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TECHNICAL NOTE

CRIMINALISTICS

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Validation Study of KPICS SpermFinder™ by NicheVision Forensics, LLC for the Identification of Human Spermatozoa*

ABSTRACT: Microscopic analysis for the identification of spermatozoa is commonly performed during the forensic examination of sexual assault evidence. Two widely utilized methods for the confirmation of the presence of spermatozoa are visualization of the cells via phase-contrast microscopy with wet mounted samples and bright field microscopy with histologically stained samples. The KPICS SpermFinder™ by NicheVision Forensics, LLC accelerates this time-consuming process via an automated microscope with an algorithm designed to locate spermatozoa on a Christmas tree histologically stained microscope slide. Upon a qualified scientist's review of the generated data, the KPICS SpermFinder™ was able to locate spermatozoa, typically finding on average 106.28% ± 115.37% more spermatozoa than with manual examinations. The KPICS SpermFinder™ provided the location of identified cells with reproducible results.

KEYWORDS: forensic science, forensic biology, sexual assault, spermatozoa, automation, KPICS SpermFinder™

In the forensic investigation of sexual assault cases, the scientist commonly examines physical evidence for the presence of seminal material. Microscopy is a powerful tool with which spermatozoa can be identified, thus confirming the presence of seminal material. Microscopic examinations are often time-consuming. In many instances, this type of examination must be conducted on numerous pieces of evidence within a case. An expedited process to perform microscopic examinations and electronically document findings would be advantageous to reducing sexual assault casework backlog and in creating an efficient form of documentation.

Semen, the fluid that is expelled during the male sex act, is comprised of glandular secretions and cellular components. Spermatozoa, the cellular component of semen, originate in the testis and contain the male's genetic information. A typical ejaculate contains 1–6 mL of seminal material, averaging 3.5 mL, and contains *c.* 50–100 million spermatozoa per milliliter. Human spermatozoa are comprised of three major structures: the head, midpiece, and tail. An intact spermatozoan measures about 50–60 μm in total length. The head and midpiece are relatively of equal length, measuring roughly 4.6 μm long. The head is *c.* 2.6 μm wide and 1.5 μm thick. Morphological characteristics of a spermatozoan head include a flattened ovoid shaped cell body with an acrosome at the apical portion. The side profile has a distinctive dolphin head shape (1–5).

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Currently, the presence of spermatozoa is confirmed in the Forensic Biology Section of the Allegheny County Office of the Medical Examiner Forensic Laboratory (ACOME FL) through viewing three spermatozoa heads or one intact spermatozoan cell via phase-contrast microscopic examination of wet mounted microscope slides prepared from questioned samples. Factors that can make microscopic examination difficult can include the presence of excessive levels of epithelial cells, bacterial and cellular debris, or extremely low levels of spermatozoa. During cases of sexual assault, spermatozoa might not be present due to the use of prophylactics, biological degradation over time, lack of ejaculation, incomplete ejaculation, vasectomy, a vas deferens obstruction, or other cases of sexual dysfunction (2–4).

This validation study investigates the utility of the KPICS SpermFinder™ detection instrument by NicheVision Forensics, LLC (NicheVision, LLC, Akron, OH) in reducing examination time, creating electronic documentation of each sample's microscopic examination, and increased spermatozoa detectability. The KPICS SpermFinder™ detection method involves histologically staining of an extract of a portion of the questioned sample on a microscope slide then covering the sample with mounting medium and a cover slip. The resulting slide is then placed onto the KPICS SpermFinder™ detection instrument. The instrument scans the slide and utilizes an algorithm to identify potential spermatozoa based on color, acrosome to nucleus color contrast density (the difference in color concentration between the clear acrosome and the red nucleus), and size. The KPICS SpermFinder™ detection instrument creates an electronic image of the slide, electronic images of spermatozoa candidates, and their *x*, *y* location on the sample microscope slide. The scientist then reviews this data to confirm the presence of spermatozoa, if they are present in the sample, and subsequently generates a report for these findings. These reports are retained electronically as case documentation, creating a permanent record of the sample examination (6–8).

