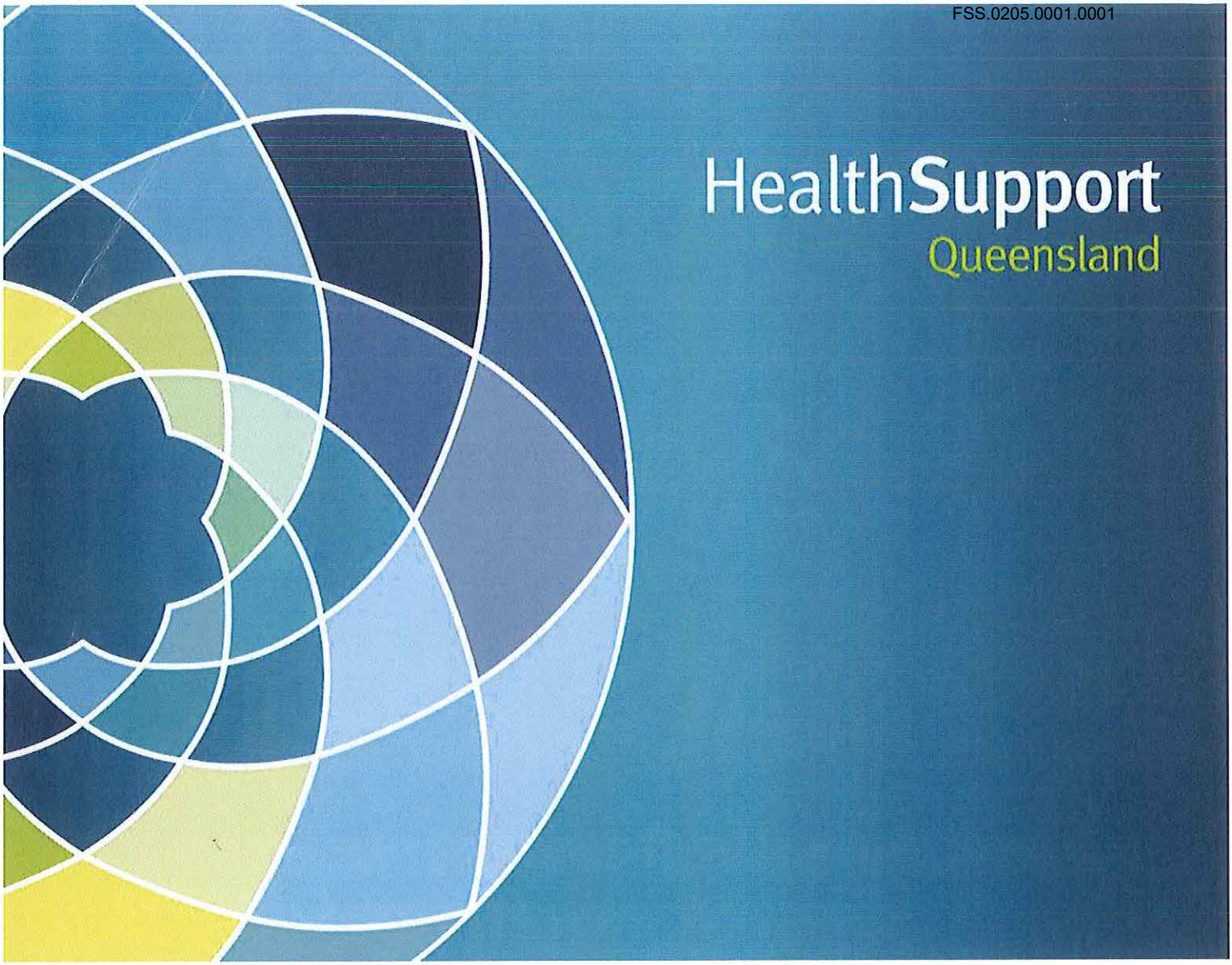

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Project#153 - Verification of Cleaning Reagents (Trigene Advance, Viraclean, Virkon, Pyroneg, Decon, Cavicide, F10SC) for use in Forensic DNA Analysis

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Forensic DNA Analysis, HSSA

April 2015

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1 Abstract

Within Forensic DNA Analysis, Trigene II has been used as an alternative cleaning agent to Bleach for corrosive surfaces. Due to changes in supply of Trigene II, Trigene Advance was used as a substitute. This project was carried out to test the effectiveness of Trigene Advance and a number of alternative cleaning agents.

