Biomarker Detection Using Mass Spectroscopy

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Outline

- Introduction to Biomarkers

- Introduction to MS

- Detailed Introduction to ICP-MS

- Biomarker Detection using ICP-MS
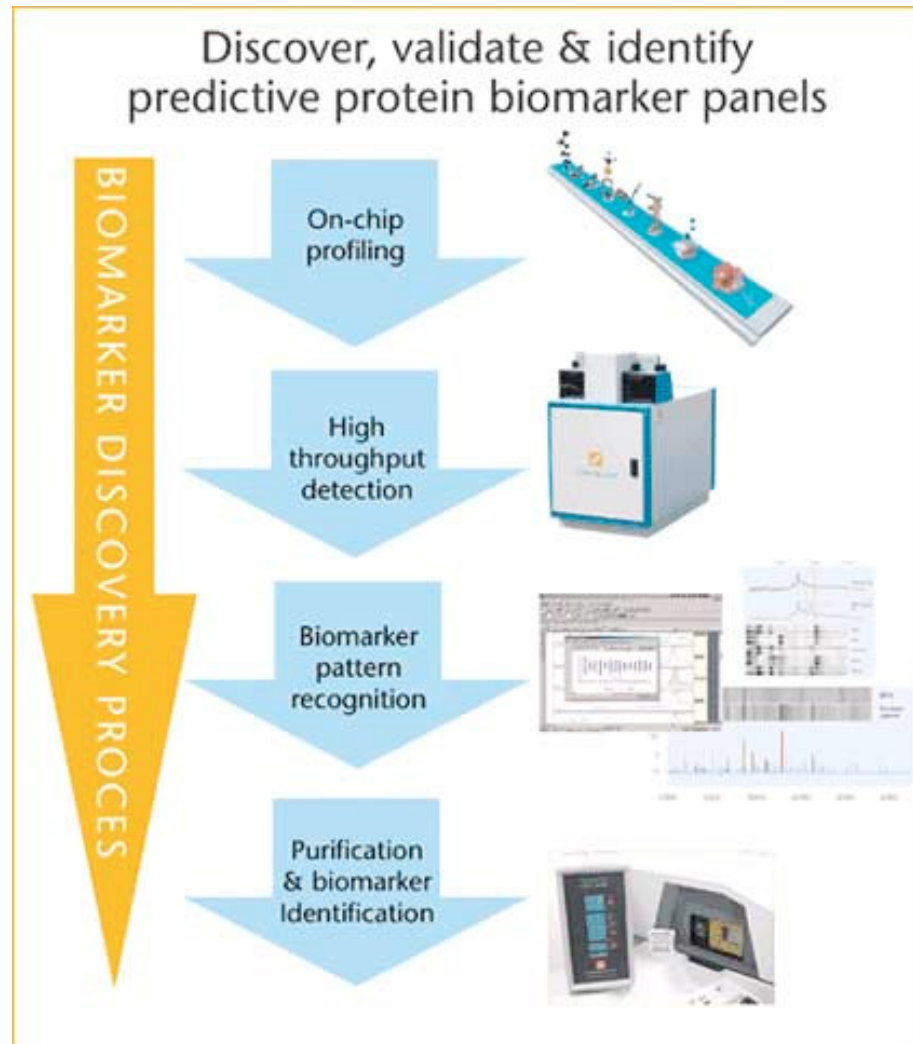
- Summary

- References

Biomarkers

- The hottest new term in Biotech industry:
  BIOMARKERS

- Defined as a characteristic objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic indication.

- Biomarker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease.

