

BECKMAN COULTER PK[®] TP SYSTEM

Microhemagglutination Test for Detection of *Treponema pallidum* Antibodies using the Beckman Coulter PK[®] Instrument

I. INTENDED USE

BECKMAN COULTER PK[®] TP SYSTEM is intended for the qualitative screening of blood donors for the detection of *Treponema pallidum* IgG and IgM antibodies in human serum or EDTA plasma using the BECKMAN COULTER PK7200 and/or PK7300, Automated Microplate System.

This assay is not intended for diagnostic use.

II. SUMMARY OF TEST

The identification of *Treponema pallidum* antibodies aids in the diagnosis of syphilis caused by the microorganisms belonging to the genus *Treponema* and provides epidemiological information on syphilis.

Serological tests for syphilis were first used in 1906 with the development of the nontreponemal complement fixation test by Wasserman. The nontreponemal tests measure both immunoglobulin G (IgG) and M (IgM) antilipid antibodies formed by the host in response both to lipoidal material released from damaged host cells early in infection and to lipid from the treponeme itself. Because of either the lipidic nature of the antigen or some unusual property of the antibodies, the antigen-antibody reaction remains suspended, and flocculation occurs, rather than agglutination or precipitation as in most other serologic tests¹.

While useful in the diagnosis of suspected syphilis infection, nontreponemal tests are nonspecific. False positive tests may account for a significant proportion of reactive results necessitating an additional test to identify the presence of specific antibodies to *T. pallidum*.

Hemagglutination (MHATP) tests for *T. pallidum* have gained wide acceptance since their emergence in the mid 1960's^{2,3,4} as a confirmatory procedure following a reactive nontreponemal assay or as a screening procedure. Automation has enhanced the value of the test by significantly reducing the amount of time and labor needed to perform the assay^{5,6}. BECKMAN COULTER PK TP SYSTEM has been developed to provide uniform reagents which are stable, easy to handle, and suitable for use on the BECKMAN COULTER PK7200 and/or PK7300, Automated Microplate System^{7,8}.

The results obtained are provided to the user in a computer generated printout. All specimens which are repeatedly reactive or indeterminate with the PK TP SYSTEM are considered positive for antibodies to *T. pallidum* by the criteria of the PK TP assay. For complete details on the setup and operation of the BECKMAN COULTER PK7200 and PK7300, refer to the PK7200 Operator's Manual or the PK7300 User's Guide.

III. PRINCIPLE OF PROCEDURE

The test is based on the principle of agglutination and pattern recognition. The BECKMAN COULTER PK TP SYSTEM utilizes fixed chicken erythrocytes sensitized with components of the pathogenic *T. pallidum* (Nichols Strain).

The test sample is diluted in a sample diluent composed of phosphate-buffered saline containing normal rabbit testicular extract and cell components of sonicated Reiter *T. phagedenis*. This sample diluent minimizes nonspecific reactions. The sensitized cells are added to the test mixture and reactants are allowed to settle in a patented, terraced microplate

well. Hemagglutination occurs in the presence of *Treponema pallidum* (TP) antibodies in specimens. Visually, a reactive test is a homogenous layer of cells. A nonreactive test would result in a compact dense button surrounded by a clear zone. The PK7200 and PK7300 instruments will read the settling patterns of erythrocytes in each well based on the threshold settings chosen for each reagent. The PK7200 and PK7300 determines the presence or absence of antibodies to *T. pallidum* using a CCD (charged coupled device) camera which captures the well image and allows differentiation of agglutinated and unagglutinated patterns.

IV. REAGENTS

BECKMAN COULTER PK TP SYSTEM is available in a kit sufficient to perform 3000 tests:

RECONSTITUTING SOLUTION (A) - 1 bottle, 130mL. This reagent is used for reconstitution of the lyophilized SENSITIZED CELLS. The reagent contains a phosphate-buffered saline solution with 0.1% sodium azide.