TABLE 1—*Sensitivity study samples comparison between KPICS SpermFinder™ scientist reviewed results and manually examined bright field microscopy results.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 2					
Sensitivity Study Samples	Preparation	Number of Confirmed Positives	Number of Confirmed Positives	Scientist 1 Results	Scientist 2 Results	Scientist 1 to KPICS SpermFinder™ Percent Difference*	Scientist 2 to KPICS SpermFinder™ Percent Difference*	Average Scientist to KPICS SpermFinder™ Percent Difference*
V1	Couple 1_0 h oral swab	516	449	412		17.11		17.11
V2	Couple 1_45 min oral swab	14	19	8	12	106.25	37.50	71.88
V3	Couple 1_12 h vaginal swab	1212	1323	649		95.30		95.30
V4	Couple 1_24 h vaginal swab	1322	1684	967		55.43		55.43
V5	Couple 1_12 h menstrual vaginal swab	618	621	321		92.99		92.99
V7	Couple 1_24 h vaginal smear	369	364	230	321	59.35	14.17	36.76
V8	Couple 1_12 h menstrual vaginal smear	973	1026	194		415.21		415.21
V10	Couple 2_6 h vaginal swab	2425	2445	1289		88.91		88.91
V12	Couple 3_24 h vaginal swab	862	889	234		274.15		274.15
V13	Couple 3_24 h vaginal swab	270	297	140		102.50		102.50
V15	Couple 3_36 h vaginal swab	495	611	328		68.60		68.60
V17	Couple 3_72 h vaginal swab	260	267	154	144	71.10	82.99	77.05
V18	Couple 3_80 h vaginal swab	9	7	2	3	300.00	166.67	233.33
V19	Couple 4_32 h vaginal swab	24	25	18	19	36.11	28.95	32.53
V21	Vaginal swab + 1:10 semen dilution	880	814	495		71.11		71.11
V22	Vaginal swab + 1:100 semen dilution	22	27	12	12	104.17	104.17	104.17
V23	Vaginal swab + 1:1000 semen dilution	2	2	0		200.00		
V24	Vaginal swab + 1:10,000 semen dilution	4	4	1	1	300.00	300.00	300.00
V25	Vaginal swab + 1:100,000 semen dilution	5	7	1		500.00		500.00
V28	Rectal swab	0	0	0		0.00		0.00
V29	Rectal swab + 1:1000 semen dilution	6	5	4		37.50		37.50
V30	Oral swab	0	0	0		0.00		0.00
V31	Oral swab + 1:1000 semen dilution	8	10	10	5	−10.00	80.00	35.00
V32	Menstruation vaginal swab	0	0	0		0.00		0.00
V33	Menstruation vaginal swab + 1:1000 semen dilution	2	4	3		0.00		0.00
V48	Vaginal swab + 1:10 semen dilution	141	132	137		−0.36		−0.36
V49	Vaginal swab + 1:100 semen dilution	44	41	43	39	−1.16	8.97	3.91
V50	Vaginal swab + 1:1000 semen dilution	3	2	1		150.00		150.00
V51	Vaginal swab + 1:10,000 semen dilution	2	3	1		150.00		150.00
V52	Vaginal swab + 1:100,000 semen dilution	2	2	0		200.00		
V53	Vaginal swab + 1:10 semen dilution	540	532	464		15.52		15.52
V54	Vaginal swab + 1:100 semen dilution	41	53	30		56.67		56.67
V55	Vaginal swab + 1:1000 semen dilution	1	0	0		100.00		
V56	Vaginal swab + 1:10,000 semen dilution	2	2	1		100.00		100.00
V57	Vaginal swab + 1:100,000 semen dilution	0	0	0		0.00		
V58	Couple 1_3.5 h oral swab	54	45	18		175.00		175.00
V59	Couple 1_36 h menstrual vaginal swab	155	169	57		184.21		184.21
V60	Couple 3_1 h oral swab	38	45	29		43.10		43.10
V62	Couple 3_90+ h vaginal swab	202	169	191		−2.88		−2.88
V63	Couple 1_48 h vaginal swab	65	68	33		101.52		101.52
V64	Couple 3_24 h oral swab	0	1	0		100.00		
Average						106.28		
Standard Deviation						115.37		

*When the instrument found more spermatozoa than the scientist(s), the percent difference is a positive number. When the instrument found fewer spermatozoa than the scientist(s), the percent difference is a negative number.

