

TD-7.2 Workshop on Antibodies to hCG

Immunoassay combinations for measurement of free hCG β

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Reference antigens

- Intact hCG (hCG) 99/688
- Nicked hCG (hCGn) 99/642
- Free hCG β -subunit (hCG β) 99/650
- Nicked hCG β (hCG β n) 99/692
- Free hCG α -subunit (hCG α) 99/720
- hCG β -core fragment (hCG β cf) 99/708
- LH Aker Hospital, Oslo



Binding studies to define the specificity of antibodies

- Microtiter wells coated with all Workshop antibodies (0.5 µg/well).
- ¹²⁵I-labelled antigens were added to all solid phase antibodies and incubated for 1 h with continuous shaking.
- Wells were washed 3 times before counting of radioactivity

Binding of ¹²⁵I-labelled antigens (B/T %)

ISOBM mAb	*hCG	*hCGn	*hCGβ	*hCGβn	*hCGβcf	LH	*hCGα	Group	Ab	Ref. Abs	Epitope code	Characteristics
435	14	12	36	20	39	0	0	A		INN-hCG-2	β1	highly specific
386	9	3	61	28	43	1	0					
403	19	17	40	22	52	0	0					
408	98	87	96	49	90	1	0					
449	76	72	83	43	82	18	0					
434	30	26	31	13	49	32	0	B		INN-hCG-22	β2	pan hCG
441	31	25	55	23	53	27	0					
445	74	63	75	38	71	21	0					
442	81	72	72	38	86	52	0					
382	77	66	76	37	77	12	0					
388	98	91	99	52	93	27	0					
390	95	88	97	49	89	15	0					
396	83	77	63	34	72	61	0					
399	97	90	98	50	93	68	0					
400	73	66	83	42	86	57	0					
401	87	79	93	49	91	64	0					
402	97	92	98	52	92	42	0					
405	94	90	99	52	95	69	0					
415	10	8	89	44	78	6	0					
416	94	85	94	47	87	17	0					
417	94	87	95	49	91	18	0					
419	94	88	91	45	83	24	0					
423	97	89	98	51	91	67	0					
426	93	88	91	50	91	22	0					
427	94	91	96	52	92	13	0					
430	71	60	73	37	64	55	0					
431	97	88	96	51	92	33	1					
428	61	60	71	35	71	65	0					
429	69	66	80	39	77	60	0					
437	2	3	74	32	46	1	0	C		INN-hCG-64	β6	free hCGβ
439	2	3	46	20	28	1	0					
407	1	1	65	23	2	0	0					
409	1	2	88	44	84	1	0					
410	1	2	94	47	86	2	0					
397	1	1	3	2	4	1	0					
418	0	0	9	2	0	0	0					
450	33	25	71	28	0	0	0					
424	95	90	58	13	0	2	0	D		h54	β8	hCGβCTP
406	71	77	62	9	0	0	0					
413	46	40	81	30	0	1	0					
420	46	34	73	26	0	0	0					
392	6	6	22	2	0	4	2					
394	71	67	30	9	0	1	0					
395	20	13	46	11	0	0	0					
447	68	13	1	0	1	27	0	E		INN-hCG-10	c1	XR hLH hCG no XR hCGn
438	68	18	5	1	1	26	0					
436	64	12	6	0	0	25	0					
446	46	39	2	1	0	0	0					
440	71	59	5	1	0	46	0					
389	91	15	12	0	1	4	0					
425	85	13	1	0	0	2	0					
422	18	6	1	0	0	0	0					
433	16	15	1	1	0	0	0					
411	21	11	1	0	0	2	0					
387	87	14	1	0	0	27	0					
414	94	26	2	2	1	72	0					
448	0	1	1	2	55	0	0	F		INN-hCG-103	β10	hCGβcf
444	0	1	2	1	62	0	0					
443	0	1	1	2	70	0	0					
384	1	1	1	1	76	0	0					
393	1	1	1	1	52	0	0					
398	30	36	10	8	13	25	51	G				hCGα
383	88	90	1	4	0	79	100					
385	94	82	1	4	0	77	92					
391	47	31	1	1	0	49	61					
412	46	31	1	1	0	43	62					
421	47	33	1	1	0	50	63					
432	29	27	1	1	0	14	17					
404	3	11	1	3	1	17	96					

Table 1. Classification of ISOBM antibodies into seven main groups by their binding characteristics to hCG-related molecules



