GUIDELINE FOR DIAGNOSIS, TREATMENT & PREVENTION OF

LEISHMANIASIS IN ETHIOPIA



2nd Edition
June 2013

Foreword

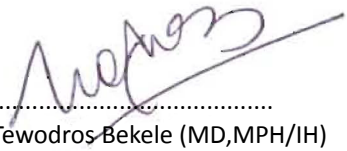
Estimated 900,000 to 1,600,000 new Leishmaniasis cases occur annually worldwide with approximately 350 million people at risk of the disease. Leishmaniasis is prevalent in more than 98 countries distributed over three major territories in the world.

In Ethiopia, both the cutaneous and visceral forms of Leishmaniasis are endemic in various localities. Visceral Leishmaniasis is found mainly in the lowlands of northwest, central, south and southwestern Ethiopia; whereas cutaneous Leishmaniasis is widely distributed all over the country. Several outbreaks occurred in the last few years. The disease is spreading to new localities due to population movement and HIV co-infection. It is estimated that the annual burden of VL ranges from 2,000 to 4,500 cases in Ethiopia.

Cognizant of this mounting burden, the Ethiopian Federal Ministry of Health has put a corner stone to establish a control program for Leishmaniasis by updating the 2006 "Visceral Leishmaniasis Diagnosis and Treatment Guideline for Health Workers in Ethiopia" in order to account for the different forms of Leishmaniasis: CL, VL and Leishmania/HIV co-infections. The new guideline was developed by a panel of experts with wide experience in prevention, control and treatment of Leishmania infections, and trainers of health professionals on different aspects of Leishmaniasis. The new guidelines also address issues related to the control of the disease.

Under the leadership of the Neglected Tropical Diseases Unit within the Agrarian Health Promotion and Disease Prevention Directorate in the Federal Ministry of Health and with regional ownership of the control programs, we are looking forward to the successful implementation of Leishmaniasis prevention and control activities.

I hope that this guideline will be a useful companion to health workers, authorities and development partners working for a prospering Ethiopia.



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Health promotion and Diseases prevention Directorate General Director

Acknowledgments

This guideline for diagnosis and treatment of Leishmaniasis was developed by the Agrarian Health Promotion and Disease Prevention Directorate team of the Federal Ministry of Health in collaboration with the World Health Organization (WHO), Addis Ababa University Faculty of Medicine, Aklilu Lemma Institute of Pathobiology at Addis Ababa University, Medicines Sans Frontiers, the Armauer Hansen Research Institute (AHRI), the International Training and Education Center for Health (I-TECH), the Drugs for Neglected Diseases initiative (DNDi) and the All African Leprosy Rehabilitation Training Center (ALERT). The Federal Ministry of Health thanks all of them for their invaluable input during the preparation of this guideline.

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Acronyms

ART	antiretroviral treatment
CD4	cluster of differentiation 4
CL	cutaneous Leishmaniasis
DAT	direct agglutination test
DCL	diffuse cutaneous Leishmaniasis
ECG	electrocardiogram
GIS	geographic information system
HCP	health care providers
HIV/AIDS	human immunodeficiency virus/acquired immunodeficiency syndrome
Hgb	hemoglobin
I-TECH	International Training & Education Center for Health
IM	intramuscular
IV	intravenous
LAB	liposomal amphotericin B
LCL	localized cutaneous Leishmaniasis
LD	*Leishmania Donovani*
LLITN	long lasting insecticide treated net
MSF	Medicines Sans Frontiers
NEDL	national essential drug list
PKDL	post kala azar dermal Leishmaniasis
RDT	rapid diagnostic test
SSG	sodium stibogluconate
TOC	test of cure
VL	visceral Leishmaniasis
WHO	World Health Organization

1- Rational of the Guideline and Introduction

Leishmaniasis is a vector-borne disease caused by protozoan parasite of the genus Leishmania. It is transmitted by the vector *phlebotomine* sandfly. Some of the Leishmania species known to cause disease in humans are: Leishmania donovani *(L. donovani)* species complex (including *L. donovani* and *L. infantum/chagasi*), *L. major, L. tropica, L. aethiopica, L. braziliensis* and *L. mexicana* species complex.

The disease is endemic in environments that range from deserts to rain forests in rural and urban settings in over 98 countries of the tropics, subtropics, and southern Europe. Globally, the population at risk is estimated to amount to 350 million people with an overall prevalence of 12 million. The estimated yearly incidence is 0.7 – 1.2 million cases of cutaneous Leishmaniasis (CL) and 0.2 – 0.4 million cases of visceral Leishmaniasis (VL-also called *kala-azar*, from Hindu for "black fever"). It is associated with about 2,357,000 disability-adjusted life years (DALYs), 946,000 in men and 1,410,000 in women, representing a significant rank among communicable diseases. A third of the global figure (770,000 DALYs) is attributable to CL and approximately 20,000 – 40,000 deaths per year.

Estimations of the burden caused by the Leishmaniasis disease in the world is challenging. Clinical and epidemiological diversity, marked geographic clustering, and a poor surveillance system leads to a lack of reliable data on incidence, duration, and impact of the various disease syndromes. 90% of CL infections occur in Afghanistan, Pakistan, Syria, Saudi Arabia, Algeria, Iran, Brazil, and Peru while with regard to VL cases 90% occur in Bangladesh, Brazil, India, Sudan, South Sudan and Ethiopia.

In Ethiopia, CL is distributed mainly in the highlands on an elevation between 1,400–3,175 meters above sea level. According to the 2012 WHO global Leishmaniasis estimate, Ethiopia is one of the ten high burden countries for CL. VL is predominantly found in the lowlands with varying degrees of endemicity. It is estimated that the annual burden of VL ranges from 2,000 to 4,500 cases. Some of the factors found to be associated with the spread include population movements to and from endemic focus areas, poverty and malnutrition associated with presence of the sandfly vector and reservoirs.

The endemicity of VL was recently extended to at least five administrative regions, namely, Amhara, Tigray, Southern Nations, Nationalities, and Peoples' Region, Oromia and Somali. In addition, there have been recent outbreaks in northern and southern parts of

the country: Libo Kemkem Woreda in Amhara region, T/Adiabo Woreda in Tigray region and Imey in Somali region. The CL burden is roughly estimated to range from 20,000 to 30,000 cases per year covering a very wide geographic area in Ethiopia. The tendency of the disease to spread to new areas was also noted, as in case of the Silte outbreak. Currently, efforts are undergoing in risk mapping of the disease throughout the country.

Moreover, the increasing *Leishmania*/HIV co-infection is an emerging challenge to be addressed as well as an important factor for the spread of the disease. While the global co-infection rate is 2-12% of all VL cases, Ethiopia has high reported rates of co-infection.

In Ethiopia, at least 3 species of sand flies (*P. orientals, P. martini* and *P. celiae*) are confirmed to be involved in the transmission of VL. Transmission is human to human (anthroponotic). Until today, no confirmed animal reservoir was identified. Moreover, detailed knowledge on the vectorial capacity as well as the breeding, resting and biting behavior of the sandfly is lacking. Such type of information needs to be gathered first in order to recommend sound sandfly control measures in a given endemic area. In addition, human habits due to economical and poverty related factors that increase the exposure to sandfly bites are not easy to address from a public health perspective

In response to the increasing public health concern, there are different efforts to account for the disease. The results of these efforts will help health authorities to design better strategies for control and the eventual elimination of the disease.

The government of Ethiopia, like other Leishmaniasis endemic countries, has developed its own control strategies so as to limit the rapid spread of the disease. Thus, this document updates the “Visceral Leishmaniasis Diagnosis and Treatment Guideline for Health Workers in Ethiopia” from 2006. It was ensured that the proposed recommendations are in agreement with other national guidelines and current international recommendations. Emphasis has been given to make the recommendations user-friendlier for the main target group, Ethiopian health professionals from the mid-level and above.

The development and implementation of this guideline is based on the following principles:

- To be used and adhered to by every organization involved, regardless of their specific mandate or mission
- To get feedback from all stakeholders involved in planning, implementing, and following-up of Leishmaniasis prevention, control and treatment programs
- To give special emphasis to surveillance of Leishmaniasis and provide a frame work to improve coordination and guided implementation of interventions among all

partners in order to develop a functional system for Leishmaniasis control in Ethiopia.

The present guideline is meant to serve as national direction for standardization and implementation of Leishmaniasis prevention, diagnosis and management. In addition, the guideline offers clear guidance for the rational use of anti-Leishmania drugs, a standardized diagnostic approach, and a standardized and simple management approach for Leishmaniasis prevention and surveillance.

Intended users of the guideline are:

- Policy makers and managers who are involved in Leishmaniasis prevention, control and treatment programs,
- Health care providers (HCPs) who manage patients with Leishmaniasis,
- Trainers who will be involved in the training of HCPs on Leishmaniasis,
- All governmental, non-governmental, private and other organizations/ institutions that are involved in Leishmaniasis prevention, control and treatment programs, and
- Institutions involved in drug procurement and distribution

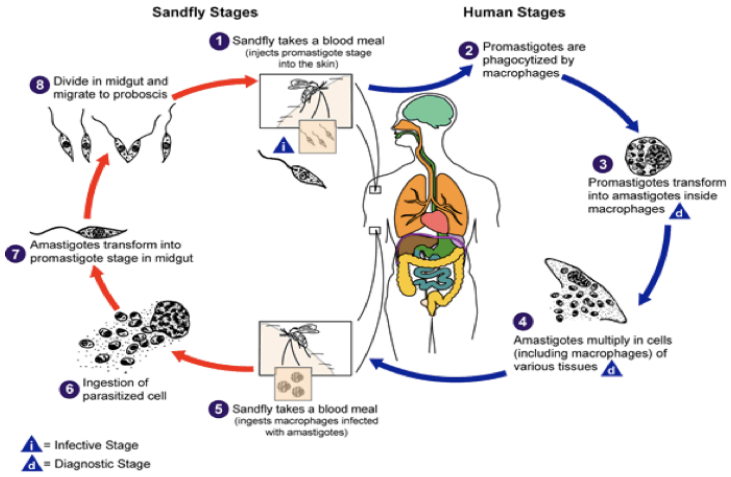
2 Life Cycle and Pathogenesis of Leishmaniasis

Leishmania parasites are digenetic parasites that need two hosts, the sandfly and a mammalian host, to complete their life cycle (see Figure 1). Over twenty Leishmania species are pathogenic to humans and thirty sandfly species are proven in the vectors. The transmission of Leishmaniasis is either **zoonotic**, which includes animal reservoir hosts, mainly dogs, in the transmission cycle, or **anthroponotic** in which humans are the sole source of infection for the vector. Moreover, different species of phlebotomine sandflies need different habitats to survive and have different biting preferences (in/outdoor, forest/ village, day/night). This has consequences for the transmission of the disease and control measures to be applied. VL in Ethiopia is caused by *L. donovani* with an anthroponotic transmission.

Table 1: Leishmania Parasite and Their Vectors in Ethiopia

Leishmania species	*Vectors*	*Reference*
L. aethiopica	P. longipes P. pedifer P. sergenti	Ashford et al., (1973) Ashford et al., (1973) Gebre-Michael et al., (2004)
L. tropica	P. sergenti P. saevus	Gebre-Michael et al., (2004) Gebre-Michael et al., (2004)
L. major	P. duboscqi	Gebre-Michael et al., (1993)
L. donovani	P. martini, P. Celiae P. orientalis	Gebre-Michael and Lane, (1996) Hailu et al., (1995)

Leishmania infections do not always equate with clinical illness. The ratio of incident asymptomatic infections to incident clinical cases varies among geographic regions. Most infections in immuno-competent individuals remain asymptomatic. Different species of Leishmania may illicit different types of responses. Both, host and parasite, seem to play a role in the course of the disease:, i.e., whether the disease is self-healing, the parasite localizes in the skin, or spreads to the viscera or mucosa. Thus, of utmost importance for vaccine development and disease control is to understand the factors that might predispose some individuals to develop the disease while others control the infection.



Picture taken from http://www.dpd.cdc.gov/dpdx

Figure 1: Life Cycle of Leishmaniasis

Figure 1 depicts the life cycle of the Leishmania parasite. The infected female sandflies inject metacyclic promastigote to a susceptible mammalian host during a blood meal. Promastigotes in the skin are phagocytized by macrophages and transform into amastigotes. Amastigotes multiply in infected cells and, depending on the infecting Leishmania species and/or the immune status of the host, the parasite can get disseminated to the viscera or remain at the site of its inoculation. This yields either the cutaneous or visceral form of clinical manifestation. When sandflies take blood meals from an infected host, they ingest macrophages infected with amastigotes. In the sandfly's mid gut, the parasites transform into promastigote which multiply and migrate to the anterior part of the gut being transformed into the metacyclic form and its life cycle continues.

3- Clinical Manifestations of Leishmaniasis

The clinical forms range from the self-healing localized cutaneous form (LCL) to the more complicated non-self limiting mucocutaneous form (MCL) and diffused cutaneous (DCL) forms to the potentially fatal visceral form (VL, also called *kala-azar*).

3.1 Cutaneous Leishmaniasis

See chapter 7

3.2 Visceral Lei shmaniasis

Visceral Leishmaniasis, also known as *kala azar,* is the severe form of the disease. The incubation period typically ranges from 2–6 months, but it can also range from a few weeks to years.

VL in Ethiopia is caused by *L. donovani* by an anthroponotic transmission. Most infections in immunocompetent individuals remain asymptomatic. The parasites replicate within the mononuclear phagocytic cells and disseminate throughout the reticulo-endothelial system when the immunity is weak. The incubation period typically ranges from 2–6 months, but it can also range from a few weeks to years.

The clinical symptoms include fever, fatigue, weakness, loss of appetite and weight. The accumulation of infected mononuclear phagocytic cells in the spleen and the kupfer cells in the liver result in the hypertrophy of the organs to a clinical apparent hepatosplenomegaly. The infection of the lymphoid tissue results in suppression of the immune response which is a predisposition for other infections. Ear infection, pneumonia, diarrhea and sep-

sis are commonly encountered infections. Anaemia could occur as a result of bone marrow infiltration by the parasite or due to bleeding. All cell lines are often affected causing pancytopenia. Diarrhoea and cough can occur due to mucosal involvement of the gastro-intestinal and respiratory tract. Signs of bacterial co-infection should be ruled out because bacterial pneumonia and gastro-intestinal infections are common. Symptoms often persist for several weeks to months before patients seek medical care or die from the bacterial co-infection, massive bleeding or severe anemia. Patients at an advanced stage of the disease become cachexis and edematous from hypoalbuminemia or congestive heart failure due to the anemia. Hepatic dysfunction, jaundice and ascitis can also occur.

The clinical manifestations of the disease are caused by multiple factors.
The most common causes are mainly due to:

- Splenomegaly resulting in
  - Sequestration of blood
  - Pressure effect of massive spleen
- Pancytopenia resulting in
  - Low hemoglobin
  - Low WBC – low immunity and high incidence of infections
  - Low platelets-bleeding

The presence of a HIV co-infection may alter the clinical presentation of patients so that atypical sites (non-reticuloendothelial organs) tend to be more involved. These include oral mucosa, gastrointestinal and concomitant diffuse skin involvement by the parasite. Generally the presence of a co-infection with HIV alters the clinical picture, the laboratory diagnosis and interpretation, the response to treatment, the occurrence of side effects, the response to therapy, and the frequency of recurrences after treatment.

Differential Diagnosis for Visceral Leishmaniasis

Hyperactive Tropical Splenomegaly

This is an immunological reaction to malaria. The patients may have had a very large spleen (often combined with an enlarged liver) for years. There is no fever. This is a diagnosis by exclusion of other differentials for patients from malaria-endemic areas with a huge spleen.

Malaria

Acute malaria normally stays for few days. However, repeated attacks of malaria can

cause chronic malaria that manifests itself with fever, severe anemia, splenomegaly, loss of weight and other constitutional symptoms. Treatment for malaria improves the patient situation.

Typhoid Fever

The classic presentation includes high fever, malaise, diffuse abdominal pain, and constipation or diarrhea. Patients usually have a slow heart rate (bradycardia), and, in severe cases, can progress to impaired mental status (confusion) or other serious complications.

Typhus

Typhus is also an acute febrile illness characterized by an abrupt onset of fever, headache and a maculopapular or petechial rash.

Brucellosis

Patients have a long history of fever, small or moderate splenomegaly and an enlarged liver. Usually there is also involvement of the joints or bones that results in musculoskeletal pains and arthritis.

Schistosomiasis (Bilharzia)

The patients can have a very large liver and spleen, which they have often had for a very long time. Patients may not have fever but can present with ascites and other portal hypertension signs. Stool or urine analysis will show eggs of *Schistosoma*.

Splenic Abscess

These patients have high fever and a very tender spleen.

Tuberculosis

The patients will present with malnutrition and a history of fever and other constitutional symptoms for several months. They may have an enlarged spleen or lymph nodes as in miliary Tuberculosis.