Need for Biomarker Detection

- Drug Discovery and Development*

- Indicate drug efficacy and toxicity

- Reduce cost and duration of clinical trials

- In Biotechnology and Pharmaceutical industry

* FDA critical path initiative

Biomarker discovery process

Discover, validate & identify predictive protein biomarker panels

BIOMARKER DISCOVERY PROCES

- On-chip profiling
- High throughput detection
- Biomarker pattern recognition
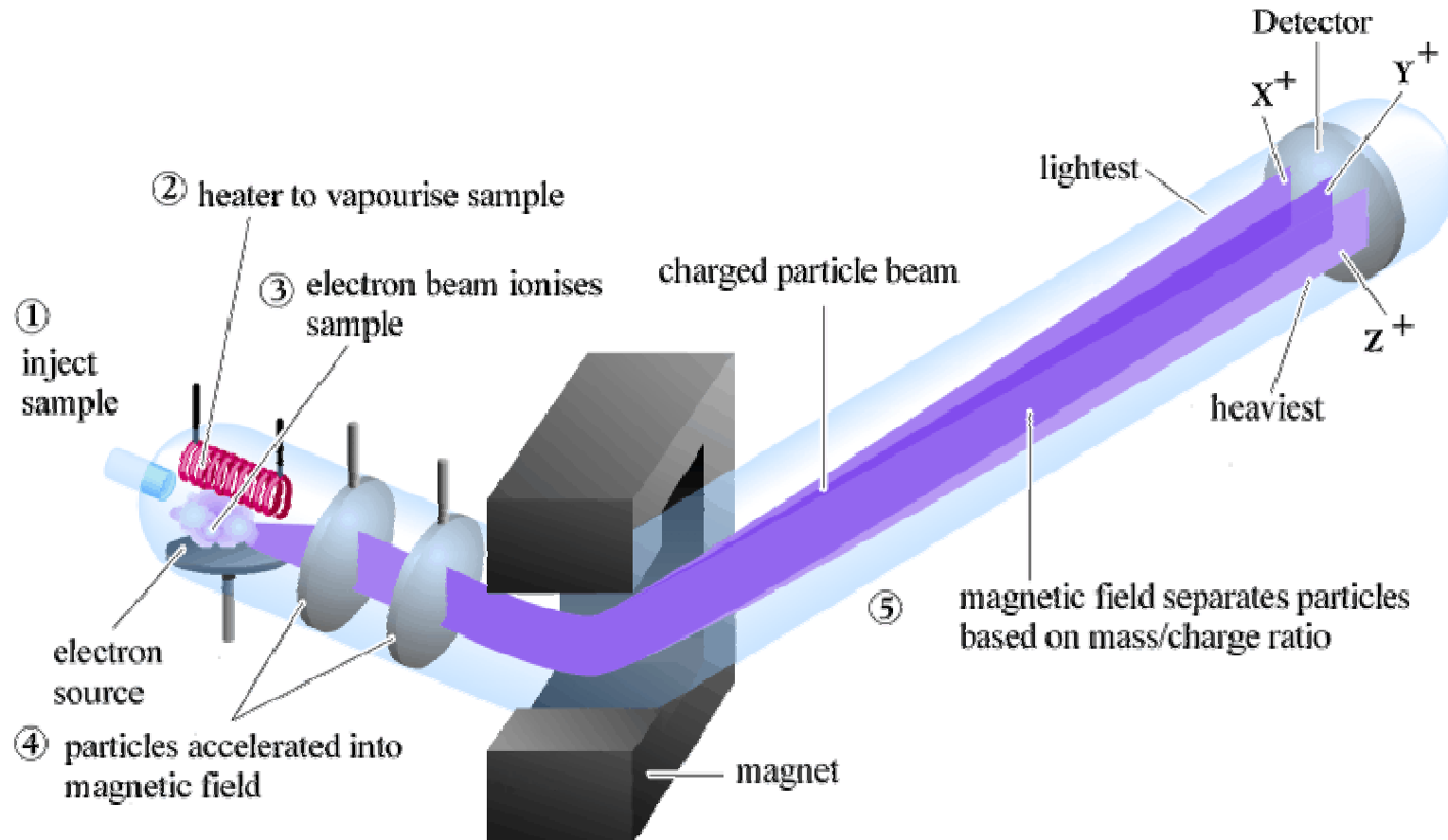- Purification & biomarker Identification

How to detect Biomarkers?

- Comparative Proteomics of Biofluids
- Immunohistochemistry Staining
- Spectroscopy-based Techniques
  - Surface Enhanced Raman Spectroscopy
  - SELDI-TOF (Surface Enhanced)
  - *ICP-MS*
- Sensing systems
  - Automatic Microfluidic Detection System
  - Carbon Nanotubes Immunosensors
  - Newly developing technologies such as arrayed imaging reflectometry, Porous silicon biosensors, etc.

Mass Spectroscopy

MECHANISM

② heater to vapourise sample

③ electron beam ionises sample

① inject sample

electron source

④ particles accelerated into magnetic field

charged particle beam

magnet

Detector

X⁺ Y⁺

lightest

Z⁺

heaviest

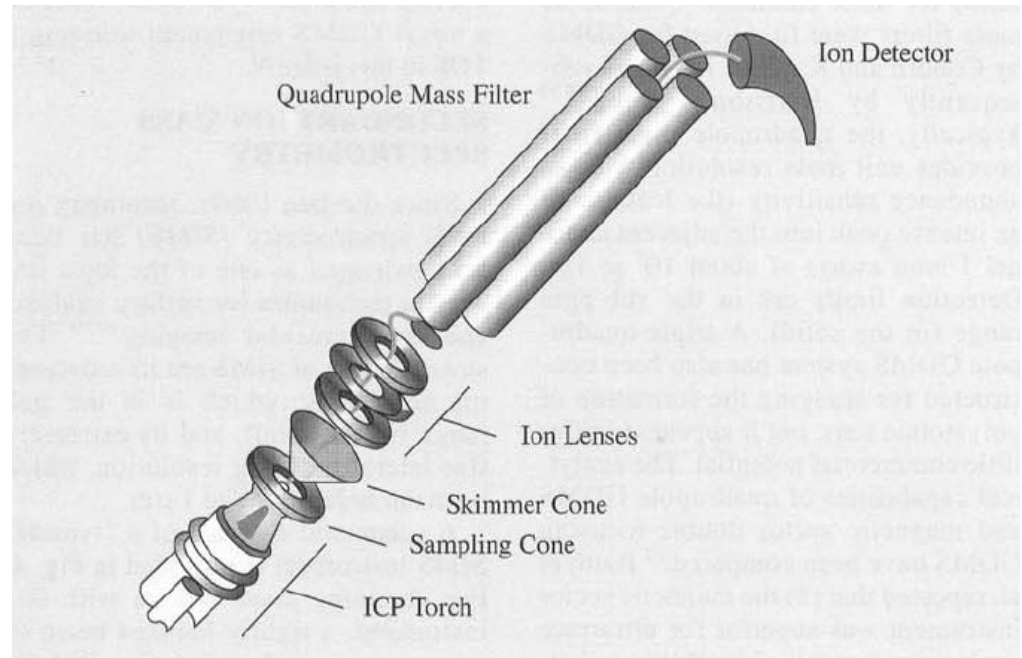⑤ magnetic field separates particles based on mass/charge ratio

