SOP for Samplie Collection, Screening and Biobank of Schistosoma or uninfected specimens

1- Consumables:

1. Sterile vacuum EDTA tubes (8ml) for blood collection.
2. Medical disposable butterfly needle for blood collection.
3. Disposable gloves, latex-free, unsterile for sample handling.
4. Disposable sterile pipette tips with filter: 10µl, 20µl, 100µl, 200µl and 1000µl.
5. Lab white coat (long sleeve coat is recommended).
6. 70% isopropyl alcohol (2-propanol).
7. Ethyl alcohol (ethanol).
8. Medical masks.
9. Safety glasses.

2- Equipments and Reagents:

1. Cryotubes of 1.8ml for whole blood and serum samples storage.
2. Circulating Schistosoma Antigen (CSA) Detection kit .
3. Dynamic Flow Schistosoma IgG Antibody Card Test
4. Whatman 903 ProteinSaver Cards for serum spots.
5. Whatman FTA Cards for blood spots.
6. Racks and boxes for 1.8ml cryotubes.
7. Pipettes of 100µl, 200µl and 1000µl.
8. Refrigerator (4ºC) and Freezer (-80°C).
9. Appropriate table-centrifuge.

3- Precautions:

1. The research personnel should wear gloves during sample collection.
2. The research personnel should use appropriate barrier precautions, such as lab coat, glove, mask, and safety glass to prevent exposure to skin and mucus membranes when working with known infectious research subjects.
3. All sample containers, equipments and reagents needed should be assembled prior to the procedure.
4. Samples should not undergo freeze-thaw cycles.

5. Freezers need to have a backup generator or other emergency system options.

4- Procedure

4.1 *S. japonicum (Sj) or S. hematobium (Sh) or S.mansoni (Sm)* infected and uninfected

Individual samples from endemic areas

The research personnel should greet the research subject, identify themselves, and then indicate the sample collection procedure to the research subject. The research subject should be correctly identified (through personal and clinical data sheet) prior to sample collection.

1. *Label sterile vacuum blood collection EDTA tubes.
2. Sterilize with isopropyl alcohol 70% the site of skin puncture (arm venipuncture).
3. Collect blood into sterile vacuum blood collection EDTA tubes, mix gently and keep specimens on ice as follow:
   - 5ml of blood from subjects over 7 years old (subjects A)
   - 3ml of blood from subjects less than 7 years old (subjects B)
4. Proceed to schistosomiasis diagnostic tests by parasitological examination and immunodiagnosis assay. The purpose of this step is to confirm whether specimens are Schistosoma infected.

   4.1 Parasitological Examination (PE): urine for Sh or stool for Sm and Sj(Appx 1)

   4.2 Immunodiagnosis Assay (IA): circulating Schistosoma Antigen (CSA) Detection kit for Sh or Sm infection (Appx 2); Dynamic Flow Schistosoma IgG Antibody Card Test for Sj infection (Appx 3).

   4.3 For Sh or Sm projects, parasitological examination will be used for the first screening; the sera of the parasitological examination negative individuals are detected by circulating Schistosoma Antigen (CSA) Detection kit.
   - Sh infected samples: PE positive or IA positive;
   - Sh uninfected samples: PE negative and IA negative;
   - Sm infected samples: PE positive or IA positive;
   - Sm uninfected samples: PE negative and IA negative;

   4.4 For Sj project, immunodiagnosis detection will be used for the first screening, the sera positive individuals continue to carry on the parasitological examination (stool) to

evaluate both egg detection and egg load quantification.

- Sj infected samples: PE positive and IA positive;
- Sj uninfected samples: PE negative and IA negative;

5. Keep Sj or Sh or Sm positive blood specimens at 4ºC (for no longer than 24 hours). We recommend carrying the following steps in the lab.

6. For each blood sample, *label 2 sterile cryotubes with the appropriate sample identification. Take an amount of each sample into the 2 cryotubes as indicated below, and keep on ice before final storage at -80ºC (biobank in Africa):

- 2ml of blood sample of subject A (aliquot 1ml in each cryotube)
- 1ml of blood sample of subject B (aliquot 0.5ml in each cryotube)

7. *Label new sterile cryotubes with the appropriate sample identification. Take from the EDTA tubes 1ml (subjects A) or 0.5ml (subjects B) of blood samples into the cryotubes. Keep on ice before storage at -80ºC (for biobank in China).

