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HIGH LEVEL ADVICE AND SERVICES RELATING TO DEVELOP VACCINE PRODUCTION CAPABILITY OF THE NEWLY DESIGNATED NATIONAL INSTITUTE OF HUMAN VIRAL DISEASES

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ARGENTINA

Technical Report : Findings and recommendations*

Prepared for the Government of Argentina
by the United Nations Industrial Development Organization
acting as the executing agency for the United Nations Development Programme

Based on the work of Messrs. N. Cucakovich and N. Harjee, UNIDO consultants
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* This document has not been edited

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PREAMBLE

1. Argentinean Hemorrhagic Fever (AHF) is an exotic severe systemic disease caused by Junin virus (Arenaviridae family, reservoirs -- sigmodontine rodents), predominant in Argentina, in the Province of Buenos Aires. The AHF has a case fatality of 30%. Immune plasma is the first line of therapy in infected individuals. An average of 500 cases of AHF were reported annually prior to the immunization programme. Since 1993, greater than 200,000 individuals have been immunized with limited availability of the vaccine. The reported incidence of AHF during this period has been remarkably reduced to an average of 60 cases per year.

2. A new vaccine "Candid 1" against AHF, has been developed with the joint collaboration of the Argentine Ministry of Health, United Nations Development Programme (UNDP), Pan American Health Organization (PAHO) and the United States Army Medical Research and Development Command (USAMRDC). The scale up of the production procedures was developed at the Salk Institute in Swiftwater, Pennsylvania. This vaccine is efficacious and safe.

3. The Argentinean vaccine industry is in its infancy. This will be the second commercially manufactured vaccine to be made in Argentina (the first being Rabies for Veterinary use), and assistance will be needed in accomplishing this project. The Argentine Ministry of Health has the commitment to manufacture and eradicate the AHF disease. The facility is nearly completed; however, funds to complete the facility, equip and progression with the manufacture have been curtailed and delayed due to budgetary constraints and other pressing priorities for the Ministry of Health.

4. Sustainable "national vaccine self-sufficiency" is critical since no commercial source of AHF vaccine is available. USAIDs, through contract work at the Salk Institute in Swiftwater, Pennsylvania, donated 200,000 doses to National Institute of Human Viral Diseases (NIHVD). Salk Institute, currently the only manufacturer of AHF vaccine, has legal, liability and other constraints to manufacture the vaccine. This reinforces the need for manufacturing the vaccine in Argentina and maintaining the control to eradicate the disease.

5. The state-owned and subsidized vaccine production can only succeed with financial and professional support from International Organizations like United Nations Industrial Development Organization (UNIDO). NIHVD performs other socially and medically essential functions in addition to their ambition of vaccine production and eradication of the AHF disease.

6. Strategic and humanitarian considerations should be given to this project as the facility can not be fully utilized after the affected population of 3.5 million inhabitants have been immunized. This project is expected to take five years from the time of funding of this project.

7. Alternative utilization of the facility should be explored as the Expanded Programme of Immunization (EPI) recommended vaccines are easily and cheaply available making manufacturing of these vaccines in the proposed plant uneconomical. A possible vaccine candidate could be Malaria.

8. UNIDO, together with NIHVD, must obtain professional help to ensure successful completion of this project as the Argentinean team is new in the vaccine manufacturing venture.

9. We recommend that UNIDO and/or another International Agency should assist in the implementation of this project and the solicitation of $4,500,000 to complete the manufacturing facility.

RECOMMENDATIONS AND CONCLUSIONS

RECOMMENDATIONS

UNIDO, the Argentinean Government and other International Agencies solicited financial cooperation for all activities related to the successful completion of the production facility. This will include equipping of facility, manufacturing know-how and distribution of the Candid 1 vaccine for the Argentinean Hemorrhagic Fever (AHF). The estimated cost of the project is $4,500,000.

If the financial funding is secured then UNIDO must:

1. give technical cooperation in providing expert advice to NIHVD.

2. in association with other Agencies form a close partnership with NIHVD in developing and implementing training, and the technology transfer programmes for the vaccine.

3. support NIHVD financially and technically until the completion of the manufacturing facility, procurement of equipment, production of 5 (five) consistency lots, neurovirulence testing, and validation.

4. give technical cooperation to the National Control Authorities (Administration Nationale de Medicamentos Alimentos y Tecnologia) in the training for control and release of vaccines.

5. allocate resources for the training in Good Manufacturing Practices (GMP) and other production requirements once the facility is built and equipped.

6. support the above activities with a full-time consultant knowledgeable in all aspects of viral vaccine manufacturing, validation, quality control, quality assurance, regulatory requirements, small animal husbandry, documentation and facility maintenance.

CONCLUSIONS

The AHF vaccine project should be supported by the Argentinean Ministry of Health, UNIDO, PAHO, UNDP, WHO, and other Institutions such as the International Development Bank (IDB). The Argentinean Ministry of Health has built a facility which is nearly completed but requires support to complete the viral vaccine production suites and to equip the facilities. Due to budgetary constraints, the Argentinean Government is not in a position to allocate funds to complete this project. The economic analysis clearly indicates a significant economic benefit to complete this project as compared to purchasing the vaccine from the Salk Institute. It should be pointed out that the Salk Institute may not be able to supply the CANDID 1 vaccine due to other constraints and legal liability issues.

Although NIHVD does not have extensive manufacturing experience, training of the staff has been conducted during the last decade. Further training, as outlined in the report, will give the staff the confidence and the know-how of the manufacturing.

Funding and completing this project gives the Argentinean government manufacturing capabilities for human vaccines and securing supply of CANDID 1 vaccine for the Argentinean population.

This project merits the soliciting of the funds to enable the Argentinean Government to eradicate AHF.

ARGENTINE HEMORRHAGIC FEVER -- A REVIEW

The following publication by authors Dr. Delia A. Enria and Maria Rosa Feuillade, Instituto Nacional de Enfermedades Virales Humanas, "Dr. Julio I. Maiztegui", cc 195. 2700 Pergamino, Argentina entitled : "Argentine Hemorrhagic Fever (Junin Virus - Arenaviridae), A review on Clinical, Epidemiological and Preventative aspects of the Disease", is a comprehensive overview on AHF disease. It deals with the etiology, epidemiology, clinical characteristics, diagnosis, pathology and pathophysiology, treatment, control and preventative measures.

SUMMARY

Argentine Haemorrhagic Fever (AHF) is a severe systemic viral disease caused by Junin virus. The etiologic agent of AHF belongs to the family Arenaviridae and is associated with sigmondontine rodents, which are the natural reservoirs. Epidemics of the disease were first recognized in the 1950 decade, although retrospective analysis concluded that there have been previous cases of a similar disease beginning in the early forties. The endemic area in located in the humid pampa, the most fertile farming land of Argentina. Since 1958, annual outbreaks of the disease have been registered without interruption, and a progressive extension of the endemic region has been documented. The disease in characterized by haematologic, neurologic, cardiovascular, renal and immunologic alterations, with a case fatality rate as high as 30%. A specific therapy is available for AHF, and consists of the early administration of immune plasma. Therapy with immune plasma in standardized doses of neutralizing antibodies reduces the case fatality rate to less than 1%. An attenuated live vaccine (Candid 1) is available for AHF. After completion of all preclinical testing, the safety, immunogenicity and efficacy of Candid 1 in human volunteers were clearly established . This vaccine is currently being used in the population exposed to the higher risk of infection.

INTRODUCTION

In the 1950 decade, a new disease named Argentine Haemorrhagic Fever (AHF) was recognized in the richest farming region of Argentina (Arribalzaga , 1955). The etiologic agent, called Junin virus (JV) was isolated in 1958 (Parodi et al, 1958) and its identity as then confirmed (Pirosky et al, 1959) . JV belongs to the Arenaviridae family, and is associated with sigmondontine rodents which are the natural reservoirs (Sabattini et al, 1977). Since 1958, annual outbreaks of the disease have been registered without interruption, with several large series of cases reported, which has allowed us to learn considerably about different aspects of the illness. An important epidemiological feature of AHF is the progressive extension of the endemic region (Maiztegui and Sabattini, 1977). The initially high case fatality rate of the disease was markedly reduced, first with adequate supportive measures and, more significantly, with the availability of a specific efficacious treatment the immune plasma (Maiztegui et al, 1979; Enria et al, 1934). The development of an attenuated live vaccine Barrera Oro and Eddy, 1982), whose safety, immunogenicity and efficacy were clearly demonstrated in human volunteers (Maiztegui et al, 1988; Maiztegui et al, 1991) is now opening the perspective of controlling the disease.

In this article, we will review the most relevant clinical, epidemiologic and preventive aspects of the disease.

ETIOLOGY

The family Arenaviridae in composed of at least 14 recognized viruses, but only six of them have been associated with human disease (Rowe et al, 1970, a; Pfau et al, 1974; Howard and Simpson, 1980; Lehmann-Grube, 1984; Salas et al, 1991; Coimbra et al, 1994, Tesh et al, 1994). Arenaviruses are a group of enveloped single stranded RNA viruses. They are divided on the basis of their geographic distribution, natural hosts and serologic relationships into the New World viruses, which contain the members of the Tacaribe complex and were isolated in the Americas, and the Old World viruses, which originated in Asia, Europe and Africa (table 1).

The Arenaviruses share morphologic, biologic, physicochemical, and serologic properties (Dalton et al, 1968; Murphy et al, 1969; Rowe et al, 1970, b; Murphy et al, 1973; Murphy and Whitfield, 1975).

The etiologic agent of AHF, Junin virus, was isolated in 1958 (Parodi et al, 1958; Pirosky et al 1959), and was found to be serologically related to the Tacaribe group (Mettler et al, 1963). Similar to other arenaviruses, its genome consists of two single-stranded segments of RNA of 33S and 25S. In addition, the purified virions yield the ribosomal RNA species 28S and 18S, and 4S and 5S RNA (Añon et al, 1976).

EPIDEMIOLOGY

All currently known arenaviruses pathogenic for man are rodent viruses. The principal epidemiologic characteristics of AHF are determined by the natural cycle of Junin virus and by the behaviour of their respective rodent reservoirs (Sabattini et al, 1977; Sabattini and Contigiani, 1982). AHF endemic area is characterized by a mosaic of highly productive croplands (principally corn, wheat and soybeans). Crop fields and borders are inhabited by six common species of small rodents. These include five sigmondontines : *Calomys musculinus, C. Laucha, Akodon azarea, Bolomys obscurus and Oligoryzomys flavescens* and the murid *Mus musculus* (Mills et al, 1991, a). *C. musculinus* has been identified as the principal reservoir for JV, although virus has also been isolated from the organs and body fluids of *C. laucha* and *A. azarae*, and occasionally from *Mus musculus*, B. obscurus, and O. flavescens (Sabattini et al, 1977; Sabattini and Contigiani, 1982).

AHF epidemics occur predominantly during the major harvesting season, with a peak incidence in the month of May. The disease is four times as prevalent in males as in females and is more prevalent among rural workers than in the urban population (Maiztegui, 1975). The seasonal distribution of the illness and the prevalence in male rural workers reflect occupational exposure of humans and the habits of the rodent hosts of JV. Virus excreted by the infected rodents is probably transmitted to human through aerosols, skin abrasions or contaminated food.

Epidemics of the disease were recognized more than 40 years ago in the north-west of the province of Buenos Aires (Fig. 1). From 1958 to 1994, more than 24,000 cases were reported with annual outbreaks of 200 to 3000 cases (Fig. 2). Since 1992, the epidemic curve has been modified by the intervention of vaccination campaigns addressed to the population at higher risk of acquiring the disease, a point that will be discussed below. During this period, and coincident with the years of greater epidemics, a steady and progressive extension of the endemic zone was observed(Maiztegui and Sabattini, 1977; Maiztegui et al, 1986) (Fig. 1). In 1958, cases were limited to an area of approximately 16,000 km^2, with a population at risk of acquiring the disease estimated in 270,000 inhabitants. In 1963, cases of AHF were confirmed in the southeast of the province of Cordoba, and between 1964 and 1967 new areas were detected in the province of Buenos Aires. Cases began to appear later in the south of the province of Santa Fe. At present, the endemo-epidemic region covers an area of approximately 150,000 km^2, with a population at risk of more than 3,000,000 inhabitants.

The incidence of AHF is not the same in different places. In general, it is higher during a period of 5 to 10 years in the new areas, where it later declines. Nevertheless, cases continue to be reported from the older locations. Several factors have been considered in relation with this geographic extension and changing incidence of AHF (Maiztegui et al, 1986). Seroepidemiological surveys demonstrated the occurrence of unapparent infections with JV, but their prevalence is low, ranging from 2 to 4% (Weissenbacher et al, 1983). Available evidence does not support a lower virulence of strains of JV.

Changes in the structure and population dynamics of rodents, fluctuations in the prevalence of JV in the reservoirs in different locations and the demonstration of JV activity in rodents captured in non-endemic areas may partially explained the geographic extension and changing incidence of AHF (Rugiero et al, 1959; Sabattini and Contigiani, 1982; Weissenbacher et al, 1985; Mills et al, 1991 a

and b; Mills et al, 1992). On the other hand, the introduction of modifications in the agricultural practices, with occupational changes , and the incorporation into the endemic region of areas with larger cities with inhabitants with different kinds of rural contact, and in consequence, different opportunities for contact with infected rodents. should also be considered.

Geographic extensions in the latest periods have been smaller than those seen in previous years, suggesting an autolimitation in the extension of the endemic area. Nevertheless, rodent studies currently in progress indicate that AHF endemic region may continue to expand northward.

AHF is not usually contagious, although human to human transmission can occur (Rugiero et al, 1962). Viraemia is present in AHF patients throughout the acute febrile period (Boxaca et al, 1965), and JV has occasionally been isolated f rom oral swabs, from urine of patients, and from mother's milk (Sabattini and Maiztegui, 1970; Maiztegui et al, 1973). A special situation to the low risk of human-to- human transmission could be the infection of wives. Secondary cases in a small proportion of AHF cases spouses have been recognized, suggesting inter-human transmission of virus (Briggiler et al, 1987 a,b).

CLINICAL CHARACTERISTICS

AHF is characterized by haematological, renal, neurological, cardiovascular and immunological alterations (Rugiero et al, 1964; Schwarz et al, 1970; Sabattini and Maiztegui, 1970; Maiztegui, 1975, a ; Biquard et al, 1977). Although sub-clinical cases occur, most infections are sufficiently severe to be recognized by the clinicians. The incubation period appears to be between seven to 14 days. The onset of illness in insidious, with chills, malaise, anorexia, headache, myalgias, and moderate hyperthermia (38° to 39 C). After several days , this gives way to further constitutional, gastrointestinal, neurological and cardiovascular signs and symptoms. Low backache, retro orbital pain, nausea or vomiting, epigastric pain, photopliobia, dizziness, constipation or mild diarrhea are common symptoms. An almost constant absence of productive cough, sore throat, or nasal congestion is helpful in distinguishing the initial symptoms of AHF from those of acute respiratory infections. During the first week of illness, physical examination reveals flushing of the face, neck, and upper chest. Conjunctival congestion and periorbital edema may also occur. The oropharyngeal membranes are congested, and there is congestion of the vessels bordering the gums, that may bleed spontaneously under slight pressure. An enanthem characterized by petechiae and small vesicles is almost invariably found over the soft palate. Most patients have cutaneous petechiae in the auxiliary regions, upper chest, and arms. Lymph nodes become enlarged, particularly in the laterocervical regions. There are no signs of pulmonary abnormalities. Relative bradycardia and orthostatic hypotension are common. Generally, there is no hepatomegaly or splenomegaly, and jaundice is very rare. At the end of the first week of evolution, there is oliguria and different degrees of dehydration occur. Neurological signs are very common. The patients may be irritable, lethargic, with a fine tremor of the hands and tongue. Moderate ataxia, cutaneous hyperaesthesia, and a decrease in deep tendon reflexes and muscular tonicity are present. In females, mild to moderate metrorrhagia is constantly present and, in some cases, is the first sign of AHF.

During the second week of illness, 70% to 80% of the AHF patients begin to improve. In the remaining 20 to 30%, severe haemorrhagic or neurologic manifestations, shock, and/or superimposed bacterial infections appear between 8 and 12 days after the onset of symptoms. Profuse bleeding may occur in the form of haematemesis, melena, haemoptysis, epistaxis, haematomas, metrorrhagia, or haematuria. The severe neurological manifestations generally begin with mental confusion, marked ataxia, increased irritability, and intense tremors; these are followed by delirium, generalized convulsions, and coma. Whether predominantly haemorrhagic, neurologic, or a combination of both, the majority of these severe clinical forms are fatal. Acute renal failure is uncommon, but may occur in terminal cases or after prolonged periods of shock and is secondary to acute tubular necrosis. Superimposed bacterial infections, such as pneumonia, septicaemia and gas gangrene can also complicate AHF. They also appear during the second week, and their diagnosis may be delayed because they are usually not accompanied by leucocytosis.

Most patients destined to survive improve by the third week of illness and experience a protected convalescence. Temporary hair loss is common. Many patients have asthenia, irritability and memory changes, but they are transitory and disappear gradually. 10% of patients treated with immune plasma develop during the convalescence period a late neurological syndrome (LNS) , that will be discussed below.

CLINICAL LABORATORY DIAGNOSIS

Clinical laboratory studies are quite helpful in establishing an early clinical diagnosis of AHF. During the acute phase, there is progressive leucopenia and thrombocytopenia, with counts falling to 1,000-2,000 white cells and 50,000-100,000 platelets per mm^3. Sedimentation rate is normal or decreased. Almost invariably, there is protenuria, with a urinary sediment in which there are hyalin-granular casts, round cells with cytoplasmic inclusions (similar to those seen in other viral infections), and less frequently, haematuria. Serum urea or creatinine are normal or, in the severely ill patient, increased in proportion to dehydration and shock (Davalos et al, 1977) SGOT, CPK and LDH elevations are common but mild. Hyperbilirubinemia or hyperamylasemia are rare (Mando, 1977). During the acute illness, cerebrospinal fluid is normal, even in patients with severe neurologic alterations.

DIFFERENTIAL DIAGNOSIS

During the first week of illness, the clinical manifestations of AHF are non-specific and can be confused with several acute febrile conditions. Among the infectious diseases, the differential diagnosis includes typhoid fever, hepatitis, infectious mononucleosis, leptospirosis, infections with hantaviruses, and rickettsial infections. Diseases presenting with haematologic or neurologic alterations, such as intoxications and blood dyscrasia, should also be considered.

ETIOLOGIC DIAGNOSIS

Viraemia occurs throughout the acute febrile period. JV can be isolated from blood of patients and from lymphoid tissues of fatal cases. Isolation of virus can be done in mice or guinea pigs, and in cell culture monolayers. Co-cultivation of lymphomononuclear cells from the peripheral blood of cases of AHF on Vero cells monolayers has been shown to be the most sensitive method of isolating JV (Ambrosio et al, 1986).

Serologic diagnosis of AHF was first done by complement fixation (CF) (Canals, 1977). CF has several disadvantages because CF antibodies are detected late and persist for no more than two years, and they cross-react with arenaviruses from the Tacaribe group. A simple and practical method, indirect immunofluorescence (IIF), replaced CF (Peters et al, 1973; Damilano et al, 1982). IIF antibodies appear much earlier and have much longer persistence than CF. The neutralization test (NT) (Webb et al, 1969) is considerably more specific and is used to determine the doses of immune plasma that is given as specific therapy. Currently, AHF serologic diagnosis is established by a sensitive and objective test, an ELISA (Meegan et al, 1986) in combination with NT.

PATHOLOGY AND PATHOPHYSIOLOGY

Examination of the gross pathology and light microscopy studies have shown non-specific alterations similar to those described in other haemorrhagic fevers, and consisting in widespread congestion,

edema, and haemorrhages (Gallardo, 1970; Elsner et al, 1970). Ultrastructural and immunohistochemical studies revealed characteristic intracellular lesions, that were observed in the majority of organs , but were more prominent in lymphatic tissues, coincident with the presence of JV antigens (Maiztegui et it.,1975, b). Several studies suggest that lymphatic tissues are the main sites of viral replication (Gonzalez et al, 1980; Ambrosio et al, 1986). Morphologic studies of the bone marrow indicate that in AHF an acute and transient arrest of haemopoiesis occurs, with bone marrow hypocellularity, but without permanent haematological sequelae in patients that overcome the disease (Ponzinibio et al, 1979). AHF is also characterized by an acute, transitory immunodeficiency (Arana et al, 1977; Bracco et al; 1978). Cell-mediated immunity is depressed (Enria et al, 1986) and there are also marked changes in the T cell subpopulation (Vallejos et al, 1989). The proportion of T helpers decreases while the T repressor lymphocytes increase, resulting in a very low T4/T8 ratio. T cell subpopulation return to normal values in early convalescence.

The mechanism of haemorrhage in AHF patients still remains uncertain. It has been demonstrated that patients with AHF have thrombocytopenia and an abnormality of the intrinsic mechanism of blood coagulation, represented on the initial days of the disease by a prolonged PTTK and decreased levels of factors VIII, IX and XI. No evidence of intravascular coagulation was found (Molinas et al 1981, a). Also, an increase in the antigenic component of factor VIII was described (Molinas and Maiztegui, 1981, b).

Studies of the endogenous interferon (IFN) levels demonstrated that very high titers of IFN are present in serum samples taken during the acute period of AHF patients. After the transfusion of immune plasma, the levels of circulating IFN decrease abruptly. Interestingly, an association between interferon titers and fever, chills, and low backache was found (Levis et al, 1984). Besides, a correlation between IFN levels and the evolution of AHF was demonstrated, IFN titers being significantly higher in fatal cases than in survivors (Levis et al, 1985). Another study suggests an association between histocompatibility antigens and the severity of AHF (Saavedra et al, 1985).

AHF pathophysiology seems to be the result of a direct viral action rather than an immunopathologic process. In this respect, several studies failed to demonstrate immune complexes, complement activation or DIC as a relevant pathogenic mechanism (Maiztegui et al , 1975; Bracco et al, 1978). Secondary mediators released or activated as a result of the virus-cell interactions, such as lymphokines, vasoactive mediators and proteolytic enzymes may also explain the alterations of AHF, specially those seen in terminal cases.

TREATMENT

AHF is one of the few viral diseases for which a specific treatment is available: the transfusion of immune plasma within the first eight days from onset of symptoms. A controlled therapeutic trial, using 500 ml of either immune plasma or normal plasma in AHF cases within the first week of illness demonstrated the efficacy of immune plasma in decreasing the case fatality rate (Maiztegui et al, 1979). This treatment was then standardized based on the amount of neutralizing antibodies given to each patient (Enria et al 1984). First, in a retrospective study, it was demonstrated that the lower doses of neutralizing antibodies were associated with a higher case fatality rate. As a result of a prospective study, a dose of 3000-4000 Therapeutic Units per Kg of body weight is currently recommended (Table 2). Immune plasma is of no benefit to patients when it is initiated after 8 days

of illness (Enria and Maiztegui, 1994).

A controlled study revealed that 8-10% of the cases of AHF treated with immune plasma develop a late neurologic syndrome (LNS) (Maiztegui et al 1979). This association has been confirmed by additional retrospective and prospective studies (Enria et al, 1985). The neurological manifestations of the LNS differ from those seen during the acute period of AHF. The LNS appears in convalescence, after an interval free of symptoms. Changes are regularly detected in the cerebrospinal fluid, these include a moderate increase in the number of cells and the presence of antibodies against JV. Specific primary humoral response is also different in patients with LNS.

Several hypotheses have been postulated to interpret the pathogenesis of this LNS (Enria et al, 1985). The study of the 2'-5' oligoadenylate synthetases in leukocytes from AHF patients may contribute to the hypothesis of viral persistence or reactivation (Ferbus et al, 1988). Recent studies using Magnetic Resonance Images in LNS cases show relatively subtle abnormalities localized to the cerebellum, where there is edema. There are no white matter lesions to suggest postinfectious encephalomyelitis (Nagel et al, 1992).

Alternative forms of specific therapy are under evaluation. In this respect, the antiviral drug ribavirin may prove useful in the treatment of AHF patients (Enria and Maiztegui, 1994).

The rest of the treatment consist of adequate hydration symptomatic measures, and proper management of the neurologic alterations, blood losses, shock, and superimposed infections. Medication should be given by oral and intravenous route. Intramuscular and subcutaneous injections are contraindicated, because of the risk of haematomas and life-threatening infections, such as gas gangrene.

CLINICAL AND LABORATORY PRECAUTIONS

All personnel working in relation with JV should be immune. Candid 1 vaccine is available for seronegative person. Universal precautions are adequate for the care of patients with AHF and when handling samples from potentially infected persons. However, when aerosols or splashes are likely to occur, use of a biologic safety cabinet is indicated. Virus cultures should be manipulated at biosafety level-3 (BSL-3) containment facilities. Outside the area where the virus is indigenous, JV should be handled under BSL-4 containment laboratories.

