Forensic Science International 302 (2019) 109907

Contents lists available at ScienceDirect

Forensic Science International

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

The use of crime scene detection dogs to locate semen stains on different types of fabric

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ARTICLE INFO

Article history:
Received 27 September 2018
Received in revised form 21 February 2019
Accepted 20 July 2019
Available online 30 July 2019

Keywords:
Dogs
Presumptive tests
Forensic
Semen detection
Fabrics

ABSTRACT

In sexual assault cases, the detection and identification of semen is extremely important as this type of evidence can be used as a source for investigative leads and contributes to case evidence. However, the detection of semen stains is often difficult, even indoors, because of different (environmental) factors, such as substrate type, coloured items and large search areas. In 2015, a project was initiated by the Dutch police to evaluate the feasibility of the use of detection dogs to locate semen stains in forensic practise. Since promising results were obtained, here, a double-blind study was designed to investigate how these detection dogs can optimally be implemented in the current work flow of crime scene investigators and to compare the dog's sensitivity and specificity with current detection methods. The performance of the detection dogs was compared to three commonly used detection methods for semen, (i) forensic light sources (FLS), (ii) the RSID semen field kit and (iii) the enzymatic Acid Phosphatase (AP)-test on semen deposited at different types of fabrics. A 100% sensitivity and specificity for the detection of semen stains using the detection dogs was obtained, compared to an overall sensitivity and specificity of 76.3% and 100% for FLS, 81.6% and 100% for RSID-test, and 92.1% and 100% for AP-test, respectively. Especially, on fabrics with a pattern or interfering fluorescent properties, detection dogs demonstrated to be of additional value to locate the semen stains. We recommend to use the following order of testing, FLS, detection dog, AP-test and RSID test in a forensic workflow.

1. Introduction

Detection and identification of human biological stains at a crime scene are important aspects in forensic casework. Human biological stains contain crucial information providing knowledge about the identity of the donor using the available DNA. Furthermore, biological stains can also be used to link individuals to criminal acts and to reconstruct criminal events that might took place at the crime scene. In sexual assault cases, the detection of human biological stains, especially, semen is crucial in reconstructing the crime and possible criminal actions. Sexual assault cases are often difficult cases, as usually only the victim and the perpetrator are involved and no witnesses are available who can support or reject statements.

Current detection and identification methods for semen stains are available and include the use of presumptive and confirmatory techniques, such as spectroscopic methods, chemical based assays, enzyme-catalytical assays and/or immunological based tests [1–5]. Forensic light sources are often used as a primarily selection tool, since they have the advantages of being rapid, contactless and non-destructive and are able to visualize the presence of semen using its intrinsic fluorescent properties. However, the detection of semen with this technique is difficult on substrates with prints and substrates with fluorescent properties. Additionally, in case of large areas, in which no location is known, the whole area needs to be investigated, which is time consuming. Spectroscopic methods are not specific for human biological stains and should be used as an indicative method. Additional testing is required to indicate the origin of the human biological stain, for instance originating from saliva, semen or urine. Different enzymatic and immunogenic methods are available to identify the presence of semen, including the enzymatic Phosphatesmo KM test (KM-test), the Acid Phosphatase printing method (AP-test) and the immunological Rapid Stain Identification (RSID) semen field kit [4,6,7]. For a good

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http://dx.doi.org/10.1016/j.forsciint.2019.109907
0379-0738/

test performance, the exact location of the stains needs to be known.

Recently, a new method to specifically detect semen stains has been introduced by a Norwegian research group using detection dogs that were specifically trained to locate blood and semen stains [8]. The use of detection dogs in forensic case work has a long history. They are used for a large variety of applications, including the detection of explosives, land mines, drugs, missing persons, fire accelerants and more recently also in the medical field to evaluate their performance in the detection of cancer [9]. In the forensic field, detection dogs are used as an indicative tool to rapidly locate these components of interest, followed by laboratory testing using confirmatory methods. The use of dogs to detect biological stains has been introduced in the 1990's, and cadaver dogs have been trained to recognize the odour of decomposing bodies successfully, even detecting residual contact odour [10]. Therefore, the use of detection dogs to locate semen stains in sexual related cases might be an interesting tool to evaluate.