SAMPLE DILUENT (B) - 5 bottles, 189mL each. The diluent consists of a phosphate-buffered saline solution containing normal rabbit testicular extract, cell components of sonicated Reiter *Treponema phagedenis*, and 0.1% sodium azide. Tartrazine (FDC No.4) [20 ppm] and Fastgreen (FDC No. 3) [2 ppm] have been added to impart a green color.

SENSITIZED CELLS (C) - 11 vials of lyophilized, fixed chicken erythrocytes. Each vial must be reconstituted with 10 mL of RECONSTITUTING SOLUTION.

Reconstituted cells are stable for 5 days and should be stored at 2-8°C for maximum stability.

V. WARNINGS AND PRECAUTIONS

The BECKMAN COULTER PK TP SYSTEM is for in vitro diagnostic use.

1) Avoid contamination of reagents or specimens with saliva, which can cause indistinguishable agglutination patterns. Do not mouth pipette any reagents.

- 2) The microplates must be clean before use. Improper washing of the microplates can adversely affect a test result by causing a false positive or false negative reaction. The suggested washing procedure can be found in the Standard Operating Procedure Manual for the PK7200 and the PK7300 User's Guide.
- 3) Avoid freezing of the reconstituted SENSITIZED CELLS.
- 4) Sodium azide is included as a preservative. Sodium azide has been reported to form explosive lead and copper azides in laboratory plumbing. To prevent azide buildup, flush with large volumes of water if solutions containing azide are disposed of in the sink.
- 5) Visible signs of microbial growth or gross turbidity in the reagent may indicate degradation and warrant discontinuance of use.
- 6) Handle all specimens, control material and serum-based reagents as if potentially infectious. Refer to the Centers for Disease Control's Guidelines on Specimen Handling⁹.
- 7) Clean pipettes should be used to reconstitute all reagents. Clean glass or plastic containers should be used for reagent preparation.
- 8) Serum is the specimen of choice for this test. EDTA plasma is suitable for screening, however, field use has demonstrated higher incidence of false positive results. Therefore, serum must be used for all repeat testing of initially reactive or indeterminate results obtained from plasma samples.
- 9) The performance of this assay has not been established with plasma samples employing sodium citrate or heparin as the anticoagulant.
- 10) The effects of specimen microbial contamination on this assay can not be predicted.
- 11) Carryover between specimens is a potential source of interference.
- 12) Positive and negative control material should be handled in the same fashion as donor samples.

- 13) When specimen fails to be added to the PK assay, the potential exists for a false negative result to occur.
- 14) Inadequate adherence to the package insert can result in erroneous results.
- 15) The use of calibrated or verified equipment is required.

VI. REAGENT PREPARATION

- 1) Reconstitute, as needed, each vial of SENSITIZED CELLS with 10 mL of RECONSTITUTING SOLUTION. Replace the stopper and gently invert to assure thorough mixing. Allow to reconstitute at room temperature (15-30°C) for a minimum of 30 minutes or overnight at 2-8°C to ensure complete rehydration.
- 2) Reconstituted cells are stable for 5 days and should be stored at 2-8°C for maximum stability.
- 3) After the reconstitution period, gently swirl (DO NOT VORTEX) the rehydrated cells to assure thorough resuspension. Follow steps under heading, "DIRECTIONS FOR USE", for use on the instruments.
- 4) SENSITIZED CELLS from the same lot may be pooled. The mixture is stable for five days from the earliest reconstitution date of the vials contained in the mixture.
- 5) SENSITIZED CELLS from one lot number should not be mixed with those of another lot number.
- 6) SAMPLE DILUENT from the same lot can be pooled for use on the instruments as long as good laboratory practices are followed.
- 7) The date of reconstitution and the reconstituted expiry should be recorded on the reagent containers.
- 8) The SAMPLE DILUENT and RECONSTITUTING SOLUTION are not matrixed to the SENSITIZED CELL lot.

Note: All reagents should be brought to room temperature (15-30°C) prior to use on the analyzer.

