Magnetic Particles in Immunoassays

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Introduction and Summary.

•Immunoassays use antibodies as analytical reagents and have had a major impact in medicine as well as in veterinary and forensic sciences, agriculture and national defence.

•Magnetic particles (or beads) already play an important part in automated immunoassays and are been used in the development of simple, near-patient, diagnostic devices.

• The lectures will cover the following topics:

1. An introduction to antibodies, antigens and different immunoassay formats including the use of magnetic particles as solid phases and as a label.
2. A review of some of the published immunoassay measurement techniques using magnetic particle labels.
3. A description of the applications of the particles in lateral-flow format immunoassays and in immunohistochemistry.

Immunoassays.

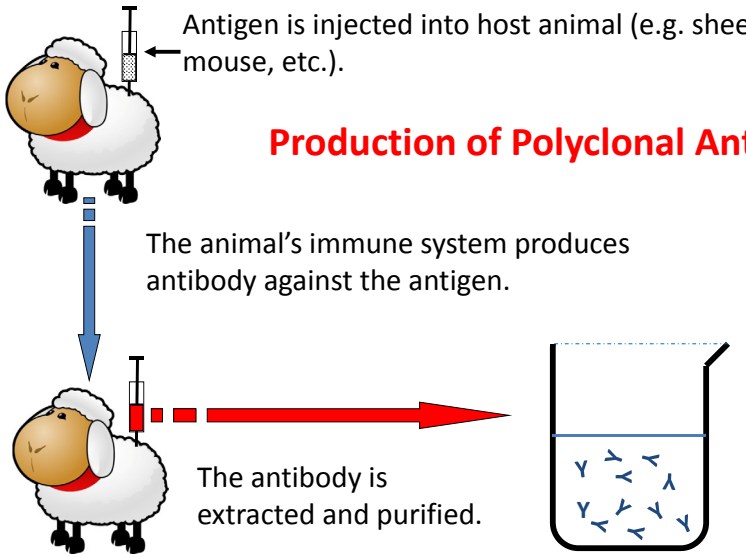
•Specific and sensitive analytical techniques.

•Thousands of immunoassays have been developed over the last 50 years.

•Use reaction between an antigen, Ag (usually the analyte) and its antibody, Ab.

$$Ag + Ab \underset{k_d}{\overset{k_a}{\rightleftharpoons}} Ag.Ab\ complex$$

•Affinity = $\frac{k_a}{k_d}$

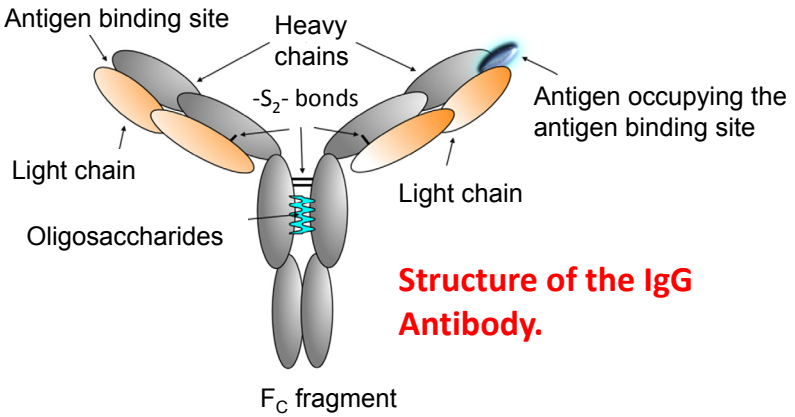


Production of Polyclonal Antibodies

Polyclonal and Monoclonal Antibodies.

•The immune response produces many different antibodies to the antigen (e.g. IgG, IgA, IgM, IgE and IgD, where Ig is immunoglobulin).

•IgG is the most commonly used in immunoassays.



Structure of the IgG Antibody.

•Antibody binds with an epitope on the antigen.
•Some antigen have two or more epitopes to which different polyclonal antibodies may bind.
• Monoclonal antibodies are very specific and bind to just one epitope on the antigen. They are more expensive to produce.

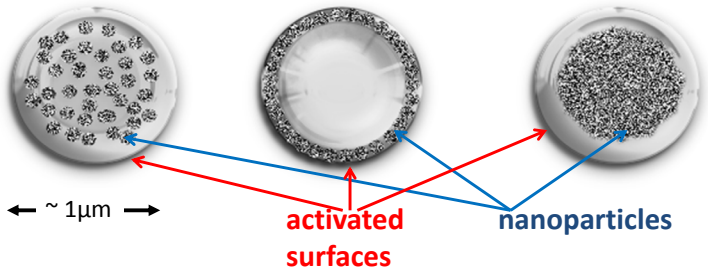
Quantifying Immunoassays.

•Numerous, very sensitive, immunoassay techniques have been developed.

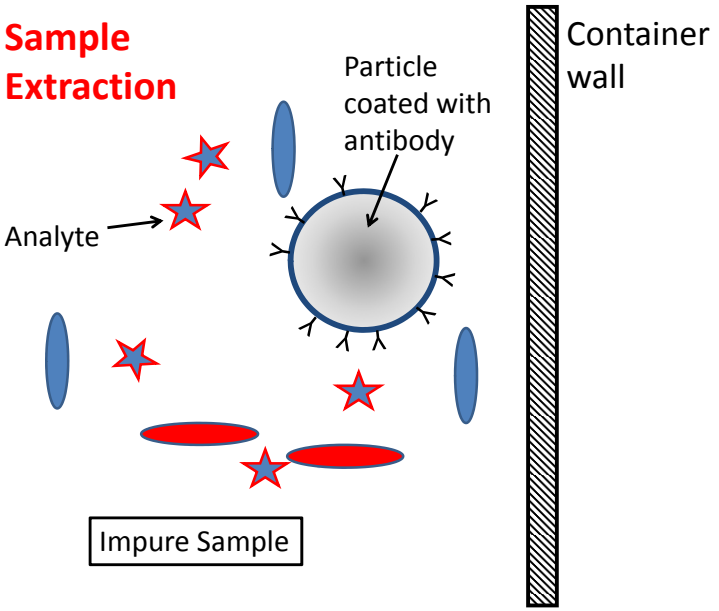
•Many automated analysers use magnetic particles in a sample extraction and purification stage.

