ReQuest® Syphilis IgG

01-580 96 Test Set

INTENDED USE

ReQuest SYPHILIS IgG is an immunoenzymatic method for the qualitative detection of IgG antibodies to *Treponema pallidum* in human serum by a manual technique. The test may be used in conjunction with non-treponemal testing to provide serological evidence of infection with *T. pallidum.*

For Qualitative Detection of IgG Antibodies to *Treponema pallidum*

Warning: A positive result is not useful for establishing a diagnosis of Syphilis. In most situations, such a result may reflect a prior treated infection; a negative result can exclude a diagnosis of syphilis except for incubating r early primary disease.

SUMMARY AND EXPLANATION OF THE TEST

Syphilis is a disease, usually sexually transmitted, caused by infection with the spirochete *Treponema pallidum* (*T. pallidum*). The infection is systemic from the outset and the disease is characterized by periods of latency.

Serological techniques play a major role in the diagnosis of syphilis and treatment follow-up because *T. pallidum* cannot be isolated in culture. The serological diagnosis of syphilis is performed by demonstrating the presence of significant levels of specific antibodies to *Treponema pallidum* in the serum sample.

Serological tests for syphilis were first used in 1906 with the development of the nontreponemal (reagin) test by Wasserman (1). Soon, many tests were developed each with its own modification of a lipoidal antigen, a crude alcohol extract of liver, heart, or other animal organs. All nontreponemal tests measure both immunoglobulin G (IgG) and M (IgM) anti-lipid antibodies formed by the host in response both to lipoidal material released from damaged host cells early in infection and to lipid from the cell surfaces of the treponeme itself.

Currently two treponemal tests are considered the standard for syphilis testing. The FTA-ABS test is an indirect immunofluorescent antibody test. The FTA-ABS test is sensitive and must be well controlled. Hemagglutination (MHA-TP) tests using treponemal antigen for *T. pallidum* have gained acceptance since their emergence in the mid-1960's (8-9) as a confirmatory procedure following a reactive nontreponemal assay or as a screening procedure.

Both assay methods described require visual reading and are limited to subjective interpretation by the operator. The use of the more specific enzyme immunoassay has received widespread acceptance due to the colorimetric results in a 96 well microplate format that can be automated and read photometrically.

Treponemal tests, such as REQUESTSYPHILIS IgG, measure antibodies specific for treponemal antigens and can be used as confirmatory tests for the diagnosis of syphilis

PRINCIPLE OF THE ASSAY

The REQUESTSYPHILIS IgG test is based on the enzyme-linked immunosorbent assay technique (ELISA).

Diluted patient sample is incubated in microplate wells coated with *T. pallidum.* During this incubation specific immunoglobulins, if present, bind to the antigen on the well. After washing, to eliminate unbound proteins, a second incubation is performed with the conjugate, composed of human IgG monoclonal antibodies labelled with peroxidase. After washing to remove unbound conjugate from the wells, the substrate is added, which will react to produce color in the presence of the peroxidase. An acidic solution is added to stop the reaction and the absorbance of the developed color is read at 450 nm.

REAGENTS

Reagents supplied are sufficient for 96 determinations.

Bring to room temperature before use.

MT PLATE MICROPLATE. 12x8 wells coated with *T. pallidum* antigen.

<u>Use</u>: open the package at the end opposite the code, N followed by the lot number, which is useful for identification purposes. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure.

CONTROL + POSITIVE CONTROL (1x1.6 mL)

Ready to use diluted human serum at known concentration of anti-Treponema IgG, in phosphate buffer 0.01 mol/L containing BSA 1% and sodium azide 0.09%.

CONTROL CUT-OFF CUT-OFF CONTROL (1 x 2.0 mL)

Ready to use diluted human serum at known concentration of anti-Treponema IgG, in phosphate buffer 0.01 mol/L containing BSA 1% and sodium azide 0.09%, liquid.

CONJ CONJUGATE. 1x16 mL.

Monoclonal antibodies labelled with Peroxidase, in phosphate buffer with phenol 0.05% and Bronidox 0.02%. Ready to use.

CONTROL – NEGATIVE CONTROL (PF93910) 1x1.6 mL
INTERCHANGEABLE BETWEEN LOTS

Ready to use human serum in phosphate buffer 0.01 mol/L, with BSA 1% and sodium azide 0.09%.

