



UNIVERSITAS GADJAH MADA

# ANALISIS KEHALAHAN PRODUK dengan IMMUNO-BASED ASSAYS

## ELISA dan IMMUNOCHROMATOGRAPHY

**Sudjadi**  
IHIS UGM/  
Sekolah Pascasarjana UGM



**Immunoassays (IAs)** merupakan tehnik yang berdasarkan pembentukan ikatan kompleks **antigen-antibodi** yang stabil

Metode ini telah telah banyak digunakan, terutama di kimia klinik, digunakan untuk deteksi protein, hormone, dan penyakit



## ■ Immunoassays menjadi penting jika:

- Diperlukan pengukuran dan evaluasi yang cepat
  - Diperlukan deteksi dipercaya
  - Jumlah sampel yang sangat banyak
  - Metode lain sulit dan mahal
- 
- Keuntungan terbesar penggunaan immunoassays untuk **penapisan** suatu penyakit, perlu validasi lebih lanjut

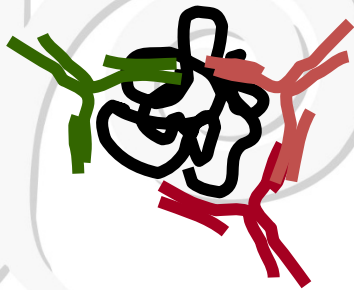


# Antibodi

## Poliklonal

Berbagai antibodi yg diambil dari sera hewan terpapar

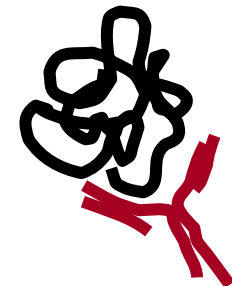
Mengenal berbagai epitop antigen



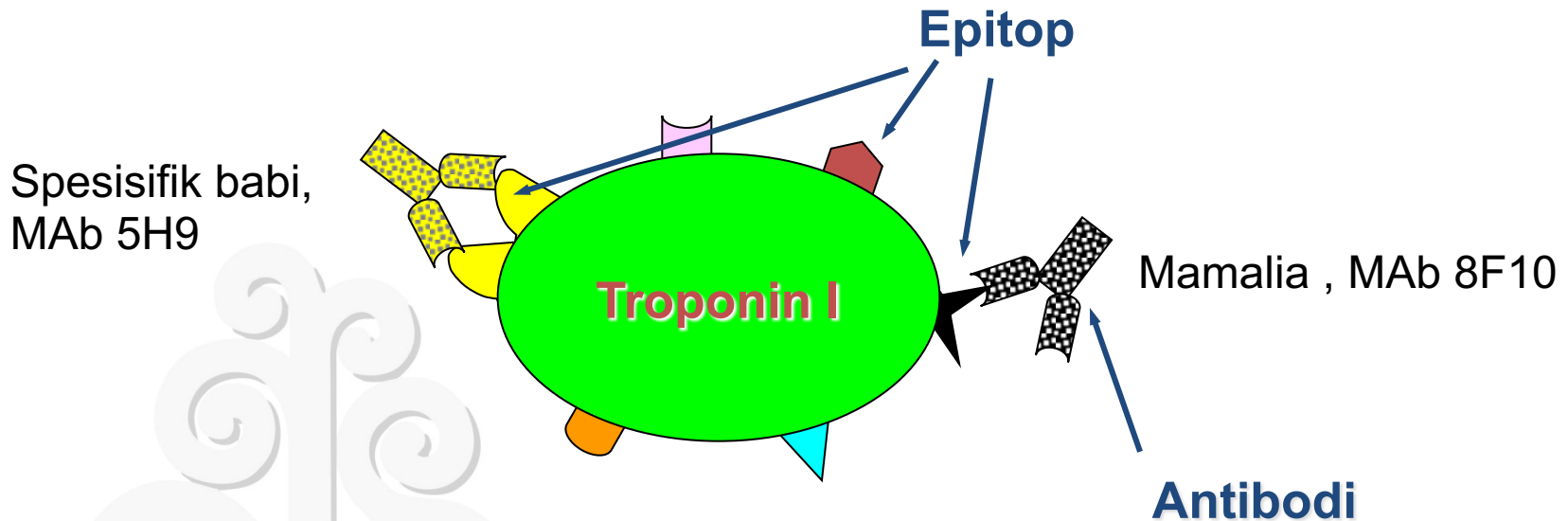
## Monoklonal

Hibridoma limposit B tunggal diklon dan dibiakkan, menghasilkan antibodi yg kumpulkan dari media