TABLE 2—*Precision study samples comparison between KPICS SpermFinder™ scientist reviewed results per automated examination run.*

Sample Designation in KPICS SpermFinder™ Database	V2 RS	V18 RS	V23 RS	V29 RS	V33 RS	V36 RS	
Total Spermatozoa Present	28	9	11	11	6	6	
Preparation	Couple 1 45 min Oral Swab	Couple 3 80 h Vaginal Swab	Vaginal Swab + 1:1000 Semen Dilution	Rectal Swab + 1:1000 Semen Dilution	Menstrual Vaginal Swab + 1:1000 Semen Dilution	Vaginal Swab + 1:1000 Semen Dilution + Vaseline™*	
KPICS SpermFinder™ results run 1							
Number of called positives	554	1931	2027	627	3354	5199	
Number of confirmed positives	18	7	8	9	3	5	
KPICS SpermFinder™ false-negative percent	35.71	22.22	27.27	18.18	50.00	16.67	
KPICS SpermFinder™ false-positive percent	96.75	99.64	99.61	98.56	99.91	99.90	
KPICS SpermFinder™ results run 2							
Number of called positives	539	1858	1918	620	3604	4995	
Number of confirmed positives	19	8	11	10	5	3	
KPICS SpermFinder™ false-negative percent	32.14	11.11	0.00	9.09	16.67	50.00	
KPICS SpermFinder™ false-positive percent	96.47	99.57	99.43	98.39	99.86	99.94	
KPICS SpermFinder™ results run 3							
Number of called positives	523	1891	1961	653	3451	5254	
Number of confirmed positives	16	8	9	9	4	5	
KPICS SpermFinder™ false-negative percent	42.86	11.11	18.18	18.18	33.33	16.67	
KPICS SpermFinder™ false-positive percent	96.94	99.58	99.54	98.62	99.88	99.90	
KPICS SpermFinder™ results run 4							
Number of called positives	513	1646	1256	491	2693	3923	
Number of confirmed positives	16	6	5	2	2	2	
KPICS SpermFinder™ false-negative percent	42.86	33.33	54.55	81.82	66.67	66.67	
KPICS SpermFinder™ false-positive percent	96.88	99.64	99.60	99.59	99.93	99.95	
KPICS SpermFinder™ results run 5							
Number of called positives	376	1821	1892	710	2543	3335	
Number of confirmed positives	17	8	9	10	4	1	
KPICS SpermFinder™ false-negative percent	39.29	11.11	18.18	9.09	33.33	83.33	
KPICS SpermFinder™ false-positive percent	95.48	99.56	99.52	98.59	99.84	99.97	
KPICS SpermFinder™ results run 6							
Number of called positives	227	1925	1963	765	2024	3296	
Number of confirmed positives	15	8	9	9	5	2	
KPICS SpermFinder™ false-negative percent	46.43	11.11	18.18	18.18	16.67	66.67	
KPICS SpermFinder™ false-positive percent	93.39	99.58	99.54	98.82	99.75	99.94	
Average number of called positives	455.33	1845.33	1836.16	644.33	2944.83	4333.66	
Standard deviation	128.89	106.07	287.92	93.11	621.44	924.76	
Average number of confirmed positives	16.83	7.50	8.50	8.16	3.83	3.00	
Standard deviation	1.47	0.83	1.97	3.06	1.16	1.67	
Average false-negative percent	39.88	16.67	22.73	25.76	36.11	50.00	31.86
Average false-negative percent total Standard deviation	5.26	9.30	17.95	27.82	19.48	27.89	21.65
Average false-positive percent	95.99	99.59	99.54	98.76	99.86	99.93	98.95
Average false-positive percent total Standard Deviation	1.38	0.03	0.07	0.43	0.06	0.03	1.50

*Vaseline™ (Unilever, Greenwich, CT).

