Ministry of Health
National Reference Laboratory

LABORATORY STANDARD OPERATING PROCEDURES FOR ANTIRETROVIRAL TREATEMENT PROGRAM IN RWANDA

July 2005

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Contents

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Introduction

The Ministry of Health recognizes the need to develop a National Public Health Laboratory system to support the Antiretroviral Treatment (ART) program as high priority. The care and treatment of HIV/AIDS requires the provision of laboratory testing services at all levels of health facility, including HIV diagnosis, CD4 count, clinical chemistry and hematology. The success of ART program is dependent on the ability to diagnose and qualify HIV-positive patients for therapy and to monitor treatment efficacy, toxicity and drug resistance.

While some existing laboratory facilities in the country are able to provide the necessary tests, currently there are several initiatives underway to ensure that a range of laboratory tests necessary for ART program are available to all Rwandans.

In order to provide quality laboratory services, the National Reference Laboratory in collaboration with Management Sciences for Health / Rational Pharmaceutical Management Plus have developed Standard Operating Procedures (SOPs) for laboratory tests in support of ART program.

This SOPs manual is useful in improving and maintaining quality laboratory services by providing laboratory personnel with standardized written information on how to perform tests. The SOPs will also provide guidance in the procurement of laboratory reagents and supplies.

Serology

Clinical significance

This test is used for screening antibodies against HIV infection.

Principles of immunochromatography (Determine method)

Determine is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. The sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilised recombinant antigens and synthetic peptides at the patient window site.

- If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site.
- If antibodies to HIV-1 and/or HIV-2 are absent, the antigen-selenium colloid flows past the patient window and no red line is formed at the patient window site.

Specimen collection and storage

Serum, plasma, and whole blood collected by venipuncture should be collected aseptically in such a way as to avoid haemolysis.

- Serum and plasma should be stored at 2–8ºC if the test is to be run within 7 days of collection. If the testing is delayed more than 7 days, the specimen should be frozen at -20 ºC .
- Whole blood should be stored at 2–8ºC if the test is to be run within 7 days.
- Whole blood collected by fingerstick should be tested immediately.

Reagents and samples should be at room temperature before testing.

Requirements

Refer to the product literature.

Equipment
Automatic precision pipettes

Reagents
Abbott Determine test kit

Procedure

Remove the protective foil cover from each test.

For Serum and Plasma

- Apply 50 µL of sample (precision pipette) to the sample pad (marked by the arrow symbol).
- Wait a minimum of 15 minutes (up to 60 minutes) and read results.

For Whole Blood

- Apply 50 µL of sample (precision pipette) to the sample pad (marked by the arrow symbol).
- Wait one minute, then apply one drop of chase buffer to the sample pad.
- Wait a minimum of 15 minutes (up to 60 minutes) and read results.

For Whole Blood (Fingerstick)

- Apply 50 µL of sample (precision pipette) to the sample pad (marked by the arrow symbol).
- Wait until blood is absorbed into the sample pad, then apply one drop of chase buffer to the sample pad.
- Wait a minimum of 15 minutes (up to 60 minutes) and read result

Quality Control

To ensure assay validity, a procedural control bar is incorporated in the assay device and is seen in the window labelled “control.”

Results

Report as either reactive or non reactive.

Interpretation of Results

• *Reactive (positive) (two bars):* Red bars appear in both the control window and the patient window of the strip. Any visible red colour in the patient window should be interpreted as reactive.
• *Nonreactive (negative) (one bar):* One red bar appears in the control window of the strip and no red bar appears in the patient window of the strip.
• *Invalid (no bar):* If there is no red bar in the control window of the strip (even if a red bar appears in the patient window), the result is invalid and should be repeated.

Test Limitations and Sources of Error

• The Abbott Determine HIV-1/2 is designed to detect antibodies to HIV-1 and HIV-2 in human serum, plasma, and whole blood. Other body fluids or pooled specimens may not give accurate results.
• Lack of colour in the patient bar does not exclude the possibility of infection with HIV. A false negative result can occur in the following circumstances:

- Low levels of antibody (e.g., early seroconversion specimens) that are below the detection limit of the test
- Infection with a variant of the virus that is less detectable by the Determine HIV assay configuration
- HIV antibodies in the patient that do not react with specific antigens utilised in the assay configuration specimen-handling conditions, resulting in loss of HIV multivalency

Whole blood or plasma specimens containing anticoagulants other than EDTA may give incorrect results.

Disposal of waste materials

All materials used in the test must be disinfected preferably in 1% sodium hypochlorite (Household bleach) before disposal.

References

Central Board of Health (CBOH). 2003. *Serology Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

User's manual for Determine HIV 1/2. Abbott Laboratories, Illinois, USA.

SOP TITLE: RAPID HIV SCREENING TEST (UNIGOLD METHOD)
SOP No.: SERO 002 / 2005
DATE: July 2005

Clinical significance

This test is used for screening antibodies against HIV infection.

Principles of immunochromatography (UNIGOLD method)

Recombinant proteins representing the immunodominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilised at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitised as a control region.

Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the colloidal gold linked antigens. The antibody protein-colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test devise.

A positive reaction is visualised by a pink/red band in the region of the device.

Specimen collection and storage

Whole blood or serum may be used. If the sample cannot be tested on the same day of collection it should be stored at 2 to 8(C. Serum samples not required for testing within 48 hours should be stored at -15 to -25(C. The use of haemolysed samples, incompletely clotted blood, or samples contaminated with bacteria must be avoided. Multiple freeze-thaw cycles of samples must also be avoided.

Reagents and samples should be at room temperature before testing.

Requirements
Refer to the product literature.

Procedure
Refer to the product literature.

Quality control
This test has an in-house quality control of both positive and negative samples.

Interpretation of results

Refer to the product literature

Test Limitations and sources of error

A negative result does not exclude the possibility for a patient being in the window period or having been exposed to HIV infection. All positive results must be repeated using another rapid test (CAPILLUS). If the results are discordant, perform the ELISA.
Refer to the product literature

Disposal of test materials

All materials used in the test must be disinfected preferably in 1% sodium hypochlorite (Household bleach) before disposal.

References

Uni-Gold HIV, TRINITY BIOTECH PLC,

Web Site: www.trinity biotech.com

SOP TITLE: RAPID HIV SCREENING TEST (CAPILLUS METHOD)
SOP No.: SERO 003 / 2005
DATE: July 2005

Clinical significance

This test is used for screening antibodies against HIV infection.

Principles of the latex agglutination (Capillus method)

The latex agglutination test uses latex beads coated with recombinant or synthetic antigen. Small amounts of whole blood or serum are mixed with latex beads and rotated on a card. After several minutes, the tests are evaluated by eye in bright light for level of agglutination relative to that of the negative control.

Specimen collection and storage

Whole blood or serum may be used. If the sample cannot be tested on the same day of collection it should be stored at 2 to 8(C. Serum samples not required for testing within 48 hours should be stored at -15 to -25(C. The use of haemolysed samples, incompletely clotted blood, or samples contaminated with bacteria must be avoided. Multiple freeze-thaw cycles of samples must also be avoided.

Reagents and samples should be at room temperature before testing.

Requirements
Refer to the product literature.

Procedure
Refer to the product literature.

Quality control
This test has an in-house quality control of both positive and negative samples.

Interpretation of results
Refer to the product literature.

Limitations and Sources of error

Refer to the product literature
A negative result does not exclude the possibility for a patient being in the window period or having been exposed to HIV infection. All positive results must be repeated using an other rapid test (UNIGOLD). If the results are discordant, perform the ELISA.

Disposal of test materials

All materials used in the test must be disinfected preferably in 1% sodium hypochlorite (Household bleach) before disposal.

References

Central Board of Health (CBOH). 2003. *Serology Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

CAPILLUS HIV-!/HIV-2, Trinitybiotech PLC,

Web Site: www.trinitybiotech.com

SOP TITLE: ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR HIV SCREENING
SOP No.: SERO 004 / 2005
DATE: July 2005

Clinical significance

The assay is used for the detection of antibodies against HIV infection.

