Report on Informal Consultation on a Diagnostics Quality Assurance Network for Visceral Leishmaniasis, Antwerp 24-26 September, 2008.

Background

Simple rapid tests with acceptable performance and operational characteristics for field use are critical for the control of visceral leishmaniasis (VL) and for the Indian subcontinent VL Elimination Initiative. A multi-centre study sponsored by TDR in Kenya, Sudan, Ethiopia, India and Nepal showed that the rK39 test (InBios, USA) has acceptable performance to be used in field settings in the Indian sub-continent but has sub-optimal sensitivity for use in Africa (Boelaert et al Transactions 2007). TDR is currently funding the development of new and improved tests for VL.

At the workshop on quality assurance of VL rapid tests, organised by the EC funded project LeishRisk and co-hosted by TDR and the Institute of Tropical Medicine (ITM) in Antwerp on 14 November 2007, it was agreed that the quality of VL diagnostics needed to be addressed on 3 levels:

i. Quality of diagnostic tests to guide test selection for procurement (evaluation)
ii. Quality of diagnostic tests after shipment and storage (lot testing)
iii. Quality of diagnostic testing (external quality assurance)

To this end, TDR, in collaboration with ITM, set out to establish a specimen bank network to facilitate VL test development, evaluation, and quality assurance. TDR posted a Request for Applications in February/March 2008 for sites interested in participating in the network. Twenty-two applications were received from 12 countries. Applications were reviewed by an ad hoc expert committee and 7 sites were selected to participate in the network.

LeishRisk, a platform which networks EC-funded consortia active in leishmaniasis, is keen to support and promote the development of the VL specimen bank network. The importance of bridging research and control has been one of the main messages LeishRisk has sought to render. An important part of this endeavour is to facilitate the development of new initiatives which are relevant for sustainable and effective surveillance and control of leishmaniasis, and to help to implement appropriate strategies and policies for prevention, control and treatment. The development of this specimen bank network would signify an important cornerstone in this policy.

Meeting Objectives

1. To discuss with participating laboratories TDR and ITM's proposed plan to enhance the quality of diagnostic tests and testing and develop a shared vision of goals and strategies with disease endemic countries to move forward
2. To share knowledge about what each laboratory can contribute to the specimen bank network
3. To develop consensus on the terms of reference for each participating network site, standard protocols for specimen collection, characterization, storage and use

4. To agree on the governance of the specimen bank, including mechanisms of access to and distribution of specimens to users within and outside of the network
5. To agree on mechanisms and priorities for test evaluation
6. To develop a workplan and timelines for moving forward
7. To review the principles and practice of Good Clinical Laboratory Practice and biobanking with all network PIs
8. Beyond the specific topic of this meeting, to identify gaps in current knowledge and expertise on diagnostics and define future multidisciplinary research (participation to open calls and advocacy for new calls)

Expected Outcomes:

1. A plan for the establishment of a specimen bank network for VL test development, evaluation and quality assurance
2. Consensus from participating sites on the governance, and operation of the VL specimen bank network
3. Consensus on access and distribution of VL specimens from the bank
4. Consensus on a workplan and timelines for moving forward

Purpose of the network

The main purpose of the VL specimen bank network, in order of priority is to :

1. Evaluate commercially available and promising pre market diagnostic tests used in endemic field settings to enable selection for procurement of VL diagnostic tests at local, national and international levels
2. Facilitate development of diagnostics quality assurance program nationally and internationally
3. Facilitate diagnostic research and development[1]

Structure of network

Over the past decade, TDR has coordinated the establishment of specimen bank networks for dengue, TB and malaria. Each bank is structured differently to reflect need, technical and operational requirements and the resources available. Each model has its strengths and weaknesses. In all cases, the bank activities are reviewed by a Steering Group comprised of technical and control program experts and rotating members of the network.

For malaria, the main purpose is to support product testing and post purchase lot-testing. Regional laboratories recruit patients, process and aliquot specimens and send a portion of each sample to the referral lab (global repository) based at the US CDC.