8. For dried blood samples, *label Whatman FTA cards with the appropriate sample identification.

9. Drop 50ul of each blood sample (after step 7) onto the card in concentric circular motions within the printed 4 circle areas. Avoid 'puddling' of the liquid sample as it will overload the chemicals on the card.

10. Allow the samples to dry for 1h at room temperature prior to punching.

11. Put dried blood samples in transparent PE zip-lock plastic bags and store at -30ºC or 4ºC.

12. For each specimen, pipette the remaining whole blood from the EDTA tubes into new 1.8ml cryotubes and *label carefully.

13. Centrifuge all the specimens for 5 minutes at 3,000rpm.

Note: If the blood is not centrifuged immediately, the tubes should be refrigerated at 4ºC for no longer than 48 hours.

14. After centrifugation, separate sera from packed cells by pipetting the sera into new sterile cryotubes.

Recommendation: do not pour! Be very careful not to pick up red blood cells. A better serum transfer can be achieved by keeping the pipette above the packed cells layer and leaving a small amount of serum in the tube.

15. Keep packed cells and serum samples on ice before storage at -80ºC.

16. *Label Whatman 903 ProteinSaver cards with the appropriate sample identification. Drop 50ul of each serum sample onto the card in concentric circular motions within the printed 5 circle areas.
17. Allow the samples to dry for 1h at room temperature prior to punching.
18. Put dried serum samples in transparent PE zip-lock plastic bags and store at -30ºC or 4ºC.
19. Freeze the remaining sera immediately at -80°C upright in a cryotube storage box.

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* **Labelling**:

- Time of sample collection
- City or district of schistosoma endemic area
- Species of Schistosoma (Sh or Sm or Sj)
- Age of the infected individuals

4-2 Uninfected individual samples from Schistosoma non-endemic areas

The research personnel should greet the research subject, identify themselves, and then indicate the sample collection procedure to the research subject. The research subject should be correctly identified (through personal and clinical data sheet) prior to sample collection.

1. *Label sterile vacuum blood collection EDTA tubes.
2. Sterilize with isopropyl alcohol 70% the site of skin puncture (arm venipuncture).
3. Collect 5ml of blood into sterile vacuum blood collection EDTA tubes, mix gently and keep specimens on ice.
4. Proceed to immunodiagnosis tests using circulating Schistosoma Antigen (CSA) Detection kit (Egypt) for Sh or Sm subjects and Dynamic Flow Schistosoma IgG Antibody Card Test for Sj subjects according to the manufacturer's instruction. The purpose of this step is to screen out Sh or Sm or Sj negative blood specimens.
5. Keep Sj or Sh or Sm negative blood specimens at 4ºC (for no longer than 24 hours). We recommend carrying the following steps in the lab.
6. For each blood sample, *label 2 sterile cryotubes with the appropriate sample identification. Take 2ml of each sample into the 2 cryotubes (aliquot 1ml in each cryotube) and keep on ice before final storage at -80ºC (biobank in Africa).
7. *Label new sterile cryotubes with the appropriate sample identification. Take from the

EDTA tubes 1ml of blood samples into the cryotubes and keep on ice before storage at -80ºC (for biobank in China).

8. For dried blood samples, *label Whatman FTA cards with the appropriate sample identification.
9. Drop 50ul of each blood sample (after step 11) onto the card in concentric circular motions within the printed 4 circle areas. Avoid 'puddling' of the liquid sample as it will overload the chemicals on the card.
10. Allow the samples to dry for 1h at room temperature prior to punching.
11. Put dried blood samples in transparent PE zip-lock plastic bags and store at -30ºC or 4ºC.
12. For each specimen, pipette the remaining blood from the EDTA tubes into new 1.8ml cryotubes and *label carefully.
13. Centrifuge all the specimens for 5 minutes at 3,000rpm.

