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**PROGRAMA REGIONAL DE BIOTECNOLOGIA PARA
AMERICA LATINA Y EL CARIBE
PNUD/UNESCO/ONUDI
RLA/83/003**

Contrato No. 89/65

**Proyecto: Producción masiva de anticuerpos monoclonales:
un esfuerzo compartido en Latinoamérica**

1er. Año de Actividades

País: Brasil

Segundo Informe Técnico (Final)

INSTITUTO BUTANTAN
CENTRO DE BIOTECNOLOGIA

PRODUCCION MASIVA DE ANTICUERPOS MONOCLONALES:

UN ESFUERZO COMPARTIDO EN LATINO AMÉRICA

DP/RLA/83/003 PROGRAMA REGIONAL DE BIOTECNOLOGIA
PNUD/UNESCO/ONUDI PARA AMÉRICA LATINA Y CARIBE

SECOND TECHNICAL REPORT: REFERENT TO THE PERIOD FROM
APRIL/1990 TO SEPTEMBER/1990.

In accordance with the established in the first
program's contract we were to perform the following tasks:

1. Signature of the contract
2. Purchasing of equipments
3. Purchasing of materials
4. Installation of project's equipment and Institute's equipment
5. Send brazilian investigator to Cuba
6. Receive cuban investigator
7. Local staff's qualifying
8. Iniciation of cultures
9. Development of monoclonal's purification
10. Qualifying of first group (Argentina, Chile and Uruguay)
11. Technical reports

1. SIGNATURE OF THE CONTRACT:

The first contract was signed by Dr. Willy Beçak, Director of Butantan's Institute, on June, 27th of 1989.

2. PURCHASING OF EQUIPMENTS:

All the equipment bought have been received by

now. The major equipment, a Bellco's airlift bioreactor and spinner flasks have arrived by the end of June. Dr. Ana Maria Nero-Furlani has gone to Bellco's for operation's training on the second week of August/1990.

3. PURCHASING OF MATERIALS:

Of the US\$ 9,000.00 liberated after the first technical report we bought nearly US\$ 6,000.00 of plastic materials from Costar (not received yet). We are in process of buying reagents with the rest of the grant.

4. INSTALLATION OF PROJECT'S EQUIPMENTS AND CENTRE OF BIOTECHNOLOGY'S EQUIPMENTS:

The Monoclonal Antibodies Laboratory at Butantan's Institute was modified in order to receive the trainees of the project. Half of one building (200 m²) is now exclusively used for cell culture, as follows:

- a) two separate air filtrated and temperature controlled areas, one for small scale cell culture and derivation of hybridomas, other for high density cell culture. The first one is equiped with laminar flow hood, inverted microscopy*, CO₂ incubator, fridge, industrial liofilizator and liquid nitrogen storage tank. The second is equiped with bioreactors (Acusyst Jr's hollow fiber, Amicon's hollow fiber and Bellco's airlift*), CO₂ incubator, inverted microscopy*, pH meter, magnetic

* purchased through UNIDO's project.

stirrers and spinners flasks^{*}, laminar flow hood, modular laminar flow above the bioreactors, liquid nitrogen storage tanks and fridge freezer.

- b) animal's facilities with two rooms, one equipped with air filtration and temperature control for inbred mouse's manutention, other for storage of clean cages and equipped with laminar flow hood for animal's manipulation. So, the animals are opened in this area and only tubes containing cells enter small scale air filtrated area.
- c) room for storage of materials and accessories for the bioreactors.
- d) assay's room equiped with spectrofotometer, microplate reader^{*}, columns, accessories for electrophoresis and blotting^{*}, microfuge, centrifuge, incubator, freezer and system for apyrogenic water.
- e) glassware cleaning facilities with osmosis reverse system for apyrogenic water, autoclaves (steam and ethylene oxide), sonicator and ovens.

Besides the above mentioned, the following table shows the equipment pertinent to the Centre of Biotechnology with the respective financial sources.

^{*} purchased through UNIDO's project.

EQUIPMENTS

UNIDO	FINEP	BUTANTAN'S INSTITUTE

RECEIVED AND INSTALLED		
MICROSCOPIES	POWER BACKUP SUPPLY	AIR FILTRATED AREA
MICROPLATE READER	LAMINAR FLOW HOOD	ANIMAL'S FACILITIES
BLOTTING SOURCE	OSMOSIS RESERVE	LAMINAR FLOW HOOD
PIPET-AIDS	WATER SYSTEM	LAMINAR FLOW MODULE
MULTICHANNEL PIPETTES	CAGES' WASHING MACHINE	MILLI Q WATER SYSTEM
MICROPIPETTES	AIR CONDICIONING	ETHYLENE OXIDE AUTO- CLAVE
	LIQUID NITROGEN	STEAM AUTOCLAVE
	STORAGE TANKS	CO ₂ INCUBATOR
	FRIDGE/FREEZER	AMICON'S HOLLOW FIBER
	MATERIALS AND REAGENTS	ACUSYST'S HOLLOW FIB.
		PH METER
		MAGNETIC STIRRERS
		SPECTROPOTOMETER
		CENTRIFUGES*
		ULTRA CENTRIFUGE*
		SCINTILATOR*
		BIOPILOT*
		FPLC* / HPLC*
		LIOFILIZATOR*
		PHAST SYSTEM*
		-40, -70 FREEZERS*
		LUMINOMETER*
		C H R O M A T O G R A P H Y
		COLUMNS/ ACESSORIES*
		MATERIALS/REAGENTS

