Verlag Dr. Kovač

2.9 Sexual assault detection dog: comparing the detection of semen by dogs, ALS and presumptive tests

Jessica de Ruiter [1]; Adee Schoon [2]; Annemieke van Dam [3]

Abstract

Semen traces at crime scenes are of major importance when investigating sexual assaults and rape cases. The identification of semen traces is often difficult as the detection of these traces is limited by the environment, especially when these traces are located outdoors, on coloured clothing or inside vehicles. In this study the sensitivity and specificity of the detection of semen using sniffer dogs was compared with two commonly used methods in the forensic field namely, i) Alternative Light Sources (ALS), which makes use of the fluorescent properties of semen stains and ii) a chemical presumptive test , Acid Phosphatase printing test (AP-test). To compare the sensitivity and specificity of the three different methods, semen traces were exposed to a large variation of different conditions, including aging for different time periods, weather conditions, deposition on different porous, semi-porous and non-porous surfaces and washing conditions. In this study we found that sexual assault detection dogs trained to detect semen traces indicated the presence of more stains compared to ALS detection and the AP-test. This suggests a higher sensitivity of sexual assault detection dogs trained to detect up to 0.005ml semen. In general, sexual assault detection dogs could be used in a wider variety of situations, which indicates that sexual assault detection dogs are potentially a useful screening tool.

Introduction

Biological traces found at crime scenes can provide significant information useful for reconstruction of the scene. Next to this, such traces can contain DNA material which can be used to generate a DNA profile to identify the donor of the trace. Biological traces are therefore essential for forensic investigations (Lee & Khoo, 2010). For example, semen traces

---

[1] VHL University of Applied Sciences, Leeuwarden, The Netherlands

[2] Canine Unit Netherlands National Police, Nunspeet, The Netherlands

[3] Biomedical Engineering & Physics, Academic Medical Centre Amsterdam, The Netherlands

are an important type of evidence and the detection of these traces plays a significant role in sexual assault cases. Different methods, including spectroscopic, chemical and immunogenic methods, are used to locate and detect biological traces, each with their own advantages and drawbacks. In the case of semen traces, the detection is often hampered by the environment at which these traces are located, for instance outdoor crime scenes, coloured clothing and inside vehicles.

Detection dogs are in use world-wide to assist in the location of substances of interest since the early 1960's. These substances are then analysed by forensic laboratories. In some countries, the dog's alert itself is presented as court evidence, but in the Netherlands the results of the forensic analysis are used in the subsequent police investigation and judicial follow-up. The dogs are used as "intelligent samplers" to rapidly indicate substances, or areas of interest, that are investigated further. The use of detection dogs in locating biological traces is relatively new. Cadaver dogs, trained on the odour of decomposing bodies as found in decomposition fluids have been in use since the 1990's but dogs trained on the odour of blood and semen have only come into use recently, for example in several Scandinavian countries during the last decade.

In 2015, the Dutch police initiated a "sexual assault detection dog" project to investigate if the use of trained dogs could assist in the investigation of sexual assault cases. Since the police already had dogs trained to detect blood traces, these dogs were trained to detect only semen. Prior research and experience with blood detection dogs had contributed to the philosophy that matching training parameters to the forensic techniques used by the police was necessary for efficient training and deployment (Schoon 2013). In Norway, research conducted by Skalleberg and Bouzga (2016) also illustrated this: the dogs were capable of locating traces that could not be confirmed by subsequent DNA analysis. Sensitivity and specificity are important factors to monitor in order to train dogs efficiently and effectively. Thus, as part of the project, research was initiated to compare the Dutch sexual assault detection dogs to other methods used in sexual assault cases.