Different kinds of MS

- Laser Microprobe Mass Spectrometry

- Inductively coupled Plasma Mass Spectrometry (ICP-MS)

- Glow Discharge Mass Spectrometry (GDMS)

- Secondary ion Mass Spectrometry

- Electrospray Mass Spectrometry
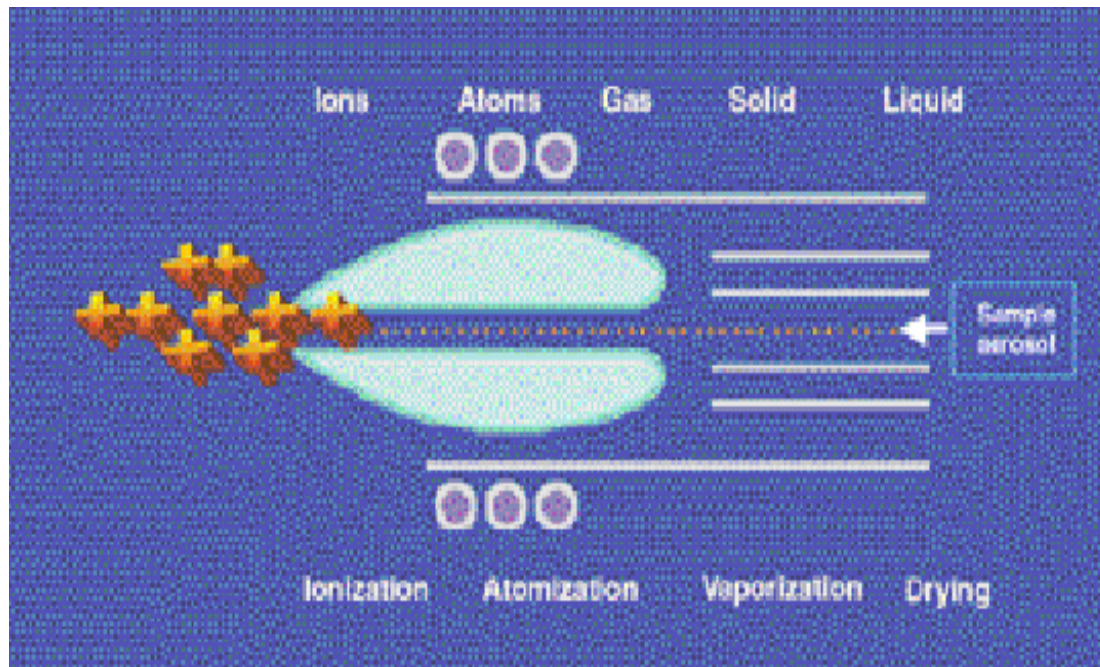
ICP-MS

- **Different Parts of ICP-MS:**
    - Principle of Ion Formation
    - Sample Introduction
    - Plasma Torch
    - Interface region
    - Ion focusing
   - Mass separation
    - Ion Detection
    - Sampling accessories

Generation of ions in the plasma

- ICP-MS is predominantly used for detection of positive ions, negative ions are also produced in plasma

- Sample is **introduced in liquid state**

- **Series of events :** Sample pumped into the sample introduction system → Sample emerges as aerosol → Sample Injector → Base of plasma → Analytical zone of plasma

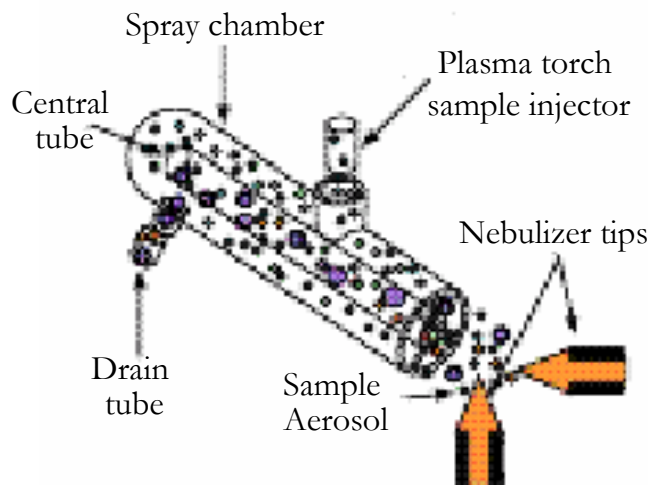- **We Get :** Liquid Sample → Solid Sample → **Gaseous sample** → **Atoms and ions**

