Molecular Diagnostics

Molecular Diagnostics

The use of molecular biology techniques to expand scientific knowledge of the natural history of diseases, identify people who are at risk for acquiring specific diseases, and diagnose human diseases at the molecular level.

Molecular Diagnostic

USE OF :
– Sequence Specific INFORMATION
in
– MACROMOLECULES
for
– Risk identification
– Diagnosis
– Prognosis
– Prediction of response to therapy
– Monitoring therapeutic responses

Characteristics of a Detection System

A good detection system should have 3 qualities:

♣ Sensitivity

♣ Specificity

♣ Simplicity

• Sensitivity means that the test must be able to detect very small amounts of target even in the presence of other molecules.

• Specificity: the test yields a positive result for the target molecule only.

• Simplicity: the test must be able to run efficiently and inexpensively on a routine basis. Molecular Diagnostics

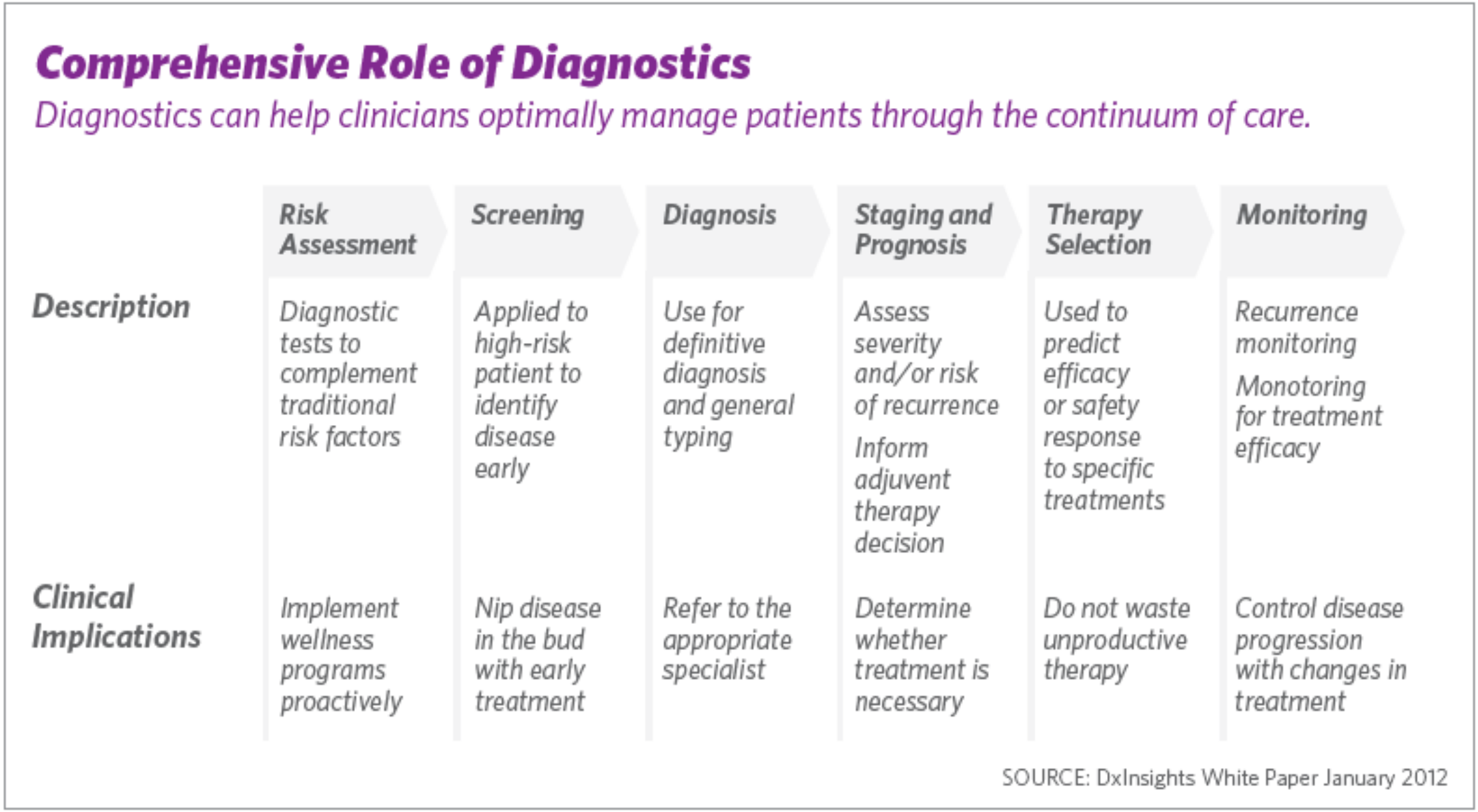
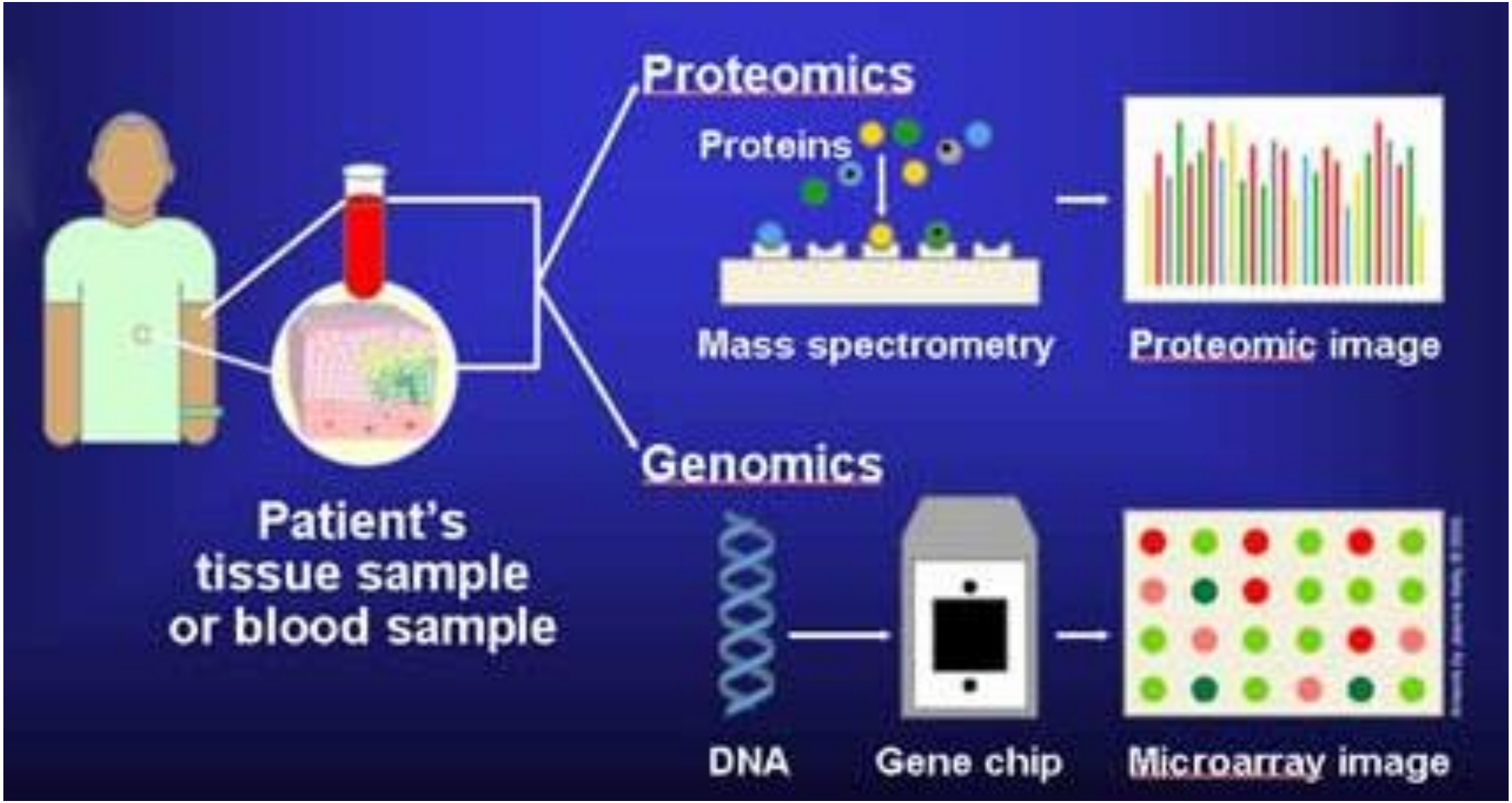


