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SUBJECT **Product Launch**
Syphagen TPHA AUTO REC plus

We are pleased to introduce the first TPHA on the market to be used in the Olympus pk-7200™ with **recombinant antigens**.

Until now our kits have been based on natural antigens from *Treponema pallidum*. These antigens have been obtained from testicular rabbit tissue culture. In spite of all the purification steps, some rabbit tissue debris can remain with the treponemal antigens and occasionally can produce non-specific results.

The new **Syphagen TPHA AUTO REC plus** reagent is a suspension of stabilised chicken erythrocytes coated with **p15, p17 and p47** *T. pallidum* antigens obtained from cellular culture of recombinant *Escherichia coli* and subsequent purification. This gives the test high specificity, since cross-reactions due to other saprophytic or pathogen treponema can be avoided without decreasing the test's sensitivity. We can also prevent any possibility of a cross-reaction against rabbit antigens.

In combination with these three recombinant antigens, another recombinant protein has been added to increase the test's sensitivity. **This fourth recombinant protein is going through the Biokit patent process.**



Kit composition

REF **3000-5709.** Kit for 5000 test.

a) Antigen reagent: 5 x 40 ml

Suspension of chicken erythrocytes sensitised with recombinant antigens.
Ready to use.

b) Diluent solution: 5 x 500 ml

Phosphate buffered saline solution containing soluble components of T. Reiter and stabilising agents.

c) Positive control: 1 x 15 ml

Immune rabbit serum.

d) Negative control: 1 x 15 ml

Diluted normal human serum.

Note: Negative control has been included in this new reagent kit, at the suggestion of several current users of Syphagen TPHA AUTO.

Olympus PK7200 application for this new product:

Sample volume μl		35	
Diluent volume μl		360	
Diluted sample volume		15	
Reagent volume		35	
Threshold	Dynamic range set	P	1-99
		C	1-99
		LIA	0-999
	Threshold set	SPC	12-13
		P/C	17-23
		LIA	75-205
	Limit set	BG/C	Middle
LIA selection	6		

Evaluations:

We herein attach two evaluations that show the performance of this new product:

1. The first is a specificity study performed in “Banc de Sang de la Vall d'Hebron - Barcelona” by Dr J.M. Hernandez. The number of screened samples was 3601 and the specificity result was 99.86%.

*Note: The Transfusion Centre and Tissue Bank “**Banc de Sang de la Vall d'Hebron - Barcelona**” is a public enterprise housed in the Vall d'Hebron complex, providing referral health services to all of Catalonia and Spain.*

2. The second is a comparative study between our new kit and the AUTO kit for the Olympus instrument by Plasmatec.

The results and conclusions were as follows:

- 2.1. 446 routine plasma samples were screened in parallel fashion. Plasmatec reported 1 false positive.
- 2.2. Our new kit showed 1.5 dilutions more sensitive than Plasmatec using the WHO standard and its dilutions.
- 2.3. Using a panel of well-known positive samples, Plasmatec reported 1 false negative and 1 indeterminate.



Specificity study of SYPHAGEN TPHA AUTO REC PLUS (Biokit)

AIM

The purpose of this study was to assess the specificity of the new kit syphagen TPHA AUTO rec plus, hemagglutination test based on recombinant antigens produced by Biokit for the detection of antibodies to *Treponema pallidum*.

MATERIALS AND METHODS

3.601 routine plasma samples from unselected blood donors were analysed with syphagen TPHA AUTO rec plus.

The tests have been automatically performed in an Olympus PK 7200 analyzer.

All the initially reactive samples have been tested for confirmation with the manual techniques syphagen TPHA from Biokit, Serodia TP-PA from Fujirebio and FTA-Abs.

RESULTS

Samples tested	Initially reactive	Confirmed positive	Specificity
3601	13	8	99.86% (3588/3593)

From 3601 samples assayed with syphagen TPHA AUTO rec plus 5 confirmed false positive samples were found

CONCLUSION:

- The specificity obtained for the kit syphagen TPHA AUTO rec plus in this study was 99.86%.
- The kit syphagen TPHA AUTO rec plus has been found to have a satisfactory performance that is appropriate for use in blood banks syphilis screening.

Barcelona, 15th July 2004

Dr. J.M. Hernández
Laboratory Manager



EVALUATION OF THE PERFORMANCE OF THE NEW SYPHAGEN TPHA AUTO REC PLUS ASSAY OF BIOKIT vs THE PLASMATEC TPHA AUTO IN AN OLYMPUS PK7200

Introduction

This study compares the performance between the Syphagen TPHA Auto Rec Plus Assay of Biokit and the TPHA Test Kit of Plasmatec for the detection of antibodies to Treponema pallidum, working both in the Olympus PK7200 auto analyzer System.