ISOBM	ISOBM	Name	Source	*hCG	*hCGn	*hCGβ	*hCGβn	*hCGβcf	*LH	*hCGα	Group	Ab	Ref. Abs	Epitope code	Characteristics
435	hCG 2	INN		14	12	36	20	39	0	0			INN-hCG-2	β1	
386	F94-8F8	Stenman		9	3	61	28	43	1	0					highly specific
403	M-INN2	Roche		19	17	40	22	52	0	0		A			
408	5016 SPRN-5	Medix		98	87	96	49	90	1	0					
449	hCG 22	INN		76	72	83	43	82	18	0			INN-hCG-22	β2	
434	hCG 111	INN		30	26	31	13	49	32	0			INN-hCG-111	β3	
441	βLH 1	INN		31	25	55	23	53	27	0			INN-hLH-1	β3	
445	hCG 24	INN		74	63	75	38	71	21	0			INN-hCG-24	β4	
442	hCG 58	INN		81	72	72	38	86	52	0			INN-hCG-58	β5	
382	F16-6G5	Stenman		77	66	76	37	77	12	0					
388	5006 SP-5	Medix		98	91	99	52	93	27	0					
390	5008 SP-5	Medix		95	88	97	49	89	15	0					
396	F132-3C10	Stenman		83	77	63	34	72	61	0					
399	F95-5C4	Stenman		97	90	98	50	93	68	0					
400	95658	Abbott		73	66	83	42	86	57	0					
401	F95-1E8	Stenman		87	79	93	49	91	64	0					
402	5004 SP-1	Medix		97	92	98	52	92	42	0					pan hCG
405	F95-1B2	Stenman		94	90	99	52	95	69	0					
415	3A11	Siemens		10	8	89	44	78	6	0		B			
416	E30	Paus		94	85	94	47	87	17	0					
417	M-INN22	Roche		94	87	95	49	91	18	0					
419	E27	Paus		94	88	91	45	83	24	0					
423	D102	Mologic		97	89	98	51	91	67	0					
426	16E2	Siemens		93	88	91	50	91	22	0					
427	34A8.1.1	Siemens		94	91	96	52	92	13	0					
430	8G5	Mologic		71	60	73	37	64	55	0					sheep IgG
431	618 (LP purif	Mologic		97	88	96	51	92	33	1					sheep poly
428	8F11	Mologic		61	60	71	35	71	65	0					sheep IgG
429	9F10	Mologic		69	66	80	39	77	60	0					sheep IgG
	4F9	Mologic		88	87	95	41	83	51	0					sheep IgG
437	hCG 64	INN		2	3	74	32	46	1	0			INN-hCG-64	β6	
439	hCG 68	INN		2	3	46	20	28	1	0			INN-hCG-68	β7	
407	F19-9C11	Stenman		1	1	65	23	2	0	0					
409	5012 SPRN-1	Medix		1	2	88	44	84	1	0		C			free hCGβ
410	M-BCG005	Roche		1	2	94	47	86	2	0					
397	F142-7F3	Stenman		1	1	3	2	4	1	0					
418	2F11	Siemens		0	0	9	2	0	0	0					
450	h54	INN		33	25	71	28	0	0	0			h54	β8	
424	1G4	Siemens		95	90	58	13	0	2	0					
406	5011 SPRN-1	Medix		71	77	62	9	0	0	0					
413	D101	Mologic		46	40	81	30	0	1	0					hCGβCTP
420	M-94.139	Roche		46	34	73	26	0	0	0		D			
392	F20-6E11	Stenman		6	6	22	2	0	4	2					
394	5014 SPTN-5	Medix		71	67	30	9	0	1	0					
395	71752	Abbott		20	13	46	11	0	0	0					
447	hCG 10	INN		68	13	1	0	1	27	0			INN-hCG-10	c1	
438	hCG 53	INN		68	18	5	1	1	26	0			INN-hCG-53	c2	
436	hCG 40	INN		64	12	6	0	0	25	0			INN-hCG-40	c2	
446	hCG 45	INN		46	39	2	1	0	0	0			INN-hCG-45	c3	XR hLH
440	hCG 26	INN		71	59	5	1	0	46	0		E	INN-hCG-26	c4	hCG no XR hCGn
389	F140-11C5	Stenman		91	15	12	0	1	4	0					
425	5E5	Siemens		85	13	1	0	0	2	0					
422	E28	Paus		18	6	1	0	0	0	0					
433	45A10	Medix		16	15	1	1	0	0	0					
411	M-1F7.9	Roche		21	11	1	0	0	2	0					
387	5009 SP-5	Medix		87	14	1	0	0	27	0					
414	E26	Paus		94	26	2	2	1	72	0					
448	hCG 103	INN		0	1	1	2	55	0	0			INN-hCG-103	β10	
444	hCG 106	INN		0	1	2	1	62	0	0			INN-hCG-106	β11	
443	hCG 112	INN		0	1	1	2	70	0	0		F	INN-hCG-112	β13	hCGβcf
384	F52-3F8	Stenman		1	1	1	1	76	0	0					
393	F52-3C11	Stenman		1	1	1	1	52	0	0					
388	F26-2G11	Stenman		30	36	10	8	13	25	51					
383	5501 SP-1	Medix		88	90	1	4	0	79	100					
385	5503 SP-1	Medix		94	82	1	4	0	77	92					
391	6601 SPR-5	Medix		47	31	1	1	0	49	61		G			hCGα
412	34/25.2.2	Siemens		46	31	1	1	0	43	62					
421	411/100.1.1.2	Siemens		47	33	1	1	0	50	63					
432	41-3-9	Medix		29	27	1	1	0	14	17					
404	F26-7E10	Stenman		3	11	1	3	1	17	96					

Table 1. Classification of ISOBM antibodies into seven main groups by their binding to hCG-related molecules.
One antibody 4F9, showing pan hCG specificity, has been added.

Immunoradiometric assays with different antibody pair combinations

- Free hCG β , 99/650, added to microtiter wells coated with all Workshop antibodies (0.5 μ g/well).
- One hour incubation with continuous shaking. Wells washed 3 times.
- 125 I-labelled tracer antibodies were added and another incubation for 1 h with continuous shaking.
- Wells were washed 3 times before counting of radioactivity



Binding of ¹²⁵I-labelled antigens (B/T %)

ISOBM	mAb	Name	Source	*hCG	*hCGn	*hCGβ	*hCGβn	*hCGβcl	LH	*hCGα	Group	Epitope code
	435	hCG 2	INN	14	12	36	20	39	0	0		β1
	386	F94-8F8	Stenman	9	3	61	28	43	1	0	A	
	403	M-INN2	Roche	19	17	40	22	52	0	0	highly	
	408	5016 SPRN-5	Medix	98	87	96	49	90	1	0	specific	
	449	hCG 22	INN	76	72	83	43	82	18	0		β2
	434	hCG 111	INN	30	26	31	13	49	32	0		β3
	441	βLH 1	INN	31	25	55	23	53	27	0		β3
	445	hCG 24	INN	74	63	75	38	71	21	0		β4
	442	hCG 58	INN	81	72	72	38	86	52	0		β5
	382	F16-6G5	Stenman	77	66	76	37	77	12	0		
	388	5006 SP-5	Medix	98	91	99	52	93	27	0		
	390	5008 SP-5	Medix	95	88	97	49	89	15	0		
	396	F132-3C10	Stenman	83	77	63	34	72	61	0		
	399	F95-5C4	Stenman	97	90	98	50	93	68	0		
	400	95658	Abbott	73	66	83	42	86	57	0		
	401	F95-1E8	Stenman	87	79	93	49	91	64	0		
	402	5004 SP-1	Medix	97	92	98	52	92	42	0		
	405	F95-1B2	Stenman	94	90	99	52	95	69	0		
	415	3A11	Siemens	10	8	89	44	78	6	0	B	
	416	E30	Paus	94	85	94	47	87	17	0	pan hCG	
	417	M-INN22	Roche	94	87	95	49	91	18	0		
	419	E27	Paus	94	88	91	45	83	24	0		
	423	D102	Mologic	97	89	98	51	91	67	0		
	426	16E2	Siemens	93	88	91	50	91	22	0		
	427	34A8.1.1	Siemens	94	91	96	52	92	13	0		
	430	8G5	Mologic	71	60	73	37	64	55	0		
	431	618 (LP purified)	Mologic	97	88	96	51	92	33	1		
	428	8F11	Mologic	61	60	71	35	71	65	0		
	429	9F10	Mologic	69	66	80	39	77	60	0		
	437	hCG 64	INN	2	3	74	32	46	1	0		β6
	439	hCG 68	INN	2	3	46	20	28	1	0		β7
	407	F19-9C11	Stenman	1	1	65	23	2	0	0		
	409	5012 SPRN-1	Medix	1	2	88	44	84	1	0	C	
	410	M-BCG005	Roche	1	2	94	47	86	2	0	free hCGβ	
	397	F142-7F3	Stenman	1	1	3	2	4	1	0		
	418	2F11	Siemens	0	0	9	2	0	0	0		
	450	h54	INN	33	25	71	28	0	0	0		β8
	424	1G4	Siemens	95	90	58	13	0	2	0		
	406	5011 SPRN-1	Medix	71	77	62	9	0	0	0		
	413	D101	Mologic	46	40	81	30	0	1	0	D	
	420	M-94.139	Roche	46	34	73	26	0	0	0	hCGBCTP	
	392	F20-6E11	Stenman	6	6	22	2	0	4	2		
	394	5014 SPTN-5	Medix	71	67	30	9	0	1	0		
	395	71752	Abbott	20	13	46	11	0	0	0		

