# DETECTION OF ANTIGLYCAN ANTIBODIES USING IMMUNOASSAY AND MAGNETIC PARTICLES



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Cancer

Heterogeneous group of diseases with uncontrolled cell division



## **Statistics 2020**

Worldwide (2020): 19.3 mil. new cases 9.9 mil. deaths One of the most common causes of death



Key to survival and quality of life - development of more sensitive diagnostic equipment



## **Biomarkers**

### **Glycans**

- o Basic biomolecules of cell
- Many important cell functions
- Aberrant structures pathological condition



Objectively measurable
 characteristic of a particular
 physiological or pathological
 condition of an organism

Different types of molecules

Healthy cellcharacteristic of aCellphysiological or ptransformationcondition of an





- Posttranslational modification
- Important balance between enzyme

expression and activity

- Imbalance = aberrant structures
- Dysfunctional COSMC chaperone T-synthase
  reduction expression of aberrant O-glycans



Ju T. Human tumor antigens Tn and sialyl Tn arise from mutations in Cosmc. Cancer Res. 2008 Mar 15;68(6):1636-46. doi: 10.1158/0008-5472.CAN-07-2345. Erratum in: Cancer Res. 2008 Apr 15;68(8):3076. PMID: 18339842

## **Autoimmunity**

The immune system responds to the presence of aberrant glycans
 Antibodies circulate in the blood - diagnostically significant

 $\circ$  5 years before clinical symptoms appear





Qiu J, Keyser B, Lin Z-T, Wu T. Autoantibodies as Potential Biomarkers in Breast Cancer. Biosensors. 2018; 8(3):67. https://doi.org/10.3390/bios8030067

## Aim

Detection of autoantibodies
 present in cancer diseases

## How?

- o Standard ELISA method
- o Modified ELISA method

### ELISA = Enzyme-Linked ImmunoSorbent Assay

#### $\circ~$ Clinically used method

- It uses a highly specific Ab-Ag interaction
- $\circ$  Indirect ELISA
- Antibody detection using an enzymeconjugated secondary antibody
- $\circ~$  Passive adsorption Ag
- Hydrophobic and electrostatic interactions
- $\circ~$  Coating buffer high pH
- Positively charged plate
- Signal color change of the substrate
- Directly proportional the more analyte, the higher the signal intensity (color)



## **ELISA = E**nzyme-Linked ImmunoSorbent Assay







- $\circ~$  Signal amplification
- Biotinylated antibodies
- Streptavidin-conjugated polyenzyme
- Higher signal for one analyte molecule







## **SUMBA = SU**spension Magnetic Bead-based Assay

- Magnetic particles
- -COOH groups on the surface
  - EDC / NHS chemistry
- MBs surface antigen immobilization
- Larger surface area compared to ELISA plate
- MBs are mostly used to concentrate analytes



of a magnetic particle





## **SUMBA**

- Comparison of secondary ab (sTn)
  - $\circ~$  conjugated to HRP and
  - $\circ~$  biotinylated ab with polyHRP streptavidin conjugate
- $\circ$  26.5 times higher slope at 1000 x dilution of polyHRP



- Comparison of standard ELISA and SUMBA (sTn)
  - $\circ$  4.06 times higher slope



## **Characterization**



### • **EDX**

- $\circ$  Altered O / Fe ratio 1.38 -> 5.32
- Presence C, N after immobilization

#### $\circ$ SEM

- $\circ~$  Slightly increased average MBs
- Tendencies of modified MBs improve in a dry environment



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