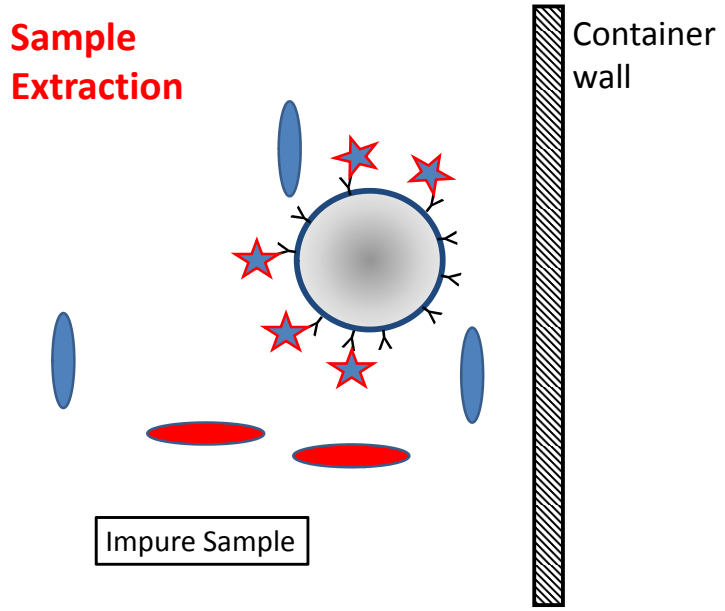
•Direct quantification of the Ag.Ab complex product is possible in a few cases although most assays use a solid phase and a label (marker, tag or reporter).

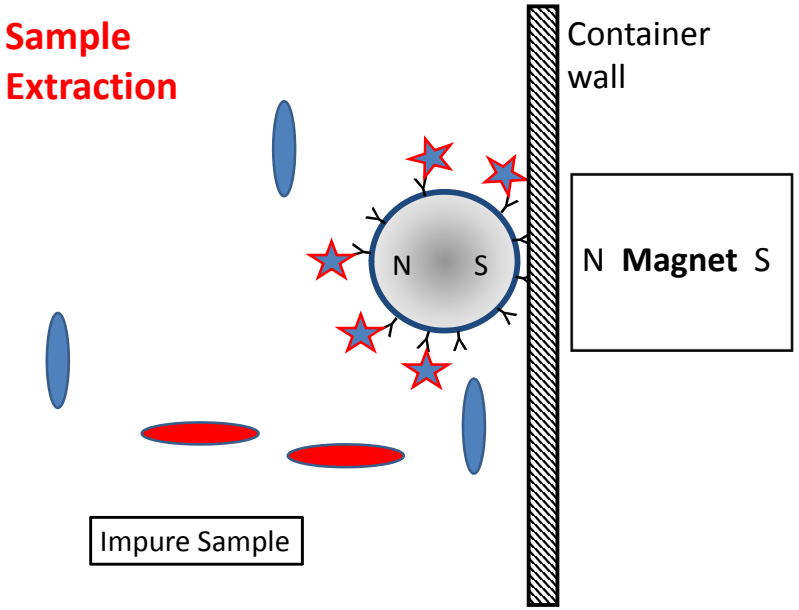
Magnetic Particles and Solid Reaction Surfaces.



•The particles contain nanoparticles of superparamagnetic magnetite (Fe_3O_4) with diameters of about 20 to 30 nm.
•The nanoparticles are embedded in a polymer matrix (e.g. polystyrene).
•Antibody is immobilised via its Fab fragment to the activated surface.


Sample
Extraction
Particle
coated with
antibody
Container
wall
Analyte
Impure Sample


Sample
Extraction
Container
wall
Impure Sample


Sample
Extraction
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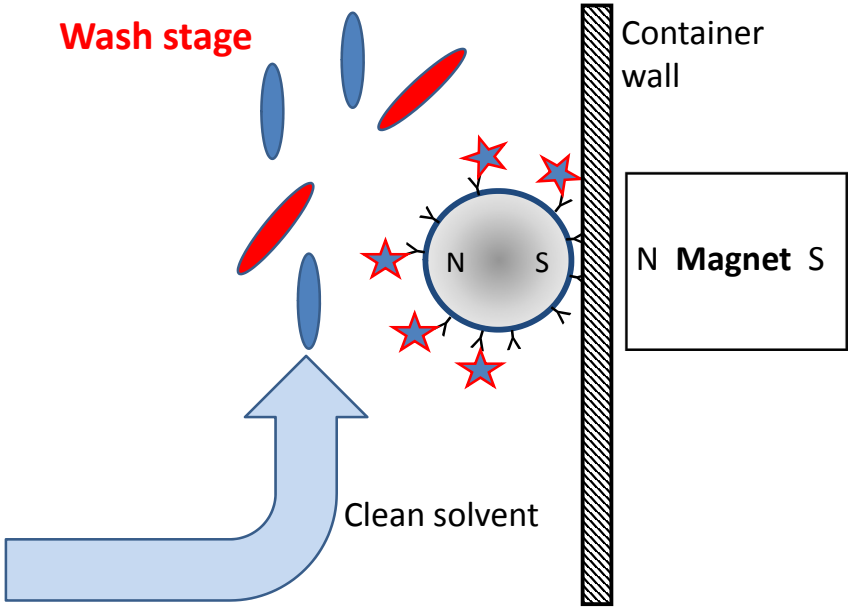
N
S
N Magnet S
Impure Sample


Wash stage
Container
wall
N
S
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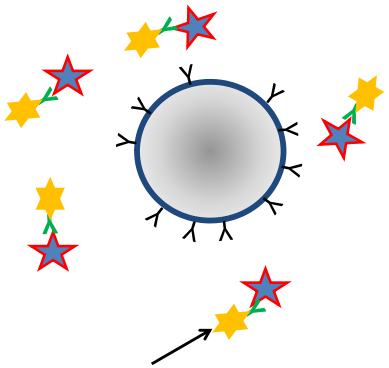
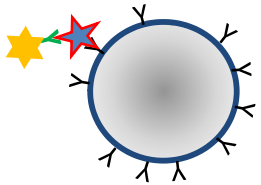
Clean solvent

•Chaotropic agent added to release analyte from particle.
• Second antibody with label added.
•Immunoassay quantified via the label.



Second antibody plus label

Container wall



•The particle could provide a solid surface for a sandwich assay to form between the analyte and the two antibodies.

•Labels used in conjunction with the magnetic particles:
Radioisotopes- now an almost obsolete technique.
Enzymes - enzyme-linked immunosorbent assay, (ELISA).
Chemiluminescent- commonly used in automated analysers.
Fluorescent - quantum dots are becoming popular.
Silver nanoparticles – an electrochemical method used .

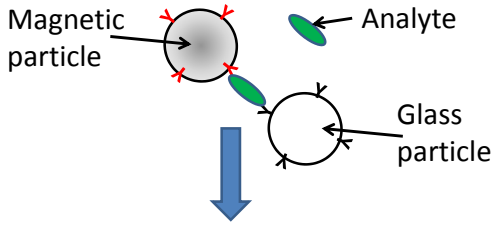
Potential Advantages of using Magnetic Particles as Labels in Immunoassays.

•There is very little magnetic material in most biological samples to interfere with the measurements.
•External magnets can be used to speed up assays by bringing antibody-coated particles to reaction surfaces.
•External magnets can be used to remove excess label.
•Sensing systems have been devised that do not respond to excess label.
•Magnetic materials do not readily degrade so samples can be archived and re-measured later.
•Simple, one step (or homogeneous), immunoassays are possible.