Sample Introduction System
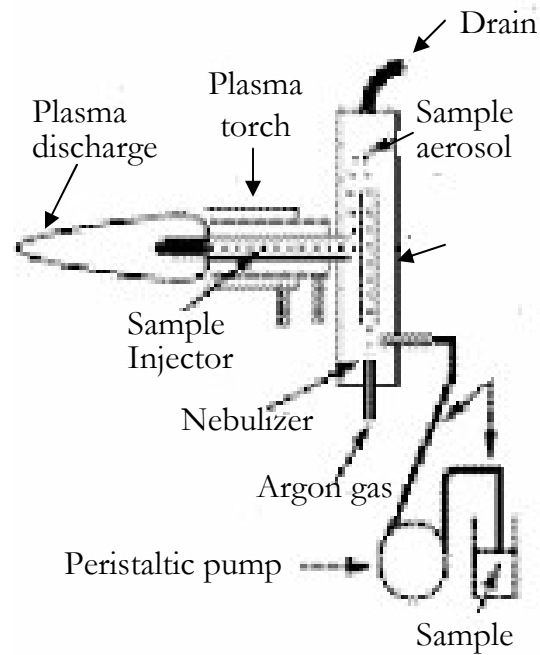
- Also named as the "ACHILLES HEEL" of ICP-MS

Droplet Selection

Why: Large droplets can't be dissociated by the plasma discharge. Hence, the allowance for small droplets
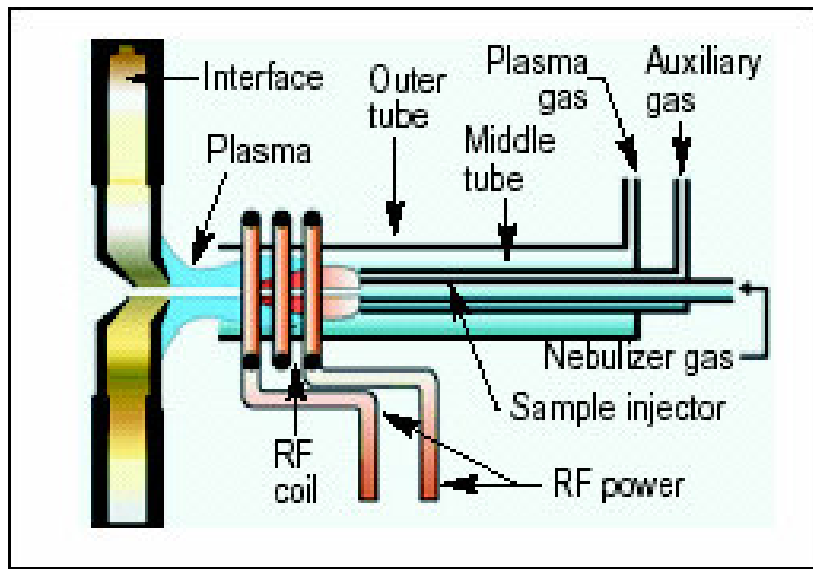