The results of this project showed that Trigene Advance, Virkon and Viraclean were effective in decontaminating work surfaces. Testing also showed that the regime tested for removing cleaning agent residue was sufficient. Additionally, investigation of retrospective extraction control results showed that there has been no detectable effect on positive extraction control profiling or inhibition since the introduction of Trigene Advance.

2 Introduction

Forensic DNA Analysis has previously been using Trigene II (CEVA DEIVET Pty. Ltd., Seven Hills, NSW, AU) (at 5% v/v concentration) for routine decontamination as an alternative to 0.5% v/v bleach, which can cause pitting and corrosion of metallic apparatus and work surfaces. Trigene II is no longer available, however a substitute product Trigene Advance (CEVA DEIVET Pty. Ltd., Seven Hills, NSW,AU) is. Trigene Advance was first received by Forensic DNA Analysis in place of Trigene II on the 12/08/2013. As part of routine decontamination process within Forensic DNA Analysis, surfaces are first wiped with 5% v/v Trigene II or 0.5% v/v bleach to decontaminate the surface and this is followed by 70% v/v Ethanol to remove cleaning agent residue and allow for rapid drying of the surface. Additionally, 5% v/v Decon (Decon Laboratories Limited, East Sussex, UK) is currently being used in Forensic DNA Analysis to decontaminate racks & other labware by soaking followed by washing with hot water.

Previously, Trigene Advance has been noted to affect post PCR processing[1]. Other forensic laboratories use alternative liquid decontaminants and there are various products on the market. This project looked at a range of products, namely Viraclean (Whitely Medical, North Sydney, NSW, AU), Virkon (DuPont Rely-On Fairfield, OH 45014), Pyroneg (Thermo Fisher Scientific Australia Pty. Ltd., Scoresby, Vic, AU), Cavicide (Bacto Labs Pty Ltd Mt Pritchard, NSW, Australia 2170.) and F10SC (Chemical Essentials Mitcham North VIC 3132).

The aims of this project were two-fold. Firstly, to test the efficacy of Trigene Advance, Viraclean, Virkon, Pyroneg, Cavicide and F10SC against bleach as liquid surface decontaminants and the efficacy of post cleaning with 70% v/v ethanol as an effective method for removing residual cleaning agent. Secondly to determine if residual cleaning agents may affect DNA processing. Due to the previously noted affects of Trigene Advance, data mining of in-house positive extraction control results prior to the use of Trigene Advance (i.e. whilst Trigene II was used within the laboratory) and after the introduction of Trigene Advance was conducted.

3 Materials and Methods

The following resources were required for this project and are currently in use within the Analytical Section:

3.1 Materials

- 0.5 % v/v Brite N White bleach (Cleantec Laundry Systems, Darra, QLD, AU)
- 5 % v/v Trigene Advance (CEVA DEIVET Pty. Ltd., Seven Hills, NSW,AU)
- 1 % w/v Virkon® (DuPont™ Rely-On™ Fairfield, OH 45014)
- 5% Decon (Decon Laboratories Limited, East Sussex, UK)
- Viraclean® (Whitely Medical, North Sydney, NSW, AU)
- 1% Pyroneg (Thermo Fisher Scientific Australia Pty. Ltd., Scoresby, Vic, AU)
- 3% Cavicide (Bacto Labs Pty Ltd Mt Pritchard, NSW, Australia 2170.)
- 1% F10SC (Chemical Essentials Mitcham North VIC 3132)
- Proteinase K (20mg/mL) (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Dithiothreitol (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Ethanol (Recochem Incorporated, Wynnum, QLD,AU)
- 0.2 and 1 % v/v Amphyl (Reckit Benckiser Pty. Ltd., West Ryde, NSW, AU)
- Sarcosyl (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Nanopure water (Forensic DNA Analysis, Brisbane, QLD, AU)
- Positive controls (Forensic DNA Analysis, Brisbane, QLD, AU)
- Positive control donor blood (CJA) (Forensic DNA Analysis, Brisbane, QLD, AU)
- TNE (Forensic DNA Analysis, Brisbane, QLD, AU)
- Hi-Di™ Formamide (Life Technologies Applied Biosystems, Foster City, CA, US)
- 3130 POP-4™ Polymer (Life Technologies Applied Biosystems, Foster City, CA, US)
- Running Buffer (Life Technologies Applied Biosystems, Foster City, CA, US)
- Promega PowerPlex® 21 system (Promega Corp., Madison, WI, US)
- Promega CC5 Internal Lane Standard 500 (Promega Corp., Madison, WI, US)
- Promega PowerPlex 5 Dye Matrix Standard (Promega Corp., Madison, WI, US)

- Promega PowerPlex® 21 Allelic Ladder Mix (Promega Corp., Madison, WI, US)
- 2800M Control DNA, 10ng/µl (Promega Corp., Madison, WI, US)
- Water amplification grade (Promega Corp., Madison, WI, US)
- DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega Corp., Madison, WI, US)
- 96-well PCR micro-plates (Axygen Scientific Inc., Union City, CA, US)
- Tape pads (Qiagen Pty. Ltd., Doncaster, VIC, AU)
- 96-well plate Septa mats (Life Technologies Applied Biosystems, Foster City, CA, USA)
- Sterile 1.5 mL & 2 mL screw-cap tubes (Axygen Scientific Inc., Union City, CA, US)
- Sterile 5 mL & 10 mL screw-cap tubes (Axygen Scientific Inc., Union City, CA, US)
- ART Filtered 1000, 300 & 20p pipette tips (Molecular BioProducts Inc., San Diego, CA, US)
- One Touch filtered 10 µL and 200 µL pipette tips (Quantum Scientific Lab Advantage, Murrarie, QLD, AU)
- Rediwipes (Cello Paper Pty. Ltd., Fairfield, NSW, AU)
- 96-well PCR plates(Axygen Inc. Union City, CA, US)
- Plate septas (Axygen Inc. Union City, CA, US)
- Adhesive film (QIAGEN, Hilden, DE)
- Sterile conductive filtered Roborack 25µL & 175µL disposable tips (PerkinElmer, Downers Grove, IL, USA)
- MicroAmp® optical 96-well reaction plates (Life Technologies Applied Biosystems, Foster City, CA, US)
- MicroAmp® optical adhesive film (Life Technologies Applied Biosystems, Foster City, CA, US)
- Nunc CryoTubes Bank-It Cryobank Vials (Thermo Fisher Scientific Australia Pty. Ltd., Scoresby, Vic, AU)
- Petri dishes (Starstedt Australia Pty. Ltd., Mawson Lakes, SA, AU)
- Sterile rayon swabs (Copan Diagnostics Inc., Murrieta, CA, USA)
- Hot-block (Ratek Instruments Pty. Ltd., Boronia, VIC, AU)
- Biological safety cabinets class II (Westinghouse Pty. Ltd., Newport, AU)
- Refrigerators and freezers (Westinghouse Pty. Ltd., AU)
- GeneMapper-IDX ver.1.1.1 (Life Technologies Applied Biosystems, Foster City, CA, USA)

