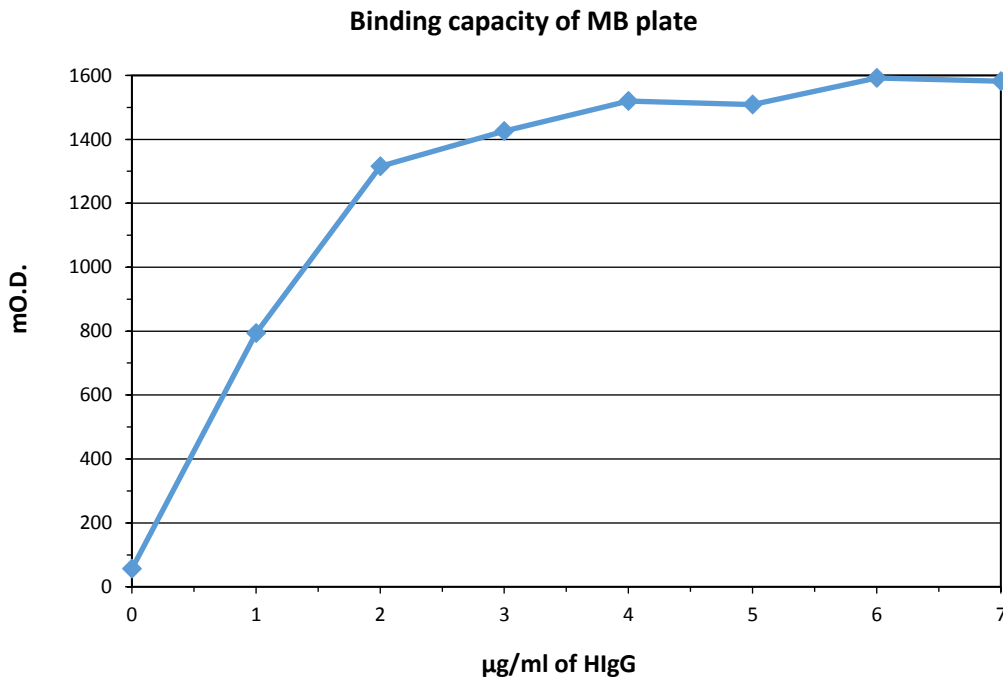


MEDIUM BINDING CAPACITY SURFACE FOR IMMUNOLOGICAL ASSAYS

Method 15

Method 15 is an indirect method with human IgG coated on medium binding plates and then revealed through an AHIgG-HRP conjugate.

1. dispense 100 μ l/well of different concentrations of human IgG diluted in 0.1M PBS pH 7.2: 1-2-3-4-5 μ g/ml and incubate overnight at 4°C
2. wash 3 times with 0.1M PBS pH 7.2 + 0.05% Tween[®] 20
3. dispense 150 μ l/well of BSA 1% in 0.1M PBS pH 7.2 and incubate 2 hours at R.T. for blocking the remaining active sites
4. wash 3 times with 0.1M PBS pH 7.2+ 0.05% Tween[®] 20
5. dispense 100 μ l/well of Goat Anti-HIgG-HRP conjugate and incubate 30' at R.T.
6. wash 3 times with 0.1M PBS pH 7.2 + 0.05% Tween[®] 20
7. dispense 100 μ l/well of TMB
8. after 30' stop the reaction with H₂SO₄ 1 N
9. reading is made at 450 nm



The data show that a plateau has got starting with an IgG concentration of 2.0 μ g/ml.

This concentration means the well binding capacity we can express as:

- μ g/well = 0.200 (200 ng/well/100 μ l)

As 100 μ l of liquid, in term of area, represent 1 cm² it is possible to state that the binding capacity is close to 200 ng/ cm². These data are well correlated with other experiments carried out with an unmodified polystyrene surface.