Principle

Diluted plasma or serum samples or controls are added to antigen coated microwells. Antibodies to the virus (env or gag antigens) if present will bind to the antigen coated wells. After removal of unbound antibody by washing, enzyme-labelled antihuman conjugate is added to each well and allowed to bind to antibody during the period of incubation. Unbound conjugate is removed by washing, followed by the addition of a substrate solution to all the wells. A colour will develop when anti HIV-1 or Anti-HIV-2 antibodies (IgG and or IgM) are present. Addition of a stop solution changes this colour to brown. The intensity of the colour produced is proportional to the amount of antibody bound to the antigen in the microwell. The intensity of the colour produced is read spectrophotometrically.

Specimen collection and storage

Serum or plasma may be used. If the sample cannot be tested on the same day of collection it should be stored at 2 to 8(C. Samples not required for testing within 48 hours should be stored at -15 to -25(C. The use of haemolysed sample, incompletely clotted blood, or samples contaminated with bacteria must be avoided. Repeated freezing and thawing of samples should also be avoided.

Reagents and samples should be at room temperature before testing

Requirements

1. ELISA reader
2. ELISA kit
3. ELISA washer
4. Water bath of incubator with relative humidity
5. Automatic pipettes (single and multi-channel)
6. Glassware
7. Timer

Procedure

Refer to the product literature.

Interpretation of results

Samples giving an absorbance value of less than the cut-off value are considered negative. Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive in the assay and should be confirmed by another, preferably a specific, method.

Quality control

The assay has an in-house quality control system of both positive and negative samples. Manufacturers always specify the lower and upper limits for cut-off validity.

Test limitation and Sources of error

- The assay is used as a screening test, or as a supplementary test if advocated for.
- A negative result does not exclude the possibility of a patient being in window period or having been exposed to HIV infection.
- Occasional false positive results have been shown to occur with this assay.

Refer to manufacturer's instruction but the following are some of the common sources of error

- Impure distilled water
- Wrong storage of reagents
- Inadequate washing and wrong dilution
- Poor technique

Disposal of test materials

Test plates and other materials used in the test should be disinfected, preferably with 1% sodium hypochlorite solution (household bleach), before disposal.
The plates should then either be burnt or buried with other waste materials.

References

Central Board of Health (CBOH). 2003. *Serology Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

Microelisa system, VIRONOSTIKA HIV Uni-Form II Plus 0, Biomerieux
Enzygnost Anti-HIV ½ Plus

SOP TITLE: RAPID PLASMA REAGIN (RPR) TEST
SOP No.: SERO 005 / 2005
DATE: July 2005

Clinical significance of the test

This is a serological test used in the screening of syphilis, a disease caused by Treponema pallidum.

Principle

RPR is a non-specific test that measures circulating antibodies against reaginic proteins that occur in the blood due to tissue damage caused by treponemal and non-treponemal infections. The test is based on the principle of flocculation. The RPR carbon antigen is a colloidal suspension of lecithin, cholesterol and cardiolipin. These compounds are coated on microparticulate carbon. The carbon enhances the difference in appearance between a positive and a negative reaction. A positive reaction is characterised by agglutination. A negative reaction is characterised by the absence of agglutination. The result is read visually or with the aid of a magnifying glass.

Specimen collection and storage

Serum and plasma samples may be used. If the sample cannot be tested on the day of collection it should be stored at 4 to 8°C. Samples not required for testing within 48 hours should be stored at -15 to -25°C. The use of haemolysed samples or samples suspected to be contaminated with bacteria must not be used to avoid false positive results. Repeated freezing and thawing of samples should be avoided

Reagents should be at room temperature before testing.

Requirements

1. RPR test kit (containing a bottle of carbon coated antigen, test cards, sample dispenser/mixers, a dropper for antigen dispensing, positive and negative control sera).
2. Mechanical rotator with a horizontal stage operating at 100rpms
3. Centrifuge
4. Timer

Procedure

1. Put serum and reagent at room temperature before testing.
2. Put a drop of well mixed antigen on the reaction card circle
3. Place a drop of patient's sample on a reaction circle containing the antigen

4. Mix the sample and antigen and spread the mixture to the full area of the

circle.
5. Rotate for 8 minutes using the mechanical rotator.
6. Immediately after 8 minutes, read the results by visual inspection in good light.
7. Record the result

It is important to refer to the product literature provided in the RPR test kit for specific instructions.

Quality control

Each run of the test should include control samples (which came with the kit, or known samples). These controls must give the expected pattern for the test run to be valid.

Interpretation of results

A positive result is indicated by the presence of clearly visible clumps of the black particles. In a negative result the carbon particles remain in even suspension, no aggregates are visible. Results should be reported as “Reactive” or “Non-reactive”. All positive results should be confirmed by specific tests to rule out false-positive results.

Test Limitation and Sources of error

RPR is not a specific antibody test against Treponema pallidum, the causative agent for syphilis. The test has been shown to give positive results in other conditions such as acute malaria, leprosy and in pregnancy. Specific tests like the Treponema Pallidum HaemAgglutination (TPHA) or Fluorescent Treponema Antibody-Absorption (FTA-Abs) tests must be used to confirm positive results.
Uneven mixing of the antigen suspension results in the carbon particles settling at the bottom of the bottle and only the watery part of antigen being dispensed.
Failure to observe specified time for reaction. The results must be read immediately after 8 minutes for them to be valid.
Test kits must not be used after expiry. Attention must be paid to expiry dates.
Any visible or particulate matter must be removed from the sample prior to testing

Disposal of waste materials

The test cards are not re-usable. The disposal of the cards is very easy as they can be burnt, or dispose of with other rubbish after adequate disinfection, preferably with 1% sodium hypochlorite (household bleach)

References

Central Board of Health (CBOH). 2003. *Serology Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

Fortress Diagnostics Limited, BT41 1QS, United Kingdom, Reviewed April 2000

SOP TITLE: PREGNANCY TESTING
SOP No.: SERO 006/ 2005
DATE: July 2005

Clinical significance of the test

Pregnancy testing is carried out to detect pregnancy in women. Tests designed to detect pregnancy can be divided into biological and immunological types. All of them detect the hormone chorionic gonadotrophin (hCG) in maternal urine. Biological tests rely on the physiological effects of hCG on a variety of animals. Immunological tests employ an antibody to hCG as an essential reagent.
There are several approaches to immunological testing and these include direct latex agglutination, latex agglutination-inhibition, haemagglutination-inhibition, agar immunodiffusion and radio-immunoassay.

Principles

Direct latex agglutination. The direct latex agglutination tests fix antibody against hCG to latex particles. The presence of hCG in the test sample will produce agglutination. A positive test, is therefore, associated with a visible agglutination reaction. As a general rule, the end point of these assays is more difficult to detect than that of the agglutination-inhibition reactions.

Latex agglutination-inhibition. Latex agglutination-inhibition tests are very popular because of their speed. The patient's urine is mixed with a reagent containing antibodies to hCG. Next the polystyrene latex particles coated with hCG are added. If hCG is present in the sample, it neutralises the antisera and no reaction will be seen when the latex is added. On the other hand, if no hCG is present in the specimen, the antisera will react with the sensitised reagent and agglutination will take place. Hence, a positive test is indicated by the absence of agglutination within a 2-minute period

Haemagglutination inhibition. This approach relies on hCG being adsorbed onto formalin-treated red blood cells. Antiserum and sample are first combined; the red blood cells are then added. If hCG is present, the antibody will be blocked and agglutination will then form a characteristic sedimentation pattern. If no hCG is present, the antibody will produce haemagglutination and diffuse homogeneous sediment will result.

Immunochromatography: is a unique two-site immunoassay on a membrane. As the test sample flows through the membrane assembly of the dipstick, the colored anti-HCG-colloid gold conjugate complexes with the HCG in the sample. This complex moves further on the membrane to the test region where it is immobilized by the anti-HCG coated on the membrane leading to the formation of a pink colored band, which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any along with rabbit IgG gold conjugate move further

on the membrane and are subsequently immobilized by the gaot anti-rabbit antibodies coated on the membrane at the control region, forming a pink band. This control band services to validate thee test results.

Specimen collection and storage

Urine/serum samples collected at any time of the day may be used. However, the first morning specimen of urine is recommended as it contains the highest concentration of hCG. If there is any delay in testing, samples may be stored at 2 to 8C but the testing should preferably be done as soon as the samples are collected. Please refer to the product literature for specific instructions.

