HIGH BINDING CAPACITY SURFACE FOR IMMUNOLOGICAL ASSAYS

TECHNICAL NOTE N. 4

Comparison of different types of High Binding capacity polystyrene strips

In order to check the performances of different polystyrene strips' surfaces we performed an extensive study comparing Biomat High Binding Capacity (HB8) strips with one of the most used High Binding Capacity type of strips currently available on the market.

To ensure the validity of results the test was performed with methods as near as possible to the standard methods which, in our knowledge, are used by manufacturers when preparing polystyrene strips as solid phase to set up diagnostic kits (e.g. ToRCH ELISA kits).

The kind of molecules used for testing were both IgM and IgG for determination of Rubella, Cytomegalovirus and Toxoplasma.

Materials and methods

I Preparation of plates

Antigens for Rubella, Cytomegalovirus and a polyclonal antibody Rabbit IgG to Human IgM (DAKO A426) were diluted in carbonate-bicarbonate buffer 0.1 M pH 9.6 and both samples of strips were coated at the same time. The coating was performed at 4° C.

After a washing step the plates were saturated with PBS 0.1 M pH 7.2 containing 1% Bovine Serum Albumin and incubated overnight at 4°C.

After a further washing step the plates were dried at 37°C for two hours, then sealed under vacuum and stored at 4°C until use.

All the sera used in this test came from hospital laboratories and were certified to be positive or negative using the commercial kits manufactured by: Behring; Biomerieux-Vidas; Sorin Biomedica.

II IgG assay

The scheme for performing the IgG assays was the following:

1. 100µl diluted samples and calibrators were incubated for 30 min at room temperature in each type of antigen- coated wells
2. a washing step with 0.1M PBS pH 7.2 + 0.05% Tween® 20 was performed
3. 100µl/well of purified goat-anti Human Fc IgG Peroxidase were added and incubated for 30 min at room temperature
4. a further washing step as that at point 2. was performed
5. 100µl/well of substrate (TMB) were added and incubated for 15 min at room temperature
6. the reaction was stopped by adding 100 µl of sulphuric acid
7. reading at 450 nm was then performed

III IgM capture assay

The scheme for performing the IgM assays was the following:

1. 100µl diluted samples and calibrators were incubated for 1 hour at room temperature in the common anti-IgM coated wells
2. a washing step with 0.1M PBS pH 7.2 + 0.05% Tween® 20 was performed
3. 100µl/well of a complex of the appropriate biotinylated purified antigen and streptavidin-peroxidase was added and incubated for 1 hour at room temperature
4. a further washing step as that at point 2. was performed
5. 100µl/well of substrate (TMB) were added and incubated for 30 min at room temperature
6. the reaction was stopped by adding 100 µl of sulphuric acid
7. reading at 450 nm was then performed

ANALYSIS OF DATA

The data obtained from the two types of samples of microplates were processed in the following way:

NEGATIVE SAMPLES	POSITIVE SAMPLES
a. min. O.D. observed for each type of strip	a. coefficient of correlation
b. Max. O.D. observed for each type of strip	b. linear regression calculated as $y = a + bx$ (1)
c. mean of Standard Deviations	with a confidence level of 95% x values were those obtained with competitor's samples y values were those obtained with Biomat HB8 samples

The results are exposed in the following tables

TABLE A IgG assays

IgG assay to Cytomegalovirus			**IgG assay to Rubella**		
TOTAL SERA TESTED	51		TOTAL SERA TESTED	81	
NEGATIVE SERA	32		NEGATIVE SERA	46	
POSITIVE SERA	19		POSITIVE SERA	35	
calibrators A.U. /ml	O.D. comp.	O.D. biomat	calibrators I.U. /ml	O.D. comp.	O.D. biomat
120	2,393	2,272	250	3,367	2,971
50	1,375	1,463	75	1,833	1,624
20	0,748	0,891	25	0,718	0,609
10	0,322	0,38	8	0,251	0,246
0	0,015	0,012	0	0,015	0,016
A.U.=Arbitrary Unit			I.U.=International Unit		
O.D. of negative sera	comp.	biomat	O.D. of negative sera	comp.	biomat
minumum	0,031	0,043	minumum	0,035	0,027
Maximum	0,183	0,173	Maximum	0,192	0,119
mean of Standard Deviation	0,111+-0,035	0,109+-0,030	mean of Standard Deviation	0,076 +- 0,032	0,061 +- 0,023
results of positive sera			results of positive sera		
R=	0.994		R=	0,961	
y=	2,644+0,996x		y=	4,567 + 0,948 x	

TABLE B IgM assays

IgM assay to Cytomegalovirus			**IgM assay to Toxoplasma Gondii**		
TOTAL SERA TESTED	32		TOTAL SERA TESTED	58	
NEGATIVE SERA	23		NEGATIVE SERA	27	
POSITIVE SERA	9		POSITIVE SERA	31	
calibrators	O.D. comp.	O.D. biomat	calibrators	O.D. comp.	O.D. biomat
HIGH POSITIVE	2,092	1,732	HIGH POSITIVE	2,673	2,353
LOW POSITIVE	0,65	0,558	LOW POSITIVE	0,931	0,665
NEGATIVE	0,146	0,14	NEGATIVE	0,225	0,17
O.D. of negative sera	comp.	biomat	O.D. of negative sera	comp.	biomat
minumum	0,153	0,142	minumum	0,228	0,174
Maximum	0,431	0,36	Maximum	0,847	0,594
mean of Standard Deviation	0,189+-0,06	0,181+-0,04	mean of Standard Deviation	0,484+-0,186	0,38+-0,131
results of positive sera			results of positive sera		
R=	0,975		R=	0,978	
y=	0,24+0,84 x		y=	0,047+0,88 x	

The above results are confirmed by the correspondence with the clinical data obtained by the hospital laboratory

DISCUSSION OF RESULTS

The analysis of the data exposed in tables A and B shows:

- a comparable binding capacity of proteins of both Biomat HB8 and competitor's strips
- the capacity of both types of samples to assure the specific binding between the coated protein and the protein to be revealed: 100% of results of our tests (on 232 sera, 94 positive and 128 negative) were confirmed stating the sensitivity and specificity of both types of samples with all the tested sera
- the result of regression analysis, whose acceptable value had been fixed at $R \geq 0.95$ has been fully respected
- the ranges of coefficients and values obtained in equation (1):

a which must not significantly differ from 0

And

b whose values ranged from $0.8 \leq b \leq 1.2$

proved the strict correspondence of results.