/wipe cycle 'mechanical action' strategies assessed using the cleaning reagent Virkon, demonstrated a comparable and significant reduction (>99.9 %) in semen DNA contamination from the vinyl work surface. Results shown in Table 29 and Fig. 11.

It is recognised that this was a limited study, without a definitive differentiation between approaches. It seems reasonable to recommend a 'best practice' mechanical action appropriate to the surface and the size of the work surface to be cleaned. This is likely to be a combination of circular and linear cleaning, with regular cloth changes, combined with a duplicate spray/wipe cycle strategy.

## 4. Discussion

In summary, the results of this study demonstrated differences in the DNA removal efficiencies between different cleaning reagents and strategies, as well as between the different surfaces tested and the biological material being removed. The results have supported conclusions from previous studies[5–7,19–22] that Presept and Virkon are highly efficient, while others were less efficient in removing DNA contamination from work surfaces.

**Mechanical action:** In the limited study conducted, directional circular motion gave statistically significantly better results than zig zag motion (Table 14) in Experiment 1, when sampling directly from the seeded area but appeared to be dispersing the contamination rather than necessarily removing more from the surface overall compared with zig-

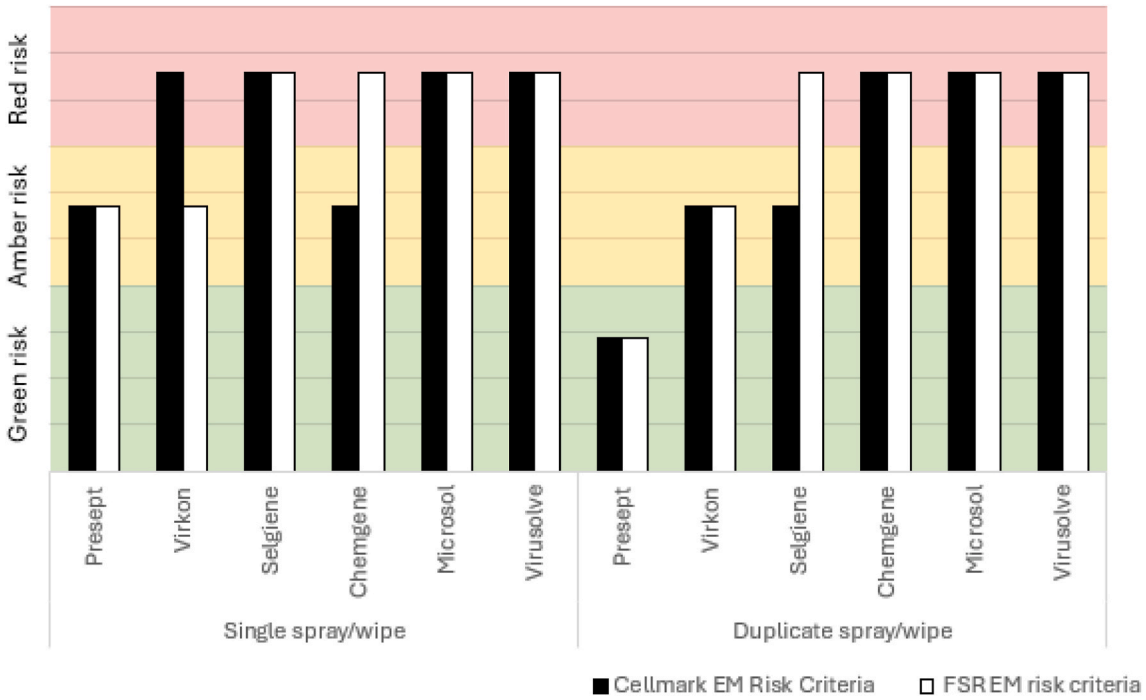


**Fig. 13.** Experiment 9: A graph to show the six cleaning reagents using a single spray/wipe strategy and a double spray/wipe strategy displayed against Cellmark and the FSR EM Indicator Guide-RAG criteria (shown in Table 3).

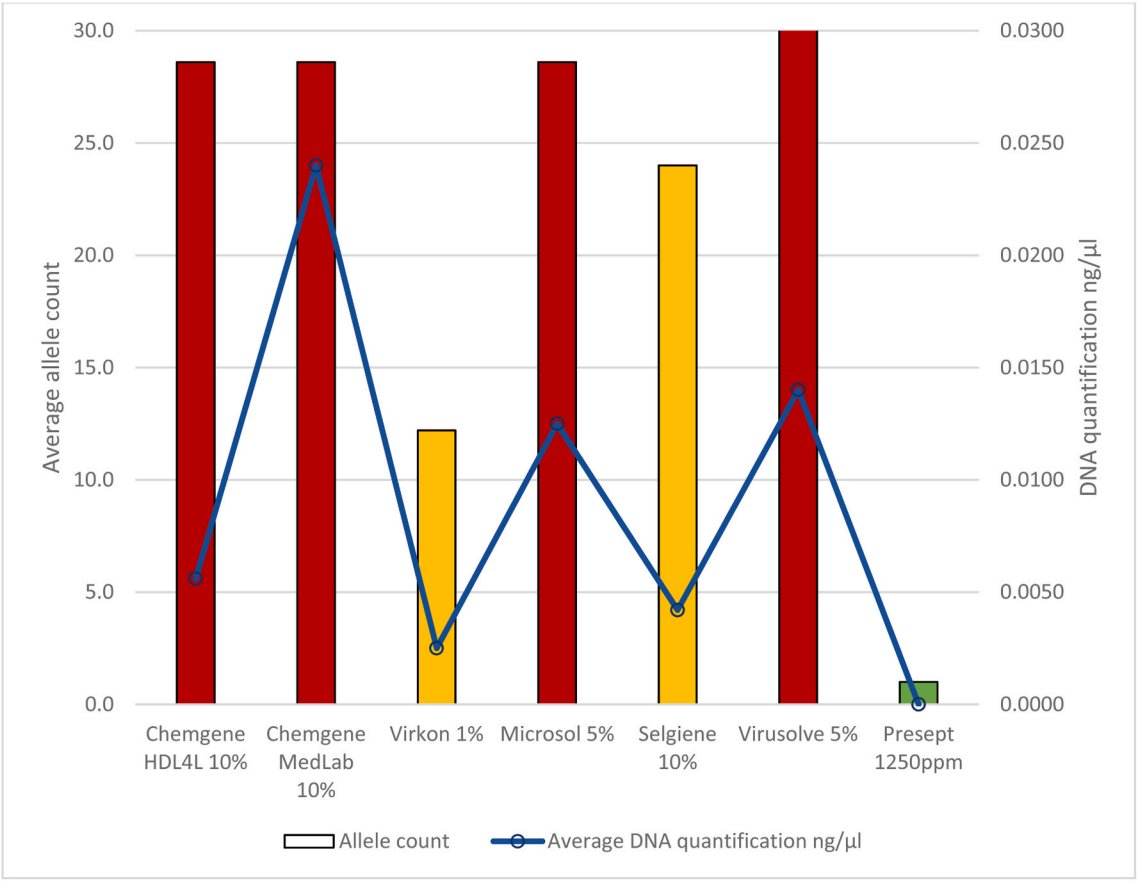


**Fig. 14.** Experiment 9. Graph to show the average allele count and reduction in DNA quantification for all cleaning products used on vinyl with a 30 s double spray/wipe strategy.

zag. The follow-up exercise (Experiment 10) demonstrated that linear wiping and linear wiping with changes of the contact surface of the cloth also gave good cleaning results, comparable to circular wiping. Given testing was limited to cleaning a flat wide surface, choice of motion may be best guided by the shape of the item being cleaned, so for instance directional circular is suitable for wider flat surfaces whilst a linear cleaning action with frequent changes of wiping surface may be more suitable for a long thin surface.

**Cleaning strategies** can significantly influence the efficiency of removing the contaminant DNA during the cleaning process (Tables 19 and 20). The most successful decontamination strategy identified in the study was the adoption of a duplicate cleaning reagent spray/mechanical wipe action to enhance the removal of DNA from the work surface. When applying this strategy, the decontamination efficiency improved for all cleaning reagents evaluated as demonstrated in Experiment 4 which assessed removal of semen from Formica. On a single wash/wipe cycle, 1 out of 6 of the reagents gave a "green, pass" score i.e. 10 or fewer alleles detectable after cleaning; four reagents gave an "amber, pass" of 24 alleles or less and one reagent gave a "red, fail" score of more than 24 alleles, based on the Cellmark EM Indicator Guide-RAG criteria. However, results with all reagents improved when a double spray/wipe was used: 5 out of 6 gave "green, pass" scores and one was an "amber, pass" score. Therefore, **all** cleans achieved acceptable cleanliness levels when combined with a double wash/wipe cycle, with Presept and Virkon being the most effective on this particular combination of body fluid and surface. This observation correlates well with previous studies[5–7,19–22] in which bleach/hypochlorite and Virkon were determined to be the most effective against cell-free DNA and blood stains, respectively.

**Contact times and concentrations** of reagents sprayed onto surfaces were assessed in Experiment 6 and Experiment 5, respectively. A range of 15 s to 5 min contact time was assessed. No overall benefit was observed when using longer periods, therefore a 'best practice' reagent contact time of approximately 30 s following spraying before wiping is recommended. Although a reagent contact time of 15 s proved to be equally effective, a recommendation of approximately 30 s allows for some slack in operator error providing a more pragmatic approach.

Assessment of the impact of varying reagent concentration did not identify any significant improvements in performance compared with the manufacturers' instructions. Therefore, the recommendation is to follow the latter and ensure that the concentration is suitable for the required use.