FIGURE 1: Role of diagnostics through the continuum of health care.

FIGURE 2: Molecular Diagnostics as a component in the global in vitro diagnostics market.

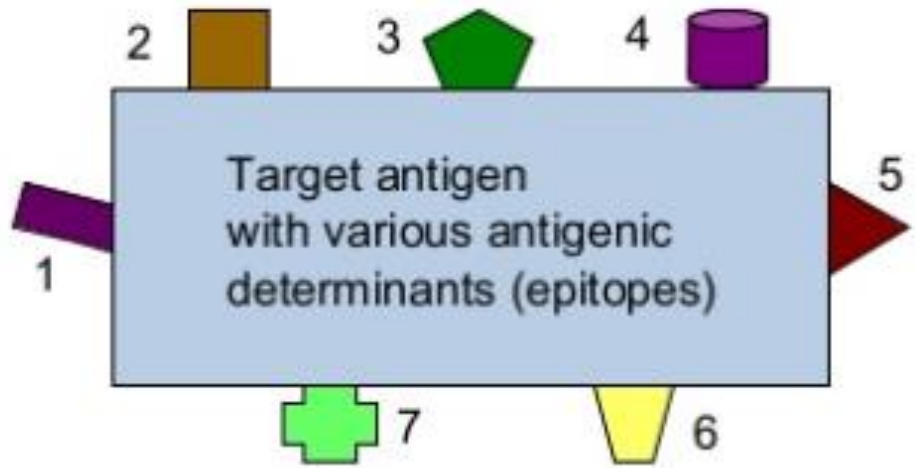
The overall global market for diagnostics was valued at $45.6 billion in 2012 and is expected to grow at about 7% annually over the next five years to reach a market size of $64.6 billion in 2017[1]. The United States and Europe account for about 60% of that market, with Asia Pacific forecast as the highest growth region over the next five years.


Proteomics
Proteins
Mass spectrometry
Proteomic image
Genomics
Patient's
tissue sample
or blood sample
DNA
Gene chip
Microarray image

