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Final Report

Studies for Dengue Viral Antigens and Antibodies in Sera of Dengue Hemorrhagic Fever (DHF) Patients

A research project

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Submitted by

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Project Profile

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Acknowledgement

I would like to express my sincere appreciation for the generous support from USAID. With this support, the Immunochemistry unit is now equipped with neccessary items for the study of immunochemistry of viruses. Our unit has expanded and more scientists have joined the team to carry on the work of both the Master and Docterate levels. To whatever extent of success has been achieved during this period, I like to mention that the technology established at our unit will continue to serve scientific program as a whole in every aspects possible. Lastly, I am very happy to express that part of my wish have come true. Thank you.

1. Introduction

Dengue infection is caused by four distinct types of dengue viruses. Clinical manifestations of infection range from inapparent infection and classical dengue fever to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Classical dengue fever is a mild form of the disease and is characterized by a sudden onset of fever, headache, lymphadenopathy, myalgias and petichial hemorrhage or maculopapular rash. Hemorrhagic fever and shock syndrome are severe-disease manifestations that may be fatal, even when treatment is given (1). These severe forms of the disease start with high fever and progress to nausea, vomitting, abdominal pain, hepatosplenomegaly, epistaxis, hematemesis, melena, and petichial hemorrhage. Shock usually occurs from day 5 to day 7 after the onset of fever.

At least 2 hypotheses for the pathogenesis of DHF/DSS have been proposed. The first hypotheses (dengue virus virulence) states that different strains of dengue viruses of all four serotypes possess varying degrees of virulence and vary in their pathogenic potential (2). The second hypothesis states that the severity of shock occuring in this disease is due to an immunopathologic mechanism caused by a second, heterotypic type, dengue infection. Timing of the second infection is also proposed to be critical in the causation of this immunological consequence (3). This later hypothesis is called the Second Infection Hypothesis.

The possibility that immunopathological processes are the factors determining the severity of clinical manifestations is suggested by the finding that most DHF/DSS are secondary, heterologus dengue virus infection (3,4,5). Observation of the anamnestic antibody responses with high concentrations of IgG antibodies in the sera of patients during their acute phase support this hypothesis (6). Because there is evidence that infections

virus and antibodies are present at the same time, antigen-antibody complexes have been postulated to exist in dengue hemorrhagic fever (5,7). Patients with DSS have low levels of complement C_3, C_4 and factor B, suggesting that complement consumption occurs in vivo via both classical and alternative pathways (7,8,9,10). The levels of depression of complement correlate with the severity of the disease and the depletion of complement is not due to extravasation (8). The complement consumption parallels signs which occurs in dengue hemorrhagic fever but not in dengue fever. These signs include an increase of vascular permeability, a decrease in plasma volume, hypotension, thrombocytopenia, and a hemorrhagic diathesis. These symptoms have a rapid onset, and are shortlasting, and thus must be a consequence of rapidly reversible pathophysiologic processes. Histopathological vascular lesions were not found which suggested that the role of active mediators is important for the generation of increased vascular permeability (10,11). These circumstances lead to a hypothesis that an activation of the complement system yielding the formation of anaphylatoxins, C3a and C5a enhance the shock syndrome (8). Evidence of disseminated intravascular coagulation (DIC); low platelet counts, reduced fibrinogen levels, presence of fibrin degradation products (FDP) and reduced Hagemann factor, have been observed (8). These phenomena appear in the milder forms and are increasingly present in the more severe forms, suggesting their primary role in the shock syndrome. The changes in both complement and clotting systems are well correlated with the severity in cases of DHF/DSS (8). Since complement could initiate blood coagulation through platelets, observed DIC may be a consequence of intravascular complement activations. Plasma enzyme inhibitors may be depleted due to the activation of proteolytic enzymes from the complement, coagulation, and possibly the kinin system. An imbalance between activated enzymes and inhibitors may produce an increase of vascular permeability and lead to the development of a shock state.

The role of the immune response (at sub-neutralizing level) in enhancing dengue virus infection is suggested to be a major determinant of the development of hemorrhage and shock (12). With In Vitro experiments, antibodies act through the Fc-mediated mechanism to enhance infection and virus yield from human monocyte culture. Experiment in animal, passive antibodies at sutneutralizing level causes higher levels of viremia in rhesus monkeys. Shock in infants is postulated to be due to maternal antibodies in a similar manner. Eventhough there are data that dengue viruses can cause primary hemorrhagic fever, there is more evidence supporting the importance of secondary antibody-mediated enhancement. These antibodies come from infection with other dengue serotypes or from passively transferred antibodies. e.g. maternal antibodies. Thereby a host-virus interaction dependent on the humoral immune response seems to be very much tied to the severity of this disease.

2. Overall aims and specific objectives.

To investigate our general hypothesis that more severe forms of dengue infection have an immunopathogenesis, we propose to:

A. Completely describe the pattern of antigen reactivity of acute and convalescent sera from patients with all clinical degrees of dengue infection, i.e. asymptomatic (subclinical), dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Additionally, the humoral response to attenuated dengue virus infection (vaccine volunteers) will be similarly described.

B. Completely describe, using collections of serially collected sera or plasma from patients with well-characterized clinical dengue syndromes, the type, the quantity and kinetics of circulating dengue antigens.

C. On the basis of these data, identify a viral antigen or anti-viral peptide antibody whose presence or absence is highly associated with the occurrence of severe symptoms.

D. Purify dengue viral polypeptide (s) using preparative gel electrophoresis and/or Fast protein liquid chromatography (FPLC) system and further reduce the peptide chain to fragments by either enzymes or chemicals.

E. Determine biological properties of the purified proteins, i.e. immunogenic property, protective inducing , and complement activation ability.

F. Develop a rapid, simple but sensitive laboratory test based on the conclusion above (#3) to prospectively select from the entire set of febrile children those likely to progress to a severe form of dengue infection. Such a test, in all likelihood, will require the preparative purification of a biologically active dengue peptide or peptide fragment, for use either as a test antigen or to produce, in animals, a diagnostic antibody reagent.

3. Relevance to development

The relevance of this research could be viewed as follows :

1. Study of the immunopathogenesis of dengue hemorrhaic fever is avery potent mechanism for scientific and technology transfer in the fields of immunology, immunochemistry, protein chemistry and molecular biology of viruses among others. These technologies will aggressively updated and utilized for research. Training and development of manpower in these various fields at all levels will be central to the activities outlined in the proposal. The scientific information should be used in the molecular understanding of the immune response to dengue infection.

2. Dengue hemorrhagic fever is am important disease of South East Asia. But a recent outbreak of this disease in Cuba in 1981 which represented a dengue 2 virus epidemic following dengue 1 outbreak in 1977. resulted in a heightened awareness of the possible spread of this disease to areas where both the vector Aedes aegypti and more than one type of dengue virus are present. Such areas include most countries in the Caribbean area, some countries in South America such as Brazil and countries in South Asia such as Sri Lanka. The information generated from this study will contribute significantly to the better understanding of the molecular immune response to dengue infection and also could be utilized for rapid diagnosis and management of patients. These efforts can be viewed as contributing to development as well as moving the frontier of knowledge about dengue far forward. The latter is obviously of global significance.

4. Innovative aspects

Three innovative aspects of this research proposal are envisaged. The first aims to clarify basic question on the human immune response to different dengue polypeptides. We want to see whether there is a basic difference in the humoral immune response of human in an endemic area who respond to dengue viral infection only with seroconversion, with milder symptomatic dengue infection or with hemorrhagic fever, mild and severe, Earlier classic epidemiologic studies support the notion that humoral immune differences may underlie different dengue disease syndromes but traditional serologic techniques have not confirmed this hypothesis. Western blotting and radioimmunoprecipitation are newer, more powerful methods, not yet applied to this question.

The description of the humoral immune response at the molecular level may identify "phlogistic" polypeptides which are crucial in the mechanism of disease i.e. related to mediators released or complement activation.

The second innovative aspect is the concept of early dianosis of severe disease through screening of a large number of febrile children during the rainy season in dengue endemic areas. Those children who have circulating dengue polypeptides could then be singled out for special observation because that they are potential shock victims. Those affected observation because that they are potential shock victims. Those affected by other infectious agents or non-dengue could be released, freeing physicians or health personal from the task of caring for an impossible number of febrile children. The children with cirulating dengue polypeptides could then be further monitored to detect certain dengue antibodies which if present or absent could be a warning signs of an impending serious manifestation. Alternatively, the children could be monitored for complement cleavage products which would indicate complement activation and warning signs for

increased permeability of the vascular system. This would put treatment of DHF on a sound scientific basis hopefully improving the existing clinical pathophysiologic approach.

The third innovative aspect is to use these new approaches to study the immune response in volunteers immunized with live attenuated viruses. Dengue vaccines are currently being developed in Dr. Natth Bhamarapravati's laboratory. The candidate dengue-2 vaccine 16681 PDK. 53, appears very encouraging after initial tests of reactogenecity and immuno-genicity in human volunteers. But short of huge field studies, the safety of these vaccines can not be ascertained. This study should complement studies in other laboratories aiming toward the definition of molecular mechanisms of attenuation. Such information would reduce the element of risk in testing new dengue vaccine, a procedure which can be done only in humans. Additonally, this study may provide a clue to the identity of a polypeptide or peptide fragments which could elicit a protective immune response without activating the complement system or causing antibody dependent enhancement of infection and thus serve as an alternate subunit vaccine.

5. Technical plan

Materials and Methods

A. Preparation of dengue viral antigen

A.1. Conventional systems for dengue viral antigen preparation

A.1.a. infected suckling mouse brain suspension

A.1.b. infected cell cultures such as infected mosquito cell (C6/36) or Rhesus monkey kidney cell (LLC-MK2)

A.2. New approaches

A.2.a. preparation of dengue viral polypeptides and peptide fragments.

A.2.a.1. Preparative gel electrophoresis to isolate viral proteins.

A.2.a.2. Western Blot.

A.2.a.3. Proteolytic digestion and chemical cleavage to identify immunogenic peptides from interesting proteins e.g. NV3 etc.

B. Characterization of prepared dengue antigen

B.1. by polyacrylamide gel electrophoresis

B.2. Viral antigen strips/Enzymeimmuno assay (VAS/EIA)

B.3. by Crossed immunoelectrophoresis

C. Preparation of anti-dengue sera

C.1. antibody raised in rabbits using different conventional sources for antigen preparation as mentioned in A.1. as immunogens

C.2. trial using each dengue viral polypeptide or its fragments from A.2 as an immunogen

C.3. human hyperimmune sera collected from dengue hemorrhagic fever (DHF) patients during acute and convalescent phases

C.4. monoclonal antibody (Mab)

D. Characterization of prepared anti-dengue sera

D.1. by Viral antigen strips/Enzymeimmunoassay (VAS/EIA)

D.2. by Crossed immunoelectrophoresis

E. Collection and Confirmation of DHF and NON-DHF cases by available diagnostic tests

E.1. Hemaglutination inhibition test

E.2. Viral isolation

E.3. Crossed immunoelectrophoresis

E.4. VAS/EIA test

F. Detection for circulating dengue viral antigen (S), antibodies and complement split products

F.1. patients

- Classical dengue fever patients
- DHF/DSS patients
- patients of other infectious diseases

F.2. healthy individual

- adults (series of tests if possible)
- children (in series if possible)
- cord blood for a maternal antibody survey
- Vaccine trial volunteers.

G. Determine biological interaction of polypeptides and polypeptide fragments

G.1. immunogenic property and protective ability

G.1.a. Immunize rabbit to elecit antibody

G.1.b. In Vivo test for protective ability using simple mouse model or rabbit model.

G.1.c. In Vitro test using monocyte culture with immunocytochemistry test to determine rate of "infectivity by counting infected cells"

G.2. Complement activation capability

G.2.a. In Vitro test using crossed immunoelectrophoresis test for determining complement split products.

H. Data collection and analysis

6. Work completed and in progress

During the past three and a half years, a lot of works have been done according to the plan. Part of the results were summarized and published. Certain portion of work has to be ceased dued to the limitation of the technique. Results obtained were still under progress to be analysed with the hope that they may be published in the near future. According to the sequence of work plan in our original proposal, they can be reported as following :

A.1. Conventional systems for dengue viral antigen preparation

A.1.a Infected suckling mouse brain suspension

Preparation of the dengue viral antigen was done for all dengue serotypes, Japanese encephalitis, and chikungunya without any problem. They were kept in infected suckling mice at -70 C until used. Part of the antigen from each viruses were extracted with sucrose acetone method (13) and were titrated by hemagglutination (HA) test (13).

A.1.b. Infected cell cultures such as infected mosquito cell (C6/36) or rhesus monkey kidney cell (LLC/MK2).

C6/36 : Infected C6/36 cell culture was done at the Center for Vaccine Development, Salaya Campus, Mahidol University. The infected cells demonstrated high density of viral antigens as seen by immunocytochemistry test which is the peroxidase-anti-peroxidase (PAP) staining (Fig 1). High titer of plaque forming units was also noticed. However when this infected cell suspension was employed with the western blot/enzymeimmunoassay using non-infected cells as negative control, we observed the few stained bands indicated certain antibody activity (Fig 2)

LLC/MK2 : Infected LLC/MK2 cells also demonstrated high intensity and density of the viral antigen by PAP staining (Fig 3).

Comparison among these sources of dengue viral antigen by western blot/enzymeimmunoassay indicated that the antigen derived from the infected

suckling mice gave a good and reproducible results (Fig 2). Therefore, this type of antigen was used in the western blot/enzymeimmunoassay through out this experiment. C6/36 and LLC-MK2 cells were still employed to isolate the circulating viruses from patients' sera. The viral isolation was done at the Center for Vaccine Development, under the supervision of Dr. Sutee Yoksan, Co-investigator.

A.2. New approaches

A.2.a. Preparation of dengue viral polypeptides and peptide fragments

a.1. Preparative gel electrophoresis

The preparative gel electrophoresis method was used. The verticle column was purchased from BRL. We are still trying to separate dengue viral antigen by this method. Three years ago, it was difficult to follow the position of the proteins while they are traveling in the electrophoretic field. Lately, the new protein markers are commercially available e.g. rainbow markers (Amersham) and prestained markers (Sigma). These markers were pre-stained and they are visible while moving in the gel allowing an easily allocation of the known molecular weight proteins. We have already ordered the markers and will try to use them for this experiment as well as replacing them in other experiment.

a.2. Western blot

This experiment was designed to used the protein attached on the surface of nitrocellulose (NC) membrane as an immunogen. The NC strip were cut and was dissolved in (0.5 ml) dimethylsulfoxide (DMSO) emulsified with an equal volume of Freund's adjuvant. After testing, only the unwanted antisera to the mouse brain were obtained. The antibody activities disappeared after absorption with the normal mouse brain. The problem may also due to the difficulty to locate exact possition of the wanted proteins and may be

improved by using the prestained molecular weight proteins markers. However, the only low titers of antibody was observed in this experiment, which discouraged us to use the protein-on-nitrocellulose membrane as the source of an immunogen.

a.3. Proteolytic digestion and chemical clevage

Since the previous experiment to purify each individual polypeptide did not work out as planned, this part of the experiment was ceased. However, in order to obtain the interesting protein, we are now using genetic engineering technology. Since the RNA sequences of dengue viruses are now available, cloning the recombinant vectors is now being done. Protein products from cell culture can be obtained. This experiment is now going on in our laboratory.

B. Characterization of prepared dengue antigen

B.1. By polyacrylamide gel electrophoresis (PAGE)

PAGE has been used as a tool to analyse all batches of prepared antigen. This technique was modified by deleting the reducing agent, 2-mercaptoethanol, from the protocol when the analysing of the viral envelope protein and other proteins besides the non-structual protein-NS1. When 2 - mercaptoethanol was included, the NS1 protein both in dimeric form (Mr of 85,000) and monomeric form (Mr of 45,000) can be detected. This experiment included an alteration of the volume of dengue antigen, and other chemicals such as the solubilizing agent (sodium dedecyl sulfate) and the reducing agent (2-mercaptoethanal) in order to preserve dengue proteins. After reaching optimum condition, the proteins were transfered from polycrylamide gel by the western blot method.

B.2. Viral Antigen Strips/Enzymeimmunoassay

The western blot following with immunoenzymatic process were applied for characterization of the separated proteins from PAGE method. This part of

the work was reported in Southeast Asian Journal of Tropical Medicine and Public Health (14), and copy of the manuscript is in the appendix section , p34-45. Shortly electrophoretic period and voltage to transfer the separated proteins from PAGE were varied to obtain maximum protein transferration. Immunoenzymatic assay was done using human hyperimmune sera, monoclonal antibody, and rabbit anti-dengue antisera as the primary antibody. The second antibody to locate the specifically bound primary antibody IgG to dengue proteins were labelled with peroxidase enzyme. The visible bands were estimated for their molecular weights by comparing with the molecular weight markers and were classified according to the nomenclature proposed by Rice et al, 1985 (15). Sensitivity and reproducibility of the methods were evaluated. Dengue viral antigen strip was standardized and was used to detect and characterize dengue antisera thereafter. At least 13 visible bands can be observed and seroconversion can also be observed in patients' sera collected during acute and convalencent phase of illness.

B.3. By Crossed immunoelectrophoresis

Crossed immunoelectrophoresis was done as described by Weeke (16). First dimension electrophoresis was performed using 10x10 cm glass slides with 15 ml of .8% agarose (Litex, Denmark) in 0.01 M EDTA-tris buffer, pH 8.6. Big wells, 1 cm in diameter, were punched at the left handside approximately 2 cm from left edge. 6-8 wells in the row can be located and dengue viral antigens, 250 ul, were used to fill the wells. Then they were electrophoresed by 10 V/cm at 4 °C for 60 min.

Second dimension was run perpendicularly in antibody-containing agarose. The antibody used was either the human hyperimmune sera or rabbit dengue antisera. After overnight electrophoresed using 2 V/cm at 4 °C, precipitin curve of dengue antigen and antibody complexes can be seen

(Fig 4a). Since dengue 'purified polypeptides are not available during the studied period these precipitin curves were still not characterized. Future planned has been made to elute each precipitin complex from the gel and identify by PAGE. Hoping that some of these protein precipitates may be useful as a starting reagent or immunogen in establishing the protocal for the screening test.

C. Preparation of anit-dengue sera

C.1. Antibody raised in rabbits

C.1.a. Using conventinal sources of antigens as an immunogen

Polyclonal dengue antisera were raised in rabbits as planned. Our first generation rabbit antisera had antibody activities toward all dengue serotypes and also to JE viral antigen. This cross-reaction are commonly observed especially in human infection. Strong antibody activities were found against E, NS3, NS5, and NS1.

In an attempt to prepare polyclonal antibody specific to each dengue serotype, the first generation antisera were absorped with other dengue serotype eg antisera to dengue type 2 was absorped with sucross acetone estracted dengue type 1 antigen. After complete absorption, polyclonal antisera specific to dengue 1, 2, 4, and JE can be obtained. The last 2 proteins found with the western blot/enzymeimmunoassay were the doublet proteins at Mr of 67 and 71 which are the NS3-nonstructural protein. This protein, NS3, was postulated to be involved in RNA replication with an enzymatic role (replicase enzyme) (15). This section of work was presented at the International Symposium on Dengue and Dengue Hemorrhagic Fever (17) and reported in Southeast Asian J. Tropical Medicine and Public Health, Dec 1990 (18). The copy of this manuscript is in the appendix section of this report (p89-97).

C.1.b. Trial using each dengue viral polypeptide or its fragments as an

immunogen.

Purification for each polypeptide can not be reached by the proposed method, therefore this part of this experiment was done differently using other method to obtained the antibody specifically bound to individual polypeptide . As mentioned in previous section, the specific antibody to NS3 of three different dengue serotypes; dengue 1, 2, and 4, are available for further used (18).

C.2. Human hyperimmune sera collected from DHF/DSS patients during acute and convalescent phases.

C.2.a. Sera will be tested by hemagglutination inhibition (HI), crossed immunoelectrophoresis, and western blot

- Hemagglution Inhibition test (HI test)

HI titers to dengue type 1-4, JE, and CHIK were titrated in all specimens including patients' sera, rabbit antisera, sera from cord blood, children infected with other disease, healthy adult sera and vaccinee, The conventional method was employed. During the first 6 months, the test was done at AFRIMS and later the test was done at our laboratory by our trained technicians.

- Western blot.

Viral antigen strip containing dengue type 1 to type 4 and normal mouse brain as negative control was used to characterize antibody to both structural proteins and non-structaral proteins. With this method, antibody in patients sera were characterized for both primary and secondary dengue infected cases. The antibodies in rabbit sera to certain proteins can also be traced after immunization.

C.3. Monoclonal antibody against peptide fragments

The monoclonal antibody to E protein specific to each type of

dengue viruses were produced in our labolatory using the purchased hybridoma cells from ATCC. These hybridomas to each dengue type are as following : dengue type 1 (15 F3-1), dengue type 2 (3H5-1), dengue type 3 (5 H4-11), and dengue type 4 (1 H10-6). These cells were passaged to obtain the healthy clones before injected intraperitoneally to the pristane-primed BALB/C mice. Monoclonal antibody rich ascites fluid were collected and the antibody activity was traced by the western blot/enzymeimmunoassay using the tested monoclonal antibody as the primary antibody. This antibody was used to confirm the E protein position in the Western blot strip.

D. Characterized of prepared anti-dengue sera

D.1. by western blot method follow by enzymeimmunoassy.

The rabbit and mouse antisera were characterized as planned. The polyclonal dengue type specific sera were characterized and reported (14,18)

D.2. by Crossed Immunoelectrophoresis

Crossed immunoelectrophoresis was applied to titrate and characterize antibodies using tested sera containing intermediate gel. Results obtained can indicate titer of antibodies as shown in Fig 4b. Since the results containing from Western blot yielded more informations, this experiment was deleted.

E. Collection and Confirmation of DHF and Non-DHF cases.

E.1. Hemagglutination Inhibition test (HI test)

HI tests for every specimens were done by our laboratory, Immunochemistry unit. Results from July 1987 to November 1990 were in "data" in appendix section (p46-88). There were 150 cases in 1987, 29 cases in 1989, 44 cases in 1989, and 40 cases in 1990. All cases were admitted to Ramathibodi Hospital.

E.2. Viral isolation

Viral isolation was done at the Center for Dengue Vaccine

Development, Mahidol University. Viruses can be isolated from sera collected during acute phase of illness as following: 20 cases in 1990 (Appendix, data, p42-86). Actually, the number of cases with isolated virus were more than those mentioned. Unfortunately, they were the cases with only one specimen collected and were excluded from the studied list. Most of them had mild clinical symptoms and the sera were mainly collected from out-patient department. In an attempt to isolate virus at our unit, we have used the suckling mice together with the western blot for detection and found that it is helpful. This paper was presented at Dengue Symposium and published in 1990 (19). The copy of the manuscript is included in appendix (P98-104).

E.3. Crossed immunoelectrophoresis

Crossed immunoelectrophoresis was initially applied to observed dengue antibodies but the western blot can give a better results, therefore only the priliminary work has been done and the result was demonstrated in Fig 6. Using the same volume of sera from the same patient, but collected from acute and convalencent phase of illness, higher titer of anitbody can be observed. Sera with no titer of antibody to dengue can't capture dengue antigen, and the dengue antigen-antibody complexes appeared in the upper gel only. Sera with low titer can bind to some dengue antigen and the precipitin complexes appeared lower between the upper and the intermediate gel. With high titer, the precipitin complexes occurred in the intermediate gel, Since soft detergent, 1% Triton X-100, was included with the dengue antigen, many curves can be demonstrated indicating mobility of different solubilized proteins. This method offered a comparison between 2 different antibody titers and may be helpful for preparation of the precipititin complexes to be used as an immunogen. The preparation of the polyclonal antibody to viral proteins with different rate of mobility is possible using these complexes.

E.4. Western blot and enzymeimmunoassay

Western blot enzymeimmunoassay was used to characterized antibody in number of cases of P-DHF and S-DHF as planned. The preliminary work was reported at the workshop to celebrate Chiangmai University 25th Anniversary named AID/SCI Funded Research in Immunology in Thailand, December 4-6, 1989, organized by Chiangmai University, BOSTID, and US Agency for International Development (USAID) and the copy of the manuscript is included in Appendix secton, P98-104 (20).

F. Detection of circulating dengue viral antigen (s), antibodies, and complement split products.

F.1.a. Patients to be studied

Patients sera were collected mainly from Ramathibodi Hospital with the collaboration with Dr. Sayomporn Sirinavin who became our co-investigator. The number of specimens collected during each year were as following :

1. 1987 ; Total number of specimens were 509. There were 409 specimens from 150 studied cases, and 100 specimens were the lost cases (single specimens)
2. 1988 ; Total number of specimens were 121. There were 82 specimens from 29 studied cases, and 39 single specimens.
3. 1989 ; Total number of specimens were 175. There were 123 specimens from 35 cases, and 52 single specimens.
4. 1990 ; Total number of specimens were 180. There were 112 specimens from 40 cases, and 68 single specimens.

The studied cases were tested for HI titers, isotype capture ELISA tests for IgM and IgG anti-dengue antibodies, Viral isolation, Western blot/enzymeimmunoassay, and complement studies. Part fo the results were shown in"data" part in Appendix, P42-86.

Patients with other disease were also included in our studied and they were also included in our studies and they were used as negative control or for comparison.

F.1.b. healthy individual

Healthy adult sera were collected from the Blood Bank unit, Ramathibody Hospital. The whole blood samples: 30 individual, men and women, were studied just like they were the patients sera. Dengue antibody by HI titers and Western blot demonstrated anti-dengue activities. At least 3 type of antibodies to dengue proteins were found. (Table 1.)

Children sera were collected from children under 10 years old whom were not ill because of viral infection. These sera were collected from the Clinical Chemistry unit, Ramathibodi Hospital. From 30 children, 23 children were found positive with western blot while 16 children had positive HI antibody titers. (Table 2).

Cord blood sera from 30 new born infants were collected from the Department of Obstetrics and Gynaecology, Faculty of Medicine, Ramathibodi Hospital and 96.67 or 29 out of 30 samples were positive with the western blot and HI tests (Table 3).

Vaccine trial volunteers

Only 5 volunteeers sera were examined and the results were summarized in Table 4. Low titer of antibodies to certain dengue proteins were found on the day they received the vaccine indicating that they already exposed to certain Flavivirus which the antibodies cross react with dengue antigen. The results were in Table 4.

F.2. Methods

The antibody analysis for primary and secondary dengue infected patients was completed and the manuscript to cover this work was submitted to

the American Journal of Tropical Medicine and Hygiene since Aug 19, 1990. No comments was given from the Journal until now. The copy of submitted manuscript is included in appendix section, P110-134.

To detect complement split products, C3c and C3d can be detected. The appearance of both proteins in circulation are much longer than C3a or C5a (Fig 5a). Verification for the presence of C3d was done by crossed immunoeletrophoresis with intermediate gel. While C3 and C3c were bound with anti-C3/C3c which was incorporated in intermediate agarose gel, the C3d pass through the part of gel and form a specific precipitin curve of C3d-anti C3d complexes as shown in Fig 5b. The presence of C3d is now under investigation and our preliminary results indicated a possibility to detect C3d protein as a parameter of severity of illness. This study is still going on with the hope to develope a simple screening test for this complement activation product.

G. Determination for the biological role of polypeptides and polypeptide fragments will be done as soon as the purified polypeptides are available.

H. Data collection and analysis

Certain portion of our data is enclosed herewith in an appendix section (P46-88). Analysis of these data is still being done. When this analysis is completed, we may be able to present more reports.

Future Plan

During the past three and a half years, we collected a lot of specimens. They were stored with the proper storage boxes (-85 C). Many tests were done to detect for the presence of antibodies to polypeptides, the presence of viruses in patients' blood circulation, and the presence of complement products. The continuation of this study will be done and it can be done rapidly and efficiently if the supporting fund is available. During 1990, we also managed to send 2 CO-investigators; Dr. Rungtiva Kongkananda, DVM, M.Sc., and Ms. Kanchana Sirisidhi, B.Sc., to be trained at Tokyo Metropolitan Research Institute for Neuroscience. Ms. Kanchana can handle the gene detection system using PCR to amplify dengue viral gene and using digoxigenine labeled probe to verified the amplified nucleic acid. With the DNA synthesizer, we are able to synthesize dengue primers for every serotypes and also synthesize the primers for other laboratories to detect abnormal DNA or DNA of other infectious agents. Dr. Rungtiva is involving with the epitope studies by preparation of monoclonal antibodies to the proteins obtained from genetic engineering.

We have sent a new preproposal to PSCT program for the development and application of a rapid, accurate and sensitive test for dengue viral infection. The aims are for the development of screening test and the sensitive confirmatory test to detect dengue virus in various type of specimens. This preproposal is attached here with in an appendix, P135-137.

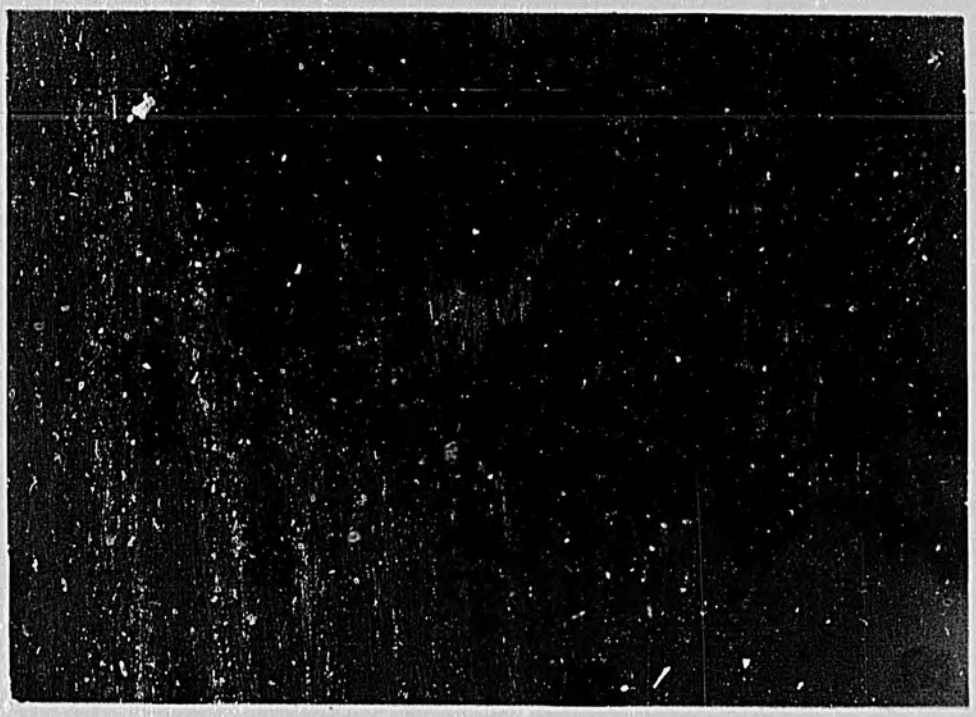


Fig 1. C6/36 cells infected with dengue type2 demonstrated viral antigens in cytoplasm. At least 2 types of stains can be seen, the big red granules and homogenous red stain in cytoplasm. The negative, non-infected cells, showed blue cytoplasm.

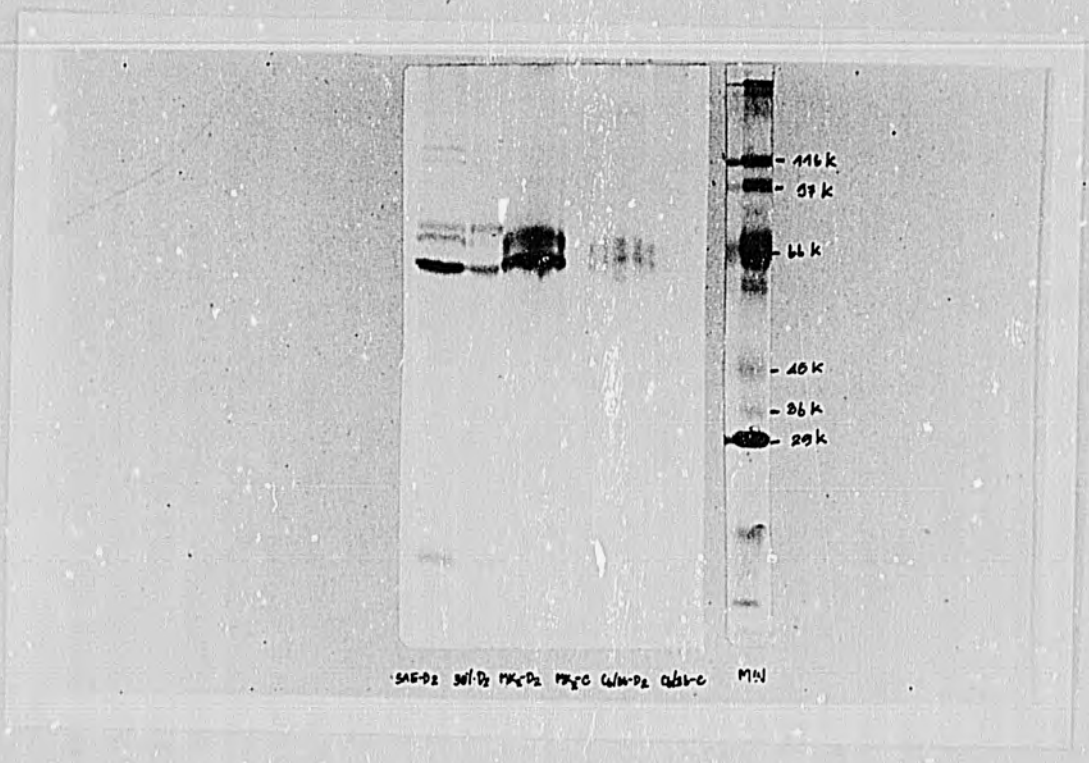


Fig 2. The comparison among different sources of dengue antigens. From left, lane 1 contains dengue type 2 sucrose acetone extracted antigen from infected suckling mouse brain showing protein bands at Mr of 92, 71, 67, 59, 55, 50, 45, and 16 kDa. Lane 2 contains 50% suspension of dengue type 2 infected mouse brain antigen showing fewer bands. Lane 3 contains dengue type2 infected LLC-MK2 cells showing protein bands at Mr of 67 and 59 kDa. Lane 4 contains non-infected LLC-Mk2 cells. Lane 5 contains dengue type 2 infected C6/36 cells showing 2 bands at Mr of 67 and 59 kDa. Lane 6 contains non-infected C6/36 cells.