VII. STORAGE

- 1) Store the BECKMAN COULTER PK TP SYSTEM test kit at 2-8°C. DO NOT FREEZE.
- 2) The BECKMAN COULTER PK TP SYSTEM should not be used after the expiration date which is printed on the outside of the package.
- 3) Reconstituted cells are stable for five (5) days and should be stored at 2-8°C for maximum stability. DO NOT FREEZE.
- 4) Visible signs of microbial growth or gross turbidity in the reagents may indicate degradation and warrant discontinuance of use.

VIII. SPECIMEN COLLECTION AND PREPARATION

BECKMAN COULTER PK TP SYSTEM may be used with serum or EDTA plasma. Serum is the preferred specimen. Specimens for repeat testing must be obtained from the same draw. The sample should be free of particulate matter. If erythrocytes or other visible components are contained in the sample, remove by centrifugation to prevent interference with the test results. The PK7200 Standard Operating Procedures and the PK7300 User's Guide require centrifugation of samples within 10 hours of analysis and centrifugation for a minimum of 10 minutes at a minimum of 1000 x g. These requirements exist for the purpose of optimizing red cell sampling. Therefore, plasma or serum samples tested using the PK TP assay do not need to comply with these requirements as long as the plasma or serum is free from particulate matter. Samples exhibiting gross lipemia, hemolysis or icterus may be compromised and may require alternative testing.

Store plasma and serum specimens at 2-8°C. ***Plasma and serum specimens may be tested for up to 5 (five) days after collection on the PK7200 and PK7300 analyzers.***

Sera may be stored at $\leq -20^{\circ}\text{C}$, if testing cannot be performed within the specimen age requirements defined above. Avoid repeated freezing and thawing of specimens. Thorough mixing of frozen samples is necessary after thawing and prior to testing.

Allow the specimens to come to room temperature before testing (a minimum of 10-30 minutes after thawing).

Inactivation of sample serum is not required but inactivated serum can also be used in the test. Serum may be heated for 30 minutes in a 56°C water bath without affecting the test outcome. Specimens to be heated should be at room temperature before placing in the water bath. Allow specimens to return to room temperature before testing (a minimum of 10-30 minutes after heating).

IX. MATERIALS

MATERIALS PROVIDED IN THE BECKMAN COULTER PK TP SYSTEM:

- RECONSTITUTING SOLUTION
- SAMPLE DILUENT
- SENSITIZED CELLS

MATERIALS REQUIRED BUT NOT PROVIDED:

- BECKMAN COULTER terraced microplates
- Pipetting devices for: 1.0 mL, 5.0 mL, and 10.0 mL
- BECKMAN COULTER PK7200 or PK7300 Automated Microplate System

- BECKMAN COULTER PK TP SYSTEM CONTROL SET (Catalog Number: PH3502)

X. DIRECTIONS FOR USE

The PK Instrument is a programmable instrument whose operation is controlled by software. Parameters validated by the manufacturer are incorporated into the operating files. The user may define panel (test) configurations. For more information about this process, please consult section 8 of the PK7200 Operator's Manual or section D of the PK7300 User's Guide.

Working files for the PK TP test are shown below for the PK7200 and PK7300. Good laboratory practice dictates that each laboratory validates the operating parameters.

RECOMMENDED PARAMETERS

Beckman Coulter has established recommended parameters for both instruments based upon application development testing with characterized samples. These parameters are listed in the tables below:

RECOMMENDED VOLUMES FOR PK7200					
Parameter Set	Sample	Diluted Sample	Sample Diluent	Reagent	Final Plasma/Serum Dilution
1	40 µL	15 µL	250 µL	35 µL	1/24
2	60 µL	10 µL	250 µL	35 µL	1/23

RECOMMENDED VOLUMES FOR PK7300						
Parameter Set	Sample	Sample Diluent	Sample/Diluent Ratio	Diluted Sample	Reagent	Final Plasma/Serum Dilution
1	21 µL*	147 µL*	8.0	20 µL	35 µL	1/22

* Sample and diluent volumes are determined by the PK7300 software based on the Sample/Diluent Ratio entered into parameters.