Double Pass Spray Chamber

- Liquid in the drain is kept at positive pressure
- Small droplets move between the outer wall and the central tube
- Tiny droplets go to the sample injector of the plasma torch
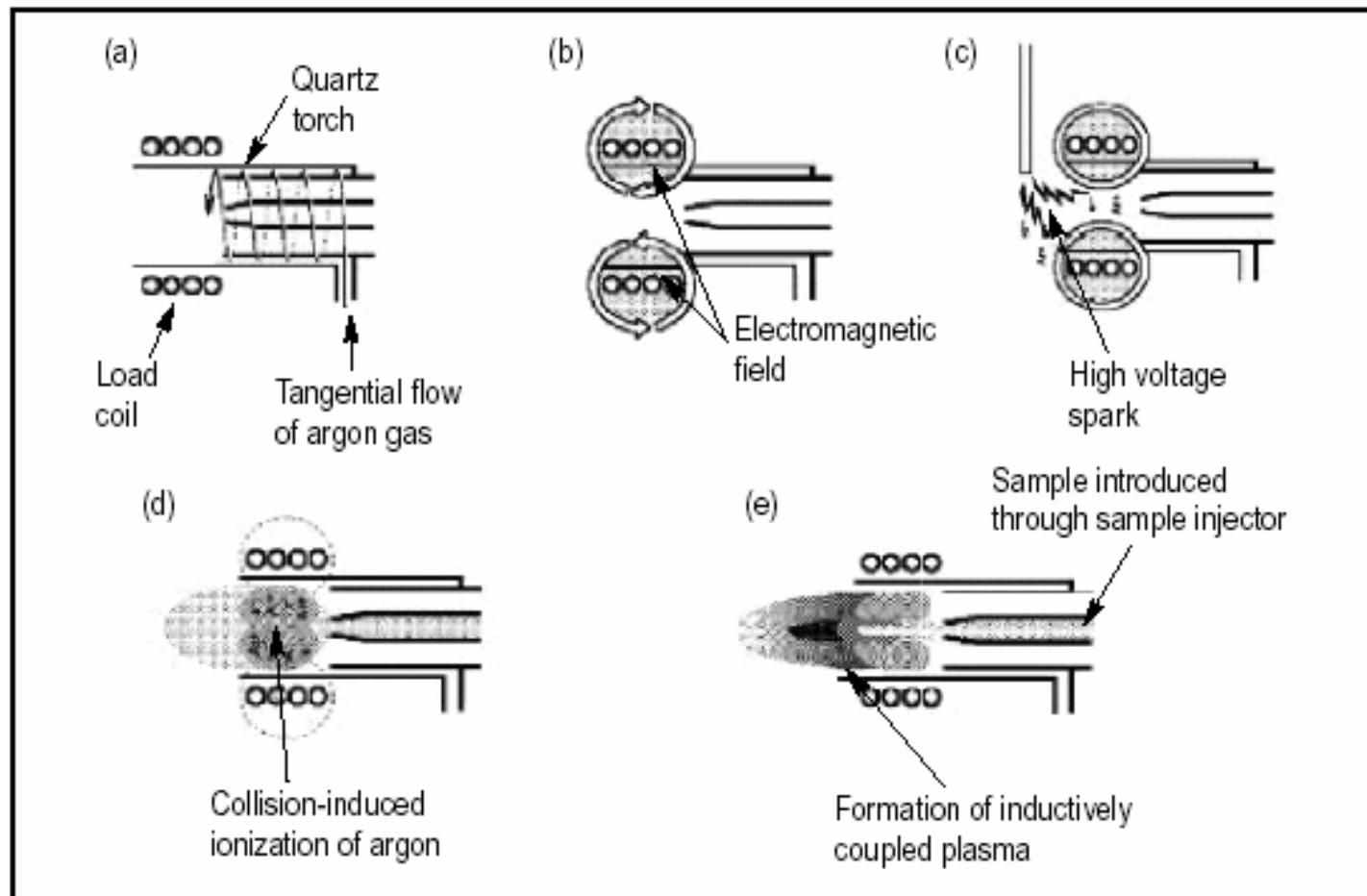
Droplet Size:
- Large droplets: >~10 μm in diameter
- Small droplets: 5-10 μm in diameter

The ICP-Plasma Torch

- Argon is used to form plasma
- Flow rate of Ar ~ **12-17 L/min**
- Auxillary gas rate ~ **1 L/min**
- Nebulizer gas rate ~ **1L/min**
- RF coil is grounded to keep the potential of interface region ~**0**

Formation of ICP Discharge

(a) Quartz torch, Load coil, Tangential flow of argon gas

(b) Electromagnetic field

(c) High voltage spark

(d) Collision-induced ionization of argon

(e) Sample introduced through sample injector, Formation of inductively coupled plasma

Mechanism

- Tangential Flow of Argon passes between the outer and the middle tube

- RF power is applied to the load coil → Intense electromagnetic field

- High voltage spark produces free electrons

- Free electrons get acceleration by the RF field → Collision and ionization of Argon

- ICP forms at the open end of the torch

The Interface region

- What happens here :