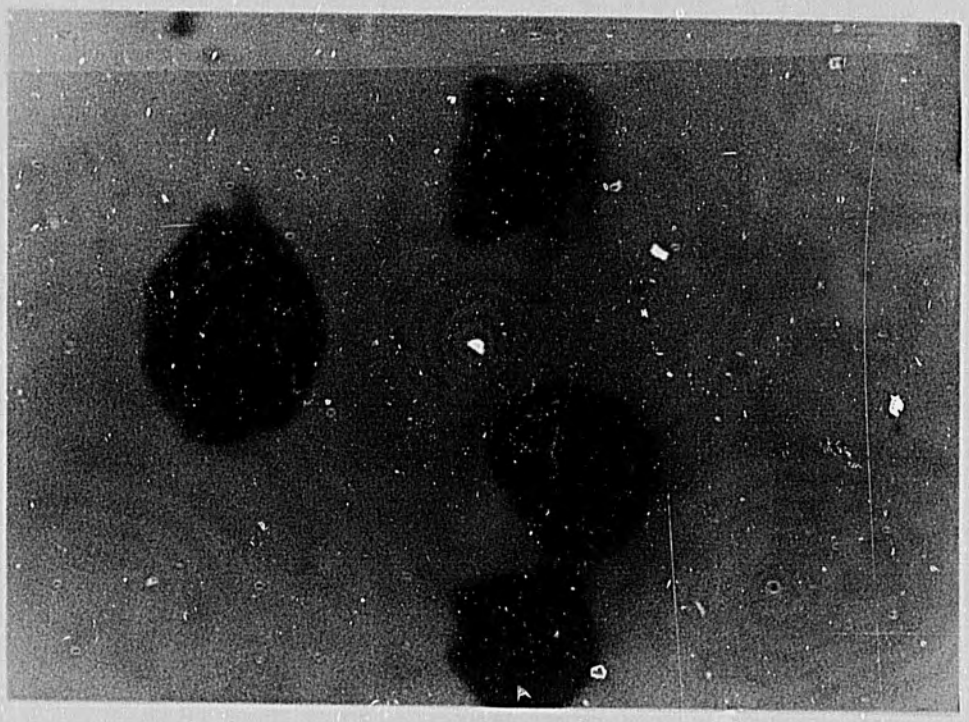
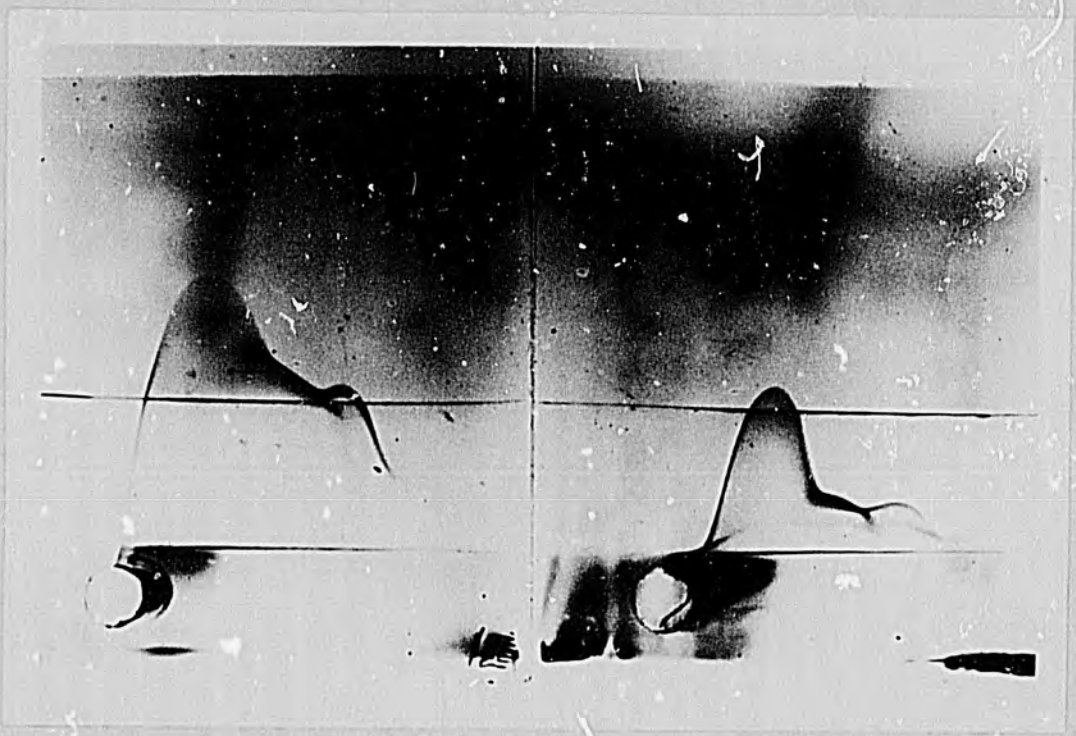


Fig 3. Dengue type 2 infected LLC-MK2 cells showing positive stain with strong red color in cytoplasm.

Fig 4 a. Crossed immunoelectropheresis of dengue type 2 and rabbit anit-dengue type 2. At least 5 curves can be seen.



Fing 4 b. Crossed immunoelectrophoresis with intermediate gels. On left side, intermediate gel contained sera collected during acute phase (Low antibody titer), while on the right side, the gel contained the convalescent sera with high anti-dengue titer. The precipitin complexes appeared in intermediate gel when the high antibody titers was tested.

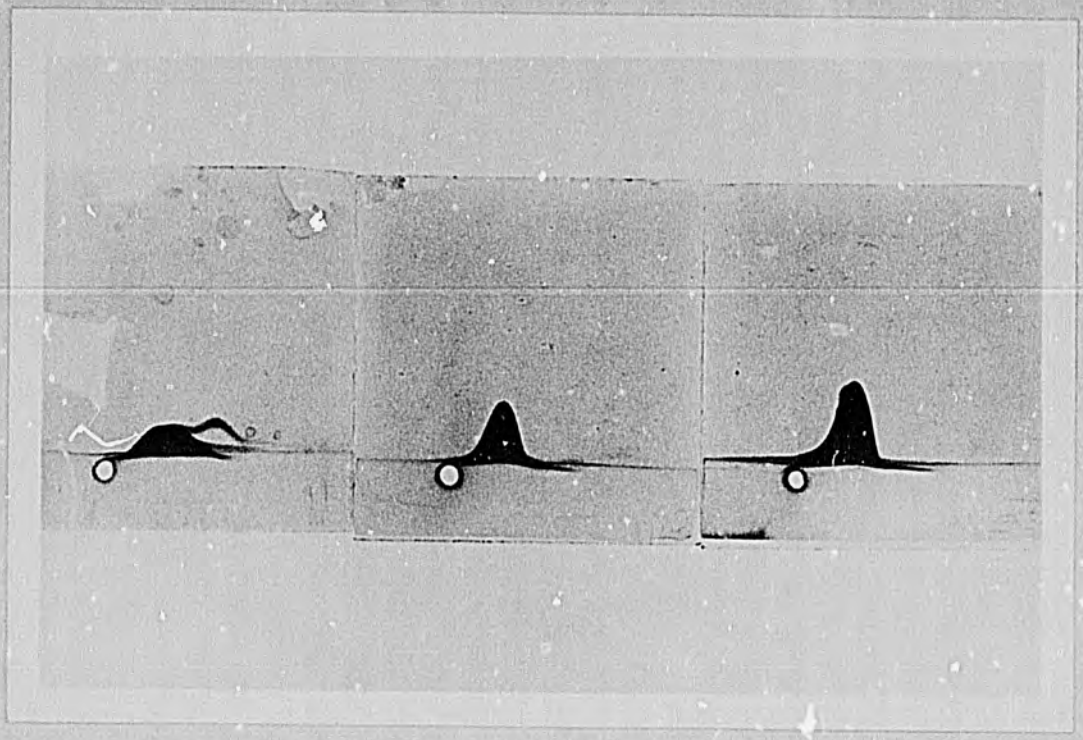


Fig 5 a. Detection of C3c and C3d in series of EDTA-plasma collected during acute (A), and convalescent phase (B,C) of one patient. C3c and C3d can be detected during acute phase.

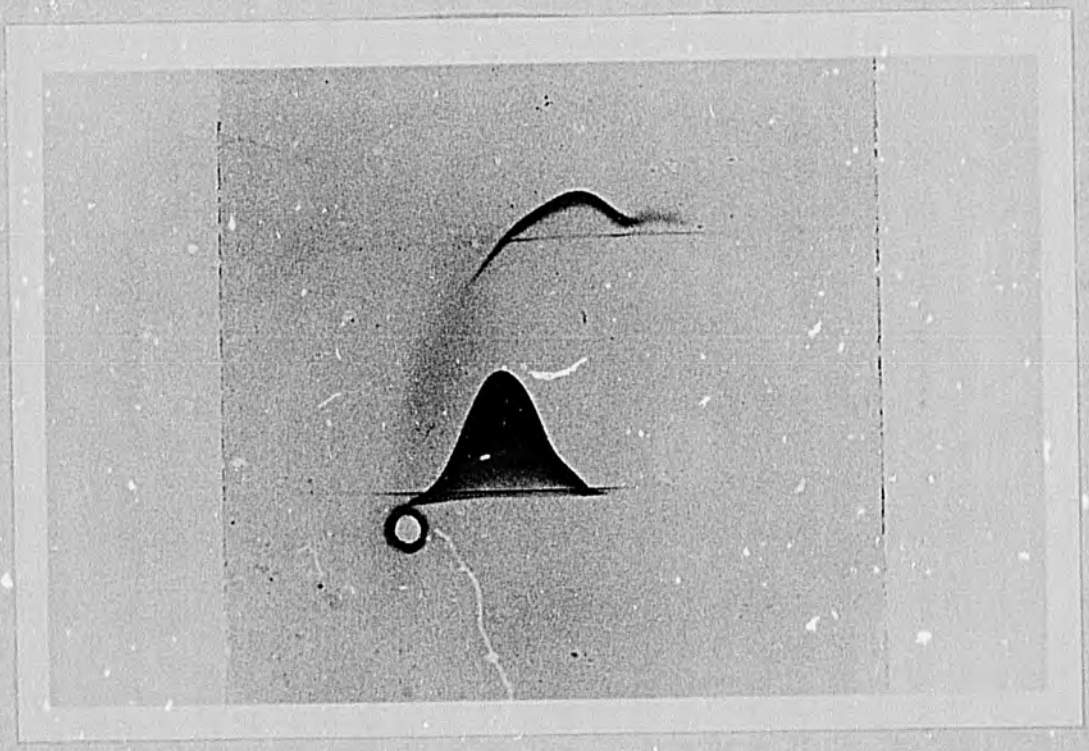


Fig 5 b. Crossed immunoelectrophoreses gel with intermediate gel showing C3d -anti C3d precipitin complex on the upper gel when C3-anti C3 complexes was in the intermediate gel containing native C3 antisera.

Table 1. Antibodies to Dengue Proteins in healthy adult Sera.

Serum No.	Intensity Score for Antibodies to Dengue Protein									H1 titers					
	NS 5	NS3 71	NS3 67	E 59	E 55	E 50	NS1	P24	P20	C	D1	D2	D3	D4	Chik	JE
1	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
2	3(d1)	1(d2,4)	1(d2,4)	3(d1,2)	1(d1,2)	1(d1)	-	-	-	1(d2)	1:20	1:20	1:40	1:20	<1:20	1:20
3	2(d1)	1(d2)	1(d2)	2(d1,2)	-	-	-	-	-	1(d2)	1:20	1:20	1:40	1:20	<1:20	1:20
4	1(d1)	2(d2)	2(d2)	2(d1,2,3)	1(d1)	1(d1)	-	-	-	-	1:40	1:20	1:40	1:20	<1:20	1:20
5	1(d1)	1(d2)	1(d2)	2(d2)	1(d2)	-	-	-	-	-	<1:20	1:20	1:80	1:20	<1:20	1:20
6	1(d1,3)	1(d2,3,4)	1(d2,3,4)	2(d1,2,3)	-	1(d1)	-	-	-	-	1:20	1:20	1:80	<1:20	1:20	<1:20
7	1(d1)	2(d2)	2(d2)	2(d2)	1(d2)	-	-	-	-	-	1:40	1:20	1:80	1:20	<1:20	1:20
8	1(d1)	1(d2,4)	1(d2,4)	2(d1,2)	1(d2)	-	-	-	-	1(d2)	1:40	1:20	1:80	1:20	<1:20	1:20
9	3(d1)	1(d2)	1(d2)	3(d1,2,3)	1(d1,2)	1(d1)	-	-	-	1(d2)	1:40	1:20	1:80	1:40	<1:20	1:40
10	2(d1)	1(d2,4)	1(d2,4)	2(d1,2,3)	-	1(d1)	-	-	-	-	1:40	1:40	1:80	1:20	<1:20	1:20
11	1(d1,2,3)	1(d2,3,4)	1(d2,3,4)	2(all)	-	-	-	-	-	1(d1,2)	<1:20	1:40	1:80	1:80	1:40	1:40
12	3(d1)	3(d2)	3(d2)	4(d1,2)	1(d1)	1(d2)	-	-	-	2(d2)	1:80	1:40	1:80	1:40	<1:20	1:80
13	1(d1)	1(d2)	1(d2)	3(all)	-	1(d1,2)	-	-	-	-	1:80	1:40	1:80	1:40	1:40	1:40
14	1(d2)	1(d2)	1(d2)	3(d2)	1(d1)	1(d1)	-	-	-	2(d2)	1:20	1:40	1:160	1:40	1:40	1:20
15	1(d1)	1(d4)	1(d4)	4(d2)	1(d2,4)	-	-	-	-	-	1:40	1:20	1:160	1:40	1:80	1:40
16	2(d1)	1(d2)	2(d2,3)	3(d1,2,3)	1(d2)	1(d1,2)	-	-	-	-	1:40	1:40	1:160	1:40	1:40	1:40
17	3(d1,2)	3(d2)	3(d2)	3(d2,3)	1(d2)	1(d1,2,3)	2(d2,3,4)	-	1(d2)	3(d2)	1:40	1:40	1:160	1:40	1:20	1:40
18	2(d1,2,3)	3(d2)	3(d2)	3(d1,2,3)	1(d2)	1(d1,2,3)	1(d2)	-	1(d2)	1(d2)	1:40	1:40	1:160	1:40	<1:20	1:40
19	3(d1)	2(d2,3,4)	2(d2,3,4)	3(d1,2)	1(d1,2,3)	1(d1,2,3)	-	-	-	-	1:40	1:40	1:160	1:40	<1:20	1:80
20	3(d1)	2(d2)	2(d2)	3(d1,2)	1(d2)	1(d1)	-	-	-	1(d2)	1:40	1:80	1:160	1:80	<1:20	1:320
21	2(d1)	-	-	3(d1,2)	1(d1,2)	-	1(d1,2)	-	-	1(d2)	1:80	1:40	1:80	1:160	1:40	1:80
22	2(d1,3)	1(d2,3)	2(d2,3)	3(d1,2,3)	1(d2)	1(d2)	1(d1)	-	-	-	1:40	1:40	1:320	1:40	<1:20	1:40
23	3(d1)	2(d2,4)	2(d2,4)	3(d2)	1(d1,4)	1(d1)	-	-	-	-	1:80	1:40	1:320	1:80	1:160	1:80
24	3(d1)	1(d1,2)	1(d1,2)	4(d1,2,3)	1(d2,3)	1(d1,3)	-	-	-	2(d1,2)	1:80	1:40	1:320	1:80	<1:20	1:80
25	2(d1)	1(d2)	2(d2)	3(d1,2,3)	1(d1,2)	1(d1)	-	-	-	-	1:80	1:80	1:320	1:40	<1:20	1:40
26	3(d1)	1(d2)	2(d2)	4(d2)	1(d1,4)	1(d1)	-	-	-	-	1:80	1:80	1:320	1:80	1:80	1:80
27	2(d1)	3(d2)	3(d2)	4(d1,2,3)	1(d2)	1(d1)	1(d2)	-	-	3(d2)	1:160	1:80	1:160	1:160	1:40	1:160
28	3(d1)	3(d2)	3(d2)	4(d1,2)	-	1(d1)	-	-	-	2(d1,2)	1:160	1:80	1:160	1:160	1:40	1:80
29	1(d1)	1(d2,4)	2(d2,4)	3(d2)	1(d2)	-	-	-	-	-	1:160	1:160	1:320	1:80	<1:20	1:160
30	2(d1)	2(d2,3)	2(d2,3)	3(d2)	1(d2)	1(d1,2)	1(all)	-	-	1(all)	1:160	1:160	1:640	1:160	1:160	1:80
Prevaience(%)	96.67	93.33	93.33	96.67	70	66.67	20	0	6.67	50						
Mean intensity score	1.93	1.47	17	2.83	0.77	0.7	0.23	0	0.06	0.77						

b : denotes boy

g : denotes girl

Table 2. Antibodies to Dengue Proteins in children sera.

Serum No.	Sex	Age (year)	Intensity Score for Antibodies to Dengue Protein										HI titers					
			NS5	NS3 71	NS3 67	E 59	E 55	E 50	NS1	P24	P20	C	D1	D2	D3	D4	Chik	JE
1	b	9	-	-	-	2(d2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
2	b	6	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
3	b	3	1(d1,3)	-	-	2(d1,2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
4	b	4	-	-	-	2(d2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
5	b	3.5	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
6	b	4.5	1(d1,3)	-	-	3(d2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
7	b	3	1(d1)	1(d4)	1(d4)	2(d2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
8	b	4	1(d1)	-	-	3(d2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
9	b	0.5	1(d1)	-	1(d2)	2(d2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
10	b	9	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
11	g	1	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
12	g	6	-	-	-	1(d2)	-	1(d1)	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
13	b	2	-	-	-	1(d2)	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
14	b	2	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
15	b	6	1(d1)	1(d2)	1(d2)	2(d2)	1(d2)	-	-	-	-	-	<1:20	<1:20	1:20	<1:20	<1:20	<1:20
16	g	2	1(d1)	1(d2)	1(d2)	2(d2)	-	-	-	-	-	-	<1:20	<1:20	1:20	<1:20	<1:20	<1:20
17	g	3	1(d1)	1(d2)	1(d2)	3(d2)	-	-	-	-	-	-	<1:20	<1:20	1:20	<1:20	<1:20	<1:20
18	b	8	1(d1)	-	-	1(d1,2,3)	-	-	-	-	-	-	<1:20	<1:20	1:20	<1:20	<1:20	<1:20
19	g	6	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	1:40	1:20	<1:20	<1:20
20	g	10	1(d1,3)	1(d2)	1(d2)	3(d2,3)	-	-	-	-	-	-	<1:20	<1:20	1:40	1:20	<1:20	<1:20
21	b	2	-	-	1(d2)	-	-	-	-	-	-	-	1:20	<1:20	1:40	<1:20	<1:20	<1:20
22	b	6	1(d1,2,3)	-	-	1(d1,2)	-	-	-	-	-	-	1:40	<1:20	1:20	<1:20	<1:20	<1:20
23	g	2	1(d1)	1(d1)	1(d2)	2(d1,2,3)	-	-	-	-	-	-	<1:20	1:20	1:40	1:20	<1:20	<1:20
24	b	2	2(d1)	1(d2,4)	1(d2,4)	2(d1,2)	-	1(d1,2)	-	-	-	-	1:20	1:20	1:40	1:20	<1:20	1:20
25	g	10	2(d1)	1(d2,4)	2(d4)	2(all)	1(d1,2)	1(d1,2)	-	-	-	-	1:40	1:20	1:40	1:20	<1:20	1:40
26	b	1	1(d1)	-	-	2(d2)	-	-	-	-	-	-	1:20	1:20	1:80	1:40	<1:20	1:40
27	b	9	2(d1,2,3)	1(d2,3)	1(d2,3)	3(d1,2,3)	1(d2)	-	-	-	-	-	1:40	1:20	1:80	1:40	1:20	1:20
28	g	6	3(d1)	1(d2,3)	1(d2)	3(d2)	1(d1,2)	1(d1,2)	-	-	-	-	1:40	1:40	1:80	1:20	1:640	1:40
29	b	4	3(d1)	1(d2,4)	2(d2,4)	2(d1,2,3)	1(d1,2)	1(d1,2)	-	-	-	1(d1,2,3)	1:80	1:40	1:80	1:40	<1:20	1:40
30	g	4	1(d1,2)	2(d2)	3(d2)	3(d2)	1(d2)	1(d2)	-	-	-	1(d2)	1:80	1:40	1:80	1:40	<1:20	1:40
Prevaience (%)			63.33	40	46.67	73.33	16.67	20	0	0	0	3.33						
Mean intensity sco			0.83	0.43	0.6	1.8	0.2	0.2	0	0	0	0.067						

b : denotes boy

g : denotes girl

Table 3 Antibodies to Dengue Proteins in Cord Blood Sera.

Serum No	Sex	Intensity Score for Antibodies to Dengue Proteins										HI titers					
		NS5	NS3 71	NS3 67	E 59	E 55	E 50	NS1	P24	P20	C	D1	D2	D3	D4	Chik	JE
1	b	-	-	-	-	-	-	-	-	-	-	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
2	b	2(d1)	1(d2,4)	1(d2,4)	3(d1)	1(d2)	1(d2)	-	-	-	-	<1:20	<1:20	1:20	1:20	<1:20	1:40
3	b	1(d1)	1(d1)	-	2(d2)	-	-	-	-	-	-	<1:20	<1:20	1:40	<1:20	<1:20	1:20
4	b	4(d1)	2(d2)	2(d2)	4(d1,2,3)	-	2(d1,2,3)	1(d4)	-	-	1(d2)	1:20	1:20	1:40	1:20	1:20	1:40
5	b	1(d1)	-	-	3(d1,2)	-	-	-	-	-	-	1:40	<1:20	1:40	1:20	<1:20	1:20
6	g	1(d1)	1(d2,4)	1(d2,4)	3(d2)	1(d1)	-	-	-	-	1(d1,2)	1:20	1:20	1:40	1:40	<1:20	1:40
7	g	2(d1)	2(d2,4)	3(d2,4)	3(d2)	1(d1,2)	1(d1)	-	-	-	1(d2)	1:20	1:20	1:40	1:40	1:40	1:20
8	b	2(d1,2)	2(d2,4)	3(d2,4)	3(d1,2)	1(d1)	1(d1)	-	-	-	2(d2)	1:20	1:20	1:40	1:40	<1:20	1:40
9	b	3(d1)	-	-	4(d1,2,3)	1(d2)	1(d1)	-	-	-	-	1:40	1:20	1:80	1:40	<1:20	1:40
10	b	3(d1)	2(d2)	2(d2)	4(d1)	1(d1)	1(d1)	1(d4)	-	-	-	1:40	1:20	1:80	1:40	1:40	1:80
11	g	4(d1)	1(d2,4)	1(d2,4)	4(d1,2)	1(d1,2,3)	1(d1,2,3)	-	-	-	1(d2)	1:40	1:40	1:80	1:40	1:20	1:40
12	g	2(d1,2,3)	2(d2)	2(d2)	3(d1,2,3)	1(d1)	-	-	-	-	1(d2)	1:80	1:40	1:80	1:20	<1:20	1:40
13	b	2(d1)	1(d2)	2(d2)	3(d2)	-	-	-	-	-	-	1:40	1:40	1:80	1:40	<1:20	1:80
14	g	3(d1)	2(d2,4)	2(d2,4)	4(d1,2,3)	-	1(d1)	-	-	-	-	1:40	1:40	1:80	1:80	1:40	1:40
15	g	2(d1)	1(d2,4)	2(d2,4)	4(d2)	1(d1,2,3)	1(d1,2)	-	-	-	2(d2,3,4)	1:40	1:40	1:80	1:80	1:40	1:80
16	b	1(d1,3)	1(d2,4)	2(d2,4)	3(all)	1(d2,4)	1(d1)	-	-	-	1(d1,2)	1:40	1:40	1:80	1:80	1:80	1:40
17	g	2(d1,3)	2(d2)	2(d2)	4(d1,2)	1(d2)	1(d1)	-	-	-	1(d2)	1:80	1:40	1:80	1:40	<1:20	1:40
18	g	3(d1)	2(d2)	3(d2)	3(d1,2)	1(d1)	1(d2)	-	-	-	1(d2)	1:80	1:40	1:80	1:80	1:40	1:80
19	b	1(d1,2,3)	1(d2,4)	1(d2,4)	3(d2)	1(d2)	-	-	-	-	2(d2)	1:80	1:80	1:80	1:40	1:80	1:80
20	g	1(d1)	2(d2)	2(d2)	3(d1,2)	1(d1,2)	1(d1)	-	-	-	-	1:80	1:80	1:80	1:80	<1:20	1:40
21	g	2(d1,2,3)	2(d2,4)	2(d2,4)	3(d1,2,3)	1(d2)	1(d1)	-	-	-	2(d2)	1:80	1:40	1:160	1:40	<1:20	1:40
22	g	2(d1)	2(d2)	2(d2)	3(d1,2,3)	1(d1)	1(d1,2)	-	-	-	-	1:80	1:80	1:160	1:40	<1:20	1:80
23	b	3(d1)	3(d2)	3(d2)	4(d1)	1(d1)	1(d1)	-	-	-	1(d1,2)	1:80	1:80	1:160	1:80	<1:20	1:320
24	g	2(d1)	1(d2,4)	1(d2,4)	2(d1,2)	-	-	-	-	-	1(d2)	1:40	1:40	1:160	1:160	<1:20	1:40
25	g	2(d1,2,3)	2(d2)	3(d2)	3(d1,2,3)	1(d1)	1(d1)	-	-	-	1(d1,2)	1:80	1:80	1:160	1:80	1:80	1:40
26	g	2(d1,2,3)	2(d2,4)	3(d2)	3(d1,2,3)	1(d1)	2(d1)	1(d1,3)	1(d1)	1(d2)	3(d2)	1:160	1:80	1:80	1:160	<1:20	1:40
27	b	2(d1)	2(d2)	2(d2)	4(d2)	1(d1,2)	1(d1)	-	-	-	-	1:160	1:80	1:160	1:80	<1:20	1:40
28	g	3(d1)	1(d2,4)	2(d2,4)	3(all)	-	1(d1)	-	-	-	1(d1,2)	1:160	1:160	1:160	1:320	1:80	1:160
29	b	2(d1)	2(d2)	3(d2)	3(d1,2,3)	1(d1)	1(d1)	1(d1)	-	-	1(d1,2)	1:160	1:160	1:320	1:320	<1:20	1:320
30	g	2(d1)	2(d2)	2(d2,4)	4(d2)	1(d2)	1(d1)	-	-	-	1(d2)	1:320	1:640	1:640	1:160	1:160	1:640
Prevolence		96.67	90	86.67	96.67	66.67	73.33	10	3.33	3.33	63.33						
Mean intensity Score		2.07	1.5	1.8	3.167	0.73	0.8	0.133	0.03	0.03	0.83						

b : denotes boy

g : denotes girl

Table 4 Antibodies to Dengue Proteins in Vaccinee.

Serum No	Day after inoculation	Intensity Score for Antibodies to Dengue Proteins										HI titers				
		NS5	NS3 71	NS3 67	E 59	E 55	E 50	NS1	P24	P20	C	D1	D2	D3	D4	JE
1a7(D1)	0	1(d1)	1(d4)	1(d4)	2(d2,4)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	14	1(all)	1(d4)	1(d4)	2(d2,4)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	30	1(all)	2(d4)	2(d4)	2(d2,4)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
1a8(D1)	0	-	-	-	1(d2)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	14	1(d1)	-	-	2(d2)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	30	4(d1)	1(all)	1(all)	4(d1,2)	2(d1)	-	-	-	-	-	1:80	1:80	1:40	1:40	1:80
1a9(D1)	0	1(d4)	-	-	2(d2)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	14	1(d1)	-	-	2(d1,2)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	30	2(d1)	-	-	3(d2)	1(d2)	-	-	-	-	-	1:40	1:40	1:40	1:40	1:80
1a3(d2)	0	1(d1,2,4)	-	-	2(d1,2)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	14	2(d1)	-	-	2(d1,2)	-	-	-	-	-	-	1:20	1:80	1:40	1:80	1:80
	30	3(d1)	1(all)	2(d2)	3(d1,2)	-	-	-	-	-	-	1:160	1:160	1:320	1:640	1:160
1a6(D2)	0	-	-	-	1(d2)	-	-	-	-	-	-	<1:10	<1:10	<1:10	<1:10	<1:10
	21	4(d1)	-	-	4(d1,2)	2(d4)	2(d1)	-	-	-	-	1:160	1:160	1:320	1:320	1:320
	30	3(d1)	-	-	3(d1,2)	1(all)	1(d1)	-	-	-	-	1:160	1:160	1:160	1:320	1:160
Prevolence (%)		86.67	33.33	33.33	100	26.67	13.33	0	0	0	0					
Mean intensity Score		1.33	0.4	0.47	2.27	0.4	0.2	0	0	0	0					

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ANTIBODIES TO DENGUE VIRAL POLYPEPTIDES I. SENSITIVITY AND SPECIFICITY OF THE VIRAL-ANTIGEN-STRIPS/ENZYMEIMMUNO ASSAY (VAS/EIA)

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INTRODUCTION

Many tests are employed for titration of antibodies to Flaviviruses, and the most frequently employed techniques are the hemagglutination inhibition (HI) test (Clarke and Casal, 1958), the plague reduction neutralization test (PRNT) (Russell *et al.*, 1967) and the antibody capture enzyme-linked immunoabsorbent assay (Burke and Nisalak, 1982). None of these tests can described the antibodies specifically react toward individual dengue viral proteins. Seventeen polypeptides were found during *in vitro* dengue infection using different kinds of target cells (Westaway and Shew, 1977 ; Westaway *et al.*, 1980; Heinz *et al.*, 1984). Three polypeptides are the structural or virion proteins including the E or major envelope glycoprotein, C or capsid protein, and M or membrane-like protein. Envelope glycoprotein (E, or VSP-3, or GP59) has molecular weight (M.W.) of 51,000-60,000 daltons, while the capsid proteins, C or VSP-2, has approximately M.W. of 13,500 daltons and the membrane-like proteins, M or VSP-1, has M.W. of 7,700 daltons. The other 13 proteins were detected in dengue-2 infected Vero cells namely P98, p82, P67, gp54, GP46, p30, p28, gp22, GP20, p18, gp16, p15 and p14. In dengue-2 infected *Aedes albopictus* C6/36 cells, gp54 and GP13 were absent (Smith and Wright, 1985); while in dengue-2 infected BHK21 cells, p130, p83, p55 and p21 were demonstrated with suggestive role of non stable precursor proteins (Ozden and Porier, 1985).

The role of the immune response (at sub-neutralizing level) in enhancing dengue virus infection was suggested to have a major role for the development of haemorrhagic and shock syndromes (Halstead, 1981). Titrations for the humoral immune respones were either based on the ability of antibodies to neutralize or to inhibit agglutination reaction, or to attach specifically to any portion of the viral antigen. Information concerning antibody response toward individual proteins is lacking and this information may lead to a better understanding for the immunopathologic mechanism of this disease. Three techniques; SDS-PAGE, western blot, and enzymeimmuno assay were employed and modified. The whole process was called "the Viral-Antigen-Strips/Enzymeimmunoassay" (VAS/EIA). We have used the classical SDS-PAGE which was described by Laemmli with western blot and enzymeimmunoassay and found that the antibody to dengue viral envelope protein (E or GP59) was almost absent in both human hyperimmune sera and in healthy population (Churdboonchart *et al.*, 1986). With this study, certain chemical and physical treatment were investigated for their effects to dengue viral polypeptides which reflect the binding of human antibodies. The VAS/EIA results were scored from 0 to 4 (Schupback *et al.*, 1985). These results can be obtained in 48 hours. The

specificity, sensitivity and reproducibility are discussed.

MATERIALS AND METHODS

Serum samples : Dengue haemorrhagic fever (DHF) patients' sera and negative control serum were provided by Dr. B.L. Innis and Dr A. Nisalak of the Virology Department, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok 10400, Thailand.

Viral antigens : Suckling mouse brain seed viruses of dengue-1(d1); Hawaii M199 passage # 17; dengue-2 (d2), New Guinea C, passage # 33; dengue-3 (d3), H-87, passage # 27, dengue-4 (d4), passage # 32, and Japanese encephalitis (JE), Nakayama, passage # 17, were obtained from the Virology Department, AFRIMS, Bangkok 10400, Thailand. Dengue viral antigens were raised in suckling mouse brain. Sucrose acetone extraction for these antigen was performed as previously described (Clarke and Casals, 1958). They were titrated by hemaglutination (HA) test and confirmed to have following HA titers : d1 (1:1280), d2 (1:1280), d3 (1:1280), and d4 (1: 1280).

Gel electrophoresis and blotting : SDS polyacrylamide gel electrophoresis (8% separating gel with 2.5% stacking gel) was performed as described by Laemmli (Laemmli, 1970). The reducing agent, 2-mercaptoethanol (2ME), was added with varying concentrations in order to evaluate its effects on dengue viral polypeptides.

The separated proteins in gels were blotted onto the surface of 0.1 μm nitrocellulose membrane (NC) (Schleicher and Schuell; PH 79, Germany), according to the method described by Towbin *et al.*, (1979). Transferring was made for 3, 6, 12 and 18 hours at 3 v/cm.

For this experiment; dengue viruses, type 1 to 4, and normal mouse brain (NMB) as a negative control were loaded in sequence. After electrophoresis, the gels were cut so that one gel strip will contain the separated proteins of dengue viruses type 1, 2, 3, 4 and NMB. The NC membranes were also cut to the same size as the gel. The NC strip after blotting will contain the separated dengue viral polypeptides and was called as the viral antigen strip or VAS.

Enzymeimmunoassay for the detection of proteins on NC : Bovine serum albumin, BSA, (Sigma, St. Louise, MO 63178, USA) and non-fat dry milk, NF, (Slim, Iverson Dairy Group, Wisconsin, USA) were used as blocking agents to prevent nonspecific background after immunochemical reaction. VAS were incubated with diluted test serum which was diluted with 10 mM Tris-hydrochloric acid (pH 7.4) with 0.9% NaCl (TBS) and BSA (5%) or NF (10%), 3 hr or overnight, at 37°C. They were washed extensively with TBS containing 0.5% (v/v) Tween 20 before incubation with peroxidase labelled antibody, rabbit-anti-human-IgG-HRP (214, Dakoimmunoglobulin, Denmark). After another washing cycle (4x, 5 min each), the strips were soaked with freshly made solution of 3-amino-9-ethylcarbazole (AEC) and H_2O_2 for 15 min (Broe and Ingild, 1983). Then they were rinsed with distilled H_2O and blot dried. The color reaction developed with different level of intensity allowing a possibility to score them as follows (Schupback *et al.*, 1985) : O or (–) was for negative (no color), 1 was for barely detected or faint pink color, 2 was for clearly positive (sharp bands with pink color), 3 was for strong positive (red), and 4 was for very strong positive reaction or dark red color.

RESULTS

Dengue viral antigen from dengue viruses type 1 to 4 were separated by SDS-PAGE

(8%), and transferred onto the surface of NC membrane. The NC containing viral antigens (VAS) were immersed in diluted test serum allowing specific antibody towards these separated proteins to bind specifically. Bounded antibodies were located by immunoenzymatic reaction using enzyme labelled antibody and substrate reaction. Different protocols were set up by varying concentration of reducing agent (2-mercaptoethanol) with or without heat treatment, varying volume and titer of viral antigens, and varying dilutions of test serum. The results were impressive and are shown in Figs. 1 to 8. Since 2-mercaptoethanol (a reducing agent) used by Laemmli was suspected to cause the lost of antigenicity for certain proteins, this reagent was excluded in the first two following experiments.

