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TECHNICAL NOTE

CRIMINALISTICS

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Validation of the Seratec® SeraQuant™ for the Quantitation of Prostate-Specific Antigen Levels on Immunochromatographic Membranes

ABSTRACT: Use of immunochromatographic membranes for the detection of prostate-specific antigen (PSA) has become commonplace in forensic laboratories. Experiments were designed to test the newly developed Seratec® SeraQuant™ for accuracy, precision, and consistency in the quantitation of PSA. PSA standards were diluted with buffers and run on the instruments. Values obtained were examined for accuracy (was the correct value obtained?) and precision (were multiple sample values consistent?). To test for variation between instruments, large volumes of diluted PSA standard were run repeatedly on six units and the values obtained were plotted against the known PSA values to obtain a standard curve for each instrument. Fifty membranes having negative or weak positive results were then run on the six units, and the adjusted values were recorded and compared. Results of these experiments indicate that the instruments are accurate and precise in the quantitation of low levels of PSA.

KEYWORDS: forensic science, SeraQuant™, prostate-specific antigen, hemoglobin, immunochromatographic membrane

Use of immunochromatographic membranes for the detection of prostate-specific antigen (PSA) and human hemoglobin has become commonplace in forensic laboratories (1–5). However, confusion arises as to the interpretation of weak results and whether or not a colored line (positive result) exists. The Seratec® SeraQuant™ detects, analyzes, and records the colored lines that precipitate on the immunochromatographic membranes, resulting in a value for the amount of PSA present (ng/mL) and an image that can be stored digitally.

SeraQuant™ offers the option of running an analysis on freshly prepared membranes (Fresh Program) and membranes that have dried (Terminated Program). The SeraQuant™ measures the intensity of the test line (first peak from left to right—blue on the monitor) and displays it along with the 4 ng/mL internal standard (second peak—red) and the control line (third peak—purple) and gives a value for the concentration of PSA in ng/mL. The image produced can be uploaded into an evidence documentation system such as Laboratory Information Management System (LIMS; Fig. 1).

Experiments were designed to evaluate three features of the instruments: accuracy, precision, and consistency between instruments.

Methods

Accuracy and Precision

PSA standard was obtained from Stanford University Medical Center (Department of Urology, Stanford, CA) and Seratec® from Gesellschaft für Biotechnologie mbH (Göttingen, Germany). The PSA from Stanford, Reference number L-500, was a lyophilized standard prepared in 1% BSA in 20 mM PBS buffer, pH 7.4. This standard resulted in a concentration of 500 ng/mL PSA from 100% Free PSA after reconstitution in 2 mL of deionized water. The PSA from Seratec® consisted of 100 μL of a 500 ng/mL PSA standard.

Dilutions of PSA were prepared using a HEPES buffer and a prepared buffer from Seratec®. The HEPES buffer was a 10.07 mM HEPES, 0.14 M NaCl solution, pH 7.2. The Seratec® buffer consisted of a 1 M Tris, 0.001% NaN_3 solution, pH 8.2. PSA was diluted with HEPES buffer or Seratec® buffer according to two protocols. Design 1 started with a PSA concentration of 50 ng/mL with subsequent halving dilutions using HEPES down to a PSA concentration of 0.5 ng/mL. The dilutions were prepared in glass test tubes and kept on ice during the experiments. Samples (200 μL) were added to Seratec® PSA SemiQuant membranes. To determine the accuracy and precision of the instruments, the diluted samples were run two times each using Fresh and Terminated Programs on a single SeraQuant™ instrument. The values obtained were recorded and plotted against the theoretical concentrations of PSA and examined for accuracy (was the correct value obtained?) and precision (were multiple sample values consistent?).

Consistency

To determine any variance between the instruments, a PSA standard of 5.0 ng/mL was diluted by halving with Seratec® buffer to a final concentration of 0.3 ng/mL, and the prepared dilutions were run using Fresh and Terminated Programs on five SeraQuant™ instruments.

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An additional experiment was designed to compare the values obtained from very weak positive membranes using the Terminated Program on six SeraQuant™ instruments. Large volumes of diluted PSA standard (*c*. 5 mL) were obtained for concentrations of 2, 1, 0.5, 0.4, and 0.3 ng/mL PSA. Eight PSA membranes were run at each dilution and allowed to dry overnight. These membranes were run on six units, and the values obtained were plotted against the known PSA values to obtain a standard curve for each instrument. Fifty membranes having negative or weak positive results were then run on the six units and the actual values were recorded. These values were plotted against the theoretical values to generate a standard curve, and a formula for the best fit line was derived for each instrument.