Materials and Methods

Diagnostic methods

1. Syphagen TPHA Auto Rec Plus, lot:070504AUTO, Exp:May-05, Biokit.
2. TPHA Test Kit, Reference: TPHA/200PK, lot:332101, Exp:2005-05, Plasmatec
3. To resolve discrepancies, the following additional tests have been used, all of them as manual assays:
 - Syphagen TPHA Rec Plus, lot:110304L, Exp:2005-03, Biokit S.A.
 - TPHA Test Kit, Ref:TPHA/020, lot:098034, Exp:2005-09, Plasmatec Laboratory Products Ltd.
 - Syphilis (TPHA), Ref:541481, lot:026088, Exp:2005-08, Randox Laboratories Ltd.
 - SERODIA TP-PA, Ref:6391, lot:WN31106, Exp:Nov-04, Fujirebio Inc.

All assays have been performed as recommended by the manufacturer.

Samples

The following samples have been used in this study:

1. The International Standard for Syphilitic Human Serum (1st preparation) from the Staten Seruminstitut (WHO International Laboratory for Biologicals Standards), with a contain of 4,9 International Units (IU) of Syphilitic Human Serum
2. 24 positive samples from the Syphilis QC Panel of Biokit S.A.
3. 446 plasmas of blood donors from the Vall Hebron Hospital Blood Bank in Barcelona.

Instrument

The comparative study has been performed in the Analyzer Olympus PK7200.

A CH-Werfen Company

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Results

Standard for Syphilitic Human Serum (WHO)

The Standard for Syphilitic Human Serum (WHO) sample with a contain of 4.,9 IU of Syphilitic Human Serum has been reporting Positive until dilution 1/75 with the Syphagen TPHA Auto Rec Plus and until dilution 1/25 with the TPHA Test Kit in the Olympus PK7200.

	Cut-off WHO dilution
Syph TPHA Auto Rec Plus BIOKIT	1/75
TPHA Test Kit PLASMATEC	1/25

These results indicate that the Syphagen TPHA Auto Rec Plus of Biokit is one and a half dilution more sensitive than the TPHA Test Kit of Plasmatec in front of this Standard.

Positive Serum Samples

		Syph TPHA Auto Rec Plus BIOKIT			TPHA Test Kit PLASMATEC		
		POS	NEG	?	POS	NEG	?
REF	POS	24	0	0	22	1	1
	NEG	0	0	0	0	0	0

All of 24 positive serum samples were Positive (P) with the Biokit Syphagen TPHA Auto Rec Plus Assay with the set parameter numbers clearly reporting positive results, whereas two of them gave a Negative (N) and an Indeterminate (?) result with the Plasmatec TPHA Test Kit Assay with the LIA and P/C parameters numbers far of the positive threshold report result. (See raw data in Annex 1)

Blood Bank Donor Plasmas

		Syph TPHA Auto Rec Plus BIOKIT			TPHA Test Kit PLASMATEC		
		POS	NEG	?	POS	NEG	?
POS	0	0	0	0	0	0	
NEG	0	446	0	1	445	0	

See raw data in Annex 2.

The discrepant plasma (sample 285 in the raw data) has been tested with the manual tests, with following results:

	Antigen Reagent	Control Reagent
Syphagen TPHA Rec Plus BIOKIT	NEG	NEG
TPHA Test Kit PLASMATEC	POS	POS
Syphilis (TPHA) RANDOX	POS	POS
SERODIA TP-PA FUJIREBIO	NEG	NEG

These results indicate that it is a false positive result of the Plasmatec Assay related to a cross reaction with the red cells of the reagents. The Randox Test also gives a false positive result for this sample.

Conclusions

The results obtained in this study demonstrate that:

1. The Syphagen TPHA Auto Rec Plus of Biokit is a sensitive and specific test for the detection of antibodies to *Treponema pallidum*.
2. The sensitivity and specificity of the Syphagen TPHA Auto Rec Plus test are superior to TPHA Test Kit of Plasmatec ones.



16/7/04
Teresa Llop
Latex, I+D Dept.
Biokit, S.A.

