WORLD REFERENCE CENTER FOR ARBOVIRUSES AND RETROVIRUSES

ANNUAL REPORT

ROBERT E. SHOPE

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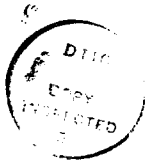

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The World Reference Center for Arboviruses and Retroviruses identified viruses from Indonesia, Ivory Coast, Viet Nam, Thailand, Angola, and USA. LaCrosse virus from an encephalitic dog was identified. A Palyam group virus, Kasba, caused arthrogryposis in cattle of Japan. ELISA was adapted to test 17D yellow fever vaccinees. H-9 and EBV-transformed lymphocyte cell lines were established to isolate HIV and other human retroviruses. Virus reference reagents for arboviruses were distributed to military laboratories and other laboratories world-wide. The low passage collection of arboviruses was augmented to over 460 strains.

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SUMMARY

Viruses were identified from Indonesia, Ivory Coast, Viet Nam, Thailand, Indonesia, Angola, the United States, Taiwan, Japan, and Brazil. LaCrosse virus was found for the first time causing encephalitis in dogs in Georgia. A Palyam virus was identified for the first time in Japan from Culicoides midges. A strain of dengue-1 isolated from the blood of a patient in Angola was genotyped by primer extension sequencing and found presumptively to be of Caribbean (rather than African) origin. Odenisrou, a new phlebovirus from the Ivory Coast was characterized serologically.

Monoclonal antibodies for identification of subtypes of Indiana vesicular stomatitis were field tested in Brazil. A mixture of monoclonal antibodies to Semliki Forest virus was blended to develop a sensitive antigen capture ELISA for alphaviruses.

An outbreak of hemorrhagic fever in Pakistan was studied. Although seroreactivity in one patient to Crimean-Congo hemorrhagic fever was detected, this virus was apparently not the only cause of the illness.

H-9 and EBV-transformed lymphocyte cell lines are established at Yale for the isolation and characterization of HIV and other human retroviruses.

Primer extension sequencing of dengue-1, dengue-2 and Japanese encephalitis viruses has shown geographic clustering of each of these flaviviruses. It is now possible to trace the origin of new cases and of outbreaks of diseases caused by the three viruses.

The yellow fever ELISA was adapted to test 17D vaccinees who are immunological virgins for flaviviruses.

The maturation of Japanese encephalitis E, NS1 and NS1' glycoproteins was demonstrated to differ between vertebrate and mosquito cells. The NS1 and NS1' proteins are not externalized in mosquito cells, and are in the extracellular fluid of vertebrate cell culture in particulate form.

The low passage virus collection has accessioned an additional 137 viruses in 1988, mostly strains of dengue and Japanese encephalitis viruses. The sequences of 9 flaviviruses are now on line in the electronic data bank and can be accessed by phone.

The Reference Center distributed 408 ampoules of virus, antigen, and antibody in 1988 to 14 foreign nations and 12 states of the United States. The reagents represented 121 different viruses.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46.

BODY OF REPORT

I. IDENTIFICATION AND CLASSIFICATION OF VIRUSES

A. Identification of Odrenisrou virus, Dak Ar A1131, as a new phlebovirus related to Arumowot virus (R. Tesh and R. Shope)

Odrenisrou virus was isolated from a pool of mosquitoes in Abidjan and was referred by the Institut Pasteur, through CDC, Fort Collins for confirmation of identity as a phlebovirus. The virus was tested by plaque reduction neutralization test (PRNT) against the following phlebovirus-specific mouse immune ascitic fluids at a 1:10 dilution: Aguacate, Alenquer, Anhanga, Arbia, Arboledas, Arumowot, Belterra, Buenaventura, Bujaru, Cacao, Caimito, Candiru, Chagres, Chilibre, Corfu, Frijoles, Gabek Forest, Gordil, Icoaraci, Itaituba, Itaporanga, Joa, Karimabad, Munguba, Naples, Nique, Oriximina, Pacui, Punta Toro, Rift Valley fever, Rio Grande, Saint-Floris, Salehabad, Sicilian, Tehran, Toscana, Turuna, Urucuri, BeAr 413570, Mariquita A, BeAr 407981, CoAr 171096, CoAr 171162, and CA Ar 170897. All were negative except for Arumowot. Cross-reactions with Arumowot by PRNT and CF are shown in Table 1.

TABLE 1

Cross reactions of Odrenisrou virus with Arumowot

Plaque reduction neutralization test:

	Mouse immune ascitic fluids	
Virus	Arumowot	Odrenisrou
Arumowot	1:5,120	<1:10
Odrenisrou	1:80	1:640

Complement fixation test:

	Mouse immune ascitic fluid
Antigens	Arumowot
Arumowot	>64/>64
Odrenisrou	4/4
Rift Valley fever	<4/<4

Odrenisrou virus appears to be a new phlebovirus, related distantly to Arumowot virus. It is of note that both of these agents have been isolated from mosquitoes.

B. Study of mosquito isolates from Indonesia (H. Kusnanto, R. Tesh, and R. Shope)

Over 200 viruses isolated from 1979-1981 from mosquitoes in Indonesia by the late J. Converse, NAMRU-II continue to be studied. These agents were recovered in mosquito tissue culture, and most do not kill baby mice.

One hundred four of the viruses were passaged in C6/36 cells. The cells were frozen and thawed and the supernatant fluid used as CF antigen. The fluids were screened with mouse ascitic fluids of group Bunyamwera, bluetongue, chikungunya, Japanese encephalitis, group Simbu, Australia grouping fluid, Koongol, and JKT 6969. JKT 6969 is an isolate which is currently ungrouped and has a 12-segmented genome.

Six of the isolates, JKT-6468, 5441, 8442, 7003, 7180, and 7887 reacted with the Japanese encephalitis ascitic fluid. IFA with JE-specific monoclonal antibody confirmed that these were JE viruses. Most of these were isolated from Culex tritaeniorhynchus, but JKT-5441 was isolated from Anopheles vagus mosquitoes collected on Bali.

Four of the isolates reacted with bluetongue antibody by CF. These were JKT-9065, 9126, 9128, and 10757.

Thirty-one of the agents reacted with JKT-6969 ascitic fluid. These agents are listed in Table 2.

TABLE 2

Viruses from NAMRU-II, Indonesia reacting by CF with JKT-6969 mouse ascitic fluid

JKT- 6423	JKT- 7879	JKT- 10081
6425	7887	10087
6429	7937	10274
6434	8385	10298
6854	8570	10304
6993	9072	10370
7003*	9086	10371
7041	9090	10395
7042	9132	10577
7043	9244	
7180*	9744	

*These specimens were also positive for Japanese encephalitis virus.

C. Identification of LaCrosse-related virus isolated from dogs with encephalitis in Georgia (S. Tirrell and R. Shope)

A virus, GA8-88, isolated from the brain of a 2-week old puppy was referred by Dr. Max Appel, Baker Institute for Animal Health, Cornell University Veterinary College. The virus was isolated in Georgia by Dr. Alfred R. Pursell, University of Georgia who informed Dr. Appel that two puppies of the same litter died.

The virus was received in A72 dog cells in which it caused CPE in 24 h and which it destroyed in 48 h. It was adapted to Vero cells in which it caused large and small plaques, and also to suckling mice which were sick or dead in 40 hours after i.c. inoculation. A complement fixation test showed that GA8-88 virus reacted with hyperimmune mouse ascitic fluids of the California serogroup and not with group Bunyamwera, rabies, EEE, Highlands J, and the flavivirus grouping fluid. Immunofluorescence of spot slides confirmed the relationship to California serogroup viruses.

Neutralization tests were carried out in baby mice inoculated i.c. A hyperimmune ascitic fluid to GA8-88 neutralized the homologous virus 6.0 log LD50 and neutralized LaCrosse virus >5.2 log. LaCrosse hyperimmune ascitic fluid neutralized the homologous LaCrosse virus >5.2 log LD50 and neutralized GA8-88 >6.2 log. Sera of California encephalitis, Keystone, San Angelo, Jamestown Canyon and trivittatus viruses neutralized GA8-88 to a lesser extent.

Plaque reduction neutralization tests in Vero cells are not complete although initial results confirm the mouse neutralization tests indicating that GA8-88 virus is identical or closely related to LaCrosse virus.

D. Dengue-1 virus identified from blood of a patient with serum yellow fever IgM reactivity in Angola (R. Tesh, R. Rico-Hesse, and R. Shope)

A virus was referred for identification from Drs. L.T. Figueiredo and H. Schatzmayr, Instituto Oswaldo Cruz, Rio de Janeiro. Serum specimens from patients in a suspected yellow fever outbreak in Angola were inoculated in C6/36 cells in Rio de Janeiro. Several of the acute phase sera were shown at the Instituto Oswaldo Cruz to have IgM antibody to yellow fever using the MACELISA, thus the isolate was also suspected of being yellow fever virus.

The virus was propagated in C6/36 cells and the infected cells were tested by IFA with monoclonal antibodies for yellow fever and the four serotypes of dengue. The cells reacted strongly with dengue-1 monoclonal antibody and were negative with the other antibodies. Since dengue-1 virus was being worked with in the Rio laboratory, there was a possibility of contamination; however, the virus was subsequently reisolated in Rio from the original specimen. In addition, primer extension sequencing showed that the Angola isolate differed from the Brazilian topotype. Surprisingly, the Angolan isolate also differed from the West African topotype, but resembled closely strains circulating in Jamaica and other parts of the Caribbean.

E. Identification of suspected flavivirus isolates from Viet Nam, Taiwan, and Indonesia (R. Tesh)

Viruses have been referred for confirmation of identity from Dr. Do Quang Ha, Pasteur Institute, Ho Chi Minh City, Viet Nam; from Mr. Ying-chang Wu, Taiwan Provincial Institute of Infectious Diseases, Taipei, Taiwan; and from Dr. Curtis R. Bartz, NAMRU-2, Jakarta, Indonesia. Most of the viruses had been identified or were suspected of being dengue or Japanese encephalitis viruses.

The agents were passaged in C6/36 cells and the cells were tested by IFA with monoclonal antibodies specific for dengue types 1-4 and JE viruses. Alphavirus antibodies were included in some of the tests. The 28 viruses (except for two which did not grow) from Indonesia reacted only with the JE antibody and are thus identified as strains of JE. An additional Indonesian isolate was identified as dengue-4 virus. The results with viruses from Viet Nam and Taiwan are shown in Table 3.

TABLE 3

Identification of suspected flaviviruses from Viet Nam and Taiwan with monoclonal antibodies

Virus	Group A	Group B	JE	DEN-1	DEN-2	DEN-3	DEN-4
VN-78	0	+	+	0	0	0	0
VN-104	0	+	+	0	0	0	0
VN-113	0	+	+	0	0	0	0
VN-118	0	+	+	0	0	0	0
VN-131	0	+	+	0	0	0	0
VN-135	0	+	+	0	0	0	0
VN5-71	+	0	0	0	0	0	0
VN9-71	+ (weak)	0	0	0	0	0	0
TAI765101				+	0	0	0
TAI765103				+	0	0	0
TAI765104				+	0	0	0
TAI765105				+	0	0	0
TAI766601				+	0	0	0
TAI76602				+	0	0	0
TAI76603				+	0	0	0
TAI76604				+	0	0	0
TAI76605				+	0	0	0
TAI76630				0	+	0	0
TAI76635				0	+	0	0
TAI76649 (not viable)							
Mosquito pool				+	0	0	0
TAI PL-001				0	+	0	0
TAI PL-002				0	+	0	0
TAI PL-003				0	+	0	0
TAI PL-004				0	+	0	0
TAI PL-046				0	+	0	0

It is interesting that both dengue-1 and dengue-2 viruses were active in the Taiwan outbreak of 1987. In addition, the identity of 9 strains suspected of JE virus from Taiwan was confirmed by IFA reaction with JE-specific monoclonal antibody.