One of the current methods to locate and detect semen traces at crime scenes and/or crime related objects uses forensic light, generically called Alternative Light Source (ALS). This method is a rapid, non-contact

and non-destructive tool to visualize biological traces based on their fluorescent properties (Virkler et al, 2009, Silenieks et al, 2002). Optimal results require a dark environment, making it less practical for outdoor crime scenes. ALS is not able to identify the origin of a trace, as different body fluids will fluoresce upon excitation with ALS. Besides this also other non-human biological traces will similarly react, therefore ALS is not a specific method to identify human biological traces, but can be used as presumptive and indicative method. In most cases, additional methods are used to get an indication of the origin of a trace, including the presumptive enzymatic Phosphatesmo KM test (KM-test) and the Acid Phosphatase printing method (AP-test). Next to these enzymatic tests, also immunogenic methods can be used to identify semen traces, for instance the Rapid Stain Identification (RSID) semen field kit. This method is based on the antibody-antigen reaction with human semenogelin, a protein unique for human semen. The KM-test, AP-test and RSID test are not able to detect traces, to apply them the exact location of a trace should be known. Therefore these methods and their potential use are limited by the visual examination of stains in forensic case work.

The goal of this study was to establish guidelines for the training and use of the dogs and compare their efficiency with the current detection and identification methods of semen. Dogs are known to be very sensitive and specific and may indicate traces that cannot easily be confirmed using other technologies, such as in human scent detection (Schoon et al 2015). To evaluate the feasibility of this project is it necessary to determine the sensitivity and specificity of the dogs and compare these results with the standard semen detection methods. As different presumptive methods are available, a first experiment was designed to investigate which of the presumptive methods has the highest specificity and sensitivity. The KM-test, AP-test and RSID-test were selected and compared. Based on the outcome of these experiments, a presumptive test was selected to compare with the performance of the sexual assault detection dog. In this latter experiment, ALS was also included as this method is used as a first screening tool to locate biological traces. To determine the specificity and sensitivity of the dogs, presumptive test and ALS, semen stains were exposed to various conditions before detection. Variables included: the amount of semen, different outdoor locations, surface type, weather and washing conditions.

Materials and methods

Sample collection

Fresh semen was obtained from healthy, anonymous donors who gave signed consent for this use of their samples. These donors visited the fertility clinic at the Academic Medical Centre (AMC) in Amsterdam. Control samples were urine, breastmilk and sweat, these were also obtained from consenting donors at the AMC. This material was stored at -80°C until use.

Dogs

Two dogs participated in the study. Cooper (dog A) had been in training since February 2016, and Evi (dog B) since September 2015. Both dogs had been trained following a back-chaining procedure based on positive reinforcement. Clicker training combined with food and play was used as reinforcer. The dogs were trained on varying amounts of semen (from 0.005 to 3ml) of varying ages (up to several months old) in a variety of circumstances both in- and outdoors by experienced instructor/handlers at the Dutch National Police Canine Unit. At the time of these experiments, the dogs had not been used operationally yet.

Experiment 1: Comparison of presumptive test methods

The 3 following presumptive test methods were used in this experiment:

a) AP-test: The Acid phosphatase printing method (President's DNA Initiative, Protocol 2.04) was used indoors and outdoors. A piece of moistened filter paper was used to sample the desired area by spreading it out over the area to be sampled and pressing it down. After spraying the paper with the acid phosphatase mix, a positive result was obtained when the stain coloured purple/pink within 60 seconds.

b) KM-test: the Phosphatesmo KM test (Machery-Nagel GmbH & Co. KG) was used in the preliminary tests on different surface types. It consists of a 15x30mm filter paper that is moistened with demineralised water, and applied directly to the stain. A positive result is obtained when the paper coloured purple within 60 seconds. This test is currently in use by the Dutch police.

c) RSID: the Rapid Stain Identification field kit (Independent Forensics) was used in preliminary tests on different surface types. The stain itself, or a swab of the stain, is put into a buffer and analysed in a cassette. A positive result was obtained when the cassette showed two red/pink lines within 10 minutes.

