**ACKNOWLEDGMENTS**

The Ministry of Health would like to acknowledge the following people and organizations that contributed to the review and finalization of these guidelines:

<table>
<thead>
<tr>
<th>Name</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Edridah Tukahebwa</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Alfred Mubangizi</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Ruiz Postigo Jose Antonio</td>
<td>WHO Geneva</td>
</tr>
<tr>
<td>Dr. Beshah, Abate Mulugeta</td>
<td>WHO AFRO</td>
</tr>
<tr>
<td>Mr. Martin Mayanja</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Mr. Gabriel Matwale</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Mr. Geoffrey Egitat</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Harriet Namwanje</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Dawson Mbulamberi</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Mr. Moses Arinaitwe</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Charles Wamboga</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Abbas Kakembo</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Patrick Turyaguma</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Mr. Tom Lakwo</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Miss Auma Anna Mary</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Mr. Tinkitina Benjamin</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Miss Carol Kyozira</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>Dr. Sagaki Patrick</td>
<td>Amudat Hospital</td>
</tr>
<tr>
<td>Dr. Andrew Munerya</td>
<td>Amudat Hospital</td>
</tr>
<tr>
<td>Mr. Daniel Kalepon</td>
<td>Amudat Hospital</td>
</tr>
<tr>
<td>Mr. Okello Lawrence</td>
<td>Amudat Hospital</td>
</tr>
<tr>
<td>Dr. Miriam Nanyunja</td>
<td>WHO Uganda</td>
</tr>
<tr>
<td>Dr. Jorge Alvar</td>
<td>WHO/HQ</td>
</tr>
<tr>
<td>Prof. Joseph Olobo</td>
<td>Makerere University</td>
</tr>
<tr>
<td>Dr. Elizabeth Sentongo</td>
<td>Makerere University</td>
</tr>
<tr>
<td>Miss Tabitha Areho</td>
<td>Sight Savers</td>
</tr>
<tr>
<td>Dr. Monique Wasunna</td>
<td>DNDi, Africa</td>
</tr>
<tr>
<td>Miss Joy Malongo</td>
<td>DNDi, Africa</td>
</tr>
<tr>
<td>Miss Linet Otieno</td>
<td>DNDi, Africa</td>
</tr>
<tr>
<td>Dr. Raymond Omollo</td>
<td>DNDi, Africa</td>
</tr>
<tr>
<td>Mr. Simon Bolo</td>
<td>DNDi, Africa</td>
</tr>
<tr>
<td>Dr. Robert Kimutai</td>
<td>DNDi, Africa</td>
</tr>
<tr>
<td>Dr. Fabiana Alves</td>
<td>DNDi, Geneva</td>
</tr>
<tr>
<td>Dr. Ahmed M Musa</td>
<td>Institute of Endemic Diseases, Sudan</td>
</tr>
</tbody>
</table>
Foreword

Visceral Leishmaniasis (Kala azar) a parasitic disease with anthropogenic transmission is one of the Neglected Tropical diseases endemic in Uganda. The disease due to the bite of an infected sand fly mainly affects the remote regions of Karamoja. If this disease is not treated may lead to death.

Though one of the most neglected tropical diseases, it can be effectively controlled using the available control strategies recommended by Ministry of Health (MoH) and WHO. As a public health problem, the disease is controlled through proper case management. Effective early diagnosis and management of Visceral Leishmaniasis (VL) cases is key to prevention of morbidity and reduction of related mortality. This should be supplemented with integrated control of sand fly vectors. Uganda has already upgraded from a first line regimen of monotherapy Sodium Stibogluconate (SSG) for 30 days to a combination therapy of Sodium Stibogluconate and Paramomycin for 17 days. This reduces on the time of hospital admission which in turn reduces hospital crowding and the risk of nosocomial transmission. The medicines for VL treatment are registered for use in Uganda by the National Drug Authority.

This manual has been designed to address diagnosis, treatment and prevention of further spread of VL in Uganda. It will therefore assist all health workers in the effective management of Visceral Leishmaniasis. MOH encourages all stakeholders involved in the management and control of VL to take advantage of these guidelines in order to control and eliminate this disease.

We acknowledge the contribution from all stakeholders: individuals, academic institutions and development partners who have participated in the development of these guidelines. Special thanks go to DNDi, LEAP, MUK, and WHO for their technical and financial support.

Dr. Henry H. Mwebesa
Ag. DIRECTOR GENERAL HEALTH SERVICES.
# Contents

Ministry of Health Uganda

1. INTRODUCTION ................................................................................................................ 10
   1.1 Background Information ............................................................................. 10
   1.2 Lifecycle and Transmission Patterns ............................................................. 11
   1.3 Human Infection and Disease ....................................................................... 12
2.0 DIAGNOSIS ...................................................................................................................... 12
   2.1. Clinical Diagnosis ...................................................................................... 12
   2.2 Laboratory Diagnosis of Visceral Leishmaniasis .............................................. 14
      2.2.1 Serological Diagnosis ............................................................................ 14
      2.2.3. Parasitological Diagnosis .................................................................... 16
   2.3. Diagnostic Algorithm Fever AND, splenomegaly and/or weight loss ............ 19
3.0 Principles and Objectives of Treatment ..................................................................... 20
   3.2. Specific chemotherapy for VL ..................................................................... 20
      3.2.1 First-Line Treatment for primary VL ..................................................... 20
      3.2.2 Second-Line Treatment for primary VL: ................................................. 23
      3.3. Amphotericin ............................................................................ 24
      3.4. VL Treatment in Uganda ................................................................. 25
   3.3 Supportive Treatment ....................................................................................... 25
   3.3.1 Treatment of Concurrent Illness ............................................................. 25
   3.3.2 Nutrition support .................................................................................... 25
4.0 Definitions of Treatment Outcomes ........................................................................... 26
   4.1 Initial Cure ...................................................................................................... 26
   4.2 Non-Responders (Primary Unresponsiveness) .................................................... 26
   4.3 Slow Responders ......................................................................................... 26
   4.4 Relapse Cases .............................................................................................. 26
   4.5 Post Kala-Azar Dermal Leishmaniasis (PKDL) ................................................. 27
   4.6 Death ............................................................................................................ 27
   4.7 Default ........................................................................................................ 27
   4.8 Lost to Initial Follow Up ................................................................................ 27
   4.9 Final (definitive) Cure .................................................................................... 27
   4.10 Drug Resistant Case ..................................................................................... 27
5.0 VL-HIV Co-Infection ................................................................................................. 27
6.0 Disease Surveillance and Epidemic Response ........................................................... 29
   6.1 Disease Surveillance ....................................................................................... 29
   6.2 Health Facilities as Reporting Units ............................................................... 29
   6.3 Report Review and Feedback .......................................................................... 29
   6.4 Surveillance of Kala – azar ............................................................................ 30
      6.4.1 Passive Case Surveillance ..................................................................... 30
      6.4.2 Active Case Surveillance ....................................................................... 30
      6.4.3 Vector Surveillance ................................................................................ 30
   6.5 Disease Outbreak Response ............................................................................. 31
      6.5.1 Detection ............................................................................................. 31
      6.5.2 Confirmation ........................................................................................ 31
      6.5.3 Response ............................................................................................. 31
      6.5.4 Post Outbreak ....................................................................................... 32
7.0 Prevention and control of Visceral Leishmaniasis ..................................................... 33
   7.1 Vector Control Interventions ............................................................................ 33
      7.1.1 Use of Long lasting Insecticide Treated Nets (LLINs) ......................... 33

Tables ................................................................................................................................. 6

Dose and Administration .................................................................................................. 21

Lost to Initial Follow Up .......................................................................................... 27

Default ...................................................................................................................... 27

Death ........................................................................................................................ 27

7.1.1 Use of Long lasting Insecticide Treated Nets (LLINs) ......................... 33
7.1.2 Indoor Residual Spraying .......................................................................................... 33
7.1.3 Personal Protection ................................................................................................. 33
7.1.4 Environmental Management .................................................................................. 34
7.1.5 Integrated Vector Management ............................................................................... 34
7.2 Other VL Prevention Measures .................................................................................. 34
7.2.1 Health Education .................................................................................................... 34
7.2.2 Community Awareness and Mobilization ............................................................... 35
7.3 Role of Community Health Workers in Prevention and Control of Leishmaniasis ...... 35
7.4 Prevention and control of Visceral Leishmaniasis in Uganda ..................................... 35
8.0 References .................................................................................................................. 38
Annexes ............................................................................................................................ 39
1. Materials needed .......................................................................................................... 44
2. Pre-operative procedures .............................................................................................. 45
3. Aspiration procedures .................................................................................................. 45
4. Post-operative procedures ........................................................................................... 46
Time .................................................................................................................................... 48
Materials needed ............................................................................................................... 48
2. Pre-operative procedures .............................................................................................. 48
3. Aspiration sites .............................................................................................................. 48
4. Aspiration procedures .................................................................................................. 49
5. Post-operative procedures ........................................................................................... 49
1. Materials needed .......................................................................................................... 50
2. Fixation ......................................................................................................................... 50
3. Staining ........................................................................................................................ 50
4. Reading Slides ............................................................................................................. 50
5. Grading of Parasite Loads ............................................................................................ 50
1. Principles ...................................................................................................................... 51
Collection of Blood Samples - Day One ........................................................................... 51
2. Materials Needed .......................................................................................................... 51
3. Procedure for Collection of Peripheral Venous Blood for Serum Samples ................ 51
4. Procedure for Collection of Blood on Filter Paper ....................................................... 52
Elution of Blood Samples from Filter Paper ................................................................... 52
5. Materials Needed .......................................................................................................... 52
6. Procedure: .................................................................................................................... 52
Setting the DAT Test from Filter Paper Elutes or Serum Samples .................................. 52
7. Materials needed .......................................................................................................... 52
8. Preparation of Diluent ................................................................................................. 52
9. Reconstitution of Freeze Dried Antigen ....................................................................... 53
10. Reconstitution of Freeze-Dried Serum Controls ......................................................... 53
11. Dilution of Samples .................................................................................................... 53
12. Reading DAT Plates .................................................................................................... 54
14. DAT Titre Scales and Titre Designations by Integers .................................................. 55
15. Important Reminders about the DAT ....................................................................... 56
16. Problems Encountered with DAT, and Troubleshooting ......................................... 56
Hb, malaria slide ............................................................................................................... 58
Malaria slide ....................................................................................................................... 58
Hb, Malaria slide ............................................................................................................... 60
Malaria slide ....................................................................................................................... 60
Annex 12: HMIS FORM 128a: VISCERAL LEISHMANIASIS OUTPATIENT
REGISTER ......................................................................................................................... 63
List of Figures

Figure 1: Lifecycle of Leishmania ................................................................. 12
Figure 2: Diagnostic Algorithm ................................................................. 19

Tables

Table 1: Validation results of different rK39 Rapid Diagnostic Tests............... 15
Table 2: Adjustment of PM dose based on weight of patient ....................... 22
Table 3: Summary Table of VL Treatment in Uganda ............................... 25
Annexes

Annex 1: Medical Form for Patients with Clinical Suspicion of VL ........................................ 39
Annex 2: Spleen Aspiration Procedures .................................................................................... 44
Annex 3: Checklist before Performing Spleen Aspiration ....................................................... 47
Annex 4: Monitoring of the patient after Spleen aspiration ..................................................... 48
Annex 5: Lymph Node Aspiration Procedures ........................................................................ 48
Annex 6: Preparation and Staining of Aspirates ..................................................................... 50
Annex 7: Procedure for Bone Marrow Aspiration .................................................................. 50
Annex 8: Direct Agglutination Test (DAT) ............................................................................. 51
Annex 9: Antimonials Treatment Table .................................................................................. 58
Annex 10: Conventional Amphotericin B Treatment Table .................................................. 60
Annex 11: Overview of treatment for concurrent illnesses in VL patients ............................. 61
Annex 12: HMIS FORM 128a: Visceral Leishmaniasis outpatient register ............................. 63
Annex 13: Visceral Leishmaniasis Health Unit Inpatient register (HMIS form 127a) .......... 64
Annex 14: Health unit VL Inpatient monthly report (HMIS form 127b) ................................. 65
Annex 15: Monthly OPD Reporting Form ............................................................................. 67
Annex 16: VL Individual Tracker ......................................................................................... 68
Abbreviations

AFB  Acid Fast Bacilli
AIDS  Acquired Immuno-Deficiency Syndrome
ARI  Acute Respiratory Infection
BCC  Behavioural Change Communication
BPM  Beats per Minute
CL  Cutaneous Leishmaniasis
DAT  Direct Agglutination Test
DDT  Dichloro-Diphenyl-Trichloroethane
DNDi  Drugs for Neglected Diseases Initiative
DOT  Directly Observed Treatment
ELISA Enzyme Linked Immuno-Sorbent Assay
ESD  Epidemiology Surveillance Division
FCS  Foetal Calf Serum
FDA  Freeze Dried Antigen
HAART  Highly Active Anti-Retroviral Therapy
Hb  Haemoglobin
HC  Health Centre
HIV/ VL  Visceral Leishmaniasis HIV co-infection
HIV  Human Immunodeficiency Virus
HMS  Hyper-reactive Malarial Splenomegaly
IDA  International Dispensary Association
IFAT  Immuno Fluorescent Antibody Technique
ITN  Insecticide Treated Mosquito Net
LEAP  Leishmaniasis East Africa Platform
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLINs</td>
<td>Long-lasting Insecticide Treated Nets</td>
</tr>
<tr>
<td>LSTMH</td>
<td>London School of Tropical Medicine and Hygiene</td>
</tr>
<tr>
<td>MoH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>MSF CH</td>
<td>Medecins Sans Frontiers Switzerland</td>
</tr>
<tr>
<td>MSF</td>
<td>Medecins Sans Frontiers</td>
</tr>
<tr>
<td>ORS</td>
<td>Oral Rehydration Salt</td>
</tr>
<tr>
<td>PKDL</td>
<td>Post Kala Azar Dermal Leishmaniasis</td>
</tr>
<tr>
<td>RDTs</td>
<td>Rapid Diagnostic Tests</td>
</tr>
<tr>
<td>SAG</td>
<td>Sodium Antimony Gluconate</td>
</tr>
<tr>
<td>Sbv</td>
<td>Pentavalent Antimonial</td>
</tr>
<tr>
<td>SSG</td>
<td>Sodium Stibogluconate</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>TOC</td>
<td>Test of Cure</td>
</tr>
<tr>
<td>TSS</td>
<td>Tropical Splenomegaly Syndrome</td>
</tr>
<tr>
<td>VL</td>
<td>Visceral Leishmaniasis</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1.1 Background Information:

Leishmaniases are caused by over 20 species of parasitic protozoa of the genus Leishmania. The disease, transmitted to humans by sand flies (Phlebotomus and Lutzomyia species), is endemic in 98 countries, affecting around 1.5 million people each year. There are three clinical forms of Leishmaniases: cutaneous, muco-cutaneous and visceral leishmaniasis. Visceral Leishmaniasis is caused by Leishmania donovani in the old world, and L. infantum in the new world and the Mediterranean region. It is estimated that some 20,000 to 40,000 people die annually due to leishmaniasis, mostly from visceral leishmaniasis (VL), which is also known as Kala Azar, a Hindi term meaning ‘black fever’ (1,2).