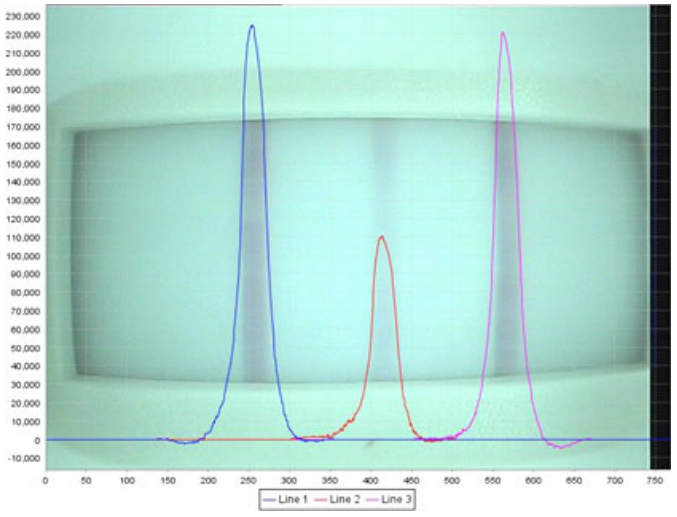

FIG. 1—*Photograph of a PSA membrane evaluated by the SeraQuant™. From left to right, the first peak is the peak corresponding to the PSA value of the sample, the second peak is the peak corresponding to the 4 ng/mL PSA internal standard, and the third peak is the peak corresponding to the control. The actual lines that developed on the membrane can be seen under the corresponding peaks.*

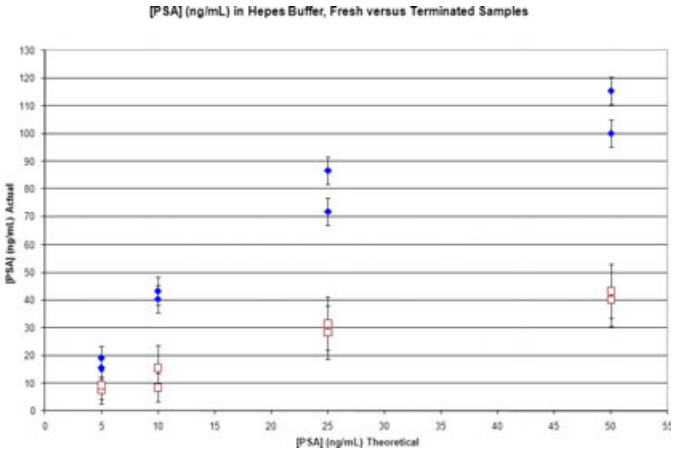

FIG. 2—*Results of the comparison between Fresh and Terminated Programs run on the same samples using the SeraQuant™ (PSA concentrations of 5, 10, 25, and 50 ng/mL). The solid diamonds represent the Fresh Program results, the open squares the Terminated Program results. The theoretical PSA concentrations (based on dilution of the standard) appear along the x-axis. The actual results obtained on the SeraQuant™ appear along the y-axis.*

Results and Discussion

Accuracy

The results of the experiments for accuracy can be found in Figs 2 and 3. Figure 2 shows the results for the higher concentrations of PSA, from 5 to 50 ng/mL. Figure 3 shows the results with lower concentrations of PSA, from 0.25 to 2.0 ng/mL.

It can be seen from the graph that results from the Terminated Program are more accurate. The Fresh Program yields results that are significantly greater than the theoretical amounts. However, quantitation at these levels is not of concern. Dark visible lines can easily be seen on the membranes and be photographed (Fig. 4).

Of concern for us is the accuracy at lower levels of PSA. Figure 3 shows the results of Fresh and Terminated Programs on samples of PSA from 0.25 to 2.0 ng/mL. Once again, the Terminated Program results are more accurate but Fresh Program results are much better than for higher levels of PSA. Visible lines on membranes can be seen down to *c*. 0.5 ng/mL PSA.

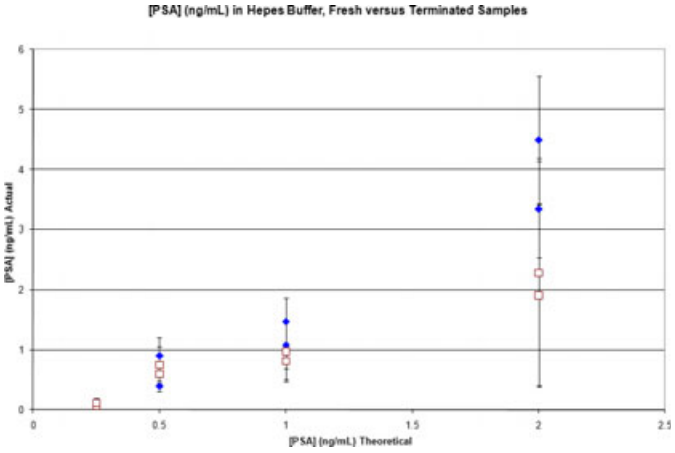

FIG. 3—*Results of the comparison between Fresh and Terminated Programs run on the same samples using the SeraQuant™ (PSA concentrations of 0.25, 0.5, 1.0, and 2.0 ng/mL). The solid diamonds represent the Fresh Program results, the open squares the Terminated Program results. The theoretical PSA concentrations (based on dilution of the standard) appear along the x-axis. The actual results obtained on the SeraQuant™ appear along the y-axis.*