For this initial comparison of the different presumptive tests the following variables were examined:

- Amounts: 0.02 ml and 0.1 ml semen
- Surface types: grass, soil, synthetic textile, cotton textile, tiles
- Ageing: 1, 6, 10, 14, 20 and 28 days old (indoors)

Experiment 2: Outdoor searches on different surfaces and the effect of rainfall

The 2 following test methods were used in this experiment:

a) Dog searching: the experimental area (1-2 m2) was delimited (Fig. 1). The handler, unaware of the position of the semen stain, allowed his dog to search the area. When the dog made an indication the handler would raise his hand. The experimenter then told the handler if the right spot was indicated. This way, the handler could reward the dog. If the dog had searched the whole area without indicating, the search was terminated by the handler.

Figure 1:
Example of search area outdoors

b) AP-test: for details see experiment 1 a.

The following variables were included in these experiments:

- Amount: 0.1ml semen; 0.1ml control fluids (urine, mother milk, sweat)
- Surface types: grass, forest soil, concrete
- Ageing: fresh, 2 days, 4 days, 7 days old
- Rainfall: 0.0 mm, 1.5 mm, 3.0 mm, 6.0 mm

Practical execution: In a first series of searches, 2m2 areas were delimited and two stains (one semen and one control) were deposited in each area. Different variables were examined: 3 surface types (grass, forest soil, and concrete), samples of different ages (fresh up to a week old with different amounts of rain having fallen on the grass and the forest soil, the concrete surface was a half-open shed with a roof that kept the surface dry). Each dog searched separate areas. Subsequently the AP test was used to detect the semen trace.

In a second series, fresh semen samples on 1m2 areas of grass and concrete were exposed to 3 controlled amounts of rainfall (amounts approximately equal to 1.5, 3 and 6 mm rainfall). Each dog searched separate 0 and 1.5mm rainfall areas, but both searched the same 3 and 6mm rainfall areas. The AP test was used to detect the semen trace after the dogs.

Experiment 3: Textiles and the effect of washing
The following 3 different methods were used to detect semen traces:

a) Dog searching: pieces of textile were searched indoors, these pieces were attached vertically to a board using magnets (Fig. 2), which allows the dogs to search the area easily. The handler, unaware of the position of the semen stain, allowed his dog to search the piece of cloth. When the dog made an indication the handler would raise his hand. The experimenter then told the handler if the right spot was indicated. This way, the handler could reward the dog. If the dog had searched the whole piece of cloth without indicating, the search was terminated by the handler.

Figure 2:
Example of textile search indoors

b) ALS: The "Crime Lite 2" (Foster & Freeman) was used indoors on the textiles. Although the instructions indicate use of 3 torches for seminal fluid (UV, violet and blue), all 5 different crime lights (UV, Violet, Blue, Blue/Green, Green) and 4 filters were used to examine the traces so the full range of light from 350 to 560nm were used. The surface was searched with the Crime Lite 2, upon excitation the presence of fluorescent spots were indicated as positive signals. Images were taken using the long pass filters supplied with the Crime Lite 2 torches using a Canon EOS 40D and a Canon Macro Lens EF 100mm f/2.8 USM.

c) AP-test: for details see experiment 1 a.

The following variables were included in these experiments:

- Amount: 0.005ml
- Textiles: different coloured and printed cotton bedsheets

- Ageing: fresh, 2 and 8 weeks
- Washing: 30°C and 60°C washing temperatures, all tumble dried the highest temperature
- Detergents: Biotex (designed to remove biological stains) and Robijn (regular detergent) washing detergents, Experimental variables

Practical execution: 0.005ml of semen was deposited on cotton bedsheets of approximately 1m2. The bedsheets were new but were washed prior to use with a regular detergent. Different types of cotton textiles were examined: 2 types of print, samples of different ages (fresh to 8 weeks old), and different washing temperatures (30°C and 60°C) with different types of washing detergents (regular (R) and specially aimed at biological traces (B)). Each piece was washed and dried separately. The stains were detected first by ALS, then by the dogs and finally by the AP-test. Each dog examined separate stains.

Results

Experiment 1: Comparison of presumptive test methods
All the presumptive tests were conducted twice per variable; the amount, age and surface type, leading to 360 tests in total. All tests on grass, synthetic and cotton textiles, and tiles were positive. On soil, some tests were negative. The positive outcomes of the 72 tests on soil are given in table 1.