**Differences in decontamination efficiencies varied depending on the body fluid** selected to artificially contaminate the work surface, as demonstrated in Experiment 2 using a Formica surface with a single spray/wipe cycle. When blood was used as the contaminant, all six tested cleaning reagents were deemed highly effective at removing the contaminant DNA from the work surface with all achieving a "green, pass" indicator score. With saliva, the resultant indicator scores were three green, two amber and one red, and with semen the indicator values were one green, four amber and one red for the six cleaning reagents. Therefore, on balance semen proved to be the hardest to remove and hence this was used in the other evaluative experiments as a worst-case scenario.

**Variances in the efficiency of cleaning different substrates** were also apparent with Formica being the easiest to clean regardless of the cleaning reagent used, whilst metal was more problematic, and vinyl was the hardest to decontaminate of the three substrates. Therefore, there is a spectrum of decontamination resistance depending on the combination of surface and body fluid, illustrated in Fig. 12, where red is the most challenging combination and green the least challenging.

Fig. 12 ranges from blood on Formica at one end as the easiest to remove, whilst semen on vinyl is the most challenging. The latter was evidenced, in Experiment 9 where all reagents were tested for cleaning effectiveness using a double spray/wipe process for cleaning the most challenging body fluid/surface combination of semen on vinyl. Presept alone gave a "green, pass" result whilst Virkon and Selgiene both achieved "amber, pass" scores using the Cellmark EM Indicator Guide-RAG criteria. Overall, the least effective cleaning reagents were Microsol, Virusolve, Chemgene $HLD_4H$ and Chemgene Medlab all of which were scored as "red, fail" in this exercise using the RAG criteria. Therefore, where these latter reagents are being used for cleaning within the SARCs, extra precautions should be taken considering additional EM of all vinyl surfaces to assess whether in practice there is an issue with maintaining appropriate cleanliness levels. In response to the outcomes of this experimentation, SceneSafe have discontinued their porous couch covers, and now only supply a non-porous version, which may help mitigate the risk of body fluids contaminating the couch during a forensic medical examination and potential cross-contamination in subsequent examinations. In the event that elevated levels if DNA are detected on the couch, the risk could be mitigated further by for example implementing a routine weekly/monthly Presept and water clean as an additional decontamination procedure. It is recognised, however that use of Presept and other bleach/hypochlorite-based reagents may not be an option for some facilities due to local variations in health and safety policy, despite their DNA decontamination effectiveness.

The FSR EM scoring criteria considers both quantification values and number of alleles above thresholds to determine scorings. The use of this new SARC specific EM criteria results in a slightly different categorisation of results as shown in Table 10 and Fig. 13, but this does not impact the overall findings or conclusions from this study.

It must be noted that for this study the amount of DNA used to simulate contamination prior to removal by the cleaning process was artificially high (10 μl of body fluid added to the work surface). This may be considered to represent a worst-case scenario especially as removal was made harder by drying the body fluids onto the surfaces. More experimentation would be of value to further assess additional contamination events that more closely mimic those observed within SARC facilities by including touch DNA deposits onto the work surfaces.

**Evaluation of pre-impregnated wipe efficiency**. At the time of conducting this exercise legislation was pending to ban plastics in pre-impregnated wipes or 'wet wipes'.[23] Therefore, whilst this study concluded that there were no significant differences between spraying/wiping and using a pre-impregnated wipe this may have to be re-visited if specifications change. In hindsight, a repeat of the cleaning exercise for sensitive instrumentation is also merited, with assessment of the removal or more real-life levels of contamination such as from touch DNA rather than the unrealistically high concentration of body fluids used in this exercise.

All experiments conducted in this study replicate the forensic medical examination room environments in police custody setting as well as SARCs, therefore all findings and conclusions from this study are equally applicable to police custody.

## 5. Conclusions

Based on the findings from this study the mechanism of the clean does have the greatest significance over its effectiveness, a combination of circular and linear cleaning, with regular cloth changes, combined with a duplicate spray/wipe cycle strategy is suitable. It is the cleaning strategy that has a far greater impact on the efficiency of the clean, which is significantly improved with a duplicate cleaning reagent spray/mechanical wipe action, enhancing the removal of DNA from the work surface compared to a single spray/wipe. There were no significant differences between spraying/wiping and using a pre-impregnated wipe. A contact time of approximately 30 s of chemical reagent with a surface before wiping is sufficient, as any longer does not provide a significant improvement in DNA reduction. Overall, the most challenging body fluid to clean was semen and most difficult surface to clean was Vinyl. Results from testing this most challenging combination using a double spray/wipe clean are shown in Fig. 14, which summarises the results from this study and the corresponding RAG rating based on Cellmark's EM Indicator Guide-RAG criteria. These results demonstrate

that Presept is by far the most effective cleaning agent for reducing DNA followed by Virkon, then Selgiene.

This study provides the validation data for SARCs and police custodies for the cleaning reagents tested, demonstrating that following the recommended cleaning strategy of a double spray/wash leaving the reagent on the surface for approximately 30 s before wiping is fit for purpose for the reduction of DNA required in a forensic medical examination setting.

## Author contribution

**Michelle Gaskell*:** Conceptualisation, Methodology, Data curation, Writing – original draft, Writing – review and editing. **Lesley Clifford:** Planning and conducting experimental work, Writing – experimental results. **Aaron Jones:** conducting experimental work review of results. **Guylaine Hanford**: Writing -validation plan and validation report **Kevin Sullivan**: Conceptualisation, Methodology, Writing -review and editing.

## Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors wish to thank June Guiness (Office of the Forensic Science Regulator) and Debbie Sharp (Forensic Capability Network) for their comments on the experimental approach and critical review of the documentation.

This research was funded by the Home Office, United Kingdom.

## Appendix

**Table 11**
List of consumables and equipment used throughout all experimentation.

| Consumable type | Batch/Lot number | Manufacturer |
|---|---|---|
| Pipette tips: Various sizes – aerosol barrier filter tips | | Fisher Scientific |
| Forensic DNA grade cotton swabs | 124127 | SceneSafe |
| Nuclease free water | 163027335 | Themo Fisher Scientific |
| Virkon solution: 50 × 5g Virkon Rely + On tablets | 2105BA0011 | LanXESS |
| Chemgene $HLD_4H$ solution: 750 ml trigger spray bottles | 220320 | Byotrol |
| Chemgene $HLD_4H$ wipes | B29178 | Byotrol |
| Microsol4 solution: 750 ml spray bottles | 220443 | Anachem |
| Microsol wipes | 1755261220937 | Anachem |
| Selgiene Extreme: 4 × 750 ml spray bottles | T197052500 | Selden Research Ltd |
| Selgiene Virucidal wipes | 72016 | Selden Research Ltd |
| Virusolve + solution: 750 ml spray bottles | OV151121 | Amity International |
| Virusolve + wipes | OZ013870-10 | Amity International |
| Presept | 22EW527 | Johnson & Johnson |
| Chlorhexidine wipes | 17J526/220937 | GAMA Healthcare |
| Microsol 4 | 731/B/2 | Anachem |
| White disposable roll | D41521 | SceneSafe |
| Isotonic Saline solution | AG12467 | Reliwash |
| EZ1 & EZ2 DNA Investigator kit | 172039661 & 172045465 | Qiagen |
| MDithiothreitol | AG12415 | Sigma Aldrich |
| Proteinase K | 172037018 & 172049217 | Sigma Aldrich |
| Investigator Quantiplex Pro kit | 172040784 | Qiagen |
| AmpFLSTR™ NGM™PCR Amplification kit | 2209113 | Themo Fisher Scientific |
| Tris-EDTA Buffer | AG12479 | Sigma Aldrich |
| 007 Human Control DNA | 2207121 | Themo Fisher Scientific |
| GS600 Liz v2.0 Size Std | 01337571 | Themo Fisher Scientific |
| Hi-Di Formamide | AG12458 | Themo Fisher Scientific |
| **Equipment type** | | **Manufacturer** |
| BioRobot® EZ1 workstation | | Qiagen |
| 7500 Real-time PCR system | | Themo Fisher Scientific |
| Quantiplex PRO RT PCR kit | | Qiagen |
| 3500 Genetic Analyser | | Themo Fisher Scientific |
| Genemapper® IDX v1.5 software | | Themo Fisher Scientific |

**Table 12**
Table of equations used in the statistical analysis of results throughout the experiments conducted.

***Equation 1: Unpaired t-test equation.***

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_p\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$S_p = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the sample means, $n_1$ and $n_2$ are the sample sizes, and $s^2_{pooled}$ is the pooled variance.

(*continued on next page*)

**Table 12** (*continued*)

***Equation 2: Kruskal Wallis equation.***

$$H = \frac{12}{N(N+1)} \sum \left( \frac{R_i^2}{n_i} \right) - 3(N+1)$$

Where $N$ is the total number of observations across all groups, $k$ is the number of groups, $R_i$ is the sum of Ranks for group $i$, and $ni$ is the number of observations in group $i$.

This equation is used to determine if there are statistically significant differences between the medians of three or more independent groups, and the calculated $H$ value is compared to a chi-square distribution with $k-1$ degrees of freedom.