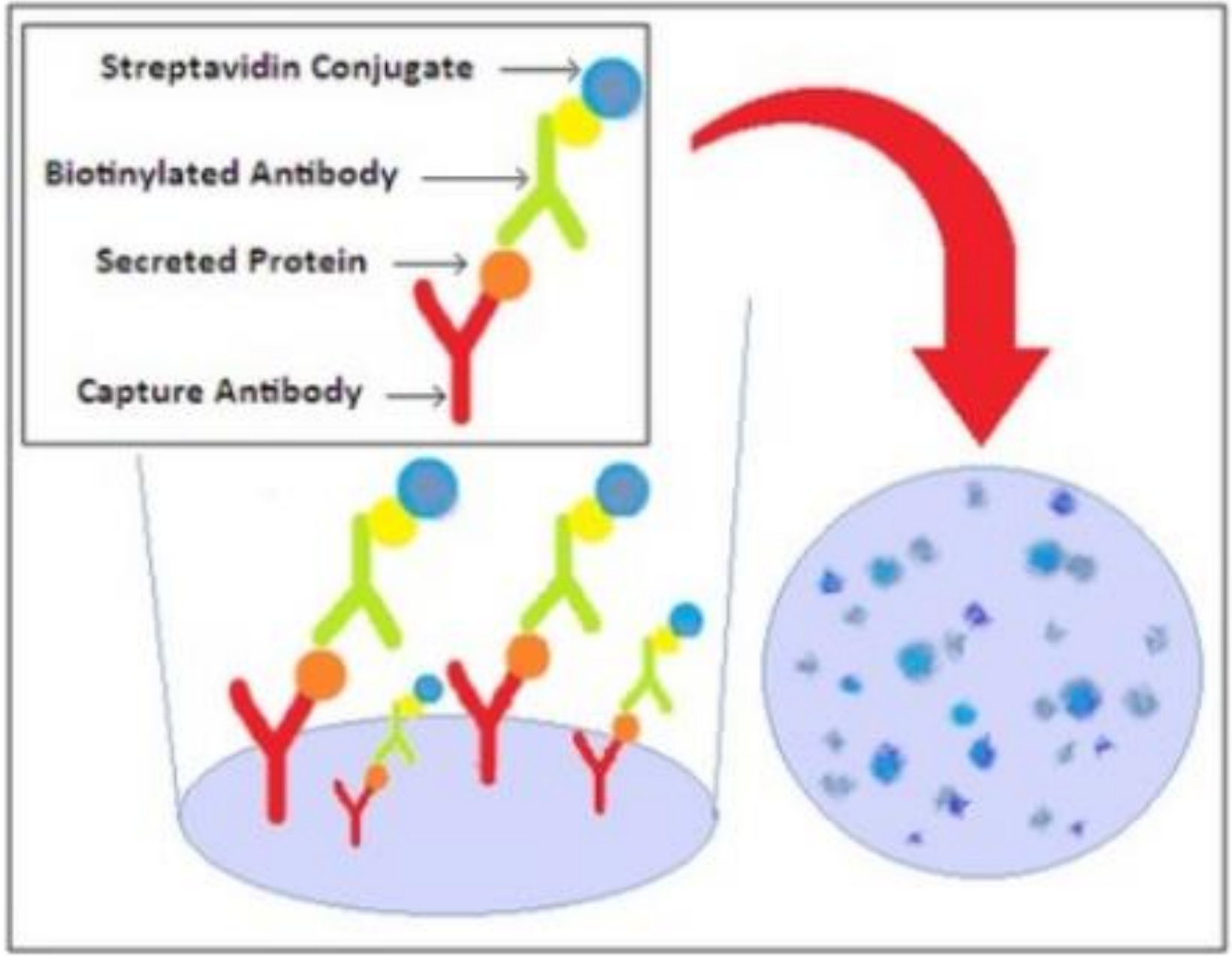
Immunological Diagnostics Methods

1. Radioimmunoassay
2. Enzyme-Linked ImmunoSorbent Assay (ELISA)
3. Western Blotting
4. Immunoprecipitation
5. Immunofluorescence
6. Flow Cytometry and Fluorescence
7. Alternatives to Antigen-Antibody Reactions
8. Immunoelectron Microscopy

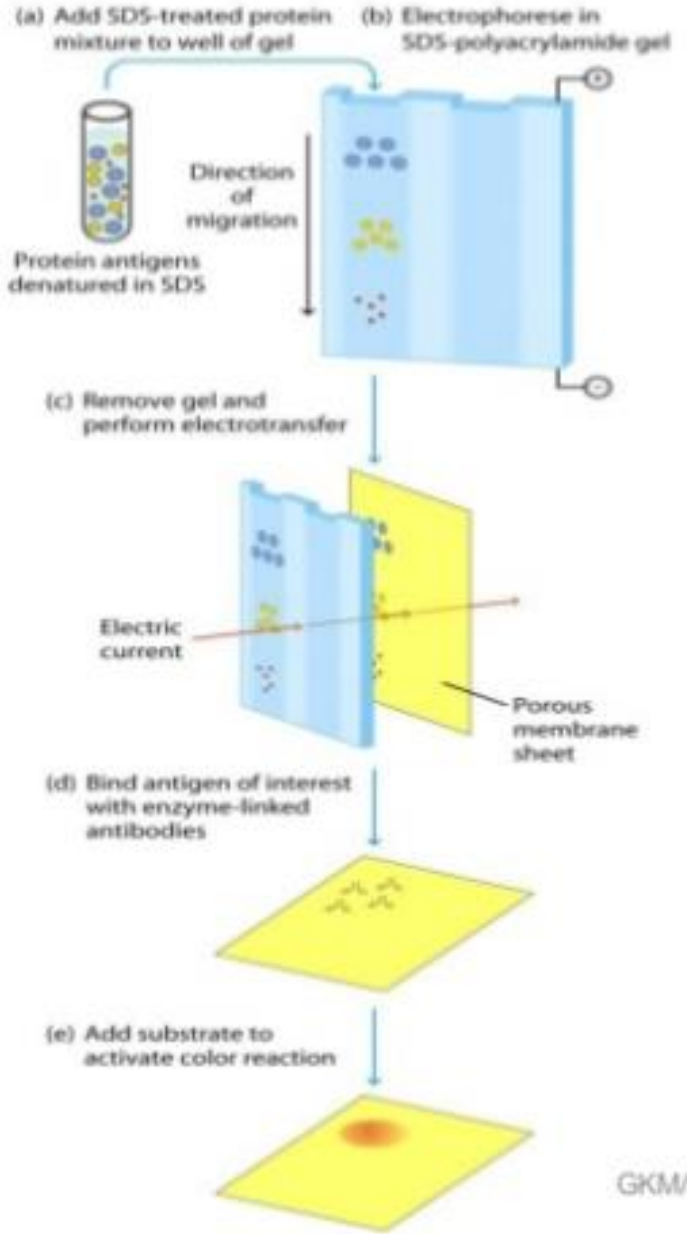
Target antigens and polyclonal versus monoclonal antibodies



Polyclonal antibodies are made against and react with multiple antigenic sites (epitopes) on a target antigen. Monoclonal antibodies are directed against a particular antigenic site.


Streptavidin Conjugate
Biotinylated Antibody
Secreted Protein
Capture Antibody

Western blot



SDS-Page: separates the components according to their molecular weight.

Blot: the proteins in the gel are transferred to the sheet of nitrocellulose or nylon by the passage of an electric current.

Immunoreaction: probed with Ab & then radiolabeled or enzyme-linked 2nd Ab.

Detection: a position is visualized by means of an ELISA reaction.

