



Trypanosoma cruzi - classification
659-7

UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR
RESEARCH AND TRAINING IN TROPICAL DISEASES

Panama City, 28-31 January 1985



7735

REPORT OF A MEETING ON THE STANDARDIZATION OF METHODS
FOR TRYPANOSOMA CRUZI CLASSIFICATION

CONTENTS

	<u>Page</u>
1. INTRODUCTION	2
2. OVERVIEW OF <u>T. CRUZI</u> CLASSIFICATION AND TAXONOMY	2
3. SIGNIFICANCE OF <u>T. CRUZI</u> DIFFERENTIATION AND SELECTION: POSSIBLE RELATIONSHIP WITH CLINICAL AND EPIDEMIOLOGICAL DISEASE VARIETIES	2
4. DESIGN OF ANALYTICAL EPIDEMIOLOGICAL STUDIES	2
5. ISOLATION AND CLASSIFICATION OF <u>T. CRUZI</u>	2
5.1 Isolation and Amplification Techniques	2
5.2 Cloning Techniques	3
5.3 Morphological and Behavioural Characterization	3
5.4 Antigen Characterization	3
5.5 Isoenzyme Characterization	3
5.6 Lectin-Interaction Analysis	3
5.7 Schizodeme Analysis	3
6. CONCLUSIONS AND RECOMMENDATIONS.	4
6.1 Interchange of <u>T. cruzi</u> Material	4
6.2 Reference Centres	4
6.3 International Reference Strains	4
6.4 Minimal Criteria for Non-Standard Strains	5
6.5 Collaborative Studies	6
6.6 Nomenclature	6
6.7 Standardization of Isolation and Amplification Procedures	6
6.8 Distribution of Reference Strains	6
6.9 Amplification and Characterization of Non-Standard <u>T. cruzi</u> Stocks	6
6.10 Glossary of Terms	7
6.11 Distribution of Data Sheets and Forms	8
6.12 Manual	8
7. REFERENCES	8

This report contains the collective views of an international group of experts convened by the UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES (TDR). It does not necessarily reflect the views of TDR/WHO. In the interests of rapid communication it has been submitted to only minimal editorial revision. Moreover, any geographical designations used in the report do not imply the expression of any opinion whatsoever on the part of TDR or WHO concerning the legal status of any country, territory, city or area or of its authorities concerning the delimitation of its frontiers or boundaries.

Ce rapport exprime les vues collectives d'un groupe international d'experts réuni par le PROGRAMME SPECIAL PNUD/BANQUE MONDIALE/OMS DE RECHERCHE ET DE FORMATION CONCERNANT LES MALADIES TROPICALES (TDR). Il ne représente pas nécessairement les vues du TDR/OMS et, en vue d'une diffusion accélérée, il n'a pas été l'objet d'une mise en forme particulièrement soignée. En outre, les noms géographiques utilisés dans le présent rapport n'impliquent, de la part du TDR ou de l'OMS, aucune prise de position quant au statut juridique de tel ou tel pays, territoire, ville ou zone, ou de ses autorités, ni quant au tracé de ses frontières.

8.	LIST OF PARTICIPANTS	9
ANNEX I	LIST OF COLLABORATING LABORATORIES	10
ANNEX II	DESCRIPTION SHEET FOR INTERNATIONAL REFERENCE STRAINS OF <u>T. CRUZI</u>	12
ANNEX III	LIST OF WORKING PAPERS PRESENTED AT THE MEETING	14

1. INTRODUCTION

A review of current techniques for the characterization and classification of Trypanosoma cruzi strains was presented at a meeting of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) Scientific Working Group on Chagas' Disease, held from 28-31 January 1985 in Panama City, Panama. Their potential for the determination of possible differences in clinical and epidemiological parameters caused by different strains was also discussed. A list of the papers presented at the meeting is given in Annex III.