Mengenal SATU epitop



# Ikatan Antibodi- Antigen

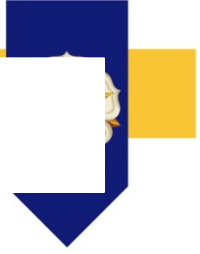


**ANTIGEN = ANTIBody GENerating**

**Setiap antigen mempunyai banyak epitop**

**Setiap antibodi mengenal satu epitop spesifik**

# Pengkuran\_Antigen



- Dua parameter yang harus diperhatikan:
  1. **Sensitivitas/kepekaan** : mampu mendeteksi antigen dalam jumlah kecil  
Metode yg sangat peka – tidak ada negatif palsu
  2. **Spesifik** : mampu membedakan molekul yang serupa  
metode yang sangat spesifik tidak ada positif palsu

# Validasi



- LoD (sensitivitas)
- Linearitas
- LoQ
- Range
- Akurasi (sesuai dengan baku)
- Presisi (repeatability, reproduktibilitas)
- Spesifisitas (Interferensi, reaktivitas silang )
- Robustness

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE. **ICH HARMONISED TRIPARTITE GUIDELINE. VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2(R1)**. Current *Step 4* version. Parent Guideline dated 27 October 1994. (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005)

US FDA, 2011; Gene Pennello, 2012

# ELISA



- EL            Enzyme-linked
- IS            Immunosorbent
- A            Assay
  - Dapat digunakan secara kualitatif (satu epitop-MAb)
  - Dapat menetapkan jumlah antigen (kuantitatif).

