Standard Operating Procedures for Laboratory Services at Integrated Health Service Centers

December 2008
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FOREWORD

Family Health International/ Nepal (FHI/Nepal), with support from the United States Agency for International Development (USAID), first developed the Rapid HIV Testing manual for FHI-supported VCT Clinics in 2005.

This revised and updated Rapid HIV Testing Standard Operating Procedure (SOP) manual includes guidelines for laboratory procedures for the diagnosis of HIV and other sexually transmitted infections. The national HIV testing strategy, laboratory quality assurance and laboratory safety are also included. This manual is compatible with the National Guidelines for Voluntary HIV/AIDS Counseling and Testing, 2007 and the National Guidelines on Sexually Transmitted Infection Case Management, 2006.

FHI/Nepal would like to express its sincere appreciation to all contributors to this manual.

Jacqueline McPherson
Country Director
Family Health International/Nepal
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acronyms</td>
<td>7</td>
</tr>
<tr>
<td>How to use this SOP</td>
<td>8</td>
</tr>
<tr>
<td><strong>LAB 1: Blood collection by Venipuncture</strong></td>
<td>9</td>
</tr>
<tr>
<td><strong>LAB 2: Preparation of serum from whole blood</strong></td>
<td>11</td>
</tr>
<tr>
<td><strong>LAB 3: National Algorithm for Rapid HIV Testing</strong></td>
<td>12</td>
</tr>
<tr>
<td><strong>LAB 4: Rapid HIV testing</strong></td>
<td>14</td>
</tr>
<tr>
<td><strong>LAB 5: Laboratory investigations for Sexually Transmitted Infections (STIs)</strong></td>
<td>19</td>
</tr>
<tr>
<td>A. Determine HIV-1/2 Rapid Test Kit</td>
<td>14</td>
</tr>
<tr>
<td>B. Uni-Gold HIV Rapid Test Kit</td>
<td>15</td>
</tr>
<tr>
<td>C. SD Bioline HIV-1/2 Rapid Test Kit</td>
<td>17</td>
</tr>
<tr>
<td><strong>LAB 6: Quality Assurance</strong></td>
<td>28</td>
</tr>
<tr>
<td>A. Use and care of laboratory equipments</td>
<td>28</td>
</tr>
<tr>
<td>B. Recording and reporting</td>
<td>32</td>
</tr>
<tr>
<td>C. External quality assessment (EQA)</td>
<td>33</td>
</tr>
<tr>
<td><strong>LAB 7: Laboratory Safety</strong></td>
<td>35</td>
</tr>
<tr>
<td>A. Infection prevention procedures</td>
<td>35</td>
</tr>
<tr>
<td>B. Hand washing</td>
<td>36</td>
</tr>
<tr>
<td>C. Glove use</td>
<td>37</td>
</tr>
<tr>
<td>D. Collection and disposal of laboratory wastes</td>
<td>37</td>
</tr>
<tr>
<td>E. Cleaning and Sterilization</td>
<td>39</td>
</tr>
<tr>
<td><strong>ANNEX</strong></td>
<td>40</td>
</tr>
<tr>
<td>1. Laboratory Report-Rapid HIV tests</td>
<td>41</td>
</tr>
<tr>
<td>2. Laboratory Report-STI tests</td>
<td>42</td>
</tr>
<tr>
<td>3. Temperature Chart</td>
<td>43</td>
</tr>
<tr>
<td>4. Laboratory Test Result Register</td>
<td>44</td>
</tr>
<tr>
<td>5. Weekly Record of Quality Control Results</td>
<td>45</td>
</tr>
<tr>
<td><strong>BIBLIOGRAPHY</strong></td>
<td>46</td>
</tr>
</tbody>
</table>
This SOP has been developed for use in Integrated Health Service (IHS) laboratories. This describes the operating procedures for blood collection, rapid HIV testing and laboratory diagnosis of sexually transmitted infections (STIs). This also describes the procedures for quality assurance of testing and laboratory safety in an IHS laboratory.

Standard operating procedures for three rapid HIV testing methods have been described. The three rapid HIV tests include Determine-HIV 1/2, Unigold HIV and SD Bioline-HIV 1/2. Similarly, standard operating procedures for RPR test, TPPA test, Gram staining, Wet mount and KOH mount have been described. Testing procedures for RPR test have been described based on BD Macro-view RPR card test and testing procedures for TPPA test have been described based on Serodia TPPA test.

This SOP will be provided to all IHS laboratories. A copy of this SOP should be kept at all times at all IHS laboratories. Laboratory technicians and laboratory assistants working in an IHS laboratory should be familiar with the procedures described in this SOP. Procedures described in this SOP should be followed at all times while performing all laboratory activities.
LAB 1: Blood Collection by Venipuncture

I. Purpose:
To obtain blood samples from patients needed for laboratory testing, with proper specimen identification and handling, while ensuring patient and staff safety.

II. Background:
A. General Considerations:
   A. Laboratory investigations are a critical part of patient management. Strict adherence to SOP requirements ensures quality laboratory results. Obtaining sufficient volumes of blood in the proper collection tubes is the responsibility of the laboratory person.
   B. It is also the duty of the laboratory person to ensure that the specimens collected are identified properly and labeled in a legible manner for the laboratory. Mislabeled specimens can jeopardize the outcome of the laboratory results, causing incorrect patient management or the discarding, and thus loss, of specimens with ambiguity that cannot be resolved.

III. Materials and equipments required:
   1. Supportive cushion
   2. Specimen collection tubes
   3. Disposable gloves
   4. Ethanol/Isopropyl alcohol
   5. Tourniquet
   6. Disposable syringe
   7. Cotton balls
   8. Waste disposal bin
   9. Labeling sticker/ Marking pen
   10. Needle Destroyer

IV. Procedures:
   1. All required materials for blood drawing should be assembled before the procedure.
   2. Label the specimen collection tube with the patient identification number.
   3. Do not prepare tubes for more than one subject at a time.
   4. Request patient to sit in a comfortable chair and stretch the forearm of the patient onto the supportive cushion on the table.
   5. Explain the blood drawing procedure to the client and reassure him/her.
   6. Tie the arm with the tourniquet above the elbow joint and ask patient to fist his/her hand.
   7. Using the tip of the index finger examine the puncture site, feel for the vein, and decide exactly where to place the puncture.
   8. After locating the site, put the gloves on.
   9. Disinfect the puncture site by swabbing the skin in small outward circles with an alcohol swab or cotton wool soaked in 70% alcohol.
   10. The area will be allowed to air dry for 30-60 seconds.
   11. Insert the needle with bevel side up and parallel to the vein.
   12. Draw 5ml of blood.
13. Release the tourniquet and ask the patient to release his/her fisted hand.
14. Remove the needle from the vein, cover the puncture site with a sterile cotton swab, and ask the patient to bend his/her forearm over the arm until adequate haemostasis is visible.
15. Destroy the needle of the syringe with the needle destroyer.
16. Transfer blood slowly into the specimen collection tube which has already been labeled with patient identification number.