- AB 7500 Real Time PCR System (Life Technologies Applied Biosystems, Foster City, CA, US)
- GeneAmp PCR system 9700 (Life Technologies Applied Biosystems, Foster City, CA, USA)
- ABI 3130xl Genetic Analyzer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- STORstar instrument (Process Analysis & Automation, Hampshire, GB)
- MultiPROBE II PLUS HT EX with Gripper Integration Platform (PerkinElmer, Downers Grove, IL, US)
- Thermomixer (Eppendorf AG, Hamburg, DE)
- MixMate (Eppendorf AG, Hamburg, DE)
- Vortex (Ratek Instruments Pty Ltd, Melbourne, VIC, AU)
- Pipettes (Eppendorf, Hamburg, DE and Thermo Fisher Scientific (Finnpipette), Waltham, MA, US)
- 50, 200 and 1000µl Pipettes (Finnpipette® Thermo Fisher Scientific Australia Pty. Ltd., Scoresby, Vic, AU)
- Promega Maxwell® 16 MDx 1 and 2 Instruments (Promega Corp., Madison, WI, USA)
- Milli-Q® Integral 3 (A10) System with Q-POD™ (Millipore™, Billerica, MA, US)
- Minifuge (CS Bio Co. (ex-Tomy Tech US Inc.), Menlo Park, CA, US)
- Tube Centrifuge (Eppendorf South Pacific Pty. Ltd., North Ryde, NSW, AU)
- Plate Centrifuge (Eppendorf South Pacific Pty. Ltd., North Ryde, NSW, AU)

3.2 Methods (Experiment 1 – testing the efficacy of cleaning agents)

3.2.1 Preparation of cleaning agents

Each cleaning reagent was made fresh, with the exception of Viraclean, Bleach and Decon. Viraclean was received ready to use, Bleach and Decon that was previously prepared for routine use within the laboratory was utilised. Stock cleaning reagents were prepared to a working concentration as outlined in Table 1 below.

Table 1 Cleaning reagent preparation.

Reagent	Working concentration	Amount of cleaning agent	Amount (mL) of nanopure water
Trigene Advance	5%	50 mL	950
Bleach	5%	500 mL	9500
Decon	5%	50 mL	950
Virkon	1%	5 g	500
Pyroneg	1%	5 mL	495
Cavicide	3%	15 mL	485
F10SC	1%	5 mL	495
Ethanol	70%	350 mL	150

3.2.2 Sample preparation

Onto a clean petri dish, 1 µL of whole blood from a known donor was spotted. This was allowed to air-dry for approximately 30 minutes. 40 petri dishes were prepared in this manner. For each cleaning agent, 5 petri dishes of the contaminated were cleaned by wiping the area with a rediwipe cleaning cloth moistened with the cleaning agent under test, followed by wiping the contaminated area with a fresh rediwipe moistened with 70% v/v ethanol.

For each of the cleaning agents under test, a petri dish was cleaned using the process outlined above with no prior addition of whole blood.

For cleaning agents Trigene Advance, Viraclean, Virkon, Pyroneg, Cavicide and F10SC, a petri dish was firstly cleaned by wiping the surface with a rediwipe cleaning cloth moistened with the cleaning agent under test. No removal of residual cleaning agent was conducted. 1 µL of whole blood from a known donor was spotted onto the cleaned surface and allowed to dry. Samples were not done for bleach and decon under this test due to them already being in use in the laboratory. We have experienced no inhibition from these two cleaning agents within our laboratory.

Table 2 below summarises the treatment of each petri dish.

For each of the 54 petri dishes prepared as above, the area under test was sampled using wet and dry rayon swabs according to the following:

- first sampled using a swab moistened with nanopure water
- then sampled using a dry swab

An additional petri dish (that was not subjected to any treatment) was also sampled. The wet and dry swabs for each dish were prepared and combined into a single tube and labelled with a unique identifying number for further processing as outlined below.

Table 2 Summary of various testing regimes (Sample 49 is negative control).