For dengue, the bank's main purpose is to validate commercially available test kits. Two regional laboratories coordinate country level laboratories.

[1] Initially through the development of bilateral agreements between test developer and a network laboratory.

For TB specimen bank the main purpose is to facilitate test development and evaluation. All collection sites ship specimens (blood, urine, sputum) to a central commercial repository. A TB strain bank was also established through regional contributions to a central repository based at ITM.
The advantages and disadvantages of each organizational structure were presented and summarized as follows:

Structure	Advantages	Disadvantages
Central repository	- All specimens in one location, facilitates all aspects of specimen management (requests, shipments etc). - No need for joint databases - easier to monitor	- Permission and funds for shipment required - Expensive maintenance costs - Extensive legal agreements to cover shipment of specimens and to protect integrity of specimens should bankruptcy or change in management occur
Regional repository(s)	- Build capacity in the region to maintain biobank - Create centres that have other functions such as lot-testing and EQA/proficiency testing. - Lower costs - shipping and maintenance - Permission for shipments locally easier to obtain	- may require larger upfront investment at the regional level - can foster regional competition instead of collaboration. - must choose database platform that can be shared to create virtual global bank.
Local repository	- no permission for or costs associated with shipment of specimens - low cost - fosters collaboration - build local capacity for evaluation and EQA	- each site must have equipment for long term reliable storage (eg. Dedicated freezers) - must choose database platform that can be shared to create virtual global bank. -requires external coordinating body to coordinate individual laboratories - requires development of bilateral agreements to support test development.

Overview of network laboratory diagnostic/research work, laboratory expertise and workload and possible contribution to specimen bank.

Institute of Medical Sciences, Banaras Hindu University, Varanasi

The institute treats over 1000 cases of visceral Leishmaniasis per year, it has a full fledged central lab in Varanasi and a lab at the field site. The institute conducted validation tests for different types of visceral leishmaniasis diagnostic tests such as rK39, DAT, Diamed and Katex, including a TDR sponsored phase III trials comparing these tests.

Rajendra Memorial Research Institute of Medical Sciences, Patna

Specialized center for visceral leishmaniasis. Apart from out-patient facilities, the center also has a 50 bed in-patient facility and laboratory. It is involved in basic, applied and operational research. Yearly the institute sees 2-3 thousand visceral leishmaniasis suspects, so far this year 766 DAT and/or rK39 positive patients were identified. Currently samples of 58 cases and 50 healthy endemic controls are available.

BP Koirala Institute of Health Sciences from Dharan, Nepal

The institute has a 700 bed hospital wth a 33 bed tropical and infectious diseases ward. It has a visceral leishmaniasis research laboratory, recognized as reference laboratory by the Nepalese government. The laboratory has been involved in various research projects related to validation of diagnostic tests for visceral Leishmaniasis, including DAT, rK39 and Katex. It currently has 134 samples positive for visceral leishmanianis and 53 samples from healthy endemic controls available.

Parasitology Laboratory of the International Centre For Diarrhoeal Disease from Dhaka, Bangladesh

The laboratory has been recognized as the national reference laboratory for visceral leishmaniasis and is currently involved in 7 projects related to visceral Leishmaniasis.

Fiocruz Laboratório de Imunoparasitologia, Belo Horizonte, Brazil.

The laboratory has been involved in validation of visceral leishmaniasis tests, however DAT is not being used in Brazil. Over the past 6 years there has been a significant increase in the reported incidence of visceral leishmaniasis in Brazil, the laboratory currently detects about 350 cases per year through its 3 outreach centers. There is a relatively high proportion of serologically positive healthy endemic controls.

Laboratório de Soroepidemiologia e Imunobiologia of the Instituto de Medicina Tropical de São Paulo, **São Paulo, Brazil**.

The laboratory is involved in a number of studies related to visceral leishmaniasis, mostly basic research. It has also conducted studies to validate diagnostic tests for visceral Leishmaniasis. Yearly about 50-80 tissue parasite positive cases of visceral leishmaniasis are identified.

Kenyan Medical Research Institute (KEMRI), Nairobi, Kenya.