RECOMMENDED THRESHOLDS AND SETTINGS FOR THE PK7200 and PK7300

	P/C		SPC		LIA		LIA Selection	BG/C	Temperature	
	(+) Limit	(-) Limit	Low	High	(+) Limit	(-) Limit			Setting	Instrument Range
PK7200	41	26	16	16	240	100	5	Low	28-32°C	±3°C
PK7300	45	35	16	16	250	150	5	Low	28-32°C	±3°C

EXAMPLES:

PK7200

PARAMETERS

Sample Volume	40 µL	
Diluent Volume	250 µL	G 0.25
Ratio	160 µL/1000 µL	
Diluted Sample Volume	15 µL	
Reagent Volume	35 µL	
Channel Name	SYP	
Channel Designation	(1-12)	
Decision Logic	+/-	
Temperature Setting	28-32°C	
Incubation Time	60 minutes	
Well	16 µm	
Thresholds:		
<u>SPC</u>	Low	16
	High	16
<u>P/C</u>	(+) Limit	41
	(-) Limit	26
<u>LIA</u>	(+) Limit	240
	(-) Limit	100
<u>LIA Selection</u>		5
<u>BG/C Limit</u>		Low

PK7300

PARAMETERS

Sample/Diluent Ratio	8.0	
Diluted Sample Volume	20 µL	
Reagent Volume	35 µL	
Channel Name	SYP	
Channel Designation	(1-12)	
Decision Logic	+/-	
Temperature Setting	28°C	
Incubation Time	60 minutes	
Well	16 µm	
Thresholds:		
<u>SPC</u>	Low	16
	High	16
<u>P/C</u>	(+) Limit	45
	(-) Limit	35
<u>LIA</u>	(+) Limit	250
	(-) Limit	150
<u>LIA Selection</u>		5
<u>BG/C Limit</u>		Low

Note: Remember to save these changes on the master program disk and on the hard drive on the PK7200.

Changes made to the PK7300 are automatically saved to the hard drive when they are made. It is recommended that when any changes are made, they also be saved to an external storage media.

All reagents, diluents, and specimens should be at room temperature (15-30°C) prior to analysis.

PK7200 Prep Procedure for the BECKMAN COULTER PK TP SYSTEM

To use the reagents on the analyzer:

- 1) Place the reconstituted SENSITIZED CELLS into the designated channel of the reagent container. Thorough, uniform mixing of SENSITIZED CELLS is important. Prior to placing on the analyzer, check reagent trough to ensure that red cells are not settling out of solution. If settling of the red cells is observed, use a pipette to carefully suspend the cells. Place the reagent container and mixing comb on the instrument. Press the MIX button on the analyzer to start the motion of the mixing comb if there is to be any delay in initiating processing.
- 2) Place the appropriate primary diluent line into the diluent container which is filled with SAMPLE DILUENT.
- 3) Remove the G stroke pins for the diluent lines only if a black rack with tubes of saline is not being processed at the beginning of the run.
- 4) Push the PREP button on the analyzer.
- 5) When the PREP cycle is complete, replace the G stroke pins, being sure to place the one marked "G 0.25" under the syringe that will be used to aspirate the SAMPLE DILUENT.
- 6) Press the DIAG button on the analyzer control panel to expel bubbles in sample and diluted sample probes.
- 7) Proceed with sample analysis as described in the BECKMAN COULTER PK7200 Operator's Manual.
- 8) Please refer to Section XI., Quality Control, for instructions about the use of control samples.

PK7300 Prep Procedure for the BECKMAN COULTER PK TP SYSTEM

- 1) Place the reconstituted, well mixed SENSITIZED CELLS into a PK7300 reagent vial. Place the reagent vial into the appropriate slot in the reagent tray. After the reagent tray is loaded into the PK7300, press the MIX button from the SYSTEM STATUS screen to mix the reagents.