Age	AP-test		KM-test		RSID	
(days)	0.02ml	0.1ml	0.02ml	0.1ml	0.02ml	0.1ml
1	2	2	0	0	2	2
6	2	2	2	2	2	2
10	0	1	0	0	2	2
14	0	1	0	0	0	2
20	0	1	0	0	2	2
28	2	0	2	2	1	2
TOTAL	6 (50%)	7 (58%)	4 (33%)	4 (33%)	9 (67%)	12 (100%)

Table 1. Positive outcomes of presumptive tests on soil. All tests were performed twice.

In general no effect of ageing on the performance of the three different tests was observed. On soil a distinctive difference could be observed between the three tests. The RSID test was found to be the most sensitive on soil, 67% and 100% of respectively the 0.02 ml and 0.1 ml semen stains were detected. The KM-test, currently in use by the Dutch police, was found to be the least sensitive (50% of the 0.02 ml semen stains, and 58% of the 0.1 ml semen stains could be detected).

In practical terms, the application of the AP-test is more versatile than the RSID or the KM-test. For the AP-test, a relatively large area can be sampled with filter paper, which allows the forensic investigator to search a large area without knowing the exact location of the trace (the examiner can select the appropriate size of paper). After sampling the filter paper is chemically analysed, The RSID test requires exact swabbing at the location of the trace. The KM-test also has to be applied directly to the stain, and grass has a tendency to stick to this (small) filter paper, making it less applicable.

Experiment 2: Outdoor searches on different surfaces and the effect of rainfall

Table 2 summarizes the results of the outdoor searches. None of the control fluids were indicated, and there were no other false alarms, so these have been left out of the table.

The results show that fresh semen was found by the dogs and the AP-test. Older traces on concrete, which had remained dry, were systematically found by both dogs and the AP-test. But ageing on grass and on forest soil might have an effect on the detection of semen traces on these surfaces. Only 1 semen stain aged for 4 days could be detected on grass by 1 dog and the AP-test, whereas no aged samples could be detected on forest soil. The results on forest soil can partly be explained by the physical disappearance of the trace. The semen had been deposited on a twig, due to weather conditions or the searching activity of the dog, the twig could have been moved from its original location.

Surface	Age	Rainfall	Dogs hit (n)	AP-test pos (n)
Grass	Fresh	0,0 mm	4 (4)	4 (4)
	Fresh	1,5 mm	2 (2)	1 (2)
	Fresh	3,0 mm	1 (2)	1 (1)
	Fresh	6,0 mm	1 (2)	0 (1)
	2 days	7,9 mm	0 (2)	0 (2)
	4 days	9,5 mm	1 (2)	1 (2)
	7 days	9,9 mm	0 (2)	0 (2)
Concrete/hard surface	Fresh	0,0 mm	4 (4)	4 (4)
	Fresh	1,5 mm	2 (2)	2 (2)
	Fresh	3,0 mm	2 (2)	1 (1)
	Fresh	6,0 mm	2 (2)	1 (1)
	2 days	0,0 mm	2 (2)	1 (2)
	4 days	0,0 mm	2 (2)	2 (2)
	7 days	0,0 mm	2 (2)	2 (2)
Forest soil	Fresh	0,0 mm	2 (2)	2 (2)
	2 days	7,9 mm	0 (2)	n/a
	4 days	9,5 mm	0 (2)	n/a
	7 days	9,9 mm	0 (2)	n/a

Table 2. Results of outdoor experiments with dogs and AP test on different surfaces, ages and conditions. n/a: no test was done.

An explanation of the limited semen stains detected on the grass surfaces might be rain fall, which could have flushed away the trace completely or partially. During the first outdoor experiment, up to 10 mm of rain had fallen during the week the samples were ageing. The older samples were exposed to more mm of rain fall and with one exception were not detected by both the dogs and the AP-test. To test the effect of rain, fresh samples on concrete and grass were exposed to rain fall under controlled conditions (rainwater was collected in rain barrels and used for this experiment). On concrete, exposure of the semen stains to various amounts of rainwater had no effect on the detectability of these stains: all semen samples were detected with the dogs and the AP-test. On grass, one dog was systematically able to detect all semen samples, in contrast the AP-test was less successful: 4/6 stains were detected by the dogs, whereas 2/4 stains were detected using the AP-test.

