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REPORT ON PAH0/WHO COOPERATIVE ACTIVITIES THROUGH THE BIOTECHNOLOGY SUBPROGRAM

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REPORT ON PAHO/WHO COOPERATIVE ACTIVITIES THROUGH THE BIOTECHNOLOGY SUBPROGRAM

Although the Pan American Health Organization (PAHO/WHO) has been carrying out activities promoting biotechnology since 1983, it is only since 1987 that a cooperative program in this field was established and a the Biotechnology Subcommittee of the Advisory Committee on Health Research (ACHR) was created to advise on its activities. Throughout these years, the program has been conducting basically three types of activities: (1) those related to country support to define policies on biotechnology development and regulation[1]; (2) training in selected technologies; and (3) support for research projects in priority areas.

In 1992, joint development of training activities began with PAHO and the UNDP/UNESCO/UNIDO Regional Program on Biotechnology. The chairman of the PAHO Biotechnology Subcommittee and the Director of this regional program met to define plans for training and course selection. The jointly financed training courses were approved on the basis of the following criteria: topic, scientific excellence, and geographic location. These courses had a minimum duration of two weeks. The previously selected students received financial assistance to attend the training courses and were evaluated upon completion of the course. Detailed reports on the courses are located in the PAHO Research Coordination Unit. In addition to the six courses detailed in Appendix I, an additional course was selected but not offered, due to local difficulties.

Through these courses, PAHO contributes to training young people in different technologies and programming their use in research and development projects undertaken in their countries. The goal was to broaden access to technologies aiding in the diagnosis and vaccine development for selected diseases.

From 1988 to the present, 26 biotechnology projects have been financed under the PAHO Research Grants Program. Twelve research and development projects on malaria, HIV, and hepatitis B, were approved (see Appendix II), and completed between 1989 and 1990.

The results of three malaria projects were evaluated. These projects focussed on the development of the immunodiagnosis of the infection produced by *Plasmodium vivax*.

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[1] Examples of this line of cooperation include: the publication of guidelines on biosafety in the management of recombinant DNA techniques and liberation of modified microorganisms in the environment, the seminar on technology transfer in biotechnology held in Caracas in 1989, and the seminar on biotechnology and biodiversity held in San José in 1994.

Procedures were developed to combine the use of monoclonal antibodies and fluorescence readings, permitting the specific identification of the species involved in the infection.

Parts of the HIV projects were devoted to the acquisition of serum panels from Brazil and Argentina. Thus, around 500 samples with an average volume of 150 ml. and characterized in all aspects known in 1989, are presently at the disposal of investigators. These sera are found partly in the AIDS Reference Laboratory of Canada, which offered to distribute them, and in the AIDS Reference Laboratories of the Oswaldo Cruz Institute of Brazil and the School of Medicine of the University of Buenos Aires in Argentina. In other projects in this area, HIV-1 was isolated from 30 patients from Argentina, Brazil, and Mexico, and approximately 15 of them were characterized. Some of the isolates from Brazil showed polymorphism with regard to the prototype HIV-1 (HTLV IIIb). In the isolates from Argentina, a structural difference was identified in the loop portion of the V 3 region of the gene in the sheath glycoprotein. In another area, support was provided to complete kit development to rule out infection by HIV, which is currently being marketed by Cuba.

With respect to hepatitis B, project support was given to develop monoclonal antibodies, which yielded a reagent that is utilized in Argentina in the Reference Center of the Carlos G. Malbrán Institute. In short, the 12 financed projects contributed a considerable body of knowledge. Eleven institutions in four countries were strengthened and two biotechnological products, three panels of monoclonal antibodies against *Plasmodium* and hepatitis B, two panels of anti-HIV sera, and three banks of well-characterized strains of HIV, in Mexico, Brazil, and Argentina, were obtained.

