## IMMUNOCHROMATOGRAPHIC METHOD:

# LATERAL FLOW STRIP TEST

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# When do you need to develop a new rapid test?

3 options required for analysis of:-

1. Market feasibility
2. Financial feasibility
3. Regulatory feasibility

## Design and Development Business Plan

- To generate idea for potential medical devices should be.

### 1 Market Research

- To provide summarised overviews of existing technologies, competitor devices, and economic considerations.

**Target:**

- **Identify your problem and set a goal/outcome for the potential intervention.**
- **Suggest solution(s) to reach the suggested goal/ outcome.**

**Methodologies:**

- Questionnaires
- Observations
- Face-to-face interviews
- Overview of data collection tools
- Cost-benefit tool
- SWOT-analysis tool
- Strategy tool PEST (Politic, Economic, Socio-Cultural and Technological-factors)
- Porter's Five Forces - Competitor's analysis
- Market Mix (4Ps)

### 2 Project Timeline and Costs

- To define timeframes, budget and solution for each stage

### 3 Prototyping

- Prototype will be the basis for feasibility studies to determine whether you should proceed with your device development project.

### 4 Regulatory Planning

- Determine device classification, which in turn can determine the level of documentation required in each market.
- Classification is based on the risk profile of the device




ชุดทดสอบสเตียรอยด์ (หลักการ TLC)


- https://website.bdn.go.th/th/service/detail/nGI4ZDWewEb3QWewEb3Q/nGy4ANWewEb3QWewEb3Q

# How to Drive Research product to the Market

**6 Steps to Drive**

Research and Development → Process verification → Prototype → Method validation → Registration → Product Launch

Design and dev. of medical Device Product

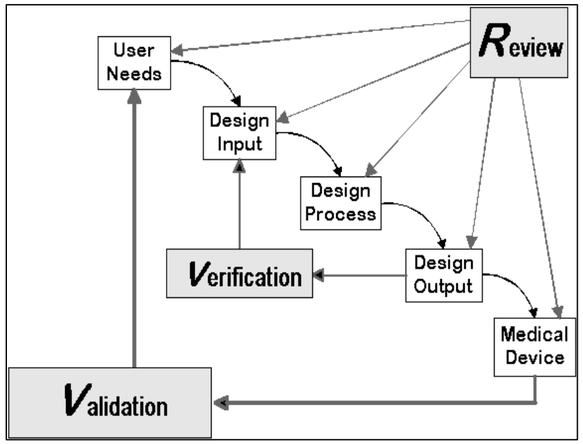
Pre-clinical data

Clinical data “Final Product”

Medical device registration

IVD = Clinical performance
Non-IVD = Clinical trial/animal trial

Prepare CSDT/
Technical document preparation




Defined “Medical device”
And
Classified “Risk group”

- Production flow process
- Risk management

Design process

Design output

Design transfer

Production of Commercialized product

Product launch

Manufacturing process

Design input
(User need and standard)

- Clinical and laboratory standard
- ISO or IEC

Following ISO 13485:2016

**Regulation vs. Research Level**

Regulatory approval
“Manufacturer” and “Scope of medical device”

Manufacturer Standard

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ISO13485 (QMS)

Regulatory approval

Post-market Surveillance

Product registration process

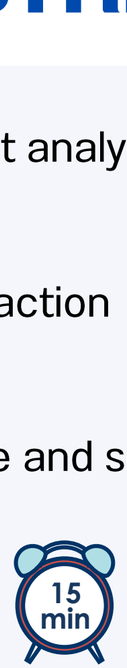
TRL level

1 2 3 4 5 6 7 8 9

# IMMUNOCHROMATOGRAPHIC METHOD:

# LATERAL FLOW STRIP TEST

- A paper-based platform to detect analyte in sample
- Based on antigen-antibody interaction
- Capillary force moving
- Applied to detect both qualitative and semi-quantitative
- Getting results in 10-20 mins




- Can be applied for:-
  - : Home testing
  - : Point of care /or
  - : Filed testing

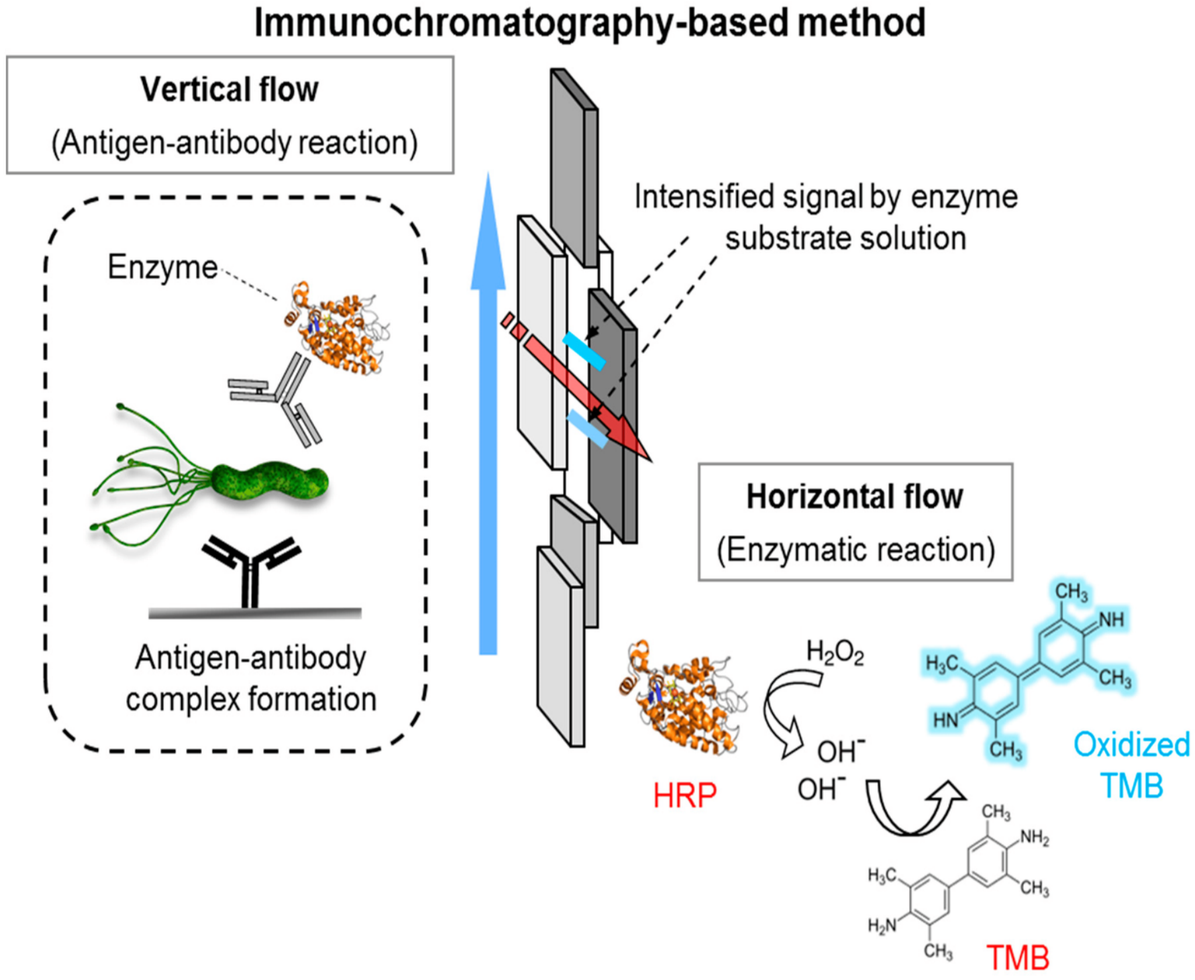



Figure 1 Chromatographic principle: schematic illustration of colorimetric analytical method associated with enzyme-based immunochromatography (e.g., horseradish peroxidase) for onsite testing (Cho and Ku, 2017).

# Advantages of the Lateral flow immunoassay

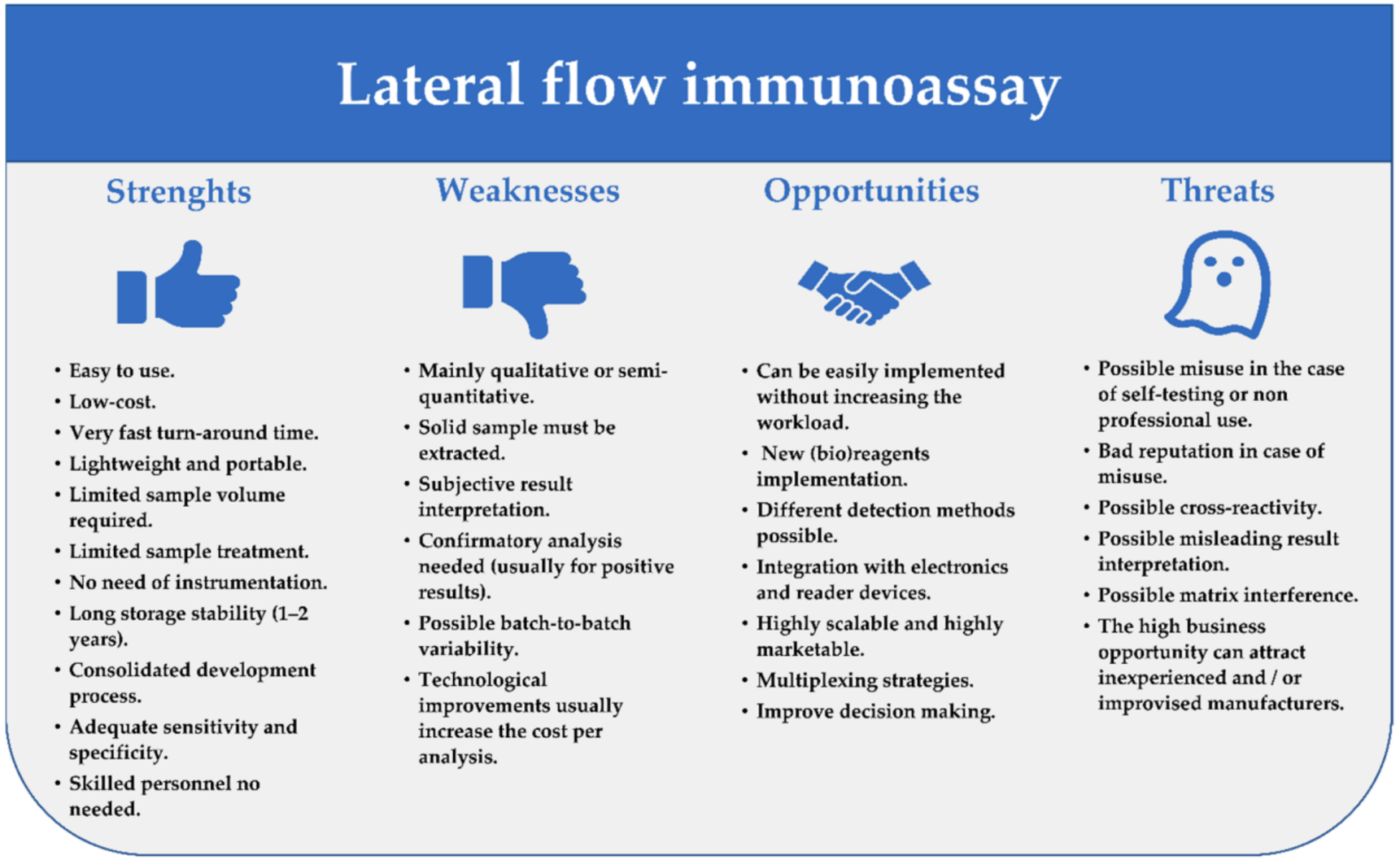



**Figure 1.** SWOT analysis of the LFIA technique considering its inherent features.

Ref. Fabio Di Nardo et al., 2021

## IMMUNOCHROMATOGRAPHIC METHOD:

# LATERAL FLOW IMMUNOASSAY

The sample flows through the device and comes in contact with immobilized reagents. The antibody and analyte migrate to a capture zone of membrane-immobilized antibody. Any unreacted tagged antibody flows past the capture zone to absorbent.

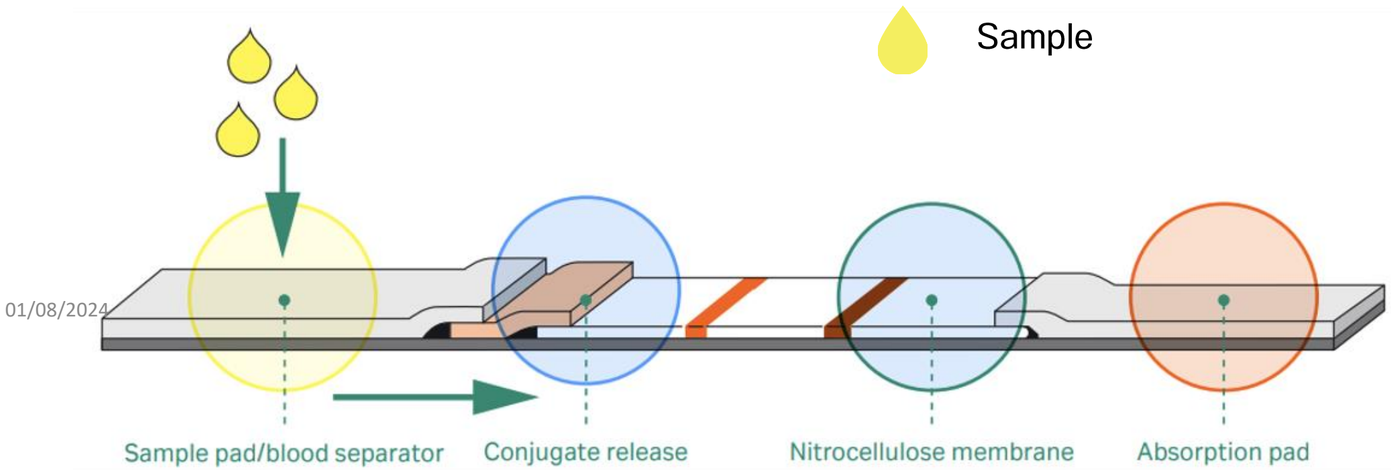



**Fig. 2** Drawing of a strip test immunoassay

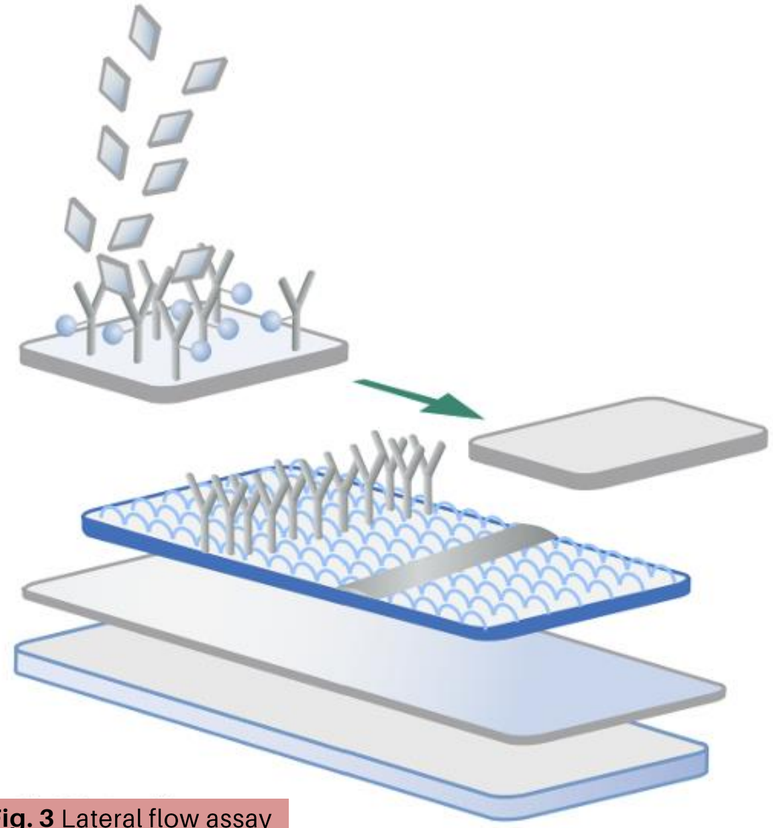
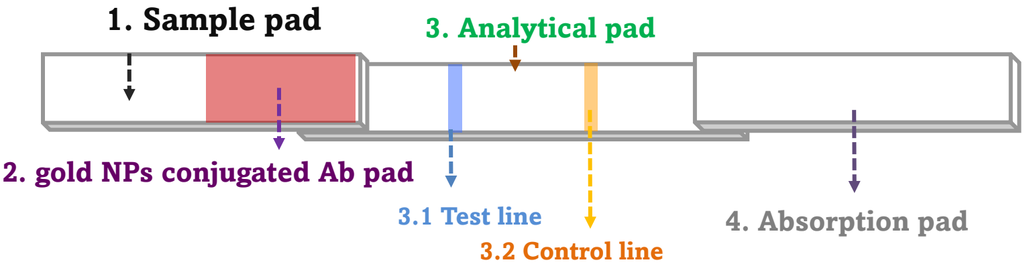


**Fig. 3** Lateral flow assay

Ref: https://cdn.cytivalifesciences.com/api/public/content/digi-16472-pdf?_gl=1*bqldzf*_gcl_au*Mjg3NDc5MjQ3LjE3MDE0OTgzNTQ.

