LAB 4
RAPID IMMUNOCHROTOGRAPHIC TECHNIQUES

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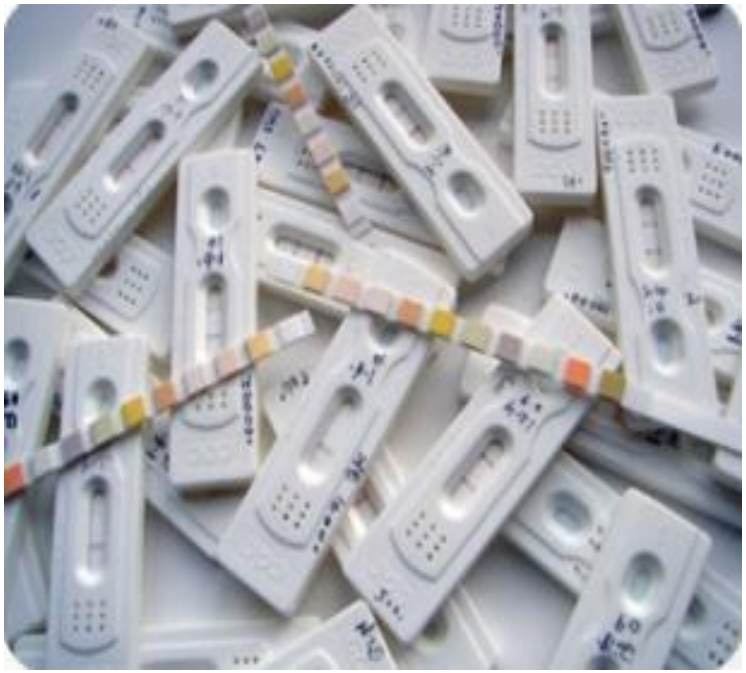
Immunochromatographic assays, also called lateral flow dipstick immunoassay or simply strip tests,

They are a logical extension of the technology used in latex agglutination tests, the first of which was developed in 1956 by Singer and Plotz.



are simple cellulose-based devices intended to detect the presence (or absence) of a target analyte in liquid sample (matrix) without the need for specialized and costly equipment, though many lab-based applications exist that are supported by reading equipment.

Typically, these tests are used for medical diagnostics either for home testing, point of care testing, or laboratory use

PRINCIPAL OF IMMUNOCHROMATOGRAPHY KIT:

Principal of immunochromatography is the same as ELISA sandwich method, only difference is in that immunological reaction is carried out on the chromatographic paper by capillary action.

For this system, two kinds of specific antibodies against antigen are used. One of the antibodies is immobilized on the chromatographic paper, and the other is labeled with colloidal gold and infiltrated into sample pad.

An immunochromatographic unit is completed by attaching the

sample pad at the end of the membrane.

BASIC COMPONENTS OF LATERAL FLOW TEST:

1. Sample pad.

2. Conjugate (detector) pad.

3. Detection conjugate.

4. Nitro-cellulose membrane.

5. Test and control reagent lines.

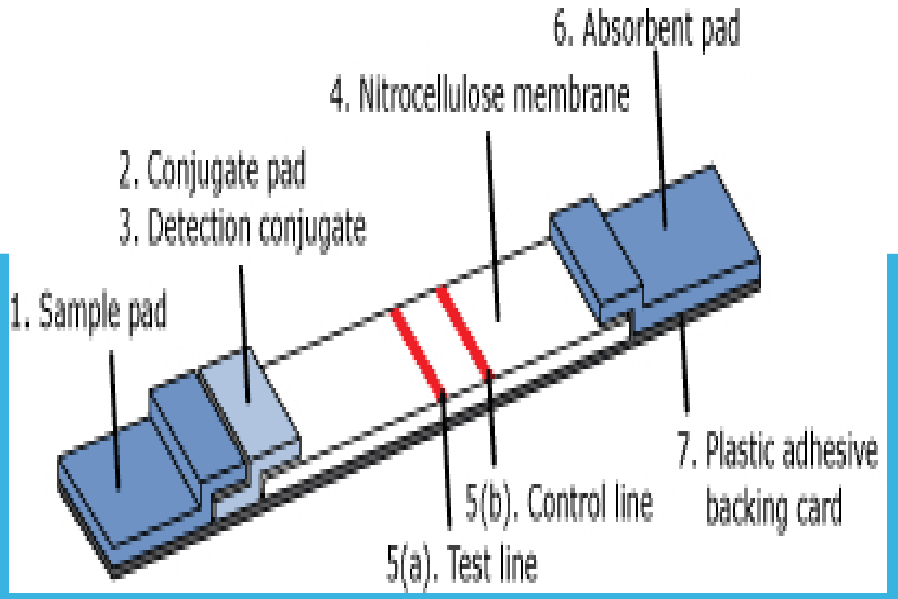
6. Absorbent (sink) pad.