HIV/AIDS

HIV damages the immune system so that the body of an infected person cannot properly fight against diseases. Patients often suffer from recurrent diarrhoea, which may lead to malnutrition/wasting and dehydration, as in a *kala azar* patient.

Leukaemia (Chronic Myeloid Leukaemia)

These patients have fever, malaise, bleeding tendencies, weight loss and splenomegaly. They are also susceptible to other infections. Blood tests in the laboratory show a high white blood cell count.

3.3 Post kala-azar Dermal Leishmaniasis (PKDL)

VL due to *L. donovani* occurs with a post treatment complication called PKDL. PKDL is characterized by the occurrence of painless skin lesions relatively common towards the end of treatment (more common in Sudan with about 50% of the cases) or shortly *after treatment*. PKDL is rare in Ethiopian VL patients but it is relatively common in *Leishmania*/HIV co-infection. PKDL patients harbor Leishmania parasites in the skin lesions and these can be potential sources of infection and disease transmission. Patients should be advised to seek medical attention and use impregnated bed nets if they develop skin rash following treatment. Depending on the severity of the lesion, PKDL can be graded which will facilitate treatment decisions.

Occasionally, PKDL may occur prior to the VL (Pre-*kala azar* dermal Leishmaniasis) or concomitantly (para-*kala azar* dermal Leishmaniasis) to the active VL disease.

Grading PKDL Lesions:

Grade 1: scattered macular, papular or nodular skin rashes mainly on the face and around the mouth, with or without some lesions on the upper chest and upper limbs.
Grade 2: dense macular, papular or nodular rashes covering most of the face and which are extending to the chest, back, upper arms and legs. If lesions are extensive or blackened, this is called PKDL grade 2 **severe** form.
Grade 3: dense macular, papular or nodular rashes covering most of the body including hands and feet. Here, crusting, ulcers, sloughing, scaling, blackening of skin and spreading of the lesions to mucosa of the lips and the palate occur.

4- Diagnosis of Visceral Leishmaniasis

4.1 Introduction to Diagnosis of Visceral Leishmaniasis

Visceral Leishmaniasis should always be suspected when an individual presents with prolonged fever from the endemic areas. The Diagnosis of VL relies on clinical, serological, parasitological and molecular findings. The definitive diagnosis is demonstration of the parasite in a tissue aspirate. However, this is an invasive procedure associated with risk of bleeding and severe pain. It also requires an hospital setting with blood transfusion service and experienced clinicians for the procedure. Thus, it may not always be applicable especially in remote regions where VL is endemic. Alternative means of diagnosis include different serology tests and molecular techniques. But these tests require a certain capacity. These tests are more important for research purposes than for the routine clinical care of patients.

Whereas early and accurate diagnosis is essential as VL is fatal if not treated, the drugs available are potentially toxic and expensive. In addition, untreated individuals might act as reservoirs putting the community at risk of ongoing transmissions.

4.2 Clinical Diagnosis

Clinical signs and symptoms associated with VL include fever for more than two weeks, fatigue, weakness, loss of appetite and weight, malaise, cough, diarrhea, epistaxis, cachexia, abdominal pain, anemia, pancytopenia, and, associated with parasitic invasion of blood and reticulo-endothelial system, enlargement of lymph nodes, spleen, and liver. Some of these clinical signs and symptoms are similar to other diseases prevalent in the areas where VL is endemic. The clinical diagnosis of VL is therefore based on the standard case definition of visceral Leishmaniasis, as given below:

VL case definition

A person who presents with fever for more than two weeks and an enlarged spleen (splenomegaly) and/or enlarged lymph nodes (lymphadenopathy), or either loss of weight, anemia or leucopenia while living in a known VL endemic area or having travelled to an endemic area.

As the clinical presentation of VL lacks specificity a definitive test is required to decide which patient should be treated. This test has to be highly sensitive (> 95%) and specific. Ideal tests should be able to make distinction between acute or asymptomatic infections. Tests available to diagnose VL include non-Leishmanial tests, parasite detection, antibody detection, antigen detection, and molecular techniques. These tests have variable sensitivity, specificity and negative and positive predictive values.

4.3 Laboratory Investigation

a) Non-Leishmanial Tests

Hematologic data, such as reduction in the number of red blood cells, white blood cells and platelets (pancytopenia), have a higher sensitivity (98%) but a lower specificity (16%) according to a study made in Nepal.

b) Parasite Detection

Definitive diagnosis of VL is made by visualization of the amastigote form of the parasite by microscopic examination of aspirates from lymph nodes, bone marrow or spleen aspiration. This diagnosis has a high specificity but the sensitivity of the microscopy varies (93-99% for spleen, 53-86% for bone marrow, and 53-65% for lymph node aspirate). The sensitivity and specificity of spleen aspirate is excellent but splenic aspiration should be performed in a hospital setting where blood transfusion is possible because the procedure is associated with a risk of fatal internal bleeding. This procedure also requires considerable technical expertise for making the aspiration and facilities for nursing surveillance, blood transfusion, and surgery.

Lymph node aspiration is safe but the sensitivity is low. However, either bone marrow or lymph node aspiration can be used whenever the condition is not prevailing for spleen aspiration. The parasitic confirmation of the diagnosis is mandatory (see Annexes 3 to 5). The accuracy of microscopic examination is influenced by the ability of laboratory personnel, reagents used, and the nature of the aspirate. Inoculation of the aspirate into a culture media (Novy, MacNeal, Nicolle (NNN) or RPMI media) under aseptic condition improves the detection of the parasites. However, this technique remains restricted to referral hospitals or research centers.

c) Antibody Detection

Immunodiagnostic techniques, like enzyme-linked immunosorbent assay (ELISA), immunofluorescent antibody test (IFAT), freeze-dried direct agglutination test (FD-DAT) or aqueous antigen direct agglutination test (AQ-DAT), Fast Agglutination Screening Test (FAST) agglutination test, indirect hemagglutination test (IHA), rK39 immunochromatographic (rk39-ICT) strip test, and rK39 ELISA, have been extensively used for the diagnosis of VL. However, all of these tests have two major limitations. First, they do not distinguish between present and past infections, as the serum antibody level remains high after successful treatment. Detection of relapse is not possible with these methods. Second, all these tests fail to differentiate between symptomatic and asymptomatic infections. As a consequence, a significant proportion of healthy individuals in the endemic area with no history of VL are positive for anti-Leishmanial antibodies.

The performance of the various serological tests in the diagnosis of VL is found to be good to excellent; their sensitivity and specificity is ranging from 70–100%. However, the facilitation of some of these tests (ELISA, IFAT, IHA) in the field where the disease is prevalent is limited because they need a well-equipped laboratory and skilled personnel. Of all these tests, DAT and rK39 have been extensively evaluated and used for the diagnosis of VL in the field and in laboratory settings.

rK39 is a 39-amino acid repeat of a kinesin-related protein cloned from *Leishmania chagasi* and conserved in *L. donovani* complex. The widely available rK39 test in the market include **Kalaazar Detect™ InBios, DiaMed-IT-Leish**, etc. (see Annex 1 for the test procedure).

DAT is a semi-quantitative test that uses V-shaped micro-titter plates in which increasing dilutions of patients' serum or blood are mixed with killed and stained freeze dried or aqueous *Leishmania donovani* promastigotes. If specific antibodies are present agglutination will be visible within 18 hours (see Annex 2).

An meta-analysis of several reports based on the performance evaluation of DAT (30 studies) and rk39 (13 studies) has calculated a combined sensitivity of DAT and rK39 at 94.8% and 93.3%, respectively. The specificity of DAT was estimated to be 85.9% (95% CI = 72.3% to 93.4%) and that of rK39 to be 90.6% (95% CI = 66.8% to 97.9%). However, initial field studies in Eastern Africa, involving the different brands of the rK39 dipstick, have

depicted variable performances in terms of sensitivity, specificity and predictive values of positive and negative test results. Currently, the main shortcoming of the rk-39 dipstick test in East Africa is its low sensitivity. The extent of this shortcoming varies between the different brands, being magnified in InBios products. This lower sensitivity of rK39 dipstick in East Africa highlights the need of careful monitoring schemes until new generations of the kits become available. In addition, the performance of this RDT in the health services should be monitored on a regular basis through a process called "External Quality Assurance". It is also recommended that the more sensitive test, DAT, should be available in health centers and hospitals treating VL. The rK39 dipstick test has several advantages over DAT, namely, ease of performance (no major equipments are required), rapid test results (10-20 minutes), cheaper cost and better reproducibility (see Annex 12). These features make the test an ideal diagnostic tool for the peripheral health facilities in VL endemic localities.

d) Antigen Detection Test

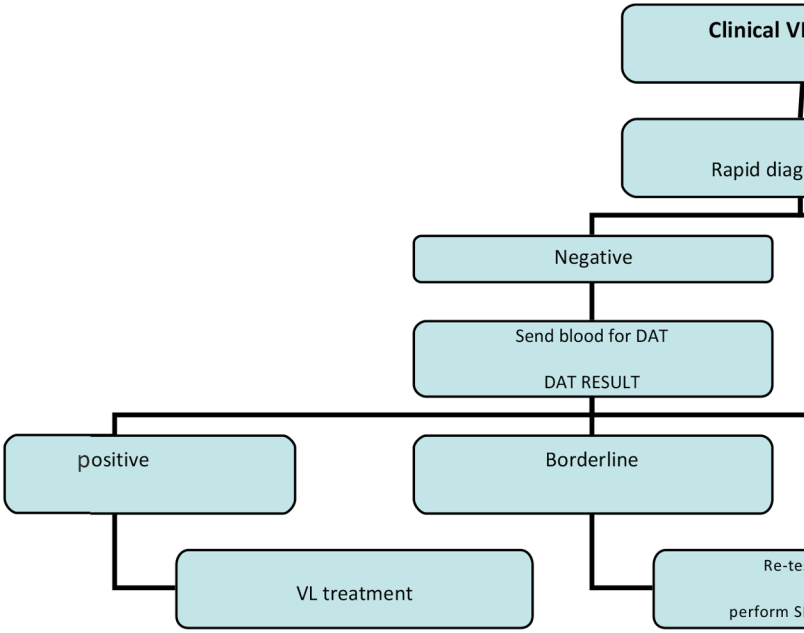
The antigen detection test is an ideal test because it is more specific than the antibody-based immunodiagnostic test. It helps to distinguish active from past infections. This method is also useful in cases with a deficient antibody production, as in AIDS patients. A urine latex agglutination (KATEX) that detects heat-stable, low molecular weight carbohydrate antigen in the urine of VL patients has been developed. The evaluation of the performance of KATEX at the Indian subcontinent and East Africa has shown that this test has a good specificity but only a low to moderate sensitivity.

e) Molecular Techniques

A number of different molecular methods have been successively evaluated for Leishmaniasis diagnosis (pulsed-field gel electrophoresis, multilocus enzyme electrophoresis and PCR-based assay). The polymerase chain reaction (PCR) based assays currently constitute the main molecular diagnostic approach of Leishmaniasis. These nucleic acid based methods answer several clinical and biological questions simultaneously. The major importance of a PCR-based technique is its high sensitivity and specificity (up to 100%) irrespective of the species or genus. Second, the burden of infection could be assessed by PCR. This may be highly relevant in monitoring disease progression and outcome of an anti-Leishmanial therapy. Third, the viability of detected parasites could be demonstrated by other forms of nucleic acid based techniques, reverse transcription real time PCR (which is expensive), or

less expensive protocol quantitative nucleic acid sequence-based amplification [QT-NASBA]. Fourth, Leishmania species and strain identification can be performed by a series of PCR-based assays. This is useful for the clinical management due to the link between Leishmania species, and disease severity and treatment outcome. Finally, a molecular diagnosis might allow defining parasite-specific features, such as virulence and drug resistance, and so-called parasite tracking, if highly discriminatory fingerprinting tools are employed. However, PCR-based protocols need standardization and optimization. Compared to the other diagnostic techniques available, the molecular approaches remain expensive and technically highly demanding. Their applicability in the endemic areas is highly questionable.

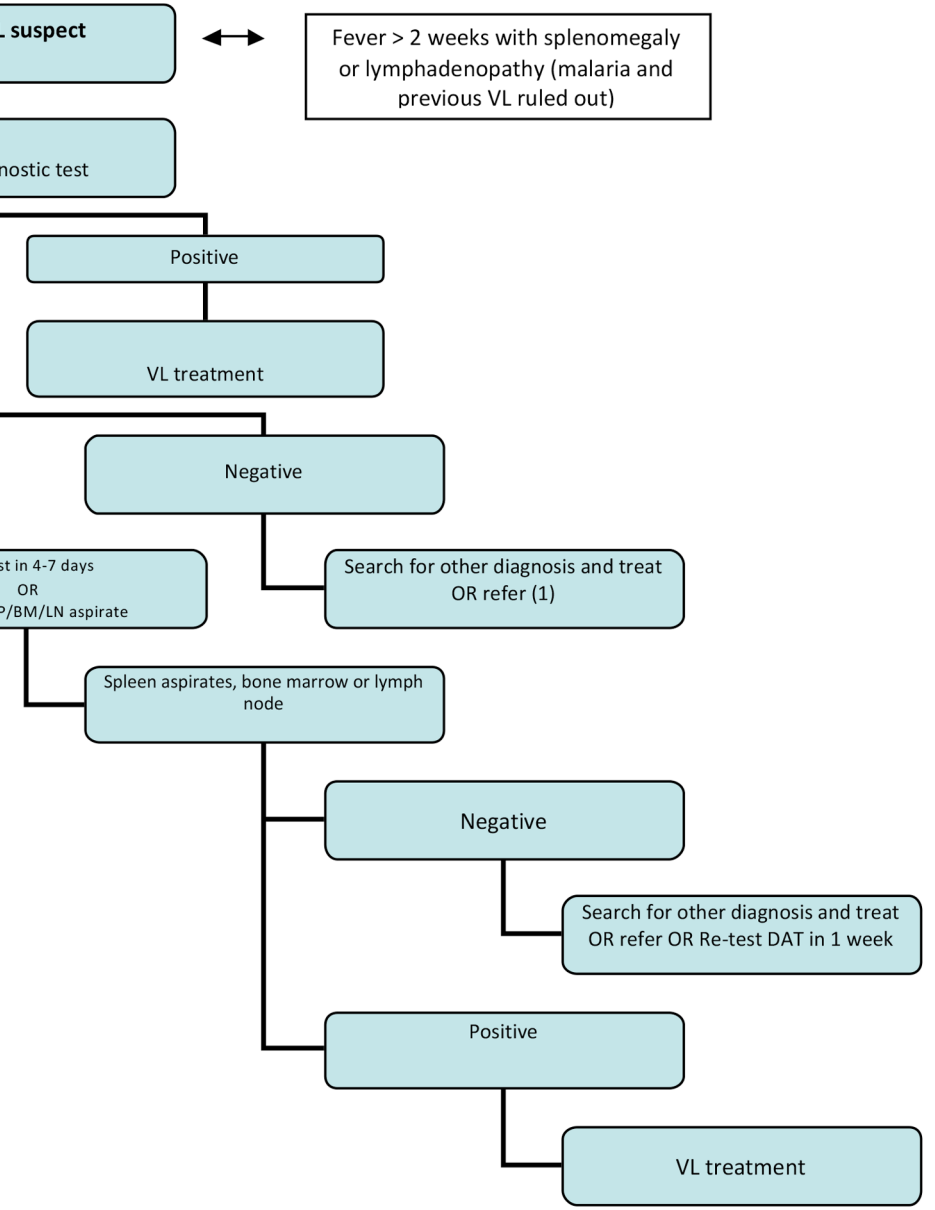
Figure 2: Diagnosis Algorithm for VL in Ethiopia



DAT	Freeze Dried
Positive:	≥ 1:3200
Borderline (BL):	1:1600; 1:800
Negative:	≤ 1:400

LN: lymph node aspirate
BM: bone marrow aspirate
SP: spleen aspirate

(1) Rarely DAT negative patients need SP (splenic)/ BM (bone marrow)/ LN lymphnode aspirate.


L suspect
Fever > 2 weeks with splenomegaly or lymphadenopathy (malaria and previous VL ruled out)
nostic test
Positive
VL treatment
Negative
st in 4-7 days
OR
P/BM/LN aspirate
Search for other diagnosis and treat OR refer (1)
Spleen aspirates, bone marrow or lymph node
Negative
Search for other diagnosis and treat OR refer OR Re-test DAT in 1 week
Positive
VL treatment

5- Treatment of Visceral Leishmaniasis

The treatment regimens should follow the national guidelines in all treatment centers in Ethiopia. Treatment should normally be given only after the confirmation of the disease based on the clinical examination and the laboratory tests. At the same time, the presence and extent of concomitant infections should be assessed because they may dictate the choice of therapy or supportive treatment.