Monoclonal antibodies from group C were chosen as solid phase mAbs for measurement of free hCGβ

Immunometric assays with antibody pairs from group C

Antigen: hCG β (WHO 99/650)

Solid phase mAb	Iodinated tracer antibody				
	407	409	410	437	439
407	0	0	53	29	0
409	0	0	63	33	0
410	37	35	1	0	0
437	27	25	0	1	0
439	1	0	1	1	0

Data expressed as % binding of tracer antibodies

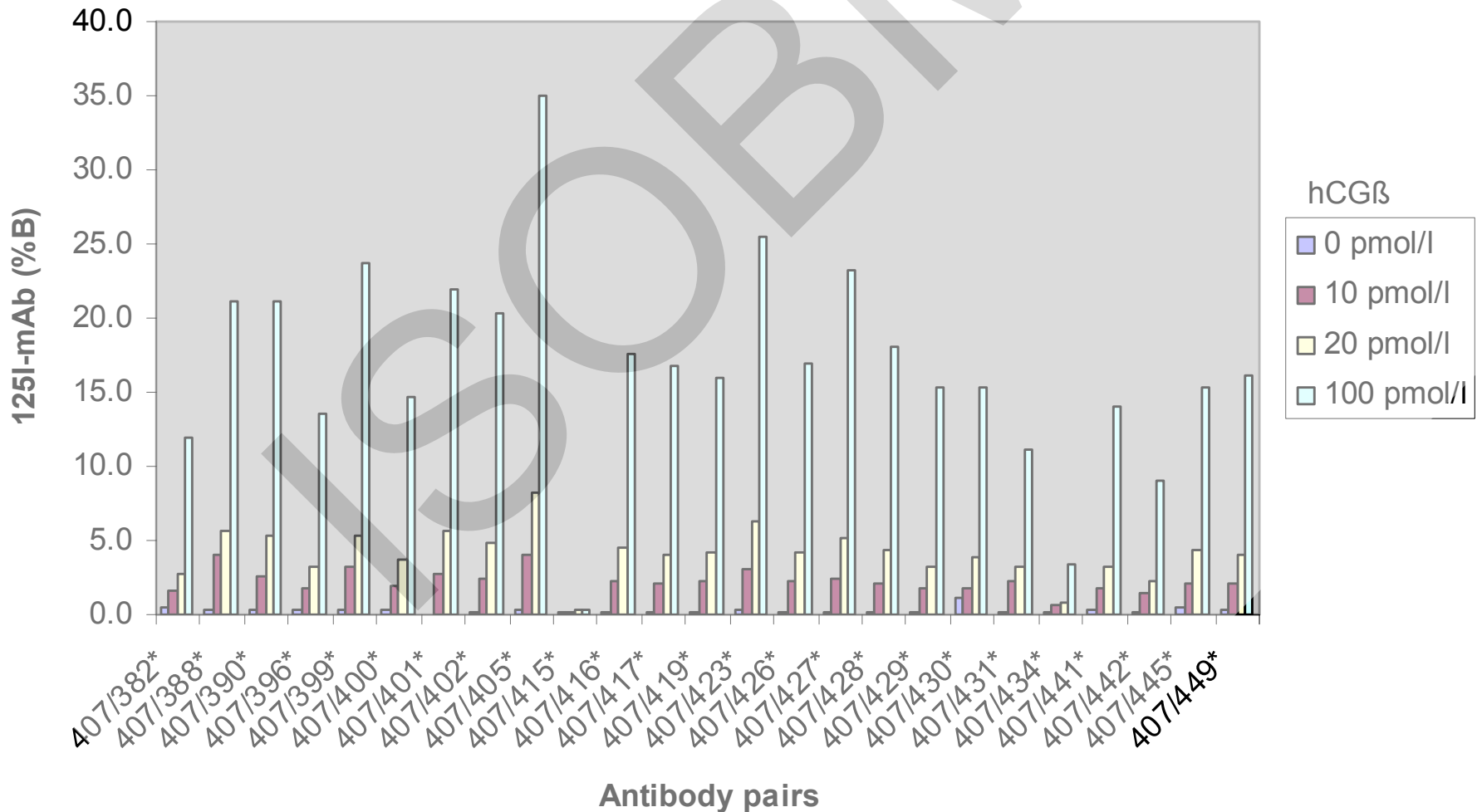


Immunoassays for free hCG β

Solid phase mAb : Group C mAb 407 (9C11) specific for free hCG β

Tracer mAbs : Group B Workshop mAbs (pan hCG)