In the period from 1990 to 1994, 14 research projects were supported. One of these--a multicenter project integrating four working groups in three countries (Argentina, Brazil, and Mexico)--was aimed at development of a kit to diagnose HIV infection. The following results were obtained: installed capacity for peptide production in the laboratories of INTEBIO, Santa Fe, Argentina, and the School of Medicine, Biomedical Research Institute of the UNAM in Mexico; an anti-IgG serum, marked with peroxidase and possessing potential commercial value, developed in the Butantán Institute in São Paulo, Brazil; and an HIV diagnostic kit by means of an immunoenzymatic reaction utilizing five selected peptides--proteins 17 and 24 and glycoproteins 36, 41, and 120--produced in the aforementioned laboratories. Testing of the diagnostic kit was conducted at the Oswaldo Cruz Institute in Rio de Janeiro, Brazil.

The discovery of monoclonal antibodies (MAb), announced in a publication by Kohler and Milstein in 1975, triggered a revolution in medicine by introducing the possibility of having unlimited amounts of immunoglobulins of high specificity. PAHO supported projects applying these MAbs to diagnose *Plasmodium* infections, as mentioned previously. Other applications include: the development of a dot-blot test to identify antigens to *Taenia solium* in the cerebrospinal fluid of patients and pigs to diagnose cysticercosis and prepare specific *Trypanosoma cruzi* antigens for the serological diagnosis of Chagas' disease (see Appendix II). The MAbs have also been applied in cancer treatment, although with less effectiveness. This may be because it is still not possible to produce sufficient volumes with consistent sensitivity or provoke an immune response or efficiently initiate the cascade of participation of human complement. PAHO approved a project which incorporated a possible solution to the clinical and diagnostic problems, based on the use of MAbs: the design of biospecific antibodies to obtain specific monovalent reagents (bsMAb), based on the fusion of two different cell lines of hybridomas in the murine cancer therapy model, for application to human tumors (see Appendix II). The investigators obtained a bsMAb-mediator of cytotoxicity against the cells of human tumor lines. Its function *in vivo* was tested by radioactive marking of the murine tumor in animals treated with different dosages of bsMAb-1251.

The PAHO Biotechnology Subcommittee recommended prioritizing human resource training in the Region in the field of genetic diagnosis, particularly in the use of the polymerase chain reaction (PCR). In this regard, the grants program approved projects related to the diagnosis of *Vibrio cholerae*, *Plasmodium* and hepatitis C virus (HCV) infections (see Appendix II). Detection by PCR in the ctx A1 region, starting from genomic DNA, manifested the expected outcome of 431 pairs of bases only in the toxigenic strains of *V. cholerae*, verifying the amplification specificity. Another project partially sequenced the 5' UT genomic region of HCV. In a sample obtained from 13 multitransfused newborns negative to ELISA tests, RNA was extracted from the serum and a fragment of 210 pairs of bases was obtained, after carrying out reverse transcription and nested PCR. The sequence obtained was identical to the HCV 1 prototype, except for positions 247 and 248; TC nucleotides were detected, instead of CT, which may indicate a virus mutation. Among the 21 samples not reactive with ELISA 2, two were positive by nested PCR, utilizing published primers corresponding to the uncoded 5' UT region of the HCV. In another aspect of the diagnosis using genetic methods, artificial targets have been produced for ribosomal genes of *Plasmodium vivax* and capture probes for *P. vivax* and *P. falsiparum*. High specificity has been demonstrated in the liberation phase, utilizing ribonuclease H and standardizing the

utilization of a nonradioactive signal to measure the chain reaction product (see Appendix II).

In addition to the regular research grant support in biotechnology, in 1994 PAHO joined with the U.S. National Institutes of Health (NIH) in establishing a special initiative, summoning countries of the Region to present joint projects with American investigators to develop biological markers and therapy, vaccines, and diagnostic reagents for important public health diseases in the Region. There is a new effort to integrate training, research, and interinstitutional collaboration (the proposal requires a 3-month training period in a NIH-laboratory or that of an affiliate institution). Three PAHO-NIH biotechnology projects were approved, valued at US$ 40,000 each.

In order to evaluate the impact of PAHO financial support for courses and research projects, a survey was conducted involving 19 principal investigators of the 26 subsidized projects and two directors of the courses already completed (see Appendix II). Only two responses were obtained from the nine investigators supported between 1988 and 1989, which was to be expected, given the time period that had elapsed. More responses were obtained from those projects financed from 1990 to 1994. Among them, nine of the 10 investigators responded, and two responses were received from directors of the courses already held. Table 1 presents the results of the responses to specific survey questions.