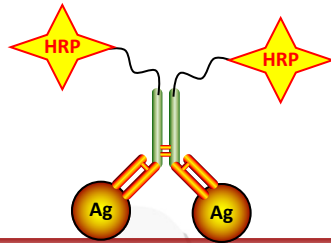




# Jenis sistem deteksi

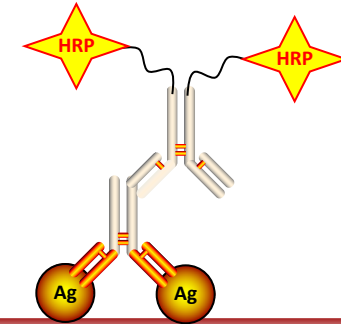
## 1. Direct immunodetection

Antibodi primer dikonjugasikan dgn sistem enzim



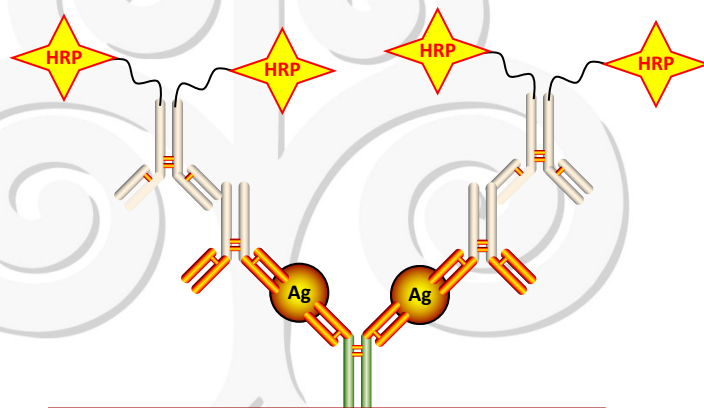
## 2. Indirect immunodetection

Antibodi sekunder dikonjugasikan dgn sistem enzim



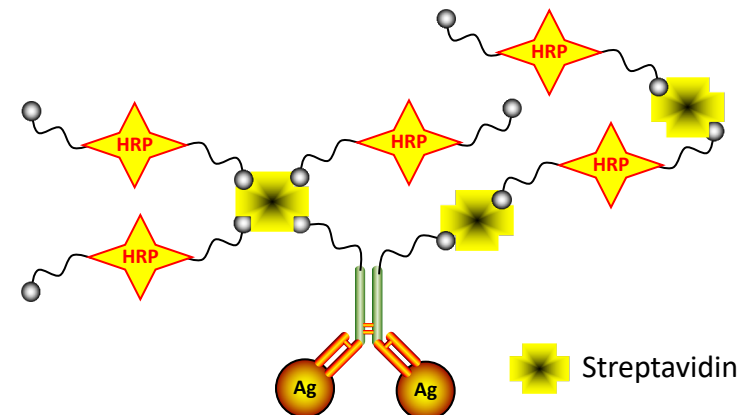
## 3. Sandwich indirect immunodetection

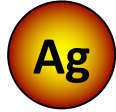


Antigen dlm bentuk terlarut