Reagents and samples should be at room temperature before testing.

Requirements

Clean, detergent-free container
Pregnancy testing kit
Timer

Procedure

Refer to product literature.

Quality control

Each test should include control samples. These are usually provided in the test kits. Please refer to the product literature.

Interpretation of results

Refer to product literature.

Test Limitation and Sources of error

A number of conditions other than pregnancy including trophoblastic and non-trophoblastic neoplasms such as hydatiform choriocarcinoma etc. causs elevated levels of HCG. Such clinical conditions must be ruled out before a diagnosis of pregnancy can be made.
Highly dilute urine specimens and specimens from very early pregnancy may not contain representative levels of HCG. If pregnancy is still suspected, repeat the test with first morning urine after 48-72 hours after the initial test.
The result must be correlated with clinical findings.
Proteinuria may interfere with the tests by entering into the immunoreaction.

Thus, it is advisable to perform a “routine” urinalysis on all specimens received for pregnancy testing.

Urine preservatives, eg boric acid

This list is not necessarily complete. Thus, the product literature must be consulted for each test used. Every commercial product is somewhat unique and changes are introduced from time to time. It is important for the laboratory technician to become familiar with the specified details of any technique he/she proposes to use.

Please refer to product literature for specific details

Disposal of waste materials

Specimen containers should be immersed in a suitable disinfectant before cleaning or disposal

References

Central Board of Health (CBOH). 2003. *Serology Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

Core Diagnostics, aspect Court, 4 Temple. Row, Birmingham B2 5HG UK, October 2004

CLINICAL CHEMISTRY

SOP TITLE: SERUM UREA (UREASE BERTHELOT METHOD)
SOP No.: CHEM 001/ 2005
DATE: July 2005

Clinical significance of the test

Urea is the main end product of protein metabolism in the body. Removal of the amino group from Amino acids from which urea is formed takes place in the liver. It is excreted by the kidney in urine. Therefore, its measurement in serum and urine can be used to assess kidney function.

Principle

Urea is hydrolyzed by the action of the urease to produce ammonia and carbon dioxide. The ammonia reacts with hypochlorite and phenol in the presence of nitroprusside to form indophenol, which in alkaline medium gives an intense blue colour. The intensity of the colour formed is directly proportional to concentration of urea in the sample.

Specimen collection and storage

- Serum or heparinised plasma
- Urine (especially 24-hour sample)

Requirements

Equipment

Water bath or heating block at 37ºC
Timer
Spectrophotometer

Reagents

- Urea testing kit
- Low and high controls

Procedures

For kit methods, follow manufacturer's instructions.

Quality Control

Include the low and high controls with each run. The values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be ¡Ü5%.

Result / Calculations of results

Concentration of Urea = Abs. SAMPLE) × Conc. STANDARD (mmol/L)/Abs.STANDARD

Linearity

Test is linear up to 33.3 mmol/L for plasma or serum. Serum samples with urea concentration higher than this limit should be diluted 1 in 5 with normal saline and the result multiplied by 5.
Urine samples must be diluted 1 in 20 with distilled water and the result multiplied by 20.

Reference Range

Serum: 2.0–8.5 mmol/L (0,15-0,45 g /L)
Urine: 333–533 mmol/24h (20-35 g/ 24h)

Interpretation of results

• Increased levels are diagnostic of nephritis, urinary tract infection, and extrarenal disorders.
• Decreased levels are found in malnutrition and are observed in pregnancies.

Test Limitations and Sources of Error

• Avoid turbid, icteric, and haemolysed samples
• Fluoride oxalate bottles must not be used because they inhibit urease.
• All glassware used must be ammonia free.

Disposal of waste materials

All disposables should be discarded in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: CBOH.

bioMerieux 1986, Urea Kit,

SOP TITLE: SERUM CREATININE (JAFFE'S REACTION)
SOP No.: CHEM 002/ 2005
DATE: July 2005

Clinical significance

Creatinine measurement is used in the diagnosis and treatment of renal diseases and monitoring patients on renal dialysis.

Principle

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The intensity of the colour formed is directly proportional to the creatinine concentration in the sample.

Specimen collection and storage

•Serum or plasma (creatinine in serum is stable for 24 hours at 2–8ºC)
• 24-hour urine sample preserved with boric acid or hydrochloric acid. Dilute urine 1 in 50 with distilled water.

Requirement

Equipment
• Spectrophotometer
• Timer
• Water bath/heat block

Reagents
• Creatinine testing kit
• Low and high controls

Note: The working reagent is made by mixing equal volumes of picric acid and sodium hydroxide solutions.

Procedure

For kit methods, follow manufacturer's instructions
Note: When using commercially available kits, prepare reagents and perform assays according to manufacturer's instructions.

Deproteinisation

Use trichloroacetic acid or tungstatic acid as deproteinisers.

Quality Control

The integrity of the reaction will be monitored by use of low and high control sera. The control values should lay within ± 2 SD of the expected range. If the results of the quality control materials are outside this range, the run must be repeated.

Results / Calculations of results

The creatinine concentration of the sample is determined by the following calculation:
Conc Creatinine = Abs (TEST) × Conc (standard)/Abs(STANDARD)

Creatinine conc in urine =Abs (TEST) × Conc of standard × Dilution factor /Abs(STANDARD)

Linearity

If the serum creatinine concentration exceeds 884 µmol/L, dilute 1 in 5 with normal saline solution and repeat the assay. Multiply the results by 5. Similarly, if the urine result exceeds 44.2 mmol/L, dilute the urine 1 in 5.

Reference Ranges

Serum, plasma creatinine Men: 53–120 µmol/L (5-15 mg/L)
Women: 44–100 µmol/L (5-12 mg/L)
Urine creatinine 8.84–13.3 mmol/24h (1-2 g/24h)

Test Limitations and Sources of Error

The determination is not specific and may be affected by the presence of reducing substances such as salicylates.

Disposal of waste materials

All disposables should be discarded in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures forHospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

bioMerieuxProduits et reactifs de laboratoire, Marcy l'Etoile 2003, Creatinine Kit,

www.biomerieux.com

SOP TITLE: SERUM BILIRUBIN TOTAL AND CONJUGATE (JENDRASSIK AND GROF)
SOP No.: CHEM 003/ 2005
DATE: July 2005

Clinical significance

Bilirubin test is used to investigate the causes of liver diseases and jaundice and to monitor patient progress e.g an infant with serious neonatal jaundice (high level of unconjugated bilirubin).

Principle

Total bilirubin is determined by its reaction with diazotised sulphanilic acid in the presence of caffeine to give a red compound, the intensity of which is directly proportional to the concentration of bilirubin.
Direct (conjugated) bilirubin is determined by the same method in the absence of caffeine.

Specimen collection and storage

Heparinised plasma or serum samples free of haemolysis are recommended. Specimens should be protected from direct light.

Requirements

Equipment
• Spectrophotometer
• Timer

Reagents for Total Bilirubin
• Total bilirubin testing kit
• Low and high controls
Reagents for Direct Bilirubin
• Direct bilirubin testing kit
• Low and high controls

Procedure

Refer to the literature instructions

Quality Control

Include the low and high controls with each run. The values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be ¡Ü5%.

Results / Calculations of results

Refer to the literature instructions

The concentration of bilirubin in a sample is determined with the following calculation:

Bilirubin (Total or Direct) = Absorbance (TEST) × Concentration of standard (µmol/L) / Absorbance (STANDARD)

Linearity

Test is linear up to 400 µmol/L. For bilirubin concentrations exceeding 400 µmol/L, dilute the sample 1 in 5 with normal saline and repeat the test. Multiply the results by 5.

Reference Ranges

Total bilirubin: up to 17.0 µmol/L (up10 mg/L)
Direct bilirubin: up to 4.0 µmol/L (up 3,5 mg/L)

Interpretation of results

• Increased levels of bilirubin are diagnostic of jaundice. Jaundice may be an indication of
liver damage or increased red cell destruction.
• Some antiretrovirals may cause liver damage while others are secreted by the liver; hence,the presence of liver damage may affect their excretion.

Test Limitations and Sources of Error

Bilirubin is light sensitive, so samples must be kept in a cool, dark place. Analyze bilirubin samples on the same day of collection. Haemolysis interferes with the test.

