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SCIENCE SERVING JUSTICE

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Project Information

Title: Comparison Study of Disinfectants for Decontamination

Evaluation Type: Comparison Study

Stakeholder: Forensic Science Community

Start Date: 05/15/2011 End Date: 07/12/2011

Manufacturer Information

Product: STERIPLEX SD

Manufacturer: sBioMed® LLC

Phone Number: (801) 922-1111

Internet address: http://www.steriplex.com/

Stakeholder Information

Contact Person: Glen Rose

Phone Number: (801) 885-1085

E-mail: glenrose@simplexbio.com

Product: Pure Blu

Manufacturer: Enamelite LLC
dba Genesis Laboratories LLC

Phone Number: 866-741-7171

Internet address: (website under development)

Contact Person: James Vinson

Phone Number: (931) 320-1471

E-mail: jvinson@enamelitellc.com

Evaluation Team

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Evaluation Summary

The purpose of this study was to evaluate the effectiveness of two disinfectants as an alternative to bleach for sterilizing forensic laboratory work surfaces and instrumentation to remove body fluids, extracted DNA and amplified DNA.

Currently the most effective disinfectant used to sterilize surfaces and instruments in a forensic laboratory is a 10% chlorine bleach solution. This solution is made daily, applied to surfaces and wiped off. While bleach solution has been shown to be effective in removing biological fluids, extracted DNA and amplified DNA, there are certain

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drawbacks. Diluted bleach loses its effectiveness after 24 hours. The bleach itself will begin to degrade after being stored for 6 months. Apart from the shelf life, bleach is corrosive; when used on surfaces or on instruments, it should be followed up with distilled or purified water to stop the formation of crystals and to reduce its corrosive effects. The use of a bleach solution requires extra time to prepare fresh dilutions daily; this puts a strain on laboratory personnel to keep up with this task since several bottles of bleach solution are required daily.

Clorox® Regular Bleach was used for this study.

STERIPLEX SD by sBioMed® LLC is a disinfectant that is currently used to destroy pathogens, both spores and bacteria. The solution comes in two parts. The manufacturer states that once activated, it is sporicidal and bactericidal for 60 days. Should this product prove effective in removal of biological fluids and DNA, then at a minimum it will only need to be replaced every 2 months as opposed to every day for the bleach solution. STERIPLEX SD also has an MSDS health rating of "0" and has no toxic fumes, no emissions into the environment and no corrosive chemicals that damage equipment or tools.

Pure Blu by Genesis Laboratories LLC is a disinfectant with anti-bacterial and anti-microbial properties that is safe for the environment, does not use harsh chemicals or known carcinogens, will not stain clothing and has a color-change indicator. Pure Blu also claims to quickly disinfect surfaces. Currently Pure Blu is being marketed for the removal of bacteria and germs.

Product Specifications

Photos

Clorox Regular Bleach

STERIPLEX SD
(Note: wipes not evaluated)

Pure Blu

Product Details

Clorox bleach: Lot Number: A41134
Cost: $3.00 for a gallon of pure bleach; $2.00 for spray bottle. Note: A dual sprayer is available for $70.60; bleach cartridges for the dual sprayer cost $81.44 for 12.
Associated costs: Diluting pure bleach requires daily labor to make new batches, since bleach breaks down and loses its effectiveness within 24 hours of being diluted.

The available dual sprayer has been found to seize up and stop working within a few months; this will require repurchasing of new dual sprayers at $70.60 each.
Storage Conditions: Room Temperature
Operational Conditions: Room Temperature

STERIPLEX SD: Cost: $44.95 for 1 gallon; $2.00 for a generic spray bottle. Note: STERIPLEX-branded spray bottles can be purchased from sBioMed at a cost of $3.95 each.
Associated costs: None
Storage Conditions: Room Temperature
Operational Conditions: Room Temperature

Pure Blu: Cost: To be determined (TBD) for 50 mL spray bottle; other sizes/costs TBD.
Associated costs: None
Storage Conditions: Room Temperature
Operational Conditions: Room Temperature

Other Materials

Applied Biosystems Quantifiler® Duo Kit

AmpFℓSTR® Identifiler® Plus Kit

Qiagen EZ1® DNA Investigator Kit

Qiagen MinElute® PCR Purification Kit

Tris Edta (TE) Buffer

Level of Operator Knowledge (Set per Manufacturer)

☒ Non-Scientist ☐ Technician ☐ Scientist

Procedure

No Mechanical Wiping

The following method was used to evaluate the effectiveness of the disinfectants without the mechanical action of wiping.

The first part of this experiment was to determine how effective each disinfectant is in the destruction of DNA without wiping. Samples of blood, extracted DNA and amplified DNA were added to the disinfectants and allowed to remain exposed to them for controlled times. At the end of this exposure, the samples were assessed to determine how much DNA was left.

Blood: To determine the ability of the disinfectants to destroy DNA in blood (biological fluids), all of the experiments were performed in triplicate. The volume of blood used was 20 µl.

It was necessary to first determine the ratio of volume of blood to volume of disinfectant needed to see a reduction in the DNA in the blood.

- 20 µl of blood was added to 20 µl of each disinfectant to give a 1 part sample to 1 part disinfectant
- 20 µl of blood was added to 40 µl of each disinfectant to give a 1 part sample to 2 parts disinfectant
- 20 µl of blood was added to 100 µl of each disinfectant to give a 1 part sample to 5 parts disinfectant
- 20 µl of blood was added to 200 µl of each disinfectant to give a 1 part sample to 10 parts disinfectant

The 10% bleach solution and STERIPLEX SD were allowed to remain in contact with the blood for 5 minutes and the Pure Blu was allowed to remain in contact with blood for 10 minutes (in accordance with manufacturer recommendations). After these times the samples were put through the quantitation process to determine if there was any reduction in the DNA content of any of the samples.

Extracted DNA: DNA from 20 µl of blood was extracted using the QIAGEN BioRobot EZ1 Workstation to a final volume of 50 µl. 10 µl of the extract was used to test the disinfectants in the following concentrations:

- 1 part sample to 5 parts disinfectant
- 1 part sample to 10 parts disinfectant

The disinfectants were allowed to remain in contact with the sample for the same time periods as before: 5 minutes for 10% bleach solution and STERIPLEX SD and 10 minutes for Pure Blu. At the end of the time period, the samples were put through the quantitation process. Since there was a possibility that the concentration of DNA would be less simply by the addition of liquid, which would dilute the sample, a TE buffer was added to a separate set of “control” extracts in the same concentrations: 1 part to 5 parts and 1 part to 10 parts.

Amplified DNA: Samples of amplified DNA were added to disinfectants in the same ratios of 1 part to 5 parts and 1 part to 10 parts and left in contact with the amplified DNA for the same times as mentioned above. 1 µl of this mixture was then added to the Applied Biosystems 3130xl Genetic Analyzer. As a control, the amplified DNA was also added to the same volumes of TE buffer to ensure any reduction in DNA was not due only to dilution.

Mechanical Wiping

The following method was used to evaluate the effectiveness of the disinfectants when accompanied by the mechanical action of wiping.

Blood: For this experiment, cream-colored ceramic tiles previously cleaned and exposed to UV light for >5 hours were used. First 20 µl of blood was placed on the tiles. A quantity of three sprays of disinfectant was applied to each tile, and the samples were allowed to sit 5 minutes for bleach solution and STERIPLEX SD and 10 minutes for Pure Blu. After this exposure, a clean paper towel was used to thoroughly wipe the stain until no visible staining could be seen on the tile. The tiles were then swabbed with a sterile cotton swab.

Extracted DNA: Control samples of 20 µl of blood were applied to clean tiles to assess how much DNA would have been retrieved using the swab. The swabs were extracted using the EZ1 DNA Investigator Kit Tip Dance protocol with a final volume of 50 µl.

Amplified DNA: For amplified DNA, cream-colored ceramic tiles previously cleaned and exposed to UV light for >5 hours were used. 25 µl of amplified DNA was added to the tiles; the target of the amplification was 1 ng/µl. A quantity of three sprays of disinfectant was applied to each tile and allowed to remain in contact with the sample

for the allotted times of 5 minutes for bleach solution and STERIPLEX SD and 10 minutes for Pure Blu. After this exposure, a swab was moistened with sterile water and the entire tile was swabbed. Before the swab could dry, the swab was cut and placed in a *Spin-EASE*™ basket and spun for 5 minutes at 15,000 rpm. The resulting liquid was put through the QIAGEN MinElute® cleanup method.

Findings

Blood with No Wiping Results

The table below presents the results of the no wiping tests. As the data in the table show, STERIPLEX SD disinfectant was most effective when used on blood samples at 1 part sample to 10 parts disinfectant. Based on these results, in all remaining experiments, 1 part sample to 5 parts disinfectant and 1 part sample to 10 parts disinfectant were tested.

