IN VITRO DIAGNOSIS OF RAPID TEST

A Internship Report submitted

for the partial fulfilment of the Degree of Master of Science

By

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[M.Sc. Biotechnology]

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2022-23

(On letterhead of the Department)

CE R T IFICATE

This is to certify that this training report entitled IN VITRO DIAGNOSIS was successfully carried out by Miss Jeni Rachhadiya towards the partial fulfilment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of her own work, carried out by her under the guidance of Miss Anjali Ranghani for a period of 3 months during the academic year of 2022-23. The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

Signature

Dr. Nutanprakash Vishwakarma

Signature

Miss Anjali Ranghani

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Abstract

In vitro diagnosis refer to medical tests that are performed outside the human body, typically in a laboratory setting, to detect or diagnose diseases and conditions. These tests use samples such as blood, urine, saliva and tissue specimens to analyze the presence of specific molecules, cells or organisms that indicate a particular disease or condition. In vitro diagnosis tests are essential in healthcare because they help healthcare professionals diagnose diseases and conditions accurately and quickly. Early detection and diagnosis can improve patient outcomes and reduce healthcare costs by enabling earlier intervention and treatment. There are various types of IVD tests including immunoassays. These tests detect specific proteins or antibodies in a patient's blood, urine, or other bodily fluids. They are commonly used to diagnose infectious diseases, such as HIV, hepatitis, and COVID. All of them are worked on the principle of enzyme-linked immunosorbent assay.

Enzyme-linked immunosorbent assay (ELISA) is a highly sensitive and specific laboratory technique used to detect and quantify the presence of an antigen (such as a protein, peptide, or antibody) in a sample. ELISA is widely used in clinical and research settings for diagnostic testing, disease monitoring and drug development. There are several types of ELISA, but the basic principles are the same. The demand for quantitative lateral flow immunoassays is growing steadily, however the development of these presents a challenging goal since quantitative assays require considerably more from the finished product in terms of reproducibility, stability, sensitivity and dynamic range than is needed from a qualitative assay.

When a lateral flow immunoassay is run, the test sample is added to a sample application pad at the end of the strip. The sample then migrates to the conjugate release pad, where a detection particle (typically gold or latex) that has been conjugated to a biological component of the assay is held. Next the sample and the detection reagent migrate to the reaction membrane; a second biological component of the assay will have been immobilized here to function as a capture reagent. The capture reagent usually exists as a test line which spans the width of the membrane; a control reagent will be immobilized in a second line further along the membrane. The analyte is either captured at the test line, or continues to migrate until reaching the absorbent wicking pad at the other end of the strip. The detection reagent binds at the control line to indicate that the assay has run successfully.

Company information

Name of company: SR Bioera

SR Bioera has been a worldwide leader partnering with rapid test manufactures since 2016. SR Bioera has been focusing on providing affordable innovation to worldwide rapid test manufacturer. SR Bioera is synonymous to competence and innovation in raw material and consumable manufacturing catering customer worldwide with finest quality product and simultaneously elevating the norms of paramount business values. In the area of Lateral flow immunoassay test strips manufacturing.

Industries	Biotechnology Research
Company size	501-1,000 employees
Type	Privately Held
Founded	2006
Specialties	Belly Spot Pregnancy Test (HCG), BioHBsAG, BioHCV, BioHRP2, BioPf/PAN, BioPf/Pv, Biosyphillis, BioHivduo, BioHIv+Syphillis, diagnostic, rapid test, point of care, lateral flow assay, biotechnology, gold nanoparticule, nitrocellulose membrane, and uncut sheet.

❖ Production material:

1. **IVD Raw materials:** Gold nanoparticles, gold chloride salt, antigen and antibodies.
2. **Consumables:** sample pad, conjugate pad, absorbent pad, PVC backing card, nitrocellulose membrane.
3. **Plastic ware:** dropper, test cassette, specimen collection apparatus.
4. **Rapid test kit:** infectious disease, drug of abuse, respiratory diseases, animal tests etc.