## 4. Indirect immunodetection with biotin linkers

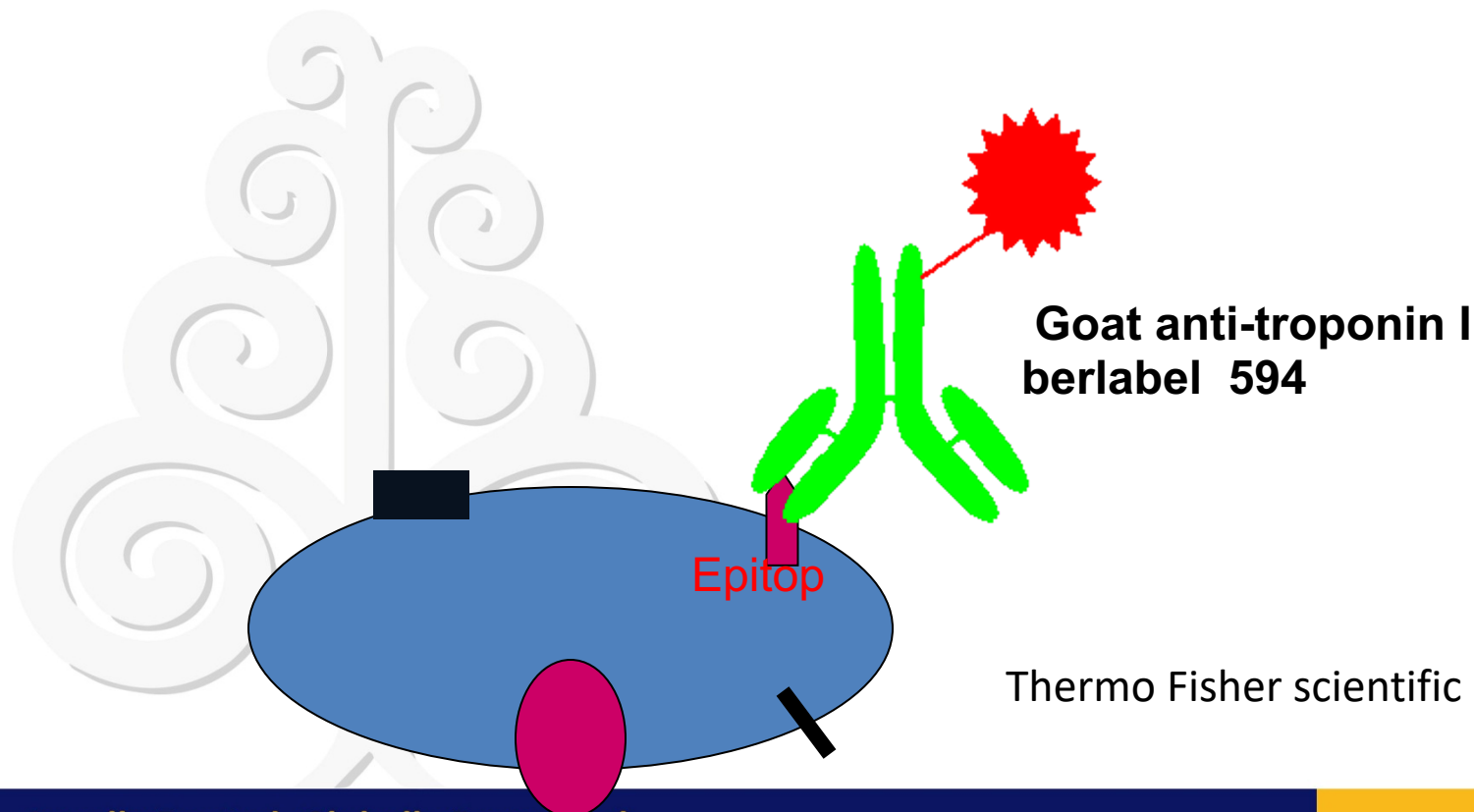
Antibodi primer dgn biotin



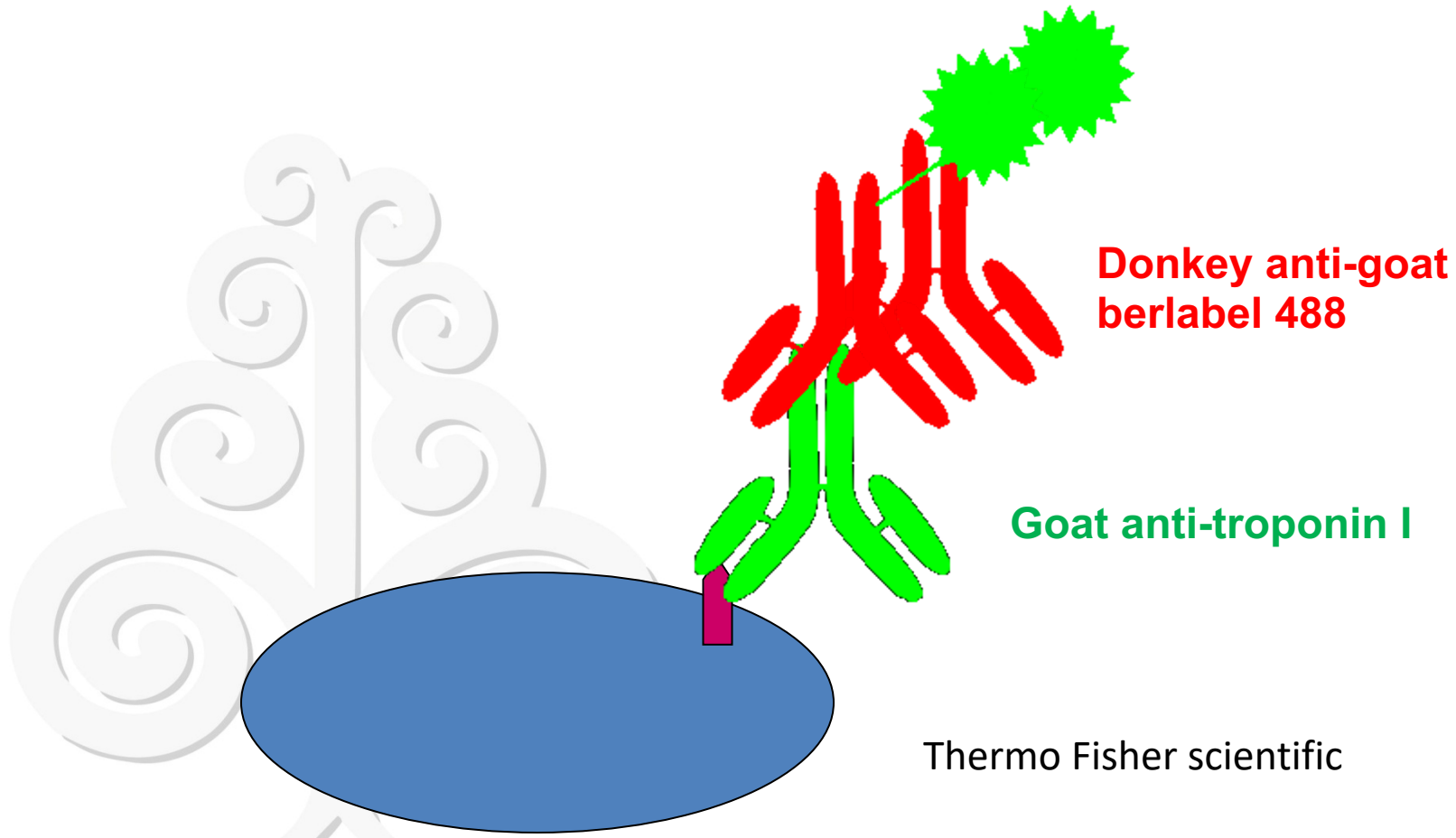
	antigen
	horseradish peroxidase
	streptavidin



# Direct method- hanya antibodi primer



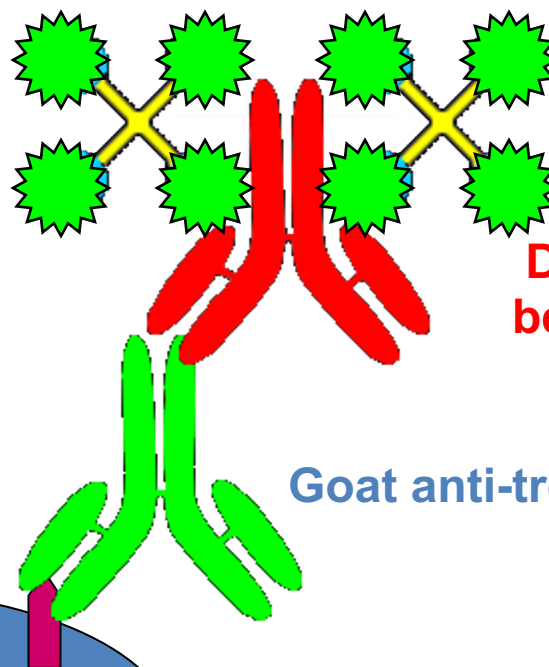
# Indirect method – antibodi primer dan sekunder



Thermo Fisher scientific



# Enzyme linkage Indirect method



Streptavidin  
terkonjugasi dgn  
fluorochrome (488)