Disposal of waste materials

All disposables should be discarded into a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

Rene caquet, Le vademecum des Examens de laboratoire, 8 eme edition2001, ed MIMI, p 63,64

bioMerieuxProduits et reactifs de laboratoire, Marcy l'Etoile 2003, Bilirubine Kit, www.biomerieux.com

SOP TITLE: SERUM TOTAL PROTEINS (BIURET REACTION)
SOP No.: CHEM 004/ 2005
DATE: July 2005

Clinical significance of the test

Protein estimation is of vital importance in the diagnosis and monitoring of diseases. Often it may be necessary to quantitate the proteins in groups and or as individual components, such as albumin, globulin etc. Although total protein estimation gives an immediate and relatively quick information about the patient, it has limited clinical value.

Principle

Cupric ions in an alkaline solution interact with peptide bonds, resulting in the formation of a coloured complex. The intensity of the colour formed is directly proportional to the concentration of protein in the sample.

Specimen collection and storage

Serum or heparinised plasma

Requirements

Equipment
- Spectrophotometer
- Timer

Reagents
- Protein testing kit
- Low and high controls

Procedure

Refer to literature instructions

Quality Control

The values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be Ü5%.

Results and calculations

Total Protein = Absorbance (TEST) × Concentration of standard (g/L)
Absorbance (STANDARD)

Linearity

The method is linear up to 150 g/L. If the total protein concentration exceeds 150 g/L, dilute the sample with an equal volume of normal saline and multiply the result by 2.

Reference Range

Total protein: 60–80 g/L

Interpretation of results

Increased serum protein levels are indicative of:
- Water depletion
- Multiple myeloma
- Infections

Decreased serum protein levels are indicative of:
- Protein deficiency
- Nephrotic syndrome
- Liver disease

Test Limitations and Sources of Error

Lipaemic sample reduces absorbance, giving false low protein levels.

Disposal of waste

All disposables should be discarded in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

bioMerieux, Produits et reactifs de laboratoire, Marcy l'Etoile 2003, Total Proteins Kit,

www.biomerieux.com

SOP TITLE: SERUM ALANINE AMINOTRANSFERASE (ALT)
SOP No.: CHEM 005/ 2005
DATE: July 2005

Clinical Significance

ALT or Glutamate Pyruvate Transaminase (GPT) is more specific for detecting liver damage and present in high concentration in liver and, to a lesser extent, skeletal muscle, kidney and heart. This enzyme is involved in transamination reactions in which an amino group is transferred from alanine alpha-ketoglutarate to form pyruvate and L - Glutamate.

Principle

- 2-oxoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-glutarate + Pyruvate (1st method)

Alanine aminotransferase (ALT) activity is measured by monitoring the concentration of
pyruvate hydrazine formed by the reaction of 2,4-dinitrophenyl hydrazine and pyruvate. This reaction is measured at 546 nm. .

- 2-oxoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-glutarate + Pyruvate (2nd method)

Pyruvate + NADH, H+ $\xrightarrow{\text{LDH}}$ Lactate + NAD+

The rate at which the NADH is consumed is measured at 340 nm. This rate is proportional to ALT catalytic activity.

Specimen collection and storage

Serum or heparinised plasma

Requirement

Equipment
• Water bath
• Spectrophotometer
• Stopwatch or timer

Reagents
Refer to literature
• ALT testing kit
• Sodium hydroxide (0.4 mol/L, 16 g in 1 L distilled water)
• Calibrator
• Low and high controls

Procedure
Refer to manufacturer instructions

Quality Control

The values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be ¡Ü5%.

Results / Calculations
Refer to manufacturer instructions

Linearity
The reagent is linear up to 290 IU/L.

Reference Range

Up to 45 RF U/mL (1^{st} method)
Up to 40 IU/L (2^{nd} method) for men
Up to 33 IU/L (2^{nd} method) for women
N.B. reference ranges are variable with working temperature.

Interpretation of results

• Increased levels are indicative of liver damage (e.g., hepatitis or carcinoma).
• Slight increases are seen in obstructive jaundice.

Test Limitations and Sources of Error

• Haemolysis interferes with the assay.
• High levels may be obtained if patient samples have high concentrations of aldehydes, ketones, or oxo acids (limitation of the first method).
• Measurement against a serum blank instead of reagent blank prevents the risk of finding such artefacts.

Disposal of waste materials

• Avoid ingestion or contact with skin or mucous membranes with reagents. Do not pipette by mouth.
• Discard all samples after use in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

bioMerieux, Produits et reactifs de laboratoire, Marcy l'Etoile 2003, Total Proteins Kit,

www.biomerieux.com

SOP TITLE: SERUM ASPARTATE AMINOTRANSFERASE (AST)
SOP No.: CHEM 006/ 2005
DATE: July 2005

Clinical significance

The test is indicative in the assessment of liver diseases, myocardial infarctions, pulmonary emboli and gangrene.

Principle

- 2-Oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ glutamate + Oxaloacetate (1st method)

Aspartate aminotransferase (AST) is measured by monitoring the concentration of oxaloacetate hydrazone formed by the reaction of 2,4-dinitrophenyl hydrazine and oxaloacetate. This reaction is measured at 546 nm. .

- 2-Oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ glutamate + Oxaloacetate (2nd method)

Oxaloacetate + NADH, H+ $\xrightarrow{\text{MDH}}$ Malate + NAD+

The rate at which the MDH is consumed is measured at 340 nm. This rate is proportional to AST catalytic activity.

Specimen collection and storage

Serum or heparinised plasma
Stability 5 days at 2-8 ^{0}C or 48 hours at room temperature

Requirement

Equipment
• Spectrophotometer
• Timer
• Water bath

Reagents
• AST testing kit
• Sodium hydroxide (0.4 mol/L, 16 g in 1 L distilled water)
• Calibrator
• Low and high controls

Procedure

Refer to the literature instructions in the kit.

Quality Control

Include both low and high controls when performing the test as shown above. Values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be ¡Ü8%.

Results / Calculations

Obtain the activity of AST in the serum from the following calculation:
Factor = AST activity of Calibrator (Standard) / Absorbance of Calibrator (Standard)
$AST_{(TEST)} = Factor \times Absorbance_{(TEST)}$ (U/L)

Linearity

If absorbance exceeds 0.170, dilute sample 1 in 10 with 0.9% NaCl solution and re-assay.
Multiply the result by 10.

Reference Range

Men: 11–41 U/L at 37ºC
Women: 11–36 U/L at 37ºC

Interpretation of results

• Increased levels are indicative of liver damage or myocardial infarction.
• ART implications to be included.

Test Limitations and Sources of Error

• Haemolysis interferes with the assay.
• Transaminase activity in some sera is stimulated by high concentrations of aldehydes, ketones, or oxo acids.

Disposal of waste materials

All disposables should be discarded in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

Product insert user's manual for urease test kit, Randox Laboratories, UK.

bioMerieux, Produits et reactifs de laboratoire, Marcy l'Etoile 2003, ASAT Kit

www.biomerieux.com

SOP TITLE: BLOOD AND CSF GLUCOSE (OXIDASE METHOD)
SOP No.: CHEM 007/ 2005
DATE: July 2005

Clinical significance

Glucose is the chief source of energy in the body. The levels of this compound are balanced by digestion and absorption of carbohydrates in the intestine, its storage and release in the liver and its utilisation in the muscle.

Apart from the screening for and monitoring of diabetes, glucose is measured in cases of pancreatic, metabolic or endocrinic disorders.

Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase.
The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and
4-aminophenazone to form a red-violet quinoneimine dye. The intensity of the colour
produced is directly proportional to the concentration of glucose in the sample.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4- amino antipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4\ H_2O$$

Specimen collection and storage

Fluoride oxalate plasma
CSF

Requirements

Equipment
- Spectrophotometer
- Water bath (optional) at 37ºC

Reagents
- Glucose testing kit
- Low and high controls

Procedure

Refer to literature instructions in the Kit
NB: For icteric or lipaemic samples, a sample blank must be prepared by adding 20 µL of sample to 2,000 µL of normal saline solution. Read absorbance against the reagent blank.

Quality Control

Include both low and high controls when performing test. The values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be Ü5%.