In 2015, a project was initiated by the Dutch police to evaluate the feasibility of the use of detection dogs to locate semen stains in forensic practise. A small pilot-study, as part of this project, was conducted to establish guidelines for the training and usage of these dogs and compare the performance of the detection dogs with the current presumptive semen detection methods [11]. As promising results were obtained, a large double-blind study was designed to investigate how these detection dogs can optimally be implemented in the current work flow of crime scene investigators and to assess the sensitivity and specificity of this new detection method.

Hence, the aim of this study was to determine the most effective way to implement the detection dogs in combination with the standard methods to detect semen stains at the crime scene. In this study, the performance of the detection dogs was compared to the performance of three commonly used detection methods for semen, (i) forensic light sources (FLS), (ii) an immunogenic test, specifically, the RSID semen field kit and (iii) the enzymatic AP-test. The different techniques were applied to semen stains deposited on different types of fabrics. First, the order of testing using the different detection methods on fabric was established to exclude/minimize the effect that each of the implemented methods could have on one another. After determining the optimal sequence of testing of the different detection methods, semen stains were deposited on different types of fabrics with varying (i) colours, (ii) patterns and (iii) sizes to test the performance of each different (presumptive) test. Sensitivity, specificity and accuracy of each method for the detection of semen was investigated.

2. Materials and methods

Overall set-up: experiments were performed using a double blind set-up in which both the researchers and dog handlers worked "blind", not having any *a priori* knowledge on which items and at which location semen stains (or negative control samples) were deposited.

2.1. Sample collection and storage

Human semen, saliva, urine and breast milk samples were collected from consenting (healthy) volunteers. Semen samples were acquired from the hospital fertility clinic. Donors gave their informed consent and all samples and donor information were anonymised. All experiments were conducted according to institutional guidelines. Semen samples were prepared freshly or stored upon use in the fridge at 4 °C. Non-target samples were collected during working hours (urine, saliva and breast milk). Saliva was captured in sterile 10 or 50 mL conical vials and urine was collected in urine collection cups and were brought to the laboratory the same day. Saliva was collected with the condition that the donor has not consumed any food or drinks for at least one hour prior to collection. Breast milk was collected from breast-feeding females. Upon arrival in the laboratory, all non-target samples were vortexed and aliquoted. All non-target samples were stored in the freezer at −80 °C until use.

2.2. Establish semen detection method sequences

To determine the optimal sequence of testing of the different detection methods, six experiments were conducted, in which the sequence in which the methods were applied, varied. Semen stains (5 μL) were deposited on twelve white cotton shirts. Samples were left to dry for at least three hours. Four detection methods were used in this experiment: FLS, crime scene dogs, AP-test and the RSID test. Experiments were performed in duplicate. In Table 1 an overview is given of the different experiments and the sequence in which each method was applied to detect the semen stains. Within each experiment, samples were analysed for the presence of semen stains with FLS at four different time points as depicted in Table 1. FLS was used as a first tool to locate the semen stain and to indicate were to apply the presumptive test methods. When positive FLS results were obtained, samples were visualized and recorded under identical circumstances to visualize the effect on the visibility of the stain after applying each detection method. Based on the overall results of each test method within each experiment, the optimal sequence of testing was determined. Optimally, after applying all different tests, positive test results are obtained for each method and the fluorescence signal is minimally affected by the different presumptive methods.

2.2.1. Detection of semen stain using forensic light source

Three different FLS were used as a first screening tool to detect semen stains on the different types of fabric: UV, blue and green Crime-lite® 82S torches (Foster & Freeman, UK). Images were obtained under identical circumstances and camera settings. Images were taken using the long pass filters supplied with the Crime-lite® 82S torches according to the scheme presented in Table 2 using a Canon EOS 40D and a Canon Macro Lens EF 100 mm f/2.8 USM and/or a Nikon D40X camera.

A positive test result means that a fluorescent stain could be identified on the substrate with one or more of the used crime-lites using the appropriate filter and goggles. A negative test result

Table 1
An overview of the experiments performed to establish the order of methods to detect semen stains on fabric. Experiments were performed in duplicate. Experiments = Exp.

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Exp. 1	FLS	RSID test	FLS	Detection dog	FLS	AP test	FLS
Exp. 2	FLS	AP test	FLS	Detection dog	FLS	RSID test	FLS
Exp. 3	FLS	Detection dog	FLS	AP test	FLS	RSID test	FLS
Exp. 4	FLS	RSID test	FLS	AP test	FLS	Detection dog	FLS
Exp. 5	FLS	AP test	FLS	RSID test	FLS	Detection dog	FLS
Exp. 6	FLS	Detection dog	FLS	RSID test	FLS	AP test	FLS

Table 2
Specifications of crime lite® 82S.