Dish number	Laboratory number	Blood spot	Trigene Advance	Bleach	Decon	Viraclean	VirkonS	Pyroneg	Cavicide	F10SC	Ethanol
1	[redacted]	+	+								+
2		+	+								+
3		+	+								+
4		+	+								+
5		+	+								+
6		+		+							+
7		+		+							+
8		+		+							+
9		+		+							+
10		+		+							+
11		+			+						+
12		+			+						+
13		+			+						+
14		+			+						+
15		+			+						+
16		+				+					+
17		+				+					+
18		+				+					+
19		+				+					+
20		+				+					+
21		+					+				+
22		+					+				+
23		+					+				+
24		+					+				+
25		+					+				+
26		+						+			+
27		+						+			+
28		+						+			+
29		+						+			+
30		+						+			+
31		+							+		+
32		+							+		+
33		+							+		+
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35		+							+		+
36		+								+	+
37		+								+	+
38		+								+	+
39		+								+	+
40		+								+	+
41			+								+
42				+							+
43					+						+
44						+					+
45							+				+
46								+			+
47									+		+
48										+	+
49											
50		+	+								
51		+				+					
52		+					+				
53		+						+			
54		+							+		
55		+								+	

3.2.3 Sample extraction, quantification and profiling

Samples prepared according to method above were then subjected to the following routine laboratory procedures:
- DNA extraction (SOP 29344 DNA IQ™ Extraction using the Maxwell®16)
- DNA quantification (SOP 19977 Quantification of Extracted DNA using the Quantifiler Human DNA Quantification Kit)
- STR DNA amplification (SOP 31511 Amplification of Extracted DNA using the PowerPlex ®21 System)
- Capillary electrophoresis (SOP 15998 Use and Maintenance of the 3130*x*l Genetic Analyzers).

3.2.4 Data analysis

All samples were analysed using Genemapper IDX (analysis parameters set to 16RFU peak detection threshold and no stutter filters). All results were exported into Microsoft EXCEL from which tables and figures were produced.

3.3 Method - (Experiment 2 – data mining of historical data)

The quantification and IPC CT values of 50 positive extraction controls that were extracted using the Maxwell 16 instrument (see section 3.2.3 above) when Trigene II was in use (extracted prior to 12/08/2013) were compared to 50 positive extraction controls that were extracted using the Maxwell 16 instrument (see section 3.2.3 above) after the introduction of Trigene Advance into the laboratory (extracted after 12/08/2013).

The data was exported from the LIMS into Microsoft EXCEL and values were compared using the student's *t*-test (T-TEST function, 2 tailed distribution, two sample unequal variance).

4 Results and Discussion

4.1 Experiment 1 – testing the efficacy of cleaning agents

For the samples 1 - 40 prepared, quantification and DNA profiling results are shown in Table 3 below.

Table 3 Samples 1-40 (donor sample added and cleaned)

Dish number	Laboratory number	Cleaning Agent	Quantifcation value (ng/µL)	IPCCT	# alleles < LOR / ≥ LOR
1	[redacted]	Trigene Advance	Undetermined	27.37	No DNA profile
2		Trigene Advance	Undetermined	27.34	No DNA profile
3		Trigene Advance	Undetermined	27.23	No DNA profile
4		Trigene Advance	Undetermined	27.23	1/0
5		Trigene Advance	Undetermined	27.22	No DNA profile
6		Bleach	Undetermined	27.27	No DNA profile
7		Bleach	Undetermined	27.32	No DNA profile
8		Bleach	Undetermined	27.57	1/0
9		Bleach	Undetermined	27.41	No DNA profile
10		Bleach	Undetermined	27.4	No DNA profile
11		Decon	Undetermined	27.32	No DNA profile
12		Decon	Undetermined	27.37	No DNA profile
13		Decon	Undetermined	27.34	No DNA profile
14		Decon	Undetermined	27.37	No DNA profile
15		Decon	Undetermined	27.40	No DNA profile
16		Viraclean	Undetermined	27.56	No DNA profile
17		Viraclean	Undetermined	27.52	No DNA profile
18		Viraclean	Undetermined	27.52	No DNA profile
19		Viraclean	Undetermined	27.41	1/0
20		Viraclean	Undetermined	27.48	No DNA profile
21		Virkon	Undetermined	27.42	No DNA profile
22		Virkon	Undetermined	27.52	No DNA profile
23		Virkon	Undetermined	27.44	No DNA profile
24		Virkon	Undetermined	27.5	No DNA profile
25		Virkon	0.00102	27.43	No DNA profile
26		Pyroneg	Undetermined	27.44	4/0
27		Pyroneg	0.00102	27.4	2/0
28		Pyroneg	Undetermined	27.38	No DNA profile
29		Pyroneg	Undetermined	27.41	2/0
30		Pyroneg	Undetermined	27.42	3/0
31		Cavicide	Undetermined	27.45	1/0
32		Cavicide	Undetermined	27.44	1/0
33		Cavicide	Undetermined	27.28	No DNA profile
34		Cavicide	0.00053	27.31	No DNA profile
35		Cavicide	Undetermined	27.31	3/1
36		F10SC	Undetermined	27.34	1/0
37		F10SC	Undetermined	27.33	1/0
38		F10SC	Undetermined	27.35	5/0
39		F10SC	0.001	27.42	1/0
40		F10SC	Undetermined	27.33	2/0