Annex 1

24 Positive Serum Samples Raw Data

Sample	Syphagen TPHA Auto Rec Plus BIOKIT				TPHA Test Kit PLASMATEC			
	thresholds 17-23	thresholds 12-13	thresholds 75-205	Assay	thresholds 26-41	thresholds 16	thresholds 100-240	Assay
	P/C	SPC	LIA	Results	P/C	SPC	LIA	Results
SP1	11	1	0	P	12	2	0	P
SP2	11	1	0	P	12	1	0	P
SP3	11	1	0	P	12	2	0	P
SP4	11	1	0	P	12	1	0	P
SP5	11	1	0	P	11	1	0	P
SP6	11	1	0	P	12	1	0	P
SP7	11	2	0	P	13	2	0	P
SP8	11	1	0	P	11	2	0	P
SP9	21	8	176	P	30	16	410	?
SP10	11	2	0	P	13	2	0	P
SP11	13	2	0	P	34	17	579	N
SP12	11	1	0	P	16	3	0	P
SP13	11	1	0	P	12	2	0	P
SP14	11	1	0	P	12	2	0	P
SP15	11	1	0	P	11	1	0	P
SP16	11	2	0	P	11	1	0	P
SP17	12	2	0	P	11	1	0	P
SP18	11	1	0	P	11	1	0	P
SP19	12	2	0	P	11	1	0	P
SP20	12	2	0	P	11	1	0	P
SP21	11	1	0	P	12	2	0	P
SP22	11	1	0	P	13	2	0	P
SP23	11	1	0	P	11	1	0	P
SP24	11	1	0	P	11	1	0	P

Annex 2

Blood Bank Donor Plasma Samples Raw Data.

#	BIOKIT			PLASMATEC			#	BIOKIT			PLASMATEC		
	17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA		17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA
1	34	30	577	36	26	600	47	34	29	544	34	26	517
2	34	27	578	35	27	559	48	34	28	558	34	26	528
3	34	28	589	35	25	584	49	34	28	572	35	24	562
4	34	28	594	35	26	584	50	34	28	567	35	25	533
5	34	28	579	35	28	584	51	34	28	564	35	26	551
6	34	30	596	35	28	583	52	34	27	585	35	23	579
7	34	30	612	35	26	626	53	34	25	572	35	26	561
8	34	29	584	36	29	596	54	34	28	564	35	26	540
9	34	29	584	35	27	593	55	34	28	551	35	26	560
10	34	26	570	35	25	571	56	34	29	559	34	25	510
11	34	28	569	35	26	584	57	34	28	549	35	27	564
12	34	29	578	35	27	553	58	34	28	557	35	27	534
13	34	26	590	35	26	528	59	34	28	567	35	26	544
14	34	27	560	35	27	546	60	30	17	533	35	26	552
15	34	28	554	35	26	552	61	34	28	564	35	27	538
16	32	25	520	35	24	610	62	34	28	569	35	26	571
17	34	30	571	35	26	561	63	34	28	575	35	26	577
18	34	28	582	35	26	536	64	34	28	561	35	26	562
19	33	27	549	34	26	541	65	34	28	563	35	24	530
20	34	28	586	35	26	573	66	34	28	556	35	28	563
21	34	27	592	35	26	568	67	34	28	531	35	26	559
22	31	21	563	35	26	593	68	34	27	537	35	27	569
23	34	28	590	35	27	577	69	34	28	566	35	24	559
24	34	29	577	35	26	582	70	34	27	555	35	26	539
25	34	29	566	35	26	580	71	34	27	564	35	26	534
26	34	28	581	35	26	561	72	34	28	549	35	26	510
27	34	29	565	35	26	567	73	34	28	568	35	26	554
28	34	28	558	35	28	561	74	34	29	565	35	26	561
29	34	27	562	35	26	538	75	34	28	557	35	26	545
30	34	26	588	35	26	517	76	34	28	532	35	26	519
31	34	27	543	35	27	531	77	34	27	548	35	26	522
32	34	27	565	35	26	548	78	34	29	560	35	27	552
33	34	28	556	35	27	534	79	34	27	538	34	26	517
34	34	28	574	35	25	551	80	34	27	569	34	25	515
35	34	28	570	35	26	555	81	34	26	577	35	25	535
36	34	28	553	35	27	546	82	34	27	589	35	26	523
37	34	29	566	35	26	520	83	34	28	575	35	27	545
38	34	29	556	35	27	527	84	34	28	564	34	24	561
39	33	27	544	35	25	530	85	34	29	573	35	27	547
40	34	28	590	35	26	540	86	34	29	562	35	26	524
41	34	28	567	35	25	539	87	34	28	569	35	26	517
42	33	27	529	35	25	522	88	34	29	572	35	27	518
43	33	26	544	35	26	532	89	34	28	574	35	26	582
44	33	28	539	34	26	528	90	34	28	565	35	26	567
45	34	27	558	35	27	541	91	34	28	565	35	26	530
46	34	29	558	34	25	526	92	34	28	555	35	27	584

