REF B11186

PK7400 TP HA REAGENT
Instructions for Use
Using the Beckman Coulter PK7400 Automated Microplate System

I. INTENDED USE

PK7400 TP HA REAGENT is intended for the qualitative screening of blood and plasma donors for the detection of *Treponema pallidum* IgG and IgM antibodies to syphilis in human serum, EDTA plasma or CPDA plasma[a] using the Beckman Coulter PK7400 Automated Microplate System. This assay is not intended for diagnostic use.

II. SUMMARY OF THE TEST

The identification of *Treponema pallidum* antibodies is useful in the diagnosis of syphilis caused by the microorganisms belonging to the genus *Treponema* and provides epidemiological information on syphilis. Syphilis is typically acquired by sexual contact, although the disease may be transmitted by transfusion of infected blood or by intrauterine infection. The infection is a chronic condition that typically progresses through distinct primary, secondary, tertiary, and quaternary stages of infection. These stages produce diverse clinical symptoms, typically producing initial sores known as chancres, then syphilitic rash, followed by long periods of dormancy. Untreated infection may eventually result in cardiovascular problems and neurosyphilis. The organism cannot be routinely cultured in artificial media, and diagnosis of the infection usually depends on the demonstration of antibodies in the blood, which appear soon after initial infection.

Serological tests for syphilis were first introduced with the development of the nontreponemal complement fixation test by Wasserman in 1906. The nontreponemal tests measure both IgG and IgM anti-lipid antibodies formed in response to lipoidal material released from damaged host cells early in infection and to lipid from the invasive treponeme. Due to the lipidic nature of the antigen or an unusual property of the antibodies, the antigen–antibody reaction remains suspended leading to flocculation, unlike most other serological tests where agglutination or precipitation occurs[1].

Although nontreponemal assays are useful in the diagnosis of syphilis, they are nonspecific which may lead to a significant number of false positives. These false positives require additional testing using a test specific for antibodies to *T.pallidum*. Hemagglutination assays have become widely accepted since their introduction in the 1960's [2,3,4] as either a confirmatory test or as a screening assay. The increasing use of automation has enhanced the use of the test by reducing the time and labor required to perform the assay[5,6]. PK7400 TP HA REAGENT has been developed to provide a consistent, liquid, stable and convenient assay on the Beckman Coulter PK7400 Automated Microplate System.

Test results are provided to the operator in an electronic format which can be printed, stored or transmitted electronically with other test data. Specimens which are repeatedly reactive or indeterminate with the PK7400 TP HA REAGENT are considered to be positive for antibodies to *T.pallidum*. For complete details on the setup and operation of the Beckman Coulter PK7400, refer to the Beckman Coulter PK7400 Instructions for Use.

III. PRINCIPLE OF ASSAY

PK7400 TP HA REAGENT test is based on the principle of agglutination and pattern recognition. The REAGENT uses preserved avian erythrocytes sensitized with extracted antigens of *T. pallidum* (Nichols strain). The EDTA plasma, CPDA plasma or serum test sample is diluted in a SAMPLE DILUENT, which uses absorbents to minimize nonspecific reactions. The REAGENT is added to the diluted test mixture and reactants are allowed to settle in a terraced microplate. Hemagglutination occurs in the presence of *Treponema pallidum* 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 PK7400 reads the settling patterns of the erythrocytes in each well based on the threshold settings chosen for each reagent. The Beckman Coulter PK7400 captures the well images using a CCD (charged couple device) camera and subsequently uses the threshold settings in its algorithm to differentiate agglutinated and non-agglutinated patterns.

IV. REAGENTS

Name	Description	Approximately 3600 Tests per Kit
REAGENT	Avian erythrocytes coated with antigens of *T. pallidum* and suspended in a saline solution containing 0.09% sodium azide	3 bottles containing 45 mL each
SAMPLE DILUENT	Saline solution containing absorbents and 0.09% sodium azide	2 bottles containing 900 mL each
PK REAGENT VIALS	Empty, capped PK REAGENT VIALS, barcode labelled with lot number and expiration, specific per kit	4 empty, barcoded PK REAGENT VIALS

Reagents contain material of animal origin. Any bovine albumin used in the manufacture of this product is sourced from donor animals that have been inspected and certified by Veterinary Service inspectors to be disease free.