TABLE 3—*Sensitivity study samples KPICS SpermFinder™ scientist reviewed false-positive results comparison between automated examination runs.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 1		KPICS SpermFinder™ Results Run 2	KPICS SpermFinder™ Results Run 2		
Sensitivity Study Samples	Preparation	Number of Called Positives	Number of Confirmed Positives	KPICS SpermFinder™ Run 1 False-Positive Percent	Number of Called Positives	Number of Confirmed Positives	KPICS SpermFinder™ Run 2 False-Positive Percent	KPICS SpermFinder™ Average False-Positive Percent
V1	Couple 1_0 h oral swab	938	516	44.99	805	449	44.22	44.61
V2	Couple 1–45 min oral swab	1476	14	99.05	1345	19	98.59	98.82
V3	Couple 1_12 h vaginal swab	9162	1212	86.77	8682	1323	84.76	85.77
V4	Couple 1_24 h vaginal swab	12,472	1322	89.40	14,152	1684	88.10	88.75
V5	Couple 1_12 h menstrual vaginal swab	5504	618	88.77	6080	621	89.79	89.28
V7	Couple 1_24 h vaginal smear	7500	369	95.08	7149	364	94.91	94.99
V8	Couple 1_12 h menstrual vaginal smear	6056	973	83.93	6215	1026	83.49	83.71
V10	Couple 2_6 h vaginal swab	7647	2425	68.29	7553	2445	67.63	67.96
V12	Couple 3_24 h vaginal swab	3774	862	77.16	3820	889	76.73	76.94
V13	Couple 3_24 h vaginal swab	649	270	58.40	664	297	55.27	56.83
V15	Couple 3_36 h vaginal swab	1641	495	69.84	1770	611	65.48	67.66
V17	Couple 3_72 h vaginal swab	2165	260	87.99	2123	267	87.42	87.71
V18	Couple 3_80 h vaginal swab	2134	9	99.58	2985	7	99.77	99.67
V19	Couple 4_32 h vaginal swab	5673	24	99.58	5429	25	99.54	99.56
V21	Vaginal swab + 1:10 semen dilution	4667	880	81.14	4586	814	82.25	81.70
V22	Vaginal swab + 1:100 semen dilution	7525	22	99.71	7483	27	99.64	99.67
V23	Vaginal swab + 1:1000 semen dilution	2435	2	99.92	2527	2	99.92	99.92
V24	Vaginal swab + 1:10,000 semen dilution	2133	4	99.81	2143	4	99.81	99.81
V25	Vaginal swab + 1:100,000 semen dilution	8914	5	99.94	9121	7	99.92	99.93
V28	Rectal swab	130	0	100.00	146	0	100.00	100.00
V29	Rectal swab + 1:1000 semen dilution	478	6	98.74	944	5	99.47	99.11
V30	Oral swab	516	0	100.00	553	0	100.00	100.00
V31	Oral swab + 1:1000 semen dilution	477	8	98.32	475	10	97.89	98.11
V32	Menstrual vaginal swab	2631	0	100.00	2654	0	100.00	100.00
V33	Menstrual vaginal swab + 1:1000	4545	2	99.96	4559	4	99.91	99.93
V48	Vaginal swab + 1:10 semen dilution	3465	141	95.93	3562	132	96.29	96.11
V49	Vaginal swab + 1:100 semen dilution	4333	44	98.98	4287	41	99.04	99.01
V50	Vaginal swab + 1:1000 semen dilution	8011	3	99.96	8578	2	99.98	99.97
V51	Vaginal swab + 1:10,000 semen dilution	5005	2	99.96	5329	3	99.94	99.95
V52	Vaginal swab + 1:100,000 semen dilution	7308	2	99.97	7457	2	99.97	99.97
V53	Vaginal swab + 1:10 semen dilution	6872	540	92.14	6974	532	92.37	92.26
V54	Vaginal swab + 1:100 semen dilution	4508	41	99.09	4738	53	98.88	98.99
V55	Vaginal swab + 1:1000 semen dilution	8559	1	99.99	8252	0	100.00	99.99
V56	Vaginal swab + 1:10,000 semen dilution	6102	2	99.97	6109	2	99.97	99.97
V57	Vaginal swab + 1:100,000 semen dilution	3412	0	100.00	3355	0	100.00	100.00
V58	Couple 1_3.5 h oral swab	706	54	92.35	682	45	93.40	92.88
V59	Couple 1_36 h menstrual vaginal swab	8325	155	98.14	8163	169	97.93	98.03
V60	Couple 3_1 h oral swab	177	38	78.53	206	45	78.16	78.34
V62	Couple 3_90+ h vaginal swab	3043	202	93.36	2849	169	94.07	93.71
V63	Couple 1_48 h vaginal swab	7430	65	99.13	7291	68	99.07	99.10
V64	Couple 3_24 h oral swab	301	0	100.00	295	1	99.66	99.83
Average								91.92
Standard Deviation								12.90

TABLE 4—*Casework study samples KPICS SpermFinder™ scientist reviewed false-positive results comparison between automated examination runs.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 1	KPICS	KPICS SpermFinder™ Results Run 2	KPICS SpermFinder™ Results Run 2	KPICS	KPICS
Casework Study Samples	Preparation	Number of Called Positives	Number of Confirmed Positives	SpermFinder™ False-Positive Percent	Number of Called Positives	Number of Confirmed Positives	SpermFinder™ False-Positive Percent	SpermFinder™ Average False-Positive Percent
V38	Case 1-rectal swab	104	1	99.04	100	1	99.00	99.02
V39	Case 2-vaginal swab	412	56	86.41	244	37	84.84	85.62
V40	Case 3-vaginal swab	5422	582	89.27	6107	1163	80.96	85.11
V41	Case 4-rectal swab	2486	512	79.40	2546	537	78.91	79.16
V42	Case 5-rectal swab	488	159	67.42	437	197	54.92	61.17
V43	Case 6-vaginal swab	226	50	77.88	274	60	78.10	77.99
V44	Case 7-vaginal swab	55	9	83.64	35	9	74.29	78.96
V45	Case 8-rectal swab	816	238	70.83	707	193	72.70	71.77
V46	Case 9-mattress cutting	1076	66	93.87	1003	70	93.02	93.44
V47	Case 10-mattress cutting	14,629	1050	92.82	12,976	1026	92.09	92.46
Average								82.47
Standard Deviation								11.25