Over 90% of the cases reported to WHO2014 were from six countries namely Bangladesh, Brazil, Ethiopia, India, the Sudan and South Sudan. In Africa, countries endemic for VL, include Ethiopia, Kenya, Somalia, Sudan, South Sudan and Uganda. VL generally affects poor and neglected populations living in remote rural areas (3).

In Uganda, historically VL has been endemic in Amudat in Karamoja sub-region (2). This is an extension of the endemic foci of West Pokot and Baringo Districts of the Rift Valley Province in Kenya. The disease is known among the Pokots as ‘Termes’, which means ‘a very enlarged spleen’ (4, 5). From the year 2000 to 2016, more than 3400 VL patients were treated in Amudat Hospital, Pokot County. Around 70% of the patients were coming from West Pokot and Baringo Districts of Kenya and 30% from Pokot County, Uganda. The male to female ratio of the patients was 3:1 and more than 60% of the patients were under 15 years of age (6).

The current geographical spread of VL in Uganda is not known, however in the past 5 years, some VL cases seen at Amudat Hospital, the VL treatment center in Karamoja region, come from several other districts in Karamoja and neighbouring Teso regions including Moroto, Kotido, Napak, Abim, Kaabong, Nakapiripirit, and Katakwi (Ministry of Health, 2017). Treatment of Kala Azar is available mainly at Amudat Hospital. Suspected or confirmed cases are referred to this hospital for management. However, due to low index of suspicion, it is possible that health workers are missing VL cases. Additionally, due to the poor health seeking behaviour in Karamoja region, it is likely that some VL cases remain and die in the community without being seen at the VL treatment centre.
1.2 Lifecycle and Transmission Patterns

Leishmaniasis is spread from man to man by infected female sand flies. While taking blood meals, the female sand flies may ingest *Leishmania* amastigotes from the blood of an infected person. Within the sandfly the parasite develops in approximately one week into an infective promastigote (flagellate form). These promastigotes when injected into the skin of a person during a blood meal, are taken up by macrophages, where they develop into the amastigote (aflagellate) form (figure 1) (7).

Different species of sand flies need different habitats to survive and have different biting patterns (in and outdoors, forest or village, day or night preferences). This has important implications for the transmission and possible control measures. The vector of VL in Pokot County, Uganda is *Phlebotomus martini*, which has a peak biting activity between 6.30 and 9.30 pm (4,8). There is no known animal reservoir of *Leishmania donovani* in Uganda (4, 8). However more animal and vector studies need to be done in the endemic districts to generate more information about this.
1.3 Human Infection and Disease

Most individuals infected by *Leishmania donovani* will not develop the disease (asymptomatic or sub-clinical infections). When the host immune system is not able to suppress the parasite, VL will develop. The incubation period is typically 2 to 6 months, but may be shorter or sometimes longer. In non-immune hosts, the incubation period could be as short as 2 weeks, which could result in an epidemic.

Patients typically present with a history of fever for 2 weeks or longer, anorexia, headache, body weakness, sometimes with cough, abdominal pain, diarrhoea, vomiting, epistaxis (nose bleeding) and symptoms of anaemia. After several weeks of illness, weight loss becomes prominent, sometimes leading to severe malnutrition. Lymphadenopathies are frequently found in Sudan but more rarely in Uganda. In Uganda the most prominent clinical finding is splenomegaly, which can be very massive. If left untreated, the disease invariably leads to death often from superimposed bacterial infection, severe anaemia or bleeding (9). The presentation is more severe and has higher mortality in patients with HIV or other immunosuppressive conditions like malignancies, people with malnutrition, and those of young age. The case fatality is about 100% if the disease is left untreated. Death is mainly due to secondary bacterial infections.

2.0 DIAGNOSIS

2.1. Clinical Diagnosis

VL should be suspected in a patient from a VL endemic area or who has travelled to an endemic area presenting with fever for more than two weeks and splenomegaly and/or weight...
loss in whom malaria has been ruled out or has not shown clinical response to effective antimalarials.

**Case definition of a clinical suspicion of VL:**
History of prolonged fever (> 2 weeks) splenomegaly and/or wasting

A typical patient will present with the following signs and symptoms:

- fever for two weeks or more
- splenomegaly
- weight loss
- anaemia
- cough
- epistaxis
- hepatomegaly
- body weakness

In rare circumstances, some patients will present with:

- oedema
- jaundice
- vomiting
- joint pains
- abdominal pains
- lymphadenopathy
- diarrhoea

Once a patient meets the clinical case definition, it is important to know if it is a primary VL case or a relapse, as the investigation and patient management is different in the two cases.

Primary Kala Azar is a patient who is diagnosed with Kala Azar for the first time. The patient had not been treated for Kala Azar before.

A relapse case is a patient who successfully completed a course of standard VL treatment and showed evidence of clinical and parasitological response (negative test of cure) but presents with clinical and parasitological evidence of VL within 6 months after completing treatment but it can also occur after six months.

Those patients meeting the clinical case definition the diagnosis needs to be confirmed serologically or parasitologically (primary KA) or parasitologically (relapses).

The main differential diagnoses in Ugandan VL patients are:

- Malaria
• Hyper-reactive malarial splenomegaly (HMS): formerly called Tropical Splenomegaly Syndrome (TSS). This condition results from multiple partially treated malaria episodes
• Schistosomiasis: the splenomegaly is caused by portal hypertension and the fever is usually caused by another condition (e.g. pneumonia)
• Brucellosis: the splenomegaly is usually not massive; hepatomegaly; joint, bone and occasionally neurological involvement
• Typhoid fever: high grade fever, bradycardia, duration of illness less than one month, impaired mental status, constipation
• Tuberculosis: usually no splenomegaly, but possible in case of miliary tuberculosis; respiratory symptoms
• Splenic abscess
• Malignancies of lymphoid origin (Leukaemias and Lymphomas)
• Chronic haemolytic anaemias
• HIV/AIDS

2.2 Laboratory Diagnosis of Visceral Leishmaniasis

2.2.1 Serological Diagnosis

Several tests have been developed to detect antibodies against *Leishmania* in the blood or serum of VL patients. Some of the antibody detecting tests are not appropriate for field use such as Immuno Fluorescent Antibody Technique (IFAT) or Enzyme Linked Immuno-Sorbent Assay (ELISA) tests. Serological tests like rK39 and Direct Agglutination Test (DAT) can be used in the field to start treatment if the VL suspect case definition is strictly followed. If serological test results of a case meeting the VL suspect case definition are negative or inconclusive, then diagnosis may be confirmed by parasitological methods or culture.

Rapid Diagnostic Tests (RDTs) in a dipstick format have been recently developed and validated in the field. They allow for diagnostic confirmation/screening at all Health Centre levels, starting of treatment and/or referral for appropriate management at specialized treatment centres, provided that the clinical case definition is strictly (appropriately) applied. The implementation of RDTs at peripheral level allows for an earlier diagnosis and treatment, and therefore an improved prognosis.

The Direct Agglutination Test (DAT) is a robust and well-validated test that requires more material and training. The procedure is fairly complex. It should be reserved for use at HC IV and hospital levels with competent and well-trained laboratory staff (10).

2.2.1.1 rK39 RDTs

The rK39 antigen-based RDT, detects specific Leishmania antibodies present in *VL patients*. There are currently commercially available rK39 dipstick types, such as the DiaMed IT-Leish (DiaMed AG, Switzerland/Bio-Rad, South Africa)) and the Kalazar Detect (Inbios Ltd, Seattle, USA). These two products have been evaluated in Amudat, Uganda among Pokot
patients with a clinical suspicion of VL. The DiaMed IT-Leish dipstick was found to be significantly more sensitive (97% versus 82%) than the Kalazar Detect dipstick but with similarly high specificity (97 % versus 99 %) (11). Similar high specificity of the DiaMed IT-Leish has been found in other studies conducted in Sudan and India (12, 13).

Validation studies of various rK 39 diagnostic test kits carried out in Kenya by KEMRI, DNDi, MSF and WHO/TDR (34) have also shown that DiaMed IT LEISH/Bio-Rad has the highest sensitivity and specificity in East Africa in the early 90s (14). Validation results of some of the RDTs are shown in the table below.

Table 1: Validation results of different rK39 Rapid Diagnostic Tests

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Sensitivity (95% CI) n=250</th>
<th>Specificity (95% CI) n=250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal KA</td>
<td>Span diagnostic Ltd</td>
<td>36.8 % (31.1 - 42.9%)</td>
<td>98.0% (95.4 - 99.1%)</td>
</tr>
<tr>
<td>Diamed – IT LEISH</td>
<td>Bio.Rad. Lab.</td>
<td>87.2% (82.5 - 90.8%)</td>
<td>96.4% (93.3 - 98.1%)</td>
</tr>
<tr>
<td>Kala azar Detect</td>
<td>InBios International Inc</td>
<td>67.6% (61.6 - 73.1%)</td>
<td>90.8% (86.6 - 93.8%)</td>
</tr>
<tr>
<td>Signal – KA (rKE16)</td>
<td>Span diagnostic Ltd</td>
<td>73.2% (67.4 - 78.3%)</td>
<td>96.4% (93.3 - 98.1%)</td>
</tr>
</tbody>
</table>

It is recommended that the DiaMed IT-Leish kits must be kept at room temperature, but not higher than 37\(^{\circ}\) C, because the test performance can be affected at temperatures of ≥45\(^{\circ}\)C (14, 34). The procedure of the DiaMed IT-Leish rK39 dipstick is simple (whole blood from finger-prick) with results available within 25 minutes. It does not require extra-material and results are stable overtime, allowing for quality control.

Based on the above evidence, the NTD control programme, Ministry of Health, recommends the use of DiaMED IT LEISH as the preferred rapid diagnostic test kit for kala azar in the country as it has a higher specificity and sensitivity compared to other test kits and is relatively easy to use. Any test should undergo similar rigorous testing and validation before being recommended for general public use.

Advantages and Disadvantages of rK39 RDTs

**Advantages**
- rK39 RDTs enable individual patients to be tested at the bedside
- Tests are individually packages and easy to store and transport
- Little training and no laboratory equipment needed
- Results available within 10 -20 minutes
- Results are clear and easy to read
- Enables decentralized screening of VL at health facility and community levels
- Easy to use in the field during active case search or an outbreak
- Kits can be transported and stored at room temperature

**Disadvantages**
- As most serological tests, they cannot distinguish between active and past infection (symptomatic or asymptomatic). Thus their results must be interpreted in combination
with clinical case definition, and diagnosis of relapse cases should rely on parasitology.

- In about 10 – 18% of cases, the test may give a negative test even if the patient has VL. In such cases, another serological or parasitological test should be performed in VL suspect cases.
- In patients with advanced HIV infection, a negative result cannot preclude the diagnosis of VL.

### 2.2.1.2 Direct Agglutination Test (DAT)

The DAT can be performed using blood (including dried blood on filter paper) or serum. It is relatively simple and can be performed under some field conditions. The DAT is technically easy to perform but requires training, cold chain, and standardization. The test measures the serological response to surface borne antigens of whole Leishmania donovani.

The DAT antigen is prepared from formalin-killed culture-derived promastigotes of *L. donovani* and methylene blue stained for visibility. DAT kits are currently manufactured at the Royal Tropical Institute, Amsterdam, the Netherlands and at the Institute of Tropical Medicine, Antwerp, Belgium. The test is semi-quantitative and gives antibody titres ranging from 1:50 (usually 1:100) up to 1:102,400 or even higher. It is a highly sensitive (>95 %) and specific (>85 %) test when performed according to standardized procedures (see Annex 8 for details about the procedure). It requires a well-trained laboratory technician to undertake the process which lasts over a period of about 18 hours to obtain results. The relative sophistication of the DAT restricts its use to Health Centre IV and Hospital levels.

The DAT cut-offs were standardized in Uganda, as follows:

- DAT negative (<1:1,600): a case of VL is very unlikely. Alternative diagnoses (Malaria, disseminated TB, Brucellosis, Typhoid fever, etc…) should be looked for and treated. However, if clinical suspicion of VL is high (i.e. fever > 2 weeks with enlarged spleen), a spleen aspiration is performed to search for the *Leishmania* parasites.

- If the DAT is positive (>1:12,800), VL is very likely and specific treatment should be initiated. Nevertheless, if parasitological exam by microscopy is available, it should be done for parasitological confirmation before treatment is initiated (provided that there is no contra-indication).

- If the DAT is borderline (1:1,600 – 1:12,800), spleen aspiration should be performed in the absence of contra-indication (see below).