❖ Departments:

1. HR
2. Quality control
3. Quality assurance
4. Production
5. R & D
6. Engineering

❖ Machine/Equipment:

1. Strip cutter machine
2. Sheet cutter machine
3. Cassette pressing machine
4. Reagent dispenser
5. Sealer machine

❖ Reagent:

1. Gold nanoparticles
2. Gold conjugates
3. Conjugation kit: Gold chloride salt, PH solution, Biomarker dilution buffer, Blocking buffer, Gold dilution buffer
4. Essential solution for lateral flow immune assay: Band enhancer
   Blocking solution
   Flow enhancer

Report

1. Plastic backing card:

Backing cards for lateral flow rapid diagnostic test kit is usually made of PVC materials with self-adhesive, with main features of good flatness, strong adhesive and ageing resistance. Compared to the common adhesive used on the market, our adhesive is a stronger type, though only small amount of adhesive coated, 20g/square meters, whereas other backing card producers have to apply 38g/squre meters on it. As a result, it will significantly lessen the cleaning time and frequency of the strip cutter and reduce the defects, then further benefit a smooth continuous large scale production, especially those engaged a lot of automatic instruments. Adhesive is non-reactive, non-volatile with a stable shelf-life of 2.5 years.

2. Sample pad or glass fibre:

This is an absorbent pad on to which the sample is applied, and is typically composed of a woven mesh or cellulose fiber. Woven meshes have good tensile strength and a low bed volume, meaning that they retain very little sample volume; they can though be rather expensive. Cellulose fiber pads have low tensile strength and a much greater bed volume; they are also relatively cheap. Irrespective of which material is chosen, the sample application pad should exhibit consistent absorbency, thickness and density so that uniform wicking rates ensure assay reproducibility. The sample application pad should also demonstrate low protein binding to avoid loss of analyte. The main function of the sample application pad is to promote even and controlled distribution of the sample, however the sample application pad can be modified to enable conditioning of the sample. By pretreating the sample application pad with components such as proteins, detergents or salts it is possible to reduce non-specific binding, increase the sample viscosity, or alter the pH. Sample conditioning is an important consideration due to the very different nature of the samples which may be tested in a lateral flow immunoassay; these can be as diverse as water, urine, serum, plasma, blood, saliva, cerebrospinal fluid, milk, amplified nucleic acids, and solubilized solid materials such as feces, food, plants and soil. Due to the low

bed volume it is impractical to pre-treat woven meshes; cellulose fibers are much more amenable to these modifications.

3. Conjugate pad:

The conjugate release pad is typically composed of non-woven glass fiber, into which the detection reagent has been dried. Once the sample application pad has been saturated, the sample flows in to the conjugate release pad where it releases the detection reagent; the detection reagent then leaves the conjugate release pad and moves with the sample in to the membrane. The role of the conjugate release pad is to ensure uniform transfer of the sample and the detection reagent, and to preserve the conjugate upon drying and re-wetting. It plays a pivotal role in controlling the performance of the lateral flow immunoassay. The conjugate release pad should exhibit low nonspecific binding so that the detection reagent does not remain trapped in the pad. A pre-treatment may be necessary to increase wettability, decrease non-specific interactions and to control the pH. It is important that the conjugate release pad has a consistent bed volume to ensure that the amount of detection reagent in each test strip remains constant.

4. Absorbent pad:

These are specially treated absorbent pads made with compressed cellulose to absorb the reaction mixture of the rapid test assay and hold the reaction mixture for a longer duration of time.

5. Cover tapes:

This cover tape is manufactured by using non-volatile, non-reactive, and stable glue which will not interfere with your assay result and provide stability to your flow of reaction mixture.

