Comparison of the ABAcard®HemaTrace (old), ABAcard®HemaTrace (YYYY-MM-DD), RSID™Blood, and SERATEC®HemDirect tests for Sensitivity, Specificity, and Detection of Blood in a Mixture.

By: Nicole Miller 6/17/24

Purpose

The ABAcard HemaTrace test is an immunochromatographic test for the detection of human hemoglobin (Hb). Due to supply chain issues related to the SARS COVID-19 pandemic, Abacus Diagnostics was unable to obtain the plastics necessary for the manufacture of the Abacus p30 card and redesigned the tests using alternate plastic housings. The new design required a different volume for the test (80 µL vs. the previous 200 µL), requiring an evaluation of the material modification.

The new lots of ABAcard HemaTrace can be differentiated by the format of the lot numbers. The original, validated lots, referred to herein as "ABAcard HemaTrace (old)" had the format of three letter month followed by the year (e.g., NOV 2023). The new, modified lots, referred to herein as "ABAcard HemaTrace (new)" have the format of YYYY-MM-DD (e.g., 2025-02-28).

This material modification was part of a wider evaluation of immunochromatographic tests including those from alternate manufacturers and testing kits for semen and saliva. This included RSID tests from Independent Forensics, and kits from SERATEC. The RSID™-Blood test (Rapid Stain identification of Human Blood) is an immunochromatographic test for the presence of human glycophorin A. The SERATEC ® HemDirect test is a chromatographic immunoassay for the presence of human hemoglobin (Hb). The material modification data was taken from sensitivity, specificity, and mixture experiments. The sensitivity test compares ABAcard HemaTrace (old), ABAcard HemaTrace(new), RSID™-Blood, and SERATEC ® HemDirect. The specificity and mixture tests compare ABAcard HemaTrace(new), RSID™-Blood, and SERATEC ® HemDirect.

The results from the sensitivity, specificity, and mixture experiments on the ABAcard®HemaTrace (old), ABAcard®HemaTrace (YYYY-MM-DD), SERATEC®HemDirect, and RSID™Blood kits will be compared for the forensic identification of human blood.

Materials and Methods

For the sensitivity experiment, three samples of human blood (blood 1, blood 2, and blood 3) were serially diluted with PCR grade water in a tenfold dilution series starting at 1/10 and ending at 1/1,000,000. These were then tested as liquid blood samples using 200 µL for ABAcard®HemaTrace (old), 80 µL for ABAcard®HemaTrace (new), 120 µL sample for SERATEC ® HemDirect, and 20 µL sample + 80 µL RSID™ -Universal Buffer for RSID™-Blood. Blood stains were prepared from the 1/10 – 1/1,000 dilutions, dried, extracted as per each kits respective protocol and SDPD Crime Lab protocol, and tested on each kit. For the ABAcard®HemaTrace (old) kit the blood stain was extracted in 100 µL of kit extraction buffer for 15 minutes. Then enough of the supernatant was added to a second microcentrifuge tube containing 200 µL of kit buffer to get a light straw yellow solution; 200 µL was tested. For the ABAcard®HemaTrace (new) kit the blood stain was also extracted in 100 µL of kit extraction buffer for 15 minutes, but due to the volume difference between tests, enough of the supernatant was added to a second tube containing 80 µL of kit buffer to get a light straw yellow solution; 80 µL was tested. For the SERATEC®HemDirect test, the blood stain cutting was added directly to the buffer vial included in the kit and extracted for 10 minutes; 120 µL was tested. For the RSID™-Blood kit, the blood stain was extracted in 100 µL RSID™ -Universal Buffer for 1-2 hours; 20 µL sample + 80 µL RSID™ -Universal Buffer was tested.

For the Specificity experiment, a sample of male urine, female urine, semen, and saliva were diluted 10-fold from neat to 1/1,000. These dilutions were tested with 80 µL sample for ABAcard® HemaTrace (new), 120 µL sample for SERATEC ® HemDirect, and 20 µL sample + 80 µL RSID™ -Universal Buffer for RSID™-Blood. Cuttings of dried animal blood from a dog, cat, pig, chicken, cow, goat, parrot, and gorilla were also extracted as described above for the sensitivity experiment. Once extracted, the sample was diluted from 1/10-1/100 and tested with each kit.

For the mixture experiment, mixtures of 20 µL semen + 180 µL of either blood semen, saliva, male urine, or female urine were collected and dried on swabs. Swabs were cut into quarters, extracted, and tested as per each kits protocol with their provided extraction buffer.