F. Identification of SP An107237 VEE complex virus from sentinel mouse, Sao Paulo, Brazil (B. Fonseca and R. Shope)

SP An107237 virus was referred by the Instituto Adolfo Lutz, Sao Paulo, Brazil for identification. This virus was recovered recently from a sentinel mouse exposed It reacted by CF test with the alphavirus grouping mouse ascitic fluid and was shown to be more closely related to VEE complex viruses than to other alphaviruses. Plaque reduction neutralization testing (Table 4) with the VEE complex virus subtypes indicated that it was not distinguishable from 78 V 3531 virus, subtype I, variety F, isolated in Brazil in 1978, and that it differed from subtype I, variety A.

TABLE 4

Plaque reduction neutralization test results of SP An107237 with VEE complex subtype viruses

	Antibodies							
Viruses Subtype	VEE TC83 IA	78V3531 IF	SP An 107237	EVE II	MUC III	PIX IV	CAB V	AG80-663 VI
VEE TC83	320	160	160	80	10	10	20	20
78V3531	160	5120	5120	80	10	20	80	320
SP An107237	40	10240	10240	160	10	40	160	320
Everglades	1280	1280	2560	10240	160	20	40	80
Mucambo	40	80	160	40	320	20	80	80
Pixuna	10	10	20	40	10	10240	40	40
Cabassou	<10	10	10	<10	<10	<10	320	10
Ag80-663	40	2560	1280	160	80	160	160	10240

Although subtype I varieties C, D, and E were not included in the PRNTs, SP An107237 was more distinct from subtype I, variety A than it was from Everglades (subtype II). This observation raises the question whether the currently classified IF viruses may not actually be distinct enough from other subtype I varieties to warrant the IF viruses being reclassified as a new subtype rather than as a variety of subtype I.

G. Identification of Kagoshima virus from Culicoides of Japan (B. Fonseca and R. Shope)

An orbivirus of the Palyam serogroup was referred for identification by Dr. Y. Inaba of the National Institute of Animal Health, Tsukuba, Japan. This virus, called Kagoshima virus, was isolated from a pool of Culicoides oxystoma collected in November, 1984 in a cowshed in Kagoshima on Kyushu Island (Kurogi, H. et al., Isolation and preliminary characterization of an orbivirus of the Palyam serogroup from biting midge Culicoides oxystoma in Japan, Vet. Microbiol., accepted for publication). This is the first Palyam group virus recognized in Japan. In tests at Tsukuba, Kagoshima virus was not neutralized by sera to D'Aguilar, and showed a one-way cross reaction with Bunyip Creek virus. Dr. Inaba did not have available other Palyam group viruses to complete the tests.

A mouse ascitic fluid was prepared to Kagoshima virus. The virus produced plaques in Vero cells. A plaque reduction neutralization test indicated a relationship to Kasba virus (Table 5). The reciprocal test has not yet been completed.

TABLE 5

Plaque reduction neutralization test results of Kagoshima virus with antibody to Palyam group viruses

Kagoshima	1:640
Bunyip Creek	<1:10
CSIRO Village	<1:10
D'Aguilar	<1:10
Kasba	>1:320
Marrakai	1:10
Palyam	<1:10
Vellore	<1:10
Nyabira	<1:10

II. CHARACTERIZATION OF MONOCLONAL ABTIBODIES

A. Field tests in Rio de Janeiro of monoclonal antibodies to separate subtypes of vesicular stomatitis, Indiana (P. Fernandez, W.R. Chen and R. Tesh)

The development of specific monoclonal antibodies which recognize subtypes of VSV Indiana was reported in the 1987 Annual Report (p. 19). During 1988, one of us (PF) carried three monoclonal antibodies to the Pan American Health Organization Foot and Mouth Disease Laboratory outside of Rio de Janeiro. There, the antibodies were tested by IFA using spot slides of VSV Indiana strains grown in a pig kidney line (Instituto Biologico Rim Suino-2).

The monoclonal antibodies were designed to be specific for Indiana (3-B10-B9), Cocal (2-3-F1-G10) and Alagoas (2-E-6-6G). Table 6 shows the results. The antibodies were quite specific for the designated subtypes. The Indiana-1 monoclonal reacted strongly with both strains available, and although it cross-reacted with 2 of the Alagoas strains (Indiana-3), the cross-reactions were not marked. The Cocal (Indiana-2) antibody was specific when tested with 7 different strains; cross-reaction was noted with the New Jersey type. This was a surprise, but this does not negate the usefulness of the monoclonal antibody. The Alagoas (Indiana 3) reacted only with 2 of the five strains typed as Indiana 3 by CF. Further work needs to be done to confirm these reactions, and possibly to select another monoclonal antibody for Alagoas.

TABLE 6

Reactivity by IFA of three monoclonal antibodies to VSV Indiana isolates at the Foot and Mouth Disease Center, Rio de Janeiro

	Monoclonal antibody		
Viral subtype	Indiana 3-B10-B9	Cocal 2-3-F1-G10	Alagoas 2-E-6-6C
Indiana 1 YARU	+++	-	-
Indiana 1 Rio	+++	-	-
Indiana 2 Cocal (Prototype)	-	+++	-
Indiana 2 Cocal	-	++	-
Indiana 2 Salto	-	+++	-
Indiana 2 Rancheria	-	+++	-
Indiana 2 Riberao	-	++	-
Indiana 2 Maraba	-	+++	-
Indiana 2 Maipu	-	+++	-
Alagoas CoAr 171044	-	-	+++
Indiana 3 Alagoas	-	-	+++
Indiana 3 Espinosa	+	-	-
Indiana 3 Sergipe	+	-	-
Indiana 3 Agulhas Negras	+	-	-
New Jersey	-	++	-

B. ELISA for the detection of alphaviruses using monoclonal antibodies (I. Greiser-Wilke, V. Moennig, O.R. Kaaden, and R. Shope)

Monoclonal antibodies raised to Semliki Forest virus and selected by testing with Sindbis virus were prepared at the Institute for Virology, Hannover, Germany. The antibodies were brought to Yale in order to test them with the full range of alphaviruses. A sensitive immunoassay for detection of alphaviruses was derived from a mixture of the antibodies. Table 7 shows the reactivity and detection limits of 5 of the antibodies. C2, C12, and C42 were not reactive with VEE complex viruses and in individual tests, none were reactive with Pixuna antigen. In addition none of the five were reactive with Barmah Forest antigen.

However, when the monoclonal antibodies were mixed, it was possible to detect Barmah Forest, Pixuna, and the other VEE complex viruses. In fact the detection system was quite sensitive. These results are shown in Table 8.

Table 7: Reactivity of monoclonal antibodies in the antigen capture test with alphaviruses

Virus	Virus titer[1*] log $TCID_{50}$/ml	Detection limit (log $TCID_{50}$/ml) MAbs SFV/C				
		C2	C12	C42	C3	C8
Eastern encephalitis	7.9	6.4	6.1	7.9	6.7	6.7
Venezuelan encephalitits						
V. encephalitis	6.5	0	0	0	5.0	4.7
Everglades	7.6	0	0	0	7.6	7.6
Mucambo	6.8	0	0	0	5.0	5.0
Pixuna	6.4	0	0	0	0	0
Bijou Bridge	6.3	0	0	0	4.8	5.4
Western encephalitis						
W. encephalitis	7.6	4.9	4.6	7.0	6.7	7.6
Y62-33	6.6	5.1	5.1	0	6.3	0
Fort Morgan	7.0	5.5	5.2	6.0	6.4	0
Sindbis	7.0	5.2	4.9	6.4	6.1	7.0
Whataroa	4.6	4.3	4.0	0	4.3	0
Kyzylagach	5.9	3.8	3.8	4.7	4.4	4.4
Semliki Forest						
Semliki Forest	8.0	6.5	6.3	6.5	7.7	8.0
Chikungunya	5.8	0	5.8	5.8	0	0
- O'nyong nyong	5.8	3.7	3.7	4.3	4.0	4.0
Getah	7.4	6.2	5.9	6.8	6.8	6.8
- Sagiyama	7.7	6.5	6.5	6.8	0	0
- Bebaru	6.9	5.7	5.7	6.0	0	6.6
- Ross River	7.3	6.4	6.1	6.4	7.0	7.0
Mayaro	7.3	6.1	5.8	6.1	6.4	6.1
- Una	7.6	7.0	6.4	7.0	6.4	7.3
Ndumu	6.8	5.6	5.3	5.9	7.1	0
Middelburg	7.3	6.4	6.1	6.4	0	6.7
Barmah Forest	6.0	0	0	0	0	0

1* lowest dilution tested

Table 8 : Reactivity of combinations of monoclonal antibodies in antigen capture tests with alphaviruses

	Virus titer[1*] log $TCID_{50}$/ml	Detection Limit (log $TCID_{50}$/ml) Mix.1-*[2] Mix.1-PO	Mix.1 Mix.3-PO	Mix.2 Mix.3-PO
Eastern encephalitis	7.9	6.4	5.8	6.1
Venezuelan encephalitits				
V. encephalitis	6.5	5.3	5.0	5.3
Everglades	6.6	5.4	5.7	5.4
Mucambo	6.8	5.9	5.0	5.6
Pixuna	6.4	4.8	5.4	4.8
Bijou Bridge	6.3	5.1	4.8	5.4
Western encephalitis				
W. encephalitis	7.6	5.5	5.5	5.8
Y62-33	6.6	5.4	4.8	5.1
Fort Morgan	7.0	6.1	5.2	5.5
Sindbis	7.0	5.5	5.2	5.8
Whataroa	4.6	3.7	3.7	3.7
Kyzylagach	5.9	4.4	4.1	4.1
Semliki Forest				
Semliki Forest	8.0	5.9	5.6	5.6
Chikungunya	5.8	5.8	5.2	5.5
- O'nyong nyong	5.8	3.7	3.1	3.4
Getah	7.4	6.2	5.9	5.9
- Sagiyama	7.7	5.9	5.6	5.6
- Bebaru	6.9	5.7	5.4	5.4
- Ross River	7.3	6.4	5.8	5.8
Mayaro	7.3	5.8	5.5	5.8
- Una	7.6	6.1	5.8	5.8
Ndumu	6.8	5.3	5.0	5.3
Middelburg	7.3	6.1	5.5	6.1
Barmah Forest	6.0	5.1	5.1	5.1

1* lowest dilution tested
2* Mixture 1: SFV/C12 + SFV/C3
Mixture 2: SFV/C2 + SFV/C12 + SFV/C42 + SFV/C3
Mixture 3: SFV/C2 + SFV/C12 + SFV/C42 + SFV/C3 + SFV/C8

III. INVESTIGATION OF OUTBREAKS

A. Hemorrhagic disease of unknown cause in Karachi, Pakistan (G. Tignor, A. Smith, R. Cedeno, and R. Shope)

Sera from 4 patients suspected of viral hemorrhagic fever were referred for testing by Maj. Gen. M.I. Burney of the National Institute of Health, Islamabad, Pakistan in January 1988. The patients were residents of Karachi City and had fever, muscle pains, and in some patients, hematemesis.

The sera were tested by IFA for Crimean-Congo hemorrhagic fever (CCHF), Hantaan, Rift Valley fever, Ebola, and Marburg. Acute and convalescent sera from patients A and C, and acute serum from patient B were negative; however, convalescent serum from patient D was strongly positive for CCHF virus. Additional sera including a repeat sample from patient D were received later. These were tested by IFA and ELISA with CCHF. Only the repeat serum from patient D was again stronly positive. A specimen of brain tissue was also sent for attempted isolation of virus in baby mice. No virus was isolated.

It was concluded that although one patient had CCHF antibody, CCHF was probably not the cause, or at least the sole cause, of the hemorrhagic fever outbreak.

B. Study of Thai patients with dengue hemorrhagic fever and hepatic coma (R. Tesh)

Specimens from 5 patients with unusual manifestations of hemorrhagic fever were referred by Dr. Prasert Thongcharoen, Faculty of Medicine, Mahidol University, Bangkok, Thailand. These patients were hospitalized in 1987. They had high fever, followed by bleeding and hepatic coma. All had hepatomegaly, thrombocytopenia, and marked elevation of transaminases. Tests for hepatitis A and B were negative. Dengue hemorrhagic fever was suspected since 4/5 patients had dengue antibody, usually in high titer, but the unusual hepatic manifestations prompted the request to rule out yellow fever.

