Measurement of super-paramagnetic nanoparticle labels in immunological point-of-care tests

Jukka Lekkala¹,², Jarkko Mäkiranta² and Mika Laitinen²

¹ Tampere University of Technology, Department of Automation Science and Engineering, PO Box 692, FI-33101 Tampere, Finland
² Magnasense Technologies Ltd, Valssikuja 5 A 8, FI-40520 Jyväskylä, Finland
Content

- Clinical diagnostics
- Immunoassays using labels
- Point-of-care testing
- Lateral flow test format
- Technological approach
  - Magnetic nanoparticles
  - RF inductance bridge
  - Measurement device
  - Test results
- Benefits of the method
- Conclusions
Clinical diagnostic tests

- A diagnostic test is any kind of medical test performed to aid in the diagnosis or detection of disease.

- Clinical chemistry is the area of pathology that is generally concerned with analysis of bodily fluids, mostly on serum or plasma → clinical diagnostics

- Most current clinical laboratories are now highly automated and use methods that are closely monitored and quality controlled.
Immunodiagnostics

- The challenge of biochemical tests is to **recognize** the target molecule (molecular structure) and **measure** its concentration in the sample.

- Immunodiagnostics uses an antigen-antibody reaction as their primary means of detection.

- The enzyme based ELISA assay and the Lateral-Flow test are currently the two predominant formats.
Examples of analytes and their normal concentration levels

Renal (Kidney) Function Tests
- Creatinine: 45-90 \( \mu \)mol/L for women and 60-110 \( \mu \)mol/L for men

Liver Function Tests
- Albumin (Alb): 3.5 to 5.3 g/dl
- Gamma glutamyl transpeptidase (GGT): 0 to 42 U/l

Cardiac (Infarct) Markers
- Troponin T: <15 ng/l
- Myoglobin: 25 - 58 \( \mu \)g/l for women and 28 - 72 \( \mu \)g/l for men
- Creatine Kinase-MB: 0-3 ng/ml
- B-type natriuretic peptide (BNP): <100 pg/ml

Miscellaneous
- Glucose: mean normal level about 4 mM (4 mmol/l or 72 mg/dl)
- C-reactive protein (CRP): < 10 mg/l
- Human Chorionic Gonadotropin (hCG): < 5 mU/ml

Highly specific and sensitive detection method is needed
\rightarrow imunoassays
Immunoassays using labels

Competitive assay
- Antigens compete with labeled conjugates for the binding sites of antibodies
- Number of the label particles is inversely proportional to the number of antigens

Biometric assay
- Assay forms a structure where antigen is sandwiched between the capture antibody and labeled trace antibody
- Number of the label particles is directly proportional to the number of antigens

→ Improving the S/N ratio in detection

Label: radio isotope (RIA), enzyme (ELISA), fluorophore (FIA), etc.
Point-of-care (POC) testing

• Point-of-care testing is defined as diagnostic testing at or near the site of patient care: doctor’s office, bed side, ambulances, workplace, home, disaster site, etc.

• POC-testing is accomplished through the use of transportable, portable, and handheld instruments (e.g., blood glucose meter) and test kits (e.g., HIV salivary assay).

• Cheaper, smaller, faster, and smarter POC-testing devices will assure its future importance in cost-effective integrated solutions to medical problems, such as diabetes and acute coronary syndrome.
Lateral flow test format

- Lateral flow tests → simple and affordable devices intended to detect the presence of a target analyte in sample.
- They are often produced in a dipstick format immunoassay (either competitive or sandwich assay) in which the test sample flows along a solid substrate via capillary action.
- The test result can be seen in the result window.
- Typical product is for example a pregnancy test which can be bought in a pharmacy.