Materials and Methods

Physiological fluid samples, including neat semen, oral swabs, vaginal swabs, rectal swabs, and postcoital samples were generously supplied by forensic laboratory personnel. Canine semen was furnished by Dr. R. V. Hutchison of the Animal Clinic Northview, Inc. Equine semen was generously provided by Dr. Nicholas G. Loutsion of the Canon Hill Veterinary Clinic Inc.

Contrived samples were prepared by depositing *c.* 30 μL of known seminal material dilutions onto cotton swabs from the indicated orifice then allowing the swabs to air-dry. Postcoital samples identified by time interval and orifice swabbed were collected onto cotton swabs and allowed to air-dry. Smears were also prepared from a selection of the postcoital samples by rolling the swab head on a microscope slide directly after collection then allowing the sample to air-dry.

Microscope slide preparation was performed through extraction of the sample on the glass microscope slide using a small amount of water and teasing the substrate with forceps to increase extraction efficiency. The extracted material was air-dried on the glass slide for *c.* 1 h at room temperature. In instances where smears were examined, the smears were air-dried at room temperature for at least 1 h prior to histological staining. Once the samples were completely dried on the glass microscope slide, the sample area was covered with Solution A: Kernechtrot Solution (Serological Research Institute, Richmond, CA) and were then allowed to incubate at room temperature for 15 min. The slide was then gently rinsed with deionized water. The sample area was then covered with Solution B: Picroindigocarmine Solution (Serological Research Institute) and allowed to incubate at room temperature for 15 sec. A final rinse was performed with absolute ethanol. One to three drops of Cytoseal™ 60 mounting medium (Richard-Allan Scientific, Kalamazoo, MI) was placed onto the sample area and covered with an appropriately sized cover slip. The resulting cellular material consisted of red nuclear material (spermatozoa head) with green or blue cytoplasm material (spermatozoa tail and midpiece) (2,7–9).

Each prepared slide was examined by one or two scientists and at least twice by the KPICS SpermFinder™ detection instrument using bright field microscopy at 400× magnification. Significant variations in staining color density and spermatozoa detectability between orifice samples and donors were not observed when evaluated by the scientist and the KPICS SpermFinder™ detection instrument. A false-negative rate (a negative percent difference value) was recorded when the detection instrument did not identify the same number of spermatozoa as the scientist when comparing the automated examination to the manual examination. When comparing an automated examination to another automated examination, a false-negative rate was recorded when the detection instrument did not identify the same number of spermatozoa as the prior run(s). Previously examined sexual assault slides were compiled from three scientists of varied experience levels to provide an estimated average time spent to manually examine one slide. Statistical analysis was generated in EXCEL (Microsoft Corporation, Redmond, WA).

Results

Sensitivity Study

Due to the KPICS SpermFinder™ detection instrument's capabilities, spermatozoa were consistently observed in higher numbers when examined with the instrument when compared to the scientist's observations. Of the 55 slides examined, 92.72% had

TABLE 5—*Contaminant study samples KPICS SpermFinder™ scientist reviewed false-positive results comparison between automated examination runs.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 1		KPICS SpermFinder™ Results Run 2	KPICS SpermFinder™ Results Run 2		
Contamination Study Samples	Preparation	Number of Called Positives	Number of Confirmed Positives	KPICS SpermFinder™ False-Positive Percent	Number of Called Positives	Number of Confirmed Positives	KPICS SpermFinder™ False-Positive Percent	KPICS SpermFinder™ Average False-Positive Percent
V34	Vaginal swab + 1:1000 + yeast cells	100,145	0	100.00	121,680	0	100.00	100.00
V35	Vaginal swab + 1:1000 + douche	7167	7	99.90	7045	7	99.90	99.90
V36	Vaginal swab + 1:1000 + Vaseline™*	3362	1	99.97	3692	2	99.95	99.96
V37	Vaginal swab + 1:1000 + KY Jelly™†	8088	14	99.83	7664	12	99.84	99.84
Average								99.92
Standard Deviation								0.07

*Vaseline™ (Unilever, Greenwich, CT).
†KY Jelly™ (Personal Products Company, Skillman, NJ).