Donkey anti-goat  
berbiotin

Goat anti-troponin I



# Protein babi tahan panas

- MAb 8F10 bereaksi dgn 20.5, 22 and 24 kDa ekstrak daging babi mentah
- MAb 8F10 & MAb 5H9 bereaksi dgn 24 kDa daging babi masak (troponin I)
- Negatif terhadap otot jantung, otot polos, darah dan organ tanpa otot

FUR-CHI CHEN, Y-H. PEGGY HSIEH, ROGER C. BRIDGMAN, 2008



# Kepekaan ELISA

- Konsentrasi antigen terendah yang dapat dideteksi diatas kontrol negatif
- 2-3 S.D diatas rata-rata signal background
- Kepekaan bervariasi tergantung pada antibodi-antigen
- Misal 10 pg/mL



# Sandwich ELISA

- Diperlukan 2 antibodi
- Harus mengenal epitope berbeda
- Antibodi pertama menangkap antigen
- Antibodi kedua mengenal antigen pada epitop beda
- Antibodi ketiga berbiotin mengenal antibodi kedua
- Enzyme yg biasa digunakan: HRP (Horse Radish Peroxidase) dan AKP (Alkaline Phosphatase)
- Substrat adalah TMB (Chromogen)



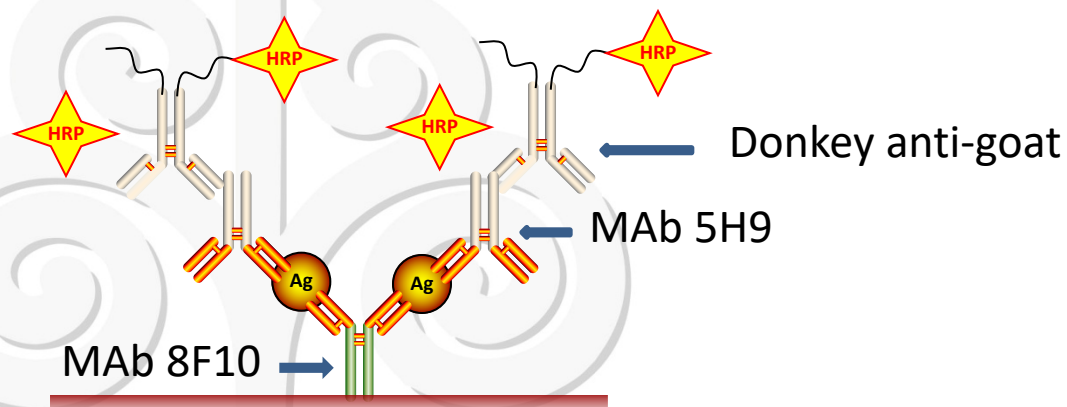
# SANDWICH ELISA

MAb 8F10 mengenal troponin I mamalia

MAb 5H9 spesifik troponin I babi

LoD : 0,5% daging babi dlm daging ayam

0,1% daging babi dlm daging sapi



FUR-CHI CHEN, Y-H. PEGGY HSIEH, ROGER C. BRIDGMAN, 2008





# Identifikasi Daging Babi

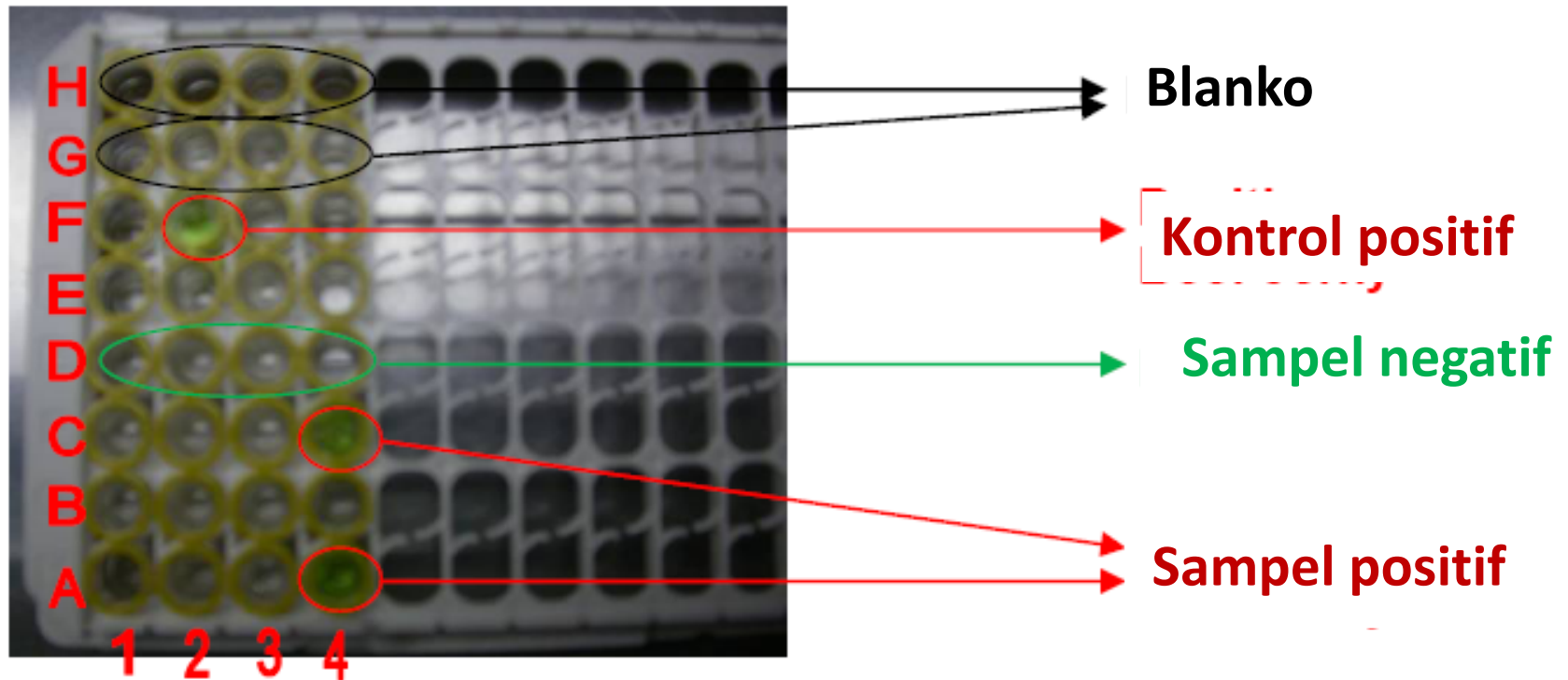
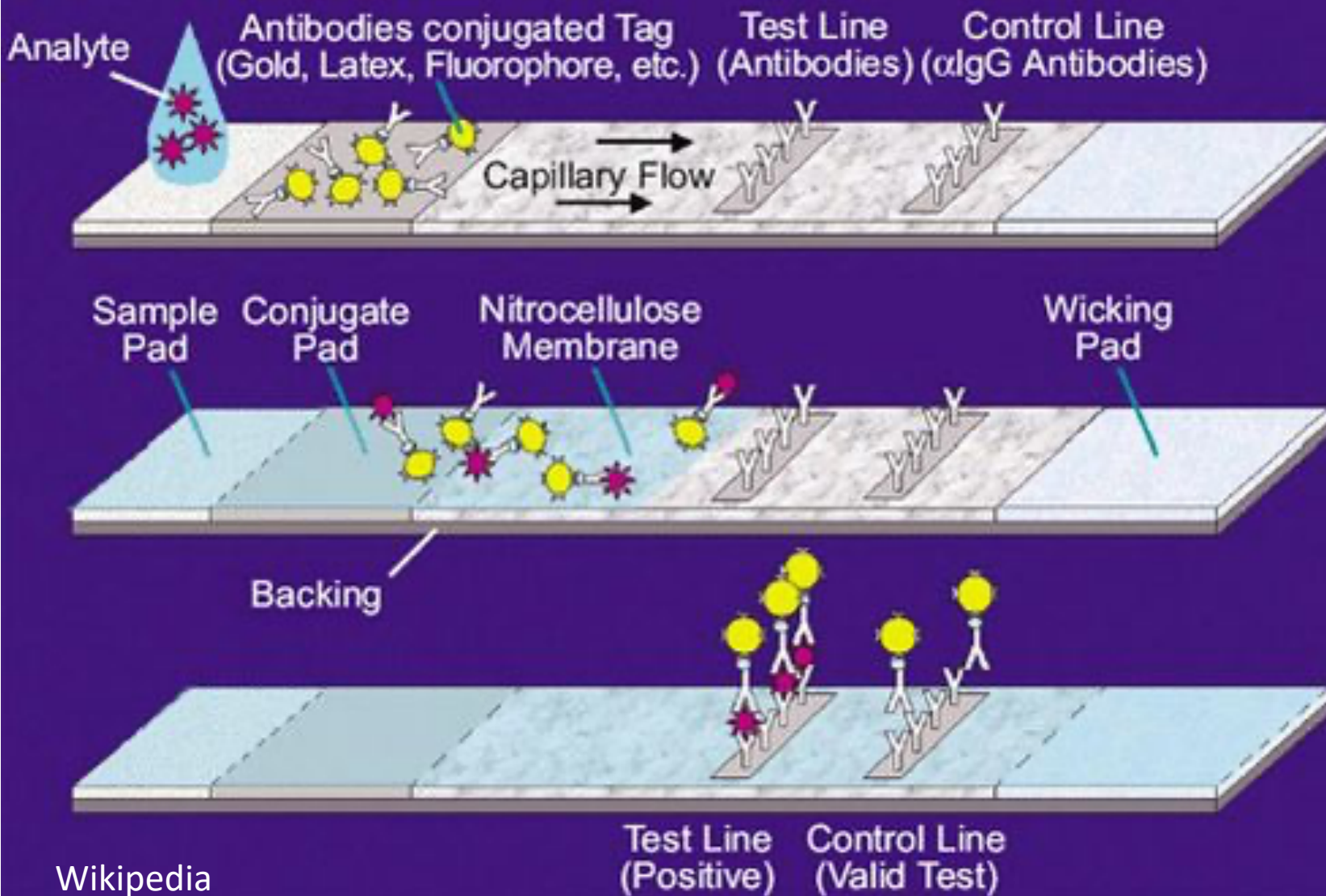