Results / Calculations

Glucose = Absorbance (TEST) × Concentration of standard (mmol/L)
Absorbance (STANDARD)

Linearity

Test is linear up to 22.2 mmol/L. Samples above 22.2 mmol/L should be diluted 1:1 with distilled water and the result multiplied by 2.

Reference Ranges

Fasting blood sugar (FBS): 3.1–6.4 mmol/L (0, 7- 1,1 g/ L)
Cerebrospinal fluid (CSF): 2.2–4.0 mmol/L (0,4 – 0,7 g/ L)

Interpretation of results

Regimens containing protease inhibitor (PI) have been associated with hyperglycaemia and even diabetes.

Test Limitations and Sources of Error

Samples collected in containers other than fluoride oxalate should be analysed within 15 minutes.

Disposal of waste materials

All disposables should be discarded in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

bioMerieux, Produits et reactifs de laboratoire, Marcy l'Etoile 2003, Glucose Kit
www.biomerieux.com

SOP TITLE: DETERMINATION OF ALPHA AMYLASE ACTIVITY
SOP No.: CHEM 008/ 2005
DATE: July 2005

Clinical Significance

Used in emergency test for patients with a painful abdominal syndrome.
Rise in serum amylase levels the diagnosis is towards pancreatic diseases used to assess for possible pancreatitis, which can occur with treatment with several ARV agents (ddI, d4T). Protease inhibitor treatment can also occasionally be associated with extremely high levels of triglycerides, which may place the patient at risk for pancreatitis.

Principle

Alpha amylase catalyses the hydrolysis of p-nitrophenyl-á-d-maltoheptaoside and of p-nitrophenyl-á-d-maltohexoside to produce glucose polymers and shorter chains of p-nitrophenyl oligosaccharides. These latter are hydrolysed by alpha glucosidase to form glucose and p-nitrophenol. The increase in absorbance at 405 nm, due to the released p-nitrophenol, is proportional to the alpha amylase activity in the sample.

$$5\ \text{CNPG3} \xrightarrow{\text{Alpha- amylase}} 3\ \text{CNP} + \text{maltose} + 2\ \text{CNPG2} + 2\ \text{glucose}$$

Specimen collection and storage

Serum or urine

Requirements

Equipment
- Timer
- Spectrophotometer
- Water bath

Reagents
- Amylase testing kit
- Calibrator
- Low and high controls

Procedure

Mix reagents to make the working reagent as recommended by the reagent kit manufacturer.

Quality Control

Include both low and high controls. The values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be ¡Ü8%. Refer to the quality control literature in the kit.

Results and Calculations

Obtain the activity of alpha-amylase in the serum from the following calculation:

Abs = Abs1 + Abs2 + Abs3

Factor = alpha-Amylase activity of Calibrator (Standard) / . Absorbance of Calibrator (Standard)alpha -Amylase (TEST) = Factor × . Absorbance (TEST) (U/L)

Linearity

For absorbance readings exceeding 0.160, repeat the assay with sample diluted 1 in 10 using normal saline and multiply the result by 10.

Reference Ranges

Serum and plasma: 22–100 U/L at 37ºC
Refer to the Kit literature.

Interpretation of results

• Levels three times higher than normal are indicative of acute pancreatitis.

• High levels are also found in severe glomerular impairment, severe diabetic ketoacidosis, and perforated peptic ulcers.

Test Limitations and Sources of Error

• Avoid haemolysis because it may decrease results.
• Avoid contamination of samples, reagents, or glassware with saliva or sweat.
• EDTA, citrate, and oxalate inhibit the enzyme.

Disposal of Waste materials

All disposables should be discarded in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.
bioMerieux, Produits et reactifs de laboratoire, Marcy l'Etoile 2003, alpha-Amylase Kit,

www.biomerieux.com

SOP TITLE: SERUM TOTAL CHOLESTEROL
SOP No.: CHEM 009/ 2005
DATE: July 2005

Clinical significance

Measurement of cholesterol is useful in detecting hyercholesterolemia, lipid and lipoprotein metabolism desorders.

Principle

Free cholesterol and cholesterol released from its esters are oxidised after enzymatic hydrolysis.
The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxide.

Specimen collection and storage

Fasting serum or heparinised plasma

Requirements

Equipment
- Spectrophotometer
- Water bath
- Timer

Reagents
- Cholesterol testing kit
- Low and high controls

Procedure

Refer to literature instructions in the kit

Quality Control
Include both low and high controls as shown in the table above. The values obtained for low and high controls must be within ± 2 SD of the given range. C.V.% must be 5%.

Refer to the literature in the kit.

Results / Calculations

Calculate the cholesterol level in the samples as follows:

Cholesterol = Absorbance (TEST) × Concentration of standard (mmol/L) / Absorbance (STANDARD)

Linearity

The method is linear up to 19.2 mmol/L. Samples with concentrations above this limit should be diluted 1 in 3 with normal saline and the result multiplied by 3.

Reference Range

Adults: up to 6.5 mmol/L (2,4 g/L)

Interpretation of results

• Protease inhibitor treatment may cause lipid elevations; extremely high triglyceride levels may occasionally be seen, which may increase risk for cardiovascular disease.
• Increased levels of lipids are diagnostic of lipid disorders.
• Decreased levels of lipids are found in malnutrition and are observed in pregnancies.

Disposal of waste material

All disposables should be discarded in a designated waste bin for incineration.

References

Central Board of Health (CBOH). 2003. *Biochemistry Standard Operating Procedures for Hospital Laboratories Level III*. Lusaka, Zambia: Mipal Printers.

bioMerieux, Produits et reactifs de laboratoire, Marcy l'Etoile 2003, cholesterol Kit,

www.biomerieux.com

HEMATOLOGY

SOP TITLE: HAEMOGLOBIN ESTIMATION (CYANMETHAEMOGLOBIN METHOD)
SOP No.: HAEM 001/ 2005
DATE: July 2005

Clinical significance

Used for the estimation of haemoglobin in whole blood for diagnosis of anemia.
Used as baseline to assess for anaemia before starting treatment and to assess possible bone marrow suppression during ARV treatment, especially with zidovudine-containing regimens.

Principle

Blood is diluted in a solution containing potassium cyanide and potassium ferricyanide.
Haemoglobin (Hb), methaemoglobin (Hi), and carboxyhaemoglobin (HbCO) (but not sulphaemoglobin [SHb]) are converted into cyanmethaemoglobin (HiCN), which is a coloured product. The colour intensity is read in the colorimeter or spectrophotometer at a wavelength of 540nm, and the absorbance is proportional to the concentration of haemoglobin in the blood.

Specimen collection and storage

Capillary or venous blood in an EDTA container

Requirements

Equipment
• Colorimeter or spectrophotometer
• Timer

Reagents
• Haemoglobin standard (commercially obtained)
• Drabkin's solution pH 7.0–7.4 prepared as follows:

COMPOUND AMOUNT FINAL CONCENTRATION

Potassium ferricyanide 200 mg 0.607 mmol/L
Potassium cyanide 50 mg 0.768 mmol/L
Potassium dihydrogen sulphate 140 mg 1.029 mmol/L
Non-ionic detergent
(e.g., Saponic 218, Triton X-100) 1 mL
Distilled or deionised water Up to 1,000 mL
Note: Solution should be clear and pale yellow in colour. Absorbance when measured against water at a wavelength of 540 nm should be zero. Solution is

stable at room temperature and should be stored in a brown bottle. If ambient temperature is above 30ºC, solution should be kept in refrigerator. DO NOT freeze.

Procedure

A. Haemoglobin Estimation of the Test Sample Using the Curve or Table

- Pipette 4 mL of Drabkin's diluting fluid into a tube.
- Pipette 0.02 mL (20 µL) of venous or capillary blood.
- Wipe the outside of the pipette.
- Add the blood to the Drabkin's fluid and rinse the inside of the pipette with the fluid.
- Mix gently and allow to stand for 5 minutes
- Zero the colorimeter with Drabkin's solution. Read absorbance of patient's sample at wavelength 540 nm or Ilford filter No. 625.
- Estimate the haemoglobin concentration using the haemoglobin calibration curve, which is prepared as shown in the box below.

Note: If cloudiness appears in the diluted blood, centrifuge the fluid before reading the colorimeter.