Western Blot Setup

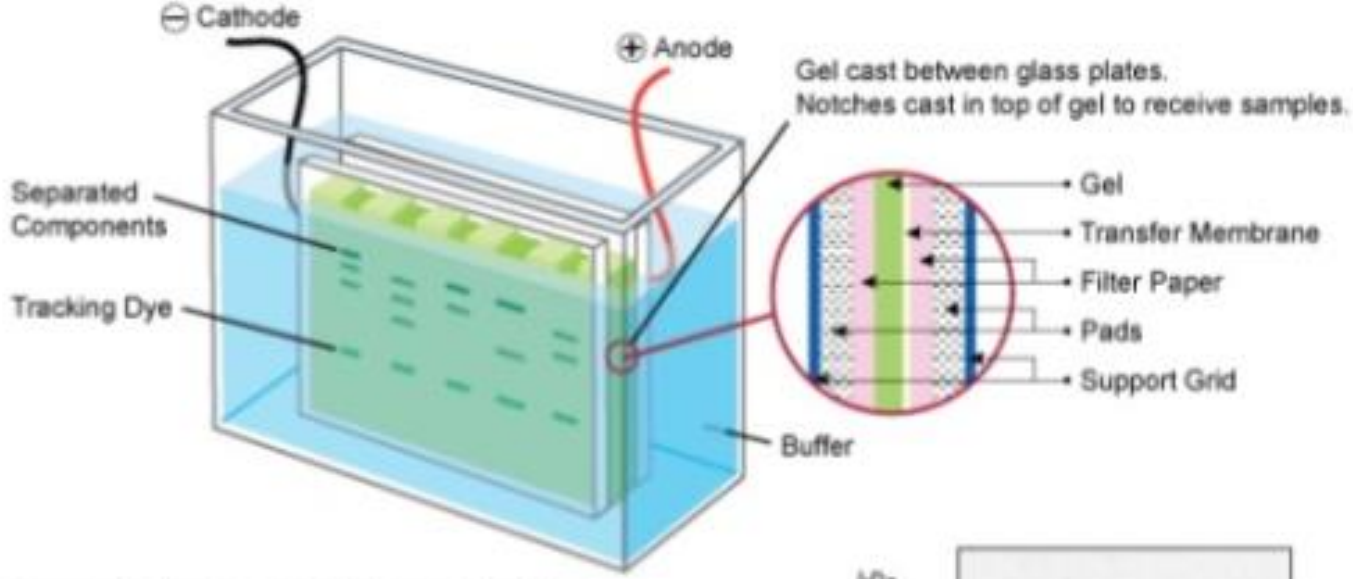
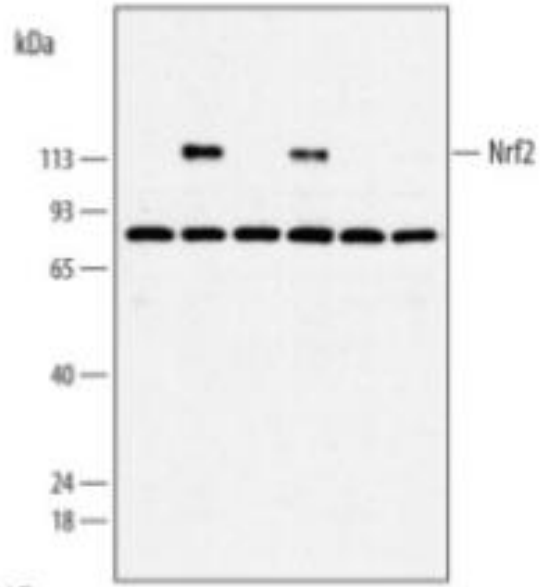


Diagram 1: *Illustration of Western Blot Setup.*



Target antigens and polyclonal versus monoclonal antibodies

- Polyclonal antibodies are made against and react with multiple antigenic sites (epitopes) on a target antigen.
- Monoclonal antibodies are directed against a particular antigenic site. Target antigen with various antigenic determinants (epitopes)1 2 3 4 5 67

Targets for diagnostic monoclonal antibodies

- Polypeptide hormones (chorionic gonadotropin, growth hormone)
- Tumor markers (Prostate-specific antigen)
- Cytokines (interleukins 1-8)
- Drug monitoring (cyclosporin)
- Miscellaneous targets (Vitamin B12)
- Infectious diseases (Chlamydia, Herpes, Rubella, Hepatitis B, Legionella, HIV)