Table 1. Results of the Blood with No Wiping Tests

Volume added to 20 µl of blood (µl)	Control (No disinfectant) (ng/µl)	Bleach solution (ng/µl)	STERIPLEX SD (ng/µl)	Pure Blu (ng/µl)	Result
20	4.99	5.07	4.53	5.72	1 part blood to 1 part disinfectant – No reduction in DNA
20	4.66	5.87	4.35	5.25	
20	4.38	6.07	4.49	5.69	
40	12.60	11.20	15.10	20.00	1 part blood to 2 parts disinfectant – No reduction in DNA
40	13.50	11.40	12.20	18.90	
40	15.70	11.60	16.70	17.50	
100	12.60	5.45	0.46	3.37	Bleach solution: 1 part blood to 5 parts disinfectant – 2-fold reduction in DNA
100	13.50	7.42	0.81	5.63	STERIPLEX SD: 1 part blood to 5 parts disinfectant – 30-fold reduction in DNA
100	15.70	7.30	0.35	4.36	Pure Blu: 1 part blood to 5 parts disinfectant – 3-fold reduction in DNA
200	12.60	1.78	0.26	2.53	Bleach solution: 1 part to 10 parts disinfectant – 7-fold reduction in DNA
200	13.50	6.63*	0.30	1.42	STERIPLEX SD: 1 part to 10 parts disinfectant – 47-fold reduction in DNA
200	15.70	1.78	0.30	1.14	Pure Blu: 1 part to 10 parts disinfectant – 9-fold reduction in DNA

* Result was not concordant with other results in triplicate set

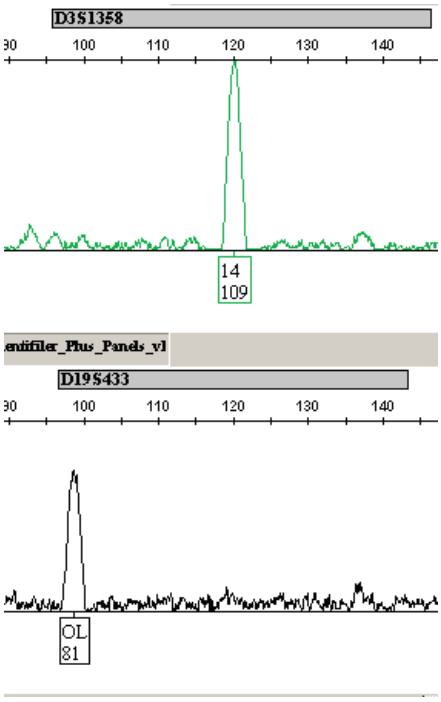
Extracted DNA with No Wiping Results

At the end of the quantitation, all of the samples mixed with disinfectants gave a negative quantitation result. This at first appeared to be successful destruction of the extracted DNA; however, on closer inspection it was determined that the disinfectants interfered with the polymerase chain reaction (PCR) process. This was seen by the action of the internal PCR control (IPC) when compared to samples that were mixed with a buffer at the same concentrations.

- The samples mixed with bleach solution did not have any IPC cross the threshold.
- In samples mixed with STERIPLEX SD, the IPC did cross, but it crossed 2 cycles after the sample with a buffer.
- In samples mixed with Pure Blu, the IPC did cross, but it crossed 7 cycles after the sample with a buffer.

This means that all the disinfectants inhibit the PCR process, with bleach solution being the most inhibitive, followed by Pure Blu. STERIPLEX SD causes the least amount of inhibition.

As a follow-up, the experiment was repeated and the samples were amplified using AmpFℓSTR® Identifiler® Plus PCR Amplification Kit and run on the 3130xl Genetic Analyzer. The samples were all treated as 0 ng/µl samples, so the full sample amount of 10 µl was added to the amplification tubes. Once again, all of the samples gave negative results. However, samples mixed with STERIPLEX SD had two anomalies. At the D3S1358 locus a peak designated a 14 by the GeneMapper software appeared in all electropherograms; the RFUs ranged from 109 to 155. Also, there was an off-ladder (OL) peak at approximately 98 base pairs (bp) just before the D19S433 locus that was also reproducible in all samples. These two anomalies are shown in the figure below.



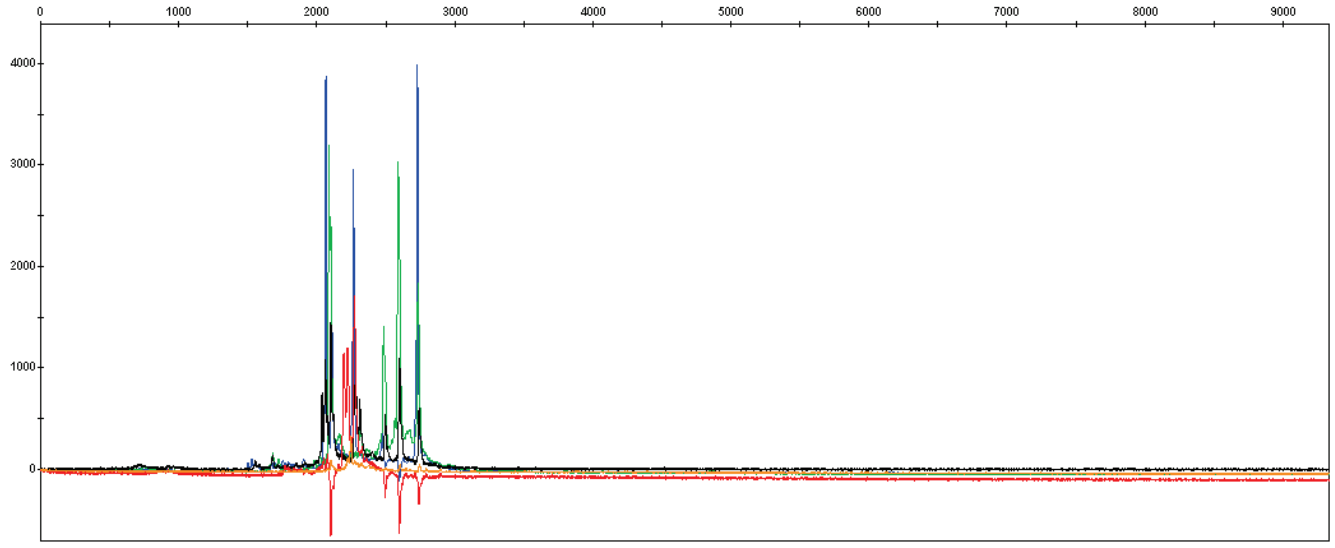
STERIPLEX SD Anomalies at the D3S1358 and D19S433 Loci

Neither of these peaks corresponded to the DNA sample used or the DNA profile of the person conducting the experiments. It is possible that a component of the STERIPLEX SD disinfectant could be causing fluorescence even though there is no DNA present.

Amplified DNA with No Wiping Results

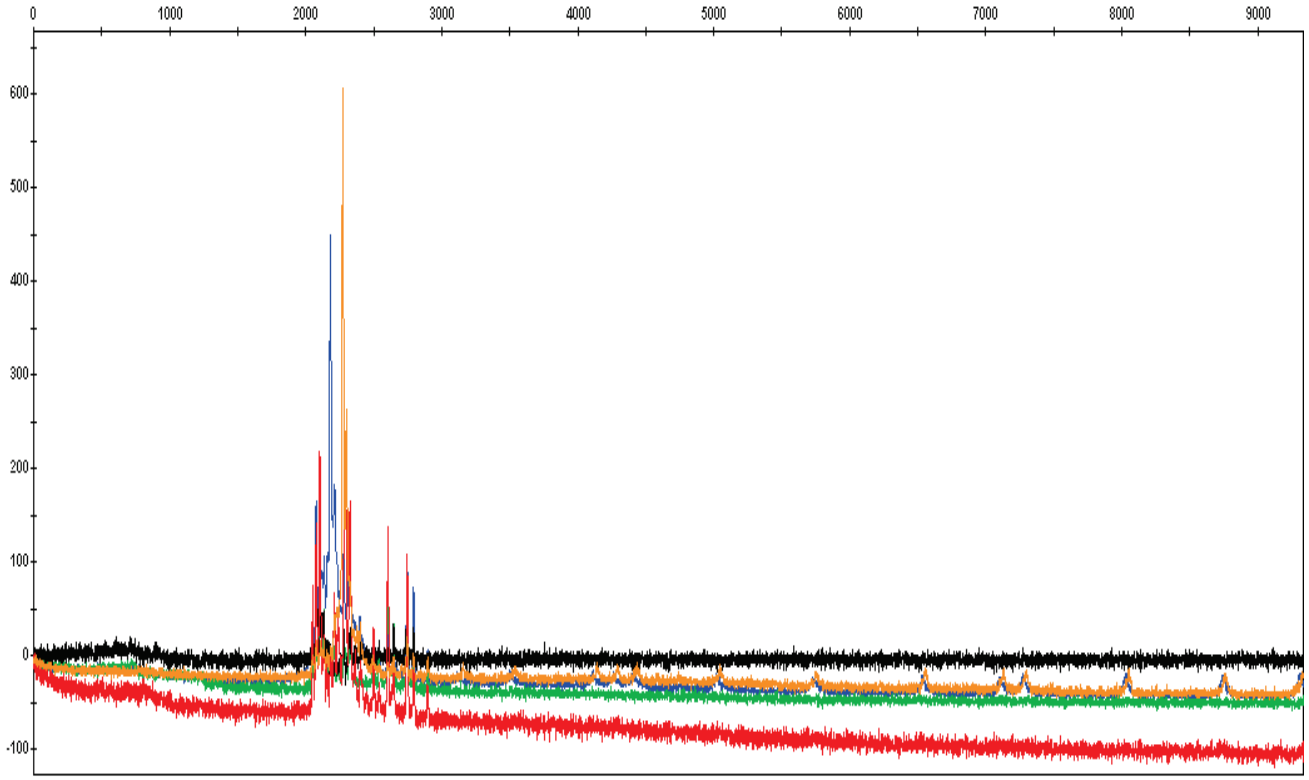
Data from the bleach solution and STERIPLEX SD showed that these disinfectants completely inhibited the entire electrophoresis process. The resulting data could not be sized because the size standard was suppressed, as shown in the raw data below.