PREPARATION OF STANDARD CALIBRATION CURVE

Note: Every effort must be made to use the calibration curve method because it saves on the standard.

1. Label five tubes as tubes 1, 2, 3, 4, and 5 dilute the standard with Drabkin's solution as follows:

Tube Standard Drabkin's % Haemoglobin

1 4.0 mL 0 mL 100%
2 3.0 mL 1.0 mL 75%
3 2.0 mL 2.0 mL 50%
4 1.0 mL 3.0 mL 25%
5 0 mL 4.0 mL 0%
2. Mix well and allow to stand for 5 minutes.
3. Zero the instrument with Drabkin's solution.
4. Read the absorbance at 540 nm against reagent blank (if using one cuvette, start with tube 5).
5. Prepare a graph plotting the absorbance readings of the diluted standards (Y axis) against their concentrations (X axis).
(Example: The reference solution concentration is calculated as 18 g/dL.)
Tube Standard Drabkin's % Haemoglobin Haemoglobin Absorbance

1 4.0 mL 0 mL 100% 18.0 g/dL
2 3.0 mL 1.0 mL 75% 13.5 g/dL

3 2.0 mL 2.0 mL 50% 9.0 g/dL
4 1.0 mL 3.0 mL 25% 4.5 g/dL
5 0 mL 4.0 mL 0% 0 g/dL
6. From the graph, make a table of hemoglobin values from 2 to 18 g/dL.

B. Alternative Method for Use Where the Graph Is Not Available

- Add 0.02 mL (20 µL) of blood to 4 mL of Drabkin's solution in a test tube.
- Mix gently and allow to stand for 5 minutes for full colour development.
- Dilute 0.02 mL (20 µL) of standard in 4 mL of Drabkin's solution and allow for full colour development.
- Zero the instrument with Drabkin's solution.
- Read the absorbance of the test at 540 nm or filter No. 625.
- Read the absorbance of the standard.
- Calculate the haemoglobin concentration in grams per decilitre using the formula shown in the Calculation section below.

Quality Control

• If possible, all specimens should be tested in duplicate; results should not differ by more than± 0.2 g/dL.
• The photometer must be checked each day by means of the haemoglobin cyanide reference material with known haemoglobin values. The value obtained must read within 2% of the reference material's stated value.
• A calibration curve must be prepared with each new batch of reagents.

Results / Calculations

Haemoglobin concentration =
Absorbance (TEST) × Concentration of standard × Dilution factor of standard / Absorbance (STANDARD)

Reference Range

Men 13.5 to 18.0 g/dL
Women 11.5 to 16.5 g/dL
Pregnant women 11.0 to 15.0 g/dL
Children 12.0 to 14.0 g/dL
Infants 11.0 to 12.5 g/dL
Neonates 13.5 to 19.5 g/dL

Interpretation of Results

• A low haemoglobin level indicates anaemia.
• A high haemoglobin level may indicate primary or secondary polycythaemia or dehydration.

Test Limitations and Sources of Error

• Not following standard procedure
• Not pipetting the correct amount of blood
• Not wiping the tip of the pipette
• Not allowing enough time for colour development
• Using the wrong filters
• Application of excess pressure to the finger during capillary blood collection
• Using dirty tubes and cuvettes
• Delayed sampling after tourniquet application
• Clots in the specimen
• Broken pipette

Disposal of waste material

Disposal of specimem containers in a plastic bag fixed in a bin ready for incineration. Drabkins solution should be disposed in sink with a lot running water.

References

Central Board of Health (CBOH). 2003. *Haematology Standard Operating Procedures for Hospital Laboratories Level III.* Lusaka, Zambia: Mipal Printers.

Lewis, S. M., B. J. Bain, and I. Bates (eds.). 2001. *Dacie and Lewis Practical Haematology*. 9th edition. New York: Churchill Livingstone, pp. 19–23.

SOP TITLE: HAEMATOCRIT METHOD
SOP No.: HAEM 002 / 2005
DATE: July 2005

Clinical significance

Haematocrit is used to calculate the mean cell haemoglobin concentration and cell volume. It measures the proportion of red blood cells to plasma. It is useful as a screening test for anaemia and to diagnose polycychaemia vera and to monitor its treatment.

Principle

The method for determining haematocrit by centrifugation is the micromethod.

In the method a column of blood is centrifuged in a tube of uniform bore, which is closed at one end.

Heamatocrit or packed cell volume is the volume of erythrocytes expressed as a fraction of the volume of whole blood in a sample.

Requirements

- Microhaematocrit centrifuge
- Microhaematocrit tubes plain and heparinised (75mm long with 1.5mm bore.)
- Specially designed scale for reading results e.g. Critocap Tube reader.
- Sealant e.g. cristaseal

Specimen collection and storage

- Venous blood in dipotassium EDTA (use plain tubes).
- Capillary blood collected directly into capillary tubes lined with a film of heparin.

Procedures

- Immerse the tip of the capillary tube just below the surface of the blood.
- Tilt the tube slightly to permit the blood to move rapidly up the capillary tube. The capillary tube should be filled by capillary action until it is about three-quarters filled.
- Seal one end of the tube with a sealant
- Place the capillary tubes in the numbered slots, making sure that the number on the slot corresponds with specimen number.
- The sealed ends of the tube should point away from the center.
- Centrifuge at (RCF 10, 000 -15,000xg) rpm for 3 - 5 minutes. The tube will show 3 distinct layers: plasma at the top, buffy layer of WBC in the middle, and RBC at bottom.

- Read the percentage of packed red cells by placing the spun capillary tube on the provided scale.

Quality control

Known samples (low and high) should be run at frequent intervals and specimens should also be run in duplicate and must agree within ± 0.01.

Results / calculations

How to use the scale

- Hold the tube against the scale and align the base of the blood in the column with zero and the bottom of the meniscus of the plasma with 100 as shown in figure 1. The value of the haematocrit is taken directly from the reader - 50% as shown in figure 1.
- The line passing through the top of the column of red cells gives the haematocrit. If the top of the column of cells is not on a line but between lines, its position can be estimated to the nearest digit.

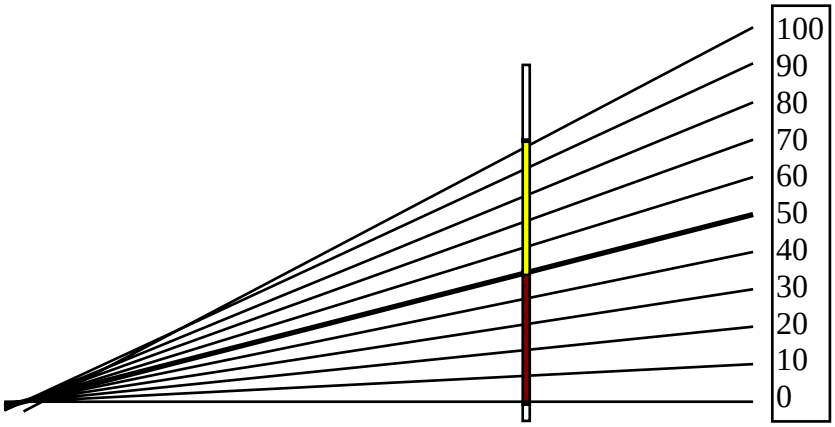

Figure 1: Microhaematocrit reading scale showing positioning of tube
Reference ranges

Normal values	**Range %**	**Ratio**
Men	40 - 50	0.40 - 0.5
Women	37 - 43	0.37 - 0.43
Children (3 - 6 yr.)	38 - 44	0.38 - 0.44
Infants (3 months)	35 - 40	0.35 - 0.40
New born	50 - 68	0.50 - 0.68

Interpretation of results

High values are found in cases of plasma depletion e.g. severe burns and dehydration due to diarrhoea and/or vomiting. Low values are found in patients suffering from anaemia and in fluid overload.

Test limitations and sources of errors

- Inadequate sealing of capillary tubes or breakage during centrifugation.
- Faulty centrifuge
- Presence of clots in the blood sample.
- Over dilution with tissue fluid by application of pressure during sampling or conditions associated with hydremia such as excessive administration of fluids, pregnancy etc.
- Improper reading of results from the scale
- Haemolysed samples
- Storing specimens beyond 6 - 8 hours.
- Haemoconcentration

Disposal of waste materials

The used capillary tubes should be discarded in hypochlorite disinfectant.