Experiment 3: Textiles and the effect of washing

Table 3 summarises the results of the detection of 0.005ml on textiles. There were no false alarms, so these have been left out of the table. In this experiment where semen stains were deposited on unwashed textiles, ageing did not affect detectability: both dogs, the AP-test and the ALS were able to detect all semen stains present on plain and printed cotton.

Surface	Age	Washing	ALS pos. (n)	Dogs hit (n)	AP test pos. (n)
Printed cotton	Fresh	unwashed	2 (2)	2 (2)	2 (2)
	Fresh	30°C, B	0 (2)	2 (2)	0 (2)
	Fresh	30°C, R	0 (2)	0 (2)	0 (2)
	Fresh	60°C, B	0 (2)	0 (2)	0 (2)
	Fresh	60°C, R	0 (2)	1 (2)	0 (2)
	2 weeks	unwashed	2 (2)	2 (2)	2 (2)
	8 weeks	unwashed	2 (2)	2 (2)	2 (2)
Plain cotton	Fresh	unwashed	2 (2)	2 (2)	2 (2)
	Fresh	30°C, B	0 (2)	0 (2)	0 (2)
	Fresh	30°C, R	0 (2)	0 (2)	0 (2)
	Fresh	60°C, B	0 (2)	2 (2)	0 (2)
	Fresh	60°C, R	0 (2)	0 (2)	0 (2)
	2 weeks	unwashed	2 (2)	2 (2)	2 (2)
	8 weeks	unwashed	2 (2)	2 (2)	2 (2)

Table 3. Results of indoor experiments on clothing with ALS, dogs and AP test. B = washed with Biotex, R = washed with Robijn

In Fig. 3 the results of a single stain deposited on a yellow cotton print exposed to different Crime Lite torches is illustrated. Although the instructions of the Crime Lites advised the use of UV, violet and blue torches (range 350 to 470nm) for the detection of semen, most semen stains were detected using the blue, blue-green and green torches (range 420 to 560nm). In preliminary experiments, we found that the colour of the textiles influenced the detectability of semen stains. Black textiles, and some printed textiles, led to no detection of the semen stains, as no fluorescence could be observed. Therefore in this experiment only textiles of lighter colours were included.