  Transportation of ions efficiently, consistently, and with electrical integrity from the plasma to the analyzer region
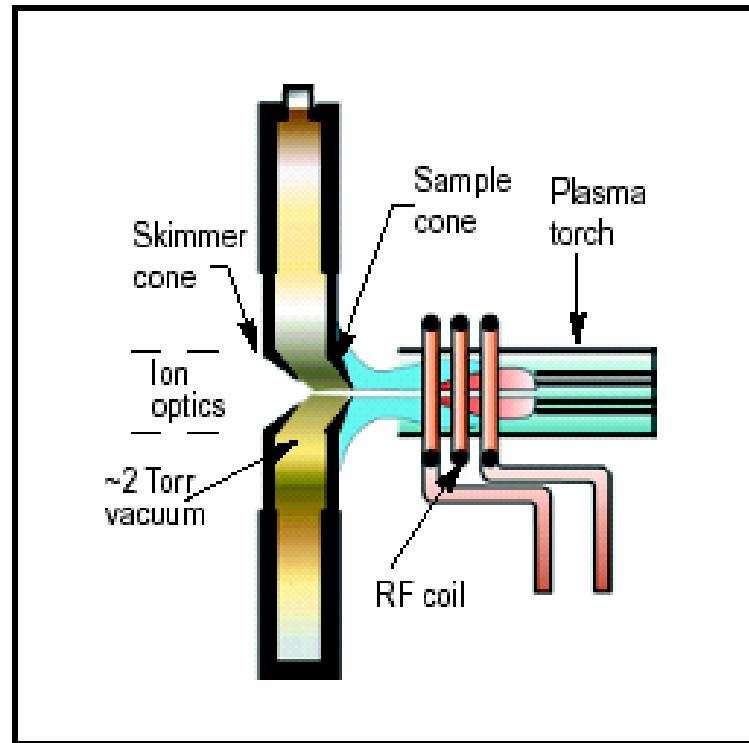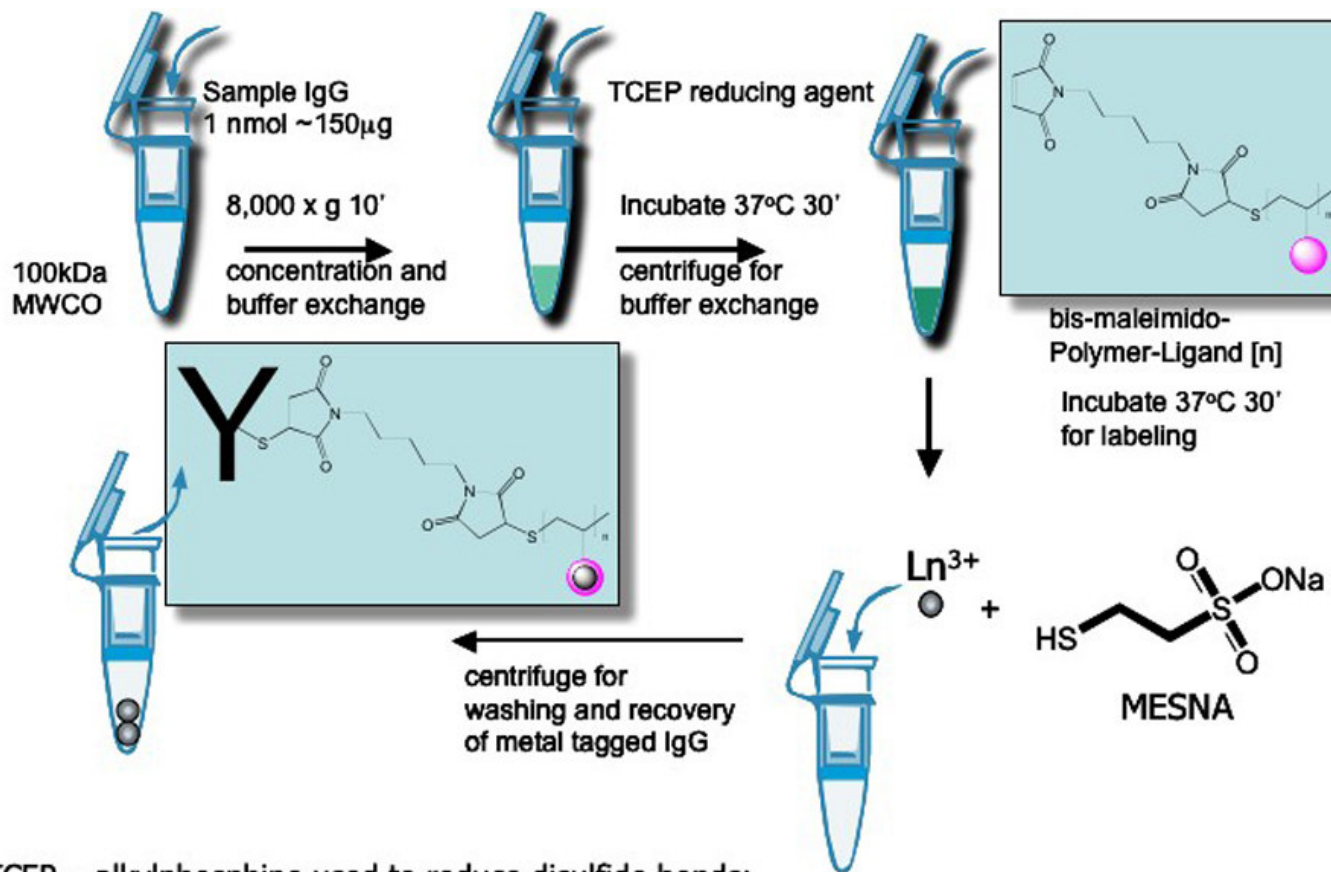
Mass Separation

