



SERODIAGNOSIS KIT
OF HUMAN LEPTOSPIROSIS AND CHAGAS DISEASE

IDEASS NICARAGUA

Innovation for Development and South-South Cooperation

Introduction

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THE CNDR/MINSA ELISA LEPTO KIT is a laboratory technique for the prompt diagnosis of human leptospirosis using samples of serum, plasma and blood from suspected leptospirosis patients. The KIT gives rapid laboratory results (2^{1/2} hours), is cheap and provides high diagnostic resolutions (100% sensitivity and 99.6% specificity).

Generally speaking, laboratory techniques are extremely useful tools in the diagnosis of infectious illnesses. If these tools are produced at the national level, a country can make big economic savings and provide a greater number of poor people with ready access to diagnosis and treatment. The Nicaraguan Ministry of Health developed a laboratory technique for the serologic diagnosis of human leptospirosis, which also provides early warning of outbreaks of this deadly disease, avoiding deaths through prompt diagnosis and effective antibiotic therapy.

Human Leptospirosis is a zoonosis caused by the genus *Leptospira* Interrogan. The illness is produced when the bacteria penetrate the organism through various natural and artificial ways, producing symptoms that are characterized by general ill health accompanied by icterohemorrhagic fever. Leptospirosis is a fatal infectious disease and can cause serious epidemics, as happened in Achuapa - El Sauce in 1995, when more than 46 people died and another 3,000 patients needed hospital treatment. Fortunately the illnesses can be cured

by antibiotics. For this reason, it is important to have good diagnostic instruments to anticipate epidemics, which occur mainly in the winter season when there are higher transmission rates. In winter, rain causes floods in which sources of water used for human consumption are polluted by rodents and domestic animals.

In 2004, more than 10,000 people were accurately tested in Nicaragua, with laboratory results that were rapidly available (2^{1/2} hours). Since this technique was introduced in Health Ministry services, in 2001, there have been no deaths from this deadly disease in Nicaragua.



The CNDR/MINSA CHAGAS KIT is another laboratory technique that provides prompt diagnosis of Chagas disease in suspected patients and can be used to screen serum, plasma and blood samples. The KIT gives quick laboratory results (2^{1/2} hours), it is cheap and provides high diagnosis resolutions (100% sensitivity and 98.4% specificity). The Nicaraguan Ministry of Health developed this laboratory technique for the serologic diagnosis of Chagas Disease. At the time, it was the only country in Central America that did not have the economic and technical resources to conduct studies on this parasite. For this reason many aspects concerning prevention and control were unknown in the country.

Chagas Disease does not produce any specific symptoms, which generally remain unobserved until, after several years, it causes death mainly as a result of heart problems. This illness caused by a hemoparasite, *trypanosoma cruzi*, which is transmitted to people by the bite of the triatomine or assassin bug. These insects are common throughout Nicaragua and their natural habitats are closely associated with the bad living conditions of poor rural areas.

The Health Ministry, through the CNDR (national diagnostics and reference centre), and economic backing from TAIWAN, guarantees screening of Chagas Disease in 19 blood banks belonging to the SILAIS (local integrated health systems) of Madriz, Nueva Segovia, Estelí, Matagalpa, Jinotega, Boaco, León, Chinandega, Granada, Masaya, Carazo, Rivas, Río San Juan and the RAAS. This procedure ensures that 70,000 patients depending on transfusions in these health units do not receive blood that is infected by hemoparasites, which quickly leads to illness and even death.

In 2004, the Leptospirosis and Chagas Disease Diagnosis KITS won a human development innovations prize, promoted by the CONYCIT-Nicaragua, in collaboration with UNDP.





What problem does it solve?

THE SERODIAGNOSIS KIT OF LEPTOSPIROSIS

Each year, there used to be more than a hundred deaths and common outbreaks of Leptospirosis in Nicaragua, with mortality rates of up to 1.5 for every 10,000 thousand inhabitants. At present, more than 10,000 people are tested each year using this reliable and quick system of diagnosis (results are available in 2^{1/2} hours).