Molecular Diagnostics

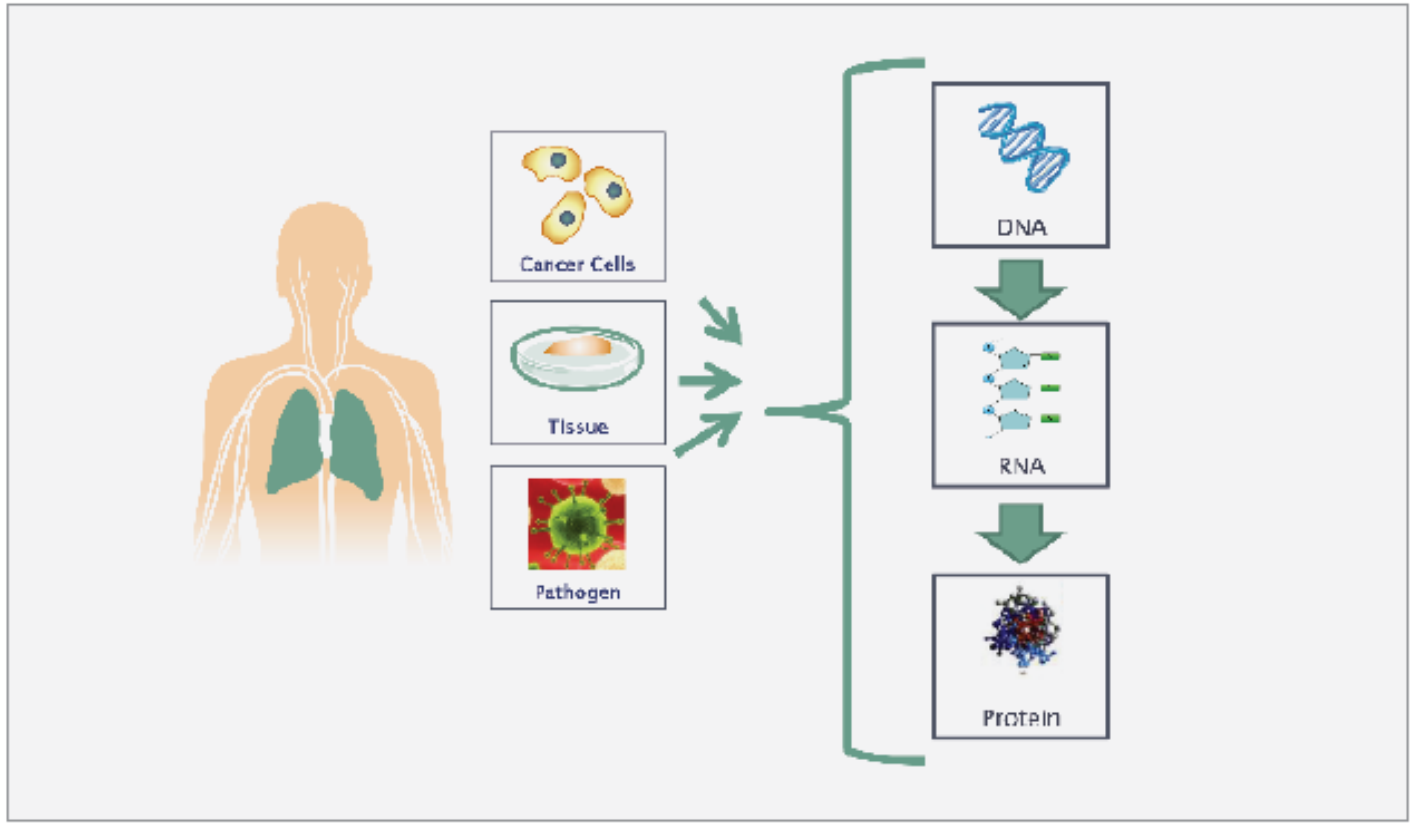


FIGURE 3: Molecular diagnostics examine the molecules in the cell, i.e. the DNA, RNA or proteins, and how their role in human biology and disease.

We must
often go to the molecular level to understand the many ways in which diseases can develop within an individual.

Macromolecules

- Peptides/proteins
- Polysaccharides
- Polynucleotides /nucleic acids

Technology of Molecular Diagnostics

DNA Sequence Detection using Polymerase Chain Reaction (PCR)

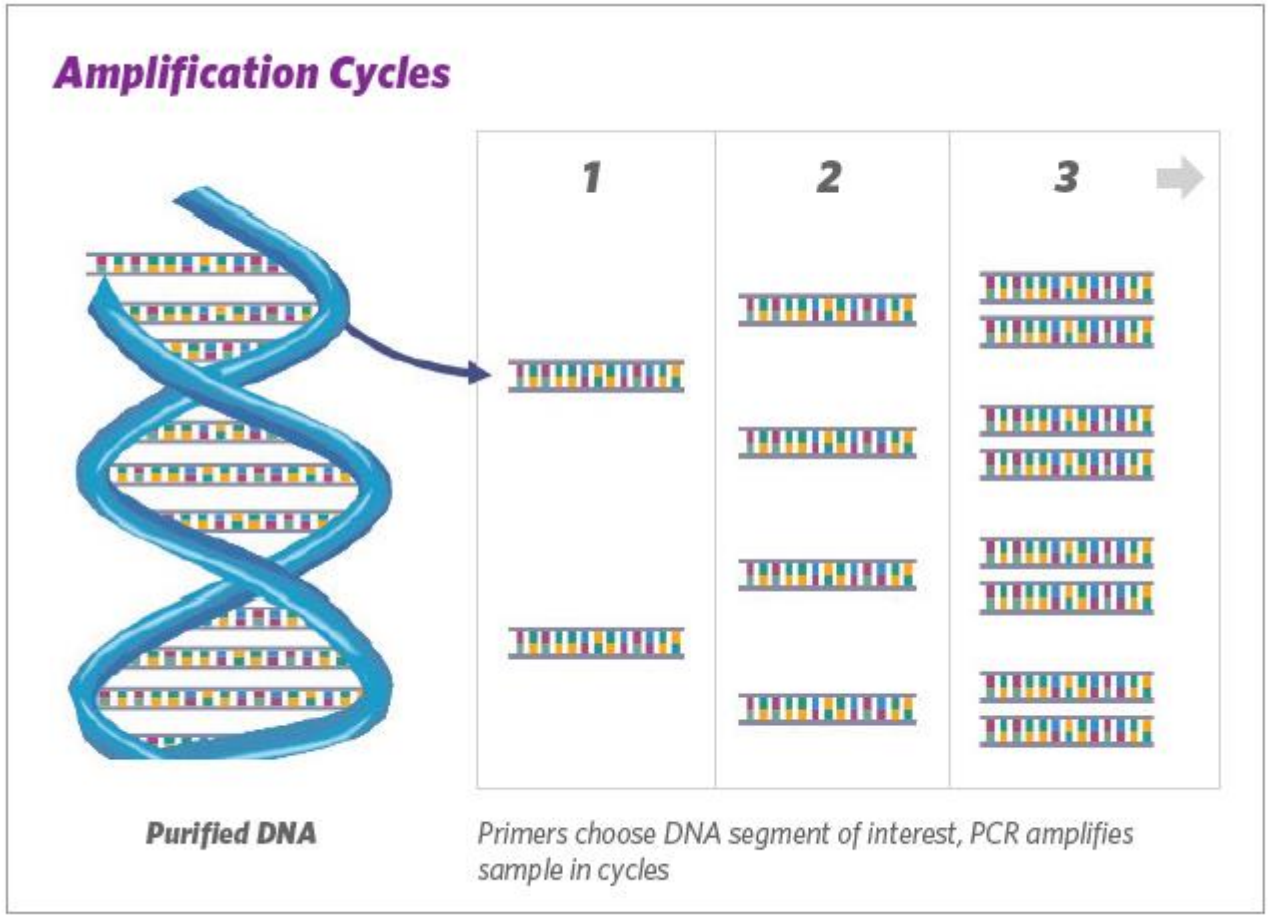


FIGURE 4: Polymerase Chain Reaction to amplify DNA by repeating cycles that each duplicates the base sequence in a specific section of the DNA strand.

Molecular Diagnostics Examples

There are hundreds of examples of molecular diagnostics across this continuum.

	Risk Assessment	Screening	Diagnosis	Staging and Prognosis	Therapy Selection	Monitoring
Description	Diagnostic tests to complement traditional risk factors	Applied to high-risk patient to identify disease early	Use for definitive diagnosis and general cancer typing	Assess severity and/or risk of recurrence Inform adjuvent chemo decision	Used to predict efficacy or safety response to specific treatments	Recurrence monitoring Monotoring for treatment efficacy
Molecular Diagnostic Example	CF carrier testing BRCA1 testing	MRSA HPV	TB CT/NG	OncoType Dx MammaPrint	BRAF KRAS Her2	BCR-ABL HIV viral load

FIGURE 6: Examples of molecular diagnostics across the continuum of care.