STERIPLEX SD: Graph showing raw data. Due to the suppression of the size standard, the raw data could not be analyzed. Therefore, no analyzed data are presented. The raw data are presented below.



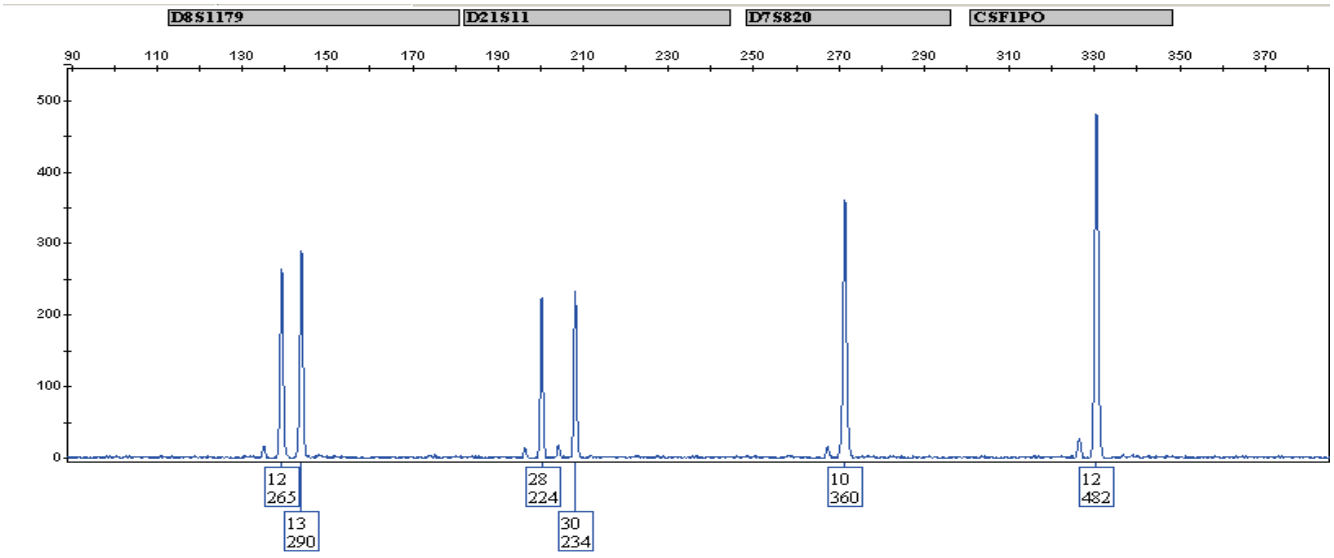
Amplified DNA with No Wiping Raw Data for STERIPLEX SD

Bleach solution: The raw data is shown below. The primer peak is smaller than expected; this could be due to the effect of the bleach. The size standard was suppressed, so the data could not be analyzed. As with the STERIPLEX SD, no analyzed data are presented. Raw data are presented below.

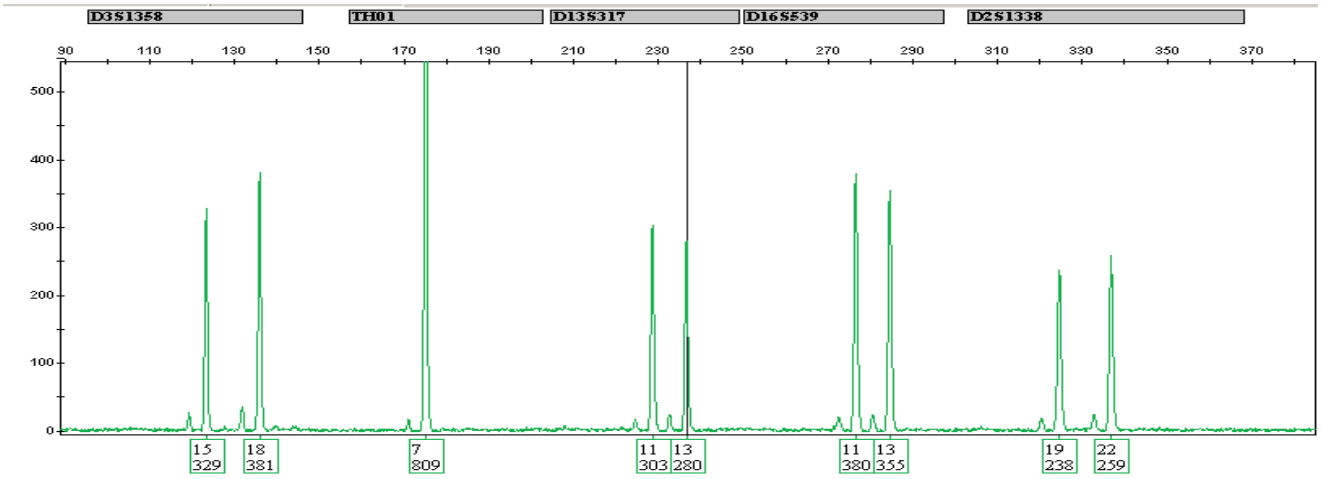


Amplified DNA with No Wiping Raw Data for Bleach Solution

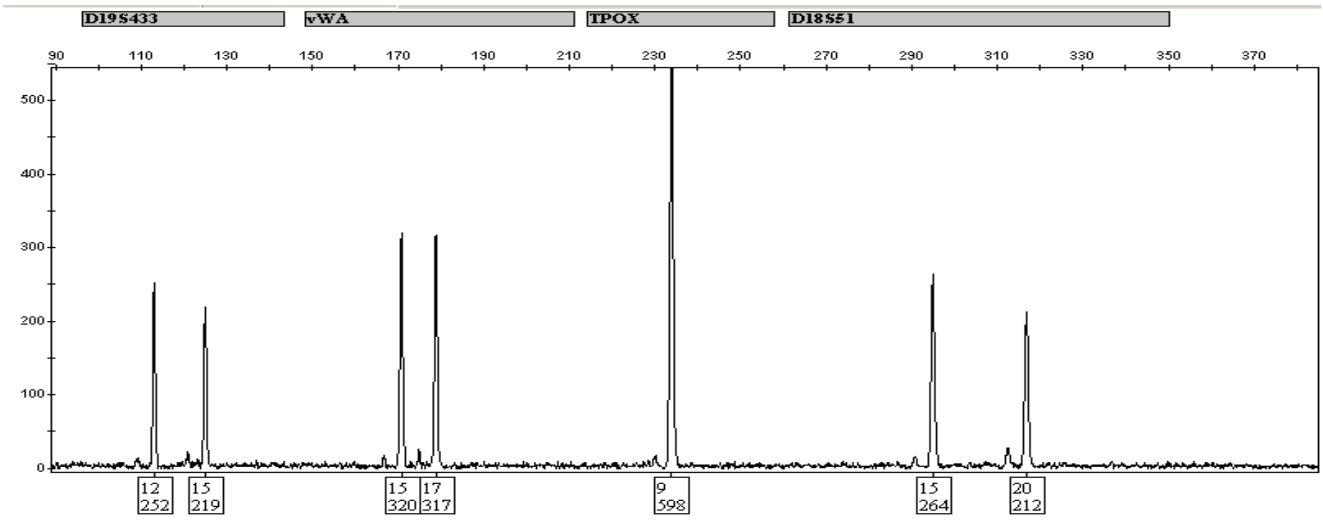
Pure Blu: Data from Pure Blu showed that this disinfectant was unable to destroy the amplified DNA. The resulting electropherograms show that the Pure Blu did have some effect because the RFUs of the peaks of the samples mixed with Pure Blu are approximately 3-fold lower than samples mixed with an equal volume of TE buffer. However, the samples mixed with Pure Blu still gave a full profile with RFUs well above 200. Pure Blu results (analyzed data) are shown below.