Fig. 1 shows the optimum condition using sucrose acetone extracted dengue-2 antigen

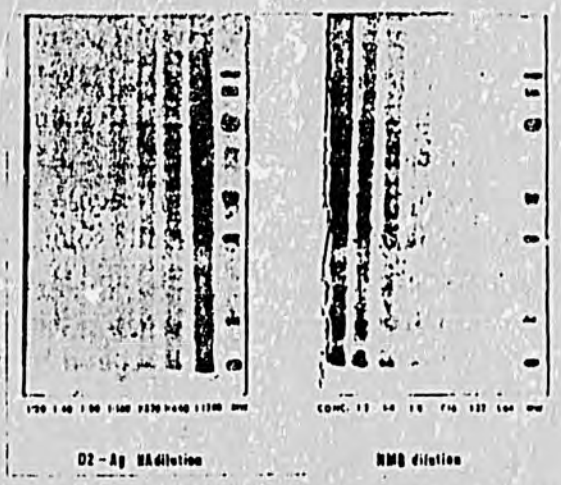

Fig. 1—SDS-PAGE results for serially diluted sucrose acetone extracted dengue type-2 antigen (HA 1:1,280 to HA 1:20) and normal mouse brain treated with the same protocol as negative control. Note the higher number of stained bands in serially diluted SAE-d2 in comparison with serially diluted mouse brain proteins. Molecular Weight markers were placed on the far left side of each gel strip.

with SDS-PAGE systems. 10µl of viral antigen and the sample buffer (1:1 ratio) were loaded into each well. The right side gel strip shows the separated dengue-2 viral proteins when serially diluted (from HA 1:1280 to 1:20), while the left side gel shows the serially diluted proteins from normal mouse brain as a negative control. The separated bands from dengue-2 with HA titer of 1:1280 were seen sharply and well separated. With larger volume of viral antigen, 20 µl and 30 µl; these bands were broader but not as sharp. At this stage, proteins from mouse brain or viral proteins can not be differentiated.

In order to determine the specificity, serially diluted dengue-2 viral antigen (form HA titers of 1:1280 to 1:20) were loaded in sequence. After blotting to NC membrane, serum with anti-dengue-2 activity proven by HI test (HI titer to dengue type 2 was 1:2560) and negative control serum, proven by both HI test and the neutralization test, were separately incubated with 2 identical twin NC strips. No specific enzyme-immunological reaction with the negative serum was found, but at least 3 positive bands with dengue-2 antigen at 1:1280 can be observed (Fig. 2). These 3 bands were compared with molecular weight markers and their molecular weights were estimated as 71,000, 66,000, and 59,000

Fig. 2—Specificity test for antibodies detected by VAS/EIA. Positive serum from dengue-2 infected patients bound to 3 polypeptides while negative serum demonstrated an absence of bound antibody.

daltons. The best blocking agent for this test was the non-fat dry milk, 10% (W/V).

In order to find out the effect of 2ME dengue-2 antigen (HA 1:1280) and non-dilute sucrose acetone extracted normal mouse brain were treated with different amount of 2ME (Fig.3). The final concentration of 2ME

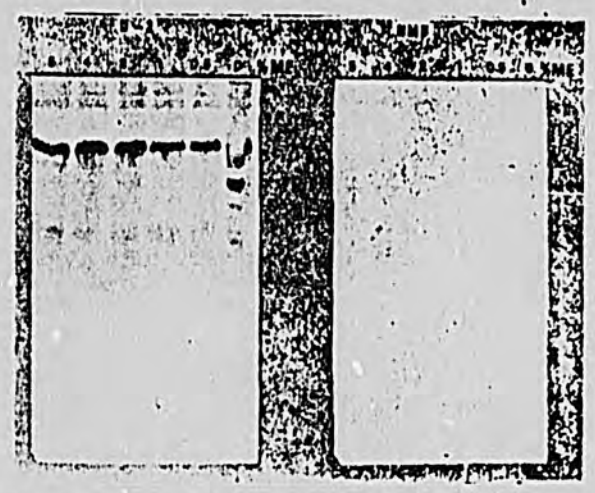

Fig. 3—Effect of 2-mercaptoethanol demonstrated by varying final concentration of this reducing agent. Normal mouse brain showed negative result while SAE-d2 (HA:1280) was sensitive to this treatment. Note the disappearance of bands at 71,000-68,000, 60,000-54,000, 50,000-48,000, 21,000-19,000, and an appearance of strongly positive band at 85,000-77,000. Also note for the higher color intensity at 85,000-77,000 and at 45,000.

were 5, 4, 2, 1 and 0.5, leaving the last well for control (without 2ME or 0%). This serum had anti-dengue-2 HI titer of 1:2560 and demonstrated at least 8 antibodies when 2ME was not included. These polypeptides are as following : between M.W. of 130,000-110,000 (p130-110), 71,000-68,000 (p71-68), 66,000-64,000 (p66-64), 60,000-54,000 (GP 60-54), 55,000-50,000 (p55-50), 50,000-48,000 (p50-48), 46,000-44,000 (GP46-44), and 21,000-19,000 (GP21-19) daltons. When 2ME was included, some of the positive bands disappeared such as the polypeptides with M.W. of 71,000-68,000, 60,000-54,000, 55,000-50,000, and 21,000-19,000 daltons. At the sametime, this treatment brought about an increasing of intensity to certain

Table 1

Antibodies to dengue 2 polypeptides by VAS/EIA in an absence and in presence of 2-mercaptoethanol.

	Intensity scores for antibodies to dengue 2 polypetides					
	%2ME					
	0	0.5	1	2	4	5
p130-110	1	1	1	1	1	1
p 85-77	-	3	3	4	4	4
p 71-68	2	-	-	-	-	-
p 66-64	2	1	1	1	1	1
GP 60-54	3	-	-	-	-	-
p 55-50	2	1	1	1	1	1
p 50-48	1	-	-	-	-	-
GP 46-44	1	1	2	2	2	2
GP 21-19	2	-	-	-	-	-

polypeptides especially the one with M.W. of 85,000-77,000 and 45,000 daltons. These results are shown in Table 1. NF is still the choice for blocking agent.

Another factor that can cause the lost of antigenicity is the heat treatment, 5 min at 100°C. This process is needed for 2ME to reduce di-sulfide bonds and normally all proteins were treated to reach a condition of being a straight single polypeptide chain for estimation of their molecular weight. A patient serum (C0909), collected 15 days after hospital admission, was used to compare the effect of 2ME with and without heat treatment, and without the 2ME. HI titers of this serum are as follows : d1 (1:5,120), d2 (1:2,560), d3(>1:10,240) and d4(>1:10,240). The results are demonstrated in Fig 4 and Table 2. With 2% of 2ME and heat, antibodies to p85-77 and GP45 can be observed. When 2ME was included, volume of all dengue viral proteins and negative control must be raised up to 20 µl, otherwise the

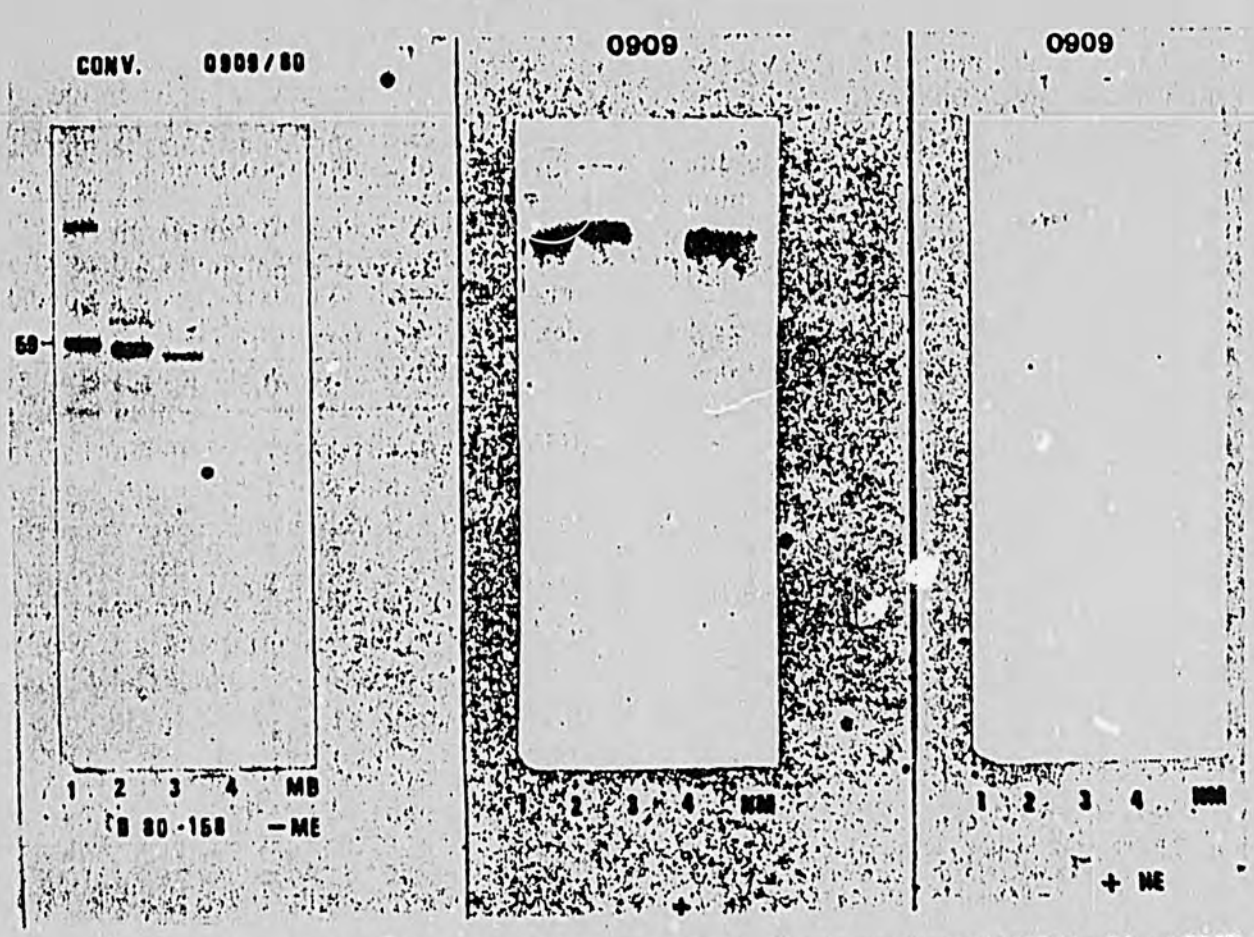

Fig. 4—Comparison of the results obtained from 3 protocols; A-strip was from VAS/EIA without 2ME, B-strip from VAS/EIA with 2% 2ME, and C-strip from 2% 2ME with heat treatment at 100°C for 3 min. Strip B and C were tested at the same time, so the bands can be compared while A-strip was run separately using different M.W. markers to determine approx M.W.. Note lower intensity of color development at 85,000-77,000 and higher intensity at 45,000 after heat treatment.

immunoenzyme reaction will be poor and can not be compared with the results obtained when 2ME was excluded.

In order to compare the rising of antibodies in DHF patients ; dengue viral antigens, from type 1 to 4, and NMB, were tested with sera collected on day 1 (0192/80) and day 15 (0317/80) after hospital admission. On day 1, HI titers were as follows: d1(1:5,120), d2 (1:2,560), d3(1:5,120) and d4(1:2,560), Dengue type 2 was isolated on day 1 indicated that the patient was secondary infected with dengue virus type 2. Antigens used in this experiment were not treated with 2ME. From Fig. 5., antibodies were found against all 4 dengue serotypes. Comparison between 2 strips was made and the scored for color intensity were assigned to each individual bands. These scores are shown in Table 3. Ten antibodies to dengue-2 polypeptides were noted in sera collected during covalescent phase.

With another pair sera, 2% of 2ME was used to treat the antigens but heat treatment was exempted. The results are shown in Fig. 6 and Table 4. Eventhough the antibodies were found to react with lesser number of polypeptides, the seroconversion can be clearly seen by visualization.

Normal adult sera were subjected to VAS/EIA with exemption of 2ME. Examples of

Table 2

Antibodies in human hyperimmune serum (C0909) to dengue viral polypeptides detected under different conditions.

Antibodies to dengue viral polypeptides	Highest VAS/EIA score to each polypeptide		
	Non-reducing condition	Reducing condition (+ 2% 2ME) without heat treatment	Reducing condition (+ 2% 2ME) with 3 min heat treatment
p130-110	3 (d1)	2 (d2)	-
p 85-77	-	4 (d1,2,4)	3 (d2)
p 71-68	2 (d2)	2 (d2,3)	-
p 66-64	2 (d2)	-	-
GP 60-54	4 (d1,2)	2 (d1)	2 (d1)
p 55-50	2 (d2)	-	-
p 50-48	2 (d1)	-	-
GP 45	-	1 (d all)	2 (d1,2,3)
p 30-28	-	-	-
p 26-24	-	-	-
GP 21-19	-	-	-
p 14	-	-	-

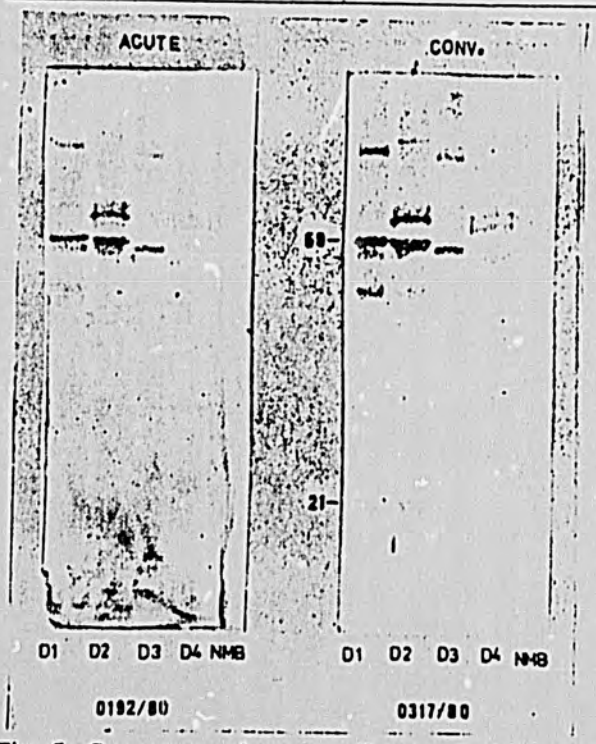

Fig. 5—Sero-conversion to all types of dengue viruses (d1, d2, d3 and d4) as seen by VAS-EIA without 2ME. 0192 is the serum collected on day 1 after hospital admission and 0317 is the serum collected on day15. Note the higher intensity of color development and number of positive bands in 0317 (Convalescent serum).

Table 3

Antibodies to dengue viral polypeptides in patients (SDS-PAGE without 2ME)

	Antibodies to dengue viral polypeptides	Serum Number 0192	Serum Number 0314
VAS/EIA results	p130-110	2 (d1)	3 (d1)
	p71-68	2 (d2)	2 (d2)
	p66-64	3 (d2)	3 (d2)
	GP60-54	3 (d2)	4 (d1,2)
	p55-50	1 (d1,2)	1 (d1,2)
	p50-48	1 (d1,2,3)	3 (d1)
	GP46-44	1 (d1,2,3)	2 (d2)
	p30-28	1 (d1)	1 (d1)
	p26-24	1 (d1)	2 (d2)
	GP21-19	1 (d2)	3 (d2)

Fig. 6—Sero-conversion to all types of dengue viruses as seen by VAS/EIA with 2% 2ME without heat treatment. Note the sero-conversion especially at d2 and at M.W. of 45,000 daltons.

Table 4

Sero-conversion between sera collected during acute and convalescent phase in presence of 2% 2-mercaptoethanol.

Antibodies to dengue viral polypeptides		Serum Number 1099/80	Serum Number 1267/80
VAS/EIA results	p130-110	1 (d1,2)	2 (d2)
	p85-77	1 (d all)	4 (d2)
	p71-68	-	1 (d2)
	GP 59	1 (d2)	1 (d2)
	p52-50	1 (d1, 2)	1 (d1,2)
	GP45	1 (d1)	2 (d1)
HI titers	d1	1:40	1:2560
	d2	1:40	1:2560
	d3	1:160	1:10240
	d4	1:40	1:5120

Table 5

Antibodies to dengue viral polypeptides in healthy adult male by VAS/EIA without 2 ME.

Antibodies to dengue viral polypeptides		Serum Number NHS 19	NHS 20	NHS 21
VAS/EIA results	p 130-110	2 (d1,3)	3 (d1)	3 (d1)
	p 71-68	2 (d2)	3 (d2)	4 (d2)
	p 66-64	3 (d2)	3 (d2)	4 (d2)
	GP 60-54	3 (d1, 2, 3)	4 (d 1, 2, 3)	4 (d 1, 2, 3)
	p 55-50	-	-	-
	p 50-48	1 (d1)	2 (d1)	1 (d1)
	GP 46-44	-	-	-
	p 30-28	-	-	-
	p 26-24	-	-	-
	GP 21-19	-	2 (d1, 2)	2 (d1, 2)
HI titers	d1	< 1 : 20	1 : 80	1 : 160
	d2	1 : 20	1 : 40	1 : 80
	d3	1 : 80	1 : 80	1 : 160
	d4	1 : 20	1 : 40	1 : 160

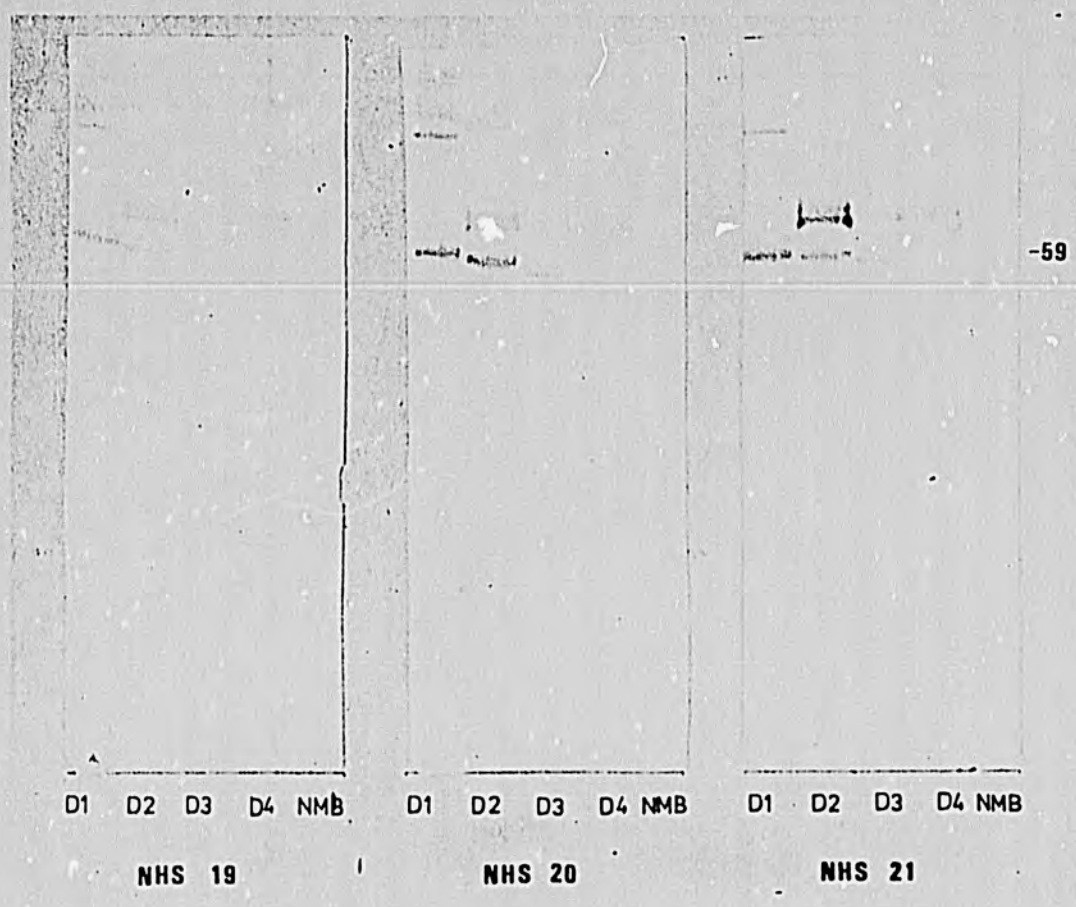

Fig 7. Antibodies to all types of dengue viruses in normal adult male sera by VAS/EIA without 2ME. Note appearance of antibodies to all dengue viruses and different level of color intensities among the sera.

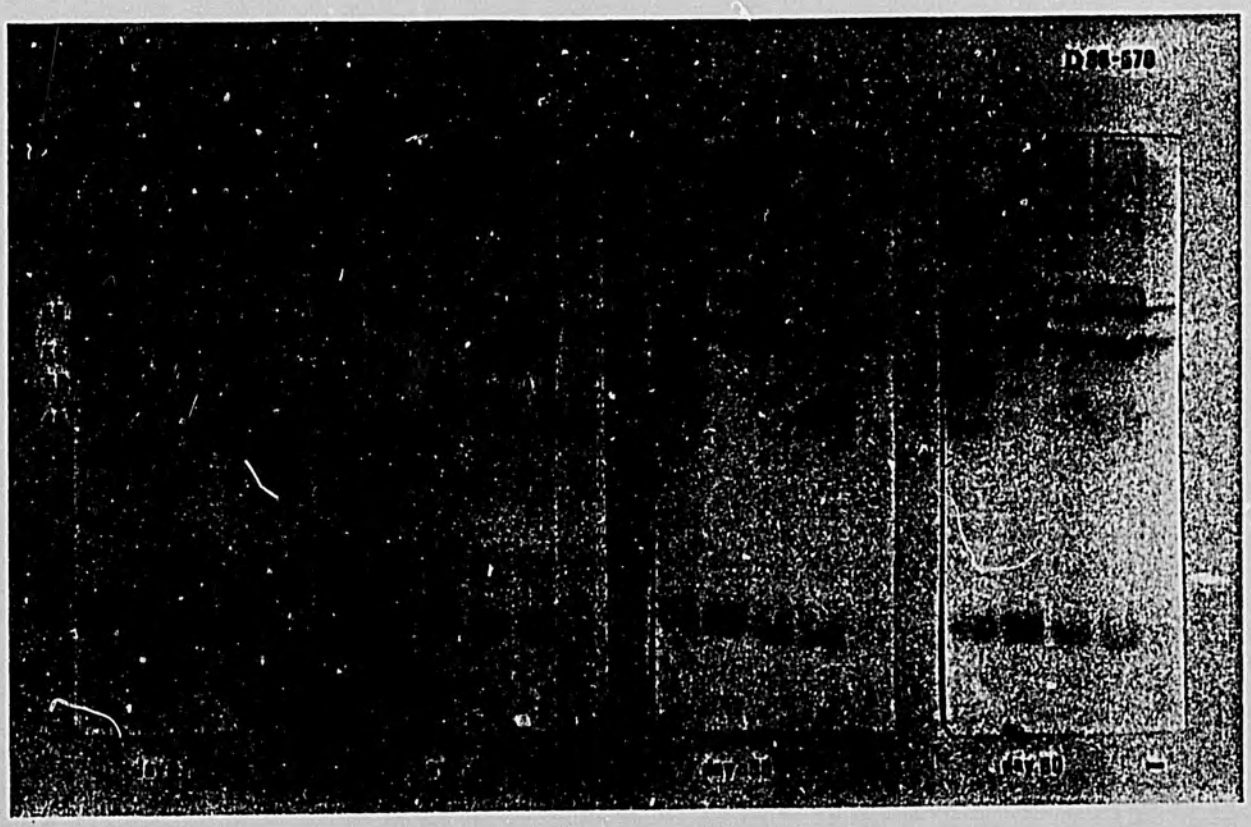

Fig 8. Sero-conversion to all type of dengue viruses in a patient's sera serially collected from day 1, day 15, day 30, to day 60. VAS/EIA was performed in non- reducing condition. Note for the appearance of antibody to polypeptide with approximate m.w. around 14,000 daltons on day 15.

this result are demonstrated in Fig 7. VAS/EIA scores and HI titers are shown in Table 5. VAS/EIA demonstrated higher sensitivity than HI test i.e. when HI titer was lesser than 1:20 for dl in NHS 19.

Antibody to protein with M.W. around 14,000 was found in a certain case. This antibody appeared on day 15 after hospital admission, and cannot be detected on day 30 and 60 in which the samples volume were increased to 20 µl (Fig. 8).

DISCUSSION

The VAS/EIA used in this study was proven useful to study dengue viral polypeptides and human humoral immune responses specifically against these individual proteins. With classical SDS-PAGE protocol (Laemmli, 1970), 2-ME was included in the sample buffer and this mixture must be heated at 100°C for 5 min. After electrophoresis, the separated proteins can be stained by Coomassie brilliant blue and observed as shown in Fig 1. After blotting, these proteins on surface of NC membrane can be traced by Indian ink. We have selected 15 hr to transfer these proteins since we found no Coomassie stain in gel and many strong Indian ink bands on NC strips.

When serially diluted sucrose acetone extracted dengue type 2 antigens were tested with positive and negative control serum (proven by HI and PRNT tests), we found that strong antigen with HA titers of 1:1,280 gave the best result while the required antigen volume is only 5 µl. After mixing this antigen with equal volume of sample buffer, the final volume loaded into each sample well is only 10 µl.)

Hyperimmune sera, diluted from 1:5, 1:10, 1:50, 1:100, 1:200, 1:500 and 1:1000, were incubated with viral antigens strip and normal mouse brain strip. Then the bound antibodies were traced by second labelled antisera, rabbit anti-human IgG. The optimum result was seen with 1:50 dilution. Therefore, all test sera were diluted to 1:50 dilution by buffer A with addition of 5% BSA or 10% NF for non-specific blocking.

When we applied the test sera to the viral antigen strips, few pinkish-red color bands developed indicating antibodies against these polypeptides. With classical Laemmli SDS-PAGE method, where 5% 2-mercaptoethanol was used as a reducing agent and heat treatment at 100°C for 5 min was required, we observed antibodies towards the polypeptides with estimated M.W. between 130,000-110,000, 85,000-77,000, 71,000-68,000 and 45,000. With BSA as non-specific blocking agents, the antibodies to smaller polypeptides (30,000-28,000, 26,000-24,000 and 21,000-19,000-daltons), were often found. One drawback of BSA is that the non-specific band can be seen with normal mouse brain strip causing difficulty to interpret the results.

Replacing BSA with NF, all non-specific background disappeared, however the intensity of positive bands were also decreased, and certain bands diminished. This problem was solved by increasing sample size from 10 µl to 20 µl. Each band was broader, but eventually brought up color intensity. However, the separation of protein bands was not as good as when the sample size was 10 µl.

To study the effect of 2ME, various concentratons of this reducing agent were mixed with the sample buffer from 0, 0.5, 1.0, 2.0, 4.0 to 5.0 percent. The samples were not heated when 2ME was not included. As seen in Fig. 3, without 2ME and without heat treatment, more antibodies can be demonstrated especially to the polypeptides at M.W. of 130,000-110,000 (P130-110), 71,000-68,000 (p71), 66,000-64,000 (p66), 60,000-54,000 (E or GP59), 66,000-64,000 (p66), 55,000-

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50,000 (p55) 50,000-48,000 (p50), 46,000-44,000 (GP45) and 21,000-19,000 (GP21 or prM). At 1-2% 2ME, antibodies at p130-110, p66, p55, and GP45 can be seen with an extra band at M.W. of 85,000-77,000 daltons (p85-77). Serum collected during convalescent phase (15 days after hospital admission) was used to compare 3 different following protocols.

(1) excluding 2ME and heat treatment;

(2) including 2ME at 2% and excluded heat treatment;

(3) including 2ME at 2% and treat sample at 100°C for 3 min.

The results indicated clearly that GP 59 is very sensitive to 2ME and also the antibodies to protein p130-110, p71-68 p66-64, p55-50, and p50-48. With 2ME and heat treatment only few antibodies can be seen with lower scores. However, the antibodies to p85-77 GP45 can be best observed.

With the same scoring system that was applied to quantitate HIV antibodies in AIDS significant seroconversion can be observed by VAS/EIA such as seen in Fig. 5. Higher color intensities were recorded and also there were antibodies towards more polypeptides. This changing pattern is interesting and allowing the analysis of antibody responses at the molecular or polypeptide level. Realizing the effect of 2ME and heat treatment investigation for interesting antibody or monoclonal antibody may be undertaken by careful selection of a proper protocol.

Sensitivity of VAS-EIA when applied with other virus such as the avian leukosis viruses (Esenman *et al.* 1980), it was calculated that a specific viral protein (p27) as little as 1-2 ng can be detected by hyperimmune rabbit serum at 1:50 dilution. In our experiment (Table 5). when antibody to dengue type 1 was found lower than 1 : 20, antibodies to dengue 1 polypeptides still can be recorded as follows : 2 for polypeptides with M.W. at p130-110, 3 for antibody to GP59, and 1 for antibody to p50. Therefore, the sensitivity can be claimed to be higher than that of HI test.

The new nomenclature for flavivirus proteins were designated according to their gene expression after the entire RNA genome of the yellow fever was described (Rice *et al.*, 1985). The first structural protein is the Capsid (C) protein with M.W. between 13,000-16,000 daltons and the antibody toward this protein can be seen with the non-reducing condition in certain hyperimmune sera (Fig. 8). The next structural protein synthesized in sequence is the virion M protein (M.W. of 8,000-8,500 daltons). This protein has not been found in infected cells and was postulated to be derived from a precursor glycoprotein (prM) which was also called as GP23, GP19, NV2, or NV2 1/2 and has estimated M.W. of 19,000-23,000 daltons. With VAS/EIA, antibodies to M was so far absent but the antibodies to protein at prM position can be detected. Envelope (E) protein follows M with M.W. between 51,000 to 60,000 daltons. Under non-reducing condition, human hyperimmune sera reacted with at least 3 polypeptides at the approximate M.W. of 60,000-54,000, 55,000-50,000 and 50,000-48,000 daltons. We can say that the antibodies to structural proteins can be detected under non-reducing condition and they are quite sensitive to 2ME and heat treatment.

The antibodies to the non-structural-proteins, NS1 (NV3 or GP45 with M.W. of 44,000-49,000 daltons) and NS3 (NV4, or p77) with M.W. of 67,000-76,000 daltons) can be seen best with the classical SDS-PAGE when the concentration of 2ME was decreased to 1-2 percent and the heat treatment period was also reduced to only 2-3 min. The ns 2a was found to have similar molecular weight to prM, therefore the antibody to this polypeptide or prM must be differentiated by the

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use of their specific antisera. This situation must also be applied to ns2b because its molecular weight is about 12,000 to 15,000 daltons and cannot be differentiated from antibody to C protein until the specific antisera is employed. With ns4a (NVX or NV2 1/2 with M.W. between 24,000-32,000 daltons, we observed at least 2 bands(at M.W. of 30,000-28,000 and. 26,000-24,000 daltons). The antibodies to ns4b (NV1 with M.W. of 10,000-11,000) was absent, while antibody to NS5 (NV5 with M.W. of 91,000-98,000 daltons) were observed with reducing condition in certain patients' sera.

Besides the proteins mentioned, VAS/EIA revealed antibodies toward at least 2 more proteins : the proteins with M.W. between 130,000-110,000 and the other with M.W. of 85,000-77,000 daltons. These antibodies are probably toward the p130 and p83 respectively.

In this study, VAS/EIA was proven as a powerful tool in monitoring antibodies to dengue viral polypeptides. It can be used to study humoral immune responses in vaccinees, patients, and in population as well. The results may lead to a better understanding of the immunopathogenesis and may as well lead to further application as rapid diagnostic method. This study is now in progress in our laboratory.

SUMMARY

The authors applied polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE), western blot, and enzymeimmunoassay (EIA) for the detection of antibodies toward individual dengue viral proteins or polypeptides. SDS-PAGE procedure as described by Laemmli *et al.*, was applied and modified. The results can be observed by visualization. The scores, from 0 to 4, can be assigned by comparing the intensity of the color development. Besides being sensitive and rapid, this technique yields information of the polypeptides or molecular level. An increasing of intensities of positive reactions indicated rising in antibodies titers and all serotypes of dengue viruses (from type 1 to type 4) can be tested together allowing reliable comparison among serotypes. With hyperimmune human sera, at least 13 polypeptides reacted with sera while negative non-immune subject showed no reaction. It is highly possible to use this technique as a rapid quantitative and for qualitative analysis of antibodies to individual viral proteins as well.

