Evaluation of a new Syphilis assay on Vitros® 5600 Integrated System

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Valutazione di un nuovo test per lo screening della Sifilide su Vitros® 5600 Integrated System

SUMMARY

Introduction. A new homogeneous immunoassay for detection of primary infection of *Treponema Pallidum* (TP) on Vitros® 5600 Integrated System was evaluated. The scope of the study was to verify analytical performances and diagnostic accuracy in comparison to commercial methods (Immunoblotting test, ELISA test, Immunoturbidimetric test).

Methods. The new Syphilis assay from SENTINEL CH. SpA, is an immunoturbidimetric assay, using microparticles coated with TP fixed on the surface of polystyrene latex particles which agglutinate by an antigen-antibody reaction when anti-TP antigen is present in the specimen. The assay was implemented on Vitros® 5600 Integrated System. Modified CLSI protocols were adopted. Acceptance criteria for total imprecision were ≤5% for negative samples (or SD ≤0.5 U/mL) and ≤4% for positive samples. In comparison to commercial methods, sensitivity must be ≥99.5% and specificity ≥99.5%.

Results. Total imprecision (22 days) gave SD at 6 U/mL lower than 0.5 U/mL, and CV% at 10 U/mL and 45 U/mL lower than 4%. Low quantitation limit is 5 U/mL. No prozone up to 13000 U/mL was found. In the on-board calibration stability study no drift was found up to 4 weeks. 153 samples were tested vs immunoblotting method and specificity was 100%, sensitivity was 100%. 495 samples were tested vs ELISA method and test specificity and sensitivity were 99.6% and 100% respectively. 521 samples were tested vs immunoturbidimetric method and specificity was 99.8%, sensitivity was 100%. Interference from Bilirubin (20 mg/dL), Hemoglobin (500 mg/dL) and Triglycerides (1000 mg/dL) was not detected. All the sample collection tubes tested (K_2EDTA, SST, LH PST II, LH, NH) did not interfere with the assay.

Conclusion. Performances of the new SENTINEL Syphilis assay on Vitros® 5600 Integrated System meet the requirements for its use as screening tool in blood bank, thus allowing consolidation with general chemistry on a single high volume chemistry analyzer, which is highly valuable for optimizing workflow and efficiency in today's laboratories.

INTRODUCTION

Syphilis is a sexually transmitted disease caused by the spirochetal bacterium *T. pallidum* subspecies *pallidum* (TP). The route of transmission of syphilis is almost always through sexual contact, although congenital syphilis caused by intrauterine transmission from mother to child is described.

Diagnosis of syphilis is currently based on several criteria: history, clinical appearance, serological tests, and identification of *T. pallidum* in lesions or tissue. Among these criteria, serological tests play an important role. The tests currently available are classified according to the antigens used. Among the tests in which *T. pallidum* is used as the antigen – such as the *T. pallidum* immobilization test, the *T. pallidum* hemagglutination (TPHA) test, and the fluorescent treponemal antibody-absorption (FTA-ABS) test – those most commonly used for clinical serodiagnosis are the FTA-ABS and the TPHA tests.

The most commonly used non-treponemal test is the rapid plasma reagin (RPR) test, in which cardiolipin is used as the antigen. The RPR test, which is a variant of the Venereal Disease Research Laboratory (VDRL) test, is as easy as the VDRL test but cannot be performed on an automated analyzers. The disadvantage of the RPR test, compared with the treponemal tests, is its lesser specificity, with an increased occurrence of false-positive reactions.

The FTA-ABS test is regarded as a confirmatory test for doubtful cases. However, the performance of the FTA-ABS test requires highly trained examiners and dedicated equipment. It is also time-consuming and expensive.

The TPHA test has high sensitivity and specificity, but is not sensitive enough for the diagnosis of primary syphilis. The animal erythrocytes that are used in the TPHA test may show nonspecific agglutination with high titers of heterophile antibodies. In addition, unpredictable quality variations in batches from the same manufacturer have been described. This is a concern because internal quality control and proficiency testing are necessary for standardization. The manual serologic tests for syphilis infection are time-consuming and interpretations may be subjective.

To overcome these problems, the *T. pallidum* latex agglutination (TPLA) test has been developed, using latex particles immobilized with purified *T. pallidum* antigens. Recently, automated assays were developed for rapid and efficient testing for syphilis infection.