The institute participated in a number of TDR sonsored validation studies of visceral leishmaniasis tests. It could contribute to the specimen bank around 75-100 samples from parasitologically confirmed cases.

Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Sudan

A leishmaniasis research laboratory was established in 2006. The lab is involved in research, training and diagnostic services. It has links to health facilities in endemic areas at 100-600 km distance, yearly 100-200 tissue parasite confirmed cases are detected. Currently 160 serum and 60 urine samples of confirmed cases are available.

Institute Endemic diseases, University of Khartoum, Sudan

The laboratory of the institute is involved in research and diagnosis. It has field laboratory sites in endemic areas at 60 and at 400km distance. Yearly from 150-300 samples of tissue parasite confirmed cases are processed.

Institute of Pathobiology, Addis Ababa, Ethiopia

Yearly in Ethiopia about 4,000 cases of visceral leishmaniasis are diagnosed and treated. The laboratory is affliated with 5 field sites in endemic areas. Currently, samples are not assembled in a formalized serum bank, however this could be done with the appropriate approvals.

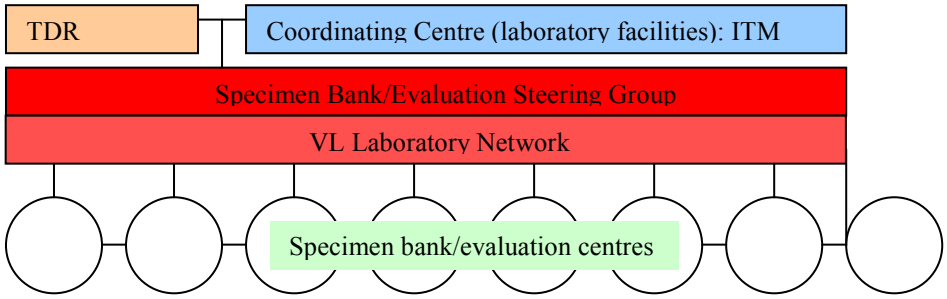
Conclusions

The network will adopt a horizontal structure in which each laboratory will house their own specimens but contribute clinical and laboratory data regarding each clinical specimen (not strains) to a virtual global electronic repository which will be compatibly designed and maintained initially by TDR. For the immediate future there will be no 'supra national reference laboratory' appointed and no shipping of specimens outside the country of origin. However, it was tentatively agreed that a round of external quality assurance (EQA)/proficiency testing will be coordinated by TDR and ITM with technical support from KIT by the end of March 2008. The global database will be housed at WHO in Geneva.

Governance

The VL specimen bank network will be overseen by an appointed Steering Group. Network laboratories will nominate individuals who can contribute clinical and diagnostic VL expertise, control program leaders, rotating representatives of the specimen bank network and WHO, TDR and ITM staff. Membership will be reviewed (renewed or replaced) every two years.

Organizational structure



Draft Steering Group Terms of Reference

The VL diagnostic specimen bank and evaluation steering group will oversee technical and logistical aspects of the VL laboratory network. It will function by email and teleconference meetings quarterly.

The Steering Group will provide recommendations to TDR on:

1. development and modification of standard operating procedures for specimen collection and use
2. content of specimen bank and characterization and maintenance
3. policy on access to specimen bank and to network laboratories on entering into bilateral agreements
4. protocols for laboratory-based testing of VL diagnostic tests
5. review and approve results of evaluations, prior to publication

Draft terms of reference for the VL laboratory network, coordinating and specimen bank/evaluation centres were developed. These TOR will be fulfilled to the best ability within resources available to support these activities.