### 2.2.3. Parasitological Diagnosis

Parasitology remains the gold standard for Visceral Leishmaniasis diagnosis. It is done by microscopical examination of stained slides of spleen, bone marrow or lymph node aspirates (see annexes 5, 6 and 7). Specificity of these tests is near 100% provided that slide staining is done properly and that the laboratory technicians are well trained. Spleen aspirate is more sensitive (96%) than bone marrow (70%) or lymph nodes (58%) aspirates (14). Bone marrow aspirate is a painful and invasive medical procedure that needs local anaesthesia, expertise
and optimal sterilization of the puncture material. The procedure of lymph node aspiration can also be painful. Despite limitations of bone marrow and lymph node aspiration, these procedures are of minimal risk for the patients.

Spleen aspiration should be limited to hospital settings or health facilities where there is adequate equipment and trained staff to manage potential complications appropriately. Transfusion facilities should be present. Provided that the test is performed properly, the rate of life threatening bleeding after a spleen puncture is around 0.1% (15). No death or major bleeding occurred in Amudat Hospital following more than 2000 spleen punctures between 2000 and 2015 (Amudat hospital records).

Please refer to Annex 2, 3 and 4 for detailed procedures on spleen aspiration; Annex 5 for lymph node aspiration procedure and Annex 7 for bone marrow aspiration procedures.

Spleen aspiration is contra-indicated in the following situations:

- Spleen barely or not palpable
- Jaundice (a sign of possible liver dysfunction)
- Signs of active bleeding (nose, skin, digestive, etc…). A history of recent nose bleeding without active bleeding is not a contra-indication for spleen aspiration
- Severe anaemia (Haemoglobin < 5 mg/dl)
- Low platelets count, < 40,000/mm³
- Pregnancy
- Patient in very poor general condition
- Low blood pressure
- Uncooperative patient or caretaker
- Patients under anti-coagulant therapy
- Any situation that predisposes the patient to bleeding e.g. NSAID use

In patients with contra-indication(s) to spleen puncture, bone marrow or lymph node aspirates can be done (provided that enlarged lymph nodes are present).

The clinical indications for parasitological diagnosis (spleen or lymph node aspiration) are the following:

1. Clinical suspect with a prior history of VL (susicion of relapse)
2. VL patient not responding to anti-VL treatment (test of cure)
3. Clinical suspect with a borderline DAT result (1:1,600 – 1:12,800)
4. Clinical suspect with a negative rK39 dipstick or DAT results but with strong clinical suspicion of VL and absence of alternative diagnosis or no response to treatment of alternative diagnosis
5. In health facilities where parasitological diagnosis is available, this test should be done to confirm the diagnosis of patients with a positive RDT or DAT.
Splenic aspirates will be done at HC IV and Hospital levels only if:

1. The Medical Officers or Clinical Officers have been trained to perform the procedure
2. Blood transfusion facilities are present
3. Referral to a hospital with surgical facilities is possible
2.3. Diagnostic Algorithm Fever AND, splenomegaly and/or weight loss

Figure 2: Diagnostic Algorithm

Note:
1. Clinical suspicion should be based on case definition, negative malaria test and coming from VL endemic area or travel history to endemic area.
2. Define Primary VL and Relapse in simple terms i.e. new case and previous VL.
3. For RDT negative, if high clinical suspicion of VL and other differential diagnoses excluded, confirmatory parasitological testing should be considered.
4. If the DAT test is positive and there is no capacity for confirmatory parasitology at the health facility, the result can be used as basis for starting VL treatment.
3.0 Treatment

The management of Visceral Leishmaniasis will depend on the patient’s age and whether the patient has primary Kala Azar, relapse, VL-HIV co-infection or other concomitant medical conditions.

3.1 Principles and Objectives of Treatment

The objectives of VL treatment are to:

- Clinically cure the patient
- Clear the parasites
- Avoid severe drug toxicity
- Support the patient’s nutrition and hydration status
- Prevent and treat complications
- Prevent the development of drug resistance
- Manage and treat concomitant medical condition(s)

The treatment of VL patients is quite complex. It should include the following components:

- Specific chemotherapy for VL: first-line treatment for primary VL cases and second-line therapy for relapse cases (17,18)
- Treatment of bacterial co-infections like pneumonia, bacterial diarrhoeas, septicaemia and tuberculosis
- Treatment of anaemia including blood transfusion
- Treatment of dehydration
- Treatment of malnutrition
- Treatment of malaria

The choice of drugs for the treatment of VL in Uganda is based on:

- Efficacy and safety
- Age
- Concomitant medical conditions
- Availability

3.2 Specific chemotherapy for VL

3.2.1 First-Line Treatment for primary VL

The first line treatment regimen for VL in Uganda is a combination Sodium Stibogluconate (SSG) and Paramomycin (PM) administered for 17 days. This is based on results of a phase III clinical trial conducted in Uganda, Ethiopia, Kenya and Sudan showing that administration of a combination of SSG and PM for 17 days could cure primary Visceral Leishmaniasis (35).
Dose and Administration

Primary Visceral Leishmaniasis

1. Combination: Sodium Stibogluconate (pentavalent antimonials)/SSG at 20 mg/kg per day given intramuscularly or intravenously plus paromomycin 15 mg [11 mg base] per kg body weight per day given intramuscularly) for 17 days.

3.2.1.1 Sodium Stibogluconate (SSG)

For SSG the dosage is 20mg/kg/day as a single daily dose. The drug can be given by slow intravenous injection over 5 minutes or intramuscular injection. Caution should be exercised when treating patients above 50 years with SSG. Instead Ambisome (Liposomal Amphotericin B), when available could be used for treatment of patients in this age group.

3.2.1.2 Paromomycin (PM)

Paromomycin, formerly called Aminosidine, is an aminoglycoside antibiotic with good antileishmanial activity. Excellent efficacy and safety results have been obtained in India with a total dose of 15 mg/kg/day intramuscularly for 21 days.

The drug was registered in India for VL use in the year 2006. In East Africa, PM was evaluated as monotherapy and in combination with SSG in a multicentre clinical trial led by the Drug for Neglected Diseases Initiative (DNDi) within the Leishmaniasis in East Africa (LEAP) Platform. WHO reviewed the results of the study and recommended the combination treatment of SSG/PM for 17 days.

Other advantages of Paromomycin are its activity against a wide variety of pathogens (bacteria and protozoa) and its low cost (5-10 US $ per treatment). Its safety during pregnancy is under evaluation (phase IV trial in India).

To administer Paromomycin:

- 1 ml and 2 ml syringes should be available to give this medicine.
- The recommended dose is 15 mg/kg sulfate (equivalent to 11 mg/kg base) with no maximum dose; monotherapy should not be used.
- Patients must remain well hydrated because paromomycin can affect the kidneys. Patients should be advised to drink enough fluids so that they pass urine at least 4 times a day.
- If patients have severe vomiting and diarrhoea, do not give the paromomycin injections.

  The medicine should not be given intravenously

- Weigh the patient weekly and adjust the dose. The table below shows how to adjust the doses based on weight. If Maximum dose = 2ml/day
Table 2: Adjustment of PM dose based on weight of patient

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Daily doses (ml)</th>
<th>Body weight</th>
<th>Daily doses (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-11 kg</td>
<td>0.3 ml</td>
<td>40-42 kg</td>
<td>1.2 ml</td>
</tr>
<tr>
<td>12-15 kg</td>
<td>0.4 ml</td>
<td>43-46 kg</td>
<td>1.3 ml</td>
</tr>
<tr>
<td>16-18 kg</td>
<td>0.5 ml</td>
<td>47-49 kg</td>
<td>1.4 ml</td>
</tr>
<tr>
<td>19-22 kg</td>
<td>0.6 ml</td>
<td>50-52 kg</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>23-25 kg</td>
<td>0.7 ml</td>
<td>53-56 kg</td>
<td>1.6 ml</td>
</tr>
<tr>
<td>26-28 kg</td>
<td>0.8 ml</td>
<td>57-59 kg</td>
<td>1.7 ml</td>
</tr>
<tr>
<td>29-32 kg</td>
<td>0.9 ml</td>
<td>60-63 kg</td>
<td>1.8 ml</td>
</tr>
<tr>
<td>33-35 kg</td>
<td>1.0 ml</td>
<td>64-66 kg</td>
<td>1.9 ml</td>
</tr>
<tr>
<td>36-39 kg</td>
<td>1.1 ml</td>
<td>67-69 kg</td>
<td>2.0 ml</td>
</tr>
</tbody>
</table>

3.2.1.3 Toxicty and Adverse-Effects

Side effects of SSG and PM are frequent, especially in malnourished patients and the elderly. These include:

- Pain at the injection site
- Muscle and joint pain
- Loss of appetite, nausea and vomiting. The vomiting can be very disturbing and should be treated aggressively with anti-emetics (Promethazine or Metoclopramide) and rehydration. If these measures fail, the antimonial must be temporarily interrupted or the dose lowered for several days
- Biochemical (frequent) or overt (rare) pancreatitis
- Others: cardiac arrhythmia’s, tremor, ataxias are rare
- Ototoxicity - Hearing disorders/Hearing losses, deafness

The risk of serious (sometimes fatal) toxicity of SSG is increased in patients who concomitantly have:

- Cardiac disease, in particular arrhythmias
- Renal failure
- Liver disease
- Severe malnutrition
- Very poor general condition
- Advanced HIV infection
- Pregnancy
- Older than 50 years

If one of these conditions is present, the patient should be closely monitored or, preferably, be treated with AmBisome.
Co-administration of quinine or any other medicines known to cause cardiac toxicity with SSG should be avoided.

Most of the side effects and toxicities do not necessitate interruption of treatment but in case of interruption the following steps are taken:

- For interruptions less than five days – resume the course until the total number of injections has been given.
- For interruptions longer than five days – restart treatment from day 1

Some conditions arising from SSG toxicity may necessitate withdrawal of SSG therapy from the combination treatment. These conditions include:

- Acute pancreatitis
- Aberrations of creatinine
- Jaundice developing during treatment
- Excessively high liver function test values e.g. more than 5 times the normal values of SGPT/SGOT
- Any evidence of cardiotoxicity e.g. cardiac arrhythmias
- Declining haematological measurements e.g. HCT, total WBC count, with symptoms suggesting treatment failure
- Uninterrupted vomiting
- Failure to respond favourably to treatment in the first weeks of treatment.

If SSG has to be withdrawn, AmBisome can be used as the rescue treatment.

Patients should be checked regularly for clinical response. The earlier signs of response are the clearance of fever (within 7 days) and the improvement of the general condition (e.g. able to walk, increased appetite). Reduction of spleen size and increased Haemoglobin (Hb) level should be assessed at the end of treatment.

### 3.2.2 Second-Line Treatment for primary VL:

#### 3.2.2.1 AmBisome® (liposomal Amphotericin B)

Liposomal Amphotericin B is an efficacious treatment against VL with a more improved safety profile compared to amphotericin B deoxycholate formulation. Mild infusion reactions (fever, chills, and rigors) and back pain may occur in some patients. Liposomal Amphotericin B circulates as particulates, making Amphotericin B far less toxic. Thus higher doses can be given. It is also the drug of choice to treat VL patients co-infected with HIV.

Liposomal Amphotericin B comes in vials of 50mg and needs to be reconstituted and diluted in 5% dextrose and given over a period of 30 – 60 minutes as an intravenous infusion.

Before use, Liposomal Amphotericin B should be stored at 2 – 4°C and should not be frozen. It should also be protected from exposure to light. The reconstituted Liposomal Amphotericin B may be stored for 15 – 24 hours at 2 – 8°C before use.

1. Liposomal amphotericin B: 3–5 mg/kg per day, dose administered by infusion given over 30 – 60 minutes, given for 6–10 days up to a total dose of 30 mg/kg
2. days, for 15–20 doses
Iposomal amphotericin B is the first line treatment for the following:

- Pregnant women with VL
- VL-HIV co-infected patients
- Patients with lack of response or relapses after SSG-PM therapy
- Severely ill VL patients
- Children less than 2 years and adults older than 50 years
- VL patients with contra-indications to SSG or PM

3.2.2.2 Toxicity and Adverse-Effects

The profile of adverse-effects are similar to conventional Amphotericin B but of milder intensity and lower frequency. Patients may develop fever, chills and a low back pain if infusion is given very fast. Hypokalaemia may occur in some patients and should be corrected using potassium chloride. Anaphylactic reactions may also occur in some patients.

Liposomal Amphotericin B is contraindicated in patients who have a history of previous hypersensitivity reactions to it.

AmBisome is an alternative to conventional Amphotericin B as second-line treatment for VL. If available, it should be the first-line treatment of choice in pregnancy, (24) in HIV co-infected and in severely sick patients.

3.3 Amphotericin

Conventional Amphotericin B has been used in the past for VL treatment, however due to its serious side effects it has been replaced by the safer Liposomal Amphotericin B.

Conventional Amphotericin B (Fungizone®, Bristol Myers Squibb, USA; Photericin B®, Cipla, India) should only be used as an alternative treatment when there is no first line treatments or Ambisome. A suitable regimen is 0.75 – 1mg/Kg per day by infusion; daily or on alternate days, for 15 – 20 doses.

The major side effects is renal impairment, thus renal function should be monitored regularly (weekly) during treatment. Renal impairment can be reduced by pre-hydrating the patient with an infusion of normal saline. Hypokalaemia and hypomagnesaemia may also occur and can managed by potassium and magnesium supplementation respectively. Other side effects include headache, fever, chills, nausea, vomiting, muscle and joint pains, GIT cramps, hypertension, hypotension, arrythmias, skin rashes, blurred vision, tinnitus, hearing loss, vertigo, liver disorders, peripheral neuropathies, convulsions, thrombophlebitis at the injection site, anaemia, and anaphylactic reaction.