Results

For the sensitivity experiment, the ABA HemaTrace (old) kit had the best sensitivity followed by SERATEC HemDirect, ABA HemaTrace (new), and RSID blood. The ABA HemaTrace (old), SERATEC HemDirect, and ABA HemaTrace

(new) consistently tested positive up to the 1/100,000 dilution while the RSID blood kit only consistently tested positive at the 1/10 dilution. The RSID blood kit did not sow any false negatives due to high dose hook effect unlike the other 3 kits.

For the specificity experiment, the RSID blood kit had the best specificity followed by ABA HemaTrace (new), and then SERATEC HemDirect. The RSID blood kit only had 1 false positive for neat saliva. The ABA HemaTrace (new) kit had false positives for neat and a 1/10 dilution of semen and was inconclusive for neat saliva. The SERATEC HemDirect kit had false positives for neat and a 1/10 dilution of semen, and 1/10 and 1/100 dilutions of saliva. When animal blood was tested, all kits were negative for dog, cat, pig, chicken, cow, goat, and parrot blood. The ABA HemaTrace (new) and SERATEC HemDirect both had a false positive for human blood when gorilla blood was tested. The RSID blood test did not have any cross reactivity with gorilla blood.

For the mixture experiment, the ABA HemaTrace (new), RSID blood, and SERATEC HemDirect kits all tested positive for human blood when mixtures of blood + either semen, saliva, male urine, or female urine were tested.

Conclusions

The ABAcard HemaTrace (new) demonstrates sensitivity that is on par with current methods. HemaTrace gave be best balance of sensitivity (liquid blood and extracted bloodstains) and specificity compared to RSID Blood and SERATEC HemDirect.

Based on the results of this study, ABAcard HemaTrace (new) testing is suitable for casework.

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Experimental Design for the Validation of the HemaTrace ABAcard for the Identification of Human Blood

1. The development of a written protocol for the HemaTrace Human Origin Test Card derived from the product insert provided with the test cards produced by Abacus Diagnostics.

2. Dilution series of liquid human blood.

   Human Blood will be serial diluted in a tenfold dilution series. The series will start with a 1/10 dilution and will extend to 1/1,000,000. Once a negative test is encountered at a particular dilution the testing of further dilutions will not be necessary. The dilution series will also be tested by our current procedure involving crossover electrophoresis.

   Purpose: To determine how sensitive the HemaTrace Human Origin Test Card is.

3. Testing of the HemaTrace Human Origin Test Card with other human body fluids.

   Urine, semen, feces (stain), and saliva will be diluted in a tenfold dilution series. The series will start as undiluted and will extend to 1/1,000.

   Purpose: To determine if other human body fluids besides blood can yield a positive result with the HemaTrace Human Origin Test Card.

4. Testing of the HemaTrace Human Origin Test Card with blood obtained from a series of animals common to the human environment. These animals may consist of chicken, cow, bird, higher primate, pig, dog, cat and goat. A dilution series of 1/10 through 1/1,000 will be tested.

   Purpose: To determine if blood from other animals can yield a positive test result with the HemaTrace Human Origin Test Card.

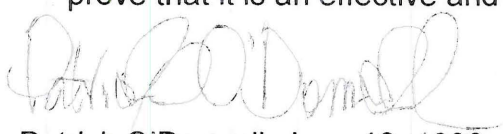
5. Testing of the HemaTrace Human Origin Test Card with human bloodstains.

   A tenfold dilution series of human blood will be prepared as described in step #2, spotted onto various clothing substrates, and the stains allowed to dry. The stains will then be dehydrated and tested with the HemaTrace Human Origin Test Card.

   Purpose: To determine if human bloodstains can be effectively tested using the HemaTrace Human Origin Test Card.

6. If needed, minor refinement of the protocol developed in step #1.

7. Implementation of the HemaTrace Human Origin Test Card if the validation experiments prove that it is an effective and reliable tool for the identification of human blood.



Patrick O'Donnell, June 10, 1999
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Summary of the ABAcard HemaTrace Validation Studies for the Identification of Human Blood

This validation study was developed to find an alternative to the method used by the San Diego Police Department Crime Laboratory in determining the origin of bloodstains collected from evidence. Currently, the Crime Laboratory utilizes crossover electrophoresis as a test to qualitatively determine if a particular bloodstain is of human origin. The "Human Origin" crossover technique is a time intensive procedure that requires approximately 24 hours to obtain results. The sensitivity of this method in detecting human blood can reach dilutions up to 1:25,000. On average, Analysts at the SDPD Crime Laboratory have been able to detect human blood up to 1:1280 dilution. The OneStep ABACard HemaTrace is designed specifically to detect the presence of human hemoglobin in quantities above 0.05 μg/ml. The results appear within 10 minutes, and with the exception of blood from higher primates, none of the non-primate animal blood the laboratory has tested produced a positive result.