The proposed assay is a latex agglutination method based on optical density measurement.

OBJECTIVES

The aim of the present study was to verify analytical performances and diagnostic accuracy in comparison to commercial methods. Accordingly studies of imprecision profile, total imprecision, on board calibration stability, interferences by endogenous substances, linearity and prozone effect were performed. Diagnostic accuracy was verified by comparisons between the results obtained with the new Syphilis assay and several commercial methods (Immunoblotting test, ELISA test, Immunoturbidimetric test).

INSTRUMENTS AND MATERIALS

The proposed assay was implemented in MicroTip Subsystem Center, a built-in module of Vitros® 5600 Integrated System that performs both incubation and photometric measurements of processed samples. SYPHILIS TP Latex is a new immunoturbidimetric assay, manufactured by SENTINEL CH. SpA, using microparticles coated with *T. pallidum* (Nichols strain) fixed on the surface of polystyrene latex particles which agglutinates by an antigen-antibody reaction when anti-TP antibodies are present in the specimen. When this agglutination is measured as a change in absorbance, the amount of change depends on the amount of

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antibodies. Based on this principle, this reagent serves to prepare a calibration curve with anti-TP antibody standard solution of known antibody titer and measure the anti-TP antibody in specimen. The results are positive for results ≥10 U/mL.

METHODOLOGY / ACCEPTANCE CRITERIA

Imprecision Profile: Imprecision of assay was evaluated on at least eight test dilutions at concentrations spanning the desired linearity range for the new Syphilis assay. A pool from human sera was prepared at a concentration about 100 U/mL. From the pool were prepared seven additional dilutions levels with saline in the ratio: 80%, 60%, 40%, 20%, 10%, 5%, 0%. For each dilution 10 replicates were carried out. For each dilution level, mean, absolute bias, standard deviation and CV% was calculated. The assessment criteria were: CV <10% or bias <1.5 U/mL from expected value for each dilution point.

Total imprecision: Imprecision was evaluated according to NCCLS (CLSI) document EP5-A2. Testing was performed over 7 days with three levels of control with two replicates per run and two runs per day. For each control level, mean, standard deviation and CV% were calculated. The assessment criteria were: CV% ≤5% (or SD ≤0.5 U/mL) for control level <10 U/mL, CV% ≤4% for control levels ≥10 U/mL.

On board calibration stability: On board reagent and calibration stability studies were performed on Vitros® 5600 Integrated System during a time interval period of 7 days with three different levels of control. Acceptance criteria was ±1.5 U/mL deviation from initial measurement.

Interferences: Interferences by endogenous substances were evaluated according to the Glick model. A pool of human sera was spiked with the potentially interfering substances and then serially diluted with unspiked human sera pool. Triplicates of the eleven concentration levels of the interfering substance from baseline serum to spiked serum were analyzed in sequence, resulting in 33 determinations. Acceptance criterion was ±1.5 U/mL or ±10% in terms of bias deviations from initial measurement.

Linearity: Linearity and analytical measuring range were evaluated on at least ten test dilutions at Syphilis concentrations spanning the desired linearity range for each assay. Data were elaborated according to CLSI document EP6-P2. Acceptance criteria were ±10% or ±1.5 U/mL in terms of bias.

Diagnostic accuracy: Evaluation of diagnostic accuracy was performed according to EP9-A2 (CLSI) by comparison of the Vitros® 5600 Integrated System with Hemagglutination Assay (TPHA) (Bio-Rad Laboratories) on Olympus systems. A test performance–qualitative analysis was used to determine the degree of agreement between the assays run on the different systems. The acceptance criteria were ≥99.5% for sensitivity, and ≥99.5% for specificity must.

Prozone: The prozone effect was assessed by testing serial dilutions of a high value calibrator for Syphilis. Prozone effect was defined as the high analyte concentration (x-axis) giving a false low result (y-axis) equal or lower than the highest standard. Acceptance criterion was no prozone effect up to 13000 U/mL.

RESULTS

Imprecision Profile: Imprecision of assay was evaluated on eight test dilutions at concentrations spanning the desired linearity range for the new Syphilis assay. The results are shown in Table 1.

The assessment criteria (CV% <10% or absolute bias <1.5 U/mL) were satisfied for each dilution point. An imprecision profile is shown in Figure I.