The Serodiagnosis KIT of Human Leptospirosis solves the following problems:

- it prevents human deaths due to an illness, such as Human Leptospirosis, which is easily treated, through a cheap and versatile laboratory technique which provides prompt, reliable and quick diagnosis.
- it is a laboratory technique that provides early warning of Leptospirosis outbreaks in Nicaragua, and reduces loss of human life from this deadly illness. The KIT ensures that every person who is suspected of being infected by

Leptospira spp bacteria, can be quickly and appropriately tested. This means that they receive prompt treatment (antibiotics), thus avoiding the complications that can cause death in a few hours or days.

- it increases diagnostic resolution levels in MINSA Health Units, which acquire the capacity to carry out epidemiological studies at the local and national levels, so that health authorities may have an up-to-date epidemiological characterization of the seriousness of the health problem that is represented by Human Leptospirosis in Nicaragua.
- reagents can be produced at the national level. Nicaragua is the only country in Central America which produces the Human Leptospirosis Diagnosis KIT and which has a functional network of laboratories providing early warning of outbreaks of the disease. In 2004, national supply was ensured by a production of 30 CNDR/MINSA LEPTO KITS, saving the country more than US\$ 160,000.00 a year, the amount which would have been spent if the country had had to buy them commercially.

The innovation was first introduced in 2001, when a series of studies were carried out to reproduce, select and adapt strains of Leptospira spp for artificial laboratory conditions. This provided the bases for sources of antigenic extracts from strains of Leptospira spp.

A second phase, spanning 2001 and 2002, involved standardizing and setting up production of ELISA (Enzyme-linked Immuno Sorbent Assay) KITS for the serodiagnosis of acute Human Leptospirosis. The technique presents intrinsic qualities such as: high diagnostic resolution (100% sensitivity and 99.6% specificity); quick laboratory results (2^{1/2} hours); simultaneous processing of several samples, since it is designed in strips that allow for the processing of between 1 and 96 samples at any one time; qualitative (colour intensity) or quantitative (ELISA readings) results; a cost that is 20 times lower than other similar techniques.



During the third stage, the technique was perfected in a standardized diagnostic KIT that can be reliably replicated by laboratory technicians working in MINSA health units. It was given the name CNDR/MINSA LEPTO KIT.

In 2002, national production of the CNDR/MINSA - LEPTO KIT enabled the Nicaraguan Ministry of Health, through the CNDR and the economic backing of the European Union, to decentralize the diagnosis of Human Leptospirosis through a network of 12 SILAIS (integrated local health care systems) and hospital laboratories, which carry out laboratory diagnosis and offer early warning of possible outbreaks of this illness.

THE SERODIAGNOSIS KIT OF CHAGAS DISEASE

In 1998, Nicaragua's CNDR/MINSA laboratory for Medical Parasitology, with the economic backing of the World Health Organisation's special program for Tropical Disease Research (TDR/WHO), began work which eventually led to the production of this KIT.

The CNDR/MINSA - Chagas Disease KIT is the result of more than five years research, which began with work to obtain local strains of *Trypanosoma Cruzi* from patients who had just caught Chagas Disease, adapting them to laboratory conditions and later using them as sources of antigens to standardize an ELISA (Enzyme-linked Immuno Sorbent Assay) type technique. Later this technique was perfected in a KIT for the diagnosis of Chagas Disease in its early acute and chronic phases.

The laboratory acquired the technical scientific know-how to isolate parasites from Chagas case patients, later adapting these national strains to artificial laboratory conditions. This provided the basis for the source of antigenic extracts, which later led to the preparation, standardization and production of this ELISA technique for the serodiagnosis of Chagas Disease.



The technique has the following intrinsic qualities: high diagnostic resolution (100% sensitivity and 98.4% specificity); quick laboratory results (2 hours); simultaneous processing of several samples, since it is designed in strips that allow for the processing of between 1 and 96 samples at any one time; qualitative (colour intensity) or quantitative (ELISA readings) results; a cost that is 8 times lower than other commercial techniques.