Crime lite® 82S	Filter goggles	Filter camera
UV (350–380)	Clear	GG420 (406 nm clear)
Blue (420–470)	Orange	OG550 (539 nm orange) GG495 (476 nm yellow)
Green (480–560)	Red	OG590 (571 nm bright red)

means that no fluorescent stains could be identified on the substrate with the three crime-lite torches.

2.2.2. Detection of semen using crime scene dogs

Five trained dogs participated with their handlers to detect the semen stains in a similar manner as when conducting casework. Fabrics were vertically fastened with magnets to a magnetic board. The use of such an experimental set-up allows the dogs to search the area easily without affecting the fabric. The handler, unaware of the position of the semen stain, allowed his dog to search the fabric. A positive result was obtained when dogs indicate the presence of semen by performing a characteristic focused sit/stare response with their nose close to the spot where they smell the semen. Such a characteristic response was identified by the handler. A negative result meant that the dog had searched the whole item, but did not show any change in behaviour while searching and did not perform the characteristic focused/sit stare response to any specific area. The search was then ended by the handler. Each fabric item was searched by only one of the available dog teams.

2.2.3. Presumptive testing for semen stains using RSID

The RSID™ semen kit (Independent Forensics, USA) was applied to the samples when positive test results were obtained with FLS or the detection dogs to identify the presence of semen on the different types of fabrics. Before applying the RSID kit, the indicated area was swabbed with a pre-wetted sterile cotton swab (Eurotubo® collection swab, Deltalab, Spain). Swabs were pre-wetted with MilliQ water (Millipore, Merk KGaA, Germany). Substrate were swabbed according to a standardized protocol to minimize the inter variability due to operation procedures within each different experiment. Samples were tested using RSID kit according to the manufacturer's protocol. A positive test result means that two red/pink lines could be identified at the cassette after 10 min of sample incubation. A negative test result means that only one red/pink line could be identified at the cassette after 10 min of sample incubation. The RSID kit was applied to the samples when positive test results were obtained with FLS or the detection dogs. All test results were visualized and recorded using the Canon Macro Lens EF 100 mm f/2.8 USM and/or a Nikon D40X camera.

2.2.4. Presumptive testing for semen stains using the AP-test

The AP-test was used as a presumptive test to indicate the presence of semen stains on different types of fabrics. Two stock solutions were made, stock A and stock B. Stock A was prepared by diluting 20 g of sodium acetate trihydrate (VWR, Netherlands) and 1 g fast blue B salt (Sigma Aldrich, Germany) in 50 mL of a 10% acetic acid (glacial) (VWR, Netherlands) solution and additionally, 60 mL of MilliQ water was added. Stock B was prepared by diluting 0.8 g of 1-naphtyl phosphate disodium salt (Sigma Aldrich, Germany) in 10 mL MilliQ water. A working solution was prepared by diluting 5 mL of stock A and 500 μL of stock B in 45 mL of MilliQ water. For each experiment the working solution was prepared freshly. A piece of filter paper (1 cm × 1 cm) was used to sample the desired area by fully covering the semen stain. Filter paper was wetted with MilliQ water using a liquid-sprayer. After wetting the filter paper, some pressure was applied to the filter paper to allow transfer of the stain to the filter paper. The filter paper was then removed from the substrate and additionally sprayed with the working solution. After spraying the paper with the acid phosphatase mix, a positive test result means that a purple/pink coloration could be observed within 60 s. A negative test result means that no coloration could be observed within 60 s. All test results were visualized and recorded using the Canon Macro Lens EF 100 mm f/2.8 USM and/or a Nikon D40X camera.

2.3. Comparison of FLS, crime scene dogs, AP-test and RSID

To determine the efficiency of the crime scene dogs on the detection of semen on different types of fabric, the sensitivity, specificity and accuracy of the four methods was determined based on the true-positive, false-positive, true-negative and false-negative results.