Total imprecision: Testing was performed over 7 days with three levels of control with two replicates per run and two runs per day. The results are shown in Table 2. The assessment criteria were satisfied: for control level <10 U/mL, SD was 0.4, for control levels ≥10 U/mL, CV% was 3.5 and 3.7.

On board calibration stability: On board reagent and calibration stability study results (Table 3 and in Figure II) met the acceptance criteria.

Interferences. Interferences by endogenous substances were evaluated according to the Glick model. The results are shown in Figure III. There are not interferences up to: Hemoglobin 500 mg/dL, Unconjugated Bilirubin 20 mg/dL, Conjugated Bilirubin 20 mg/dL, Rheumatoid Factor (RF) 500 IU/mL. Acceptance criteria were satisfied based on a deviation less than ±1.5 U/mL or ±10% in terms of bias deviations from initial measurement.

Linearity: Linearity and analytical measuring range (AMR) were evaluated on twelve test dilutions at Syphilis concentrations spanning the desired linearity range for each assay. Data were elaborated according to CLSI document EP6-P2. The results are shown in Table 4 and in Figure IV. The measured range was from 1 U/mL to 54 U/mL. The clinical decision level is 10 U/mL. Acceptance criteria (±10% or ±1.5 U/mL in terms of bias) were satisfied for each dilution point.

Diagnostic accuracy: Diagnostic accuracy was evaluated using a protocol based on NCCLS (CLSI) document EP9-A2. This testing was performed by comparison of the Vitros® 5600 Integrated System results with Hemagglutination Assay (TPHA) (Bio-Rad Laboratories) on Olympus systems. In Table 5, the results of the statistical analysis of the comparison between the results obtained with the different methods are shown. The assessment criteria (sensitivity ≥99.5%, specificity ≥99.5%) were satisfied. In Figure V, the frequency distribution of *Treponema* antigen on positive samples (immunoblotting characterization) is shown. In Table 6, the immunoblotting characterization of positive samples and Sentinel assay results are shown.

Prozone: The prozone effect was studied by testing 10 serial dilutions of a high value calibrator for Syphilis. In Figure VI, the obtained profile is shown. Fulfilment of acceptance criteria (no prozone effect up to 13000 U/mL) was found.

CONCLUSIONS

Performance of the new SENTINEL Syphilis assay on Vitros® 5600 Integrated System meets the requirements for its use as screening tool in blood bank, thus allowing consolidation with general chemistry on a single high volume chemistry analyzer, which is highly valuable for optimizing workflow and efficiency in today's laboratories.

Table I. *Imprecision profile.*

	Syphilis TP Latex					
	n	Target Conc. U/mL	Found Conc. Mean U/mL	Bias U/mL	SD U/mL	CV%
1	10	-1.04	2.59	3.63	0.24	9.4%
2	10	4.33	5.37	1.04	0.63	11.7%
3	10	9.69	9.31	-0.39	0.34	3.7%
4	10	20.42	17.61	-2.81	0.48	2.7%
5	10	41.88	38.34	-3.54	0.57	1.5%
6	10	63.34	62.09	-1.24	0.91	1.5%
7	10	84.80	87.83	3.03	1.22	1.4%
8	10	106.25	106.54	0.28	1.37	1.3%

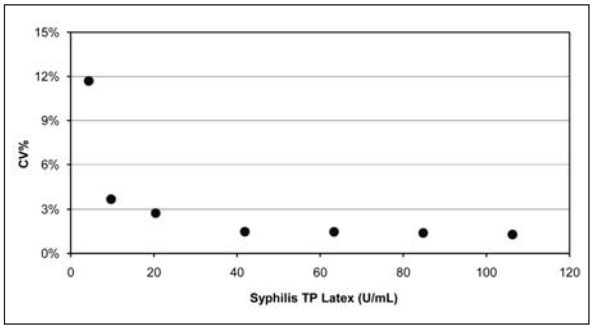


Figure I. *Imprecision profile of Syphilis TP Latex on Vitros® 5600.*

Table 2. *Total Imprecision.*

Level			Total imprecision		Between days		Within run	
	N	Mean (U/mL)	SD (U/mL)	CV%	SD (U/mL)	CV%	SD (U/mL)	CV%
1	28	6.3	0.4	6.0	0.0	0.0	0.0	0.0
2	28	10.1	0.4	3.5	0.2	1.9	0.1	1.4
3	28	45.4	1.7	3.7	1.4	3.2	0.6	1.3