7. Plastic-adhesive backing card.

The following components are “optional” and are not necessary or included in many lateral flow platforms:

8. Laminate Tape.

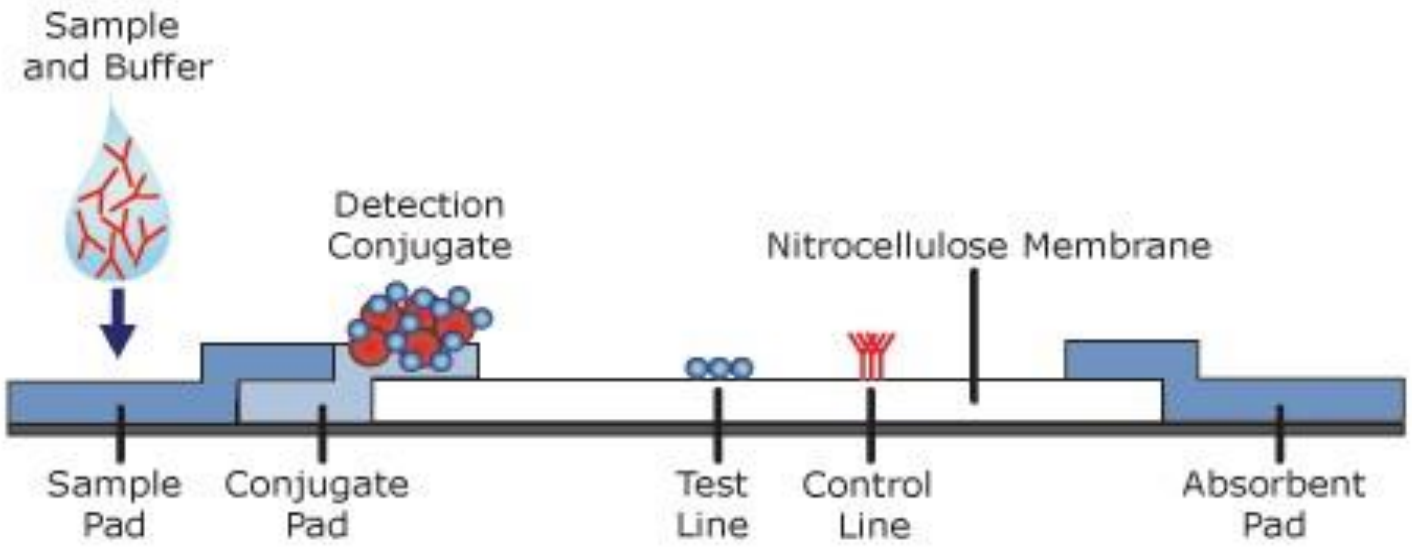
9. A Strip housing / Cassette.