# IMMUNOCHROMATOGRAPHIC METHOD: LATERAL FLOW STRIP TEST

## Test strip components




**Function of each components**

- 1 = sample application pad

**To drop sample and reduce non-specific binding of sample**

- 2 = gold nanoparticles conjugated pad

**To hold the detector particles**

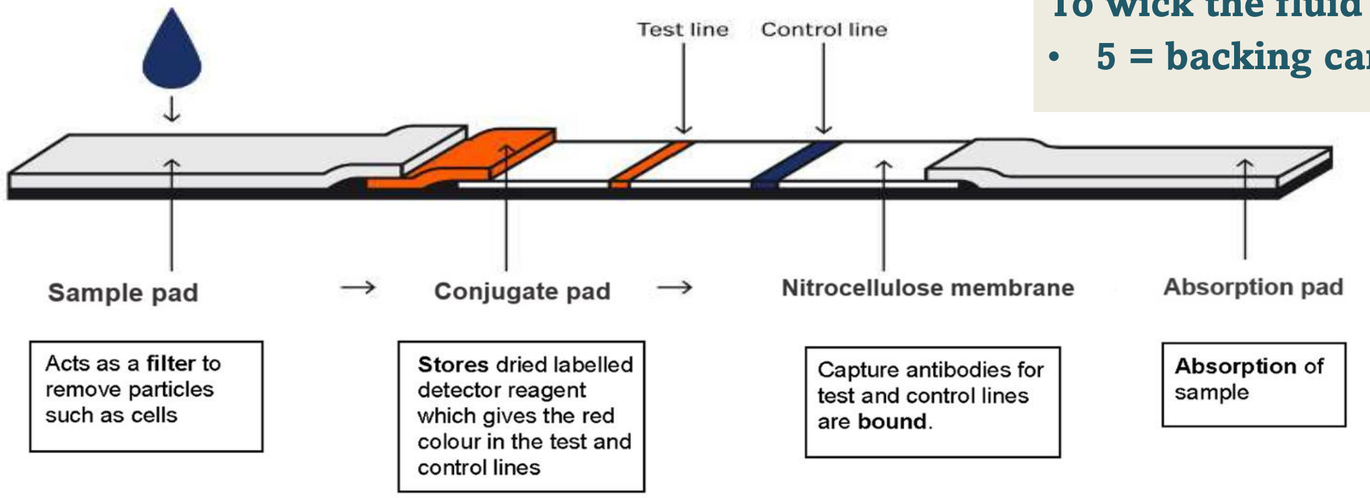
- 3 = Analytical pad as detection zone

**To immobilize targeted antigen or antibody for analyzing**

- 4 = absorption pad

**To wick the fluid through the membrane and collect excess liquid**

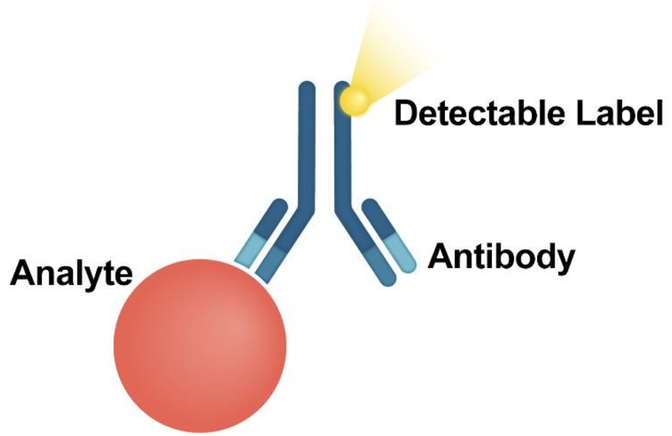
- 5 = backing card for supporting strip components




Picture from: https://www.cytivalifesciences.com/en/us/news-center/lateral-flow-assay-format-sandwich-or-competitive-10001

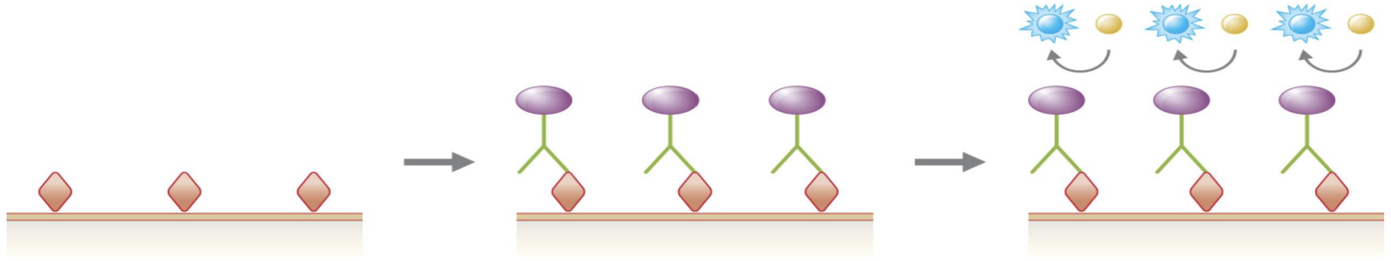
# Principle of immunoassay

- Immunoassay is a method for determining the content of a substance to be tested from a sample using the principle of immunology
- This assay is a procedure for detecting or measuring specific protein or other substances through their properties as antigen and antibody.
- Several methods are divided according to different labeling techniques: fluorescent immunoassay, radioimmunoassay, enzyme immunoassay, colloidal gold immunoassay, and chemiluminescent immunoassay

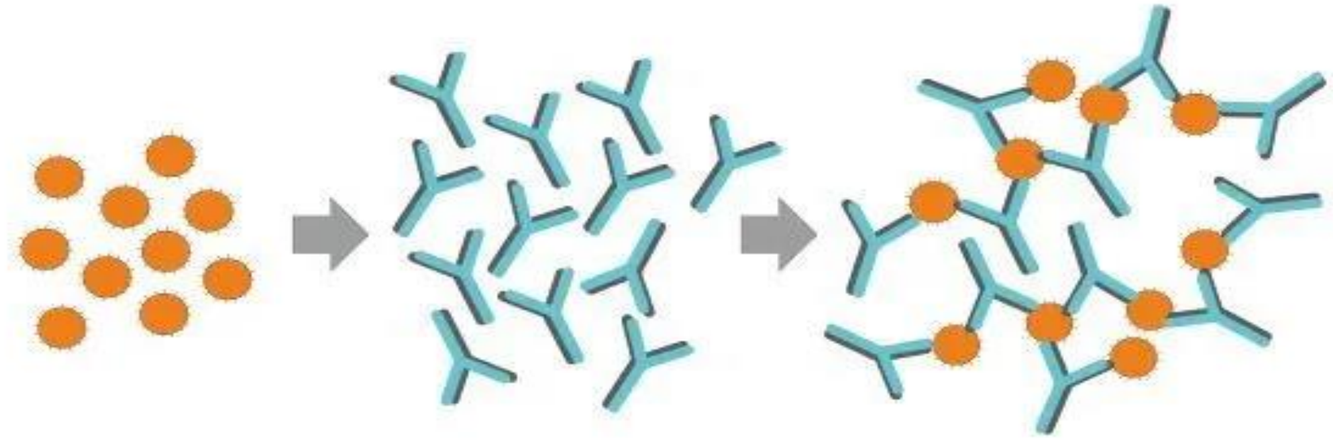



- Based on specific immuno-reaction between antibody (Ab) and antigen (Ag)

- The most widely used are enzyme-linked immunoassay (ELISA) and colloidal gold immunochromatographic strip test



# Antigen vs. Antibody



Antigen | Antibody | Antigen-antibody complex

**An antigen** is any substance that stimulates an immune response.

- Foreign antigens come from outside the body and may be pathogens (like disease-causing viruses or bacteria) allergens (like pollen), or toxic substances (like venom or chemicals).
- Autoantigens originate in the body and do not usually provoke an immune response, except in individuals with autoimmune disorders.

**An antibody** is a Y-shaped protein that is produced by white blood cells, and that tags antigens for destruction by immune cells.

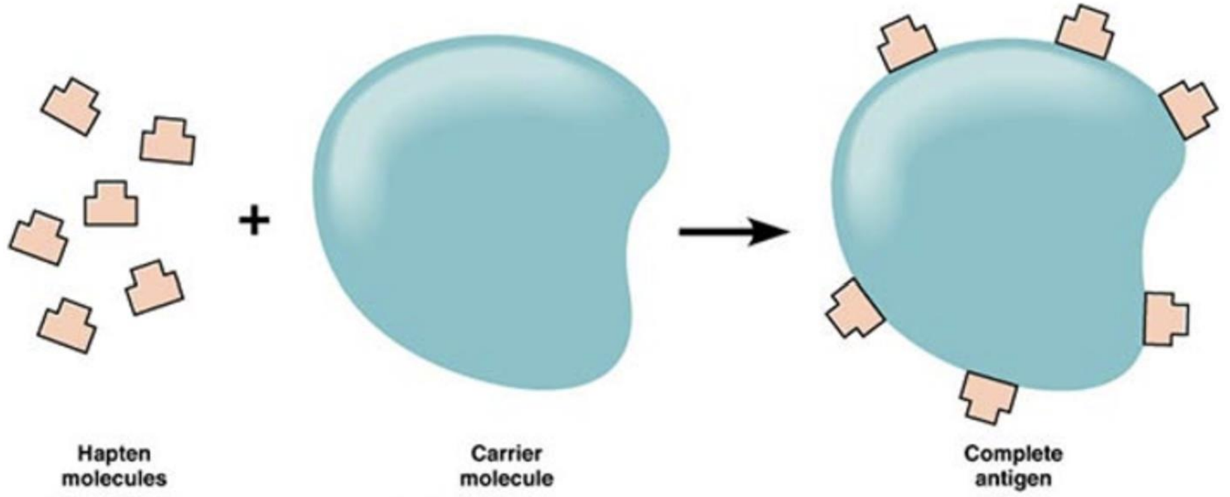
- When an antibody binds to an antigen, an antigen-antibody complex is formed.
- The formation of the antigen-antibody complex stimulates an immune response against the foreign substance.

| Characteristics | Antigen | Antibody |
|---|---|---|
| Molecule Type | Usually, proteins may also be polysaccharides, lipids or nucleic acids. | Proteins |
| Definition | These are substances that provoke an immune response. | These are Glycoproteins that are secreted by immune cells (plasma cells) in response to a foreign substance (antigen). |
| Effect | Cause disease or allergic reactions. | Protect the system by lysis of antigenic material. |
| Origin | Within the body or externally. | Within the body. |
| Parts | Highly variable with different structural conformations and is usually composed of different epitopes. | Composed of three main parts:<br>-Two light chains<br>-Two heavy chains<br>-Four polypeptides |
| Prevalence | Exists in all types of cells; mostly found in viruses, bacteria, and fungi. | Only present in some types of cells. |
| Synonyms | Immunogens | Immunoglobulins |
| Specific binding site | Epitope | Paratope |
| Complexity | Medium; exists due to random mutations in the cell's gene. | Very High; Complex chemical that bonds to a very specific Antigen. |
| Source | Usually from a foreign substance (viruses, and bacterial and fungal toxins). | Naturally produced by the body (B lymphocytes or B cells). |
| Kinds | There are three basic kinds of antigens (Exogenous, Endogenous, and Autoantigens) | There are five basic kinds of antibodies (IgG, IgM, IgA, IgE, and IgD). |
| Examples | **Exogenous antigens**: bacteria, viruses, fungi, etc.<br>**Endogenous antigens:** Blood group antigens, HLA (Histocompatibility Leukocyte antigens), etc.<br>**Autoantigens:** Nucleoproteins, Nucleic acids, etc. | Breast milk, tears, saliva, sweat, and mucus. |

# Hapten

Hapten is a molecule that reacts with specific antibody but is not immunogenic by itself, it can be made immunogenic by conjugation to a suitable carrier.

Many drugs like penicillins are haptens. A hapten is essentially an incomplete antigen. These small molecules can elicit an immune response only when attached to a large carrier such as a protein; the carrier typically does not elicit an immune response by itself.



# Monoclonal antibody VS Polyclonal antibody

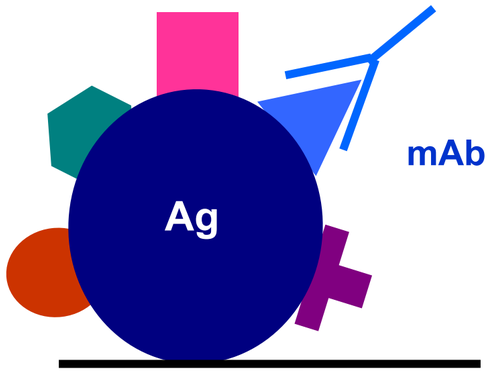
## Monoclonal antibodies (mAb)

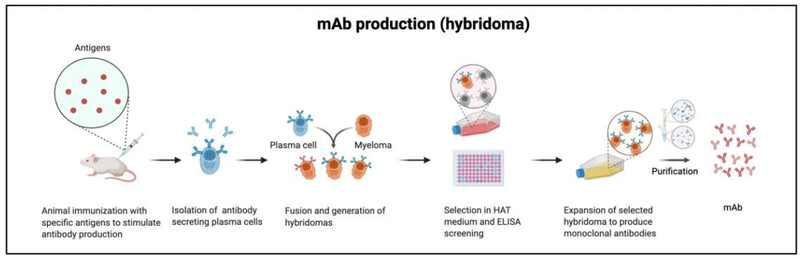
- A homogenous antibody population.
- Single antigenic determinant
- Interact with a particular epitope on the antigen (mAb is antibody against only one epitope carried on antigen/immunogen)
- Refer to a homogenous population of antibodies that are produced by a single clone of plasma B cells.
- Produced by the same clone of plasma B cells.

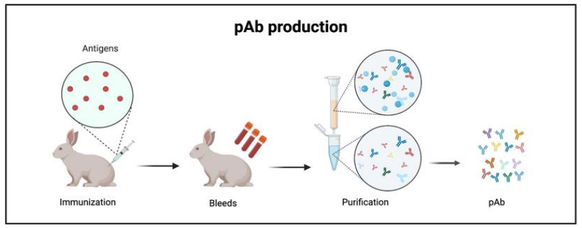
## Polyclonal antibodies (pAb)

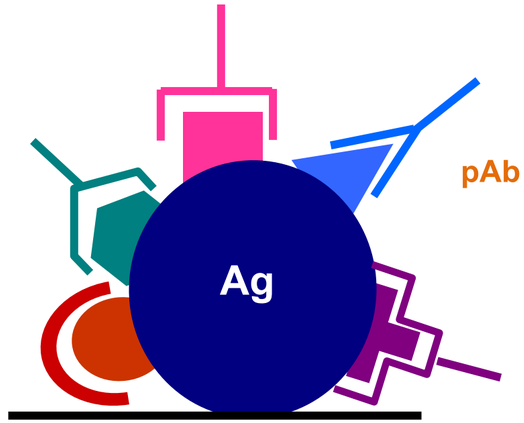
- A heterogeneous antibody population.
- Interact with different epitopes on the same antigen.
- Refer to a mixture of immunoglobulin molecules that are secreted against a particular antigen.
- Produced by different clones of plasma B cells.

****Epitope is the site of antigen which antibody come to attach.**



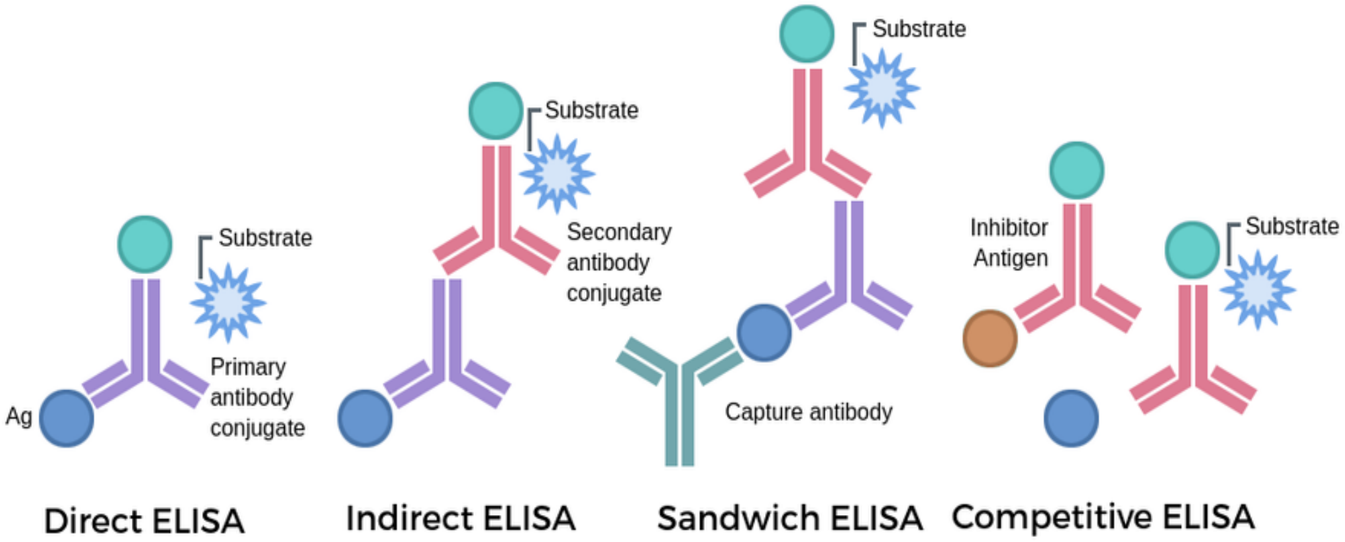

https://www.szabo-scandic.com/en/information/news/polyclonal-monoclonal-recombinant-antibodies/

# Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA (which stands for enzyme-linked immunosorbent assay) is a technique to detect the presence of antigens in biological samples. An ELISA, like other types of immunoassays, relies on antibodies to detect a target antigen using highly specific antibody-antigen interactions.