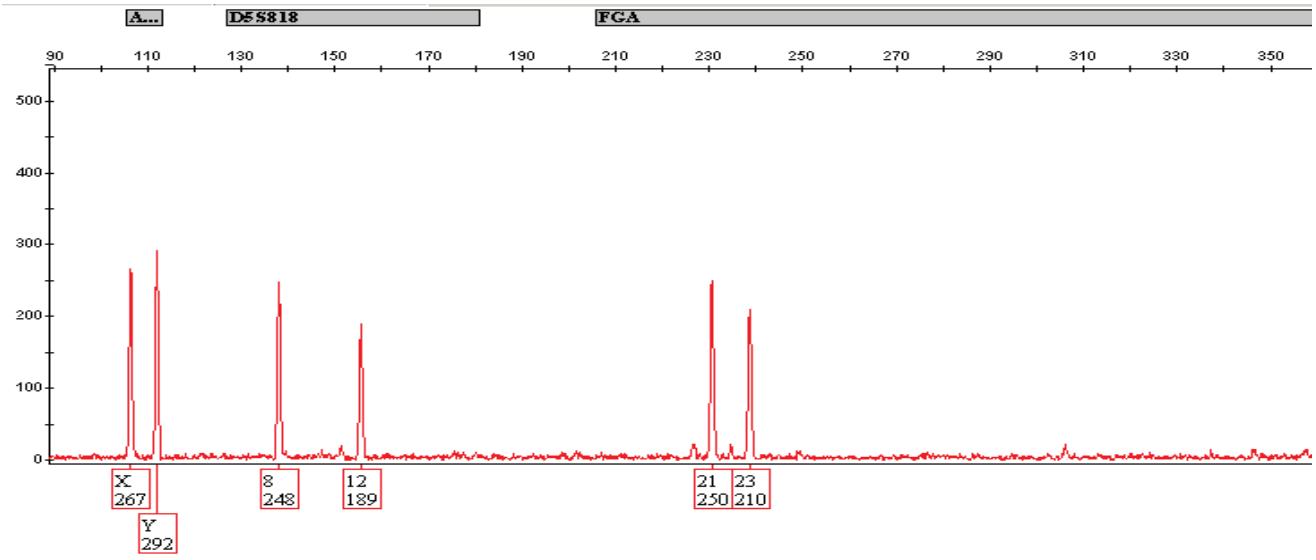
Amplified DNA with No Wiping Results for Pure Blu: Blue Dye for D8S1179, D21S11, D7S820 and CSF1PO Loci



Amplified DNA with No Wiping Results for Pure Blu: Green Dye for D3S1358, TH01, D13S317, D16S539 and D2S1338 Loci



Amplified DNA with No Wiping Results for Pure Blu: Yellow Dye for D19S433, vWA, TPOX and D18S51 Loci



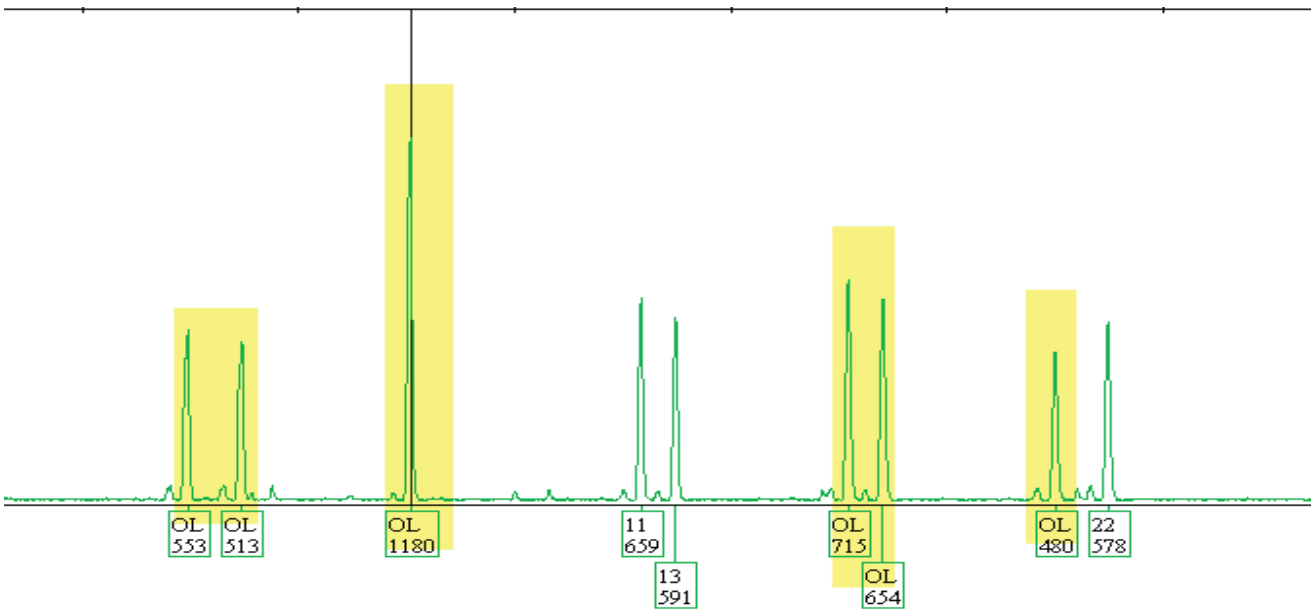
Amplified DNA with No Wiping Results for Pure Blu: Red Dye for Amelogenin, D5S818 and FGA Loci

To determine the effectiveness of the STERIPLEX SD and also the bleach solution, the experiment was repeated, but after the time limit of 5 minutes the samples were put through the MinElute® cleanup process. The goal was to remove the disinfectants from the sample to prevent them from affecting the electrophoresis process in order to visualize any DNA present.

After cleanup, the samples with bleach solution did not show any DNA present, and based on the size standard peaks the electrophoresis process was not affected. A spike occurred in all colors in the samples with 1 part sample to 10 parts bleach solution. One of the triplicate samples did give a full profile. The samples with 1 part sample to 5 parts bleach solution did not give a profile; however, there was a spike present in the same position in some of these samples.

The samples put through the MinElute® cleanup after being mixed with STERIPLEX SD disinfectant gave very unusual results. These results were reproducible in all samples with both concentrations of STERIPLEX SD.

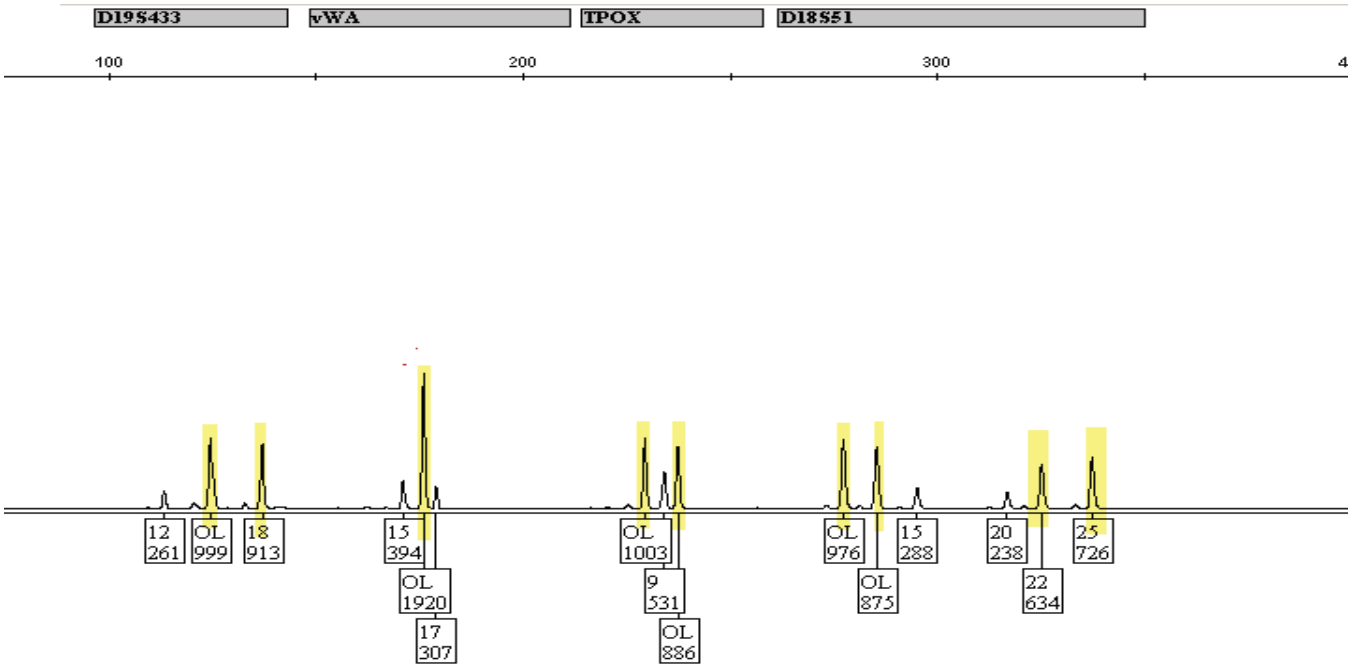
- The RFUSs of the peaks present were lower than comparable sample mixed with TE buffer.
- Loci in blue and red dyes all gave a profile that was consistent with the sample used.
- Some loci in green dye were called OL. The pattern at the different loci was the same as the sample used (homozygotes and heterozygotes in their respective places), but some of the peaks were shifted just "outside of the bin" (outside of the range where they would get called), so these peaks were not assigned an allele call. See the figure below; the OL peaks are highlighted in yellow. Other called peaks were consistent with the profile of the sample used.