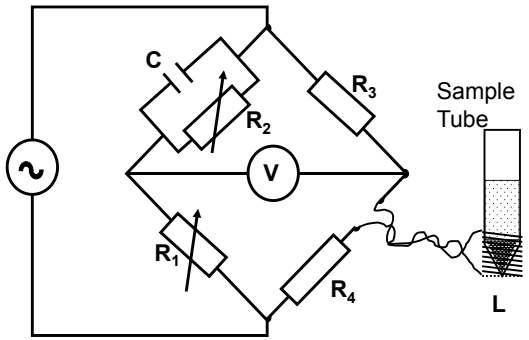
•Several ingenious techniques have been developed to quantify immunoassays using magnetic particles as labels.

1. Technique where the analyte bridges two different particles.

•Technique used by LifeAssays, Lund, Sweden.



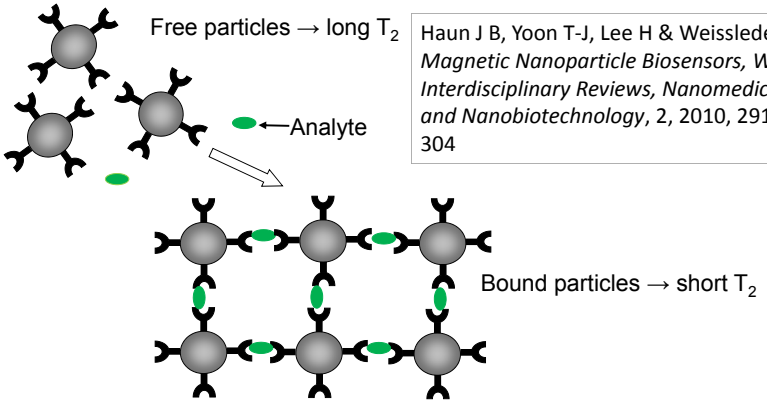
Cross-linked particles sink under gravity.



•Maxwell – Wein Bridge is balanced by adjusting R_1 and R_2.
•Cross-linked particles sink to the bottom of the tube, increasing the inductance of L and unbalancing the bridge.

Kriz C B, Rådevik K & Kriz D, Magnetic Permeability Measurements in Bioanalysis and Biosensors, *Analytical Chemistry,* 68, 1996, 1966-1970.

2. Technique using several cross-linked particles.



Haun J B, Yoon T-J, Lee H & Weissleder R, *Magnetic Nanoparticle Biosensors, Wiley Interdisciplinary Reviews, Nanomedicine and Nanobiotechnology*, 2, 2010, 291-304

Clusters of cross-linked particles (diameter ≈300nm) decrease the transverse time constant T_2 for the proton magnetic resonance of the water molecules. Unbound particles (diameter ≈38nm) have a smaller effect on T_2.

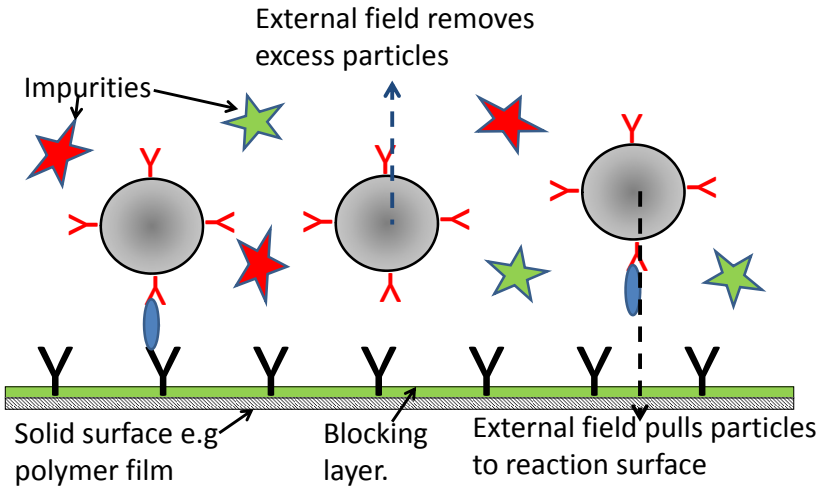
Competitive and Non-competitive Assays on a Reaction Surface.

•Many methods use a polymer, glass or ceramic reaction surface on which capture antibody is immobilised via its F_c fragment.
• A sensor is usually placed beneath the reaction surface.

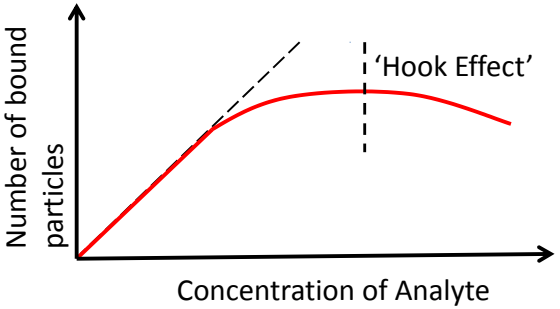
1. Non-competitive (or Sandwich) Assays are used with analytes having at least 2 epitopes.

2. Competitive Assays are used with small analyte molecules having only 1 epitope.

Magnetic particle labels in Sandwich (Non-competitive) Immunoassays.

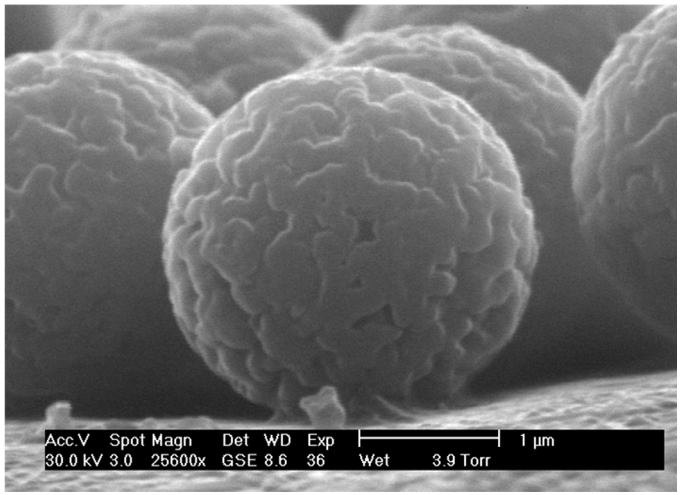


Typical response of a sandwich immunoassay using magnetic particles as the label.



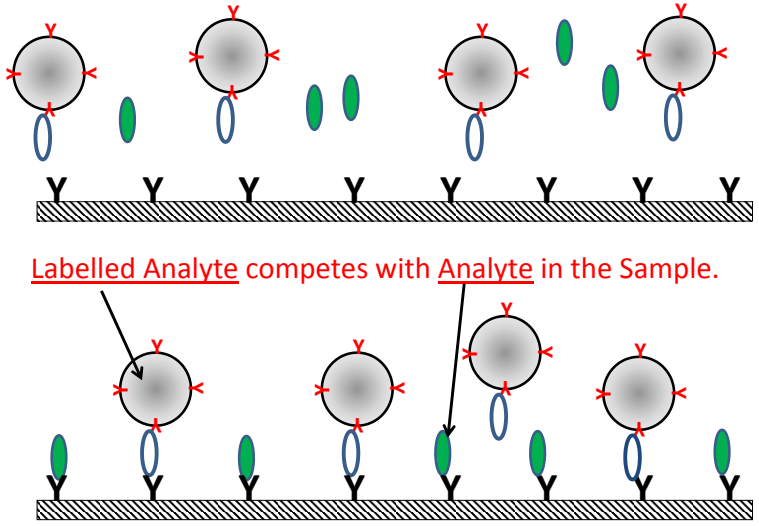
•The response becomes non-linear as capture antibody on the reaction surface is used up.