Antigen : hCG β WHO 99/650

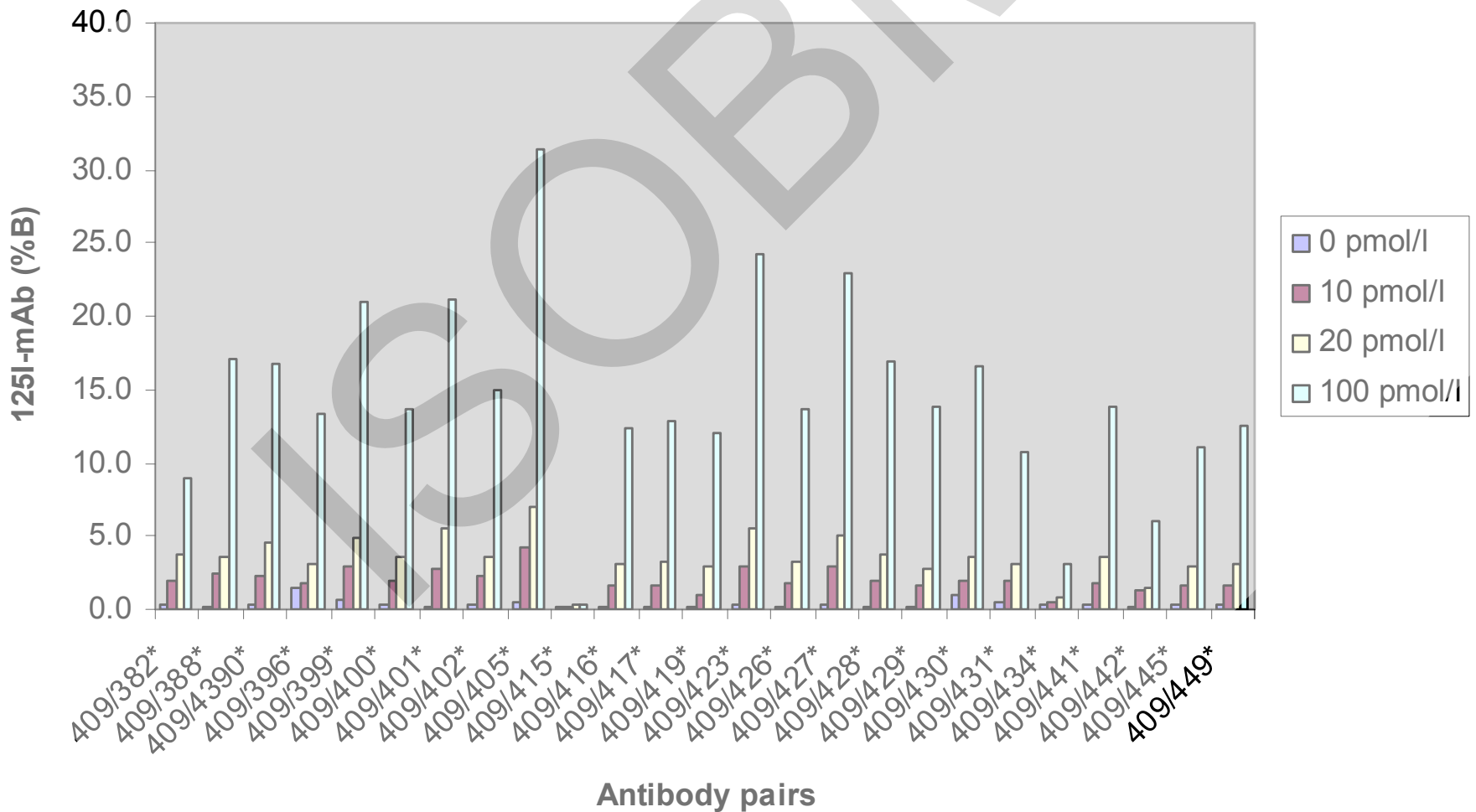


Immunoassays for free hCG β

Solid phase mAb : Group C mAb 409 (9C11) specific for free hCG β

Tracer mAbs : Group B Workshop mAbs (pan hCG)

Antigen : hCG β WHO 99/650

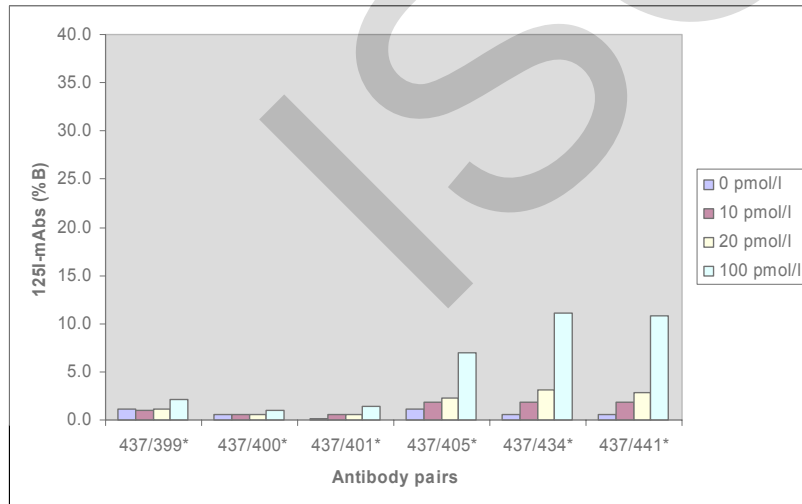
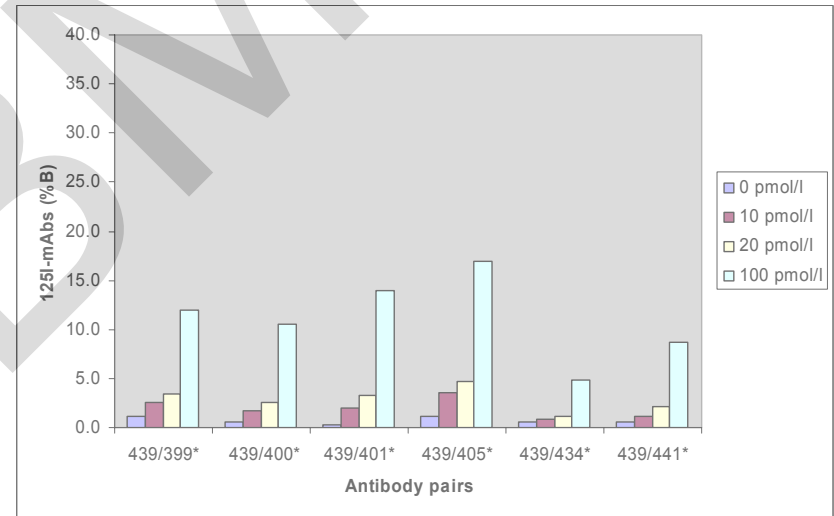
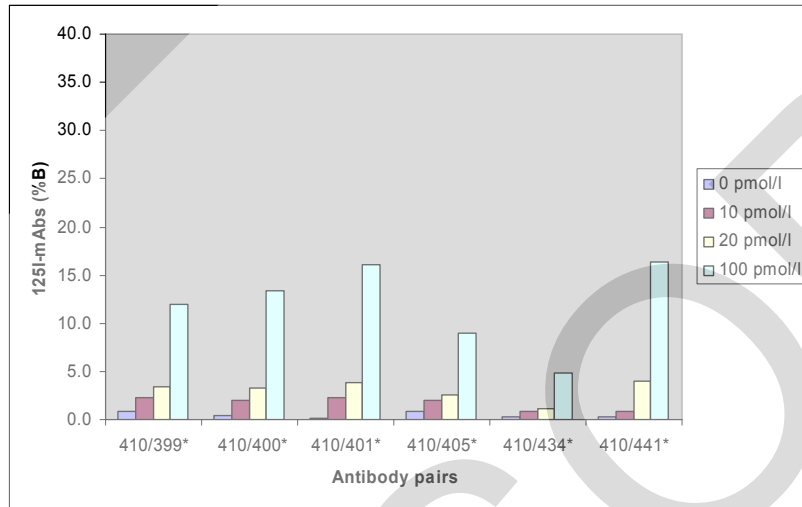