- Ions of selected mass pass through the rods of the detector while others are ejected from the quadrupole on application of dc current on one pair of rods and radio frequency field on the other pair.
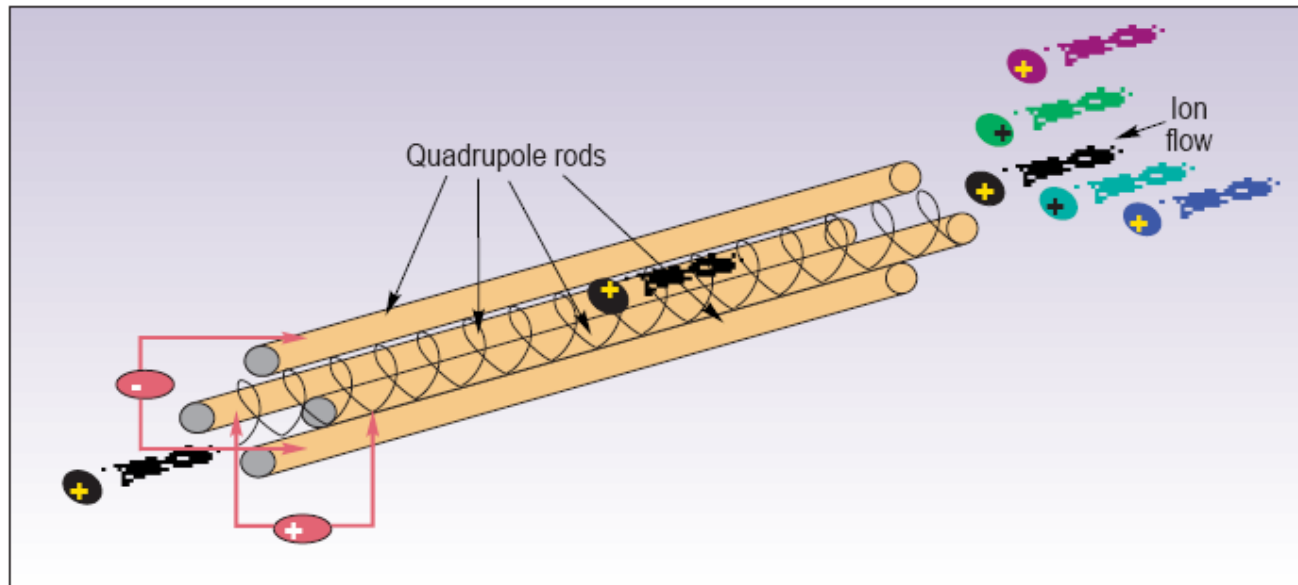
Application of ICP-MS in analysis of biological samples

- For quantification of toxic elements
- Study of metal transport
- Adsorption and metabolic studies of metals and metallo-drugs
- Affinity products having metal tag are used for ICP-MS immunoassays e.g. Antibodies raised against antigens of interest and tagged with metal
- Means of detection by fluorochromes and radioisotopes suffer from limited dynamic range and limited ability to detect multiple proteins simultaneously So, this provides a good alternative

Metal tagging to 2^0 antibodies

Sample IgG 1 nmol ~150μg

100kDa MWCO

8,000 x g 10'

concentration and buffer exchange

TCEP reducing agent

Incubate 37°C 30'

centrifuge for buffer exchange

bis-maleimido-Polymer-Ligand [n]

Incubate 37°C 30' for labeling

Ln^{3+} +

MESNA

centrifuge for washing and recovery of metal tagged IgG

TCEP – alkylphosphine used to reduce disulfide bonds;
MESNA – mercaptoethanesulfonate used to quench

Advantages of ICP-MS in enhancing performance of immunoassays

- High Precision
- Low detection limits
- Large dynamic range, both for each antigen and between antigens
- Lower matrix effects from other components of biological sample
- Lower background from plastic containers and plates
- Independence of non-specific background and analytical response from incubation or storage times
- Large Multiplexing Potential
- Better Spectral Resolution

Immunolabeling Cellular Components

- **Three Basic Stages**
    - Harvesting of cells of interest → Separation from contaminants → React with specific primary antibodies
    - Addition of secondary antibodies with the metal tag directed against 1^0 antibody specifically labeling the biomarker
   - Quantification by ICP-MS
- **ICP-MS used in :**
    - Surface antigen detection
    - Concordance of ICP-MS elemental signal to cell numbers
    - Simultaneous cell surface and intracellular detection
    - Multiplex identification

ICP-MS detection of a surface biomarker

- Target: Myeloid cell surface antigen CD33 (biomarker)
- Antibodies considered:
  - Anti-CD33 antibody A (BD biosciences)
  - Anti-CD33 antibody B (Immunotech Inc.)
  - Anti-mouse-Au
- Three cell systems:
  - Cells stained with antibody A (3 cell populations)
  - Cells stained with antibody B (3 cell populations)
 - Control cells stained with IgG1 (3 cell populations)