**There are four major types of ELISA:**

1. Direct ELISA (screening antigen)
2. Indirect ELISA (antigen-coated plate; screening antigen/antibody)
3. Sandwich ELISA (antibody-coated plate; screening antigen)
4. Competitive ELISA (screening antibody)




https://theory.labster.com/types-elisa/

## Diagnostic Tests

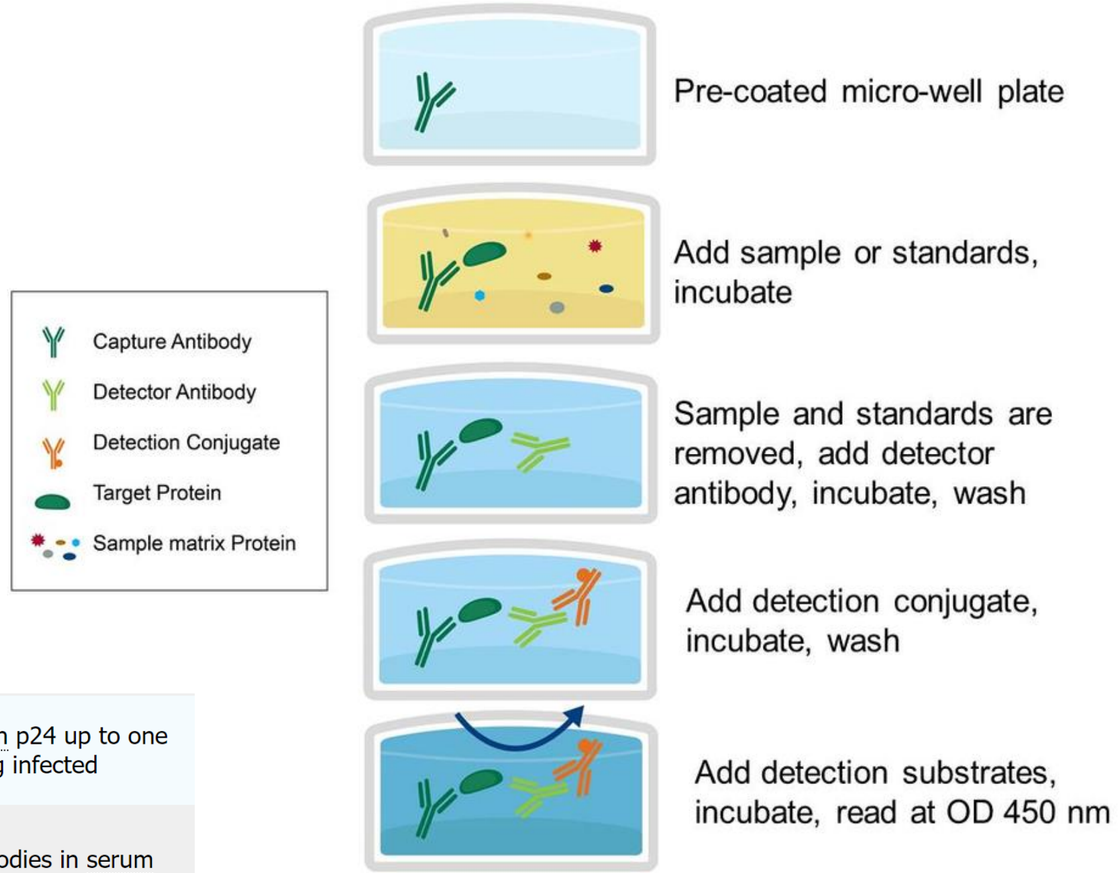
| | |
|---|---|
| **Detect and Measure the Presence of Antibodies in the Blood** | • Autoantibodies (anti-dsDNA, anti-dsg1, ANA, etc.)<br>• Antibodies against infectious disease (antibacterial, antiviral, antifungal)<br>• Hepatitis A, B, C, HIV, etc. |
| **Detect and Estimate the Levels of Tumor Markers** | • Prostate-specific antigen (PSA)<br>• Carcinoembryonic Antigen (CEA) |
| **Detect and Estimate Hormone Levels** | • Luteinizing hormone<br>• Follicular stimulating hormone<br>• Prolactin<br>• Testosterone<br>• Human chorionic gonadotropin (hCG) |
| **Tracking Disease Outbreaks** | • Cholera<br>• HIV<br>• Influenza<br>• Covid-19 |
| **Detecting Past Exposures** | • HIV<br>• Lyme disease<br>• Hepatitis |
| **Screening Donated Blood for Possible Viral Contaminants** | • Anti-HIV-1/2<br>• Anti-HCV<br>• HBsAg |
| **Detecting Drug Abuse** | • Amphetamine<br>• Methamphetamine<br>• 3,4-methylenedioxymethamphetamine<br>• Cocaine<br>• Benzoylecgonine |

https://www.ncbi.nlm.nih.gov/books/NBK555922/

# Enzyme-Linked Immunosorbent Assay (ELISA)

There are four main general steps to completing an ELISA immunoassay. These steps are:

1. Coating (with either antigen or antibody)
2. Blocking (typically with the addition of bovine serum albumin [BSA])
3. Detection
4. Final read



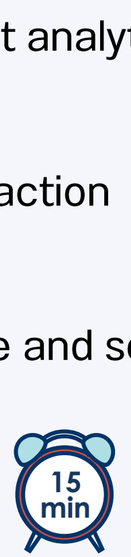

| | | |
|---|---|---|
| Uses enzyme-antibody conjugates to quantify target molecules | Direct ELISA: Uses a single antibody to detect the presence of an antigen | Detection of HIV antigen p24 up to one month after being infected |
| | Indirect ELISA: Measures the amount of antibody produced against an antigen | Detection of HIV antibodies in serum |

https://www.avivasysbio.com/technical-resources/protocols-procedures/elisa

# IMMUNOCHROMATOGRAPHIC METHOD:

# LATERAL FLOW STRIP TEST

- A paper-based platform to detect analyte in sample
- Based on antigen-antibody interaction
- Capillary force moving
- Applied to detect both qualitative and semi-quantitative
- Getting results in 10-30 mins




- Can be applied for:-
  - : Home testing
  - : Point of care /or
  - : Filed testing

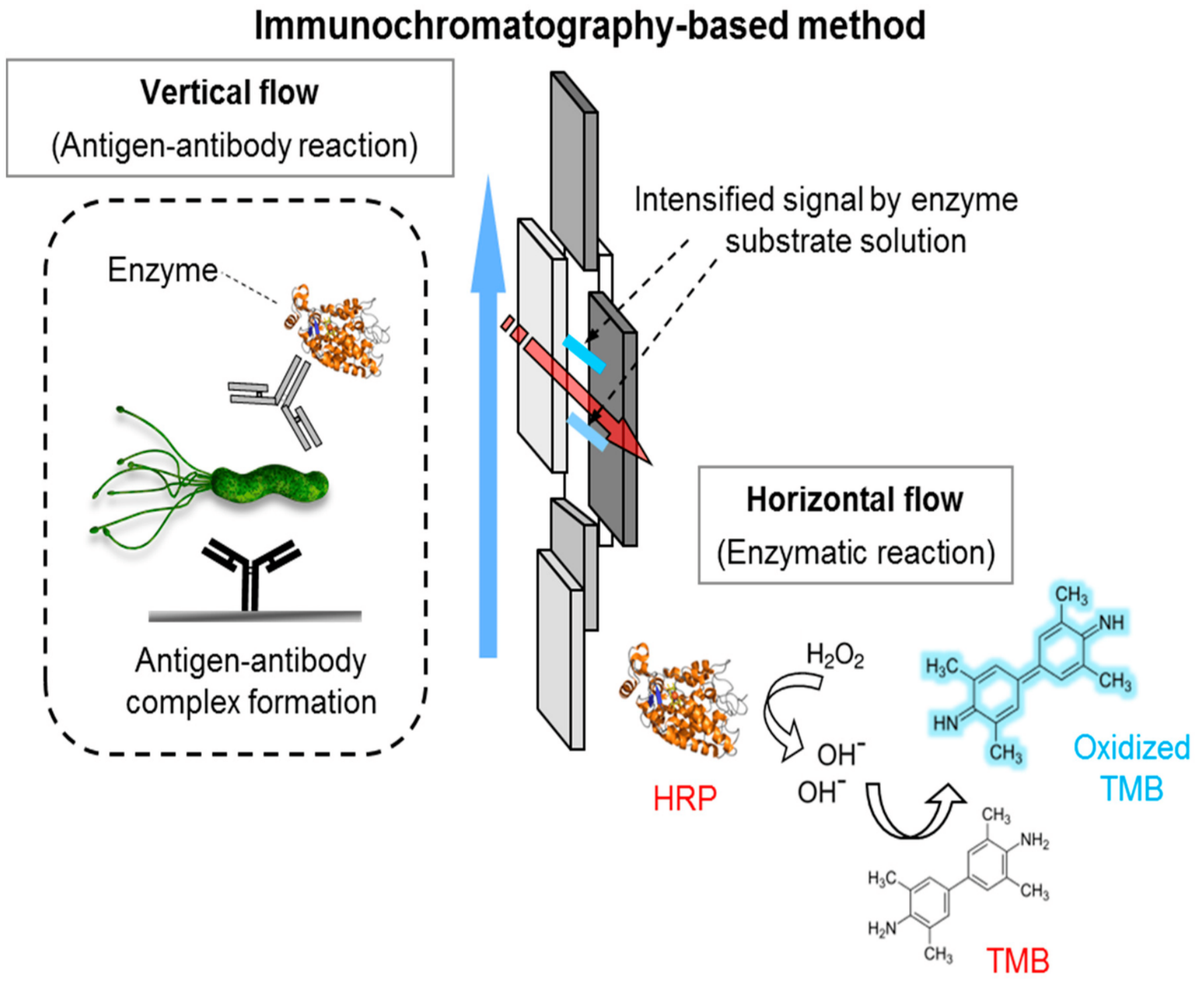



Figure 1 Chromatographic principle: schematic illustration of colorimetric analytical method associated with enzyme-based immunochromatography (e.g., horseradish peroxidase) for onsite testing (Cho and Ku, 2017).

## IMMUNOCHROMATOGRAPHIC METHOD:

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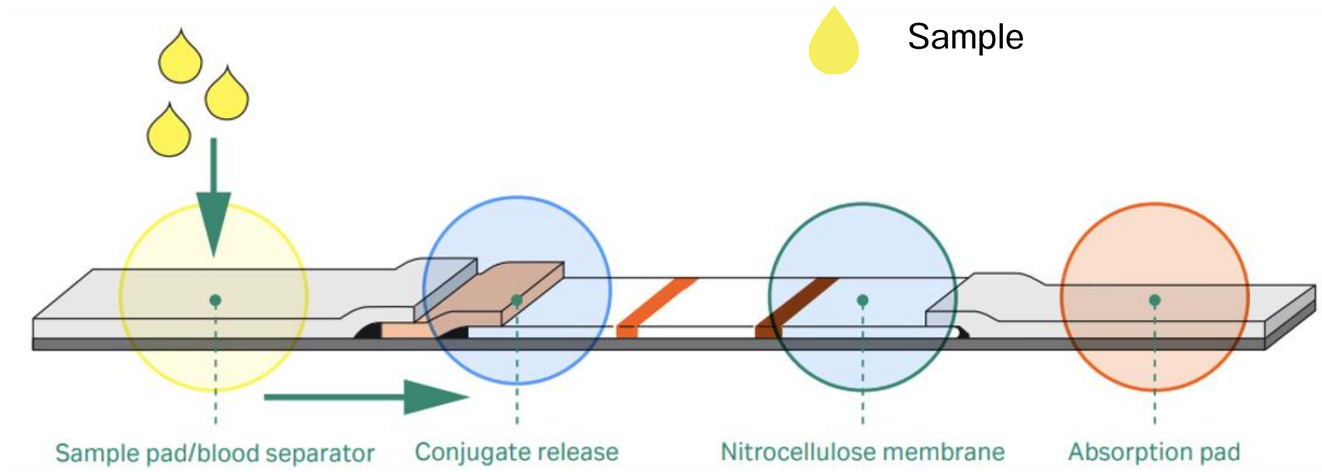



**Fig. 2** Drawing of a strip test immunoassay

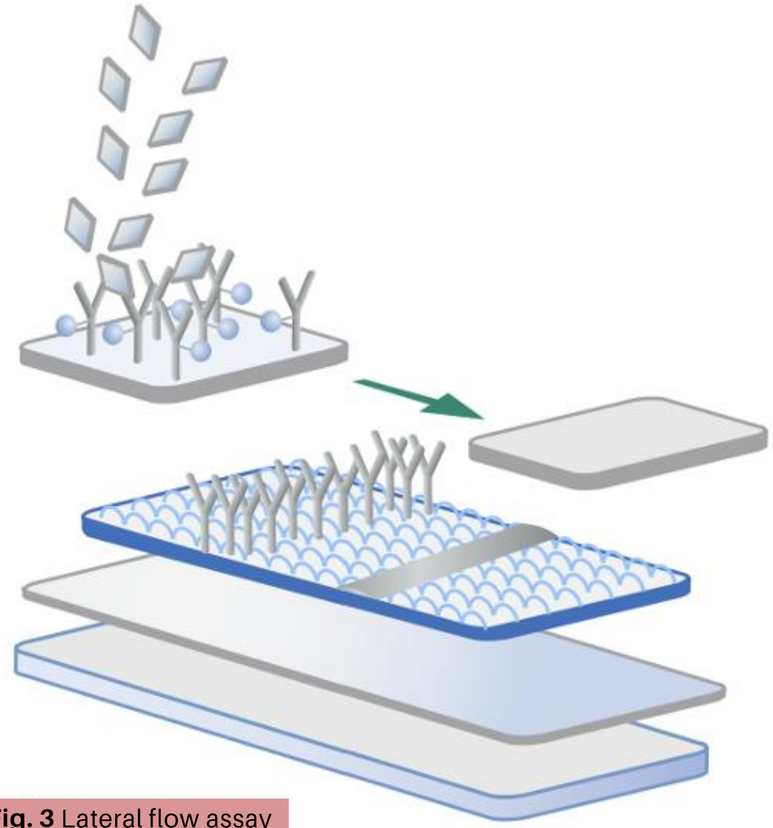


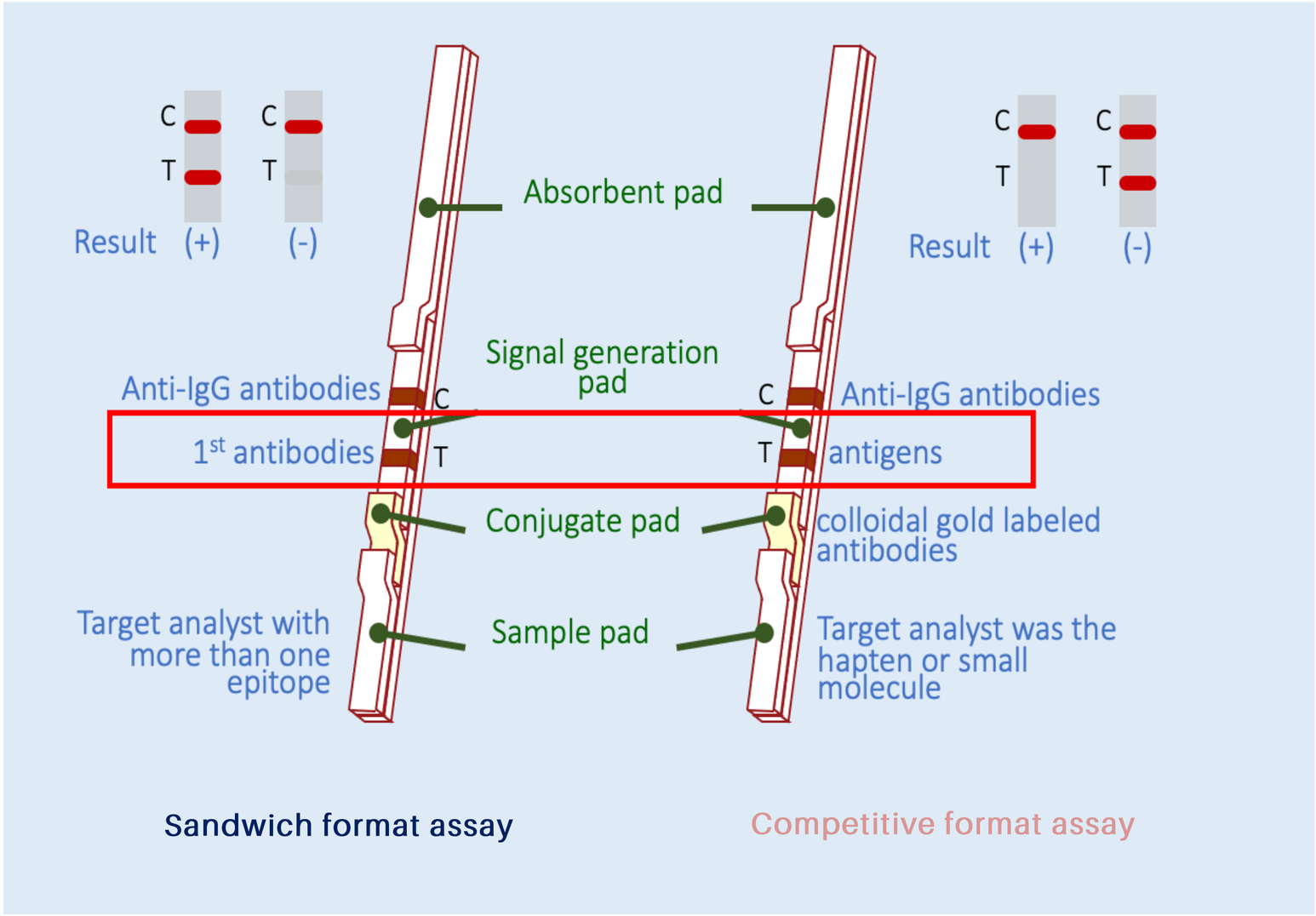
**Fig. 3** Lateral flow assay

Ref: https://cdn.cytivalifesciences.com/api/public/content/digi-16472-pdf?_gl=1*bqldzf*_gcl_au*Mjg3NDc5MjQ3LjE3MDE0OTgzNTQ.

# TYPES OF IMMUNOCHROMATOGRAPHIC METHOD

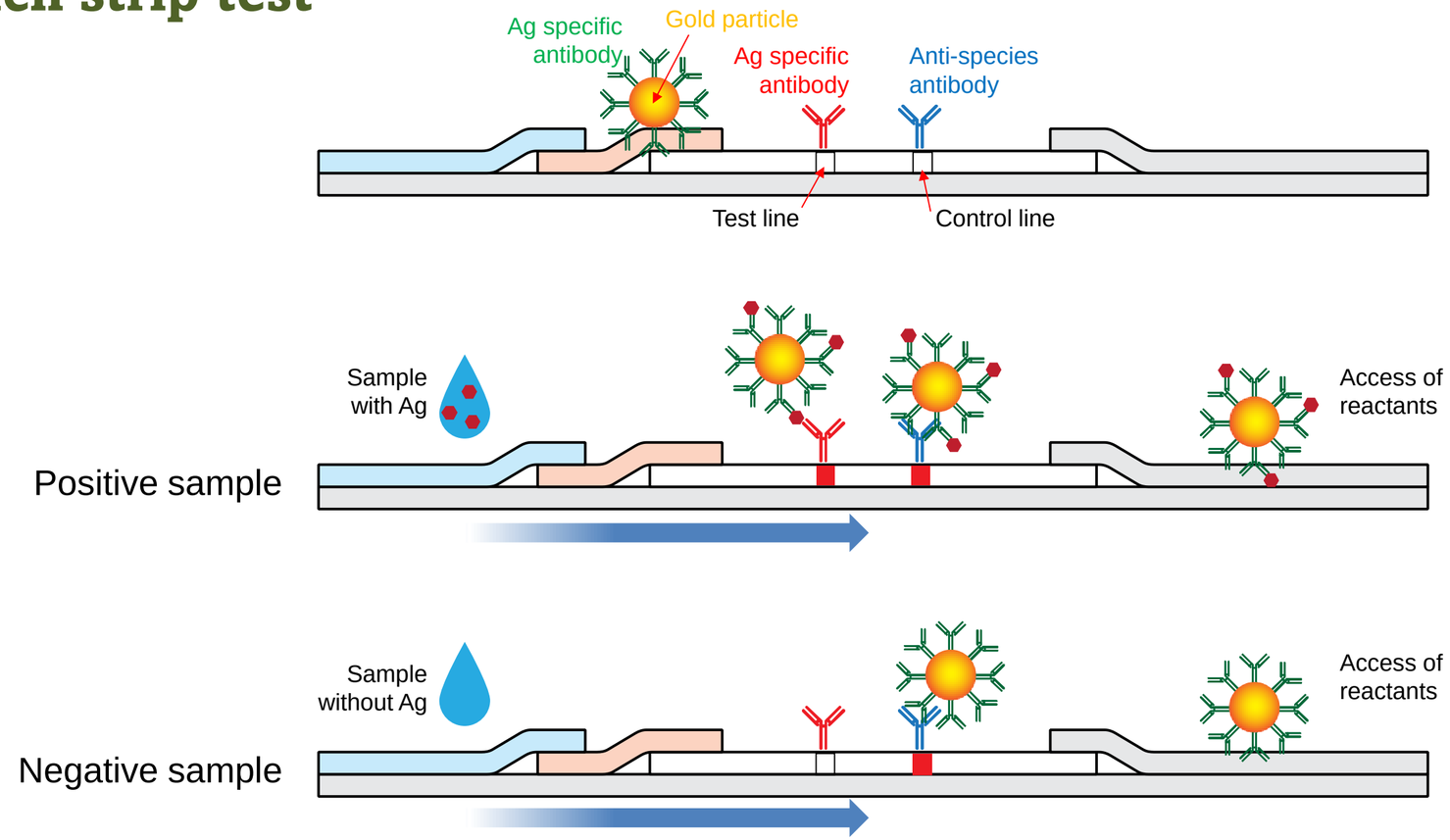
1. Direct format (Non-competitive/Sandwich assay)
   - Large analyze with several antigenic sites