CONTROL AND PREVENTIVE MEASURES

Rodent control, or the control of human contact with infected rodent population is impracticable. Therefore, several efforts have been directed toward obtaining a vaccine against AHF (Guerrero, 1977). A live attenuated JV vaccine (Candid I) has been developed through an international cooperative project involving the Government of Argentina, the Pan American Health Organization, the United Nations Development Programme, and the United States Army Medical Research and Development Command (Barrera Oro and Eddy, 1982). After completion of the preclinical testing (Lupton et al, 1984; Barrera Oro et al, 1984) , the safety and immunogenicity of this vaccine were established in more than 300 volunteers immunized between 1985 and 1988 in Frederick, MD, United States and Pergamino, Argentina (MacDonald et al, 1986; Barrera Oro et al, 1986; Maiztegui et al, 1988).

Between 1988 and 1990, a prospective, randomized, double blind placebo controlled trial in 6500 human volunteers from 41 localities of the endemic area clearly established the efficacy of the vaccine in preventing AHF. Candid 1 efficacy was estimated in 95.5 % (Maiztegui et al, 1991). In 1991, vaccination campaigns were initiated to provide coverage to high risk populations. Limited quantities of vaccine determined restrictions in the geographic area and populations targeted by this vaccination campaign. A total of 137,421 persons selected on the basis of their frequent exposure and/or rural residence in areas of high incidence were vaccinated to 1994. Although the definite impact of vaccination is still under evaluation , the 1992, 1993 and 1994 epidemics outbreaks were the lowest registered since the description of the disease (Fig. 2, Table 3). Five cases of AHF have been detected among vaccines, and all were mild forms of the disease, with a favourable outcome.

Ongoing studies continue to reinforce previous observations concerning the safety and immunogenicity of Candid 1 vaccine. Persistency of the specific immune response is still under evaluation. Recent studies have shown that, five years after vaccination, more than 85% of the vaccines have neutralizing antibodies against JV (Levis et al, 1993).

With sufficient supplies of Candid 1 vaccine to protect the whole population at risk, AHF is a disease that can be controlled. Eradication is not an objective achievable at present. A reinforced epidemiological surveillance, together with studies of JV activity in rodent populations should play an important role in AHF control.

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Figure 1

AHF Endemic area, with Progressive extension

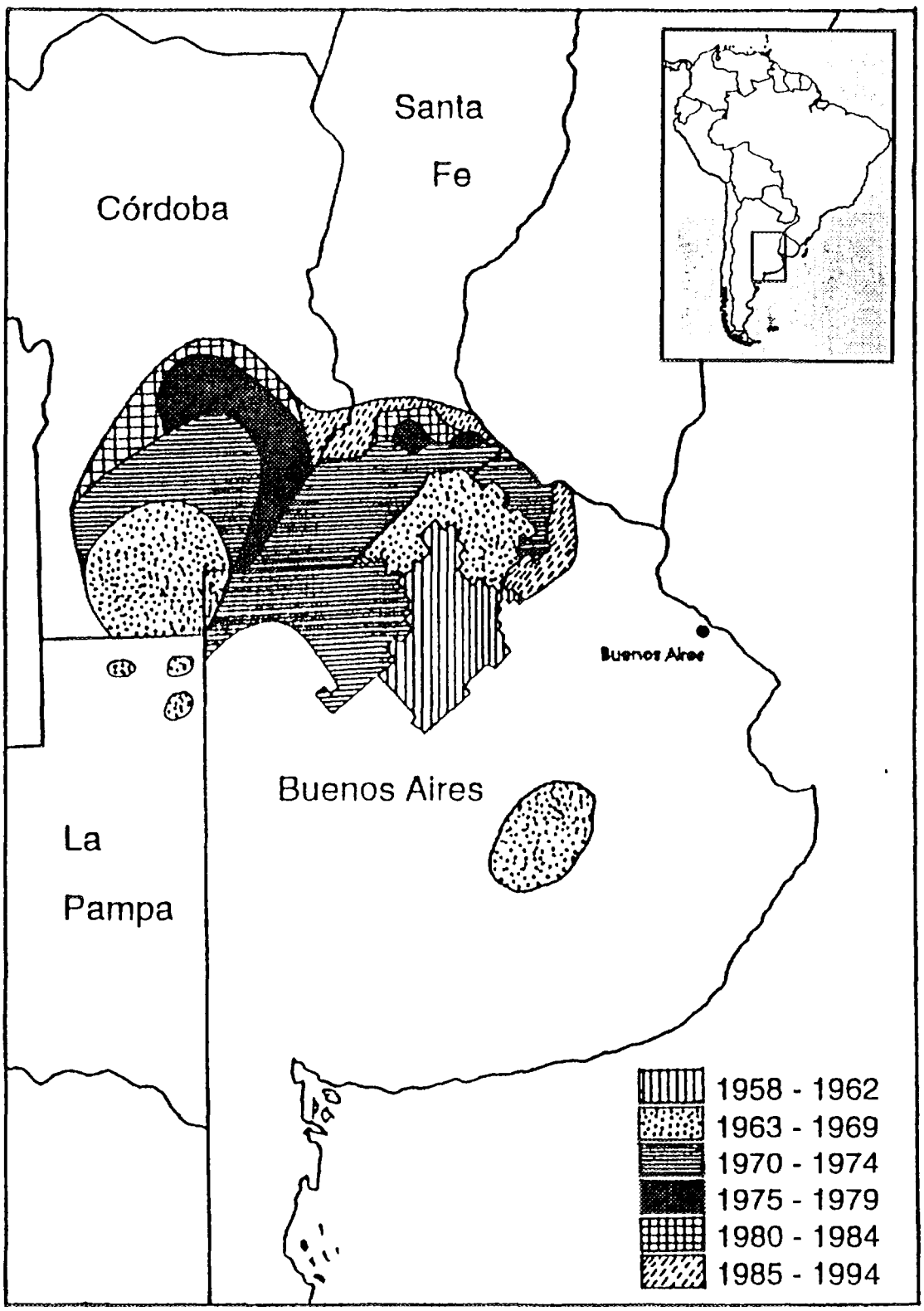


Figure 2

Annual Distribution of AHF Cases

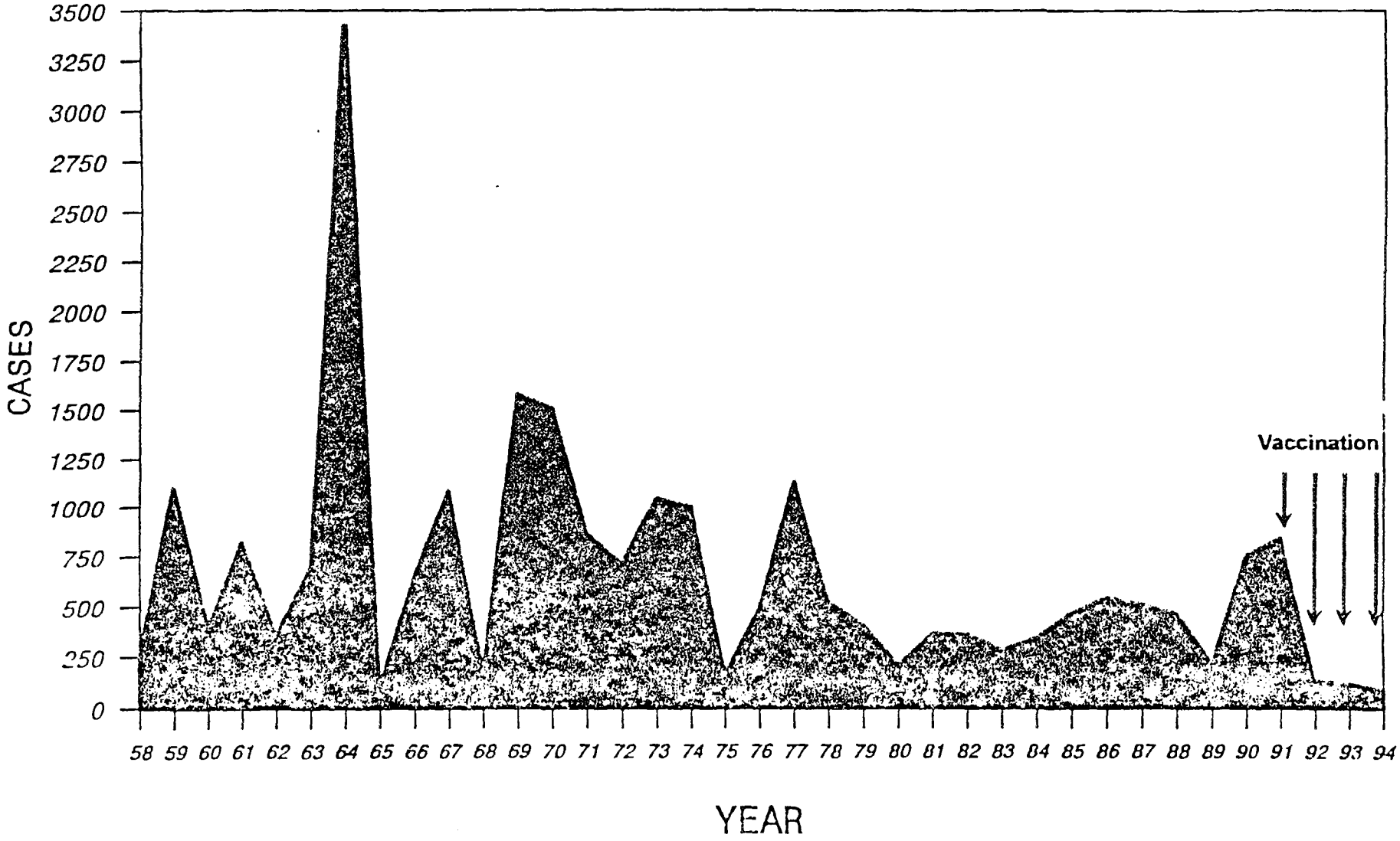


Table 1
Arenaviruses

Virus	Geographic Distribution	Natural Host	First Isolation	Naturally Occurring Human Disease
New World				
1 - Junin	Argentina	*Calomys musculinus*	Parodi et al, 1958 Pirosky et al, 1959	Argentine haemorrhagic fever (AHF)
2 - Machupo	Bolivia	*C. callosus*	Johnson et al, 1965	Bolivian haemorrhagic fever (BHF)
3 - Tacaribe	Trinidad, West Indies	*Artibeus bats*	Downs et al, 1963	None detected
4 - Amapari	Brazil	*Oryzomys gaeldi* *Neacomys guianae*	Pinheiro et al, 1966	None detected
5 - Parana	Paraguay	*0. buccinatus*	Webb et al, 1970	None detected
6 - Tamiami	United States (Florida)	Sigmodon hispidus	Calisher et al, 1970	Antibodies detected
7 - Pichinde	Colombia	*0. albigularis*	Trapido & Sanmartin, 1971	None detected
8 - Latino	Bolivia	*C. callosus*	Webb et al, 1973	None detected
9 - Flexal	Brazil	*O. spp*	Pinheiro et al, 1977	None detected
10 - Guanarito	Venezuela	*Sigmodon hispidus*	Salas et al, 1991	Venezuelan haemorrhagic Fever
11 - Sabia	Brazil	–	Coimbra et al, 1994	Haemorrhagic fever
Old World				
1 - Lymphocytic choriomeningitis (LCM)	Americas, Europe	*Mus musculus*	Armstrong & Lillie, 1934	Undifferentiated febrile illness, aseptic meningitis
2 - Lassa	West Africa	*Mastomys natalensis*	Buckley & Casals, 1970	Lassa fever
3 - Ippy	Central African Republic	*Arvicanthus*	Digoutte, 1970 Swanepoel et al, 1985	Unknown
4 - Mopeia	Mozambique, Zimbabwe	*Mastomys natalensis*	Wulff et al, 1977 Johnson et al, 1981	Unknown
5 - Mobala	Central African Republic	*Praomys*	Gonzalez et al, 1983	Unknown

TABLE 2

CASE FATALITY RATE IN AHF PATIENTS TREATED WITH IMMUNE PLASMA WITH DIFFERENT THERAPEUTIC DOSES OF NEUTRALIZING ANTIBODIES

OUTCOME	TU/Kg*		
	1000-1999	2000-2999	3000-3999
DIED	2	3	5
IMPROVED	24	46	908
TOTAL	26	49	913
Case fatality rate	7.69%	6.12%	0.55%

X^2: 26.32; p = 0.0002

* TU/Kg: $\left(\text{Tu/Kg} = \frac{\sum \text{weight immune plasma unit X NT antibodies titer}}{\text{Body weight in Kg}}\right)$

TABLE 3

ANNUAL DISTRIBUTION OF AHF CASES FROM 1990 TO 1994

	YEAR				
	1990	1991	1992	1993	1994
TOTAL AHF CASES (1)	597	578	94	88	36
VACCINE COVERAGE PRIOR TO EPIDEMIC SEASON	3500	7000	75000	105000	123000
CASES OCURRING AMONG VACCINES	1	2	0	2	1

(1) Notified - negative by serology

OBJECTIVES

The objectives of this mission were clearly defined by UNIDO which were as follows:

I. Current status at NIHVD for the manufacture of AHF vaccine.

II. Market analysis of vaccine demands which include alternative usage of the manufacturing plant.

III. Environmental and safety assessment relating to the BL-3 biocontainment facility.

IV. Outline of the master validation plan for the production and quality control facility.

V. Process Description Summary.

VI. Product Stability.

VII. Maintenance and Validation of the production strains and establishment of the seed lot systems (master and working seed).

VIII. Control for the supply of culture media with specific reference to Fetal Bovine Serum (FBS) and other media components of bovine or human origin.

IX. Assessment of training and resource requirements.

X. Organization and Personnel Plan.

XI. Patent Situation and Intellectual Property Rights and other relevant legal and public relation issues.

XII. Evaluation of the Specific Pathogen Free (SPF) animal facility.

XIII. Proposed Facility.

XIV. Capital Requirements.

XV. Economic Analysis.

XVI. 5-Year Business Plan.

CURRENT STATUS AT NIHVD

The National Institute of Human Viral Diseases, in Pergamino, is 240 km from the capital of Buenos Aires. The Institute has been involved in the development of the vaccine for Junin virus for over a decade. The case fatality rate of AHF ranges from 15 to 30%. About 300-1000 cases are reported from the endemic area each year. Recently a live attenuated vaccine, termed "Candid 1" was developed jointly with the Argentine Ministry of Health, UNDP, PAHO, USAMRDC. The process was scaled up to manufacturing levels at The Salk Institute. This vaccine in clinical trials was found to be highly efficacious, very safe and with minimal side effects.

The new facility is classed as BL-3, and was constructed in compliance with the US Code of Federal Regulations and Good Manufacturing Practices. This facility is approximately 1500 square meters with four (4) distinct suites:

- SPF animal breeding colony (400 square metres), is currently utilized for the breeding of mice for testing purposes and for other vaccine development projects.

- Quality control laboratory (180 square metres), some sections of the quality control laboratory are utilized for research on Haantan and Dengue diseases.

- Cell culture propagation, reagents, and washing and sterilization laboratory (180 square meters), is utilized for the manufacture of reagents and preparation of laboratory equipment and glassware. Cell propagation had not started in earnest.

- Viral infection and filling and freeze-drying suite (500 square metres), this facility has no equipment to proceed with the needs of manufacturing and is the suite that will be renovated.

The Argentine government recognized the need for the AHF vaccine and, on a yearly basis, budgeted to complete the facility and equip it to start manufacturing the vaccine. However, monetary constraints and other more pressing priorities have slowed this project.

In 1992, NIHVD launched a vaccination programme with 200,000 doses of Candid 1 vaccine donated by USAID. Between 1992-1995, more than 140,000 persons in the endemic area were vaccinated with a marked decrease in incidence of the disease. Recently, the Argentine Government purchased 50,000 doses of Candid 1 from the Salk Institute at a price of $5 per dose for emergency inventory purposes. Salk Institute, with legal and budgetary constraints, will not be able to manufacture the Candid 1 vaccine in the future, and this further reinforces the need for self sufficiency for the Argentinean Ministry. The 50,000 doses will be used very critically to immunize only those who are at high risk.

During the manufacturing of Candid 1 at the Salk Institute, various personnel from the NIHVD were trained at Salk on the manufacturing procedures, testing, freeze-drying, filling, formulations and testing. However this was done some time ago and a refresher training must be considered.

Presently no vaccine can be manufactured at NIHVD as the facility requires modifications to allow for proper flow of the product, extension of the facility to accommodate the processes, and capital equipment that must be purchased.

In the meantime there is an ongoing training at NIHVD to ensure that the personnel are still familiar with the techniques that will be required once the facility is fully operational.

MARKET ANALYSIS

Figure 1, AHF endemic area, with progressive extension demonstrates how the disease has spread in the province of Buenos Aires since 1958 to present. The estimated population of the affected area is 3.5 million inhabitants. The immunization consists of a single dose and administration to patients 15 years of age and over (pregnant women excluded). To date, the vaccine has shown to be greater than 95% efficacious for at least 8 years. Continued monitoring is ongoing to evaluate the need for a booster shot.

It is estimated that the birth cohort of approximately 2% will require about 100,000 maintenance doses per year after the total 3.5 million population has been immunized. It is estimated that from the onset of manufacturing, at the rate of approximately 1 million doses per year, the affected population be immunized in a period of 4 years. The production of the vaccine will increase should the need for booster shots be required (assuming after the 10th year of immunization).

The spread of the disease is slowly increasing demographically and since the manufacturing plant has excess capacity, it can meet any increase in demand. Furthermore, since the Junin virus does cross-react with the Machupo virus (Bolivian Hemorrhagic Fever), there is a potential of selling some of the vaccine to Bolivia. It is estimated that Bolivia may have a demand of 20,000 doses a year.

There is no commercial value to the vaccine as it is given free of charge by the Argentinean Ministry of Health. No other demand for this vaccine exists since the virus is endemic only to the Pampas region of the province of Buenos Aires. There is no commercial value other than the value of increased productivity of the affected population.

Alternative usage of the facility has been evaluated and the concept of manufacturing Measles for Argentinean self-sufficiency was considered. The cost to the Argentinean Government to manufacture Measles will increase 3-4 times the current cost of purchasing from PAHO. This is due to the subsidy of the large manufacturers when tendering for the PAHO and the UNICEF contracts.

Another alternative is to solicit from the Bolivian Government for NIHVD to manufacture the Machupo vaccine, but here the demands are small, and some development work has to be committed for an efficacious vaccine.

Currently WHO is looking for partners for the manufacture of Malaria vaccines. This would be a viable proposition and NIHVD should pursue this avenue.

RECOMMENDATION

Once the high risk population has been immunized, the facility should be utilized for other vaccine or biological manufacture. As the WHO is soliciting partners for the Malaria vaccine manufacture, this opportunity should be explored.

ENVIRONMENTAL AND SAFETY ASSESSMENT

Laboratory Biosafety Guidelines place Junin Virus in risk group 4 requiring a BL-4 containment. Since the Junin Virus for the manufacture of the CANDID 1 vaccine is attenuated, it is a common practice to operate under classification BL-3.

The NIHVD facility could not be fully evaluated for class BL-3 as all the 4 suites were not fully operational and there are modifications and extensions still planned for the facility. It can be safely stated that the facility can easily meet BL-2 and an assessment has to be completed for the BL-3 classification.

Air pressure differentials in the live virus laboratory in the Quality Control (QC) and virus manufacturing facility have to be validated and monitored regularly to maintain the integrity of the system. Air locks must be installed in the live virus laboratories to control entry and egress. It is important to maintain the live virus laboratories under negative pressure relative to the surrounding areas at all times. The air discharged from these live virus laboratories cannot be recirculated back into either the air supply system of the laboratory itself or into the building or adjacent buildings. The appended laboratory Biosafety Guidelines manual (complements of the Health and Welfare Canada and Medical Research Council of Canada) is a good reference that should be adhered to during the new construction, validation and operation of the facilities.

The SPF animal colony can easily meet the BL-3 requirements. The controls and the procedures were adequate to ensure a safe environment and maintain the integrity of the animals. Standard Operating Procedures (SOP's) for all aspect of the operation of the facility should be in place and the personnel trained accordingly.

The QC facility needs to be assessed critically, as the intentions of NIHVD are to use this facility for the purpose of both in-vitro and in-vivo testing and all immunological and physiochemical testing of CANDID 1 in addition to its regular research and development on the Haantan and Dengue viruses. Since individual laboratories within the QC facility are not controlled independently with HEPA filter (air entering and egressing) and as they are not individually air-locked, the suggestion to use QC facility for other viruses may breach integrity of the test being performed. Secondly, since the proposed laboratories for the Haantan and Dengue viruses research are not individually air-locked, any failure in the pressure differentials will compromise the safety of the personnel and the system. During our visit, one of the live virus negative pressure rooms was found to be under positive pressure, resulting in a reverse air flow into the corridor. These laboratories must have alarmed individual air differential monitors to ensure that such air reversals are detected and appropriate actions executed.

The QC facility is a good solid facility and the intentions to perform all required tests in this area is a viable approach. However, the above concerns must be addressed before any live Junin virus testing is performed in this facility.

The cell culture and media reagent preparation facility need not be class BL-3. This facility is well

maintained. Interlocks between this facility and the live viral manufacturing are essential to ensure proper entry and egress of raw materials, cell cultures and personnel. Air differential validation and routine monitoring are essential to maintain the integrity of the facility.

The viral infection laboratories and the filling suites could not be properly evaluated as they were not functional and there are further modifications planned for these areas. However, it is imperative that the Laboratory Biosafety Guidelines for BL-3 classification be emphasized during the modifications and construction of this facility.

In general, regular validation of the systems and certification of the temperature controllers and monitors, especially for the steam sterilize autoclaves, is essential in ensuring proper integrity of the class BL-3. This appears to be a weak point and must be strengthened. Overall, with proper planning, validation, monitoring, appropriate personnel training and procedures, this facility can meet the class BL-3 requirements.

MASTER VALIDATION PLAN

It is a cGMP requirement for any vaccine manufacturing facility to demonstrate a Master Validation Plan that encapsulates the Installation Qualification (IQ), Operational Qualification (OQ) and the Process Qualification (PQ) for the facility, utilities, air handling system, equipment, manufacturing processes, quality control testing, raw material and vendor certification.

The attached Master Validation Plan summarizes the sequence and schedule for the facility, utilities, equipment and processes that need to be validated and the approximate time to complete. Validation is a large undertaking and professional help will be required to accomplish this task. A total of $150,000 has been incorporated for this endeavor, and a further $50,000 has been budgeted for consultants to assist in the validation and guidance during the commissioning of the facility and manufacturing of the 5 consistency lots. The 5 manufacturing lots are for the consistency of manufacture of which 3 have to be monitored for stability and expiry of the product.

The Master Validation time line assumes that the SPF facility air handling system validation can be started as early as October 1996, or as soon as the funding is approved. It is essential that the facility and equipment validation is completed prior to commencing of the 5 consistency production lots. The process validation will occur concurrently as the 5 consistency lots are being manufactured. Personnel training and validation will also occur during this period.