#	BIOKIT			PLASMATEC			#	BIOKIT			PLASMATEC		
	17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA		17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA
93	34	27	548	35	26	555	145	34	28	574	35	26	553
94	34	28	535	35	26	556	146	32	26	543	35	26	515
95	34	27	558	35	26	542	147	34	27	557	35	28	579
96	34	28	552	35	27	555	148	34	28	549	35	26	546
97	34	29	553	35	27	537	149	34	28	571	35	26	565
98	34	28	542	35	27	532	150	34	28	565	35	25	557
99	32	27	531	34	23	542	151	34	28	562	35	27	542
100	32	25	553	35	25	565	152	34	28	572	35	26	556
101	33	28	549	35	26	541	153	34	30	589	35	27	587
102	34	27	562	35	26	546	154	34	28	571	34	24	547
103	34	27	554	35	27	581	155	34	29	581	35	27	572
104	33	28	538	35	27	565	156	34	28	569	35	26	557
105	33	28	540	35	27	596	157	32	26	544	34	26	543
106	33	28	535	35	26	543	158	34	28	561	35	27	563
107	34	28	562	34	26	552	159	32	27	533	35	26	576
108	33	28	539	34	26	564	160	34	28	548	35	26	533
109	34	28	556	35	26	585	161	34	28	572	35	26	569
110	34	28	560	35	26	575	162	34	28	570	35	26	566
111	34	26	584	35	25	599	163	34	28	574	35	25	592
112	34	28	569	35	26	585	164	34	29	608	35	26	557
113	32	26	571	35	24	543	165	34	28	579	35	25	561
114	34	28	545	35	26	563	166	34	28	566	34	26	532
115	34	28	558	35	28	554	167	34	29	579	35	27	551
116	34	29	566	35	26	555	168	34	29	568	35	28	576
117	34	30	559	35	27	560	169	34	28	553	35	26	537
118	34	28	559	35	27	552	170	34	28	570	35	26	550
119	34	27	578	35	26	570	171	34	28	563	35	26	526
120	32	26	560	35	25	555	172	34	26	580	35	26	526
121	34	28	563	35	26	552	173	34	28	566	35	26	537
122	34	28	555	35	27	576	174	34	28	565	35	24	541
123	34	29	558	35	26	576	175	34	29	545	34	25	539
124	34	29	558	35	26	590	176	34	29	541	35	27	531
125	34	28	555	35	25	565	177	34	28	538	35	26	545
126	34	28	546	35	26	572	178	34	29	547	35	26	545
127	34	28	562	35	27	579	179	34	28	546	35	25	536
128	34	28	550	35	27	551	180	34	28	566	35	26	539
129	34	27	562	35	25	538	181	34	28	559	35	27	546
130	34	28	547	34	25	511	182	34	27	560	35	25	533
131	34	26	570	35	26	546	183	34	30	566	35	27	589
132	34	28	549	32	25	528	184	34	26	571	35	26	537
133	34	26	583	34	25	524	185	34	28	546	35	27	521
134	34	28	567	35	27	576	186	34	26	559	34	25	539
135	34	29	573	35	25	550	187	34	29	548	35	26	508
136	34	80	561	35	25	568	188	34	27	558	35	26	528
137	34	28	569	35	27	566	189	33	27	534	35	26	574
138	34	30	565	35	28	564	190	34	26	551	35	27	566
139	34	28	544	35	27	537	191	34	28	535	35	26	556
140	34	28	536	35	26	563	192	34	26	541	35	26	553
141	34	28	550	35	26	525	193	34	28	536	35	27	542
142	34	27	573	35	27	565	194	34	28	546	35	26	547
143	34	28	559	35	27	566	195	34	28	547	35	26	539
144	33	27	547	34	24	519	196	34	28	545	34	24	536