V. **Safety Precautions:**
1. Always put on laboratory protective clothing and gloves and adhere to the laboratory safety practices to avoid viral and/or other infectious disease transmissions.
2. Needles should not be recapped, but should be destroyed using needle destroyer.
3. Any blood-soiled materials should be placed in an infectious waste container for proper disposal.
4. Visibly soiled or splashed tourniquets should be discarded in proper containers and a new tourniquet should be used.
5. Any blood spills or splashes should be immediately cleaned up with absorbent material using an approved disinfectant.
6. No food or drink is permitted in the blood collection area.

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LAB 2: Preparation of serum from whole blood

I. Purpose:
To get serum specimens (from whole blood obtained by venipuncture) to be used for a variety of serological investigations such as rapid HIV testing, RPR test, TPPA test, etc.

II. Principle:
Serum is obtained when whole blood is allowed to clot and then centrifuged. Separation activity is a function of both centrifugal force and timing.

III. Materials and equipments required:
1. Centrifuge machine
2. Test tubes
3. Tube holding rack

VI. Procedures:
1. Keep the collection tubes with blood on tube-holding rack.
2. Keep the collected whole blood in the collection tube for 15 minutes in order for clotting to take pace.
3. Plug the test tube.
4. Keep the collection tube in centrifuge.
5. Balance the contents of centrifuge (if required, with a separate test tube containing plain water).
6. Cover centrifuge with its lid.
7. Switch on the electricity supply.
8. Increase the speed slowly until speed reaches 3000 rotation per minute (RPM).
9. Allow centrifuge to spin for about 15 minutes.
10. Switch off the electricity supply.
11. Allow centrifuge to come to rest.
12. Remove the test-tube from centrifuge.
13. Transfer the separated serum with the help of a transfer pipette into a clean test tube.

VII. Safety Precautions:
1. Always operate the centrifuge with the lid closed.
2. Balance contents before turning on.
3. Check for vibration.
4. Do not open the lid until the rotor has come to a complete stop.
5. Keep lids on tubes when spinning.

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Serial rapid HIV testing algorithm

I. Principle:
Algorithm is defined as the combination and sequence of specific tests used in a given testing strategy. An HIV Positive Status should be based upon the outcome of 2 or more tests.

Serial testing means samples tested by a first test. The results of the first test determine whether additional testing is required. If the first test shows a non-reactive result, the tested sample will be reported as "HIV Negative." If the first test shows a reactive result, the sample will be tested further by a second test; if the second test shows a reactive result, the tested sample will be reported as "HIV Positive."

When two test results disagree (the first is reactive and second is non-reactive), the finding is called "discordant." In this case, a third test must be performed; the result of the third test will be the final test result.

The first test used in a serial HIV testing algorithm should be highly sensitive so that all the positive samples will be identified as positive. The second test should be highly specific so that all true negative samples will be identified as negative.

II. Procedure and Interpretation of test results:
1. Test the serum specimen using first rapid HIV test kit (Determine HIV 1/2).
2. If first rapid HIV test kit shows a non-reactive result, report tested sample as "HIV Negative."
3. If the first rapid HIV test kit shows a reactive result, test the specimen using a second rapid HIV test kit (Uni-Gold HIV 1/2).
4. If the second rapid test shows a reactive result, report tested sample as "HIV Positive."
5. If the second rapid test shows a non-reactive result, a third, tie breaker, rapid HIV test kit (SD Bioline HIV 1/2) should be used to test the sample.
6. If the third test shows a reactive result, report tested sample as "HIV Positive."
7. If the third test shows a non-reactive result, report tested sample as "HIV Negative."

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Figure: Diagrammatic representation of rapid HIV testing algorithm.

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>A1 (First test):</strong> Determine HIV 1/2</td>
</tr>
<tr>
<td><strong>A2 (Second test):</strong> Uni-Gold HIV</td>
</tr>
<tr>
<td><strong>A3 (Third test):</strong> SD Bioline HIV 1/2</td>
</tr>
<tr>
<td>&quot;+&quot; : Reactive</td>
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<tr>
<td>&quot;-&quot; : Non-reactive</td>
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LAB 4: Rapid HIV Testing

A. Determine HIV-1/2 Rapid Test Kit

I. Principle:
HIV-1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2.

II. Description of Kit:
- Kit contents: Serum assay contains 100 tests
- Run time of test: 15 minutes
- Storage conditions: 2-30°C
- Quality control (QC): Procedural QC is incorporated in the device
- Shelf life: Shelf life is indicated by the expiration date printed on the test package

III. Materials Required:
- Pipette, pipette tips and disposable gloves

IV. Test Procedure:
1. Collect blood specimen by venipuncture and prepare serum from whole blood.
2. If the test kit has been stored in the refrigerator, keep it on the table for 20-25 minutes until it acquires the room temperature (15-30°C).
3. Check for expiry date of the test kit.
4. Remove the protective foil cover for each test.
5. Label the test device with the patient's identification number.
6. Bring the serum to room temperature, if needed (if the serum has been stored in refrigerator).
7. Place 50 µl of serum to the sample pad marked by the arrow symbol using a precision pipette.
8. Wait until it is absorbed by the sample pad.
9. Set the electronic timer countdown for 15 minutes. Wait for at least 15 minutes and note down the result. Do not read the results after 60 minutes following sample addition.

V. Interpretation of Test Result:
1. Positive (Two Bars): Red bars appear in both the control window (labeled "Control") and the patient window (labeled "Patient") of the strip. Any visible red colored bar in the patient window should be interpreted as positive, even if the line is lighter or darker than the control line.
2. Negative (One Bar): One red bar appears in the control window of the strip (labeled "Control"), and no red bar appears in the patient window of the strip (labeled "Patient").

[Images of test results: Positive, Negative, Invalid]
3. Invalid (No Bar): If there is no red bar in the control window of the strip, regardless of whether a red bar appears in the patient window of the strip, the result is invalid and should be repeated. If problem persists, contact concerned center.

VI. Quality Control (QC):
A built-in procedural control on the test device indicates whether or not the test is functioning correctly. If there is no line visible in the control window, the test is invalid and must be repeated.

VII. Disposal of the used Devices:
1. Collect the used devices in a waste container.
2. Incinerate the used devices collected in the waste container.

B. Uni-Gold HIV 1/2 Rapid Test Kit

I. Principle:
The Trinity Biotech Uni-Gold HIV test is a rapid immunoassay based on the immunochromatographic sandwich principle for detection of antibodies to HIV-1 and HIV-2 in serum, plasma or whole blood.

II. Description of Kit:
Kit contents: 20 test devices, wash reagent, pipettes and package insert
Run time of test: 10 minutes
Storage conditions: 2-27°C, Do not freeze
Quality control (QC): Procedural QC is incorporated in the device
Shelf life: Shelf life is indicated by the expiration date printed on the test package

III. Materials required:
Pipette, pipette tips and disposable gloves

IV. Test procedure:
1. Take the test kit out of the refrigerator and keep it on the table for 20-25 minutes so that it acquires the room temperature (15-30°C).
2. Take out the required number of test devices depending on the number of tests to be done.
3. Check for the expiry date of the test kit.
4. Remove the test device from its protective wrapper.
5. Label the test device with the patient’s identification number.
6. Bring the serum to room temperature, if needed (if the serum has been stored in refrigerator).
7. Place 60 µl of serum over the sample port.
8. Place two drops of wash buffer reagent (about 60 µl) over the sample port.
9. Start the electronic timer countdown at 10 minutes. Wait for 10 minutes and note down the result. Do not read results after 20 minutes following sample addition.