The plasma from each case and the liver of one patient taken at necropsy were inoculated into C6/36 cells. The cells did not show cytopathic effect. Seven days after inoculation, the cells were examined for flaviviruses using monoclonal antibodies and IFA. All tests were negative.

IV. RETROVIRUS REFERENCE STUDIES

A. Establishment of HIV diagnostic capability (R. Shope)

H-9 cells and a line of EBV-transformed human lymphocytes are being established in collaboration with the laboratory of Dr. I. George Miller, Department of Pediatrics, Yale University School of Medicine. The EBV-transformed cells are more sensitive for isolation of HIV. An arrangement has been made with Dr. Jorge Boshell, Instituto Nacional de Salud, Bogota, Colombia to receive materials from AIDS patients and to receive HIV isolates for confirmation of identity and for storage as reference strains.

V. DEVELOPMENT OF NEW TECHNIQUES

A. Primer extension sequencing of dengue viruses and application to epidemiology (R. Rico-Hesse and R. Tesh; supported in part by The Rockefeller Foundation)

This project aims to develop the methodology through primer extension sequencing of dengue virus RNA to determine the worldwide transmission patterns of dengue viruses in order to understand their epidemiology and improve control of disease. The methodology will also permit identification of viral factors which influence the severity of dengue infection.

Primer extension sequencing of RNA templates can specifically target selected genomic regions for comparison, such that areas on the genome having different rates of evolution may be independently examined. Thirty-two strains of dengue-1 and dengue-2 isolated over a 20 year span, vary sufficiently at the nucleotide level to make the approach applicable to the study of dengue virus epidemiology. A portion of the NS1/E junction region of the dengue genome showed fixation of mutation rates of approximately 7% across isolates from different geographic areas. The majority of these mutations occurred in the third position of the codon. Other NS1 and capsid sequences showed an equivalent rate of mutation, but the sites were not limited to the third position, and many amino acid changes occurred across strains.

Analysis of <5% of the genome revealed that the evolutionary patterns of dengue viruses of serotypes 1 and 2 are different, as are the transmission pathways of these viruses across the world. These relationships follow those defined by oligonucleotide fingerprinting, although much broader relationships were revealed. So far the research has shown 1) that limited genomic sequencing for the determination of transmission pathways is applicable to RNA viruses other than polio, even though mutation rates are lower, 2) that the results are easy to interpret and are directly comparable across serotypes as well as within serotypes, and 3) that the technique allows the definition of broader dengue virus genetic relationships than was possible with oligonucleotide fingerprinting.

Sixteen strains of dengue-1 and 16 of dengue-2 initially were used from the low passage collection maintained in the Reference Center. The viruses were passaged twice in C6/36 cells and dengue serotype was confirmed using type-specific monoclonal antibodies. One spinner culture bottle containing 300 ml of C6/36 cells in suspension was inoculated with approximately 1 pfu/cell of each of the 32 viruses. The culture fluid was harvested by low speed centrifugation on day 6 post inoculation.

Viral RNA was prepared for sequencing using the technique previously used for polio virus (Rico-Hesse, R. et al., Natural distribution of wild type 1 poliovirus genotypes. In: Positive Strand RNA Viruses (R.R. Rueckert and M.A. Brinton, Eds.), pp. 477-486. Alan R. Liss, New York). The virus was precipitated by stirring 8-12 h in polyethylene glycol and collected by centrifugation. The virions were then pelleted by ultracentrifugation through a sucrose cushion and dissociated with 1% SDS and 1% 2-mercaptoethanol. The suspension was then extracted 3 times with a

phenol:chloroform mixture, and LiCl was added to precipitate the RNA in ethanol. The RNA pellet was washed 2X in ethanol and dried in vacuo. The RNA was resuspended in buffer and reaction mixes. This crude RNA was used in primer-extension experiments, without generating artifacts or other problems in interpreting the data. The estimated RNA yield was approximately 25 ug per virus, sufficient for approximately 5 primer-extension sequencing reactions. The procedure was repeated whenever more RNA was needed, using the same seed virus.

Sequencing reactions used reverse transcriptase to extend synthetic DNA primers in the presence of the Sanger dideoxy chain-terminating inhibitors, as modified for RNA templates. The sequences to which the primers bind must be known. At the beginning of the project, sequences for two different strains of type 2 and one of type 1 had been reported. Tabulaton of these data, and those from a serotype 4 strain, pointed to areas conserved across serotypes which would serve as good targets for synthetic primers. Six different primers were designed and tested in primer-extension experiments on 6 viruses of serotype 1 initially. Later, other primers were designed for dengue type 2 strains, binding to the equivalent genomic areas as those for type 1 strains.

Three distinct functional domains of the dengue virus genome were explored by sequencing: the capsid, C; the envelope, E; and the non-structrual protein, NS1.

The 6 primers used to obtain RNA sequence information and their target regions are: (1) PR3, a 22-mer, 5'-CTGTTGGTGGGATTGTTAGGAA-3', binds to the center of the C gene; (2) VD2, a 17-mer, 5'-TCCACATTTGAGTTCTC-3', binds to the 3' end of the E gene; (3) NAU1, a 25-mer, 5'-TGGCTGATCGAATTCCACACACACC-3', hybridizes to the 5' end of NS1; (4) NAU4, a 15-mer, 5'-TCTATCCAGTACCCC-3', binds to the middle of hte NS1 gene; (5) D1234, a 14-mer, 5'-CCGTACCAGCACCC-3', binds to the 3' end of NS1, and primes dengue viruses of all four serotypes; 96) NS1/920, a 23-mer, 5'-GTGCAAGATCGGCAGCACCATTC-3', binds to the 3' end of NS1, and seems to be conserved across all flaviviruses. Nucleotide sequences obtained with these synthetic DNA primers begin 10-20 nucleotides upstream of the primer binding site.

The E/NS1 junction of the genome was chosen as the best source of nucleotide sequences for comparison across strains of the same serotype and across the 4 serotypes, to derive evolutionary information which can be interpreted epidemiologically. This choice was based on the following:

1. This area of the genome showed a uniform rate of random mutation, without hypervariable regions that might be affected by immune selection of epitopes.

2. The large majority of the mutations occurred in teh third position of the codon, and are therefore silent, probably random mutations.

3. Complete E kgene nucleotide sequences of type 1 dengue viruses obtained at CDC, Fort Collins have shown this region to vary in a non-uniform manner. Limited sequences done at Yale confirmed these results.

4. Nucleotide sequences encoding the capsid showed very little variation across strains within a serotype.

5. Comparisons of nucleotide sequences across the E/NS1 junction established genetic relationships among strains which seem to correlate with geographic origin and with previous oligonucleotide fingerprinting studies. A sample of the obtained nucleotide sequences is shown in Figs. 1 and 2. Sixty nucleotides are shown, from the indicated map sites, from 16 isolates of serotypes 1 and 2, respectively. The viruses were compared to an arbitrary reference strain (underlined), a previously published sequence obtained by molecular cloning and sequencing. Nucleotide differences are indicated by letters; dashes indicate identities.

The alignment of approximately 150 nucleotides of sequence information for dengue isolates of serotypes 1 and 2, obtained with the NAU1 primer, gives some information as to the evolutionary relationships of strains. A rough estimate of 7% nucleotide sequence divergence across strains of a serotype was derived from these data, by averaging the medians of divergence within each serotype. A portion of these data is shown in Figs. 1 and 2, and represents regions where visual comparison across isolates demonstrates these relationships (these regions showed higher amounts of variation across strains, or are "hot spots"). Note that the compared areas are different regions of the genome for the two serotypes; strains of the two serotypes show different rates of mutation aross the same NS1 region of the genome, although they were aligned by amino acid sequence. The reason(s) for these differences are unknown and may represent three-dimensional structural differences between the proteins of types 1 and 2.

Based on only 60 nucleotides shown here, it is evident that type 1 and type 2 strains can be grouped according to geographic origin. For type 1 strains, viruses obtained from Southeast Asia, the Americas, and Africa fall into separate groups, with the direct relationships among some strains evident (e.g., Thailand, 1980, and Taiwan, 1987). The circulation of two distinct genotypes in one country (e.g., Mexico, 1980, 1982, and 1983) is suggested by this comparison, while the relationship between two geographically isolated strains is unclear (Burma, 1976, and Haiti, 1983). The highest amount of nucleotide difference across this region can be calculated at approximately 11% (7/60).

For type 2 strains, where more variation is seen across isolates, genetic relationships are more obvious, and geographic groups can also be distinguished. Africa is represented by strains from Ivory Coast and the Republic of Guinea; isolates from the South Pacific show identity with each other and with the Puerto Rican passaged vaccine strain; isolates from the Americas (Mexico, Colombia, and Puerto Rico) show similarity to those from the South Pacific, and suggest a possible origin of these strains; the close relationship between a strain from Taiwan (PL-001, 1981) and one from the Philippines (1983), suggests direct transmission routes. The highest amount of sequence divergence seen across this area is 20% (12/60).

The only point of disparity between the sequencing data and oligonucleotide fingerprinting data occurs when comparing West African type 2 isolates (IVOR 80, GUIN 81) with a Jamaican isolate from 1982. By

Figure 1. Nucleotide sequences of dengue type 1 isolates

```
                    2302                                                2361
16299    NAUR  77   GCUAGGAUUAAACUCAAGGAGCACGUCCCUUUCAAUGACGUGUAUCGCAGUUGGCAUGGU
8682     PHIL  74   ------------------------------------------------------------
2683     FIJI  75   ------------------------------------------------------------
8686     BURM  76   ----------U----------A-----G----------U----------
8690     JAMA  77   -U-G--------U----------A-----G----------U----------
1186     INDO  77   ------------------------------------------------------------
1236     INDO  78   --------------------U---------------------------------------
IBH689   NIGE  78   ----------U----------G-----U--C--U----------
29177    SENE  79   ----------U----------A-----G--------C--U----------
PUO359   THAI  80   ----------------------------------G----------U---------C----
1298     MEXI  80   ----------U-------------------------------------------------
1344     MEXI  82   -U-G--------U----------A-----G--------C--U----------
1351     COLO  82   -U-G--------U------G-------A-----G--------C--U----------
1412     MEXI  83   -U-G--------U----------A--A----------C--U----------
1413     HAIT  83   ----------U----------A-----G----------
347869   COLO  85   -U-G--------U----------A-----G--------C--U----------
765101   TAIW  87   ----------------------------------G----------U---------C----
```

Figure 2. Nucleotide sequences of dengue type 2 isolates.

```
                    2432                                                2491
PR159S1  PUER  69   AAAUGUGGCAGUGGAAUAUUCGUCACAGAUAACGUGCAUACAUGGACAGAACAAUACAAG
NGC      NEWG  44   --G----------G--U---A-------C--------C----------
PR159    PUER  69   ----------------U----------C--U-----C----------
28741    TAHI  71   ------------------------------------------------------------
NC9163   NEWC  72   ------------------------------------------------------------
8720     INDO  73   ----------G--U--UA-------C--------C----------A
1251     TONG  74   ------------------------------------------------------------
DakA578  IVOR  80   --------A-----G--C--U--A--U--C--U-----C--U-----G----------A
1318     PUER  81   ------------------------------------C----------
PM33974  GUIN  81   --------A-----G--C--U--A--U--C--U-----C--U-----G----------A
PL-001   TAIW  81   --------U-----GU-U---A-------C--U-----C----------
PL-046   TAIW  81   ----------G--U---A-------C--U-----C----------
1329     JAMA  82   ----------G--C--UA----------C----------U---
PhH2172  PHIL  83   --------U-----GU-U---A-------C--U-----C----------U---
044      MEXI  83   ------------------------------------------------------------
975      SRIL  85   ----------G--U--U--------C--U-----C----------A
766635   TAIW  87   ----------G--U---A-------C--------C----------
351863   COLO  88   ----------------------------C----------
```

fingerprinting, two Jamaican strains from 1981 were shown to be similar to an isolate from Upper Volta (1980) and it was suggested that West Africa was the source of strains circulating in the Caribbean. The nucleotide sequence data suggest that a Jamaican isolate (#1329) shares a common progenitor with an Indonesian isolate (1973) and shows little relationship to two isolates from West Africa. In fact, isolates from West Africa are very distinct in their patterns of mutation. The inclusion of more isolates in this study should clarify this matter.