Principle Behind Human Origin Crossover Electrophoresis:

The "Human Origin" crossover electrophoresis technique is utilized to test for origin of species. Using agarose gel solidified on a gel bond, a pair of wells is punched on the gel. One well is loaded with human serum and the other well with human antiserum. Through electrophoresis, the serum and the antiserum migrate towards each other forming a precipitin band. Formation of this band confirms that the bloodstain is of human origin. This technique is time consuming. It requires preparation of four different reagents, and a multi-step procedure that takes almost a day and a half before results can be obtained.

Principle Behind the OneStep ABACard HemaTrace:

The OneStep ABACard HemaTrace is an immunochromatographic test for the detection of human blood, specifically human hemoglobin. Human hemoglobin (Hb) reacts with a mobile monoclonal antihuman Hb antibody forming a mobile antigen-antibody complex. This complex migrates through the test membrane, towards the test area of the card labeled (T) where a bound polyclonal antihuman Hb antibody captures this complex thus forming an antibody-antigen-antibody sandwich. When the amount of hemoglobin in a sample exceeds 0.05 μg/ml, a formation of two colored lines in the (T) and (C) area of the test strip indicates a positive reaction, while a single line in the (C) area indicates a negative result. The card has a built in control mechanism that allows the analyst to determine whether proper procedures were followed.

The HemaTrace kit contains individually sealed test cards and pre-aliquoted buffer in separate extraction tubes. The results are consistent and, and with the exception of the time required to extract samples, can be obtained in ten minutes.

Results/Discussion:

This study focused on the sensitivity and specificity of the HemaTrace Card as a potential alternative to the methods currently in place at the SDPD Crime Laboratory. Several trials were performed on various specimens such as human blood, other human body fluids, and different animal bloods. The methods and procedures used are outlined in the individual trials.

The HemaTrace Test Card is capable of detecting human hemoglobin in liquid blood samples at concentration levels that reached parts per million. This value agrees with the detection limit of 0.05 μg/ml as stated by the manufacturer. The sensitivity of the card to human bloodstains was observed at concentrations of 1:10,000 dilution.

With respect to other human body fluids tested, the test card was found sensitive to trace levels of hemoglobin present in urine, semen, and saliva samples. Liquid saliva samples gave a positive result at the 1:10 dilution, while that of liquid semen and urine showed a weak positive at the 1:100 dilution. Further dilution of the liquid samples to 1:1000 all yielded negative results. Semen and saliva stain extracts gave weak positive results, and the urine stain extract was negative. Reactivity of the stain extracts diminished upon further dilution to 1:10.

One of the limitations of the HemaTrace Test Card is its ability to react with primate blood. The extracted primate bloodstains gave positive result up to the 1:100 dilution. Twenty-two bloodstain samples from 13 different animals were also used to test the specificity of the cards. With the exception of the primate blood, all gave negative results.

In many of the samples tested, noticeable "false positive" results were visible after the maximum reading time of ten minutes has lapsed. The manufacturer has cautioned against reading the test card after this time since non-specific reactions may take place that could result in a "false positive" reading.

Although no "high dose hook effect" was observed, it can result when the sample concentration is too high. Competitive binding between the antigen (human hemoglobin) and the limited number of antibody present in the test membrane occurs. Therefore, it is suggested that analyst dilute blood samples until it is "straw-color" in appearance.

Conclusion:

The HemaTrace Test Card was found to be sensitive and specific to human and higher primates. The sensitivity of the HemaTrace Test Card far exceeds the sensitivity of the Human Origin Crossover Electrophoresis. The test procedure requires a minimal amount of time and produces consistent results.

References:

1. OneStep ABACard HemaTrace for the Forensic Identification of Human Blood. Technical Information Sheet.

2. Swander, C.J., Stites, J.G. Evaluation of the ABACard HemaTrace for the Forensic Identification of Human Blood. MAFS 1998 Annual Meeting.

3. Spear, T.F., Binkley, S.A. The Hemeselect Test; a Simple and Sensitive Forensic Species Test. Journal of Forensic Science Society, V34 (1), p41-46, 1994.

4. Hochmeister, M.N., (et al). Validation Studies of an Immunochromatographic 1-Step Test for the Forensic Identification of Human Blood. Journal of Forensic Science. V44 (3), p597-602, 1999.

Acknowledgment:

The following were instrumental in providing the laboratory with animal blood samples.