HOW THEY WORK:
STEP 1: SAMPLE PLACEMENT

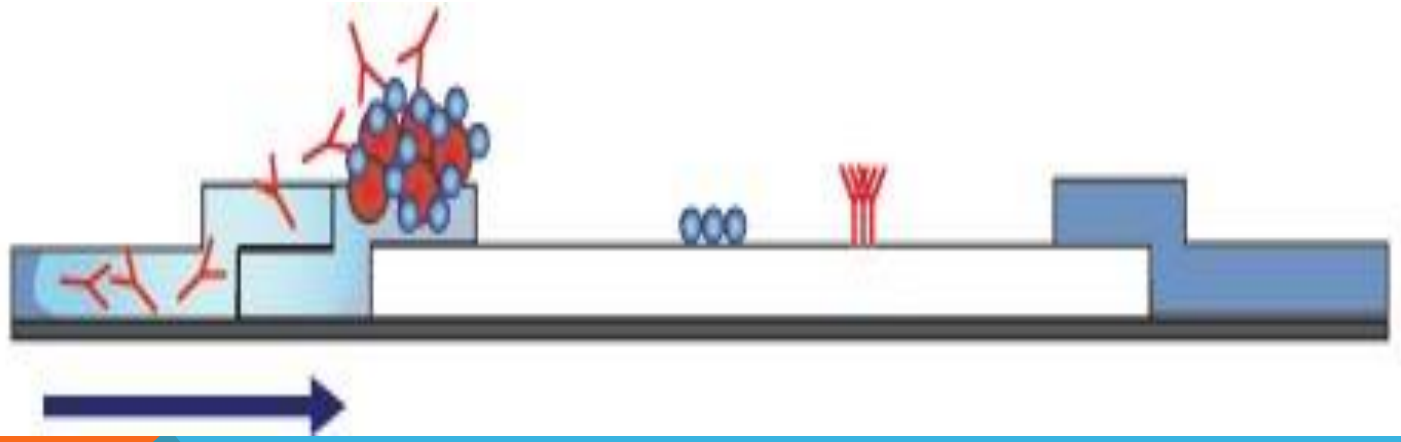
Step 1: Sample placement

To perform the test, a sample is placed on the sample pad at one end of the strip. The sample may be used alone as is commonly done with urine or serum or whole blood or plasma compatible tests, or it may be mixed with a buffer specific to the test.



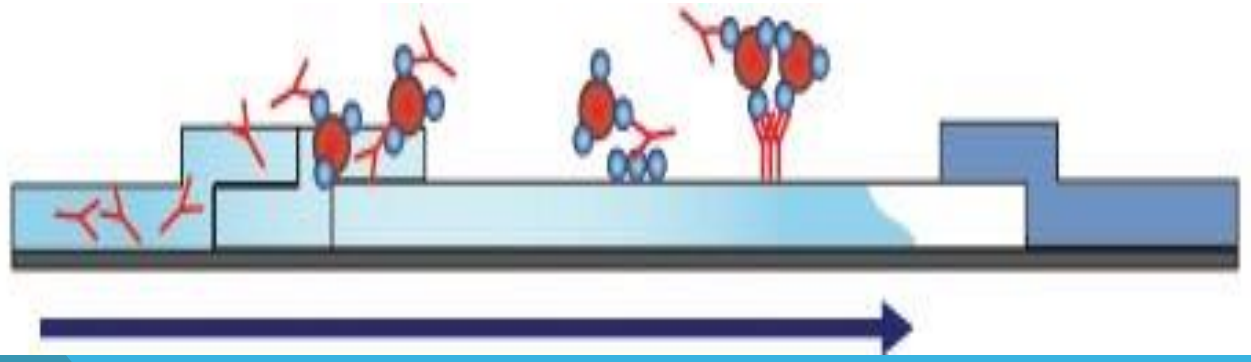
Step 2: Molecules solubilized

With the addition of the sample, the detector molecules are solubilized. When solubilized the detector molecules mix with and bind to the analyte in the sample (if analyte is present).