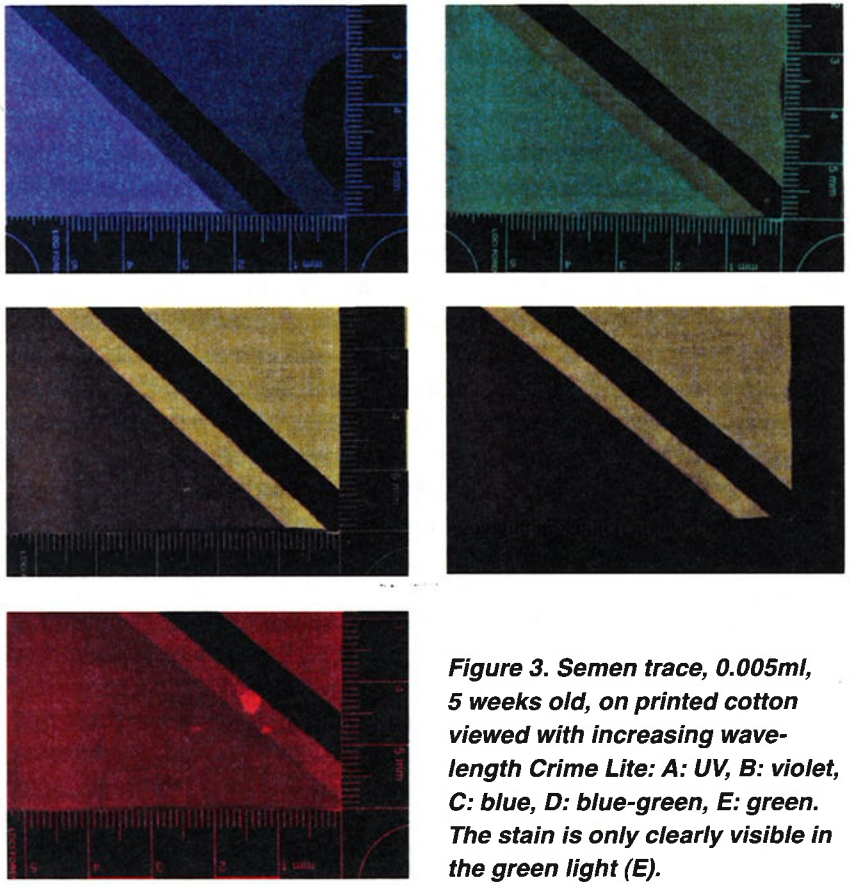

Figure 3. Semen trace, 0.005ml, 5 weeks old, on printed cotton viewed with increasing wavelength Crime Lite: A: UV, B: violet, C: blue, D: blue-green, E: green. The stain is only clearly visible in the green light (E).

Although no effect of ageing on the detection of semen stains was observed, an effect of washing of the textiles on the detection was observed. The dogs were able to detect semen traces in about 31% of the semen stains deposited on textiles after washing, but no stains could be found using ALS or the AP-test. There was no correlation between washing temperature and the detection of semen stains left on textiles (the dogs found 2/8 stains washed at 30 degrees, vs 3/8 stains washed at 60 degrees). Washing with Biotex, especially meant to wash away biological stains, was less effective in removing the stains completely than washing with a regular detergent (the dogs found 4/8 Biotex stains, but only 1/8 Robijn stains).

Conclusion and discussion

Based on the comparison of the different presumptive tests in the first experiment, it was decided to use the AP-test as presumptive test for the other experiments based on a combination of detection rate and practical use. The KM-test and RSID field kit both required pinpoint location of the stain for sampling, which was unachievable outdoors. The AP-test could be conducted on a larger filter paper that could sample (through printing) a more general area and provided better detectability of the semen stains compared to the KM-test, however had a lesser sensitivity compared to the overall performance of the RSID field kit.

During this research it was found that the method used to train the sexual assault detection dogs was of good quality, which made it possible to deploy the dogs as a useful detection tool when comparing them with the other common forensic techniques tested. Dogs were trained to detect amounts of semen varying from 0.005ml to 3 ml, semen stains aged for different periods of time and deposited on a large variety of surfaces. The dogs could indicate the presence of more semen stains compared to the ALS and the AP-test. Comparing the detection of outdoor stains using dogs and the AP-tests, dogs were able to locate 79,4% of the experimental stains and the AP-test 73,3%. On textiles the dogs detected 60,7% of the experimental stains, whereas ALS and the AP-test identified only 42,9% of the experimental stains. Noteworthy, all stains detected by ALS were detected by at least one of the two dogs used in the study. The results are in line with those obtained by Skalleberg & Bouzga (2016) who also found that in 16/18 tests conducted with 0.1ml semen outdoors, the AP-test confirmed the presence of semen the dogs had indicated. However, in their study, they did not perform AP-tests on stains the dog had missed, therefore no firm conclusions can be drawn about the sensitivity of these Norwegian dogs.

Outdoors stains are subject to a variety of influences. Skalleberg & Bouzga (2016) found that ageing had an effect on the detection of semen stains by the dogs. They used 0.1, 0.5 and 1.0ml and found that the stains could be detected upon 48 hours after deposition on grass and forest soil. Additionally, on grass, semen stains of large volumes could even be detected for a longer period of time. In our study, we also looked at the effect of ageing, and found that all stains deposited on concrete and aged up to a 1 week period, could be detected by

either the dogs and/or the AP-test. However, this was not the case on grass: the fresh stains were detected by the dogs and the AP-test but with one exception, the older samples were not detected. A first explanation for this decrease in detection of semen stains, was thought to be rain fall. Rain fall was indicated as one of the influences that might affect the stains and lead to a decrease in detection. However, in an experiment where we exposed the semen stains to different amounts of artificial rain fall, we found this did not lead to a dramatic decrease of the detection of the stains. The detection of stains deposited on grass was minimally affected, whereas no effect of rain fall was noticed on the detection of stains deposited on concrete. The amount of artificial rain was increased up to 12mm on one stain on concrete, and even this could easily be found by the dogs the next day.

