MULTI-ANALYTE IMMUNOASSAYS (MAIA) – a concept put forth by Prof. Ekins over a decade ago – promises to be a cutting edge technology in clinical chemistry. It will offer the advantage of estimating many analytes in one assay as compared to present assays where each assay can estimate only one analyte. MAIA, based on antibody chips (similar to DNA chips) will use miniscule amounts of reagents and patient sample compared to the latter. By enabling simultaneously, a battery of tests related to a given disease or associated diseases, it can save valuable time for patient management. In short, antibody-chip MAIA can revolutionize immunoassay technology just as the DNA chip is revolutionizing gene analysis.

However, putting MAIA into practice needs team-effort from immunoassayists, chemists, physicists, instrumentation and robotic engineers.

Objective

At the core of MAIA is the ‘antibody chip’ a small inert glass or plastic matrix on which a number of antibodies, each specific to a different analyte, is immobilized at spatially determined sites.

We have plans to develop this technology and have carried out preliminary experiments to standardize and gain expertise in making antibody coated matrix.

Materials and Methods

Standard microscope cover slips, 13 mm dia., were used as the substrate. They were silanized and activated with glutaraldehyde as the bifunctional agent.

Polyclonal antibodies to T4 and monoclonal antibodies to TSH were spotted on the activated glass surface, incubated for 1 h, unreacted antibodies washed off and surface blocked with BSA.

Fixed amounts of $^{125}$I-T4 mixed with different concentrations of T4 standards in serum (RIA format) were used to test the viability of the coated anti-T4 antibody spots.

Using TSH standards and a second anti-TSH antibody labeled with $^{125}$I, the coated anti-TSH monoclonal antibodies were tested in a sandwich (IRMA) assay.

Fig. 1 Anti-T4 antibody spots made by touching activated glass disc with a pipette tip, containing antibody solution (1mg/ml). Entire disc reacted with 200 µl of $^{125}$I labeled T4 tracer (1,20,000 cpm) Autoradiography exposure time - 3 days.
Fig 2 Anti-T4 polyclonal antibody spotted - 2 µl (1mg/ml).
1µl of T4 standard (20,10,5,2.5,0 µg/dl) was mixed with 1µl I^{125}-T4 (30,000-cpm/µl) placed on antibody spots. Incubated for 1 hour and rinsed with PBS-Tween 20.
Autoradiography exposure time - 2 days

Fig 3  Stability study. 2 µl of anti-T4 antibody (polyclonal) was spotted
Assay done 14 days after antibody spotting. Procedure same as in Fig.2
For testing of non-specific binding 1 µl of 100 µg/dl of T4 standard mixed with 1 µl I^{125} T4 (20,000 cpm/µl) was used.
Autoradiography exposure time - 2 days

Fig 4 Anti –TSH monoclonal antibody spotted  – 2 µl.
Incubated for 1 hour with 3 µl of different TSH standards (100,50,25 µIU /ml) and rinsed with PBS.
Incubated for 2 hrs with 200 µl of TSH tracer (1,40,000cpm/disc) and rinsed with PBS-Tween 20. Autoradiography exposure time - 4 days.
After washing away the unreacted tracer, the amount of tracer that was bound was visualized by autoradiography using AGFA X-ray film.

**Results**

Using our procedure we could spot antibodies at multiple locations. They were covalently bonded, with negligible background. The antibodies retained their antigen binding property.

The amount of tracer that reacted was visualized by autoradiography. Dark spots were seen with an intensity inversely relative to the concentration of T4 (RIA) and directly relative to the concentration of TSH (IRMA).

**Discussion**

Our experiments are preliminary, but show that we have standardized the chemistry required for covalently coupling antibodies to small glass discs, which could be used as the ‘antibody-chip’ matrix.

Using autoradiography we have demonstrated the validity of our process although radioactivity is not the tracer of choice for multi-analyte immunoassays.