Immunoassays for free hCG β

Solid phase mAb : Group C mAbs 410, 437 and 439, specific for free hCG β

Tracer mAbs : Group B Workshop mAbs (pan hCG)

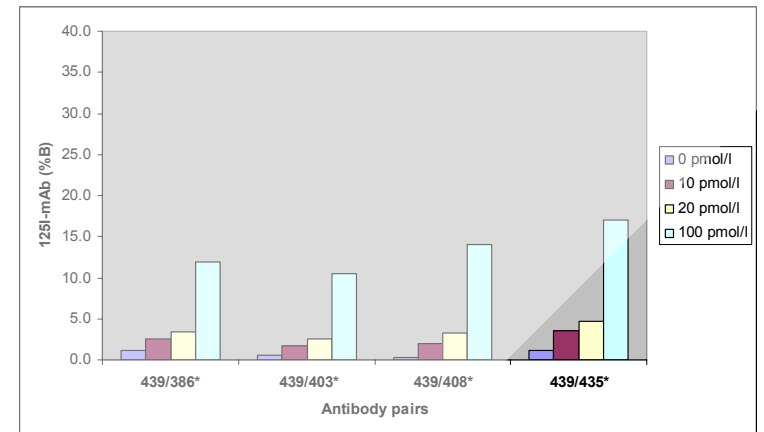
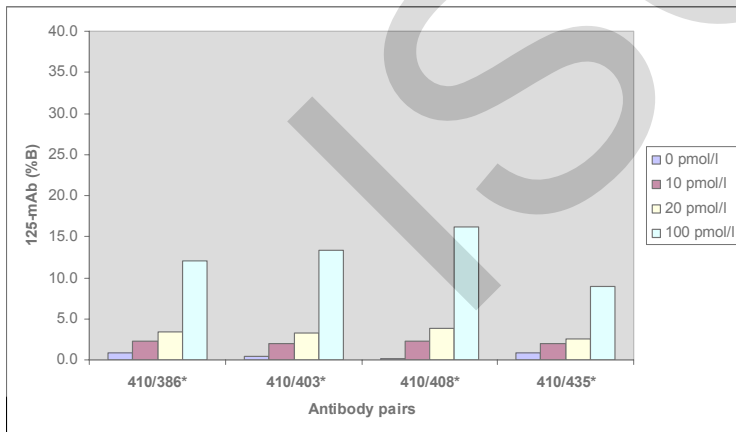
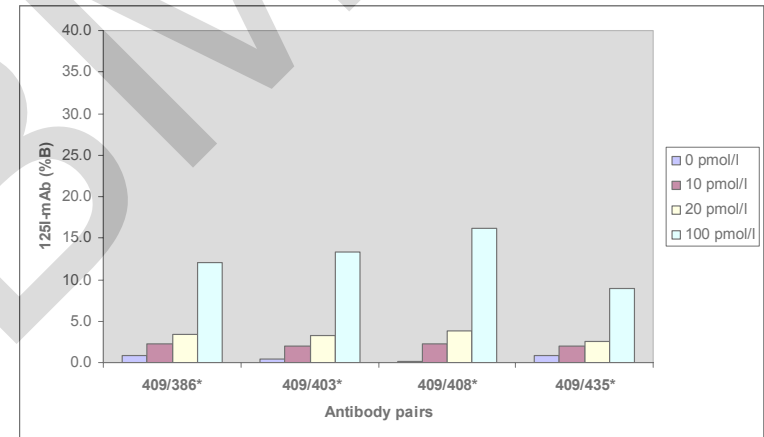
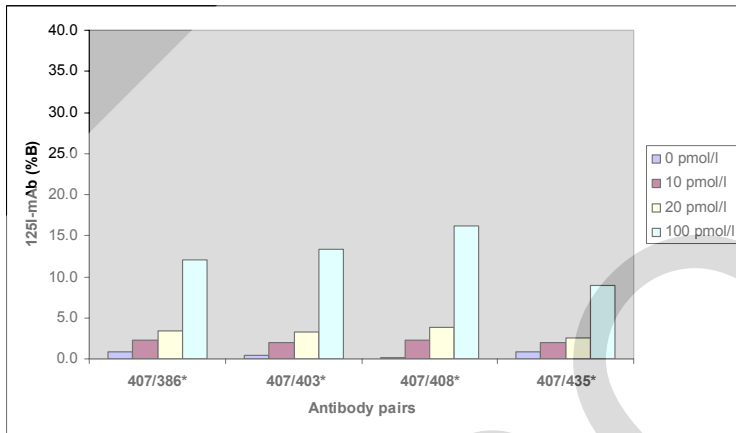
Antigen : hCG β WHO 99/650



Assay with antibodies 410, 437 and 439, (group C) did not have acceptable sensitivity in IRMAs for free hCG β

Immunoassays for free hCG β

Solid phase mAbs : Group C mAbs specific for free hCG β
Tracer mAbs : Group A specific for hCG,
Antigen : hCG β WHO 99/650



Assays for free hCG β with antibodies from group A (highly specific for hCG) did not have acceptable sensitivities in combinations with group C antibodies.