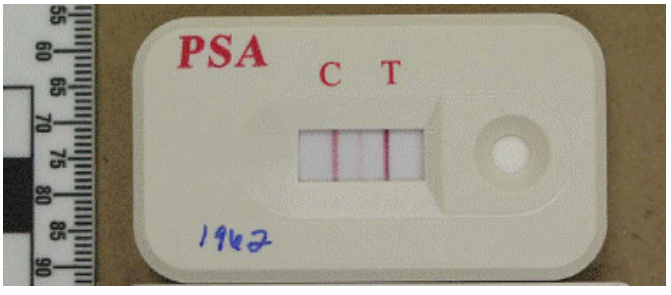

FIG. 4—*Seratec® PSA membrane card after the addition of an extract from a seminal stain deposited on cloth and stored at room temperature since 1962. The first line (from the left) is a control line that must develop to ensure the test is working properly. The second line is an internal standard that is adjusted to the color intensity of a PSA stain of 4 ng/mL concentration. The third line is the test line and is quite obviously positive with a concentration of PSA in excess of 4 ng/mL.*

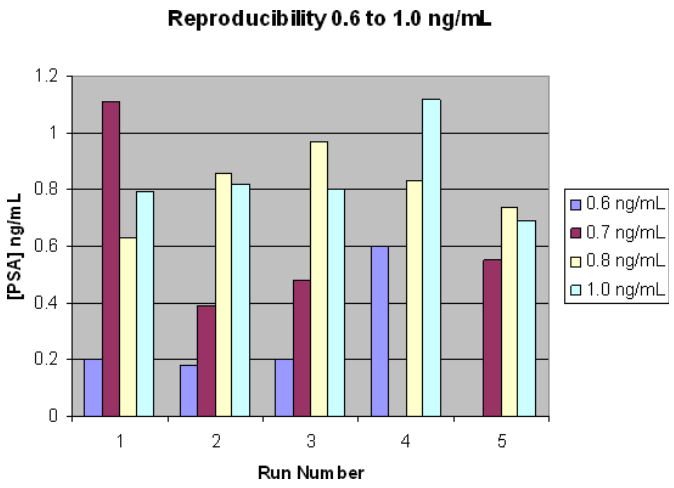

FIG. 5—*Results of Fresh Program multiple runs on samples with PSA concentrations of 0.6, 0.7, 0.8, and 1.0 ng/mL.*

Precision

Figures 5 and 6 show the results of multiple runs of the same diluted sample using the Fresh Program. All the membranes used in the data from Fig. 6 had visible "T" (test) lines. Accuracy and precision were best for the lower concentrations of PSA. Accuracy and precision were the worst for the sample at 20 ng/mL; however, once again, visible "T" lines were easily seen and would have been recorded as positive. Two results in Fig. 5 stand out. Run number 4 of the 0.6 ng/mL sample read true at 0.6 ng/mL and would have been recorded as a positive result (>0.55 ng/mL) based on the following protocol that was used for interpretation: <0.35 ng/mL = negative, 0.35–0.55 ng/mL = inconclusive, >0.55 ng/mL = positive. The other samples read 0.2 ng/mL or less and would have been recorded as negative (<0.35 ng/mL). The results for the 0.7 ng/mL samples require discussion. Runs 1 and 5 would have been recorded as positive with results of 1.11 and 0.55, respectively. Runs 2 and 3 would have been found inconclusive with values of 0.39 and 0.48, respectively (0.35–0.55 ng/mL). These samples would have required Terminated Program runs.