Within the serotype 2 strains tested, one isolate from Thailand (PUO-293, from 1980) and one from Jamaica (124, from 1983), obtained from patients with DHF/DSS (S. Kliks) were not bound by the NAU1 primer used to obtain sequence information from all other strains, and no sequences for comparison were obtained from this region. Thus, these two strains vary in sequence across the 25 nucleotides to which the primer hybridizes, when compared to all other dengue 2 strains. One additional primer, D1234, which binds downstream from the NAU1 site, did produce sequence data, confirming the presence of RNA in the reaction mixes and the fact that these strains are indeed dengue 2-like at the nucleotide level. This information serves as preliminary evidence for distinguishing dengue isolates associated with hemorrhagic disease from those causing only dengue fever and this characteristic is shared by geographically independent isolates.

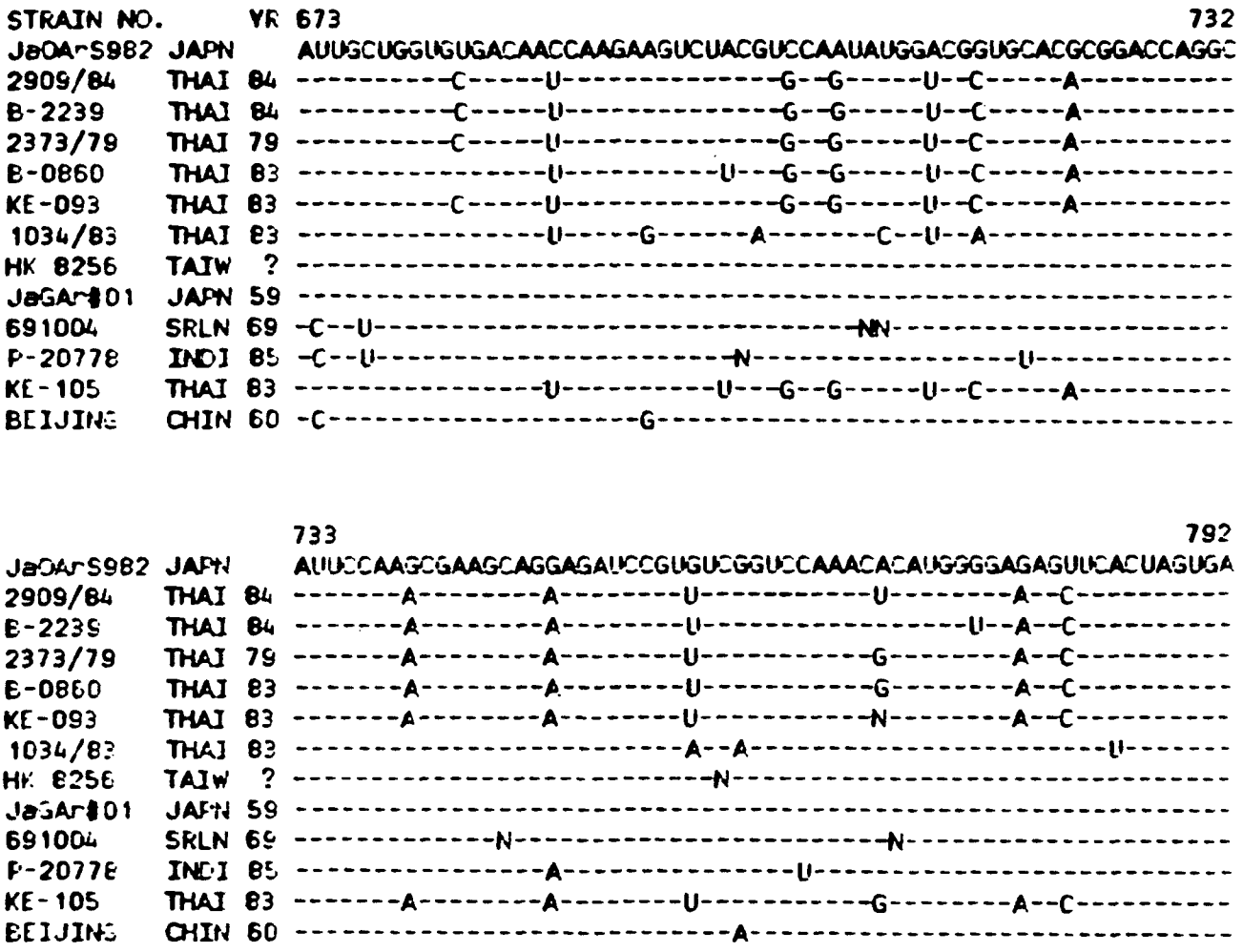
B. Primer extension sequencing of Japanese encephalitis virus strains and application of the technique to epidemiology (W. Chen, R. Rico-Hesse, and R. Tesh)

Japanese encephalitis (JE) virus strains from the Reference Center low passage collection, isolated from humans, pigs, and mosquitoes, and from a number of different geographic regions (Japan, China, Taiwan, Thailand, and India) were compared by primer-extension sequencing. The long-term goal of this project is to study the geographic distribution of JE virus genotypes and to gain information on the epidmiology of this virus.

Synthetic oligonucleotide primers were made to hybridize between E glycoprotein and NS1 areas of the genome (B-33, B-31, B-10); these areas showed variation across dengue viruses. Comparison of the resulting JE sequences did not show much difference among these viruses. Other primers were made to hybridize to the NS1 region. One primer did not work; primer number NS1-1 connected with number B-10 and provided 300 nucleotides of continuous information. Because NS1-1 and B-10 overlap, primer number B-31 and NS1-1 were used primarily during the continuation of the study; these two primers were used to obtain sequences of more than 10 strains of JE, but the results still did not show much variation among strains. The 3' end of the genome, which does not code for protein, was explored also. JE virus strains from different geographic distributions were compared across this region of the genome; again the results did not show much variation among strains. These data suggested that JE might be a very conserved virus; that is, this virus does not fix random mutations at a very high rate.

FIGURE 3

Partial RNA sequence analysis of Japanese encephalitis virus strains from different geographic origins



A strain from India (P-20778) gave more sequence information than any other strain with the B-33 primer. The results suggested that variation might occur in the M area and a new primer (M-100) was designed. Using this new primer, a strain from Thailand (KE-105/83) showed a large degree of nucleotide sequence difference when compared to other strains. This result agrees with the findings of a previous JE fingerprint study; the Thai strains seem to be unique virus variants. More Thai JE strains were selected and studied, using the B-3 and M-100 primers, to obtain data from the M gene region. The data showed some unique differences among Thai strains and these differences were not seen in strains from other geographic areas. Other strains also showed unique variation across this area of the genome, as shown in Fig 3. Therefore, this area will be explored further, using virus strains from other, distant geographic regions, in an attempt to confirm that this is an area of the genome which will be of wider use in distinguishing JE viruses. Table 9 shows the currently available JE strains which have been grown in cell culture and reidentified by JE-specific monoclonal antibody.

TABLE 9

Currently available strains of Japanese encephalitis virus which have been propagated in Vero cells and reidentified by monoclonal antibody

China			Japan		
SA-14	1960	mosquito	Nakayama	1935	human
			M5-596	1955	mosquito
India			55-1456	1955	human
63498	1963	mosquito	281801	1955	bird
InAr633759	1963	mosquito	224052	1956	mosquito
InAr641686	1964	mosquito	M7-271	1957	mosquito
InAr724038	1972	mosquito	M7-287	1957	mosquito
InAr755723	1975	mosquito	M7-292	1957	mosquito
InH78668A	1978	human	JaGAr01	1959	mosquito
P-20778	1978	human	JaAr245980	1979	mosquito
InH7812474	1978	human	Osaka	1979	mosquito
InAr803830	1980	mosquito			
InH826309	1982	human	Nepal		
			B-2524/85	1985	pig
Indonesia			9548/85	1985	human
JKT- 654	1978	mosquito	8631/86	1986	human
IndAr220507	1979	mosquito			
222682	1979	mosquito	Philippines		
JKT-1105	1979	mosquito	Ph.An 1242	1984	pig
JKT-1724		mosquito			
JKT-2254		mosquito	Sarak		
JKT-2363	1979	mosquito	JE827	1982	mosquito
JKT-4887		mosquito			
JKT-5441	1980	mosquito	Sri Lanka		
JKT-6468	1981	mosquito	69/004	1969	human
JKT-7003		mosquito	SL H86596	1986	human
JKT-7180		mosquito			
JKT-7887		mosquito			
JKT-8110		mosquito			
JKT-8442	1980	mosquito			

TABLE 9 (continued)

Taiwan			Thailand (continued)		
TaAr225492	1972	mosquito	1070/82 KE008	1982	human
TaAr229140	1981	mosquito	3076/83 KE093	1983	human
TaAr229246	1981	mosquito	3094/83 KE094	1983	human
TaAr242154	1981	mosquito	B-0860/83	1983	pig
HK 8256	1982	mosquito	KE 105/83	1983	human
TaAr603525	1982	mosquito	KE 094/83	1983	human
TaAr249781	1982	mosquito	B-1065/83	1983	pig
CC-27	1983	mosquito	B-1034/83	1983	pig
TaArP-61780	1983	mosquito	KP-245	1984	mosquito
TaArP-61779	1983	mosquito	KP-0269	1984	mosquito
CC-94	1984	mosquito	KP-0270	1984	mosquito
NT-90	1984	mosquito	KPO-439/84	1984	mosquito
ML-117	1985	pig	KP-0252	1984	mosquito
CN-80	1985	mosquito	B-2239/84	1984	pig
NT-113	1985	mosquito	2632/84	1984	human
NT-109	1985	mosquito	2909/84	1984	human
CH-109	1986	mosquito	KPO-561/84	1984	mosquito
CC-223	1987	mosquito	B-0005/85	1985	pig
CH-392	1987	mosquito	B-2582/85	1985	mosquito
			MNI-75ct	1986	mosquito
Thailand					
2372/79	1979	human	Viet Nam		
B-1208/82	1982	pig	VN-78		
B-0922/82	1982	pig	VN-113	1979	mosquito
B-105/82	1982	pig	VN-118	1979	mosquito
MNI-93ct	1986	mosquito	VN-131		
			VN-135	1980	mosquito

C. Adaptation of yellow fever ELISA to test volunteer subjects for antibody (S. Tirrell, B. Ratcliff, M. Barry, and R. Shope)

A study is underway to test volunteer subjects by plaque reduction neutralization test for their response to 17D yellow fever vaccine while they are taking chloroquine. Control subjects are also vaccinated and do not receive chloroquine. The study is sponsored in part by Connaught Laboratories. Sixty medical students and other volunteers were enlisted for the study. So far the subjects have been bled prevaccination and 14 and 35 days postvaccination. The study will be terminated after 6 months; neutralization tests are not yet completed and therefore the data are still under code.

The pre vaccination sera and sera taken at 14 and 35 days were tested in the 1:200 dilution by ELISA using flavivirus monoclonal DEN 4G2-15 (1:1600) as capture antibody, French neurotropic virus sucrose-acetone extracted mouse brain (1:40) as antigen, and a polyvalent antihuman peroxidase conjugate. The results shown in Table 10 indicate that all 60 subjects responded to the vaccine, although three (#23, 56, and 64) were still low-titered at 35 days or antibody had disappeared. Seven of the subjects had flavivirus antibody prior to vaccination. When the results of the neutralization tests are available, it will be possible to determine if the correlation is sufficiently good to enable the ELISA to be substituted for PRNT in screening non-flavivirus immune subjects for 17D antibody response.