The following variables were included in this experiment:

- Types of fabric: cotton, polyester and denim
- Colour of fabric: white, white with pattern, red, red with pattern, black and black with pattern
- Size: small sizes (underwear), large sizes (bed sheets)

Sample preparation was performed according to a standardized protocol to minimize inter-donor variation. 20 μL of sample material was deposited at the different types of fabric. Samples were prepared by a researcher who was not further involved in this study to guarantee a double-blind set-up, one week before the experiments were conducted. The researcher was asked to deposit the semen, urine, saliva or breast milk samples on to the substrates (20 μL for each body fluid). Urine, saliva and breast milk served as potential biological material that could give false-positive results with one or more of the methods. A blank control was also included, in which no semen or other biological material was deposited at the substrate. The researcher was asked to describe the precise location of the deposition of the sample material in a table. Samples were left to dry for one hour, subsequently samples were packed and folded into sterile paper bags, normally used by the Dutch police to secure evidence. When samples were folded, sterile sheets were used to prevent stamping of the stains to other sites of the substrate. A total of 44 samples were prepared as specified in Table 3.

Samples were analysed in the following sequence: (1) FLS, (2) crime scene dogs, (3) AP-test and (4) RSID as described in Sections 2.2.1–2.2.4.

3. Results

3.1. Determine the sequence of testing

To determine the sequence of the different (presumptive) methods, six different experiments were conducted. In all cases, positive results were obtained independent in which sequence the methods were applied to the semen stains (Fig. 1). FLS was used to observe differences in fluorescence signal before and after applying the different methods. A decrease in fluorescent signal was observed in all experiments after applying one of the three methods, as depicted in Fig. 1 (step 1, 3, 5 and 7). Searching the substrate for semen stains with the crime scene detection dogs affected the fluorescence signal of the stain minimally. The RSID test and AP-test did not negatively affect the detection of semen using the crime scene dogs. In all samples, the crime scene detection dogs were able to indicate the location of the semen stain. The crime scene detection dogs did also not

Table 3
Overview of samples prepared for the method comparison.

Fabrics	Colours	Further specifications	Type of stain
Cotton	White	No pattern (n = 2)	Semen (n = 2)
		Pattern (n = 2)	Semen (n = 1); spittle (n = 1)
	Red	No pattern (n = 2)	Semen (n = 2)
		Pattern (n = 2)	Semen (n = 1); blank (n = 1)
	Black	No pattern (n = 2)	Semen (n = 1); urine (n = 1)
		Pattern (n = 2)	Semen (n = 2)
Polyester	White	No pattern (n = 2)	Semen (n = 2)
		Pattern (n = 2)	Semen (n = 2)
	Red	No pattern (n = 2)	Semen (n = 2)
		Pattern (n = 2)	Semen (n = 1), breast milk (n = 1)
	Black	No pattern (n = 2)	Semen (n = 2)
		Pattern (n = 2)	Semen (n = 1); spittle (n = 1)
Denim	Light (n = 2)		Semen (n = 2)
	Dark (n = 2)		Semen (n = 1); spittle (n = 1)
Cotton	White	Bedsheets (n = 4)	Semen (n = 4)
		Underwear (n = 4)	Semen (n = 4)
	Dark	Bedsheets (n = 4)	Semen (n = 4)
		Underwear N = 4)	Semen (n = 4)

influence the outcomes obtained with RSID or the AP-test. In all experiments, semen could be detected using the four methods, no remarkable differences were obtained between the different experiments.

To minimally affect the current forensic workflow, it was decided to basically follow the sequence of testing that is at present used by the Dutch police and the Netherlands Forensic Institute and include the detection dog in the second step, see experiment 3 in Table 1 (FLS, detection dog, AP-test and RSID).

3.2. Comparison of FLS, crime scene dogs, AP-test and RSID

To evaluate the performance of the four different methods, sensitivity and specificity were calculated for each method. In this