WASH BUF 10X WASH BUFFER 10X (PF93603). 2X50 mL
INTERCHANGEABLE BETWEEN LOTS

Phosphate buffered saline concentrated 10 times; contains Brij 0.5%.

<u>Preparation</u>: dilute the required volume 1:10 with distilled water to obtain the working wash buffer ready for use. If crystals are present, they should be dissolved at 37°C before dilution.

SAMP DIL DILUENT (PF93621). 2 X30 mL. For dilution of serum samples. **INTERCHANGEABLE BETWEEN LOTS**

Proteic solution in phosphate buffer with sodium azide 0.09%. Ready to use

SUBS | TMB SUBSTRATE (PF93619). 12 mL. Ready to use.
INTERCHANGEABLE BETWEEN LOTS

Tetramethylbenzidine 0.26 mg/mL and hydrogen peroxide 0.01% stabilised in citrate buffer 0.05 mol/L (pH 3.8).

STOP SOLN STOP SOLUTION (PF93602). 1x16 mL
INTERCHANGEABLE BETWEEN LOTS

H_2SO_4 0.3 mol/L, in solution ready to use.

ADHESIVE FILMS (2).

POLYTHENE BAG (1).

MATERIALS REQUIRED BUT NOT PROVIDED.

- 37°C Incubator
- Microplate reader, wavelength 450 or 450/620 nm, with OD linearity up to 2,000 (at least).
- Microplate washer (preferable) able to dispense volumes in the range 225-375 µL
- Distilled or deionized water
- Normal laboratory glassware: cylinders, test-tubes etc.
- Micropipettes for the accurate collection of 10, 100, 1000 µl solution
- Disposable gloves

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- Timer
- Sodium Hypochlorite solution (5%)
- Containers for collection of potentially infectious materials
- Absorbent tissue.

STORAGE AND STABILITY OF REAGENTS

Reagents must be stored at 2-8°C.

The expiration date is printed on each component and on the box label.
Reagents have a limited stability after opening and/or preparation

REAGENT	CONDITIONS
Microplate	8 weeks at 2-8°C, polythene bag
Controls	5 weeks at 2-8°C
Conjugate	5 weeks at 2-8°C
Substrate	up to the expiration date at 2-8°C,
	1 week at 15-30°C; store in the dark
Sample Diluent	up to the expiration date at 2-8°C
Wash Buffer	2 weeks at 2-8°C, 5 days at 15-30°C.
Stop Solution	up to the expiration date at 2-8°C

PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE ONLY

This kit contains materials of human origin, which have been tested and gave negative test results for the presence of HbsAg, anti-HIV-1, anti-HIV-2 and anti-HCV antibodies by FDA-approved methods. All material of human origin must be handled as potentially infectious as no diagnostic test can offer a complete guarantee regarding the absence of infective agents. All precautions normally adopted in laboratory practice should be followed when handling material of human origin.

WARNINGS AND PRECAUTIONS

1. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
2. The following reagents contain low concentrations of harmful or irritant substances:
   a) The Wash Buffer contains detergents
   b) The conjugate contains phenol
   c) The substrate is acid
   d) The controls contain 0.09% sodium azide which can react with lead and copper in plumbing forming highly explosive deposits of metal azides; dilute with large amounts of water to prevent azide build up.

   If any of the reagents come into contact with the skin or eyes, wash the area extensively with water.
3. Non-disposable equipment should be sterilized after use. The preferred method is to autoclave for 1 hour at 121°C; disposables should be autoclaved or incinerated.
4. Sulphuric acid required for the Stop Solution and hydrochloric acid used for washing glassware are corrosive and should be handled with appropriate care. If they come into contact with the skin or eyes, wash thoroughly with water.
5. Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1% sodium hypochlorite may be necessary to ensure effective decontamination.
6. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% sodium hypochlorite before work is continued. Sodium hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed of as potentially biohazardous waste. Do not autoclave materials containing sodium hypochlorite.