V. WARNINGS AND PRECAUTIONS

1. The PK7400 TP HA REAGENT is for *in vitro* diagnostic use only and designed for use only with the PK7400 TP HA CONTROLS.
2. REAGENT and SAMPLE DILUENT contain sodium azide (< 0.1% w/v) as a preservative, which can accumulate in lead or copper pipes to form potentially explosive azides. To prevent azide build-up, flush with large volumes of water after disposing of solutions containing azide in the sink.
3. Do not freeze REAGENT or SAMPLE DILUENT.
4. REAGENT must be thoroughly re-suspended prior to decanting into the PK REAGENT VIALS. Failure to do so could result in an inadequate dilution and erroneous results.
5. REAGENT erythrocytes should be covered by suspension medium during storage, where this has not been the case then erythrocytes should be re-suspended. See section VI Reagent Preparation step 3. Failure to do so could result in clumping in the test well image observed during microplate review when performed as described in the Beckman Coulter PK7400 Instructions for Use.
6. REAGENT from the same lot may be pooled using good laboratory practices. See Section VII. Storage for additional storage and pooling directions.
7. Clean pipettes should be used to transfer all reagents between the REAGENT bottle and the PK REAGENT VIALS. The PK REAGENT VIALS are barcode labeled for a specific PK7400 TP HA REAGENT lot number. Use only the PK REAGENT VIALS designated for the accompanying PK7400 TP HA REAGENT lot number.
8. REAGENT or SAMPLE DILUENT showing visible signs of microbial growth or gross turbidity may indicate degradation and should be discarded according to local rules.
9. The effects of microbial contamination in specimens cannot be predicted.
10. Do not use reagent, diluent, or controls after the expiration date.
11. PK7400 TP HA CONTROLS, (REACTIVE and NONREACTIVE CONTROLS) should be handled the same as donor samples.
12. Clean, dry microplates must be used for testing. Improper washing of the microplate can adversely affect the test results. The recommended washing procedure is found in the Beckman Coulter PK7400 Instructions for Use.
13. Samples exhibiting gross lipemia, hemolysis or icterus may be compromised and may require alternative testing. Samples containing hemoglobin >5g/L could produce erroneous results.
14. When a specimen fails to be added by the PK7400 instrument, the potential for a false negative exists.
15. Carryover between specimens is a potential source of interference.
16. Deviations from the Beckman Coulter PK7400 Instructions for Use can lead to erroneous results.
17. Deviations from the PK7400 TP HA REAGENT or the PK7400 TP HA CONTROLS Instructions for Use can lead to erroneous results.
18. Dispose of leftover REAGENT and SAMPLE DILUENT in a safe manner, in accordance with local regulations.

VI. REAGENT PREPARATION

1. Equilibrate all reagents, diluent, controls and specimens to room temperature (18 – 28°C) before use.
2. SAMPLE DILUENT is ready to use in a barcoded PK diluent bottle.
3. Ensure REAGENT is thoroughly re-suspended by gentle hand mixing prior to decanting into the PK REAGENT VIALS. Do not vortex.
4. Once REAGENT has been decanted into PK REAGENT VIALS, it is stable for 5 days when stored upright at 2–8°C in capped PK REAGENT VIALS.
5. Ensure REAGENT is thoroughly re-suspended by hand prior to placing an open PK REAGENT VIAL back on the Beckman Coulter PK7400 instrument. Select MIX REAGENTS (F6) from the ANALYZER STATUS screen to mix the reagents as soon as they are placed on the PK7400.

VII. STORAGE

1. REAGENT and SAMPLE DILUENT must be stored in an upright position at 2–8°C. Do not freeze REAGENT or SAMPLE DILUENT.
2. After opening, REAGENT and SAMPLE DILUENT are stable, in their primary bottles, for up to 2 months when stored at 2–8°C in an upright position.
3. REAGENT that has been decanted into PK REAGENT VIALS is stable for up to 5 days from the first date the REAGENT was decanted into PK REAGENT VIALS, when stored in the capped PK REAGENT VIAL, in an upright position, at 2–8°C. Do not return decanted REAGENT back into the primary bottles.