#	BIOKIT			PLASMATEC			#	BIOKIT			PLASMATEC		
	17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA		17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA
197	34	29	546	35	27	579	249	32	23	582	35	25	536
198	34	28	545	35	26	549	250	34	27	572	35	25	532
199	34	28	546	35	24	547	251	34	28	563	35	25	554
200	34	28	551	35	24	567	252	34	26	569	35	26	547
201	32	26	574	35	24	569	253	34	27	545	35	26	562
202	34	28	573	35	26	576	254	34	28	553	35	26	557
203	34	28	570	35	26	589	255	34	28	561	34	25	531
204	34	29	568	35	26	565	256	34	29	573	35	27	582
205	34	28	538	35	26	587	257	34	29	552	35	27	586
206	32	26	543	35	26	560	258	32	26	551	35	26	564
207	32	27	563	35	28	562	259	34	28	554	35	26	559
208	34	28	539	35	27	561	260	34	27	548	35	25	543
209	34	27	564	35	26	556	261	34	27	560	35	26	553
210	34	27	567	35	26	515	262	34	28	559	35	26	567
211	34	28	567	35	26	528	263	34	28	557	35	27	537
212	34	29	558	35	26	552	264	34	28	552	35	26	526
213	34	28	556	35	26	546	265	34	28	565	35	26	556
214	34	28	534	35	26	569	266	34	28	562	35	26	552
215	34	30	555	35	28	544	267	34	27	548	34	26	537
216	32	26	564	35	26	519	268	32	26	535	34	25	506
217	34	29	543	34	26	533	269	34	29	554	35	25	533
218	34	28	560	35	27	568	270	34	28	554	35	25	526
219	34	28	566	35	27	598	271	32	22	586	34	24	523
220	34	26	564	35	26	563	272	32	24	534	34	24	534
221	34	28	568	35	24	551	273	33	28	548	34	24	548
222	34	28	576	35	26	583	274	33	27	530	35	26	534
223	34	27	551	35	26	561	275	34	29	548	35	27	525
224	34	28	565	35	27	574	276	34	28	536	35	27	521
225	34	27	560	35	27	584	277	34	29	567	35	26	551
226	34	27	570	35	27	573	278	34	28	549	34	24	531
227	34	28	543	35	27	534	279	34	28	563	35	25	542
228	32	26	587	35	25	578	280	34	28	565	35	24	555
229	34	28	573	35	25	588	281	34	28	567	35	27	528
230	34	27	575	35	26	575	282	34	29	550	35	26	580
231	34	26	557	35	26	568	283	34	29	557	35	26	544
232	34	27	574	35	26	581	284	32	28	563	35	28	570
233	34	28	551	35	27	557	285	34	29	545	11	1	0
234	34	28	552	35	26	562	286	34	28	549	35	27	553
235	34	29	555	35	27	579	287	32	27	550	32	24	492
236	34	28	563	35	28	560	288	34	28	547	35	25	530
237	32	27	553	35	27	586	289	34	27	564	35	26	558
238	34	28	558	35	26	598	290	34	28	560	35	26	547
239	34	27	569	35	27	585	291	34	28	577	35	26	557
240	34	29	568	35	27	575	292	34	27	558	35	26	563
241	34	28	564	35	27	584	293	34	30	560	35	26	569
242	34	28	560	35	26	601	294	31	25	511	35	25	553
243	34	28	555	36	28	597	295	34	28	558	35	26	553
244	34	29	569	36	28	591	296	34	30	543	35	27	550
245	34	27	566	35	28	581	297	33	28	553	35	26	556
246	34	29	563	35	27	571	298	32	26	558	35	26	538
247	34	28	561	35	27	571	299	34	28	554	35	26	543
248	34	27	572	35	26	585	300	34	28	556	35	27	555