Having acquired a highly reliable ELISA diagnostic technique, in 2000, MINSA laboratories succeeded in perfecting it into a standardized diagnostic KIT, which can be readily replicated by laboratory technicians in all national health units. It was given the name CNDR/MINSA - Chagas KIT.

This technological advance enabled the Health Ministry, through to the CNDR and with the economic backing of TAIWÁN, to introduce, within in six months, the screening of Chagas Disease in 19 Blood Banks belonging to the SILAIS (integrated local health care systems) of Madriz, Nueva Segovia, Estelí, Matagalpa, Jinotega, Boaco, León, Chinandega, Granada, Masaya, Carazo, Rivas, Río San Juan and the RAAS.

This process enabled more than 70,000 patients, whose lives depend on transfusions in these health units to receive blood that contained no hemoparasites which cause rapid illness and even death. The CNDR/MINSA - CHAGAS KIT is used to screen Chagas Disease in blood banks to prevent transmission of the disease through transfusions. By having this technique at their disposal, health units throughout the country can carry out on site diagnosis, epidemiological studies and assessments on the effectiveness of the treatment of Chagas patients.



The CNDR/MINSA - Chagas KIT solves the following problems:

- prevents the transmission of Chagas Disease through blood transfusions by screening blood donors, in accordance with Law no.369 concerning 'transfusion security' in Nicaragua.
- increases diagnostic resolution levels in national health system laboratories, providing them with a prompt diagnosis technique for Chagas Disease, which gives cheap, rapid, and reliable laboratory results, and which is accessible to the poorest sectors of the Nicaraguan population.

The Leptospirosis and Chagas KITS in practice

THE LEPTOSPIROSIS KIT

The Leptospirosis KIT is a laboratory technique prepared specifically for the in vitro diagnosis of human Leptospirosis in the acute or early stage of the illness from serum of suspected leptospirosis patients. The KIT comes in a commercial diagnostic packet.

The way the KIT is designed means it can be easily replicated in any regional or local laboratory that has a minimum amount equipment and technical personnel with some basic experience. Among the most important intrinsic characteristics of the Leptospirosis KIT is that it utilizes, as the source of the antigens, a somatic extract of *Leptospira* serovar Copenhageni strain Wijnberg, which has entered the exponential phase. The extract is useful in the diagnosis of acute Human Leptospirosis after the first few days of infection.

The Leptospirosis KIT is a technique providing high diagnostic resolution, with a 100% sensitivity and 99.6% specificity. It gives quick laboratory results (2^{1/2} hours), enables several samples to be processed simultaneously, since it is designed in strips that allow for the processing of between 1 and 96 samples at any one time. The results can be attained qualitatively from colour intensity, which means that the analysis of the samples can easily be carried out in peripheral laboratories. Results can be attained quantitatively when laboratories are equipped with ELISA readers. The KIT is a low cost technique, since it is nationally produced. In Nicaragua the KIT costs 20 times less than other commercial techniques.

THE CHAGAS KIT

The CNDR/MINSA – Chagas KIT is an enzyme immunoassay (ELISA) for the in vitro diagnosis of Chagas Disease, based on the detection of *T.cruzi* antibodies in serum of suspected Chagas patients. The antigen used is a somatic extract of the tripomastigotes

parasite, useful in the diagnosis of chronic and acute American trypanosomiasis after the first week of infection.

When a patient is clinically suspected of suffering from the illness, a blood sample is taken, which is analysed using the CNDR/MINSA - CHAGAS KIT. Results can either confirm (Chagas positive) or exclude (Chagas negative) infection.