4. Do not use after the expiration date.

5. After placing on the PK7400, REAGENT and SAMPLE DILUENT can be left on board the instrument for up to 12 hours. If the REAGENT is left on the analyzer without the reagent tray mixing, completely resuspend the REAGENT in the vial before commencing testing again. Reagent left at room temperature for longer than 12 hours should be discarded.

VIII. SPECIMEN COLLECTION AND PREPARATION

PK7400 TP HA REAGENT may be used for testing with either human serum, EDTA plasma or CPDA plasma specimens on the Beckman Coulter PK7400 instrument for up to 7 days after collection. Specimens should be free of particulate matter to prevent interference with the assay result. If erythrocytes or other visible components are present in the specimen, remove by centrifugation to prevent interference with the test results. The Beckman Coulter PK7400 Instructions for Use require centrifugation of specimens 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, specimens tested only using the PK7400 TP HA REAGENT do not need to comply with these requirements as long as the plasma or serum is free from particulate matter. Store EDTA plasma, CPDA plasma and serum specimens at 2-8°C up to 7 days. EDTA plasma, CPDA plasma and serum specimens can be frozen at less than -20°C for up to one month, thawed and mixed thoroughly prior to testing. Specimens may be frozen and thawed up to 5 times.

Allow all specimens to equilibrate to room temperature (18 – 28°C) before use.

IX. MATERIALS

Materials provided:	REAGENT SAMPLE DILUENT Capped PK REAGENT VIALS – barcoded
Materials required but not provided:	Beckman Coulter 16µm terraced microplates P3 or P4 Beckman Coulter PK7400 Automated Microplate System PK7400 TP HA CONTROLS (Ref B11187)

X. DIRECTIONS FOR USE

The Beckman Coulter PK7400 is a programmable instrument whose operation is controlled by software. Instrument parameters have been validated by the manufacturer and are incorporated into the operating files. The operator may define panel (test) configurations. For more information about this process, please consult the Beckman Coulter PK7400 Instructions for Use.

RECOMMENDED PARAMETERS

Newmarket Biomedical has established parameters for the Beckman Coulter PK7400 by application development testing with characterized samples. The parameters are listed in the tables below.

REAGENT NAME	TPHA
DILUENT NAME	TPDIL
REAGENT PARAMETERS	
REAGENT VOLUME	35µl
SAMPLE	PLASMA or SERUM
DILUENT	TPDIL
SAMPLE/DILUENT RATIO	7.2
DILUTED SAMPLE VOLUME	20µl
MICROPLATE WELL	16µm
REACTION TIME	60 min.
THRESHOLDS	
DYNAMIC RANGE SET	
P DYNAMIC RANGE	Low 0, High 99
C DYNAMIC RANGE	Low 0, High 99
LIA DYNAMIC RANGE	Low 0, High 950
SPC DYNAMIC RANGE	Low 0, High 53
THRESHOLD SET	
SPC THRESHOLD	Low 16, High 16
P/C THRESHOLD	(+ Limit) 45, (- Limit) 26
LIA THRESHOLD	(+ Limit) 250, (- Limit) 100
LIMIT SET	
BG/C LIMIT	Low
LIA SELECTION	5
PANEL DETAILS	
REAGENT MIX	ON
PLATE MIXING METHOD	Air or Vibration

Preparation Procedure for PK7400 TP HA

1. Thoroughly resuspend the REAGENT and decant into the accompanying PK REAGENT VIAL, confirming the lot number on the PK REAGENT VIAL and the REAGENT bottle match. The maximum volume allowable in the PK REAGENT VIAL is 20 mL, a red guideline is provided on the vial to indicate a volume of 18mL. Thoroughly resuspend the REAGENT in the PK REAGENT VIAL. Place the PK REAGENT VIAL in the appropriate slot in the reagent tray. Load the reagent tray on to the Beckman Coulter PK7400 instrument and press the MIX REAGENTS (F6) from the ANALYZER STATUS screen, to mix the REAGENTS. If the REAGENT is left on the analyzer without the reagent tray mixing, resuspend the REAGENT in the vial before commencing testing again.