2. Competitive (Competitive inhibition)
   - Small molecule with a single antigen



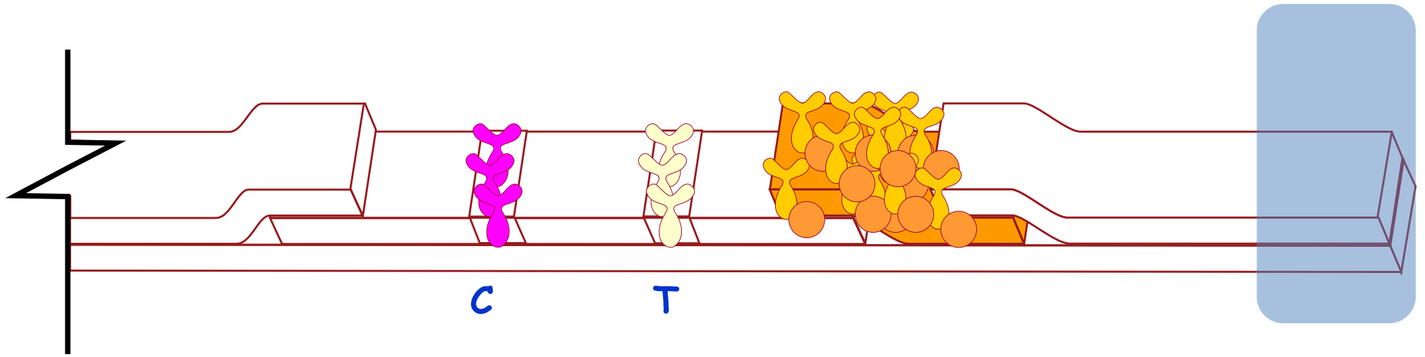
# 1. Direct format (Non-competitive/Sandwich assay)

## Sandwich strip test



# Test strip: Sandwich format assay

- **Sample without antigen**



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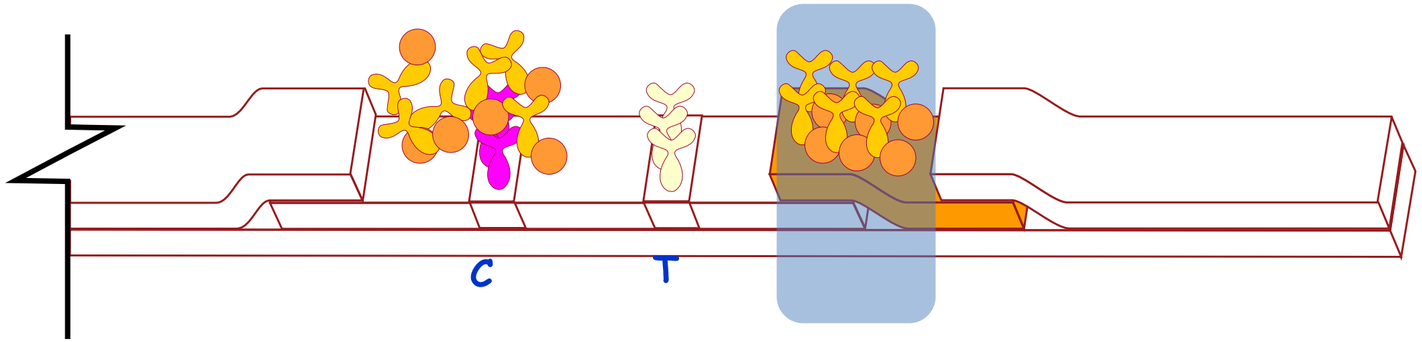
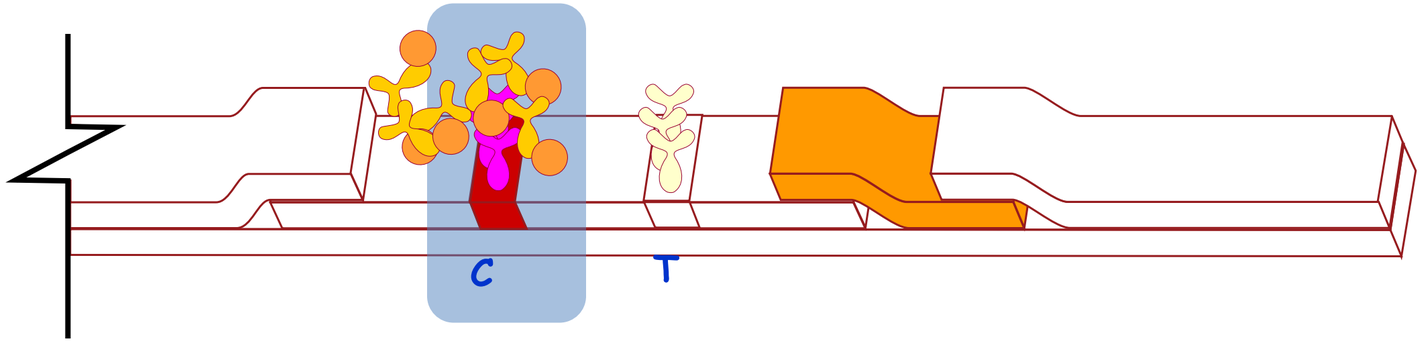


# 1. Direct format (Non-competitive/Sandwich assay)

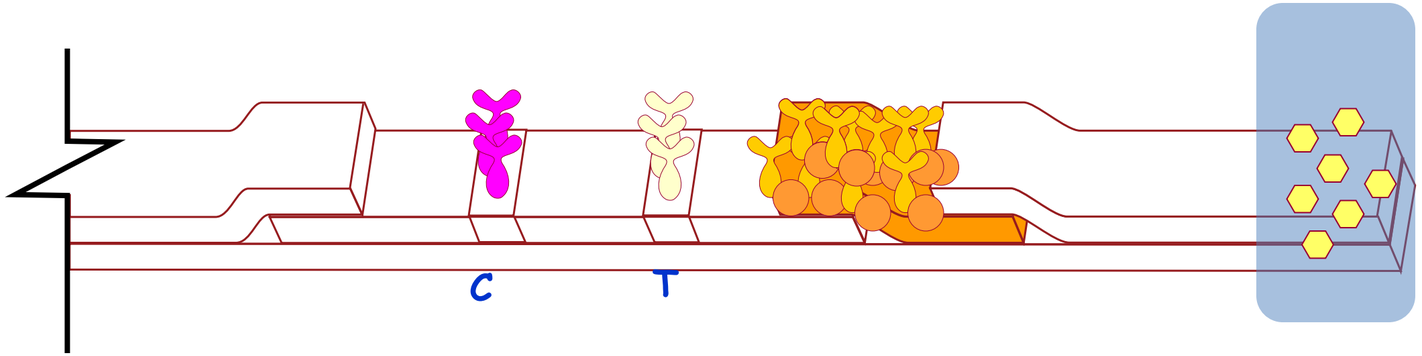
- **Sample without antigen**




Negative result

# 1. Direct format (Non-competitive/Sandwich assay)

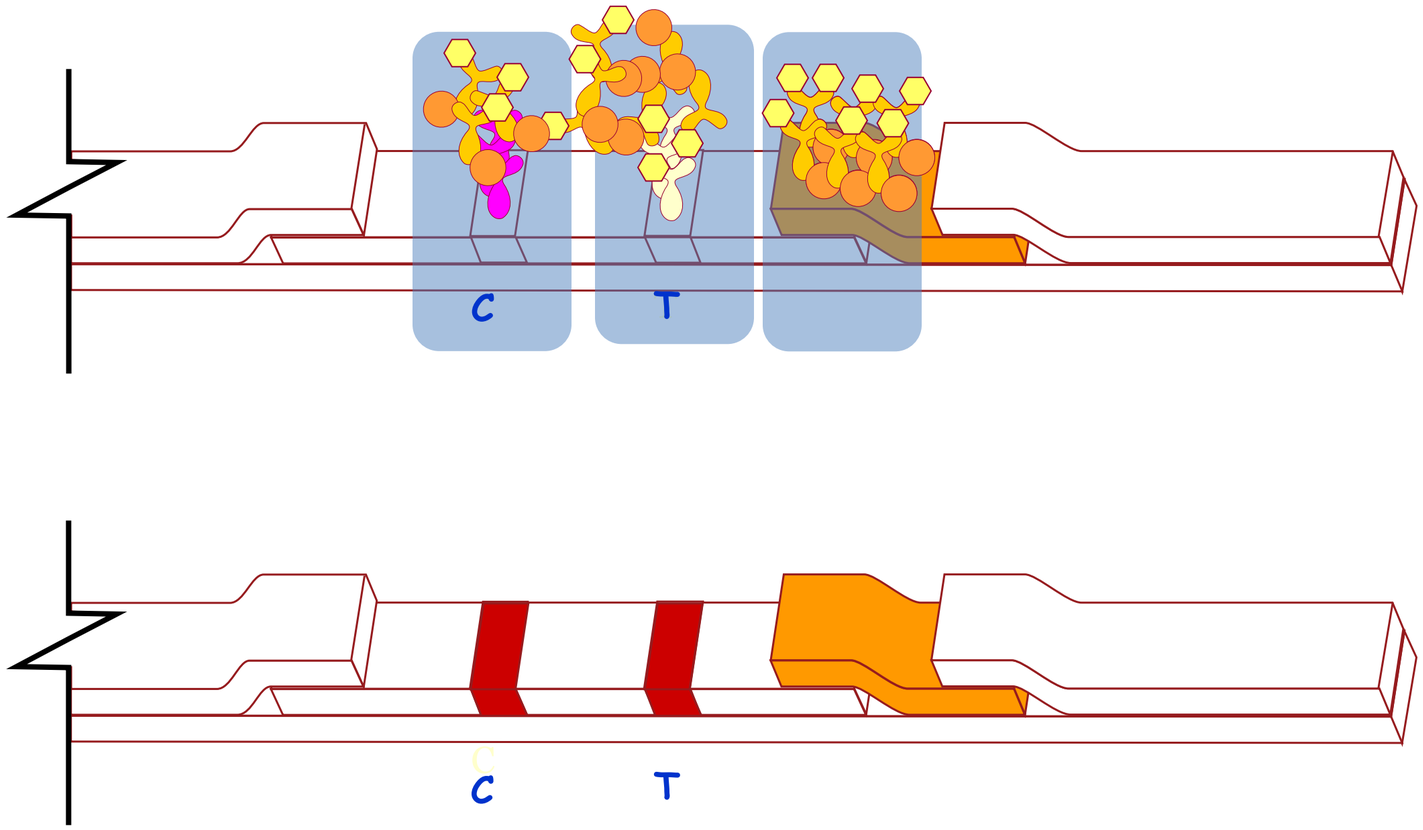
- **Sample with antigen**



# 1. Direct format (Non-competitive/Sandwich assay)

**Test strip: Sandwich format assay**

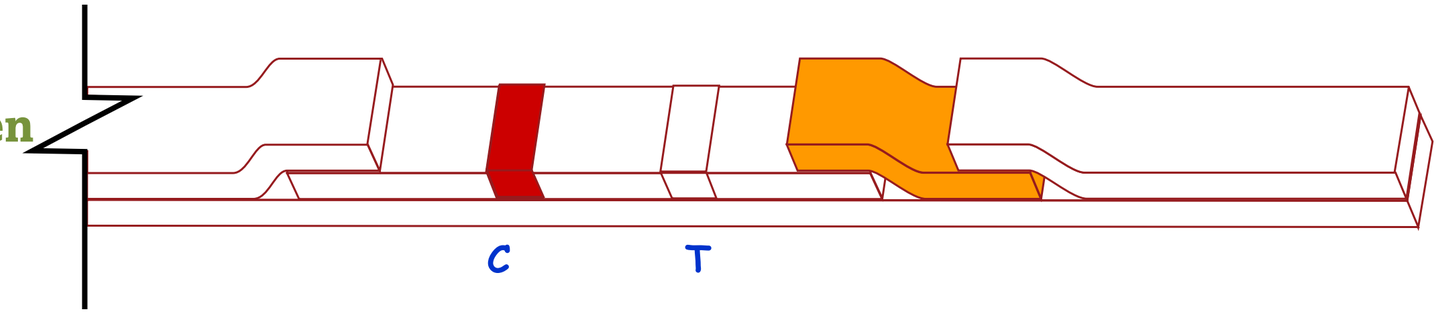
- **Sample with antigen**




Positive result

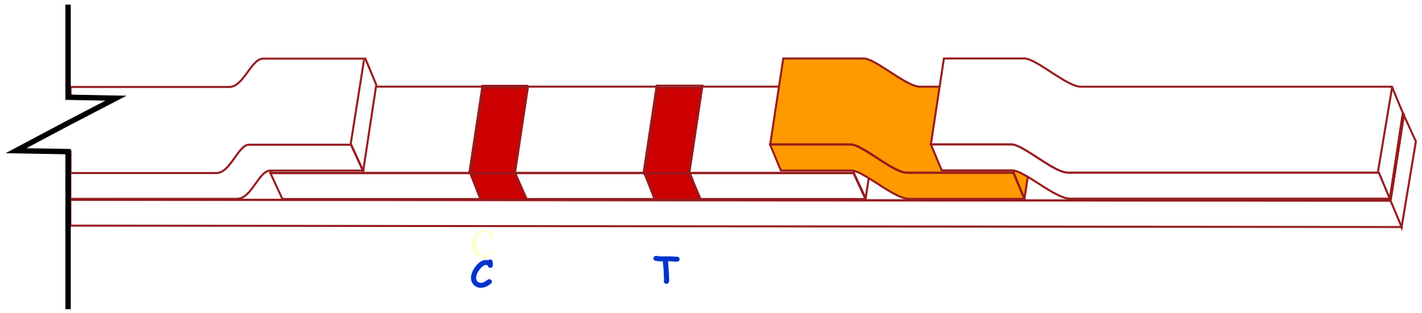
# 1. Direct format (Non-competitive/Sandwich assay)