Administration

Amphotericin B is administered in 1 litre of 5% dextrose infusion running over 2 – 12 hours. Before starting therapy, hydrate the patient and maintain hydration with ORS and if needed, IV fluids. This is important to reduce the risk if renal toxicity. Give potassium supplementation to adults (1 tablet 3 times a day for adults).
3.4.3 VL Treatment in Uganda

Table 3: Summary Table of VL Treatment in Uganda

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary VL</td>
<td><strong>First line treatment</strong> Combination: Sodium SSG at 20 mg/kg per day intramuscularly or intravenously plus Paromomycin 15 mg [11 mg base] per kg body weight per day intramuscularly) for 17 days</td>
<td></td>
</tr>
<tr>
<td>1st Relapse</td>
<td>AmBisome 3-5 mg/kg/d for 10 days</td>
<td></td>
</tr>
<tr>
<td>Primary VL in HIV+</td>
<td>AmBisome 5 mg/kg/d for 10 days</td>
<td></td>
</tr>
<tr>
<td>VL with Pregnancy</td>
<td>AmBisome 3 mg/kg/d for 10 days</td>
<td></td>
</tr>
</tbody>
</table>

3.3 Supportive Treatment

3.3.1 Treatment of Concurrent Illness

Patients with VL are immuno-compromised and pancytopenic. The most common causes of death in these patients are inter-current illnesses like bacterial infections (e.g. pneumonia, diarrhoea and meningitis), severe anaemia or bleeding. These conditions have to be looked for and adequate treatment must be started without delay if detected.

- Treat Pneumonia and otitis media with appropriate antibiotics
- Maintain oral hygiene to prevent mouth and gum infections and rapidly treat any oral infections should they occur, with metronidazole and ampicillin
- Maintain skin hygiene and treat any skin infections that may arise
- Treat malaria and/or tuberculosis if present.

Treatment must be far more aggressive in these patients than other patients.

3.3.2 Nutrition support

Patients should receive adequate nutrition support during treatment, and vitamin supplements where indicated.

**Anaemia**

Occasionally blood transfusion may be required if the patient develops severe anaemia or bleeding due to thrombocytopenia.
4.0 Definitions of Treatment Outcomes

4.1 Initial Cure
When a patient has completed a full course of treatment and has clinically improved. Clinical criteria for initial cure are “no fever + regression of splenomegaly + return of appetite and/or gain in body weight”. Other changes that are evident in initial cure cases are improvement in anemia and a rise in haemoglobin, and an increase in White Blood Cell count.

4.2 Non-Responders (Primary Unresponsiveness)
Probable non responders – signs and symptoms still persist or recur during treatment without parasitological confirmation.

Confirmed non responders - Patients who do not show any decline or show increase in parasite load (if parasitology was done before treatment to allow for comparison) or patients with persistent clinical symptoms/signs and a positive test-of cure (TOC)

The TOC is a spleen or lymph node aspiration performed after completion or near completion of treatment (a minimum of 25 days). Clinical evaluation should be prioritized over test of cure for every patient because the latter is an invasive procedure. TOC should be reserved for cases where response is in doubt, in treatment of relapses, and for monitoring emergence of drug resistance. TOC can also be done to assure that a patient is due for discharge, especially for patients likely to be difficult to follow up. Negative TOC is when there are no amastigotes in a tissue slide taken from a VL patient while a positive TOC is when amastigotes are seen in a tissue slide taken from a VL patient. For relapse cases, there must be two negative TOC results, one week apart, before discharge.

Non-responders will be treated with a second-line treatment.

4.3 Slow Responders
Patients who show a decrease but not a disappearance of parasite load on spleen aspirate examination after completion of first-line therapy.

These patients should be treated with an extended course of pentavalent antimonials. The TOC should be repeated weekly and the treatment can be stopped when the TOC is negative. Antimonials should not be given for more than 60 days because of the dose-dependent risk of cardiotoxicity. If parasites are still seen after 60 days treatment, a second-line drug should be given.

4.4 Relapse Cases
Patients presenting with VL who have previously received a complete course of therapy with a good initial clinical response.

- Relapse cases can only be diagnosed parasitologically by spleen or lymph node puncture and should be treated with the second-line therapy.
- A clinical assessment and a TOC should be performed after the completion of therapy.
Patients with persistent parasites after second-line therapy and patients presenting with a second or more relapses should be tested for HIV.

Most relapse cases occur within 6 months after completion of therapy, but a patient diagnosed with VL several years after proven adequate anti-leishmanial therapy must be considered as a relapse case.

4.5 Post Kala-Azar Dermal Leishmaniasis (PKDL)

PKDL occurs weeks, months or years after the treatment of VL. The incidence of PKDL is very low among VL patients in Uganda and Kenya.

The treatment of severe PKDL relies on SSG 20 mg/kg/day until clinical cure. Several weeks or even months of treatment are necessary. Treatment on an Outpatient basis would be the best option if it is feasible for the patient.

4.6 Death

Death of a VL patient as a treatment outcome refers to death due to any cause, whether related to VL or not. Death should additionally be reported under the following sub-categories: i) due to VL; ii) due to a concomitant disease; iii) due to treatment (iatrogenic); iv) due to non-medical reasons (e.g. accident); v) unknown

4.7 Default

Refers to a patient who does not complete treatment

4.8 Lost to Initial Follow Up

This refers to when a patient does not present for assessment after completion of treatment. A patient who does not report for assessment 6 months after completion of treatment is considered to be lost to follow up.

4.9 Final (definitive) Cure

This is when a patient who after initial cure remains symptom free at 6 months after the completion of treatment. Note that in some cases a relapse can occur after 6 months.

4.10 Drug Resistant Case

A VL patient from which a laboratory confirmed drug resistant parasite is isolated.

5.0 VL–HIV Co-Infection

HIV and VL influence each other reciprocally. VL stimulates the replication of HIV and aggravates the immune-suppression; it accelerates the onset of full-blown AIDS and could shorten the life expectancy of HIV infected people.

HIV suppresses cell-mediated immunity and clinical VL occurs with an increased incidence after infection with *Leishmania donovani*. The clinical picture of VL in HIV positive patients is sometimes atypical and splenomegaly can be mild or absent. Non-specific symptoms like fever and wasting can be the only signs on admission.
The treatment of VL in HIV positive patients is complicated. Clinical improvement occurs in most cases with first-line or second-line therapy but relapses occur in the large majority of patients within months. Patients are less responsive to treatment with each relapse and finally become resistant to antimonials and all other anti-leishmanial medicines. Patients with HIV-VL co-infection should not be treated with SSG unless no less toxic alternative drugs are available. Liposomal Amphotericin B is the first-line drug of choice in these patients. If this drug is not available, conventional Amphotericin B or Miltefosine are suitable alternatives.

All HIV positive patients with VL should be classified as WHO stage IV - AIDS defining disease and should receive Highly Active Anti-Retroviral Therapy (HAART) (36). Monitor patients closely and ensure adequate HIV care. If available use Pentamidine to prevent VL relapses.

In case of relapse, anti-leishmanials should be given only for symptom relief. There are no current recommendations for maintenance treatment as more data are needed. Because of the high parasite load in the blood and skin, it is particularly important that these patients sleep under impregnated bed nets!

Since a VL patient co-infected with HIV requires special care, all VL patients should be tested for HIV. Provider initiated HIV counselling and testing should be conducted for all VL patients.
6.0 Disease Surveillance and Epidemic Response

6.1 Disease Surveillance
Surveillance is defined as the systematic collection, analysis, and interpretation of health data, and includes timely dissemination of the data for action. Surveillance can be summarized as collecting information for action. It is a key component of Kala-azar control as it helps to assess the annual trends, disease burden and cost-effectiveness of interventions. VL surveillance includes reporting of all cases of Kala-azar and PKDL. To make disease surveillance effective, it is necessary to establish a system for regular reporting on the disease. This should be within health facilities and linked to higher authorities to facilitate and rationalize the planning, implementation and evaluation of control measures.

6.2 Health Facilities as Reporting Units
All health facilities within the endemic districts should report any VL cases seen. The health facilities without diagnostic capacity should report suspected VL cases and refer the cases to the facilities with diagnostic capabilities. The facilities with diagnostic capacity should report all suspected cases seen (at the facility plus those referred from other facilities) as well as the confirmed cases. The confirmed cases should then be referred to the treatment center for further management. Appropriate tools for registering and reporting cases have been developed (See Annexes 12 outpatient reporting tool and outpatient register). These forms are to be filled in and submitted to the district monthly, by the 7th of the next month. The district biostatisticians should enter the data into HMIS/DHIS2 platform and share the national with the national level. At the national level, the National Surveillance Officers should access the data, analyze and interpret it, and transmit it to the relevant authority in the country and national level for action.

In health facilities that are treatment centers and have internet facilities, the data on every VL case should be recorded in the in-patient register (see Annex 13) and then entered in the “Individual Tracker” DHIS platform. The data will be transmitted to the district level for validation and then to the National level for analysis, use in planning interventions, and sharing with WHO and other partners.

The advantage of using a dedicated software e.g. the HMIS/DHIS 2 is that surveillance officers and authorities at district and national level would have access to the data in real-time, as soon as the data are entered. Data entered at health facility using the individual tracker would allow data analysis and producing reports in a very easy and standardized manner.

6.3 Report Review and Feedback
The country surveillance officers and VL focal person, after compilation of the monthly report, should submit it to the National Leishmaniasis Control Programme for review and use. The programme will review the report and take any follow up actions needed. The National VL Programme should compile the monthly reports from the different districts and develop a quarterly report to present to the National NTD Technical Committee on a quarterly basis. The National VL Programme should provide feedback to the districts reporting about their data and key actions the district needs to take; ensuring that the District Health Officer and District Surveillance Officer are all copied on the feedback messages. The district surveillance officer should provide regular feedback (verbal and written feedback) to the health facilities. Review and feedback should be taken seriously at all levels and recommended actions should be executed. A supervisory visit should be made to mentor health workers and follow up recommended actions.
6.4 Surveillance of Kala-azar
Surveillance comprises passive and active surveillance of cases as well as vector surveillance. Integration of pharmacovigilance and treatment outcome monitoring should also be part of the routine surveillance.

6.4.1 Passive Case Surveillance
This is the main method used and is health-facility based. This means timely, regular, and accurate reporting of patients who seek diagnosis and treatment in the health facilities. Patient cards should be the starting point for passive surveillance. All relevant information in the card should be entered in the case and laboratory reporting tools that have been provided by the Programme. The main disadvantage of passive surveillance is that it is a slow process as one has to wait for the patients to report to the health facility. This type of surveillance misses the patients that do not report to the health facilities.

6.4.2 Active Case Surveillance
Active surveillance implies active search for cases. Health workers and community health workers/volunteers are oriented in the process of active case search through campaigns and house-to-house visits. During active surveillance health workers/community health workers visit the households to detect cases of fever of more than 2 weeks, splenomegaly, or abdominal pain and screen them with rK39 diagnostic test kit and the malaria RDT. Those who test positive on rK39 and negative for malaria should be referred to the nearest treatment center for treatment of Kala-azar. The health facility should ensure there are adequate materials for the diagnosis and treatment of VL cases. The health facility team should also organize to provide services or refer patients who may not be suffering from Kala-azar. This process is disadvantaged by the unavailability of funds to facilitate movement of the health teams to communities.

6.4.3 Vector Surveillance
In most endemic areas, VL is characterized by a patchy distribution with discrete transmission foci. This focal distribution of VL transmission sites is due to micro-ecological conditions that affect vector dynamics (density, parity, infectivity rate, feeding and resting behaviors). An increase in vector density will directly result in an increase in parasite transmission. Thus frequent monitoring of vectors will give a trend of their occurrence over a particular period. These activities would allow establishing a vector population density threshold above which control measures should be put in place or strengthened in order to reduce transmission.

6.4.3.1 Methods of Vector Surveillance
Vector surveillance requires sampling of sandflies frequently using uniform trapping methods as certain species may only appear at certain times of the year. Sand fly species densities are influenced by ecological factors such as climatic conditions, land cover, vegetation, and rainfall. For sand fly trapping, it is important to use several collection methods to minimize biases in individual techniques as well as to allow sampling of populations with diverse behavioral characteristics from various habitats. Some of the sampling methods used for sand fly collection are:

1) Human-landing catches
2) Aspirations
3) Pyrethrum spray catches
4) Light trap catches
5) CO₂ baited traps
6) Sticky paper traps

These methods can be used under different settings that include indoors and outdoors.

6.5 Disease Outbreak Response

When more than expected number of cases of VL is reported in a given area, the situation should be investigated and verified. Weekly data should be collected and filled in the right reporting form. An outbreak should be declared and a prompt response should launched if cases are confirmed. An outbreak may be restricted to one village or location and rarely is the whole sub-county affected. Some endemic foci can erupt into epidemics, or new foci can appear where VL has not previously been reported.

6.5.1 Detection

Detection and confirmation of an outbreak depend on accurate surveillance data that show an increase in the number of confirmed VL cases, more than expected. Outbreaks may be detected by the existence of large numbers of cases, health clinic attendances, admissions to hospitals, deaths or media reports.

6.5.2 Confirmation

When an outbreak is suspected:

- A preliminary case investigation must be carried out to confirm diagnosis, assess the extent of the outbreak, and identify population at risk. This is best done by health workers using a standard outbreak form, seeking details on the case (see outbreak form)
- The outbreak is confirmed by comparing current and previous incidence of the disease, while allowing for seasonal variation, potential changes in completeness of reporting due to alteration of local conditions e.g. insecurity affecting access to health facilities.

6.5.3 Response

- The health worker using the outbreak form the District Surveillance Officer and the District Health Officer. The District Health Office should inform the national level (ESD and NTD Programmes) about the outbreak.
- The District Rapid Response Team (DRRT) should be constituted to address and implement the various response activities. The District Epidemic Response Committee and the National Task Force for Epidemic Response should be activated to coordinate outbreak response at district and national level respectively. These should mobilize the resources needed for effective response including:
  - Drugs for treatment of VL and management of opportunistic infections
  - Rapid Diagnostic Tests
  - Laboratory and parasitological diagnostic equipment and reagents
  - Weekly surveillance materials
  - Transport logistics
  - Human resources
  - Set up of emergency diagnostic and treatment centre (s).
o Training on case identification and management
o Identification of risk factors for the outbreak (e.g. increase in sand fly population, malnutrition etc.)