The uniform darkening, within the spot, shows that quantitative information is independent of the size of the spots. Hence, very minute spots could be used and antibody chips, until recently just a concept, can be realized in practice.

A lot more work involving immunoassayists, chemists, physicists, instrumentation and robotic engineers is required before MAIA comes into routine use.

**References**


**Addendum**

Following the above study, there was a continued search for a better substrate which was flexible, porous and required minimal activation for coating the antibodies. We tested track etched membranes (TEM) prepared by Dr. R.H. Iyer, Emeritus Scientist, Nuclear Recycle Group, B.A.R.C., as potential substrates. These are thin polycarbonate and bombarded with heavy ions in a Pelletron accelerator to a pore density of $10^5 - 10^8$ pores/cm$^2$. Further these membranes are etched in nitric acid to give very uniform pore dimensions.

Although plastics have been used for coating antibodies with polystyrene being the most popular, it is not suitable for MAIA because of its physical, chemical and background fluorescent properties. Our preliminary experiments show that polyethylene terephthalate films (10 - 25 µ metres) thick with a pore density of $10^8$/cm$^2$ and pore diameter of 1µ was quite suitable as a substrate for coating antibodies as shown in Fig.5. Further work is in progress.

Fig 5. 25 µ thick polyethylene terephthalate TEM $10^8$ pores/cm$^2$ and 1µ dia were activated with glutaraldehyde and antibody was spotted on them as was done for the glass discs. Good coupling of plain and FITC conjugated antibodies are seen. The background and non-specific binding is insignificant.
About the authors ...

**Dr M.G.R. Rajan**, Head, Laboratory Medicine Section, Bio-Medical Group, BARC, joined BARC in 1978 through the 21st batch of Training School. He underwent training during May-July 1984 in the culturing of malarial parasites and extracting antigens for immunodiagnosis at Guys Hospital, London. He also studied the theory and practice of Radioimmunoassay, particularly the concept of free thyroid hormones and multianalyte immunoassays at Middlesex Hospital Medical School, London. He underwent an IAEA-sponsored course on preparation, storage and distribution of bulk reagents for radioimmunoassay in October 1989 at National Institute of Health, Nonthamburi, Bangkok, Thailand. The course gave practical and theoretical training in all the aspects pertinent to producing and preparing and quality control of reagents required for radioimmunoassay.

Dr Rajan has been involved in the development of new RIAs for thyroid disorders and other diseases. His field of work has been in pursuing the objective of developing a comprehensive set of assays for a thorough in-vitro evaluation of thyroid disorders and, presently, for diabetes.

His current interest is in developing multi-analyte immunoassays (MIA) to assay all analytes relevant to a given disease. He supervises the routine RIAs for thyroid related hormones carried out on patients coming to RMC for diagnosis and treatment.

Dr Rajan also engages himself in teaching of radioimmunoassay and statistics to students doing the DRM and DMRIT course at RMC and BARC Training School.

He received his Ph.D. for his work entitled, "Studies in the estimation of free (non-protein bound) thyroid hormones", from Mumbai University in 1993. He has 21 papers published to his credit and has presented 30 papers at Conferences. He has delivered the Brig S.K. Muzumdar Oration at the 30th Annual Conference of the Society of Nuclear Medicine - India on December 3, 1998. He is a Life Member of the Society of Nuclear Medicine (India), Endocrine Society of India, Association of Clinical Biochemists of India, Indian Association for Nuclear Chemistry and Allied Sciences, Indian Association for Radiation Protection, Indian Immunological Society and Action Council against Tobacco.

**Ms Bharti Gupta**, M.Sc. (Biochemistry) from Jamia Hamdard University, is actively involved in development of multianalyte immunoassay, which allows simultaneous measurement of several analytes related to a given disease. This will save time in patient management.

Patient oriented services involve serum measurement of T4, TSH and Tg by RIA and IRMA.