- 2) Place the appropriate diluent line into the diluent bottle which is filled with SAMPLE DILUENT.
- 3) Perform Diluent Priming by selecting the Preparation button on the SYSTEM STATUS screen.
- 4) Perform a Diluent and Reagent Check after selecting the Reagent/Diluent Status button from the SYSTEM STATUS screen.
- 5) Proceed with sample analysis as described in the BECKMAN COULTER PK7300 User's Guide.
- 6) Please refer to Section D of the PK7300 User's Guide for instructions about the use of control samples.

XI. QUALITY CONTROL

The PK TP SYSTEM CONTROL SET must be tested at the beginning and end of each batch of samples assayed, after the addition of reagents, and after interruption or delays in processing. Additional QC testing may be performed by the user by including other well-characterized specimens or referenced sera.

Perform the test as described under Section X, DIRECTIONS FOR USE, using the reactive and non-reactive controls as the specimens. The reactive control should produce a positive (+) reaction and the nonreactive control should produce a negative (-) reaction with the test. If the appropriate results are not obtained with the controls, all assay results within that batch are invalid and must be retested. Repeat testing making sure that the volume of control is sufficient for adequate instrument sampling (>1.5 mL). When control material repeatedly fails to perform as expected, contact Beckman Coulter Immunohematology Technical Services at 1-800-447-5852.

XII. INTERPRETATION

The PK7200 and PK7300 instrument will read the settling patterns of erythrocytes in each well based on the threshold settings chosen for each reagent. See the BECKMAN COULTER PK7200 Standard Operating Procedure Manual or the PK7300 User's Guide, for complete details of the analyzer's interpretation of reactions. The threshold limits are programmed into the PK7200 and PK7300 under the current operating conditions.

As soon as possible after analyzer interpretation, results should be verified by visual judgment of the reaction pattern against the photometric data. All photometer results should be visually reviewed if tested on the PK7200. The PK7300 stores the reaction patterns on the hard drive and plate review may be performed either manually or on-line. Visually, a reactive test is a homogeneous layer of cells. A non-reactive test would result in a compact dense button surrounded by a clear zone. Additional testing must be performed on any sample for which visual and analyzer interpretations do not agree.

The presence or absence of antibodies to *Treponema pallidum* is determined by the PK7200 and PK7300 by a CCD camera which analyzes the well images and differentiates agglutinated and non-agglutinated patterns. The PK7200 and PK7300 employs three assessment parameters for each microplate well containing BECKMAN COULTER PK TP SYSTEM reagents and test specimens:

- SPC Sharpness of the edge of the cell button
- LIA Quantity of cells in the center of the well
- P/C Ratio of the average light transmittance of the peripheral and central values

The parameters, SPC, LIA and P/C, are compared to programmable thresholds to obtain an interpretation (+, -, ?) for each reaction.

The most important parameter resulting from the image analysis system is SPC. If the SPC is determined positive, then either a positive or indeterminate LIA or P/C value will result in an overall positive results interpretation for the reaction. A positive SPC value together with a negative value for either the LIA or P/C will cause the channel result to be an indeterminate result. If the SPC is determined negative, then either a negative or indeterminate LIA or P/C value will result in an overall negative result interpretation for the reaction. A negative SPC value together with a positive value for either the LIA or P/C will cause the channel result to be an indeterminate result. Please refer to Table 1 for further clarification.

TABLE 1: DECISION LOGIC FOR PK7200 and PK7300 RESULTS INTERPRETATION

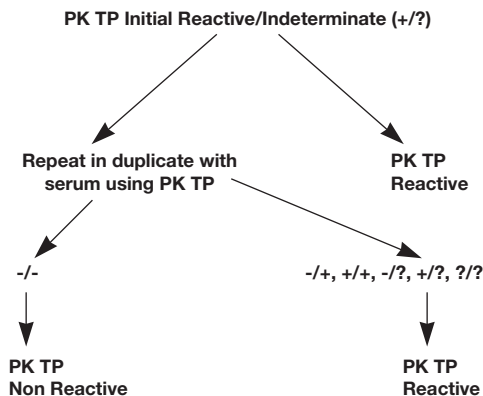
Channel Results Interpretation	SPC	LIA	P/C
Positive	+	+ or ?	+ or ?
Negative	-	- or ?	- or ?
Indeterminate	+	-	-
	+	-	+
	+	+	-
	-	+	+
	-	+	-
	-	-	+
?	+, -, or ?	+, -, or ?	