VL Laboratory Network Members

1. Provide VL diagnostic expertise and technical support, to network members
2. Establish, maintain and expand collaborative links between VL diagnostic and research laboratories nationally, regionally and globally.
3. Promote best practice in VL diagnosis
4. Work collaboratively with and provide strategic advice to TDR to ensure optimal use of VL laboratory/diagnostic resources and to attract additional funding.
5. Assist TDR to develop and evaluate its strategy and methods for promoting quality VL diagnosis

VL Specimen Bank Coordinating Centre

In collaboration with TDR, the coordinating centre (ITM) will organize the design and conduct of proficiency testing for network laboratories before any product evaluation is undertaken. They will agree to and follow the Terms of Reference as defined by the Working Group which includes the following:

1. be responsible for assembly and validation of appropriate clinical specimens to form an proficiency testing panel for VL specimen bank network, evaluation centres;

2. ship the proficiency panel and standardized testing materials to evaluation laboratories in the VL specimen bank network;

3. contribute to the development of a consensus protocol for evaluating VL rapid serological diagnostic tests and corresponding data collection tools;

4. assist TDR in the development of an electronic data collection, storage, and management system and collaborate in data analysis and publication.

VL Specimen Bank/Evaluation Centres

The specimen banking and evaluation centres were selected through a process of Request for Application and subsequent assessment of the applicants. The evaluation laboratory roles and responsibilities can be found in the Working Group Terms of Reference which includes the following:

1. characterize specimens using standard tests for VL including rK39 ICT, direct agglutination test (DAT) or rK39 ELISA, tissue/aspirate culture under GLP-GCP conditions;

2. practice quality assurance (QA) by participating in QA programs and proficiency testing organized by TDR and the coordinating centre;

3. have the capacity to receive proficiency panels in a timely fashion from an international sender;

4. storage (-80C) and archiving of specimens;

5. maintain electronic database of archived specimens and test results;

6. conduct freezer inventory of archived samples and contribute, at regular intervals, to a shared electronic database system;

7. have a mechanism for human subject and institutional review of evaluation protocol as well as ethics approval;

8. perform testing of VL diagnostics on request from TDR, according to an agreed protocol and within two (2) months of receiving the kits;

9. collaborate in establishing a protocol for product testing;

10. work in a collaborative way with other specimen bank network laboratories;

11. approve the final evaluation report and, if applicable, participate in the publication of evaluation results.

Network laboratories will retain ownership of all specimens prospectively collected and stored, and any archived specimens used for product evaluations.

Next steps

Consensus was reached on the following:

1. The purpose of the network is the following:
    a. Evaluation of commercially available and promising pre market diagnostic tests used in endemic field settings to inform selection for procurement of VL diagnostic tests at local, national and international levels
    b. Facilitate development of diagnostics quality assurance program nationally and internationally
    c. Facilitate diagnostic research and development[2]
2. The network will adopt a horizontal structure in which each laboratory will house their own specimens but contribute clinical and laboratory data regarding each clinical specimen (not strains) to a virtual global electronic repository which will be compatibly designed and maintained initially by TDR. For the immediate future there will be no 'supra national reference laboratory' appointed and no shipping of specimens outside the country of origin. However, it was tentatively agreed that a round of external quality assurance (EQA)/proficiency testing will be coordinated by TDR and ITM with technical support from KIT by the end of March 2008.

3. Network priorities: i) evaluation of rapid diagnostic tests used in endemic field settings to inform selection for procurement of VL diagnostic tests at local, national and international levels ii) support development of diagnostics quality assurance program nationally and internationally iii) facilitate discovery and test development through bilateral agreements (network laboratory and test developer or scientist).

4. Terms of reference for each contributing laboratory and the network including governance to be finalized.

5. A Steering Group (SG) for the VL specimen bank network activities, will be assembled to guide access to specimens. The SG will be comprised of technical experts, control program representatives and rotating members of the network.

6. The first round of product testing to include commercially available and promising pre market rapid antibody detection tests for VL, using archived blood (serum) samples. Contracts will be issued to each TDR sponsored network laboratory to conduct an inventory of current specimens and enter those meeting case definitions and required criteria in a central database. These specimens will then be validated to construct the evaluation panel, using standardized test materials (DAT, rK39 ELISA). Funds will also be used to support preparations for the evaluation including site specific modifications to master protocol, development of protocol for prospective specimen collection.

[2] Initially through the development of bilateral agreements between test developer and a network laboratory.

