

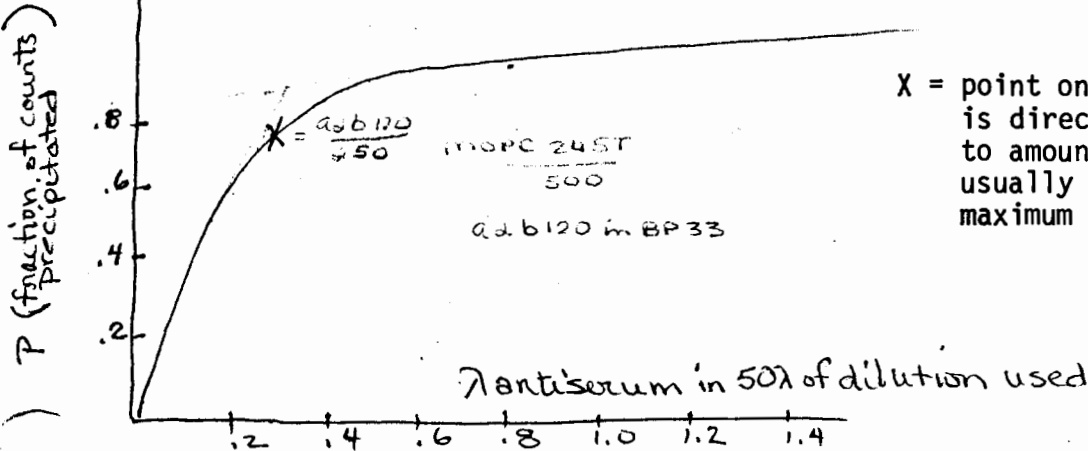
Radioimmunoassay

I. ANTIBODY TITRATION CURVE

The first step in the set-up of the radio immunoassay is to choose an antibody concentration at which the known amount of Ag* will be precipitated in direct proportion to the amount of antibody available.

The antigen is used at a concentration to yield approximately 10,000 - 20,000 cpm in the maximum precipitate tube supernatant. This is usually a 1/500 dilution of I¹²⁵ labelled antigen using our labelling technique.

1/350 if extra purification steps taken in labeling procedure
 The antibody concentrations used are 1:2 dilutions beginning with $\approx 25 \lambda$ total Ab present.



X = point on curve when ppt is directly proportional to amount of Ab added, usually chosen at 2/3 maximum precipitation.

Maximum ppt 100% is determined by count of tube to which no antiserum is added since no ppt occurs and all radioactivity is in the supernatant.

$1/40 \rightarrow 1/5 \cdot 120$

$10 \times / 100 \times$

Not too clean - SJL P1 - Ig 1b
 MOPC 245 T - Ig 4b
 GPCB - Ig 1A
add quite clean

use
 a x b 120 A (specific for Ig 1b)
 a x b 120 (x Ig 1b + Ig 4b)

EXAMPLE AB TITRATION CURVE

12-8-71

WELLS	1:500 # M3PC245T	BP 33	uL 120 diln's	λ AB	C/min	AV C/ min	% PPT
1	50 λ	50 λ	-	-	9046	9850	
2		50 λ	-	-	9108		
3		-	1/40	1.25	1908	1893	~80
4		-	"	"	1883		
5		-	1/80	.625	1902	1920	~80
6		-	"	"	1925		
7		-	1/160	.312	2018	2025	~74
8		-	"	"	1820		
9		-	1/320	.156	2001	4090	51
10		-	"	"	1800		
11		-	1/640	.078	4033	6350	30
12		-	"	"	4131		
13		-	1/1280	.04	6881	7930	19
14		-	"	"	6792		
15		-	1/2560	.02	8074	8870	10
16		-	"	"	7807		
17		-	1/5120	.01	8671	8800	10
18		-	"	"	9065		
19		50 λ	-	-	10,077		
20		50 λ	-	-	9,633		