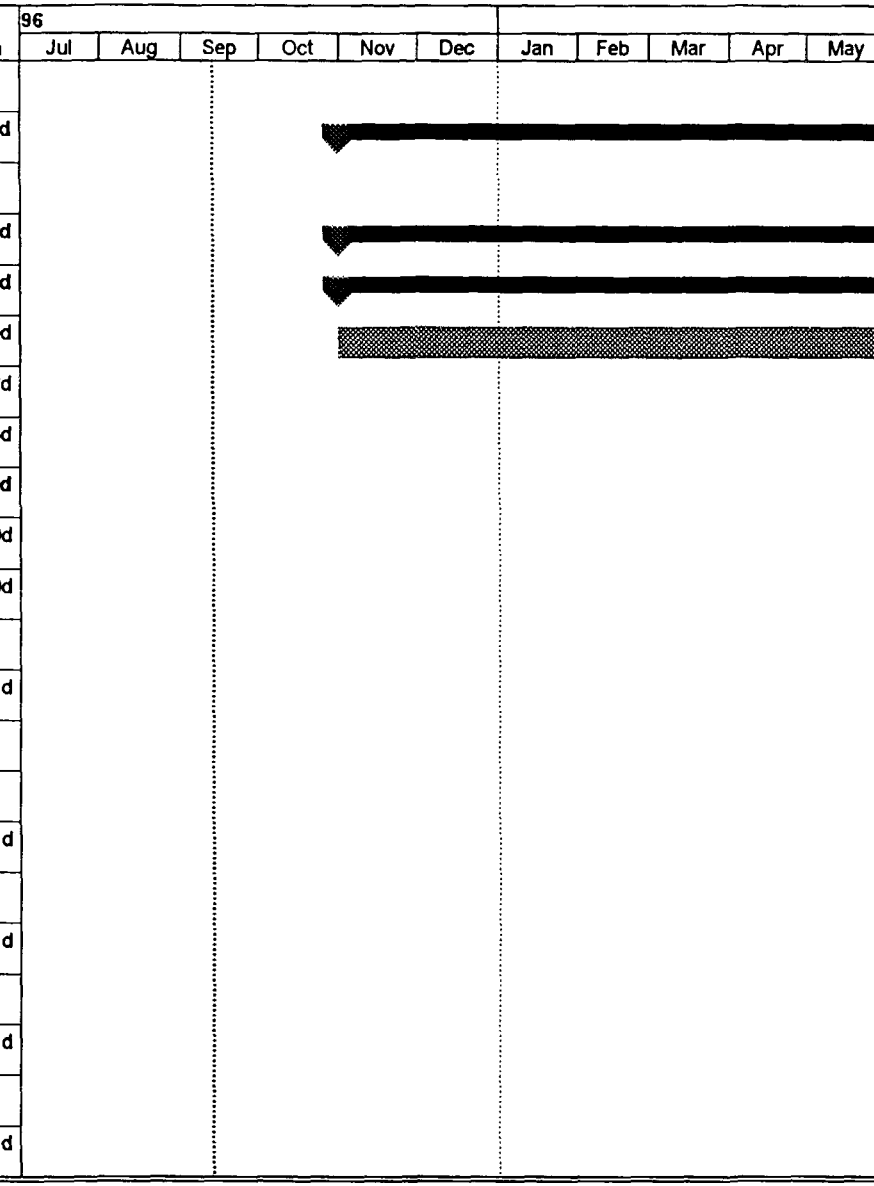
Training in operations and validation for the current maintenance engineer and the new proposed engineer to be hired is imperative for the success of this project. The engineers will be instrumental in heading the validation. All employees must be involved in the validation as the time frame assigned will be demanding.

Validation of this magnitude is an ambitious undertaking, and the success will depend on the professional assistance of a consultant(s) as the NIHVD group has no real experience and training in this aspect. The need for a contract consultant with a broad knowledge in cGMP, validation, viral manufacture, regulatory, quality control, quality assurance, engineering and training experience cannot be overly emphasized. The success of this project depends on the hands-on approach of the consultant(s). It is envisaged that the consultant may have to spend as much as a year at the site to ensure success of this project.

Time line for the estimated period for the validation are based on minimum requirements for validation. If an aggressive, thorough validation is envisioned than the time frames must be extended appropriately, resource requirements redefined and the budget to reflect accordingly.

MASTER VALIDATION PLAN (DETAILED)

ID	Task Name	Duration
1		
2	VALIDATION MASTER PLAN	429d
3		
4	- FACILITY VALIDATION	264d
5	- AIR HANDLING	244d
6	AIR PRESSURE AND DIFFERNTIAL MAPPING	239d
7	MAGNEHELICS	2d
8	AIR VELOCITY	3d
9	- AIR QUALITY(PARTICULATE AND BIOBURDEN)	20d
10	STATIC	10d
11	DYNAMIC	10d
12		
13	- RAW MATERIAL FLOW	1d
14		
15		
16	- WASTE FLOW	1d
17		
18	- PRODUCT FLOW	1d
19		
20	- PERSONNEL FLOW	1d
21		
22	- EQUIPMENT FLOW	1d



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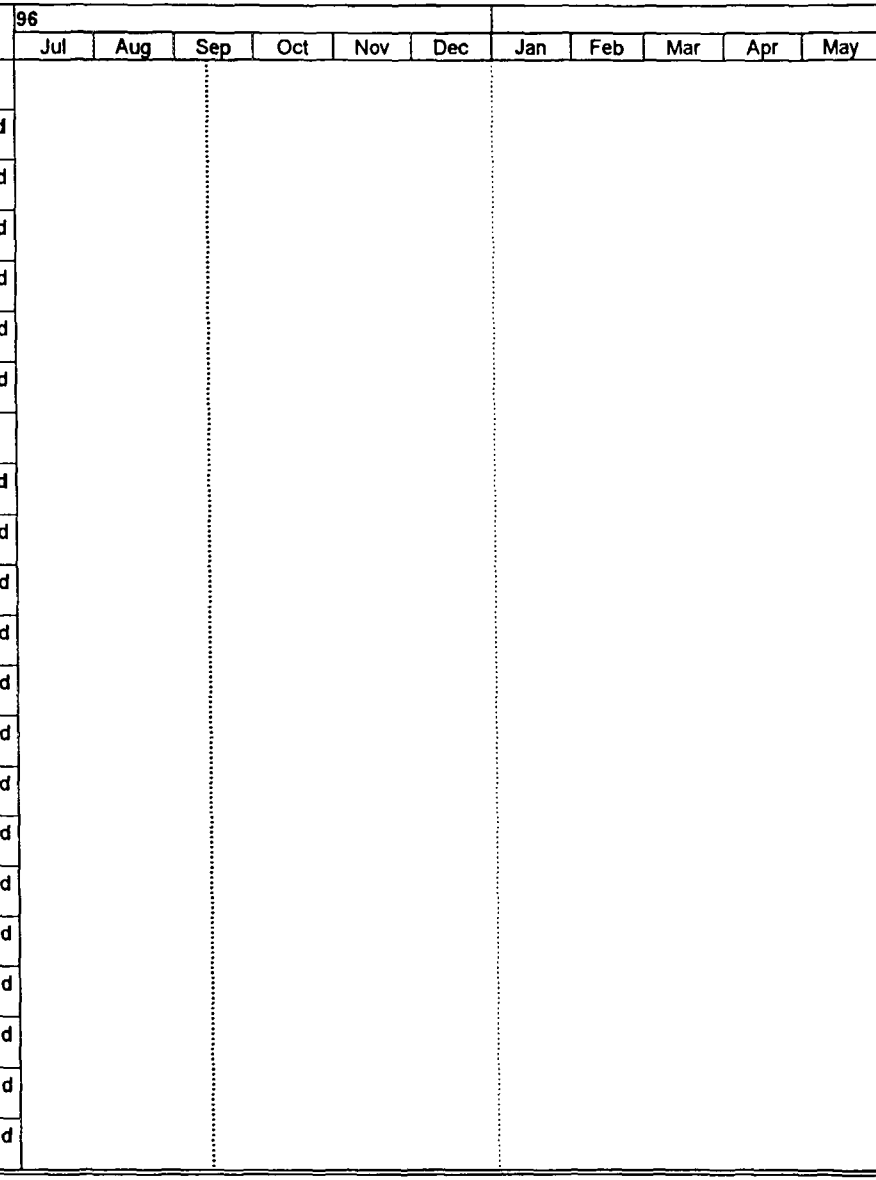
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name	Duration
23		
24	**- UTILITIES**	**10d**
25	HYDRO	1d
26	POTABLE WATER	1d
27	WATER FOR INJECTION	2d
28	VACUUM AND PRESSURIZED AIR	3d
29	AIR HANDLING SYSTEM	3d
30		
31	**- EQUIPMENT VALIDATION IQ, OQ, PQ**	**38d**
32	AUTOCLAVE	10d
33	HOT PACK OVEN	10d
34	VIAL & STOPPER WASHER	10d
35	FREEZEDRYER	28d
36	FILLING EQUIPMENT	10d
37	WATER FOR INJECTION	10d
38	FUME HOODS	2d
39	LAMINAR FLOW HOODS	2d
40	BALANCES	1d
41	PH METER	1d
42	UV SPECTROPHOTOMETER	1d
43	OSMOMETER	1d
44	CENTRIFUGE	1d



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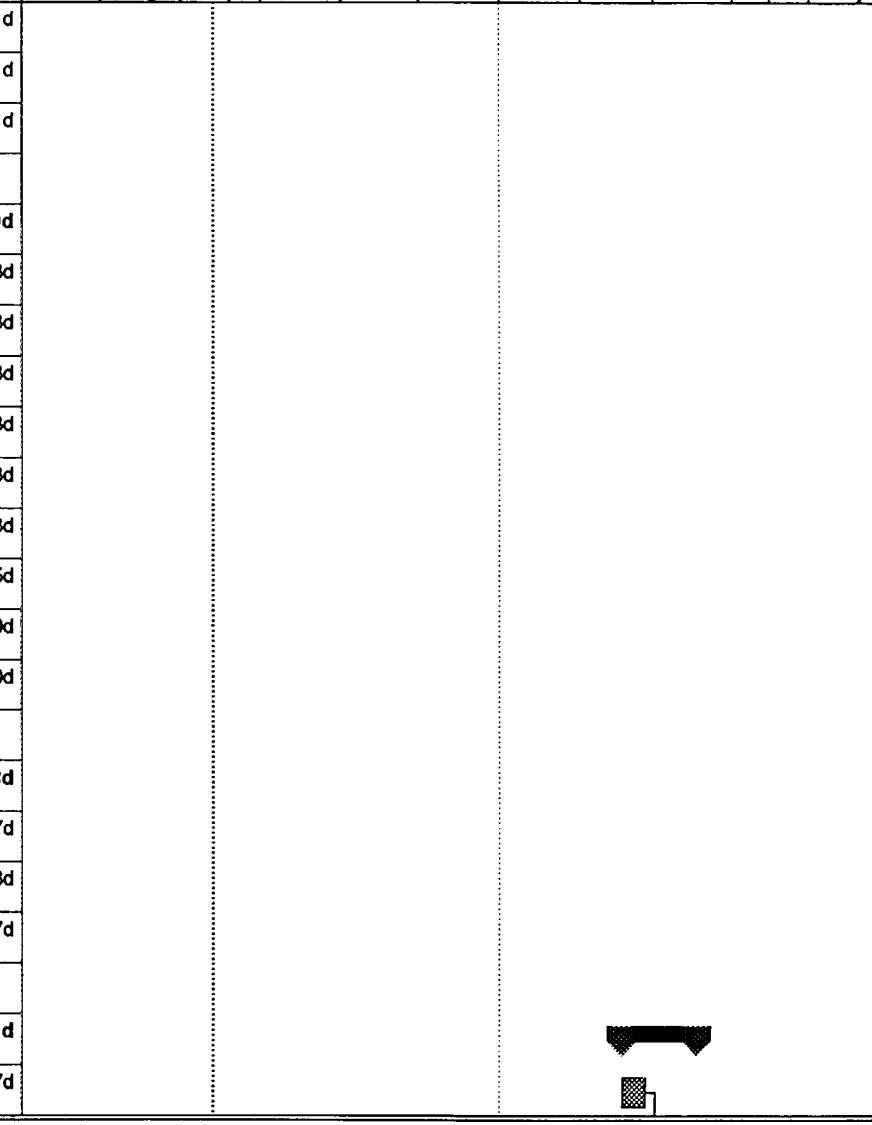
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name	Duration
45	HOT PLATES	1d
46	INCUBATOR (STABILITY PROGRAM)	1d
47	REFRIGERATOR (STABILITY PROGRAM)	1d
48		
49	**- PROCESS QUALIFICATION**	**110d**
50	STEAM STERILIZATION LOADS	28d
51	FILLING AND FREEZEDRYING	28d
52	FILLING ROOM - STATIC AND DYNAMIC AIR AND BIOBURDEN TEST	28d
53	CELL PROPAGATION	28d
54	VIRAL INNOCULATION AND HARVEST	28d
55	BLENDING AND FORMULATION	28d
56	GLASSWARE WASHING	15d
57	VIAL DEPYROGENATION	10d
58	QC TESTING	100d
59		
60	**- PERSONNEL QUALIFICATION**	**28d**
61	GMP	7d
62	PROCESS TRAINING	28d
63	DOCUMENTATION TRAINING	7d
64		
65	**- SAFETY**	**21d**
66	STORAGE OF FLAMMABLE SOLVENTS	7d



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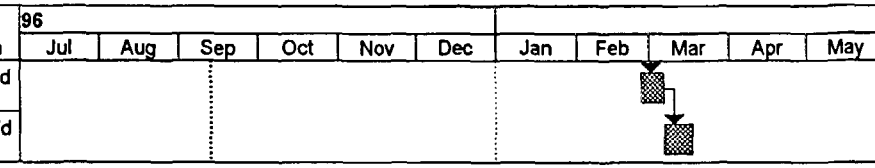
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name	Duration
67	DISPOSAL AND STORAGE OF USED SOLVENTS	7d
68	SAFETY TRAINING IN FOR THE LAB.	7d



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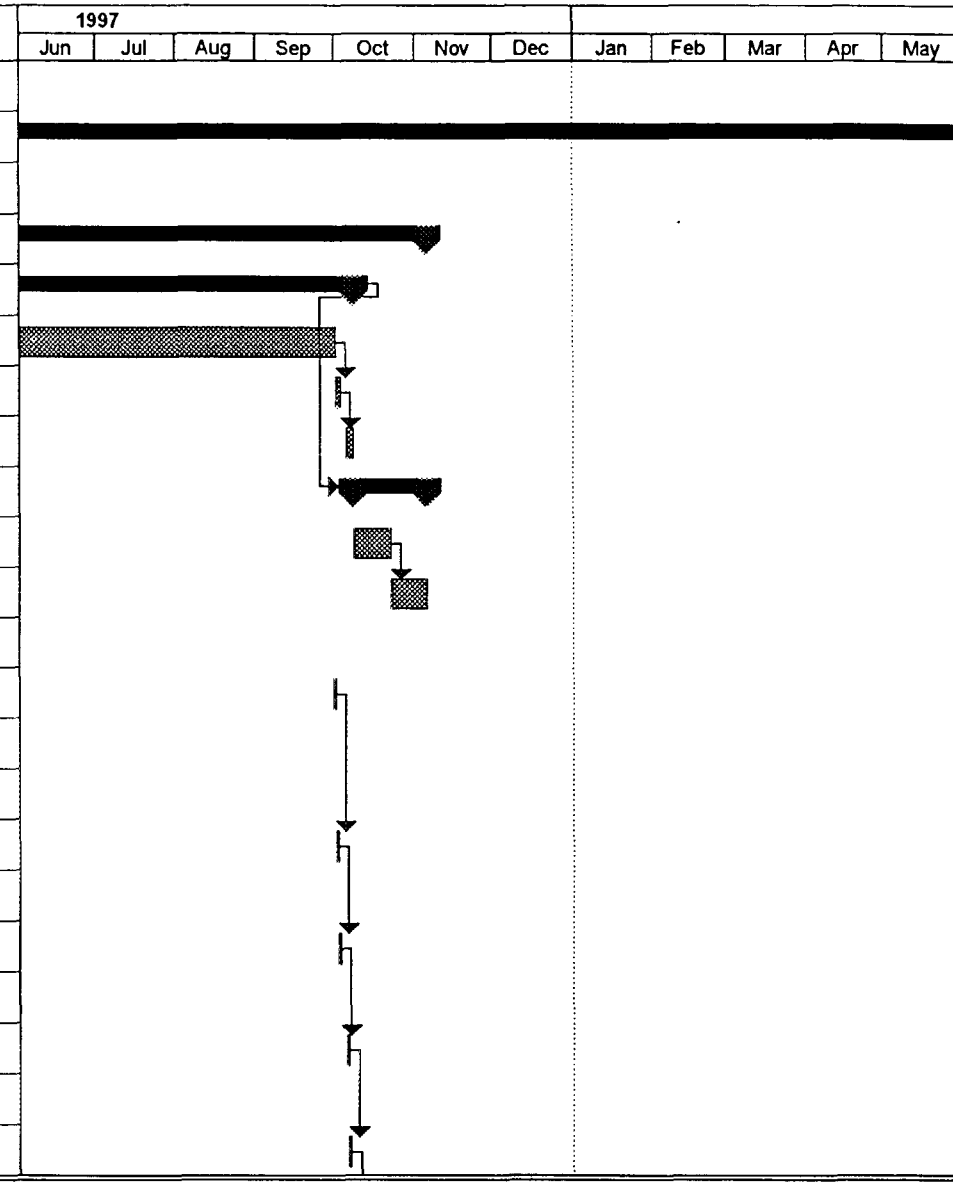
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name
1	
2	VALIDATION MASTER PLAN
3	
4	- FACILITY VALIDATION
5	- AIR HANDLING
6	AIR PRESSURE AND DIFFERNTIAL MAPPING
7	MAGNEHELICS
8	AIR VELOCITY
9	- AIR QUALITY(PARTICULATE AND BIOBURDEN)
10	STATIC
11	DYNAMIC
12	
13	- RAW MATERIAL FLOW
14	
15	
16	- WASTE FLOW
17	
18	- PRODUCT FLOW
19	
20	- PERSONNEL FLOW
21	
22	- EQUIPMENT FLOW



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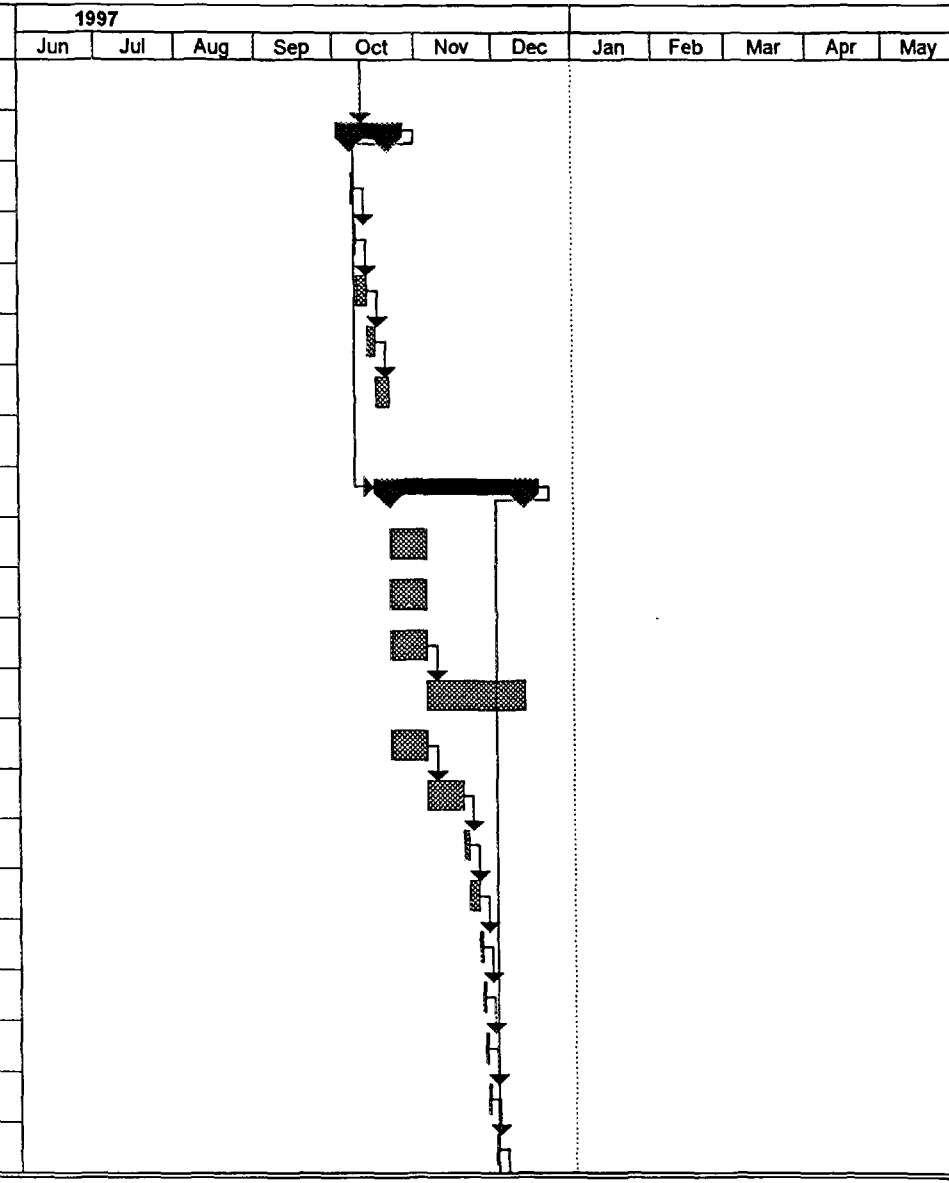
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name
23	
24	- UTILITIES
25	HYDRO
26	POTABLE WATER
27	WATER FOR INJECTION
28	VACUUM AND PRESSURIZED AIR
29	AIR HANDLING SYSTEM
30	
31	- EQUIPMENT VALIDATION IQ, OQ, PQ
32	AUTOCLAVE
33	HOT PACK OVEN
34	VIAL & STOPPER WASHER
35	FREEZEDRYER
36	FILLING EQUIPMENT
37	WATER FOR INJECTION
38	FUME HOODS
39	LAMINAR FLOW HOODS
40	BALANCES
41	PH METER
42	UV SPECTROPHOTOMETER
43	OSMOMETER
44	CENTRIFUGE



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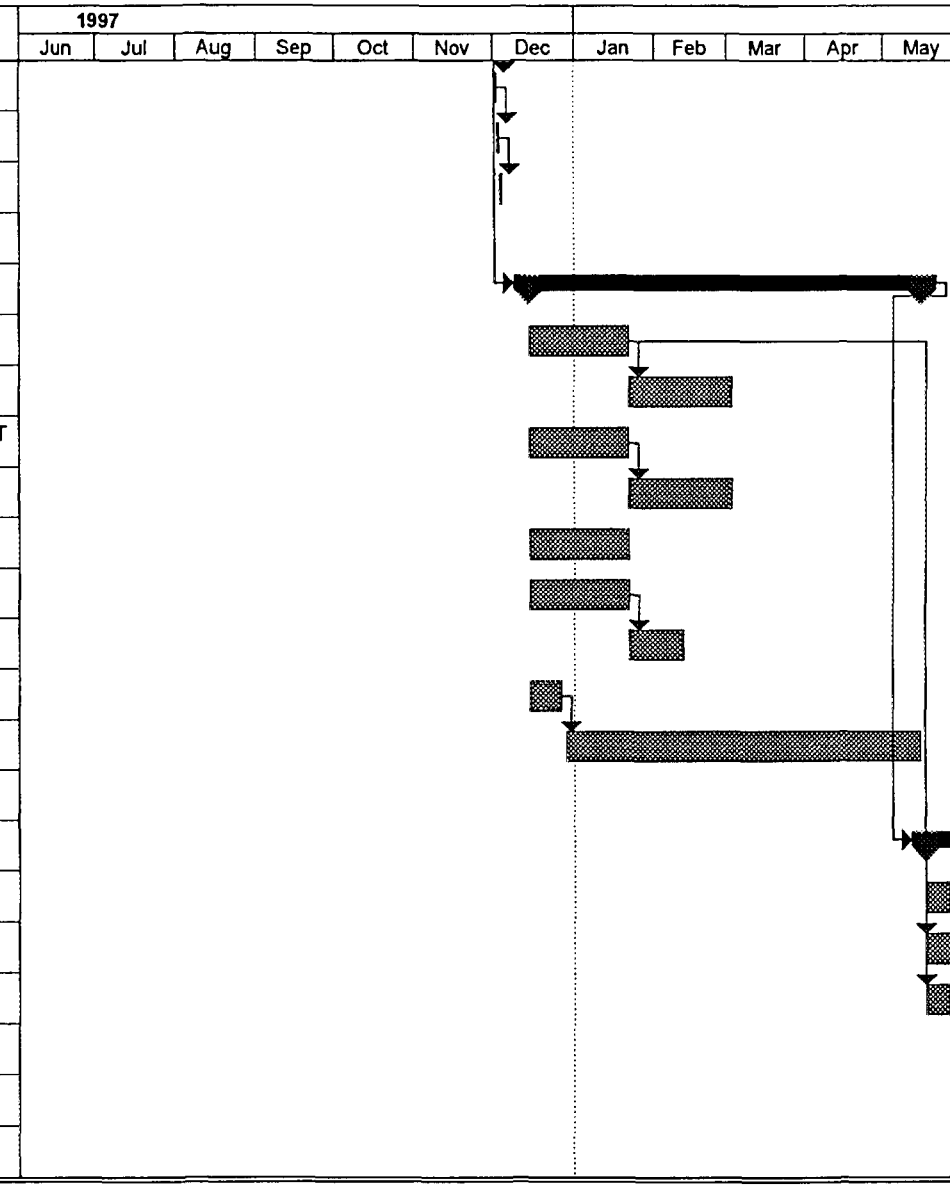
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name
45	HOT PLATES
46	INCUBATOR (STABILITY PROGRAM)
47	REFRIGERATOR (STABILITY PROGRAM)
48	
49	**- PROCESS QUALIFICATION**
50	STEAM STERILIZATION LOADS
51	FILLING AND FREEZEDRYING
52	FILLING ROOM - STATIC AND DYNAMIC AIR AND BIOBURDEN TEST
53	CELL PROPAGATION
54	VIRAL INNOCULATION AND HARVEST
55	BLENDING AND FORMULATION
56	GLASSWARE WASHING
57	VIAL DEPYROGENATION
58	QC TESTING
59	
60	**- PERSONNEL QUALIFICATION**
61	GMP
62	PROCESS TRAINING
63	DOCUMENTATION TRAINING
64	
65	**- SAFETY**
66	STORAGE OF FLAMMABLE SOLVENTS



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MASTER VALIDATION PLAN (DETAILED)

ID	Task Name	1997 Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
67	DISPOSAL AND STORAGE OF USED SOLVENTS												
68	SAFETY TRAINING IN FOR THE LAB.												