It is worth discussing some of the results of the analysis of the 13 responses received. PAHO has contributed to the fact that biotechnology research groups obtain external financing from other sources (Questions 7 and 36), and has promoted graduate study for doctoral and master's degrees (Questions 9 and 23). PAHO's support has contributed to the education of other research groups and the continuity of original research teams (Questions 11 and 12). The research and development grants for biotechnology projects, as well as courses, are applied to purchase laboratory research supplies; computer equipment has not been acquired, and bibliographic material purchases (Questions 13, 14, and 16) have not multiplied. Research gave rise to publications, and the results were widely disseminated, even though less than five years have passed (Questions 17, 20, 21, and 22). PAHO-financed research was widely applicable, as the survey demonstrates (Questions 24, 25, and 26). Most of the responses indicate that the investigators presented projects responding to PAHO's announcement for project submissions, while the majority of investigators or members of their group did not request new support from PAHO (Questions 29 and 36).

Most of the investigators believe that the results have helped explain or address priority health problems that require research in their respective countries (39). In their responses to this question, investigators indicated that there was a positive contribution, evidenced by the concrete application of results. PAHO's efforts during the last nine years--mainly in the past five years (primarily because of the number of responses)--demonstrates that there was a corresponding strong commitment by the investigators, as can be deduced from the survey results.

With regard to suggestions, the investigators consider it important that formal announcements to submit projects on a specific subject be issued (a promotion mechanism that is already being adopted in other areas and was utilized to a great extent for the PAHO-NIH Biotechnology Program). The members of the Biotechnology Subcommittee and the investigators themselves would also be important channels to disseminate research requests.

As for future topics, scientific training/education is a recognized need in disciplines such as molecular epidemiology and molecular entomology. It is also considered very important to give priority to the practice of PCR in the joint PAHO-UNDP/UNESCO/UNIDO biotechnology training courses and PAHO-NIH biotechnology projects. The initiative to eliminate the transmission of Chagas' disease in Southern Cone countries is promoted through coverage of affected areas with insecticide treatment, entomological surveillance, and blood control for transfusion purposes. In this situation, the detection and treatment of recent cases, as in the case of the children under 5, is very useful. Besides serology, there is no technological replacement for xenodiagnosis, different from PCR. The laboratories of the Oswaldo Cruz Institute in Rio de Janeiro and those of the Fatala Chabén Institute in Buenos Aires, among others, are testing this technology in hospitals and rural areas. PAHO may want to take into account research projects to apply PCR, which might interest several countries of the Region. In addition, it would be useful to investigate the persistence of components of hepatitis C virus in patients under treatment. It is recommended that genetically based diagnostic tests be developed for several diseases whose transmission is intended to be eliminated by the year 2000. The application of biotechnologies is also a priority in order to diagnose, and test and develop malaria vaccines, a disease for which there continue to be considerable new cases in the Region.

APPENDIX I

PAHO-UNESCO/UNDP/UNIDO Jointly Financed Courses, under the Biotechnology Initiative: 1993-1995

1. Scale-up in Recombinant Protein Production. Course conducted in October 1993 at the Institute of Biotechnology of the UNAM in Cuernavaca, Mexico. Directed by Dr. Rodolfo Quintero Ramírez.

2. Systems of Expression in Eukaryotes for Recombinant Protein Production. Course held from March to April 1994 at the Institute of Biochemistry and Molecular Biology, School of Exact Sciences, National University of La Plata, Argentina. Directed by Dr. Victor Romanowski.

3. Polymerase Chain Reaction in Research, Diagnosis, and Environmental Surveillance. Two-week course conducted in the summer of 1994 at the Center for Genetic Engineering and Biotechnology of Havana, Cuba (CIGBH). Directed by Dr. Gavilondo of Havana, Cuba.

4. Utilization of Monoclonal Antibodies in Immunodiagnosis. Course held at the Immunology Laboratories of the School of Medical Sciences, Central University of Ecuador and of Immunopathology Laboratories of the IESS of Guayaquil, Ecuador. Directed by Drs. Washington Benítez Ortíz and Fernando Espinoza.