V. Interpretation of test result:
1. Positive (Two Bars): Red bars appear in both the control area (labeled "C") and the patient area (labeled "T") of the device. Any visible red color in the patient area should be interpreted as positive, even if the line is lighter or darker than the control line.
2. Negative (One Bar): One red bar appears in the control area of the device (labeled "C"), and no red bar appears in the patient area of the strip (labeled "T").
3. Invalid (No Bar): If there is no red bar in the control window of the strip, regardless of whether a red bar appears in the patient window of the strip, the result is invalid and should be repeated. If problem persists, contact concerned center.

VI. Quality Control:
A built-in procedural control on the test device indicates whether or not the test is functioning correctly. If there is no line visible in the control window, the test is invalid and must be repeated.

VII. Disposal of the Used devices:
1. Collect the used devices in a waste container.
2. Incinerate the used devices collected in the waste container.

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C. SD Bioline HIV 1/2 3.0 Rapid Test Kit:

I. Principal:
The SD Bioline HIV 1/2 3.0 test is an immunochromatographic (rapid) test for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific to HIV-1 and HIV-2 simultaneously in human serum, plasma or whole blood.

II. Description of Kit:
Kit contents: 20 test devices, assay diluent and package insert
Run time of test: 5 minutes
Storage conditions: 1-30°C, Do not store at refrigerator
Quality control (QC): Procedural QC is incorporated in the device
Shelf life: Shelf life is indicated by the expiration date printed on the test package

III. Materials required:
Pipette, pipette tips and disposable gloves

IV. Test procedure:
1. Take out the required number of test devices depending on the number of tests to be done.
2. Check for the expiry date of the test kit.
3. Remove the test device from foil pouch; place it on a flat, dry surface.
4. Label the test device with the patient's identification number.
5. Bring the serum to room temperature, if needed (if the serum has been stored in refrigerator).
6. Using a micropipette, add 10 µl of serum specimen into the sample well.
7. Add 4 drops of assay diluent into sample well.
8. Start the electronic timer countdown at 5 minutes. Wait for 5 minutes and note down the result. Do not read results after 20 minutes.

V. Interpretation of test result:
1. Negative: The presence of only control line (C) within the result window indicates a negative result.
2. Positive: The presence of two lines as control line (C) and test line 1 (1) within the result window indicates a positive result for HIV-1.
The presence of two lines as control line (C) and test line 2 (2) within the result window indicates a positive result for HIV-2.
The presence of three lines as control line (C), test line 1 (1) and test line 2 (2) within the result window indicates a positive result for HIV-1 and/or HIV-2.
   >>> If the color intensity of the test line 1 is darker than the one of test line 2 in the result window, interpret the result as HIV-1 positive.
   >>> If the color intensity of the test line 2 is darker than the one of test line 1 in the result window, interpret the result as HIV-2 positive.
3. Invalid: No presence of control line (C) within the result window indicates an invalid result. Test should be repeated in case of invalid result. If problem persists, contact concerned center.
VI. Quality Control:
   A built-in procedural control on the test device indicates whether or not the test is functioning correctly. If there is no line visible in the control window, the test is invalid and must be repeated.

VII. Disposal of the Used devices:
   1. Collect the used devices in a waste container.
   2. Incinerate the used devices collected in the waste container.

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A. BD Macro-view RPR card test for diagnosis of Syphilis

I. Principle:
The Rapid Plasma Reagin (RPR) is a "Non-treponemal" test in that the antibodies detected are not specific for Treponema pallidum, although their presence in patient's serum or plasma is strongly associated with infection by Treponema pallidum. These antibodies tend to disappear after successful cure of infection. RPR kits use carbon particles coated with a mixture of lipid antigens, which will combine with antibodies present in patient's serum or plasma. Positive reactions are shown by macroscopic aggregation of the particles.

II. Description of Kit:
Kit contents: BD Macro-view RPR card test contains 500 test circles, 3 vials of carbon particles coated with antigen and dispentirs
Run time of test: 8 minutes
Storage conditions: 2-8°C
Shelf life: Shelf life is indicated by the expiration date printed on the test package
Disposal Method: incinerate/autoclave the used devices

III. Materials required:
RPR rotator, Micropipette, Pipette tips, Controls, Saline and Disposable gloves

IV. Test procedure:
a) Qualitative test:
1. Take the test kit out of the refrigerator and keep it on the table for 20-25 minutes so that it acquires the room temperature (15-30°C).
2. Check for the expiry date of the test kit.
3. Label a test circle on the test card with the patient's identification number.
4. Bring the serum to room temperature, if needed (if the serum has been stored in refrigerator).
5. Place 50 µl of specimen into a circle on the test card.
6. Using a new stirrer (broad end), spread the specimen evenly over the test circle area. Discard stirrer.
7. Shake the vial of RPR antigen to ensure thorough mixing.
8. Holding the dropping bottle vertically over the test specimen, dispense a single "free-falling" drop of antigen onto each test area. Do not restir.
9. Place test card on a card rotator and rotate at 100 rpm for 8 minutes.
10. Following rotation, make a brief rotating and tilting of the card by hand (3 or 4 to-and-fro motions).
11. Immediately read in the "wet" state and interpret results visually in good light.

Positive and Negative controls must be run at least once a week.

b) Quantitative test:
All the serum specimens reactive in the qualitative test should be further analyzed by quantitative test to find the titer of the specimen.
1. Label five circles on the test card with numbers 1 to 5.
2. Place 50 µl of saline serially into circles no.1 through 5.
3. Add 50 µl of specimen to circle no.1.
4. Mix the contents of circle no.1 thoroughly by filling and discharging with a micropipette 5-6 times. Avoid formation of air bubbles during mixing.
5. Transfer 50 µl of the mixture of specimen and saline into circle no. 2. Then mix well and transfer 50 µl of the mixture to circle no. 3.
6. Repeat this procedure again with circles no. 3, 4 and 5 to obtain serial doubling dilutions (i.e. 2, 4, 8, 16, 32 fold dilutions).
7. Using a new stirrer for each specimen, spread contents of each circle evenly over the test circle area starting at highest dilution of serum (circle 5) and then proceeding to circles 4, 3, 2 and 1.
8. Shake the vial of RPR antigen to ensure thorough mixing.
9. Holding the dropping bottle vertically over the test specimen, dispense a single “free-falling” drop of antigen to circles no. 1 through 5. Do not restir.
10. Place test card on a card rotator and rotate at 100 rpm for 8 minutes.
11. Immediately read in the “wet” state and interpret results visually in good light.

V. Interpretation of test results:

a) Qualitative test:
   - **Reactive:** Showing characteristic clumping ranging from slight but definite (minimum-to-moderate) to marked and intense.
   - **Non-reactive:** No clumping (A smooth grey pattern or a button of non-aggregated carbon particles in the center of the test circle)

   *Note: There are only two possible final reports with Card Test: Reactive or Non-reactive regardless of the degree of reactivity.*

b) Quantitative test:
   Express the titre of the specimen as the reciprocal of the highest dilution showing aggregation of the carbon particles i.e. if the 4 fold dilution shows aggregation of carbon particles but the 8 fold dilution doesn’t show aggregation of carbon particles, the titre of the specimen will be 1:4.