TABLE 10

The ELISA response of volunteers to 17D antigen expressed as difference of O.D. between negative antigen and yellow fever antigen

Subject #	Pre	14 day	35 day	Subject #	Pre	14 day	35 day
1	.02	.42	.89	33	.03	.27	.57
2	.01	.47	1.00	34	.02	.54	.70
3	.03	.38	.93	35	.02	.07	.51
4	.13*	.62	.63	37	.01	.03	.34
5	.02	.22	.88	38	.04	.35	.93
6	.10	.63	.84	39	.03	.14	ND
7	.00	.36	.85	41	.23	.62	.65
8	.03	.54	.93	42	.01	.34	.55
9	.00	.39	.58	43	.02	.13	.22
10	.00	.54	.71	44	.02	.27	.84
11	.02	.36	.70	45	.03	.13	.64
12	.04	.26	.36	46	.01	.14	.44
13	.10	.53	.73	47	.00	.22	.48
14	.50*	.67	.58	48	.00	.10	.40
15	.00	.19	.52	49	.16*	.61	.27
16	.00	.34	.60	50	.45*	.86	.68
17	.14*	.79	.46	51	.00	.01	.22
18	.01	.18	.42	52	.02	.01	.74
19	.00	.52	.44	53	.02	.01	.77
20	.01	.33	.50	54	.02	.46	.64
21	.00	.50	ND	55	.00	.14	.30
22	.00	.20	.18	56	.02	.08	.13
23	.00	.17	.00	57	.02	.13	.38
24	.02	.39	.45	58	.01	.23	.48
25	.04	.18	.56	59	.14*	.55	.61
26	.01	.33	.76	60	.02	.33	.78
27	.02	.41	.94	61	.06	.20	ND
30	.03	.10	.73	62	.01	.19	.24
31	.07	.35	.96	63	.00	.17	.23
32	.34**	.48	.66	64	.00	.09	.11

* Positive in the plaque reduction neutralization test (PRNT)
**Not tested by PRNT; ND = not done.

Positive control serum 1:200 reacted with O.D. .70, .65, .74, and .77 in four ELISAs.

VI. ATTEMPTS TO ISOLATE CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS RNA (P. Mason and W. Fan)

As a preliminary step to molecular cloning of Crimean Congo hemorrhagic fever virus, the IbAn 10200 strain of virus was grown in CER cells in 75 cm sq. flasks, then subcultured into CER cells which were adapted to spinner cultures. The CER cells were monitored for antigen by IFA, and titered. Supernatant fluids titered as high as 7 log ID50/ml. When between 50% and 100% of the cells fluoresced, the cells were harvested, treated with NP-40 and centrifuged in a cesium chloride gradient. The fractions containing nucleocapsids were determined by western blotting with a polyvalent CCHF mouse ascitic fluid which stained the nucleoprotein. The nucleocapsid fraction was then extracted with phenol and electrophoresed in agarose. Several bands were visualized which were present in the preparation from infected cells and not in the preparation from mock infected cells. Hybridization has not yet been carried out to confirm that these bands are RNA segments.

VII. MATURATION OF JAPANESE ENCEPHALITIS E, NS1, AND NS1' GLYCOPROTEINS IN VERO AND MOSQUITO CELL LINES (P. Mason)

In an attempt to understand the maturation process of the Japanese encephalitis virion, the glycoproteins E, NS1 and NS1' were studied in Vero and C6/36 cells. The three glycoproteins were processed differently in the JE-infected Vero and C6/36 cell lines. All three proteins were released slowly from Vero cells. The time to release of half of the labeled protein was >6 h. The release of E was also slow (>8 h) in C6/36 cells, while, surprisingly, NS1 and NS1' were not released from the mosquito cells and were retained in an undegraded form in the cell layer. AP61 and TRA284 mosquito cell lines gave similar results, indicating that the non-release of NS1 and NS1' is a general phenomenon in mosquito cells.

The proteolytic processing of the three proteins appeared identical in Vero and C6/36 cells, but some differences in N-linked glycosylation were observed. E, NS1, and NS1' found within infected cells of both types contained high-mannose oligosaccharide groups for more than eight hours after synthesis. Additional sugar residues were added to the single E protein oligosaccharide group prior to release from Vero cells, while sugar residues were trimmed from the E protein oligosaccharide group prior to release from mosquito cells. The forms of NS1 and NS1' found in the culture fluid of infected Vero cells contained one complex and one high-mannose oligosaccharide.

All three glycoproteins released from JE-infected Vero cells were associated with extracellular particles, the virion in the case of E and a low density particle in the case of NS1 and NS1'. Furthermore, E, NS1, and NS1' exhibited amphipathic properties in Triton X-114 extraction experiments. Taken together, these results suggest that both the structural (E) and non-structural (NS1 and NS1') glycoproteins were accumulated within the secretory pathway of the infected Vero cells, assembled into particles, and then released into the extracellular fluid.

VIII. LOW PASSAGE ARBOVIRUS COLLECTION (R. Tesh, R. Shope, S. Tirrell)

In collaboration with the American Committee on Arboviruses' Subcommittee for the Collection of Low Passage Arbovirus Strains (SCLAS), the Reference Center continues to collect low passage strains of arboviruses of medical or veterinary importance from representative geographic regions of the world and from different time periods and hosts. These strains are proving useful in studies of arboviral genetics, pathogenesis, epidemiology, and vaccine development. Arbovirus field laboratories around the world have generously responded to the request for strains.

Virus specimens submitted were subsequently passaged in C6/36 or Vero cells. This material was used to prepare virus stocks which were lyophilized and stored. Because of the large number of virus specimens received, the identification of each strain has not yet been comfirmed. The identity listed for each strain is that attributed by the donor. During 1988, however, the strains of dengue and Japanese encephalitis viruses have been tested by type-specific monoclonal antibody in IFA to confirm the identity.

A complete listing of arbovirus strains now lyophilized is given in Table . Ampoules of these agents are available on request. There are now 457 strains listed, an increase during 1988 of 137 low passage strains. During the year the Reference Center has had requests for multiple strains of yellow fever virus for a study in Africa, and for multiple strains of dengue 3 virus for a study in Asia to select a candidate vaccine strain. In addition, molecular examination of nearly all of the strains of dengue-1, dengue-2, and Japanese encephalitis by primer extension sequencing is described in sections V, A and B of this report.

Abbreviations shown under passage history are as follows: suckling mouse (SM), Vero cells, (Vero), C6/36 clone of Aedes albopictus cells (C6/36), Aedes pseudoscutellaris cells (AP-61), primary chicken embryo (PCE), and inoculation of live mosquitoes (MOSQ). The number following the abbreviation refers to the frequency of passage. For example, SM #1, Vero #1 would mean one passage each in suckling mice and in Vero cellls.

IX. ARBOVIRUS BULLETIN BOARD, REFERENCE, AND DATA ACCESS (P. Mason)

A phone-accessible computer network is available by calling 203/785-2912. Prospective users may request a password at the time of initial contact. Arbovirologists and other interested persons are encouraged to use this free service.

During 1988, flavivirus sequences (under separate funding by WHO) were added to the existing data bases. Sequences of West Nile, 17D yellow fever, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Japanese encephalitis, and dengue 1, 2 and 4 strains are now on line and accessible by phone. Floppy discs containing flavivirus sequences may also be obtained on request.

TABLE II

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
Alajuelva	76V-2441	Mosq #1,C6/36#1	Aedeomyia squamipennis	Vinces, Ecaudor	May. 1975
Arboledas	CoAr 171000	Vero #1	Lutzomyia spp.	Arboledas,N.S.,Colombia	1986
Arboledas	CoAr 170150	Vero #2	Lutzomyia spp.	Arboledas,N.S.,Colombia	1984
Cache Valley	69V-2152	C6/36 #1	Culiseta inornata	Umatilla Co.,Oregon	1969
Cache Valley	RU-68	Vero #1	Aedes sollicitans	Dennisville,New Jersey	Sept.1982
Calif.enceph.	BFN-2130	C6/36 #1	Aedes melanimon	Butte Co., California	May 1970
Calif.enceph.	BFN-3931	C6/36 #1	Aedes melanimon	Butte Co., California	Aug.1971
Calif.enceph.	E-19032	C6/36 #1	Aedes melanimon	Kern Co., California	Sept. 1981
Calif.enceph.	Kern 175-82	C6/36 #1	Aedes melanimon	Kern Co., California	Sept. 1982
Chagres	PaAr3419	Vero #2	Lu. sanguinaria	Bayano, Panama	Oct.1976
CHIK	RSU1	Vero #2	human serum	Ambon Island, Indonesia	1985
CHIK	37941	C6/36 #2,Vero#1	Aedes furcifer	Kedougou, Senegal	198?
CHIK	37937	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou,Senegal	1983
CHIK	37950	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou,Senegal	1983
CHIK	37997	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou,Senegal	1983
CHIK	37953	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou,Senegal	1983
CHIK	37963	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou,Senegal	1983
CHIK	1455/75	Mosq #2,C6/36 #1	human serum	Bangkok,Thailand	1975
CHIK	PO-731460	Vero #1,Mosq #1	" "	Barsi, India	1973
CHIK	UgSg 41855	SM#3, Vero#1	" "	Mukono Dist.,Uganda	1982
DEN-1	INS-347869	C6/36 #3	" "	Caqueta, Colombia	1985
DEN-1	Fiji 40130	Mosq?,C6/36 #1	" "	Fiji	1975
DEN-1	Manila 19076	Mosq?,C6/36 #1	" "	Manila,Philippines	1974
DEN-1	Burma 10378	Moq.?, C6/36 #1	" "	Burma	1976
DEN-1	Jamaica 44684	Mosq.?, C6/36 #1	" "	Jamaica	1977

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-1	PUO-359	C6/36 #1	" "	Bangkok, Thailand	1980
DEN-1	Nauru 16299	Mosq.C6/36 #1	" "	Nauru	1974
DEN-1	Dak H29177	Mosq #1,C6/36#1	" "	Bandia, Senegal	1979
DEN-1	1186	Mosq.#2,C6/36 #1	" "	Jakarta,Indonesia	1977
DEN-1	1236	"	" "	Jakarta,Indonesia	1978
DEN-1	1298	"	" "	Mexico	1980
DEN-1	1318	"	" "	Puerto Rico	1981
DEN-1	1335	"	" "	Colombo,Sri Lanka	1981
DEN-1	1344	"	" "	Mexico	1982
DEN-1	1351	"	" "	Colombia	1982
DEN-1	1378	"	" "	Mexico	1983
DEN-1	1412	"	" "	Mexico	1983
DEN-1	1413	"	" "	Haiti	1983
DEN-1	INS 351094	C6/36 #3	" "	Guaviare, Colombia	July 1987
DEN-1	765104	C6/36 #4	" "	Pingtung Co., Taiwan	Nov.1987
DEN-1	765105	C6/36 #4	" "	Pingtung Co., Taiwan	Nov.1987
DEN-1	766603	C6/36 #4	" "	Pingtung Co., Taiwan	Nov.1987
DEN-1	765101	C6/36 #4	" "	Pingtung Co., Taiwan	Nov.1987
DEN-1	766602	C6/36 #4	" "	Pingtung Co., Taiwan	Nov.1987
DEN-1	Dak HD 29177	SM#2,C6/36#1	human serum	Bandia,Senegal	Nov.1979
DEN-1	Dak Ar A 15120	? C6/36#1	Aedes aegypti	Dabakala,Ivory Coast	June 1985
DEN-1	CAREC 775280		human serum	Dominica	1977
DEN-1	CAREC 778156		"	Grenada	1977
DEN-1	CAREC 778160		"	Grenada	1977

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-1	CAREC 777921		human serum	Nassau	1977
DEN-1	CAREC 776787		"	Turks & Caicos	1977
DEN-1	CAREC 778774		"	St. Kitts	1977
DEN-1	CAREC 778775		"	St. Kitts	1977
DEN-1	CAREC 778546		"	Antigua	1977
DEN-1	CAREC 778554		"	Antiguq	1977
DEN-1	CAREC 778558		human serum	Antiqua	1977
DEN-1	CAREC 778108		"	Grenada	1977
DEN-1	CAREC 780894		"	Trinidad & Tobago	1978
DEN-1	CAREC 780572		"	Trinidad & Tobago	1978
DEN-1	CAREC 780874		"	Trinidad & Tobago	1978
DEN-1	CAREC 780590		"	Trinidad & Tobago	1978
DEN-1	CAREC 8110979		"	Suriname	1981
DEN-1	CAREC 818001		"	Barbados	1981
DEN-1	CAREC 8110498		"	Suriname	1981
DEN-1	CAREC 816879 D287		"	Suriname	1981
DEN-1	CAREC 816885 D294		"	Suriname	1981
DEN-1	CAREC 822964 TPHL 2718		"	Trinidad & Tobago	1982
DEN-1	CAREC 852679		"	Aruba	1985
DEN-1	CAREC 852791		"	Aruba	1985
DEN-2	CAREC 775283		"	Dominica	1977
DEN-2	CAREC 8110827		"	Jamaica	1981
DEN-2	CAREC 828902		"	St. Vincent	1982
DEN-2	CAREC 835171		"	Trinidad & Tobago	1983
DEN-2	CAREC 8511995 TPHL 9673		"	Trinidad & Tobago	1985