The best strategy to control anthroponotic VL is through early case detection and adequate treatment. VL is invariably fatal if left untreated. The treatment options for VL are not safe and often unaffordable. The objectives of VL treatment are to:

1. Reduce the parasite burden,
2. Prevent drug resistance,
3. Avoid toxic drug effects, and
4. Improve the clinical condition of patients and to manage complications (anemia, malnutrition and secondary infections)

Patient monitoring is vital during VL treatment as the currently used antileishmanial drugs are toxic. Antimonials, the antileishmanial drug used most frequently, are toxic causing sudden death due to cardiac complication or may result in severe acute pancreatitis although these are very rare complications. Clinical follow-up of complications (symptoms or signs) and basic laboratory tests including hematology, chemistry and ECG are required when using antimonies. Hence, patients should be invariably admitted for visceral Leishmaniasis treatment, close monitoring of drug adverse effects, supportive management, and follow-up of patient's progress.

Supportive treatment is important. Patients should be properly hydrated and given nutritional therapy before starting the VL treatment. Severe anemia should be corrected with blood transfusions, and concomitant infections should be treated with appropriate anti-infective agents.

Sterile cure by drugs in VL treatment is not always possible – at least by the currently available antileishmania drugs. The drugs enable reduction in parasitic burden and help the stimulation of the weakened cell-mediated immunity to clear the remaining parasites from the body. There are occasions where the patient's cell-mediated immunity is slow to develop as seen in those co-morbidities like HIV/AIDS, malnutrition and

tuberculosis leading to ineffective parasite clearance predisposing to relapse, treatment failure and drug resistance.

5.1 First-Line Regimens for Primary VL

The first-line drugs for the management of primary visceral Leishmaniasis in Ethiopia are a combination of antimonials with aminiglycosides and Liposomal Amphotericin B for special situations as described below. The combination therapy of SSG and Paromomycin is the preferred choice for its better safety and affordability. The efficacy of the combination regimen is comparable with that of the SSG monotherapy. Although liposomal Amphotericin B is the safest drug, it is also the most expensive one.

a) Combination Therapy: Sodium Stibogluconate (SSG) and Paromomycin

In the combination therapy, **sodium stibogluconate** (20mg/kg body weight/day), and **paromomycin** (15mg/kg body weight/day) injections are given intramuscularly for 17 days (see Annex 8).

This combination treatment has been recommended as one of the treatment regimens for VL caused by *L. donovani* in East Africa by the WHO. Therefore, it should be one of the first choices if both drugs are available. The efficacy of the combination therapy is similar to that of the pentavalent antimonials monotherapy, with the advantage of a decreased toxicity, shorter treatment duration and cheaper cost.

The combination treatment has the potential advantages of: (i) shortening duration of treatment, thereby increasing compliance, (ii) reducing the overall dose of drugs, thereby reducing toxic effects of drugs and cost, and (iii) reducing the probability of selection of drug resistant parasites, thereby prolonging the effective life of the available drugs.

The combination of SSG at 20 mg/kg Sb^{5+} plus Paromomycin given at 15mg/kg sulphate (11 mg/kg/d of base) for 17 days had shown an efficacy of 90-94% in Ethiopia.

Note:

A challenge for the future remains to constitute the combination therapy in a single injection. Currently SSG and paromomycin can be administered at the same time but in two separate injections, i.e., one in either buttock or the PM injection in another IM area. Please, also note that the Paromomycin injection is a very small volume injection.

Several studies in India have recently shown that three separate combinations – liposomal amphotericin B (LAmB) plus miltefosine, or LAmB plus Paromomycin, or Miltefosine plus Paromomycin – demonstrate high cure rates. These have yet to be investigated in the Ethiopian context.

b) Sodium Stibogluconate or Meglumin Antimoniate (Monotherapy)

SSG in monotherapy is administered as intramuscular injection of 20mg/kg/day for 30 days. In the absence of or in case of stock ruptures of Paromomycin, Pentavalent antimonials can be used in monotherapy (see Annexes 7 and 9).

Pentavalent antimonials have been the first-line drug used for the treatment of visceral Leishmaniasis in Ethiopia for the last few decades. The generic form of the drug (Sodium Stibogluconate) is widely used, rather than its brand form (Pentostam), mainly due to cost related reasons. The efficacy of both forms is comparable and acceptable as well. The antimonials include Sodium Stibogluconate (generic SSG), and meglumine antimoniate (Glucantime). The half-life of antimonials is about 2 hours and their elimination is through the kidneys. SSG and Glucantime have similar mechanism of action, efficacy and side effects.

Preparation and Dosage:

Sodium Stibogluconate 100mg/ml or Glucantime 81mg/ml

The recommended dose is 20mg/kg/day for 30 days either intramuscular or by a slow intravenous infusion within 5 minutes. The dose depends on the weight of the patient but the minimum recommended dose of SSG is 2ml. It is also recommended to split the dose of it is more than 10ml. Consideration should be given in Leishmaniasis patients with edema to avoid overdosage. Weekly weight measurements need to be made and the dosage should be recalculated accordingly.

Although resistance to antimonials is an issue in other countries, the response rate for SSG in Ethiopia is good currently especially for those who are not co-infected with HIV.

HOW TO CALCULATE THE DOSE OF SSG

SSG vials contain 100mg/ml so it is calculated as follows:

Dose in ml = Body weight in kg x 0.2

EXAMPLES: If your patient weights 40 kg: 40 x 0.2 = 8 - so give 8ml!

Toxicity: Please, see Annex 7!

Precautions during the use of antimonials which require to withdraw or stopping VL treatment:

Patients with a very high or low age, severe illness, severe anemia, HIV co-infection or severe malnutrition require a close follow-up during treatment with antimonials. Necessary precautions should be taken for patients with visceral Leishmaniasis. These may require stoppage of the drug and a possible shift of treatment to AmBisome :

1. Acute pancreatitis with abdominal pain and vomiting
2. Jaundice developing while on treatment
3. Elevated liver function tests (5 times of the normal value)
4. Elevated creatinine level
5. Evidence of cardio-toxicity
6. Uninterrupted severe vomiting
7. Declining hematologic parameters, and
8. Failure to respond to treatment after two weeks of drug treatment

c) Liposomal Amphotericin B (LAmB, AmBisome)

Liposomal Amphotericin B is the first-line treatment for VL in special situations. As the use of antimonials is not safe in pregnant women due to the higher risk of miscarriage, its use is not recommended where there is an alternative drug to use. Similarly, the use of antimonials in *Leishmania*/HIV co-infected patients is found to be less effective with higher and frequent relapse rates and severe (fatal) toxicity. Hence, Liposomal Amphotericin B is recommended in those patients with pregnancy, HIV-co-infection, severe illness, severe anemia, severe malnutrition and extremes of age (below 2 years or above 45 years). In special situations with severe risk factors for death at the patient's admission, antimonials toxicity has proved to be very high and, therefore, LAmB is preferable if available for these patients.

5.2 Second-Line Treatment for Primary Visceral Leishmaniasis

The second line treatment for VL in Ethiopia is Liposomal Amphotericin. Indications for the use of second-line VL treatment are drug toxicity, relapse, treatment failure, very severe illness, pregnancy and VL/HIV co-infection.

a) Liposomal Amphotericin B (AmBisome)

Liposomal Amphotericin is a lipid formulation of Amphotericin B in which the drug is packaged with cholesterol and other phospholipids within a small unilamellar liposome. This is an extremely safe and efficacious drug for the treatment of visceral Leishmaniasis.

According to the report of a WHO informal consultation in Rome in 2005, AmBisome has the highest therapeutic index of existing antileishmania drugs with moderately long half-life (7 hours in the plasma) and sustained presence in the tissues (several weeks) after treatment. Beside its high therapeutic index, AmBisome has a relatively short treatment course and minimal side effects; its main disadvantage is its high cost.

Mechanism of Action:

The mechanism of Leishmanicidal action of AmBisome is thought to be drug binding to parasite ergosterol precursors causing disruption of the parasite membrane. This formulation has several characteristics that increase its efficacy against VL while minimizing its toxicity such as its small size promoting wide distribution, high transition temperature helping stability and its high affinity and tissue penetration ability.

Dosage and Administration:

The recommended dose of AmBisome for treatment is 5mg/kg/day over a period of 6 days (i.e. 30mg/kg in total). It is administered by reconstitution with 5% D/W with a volume of 100ml of D/W for 50mg of AmBisome (for 100mg or 2 vials of AmBisome 200ml of D/W, for 3 or more vials use all the 500ml D/W). It is advised to use whole vials to avoid wastage but the drug should be discarded after 24 hours of reconstitution. The infusion can run over 30 to 60 minutes (see Annex 10).

HOW TO CALCULATE THE DOSE OF Liposomal Amphotericin (AmBisome)

AmBisome vials contain 50 mg/ml. It is calculated as follows:

Dose in mg = Body weight in kg x 5/day

EXAMPLE: If your patient weighs 40kg: 40 x 5 = 200 mg per day.

This will be given IV reconstituted with 500ml of 5% D/W to run for 30 to 60 minutes.

Side Effects and Precautions:

It is a very safe drug which rarely may cause a transient rise in the creatinine level. However, acute and chronic toxicity from AmBisome is low. AmBisome requires a fairly reliable cold chain as exposure to temperatures above 25 oC or below 0 oC will alter the characteristics of the drug which may increase its toxicity or decrease its efficacy.

b) Miltefosine

Miltefosine is an alkyl phospholipid (hexadecacylphosfocholine), originally developed as an oral anticancer drug that has shown to have anti-leishmanial activity. This is the only

oral anti-leishmanial drug taken at a dose of 2-3mg/kg per day (100mg/day for patients weighting more than 25kg) for 28 days. In one study in Ethiopia, Miltefosine was found to be less effective but safer than antimonials in HIV/VL co-infected patients.

Miltefosine commonly induces gastrointestinal side effects, such as anorexia, nausea, vomiting (38%) and diarrhea (20%). These side effects can be reduced if it is taken with or after a meal. Most often, episodes are brief and resolve as the treatment is continued. Occasionally, side effects can be severe and require the treatment to be stopped. Skin allergy, elevation of hepatic transaminases, and rarely renal insufficiency can be observed. Miltefosine is potentially teratogenic and, thus, it is contraindicated during pregnancy. Lactating women and women of childbearing age must use effective contraception during the treatment and for 3 months afterwards.

c) Paromomycin (Aminosidine)

Paromomycin is an aminoglycoside antibiotic. The 15mg/kg sulphate is equivalent to 11mg/kg base, whereas 20mg/kg sulphate is equivalent to 16mg/kg base. Pain at the injection site is the most common adverse event. Reversible oto-toxicity is the main secondary effect. Renal toxicity is rare. Some patients may develop hepatotoxicity indicated by raised hepatic enzymes; tetany has also been occasionally reported. Rare toxicities due to Paromomycin include renal toxicity and hepato-toxicity; tetany has also been reported.

5.3 Treatment of VL Relapse

(Refer to the Treatment Algorithm!)

A patient who is diagnosed with visceral Leishmaniasis for the first time is called a **primary VL case**. A definitive cure is the absence of visceral Leishmaniasis signs and symptoms and a negative test of cure 6 months after **initial cure**, i.e., 6 months after active diseases treatment. Patient follow-up is important to establish a definite cure with proper evaluation on appointment or when presented with fever, loss of weight, anemia and splenomegaly. For definitive cure, one looks at the clinical picture of the patient; tissue aspirate may not be necessary at follow-up sessions unless a relapse is clinically suspected.

If a person returns with clinical features and a positive parasitology consistent with visceral Leishmaniasis, after having been successfully treated for primary VL and discharged improved or with a negative test of cure (TOC), the patient is known as **relapse VL case.** It is impossible to differentiate relapse from new infection but various molecular studies proved relapse to be more common than re-infection.

In immune-competent individuals, VL relapses can occur in up to 5% of the patients treated, whereas VL relapses can occur in up to 50% in HIV/VL co-infected cases. Most relapses occur within the first 6 months of initial treatment and are referred to as a first relapse if the relapse occurs for the first time and subsequent relapses are called second, third and so on depending on the number of relapses. Risk factors for a visceral Leishmaniasis relapse in HIV patients are: AIDS patients not on ART, low CD4+ count, previous VL episodes and failure to achieve clinical or parasitological cure during the first episode. ART could help to delay VL relapse but it does not prevent it. Sometimes relapses tend to have a higher parasite load and are often difficult to treat.

Treatment Regimens for First VL Relapse (Refer to the Treatment Algorithm!)

If possible, the regimen used for the treatment of relapses should be a different drug than that used for primary VL. The use of combination therapy with SSG and Paromomycin is recommended if it was not used for the treatment of prior VL episodes. When choosing a drug for treating a relapse the response to the initial treatment should be considered.

The following regimens are recommended for the treatment of visceral Leishmaniasis relapses (refer to the treatment algorithm in Annex 10):

1. Liposomal amphotericin B 5mg/kg/day for a period of 6 days. The treatment period can be extended up to 14 doses for better therapeutic advantages.
2. SSG 20 mg/kg/day for 40days until 2 consecutive weekly aspirates for parasitology are negative. If TOC is still positive, SSG should be given for a total of 60 days with close monitoring of drug toxicity and TOC checked again, if positivity persists, then 2nd line treatment must be used.
3. SSG/PM combination: SSG (30 days) and Paromomycin (17 days) can be used for VL relapse treatment.

5.4 Patient Follow-Up and Management of VL Treatment Failure

During treatment patients require a strict follow-up of their response to treatment and drug side effects. An follow-up on clinical parameters including fever, spleen size, and patient wellbeing is important. Besides, laboratory tests, like a hematologic profile, chemistry and radiography are necessary for follow-up. Prevention and treatment of secondary infections, diagnosis and treatment of complications and nutritional supplementation are important during the treatment of visceral Leishmaniasis.

At the end of therapy, the outcome of the treatment should be recorded based on the status of the patient as discharged/improved, cured, treatment failure, relapse, referred or defaulted. At discharge, the patient should be given regular appointments 3 and 6 months after the treatment. The patient should be advised to visit the health facility for recurrence of the clinical features and/or appearance of skin rash.

Evaluating Cure

At the end of VL treatment, patients should be re-assessed for response clinically or using laboratory tests.

Clinical Response

Many patients get worse during the first few days of treatment with SSG. After 7 to 10 days, patients become afebrile and begin to look stronger with increased alertness and appetite. By day 14, the spleen size regresses, the Hgb level rises and there is weight gain in the absence of edema.

At the end of successful treatment, patients look improved, afebrile, and usually have a smaller spleen size than on admission and have an increased Hgb level. Look for signs of co-existing TB or HIV, both of them will increase the risk of treatment failure.

Cure is best defined as the absence of clinical features of the disease after completion of the recommended dose and duration of treatment for VL in addition to a negative parasitological test for LD bodies.

Initial Parasitological Cure

Test of cure (TOC) is performed at the end of treatment when there is no or insufficient clinical improvement and in treatment of relapse for decision to stop treatment. There is no clinical sign that best correlates with a positive test of cure or that predicts an increased risk of relapse. Clinical evaluation should get the priority over a test of cure for every patient under the currently available invasive procedures. TOC should be reserved for cases where response is in doubt, in the treatment of relapses and for monitoring emergence of drug resistance. In addition, TOC is done to assure that discharge is appropriate for patients who may have difficulty returning for follow-up.

There must be 2 negative tests of cure (TOC) results before discharging patients with VL relapse. The tests are done one week apart to ensure that there is at least a week of treatment after the first negative TOC. The likelihood for a positive TOC after treating a relapse is higher than 10% and any further relapses will be more difficult to cure.

In some situations, it is better to refer patients with VL relapse for treatment into a hospital setting.

Positive Parasitology (TOC) at the End of Treatment

If the TOC is scanty positive, continue the same treatment until two consecutive weekly aspirates are negative. The limit of duration of therapy is 60 days for SSG and a total dose of 40mg/kg for AmBisome , if at this point the TOC is still positive, use a 2nd line treatment. *Non-response* is defined as failure to decrease the parasitological grade after adequate treatment.

Definitive Test of Cure

It is not a routine practice to do TOC at the 6th month of patient follow-up if the patient does not fit for clinical visceral Leishmaniasis. Relapse is diagnosed when a patient with VL treatment history presents with clinical visceral Leishmaniasis and is diagnosed with positive parasitology (LD bodies) after successful completion of the treatment. Re-infection is possible for VL but most cases are considered as relapse and treated accordingly.

Research is needed for less invasive but reliable procedures of test of cure. Clinical judgement will always play the final role in any decision-making process given the variability of individual VL cases.

5.5 Treatment of VL by Drug Interruption

VL treatment can be interrupted by the treating clinician due to a medical reason by the treating clinician or by a failure of the patient. These patients are at high risk and also potential sources of disease transmission. Therefore, they should be treated. The treatment of VL after drug interruption depends on the duration of the interruption period as summarized below:

- If the duration of treatment interruption is less than 5 days, continue the same treatment until the full course without compensating the missed treatment days.
- If the duration of treatment interruption is between 5–10 days, continue the same treatment until the full course but with compensation.
- If the duration of treatment interruption is more than 10 days, restart treatment as day one.