← Ab dilution yielding 2/3% ppt lies between these 2 dilutions

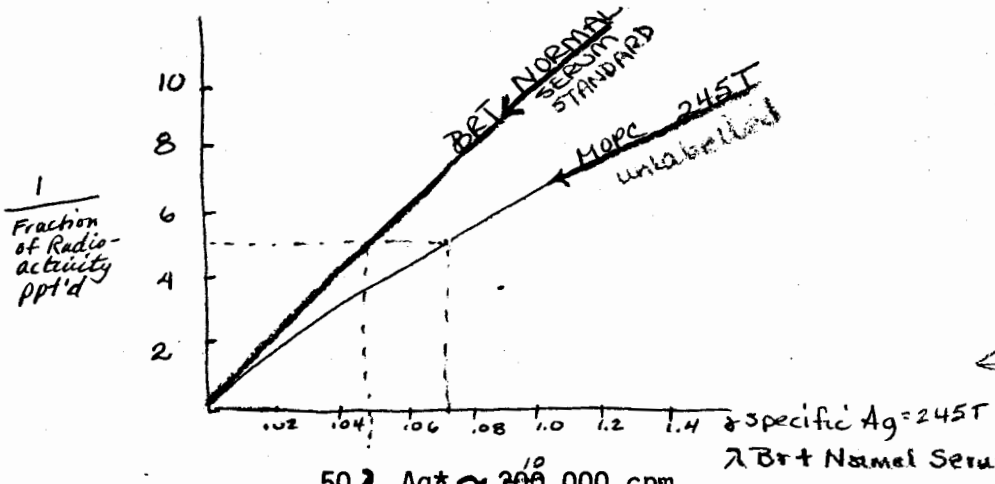
.2 λ total Ab was chosen as amount to be used in assays = 1/250 dilution

averaged above with tube #1,2

M3PC 245T made 1:500 400 λ 120 mL
 a + b 120 dil 1:40 10 λ 100 λ serial 1:2 dilutions to 1/5120 with 500 λ
 λ AB in 1/40 assay tube = $\frac{50\lambda \text{ sampled} \times \text{conc of } 10\lambda \text{ Ab}}{400\lambda \text{ solution}} = 1.25\lambda$

II. STANDARD PRECIPITATION CURVE [INHIBITION ASSAY]

Normal serum standard



Inhibitors @ conc range
 1/20 - 1/520 range of
 antigen expected in normal
 serum.

50 λ Ag* \approx 200,000 cpm
 5 λ standard Ag at varying dilutions $1/5 - 1/500$
 50 λ Ab @ dilution which ppt \approx 2/3 maximum number of
 counts precipitable by that antiserum

$\frac{1}{X}$ = Maximum fraction of labelled Ag precipitable
 by amount of Ab used

Minimum Ag detectable \sim .02 mg/ml serum

e.g., Brt serum as inhibitor of MPOC245T Ag* + a**ad**b120 @ 2/3 ppt. conc.
 for 20% ppt, 1/20% = 5. Reading μ gm inhib. to give 20% ppt =

$$\frac{.07 \mu\text{gm Specific Ag}}{X \lambda \text{ BRT}} = \text{mg Ig 4}^b / \text{ml serum}$$

8-21-72 Ig 4^b assay

EXAMPLE STANDARD PRECIPITATION CURVE

1-3-72

Inhibitors = unlabelled 245T and BRT NORMAL SERUM STANDARDS

in BP33

UBS	MOPC 245T 500	BP33	Inhibitors	in 3% BSA	245T 250	1/2 min	A ₄₁₅	% ppt	1/P	
1	500	500	500	3% BSA	=	3233	4500	0	/	CONTROLS
2		500	"			3931				
3		-	"		500	1022	1000	75	1.37	
4		-	"		500	1022				
5		-	BRT 4154	1/40 = .025		3498			6.6	BRT
6		-				3445			5.1	
7		-	1/80	.063		3305				
8		-				3225			4.3	
9		-	1/120	.042		3155				
10		-				3142			3.5	
11		-	1/160	.031		2951				
12		-				2777			2.7	
13		-	1/240	.021		2577				
14		-				2502			2.3	
15		-	1/320	.016		2346				
16		-				2246			1.8	
17		-	1/640	.008		1904				
18		-				1715			1.5	
19		-	1/1280	.004		1326				
20		-				1092			1.0	
21		-	1/2560	.002		727				
22		-				512			0.5	
23		-	MOPC 245T .025 ml/ml	.1250		3877				
24		-	0/λ			3501			5.1	
25		-	.0125 ml/λ	.063		3435				
26		-				3303			3.2	
27		-	.0063	.031		2823				
28		-				2728			2.2	
29		-	.0031	.016		2254				
30		-				2165			1.7	
31		-	.0016	.008		1689				
32		-				1673			1.5	
33		-	.0008	.004		1208				
34		-				1364			1.4	
35		-	.0004	.002		1160				
36		-				1247			1.3	
37		-	.0002	.001		1267				
38	V	-			V	1154				