5. Probes and PCR to Diagnose the Most Prevalent Tropical and Infectious Diseases in the Central American subregion (PCR 95); the immunology module, to be held in July 1995 at the Institute of Nutrition of Central America and Panama (INCAP), Guatemala. Directors: Olga Torres and Drs. Omar Dary and Ricardo Luján.

6. Molecular Methods to Diagnose the Rotavirus. Course to be held at the University of San Andrés, La Paz, Bolivia. Director: Dr. Volga Iñiguez.

Appendix II. List of PAHO-Financed Biotechnology Projects, 1986-1994

RGP #	Año - Investigador Principal	Institución	Respondente	Título del Proyecto	Resp.
30	1988 Santos Sileone	Instituto de Virología, Depto. de Microbiología, Buenos Aires, Argentina		Aislamiento y Caracterización de Estirpes Regionales del Virus de la Inmunodeficiencia Humana	-
31 37	1993 Mercedes Weissenbacher	Depto. de Mibrobiología UBA, Argentina	O. Libonatti	Desarrollo de Paneles de Sueros de Referencia para Evaluación y Control de Reactivos de Diagnóstico Serológico del HIV	si
32	1987 Roberto Hosokawa	Inst. Malbrán, Argentina	J. González	Obtención de Anticuerpos Monoclonales Anti HBs. Kit para HBsAg Sérico	si
36	1989 Diego de Mendoza	Centro de Tecnología en Salud Pública, Univ. Nac. de Rosario, Argentina	-	Expresión de las Distintas Formas del Antígeno de Superficie del Virus de la Hepatitis B en *Bacillus Subtilis*	-
39	1992 Alberto L. Horenstein	CNEA - Argentina	A. Horenstein	Generación de Anticuerpos Monoclonales Bifuncionales y su Aplicación en el Diagnóstico y Terapia del Cáncer	si
40	1993 José Raúl Oubiña	Depto de Mibrobiología UBA, Argentina	J.R. Oubiña	Caracterización Genómica del Virus de la Hepatitis C en la Argentina	si
41	1992 Alberto C.C. Frasch	Fundación Campomar, Buenos Aires, Argentina	A.C.C. Frasch	Diagnóstico de *Vibrio Cholerae* por PCR	si
47	1986 Bernardo Galvao Castro	Fundación Oswaldo Cruz, Rio de Janeiro, Brasil	-	Isolamento e Caraterização do HIV de Pacientes Brasileiros	no
48	1988 Antonio Walter Ferreira	Inst. de Medicina Tropical São Paulo, Brasil	-	Human Malaria: Detection and Characterization of *P.vivax* and *P. Falciparum* Circulating Antigens in Sera or Urine, with Monoclonal Antibodies Using an Enzime mmunoassay	no
50	1989 Jairo Ivo Dos Santos	Fundación Oswaldo Cruz Rio de Janeiro, Brasil	-	Preparo de Paineis de Soro para Avaliação e Padronização de Kits para Detecção de Anticorpos Anti Virus da SIDA/AIDS	no