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Progress | Rolled Up Task

Milestone | Rolled Up Milestone

MASTER VALIDATION PLAN (DETAILED)

ID	Task Name
1	
2	**VALIDATION MASTER PLAN**
3	
4	**- FACILITY VALIDATION**
5	**- AIR HANDLING**
6	AIR PRESSURE AND DIFFERNTIAL MAPPING
7	MAGNEHELICS
8	AIR VELOCITY
9	**- AIR QUALITY(PARTICULATE AND BIOBURDEN)**
10	STATIC
11	DYNAMIC
12	
13	**- RAW MATERIAL FLOW**
14	
15	
16	**- WASTE FLOW**
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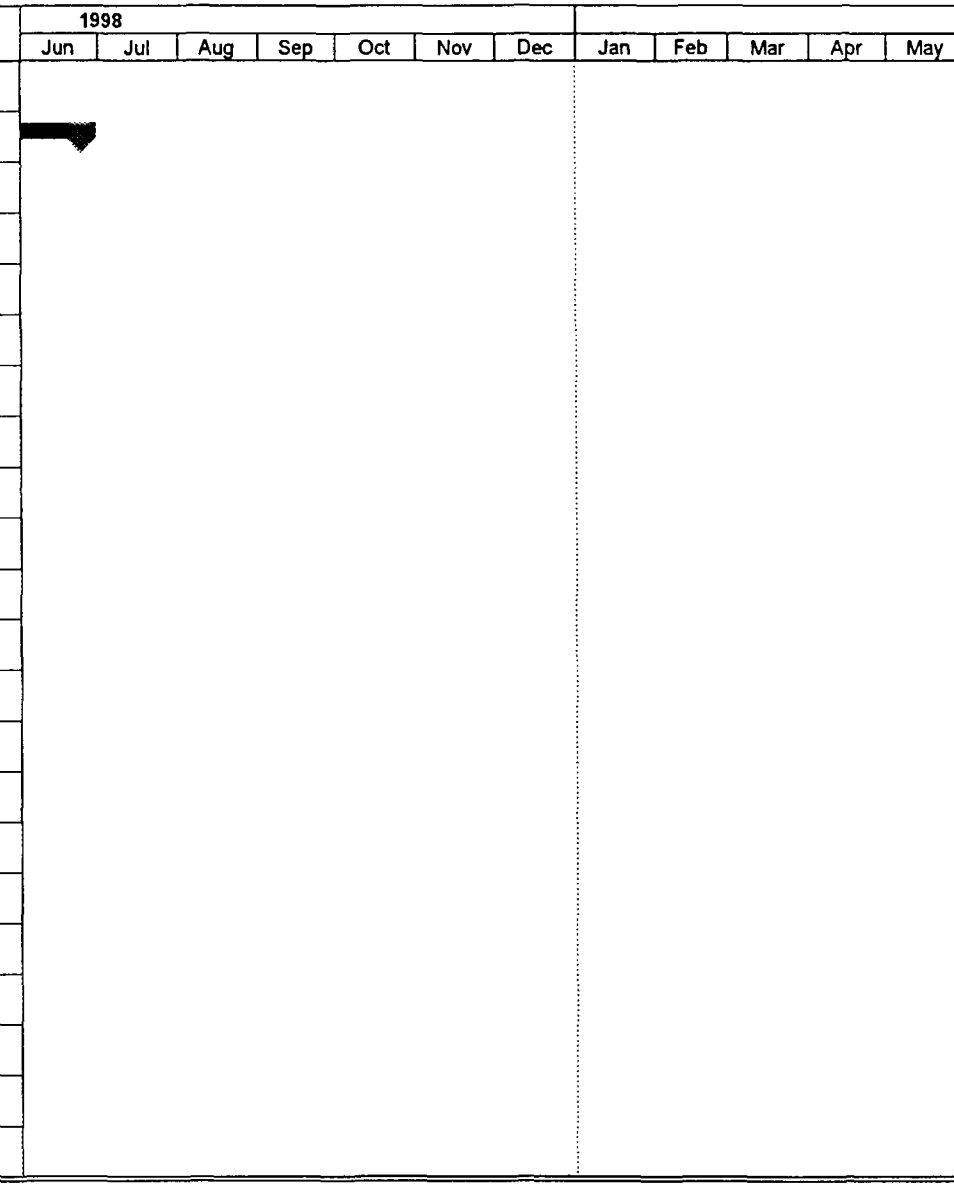
18	**- PRODUCT FLOW**
19	
20	**- PERSONNEL FLOW**
21	
22	**- EQUIPMENT FLOW**



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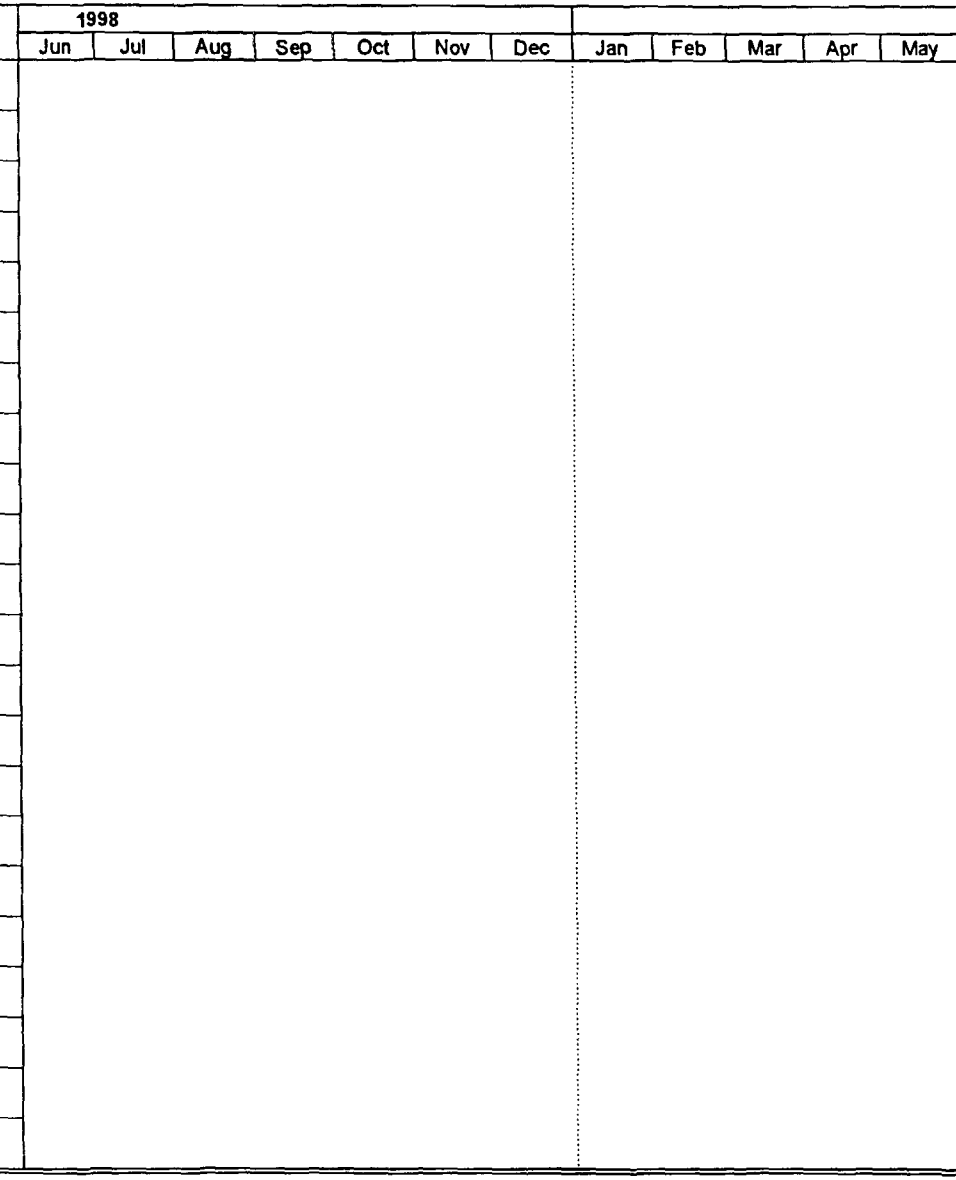
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name
23	
24	- UTILITIES
25	HYDRO
26	POTABLE WATER
27	WATER FOR INJECTION
28	VACUUM AND PRESSURIZED AIR
29	AIR HANDLING SYSTEM
30	
31	- EQUIPMENT VALIDATION IQ, OQ, PQ
32	AUTOCLAVE
33	HOT PACK OVEN
34	VIAL & STOPPER WASHER
35	FREEZEDRYER
36	FILLING EQUIPMENT
37	WATER FOR INJECTION
38	FUME HOODS
39	LAMINAR FLOW HOODS
40	BALANCES
41	PH METER
42	UV SPECTROPHOTOMETER
43	OSMOMETER
44	CENTRIFUGE



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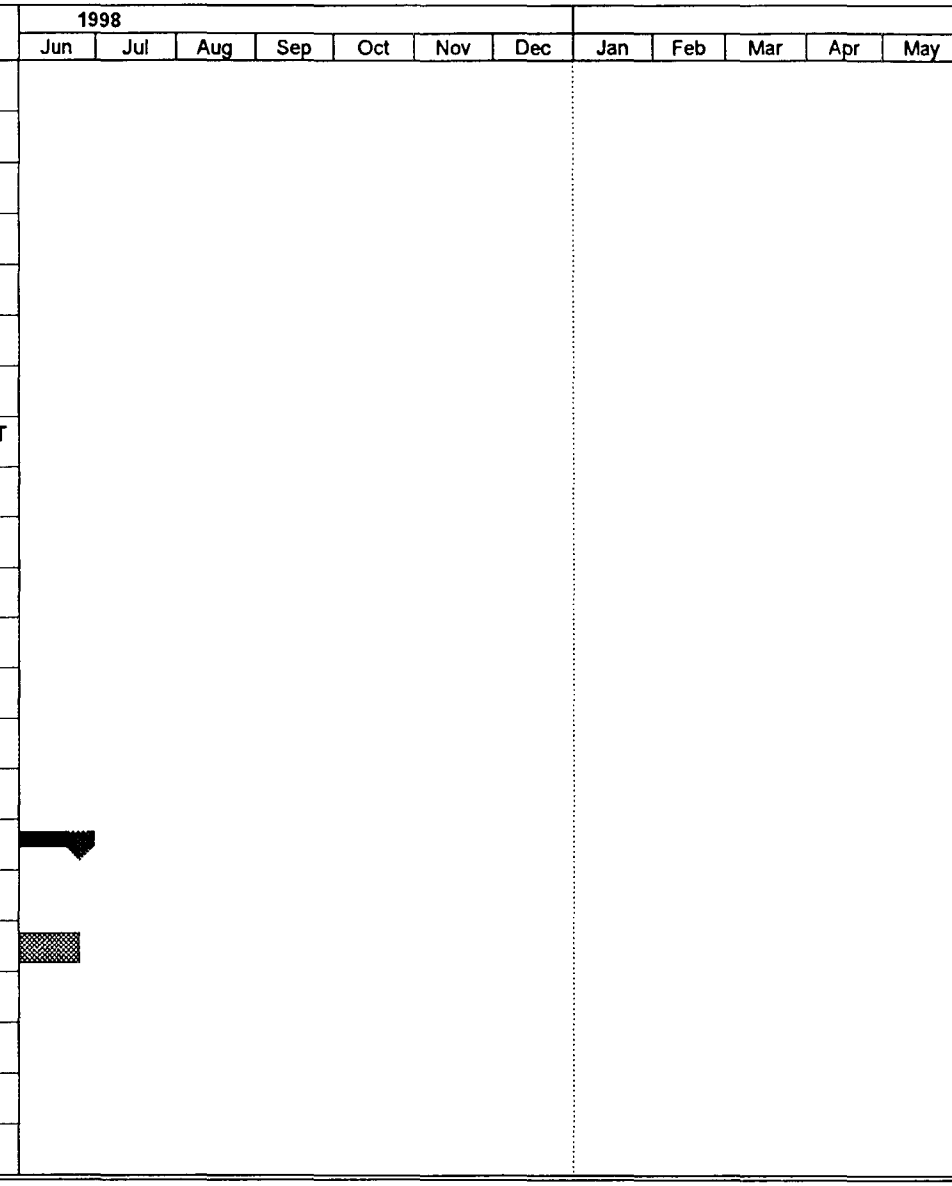
MASTER VALIDATION PLAN (DETAILED)

ID	Task Name
45	HOT PLATES
46	INCUBATOR (STABILITY PROGRAM)
47	REFRIGERATOR (STABILITY PROGRAM)
48	
49	**- PROCESS QUALIFICATION**
50	STEAM STERILIZATION LOADS
51	FILLING AND FREEZEDRYING
52	FILLING ROOM - STATIC AND DYNAMIC AIR AND BIOBURDEN TEST
53	CELL PROPAGATION
54	VIRAL INNOCULATION AND HARVEST
55	BLENDING AND FORMULATION
56	GLASSWARE WASHING
57	VIAL DEPYROGENATION
58	QC TESTING
59	
60	**- PERSONNEL QUALIFICATION**
61	GMP
62	PROCESS TRAINING
63	DOCUMENTATION TRAINING
64	
65	**- SAFETY**
66	STORAGE OF FLAMMABLE SOLVENTS



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MASTER VALIDATION PLAN (DETAILED)

ID	Task Name	1998 Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
67	DISPOSAL AND STORAGE OF USED SOLVENTS												
68	SAFETY TRAINING IN FOR THE LAB.												

Project:
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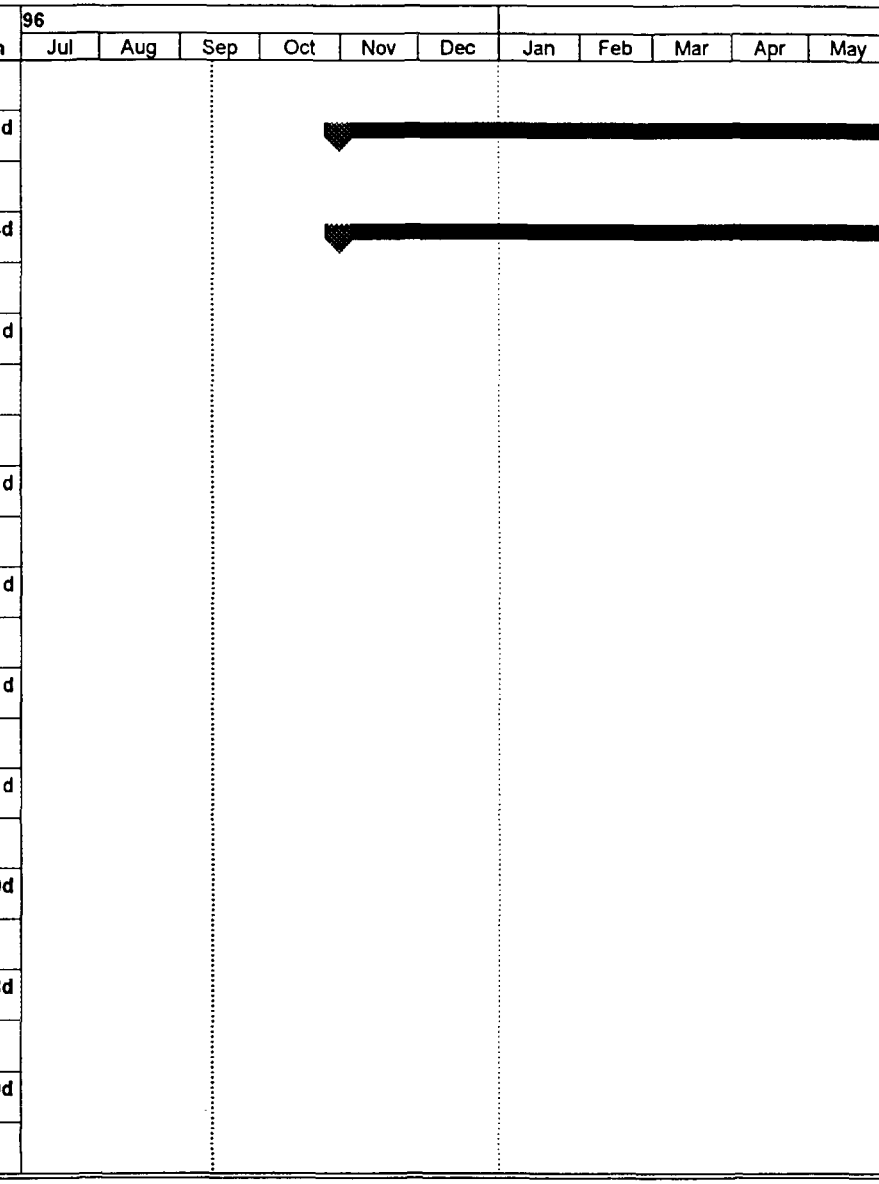
MASTER VALIDATION PLAN (SUMMARY)

ID	Task Name	Duration
1		
2	VALIDATION MASTER PLAN	429d
3		
4	- FACILITY VALIDATION	264d
12		
13	- RAW MATERIAL FLOW	1d
14		
15		
16	- WASTE FLOW	1d
17		
18	- PRODUCT FLOW	1d
19		
20	- PERSONNEL FLOW	1d
21		
22	- EQUIPMENT FLOW	1d
23		
24	- UTILITIES	10d
30		
31	- EQUIPMENT VALIDATION IQ, OQ, PQ	38d
48		
49	- PROCESS QUALIFICATION	110d
59		



Project:
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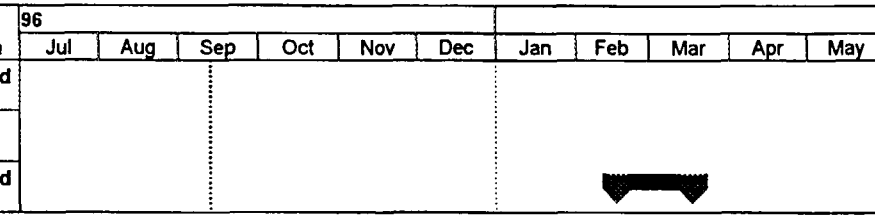
MASTER VALIDATION PLAN (SUMMARY)

ID	Task Name	Duration
60	- PERSONNEL QUALIFICATION	28d
64		
65	- SAFETY	21d



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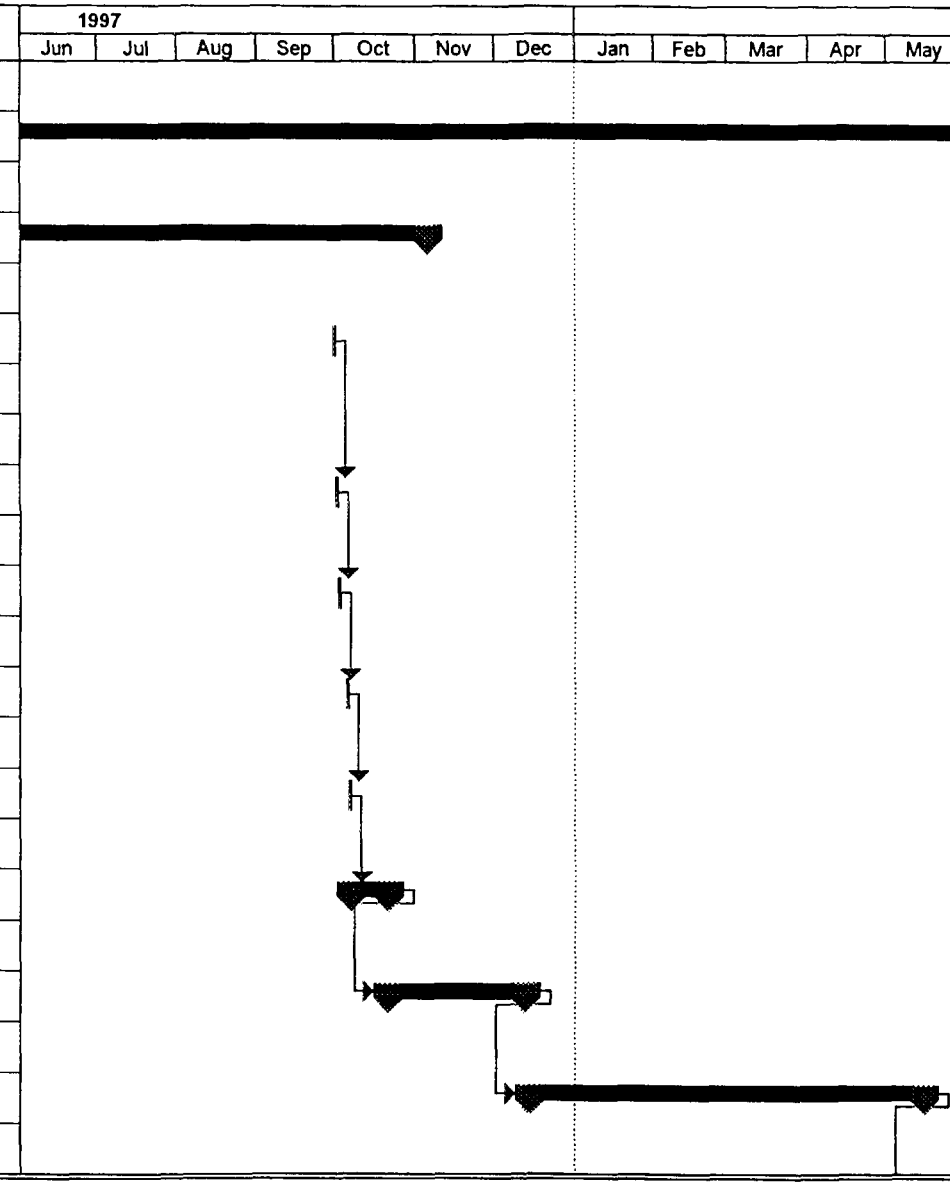
MASTER VALIDATION PLAN (SUMMARY)

ID	Task Name
1	
2	VALIDATION MASTER PLAN
3	
4	- FACILITY VALIDATION
12	
13	- RAW MATERIAL FLOW
14	
15	
16	- WASTE FLOW
17	
18	- PRODUCT FLOW
19	
20	- PERSONNEL FLOW
21	
22	- EQUIPMENT FLOW
23	
24	- UTILITIES
30	
31	- EQUIPMENT VALIDATION IQ, OQ, PQ
48	
49	- PROCESS QUALIFICATION
59	



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MASTER VALIDATION PLAN (SUMMARY)

ID	Task Name
60	- PERSONNEL QUALIFICATION
64	
65	- SAFETY

1997: Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May

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Milestone — Rolled Up Milestone

MASTER VALIDATION PLAN (SUMMARY)

ID	Task Name
1	
2	VALIDATION MASTER PLAN
3	
4	- FACILITY VALIDATION
12	
13	- RAW MATERIAL FLOW
14	
15	
16	- WASTE FLOW
17	
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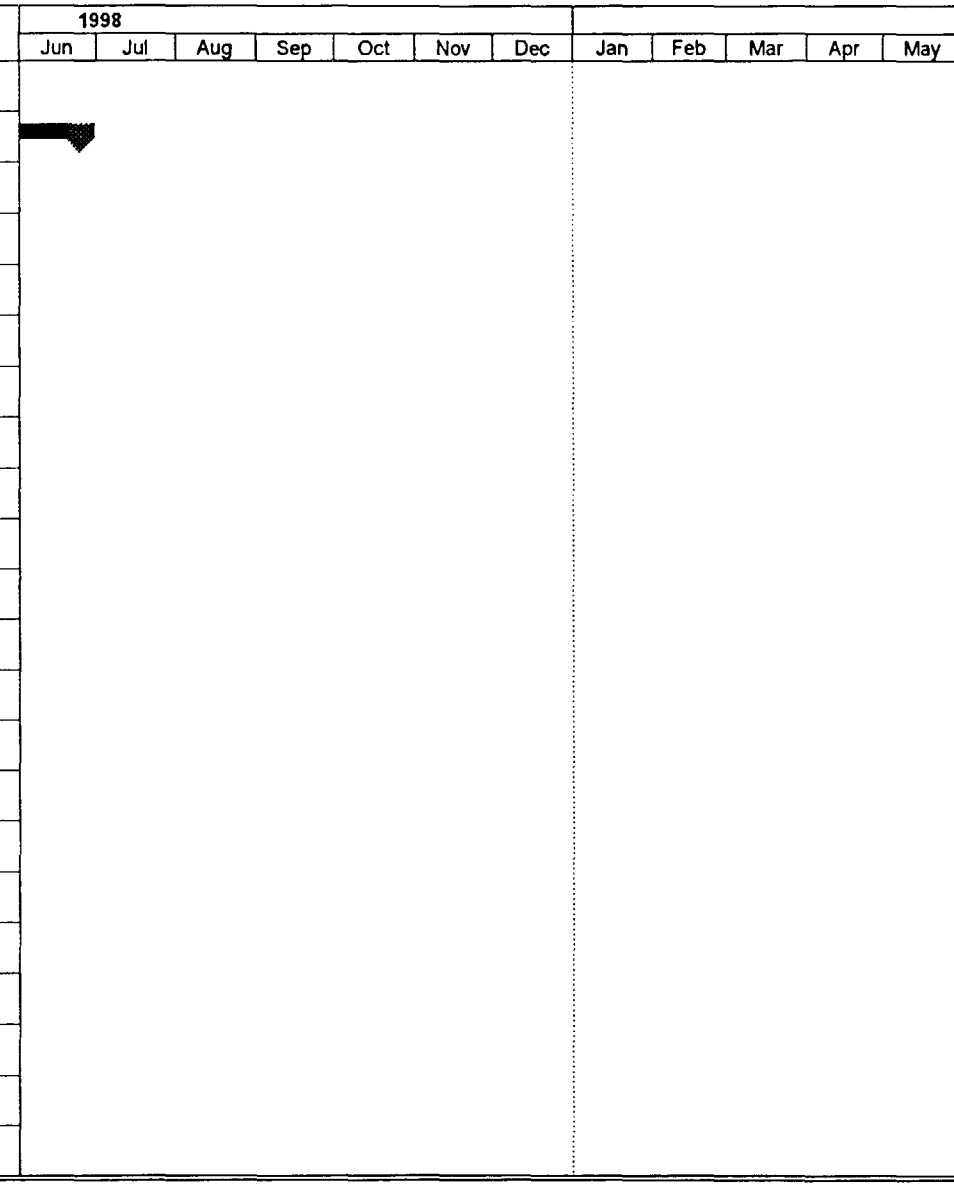
24	- UTILITIES
30	
31	- EQUIPMENT VALIDATION IQ, OQ, PQ
48	
49	- PROCESS QUALIFICATION
59	



Project:
Date: 9/13/96

Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress

MASTER VALIDATION PLAN (SUMMARY)

ID	Task Name	1998 Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
60	- PERSONNEL QUALIFICATION												
64													
65	- SAFETY												

Project:
Date: 9/13/96

Task | Summary | Rolled Up Progress

Progress | Rolled Up Task

Milestone | Rolled Up Milestone

PROCESS DESCRIPTION

SUMMARY

The manufacturing of the CANDID 1 vaccine is a classical viral vaccine procedure using individual stationary, 150 square centimeter flasks. One hundred and ten flasks each containing 45 ml of EMEM-NEAA media are inoculated with 1.2 x 106 Fetal Rhesus Lung-2 (FrhL-2) cells. The cultures are incubated at 37 °C in 5% CO_2. After 24 hours, the cultures are fluid changed with 45 ml growth media and incubated for a further 4 days.