Sample (a few drops of blood, serum, saliva, urine etc.)
Lateral flow test format

Porous cushion, that sucks and filters the sample

Direction of solution flow

At the end of the strip there is sucking cushion which ensures that the sample solution will flow over the reaction areas

Porosity of the strip (width 4-6 mm, thickness 10 mm)

Reaction areas

Top view

Side view (opened)
Lateral flow test format

- The test result can be seen as colour lines (detection and reference zones) in the result window

**Negative (-)**
- Only the reference line

**Positive (+)**
- Both lines

- In visual reading (a qualitative test like pregnancy test) any coloured particle can be used as a label
  - commonly either latex (blue colour) or nanometer sized particles of gold (red colour) are used.

- However, in a *quantitative* test an optical reader instrument is needed to obtain a *numerical value of the measurement quantity* that is proportional to the analyte concentration
Our technological approach

- Measurement is based on the use of magnetic nanoparticles (MNP) as labels in a Lateral flow test format.
- The change of magnetization in the detection zone is measured using an instrument, which uses an arrangement of coils and a radio frequency magnetic field.
Superparamagnetic nanoparticles

- The quantitative measurement of the test is based on superparamagnetic nanoparticles.
- Paramagnetism → magnetism occurs only in the presence of an externally applied magnetic field.
- Superparamagnetic materials → no residual magnetization because thermal fluctuations (Brownian motion) reorientate the domains → no aggregates → precondition for the biochemical reactions.
Magnetic nanoparticle labels

- Typical superparamagnetic nanosphere labels range from nanometers to a few micrometers in diameter and are made of a polystyrene matrix where iron oxide crystals are uniformly distributed.
- Chemically these particles behave similarly to latex or polystyrene particles, allowing for easy modification with covalent coupling on carboxyl or amino groups, as well as by passive adsorption of antibodies on to the surface.*

* Estapor® Super Paramagnetic Microspheres, Merck
Inductance bridge for detection

A set of four planar micro coils symmetrically aligned in an impedance bridge (Wheatstone bridge) is used for quantitative measurement of magnetic nanoparticles in the detection zone.

The sample is placed on one of the four identical coils (A) and the control area of the lateral flow strip is placed on the other symmetrical coil (B).

The two other compensation coils (C, D) read the background from the surroundings.
Inductance bridge for detection

- The inductance of the measurement coil is changed due to the change of the relative permeability of the nanoparticles.
- This unbalances the bridge at high frequencies. An output signal is proportional to the amount of the particles.
In a real bridge the inductances are LCR circuits including in addition to the inductance also a stray capacitance and the resistance of the conductor; thus the inductance is

\[ Z = \sqrt{R^2 + (X_L^2 - X_C^2)} \]

The coils are made of copper on FR-4 substrate. The copper layer is 36 µm thick and coated with gold to prevent oxidation.
Designing the sensing coil

- Sensitivity of the detection system depends on the inductance (inductive reactance) and resistance of the coil.

- Inductance of a planar coil*
  - $N$ is number of turns,
  - $d_{out}$, $d_{in}$ and $d_{avg}$ are outer, inner and average diameters
  - $\rho$ is fill ratio and
  - $c1 - c4$ are coil shape depending constants

\[
L = \frac{\mu_0 N^2 d_{avg} c_1}{2} \left( \frac{c_2}{\rho} + c_3 \rho + c_4 \rho^2 \right),
\]

\[
d_{avg} = 0.5(d_{out} + d_{in}),
\]

\[
\rho = \frac{d_{out} - d_{in}}{d_{out} + d_{in}},
\]

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<th>Shape</th>
<th>$c_1$</th>
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</table>

Designing the sensing coil

Resistance of the coil is

\[ R_s = \frac{\rho l}{A} = \frac{\rho l}{wt} \]

- where \( \rho \) is the resistivity of the material
  (for copper \( 1.678 \cdot 10^{-8} \text{ } \Omega \text{m} \))
- \( w \) is the width of the wire and
- \( t \) is the thickness of the wire.