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-2	CAREC 867850	D 2098	human serum	Suriname	1986
DEN-2	CAREC 867744	D 1945	"	Suriname	1986
DEN-2	CAREC 860435	TPHL 9747	"	Trinidad & Tobago	1986
DEN-2	CAREC 877764	TPHL 14870	"	Trinidad & Tobago	1987
DEN-2	CAREC 877765	TPHL 14871	"	Trinidad & Tobago	1987
DEN-2	CAREC 876775	TPHL 14492	"	Trinidad & Tobago	1987
DEN-2	CAREC 881527	B'dos 384	"	Barbados	1988
DEN-2	CAREC 880720	TPHL 15803	"	Trinidad & Tobago	1988
DEN-2	CAREC TPHL 3963		"	Trinidad & Tobago	19?
DEN-2	PL-003	C6/36 #4	" "	Pingtung Co., Taiwan	Sept.1981
DEN-2	Bangkok 16803	Mosq ? ,C6/36#1	" "	Bangkok,Thailand	1974
DEN-2	Indonesia 10410	" "	" "	Java, Indonesia	1973
DEN-2	JA-TVP-496	C6/36 #1	" "	Jamaica	Aug. 1982
DEN-2	NC 9163	C6/36 #1	" "	New Caledonia	1972
DEN-2	Burma 40479	C6/36 #1	" "	Burma	1976
DEN-2	PM 33974	Mosq#1,C6/36#1	Aedes africanus	Rep. of Guinea	Nov. 1981
DEN-2	PR-159	C6/36 #1	human serum	Puerto	1969
DEN-2	Ph.H 2172	AP-61 #2,C6/36 #1	human serum	Manila, Philippines	1983
DEN-2	766635	C6/36 #5	" "	Pingtung Co., Taiwan	Nov.1987
Den-2	PL-001	C6/36 #4	" "	Pingtung Co., Taiwan	Sept.1981
DEN-2	PL-046	C6/36 #4	" "	Pingtung Co., Taiwan	Sept.1981
DEN-2	Dak HD 10674	?, C6/36 #1	" "	Bandia, Senegal	Feb. 1970
DEN-2	Dak ArD20761	SM#8, C6/36 #1	Aedes luteocephalus	Kedongon, Senegal	Nov. 1974
DEN-2	Dak ArA578	SM#8, C6/36 #1	Aedes taylori	Dabakala,Ivory Coast	June 1980
DEN-2	Dak ArA2039	SM#6, C6/36 #1	Aedes luteocephalus	Somousso,Bukina Faso	Sept.1980

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-2	Dak ArA510	SM#4, C6/36 #1	*Aedes taylori*	Dabakala, Ivory Coast	July 1980
DEN-2	Dak ArA1247	SM#5, C6/36 #1	*Aedes taylori* (males)	Dabakala, Ivory Coast	Oct. 1980
DEN-2	Dak ArA2022	SM#6, C6/36 #1	*Aedes africanus*	Nasso, Burkina Faso	Sept. 1980
DEN-2	Dak ArA6894	? , C6/36 #1	*Aedes aegypti*	Bobo-Dioulasso Burkina Faso	Jan. 1980
DEN-2	INS 348600	C6/36 #3	human serum	Tomaco, Narino, Colombia	Jan. 1986
DEN-2	1232	Mosq.#2, C6/36 #2	human serum	Jakarta, Indonesia	1978
DEN-2	1251	"	" "	Tonga	1974
DEN-2	1268	"	" "	Jogyakarta, Indonesia	1978
DEN-2	1328	"	" "	Puerto Rico	1977
DEN-2	1329	Mosq.#1, C6/36 #2	human serum	Jamaica	1982
DEN-2	1334	"	"	Colombo, Sri Lanka	1981
DEN-2	1349	"	"	Upper Volta	1982
DEN-2	1353	"	"	Colombo, Sri Lanka	1982
DEN-2	1408	"	"	Jamaica	1983
DEN-2	1421	"	"	Mexico	1983
DEN-2	INS 351863	C6/36 #3	"	Tolima, Colombia	Jan. 1988
DEN-3	Burma DHF 190	Mosq?, C6/36#1	"	Burma	1976
DEN-3	Tahiti 18	"	"	Tahiti	1964
DEN-3	PR-9311	"	"	Puerto Rico	1963
DEN-3	Thailand 49080	"	"	Thailand	1971
DEN-3	Singapore 16182	"	"	Singapore	1973
DEN-3	1245	Mosq.#2, C6/36 #2	"	Sleman, Indonesia	1978
DEN-3	1259	"	"	Jakarta, Indonesia	1978
DEN-3	1301	"	"	Malaysia	1975
DEN-3	1309	LLC/MK_2#1, Mosq.#2, C6/36 #1	"	Bangkok, Thailand	1978

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-3	1325	Mosq. #2, C6/36 #1	human serum	Colombo,Sri Lanka	1981
DEN-3	1339	"	"	Puerto Rico	1977
DEN-3	1359	"	"	Colombo,Sri Lanka	1982
DEN-3	1363	"	"	Colombo,Sri Lanka	1982
DEN-3	D83-144	C6/36 #1	"	Bangkok, Thailand	1983
DEN-4	Dak HD 34460	SM#3, C6/36 #1	human serum	Dakar, Senegal	Nov. 1981
DEN-4	Dak ArNC599	SM#6, C6/36 #1	Aedes vigilax	Koumac,New Caledonia	Jan. 1981
DEN-4	1228	Mosq. #2, C6/36 #1	"	Jogyakarta,Indonesia	1978
DEN-4	1229	"	"	Jakarta,Indonesia	1976
DEN-4	1315	"	"	Puerto Rico	1981
DEN-4	1331	Mosq.#2,C6/36 #1	human serum	Puerto Rico	1982
DEN-4	1332	"	"	Puerto Rico	1982
DEN-4	1385	Vero #1,Mosq.#2,C6/36 #1	"	Boa Vista,Brazil	1982
DEN-4	1411	Mosq. #2, C6/36 #1	"	San Salvador,El Salvador	1983
DEN-4	1414	Mosq. #2, C6/36 #1	"	Mexico	1983
DEN-4	1415	"	"	Mexico	1983
DEN-4	Medan 12524	"	"	Sumatra,Indonesia	1973
DEN-4	Tahiti 79	"	"	Tahiti	1979
DEN-4	Sri Lanka	"	"	Sri Lanka	1978
DEN-4	Niue	"	"	Niue	1980
DEN-4	Gilberts 49367	"	"	Kiribati,Gilberts	1980
DEN-4	PR-TVP 376	C6/36 #1	"	Puerto Rico	Feb.1982
DEN-4	SH 38549	Mosq.#1,C6/36 #3	"	Dakar, Senegal	Nov.1983
DEN-4	38549	AP-61 #1,C6/36 #1	"	Dakar, Senegal	1977
DEN-4	38550	AP-61 #1,C6/36 #1	"	Dakar, Senegal	1980

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-4	INS 351785	C6/35 #3	human serum	Tolima, Colombia	Nov.1987
EEE	ME-77132	Mosq #1,C6/36 #1	Culiseta melanura	Carver Cdr Swmmp,MA	Aug.1977
EEE	78-3372	C6/36 #1	" "	Raynham,Mass.	Sept.1978
EEE	79-2138	C6/36 #1	" "	Westport,Mass.	Aug.1979
EEE	DV-260-82	C6/36 #1	Parus bicolor	Dennisville,NJ	July 1982
EEE	VO80784/1	Vero #1	horse brain	Florida	1984
EEE	VO82085/2	Vero #1	horse brain	Florida	1985
EEE	44-84	Vero #1	bobwhite quail	Pocomoke Swamp, MD	1984
EEE	323-85	Vero #1	Culiseta melanura	Pocomoke Swamp,	1985
EEE	187-85	Vero #1	" "	" "	1985
EEE	215-85	Vero #1	" "	" "	1985
EEE	GML 903866	Vero #3	sentinel chicken	Bayano, Panama	1984
EEE	GML 900188	SM#2,Vero#1	horse brain	Gatuncillo, Panama	1962
EEE	lung #72	Vero #1	whooping crane	Patuxent Wildlife, Research Center,MD	1984
EEE	FD #7829	Vero #2	Culiseta melanura	Pocomoke Cypress, Swamp, MD	1983
EEE	FD #7830	Vero #2	" "	Pocomoke Cypress, Swamp, MD	1983
EEE	Ma 2494	PCE #1, Vero #1	" "	Raynham, MA	Sept.1977
EEE	Ma 2020	PCE #1, Vero #1	" "	Halifax, MA	Aug. 1978
EEE	Ma 1058	PCE #1, Vero #1	" "	Halifax, MA	July 1979
EEE	Ma 1833	PCE #1, Vero #1	" "	Halifax, MA	Aug. 1980
EEE	Ma 396	PCE #1, Vero #1	" "	New Bedford, MA	Aug. 1982
EEE	Ma 848	PCE #1, Vero #1	" "	Easton, MA	Sept.1983

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
EEE	Ma 1313	PCE #1, Vero #1	Culiseta melanura	Raynham, MA	Sept.1984
EEE	MARU 435731	Vero #2	horse brain	Chepo, Panama	July 1986
EEE	M-210-83A	Original	horse brain	Colchester, CT	Sept.1983
EEE	MP-9	C6/36 #1	Culiseta melanura	Colchester,CT	Sept.1979
EEE	R-35108	C6/36 #1	horse brain	Three Rivers,Michigan	1980
EEE	70U1104	Vero #1	sentinel hamster	Iquitos, Peru	1970
EEE	M-649-84	original	horse brain	Waterford, CT	1984
Highlands J	NJO-111D	C6/36 #1	Culeseta melanura	S.R.Game Farm,New Jersey	1960
Highlands J	78-3331	C6/36 #1	Culiseta melanura	Canton, Mass.	Sept. 1978
Highlands J	79-2137	C6/36 #1	" "	Westport,Mass.	Aug. 1979
Highlands J	WC-431	C6/36 #1	Dumetella carolinensis	West Creek,NJ	Sept. 1981
Isfahan	91025-C	Vero #2	" "	Isfahan, Iran	Aug. 1975
Isfahan	91026-167	Vero #3	" "	Isfahan, Iran	Aug. 1975
Jamestown Canyon	MP-935	C6/36 #1	Aedes canadensis	Isfahan, Iran	1979
JE	Osaka 222681	Mosq?,C6/36#1	Culex tritaeniorhynchus	Osaka,Japan	1979
JE	Sagiyama 224052	" "	" "	Sagiyama, Japan	1956
JE	Java 222682	" "	" "	Java, Indonesia	1979
JE	HK 8256	Mosq#7,C6/36#1	Culex annulus	Taiwan	19?
JE	Ph.An 1242	Vero #1,C6/36#1	pig blood	Santo Cristo,Philippines	1984
JE	KE-105/83	C6/36 #1	human brain	Kampangphet, Thailand	1983
JE	KE-094/83	C6/36 #1	human brain	Kampangphet, Thailand	1983
JE	B-1080/83	C6/36 #1	pig serum	Choomporn, Tahiland	1983
JE	KP0035-114CT	C6/36 #2	Culex tritaeniorhynchus	Kampangphet, Thailand	1982
JE	JKT-8111			Indonesia	19
JE	JKT-8110			Indonesia	19