OL Peaks in Green Dye Loci

Loci in the yellow dye had a mixture of OL peaks and peaks with allele calls. However, the true DNA peaks that were called were much lower than other peaks.

For example, the D19S433 true peak was at 261 RFUs while other peaks were at >900 RFUs. The vWA true DNA peaks were at 394 RFUs and 307 RFUs for the heterozygote profile, while the other peak appeared as a homozygote at 1920 RFUs. The TPOX true peak (homozygote) was at 531 RFUs, while other peaks called OL were at 1003 RFUs and 886 RFUs (heterozygote). At D18S51 there were two true peaks (heterozygote), at 288 RFUs and 238 RFUs. There were also four other peaks; two were called OL at 976 RFUs and 875 RFUs and the other two were assigned allele calls of 22 and 25 at 634 RFUs and 726 RFUs, respectively. The STERIPLEX SD disinfectant in some way affects the fluorescent tags attached to the amplified DNA, or perhaps the disinfectant has some fluorescent properties of its own, or it could be a combination of both. The figure below shows an example of the yellow dye loci with all of the peaks that are not consistent with the sample highlighted in yellow.



Yellow Dye Loci

Blood with Wiping Results

Samples with bleach solution gave a negative quantitation result. The IPC was compared with the control sample that contained blood with no disinfectant. There was no significant change in the cycle where the IPC crossed, so the negative result is a true negative and not a result of inhibition.

In samples with STERIPLEX SD, one of the triplicates was negative and the other two had results of 0.00564 ng/µl and 0.00473 ng/µl of DNA. These quantities are typically considered negative results since there is not enough DNA to give a profile. The IPCs showed no signs of inhibition.

In samples with Pure Blu, one of the triplicates was negative, and the other two had quantities of 0.00166 ng/µl and 0.00125 ng/µl of DNA. Similarly to the STERIPLEX SD results, these low quantities are usually considered negative results. The IPC did not show any signs of inhibition.

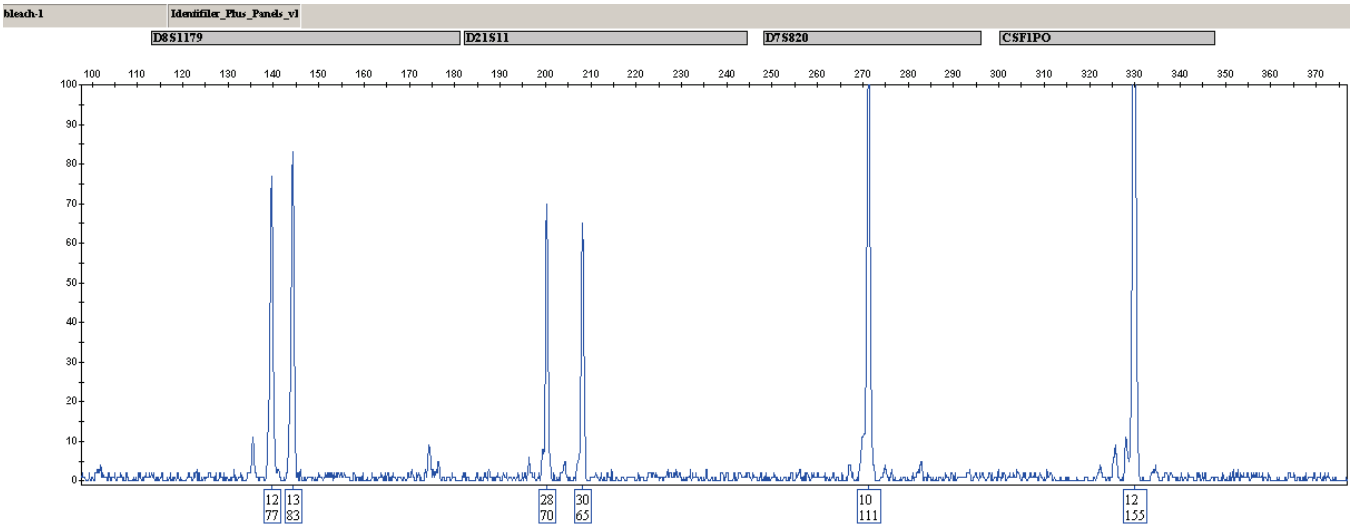
From these results it can be concluded that wiping greatly enhances the cleaning effect of the disinfectants.

Extracted DNA with Wiping Results

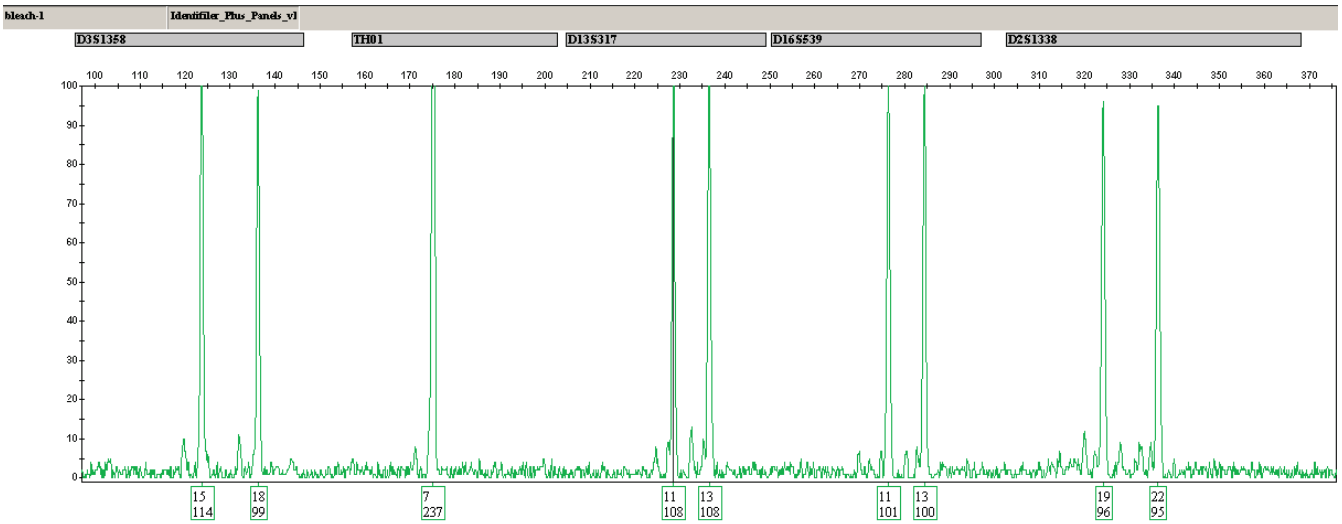
Since the results of the no wiping test for extracted DNA were successful, there was no need to perform the test with wiping.

Amplified DNA with Wiping Results

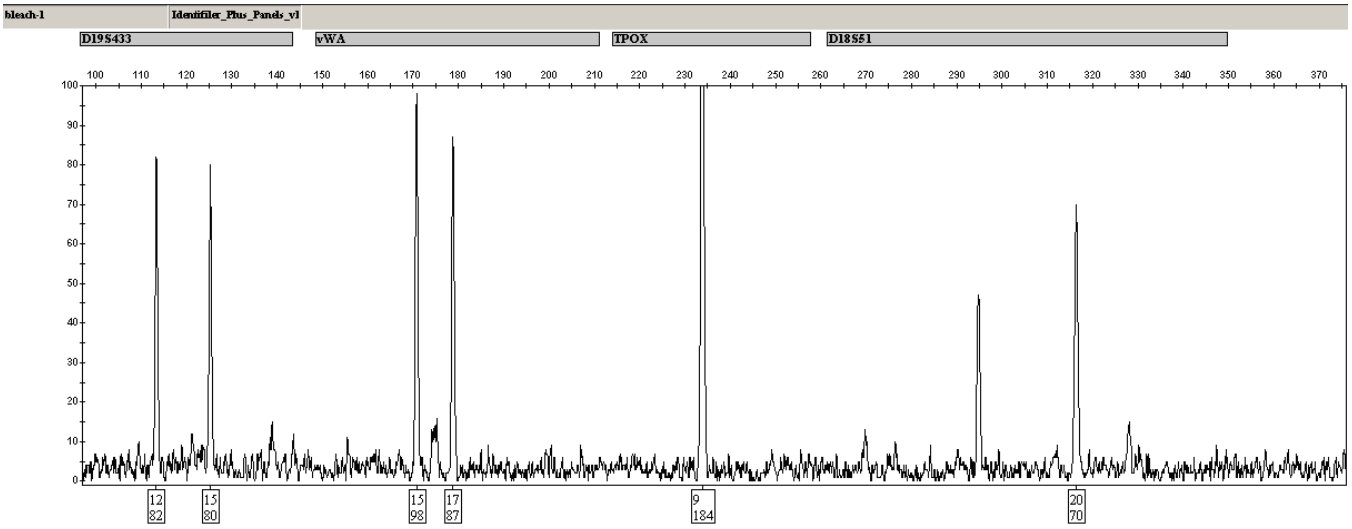
The samples with bleach solution gave partial DNA profiles with peak heights ranging from 60 RFUs to 300 RFUs for some homozygotes. It should be noted that the MinElute® process on average increases peak heights by 10-fold; therefore, without this method the majority of these peaks would not be called. See the figures below.