The cultures are inoculated with 2 ml of 105.0/ml of the Junin virus strain # C-1 and the virus is adsorbed for 1 hour at 30-35 °C at which point 60 ml growth media containing 0.25% of Human Serum Albumin (HSA) is added. Cultures are placed in the incubator for an additional 4 days during which time they are observed microscopically. After the completion of incubation, the viral fluid is harvested and stabilized with 7% HSA. After removal of samples for quality control testing, the harvested virus is stored at -70 °C until completion of testing.

For preparation of the final bulk, 4 L of the Junin vaccine is supplemented with an additional 23% HSA and further diluted 1:1 with stabilizer for a total 9.6 L.

Vaccine is filled into freeze-drying containers with 2.75 ml/vial. A normal batch yields 3500-10 dose containers. The vaccine is freeze-dried using a 48-72 hr cycle. Each batch yields approximately 35000 doses.

The following process flow identifies the major steps and the required testing controls at each point.

RECOMMENDATIONS

- Consideration should be given to coarse filter the virus harvest through a 0.45 μm filter to minimize any cellular debris. The current procedure does not have this step.

- Change the production methodology from the present "open system" individual flask to safer "close system" technology.

FLOW CHART - CANDID 1 MANUFACTURE

Step	Controls
Set up Cultures	Cell count
Fluid Change	
Cell Cultivation	Sterility Extraneous Agents
Virus Infection	Virus Titre of Inoculum
Harvest	Sterility Mycoplasma Tissue Culture Safety Virus Titre Identity TB *in vitro* Adult Mice Suckling Mice Guinea Pig Safety
Clarification*	Sterility Virus Titre
Final Bulk Preparation	Sterility Virus Titre
Filling	Sterility Virus Titre Fill Volume
Freeze-Drying	Sterility Virus Titre Identity Moisture Content Osmolality pH General Safety Stability
Capping & Labeling	
Storage @ -20 ° C	

Note: This step is presently being performed and it is highly recommended that clarification of the virus harvests is done through a 0.45μm membrane filter.

PRODUCT STABILITY

Currently, limited stability data of the freeze-dried vaccine is available.

Accelerated and -20°C storage stability of the product appears to be satisfactory. New stability programme for the vaccine to be produced at NIHVD has to take into consideration the post reconstitution and 4 °C product stability.

The minimum potency requirement for the vaccine is 104 pfu.

ACCELERATED STABILITY

Lot 2-1-88

		Potency pfu
Final Container (4/13/88)		$10^{5.25}$
Final Container (5/13/88)		$10^{5.05}$
Final Container (3/29/89)		$10^{4.98}$
Accelerated Stability Test (6/5/89)	24h @ 37 °C	$10^{4.39}$
	72h @ 37 °C	$10^{2.77}$
	168h @ 37 °C	$10^{2.55}$
Accelerated Stability Test (3/1/90)	24h @ 37 °C	$10^{4.64}$
	72h @ 37 °C	$10^{4.02}$
	168h @ 37 °C	$10^{2.71}$

Lot 3-1-89

		Potency pfu
Final Container (10/12/89)		$10^{5.00}$
Accelerated Stability Test (10/20/89)	24h @ 37 °C	$10^{4.62}$
	72h @ 37 °C	$10^{4.08}$
	168h @ 37 °C	$10^{3.23}$
Accelerated Stability Test (3/1/90)	24h @ 37 °C	$10^{4.44}$
	72h @ 37 °C	$10^{3.91}$
	168h @ 37 °C	$10^{3.42}$

-20 °C STORAGE STABILITY

	Test Date	Potency
Lot 1-85	8/13/85	$10^{4.61}$
	2/19/87	$10^{4.77}$
	8/11/87	$10^{4.40}$
	Average of 3	$10^{4.62}$
Lot 2-1-88	4/18/88	$10^{5.23}$
	6/2/88	$10^{5.12}$
	4/27/89	$10^{4.98}$
	12/17/90	$10^{5.01}$
	7/15/91	$10^{5.09}$
	8/24/92	$10^{5.17}$
	Average of 6	$10^{5.11}$
Lot 3-1-89	10/27/89	$10^{5.00}$
	3/1/90	$10^{4.89}$
	7/15/91	$10^{4.92}$
	11/13/91	$10^{4.95}$
	8/24/92	$10^{4.84}$
	Average of 5	$10^{4.93}$
Lot 4-1-91	2/13/92	$10^{4.70}$
	8/24/92	$10^{4.80}$
	9/30/93	$10^{4.76}$
	Average of 3	$10^{4.76}$
Lot 5-1-92	12/21/92	$10^{4.77}$
	9/30/93	$10^{4.76}$
	10/3/94	$10^{4.82}$
	Average of 3	$10^{4.79}$

HISTORY OF VIRAL SEED

The Junin XJ isolate was attenuated at USAMDRC in 1980 together with the collaboration of the Argentine Ministry of Health. The following passage sequences were used to attenuate the virus and to formulate the Master Seed:

Junin XJ isolate was passaged in Guinea Pig brain for 2 passages.....XJ GP2

A further 44 passages were performed in Mouse brain.....XJ GP2 MB44

Subsequently 16 passages in Tissue Culture, cell line FRhL-2 were performed.....XJ GP2 MB44 FRhL16

From this the **Master Seed**, and the **Working Seed** were produced using FRhL-2 stationary cultures.

Therefore, CANDID 1 live, attenuated vaccine is considered at passage level.

XJ GP2 MB44 FRhL16

The Master and Working Seed were produced by the Salk Institute by 14 passages of the above. The following seeds were supplied to NIHVD in the early 1980's.

Master Seed: 41 x 1 ml vials

Working Seed: 55 x 2 ml vials

The seeds have been validated by the Salk Institute that included the Neurovirulence tests in monkeys. Several production lots have been manufactured and have been tested satisfactorily as per the CFR requirements.

RECOMMENDATIONS

NIHVD must obtain additional seed vials of both the Master and Working levels to ensure long-term consistency of production with the use of the original Seed. This strategy will minimize extensive tests and revalidation required when a new Seed is manufactured.

It is also our recommendation that when production is initiated at NIHVD, that the first 3 lots manufactured are tested for neurovirulence in monkeys as this will validate manufacturing procedures at NIHVD.

The current Master and Working Seed inventory should last for 10 to 12 years based on 30 production runs per year. This will provide for the production of 15 million doses of vaccine in total.

CELL SEED

For the production of the attenuated Junin virus, FRhL-2 cell line is being used. The following are the inventories as of this report:

Master Seed	@ passage 14	40 vials
Working Seed 1	@ passage 17	50 vials
Working Seed 2	@ passage 24	120 vials

All of the Seeds are stored in liquid Nitrogen in 1 ml aliquots at the concentration of 1.2×10^6 cells/ml.

At the Working Seed 2 level (FRhL-2), the contents of one vial is used to inoculate one T150 flask. Therefore, for each routine production lot consisting of 110 culture flasks, 110 vials of the working seed will be used.

RECOMMENDATIONS

Consideration be given to produce the new working cell seed at the appropriate passage level and quantity to secure future production.

To ensure quality and consistency of production, the cell seeds at the Master and Working seed levels must be tested for:

- Sterility
- Mycoplasma
- Viability
- Identity
- Susceptibility
- Extraneous Agents

CONTROL OF FETAL BOVINE SERUM AND HUMAN SERUM ALBUMIN

As both bovine and human sera are utilized during the manufacture of the AHF vaccine, it is important that NIHVD take the appropriate precautions when procuring and testing these raw materials. Heat inactivated sera is a preferred option if the production process is not adversely affected. Substituting with serum free media is ideal, although in many processes is impractical.

Fetal Bovine Serum must be source from Bovine Spongiform Encephalopathy (BSE) free designated countries. Argentina falls within this category and so does the United States of America. The serum should be tested for Adventitious Agents and the following Bovine viruses and Mycoplasma:

- Infectious bovine Rhinotracheltis (IBR)
- Bovine Viral Diarrhea (BVD)
- Parainfluenza Virus Type 3 (PI3)
- Bovine Adenovirus
- Bovine Parvovirus
- US Food and Drug Administration (FDA), Code of Federal Regulations (CFR) testing includes Rabies and Reovirus Type 3
- Mycoplasma

Human Serum Albumin must be tested as per the CFR requirements, which include tests for the absence of Mycoplasma, Hepatitis B, Hepatitis A Surface Antigen and Human Immunodeficiency Virus (HIV). These tests are detailed in the 21 CFR sections 610.30, 610.40, and 610.45.

It is recommended that alternatives to Human Serum Albumin be evaluated as replacement for the stabilization of the vaccine during freeze-drying.

TRAINING AND RESOURCE REQUIREMENTS

NIHVD will be attempting to manufacture a viral vaccine with no previous experience in vaccinology. This is a handicap and requires extensive training for the next couple of years to fully prepare for the challenge of manufacturing and compliance with cGMP.

It is recommended that two types of training be implemented; at a collaborative vaccine manufacturer for practical experience such as Salk Institute, and in-house theoretical and practical training using qualified consultants. The staff will need to be trained in the following:

- Production procedures for the manufacturing of the Candid 1 vaccine must include the preparations of the working seeds for the cells and virus, preparation and infection of the cultures, processing of virus harvest, preparation of the final bulks, filling and freeze drying, labeling and packaging.
- Training for all the required testing for the in-process and final release of vaccines.
- Preparation for the writing and the management of Standard Operating Procedures.
- Training in cGMP procedures.
- Validation procedures and documentation.
- Principal and operation of freeze-dryer.
- Preventative maintenance of essential equipment.
- Setting of stability programme and the maintenance of this programme.
- Preparation of protocols for release of products.
- Designing of Batch Production Record and the maintenance of these records.
- Statistical analysis of production information.

This training should be of high priority and must commence immediately after the budget has been approved and must be completed prior to initiating any production or testing activities.

The cost of the training and consultant fees, totaling $200,000, has been included in the summary of the budget.

THE NATIONAL CONTROL AUTHORITY

It is our understanding that the National Control Authority, Administracion National de Medicamentos Alimentos y Tecnologia is involved mainly with the release of "Over the Counter" drugs and is not equipped to control, test and release vaccines. Therefore, it will be in the best interest of NIHVD to take an active role in the training of the NCA regulators in the requirements for the release of the vaccine. This early interaction with NCA will facilitate timely release of the vaccine and will give credence and build trust between the two institutions so that the vaccine could be released on protocol.

It is also advisable that UNIDO take an active role in the training of the NCA staff for the release and control of biologicals.

PROPOSED ORGANIZATION AND PERSONNEL PLAN

The attached organizational chart represents a typical hierarchy in a biological manufacturing operation. Dr. Delia Enria is currently the Director of the Institute and we highly recommend her to manage this project. As per the organizational chart, the Heads of Manufacturing, Regulatory Affairs, Facility Engineer, and the SPF colony report to the Director.

Reporting to Manufacturing are the 3 production areas: Cell Propagation, Viral Manufacturing (to which includes Filling and Freeze-Drying), Reagent Preparation, and Washing and Sterilizing.

Reporting Quality Assurance are Validation and Metrology department, Compliance and Product Release.

Reporting to Quality Control are In Vivo and In Vitro Testing and Physicochemical Testing.

The following are the recommendations for the personnel plan:

1. Currently the SPF facility is adequately staffed with a maximum load of 1000 mice and 250 guinea pigs per year. We recommend no new hiring. However, should the demand increase substantially due to demands from the research department, increasing the staff must be considered.

2. Quality Control department needs to be established from scratch. The head of QC needs to be appointed and must report to Regulatory Affairs. Two technicians will report to the QC head.

3. Cell Culture and Reagent Manufacturing Department is currently a well-established area and is capable of meeting all of the needs of the institute. However, 3 additional technical help, 1 for Washing and Sterilizing and the other 2 for Cell Manufacture, will be required once the Viral Production Department becomes fully operational.

4. Viral Production Department currently does not exist and has to be newly established. Due to the nature of manufacturing, viral production, bulk preparation, filling, freeze-drying, capping and labeling can be scheduled on a campaign basis. This will allow for optimal usage of the same personnel within this area. We recommend the hire of a virologist for the position of Supervisor, 2 technical staff and 2 technical assistants.

5. In Engineering and Maintenance, in addition to the current staff, we strongly recommend the recruitment of an engineer whose responsibilities will include operation of the complex freeze-dryer, maintenance of all large production equipment and validation.

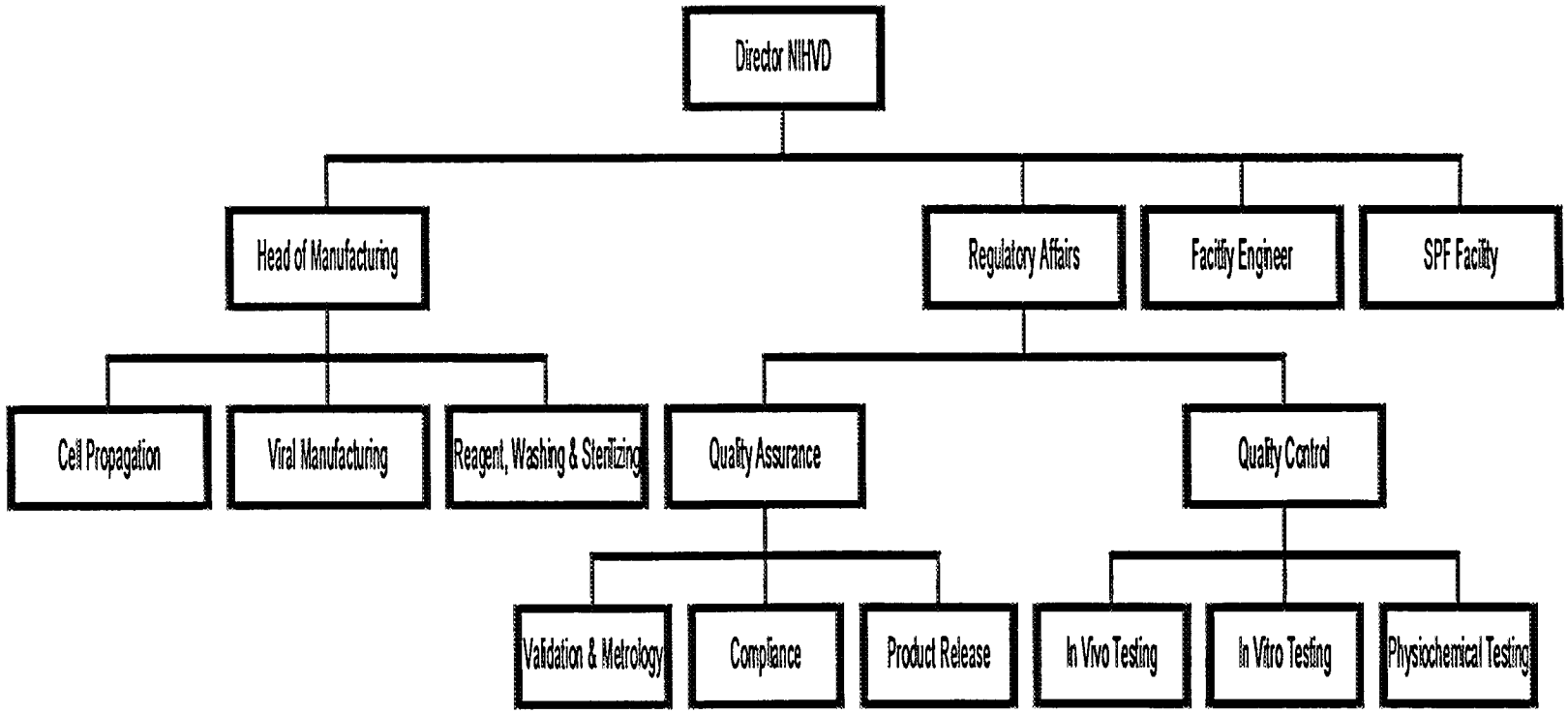
6. Regulatory Affairs department needs to be established for the purposes of preparing all the documentation and protocols for submission to the National Control Authorities (NCA). In addition to placement of the Head Regulatory Affairs, a technical writer with sound knowledge of science is required.

7. Quality Assurance needs to be established as well. Two technical staffs are required whose duties and responsibilities will include validation and certification of facilities, equipment, cGMP training, inspection, vendor certification, compliance, product release, product complaint and complete documentation.

Summary:

SPF facility	no new hires
Quality Control	1 supervisor + 2 technicians
Cell Culture, Media and Washing Sterilizing	2 supervisors + 1 technician
Virus Production and Processing	1 supervisor + 2 technicians + 2 assistants
Engineering and Maintenance	1 supervisor
Regulatory Affairs	1 technical writer
Quality Assurance	2 technician
Total	**15 additional staff**

PROPOSED ORGANIZATION CHART



PATENT AND INTELLECTUAL PROPERTY RIGHTS

The vaccine was developed by the Argentinean Government with the collaboration of USAMRDC. It is our understanding that no patents or Intellectual Property Rights were registered by any of the above.

It is our recommendation that NIHVD obtain, in writing, an agreement from USAMRDC and The Salk Institute giving NIHVD the total rights for manufacturing and marketing for the Candid 1 vaccine.

EVALUATION OF THE SPF ANIMAL COLONY

The SPF colony is a well-designed, 400 square metres, modern facility. The purpose of this facility is to breed mice and guinea pigs for testing and research requirements.

The air system supplying this facility is independent and the air supply to the six rooms housing the animals is filtered in situ.

The personnel entering the clean side of this facility must shower and gown prior to entry. The personnel flow is unidirectional and should meet cGMP requirements.

The flow of waste materials is via the dirty corridor into the room housing the double- ended autoclave for decontamination and sterilization. Material and equipment utilized for the clean breeding suites are sterilized through a double-ended autoclave into the clean area.

The six suites housing the animals can sufficiently accommodate the requirement for all the testing needs for the AHF vaccine and the additional requirement for the diagnostic research.

This facility is in good control and the only recommendations are for institution of SOP's and proper validation.

THE PROPOSED FACILITY

Changes to the current facility will be localized in the Viral Production department with upgrades in other departments. Additions to the facility will consist of the new filling and freeze-drying area, capping and labeling area, quarantine storage and final product storage freezers.

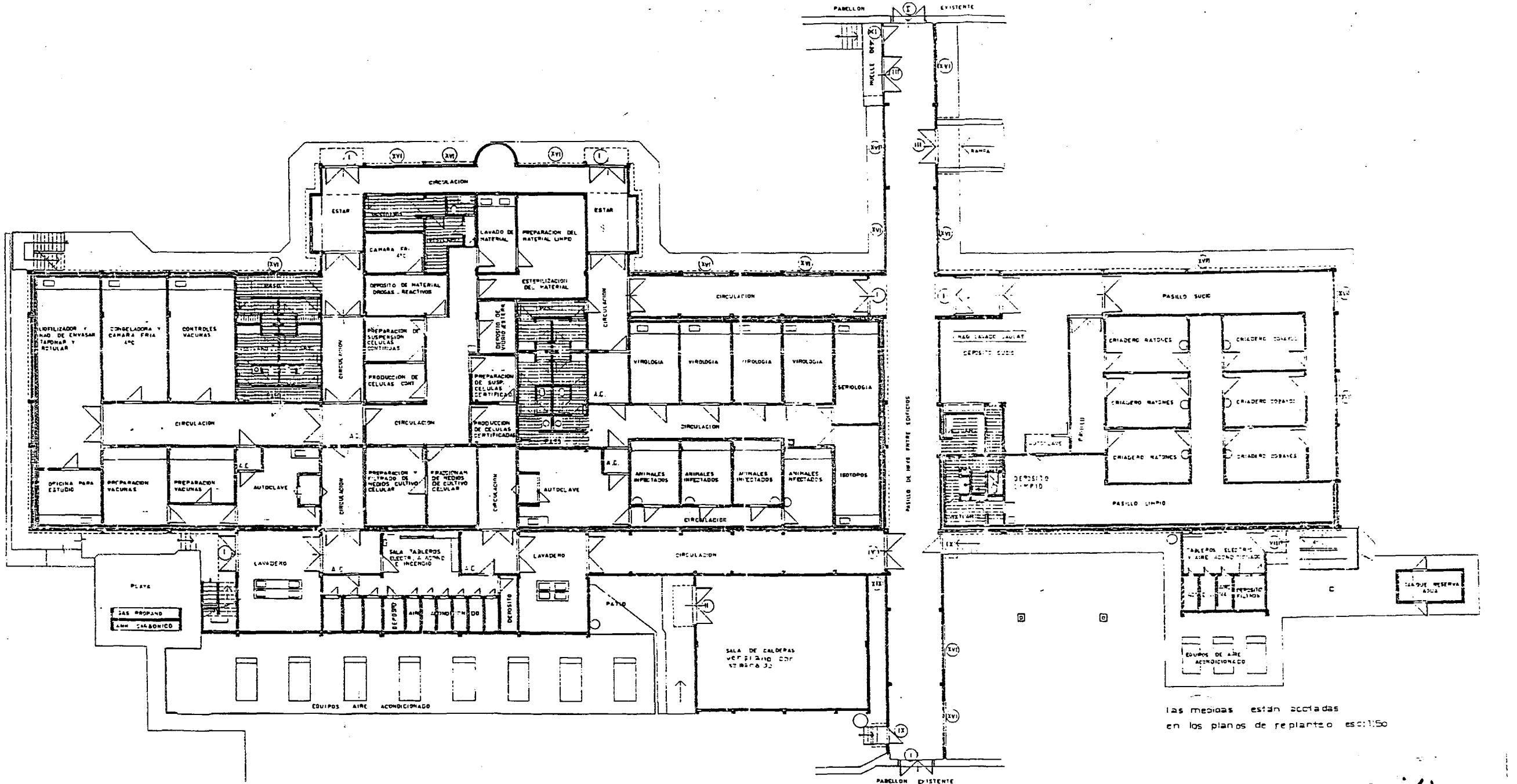
In addition, Water For Injection (WFI), an emergency dry season water reservoir, and complete air handling systems will be installed in this facility. Throughout the facility, recommended interlock doors must be installed to increase product and personnel safety.