The Chagas KIT produces the following results:

- **Prompt diagnosis of Chagas Disease.**

It is very important to detect Chagas patients as quickly as possible, especially in rural areas where the illness is endemic because of the conditions of extreme poverty in which the

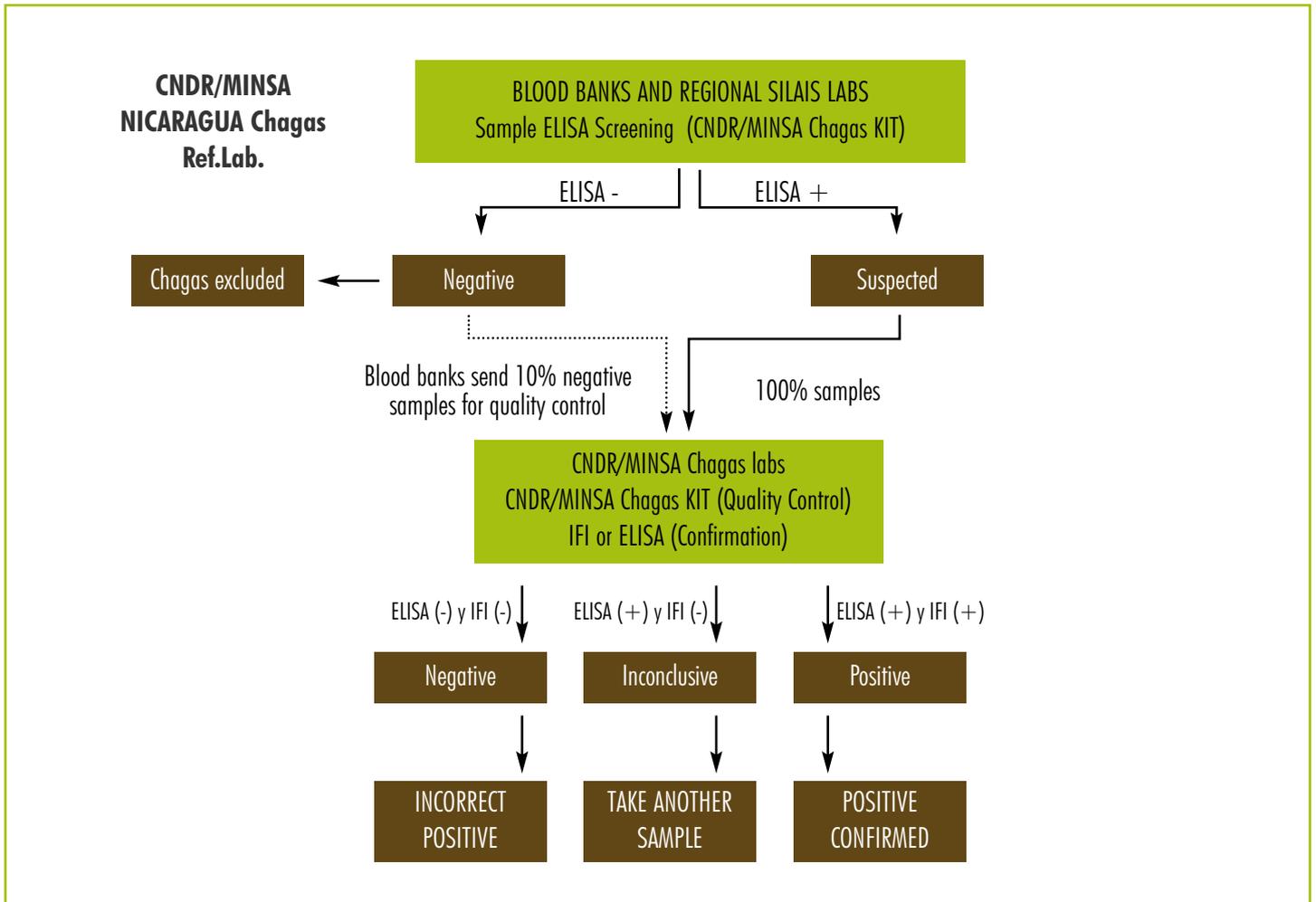


population lives. Early detection allows the Health Ministry to provide patients with prompt treatment. International studies show that treatment is very effective in the early stages of the illness, mainly in children under 15 years.