#	BIOKIT			PLASMATEC			#	BIOKIT			PLASMATEC		
	17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA		17-23 P/C	12-13 SPC	75-205 LIA	26-41 P/C	16 SPC	100-240 LIA
301	34	28	537	35	25	538	353	34	28	546	35	26	530
302	34	27	568	35	25	529	354	34	28	537	35	26	526
303	34	28	544	35	26	563	355	34	28	534	35	26	509
304	32	27	553	33	24	533	356	34	28	534	35	28	549
305	34	29	572	35	26	547	357	34	28	542	35	26	520
306	34	28	566	35	26	522	358	34	25	568	35	25	546
307	32	27	549	35	26	542	359	34	28	558	35	25	531
308	34	26	563	35	26	556	360	34	28	548	35	26	537
309	34	27	569	35	26	547	361	34	26	545	35	26	528
310	34	27	557	35	25	530	362	34	28	549	35	26	556
311	34	28	543	35	27	553	363	32	25	541	34	25	533
312	34	27	554	34	26	511	364	34	29	554	35	25	535
313	34	28	533	35	26	521	365	34	28	546	35	26	523
314	34	28	547	35	27	564	366	34	30	567	34	26	519
315	32	28	539	35	26	541	367	32	24	547	34	25	519
316	34	27	549	35	26	549	368	34	28	557	35	26	517
317	32	26	563	35	25	524	369	34	26	551	35	27	565
318	34	28	558	35	26	529	370	34	28	570	35	26	566
319	32	25	564	35	25	519	371	34	28	552	35	27	562
320	34	28	553	35	26	525	372	34	28	544	35	26	545
321	34	27	543	35	27	545	373	34	28	547	35	26	517
322	34	29	549	35	27	543	374	34	28	533	35	27	567
323	34	28	553	35	26	539	375	34	28	540	35	27	557
324	34	29	556	35	26	516	376	34	28	546	35	26	535
325	32	28	540	35	26	496	377	34	28	537	35	26	538
326	34	28	541	35	27	528	378	34	29	555	35	25	546
327	34	28	548	35	24	510	379	34	27	546	35	26	566
328	34	27	552	35	25	523	380	34	26	548	35	27	565
329	34	26	556	35	27	516	381	34	26	559	35	26	535
330	34	28	557	35	27	557	382	34	28	543	34	25	538
331	34	26	562	35	25	543	383	34	28	549	35	26	549
332	34	29	558	35	26	522	384	34	28	548	35	26	515
333	34	29	550	35	27	540	385	34	29	546	35	26	556
334	34	28	555	35	26	499	386	34	28	526	35	27	544
335	34	28	550	34	26	503	387	34	27	544	35	24	538
336	34	29	549	34	26	551	388	34	28	566	35	26	529
337	32	22	536	35	26	531	389	34	28	558	35	26	568
338	34	27	550	35	25	542	390	34	28	560	35	26	546
339	34	28	523	34	25	514	391	34	26	558	35	26	560
340	32	26	552	35	26	546	392	34	28	571	35	27	569
341	34	27	557	35	27	540	393	34	29	558	35	27	554
342	34	28	568	35	26	529	394	34	29	561	35	26	578
343	34	27	574	34	25	524	395	34	28	546	35	26	559
344	34	29	564	35	26	529	396	34	28	578	35	26	545
345	32	26	538	35	27	539	397	32	26	524	35	25	510
346	34	28	557	35	26	555	398	33	28	545	35	26	548
347	32	24	576	34	23	548	399	34	29	555	35	24	571
348	34	28	560	35	26	557	400	34	27	552	35	25	534
349	34	28	563	35	25	561	401	34	29	546	35	26	547
350	33	26	540	34	24	523	402	34	29	558	35	25	566
351	34	29	554	35	26	532	403	34	28	555	35	26	542
352	34	29	549	35	26	525	404	34	30	541	35	27	558

#	BIOKIT			PLASMATEC		
	17-23 P/C	12-13 SPC	75- 205 LIA	26-41 P/C	16 SPC	100- 240 LIA
405	34	29	535	35	27	542
406	33	29	534	35	25	533
407	34	28	548	35	24	577
408	34	28	553	35	26	558
409	34	27	547	35	26	539
410	34	27	550	35	26	553
411	34	27	547	35	26	567
412	34	26	539	35	26	534
413	32	26	546	35	25	516
414	34	28	538	35	27	512
415	34	28	536	35	27	556
416	32	27	522	35	26	529
417	32	28	532	35	26	529
418	34	26	549	35	26	536
419	34	28	541	35	25	554
420	34	27	537	35	24	528
421	34	27	537	35	27	558
422	34	28	547	35	26	551
423	34	28	537	35	27	545
424	34	30	555	35	26	547
425	34	29	525	35	24	546
426	33	28	538	35	26	543
427	32	28	547	35	26	542
428	34	28	546	34	24	522
429	34	28	554	35	27	554
430	34	28	543	35	26	543
431	34	28	529	35	25	547
432	34	27	526	35	26	558
433	34	28	552	35	26	545
434	34	28	537	35	27	551
435	32	28	538	35	26	524
436	34	29	531	35	27	551
437	32	27	550	35	26	549
438	34	28	545	35	26	531
439	34	28	543	34	25	519
440	34	26	549	35	26	520
441	34	28	550	35	26	545
442	33	27	537	35	24	539
443	34	28	545	35	27	549
444	34	28	550	35	26	541
445	34	28	534	35	27	555
446	34	27	539	35	26	512

syphagen TPHA AUTO REC plus

Indirect hemagglutination test for the detection of specific antibodies to *Treponema pallidum* in human serum or plasma using the OLYMPUS PK7200^R.

Summary

Syphilis, one of the most important sexually transmitted diseases worldwide, is caused by the *Treponema pallidum* infection. However, it can also be transmitted congenitally by transplacental passage of *T. pallidum* to the foetus and by blood transfusion. The serological diagnosis of syphilis is performed demonstrating the presence of specific antibodies to *T. pallidum*.

TPHA, the hemagglutination treponemal test for excellence has the advantage of the higher sensitivity but the antigenic similarity between *T. pallidum* and other non pathogenic treponemes can give some cross reactions to the test that will be reported as false positive results. For the screening of a high number of samples is necessary to use a test with a good level of specificity in order to reduce the number of non productive tests (samples that give positive result with the screening test and negative with the confirmatory test).