These group of patients require test of cure (ToC) at the end of the treatment

5.6 Treatment of PKDL (Pre-, Para-, Post-*Kala Azar* Dermal Leishmaniasis)

Not all PKDL patients need treatment; decision can be made depending on severity of the lesion (PKDL Grade). As PKDL patients harbor the parasite, they can be a potential sources of infection and disease transmission. Patients should be advised to seek medical attention and use impregnated bed nets (ITNs) if they develop skin rash following VL treatment (see grading of PKDL in section 3.3).

Most PKDL cases are Grade 1. The majority of the lesions heal spontaneously within 12 months but need close follow-up. However, PKDL patients with a **severe** form of **Grade 2** and **Grade 3** lesions and/or disfiguring disease, those with lesions that have existed for more than 6 months, those with concomitant anterior uveitis/conjunctivitis and young children with oral lesions that interfere with feeding need treatment.

Antimonials:

SSG 20mg/kg/day for 30 days. Treat until lesions are flattened or are no longer palpable but discoloration can still be visible. Do not wait until lesions disappear, sometimes longer treatment courses are needed. Follow-up is passive as there are no parasite criteria for cure due to the difficulties of demonstrating the parasite in the lesions. SSG toxicity is rare in PKDL treatment.

Miltefosine:

Miltefosine has shown a beneficial effect for the management of PKDL in large series of Indian patients. It demonstrated a shorter duration of therapy and a better compliance by increasing the dose or duration of therapy. Instead of 100mg daily for 12 weeks, increasing the daily dose by using 50mg twice or trice per day could shorten the duration of therapy. In Ethiopia, two cases of PKDL have been successfully treated with Miltefosine 100mg daily for 28 days. Thus, Miltefosine may provide an opportunity for treating PKDL.

5.7 VL Treatment in Special Groups

1. Pregnancy:

Evidence for the safety of anti-Leishmanial drugs is scarce. No VL drug is proven to be safe in pregnancy. Pregnant women should, therefore, be treated with the safest available anti-Leishmanial drug as the disease is life threatening for the mother and may have consequences for the fetus. Liposomal Amphotericin B is relatively safe and effective, while conventional amphotericin B is nephrotoxic. Pregnant women are particularly vulnerable to this. Dosage and administration are similar to other group of patients. In situations where there is no option, risk-benefit has to be assessed in the use of SSG especially in the first trimester.

2. Malaria and VL:

Due to the overlap in epidemiologic occurrence of both diseases, it is not uncommon to find these fatal diseases occurring simultaneously. Malaria will be severe in patients with VL because VL patients are already weak, with an ineffective spleen function and are already anemic. Screening for malaria is recommended for all VL patients admitted from malaria endemic areas. Treatment of both diseases at a time needs attention due to the overlap of drug side effects. The following points should be taken into considerations during the management of malaria-VL co-infection:

1. SSG should not be used with quinine (due to the cardio-toxicity of both drugs).
2. Use ACT (Coartem) in uncomplicated falciparum malaria cases or Artemether (intramuscular) in severe malaria cases.
3. If treatment with quinine is inevitable, stop SSG while quinine is being given. After quinine treatment is finished, resume SSG to complete the recommended duration of treatment starting 24 hours after the last dose of quinine.
4. If quinine needs to be used, consider shortening quinine usage for 3-5 days and finish with Coartem full dose to help to resume SSG within a short period. Consider Artemether as a suitable alternative for quinine.

5.8 Management of Concomitant Infections in Visceral Leishmaniasis

Patients with visceral Leishmaniasis are generally immuno-compromized and can get infections with bacteria, parasites and mycobacteria.

Pneumonia is one of the commonest co-morbidities with VL leading to increased morbidity. VL patients with pneumonia require vigorous treatment with broad-spectrum antibiotics. Consider also tuberculosis if there is no response to an appropriate antibiotic treatment.

Diarrhea is the principal cause of death in VL patients. The diarrhea may be watery, mucoid or bloody with variable causative agents. It needs aggressive therapy with hydration and proper antibiotics. The choice of antibiotics is based on the result of stool examination. Other infections that can occur with visceral Leishmaniasis, like skin infections, cancrum oris, and otitis media, also require treatment.

5.9 Supportive Management

Nutrition:

Over one third of VL patients present with moderate to severe state of malnutrition which further predisposes them to infections and affects the prognosis of VL treatment.

VL patients require adequate nutrition, vitamin and micro-nutrient supplementation to speed up recovery, improve treatment response and decrease or avoid the risk of relapse. Measure nutritional status with BMI (body mass index) or weight for height and follow the national nutrition guideline (national protocol) for management.

Management of Anemia:

VL patients often present with moderate to severe degree of anemia due to bone marrow infiltration by the Leishmania parasite, hypersplenism, auto-immune reactions or bleeding. Epistaxis could occur due to thrombocytopenia and mucosal infection. If it is severe nasal packing and posterior epistaxis balloon might be required. The anemia may require transfusion if severe.

5.10 Prognosis of VL Treatment

If untreated, VL is invariably fatal. In a well-established treatment center, the mortality of patients with VL treatment is below 10%. Predictors of bad prognosis are AIDS patients with a low CD4 count, very young or very old patients, patients with suffer from the disease for more than 5 months prior to their presentation, severe anemia, severe malnutrition, severe opportunistic infections, thrombocytopenia, relapsing course of the disease, patients with vomiting, patients in a state of collapse, and patients without secondary prophylaxis.

5.11 Challenges in VL Treatment

There are lots of challenges associated with VL treatment. Challenges may be related to the preparation of the available drugs, treatment choice and availability of the drugs and also the evaluation of cure. The drugs widely used are injectables (painful and not user friendly), very expensive, with high toxicity and inferior efficacy for HIV co-infected VL patients. Hence, it is crucial to protect the available drugs with rational use and proper surveillance. Effort has to be made to find new effective treatment options (including combination therapy) and effective tools for TOC.

6 VL/HIV Co-Infection

The HIV/AIDS pandemic has modified the natural history of the Leishmaniasis disease. HIV infection increases the risk of developing VL by a factor between 100 and 1000 in endemic areas. It reduces the likelihood of therapeutic response, and greatly increases the probability of relapse. At the same time, VL promotes the clinical progression of the HIV disease and the development of AIDS-defining conditions. As the two diseases target similar immune cells, together they exert a synergistic damaging effect on the cellular immune response with a significant reduction of the TLC and CD4 cells. Atypical presentations of Leishmaniasis are reported in HIV patients, especially in those with a CD4 count less than 200-cells/µL. Relapses are more common in this group of patients.

VL is an AIDS-defining condition and co-infected patients present the WHO stage 4 (CDC stage C) of the disease. They have very low CD4+ cell counts and high viral loads (1000-1 million copies). In some parts of Ethiopia (e.g. Humera), about 30% of all VL patients are co-infected with HIV.

6.1 Pathogenesis in VL/HIV Co-Infected Patients

HIV can enhance Leishmania growth and inhibit macrophage-killing capacity. This increases the risk of active VL and explains the huge parasite burden in HIV-infected patients. The HIV infection progressively weakens the Th1 immune response and switches the immune response Th1 to Th2. HIV can diminish the effective cellular immune responses during natural infections; it may be associated with reactivation of VL in previously asymptomatic persons through reduced anti-Leishmanial activity of macrophages (as evidenced by enhanced intracellular growth of *L. donovani* in monocyte-derived macrophages). Co-infection with HIV reduces in-vitro cellular and Th1 cytokine responses to Leishmania and increases Th2 cytokine responses.

6.2 Diagnosis

Disease manifestations in HIV-infected individuals without severe immuno-suppression are similar to those in immuno-competent persons. Among those with advanced immuno-suppression and low CD4+ T-lymphocyte counts, manifestations of Leishmaniasis may be more severe, atypical and with a chronic and relapsing course after treatment.

Although a deficit in host humoral and cellular response induced by both, HIV and Leishmania infections, results in a somewhat reduced sensitivity of serological and delayed-type IV hypersensitivity-based tests, studies in Ethiopian co-infected patients have demonstrated acceptable sensitivity.

The gold standard for the diagnosis of Leishmaniasis in HIV-infected patients remains the isolation or identification of the parasite in spleen, lymph node or bone marrow aspiration. Amastigotes can also be found in the peripheral blood of a proportion of HIV-infected individuals.

Where HIV counseling and access to antiretroviral treatment are available, all VL patients should be screened for HIV (WHO, TRS_949, 2010). Vice versa, in endemic foci for VL, the HIV services should suspect VL in patients with clinical symptoms for VL or unexplained deterioration of an HIV patient after other opportunistic infections are ruled out.

6.3 Treatment

(Refer to the Treatment Algorithm in Annex 10!)

If VL is present in an HIV-positive patient, ART should be started after VL treatment, regardless of the CD4+ cell count. When there is a history of VL, but the patient is currently asymptomatic, a CD4+ count should be done and the national HIV treatment guidelines should be followed.

It is crucial to try to restore cell-mediated immunity with ART as soon as possible to prevent further opportunistic infections and VL relapses, although ART alone will not prevent VL relapses. All anti-Leishmanial drugs are less effective in HIV-positive patients and the risk of relapse is high with them. Most relapses occur within 3-9 months, successive relapses become less typical and less acute, but more frequent. Moreover, with each relapse, patients become less responsive to treatment and eventually unresponsive to all drugs.

Due to the overlapping toxicity between drugs (SSG and Amphotericin B) and some of the ARVs, ART should be initiated after completing VL treatment, unless the risk of delaying ART outweighs the risk of potential drug toxicity. In patients already on ART, VL treatment with AmBisome is preferred and ART can be continued. SSG is more toxic in HIV patients, necessitating careful monitoring. It is advisable to provide bed nets to VL/HIV co-infected patients because it might help in reducing the risk of transmission.

Anti-leishmanial drug combination therapy may be the way to delay the emergence of resistance and increase antimicrobial activity. However, it is probable that even drug combinations will not prevent relapses altogether, leaving ART as the key in reducing and postponing relapses.

First-Line Drugs for First Episode (Primary) *Kala Azar* in VL/HIV Co-Infected Patients: AmBisome (LAmB)

Newer lipid formulations of Amphotericin B, such as liposomal amphotericin B (LAmB) and lipid complex amphotericin B (AmBLC) are of similar efficacy like Amphotericin B, but are much less toxic and much better tolerated. Hence, AmBisome with a total dose of 40mg/kg in divided dose can be used for the treatment of VL in HIV co-infected individuals.

If TOC is still positive but with a significant parasite load reduction, the treatment with AmBisome can be repeated until TOC becomes negative. If TOC positivity persists after repeating AmBisome , a compassionate treatment regimen should be used. However, if the parasite load does not decrease significantly after the initial AmBisome 40mg/kg therapy, SSG for 60 days and above should be considered as therapy bearing in mind that safety monitoring by qualified medical personnel is necessary. If even after the SSG therapy TOC is still positive, a compassionate regimen should be used (refer to Annex 10).

Second-Line Drug for Primary *Kala Azar* in VL/HIV Co-Infected Patients: Pentavalent antimonials

It is administered IV or IM at a dose of 20mg antimony/kg of body weight per day for 30 days. Adverse effects of the drug are more frequent in HIV infected patients.

Clinical trials on combination therapy for relapse cases of VL are currently undertaken in India. Combination treatments are cost effective and, given their expected impact on the emergence of drug resistance, a switch to combination therapy can be considered after the final results from the trials are available.

Risk factors for death that should be considered for all VL patients (with more emphasis for VL/HIV co-infected individuals) include malnutrition, concomitant opportunistic infection (TB), pneumonia, diarrhea, vomiting, anemia, bleeding and signs of toxicity during treatment (heart failure, arrhythmia, pancreatitis, jaundice, kidney failure, anemia, severe vomiting or diarrhea).

After treatment of the first episode, assess for fever, weight, general condition, spleen size, and hematologic values to see the clinical improvement and ideally demonstrate parasitological cure (TOC) to endure that discharge is appropriate. If the test is positive, continue treatment, or substitute to a second-line regimen and exclude other opportunistic infections.

6.4 Management of VL Relapses in VL/HIV Co-Infected Patients

Relapse of visceral Leishmaniasis is a common phenomenon in HIV co-infected cases. This poses challenges to the control of Leishmaniasis. The drug chosen to treat relapse should be different from that used to treat the first episode. However, in case of multiple relapses, the drug of choice should not be a drug that has shown to be ineffective during any of the previous treatment episodes. To avoid the development of drug resistance as a consequence of repeated relapses, and to increase treatment efficacy, combinations of drugs should ideally be used. However, there is still a lack of evidence for the best combination for co-infected patients. The physician's decision on combination treatment or compassionate treatment should, therefore, be made on an individual basis.

Due to lower efficacy of anti-Leishmanial drugs in HIV/VL extended treatment courses may be required to achieve cure. In order to distinguish between drug unresponsiveness and partial or slow drug responsiveness, it is recommended to do a TOC at the end of the standard treatment.

- If there has been no parasitological response to treatment (demonstrated by the same parasite grade found in the TOC as for diagnosis, or, alternatively,) drug unresponsiveness is demonstrated, and treatment should be continued with another anti-Leishmanial drug
- If standard treatment has resulted in a partial but significant parasitological response (demonstrated by parasite density in the TOC being at least two grades lower compared to the diagnostic assessment) treatment with the same drug may be continued until two subsequent negative TOCs have been obtained.

6.5 Secondary Prophylaxis – Maintenance Therapy

Experience with L. infantum/HIV co-infected patients in Mediterranean areas indicates that secondary prophylaxis should be started ideally after treatment of the first episode. There is an ongoing trial in north Ethiopia, in which a monthly injection of Pentamidine is being evaluated for secondary prophylaxis. Maintenance therapy may be stopped when the clinical status is stable with no deterioration and with a CD4+ count exceeding 200 cells/µl for more than 6 months. In anthroponotic transmission areas, treatment options are limited and account must be taken of the fact that frequent relapses significant increase the risk of resistance development. Drugs used to treat relapses should, therefore, be avoided for secondary prophylaxis. Relapsing patients should be retreated according to the first- and second-line recommendations described above as appropriate for individual patients.

7 Cutaneous Leishmaniasis in Ethiopia

L. aethiopica causes more than 99.9% of the CL cases in Ethiopia. *L. major* and *L. tropica* have been isolated from sandfly vectors and one case of CL due to *L. tropica* was reported.

7.1 Clinical Manifestations

a) Localized Cutaneous Leishmaniasis (LCL)

LCL starts as a small red papule that enlarges gradually up to 2 cm in diameter forming nodules or plaques. The surface forms curst in the center; the lesion can be single or multiple. The distribution of initial lesions corresponds to the sites of bite by the sandfly. Thus, they occur on exposed parts, such as the face (over 90%) and extremities. The clinical incubation period varies from a few weeks to several months.

LCL lesions vary in size, clinical appearance and time to spontaneous healing. The lesions are usually self-healing in about 3–6 months leaving a flat atrophic scar, but may last 1–5 years. Spontaneous healing usually results in lifelong immunity to re-infection with *L. aethiopica*. Depending on its size and location, the scar may cause disfigurement, psychological distress, stigma, and even disability if situated over a joint.

b) Diffuse Cutaneous Leishmaniasis (DCL)

Diffuse cutaneous Leishmaniasis is a rare presentation of CL due to *L. aethiopica* occurring in areas where LCL is common. It results from a defect in specific cell-mediated immunity.

DCL starts with few papular or nodular lesions followed by a gradual dissemination of the infection leading to multiple papular, nodular and plaque lesions involving larger areas of the skin. Local dissemination begins at once in 30% of cases. New metastatic lesions appear at distant sites on the skin, presumably resulting from a spread through the bloodstream. The face, ears, extensor surfaces of the limbs, buttocks penile and scrotal skin can be affected. DCL is chronic and progressive. It does not heal spontaneously and is difficult to cure requiring a long duration of treatment.

c) Mucocutaneous Leishmaniasis (MCL)

If the sandfly bite has been on the mucosal border of the nose or mouth, primary mucocutaneous Leishmaniasis may develop with the progression of the disease to the mucosa producing

swelling of lips or nose which persist for many years. It may also follow a contiguous spread from a near-by cutaneous lesion. In Ethiopia, MCL most frequently remains confined to the borders of the nose and the mouth. However, it can also spread deeply into the nose, the palate or the pharynx, where it may cause extensive and disfiguring destructive lesions. MCL does not heal spontaneously and, thus, requires treatment.

7.2 Diagnosis of Cutaneous Leishmaniasis

Clinical signs and symptoms are important in the diagnosis of cutaneous Leishmaniasis although confirmation is required through demonstration of the parasite in the lesions.

A. Clinical Diagnosis

Localized Cutaneous Leishmaniasis (LCL):

A clinically suspicious lesion is defined as a skin nodule or ulcer with a raised edge appearing on someone who lives in an area known to be endemic for cutaneous Leishmaniasis or visited such an area in the last two years. In rare cases, particularly with HIV co-infection, manifestations of cutaneous Leishmaniasis may be altered. Flat plaques or hyperkeratotic wart-like lesions, for example, may develop. There may also be involvement of draining lymphatic vessels or proximal lymph nodes.

Mucocutaneous Leishmaniasis (MCL):

MCL typically causes infiltrative inflammation of the mucocutaneous borders of the nose and/or lips, or swelling and expansion of the nose.