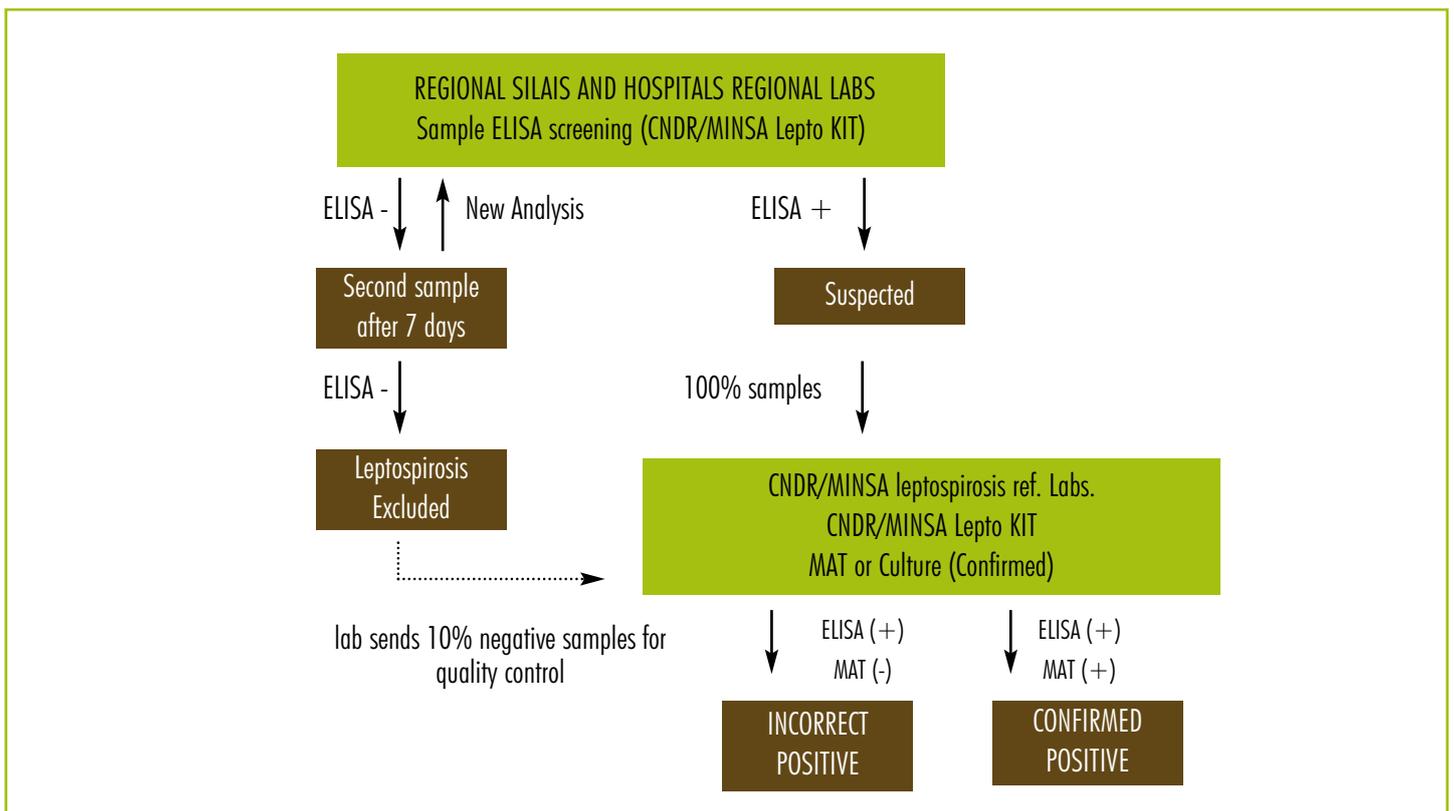
- **Guarantees safe blood.** The Chagas KIT enables the Health Ministry to screen all blood banks belonging to the national health system, guaranteeing that patients whose lives depend on blood transfusions or blood products do not get infected. *Tripanosoma cruzi* parasites can produce irreversible lesions in patients, leaving them incapacitated for the rest of their lives and even leading to death after a few years.
- **Availability of diagnosis at the local level.** The Chagas KIT allows a greater number of Nicaraguans to access the test free of charge in the health units of their respective territories. It allows local health personnel to follow and evaluate the efficiency Chagas treatment in patients treated in respective units.
- **Capacity to carry out epidemiological studies.** The studies enable samples to be carried out in areas of the country that are difficult to reach so that active transmission of the disease can be detected and prompt treatment provided. Health personnel also acquire a higher capacity to evaluate the effectiveness of vector control activities in endemic zones.