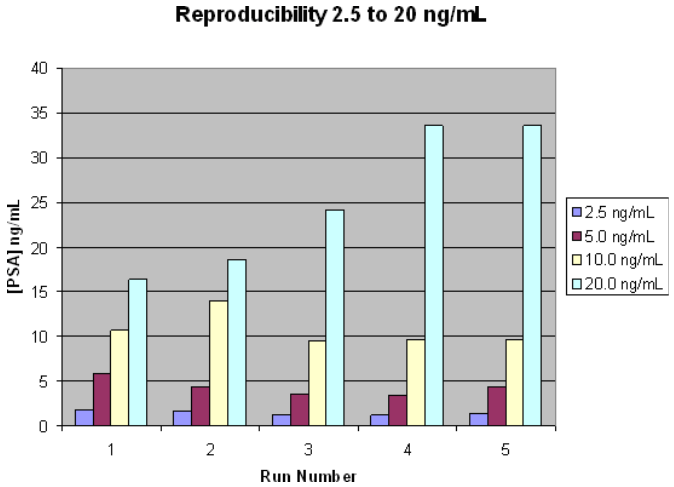

FIG. 6—*Results of Fresh Program multiple runs on samples with PSA concentrations of 2.5, 5.0, 10.0, and 20.0 ng/mL.*

Results like these may be due to pipette sampling error or insufficient mixing of samples before successive runs. For the most part, results were consistent between runs, especially for the lower concentrations of PSA.

Of greatest concern to us are the extremely faint "T" lines observed on some cards making interpretation very difficult. Faint lines visible with the naked eye often do not appear in photographs. Transfer of the images to LIMS also results in a decrease in resolution of the image.

Figure 7 shows the results of experiments performed in attempt to analyze the weakest lines visible to the human eye. Samples having Fresh Program results between 0.35 and *c*. 1.0 ng/mL were allowed to dry overnight and run multiple times using the Terminated Program the following day.

The Terminated Program values obtained for samples with a fresh PSA concentration between 0.35 and 0.5 ng/mL were mostly below 0.2 ng/mL and would have been recorded as negative. When the fresh PSA concentrations were >0.5 ng/mL, a significant increase occurred in the PSA values obtained using the Terminated Program. The Terminated Program values obtained for samples with fresh PSA concentrations >0.5 ng/mL were all above the 0.55 ng/mL level and would have been recorded as positive.

Another jump in the Terminated Program values took place when fresh PSA concentrations were *c*. 1.0 ng/mL. These membranes averaged 1.6 ng/mL PSA using the Terminated Program.

Seratec® states that "samples containing <2 ng PSA/mL may also produce faint positive results so that 0.5 ng PSA/mL are most of the times still detectable with the test" (6, p. 1). It is apparent that the SeraQuant™ would be desirable to use on membranes that have fresh readings <1.0 ng PSA/mL.

Consistency

Multiple samples of known dilutions of PSA were run on six SeraQuant™ instruments using the Terminated Program (Table 1). The values obtained were plotted against the known PSA values, and a standard curve was generated for each unit (Fig. 8).

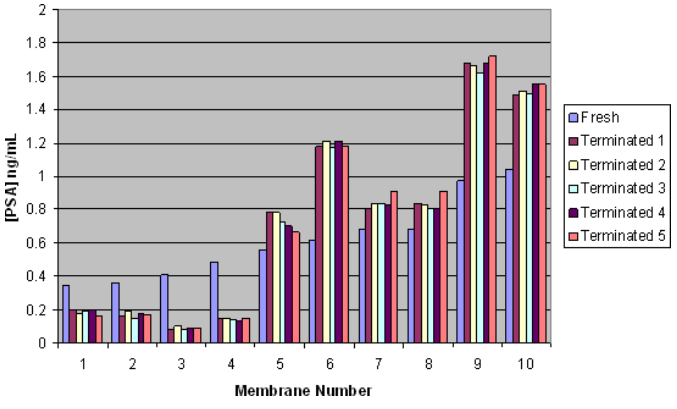

FIG. 7—*This chart shows the results of multiple Terminated Program runs after Fresh Program runs of relatively low concentrations of PSA. The first column in each series (left to right) represents the value obtained for the sample using the Fresh Program. The five columns following are successive runs of the same membrane using the Terminated Program. For samples having Fresh Program values of PSA between 0.35 and 0.5 ng/mL, Terminated Program values were mostly below 0.2 ng/mL and would have been recorded as negative. For Fresh Program values of PSA >0.5 ng/mL, Terminated Program values were all above the 0.55 ng/mL value and been recorded as positive.*

TABLE 1—*Prostate-specific antigen (PSA) values obtained by running dilutions multiple times on each of six SeraQuant™ instruments.*