- **Sample without antigen**




Negative result

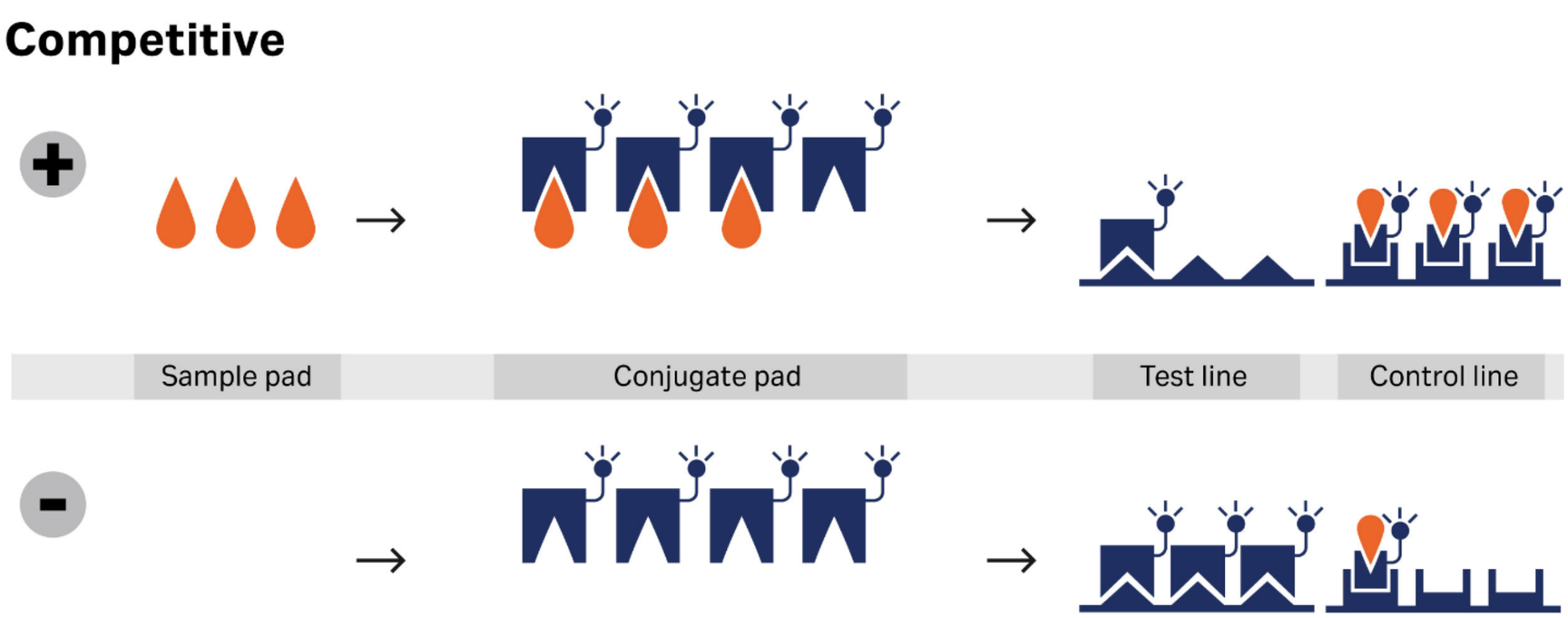
- **Sample with antigen**

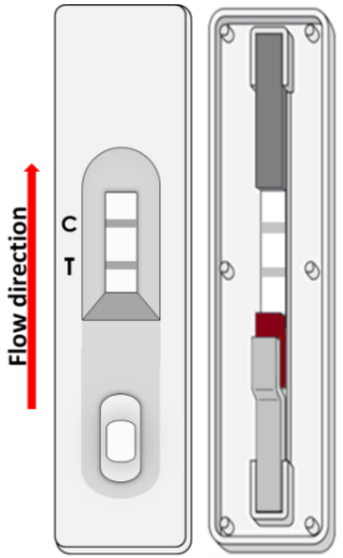


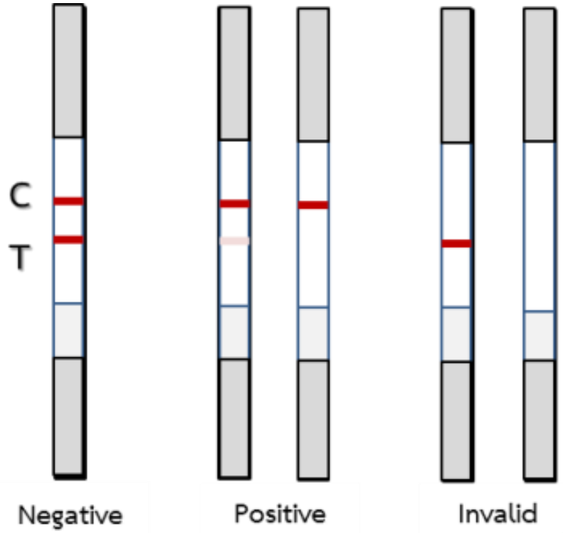

Positive result

# Competitive Strip test (Competitive inhibition)

Methods



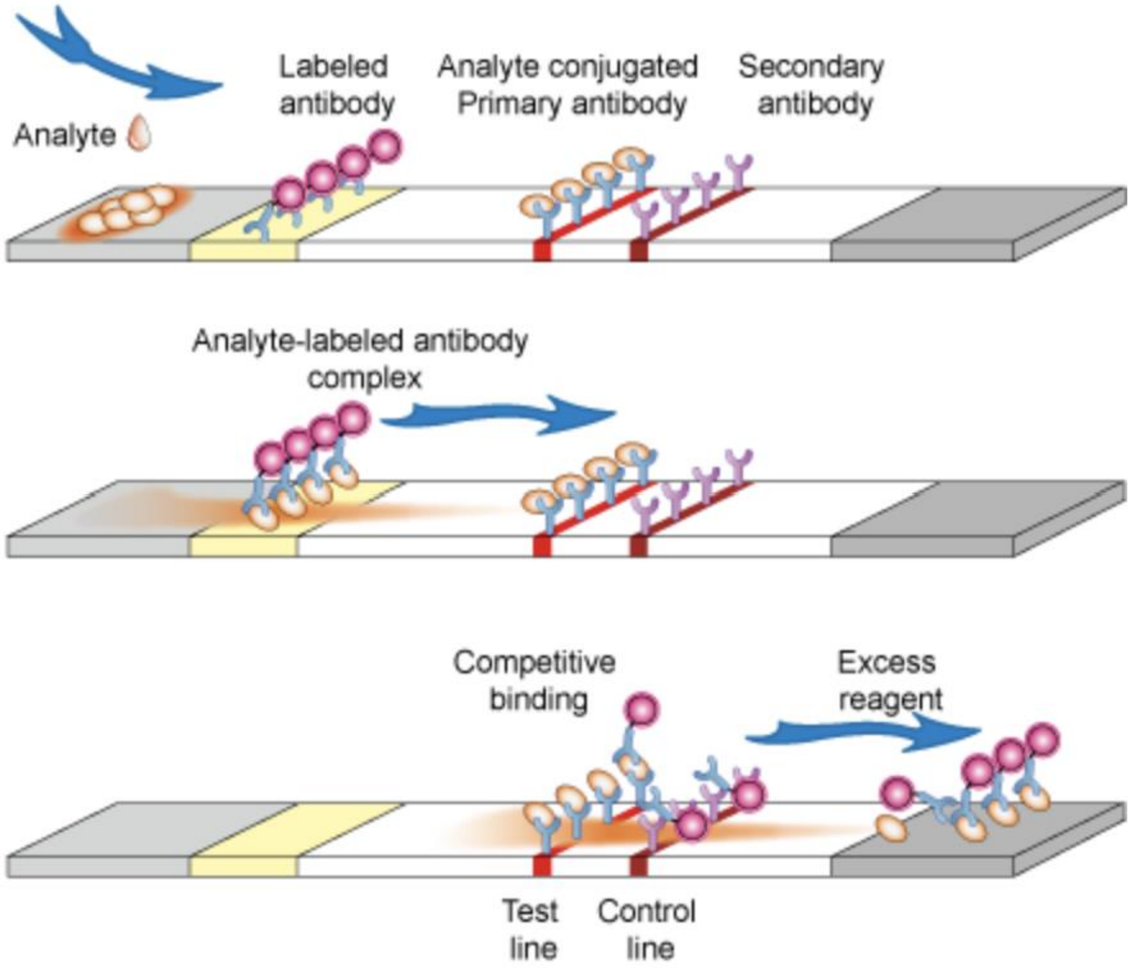




Ref. https://www.cytivalifesciences.com/en/us/news-center/lateral-flow-assay-format-sandwich-or-competitive-10001

# Competitive Strip test (Competitive inhibition)



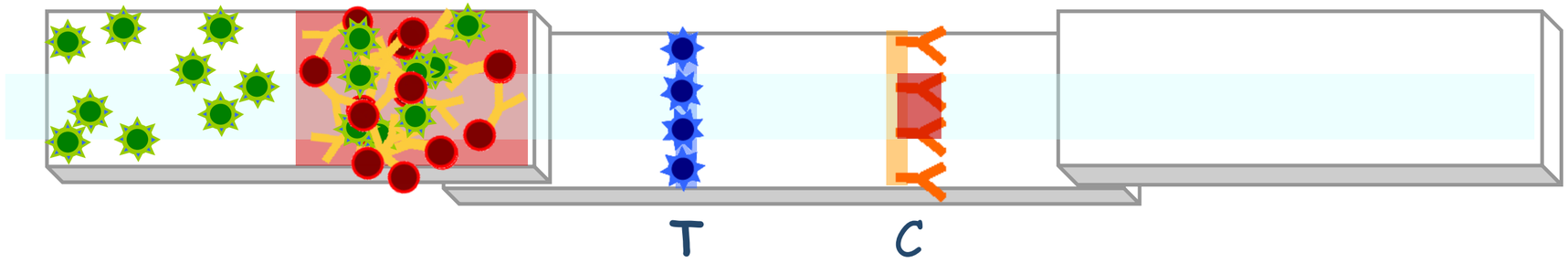


# Methods

## Test strip: Competitive format assay

- **Sample with antigen**

**10 min**



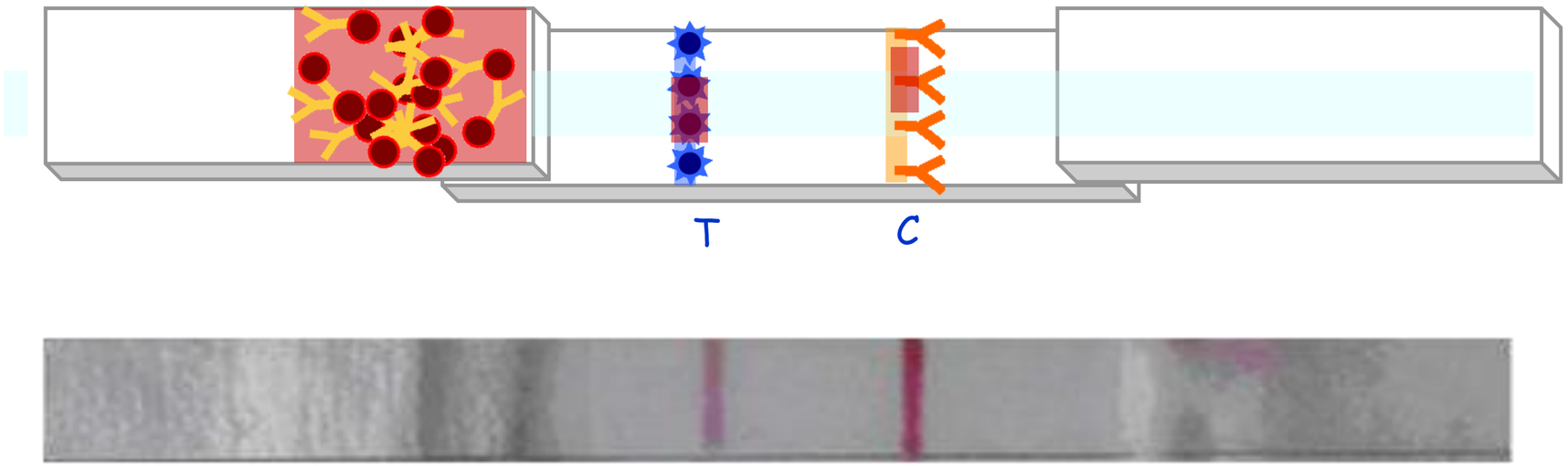

**Positive reaction**

# Methods

## Test strip: Competitive format assay

- **Sample without Antigen**

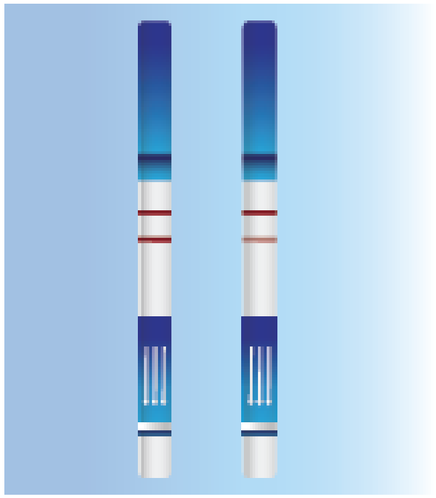
**10 min**



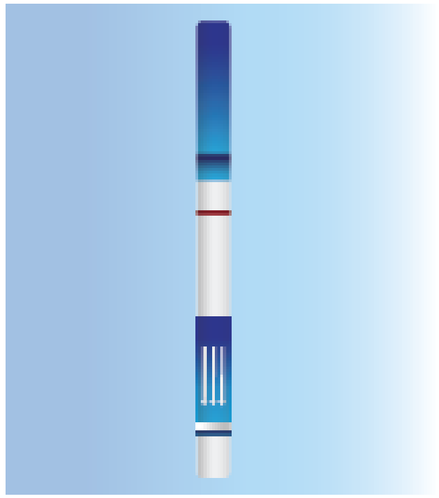

**Negative reaction**

# How different of the result between Direct and Indirect assay

1. Direct format (Non-competitive/Sandwich assay)
   - Large analyze with several antigenic sites



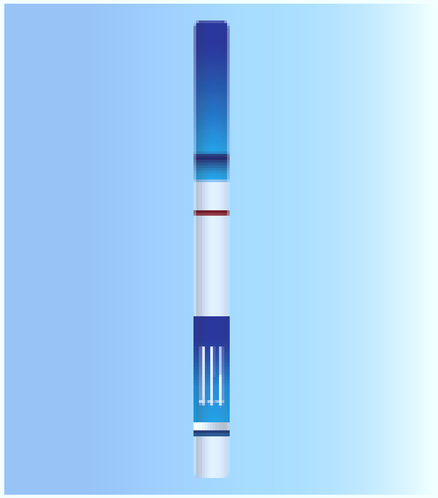
Positive result



Negative result

2. Competitive (Competitive inhibition)
   - Small molecule with a single antigen

Negative result

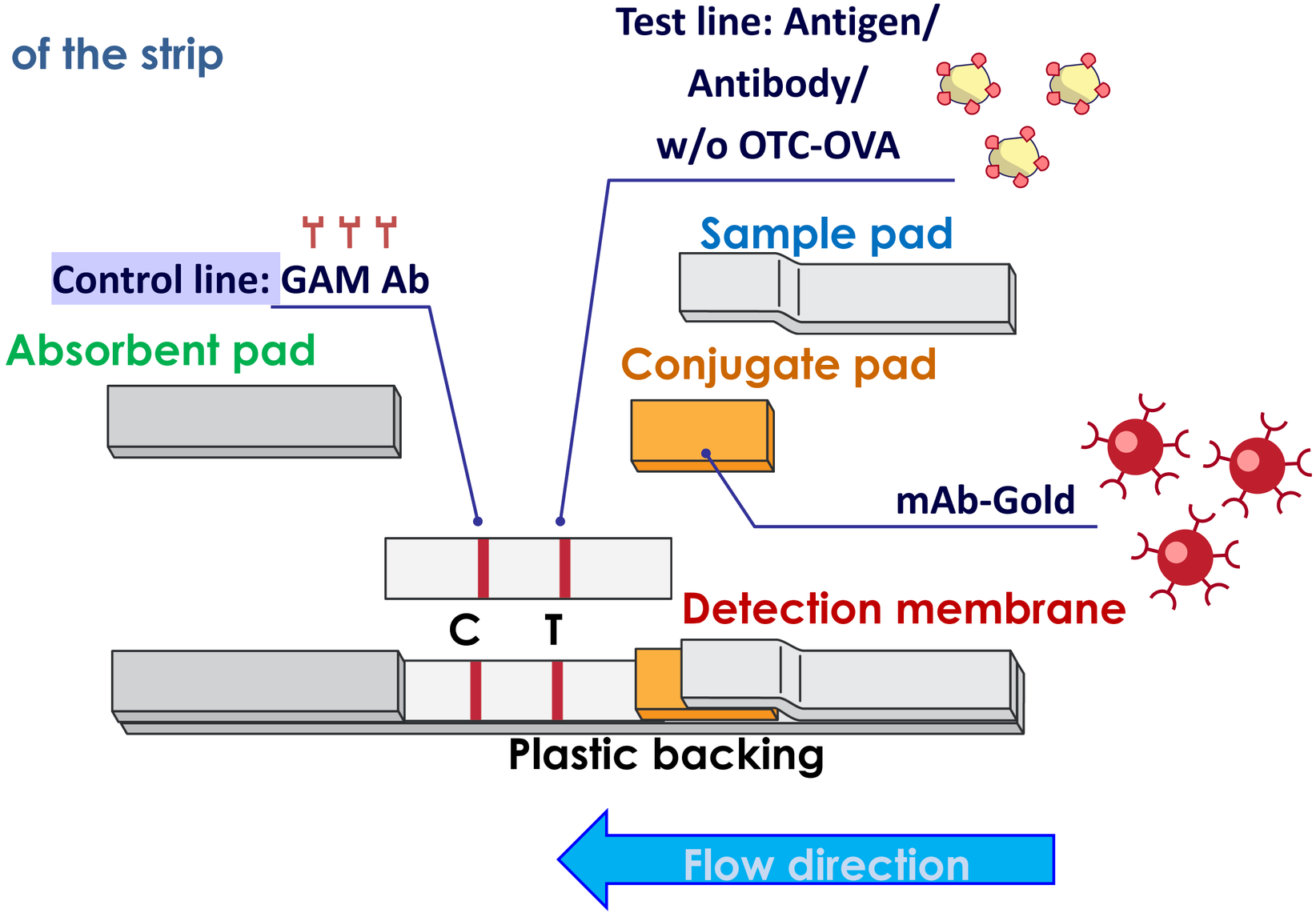


Positive result

# Development of Strip test



- Composition of the strip



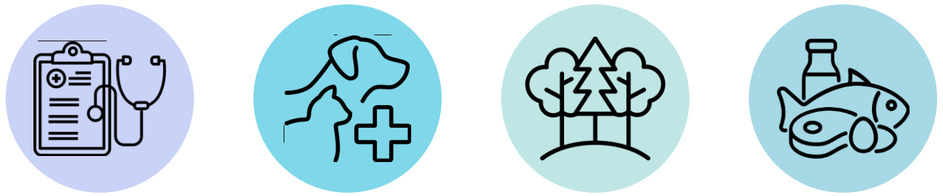
# DESIGN AND DEVELOPMENT

## Target product profile

**1** **To desire characteristic and acceptable criteria**

- Intended use
- Target analyte
- Specificity, sensitivity, and accuracy
- Sample type
- Time to get the result
- Type of analysis (qualitative/quantitative)
- Limit of detection/cut-off value
- Target/patient population

**Intended use:**

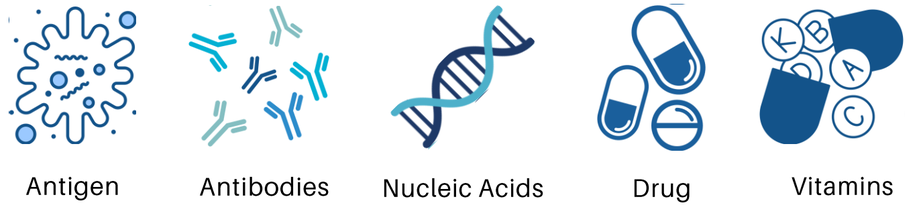



## Design of the device

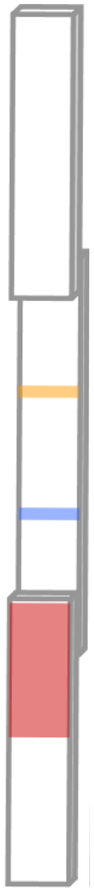
**2** **To define type of device**

- Analyte Sample
- Material components
- Compatibility
- Recognition molecule such as antibody, aptamer
- Detection method

**Analyte Sample:**



# To Develop A Rapid Diagnostic Kit Using Immunochromatographic Method

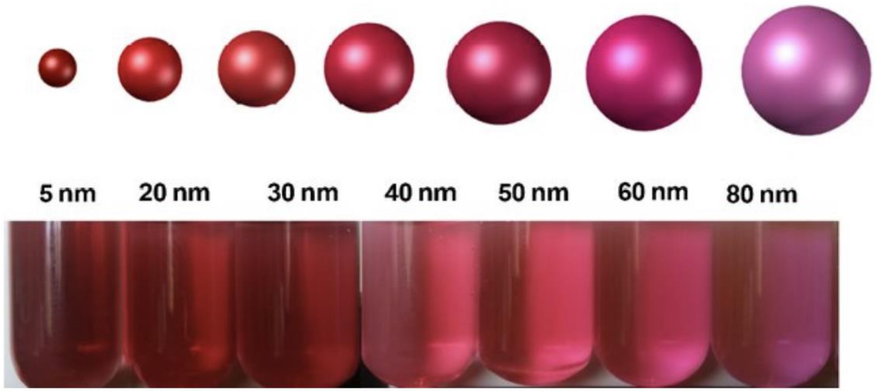


1. Production of antibody against antigen >> For detection
   - Immunization
2. Synthesis of colloidal gold >> For signal reporter
   - Tag-labeled antibody probe (Nanogold colloidal particle)
3. Formation of antibody-colloidal gold conjugates
   - Optimization antibody concentration to attach colloidal gold
4. Preparation of immunochromatography test strip
   - Optimization of antigen and antibody concentration to code on the analytic pad
5. Prototype testing
   - Process verification (partial method validation)
6. Performance testing (Method validation)
   - Performance testing (for finding: accuracy, specificity, sensitivity, false positive rate, and false negative rate)
   - Cross-reactivity testing
   - Stability testing

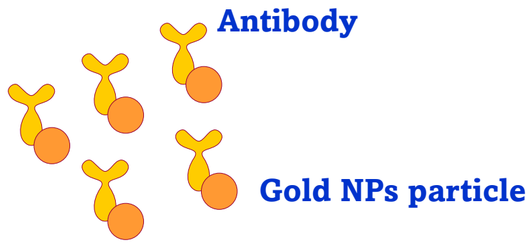
# Synthesis of colloidal gold

## Colloidal gold probe

- Accelerate antibody-antigen reaction
- Provide an amplified signal for immunoassay
- Read results directly by naked eyes
- Convenience
- Test without the handling of reagents
- One-step assay




Gold nanoparticle colloidal suspensions (size of the gold colloids: 5 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, and 80 nm)




Abrica-González, Paulina, J. A. Zamora-Justo, Blanca Estela Chavez-Sandoval, G. R. Vázquez-Martínez, and J. A. Balderas-López. "Measurement of the optical properties of gold colloids by photoacoustic spectroscopy." *International Journal of Thermophysics* 39 (2018): 1-7.