    Note: If the blood is not centrifuged immediately, the tubes should be refrigerated at 4ºC for no longer than 48 hours.
14. After centrifugation, separate sera from packed cells by pipetting the sera into new sterile cryotubes as descrived above.
15. Keep packed cells and serum samples on ice before storage at -80ºC.
16. *Label Whatman 903 ProteinSaver cards with the appropriate sample identification and drop 50ul of each serum sample onto the card as described above.
17. Allow the samples to dry for 1h at room temperature prior to punching.
18. Put dried serum samples in transparent PE zip-lock plastic bags and store at -30ºC or 4ºC.
19. Freeze the remaining sera immediately at -80°C upright in a cryotube storage box.

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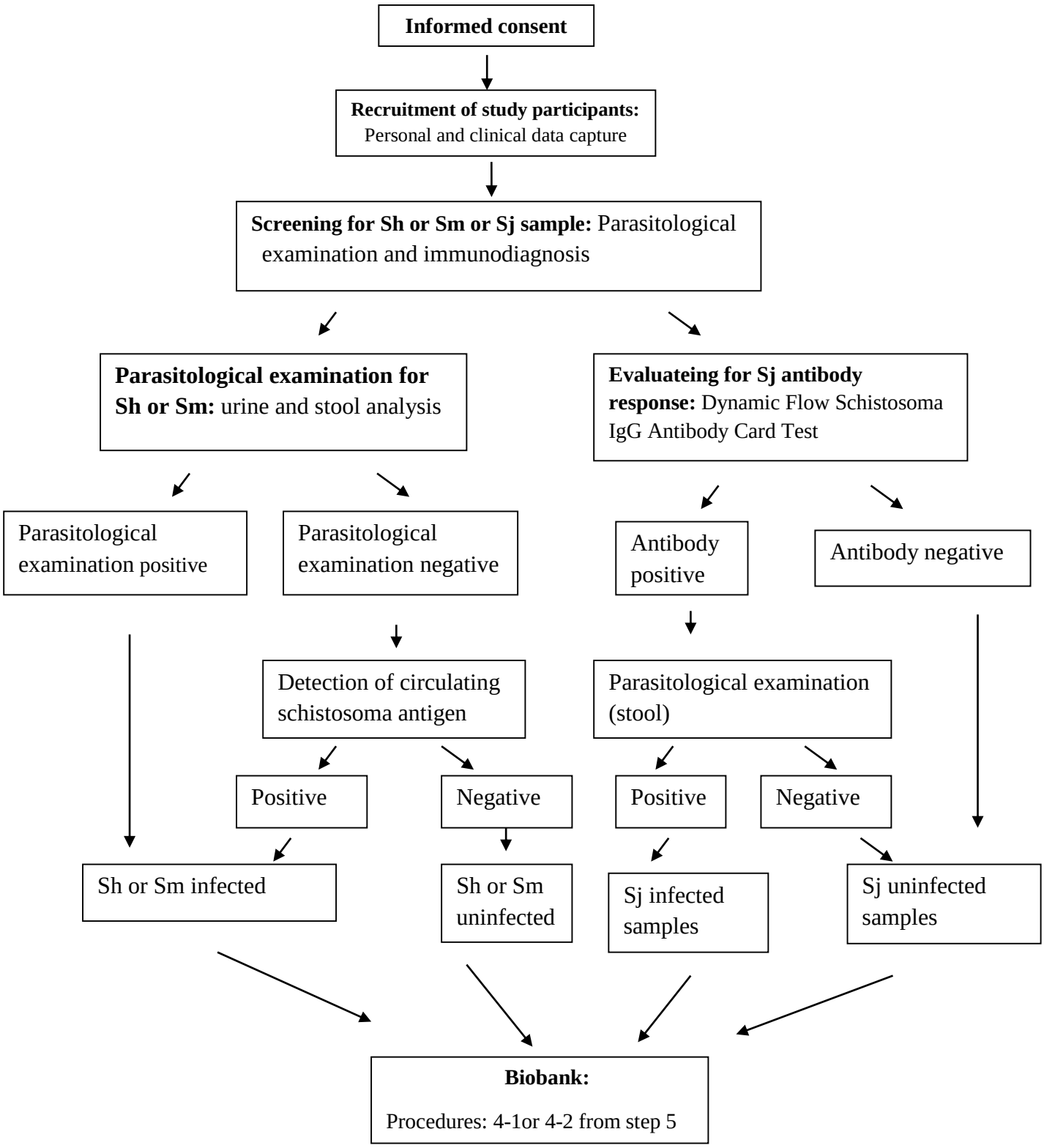
* **Labelling**:

- Time of sample collection
- City or district of schistosoma non-endemic area
- Age of the uninfected individual

5. Storage of specimens:

1. Before storage, check that all aliquot cryotubes, Whatman FTA and 903 cards are well labelled.

2. Place all aliquots of whole blood samples upright in a specimen box and store at -80ºC.
3. Put all dried samples in transparent PE zip-lock plastic bags at -30ºC or 4ºC.
4. Place all aliquots of packed cells and serum samples upright in a specimen box for cryostorage.