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	HN	Serum No.	Name	HI D1	HI D2	HI D3	HI D4	HI CHIK	HI JE	Age	Day	Grade	CBC HCT	WBC	NEU	LYM	ATY	MON
1. (OPD) ✓	1792015	RS-001 (Jul-8)	ด.ญ. จิตต ภาคภูวิถี (10.30)	1:80	1:80	1:80	1:160	<1:20	1:80	6 ปี 2 เดือน 4.11	5	(2)	39.0	9100	65	28	—	5
น.ส. ...		RS-004 (Jul-8)	(5-DHF) (12.00)	1:160	1:160	1:160	1:320	<1:20	1:320		7		38.0	4000	43	53		4
		RS-009 (Jul-9)	(9.00)	1:640	1:640	1:640	1:640	<1:20	1:1280		6		38.0	3800	12	83	(3)	2
		RS-008 (Jul-10)	(15.30)	1:5120	1:1280	1:5120	1:5120	<1:20	1:1280		12		32.0	12500	45	53		2
	dengue (2)					m / DEN / G		m / JE / G										
					RS 004	55	116	42	116									
					RS 005	133	207	67	147									
					RS 048	253	240	87	962									
2. (OPD)	390393	RS-002 (Jul-8)	ด.ญ. [illegible] (10.30)	1:80	1:40	1:80	1:80	<1:20	1:40	11	3		45.0	11300	78	22		
		RS-011 (Jul-13)	(14.20)	1:80	1:40	1:80	1:80	<1:20	1:20		8							
		not - DHF				m / DEN / G		m / JE / G										
					RP-011	9	51	0	15									
3. (OPD)	1262564	RS-003 (Jul-8)	ด.ญ. [illegible] (10.30)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20			not DHF						
	*	RS 012 (Jul-13)	[illegible]	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
						m / DEN / G		m / JE / G										
					RS-013													
					RS-042	0	0	0	0									
4. (OPD) ✓	1793476	RS-007 (Jul-9)	ด.ญ. [illegible] (8.00)	1:1280	1:1280	1:2560	1:2560	<1:20	1:2560	2.07 / 15/6/22	7 / 8	(2)	43.0	5700	22	74	10	1
น.ส. 24.5 กก.		RS-044 (Jul-10)		1:640	1:640	1:2560	1:2560	<1:20	1:1280		16							
						m / DEN / G		m / JE / G										
					RP-007	94	916	51	534									
					RS-044	91	263	40	254									
5. (OPD)	1411426	RS 008 (Jul-10)	ด.ญ. [illegible] (8.00)	1:1280	1:1280	1:640	1:1280	<1:20	1:1280	10	6	2 y	42.0	9100	34	60 / 35	25	2
น.ส. 27.8 กก.		RS 009 (Jul-10)	(12.00)	1:5120	1:640	1:1280	1:1280	<1:20	1:320		6							
		RS 043 (Jul-22)	(15.10)	1:1280	1:1280	1:5120	1:2560	<1:20	1:320		17							
						m / DEN / G		m / JE / G										
					RP 006	141	24	35	271									
					RS 009	139	339	40	217									
					RS 043	165	959	40	231									
6. (OPD) ✓	1495529	RS-019 (Jul-13)	ด.ญ. [illegible] (9.30)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	11	1 (9 กก.) / 3 (14 กก.) / 4 (15 กก.) / 5	2	38.0 / 40.0	6200 / 4700	54 / 41	4 / 41	2 / 1	4 / 1
น.ส. 27 กก.		RS-015 (Jul-14)	(P-DHF)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20		6		37.0	4700	13	50	7	8
		RS-040 (Jul-20)	[illegible] (14.30)	1:40	1:40	1:80	1:80	<1:20	1:40		12		38.0	10400	22	72	10	1
	dengue (3)		negative ab/pt			m / DEN / G		m / JE / G										
					RS 013	22	4	13	13									
					RS 015	134	4	113	8									
					RS 040	220	55	43	16									
7. (OPD) ✓	1391650	RS-013 (Jul-6)	ด.ช. [illegible] (10.30)	1:40	1:20	1:40	1:80	<1:20	1:20	(3 กก.) 5 / (4 กก.) 6 / (5 กก.) 8		(2)	40.0 / 44 / 38	3,180 / - / -	39 / 66 / 31	55 / 44 / 58	6 / 22 / 7	- / 3 / 3
น.ส. 25.3 กก.		RS 014 (Jul-13)	(P-DHF)	1:80	1:40	1:80	<1:20	<1:20	1:40		15							
						m / DEN / G		m / JE / G										
					RS 013	274	39	102	11									
					RS 014	245	56	151	41									
8. (OPD) ✓	1502394	RS-016 (Jul-14)	ด.ช. [illegible] (10.30)	<1:20	<1:20	<1:20	1:640	<1:20	<1:20	2.09	7	1	33.0	3400	20	70	5	4 (101)
น.ส. 14 กก.		RS 039 (Jul-20)	[illegible] (13.50)	<1:20	<1:20	<1:20	<1:20	<1:20	1:20		13		35.0	14400	45	21	1	1
		[illegible]				m / DEN / G		m / JE / G										
	Elisa IgM + JE (P-JE)			RS 016	32	0	148	0										
				RS 039	20	0	63	0										

Best Available Document

ต่างกัน 100,000 = inadequate.

B.T.	platelets	Complement	
40.0 40.0	decrease adequate	RR-5 RR-4 RR-4	admit 6 กรกฎาคม (ไข้ 6 วัน), TT⁻ / 7 กค ไข้ลดลง / 8 กรกฎาคม BT 40.0° + ผื่นแดง + NSS(+) ไม่มีอาการ ตับ ม้าม, ตับ 2 cm
39.0	88000		
37.0	378000		
	266000		ไข้ลง 8 กค 2540 ไม่มีอาการใดๆ ตื่นนี้ 9 กค 2540 เกิดอาการ 13 กค ตายแล้ว
40.0 39.2 36.2	46000	50%	admit 9 กค, ไข้ขึ้นสูง, เพิ่งกลับจากต่างจังหวัด, ไข้ 40°C, TT⁺, L 9cm, NSS(+), 9 กค อาการเปลี่ยนแปลงอย่างรวดเร็ว ซึมลง เกิดภาวะช็อกจากเลือดออก
41.0 39.6	400 157,000 20,000		6 กค เริ่มมีอาการไข้ ผื่นแดง ตาแดง ไม่มีอาการอื่นๆ ไม่มีอาการเลือดออก ไม่มีอาการ GI BT 39.9° not acceptable not respond to verbal command ตรวจ Encephalitis admit เช้า 7 LP: ICP 220 Hemo 10/mm³ Sugar 80 BS 120 ปกติ 15 ไม่มี chemotherapy 7 กค ตรวจร่างกาย BT 39.2° 8 กค อาการเปลี่ยนแปลง BT 37.6° / 9 กค CXR Rt Pleural effusion Liver fm EEG diffuse Slow wave 10 กค เสียชีวิต
40.0 39.8 39.4 38.0 33.0	— → 10 กค/BT 40.1° 54,000 40000 10000	50%	9 กค 37.8°C ไข้ขึ้นสูง 1 วัน, ตาแดงทั้งสองข้าง, L 1 cm, 11 กค ไข้ลง ผื่นขึ้นทั่วตัว ตาแดงทั้งสองข้าง TT⁺, ตับ ม้ามโต L 3 cm ซึมลง NSS(+) 12 กค ซึมลงมาก BT 39.8°C, CXR ปกติ, CSF/ไขสันหลังไม่ขุ่น 13 กค ชูคอได้, 39°, 14 กค ไข้ลง ผื่นขึ้นทั่วตัว, ตับ ม้ามโต + petechiae, L 3 cm. 30 กค เสียชีวิต
38.6 38.0 37.2	54,000 36,000 150,000	RR-14 RR-14	5 วันก่อนมีไข้สูง ปวดท้อง ท้องเสีย ถ่ายเหลว BT 39.7°C (38 วัน) Hct 40° admit 3 กค ไข้ 38.6°C ซึมลง ตับ ม้ามโต L 3 cm, 4 กค ซึมลงมาก L 3 cm. BP ต่ำ 5 กค BT 37.2°
38.0 37.0	65,000 43,000	⅓	10 กค ไข้ ปวดท้อง 4 วัน มีอาการเลือดออกทางเดินอาหาร Dx lang cupolitis (Bacillary), BT 38.9° 12 กค ไข้ลงเล็กน้อย ไม่มีอาการเลือดออก ปวดท้อง BT 37.5° ซึมลง เสียชีวิต TT⁻ x-ray chest interstitial infiltration 14 กค ไข้สูง ปวดท้อง ท้องเสียมากขึ้น liver 1 cm

	HN	Serum No.	Name	HI D1	HI D2	HI D3	HI D4	HI CHIK	HI JE	sig	Day	Grade	CBC HCT	CBC WBC	CBC NEU	CBC LYM	CBC ATY	CBC MON
9.(OPD.)	39 1312624	RS-018 (Jul-15)	ร.ศิกริช ศรีสมร์ (9.15)	<1:20	1:20	1:40	1:160	<1:20	<1:20	1.04	4 5	(2)	36.0 40.0	3000 4100	50 25	45 60	2 14	1
H.N 16.5		RS-021 (Jul-16)	S-DHF (10.00)	1:1280	1:2560	1:2560	<1:20	<1:20	1:640		6 10		35.1 47.0 33.0	4270 7900 8000	24 22 40	66 72 59	38	9 50 1
	dengue (3)		(P-DHF)		RS-012 RS-021	m 27 46	DEN 6 225	m 115 110	JE 6 242									
	P-d3.																	
10.(OPD)	39 1795347	RS-019 (Jul-15)	ร.เกษจิรา ไพรวิทย์	1:80	1:160	1:160	1:1280	<1:20	1:80	14	5	(3)	30.0	5200	34	52	8	
H.N. 37 กก		RS-027 (Jul-16)	S-DHF (14.05)	1:160	1:160	1:160	1:640	<1:20	1:160		6		37.0	4900	27	51	20	
		RS-029 (Jul-17)	(9.10)	1:640	1:640	1:640	1:640	<1:20	1:640		7		35.0	10400	28	55	30	4
		RS-042 (Jul-29)	(14.20)	1:1280	1:640	1:1280	1:10240	<1:20	1:640		17		32.0	16200	89	9		2
						m	DEN 6	m	JE 6									
					RS-019	51	51	144	25									
					RS-027	99	201	58	168									
					RS-029	142	346	79	239									
					RS-042	48	340	27	297									
11.(OPD)	39 1795321	RS-020 (Jul-15)	ร.ศิริพร หฤทัยจิต	1:640	1:640	1:1280	1:1280	<1:20	1:640	11.05	8	3	30.0	5100	40	46	4	5 band
H.N. 42		RS-022 (Jul-16)	S-DHF	1:20	<1:20	1:40	1:20	<1:20	<1:20		9 10		45.0	7900	22	34 32	(35)	band 4
		RS-022 (Jul-20)	(15.00)	1:1280	1:5120	1:80	1:5120	<1:20	1:80		13		42.0	11300	22	71	6	
						m	DEN 6	m	JE 6									
					RP 040	49	165	42	165									
					RP 042	440	6	21	4									
					RP 042	43	322	80	242									
12.(OPD)	52 1794161	RS-024 (Jul-16)	ร.ศิริพร หาดชัยเวช (10.30)	1:160	1:160	1:320	<1:20	<1:20	1:160	12	5	1	37.0	4000	41	54	1	
H.N 39.7		RS-066 (Jul-23)	S-DHF (11.45)	1:5120	1:1280	1:2560	1:1280	<1:20	1:2560		12		35.0	7800	40	47	3	5
							DEN		JE									
					RS 024	64	137	96	119									
					RS 066	61	560	46	354									
13.(OPD)	91 1795676	RS-035 (Jul-16)	ร.นิธิพล จำปาทอง (10.40)	1:20	1:20	<1:20	<1:20	<1:20	<1:20	12	4	H/DF	31.0	6800	61	32		7
		RS-041 (Jul-20)	[illegible] (14.45)	<1:20	1:20	<1:20	<1:20	1:20	<1:20		8		33.0	6700	53	39	1	6
							DEN		JE									
					RS 035	24	0	131	0									
					RS 041	99	6	54	6									
14.(OPD)	53 1484639	RS-036 (Jul-16)	ร.กฤษณ์ ศรีวร (11.40)	1:20	<1:20	<1:20	<1:20	<1:20	<1:20	2.10 31 ม.ค.	9	H/DF	35.0	4100	24	50 68	18	8
HN 13.9		RS-047 (Jul-20)	(P-DHF) (14.40)	1:160	1:160	1:160	1:640	<1:20	1:160		9		36.0	7400	40	56		2
dengue-1			กลุ่มเสี่ยง ab/pt		RS 036	32	DEN 8	52	JE 5									
					RS 047	575	2	36	938									
15.(OPD)	17 804091	RS-029 (Jul-17)	ร.ปิยนุช เอี่ยมกำ (10.10)	1:640	1:320	1:640	<1:20	<1:20	1:160	9	4	3	45.0	7900	59	36	4	7
H.N 24 ม VAS/		RS-031 (Jul-18)	S-DHF (11.35)	1:1280	1:640	1:1280	1:1280	<1:20	1:160		9	3	35.0	5200		62		3 band 6
		RS-035 (Jul-19)	(9.30)	>1:20480	>1:20480	>1:20480	>1:20480	<1:20	1:5120		6		24.0	8900	7	81		6
		RS-100 (Jul-24)	(13.40)	1:2560	1:1280	>1:20480	1:5120	<1:20	1:10240		14		33.0	5300	38	60		
							DEN		JE									
					RS 029	62	119	86	98									
					RS 031	143	242	52	307									
					RS 035	835	859	182	844									
					RS 100	119	366	62	396									
16.(OPD)	30 1138314	RS-030 (Jul-17)	ร.ปิโรจน์ ปิกุลทอง (10.30)	1:40	<1:20	<1:20	<1:20	<1:20	<1:20	5.10	7	1	42.0	6600	27	54	9	10
		RS-036 (Jul-27)	(P-DHF)	1:160	<1:20	1:80	<1:20	<1:20	<1:20		17		38.0	5900	34	54	5	1
							DEN		JE									
					RS 030	288	2	197	2									
					RS 036	172	30	87	14									

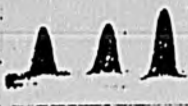
41a

B.T.	Platelets	Cx.	
38.8	20,000		
37.5	36000		กลางวัน 14 กค ตกใจ ER เจ็บอกเรื้อรัง 4 วันก่อนมาโรงพยาบาล ปัสสาวะ ถ่ายเป็นสีดำ เอาเลือด 5 ครั้งต่อวัน ไม่มีอาการ จึงมาตรวจที่ รพ.ร.ร. 43cm
37.0	14000		1 วันก่อน ร่วมโลหิต ไข้ 39
37.6	238,000		11 กค เจ็บคอ ไข้สูง ปวดเมื่อยตามตัว (กลืนลำบาก)
			20 กค มีปัสสาวะสีดำ เป็น 43cm
36.0	24000		ad 15 กค มีไข้ต่ำๆ อาบมาตลอด ตามไม่ได้เอาเรื้อรัง TT⁻, BP อยู่ในเกณฑ์ต่ำ ไข้ 100 mm, pulse เร็ว 120/min, L-neg
37.0	30000		petechiae ที่แขนขา ไข้ 39 แล้วลดลง *
36.8	37000	ต่ำ	16 กค liver palpable, 17 กค vital sign stable กลืนลำบาก
37.0	380000	สูงปกติ	
38.1	decrease	สูง	Y อุ้มท้องเดือนที่ 7 ออกอาการ เลือดออกทางช่องคลอด ปวดท้องน้อย มีมาตั้งแต่ 2 วันก่อน เคยเป็นไข้เลือดออก 3 ปีก่อน
37.0	16,000	ต่ำ	15 กค admit BP 90/70 P 125, BT 38 ไข้ 39 เป็น ON, L 1 cm (เดิม 0 → เป็น 1 cm)
37.9			16 กค อาการเหมือนเดิม ตามร่างกายมีจ้ำเลือด liver 2cm BT 37 ตลอดมา 5 วัน
37.0	212000	สูง	20 กค
36.0	200000		ไข้ 2 วัน ตั้งแต่ 13 กค TT⁻, BT 39.4, L-neg, ไม่รับประทาน
37.0	268000		16 กค BT มีไข้เล็กน้อย เจ็บคอ, TT⁺, injected pharynx
38.0	ต่ำมาก ~~24000~~		ตั้ง OPD ไม่ได้ admit. 4 วันก่อนมีไข้ เบื่ออาหาร ปวดศีรษะ ไม่ไอ เวียนหัว ปวดกล้ามเนื้อ ปวดเมื่อย กลืนไม่
	264000		ไม่ถ่ายเหลว BT 38°, liver ไม่โต, TT±
	ปกติ / ลง %		20 กค negative หมด
38.5	ADQ	50%	ตั้ง 13 กค ตัวร้อน ถ่ายเหลว ปวดท้อง เบื่ออาหาร BT 39°C, liver 0 DX viral infection ให้กลับบ้าน
37.5	484000		16 กค กลับมาใหม่ ไม่ไอ ไม่ถ่ายเหลว ท้องเสีย 1 ครั้ง Liver 1cm
			20 กค กลืนลำบาก คนไข้ดี.
37.7	100000	ต่ำ	ตั้ง 14 กค (มีไข้เรื้อรัง) อาเจียน 2-3 ครั้ง เจ็บคอ ไม่มีน้ำมูก ไม่มีไอ ไข้ 37.7° Liver 0 cm, exploding rash
37.2	34000	สูงมาก	17 กค admit. ไม่มีไข้ ขึ้นผื่น ตามตัว ตามแขนขา ไม่ค่อยไอ ตามตัว BP 90/70, เจ็บคอ 2cm การเจาะเลือด 17.5
37.0	24000	90.	21.00 Hct ลด 32%
	372000	ปกติ	18 กค BT 37.5 ขึ้นผื่นตามแขน ไม่มี bleeding. เริ่มมีผื่นตอนเย็น (Typical)
12.0	ADQ		
	34000		
	400000		
	ฟื้นตามระดับ Cr.		

	HN.	Serum No.	Name		HI D1	HI D2	HI D3	HI D4	HI CHIK	HI JE	Age	Day	Grade	CBC HCT	WBC	NEU	LYM	ATY	MON
												1		34.0	2400	50	35	8	
17. (นอกเวร)	92 1796493	RS-032 (Jul-19)	ด.ญ. เบ[illegible] คันทอง	(9.25)	1:1280	1:640	1:1280	1:1280	<1:20	1:640	10.00	5	I y.	38.0	2300	20	61	33	..
		RS-039 (Jul-20)	S-DHF	(9.00)	1:2560	1:2560	1:2560	1:10240	<1:20	1:2560		6		34.0	7000	27	39	18	10
												9		35.0	8200	30	58	10	1
		RS-090 (Jul-27)		(14.30)	1:2560	1:2560	>1:20480	1:10240	<1:20	1:2560		13		30.0	6400	93	42		3
							DEN			JE									
						RS032	349	186	84	242									
						RS035	55	251	87	232									
						RS090	25	344	17	258									
18. (OPD)	93 1796510	RS-034 (Jul-19)	ด.ชายชื่อ วิสาหกิจ		1:160	1:160	1:160	1:160	<1:20	1:80	10	1	(II)	43.0	4300	72	15	(L)	1.0
Dengue (3)		RS-036 (Jul-20)	S-DHF	(9.30)	1:160	1:160	1:160	1:160	<1:20	1:80		7	III	33.0	3800	37	52	20	
		RS-049 (Jul-21)		(9.45)	1:2560	1:1280	1:1280	1:1280	<1:20	1:640		6		38.0	8400	10	74	30	
(O.ds)		RS-099 (Jul-27)		(13.45)	1:1280	1:2560	>1:20480	1:2560	<1:20	1:5120		12		37.0	6500	37	46		3
							DEN			JE									
						RS034	87	69	52	64									
						RS036	235	118	90	114									
						RS049	438	227	197	222									
						RS099	31	356	312	358									
19. (O.D)	18 1616535	RS-038 (Jul-20)	ด.ชายชื่อ [illegible]		1:640	1:1280	1:640	1:1280	<1:20	1:640	3.11	5	II.	46.0	4000	22	69	30	
(OH.1)		RS-050 (Jul-21)	S-DHF	(10.00)	1:1280	1:2560	1:1280	1:1280	<1:20	1:320		6		40.0	5900	27	68		1
		RS-101 (Jul-27)		(13.35)	1:640	1:640	1:5120	1:1280	<1:20	1:1280		12		37.0	7600	42	50		2
							DEN			JE									
						RS038	221	193	149	144									
						RS050	263	829	79	174									
						RS101	149	340	36	281									
	94	D3																	
20. (OPD)	179 9303	RS-051 (Jul-21)	ด.ชายชื่อ [illegible]		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	6	9	(I)	37.0	2400	42	45	2	
dengue-3		RS-058 (Jul-22)	(P-DHF)	(11.10)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20		6	(III) (Hct ↓ ↑)	37.0	2600	29	60	3	9
		RS-093 (Jul-27)		(14.15)	1:40	1:20	1:160	1:160	<1:20	1:20		11		35.0	11100	79	18		5
							DEN			JE									
						RS051	142	4	50	7									
						RS058	142	4	36	7									
						RS093	949	62	129	17									
21. (OPD)	95 1148945	RS-052 (Jul-21)	ด.ชายชื่อ [illegible]		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	7	4	(I) (II) (III)	36.5	2600	38	60		
dengue-3		RS-059 (Jul-22)	(P-DHF)	(10.00)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20		9		41.0	3500	44	47	22	5
(OH.1)		RS-062 (Jul-23)		(10.30)	1:20	1:40	1:20	<1:20	<1:20	<1:20		6		38.0	4700	29	54	4	7
							DEN			JE									
		Neg Ab/pt	Repeat ELISA				RP052	90	2	317	6								
							RP059	66	7	43	12								
							RS062	156	12	42	17								
22. (OH.1)	97 1797975	RS-055 (Jul-22)	ด.ชายชื่อ [illegible]		1:40	1:160	1:20	1:80	<1:20	1:20	11	6	2	38.0	3300	44	47	(AT.2) 62,	5
		RS-063 (Jul-23)	S-DHF		1:1280	1:1280	1:2560	1:640	<1:20	1:320		7		41.0	7700	21	45	25	4
		RS-097 (Jul-27)		(13.55)	1:1280	1:5120	1:2560	1:5120	<1:20	1:640		11		35.0	8000	30	63	—	2
							DEN			JE									
						RS055	17	48	38	43									
						RS063	28	313	21	257									
						RS097	36	313	11	274									
				(10.00)															
23. (OPD)	1117646	RS-056 (Jul-22)	ด.ชายชื่อ [illegible]		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	10 8/12	4	I	40	5600	53	40	Eo1	
dengue-3		RS-076 (Jul-24)			<1:20	<1:20	<1:20	<1:20	<1:20	<1:20		6	แทงเลือด	42.5	3300	48	46	05/AT9	
		Neg Ab/pt	P-DHF				DEN			JE									
						RS056	67	1	2011	4									
(P-3)						RS076	29	13	13	23		9		45.0	[illegible]	15	[illegible]		
				(11.00)															
24. (นอกเวร)	900867	RS-057 (Jul-22)	ด.ชายชื่อ [illegible]		1:5120	1:5120	1:1280	>1:20480	<1:20	>1:20480	(5)	6	2	34.0	10900	32	66	4	
		RS-098 (Jul-27)		(13.50)	1:5120	1:5120	1:5120	>1:20480	<1:20	1:640		11	(III) Hct ↓ ↑	31.0	4800	24	75		
							DEN			JE									
						RS057	[illegible]	361	185	254									
						RS098	[illegible]	324	50	317									

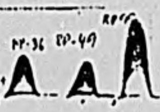
B.T. Platelets C_3

37.4 ALQ
37.4 54000
36.9 68000
36.9 22000



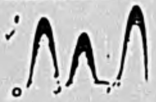
8 กค [illegible] Toxic, BP [illegible] [illegible]
[illegible] fluid. [illegible] Jaundice.

37.1 220000
37.2 44000
37.4 98000
394000



19 กค 2530 [illegible] BT 37.1 P120, BP [illegible] TT+ [illegible] dehydration [illegible] fluid
[illegible] Grade II [illegible]

36.0 50000
37.0 24000
37.0 474000

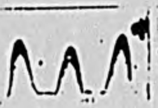


20 กค 30 [illegible] 16.15 [illegible] weak [illegible] BP 110/70 P104 [illegible] fluid
21 กค 30 BP [illegible] 90/52.60 [illegible] Grade II [illegible].

38.4 70000
37.1 50000
37.0 362000

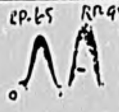
21 กค. [illegible] 5 [illegible] TT−, [illegible] Sign [illegible] Impending shock. [illegible] Hct 37% BP 51/40 [illegible] Grade II.

41.8 39000
37.4 76000
37.5 76000



21 กค 2530 [illegible] ER 16.30 [illegible] weak [illegible]
mild dehydrate P112/m RR 52 BP 100/60 no active bleeding
22 กค 2530 BP 90/60, P76, R20, [illegible] 24 ชั่วโมง Hct 47% [illegible] fluid [illegible] D5NSS [illegible]
[illegible] BP 110/90 [illegible] 14 [illegible].

41.3 162000
37.0
37.0 52000
37.7 202000



21 กค 2530 [illegible] 5 [illegible] TT+, liver [illegible], [illegible]

176000
125000

22 กค 2530 [illegible] erythematous nodule [illegible]
1 [illegible] liver [illegible] − TT (+)
24 [illegible]
26 [illegible] BP 110/70 PR 80. [illegible] admit. [illegible] 37.3 [illegible]
fluid [illegible] 27. [illegible].

31000
36.8 34000
40000

[illegible] 21 กค 2530 [illegible] bleeding [illegible] liver 2 cm, TT+
[illegible] sign [illegible] impending shock BP [illegible] P120 [illegible]
[illegible] 22 กค [illegible], Hct [illegible], platelet [illegible]

ที่ 7

HN.	Serum No.	Name	HI D1	HI D2	HI D3	HI D4	HI CHIK	HI JE	Day	Day	grade	CBC HCT	CBC WBC	CBC NEU	CBC LYM	CBC A/Y	CBC MON
25. (OPD) 1792782	RS-061 (Jul-22)	ย. จรรจิตต์ ศาลายาน (12.10)	1:320	1:640	1:160	1:640	<1:20	1:160	14	1	I. (นอน)	42.0	30800	89	11		
105/61	RS-067 (Jul-23)	S-DHF	1:160	1:80	1:320	1:320	<1:20	1:320		1		37.0	21300	72	16		2
	RS-019 (Jul-27)	(14.09)	1:160	<1:20	1:320	1:2560	<1:20	1:160		6		33.0	8700	57	37		5
						DEN		JE									
				RS061	12	114	11	93									
				RS069	11	112	12	87									
				RS095	13	94	6	83									
26. (OPD) 1721100	RS-060 (Jul-22)	ย. ฉันทุกข์ ชุมทาง (12.00)	<1:20	1:20	<1:20	<1:20	<1:20	<1:20	11	7		36.0	8200	25	71	16	
	RS-094 (Jul-27)	Not Dengue (14.10)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20				37.0	5600	44	50		
						DEN		JE									
				RS060	8	9	20	7									
				RS094	3	0	3	3									
22. (OPD) 1798050	RS-067 (Jul-23)	ย. สุรสิทธิ์ ปวงแสงวรี	1:2560	1:640	1:2560	1:640	<1:20	1:1280	6	6	?	32%					
	RS-072 (Jul-24)	(10.10)	1:5120	1:5120	1:20480	1:20480	<1:20	1:5120				29	34800	4	85		1
	RS-091 (Jul-27)	เดือน 3/พ.ศ. 24 (14.25)	1:2560	1:1280	1:2560	1:10240	<1:20	1:5120									
						DEN		JE									
				RS067	216	353	257	285									
				RS072	240	369	265	413									
				RS091	339	336	222	391									
28. (OPD) 1797503	RS-068 (Jul-23)	ย. วีระ วิทยาปุเรืองศรี	<1:20	1:40	<1:20	<1:20	<1:20	<1:20	7	4	I.	34%	5400	79	16		5
	RS-077 (Jul-24)	22 kg	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20				37%		59	32		8
	(Jul-26) (P-DHF)					DEN		JE									
				RS065	29	4	18	8				36.8%	2700	36	48	(42)	(8)(E4)
				RS077	65	12	21	19									
29. (เด็กชาย) 1019399	RS-070 (Jul-24)	ย. อนุทิน ทันหนองพังแก้ว (9.10)	1:40	1:40	1:40	1:20	<1:20	1:40	8	2	II y	30	4200	80	12		5
	RS-079 (Jul-25)	S-DHF (9.40)	1:40	1:80	1:80	1:40	<1:20	1:20		3		32.0	4700	60	22		12
น.น. 18 kg												32.0	2800	67	32	5	1
	RS-083 (Jul-26)	(9.30)	1:320	1:80	1:640	1:160	<1:20	1:160		4		39.0	3100	54	31	7	7
	RS-089 (Jul-27)	(10.40)	1:640	1:1280	1:640	1:640	<1:20	1:320		7		39.0	7000	39	48		3
	RS-111 (Jul-28)	(13.25)	1:1280	1:5120	1:5120	1:1280	<1:20	1:1280		6		36.0	8400	28	71		1
						DEN		JE									
				RS076	34	37	14	30									
				RS074	30	29	29	33									
				RS083	13	108	39	107									
				RS089	35	238	33	319									
				RS111	42	300	42	228									
30. (OPD-1) 1017970	RS-073 (Jul-24)	ย. ปรีดา ศาลาจีน	1:20	<1:20	1:20	1:20	<1:20	<1:20	(7)	15	(III)	30	1150				
	RS-084 (Jul-25)	(P-DHF) (10.30)	1:80	1:40	1:320	1:320	1:20	1:40				40	[illegible]	14	63	(8)	2
	(Pneumatic baby)	เกิด 20 ก.ค. 2523										34	4200	40	58		1
						DEN		JE									
				RS073	162	7	55	9									
				RS084	212	45	207	39									
31. (เด็กชาย) 838292	RS-074 (Jul-24)	ด.ช. ชัยพร เชียงสะอาด (10.20)	1:320	1:640	1:2560	1:1280	<1:20	1:640	13	5	II+	39.0	9000	48	38	8	
	RS-078 (Jul-25)	S-DHF (9.25)	1:1280	1:640	71:20480	1:2560	<1:20	1:1280		6	(มาก 4.9)						
	RS-089 (Jul-27)	มีน AGN บ่าย (15.00) (295)	1:2560	1:2560	71:20480	1:5120	<1:20	1:1280		8							
						DEN		JE									
				RS074	118	395	25	212									
				RS076	44	332	104	341									
				RS089	128	335	37	283									
32. (OPD) 1798306	RS-075 (Jul-24)	ย. วิชาญ เชียงตวงศาวัน	<1:20	1:20	<1:20	1:20	<1:20	<1:20	½	3	(III)	(49.7%)	11500	15	85	(3)	
	RS-148 (Aug-3)	(P-DHF)	1:20	1:80	1:80	1:160	<1:20	1:40									
dengue (?)	(เกิด 24 ก.ค. 2530)					DEN		JE									
				[illegible]	116	3	37	4									
				[illegible]	846	59	72	19									

49a

B.T.	Platelets	C_3	
43.4	300000		เสียชีวิตด้วย ไข้ Infection ตั้ง ๆ ๆ วัน. (Fever of Encephalopathy) มีอาการ ตั้งแต่ 24-34 x ในเวลา 82 กก Hct 43% TT⁻ อาเจียน liver sp⁻ แพทย์ให้ Gr I. (Comk's)
	190000		25 กก. ตรวจเจ้า ออกจากโรงพยาบาล
	360000	100	
36.8 37.6 31.0	194000 ✓ 182000 150000		admit. 21 กก 2530 Observe + Hydration ตั้งไข้ 6 ชั่วโมงก่อนมีไข้ 39°C TT⁻ BP 110/40, 110-120/50-60, PLS หมดสติไว้ เป็น Acute Viral Syndrome หรือ DHF. ปวดเหนื่อย ผล HI สงสัยว่าไข้ DHF/DF
37.3	24,000 ✓		23 กค 2530 BT 37.3 R 32 P 80/50 ร้อนเหงื่อออก มีไข้ 5 วันก่อนมาโรงพยาบาล แพทย์ให้ DHF grade III ให้ 5% D/NSS ให้ภายใน 100 ml เพื่อ load. Ct/XN. 23.24 g albu SP ไม่แดง สภาพดีขึ้น. ✓
	Adequate 178,000 ✓ 190,000		ไม่ admit 21 กค ตัวร้อนมาก เดิน ปวดหัว ไม่ไข้ อาเจียนเรื่อยๆ 3 ครั้ง ถ่ายเหลว 3 ครั้ง 22 กค ปวดศีรษะ ตามองไม่เห็น good ตาแดง, look weak, liver⁻, Hct ไม่สูง, PLS (3+1) 24 กค. เริ่มตาสว่าง, active, look well, ตับโต 2 cm, 27 กค. Hct 36.8 ไม่ซีดจาง
39 41.0 40.0 41.0 35.0 6.0	372,000 192000 248000 65000 24000 30000		22 กค มีไข้สูงเกิน มีไข้ต่อเนื่อง ซึม เห่อ ทำอย่างไรก็ไม่ได้ แต่ตาแดง อาเจียน ไม่ถ่ายปัสสาวะ ปวดศีรษะ: BT 39.2° ตาแดง Liver สูง clot generalization L.P: ICP 18 cm H_2O (L) 2 ml BS/CSF Sugar 107/178 mg/dl อดน้ำ เกิดขึ้น 24 กค. BT 40.8° เริ่ม ท้องเสีย TT⁻ 25 กค. BT 40° Consciousness ต่ำลง Liver 0⁺ 26 กค BT 40.2° มี petechia, ตามแขน ขา มี epistaxis 27 กค มี epistaxis BT 38° ตกเลือด NSS ทดแทน 28 กค อาการดีขึ้น ไม่มีอ่อนเพลีย อาเจียน ตรวจเลือด (N.B: ผป มี confusion ตั้งแต่ T. 40-40.6°C อาจเกิดภาวะ fulmine encephalopathy ได้)
	32,000 ✓ 66,000 152,000		24 กค 2530 admit แพทย์ให้ Gr III. มีไข้ต่อเนื่อง ซึมมาก อาเจียน ไม่ถ่ายปัสสาวะ ตกเลือดมีจุดแดงที่ตัว TT⁺, ESR 23. Hx Gr I. BP แกว่ง 110/60 PLS 120 ครั้ง Hct 45-41 Liver 3 FB แต่งาน ให้ fluid แก้ SP ไม่แดง.
	7200 50000 ✓		admit 22 กค. ตี 19.45 BT 40, P 100, BP 110/50 มีไข้สูง อาเจียน ถ่ายเหลว ไข้ไม่ลดลงแม้ให้ยา เป็นตลอด 3 วัน, liver ⁻, มี Hemoconcentration 39 → 42 % 23 กค 2530: มี BP แกว่ง 1 ครั้ง (100/60) ใน ward 18.00 น. หลังจากนี้ มี petechiae ผิวหนัง TT⁺
	76,000		24 กค มีไข้ต่อ 2 วัน ตัวร้อน look sick, Fever แพทย์ DHF + Shock + impending shock petechia ตามแขนขา. Hct 47.5% not pale, not cyanotic, liver 3 cm drowsy SP 100/110 แพทย์ให้ Grade II-III. BP ตั้งแต่ 120-150/70-80 มีเป็นพักๆ แต่ตัวเย็น ให้ fluid ได้ 25 Hy.