2. OVERVIEW OF T. CRUZI CLASSIFICATION AND TAXONOMY

Sensitive probes (e.g. DNA probes or monoclonal antibodies) are needed to analyse parasite heterogeneity within host or vector before amplification. An ideal, rapid drug-susceptibility test is also required, and drug susceptibility between strains has to be studied. Some immunological characterization methods (e.g. ¹²⁵I-labelling of surface proteins) yield complex patterns. Although this complexity may introduce difficulties in classification, these methods could none the less be useful. A major initiative is called for to compare the reactivity of clones/strains to monoclonal antibodies.

3. SIGNIFICANCE OF T. CRUZI DIFFERENTIATION AND SELECTION; POSSIBLE RELATIONSHIP WITH CLINICAL AND EPIDEMIOLOGICAL DISEASE VARIETIES

The use was discussed of 'subset analyses' in 'polar' epidemiological areas and of clinical pictures for assessing the influence of strain heterogeneity. The epidemiological studies sponsored by the Steering Committee on the Epidemiology of Chagas' Disease (EPICHA) could provide a suitable basis for new 'strain-related' research.

4. DESIGN OF ANALYTICAL EPIDEMIOLOGICAL STUDIES

Concurrent case-control studies might help to elucidate the influence of an infecting strain on clinical variations of the disease, but it would be necessary to carefully re-evaluate the definition of clinical groups and sampling techniques.

5. ISOLATION AND CLASSIFICATION OF T. CRUZI

5.1 Isolation and Amplification Techniques

The use of local vector species for xenodiagnoses (and perhaps other species for follow-up of patients) is important, as there are clear differences in the susceptibility of vectors to infection by T. cruzi.

The study of parasite/invertebrate host relationships should be given high priority.

5.2 Cloning Techniques

Computer simulation of the growth of mixtures of T. cruzi clones demonstrates graphically the considerable influence of culture methods on the population profile or heterogeneity of the parasite. This was confirmed by experimental studies and by monitoring of clinical isolates.

The direct relationship between the epimastigote and amastigote growth phases of the life-cycle can be used, on the basis of readily available epimastigote growth data, to predict parameters of the complex intracellular cycle.

5.3 Morphological and Behavioural Characterization

Morphobiological classification into three types [Andrade (1)] and isoenzyme analysis (2) showed the following correspondence with the principal zymodemes: Z1 = Type III; Z2 = Type II; and Z2 'heterozygous' = possibly Type I (to be confirmed).

The newly discovered phase of the T. cruzi life-cycle which takes place in opossum anal glands might be explored as an additional source of Chagas' disease transmission (3).

5.4 Antigen Characterization

Relevant monoclonal antibodies might provide a basis for rapid strain classification in simply equipped laboratories. The wish to simplify and unify the concept of T. cruzi diversity has to be balanced against the need to take into account inherent T. cruzi complexity during analyses of data sets. New insight into T. cruzi strain diversity has arisen from complex multivariate and intuitive approaches.

5.5 Isoenzyme Characterization

Due to limited sample sizes and potentially biased sampling methods, caution is required in deducing zymodeme/transmission/disease correlations.

The possibility of a sexual phase in the T. cruzi life-cycle should be investigated.

5.6 Lectin-Interaction Analysis

Lectins have a potential role in strain classification. However, lectin-purified glycoconjugates/antigens are likely to be heterogeneous. Monoclonal antibodies and affinity chromatography should be used for antigen purification.

5.7 Schizodeme Analysis

Schizodeme analysis is highly discriminatory and suitable for qualitative and possibly quantitative comparisons. Sensitivity could be further improved with selected labelled DNA probes. All reliable evidence collected over several years indicates a high degree of stability in clonal schizodeme and zymodeme characteristics.

5.7.1 Data from a specific epidemiological situation

Preliminary evidence was presented of selection operating during the transfer of T. cruzi stocks between different hosts in Bolivia. The possible sylvatic and domestic histories of T. cruzi zymodemes were described.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Interchange of T. cruzi Material

6.1.1 International Reference Strains should be transported in the form of stabilates in dry ice. Provision of multiple stabilates would guard against instability or confusion of strain characteristics.