As can be seen from Table 3, all samples tested showed quantification values less than the in-house derived limit of detection (0.00214 ng/µL). For Trigene Advance, Bleach and Viraclean, one allele below reporting threshold was noted in one sample out of five. For Virkon and Decon, no alleles were present in all five samples above or below reporting threshold. For the remaining cleaning agents, more than one test sample showed the presence of DNA, with F10SC showing the presence of DNA in all test samples. This is shown in Figure 1 below.

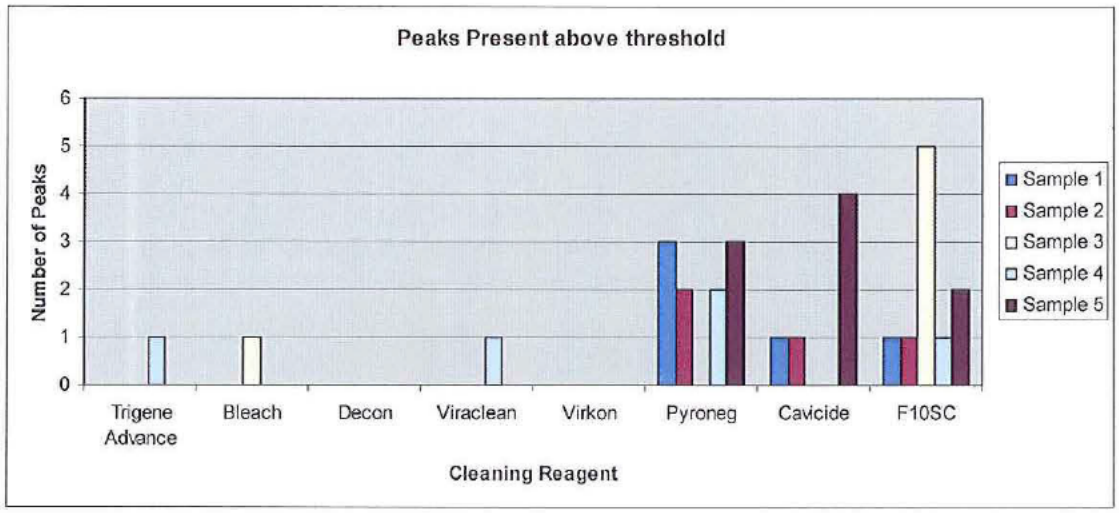

Figure 1 Number of allelic peaks in each sample for each cleaning reagent

For Pyroneg, Cavicide and F10SC, although at a low level, the amount of DNA present indicates that these 3 reagents would not be recommended for regular use within the Forensic DNA Analysis Laboratory.

Table 4 below shows the results obtained from petri dishes 41 – 49, where dishes 41-48 were each cleaned without the addition of donor blood and dish 49 was an untreated plate.