***Equation 3: Percentage yield calculation***

$$Percentage\ yield = \frac{Test\ DNA\ quantification\ (ng/\mu l)}{Control\ DNA\ quantification\ (ng/\mu l)} \times 100$$

***Equation 4: Allele count percentage reduction***

$$Allele\ count\ percentage\ reduction = \frac{(Control\ allele\ count - test\ recovered\ allele\ count)}{Control\ allele\ count} \times 100$$

**Table 13**

Experiment 1. Experimental design and results for the assessment of different cleaning actions on a Formica surface dosed with semen using a saline solution or Chemgene with a white paper cloth.

| Experimentational design | | | | | Results | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Expt No. | Cleaning Reagent | Replicates | Sampled area | Cleaning action | Average Peak height | Average number of alleles | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 1.1 | Saline solution | 5 | Semen spot | Circular wipe | 3712.85 | 25.2 | 0.8062 | 1.1129 | 26.17% |
| 1.2 | Saline solution | | | Linear wipe | 2567.42 | 29 | 0.0071 | 0.0069 | 0.23% |
| 1.3 | Control: not cleaned | | | No wipe | 8087.37 | 28 | 3.0805 | 0.9628 | 100% |
| 1.4 | Control area: not cleaned | 3 | Control area 10cm from spot in Experiment 1.3 | No wipe | 123.33 | 1.33 | 0.0000 | 0.0000 | N/A |
| 1.5 | Saline solution | | Control area 10cm from spot in Experiment 1.2 | Linear wipe | 77.67 | 0.33 | 0.0000 | 0.0001 | |
| 1.6 | Saline solution | | Control area 10cm from spot in Experiment 1.1 | Circular wipe | 312.28 | 16.33 | 0.0007 | 0.0006 | |
| 1.7 | Chemgene $HLD_4H$ | 5 | Semen spot | Circular wipe | 3017.62 | 29.2 | 0.0163 | 0.0231 | 0.53% |
| 1.8 | Chemgene $HLD_4H$ | | | Linear wipe | 7553.99 | 28.8 | 0.0894 | 0.0308 | 2.90% |
| 1.9 | Chemgene $HLD_4H$ | 3 | Control area 10cm from spot in Experiment 1.8 | Linear wipe | 0.00 | 0 | 0.0000 | 0.0000 | N/A |
| 1.10 | Chemgene $HLD_4H$ | | Control area 10cm from spot in Experiment 1.7 | Circular wipe | 1835.95 | 14 | 0.0049 | 0.0080 | |
| 1.11 | Post bleach /water clean EM | | Semen spot | Standard clean | 84.11 | 6 | 0.0003 | 0.0005 | 0.01% |

**Table 14**

Experiment 1. Statistical analysis, unpaired *t*-test calculations to determine if the difference in quantification score for circular wiping with chemgene is significantly different to linear wiping. The outcome of the *t*-test is that the difference between the means of these 2 sets of results are statistically significantly different.

| Results | Chemgene $HLD_4H$ - ***circular wipe*** | Chemgene $HLD_4H$ - ***linear wipe*** | St. Dev |
|---|---|---|---|
| Quantification Score ng/µl | 0.013 | 0.090 | 0.054 |
| | 0.057 | 0.086 | 0.020 |
| | 0.003 | 0.041 | 0.027 |
| | 0.005 | 0.122 | 0.083 |
| | 0.004 | 0.109 | 0.074 |
| Total Quantification Score ng/µl | **0.016** | **0.089** | **0.052** |
| Unpaired T -test | | | |
| p value 0.05 | | | 0.0034 |

The difference between the means of the two independent groups is statistically significant.

**Table 15**
Experiment 2: Impact of cleaning reagents on Formica dosed with different body fluids using a circular wipe method after a 30 s contact time.

| Experimental set up | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Body fluid | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/μl) | DNA quantification standard deviation | DNA quantification % yield |
| 2.1 | Chemgene HLD$_4$H 10% | 5 | Blood | 233.78 | 7.4 | 73.6% | 0.0007 | 0.0013 | 0.39% |
| 2.2 | | | Semen | 510.01 | 22.4 | 22.2% | 0.0013 | 0.0015 | 0.03% |
| 2.3 | | | Saliva | 411.22 | 21.6 | 32.5% | 0.0025 | 0.0023 | 0.17% |
| 2.4 | Virkon 1% | 5 | Blood | 36 | 0.2 | 99.3% | 0 | 0 | 0.00% |
| 2.5 | | | Semen | 302 | 21 | 27.1% | 0.001 | 0.0004 | 0.02% |
| 2.6 | Post bleach/water clean EM | 3 | n/a | 37.67 | 0.3 | N/A | 0 | 0.0001 | N/A |
| 2.7 | Virkon 1% | 5 | Saliva | 162.52 | 4 | 87.5% | 0.0002 | 0.0002 | 0.01% |
| 2.8 | Microsol 5% | 5 | Blood | 92.2 | 1.6 | 94.3% | 0 | 0 | 0.00% |
| 2.9 | | | Semen | 391.95 | 19 | 34.0% | 0.0008 | 0.0006 | 0.02% |
| 2.1 | | | Saliva | 1000.61 | 29.4 | 8.1% | 0.0065 | 0.0064 | 0.44% |
| 2.11 | Control | 5 | Blood | 12805.39 | 28 | 0.0% | 0.1811 | 0.0718 | 100.00% |
| 2.12 | Post bleach/water clean EM | 3 | n/a | 0 | 0 | N/A | 0 | 0.0001 | N/A |
| 2.13 | Control | 5 | Semen | 11087.55 | 28.8 | 0.0% | 4.6936 | 0.3174 | 100.00% |
| 2.14 | | | Saliva | 9437.79 | 32 | 0.0% | 1.4806 | 0.304 | 100.00% |

| Experimental set up | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Body fluid | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/μl) | DNA quantification standard deviation | DNA quantification % yield |
| 2.1 5 | Selgiene 10% | 5 | Blood | 0 | 0 | 100.0% | 0 | 0.0001 | 0.00% |
| 2.16 | | | Semen | 330.79 | 12.6 | 56.3% | 0.0004 | 0.0006 | 0.01% |
| 2.17 | | | Saliva | 175.79 | 9.8 | 226.5% | 0.0003 | 0.0001 | 0.02% |
| 2.18 | Post bleach/water clean EM | 3 | n/a | 71 | 1 | N/A | 0 | 0.0001 | N/A |
| 2.19 | Virusolve 5% | 5 | Blood | 174.3 | 1.4 | 95.0% | 0 | 0.0001 | 0.00% |
| 2.2 | | | Semen | 986.63 | 25.4 | 11.8% | 0.0024 | 0.0024 | 0.05% |
| 2.21 | | | Saliva | 263.7 | 15.4 | 51.9% | 0.0007 | 0.0004 | 0.05% |
| 2.22 | Presept 1250ppm | 5 | Blood | 29.6 | 0.2 | 99.3% | 0 | 0 | 0.00% |
| 2.23 | | | Semen | 93.4 | 1.2 | 95.8% | 0 | 0 | 0.00% |
| 2.24 | Post bleach/water clean EM | 3 | n/a | 0 | 0 | N/A | 0 | 0.0001 | N/A |
| 2.25 | Presept 1250ppm | 5 | Saliva | 20.8 | 0.2 | 99.4% | 0 | 0 | 0.00% |
| 2.26 | Post bleach/water clean EM | 3 | n/a | 0 | 0 | N/A | 0 | 0 | N/A |

**Table 16**
Experiment 3. Experimental design and results for the comparison of the effectiveness of spraying and then wiping versus wiping with a pre-impregnated wipe for 30 s on Formica dosed with semen using a circular wipe method.

| Experimental Design | | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Expt No. | Reagent | Replicates | Body fluid | Wipe type | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/μl) | DNA quantification standard deviation | DNA quantification % yield |
| 2.13 | Control | 5 | Semen | N/A | 11087.55 | 28.8 | 0.0% | 4.6936 | 0.3174 | 100.00% |
| 3.1 | Chemgene HLD$_4$H | 5 | Semen | Pre-impregnated | 336.56 | 18.2 | 36.8% | 0.0018 | 0.002 | 0.04% |
| 3.2 | Virkon | | | | 277.39 | 20.6 | 28.5% | 0.001 | 0.0005 | 0.02% |
| 3.3 | Microsol | | | | 374.31 | 24.4 | 15.3% | 0.0014 | 0.0005 | 0.03% |
| 3.4 | Selgiene | | | | 99.71 | 4.8 | 83.3% | 0.0002 | 0.0002 | 0.00% |
| 3.5 | Virusolve | | | | 1043.61 | 28.2 | 2.1% | 0.004 | 0.0024 | 0.09% |
| 3.6 | Post bleach clean EM | 3 | N/A | N/A | 0 | 0 | 100.0% | 0 | 0 | N/A |
| 3.7 | Presept | 5 | Semen | Paper towel sprayed with reagent | 0 | 0 | 100.0% | 0 | 0 | 0.00% |
| 3.8 | Chemgene HLD$_4$H | | | | 818 | 20.5 | 28.8% | 0.003 | 0.0048 | 0.06% |
| 3.9 | Post bleach clean EM | 3 | N/A | N/A | 30.33 | 1 | 96.5% | 0.0001 | 0 | N/A |

**Table 17**
Experiment 3. Statistical data for unpaired t-tests to show the difference between spray verses impregnated wipe and spray verses sprayed paper towel.