VI. Quality Control:

1. Run both the positive and negative controls at least once a week.
2. Report the test results only when the controls give the expected results.

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B. Serodia TPPA Test

I. Principle:
TPPA test is specific for the diagnosis of syphilis. This test is carried out in a microwell plate. The antigens, in this TPPA test, are coated on gelatin particles and are known as sensitized particles. The test is based on the principle that sensitized particles are agglutinated by the presence of antibodies to Treponema pallidum in human serum. Antibodies against Treponema pallidum become detectable after 3-4 weeks of infection and may remain detectable for long periods.

II. Description of Kit:
Kit contents: Serodia TPPA test contains 5 vials of sensitized particles, 5 vials of unsensitized particles, 1 vial of reconstituting solution, and 1 vial of sample diluent
Run time of test: 2 hours
Storage conditions: 2-10°C, Do not freeze
Quality control: Positive control is provided with the kit
Shelf life: Shelf life is indicated by the expiration date printed on the test package
Disposal Method: incinerate/autoclave the used devices

III. Materials required:
Micropipette, Pipette tips, Microwell plate having U- shaped bottom, disposable gloves, Plate mixer (optional)

IV. Preparation:
Reconstitution of lyophilized particles: Reconstitute Sensitized and Unsensitised particles, respectively, with prescribed quantity of reconstituting solution (0.6 ml) at room temperature (15-30°C) 30 minutes prior to use. In order to obtain suitable test results, make sure to mix the Sensitized and Unsensitized Particles thoroughly before testing. Under proper storage condition at 2-10°C, reconstituted sensitized and unsensitized particles can be used for 7 days after reconstitution. Reconstituted sensitized and unsensitized particles should be sealed with sealing film to prevent contamination from any foreign bodies during storage.

V. Test Procedure:
1. Take the test kit out of the refrigerator and keep it on the table for 20-25 minutes so that it acquires the room temperature (15-30°C).
2. Bring the serum to room temperature.
3. Select four wells of the Microwell plate serially and label with patient identification number at one end.
4. Place 100 µl of sample diluent in well no.1.
5. Place 25 µl of diluent into the well no. 2, 3 and 4.
6. Add 25 µl of patient serum to well no.1 and mix thoroughly by filling and discharging with a micropipette 4-5 times. Transfer 25 µl of the mixture of specimen and sample diluent into well no.2. Then mix well and repeat this procedure again with wells no.2, 3 and 4 to obtain serial doubling dilutions.
7. Place 25 µl of Unsensitized particles in well no.3 and 25 µl of Sensitized particles in well no.4.
8. Mix the contents of the well thoroughly (for approximately 30 seconds) using a plate mixer, or tap the plate sharply with your finger to assure proper mixing. DO NOT USE A ROTATOR.
9. Cover the plate and let it stand at room temperature (15-30°C) for 2 hours before reading.
VI. Reading test results:

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<thead>
<tr>
<th>SETTLING PATTERNS OF PARTICLES</th>
<th>READING</th>
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<tbody>
<tr>
<td>Particles concentrated in the shape of a button in the center of the well with a smooth round outer margin</td>
<td>( - )</td>
</tr>
<tr>
<td>Particles concentrated in the shape of a compact ring with a smooth round outer margin</td>
<td>( ± )</td>
</tr>
<tr>
<td>Definite large ring with a rough multiform outer margin and peripheral agglutination</td>
<td>( + )</td>
</tr>
<tr>
<td>Agglutinated particles spread out covering the bottom of the well uniformly.</td>
<td>( ++ )</td>
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I. Criteria for Interpretation:
1. **Positive**: Interpret a specimen showing ( - ) with Unsensitized particles but demonstrating ( + ) or ( ++ ) with Sensitized Particles as POSITIVE.
2. **Negative**: Regardless of the reading of the reaction pattern with Unsensitized Particles, interpret a specimen showing ( - ) with Sensitized Particles as NEGATIVE.
3. **Indeterminate**: Interpret a specimen showing ( - ) with Unsensitized particles and demonstrating ( ± ) with Sensitized particles as INDETERMINATE. Specimens which show an indeterminate result should be retested.

II. Quality control:
1. Process Positive Control at least once on the day of testing. The control should yield the expected results.
2. Confirm that the reaction with Unsensitized Particles is Negative (-) for each patient sample.
3. If an assay does not meet Quality Control parameters listed above, the patient results from that assay should not be reported.
4. Ideally, lyophilized reagents contained in the kit should be used with in the same day of reconstitution. However, under proper storage conditions at 2-10 ºC, they will remain stable for 7 days after reconstitution.

III. Absorption Procedure:
If a specimen causes agglutination with both Sensitized and Unsensitized particles, or shows an indeterminate result, it should be retested after the following absorption procedure.
1. Place 0.95 ml of reconstituted Unsensitized Particles in a small test tube.
2. Add 50 µl of specimen into the tube and mix thoroughly. Then incubate at room temperature (15-30ºC) for 20 minutes or more (mix once or twice during incubation).
3. Centrifuge for 5 minutes at 2000 rpm. Remove the supernatant (absorbed 1:20 diluted specimen) carefully, and then place 50 µl in well no.3 of the microplate.
4. Place 25 µl of Sample Diluent in wells no.4 through no.12. Using a micropipette, prepare serial doubling dilutions from wells no.3 through no.12.
5. Place 25 µl of Unsensitized Particles in well no.3 and 25 µl of Sensitized Particles in wells no. 4 through no.12.
6. Mix the contents of the well thoroughly (for approximately 30 seconds) using a plate mixer, or tap the plate sharply with your finger, to assure proper mixing. **DO NOT USE A ROTATOR.**
Cover the plate and let it stand at room temperature (15-30 ºC) for 2 hours before reading.
C. Gram Staining for diagnosis of Neisseria gonorrhoeae in urethral or cervical smear

I. Principle:
Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls.
The Gram stain is useful in the diagnosis of gonorrhea and many other infections. Both the numbers of polymorphonuclear leukocytes (PMLs) and microbial flora present can be assessed.

II. Materials Required:
1. Cotton-tipped swabs
2. Microscope
3. Glass slides
4. Methanol
5. Gram stain reagents
   a. Crystal violet
   b. Gram's iodine
   c. Decolorizer (50:50 mixture of acetone and ethanol)
   d. Safranin
6. Sink or staining tray with water source
7. Paper towels or blotting paper
8. Immersion oil.

III. Preparation of Smears:
Clinicians will collect samples from the patients, prepare smears on slides and send the smears to the laboratory.
Carefully roll the swab (urethral or cervical) onto a slide to avoid disrupting cells.

IV. Fixation of smear:
1. Let the smear air-dry rather than drying it over a flame.
2. Fix the smear with 2-3 drops of absolute methanol. Allow the methanol to dry on the smear (for about 2 minutes).

V. Staining procedure:
1. Flood the slide with crystal violet for 1 min, and then rinse with a gentle stream of tap water.
2. Flood the slide with Gram's iodine for 1 min, and then rinse with a gentle stream of tap water.
3. Rinse the slide with decolorizing solution until purple no longer runs from the thinnest part of the smear.
4. Flood the slide with safranin for approximately 1 minute, and then rinse with water.
5. To dry the smear, blot it gently on a clean paper towel (do not rub).