- Health workers in the affected area should be oriented and provided with appropriate training to equip them with the necessary skills to detect, diagnose, manage, and report VL cases.
- Work with peripheral health facilities and communities in identifying and referring clinically suspect cases to a VL diagnostic center and treatment center for confirmatory testing and treatment.
- When there is high mortality from VL, active case search in the areas where the victims came from should be conducted, as a high case fatality rate is a result of advanced disease and indicates that patients had difficulty or delay in accessing treatment.
- Provide information to all levels (health centers, hospitals, and communities) in messages that:
  o Contain clear, simple instruction to the population at risk to consult a health facility at an early stage of the disease
  o Indicate the locations of the diagnostic and treatment centers
  o Highlight epidemiological data and practical measures for prevention.
- Community engagement for awareness creation (media, community leaders etc.), early detection and treatment through understanding of signs and symptoms, and location of treatment centers. Community engagement is key in creating sustainability of the activities for the control of the outbreak
- Mobilize resources, if available, from international technical expertise to assist where needed, ensuring multi-sectoral and multi-partner involvement.

6.5.4 Post Outbreak

After an outbreak, the District Epidemic Management Committee should carry out a thorough evaluation of the following:

- Cause and magnitude of the outbreak
- Epidemiological characteristics of the outbreak
- Risk factors for the outbreak
- Surveillance of VL and detection of the outbreak
- Preparedness for the outbreak
- Management of the outbreak – the measures taken to coordinate the response and to control the outbreak

The findings of this evaluation should be documented in a written report containing clear recommendations regarding:

- Enhancing VL surveillance in the affected and other endemic areas
- Improving preparedness for VL outbreak
- Improving outbreak response in future.

Ongoing surveillance should be instituted or strengthened in endemic sub-counties using the monthly reporting tools to detect any changes in trends of the disease incidence.
7.0 Prevention and control of Visceral Leishmaniasis

Leishmaniasis control is primarily based on finding and treating cases; where feasible vector control can be undertaken, and in some zoonotic foci, control of animal reservoirs. However, there is no single tool or approach that is appropriate for controlling vectors in all situations due to variations in geography, ecology, human and vector behaviour.

7.1 Vector Control Interventions

The main vector control methods available are the following:

7.1.1 Use of Long lasting Insecticide Treated Nets (LLINs)

Long lasting insecticide treated nets (LLINs) are currently used as the most available and practical method to reduce human-vector contact. LLINs form a physical protective barrier around people sleeping under them. LLINs are most effective where the sand flies tend to bite people from inside houses. The insecticides impregnated in the LLINs kill sand flies, mosquitoes and other insects. The pyrethroids also repel sand flies reducing the number of sand flies that enter the house and attempt to feed on people inside. In addition, if high community coverage with LLINs is achieved, the number of sand flies and their survival time will be reduced. When this happens, the members of the community are protected regardless of whether they are using an LLIN or not. To achieve such effects, more than half of the people in a community must use an LLIN. The use of LLINs for malaria has been shown to reduce VL transmission in areas where the 2 diseases coexist.

7.1.2 Indoor Residual Spraying

Insecticide play an important role in control of sand flies, especially in domestic and peri-domestic transmission situations. Spraying the internal walls of appropriate houses is a cost-effective way to control endophillic vectors and can have a long lasting effect depending on the insecticide used, the surface treated, the dosage and method of application. Moreover the spraying coverage has to be high as low coverage may contribute to insecticide resistance. If vectors involved are partly exophillic or peri-domestic, outer surfaces of the shelter and other potential outdoor resting sites could be sprayed. Community involvement should be ensured during such an exercise.

7.1.3 Personal Protection

Indoor protection from sand flies can be obtained by use of fine-mesh screens on windows and doors, insecticide treated curtains, mosquito coils, and burning of traditional leaves known to be sand fly repellents. Individual protection measures in outside areas include application of repellents to skin or clothing to reduce human-vector contact, and wearing of appropriate clothing to minimize areas of exposed skin. People living in or traveling to VL endemic areas should wear long-sleeved shirts, long trousers, boots, and hats. Tucking in shirts, tucking trousers into socks, and wearing closed shoes instead of sandals may also reduce risk.
7.1.4 Environmental Management
Modification of the physical environment may have dramatic effect on the relative abundance of the sand flies and on the levels of transmission. House structure improvement by sealing open spaces may greatly minimize the entry and resting sites for sand flies. The clearing of vegetation around settlements and villages may effectively reduce or eliminate vector-human contact and disease transmission. Similarly, destruction of animal burrows and inactive termite hills near households may also reduce significantly the sand fly population. This can be achieved through community involvement in rural areas.

7.1.5 Integrated Vector Management
In most situations, no single effective measure to reduce transmission is guaranteed. Integrated vector management (IVM) entails the utilization of all appropriate technological and management techniques to bring about an effective degree of vector suppression in a cost-effective manner. The complementary or synergistic effect of two or more methods always gives a better result in vector control. IVM involves the use of a range of locally appropriate and effective vector control interventions, often in combination, to reduce or interrupt transmission. It is important that the selection of control methods be based on knowledge of local vector biology, disease transmission, ecological, environmental, safety, and cost-benefit consideration. IVM has an advantage in that it can be implemented through community participation. Partnership and collaboration with various stakeholders and other vector-borne disease control programmes are key in implementing IVM. Nevertheless, continuous research is needed to guide planning and implementation of vector control activities, especially those aimed at IVM.

Vector control is rarely carried out as a specific approach to Leishmaniasis control, and cost-effectiveness estimates are not available. Generally, strategies for vector and reservoir control need to be deployed depending on the local context. In foci of peri-domestic or intra-domestic transmission, indoor residual spraying may be used. Individual protection using Long Lasting Insecticide treated Nets could also be useful in such areas.

In general, domestic and peridomestic sand fly vectors are more susceptible to indoor residual spraying than are other domestic vectors, such as anopheline mosquitoes or triatomine bugs, so that transient suppression of sand fly populations is seen as an additional benefit of malaria or Chaga’s disease vector control in areas where these vectors coincide (26). However, LLINs which are becoming widely deployed against malaria transmission, may also be cost-effective for reducing Leishmaniasis in areas of domestic transmission.

7.2 Other VL Prevention Measures

7.2.1 Health Education
Health education is an essential component in any disease control programme. In VL control, the main targets are the health personnel in the local health facilities, the community health workers/volunteers, and the local community at risk. The success of the Leishmaniasis control programme in any endemic area will depend on the local community who should support and won the control activities for their long-term use and sustainability.
7.2.2 Community Awareness and Mobilization
Communities living in endemic areas should be mobilized and sensitized on the global burden of leishmaniasis. Individuals should be able to recognize the disease signs and symptoms as well as prevention and control measures. Communities should be made aware of health services for disease prevention and case management.

7.3 Role of Community Health Workers in Prevention and Control of Leishmaniasis
Community health workers or volunteers can participate in the control of Leishmaniasis using the IVM strategy by educating members to take the following steps:

1. All people living in leishmaniasis endemic communities to sleep under a LLIN to prevent human–vector contact.
2. Use of long sleeved shirts/bloses and long trousers when outside the house in the evenings
3. Spray appropriate houses with insecticide to reduce on sand fly population
4. Apply insect repellents on exposed parts of their body when outside
5. Avoid sand fly breeding and resting sites especially for the boys when grazing animals
6. Repair cracks in walls of houses to minimize entry and resting sites of sand flies
7. Clear vegetation around the homestead to reduce on resting sites for sand flies
8. Destroy inactive termite hills and animal burrows around homesteads to reduce breeding sites for sand flies
9. Recognize early symptoms and signs of leishmaniasis and seek health services as soon as possible (early diagnosis and treatment).

Before embarking on any control strategy, it is important to discuss the implications with the community and the leaders. It is also crucial to have baseline data and to monitor and evaluate control interventions to later assess the extent to which they have to be maintained or modified.

7.4 Prevention and control of Visceral Leishmaniasis in Uganda
To date, no preventive strategy for VL has been deployed in Uganda. This is partly due to the limited information on vector behaviour and risk factors, which made it unclear what potential interventions are appropriate in the given context. An entomological study conducted by MSF and LSHTM in 2004, showed that termite mounds are important vector breeding and resting sites and that humans may be protected from transmission when in close proximity to livestock due to diversion of sand flies from humans to domestic animals. However another suggestion was that the practice of sitting on termite mounds (resting sites) while guarding livestock results in an increased risk of infection. The study also indicated that other human behaviour such as lighting fires indoors and building household structures with wood or grass rather than mud, may reduce the risk of contracting of VL. Although such entomological studies revealed vital information, they were limited to only Amudat district. Given the wider geographical spread of VL in the entire Karamoja and neighboring Teso regions, more comprehensive entomological surveys should be conducted to guide evidence based interventions. Entomological studies should be conducted to provide the following information:
• The vector species involved in VL transmission
• Vector and seasonal abundance
• Biology of the sand fly vector (biting behavior, breeding habitats and peak of biting activity)
• Possible animal reservoirs (through doing blood meal analysis to rule out possibility of zoophilic nature)
• Confirm anthroponotic transmission.
• The benefits of the use of ITNs in the control of VL and modernization of Agriculture in the endemic region

Furthermore entomological surveillance (pre and post spraying) should be conducted as well as monitoring insecticide resistance in all the communities implementing indoor residual spraying.

There is also need to design Information Education and communication (IEC) materials for use in health education and community mobilization. For example, IEC materials are needed on:

• Mode of transmission of Leishmaniasis
• Appropriate usage of LLINs
• -The danger of sleeping outside without being protected by a mosquito net
• The signs and symptoms of Leishmaniasis
• The availability and location of diagnostic and treatment centers aiming at improving early reporting of symptomatic cases

In addition to passive case detection, there is need to carry out active case detection using any one of the following methods:

• Blanket approach
• Camp approach
• Index case based approach

Epidemiological mapping of the entire Karamoja region should be using rK39 kits to determine:

• Magnitude and disease distribution
• Population characteristics of the most at risk age group
• Pre-disposing factors

We should also increase awareness of VL among the population and advocate for more support at all levels:

• Advocacy at international and national level through Social services committee of the Parliament
• Advocacy and sensitization of district and religious leaders
• Advocate for financial support of VL activities in the district local Government budgets
• Sensitization of Sub-county leaders
• Increase support through use of more IEC materials such as radio talk shows about VL, use of Megaphones, flipcharts, leaflets in local languages
• Hold bilateral meetings to strengthen cross border collaboration for the control of VL
8.0 References

6. Final Report of MSF Switzerland’s Kala Azar Project in Amudat Hospital, Uganda 2007 (in preparation)
8. Stevenson J, Kala azar entomology study final report: Household-level risk factor for Phlebotomus martini, vector of kala azar, and Anopheles vectors of malaria in Pokot County, Uganda.2004


Annexes

Annex 1: Medical Form for Patients diagnosed and treated for VL

A. UNIQUE IDENTIFICATION

Name: __________________________ Sex: M / F Age (y): ____

Date of admission: ___/___/___

Current place of stay: Village: ____________ Subloc../Parish: ____________
HISTORY

Duration of sickness (months) (time elapsed between onset of symptoms and treatment or diagnosis): ______

Patient pregnant: yes / no / unknown

<table>
<thead>
<tr>
<th>Signs and Symptoms</th>
<th>Y</th>
<th>N</th>
<th></th>
<th>Y</th>
<th>N</th>
<th></th>
<th>Y</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose bleeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal swelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of appetite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Previous Kala Azar treatment: Y / N / unknown
If Y: Location __________ Drug ___________ complete / incomplete
Date (month/year): _________/_____ Previous pt number: ________

Remarks: _______________________________________________________

PHYSICAL EXAMINATION

Weight (kg): _______ Height (cm): _______

Temperature (°C): _______ Came walking: Y / N / Unknown

Oedema: Y / N / Unknown Jaundice: Y / N / Unknown

Spleen size (cm): _______ Lymph node enlargement: Y / N / Unknown
Skin signs consistent with PKDL: Y / N

Severity score/grade/status (because it is one of the criteria to use 2nd line treatment

LABORATORY (ADMISSION)

DAT: DAT no: __________ result (titre): 1: _______
Dipstick Opti-Leish (rK39): date: __________ result: pos / neg / inconclusive / not done

Lymph node aspiration: date: __________ result: pos / neg / not done
Spleen puncture: date: __________ result: pos / neg / not done
Bone marrow aspiration: date: __________ result: pos/ neg / not done

Complication: Y / N; if Y: ______________

Hb (g/dl): date: __________ result: ______
Malaria smear: date: __________ result: pos / neg
Sputum/pus AFB staining: date: __________ result: pos / neg
Serology Brucellosis: date: __________ result (titre): ______
HIV: date: __________ Test 1 result: pos / neg Test 2 result: pos / neg
Other test(s): __________________________________________________

DIAGNOSIS

☐ KALA-AZAR ☐ Serologically proven
☐ Parasitologically proven
☐ Clinically suspected

☐ PKDL
☐ Other diagnosis: 1. _______________________ certain / probable / possible
2. _______________________ certain / probable / possible
3. _______________________ certain / probable / possible

Remarks: _______________________________________________________

TREATMENT # 1
Drug: □ Pentostam □ generic SSG □ liposomal Ampho B □ Ampho B deoxycholate □ liposomal Ampho B

□ Other: _______________________________

Date of start: __/__/__  daily dose (ml): _______  number of doses: ____

Complications during treatment: Y / N; if Y, describe:

________________________________________________________________

OUTCOME (AT END OF TREATMENT #1)
Persistent fever: Y / N  Weight (kg) ________  Hb (g/dl): __________  spleen size (cm) ________
TOC 1: not done / neg / pos (intensity: _______)
Issue of treatment # 1: □ Alive, treatment completed (clinical recovery)
□ Alive, treatment completed (no clinical recovery)
□ Alive, treatment not completed
□ Alive, treatment not started
□ Died, treatment completed
□ Died, treatment not completed
□ Died, treatment not started
□ Died, due to VL
□ Died, due to concomitant disease
□ Died, due to treatment (iatrogenic)
□ Died, due to accident
□ Died, unknown reason
□ Unknown

If dead, cause of death: _______________________________

TREATMENT # 2
Drug: □ Pentostam □ generic SSG □ Ampho B
□ Other: _______________________________

Date of start: __/__/__  daily dose (ml): _______  number of doses: ____
Complications during treatment: Y / N; if Y, describe: _______________________________

TOC 2: not done / neg / pos (intensity: ______)

Issue of treatment # 2: □ Alive, treatment completed (clinical recovery)
□ Alive, treatment completed (no clinical recovery)
□ Alive, treatment not completed
□ Alive, treatment not started
□ Died, treatment completed
□ Died, treatment not completed
□ Died, treatment not started
□ Unknown

If dead, cause of death: ______________________________

Date of discharge: ___ / ___ / ____ Date of expected f-up: ___ / ___ / ____

Remarks: _______________________________________________________

43
FOLLOW-UP:

Date: ___ / ___ / ___
Follow-up number: 1 - 2 - 3 - 4

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Y</th>
<th>N</th>
<th>Y</th>
<th>N</th>
<th>Y</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose bleeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal swelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of appetite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Weight (kg): _____**
- **Temperature (°C): _____**
- **Oedema: Y / N / Unknown**
- **Jaundice: Y / N / Unknown**
- **Spleen size (cm): _____**
- **Lymph node enlargement: Y / N / Unknown**
- **Skin signs consistent with PKDL: Y / N**
- **Hb (g/dl):**
  - date: ________
  - result: ________
- **Clinical suspicion of relapse:** Y / N
- **Spleen puncture:**
  - done / not done
  - date: ___/___/___
  - result: pos / neg
  - Complication: Y / N; if Y: ____________________
- **Lymph node puncture:**
  - done / not done
  - date: ___/___/___
  - result: pos / neg

- **Follow-up outcome:**
  - ☐ Well, no sign of kala-azar
  - ☐ Unwell, no sign of kala-azar; other diagnosis: ________ certain / probable
  - ☐ Kala-azar relapse
  - ☐ PKDL
  - ☐ Died, medically related to Kala-azar
  - ☐ Died, medically not related to kala-azar: cause: ____________________
  - ☐ Did not return to follow-up
  - ☐ Unknown

Annex 2: Spleen Aspiration Procedures

1. **Materials needed**
   - 5 ml syringes and needle (1¼-inch x 21-gauge/32 x 0.8 mm)
   - Clean microscope slides
   - Wooden applicator or tooth picks
   - Spirit lamp with sufficient flame
   - Drapes (clean and sterile if available)
   - Sterile gloves
   - Sterile cotton wool and gauze
Plaster
• Labels
• Pen and pencil/marker
• Antiseptic solution (iodine tincture or alcohol swab)

2. Pre-operative procedures
Patient is physically examined and the contraindications excluded (see section 2.3 for details):

3. Aspiration procedures
1. Label a slide with the patient’s reference number using a diamond pencil and clean the slide with gauze or dry cotton wool.
2. Put on sterile gloves and cover the aspiration site with sterile drapes
3. Palpate the spleen and outline its margins on the patient’s abdomen. A pencil may be used to mark the margins. Alternatively a sterile drape may be used to cover the aspiration site.
4. Clean the skin at site of aspiration with alcohol swab and allow to dry [site of aspiration is 2–4 cms below the costal margin at the mid-line of the spleen on the anterior surface]
5. With the needle attached to the syringe, penetrate the skin mid-way between the edges of the spleen
6. Aim the needle cranially at an angle of 45° to the abdominal wall
7. While in the skin, create a vacuum up to 1.0 cc mark of the syringe by pulling the plunger
8. While maintaining a 1.0 cc vacuum, order the patient to stop breathing and during inspiration push the needle to its full length (3 cm) into the spleen and pull out completely with a quick in-and-out movement (less than one second)

N.B.
• Carry out the procedure using the same landmarks, angles and suction
• The axes of entry and exit of the aspirating needle should be identical
• Maintain suction throughout the procedure
• Carry the aspiration in a single-step procedure all in one quick motion (about one second)
• In restless children, arms should be folded across chest and held with an assistant in addition to the pelvis, which should also be held firmly by a second assistant
• The insertion of the needle should be timed with the patient’s breathing so that the diaphragm is not moving, i.e., during fixed expiration in a crying child
• Once the aspirating needle is withdrawn, pull the plunger slowly to the 2–3 cc mark [Note that only minute amounts of splenic material is visible in the syringe, lumen of the needle or end of the plunger]
9. The material can then be expelled on to clean slides
   For smears, expel any remaining material gently on clean glass slides holding tip of needle on the surface of slide, and spread evenly into a smear immediately 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 needle. Tooth picks or wooden applicators may be used.
10. Slides can be stained with Leishman, Giemsa or Wright’s stain.
4. **Post-operative procedures:**

1. Clean the puncture site with antiseptic soaked sterile cotton wool and apply strapping on it.
2. Record time of aspiration on the patient’s chart.
3. Record pulse and blood pressure ½ hourly for 2 hours and hourly for 6 hours.
4. Patient should remain in bed for at least 8 hours and observed.

**N.B.**

- Facilities for blood transfusion and surgical intervention are may be needed during splenic aspiration procedures.
- Experience shows that risk of mortality from aspiration procedure may happen 1 in 1000 cases aspirated
Annex 3: Checklist before Performing Spleen Aspiration

- Spleen palpable: NO → Spleen puncture contraindicated
- Pregnant woman or amenorrhea > 24: YES → Spleen puncture contraindicated
- Very bad general condition: NO → NO → Spleen puncture contraindicated
- Active bleeding: NO → NO → Spleen puncture contraindicated
- Anemia (Hb < 5.5 g/l): YES → Spleen puncture contraindicated
- Jaundice: NO → NO → Spleen puncture contraindicated
- Advanced HIV disease: YES → Spleen puncture contraindicated
- NO or unknown

SPLEEN ASPIRATION ALLOWED
Annex 4: Monitoring of the patient after spleen aspiration

<table>
<thead>
<tr>
<th>Time</th>
<th>Blood pressure (mmHg)</th>
<th>Heart rate (bpm)</th>
<th>Symptom(s) or sign(s)</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 0 (before procedure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 1 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 1 ½ h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 2 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 3 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 4 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 5 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Increase of heart rate equal or more than 20/minutes**

**Drop of blood pressure equal or more than 15 mm Hg**

**Patient complains of**
- Abdominal pain
- Dizziness, sweating

**IMMEDIATELY CALL THE CLINICAL OFFICER OR THE MEDICAL DOCTOR**

Annex 5: Lymph Node Aspiration Procedures

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

**2. Pre-operative procedures**
No specific procedures are needed, except that inguinal or epitrochlear lymph nodes should be palpable.

**3. Aspiration sites**
The inguinal and epitrochlear lymph nodes are most convenient for the procedure.
4. **Aspiration procedures**

1. Label a slide with the patients’ reference number using a diamond pencil and clean the slide with gauze or dry cotton wool.
2. Rest the patient on the back with the legs stretched out. Another person can hold down the patient if he/she is restless. If it is a small child, the mother can hold him/her on her laps.
3. Put on sterile gloves, cover the aspiration site with sterile drapes, and disinfect the skin over the lymph node with cotton wool soaked in 70% alcohol or iodine and allow to dry.
4. Grasp the lymph node between the thumb and index finger of the left hand and insert a sterile 21-gauge needle attached with a 5 ml syringe into the center of the gland at right angles to the skin. Avoid adjacent blood vessels.
5. Gently squeeze the node with the left hand and twirl the needle in the right hand, at the same time pushing the needle in and out, and pull the plunger to maintain suction [This may be done for a few minutes, until some tissue is visible at the end of the lumen of the needle]

**N.B.**
The procedure may be painful, but tolerable (no anaesthesia is needed)
- Big lymph nodes may fill the needle with lymph and dilute the tissue; and so smaller but palpable lymph nodes are preferred.
- Lymph nodes might be difficult to grasp by two fingers, hence caution should be made not to sample the surrounding fatty tissues. Palpable lymph nodes (size more than 1x1 cm) are usually felt in the inguinal, femoral and epitrochlear regions.
6. The material can then be expelled on to a clean glass slide for microscopical examination.

**N.B.**
For smears, expel any remaining material gently on clean glass slides holding tip of needle on the surface of slide, and spread evenly into a smear immediately 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 needle. Tooth picks or wooden applicators may be used for this purpose.
7. Slides can be stained with Leishman, Giemsa or Wright’s stain (See Annex 3).

5. **Post-operative procedures**

No specific procedures are needed
Annex 6: Preparation and Staining of Aspirates

1. Materials needed
Slides rack, staining rack or staining trough, 100% methanol, filtered stock of Giemsa stain and glass slides. It is best to use day fresh Giemsa staining rather than filtering a stock.

2. Fixation
- Place the slides horizontally on the slide 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.

3. Staining
- Stain the slides with Giemsa stain 1:10 concentration; 1 ml of stock Giemsa stain to 9 ml buffer solution pH 7.2. In the absence of buffer solution, tap, rain or filtered water can be used provided the pH is 7.2. The slides can either be stained in a staining trough or on a staining rack. When the stain concentration is 1:10 the staining time is 10 minutes.
- At the end of the staining rinse the slides briefly with tap water or filtered water and place them in a vertical position on a slides rack to dry.

4. Reading Slides
Examine at least 1000 microscopic fields for amastigotes using an x100 oil immersion lens. Dispersed platelets and artefacts (i.e., Giemsa precipitates) are more likely to be taken for a parasite if the microscopists are overloaded (more than 4 hours of microscopy per day), have poor microscope light, or if dirty (unfiltered) Giemsa is used. It takes at least twenty minutes or 1000 microscopic fields of reading to label a sample smear as negative.

5. Grading of Parasite Loads

<table>
<thead>
<tr>
<th>Average parasite density</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 in 1000 microscopic fields</td>
<td>0 (negative)</td>
</tr>
<tr>
<td>1-10 parasites in 1000 fields</td>
<td>1 +</td>
</tr>
<tr>
<td>1-10 parasites in 100 fields</td>
<td>2 +</td>
</tr>
<tr>
<td>1-10 parasites in 10 fields</td>
<td>3 +</td>
</tr>
<tr>
<td>1-10 parasites per field</td>
<td>4 +</td>
</tr>
<tr>
<td>10-100 parasites per field</td>
<td>5 +</td>
</tr>
<tr>
<td>&gt; 100 parasites per field</td>
<td>6 +</td>
</tr>
</tbody>
</table>

Annex 7: Procedure for Bone Marrow Aspiration

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 with ink or thumb nail pressure
3. Using sterile technique, prepare the skin with anti-septics and drape
4. Using sterile syringe, apply/infiltrate the marked area with anaesthetic 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 in 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 presence/absence of marrow material. If not, proceed gently to bore until marrow is found at the tip of the stylet.
11. Using a 10cc syringe, locked into the proximal portion of the bone marrow needle aspirate for bone marrow material.
12. The material can then be expelled onto a clean slide. Presence of bone marrow material can be detected as granules on a glass slide.

Annex 8: Direct Agglutination Test (DAT)

1. Principles
   Blood can be collected from by finger prick and blotted on to filter paper (Whatman No.3) or from a peripheral vein to obtain serum. Serial dilutions of the patients blood samples eluted from filter paper, or directly from serum, are incubated with Leishmania antigen in V-shaped bottom micro titre plates. The plates are incubated at room temperature for 8-12 hours and then read visually. If no anti-Leishmania antibodies are present, the antigen will sediment to the bottom of the well and form a small sharp blue dot. If anti-Leishmania antibodies are present in the blood, they will react with the antigen and the agglutination will be visible as a mat, a dot with frayed edges or an enlarged dot.

Collection of Blood Samples - Day One

2. Materials Needed
   Filter paper (Whatman’s No.3), sterile blood lancets or needles, syringes, test tubes, gloves, registration book, scissors, hard card, clips, disinfectant, cotton wool and plastic dispensing bags

3. Procedure for Collection of Peripheral Venous Blood for Serum Samples
   This is a standard venipuncture procedure. Three ml of whole blood should permit collection of serum sufficient to test and re-test the sample.
4. Procedure for Collection of Blood on Filter Paper
   - Cut circles or rectangles of filter paper into small pieces enough to make two punch outs
   - Write the patient’s number (DAT number) on the piece of filter paper
   - Take blood from the patient by finger pricking
   - Blot blood on the filter paper. Make sure the blood is soaked through the filter paper
   - Fix the blood sample with a clip on the hard card and leave to air dry thoroughly
   - Place the samples in plastic bags and store cool and dry if they are to be stored or transported.

Elution of Blood Samples from Filter Paper

5. Materials Needed
   DAT registration book, 5 mm paper puncher, microtitration plates, normal saline, multipipette (Eppendorf repeater), and Combitip 1.25 ml

6. Procedure:
   - Make a list of samples, and label the positions of the samples in the microtiter plates and in the DAT book
   - Punch out 5 mm from the filter paper leaving enough material for another punch
   - Place the punches of filter paper (in order of registration) in the wells of the f column 2 of the microtiter plates. The punches of filter paper blood may need to be folded to fit into the well.
   - Reserve column 1 for buffer control
   - Add 125 µl of normal saline to the filter paper samples immersed in column 2 (multipipette dial at 5 + Combitip 1.25 ml)
   - Cover the plates with another microtitration plate and incubate overnight for at least 8 hours in the fridge at 4°C

Setting the DAT Test from Filter Paper Elutes or Serum Samples

7. Materials needed
   Measuring cylinder, multipipette, multi-channel pipette, research pipette, standard tips 100 µl (yellow tips), standard tips 1 000µl (blue tips), Combitip 2.5 ml, absorbent paper, normal saline, DAT antigen aqueous or Freeze Dried Antigen (FDA), 2-mercaptoethanol, Foetal Calf Serum (FCS) or gelatin, freeze-dried control sera, 5 ml syringe.