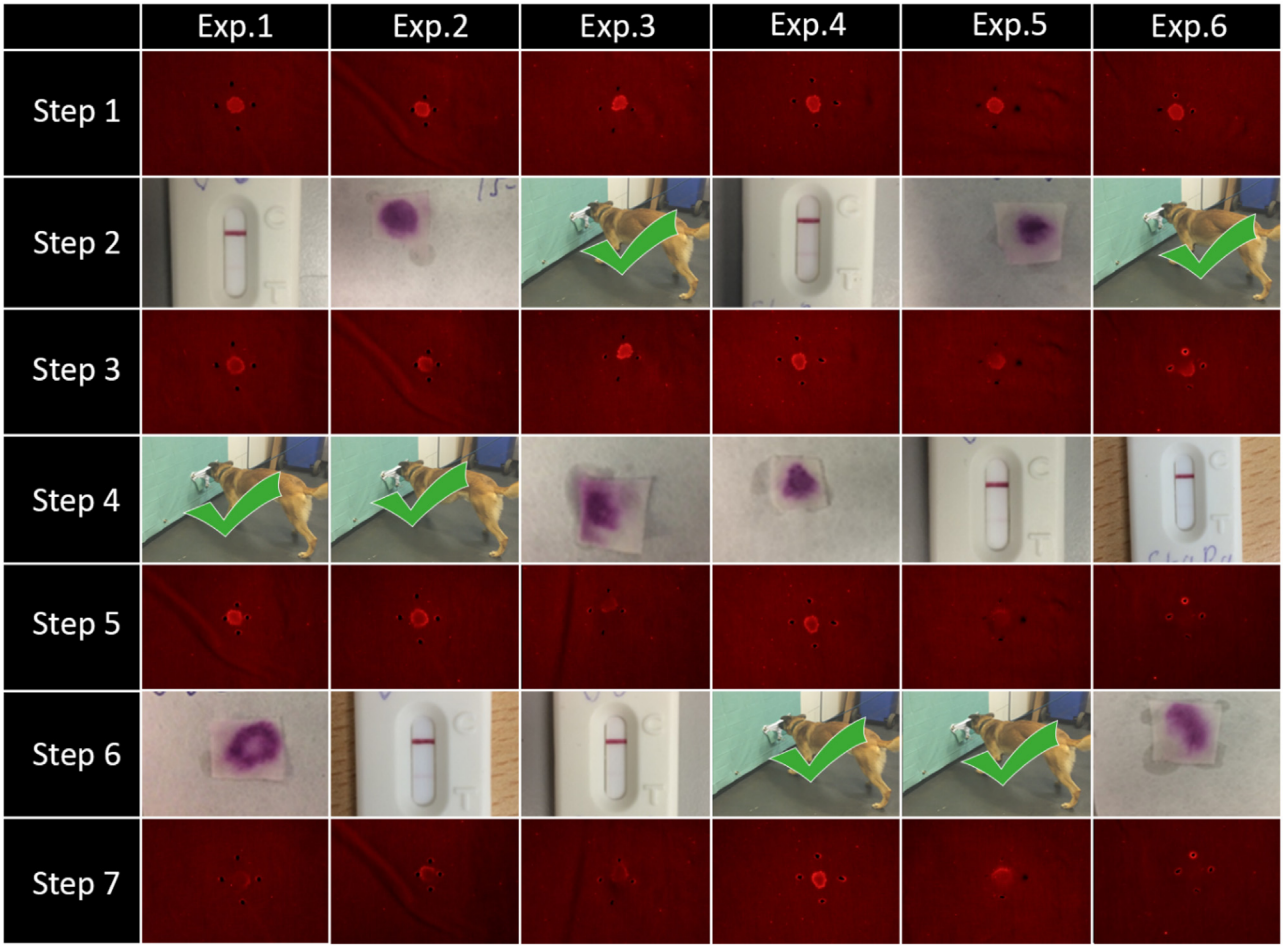

Fig. 1. Results of the sequence of testing. Positive detection of semen stains was possible using the four different methods independent of sequence of testing. A decrease of autofluorescence intensity of the semen stain is observed in all experiments after the different methods have been applied (step 1, 3, 5 and 7).

study, a total of 44 samples were investigated on the presence of semen. Thirty-eight samples were prepared on which semen was deposited on the fabric and six negative control samples were included. These six control samples comprised one blank sample (no body fluid was deposited on the fabric) and five samples, whereby a body fluid other than semen was deposited on the fabric, specifically, three saliva samples, one urine sample and one breast milk sample. Using FLS, nine semen stains could not be detected, resulting in a sensitivity of 76.3%, with a specificity of 100%. The crime scene dogs were able to locate all the semen stains and no false-positive results were obtained, resulting in a sensitivity and specificity of 100% (Table 4). Using the RSID test seven samples were false-negative (sensitivity of 81.6% and specificity of 100%). Three samples could not be detected with the AP test (sensitivity of 92.1% and specificity of 100%). No false-positive results were obtained with one of the four methods. The overall sensitivity and specificity of the different testing methods is given in Table 4.