RGP #	Año - Investigador Principal	Institución	Respondente	Título del Proyecto	Resp.
62	1988 Eduardo Penton Arias	CIGB - Cuba	-	Desarrollo de una Combinación de Reactivos para el Diagnóstico de Seroconversión para HIV Empleando dos Antígenos Virales de Origen Recombinante	no
67	1987 Librado Ortiz Ortiz	UNAM - México	-	Desarrollo de una Prueba Diagnóstica para Detectar *Plasmodium vivax*	no
69	1988 Carmen Soler	UNAM - México	-	Detection. Isolation and Characterization of HIV Viruses Infecting Individual of High Risk Groups in Mexico	no
74	1994 Mario H. Rodríguez	Centro de Investigación de Paludismo - México	M. Rodríguez	Development of a Rapid, non-Radioactive Assay for the Detection of *Plasmodium Falciparum* and *Plasmodium Vivax* in Blood	si
76	1993 María D. Correa Beltrán	INDRES - México	Correa Beltrán	Desarrollo de Tecnología Moderna para el Diagnóstico de a Teniasis Humana y de la Cisticercosis Humana y Porcina	si
84	1988 José Azocar	Instituto Venezolano de Investigaciones Científicas (IVIC) Caracas, Venezuela	-	Isolation and Characterization of HIV in Venezuela	no
85	1988 Hilda Pérez-Carvajal	Instituto Venezolano de Investigaciones Científicas (IVIC) Caracas, Venezuela	-	Desarrollo de una Prueba Inmunológica para el Diagnóstico Epidemiológico de *Plasmodium vivax*	-
92	1991 Tania Margarita Aguirre	Centro Médico Docente la Trinidad, Unidad Investig. Caracas, Venezuela	Romano Piras	Desarrollo de un Dot-blot para Serodiagnóstico de la Enfermedad de Chagas Usando un Antígeno *T.cruzi* Específico Obtenido por Inmunocromatografía con un Anticuerpo Monoclonal	si
667	1993 Hernán Speisky	Inst.Nutrición y Tecnología Universidad de Chile Santiago, Chile	-	Pharmacokinetics of Boldine	no
989	1994 Garry T. Cole	Dpt. of Botany, Univ. of Texas - Austin, Texas	Guadalupe E. Rodríguez	Development of a Vaccine Against South American Blastomicosis	si

RGP #	Año - Investigador Principal	Institución	Respondente	Título del Proyecto	Resp.
1015	1994 Betty Matsuhiro	Fac. de Medicina, Univ. de Chile, Santiago, Chile	-	Conjugación del Polisacarido Vi de *Salmonella Typhi* a Proteinas	-
1052	1994 Felipe C. Cabello	New York Medical College Dpt. Microbiology & Immunology, Valhalla, NY	F.C. Cabello	*S. typhi* Vi Antigen Porin Vaccine: Improved Production of the *S.typhi* 36 kDa Porin	si
1059	1994 Abraham Landa Piedra	UNAM - Depto. de Inmunología, México	-	Recombinant Fragments of AgB as a Cestode Vaccine and Therapeutic Agent	-
1105	1994 Luis Carballo	Instituto de Investigaciones Inmunológicas, Cartagena, Colombia	-	Mapeo de Epitopes B de un Alergeno Recombinante del Acaro *Blomia Tropicalis*	-
n/r	1990 Alberto Marcipar	INTEBIO - Argentina	A. Marcipar	Programa de Biotecnología Aplicada a la Salud OPS-OMS, Proyecto Multicéntrico para el Desarrollo de un Estuche de Diagnóstico para el HIV	si
n/r	1993 Víctor Romanowsky	UNLP - Argentina	Romanowsky	Curso Intensivo Internacional "Sistemas de Expresión en Eucariotes"	si
n/r	1993 Rodolfo Quintero-Ramírez	UNAM - Mexico	Quintero-R	Curso Internacional: Escalamiento de la producción de proteínas recombinantes	si

Table 1. Impact of PAHO Cooperation on Research in Biotechnology, 1986-1994.

Question no.	Subject	Yes	No	Unknown/ NA
4	Other financing, in addition to PAHO funds	6	7	0
7	Acquisition of other resources as a result of PAHO's actions	8	3	2
8	Granted by PAHO	1	8	4
9	Training of team members	5	7	1
11	Continue to work together	10	1	2
12	Training of other work teams	9	2	2
13	Purchase of books or journals	3	8	2
14	Subscription to scientific journals	1	11	1
16	Purchase of computer equipment	2	10	1
17	Research resulted in publications	8	3	2
20	Research results were presented at conferences	8	3	2
21	Research was cited in other research	4	7	2
22	Used as bibliography	6	5	2
23	Resulted in master's or doctoral theses	5	6	2
24	Results utilized in other countries	9	2	2
25	Immediate application of the results	10	1	2
26	Knows of application of the results	10	0	3
29	Formal PAHO announcement for project submissions	9	2	2
31	Met anticipated schedule	7	5	1
32	Support from local PAHO Representative Office	10	2	1
33	Support from PAHO technical programs	4	6	3
34	PAHO presence during development	3	8	2
35	PAHO utilized the results	1	10	2
36	Participation in new PAHO projects	1	10	2 *
39	Positive contribution	9	1	3