•'Hook effect': unlabelled analyte competes with labelled analyte for antibody on the reaction surface.



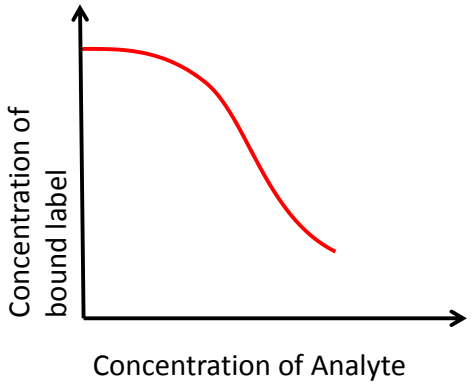
An EM image showing immobilised 2.8 µm-diameter particles on a plastic strip in a sandwich assay.

Magnetic Particles in Competitive Immunoassays



Labelled Analyte competes with Analyte in the Sample.

Typical response of a competitive immunoassay using magnetic particles as the label.



•The concentration of bound, labelled analyte decreases non-linearly with increasing concentration of the analyte.

Techniques that can distinguish between particles bound to the surface and unbound magnetic particles.

1. Decay in Magnetic Remanence.

•The particles are aligned by an external magnetic field.

•The field is removed and the particles are randomised by two mechanisms:

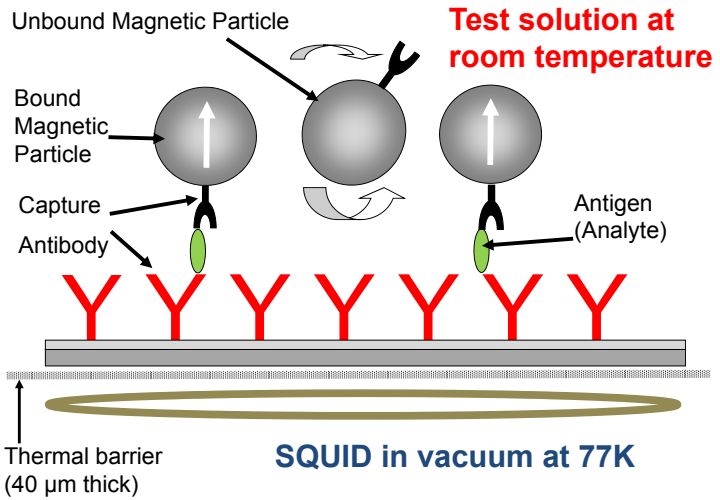
Brownian motion time constant, $\tau_B \approx 1$ ms

Néel relaxation time constant, $\tau_N \approx 1$ s

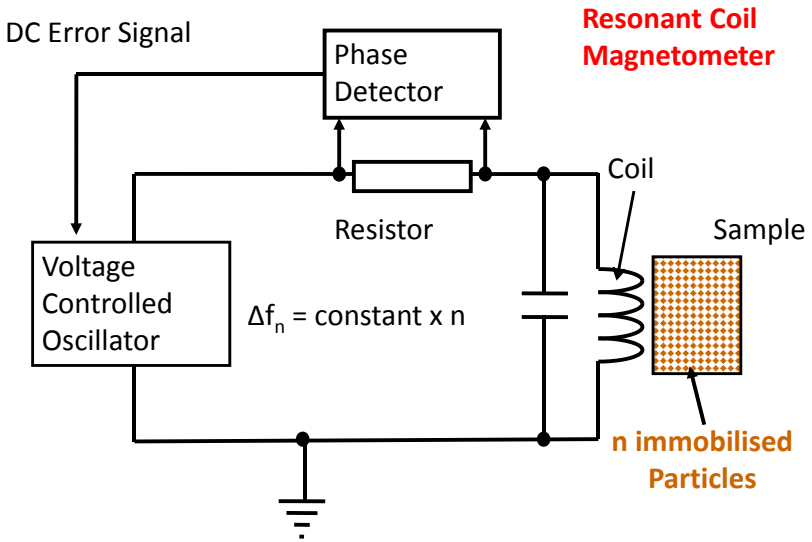
•The bound magnetic particles are not subject to Brownian motion so the slower decay in remanence measured by an external detector comes only from the bound particles.

2. Limited response range of the Detector.

•The response of detectors often falls off rapidly with distance so particles in suspension produce a weaker signal.

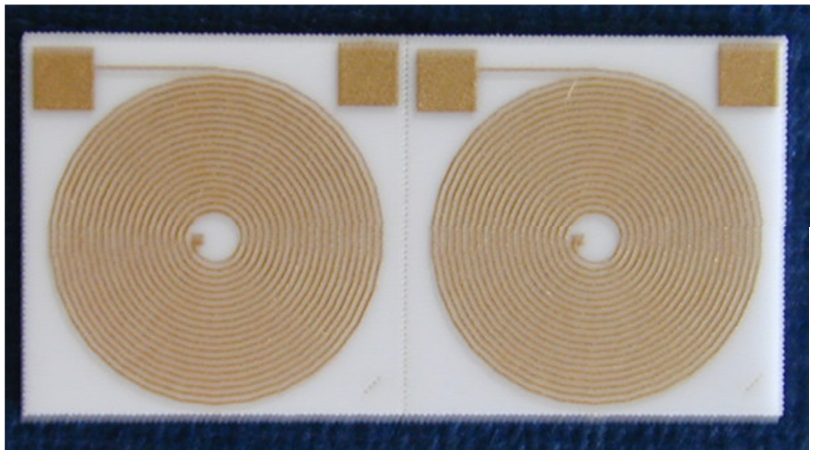


Chemla Y R, Grossman H L, Poon Y, McDermott R, Stevens R, Alper M D, & Clarke J, Ultrasensitive magnetic biosensor for homogeneous immunoassay, *Proceeding of the National Academy of Science of USA*, 97, 2000, 14268-14272



Hawkins P, Luxton R, and Macfarlane J, Measuring system for the rapid determination of the concentration of coated micrometer-sized paramagnetic particles suspended in aqueous buffer solutions, *Review of Scientific Instruments*, 72, 2001, 237-242.