| Reagent/Spray or wipe | Replicates (DNA quantification ng/μl) | Average DNA quantification (ng/μl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T test Outcome |
|---|---|---|---|---|---|
| Chemgene HLD$_4$H 10 % spray (Experiment 2) | 0.0021<br>0.0009<br>0.0007<br>0.0044<br>0.0012 | **0.0019** | **0.002** | **0.9303** | The difference is not statistically significantly different |
| Chemgene HLD$_4$H pre-impregnated wipe | 0.0006<br>0.0022<br>0.0004<br>0.0006<br>0.005 | **0.0018** | | | |
| Chemgene HLD$_4$H 10 % spray (Experiment 2) | 0.0021<br>0.0009<br>0.0007<br>0.0044<br>0.0012 | **0.0019** | **0.018** | **0.4133** | The difference is not statistically significantly different |
| Chemgene HLD$_4$H sprayed paper towel | 0.0006<br>0.0004<br>0.0102<br>2.2197<br>0.0006 | **0.0259** | | | |
| Virkon 1 % spray (Experiment 2) | 0.0009<br>0.001<br>0.0008<br>0.0007<br>0.0016 | **0.0010** | **0.0000** | **1.0000** | A statistically insignificant difference in results. |
| Virkon pre-impregnated wipe | 0.0016<br>0.0009<br>0.0014<br>0.0004<br>0.0007 | **0.0010** | | | |
| Microsol 5 % spray (Experiment 2) | 0.0015<br>0.0001<br>0.0006<br>0.0012<br>0.0004 | **0.0008** | **0.001** | **0.1087** | The difference is not statistically significantly different |

(*continued on next page*)

**Table 17** (*continued*)

| Reagent/Spray or wipe | Replicates (DNA quantification ng/µl) | Average DNA quantification (ng/µl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T test Outcome |
|---|---|---|---|---|---|
| Microsol pre-impregnated wipe | 0.001<br>0.0008<br>0.0013<br>0.0018<br>0.0021 | **0.0014** | | | |
| Selgiene 10 % spray (Experiment 2) | 0.0015<br>0<br>0.0006<br>0.0001<br>0 | **0.0004** | **0.0001** | **0.4280** | The difference is not statistically significantly different |
| Selgiene pre-impregnated wipe | 0.0002<br>0.0001<br>0<br>0.0001<br>0.0005 | **0.0002** | | | |
| Virusolve 5 % spray (Experiment 2) | 0.0066<br>0.0019<br>0.001<br>0.0018<br>0.0008 | **0.0024** | **0.002** | **0.3366** | The difference is not statistically significantly different |
| Virusolve pre-impregnated wipe | 0.0053<br>0.0024<br>0.0074<br>0.0012<br>0.0036 | **0.0040** | | | |
| Presept (1250 ppm) spray (Experiment 2) | 0<br>0.0001<br>0<br>0<br>0 | **0.0000** | **0.0000** | **0.3739** | The difference is not statistically significantly different |
| Presept (1250 ppm) Sprayed on paper towel | 0<br>0<br>0<br>0<br>0 | **0.0000** | | | |

**Table 18**
Experiment 4. Experimental design and results for the assessment of a single spray/wipe cycle versus double spray/wipe cycles on Formica dosed with semen with a 30 s reagent contact time. Please note: The single spray/wipe cycles were carried out in Experiment 2, but results included below for ease of review.

| Experiment set up | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Duplicate Spray/ Wipe method | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 2.13 | Control | 5 | No action - Control | 11087.55 | 28.8 | 0.0% | 4.6936 | 0.3174 | 100.00% |
| 4.1 | Chemgene HLD4H 10% | 5 | 2x reagent spray/wipes | 153.04 | 7.2 | 75.0% | 0.0001 | 0.0001 | 0.00% |
| 2.2 | | | Single spray/wipe | 510.01 | 22.4 | 22.2% | 0.0019 | 0.0015 | 0.04% |
| 4.2 | Virkon 1% | 5 | 2x reagent spray/wipes | 56.8 | 0.4 | 98.6% | 0 | 0.0001 | 0.00% |
| 2.5 | | | Single spray/wipe | 302 | 21 | 27.1% | 0.001 | 0.0004 | 0.02% |
| 4.3 | Microsol 5% | 5 | 2x reagent spray/wipes | 217.62 | 11.2 | 61.1% | 0.0003 | 0.0002 | 0.01% |
| 2.9 | | | Single spray/wipe | 391.95 | 19 | 34.0% | 0.0008 | 0.0006 | 0.02% |
| 4.4 | Selgiene 10% | 5 | 2x reagent spray/wipes | 165.97 | 9 | 68.8% | 0.0003 | 0.0002 | 0.01% |
| 2.16 | | | Single spray/wipe | 330.79 | 12.6 | 56.3% | 0.0004 | 0.0006 | 0.01% |
| 4.5 | Virusolve 5% | 5 | 2x reagent spray/wipes | 237.99 | 7 | 75.7% | 0.0002 | 0.0002 | 0.00% |
| 2.2 | | | Single spray/wipe | 986.63 | 25.4 | 11.8% | 0.0042 | 0.0042 | 0.09% |
| 4.6 | Post bleach clean EM | 3 | Bleach/water clean | 0 | 0 | N/A | 0 | 0 | N/A |
| 4.7 | Presept 1250 ppm | 5 | 2x reagent spray/wipes | 82.76 | 1.2 | 95.8% | 0 | 0 | 0.00% |
| 2.23 | | | Single spray/wipe | 93.4 | 1.2 | 95.8% | 0 | 0 | 0.00% |
| 4.8 | Virkon 1% | 5 | 2x reagent spray/wipes & wipe dry | 0 | 0 | 100.0% | 0 | 0 | 0.00% |
| 4.9 | Chemgene HLD4H 10% | 5 | 1x reagent spray/wipe & 1 water spray and wipe dry | 261.83 | 3.6 | 87.5% | 0 | 0 | 0.00% |

| Experiment set up | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Duplicate Spray/ Wipe method | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 4.1 | | | 1 x spray, then wipe as per 3.7, and then mist with reagent spray | 205.42 | 5.5 | 80.9% | 0.0002 | 0.0002 | 0.00% |
| 4.11 | Post bleach clean EM | 3 | n/a | 51.11 | 1 | N/A | 0 | 0 | |

**Table 19**
Experiment 4. Statistical comparison of results from assessment of a single spray/wipe cleaning strategy versus double spray/wipe cleaning. Comparison of efficiency determined using an unpaired student's t-test.

| Reagent/Spray or wipe | Replicates (DNA quantification ng/µl) | Average DNA quantification (ng/µl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T test Outcome |
|---|---|---|---|---|---|
| Virkon single *spray/wipe* | 0.0009<br>0.0010<br>0.0008<br>0.0007<br>0.0016 | **0.0010** | **0.001** | 0.0028 | The difference is statistically different |
| Virkon - *duplicate spray/wipe* | 0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0002 | **0.0000** | | | |
| Virkon single spray/wipe | 0.0009<br>0.0010<br>0.0008 | **0.0010** | **0.001** | 0.0032 | The difference is statistically different |

(*continued on next page*)

**Table 19** (*continued*)

| Reagent/Spray or wipe | Replicates (DNA quantification ng/µl) | Average DNA quantification (ng/µl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T test Outcome |
|---|---|---|---|---|---|
| | 0.0007 | | | | |
| | 0.0016 | | | | |
| Virkon - duplicate spray/wipe + wipe dry | 0.0000 | **0.0000** | | | |
| | 0.0000 | | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| Chemgene single *spray/wipe* | 0.0021 | **0.0019** | **0.001** | 0.0621 | The difference is not statistically significantly different |
| | 0.0009 | | | | |
| | 0.0007 | | | | |
| | 0.0044 | | | | |
| | 0.0012 | | | | |
| Chemgene - *duplicate spray/wipe* | 0.0002 | **0.0001** | | | |
| | 0.0003 | | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| Chemgene single spray/wipe | 0.0021 | **0.0019** | **0.001** | 0.0534 | The difference is not statistically significantly different |
| | 0.0009 | | | | |
| | 0.0007 | | | | |
| | 0.0044 | | | | |
| | 0.0012 | | | | |
| Chemgene - *1x chemical spray/ wipe, 1 x water spray/wipe dry* | 0.0000 | **0.0000** | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| Chemgene single spray/wipe | 0.0021 | **0.0019** | **0.001** | 0.0747 | The difference is not statistically significantly different |
| | 0.0009 | | | | |
| | 0.0007 | | | | |
| | 0.0044 | | | | |
| | 0.0012 | | | | |
| Chemgene - *1x chemical spray/ wipe, 1 x chemical mist* | 0.0000 | **0.0002** | | | |
| | 0.0000 | | | | |
| | 0.0002 | | | | |
| | 0.0005 | | | | |
| | 0.0005 | | | | |
| Microsol single *spray/wipe* | 0.0015 | **0.0008** | **0.000** | 0.1284 | The difference is not statistically significantly different |
| | 0.0001 | | | | |
| | 0.0006 | | | | |
| | 0.0012 | | | | |
| | 0.0004 | | | | |
| Microsol - *duplicate spray/wipe* | 0.0006 | **0.0003** | | | |
| | 0.0003 | | | | |
| | 0.0000 | | | | |
| | 0.0002 | | | | |
| | 0.0002 | | | | |
| Selgiene single *spray/wipe* | 0.0015 | **0.0004** | **0.000** | 0.5780 | The difference is not statistically significantly different |
| | 0.0000 | | | | |
| | 0.0006 | | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| Selgiene - *duplicate spray/wipe* | 0.0003 | **0.0003** | | | |
| | 0.0005 | | | | |
| | 0.0004 | | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| Virusolve single *spray/wipe* | 0.0066 | **0.0024** | **0.002** | 0.1036 | The difference is not statistically significantly different |
| | 0.0019 | | | | |
| | 0.0010 | | | | |
| | 0.0018 | | | | |
| | 0.0008 | | | | |
| Virusolve - *duplicate spray/wipe* | 0.0006 | **0.0002** | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| | 0.0001 | | | | |
| | 0.0001 | | | | |
| Presept (1250 ppm) single *spray/ wipe* | 0.0000 | **0.0000** | **0.000** | **1.0000** | A statistically insignificant difference in results. |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| Presept (1250 ppm) - *duplicate spray/wipe* | 0.0000 | **0.0000** | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |

(*continued on next page*)

**Table 19** (*continued*)

| Reagent/Spray or wipe | Replicates (DNA quantification ng/μl) | Average DNA quantification (ng/μl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T test Outcome |
|---|---|---|---|---|---|
| | 0.0000<br>0.0000 | | | | |

**Table 20**
Experiment 4. Kruskal-Wallis Nonparametric ANOVA statistical analysis of results.

| Test Information | | | | |
|---|---|---|---|---|
| $H_0$: Median 1 = Median 2 = … = Median k<br>$H_a$: At least one pair Median i ≠ Median j | | | | |
| Results: | Chemgene single spray/wipe | Chemgene - 1x chemical spray/wipe, 1 x water spray/wipe dry | Chemgene - 1x chemical spray/wipe, 1 x chemical mist | |
| **Count (N)** | 6 | 6 | 6 | |
| **Median** | 22.5 | 3.3 | 5.25 | |
| **UC Median (2-sided, 95 %)** | 27.286 | 6.286 | 9.643 | |
| **LC Median (2-sided, 95 %)** | 16.500 | 1.357 | 3.357 | |
| **Z** | 3.372 | −2.622 | −0.749269 | |
| **Kruskal-Wallis Statistic (H):** 12.564<br>**DF:** 2<br>**P-Value (2-sided, adjusted for ties): 0.0019** | | | | |
| Results: | | Virkon single spray/wipe | Virkon - duplicate spray/wipe | |
| **Count (N)** | | 6 | 6 | |
| **Median** | | 21.5 | 0.2 | |
| **UC Median (2-sided, 95 %)** | | 23.643 | 1 | |
| **LC Median (2-sided, 95 %)** | | 17.714 | 0 | |
| **Z** | | 2.882 | −2.882 | |
| **Kruskal-Wallis Statistic (H):** 8.456<br>**DF:** 1<br>**P-Value (2-sided, adjusted for ties): 0.0036** | | | | |
| Results: | Microsol single spray/wipe | Microsol - duplicate spray/wipe | Selgiene single spray/wipe | Selgiene - duplicate spray/wipe |
| **Count (N)** | 6 | 6 | 6 | 6 |
| **Median** | 19 | 11 | 9.5 | 9.5 |
| **UC Median (2-sided, 95 %)** | 28.214 | 18.500 | 26.500 | 15.643 |
| **LC Median (2-sided, 95 %)** | 9.786 | 4.143 | 2.714 | 1.429 |
| **Z** | 1.933 | −0.466667 | −0.300000 | −1.167 |
| **Kruskal-Wallis Statistic (H):** 4.066<br>**DF:** 3<br>**P-Value (2-sided, adjusted for ties):** 0.2545 | | | | |
| Results: | Virusolve single spray/wipe | Virusolve - duplicate spray/wipe | Presept (1250 ppm) single spray/wipe | Presept (1250 ppm) - duplicate spray/wipe |
| **Count (N)** | 6 | 6 | 6 | 6 |
| **Median** | 26.5 | 3.5 | 0 | 0 |
| **UC Median (2-sided, 95 %)** | 29 | 19.214 | 0.714286 | 3.571 |
| **LC Median (2-sided, 95 %)** | 20.357 | 0.714286 | 0 | 0 |
| **Z** | 3.400 | 0.666667 | −2.233 | −1.833 |
| **Kruskal-Wallis Statistic (H):** 16.120<br>**DF: 3**<br>**P-Value (2-sided, adjusted for ties): 0.0011** | | | | |

**Table 21**
Experiment 5. Experimental design and results for the assessment of the impact of different cleaning reagent concentrations on Formica dosed with semen after 30 s contact time and using a single spray and circular wipe. The manufacturers' recommended concentrations were used in experiment 2 and included in this table for ease of comparison.

| Experimental set up | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/μl) | DNA quantification standard deviation | DNA quantification % yield |
| 2.13 | Control | 5 | 11087.55 | 28.8 | 0.0% | 4.6936 | 0.3174 | 100.00% |
| 5.1 | Chemgene $HLD_4H$ 1% | 5 | 200.62 | 9.8 | 66.0% | 0.0001 | 0.0001 | 0.00% |
| 2.2 | Chemgene $HLD_4H$ 10% | | 510.01 | 22.4 | 22.2% | 0.0019 | 0.0015 | 0.04% |
| 5.2 | Virkon 3% | 5 | 322.11 | 17 | 41.0% | 0.0015 | 0.0016 | 0.03% |
| 2.5 | Virkon 1% | | 302 | 21 | 27.1% | 0.001 | 0.0004 | 0.02% |
| 5.3 | Microsol 10% | 5 | 373.15 | 25.2 | 12.5% | 0.0009 | 0.0005 | 0.02% |
| 2.9 | Microsol 5% | | 391.95 | 19 | 34.0% | 0.0008 | 0.0006 | 0.02% |
| 5.4 | Selgiene 2.5% | 5 | 1506.26 | 28.8 | 0.0% | 0.0033 | 0.0026 | 0.07% |
| 2.16 | Selgiene 10% | | 330.79 | 12.6 | 56.3% | 0.0004 | 0.0006 | 0.01% |
| 5.5 | Virusolve 0.5% | 5 | 380.55 | 15.6 | 45.8% | 0.0005 | 0.0006 | 0.01% |
| 2.2 | Virusolve 5% | | 986.63 | 25.4 | 11.8% | 0.0042 | 0.0042 | 0.09% |
| 5.6 | Post bleach clean EM | 3 | 0 | 0 | N/A | 0 | 0 | N/A |
| 5.7 | Presept 10,000 ppm | 5 | 96.92 | 1.8 | 93.8% | 0 | 0 | 0.00% |
| 2.23 | Presept 1250 ppm | | 93.4 | 1.2 | 95.8% | 0 | 0 | 0.00% |

**Table 22**
Experiment 5: Statistical analysis of the difference between lower and higher concentrations of cleaning products using an unpaired statistical *t*-test.

| Reagent/Spray or wipe | Replicates (DNA quantification ng/μl) | Average DNA quantification (ng/μl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T test Outcome |
|---|---|---|---|---|---|
| Chemgene 1 % | 0.0000<br>0.0001<br>0.0003<br>0.0002<br>0.0000 | **0.0001** | **0.001** | 0.0621 | The difference is not statistically significantly different |
| Chemgene 10 % | 0.0021<br>0.0009<br>0.0007<br>0.0044<br>0.0012 | **0.0019** | | | |
| Virkon 1 % | 0.0009<br>0.0010<br>0.0008<br>0.0007<br>0.0016 | **0.0010** | **0.001** | 0.5658 | The difference is not statistically significantly different |
| Virkon 3 % | 0.0042<br>0.0005<br>0.0015<br>0.0010<br>0.0001 | **0.0015** | | | |
| Microsol 5 % | 0.0015<br>0.0001<br>0.0006<br>0.0012<br>0.0004 | **0.0008** | **0.000** | 0.7360 | The difference is not statistically significantly different |
| Microsol 10 % | 0.0010<br>0.0017<br>0.0006<br>0.0007<br>0.0004 | **0.0009** | | | |
| Selgiene 2.5 % | 0.0013<br>0.0021 | **0.0033** | **0.002** | 0.0656 | The difference is not statistically significantly different |

(*continued on next page*)

**Table 22** (*continued*)

| Reagent/Spray or wipe | Replicates (DNA quantification ng/μl) | Average DNA quantification (ng/μl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T test Outcome |
|---|---|---|---|---|---|
| | 0.0072 | | | | |
| | 0.0013 | | | | |
| | 0.0047 | | | | |
| Selgiene 10 % | 0.0015 | **0.0004** | | | |
| | 0.0000 | | | | |
| | 0.0006 | | | | |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| Virusolve 0.5 % | 0.0000 | **0.0005** | **0.002** | 0.1447 | The difference is not statistically significantly different |
| | 0.0006 | | | | |
| | 0.0015 | | | | |
| | 0.0001 | | | | |
| | 0.0002 | | | | |
| Virusolve 5 % | 0.0066 | **0.0024** | | | |
| | 0.0019 | | | | |
| | 0.0010 | | | | |
| | 0.0018 | | | | |
| | 0.0008 | | | | |
| Presept 1250 ppm | 0.0000 | **0.0000** | **0.000** | 0.3739 | The difference is not statistically significantly different |
| | 0.0001 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| Presept 10000 ppm | 0.0000 | **0.0000** | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |
| | 0.0000 | | | | |