Diffuse Cutaneous Leishmaniasis (DCL):

The gross appearances of DCL may resemble lepromatous leprosy, but peripheral nerves, eyes, oro-nasal mucosa and internal organs are not involved. Very rarely, both diseases may be present in the same patient.

Table 2: Differential Diagnosis of Cutaneous Leishmaniasis

Cutaneous Leishmaniasis	***Mucosal Leishmaniasis***
Bacterial skin infections	Behçet's syndrome
Blastomycosis	Discoid lupus erythematosus
Cutaneous anthrax	Histoplasmosis
Eczema	Lethal midline granuloma
Fungal skin infections	Neoplasms
Leprosy	Paracoccidioidomycosis

Mycobacterium marinum
Myiasis
Sarcoidosis
Skin cancer
Sporotrichosis
Syphilis
Tuberculosis
Yaws
Verruco
Rhinoscleroma
Sarcoidosis
Syphilis
Tuberculosis
Wegener's granulomatosis
Yaws

B. Parasitological Diagnosis

Clinical suspicion of CL should, if possible, be confirmed parasitologically. Samples from suspect lesions may be collected through aspiration, scraping or tissue biopsy. Samples should be taken from active lesions but not from re-epithelialised lesions or scars. Slit skin smear examinations with Giemsa's stain is the simplest test that could be performed at the health center level.

1. Aspiration:

Aspirations are performed from the raised edge or nodular part of a lesion. A 0.5 mm diameter needle is tightly connected to an empty syringe. The needle is slowly advanced, with suction applied, in a straight line along the edge of an ulcer or into the center of a solid lesion. It is withdrawn in a similar manner. The aspirate should be gently spread on the slide using the tip of the needle. The slide should be labeled to identify the patient and the sampling technique used before transported to the laboratory.

2. Scraping:

Scrapings are performed either from the surface of an ulcer or from skin slits. Ulcer scrapings should be taken from beneath the edge and from the center of the lesion by using a small scalpel blade. To make a slit skin smear, squeeze the nodule or nodular edge firmly between finger and thumb to prevent bleeding and make small incision into the dermis with a point of a scalpel blade. Then turn the blade through 90o and scrape the cut edges to obtain tissue juice. A bloody scrape is useless. Either type of scraping should be smeared onto a clean, polished and alcohol-free microscope slide. The slide should be labeled to identify the patient and the sampling technique used before transported to the laboratory.

3. Biopsy:

Ideally, two biopsy samples are obtained – one for culture and another for histopathology. Standard 4 mm punch biopsy is performed from a raised edge or nodular part of a lesion.

C. Leishmanian Skin Test (LST) and Serological Tests:

These tests are not useful in the diagnosis of cutaneous Leishmaniasis.

7.3 Treatment of CL

The **goals of therapy in cutaneous Leishmaniasis** are to kill the parasite, to control its spread, especially in the mucosal disease, to accelerate healing and to reduce scarring, especially in cosmetic sites. Adequate treatment should cure the patient and prevent a relapse.

Treatment Options

There are 3 options for treatment of CL, which should be discussed with the patient:

1. To withhold treatment,
2. Topical or local treatment, and
3. Treatment with systemic drugs.

The decision which option to offer depends on the nature of the lesions and the ability of the patient to attend for treatment and to return for follow-up.

a) Withhold Treatment

As the majority of cutaneous Leishmaniasis lesions heal spontaneously within a year, the best treatment for patients who fulfill the following criteria are to withhold treatment or to accept local treatment:

- Fewer than 2 lesions requiring immediate treatment;
- Lesions are smaller than 5 cm in diameter;
- Absence of indurations or firmness of surrounding skin, on palpation no mucosal involvement, or lesions are not close to the nose or the lips;
- Lesions are not on the border of the lip, the nostrils or the eyes;
- No potentially disfiguring or disabling lesion (on the nose, the joints, the toes, or the fingers);
- No immuno-suppression

Patients who fulfill the above criteria need follow-ups to evaluate the progress of the lesion(s) and to decide on their subsequent treatment.

The relative benefits and adverse effects of the two options should be discussed with the patient. If the decision is to withhold treatment, the patient should agree to return for further evaluation after 3 months when the decision for treatment can be reviewed.

b) Local Treatments – Suitable for Health Centers and Referral Centers

When withholding treatment is not considered appropriate or is unacceptable to the patient, local treatment may be used for the category of patients listed under A) above. A bacterial superinfection is uncommon, but when suspected, it should be managed with appropriate antibiotics and wound care before starting the CL treatment.

There are no published reports on the results of any method of local treatment for CL in Ethiopia, nor of studies comparing the outcomes of local treatment with withholding treatment. Nor is it clear whether treatment leads to less scarring than withholding treatment. In the absence of such studies, firm recommendations cannot be made. Two methods are commonly used in Ethiopia, intra-lesional administration of Sodium Stibogluconate (SSG) and cryotherapy.

1) Intra-Lesion Administration of SSG:

For a few large lesions (< 3), weekly intra-lesional SSG for four to six weeks can be used but training and meticulous technique are essential for this method, in which *the injection must be administered intralesionally* and *NOT beneath or around the lesion*.

Using a 1ml syringe with a fine intradermal needle, undiluted SSG is injected into the lesion until it blanches. About 0.2-0.5ml may be adequate for the first injection of a small nodule. Weekly reassessment is recommended with SSG re-injections if clinically indicated. Once the lesion has been disrupted, up to 2ml SSG may be needed to infiltrate residual thickened areas. Injections may be painful, especially for children.

2) Cryotherapy:

Specialized equipment, liquid nitrogen, training and meticulous attention to detail are necessary for the successful application of this method. The method may be applied by suitably trained nurses. It is labor intensive and not appropriate for multiple lesions. Liquid nitrogen is gently applied to the lesion with cotton swab but evidence on the duration and frequency of the application is lacking.

3) Curettage Under Local Anesthetic:

This method (not yet used in Ethiopia) has been trialed successfully in Pakistan. It is simple and inexpensive, and appropriate for single and small lesions.

4) Thermotherapy under Local Anesthetic:

This method has not yet been used in Ethiopia. Radio waves delivered by the Thermomed device (name of manufacturer) produce an intra-lesional temperature of 50ºC that is maintained for 30 seconds. It is as effective as intra-lesional SSG with a 70% cure rate in Afghanistan (*L. tropica*) and, thus, more effective than systemic SSG in *L. major* CL. The Thermomed device is expensive but can be run off a rechargeable battery; it may be suitable for field deployment.

5) Topical Ointments:

This method has not yet been used in Ethiopia. Two formulations of paromomycin ointment have shown the same efficacy as that of intra-lesional SSG with a 70% cure rate in Iran, Israel and Sudan. A formulation of gentamicin/paromomycin was more efficient than a placebo for *L. major* CL treatment in Tunisia.

There is an urgent need for trials comparing the different methods of local and topical treatment in Ethiopia.

c) Systemic Treatment – Suitable for Referral Centers

It is indicated for:

- MCL or DCL
- Patients at risk of mucosal Leishmaniasis (lesions on the margin of the nostril or lip)
- Lesions which are bothersome to the patient but unsuitable for local treatment
- More than 4 lesions
- Large lesions unsuitable for local treatment
- Lesions located over joints
- Lesions accompanied by nodular lymphangitis
- Immuno-compromised patients (HIV/AIDS)
- Patients not responding to topical treatment

There are no published reports on the results of trials of systemic treatment for CL in Ethiopia, nor any critical appraisal of the results of any method of treatment. Published accounts of the treatment of patients with DCL in Ethiopia, and clinical experience with treatment of patients with DCL and MCL, have shown SSG to be ineffective. In one study, paromomycin has been shown to be effective in Ethiopian DCL and MCL patients, and isolates of *L. aethiopica* have proved to be sensitive in vitro. Paromomycin can be a treatment of choice for systemic treatment of CL due to *L. aethiopica.*

1) Paromomycin

Paromomycin has been used extensively in the treatment of VL and in a single small-scale study of DCL in Ethiopia. Its action is synergistic with SSG against isolates of *L. aethiopica.* Prolonged treatment with paromomycin may result in loss of sensitivity or even resistance.

Paromomycin is administered by intramuscular injection in a single daily dose of 14–15mg (sulphate)/ kg for 20–30 days. Mild pain at the injection site is the commonest adverse event (55%). Reversible oto-toxicity occurs in 2% of the patients. Renal toxicity is rare. Some patients may develop hepatotoxicity indicated by raised hepatic enzyme concentrations.

Treatment of DCL

Patients with DCL have a defect in specific cell-mediated immunity and relapse after conventional treatment. They should be treated with paromomycin plus SSG in a synergistic dose of 10 mg/kg/day, until amastigotes are no longer found in slit skin smears and then for several more weeks or months.

2) Pentavalent Antimony Compounds (SSG) or Meglumine Antimoniate (MA)

Dosage: 20mg Sb/kg/day IM or IV for 4–8 weeks.

3) Miltefosine and Liposomal Amphotericin B

Miltefosine and Liposomal Amphotericin B are effective in the treatment of CL in several countries, but have not yet been used for *L. aethiopica* infections.

There is an urgent need for trials to evaluate the relative merits of paromomycin, miltefosine and liposomal amphotericin B in the treatment of CL in Ethiopia and to compare them against SSG.

Response to Treatment

The signs of therapeutic response or natural healing are flattening followed by re-epithelization of the lesion. Clinical reactivation usually begins at the margins of the old lesions. The response is generally poor in HIV co-infections resulting in high rates of recurrence, treatment failure and relapse.

Follow-Up

It is recommended that patients are routinely followed up 6 weeks and 6 months after the completion of treatment. Patients should be advised that recurrence is possible and that they should return if that happens. Most relapses occur within 6 months after treatment.

8 Prevention and Control

8.1 Vector Control

A. Control against Immature Stages

Control measures against the immature stages of sandflies are difficult because their breeding sites are not fully understood, highly dispersed and inaccessible and – unlike mosquito larvae in their microhabitats.

B. Control Against Adult Sandflies

Environmental Management

Environmental management includes physical modification of the sandfly habitat, relocation of human settlements away from known vector habitats, and the implementation of environmental sanitation programs to eliminate actual or potential breeding sites. Successful control has been achieved in some countries by complete destruction of the breeding/resting habitat of the sandfly followed by exploitation of the land to ensure that conditions are unsuitable for the sandflies to return. This method was used in Russia and other Asian countries to control *Phlebotomus papatasi* (vector of *L. major)* and the reservoir host (*Rhombomys spp.*) which live in the same rodent burrow. Similarly, in termite hill *kala-azar* areas in southern Ethiopia, manual or mechanized destruction (e.g. bulldozing) of the termite hills could be an option. However, this would be a tremendous task which needs systematic evaluations.

Insecticide Application

Insecticide application is only effective in indoor or peridomestic transmission (i.e. when vectors are endophilic/endophagic) and not in sylvatic or outdoor situations. Most sandfly vectors are susceptible to insecticides. The susceptibilities of the sandfly vectors in Ethiopia need to be established in order to use the appropriate insecticide. Residual spraying in houses and animal shelters with DDT has been successful to control *P. argentipes* in India (vector of VL) for about 8–9 months followed by a dramatic decrease in the number of *kala-azar* cases. DDT is no longer used for IRS for malaria control in Ethiopia. Therefore, it cannot be used against *Leishmania* vectors in Ethiopia. Indoor residual spraying in Ethiopia should be coordinated with the malaria control program according to the principle of Integrated Vector Control Strategy. DDT or the alternative insecticide (possibly deltamethrin) could be less effective due to the predominant outdoor biting behavior of the vectors in Ethiopia and East Africa in general. However, systematic evaluation of the sandfly biting behavior is lacking and also needs further clarifications.

Use of Long-Lasting Insecticide Treated Nets (LLITN)

LLITNs are useful in malaria control and are probably also useful for sandfly control in indoor transmission. An advantage of LLITNs is that they can be integrated with malaria control as both diseases (VL and malaria) are found in the same general area in Ethiopia. However, like IRS, the usefulness of LLITNs very much depends on the biting behavior of the vectors (indoor vs. outdoor). Another issue regarding the use of nets against sandflies is that much sandfly biting activity occurs during early evening between 19-21 o'clock before most people go to sleep so that exposure to sandfly bites is only reduced but not eliminated. Therefore, the use of impregnated curtains on doors and windows might also be useful. A few risk factors assessment studies conducted in endemic areas of Uganda, Ethiopia and Sudan have shown protective effects of the use of insecticide treated nets.

Repellents (Chemical or Natural)

In areas where Leishmania transmission occurs outdoor and where Leishmaniasis is an occupational hazard, the use of repellents (synthetic or botanical) may be useful to prevent biting sandflies. Repellents are also useful even in indoor or peri-domestic transmission situations as most sandflies have an early peak in their biting activity in the evening. Commercially available synthetic repellents, such as DEET (N, N-diethyl-3-toluamide), DEPA (N,N-diethylphenylacetamide), or natural (plant-based) repellents (PDM (p-methane-3,8-diol), citronella oil, neem oil, etc.) are effective broad-spectrum repellents for about 6–8 hours. Their cost may preclude the use of synthetic repellents but natural repellents (e.g. neem oil) are relatively cheap and accessible for possible use in endemic areas of Ethiopia.

Integrated Control

As in all other vector control systems, the most successful methods of sandfly vector control is the use of a combination of several control methods in accordance with the local conditions as well as with the behavior and ecology of the vector. No single method is effective in controlling Leishmaniasis. Vector control must also be combined with control of the reservoir hosts (if any) and treatment of patients.

8.2 Health Education and Social Mobilization through health development army

Health education or promotion is a core element of any disease prevention and control program. The target group for health education might include public health managers, health staff, community health workers and leaders, people living in endemic areas and patients. In Ethiopia, the introduction of the health extension program is quite helpful for disease control programs with strong activities on the community level.

The main objectives of health education promotion in Leishmaniasis control include to:

- Attain strong commitment at all levels for active participation in prevention and control activities;
- Enable the community to obtain clear and correct information (preferably in their own languages) and to develop their capacity to make the right decisions;
- Educate local communities, especially women, and school children about control measures;
- Enable the community to understand problems related to Leishmaniasis and analyze them;
- Positively change behavior and practice through motivating and leading the community in the right direction to effect the desired change; and
- Establish links and collaboration with people concerned with health promotion and disease control.

It is essential to sensitize decision-makers at each level about the importance of Leishmaniasis as a public health problem so that they will support the control program in their capacity. In endemic Woredas, training courses for health authorities and health personnel should be organized before the full implementation of the control program commences. Primary health education programs should be organized for community health workers and leaders, school teachers and communities in endemic areas so that they are able to recognize the disease and guide patients on what to do, to be proactive and to participate in prevention and control campaigns. Health education applies equally to personnel in non-endemic areas where infections occurred in people who have travelled to endemic areas. The Leishmaniasis health education approach should follow the national overall health education and behavioral change communication strategies and plans on the basis of the local context

Communication is a key component of community empowerment and mobilization. The Health Extension Programme educates, mobilizes and involves the community in all aspects and stages of leishmanisis control and leads to increased ownership of the programme. It is recognized that it is only at community level that the control will be achieved. This will be effected by the health extension program through the health development army; a new initiative which creates a network to expand the best practices in health at scale. With the help of Health Extension Workers, the health development army members will conduct regular meetings; identify bottlenecks in the prevention and control activities and fill gaps as per the discussion. The health development army under their network and development teams mobilize communities on the uptake and utilization of

NTDs control services; environmental management, utilization of LLINs, early treatment seeking. By doing these activities in a sustainable manner it is possible to create a resilient and empowered community. This will be complemented with integrated social behaviour change communication (SBCC). SBCC materials will be customized and prepared based on previously identified gaps at the community level. IEC/BCC provides information and skills to populations at risk of the diseases so that they can make informed decisions and participate in prevention, treatment, and control.

8.3 Information System

Information regarding Leishmaniasis is inadequate in developing countries and surveillance systems for Leishmaniasis are poorly established. Therefore, data collection and their analysis for monitoring and evaluation of the program is a crucial aspect.

Integrated Disease Surveillance (IDS) is the major strategy and mainstay of the Disease Surveillance in Ethiopia. Leishmaniasis is a disease with common outbreaks and should be included in the IDS system of the endemic regions.

This guideline includes reporting formats standardized for the endemic regions of the country in order to strengthen the surveillance system of Leishmaniasis. In addition to the register books and patient's forms, data should be summarized in the monthly report forms and then submitted to the MOH (see Annex 11).

9- Annexes

Annex 1: rK39 Rapid Diagnostic Test Procedure

The utility of a rapid diagnostic test for visceral Leishmaniasis lies in its simplicity. Several brands of tests with rK39 antigen are available. Operators should always read the package inserted carefully and follow the manufacturer's instructions. This is especially important with regard to the type of specimen used. Some brands can be used only with serum, while others can be used with whole blood collected by finger prick.