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
JE	JKT-1105	SM#1,Vero#4	Culex gelidus	Java, Indonesia	Feb. 1979
JE	JKT-2363	SM#1,Vero#4	Culex tritaeniorhynchus	Java, Indonesia	Nov. 1979
JE	JKT-654	SM#1,C6/36#1,Vero#2	"	Java, Indonesia	1978
JE	JKT-1724	SM#1, Vero#4	"	Java, Indonesia	March 1979
JE	VN-135			Viet Nam	19
JE	VN-131			Viet Nam	19
JE	VN-104			Viet Nam	19
JE	JKT-6468	C6/36#2,AP-61#2,Vero#1	Culex tritaeniorhynchus	Flores,Indonesia	May 1981
JE	JKT-8442	AP-61#4,Vero#1	"	Bali, Indonesia	Dec.1980
JE	JE 827	SM#1, AP-61#3,Vero #1	Culex tritaeniorhynchus	Sarawak	19?
JE	78668	?	human brain	Lucknow, India	1970
JE	Beijing-1	?	human brain	China	1949
JE	63498	SM#11, Vero #1	Culex tritaneiorhynchus	Vellore, India	1963
JE	7812474	SM#5, Vero #1	human brain	Dibrugarh,Assam,India	1978
JE	803830	SM#9, Vero #1	Culex pseudovishnui	Kolar,Kanataka,India	1980
JE	826309	SM#5, Vero #1,	human brain	Goa, India	1982
JE	86596	SM#3, Vero #1	human brain	Sri Lanka	1986
JE	242154	?	Culex tritaeniorhynchus	Taiwan	Sept. 1981
JE	P-61779	?	"	Taiwan	June 1983
JE	249781	?	"	Taiwan	July 1982
JE	225492	?	"	Taiwan	May 1981
JE	229246	?	"	Taiwan	June 1981
JE	P-61780	?	"	Taiwan	June 1983
JE	603525	?	"	Taiwan	Oct. 1982
JE	P-20778	?	human brain	India	1985

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
JE	691004	?	human brain	Sri Lanka	1969
JE	55-1456	SM#1, Vero #1	human brain	Tokyo, Japan	Aug. 1955
JE	M5/596	?	Culex tritaeniorhynchus	Sagiyama, Japan	Aug. 1955
JE	281801	?	bird blood	Sagiyama, Japan	July 1955
JE	JKT-7180	?	mosquitoes	Indonesia	
JE	JKT-7887	?	mosquito	Indonesia	
JE	JKT-7003	?	mosquito	Indonesia	
JE	B-1208/82	?,Vero #1	pig blood	Kampangphet Prov.,Thailand	Aug.1982
JE	B-0860/83	"	" "	" " "	July 1983
JE	B-2239/84	"	" "	" " "	June 1984
JE	B-0005/85	"	" "	" " "	Jan. 1985
JE	B-2582/85	"	" "	" " "	Nov. 1985
JE	3094/83 (KE-094)	"	human brain	" " "	July 1983
JE.	3076/83 (KE-093)	"	human brain	" " "	July 1983
JE	KPO 561/84	"	mosquito	" " "	July 1984
JE	KPO 439/84	"	mosquito	" " "	July 1984
JE	MNI-93CT	"	mosquito	Bangkok, Thailand	June 1986
JE	MNI-75CT	"	mosquito	" "	June 1986
JE	2372/79	"	human brain	" "	June 1979
JE	B-1034/83	"	pig blood	Chumporn Prov.,Thailand	July 1983
JE	B-1065/83	"	pig blood	Chumporn Prov.,Thailand	July 198
JE	B-2524/85	", C6/36 #1	pig blood	Biratnagar, Nepal	Sept.1985
JE	9490/85	"	human CSF	" "	Oct. 1985
JE	9548/85	", "	human CSF	" "	Oct. 1985
JE	8631/86	", "	human CSF	" "	Sept.1986

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
JE	2909/84 (CR84-10)	?, C6/36 #1	human brain	Chiang Rai Prov.,Thailand	July 1984
JE	2632/84 (CR84-12)	", "	human brain	Chiang Rai Prov.,Thailand	July 1984
JE	B-0922/82	", "	pig blood	Kampangphet Prov.,Thailand	June 1982
JE	B-1053/82	", "	pig blood	" " "	July 1982
JE	KP-0245	", "	mosquito	" " "	June 1984
JE	KP-0269	", "	mosquito	" " "	June 1984
JE	KP-0270	", "	mosquito	" " "	June 1984
JE	KP-0252	", "	mosquito	" " "	June 1984
JE	1070/82 (KE82-008)	", "	human brain	" " "	Aug. 1982
JE	VN-78			Viet Nam	19
JE	VN-118			Viet Nam	19
JE	M7/292		Culex tritaeniorhynchus	Sagiyama,Japan	1957
JE	M7/287		"	Sagiyama,Japan	1957
JE	M7/271		"	Sagiyama,Japan	1957
JE	InAr 633759	SM#4, Vero #1	Culex sp.	Tamil Nadu,India	1963
JE	InAr 641686	SM#11, Vero #1	Culex tritaeniorhynchus	Tamil Nadu,India	1964
JE	InAr 724038	SM#3, Vero #1	Culex whitmorei	Andhra Pradesh,India	1972
JE	InAr 803830	SM#8, Vero #1	Culex pseudovishnui	Karnataka,India	1980
JE	In H 826309	SM#4, Vero #1	human brain	Goa, India	1982
JE	In Ar 755723	SM#6, Vero #1	Culex epidesmus	West Bengal,India	1975
Karimabad	91045-AG	Vero #3	Phlebotomus papatasi	Isfahan,Iran	Aug.1975
Keystone	MB7-34EJ	Mosq#1,C6/36#1	A.atlanticus-tormentus	Bay St.Louis,Ms.	Sept.1967
Keystone	FD-BHK	2BHK#2,C6/36#1	Aedes atlanticus	Pocomoke Swamp,MD	1975
Kokobera	CH 19620	Mosq#2,C6/36#1	Culex annulirostris	Charleville, Aust.	Feb.1976
Kunjin	CH 16532	C6/36 #2	Culex annulirostris	Charleville, Aust.	Mar.1974

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
La Crosse	78V 13193	SM#1,Vero#2	Aedes triseriatus (larvae)	North Carolina	1978
La Crosse	prototype	C6/36 #1	human brain	La Crosse, Wisconsin	1960
La Crosse	78134	C6/36 #1	human brain	Wisconsin	1978
Punta Toro	PaAr 2381	Vero #2	human brain	Bayano, Panama	Nov.1975
Punta Toro	Adames	Vero #3	man	Darien Pr., Panama	Apr.1972
Ross River	S-48325	C6/36 #4	human serum	Pago Pago,Am Samoa,	Dec.1979
Ross River	Aus.Ar.96614	SM#1,C6/36#1	Aedes sp.	New South Wales,Australia,	1983
San Angelo	72V-4089	Mosq #2,C6/36#1	Psorophora signipennis	Las Cruces,N.M.	1972
Sicilian	91025-B	Vero #3	" "	Isfahan, Iran	1975
Sicilian	OSS-42	Vero #1	Phlebotomus sp.	Imbaba,Giza,Egypt	1986
Sicilian	91045-I	Vero #2	Phlebotomus papatasi	Isfahan Prov.,Iran	Aug.1975
SLE	Ft.Wash.-4	Mosq #2,C6/36#2	Culex pipiens	Ft.Washington,MD.	Jan.1977
SLE	BFS-508	C6/36 #1	Culex tarsalis	Kern Co., CA	Aug.1950
SLE	Ft. Wash.-4	C6/36 #1	Culex pipiens	Pocomoke Swamp MD	Jan.1977
SLE	FL 79-411	C6/36 #1	Culex nigripalpus	Lee County,Florida	1979
SLE	BFS-2874	C6/36 #1	Culex tarsalis	Kern Co., CA	Sept.1960
SLE	BFN-1324	C6/36 #1	Culex tarsalis	Butte Co., CA .	July 1970
SLE	E-2819	C6/36 #1	Culex tarsalis	Riverside Co.,CA	July 1980
Snowshoe hare	82-Y-21	C6/36 #1	Aedes nigripes	Yukon Ter.,Canada	July 1982
VEE (1-D)	71D 1394	SM #1,C6/36 #1	Mixed mosquitoes	Peru	1971
VEE (1-D)	310979	C6/36 #1	Sentinel hamster	Puerto Boyaca,Colombia	June 1974
VEE (1-E)	68U 200	C6/36 #1	Sentinel hamster	Avellana,Guatemala	May 1977
VEE (1-E)	64A 87	SM #1,C6/36 #1	Culex opisthopus	Sontecomapan,Mexico	1964
VEE	64U 60	SM #1	sentinel hamster	Santecomapan, Mexico	1964
VEE	83U 12	SM #1	sentinel hamster	Rio de Oro, Colombia	1983

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
VEE-IC	CBS1-9	Vero #2	Anopheles triannulatus	Sotillo, Venezuela	1963
VEE-IE	68U 201	Vero #1	sentinel hamster	La Avellana,Guatemala	1968
VEE-ID-E	71D 1249	SM #1,Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-ID-E	70U 1139	Vero #2	sentinel hamster	Iquitos, Peru	1970
VEE-IE	68U 201	SM #1,Vero #1	sentinel hamster	La Avellana, Guatamala	1968
VEE-IE	71U 338	SM #1,Vero #1	sentinel hamster	Monte Rico, Guatemala	1971
VEE-V	CaAr 4389	SM #4,CEC #1,Vero#1	Culex spp. (pool)	Cabassou, French Guyana	1974
VEE-III	TVL 52049	Vero #2	Zygodontomys brevicauda	Bush-Bush, Trinidad	1963
VEE-IB	541/73	SM #1,CEC #2,Vero#1	human	Guajira, Venezuela	1973
VEE-IA	E1/68	SM #1,CEC #1,Vero#1	human	Guajira, Venezuela	1968
VEE-ID	202330	SM #1,Vero #2	Proechimys semispinosus	Gamboa, Panama	1963
VEE-IC	V-198	SM #1,Vero #2	human serum	Guajira, Colombia	1962
VEE-IC	V-178	SM #1,Vero #2	human	Cundinamarca, Colombia	1961
VEE-IE	63A 216	SM #2,Vero #1	Culex spp. (pool)	Sontecomapan, Mexico	1963
VEE-ID	3880	SM #2,Vero #3	human serum	Canito, Panama	1961
VEE-III	Fe 37C	SM #6,Vero #1	Culex spp. (pool)	Florida, USA	1963
VEE-ID-E	Tumaco An9004	SM #3,Vero #1	sentinel hamster	Pacific coast, Colombia	1969
VEE-IE	71U 384	SM #1,Vero #1	sentinel hamster	La Avellana, Guatemala	1971
VEE(IAB)	G 8419	SM #2,Vero #2	horse	Sonora, Mexico	1972
VEE-IA	71D 1252	SM #1,Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-IA	E123/69	SM #1,CEC #1,Vero#1,	human	Mara, Venezuela	1969
VEE-IB	69U332	Vero #1	sentinel hamster	La Avellana,Guatemala	1969
VEE-ID	V-209A	SM #2,Vero#1,Vero#2,	sentinel mouse,bov.teat	Alta Verapaz, Guatemala	1986
VEE-IV	BeAr 35645	SM #4,Vero#1	Anopheles nimbus	Belem, Brazil	1961
VEE-IA	52/73	SM #2,Vero #1	burro	La Libertad, Peru	1973