2. Set the diluent bottle in the diluent bottle set-up unit. Place the appropriate diluent tube into the SAMPLE DILUENT bottle provided in this kit.

3. Perform VERIFY REAGENTS (F2), VERIFY DILUENTS (F3), and PRIME and WASH (F4) from the REAGENT AND DILUENT STATUS screen as defined in the Beckman Coulter PK7400 Instructions for Use.

4. PK7400 TP HA CONTROLS, REACTIVE and NONREACTIVE CONTROLS (Ref B11187) must be run at the beginning and end of each test run and test as expected. For more information about this process, please consult the PK7400 TP HA CONTROLS Instructions for Use.

5. Proceed with sample analysis as described in the Beckman Coulter PK7400 Instructions for Use.

XI. QUALITY CONTROL

The PK7400 TP HA CONTROLS (Ref B11187) must be tested at the beginning and the end of each batch of samples assayed, after the addition of reagents and after interruptions or delays in processing. Additional QC testing may be performed by the operator by the inclusion of other characterised specimens or reference material.

The REACTIVE CONTROL should produce a positive (+) result and the NONREACTIVE CONTROL should produce a negative (-) result with the test. If the appropriate results are not obtained with the controls, the assay is considered invalid and all samples within that batch should be retested. Where control material repeatedly fails to perform as expected contact your local Beckman Coulter Representative.

XII. INTERPRETATION

The presence or absence of *T.pallidum* antibody is determined by the Beckman Coulter PK7400 instrument which analyzes and differentiates agglutinated and non-agglutinated patterns. The Beckman Coulter PK7400 instrument uses three assessment parameters for each microplate well;

SPC	Sharpness at the edge of the cell button at the border of the peripheral (P) and central (C) parts of the well
LIA	Low intensity area which indicates the size and density of the cell button
P/C	Ratio of transmitted light between the peripheral (P) and central (C) parts of the well

SPC, P/C, and LIA work in conjunction to determine a reaction interpretation. The following threshold values define the interpretation method of SPC, P/C, and LIA.
- Threshold Set (High)
- Threshold Set (Low)
- Threshold Set (+ Limit)
- Threshold Set (– Limit)

The system determines the reaction interpretations for each reagent from the values for SPC, P/C, and LIA. The following figures show the reagent interpretation conditions.

SPC

+	?	-
Less than 16	**16**	**Greater than 16**

P/C and LIA

Result	**+**	**x**	**-**
	1) Positive Readings	**x**	
		x	2) Negative Readings

Limits	**(-)**	**(+)**
P/C	**26**	**45**
LIA	**100**	**250**

The system uses SPC, P/C and LIA readings in the determination of a reagent result. SPC is the predominant value used to establish pattern interpretation with LIA and P/C used in support of SPC. However, it does not use P/C and LIA readings that fall within the range (x) between the two limits for the reagent result determination. The following table shows this relationship.

Reagent Result Interpretation Relationships

Reagent Result Interpretation	SPC	P/C	LIA
+	+	+ or x	+ or x
-	-	- or x	- or x
?	?	+ or - or x	+ or - or x
	Any combination of results other than the preceding defined results		

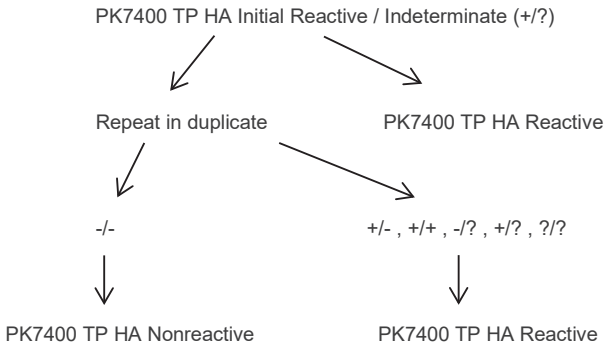
XIII. INTERPRETATION OF RESULTS

A sample which gives a reagent result interpretation of nonreactive (-) should be considered as negative for *T.pallidum*.