The following 7 facility floor plans give an overview of:

- proposed facility
- personnel flow
- material flow
- clean equipment flow
- dirty equipment flow
- product flow
- interlocking door locations

It is recommended that for cGMP compliance, additional modifications to the corridor system in the Viral Production facility be implemented so that the flow of clean and dirty equipment do not crossover. This is shown in the attached facility drawings.

Overall, the whole facility is solidly built and has a great potential for producing biological vaccines. The Argentinean Ministry of Health has to be commended for their commitment and overwhelming support for this project.



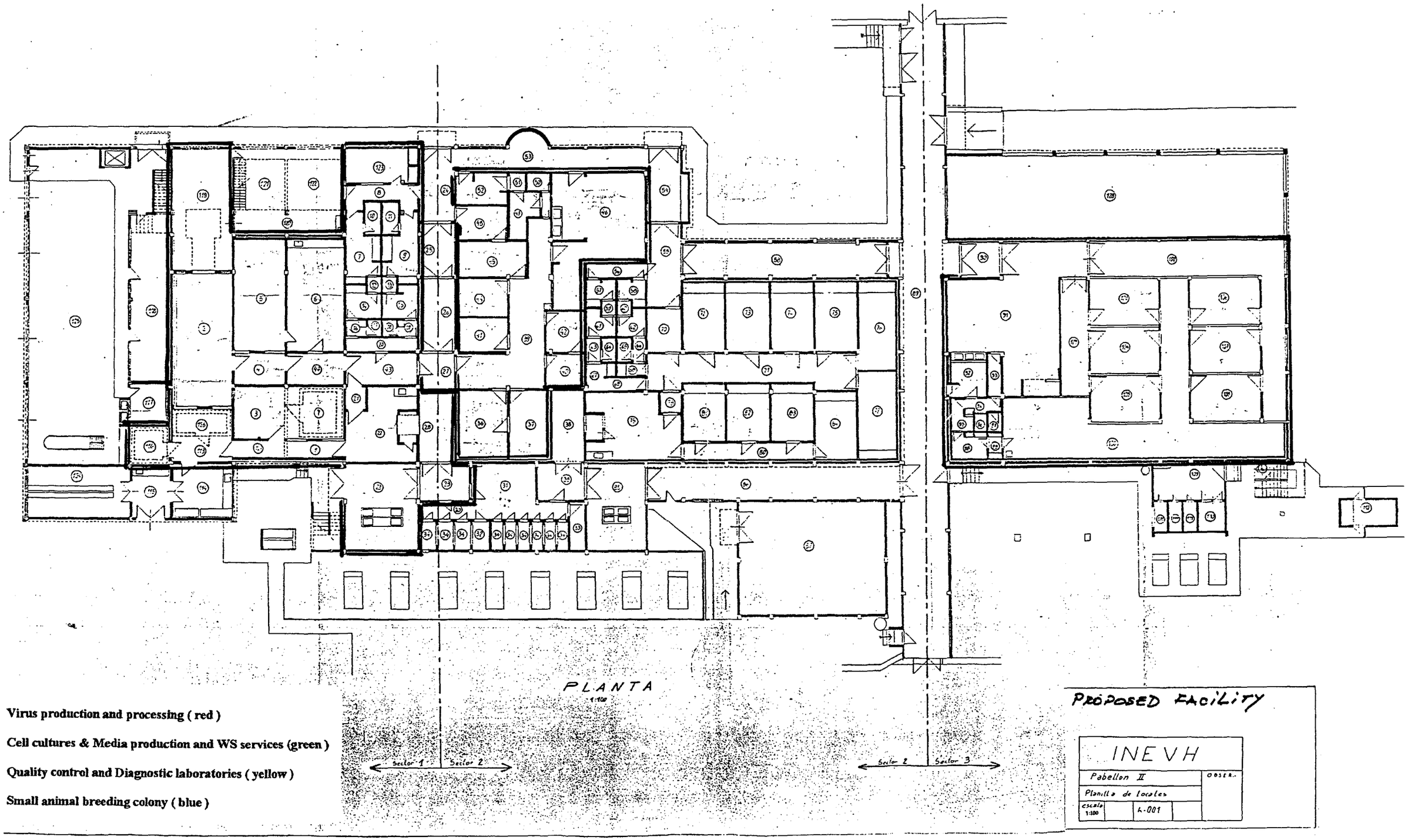
PLANTA

ESCALA 1:100

las medidas estan acotadas en los planos de replanteo esc: 1:50

CURRENT FACILITY

MINISTERIO DE SALUD Y ACCION SOCIAL DE LA NACION DIRECCION GENERAL DE ADMINISTRACION-DEPARTAMENTO DE ARQUITECTURA	
SECRETARIA DE SALUD INSTITUTO NACIONAL DE ENFERMEDADES VIRALES HUMANAS	
OBRA CENTRO DE BIO SEGURIDAD	
LOCALIDAD PARTIDO DE PILAR BUENOS AIRES	
DIBUJO / REVISO	TITULO: PLANTA EXISTENTE PABELLON 2 — PLANO Nº
ESCALA / FECHA	MODIFICACIONES

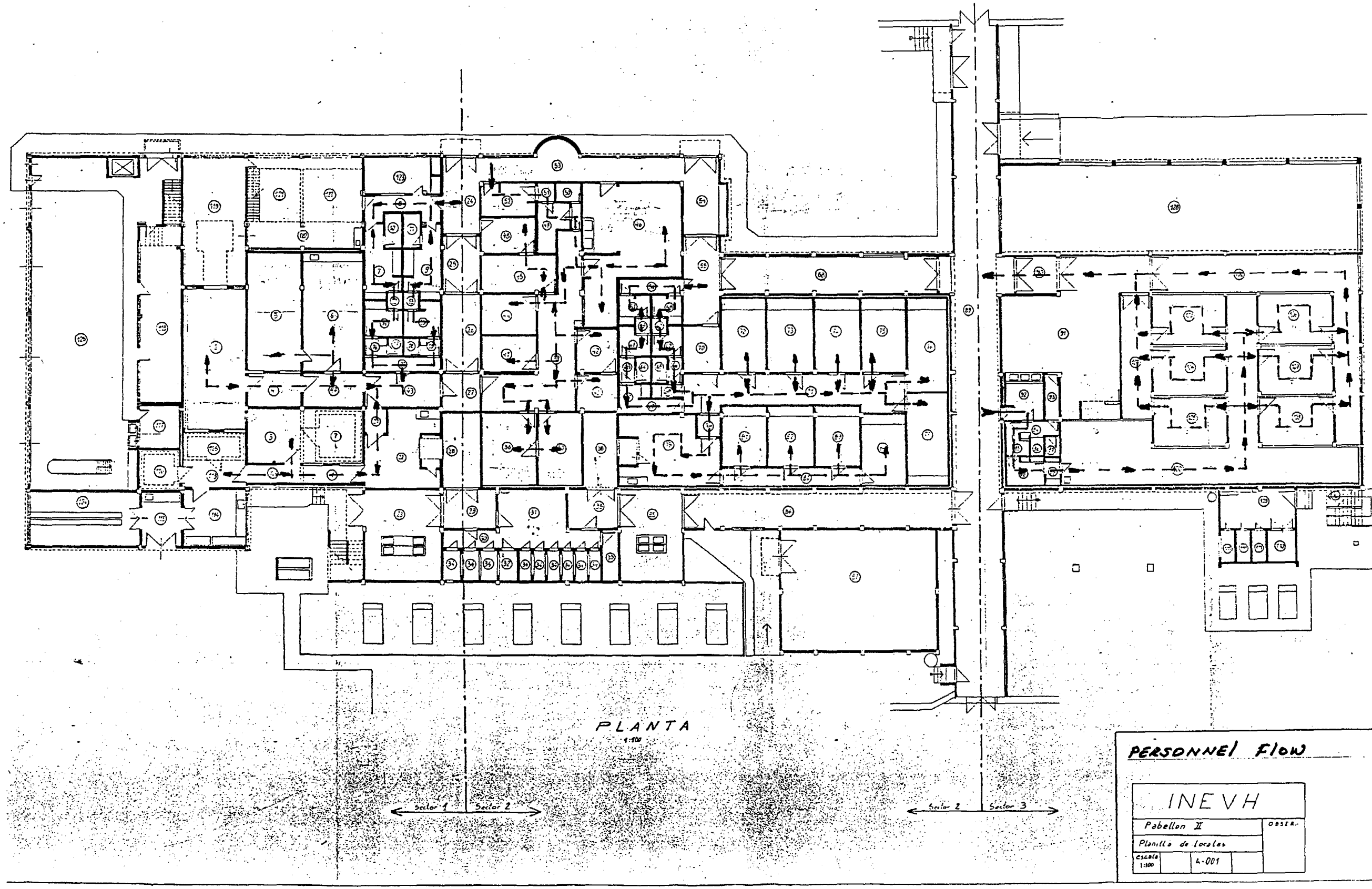


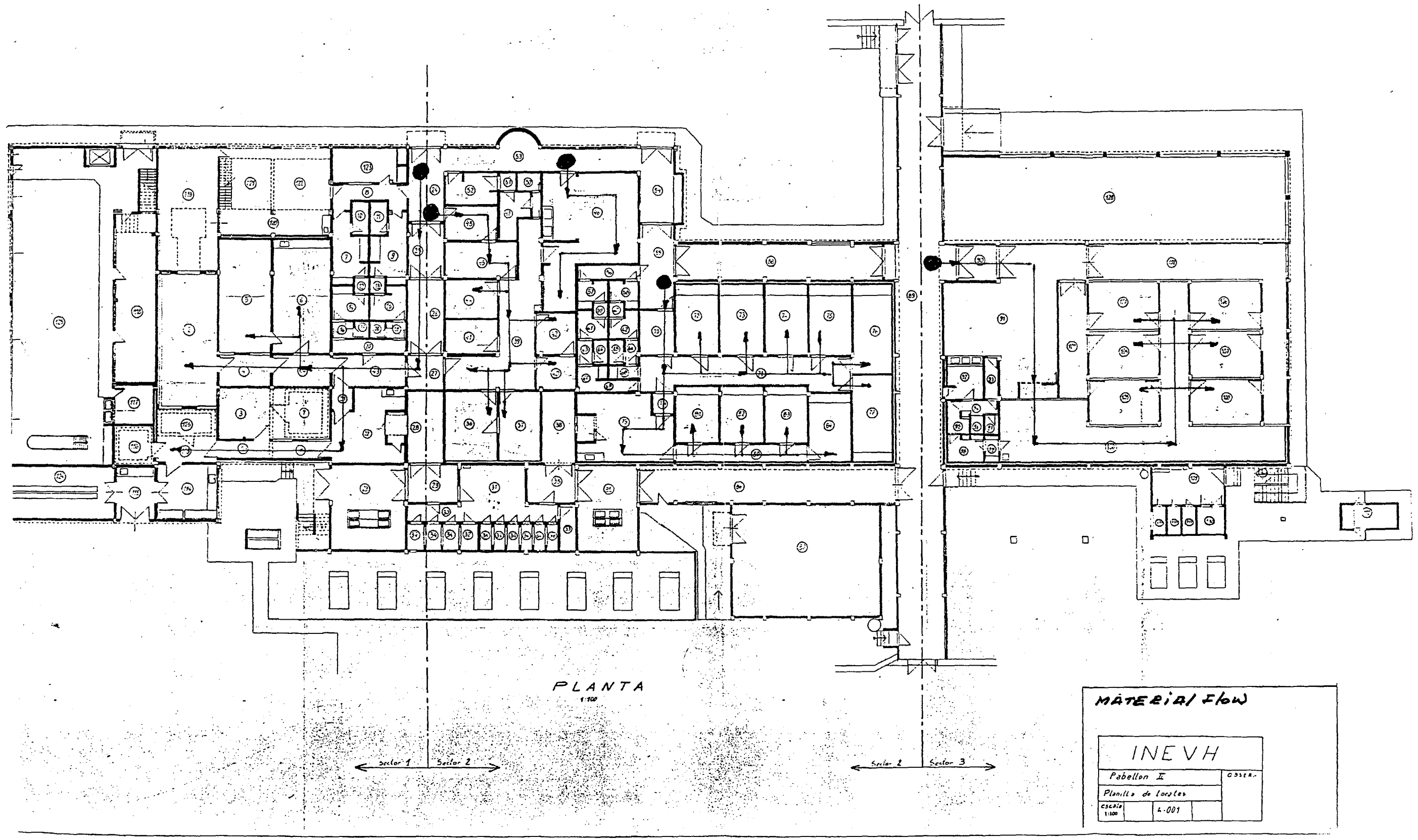
Virus production and processing (red)

Cell cultures & Media production and WS services (green)

Quality control and Diagnostic laboratories (yellow)

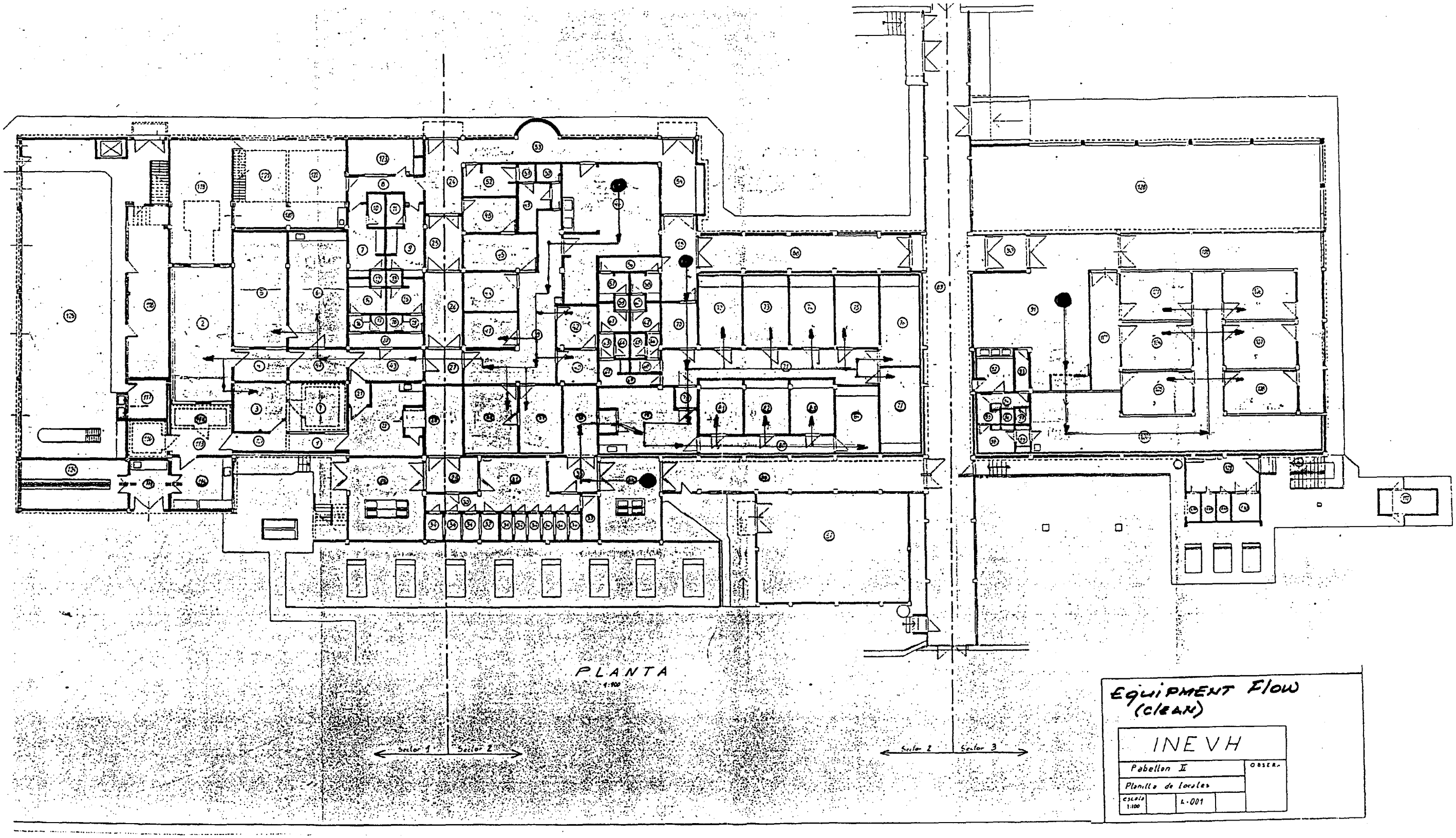
Small animal breeding colony (blue)

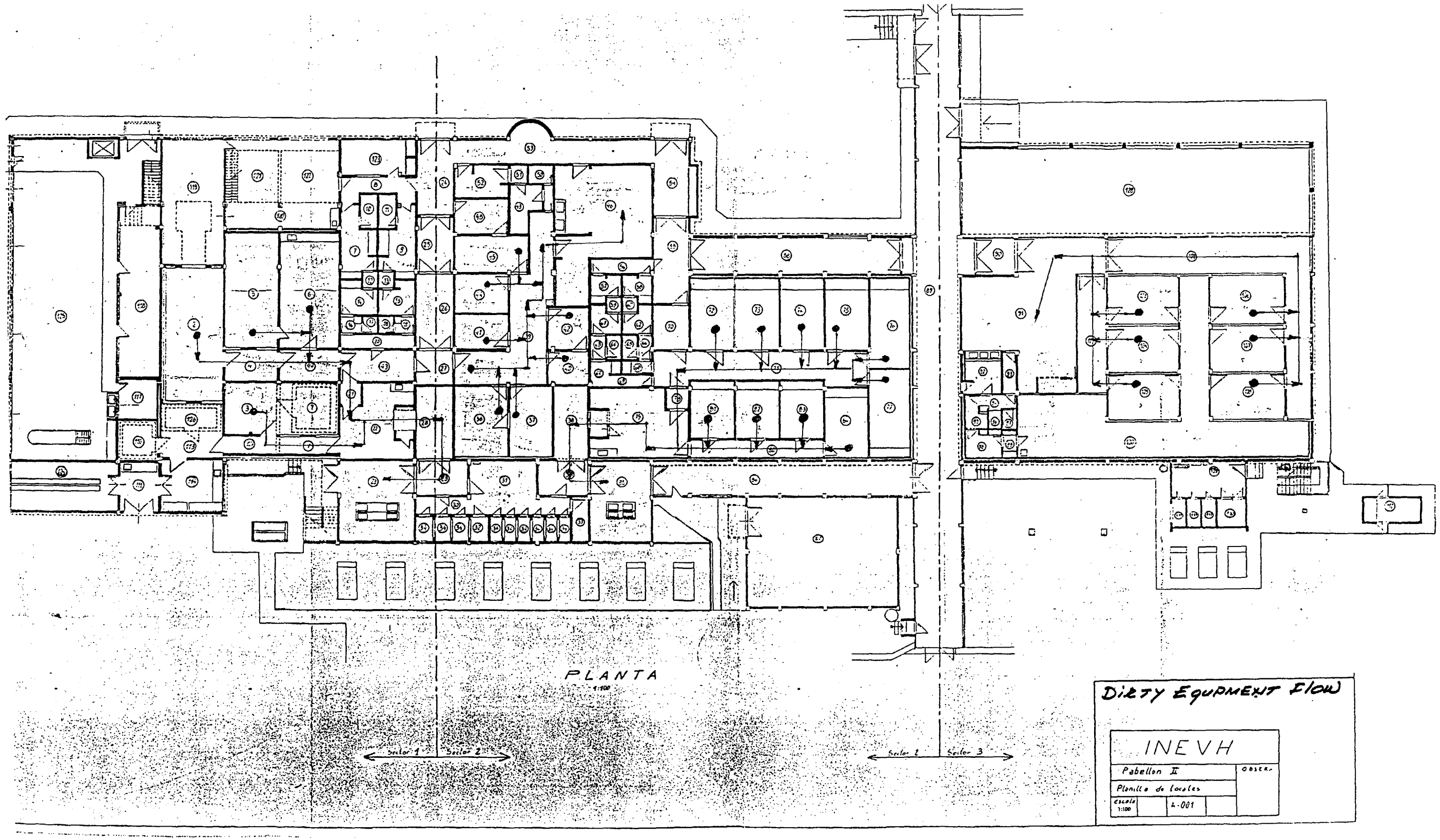

PLANTA
1:100
Sector 1
Sector 2
Sector 2
Sector 3
PERSONNEL FLOW
INEVH
Pabellon II
OBSER.-
Planilla de Locales
escala 1:100
L-001