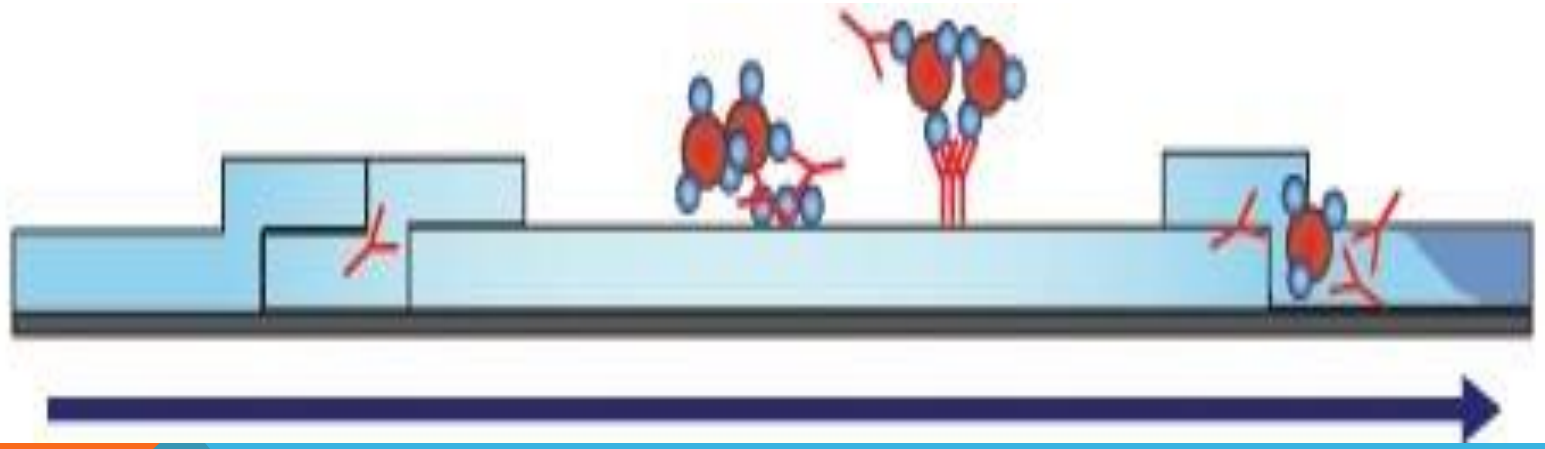
STEP 3: CAPILLARY ACTION

Then capillary action draws the fluid mixture up the sample pad and into the membrane. The sample/detector molecule mix continues to move up the membrane until it reaches the analyte capture molecule. In these lines a second (and third) antibody or antigen, immobilized as a thin stripe in the nitrocellulose will then capture the complex if it is positive for the target analyte.
The control line should always show as a visible line, otherwise the test is invalid and must be repeated. If the test is positive, a colored (typically pink or purple) line develops along with the control line.



STEP 4: EXCESS ABSORBED

Excess buffer along with any reagents not captured at the test of control line will then move into the absorbent wicking pad



The technology is based on a series of capillary beds, such as pieces of

-porous paper,

-microstructured polymer,

-sintered polymer.

- **Each of these elements has the capacity to transport fluid (e.g., urine) spontaneously.**