		Instrument Number					
[PSA] Theoretical (ng/mL)	Card Number	224155	224157	224156	224153	224152	224154
2	1	8.83	9.58	6.67	12.82	8.97	9.07
2	2	9.12	10.09	7.52	12.27	9.61	13.39
2	3	6.27	7.03	3.73	7.81	5.86	6.99
2	4	9.18	11.67	6.34	12.73	9.31	9.92
2	5	8.36	9.01	4.12	9.26	7.55	9.71
2	6	7.93	8.37	5.05	9.44	7.83	8.26
2	7	8.56	8.81	6.23	9.92	8.43	9.11
2	8	8.47	8.76	5.85	9.68	8.43	9.51
1	1	3.41	3.96	1.84	4.50	3.48	4.43
1	2	3.23	4.67	1.84	4.46	3.38	4.33
1	3	4.84	6.16	3.20	6.13	4.73	5.38
1	4	3.40	3.54	1.99	4.16	3.27	3.46
1	5	4.58	5.46	2.67	6.66	5.02	6.25
1	6	2.97	3.87	1.89	4.26	3.15	4.09
1	7	3.66	5.16	1.93	4.98	3.72	5.30
1	8	2.65	2.98	1.99	3.59	2.77	2.76
0.5	1	1.42	1.70	0.84	1.78	1.39	1.62
0.5	2	1.29	1.56	0.77	1.75	1.44	1.56
0.5	3	1.33	1.74	0.68	1.59	1.41	1.59
0.5	4	1.30	1.53	0.86	1.67	1.37	1.63
0.5	5	1.25	1.66	0.73	1.71	1.50	1.59
0.5	6	1.63	1.85	0.81	1.71	1.63	1.76
0.5	7	1.69	1.95	0.97	1.99	1.67	1.84
0.5	8	1.37	1.68	0.77	1.65	1.48	1.65
0.4	1	1.26	1.38	0.80	1.39	1.32	1.54
0.4	2	0.89	1.06	0.44	1.08	0.94	1.09
0.4	3	1.13	1.56	0.53	1.47	1.19	1.53
0.4	4	0.91	1.11	0.46	1.29	1.07	1.19
0.4	5	1.03	1.35	0.45	1.30	1.16	1.25
0.4	6	1.13	1.47	0.45	1.20	1.28	1.36
0.4	7	1.12	1.20	0.66	1.45	1.11	1.20
0.4	8	1.18	1.57	0.62	1.54	1.33	1.44
0.3	1	0.62	0.75	0.30	0.84	0.77	0.70
0.3	2	0.63	0.93	0.30	0.82	0.72	0.79
0.3	3	0.49	0.69	0.24	0.75	0.63	0.62
0.3	4	0.49	0.81	0.22	0.86	0.68	0.71
0.3	5	0.38	0.58	0.13	0.60	0.44	0.53
0.3	6	0.57	0.83	0.26	0.70	0.57	0.84
0.3	7	0.57	0.57	0.15	0.73	0.50	0.61
0.3	8	0.46	0.82	0.19	0.79	0.56	0.62

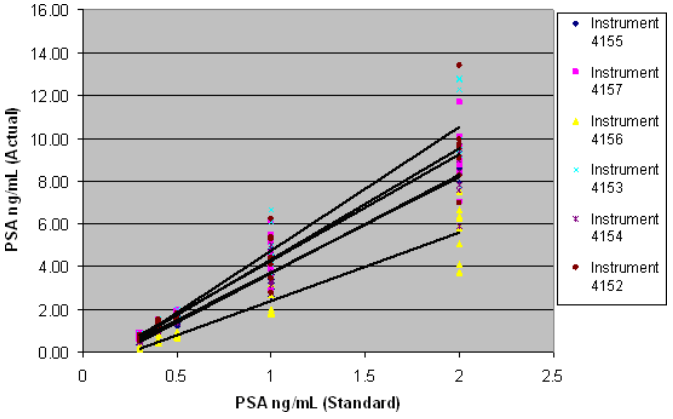

FIG. 8—*PSA of known concentrations (x-axis) plotted against actual values obtained using the Terminated Program on six SeraQuant™ instruments. The best fit line was then generated through each set of data which was used to generate standard curves for each instrument.*

Fifty PSA SemiQuant membranes used in casework that had weak or negative results were retained and run on six SeraQuant™ instruments using the Terminated Program. The values obtained were then entered into the equation for the best fit line that was generated for each instrument's standard curve resulting in adjusted values for concentration of PSA (Table 2).

As can be seen from the data in Table 2, variation existed in the data obtained from each instrument for each membrane. But after application of the standard curve for each instrument, the data obtained was consistent (shaded values). After examining the data, guidelines for the interpretation of the results obtained from the SeraQuant™ using the Terminated Program were developed (<0.35 ng/mL = negative, 0.35–0.55 ng/mL = inconclusive, >0.55 ng/mL = positive). Certain criteria were used in establishing these guidelines. We did not want a "positive" result on a membrane having no visible line. We felt that the term "inconclusive" was suitable for membranes having very faint lines that were typically visible by the analyst but did not appear in photographs and hence, were not recordable. And, most importantly, we wanted to be confident that the same sample run in all of our laboratories gave the same results. The guidelines developed met these requirements. Using these guidelines, only 12 results differed from the majority (the remaining values determined on other instruments) out of 300 runs. For example, the analysis of sample 2 resulted in "inconclusive" results with four instruments and "negative" results with two instruments. In the analysis of sample 7, results were "positive" on five instruments and "inconclusive" on one instrument.