Table 3. *On Board calibration stability.*

	On Board Reagent Stability	On Board Calibration Stability
Target	up to 7 days	up to 7 days
Criteria	Max bias vs initial measurement: 1 U/mL Max % bias vs initial measurement: 10%	

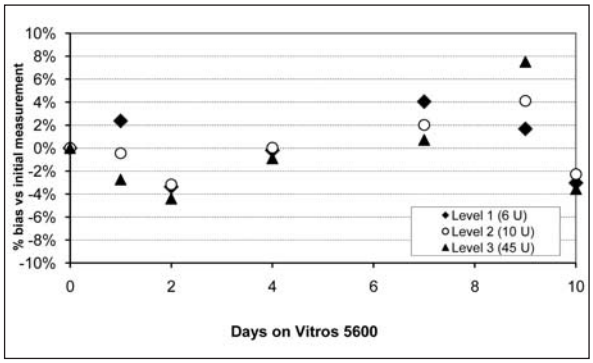


Figure II. *On Board calibration stability.*

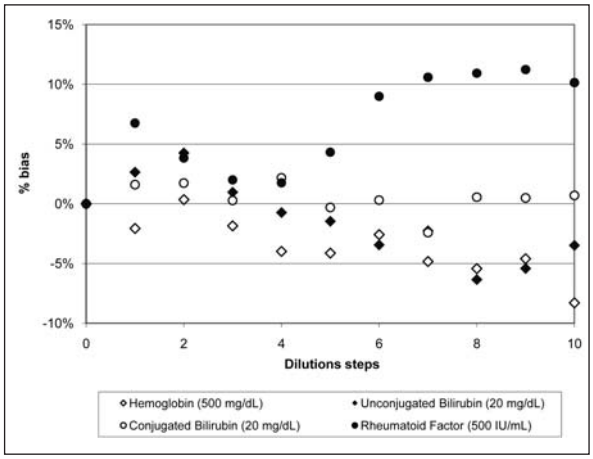


Figure III. *Interferences.*

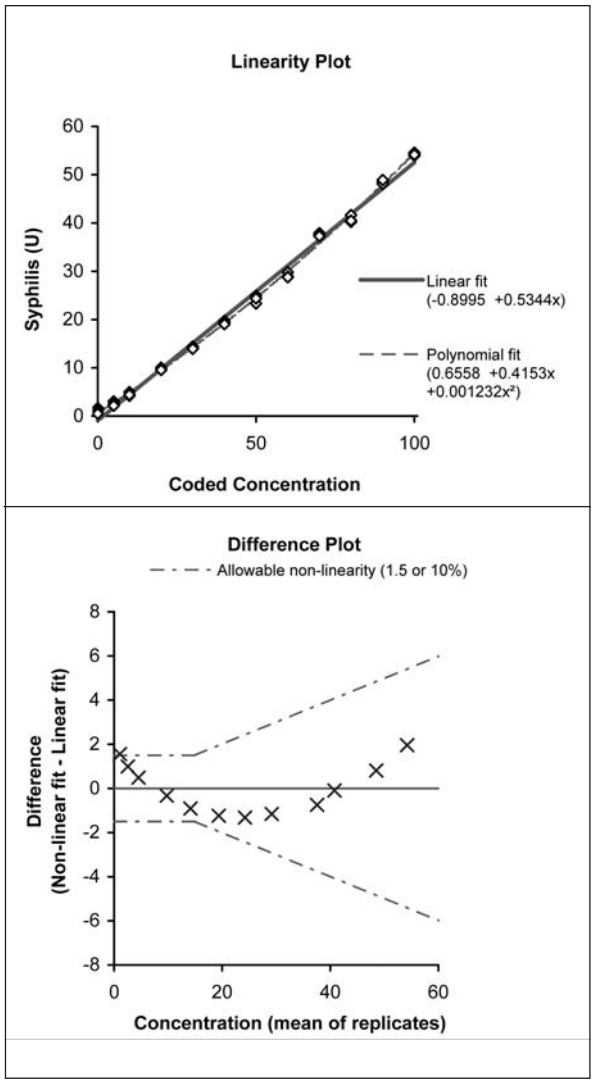


Figure IV. *Linearity on clinically relevant range.*

Table 4. *Linearity on clinically relevant range*

Measured range:	from 1 U/mL to 54 U/mL
Clinical decision level:	10 U/mL

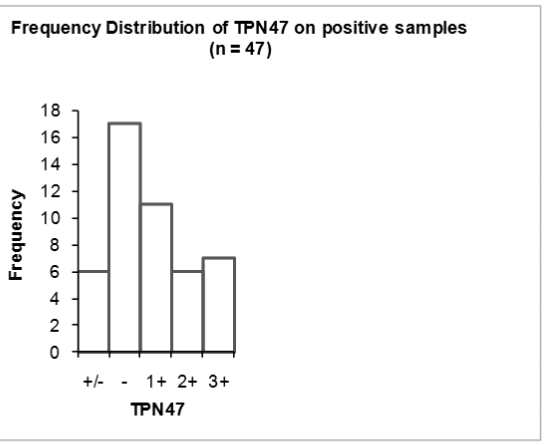
(3 replicates x 12 dilution levels)
(Elaboration according to CLSI Ep6-P2 – Analyse-it vs. 2.12)

Table 5. *Diagnostic accuracy.*

	n 153 Hemagglutination Assay		
Syphilis TP Latex	**POS (+)**	**NEG (-)**	**Total**
Positive test ≥10	69	0	69
Negative test < 10	0	84	84
Total	69	84	153
Sample prevalence	0.451	95%	CI