Results
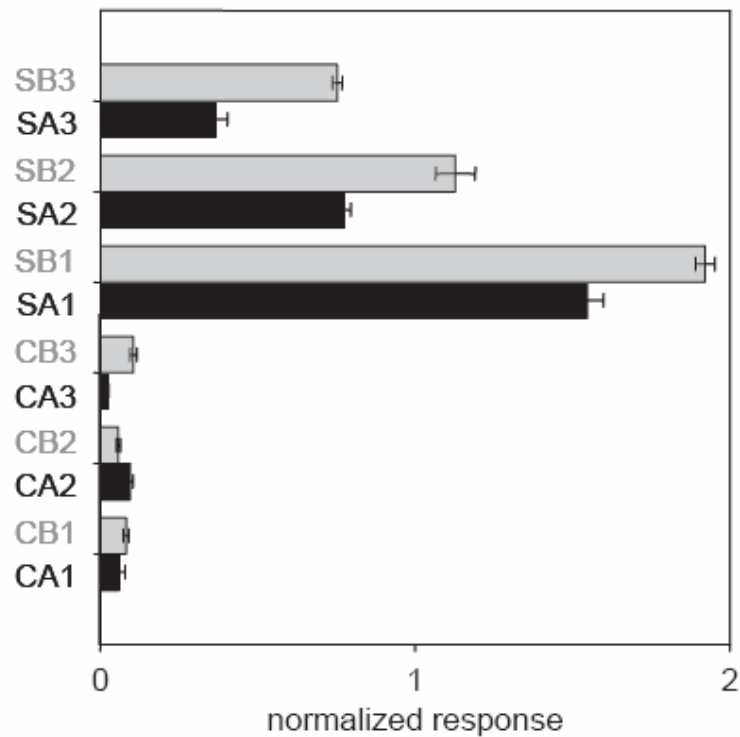
❑ **Black bars: Antibody A**

❑ **Grey bars: Antibody B**

▪ Anti-CD33 Antibody B shows higher response as compared to Anti-CD33 Antibody A

Simultaneous cell surface and intracellular biomarker detection

- Target: 2 cell surface biomarkers (CD33 and c-Kit) and 2 intracellular biomarkers (BCR and p210BCR/Abl)

- Antibodies:
  - Anti-CD33
  - Biotinylated-anti-c-Kit
  - Anti BCR

- Metal tagged reagents:
  - Anti-mouse - Au
  - Streptavidin - Tb
  - Anti-rabbit - Eu

Results
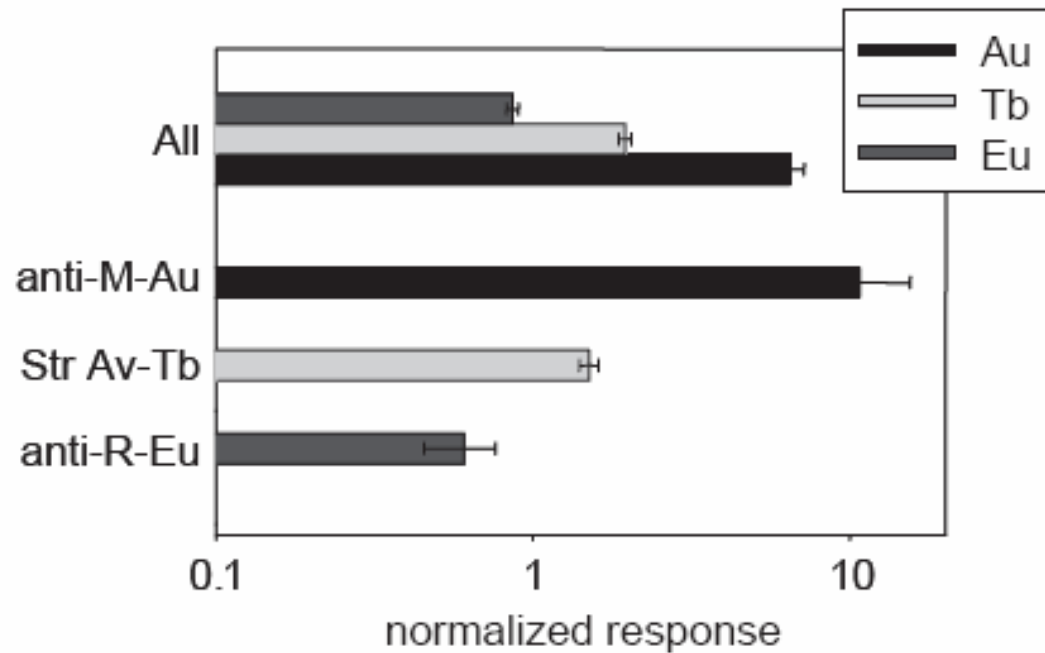
- The signal obtained are comparable in cases where the secondary tags were added all at once as to sample "All" and where they are added separately.
- So there is no interference from each metal tag when used in a multiplex assay.

Summary

- Biomarker basics

- Introduction to MS and ICP-MS

- Detailed description of ICP-MS

- ICP-MS in Biomarker detection

- Various studies

References

- O.Ornatsky, V.I.Baranov, D.R.Bandura, S.D.Tanner and J.Dick (2006), Multiple cellular antigen detection by ICP-MS. *J. Immuno. Met.,* **308**:68-76

- V.I.Baranov, Z. Quinn, D.R.Bandura and S.D.Tanner (2002), A senstitive and quatitative element-tagged immunoassay with ICPMS detection. *Ana.Chem.,* **74**:1629-36

- M.W. Blades (1994). Atomic Mass Spectrometry. *Applied Spectroscopy* **48** (11) 12 A.

- A beginners guide to ICP-MS, Robert J. Thomas
  - Part I
  - Part II: The Sample-Introduction System
  - Part III: The Plasma Source
  - Part IV: The Interface Region
  - Part V: The Ion focussing system
  - Part VI: The Mass Analyzer

Acknowledgements

- Prof. James F.Rusling

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