Table 4 Samples 41-48 (no donor sample added and cleaned) and 49 (no treatment)

Dish number	Laboratory number	Cleaning Agent	Quantifcation value (ng/µL)	IPCCT	# alleles ≥ LOR / < LOR
41	[redacted]	Trigene Advance	Undetermined	27.20	No DNA profile
42		Bleach	Undetermined	27.23	No DNA profile
43		Decon	Undetermined	27.20	No DNA profile
44		Viraclean	Undetermined	27.20	No DNA profile
45		Virkon	Undetermined	27.20	No DNA profile
46		Pyroneg	Undetermined	27.20	No DNA profile
47		Cavicide	Undetermined	27.28	No DNA profile
48		F10SC	Undetermined	27.16	No DNA profile
49		Nil	Undetermined	27.15	No DNA profile

As per manufacturer specifications, IPC CT values of ≥ 30 from quantification indicate the presence of inhibitors. These results indicate that the cleaning regime employed did not leave sufficient cleaning agent residue to produce detectable PCR inhibition. Additional to this, the presence of detectable DNA, IPC CT values of < 30 and expected DNA profiles following cleaning and the addition of donor sample (see Table 5 below) also indicate that the amount of cleaning agent employed does not leave sufficient residue to be detectable in PCR post sampling.

Table 5 Samples 50-55 (cleaning followed by donor sample addition)

Dish number	Laboratory number	Cleaning Agent	Quantifcation value (ng/µL)	IPCCT	DNA profile	Comment
50	[redacted]	Trigene Advance	0.123	27.11	Full Profile	Expected profile
51		Viraclean	0.0846	27.08	Full Profile	Expected profile
52		Virkon	0.0749	27.09	Full Profile	Expected profile
53		Pyroneg	0.186	27.05	Full Profile	Expected profile
54		Cavicide	0.117	27.12	Full Profile	Expected profile
55		F10SC	0.101	27.17	Full Profile	Expected profile

In table 5, samples 50-55 resulted in a full profile as we expected. There was no sign of degradation with any of these profiles. Figure 2 below shows the quantification internal positive control results for plates 41, 44-48 (cleaned then sampled) and 50-55 (cleaned, known blood added then sampled).

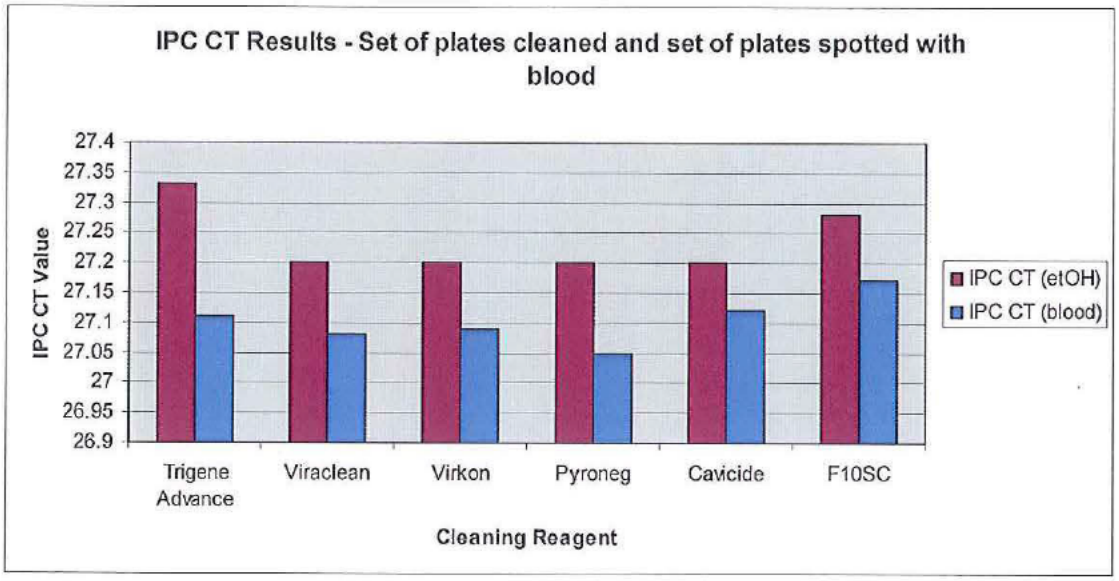

Figure 2 **IPC CT results of dishes cleaned with cleaning reagent and dishes that were spotted with 1uL positive control blood.**