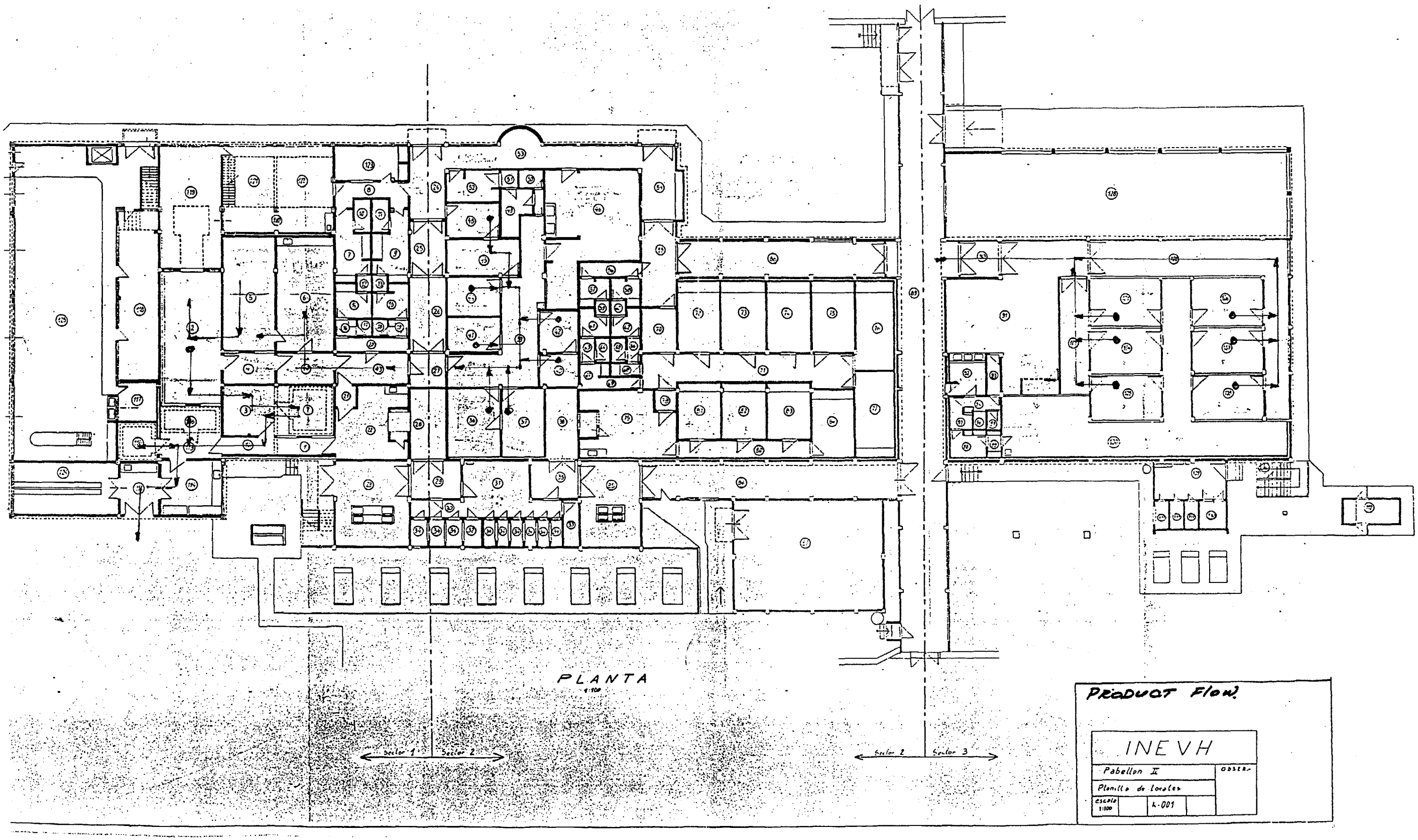


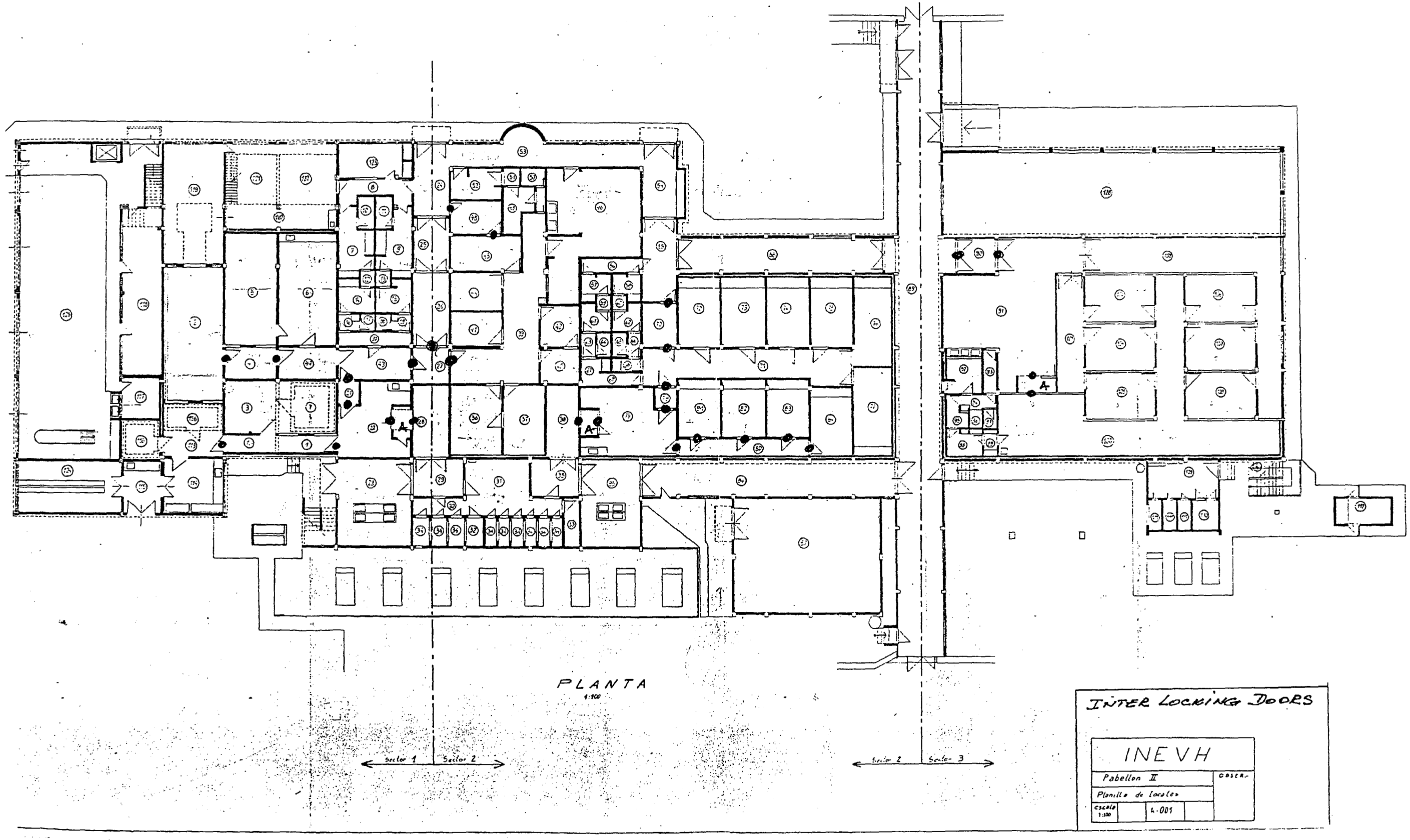
MATERIAL FLOW

INEVH		
Pabellon II		OSSER.
Planilla de tarjetas		
escala 1:100	L-001	


PLANTA
1:100
Sector 1
Sector 2
Sector 2
Sector 3
EQUIPMENT FLOW
(CLEAN)
INEVH
Pabellon II
OBSER.
Planilla de Locales
escala 1:100
L-001


PLANTA
1:100
Sector 1
Sector 2
Sector 2
Sector 3
DIRTY EQUIPMENT FLOW
INEVH
Pabellon II
OBSER.
Planilla de locales
escala 1:100
L-001


PLANTA
1:100
Sector 1
Sector 2
Sector 2
Sector 3
PRODUCT FLOW
INEVH
Pabellon II
Planilla de locales
OBSER.
ESCALA 1:100
4-001



INTER LOCKING DOORS

INEVH		
Pabellon II		COSER-
Planilla de Locales		
escala 1:100	A-001	

CAPITAL REQUIREMENTS

The attached Summary of Capital Costs totaling approximately $4.5 million identifying the individual capital requirements for designated production and testing areas are listed in detail. The highlights are:

- $850,000 construction cost to modify and renovate the existing Viral Production Facility which will also include the Filling and Freeze-Drying area, Capping and the Labeling area and the Vaccine Warehouse.

- $470,000 has been included for the installation and upgrading of the Air Handling System for the new construction and existing facilities to meet the class BL-3 requirements.

- Equipment cost of $2,005,770, which has to be expensed in the first year of this project. The detailed equipment list is identified as per the area of manufacturing where they will be required.

- $290,000 have been reserved for the upgrading of the Sewage Treatment Plant, the Water Reservoir which is required due to water shortage during the summer period, and the upgrading of gas supply lines.

- As emphasized, a total budget of $300,000 has been included for Personnel Training, Validation Costs and Short-term Consultant Fees. This expenditure is crucial for the success of this project.

SUMMARY OF CAPITAL COSTS

	US$
Capital Expenditure General	695,000
Capital Expenditure Production Facility	
Cell Culture Area	80,000
Vaccine Production (Bulk) Area	114,000
Filling, Freezing, Drying & Packaging Area	898,000
Capital Expenditure Quality Control and Quality Assurance	
Quality Control	187,200
Quality Assurance	31,570
Spare Parts (10% of total)	200,577
Freight & Insurance (15% of total)	300,866
-70 °C Freezer Warehouse	70,000
Production Facility Construction	850,000
Sewage Treatment	120,000
Water Reservoir	150,000
Gas Supply Lines	20,000
Personnel Training	100,000
Short Term Consultants	50,000
Facility Maintenance/Spare Parts	50,000
Validation Costs	150,000
Upgrading and New installation of air handling	470,000
Total	**4,537,213**

Note: Equipment costs are based on actual quotations from Canadian suppliers, as requested in July 1996, for delivery to Argentina. Construction costs are based on Canadian costs and may be cheaper in Argentina. All other costs are estimates based on previous experiences.

FACILITY CAPITAL REQUIREMENTS

Requirements for completion of production plant to specifications:

Light Fixtures
Laboratory Case Work
Laboratory Sewage
Automatic Airlock System
Polyurethane Sealing of Electrical Conduits
Water for Injection
Rain Water Drainage
Installation of New Doors for Proper Flow of Material and Personnel
Installation of Filter Testing Chambers for HEPA Filter Equipment Rooms
Construction for Water Reservoir
Construction of Walk-in Freezers
Extension of Facility for Freeze-Dryer and Mechanicals

Total **US$ 850,000**

CAPITAL EXPENDITURE GENERAL

EQUIPMENT	UNIT COST US$	QUANTITY	TOTAL COST
Glassware Washer	14,500	1	14,500
Stopper Washer	15,000	1	15,000
Vial Washer	22,500	1	22,500
Autoclave (GMP)	300,000	1	300,000
Filter Integrity Testing Equip.	10,000	1	10,000
Clean Steam Generator	130,000	1	130,000
Water: Demineralizer	28,000	1	28,000
Still (720 l/hr)	130,000	1	130,000
Holding Tank (with recycling pump, 80 °C, 4000 l)	45,000	1	45,000
Total			**695,000**

CAPITAL EXPENDITURE PRODUCTION FACILITY

	UNIT COST US$	QUANTITY	TOTAL COST US$
CELL CULTURE AREA			
Controlled-Rate Freezing Device	7,000	1	7,000
Refrigerated Centrifuge Low-Speed	7,000	1	7,000
Inverted Microscope	6,000	1	6,000
Laminar Flow Hood	8,000	2	16,000
Refrigerator (4 °C)	10,000	1	10,000
Freezer (-70 °C)	8,000	2	16,000
Liquid Nitrogen Freezer	8,000	2	16,000
Large Water-Bath	2,000	1	2,000
Subtotal			80,000
VACCINE PRODUCTION (BULK)			
Inverted Microscope	6,000	2	12,000
Biohood	10,000	1	10,000
Freezer (-70 °C)	10,000	2	20,000
Walk-In (40 °C)	20,000	2	40,000
Walk-In (37 °C)	15,000	1	15,000
Walk-In (4 °C)	15,000	1	15,000
Water-Bath	2,000	1	2,000
Subtotal			114,000
FILLING, FREEZING, DRYING & PACKAGING AREA			
Freeze-Drier (11000 x 32 mm vial)	700,000	1	700,000
Labeling Machine	15,000	1	15,000
Overhead Laminar Flow Hood (Filling)	20,000	1	20,000
Vial Capper	12,000	1	12,000
Mixing Tank (50 l)	10,000	2	20,000
Filling Machine with Multiple Heads	105,000	1	105,000
Overhead Laminar Flow (Vials)	10,000	1	10,000
Airborne Particle Counter	6,000	1	6,000
Stainless Steel Vial Container	100	100	10,000
Subtotal			898,000
Grand total			**1,092,000**

CAPITAL EXPENDITURE QUALITY CONTROL AND QUALITY ASSURANCE

	UNIT COST US$	QUANTITY	TOTAL COST
QUALITY CONTROL			
Egg Incubator	2,500	2	5,000
CO2 Incubator	5,000	6	30,000
Refrigerator (4 °C)	1,200	3	3,600
Freezer (-70 °C)	12,000	5	60,000
Laminar Flow Hood	10,000	2	20,000
Inverted Microscope	6,000	1	6,000
Microscope	6,000	1	6,000
Fluorescence Microscope	14,000	1	14,000
Gas Pack Anaerobic system	600	1	600
Water Bath	1,000	2	2,000
Karl Fisher Moister Detector	12,000	1	12,000
Analytical Balance	5,000	1	5,000
pH Meter	1,000	2	2,000
Osmometer	4,000	1	4,000
Chemical Fume Hood	12,000	1	12,000
ELISA Recorder	5,000	1	5,000
			187,200
QUALITY ASSURANCE			
Multipoint Recorder	8,000	1	8,000
Thermocouple Wire	460	2	920
Constant Temperature Oil Bath	900	1	900
Standard Thermometer	75	10	750
Thermoanemometer	1,000	1	1,000
Airborne Particle Counter	6,000	1	6,000
DOP Aerosol Photometer	6,000	1	6,000
Pressure Calibrator	1,400	1	1,400
Temperature/Relative Humidity/Differential Pressure Meter	3,400	1	3,400
Smoke Generator	1,600	2	3,200
Subtotal			31,570
Total			**218,770**

ECONOMIC ANALYSIS

The economic analysis for this project has been simplified to take into consideration the fact that CANDID 1 vaccine is not a commercial product and the intentions of the Argentinean Ministry of Health is to supply the vaccine free of charge to its population.

This analysis does not take into consideration capital spent to date by the Argentinean Ministry of Health in constructing and equipping the facility as it stands today. This analysis also takes into consideration that once the affected population of 3.5 million inhabitants has been immunized the maintenance requirement will be approximately 150,000 doses per year. This should be enough to cover the 2% birth cohort and a certain allowance for the increase in the diseased demographic. Current data for patients who had received the vaccine at least 8 years ago indicate good protection levels to date. However, should the antibody levels decrease over a 10 year period, booster shots for this population will be required at that point in time. The economic savings of the booster shot has not been taken into consideration as this is an unknown factor.

It is also assumed that the purchase price of the CANDID 1 vaccine will remain at $5/dose over the next 5 years. However, it should be understood that the Salk Institute may not be in the position to supply the CANDID 1 vaccine in the future as legal constraints and other projects take priorities.

The total required capital to complete this project is estimated at $ 4,537,213. The attached economic analysis model takes into consideration the depreciation cost of approximately $1 million dollars a year for the first three years and thereafter reduced to reflect lower production outputs. The total cost per dose is estimated at $2.03 for the first four years, and thereafter at a cost of $2.36 per dose which reflects the reduced production rate. This cost of $2.03/dose reflects material, wages and overhead costs which equals the incremental operating cost of $1000,000 annually.

It is cost effective to manufacture the vaccine at NIHVD, as this model shows the return on investment in less than two years.

Recommendations are for the Argentinean Government to fund or find International Agencies to help fund this project as the financial justifications are favorable. Also, the manufacturing capability provides the Argentinean Government with the opportunity to eradicate the AHF disease, to become self-sufficient in production of AHF, and sows the seeds for a human vaccine manufacturing industry in the country.

ECONOMIC ANALYSIS

years	1	2	3	4	5	6	7	8	9	10
# of doses	1000000	1000000	1000000	500000	150000	150000	150000	150000	150000	150000
Cost/dose from SALK	$5	$5	$5	$5	$5	$5	$5	$5	$5	$5
Total Cost/year	$5,000,000	$5,000,000	$5,000,000	$2,500,000	$750,000	$750,000	$750,000	$750,000	$750,000	$750,000

Capital Cost of Project **$4,537,213**										
yearly depreciation	$1,031,185	$1,031,185	$1,031,185	$515,592	$154,678	$154,678	$154,678	$154,678	$154,678	$154,678
incremental operation cost	$1,000,000	$1,000,000	$1,000,000	$500,000	$200,000	$200,000	$200,000	$200,000	$200,000	$200,000
total operating cost	$2,031,185	$2,031,185	$2,031,185	$1,015,592	$354,678	$354,678	$354,678	$354,678	$354,678	$354,678

Cost savings	$2,968,815	$2,968,815	$2,968,815	$1,484,408	$395,322	$395,322	$395,322	$395,322	$395,322	$395,322
Cost/dose	$2.03	$2.03	$2.03	$2.03	$2.36	$2.36	$2.36	$2.36	$2.36	$2.36
Cost saving/dose	$2.97	$2.97	$2.97	$2.97	$2.64	$2.64	$2.64	$2.64	$2.64	$2.64

5 YEAR BUSINESS PLAN

The attached 5 Year Facility and Manufacturing Plan Time line gives a comprehensive overview of all functions that need to be accomplished in order to achieve the goals set by NIHVD to immunize 3.5 million high risk inhabitants of the endemic region. The highlights of this plan are:

- Protocols of all existing clinical data pertaining to safety and efficacy will have to be submitted to the NCA for their acceptance. We have estimated that within a period of six months this goal can be accomplished. This action point will be the responsibility of Dr. Enria and it is expected that this task will start immediately.

- The facility design and construction approval once the funding has been approved should be completed in less than 3 months and thereafter, construction should be completed by the first quarter of 1997.

- It is envisaged that all equipment specification, tender, delivery and installation will be accomplished within a period of 1 year. As with the facility, the validation can commence as the equipment is being installed and tested for operation. It is expected that the facility and the equipment validation as per the Master Validation Plan will be completed in time for manufacture of the 5 Consistency Lots of which three will be for the stability programme.

- Establishment of technology and training is the key to the success of this project. It has become very clear to the consultants that NIHVD will be attempting to manufacture the CANDID 1 vaccine with no previous experience in cGMP manufacturing. Ms. Ambrosia and some of her staff had the opportunity of being trained at the Salk Institute during the period when Salk was manufacturing the CANDID 1 vaccine. However, a 5 week training period does not encompass the art cGMP manufacturing. It is our recommendation that negotiations with SALK Institute in Pennsylvania to accommodate further training be conducted for some of the key employees at NIHVD. It is also imperative that a full-time consultant be available at NIHVD to assist in the whole aspect of facility renovation and construction, validation, cGMP and manufacturing. It is envisioned that training and validation will be concurrently ongoing through the manufacture of the 5 Consistency Lots which is scheduled to be completed in early 2nd Quarter, 1998.

- Validation of the facility, equipment, process and testing is an ambitious goal and will initiate when the facility is completed and as the equipment begins to arrive. This is expected to start when the funding is approved as the SPF and the Cell Culture/Media preparation/ Washing and Sterilization facilities are ready for validation now. Due to the size of this project, all the staff at NIHVD will be involved in the validation with supervision from the assigned project leaders. It is also important that training in validation commence prior to starting the validation. It is also highly recommended that a consultant in validation is available to guide and train the staff. A total of $150,000 has been assigned for basic validation. Process and QC testing validation will occur concurrently with the manufacture of the 5 consistency lots. It is expected that the validation project will take up to the second quarter of 1998.

- Neurovirulence testing, the seed supplied by USAMRDC, has been tested for Neurovirulence and shown to be safe. However, if a new seed lot needs to be manufactured at NIHVD using

the Master Seed from USAMRDC, it is advisable to initiate a reduced Neurovirulence test. This gives the assurance for the attenuation of the virus strain. A reduced Neurovirulence test will cost approximately $100,000, whereas a full test for three samples can cost as much as $400,000. The cost of the neurovirulence test has not been incorporated in the budget as this is optional.

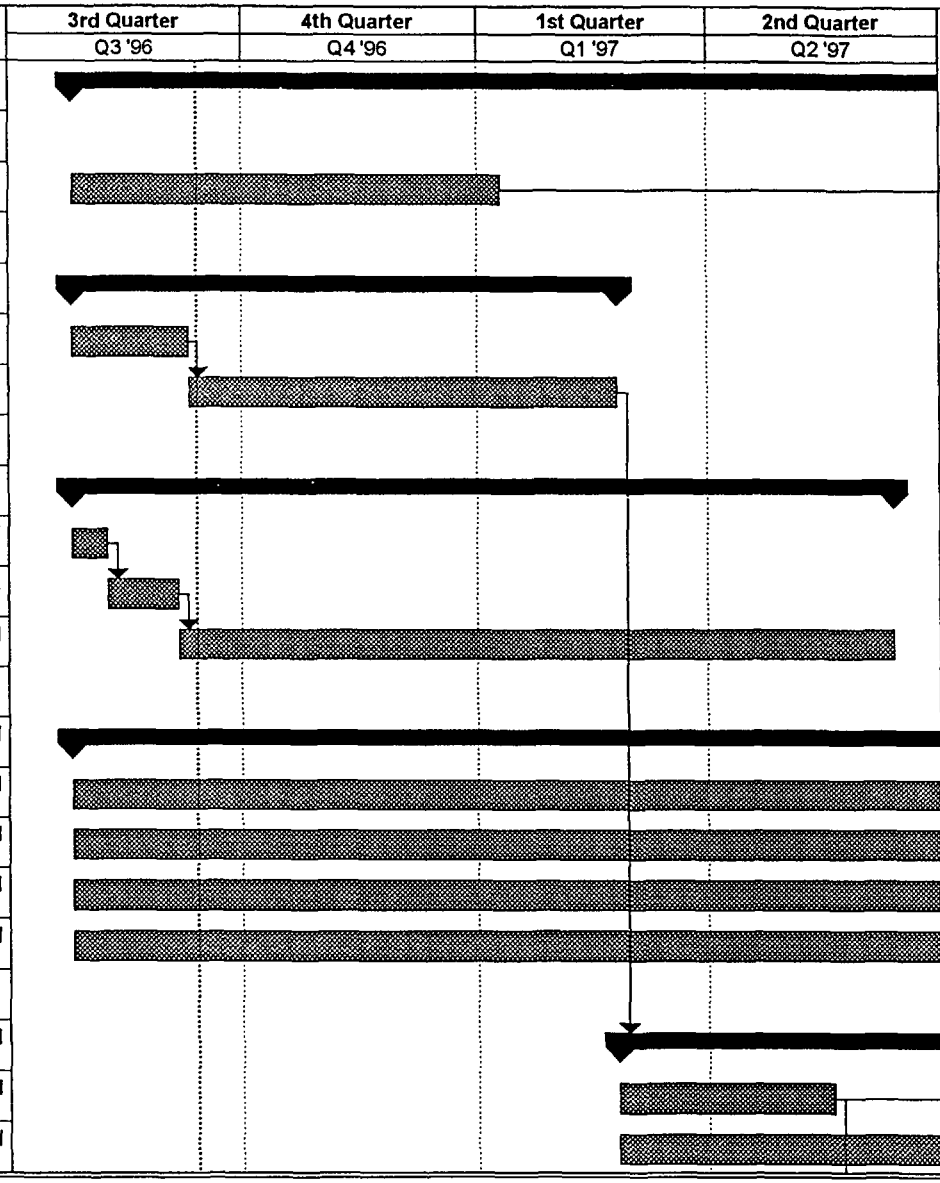
- Regulatory Approvals from NCA. It is expected that as the facility, equipment and process validation is completed the protocols are promptly prepared and submitted to the NCA for their approvals. It is planned that all approvals including the license for Candid 1 are received by mid 1998 and that launch of the product can commence immediately thereafter.

- It is planned that manufacturing can produce one million doses of vaccine a year for three to four years, and thereafter the manufacturing capacity will be reduced to meet the demands of the new birth cohorts at 150,000 doses a year. This can be easily accomplished by stock-piling the bulk concentrate and holding it at -70 °C.

- It is important that a stability plan be established as it is critical that the potency of the vaccine is maintained up to the expiry date.