7. Evaluation results will be pooled across regions, if necessary, to achieve the required sample size.

8. A draft protocol for the evaluation of rapid VL diagnostic tests using archived serum was circulated for comments.

9. A work-plan providing timelines to ensure that by the end of 2009, the first results on evaluation of test kits will be available.

VL Research Priorities and Network Workplan

The 2007 EU call on vaccine development and disease control for neglected diseases was modified in response to a letter from ITM. On behalf of LeishRisk, participants completed a survey to inform a position paper to influence the 2009 call.

Contracts will be issued to each TDR sponsored network evaluation laboratory to conduct an inventory of currently archived specimens and enter those meeting case definitions and required criteria in a central database (Annex 2).

Those specimens meeting criteria will be revalidated using standardized test materials and be used to construct and 'evaluation' panel.

The essential composition of the evaluation panel was discussed and it was agreed that the following were essential: i) HIV negative parasitologically confirmed visceral leishmaniasis cases (150), healthy endemic controls serologically (DAT, rK39 ELISA) negative (150) and confirmed potentially cross reacting diseases including diseases with similar presentation to VL (e.g. typhoid fever, malaria, TB, brucellosis, systemic mycosis) and those with potentially cross reacting antibody responses (cutaneous leishmaniasis, post kala-azar dermal leishmaniasis (PKDL), Chagas disease),with negative serology (DAT, rK39 ELISA) for visceral leishmaniasis (100).

In parallel, one round of proficiency testing using well characterized specimens and materials will be coordinated by TDR and ITM with technical support from KIT.

The first round of product testing will include commercially available and promising pre market rapid antibody detection tests for VL, using archived blood (serum) samples.

Tests to be included in the evaluation will be identified through an Expression of Interest issued by TDR and distributed to known VL rapid test manufacturers and posted on relevant websites (TDR, WHO, manufacturer associations (Asia, EU, South America, Africa, North America).

Since no individual site will easily achieve the required sample size, it was decided to do a pooled analysis across regions. There will be three regions: the Indian subcontinent (Bangladesh, India, Nepal), Africa (Sudan, Kenya and Ethiopia if funds can be found) and Latin America (different sites in Brazil).

Despite pooling of results, prospective collection may still be required, to this end, a collection protocol will be developed and submitted for ethics review. Then if funds are secured sites will collect and store serum, urine, saliva and aspirates from bone marrow, spleen or lymph glands. Specific specimen processing requirements (eg. buffy coats, boiled urine) could be accommodated through bilateral agreements between network members and test developers and/or discovery scientists.

The draft evaluation protocol is included in **Annex 3**.

A work plan with associated timelines was developed by the network with the ultimate goal of to have 2 rapid VL diagnostic test kits included in WHO bulk procurement scheme by the end of 2009.

Results/Activity	Timeline
1. Agreement on governance of network	By end 2008
1.1 Network agreement drafted	
1.2 Network agreement signed	
2. A generic protocol for evaluation of rapid tests using archived samples approved by IRB of each site and WHO ERC	By end 2008
2.1 Review the Sep 26 version and send feedback to JC	Oct 15, 2008
2.2 Produce final protocol and send it to the PIs	Oct 31, 2008
2.3 Submit the final protocol to IRB for each site and WHO ERC in order to obtain approval by end 2008	Nov 1, 2008
3. Selection and validation of archived sera for the Evaluation Panel	By Q1 2009
3.1 To send a table for sample inventory to each PI	Sept 30, 2008
3.2 To select sera eligible for inclusion in the evaluation panel and return table to JC	Nov 15, 2008
3.3 SOP for validation of archived samples developed	End 2008
3.4 To have each site with archived samples validated	March 31, 2009
4. Sites ready for prospective collection of specimens	By Q2 2009
4.1 Protocol for prospective specimen collection developed	Nov 1, 2008
4.2 Protocol approved by sites IRB	By March 31, 2009
4.3 Additional funding needs identified/funding secured	By March, 2009
5. Data management system operational	By end 2008
5.1 To decide on coding system	
5.2 To develop the network's database (EPI-INFO)	Dec 1, 2008
5.3 To send the status of site's archived specimen bank to JC	Dec 15, 2008
6. Each site certified by one round of EQA	By March 31 2009
6.1 To constitute test panel (20 specimen plus DAT	end 2008