Test procedure

In general, the test procedure is as follows:

1. Remove the test strip from the pouch and place it on a flat surface.
2. Place a specified amount of patient specimen (serum or finger-prick blood) on the absorbent pad on the bottom of the strip.
3. Add the specified amount of buffer provided.
4. Read the result after 10–20 minutes according to the manufacturer's instructions.

Some brands require a slightly different procedure, for example:

1. Take a test tube or a U-bottom microtitre plate.
2. Add a specified amount of buffer to the tube or well.
3. Add a specified amount of specimen (blood or serum) to the tube or well and mix.
4. Immerse the test strip into the buffer-specimen mixture.
5. Read the result after 10–20 minutes according to the manufacturer's instructions.

Points to consider for optimizing use of rapid diagnostic tests:

- Have a clear management plan to deal with positive and negative results.
- Follow biosafety standards and precautions for handling blood and other body fluids.
- Ensure proper storage conditions.
- Do not use damaged or expired tests.
- Adhere strictly to the manufacturer's instructions.
- Use test kits within 1 hour of removal from the pouch.
- Read the results within the time specified by the manufacturer.
- Do not reuse a test.

Interpretation of the test

Positive result: When both, control and test lines, appear, the sample tested has antibodies against recombinant K39 antigen of Leishmania. Even a faint line should be considered positive.

Negative result: When only the control line appears, there are no antibodies against recombinant K39 antigen of Leishmania present in the patient's sample.

Invalid result: When no control line appears, a fresh patient sample should be tested with a new strip.

Advantages and disadvantages of the rK39 test
Advantages

- Simple to perform with minimal training.
- Does not require a laboratory.
- Can be performed with finger-prick whole blood, serum or plasma.
- Kits can be transported and stored at ambient temperature (up to 30 °C).
- Results are available within 10–20 minutes

Disadvantages

- Cannot distinguish between active cases and relapse in previously treated cases. Therefore, interpretation must always be accompanied by clinical case definition.
- In patients with advanced HIV infection, a negative result does not rule out VL diagnosis.

Annex 2: Direct Agglutination Test Procedure

Principle of DAT

Infections with *L. donovani* yield the production of antibodies against the parasite. These antibodies can be demonstrated in blood or serum by the agglutination test. The DAT antigen is a whole, killed, promastigotes from cultures of *L. donovani* which have been stained blue for visibility. These are suspended in solution. When left in a V-shaped well, they will slowly fall to the tip of the V giving a dense blue dot. If anti-Leishmania antibodies are present in the blood or serum, the blue-stained parasites (DAT antigen) will become cross-linked by the antibodies (directly agglutinated) and settle to the bottom of the well as a hazy blue mat or a cloud, but **NOT** as a dot.

By diluting the serum 2-fold in each well starting at 100 x dilutions, the titer (quantity of antibody) can be measured.

These procedures are for freeze-dried presentation of DAT.

Collection of blood specimen for DAT

Collect capillary blood from the finger or the toe or heel in infants.

Method:

Requirements:

- DAT request form
- DAT registration book
- Whatman **No. 3** filter paper
- Sterile lancet
- Disinfectant, e.g. iodine, alcohol, etc.
- Cotton wool
- Scissors
- Plastic bag
- Pen (ball point)
- Paper clips

Procedure:

- Cut the circular filter paper into 8–16 segments depending on the size. Each segment can be used for one patient's test only.
- Draw a circle of approximately 1cm in radius on the segment of the filter paper.
- Record the patient's details in the laboratory registration book (see Annex 7).
- Write the DAT number on the patient's request form and also on the segment.
- Soak a piece of cotton wool in iodine or alcohol and disinfect finger, toe or heel thoroughly. Allow the skin to dry.
- Using the sterile lancet, prick the finger firmly so that blood flows freely without excessive squeezing.
- Wipe the first drop of blood with a plug of dry cotton wool. The first drop of blood is normally contaminated with dirt and tissue fluid.
- Squeeze the finger gently and collect the next drop of blood into the circle on the segment of the filter paper. Check the DAT number on the filter paper before collecting the blood.
- Make sure the blood **soaks through** both sides of the filter paper and fills the circle
- Allow the filter paper to dry.
- Apply pressure to the finger prick with a dry cotton wool.
- Discard the used cotton wool into the waste bucket and the lancet into the sharps container.

- When dry, **very dry**, clip the filter paper with the request form and put into a plastic bag.
- Store in a refridgerator or cool box until ready to send for testing or until ready for performing the test.

Elution of samples – Day 1

Requirements

- Laboratory register, Microtitre plates (V-shaped), Sample (dry in filter paper), Micropipette to measure 125 µl, Normal saline, Paper punch (5mm), Scissors, Marker (permanent), Forceps and Refrigerator

Procedure

- Write the number of the samples in order in the laboratory register. The list should be labeled to show which row of which microtitre plate corresponds to which sample, e.g. the list can be labeled A, B, C, D, E, F, G, and H in order.
- The samples need be grouped by 8 samples in each group or less. Group one is called Plate I, group two is called Plate II, and so on.
- Label the microtitre plates to correspond with the laboratory register, i.e., get out the micro-titer plates and write on the plate itself the plate number. The first one is plate I.
- Using the paper punch, punch out a sample of filter-paper blood.
- Using the forceps, put the punched sample of filter-paper blood into the well of the first column of the microtitre plates corresponding to the position recorded on the patient laboratory register. Ensure that the punched filter-paper blood is properly inserted in the well, e.g. the sample listed as A should go in the first row labeled A. The sample listed as B should go in the second row labeled B, and so on.
- Take the micropipette and adjust it to measure 125µl, fix the pipette tip firmly and pipette 125µl of normal saline. Add the 125µl of normal saline to each well with a sample paper. Make sure that the punched filter paper blood is completely immersed in the saline.
- Cover the plates with another microtitre plate; incubate in the refridgerator at 40C overnight for at least 8 hours.

Dilution and titration – Day 2

Requirements

- Measuring cylinder
- 50ml container (plastic or glass bottle or conical tubes)

- Research (repetitive) pipette 100–1000µl, adjustable; brand Handy-Step or Eppendorf with volume display, Combitip ejection.
- Multipipette 100–1000µl Eppendorf or Handy-Step adjustable with volume display and tip ejection.
- Multichannel pipette, 8 channels, 5–50µl Eppendorf, adjustable volume and tip ejection.
- Micropipette tips (yellow tips for 100µl Multipette and the Multichannel pipette, blue tips for up to 1000µl research pipette, combitips standard 1.25ml and combitips plus 2.5 ml)
- 5–10ml syringe
- 2-mercaptoethanol (2-ME)
- Normal saline
- DAT antigen (freeze dried antigen OR liquid antigen)
- Freeze dried control sera

Preparation of diluents

1. To be used with freeze-dried antigen (FDA)

- Measure 50ml of normal saline and put it into the container
- Adjust the multipipette to measure 390µl, pipette 390µl of 2-ME and add to the normal saline then mix

Reconstitution of the freeze-dried antigen (FDA)

- Add 5ml of fresh normal saline to the vial of the antigen
- Mix gently by rotating and tilting the vial. Do not shake
- Let it stand for about 10 minutes before use

Freeze-dried antigen is kept at room temperature!

Reconstitution of the freeze-dried control

Use a new set of control sera with every new batch of DAT antigen. Make sure that all the freeze-dried powder is on the bottom of the vial. (The control kits made in Amsterdam contain 2µl of serum each)

Strictly follow the manufacturer's instructions on the procedure for reconstitution especially the amount of normal saline or diluents to be added to the vial.

- Either add 100µl or 200µl of normal saline or diluent to the vial of the control sera depending on the manufacturer's instructions.
- Mix gently by rotating.
- Let it stand for at least 10 minutes before use.

Dilution of samples

- *Filter paper blood*

  Take the microtitre plates with the eluted blood out of the refridgerator and allow it to come to room temperature (Serum dilution in column 1 is 1:50).

- *Reconstituted control sera*

Fill the control well(s) in column 1 with 100µl of reconstituted control serum.
This is a 1:50 dilution.

- Adjust the pipette to measure 50µl.
- Fill the wells from columns 2 to 12 with 50µl diluent.
- Adjust the multichannel pipette to measure 50µl.
- Place 8 standard tips (yellow tips) on the multichannel pipette and make sure they are firmly fixed to avoid pipetting errors. If the multichannel pipette is not available, you may use a single channel pipette. Pipette each row separately.
- Mix the contents in column 1 by pipetting in and out at least 5 times. Avoid forming bubbles by expelling air prior to inserting the pipette tips into the wells and using a slow action.
- Pipette 50µl from column 1 and transfer to column 2. Continue this mixing and transferring until column 11. Discard the last 50µl from column 11. Do not add to column 12. (Serial dilution). Column 12 is the negative control.

Adding antigen

- Gently rotate the antigen bottle to mix it. Do not shake the bottle as this may destroy the antigen.
- Adjust the pipette to measure 50µl and fit the pipette tips.
- Add 50µl antigen to every well except wells in column 1. It is advisable to start with wells in column 12 (negative control) to avoid contamination, and then to add row by row. You should also change the pipette tip every time antigen is taken out of the bottle.
- Rotate the plates gently clockwise and anticlockwise.
- Cover the plates with a spare microtitre plate, leave them on a level surface at room temperature for about 12 to 18 hours.

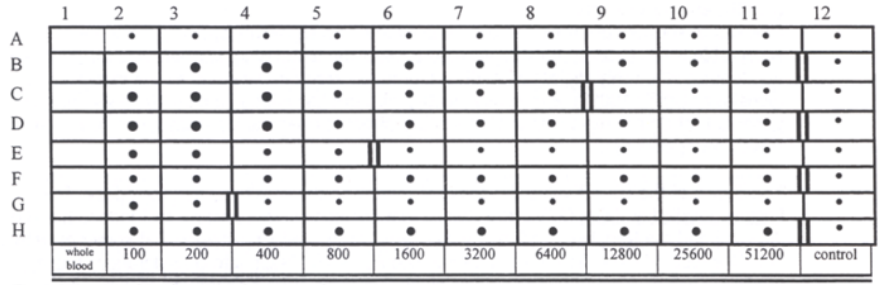
Well →	1	2	3	4	5	6	7	8	9	10	11	12
A	o	o	o	o	o	o	o	o	o	o	o	o
B	o	o	o	o	o	o	o	o	o	o	o	o
C	o	o	o	o	o	o	o	o	o	o	o	o
D	o	o	o	o	o	o	o	o	o	o	o	o
E	o	o	o	o	o	o	o	o	o	o	o	o
F	o	o	o	o	o	o	o	o	o	o	o	o
G	o	o	o	o	o	o	o	o	o	o	o	o
H	o	o	o	o	o	o	o	o	o	o	o	o
dilution →	blood	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600	1:51200	control

Reading the DAT results – Day 3

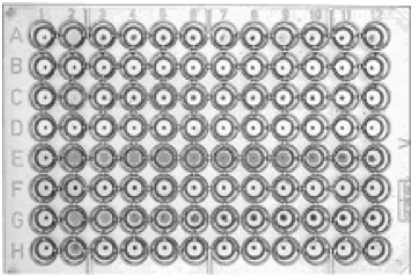
- Put the plates against a white background.
- Estimate the titre by comparing the dots in column 12 (negative control) to those of the samples in the other columns.
- The titre is expressed as the last dilution that shows a difference compared to the negative control. A dark blue dot indicates that the result is negative and no reaction took place whereas a hazy blue mat or cloudy appearance indicates that reaction took place. The highest titre will be last dilution that still shows a hazy blue mat or cloud.
- Record the result in the lab book by titre and meaning. For example, record "well 9, 12800 positive". If you are not sure of the meaning (positive, negative or borderline) simply record the titre. Currently, the positive titre is well 7 or 3200 with the FD antigen.
- Report the results in the patient's request form.

The titres are as follows:

Column:	1	2	3	4	5	6	7	8	9	10	11
Dilution:	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600	1:51200



See below an example of a positive result: sample E: well 11, titre 1:51200, positive



Annex 3: Lymph Node Aspirate Procedure

Collection of lymph node aspirate

The most common site for collection of the lymph node aspirate for diagnosis of *ka-la-azar* is the inguinal gland. Infected glands will be swollen.

Method:

Items required:

- Sterile needle (21G)
- Syringe (10ml)
- Clean glass slide
- Iodine (disinfectant) or sterile cotton swabs
- Cotton wool

Procedure

- Allow the patient to lie comfortably. Prepare the syringe by pulling the piston back as far as possible.
- Feel both sides of the inguinal area to locate a swollen gland.
- Disinfect the chosen site with swollen glands using a piece of cotton wool soaked in iodine or another suitable disinfectant.
- Take the gland between the thumb and index finger of the left hand. Hold it steady and make it at the same time standing out.
- Introduce the needle with a right angle into the center of the gland in two stages:
  a) First pierce the skin
  b) Second penetrate the gland
- With your left hand, gently knead the gland. With your right hand, revolve the needle in both directions. The glandular fluid will ooze into the needle.
- Withdraw the needle in one rapid movement while holding the thumb over the hub. Then apply a swab dipped in iodine to the point of entry.
- Attach the syringe (piston pulled back) to the needle. Place the needle on the slide. Push the piston gently down the barrel to discharge the glandular fluid contained in the needle onto the slide.
- Make a thin film using the fluid on the slide. The fluid can be discharged in more than one slide.

Annex 4: Bone Marrow Aspiration Procedures

Materials needed

Sterile BM needle, 10ml syringe, clean microscope slides, NNN or any other suitable culture medium, wooden applicator or tooth picks, spirit lamp with sufficient flame, drapes, sterile gloves, sterile cotton and gauze, plaster, labels, pen and pencil/marker.

Biopsy aspiration needles (recommended sizes):

Regular/Adults:	4-inch, 11-gauge
Adults:	4-inch, 8-gauge
Orthopedic:	6-inch, 10/11-gauge
Pediatric:	3½-inch, 13-gauge
Infant:	2-inch, 13-gauge

Pre-operative procedures

No specific procedures are needed.

Aspiration procedures

1. Place the patient in a right or left lateral decubitus position with the back comfortably flexed and the top knee drawn toward the chest.
2. Locate the posterior iliac spine and mark it with ink or thumb nail pressure.
3. Using sterile technique, prepare the skin with anti-septics and drape.
4. Using a sterile syringe, infiltrate the marked area with local anesthesia especially the periosteum.
5. Make a 3-mm skin incision with a scalpel blade over the marked area.
6. Hold the needle with the proximal end between the palm and the index finger against the shaft near the tip.
7. With the stylet locked in place, introduce the needle through the incision pointing toward the anterior superior iliac spine and bring it into contact with the posterior iliac spine.
8. Using gentle but firm pressure, advance the needle to bore through the iliac spine.
9. Rotate the needle in an alternating clock-wise and counter-clockwise motion. Entrance into the marrow cavity is generally detected by decreased resistance.
10. Remove the stylet, and check for marrow material. If not present, proceed to bore until marrow is found in the tips of the stylet.
11. With a syringe locked into the proximal portion, apply a negative pressure.
12. The material can be expelled onto clean slides and also inoculated into an appropriate culture medium (preferably NNN medium).

N.B. For cultures, insert needle into a tube containing a culture medium and push the plunger into the barrel to expel contents of the needle onto the side walls of the tube or directly into the liquid phase of the medium. You may repeat this once or twice until the LN material is visible in the tube. For safety purposes, inoculate 2 culture tubes.

For smears, expel any remaining material gently on clean glass slides holding the tip of the needle on the surface of the slide, and spread evenly into a smear by using a linear motion. More material can be obtained at the end of the plunger or the needle (or tip of syringe) after removing the plunger and the needle. Tooth picks or wooden applicators may be used for this purpose.

It is important that culture tubes are not over loaded by large amounts of inoculum, which is not uncommon with BM aspirates.

Slides can be stained with Leishman, Giemsa or Wright's stain (See Annex 4). NNN cultures should be incubated at 25 0C for up to 2 weeks.

Post-operative procedures: No specific procedures are needed

Annex 5: Procedures for Splenic (Spleen) Aspiration

Splenic aspiration should be performed only if the following conditions are met:

- **absence of clinical contraindication(s):**
  - signs of active bleeding (e.g. epistaxis, rectal bleeding, skin bruises)
  - jaundice (a potential marker of liver dysfunction)
  - pregnancy – spleen barely palpable
  - bad general condition (e.g. cardiovascular shock, altered consciousness)
- **absence of biological contraindication(s):**
  - severe anemia (hemoglobin count ≤ 5 g/l) – platelet count < 40,000/ml
  - difference in prothrombin time between patient and control > 5 s
- **rapid access to blood transfusion in case of bleeding**

The two important prerequisites for the safety of the procedure are **rapidity** so that the needle remains within the spleen for less than l second and **precision** so that the entry and exit axes of the aspirating needle are identical to avoid tearing the splenic capsule.

The procedure is as follows:

1. Clean three glass slides and label them. Have culture medium ready (if available) and labeled in the same way as the slides. Attach a 11/4-inch × 21-gauge (32 × 0.8-mm) needle to a 5ml syringe.