III. INTERPRETATION OF RESULTS (Table 2)

A sample reported as nonreactive on initial screening is considered nonreactive for antibodies to *T. pallidum* and needs no further testing.

An EDTA plasma sample which is reactive or indeterminate (?) on initial screening with the PK TP SYSTEM, is considered initially reactive by the PK TP SYSTEM, but prior to interpretation, the test may be repeated in duplicate using a serum specimen from the same draw. If serum is the initial sample tested, retesting in duplicate on serum is optional. The duplicate tests must occur in the same run. If either duplicate is reactive or indeterminate, the specimen is to be interpreted as repeatedly reactive for antibodies to *T. pallidum* by the criteria of the PK TP SYSTEM. Initially reactive plasma or serum specimens which are negative in both of the duplicate re-tests are considered negative by the criteria of the PK TP SYSTEM.

TABLE 2: INTERPRETATION OF RESULTS FOR PK[®] TP



XIV. EXPECTED VALUES

A sample reported as reactive on the BECKMAN COULTER PK TP SYSTEM is considered to be reactive for IgG and/or IgM antibodies to *T. pallidum* by the criteria of the PK TP System. Reactive results may indicate active, past, or successfully treated syphilis infections. The diagnosis of syphilis depends not only on the laboratory findings, but also on a carefully obtained history and a thorough physical examination.

Studies performed on 3,440 random blood donors have shown the initial plasma reactive rate to be 1.48% (51/3440) and the repeat reactive rate to be 0.47% (16/3432) when samples were tested on the PK TP System on the PK7200. When tested against samples from individuals at varying stages of the disease (primary, secondary and tertiary), the overall reactive rate was 93.9% (447/476) by the PK TP System. By comparison, RPR positively identified 88.2% (420/476) of the samples. FTA-ABS testing showed 94.1% of the population (444/472) to be reactive.

One hundred percent (100%) of samples tested from both treated and untreated patients in both the primary

and secondary stages of the disease were detected by the PK TP System. Samples from both treated and untreated tertiary or latent stage patients were also tested with the PK TP System. Ninety-two percent (92%) of the samples were positively identified.

Of samples from individuals with unknown disease status, 77% were identified by the PK TP System as compared to 69.9% by RPR.

Samples from individuals with autoimmune disease, Lyme disease, Legionella, infectious mononucleosis and Rubella, including drug addicts and multiparous women, have shown the reactive rate to be 4.37% (10/229) when the PK TP System was employed. Eight of these ten samples reactive with PK TP were also reactive by either RPR and/or FTA-ABS. A set of known syphilis negative samples from individuals demonstrating reactivity for antinuclear antibodies, rheumatoid factor and Lyme disease were tested on the PK7300 with the PK TP System. All samples tested negative as expected with no evidence of interference or crossreactivity.

Some institutions may encounter a higher rate of false positive results with apheresis donors. These results seem to be related to the mechanism of sample collection and preparation for some apheresis donors. BECKMAN COULTER'S experience with this situation reveals that the serum repeat algorithm minimizes this false positive situation. An alternative screening method may be preferred.

XV. LIMITATIONS OF THE PROCEDURE

BECKMAN COULTER PK TP SYSTEM has been shown to be safe and effective for the large scale detection of antibodies to *Treponema pallidum* in serum or EDTA plasma from blood donors when used in accordance with the instructions provided with the kit. Serum must be used for any repeat testing on initially reactive or indeterminate results obtained from plasma samples. As with all serological tests for syphilis, interpretation of results obtained with the BECKMAN COULTER PK TP SYSTEM must take into consideration the donor's history and other clinical and/or laboratory findings.