TABLE 2—*Instrumental (unshaded rows) and adjusted (shaded rows) prostate-specific antigen values on each of six SeraQuant™ instruments using the Terminated Program on 50 membranes. The adjusted values were obtained using the standard curve prepared for each instrument. The bold numbers are the values that differed from the majority (the remaining numbers in the row) with regard to the interpretation guidelines*. For example, Sample 7 was recorded as a "positive" result by five instruments and was recorded as an "inconclusive" result by instrument no. 4155. Some data are not shown.*

	Instrument Number												
Sample No.	4155	4157	4156	4153	4154	4152	Sample No.	4155	4157	4156	4153	4154	4152
1	0.03	0.24	0	0.19	0.14	0.15	26	0	0.09	0	0	0	0.1
	0.19	0.19	0.25	0.21	0.19	0.19		0.19	0.15	0.25	0.17	0.16	0.18
2	0.73	0.96	0.42	0.9	0.7	0.86	27	0.4	0.35	0	0.5	0.31	0.53
	0.35	0.35	0.38	**0.33**	**0.29**	0.35		0.27	0.21	0.25	0.26	0.22	0.28
3	0.01	0.85	0.25	0.26	0.2	0.26	28	0	0	0	0	0	0
	0.19	0.31	0.25	0.26	0.2	0.26		0.19	0.14	0.25	0.17	0.16	0.16
4	0.15	0.37	0.25	0.33	0.2	0.28	29	0	0.9	0.04	0.17	0.3	0.4
	0.22	0.21	0.25	0.23	0.2	0.22		0.19	0.32	0.26	0.2	0.22	0.25
5	1.17	1.56	0.97	1.28	1.03	1.27	30	0	0.29	0	0	0.08	0.11
	0.44	0.45	0.55	0.4	0.36	0.44		0.19	0.2	0.25	0.17	0.17	0.18
6	0.78	1.47	0.54	1.27	1.19	1.11	31	0	0.17	0	0.06	0.26	0.29
	0.36	0.43	0.42	0.4	0.39	0.41		0.19	0.17	0.25	0.18	0.21	0.22
7	1.53	2.45	1.46	2.37	2.48	1.94	32	0.03	0.32	0	0.11	0.34	0.31
	0.52	0.63	0.71	0.59	0.64	0.59		0.19	0.2	0.25	0.19	0.22	0.23
8	0.67	0.73	0.34	0.83	0.32	0.7	33	0	0	0	0	0	0
	0.33	0.28	**0.36**	0.32	0.22	0.32		0.19	0.14	0.25	0.17	0.16	0.16
9	0.69	0.6	0.3	0.95	0.6	0.6	34	0	0.05	0	0	0.12	0
	0.34	0.26	0.34	0.34	0.27	0.28		0.19	0.15	0.25	0.17	0.18	0.16
10	1.15	1.61	0.68	1.24	1.06	1.2	35	0.19	0.19	0	0.11	0.18	0.21
	0.44	0.46	0.46	0.39	0.36	0.43		0.23	0.17	0.25	0.2	0.19	0.21
11	1.21	1.4	0.42	1.18	1.13	1.2	36	0.22	0.26	0	0.15	0.19	0.29
	0.45	0.42	0.38	0.38	0.38	0.43		0.23	0.19	0.25	0.2	0.2	0.22
12	0.16	0.23	0.25	0.21	0.14	0.25	37	0	0.09	0	0	0	0.16
	0.22	0.18	0.25	0.17	0.19	0.21		0.19	0.15	0.25	0.17	0.16	0.19
13	0.55	0.12	0.24	0.28	0.17	0.52	38	0.62	0.83	0.34	0.7	0.49	0.72
	0.31	0.16	0.33	0.21	0.19	0.28		0.32	0.3	**0.36**	0.3	0.25	0.32
14	0.94	0.69	0.49	0.76	0.61	0.79	39	0	0.01	0	0	0	0
	0.39	0.28	**0.4**	0.31	0.28	0.34		0.19	0.14	0.25	0.17	0.16	0.16
15	0	0	0.03	0.08	0	0.14	40	0	0	0	0.04	0	0.13
	0.19	0.14	0.26	0.19	0.16	0.19		0.19	0.14	0.25	0.18	0.16	0.19
16	1.44	1.9	0.69	1.77	1.56	1.68	41	1.13	2.26	0.57	1.52	1.33	1.48
	0.5	0.52	0.47	0.48	0.46	0.54		0.43	**0.59**	0.43	0.44	0.42	0.49
17	0.2	0.17	0	0.36	0.21	0.28	42	0.42	0.71	0.23	0.5	0.71	0.65
	0.23	0.17	0.25	0.24	0.2	0.22		0.28	0.28	0.32	0.26	0.3	0.3
18	0.41	0.34	0	0.32	0.24	0.36	43	0.41	0.87	0.27	0.56	1.02	0.77
	0.28	0.21	0.25	0.23	0.21	0.24		0.28	0.31	0.33	0.27	**0.36**	0.33
19	0	0	0	0.1	0	0.17	44	0.32	0.86	0.18	0.54	1.03	0.7
	0.19	0.14	0.25	0.19	0.16	0.2		0.26	0.31	0.31	0.27	**0.36**	0.32