1. Dr. Rosanne Brown, Rancho San Diego Animal Hospital, El Cajon.
2. Dr. Scott Humphries Broadway Animal Hospital, El Cajon.
3. Dr. Don Janssen, San Diego Zoo Hospital, San Diego.
4. Mark Traughber, DOJ Riverside

San Diego Police Department Crime Laboratory
Forensic Biology Unit
The ABAcard® p30 Test using Concentrated Samples

Analysts: Brian Lew & DeKenya Williams
Date: 11/8/2012

INTRODUCTION

The ABAcard® p30 Test (Abacus Diagnostics) is one of two methods currently employed by the San Diego Police Department (SDPD) Crime Laboratory's Forensic Biology Unit as a confirmatory test for the identification of semen. The Y-screen male DNA detection assay[1] was developed to address an increased volume of sexual assault evidence requiring DNA testing. DNA testing of sexual assault evidence traditionally involved identifying the presence of semen, differentially extracting each sample, and conducting the highly labor-intensive microscopic search for the presence of sperm cells. In an effort to increase the efficiency and throughput for which sexual assault evidence is analyzed, the ABAcard® p30 Test is being utilized as an alternative ~~conformational~~ confirmatory sl 5/16/13 test for the presence of semen to microscopic examination.

In the original validation of the ABAcard® p30 Test[2], it was determined that a 1:10 dilution of every sample extract should be used for optimal results. In casework, it has been observed that a portion of sexual assault evidence is composed of stains with a minimal number of sperm cells or low amounts of male DNA. This study is an effort to identify the necessity of diluting sample extracts when low amounts of p30/semen are suspected and to evaluate the utility of the ABAcard® p30 Test using larger sized samples.

MATERIALS & METHODS

Materials

- ABAcard® p30 Test (Abacus Diagnostics, Catalog #308322)
- Nanopure water
- Human semen
- Human male urine

Concentration Test Samples

The two-fold human semen dilution series from 1:200 to 1:25600 was generated during validation of the Y-screen method[1]. A 1:1 mixture of each semen dilution with female blood was generated and 140 µL of each mixture was dried onto individual cotton swabs for testing.

Additional test samples were generated as during validation of the Y-screen method[1] and using the same semen sample used previously. Instead of generating 1:1 mixtures with female blood, 1:1 mixtures with nanopure water were generated. Human semen dilutions (1:2, 1:3, 1:4, 1:5, 1:10, and a two-fold dilution series from 1:25 to 1:25600) were generated and dried onto cotton swabs for testing.

Re-test samples were generated as before*. A two-fold human semen dilution series from 1:50 to 1:25600 was generated from a 1:25 dilution of the human semen used previously and subsequently dried onto cotton swabs for re-testing.

Male Urine Test Samples

The following dilutions were generated from neat male urine (single source) sl 5/16/13: 1:2, 1:3, 1:4, 1:5, 1:10, and a two-fold dilution series from 1:25 to 1:3200. 140µL of each dilution was absorbed onto cotton swabs, which were dried prior to testing.

Methods

ABAcard® p30 Test

As per the Technical Manual[3], except the 1:10 dilution (20µL into 200µL) was optional.

* The 1 3/4 swab study was repeated due to faulty test cards. (selected dilutions only) sl 5/16/13

San Diego Police Department Crime Laboratory
Forensic Biology Unit
The ABAcard® p30 Test using Concentrated Samples

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Concentrated Extracts from ¾ Swab
¼ of each swab was taken for p30 testing via the existing SDPD method while the remaining ¾ swab was tested using the concentrated method. The remaining ¾ swab was extracted with 250µL nanopure water and 200µL of the supernatant was used for testing. All samples were tested in triplicate.

Concentrated Extracts from 1 ¾ Swabs
1 ¾ swabs were extracted with 300µL nanopure water and 200µL of the supernatant was used for testing. All samples were tested in triplicate.

Microscopy
Microscopic examination was conducted as during the Y-screen validation[1]. Briefly, a water extraction was conducted. Instead of performing microscopy on the water extracted samples, 10 µL of the ~50 µL cell pellet was discarded to mimic what is conducted in actual casework. The original substrates were digested with the remaining cell pellet and all ~50 µL was spotted for microscopic examination.

Y-Screen
The remaining ¼ swab from the neat through 1:100 semen samples used in the 1 ¾ swabs study was taken for Y-screening. Extraction and quantification were conducted as per the Technical Manual[3]. All samples were tested in triplicate.

False Positives from Male Urine
¼ swab was extracted with 250µL nanopure water and 200µL was used for testing. All samples were tested in triplicate.