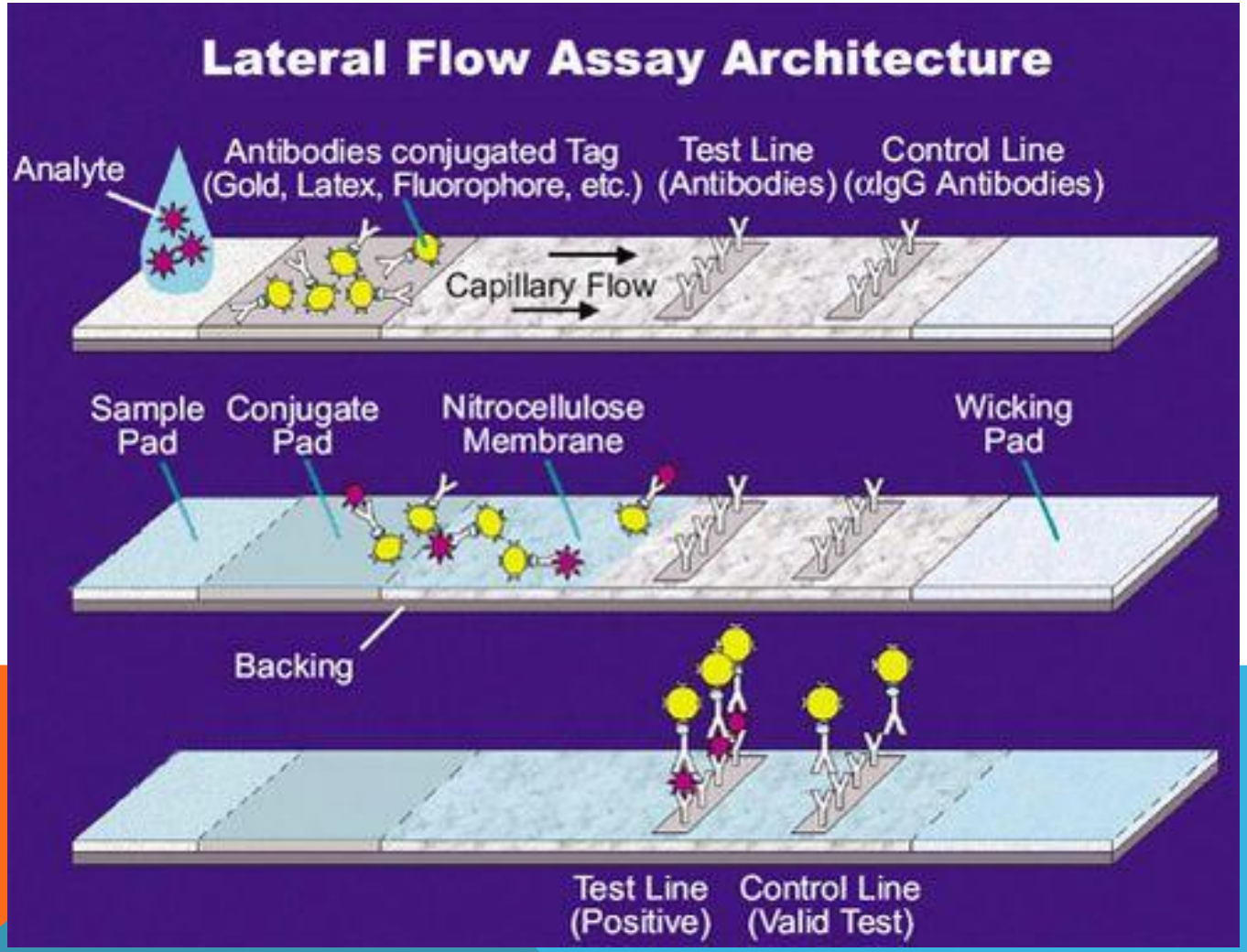
The first element (the sample pad) acts as a sponge and holds an excess of sample fluid. Once soaked, the fluid migrates to the second element (conjugate pad) in which the manufacturer has stored the so-called conjugate, a dried format of bio-active particles (see below) in a salt-sugar matrix that contains everything to guarantee an optimized chemical reaction between the target molecule (e.g., an antigen) and its chemical partner (e.g., antibody) that has been immobilized on the particle's surface.

While the sample fluid dissolves the salt-sugar matrix, it also dissolves the particles, and in one combined transport action, the sample and conjugate mix while flowing through the porous structure.

In this way, the analyte binds to the particles while migrating further through the third capillary bed. This material has one or more areas (often called stripes) where a third molecule has been immobilized by the manufacturer. By the time the sample-conjugate mix reaches these strips, analyte has been bound on the particle and the third "capture" molecule binds the complex. After a while, when more and more fluid has passed the stripes, particles accumulate and the stripe-area changes color.

Typically, there are at least two stripes: one (the control) that captures any particle and thereby shows that reaction conditions and technology worked fine and one that contains a specific capture molecule and only captures those particles onto which an analyte molecule has been immobilized.

After passing these reaction zones, the fluid enters the final porous material, the wick, that simply acts as a waste container.


Lateral Flow Assay Architecture
Analyte
Antibodies conjugated Tag
(Gold, Latex, Fluorophore, etc.)
Test Line
(Antibodies)
Control Line
(αIgG Antibodies)
Capillary Flow
Sample
Pad
Conjugate
Pad
Nitrocellulose
Membrane
Wicking
Pad
Backing
Test Line
(Positive)
Control Line
(Valid Test)

Coloured particles

In principle, any coloured particle can be used, however latex (blue colour) or nanometer sized particles of gold (red colour) are most commonly used. The gold particles are red in colour due to localised surface plasmon resonance. Fluorescent or magnetic labeled particles can also be used, however these require the use of an electronic reader to assess the test result.

Sandwich assays

As the sample migrates along the assay it first encounters a conjugate, usually colloidal gold, which is labelled with antibodies specific to the target analyte. If the target analyte is detected within the sample the conjugate antibodies will bind and subsequently reach the test line which also contains antibodies specific to the target. Once the sample reaches the test line and the target analyte is present a visual change, normally a line appearing, will occur allowing the test to be read as a positive. The majority of sandwich assays also have a control line which will appear regardless of whether or not the target analyte is present.