Analytical precautions

1. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assay not falling within the established time and temperature ranges must be repeated.
2. Allow all reagents and samples to come to room temperature (18-30°C) before use. Immediately after use return reagents to the recommended storage temperature. **It is important to work at the correct temperature. Check that the thermostat does not go below 35°C or over 39°C**. Open the envelope containing the strips after at least ½ hr at room temperature.
3. Do not use the reagents beyond the stated expiration date. Microbiological contamination of reagents must be avoided as this may reduce the dating of the product and cause erroneous results.
4. Do not modify the Test Procedure or substitute reagents from other manufacturers or other lots unless the reagent is stipulated as interchangeable. Do not reduce any of the recommended incubation times.
5. Any glassware to be used with the reagents should be thoroughly washed with 2M hydrochloric acid and then rinsed with distilled water or high quality deionized water.
6. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
7. Do not allow wells to dry during the assay procedure.
8. Care must be taken not to cross-contaminate reagents. It is important that pipettes are dedicated for exclusive use with the various reagents.
9. Care should be taken to avoid touching or splashing the rim of the well with conjugate. Do not "blow-out" from microplates.
10. Enzyme immunoassays can occasionally exhibit an "edge effect" which must be minimized by increasing the humidity during incubation steps. Plates must be covered and incubated at 37°C either in a water bath with a rack or float to support the plates if necessary, or in an incubator. Alternatively, plates can be incubated in an approved analyzer. See the appropriate operating manual for further details. CO_2 incubators must not be used.
11. Ensure that the bottom of the plate is clean and dry, and that no bubbles are present on the surface of the liquid before reading the plate.
12. Use of highly hemolyzed samples, incompletely clotted sera, or samples with microbial contamination may give rise to erroneous results.
13. For each instrument used, read the manufacturer's instructions manual carefully to obtain additional information on the following points:
    - installation and particular requisites

- operating principles, instructions, precautions and risks
- manufacturer's specifications and instrument performance
- servicing and maintenance.

SPECIMEN COLLECTION AND STORAGE

Aseptically draw blood by venipuncture and collect the serum using standard techniques. The fresh serum may be stored for 4 days at 2-8°C, or frozen for longer periods at –20°C, and can be thawed a maximum of 3 times. Thawed samples must be carefully mixed before performing the test. Heat inactivation can lead to erroneous results. The quality of the sample can be seriously affected by microbial contamination, which leads to erroneous results.

Strongly lipemic, icteric or contaminated samples should be avoided. If a new sample cannot be obtained, such samples should be clarified by filtration (0.45 µm) or centrifugation (3000 rpm x 10').
The test cannot be performed on human plasma.

ASSAY PROCEDURE

Summary of the procedure

Manual Technique

1. Prepare 1:26 dilutions of patient samples in sample diluent. Note that Controls are ready-to-use.
2. Add 100 µL of controls and diluted patient samples into the wells. Controls must always be used.
3. Incubate at 37 ± 2 °C for 45 ± 5 min.
4. Discard contents of the wells. Wash the wells 4 times with Wash Solution.
5. Add 100 µL of conjugate to each well except the blank well.
6. Incubate at 37 ± 2 °C for 45 ± 5 min.
7. Wash the wells as in # 4 above.
8. Add 100 µL of Substrate to each well.
9. Incubate for 15 ± 3 min. at R.T.
10. Add 100 µL of Stop Solution to each well.
11. Read absorbance at 450 nm or 450/620nm within 30 min

Test Procedure

- Prepare the working-strength wash buffer by diluting the Wash Buffer 10X (100 mL + 900 mL distilled water).

1. Dilute samples 1:26 by adding 20 µL of serum to 500 µl of diluent.
2. Add 100 µL diluted sample per well (duplicate testing is recommended). Add 100 µL UNDILUTED controls to the appropriate wells. The minimum requirement is 1 Negative Control, 2 Cut-Off Control and 1 Positive control. Leave one well for the blank (100 µL of substrate).
3. Cover the wells with a protective film and incubate for 45 ± 5 minutes at 37 ± 2°C.
4. Discard contents of the wells and wash the wells four times.
5. Add 100 µL of conjugate to each well except the blank well. Cover the wells.
6. Cover the wells with a protective film and incubate for 45 ± 5 minutes at 37 ± 2°C
7. Wash 4 more times as described above.
8. Add 100 µL of substrate per well
9. Incubate at RT for 15 minutes.
10. Add 100 µL of Stop Solution to each well.
11. Read absorbance (O.D.) at 450 nm or 450/620 nm within 30 min.