This product is for use only in screening blood donors and has not been evaluated as a serological test for

syphilis outside the blood bank setting. The product cannot be considered a standard test for syphilis in other test settings. The BECKMAN COULTER PK TP SYSTEM may not be used to monitor the efficacy of therapy or reinfection.

XVI. SPECIFIC PERFORMANCE

The relative sensitivity and specificity of the BECKMAN COULTER PK TP SYSTEM was established by testing 3440 random blood donors on the PK7200 (Tables 3 & 4), and 6095 random blood donors on the PK7300 (Tables 5 & 6).

Random Blood Donors

PK7200 (Tables 3 & 4)

Random blood donor specimens (3,440) were tested using the BECKMAN COULTER PK TP SYSTEM on the PK7200 instrument at two major blood centers in comparison with their test of record for screening blood donors for antibodies to *T. Pallidum* (BECKMAN COULTER PK TP SYSTEM on the PK7100 instrument). Donors who tested initially reactive with plasma samples were tested in duplicate with serum samples. Repeat reactive samples from testing on either instrument were confirmed with either the FTA or MHATP test. An additional twenty-four known reactive samples gave positive results when tested with the PK TP System on the PK7200.

TABLE 3: BECKMAN COULTER PK TP SYSTEM RANDOM DONOR RESULTS: PK7200 & PK7100 INITIAL, SERUM REPEAT AND CONFIRMATORY TESTING

PK TP SYSTEM		NUMBER		FTA/MHATP	
PK7200	PK7100	INITIAL (PLASMA)	REPEAT*	R	N
N	N	3339	3415		
N	R	50	1	0	1
R	N	24	7	2	5
R	R	27	9	6	3
TOTAL		3440	3432 ^a	8	9
PK7200 REACTIVE RATE		1.48%	0.47%	0.23%	
PK7100 REACTIVE RATE		2.24%	0.29%	0.17%	

R REACTIVE & INDETERMINATE

N NONREACTIVE

^a Eight (8) samples could not be retested on the PK7200 and were removed from the analysis. Four (4) were initially reactive on the PK7100 and PK7200. All were nonreactive in duplicate when repeated with serum on the PK7100. They were not retested on the PK7200. Four (4) were nonreactive on the PK7100, initially reactive on the PK7200 but could not be repeated on the PK7200 due to mechanical problems. The samples were not retained for further testing. All four (4) samples occurred on the same day of testing.

* Following serum repeat testing of initial plasma reactives.

TABLE 4: RESULTS OF PK7200 REPEAT TESTING WITH SERUM SAMPLES AND CONFIRMATORY TESTING OF RANDOM DONORS

PK7200 (REPEAT TESTING ON SERUM)	PK7100		REACTIVES AND DISCORDANTS RECONCILED BY FTA-ABS OR MHATP	
	R	N	R	N
REACTIVE	9	7	8	8
NONREACTIVE	1	3415	0	3416
% CONCORDANCE	3424/3432	= 99.8%	3424/3432	= 99.8%
RELATIVE SENSITIVITY	9/10	= 90.0% ^a	8/8	= 100%
RELATIVE SPECIFICITY	3415/3422	= 99.8% ^b	3416/3424	= 99.8% ^c

R REACTIVE & INDETERMINATE

N NONREACTIVE

^a 95% Confidence interval is 0.555-0.97

^b 95% Confidence interval is 0.992-0.998

^c 95% Confidence interval is 0.992-0.998

PK7300 (Tables 5 & 6)

Random blood donor specimens (6095) were tested using the BECKMAN COULTER PK TP SYSTEM on the PK7300 instrument at two major blood centers and at Olympus America, Inc. in comparison with their test of record for screening blood donors for antibodies to *T. Pallidum*. Donors who tested initially reactive with plasma samples were tested in duplicate with serum samples. Repeat reactive samples from either instrument were tested with an approved confirmatory assay for syphilis.