6.1.2 The Pan American Health Organization (PAHO) should, where appropriate, be asked to facilitate customs clearance.

6.1.3 Packaging should be in accordance with the strictest regulations of any of the countries involved, probably those of the United States of America. In countries where regulations for packaging and shipment of T. cruzi are non-existent, these should conform to the P-2 requirements for infectious agents as established by the Centers for Disease Control (Atlanta, GA, USA). These regulations include construction and design of shipping containers.

The parasite material should be shipped frozen, in dry ice. The external container should be similar to that used by the American Type Culture Collection (Rockville, MD, USA), i.e. a closed cell contained in a cardboard shipping box.

6.1.4 WHO should be asked to finance a supply of packaging containers for participating laboratories.

6.2 Reference Centres

The laboratories indicated in Annex I were suggested as reference centres, to act as donors, recipients and distribution centres for International Reference Strains of T. cruzi.

6.3 International Reference Strains

6.3.1 T. cruzi International Reference Strains were selected on the following criteria: representative known zymodemes and schizodemes available; behaviour patterns known in mice; growth rates determined; DNA contents known; reactivities to monoclonal antibodies characterized; and susceptibility to drugs known.

Cloned reference strains should be included, in view of the known heterogeneity of T. cruzi populations.

6.3.2 The following International Reference Strains were selected:

- M/HOM/PE/00/Peru
- M/HOM/BR/00/12 SF
- M/HOM/CO/00/Colombia
- M/HOM/BR/00/Y strain
- M/HOM/BR/00/CL strain
- M/HOM/CH/00/Tulahuen
- M/HOM/AR/74/CA-I
- * M/HOM/AR/74/CA-I/72
- * M/HOM/AR/00/CA-I/78
- * M/HOM/AR/00/Miranda 83
- * M/HOM/AR/00/Miranda 88
- * M/HOM/BR/82/Dm 28c

* Derived from clonal populations

- * M/HOM/BR/78/Sylvio-X10-CL1
 - * M/HOM/BR/78/Sylvio-X10-CL4
 - * M/HOM/BR/77/Esmeraldo CL3
 - * M/HOM/BR/68/CAN III CL1
 - * M/HOM/BR/68/CAN III CL2
 - * M/HOM/BO/80/CNT/92: 80 CL1
 - * I/INF/BO/80/SC43 CL1
 - * I/INF/PY/81/P63 CL
- Low virulence strains
M/HOM/AR/78/RA

6.3.3 It was suggested that T. rangeli reference strains be added to the list at some future date [e.g. M/HOM/SV/00/R1625 (4) representing T. rangeli isolated in Panama, Venezuela, etc. from man].

6.3.4 A description of each T. cruzi International Reference Strain should be produced on a standardized data-entry form for distribution to reference centres (see Annex II).

6.3.5 This description sheet should be designed in part as an International Request Form for T. cruzi characterization.

6.4 Minimal Criteria for Non-Standard Strains

The minimum information required on newly isolated T. cruzi strains to be included in the bank and used for studies of T. cruzi strains in different clinical and/or epidemiological situations would fall into three categories:

Clinical:

- a) Acute phase
 - inapparent
 - apparent (to be specified)
- b) Chronic phase
 - indeterminate
 - cardiac
 - digestive (megaesophagus or megacolon)
 - nervous
 - mixed

Geographical:

- a) From patients with differing morbidity patterns ("polar" situations)
 - with and without cardiac forms
 - with and without digestive forms
 - with and without nervous forms
 - with high congenital transmission
- b) Preferably from areas where follow-up studies have been carried out

Epidemiological:

- a) From patients infected after exposure to a sylvatic cycle
- b) From areas where the disease was newly introduced
- c) From areas with differing transmission mechanisms.

6.4.1 It was recommended that new T. cruzi isolates should be obtained from regions with differing geographical and epidemiological characteristics, such

* Derived from clonal populations

as regions north and south of the Amazon Basin, where disease characteristics and some strain characteristics appear to differ.

6.5 Collaborative Studies

6.5.1 The following collaborative studies are to be encouraged:

- a) Assembly of complete data sets on selected International Reference Strains (zymodemes, schizodemes, susceptibility to drugs, etc.).
- b) Collaboration between clinicians, epidemiologists and biologists working in field studies and on laboratory analyses of T. cruzi characteristics.
- c) Investigations of the significance of selection during isolation and amplification procedures.