It is likely that some interaction between the environment and the stain occurs that influences the degradation process. Virkler et al (2009) described that mould, putrefaction and heat are able to degrade the acid phosphatase enzyme present in semen to which the AP-test responds to. The dry concrete surface in our experiment was a hostile environment for the mould and bacterial activity, suggesting that the degradation process of the semen stain was slower compared to the other outdoor surfaces. The grass and (forest) soil conditions were more friendly environments in that respect at the start of the experiment, and biological activity would have been provided with sufficient moisture through the rain fall during that week to degrade the acid phosphatase enzyme.

Washing of textiles seems to have a disastrous effect on the detection of semen stains using ALS and the AP-test. Vandenberg et al (2006) described that even gentle washing with cold water of a stain on nylon fabric made it impossible to detect semen stains with ALS (Polilight), although they discussed that washing could still lead to a weak AP-result. Silenieks et al (2002) also found that washing led to less detectability. New, unwashed textiles retained 0.5ml stains better than previously washed textiles, and a 6 month old stain was more resistant to washing than 1 day old stains. Contrary to Vandenberg, Silenieks et al. found that ALS was able to detect more washed and aged stains than the AP-test and suggested the fluorescent components were less easily removed by washing. The stains in our experiment were minute compared to the stains used in the experiment of Silenieks et al., in our experiments only 0.005 ml of semen stain was deposited on the textiles. No positive detection of semen stains was possible using ALS or the AP-test on washed textiles in our experiments.

The major problem that influences the detection of semen on textiles using ALS is caused by the background of the surface. Silenieks et al (2002) confirmed our findings that dark backgrounds lead to poor results (meaning less semen stains detected) using crime lights, and noted that detergents can contribute to the background fluorescence, making it more difficult to use crime lights. Lee & Khoo (2010) described that highly fluorescent material reduces the contrast between the background and the semen stain, and Vandenberg et al (2006) noted that seminal stains on particular colour/material combinations and checked patterns led to poor results. Searching the area and surfaces using ALS is time consuming, as the use of different combinations of light sources and filters is necessary to obtain the best results and detect most of the fluorescent stains (Silenieks et al 2002). Therefore, experience with the method is required to detect biological stains in forensic practice as fast as possible.

The type of material is hypothesized to play a role in the detection of semen, however contrary results are presented by different studies. For saliva and blood stains the absorbance of the material has been reported to have an effect on the detection using ALS (Vandenberg et al 2006) and dogs (Schoon 2013). Thick fleecy fabrics do not absorb semen, as a result semen stains can be easily identified - in contrast to blood and saliva. The dilution of the stain with water causes the stain to be absorbed by the surface, resulting in the invisibility of the stain using ALS (Silenieks et al 2002). Vandenberg et al (2006) reported that seminal stains on highly absorbent velour and polar fleece could be detected with ALS, however in their study the stains have not been diluted with a liquid. In our experiments we did not examine the effect of dilution. During training with the dogs, it was found that the type of material may have an effect on their detection of the semen stains. Fresh semen stains on the terry cloth of a thick bath towel could easily be detected, however after two days of ageing these stains could not be detected anymore. In future research, the effect of the surface on the detection of semen stains with the different methods needs to be further explored.