RESULTS

Concentrated Extracts from ¾ Swab

Dilution Factor	Amount of DNA (ng/4µL)	# of Sperm (per field)	p30 Test Result (existing method)	p30 Test Result (¾ swab)	p30 Test Result (1 ¾ swab)
Neat	83.776	TMTC	+	-	-
		TMTC	+	-	-
		TMTC	+	-	-
2	55.191	TMTC	+	-	-
		TMTC	+	-	-
		TMTC	+	-	-
3	40.749	TMTC	+	-	-
		TMTC	+	+	-
		TMTC	+	-	-
4	22.309	TMTC	+	+	-
		TMTC	+	+	-
		TMTC	+	+	-
5	22.103	TMTC	+	+	+
		TMTC	+	+	-
		TMTC	+	inc.	-

↑ 5/16/13
Faulty test cards observed; re-testing conducted (see Table 2)

San Diego Police Department Crime Laboratory
Forensic Biology Unit
The ABAcard® p30 Test using Concentrated Samples

Analysts: Brian Lew & DeKenya Williams
Date: 11/8/2012
(see note on previous page) BL 5/16/13

10	9.056	TMTC	+	+	-
		TMTC	+	+	+
		TMTC	+	+	-
25	3.317	TMTC	+	+	+
		TMTC	+	+	+
		TMTC	+	+	+
50	1.753	~60	+	+	-
		~45	-	+	+
		~50	-	+	-
100	0.868	~15	-	+	-
		~17	-	-	-
		~20	-	+	-
200	1.0250†	~15†	+	+	-
			+	+	+
			+	+	-
400	0.6143†	~5†	+	+	-
			+	+	+
			+	+	-
800	0.3077†	~2†	+	+	-
			+	+	-
			+	+	-
1600	0.1012†	~38*†	-	+	-
			-	+	-
			-	+	-
3200	0.0208†	~6*†	-	+	-
			-	+	-
			-	+	-
6400	0.0338†	~4*†	-	+	-
			-	+	-
			-	+	-
12800	0.0195†	3*†	-	-	inc.
			-	-	-
			-	-	-
25600	0.0121†	2*†	-	-	-
			-	-	-
			-	-	inc.

Table 1. *Increased sensitivity from p30 test on concentrated extracts from larger samples.*

** Sperm cells observed per well*

† The amount of DNA from each sample and the number of sperm cells observed were determined during validation of the Y-screen method[1]. The averages at each dilution are shown. (NOTE: 2 of the 3 replicates at 1:12800 yielded an undetermined quantitation result; therefore, the concentration above is the quantitation result from one replicate.)

San Diego Police Department Crime Laboratory
Forensic Biology Unit
The ABAcard® p30 Test using Concentrated Samples

Analysts: Brian Lew & DeKenya Williams
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Concentrated Extracts from 1 ¾ Swabs

Dilution Factor	Amount of DNA (ng/4µL)	# of Sperm (per field)	p30 Test Result (1 ¾ swabs)	p30 Re-Test Result (1 ¾ swabs)
Neat	83.776	TMTC	-	
		TMTC	-	
		TMTC	-	
2	55.191	TMTC	-	
		TMTC	-	
		TMTC	-	
3	40.749	TMTC	-	
		TMTC	-	
		TMTC	-	
4	22.309	TMTC	-	
		TMTC	-	
		TMTC	-	
5	22.103	TMTC	+	
		TMTC	-	
		TMTC	-	
10	9.056	TMTC	-	
		TMTC	+	
		TMTC	-	
25	3.317	TMTC	+	
		TMTC	+	
		TMTC	+	
50	1.753	~60	-	+
		~45	+	
		~50	-	
100	0.868	~15	-	+
		~17	-	
		~20	-	
200	1.0250†	~15†	-	+
			+	
			-	
400	0.6143†	~5†	-	+
			+	
			-	
800	0.3077†	~2†	-	+
			-	
			-	
1600	0.1012†	~38*†	-	+
			-	
			-	
3200	0.0208†	~6*†	-	-
			-	
			-	

(Table 2 cont'd on next page) el 5/16/13

6400	0.0338[†]	~4*[†]	-	-
			-	-
			-	-
12800	0.0195[†]	3*[†]	inc.	-
			-	-
			-	-
25600	0.0121[†]	2*[†]	-	-
			-	-
			inc.	-

Table 2. *The p30 detection threshold is not increased by using 1 ¾ swabs instead of ¾ swab.*