QUALITY CONTROL VALUES

- Additional control may be tested according to guidelines or requirements of local, state and/or federal regulation or accrediting organization.
- The O.D. value of the blank must be less or equal to 0.150; this value is for validation purposes only.
- The O.D. values of the Cut-Off Control must be within 25% of the mean value. Disregard any abnormal value and recalculate the mean.
- The Positive control must have an O.D. at least 1.5 times that of the Cut-Off Control. This value is for validation purposes only.
- The ratio between O.D. Negative Control and O.D. Cut-Off Control must be less than 0.6.
- The O.D. of the Cut-Off Control must be greater than or equal to 0.2 at 450 nm and greater than or equal to 0.16 at 450/620 nm.
- Not report patient results if QC is out of range.

INTERPRETATION OF THE RESULTS

Qualitative results

Note: The Magnitude of the measured result above Cut Off is not indicative of the total amount of Antibody present.
If the adsorbance of the sample is higher than that of the Cut-Off Control, the sample is positive for the presence of specific IgG.

Calculate the ratio between the average O.D. value of the sample and that of the Cut-Off Control (INDEX). The sample is considered:

Positive: ratio is greater than 1.2.

Equivocal: ratio is equal to or greater than 0.8 and equal to or less than 1.2

Negative: ratio is less than 0.8.

If the result is equivocal, repeat the test. If it remains equivocal, collect a new serum sample.

nontreponemal result (NT)	treponemal result	Report/interpretation for all except neonates or infants**
nonreactive	Negative (Nonreactive)	No serologic evidence of infection with *T. pallidum* (incubating or early primary syphilis cannot be excluded).
reactive	Negative (Nonreactive)	Current infection unlikely; probability of BFP secondary to other medical conditions (febrile diseases, immunizations, IVDU, autoimmune diseases, etc.). Recommend repeat testing (nontreponemal, and treponemal by other test method.)
nonreactive	Positive (Reactive)	probably past infection or potential cross-reactivity with other spirochetes/related antigens; additional testing appropriate to clinical findings/history;* possibility of false negative NT due to prozone and late latent syphilis or neurosyphilis.
reactive	Positive (Reactive)	presumptive evidence of current infection (or inadequately treated infection, persistent infection, reinfection, or BFP if prior history); additional testing consistent with clinical assessment.*
nonreactive	Not done	Current infection unlikely; effectively treated infection if previous diagnosis and treatment; cannot exclude incubating or early primary syphilis; cannot exclude latent or neurosyphilis. Treponemal testing advised if clinical suspicion of latent or neurosyphilis.
not done	Negative (Nonreactive)	Current or past infection unlikely; cannot exclude incubating or early primary syphilis.

*Quantitative nontreponemal testing; clinical history; repeated (sequential) serological testing for changes in titer

**HIV-infected individuals may have delayed seroreactivity or negative serology.

LIMITATIONS OF THE PROCEDURE

1. A serum sample obtained during the acute phase of infection, when only IgM antibodies are present, may be negative by this procedure.
2. The *Treponema pallidum* IgM level should be determined using a *Treponema pallidum* IgM kit. Alternatively, a second serum sample obtained 8-14 days later should be tested in parallel to determine an increase in the IgG antibody level.
3. The test result should be used in conjunction with information available from the evaluation of other diagnostic procedures.
4. Although the control sera used in the kit are calibrated to WHO reference serum, certain discordances of results may be observed when the same serum is tested by different serological techniques.
5. This test has not been cleared for blood and/or plasma screening.
6. Strongly lipemic, icteric or contaminated samples should be avoided.
7. This test has not been evaluated in patients with other treponemal diseases such as pinta, yaws, Lyme disease and leptospirosis.
8. This test has not been evaluated in patients with connective tissue diseases such as rheumatoid arthritis and lupus erythematosus.

PERFORMANCE CHARACTERISTICS

A total of 525 samples were collected and tested to study the performance of the REQUESTSYPHILIS IgG kit.

FIRST group: 125 serum samples from both pediatric and adult male and female patients with syphilis.

SECOND group: 300 negative sera; 150 of these serum samples came from clinical sources and/or from a blood donor facility and 150 samples from normal donors.

THIRD group: 100 samples from subjects with no known history or serological evidence of syphilis and suspected for different kinds of infective or clinical pathology.

SENSITIVITY AND SPECIFICITY

In the first group of the 125 syphilitic sera there was no disagreement between two comparative testing methods; therefore the reference method FTA-ABS was not used.
In the second group of the 300 syphilis-negative sera there was one sample not in agreement. It was equivocal with the REQUESTSYPHILIS IgG test and negative with the CAPTIA Syphilis - G test. The confirmatory FTA-ABS test was negative (it was also negative with TPHA and VDRL test).