6.5.2 Funding should be provided urgently to set up facilities for growing T. cruzi in bulk. Lack of such logistical support is a major obstacle to progress.

6.6 Nomenclature

6.6.1 The SWG produced a glossary of commonly used terms (see section 6.10). The terms 'isolate', 'stock' and 'strain' would be used as defined in ref. (5) and (6).

6.6.2 The coding system recommended for Leishmania would be adopted for T. cruzi International Reference Strains [see ref. (7)].

6.6.3 The numbering/naming system for principal zymodemes should be standardized as soon as possible. A flexible, comprehensive system of describing and naming all zymodemes (and schizodemes) should be sought.

6.7 Standardization of Isolation and Amplification Procedures

6.7.1 In view of the possible selective effect of isolation procedures, no single method could be recommended. The use of more than one technique (xenodiagnosis, haemoculture, mouse inoculation) was to be encouraged.

6.7.2 Any method producing adequate growth could be used to amplify parasite populations. The heterogeneity of uncloned stocks must be considered.

6.7.3 Clones should be prepared as soon as possible after isolation in order to recover parasites of the original T. cruzi population. The single-cell manipulation method, described by Dvorak (8), should be used to prepare reference clones.

6.8 Distribution of Reference Strains

The following measures were approved to make standard reference strains of T. cruzi available to national centres.

6.8.1 Reference strains should be collected and sent as stabilates to the Gorgas Memorial Laboratory (Panama).

6.8.2 The strains should be amplified at the Gorgas Laboratory and distributed as stabilate sets to all national centres.

6.9 Amplification and Characterization of Non-Standard T. cruzi Stocks

6.9.1 Additional resources should be provided (for reagents and technical

assistance, for example) to individual laboratories or linked groups of laboratories to strengthen amplification facilities.

6.9.2 Resources should be sought to allow selected centres in different countries to compare T. cruzi characterization data.

6.10 Glossary of Terms

(Primary) Isolate: the viable organisms present in a culture or in an experimental animal host following the introduction of a sample or part of a sample from a naturally infected host.

Stock: the population derived by serial passage in vivo and in vitro from a primary isolate without any implication of homogeneity or characterization.

Strain: a population set originating from a group of trypanosomes at a given time in a given host or culture, defined by the possession of one or more designated characteristics.

Zymodeme: trypanosome populations that differ from others of the same species in a specified isoenzyme property or set of isoenzyme properties.

Principal zymodeme: zymodemes believed (on the basis of intuitive analysis, hierarchical clustering or ordination) to form a distinct group.

Schizodeme: trypanosome populations with similar kDNA restriction fingerprints.

Principal schizodeme: schizodemes believed (on the basis of intuitive analysis, hierarchical clustering or ordination) to belong to related principal groups.

Isoenzyme pattern: the pattern of isoenzyme bands observed with a given T. cruzi population and a given single enzyme-staining procedure.

Isoenzyme profile: the combination of isoenzyme patterns seen with a given T. cruzi population and several enzyme-staining procedures.

DNA probe: a labelled DNA preparation used to detect complementary DNA sequences in different samples. The DNA may be of nuclear or kinetoplast origin and isolated by biochemical, gene cloning or synthetic techniques. Labelling of the probes may use radioactive (e.g. ³²P) or nonradioactive (e.g. biotin) methods.

Virulence: the capacity of the parasite to multiply within the experimental host (this is influenced by several factors).

Pathogenicity: the ability to produce tissue lesions and mortality. Highly virulent strains (e.g. Y and Peru) are, in general, highly pathogenic. However, other strains with slower multiplication rates and lower virulence (e.g. Colombia) can also cause prominent tissue lesions. Pathogenicity may be independent of parasitaemia levels (9) and animals may die with relatively low parasitaemia levels (10).

Experimental pathology types:

Type I: strains with rapid multiplication rates; maximum parasitaemia and mortality from 7 to 12 days after infection; predominance of slender forms and macrophagotropism in the early phase of infection (e.g. Y and Peru).