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
VEE-II	Fe5-47 et	SM #2,Vero #1	Aedes taeniorhynchus	Florida, USA	1965
VEE-IB	6921	SM #2,Vero #1	human serum	Montufar, Guatemala	1969
VEE-ID-E	CoAn 59145	? Vero #1	sentinel hamster	Santander,Colombia	1970
VEE-IA	CoAn 5384	SM #2,CEC #1,Vero #1	horse	Carmelo, Colombia	1967
VEE-V	CoAn 410d	SM #3, Vero #1	Psaracolus decumanus	Tonate, French Guyana	1973
VEE-IA	71D 1252	SM #1,Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-ID-E	75D 143	SM #2,Vero #1	Mosquito pool	Iquitos, Peru	1975
VEE-IV	BeAr 40403	SM #6,Vero #1	Trichoprosopon digitatum	Belem, Brazil	1961
VSV-Alagoas	CoAr 171638	Vero #2	Lutzomyia sp.	Durania, N.S.,Colombia	Dec.1987
VSV-IND	L53-85	Vero #1	bovine mouth	Cartago, Costa Rica	1985
VSV-IND	L28-87	Vero #1	bovine mouth	Sta. Ana, San Salvador	1987
VSV-IND	L274-86	Vero #1	bovine mouth	Chalatenango,San Salvador	1986
VSV-IND	L96-87	Vero #1	bovine teat	Alajuela, Costa Rica	1987
VSV-Ind	GML 903816	Vero #4	human(throat swab)	Panama City, Panama	1984
VSV-Ind	L5-85	Vero #1	bovine teat	Guatemala	1984
VSV-Ind	L30-84	Vero #1	bovine mouth	Costa Rica	1984
VSV-Ind	L125-84	Vero #1	bovine teat	Panama	1984
VSV-Ind	L2-83	?,Vero #1	"	Honduras	1983
VSV-Ind	L51-85	Vero #1	"	Costa Rica	1985
VSV-Ind	L111-85	Vero #1	"	Salvador	1985
VSV-Ind	L134-85	Vero #1	cow	?	?
VSV-N.J.	Jardin 12-IV	bovine #1,Vero #1		Veracruz, Mexico	1982
VSV-N.J.	Ossabaw	Vero #2	feral pig	Ossabaw Island,Georgia	1983
VSV-NJ	L32-85	Vero #1	bovine epithelium	Managua,Nicaragua	1985
VSV-NJ	L7-82	Vero #2	bovine epithelium	Honduras	1982

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
VSV-NJ	L11-85	Vero #1	bovine teat	Panama	1985
VSV-NJ	L14-85	Vero #1	bovine teat	Costa Rica	1984
VSV-NJ	L35-85	Vero #1	bovine mouth	Nicaragua	1985
VSV-NJ	L264-84	Vero #1	bovine teat	Honduras	1983
VSV-NJ	82A175	Vero #1	*Equus caballus*	Loveland,Colorado Sept.	1982
VSV-NJ	L270-84	Vero #1	bovine mouth	Guatemala	1984
VSV-NJ	L243-84	Vero #1	bovine mouth	Belize	1984
VSV-NJ	L8-82	Vero #1	bovine epithleium	Honduras	1982
VSV-MJ	L14-82	Vero #1	"	Costa Rica	1982
VSV-NJ	L67-82	Vero #1	"	Guatemala	1982
VSV-NJ	L153-83	Vero #1	"	Nicaragua	1983
VSV-NJ	L183-83	Vero #1	bovine epithleium	Panama	1983
VSV-NJ	L177-85	Vero #1	"	Honduras	1985
VSV-NJ	L172-86	Vero #1	bovine mouth	Managua,Nicaragua	1986
VSV-NJ	L123-87	Vero #1	bovine mouth	Managua,Nicaragua	1987
VSV-NJ	L44-87	Vero #1	bovine mouth	Alta Verapaz,Guatemala	1987
VSV-NJ	L130-87	Vero #1	bovine mouth	Comayagua,Honduras	1987
VSV-NJ	L212-86	Vero #1	bovine mouth	Chiriqui,Panama	1986
VSV-NJ	L204-86	Vero #1	bovine teat	Alta Verapaz,Guatemala	1986
WEE	NM5-7ET	C6/36 #1	*Aedes vexans*	Rancho de Albiquin,New Mexico	1965
WEE	BFS-1428	C6/36 #1	*Culex tarsalis*	Kern Co.,CA June	1952
WEE	DLAN-23-82	C6/36 #1	*Culex tarsalis*	Tulane Co.,CA July	1982
WEE	BFS-4143	C6/36 #1	*Culex tarsalis*	Kern Co.,CA July	1962
WEE	BFN-3258	C6/36 #1	*Culex tarsalis*	Glenn Co.,CA Aug.	1971
YF	1362/77	C6/36 #1	human serum	Mutucana,Peru	1977

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
YF	5384	SM #2,CEC #1,	horse	Carmelo, Colombia	1967
YF			human serum	Antioquia, Colombia	1985
YF	HD 38564	C6/36 #2	" "	Upper Volta	198?
YF	Ar B 9005	SM_5, $MOSQ_1$	Aedes africanus	Bozo,Cent.Afr.Rep.	Nov 1977
YF	Ar B 8883	SM_5, $MOSQ_1$	Aedes africanus	Bozo,Cent.Afr.Rep.	Oct 1977
YF	P128M_c SMB/IVIC	SM_3, $MOSQ_1$	liver of Alouatta seniculus	Las Claveles, Cojedes, Venezuela	Oct 1959
YF	INS-347613	C6/36 #3	human serum	Antioquia, Colombia	1985
YF	H117505	SM #4	human serum	Ogbomosho,Nigeria	4/22/87
YF	H117491	SM #4	human serum	Ogbomosho,Nigeria	2/26/87
YF	BA55	SM #3	human serum	Ogbomosho,Nigeria	5/23/87
YF	PHO-42H,SMB/IVIC-2	SM_2,$MOSQ_1$	human liver	San Rafael de el Pinal,Tachira,Venezuela	Nov 1961
YF	Asibi	Monkey 4	human serum	Kpeve, Ghana	Jun 1927
YF	R35740	SM1, Mosq.1	human liver	Ayacucho Dept.,Peru	Feb 1979
YF	Jiminez	Aotus 1	human liver	Panama	1974
YF	Serie	SM 7,Mosq.1	?	Ethiopia	1961
YF	V-528A	Alouatta 1,Mosq.1	human serum	Colombia	1979
YF	14 FA	SM 7,Mosq.1	human serum	Luanda, Angola	1971
YF	E-1337	SM 1,Mosq.1	human serum	Guayzimi,Ecuador	1979
YF	PM 27340	Mosq. 3	Aedes furcifer-taylori	Kedougou, Senegal	1978
YF	M 37	Mosq. 3	human serum	Toubacouta, Senegal (patient died in Paris)	1979
YF	Ar B 11059	AP-61 #1,C6/36 #1	Aedes africanus	Bozo, Cent.Afr.Rep.	1977
YF	Ar B 17239	AP-61 #1,C6/36 #1	Aedes africanus	Bozo, Cent.Afr.Rep.	1980

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
YF	SH 38556	AP-61 #1,C6/36 #1	human blood	Burkina Faso	1983
YF	Ar D 25865	SM 5,Mosq.#1	Aedes furcifer-taylori (males)	Kedougou, Senegal Dec	1977
YF	T 797984	Mosq.1	human liver	Trinidad	1979
YF	T 790882	Mosq.1	Haemagogus janthinomys	Trinidad	1979
YF	Ar 232869	SM 2, Mosq.1	Haemagogus sp.	Brazil Mar	1973
YF	Ar 350397	SM 2, Mosq.1	Haemagogus sp.	Belterra,Para,Brazil Aug	1978
YF	H 3509698	SM 2, Mosq.1	human liver	Tome-Acu,Para,Brazil Aug	1978
YF	Be Ar 233164	Mosq.4	Haemagogus sp.	Goias, Brazil	1973
YF	TRVL 4205	$Aotus_1$,$MOSQ_2$	liver of dead Alouatta seniculus	Trinidad	1954
YF	79 H 327	$MOSQ_2$	human serum	Minteh Kunda,N.Bank Div., Gambia Jan	1979
YF	38578	AP-61 #1,C6/36 #1	human blood	Burkina Faso	1983
YF	38580	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38581	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38557	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38558	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38564	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38566	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38570	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38571	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38572	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38574	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38576	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
YF	38577	AP-61 #1,C6/36 #1	human liver	Burkina Faso	1983
YF	38329	AP-61 #1,C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38334	AP-61 #1,C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38335	AP-61 #1,C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38390	AP-61 #1,C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38400	AP-61 #1,C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38087	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
YF	38088	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
YF	38089	AP-61 #1,C6/36 #1	Ae. taylori	Kedougou, Senegal	1983
YF	31104	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
YF	38693	AP-61 #1,C6/36 #1	Ae. furcifer & africanus	Kedougou, Senegal	1983
YF	37961	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou,Senegal	1983
YF	M-37	MOSQ #2, C6/36 #1	Human serum	Kedougou,Senegal	Nov. 1979
YF	PM 27340	MOSQ #2, C6/36 #1	Ae. furcifer-taylori	Kedougou, Senegal	Oct. 1978
YF	Ar B 8883	SM_5, $MOSQ_1$	Ae. africanus	Bozo, Cent.Afr.Rep.	Oct. 1977
YF	Ar D 25865	SM_5, $MOSQ_1$	Ae. furcifer-taylori(males)	Kedougou,Senegal	Dec. 1977
YF	T 797984	$MOSQ_1$	human liver	Trinidad	1979
YF	T 790882	$MOSQ_1$	Haemagogus janthinomys	Trinidad	1979
YF	Ar 232869	SM_2, $MOSQ_1$	Haemagogus sp.	Brazil	Mar. 1973
YF	Ar 350397	SM_2, $MOSQ_1$	Haemagogus sp.	Belterra,Para,Brazil	Aug 1978
YF	H 3509698	SM_2, $MOSQ_1$	human liver	Tome-Acu,Para,Brazil	Aug 1978
YF	Be Ar 233164	$MOSQ_4$	Haemagogus sp.	Goias, Brazil	1973
YF	TRVL 4205	$Aotus_1$,$MOSQ_2$	liver of dead Alouatta seniculus	Trinidad	1954
YF	79 H 327	$MOSQ_2$	human serum	Minteh Kunda,N.Bank Div., Gambia	Jan 1979

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
YF	P128M_c SMB/IVIC	SM_3, $MOSQ_1$	liver of *Alouatta seniculus*	Las Claveles, Cojedes, Venezuela	Oct 1959
YF	PHO-42H,SMB/IVIC-2	SM_2,$MOSQ_1$	human liver	San Rafael de el Pinal,Tachira,Venezuela	Nov 1961
YF	Asibi	Monkey 4	human serum	Kpeve, Ghana	Jun 1972
YF	R35740	SM_1,$MOSQ_1$	human liver	Ayacucho Dept.,Peru	Feb 1979
YF	Jiminez	*Aotus*$_1$	human liver	Panama	1974
YF	Serie	SM_7,$MOSQ_1$	?	Ethiopia	1961
YF	V-528A	*Alouatta* 1,Mosq.1	human serum	Colombia	1979
YF	14 FA	SM 7, Mosq.1	human serum	Luanda, Angola	1971
YF	E-1337	SM 1, Mosq.2	human serum	Guayzimi,Ecuador	1979
YF	PM 27340	Mosq. 3	*Aedes furcifer-taylori*	Kedougou, Senegal	1978
YF	M 37	Mosq. 3	human serum	Toubacouta, Senegal (patient died in Paris)	1979
Zika	41662	AP-61 #1,C6/36 #1	*Ae. taylori*	Kedougou,Senegal	1984
Zika	41667	AP-61 #1,C6/36 #1	*Ae. taylori*	Kedougou,Senegal	1984
Zika	41671	AP-61 #1,C6/36 #1	*Ae. africanus*	Kedougou,Senegal	1984
Zika	41519	AP-61 #1,C6/36 #1	*Ae. africanus*	Kedougou,Senegal	1984
Zika	41524	AP-61 #1,C6/36 #1	*Ae. africanus*	Kedougou,Senegal	1984
Zika	41525	AP-61 #1,C6/36 #1	*Ae. africanus*	Kedougou, Senegal	1984

X. DISTRIBUTION OF REAGENTS, WHO COLLABORATING CENTRE FOR ARBOVIRUS REFERENCE AND RESEARCH (R. Shope, R. Tesh, G. Tignor, S. Tirrell)

The WHO Centre distributed a total of 408 ampoules of arboviral reagents during 1988. These consisted of 191 virus stocks, 103 antigen preparations, and 114 antibody preparations. Distributions were made to laboratories in 12 states of the United States and in 14 other nations. The reagents represented 121 different viruses in the Arbovirus Unit's collection.

In addition several hundred larvae of colonized arthropods were supplied to other laboratories; these included Lutzomyia longipalpis, Phlebotomus papatasi, and Aedes taeniorrhynchus. Seven different vertebrate and invertebrate cell lines were distributed to 12 laboratories.

XI. PUBLICATIONS - 1988

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