Figure 1. Enzyme immunoassay result

R. Balia dkk, 2009



# Lateral Flow Assay Architecture



Wikipedia

# Usage of the "Easy to use Pork Detection Kit"

①



②



③



④



Neg Pos



Hasil

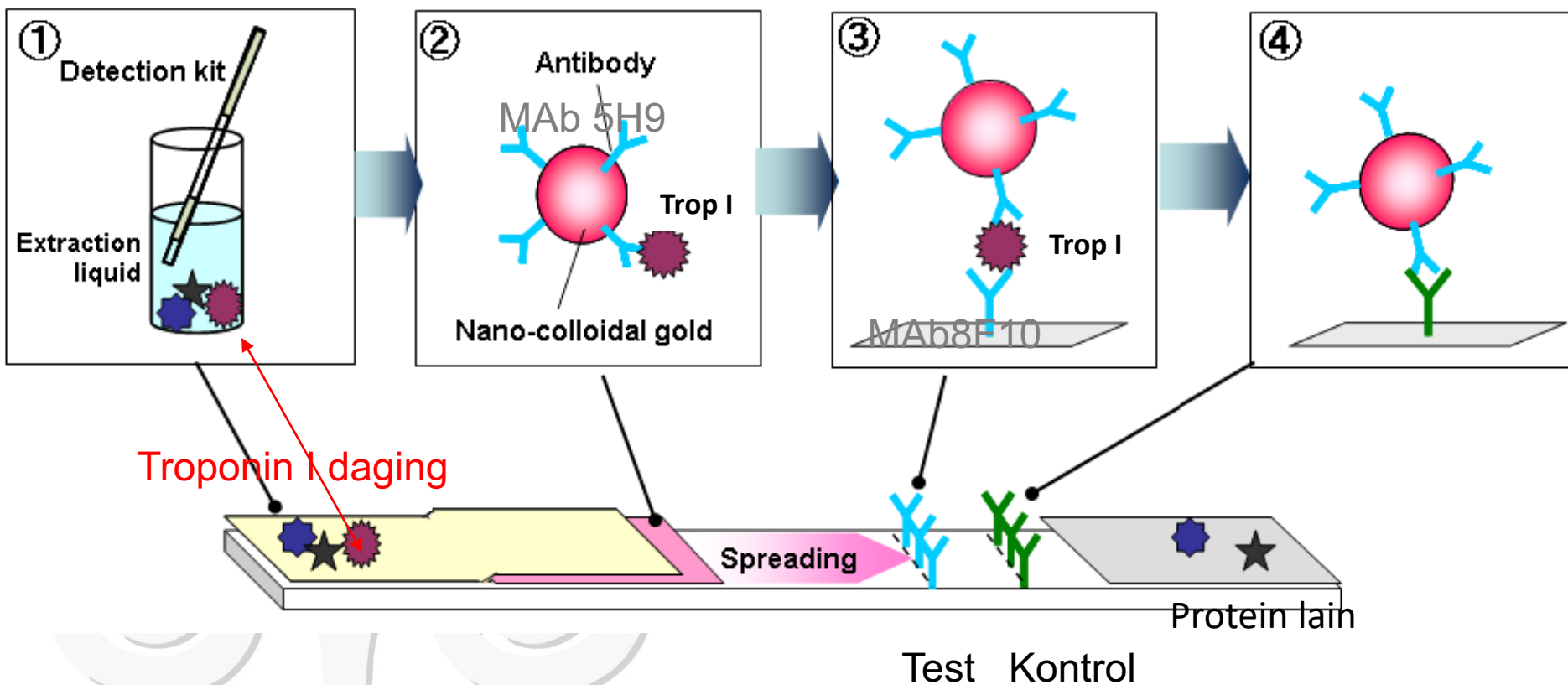


Pre-processing (5 minutes)