8 9

HN	Serum No.	Name		HI D1	D2	D3	D4	CHK	JE	Age	Day	grade	CBC HCT	WBC	NEU	LYM	ATY	MON
33 (เด็กชาย) 1398456	RS-080 (Jul-29)	ร.ว. [illegible]	(9.45)	1:320	1:320	1:20480	1:2560	<1:20	1:640	6	7	2	42.0	3400	38	49		
													46.0	8500	39	53	12	8
	RS-082 (Jul-26)	S-DHF	(9.19)	1:2560	1:1280	1:20480	1:10240	<1:20	1:2560		8	II-III	44.0	1170	30	36	27	7
	RS-086 (Jul-27)		(10.40)	1:640	1:2560	1:20480	1:20480	<1:20	1:1280		9	III	35.0	7500	27	64	3	9
							DEN		JE									
					RS060	46	296	55	323									
					RS082	94	377	119	416									
					RS086	120	360	118	361									
34. (เด็กชาย) 1553552	RS-081 (Jul-24)	ร.ว. [illegible]	(9.10)	1:80	1:80	1:320	1:320	<1:20	1:80	8	3	3?	43.0	5600	58	33		4
	RS-088 (Jul-27)	S-DHF	(13.00)	1:640	1:320	1:320	1:1280	<1:20	1:640		4		49.5	9000	39	48	11	3
											5		30.0	12800	[illegible]	[illegible]		3
	RS-114 (Jul-29)			1:1280	1:20480	1:2560	1:20480	<1:20	1:640		6		32.0	10900	39	84		
	RS-119 (Jul-30)		(10.30)	1:1280	1:20480	1:20480	1:20480	<1:20	1:5120		7		28.0	11000	66	26		8
(OPD)	RS-217 (Aug-10)			1:10240	1:1280	1:5120	1:1280	<1:20	1:320		16							
							DEN		JE									
					RS 061	29	90	18	78									
					RS 066	132	311	37	258									
					RS 114	174	360	57	347									
					RS 119	171	418	69	434									
					RS 217	84	533	83	373									
35. (ชาย-เด็ก) 1398926	RS-087 (Jul-26)	ร.ว. [illegible]	(21.00)	1:640	1:640	1:10240	1:2560	<1:20	1:640	4	6	IV	38%	26,000	55	27		6
(เด็กชาย)	RS-193 (Aug-7)	S-DHF		1:640	1:320	1:20480	1:1280	<1:20	1:1280				44%					
													30%					
(OPD)	RS-423 (Oct-19)			1:80	1:80	1:320	1:640	<1:20	1:1280									
							DEN		JE									
					RS087	181	315	154	323									
					RS193	225	327	78	332									
					RS423													
36. (เด็กชาย) 1791884	RS-104 (Jul-27)	ร.ว. [illegible]	(15.45)	1:80	1:320	1:160	1:160	<1:20	1:640	7	5	2	48.0	2500	18	64	14	4
1196049																		
	RS-107 (Jul-28)	S-DHF	(9.20)	1:80	1:20480	1:10240	1:10240	<1:20	1:1280		6							
	RS-113 (Jul-29)			1:2560	1:20480	1:10240	1:20480	<1:20	1:640		8		38.0	4900	24	73	3	1
							DEN		JE									
					RS107	166	266	120	268									
					RS107	317	301	168	370									
					RS113	223	395	199	340									
37 (เด็กชาย) 1394328	RS-108 (Jul-28)	ร.ว. [illegible]	(9.30)	1:20	<1:20	1:160	1:20	<1:20	<1:20		(24 mo) 3		37.0	7300	57	29		
											(36 mo) 5		39	5900	69	36		4
											(27 mo) 6		33.0	3100	47	28	35	
ป.น 28 ก.ค.	RS-116 (Jul-29)	R-DHF		1:40	<1:20	1:160	1:40	<1:20	<1:20		4.10 7	2 Y	34.0	3100	46	39	5	3
											8		39.0	10000	68	24		8
	RS-117 (Jul-30)		(9.20)	1:40	1:20	1:320	1:20	<1:20	<1:20		9	1	31.0	4800	25	71		2
							DEN		JE									
					RS106	149	4	36	4									
					RS116	313	14	68	3									
					RS117	238	28	104	5									
38 (OPD) 265030	RS-103 (Jul-28)	ร. [illegible]		1:160	1:320	1:1280	1:2560	<1:20	1:160			II-III	52%	4300	44	40	5	10
(เด็กชาย)	RS-115 (Jul-29)	S-DHF		1:640	1:2560	1:1280	1:5120	<1:20	1:160									81
	RS-132 (Jul-31)		(10.30)	1:20480	1:320	1:20480	1:10240	<1:20	1:640			III						
							DEN		JE									
					RS103	27	137	60	144									
					RS115	55	269	59	261									
					RS132	63	410	70	415									
39. (เด็กชาย) 109245	RS-112 (Jul-29)	ร.ว. [illegible]	(9.15)	1:160	1:640	1:320	1:1280	<1:20	1:80	13	5	2	47.0	3900		36	10	
	RS-131 (Jul-31)	S-DHF	(10.40)	1:1280	1:640	1:1280	1:320	<1:20	1:40		6	?	43.0	3500		40	5	
											7		28.0		19	16	13	2
	RS-211 (Aug-10)	[illegible] ?		1:5120	1:20480	1:2560	1:640	<1:20	1:640		11							
(OPD)	RS-431 (Oct-19)			1:160	1:2560	1:320	1:2560	<1:20	1:640									
							DEN		JE									
					RS112	47	128	51	96									
(R - d3)					RS131	93	356	26	396									
					RS211	55	306	7	288									
					RS431													

SDa

7 Repeat.
เริ่มเจ็บหลัง (ตอนนี้)

B.T.	Platelets	C3
39.6	140000	
37.5	86000	
38.0	18000	
37.6	100	

24 กค. 1 ชั่วโมงก่อน 5 เดือนก่อน · Anorexia, vomiting, abdominal pain, เจ็บคอ, ไข้, G.I. Bleeding (ไม่ชัด)
hepatomegaly. TT+, vomiting stick (ไม่ชัด sign intrapleural eff...)
25 กค. BP แกว 100/60 Hct 45% L53% ATL 12% ม.ค. Hct ขึ้นถึง 47%
26 กค. เริ่ม BP ไม่เกน Hct ลดลง 43% 27 กค. Hct ลดลงเหลือ 35% L64 ATL 3%
Hct adequate.

37-38	188000	
36-37	20000	
	34000	
36	66,000	
	134000	

25 ส.ค. 20 ไข้ลงเดือน 3 วัน BP 80 กว่า ฟังเห็นเสียง ascites ได้ liver 2cm, SP 10cm, no petechiae, Dx. DHF + shock (Grade III) ให้ Hand ให้ treatment BP 90/70
27. BP 80 แรง 30 FFP เกิดภาวะเกือบ Ground office ก็-ตัน, BP ขึ้นอยู่ระหว่าง 70-100/80-90
วันที่ 105/100
28. Weak เริ่มเป็นช่วงใหญ่ BP ดี. Petechiae ลดลง
30 กค. อยู่ดีไม่ค่อยเหนื่อย.

37-40	230,000	
	40,000	
37.5	liver 2cm	

admit 26 July 17.50 DHF ⓝ hepatic encephalopathy. อยู่ในตึก 10 ปีก่อน ตรวจพบ HBsCarrier
ต้องส่งเลือดให้ exchange transfusion, GI bleeding, DIC 6 กค. พบว่า HB carrier ไม่ใช่ manual
วันที่ 26 กค., 1 กค. ไข้ Cellulitis LT leg, 3 กค. Oral moniliasis

37.1	50,000
37.0	
	90000

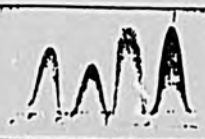
39.0	255,000
39.7	276,000
37.7	64000
37.8	38000
36.7	240000
37.5	78000

24 กค. ไข้ลง 3 วัน อาเจียน ปวดท้องมาก ... ตรวจพบ ... ตับโต 2 cm ... TT+ BP 30/...
25 กค. ซึมลง หมดสติ ไข้ สูงมาก Liver 2 cm ... ataxia EEG ไม่ (diffuse slow wave) ... 26 กค. 27 กค.
5 กค. ... ไม่ปรากฏ ...

37.2	46,000

28 กค. OPD 15.45 น. 5 วันก่อนหน้านี้ปวดท้อง ไข้สูง อาเจียน ถ่ายเหลว TT+ เหลือง ซึมลง Plt - 96
R 28 BP [100/80 – 110/90] ไม่มีไข้ ไข้ 5 วันแล้ว ไม่มี active bleeding liver 3 cm ไม่มี petechiae Hct ... 17.00น.
แพทย์ให้ TT-III เกรงว่า BP แกว pulse เร็วขึ้น R 30. 31. ไข้ขึ้น

37.7	400,000
38.7	80000
	110000
37.3	240,000



ตั้งแต่ 26 กค. มีไข้ 3 วันก่อนมา (OP). ซึมมาก, ใช้เท้าเตะ ... ปวดท้อง
มีอาเจียนบ้าง ไม่ถ่าย, bleeding ดำ (ตรวจพบเมื่อวันที่) Liver 4 cm, TT+ ให้ fluid
มากๆ ให้ Grade I. BP แกว เกือบ (1 ครั้ง วันที่ 26) ... ซึมลงเรื่อย BP 100/50
วันที่ 30. มี pleural effusion sign วันที่ 30.1.

UI 11

	HN	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Day	Grade	CBC HCT	WBC	NEU	LYM	ATY	MON
40 (ผ่าตัด)	1799131	RS-118 (Jul-30)	ด.ดิศรัณ์ ศิริพร (9.30)	1:20	<1:20	1:20	1:40	<1:20	1:40	7	5	Not DHF	37%	6400	33	54		5
		RS-130 (Jul-31)	Not DHF (9.29)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20			DHF	34%	7600	56	38		81
		RS-141 (Aug-1)	(9.15)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20			@ CF Reply 70%						
(OPD)		RS-219 (Aug 10)		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
							DEN		JE									
					RS 118	8	7	16	26									
					RS 130	11	4	36	18									
					RS 141	6	9	10	4									
					RS 219	0	0	0	0									
41. (OPD)	1798932	RS-122 (Jul-29)	ด.ชัยวรา จุ่มโต	1:80	1:160	1:320	1:80	<1:20	1:160	13	6	DF.	40%	3200	53	30	3	51
		RS-133 (Jul-31)	S-DHF	1:2560	1:640	1:10240	1:1280	<1:20	1:320			ช็อค I	34%	3900	28	72	20	
							DEN		JE									
					RS 122	13	98	25	92									
					RS 133	25	360	23	583									
42. (OPD)	1799973	RS-123 (Jul-29)	ด.อัครวรรณ์ แป้งบุกต์	1:20	1:20	1:80	1:80	<1:20	1:20	14.11	2	I	42.0	6900	75	4		
		Jul 31 RS-219 (Aug-10)	P-DHF	1:160	1:40	1:80	1:80	<1:20	1:40		4	Not DHF ?	38.0	3600	29	69		1
							DEN		JE									
					RS 123	10	28	24	38									
					RS 219	8	20	1	22									
43. (OPD)	898027	RS-125 (Jul-29)	ด.ศุภชัย ชนะสุนทร	1:320	1:80	1:320	1:160	<1:20	1:40	8½	4	I.	39%	5500	33	58		3
		RS-135 (Jul-31)	S-DHF	1:320	1:80	1:640	1:40	<1:20	1:40									35
		(Q. = d3)					DEN		JE									
					RS 125	11	60	33	54									
					RS 135	7	63	8	50									
44. (OPD)	785235	RS-126 (Jul-30)	ด.ศุภกร เพียงจิตต์ (11.00)	1:40	1:20	1:80	1:80	1:40	1:20	13	4	II.	สั่นๆ	6200	68	30		2
		RS-140 (Jul-31)	S-DHF	1:160	1:160	1:1280	1:640	1:80	1:640					2800	44	49	4	
							DEN		JE									
					RS 126	17	24	35	31									
					RS 140	18	170	24	159									
45. (OPD)	788918	RS-127 (Jul-30)	ด.วิจิตรกรณ์ ชุติอนันต์	1:640	1:80	1:1280	1:80	<1:20	1:80	9.06	7	I	38.0	4400	51	31	5	4
(OPD)		RS-205 (Aug-10)	S-DHF	>1:20480	1:640	1:2560	1:1280	<1:20	1:1280		17							
							DEN		JE									
					RS 127	108	189	65	175									
					RS 205	90	455	29	359									
46. (OPD)	1800229	RS-128 (Jul-30)	ด.นิรัช ศักดาศิลป์	<1:20	1:20	<1:20	<1:20	<1:20	<1:20	9 ขวบ	2	II	38.0	7600	87	5		8
(OPD)		RS-139 (Jul-31)	S-DHF	1:40	1:20	1:80	1:40	<1:20	1:20				39.0	3400	52	30	2	10
* (Q = d3)		RS 139	อยู่ห้องหลัง P. 37				DEN		JE									
					R. 128	19	5	45	38									
					RS 139	23	14	35	40									
47. (OPD)	801318	RS-134 (Jul-31)	ด.วรรณวรา ศรีสุข	1:2560	1:640	>1:20480	1:2560	1:160	1:320	12	6	I	ALS	2,800 5,100	48 37	30 20	10% 30%	12 13
		RS-201 (Aug-10)	S-DHF	1:640	1:1280	1:10240	1:1280	1:80	1:1280									
							DEN		JE									
					RS 134	40	373	39	343									
					RS 201	40	377	35	317									
48. (ผ่าตัด)	1093217	RS-142 (Aug-1)	ด.รุจิรา เหล็กศุภวิทิตร (12.10)	<1:20	<1:20	1:80	<1:20	<1:20	<1:20	6	8	I						
125/1.8		RS-203 (Aug-2)	P-DHF (9.30)	<1:20	<1:20	1:80	1:20	<1:20	<1:20				35	6,200	15	53	19	
(OPD)		RS-202 (Aug-10)		1:80	1:80	1:640	1:160	<1:20	1:40									
							DEN		JE									
					RS 142	133	7	33	0									
					RS 203	122	2	20	0									
					RS 202	200	24	124	9									

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BT	Platelet	Cs
37.8	136000	เช้า 30 ก.ค. 30 อาเจียนเลือด ถ่ายดำ 1 ครั้ง ซึมลงบ่าย ส่ง ER. Hct 24,000, BP 100/60 pulse 100/min TT- ให้เลือดไปตรวจ DHF + GI bleeding ให้เลือด stace → Hct 29% liver - (1 ส.ค. 30)
	130,000	
	213,000	28 ก.ค. (19.45) มีไข้มา 2 วัน อาเจียน มาด้วยปวดท้อง TT+ WBC 4300 N29 L17
	220,000	PLT 260,000 ผลวันที่ 28 29 ก.ค. ซึมลง ไม่รู้สึกตัว อาเจียน ถ่ายเหลวไม่มีมูกเลือด มีตาเขียว pulse 72/min BT 38.5 BP 140/90 good consciousness admit ผลวันที่ 30 ก.ค. ป่วยวันที่ 6 BP 110/70 การวินิจฉัย Acute viral infection
38.8	164000	29 ก.ค. มีไข้มา 2 วัน อาเจียน 1 ครั้ง ไม่มีปวดท้อง TT- ไม่มีเลือดออก bleeding ไม่ค่อยรู้สึกตัว ก่อนวันที่ 29 ก.ค. ผลตรวจ OK
36.2	296000	
37.0		
	276,000	เช้าวันที่ 27 ก.ค. 2530 (21.15) T38.8, P60, BP 100/70 มีไข้มา 1 วัน มีอาเจียนเล็กน้อย การวินิจฉัย TT-II TT+, liver just palpable, heart ตามปกติ ฟังปอด ปกติ, การวินิจฉัย Acute viral Infection, fever cause,
38.5	132000 ส่งตรวจ	15.30 น. ผลตรวจวินิจฉัยจากเลือด DHF เหมือนกัน ไม่มีอาการ viral infection, fever + vomiting
41.0		27 ก.ค. 2530 ER 15.30 น. เป็นไข้มา 3 วัน ไม่มีปวดท้อง อาเจียน ถ่ายไม่ค่อยออก 2 วัน ตอนนี้ซึมลง
41.5 (ส.ค.)		ตอนนี้ TT- 20 ml 2 ครั้ง Hct 38% Platelet ต่ำ Extremely มีอาเจียนเป็นน้ำสีกาแฟ coffee ground BP 110/70 weak
42.0		29. → Hct. สูงขึ้น ไข้ลดลงตลอด เสียชีวิตเมื่อวันที่
ACL 39.5		30 ก.ค. (3.10 น.) pulse 120 TT+ Grade I-II admit OW อาการทั่วไปดี liver -
37.8	138000	Hct 39. ผลวัน FU ปกติ
	148,000	29 ก.ค. 2530 (20.00) เริ่มมีไข้เมื่อวันที่ 27 ก.ค. ถึงวันที่ 29 ก.ค. ไม่อาเจียน ไม่ถ่ายเหลว ปอดปกติ 100/60 pulse 140/
	170,000	BT 38.5 เพลีย TT- ซึมลง ตกตัวลดลง 30 ก.ค. 2530 ER (13.15) ไข้ลดลง เหงื่อออก
		P 140, BP 100/70, no petechiae, TT+ ให้ 5% D/N/2 500 cc แผน 5 ธ.ค. (อาเจียน, ถ่ายเหลว)
	136,000	27 ก.ค. 2530 แผนก ER 18.30 น. เป็นไข้มา 2 วัน ไม่ถ่ายเลือด หรือดำ ถ่ายวันละ 1 ครั้ง BT 38.2
	96,000	P 100, BP 120/80 เริ่มวันที่ 30 ก.ค. เหนื่อย ไม่มีแรง ปวดท้อง หรือดำ TT+ วันที่ 31 ก.ค. เหนื่อยไม่มีแรง
		TT-, DHF อาการดีขึ้น ผลตรวจ follow up ครั้งต่อไป (ถ้ามี) ไม่ต้อง admit
38.7		เหนื่อยง่าย Grade I ไข้สูง มา 7 วัน ก่อนมา รพ. BP 100/70, P120, ใจสั่น, liver 4 cm
88,000		มีอาเจียน, ถ่ายเหลว เล็กน้อย ปอดแดง. ความดัน BP ต่ำ. มี Convalescent
95%		วันที่ 3. ให้ IV fluid เกลือ 2 วัน
35% (ส.ค.)	Cs 107 + Cs 1/P 1/1	
	Cs 137 + Cs 1/P 2/1	
	Cs + N	

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	HN.	Serum No.	Name		HI D1	D2	D3	D4	CHIK	JE	Age	Dog	Grade	CBC HCT	WBC	NEU	LYM	ATY	MON
49. (OPD)	72 1143239	RS-144 (Aug-3)	ช. บุษบา หอมหวล	(9.10)	1:320	1:1240	1:1920	1:640	<1:20	1:640	9.11	5	-b	32.0 / 36.0	7100 / 8300	74 / 94	39 / 89	13 / 19	6 / 9
		RS-151 (Aug-4)		(9.00)	1:10240	1:1280	1:2560	1:2560	<1:20	1:640		1	II?	32.0	12900	82	64	6	2
		RS-159 (Aug-5)	DEN JE	(9.10)	>1:20480	1:2560	1:5120	>1:20480	<1:20	1:1280		7		33.0	8800	11	76	9	
(OPD)		RS-210 (Aug-10)	M G / M G		>1:20480	1:1280	1:5120	1:10240	<1:20	1:2560		12							
			RS144: 240 263 / 216 240																
			RS151: 258 353 / 278 343																
			RS159: 321 363 / 236 314																
			RS210: 222 566 / 196 373																
50. (OPD)	67 1123047	RS-149 (Aug-3)	ช. สุวิทย์ สุวรรณรัง	(13.15)	1:1280	1:640	1:10240	1:1280	<1:20	1:1280	7	8	X	37.0	4900	40	52	5	6
		RS-152 (Aug-4)	S-DHF	(9.16)	1:2560	1:1280	1:5120	1:2560	<1:20	1:640		9		32.0	11800	46	91		3
		RS-160 (Aug-9)	D: M G / JE: M G	(9.15)	>1:20480	1:2560	1:1280	1:640	<1:20	1:1280		10		34.0	8700	50	34		16
(OPD)		RS-213 (Aug-10)	RS147: 139 360 / 34 245; 131 322 / 22 361; 160: 426 392 / 30 343; 213: 68 469 / 19 359		1:10240	1:1280	1:5120	1:1280	<1:20	1:2560		19							
51. (OPD)	31 1138600	RS-149 (Aug-3)	ช. [illegible]		1:160	1:160	1:320	1:80	<1:20	1:160	8	4	2	38.0	3800	70	23		5
(เด็กชาย)		RS-150 (Aug-4)	S-DHF	(9.00)	1:1280	1:160	1:640	1:320	<1:20	1:640		5	เด็กชาย III	44.0	7300	17	76	12	2
		RS-161 (Aug-7)		(9.10)	1:5120	1:1280	1:1280	1:640	<1:20	1:1280		6		43.0	9000	27	59	9	2
		RS-178 (Aug-6)	D: M G / JE: M G		1:5120	1:1280	1:1280	1:2560	<1:20	1:1280		7		39.0	6300	40	41	6	11
(OPD)		RS-208 (Aug-10)	149: 60 136 / 21 99; 160: 110 246 / 69 225; 161: 104 322 / 63 303; 178: 93 351 / 71 320; 201: 72 431 / 69 353		1:2560	1:160	1:5120	1:1280	<1:20	1:1280		11							
52 (OPD)	80 1331615	RS-133 (Aug-2)	ช. [illegible]		1:320	1:80	1:320	1:320	<1:20	1:80	6.03	5	1	39.0	3800	39	47	10	4
✓ DEN-2		RS-138A (Aug-5)	S-DHF		1:5120	1:320	1:640	1:160	<1:20	1:320		6		39.0	6700	10	84	6	
(เด็กชาย) โรงเรียน		RS-176 (Aug-6)	D: M G / JE: M G	(9.00)	<1:20480	1:640	1:640	1:640	<1:20	1:320		7							
			151: 99 / 3 38; 174: 95 235 / 16 137; 176: 117 274 / 16 167																
(a = d2)																			
53 (OPD)	1801030	RS-184 (Aug-4)	ช. [illegible]		1:640	1:160	1:640	1:640	<1:20	1:320	11	5	10	47.0	[illegible]	36	58		6
		RS-184 (Aug-7)	S-DHF		1:2560	1:1280	>1:20480	1:1280	<1:20	1:2560									
		RS-236 (Aug-14)	D: M G / JE: M G		1:640	1:1280	1:5120	1:1280	<1:20	1:2560									
			154: 24 163 / 10 112; 154: 52 323 / 52 358; 236: 114 410 / 29 353																
54. (OPD)	1801626	RS-155 (Aug-4)	ช. [illegible]		1:1240	1:160	1:640	1:640	<1:20	1:320	6	5	II	36.0	8600	21	62	10	[illegible]
		RS-236 (Aug-13)	D: M G / JE: M G		1:320	>1:20480	>1:20480	1:2560	<1:20	1:2560									
			155: 84 195 / 6 177; 236: 76 474 / 57 378																
55. (เด็กชาย)	40 1132250	RS-153 (Aug-4)	ช. [illegible]	(15.45)	1:320	1:320	1:320	1:160	<1:20	1:320	3.05	3	X	42.0	6900	49	32	15	
		RS-162 (Aug-9)	S-DHF	(10.40)	1:5120	1:640	1:160	1:320	<1:20	1:640		8	IV	39.0	10000	33	63	13	
(OPD)		RS-221 (Aug-10)	D: M G / JE: M G		1:640	1:160	1:1280	1:640	<1:20	1:160		13							
			157: 123 216 / 23 249; 162: 142 269 / 53 283; 201: 98 362 / 11 232																

52a

O.T. Phalada C3 L

37.8 136000
39.5 92000
37.0 96000
37.0 78000

วันแรก ER. 0 หด (21/43) Hct 55% ปลายก้อนมีน้ำ, TT+, ซีด เหลือง, BP 90/50 ให้ fluid ตลอดคืน (3วัน) มีอาเจียน กลืนไม่ได้. BP 90/60 – 100/60, P 104-132, R 24-32 วันที่ 4 รับอาหารเหลว. ไม่มีไข้. ไม่มีอาเจียน BP 80/50 – 90/60, P 98-120, R 18-12, 5 หด ท้องเสียไม่มาก ปลายคอ มีน้ำมูกเกาะเรื้อง ให้ IV ตลอดวันเรื่อยๆ ใน ward.

37.2 1000
50000
166000
37.0

วันที่ มีไข้ต่ำ 7 วัน อาเจียนไม่ได้ ไม่ถ่าย ปวดท้องน้อยด้านขวา แพทย์วินิจฉัย ลำไส้อักเสบ 2 หด (อุจจาระ) อาเจียน เวลา 2-3 ครั้ง Hct ลดลงเรื่อย 37% ให้เลือดทดแทนทางเส้นเลือด. ตาบวม, puffy eye lid, แพทย์ให้ DHE ตาหนังตาบวม ปรากฏ @ pleural effusion. เดิม 5 ตอน แต่ตอนนี้ท้องอืด ท้องผูกมาก ปวดท้องเรื่อยๆ ปวดแก้ไม่ได้ ปวดบริเวณ 120-124 ครั้งต่อนาที R 30-40/ BP 100/60 – 103/65 ต่อมาเป็นไข้ BP 90-100/60, P 120-124, R 24-32 ไม่มีไข้, BP ต่ำ.

134000
102000
12000
50000

มีไข้ 4 วัน ต่อมา Aug 3. อาเจียน ถ่ายเหลว T35, BP 110/70 (100/60), PR 120, liver 3 cm, ตับโตมาก ต่อมาเริ่ม Jaundice ต่อมาก็ BP ไม่แสดงอาการเกี่ยวกับ RR (26/min) Cavity-filling ไม่ได้ วันที่ 6 อาการไม่ดีขึ้น เลยตัดสินใจส่งต่อ. เลยให้ Gluc III ? ให้ IV ตลอดเวลา. 5% D/N/SS → 140 ml/hr (m+10/D) ปัสสาวะ เริ่มลด เหลือ 120 ml/hr ตลอดวันที่ 6 ครั้ง

39.0 20000
40000

เช้าวัน 3 (15.20) หด 2530 ไข้ ตัวร้อนจัด กลางคืน ถ่ายเหลว มีน้ำมูกเล็กน้อย อาการซึมไม่ได้ BP 38.3°C L 3 cm 4 หด 2531 ซึมมากขึ้น ปัสสาวะ และอุจจาระ ไม่ได้ Liver 3 cm Hb 12.7, Hct 39, Platelet 90,000 5 หด มีไข้ ซึมลง ชักเกร็งเล็กน้อย 10 หด 2530 หมดสติ ตลอด

40.3 96,000

วันที่ 3 หด มีไข้ต่ำๆ 3 วัน ปวดท้อง ถ่ายเหลว ไม่มีน้ำมูก อาเจียน ซึมลง ปัสสาวะน้อย ไม่ถ่าย ท้องอืด ไม่ค่อยรู้สึกตัว 3 วันก่อนหน้านี้ BP 110/70, T 40.3, P 84/m, R 20/m BP แกว่ง look weak, no dyspnea, not pale, no jaundice, no petechiae ตื่นเต้น 4 หด TT+ 12-13/m² liver 1 cm, 7 หด ชักเกร็ง มีน้ำลายฟูมปาก (ปากเบี้ยวทางขวา, ตาซ้ายเล็ก เล็กน้อย) อาเจียน แกล้งไม่ได้ 2 ครั้ง ตลอดวัน
Imp. • oliekungunya/DHF weak

38.5 62,000

Aug 4 ป่วยมา 5 วัน ซึมลงไม่ได้ เบื่ออาหาร ตัวร้อนจัด อาเจียนมากขึ้น ถ่ายเหลว BP 100/60, TT+ มีน้ำมูกเล็กน้อย ซึมลง look dull looking Aug 6. ตัวเหลืองมาก ตับโตมาก Liver – Aug 13. ดีขึ้น ตอน 3-4 วัน.

22000
26000

ตอน Repeat

ซึมลง → เหมือนเดิม

BR 13.45 น. Aug 4. อาเจียนมาก 5 วัน ถ่ายเหลว ไข้ต่ำๆ ไม่ได้ Hct 43.5% มีไข้ 1 ครั้งตอนเช้า P 120/min, BP กลางๆ 80, liver 3 cm. ไข้ลดลงตลอด ตัวเย็น มีรู้ตัว ชีพจรเต้นเร็ว BP ต่ำ ม่าน weak แต่ BP 110/90 ตุ่มนูน 2 วัน 4-5. วันที่ 5 ผื่นแทนที่ด้วยไข้ ตัวเย็น

	HN	Serum No.	Name	HI D1	D2	P3	D4	CHIK	JE	Mp	Day	grade	CBC HCT	HBC	NEU	LYM	ATY	MON
56. (ผู้ป่วย)	100 1801914	RS-138 (Aug-4)	ญ. กานต์ ศักดิ์กรม (16.00)	<1:20	1:20	1:20	1:40	<1:20	<1:20	0,10	5 6	2	40.0 34.0	9,000 9000	19 19	84 73	7	1 1
ผล/EIA ผน. ล.=ก		RS-163 (Aug-9)	P-DHF (10.49)	1:40	1:80	1:40	1:80	<1:20	1:20		11	5						
(OPD)		RS-219 (Aug-10)		1:80	1:80	1:160	1:160	<1:40	1:40		16							
											38							
57. (OPD)	135 1294196	RS-164 (Aug-9)	ญ. คุณหา หนาจี	1:2560	1:640	>1:20480	1:640	<1:20	1:640	11	3 6	III	40.0	3600	52	32		
(ผู้ป่วย)		RS-172 (Aug-9)	S-DHF (13.19)	>1:20480	1:2560	1:5120	1:320	<1:20	1:320									
		RS-177 (Aug-6)	(9.10)	1:10240	1:2560	1:640	1:5120	<1:20	1:1280		7	1	41.0	7200	26	38	28	
		RS-185 (Aug-7)	(9.20)	1:1280	1:1280	>1:20480	1:5120	<1:20	1:1280		8		39.0	5200	7	73	12	6
(OPD)		RS-232 (Aug-12)		>1:20480	1:2560	>1:20480	1:5120	<1:20	1:320		15							
58. (OPD)	90 1462782	RS-169 (Aug-9)	ญ. ณนิต ผ่องโรค	<1:20	<1:20	<1:20	<1:20	<1:20	1:20	3.12	1	1						
ไข้ไม่ทราบ		RS-179 (Aug-6)	ถ้วิต	<1:20	<1:20	1:20	1:40	<1:20	1:20		2		36.0	6000	28	47	1	11
(a=d3)		RS-240 (Aug-13)		1:320	>1:20480	1:5120	1:1280	<1:20	1:1280		9							
59. (OPD)	102 1801993	RS-167 (Aug-9)	ด. สันทนีย์ ชิปปักขา	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	9	2	II	34.0	4000	73	16		
		RS-179 (Aug-6)	NSD - (Aug 9)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20		4							
(OPD)		RS-214 (Aug-10)		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20		8		36.0	7200	69	29		
60. (OPD)	60 1934996	RS-169 (Aug-9)	ด. ปรมาจันท์ ศิลปุมิวิถี	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	2.09	6	D-DHF	33.7	4400	18	77		9
		RS-187 (Aug-7)	NSD - (Aug 9)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
		RS-266 (Aug-12)		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
61. (OPD)	65 1996969	RS-170 (Aug-9)	ญ. ดุจา ตั้งสัมพัน	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	1.10	2	นอนไข้ fever	36.7	18,100	48	47	2	3
		RS-238 (Aug-13)	NSD ต่อ N-DHF	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
62. (OPD)	29 1039269	RS-173 (Aug-9)	ด. วีรศักดิ์ ทศวา	1:1280	1:640	1:640	1:320	<1:20	1:640	7	4 5 6	1	39.0 40.0 39.0	5000 3900	45 38	52 40	1	13
(OPD)		RS-220 (Aug-10)	S-DHF	1:2560	1:160	>1:20480	1:5120	<1:20	1:2560		10							
63. (OPD)	13 1701205	RS-168 (Aug-9)	ญ. นฤมล อนาศิริวาศ์	1:40	1:80	1:160	1:160	<1:20	1:40	0.10	5		36.0	9000	5	74	12	4
		RS-216 (Aug-10)	P-DHF	1:160	1:160	1:320	1:320	<1:20	1:80	10/20V	10							

	D3 Mpx		D3 B	
	m	ข	m	ข
168	265	5	120	0
216	304	150	270	76

D.T.	Platelets	Cs
37.2°	36,000	
37.5°	14000	
		Cs 4 N.
		เกล็ดเลือดกลับเป็นปกติ
		Rapid C-T
40.0	100000	
39.8	42,000	
38.8	88,000	
37.0	160000	Cs วัณโรค + วัณโรค
37.0		Cs 4 N.
38.3		
39.8	158000	
37.0		Cs. N.
	28,000	
	296,000	Cs-N
	135,000	
	234,000	
	166000	
5	142000	
	(24000)	

หลังจากต่อมาไข้ ปวดศีรษะ Hct 45% WBC 5900 Plan 12, 6 ง, Lym 65, Mty 3, Promel, plt 21,000

Aug 3: รู้สึกร่างอ่อนเพลีย ปวดศีรษะ ตาลาย BT 37.2° ฝน+(ตกใจ) ตกเลือดใต้ผิวหนัง Liver 2 cm TT⁻ VSS (IV)

Aug 4 ตื่นขึ้น มีผื่นแดง

Aug 5. กลับบ้าน

ตกเลือดใต้ผิวหนัง Hct 43 % ซีดเล็กน้อย ไข้ 38.4/m, P 72/m, BP 100/70, BP ↓ 70/50 – 80/60 ตกเลือด Hct ลด 41 % มีไข้ 6 ชั่วโมง ATA 1:16 BP 80/50 – 100/30 Hct 42 % BP ดีขึ้นเล็กน้อย มีไข้ Hct ลด 34 % มีไข้ 2. เดือนต่อมา Hct 37% มีไข้ 5 Hct 46 % BP drop 80/35 แทบช็อค ต่อเป็น Shock stage

5. ลด มีไข้ปวดศีรษะ ไม่ผื่น ไม่ไอ ไม่เจ็บคอ ต่อมาน้ำเหลืองโตทั่วตัว แทบไม่มีไข้ Fever: viral infe R/O DHF ไม่ใช่ 6 ลด พบเกล็ดเลือดต่ำ epigastrium ถ่ายดำ 1 ครั้ง 13 ลด ตกเลือด

5 ลด ไข้ลง 3 วัน ไข้ ขึ้นอีก ไม่มีไอ เจ็บคอ ไม่มีน้ำมูก ผื่นขึ้น มีไข้ 3 วัน clinic ลด TT⁺ (+) skin petechiae แพทย์ให้ Gr II. liver 1 cm. ↓ 2 cm

Aug 4 Hct 120,000, WBC 4600, ไข้ 4 วัน ถ่ายเหลว อาเจียน. R/O DHF ส่ง follow up.
Aug 5. กลับจาก ไม่มีไข้ liver not palpable,
Aug 7 ไข้ขึ้นใหม่ เบื่อ เซื่องซึม ไม่ค่อยพูด ไม่มีรายที่ออกมา. แพทย์ให้ Viral Infection.
Aug 17. follow up อาการดี 24 ลด. Scrotum ผ่าตัด / Lt chronic ส่ง surgeon.

18.45 น. มีอาเจียน 1 ครั้งก่อนมา มีไข้ Febrile convulsion. ให้ paracet, phenobarb. ส่งมา follow up. 1 wk เมื่อวาน ตื่นขึ้น 13 แพทย์ให้กลับบ้าน 5 วัน.