6. Nitrocellulose membrane:

The membrane is considered to be the most important element in a lateral flow immunoassay. Nitrocellulose is the most commonly-used material, although cellulose acetate, polyvinylidene fluoride (PVDF), charge modified nylon and polyethersulfone (PES) may also be employed. Nitrocellulose membranes exhibiting a range of pore sizes (0.05 to 12μm) are commercially available, but since the pores do not have an even distribution it is more appropriate to consider capillary flow time when selecting a membrane for the lateral flow immunoassay. Capillary flow time is typically expressed as seconds/cm and is defined as the time taken for the sample liquid to travel to and completely fill the strip of membrane. The capillary flow time can affect the sensitivity and specificity of the lateral flow immunoassay, as well as the consistency of the test line. Capture antibodies are immobilized across the membrane, typically in two lines. The test line is used to bind the sample protein, while the control line consists of species-specific antibodies for the detection antibodies (e.g. anti-mouse antibodies) and is used to demonstrate that the lateral flow immunoassay is performing as it should be. Instrumentation is required for precise application of the capture antibodies;

7. Gold nanoparticle:

Commercializes highly spherical and stable citrate-stabilized gold colloids specifically adapted to lateral flow test format. Their unique sphericity combined with their size homogeneity and their highly contrasting ruby red color make Gold nanoparticle ideal for the development of rapid tests showing high sensitivity and precision.

Principle

Lateral flow immunosorbent assay

Lateral flow immunoassays, also known as immunochromatographic assays or strip tests, are immunoassays which have been designed to operate along a single axis. Although there are a number of different variations of the technology, they all operate using the same basic concept. When a lateral flow immunoassay is run, the test sample is added to a sample application pad at the end of the strip. The sample then migrates to the conjugate release pad, where a detection particle (typically gold or latex) that has been conjugated to a biological component of the assay is held. Next the sample and the detection reagent migrate to the reaction membrane; a second biological component of the assay will have been immobilized here to function as a capture reagent. The capture reagent usually exists as a test line which spans the width of the membrane; a control reagent will be immobilized in a second line further along the membrane. The analyte is either captured at the test line, or continues to migrate until reaching the absorbent wicking pad at the other end of the strip. The detection reagent binds at the control line to indicate that the assay has run successfully.

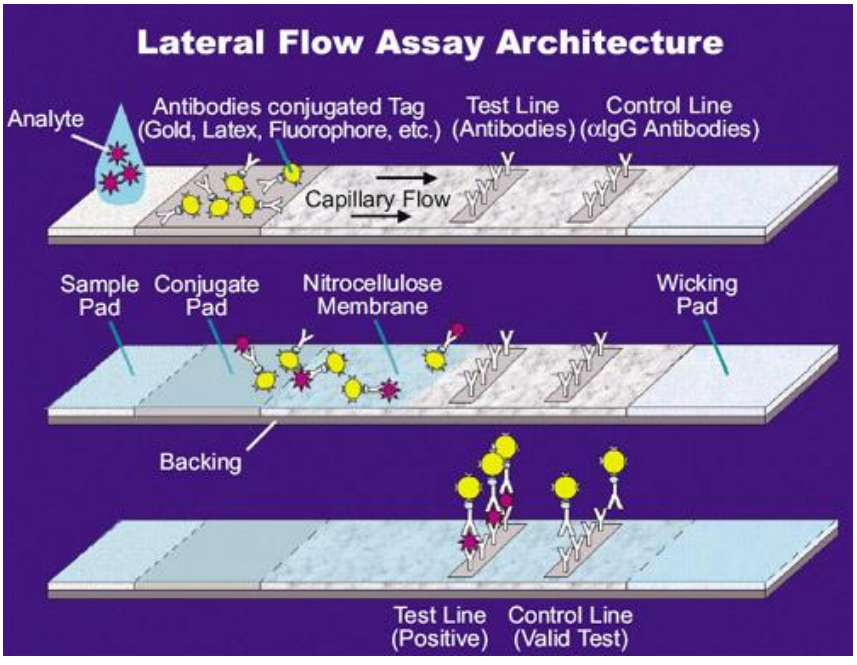

Fig. 1 Lateral flow immunosorbent assay

Rapid test kit

1] COVID - 19 Ag Test:

- Corona virus disease is an infectious disease caused by the SARS-CoV-2 virus. Most people infected with the virus will experience mild to moderate respiratory illness and recover without requiring special treatment. However, some will become seriously ill and require medical attention. Older people and those with underlying medical conditions like cardiovascular disease, diabetes, chronic respiratory disease, or cancer are more likely to develop serious illness. Anyone can get sick with COVID-19 and become seriously ill or die at any age.
- Detect variants: progressive studying on mutant detection
- 3 in 1 nasal or nasopharyngeal or oropharyngeal swab available
- Fast result within 10 – 20 minutes
- Reliable with high sensitivity and specificity
- Easy and convenient to use.

2] Belly spot - pregnancy test:

- HCG is glycoprotein hormone secreted by the placenta shortly after implantation. In normal pregnancy, HCG can be detected in both urine and serum as early as 7 to 10 days after fertilization. The appearance of HCG and its subsequent rapid rise in urine and serum after conception during early gestational growth makes it an excellent marker.
- Detection time: 10 mIU/ml
- Specimen: Urine
- Shelf life: 24 months

3] Dengue test:

- Dengue is caused by Aedes mosquitoes, particularly A. Albopictus. Dengue is found well in tropical and subtropical area. The difference between Dengue and Malaria is that Dengue is as prevalent in the urban districts of its range as in rural area. According to WHO, around

2.5 billion people are at risk from dengue. Dengue menifests as fever with headache, muscle and joint pains and rash. There are four serotypes of Dengue and there is no cross - protection. So it is really important to treat it within proper time since it can be the life – threatening disease.

A] **Dengue NS1 Antigen Test:** It is an immunochromatographic test for qualitative detection of dengue virus NS1 antigen in human serum, plasma or whole blood.

Features: Early detection right after the inset of symptoms.

-Highly sensitive and easy to use.

-Detection of infection prior to seroconversion.

B] **Dengue IgG / IgM Antibody Test:** Dengue IgG/IgM Antibody Test is an immunochromatographic for qualitative detection of dengue virus serotype DEN-1,2,3 and 4.

Features: To show high sensitivity and specificity.

-To detect dengue lgG/IgM at early stage.

-To Require no other reagent.

C] Dengue COMBO Test: Dengue COMBO test is one step assay designed to detect both dengue virus NS1 antigen and different IgG/IgM antibodies to dengue virus in human serum, plasma or whole blood. It contains two devices (left side: Dengue NSI Ag test, right side: Dengue IgG/IgM test).

General information: Qualitative detection of NSI Antigen and IgG/IgM antibody to dengue.

-Specimen: Serum, Plasma, Whole Blood

- Differentiation between primary and secondary dengue

-Test Result: 15 minutes

4] Malaria Test:

- In 2020, there were an estimated 241 million cases of malaria worldwide. Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes.

A] Malaria P.f/Pan Antigen Test: Pf/Pan Antigen Test is a one step in-vitro diagnostic test based on immunochromatographic assay. It is designed for detection of P. falciparum(HRP-II) and PLDH(Pfalciparum, P.vivax, Povale, P.malariae) in human blood.

Features: Detect HRP-II Ag to P. falciparum and PLDM to Plasmodium species.

-Distinguish the infection between P. falcipuram and other species.

B] **Malaria P.f/P.v Antigen Test:** Malaria Pf/Pv Antigen Test is a one step in vitro diagnostic test based on immunochromatographic assay. It is designed for detection of P. falciparum(HRP) and P.vivax(PLDH) in human blood.

Features: Detect HRP-II AG to P.falciparum and PLDH to Plasmodium species.

-Distinguish the infection between P. falcipuram and other P.vivax.