2. Inform the patient about the procedure. Check all clinical and biological contraindications again. Palpate the spleen and outline its margins on the patient's abdomen with a pen. For safety, the spleen should be palpable at least 3 cm below the costal margin on expiration. Use an alcohol swab to clean the skin at the site of aspiration and allow the skin to dry.

3. With the 21-gauge (0.8 mm) needle attached to the 5ml syringe, just penetrate the skin, midway between the edges of the spleen, 2–4cm below the costal margin. Aim the needle cranially at an angle of 45° to the abdominal wall. The actual aspiration is done as follows: pull the syringe plunger back to approximately the 1ml mark to apply suction and with a quick in-and-out movement push the needle into the spleen to the full needle depth and then withdraw it completely, maintain suction throughout.

4. For young, restless children, have two assistants hold the child (arms folded across the chest, with shirt raised to obstruct the line of vision, and pelvis held firmly). Carry out the aspiration as a single-stage procedure using the same landmarks, angles and suction as in step 3 – all in one quick motion. The insertion should be timed with the patient's breathing so that the diaphragm is not moving. This should be done during fixed expiration if the child is crying. Only a minute amount of splenic material is obtained for culture and smear.

5. If culture is available: *slowly pull* the plunger back to the 2–3ml mark and, by using sterile techniques, insert the needle into a tube containing culture medium and *briskly push* the plunger into the barrel to expel the contents of the needle onto the side walls of the tube. If necessary, repeat once or twice until splenic material is visible in the tube. Replace the cap on the tube and invert to wash splenic material on the side of the tube. Repeat the procedure for the second tube of culture medium. Sterile techniques are essential throughout.

6. Expel material gently onto glass slides, holding the needle tip on the surface of the slide. Immediately spread evenly with the needle, using a linear (not circular) motion. The smear should be slightly thinner than a thick blood film for malaria. Remove the needle and use the end of it to obtain additional material from the tip of the syringe and spread it on slides. Further material found on the end of the plunger may be dabbed directly onto a slide and spread. Allow the slides to dry.

7. Write the time of aspiration on the patient's chart with the instructions: "Record pulse and blood pressure every half hour for 4 hours, then every hour for 6 hours. Patient must remain in bed for 12 hours!" Ensure that the patient understands the instructions. Enter the procedure in the notes and sign.

8. Take the slides (and medium) to the laboratory for preparation and microscopic examination.

Annex 6: Preparation and Examination of Aspirates

Preparation of the aspirates

Prepare thin films of the splenic aspirate material or lymph gland fluid.
Spread the films on a clean glass slide immediately after collection before the material clots.
Slides are stained with Giemsa as for a thin malaria film and examined under oil immersion.

Items required:

- Methanol, Glass slide, Slide rack, Giemsa, Buffer solution pH 7.2 and Microscope

Thin films

- Collect a drop of the aspirate/fluid on one end of the slide, about 1–2cm from one end.
- Place the slide horizontally on a flat surface.
- Hold the sides of second slide (spreader) or coverslip on to the center of the specimen slide and move it backwards until it touches the drop of the fluid. Let the fluid spread along its base.
- At an angle between 30-450, move the spreader firmly and steadily across the specimen slide to make a film. A good film should have a conical 'tail' and should cover about two-third of the slide.
- Let the film dry.
- Fix the dried smear by dipping it in absolute methanol for a few seconds and allow to air-dry on a drying/draining rack.

Fixation:

- Place the slides horizontally on the slides rack and leave to air dry.
- Fix the slides by dipping them in 100% methanol for 1 minute. The methanol must be stored in a tightly closed bottle to prevent absorption of water.

Staining:

- Stain the slides with Giemsa stain 1:10 concentration; 1ml of stock Giemsa stain to 9ml buffer solution pH 7.2. The slides can either be stained in a staining trough or on a staining rack.

Staining in trough

- Place the slides in a staining trough.
- Pour the stain gently into the trough until the slides are totally covered. Avoid pouring the stain directly onto the film.
- Leave the slides to stain for 10–15 minutes.
- Pour clean water into the trough to float off the scum on the surface of the stain. The water should be poured into the end of the trough.
- Gently pour off the stain and rinse again in clean water. Then pour off the water.
- Remove the slides one by one and place them in vertical position on slides rack to dry.

Staining on a rack

- Use a test tube or a small container to hold the prepared stain.
- Gently pour the stain onto the slide or use a pipette to drop the stain on to the slide.
- Leave the slides to stain for 10–15 minutes.
- Gently flush the stain off the slide by adding drops of clean water. Never pour the stain off the slides, otherwise the surface scum will stick to the film and spoil if for microscopic examination.
- Place the slides in vertical position on a slides rack to dry.

Microscopic examination of stained aspirates

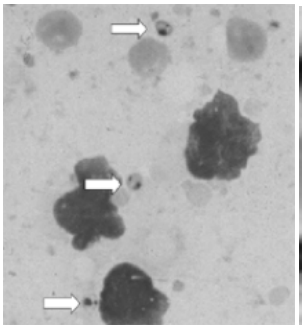
Method

- Place the microscope on a firm bench, free from vibration.
- Switch on the light source. If there is no inbuilt light source, adjust the flat side of the mirror to reflect the light up through the condenser.
- Adjust the eyepieces by sliding them horizontally until they fit both eyes comfortably and the two fields merge.
- Center the condenser if applicable.
- Prepare the slide for examination by putting a drop of oil immersion on the smear.
- Clean and dry underneath of the slide by wiping with cotton gauze/wool or tissue paper.
- Rotate the nosepiece until the low power objective is in position (x10). A slight resistance and a click are felt as the objective moves to the correct position.

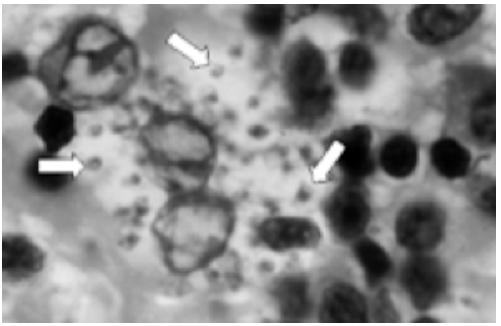
- Place the slide carefully on the stage. **Never place the slide on the stage when the x40 or x100 (oil immersion) objectives are in position as this may scratch the lenses.**
- Adjust the illumination.
- Focus the specimen by racking the stage up to the top and then while observing through the eyepiece, rack the stage down slowly using the coarse adjustment knob, until the image comes into view. Use the fine adjustment knob to focus the image sharply. Scan the film and select a part that is well stained, free of staining debris and well populated with white blood cells.
- Swing the required objective into position, i.e. oil immersion (x100) objective. Focus using the fine adjustment knob. Never use the coarse adjustment knob because the objectives are perfocal.
- Adjust the illumination by opening the iris diaphragm as required, for oil immersion objective open the iris diaphragm fully.
- Examine the specimen systematically, moving from field to field using the knobs that control the mechanical stage. For example, start at the selected site and move horizontally to the top right hand corner. Move the slide down by one field and then horizontally in the other direction to the end of the smear. Continue until the whole specimen has been examined.
- After examination, lower the stage or swing the lowest power objective into position before removing the slide from the stage. **Never remove the slide from the stage when x40 or x100 objectives are in position, as this may scratch the lenses.**
- Wipe the oil immersion objective using lens cleaning tissue.
- Switch off the microscope.

Identification of the Leishmania parasite

Leishmania amastigotes are oval or round and are usually seen in the cytoplasm of monocytes. Free parasites may be seen if the host cells are ruptured during preparation of the film.



Free amastigotes



Intra-cellular amastigotes

In stained preparations, Leishmania amastigotes contain two visible structures:

- Nucleus
- Kinetoplast

The nucleus and kinetoplast stain dark reddish. The cytoplasm stains pale pink. The nucleus of the host cell may be pushed to one side by the multiplying parasites.

Leishmania amastigotes are sometimes referred to as L.D. bodies.

If Leishmania amastigotes are seen, report the slide as:

Lymph node or splenic aspirate: Leishmania amastigotes seen and indicate the grade.

Examine at least 1000 fields before declaring a film to be negative.

Grading of slides:

Parasite grading has several uses. It increases the sensitivity of parasite detection, provides an objective measure of the speed of response to treatment, distinguishes quickly between slow responders and non-responders, and provides an indication of parasite load that is useful in research.

The average amastigote density is graded as follows:

6+: > 100 parasites per field (viewed with a 10× eyepiece and 100× oil-immersion lens)
5+: 10–100 parasites per field
4+: 1–10 parasites per field
3+: 1–10 parasites per 10 fields
2+: 1–10 parasites per 100 fields
1+: 1–10 parasites per 1000 fields
0: 0 parasite per 1000 fields

Annex 7: Dosage and Precautions for the Use of Sodium Stibogluconate (SSG)

Presentation:

Solution for injection vials 30ml.

Contains 33% (= 9.9g/30ml) SSG corresponding to 10% Sb^{5+} which is 100mg Sb^{5+}/1ml or 3000mg Sb5+/30ml.

Table of SSG volume per body weight (20mg Sb5+/kg/day)

Weight in kg	*SSG dose in ml*	*Weight in kg*	*SSG dose in ml*	*Weight in kg*	*SSG dose in ml*
1	2	26	5.2	51	10.2
2	2	27	5.4	52	10.4
3	2	28	5.6	53	10.6
4	2	29	5.8	54	10.8
5	2	30	6	55	11
6	2	31	6.2	56	11.2
7	2	32	6.4	57	11.4
8	2	33	6.6	58	11.6
9	2	34	6.8	59	11.8
10	2	35	7	60	12
11	2.2	36	7.2	61	12.2
12	2.4	37	7.4	62	12.4
13	2.6	38	7.6	63	12.6
14	2.8	39	7.8	64	12.8
15	3	40	8	65	13
16	3.2	41	8.2	66	13.2
17	3.4	42	8.4	67	13.4
18	3.6	43	8.6	68	13.6
19	3.8	44	8.8	69	13.8
20	4	45	9	70	14

21	4.2	46	9.2	71	14.2
22	4.4	47	9.4	72	14.4
23	4.6	48	9.6	73	14.6
24	4.8	49	9.8	74	14.8
25	5	50	10	75	15

No upper limit for SSG!! If the patient's weight is higher than 75, then calculate accordingly.

Contraindications

There are no absolute contraindications to its use. Under ideal circumstances patients with underlying renal, hepatic or cardiac disease should be well monitored. Elderly may have age-related decreased kidney function and may be at risk of increased toxicity.

Toxicity and side effects

Prevention of SSG toxicity:

The most important way to prevent SSG accumulation between doses is to ensure adequate hydration. SSG is cleared in the urine. Patients should repeatedly be told to drink enough fluids so that they pass urine at least 4 times a day. Babies should pass urine every hour or so while awake.

Minor side effects:

Symptoms: nausea, anorexia, arthralgias, myalgias, injection site pain, fatigue and abdominal pain.

Laboratory toxicity: elevated amylase (biochemical pancreatitis), elevated liver enzymes (biochemical hepatitis), leucopenia/anemia/thrombocytopenia. Occasionally, renal failure occurs.

Electrocardiograph changes (ST segment and T wave).

Nausea and anorexia are substantial problems when patients are already malnourished and dehydrated. The nausea and anorexia subside somewhat in the later weeks of treatment.

Serious toxicity:

Severe vomiting and abdominal pain (pancreatitis): Vomiting is relatively common and should be treated aggressively. Treat with anti-emetic medications and push sips of fluids. When anti-emetic treatment fails the SSG should be withheld for 2 to 5 days as needed.

If vomiting is associated with other risk factors, especially extremes of age, low Hb, severe malnutrition, withholding of SSG is even more imperative.

In hospitals with chemistry available, patients vomiting from known pancreatitis should get SSG withheld. Note that when the pancreatic enzymes return to normal and the patient is re-challenged with SSG, the amylase may remain normal.

Electrocardiograph abnormalities (QT prolongation), and sudden death (rare): Sudden death occurs rarely – possible explanations are cardiac arrhythmias or intra-cerebral bleeds. ECG changes are common. Sudden death is associated with high doses of SSG (over 30mg/kg/day). However, cardiotoxicity and sudden deaths are not seen in PKDL patients, so toxicity may be a combination of SSG and a weak individual.

Other points of interest:

- Blindness is NOT a toxicity of SSG – if a patient complains of loss of vision after treatment then it could be iritis (which can occur in isolation or with PKDL). The iritis requires further treatment with SSG. Apparently retinal haemorrhages occur with KA (not associated with SSG) as well.
- Injection abscesses from the IM route are uncommon – but when present they need aggressive treatment (antibiotics, drainage of pus).
- Neurological toxicity: not reported elsewhere as a toxic effect of SSG. Before or during treatment some patients have ataxia and severe tremors with or without headache. Neuropathy, psychosis and epilepsy are other occasional neurological features. It is unclear whether any of these are an effect of SSG in patients with KA (it never occurs in patients undergoing treatment for PKDL), if it is KA itself, or if it represents bleeding into areas of the brain. The ataxia, tremor and neuropathy may all remain for months after cure.

Annex 8: Dosage and Precautions for the Use of Paromomycin (Aminosidine)

Presentation: solution for injection vials of 500mg/ml, 2ml (1gr)

Table of paromomycin (PM) volume per body weight (15mg/kg/day)

Weight in kg	*PM dose in ml*	*Weight in kg*	*PM dose in ml*	*Weight in kg*	*PM dose in ml*
1	0.03	26	0.78	51	1.53
2	0.06	27	0.81	52	1.56
3	0.09	28	0.84	53	1.59
4	0.12	29	0.87	54	1.62
5	0.15	30	0.9	55	1.65
6	0.18	31	0.93	56	1.68
7	0.21	32	0.96	57	1.71
8	0.24	33	0.99	58	1.74
9	0.27	34	1.02	59	1.77
10	0.3	35	1.05	60	1.8
11	0.33	36	1.08	61	1.83
12	0.36	37	1.11	62	1.86
13	0.39	38	1.14	63	1.89
14	0.42	39	1.17	64	1.92
15	0.45	40	1.2	65	1.95
16	0.48	41	1.23	66	1.98
17	0.51	42	1.26	67	2.01
18	0.54	43	1.29	68	2.04
19	0.57	44	1.32	69	2.07
20	0.6	45	1.35	70	2.1
21	0.63	46	1.38	71	2.13
22	0.66	47	1.41	72	2.16
23	0.69	48	1.44	73	2.19

24	0.72	49	1.47	74	2.22
25	0.75	50	1.5	75	2.25

Toxicity and side effects

Oto-toxicity: PM is probably less toxic than other aminoglycosides (streptomycin, amikacin or gentamicin). PM should be avoided in patients who complain of deafness before treatment. Like streptomycin, PM could theoretically affect the hearing of the newborn in pregnant women.

Renal toxicity: Renal toxicity is increased in patients who are dehydrated, hypokalemic (e.g. patients with vomiting or profuse diarrhea), or who have underlying renal impairment. All patients should be encouraged to take adequate fluid while on PM or SSG. Patients should be told to drink until they have passed urine at least 4 times a day; babies should pass urine about each hour. Old patients are more vulnerable to renal toxicity.

Annex 9: Dosage, Administration and Precautions for Meglumine Antimoniate

Dosage

Meglumine antimoniate and sodium stibogluconate are the pentavalent antimony (Sb5+) compounds used to treat Leishmaniasis:

Meglumine antimoniate is commercialized by SANOFI-AVENTIS as solution for injection in 5ml ampoules (Glucantime) containing 405mg of pentavalent antimony (Sb5+), it means 81mg of Sb5+/1 ml. The dose of meglumine antimoniate is based on the amount of pentavalent antimony the presentation contains and is 20mg/kg/day.

The minimum dose is 2ml (162mg) for children weighting less than 10kg.

Table of Meglumine Antimoniate volume for injection of 20mg/kg/day

Weight in kg	***Meglumine antimoniate dose in ml***	***Weight in kg***	***Meglumine antimoniate dose in ml***	***Weight in kg***	***Meglumine antimoniate dose in ml***
< 10	2	34	8.4	59	14.6
10	2.6	35	8.6	60	14.8
11	2.8	36	9.0	61	15.0
12	3.0	37	9.2	62	15.4
13	3.2	38	9.4	63	15.6
14	3.4	39	9.6	64	15.8
15	3.8	40	9.8	65	16.0
16	4.0	41	10.2	66	16.2
17	4.2	42	10.4	67	16.6
18	4.4	43	10.6	68	16.8
19	4.8	44	10.8	69	17.0
20	5.0	45	11.2	70	17.2
21	5.2	46	11.4	71	17.6
22	5.4	47	11.6	72	17.8
23	5.8	48	11.8	73	18.0
24	6.0	49	12.2	74	18.2
25	6.2	50	12.4	75	18.6
26	6.4	51	12.6	76	18.8
27	6.6	52	12.8	77	19.0
28	7.0	53	13.0	78	19.4
29	7.2	54	13.4	79	19.6
30	7.4	55	13.6	80	19.8
31	7.6	56	13.8	>80	20
32	8.0	57	14.0		
33	8.2	58	14.4		

Route of administration

Intravenous or intramuscular

Sb^{5+} pharmacokinetics are almost identical by the IM and IV routes. Choice of IV or IM depends on the setting. IM is most logical in the bush. The drug may be given by a deep intramuscular injection. Consider also the big volume that should be injected (if the volume of injection exceeds 10ml, it should be divided in 2 doses: one in each buttock or thigh). During a polio outbreak consider giving all the children less than 3 years of age IV injections (IM injections increase the rate of paralytic disease in those incubating with polio). IV is less painful. It should be given slowly over 5–10 minutes or longer using small butterfly-style needles. Another possibility is to dilute the drug in 5% glucose solution 500ml in adults and give it slowly (30 minutes to 1 hour). In children between 10-25kg body weight, use 100ml and if less than 10kg use 50ml.