TABLE 6—*Specificity study samples KPICS SpermFinder™ scientist reviewed false-positive results comparison between automated examination runs.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 1		KPICS SpermFinder™ Results Run 2	KPICS SpermFinder™ Results Run 2		
Specificity Study Samples	Preparation	Number of Called Positives	Number of Confirmed Animal Spermatozoa Positives	KPICS SpermFinder™ False-Positive Percent Run 1	Number of Called Positives	Number of Confirmed Animal Spermatozoa Positives	KPICS SpermFinder™ False-Positive Percent Run 2	KPICS SpermFinder™ Average False-Positive Percent
V26	Vaginal swab + 1:100 horse semen dilution	6082	22	99.64	5364	21	99.61	99.62
V27	Vaginal swab + 1:100 dog semen dilution	9222	57	99.38	9012	48	99.47	99.42
Average								99.52
Standard Deviation								0.12

spermatozoa identified in numbers equal to or greater than scientist's results when the KPICS SpermFinder™ detection instrument was employed. In 7.27% of the slides examined, the KPICS SpermFinder™ detection instrument found less spermatozoa than the scientist, but spermatozoa were positively identified on each slide (Table 1; V31, V48, V49, and V62 exhibited false-negative rates). The KPICS SpermFinder™ detection instrument finds spermatozoa within a range of 500% to −10% of the scientist's resulting numbers by comparison. There was no instance where a false-negative slide was observed during this study; the required threshold to report a positive slide was reached in each slide containing spermatozoa.

Casework Sample Study

Ten slides, which had been examined with phase-contrast microscopy prior to utilization in this validation study, were prepared by removing the existing cover slip once the sample had air-dried then staining according to the above protocol. The KPICS SpermFinder™ detection system found spermatozoa at numbers equal to or higher than the manual examination of the sample. When compared to examinations performed with phase-contrast microscopy, the KPICS SpermFinder™ detection system found on average 1770.68% more spermatozoa. When compared to the manual examination with bright field microscopy, the KPICS SpermFinder™ detection system found on average 214.10% more spermatozoa.

Contaminant Study

Contrived samples containing contaminants such as lubricants, cleansers, and yeast cells combined with known seminal material dilutions were prepared according to protocols outlined in the Materials and Methods section. The KPICS SpermFinder™ detection system found spermatozoa at numbers equal to or higher than the manual examination of the sample averaging 52.68% more spermatozoa observed.

Specificity Study

Contrived samples of diluted canine and equine seminal material were prepared with the preparation protocols outlined in the

TABLE 7—*Casework study samples comparison between KPICS SpermFinder™ scientist reviewed results and manually examined bright field microscopy results.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 2				
Casework Study Samples	Preparation	Number of Confirmed Positives	Number of Confirmed Positives	Scientist 1 Results	Scientist 1 to KPICS SpermFinder™ Percent Difference*	Phase-Contrast Positives	Phase-Contrast to KPICS SpermFinder™ Percent Difference*
V38	Case 1-rectal swab	1	1	1	0.00	1	0.00
V39	Case 2-vaginal swab	56	37	30	55.00	16	190.63
V40	Case 3-vaginal swab	582	1163	338	158.14	18	4747.22
V41	Case 4-rectal swab	512	537	108	385.65	18	2813.89
V42	Case 5-rectal swab	159	197	68	161.76	18	888.89
V43	Case 6-vaginal swab	50	60	11	400.00	3	1733.33
V44	Case 7-vaginal swab	9	9	3	200.00	3	200.00
V45	Case 8-rectal swab	238	193	120	79.58	11	1859.09
V46	Case 9-mattress cutting	66	70	14	385.71	9	655.56
V47	Case 10-mattress cutting	1050	1026	250	315.20	22	4618.18
					Phase-contrast to KPICS SpermFinder™	Average	1770.68
						Standard deviation	1769.67
					Scientist to KPICS SpermFinder™	Average	214.10
						Standard deviation	148.69

*When the instrument found more spermatozoa than the scientist(s), the percent difference is a positive number. When the instrument found fewer spermatozoa than the scientist(s), the percent difference is a negative number.