A sample which gives a reagent result interpretation of reactive (+) or indeterminate (?) in initial screening is considered as initially reactive and may be repeated in duplicate using the plasma and/or serum sample from the same donation. Results obtained with serum or plasma are of equal validity and repeat test may use either sample type.

If either repeat duplicate is reactive or indeterminate then the sample is determined as repeatedly reactive for *T.Pallidum* antibodies.

If both duplicate repeats are nonreactive then the sample is determined as nonreactive.



XIV. EXPECTED VALUES

A sample reported as reactive by PK7400 TP HA REAGENT is considered to be reactive for IgG and/or IgM antibodies to *T.pallidum*. Reactive results may indicate active, past or successfully treated syphilis infections.

XV. LIMITATIONS OF PROCEDURE

PK7400 TP HA REAGENT may be used for serum, EDTA plasma or CPDA plasma samples.

The assay is not intended for diagnostic use.

XVI. SPECIFIC PERFORMANCE

Negative Percent Agreement

A total of 6663 random blood donor specimens (3474 EDTA and 3189 serum) were tested using the PK7400 TP HA Reagent on the PK7400 Automated Microplate System at three major blood donor centers in the US in comparison with the Beckman Coulter PK TP system on the PK7300, their test of record for screening blood donors for antibodies to *T. pallidum.* Donor samples which tested initially reactive were retested in duplicate in both methods. Repeat reactive samples in either method were tested with FTA-ABS as a confirmatory assay. Samples that were nonreactive on repeat testing were considered true negatives for the purposes of calculating agreement.

Initial TP HA results comparison – EDTA plasma

	PK7400		
PK7300	**IR**	**NR**	**Total**
IR	0	5	5
NR	1	3468	3469
Total	1	3473	3474

Initial TP HA results comparison – serum

	PK7400		
PK7300	**IR**	**NR**	**Total**
IR	0	1	1
NR	1	3187	3188
Total	1	3188	3189

Statistical summary by sample type - Initial TP results

Sample	All Sites	Agreement N=	Total N =	ROA	LCB
Plasma	NPA	3468	3469	99.97%	99.84%
Serum	NPA	3187	3188	99.97%	99.83%
Combined	NPA	6655	6657	99.97%	99.89%

Repeat TP HA results comparison – EDTA plasma

	PK7400		
PK7300	**RR**	**NR**	**Total***
RR	0	0	0
NR	1	3472	3473
Total	1	3472	3473

* Repeat testing was not performed for one IR sample on PK7300

Repeat TP HA results comparison – serum

	PK7400		
PK7300	**RR**	**NR**	**Total**
RR	0	0	0
NR	1	3188	3189
Total	1	3188	3189

Statistical summary by sample type – after repeat testing

Sample	All Sites	Agreement N=	Total* N =	ROA	95% CI
Plasma	NPA	3472	3473	99.97%	99.84 – 100.0%
Serum	NPA	3188	3189	99.97%	99.83 – 100.0%

* Repeat testing was not performed for one IR sample on PK7300

PK7400 TP HA Reagent comparison to clinical status for EDTA plasma

	PK7400		
Clinical status	**R**	**NR**	**Total***
R	0	0	0
NR	1	3472	3473
Total	1	3472	3473

* Repeat testing was not performed for one IR sample on PK7300

PK7400 TP HA Reagent comparison to clinical status for serum

	PK7400		
Clinical status	**R**	**NR**	**Total**
R	1	0	1
NR	0	3188	3188
Total	1	3188	3189

Statistical summary by sample type against clinical status — after repeat testing

Sample	All Sites	Agreement N=	Total* N =	ROA	95% CI
Plasma	Specificity	3472	3473	99.97%	99.84 – 100.0%
Serum	Specificity	3188	3188	100.0%	99.88 – 100.0%

* Repeat testing was not performed for one IR sample on PK7300

The rate of indeterminate (?) results for this study was 0.015% (1/6663) on initial testing. The sample gave a repeat reactive result when retested in duplicate.