# Comparison of the characteristics of labels commonly used in rapid tests

| Feature | Gold | Silver | Carbon | Latex | Dye | Enzyme |
|---|---|---|---|---|---|---|
| Visibility | *** | * | *** | *** | *** | *** |
| Sensitivity | *** | * | ** | ** | ** | *** |
| Stability | *** | *** | ** | ** | ** | * |
| Colors | * | — | * | *** | *** | ** |
| Reproducibility | *** | *** | * | * | * | ** |
| Scale-up | *** | *** | ** | * | * | * |
| One step | *** | *** | *** | *** | *** | — |
| Multianalyte detection | *** | *** | ** | ** | ** | ** |
| Clean result | *** | *** | * | ** | * | * |
| Ease of preparation | *** | *** | ** | *** | ** | ** |
| Ease of use | *** | *** | ** | *** | ** | * |
| Adaptability | ** | ** | ** | ** | ** | *** |
| Low cost | *** | *** | *** | *** | *** | ** |

*Limited application

**Acceptable for some applications

***Outstanding and applicable to most tests

# • Synthesis of colloidal gold

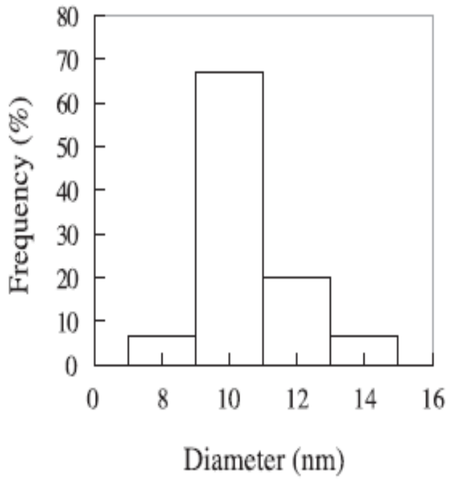


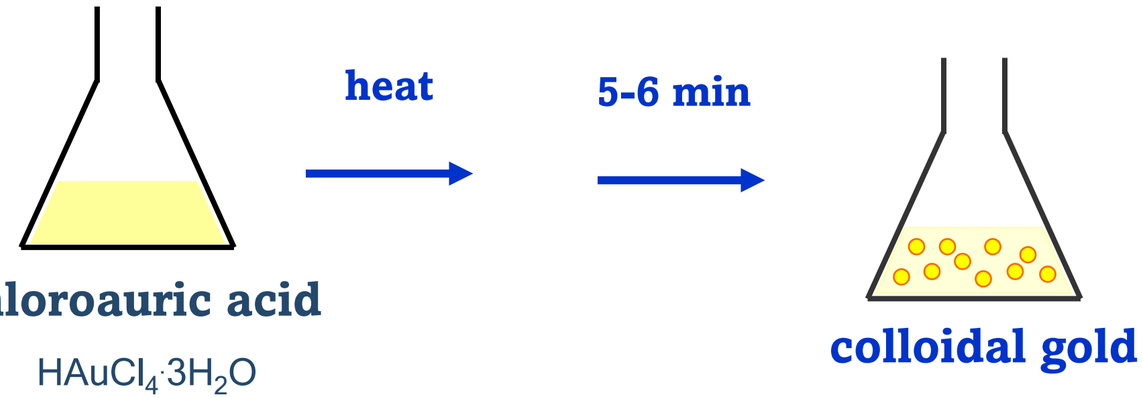
## Nanogold colloidal particle

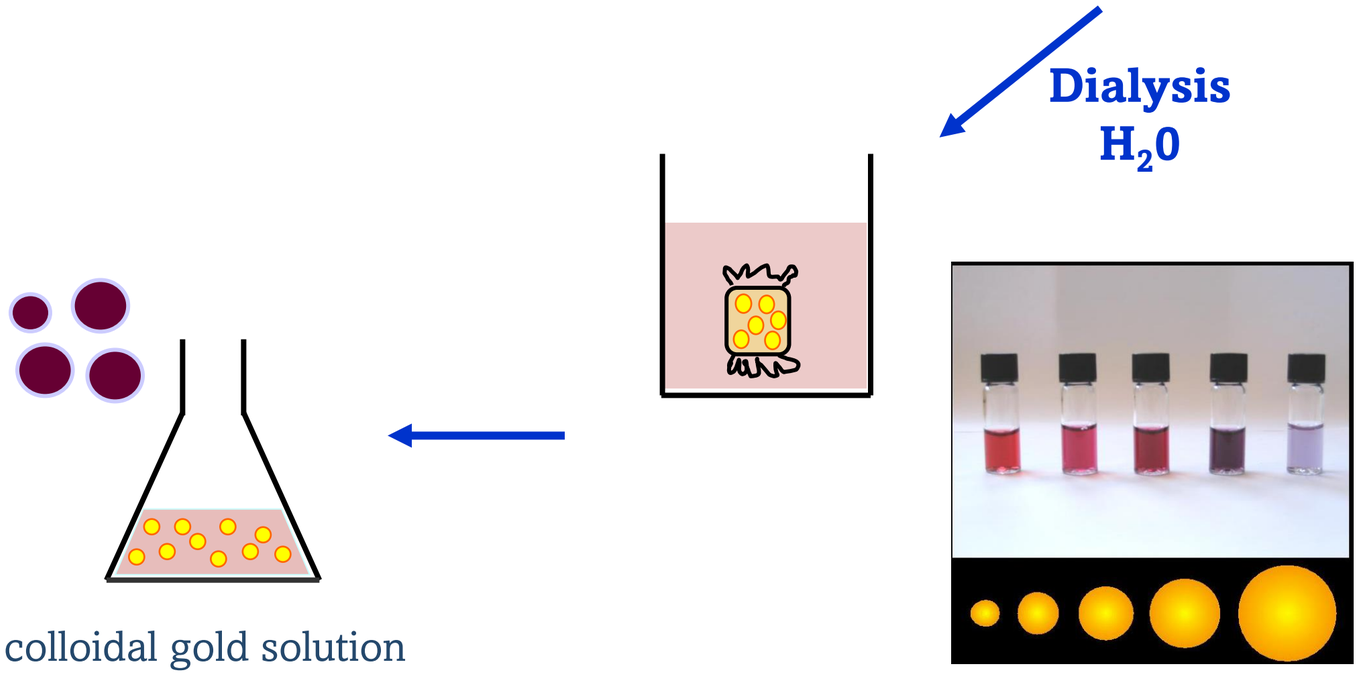
- Stable and easy to use
- Absorb at different wavelengths

Formation of antibody-colloidal gold conjugates
: Measured the size distributions
Transmission Electron Microscopy(TEM)
UV-vis spectroscopy

**Average diameter 10.7±1.5 nm**

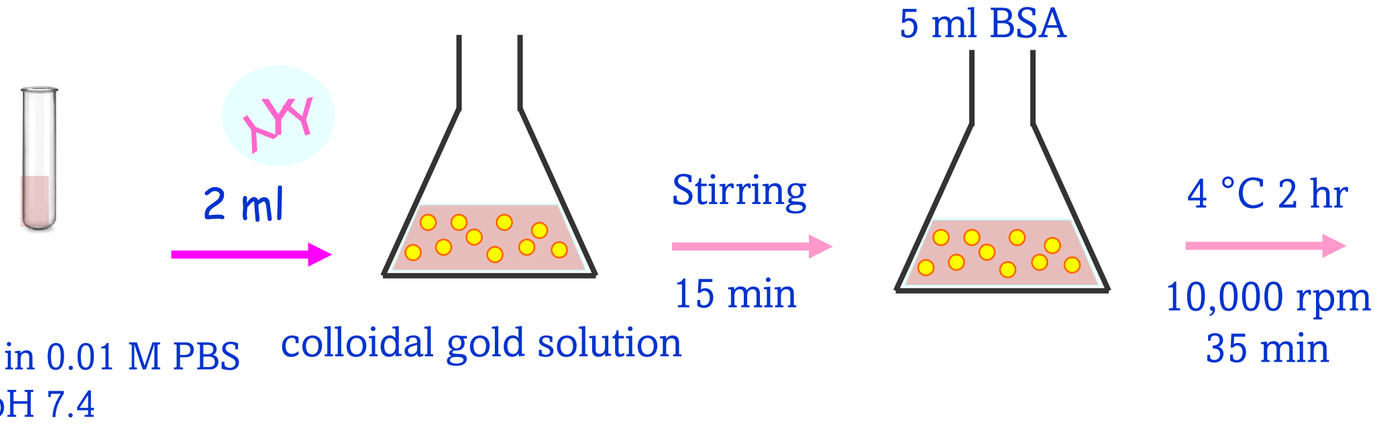


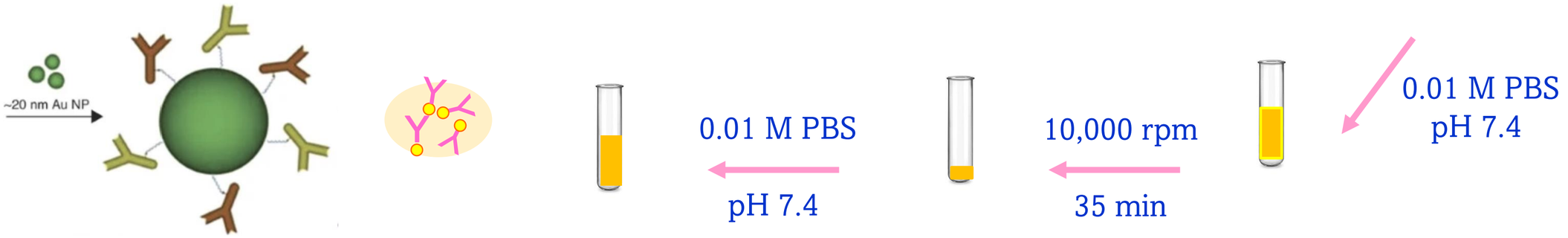



# • Formation of antibody-colloidal gold conjugates






****Optimization of Antibody concentration****



# Lateral flow strip test components

- Sample pad
- Conjugate pad
- Analytical pad
- Absorption
- Backing card

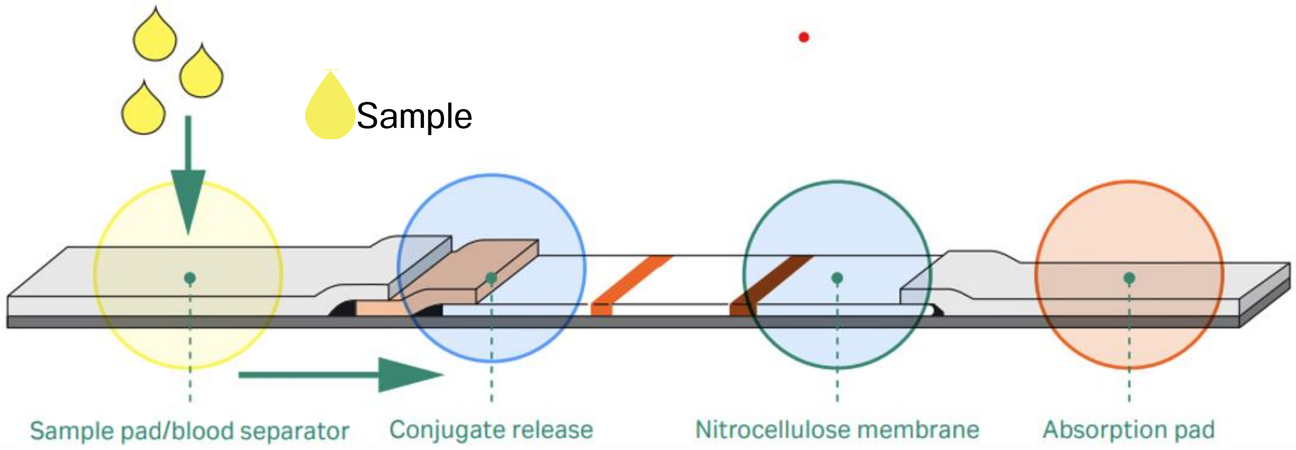



**Fig. 2** Drawing of a strip test immunoassay

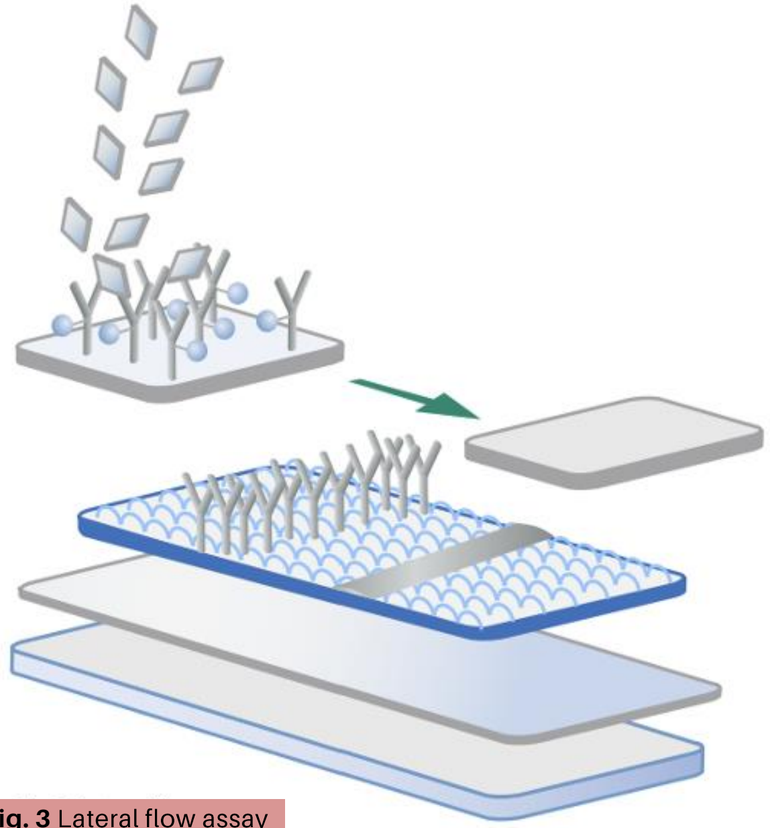


**Fig. 3** Lateral flow assay

Ref: https://cdn.cytivalifesciences.com/api/public/content/digi-16472-pdf?_gl=1*bqldzf*_gcl_au*Mjg3NDc5MjQ3LjE3MDE0OTgzNTQ.

# SAMPLE PAD

Choosing a sample pad with the properties are suitable for your sample (volume and type of samples)

**Chemical properties**

- pH
- Ion strength

**Physical properties**

- Sample or target size
- Viscosity
- Volume

Whatman™ sample pads are available in a wide range of thicknesses, absorbencies and wicking rates.

| Volume per sq cm | Grade | Properties | Thickness (µm @ 53 kPA) | Wicking rate (s/4 cm) | Water absorption (mg/cm$^2$) |
|---|---|---|---|---|---|
| < 50 µL | CF1 | A thin, smooth-surfaced cotton linter paper with a liner flow rate, suitable for small volumes. | 176 | 187 | 16 |
| 50–100 µL | CF3 | A medium thick cotton linter paper, originally used for separation of inorganic compounds. Larger sample volume than CF1. | 322 | 161 | 31 |
| 100–150 µL | CF4 | A medium thick cotton linter paper with acid treatment to improve wet strength and reduce trace impurity content. Similar weight and thickness to CF3 with faster wicking. | 482 | 65 | 46 |
| > 150 µL | GR470 | Untreated bound cellulose suitable for whole blood or serum. Performs well with one or two drops of whole blood. | 840 | 77 | 78 |
| > 50 µL | Standard 14 | Untreated bound glass fiber for faster flow than cotton with lower sample retention. Higher absorption capacity than Standard 17. | 355 | 23.1 | 50.9 |
| > 50 µL | Standard 17 | Untreated bound glass fiber for faster flow than cotton with lower sample retention. Greater tensile strength than Standard 14. | 370 | 34.5 | 44.9 |
| > 50 µL | GF/DVA | Untreated bound glass fiber particularly suitable for saliva samples and raw milk samples. | 785 | 28.2 | 93 |
| > 50 µL | VF2 | Bound glass fiber used as a single or multiple layers for separation. | 785 | 23.8 | 86.2 |

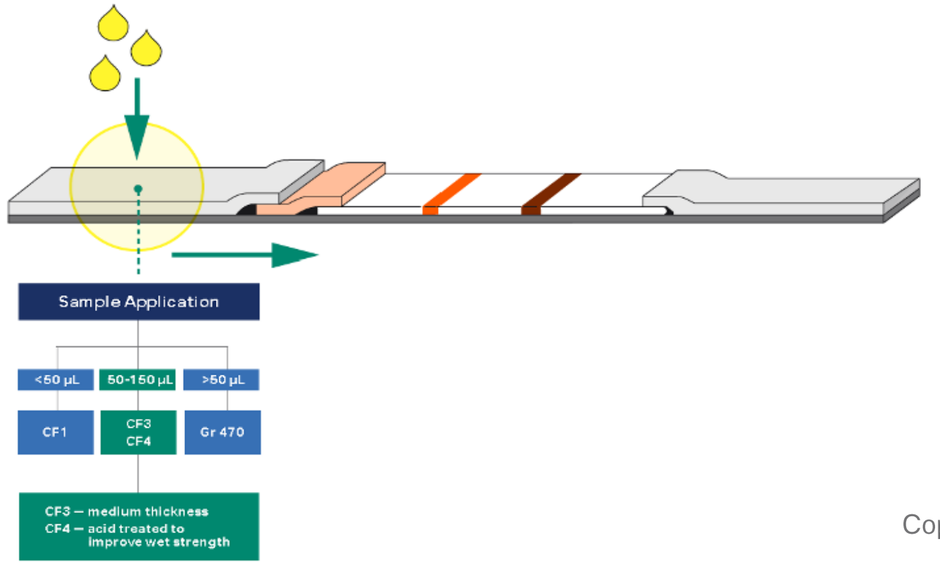
Ref: https://cdn.cytivalifesciences.com/api/public/

** Define sample pad and pretreatment

# SAMPLE PAD

## Technical properties

| Product | Material | Properties | Thickness (μm @ 53 kPA) | Wicking Rate (s/4 cm) | Water absorption (mg/cm²) |
|---|---|---|---|---|---|
| CF1 | 100% cotton linter | Light, thin grade suitable for small volume | 176 | 207.3 | 18.7 |
| CF3 | | Medium weight | 322 | 174.3 | 34.6 |
| CF4 | | | 482 | 67.3 | 49.9 |
| 470 | | | 840 | 77 | 78 |
| Standard 14 | Bound glass fiber | Faster flow than cotton, with lower sample retention | 355 | 23.1 | 50.9 |
| Standard 17 | | | 370 | 34.5 | 44.9 |
| GF/DVA | | Works well with saliva samples and can act as a blood separator as well | 785 | 28.2 | 93 |
| LF1 | | Works well with whole blood or serum samples and can act as a blood separator as well | 247 | 35.6 | 25.3 |
| MF1 | | | 367 | 29.7 | 39.4 |
| VF2 | | | 785 | 23.8 | 86.2 |




## BLOCKING AGENTS FOR SAMPLE PAD

Blocking agents can be soaped to reduce the non-specific binding of the analyte or detection reagents to the reaction membrane such as

- BSA
- Detergent >> Tween 20/Tween 80
- Buffer substance

# CONJUGATE RELEASE PAD

- Zone for immobilized Specific Mab conjugated Gold
- First place of conjugate and target sample interaction
- Glass fiber is a popular material.
- Conjugate buffer is used as a preservative (especially Carbohydrate).