TABLE 8—*Contaminant study samples comparison between KPICS SpermFinder™ scientist reviewed results and manually examined bright field microscopy results.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 2					
Contamination Study Samples	Preparation	Number of Confirmed Positives	Number of Confirmed Positives	Scientist 1 Results	Scientist 2 Results	Scientist 1 to KPICS SpermFinder™ Percent Difference*	Scientist 2 to KPICS SpermFinder™ Percent Difference*	Average Analyst to KPICS SpermFinder™ Percent Difference*
V34	Vaginal swab + 1:1000 semen dilution + yeast cells	0	0	0		0.00		0.00
V35	Vaginal swab + 1:1000 + douche	7	7	4	6	75.00	16.67	45.83
V36	Vaginal swab + 1:1000 + Vaseline™[†]	1	2	1	1	50.00	50.00	50.00
V37	Vaginal swab + 1:1000 + KY Jelly™[‡]	14	12	7	4	85.71	225.00	155.36
Average						52.68		
Standard Deviation						38.17		

*When the instrument found more spermatozoa than the scientist(s), the percent difference is a positive number. When the instrument found fewer spermatozoa than the scientist(s), the percent difference is a negative number.

[†]Vaseline™ (Unilever, Greenwich, CT).

[‡]KY Jelly™ (Personal Products Company, Skillman, NJ).

Materials and Methods section. Animal spermatozoa were typically not detected because of their unique morphological characteristics that are inconsistent with the morphological characteristics of human spermatozoa. Examination by the KPICS SpermFinder™ detection instrument lead to human-specific identification of spermatozoa upon data review by a qualified scientist.

Precision Study

Six samples (V2, V18, V23, V29, V33, and V36) were selected from the prepared validation samples and run on the detection instrument six times each. It was determined that the KPICS SpermFinder™ detection instrument found spermatozoa in higher numbers than with manual examinations (Table 1; 106.28% more spermatozoa were found on average when using the detection instrument compared with manual examinations). On average 31.86% of the total number of spermatozoa on each slide, as identified through multiple runs, were not identified in each repeated run. Despite this discrepancy, higher numbers of spermatozoa are typically observed in each run when compared to manual examinations (Tables 1 and 2).

Discussion

The KPICS SpermFinder™ detection instrument employs an algorithm specific for the identification of the size, color contrast density

TABLE 9—*Specificity study samples comparison between KPICS SpermFinder™ scientist reviewed results and manually examined bright field microscopy results.*

Sample Designation		KPICS SpermFinder™ Results Run 1	KPICS SpermFinder™ Results Run 1					
Specificity Study Samples	Preparation	Number of Confirmed Animal Spermatozoa Positives	Number of Confirmed Animal Spermatozoa Positives	Scientist 1 Results	Scientist 2 Results	Scientist 1 to KPICS SpermFinder™ Percent Difference*	Scientist 2 to KPICS SpermFinder™ Percent Difference*	Average Analyst to KPICS SpermFinder™ Percent Difference*
V26	Vaginal swab + 1:100 horse semen dilution	22	21	23	21	−6.52	2.38	−2.07
V27	Vaginal swab + 1:100 dog semen dilution	57	48	1397		−96.24		−96.24
Average						−51.38		
Standard Deviation						63.44		

*When the instrument found more spermatozoa than the scientist(s), the percent difference is a positive number. When the instrument found fewer spermatozoa than the scientist(s), the percent difference is a negative number.

between the acrosome and nucleus of the spermatozoa, and color produced by Christmas tree staining of spermatozoa. The algorithm's parameters took into account differences in staining lot products (color density variations because of different lot numbers), sample characteristics (location collected from and presence of contaminants), viewed morphological characteristics (spermatozoa degradation and orientation), and variations between scientist's staining technique. These less stringent parameters led to fewer false-negative rates, and high false-positive percentages. High false-positive percentages were not detrimental to the analysis of the sample (Tables 3–6). Through verification of the focus confidence and the slide scan area, the resulting candidate images were representative of the sample area examined. Discrepancies observed in comparisons between manual and automated examinations (Table 1) and the precision study (Table 2), where all spermatozoa were not identified in each run, could have been due to environmental factors such as vibrations during the sample's data collection. When the instrument was capturing images of the sample for analysis, any movement that would cause a blurred image was detrimental, thus variations in false-positive numbers were observed between automated examination runs. These vibrations could not be eliminated but were reduced with the installation of an antivibration platform to the microscope.