Type II: strains with relatively slow multiplication rates; irregular parasitaemia peaks from 12 to 20 days after infection (when mortality reaches

a maximum); predominance of broad forms with a low percentage of slender forms in the initial phase of infection; myotropism with predominant involvement of myocardium (e.g. 12 SF).

Type III: slow multiplication rates; late and high parasitaemia peaks (20 to 30 days after infection); low rates of mortality from the 50th day of infection; predominance of broad forms throughout the course of infection; myotropism predominantly of skeletal muscle (e.g. Colombia).

6.11 Distribution of Data Sheets and Forms

A draft data sheet on International Reference Strains was distributed to all participants for comments and inclusion of data. A standard data-entry form will be designed to ensure compatibility among the computer facilities located at the various institutions involved in the project.

6.12 Manual

Additional technical descriptions will be included in the third edition of the laboratory manual, Genes and Antigens of Parasites: Proceedings of a Course Sponsored by UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases and the Fundação Oswaldo Cruz [2nd ed. (C. Morel, ed.), Rio de Janeiro: Fundação Oswaldo Cruz, 1984].

7. REFERENCES*

- (1) ANDRADE, S. Morphological and behavioural characterization of Trypanosoma cruzi strains. Document TDR/EPICHA-TCC/84.9.
- (2) ANDRADE, V., BRODSKYN, C. & ANDRADE, S.G. Correlation between isoenzyme patterns and biological behaviour of different strains of Trypanosoma cruzi. Trans. Roy. Soc. Trop. Med. Hyg., 77: 796-799 (1983).
- (3) DEANE, M.P., LENZI, H.L. & JANSEN, A. Trypanosoma cruzi vertebrate and invertebrate cycles in the same mammal host, the opossum Didelphis marsupialis. Mem. Inst. Oswaldo Cruz, 79: 513-515 (1984).
- (4) MILES, M.A. ARIAS, J.R., VALENTE, S.A.S., NAIFF, R.D., DE SOUZA, A.A., POVOA, M.M., LIMA, J.A.N. & CEDILLOS, R.A. Vertebrate hosts and vectors of Trypanosoma rangeli in the Amazon Basin of Brazil. Amer. J. Trop. Med. Hyg., 32: 1251-1259 (1983).
- (5) Serotyping of African Trypanosomes: A Meeting of the Scientific Working Group on African Trypanosomiasis. Document TDR/TRY-SWG(Sero)/82.3.
- (6) *LUMSDEN, W.H.R. & KETTERIDGE, D.S. (eds.). Proposals for the nomenclature of salivarian trypanosomes and for the maintenance of reference collections. Bull. Wld. Hlth. Org., 56: 467-480 (1978).
- (7) *The leishmaniasis. Technical Report Series, No. 701, 1984.
- (8) DVORAK, J. Single cell isolates of T. cruzi. How and why? Document TDR/EPICHA-TCC/84.8.
- (9) PHILLIPS, N.R. Experimental studies on quantitative transmission of Trypanosoma cruzi: Considerations regarding standardization of material. Ann. Trop. Med. Parasitol., 54: 60-70 (1960).

* WHO publications are available through the WHO network of designated sales agents (listed in all WHO publications) or may be obtained directly from the World Health Organization, Distribution and Sales Service, 1211 Geneva 27, Switzerland.

- (10) ANDRADE, V. Estudo imunopatológico de camundongos de seis diferentes linhagens isogênicas à infecção por três tipos de cepas do Trypanosoma cruzi. Master's Thesis, University of Bahia, Salvador, Bahia, Brazil 1984.