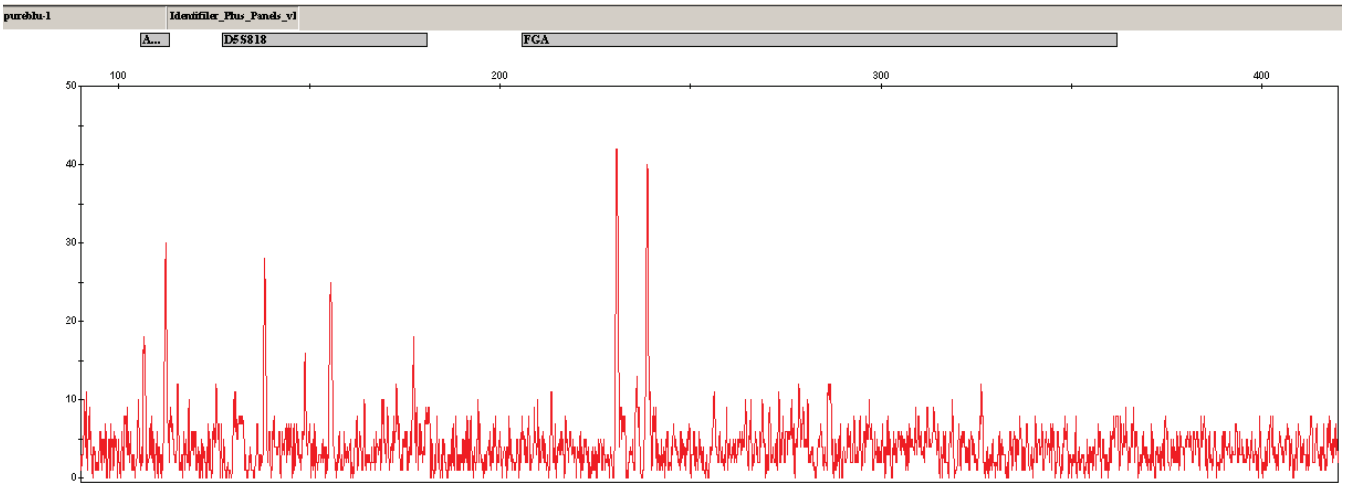
Amplified DNA with Wiping Results for Bleach Solution: Blue Dye for D8S1179, D21S11, D7S820 and CSF1PO Loci



Amplified DNA with Wiping Results for Bleach Solution: Green Dye for D3S1358, TH01, D13S317, D16S539 and D2S1338 Loci

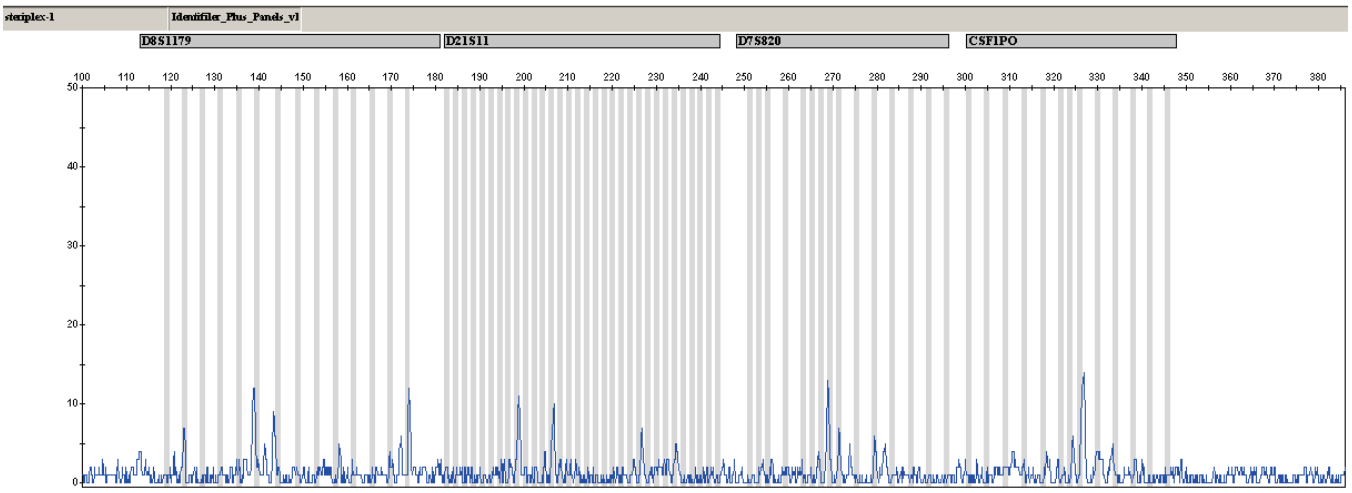


Amplified DNA with Wiping Results for Bleach Solution: Yellow Dye for D19S433, vWA, TPOX and D18S51 Loci

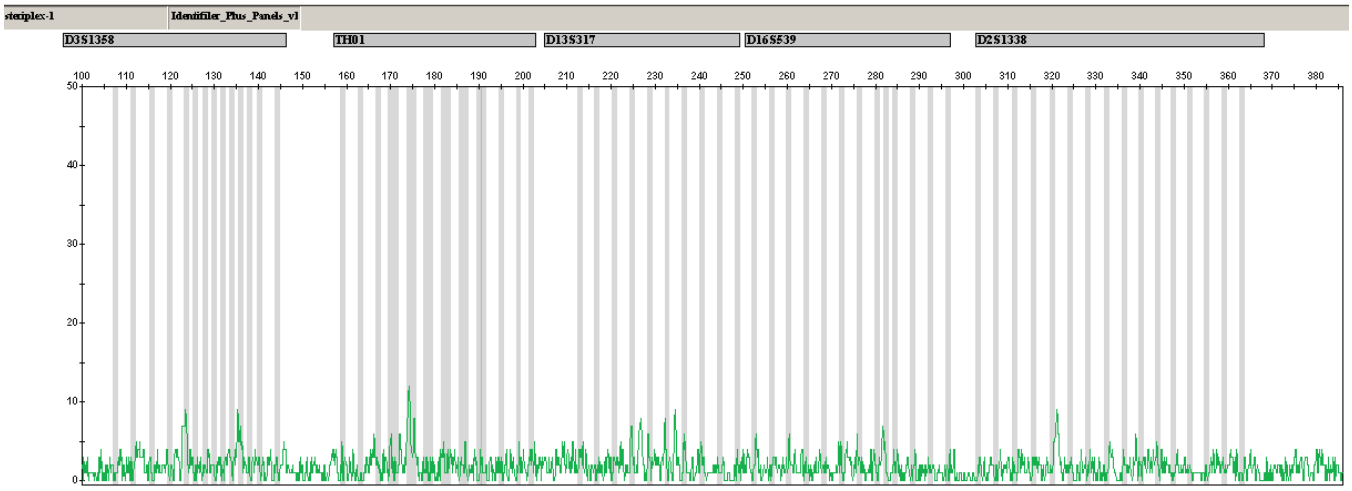


Amplified DNA with Wiping Results for Bleach Solution: Red Dye for Amelogenin, D5S818 and FGA Loci