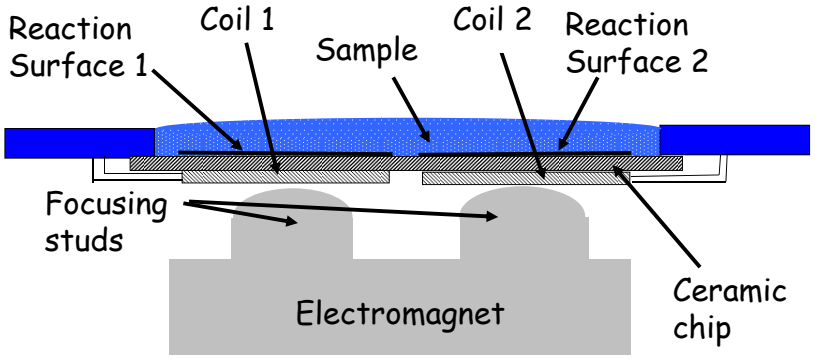
Flat Spiral Coils in a Two-analyte Measuring System.



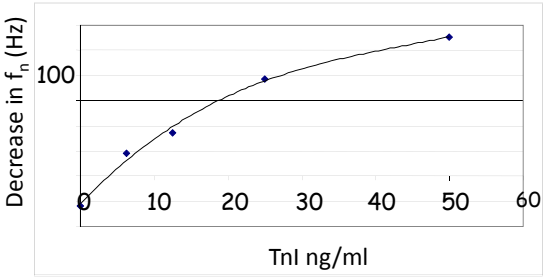
Coil 1 Coil 2

The Reaction Cell

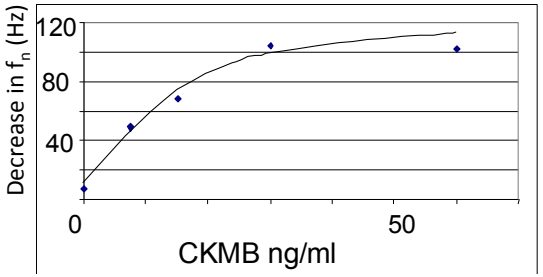
Randox Laboratories,
County Antrim, UK



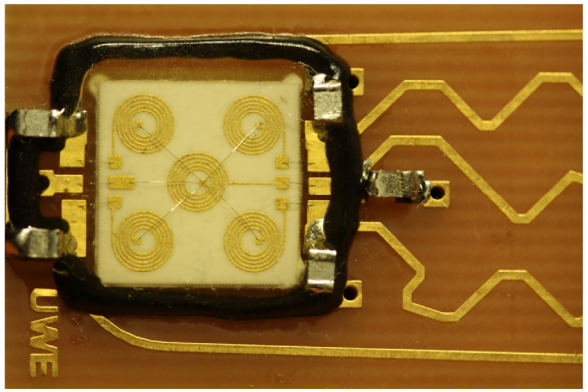
Kiely J, Hawkins P, Wraith P & Luxton R, Paramagnetic particle detection for use with an immunoassay based biosensor, *IET Science, Measurement & Technology*, 1, 2007, 270-275.



Simultaneous Measurements using two Coils

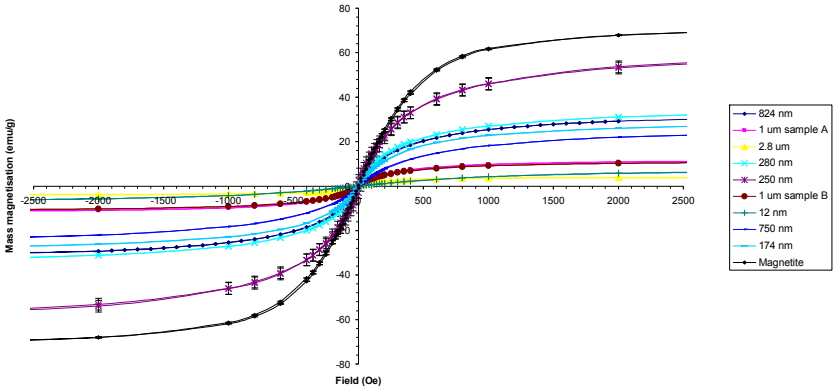


Troponin I and Creatine Kinase-MB are cardiac markers.

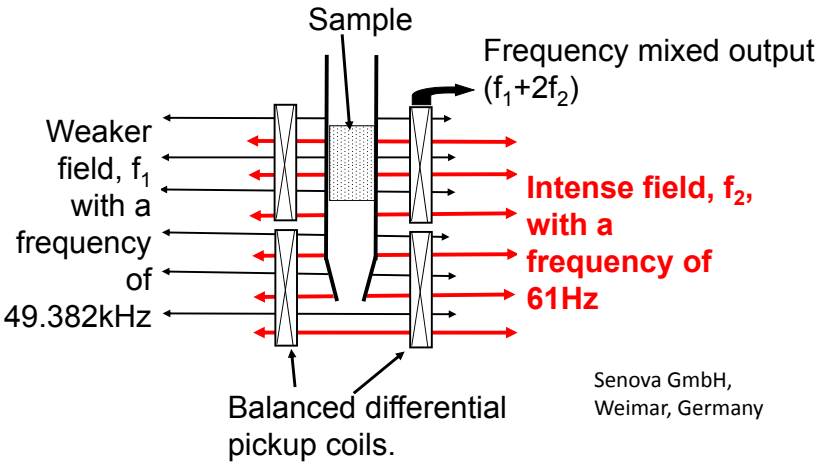


A five-coil measurement system.

Measurement Systems based on Magnetic Saturation.



Commercially available particles saturate at fields greater than about 2000 Oe.



Senova GmbH, Weimar, Germany

Meyer M H F, Hartmann M, Krause H-J, Blankenstein G, Mueller-Chorus, Oster B J, Miethe P & Keusgen M, CRP determination based on a novel magnetic biosensor, *Biosensors and Bioelectronics,* 22, 2007, 973-979.

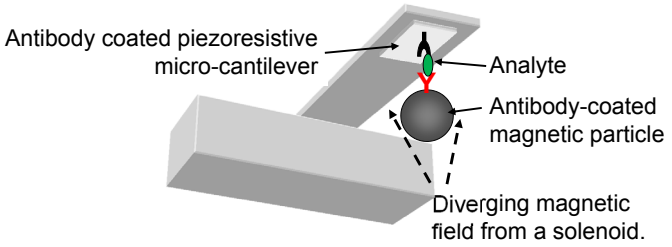
Magnisense, San Francisco, USA

- In a variation of the previous method, a simpler coil arrangement is used.
- f_1 is 24.4 kHz and has a fixed amplitude of 8.88 Oe.
- f_2 is 0.025 Hz and has a variable amplitude, H, up to 452.4 Oe.
- A signal proportional to d^2M/dH^2 is derived.
- The second derivative is related to the composition of the particle.
- Can be used in a multi-analyte assays.