Appx 1

Parasitological Examination (PE): urine for Sh

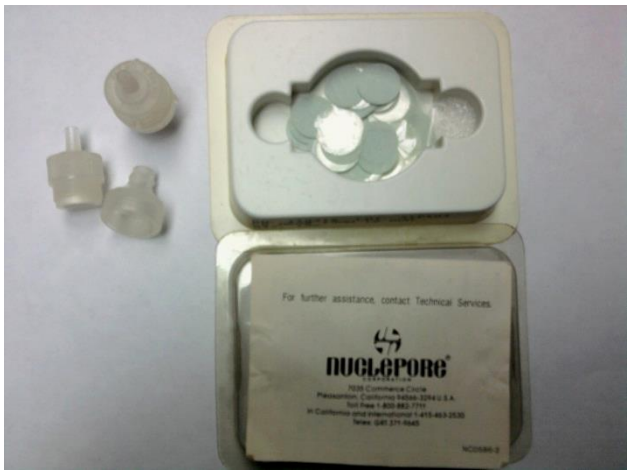
Examination of urine samples for *S. haematobium* ova by centrifugation and by nuclepore filtration as follows:

a- Centrifugation: 10 ml of well-mixed urine are transferred to 15 ml centrifuge tube, and the tube is centrifuged for 5 min at 2000 g. After centrifugation, a drop of the sediment is placed on a slide and examined microscopically for *S. haematobium* ova.

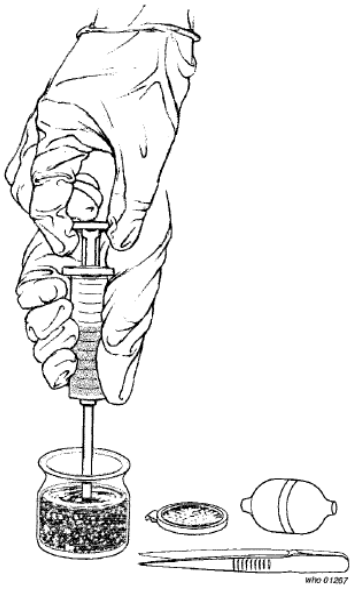
b- Nuclepore filtration: this technique is used to quantify S. haematobium eggs in urine samples as follows:

- Urine is collected from patients with positive sediment for *S. haematobium* eggs between 10 am and 2 pm.
- For every patient, three samples each of 10 ml, of well mixed urine are withdrawn by a syringe and injected through a PT-013 chamber (Micropore filter holder) containing a Nucleopore filter (13mm in diameter with a pore size 8 um)
- The syringe is then removed and filled with air, which was injected through the filter chamber, the filter is removed and placed face down on a drop of saline on a glass microscope slide. Using x40 magnification, each Nucleopore filter is completely examined without staining. The three slides are counted and average number is calculated. Results are expressed as mean number of eggs per 10 ml urine:
  - Light infection: (1- 49) eggs per 10 ml of urine.
  - Moderate infection: (60-100) eggs per 10 ml of urine.
  - Heavy infection: (≥ 100) eggs per 10 ml of urine.

- Filters are removed immediately after use and are soaked overnight in a 1% hypochlorite solution (domestic bleach). After soaking, the filters are washed thoroughly with detergent solution, and then rinsed several times with clean water. Filters are checked under the microscope to ensure that it is free of eggs before reusing it.