To illustrate the effect of fabric type, colour of fabric and pattern on the sensitivity of the detection method for the detection of semen stains, the data was regrouped into four different groups, including fabric type, colour, pattern and size (Tables 5–7).

The sensitivity of FLS, detection dogs and AP-test was 100% for the detection of semen on denim, although a limited amount of samples were included (four samples, including one blank sample). However, in one of these three semen positive samples the RSID test resulted in a negative result.

No remarkable differences could be observed between the results obtained on the different types of fabric, nor on the colour. Pattern had a much stronger impact on the detection of the semen stains using the different methods, particularly on the sensitivity of the use of FLS (χ^2, p = .062). However the number of patterned items investigated in this study was limited.

4. Discussion

The objective of this study was to determine the most effective way to implement detection dogs in combination with the standard methods to detect semen at the crime scene and to compare the sensitivity and specificity of this new technique with the current detection methods.

In the Netherlands, the use of detection dogs to detect semen stains at the crime scene is relatively new and no guidelines or standard operational procedures are available. For optimal use of the detection dogs, guidelines need to be established to assist the forensic investigator in forensic casework. The first step in this study, was to determine in which step of the forensic investigation the detection dogs can be implemented to be the most effective and without affecting future additional analysis of sample material. Since semen could be detected in the six experiments using the four different methods, independently of the sequence of testing, we recommend to use the sequence of testing that is currently used by the Dutch police and the Netherlands Forensic Institute and implement the detection dog after FLS have been applied to search the crime scene for semen stains. If no stains are detected at the crime scene or on crime-related objects, the forensic investigator has no clue where to recover the evidence. The detection dogs are able to search larger areas in a relative short time period. If the dogs indicate a particular spot at the crime scene/object, the forensic investigator has a lead to search for forensic evidence and can easily apply a presumptive method, such as the AP-test or RSID-test to determine the presence of semen.

In the canine world, it is accepted that handler expectations have a significant effect on the results of the dogs [12]. Therefore, the Scientific Working Group on Dog and Orthogonal detector Guidelines (www.swgdog.org) advises to perform double-blind assessments with dogs to minimally affect their search results. In order to simulate operational reality, including all interpretation and operational errors as much as possible, we decided to compare all the different semen detection techniques included in this study on physically the same sample set in a double blinded manner, both the dog handler as the researcher who applied the detection methods did not know whether and where semen stains were located at a investigated items. The techniques were performed consecutively following the most logical operational protocol as tested and described in this study.

FLS was used a first detection tool to search the substrates for possible semen stains using their autofluorescent properties. Due to autofluorescence properties of the fabric and/or the presence patterns, not all semen stains could be detected with FLS. A specificity and sensitivity of 100% was obtained using the detection dogs to locate semen stains at fabric. However, the dogs were not always able to indicate the exact location of the semen stain. If the dog's response was within 10 cm of the exact location of the stain, a positive response was recorded by the investigator. Once the dogs had indicate the location of the semen stain, FLS was used to determine the exact location of the semen. However, combining both, FLS and detection dog, did not always lead to the exact spot of the semen stain. In these cases, the exact location of the semen stains was broadly estimated. The RSID and AP test were then performed without exact knowledge on the location of the semen stain, which might have influenced the outcome of the results of the RSID and AP. Swabs taken from these samples might have missed the exact semen spot, leading to negative results for these two tests. Although the amount of semen used in this study (20 μL) corresponds within the optimal range of these two tests as indicated by the manufacturer, the results of this study reflect the operational reality more objectively.

Noteworthy, a total of six potentially semen positive stains were not detected with FLS. These samples were later re-evaluated by one of the experts in this field at the Netherlands Forensic Institute. The expert was not able to pinpoint the exact location of the semen

Table 4
Sensitivity and specificity of test methods.

Overall	FLS	Dog	RSID	AP
Sensitivity (38)	76,3%	100,0%	81,6%	92,1%
Specificity (6)	100,0%	100,0%	100,0%	100,0%

Table 5
Sensitivity of test methods for cotton and polyester material (three denim sample are disregarded).

Material	FLS	Dog	RSID	AP
Cotton (25)	76.0%	100.0%	88.0%	92.0%
Polyester (10)	70.0%	100.0%	70.0%	90.0%

Table 6
Sensitivity of test methods for different colours.

Colour	FLS	Dog	RSID	AP
White/light (17)	94.1%	100.0%	82.4%	94.1%
Black/dark (15)	66.7%	100.0%	80.0%	86.7%

Table 7
Sensitivity of test methods for textiles with or without patterns.