8. LIST OF PARTICIPANTS

- ANDRADE, Dr S.G., FIOCRUZ, Instituto de Pesquisas 'Gonzalo Moniz', rua Valdemar Falcao 121 - Brotas, 40 000 Salvador, Bahia, Brazil
- ANTUNES, Dr C.M., Department of Parasitology ICB, Federal University of Minas Gerais, Caixa Postal 2486, 30 000 Belo Horizonte, Brazil
- BRENER, Prof Z., FIOCRUZ, Centro de Pesquisas 'René Rachou', Avenida Augusto de Lima 1715, Caixa Postal 1743, 30 000 Belo Horizonte, Brazil
- DVORAK, Dr J., Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205, USA
- GONZALEZ-CAPPA, Dr S.M., Department of Microbiology and Parasitology, Faculty of Medicine, University of Buenos Aires, Paraguay 2155, Buenos Aires, Argentina
- LA FUENTE, Dr C., Centro Nacional de Enfermedades Tropicales (CENETROP), Ministerio de Prevision Social y Salud Publica, Casilla 2974, Santa Cruz, Bolivia
- MILES, Dr M.A., London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT, UK
- MOREL, Dr C.M., Biochemistry and Molecular Biology Department, Instituto Oswaldo Cruz, Avenida Brasil 4365, Manguinhos, CEP 21040, Rio de Janeiro, Brazil
- PONCE, Dr C., Laboratorio Central, Ministerio de Salud, 3° piso, Centro de Salud 'Dr Alonso Suazo', Tegucigalpa DC, Honduras
- PRATA, Prof A., Department of Tropical Diseases, University of Brasilia, Faculdade de Ciencias da Saude, 70 000 Brasilia, Brazil
- SCHENONE, Dr H., Universidad de Chile, Facultad de Medicina, Casilla 9183, Santiago, Chile
- SEGURA, Dr E.L., Instituto 'Dr M. Fatala Chaben' (INDIECH), Av. Paseo Colon 568, 7° piso, Buenos Aires 1063, Argentina
- SOUSA, Dr O.E., Gorgas Memorial Laboratory, Apartado Postal 6991, Panama City 5, Republic of Panama
- * TIBAYRENC, Dr M., Instituto Boliviano de Biologia de la Altura, Casilla 824, La Paz, Bolivia

WHO Secretariat

- MONCAYO, Dr A., Trypanosomiasis and Leishmaniases Unit, Geneva, Switzerland

* Unable to attend

ANNEX I

LIST OF COLLABORATING LABORATORIES

- Argentina: Dr E.L. Segura
Instituto 'Dr M. Fatała Chaben' (INDIECH)
Av. Paseo Colon 568
7° piso, Buenos Aires 1063
Tel: 33-7732; 33-2330
- Dr A.C.C. Frasch
Fundación Campomar
Antonio Machado 151
1405 Buenos Aires
Tel: 884013. Telex: 18694 IBUBA-AR
- Bolivia: Dr C. La Fuente
Centro Nacional de Enfermedades Tropicales (CENETROP)
Casilla 2974
Santa Cruz
Tel: 39101
- Brazil Dr C.M. Morel
Biochemistry and Molecular Biology Department
Instituto Oswaldo Cruz
Avenida Brasil 4365 - Manguinhos
Cep. 21040, Rio de Janeiro, RJ
Tel: (021) 2907549; (021) 2702946
Telex: (021) 23239 FUOC BR
- Chile: Dr A. Solari
Depto. de Biología Celular y Genética
Facultad de Medicina-Norte
Universidad de Chile
Casilla 6556
Santiago-7
Tel: 370081/776560 (Ext. 5214)
- Colombia: Dr N. Saravia
Centro Internacional de Investigaciones Médicas (CIDEIM)
Apartado Aéreo No. 5390
Cali
- Costa Rica: Dr R. Zeledon
Escuela de Medicina Veterinaria
Universidad Nacional
Apartado 86
Heredia
- Honduras: Dr H. Cosenza
Departamento de Microbiología
Universidad Nacional Autónoma de Honduras
Tegucigalpa DC
- Dr C. Ponce
Ministerio de Salud
Laboratorio Central
Centro de Salud 'Dr Alonso Suazo', 3° piso
Tegucigalpa DC
Tel: 32 11 35

Mexico: Dr L. Zarate
Centro de Investigaciones Ecologicas del Sureste (CIES)
Calle Real de Guadalupe 55
San Cristobal de las Casas
Chiapas