** Requires equipment to spray

## Conjugate Release Pad: Pretreatment

- Buffer substance
- Non-ionic surfactant
- Protein and/or polymer (reduce non-specific binding)
- Need Sugar (Trehalose or Sucrose)

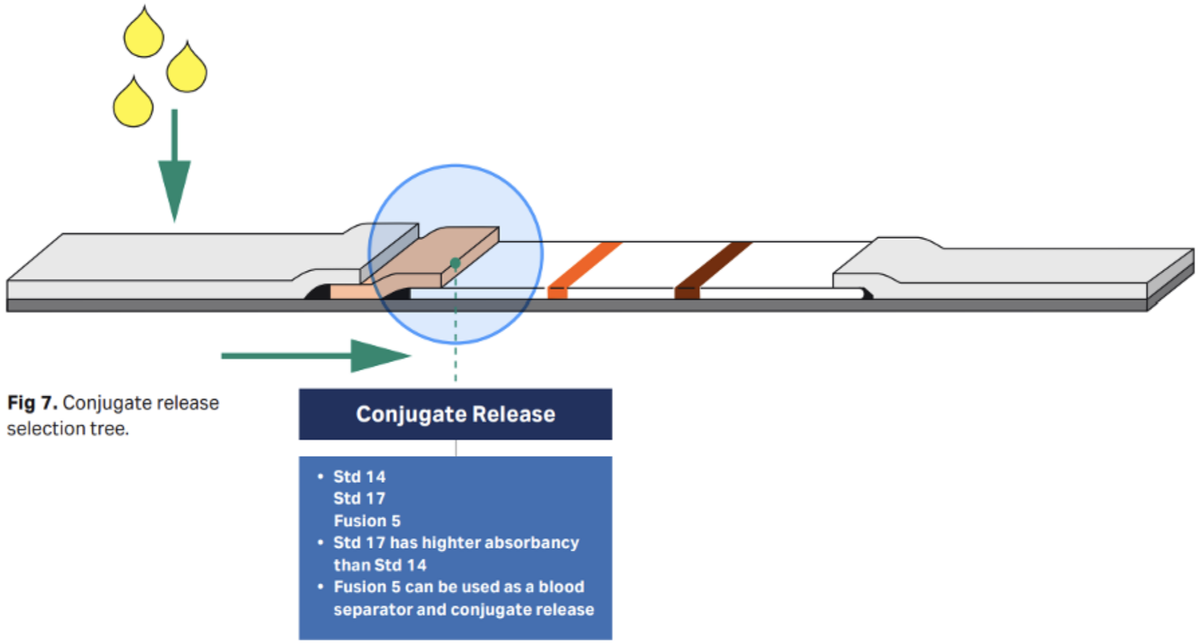



**Fig. 7.** Conjugate release selection tree.

**Fig. 4** Conjugate release pad

### Technical properties

| Grade | Thickness (µm @ 53 kPA) | Wicking rate (s/4 cm) | Water absorption (mg/cm²) | Percent release of gold conjugate (after 90 s) |
|---|---|---|---|---|
| Standard 14 | 355 | 23.1 | 50.9 | 75 |
| Standard 17 | 370 | 34.5 | 44.9 | 75 |
| Fusion 5 | 370 | 43.9 | 42.3 | >94 |

Ref: https://www.cytivalifesciences.com/en/us/solutions/lab-filtration/knowledge-center/conjugate-release-pads

Define pad material, dimensions, pretreatment

# ANALYTICAL PAD: MEMBRANES

Analytical pad Selection to Strip test development generally is selected by

- Capillary flow times
- Surfactant contents

Fig 1. Basic set-up of membrane capillary flow time measurement. Strip width is 1 cm, strip length 4.5 cm, with triangular marks at 4 cm strip length. The time the liquid (water) needs to reach these marks is taken and documented. Water volume is 100 µL.

## Base properties of Cytiva lateral flow membranes

**Table 1.** Summary of Cytiva membrane properties

| Membrane grade | Backed/unbacked | Capillary flow time (seconds/4 cm) | Relative surfactant concentration | Use recommended for sample | Additional information |
|---|---|---|---|---|---|
| FF80HP | Backed | 60–95 | Low | Serum, plasma | In general, used for high viscosity samples, should be blocked in a separate manufacturing step |
| FF120HP | Backed | 90–140 | Low | Diluted serum, plasma, urine | Membrane of choice for lateral flow arrays. Requires blocking in a separate manufacturing step |
| FF170HP | Backed | 140–200 | Low | Low viscosity samples, urine, water | Is also being used for high viscosity samples and capture reagents with slow on-rates |
| FF80HP Plus | Backed | 60–95 | Medium | Serum, plasma, milk | |
| FF120HP Plus | Backed | 90–140 | Medium | Diluted serum, plasma, urine | |
| FF170HP Plus | Backed | 140–200 | Medium | Low viscosity sample, urine, water | |
| Immunopore RP | Backed | 90–150 | High | Serum, plasma, urine | |
| Immunopore FP | Backed | 140–200 | High | Diluted serum, urine water | |
| Immunopore SP | Backed | 200–300 | High | Low viscosity samples in general | Membrane of choice for capture reagents with very slow rates. It will result in longer test duration times |
| Prima 40 | Backed | 38–52* | Very high | Very high viscosity samples, milk | Originally developed for test systems aiming for the detection of whole cells in lateral flow tests |
| AE 100 | Unbacked | 90–120 | Medium | Serum, plasma | |
| AE 99 | Unbacked | 120–160 | High | Urine, diluted serum | |
| AE 98 | Unbacked | 140–200 | Medium | Urine, low viscosity samples | |

*The capillary flow time in Prima 40 is determined by a method that is different from the one described in Figure 1

Please note that the use recommendations only refers to first experiments. It may be that a developer can or must use a membrane with a faster or slower capillary flow time, depending on the kinetic properties of the reagents that are to be used in the test system. Whole blood has not been listed in the table as a sample liquid since the blood cells in lateral flow tests are usually retained in the sample pad, and the liquid that runs through the membrane will be serum or plasma even in that case.

Capillary flow times

Ref: https://www.cytivalifesciences.com/en/us/solutions/lab-filtration/knowledge-center/conjugate-release-pads

# ANALYTICAL PAD: MEMBRANES

## Membrane selector according to sample type

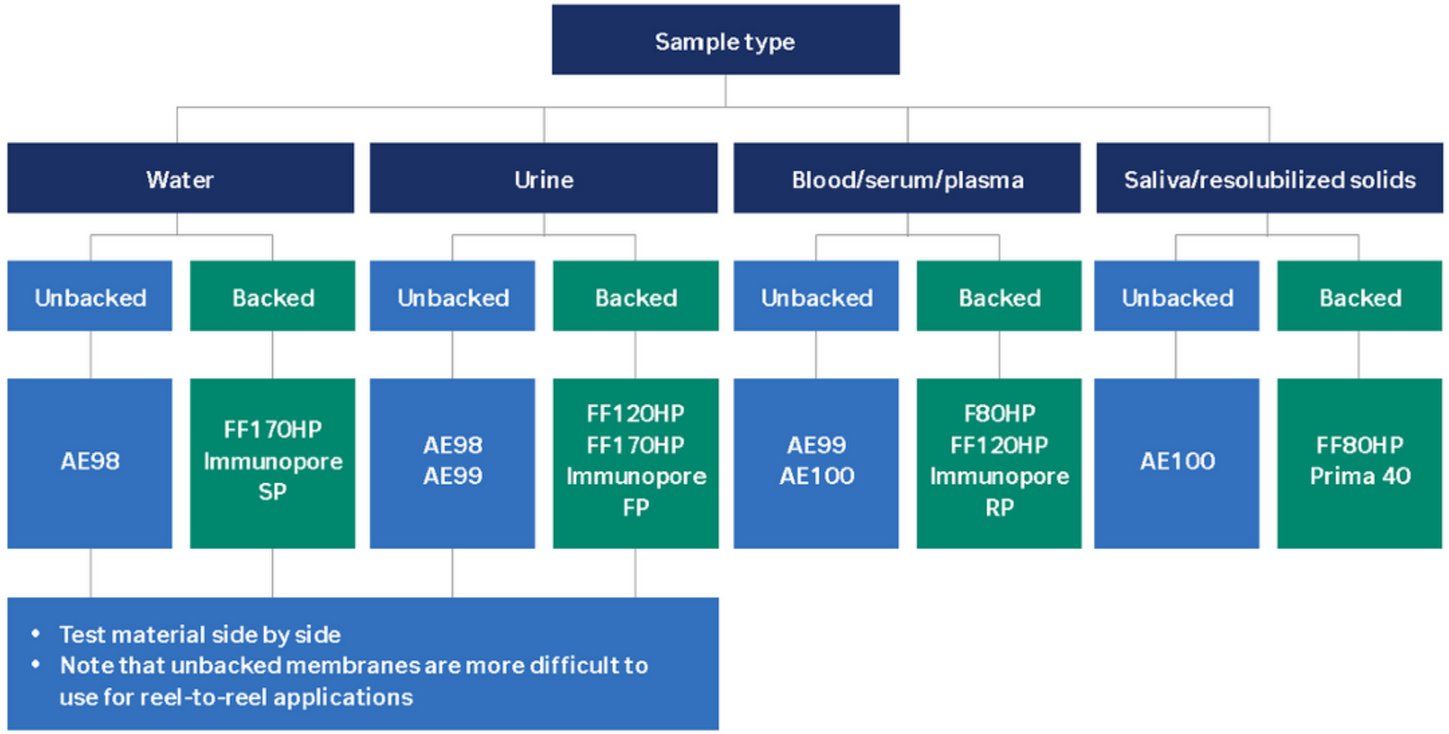
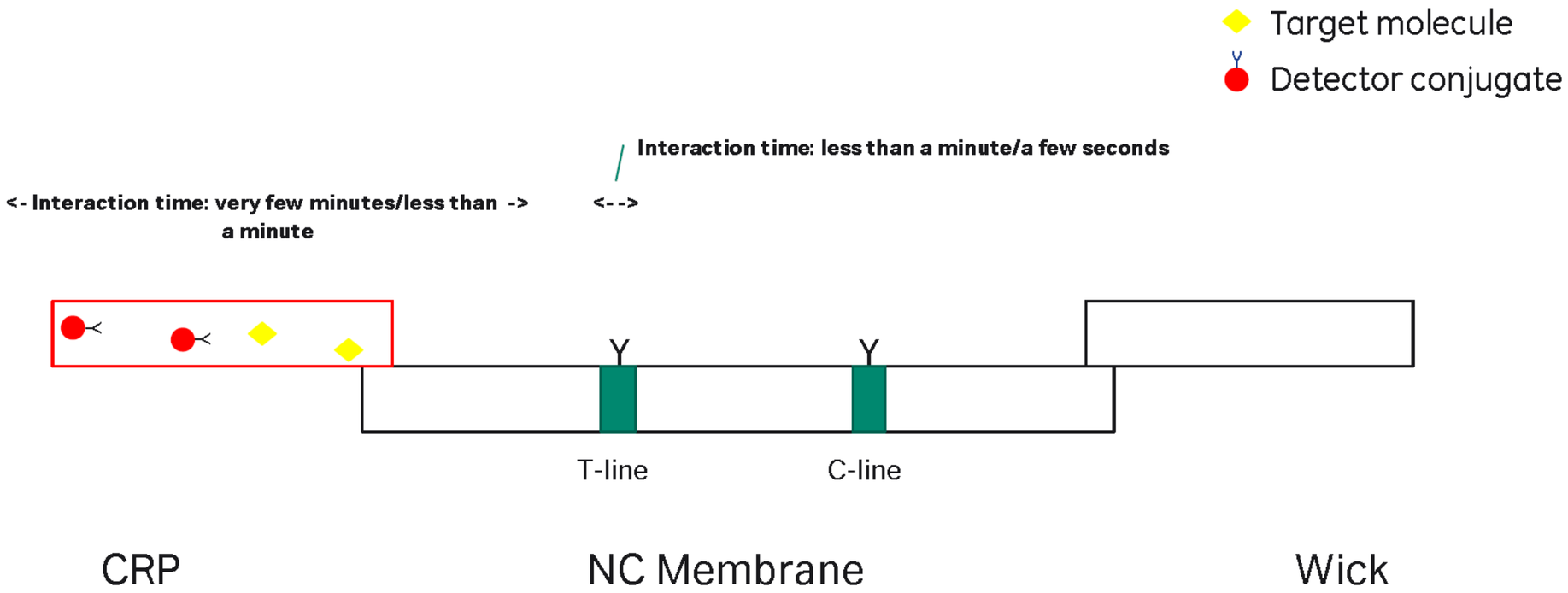



**Table 2:** Comparison between backed and unbacked membrane features

| Backed membrane | Unbacked membrane |
|---|---|
| • Increased mechanical strength of the membranes, simplifying use in reel-to-reel machines<br>• Direct contact is prevented between the nitrocellulose material and the adhesive from the lamination card where the test elements are mounted | • Enables assay suitability tests of both air and belt side of the membrane |

Ref: https://www.cytivalifesciences.com/en/us/solutions/lab-filtration/knowledge-center/

# Interaction times




**ISSUES WITH SLOW MEMBRANES:**
False positive signals, high background

**ISSUES WITH FAST MEMBRANES:**
False negative results

# ABSORBENT PAD

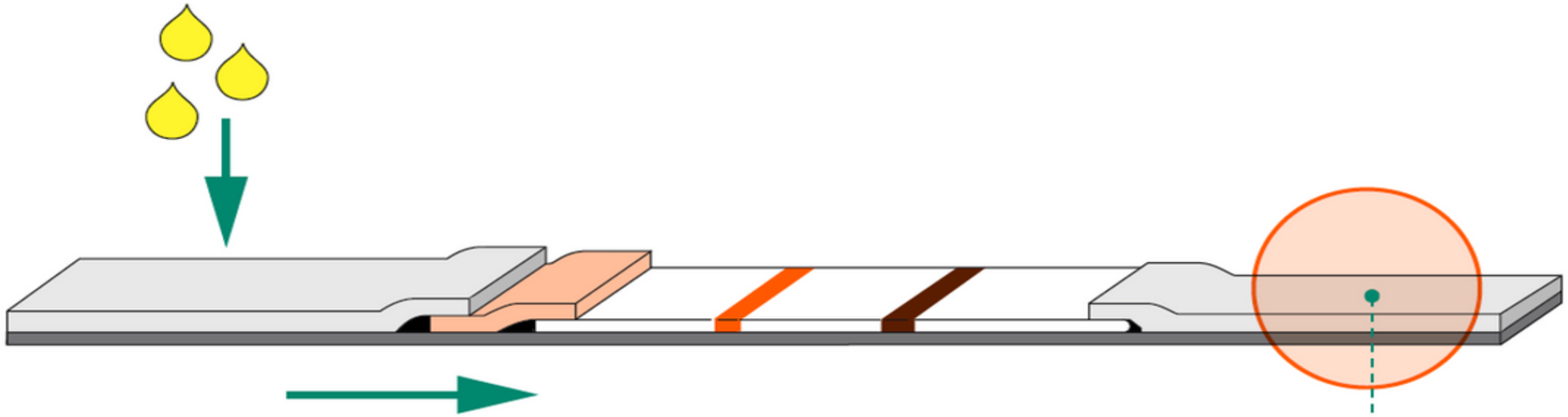
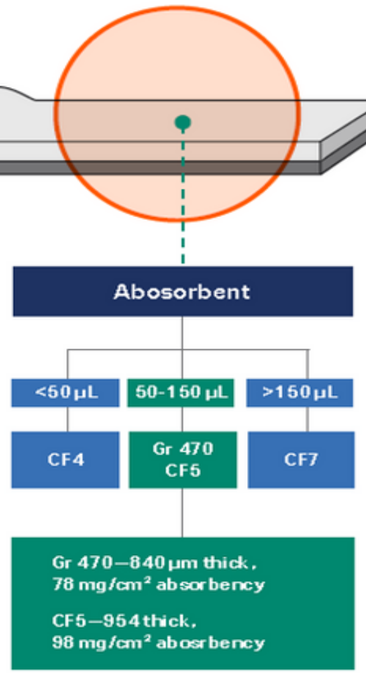
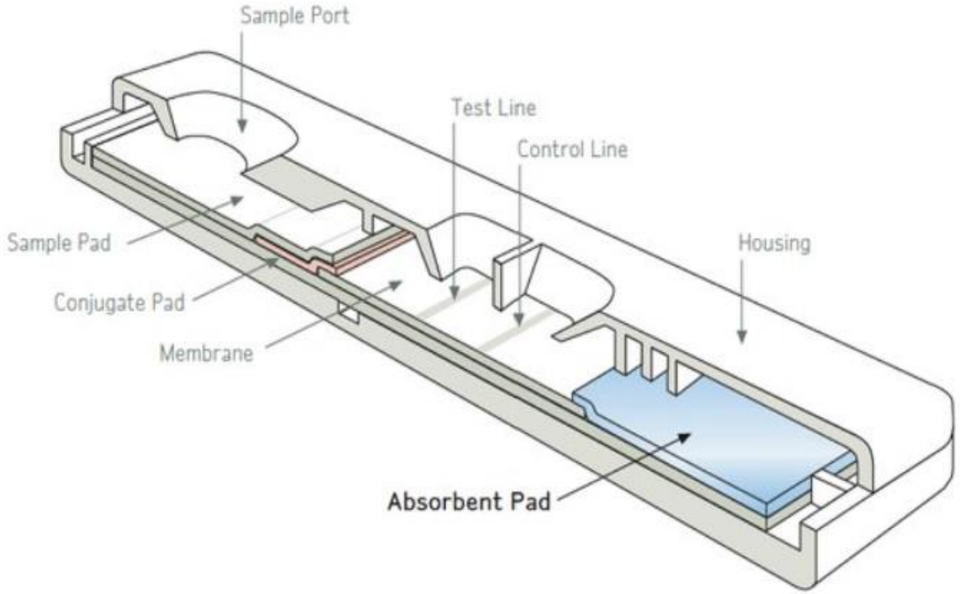