The internal positive control CT results are all below 30, indicating no detectable inhibition. There is a trend where for all results shown in Figure 2, the IPC CT for those plates cleaned with the cleaning agent followed by 70% v/v ethanol is higher in all cases, compared to those plates that were cleaned with cleaning agent only followed by the addition of the known blood sample. As discussed above, the results for samples 50-55 as shown in Table 5 indicate there has been no inhibition of the downstream processing by the cleaning agent; therefore, there may be a small inhibitory effect from the post cleaning treatment with 70% v/v ethanol. However, the IPC CT differences are quite small.

All samples were compared to the results obtained for bleach by carrying out a *t*-test as seen in Table 6 below.

Table 6 ***t*-test results of each cleaning agent compared to Bleach**

Cleaning Agent	*t*-test Results
Trigene Advance	0.097
Viraclean	0.119
Virkon	0.268
Pyroneg	0.772
Cavicide	0.582
F10SC	0.491

The results from the *t*-test, as seen in Table 6 above, shows that the IPC CT values for all cleaning agents are not significantly different when compared with bleach. All IPC CT results were below 30 which indicate each of the cleaning agents have no inhibitory effect on the DNA processes used within this laboratory.

4.2 Experiment 2

The average quantification values for 50 positive extraction controls before using Trigene Advance were compared to 50 positive extraction controls quantification values after Trigene Advance was implemented. Results from the *t*-test are seen in Table 7 below.

Table 7 ***t*-test results before and after introduction of Trigene Advance**

T-test Quantification	0.7751080
T-test IPC CT	0.0000424

The results from the *t*-test showed that the quantification values of the 50 positive extraction controls prior to the use of Trigene Advance are not significantly different (0.775) to after Trigene Advance was implemented as seen in Table 7 above.

The IPC CT values, however, were significantly different prior to and after Trigene Advance was introduced. Note, all IPC CT values were less than 30 as per manufacturer's specifications and indicate there was no inhibition by either cleaning agent. However, the trend of lower IPC CT values from post Trigene Advance implementation indicates that, if there is an effect, that Trigene II may have had a slight inhibitory effect on the quantification reaction compared to Trigene Advance. It must be noted though, that laboratory staff have not noted a change in positive extraction control appearance, where for both time periods, positive extraction control DNA profiles have not shown any indication of any PCR inhibition.

5 Conclusions and Recommendations

The findings of this project do not show any indication that Trigene Advance has any inhibitory effect on current DNA profiling processes when used in the manner described and that there has been no discernable change in positive extraction control outcomes since the introduction of this cleaning agent into the laboratory. Comparisons to the other reagents were shown that Trigene Advance performs as well as or better than the other agents tested. Therefore, the results support the continued use of Trigene Advance within the laboratory.

1. Continue using Trigene Advance in the laboratory as a liquid decontaminant
2. Continue using 70% Ethanol to remove any cleaning reagent residue
3. Results show Virkon is an acceptable alternative as a cleaning reagent to use within the laboratory
4. Pyroneg, Cavicide and F10 SC are not recommended for use as cleaning agents.

6 Abbreviations

LOD = Limit of Detection

LOR = Limit of Reporting

RFU = Relative Fluorescence units

7 References

1. Bright, J.-A., Cockerton, S., Harbison, S., Russell, A., Samson, O. and Stevenson, K. (2011), *The Effect of Cleaning Agents on the Ability to Obtain DNA Profiles Using the Identifiler™ and PowerPlex® Y Multiplex Kits*. Journal of Forensic Sciences, 56: 181–185.
2. Kaity, A., Mathieson, M., Ryan, L., Allen, C., *Project Proposal #153 Verification of Trigene Advance, Viraclean and Virkon S.* 2014

Note: Virkon is an "orange" product in Chemalert while Trigene Advance is a "green" product. Due to Trigene having fewer health hazards for use than Virkon: Virkon is not considered suitable to replace Trigene Advance at this time. KIRSTEN SCOTT [redacted] 19/06/2015