VI. Examination of stained smears:
1. Focus at low power on a region of a smeared and stained specimen which is well-spread and stained (not too thin, nor too thick).
2. Rotate nosepiece to 40x objective, locate desired portion of specimen in the center of the field. Refocus very carefully so that the specimen is focused as sharply as possible. (Do not alter focus for the following steps)
3. Partially rotate nosepiece so that 40x and 100x objectives straddle the specimen.
4. Place a small drop of immersion oil on the slide in the center of the lighted area.
5. Rotate nosepiece so that the 100x oil immersion objective touches the oil and clicks into place.
6. Focus only with fine adjustment. Hopefully, the specimen will come into focus easily. Do not change focus dramatically. If you still have trouble, move the slide slightly left and right, looking for movement in the visual field, and focus on the object which moved.
7. Never go back to the 10x or 40x objectives after you have applied oil to the specimen since oil can ruin the lower power objectives.
8. When you have finished for the day, wipe the 100x oil immersion objective carefully with lens paper to remove all oil. Cleanse stage. Recap the immersion oil container securely, replace in drawer.

VII. Interpretation:
Cells and mucus should stain Red. Yeast stains violet. Bacteria are characterized as Gram-positive (violet) or Gram-negative (red), and as cocci (round), bacilli (rod-shaped), or coccobacilli (small in size with morphology in between rods and cocci).
Gonococci appear as Gram-negative diplococci within polymorphonuclear leukocytes.

1. Examine a slide for at least 2 minutes before concluding it does not contain any Gram-negative intracellular diplococci.
2. In the case of cervical smears, express number of PMLs present in the slide as more than, or less than, 25 per oil immersion high power field. This should be arrived at by averaging the PML count in five different oil immersion HPFs.
3. In the case of urethral smears, express number of PMLs present in the slide as more than, or less than, 5 per oil immersion high power field. This should be arrived at by averaging the PML count in five different oil immersion HPFs.

VIII. Sources of error:
1. "Scrubbing" rather than rolling the swab across the slide may destroy cellular morphology.
2. Failure to fix the slide may cause material to wash off during staining.
3. Over-decolorizing the slide may cause Gram-positive bacteria to appear Gram-negative.
4. Under-decolorizing the slide may cause Gram-negative bacteria to appear Gram-positive.
5. Reagents contaminated with bacteria or yeast may give spurious results.
6. Sedimentation of crystal violet.
D. Wet Mount

I. Principle:
The saline wet preparation of vaginal discharge is easily prepared and is used for the rapid detection of Trichomonas vaginalis and "clue" cells associated with bacterial vaginosis.

II. Materials required:
1. Microscope
2. Slides
3. Coverslips (22 x 22 mm)
4. Saline
5. Cotton-tipped swabs
6. Brightfield microscope

III. Procedure:
Clinicians will collect samples from the patients, prepare mounts on slides and send the mounts to the laboratory.
1. Place a drop of saline on a glass slide; then mix with a drop of vaginal fluid collected on swab.
2. Cover the wet preparation with a coverslip avoiding the formation of air bubbles.
3. Send the prepared wet mount slide to the laboratory immediately after preparation.

IV. Examination:
Laboratory person will examine the wet mount slide immediately after he or she receives it in the laboratory.
1. Examine the prepared slide, microscopically, under 10x objective of microscope (low power objective).
2. Confirm the presence of Trichomonads or Clue cells under 40x objective of microscope (high dry objective).

V. Interpretation:
1. Trichomonads: Trichomonads are best recognized by their typical jerky motility. Trichomonas vaginalis is a pear shaped parasite. It contains a central nucleus, four anterior flagella and an undulating membrane.
2. Clue cells: Clue cells are squamous epithelial cells covered with many small coccobacillary organisms, giving a stippled, granular aspect; the edges of these cells are not clearly defined, owing to a large number of bacteria present and the apparent disintegration of the cells. In most patients with BV, a mixture of normal exfoliated vaginal epithelial cells and 20% or more clue cells will be seen.

E. KOH Mount/Whiff test:

I. Principle:
The KOH preparation is used to detect yeast. In addition, a characteristic amine odor may be observed in patients with Bacterial Vaginosis when vaginal secretion is mixed with 10% KOH.

II. Materials required:
1. Microscope
2. Slides
3. Coverslips (22 x 22 mm)
4. 10% KOH
5. Cotton-tipped swabs
6. Brightfield microscope

III. Preparation of 10% KOH:
1. Weigh 10 gram of potassium hydroxide (KOH) using a digital balance and pour in a measuring cylinder.
2. Add distilled water in the cylinder to make the final volume 100 ml.
3. Shake the mixture well.
4. Pour the solution in a bottle and store at room temperature.
5. Thus prepared solution can be used for 1 month.

IV. Procedure:
Clinicians will collect samples from the patients, prepare mounts on slides and send the mounts to the laboratory.
1. Collect the vaginal specimen on a swab, and then roll the swab on a small area of the slide.
2. Add a drop of 10% KOH (potassium hydroxide) and mix with a wooden applicator or swab.
3. Sniff for a "fishy" odor (Whiff test).
4. Cover with a coverslip; avoid trapping air bubbles.
5. Send the prepared KOH mount slide to the laboratory immediately after preparation.

V. Examination:
Laboratory person will examine the KOH mount slide immediately after he or she receives it in the laboratory.
1. Examine the prepared slide, microscopically, under 10x objective of microscope (low power objective).
2. Confirm the presence of Yeast cells and Pseudohyphae under 40x objective of microscope (high dry objective).

VI. Interpretation:
Yeasts may be found in two forms:
1. Budding yeast cells: round to ovoid cells, approximately 4μm in diameter, showing typical budding
2. Pseudohyphae: tubular and branching form

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A. Use and Care of Laboratory Equipments:

I. Background:
Good functioning laboratory equipment is an important part of quality assurance. All our equipment has to be kept in good order, which is achieved by maintenance. Maintenance includes appropriate setting up of new equipment, proper daily handling, regular cleaning, routine maintenance, preventive maintenance and appropriate repairs. Microscope, Centrifuge, Micropipette and Refrigerator are the most used laboratory equipments in STI/VCT laboratory.