STEPS IN THE SERODIAGNOSIS OF CHAGAS DISEASE



STEPS IN THE SERODIAGNÓIS OF HUMAN LEPTOSPIROSIS



Results

LEPTOSPIROSIS KIT

In a short time, Nicaragua has made great progress in terms of knowledge and prevention of Human Leptospirosis. Nicaragua is now the only country in Central America that produces a standardized CNDR/MINSA - LEPTO KIT, which guarantees diagnosis of the disease and early warning of possible outbreaks through a network of nationwide laboratories.

The leptospirosis KIT enables regional and hospital laboratories to increase diagnostic resolution levels. Each year, more than 10,000 poor people living in isolated zones have prompt access to this laboratory service, free of charge. The illness can be promptly detected in people that live in high-risk areas, or whose jobs make them susceptible to Leptospiras, providing them with immediate care and treatment through the prescription of specific medicine (antibiotics), avoiding later complications which can cause severe health risks or even death in a few hours or days.

Prior to this there were more than a hundred deaths and outbreaks of Leptospirosis each year in Nicaragua, with death rates of up to 1.5 per 10,000 thousand inhabitants.

Decentralization has led to the establishment of an early warning network for epidemiological outbreaks, which has brought great benefits in terms of health. Firstly, decentralization has raised the level of diagnostic resolution of Human Leptospirosis and early warning of epidemiological outbreaks in the 12 MINSA local integrated health care systems (SILAIS). Secondly, each SILAIS acquired the technical capacity to carry out studies at the local and national level, so that health authorities can have an up-to-date epidemiological characterization of the magnitude of the public health problem that is represented by Human Leptospirosis in Nicaragua.

The conventional technique of using live antigens in Microscopic Agglutination Tests (MAT) can only be carried out in laboratories that meet all biosecurity requirements. Furthermore, conventional techniques can only process a few samples (250 to 300 a year) and results take up to two weeks to come out. With the introduction of the ELISA Leptospirosis KIT, a great improvement has been made. This technique can be implemented in any laboratory with the most basic laboratory resources and conditions. Laboratory results take just two hours and up to 96 samples can be processed at any one time.

This technological advance, which has come about in the last few years, has had a great impact on the health of the poorest section of the population, who live in the most isolated parts of the country. If patients are not diagnosed in time, they may die from the consequences of leptospirosis infection.



Since this new technology was introduced in national health services, in 2001, there have been no deaths in Nicaragua from this illness.

THE CHAGAS KIT

In Nicaragua, the CNDR/MINSA - Chagas KIT was given a positive scientific assessment by the Nicaraguan Red Cross (National Blood Centre, Managua); the blood banks of the Nicaraguan Red Cross in Estelí, Matagalpa and Juigalpa; the MINSA laboratories in Granada and León.



The Chagas KIT has enabled the Health Ministry to achieve the following results in Nicaragua:

- for the first time, the Ministry was able to guarantee safe blood in Nicaragua, through the screening of Chagas Disease in all blood donated in 19 blood banks in the National Health System. This prevents the transmission of this deadly disease to more than 1,500 people depending on blood transfusions and blood products every year.
- increased diagnostic resolution levels in all MINSA Health Units. By developing the capacity to carry out studies at the local and national levels, health authorities can have an up-to-date epidemiological characterization of the magnitude of the public health problem represented by Chagas Disease in the country.
- increased capacity to recognise Chagas case patients in the early stages of the illness, mainly in children under 15 years of age, allowing for the prescription of effective treatment. The capacity of local health personnel to evaluate and keep track of controlled treatments has also increased.