Table n°1
Results obtained with ENZYWELL vs. TRINITY testing 125(First Group) Syphilitic sera:

		CAPTIA Syphilis - G		
		Negative	Equivocal	Positive
REQUEST SYPHILIS IgG	Negative	0	0	0
	Equivocal	0	0	0
	Positive	0	0	125

Results:
Sensitivity = 100 %.
95% CI: 100% <Se <100%

Table n°2
Results obtained testing 300 sera (Second Group): 150 sera from clinical source and 150 sera from normal donor, both groups were negative to Syphilis

		CAPTIA Syphilis -G		
		Negative	Equivocal	Positive
REQUEST SYPHILIS IgG	Negative	299	0	0
	Equivocal	0	0	0
	Positive	1*	0	0

* The test gave a positive result even after repeat testing. Both the confirmatory tests (FTA-ABS and TPHA) gave negative results.

Specificity = 99.6 %.

95% CI: 99%<Sp<100%

In the third group of 100 sera with different pathological diseases, two sera gave equivocal results with both methods, also after repeating the test. The confirmatory test gave a positive result with a titer of 1280; the FTA-ABS test also gave positive results.

The specificity of REQUESTSYPHILIS IgG kit in this group is 100%

In addition, clinical studies performed at two independent clinical laboratories with a total of three hundred and eighty-seven specimens, comparing the Diesse REQUEST Syphilis IgG test with two other commercially available tests.

Lab B

	REQUEST			% Agreement (Pos.or Neg.)	
FTA	Pos	Eqv	Neg	%	95 % C.I.
Reactive	29	3	1	96.7	90.2 to 100
Non-Reactive	5	1	75	88.4	90.3 to 99.0
				94-5	90.3 to 98.8

Lab C

	EIA			% Agreement (Pos. or Neg.)	
REQUEST	Pos	Eqv	Neg	%	95 % C.I.
Positive	7	2	2	77.8	50.6 to 100
Equivocal	0	1	0		
Negative	2	2	257	99.2	98.2 to 100
				98.5	97.1 to 99.9

Excluding equivocal results, a total of 10 samples gave discordant results. When these samples were tested by a third commercially available test, the referee test agreed with the Diesse test for 8 of the 10 discordant samples tested.

EXPECTED VALUE

In the first study 525 sera were evaluated in the REQUEST Syphilis IgG kit . Of these samples 126 (24 %) resulted positive to Syphilis and 399 (76 %) resulted negative to Syphilis.
Of the 125 positive samples, 32 (25.6%) samples were in the latent stage of Syphilis, 26(20.8%) samples were in Primary stage of Syphilis, 37 (29.6%) were in the Secondary stage of Syphilis, 1.7% were affected by both I & II Syphilis, 1(0.8%) were in tertiary stage of Syphilis, 7 (5.6%) were in Advanced stage of Syphilis, 8 (6.4%) affected by Suspected Neurosyphilis, 12 (9.6%) were not defined stage.
Comparable results were obtained with CAPTIA syphilis IgG

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PRECISION

All samples (Cut-Off Control, Positive Control and Negative Control) were tested in triplicate in two separate runs on three different days. CV lower than 15% are accepted.

Within run Precision

DAY 1	Replicates	RUN 1			RUN 2		
SAMPLES	3	O.D.	S.D.	CV%	O.D.	S.D.	CV%
CutOff	3	353	22	6.2	324	23	7.1
Pos. control.	3	1171	20	1.7	1091	91	8.4
Neg. Control	3	49	1	2.3	49	2	3.5
Pos serum 1	3	1687	28	1.7	1524	137	9.0
Pos serum 2	3	2141	89	4.1	2184	50	2.3
Pos serum 3	3	383	13	3.3	329	32	9.8
Pos serum 4	3	838	18	2.1	766	59	7.7
Neg serum1	3	75	3	4.1	67	5	7.0
Neg serum2	3	86	11	12.4	80	3	4.3
DAY 2	**Replicates**	**RUN 1**			**RUN 2**		
SAMPLES	3	O.D.	S.D.	CV%	O.D.	S.D.	CV%
CutOff	3	386	27	6.9	314	14	4.5
Pos control.	3	1235	83	6.7	1233	60	4.9
Neg. control	3	37	1	2.7	35	0	0.0
Pos serum 1	3	1787	82	4.6	1706	43	2.5
Pos serum 2	3	2408	59	2.4	2351	94	4.0
Pos serum 3	3	360	21	5.7	322	24	7.4
Pos serum 4	3	879	84	9.6	775	57	7.4
Neg serum1	3	68	9	12.7	52	3	5.8
Neg serum2	3	80	4	5.4	76	21	27.4