II. Microscope:

a. Installation and storage:
1. Install the microscope on a sturdy, level table. Equipment and instruments which generate vibrations, such as centrifuges and refrigerators, should not be placed on or near this table.
2. The height of the table should be convenient for the user.
3. The table should be away from water, sinks, and racks containing chemicals, to prevent damage to the microscope from splashes or spills.
4. As much as is possible, the microscopy room should be free from dust and should not be damp.
5. If the microscope is to be used every day, do not remove it from the site of installation, provided security is assured.
6. When the microscope is not in use, keep it covered with a polythene or plastic cover and take necessary precautions against fungus.
7. In humid areas, store the microscope every night in a cabinet fitted with an electric bulb (5 W or 40 W). This is switched on at night to reduce humidity.
8. If the microscope is used intermittently and requires storage for prolonged periods, keep it in an air-tight plastic bag with about 100g of drying agent. Remember to regenerate/replace drying agents (silica gel or dry rice) fortnightly or as needed.
9. If only a wooden box is available, keep the microscope in it with some dry silica gel or dry rice.

b. Routine Operation of the Microscope:
1. Ensure that the voltage supply in the laboratory corresponds to that permitted for the microscope; use a voltage protection device, if necessary.
2. Turn on the light source of the microscope.
3. With the light intensity knob, decrease the light while using the low magnification objective.
4. Place a specimen slide on the stage. Make sure the slide is not placed upside down. Secure the slide to the slide holder of the mechanical stage.
5. Rotate the nosepiece to the 10x objective, and raise the stage to its maximum.
6. Move the stage with the adjustment knobs to bring the desired section of the slide into the field of view.
7. Focus the specimen under 10x objective using the coarse focusing knob and lowering the stage.
8. To switch to the high power objective lens, look at the microscope from the side. CAREFULLY revolve the nosepiece until the high-power objective lens clicks into place. Make sure the lens does not hit the slide.
9. Looking through the eyepiece, turn the fine adjustment knob until the specimen comes into focus.
Process of using the oil immersion lens:

1. Focus at low power on a region of a smeared and stained specimen which is well-spread and stained (not too thin, nor too thick).
2. Rotate nosepiece to 40x objective and locate desired portion of specimen in the center of the field. Refocus very carefully so that the specimen is focused as sharply as possible. (Do not alter focus for the following steps)
3. Partially rotate nosepiece so that 40x and 100x objectives straddle the specimen.
4. Place a small drop of oil on the slide in the center of the lighted area.
5. Rotate nosepiece so that the 100x oil immersion objective touches the oil and clicks into place.
6. Increase the light by turning the intensity knob until a bright but comfortable illumination is achieved.
7. Focus the specimen by turning the fine focusing knob.
8. When the reading/observation has been recorded, rotate the objective away from the slide.
9. Release the tension of the slide holder, and remove the slide.
10. Wipe oil from the objective with lens paper at the end of each session of use.
11. Turn off the light.
12. Cover the microscope when not in use and take necessary precautions against fungus.

c. Maintenance of lenses:

1. Always keep the eye-pieces in place to protect the inner surface of the objective.
2. Close the holes of missing objectives in the nosepiece by using special caps that are provided, or by sealing with adhesive tape.
3. Check for dust or dirt on the lenses (eye-pieces, objective, condenser and illuminator lenses) by observing whether the image appears hazy or with black dots. If the black dot moves when the eye-piece is rotated, this means that the dust is on the eye-piece. If the black dot moves when the slide moves then the dust is present on the slide. If these two are ruled out, presume that the dust is on the objective. Dust on objectives shows as dots if it is on the inside. If the dust is on the outside of the objective, it shows as a hazy image.
4. Do not remove the dust from the lenses by wiping with a cloth as this can scratch the lens and damage it permanently.
5. Use an airbrush or a camel-hair/artist's brush.
6. Dust can be removed with a camel-hair/artist's brush or by blowing air over the lens with an airbrush. Dust on the inner surface of the objective can be removed by using a soft camel-hair brush (artist's brush).
7. The presence of oil on the lense produces a hazy image. The localization of oil can be done by the same method as has been described above for localization of dust.
8. Oil should be removed with the help of lens paper using lens cleaning fluid as recommended by the manufacturer. This can be applied gently with lens paper. Don't use force to remove oil as this might result in scratches on the lens.
9. If the field of view is not clear despite cleaning, and the microscope works well with another lens, then the lens has been permanently damaged and must be repaired or replaced.
10. If the field of view is not clear even after changing the lenses (objective and eye-piece) there is probably dirt or fungus on the tube prisms. These can be checked by removing the eye-pieces, and examining the upper part of the microscope tube with the light fully open. Fungus is seen as threads, dots or a woolly layer.
III. Centrifuge:
Appropriate use of the centrifuge will maintain it in good condition for a long time.
1. Place it on a firm level bench, away from the edge.
2. Make sure the load is balanced at all times.
3. Turn the speed control up and down slowly.
4. Stop the centrifuge immediately if it makes an abnormal noise.
5. Never use a higher speed than necessary.
6. Always close the lid during centrifugation to avoid dangerous aerosols.
7. Never attempt to stop a moving centrifuge by hand.
8. Clean any sample spillage and disinfect immediately.
9. Clean regularly with disinfectant (once a week).
10. Check for corrosion and cracks every 3 months; clean and paint with anticorrosive paint when necessary.

IV. Micropipette:
Pipetting procedure:
1. Preparation: Hold the pipette in a vertical position. Depress the plunger smoothly to the first stop position.

2. Aspiration: Immerse the pipette into the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip. Keeping the pipette upright remove the pipette from the liquid.
3. **Distribution**: Place the pipette tip at an angle against the inside wall of the receiving tube. Depress the plunger smoothly to the first stop position.

4. **Purge**: Wait one second, then depress the plunger to the second stop position; this removes any remaining sample from the tip. Remove the pipette tip end from the sidewall by sliding it up the wall.

5. **Home**: Allow the plunger to move up to the rest position.

**Precautions:**
1. Select the appropriate pipette for the volume required.
2. While pipetting, ensure that the pipettor, tips, and specimen are at the same temperature.
3. While pipetting, firmly attach tip and avoid air bubbles.
4. Never lay the pipette on its side when liquid is in the tip - doing so will cause the specimen to flow into the pipette shaft and damage the pipette.
5. Never re-use a pipette tip; this will cause cross contamination and compromise patient results. A fresh tip should be used for each sample.

V. Refrigerator:
A laboratory refrigerator is normally an explosion-proof insulated cabinet capable of reducing and holding temperatures to levels above the freezing point of water. There is usually an adjustable control for varying the internal temperature. This unit is used to store reagents and blood products.

Use and Care of Refrigerator:
1. Keep refrigerator organized
2. Periodically clean inside and outside
3. Ensure door is completely sealed when closing
4. Do not store food items or beverages in laboratory refrigerator or freezer
5. Monitor internal temperature of refrigerator daily and make sure it is within the acceptable temperature range (2°C to 8°C).
6. If the temperature range is unacceptable, adjust and record.
7. When adjustments fail to correct a problem, or if gaskets and/or seals are faulty, notify supervisor and medical maintenance staff.
8. Attach the form for recording temperatures directly on the front of the refrigerator for easy access. Inserting it into a protector page will guard against tearing.
9. Attach, on the door of the refrigerator, a list of materials kept in the refrigerator.
10. Use a voltage stabilizer to supply electricity to the refrigerator.
1. Keep these records at your test site:
   - Client test result
   - Lab/Test register
   - Temperature logs
   - Equipment maintenance logs
   - Inventory records
   - Specimen transfer logs
   - Daily record of quality control results

2. Keep facilities where records are kept secure to maintain patient/client confidentiality. Prevent unauthorized access.

3. Records should be permanent, secure, and traceable.

4. To keep records secure, limit access and protect them from environmental hazards. To keep records traceable, make sure every record is signed and dated.

5. The reporting pattern should be as described in the standard operating procedure (SOP).

6. A laboratory report is produced by recording the test results and placing it in the patient's file. The VCT counselor or clinician is provided with the patient's file.