The "antigens" used just now to manufacture the treponemal tests have been obtained from testicular rabbit tissue culture and were a mixture of treponemal antigens and tissue debris that occasionally produce non-specific results. The **syphagen TPHA AUTO REC plus** reagent uses a combination of recombinant antigens that give high specificity to the test since cross reactions due to others saprophytic or pathogen treponemes can be removed without decreasing the sensitivity of the test.

Principle

The **syphagen TPHA AUTO REC plus** is a TPHA test for detection of antibodies to *T. pallidum*. The reagent is a suspension of stabilized chicken erythrocytes coated with p15, p17 and p47 *T. pallidum* antigens obtained from cellular culture of recombinant *Escherichia coli* and subsequent purification. In combination with these three antigens, another recombinant protein has been added to increase the sensitivity of the test.

Components

REF 3000-5709.

- a) **Antigen reagent:** 5 x 40 ml.
Suspension of sensitized chicken erythrocytes. Ready to use.
- b) **Diluent solution:** 5 x 500 ml.
Phosphate buffered saline solution containing soluble components of T. Reiter and stabilizing agents.
- c) **Positive control:** 1 x 15 ml.
Immune rabbit serum.
- d) **Negative control:** 1 x 15 ml.
Diluted normal human serum.

All reagents contain < 0.1% sodium azide.

Precautions

syphagen TPHA AUTO REC plus is intended for IN VITRO diagnostic use.

The reagents contain sodium azide as a preservative. Sodium azide may react with lead or copper pipes and plumbing creating highly explosive metal azides. Flush drains with water thoroughly after disposing of the remains of reagents.

Do not interchange reagents from different lots.

The microplates must be cleaned before use following manufacturer instructions.

All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 and HCV antibodies, as well as for the hepatitis B surface antigen, using a method approved by the Food and Drug Administration (USA).

WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.

Because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution.

Dispose all used materials in a suitable biohazardous waste container.

Storage

The reagents will remain stable until the expiration date shown on the label if stored between 2-8°C. Do not freeze.

Material required not provided

- OLYMPUS «green» type microplates.
- OLYMPUS PK7200 automated pretransfusion blood testing system.
- Automatic pipettes.

Sample collection

Plasma:

Although serum is the specimen of choice for all tests for syphilis, for screening purposes in blood banks, EDTA plasma samples may be used. Other anticoagulants should be evaluated before use. Plasma can be stored at 2-8°C and tested within 72 hours of blood collection.

Serum:

Use fresh serum. Serum can be stored at 2-8°C for 5 days. For longer periods serum samples should be frozen (-20°C).

PROCEDURE

Quality control:

Allow reagents to reach room temperature. Ensure that antigen reagent is thoroughly resuspended.

Include the positive and negative controls in each series of samples. If expected results are not obtained, do not use the kit.

Directions for use

The PK7200 analyzer is a programmable instrument whose operations are controlled by the Test Parameter Files defined and established by the user. Validated parameters may be incorporated with routine operating files or defined in a separate file. For complete details on the set up and operations of the OLYMPUS PK7200, please refer to the Operator's Manual.

Olympus PK7200 Application:

Sample volume µl		35	
Diluent volume µl		360	
Diluted sample volume		15	
Reagent volume		35	
Thresholds:	Dynamic range set	P	1-99
		C	1-99
		LIA	0-999
	Threshold set	SPC	12-13
		P/C	17-23
		LIA	75-205
	Limit set	BG/C	Middle
	LIA selection		6

The most important parameter resulting from the image analysis system is SPC.

Interpretation of the results

Results are determined by the threshold parameters defined previously in the Olympus PK7200. The decision logic used for the Olympus PK7200 for the interpretation of the results is the following:

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

If a plasma specimen is positive or indeterminate on initial screening, the test should be repeated using a serum specimen and if same result is obtained the sample should be considered positive for antibodies to *T. pallidum* and should be confirmed by using a confirmatory test. If repeated sample is negative it should be considered negative for antibodies to *T. pallidum*.

A positive result indicates the presence of antibodies to *T. pallidum*, resulting from a past or present infection. A negative result indicates the absence of antibodies to *T. pallidum* (see limitations of the procedure).

Limitations of the procedure

- Although serum is the specimen of choice for all tests for syphilis, for screening purposes in blood banks, EDTA plasma samples may be used. However, some incidence of false positive reactions has been reported. Therefore, serum must be used for all repeat testing of initial positive or indeterminate results obtained from plasma samples and for any further confirmatory testing.
- Specific antibodies may persist for a long period of time, even after successful treatment of the disease. In order to assess the response to treatment, the use of a reagin test (e.g. RPR reditest) is recommended.
- The TPHA technique can give cross reactions with other forms of treponemal infections and false positive reactions with samples from patients with infectious mononucleosis, leprosy, autoimmune diseases and drug addiction.
- Occasionally, in early primary syphilis, the specific antibodies could not be detected by the TPHA technique.

