# Improving treatment of Cutaneous Leishmaniasis (CL) based on species differentiation

***Part 2***

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## Tasks & Problems to solve

**Problem I :** some Leishmania species are **not valid**, taxonomy is currently reassessed Lukeš et al. 2007 PNAS

*e.g. L. infantum = synonymous to L. chagasi; L. archibaldi is not a valid species*

**Problem II:** differention among **closely related** species and **species hybrids**

*e.g. differentiation of sympatric L. braziliensis (MCL) from L. peruviana*

**inter-laboratory collaboration required**

„**Gold standard**" for classification of Leishmania isolates = **MLEE** = Multi Locus Enzyme Electrophoresis

Single Nucleotide Polymorphism (SNPs) in house keeping genes (enzymes) change mobility in gel electrophoresis

**Enzyme patterns = zymodemes**

Critical points:
- culture required ➡ slow & costly
- only synonymous mutations detected
- within species polymorphism
- tool for epidemiology

## Molecular markers currently used

### Species discrimination by PCR- RFLP on multigene families:

- Mini-exon SL Fernandes et al. 1994, Marfurt et al. 2003
- Glycoprotein 63 (gp63) Victoir/Dujardin et al. 2003
- kDNA
- rDNA internal transcribed spacer

### Population & MolEpi studies:

- Microsatellites MLMT
- Kinetoplast DNA combined with RFLP

## Aims of Mol. Diagn. part in project

- **compare current Leish species typing assays**
  QC, inter-laboratory comparison
- **collect & distribute well documented isolates** *(culture, DNA)*
- **gather sequences/SNPs data of potential marker genes**
  current polymorphic genotyping markers plus enzyme genes ("extended MLST" )
- **identify most relevant diagnostic SNPs for each species**
  (Old and New World)
- **develop new techniques** fast, robust and cheap
  - Allelic discrimination (SNP-spec. Probe; multiplex qPCR)
  - SNP microarray
  - Sequencing
  - other

## Lab procedures

**1. step: QC on archived samples collected among all collaborators**

- compare genotyping results from methods used in each routine lab

**> set of DNA aliquots distributed through STI**

**2. step: species differentiation in samples from joint study**

- genotype samples from project with as many techniques as possible
- synthesize a solid species differentiation for clinical research from combined results of several markers

**> devide specimen: ½ for DNA extraction, ½ for culture**
**> DNA from culture/remaining DNA distributed through STI**

**3. step: R&D new technique**

**4. step: transfer of new MLST assay and multi-center evaluation**

**> establish and continue genotype database**

## Funding options

| | Clinic | Assay development | Requirements |
|---|---|---|---|
| **No major funding** | as presented by JB | ▪ QC for routine tests<br>▪ Exchange of samples | DNA |
| **Local small scale (SNF)** | | ▪ Create dbf of diagnostic SNPs in current markers (RFLP data)<br>▪ Identify robust species-specific SNPs from multiple loci | DNA & culture |
| **EU** | | ▪ Sequencing of new markers in search of small no. of diagnostic SNPs<br>▪ Develop new SNP-based MLST assay<br>▪ Chose new platform technology<br>▪ Include South Partners | DNA & culture |

## Topics for discussion

- **which Leish species to investigate NW/OW**
- **culture facilities**
- **new multi locus SNP typing platform:**
  - sequencing
  - QPCR
  - microarray
  - other
- **funding**

## FP7 Health-2010

**Main focus : "***Providing tools for translational research"*

***Systems biology***

***Cell therapy***

➡ ***Diagnostics***

**1.2. Detection, diagnosis and monitoring**

The objectives are to develop visualisation, imaging, detection and analytical tools and technologies for biomedical research, **for prediction, diagnosis, monitoring and prognosis of diseases, and for support and guidance of therapeutic interventions**. The focus will be on a multidisciplinary approach integrating areas such as: molecular and cellular biology, physiology, genetics, physics, chemistry, biomedical engineering, nanotechnologies, microsystems, devices and information technologies. Non- or minimally- invasive and quantitative methods and **quality assurance aspects** will be emphasised.

**HEALTH.2010.1.2-1: Tools for the identification and the detection of biomarkers in clinical samples and patients.**

**International Cooperation:**

Special international cooperation actions (**SICA**s) particularly **targeted for research activities in the areas of infectious diseases**

**2.3.4. Neglected infectious diseases**

The aim of this area is to establish an integrated approach for the development of preventive, therapeutic and diagnostic, tools for neglected infectious diseases. Activities will address, but not be limited to, parasitic diseases caused by Trypanosomatidae species (Trypanosomiasis, Chagas Disease, Leishmaniasis), bacterial diseases such as Buruli ulcer, leprosy and trachoma, helminth diseases such as schistosomiasis, as well as health conditions that can be caused by several pathogens, such as childhood diarrhoea.

**HEALTH.2010.2.3.4-2: Comprehensive control of Neglected Infectious Diseases (NID) deadline; 19. Nov 2009**

## HEALTH.2010.2.3.4-2: Comprehensive control of Neglected Infectious Diseases (NID)

Research should aim at **developing an improved system for delivering primary health care in resource poor settings.** This project should aim to overcome the vertical approach of many disease-specific control programmes, and develop methods that are more adapted to a primary health care setting. The proposed research should address several of the NID priority diseases, while not necessarily be limited to these. The project should aim at integrating existing prevention methods with **new diagnostic methodology and diagnosis-treatment algorithms** adapted to resource-poor settings. The project can include the development of new or optimized diagnostic platform for multiple infectious diseases, but the proposed prevention and treatment options should be based on existing and affordable tools, vaccines and medication. Innovative research on intervention delivery may be included. Finally, the project should also aim to develop primary health care recommendations for use by policy makers and health care managers in disease endemic countries. Active participation of SMEs could lead to an increased impact of the research proposed and this will be considered in the evaluation of the proposal.

## HEALTH.2010.2.3.4-2: Comprehensive control of Neglected Infectious Diseases (NID) *cont.*

**Funding scheme:** Specific International Cooperation Action (**SICA**) Collaborative Project (Small- or medium scale focused research project) Target Region: all International Cooperation Partner Countries (ICPC)
**EC contribution per project:** max. EUR 6 000 000
**One or more proposals can be selected.**
**Expected impact:** Research must bridge vertical, disease-specific approaches and must use state-of-the-art epidemiological and diagnostic knowledge and technology to develop 1) a single comprehensive, low-cost diagnostic system that is suitable for use in resource poor settings and 2) diagnosis-treatment algorithms on the basis of existing prophylactics and medication, and 3) document the cost-savings and increased efficacy of a comprehensive, horizontal approach to NID, and develop recommendations for broad implementation to policy makers, relevant international organisations, and health care managers.