DAY 3	Replicates	RUN 1			RUN 2		
SAMPLES	3	O.D.	S.D.	CV%	O.D.	S.D.	CV%
Cut-Off	3	342	9	2.7	339	14	4.0
Pos Control.	3	1218	26	2.1	1204	83	6.9
Neg Control	3	34	4	11.2	34	1	3.4
Pos serum 1	3	1613	57	3.6	1633	40	2.4
Pos serum 2	3	1958	228	11.7	2093	176	8.4
Pos serum 3	3	365	19	5.1	370	23	6.3
Pos serum 4	3	769	48	6.2	801	8	1.1
Neg serum1	3	61	3	5.7	101	10	10.0
Neg serum 2	3	66	5	7.2	68	6	8.6

Between run Precision

	INDEX		
SAMPLE	O.D. AVERAGE	SD	CV%
CUTOFF	343	18	5.2
Pos. Control.	1192	61	5,1
Neg. Control	40	1	3.9
Positive serum 1	1658	65	4.0
Positive serum 2	2189	116	5.5
Positive serum 3	355	22	6.3
Positive serum 4	805	46	5.7
Negative serum 1	71	5	7.5
Negative serum 2	76	8	10.9

In addition, the kit positive and negative controls, plus six additional samples, including 2 negatives and four positives, were assayed in triplicate, in three different runs, at three independent laboratories, using automated analyzers.

Within Run Precision

Lab A

	Run 1			Run 2			Run 3		
ID	O.D.	S.D.	CV%	O.D.	S.D.	CV%	O.D.	S.D.	CV%
PC	1870	211	11.3	2108	227	10.8	2358	114	4.8
NC	5	2	NA	0	0	NA	0	0	NA
A	52	20	NA	0	0	NA	0	0	NA
B	55	6	NA	0	0	NA	0	0	NA
C	617	44	7.2	398	61	15.2	501	48	9.5
D	603	105	17.4	463	101	21.8	543	60	11.0
E	1013	38	3.7	1004	56	5.5	1222	67	5.5
F	1010	100	9.9	890	53	5.9	1224	74	6.0

Lab B

	Run 1			Run 2			Run 3		
ID	O.D.	S.D.	CV%	O.D.	S.D.	CV%	O.D.	S.D.	CV%
PC	1802	32	1.8	1740	29	1.6	1825	17	0.9
NC	142	104	NA	96	61	NA	136	92	NA
A	138	17	NA	94	28	NA	136	55	NA
B	96	47	NA	67	37	NA	86	73	NA
C	637	81	12.7	597	58	9.7	614	51	8.4
D	782	39	5.0	691	17	2.5	770	48	6.3
E	1202	101	8.4	1050	60	5.8	1177	77	6.6
F	1010	89	8.8	995	94	9.5	981	69	7.0

Lab C

	Run 1			Run 2			Run 3		
ID	O.D.	S.D.	CV%	O.D.	S.D.	CV%	O.D.	S.D.	CV%
PC	2766	30	1.1	2747	116	4.3	2850	49	1.7
NC	0	30	NA	0	0	NA	2	0	NA
A	13	0	NA	14	9	NA	14	8	NA
B	57	10	NA	59	6	NA	54	2	NA
C	824	10	1.3	887	70	7.9	823	74	9.1
D	909	38	4.1	942	19	2.0	937	28	3.0
E	1502	70	4.7	1489	42	2.8	1567	41	2.6
F	1469	28	1.9	1659	63	3.8	1470	65	4.4

Between Run Precision

	Lab A			Lab B			Lab C		
ID	O.D.	S.D.	CV%	O.D.	S.D.	CV%	O.D.	S.D.	CV%
PC	2112	271	12.8	1789	45	2.5	2788	83	3.0
NC	2	3	NA	125	85	NA	1	1	NA
A	17	28	NA	123	43	NA	14	9	NA
B	18	28	NA	83	055	NA	57	7	NA
C	506	106	21.0	616	61	9.9	845	68	8.1
D	536	102	19.0	748	56	7.5	929	043	4.6
E	1080	119	11.0	1143	103	9.0	1520	51	3.3
F	1041	164	15.7	995	078	7.9	1533	113	7.4