*<0.35 ng/mL = negative, 0.35–0.55 ng/mL = inconclusive, >0.55 ng/mL = positive.

All of these membranes had very weak lines with results below 1.0 ng PSA/mL. These membranes were selected as they are the most troublesome to interpret. It should be pointed out that in not one instance was a membrane run on two instruments resulting in conflicting "positive" and "negative" results. All the differences were from a "positive" or "negative" result to an "inconclusive" result.

Our laboratory reports a positive result on the Seratec® PSA SemiQuant membrane as confirmation of the presence of semen. We are well aware of the presence of PSA in other body fluids but remain confident that our extraction procedure dilutes the sample to the point that only semen will give a positive result on the membrane (see 7).

We extract ¼ from 1 or 2 body orifice swabs for a total of ½ swab at most in 1 mL HEPES buffer. Cuttings from clothing and bedding are smaller than 1 cm^2 and are extracted in 1 mL HEPES buffer. Swabs absorb on average 150 μL volume and a 1 cm^2 cutting averages <10 μL volume depending on composition. This corresponds to a 0.04 and 0.01 dilution factor of PSA levels for ¼ swab and cuttings, respectively. Taking into consideration an extraction efficiency reported at <1% (8) to 16% (7) and the sensitivity threshold of Seratec® SemiQuant membranes at 0.5 ng/mL PSA, then the minimum amount of PSA required to elicit a response on the membranes is 78–1250 ng/mL PSA for ¼ swab and 300–5000 ng/mL PSA for a 1 cm^2 stain. Only semen with a mean value of 820,000 ng/mL PSA (9) will register a positive result (two rare cases of elevated PSA levels in breast milk reported, Lovgren et al. [9] and Filella et al. [10]).

The results in Table 2 correspond quite well with the visible results on membranes (Fig. 9).

We suggest the following protocol in using the SeraQuant™. Individual samples can be run on the SeraQuant™ using the Fresh Program. Prior to using the Fresh Program, a standard curve should be prepared and utilized for each instrument using multiple samples of serially diluted PSA standard. The data obtained from the SeraQuant™ including a photograph of the membrane can be uploaded into an evidence management system such as LIMS.

Multiple fresh samples can be run one after another with the ability to get results on four membranes per hour. Calibration of the instrument between runs is not necessary. Multiple samples can also be run on the laboratory bench at one time and for those membranes with negative (at 15 min) or clearly positive results, the results can be documented and photographed conventionally.

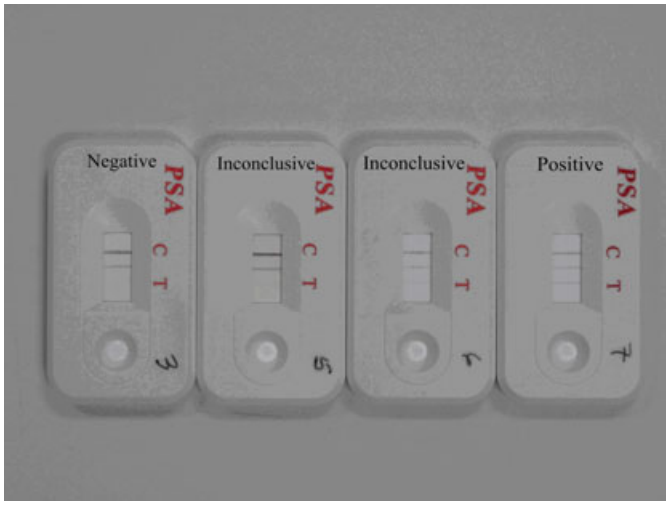

FIG. 9—*Photograph illustrating various results obtained from the analysis of serial dilutions of a minimal PSA standard solution. No test line can be seen in the membrane at far left (Terminated Program PSA concentration <0.35 ng/mL) and this result would be recorded as "negative." Slightly more concentrated solutions result in very weak test lines that have Terminated Program results between 0.35 and 0.55 ng PSA/mL (middle two membranes). These results would be recorded as "inconclusive." At far right, a slightly more concentrated solution (Terminated Program PSA result >0.55 ng/mL) results in a visible test line that is strong enough to be recorded easily by photography which would be recorded as "positive."*

For membranes with weak positive bands such as those found in Fig. 9 (inconclusive), it is suggested that the membranes dry overnight and be processed on the SeraQuant™ using the Terminated Program. This makes interpretation of the weak bands highly reliable and consistent between laboratories and analysts.