The rapid, low-cost sandwich-based assay is commonly used for home pregnancy tests which detects for human chorionic gonadotropin, hCG, in the urine of women.

Competitive assays

The sample first encounters coloured particles which are labelled with the target analyte or an analogue. The test line contains antibodies to the target/its analogue. Unlabeled analyte in the sample will block the binding sites on the antibodies preventing uptake of the coloured particles.

The test line will show as a coloured band in negative samples.

QUANTITATIVE TESTS

Most tests are intended to operate on a purely qualitative basis. However it is possible to measure the intensity of the test line to determine the quantity of analyte in the sample.

Handheld diagnostic devices known as lateral flow readers are used by several companies to provide a fully quantitative assay result. By utilizing unique wavelengths of light for illumination in conjunction with either CMOS or CCD detection technology, a signal rich image can be produced of the actual test lines. Using image processing algorithms specifically designed for a particular test type and medium, line intensities can then be correlated with analyte concentrations. One such handheld lateral flow device platform is made by Detekt Biomedical L.L.C. Alternative non-optical techniques are also able to report quantitative assays results. One such example is a magnetic immunoassay (MIA) in the lateral flow test form also allows for getting a quantified result.

Control line

While not strictly necessary, most tests will incorporate a second line which contains an antibody that picks up free latex/gold in order to confirm the test has operated correctly.

Speed and simplicity

Time to obtain the test result is a key driver for these products. Tests can take as little as a few minutes to develop. Generally there is a trade off between time and sensitivity – so more sensitive tests may take longer to develop. The other key advantage of this format of test compared to other immunoassays is the simplicity of the test – typically requiring little or no sample or reagent preparation.

Patents

This is a highly competitive area and a number of people claim patents in the field, most notably Alere (formerly Inverness Medical Innovations, now owned by Abbott) who own patents[16] originally filed by Unipath. A group of competitors are challenging the validity of the patents.[17] A number of other companies also hold patents in this aren

Basic Components of Lateral flow test:

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3. Detection conjugate.
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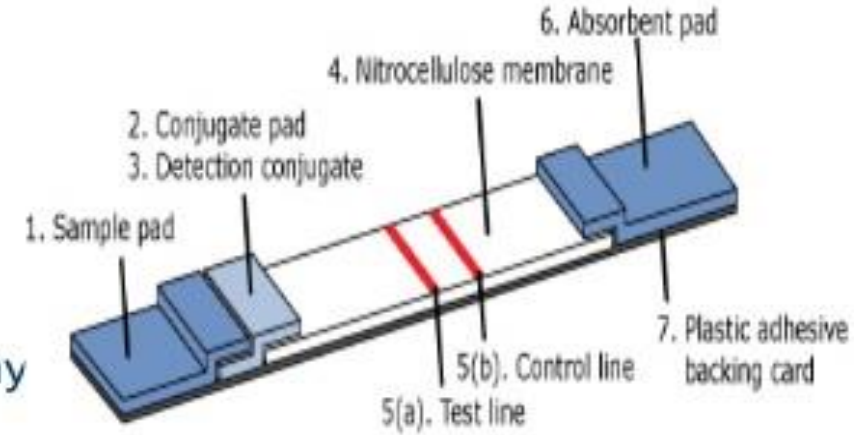


Typical lateral flow test strip configuration (Card, Cassette, Dipstick, Strip)

THE BENEFITS OF IMMUNOCHROMATOGRAPHIC TESTS INCLUDES

1. Can detect antigen or antibody.
2. Commercially available.
3. Easy to perform.
4. Limited/no instrumentation.
5. Single use, rapid test.
6. User friendly format.
7. Very short time to get test result.
8. Long-term stability over a wide range of climates.
9. Relatively inexpensive to make.

Limitations:

1-Results are qualitative.

2-Rapid tests can be less sensitive or less accurate compared to existing tests.