\( l \) is the length of the current sheet

- where \( N \) is the number of turns
- \( N_{\text{sides}} \) is the number of sides (for square 4)

For high frequency excitation

skin depth \( \delta \) must be taken into
account as effective thickness

\[ t_{\text{eff}} = \int_{0}^{t} e^{-\frac{z}{\delta}} \, dz = \sqrt{\frac{1}{\pi f \mu \sigma}} \left( 1 - e^{-\frac{t}{\pi f \mu \sigma}} \right) \]
Numerical methods like FEM (Finite Element Method) can be used for describing the interactions between the sensing coil and magnetic nanoparticles. The model above was built using 2D axial symmetry. Maxwell’s equations were solved using FEM and the inductance of the coil was integrated from the solution. The change of impedance for different coil geometries and number of particles can be approximated.
Measurement device

Phase sensitive measurement system

- DDS (Direct Digital Synthesis) quadrature oscillators for adjustable sinusoidal excitation signal.
- Transformers in the bridge drive and measurement to maximize noise immunity
- High gain (30000), low noise and wideband amplifier (LNA)
- A quadrature detection was used to reduce low frequency noise, 50 Hz pick-ups signal, and to be able to measure phase differences.
- Mixer outputs are filtered, amplified and fed to 16b ADCs.
Measurement instrument

www.magnasense.com
Detection limit

- Detection sensitivity of the measurement system for magnetic nanoparticles has been tested using MNP calibration samples.
- Using a calibration sample of total number of 1.75 millions of particles the output signal of the bridge is 14.7 µV with S/N ratio of about 29 (amplitude of the noise about 500 nV (rms)).
- The detection limit of the sensing system is 60,000 particles (with the area of 1 mm x 1 mm and particle about 400 nm in diameter).
Dynamic range

Dynamic range of the Instrument is 5 decades

Digital signal versus dilution of Estapor-particles

R² = 0.99578

Detection limit

Visual limit

Dynamic range

Noise level of electronics

Stock solution = 1

Signal/Bit
Benefits of the method

- Using inductive bridge for detection of magnetic nanospheres as labels creates an innovative way to measure analyte concentrations.
- The measurement of the magnetic label is substantially more affordable and more sensitive when compared to optical measurement methods currently used.
- Absence of optics means decreases vulnerability to dust and other contaminants of dirty or otherwise coloured samples.
- Measurement is fast and enables the assessment of different tests consecutively.
- The dynamic range of the measurement is large. For example the range of 0.1 – 100 mg/ml of CRP can be measured regardless of the changes in the dipstick test lots.
Wide application areas

- The detection method is widely applicable to different analytes as well as different sample materials such as; proteins, steroids, pharmaceutical compounds, nucleic acids, bacteria and viruses.
- Magnasense technology can be applied in
  - Clinical testing
  - Drugs of abuse
  - Veterinary testing
  - Food industry testing
  - Environmental testing
Future

• The measurement method can be utilized in a multichannel device to detect a panel of different analytes simultaneously.

• This measurement technology has a great potential outside of lateral flow tests as well, such as in the field of microfluidistics.

• Optimization of the coil structure, effective shielding against external disturbances and reduction of the electronic noise of the measurement instrument are the main targets for future development.
Conclusions

- The use of biosensor devices in clinical diagnostics has increased rapidly during the last years.
- Key issue in POC diagnostics is specific and accurate sensing of the target analyte molecules using sophisticated immunodiagnostics.
- Using magnetic nanoparticles (MNP) as labels creates a novel way to quantitatively measure analyte concentrations.
- We have developed a sensitive, reliable and low-noise method to measure the change of magnetization in the capture zone of the test. This method together with an instrument forms an easy to use quantitative biosensor when combined with disposable test cartridges.
- The measurement is fast, affordable and enables the efficient consecutive assessment of different tests.
References


Thank you!

More information available: 
www.magnasense.com and www.tut.fi/ase