Interlaboratory Precision

ID	O.D.	S.D.	CV%
PC	2230	510	23
NC	43	71	NA
A	51	62	NA
B	53	33	NA
C	656	173	26
D	738	197	27
E	1248	238	19
F	1190	298	25

TROUBLE SHOOTING GUIDE

PROBLEM	PROBABLE CAUSE	TEST OR ACTION
Invalid run (all negative)	One or more reagents not added or added in wrong sequence	Recheck procedure Check for unused solutions. Repeat test.
	Unreactive plate	Check the code on the package containing the plate (see instructions for use – Kit contents/MT PLATE for correct code).
		Check for moisture in unused plate. (Silica gel desiccant must be pale yellow).Repeat test
Invalid run The O.D. value of the blank is greater than 0.150	Contamination of substrate	Use a new vial of substrate.
Invalid run (all positive)	Contamination of STOP SOLUTION	Use a new vial of STOP SOLUTION
	Inadequate washing	Ensure that wash apparatus works well
Poor precision	Incomplete washing of wells	Ensure that wash apparatus works well
	Inadequate aspiration of wells	Ensure that wash apparatus works well
	Pipetting error	Check pipette function
	Reagent addition too slow	Avoid drying of the plate after washing step. Add reagents immediately
	Presence of bubbles	Avoid air bubbles during pipetting.
	Optical pathway not clean	Check instrument light source and detector for dirt. Wipe bottom of plate with soft tissue.
Inadequate color development	Incorrect incubation time or temperature	Check for temperature control and time monitoring
		Adhere to recommended instructions for use.

CROSSREACTIVITY & INTERFERENCE STUDIES

In order to demonstrate Analytical Specificity and Interferences an internal experimentation was performed using a total of 332 sera with known disease. Experimentation was performed in two times. The first time the following 131 sera, collected from seroteque, were tested:
73 sera from adults females, 58 from adult males. All these were characterized as follows:

CATEGORY OF SPECIMENS	n
ALT	20
HCV Positives	21
HCV Ab Reactive	9
Hypergammaglobulinemia	19
Pregnant	50
Total Bilirubine	2
Lipemic	2
Mono test (MT)	8

In a second day 201 sera were tested. Sera summarized below:

CATEGORY OF SPECIMENS	n
HCV Positives	46
Pregnant	110
HBSAg Vaccinated	30
HBSAg Positives	15

Obtained Results:

CATEGORY OF SPECIMENS	n	REQUESTSYPHILIS IgG Reactive
HbsAg Vaccinated	30	0
HBsAg Positives	15	0
ALT	20	0
Sera from HBV Vaccines	11	0
HCV Positives	64	1*
HCV Ab Reactive	9	0
Hypergammaglobulinemia	19	0
Pregnant	160	0
Total Bilirubine	2	0
Lipemic	2	0
Total Specimen	332	

Note: Confirmed positive with TPHA

Conclusion:
Only one sample that results reactive when tested with ENZY- WELL Syphilis IgG was confirmed to be positive with TPHA (Confirmatory test)

Analytical specificity= 100%

No interferences are shown.

REFERENCES

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ReQuest® Syphilis IgG

Manufacturer:
Quest International, Inc.
8127 NW 29th Street
Miami, FL 33122
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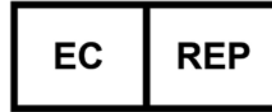

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Symbols Glossary

Symbol	Standard Title and Number	Title of Symbol	Symbol reference #	Explanatory Text
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Manufacturer	5.1.1	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
EC REP	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Authorized representative in the European Community	5.1.2	Indicates the Authorized representative in the European Community.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Use-by-date	5.1.4	Indicates the date after which the medical device is not to be used.
LOT	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Batch code	5.1.5	Indicates the manufacturer's batch code so that the batch or lot can be identified.
REF	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Catalog number	5.1.6	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Temperature limit	5.3.7	Indicates the temperature limits to which the medical device can be safely exposed.
i	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Consult instruction for use	5.4.3	Indicates the need for the user to consult the instructions for use.
IVD	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	*In vitro* diagnostic medical device	5.5.1	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
Σ 96	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Contains sufficient for 96 tests	5.5.5	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
Rx Only	Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements	Rx Only	N/A	Caution: Federal law prohibits dispensing without prescription.

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