C] **Malaria P.f Antigen Test:** Malaria PT Antigen Test is a one step in vitro diagnostic test based on immunochromatographic assay. It is designed for detection of P. falciparum(HRP-II) in human blood.

Features: Detect HRP-II Ag to P. falciparum and PLDH to Plasmodium species.

-Distinguish the infection between P. falcipuram and other species.

5] H. Pylori Test:

- Helicobacter pylori is a helical shaped gram-negative bacterium that infects various area of the stomach and duodenum. Many cases of peptic ulcers, gastritis, duodenitis, and perhaps some cancers are caused by H.pylori infection.
- However, many who are infected do not show any symptoms of disease. Helicobacter spp. are the only known microorganisms that can thrive in the highly acidic environment of the stomach. Its helical shape (from which the genus name is derived) is thought to have to penetrate and favor its mortility in the mucus gel layer.
- Helicobacter pylori (H. pylori) infection occurs when H. pylori bacteria infect your stomach. This usually happens during childhood.
- **General Information:** Qualitative detection of antibodies to H. Pylori.Whole blood or serum / plasma can be used as specimen.Resul in 10 min or less.

6] Hepatitis C Virus Test:

- HCV is a positive, single-stranded RNA virus in the Flaviviridae family. Approximately 170 million people worldwide are infected with HV. The virus is transmitted primarily by blood and blood products. It is generally believed that the majority of HCV infections give rise to an acute illness up to 80% which may develop into chronic hepatitis.

7] HIV Test:

- Human immunodeficiency virus (HIV) is a retrovirus that can lead to acquired immunodeficiency syndrome(AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Infection with HIV occurs by the transfer of blood, semen vaginal fluid, pre-ejaculate or breast milk AIDS has killed more than 25 million people since it was first recognized on December 1, 1981, making it one of the most destructive pandemics in recorded history.

8] Syphilis Test:

- Syphilis is curable sexually transmitted disease caused by the Treponema pallium spirochete. The route of syphilis is almost always by sexual contact. However, there are examples of congenital syphilis via transmission from mother to fetus.
- **General information:** One Step qualitative immunochromatographic assay.

  -Specimen: Serum, Plasma.

  -The optimal choice for mass screening program.

  -Room temperature storage.

9] HBsAg Test:

- Hepatitis B Virus Test.
- Hepatitis B is a widespread serious liver disease. Hundreds of millions of people, mostly from regions with poor medical care, are chronically infected with the virus and face an elevated risk of acquiring liver cancer. The hepatitis B virus (HBV) is made up of an inner core surrounded by an outer capsule. HBsAg is also found within the core.

Product name	Product description
Covid-19 Ag Test	Recombinant receptor binding domain (RBD) of SARS-COV-2 splike protein with the mouse Fc region.
Belly spot – pregnancy Test	Anti alpha HCG Antibody
Dengue Test	Recombinant dengue virus (DENV – serotype) Antigen
H. pylori Test	Mouse anti – Helicobacter pyroli monoclonal Antibody
Hepatitis C virus Test	Recombinant hepatitis C virus Antigen
HIV Test	Recombinant human immunodeficiency virus (HIV – gp41) Antigen
Syphilis Test	Recombinant treponema pallidum (TpN62) Antigen
HBs Ag Test	Mouse anti HBs Ag monoclonal Antibody
Malaria Test	Recombinant malaria p. Falciparum HRP 2 Antigen

Result

1] COVID - 19 Ag Test:

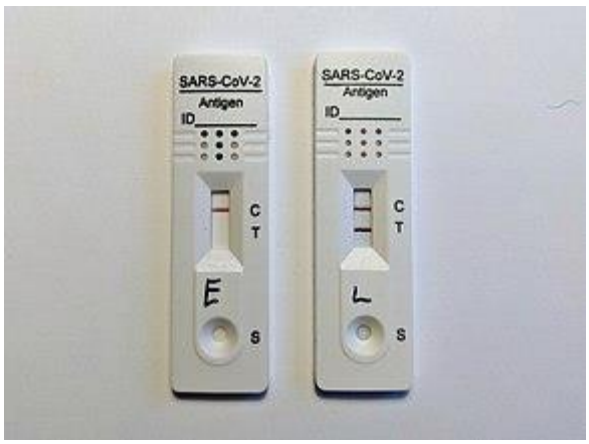

Fig. 2– COVID test kit result

Status	Test line	Control line	Test result
E	+	+	Positive
L	-	+	Negative

- **Interpretation:**

- **Positive Test:** Pink bands appear in the Control (top line) and IgG (bottom line). The test is positive for IgG antibodies to COVID19 virus.

- **Negative Test:** Pink bands appear in the Control (top line) only. The negative test does not exclude COVID-19 infection.

- **Invalid Test:** No pink band appear in the Control (top line). The test is invalid and should be repeated.

2] Belly spot – pregnancy Test:

FIG. 3 – HCG Test kit result

Status	Test line	Control line	Test result
1	-	+	Negative
2	+	+	Positive

- **Interpretation:**

- **Negative Test:** A coloured line appears in control line. No coloured line appears in test line.

- **Positive Test:** Two coloured lines appear, one in control area C and one in test area T. The intensity of the test line T may vary depending on the concentration of the antigen in the sample. Any sign of a line should be considered a positive result.

- **Invalid Test:** In the control area C, a red line must always appear when the test is performed. If this line does not appear, the test is invalid in any case. Please repeat the test with a new test cassette.

3] Dengue test:

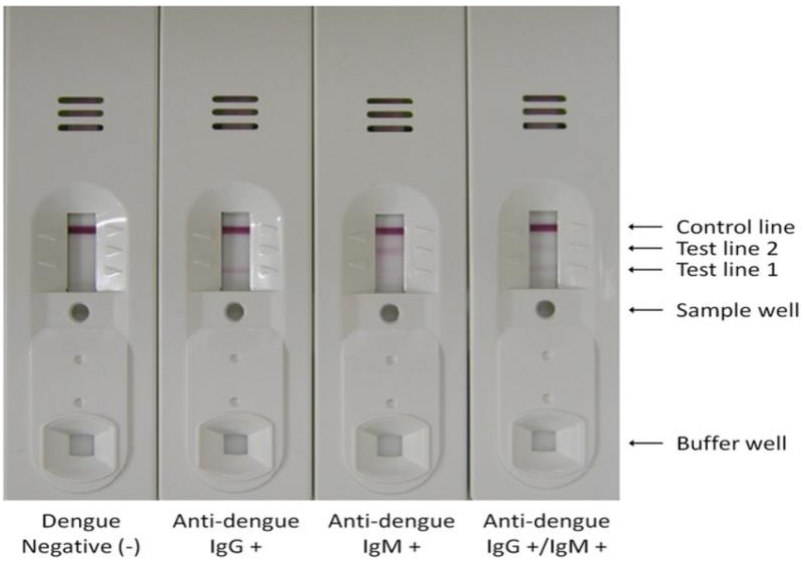

FIG.4–Dengue Test kit result

Status	Test Line		Control line	Test result
	IgM	IgG		
1	-	-	+	Negative
2	-	+	+	Anti dengue IgG+
3	+	-	+	Anti dengue IgM+
4	+	-	+	Anti dengue IgG+/IgM+

- **Interpretation:**

- **IgM Positive:** The colored line in the control line region (C) changes from red to blue, and a colored line appears in test line region 1(IgM). The result is positive for Dengue virus specific-IgM and is probably indicative of primary Dengue infection.

- **IgG Positive:** The colored line in the control line region (C) changes from red to blue, and a colored line appears in test line region 2 (IgG) The result is positive for Dengue virus specific-IgG antibodies and is indicative of secondary Dengue infection.