Sensitivity - TP proportion	1.000	0.948	to 1.000
Specificity - TN proportion	1.000	0.957	to 1.000
FP proportion	0.000	0.000	to 0.043
FN proportion	0.000	0.000	to 0.052
Likelihood ratio (+)	+ ∞		
Likelihood ratio (-)	0.00		

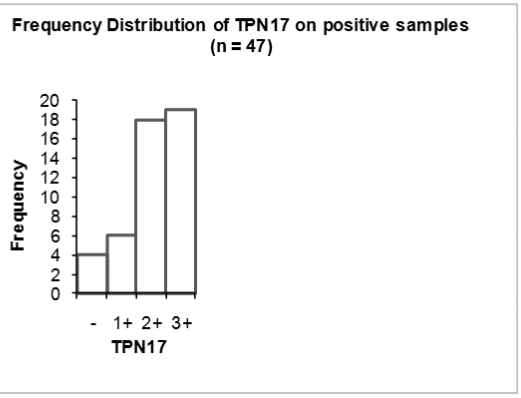
TPN47

n	47	
Categories	5	
Mode	-	
TPN47	**N**	**Proportion**
+/-	6	0.128
-	17	0.362
1+	11	0.234
2+	6	0.128
3+	7	0.149



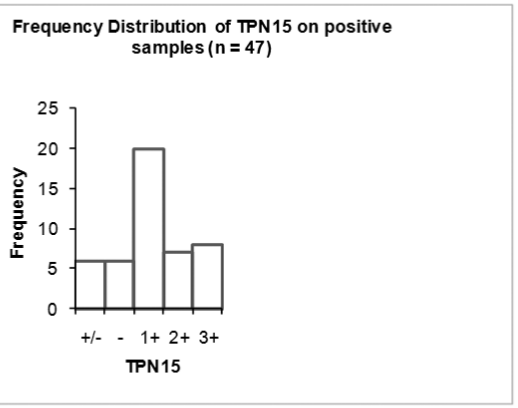
TPN17

n	47	
Categories	4	
Mode	3+	
TPN47	**N**	**Proportion**
-	4	0.085
1+	6	0.128
2+	18	0.383
3+	19	0.404



TPN15

n	47	
Categories	5	
Mode	1+	
TPN47	**n**	**Proportion**
+/-	6	0.128
-	6	0.128
1+	20	0.426
2+	7	0.149
3+	8	0.170



TmpA

n	47	
Categories	5	
Mode	-	
TPN47	**n**	**Proportion**
+/-	9	0.191
-	18	0.383
1+	10	0.213
2+	7	0.149
3+	3	0.064

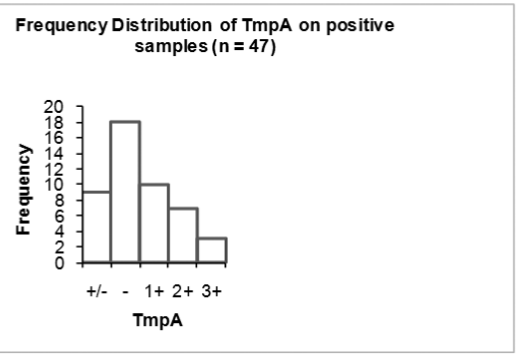


Figure V. *Frequency distribution of Treponema antigen on positive samples - Immunoblotting characterization.*