Alphandéry E, Lijeour L, Lalatonne Y, & Motte L, Different signatures between chemically and biologically synthesized nanoparticles in a magnetic sensor: A new technology for multiparametric detection, *Sensors and Actuators B*, 147, 2010, 786–790

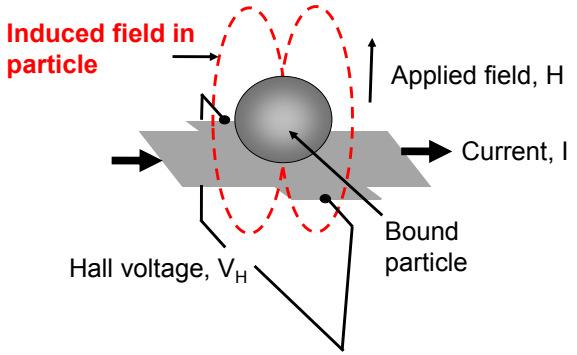
Techniques capable of detecting a Single Particle.

1. Micro Cantilever Based Force Amplified Biological Sensor



Baselt D R, Lee G U, and Colton R J, Biosensor based on force microscope technology, *Journal of Vacuum Science and Technology B*, 14, 1996, 789-793.

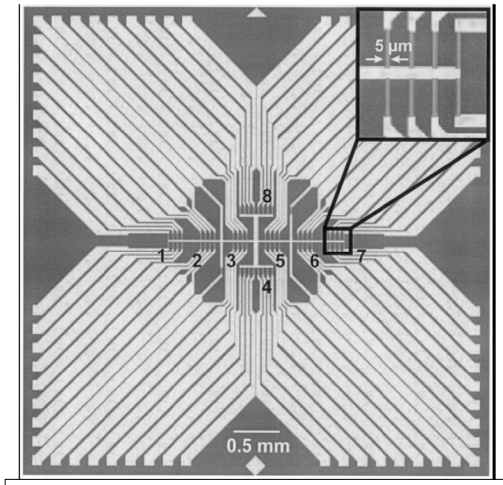
2. Thin-Film Hall Effect Sensors.



$V_H = C (I \times H)$, where C is a constant for the sensor

Besse P-A, Boero G, Demierre M, Pott V, & Popovic R, Detection of a single magnetic microbead using a miniaturized silicon Hall sensor, *Applied Physics Letters*, 80, 2002, 4199–4201.

3. Thin film Gigantic Magneto-Resistive Sensors.



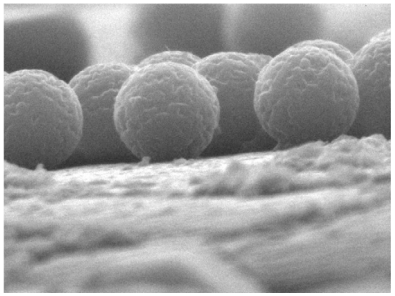
•Several research groups have worked, or are working, on this approach including Diagnostic Biosensors, Minneapolis.

•Used in the US Naval Research Laboratory, cBASS measurement system.

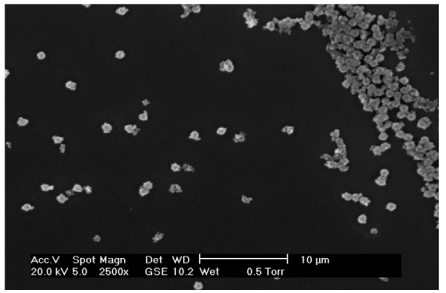
Tamanaha C R, Mulvaney J C, Rife J C & Whitman L J, Magnetic labelling, detection, and system integration, *Biosensors and Bioelectronics*, 24, 2008, 1–13.

Problems associated with Measurement Systems using a small Number of Magnetic Particles.

1. Variations between particles in size, Fe_3O_4 content and amount of bound and active antibody on the surface.



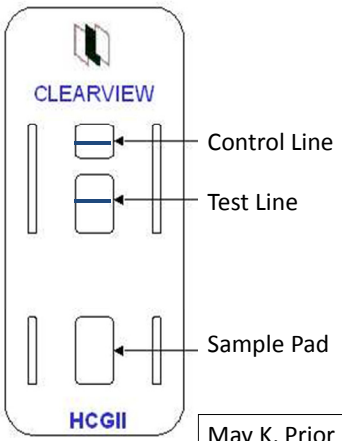
2.8µm diameter particles



760nm diameter particles

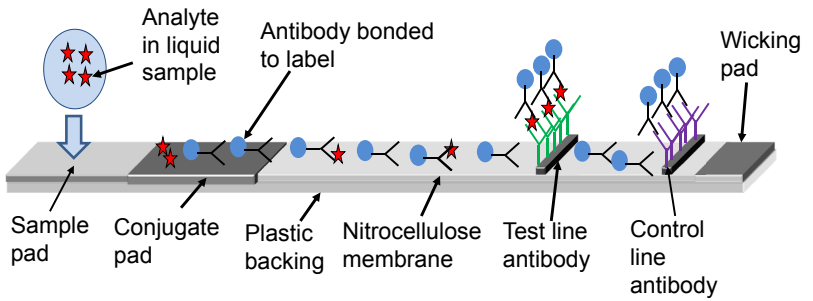
2. Difficulty in directing the magnetic particles and analyte to the measurement site.

Lateral Flow Immunoassays (or Immunochromatography).



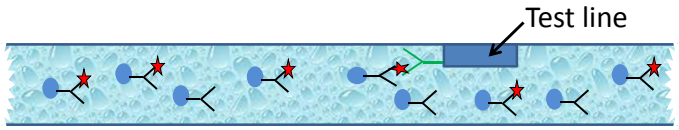
•Simple devices not requiring a skilled operator or sample preparation.
•Mainly used in applications to determine if the analyte concentration is above a threshold value.
•Pregnancy test for human chorionic gonadotropin hormone now widely accepted.

May K, Prior M E & Richards I, *UK patent* GB 2204398, 1988.



Direction of flow of liquid and reagents ----------→

•Sample pad contains a filter to remove unwanted solids.
•Conjugate pad contains the dried label and antibody to the analyte which are reconstituted by the sample liquid.
•Average pore size of membrane ≈ 10 × the diameter of the label.
•Test line contains a second antibody to the analyte.
•Control line contains an antibody to the antibody attached to the label.



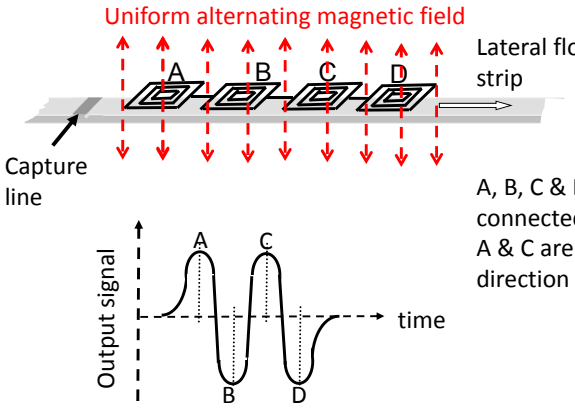
Direction of flow ------->

Some problems in making lateral flow devices more quantitative:

1. Test line does not capture all labelled analyte.
2. Particles trapped in the nitrocellulose membrane cause difficulties in determining baseline measurements.
3. Not all free label is captured by the control line making it difficult to determine total number of particles involved in the assay.