**Table 23**
Experiment 5: Kruskal-Wallis Nonparametric ANOVA statistical analysis of results.

| Test Information | | | |
|---|---|---|---|
| $H_0$: Median 1 = Median 2 = … = Median k<br>$H_a$: At least one pair Median i ≠ Median j | | | |
| Results: | Formica Av.DNA (ng/μl) | Metal Av.DNA (ng/μl) | Vinyl Av.DNA (ng/μl) |
| **Count (N)** | 6 | 6 | 6 |
| **Median** | 0.0009 | 0.00265 | 0.00845 |
| **UC Median (2-sided, 95 %)** | 0.002221429 | 0.00595 | 0.031035714 |
| **LC Median (2-sided, 95 %)** | 0.000142857 | 0.000142857 | 0.001607143 |
| **Z** | −1.873 | −0.468293 | 2.341 |
| **Kruskal-Wallis Statistic (H):** 6.153 | | | |
| **DF: 2** | | | |
| **P-Value (2-sided, adjusted for ties): 0.0461** | | | |
| Results: | Formica Mean allele count | Metal Mean allele count | Vinyl Mean allele count |
| **Count (N)** | 7 | 7 | 7 |
| **Median** | 21 | 28.4 | 29 |
| **UC Median (2-sided, 95 %)** | 26.093 | 29.200 | 29.773 |
| **LC Median (2-sided, 95 %)** | 9.560 | 5.240 | 20.080 |
| **Z** | −1.641 | −0.149209 | 1.791 |
| **Kruskal-Wallis Statistic (H):** 3.961 | | | |
| **DF: 2** | | | |
| **P-Value (2-sided, adjusted for ties):** 0.1380 | | | |

**Table 24**
Experiment 6: Impact of different reagent contact times on Formica dosed with semen and using a single spray with circular wipe method. Contact times of 30 s were conducted as part of experiment 2 and these have been included in this table to ease comparison.

| Experimental set up | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Contact time | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 2.13 | Control | 5 | N/A | 11087.55 | 28.8 | 0.0% | 4.6936 | 0.3174 | 100.00% |
| 6.1 | Chemgene HLD$_4$H 10% | 5 | 15 secs | 518.96 | 19.2 | 33.3% | 0.0012 | 0.0018 | 0.03% |
| 2.2 | | | 30 secs | 510.01 | 22.4 | 22.2% | 0.0019 | 0.0015 | 0.04% |
| 6.2 | | | 1 min | 276.82 | 20 | 30.6% | 0.0004 | 0.0004 | 0.01% |
| 6.3 | | | 5 mins | 346.37 | 21.6 | 25.0% | 0.0007 | 0.0005 | 0.01% |
| 6.4 | Virkon 1% | 5 | 15 secs | 304.68 | 12 | 58.3% | 0.0007 | 0.001 | 0.01% |
| 2.5 | | | 30 secs | 302 | 21 | 27.1% | 0.001 | 0.0004 | 0.02% |
| 6.5 | | | 1 min | 240.01 | 18.4 | 36.1% | 0.0013 | 0.0007 | 0.03% |
| 6.7 | | | 5 mins | 151.88 | 10.4 | 63.9% | 0.0003 | 0.0004 | 0.01% |
| 6.8 | Microsol 5% | 5 | 15 secs | 367.11 | 17 | 41.0% | 0.0006 | 0.0007 | 0.01% |
| 2.9 | | | 30 secs | 391.95 | 19 | 34.0% | 0.0008 | 0.0006 | 0.02% |
| 6.9 | | | 1 min | 334.71 | 17.2 | 40.3% | 0.0004 | 0.0006 | 0.01% |
| 6.1 | | | 5 mins | 325.37 | 22 | 23.6% | 0.0008 | 0.0003 | 0.02% |
| 6.11 | Selgiene 10% | 5 | 15 secs | 791.06 | 29.2 | -1.4% | 0.0028 | 0.0015 | 0.06% |
| 2.16 | | | 30 secs | 330.79 | 12.6 | 56.3% | 0.0004 | 0.0006 | 0.01% |
| 6.13 | | | 1 min | 216.4 | 16.8 | 41.7% | 0.0004 | 0.0002 | 0.01% |
| 6.14 | | | 5 mins | 225.63 | 15.6 | 45.8% | 0.0006 | 0.0003 | 0.01% |
| 6.15 | Virusolve 5% | 5 | 15 secs | 185.56 | 13.8 | 52.1% | 0.0004 | 0.0003 | 0.01% |
| 2.2 | | | 30 secs | 986.63 | 25.4 | 11.8% | 0.0024 | 0.0024 | 0.05% |
| 6.16 | | | 1 min | 574.56 | 20.6 | 28.5% | 0.0016 | 0.0017 | 0.03% |
| 6.17 | | | 5 mins | 394.83 | 20.6 | 28.5% | 0.0015 | 0.0015 | 0.03% |
| 6.19 | Presept 1250ppm | 5 | 15 sec | 72.4 | 5.6 | 80.6% | 0.0004 | 0.001 | 0.01% |
| 2.23 | | | 30 secs | 93.4 | 1.2 | 95.8% | 0 | 0 | 0.00% |

| Experimental set up | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Contact time | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 6.2 | | | 1 min | 86.4 | 1.4 | 95.1% | 0 | 0.0001 | 0.00% |
| 6.21 | | | 5 mins | 97.7 | 0.8 | 97.2% | 0.00002 | 0.00004 | 0.00% |
| 6.6 | Post bleach/water clean EM | 3 | N/A | 0 | 0 | N/A | 0 | 0 | N/A |
| 6.12 | | | | 25.67 | 0.333 | | 0 | 0.0001 | |
| C6.18 | | | | 0 | 0 | | 0 | 0.0001 | |

**Table 25**
Experiment 7. Experimental design and results for the effectiveness of cleaning on different substrates dosed with semen, with a reagent contact time of 30 s, cleaned with a single spray/wipe using a circular wipe. The results for this cleaning assessment on Formica was completed in Experiment 2, these results have been included in this table for ease of comparison.

| Experimental design | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Substrate | Average peak height (RFU) | Average number alleles | % Allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 2.13 | Control | 5 | Formica | 11087.55 | 28.8 | 0.0% | 4.6936 | 0.3174 | 100.00% |
| 7.3 | Chemgene $HLD_4H$ 10% | 5 | metal | 259.6 | 7 | 76.0% | 0.0004 | 0.0003 | 0.01% |
| 7.4 | | | vinyl | 1615.59 | 22 | 25.2% | 0.0096 | 0.0117 | 0.21% |
| 2.2 | | | Formica | 510.01 | 22.4 | 22.2% | 0.0019 | 0.0015 | 0.04% |
| 7.5 | Virkon 1% | 5 | metal | 237.21 | 15.8 | 45.9% | 0.0014 | 0.001 | 0.04% |
| 7.6 | | | vinyl | 562.58 | 25.6 | 12.9% | 0.0036 | 0.0009 | 0.08% |
| 2.5 | | | Formica | 302 | 22 | 23.6% | 0.001 | 0.0004 | 0.02% |
| 7.9 | Microsol 5% | 5 | metal | 853.26 | 29.2 | 0.0% | 0.0039 | 0.002 | 0.12% |
| 7.1 | | | vinyl | 6834.59 | 30.8 | -4.8% | 0.0415 | 0.02 | 0.91% |
| 2.9 | | | Formica | 391.95 | 19 | 34.0% | 0.0008 | 0.0006 | 0.02% |
| 7.11 | Control | 5 | metal | 7826 | 29.2 | 0.0% | 3.3668 | 0.7485 | 100.00% |
| 7.12 | | | vinyl | 8175.39 | 29.4 | 0.0% | 4.5417 | 2.3791 | 100.00% |
| 7.13 | Post bleach/water clean | 3 | metal | 30.83 | 2 | N/A | 0.0002 | 0.0003 | N/A |
| 7.14 | | | vinyl | 133.93 | 3 | N/A | 0.0001 | 0.0002 | N/A |
| 7.15 | Selgiene 10% | 5 | metal | 1149.98 | 28.4 | 2.7% | 0.0055 | 0.0025 | 0.16% |
| 7.16 | | | vinyl | 2510.62 | 29.4 | 0.0% | 0.0122 | 0.004 | 0.27% |
| 2.16 | | | Formica | 330.79 | 12.6 | 56.3% | 0.0004 | 0.0006 | 0.01% |
| 7.17 | Virusolve 5% | 5 | metal | 1582.36 | 29.2 | 0.0% | 0.0062 | 0.0049 | 0.18% |
| 7.18 | | | vinyl | 1313.25 | 29 | 1.4% | 0.0073 | 0.005 | 0.16% |
| 2.2 | | | Formica | 986.63 | 25.4 | 11.8% | 0.0024 | 0.0024 | 0.05% |
| 7.21 | Presept 1250ppm | 5 | metal | 38 | 0.4 | 98.6% | 0 | 0 | 0.00% |
| 7.22 | | | vinyl | 241.73 | 14.8 | 49.7% | 0.0005 | 0.0003 | 0.01% |
| 2.23 | | | Formica | 93.4 | 1.2 | 95.8% | 0 | 0 | 0.00% |

**Table 26**
Experiment 8. Experimental design and results for the assessment of wiping instrumentation and small items dosed in semen with a reagent contact time of 30 s. The wipe action needed to be adapted to suit the contours of the chosen equipment (in this experiment a ridged control knob to reflect those used on adjustable lights and colposcopes). A control swab was taken ahead of each clean before then spraying, wiping and swabbing for post clean results.