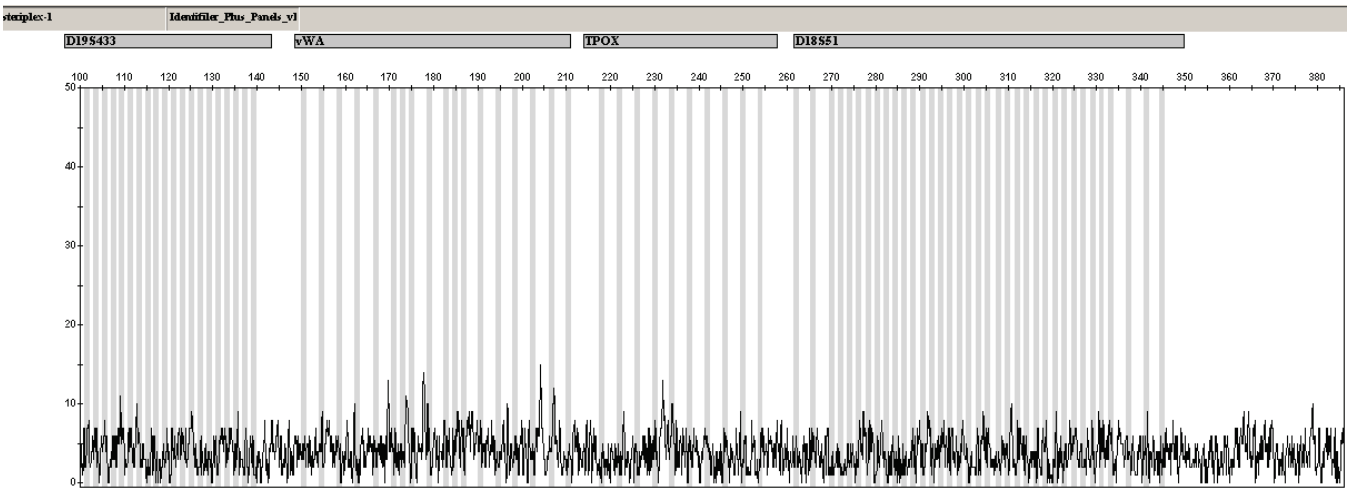
For STERIPLEX SD, the samples appeared to be negative. There were some peaks around 20 RFUs that were consistent with the profile, but nothing was called. Without MinElute® these peaks would be well within the baseline and would not be noticeable. See the figures below.



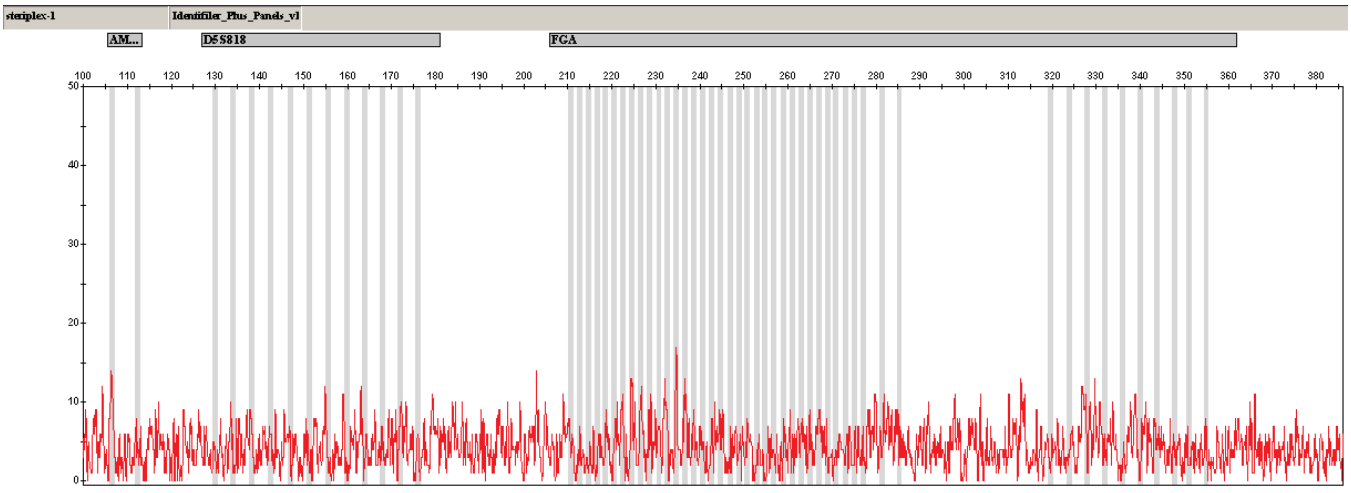
Amplified DNA with Wiping Results for STERIPLEX SD: Blue Dye for D8S1179, D21S11, D7S820 and CSF1PO Loci



Amplified DNA with Wiping Results for STERIPLEX SD: Green Dye for D3S1358, TH01, D13S317, D16S539 and D2S1338 Loci

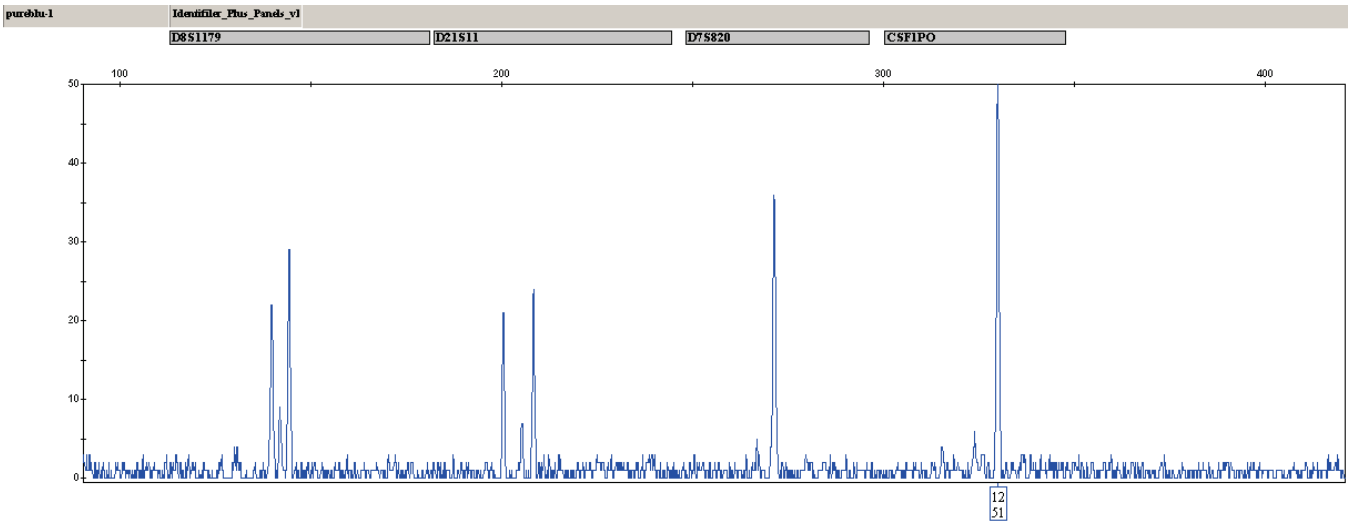


Amplified DNA with Wiping Results for STERIPLEX SD: Yellow Dye for D19S433, vWA, TPOX and D18S51 Loci

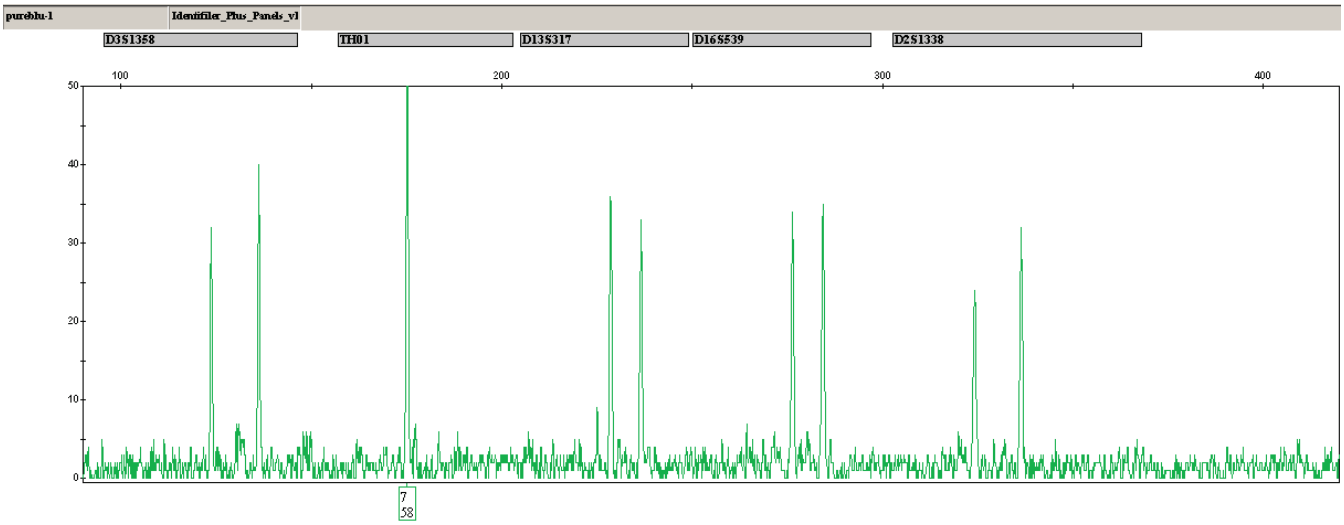


Amplified DNA with Wiping Results for STERIPLEX SD: Red Dye for Amelogenin, D5S818 and FGA Loci