References

Central Board of Health (CBOH). 2003. *Haematology Standard Operating Procedures for Hospital Laboratories Level III.* Lusaka, Zambia: Mipal Printers.

Lewis, S. M., B. J. Bain, and I. Bates (eds.). 2001. *Dacie and Lewis Practical Haematology*. 9th edition. New York: Churchill Livingstone, pp. 19–23.

SOP TITLE: AUTOMATED FULL BLOOD COUNT (QBC AUTOREAD Plus)
SOP No.: HAEM 002/ 2005
DATE: July 2005

Clinical significance

Automated analyzer for assessment of hematological disorders.

Principe

The QBC capillary tubes containing pre-incorporated with acridine orange fluorochrome stain are filled with venous or capillary blood and are centrifuged at high speed, then placed in QBC AUTOREAD PLUS analyzer where it is automatically scanned and fluorescence and absorbance readings are made to identify the expended layers of differentiated cells.

Specimen collection and storage

Venous blood collected in EDTA container.
Capillary blood collected in QBC capillary tubes.

Requirements

Equipment

QBC Autoreader Plus
QBC Centrifuge
QBC Printer
QBC pipette

Reagents

QBC ACCUTUBES

Procedure

- Check the expiry date of the tubes
- Avoid the air bubbles while filling up the tubes
- Mix well with the incorporated reagent
- Do not allow the blood to come in contact with the stopper while filling up the tube
- Do not use the broken tubes or having any other anomaly
- Homogenise the sample by turning the tube 8 times or during 5 minutes in a mixer
- Lean the tube containing the blood
- Put the open end of ACCUTUBE in contact with the blood
- Fill up to the first filling up line (between 2 black lines)
- Wipe the outside of the ACCUTUBE with hygienic paper

- Fix the stopper of the other end of the ACCUTUBE
- Insert the floater
- The floater should not be touched with the fingers
- Slip the open end of the ACCUTUBE on the floater up to a partial insertion
- Finish the insertion by pushing the floater against the cover
- Mark the tube
- Centrifuge the tubes for five minutes

NB: The insertion of the floater is easier by using a grip

- mix the blood with orange acridine
- Hold the tube vertically in the medium of the fingers
- Mix by turning over the tube 5 times
- Hold the tube by pressing the stopper against a solid surface
- Select the QBC mode of QBC AUTOREAD PLUS
- Open the door of the apparatus
- Insert the capillary tube, the plastic part of the stopper at left
- Close the door to start the analysis
- The results are displayed at the end of the analysis
- Print out the results
- Open the door of the analyzer and remove the tube
- Throw the tubes in an appropriate container

Quality control

QBC ACCUTUBE control or use in house sample

Reference ranges

. Haemoglobin	Man	from 14 to 18 g/dl
	Woman	from 12 to 16 g/dl
	Child	from 12 to 16 g/dl
. Haematocrit	Man	from 40 to 50 %
	Woman	from 37 to 47 %
	Child	from 36 to 44 %
. CCMH		from 32 to 36 g/dl
. Platelets		from 150 to 500 x 10^9 / litre
. White blood cells		from 4 to 10 x 10^9 / litre
. Granulocytes		from 1.8 to 7.2 x 10^9 /litre
. Lymphocytes-Monocytes		from 1.7 to 4.9 x 10^9 /litre

Test limitation and sources of error

- Red blood cells count from 1.6 to 99.9 x 10^9 / litre
- Platelet count from 20 to 999 x 10^9 / litre
- Estimation of Haemoglobin from 5 to 20 g/dl
- Haematocrit from 15 to 65 %
- Granulocytes count from 0.8 to 70 x 10^9 / litre
- Lymphocytes-monocytes count from 0.8 to 99.9 x10^9 / litre

The results beyond these intervals flicker and are preceded by asterisk sign

- The results apart from the intervals accepted by the machine must be found by other techniques
- The ACCUTUBES are made to optimise the results in case of layers of normal cells
- The errors due to technician or material used can lead to a non display of results
- The QBC AUTOREAD PLUS cannot detect the qualitative abnormalities of blood cells
- The QBC AUTOREAD PLUS cannot replace the leukocytic formula done manually
- The QBC does not calculate the number of red blood cells

References

Becton Dickinson; QBC AUTOREAD PLUS Operator's Manual, 1996

Becton Dickinson; QBC Centrifuge System Operator's Manual, 1996

Lewis, S.M; Bain, B.S; Bates, I; Dacie and Lewis practical Hematology; 9th Ed. 2003

SOP TITLE: MAKING A THIN BLOOD FILM
SOP No.: HAEM 004/ 2005
DATE: July 2005

Clinical significance

The thin blood film is important in the investigation and management of anemia infections and other conditions which produce changes in the appearance of blood cells and differencial white cell count. The test examine the cell morphology all blood cells and perform a differential blood cell count.

Principle

A drop of blood is placed on one end of a slide. It is spread into a thin film while holding a spreader at a 45º angle.

Specimen collection and storage

Freshly collected venous blood in an EDTA container or capillary blood is recommended.

Equipments and Reagents

Equipment
• Glass slides
• Spreader
• Orange stick
• Cotton wool

Reagents
• Methanol

Procedure

- Soak glass slides in methanol.
- Clean the glass slide using cotton wool.
- Place a small drop of blood about 2 cm from the end of the slide.
- Without delay, place the spreader at an angle of 45º to the slide. Move it back to touch the drop of blood.
- The drop spreads out quickly along the line of contact of the spreader with the slide.
- Then spread the film with a rapid, smooth, forward movement of the spreader.

Results / Calculations

A well-made slide will have three distinct regions: a head, body, and tail.

Test limitation and source of error

- A ragged spreader or one that is not smooth can ruin a slide.
- Make sure the drop of blood is not too big or too small.
- Hold the spreader at the correct angle.
- Do not use a glass slide that is dirty or oily.

Disposal of waste materials

Dispose of specimen containers and orange sticks in a plastic bag fixed in a bin ready for incineration.

References

Central Board of Health (CBOH). 2003. *Haematology Standard Operating Procedures for Hospital Laboratories Level III.* Lusaka, Zambia: Mipal Printers.
Lewis, S. M., B. J. Bain, and I. Bates (eds.). 2001. *Dacie and Lewis Practical Haematology*. 9th edition. New York: Churchill Livingstone.

SOP TITLE: MANUAL DIFFERENCIAL LEUKOCYT COUNT (THIN BLOOD FILMS)

SOP No.: HAEM 005/ 2005
DATE: July 2005

Clinical significance

To determine the proportions of different white blood cells types in blood and look at the morphology of white blood cells, red blood cells and platelets**.**

Principle

A drop of blood is spread on a slide and then fixed, stained, and examined under the microscope. In this way, red blood cells, leucocytes, and platelets may be studied.
The Romanowsky stains contain eosin Y, which is an acidic anionic dye, as well as azure B and other thiazine dyes, which are basic cationic dyes. When these dyes are diluted in buffered water, ionization occurs. Eosin stains the basic components of blood cells; for example, haemoglobin stains pink-red, and other methylene blue–derived dyes stain the acidic components of the cells. Nucleic acids and nucleoproteins stain various shades of mauve-purple and violet, the granules of basophils stain dark blue-violet, and the cytoplasm of monocytes and lymphocytes stains blue or blue-grey. The staining reactions of Romanowsky stains are pH dependent, which is why the stains are diluted in buffered water with a specific pH.

Specimen collection and storage

Freshly collected venous blood in an EDTA container or capillary blood is recommended.

Requirements

Equipment
• Differential counter
• Timer
• Microscope
• Staining racks/staining jars

Reagents
• Romanowsky stains (e.g., May-Grunwald-Giemsa, Leishman)
• Absolute methanol
• Buffered water at pH 6.8
• Immersion oil
• Thin blood films

Procedure

A. May-Grunwald-Giemsa Stain Technique

- iFix in absolute methanol for 10–15 minutes.
- Transfer the slides to a staining jar containing May-Grunwald stain freshly diluted with anequal volume of buffered water and allow to stain for 5 minutes.
- Transfer slides without washing or drying into a jar containing Giemsa stain freshly diluted with 9 volumes of buffered water and stain for 10–15 minutes.
- Transfer the slides into a jar containing buffered water and rapidly wash; then allow them to stand undisturbed for 2 minutes for differentiation to take place
- .Air dry the slides.