Pattern	FLS	Dog	RSID	AP
No (30)	90.0%	100.0%	93.3%	100.0%
Yes (8)	25.0%	100.0%	37.5%	62.5%

spot, but did locate a few spots of interest, of which none of them were semen stains. Especially, in these cases the detection dogs can be applied to indicate whether semen is present at a substrate.

In forensic science, knowledge on the sensitivity of the techniques used to recover evidence from the crime scene is an important factor in evaluating the evidence. Studies undertaken to assess the sensitivity of techniques usually focus on technical aspects in standard circumstances and do not take operator mistakes or operational circumstances into account. However, when these operator mistakes and/or operational circumstances are included, for example in the proficiency testing offered by Collaborative Testing Services, the achieved sensitivity and specificity is lower and reported on anonymously (https://cts-forensics.com/index-forensics-testing.php). Similarly, dogs can be trained to be very sensitive to particular odours in laboratory circumstances. But then again, in field studies variable results can be obtained, depending on the way the dogs are trained, the study design, undiagnosed health issues, day to day variability, and handler issues [9,13,14]. Direct comparisons between dogs and other instruments or techniques are scarce. A few have been done particularly in the field of explosive detection, where the dogs are still considered as the gold standard [13–16]. Such direct comparisons, using the same sample set and including handler/operator variables, are necessary to fairly evaluate the capabilities and limitations of the different systems and techniques.

Using dogs as "intelligent samplers" to pinpoint areas of interest is a useful approach that maximises the canine potential whilst observing the legal requirements of evidence collected. In some countries, dogs are trained to detect several body fluids, for example blood and semen (Norway), or even a combination of fire accelerants and semen (Sweden). Whilst this may seem more economical, it may complicate follow-up procedures since it may not be clear what the dog is alerting on. Even if dogs are trained to respond differently, or only respond to one particular trace based on a command, such indications are not always reliable [17]. For this reason, dogs in The Netherlands are trained only on semen.

To observe legal requirements, one has to bear in mind that dogs might be a potential risk of contamination at the crime scene, and special attention needs to be given to minimize/exclude the transfer of semen or DNA from scene to scene. Also, the detection dogs should not remove traces from the crime scene. Preliminary work within our group has shown that the risks of contamination is minimal, however a larger validation study needs to be performed. Additionally, the sensitivity of the dogs to locate semen should match the detection level of follow up technology. An earlier study on blood residue detection [18] illustrated that dogs were more sensitive than presumptive blood tests on carpet, but not on vinyl. Earlier work on arson also showed dogs being more sensitive than ASTM laboratory techniques used at the time [19]. Being aware of these differences in sensitivity leads to a better understanding and thus a better use of canine detection. For semen, we have yet to investigate the limits of their detection and combine this with the sensitivity of current DNA methods. DNA profiling is possible with little cell material, however to allow DNA analysis the location of the stain and additional extraction of the stain need to be performed.

In the Netherlands, crime scene dogs trained to detect semen traces are now used in forensic casework. They have already proven their potential in several Dutch cases, locating semen traces on leaves beside a cycle path where a woman had been assaulted, and on the clothing of a child who was initially very reluctant to testify.

5. Conclusion

In conclusion, this study demonstrates the potential of the use of detection dogs to locate semen stains at crime-scene related objects, in particular different types of fabric. A 100% sensitivity and specificity of detecting semen stains on different types of fabric was obtained using the detection dogs, compared to an overall sensitivity and specificity of 76.3% and 100% for FLS, 81.6% and 100% for the RSID-test and 92.1% and 100% for AP-test respectively. Detection dogs are therefore a valuable tool to use for the detection of semen on difficult substrates or with difficult patterns/fluorescent backgrounds. The crime scene detection dogs can easily be integrated in current forensic workflow, and we recommend to use the sequence of testing that is currently used by the Dutch police and the Netherlands Forensic Institute and implement the detection dog after FLS have been applied to search the crime scene for semen stains.

Acknowledgements

The authors would like to thank the dog handlers from the Netherlands National Police Force who participated in the study. We also thank Dennis Boon from the Netherlands Forensic Institute for his help with looking at the samples as an expert in the field. We also acknowledge the many donors who donated their semen for this study through the kind cooperation of the fertility clinics of the Amsterdam Medical Center and the Isala Hospital.

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