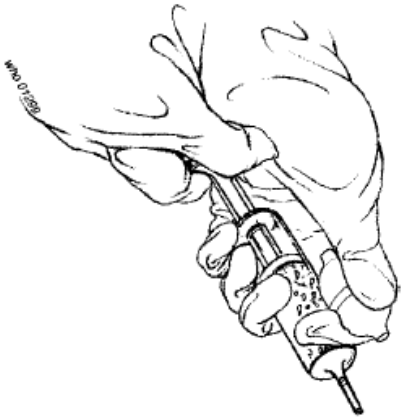


Nuclepore® filters and Milipore® filter holders



a. 10ml of urine is drawn

b. Urine is slowly expelled into the syringe.



c. Air is drawn into the syringe. d. Air is expelled through the filter

Figure: Urine filtration technique ***(WHO, 2003)***

Parasitological Examination (PE): stool for Sm or Sj

Kato-Katz technique, for both egg detection and egg load quantification:

- The Kato Katz (KK) method is recommended by WHO for the quantitative diagnosis of *Schistosoma mansoni and Schistosoma japonicum* It is the most widely used technique in epidemiological surveys because it is simple, quantitative and relatively inexpensive.

-The glycerin, in the malachite green in Kato Katz's technique, functions as a clearing agent while the malachite green besides being a dye, is bactericidal

- The Kato-Katz methods require between 1 to 2 hours before the glycerin clears the background of the stool smear on the slide for accurate visualization of most helminth eggs.

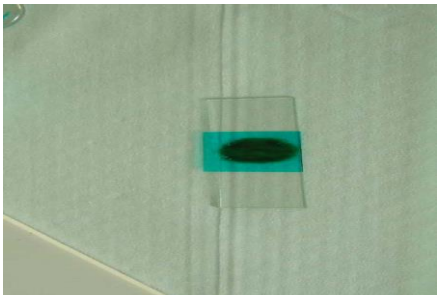
- After the collection of stool specimen, sample examination is performed as in the following figure:

1-Place a small amount of fecal material on the newspaper or the glazed tile.

2-Press the screen on top so that some of the feces filters through and scrap with the flat spatula across the upper surface to collect the filtered feces.

3-Cover the faecal material with the pre-soaked cellophane strip in glycerol-malachite green solution.

4-Invert the microscope slide and firmly press the faecal sample against the cellophane strip on a smooth hard surface such as a tile. The material will be spread evenly.



5- Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip. Place the slide with the cellophane upwards.

Figure: Kato-Katz technique, for stool analysis.

Appx 2 circulating Schistosoma Antigen (CSA) Detection kit

Appx 3 Dynamic Flow Schistosoma IgG Antibody Card Test for Sj infection

Dynamic Flow

Schistosoma IgG Antibody Card Test

Rapid Detection of IgG Antibodies to Schistosoma in Human Serum/Plasma

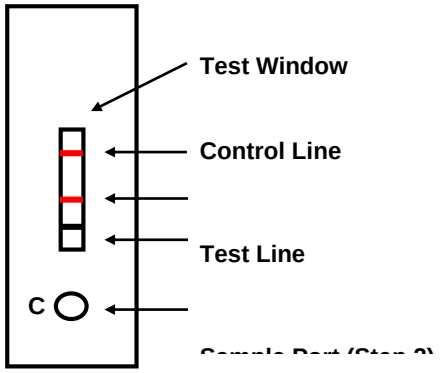
Catalog No.: 1-127G-DF

SUMMARY OF TEST PROCEDURE

Step	Description	Time
1	Apply 2 drops of conjugate to the **reagent port (R)*** to wet the membrane of **sample port (S)***	A few seconds
2	Apply 5 µl sample in the **sample port**	
3	Read result	**10 minutes** after sample application

*Refer to illustration 1 and the package insert for detail

Illus. 1: Test Device (Card)



INTENDED USE

Dynamic Flow Schistosoma IgG Antibody Test (The Test) is a rapid immuno-assay intended as a screening test for qualitative detection of IgG antibodies to ***Schistosoma*** in human serum or plasma. The Test is designed for investigational use only.

INTRODUCTION

A genus of trematodes, ***Schistosoma***, commonly known as **blood-flukes** and **bilharzia**, includes flatworms which are responsible for the most significant parasitic infection of humans by causing the disease schistosomiasis, and are considered by the World Health Organization as the second most important parasitic disease, next only to malaria, with hundreds of millions infected worldwide. Diagnosis of the disease is based on a series of laboratory studies including egg detection, complete blood count and coagulation studies, urinalysis, liver function tests, and serologic tests.