Peru: Dr H. Lumbreras
Instituto de Medicina Tropical
'Alexander von Humboldt'
Universidad Cayetano Heredia
Calle Honorio Delgado No. 932, Ap. 5045
Lima

Republic of Panama: Dr O.E. Sousa
Laboratorio Conmemorativo Gorgas
(Gorgas Memorial Laboratory)
Depto. de Parasitologia
Apartado Postal 6991, Panama City 5
Tel: 27-4111
Telex: 3433

United Kingdom: Dr M.A. Miles
London School of Hygiene and Tropical Medicine
Keppel Street (Gower Street)
London WC1E 7HT
Tel: (01) 636-8636 (399)
Telex: 8953474

United States: Dr J. Dvorak
Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, MD 20205
Tel: (301) 4964880

Venezuela: Dr J. Urbina
Centro de Biologia Experimental
Calle Suapure - Colinas de Bello Monte
Universidad Central de Venezuela
Caracas 106
Tel: 751.0111

ANNEX II

DESCRIPTION SHEET FOR INTERNATIONAL REFERENCE STRAINS OF T. CRUZI

WHO NOMENCLATURE:

OTHER CODES:

ISOLATION:

- Triatomine - species
 - region and site of isolation (house, nest, etc.)
 - natural infection or after xenodiagnosis
- Reservoir - species
 - region and locality
- Patient - name, age, sex, colour
 - locality where infection took place
 - duration of infection before isolation

CLINICAL FORM:

- Acute - apparent
 - inapparent
- Chronic - indeterminate
 - cardiac
 - digestive
 - nervous (denervation)
 - mixed (specify)

THERAPY (if any):

When, which drug, etc.

ADDITIONAL INFORMATION:

If disease was due to transfusion or congenital transmission

If patient died

Isolator/method of isolation

Donor

Available from

LABORATORY DATA:

Replication time (specify medium, temperature)

Nuclear and kinetoplast DNA content

Drug susceptibility data (survival, mortality, cure rates)

- nifurtimox
- benznidazole

Course of infection in experimental animals (provide detailed data)

Zymodeme (specify enzymes used)

Schizodeme analysis (Eco RI) (others if possible)

Antigen characterization

MAB characterization

Lectin reactivity data

Other data

REASONS FOR INCLUSION IN REFERENCE STRAIN BANK:

REFERENCES:

ANNEX III

LIST OF WORKING PAPERS PRESENTED AT THE MEETING*

- "Overview of Trypanosoma cruzi Classification and Taxonomy"
Z. Brener (TDR/EPICHA-TCC/84.4)
- "The Significance of Trypanosoma cruzi Differentiation and Selection:
Possible Relationship with Clinical and Epidemiological Disease Varieties"
A. Prata (TDR/EPICHA-TCC/84.5)
- "Design of Analytical Epidemiological Studies to Determine Possible
Associations between Strains and Clinical Varieties"
C.M. Antunes (TDR/EPICHA-TCC/84.6)
- "Isolation and Amplification Techniques" O.E. Sousa (TDR/EPICHA-TCC/84.7)
- "Single cell isolates of Trypanosoma cruzi. How and why?" J. Dvorak
(TDR/EPICHA-TCC/84.8)
- "Morphological and Behavioural Characterization of Trypanosoma cruzi
Strains" S.G. Andrade (TDR/EPICHA-TCC/84.9)
- "Antigen Characterization of Trypanosoma cruzi"
E.L. Segura and J.J. Cazzulo (TDR/EPICHA-TCC/84.10)
- "Isoenzyme Characterization" M.A. Miles (TDR/EPICHA-TCC/84.11)
- "Trypanosoma cruzi: Lectin-Interaction Analysis"
S.M. Gonzalez-Cappa and A.M. Katzin (TDR/EPICHA-TCC/84.12)
- "Schizodeme Analysis of Trypanosoma cruzi"
A.M. Gonçalves, N.S. Nehme and C.M. Morel (TDR/EPICHA-TCC/84.13)

* These unpublished documents are not available from TDR but will be published in a special issue of the Revista da Sociedade Brasileira de Medicina Tropical.

.....