C. External Quality Assessment (EQA):

   External Quality Assessment (EQA) is the objective assessment of a test site’s operations and performance by an external agency or personnel. EQA allows comparison of performance and results among different test sites offering not only an opportunity for performances checks, but an opportunity to systematically identify problems with kits or operations. Additionally, EQA also provides objective evidence of testing quality, indicates areas that need improvement and identifies training needs.

Dried Blood Spot (DBS) Specimens: Dried Blood Spots (DBS) are whole blood collected on filter paper and dried. They are made directly from the client's whole blood. DBS are used for re-testing at a reference laboratory. Testing site results are compared to reference laboratory results. DBS samples are useful for re-testing as they are easy to collect, store, and transport.

1. Collect valid specimens according to standard operating procedures (SOP) for collection of DBS specimens.
2. Label and store appropriately until transported for re-testing.
3. Ensure records are properly maintained.
4. Avoid transcription errors.
5. Send the collected specimens to reference laboratory appropriately. Include appropriate documentation with the shipment.

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Laboratory safety precautions are essential and should be followed at all times to minimize occupational risks. All laboratory staff must be familiar with, and follow the Universal Precautions for the prevention of transmission of diseases.

Laboratory staff must follow all laboratory safety procedures in order to:

- Prevent injury or infection to laboratory staff and others.
- Maintain a safe laboratory environment for effective laboratory services.

A. Infection prevention procedures:

I. Background:
   Biological infections or injury among laboratory staff may occur due to accidental inoculation by needles, cuts by contaminated instruments, and spillage or splattering of clinical specimens on floors, bench tops, table tops etc. Infection prevention procedures help to minimize the risk of injury and transmission of infection to laboratory staff.

II. Procedures:
   1. Maintain cleanliness in the laboratory.
   2. Always work in sufficient light to minimize error in laboratory procedures.
   3. Do not eat, drink or store food in the laboratory.
   4. Wear a laboratory coat over clothing for additional protection clothing while working in the laboratory.
   5. Do not pipette by mouth.
   6. Handle sharp, pointed materials carefully and dispose of them separately in a puncture proof container.
   7. Wear gloves while performing the finger prick or venipuncture procedure and performing tests. Gloves must be changed for each new patient to reduce the risk of cross infection.
   8. Always wear gloves while handling clinical specimens.
   9. Always wear gloves while handling soiled instruments.
   10. Always wear gloves while handling contaminated waste (e.g. cotton gauze).
   11. After finishing laboratory work, disinfect the working table surface. For disinfection, wipe the working table surface with a piece of cloth soaked in 0.5% sodium hypochlorite.
   12. Incinerate laboratory waste by controlled burning in an incinerator that can be made locally.
   13. Wash hands thoroughly after completing laboratory work.
   14. Do not wear jewelries while working in the laboratory.
   15. Tie long hair on the back.
B. Hand Washing

I. Background

Hand washing is the most practical procedure for preventing cross-contamination (person to person). Hand washing may be the single most important procedure for preventing infection.

1. Laboratory staff must wash their hands after handling infectious materials, after removing gloves and immediately after accidental skin contact with blood, body fluids or tissues.
2. If contact occurs through breaks in gloves, the gloves should be immediately removed and hands thoroughly washed.
3. Hands should be washed at the completion of work and before leaving the laboratory.
4. Hands should be washed before all other activities which entail hand contact with mucous membranes, eyes and breaks in the skin.

II. Simple hand washing procedure:

1. Remove all jewelry
2. Thoroughly wet hands
3. Apply plain soap
4. Vigorously rub all areas of the hands and fingers together for 10-15 seconds paying special attention to areas under fingernails and between fingers
5. Rinse hand thoroughly with clean running water
6. Dry hands with paper towel

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C. Use of Gloves and other protective barriers
I. Gloves
   All laboratory personnel who come into contact with blood and body fluids must wear gloves.
   1. Wear gloves while collecting specimens.
   2. Change gloves between each patient.
   3. Remove gloves when visibly contaminated.

II. Laboratory coats, gowns and shoes:
   1. Wear a long-sleeved white laboratory coat, which is buttoned closed.
   2. Wear laboratory coat at all times while at the workstation or at times when the possibility of splashing of blood or body fluids exists.
   3. Change Laboratory coats and/or gowns immediately if grossly contaminated with blood or body fluids.
   4. If one’s personal clothing becomes contaminated, remove the article of clothing and send for sterilization.
   5. Do not wear Laboratory coat and gowns outside the laboratory. The only time a laboratory coat is allowed outside the laboratory is for phlebotomy or other technical procedures.
   6. Remove all personal protective equipment prior to leaving the work area.

III. Mask and goggles:
   1. Wear masks and goggles while opening tubes of blood or body fluids.
   2. When opening tubes of such specimens there is a risk of splatter or aerosolization. To reduce this risk, place a gauze pad over the top of the tube while removing the stopper.
   3. Eyeglasses do not provide adequate splash protection. Wear goggles or face shields over eyeglasses.

D. Collection and disposal of laboratory wastes
I. Background
   Much of the waste from health care facilities is contaminated. Contaminated wastes may carry high loads of microorganisms, which are potentially infectious to any person who contacts or handles the waste, and to the community at large, if not disposed of properly. Contaminated wastes include blood, pus, urine, stool and other body fluids, in addition to items which contacted them, such as used dressings.

   Proper handling of contaminated waste is required to minimize the spread of infection to clinic personnel and to the local community. All contaminated materials should be decontaminated (by disinfecting or incinerating) before disposal. Contaminated materials include specimens of body fluids, cotton gauze, broken glassware and used needles. If materials are decontaminated or disposed of outside the laboratory they should be placed in a strong, leak-proof and puncture proof container to transport them from the laboratory to the decontamination site.

II. Preparation of 0.5% Sodium Hypochlorite solution from 5% Sodium Hypochlorite solution
   1. Measure 1 part (e.g. 100ml) of 5% sodium hypochlorite solution with a measuring cylinder and pour in a jar.
   2. Measure 9 parts (e.g. 900ml to make 1 liter of 0.5% solution) of clean water with a measuring cylinder and pour in the jar containing 1 part of 5% sodium hypochlorite solution.
   3. Mix the contents in the jar well.

   Thus prepared solution can be used for up to 24 hours.

   If Sodium Hypochlorite solution available in the market has concentration other than 5% use the following formula to prepare the 0.5% sodium hypochlorite solution:

   $\text{Parts of water to be added to 1 part of concentrated sodium hypochlorite solution} = \left[ \frac{\% \text{ Concentrate}}{\% \text{ Dilute}} \right] - 1$
III. Collection of different types of wastes:
There should be different types of containers for collections depending on types of wastes generated in the clinic and laboratory settings. The person who generates the waste is responsible for putting it in the appropriate containers. Containers for collecting wastes should be designated using defined color-coding.
1. Red color: For collecting contaminated hazardous wastes other than sharps. Used test kits, pipette tips, infected dressing material etc. are solid wastes and collected separately from liquid wastes.
2. Yellow color: For syringes and other sharp wastes generated in the facility. Sharps can be kept in a puncture-proof container with a small hole on the top which allows personnel to put the materials, mainly syringes, into the container.
3. Blue color: For hazard free wastes like paper, plastic covers of syringes and other uninfected materials.
4. Liquid wastes are collected in a container with 0.5% Sodium Hypochlorite solution. There must be enough solution in the container so that even when liquid waste is added, the concentration of the solution remains approximately the same.