			Bound tracer mAb (%)						
			hCGβ, 99/650						
Solid Phase antibody			Tracer antibody		100pM	20pM	10pM	0pM	
407	Stenman	F19-9C11	Stenman	F95-1B2	405*	34.6	7.9	3.7	0
407	Stenman	F19-9C11	Mologic	D102	423*	25.2	6.0	2.8	0
407	Stenman	F19-9C11	Stenman	F95-5C4	399*	23.3	5.0	2.8	0
407	Stenman	F19-9C11	Siemens	34A8.1.1	427*	23.0	5.0	2.2	0
407	Stenman	F19-9C11	Stenman	F95-1E8	401*	22.0	5.6	2.8	0
407	Stenman	F19-9C11	Medix	5006 SP5	388*	20.8	5.4	3.7	0
407	Stenman	F19-9C11	Medix	5008 SP-5	390*	20.8	5.0	2.3	0
407	Stenman	F19-9C11	Medix	5004 SP-1	402*	20.1	4.6	2.3	0
407	Stenman	F19-9C11	Mologic	8F11SheepMono	428*	17.8	4.1	1.9	0
407	Stenman	F19-9C11	Paus	E30	416*	17.4	4.3	2.1	0
407	Stenman	F19-9C11	Siemens	16E2	426*	16.8	4.1	2.1	0
407	Stenman	F19-9C11	Roche	M-INN22	417*	16.6	3.9	1.9	0
407	Stenman	F19-9C11	Paus	E27	419*	15.9	4.1	2.1	0
407	Stenman	F19-9C11	Berger	hCG 22	449*	15.8	3.7	1.8	0
407	Stenman	F19-9C11	Mologic	9F10	429*	15.3	3.2	1.6	0
407	Stenman	F19-9C11	Berger	hCG 24	445*	14.8	3.8	1.6	0
407	Stenman	F19-9C11	Abbott	95658	400*	14.4	3.4	1.6	0
407	Stenman	F19-9C11	Mologic	8G5SheepMono	430*	14.2	2.7	0.7	0
407	Stenman	F19-9C11	Berger	βLH 1	441*	13.7	3.0	1.4	0
407	Stenman	F19-9C11	Stenman	F132-3C10	396*	13.2	2.9	1.4	0
407	Stenman	F19-9C11	Stenman	F16-6G5	382*	11.4	2.3	1.1	0
407	Stenman	F19-9C11	Mologic	618SheepPoly	431*	11.0	3.1	2.0	0
407	Stenman	F19-9C11	Berger	hCG 58	442*	8.8	2.1	1.3	0
407	Stenman	F19-9C11	Berger	hCG 111	434*	3.2	0.6	0.4	0
407	Stenman	F19-9C11	Siemens	3A11	415*	0.1	0.1	0.0	0
409	Medix	5012 SPRN-1	Stenman	F95-1B2	405*	30.9	6.5	3.8	0
409	Medix	5012 SPRN-1	Mologic	D102	423*	24.0	5.3	2.7	0
409	Medix	5012 SPRN-1	Siemens	34A8.1.1	427*	22.7	4.8	2.7	0
409	Medix	5012 SPRN-1	Stenman	F95-1E8	401*	21.0	5.3	2.6	0
409	Medix	5012 SPRN-1	Stenman	F95-5C4	399*	20.4	4.3	2.4	0
409	Medix	5012 SPRN-1	Medix	5006 SP5	388*	16.9	3.3	2.3	0
409	Medix	5012 SPRN-1	Mologic	8F11SheepMono	428*	16.7	3.5	1.7	0
409	Medix	5012 SPRN-1	Medix	5008 SP-5	390*	16.4	4.2	2.0	0
409	Medix	5012 SPRN-1	Mologic	8G5SheepMono	430*	15.6	2.6	0.9	0
409	Medix	5012 SPRN-1	Medix	5004 SP-1	402*	14.6	3.3	1.9	0
409	Medix	5012 SPRN-1	Mologic	9F10	429*	13.7	2.7	1.5	0
409	Medix	5012 SPRN-1	Siemens	16E2	426*	13.6	3.2	1.7	0
409	Medix	5012 SPRN-1	Berger	βLH 1	441*	13.5	3.3	1.5	0
409	Medix	5012 SPRN-1	Abbott	95658	400*	13.3	3.2	1.6	0
409	Medix	5012 SPRN-1	Roche	M-INN22	417*	12.7	3.0	1.5	0
409	Medix	5012 SPRN-1	Berger	hCG 22	449*	12.2	2.8	1.4	0
409	Medix	5012 SPRN-1	Paus	E30	416*	12.1	2.9	1.5	0
409	Medix	5012 SPRN-1	Paus	E27	419*	12.0	2.9	0.8	0
409	Medix	5012 SPRN-1	Stenman	F132-3C10	396*	11.9	1.7	0.4	0
409	Medix	5012 SPRN-1	Berger	hCG 24	445*	10.6	2.6	1.3	0
409	Medix	5012 SPRN-1	Mologic	618SheepPoly	431*	10.3	2.6	1.5	0
409	Medix	5012 SPRN-1	Stenman	F16-6G5	382*	8.6	3.3	1.6	0
409	Medix	5012 SPRN-1	Berger	hCG 58	442*	5.8	1.3	1.1	0
409	Medix	5012 SPRN-1	Berger	hCG 111	434*	2.7	0.4	0.1	0
409	Medix	5012 SPRN-1	Siemens	3A11	415*	0.1	0.1	0.0	0
437	Berger	hCG 64	Berger	hCG 111	434*	10.6	2.6	1.4	0
437	Berger	hCG 64	Berger	βLH 1	441*	10.3	2.3	1.4	0
437	Berger	hCG 64	Stenman	F95-1B2	405*	5.8	1.1	0.7	0
437	Berger	hCG 64	Stenman	F95-1E8	401*	1.2	0.4	0.3	0
437	Berger	hCG 64	Stenman	F95-5C4	399*	0.9	0.0	-0.2	0
437	Berger	hCG 64	Abbott	95658	400*	0.5	0.0	0.0	0
439	Berger	hCG 68	Stenman	F95-1B2	405*	15.9	3.6	2.4	0
439	Berger	hCG 68	Stenman	F95-1E8	401*	13.7	3.0	1.7	0
439	Berger	hCG 68	Stenman	F95-5C4	399*	10.7	2.2	1.3	0
439	Berger	hCG 68	Abbott	95658	400*	10.0	2.1	1.2	0
439	Berger	hCG 68	Berger	βLH 1	441*	8.2	1.7	0.7	0
439	Berger	hCG 68	Berger	hCG 111	434*	4.3	0.7	0.3	0
410	Roche	M-BCG005	Berger	βLH 1	441*	16.1	3.7	0.5	0
410	Roche	M-BCG005	Stenman	F95-1E8	401*	15.9	3.6	2.1	0
410	Roche	M-BCG005	Abbott	95658	400*	13.0	2.9	1.6	0
410	Roche	M-BCG005	Stenman	F95-5C4	399*	11.2	2.6	1.5	0
410	Roche	M-BCG005	Stenman	F95-1B2	405*	8.2	1.8	1.2	0
410	Roche	M-BCG005	Berger	hCG 111	434*	4.6	0.9	0.5	0

- Immunometric assay combinations measuring free hCGβ.
- The results are sorted by assay responses obtained at three different concentrations of hCGβ.