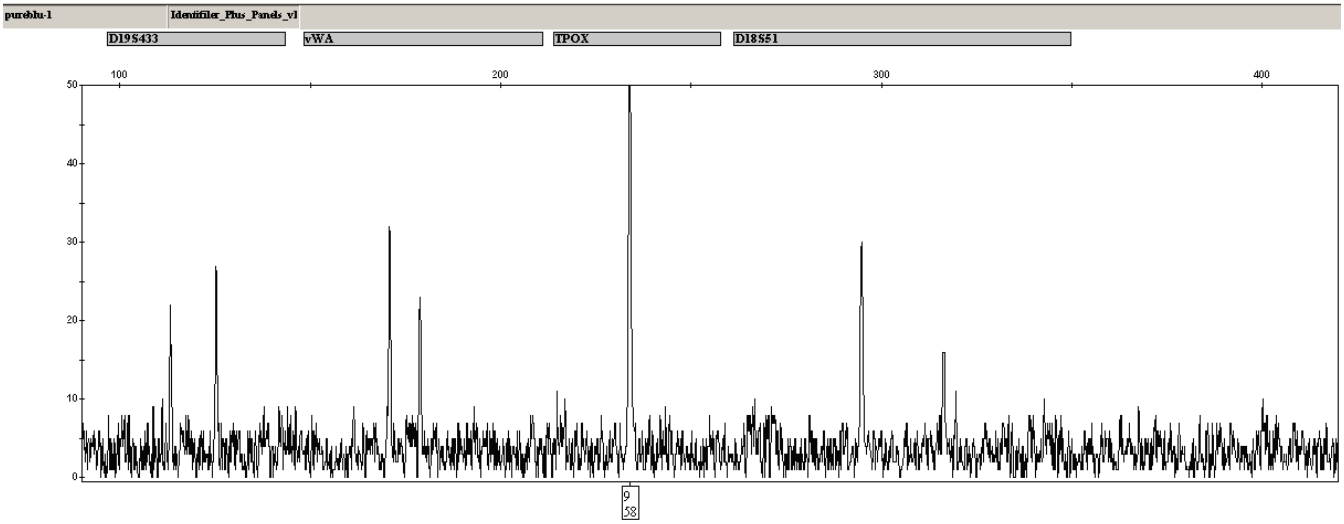
For Pure Blu, the majority of peaks were not called; two or three peaks were called in each triplicate sample. The highest peak height seen was 107 RFUs. Once again, without MinElute® cleanup these peaks would be closer to 10 RFUs. See the figures below.



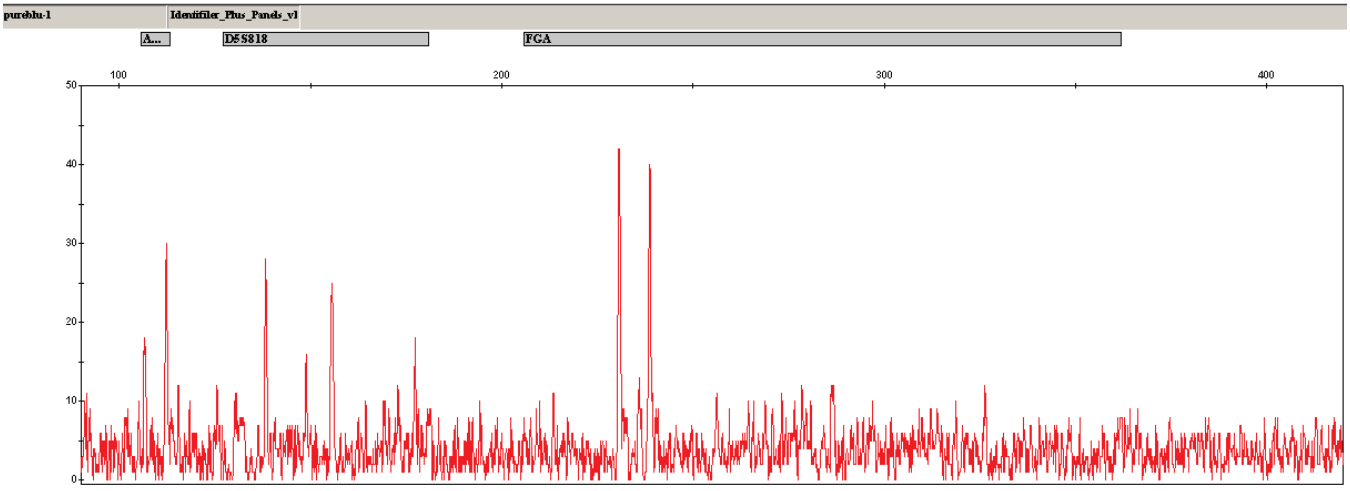
Amplified DNA with Wiping Results for Pure Blu: Blue Dye for D8S1179, D21S11, D7S820 and CSF1PO Loci



Amplified DNA with Wiping Results for Pure Blu: Green Dye for D3S1358, TH01, D13S317, D16S539 and D2S1338 Loci



Amplified DNA with Wiping Results for Pure Blu: Yellow Dye for D19S433, vWA, TPOX and D18S51 Loci



Amplified DNA with Wiping Results for Pure Blu: Red Dye for Amelogenin, D5S818, and FGA Loci

When amplified DNA was placed on a clean ceramic tile and retrieved using the same method as the sample with disinfectant, the peak heights ranged from 2000 RFUs to >8000 RFUs. Based on this, it can be concluded that the disinfectants plus mechanical action of wiping was effective in cleaning the surface.

The differences in the results for the bleach solution plus wiping compared to the bleach solution without any wiping could be due to the spray bottle, since the spray was spread out over the entire tile, whereas in the previous experiment the full volume of bleach solution was placed directly on the sample.

Conclusions

- The disinfectants in general were effective in destroying the DNA present, whether it is encased within cells like blood, exposed like extracted DNA or exponentially increased as in amplified DNA.
- The key to proper cleanup is using a good disinfectant and allowing it to remain in contact with the surface for the time recommended by the manufacturer. Since this still does not guarantee removal of the DNA, the action of wiping should be performed, perhaps followed by a secondary wiping with water.
- Disinfectants need to be completely removed because, as shown by the data, they could have a negative effect on the PCR and electrophoresis processes. To ensure this does not occur, analysts should pay attention to the IPC in the quantitation step to determine if the PCR process was affected negatively by the disinfectant. The size standard would be a good way to determine if the electrophoresis process was affected by the disinfectants.
- **Bleach:** 10% bleach solution was effective as a cleaning agent; however, it does have drawbacks. It is corrosive, quickly loses activity, can affect the PCR process and is time-consuming to prepare, especially in large laboratories, which may require several bottles to be used on a daily basis. The dual spray bottles are expensive and in time do seize up and have to be replaced, which can become costly.

- **STERIPLEX SD:** Was effective as a cleaning agent; it appears to work better than bleach solution when left in contact with blood. It is not corrosive and once activated is at full strength for 2 months. Its drawbacks include the adverse effects on the PCR and electrophoresis processes by creating false peaks that can be misconstrued as real data; however, this only occurred without wiping action. When the disinfectant was used plus the wiping action, these peaks were not seen. Because STERIPLEX SD is not corrosive, regular spray bottles can be used, and they would not seize up and stop working over time.
- **Pure Blu:** Was an effective cleaning agent when followed up with wiping. The disadvantage is that the color fades before the time it is recommended to stay on the surface, and the liquid evaporates as well, leaving the surface dry. The time required for the Pure Blu to be effective is 10 minutes, which is twice as long as for bleach solution and STERIPLEX SD. After 10 minutes it would be hard to determine where exactly the Pure Blu was sprayed. Pure Blu is not corrosive and would not have to be made fresh daily.

Strengths

- **Bleach:** Effective cleaning agent when combined with wiping. Is readily available; start-up cost is low.
- **STERIPLEX SD:** Most effective cleaning agent when combined with wiping. Activated solution is at peak effectiveness for 2 months after activation. It is not corrosive or toxic; no emissions into the environment. Long-term cost savings can be expected due to reduction in labor and replacement parts.
- **Pure Blu:** Effective cleaning agent when combined with wiping. Product is non-corrosive and safe for the environment. It does not use harsh chemicals or known carcinogens, will not stain clothing and has a color-change indicator.

Limitations

- **Bleach:** Is toxic and corrosive. It may cause long-term damage to surfaces and instrumentation, it loses its effectiveness quickly in a diluted state, and long-term costs can be incurred with preparation time and replacement of specialized spray bottles.
- **STERIPLEX SD:** May have fluorescent properties, which may affect results, but only if pure disinfectant gets into detection system. Initial cost may be high.
- **Pure Blu:** Not a very effective disinfectant without wiping. Is flammable. Cost unknown at this time. Needs to remain in contact with stains longer than other disinfectants assessed.

Health and Safety Issues

- Bleach is toxic and corrosive; it should not be inhaled or come into contact with skin.
- Pure Blu is flammable; it should not be used around open flame or while smoking.
- STERIPLEX SD has an MSDS rating of “0” and does not pose any health or safety issues.

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