IV. Disposal of wastes:
1. Wear utility gloves.
2. Decontaminate all the contaminated waste in the clinic before disposal; contaminated waste includes specimens of body fluids, broken glassware, and containers of contaminated needles.
3. Place materials that are to be decontaminated or disposed of outside into a strong leak-proof covered container prior to transporting them outside.
4. Transport sharp items in puncture-resistant containers.
5. Carefully pour decontaminated liquid waste down a utility drain or flushable toilet.
6. Burn contaminated solid waste in a locally made incinerator.
7. When the sharp container is 3/4 full; cap, plug or tape it tightly closed and send for incineration or burying. Needles and other sharp objects may not be destroyed by burning, and may later cause injuries, which can lead to a serious infection.
8. Decontaminate sharps by dipping in 0.5% hypochlorite solution and then bury.
9. If medical waste cannot be burned, dispose waste by onsite burial.
10. Tubes used for collection of blood should be decontaminated in 0.5% hypochlorite solution first and then disposed by burial (for glass tubes) or by incineration (for plastic tubes).
11. Use the needle destroyer for destruction of used syringe needles.
12. Wash hands, gloves and containers after disposal of infectious waste.
E. Cleaning and Sterilization:
1. Disinfect the laboratory working surfaces twice a day with 0.5% sodium hypochlorite solution; allow the solution to remain in contact with the surface for at least 10 minutes.
2. Wipe refrigerators thoroughly with 0.5% sodium hypochlorite.
3. For materials to be sterilized, immerse them completely into the hypochlorite solution for 10 minutes, then wash with water.
4. Hypochlorite solutions should be prepared fresh and all containers should be refreshed every day.
5. To decontaminate the spillage of blood and body fluids:
   - wear disposable gloves
   - cover visible blood or body fluids with paper towels or newspaper and
   - soak with 0.5% sodium hypochlorite
   - allow it to stand for at least 10 minutes
1. Laboratory Report- *Rapid HIV tests*
2. Laboratory Report- *STI tests*
3. Temperature Chart
4. Laboratory Test Result Register
5. Wekly Record of Quality Control Results
## LABORATORY REPORT - RAPID HIV TESTS

### Client Test Result

**CONFIDENTIAL**

**Laboratory ID:**

**Date:** __/__/__

**Client Name (Optional):**

### LABORATORY REPORT - RAPID HIV TESTS

<table>
<thead>
<tr>
<th>Test name</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Determine™ HIV-1/2</td>
<td>☐ REACTIVE ☐ NON REACTIVE</td>
</tr>
<tr>
<td>2. Uni-Gold™ HIV</td>
<td>☐ REACTIVE ☐ NON REACTIVE</td>
</tr>
<tr>
<td>3. SD Bioline HIV-1/HIV-2</td>
<td>☐ REACTIVE ☐ NON REACTIVE</td>
</tr>
</tbody>
</table>

### FINAL RESULTS: *(To be reported to client)*

☐ POSITIVE ☐ NEGATIVE

**NOTE:** A negative result does not necessarily indicate that the individual is not infected. If the individual had unprotected sex, shared injecting equipment, or received unscreened blood products, or had an occupational exposure in either the three month period before the test was performed or after blood was drawn, they may be infected. The HIV antibody test may not detect recently acquired HIV infection.

---

**Authorized Signature**

---

**Site Name**

**Address**

**Phone Number**
## LABORATORY REPORT - STI TESTS

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Gram staining</td>
<td><strong>GNID (Gram Negative Intracellular Diplococci):</strong> Seen ☐ Not Seen ☐</td>
</tr>
<tr>
<td></td>
<td><strong>PMN Leukocytes:</strong>_________/HPF</td>
</tr>
<tr>
<td>2. KOH Mount</td>
<td><strong>Fungal element:</strong> Seen ☐ Not Seen ☐</td>
</tr>
<tr>
<td>3. Wet Mount</td>
<td><strong>Clue Cells:</strong> Seen ☐ Not Seen ☐</td>
</tr>
<tr>
<td></td>
<td><strong>Trichomonas vaginalis:</strong> Seen ☐ Not Seen ☐</td>
</tr>
<tr>
<td>4. RPR Test</td>
<td><strong>Reactive ☐ Non reactive ☐</strong> Titer: __________</td>
</tr>
<tr>
<td>5. TPPA Test</td>
<td><strong>Positive ☐ Negative ☐</strong></td>
</tr>
</tbody>
</table>

---

Client Test Result
CONFIDENTIAL

laboratory ID: ___________ Date: __/__/__

Client Name (Optional):

---

Authorized Signature

---

Site Name ___________ Address ___________ Phone Number ___________
# TEMPERATURE CHART

Name of organisation: ____________________________

| Date | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Temp.| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10| 10|
| 10°C |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 9°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 8°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 7°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 6°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 5°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 4°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 3°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 2°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 1°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 0°C  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| -1°C |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| -2°C |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

Note: record the temperature every day at 10:00 am and 4:00 pm and tick in the appropriate temperature box.

Initials: ____________________________

Supervisor: ____________________________
Name: ____________________________
Date: ____________________________
## LABORATORY TEST RESULT REGISTER

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Date</th>
<th>Client ID</th>
<th>Age/Sex</th>
<th>Type of Visit</th>
<th>Determine HIV-1/2</th>
<th>Uni-Gold HIV</th>
<th>Capillus HIV-1/2</th>
<th>Final Test Result</th>
<th>RPR Titer</th>
<th>TPPA</th>
<th>Gram Staining</th>
<th>Wet Mount</th>
<th>KOH Mount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Gnid</td>
<td>PMN</td>
<td>Clue cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fungal elements</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Laboratory test results**

- **rapid HIV testing**
- **Syphilis Serology**
- **Microscopic Investigations**

- **Laboratory tests:**
- **Unigold HIV**
- **Capillus HIV-1/2**
- **Final Test Result**
- **RPR Titer**
- **TPPA**
- **Gram Staining**
- **Wet Mount**
- **KOH Mount**
- **Gnid**
- **PMN**
- **TV**
- **Clue cells**
- **Fungal elements**
## Weekly Record of Quality Control Results

<table>
<thead>
<tr>
<th>Kit:</th>
<th>Lot #:</th>
<th>Expiration Date:</th>
<th>Date:</th>
<th>Negative Control result</th>
<th>Acceptable?</th>
<th>Y/N</th>
<th>Pos Control Lot #:</th>
<th>Positive Control Result</th>
<th>Low Pos</th>
<th>Pos</th>
<th>Acceptable?</th>
<th>Y/N</th>
<th>Corrective Actions</th>
<th>Action Taken</th>
<th>Date:</th>
<th>Reviewed by &amp; Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ANNEX 3: Weekly Record of Quality Control Results**
BIBLIOGRAPHY:

8. WHO, Regional Office for South-East Asia, New Delhi, India. The Microscope, A practical guide 1999.
Standard Operating Procedures for Laboratory Services at Integrated Health Service Centers

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