TD-7.2 antibodies working best in immunoradiometric assays for free hCG β

Solid Phase antibody			Tracer antibody			Bound tracer mAb (%)			
						hCG β , 99/650			
						100pM	20pM	10pM	0pM
407	F19-9C11	Stenman	405*	F95-1B2	Stenman	34.6	7.9	3.7	0
407	F19-9C11		423*	D102	Mologic	25.2	6.0	2.8	0
407	F19-9C11		399*	F95-5C4	Stenman	23.3	5.0	2.8	0
407	F19-9C11		427*	34A8.1.1	Siemens	23.0	5.0	2.2	0
407	F19-9C11		401*	F95-1E8	Stenman	22.0	5.6	2.8	0
407	F19-9C11		388*	5006 SP5	Medix	20.8	5.4	3.7	0
407	F19-9C11		390*	5008 SP-5	Medix	20.8	5.0	2.3	0
407	F19-9C11		402*	5004 SP-1	Medix	20.1	4.6	2.3	0
409	5012 SPRN-1	Medix	405*	F95-1B2	Stenman	30.9	6.5	3.8	0
409	5012 SPRN-1		423*	D102	Mologic	24.0	5.3	2.7	0
409	5012 SPRN-1		427*	34A8.1.1	Siemens	22.7	4.8	2.7	0
409	5012 SPRN-1		401*	F95-1E8	Stenman	21.0	5.3	2.6	0
409	5012 SPRN-1		399*	F95-5C4	Stenman	20.4	4.3	2.4	0



Binding of ¹²⁵I-labelled antigens (B/T %)

ISOBM	mAb	Name	Source	*hCG	*hCGn	*hCGβ	*hCGβn	*hCGβcl	LH	*hCGα	Group	Epitope code
435	hCG 2	INN		14	12	36	20	39	0	0		β1
386	F94-8F8	Stenman		9	3	61	28	43	1	0	A	β1
403	M-INN2	Roche		19	17	40	22	52	0	0	highly specific	β1
408	5016 SPRN-5	Medix		98	87	96	49	90	1	0	specific	β1
449	hCG 22	INN		76	72	83	43	82	18	0		β2
434	hCG 111	INN		30	26	31	13	49	32	0		β3
441	βLH 1	INN		31	25	55	23	53	27	0		β3
445	hCG 24	INN		74	63	75	38	71	21	0		β4
442	hCG 58	INN		81	72	72	38	86	52	0		β5
382	F16-6G5	Stenman		77	66	76	37	77	12	0		
388	5006 SP-5	Medix		98	91	99	52	93	27	0		
390	5008 SP-5	Medix		95	88	97	49	89	15	0		
396	F132-3C10	Stenman		83	77	63	34	72	61	0		
399	F95-5C4	Stenman		97	90	98	50	93	68	0		
400	95658	Abbott		73	66	83	42	86	57	0		
401	F95-1E8	Stenman		87	79	93	49	91	64	0		
402	5004 SP-1	Medix		97	92	98	52	92	42	0		
405	F95-1B2	Stenman		94	90	99	52	95	69	0		
415	3A11	Siemens		10	8	89	44	78	6	0	B	
416	E30	Paus		94	85	94	47	87	17	0	pan hCG	
417	M-INN22	Roche		94	87	95	49	91	18	0		
419	E27	Paus		94	88	91	45	83	24	0		
423	D102	Mologic		97	89	98	51	91	67	0		
426	16E2	Siemens		93	88	91	50	91	22	0		
427	34A8.1.1	Siemens		94	91	96	52	92	13	0		
430	8G5	Mologic		71	60	73	37	64	55	0		
431	618 (LP purified)	Mologic		97	88	96	51	92	33	1		
428	8F11	Mologic		61	60	71	35	71	65	0		
429	9F10	Mologic		69	66	80	39	77	60	0		
437	hCG 64	INN		2	3	74	32	46	1	0		β6
439	hCG 68	INN		2	3	46	20	28	1	0		β7
407	F19-9C11	Stenman		1	1	65	23	2	0	0		
409	5012 SPRN-1	Medix		1	2	88	44	84	1	0	C	
410	M-BCG005	Roche		1	2	94	47	86	2	0	free hCGβ	
397	F142-7F3	Stenman		1	1	3	2	4	1	0		
418	2F11	Siemens		0	0	9	2	0	0	0		
450	h54	INN		33	25	71	28	0	0	0		β8
424	1G4	Siemens		95	90	58	13	0	2	0		
406	5011 SPRN-1	Medix		71	77	62	9	0	0	0		
413	D101	Mologic		46	40	81	30	0	1	0	D	
420	M-94.139	Roche		46	34	73	26	0	0	0	hCGβCTP	
392	F20-6E11	Stenman		6	6	22	2	0	4	2		
394	5014 SPTN-5	Medix		71	67	30	9	0	1	0		
395	71752	Abbott		20	13	46	11	0	0	0		

Monoclonal antibodies forming the best immunometric assay pair for measurement of free hCGβ are from group C and group B

The best antibody pair is ISOBM mAb 407, **F19-9C11** and ISOBM mAb 405, **F95-1B2**

Binding of ¹²⁵I-labelled antigens (B/T %)

ISOBM mAb	Name	Source	*hCG	*hCGn	*hCGb	*hCGβn	*hCGβcf	LH	*hCGα	Group	Epitope code
447	hCG 10	INN	68	13	1	0	1	27	0	E XR hLH no XR hCGn	c1
438	hCG 53	INN	68	18	5	1	1	26	0		c2
436	hCG 40	INN	64	12	6	0	0	25	0		c2
446	hCG 45	INN	46	39	2	1	0	0	0		c3
440	hCG 26	INN	71	59	5	1	0	46	0		c4
389	F140-11C5	Stenman	91	15	12	0	1	4	0		
425	5E5	Siemens	85	13	1	0	0	2	0		
422	E28	Paus	18	6	1	0	0	0	0		
433	45A10	Medix	16	15	1	1	0	0	0		
411	M-1F7.9	Roche	21	11	1	0	0	2	0		
387	5009 SP-5	Medix	87	14	1	0	0	27	0		
414	E26	Paus	94	26	2	2	1	72	0		
448	hCG 103	INN	0	1	1	2	55	0	0	F hCGβcf	β10
444	hCG 106	INN	0	1	2	1	62	0	0		β11
443	hCG 112	INN	0	1	1	2	70	0	0		β13
384	F52-3F8	Stenman	1	1	1	1	76	0	0		
393	F52-3C11	Stenman	1	1	1	1	52	0	0		
398	F26-2G11	Stenman	30	36	10	8	13	25	51	G hCGα	
383	5501 SP-1	Medix	88	90	1	4	0	79	100		
385	5503 SP-1	Medix	94	82	1	4	0	77	92		
391	6601 SPR-5	Medix	47	31	1	1	0	49	61		
412	34/25.2.2	Siemens	46	31	1	1	0	43	62		
421	411/100.1.1.200	Siemens	47	33	1	1	0	50	63		
432	41-3-9	Medix	29	27	1	1	0	14	17		
404	F26-7E10	Stenman	3	11	1	3	1	17	96		

hCGβcore fragments
can be measured with
antibody pairs from
group C and group F



Immunometric assay combinations of group C and F antibodies

Antigen: hCG β (WHO 99/650)