TABLE 5: BECKMAN COULTER PK TP SYSTEM RANDOM DONOR RESULTS: PK7200 & PK7300 INITIAL, SERUM REPEAT AND CONFIRMATORY TESTING

PK TP SYSTEM		NUMBER		FTA/MHATP	
PK7300	PK7200	INITIAL (PLASMA)	REPEAT*	R	N
N	N	6013	6025		
N	R	9	0	0	0
R	N	3	1	0	0
R	R	70**	69**	1***	1
TOTAL		6095	6095	1	1
PK7300 REACTIVE RATE		1.20%	1.15%	0.02%	
PK7200 REACTIVE RATE		1.30%	1.13%	0.00%	

R REACTIVE & INDETERMINATE

N NONREACTIVE

* Repeat testing of initial donor plasma reactives with serum.

** 68 of 69 reactive samples were known positive (FTA-ABS) samples. Repeat testing was not performed.

*** Sample uninterpretable by FTA-ABS. True status not determined.

TABLE 6: RESULTS OF PK7300 REPEAT TESTING WITH SERUM SAMPLES AND CONFIRMATORY TESTING OF RANDOM DONORS

PK7300 (REPEAT TESTING WITH SERUM)	PK7200		REACTIVES AND DISCORDANTS RECONCILED BY FTA-ABS OR MHATP	
	R	N	R	N
REACTIVE	69*	1**	0	1
NONREACTIVE	0	6025	0	6026
% CONCORDANCE	6094/6095 =	99.9%	0/6026 =	100%
RELATIVE SENSITIVITY	69/69 =	100%	0/0 =	100%
RELATIVE SPECIFICITY	6025/6026 =	99.9%	6026/6026 =	100%

* 68 known positive FTA-ABS samples (known positives were not repeated with serum)

** Sample uninterpretable by FTA-ABS

Reproducibility:

PK7200 (Table 7)

The reproducibility of the BECKMAN COULTER PK TP SYSTEM on the PK7200 was evaluated at two blood centers. One center tested 100 nonreactive random donor EDTA specimens and 11 confirmed reactive samples on each of three days. The other center tested 140 nonreactive random donor EDTA specimens and 12 confirmed reactive samples on each of three days. Results are summarized in Table 7.

TABLE 7: REPRODUCIBILITY OF THE BECKMAN COULTER PK TP SYSTEM ON THE PK7200

	n=	CORRELATION			
		DAY 1	DAY 2	DAY 3	TOTAL
REACTIVE	23	100%	100%	100%	100%
NONREACTIVE	240	99.6%	97.5%	97.5%	98.2%
COMBINED REACTIVE & NONREACTIVE	263	99.6%	97.7%	97.7%	98.4%

PK7300 (Table 8a & 8b)

The reproducibility of the BECKMAN COULTER PK TP SYSTEM on the PK7300 was evaluated at two blood centers and also at Olympus America, Inc. All sites tested random EDTA donor samples. Also tested were 3 known non-reactive and 26 known reactive samples. (One site tested only 16 known reactive samples). All samples were tested on days 1-3, 4 and 6. Agreement between instruments for the known samples was 100%. The unknown samples are evaluated for reproducibility on each analyzer for the 3 days tested.

TABLE 8a: REPRODUCIBILITY OF THE BECKMAN COULTER PK TP SYSTEM ON THE PK7300

	n=	DAY 1-3	CORRELATION		
			DAY 4	DAY 6	TOTAL
KNOWN REACTIVE	68	100%	100%	100%	100%
KNOWN NON-REACTIVE	9	100%	100%	100%	100%

TABLE 8b: RATE OF AGREEMENT FOR SAMPLE AGE SUBSET ON THE PK7300

TEST DAY	NUMBER IN AGREEMENT	RATE OF AGREEMENT	LOWER 95% CONFIDENCE BOUND
INITIAL	1134/1137	99.74%	99.32%
DAY 4	1136/1137	99.91%	99.58%
DAY 6	1135/1137	99.82%	99.45%

Due to insufficient sample quantity, not all samples processed in the initial run were retested on Day 4 and Day 6.

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FUJIREBIO INC.
Tokyo, Japan

Customer Service: 800-223-0125
Technical Support: 800-447-5852

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Brea, CA 92821

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