| Experimental design | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Substrate | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 8.1 | Chemgene HLD4H 10% | 5 | Control Knob (Plastic) | 1659.9 | 29.8 | 0.0% | 0.0051 | 0.0026 | 2.12% |
| 8.2 | Pre clean control (Chemgene HLD4H 10%) | | | 6962.39 | 29.8 | N/A | 0.2402 | 0.1282 | N/A |
| 8.3 | Virkon 1 % | | | 2060.17 | 26.2 | 12.7% | 0.0072 | 0.0045 | 8.39% |
| 8.4 | Pre clean control (Virkon 1 %) | | | 10386.98 | 30 | N/A | 0.0858 | 0.0647 | N/A |
| 8.5 | Microsol 5% | | | 778.03 | 28.2 | 1.4% | 0.0045 | 0.0021 | 5.53% |
| 8.6 | Pre clean control (Microsol 5%) | | | 5616.41 | 28.6 | N/A | 0.0814 | 0.0547 | N/A |
| 8.7 | Selgiene 10% | | | 556.28 | 23.8 | 15.0% | 0.0027 | 0.0027 | 1.99% |
| 8.8 | Pre clean control (Selgiene 10%) | | | 6789.98 | 28 | N/A | 0.1358 | 0.0507 | N/A |
| 8.9 | Virusolve 5% | | | 1324.24 | 28.8 | 0.0% | 0.0058 | 0.0055 | 7.28% |
| 8.1 | Pre clean control (Virusolve 5%) | | | 6968.87 | 28.8 | N/A | 0.0797 | 0.0504 | N/A |
| 8.11 | Presept 1250pmm | | | 237.42 | 20 | 32.9% | 0.0008 | 0.0003 | 0.86% |
| 8.12 | Pre clean control (Presept 1250 ppm) | | | 8701.54 | 29.8 | N/A | 0.0929 | 0.0437 | N/A |
| 8.13 | Chlorhexidine | | | 353.57 | 24.4 | 17.0% | 0.0012 | 0.0002 | 0.74% |
| 8.14 | Pre clean Chlorhexidine control | | | 10533.15 | 29.4 | N/A | 0.1622 | 0.0814 | N/A |

| Experimental design | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment number | Reagent | Replicates | Substrate | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 8.15 | Post bleach /water clean EM | 3 | | 0 | 0 | N/A | 0 | 0 | N/A |

**Table 27**
Experiment 9. Performance of reagents on most challenging body fluid/substrate combination from experiment 7 (semen on vinyl) using both a single and double spray wipe with 30 s contact time after each spray. This experiment included a small assessment of the new Chemgene Medlab on the most difficult combination. All other reagents include the results from the single wipe and spray that was completed in Experiment 7 and included here to ease comparison.

| Experimental design | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Expt. No. | Cleaning Reagent | Reps | Wipe method | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/µl) | DNA quantification standard deviation | DNA quantification % yield |
| 7.12 | Control | 5 | N/A | 8175.39 | 29.4 | 0.0 % | 4.5417 | 2.3791 | 100.00 % |
| 9.1 | Chemgene Medlab 10 % | | Single spray/wipe | 7517.83 | 28.8 | 2.0 % | 0.0463 | 0.0189 | 1.02 % |
| 9.2 | | | Double spray/wipe | 5543.76 | 28.6 | 2.7 % | 0.024 | 0.012 | 0.53 % |
| 7.4 | Chemgene HLD4H 10 % | | Single spray/wipe | 1615.59 | 22 | 25.2 % | 0.0096 | 0.0117 | 0.21 % |
| 9.3 | | | Double spray/wipe | 1624.91 | 28.6 | 2.7 % | 0.0056 | 0.0055 | 0.12 % |
| 7.6 | Virkon 1 % | | Single spray/wipe | 562.58 | 25.6 | 12.9 % | 0.0036 | 0.0009 | 0.08 % |
| 9.4 | | | Double spray/wipe | 311.48 | 12.2 | 58.5 % | 0.0025 | 0.0013 | 0.06 % |

(continued on next page)

**Table 27** (*continued*)

| Experimental design | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Expt. No. | Cleaning Reagent | Reps | Wipe method | Average peak height (RFU) | Average number alleles | % allele reduction | Average DNA quantification (ng/μl) | DNA quantification standard deviation | DNA quantification % yield |
| 7.12 | Control | 5 | N/A | 8175.39 | 29.4 | 0.0 % | 4.5417 | 2.3791 | 100.00 % |
| 9.1 | Chemgene Medlab 10 % | | Single spray/wipe | 7517.83 | 28.8 | 2.0 % | 0.0463 | 0.0189 | 1.02 % |
| 9.2 | | | Double spray/wipe | 5543.76 | 28.6 | 2.7 % | 0.024 | 0.012 | 0.53 % |
| 7.4 | Chemgene HLD4H 10 % | | Single spray/wipe | 1615.59 | 22 | 25.2 % | 0.0096 | 0.0117 | 0.21 % |
| 9.3 | | | Double spray/wipe | 1624.91 | 28.6 | 2.7 % | 0.0056 | 0.0055 | 0.12 % |
| 7.6 | Virkon 1 % | | Single spray/wipe | 562.58 | 25.6 | 12.9 % | 0.0036 | 0.0009 | 0.08 % |
| 9.4 | | | Double spray/wipe | 311.48 | 12.2 | 58.5 % | 0.0025 | 0.0013 | 0.06 % |
| 7.16 | Selgiene 10 % | | Single spray/wipe | 2510.62 | 29.4 | 0.0 % | 0.0122 | 0.004 | 0.27 % |
| 9.5 | | | Double spray/wipe | 945.7 | 24 | 18.4 % | 0.0042 | 0.0047 | 0.09 % |
| 7.18 | Virusolve 5 % | | Single spray/wipe | 1313.25 | 29 | 1.4 % | 0.0073 | 0.005 | 0.16 % |
| 9.6 | | | Double spray/wipe | 2499.94 | 30.2 | −2.7 % | 0.014 | 0.0085 | 0.31 % |
| 7.1 | Microsol 5 % | | Single spray/wipe | 6834.59 | 30.8 | −4.8 % | 0.0415 | 0.02 | 0.91 % |
| 9.7 | | | Double spray/wipe | 2491.18 | 28.6 | 2.7 % | 0.0125 | 0.0141 | 0.28 % |
| 7.22 | Presept 1250 ppm | | Single spray/wipe | 241.73 | 14.8 | 49.7 % | 0.0005 | 0.0003 | 0.01 % |
| 9.8 | | | Double spray/wipe | 46.6 | 1 | 96.6 % | 0 | 0.0001 | 0.00 % |
| 7.16 | Selgiene 10 % | | Single spray/wipe | 2510.62 | 29.4 | 0.0 % | 0.0122 | 0.004 | 0.27 % |
| 9.5 | | | Double spray/wipe | 945.7 | 24 | 18.4 % | 0.0042 | 0.0047 | 0.09 % |
| 7.18 | Virusolve 5 % | | Single spray/wipe | 1313.25 | 29 | 1.4 % | 0.0073 | 0.005 | 0.16 % |
| 9.6 | | | Double spray/wipe | 2499.94 | 30.2 | −2.7 % | 0.014 | 0.0085 | 0.31 % |
| 7.1 | Microsol 5 % | | Single spray/wipe | 6834.59 | 30.8 | −4.8 % | 0.0415 | 0.02 | 0.91 % |
| 9.7 | | | Double spray/wipe | 2491.18 | 28.6 | 2.7 % | 0.0125 | 0.0141 | 0.28 % |
| 7.22 | Presept 1250 ppm | | Single spray/wipe | 241.73 | 14.8 | 49.7 % | 0.0005 | 0.0003 | 0.01 % |
| 9.8 | | | Double spray/wipe | 46.6 | 1 | 96.6 % | 0 | 0.0001 | 0.00 % |
| 9.9 | Post bleach/water clean EM | 8 | Bleach spray and wipe/Water spray and wipe | 43 | 0.375 | N/A | 0 | 0 | N/A |

**Table 28**
Experiment 9. Statistical comparison of residual DNA quantities after single spray/wipe cycle strategy verses duplicate spray/wipe strategy using an unpaired students t-test. Including a comparison of Chemgene HDL$_4$L and Chemgene Medlab results.

| Reagent/Spray or wipe | Replicates (DNA quantification ng/μl) | Average DNA quantification (ng/μl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T-test Outcome |
|---|---|---|---|---|---|
| Chemgene HDL$_4$L - single spray/wipe | 0.0002<br>0.0019<br>0.0123<br>0.0289<br>0.0047 | **0.0096** | **0.005** | 0.5180 | The difference is not statistically significantly different |
| Chemgene HDL$_4$L - *duplicate spray/wipe* | 0.0046<br>0.0027<br>0.0154<br>0.0025<br>0.0028 | **0.0056** | | | |

(*continued on next page*)