Note: Methanol should be free of water, and films should not be allowed to dry between steps and

Quality Control

Repeat counts on selected slides on subsequent days because this will give an indication of the range in the variation of the results. (Include note on reagent quality.)

Procedure

- Examine with 10× objective to check if the film is well made and for any extracellular organisms.
- Examine with the 40× objective and with the 100× objective if necessary.
- Examine the body of the smear where the cells are lined singly and are evenly distributed.
- Count a total of 100 leucocytes and record the number of each type of leucocyte seen. Present as a percentage of each type.
- Comment on any abnormalities seen in the WBC (e.g., blastoid features of lymphocytes, neutrophil shift left or right, toxic granulation of polymorphs, vacuolation of cytoplasm).
- Comment on the shape, size, and colour of red blood cells
- .Comment on the number and morphology of platelets seen.

Report the percentages, number, and morphology of cells counted.

Reference ranges

EXPECTED VALUES PERCENTAGE COUNT AND ABSOLUTE COUNT

Adults

Neutrophils 45–75% 2.0–7.5 x 10^9/L
Lymphocytes 15–45% 1.5–4.0 x 10^9/L
Monocytes 2–10% 0.2–0.8 x 10^9/L
Eosinophils 1–6% 0.04–0.4 x 10^9/L
Basophils 0–1% <0.01–0.1 x 10^9/L

Children (2-6years)

Neutrophils 20–45% 1.5–6.5 x 10^9/L
Lymphocytes 45-70 % 6.0–8.5 x 10^9/L
Monocytes 2–10% 0.1–1.0 x 10^9/L
Eosinophils 1–6% 0.3–1.0 x 10^9/L
Basophils 0.1–1% 0.01–0.1 x 10^9/L

Interpretation of results

• Elevated white blood cell count may mean infection.
• Decreases in white blood cell count may occur with disease progression or may indicate bone marrow suppression from ARV therapy.
• Total lymphocyte count: After a patient is on ARV therapy, a decrease in absolute lymphocyte count may reflect bone marrow suppression from treatment. A total lymphocyte count of <1,200/mL has been correlated with a CD4 count of <200 cells/mL. However, the total lymphocyte count alone should not be used in asymptomatic patients when deciding whether to start ARV therapy.
• An increase in neutrophils may be due to an acute bacterial infection or haematological malignancies such as myeloid leukaemia.
• An increase in eosinophils may be due to a parasitic infection or an allergic reaction.
• An increase in lymphocytes may be due to viral infections or chronic infections such as tuberculosis or lymphocytic leukaemia.
• An increase in monocytes is found in haematological malignancies such as chronic myelomonocytic leukaemia and certain bacterial and parasitic infections (e.g., typhoid fever, malaria).

Interpretation of results

Morphologic Reports
• Red cell colour: normochromic, hypochromic, hyperchromic
• Red cell size (anisocytosis): normocytic, macrocytic, and microcytic
• Red cell shape (poikilocytosis): normal or abnormal; report the presence of sickle cells, target cells, spherocytes, etc.
• Red cell inclusion: RNA, polychromasia, punctate basophilia, Howell-Jolly bodies, etc.
• Report the presence of parasites such as sporazoa, nematodes, and trypanosomes as well as bacteria such as spirochetes
• Platelets: numbers and morphology

Test Limitations and Sources of Error

• Poorly made smear
• Squeezing the site of the capillary blood puncture
• Insufficient mixing of blood in EDTA container
• Unfiltered stain
• Dirty glassware
• Incorrect buffer pH
• Films made from blood that had been standing for more than 6 hours
• Examining the wrong end of the film

Disposal of waste materials

• Clean oil off slide with xylene. Store cleaned slides for 7 days before specimen disposal or keep slides of interest in slide racks for future reference.
• Autoclave specimen containers and dispose of them.

References

Central Board of Health (CBOH). 2003. *Haematology Standard Operating Procedures for Hospital Laboratories Level III.* Lusaka, Zambia: Mipal Printers.

Lewis, S. M., B. J. Bain, and I. Bates (eds.). 2001. *Dacie and Lewis Practical Haematology*. 9th edition. New York: Churchill Livingstone.

SOP TITLE: ENUMERATION OF CD4+ T LYMPHOCYTES (FACSCount FLOW CYTOMETER)
SOP No.: HAEM 006 / 2005
DATE: July 2005

Clinical significance

The test is mainly used for baseline assessment and monitoring response to treatment.

Principle

A single test requires one convenient, ready-to-use reagent tube pair. When whole blood is added to the reagents, fluorochrome-labelled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. Here, the cells come in contact with the laser light, which causes the fluorochrome labelled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count cells. In addition to containing the antibody reagent, the tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantification standard for enumerating the cells.

Specimen collection and storage

Use whole blood specimen collected by venipuncture in evacuated blood collection K3-EDTA or K2EDTA containers. Samples must be transported as soon as possible after collection at 18–22ºC, and the tests should be performed within 48 hours but no later than 72 hours after the blood specimen (kept at room temperature) is drawn.

Requirements

Equipment

• BD FACScount instrument
• Automatic electronic pipette and tips
• Vortex mixer
• Coring station
• Cleaning tubes
• FACScount workstation
• Disposable clothing
• Biohazard waste container or bag
• Safety Cabinet class II (optional)

Reagents

• BD FACScount reagent kit
• BD FACScount control kit
• BD multicheck control
• BD FACScount sheath fluid
• BD FACScount rinse
• BD FACScount clean

Procedures

- Label the tab of one reagent tube pair with patient laboratory number.
- Vortex the reagent tube pair upside down for 5 seconds, then upright for 5 seconds.
- Open the reagent tube pairs with the coring station.
- Transfer the reagent tube pair from the coring station to the workstation, keeping the tubesupright.
- Close the workstation cover to protect the reagents from light.
- Mix the whole blood by inverting the BD Vacutainer tubes five times.
- Pipette 50 µL of blood into each of the four reagent tubes. Change the tips between each tube.
- Cap the reagent tube pairs and vortex upright for 5 seconds.
- Replace the reagent tube pairs in the FACScount workstation, close the cover to protect reagent from light, and incubate for 60–120 minutes at room temperature (20–25ºC).
- After the incubation step is complete, uncap the tubes and pipette 50 µL of fixative solution into each reagent tube. Change tips between tubes.
- Seal the reagent tube pair with new caps and vortex upright for 5 seconds. (Fixed samples can be held up to 12 hours before adding the control beads.)
- Run the tubes on the FACScount instrument within 2 hours of adding control beads to the reagent tubes.
- Store samples at room temperature in the workstation until they are run on the instrument. Vortex upright for 5 seconds immediately before running and run on the BD FACScount instrument following the instructions in the user's manual.

Quality Control

• Commercially available BD multicheck controls or fresh peripheral blood from blood donors must be run every morning to verify both the reagents and methodology.
• Control specimens must be tested in the same manner as patient samples.
• Control samples that fall out of range need to be investigated, and patient results from the same test run are suspect until the reason for the control sample failure is resolved.
• All control data must be documented and the results verified for acceptability before
reporting results.

Results

Important immunological evaluation includes:
• CD4 absolute count
• CD8 absolute count
• Percentages of CD4 and CD8 in special laboratories, especially for paediatric and pregnant patients
• Ratio CD4/CD8

Reference ranges

Normal values range from 500–1,500 cells/mL of blood.

Interpretation of Results

• The lower the CD4 count, the more the disease has progressed. Treatment with ARVs will be initiated when counts are below 500 cells/mL.(For Rwanda policy is below 350 cells/mL)
• AIDS is diagnosed when CD4 cell counts are below 200 cells/mL.

Tests Limitations and Sources of Error

Upon receipt, check for:
• Clots
• Gross haemolysis
• Lipaemic samples for rejection
Also refer to the product literature.

Disposal of waste materials

Materials used in the test must be decontaminated, preferably with 1% sodium hypochlorite,before disposal.

References

User's manual for BD FACScount System. BD Biosciences, California, USA.

Standard Operatating Procedures for Laboratory Services in support of the ART Programme, Zambia., December 2004