

Testing of ATP Loaded Anti Strep Group A Liposomes Using Sandwich Assay on a PVC Plate

1. A polyvinylchloride ELISA plate was precoated with rabbit anti Group A streptococci antibody in 0.05M carbonate-bicarbonate buffer pH 9.5 for 18 hours at 4° C., then blocked with 1% BSA, then washed with 0.05M phosphate buffered saline, and 0.05% Triton X-100, pH 7.5.
2. Fifty microliters of dilutions of nitrous acid extracts of Group A and Group C streptococci were reacted for 30 minutes in the ELISA plate. The plate was then washed with the PBS-triton buffer.
3. Fifty microliters of a 1:25 dilution of anti strep Group A liposomes were added to each well and incubated for 30 minutes at room temperature. The plate was then washed with PBS Triton X buffer.
4. Fifty microliters of ATP releasing reagent (saponin detergent) was added to each well and then reacted with fifty microliters of a luciferin-luciferase reagent.
5. The light emitted was read in a luminometer.

DILUTION OF CARBOHYDRATE (CHO)	Light Units Emitted/Min	
	A CHO	C CHO
10 ⁻²	15,384	389
10 ⁻³	17,976	459
10 ⁻⁴	7,552	587
10 ⁻⁵	1,492	417
10 ⁻⁶	556	458

The presence of Group A strep is indicated by the higher light emission of the Group A infected sample. This corresponds to the significant presence of ATP.

EXAMPLE 2

The procedure of Example 1 was followed for preparing the Fab' fragments, and coupling of Fab' to PDP-Liposomes. A filtration assay using capture particles was then performed as follows:

1. One hundred microliters of anti Group A strep or control rabbit immunoglobulin-coupled polystyrene particles (0.25%) were added to a 0.22 microfiltration tray.
2. Fifty microliters of Group A carbohydrate dilutions were added and incubated for 30 minutes at room temperature.
3. The particles were washed in the plate with 0.05M phosphate buffered saline, pH 7.5, containing 0.05% Triton X-100 under vacuum pressure.
4. Fifty microliters of strep liposomes were added and incubated for 30 minutes at room temperature.
5. The particles were washed again in the same PBS Triton buffer.
6. One hundred microliters of releasing reagent (saponin) were added to each well.
7. Fifty microliters of released ATP were added to a cuvette and fifty microliters of luciferin-luciferase reagent were added.
8. The light emitted was read in a luminometer.

DILUTION OF CARBOHYDRATE	Light Units Emitted/Min	
	Anti Gp A	Control
10 ⁻²	32,565	5868
10 ⁻³	23,235	5333
10 ⁻⁴	10,275	5243
10 ⁻⁵	7,555	3224
10 ⁻⁶	6,141	3003

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DILUTION OF CARBOHYDRATE	Light Units Emitted/Min	
	Anti Gp A	Control
10 ⁻⁷	5,696	4004

The presence of Group A strep antigen is indicated by the significant presence of ATP, evidenced by significantly greater light emission of the Group A strep sample than the control, especially at low dilution.

EXAMPLE 3

The procedure of Example 1 was followed for preparing the Fab' fragments, and coupling of Fab' to PDP-liposomes. A rapid immunoassay using capture particles and centrifugation was then performed as follows:

1. One hundred microliters of anti Group A Strep-coupled polystyrene particles (0.25%) were reacted with fifty microliters of Group A or Group C streptococcal extract dilutions, and fifty microliters of anti Group A liposomes (1:5) in microfuge tubes. The reactants were vortexed and incubated for twenty minutes at room temperature.
2. The reactants were centrifuged and washed with PBS Triton buffer.
3. Fifty microliters of releasing reagent were added and mixed with fifty microliters of luciferin-luciferase reagent.
4. The light emitted was read in a luminometer.

DILUTION OF CARBOHYDRATE	Light Units Emitted/Min	
	Gp A CHO	Gp C CHO
10 ⁻²	15,032	1415
10 ⁻³	6,888	1517
10 ⁻⁴	2,558	1277
10 ⁻⁵	2,605	1707
Buffer	1179	

Again, the presence of Group A strep is indicated by the significant presence of ATP, manifested by the significantly greater light emission by the Group A strep sample.

What is claimed is:

1. A process for assaying an analyte, said analyte being a member of a specific binding pair selected from the group consisting of ligand and antiligand, wherein said process comprises the steps of:
 - obtaining a first fluid suspected of containing the analyte to be determined,
 - combining the first fluid with a solid support, said support having been sensitized with receptors that will bind the analyte to be determined,
 - contacting the support with a second fluid comprising ATP encapsulated within the walls of liposomes, said liposomes having bonded thereto a compound, wherein said compound is ligand, ligand analog, or antiligand, and
 - testing for the presence of ATP associated with the support.
2. The process of claim 1, wherein the step of testing for the presence of ATP includes the steps of:
 - contacting the solid support and bound immunological particles thereon with a reagent capable of releasing the ATP from the liposomes,