8. Preparation of Diluent
   - Take the antigen, FCS and DAT plates out of the fridge before handling them
   - Mix 50 ml of normal saline with 500 µl (0.5 ml) of FCS and 390 µl of 2-mercaptoethanol or mix 50 ml of normal saline with 0.1 g gelatin and heat in a water bath for 10 minutes; leave to cool to room temperature and add 390 µl of 2-ME (research pipette adjusted to 390µl)

NB. Avoid adding FCS or gelatin, if you are running DAT with FDA, which has FCS, incorporated. The solutions are stable for 24 hours when stored at 4°C.
9. Reconstitution of Freeze Dried Antigen
- Add 5 ml of fresh normal saline to the vial of antigen and mix gently by rotation, **DO NOT SHAKE**!
- Leave the antigen for a minimum of 10 minutes before use

10. Reconstitution of Freeze-Dried Serum Controls
- Use different control sera every time a new batch of DAT antigen is used
- Make sure all the freeze-dried material is on the bottom of the vial. This usually contains 2 µl of serum (Please refer to the inserts of the manufacturer)
- Either add 100 µl of normal saline or diluent (multipipette dial on 2, Combitip 2.5 ml), or add 200 µl of normal saline or diluent (multipipette dial on 4, Combitip 2.5 ml) depending on the instruction of the manufacturer. Mix gently. These make 1:50 and 1:100 dilutions respectively
- Leave for at least 10 minutes before use
- Use high-titre positive, low-titre positive and negative control sera (usually provided in the kit)

11. Dilution of Samples
   a. Filter Paper Blood
      - Take the microtiter plates with the eluted blood out of the refrigerator (*serum dilution in column 2 is 1:50*)
      - Fill the wells in column 1 and those in columns 3-12 with 50 µl of diluent (multipipette dial on 1, Combitip 2.5 ml)
      - Adjust the multi channel pipette reading to 50 µl, and place 8 standard tips 100 µl (yellow tips) on the multi-channel pipette. Make sure the yellow tips are firmly fixed to avoid PIPE TTING ERRORS
      - Mix the contents of the wells of column 2 by pipetting in and out up to 5 times and transfer 50 µl to column 3
      - Mix again and transfer 50 µl to the next column – continue up to column 12 from which 50 µl must be discarded. Column 1 is the buffer control, it does not contain blood/serum.

   b. Sera
      - Fill the wells in column 2 with 100 µl of diluent and add 2 µl of serum (*serum dilution is 1:50*)
      - Fill the wells in column 1 and those of columns 3-12 with 50 µl of diluent (multipipette dial on 1, Combitip 2.5 ml)
      - Adjust the multi-channel pipette reading to 50 µl, and place 8 standard tips 100 µl (yellow tips) on the multi-channel pipette. Make sure the yellow tips are firmly fixed to avoid PIPE TTING ERRORS
      - Mix the contents of the wells of column 2 by pipetting in and out up to 6 times and transfer 50 µl to column 3
      - Mix again and transfer 50 µl to the next column – and continue up to column 12 from which 50 µl must be discarded. Column 1 is the buffer control, it does not contain blood/serum.

12. Adding Antigen
• Mix the bottle of antigen gently (by rotation) to resuspend the sedimented promastigotes. Mixing the antigen by shaking vigorously will damage the promastigotes
• Connect a fresh Combitip 2.5 ml to multipipette dial on 1 and fix a yellow tip on the Combitip
• Suck antigen into the Combitip and add 50 ul to every well except the wells of column 1, which contain the samples. GET RID OF AIR BUBBLES AND EXTRA ANTIGEN AT THE TIP OF THE YELLOW TIP BEFORE DISPENSING INTO THE WELLS
• Start with the wells in column 1 (*buffer control*) and then add row by row from left to right to avoid contamination
• Change yellow tips every time antigen is taken out of the bottle to avoid contamination
• Discard the Combitip at the end of the process
• Cover the V-shaped plates and rotate gently clockwise and anticlockwise a few times
• Leave the plates at room temperature on a level surface for at least 12 or 18 hours

13. Reading DAT Plates

• Put the plates against a white background and estimate the titre by comparing the dots in the control column, i.e. column 1 with those of the samples (columns 2 or 3 through 12)
• Take notice of any contamination
• Record the titre of the sample against the corresponding code numbers of patients in the DAT logbook
• Record the results in the VL registration book as well
• Collect the investigation papers from the patients and write down the titres [The titre is expressed as the last dilution that shows agglutination or a difference in size of dots compared to the buffer control]

The following are titre designations, with a starting dilution of 1:50 in column 2 of the microtitration plates:

<table>
<thead>
<tr>
<th>Columns endpoints showing endpoints</th>
<th>Titres</th>
<th>Titre designation by integer numbers</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>-</td>
<td>-</td>
<td>Buffer control</td>
</tr>
<tr>
<td>Column 2</td>
<td>1:50</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>Column 3</td>
<td>1:100</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>Column 4</td>
<td>1:200</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Column 5</td>
<td>1:400</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Column 6</td>
<td>1:800</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Column 7</td>
<td>1:1 600</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Column 8</td>
<td>1:3 200</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Column 9</td>
<td>1:6 400</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Column 10</td>
<td>1:12 800</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Column 11</td>
<td>1:25 600</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Column 12</td>
<td>1:51200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The following are titre designations, with a starting dilution of 1:100 in column 2 of the microtitration plates:

<table>
<thead>
<tr>
<th>Columns endpoints showing endpoints</th>
<th>Titres</th>
<th>Titre designation by integer numbers</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Column 2</td>
<td>1:100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Column 3</td>
<td>1:200</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Column 4</td>
<td>1:400</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Column 5</td>
<td>1:800</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Column 6</td>
<td>1:1 600</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Column 7</td>
<td>1:3 200</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Column 8</td>
<td>1:6 400</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Column 9</td>
<td>1:12 800</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Column 10</td>
<td>1:25 600</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Column 11</td>
<td>1:51 200</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Column 12</td>
<td>1:102 400</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>No end points in Column 12</td>
<td>&gt;1:102 400</td>
<td>&gt;10</td>
<td></td>
</tr>
</tbody>
</table>

**14. DAT Titre Scales and Titre Designations by Integers**

<table>
<thead>
<tr>
<th>Titres</th>
<th>Integer scales</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50</td>
<td>-1</td>
<td>No agglutination at starting dilution of 1:100</td>
</tr>
<tr>
<td>1:100</td>
<td>0</td>
<td>Agglutination at starting dilution of 1:100 (first dilution)</td>
</tr>
<tr>
<td>1:200</td>
<td>1</td>
<td>End of agglutination in the well after the starting well (second dilution)</td>
</tr>
<tr>
<td>1:400</td>
<td>2</td>
<td>End of agglutination in the well after the 2\textsuperscript{nd} dilution (third dilution)</td>
</tr>
<tr>
<td>1:800</td>
<td>3</td>
<td>End of agglutination in the well after the 3\textsuperscript{rd} dilution (fourth dilution)</td>
</tr>
<tr>
<td>1:1 600</td>
<td>4</td>
<td>End of agglutination in the well after the 4\textsuperscript{th} dilution (fifth dilution)</td>
</tr>
<tr>
<td>1:3 200</td>
<td>5</td>
<td>End of agglutination in the well after the 5\textsuperscript{th} dilution (sixth dilution)</td>
</tr>
<tr>
<td>1:6 400</td>
<td>6</td>
<td>End of agglutination in the well after the 6\textsuperscript{th} dilution (seventh dilution)</td>
</tr>
<tr>
<td>1:12 800</td>
<td>7</td>
<td>End of agglutination in the well after the 7\textsuperscript{th} dilution (eighth dilution)</td>
</tr>
<tr>
<td>1:25 600</td>
<td>8</td>
<td>End of agglutination in the well after the 8\textsuperscript{th} dilution (ninth dilution)</td>
</tr>
<tr>
<td>1:51 200</td>
<td>9</td>
<td>End of agglutination in the well after the 9\textsuperscript{th} dilution (tenth dilution)</td>
</tr>
<tr>
<td>1:102 400</td>
<td>10</td>
<td>End of agglutination in the well after the 10\textsuperscript{th} dilution (eleventh dilution)</td>
</tr>
<tr>
<td>&gt;1:102 400</td>
<td>&gt;10</td>
<td>End of agglutination in the well after the 11\textsuperscript{th} dilution (twelfth dilution)</td>
</tr>
</tbody>
</table>
15. Important Reminders about the DAT

- Due to the batch-to-batch variations of DAT antigens VL control programs should ensure that DAT antigen of acceptable quality is procured
- The DAT does not distinguish between previously treated cases of VL and active VL as the antibodies remain in the blood for a number of years (up to 10 or more years) after successful treatment. Therefore DAT is not useful for diagnosing relapses and is substituted by parasitology
- The aqueous antigen, containing formalin fixed Leishmania promastigotes, requires a cold chain
- The DAT antigen may be shock-sensitive, and shaking too much especially during transport can reduce its quality
- The DAT liquid antigen is expensive: 2500 US $ for 1 litre of Amsterdam (Royal Tropical Institute - KIT) antigen, and 2000 US $ for Antwerp (Prince Leopold Institute for Tropical Medicine) produced antigen. FDA is more expensive, 5000 US $ per litre

16. Problems Encountered with DAT, and Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Probable cause(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Low titres of controls</td>
<td>Pipetting error; check pipette (volume too small); not properly diluted, tip not tightly fitted</td>
</tr>
<tr>
<td>2. High titres of controls</td>
<td>Pipetting error; check pipette (volume too small); not properly diluted, tip not tightly fitted</td>
</tr>
<tr>
<td>3. Irregular readings</td>
<td>Contamination of positive serum with negative serum wells during dilution or adding antigen</td>
</tr>
<tr>
<td>4. Unclear readings</td>
<td>Tests are read too early</td>
</tr>
<tr>
<td>5. Auto-agglutination</td>
<td>FCS/gelatin not added to diluent; aqueous antigen too old (compare with FDA); contamination in diluent (every time rinse the container for the diluent and let dry)</td>
</tr>
<tr>
<td>6. Low or negative titres with filter paper blood</td>
<td>Problems with the storage of filter paper (humidity)</td>
</tr>
<tr>
<td>7. Blood does not elute from the filter paper</td>
<td>Filter paper stored at too high a temperature or wrong type of filter paper used</td>
</tr>
</tbody>
</table>

17. Washing the Micro-titre Plates
New plates are preferred. When there are no enough microtiter plates, used ones can be washed, but should this be done immediately after reading

18. Materials Needed

- Bleach (house hold bleach)
- Tap water or filtered water and distilled water
- Acid alcohol 3%
- Washing powder
- Wooden spatula
- Cotton wool
- Pipette (Eppendorf multipipette, Combitips 2.5 ml and 1.25 ml)
19. Procedure

- Fill all the wells with 200 µl bleach diluted 1: 5 in tap water or filtered water. Use the Eppendorf multipipette, dial at 4 and Combitip 2.5 ml. keep the Combitip for later use. Leave overnight for disinfection, in the shade **KEEP AWAY FROM THE SUN**
- Empty the plates next morning and soak them in water with some washing powder for 1-2 hours
- Then empty the plates again and fill them with 25 µl acid alcohol 3 %. Use the Eppendorf multipipette, dial at 1, Combitip 1.25 ml. Keep the Combitip for later use
- Clean all plates with cotton wool wrapped around a small wooden spatula. Be careful not to scratch the wells with the wood, a soft paintbrush will do well
- Rinse the plates 10 times with tap water or filtered water if there is no tap water available. After this also rinse 3 times with distilled water
- Let the plates dry upright against a stand
- When the plates are dry, inspect them thoroughly whether they are really clean. If not repeat steps 3, 4, 5 and 6
- Store the dry plates upside-down, in order to prevent dust settling on plates
**Annex 9: Antimonials Treatment Table**

Patient number: _______  Patient name: ______________________________

Dosage schedule: 20 mg/kg/day x 30 doses

Weight: _____ Dose (mg): _____ Dose (ml): _____

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Dose (ml)</th>
<th>Comments</th>
<th>Day</th>
<th>Date</th>
<th>Dose (ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>Hb, malaria slide</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td></td>
<td></td>
<td>Hb, Malaria slide, splenic size</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>Malaria slide</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
<td>Hb, splenic size</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td>37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **For all patients:**
  - On day 1: VITAMIN A
  - On day 1 to 3: Albendazole
  - Day 1 to 30: MULTIVITAMIN

- For patients with suspected or proven anaemia:
  - Day 1 to discharge: FERROlic (Ferrous sulphate + Folic acid)
  - Day 1 to discharge: VITAMIN C

- **Other drugs:**
  - Dose: _______ From: ____ To: ____ Reason: _________________
  - Dose: _______ From: ____ To: ____ Reason: _________________
  - Dose: _______ From: ____ To: ____ Reason: _________________
  - Dose: _______ From: ____ To: ____ Reason: _________________
  - Dose: _______ From: ____ To: ____ Reason: _________________

Side-effects of antimonials:
### Patient category: Primary KA..... PKDL........ Relapse.........