Four-Coil Gradiometer Detection System

MagnaBiosciences, San Diego, USA

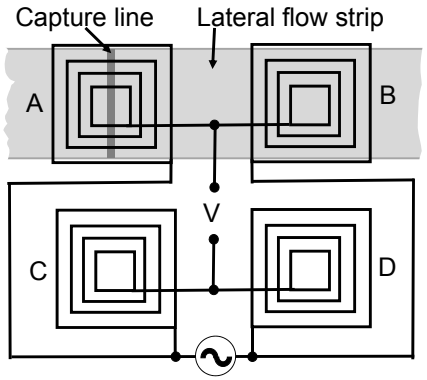


A, B, C & D are identical coils connected in series.
A & C are wound in opposite direction to B & D.

LaBorde R T, & O'Farrell B, Paramagnetic labeling offers an alternative method for analyte detection, *IVD Technology*, April 2, 2002

Four-Coil Balanced Bridge Detection System

Magnasense, Survontie, Finland



A, B, C & D are identical coils connected in a bridge circuit.
A & C are wound in opposite direction to B & D.
The lateral flow strip does not move and coil A is positioned over the capture line.

Mäkiranta J. and Lekkala J, Optimization of a novel magnetic nanoparticles sensor, *XVIII IMEKO World Conference, Metrology for a Sustainable Development, Rio de Janeiro, Brazil, 2006*.

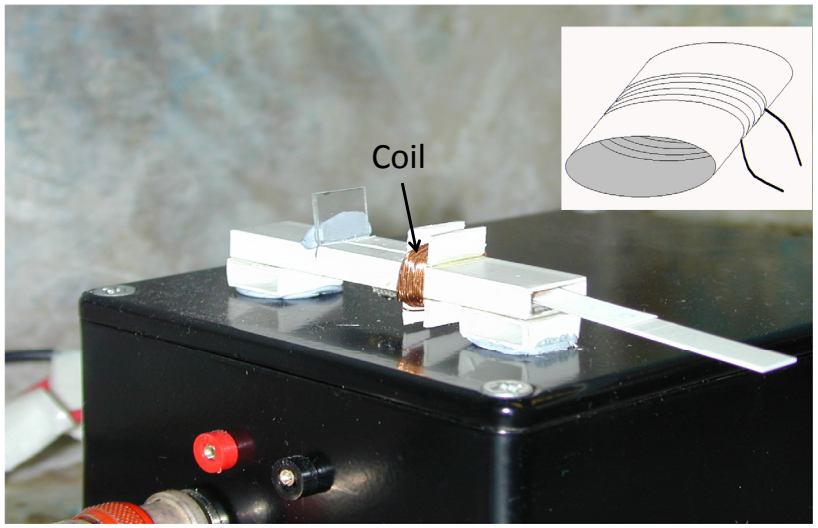
Integrated Giant Magneto-Resistive Strip

Diagnostic Biosensors , Minneapolis, USA

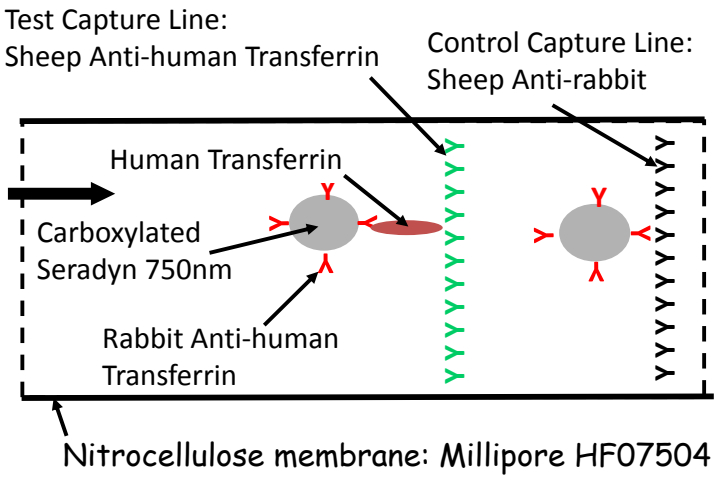
•A GMR strip is integrated into the membrane to detect the presence of magnetic labels in capture spots.

Taton K, Johnson D, Guire P, Lange E & Tondra M, Lateral flow immunoassay using magnetoresistive sensors, *Journal of Magnetism and Magnetic Materials*, 321, 2009, 1679-1682

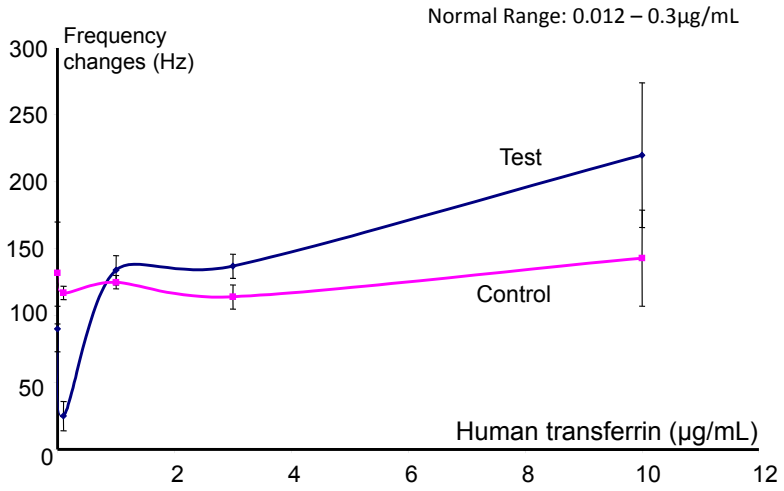
Resonant Coil Magnetometer in Lateral Flow Measurements



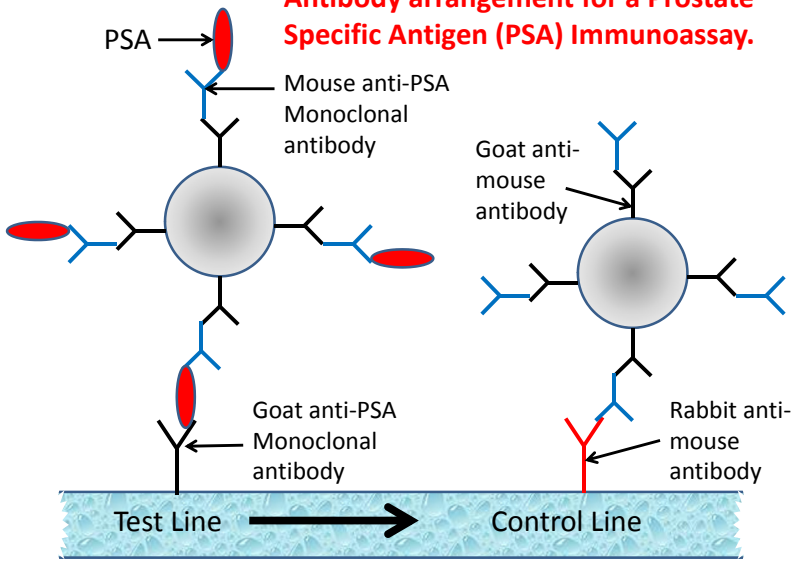
Antibody arrangement for Human Transferrin immunoassay.