Contra-indications

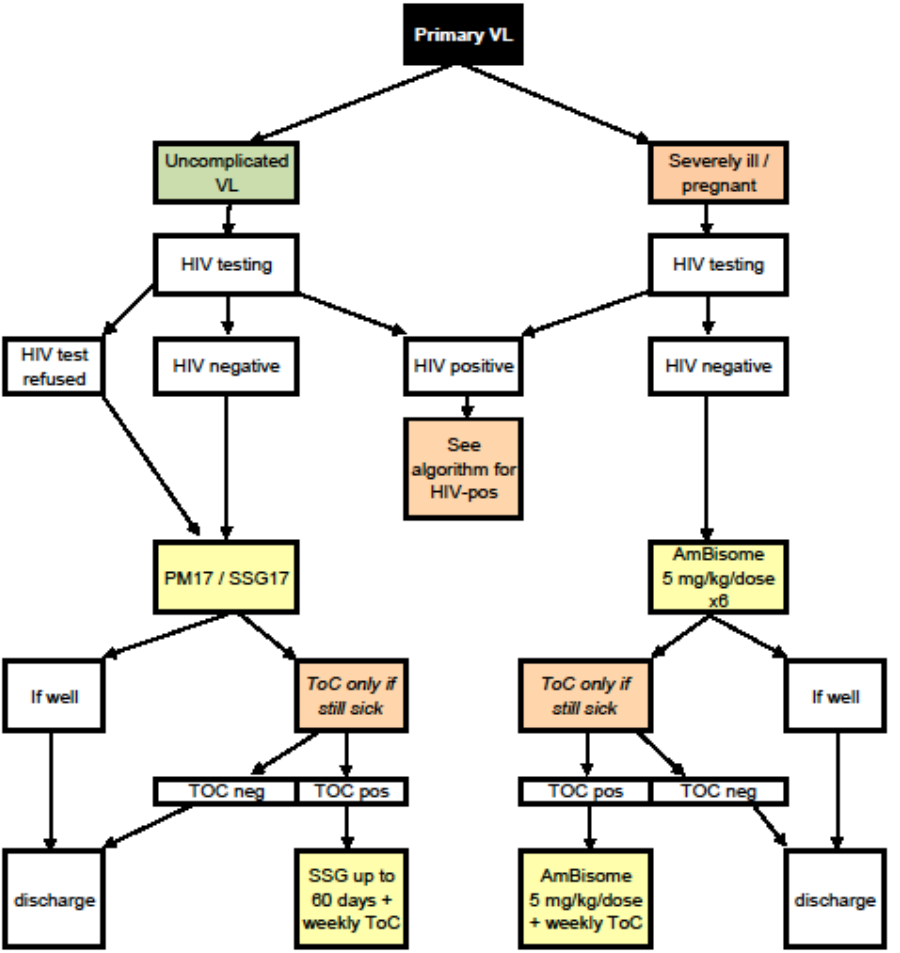
Severe cardiac, liver and kidney disorders, and during breastfeeding.

Precautions

The risk of serious, even fatal, toxicity of pentavalent antimonials is increased in patients who concomitantly present with cardiac disease, in particular arrhythmia; renal failure, liver disease, severe malnutrition/severely impaired general condition; advanced HIV infection; pregnancy. If one of these conditions is present, provide protein-rich diet and a good hydration throughout treatment and, if possible, correct iron and other nutritional deficiencies; renal and hepatic impairment; monitor cardiac, renal and hepatic function; treat concomitant infection (for example pneumonia), and check regularly the patient (ECG and renal, liver, pancreatic function). If possible, an alternative drug should be used.

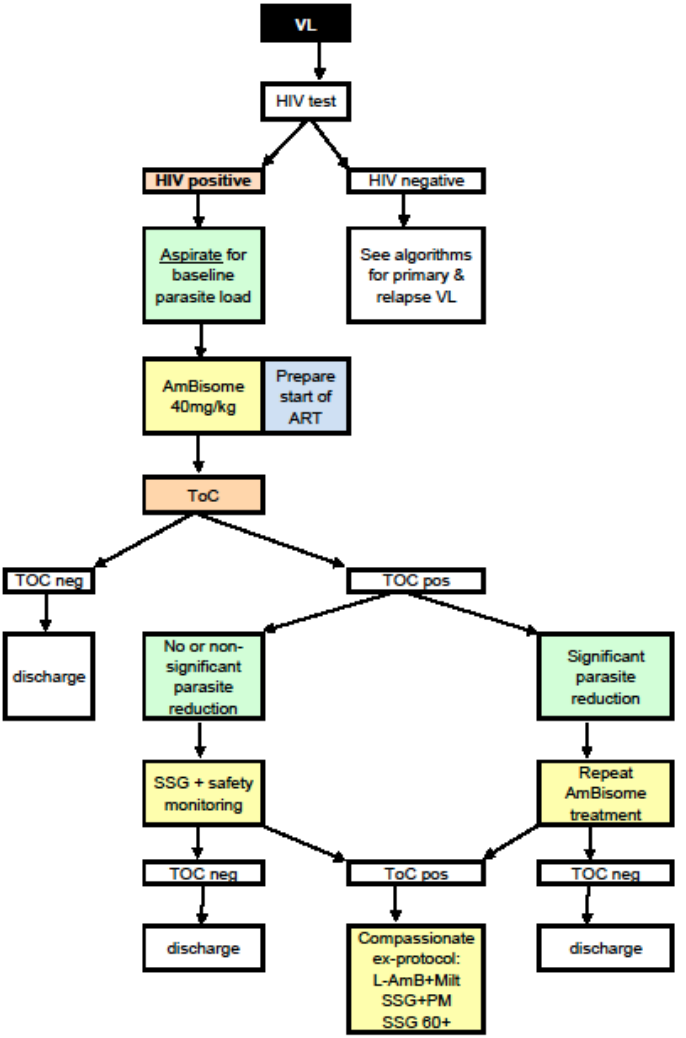
Annex 10: Visceral Leishmaniasis Treatment Algorithm

Visceral Leishmaniasis treatment algorithm: Primary VL, immunocompetent



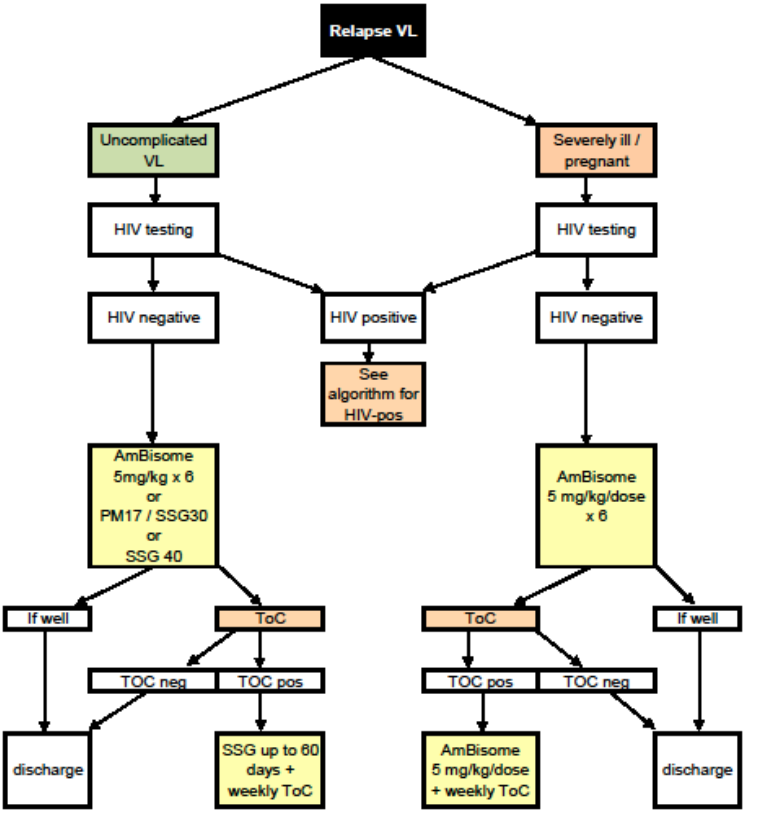
Note: Treatment of SSG can be extended to 60 days but with precaution in safety monitoring.

Visceral Leishmaniasis treatment algorithm: HIV co-infected VL



Note: In VL/HIV co-infected individuals, SSG 60+ may be considered while safety monitoring is warranted to the patient. The 40mg/kg AmBisome schedule is on days 1 to 5 and then on the 10th, 17th and 24th day. If the TOC is positive after treatment with AmBisome 40mg total dose, compassionate treatment may be required. These include SSG/PM, SSG 60+, AmBisome /Miltefosine combinations, etc.

Visceral Leishmaniasis treatment algorithm: Relapse VL



Note: Severely ill VL patients may require an extended dose of AmBisome if the TOC is positive after the end of 40mg/kg total dose of AmBisome . Patients with an extended dose of SSG require close safety monitoring.

Annex 11.1: Leishmaniasis Disease Record Form

PATIENT'S CARD Center name: Patient Id:

This form is completed by: Initials: _____ Position: Physician: ☐ Nurse: ☐ Other, describe: _____

Date of completion of form (dd-mm-yyyy): __ __-__ __-__ __ E.C. Data entered: Yes ☐ No ☐ by: _____

PATIENT DEMOGRAPHIC INFORMATION
Patient Name: _____ Age: ____ Gender: Male: ☐ Female: ☐
Occupation _____ Migration Status: Migrant ☐ Resident ☐ Re-settler ☐
Current Kebele: _____ Current Woreda: _____ Region:_____
How long in the current address: _____ (months) Original Kebele & Woreda: _____

BASIC CLINICAL INFORMATION AT ADMISSION	
General condition: able to walk: ☐ unable to walk: ☐ Number of months sick before treatment: ____	Clinical conditions: Fever ☐ Weight loss ☐ Jaundice: ☐ Lymphadenopathy : ☐ Vomiting: ☐ Bleeding: ☐
HIV status ☐ unknown ☐ known If known, Positive ☐ Negative ☐ If known, PITC offered & counseled Yes No Tested Yes ☐ No ☐ Test Result Positive ☐ Negative ☐	Spleen size (cm) _____ Haemoglobin (g/dl): _____ Platelet count (if done) _____
Presence of concomitant infection: No: ☐ Yes: ☐ If yes specify: Tuberculosis ☐ Malaria ☐ Diarrhea ☐ Pneumonia ☐	

NUTRITIONAL STATUS AT ADMISSION

Weight (kg): _____.___ Height (cm). _____ B.M.I _____or Wt/Ht_____ Oedema: Yes ☐ No ☐

LEISHMANIASIS DIAGNOSIS

New case: ☐ Relapse: ☐
If relapse: First ☐
Second: ☐
Other: ___

DAT: Done: ☐ Not done: ☐ DAT titre:_____

rk39: Positive: ☐ Negative: ☐ Not done ☐

Aspirate: Done: ☐ Not done: ☐

Aspirate source: Bone marrow : Positive ☐ Negative ☐
Spleen: Positive ☐ Negative ☐
Lymph nodes: Positive ☐ Negative ☐

DISEASE CATEGORY

VL: ☐
CL: ☐
MCL: ☐
PKDL ☐

TREATMENT

Date treatment started (dd-mm-yyyy): __ __-__ __-__ __ __ __ E.C.
1st treatment: SSG: ☐ Ambisome: ☐ SSG + Paromomycin ☐
Other: _____ No. of doses: _____
2nd treatment: SSG: ☐ Ambisome: ☐ SSG + Paromomycin ☐
Other _____ No. of doses: _____

Toxicity during treatment:
Arrhythmia: ☐
Pancreatitis: ☐
Jaundice: ☐
Kidney failure: ☐
Others: _____

DISCHARGE STATUS

Initial cure: ☐ Date of initial cure: (dd-mm-yyyy): ___-___-_____EC
Final cure: ☐ Date of final cure: (dd-mm-yyyy): ___-___-_____EC
Defaulter: ☐ Date last seen (dd-mm-yyyy): ___-___-_____EC
Referred: ☐ Date referred: (dd-mm-yyyy): ___-___-_____EC
Died: ☐ Date of death: (dd-mm-yyyy): ___-___-_____EC
If patient died, cause of death:_____

Test of cure : Done ☐
Not done ☐
Source _____
Result _____
Discharge weight (kg): _____.___
Discharge spleen size (cm): _____
Discharge hemoglobin (g/dl): _____

HIV Co-infection:
CD4 count (if done) _____
On ART on admission ☐ Regimen _____

Initial cure: eradication of parasites and/or improvement in clinical signs and symptoms (defervescence, weight gain, spleen size decrease) at the end of treatment. **Final cure:** initial cure followed by 6 months follow-up without relapse and absence of clinical signs and symptoms attributable to VL (defervescence, weight gain, spleen size decrease).

Date	Dose SSG (in ml)	Dose PM	Dose Amb.	No	T°	DI	DI wb	Vo	Co	RD	BL	Other meds given including ORS	Others specify

REMARKS: _____

Annex 11.2: Back Page of Patient's Card

No: = Day number
T° = Temperature
DI = Diarrhea (how many times)
DI wb = Diarrhea with blood (how many times)
Vo= Vomiting (how many times)
RD = Respiratory distress
BL = Bleeding
Co = Coughing
Other = Any other sign (e.g. abdominal discomfort, pain/abscess at injection site etc)

Annex 11.3: KA Patient Follow-Up and Discharge Card

KA PATIENT Follow up Card

Health Facility ----------
Name ---------- Age ------ Sex ---

Admission date ----------
Treatment ----------

Discharge date ----------
Appointment date: 3rd month ---------
6th month ----------
Discharged by ----------

Sign ----------

Annex 11.4: Quarterly Visceral Leishmaniasis (VL) Health Facility Report

Region_____ Zone_____ District_____ Health Facility_____ Quarter/Year _____

1. VL Diagnosis and admissions during the reporting quarter

Age Group	*Sex*			*Admission by VL type*			
	M	F	Total	Primary	Relapse	PKDL	Remark
< 5 years							
5–9 years							
10–14 years							
≥ 15 years							
Total							

2. VL data on pregnant women during the quarter:

	Number of cases
Primary VL	
Relapse VL	
PKDL	
Total	

3. PKDL case profile during the reporting quarter

	Number of cases
Total PKDL cases screened	
Total PKD cases treated	

4. Cutaneous Leishmaniasis (CL/MCL) cases treated in the health facility during the quarter

Male	***Female***	***Total***

5. VL treatment outcome

	Primary	***Relapse***	***PKDL***	***Total***
Discharged/Cured				
Defaulted				
Died				
Treatment failure				
Referred				
Total exits				

6. VL exits (discharges, deaths, defaulters and referred cases) by age:

	No.	***%***
< 5 years		
5-14 years		
≥ 15 years		

7. Description of VL deaths

Type of VL (1^0, Relapse, PKDL)	***VL treatment (drug) and No. of doses taken***	***Complications***

8. Proportion of malnourished VL cases (SAM rate) from the total exits: _____

9. Laboratory:

9a. Leishmaniasis diagnostic tests performed

Type of test	***No. Positive***	***No. Negative***	***Total***	***% Positive***	***Remark***
rK39					
DAT					
Aspirate					
Skin scraping					
Total					

9b. HCT and HIV care for co-infected patients

Age	***No. pre-test counseled***		***No. tested***		***No. post-test counseled***		***No. positive***		***No. positive referred***	
group	M	F	M	F	M	F	M	F	M	F
0-4										
5-14										
15-19										
20-24										
25-49										
50+										
Unre-corded										
Total										

10. General narrative on VL, HCT for VL patients and the Leishmaniasis control activities in the area:

Compiled by _____ Responsibility_____ Date_____

Annex 11.5: Registration Format

S.N	*Card No.*	*Patient Full Name*	*Age*	*Sex*	*Patient's Address (Z: zone, D: district, K: kebelle, V: village)* *Z D K V*	*Travel history*	*Diagnosis and Lab Result (DAT/RDT/ Aspirate/ Skin scrap-ing*	*Nut-Status*	*Sero-status*

	Date of admission	*Months sick before admission*	*Pregnancy (Yes/ No/ NA)*	*Other OI & its RX*	*Complications or drug side effect*	*Treatment*	*Date discharge*	*Treatment outcome*	*Remark*