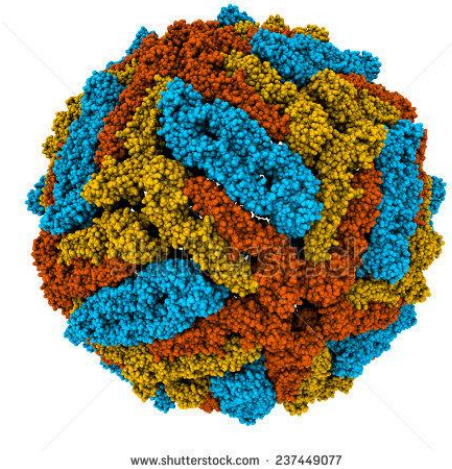
PADJADJARAN UNIVERSITY
INDONESIA

RISTEKDIKTI

大阪府立大学
OSAKA PREFECTURE UNIVERSITY

Single Chain Fragment Variable Discovery for Development of a Serotype-2 Dengue Virus NS1 Detection Kit

DENGUE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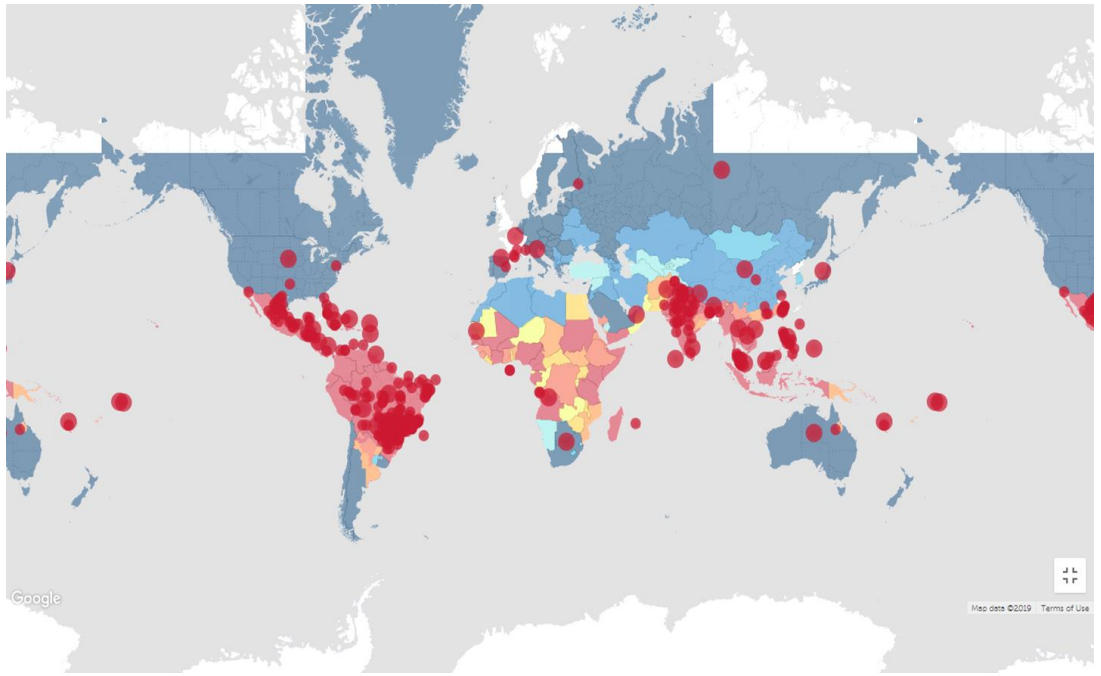
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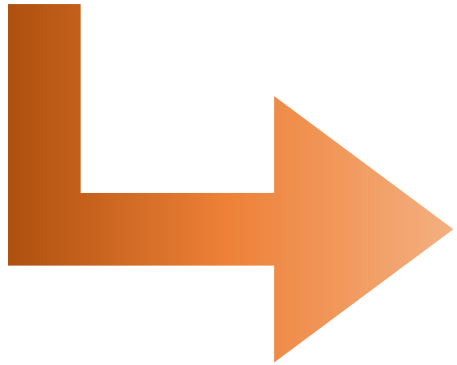
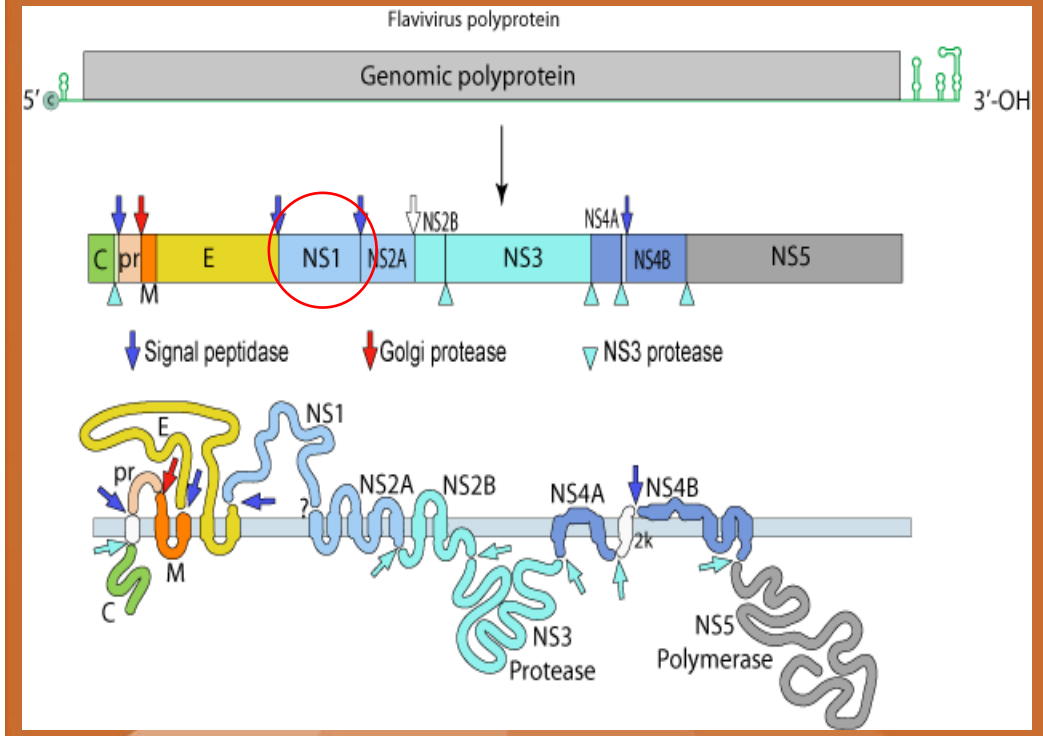



