Evaluation of the SeraQuant Reader for Objective Interpretation of Single- and Dual-Analyte Forensic Lateral Flow Tests

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Background

Forensic lateral flow assays (LFA) are routinely interpreted visually, but weak or borderline test lines may be subject to examiner-dependent variation. This is particularly relevant for duplex assays, where two analyte-specific test lines must be evaluated independently. The SeraQuant Reader provides more objective and standardized evaluation of SERATEC® LFA using lot-specific calibration, numerical signal analysis, and qualitative result assignment. Standardized and traceable interpretation is particularly relevant for laboratories operating under ISO/IEC 17025-accredited quality systems.

Objective

To evaluate a more objective and standardized interpretation of the dual-analyte SERATEC® PAM Test, which simultaneously detects prostate-specific antigen (PSA) and alpha-amylase, using the SeraQuant Reader, with particular emphasis on reproducibility between instruments. Three complementary studies assessed agreement with visual interpretation, paired same-cassette reader-to-reader reproducibility under challenging test conditions, and instrument agreement using dried cassettes with stable line intensities.

Study design

Study 1: Method comparison | N=140
Randomized PAM cassettes covering negative, low-level, and high-level PSA/α-amylase conditions were evaluated visually by two observers (P1 and P2) and measured using the SeraQuant Reader to assess agreement between visual and reader-based interpretation.

Study 2: Reader-to-reader reproducibility | N=66
The same PAM cassettes were measured consecutively on two SeraQuant Readers (within 1 minute) to evaluate reproducibility under challenging conditions, including weak signals and asymmetric dual-analyte responses.

Study 3: Instrument agreement at stable line intensities
Fully developed, dried PAM cassettes were measured on both SeraQuant Readers to assess instrument agreement while minimizing the effects of ongoing line development and measurement timing.

SERATEC® PAM Test
Specified analytical cut-offs
PSA: 0.5 ng/mL | α-amylase: 50 mIU/mL



Image 1: Example of a reacted PAM Test



Image 2: SeraQuant Reader

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Study 1: Method comparison | N = 140

Detection frequency by nominal concentration. Each cassette was measured on one SeraQuant Reader and independently assessed by P1 and P2; this study describes overall detection performance, not paired reader-to-reader reproducibility.

Analyte	Nominal	Reader detected*	Visual P1 detected	Visual P2 detected
PSA	0	0/56	0/56	0/56
PSA	0 .15	7/46	3/46	2/46
PSA	0.2	14/20	11/20	5/20
PSA	10	18/18	18/18	18/18
AMY	0	0/56	0/56	0/56
AMY	10	21/46	20/46	14/46
AMY	20	20/20	20/20	20/20
AMY	500	18/18	18/18	18/18

*Reader detected = numerical output >0. Visual line detected = visible analyte-specific line. Concentrations below the specified analytical cut-offs are exploratory and are not performance claims.

Study 2: Reader-to-reader reproducibility | N = 66

Numerical reader results are shown as mean (range) for each nominal analyte concentration. Each cassette was measured consecutively on Reader A and Reader B.

Analyte	Nominal	Reader A (range)	Reader B (range)	n
PSA	0	0.00 (0.00–0.00)	0.00 (0.00–0.00)	24
PSA	0.15	0.15 (0.13–0.19)	0.16 (0.13–0.19)	6
PSA	0.2	0.16 (0.13–0.18)	0.17 (0.14–0.19)	6
PSA	0.25	0.25 (0.21–0.30)	0.25 (0.21–0.30	18
PSA	10	7.31 (4.56–11.75)	7.44 (4.99–11.14)	12
AMY	0	0.00 (0.00–0.00)	0.00 (0.00–0.00)	24
AMY	10	8.70 (7.12–10.71)	8.85 (7.42–10.82	6
AMY	20	8.79 (7.41–10.48)	9.00 (7.60–10.44)	6
AMY	25	14.22 (7.98–25.20)	14.46 (8.75–26.25)	18
AMY	500	mostly >250 (2 quant.)	mostly >250 (2 quant.)	12

AMY 500 mIU/mL frequently exceeded the quantitative reader range (>250 mIU/mL); censored results were not averaged.

Study 2: Reader-to-reader agreement

PSA: very high same-cassette agreement, r = 0.998; 42 non-zero paired measurements; mean absolute paired relative difference ≈2.8%).

α-Amylase: excellent same-cassette agreement across quantifiable measurements (r = 0.997; mean absolute paired relative difference ≈3.2%). Samples at 500 mIU/mL frequently exceeded the quantitative reader range (>250 mIU/mL).

Study 2: Dual-analyte challenge

PSA/AMY nominal	PSA result	AMY result
0 / 0	negative	negative
0.25 / 0	weak +	negative
0 / 25	negative	weak +
0.25 / 25	weak +	weak +
0.25 / 500	weak +	strong +
10 / 25	strong +	weak +
10 / 500	strong +	strong +

Weak and strong PSA / α-amylase signals were combined to challenge independent interpretation of both channels. No relevant cross-analyte effect was observed.

Study 2: Visual comparison

Both visual evaluators gave identical qualitative assessments across all 66 cassettes. For the 42-cassette dual-analyte challenge, visual and reader interpretation were concordant for both analytes across all tested concentration combinations. Consistent reader performance was also observed under low-signal conditions below the specified analytical cut-offs. These exploratory measurements were not used to redefine the validated performance claims.

Study 2: Low-level performance

The paired evaluation included PSA concentrations of 0.15 and 0.20 ng/mL and α-amylase concentrations of 10 and 20 mIU/mL, all below the specified analytical cut-offs. Consistent reader detection was observed across these low-signal conditions, including all six α-amylase samples at 10 mIU/mL. These exploratory measurements were included to characterize reader behavior at very low analyte levels and were not used to redefine the specified performance claims.

Study 3: Reader comparison using stable line intensities

Paired measurements of fully developed, dried cassettes showed very close agreement between the two reader prototypes under stable line-intensity conditions. Excellent agreement was observed for PAM PSA measurements (r = 0.9994) and for quantifiable PAM α-amylase measurements (r = 0.9998).These results provide complementary evidence of instrument-to-instrument reproducibility while minimizing potential effects of ongoing test-line development.

Conclusion

Study 1 demonstrated agreement between visual and reader-based interpretation across 140 PAM cassettes covering negative, low-level, and high-level PSA/α-amylase conditions.

Study 2 demonstrated close agreement between two SeraQuant Readers measuring the same cassettes, including challenging dual-analyte combinations. Exploratory measurements below the specified cut-offs further characterized reader response at very low analyte levels without extending validated performance claims.

Study 3 confirmed reader-to-reader agreement under stable line-intensity conditions, minimizing the influence of measurement timing.

Overall, the findings support the use of the SeraQuant Reader for objective, standardized, and traceable interpretation of forensic lateral flow tests. Such capabilities are particularly relevant to forensic laboratories operating under ISO/IEC 17025, where reproducibility, standardization, and traceability are essential components of quality assurance.

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