5 YEAR FACILTY AND MANUFACTURING PLAN (DETAILED)

ID	Task Name	Duration
1	**TIME LINE - AHF VACCINE**	**527d**
2		
3	**CLINICAL DATA TO REGULATORY AUTHORITY**	120d
4		
5	**FACILITY**	**152d**
6	DESIGN AND CONSTRUCTION APPROVAL	32d
7	FACILITY CONSTRUCTION	120d
8		
9	**EQUIPMENT**	**230d**
10	SPECIFICATIONS	10d
11	TENDER	20d
12	DELIVERY AND INSTALLATION	200d
13		
14	**ESTABLISMENT OF TECHNOLOGY &TRIANING**	**260d**
15	MANUFACTURING	260d
16	QUALITY CONTROL- TESTING	260d
17	QUALITY ASSURANCE	260d
18	CGMP AND VALIDATION	260d
19		
20	**VALIDATION**	**120d**
21	FACILITY	60d
22	EQUIPMENT	90d



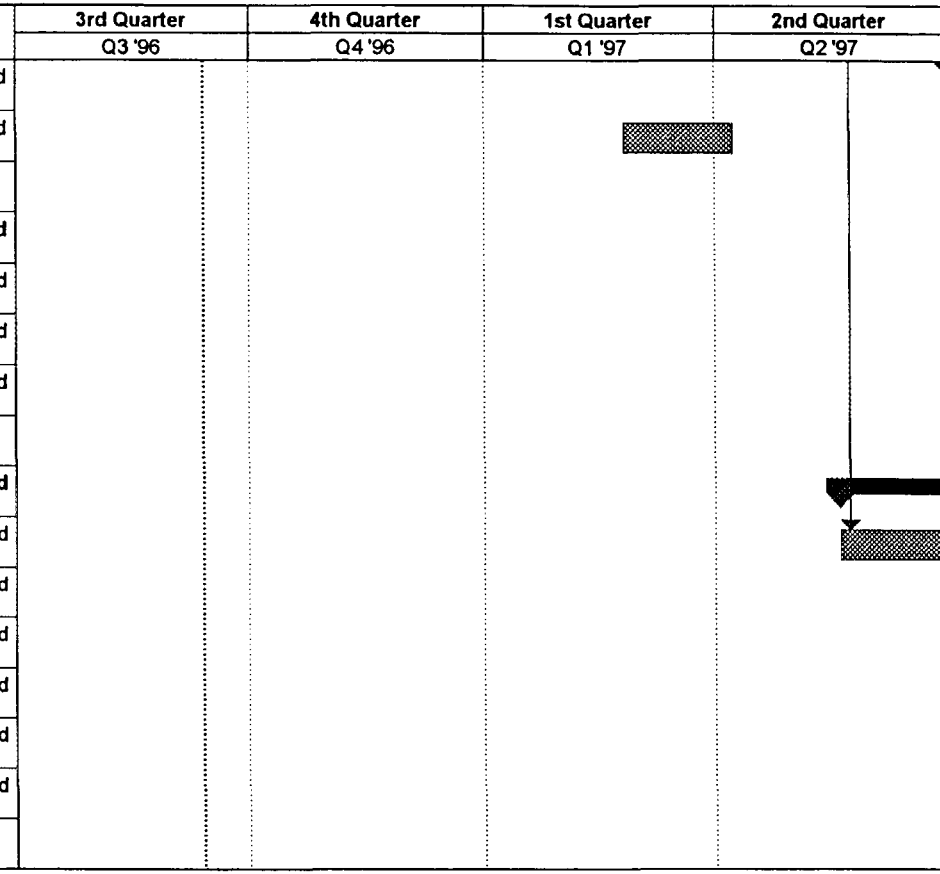
Project:
Date: 9/13/96

Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress

PRARGEN

5 YEAR FACILTY AND MANUFACTURING PLAN (DETAILED)

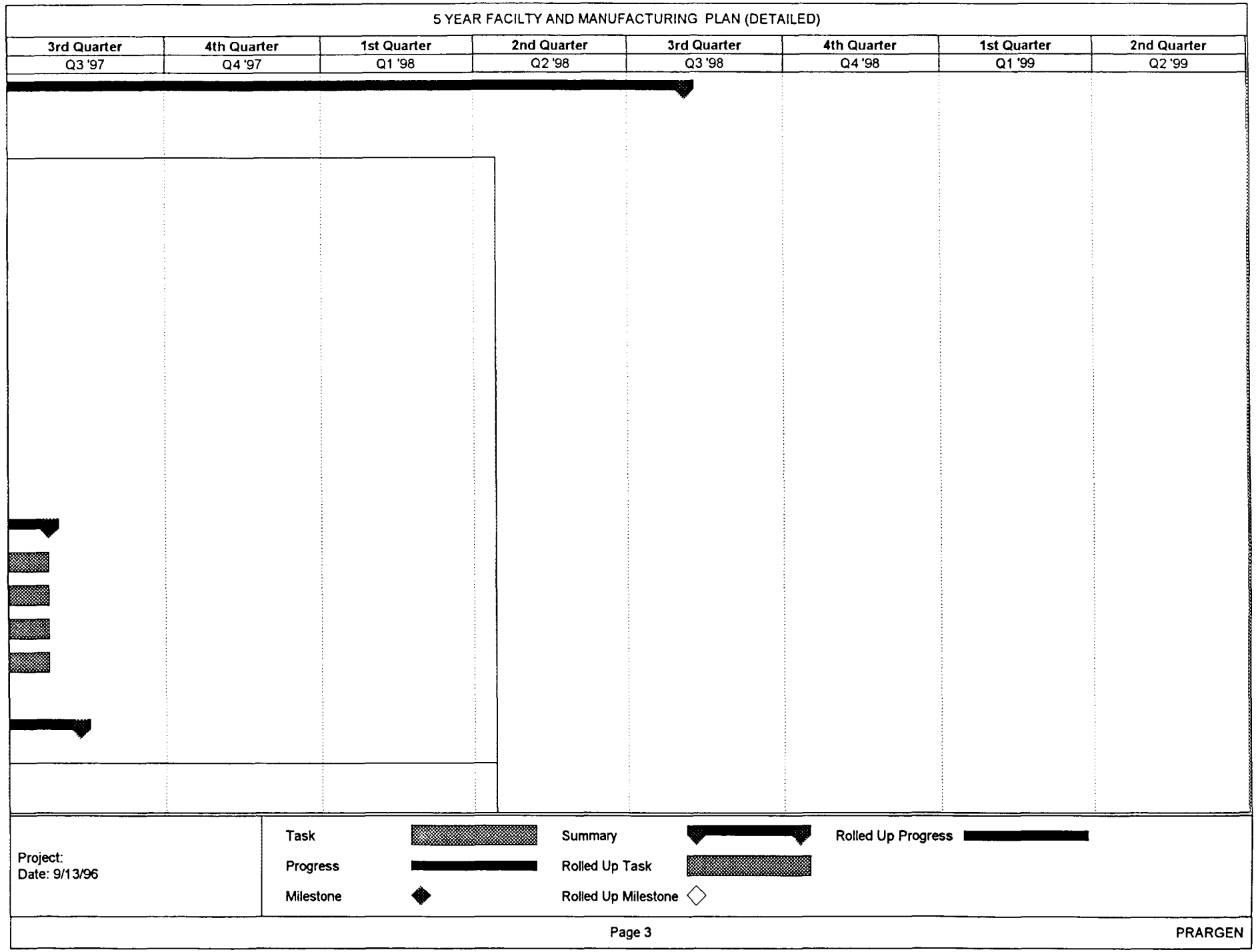
ID	Task Name	Duration
23	PROCESS	30d
24	TESTING	30d
25		
26	**5 LOTS CONSISTENCY**	**175d**
27	MANUFACTURING	80d
28	TESTING	35d
29	NEUROVIRULENCE	60d
30		
31	**REGULATORY APPROVALS**	**315d**
32	FACILTY APPROVAL	60d
33	PROCESS APPROVAL	60d
34	LICENCE APPROVAL	60d
35	LAUNCH PRODUCT	20d
36	**MANUFACTURING-1MILLION DOSES/YEAR**	20d
37	**STABILITY PROGRAM**	20d

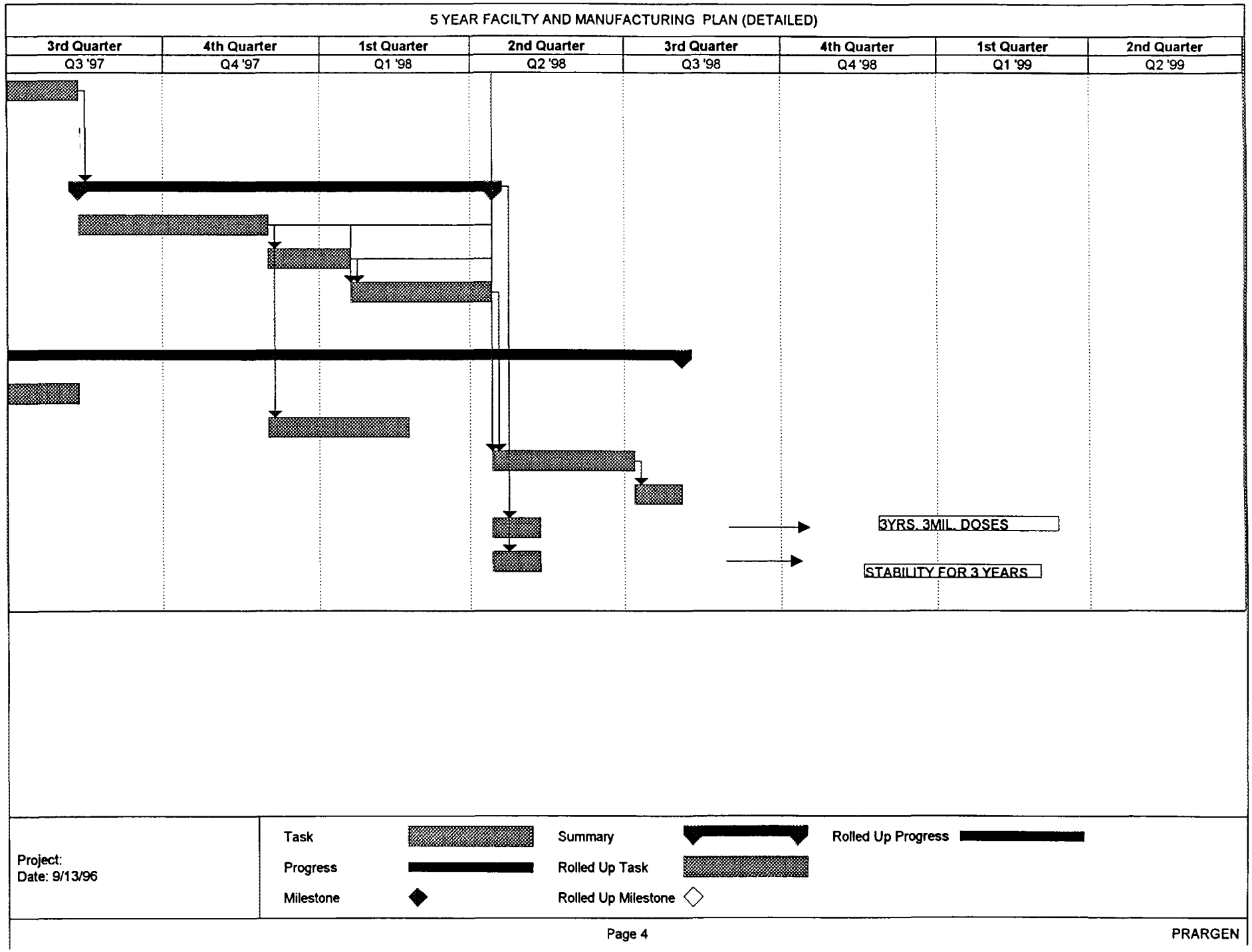


Project:
Date: 9/13/96

Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress

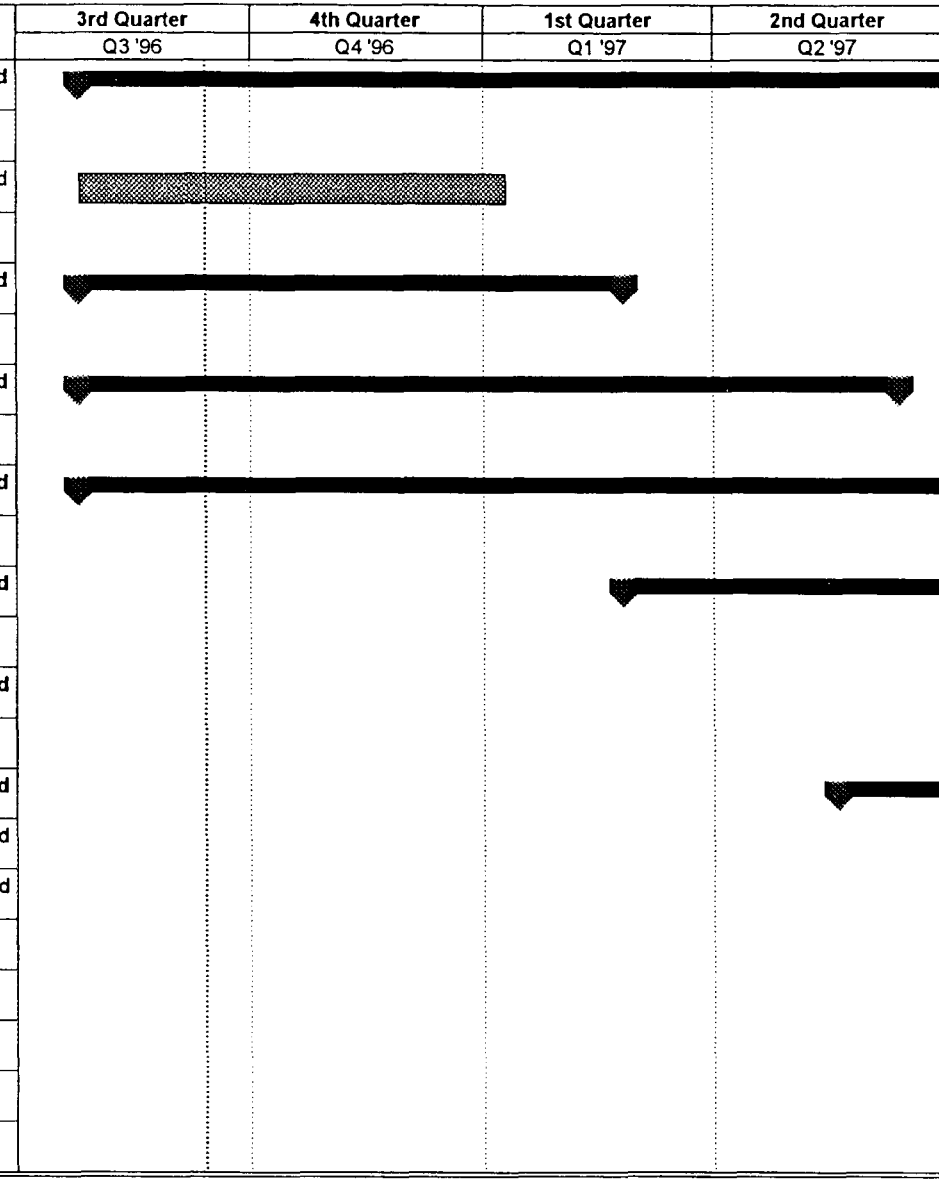
PRARGEN


5 YEAR FACILTY AND MANUFACTURING PLAN (DETAILED)
3rd Quarter
Q3 '97
4th Quarter
Q4 '97
1st Quarter
Q1 '98
2nd Quarter
Q2 '98
3rd Quarter
Q3 '98
4th Quarter
Q4 '98
1st Quarter
Q1 '99
2nd Quarter
Q2 '99
Project:
Date: 9/13/96
Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress
Page 3
PRARGEN


5 YEAR FACILTY AND MANUFACTURING PLAN (DETAILED)
3rd Quarter
Q3 '97
4th Quarter
Q4 '97
1st Quarter
Q1 '98
2nd Quarter
Q2 '98
3rd Quarter
Q3 '98
4th Quarter
Q4 '98
1st Quarter
Q1 '99
2nd Quarter
Q2 '99
3YRS. 3MIL. DOSES
STABILITY FOR 3 YEARS
Project:
Date: 9/13/96
Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress
Page 4
PRARGEN

5 YEAR FACILTY AND MANUFACTURING PLAN (SUMMARY)

ID	Task Name	Duration
1	TIME LINE - AHF VACCINE	527d
2		
3	CLINICAL DATA TO REGULATORY AUTHORITY	120d
4		
5	FACILITY	152d
8		
9	EQUIPMENT	230d
13		
14	ESTABLISMENT OF TECHNOLOGY &TRIANING	260d
19		
20	VALIDATION	120d
25		
26	5 LOTS CONSISTENCY	175d
30		
31	REGULATORY APPROVALS	315d
36	MANUFACTURING-1MILLION DOSES/YEAR	20d
37	STABILITY PROGRAM	20d



Project:
Date: 9/13/96

Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress

PRARGEN

5 YEAR FACILTY AND MANUFACTURING PLAN (SUMMARY)

ID	Task Name	Duration	3rd Quarter Q3 '96	4th Quarter Q4 '96	1st Quarter Q1 '97	2nd Quarter Q2 '97

Project:
Date: 9/13/96

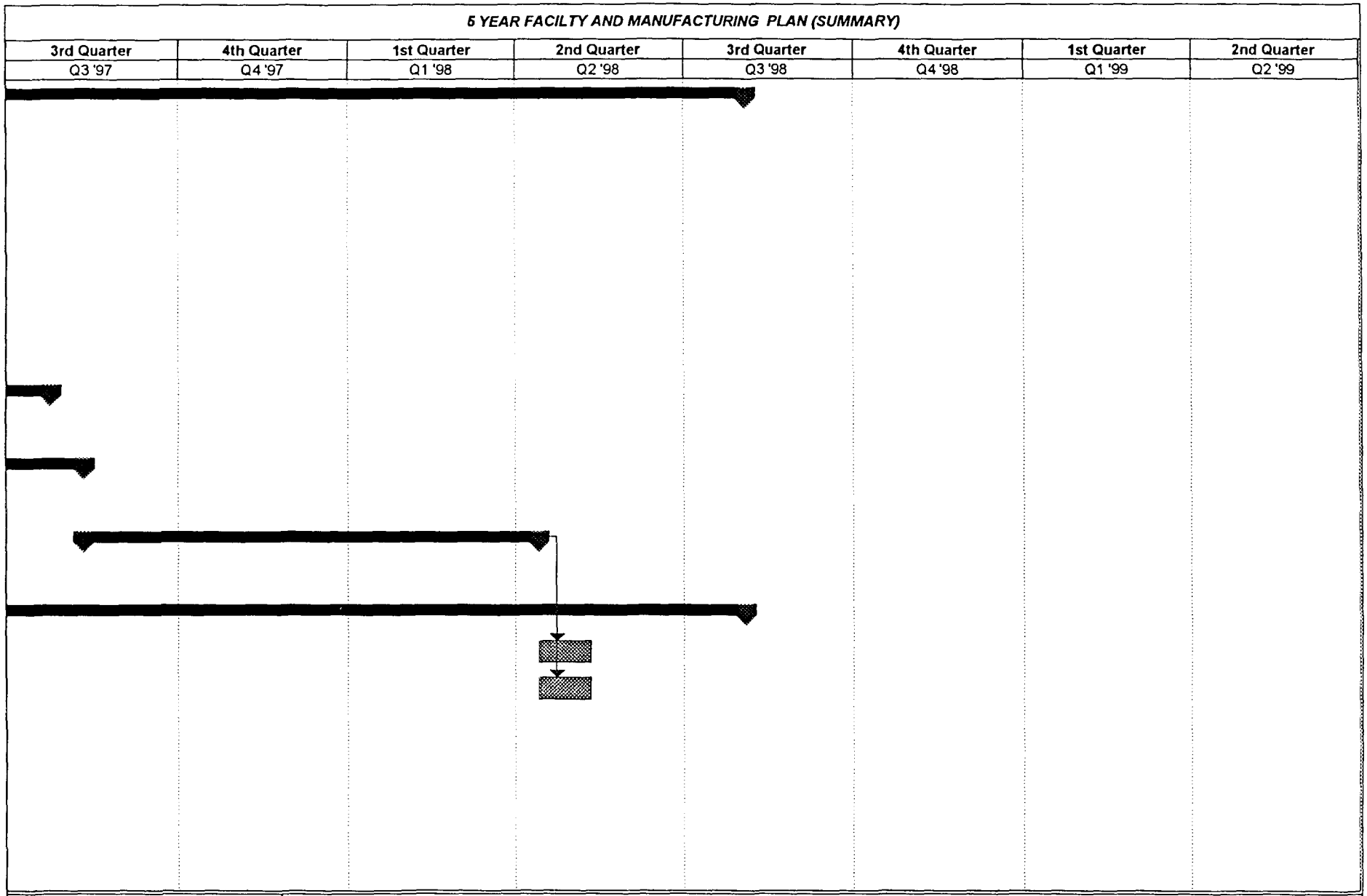
Task — Summary — Rolled Up Progress

Progress — Rolled Up Task

Milestone — Rolled Up Milestone

PRARGEN

5 YEAR FACILTY AND MANUFACTURING PLAN (SUMMARY)



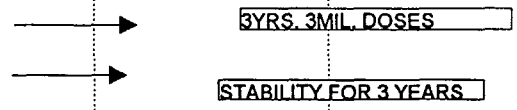
Project:
Date: 9/13/96

Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress

PRARGEN

5 YEAR FACILTY AND MANUFACTURING PLAN (SUMMARY)

3rd Quarter	4th Quarter	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter	1st Quarter	2nd Quarter
Q3 '97	Q4 '97	Q1 '98	Q2 '98	Q3 '98	Q4 '98	Q1 '99	Q2 '99



Project:
Date: 9/13/96

Task | Summary | Rolled Up Progress
Progress | Rolled Up Task
Milestone | Rolled Up Milestone

PRARGEN

UNIDO Project Manager's comments to the report

I. General comments

The following comments refer to the publication: Biosafety in Microbiological and Biomedical Laboratories; Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH); 3rd Edition May 1996.

1. Position of Candid I

Junin virus is an arenavirus classified as BSL-4 (Biosafety level 4). The definition of BSL-4 virus is the following: dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted laboratory infections; or related agents with unknown risk of transmission.

Candid 1 is a live attenuated virus derived from Junin virus. The safety and the immunogenicity of this vaccine were established in more than 300 volunteers immunized between 1985 and 1988 in the USA. In accordance with the CDC-NIH publication Candid I is considered as BSL-2 agent like Yellow fever 17D (see table below). There is no need to use BSL-3 facilities or BSL-3 practices. But Technical staff working with Candid 1 have to be vaccinated, and neutralizing antibodies checked at regular intervals.

Table 1
Vaccine Strains of BSL 3/4 Viruses which may be handled at BSL 2

Virus	Vaccine Strain
Chikungunya	131/25
Junin	Candid 1
Rift Valley fever	MP-12
Venezuelan equine encephalomyelitis	TC-83
Yellow fever	17-D

Agent Summary Statements - Arboviruses

Biosafety in Microbiological and Biomedical Laboratories, 3rd edition, May 1993, HHS Publication No. (CDC) 93-8395

Candid 1 may be handled at BSL-2

Standard and special pratices, safety equipments and facilities described in the above referred CDC-NIH publication can be applied to handled Candid 1 (annex 1). On the other hand, taking into consideration the non-pathogenic properties of Candid I, like all the live attenuated vaccines (measles,

rubella, mumps, yellow fever, varicelia, oral polio, etc.) this virus can be handled in a facility with positive pressure to prevent bacterial contamination during the process.

2. Prevention of cross contamination

The goal of this project is to produce a large quantity of Candid 1 vaccine. No other infectious agents can be handled in the same building. **The facilty has to be dedicated to the vaccine production.**

Particulary viruses like Hantaan or Junin (wild strain) which need to be handled at BSL-3/4 can not be introduce in the vaccine building.

No other pathogenic viruses can be handled in the vaccine production building.

II. Specific comments

These comments refer to the technical report: finding and recommendation from N. Cucakovich et al.

Page 30: Current Status at NIHVD

Hantaan and dengue viruses can not be utilized for research purpose in the QC laboratory. This laboratory like others need to be dedicated specifically to Candid 1 virus.

Page 33: Environmental and Safety Assessment

If all the buildings are dedicated to the vaccine production there is no need to develop BSL-3. It would be very dangerous to work with pathogenic strain like Junin or Hantaan viruses in the vaccine production area. Hantaan and Junin are aerosol transmitted viruses. Only 2 or 3 lab around the world are BSL-4 and of course these labs are not involved in the field of vaccine production. Cross contamination of Candid 1 vaccine by Junin virus will be probably not detected by QC.

Page 54: Process Description

Use 300 square centimeter flasks instead of 150.

To increase the volume of each batch it would be more convenient to perform 2 or 3 harvests if the virus is not cytolytic.

A filtration step (0.45 μm) have to be performed on the crude harvests . Therefore, taking into consideration the size and the pleiomorph properties of arenaviruses the filtration step need to be

validated before use routinely.

There is no need to change the production methodology to a close system. The use of 300 square centimeter flasks or roller bottles will reduce the contamination risk.

Page 56: Product Stability

Accelerated stability lot 2-1-88 and lot 3-1-89 show an important loss of titer at 37 °C : 1.6 to 2.5 log l0 after 168 hrs at 37 °C. In accordance with WHO requirements, live attenuated vaccine like measles , rubella, yellow fever, include a thermal stability. The virus concentration after one week at 37 °C is no more than 1 log10 lower than the initial value and in any case, must not be less than 1×10^{3} CCID50 per dose. Candid 1 vaccine does not pass this requirement. There is a need to develop a specific stabiliser.

Page 58: History of Viral Seed

I agree that there is a need to produce a large working seed from the master seed. If the master seed have been qualified at Salk Institute, including neurovirulence test, the new working seed can be qualified with a minimun of tets, in accordance with CFR.

Page 59: Cell Seed

I do not think that the strategy used is effective. To produce 110 culture flasks, 110 vials of the working seed are used. In this condition there is no consistency, and the metabolism of cells is poor. There is a need to use classical approach, by expanding the cells from passage level 17 , until to obtain 110 flasks. In this condition, a better activity of the cell metabolism is expected and probably a significant increase of the virus yields.

Page 60: Control of Fetal Bovine Serum and Human Serum Albumin

Use gamma-irradiated calf sera from international manufacturer like Hyclone. Gamma- irradiation will be performed by the manufacturer.

Trypsine which will be use to expand the cells need also to be gamma irradiated.

Human albumin: I agree an alternative stabilizer have to be develop . Human albumin will be used only to grown virus.

Conclusion

The main point to reconsidered is the classification of Candid 1 as a BSL-2 virus instead of BSL-3. In this condition the actual facilties can be used without major modification. The production laboratory has to be in a positive pressure to limit bacterial contamination.

No other agent can be handled in the manufacturer building.

The process is adapted to the quantity of vaccine needed. The main point is the development of a specific stabiliser.

ANNEX 1

Biosafety Level 2

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in :

(1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists,

(2) access to the laboratory is limited when work is being conducted,

(3) extreme precautions are taken with contaminated sharp items, and

(4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. All procedures are performed carefully to minimize the creation of splashes or aerosols.

6. Work surfaces are decontaminated at least once a day and after any spill of viable material.

7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated at off-site from the laboratory are packed in accordance with applicable local, state, and federal regulations, before removal from the facility.

8. An insect and rodent control programme is in effect.

B. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) could enter the laboratory or animal rooms.

3. When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., immunization), a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

6. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

7. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.

8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

    a. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used

disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

b. Syringes which re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.

c. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. Spills and accidents which result in over exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

12. Animals not involved in the work being performed are not permitted in the laboratory.

C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intra-nasally, and harvesting infected tissues from animals or eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guards) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed. Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and are not worn outside the laboratory. Disposable gloves are not washed or reused.

D. *Laboratory Facilities* (Secondary Barriers)

1. Each laboratory contains a sink for hand washing.

2. The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.

3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

5. If the laboratory has windows that open, they are fitted with fly screens.

6. A method for decontamination of infectious or regulated laboratory wastes is available (e.g., autoclave, chemical disinfection, incinerator, or other approved decontamination system).

7. An eyewash facility is readily available.