- **IgM and IgG Positive:** The colored line in the control line region (C) changes from red to blue, and two colored lines should appear in test line regions 1 and 2 (IgM and IgG). The color intensities of the lines do not have to match. The result is positive for IgM & IgG antibodies and is indicative of secondary Dengue infection.

- **NEGATIVE:** The colored line in the control line region (C) changes from red to blue. No line appears in test line regions 1 or 2 (IgM or IgG).

- **INVALID:** Control line (C) is still completely or partially red, and fails to completely change from red to blue. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

4] Malaria test:

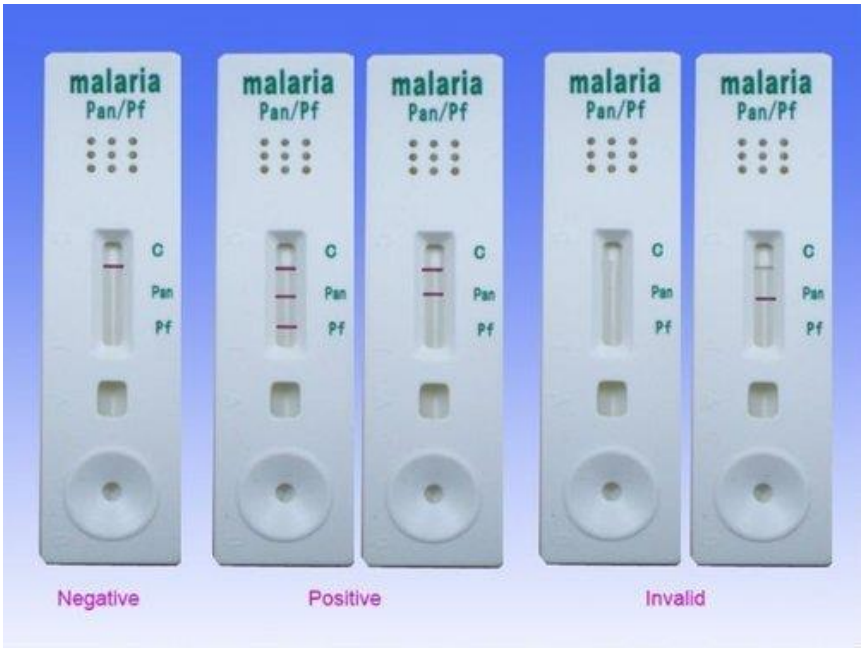

FIG.5 – Malaria Test kit result

Status	Test Line		Control line	Test result
	Pan	p.f.		
1	-	-	+	Negative
2	+	+	+	Positive
3	+	-	+	Positive
4	-	-	-	Invalid
5	+	-	-	Invalid

- **Interpretation:**

- **Positive:** One line appears in the control line region, and one line appears in the test line region.

- **Negative:** Only one colored line appears in the control region.

- **Invalid:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

5] H. Pylori Test:

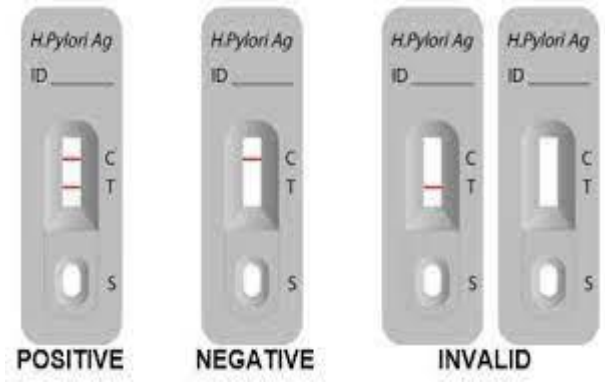

FIG.6 – H. Pylori Test kit result

Status	Test line	Control line	Test result
1	+	+	Positive
2	-	+	Negative
3	+	-	Invalid
4	-	-	Invalid

- **Interpretation:**

- **Positive:** Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

- **Negative:** One colored line appears in the control line region(C). No line appears in the test line region (T).

- **Invalid:** Control line fails to appear.

References

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