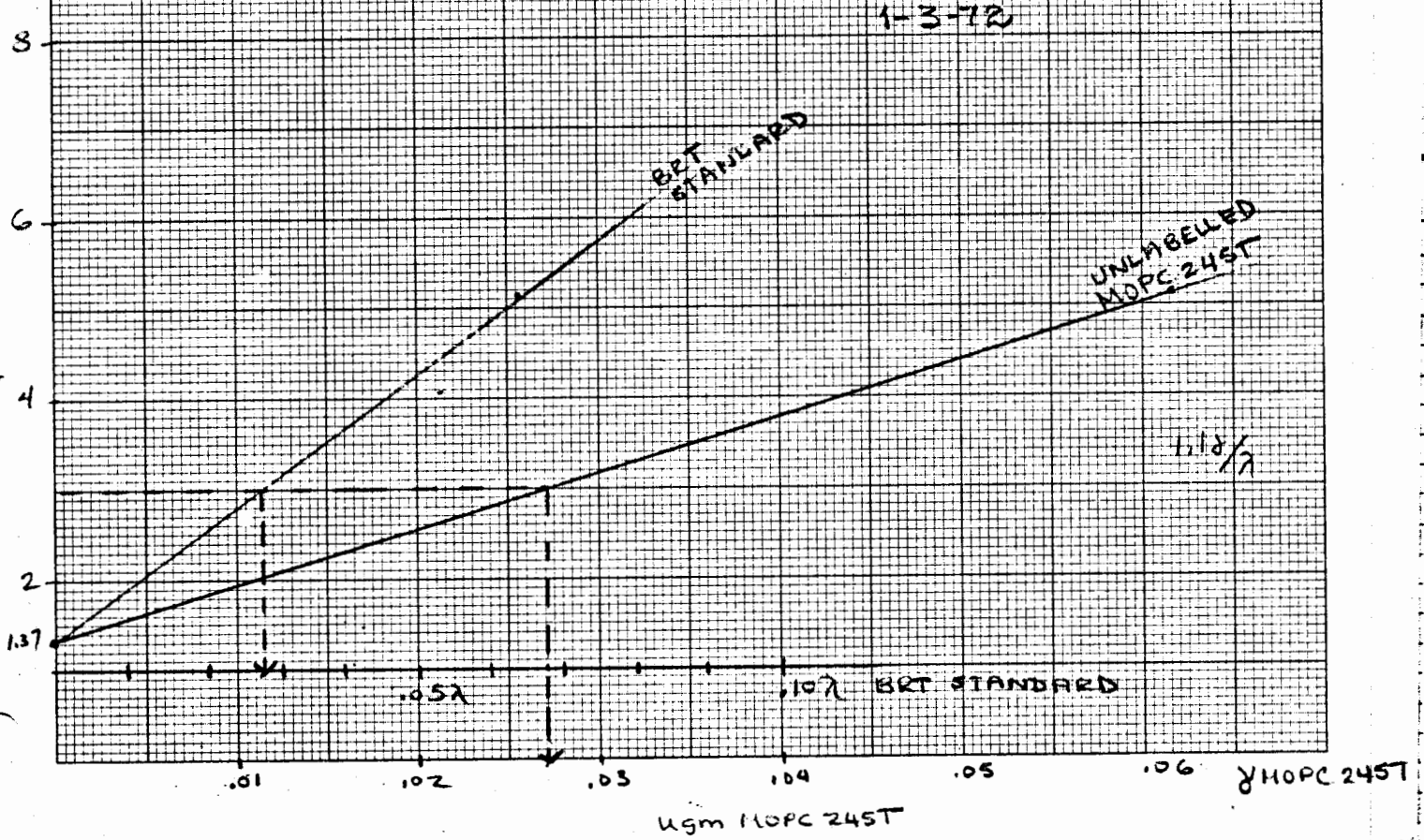
$106120/250 = 607 \text{ } \mu\text{g/ml}$ $381 \mu\text{g/ml} \times 500 = 19050 \sim 2 \text{ mg}$