4 ลด มา ER (14.05 น.) T 39.2° มีไข้ 4 วัน fever (+) lymphadenopathy TT ใต้ขาก เด็กดูเจ็บป่วยมาก TT⁺ edema obtuse ตื่นขึ้น 5 ไข้ต่อเนื่อง viral ผื่นขึ้น ตื่นขึ้น

5 ลด ชัก ก่อนมา 5 วัน ไข้ลงแล้ว มีผื่นขึ้นทั่วตัว ไข้ มี erythematous maculo-papular rash ตับ 3-4 cm ↓ R/O DHF – chickungunya – Shock, Viral Exanthema

	HN	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Day	grade	CBC HCT	WBC	NEU	LYM	ATY	MON
68. (OPD)	108 1802903	RS-185 (Aug-7)	ด.ด็กชาย ต้นหาใบเล่ม	<1:20	<1:20	1:20	1:40	<1:20	<1:20	6	4	III-IV	37.0	11900	18	12		8
		RS-316 (Aug-24)	S-DHF (VAS/EIA)	1:2560	1:640	1:10240	1:1280	<1:20	1:1280		5 6 7 8	(III)	37.0 43.0 34.0	8800 7100 11400	43 37 49	30 10 49		6
69. (OPD)	70 1631171	RS-186 (Aug-7)	ด.ชัยพลกิน แก้วคำ	1:160	1:80	1:2560	1:320	<1:20	1:160	9	21 5	II	44.6	11000	8	89		
(OPD)		RS-196 (Aug-8)	S-DHF	1:1280	1:320	1:5120	1:1280	<1:20	1:1280		6		42.0	11000	8	84	13	1
(OPD)		RS-249 (Aug-14)		1:5120	1:5120	1:5120	1:10240	<1:20	1:2560		12		32.0	10900	18	74	1	2
66. (OPD)	46 1449676	RS-188 (Aug-7)	ด.ศศิกาล์ ภาคไชย	1:640	1:1280	>1:20480	1:640	<1:20	1:320	9.09	4	II	37.0	5900	48	39		6
(ส่งกรุ)		RS-198 (Aug-8)	S-DHF (9.10)	1:640	1:1280	>1:20480	1:1280	<1:20	1:640	วันสิ้นเดือน	6		37.0	6900	33	67		
(OPD)		RS-207 (Aug-10)		1:5120	1:640	>1:20480	1:1280	<1:20	1:640		8							
67. (OPD)	26 1053104	RS-189 (Aug-7)	ด.ทรงพล พงษ์ธกิน	1:320	1:640	1:1280	1:640	<1:20	1:640	12	4	II	30.0	4200	66	33	4	
(OPD)		RS-231 (Aug-13)	S-DHF	>1:20480	1:1280	>1:20480	1:5120	<1:20	1:640		7		40.0	3100	46	54		
											6		42.0					
68. (ส่งกรุ)	101 1801951	RS-190 (Aug-6)	ด.ธนภัทร์ ศีหนาท	1:640	1:1280	1:1280	1:1280	<1:20	1:2560	2 1/2	5	III	35% 31.5	17000 12200	30 30	54 54	10 13	6 6
		RS-192 (Aug-7)	S-DHF	1:1280	1:2560	1:5120	1:2560	<1:20	1:10240		6	BP 150	28.0	10800	70			1
(OPD)		RS-212 (Aug-10)		>1:20480	1:640	>1:20480	1:640	<1:20	>1:20480		9	70						
69. (ส่งกรุ)	72 1246232	RS-191 (Aug-6)	ด.ศิริกา เลี่ยงยุค	1:80	1:40	1:160	1:80	<1:20	1:40	5	3 5 6	2	32.0 37.0	9[illegible] 100	56 56	41 34		3 3
		RS-193 (Aug-7)	S-DHF	1:320	1:320	1:1280	1:640	<1:20	1:640		7		38.0	7800	29	52	6	13
(OPD)		RS-206 (Aug-10)		>1:20480	1:640	1:5120	1:1280	<1:20	1:640		10							
70. (OPD)	628228	RS-194 (Aug-7)	ด.คมศักดิ์ ศรีศิริ	1:160	1:640	1:640	1:640	<1:20	1:1280	10 8/12	6	I			32	45	1	1
		RS-240 (Aug-13)	S-DHF	>1:20480	1:1280	>1:20480	>1:20480	<1:20	1:640			[illegible] 7						61
71. (ส่งกรุ)	1803137	RS-197 (Aug-8)	ด.วุฒิพร ศุภโชคชัย (9.10)	1:80	1:40	1:160	1:160	<1:20	1:160	13	6	II	43%	5,200 20,000	59 60	41 28	1	2
		RS-198 (Aug-9)	S-DHF (9.20)	1:160	1:160	1:1280	1:320	<1:20	1:640		7		41%					89
		RS-199 (Aug-10)	(9.15)	1:320	1:640	1:2560	1:1280	<1:20	1:640									

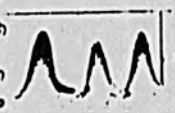
B.T. | Platelets | Cs

[illegible] 7 สค 17.4 [illegible] coffee ground ไข้ TT+ DHF II ⊕ [illegible]
BP 100/60 PR 80 [illegible] 12 [illegible] ไม่มี Hemoconcentration
[illegible] Hct 43% [illegible] DHF [illegible] CAC มี slight
to the left. 13 สค [illegible] ดีขึ้น BP ปกติ [illegible]

246000
132000
150000
106000
90000
Hct 43,000

5 สค 2530 เริ่ม T 40.7°C BP 120/70 มีไข้ + สค ไข้ลง ไม่อาเจียน 1 ครั้งก่อนมาโรงพยาบาล มีไข้
liver 1 cm ↓ TT+ 2 opt. 7 สค 2530 BT 39.3. BP 90/60 ไข้ลดลง 200 cm ปัสสาวะ
ไข้ ปวดท้อง liver 3 cm. TT+ ให้ยาลดไข้ไม่ได้ผล 23.53 น. ส่ง ER pulse 120 ครั้ง
BP 110/50 Hct 42.5 ✓ DHF II. admit.

92000
90000
92000
492000
Cs sp+ Cs 90% + Cs sp 10%
Cs sp− Cs 90%
Cs sp− Cs 9 11

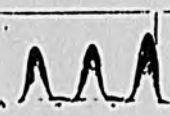


6 สค 2530 12.15 น. อาเจียนเป็นน้ำเรื่อย 3 ครั้งก่อนมาถึง มีไข้ 5 วัน ไม่ได้ไข้ลด
TT−

58000 6 สค
16000 7 สค

ER 18.50 น. ไข้ลดลง 4 วัน อาเจียน petechiae กระหายน้ำ good consciousness, tachycardia, liver 2 cm
แพทย์ให้ grade II ให้ 5% D/N/2 500 ml. 7 สค ไข้ลดลงเรื่อยๆ

2800(6)
2200(6)



4 สค 18.55 ER มีไข้ 4 วัน PTA ไม่ได้ไข้ลด ปวดท้อง pulse 140. BP 100/70 liver 1 FB ↓
no petechiae TT+ ให้ [illegible] 5 สค liver 3 cm ซึมลงบ้าง ไม่อาเจียน
ปวดท้อง admit. ONI 6 สค VS stable ไข้ลดลง 7 สค ซึมไม่มากขึ้น BP ปกติ
BP 100/50

47,000
25000

170,000
40000
8000

ซึมมาก 7 สค 30 มีไข้ลด 5 วัน ไม่ได้ไข้ลด

29,000

85,000
102,000
46,000
38,000

มีไข้ลด 4 วัน ก่อนมาร.พ. (8 สค) เพลีย ไม่ทุกข์ทรมาน มีเลือดออกทางช่องคลอด PR 142
RR 24 BP 110/80 [illegible] Hct. [illegible] 46,000 [illegible]
ตกเลือดเล็กน้อย

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	HN	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Day	Grade	CBC HCT	WBC	NEU	LYM	ATY	MON
72. (OPD)	1503292	RS:219 (Aug-9)	ร. ธารีสกุล ทุกข์ทั่วไป	1:40	1:160	1:640	1:320	<1:20	1:80	3/12	3	(III)	39	3000	50	30		
VAS/EIA		RS:220 (Aug-10)	S-DHF	1:320	1:640	1:2560	1:1280	<1:20	1:320				34	5600	11	13	22	3
		RS:224 (Aug-11)		1:640	1:640	1:5120	1:1280	<1:20	1:640					1:800	25	48	11	2
73. (OPD)	104 1802402	RS:203 (Aug-10)	ร. จิรพุทธ ศรีโท	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	7	6	II	32.0	1200	74	15	2	
		RS:223 (Aug-11)	(P-DHF)	<1:20	<1:20	1:40	<1:20	<1:20	<1:20		7		34.0	4300	15	35	24	1
					Dengue		JE											
			803	M 68	G 0	M 7	G 0											
			228	114	6	10	0											
74. (OPD)	1992111	RS:209 (Aug-10)	ร. ชูวิทย์ ศรีวาสศรี	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
		RS:229 (Aug-11)	NSD	<1:20	<1:20	<1:20	1:20	<1:20	<1:20									
75. (OPD)	104 1803925	RS:209 (Aug-10)	ร. ภาณุวัฒน์ จ่อยุทธ	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	4.10	5	I	33.0	4100	28	52		4
		RS:243 (Aug-13)	(P-DHF)	1:20	<1:20	1:160	1:40	<1:20	<1:20		6							
		day 30			Dengue		JE											
			209	23	4	0	2											
			243	198	23	16	0											
76. (OPD)	106 1803300	RS:226 (Aug-11)	ร. เจริญศรี จงบำเพ็ญ	1:80	1:40	1:320	1:160	<1:20	1:40	7	4.5	I				34		
DHF		RS:287 (Aug-20)	S-DHF	>1:20480	1:1280	>1:20480	1:5120	<1:20	1:2560									
		(a=d2)																
77. (OPD)	108 1804008	RS:228 (Aug-11)	ร. ชัยวัฒน์ กรุณศรีพิทักษ์	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	3.06	4	I	35.0	7000	43	53	6	1
(OPD)		RS:234 (Aug-12)	P-DHF (10.00)	<1:20	1:20	<1:20	<1:20	<1:20	<1:20		5		33.0	5300	42	44		
		day 30			DEN		JE											
			228	18	14	16	0											
			234	13	22	0	0											
(a=d3)																		
78. (OPD)	1069843	RS:230 (Aug-11)	ร. ทรงพล ตั้งจันทร์ (9.50)	1:2560	1:80	1:320	1:80	<1:20	1:40	6.07	4	I	40.0	3200	56	40	3	3
		RS:233 (Aug-12)	S-DHF	1:5120	1:160	1:2560	1:640	<1:20	1:640		5		40.0	3400	13	33	15	3
		RS:273 (Aug-17)		>1:20480	1:2560	>1:20480	1:5120	<1:20	1:1280		10							
79. (OPD)	74 1706494	RS:231 (Aug-11)	ร. วิจิตร ศรีแพทย์	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	~~4.01~~ 43/12	4	I						
		RS:242 (Aug-12)	(P-DHF)	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20				38	3200	65	40	2	5
		RS:264 (Aug-17)		1:40	1:20	1:320	1:40	<1:20	<1:20									
(a=d3)					D		JE											
			231	9	12	4	1											
			242	52	8	3	0											
			264	320	32	40	0											

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B.T. Hakkelo C3

[illegible]	310000	9 ธค ไข้ขึ้นวันที่ 3 เริ่มต้น ไข้ขึ้นเดือน ตกเช้า ไข้ขึ้นเดือน ไข้ขึ้นเดือน ไข้ขึ้นเดือน ไข้ขึ้นเดือน Blood TT !
[illegible]	[illegible]	Liver 3 cm. เจ็บ IV admit วันที่ 5 ธค ตั้งแต่ 110/80 [illegible]
[illegible]	[illegible]	12 ธค กลืนลำบาก
[illegible]	[illegible]	→ ตรวจพบเกล็ดเลือด Gr I.

37.1	118000	6 ธค 11.00 น. T 40°C ไข้ 1 วัน ดูไม่ค่อย ตาสีเหลืองเล็กน้อย ลิ้นเป็นฝ้าขาวหนา เกร็งคอเล็กน้อย
37.3	22000	ให้ Penicillin กลืนลำบาก 7 ธค 2530 9.30 น. ตรวจพบไข้ Hct 33% Hb 15,000 DHF grade I กลืนลำบากน้อยลง
		10 ธค ไข้ลดลง หลับ BP 90/60, P96, TT+, เบื่ออาหาร ตาเหลือง ปวดท้อง liver 4 cm Hct 32% ATL 2%

38.5°C	178000	10 ธค 30 ไข้ขึ้นวันที่ 2 ตัวร้อน ตาเหลือง เจ็บคอ ไม่ค่อยไอ ไม่มีน้ำมูก dry lip, TT+ 100/min
		13 ธค 2530 ติดเชื้อไข้เลือดออกตั้งแต่ 11 ธค ไม่ได้ admit กลืนลำบาก Fu และ ติดเชื้อ

40.5		ส่ง ER 9 ธค 2530 (23.30 น.) ให้น้ำเกลือ ไข้ไม่มีน้ำมูก แต่ไม่ไอ ปวดท้อง ปวดท้องมาก เดินตรงไม่ได้
		30 น. อ็อกซิเจน Good consciousness, not pale, TT−, เช้าวันที่ 11 ธค BP 90/60, TT−, แทงเกล็ดเลือด Gr I.

37.8	194000	11 ธค 2530 ตาเหลืองเล็กน้อย ไข้ 3 วัน ไข้ลดลง BP 110/60 ตกเช้า เหงื่อแตกเล็กน้อย
37.1	202000	ให้ 5% D/N/2 drip 60 cc/hr แพทย์ ตรวจ เห็นน้ำมูกเล็กน้อย

[illegible]	302000	
38.7	210000	11 ธค 2530 4 วันก่อนมีไข้ขึ้นสูง ไข้ลดลงเล็กน้อย ตาเหลือง เจ็บคอ ไม่มีน้ำมูก เจ็บคอ ไอ
38.2	198000	Liver 2+ @ ~~[illegible]~~ tenderness, TT. ไม่เต็ม ~~[illegible]~~
37.0		

C3sp+ liver, C3 80%
C3sp+ liver, C3 80%
C3sp−, C3 9 %

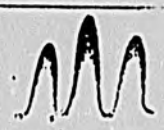
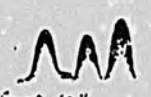
40.5		วันที่ 11 ธค. มีไข้ขึ้นเป็นวันที่ 3 วัน ปวดแขนขา ปวดเมื่อยมาก TT 3.300
	192,000	liver palpable 13 ธค เด็กมีไข้ ซึมลง ทรมาน ตรวจพบ organomegaly not ไข้เลือด
		fever cause – most likely viral infection.

C3sp− C3N
C3sp− C3N
C3sp C3 [illegible]

21 ปี

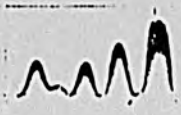
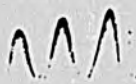
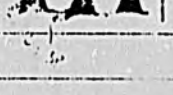
	HN	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Dat	grade	CBC HCT	WBC	NEU	LYM	ATY	MON
80 (OPD)	110 1804399	RS-232 (Aug-12)	ช. [illegible] (9.45)	1:10240	1:640	1:9120	1:1280	<1:20	1:1280	11	4	(Aug 11)	37	5700	20	75	20	
(OPD)		RS-270 (Aug-13)	S-DHF	>1:20480	1:2560	>1:20480	1:5120	<1:20	<1:20		5	1	38.0	6200	45	45	2	
													37.0 37.5	9100	20	79	20	5
81 (OPD)	17 1699880	RS-235 (Aug-13)	ช. [illegible]	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	2.02	3	1	33.5	7900	69	25		5
		RS-268 (Aug-19)	(R-DHF ?)	<1:20	<1:20	1:80	1:40	<1:20	<1:20	2.2/12	4							
		RS-376 (Sep-14)		1:160	1:160	1:1280	1:320	<1:20	1:160		32							
				D		JE												
				995	14	20	1	2										
				268	226	5	44	0										
				376	197	58	33	51										
82 (OPD)	76 1726409	RS-237 (Aug-13)	ช. [illegible]	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	12	4	1	38 %	6500	56	33		506 5
IAS/EIA		RS-263 (Aug-17)	R-DHF	<1:20	<1:20	1:80	1:40	<1:20	<1:20									
				D		JE												
				937	7	17	2	0										
				263	168	5	29	0										
83 (OPD)	1176046	RS-239 (Aug-13)	ช. [illegible]	1:40	1:160	1:640	1:320	<1:20	1:160	5 7/12	6	1	38 %	3100	63	33	6	
		RS-291 (Aug-14)	S-DHF	1:1280	1:640	1:5120	1:2560	<1:20	<1:1280									
84 (ผู้ป่วย)	111 1804617	RS-244 (Aug-14)	ช. [illegible] (9.00)	1:1280	1:640	1:5120	1:2560	<1:20	1:2560	12	4		41.0	2100	40	40		6
		RS-254 (Aug-19)	S-DHF (9.30)	1:10240	1:1280	>1:20480	1:2560	<1:20	1:1280		5	(II)	48.0	4900	20	60	(8)	10
											6	III	42.0	4000	17	19		12
(OPD)		RS-310 (Aug-24)		1:1280	1:640	1:1280	>1:20480	<1:20	1:640		17							
85 (OPD)	45 1812667	RS-245 (Aug-14)	ช. [illegible] (9.30)	1:640	1:5120	1:1280	1:640	<1:20	1:1280	3.07	6		50.0	1,000	31	52	8	
(OPD)		RS-269 (Aug-17)	S-DHF	1:10240	1:2560	>1:20480	1:5120	<1:20	1:1280		7	(III)	42.0	12000	22	74	18	4
											10							
86 (OPD)	722666	RS-247 (Aug-14)	ช. [illegible]	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
IAS/EIA		RS-265 (Aug-17)	(R-DHF) ?	1:20	<1:20	1:160	1:40	<1:20	<1:20									
				D		JE												
				247	66	0	0	0										
				265	254	17	53	0										
87 (OPD)	14 991177	RS-248 (Aug-14)	ช. [illegible]	1:160	1:640	1:1280	1:160	<1:20	1:640	11	4		39.0	2400	38	36		
		RS-288 (Aug-20)	S-DHF	>1:20480	1:2560	>1:20480	1:5120	<1:20	1:1280		5	(?)	38.0	4000	60	30		10
											11	I						
88 (OPD)	19 923640	RS-252 (Aug-19)	ช. [illegible] (9.30)	<1:20	<1:20	1:40	1:20	<1:20	1:20	8	4		39.0	4300	62	23	24	
(OPD)		RS-313 (Aug-24)	(R-DHF) ?	1:80	1:40	1:160	1:640	<1:20	1:80		5	(?)	39.0	2300	44	46	4	6
											14	I						
				D		JE						DF						
				252	111	0	17	0										
				313	59	13	23	24										

56a

O.T.	Platelets	C3	
36.6	100000		11 ส.ค. 2530 16.15 น. ER BT 38.6. P120, BP 100/60 ไข้ต่ำเรื้อรัง 3 วัน หลังจาก แพ้ยาฉีด ไข้ลงวันที่ 3 วัน no petechiae, liver just palpable, TT −, ไม่มีเลือดออก Alg 13. Liver 3 cm. Hct. ลง น้อย 40%. 63 ตกเลือด 37.5% แพ้ยา grade I
37.8	61000		
37.2			
37.5			
38.8			11 ส.ค. 30 หลังจากไข้ลงวันที่ 4 ประมาณ: ตกเลือด 13 ส.ค. ตกเลือดมาก ตกเลือดต่อเนื่อง ตกเลือดออกมาก ไข้ลงหลังจากนั้น TT − liver just palpable มีผื่น of hyperpigmental macule @ ผื่น of erythematous papule ตกเลือดวันที่ 15 ส.ค. 2530 ตกเลือดมาก
37.7			
38.0		C3sp− ต่ำ 55% C3sp ปกติ C3sp ?	
	334000		13 ส.ค. ไข้ลงวันที่ 3 วัน ไข้ลงวันที่ 3 mild dehydrate, lung clear, P 90/min BP 100/70 TT neg Impr − Acute viral infection. N/O DHF กลับบ้าน วันที่ 17 ส.ค. FP ไข้ลง OK.
	108000		13 ส.ค. 2530 มีไข้ลงวันที่ 9 ส.ค. (4-5 วัน) ตกเลือดวันที่ 13 ส.ค. P100/min full petechiae chest wall ทั่วตัว TT+, DHF febril stage. 14 ส.ค. ไข้ลง liver 2cm.
38.5	104000		13 ส.ค. 2530 ไข้ลงวันที่ 4 วัน ตกเลือดวันที่ 13 ส.ค. T 38, P72 R20, BP 100/80, liver just palpable แพ้ยา grade II − III petechiae+, TT+ BP ต่ำลงมาก wind 100/100 แรง
37.8	88000		
37.2	78000		
		C3sp+ 60% C3sp ต่ำ C3sp+ (N.)	
36.7	52000		
36.0	20000		10 ส.ค. 2530 ตกเลือด 17 ส.ค. ไข้ลงวันที่ (4-5 วัน) X 13 ส.ค. 2530 ไข้ลง ตกเลือดวันที่ 13 ส.ค. Hct ลง [illegible] 1 [illegible] ไข้ลง petechiae ไข้ลงวันที่ 14 ส.ค. 2530 มี petechiae [illegible]
36.0			
37.0	83000		
	68000		Aug 13, 14.30 น., ER ST 38.7, P88, BP 100/60, RR 20 ไข้ลงวันที่ 4 วัน, injected conjunctiva (petechiae) on chest wall, liver 1 cm. Impr. − DHF. Hct 35%. Aug 14 liver 3cm
36.5			
37.3	210000		
37.9	110000		Fever. C vomiting @ petechiae Aug 6 อ่านผล E.R. 3 วันไข้ PTT ตกเลือด DHF II ตกเลือดออกมาก

	HN	Serum No.	Name	HI D1	D2	D3	DA	CHIK	JE	Age	Day	grade	CBC HCT	WBC	NEU	LYM	ATY	MON
89. (เด็ก2)	1239639	RS-253 (Aug-13)	ด.ด้วงกล้า นนทบุรี (9.15)	1:320	1:320	1:2560	1:320	<1:20	1:320	5	6	(III)	49. 47	6600 7400	35 71	34 21	(1)	7 7
		RS-255 (Aug-16)	S-DHF (9.30)	1:1280	1:640	1:10240	1:1280	<1:20	1:640				44	12000	[illegible]	[illegible]	(6)	5
		RS-258 (Aug-17)	(9.30)	1:640	1:1280	>1:20480	1:1280	<1:20	1:1280				38	[illegible]	49	30		11
90. (SCU-นอน) (เด็ก2) VAS/EIA	115 1805420	RS-256 (Aug-15)	ด.ณัฐชาติ ภูเก็ต	1:80	1:160	1:320	1:160	<1:20	1:20	14	7	2-3	43.0	7700	59	29	2	2
		RS-257 (Aug-17)	S-DHF (9.05)	1:640	1:640	1:2560	1:1280	<1:20	1:640		6	(III)	28.0	6900	34	57	19	7
		RS-276 (Aug-19)	(10.05)	1:5120	1:640	>1:20480	1:1280	<1:20	1:640		8		30.0	9400	38	42	5	12
(OPD)		RS-312 (Aug-24)		1:1280	1:1280	1:1280	>1:20480	1:20	1:1280		13		31.0	5100	25	41	4	12
91. (OPD)	933980	RS-299 (Aug-17)	ด.จิรพงศ์ ศิริชัย	1:40	<1:20	1:40	1:20	<1:20	<1:20	7								
		RS-274 (Aug-18)	NSD (9.30)	1:40	<1:20	1:20	1:20	<1:20	<1:20									
92. (เด็ก2)	119 180 1075	RS-279 (Aug-18)	ด.ปานศิริ พรมชื่น (10.00)	1:10240	1:1280	>1:20480	1:2560	<1:20	1:1280	14	4 5	?	[illegible] 43.0	3500 6700	39 39	49 39	2 19	6 6
		RS-282 (Aug-14)	S-DHF (9.20)	1:20480	1:2560	>1:20480	1:10240	<1:20	1:5120		6							
(OPD)		RS-309 (Aug-24)		1:2560	1:1280	1:2560	>1:20480	<1:20	1:2560		10	(III)						
93 (OPD) (เด็ก2)	180 6492	RS-277 (Aug-14)	ด.กล้าหาญ พรมจุก	1:640	1:640	1:2560	1:640	<1:20	1:640	14	6	(II)	44.0	13200	82	15	5	3
		RS-281 (Aug-14)	S-DHF (9.10)	1:10240	1:2560	1:5120	1:2560	<1:20	1:1280			(III)?	36	9500	66	30		4
		RS-284 (Aug-20)	(9.10)	1:20480	1:1280	>1:20480	1:2560	<1:20	1:1280									
94 (OPD)	1961973	RS-278 (Aug-18)	ด.[illegible] จิตรพันธ์	1:20	1:20	<1:20	<1:20	<1:20	<1:20	7.03/12	4	I	36.0	4200	66	34		
		RS-283 (Aug-19)	P-DHF	1:40	<1:20	1:40	<1:20	<1:20	<1:20		5		39.0	4800	26	63		5
		RS-314 (Aug-24)		1:160	1:40	1:40	1:160	<1:20	<1:20		14							
					D		JE											
				278	25	1	27	0										
				283	106	26	171	43										
				314	306	40	107	14										
95. (เด็ก2)	993005	RS-279 (Aug-19)	ด.กำชนพงษ์ หลวงดา (9.00)	1:640	1:160	1:640	1:160	<1:20	1:80	7	5	3	49.0	5300	77	20		2
		RS-285 (Aug 20)	S-DHF (9.20)	>1:20480	1:320	>1:20480	1:160	<1:20	1:320		6		40.0	7000	39	45	17	1
		RS-292 (Aug-21)	(9.40)	1:80	1:640	>1:20480	1:1280	<1:20	1:5120		7		39.0	6500	18	76	29	4
							HEN		JE									
				RS 279	40	111	63	[illegible]										
				RS 285														
				RS 292														

5/a

B.T.	Platelets	Cs
	154,000 51,000 59,500	
38.0 38.0 38.0 38.0 37.0	18000 38000 20000 20000 112000	
38.8 41 ง		
37.0 37.2 34.0 32.5	114000 36000	 Cs ↑ sp⁻ @ 20% Cs ↑ sp⁻ @ 10% Cs N
	38000 38000	
39.0 37.0 37.0	220000 172000	 Cs 95% Cip⁻ Cs 95% Trou sp Cs + N
32.2 34.1 36.0	158000 140000 12000 18000	

17 ส.ค. 2536 ปัสสาวะออก 19.30 น. 8 วันหลังไข้ ... ~ สะอึกก่อนมาด้วย + มีอาเจียน
Hct 48 % TT⁻ admit. BP 110/60 ... BP 100/60 - 100/70

ตอนวันที่ Aug 17 BP ลดลง ปัสสาวะน้อยกว่าวันก่อนๆ มี imp. shock มีอาการแน่น Hypotension จาก low intake
ตั้งแต่ 17 - 19 ส.ค. เป็นไข้ 4 วัน ไข้ลง ไข้เป็นวัน ... ตอนนี้มีสะอึก ท้องเป็น
ซึมลงเรื่อยๆ T 38°C P 108 BP 130/80 petechiae ตัว. Rt 3rd tel fasciae + Lt Ankle
Slight dry lip Heart/lung clr abd soft, liver –, spleen 9 cm. Hct 49 %, BP 100/70, plt 114000
เป็นปัสสาวะ Plt เหลือ 36000 Imp. DHF (Grade II) toxic stage Chi fluid Hct. จาก 48 → 49

เช้าวาน 18 ส.ค. 2536 5 วันหลังไข้ ไข้เพิ่งลง ... 20 วัน อาเจียนไม่ดี
1 ชั่วโมงมานี้ไข้ลง ... Hct 44 % DHF toxic stage gr II - III
P 120 BP 130/80 admit ward I petechiae at both arm, liver 1 cm., มีเลือดออก

18 ส.ค. 2536 เป็นไข้มา 3-4 วัน ไม่ค่อยกินอาหาร อาเจียนบ่อย TT⁺, BP 100/60 ไข้สูง, ซึม
ไม่ admit ... 11 วัน drink well. lung ok liver 2 cm. มี
Impetigo.

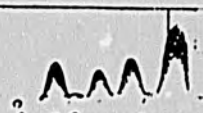
@ 4 ม.ค. 16 - 22 ส.ค. 30 เกณฑ์ DHF gr II. ปวดท้อง BP 83/40 น. เป็นไข้มา 3 วัน ซึมลง 1 วัน ... อาเจียนบ่อย
ไม่ค่อยมีปัสสาวะ อาเจียนเป็นน้ำ ... P 140/min BP 90 mm T 37.7°C liver 4 cm +
Imp. DHF @ impending shock. วันที่ 19 BP ... 30-21 ... มีปัสสาวะ, ...
... rash ...

	HN	Serum No.	Name		HI D1	D2	D3	D4	CHIK	JE	kg	Day	Grade	CBC HCT	MBC	NEU	LYM	ATY	MON
96. (เด็กชาย)	119 1806506	RS-280 (Aug-19)	ด.ญ.เศวรินท์ คล้ายพิลิศาร์	(9.09)	1:2560	1:640	1:2560	1:640	<1:20	1:320	8	4	(2)	43.0	[illegible]	71	[illegible]	[illegible]	[illegible]
														49.0	4400	99	39		6
		RS-286 (Aug-20)	S-DHF	(9.39)	>1:20480	1:1280	>1:20480	1:640	<1:20	1:1280		5	?	42.0	8000	42	30	29	3
		RS-291 (Aug-21)		(9.35)	1:10240	1:5120	>1:20480	1:10240	<1:20	1:1280		6		39.0	9600	38	60	1	1
		RS-311 (Aug-24)			1:2560	1:1280	1:2560	>1:20480	<1:20	1:1280		9							
(OPD)						R: 240 RS 286. RS 291	78	DEN 350	66	JE 293									
						RS 311	138	481	93	499		4 9		19.0 33.0	[illegible] 24.0	10 32	60 32	10	
97. (OW1)	31 1168246	RS-293 (Aug-21)	ด. ธวรภพย์ ทิพย์อัครา		1:20	<1:20	1:20	1:20	<1:20	1:20	6.02	6	1	23.0	4100	19	78	3	3
		RS-302 (Aug-24)	(P-DHF) ?		<1:20	1:40	1:20	1:20	<1:20	<1:20		9	?	17.0	4000	20	64	14	4
			เด็กเป็น β-Thal major มี thumbacy hyperemia, dimpling			RS 293	44	DEN 18	92	JE 58									
						RS 302	33	19	51	40									
98. (OW1)	153994	RS-294 (Aug-21)	ด. สิทธิกร แม้นเหม้ว		1:20	<1:20	1:20	<1:20	<1:20	1:20									
		RS-299 (Aug-22)	HSV	(9.30)	1:20	<1:20	1:20	<1:20	<1:20	1:20									
						RS 294 RS 299	36	DEN 34	81	JE 59									
99. (OPD)	15 1412994	RS-296 (Aug-20)	ด.ญ.จิรภาสี ทนะเจริญ		1:20	<1:20	1:20	<1:20	<1:20	1:20	7.11	3	I9	37%	700	79	22		1
		RS-308 (Aug-24)	HSV		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
						RS 296. RS 308	41	DEN 29	129	JE 99									
100. (OW1)	977461	RS-297 (Aug-21)	ด. ศรายุทธ มุสิกชื่อง	(10.00)	1:80	1:20	1:320	1:80	1:80	1:40	11		III	37%	[illegible]	63	30	(3)	5
(เด็กชาย)		RS-298 (Aug-22)	S-DHF		1:320	1:160	1:10240	1:160	1:80	1:160				(Aug 11) 42% A [illegible] Aug 21 [illegible]					
			[illegible]			RS 297 RS 298	36	DEN 50	115	JE 155									
101. (เด็กชาย)	1807897	RS-300 (Aug-21)	ด. สุรพงษ์ ชูนท์	(9.15)	1:640	1:1280	1:640	1:1280	<1:20	1:320	8	6	III	45	1,700	61	31	(4)	4
		RS-301 (Aug-24)	S-DHF	(9.00)	1:640	1:2560	1:2560	1:5120	<1:20	1:640				42 / 37	[illegible]	[illegible]	[illegible]	(4) (10)	4
						RS 300 RS 301	67	DEN 305	35	JE 197									
102. (OW1)	12 1808231	RS-318 (Aug-24)	ด. เนตรนภา อ่อนจิตต์		1:1280	1:40	1:640	1:160	<1:20	1:40	8	4	3	41.0	5000	28	64	1	
(เด็กชาย)		RS-319 (Aug-28)	S-DHF		1:2560	1:80	1:1280	1:320	<1:20	1:80		6		35.0	5500	27	50	15	
		RS-323 (Aug-26)		(9.15)	>1:20480	1:320	1:5120	1:640	<1:20	1:320		1		36.0	5800	19	78	3	
(OPD)		RS-341 (Aug-31)			>1:20480	1:320	1:640	1:10240	<1:20	1:640		12							
						RS 318 RS 319 RS 323 RS 341	324	DEN 90	97	JE 118									
103. (OPD)	948190	RS-321 (Aug-29)	ด. สุชาติ เอี่ยมศิริพร		1:640	1:40	1:320	1:80	<1:20	1:40									
		RS-334 (Aug-29)	S-DHF		>1:20480	1:5120	>1:20480	1:2560	<1:20	1:640									
						RS 321 RS 334	68	DEN 307	134 / 78	JE 329 / 336									
104. (เด็กชาย)	504949	RS-290 (Aug-21)	ด. พิชิตศักดิ์ ทาสกาน	(9.30)	1:160	1:20	1:40	1:80	<1:20	1:20	12	6	II	[illegible]	6500	84	10		5
(OPD)		RS-349 (Sep-3)	(Kosum 116H ดีขึ้น) ๊ ดีขึ้นมาก		1:2560	1:5120	1:2560	1:1280	<1:20	1:2560			?	34%					
						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]									

586

Patient C_3

84000
12000
36000



C_3 sp^3 C_3 30% + C_3 sp^3 3-37
C_3 sp^3 C_3 10%
C_3 sp^3 C_3 257 + lingual
C_3 sp^3 C_3 + N

Admit 15 ม.ค. 21.00 ด้วยไข้สูง ไม่มีอาการปัสสาวะ ปวดท้องน้อยทั่วๆ ไม่ปวดเฉพาะที่ ไม่ปวดเอว
1 วันก่อนรับเข้าเริ่มเจ็บแน่นท้องน้อยเล็กน้อย ถ่ายปกติ ไม่มีอาการปัสสาวะแสบขัด ไม่ถ่ายบ่อย ไม่ปวดเอว
15.00 น. BP 90/60 T 36.2, TT+, admit ตั้ง 16 ∞, Hct 20%, ให้ IV ตามด้วยน้ำเกลือ
19. - ตื่นเช้า 2 ครั้ง 37.3, 20 ปัสสาวะขุ่น Liver 5 cm, 21 T 37.4 กลับบ้าน

21000
14,000
26000
48000

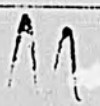
admit + H ตอน 20 BP 120/70 ตื่นเช้า 22. มีไข้ ปัสสาวะ ปวดเอว 120
ตอน admit 24 ชม. รับไว้ใน (IPD) 15 น.
แพทย์ตรวจ แต่ไม่ตรวจ เพราะไม่ได้ admit

Known B Hel. Dwyer case

244,000

ป่วย ม.ค. 2530 ไข้สูง 2 วัน ไม่ถ่าย ไม่ปวดเอว ไม่ปวดท้องน้อย BP 110/70 P 120 TT+
R/O DHF Aug 24 ไม่ปรากฏ ไม่ได้ admit เดินทาง R/O DHF.