Dilution Factor	Male DNA Detected (ng/4µL)	Sperm (per field)	p30 Result (existing)	p30 Result (3/4 Swab)	p30 Result (1 3/4 Swabs)
Neat	83.776	TMTC	(+)	(-)	(-)
2	55.191	TMTC	(+)	(-)	(-)
3	40.749	TMTC	(+)	(+)	(-)
4	22.309	TMTC	(+)	(+)	(-)
5	22.103	TMTC	(+)	(+)	(+)
10	9.056	TMTC	(+)	(+)	(+)
25	3.317	TMTC	(+)	(+)	(+)
50	1.753	~52	(+)	(+)	(+)
100	0.868	~17	(-)	(+)	(+)
200	1.025	~15	(+)	(+)	(+)
400	0.6143	~5	(+)	(+)	(+)
800	0.3077	~2	(+)	(+)	(+)
1600	0.1012	~38*	(-)	(+)	(+)
3200	0.0208	~6*	(-)	(+)	(-)
6400	0.0338	~4*	(-)	(+)	(-)
12800	0.0195	3*	(-)	(-)	(-)
25600	0.0121	2*	(-)	(-)	(-)

Legend	
	Enough for ID+ using ¾ swab or less
	Enough for ID+ using 1 ¾ swabs
	Not enough for DNA

Calculations based on 100 sperm or 1.00ng DNA total for ID+

Table 3. *p30 from extremely dilute semen samples may not be detected.*

False Positives from Male Urine

Dilution Factor	p30 Test Result (¼ swab)
Neat	-
	-
	-
2	-
	-
	inc.
3	-
	-
	-
4	-
	-
	-
5	-
	-
	-
10	-
	-
	-
25	-
	-
	-

Table 3. *p30 was not detected in male urine.*
4 SR 5/16/13

DISCUSSION/CONCLUSION

Our current methods dictate that a 1:10 of the sample extract be applied to the test card. With samples where a low amount of male DNA (as is commonly seen in sexual assault evidence) is expected, the option to not dilute is desirable so as to avoid a false negative test result. This validation shows that using concentrated extracts from ¾ swab instead of a 1:10 extract from a ¼ swab increases the sensitivity of p30 detection by 8-fold and is favorable for samples where dilute semen is suspected. The detection threshold was approximately equal, regardless of whether ¾ swab or 1 ¾ swabs were tested. Because a low number of sperm cells were previously observed in samples where p30 was not detected, it is recommended that microscopic examination be conducted if semen is suspected when male DNA is detected and a negative p30 test result is obtained. Lastly, because no p30 was detected in male urine, it is no longer recommended as a necessary standard control for the test.

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[1] "Dried Blood/Semen Mixture Study 2" (2012). *Validation of Y-Screen Method for Screening Evidence of Sexual Assault*. San Diego Police Department Crime Laboratory: Forensic Biology Unit, San Diego, CA.
[2] *Validation of p30 ABAcard for Identification of Human Semen* (1999). San Diego Police Department Crime Laboratory: Forensic Biology Unit, San Diego, CA.
[3] *Unit Technical Manual: Forensic Biology* (2012). San Diego Police Department Crime Laboratory: Forensic Biology Unit, San Diego, CA.

5/13/2013

Material Modification of Abacus Diagnostics ABAcard® p30 Test

Purpose

The ABAcard p30 test (Abacus Diagnostics) is an immunochromatographic test for the presence of Prostate Specific Antigen (PSA or p30) that was validated by the SDPD Crime Laboratory in 1999 and has been approved for the confirmation of semen stains since that time. Due to supply chain issues related to the SARS COVID-19 pandemic, Abacus Diagnostics was unable to obtain the plastics necessary for the manufacture of the Abacus p30 cards and redesigned the tests using alternate plastic housings. The new design required a different volume for the test (80µL vs. the previous 200µL), requiring an evaluation of the material modification.

The new lots of ABAcard p30 can be differentiated by the format of the lot numbers. The original, validated lots, referred to herein as "ABAcard p30 (old)" had the format of three letter month followed by the year (e.g. NOV 2023). The new, modified lots, referred to herein as "ABAcard p30 (new)" have the format of YYYY-MM-DD (e.g. 2025-02-28).

This material modification was part of a wider evaluation of immunochromatographic tests including those from alternate manufacturers and testing kits for blood and saliva. The material modification data was taken from the sensitivity experiments comparing ABAcard p30 (old) to ABAcard p30 (new). Current SDPD protocol incudes extraction of semen stains in PCR grade water. ABAcard p30 (new) tests include an extraction buffer that is not used by SDPD. This material modification compared the new test cards with stains extracted in water and extracted in the buffer.

Materials and Methods

SERI Semen Standard and 2 additional samples of semen ("Semen 1" and "Semen 2") were serially diluted tenfold (1/10 – 1/1,000,000) in PCR grade water. The six dilutions were tested using 200µL for ABAcard p30 (old) and 80µL for ABAcard p30 (new).