- a low cost nationally produced KIT that is always available. This has enabled the Health Ministry to decentralize the serodiagnosis of Chagas through the country's nationwide network of health units. In this way, Nicaragua now boasts prompt diagnosis of this illness through a service which is also accessible to the poorest people.
- annual national production of an average of 50 Chagas Diagnosis KITS earns the country US\$ 80,000.00 a year in monetary savings, which would have been spent on importing the product.



International interest

LEPTOSPIROSIS KIT

In **2003**, the leptospirosis KIT was given international recognition for its diagnostic qualities by the International Leptospirosis Reference Centre in Holland.

PAHO/WHO has provided the Nicaraguan Health Ministry with special support in its efforts to find a solution to the problem of Human Leptospirosis. Since 2001, it has actively supported all the innovation process. In particular, it supported the development of all field and laboratory studies, with scientific advice from international experts, materials, reagents, and professional training in countries such as Brazil, Cuba, the United States and Venezuela.



The European Union actively supported the process of decentralizing the diagnosis of Leptospirosis in the 12 regional SILAIS laboratories, by providing materials, capacity building, and technical training for laboratory technicians in all the country's health units.

In **2004**, the innovation won a prize for human development innovation in a competition promoted by CONYCIT, in collaboration with UNDP.

CHAGAS KIT

The CNDR/MINSA Chagas KIT received highly positive evaluations in scientific tests carried out by the following institutions:

- Laboratorio de Referencia para Chagas, sub-región de Centroamérica, Dr. Ponces - Honduras, 1999
- Laboratorio Central de Guatemala, 2001

In **2004**, the innovation received a prize in the National Competition of innovation for Human Development promoted by CONYCIT, in collaboration with PNUD.



Using the LEPTOSPIROSIS AND CHAGAS KIT in other countries



In general, technology is making rapid progress. This is particularly true in the developed world, but not in the case of developing countries where economic resources are few or even non-existent. For this reason, the perfection of new biomedical technologies is vital for rapid progress and health improvement.

LEPTOSPIROSIS KIT

The innovative technology behind the leptospirosis KIT can be transferred to any health ministries in Latin American countries that want to effectively deal with the problem of Human Leptospirosis and create an early warning system for outbreaks. The KIT can be produced and applied directly by health ministries in any country, with no need for commercial intermediaries.

The PAHO/WHO, through their national and representative offices, can support the process of transferring the technology from Nicaragua.

Despite the fact that the technical and scientific bases of ELISA type assays date back to the 1980s, modifications and applicability are subject to restriction from international legislation as a result of commercialisation. Restrictions do not apply if the assays are applied locally and not produced for commercial ends.

The KIT for the serologic diagnosis of Human Leptospirosis is an innovative product that can be easily replicated in other Latin American countries with laboratories that have the capacity for diagnostic resolution and meet requirements of biosecurity.

THE CHAGAS KIT

Despite the fact the technical and scientific bases of ELISA type assays date back to the 1980s, modifications and applicability are subject to restriction from international legislation as a result of commercialisation. Restrictions do not apply if the assays are applied locally and not produced for commercial ends.

The innovative technology behind the KIT can be transferred to any health ministries in Latin American countries that want to effectively deal with the problem of Chagas Disease. The KIT can be produced and applied directly by health ministries in any country, with no need for commercial intermediaries.

The CNDR/MINSA Chagas KIT is a finished product which can be easily replicated in other Latin American countries that have laboratories meeting diagnostic resolution standards.