JUST ARRIVED OR TO ARRIVE		
BELLCO'S AIRLIFT SPINNERS	FLUOSKAN GAMA COUNTER	CELLIGEN -100 FREEZER*
MAGNETIC STIRRERS	CO ₂ INCUBATOR	
MATERIALS/REAGENTS	ELECTROPHORESIS APPARATUS	
	CHROMATOGRAPHY DEVICES	
	MICROPIPETTES	
	MICROPLATE WASHER	
	MATERIALS/REAGENTS	

US\$ 94,300	US\$ 245,000	US\$ 300,000

* NOT EXCLUSIVELY FOR MONOCLONALS' PROJECT

5/6. BRAZIL AND CUBA RECIPROC VISITS:

See previous report for details. We would only like to comment on the fact that the two visits had to take place before beginning the training, when not all the equipments (mainly bioreactors) had been installed. Notwithstanding the exchanging of information were very productive, it could had been still better with a more realistic programmation.

7. LOCAL STAFF'S QUALIFYING:

Dr. Ana Maria Moro-Furlani is the reseacher responsible for the monoclonal's laboratory. She has been working at the Centre of Biotechnology for three years.

Maria Teresa Alves Rodrigues has a master degree in Quality Control and is a doctorate student on the same subject. Besides collaborating in cell culture, is responsible for the quality control of the areas, water and product.

Mirian Nakamura is a master degree student in Biochemistry and works mainly in chromatography and assays.

Mara Lúcia Zucheran Silvestri is graduated in Biomedicine and works in cell culture and assays.

Marisa Sumiko Baba is a pharmaceutical student and collaborates in chromatography and assays.

Besides the specific committments everyone performs other's tasks when it is necessary. A graduated

technician is being selected.

8. INITIATION OF CULTURES:

We have already cultured some hybridomas in Amicon's hollow fiber. As this system is very simple, with no control of parameters, even temperature, the cultures ceased after 2 - 3 weeks. When each culture was attaining high density, the cells died. Once we had good results during a short period of time and the antibody harvested was purified.

The Endotronics' hollow fiber system (Acusyst Jr.) is temperature, pH, dissolved O₂, media and harvest flow rates controlled. The bioreactor arrived with many problems and only after a year (March/1990) we could start operation. Since then only one culture was seeded and was running very well when contaminated because of tube leakage. The supernatant was purified and we are preparing to seed again.

The Bellco's airlift bioreactor is being installed and will enter in operation soon.

Other cultures were performed in spinners, encapsulated in calcium alginate or free suspensions.

9. DEVELOPMENT OF MONOCLONAL'S PURIFICATION:

We have first tried to purify α -digoxina and OKT3 monoclonal antibodies through ionic exchange resins with

poor results. Just now we received isoelectric focusing standards and are able to know the antibodies' pI in order to plan a better ionic exchange chromatography.

Using protein A sepharose we are purifying without greater problems. The electrophoresis of the samples in reduced conditions show two bands, corresponding to, approximately, 50 and 25 Kd when coomassie or Ag-stained.

10. QUALIFYING OF FIRST GROUP:

The first training group received at Butantan's Institute was composed of three investigators: Leonardo Matteo Terra (Uruguay) started on the 7th of March and stayed approximately for 45 days; Gabriel Fishman (Argentina) and Rodrigo Ramos Mora (Chile) started on 1st of April and stayed until the end of June.

The training program was mainly practical, comprising of the following activities:

- thawing, stationary culture and freezing of hybridomas.
- metabolic assays (glucose consumption and lactate production) of the cultures' supernatant.
- antibody's activity assays (ELISA or cytotoxicity).
- seeding the hybridomas into the hollow fiber bioreactor and alginate encapsulation.
- daily monitoring of bioreactor's culture, including metabolic and antibody's activity assays.

- purification of antibodies and purity avaluation.
- report confection (included in this report).

Besides the experimental work, we had meetings for discussing each's program part or seminars on the themes:

- aplication of monoclonal antibodies in biotechnology.
- large scale production of monoclonal antibodies.
- purification methods for monoclonal antibodies.
- human, bifunctional and genetic engeeniered monoclonal antibodies.
- themes of students' intèrests.

On the arrival, the students were asked to make an exposition of theirs works at the institutions of origin. The students could also attend to the Centre of Biotechnology's seminars on other themes.

Considerations:

Due to differences on the project subject's experience among the students, the training couldn't advanced exactly as pretended. Notwithstanding one's dedication, which resulted in a certain degree of sucess, it is convenient that the trainees have experience in small scale hybridoma's culture; otherwise could be joined in one group.

Notwithstanding the difficulties, also because part of the equipment and materials didn't arrive in time, the first group worked in a integrated mode, operated hollow's fiber bioreator on their own, purified antibodies

through affinity columns and held control of the results. As they participated in all phases of the program, we think they acquired a good idea of the whole process, including problems (which they are likely to face in their countries) and are prepared for the second phase of the project.

11. TECHNICAL REPORTS:

Sending this second report we would like to have the grants' second part (US\$ 9100.00) released in order to buy reagents to continue the training of other groups as follows:

- beginning 1st of October 1990: Colombia, Costa Rica and México.
- beginning 1st of February 1991: Bolivia, Ecuador and Venezuela.

August 21/90
Frank