Conclusions

The SeraQuant™ instruments have been developed to quantitate the intensity of the bands that develop on the immunochromatographic membranes used to detect the presence of seminal fluid in extracts. The amount of PSA in an extract is related to the intensity of the line that develops in the test region. The darker and more intense the line, the greater the amount of PSA in the extract. Conversely, very weak lines possess smaller amounts of PSA. The absence of a line indicates that no PSA is present or the level is below the sensitivity threshold of the membrane. The sensitivity of the membranes is *c*. 0.5 ng/mL PSA. At this level, a very faint line may be visible by sight on the membrane by some analysts and not others. Also, photodocumentation of such lines and upload into computers can be difficult if not impossible.

These studies have evaluated the SeraQuant™ instruments in the detection and quantitation of the lines that develop on the membranes. Two methods of analysis are available. A Fresh Program determines the quantity of PSA in an extract in 15 min, records a picture, and displays the value in ng/mL. A Terminated Program analyzes the intensity of a line on a dry membrane in 20 sec and also records a picture and displays the value of PSA in ng/mL.

From this study, it is apparent that these instruments can estimate the amount of PSA in samples using the Fresh Program for samples greater than the internal standard of 4 ng/mL. For samples containing <4 ng/mL PSA, the Terminated Program gave accurate and consistent results after a standard curve was established for the instrument.

Currently, the use of immunochromatographic membranes in semen analysis is to detect the presence of PSA in cases where sperm are absent, thereby indicating or confirming the presence of semen. Generally, quantitation of the amount of PSA present is not necessary, just determination of its presence. But determining the presence at very low levels is not an easy and clear-cut task and can be quite subjective if left to an analyst's visual examination. Quantitation at these low levels is suggested as a way to alleviate ambiguity and offer a sensitive and consistent means of interpretation. Laboratories can establish their own thresholds for interpretation of band intensities and be certain that the reports that are generated are consistent and reflect the results of the data analyzed.

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References

1. Hochmeister MN, Budowle B, Rudin O, Gehrig C., Borer U, Thali M, et al. Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid. J Forensic Sci 1999;44(5):1057–60.
2. Koch K, Maddox LO, Suit B, Higgins J, Word CJ, Cotton RW. Forensic use of Abacus OneStep ABAcard test for the identification of the p30 antigen. Genetic Identity Conference Proceedings of the Eleventh International Symposium on Human Identification. Phoenix, AZ: Promega Corporation, 2000, http://www.promega.com/geneticidproc/ussymp11proc/abstracts/koch.pdf (accessed September 8, 2010).
3. Kearsey J, Louie H, Poon H. Validation study of the "OneStep ABAcard PSA Test" kit for RCMP casework. Can Soc Forensic Sci J 2001;34(2):63–72.
4. Baranovienë R, Petruďkienë O. Possibilities of use of prostate-specific antigen (PSA) membrane test for detection of semen in forensic stains. Medicinos Teorija Ir Praktika 2005;43(3):189–91.
5. Hochmeister MN, Budowle B, Sparkes R, Rudin O, Gehrig C, Thali M, et al. Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. J Forensic Sci 1999;44(3):597–602.
6. Seratec® PSA Semiquant user information, 2009 June, http://www.seratec.com/docs/user_instructions/psm400f_en.pdf (accessed September 8, 2010).
7. Laux DL, Custis SE. Forensic detection of semen III: detection of PSA using membrane based tests: sensitivity issues with regards to the presence of PSA in other body fluids. Midwestern Association of Forensic Scientists Newsletter 2004 Winter;33(1):33–39.
8. Gartside BO, Brewer KJ, Strong CL. Estimation of prostate-specific antigen (PSA) extraction efficiency from forensic samples using the Seratec® PSA semiquant semiquantitative membrane test. Forensic Science Communications 2003 April;5(2), http://www.fbi.gov/hq/lab/fsc/backissu/april2003/gartside.htm (accessed September 8, 2010).
9. Lovgren J, Valtonen-Andre C, Marsal K, Lilja H, Lundwall A. Measurement of prostate-specific antigen and human glandular kallikrein 2 in different body fluids. J Androl 1999;20(3):348–55.
10. Filella X, Molina R, Alcover J, Carretero P, Ballesta AM. Detection of nonprostatic PSA in serum and nonserum samples from women. Int J Cancer 1996;68(4):424–7.

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