To learn more

LEPTOSPIROSIS BIBLIOGRAPHY

- Hartskeerl, R. Smits, H. Korver, H. Goris, H. and Terpstra WJ. 2000. **International course on laboratory methods for diagnosis of leptospirosis**. Royal Tropical Institute. Amsterdam.
- Acha Pedro N, Boris Szufres. 2000. **Zoonosis y enfermedades transmisibles comunes al hombre y a los animales**. Second edition. Scientific publication No. 503.
- Murray Patrick R. Baron Ellen Jo, Pfaller Michael A, Tenover Fred C, Tenover Robert H. 1, 999. **Manual of Clinical Microbiology**, 7th Edition, ASM PRESS. 3 (1982), pag 739-745.
- Faine, S, Adler, B. Bolin, C. Perolat, p. 1, 999. **Leptopira and Leptospirosis**, MediSci. Melbourne, Australia, 2nd Edition.
- Trevejo, et al. 1998. Epidemic Leptospirosis associated with pulmonary hemorrhage- Nicaragua, 1995. *The Journal of infectious Diseases*, 178:1457-63.
- Terpstra, WJ. Ligtharta, GS, Schoone GJ, 1985 **Serodiagnosis of human leptospirosis for ELISA**. *Zbl. Bact. I. Abt.* A247, 400-405.
- Terpstra, WJ. Ligtharta, GS, Schoone GJ, 1985. **ELISA for detection of specific IgM and IgG in human leptospirosis**. *J. Gen. Microbiol.* 131, 377-385.
- Faine S. et al. 1982. Guidelines for the control of leptospirosis. WHO Geneva. 111 -113.
- Turner LH. 1968. Leptospire II: Serology. *Trans R Soc Trop Med Hyg.* 62, 880-899.

CHAGAS BIBLIOGRAPHY

- Moncayo, A and O. Luqueti, 1990. Multicentre double blind study for evaluation of tripanosoma cruzi defined antigens as diagnostic reagent. *Mem. Inst. Oswaldo Cruz.* 85:489-495.
- Schmunis, G.A, 1991. Tripanosoma cruzi, the etiologic agent of Chagas disease: status in the blood supply in endemic and non endemic countries. *Transfusion* 31: 547-555.
- Pastini, A.C, Iglesias, S.R, Carricarte, V.C, Guierin, M.E, Sanchez, D.O and Frasch, A.C. 1994. Immunoassay with recombinant Tripanosoma cruzi antigens potentially useful for screening donated blood and diagnosing Chagas disease. *Clin. Chem.* 40:1893-1897.
- Schmunis, G.A, 1999. Prevention of transfusional T. cruzi infection in Latin America. *Mem. Inst. Oswaldo Cruz.* 94: 93-101.
- Umezawa, E.S, M.S Nacimiento, and A.M, Stolf. 2001. Enzyme-Linked immunosorbent assay with T. cruzi excreted-secreted antigens (TESA-ELISA) for serodiagnosis of acute and chronic Chagas disease. *Diagn. Microbiol. Infect. Dis.* 39:169-176.
- WHO, 2002. Control of Chagas Disease. 2nd experts commission report.

Contacts

The CNDR/MINSA Leptospirosis and Chagas laboratories have two experienced technical and scientific teams, who can give technical advice and help transfer the innovation to other laboratories in interested regions.

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The IDEASS Programme – Innovation for Development and South-South Cooperation – is part of the international cooperation Initiative ART. IDEASS grew out of the major world summits in the 1990s and the Millennium General Assembly and it gives priority to cooperation between protagonists in the South, with the support of the industrialised countries.

The aim of IDEASS is to strengthen the effectiveness of local development processes through the increased use of innovations for human development. By means of south-south cooperation projects, it acts as a catalyst for the spread of social, economic and technological innovations that favour economic and social development at the local level. The innovations promoted may be products, technologies, or social, economic or cultural practices. For more information about the IDEASS Programme, please consult the website: www.ideassonline.org.

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Innovation for Development and South-South Cooperation



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It is in the framework of ART GOLD Programmes where IDEASS innovations are promoted and where cooperation projects are implemented for their transfer, whenever required by local actors.