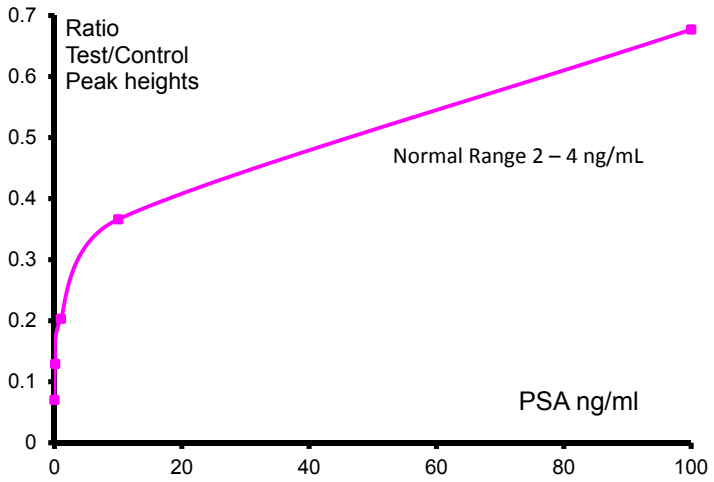
Lateral flow assay for human Transferrin



Antibody arrangement for a Prostate Specific Antigen (PSA) Immunoassay.



Lateral flow assay for PSA



Application of Magnetic Particles in Immunohistochemistry in HER2 breast cancer.

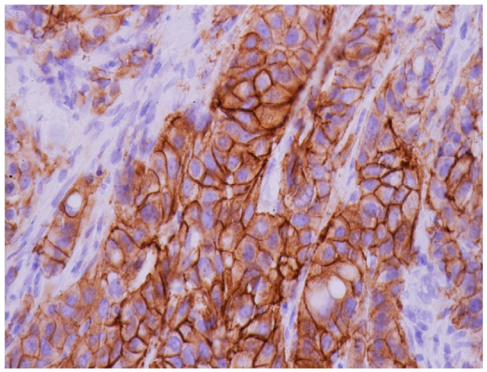
•20-30% of breast cancers are caused by over-expression of the human epidermal growth factor receptor-2 (HER2).

•Advanced stages of HER2 can be treated with monoclonal antibody trastuzumab (marketed as Herceptin). Other treatments will soon be available.

•The disease is diagnosed by making a microscopic slide of a biopsy and applying anti-human HER2 labelled with an enzyme.

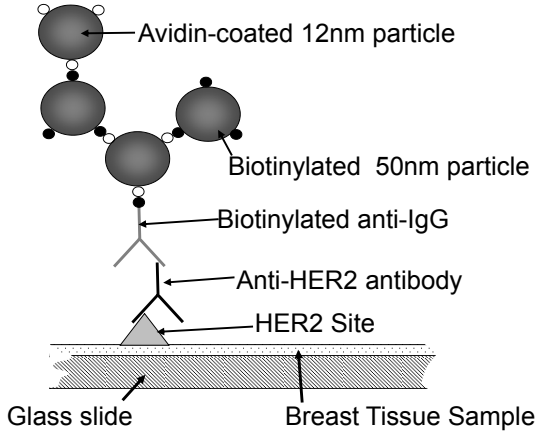
•The enzyme is used to precipitate a brown dye on to the slide.

•The intensity of the staining is determine by eye using a microscope. The brown coloration is scored (0, 1+, 2+ or 3+) which approximately equates to expression level.

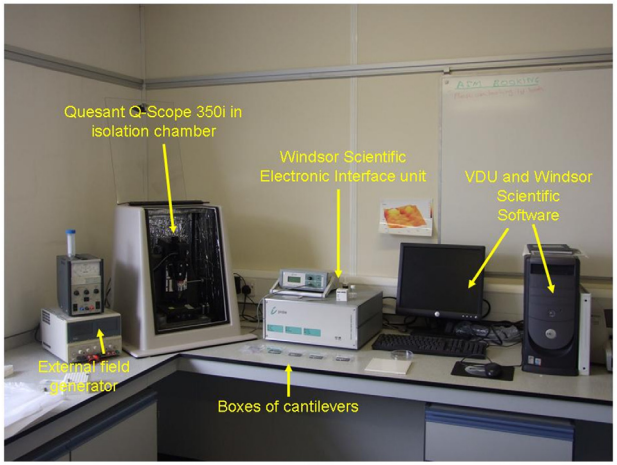


•Slides scoring 2+ or 3+ are assessed further using a fluorescent labelled antibody (fluorescent *in situ* hybridisation, FISH).

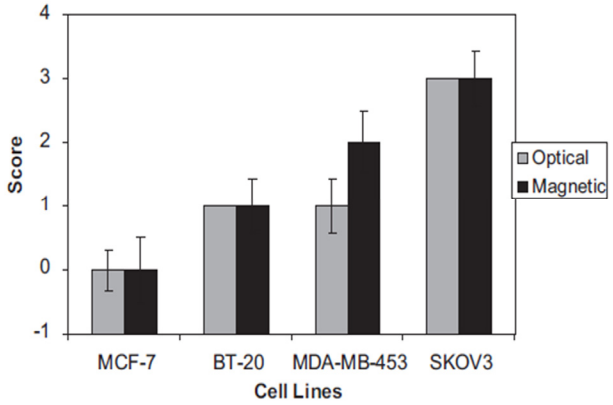
•In this investigation, magnetic particles were used as label and an amplification technique used to produce a stain on commercially-available slides with known scores of HER2.



The slides were then scanned using a magnetic force microscopy in the presence of a magnetic field.



•The normalised results were in good agreement with the optical scoring and known characteristics of the cell lines.



Mitchels J, Hawkins P, Luxton R & Rhodes A, Quantification in histopathology—Can magnetic particles help? *Journal of Magnetism and Magnetic Materials*, 311, 2007, 264–268.

Further Reading:

Magnetic Nanoparticles in Immunoassays, Chapter 9, p 243-276, *Magnetic Nanoparticles From Fabrication to Clinical Applications*, Edited by Nguyen T K Thanh (2012), CRC press.

A Big Thank You to:

Richard Luxton
Janice Kiely
Jasvant Badesha
John Sullivan
Keith Hewett
Julie Richardson
Nicholas Haji-Ioannu
Tony Rhodes
John Mitchels
Patrick Wraith
Jackie Barnett
John Eveness
Andrew Hill
Sabine Barakat
Elham Mohd Sharif
Bing Han
Mark Colclough (University of Birmingham)
+ numerous BSc and MSc Students