Solid phase mAb	Iodinated tracer antibody									
	Group C					Group F				
	407	409	410	437	439	384	393	443	444	448
Group C										
407	0	0	53	29	0	0	0	0	1	1
409	0	0	63	33	0	0	0	0	1	1
410	37	35	1	0	0	0	0	0	1	1
437	27	25	0	1	0	0	0	0	1	1
439	1	0	1	1	0	0	0	0	1	1
Group F										
384	0	0	0	0	0	0	0	0	0	0
393	0	0	0	0	0	0	0	0	0	0
443	0	0	0	0	0	0	0	0	1	0
444	0	0	0	0	0	0	0	0	1	1
448	0	0	1	1	0	0	0	0	1	1

ISOBM mAb 407, F19-9C11 seems to be the most specific monoclonal antibody for free hCG β

Immunometric assay combinations of group C and F antibodies

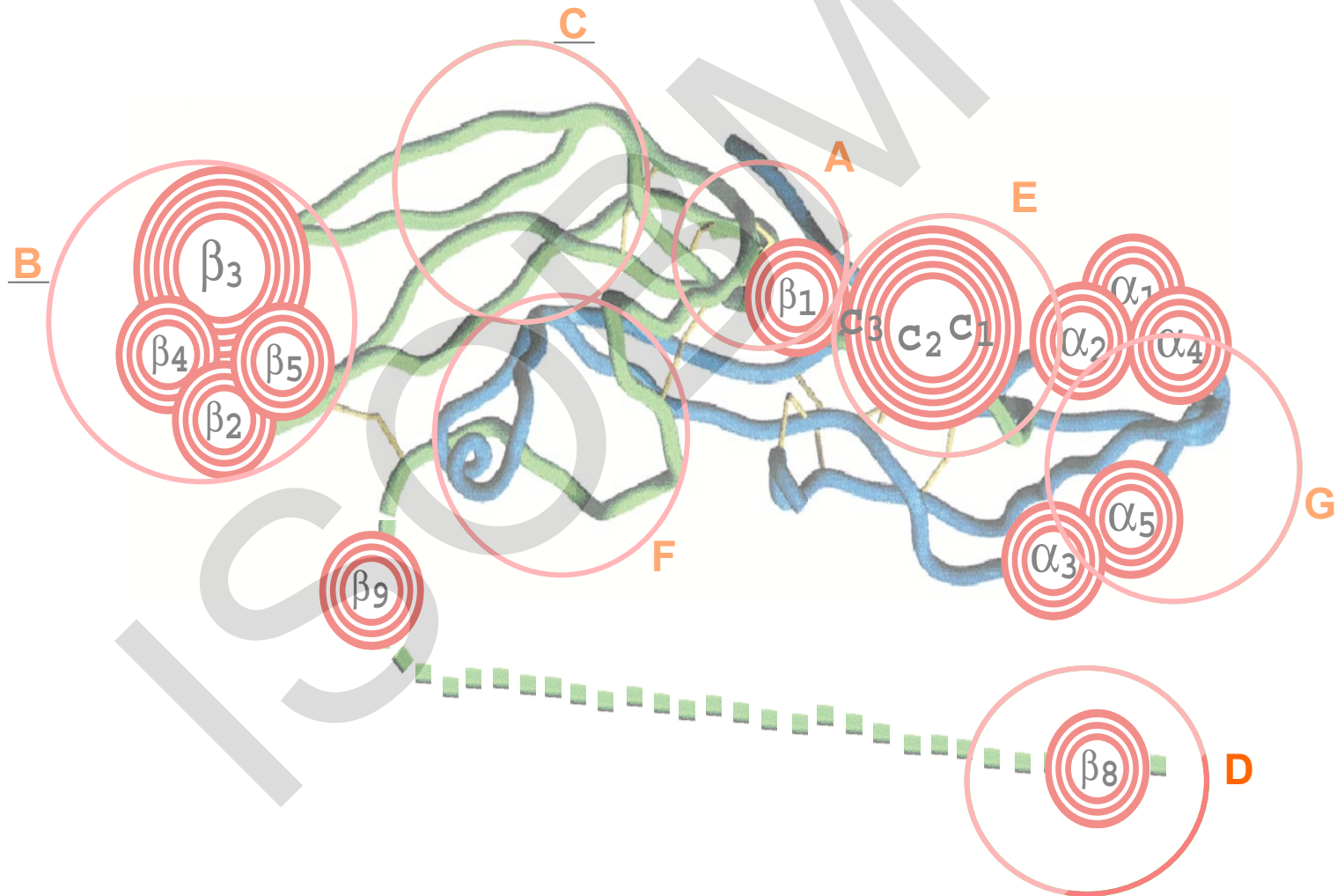
Antigen: hCG β core (WHO 99/708)

Solid phase mAb	Iodinated tracer antibody									
	Group C					Group F				
	407	409	410	437	439	384	444	443	448	393
Group C										
407	0	0	1	0	0	0	1	0	0	0
409	0	0	62	34	0	1	1	0	0	0
410	12	51	0	0	0	10	1	66	61	20
437	2	30	0	0	0	1	1	35	32	4
439	0	0	2	1	0	26	31	1	0	1
Group F										
384	0	0	8	0	13	4	1	1	1	37
444	0	0	0	0	19	2	1	0	0	36
443	0	0	64	34	0	3	0	0	62	36
448	0	0	66	41	0	1	1	0	0	0
393	0	0	27	2	0	46	42	40	0	0

hCG β core fragments can be measured with antibody pairs from group C and group F



3D Epitope map of hCG. Berger et al 2002



Summary

- **Seventy antibodies to hCG were classified using binding data to labelled hCG-related molecules.**
- **The antibodies were sorted into seven main groups.**
- **Specific immunometric assays for free hCG β were possible with mAbs from group C (free hCG β) and group B (pan mAbs).**
- **These preliminary studies show that the most sensitive and specific immunometric assay for free hCG β was obtained with mAbs F19-9C11 and F95-1B2.**
- **Assays for hCG β core fragment could be made using mAbs from group C and F.**