40,000

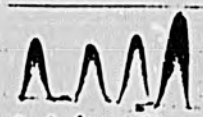


มีไข้ Viral Infection 2535 เดือน ธ.ค. 2540 การเจ็บป่วย DHF + diarrhea
ก่อนวัน Aug 18 เดือน Hypokalemia ไข้ต่ำ (37) BP 90/60, TT+ วันที่ 20/8/2540 เวลา
(90/70 ถึง 110/70). ตื่นเช้า วันที่ Aug 25 ปรากฏ 24 Aug.

54,000
52,000
15,000

admit (Aug 22) ผลตรวจเลือดไข้ ปัสสาวะปกติ ตรวจ ไข้ต่ำ bleeding T 38.4, รับไว้ใน
R 24/min (BP 90/60 ถูกส่งตรวจ Lab 5 cm, BP 120 mm ตรวจ DHF III. ต่อมา,
BP เพิ่ม ตั้ง Aug 24, Aug 23 100/70

90000
80000
62000



C_3 sp^3 C_3 50% + C_3 sp 5%
C_3 sp^3 C_3 50% - C_3 sp - 1 mm
C_3 sp^3 C_3 75%
C_3 sp^3 C_3 N

24 พ.ค. 2530 ตื่นเช้า 12.30 น. มีไข้ [illegible] BP 110/60, [illegible]
เริ่ม [illegible] BP 100/70, P 120, TT+, [illegible] ให้ [illegible] Gr. III.
ตรวจพบ [illegible] Gr. II - III ไม่มีอาการ เช่น [illegible] no [illegible] no [illegible]
ให้ active bleeding ตรวจ ก่อน 26 ก.ค.

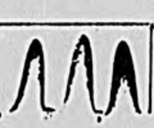
87,000

20 ก.พ. 2530 มีไข้สูง 4 วัน ไม่ถ่ายปัสสาวะ ตรวจร่างกายไม่ปรากฏ ตรวจ Hind Trace 2 ครั้ง ตรวจเลือด
เช้า 2 ครั้ง ไม่ปรากฏ ไข้ต่ำ (ไข้ต่ำ), [illegible] เช้า
เวลา 4 ชม. ถึง 2V BP ไม่ปรากฏ [illegible] Gr. II. [illegible]
รับ [illegible] ตรวจ.

	HN	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Dang	grade	CBC HCT	WBC	NEU	LYM	ATY	MON
105 (ward)	119 1807090	RS-328 (Aug-26)	ย. บุญตา นักเรียน (11.00)	1:640	1:2560	1:10240	1:10240	<1:20	1:640	2	7	1.	35.0 / 39.0	11200	41 / 57	42 / 41	10	4 / 6
		RS-330 (Aug-27)	S-DHF	1:640	1:1280	>1:20480	1:5120	<1:20	1:1280		8		25.0	3600	18	55	4	9
(OPD)		RS-339 (Sep-7)		1:1280	1:2560	1:2560	1:2560	<1:20	1:2560		18							
						RS 324	145	451	105	572								
						RS 330 / RS 339	152	416	113	560	6	6	1	34.0	3,200	34	30	8 / 13
106 (OPD)	1143221	RS-329 (Aug-26)	ย. ดวงกมล ชื่นชม	1:160	1:160	1:640	1:640	<1:20	1:160									
		RS-337 (Aug-28)	S-DHF	1:320	1:2560	>1:20480	1:2560	<1:20	1:640									
						RS 325	148	140	118	202								
						RS 337	353	449	222	536								
107 (OPD)	701394	RS-326 (Aug-26)	ย. ดวงจันทร์ กลิ่นหนู	1:80	1:80	1:640	1:320	<1:20	1:80	10	4	1.	41.0	2700	74	20	4	1
(OW1)		RS-327 (Aug-27)	S-DHF	1:160	1:320	1:2560	1:1280	<1:20	1:160		5		49.0	4100	54	42		2
VAS/EIA		RS-332 (Aug-28)	(10.00)	>1:20480	1:2560	1:5120	1:1280	<1:20	1:320		6		40.0	6400	43	43	14	6
(OPD)		RS-342 (Aug-31)		1:5120	1:640	1:640	>1:20480	<1:20	1:2560		9							
		RS-379 (Sep-14)		1:1280	1:1280	1:5120	1:5120	<1:20	1:1280		23							
						RS 326	58	115	56	161								
						RS 342	176	391	94	347								
108 (OPD)	122 1809495A	RS-328 (Aug-27)	ย. จันทร์เพ็ญ สกวงศ์ตันต์	1:80	1:160	1:320	1:320	<1:20	1:80	6	4	1 y	33.0	2000	54	46	10	
		RS-33) (Aug-28)	S-DHF (10.00)	>1:20480	1:640	1:2560	1:1280	<1:20	1:320		7 / 6		32.0 / 33.0	3600 / [illegible]	22 / 10	76 / 66	20 / 14	2 / 10
		RS-340 (Aug-31)		>1:20480	1:5120	1:1280	>1:20480	<1:20	1:5120		8							
						RS 328	34	95	63	111								
						RS 340	124	395	131	524								
109 (OPD)	1391068	RS-329 (Aug-27)	ย. วิทัช พวงศรีใหญ่	1:20	<1:20	<1:20	<1:20	<1:20	<1:20	6	3	I	35	7300	71	19	8	2
(OW1)		RS-336 (Aug-29)	[illegible]	1:20	<1:20	<1:20	<1:20	<1:20	<1:20				31	5100	22	69	8	3
						RS 329	49	90	100	166								
						RS 336												
110 (ward)	1809340	RS-331 (Aug-27)	ย. แอม จำนียงศาสา	1:320	1:320	1:5120	1:1280	<1:20	1:160	3/9 months	4	2	40	4500	40	52	5	3
(OPD)	11 mn.	RS-341 (Sep-8)		>1:20480	1:2560	1:5120	1:2560	<1:20	1:2560	3 months		3	37	3400	24	67	7	2
						RS 331	72	209	119	255								
						RS 341	[illegible]	372	61	392								
111 (OPD)	1119786	RS-335 (Aug-31)	ย. บัวศรี จารจารวุฒิ	1:320	1:160	1:160	1:640	<1:20	1:320	4	5	I.		3500	65	35		
(OW1)		RS-345 (Sep-1)		1:1280	1:1280	1:1280	1:1280	<1:20	1:1280					3200	23	55	9	
(OPD)		RS-360 (Sep-7)		1:1280	1:2560	1:5120	1:2560	<1:20	>1:20480									
						RS 335	38	132	86	167								
						RS 360	16	372	42	475								
112 (ICU-ward)	1810204	RS-339 (Aug-30)	ย. อรัญญา ศรีจันทร์	1:80	1:80	1:160	1:320	<1:20	1:80	11								
(ward)		RS-346 (Sep-11)	(10.10)	1:320	1:320	1:1280	1:2560	<1:20	1:1280									
(ICU)		RS-438 (Oct-21)		1:640	1:640	1:640	1:1280	<1:20	1:2560									

83 74 54 67 — 26 Oct 2543 — 2% DHF # bleeding.
[illegible] 225 110 145 — ex change transfusion

59a

B.T.	Platelets	C_3	
3?.? / 3?.0 / 38.0	135000 / 92000 / 80000		20 ?? 2530 [illegible] gen'l convulsion, [illegible]
37.0	20000		Pale, no jaundice,
			24 ?? 2530 [illegible] liver 3 cm TT⁺, admit [illegible] petechiae, no bleeding
			[illegible] Convalescent [illegible]
	83?,000		26 ?? 2530 [illegible] P 10?/m full, BP 90/60 [illegible] no petechiae, Liver 1 FB, TT⁺ 10 [illegible]
			26 ?? [illegible]
38.7	40000		admit 26 ??: 4 [illegible]
39.0	91000		2 [illegible] weak BP 110/60 Liver 3 cm TT⁺ admit [illegible] IV NSS
38.0	56000	$C_3 sp^+$ 60% (+sp 3%)	27 ??: [illegible], Liver 3 cm, [illegible] Hct 45% 11.00 [illegible] / 28 ??
?.0		$C_3 sp^+$ 55% (+sp 2-3%)	[illegible] 28 [illegible] 31. [illegible] liver 2 cm [illegible]
		$C_3 sp^-$ C_3↑ 10%	
		$C_3 sp^-$ C_3 (N)	([illegible] admit [illegible] narcosis) 14 ?? Liver [illegible]
38.3	65000		27 ?? 2530 [illegible] 39.5 - 40.2 (27-29 ??) [illegible]
37.8 / 36.5	40000 / 100000		[illegible] liver⁻ [illegible]
38.0		$C_3 sp^+$ 90%	29 ?? liver 3 cm [illegible], [illegible] liver⁻, [illegible], 5 ?? [illegible]
		$C_3 sp^+$ 90% + sp 10%	30 ?? [illegible] 13 - 15 ?? TT⁺, liver 3 cm, [illegible] maculopapular rash [illegible]
		$C_3 sp^-$	[illegible] Koplik's [illegible] Mycoplasma [illegible] CF 1:28 - 1:256.
			[illegible] (Koch? [illegible]) [illegible] negative
			[illegible] Mycoplasma pneumonia [illegible] CF 1:256 [illegible]
	130,000		— [illegible] 27 ?? 2530 [illegible]
			[illegible], (TT⁻), Hct 35%, [illegible] 4,400, [illegible]
			— 29 ?? 2530 [illegible]
36.6	23,000 / 20,650		admit 27 ?? 00.00 [illegible]
			[illegible], liver 1.5 cm, BP 90/60 [illegible] grade II-III
	135,000 / 94,000		31 ?? 2530 [illegible] TT⁺ [illegible]
			liver just palpable, P 108, BP 110/70 [illegible]
	?,???		[illegible]

	HN	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Dengue grade		CBC HCT	WBC	NEU	LYM	ATY	MON
113 (OPD)	468909	RS-343 (Sep-1)	ด. เทวภิทย์ ศรีวิเศษ	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	7	4	I			41	55		4
(OPD)		RS-346 (Sep-2)	x เม่น.	1:1280	1:640	1:640	1:320	<1:20	1:640		5				33	53	11	
(OPD)		RS-348 (Sep-3)		1:1280	1:1280	1:640	1:1280	<1:20	1:1280		6							
(OPD)		RS-387 (Sep-7)		1:2560	1:2560	1:320	1:2560	<1:20	1:5120		10							
VP3/1.2					RS343	34	DEN 21	75	JE 122									
					RS347	228	362	46	441									
114 (OPD)	1296934	RS-350 (Sep-5)	ด. ศรีรัตน์ จ้อง	1:20	<1:20	<1:20	<1:20	<1:20	<1:20	5	4	1-3?			35	57	(12)	7
(OPD)	DEN-3 (NS1)	RS-398 (Sep-7)	25 ส.ค. 25 4.m. 16.00	1:10	1:80	1:10	1:80	<1:20	<1:20									
(OPD)		RS-362 (Sep-8)	(RDHF) (11.00)	1:20	1:40	1:40	1:40	<1:20	1:20									
	dengue-3				RS-350	17	DEN 22	50	JE 73									
	(Q=d3)				RS336	346	17	124	39									
115 (OPD)	1002479	RS-351 (Sep-2)	ด. ศศิธร เรือนไพรเล็ก	1:20	1:40	1:20	1:40	<1:20	1:20	11	3	(3)		6100	43	12	4	1
(OPD)	24.08	RS-382 (Sep-4)		1:40	1:40	1:40	1:40	<1:20	1:20									
VAS/E.A	DEN-3 (NS1)	RS-353 (Sep-5)	257 30 32 / 258 59 (9.00)	1:160	1:320	1:160	1:160	<1:20	1:320		4			31.00	64	24	8(5)	1
		RS-354 (Sep-6)	262 (9.10)	1:160	1:320	1:160	1:320	<1:20	1:160		5				52	38	13	
		RS-359 (Sep-7)	263 (13.30)	1:320	1:2560	1:320	1:320	<1:20	1:640		6				29	40	18	
		RS-363 (Sep-8)	264 (11.00)	1:1280	1:640	1:640	1:1280	<1:20	1:2560		7							
(OPD)		RS-374 (Sep-14)	277 161 102 / 105 463	1:5120	1:10240	1:5120	>1:10480	<1:20	1:5120		13							
			363		RS351	30	DEN 22	32	JE 59									
			374		RS374	161	438	102	463									
116 (เด็กชาย)	1812706	RS-361 (Sep-9)	ด. ธรุณี วงษาเกาะ (9.30)	1:20480	1:1280	>1:20480	1:2560	<1:20	>1:20480	5	6 7	III	50	18,600 11,700	14 25	50 58	18 9	2 5
		RS-369 (Sep-11)	(9.00)	1:20480	1:1280	1:2560	1:5120	<1:20	>1:20480									
					RS361	169	DEN 417	16	JE 427									
					RS362													
117 (เด็กชาย)	1194026	RS-369 (Sep-12)	ด. รัตนช่วงรอด วงษาสุวรรณ	1:640	1:160	1:640	1:80	<1:20	1:160	12	5	(3)						
(เด็กชาย)		RS-369 (Sep-13)		1:1280	1:1280	1:10240	1:1280	<1:20	1:80					45,500	30	28	34	8
		RS-371 (Sep-14)		1:10240	1:5120	1:10240	>1:20480	<1:20	1:1280									
					RS366	50	DEN 102	5	JE 98									
					RS371	145	344	27	317									
118 (เด็กชาย)	1441646	RS-370 (Sep-13)	ด. ศุภกร เหมือง	<1:20	1:20	<1:20	<1:20	<1:20	<1:20									
		RS-373 (Sep-14)		<1:20	1:40	<1:20	<1:20	<1:20	<1:20									
		RS-377 (Sep-15)		<1:20	1:20	<1:20	<1:20	<1:20	<1:20									
		RS-379 (Sep-16)		<1:20	1:20	<1:20	<1:20	<1:20	<1:20									
(OPD)		RS-402 (oct-14)		<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
					RS370	7	DEN 7	15	JE 16									
119 (เด็กชาย)	1011158	RS-372 (Sep-14)	ด. จรรยาพร ศาตาโพธิ์	1:20	1:40	1:40	1:40	<1:20	1:20	7	4	I		3.0	58	36		
(เด็กชาย)	DEN-2 (NS1)	RS-378 (Sep-15)		1:160	1:20	1:160	1:160	<1:20	1:640		5			50.0	52	31		
(OPD)		RS-418 (oct 14-41)		1:5120	1:2560	1:20480	1:5120	<1:20	1:20480									
	(Q=d2)						DEN		JE									

60a

B.T.	Platelets	C3
[illegible]	DEC	
[illegible]	17000	
[illegible]	28000	
[illegible]		

1 กย 2520 BP [illegible] 12/10 [illegible] RC 26 [illegible] Hct 37% BP 110/60

*. เมื่อ 24 มี.ค. 2525 [illegible] DHF grade I.

	46000

OPD [illegible] 5. [illegible] DHF [illegible] Hct 59% [illegible] 104/80, P 96/min. Liver just palpable, Hct 40% (14-30 u), [illegible] 51/0/1250 ml, เลือดกำเดาไหล, TT +

8 กย BP 110/60, P 90, [illegible]

10.0	124,000
37.8	5500
36.5	32000
34.4	30000
37.0	

[illegible] 4 : 2-3 [illegible] ก่อนเดือน 2 ครั้ง ก่อนถึง [illegible] ผื่นคัน

5 กย: เลือดกำเดาไหล, mild dehydration, look toxic

6 กย: ไข้สูง [illegible] Liver 1 cm. [illegible] 2 [illegible] ไข้ลงเร็ว

BP 100/60, [illegible] 1 m, ไข้สูง มีเลือดออกตามผิวหนัง (ตั้ง 5 กย)

7 กย ไข้ลง, เลือดกำเดาไหล มีปัสสาวะน้อยมาก (anuria) BP 100/70 Liver 2 cm off flank

8 กย liver 1 cm.

	84,000
	30,000

วันที่ 7-9 กย แทรกซ้อน Shock - DHF. [illegible] 5 [illegible] BP 50/50 PR 130, Cold clammy [illegible] BT 37.4 P 120 RR 28 Hct 50 WBC 16600 [illegible] load fluid ไม่ได้. 8 กย [illegible] BP 100/70 Liver 2 cm. [illegible] BP [illegible]

38.5	[illegible]

OPD 11 กย BT 37.3 Hct 35% BP 90/50 [illegible] Viral gastritis.

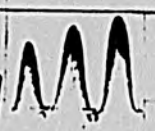
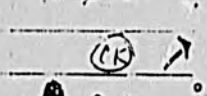
[illegible] 10 [illegible] 11 กย [illegible] Hct 42%, WBC 3500, PMN 76 [illegible] 13 กย. [illegible] BT 35.5, BP 120/55 [illegible] 90/70 [illegible]

1. Salmonella [illegible]

38.3	[illegible]
37.3	122000
37.2	175000
38.5	206000

13 กย [illegible] BT [illegible] 120/70 [illegible] 3 กย [illegible] 14 กย. [illegible] BT 36.5, P 80 RR 20 BP 120/70 [illegible] 4 วัน BP [illegible] Still [illegible] TT

	HN	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Day	Grade	CBC HCT	WBC	NEU	LYM	ATY	MON
120 (OHI)	1234766	RS-350 (Sep-16)	ด.ญ. จิตติมา เชื่อมปง.ร.1	1:320	1:640	1:640	1:320	<1:20	1:160	5.01	5	II	43.0	7000	61	35	4	8
(OHI)		RS-351 (Sep-17)		1:1280	1:1280	1:2560	1:5120	<1:20	1:2560		6							
(OPD)		RS-340 (Sep-21)		1:1280	>1:20480	1:640	>1:20480	<1:20	1:2560		10							
					DEN			JE										
				RS 340	245	346	124	352										
				RS 390	369	442	201	456										
121 (OHI)	1819146	RS-391 (Sep-16)	ด.ญ.จันทร์จิรัศ เพชรวะเกิด	1:320	1:320	1:640	1:160	<1:20	1:320	5.06	6	II	35					
(OPD)		RS-392 (Sep-21)		1:1280	>1:20480	>1:20480	>1:20480	<1:20	>1:20480		10							
					DEN			JE										
				RS 381	91	385	65	396										
				RS 392	159	457	140	469										
122 (OPD)	1816092	RS-386 (Sep-16)	ด.ญ. จิตติพรพร จันทร์ไพรินทร์	1:320	1:320	1:160	1:160	1:20	1:160	12	6	III	49					
		RS-387 (Sep-19)	ไข้ 1 วัน	1:1280	1:1280	1:640	1:640	1:20	1:640									
		RS-424 (Oct-19)		1:640	1:640	1:1280	1:1280	<1:20	1:2560									
						DEN		JE										
				X RS 386	19	72	27	91										
123 (OPD)	1534117	RS-393 (Sep-23)	ด.ญ.พิมพ์ดาว ดวงเกื้อ	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	3 1/2	2	I	40	12400	67	26		7
		RS-397 (Sep-24)	ไข้ 14.7	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20									
			P-DHF			DEN		JE										
				RS 393	101	42	115	99										
				X RS 397														
124 (OHI)	1817054	RS-395 (Sep-23)	ด.ญ. วราภรณ์ กระต่ายทอง	1:320	1:320	1:2560	1:2560	1:20	1:1280	11	6	III	46%					
		RS-396 (Sep-24)		1:640	1:640	1:2560	1:2560	<1:20	1:1280									
(OPD)		RS-417 (Oct-16)		1:2560	1:2560	>1:20480	1:10240	<1:20	>1:20480				28%		60	34		
					DEN			JE										
				RS 395	74	396	68	362										
				RS 407	116	399	114	365										
				RS 417	65	374	46	379										
125 (พ้อง2)	1817597	RS-398 (Sep-29)	ด.ญ.เอกรัตนกร แก่งคี	1:640	1:320	1:1280	1:640	<1:20	1:640	12	7	III	42%					
		RS-401 (Sep-25)		1:5120	1:10240	1:10240	1:1280	<1:20	1:5120						13	80		5
		RS 405 (Oct-5)		1:10240	1:640	1:1280	1:2560	<1:20	1:10240									
						DEN		JE										
				RS 398	[illegible]	334	44	316										
				RS 401	103	355	56	338										
				RS 405	[illegible]	[illegible]	36	331										
126 (พ้อง2)	918493	RS-399 (Sep-29)	ด.ญ. วรรณรัตน์ หนองหมา	1:640	1:1280	>1:20480	1:1280	<1:20	1:640	8	5	III	47%					
		RS-402 (Sep-30)		1:5120	1:2560	>1:20480	1:5120	<1:20	1:5120									
(OPD)		RS 410 (Oct-5)		1:2560	1:640	1:2560	>1:20480	<1:20	1:10240									
					DEN			JE										
				RS 399	193	451	[illegible]	439			26	ND	52%	8000	39	28	19	(Ba) 5
				RS 402	172	463	199	423										
				RS 410	118	445	109	437			27	ND	51%					
127 (พ้อง2)	614074	RS-403 (Sep-28)	ด.ญ. กัณฑรา สุภาภาคุณ	1:2560	1:5120	1:10240	1:2560	<1:20	1:5120	11	8	III	33%	4600	32	65		-
		RS-405 (Sep-29)		1:2560	1:2560	>1:20480	1:5120	<1:20	1:5120									
						DEN		JE										
				RS 403														
				RS 405														
128 (OHI)	1819966	RS-355 (Sep-18)	ด.ญ.จันทร์จิรา ทาง	1:640	1:2560	1:1280	>1:20480	<1:20	1:640	12	7	III	39%	3450	53	38	5	7
(OPD)		RS-409 (Oct-5)		1:2560	1:640	1:2560	>1:20480	<1:20	1:10240				45%	9300	44	44	6	6
						DEN		JE										
				RS 355	61	265	61	372										
				[illegible]	[illegible]	433	71	433										

ปีพ.ศ.	C3
140000 / 120000 / 90000	 ไข้ไม่ได้ระยะเป็นเวลา — 4 กย 30.10 มา ER ไข้ 3 วัน 15 กย 3530 ซึมมาก ER เท่า 30 ม. 4 วัน เริ่ม เบื่ออาหาร ท้องผูก ถ่ายเหลวเป็นน้ำ ไข้สูง 40.2 BP 90/60 P96 RR 24 16 กย เริ่มมีผื่นขึ้น 35.2 ผื่นเริ่มเลือดออก petechiae เริ่มซึม เพ้อ 17 กย mild dehydration. BP 100/60 เกณฑ์วิน grade II ตอน 15 Sept.
100000 / 40000 / 110000	วันที่ 15 กย 3530. ER 38.30 น. ไข้สูง ไข้ลงวันที่ ตกเลือด อาเจียน ต้องรับ grad II. BP 80/50, P 36, หัวใจ 16 กย ซึมเพ้อ ไม่ได้ไข้. ท้องผูก ถ่ายดำ stool. เกณฑ์ BP ไม่แตกต่างกัน
	 CK ↗
	มา 6 กย: ไข้ลง 5-6 วันแล้ว อาเจียนหนัก เอาเข้าไปไม่ได้ ไม่ถ่ายอุจจาระ ไม่มีปัสสาวะ look weak, toxic, dehydrate, BP 100/50 (Used DPD), TT+, liver mild tender DHF @ impending shock แก้ไขแล้ว Ward แล้ว BP ต่ำตลอด liver 2 cm อันดี 14 เริ่ม convalescent
sm. udg.	มา 23 กย ไข้ลงไม่มีอาการ ไข้ลง 5 วัน อาเจียน ถอดเลือดเล็กน้อย liver 2 cm, เป็นไข้อีกเล็กน้อย หมดสติอาเจียน visual infection คล้าย DHF.
110,000	22 กย ตื่นตกใจมีไข้ 4 วัน. ไข้ลง เบื่ออาหาร เพลียมาก ไม่ถ่ายอุจจาระ ไม่ปัสสาวะ BP 110/60, TT+ H.O. 23 กย 7.30 น BP 100/55 ไข้ลง เบื่ออาหาร liver 2 cm petechiae ตกเลือดมาก ซึมลงมาก ท่าทางไข้ Gr. fever BP 110/60 (ก. 60 น)
330,000	24 กย ไข้ลง เบื่ออาหาร
15,000	Repeat ซ้ำอีกที. เป็นไข้มา 5 วัน อาเจียน ซึมลง ถ่ายเหลว เบื่ออาหาร BP 150/40, pulse 80 เกิด 4 ชม 220 BP 110/95 เอาเข้าวันที่ 25 ซึมลงมาก เริ่มหอบ ต้องให้ oxygen 10 cc/kg 80 ml/min Sept 25. ตกเลือด ไข้ลง BP 80/40, RR 50, clinical ช็อค/15 static
12,000	DPD 21 กย (Emergency) BT 39.2°C มีไข้มา 1 วัน ไข้ลงไม่ได้ ซึมลงมาก อาเจียน ถ่ายเหลว TT+ acute pharyngitis 22 กย ไข้ 4 วัน. ไข้ลงเป็นระยะ เริ่มซึมมากขึ้น เป็นมาตั้งแต่เกิดเหตุไข้ clinic BP 100/50, หมดสติ อาเจียน. BP 100/90. liver 2 cm, spleen ↗. 21 80 BP 70/75
40,000	ตั้งแต่ 26 กย – 29 กย. ยากลับ 26 กย เป็นไข้มา 5 วัน. ไข้ลง ถ่ายไข้ 1 ครั้งก่อนมาไข้ลง ถ่ายไม่ได้ ซึมลง ท้องผูก อาเจียน hepatomegaly @ tender เหลือง viral hepatitis. no petechiae BP ↓ ที่ ER load IV P130 BP 60/n ต่ำ Hct 35% ที่ ward BP 100/80 P120 เหนื่อย Sept 30 ซึมลงมาก ไข้ลง ไม่ได้ปัสสาวะ BP ไม่แตก.
CK → เหนือนเดิม	
68,000(17) / 76,000(18) / 70,000(19)	เข้า รพ Sept 17. ไข้ลง 5 วัน ซึมลงมาก BT 37.5°C, PR 100/min, RR 20, BP 100/70, good oxygenation, afebrile, liver 1 cm Hct 37%, WBC ต่ำลง ต่อไป BP 90/80 Hct 45% (Sept 18) เหนื่อย dehydrate, ไข้ 39°C, petechiae 7.12 น ไข้ 37.7, PR 100/min, R 20, BP 100/70, ซึม flush 13 น BP 70/60 ให้ IV 760 cc/hr P 100/min, หอบ, ซึมเข้าเต็ม ๆ ตกเลือดเกล็ด ผลเท่า BP 100/50 ถึงวันที่ Sept 20.

	HN.	Serum No.	Name	HI D1	HI D2	HI D3	HI D4	HI CNIK	HI JE	Age	Day	Grade	DRC HCT	HBG	NUC	LYM	MY	PM
129 (OPD)	1818195	RS 400 (Sep 24)	6. เกาทุติ์ ชนแดนพก	1:80	1:80	1:320	1:160	<1:20	1:160	45/12	3	I						
		RS-444 (oct 24)		1:80	1:80	1:320	1:320	<1:20	1:640									
						DEN			JE									
					RS 400	37	76	109	123									
					RS 444	49	73	44	122									
130 (OPD)	1840900	RS-411 (oct 24)	6. ชาย แซ่ตั้ง	1:5120	1:1280	1:1280	1:5120	<1:20	1:10240	7	6	III (I)	46/	4,100	10	84	3/	2
		RS-416 (oct 24)		1:1280	1:2560	>1:20480	1:10240	<1:20	>1:20480									
						DEN			JE									
					RS 411	269	336	204	355									
					RS 416	362	315	176	422									
131 OPD	1424147	RS-412 (oct 24)	ญ พลิกา เกษรศิริ	1:40	1:40	1:80	1:160	<1:20	1:160	3 7/12	4							
		RS-424 (oct 24)	Not DHF	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20				9 วัน	85	15,500	61	35	
						DEN			JE									
					RS 412	33	22	93	73									
					RS 424	10	14	56	61									
132 OPD	1819776	RS-413 (oct 24)	ญ เอ้พิทักษ์ ทนทนน	1:640	1:80	1:320	1:320	<1:20	1:160	4	2	I	40/	4,500	79	21		
		RS-44 (oct 24)		1:320	1:640	1:2560	1:1280	<1:20	1:10240									
						DEN			JE									
					RS 413	31	67	130	119									
					RS 414													
133 OPD	1842390	RS-415 (oct 24)	6. วันชัย แซ่ตั้ง	<1:20	1:40	1:80	1:20	<1:20	<1:20	10 5/12	5	I						
		RS 428 (oct 24)	P-DHF	1:80	1:80	1:320	1:640	<1:20	1:640			DF.						
						DEN			JE									
					RS 415	59	[illegible]	[illegible]	50									
				X	RS 428													
134 OPD	1844062	RS-422 (oct 24)	ญ กรรณิการ วะกาทัศ	1:110	1:320	1:2560	1:640	<1:20	1:1280	14 (2516)	4	(3)	15 กย 40/	5300	62	33	1	3
		RS-442 (oct 24)		1:280	1:2560	>1:20480	1:5120	<1:20	1:2560				16 พค 44/	3700	59	41	2	
													1 ตค 43/	2500	38	66	18	
					RP 422	56	242	[illegible]	[illegible]									
					RP 442	[illegible]	323	10	[illegible]									
134 (OPD)	730812	RS-443 (oct 24)	6. ชัยพันธ์ ตั้งเทียร	1:640	>1:20480	>1:20480	>1:20480	<1:20	>1:20480	10								
		RS-443 (oct 24)		1:10240	1:80	>1:20480	1:10240	<1:20	1:10480									
						DEN			JE									
					RP-435	123	564	21	[illegible]									
					RP-443	45	471	47	[illegible]									
135 (OPD)	1498289	RS-437 (oct 24)	ญ พิมพรรณ กาญจนโชคชัย	1:80	1:160	1:640	1:2560	<1:20	1:2560	9	6	III	37/	4,500	70	18	A-(10)	
(เด็กป่วย)		RS-439 (oct 24)	* BP [illegible]	1:1280	1:1280	1:1280	1:2560	<1:20	[illegible]				34/	3,600	42	40	AL (17)	1
(OPD)		RS-458 (nov 24)		1:1280	1:2560	1:5120	>1:20480	<1:20	>1:20480									
					[illegible]	[illegible]	124	[illegible]	[illegible]									
					[illegible]	208	405	119	334									
					[illegible]	24[illegible]	[illegible]	176	519									
136 (OPD)	1076920	RS-445 (oct 24)	6. เกปิ่นโกะ จู่ทัน	1:640	1:2560	1:1280	1:5120	<1:20	1:10240	11	5	III	41/ 48/					
		RS 453 (nov 24)		1:1280	1:1280	1:2560	1:10240	<1:20	1:2560									
					[illegible]	123	234	20	277									
					[illegible]	[illegible]	535	57	407									

6วัน

BT	Platelets	C3
38.3		25 no 2536 2 เดือนก่อน ตามที่ clinic เคยเจาะเลือดไปตรวจที่โรงพยาบาล ไม่ได้ admit. liver - clinic บันทึกว่า Hct 44% WBC 7,950 N50 L30 เม็ดเลือดแดงปกติ Hct 38% WBC 5,300, P85 Lym 15%. คนไข้ไม่มีประวัติมาก่อน ประวัติไม่ชัดเจนเกี่ยวกับ Platelet
70,000		6 ม.ค. 26 ป่วยวันที่ 5 ปวด. ซึมลง ไข้สูงขึ้น ตรวจพบ petechiae ตามตัวและขาไม่มาก TT+, spl enlarged, not palpable not icteric, no jaundice, liver 10 cm ใต้ชายโครง ไม่ได้ admit ให้ fluid. BP ให้ mild 110/60, Petechiae เพิ่มขึ้น P. 120-130, BP 110-120/70-60 Hct 41%
1		Oct 7. อาจารย์ แต่งตั้งพัน - ตรวจ OPD ใน ตามร่างกายมีจุดเลือดไหลออกกระปริบ. pale ตรวจไม่พบ C lines 3 cm กดเจ็บเล็กน้อย บวมเล็กน้อยตามขา ไม่มีอาการ หรือ DHF TT+ ไม่มาก 6-7 เส้น ไม่ชัดเจนเท่าเมื่อวานนี้ ให้ admit วันที่ Oct 8. ไม่มี Liver sp., ผลวัน 9 ตค. Hct 36% ไม่ดำ, ตกตะกอน (7 ตค.) titer negative
184,000		เช้า Oct 8. มีไข้ขึ้นอีก ไม่มาก ไม่ค่อยเจ็บ liver -, no bleeding, Imp. DHF ให้ mild rehydration. Hct 40%. TT+, วันที่ Oct 9. อาการไข้ลดลง ตรวจเลือดออกกระปริบ ดีขึ้น ไข้ไม่ admit มีไข้ขึ้นอีก Oct 11, เพราะมีอาการ DHF ให้ mild dehydration, BP 100/60, PR 88 pulse full, no petechiae, Heart-lung clear, วันที่ 11 Hct เพิ่มขึ้น 39 → 42% ไข้ลดลง Oct 12
38.6 38.8		Oct 10, 8.56, ER มีไข้ขึ้นสูง 2 วัน ก็แสดงอาการเพิ่ม มาตรวจที่ clinic ส่งมาที่ ER (เช้าวันที่ 10) Oct 11, ไม่มีไข้ ไข้ลดลง อาการดีขึ้น ไม่มีไข้ TT+ ไม่มาก Imp. DHF stage ไข้ลดลง Oct 13. มีอาการไข้ขึ้นมาใหม่, BT 36.9 ตกตะกอน ให้ DHF stage I Oct 14 สบายดี ไม่มีไข้ ไม่มี liver
128,000 47,000		admit 15 Oct. เป็นไข้ 3 วัน เริ่ม, ตรวจ ER ไข้ 38 ตรวจพบ petechiae TT+ → 16 Oct ไม่มีไข้ลด เบื่อหน่าย ตรวจพบ liver just palpable, no pulse petechiae ตรวจพบมากขึ้น (Clinician ให้ยา I.) มีเลือดไหลที่ Hct 72,000 Hct 37% BP ต่ำ 90/60 17 Oct. มีไข้ดีขึ้น ไม่ค่อยเจ็บตามตัว BT 36°C petechiae ตรวจ ... back. liver ตกลงไม่ได้ 18 Oct ไม่มีไข้ อาการดีขึ้นมาก Hct 41%.
62,000		21 ตุลาคม ไข้ขึ้นมาตั้งแต่ 5 วัน ตรวจที่ ER เมื่อ 2 วันก่อน กับ petechial ตามตัวไม่มาก อาการไข้ไม่ดี liver 2 cm ↓ Imp. DHF stage II-III admit ให้ IV fluid BP drop เช้าวันที่ 2. P120, R24 BP 90/50 liver 2cm BP 90/50, flushing (23 ตุลาคม) Hct ↑ 39-40 BP 100/70 - 80/60 liver 4cm Imp. DHF toxic stage. 21 ตุลาคม ไข้ลดลง BP 90/60
5 5		21 ตค 15.23 รับจาก ER 21.20 น. 38°C ไข้สูง อาเจียน ซึมลง อาเจียนดำ TT+, Hct 43% จึงให้ DHF admit. OW1 Oct 27. เพิ่งมีเลือดออกตามตัว P 36-40, BP 110-120 / 80-90 ตอนเช้า 110/70 Hct 45% BP 90/60-70/50 ไม่มี active bleeding. 27-28 BP แกว่งมาก. ตอนเช้า Hct 35% อาการดีขึ้น กลับบ้าน.