The Test is a rapid visual assay based on principles of immuno-chromatography and fluid dynamics to qualitatively detect the presence of **IgG** antibodies to ***Schistosoma*** in human serum or plasma specimen.

PRINCIPLES OF THE TEST

The Test is based on a proprietary technology that combines the principles of immuno-chromatography and fluid dynamics. The device of the Test has ***Schistosoma*** antigen immobilized on the membrane within the test zone. The liquid **Protein A - gold conjugate** applied to the device through the reagent port (marked ”R”) in the first step serves to prime the device and to facilitate the migration of samples applied in the sample port (marked “S”) in the second step. As the sample migrates through the test zone, the **IgG** antibodies to the ***Schistosoma*** antigen present in the specimen are captured by the immobilized antigen and subsequently visualized by the conjugate in the form of a magenta test line. The absence of the test line indicates a negative test. In the control zone of the membrane, a magenta control line appears in every valid test indicating that the Test is properly performed and reagents are functional as specified.

MATERIAL PROVIDED

1. Test Device (card)
2. Conjugate Reagent (Protein A - gold conjugate)

MATERIAL REQUIRED BUT NOT PROVIDED

1. Micropipette for 10 µl
2. Timer

STORAGE AND STABILITY

1. **Store unopened kit at 4 temperature (4ºC).**

   Test Card: Store at 2-30ºC.

   Conjugate: Store unopened bottle at 4 ºC.

2. **Keep opened conjugate refrigerated at 2-8ºC.**

   DO NOT FREEZE CONJUGATE.

3. Do not use all of the above components beyond expiration date.

PRECAUTIONS

1. Do not use the test device and reagent beyond expiration date.
2. Treat all specimens as infectious. Practice universal precautions and wear protections throughout the procedure. Properly dispose of specimens and other testing materials according to standard procedures.
3. Bring testing materials including specimen to room temperature before testing.

SPECIMEN COLLECTION AND PREPARATION

1. The Test can be performed on either serum or plasma. It is recommended that fresh specimens be used if possible. Specimens may be stored at 2-8ºC for up to 3 days before testing. For long-term storage, specimens should be kept below -20°C.
2. Separation of serum or plasma from blood should be performed as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
3. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

TEST PROCEDURE

(Please refer to Illustration 1.)

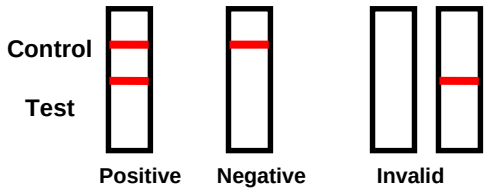
- Allow test card, reagent, specimen, and controls to equilibrate to room temperature (15-30°C) before testing.
- Place the test card on a clean and level surface.

1. Hold the conjugate dropper bottle vertically and transfer 2 full drops (-100 µl) of conjugate into the reagent port (marked "**R**"). Wait for the conjugate to pass the sample port (marked "**S**") as indicated by the red liquid front passing through.
2. Transfer 5µl of sample onto the membrane in the sample port.
3. Read result within **10 minutes** after the sample application. Do not attempt to interpret result after **15** minutes.

RESULT INTERPRETATION

(Please refer to Illustration 2.)

Illus. 2: Result Interpretation



Positive:	**Both test and control lines appear.** Note: Low titers of antibody may result in a faint test line.
Negative:	**Only control line appears.**
Invalid:	**Control line fails to appear.** The test should be repeated on a new device.

QUALITY CONTROL

The control line in the control zone is a built-in procedural control in the Test. The control line appearing as specified indicates that the test is properly performed and reagents are functional.

LIMITATIONS

1. The Test (Serum/Plasma) is for *in vitro* use only.
2. The Test is a qualitative test.
3. Positive results should be confirmed by independent confirmatory tests.

4. In cases where the test result is negative while clinical symptoms persist, further consultation with a physician and additional tests of other methods should be followed.
5. Optimal assay performance requires strict adherence to the assay procedures described in this insert sheet. Deviations may lead to aberrant results.

REFERENCES

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Email: customer.service@ease-medtrend.com

CNINESE CENTER FOR DISEASE CONTROL AND

PERFORMANCE CHARACTERISTICS

The Test showed concordance with other commercial tests when tested with commercial performance panels.