Vaccine
SIN DBD
MAM BERDARAH DENGUE)
piogamaugm.wordpress.com

DENGUE VIRUS



Category 1
Add Text here

Category 2
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Category 3
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Category 4
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64%

Sensitivity

100%

Specificity
(Guzman et al., 2010)

Diagnostic of

NS1

48 Indonesian NS1 antigen

(Aryati et al., 2013)

In-silico Analysis and Homology Modeling of Indonesian NS1 Antigen on Diagnostic Sensitivity Test of Dengue

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ABSTRACT

Aim: The non-structural glycoprotein-1 (NS1) antigen is one of the device options in laboratory diagnostic test, that can be used to detect either primary or secondary infections in the earliest stages [13]. Nowadays, the sensitivity of the kit currently diagnosed with DHF during surveillance in Indonesia is less than 70% [1]. The aim of this study was to analyze 48 structures of Indonesian NS1 antigenes of DENV1-4 isolates.

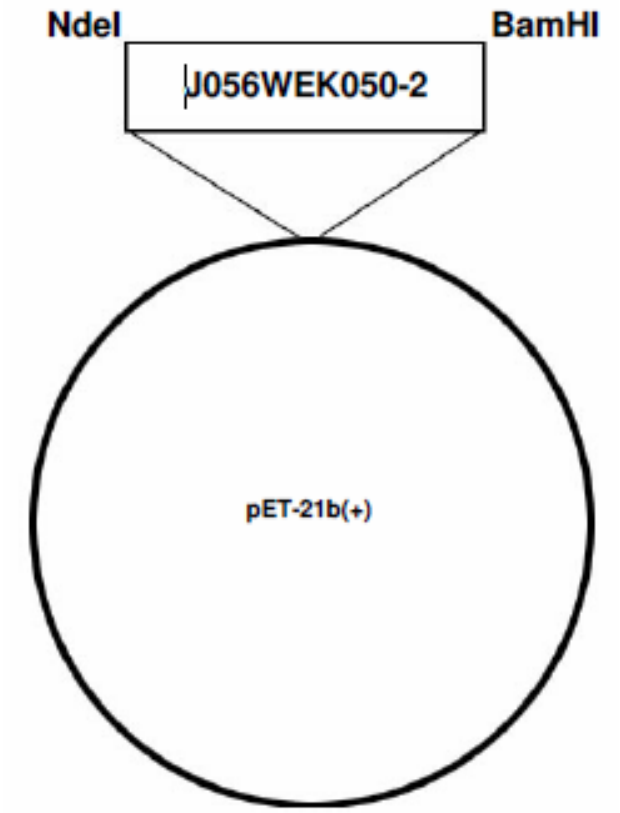
Methods: In this study, bioinformatics approach was performed on the 48 structures of Indonesian NS1 antigenes of DENV1-4 to obtain the best model using BLAST and Pyre2. Homology modeling was carried out using Modeller 9.19 through templates which have a high homology level with the amino acid sequence NS1 antigen.

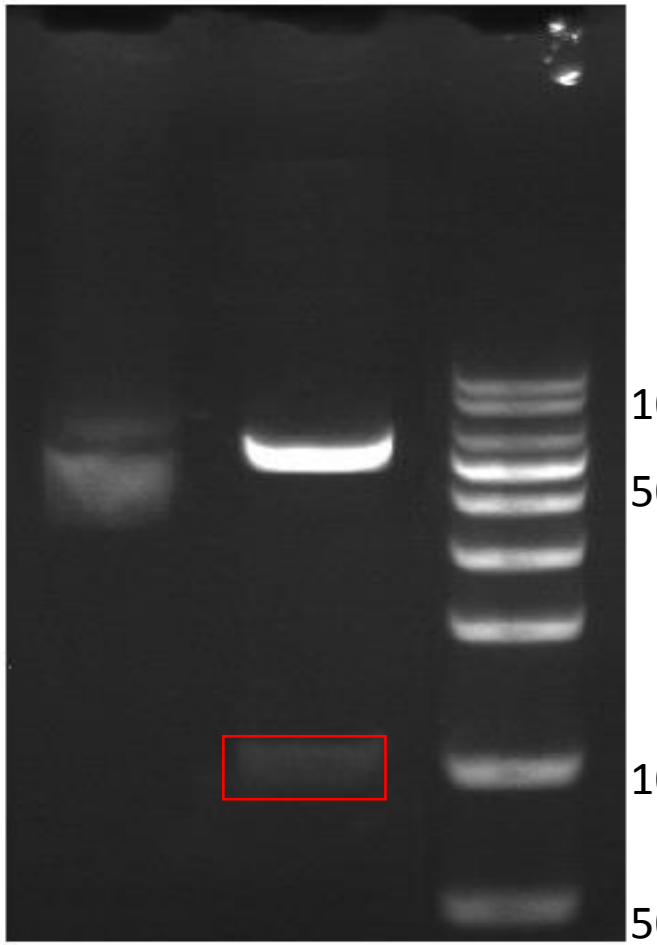
Results: We obtained the best template of NS1 antigen Indonesia with PDB ID 4O6B_A. The best model of DENV-1, DENV-2, DENV-3, and DENV-4 were NS1/ID/DENV1-20, NS1/ID/DENV2-2, NS1/ID/DENV3-6 and NS1/ID/DENV4-1 with Ramachandran plot value 91.3%, 90.5%, 90.6% and 90.2%, respectively.

Conclusion: The results showed that the differences of structures, amino acid residues, and epitopes from templates and each serotype are valuable tools for designing molecular antigen as diagnostic agent to enhance the sensitivity value of dengue diagnostic kits in Indonesia.

Keywords: dengue virus, NS1 antigen, homology modeling, sensitivity, dengue diagnostic kit.