Performance characteristics

3593 negative plasma samples from blood bank donors previously characterized were tested on the Olympus PK7200 with the **syphagen TPHA AUTO REC plus**. The initial specificity found was 99.44%, 5 samples were reported as positive and 15 samples as indeterminate. Once repeated these samples, the specificity found was 99.89%.

References

1. Biosafety in Microbiological and Biomedical Laboratories, Richmond JY, McKinney RW eds. US Department of Health and Human Services, 4th Edition, 1999.
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3. Hagedorn HJ, Naumann P. Moderne Serodiagnostik der Syphilis. Dtsch med Wschr 104: 209-214, 1979.
4. Larsen SA, Hambie EA, Pettit DE, Perryman MW, Kraus SJ. Specificity, Sensitivity, and Reproducibility Among the Fluorescent Treponemal Antibody-Absorption Test, the Microhemagglutination Assay for *Treponema pallidum* Antibodies, and the Hemagglutination Treponemal Test for Syphilis. J Clin Microbiol 14: 441-445, 1981.
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6. Rathlev T. Haemagglutination Test Utilizing Pathogenic *Treponema pallidum* for the Sero-diagnosis of Syphilis. Brit J Vener Dis 43: 181-185, 1967.
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9. Tomizawa T, Kasamatsu S, Yamaya S. Usefulness of the Hemagglutination Test Using *Treponema pallidum* Antigen (TPHA) for the Serodiagnosis of Syphilis. Jap J Med Sci Biol 22: 341-350, 1969.

OLYMPUS PK7200® is a trademark of the Olympus Corporation.



MATERIAL SAFETY DATA SHEET

1. COMPANY IDENTIFICATION

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Telephone : +34-93-860 9000
Fax : +34-93-860 9009

2. PRODUCT NAME

Syphagen TPHA AUTO REC Plus

Code # 3000-5709

3. HAZARDOUS COMPONENTS

All reagents contain less than 0.1% wt of Sodium Azide (NaN_3) as preservative.

CAS-No.: 26628-22-8

EINECS-No.: 247-852-1

4. INTENDED USE

Indirect hemagglutination test for detection of specific antibodies to *Treponema pallidum* in human serum or plasma using the OLYMPUS OLYMPUS PK7200^R.

5. PHYSICAL AND CHEMICAL PROPERTIES

Antigen reagent: Suspension of sensitized chicken erythrocytes.
Diluent solution: Soluble components of T. Reiter in phosphate buffered saline and stabilizing reagents.
Positive control: Diluted rabbit serum containing specific antibodies to *T. pallidum*.
Negative control: Diluted normal human serum.

A CH-Werfen Company

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6. HAZARDS TO HEALTH

When used in accordance with the Principles of Good Laboratory Practice, Good Standards of Occupational Hygiene and the instructions stated in the product insert, these products are not considered to present a hazard to health.

7. PRECAUTIONS

The storage must be between 2 and 8°C.

For IN VITRO diagnostic use.

The reagents in this kit contain sodium azide as a preservative. Sodium azide has been reported to form lead or copper azide in laboratory plumbing which may explode on percussion. Flush drains with water thoroughly after disposing of fluids containing sodium azide.

Each donor unit used in the preparation of the negative control of this kit was tested by an approved method for the presence of the antibody to HIV and HCV (negative control) as well as for HBsAg and found to be negative.

WARNING: Because no test method can offer complete assurance that HIV, HCV, HBsAg or other infectious agents are absent, the controls of this kit should be handled carefully.

8. EMERGENCY ACTION

If the material comes into contact with the skin or is splashed into the eyes, rinse immediately with plenty of water.

Wipe up spills with absorbant tissues and dispose of appropriately.

9. FIRE HAZARDS

Extinguishing media: Carbon dioxide, dry chemical powder or polymer foam.

Special firefighting procedures:

wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

10. DISPOSAL REQUIREMENTS

Remains of samples, reagents and controls should be collected in a recipient for this purpose.

Observe all Federal, State and local laws.

11. TRANSPORT PRECAUTIONS

Special requirements: NONE.

THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT, BUT DOES NOT PURPOSE TO BE ALL INCLUSIVE AND SHOULD BE USED ONLY AS A GUIDE. biokit SHALL NOT BE HELD FOR ANY DAMAGE RESULTING FROM HANDLING OR USE OF THE PRODUCT.

Quality Assurance Department.