Semen stains were prepared from tenfold serial dilutions (1/10 – 1/1,000). For ABAcard p30 (old), dried semen stains were extracted in 350µL PCR grade water and 200µL was tested. For ABAcard p30 (new), the dried semen stains were alternatively extracted in 350µL PCR grade water or 750µL of the extraction buffer supplied by the manufacturer in the testing kit; 80µL was used for ABAcard p30 (new).

Results

In general, ABAcard p30 (new) performed better than or the same as ABAcard p30 (old) in the sensitivity experiments. Extraction with ABAcard p30 (new) in water was generally as good or better than extraction in the supplied buffer.

The data from the cards is present at the end of this document, but can is summarized in the following tables.

Serial Dilutions of Semen

Semen Standard Dilutions	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000
ABAcard p30 (old)	+	+	+	-	-	-
ABAcard p30 (new)	+	+	+	+	-	-

Semen Stain 1 Dilutions	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000
ABAcard p30 (old)	+	+	+	-	-	-
ABAcard p30 (new)	+	+	+	+	-	-

Semen Stain 2 Dilutions	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000
ABAcard p30 (old)	+	+	+	-	-	-
ABAcard p30 (new)	+	+	+	-	-	-

Extractions of Semen Stains

Semen Standard Dilutions	1/10	1/100	1/1,000
ABAcard p30 (old)	+	+	-
ABAcard p30 (new - water)	+	+	+
ABAcard p30 (new - buffer)	+	+	+

Semen Stain 1 Dilutions	1/10	1/100	1/1,000
ABAcard p30 (old)	+	-	-
ABAcard p30 (new - water)	+	+	+
ABAcard p30 (new - buffer)	+	+	+

Semen Stain 2 Dilutions	1/10	1/100	1/1,000
ABAcard p30 (old)	+	-	-
ABAcard p30 (new - water)	+	+	+
ABAcard p30 (new - buffer)	+	+	-

Conclusions

ABAcard p30 (new) demonstrates sensitivity that is on par with current methods. Extraction of semen stains in 350µL PCR grade water demonstrated improvement with ABAcard p30 (new) over current methods. Extraction with 750µL of the

supplied extraction buffer did not show improvement using ABAcard p30 (new) over extraction in 350µL PCR grade water.

Based on the results of this study, ABAcard p30 (new) testing using 80µL of extract from samples extracted in 350µL of PCR grade water is suitable for casework.

Nicole Miller 11/3/23
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Forensic Biology Intern

11/3/23
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Experimental Design for the Validation of the p30 ABAcard for the Identification of Human Semen

1. The development of a written protocol for the p30 Test Card derived from the product insert provided with the test cards produced by Abacus Diagnostics.

2. Dilution series of SERI semen standard.

   SERIS semen standard will be serial diluted in a tenfold dilution series. The dilution series will start at 1/10 and will extend to 1/1,000,000 (this dilution series does not take into account that the standard is shipped as a 1/50 dilution). Once a negative test is encountered at a particular dilution the testing of further dilutions will not be necessary. The dilution series will also be tested by our current procedure involving crossover electrophoresis.

   Purpose: To determine how sensitive the p30 Test Card is.

3. Dilution series of liquid human semen (two semen donors will be used).

   Human semen will be serial diluted in a tenfold dilution series. The series will start with a 1/10 dilution and will extend to 1/1,000,000. Once a negative test is encountered at a particular dilution the testing of further dilutions will not be necessary. The dilution series will also be tested by our current procedure involving crossover electrophoresis.

   Purpose: To determine how sensitive the p30 Test Card is.

4. Testing of the p30 Test Card with other human body fluids (three donors will be used).

   Urine, blood, feces (stain), and saliva will be diluted in a tenfold dilution series. The series will start as undiluted and will extend to 1/1,000.

   Purpose: To determine if other human body fluids besides semen can yield a positive result with the p30Test Card.

5. Testing of the p30 Test Card with semen stains.

   A tenfold dilution series of human semen stains will be prepared as described in step #2, spotted onto various clothing substrates, and the stains allowed to dry. The stains will then be dehydrated and tested with the p30 Test Card.

   Purpose: To determine if human semen stains can be effectively tested using the p30 Test Card.

6. If needed, minor refinement of the protocol developed in step #1.

7. Implementation of the p30 Test Card if the validation experiments prove that it is an effective and reliable tool for the identification of human semen.