Plasmid Construct Map of scFv-BAD DENV

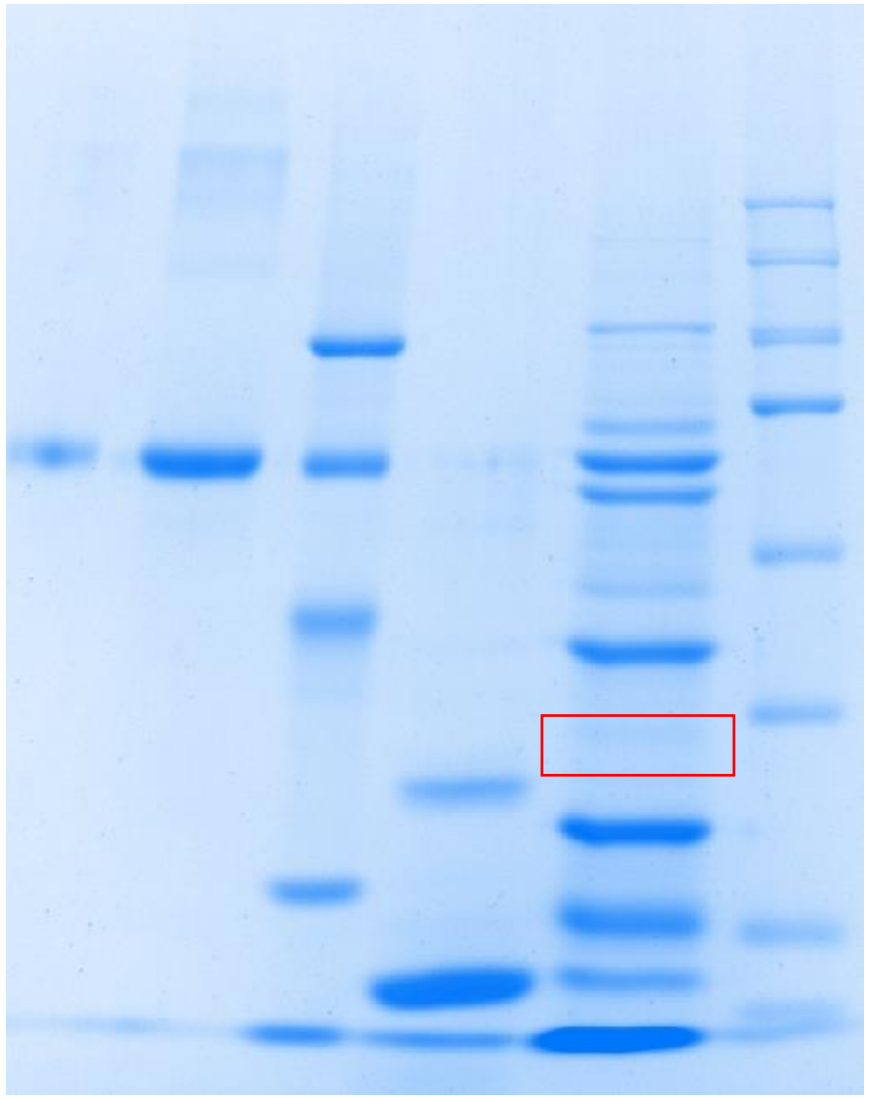


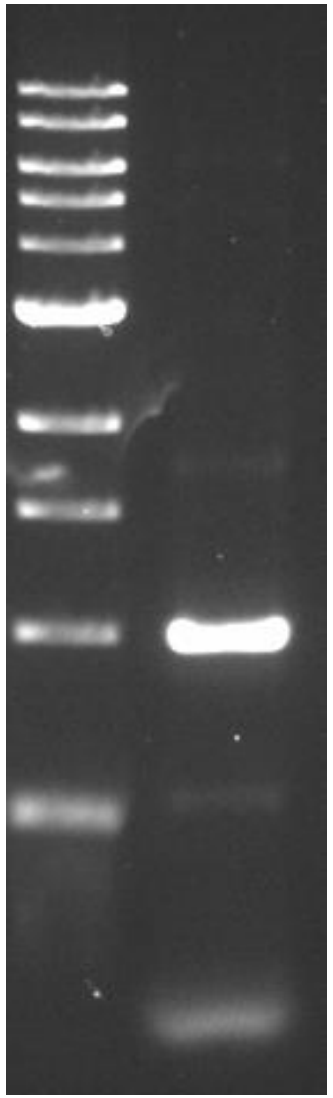


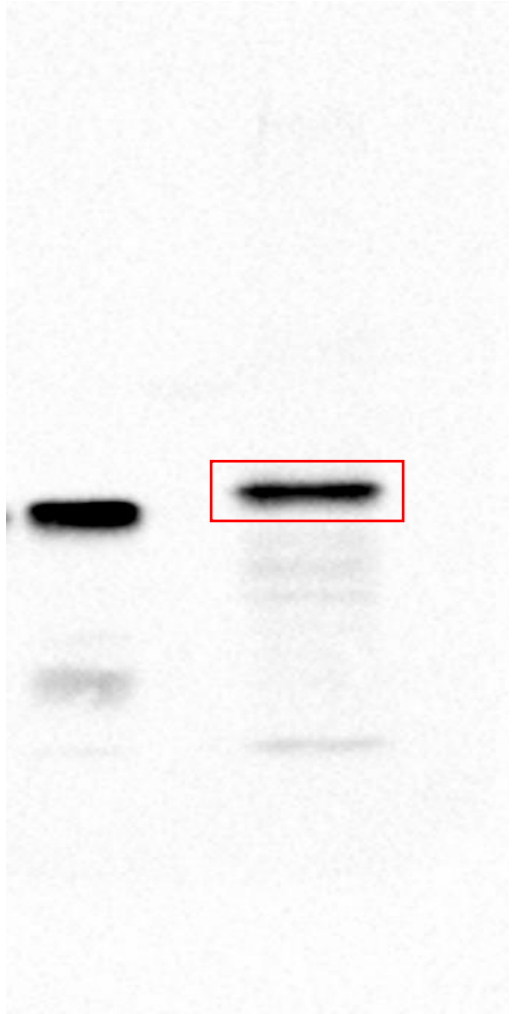
Lane M: KB Ladder
Lane 1:J056WEK050-2 plasmid
Lane 2:J056WEK050-2 plasmid digested by NdeI and BamHI

Digestion Conditions:
About 300ng plasmid digested
Digestion in water-bath,37°C for 40 minutes
1% Agarose Gel

Western Blotting







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