– antigen & kit)	
6.2 To send test panel to all labs	Jan 1, 2009
6.3 To have panel tested and send results to JC	Jan 31, 2009
6.4 To analyse EQA results	March 1, 09
7. Candidate brands of rK39 dipstick and included in WHO bulk procurement scheme	By end 2009
7.1 Call for expression of interest	Q4 2008
7.2 Each site evaluating products	April 2009
7.3 Data pooled at regional level and analysed	Q4 2009

Annex 1: Meeting Participants

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Annex 2 : Case definitions & Central Database Contents

Case definitions

Code	Category	Details
1	Confirmed VL, primary cases	positive parasitology for VL on a splenic or lymph node or bone marrow aspiration. With variable DAT titers: – high, medium, low
2a	Healthy endemic controls, with follow up	a healthy subject living in an area with transmission of leishmania infection, who has negative serology (DAT and rK39 ELISA negative) who stays asymptomatic for VL during several months follow up.
2b	Healthy endemic controls, no follow up	a healthy subject living in an area with transmission of leishmania infection, who has negative serology (DAT and rK39 ELISA negative), no follow up assessment.
3	Confirmed VL, relapse cases	Past history of VL positive parasitology for VL on a splenic or lymph node or bone marrow aspiration. With variable DAT titers: – high, medium, low
4	Confirmed other disease states	Typhoid fever, tuberculosis, malaria, brucellosis, cutaneous leishmaniasis, PKDL, trypanosomiasis, chagas, etc . DAT/ELISA neg
5	All others	

Central Database Fields

Required entries for archived specimens in evaluation panel are denoted with an asterix "*".

- Code*: All codes must begin with a two-digit, site identification number
- Specimen type:
    - blood *(serum/plasma/buffy coat)
    - urine (details of processing/unknown)
    - aspirate (bone, spleen, lymph node)
    - tissue (skin/bone/spleen/ lymph node/unknown)
    - saliva
    - culture
    - DNA
    - RNA
    - DNA/RNA
    - Other (specify)
- Specimen volume:
    - Total volume* (ml)/not applicable
    - Per aliquot (µl)/not applicable

- Species: select from list/unknown
- Storage conditions*:
    - Freezer -20°C, -70°C, -80°C
    - refrigerator (4°C)
    - liquid nitrogen
    - other
- Storage location*: All codes must begin with a two-digit, site identification number (as above)
- Date of sample collection*: dd/mm/yyyy/unknown
- Date of storage: dd/mm/yyyy/unknown
- Sex*: male/female/unknown
- Age or date of birth*: __years, or dd/mm/yyyy/unknown
- Country of specimen origin*: all endemic countries/unknown
- Past history of VL*: yes/no/unknown
- Duration of fever (weeks) : number/unknown
- Direct tissue microscopic examination*: positive(1+-6+)/negative/unknown/not done
- Tissue culture examination: positive/negative/unknown/not done
- DAT result 1*:
    - Liquid DAT/freeze dried DAT/unknown
    - negative/positive, if yes, titer = ≥1: XXXX /unknown
- Date & DAT result 2:
    - dd/mm/yyyy;
    - Liquid DAT/freeze dried DAT/unknown
    - negative/positive, if yes, tite r= ≥1: XXXX /unknown
- rk39 ELISA*[3] result-1: positive, if yes, O.D./negative/unknown/not done
- Date & rK39 ELISA result-2: positive, if yes, O.D./negative/unknown/not done
- rK39 RDT result: positive, negative, indeterminant/unknown/not done
- Concomitant diseases (specify)
- Disease category*: see above case definitions.
- HIV status: positive/negative/indeterminant/unknown/not done
- Spleen size (cm): XXcm/unknown
- Malaria smear results: positive/negative/unknown/not done

[3] Either DAT or rK39 ELISA result must be available

Annex 3: Draft VL RDT Evaluation Protocol

See Word document