For forensic purposes, it is important to establish an optimal sequence of using different detection technologies during the process from locating a trace at the crime scene to the linking of the evidence to a suspect through DNA analysis. Such technologies should not interfere with each other, be able to be used sequentially, and not be destructive in any way. In general a technique like ALS is a good first screening tool: quite sensitive, not specific, and non-destructive - although prolonged exposure with UV light may lead to photo degradation (Virkler et al 2009). However, the use of ALS is limited by i) the environment, outdoor scenes need to be screened in the dark (evening), ii) the fabrics, type and colours, iii) effect of washing and other cleaning procedures. Silieniks also described that routinely screening of underpants, tainted with blood, vaginal deposits and faeces, is difficult using ALS since these deposits might mask the seminal fluorescence, in these cases it is advised to use the AP-test.

However, the AP-test and other presumptive tests are not ideal screening tools: they are only useful when the exact location of the stain is known. A follow-up confirmatory testing is necessary to identify the stain as these tests can only be used as an indicative tool and they are not (human) specific.

Basically detection dogs can be integrated into the investigation process as a first screening tool: as intelligent samplers that will quickly and specifically respond to semen stains of different ages in a variety of circumstances deposited on a many different backgrounds and surfaces. Arson dogs and blood detection dogs are similarly deployed successfully by the Dutch police. A comparison between GC/MS technology and Dutch arson dogs in 1999 showed that the dogs exhibited better discriminatory powers in detecting trace amounts of gasoline on burned foam carpet. In consequence, the dogs were not trained on amounts below the detection limit of the GC/MS in such cases, as this would be marked as false positive results by GC/MS (unpublished data, Dutch National Police Canine Unit). A comparison between presumptive tests and blood detection dogs showed that on smooth surfaces, presumptive tests were more sensitive than the dogs, but on carpets the dogs were much more sensitive (Schoon 2013). It is important to match the sensitivity of the dogs as much as possible to validated technologies, these methods are used as the gold standard and meet the requirements necessary for the use in court.

In future work, the effect of absorbance of the material on the detection of semen stains by the dogs needs to be explored. Additionally, the influence of contamination using the dogs (especially adding another person's DNA to the stain found at the crime scene, for example from a seminal stain used in training the dog earlier), and possibly the loss of trace material needs to be investigated. The use of stronger, more powerful ALS might lead to the detection of more stains on surfaces that are troublesome, for instance outdoor locations. Establishing the sensitivity, specificity, and costs involved using different presumptive methods in different methods in a direct comparison is also necessary to understand how these methods can be used effectively. Research in this area will continue. In this study, we have shown that well-trained dogs might be a valuable detection tool, when the detection is limited by the current detection techniques.

Acknowledgements
The authors would like to thank the Fertility clinic of the Academic Medical Centre for their active cooperation in finding donors for this project. They would also like to thank the forensic unit of the Amsterdam police force who were host to the initial part of the study. And they are grateful to the handlers, dogs and management of the Canine Unit of the Netherlands National Police for their willingness to participate in the experiments presented here.

References

Lee, W. C., & Khoo, B. E. (2010): Forensic light sources for detection of biological evidences in crime scene investigation: a review. Malaysian J Forensic Sci, 1, 17-28.

Schoon, A. (2013): A comparison between canine detection of blood residue and some blood presumptive tests. Journal of Forensic Identification, 63(3), 255-262.

Schoon, A. A., Curran, A. M., & Furton, K. G. (2015): Odor biometrics. Encyclopedia of Biometrics, 1178-1183.

Silenieks, E., Kobus, H. J., & Scharnberg, J. (2002): Improving the effectiveness of fluorescence for the detection of semen stains on fabrics. Journal of Forensic Science, 47(4), 1-5.

Skalleberg, A. G., & Bouzga, M. M. (2016): Detecting and collecting traces of semen and blood from outdoor crime scenes using crime scene dogs and presumptive tests. Forensic science international, 264, 146-152.

Vandenberg, N., & Oorschot, R. A. (2006): The Use of Polilight® in the Detection of Seminal Fluid, Saliva, and Bloodstains and Comparison with Conventional Chemical_Based Screening Tests. Journal of forensic sciences, 51(2), 361-370.

Virkler, K., & Lednev, I. K. (2009): Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic Science International, 188(1), 1-17.