**Fig 12.** Absorption pads selection tree.



# ABSORBENT PAD

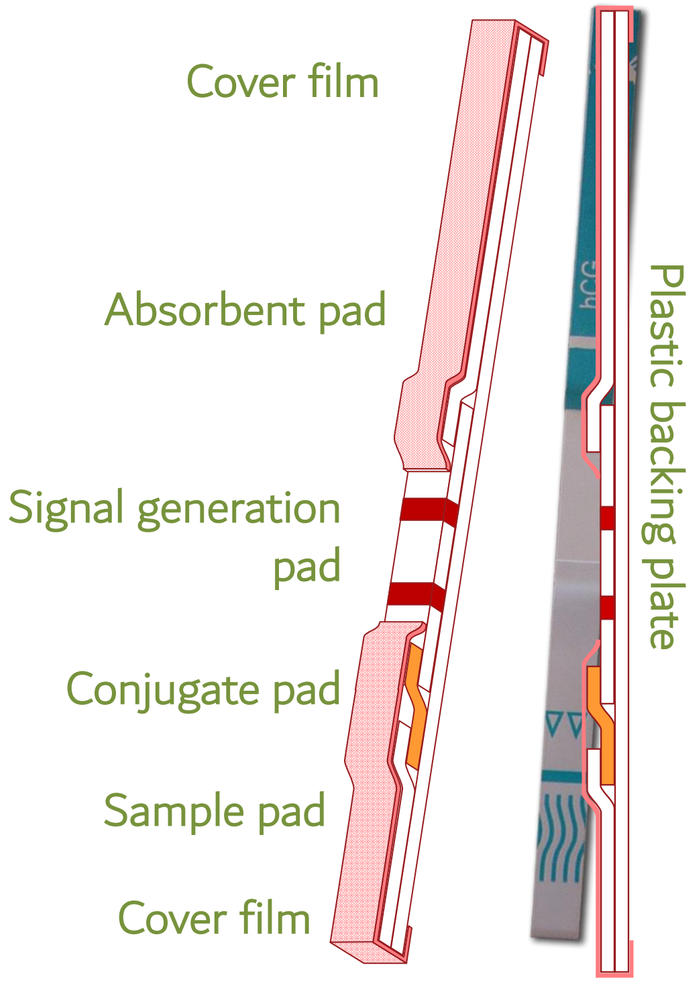
- To wick the fluid through the membrane
- To collect the processed liquid



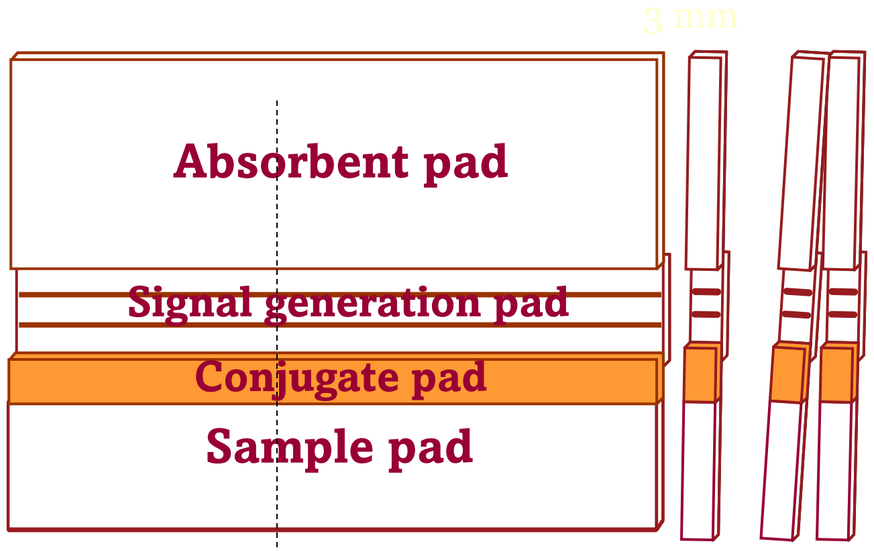

Ref. Clayton, Katherine Noel. "Comparing Anti-VEGF Antibodies and Aptamers on Paper Microfluidic-based Platforms." PhD diss., California Polytechnic State University, 2012.

| Grade | Properties | Thickness (um @ 53kPA) | Wicking rate (s/4cm) | Water absorption ($mg/cm^2$) |
|---|---|---|---|---|
| CF3 | A medium thick cotton linter paper, originally used for separation of inorganic compounds. Larger sample volume than CF1. | 322 | 174.3 | 34.6 |
| CF4 | A medium thick cotton linter paper with acid treatment to improve wet strength and reduce trace impurity content. Similar weight and thickness to CF3 with faster wicking. | 482 | 67.3 | 49.9 |
| CF5 | 100% cotton linter can handle medium/high volumes | 954 | 63.3 | 99.2 |
| CF7 | 100% cotton linter can handle medium/high volumes | 1873 | 35 | 252.3 |
| GR470 | Untreated bound glass fiber suitable for whole blood or serum. | 840 | 77 | 78 |

# METHODS



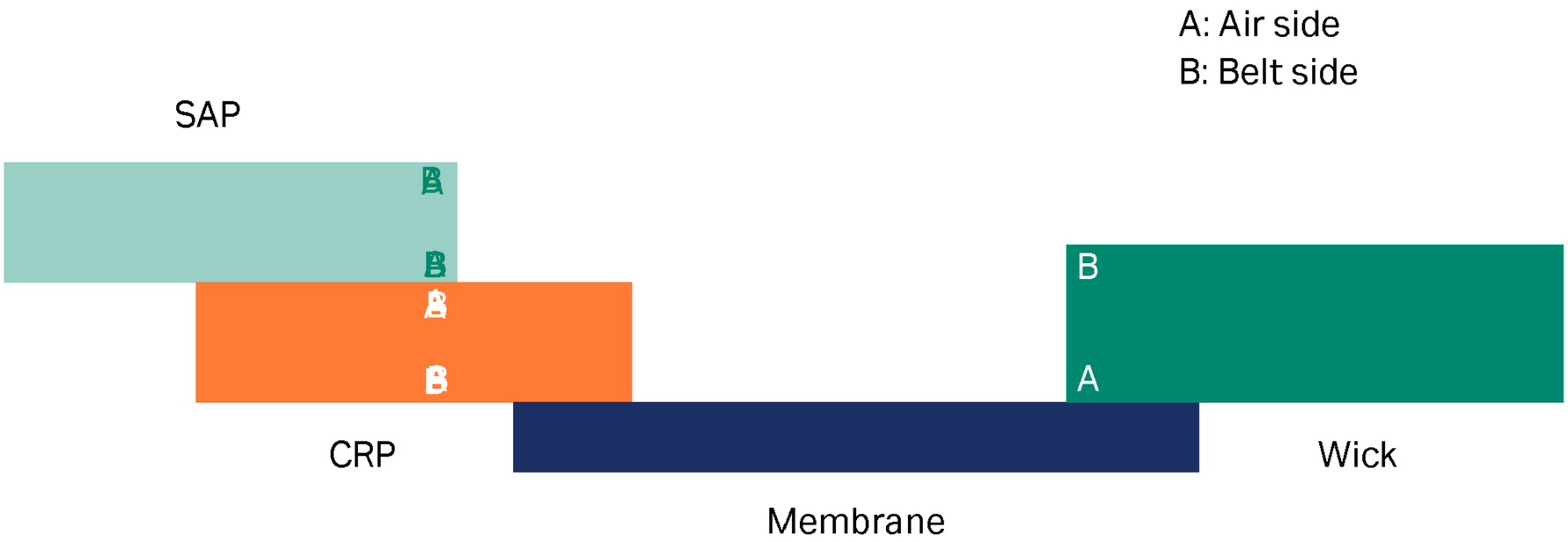

# ASSEMBLING



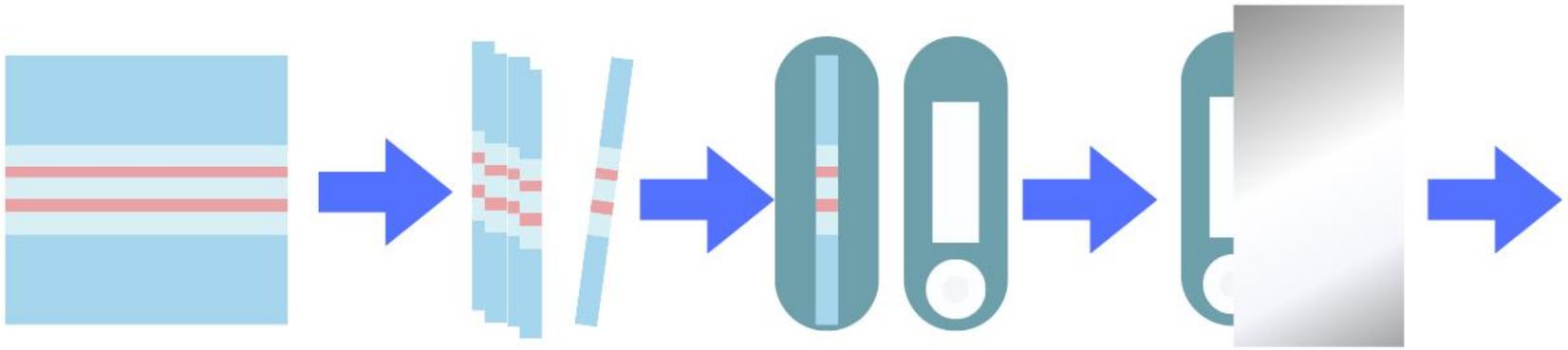

- **Cut strip using a CM4000 cutter**
- **Store at 4˚C**

# Warning !!

## Paper orientation in lateral flow tests



# ASSEMBLING



Assemble >> all components of the strip test

Cut to 4 cm

Put to the cassette

Put into aluminum foil


Pack to a box

# Information/Instruction for use (IFU)

## Information needs

**Manufacturer information**

Produce name :

Application:

Country of Origin:

Code of product:

Manufacturer address:

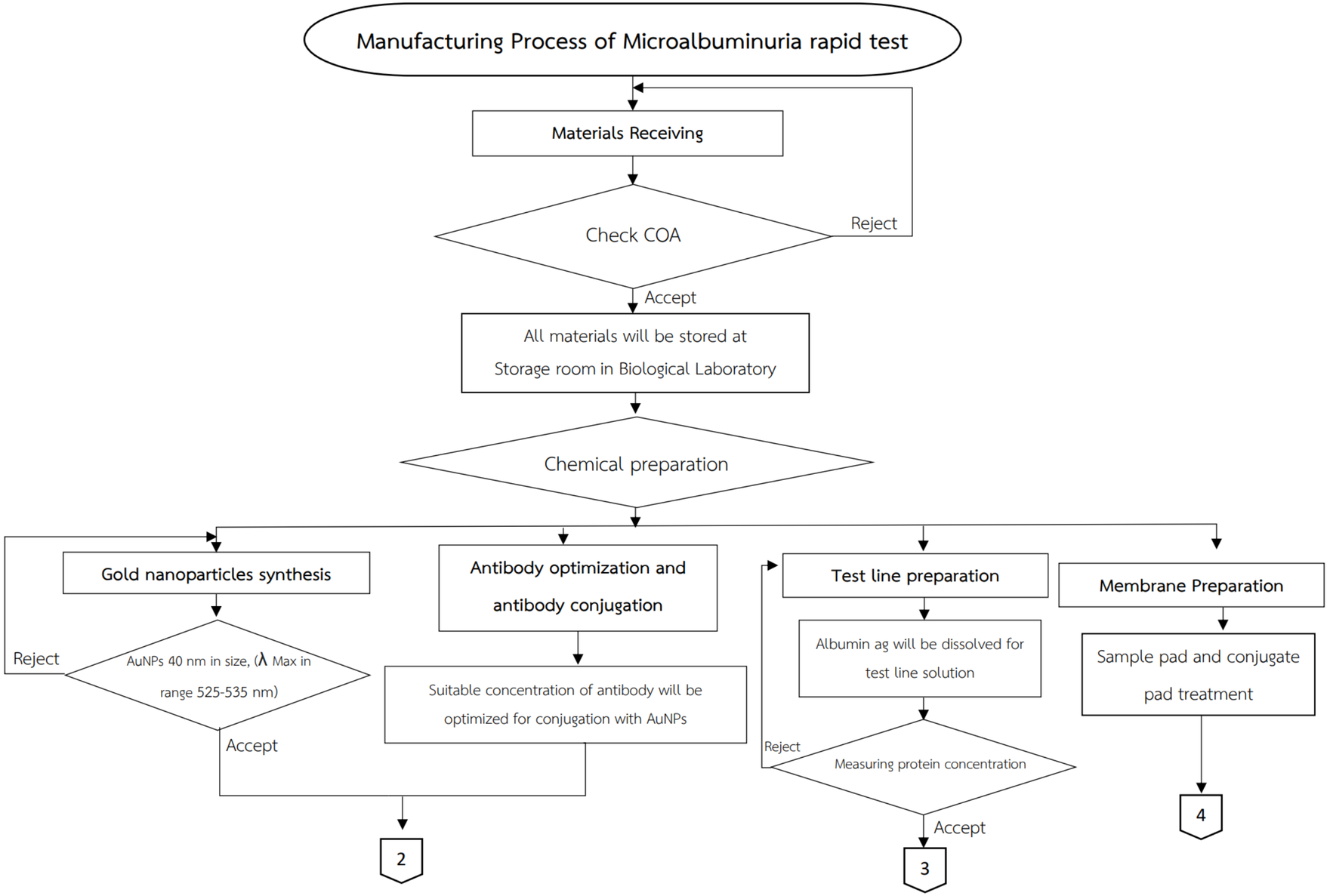
**Performance characteristics**

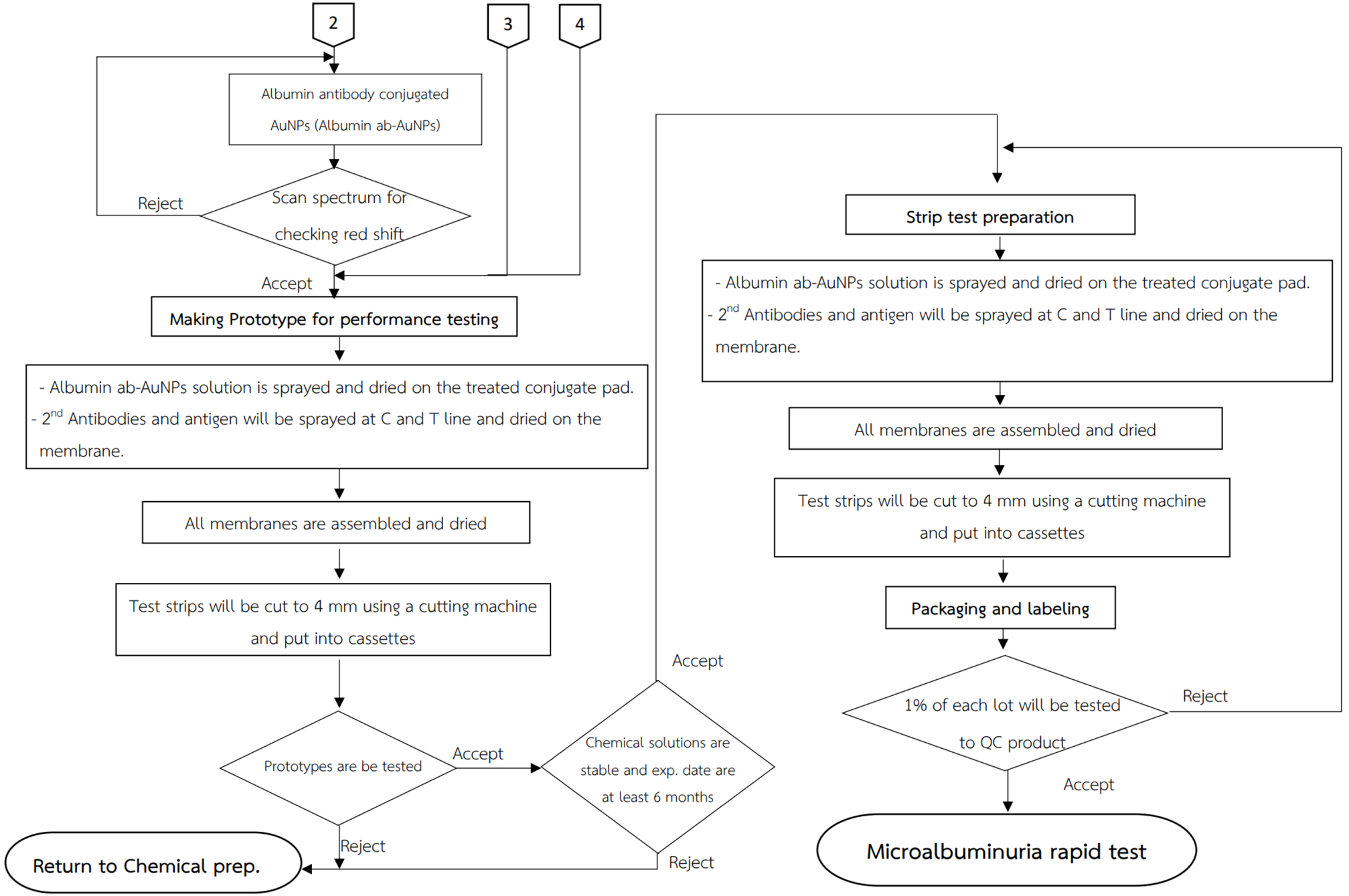
1. Test Verification
2. Cut off value
3. Cross-reactivity
4. Interference substance
5. Precision
6. Hook effect

**Product Description**

1. Intended use
2. Summary and explanation
3. Device Description and Features
4. Medical device contents
5. How to storage
6. Shelf-life
7. Test procedure
8. Internal Quality Control
9. Indications
10. Warnings and Precautions (this information should be related with risk analysis report)
11. Limitations
12. Materials and components
13. Other relevant specifications

# Immunochromatographic strip test Production




2
3
4
Albumin antibody conjugated AuNPs (Albumin ab-AuNPs)
Scan spectrum for checking red shift
Reject
Accept
Making Prototype for performance testing
- Albumin ab-AuNPs solution is sprayed and dried on the treated conjugate pad.
- 2nd Antibodies and antigen will be sprayed at C and T line and dried on the membrane.
All membranes are assembled and dried
Test strips will be cut to 4 mm using a cutting machine and put into cassettes
Prototypes are be tested
Accept
Chemical solutions are stable and exp. date are at least 6 months
Accept
Reject
Reject
Return to Chemical prep.
Strip test preparation
- Albumin ab-AuNPs solution is sprayed and dried on the treated conjugate pad.
- 2nd Antibodies and antigen will be sprayed at C and T line and dried on the membrane.
All membranes are assembled and dried
Test strips will be cut to 4 mm using a cutting machine and put into cassettes
Packaging and labeling
1% of each lot will be tested to QC product
Reject
Accept
Microalbuminuria rapid test