Includes

Strip

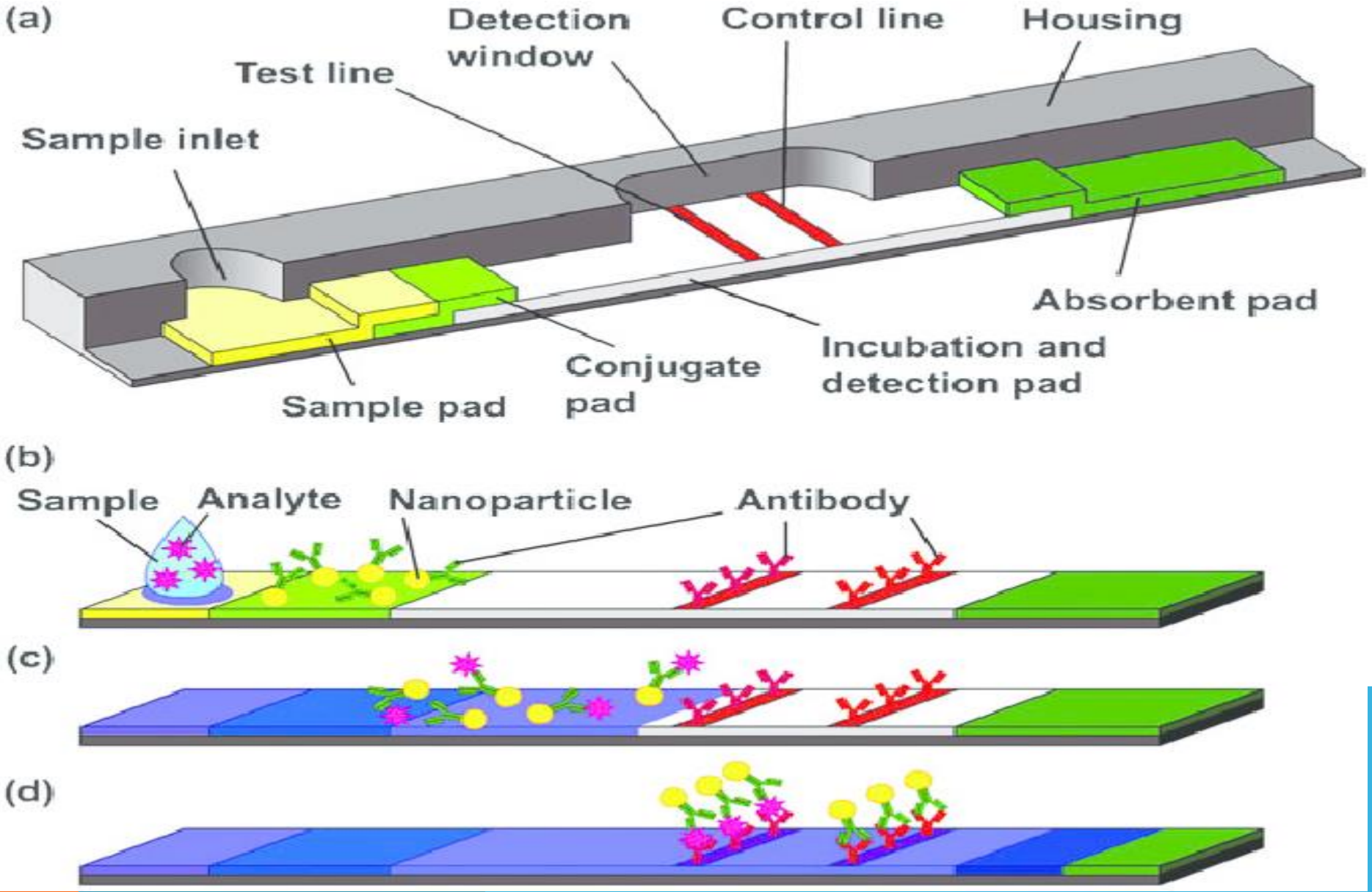
or Cassette

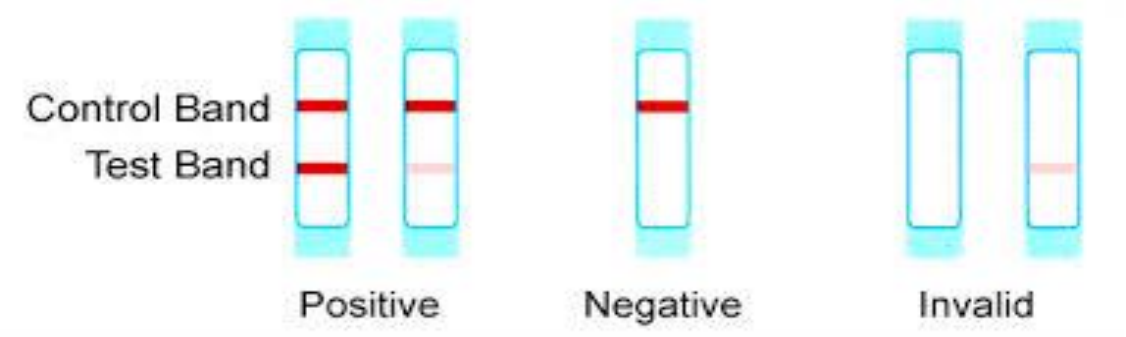
or Multi-devices

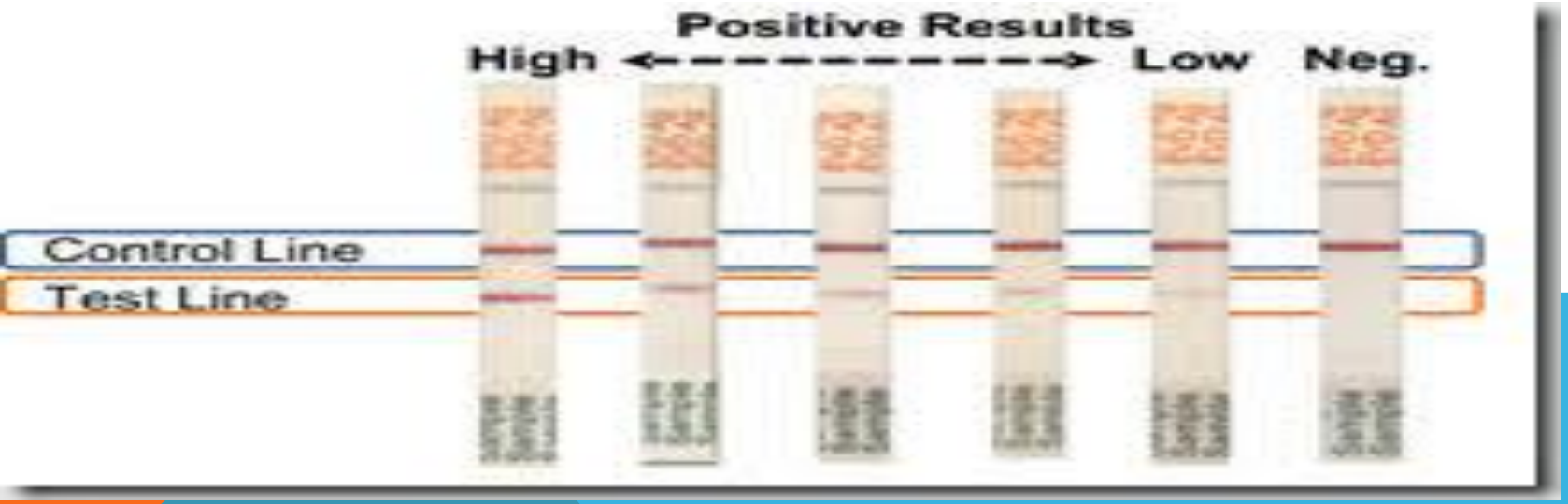
or Midstream (hCG & LH only)

APPLICATIONS OF IMMUNOCHROMATOGRAPHIC ASSAYS:

It can be applied for multiple test platforms for liver, sexually transmitted diseases, cardiac markers, as well as women's and men's health (hCG, VDRL, CMV, PSA , H. Pylori, Troponin I, TORCH, HIV, HBsAg, HBV, HCV, FOB, RA, CRP, ASO, SLE, IM, Salmonella IgM & IgG , Toxoplasma IgG & IgM, Rubella IgG & IgM …).


(a)
Detection window
Control line
Housing
Test line
Sample inlet
Absorbent pad
Incubation and detection pad
Conjugate pad
Sample pad
(b)
Sample
Analyte
Nanoparticle
Antibody
(c)
(d)


Control Band
Test Band
Positive
Negative
Invalid


Positive Results
High
Low
Neg.
Control Line
Test Line