It was observed that the KPICS SpermFinder™ detection instrument provided significantly clearer contrast when compared to the scientist's bench top microscope (Leica DMLS; Leica Microsystems, Wetzlar, Germany), leading to the identification of more spermatozoa. Samples with high levels of debris and dense cellular material were examined with the detection instrument and spermatozoa were observed between these layered cells. Due to the improved optics, the detection instrument found spermatozoa in samples where the scientist originally reported the sample to be negative or inconclusive with manual examination (Tables 1 and 7). In the casework sample study, it was concluded that when comparing phase-contrast manual examinations to the detection instrument, the detection instrument on average found 1770.68% more spermatozoa. When comparing bright field manual examinations to the detection instrument, it was determined that on average 214.10% more spermatozoa were observed (Table 7). With scientist review of the morphological characteristics of spermatozoa in generated candidate images, it was concluded that the KPICS SpermFinder™ detection instrument equipped with the Leica DM5500B microscope was superior to the current manual examinations. The nature of the slide preparations could offer an additional explanation for the observed discrepancies besides the previously discussed contrast differences between microscopes. In phase-contrast microscopy a wet mounted slide is prepared from the sample, thus allowing cellular material to move within the water under the cover slip during the manual examination. Spermatozoa present in the wet mounted slide could have been moving in the area outside of the scientist's field of view or moving under other cellular material or debris. This movement may explain why a sample with low concentrations of spermatozoa would be identified as being negative for spermatozoa. In histologically stained slides, the sample is fixed to the slide by air drying, preventing the movement of cellular material. Due to the stationary cellular material a higher number of spermatozoa were observed when compared to wet mount samples.

Chemical contaminants present in some samples make identification of spermatozoa challenging for the scientist. Samples containing chemical contaminants, such as lubricants and cleansers, were examined by the KPICS SpermFinder™ detection instrument to determine the chemical's impact on results (Table 8). No significant effects were observed. Inconsistent staining results may be produced in the presence of petroleum-based contaminants because of hydrophobic interactions preventing contact between the cellular material and the water-based staining solutions, but do not adversely affect results. In samples containing petroleum-based lubricants, sampling was conducted to limit the amount of lubricant present to minimize this issue. Significantly, higher false-positive results were observed when evaluating samples with high levels of yeast cells on the detection instrument. This was because of the manner in which yeast cells stained red similar to a spermatozoan nucleus. Yeast cells could be differentiated from spermatozoa when reviewed by a qualified scientist.

The detection system's algorithm considered the contrast between the acrosome and the nucleus of the human spermatozoan head. This contrast can occur in objects other than spermatozoa resulting in a false-positive finding. In some instances, the contrast between the animal spermatozoa and the surrounding materials produced a false-positive result (Table 9). Animal spermatozoa have unique morphological characteristics and thus do not consistently cause a false-positive result to be reported. Although the detection system is capable of detecting human spermatozoa, the generated candidate images must be reviewed by a qualified scientist to determine the species origin of the spermatozoa.

A precision study was conducted on the detection system by scanning the same sample area of six slides six times each. It was determined that the KPICS SpermFinder™ detection instrument performed to a reproducibility rate of 68.14%. While the instrument did not find each spermatozoan in each run, it did find spermatozoa in each run typically in higher numbers than the scientist did manually (Tables 1 and 2). It was found that the instrument generated on average 98.95% false-positive results during the precision study. This false-positive average varied from sample to sample and continually changed within multiple runs of the same sample. These discrepancies in reproducibility and false-positive results could be due to previously discussed vibration issues during the image collection process.

The required amount of scientist time to examine sample slides was evaluated through compiling an estimated average time spent per slide from three scientists of varied experience levels. Manual examination of a sample slide takes on average 1–2 h of a scientist's time to complete. The automated detection instrument typically examines one slide in *c.* 2 h and can be run overnight thus allowing the scientist to perform other tasks during data collection. Review of the generated data by the scientist typically takes *c.* 20 min but can take up to 90 min. Variations in data review times are largely due to the number of generated positive results per slide. This number is expected to change between samples, based on the analysis parameters of the instrument and variation in sample size. As with manual examinations, review of data generated by the instrument may also be lengthened by high levels of epithelial cells, bacteria, yeast cells and debris present in the sample.

Conclusions

The KPICS SpermFinder™ detection instrument by NicheVision Forensics, LLC validation study determined that the detection instrument performs as well as, and in most cases better than, a qualified scientist utilizing phase-contrast microscopy or manual bright field microscopy for identification of spermatozoa. Automated microscope slide examinations typically took less scientist time, permitted the scientist to perform other tasks during data collection, could be run overnight, and detected significantly more spermatozoa. The detection instrument documented the *x*, *y* coordinates of spermatozoa with reproducible results. When necessary, the reexamination of samples was facilitated with the use of the electronic record produced by the detection instrument and the availability of the histologically stained slide. The detection instrument was capable of detecting spermatozoa in the presence of chemical contaminants. With data review by a qualified scientist, spermatozoa species identification is confirmed. The utility of the KPICS SpermFinder™ detection instrument has been proven to provide superior analysis and documentation of microscopically examined samples and is a valuable alternative to the current manual phase-contrast microscopy method.

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