Patrick O'Donnell, June 14, 1999
Supervising Criminalist, Forensic Biology

Summary of the Abacus *OneStep* p30 ABAcard Validation Studies Performed by the San Diego Police Department

Intended Use

The San Diego Police Department currently utilizes the crossover gel electrophoresis technique for detection of the p30 protein found in human semen. The p30 crossover technique requires approximately twenty-four hours to obtain results that yield a relatively low degree of sensitivity (1:8 fold dilution for semen stains, 1:16 fold dilution for SERI p30 standard). In efforts to increase the efficiency and sensitivity of semen detection, the San Diego Police Department has performed a validation study of the Abacus OneStep p30 ABAcard. The OneStep ABAcard is designed to detect semen in samples containing p30 concentrations exceeding 4ng/ml, including samples collected from vasectomized or azoospermic individuals. The results of the ABAcard test appear within ten minutes. Serial dilutions of liquid semen, liquid body fluids, semen stains, and body fluid stains were used in this experiment to determine if the OneStep ABAcard would provide reliable, specific results that exceed the efficiency and sensitivity of the current p30 crossover technique.

Principle Behind p30 Crossover Electrophoresis

The San Diego Police Department currently utilizes the p30 gel electrophoresis technique to detect the presence of semen in a given sample. In crossover electrophoresis, antigen is placed in a well of an agarose gel that is parallel to a well that holds an antibody (SERI Anti-p30). The antigen is placed in the well on the cathodic side of the gel, while the antibody is placed in the anodic well. An electrical current is applied to the gel medium, causing the antigen and antibody to migrate towards each other. The formation of a visible line midway between the two wells signifies the presence of p30 in the sample. The p30 crossover method requires approximately twenty-four hours to obtain results.

Principle Behind OneStep p30 ABAcard

Each test card includes a test area (S) labeled with the monoclonal p30 antibody, a test area (T) labeled with a polyclonal antihuman p30 antibody, and a positive control area (C) labeled with an immobilized anti immunoglobulin antibody. If p30 is present in a semen sample, it will react with the monoclonal p30 antibody to form a mobile antigen-antibody complex. The antigen-antibody complex migrates from the test area (S) towards the polyclonal antihuman p30 antibody in the test area (T). An antibody-antigen-antibody sandwich is formed upon contact with the test area, producing a conjugated pink dye band when the amount of p30 present exceeds 4ng/ml.
An internal positive control in area (C) consists of an immobilized anti immunoglobulin antibody that binds all p30 antibody-dye conjugates that are unable to bind to the antibody in the test area (T). The presence of a colored line in both the test area (T), and the control area (C) indicates a positive reaction. A negative result is indicated by a single colored line that appears in the control area (C).

Reagents and Materials Required

1. Test Device card
2. Nanopure water
3. Swatch stain cards
4. Cotton swabs
5. 1.5 ml microcetrifuge tubes
6. Centrifuge
7. Clock or timer

Results

The validation study performed on the Abacus OneStep p30 ABAcard proved the test cards successful in detecting the p30 protein at a considerably higher sensitivity than the current p30 gel electrophoresis method. The ten-minute test yielded a positive result at a 1:3125 fold dilution for the semen swatch stains, while the liquid semen samples produced a positive reaction at a 1:10,000 fold dilution. The human and SERI standard liquid semen samples produced slightly weaker positive reactions in the more heavily concentrated samples. This result is explained by the "high dose hook effect" that occurs when there is too much p30 present in the sample. Consequently, the excess p30 that is unable to bind to the antibody migrates towards the test area (T), blocking the antibody from binding to the antibody-antigen-pink dye complex. A false negative result is produced even though there is a large amount of p30 present in the sample.

Blood, saliva, and feces were eliminated as detectable human body fluids, yielding negative results at a 1:5 fold dilution. Male urine was detectable at a 1:75 fold dilution in the liquid urine samples. This was predicted as a potential result, given that p30 has been detected in male urine at 260ng/ml. However, the male urine stain samples did not yield a positive result at any dilution. The male urine stain sample results are more informative because they closely mimic the casework samples received by the department.

Finally, four p30 crossover gels were run to provide a reference to measure the sensitivity of the Abacus test cards. Each gel produced a reaction at a 1:8 fold dilution of the semen stain samples. The SERI standard was visible at a 1:8 fold dilution but has demonstrated visible detection at a 1:16 fold dilution in previous work performed by analyst Zach Gaskin. This is considerably less sensitive than the results given by the ABAcards.

Conclusion

The goal of this validation study was to determine the efficiency and sensitivity of the Abacus Onestep p30 ABAcard when applied to samples that mimic casework samples

received by the forensic laboratory at the San Diego Police Department. When compared to the p30 crossover electrophoresis method currently utilized by the department, the ABAcard successfully demonstrated a higher degree of sensitivity and efficiency. Reliable results were obtained within ten minutes, significantly decreasing the amount of time currently necessary for semen detection by crossover electrophoresis.

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