**Table 28** (*continued*)

| Reagent/Spray or wipe | Replicates (DNA quantification ng/μl) | Average DNA quantification (ng/μl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T-test Outcome |
|---|---|---|---|---|---|
| Chemgene Medlab - single spray/wipe | 0.0329<br>0.0570<br>0.0287<br>0.0740<br>0.0389 | **0.0463** | **0.020** | 0.0627 | The difference is not statistically significantly different |
| Chemgene Medlab - *duplicate spray/wipe* | 0.0280<br>0.0180<br>0.0434<br>0.0144<br>0.0164 | **0.0240** | | | |
| Presept single *spray/wipe* | 0.0007<br>0.0004<br>0.001<br>0.0003<br>0.0002 | **0.0005** | **0.000** | 0.0281 | The difference is statistically different |
| Presept - *duplicate spray/wipe* | 0<br>0<br>0<br>0<br>0.0002 | **0.0000** | | | |
| Microsol single *spray/wipe* | 0.0264<br>0.0444<br>0.0231<br>0.0735<br>0.0402 | **0.0415** | **0.024** | 0.0321 | The difference is statistically different |
| Microsol - *duplicate spray/wipe* | 0.0064<br>0.0048<br>0.0364<br>0.0137<br>0.0011 | **0.0125** | | | |
| Selgiene single *spray/wipe* | 0.0127<br>0.0082<br>0.0187<br>0.0107<br>0.0105 | **0.0122** | **0.006** | 0.0216 | The difference is statistically different |
| Selgiene - *duplicate spray/wipe* | 0.0019<br>0.0007<br>0.0012<br>0.0121<br>0.0052 | **0.0042** | | | |
| Virkon single *spray/wipe* | 0.0029<br>0.0036<br>0.0049<br>0.0039<br>0.0027 | **0.0036** | **0.001** | 0.1464 | The difference is not statistically significantly different |
| Virkon - *duplicate spray/wipe* | 0.0022<br>0.0046<br>0.0012<br>0.0022<br>0.0022 | **0.0025** | | | |
| Virusolve single *spray/wipe* | 0.0032<br>0.0039<br>0.0045<br>0.0105<br>0.0145 | **0.0073** | **0.005** | 0.1761 | The difference is not statistically significantly different |
| Virusolve – duplicate spray/wipe | 0.0085<br>0.0047<br>0.0207<br>0.0112<br>0.0249 | **0.0140** | | | |
| Comparison of Chemgene $HDL_4L$ with Chemgene Medlab results | | | | | |
| Chemgene $HDL_4L$ - single spray/wipe | 0.0002<br>0.0019<br>0.0123<br>0.0289<br>0.0047 | **0.0096** | **0.026** | 0.0084 | The difference is statistically different |
| Chemgene Medlab - single spray/wipe | 0.0329<br>0.0570<br>0.0287<br>0.0740<br>0.0389 | **0.0463** | | | |
| Chemgene $HDL_4L$ - *duplicate spray/wipe* | 0.0046<br>0.0027<br>0.0154<br>0.0025 | **0.0056** | **0.013** | 0.0226 | The difference is statistically different |

(*continued on next page*)

**Table 28** (*continued*)

| Reagent/Spray or wipe | Replicates (DNA quantification ng/μl) | Average DNA quantification (ng/μl) | Average DNA Quantification SD | Unpaired T test (p value = 0.05) | T-test Outcome |
|---|---|---|---|---|---|
| Chemgene Medlab - *duplicate spray/wipe* | 0.0028<br>0.0280<br>0.0180<br>0.0434<br>0.0144<br>0.0164 | **0.0240** | | | |

**Table 29**
Experiment 10. Experimental design and results for the additional mechanical action assessment of Virkon on vinyl dosed with semen.

| Experimental design | | | | | | | | Results | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Expt. No. | Cleaning Reagent | Reps | Wipe method | Contact time | Average peak height (RFU) | Average number alleles | Average DNA quantification (ng/μl) | DNA quantification standard deviation | % DNA quantification Yield | % Allele reduction |
| **7.12** | Control | 5 | N/A | N/A | 8175.39 | 29.4 | 4.5417 | 2.3791 | 100% | N/A |
| **7.6** | Virkon 1% | | Single spray circular wipe | 30 secs | 562.58 | 25.6 | 0.0036 | 0.0009 | 0.08% | 12.9% |
| **9.4** | | | Exp 9.4 Double spray/wipe | 2 x 30 secs | 311.48 | 12.2 | 0.0025 | 0.0013 | 0.06% | 59% |
| 10.1 | | | Unidirectional Linear | | 249.27 | 17 | 0.0029 | 0.0036 | 0.06% | 42% |
| 10.2 | | | 10.1 + cloth changes | | 336.68 | 18.6 | 0.0051 | 0.0040 | 0.11% | 37% |
| 10.3 | | | 9.4 + extra wipe dry | | 570.45 | 20.6 | 0.0058 | 0.0063 | 0.13% | 30% |
| 10.4 | Post bleach / water clean EM | 1 | Standard Cellmark | | 0.0 | 0 | 0.0000 | 0.0000 | 0.00 | N/A |

## References


1. Toom V, M'charek A, Wienroth M, eds. *Law, Practice and Politics of Forensic DNA Profiling: Forensic Genetics and Their Technolegal Worlds*. Abingdon, Oxon ; New York, NY: Routledge; 2023.
2. Alketbi SK. Emerging technologies in forensic DNA analysis. *Perspect Leg Forensic Sci*. Sep. 2024;1(1). https://doi.org/10.70322/plfs.2024.10007pp.10007–10007.
3. Haddrill P. Developments in forensic DNA analysis. *Emerg Topics Life Sci*. 2021;5(3): 381–393. https://doi.org/10.1042/ETLS20200304. ISSN 2397-8562.
4. Butler JM. *Recent advances in forensic biology and forensic DNA typing*: INTERPOL review 2019-2022. *Forensic Sci Int Synergy*. 2023;6, 100311. https://doi.org/10.1016/j.fsisyn.2022.100311.
5. Kampmann M-L, Børsting C, Morling N. Decrease DNA contamination in the laboratories. *Forensic Sci Int: Genetics Supplement Series*. 2017;6:e577–e578. https://doi.org/10.1016/j.fsigss.2017.09.223.
6. Ballantyne KN, et al. DNA contamination minimisation – finding an effective cleaning method. *Aust J Forensic Sci*. 2015;47(4):428–439. https://doi.org/10.1080/00450618.2015.1004195.
7. Nilsson M, De Maeyer H, Allen M. Evaluation of different cleaning strategies for removal of contaminating DNA molecules. *Genes*. 2022;13(1):162. https://doi.org/10.3390/genes13010162.
8. Forensic Science Regulator. *Lessons Learnt Evidence Handling Error*; 2019. Available at: Evidence handling error . Accessed October 25, 2025.
9. ISO. ISO 15189:2022. ISO Standard Medical lab Requirements Quality Competence. 2022. Edition 4. P.1-64. Available at: https://www.iso.org/standard/76677.html. [Accessed 13 October 2024].
10. *Forensic science regulator: code of practice. Forensic Science Activities: Statutory Code of Practice - GOV.UK*; 2023. . Accessed October 25, 2025.
11. ISO. ISO/IEC 17025:2017, ISO standard. *General Requirement Competence Test Calibration lab*; 2017. Available at: ISO/IEC 17025:2017 - General requirements for the competence of testing and calibration laboratories . Accessed October 25, 2025.
12. FSR-GUI-0020 Forensic Science Regulator Guidance: *Forensic Medical Examination of Sexual Offence Complainants*. Available at: Forensic medical examination of sexual offence complainants (FSR-GUI-0020) - GOV.UK (Accessed: 25 October 2025)..
13. FSR-GUI-0017 Forensic Science Regulator Guidance: *DNA Contamination Controls: Forensic Medical Examinations*. DNA contamination controls: forensic medical examinations (FSR-GUI-0017) - GOV.UK (Accessed: 25 October 2025)..
14. Brayley-Morris H, Sorrell A, Revoir AP, Meakin GE, Court DS, Morgan RM. Persistence of DNA from laundered semen stains: implications for child sex trafficking cases. *Forensic Sci Int: Genetics*. 2015;19:165–171. https://doi.org/10.1016/j.fsigen.2015.07.016.
15. Medina-Paz F, Kuba B, Kryvorutsky E, Roca G, Zapico SC. Assessment of blood and semen detection and DNA collection from swabs up to three months after deposition on five different cloth materials. *Int J Mol Sci*. 2024;25(6). https://doi.org/10.3390/ijms25063522, 3522–3522.
16. Gaskell M, Guiness G, Sullivan K. Understanding and mitigating the risks that environmental DNA contamination poses to the recovery of forensic evidence from victims and suspects of rape and sexual assault. *J Forensic Legal Med*. 2025;114, 102911. ISSN 1752-928X.
17. The Scottish Government. Forensic medical examinations: DNA decontamination guidelines - october 2019. https://www.gov.scot/publications/forensic-medical-examinations-dna-decontamination-guidelines/; 2019. Accessed October 27, 2025.
18. Faculty of Forensic & Legal Medicine. *Recommended Equipment for Obtaining Forensic Samples*. ARCHIVED-Recommended-equipment-for-obtaining-forensic-samples-FSSC-Jan-2023.pdf (Accessed: 25 October 2024)..
19. Kampmann M-L, Tfelt-Hansen J, Børsting C. Cleaning protocols in forensic genetic laboratories. *Int J Leg Med*. 2024;138(5):1787–1790. https://doi.org/10.1007/s00414-024-03232-0 [online].
20. Vandewoestyne M, Van Hoofstat D, De Groote S, Van Thuyne N, Haerinck S, et al. Sources of DNA contamination and decontamination procedures in the forensic laboratory. *J Forensic Res S*. 2011;2:1. https://doi.org/10.4172/2157-7145.S2-001, 10.4172/2157-7145.S2-001.
21. Gršković B, Zrnec D, Popović M, Petek MJ, Primorac D, Mršić G. Effect of ultraviolet C radiation on biological samples. *Croat Med J*. 2013;54(3):263–271. https://doi.org/10.3325/cmj.2013.54.263, 10.3325/cmj.2013.54.263.
22. Kampmann ML, Simonsen BT, Børsting C. Test of chlorine wipes for efficient removal of DNA from forensic genetics laboratories. *Forensic Sci Int: Genet Supplement Ser*. 2022;8:149–150. https://doi.org/10.1016/j.fsigss.2022.10.016. ISSN 1875 – 1768. 10.1016/j.fsigss.2022.10.016.
23. UK Government Legislation. The environmental protection (wet wipes containing plastic) (England) regulations 2025 draft SI. https://www.gov.uk/government/publications/the-environmental-protection-wet-wipes-containing-plastic-england-regulations-2025-draft-si; 2025. Accessed October 26, 2025.