TechNote 5 : Spherotest-Directions for use

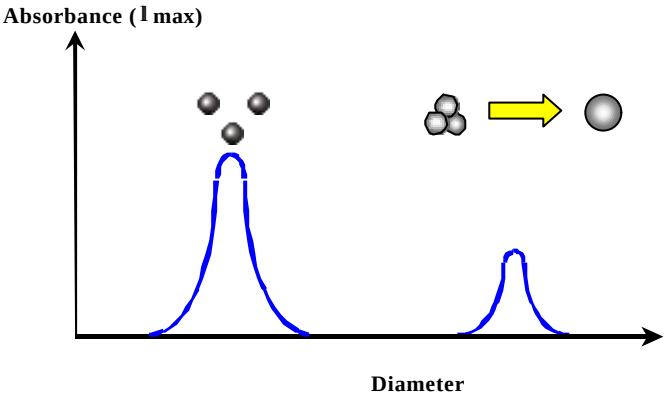
I. PRINCIPLE OF THE TEST

The test uses calibrated and monodispersed polystyrene microspheres sensitised with affinity purified goat antibodies to mouse IgG (H+L).

The reaction is performed in a 96-well microplate under agitation.

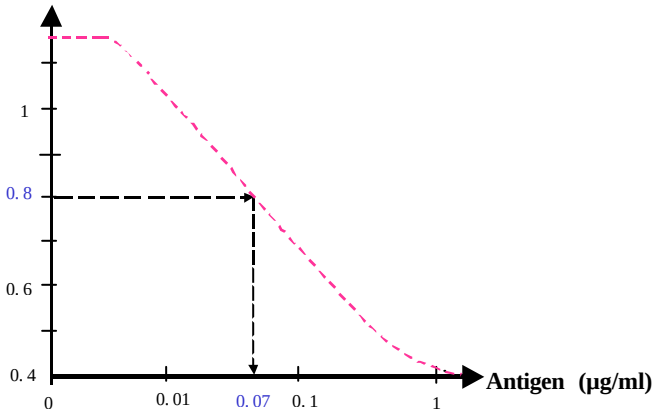
Single non agglutinated submicron microspheres present a maximum of absorption at their ë max which depends on the refractive indices of both particles and environmental buffer, and on the diameter of the particles.

In contact of mouse IgG, sensitised microspheres agglutinate to form clusters of bigger apparent diameter where they do not absorb as well at the same ë max.



Therefore, the decrease of the number of single microspheres can be optically monitored and quantified.

An unknow concentration of mouse IgG can be determinated on a standard curve constructed with serial dilutions of standard sample supplied with the kit.



II. PRODUCT SPECIFICATIONS

- Specificity : mouse IgG (H+L)
- Sensitivity : Limit of detection 2ng/ml
  Standard curve 5 to 320 ng/ml
- Total time of reaction : 10 minutes
- CV : < 5%

III. COMPOSITION OF THE KIT

SHEROTEST reagent
1 tube containing 2 ml of anti-mouse IgG sensitised particles sterically stabilized in a phosphate buffer pH 7.4.
Contains bovine albumin and azide 0.09%.

Dilution buffer
1 vial containing 30 ml of ready to use solution (azide 0.09%)

Blocking solution
1 vial containing 15 ml of ready to use solution (azide 0.09%)

Standard sample
1 vial containing 100 µl of purified mouse IgG (purity 99.5%) at 96 µg/ml (Lowry method).
Contains bovine albumin and azide 0.09%.

One 96-well microplate

IV. INSTRUCTIONS FOR USE

All reagents should be brought to room temperature before use.

1. Dilutions

a) Dilute the samples in the dilution buffer to obtain a final concentration within the range of 5-3520 ng/ml.(Samples expected to contain high level of IgG can be prediluted in deioized water).
b) Dilute the standard sample (mouse IgG 96 µg/ml) 3 fold in deionised water, then 50 fold in the dilution buffer, to obtain a final concentration of 640 ng/ml.

2. Standard curve

a) Add 20 µl of the dilution buffer in each well of the first column of the microplate (8 wells).
b) Add 20 µl of the diluted standard to first well (A1) and mix.
c) Transfer 20 µl from the first well to the second and mix.
d) Proceed in the same manner until the 7th well (G1) and discard the last 20 µl.
e) Keep the last well (H1) free of sample (20 µl of dilution buffer only).

3. Samples

Distribute 20 µl of each sample in the other wells (samples from which IgG concentration cannot be approximately assessed can be serially diluted as indicated for the standard sample).
Note: Keep one well free of sample for the blank (example H12).

4. SPHEROTEST reagent

a) Mix thoroughly the Spherotest reagent for 1 minute with a vortex.
b) Add 20 µl of the Spherotest reagent to each well (standards and samples), excepted in blank.

5. Reaction

a) Place the microplate on a mechanical agitator (1000/rpm) for 5 minutes at room temperature
b) After incubation, stop the agitation and add 100 µl of the blocking solution to each well.
c) Place the microplate on the agitator (600/rpm) for 5 minutes at room temperature.
d) Read the microplate at 340 (or 405) nm.

Note: the blank is obtained with an empty well or in a well containing 40 µl of dilution buffer and 100 µl of blocking solution.

V. INTERPRETING THE RESULTS

1. For each sample and standard, subtract the blank value to the OD value.
   OD= OD sample - OD blank
2. Construct the standard curve OD = f (concentration) on a semi-logarithm diagram.
3. Report the sample values on the standard curve and read the concentration.
4. Calculate the concentration by multiplying the result by the dilution factor D.

Note: all the calculations can be performed with current softwares from your microplate reader supplier.

VI. PRECAUTIONS FOR USE

- Reagents of the kit must be stored at 4-8 °C.
- Reagents must be brought at room temperature before use.
- Use reagents from the kit only.
- For prediluted samples or standards, the final content of deionised water should not exceed 10 %.
- Do not dilute Spherotest reagent.
- Use precise pipettes.
- Careful pipetting and timing are required
- For special samples other than sera, a control assay with the same buffer (free of IgG) as that of the sample should be performed.
- For research use only.