Positive Percent Agreement

Due to the low prevalence of TP reactive samples in the blood donor population, a panel of 826 commercially sourced, known TP positive samples (382 serum, 205 EDTA plasma and 239 CPDA plasma) were tested using the PK7400 TP HA Reagent on the PK7400 Automated Microplate System at Newmarket Biomedical in comparison with the Beckman Coulter PK TP system on the PK7300. Samples which tested initially reactive were retested in duplicate on the PK7400. The true clinical status for the commercially obtained syphilis positive samples was presumed to be that defined by the vendor assay results. In the case of discordant or concordant negative results, confirmatory testing was performed using Syphilis Total Ab EIA.

Agreement matrix for Initial testing on known serum positive samples

	PK7400		
PK7300	**IR**	**NR**	**Total**
IR	381	1	382
NR	0	0	0
Total	381	1	382

Agreement matrix for Initial testing on known CPDA plasma positive samples

	PK7400		
PK7300	**IR**	**NR**	**Total**
IR	239	0	239
NR	0	0	0
Total	239	0	239

Agreement matrix for Initial testing on known EDTA plasma positive samples

	PK7400		
PK7300	**IR**	**NR**	**Total**
IR	204	0	204
NR	0	1	1
Total	204	1	205

Positive percent agreement of TP HA assay on PK7400 *vs* PK7300 on Initial testing

Stratified sample group	Point estimate	95% CI
Serum	99.74%	98.55 – 99.99%
CPDA	100.0%	98.47 – 100.0%
EDTA	100.0%	98.21 -100.0%
All samples	**99.88%**	**99.33 – 100.0%**

PK7400 TP HA Reagent results comparison to clinical status for known serum positive samples after repeat testing

	PK7400		
Clinical status	**R**	**NR**	**Total**
R	381	1	382
NR	0	0	0
Total	381	1	382

PK7400 TP HA Reagent results comparison to clinical status for known CPDA plasma positive samples after repeat testing

	PK7400		
Clinical status	**R**	**NR**	**Total**
R	239	0	239
NR	0	0	0
Total	239	0	239

PK7400 TP HA Reagent results comparison to clinical status for known EDTA plasma positive samples after repeat testing

	PK7400		
Clinical status	**R**	**NR**	**Total**
R	204	1	205
NR	0	0	0
Total	204	1	205

Sensitivity of PK7400 TP HA Reagent after repeat testing

Stratified sample group	Point estimate	95% CI
Serum	99.74%	98.55 – 99.99%
CPDA	100.0%	98.47 – 100.0%
EDTA	99.51%	97.31 – 99.99%
All samples	**99.76%**	**99.13 – 99.97%**

The rate of indeterminate (?) results for this study was 0.12% (1/826) on initial testing.

Limit of Detection

The PK7400 TP HA Reagent has an expected limit of detection of ≤0.1 IU/mL against the WHO 1st IS for human syphilitic plasma IgG and IgM NIBSC code:05/132.

Prozone effect

PK7400 TP HA Reagent is not affected by prozone at high levels of TP antibodies up to 100 IU/mL.

Microbial interferences

Interference studies were conducted using recommendations contained in CLSI standard EP7-A2. Five TP-positive and five TP-negative plasma samples were inoculated with a mixed suspensions of *Candida sp., Escherichia coli, Pseudomonas sp., Staphylococcus sp*. and *Aspergillus sp*. TP-positive samples were generated by spiking normal plasma samples with a known syphilis positive serum at a level of ~0.7 IU/mL. Inoculated and un-inoculated controls were tested in triplicate at 16 days post inoculation using three lots of PK7400 TP HA reagents.

Results demonstrated 100% concordance between all triplicate runs, indicating that there was no interference from the mixed suspension of organisms listed at 16 days post inoculation.