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose SSG (ml)</th>
<th>Dose PM</th>
<th>Dose AmB.</th>
<th>No</th>
<th>T°</th>
<th>DI</th>
<th>DI wb</th>
<th>Vo</th>
<th>Co</th>
<th>RD</th>
<th>BL</th>
<th>Other medicines given including ORS</th>
<th>Others specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Condition on discharge: Date: Weight: Spleen: cm
TOC result: Date: Outcome: Discharged clinically cured......... Died.......... Default.............. Transferred............
No=Day number
T°= Temperature
DI= Diarrhoea (how many times)
DI wb=Diarrhoea with blood (how many times)
Vo=Vomiting (how many times)
Others= Any other sign (e.g. Abdominal discomfort, pain/abscess at injection site etc.
RD=Respiratory distress
BL=Bleeding
Co=Coughing
Annex 10: Conventional Amphotericin B Treatment Table

Patient number: ________  Patient name: ______________________________

Dosage schedule: 1 mg/kg every other day x 15 doses

Weight (kg): _____  Dose (mg) ______

Dilution: in _____ ml of Dextrose 5 % (NEVER in Saline solution!!) given in 8 hours

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Dose (mg)</th>
<th>Comments</th>
<th>Day</th>
<th>Date</th>
<th>Dose (mg)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1</td>
<td>Hb, Malaria slide</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td></td>
<td></td>
<td>3</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td></td>
<td></td>
<td>5</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td></td>
<td></td>
<td>7</td>
<td>22</td>
<td>Malaria slide</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td></td>
<td></td>
<td>9</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td></td>
<td></td>
<td>11</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td></td>
<td></td>
<td>13</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>29</td>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
<td></td>
<td>Hb, splenic size</td>
</tr>
</tbody>
</table>

HYDRATION: correct dehydration before starting Amphotericin B then maintain hydration with ORS (or intravenous Ringer Lactate) if needed.

- **For all patients:**  Day 1: VITAMIN A and Albendazole  
  Day 1 to 30:  MULTIVITAMIN, POTASSIUM SUPPLEMENTATION

- **For patients with suspected or proven anaemia:**  
  Day 1 to discharge: FERROLIC (Ferrous sulphate + Folic acid)

- **Other drugs:**
  
  ______ Dose: ________  From: ___ To: ___  Reason: ____________
  
  ______ Dose: ________  From: ___ To: ___  Reason: ____________
  
  ______ Dose: ________  From: ___ To: ___  Reason: ____________

- **Remarks:** - avoid Gentamicin because of renal toxicity

- **Side-effects of Amphotericin B:** ______________________________

_N.B._ Give corticosteroids only if severe infusion-related side effects
**Annex 11: Overview of treatment for concurrent illnesses in VL patients**

List of common diseases in Kala Azar patients. This is slightly different than what is commonly done in health facilities because often VL patients come when they are severely malnourished and immune compromised.

<table>
<thead>
<tr>
<th>Disease</th>
<th>First line treatment</th>
<th>Second line treatment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea, bloody with fever and the patient is severely malnourished</td>
<td>Tinidazole 3-10 days plus ORS. Consider KCl for severe disease</td>
<td>Metronidazole 5-10 days plus Ciproflaxacin Ciproflaxacin for 3-5 days plus ORS</td>
<td>Tinidazole causes less vomiting than Metronidazole and is given once a day</td>
</tr>
<tr>
<td>Vomiting with nausea. This can be a side effect of SSG. You may need to stop the SSG for 2-5 days</td>
<td>Promethazine orally or IM or IV ORS</td>
<td>Metoclopramide oral or IM or IV Diazepam oral or IM or IV Hydration with ORS or by IV A combination of all the medicines may be necessary</td>
<td>You must make sure that you did not miss out any diagnosis such as malaria or meningitis. Vomiting can be a sign of serious illness especially children. Young children can easily get an overdose. Make sure older children do not give their tablets to babies The major overdose effect of promethazine and metoclopramide is a stiff neck, sometimes the tongue sticks out. Treatment with IV or rectal diazepam helps overdose</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Amoxicillin for mild to moderate pneumonia Ceftriaxone if seriously ill</td>
<td>Chloramphenicol: for serious pneumonia or treatment failure oral is as good or better than IV or IM, however it is not a good choice if the patient is severely anaemic Cotrimoxazole: for patients who are allergic Erythromycin: for patients &gt; 5 who fail Amoxicillin</td>
<td>Many Kala-Azar patients especially &lt; 5 years old have aspirations because of vomiting or a febrile seizure. Amoxicillin, Ceftriaxone and Chloramphenicol will treat aspiration pneumonia Chloramphenicol suppresses blood formation so it is not recommended for severely anaemic patients. Erythromycin can cause stomach pain or vomiting so be careful with KA patients</td>
</tr>
<tr>
<td>Malaria</td>
<td>Amodiaquine plus Artesunate</td>
<td>Quinine for blood film with positive malaria within 2 weeks of treatment with Amodiaquine plus Artesunate for serious malaria</td>
<td>All Kala Azar patients are treated for malaria within 1 week of admission. The first week of Kala Azar treatment, the patient has fever from Kala Azar so diagnosis is difficult</td>
</tr>
<tr>
<td>Ear infection</td>
<td>Amoxicillin</td>
<td>Cotrimoxazole</td>
<td>Chronic draining ears need to be</td>
</tr>
<tr>
<td>Condition</td>
<td>Treatments</td>
<td>Cleaning Notes</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>recent infection with or without draining</td>
<td>Amoxicillin plus Metronidazole. Ceftriaxone if seriously ill. Amoxicillin, Metronidazole and Gentamycin IM.</td>
<td>Saline or vinegar (half strength) are good for cleaning. Antibiotics are not useful. Gentian violet in the ear canal can be used if there is an ear canal infection with itching.</td>
<td></td>
</tr>
<tr>
<td>Jaundice</td>
<td>Vitamin K IM (for 5 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gum pain</td>
<td>Penicillin and Vitamin C</td>
<td>Penicillin plus Metronidazole for cancrum oris. Educate about good oral hygiene.</td>
<td></td>
</tr>
<tr>
<td>Wounds</td>
<td>Penicillin and Gentian violet in the ear canal can be used if there is an ear canal infection with itching.</td>
<td>Penicillin and Gentian violet in the ear canal can be used if there is an ear canal infection with itching.</td>
<td></td>
</tr>
<tr>
<td>Herpes Zoster</td>
<td>Paracetamol for 5 days minimum. Gentian violet if blisters open. Ibuprofen is first line if the patient is at the end of Kala Azar treatment. Tramadol for severe pain.</td>
<td>At the end of treatment, Ibuproxen is superior to Paracetamol for treatment as it has more pain relief than Paracetamol. Ibuprofen should not be used early in treatment because of risk of bleeding.</td>
<td></td>
</tr>
</tbody>
</table>
| Eye pain                        | Tetracycline Eye Ointment until 2 days after symptoms have gone. For 1 month if there is trachoma. Vitamin A, if not given, may need 3 doses if severely malnourished. Gentamycin or Chloramphenicol eye drops. Use first line for excessive pus. | Pain
| Pain                            | Paracetamol for severe pain                                                 | No aspirin as it increases the risk of bleeding. Ibuprofen may also increase the risk of bleeding. |
### Annex 12: HMIS FORM 128a: VISCERAL LEISHMANIASIS OUTPATIENT REGISTER

<table>
<thead>
<tr>
<th>SERIAL NUMBER</th>
<th>NAME OF PATIENT</th>
<th>NATIONALITY (N/F)</th>
<th>AGE</th>
<th>MUAC</th>
<th>BMI</th>
<th>BLOOD PRESSURE</th>
<th>NEXT OF KIN</th>
<th>PHONE NO</th>
<th>TICK CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIRST NAME</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PRIMARY</td>
</tr>
<tr>
<td>SURNAME</td>
<td>VILLAGE</td>
<td>PARISH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RELAPSE</td>
</tr>
<tr>
<td></td>
<td>SUBCOUNTY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UNSPECIFIED</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WEIGHT</th>
<th>Weight for Age Z Score</th>
<th>HEIGHT/LENGTH</th>
<th>Height/Length for Age Z Score</th>
<th>BLOOD SUGAR</th>
<th>VHT</th>
<th>PHONE NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cm</td>
<td>5 cm</td>
<td>1 cm</td>
<td>1 cm</td>
<td>1 cm</td>
<td>1cm</td>
<td>5 cm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PASSIVE RDT TEST</th>
<th>NEW FOCI</th>
<th>DAT</th>
<th>PARASITOLOGY</th>
<th>HIV STATUS</th>
<th>PREGNANCY</th>
<th>REF. IN</th>
<th>REF. OUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>NEGATIVE</td>
<td>INCONCLUSIV E</td>
<td>(Y/N)</td>
<td>&lt;1:800 – NEGATIVE</td>
<td>SPLENIC ASPIRATE</td>
<td>BONE MARROW ASPIRATE</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>NEW FOCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTIVE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ACTIV</th>
<th>RDT TEST</th>
<th>NEW FOCI</th>
<th>DAT</th>
<th>PARASITOLOGY</th>
<th>HIV STATUS</th>
<th>PREGNANCY</th>
<th>REF. IN</th>
<th>REF. OUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>NEGATIVE</td>
<td>INCONCLUSIV E</td>
<td>(Y/N)</td>
<td>&lt;1:800 – NEGATIVE</td>
<td>SPLENIC ASPIRATE</td>
<td>BONE MARROW ASPIRATE</td>
<td>POSITIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>NEW FOCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Annex 13: VISCERAL LEISHMANIASIS HEALTH UNIT INPATIENT REGISTER (HMIS FORM 127a)

**Name of Health Unit: ………………………Month: ……………… Financial Year: …………...**

<table>
<thead>
<tr>
<th>(1) IPD No.</th>
<th>(2) Name + Phone No</th>
<th>(3) Residence Subcounty+ Village+ Parish</th>
<th>(4) Age (Years)</th>
<th>(5) Sex</th>
<th>(6) Next of Kin LC 1/Person VHT</th>
<th>(7) Phone No</th>
<th>(8) Ref In? (Y/N)</th>
<th>(9) Referred From</th>
<th>(10) Date In</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-4</td>
<td>5-14</td>
<td>15-49</td>
<td>50 and above</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(10) Diagnosis</th>
<th>(11) Treatment</th>
<th>(12) Treatment Outcome</th>
<th>(13)</th>
<th>(14)</th>
<th>(15) Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL/HIV Co-infection</td>
<td>SSG &amp; Paromomycin &amp; AmBisome</td>
<td>Other medicines</td>
<td>Cured</td>
<td>Slow Responder</td>
<td>Failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

64
### HMIS FORM 127B: HEALTH UNIT VL INPATIENT MONTHLY REPORT  Page 1

| Health Unit ___________________ Level _____ Code ___ District ________________ Health Sub-district _____________ |
|---|---|---|---|---|---|
| Sub county____________________ Parish _________________ Reporting Period: Month ____________ Year _______ |

<table>
<thead>
<tr>
<th>DIAGNOSIS</th>
<th>TREATMENT TYPE</th>
<th>TREATMENT OUTCOME</th>
<th>0-4</th>
<th>5-14</th>
<th>15-49</th>
<th>50 and above</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSG &amp; Paramomycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow Responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AmBisome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow Responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other medicines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSG &amp; Paramomycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow Responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AmBisome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow Responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other medicines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AmBisome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIAGNOSIS</td>
<td>TREATMENT TYPE</td>
<td>TREATMENT OUTCOME</td>
<td>0-4 M</td>
<td>0-4 F</td>
<td>5-14 M</td>
<td>5-14 F</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow Reponders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL/HIV Co-infection</td>
<td>AmBisome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow Reponders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKDL</td>
<td>Discharged</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKDL</td>
<td>Run away</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKDL</td>
<td>Cured</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKDL</td>
<td>Relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKDL</td>
<td>Lost to follow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKDL</td>
<td>Failed to turn up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6 MONTHS FOLLOW-UP
### Annex 15: Monthly OPD Reporting Form

**HMIS FORM 128B: HEALTH UNIT VL OUTPATIENT MONTHLY REPORT**  Page 1

Health Unit _________________ Level _____ Code ___ District ________________ Health Sub-district _____________

Sub county____________________ Parish _________________ Reporting Period: Month _______ Year _______

<table>
<thead>
<tr>
<th></th>
<th>0-4</th>
<th>5-14</th>
<th>15-49</th>
<th>50 and above</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RDT SCREENING</strong></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Passive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inconclusive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inconclusive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Number of New Foci**

<table>
<thead>
<tr>
<th></th>
<th>0-4</th>
<th>5-14</th>
<th>15-49</th>
<th>50 and above</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT - Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1:1600-Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1600-1:12800-Borderline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1:12800-Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PARASITOLOGY**

<table>
<thead>
<tr>
<th></th>
<th>0-4</th>
<th>5-14</th>
<th>15-49</th>
<th>50 and above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenic Aspirate(SA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Marrow Aspirate(BMA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HIV STATUS (for only VL Positive)**

<table>
<thead>
<tr>
<th></th>
<th>0-4</th>
<th>5-14</th>
<th>15-49</th>
<th>50 and above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PREGNANCY (for only VL Positive)**

<table>
<thead>
<tr>
<th></th>
<th>0-4</th>
<th>5-14</th>
<th>15-49</th>
<th>50 and above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Annex 16: VL Individual Tracker

Visceral Leishmaniasis (VL-IPD) Tracker Model

Registration:
- IPD No
- Enrolment date
- Incidence Date

Diagnosis:
- Primary VL
- Clinical VL
- Relapse VL
- VL/HIV Co-infection

Treatment:
- SSG & Paramomycin
- AmBisome
- Other medicines

Treatment outcome:
- Cured
- Slow Responder
- Failure
- Died
- PKDL
- Discharged
- Runaway

6 months Follow-up:
- Cured
- Relapse
- Lost to Follow-up

IPD No
- Name
- Phone
- Age
- Sex
- Address
- Next of Kin/Phone
- VHT/Phone
- Referral from

Visceral Leishmaniasis (VL-IPD) Tracker Model

Entity Registration:
- IPD No
- Enrolment date
- Incidence Date

Program stage:
- Treatment
  - SSG & Paramomycin
  - AmBisome
  - Other medicines

Treatment outcome:
- Cured
- Slow Responder
- Failure
- Died
- PKDL
- Discharged
- Runaway

6 months Follow-up:
- Cured
- Relapse
- Lost to Follow-up