10 minutes later



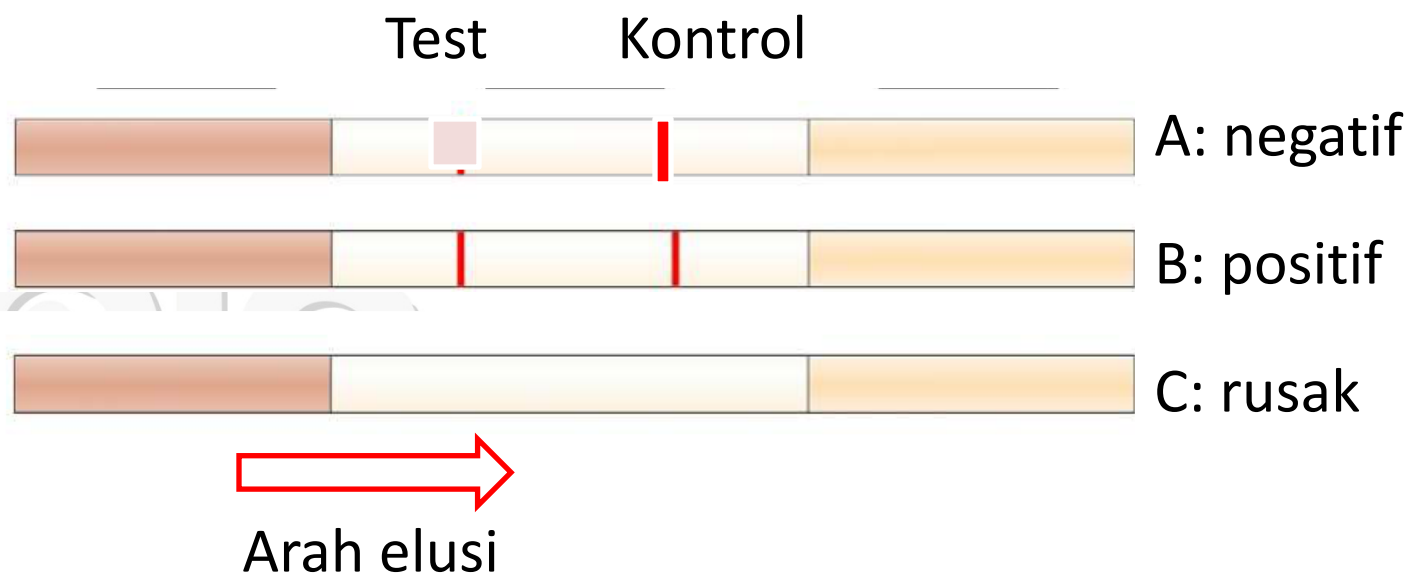
## Principle of Detection Using Immunochromatography



Tanaka Precious Metals, 2010



# Hasil



**Table 1. The cut off level of the IC tests for pork components in adulterated meats**

Sample ratio mix (w/w)	Colour intensity
<b>A. Pork/Chicken</b>	
0/1	-
1/10,000	+/-
1/5,000	+
1/1,000	++
1/100	+++
1/0	+++
<b>B. Pork/Beef</b>	
0/1	-
1/10,000	+/-
1/5,000	+
1/1,000	++
1/100	+++
1/0	+++

Note: - negative results; +/- in doubt result; +: positive results; ++ strong positive results; +++: very strong positive results relative to the control line. (n=6).





- Coba difikirkan untuk mendesain utk deteksi daging babi dan sapi dalam satu kit imunokromatografi



<Comparison with Existing Test Methods>

	Easy to use Pork Detection Kit	Existing Methods	
		PCR	ELISA
<b>Time</b> (From sample extraction to obtaining results)	15 minutes	6 hours	5 hours
<b>Detection Limit</b>	0.1% (processed meat) 0.005% (raw meat)	0.001-0.1% * varies with the types of the materials and sample processing conditions recommended by each manufacturer	1%

Spesifik jaringan ttt  
Rutin

Jaringan tdk berpengaruh  
LoD kecil

Tanaka Precious Metals, 2010





*If you believe in yourself,  
if you really stick to things,  
and if you always pray,  
there is very little that is really impossible*



Thank you for your attention