Precision

Lot to lot variability was assessed with three assay lots at one site using a panel of 826 positive and 91 negative samples

Precision study—rate of agreement

		Agreement N=	Total N=	ROA (95%, 2-sided LCI)	Mean	SD	CV (%)
PPA	Lot 1	824	826	99.76% (99.12%)	99.80%	0.069	0.07
	Lot 2	825	826	99.88% (99.32%)			
	Lot 3	824	826	99.76% (99.12%)			
NPA	Lot 1	91	91	100% (96.27%)	100%	-	-
	Lot 2	91	91	100% (96.27%)			
	Lot 3	91	91	100% (96.27%)			

Reproducibility

Assay reproducibility was assessed at three US blood donor centers using a characterized, mixed titer panel comprising 25 syphilis positive and 5 syphilis negative samples. The panel was tested using multiple Reagent lots on 5 testing days over a 7 day period, in duplicate with two separate runs on each testing day.

Reproducibility Study – rate of agreement

Samples	Agreement N=	Total N=	Rate of Agreement	95% CI
Syphilis positive	1500	1500	100.00%	99.75 - 100%
Syphilis negative	300	300	100.00%	98.78 - 100%
Overall	1800	1800	100.00%	99.80 - 100%

Cross reactivity

A total of 121 samples which were positive for potential cross reactants were tested in the PK7400 TP HA Reagent on the PK7400 Automated Microplate System at Newmarket Biomedical in comparison with the Beckman Coulter PK TP system on the PK7300. Samples which tested initially reactive were retested in duplicate according to the instructions in the product IFUs. Repeat reactive samples in either method were tested using a Syphilis Total Ab EIA.

List of potential cross reacting samples

Potential cross reactant	No of samples
Toxoplasma	10
Rubella IgG	10
Lyme disease (*Borrelia sp.*)	10
SLE	10
Rheumatoid Factor	10
Epstein Barr virus (EBV)	10
HCV	10
HIV 1/2	10
HTLV	10
HBsAg	10
HAV	11
Pregnant and Multiparous	10
Total	121

PK7400 TP HA Reagent comparison to clinical status for potential cross reactants

	PK7400		
Clinical status	**RR**	**NR**	**Total**
R	1*	0	1
NR	1**	119	120
Total	2	119	121

*HIV positive sample co-infection with syphilis
**Rubella sample

Samples previously tested for cross reactivity in the PK7400 TP HA Reagent assay were also spiked with TP antibodies using a known positive sample. One sample from the Rubella group was excluded from the additional study as this sample had previously shown a positive result in the PK7400 assay.

22 real samples which were supplied certified as positive for both HIV and syphilis were included. A total of 132 samples were tested. 100% concordance was observed.

PK7400 TP HA and clinical status	**Point estimate**	**95% Confidence Interval**
NPA	99.2% (120/121)	95.5 – 100%
PPA	100% (132/132)	97.24-100%

Mixing mode equivalence

Air mix and vibration mix modes for the PK7400 were compared using a panel of 603 known TP positive samples with mixed titers (serum and plasma) and 100 known TP negative samples (serum and plasma).

	PK7400 vibration mix mode		
PK7400 air mix mode	**IR**	**NR**	**Total**
IR	602	0	602
NR	1[b]	100	101
Total	603	100	703

Concordance of the PK7400 TP HA Reagent between mixing Modes

Sample group	**Point estimate**	**95% CI**
Known positives	99.83%	99.08 – 100.0%
Known negatives	100.0%	96.38 – 100.0%

Analytical interference

Maximum levels of interferents tested with no interference

Substance	Maximum concentration
Human serum albumin (HSA)	120g/L
Bilirubin	0.342mmol/L
Conjugated bilirubin	0.342mmol/L
Triglyceride	37mmol/L
Intralipid	1000mg/dL
Hemoglobin	5g/L

XVII FOOTNOTES

a. Citrate Phosphate Dextrose Adenine plasma

b. Discordant sample was close to assay cut off threshold and performed inconsistently across 3 Reagent lots. Results from a single lot have been used as representative.

XVIII REFERENCES

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XIX KEY TO SYMBOLS

REF	Catalogue number
IVD	IVD In Vitro Diagnostic Medical Device
	Manufactured by
EC REP	Authorized representative in the European Community
	Temperature limitation
	Use by
LOT	Batch code
	Consult instructions for use
Rx only	Prescription device symbol (US)

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