Table 6. *Immunoblotting characterization of positive samples and Sentinel assay results.*

ID	Immunoblotting characterization					Syphilis TP Latex
	TPN47	TPN17	TPN15	TmpA	Conclusion	[U/mL]
001	-	-	I+	-	POSITIVE	27.3
002	I+	2+	I+	-	POSITIVE	69.6
003	I+	2+	+/-	-	POSITIVE	132.7
004	I+	I+	+/-	I+	POSITIVE	140.6
005	3+	3+	3+	I+	POSITIVE	128.3
006	3+	3+	3+	2+	POSITIVE	94.2
007	I+	3+	+/-	I+	POSITIVE	123.8
008	-	2+	I+	-	POSITIVE	47.8
009	2+	2+	I+	2+	POSITIVE	104.3
010	+/-	3+	+/-	+/-	POSITIVE	131.0
011	I+	3+	2+	I+	POSITIVE	142.0
012	2+	3+	3+	+/-	POSITIVE	102.2
013	2+	2+	I+	I+	POSITIVE	137.5
014	I+	2+	2+	+/-	POSITIVE	137.9
015	I+	2+	I+	+/-	POSITIVE	140.1
016	-	2+	I+	2+	POSITIVE	111.7
017	I+	2+	2+	I+	POSITIVE	137.9
018	-	I+	-	-	POSITIVE	24.0
019	-	I+	-	-	POSITIVE	16.8
020	-	I+	-	-	POSITIVE	139.2
021	-	-	I+	-	POSITIVE	25.3
022	I+	2+	I+	-	POSITIVE	67.6
023	-	I+	-	-	POSITIVE	23.2
024	-	2+	I+	-	POSITIVE	39.9
025	2+	3+	2+	-	POSITIVE	116.5
026	3+	3+	3+	2+	POSITIVE	58.8
027	2+	3+	2+	I+	POSITIVE	110.3
028	3+	3+	3+	2+	POSITIVE	87.5
029	+/-	3+	I+	-	POSITIVE	14.1
030	+/-	2+	I+	+/-	POSITIVE	104.0
031	-	-	I+	+/-	POSITIVE	13.4
032	-	2+	I+	-	POSITIVE	66.0
033	+/-	2+	+/-	+/-	POSITIVE	74.9
034	-	2+	I+	+/-	POSITIVE	22.2
035	2+	3+	2+	2+	POSITIVE	115.1
036	3+	3+	3+	3+	POSITIVE	95.8
037	I+	3+	I+	I+	POSITIVE	119.9
038	-	3+	+/-	-	POSITIVE	77.0
039	-	2+	I+	+/-	POSITIVE	34.3
040	I+	3+	2+	I+	POSITIVE	117.8
041	3+	3+	3+	3+	POSITIVE	45.9
042	+/-	3+	I+	2+	POSITIVE	118.5
043	3+	3+	3+	3+	POSITIVE	48.3
044	-	2+	-	-	POSITIVE	29.8
045	-	-	I+	-	POSITIVE	11.7
046	-	2+	-	-	POSITIVE	42.1
047	+/-	I+	I+	I+	POSITIVE	94.0

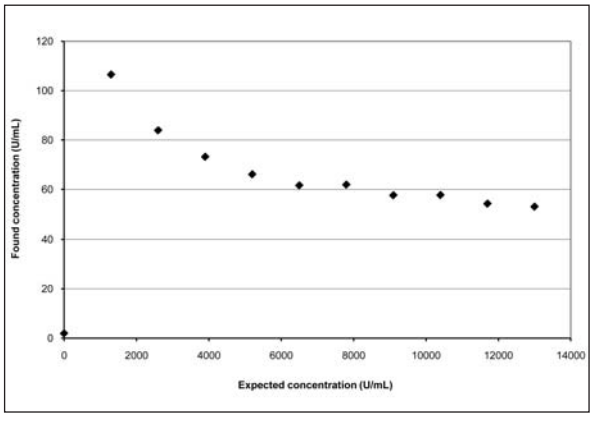


Figure VI. *Prozone effect.*

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