MOPC 245T TOTAL PROTEIN \times .0031 λ \times 500 λ sampled = .0168 specific protein conc

Normal BRT λ = 2.2 λ = 2.3 λ = 2.4 λ = 2.5 λ = 2.6 λ = 2.7 λ = 2.8 λ = 2.9 λ = 3.0 λ = 3.1 λ = 3.2 λ = 3.3 λ = 3.4 λ = 3.5 λ = 3.6 λ = 3.7 λ = 3.8 λ = 3.9 λ = 4.0 λ = 4.1 λ = 4.2 λ = 4.3 λ = 4.4 λ = 4.5 λ = 4.6 λ = 4.7 λ = 4.8 λ = 4.9 λ = 5.0

λ BRT-standard # 54

STANDARD PPTN CURVE



III. DIRECT PRECIPITATION

Qualitative assay where you are measuring unknown for its antigen binding activity. Detection of antibody.

Ag conc - 1/500 of labelled goody by hand
1/250 " " " " compupet

Ab unknown 1/5 → 1/100 or at concentration
determined from Ouchterlony
strength estimates.

Can choose comparisons of sera's binding ability as to amount necessary to ppt 50% counts.

α Mouse Ig (R65) will show max. ppt obtainable with labelled preparation.

IV. INHIBITION ASSAY (test for presence of classes - heavy chain)

Unknown acting as antigen to inhibit pptn of Ag* with Ab @ 2/3 ppt point
Quantitative

1. Series with known inhibitor = normal serum standard
2. Unlabelled Ag is plotted on 1/p scale with ugm inhibitor on bottom scale.

It is best to use antiserum which has previously been absorbed and made specific. However, if a purified myeloma protein is used as the labelled antigen, an antiserum may be usable even if it has several specificities. This is because only the reaction with the labelled protein is relevant. However, it is extremely important in this situation to ensure that the Clq supply is not consumed by these irrelevant complexes.

EXAMPLE I_{94}^b assay in Suppressed Mouse Serum

Example Ig 4b assay

05710345A

	Mape-245T*G 250	BP33	Inhibitor		a+b 300	%min	Ave	% of rest	1/p
controls		50λ	5λ 3% BSA		—	7073	6976	63%	
		50λ	"		—	6877			
		—	"		50λ	2452	4607		
		—	"		"	2671			
		—	Bnt Std #54	1/80	"	5819			
		—			"	5569			
		—		1/120	"	6578			
		—			"	6170			
		—		1/160	"	4607			
		—			"	4452			
4b protein standards				1/240	"	5290			
					"	5108			
				1/320	"	3954			
					"	3844			
				1/640	"	3535			
					"	3565			
				1/1280	"	3040			
					"	3053			
		Supp 93 5/30/73	40420	1/10	"	3954	} Reject		
					"	3844			
			40421	1/10	"	3535	} Reject		
					"	3565			
			40422	1/100	"	6647	} Suppressed		
					"	7194			
			40423	1/10	"	5613	} ? Reject		
				"	5139				
		40424	1/100	"	6732	} Suppressed because all counts in supernatant, the test sera did inhibit 24b precipitation of labeled 4b antigen			
				"	6735				
		etc	1/100	"	5798				
				"	5709				
				"	5352				
				"	5257				
				"	6054				
				"	6240				
				"	5595				
				"	5939				

Add 25λ HOPC 245T*G/250
 5λ Inhibitor @ 1/100, wash 20λ
 50λ Antiserum @ 1/20/300 in BP33