	HN.	Serum no	Name	D1	D2	D3	D4	CHIK	JE	Age	day	grade	HCT	WBC	NEU	LYM	ATY	MCN
				HI														
137	[illegible]	RS-458 (NOV 1)	ญ [illegible]	1:160	1:160	1:1280	1:5120	1:20	1:10240	11	5	IV	50%	5900	59	32	19	9
		RP-455 (NOV 3)		1:2560	1:320	1:320	1:2560	1:20	1:10240				23%	3200	64	29	11	3
		RS-456 (NOV 4)	451 42 377 / 96 341	1:160	1:320	1:1280	1:5120	1:20	1:20480				49%					
		RS-460 (NOV 4)	455 4 384 / 33 322	1:10240	1:640	1:1280	1:5120	1:20	1:10240				34%					
			456 44 355 / 26 235															
		RS 474	460 29 226 / 15 250	1:20	1:20	1:20	1:20	1:20	1:20									
138	OPD 1734449	RS 447 (OCT 28)	ช [illegible]	1:160	1:160	1:320	1:640	1:20	1:1280	57	5	I	35%	5900	63	30	16%	
		RS 466 (NOV 16)	VAS/EIA IgG	1:5120	1:5120	1:10240	1:10240	1:20	1:20480									
				RP-447	21	100	36	106										
				RP-446	30	170	15	274										
139	OPD 1326646	RS 464 (NOV 10)	ญ [illegible]	1:5120	1:640	1:1280	1:10240	1:20	1:320	12	7	III	40% 34%	4300	87	64		1
		RS 467 (NOV 17)		1:5120	1:2560	1:10240	1:2560	1:20	1:20480									
				RP-421	40	411	56	227										
				RP-467	65	394	20	427										
140	OPD 1533888	RS 464 (NOV 16)	ช [illegible]	1:40	1:40	1:40	1:80	1:20	1:20	14	4	I	49%	6400	36			
		RS 477 (NOV 23)	VAS/EIA ✓	1:5120	1:20480	1:10240	1:20480	1:20	1:20									
				RP-464	24	43	39	45										
				RP-477	124	256	12	356										
141	OPD 1620069	RS 470 (NOV 17)	ช [illegible]	1:160	1:80	1:5120	1:160	1:20	1:160	11	5	III	47 487	7100 3400	74 32	16 48	(16)	3
		RS 471 (NOV 18)	[illegible]	1:1280	1:1280	1:10240	1:20480	1:20	1:5120				33%	3450	31	67	(16)	
	OPD	RS 478 (NOV 23)	VAS/EIA IgG ✓ IgM	1:5120	1:20480	1:20480	1:20480	1:20	1:2560									
				RP-470	35	37	43	[illegible]										
				RP-471	51	318	34	225										
				RP-478	62	440	23	3[illegible]										
													43%	5600	30	70	(17%)	
142	KU 1534144	RS 473 (NOV 17)	ช [illegible]	1:80	1:40	1:40	1:160	1:20	1:40	8 เดือน	8	IV	41%	5				
		RS 481 (NOV 30)	(LP-DHP)	1:80	1:40	1:160	1:160	1:20	1:40									
			VAS/EIA IgG ✓ IgM	RP-473	220	13	30	22										
				RP-481	451	26	47	9										
143	OPD 1617563	RS 476 (NOV 23)	ช [illegible]	1:1280	1:2560	1:10240	1:20480	1:20	1:2560	5	6	III	41%	(NOV 22) [illegible]	35	81	(12)	5
	OPD	RS 482 (NOV 30)		1:5120	1:2560	1:2560	1:5120	1:20	1:5120								41% 27%	
				RP-476	256	128 ✓	158	267										
				RP-482	456	517 ✓	106	576										

BT	Platelets	Cg
		→ แม่อนาถิ่น (R)
37°C	100,000 29,000 22,500	- 1 พ.ย. 2530 ปวดหลัง เริ่ม PTA 4 วัน PTA มีไข้ ตลอด ER ตลอด DHF ซึมไม่ร้องไห้มาก อาเจียน ท้อง ไม่ถ่ายเหลวมาก ไข้ก่อน ขึ้น ทุกอย่างเรื่อง Tachycardic liver 2cm Imp. DHF + shock BP ต่ำไม่ได้ PR วัด ให้ 5% D/W 55 cc/hr/ใน ขึ้นมาเป็น 40/60 ถึง 80/60 Hct 50 ทำ blood exchange transfusion ที่ 1 ขวด ตกเลือด ตั้งแต่ตรวจ 6 ชม แล้ว 14.00 ก็เพิ่ง อาเจียน → bleeding GI. * C3 - ลดลง
37.2-38.6°C	160,000	เริ่มมีประวัติไข้ตั้งแต่ 1 ขวบ มีน้ำหนักน้อย, ตั้งท้อง Oct 28 อายุ 5½ ปี หลังจากนี้ เมื่อคืนนี้มีไข้ถึงตอน 4 ชั่วโมง ไม่อาเจียน ท้องไม่ liver 1.5cm ↓, AP⁻, BP 110/70, PR 100, RR 24, TT⁻, ซีด malnutrition Hct 35 อายุ 1 ปี 29 กรัมเท่านั้น ไม่มี Hemoconcentration ตับแข็งดี ไข้.
(~38.3) 30,000		9 พ.ย. ขึ้นจาก ER 21-30 มีไข้ 5 วัน ไม่ถ่ายเหลว แต่ง ตรงไข้ หลังจากนี้ ซึมมาก เดือนสอง ไม่มี petechiae, spleen liver OK. BP แกว่ง 70/40, P 92 Lyad TV, TT⁺, DHF 3 impending shock 10 พ.ย. V.S ปกติ BP 100/60 ไม่ต่อเนื่อง
39°C (ตามเนื้อ)	76,000	ตั้งแต่ 16 พ.ย. เป็นไข้มาก่อน 3 วัน หลังมื้น ปวดเมื่อย ไม่ถ่าย ไม่มีปัสสาวะ เดิน หน้าซีด BP 100/70 ไม่ต่อเนื่องมาก ตอน CBC หนาเรื่อง. ก่อนหน้า 23 พ.ย. ตรวจ Cm. ไม่ถ่ายเป็นเวลา 5 วัน. ไม่ทำ TT.
38 8	3.4×10³ 7.6×10³ 36,000	16 พ.ย. 2530 4 วันไข้ ปวดท้อง เบื่อ ซึม เป็นมาก อาเจียนเป็น, ปวดท้อง ตับโต liver 3 cm, T 38 เหงื่อ P110, R28 BP 90/60 แกว่งไข้ ดูแล II Hct 40% ให้ fluid ให้ยาแก้ปวด ตั้งแต่ admit วันที่ 17 พ.ย. ตลอดเวลาวัดได้ 110/70 / 100/60 ห้องแต่ให้ fluid (ให้ยาแก้ไข้ fluid เรื่อง) วันที่ 19 ตุลาคม BP ต่ำลง 110-100/50-70 ให้เลือด FFP, Plasma milk, platelet.
4,000 20,000 12,500 104,000		16 พ.ย. 2530 ตลาดแดง 19.15 BT 38.7. BP 80 กลม PR 100/min. liver 4 cm Hct 41. เป็นไข้ 5 วันแล้ว BP 80-85 / 50-60 P เหงื่อ 156-66. มีอาเจียน 17 พ.ย. BP 80/50 → 40/20 ให้ยาเข้าทาง IV Hct ลด 34%. 19 พ.ย. OK IV fluid. BP 90-96/40-52 อาเจียนมาก ตั้งแต่ 20 พ.ย. เริ่มเป็นเลือดใหญ่. ตับโต. OK ตับ. ขึ้น Serum.
44,000		วันที่ 19 พ.ย. 2530 ตับแข็งไม่ชัด 1 วัน ไม่ถ่าย อาเจียน ปวดท้องมาก ถ่ายเป็นเลือด อีก 2 ครั้ง เช้า ขึ้นมา 23 พ.ย. ad DWI. pulse 120., BP 100/60 → 80/50 P → 130 ตั้งแต่ load .5% D/N 50 cc. BP 90/60 → 110/70 ให้ 112 ml fluid Hct. เหลือ 40% ซีด grade II-III เหงื่อไม่ IV ตลอด.

	HN.	SERUM NO	NAME	HI D1	D2	D3	D4	CHEK	JE	AGE	PAY	GRADE	HCT	WBC	NUE	LYM	MY	MON
144 OPD	1838633	RS 80-483 (Dec 1,81)	ส. วิชัย เลขที่	1:160	1:640	1:640	1:5120	1:20	1:640	9	5	III	[illegible]	4400	40	53	14%	
เช้า ส		RS 51-451 (Dec 4)		1:320	1:1280	1:2560	1:10240	1:20	1:1280									
		RS-507 (Dec 24)		1:640	1:1280	1:2560	1:5120	1:20	1:640									
				RP-413	67		344	72	232									
				RP-417	14		470	42	395									
				RP-507	23		390	8	329									
145 เช้า	1838455	RS 484 (Dec 4)	ก. [illegible]	1:2560	1:10240	1:5120	1:10240	1:20	1:10240	8	7	II	41%	5900	30	20	40 (10)	6
OPD		RS 494 (Dec 7)	ส. [illegible]	1:5120	1:10240	1:10240	1:10240	1:20	1:10240									
				RP-484	112		510	49	538									
				RP-492	253		543	42	609									
146 เช้า	1838457	RS 485 (Dec 4)	ด. [illegible]	1:5120	1:10240	1:1280	1:10240	1:20	1:5120	13	8	II						
OPD		RS 498 (Dec 7)		1:2560	1:1280	1:20480	1:10240	1:20	1:5120									
				RP-485	265		506	48	466									
				RP-496	326		574	74	532									
147 OPD	1839133	RS 489 (Dec 4)	ด. [illegible]	1:20	1:20	1:20	1:20	1:20	1:20	11	6	(9)	40.5% 35.8 1500					
		RS 491 (Dec 7)		1:40	1:40	1:320	1:320	1:20	1:80									
			(P-DHP)	RP-489	111		34	23	38									
				RP-491	433		56	47	20									
148 เช้า	1667695	RS 496 (Dec 14)	ด. [illegible]	1:2560	1:5120	1:10240	1:10240	1:20	1:5120	5	4	III	47%	6100	37	35	18	7
		RS 497 (Dec 15)	ด.	1:2560	1:256	1:10240	1:10240	1:20	1:5120				40%	4200	23	50	19	6
		RS 498 (Dec 14)		1:10240	1:1024	1:5120	1:10240	1:20	1:10240				37%	5400	56	38		6
		RS 503 (Dec 4)		1:2560	1:10240	1:10240	1:5120	1:20	1:640									
				RP-496	122		511	110	502									
				RP-497	160		526	42	544									
				RP-498	171		533	09	632									
				RP-503	184		526	61	530									
149 OPD	1800264	RS 149 (Dec 30)	ด. [illegible]	1:20	1:20	1:20	1:20	1:20	1:20	13 5/12	4	?	38%	5800	85	13	2	2
		RS 443 (Aug 11)		1:1280	1:1280	1:10240	1:5120	1:20	1:1280				Hct 40%	5300	57	33	4	6
Typhoid & Weil-Felix blot มาด้วย AL				429 7 0 92 10 / 247 176 415 55 315														
150 (เช้า)	1843618	RS-501 (Dec 20)	ง. [illegible]	1:640	1:2560	1:5120	1:10240	1:20	1:640	14	8	III	42.8 36	4600 3800	17 43	59 47	1 (9)	7 1
		RS-502 (Dec 21)	VAS/EIA ✓	1:2560	1:5120	1:5120	1:10240	1:20	1:1280				34	4900	36	53		59 1
OPD		RS-506 (Dec 28)		1:640	1:2560	1:5120	1:10240	1:20	1:2560									
				RP-501	155		421	74	474									
				RP-502	176		521	110	614									
				RP-506	200		513	67	580									

วัน	PLATELETS	C3	
1.6	45,000		[illegible] Hct [illegible] WBC 4300 [illegible] 115,000 [illegible] DHF 1 วัน. [illegible] admit [illegible] BP 100/60 liver 3 cm. (Impending shock). [illegible] BP 95/65 → 80/60 → [illegible]
2	50,000		30 ก.ค. 2530 1001 19.30 น. ER [illegible] 4 วัน + คนไข้ 2 วันก่อนมาอาเจียนมาก [illegible] liver 1 cm, BP 110/50 R 100, R 66 มี petechiae ทั่ว. Chest wall Imp DHF Stage II Grade II. discharge 2 ก.ค. เกร็ด [illegible]
			รับ 30 ก.ค. ไข้มา 5 วัน 2 วันก่อนไข้ลงแล้ว 1 วันอาเจียนมาก BT 37.5 RR 24 BP 110/80 Hct 40% WBC 4300, P 18, L 59, ATL 12, B 3% PLT 46,000 cell/mm³ เกณฑ์ DHF grade II No petechiae, mild injected conjunctiva, maculopapular rash (ตั้งแต่ wall) BP ไม่ต่ำลงตลอดเวลา 36 ชม. – 2 ชม. เกิดมีอาการ weak - dry lip.
ก่อนเข้ม 40,000			ก. 2 ส.ค. 2530 ไข้มาแล้ว 5 วัน, หน้าแดงมาก มีตาแดง เกร็ดเลือด. ซึม, P 72, weak, dry lips, liver 2 cm, SPO Imp Fever c hepatomegaly BP 110/70. PT – ไข้ลงวันที่ 3. Hct วันที่ 3 มี 41.3. WBC ภายหลัง 3350 N 27, L 66 M 1, E 1 plat 76,000 AL (5)
136,000			[illegible] Dec 12-15. Dec 12 รับจาก ER เวลา 14.10 น. [illegible] BP 70/50 Dx DHF grade III [illegible]
50,000			[illegible] Dec 13 [illegible] petechiae BP [illegible] 100/70 ไม่มี active bleeding Dec 14 เกร็ด [illegible] petechiae [illegible] Hct [illegible]
122,000			Dec 15 [illegible] weak เดินไม่ออก [illegible] BP 70/50 [illegible] admit [illegible] 100/70 [illegible] 130 [illegible] weak [illegible]
114,000			[illegible] BP 110/70 Pulse 110/m Resp 111 Dx DHF + hypoglycemia, [illegible] pulse [illegible] 100 mild drip [illegible] ER. (เวลา [illegible] 10.11 น.) PT – Hct 40% [illegible] 4 cm [illegible] BP 110/60 (Macular rash)
49,000			Dec 19 admit. BP 90/60, P 88. Hct 33% (Al 30 %) [illegible] 6 วัน
4 36,000			Dec 20 [illegible] Shock stage Hct 40% BP 70-50/50-60 pulse [illegible] fluid. [illegible] Hct 35% [illegible] petechiae [illegible] BP 80/60 – [illegible]

1988.

	HN.	Serum No	NAME	HI D1	D2	D3	D4	Ch/A	JE	Age	day	grade	Hct	WBC	Mc	Ly	HTY	Mono
1. (OWI)	1993551	RS-001(May 18)	ด. [illegible]	1:1280	1:1280	1:1280	1:10240		1:640	10	4	I	17 → 40 / 38 (4/21 → 11)	2700 / 2300	67 / 62	30 / 72	20%	3 / 3
		RS-004(May 19)		1:540	1:5120	1:10240	1:10240		1:1280				40%	3600	6	24	51%	6
		RS-003(May 20)		1:5120	1:5120	1:10240	1:10240		1:5120				40	5100	13	81	21%	
2. (OWI)	1891/13/19	RS-004(May 22)	ด. [illegible]	1:640	1:640	1:10240	1:1280		1:40	12	5	II	45%					
		RS-005(May 24)		1:5120	1:2560	1:10240	1:5120		1:160									
		RS-003(May 25)		1:5120	1:2560	1:10240	1:5120		1:160									
3. (OWI)	1869991	RS-006(May 24)	ด. [illegible]	1:160	1:40	<1:20	<1:20		<1:20	9								
		RS-008(May 25)		1:80	1:20	1:20	1:20		<1:20									
4. (OWI)	1897244	RS 007(June 2)	ด. [illegible]	1:80	1:160	1:80	1:160		1:20	14			44 / 44→42	2,900	57 / 43	43 / 53		8 / 2
		RS 010(June 3)		1:320	1:320	1:640	1:1280		1:80									Reject
(C2d3)																		
5. (OWI)	1905892	RS 008(June 23)	ด. [illegible]	1:5120	1:1280	1:5120	1:1280		1:40	12	7	III	39% / 47% / 39%	44,000 / 7,000	42 / 9	48 / 41	21 11% / .2%	1
		RS 011(June 24)		<1:10240	<1:10240	<1:10240	1:5120		1:160									

652

87	Platelet	C_3

145,000
123,000
159,000

แทงอัด DHF gr II

เข้าวัน 17 - 20 ก.ค. เกิด ER ไข้ 3 PTA ตลอด ไม่มีอาการ ปอดสั่นสะท้อน, liver just palpable, spleen ok. TT+ HR 80-40/min BP 110/60

45.1°C
38.5°

วัน 22 ก.ค. 2531 4 เดือนก่อนคลอด มีไข้ อ่อนเพลีย ปวดศีรษะ ไม่คลื่นไส้อาเจียน ไม่ปวดท้อง TT+ 14 ชม. ไข้ 40°C ไม่มี active bleeding P108/m BP ไม่ลด

ปรากฏไม่ต่อเนื่องกับ 2ab มีแต่ความแตกต่างกับ DHF profile

เข้าวัน 23 ก.ค. - 26 ก.ค. มีไข้ ๆ 15 ม. วันแรก ER ร่วมด้วยปวดท้องน้อย ไม่คลื่นไส้ อาเจียน ปวดศีรษะ ปวดเมื่อย ไข้ไม่ลด tonsil 2+ มีแต่แดง BP ไม่แดง Hct วันที่ 4 : 1 (25 ก.ค.) เมื่อกลับไป เกร็ด หมดเม็ดเลือด

ไข้ Hct alger.
1 6 185,000

วันแรก ER 1 วัน ไข้ หลังจากนี้ อาเจียนเล็กน้อย weak BP 120/80 m/mg pulse 100/min ตลอดวัน ให้ IV + ยาแก้ปวด อาการดีขึ้น ไม่มี active bleeding 3 วัน Hct 44% อาการดีขึ้น 110/70 37.8°C BP ก่อนจะหายวันที่ 3 100/60 ไม่มี active bleeding sign.

12.40 น. วันที่หลังจาก ER 6 วัน ก่อนคลอดวันเดียว ไม่มีอาการ ไข้ต่ำๆ ต่อมาพบ petechiae ทั่วไป เช่น DHF (ไม่ impending shock ให้ IV. 36 ชั่วโมง BP 90/70 37 ชั่วโมง BP 100/50 ไม่มี active bleeding ภายหลังมี Hemoconcentration + leak fluid ออกจาก vessel

HN.	Serum no	Name	HI D1	D2	D3	D4	CMIA	JE	Ag dry yeast 6		HCT	WBC	PLC	Lym	Mny	Mono
6 (ชาย) 4417143	RS-30(Jun 30)	ง วิเชียรจันทร์	1:20	<1:20	1:40	1:80		<1:20	6	9	45%	6400	67%	30%	4	
OPD	RS-34(Jul 9)		1:20	1:20	1:20	1:160		<1:20		DHF						
				Den M 20	G 11	M 6	G 22	7								
7. (ชาย) 1899174	RS 45(Jun 28)	ง สิงคาล	1:20	1:20	1:40	1:80		1:20	2 9/12	4 DF	35%	5,000	52	48		
	RS-46(Jun 29)		1:80	1:80	1:320	1:1280		1:80			33%	4,300	68	30	Baso 1	1
	RS-47(Jun 30)	ไข้ 7 วัน	1:1280	1:640	1:2560	1:5120		1:1280			41%	7,500	40	58	Mg 1	
	RS-50(Jul 1)		>1:20480	1:10240	>1:20480	>1:20480		>1:20480			39%	8,400	24	72	Baso 1	3
(2.d3)																
*																
8. (ชาย) 1908403	RS 48(Jun 30)	ง วิเชียร	1:20	<1:20	1:40	1:40		<1:20	18/9 6	IV 7A. 54.3% 43%	15600	74	16	68	2	
	RS 49(Jul 1)		1:80	1:20	1:80	1:320		1:40								
	RS 31(Jul 2)		1:20	<1:20	1:80	1:160		<1:20								
	RS 32(Jul 3)		1:20	<1:20	1:40	1:160		<1:20								
	RS 33(Jul 4)		1:40	1:20	1:40	1:160		<1:20								
	RS 37(Jul 5)		1:40	1:20	1:40	1:320		<1:20								
	RS 39(Jul 6)		1:20	<1:20	1:40	1:160		<1:20								
หลัก 3	RS 46(Jul 10)		1:20	<1:20	1:80	1:160		<1:20								
หลัก 2	RS 83(Aug 4)		<1:20	<1:20	1:20	1:20	<1:20	<1:20								
9 (OPD) 723193	RS 41(Jul 5)	ง ตาวิทย์	1:160	1:160	1:320	1:1280		1:80	10 8/12 5		37%	4800	51	38		11
ON	RS 44(Jul 9)	สุขาเพิ่งรู้	1:320	1:640	1:2560	>1:20480		1:1280			41%					
OPD	RS 84(Jul 14)		>1:20480	>1:20480	>1:20480	>1:20480		>1:20480			10วัน 38% 37%	5400 4,200	28 64	45 33	29%	4 1
10 (OPD) 430072	RS 95(Jul 13)	ด ฐิติ	<1:20	<1:20	<1:20	1:20		<1:20	1	5	13 7/12 41%	5,400	43	36	Bal 8	13
	RS-97(Jul 15)		<1:20	<1:20	<1:20	<1:20		<1:20		4						
				DEN M 1	G 0	JE M 2	G 0									

Glow

เด็กชาย C3

รับไว้วันที่ 25 มี.ค. 2531 เวลา 1 ชั่วโมงหลัง คลื่นไส้ ซึม BP 110/70 Hct 36%
WBC 12600, N77, L11, M12, E4, Platelet 250000, ตรวจพบว่ามี pulse pressure
แคบ และ Hct 44 รับ admit (+ shock) มีประวัติ Bronchial asthma
อาการไข้ต่อเนื่องเหมือน DHF แต่ Hct สูง อาจเกิดจาก dehydration. TT⁻ shock อาจเกิดจาก
hypovolemic (dehydration) ร่วมกับ + vomiting + shock.
เจาะเลือดซ้ำ Hct 35%, 37%, 32, 31 BP แกว่งไปมาตลอดวัน

214,000

วันที่ OPD 28 มี.ค. 1031 15.น. เดินมา OPD เริ่ม ซึม 31. ต่อมาสั่นหัก
10 นาทีต่อมา เริ่มมีชักกระตุก ไข้ มีอาเจียน ตัวเขียว. ให้ออกซิเจนมีชีพ ตัวร้อนมาก
ไข้. ไม่ตอบสนองต่อการ ก่อนหน้านี้ 37° C ห้อง TT+ 7400 g/dl.
ในห้องกดตามตัวเขาเย็น ให้ IV fluid ไว้ ไข้ลด 37.9°C P.124/ R.28
ซึม. weak BP 110/80 liver ใต้ เท่าไม่มี Hemoconcentration.
สรุปว่า เป็น DF แต่ต้องรีบให้ IV ตลอด.

213,000
142,000
109,000
69,000

2.3/02

อายุครบ 3 วัน - 16 กค. หลังก่อนมาโรง 5 วัน
เป็นลูกมากขึ้น มีไข้ 5 วัน ไข้ ซึมลงเรื่อย ตม
Hemoconcentration ค่า 180,000 เด็กซึมมาก ตม
infiltration ปอด รับ refer มา. RE ตรวจไม่พบ
Restless, sweating, tachycardia, liver+ 3FB, spleen 1 cm
Hepatitis profile⁻, BP ↑ ควบคุมได้ดี, ปาเจ็บ coffee ground 40-5 ml
วันที่ 11 ก.พ. เวลา 22. Hct ขึ้นถึง 37% platelet
count 30,000 มี maculo-papular rash

(1) DHF (2) Shock (3) Pneumonia + pleural effusion
DHF with ARDS
DSS with cardiovascular collapse
มี Renal failure. Rising BUN, CT, hematuria

134,000
low No adequate

adequate
400,000

ให้ Grade I DHF TT+. liver 1½ cm/RCM
วันที่ ER 1031 11.40 น. มีไข้ 4 วัน ไข้เล็กน้อย ท้องเสียบ่อย ไม่ธาตุไว้. เด็ก
กลับมาอีกครั้ง 7.15 น. วันที่ 9. ซึม ไข้ เริ่มตอ ตม Hct ขึ้นจาก 37→41.% รีบ
ให้ admit. ไข้ 38°C VS stable วันที่ 10.

164,000

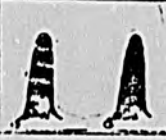
13 กค. 2531. เด็กมีไข้ 4 วัน หมดแรง ซึมลงเรื่อย ไข้ ไข้สูงตลอด 4 วัน หลังจาก
ไข้ไม่ลด 9 วัน แล้วไข้ขึ้นมาอีก 2 วัน. เมื่อกลับมาอีกครั้งดูซีดมาก ไข้
ตก HP เดิม 2 วัน ไม่ธาตุมาก dx ไข้.

HN	Sample No	Name	III D1	D2	D3	D4	Chik	TC	Mp	Neg grade		Hct	WBC	RBC	Lym	AM	Memo
1. (OPD) 1919833	RS 50 (30/12)	ส. คนสิน อภาวุฒิ	1:1250	1:160	1:5120	>1:20480		1:1280	8	4	DF.	39 %	3,700	44	32		1
	RS 56 (30/01)		1:10240	1:10240	>1:20480	>1:20480		>1:20480									
12) (OPD) 1806438	RS 84 (30/11)	ด. สิทธิศาร ศักดาน้อย	1:160	1:80	1:640	1:640		1:80	4	3	~	38%	8400	8.3	13		
	RS 89 (30/05)		1:320	1:40	1:640	1:640		1:40			DF?						
13) OPD 1904557	RS 15 (30/04)	ด. วิไลลักษณ์ สันตติ	<1:20	<1:20	<1:20	1:80		<1:20	12	3	II.						
	RS 16 (30/05)		<1:20	<1:20	<1:20	1:80		<1:20									
(Q = d3)																	
14) OPD 1914138	RS 55 (30/10)	ด. ฟันธีรักษ์ พงษ์ธนิตา	>1:20480	1:10248	>1:20480	>1:20480		1:1280									
	RS 57 (30/11)	ศิลป์	>1:20480	>1:20480	>1:20480	>1:20480		1:5120									
OPD	RS 60 (30/12)		>1:20480	>1:20480	>1:20480	>1:20480		1:10240									
15) OPD 1899699	RS 18 (30/03)	ด. ปราจวล ปลายเกว้	1:80	1:80	1:160	1:160	<1:20	1:40	182	6	III	35% 44	2300	76	21	ALL	1 blast
OPD	RS 46 (30/07)		1:320	1:320	1:640	1:1280	<1:20	1:320					1800				
เด็ก 2	RS 46 (30/31)	ด.	1:2560	>1:20480	>1:20480	>1:20480	<1:20	>1:20480					7800	10	85	AL 85%	54

BT	Platelet	Cs
210,000		เริ่มป่วย 16 กค 2532 มีไข้สูง ปวดศีรษะมาก อาเจียน มีอาเจียนหลังทานยา ปวดท้อง ตาแดง TT -, อัตราเต้นหัวใจช้ากว่าไข้ เป็น Viral Infection ให้ยาลดไข้ แก้ปวด ให้น้ำเกลือ มาหายเองได้ ไม่ได้ไปไหน - 20 กค มีผื่นขึ้นตามตัว กลืนไม่เป็น 2 วัน ปัสสาวะน้อยลง มีอาเจียน เพ้อ กด P 80/min RR 20/m T 36.6°C ท้องผูก ถ่ายดำ DHF ไม่ได้ไปที่ไหนเลยนอกจากที่ รพ.
180,000		8 กค 2532 ไข้มา 2 วัน ไข้ สั่นเล็กน้อย. อาเจียน ถ่ายเหลว 2-3 ครั้ง. TT - ส่งมาเช็ค RO - DHF 5 กค ทนไม่ไหว ไข้สูง ตัวร้อนจัด. liver 3 cm b. f.m.
38.8°	 แพทย์ให้ Fever @ Vomiting	อายุ 12-13 ปี. BP ไม่แดง ส่งจาก ER วันที่ 12 เวลา 11.15 น. ปวดท้อง มีไข้สูง อาเจียนเยอะ TT+ เหนื่อย mild dehydrate T 38. P 108/min R 24/min BP 130/60 ไม่มี petechiae
6 วัน หลังป่วย 60,000	 15/8 15/11 15/14	5 วันก่อนมาถ่ายไม่ได้ เพลียมาก ปวดท้อง ไม่ได้ไปไหนมาทั้งสิ้น ไข้สูง 38.5°C liver 1 FB, spleen not palpable, no petechiae, TT+ Hct เพิ่มจาก 35 → 47 ให้น้ำเกลือแก้ ให้ adm. BP 90/60 WBC 1600, plt 62,000 มี hepatomegaly liver 2 FB ต่อมา 3 วัน BP อยู่ระหว่าง 90/65-70, pulse > 100

HN.	Serum no	Name	HI D1	D2	D3	D4	CHIK	JE	Rx Dry gude		Hct	WBC	Neu	Lym	Aty	Mono
N.OPD 1300719	25-42 (Aug 1)	ด. ศศิธิมาศ ผดุงศรี	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	5½ 2	I	27%	4,600	66	31		2
	25-71 (Aug 3)	สมัครดี	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20		NDF.						
	25-78 (Aug 6)		1:20	1:40	1:50	1:110	<1:20	1:40								
	(D2 d3)															
458488																
1290PD 458488	25-17 (Aug 2)	ด. อาทุต กาฬวัง	1:1280	1:640	1:160	1:160	<1:20	1:80	ปี 3							
OPI	25-13 (Aug 4)		>1:20480	>1:20480	>1:20480	>1:20480	<1:20	1:1280		25	34%	4900	81	8		M 10
		DHF @ Convulsion.								28	37%	3300	62	36		EO 3
										28	39%	6300	30	58	AT 7%	M 4
										30	30%					B 1
ห.เด็ก 1917353	25-65 (3/31) กพ	ด. ศิริวัฒ เสือกวงเมือง	1:640	1:320	1:640	1:320	<1:20	1:80	7 5	IV	52% 47% 44%	6,200				
OPD	25-79 (Aug 5)		>1:20480	>1:20480	>1:20480	>1:20480	<1:20	1:1280								
9 OPI 1917154	25-19 (3/30)	ด. ชุติกา แก้วตั้ง	1:1280	1:2560	1:2560	1:7680	<1:20	1:1280	4 ปี 6 เดือน	5. III	36.5. 38% 35% 35% 35%	8,700	19	81%	5%	2
ICU	25-74 (Aug 1)		1:1280	1:1280	1:2560	>1:20480	<1:20	1:1280			35%	5,900	44	49.	0%	5
	25-75 (Aug 2)		1:2560	1:2560	1:10240	>1:20480	<1:20	1:10240			43%	7,300	51	42	15%	6
	25-76 (Aug 3)		1:5120	1:2560	1:10240	>1:20480	<1:20	>1:20480			38%	8,100	42	40		2
OPD	25-81 (Aug 15)		1:10240	1:10240	>1:20480	>1:20480	<1:20	>1:20480							19%	
ห.ชาย 1900187	25-32 (Aug 4)	ด. สมัคร์ ศิริพันธ์	<1:20	<1:20	<1:20	1:20	<1:20	<1:20	4		35%	11,400	73	19		5
OPI	25-41 (Aug 1)		<1:20	<1:20	1:20	1:20	<1:20	<1:20								

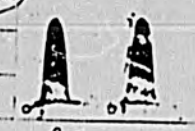
	DEN.		JE	
	M	G	M	G
75	30	10	11	2
15				

BT	Result	C3.
	320,000 [illegible] [illegible] [illegible]	มีไข้ 1 วัน ไม่เจ็บคอ ซึมลงมาก ไม่ค่อยรู้สึกตัว ไม่ค่อยรู้เรื่อง ไม่ค่อยพูด เหนื่อยง่าย spiderlaw. ตับ 1 ซม. hot wet, not pale TT – no bleeding tendency ไม่ค่อยรู้เรื่อง ward
45	adjust 49000	25 – 30 กค 2531 รับไว้ในแผนก ER 3 ม. (25 กค) ประวัติก่อนมาปีที่ ไม่ค่อยไม่สบาย ปลายปีนี้ มีไข้ออกบ่อยๆ ๆ วัน ก่อนมา รพ มีชักเกร็ง ตาค้าง ๆ ๆ ครั้ง ไม่ค่อยรู้สึกตัว 2 วัน good consciousness not pale liver spleen – มี Convulsion อาเจียน ง่วง drowsy เหม่อๆ ลืมๆ ตาๆ ติดเชื้อ encephalitis มี Seizure disorder ตับ 30 ซม มี Liver โต + 1.5 cm.
36.8		อุจจาระ 1-4 ซ.ม. อุจจาระเหลว ไข้ก่อนมา 5 วัน ไข้เรื้อรัง ไม่สบายๆ 4 วันก่อนมาเริ่มอาเจียน ซึมลงมาก มีเลือดกำเดาไหลบ่อยๆ TT + มองว่าน่าจะมีเลือดออกในทางเดินอาหาร ไม่ค่อยรู้สึกตัว ไม่ค่อยรู้เรื่อง ไม่ค่อยพูด เหนื่อยง่าย ตับ 2 ซม. Liver โต ตับมีก้อนมาก ~ 10 วัน/เม รับ ~ BP 90/60 1 เม.ย./มาก Liver โต ตับมีก้อน มีอาการเหมือน dengue shock syndrome | Hct 48% → 52% จาก 5 05 น มีเลือดไม่ค่อยแข็งตัว
31,000 19,000 10,000 100,000	19.6.9	31 กค 2531. 34.00 Hct 35.5% รับมาจาก ER ไข้สูง อาเจียน ท้องเสีย รับไว้ 38 ชั่วโมง. BP 40/70 P 120/นาที R 28/นาที, มาถึงแล้ว ก่อนตาย 7.15 น. (1 ชม) BP 40/70 เกร็งๆ กลายเป็นเลือดออก, ผื่นขึ้นมาก มีเลือดออก อาเจียน อาเจียนสีน้ำตาลปนเลือด petechiae. แขน 2 ข้าง เขียวช้ำ กดเจ็บ เลือดไม่ค่อยออก Hct 34% 129 มี thus emotions. BP แขนซ้าย 15-34 น. 88/45 – 51/60 ห้องปัสสาวะ เป็น late IV. 5 % 3/HSS late 50 / –
40°		 3 กค 2532 ผู้ป่วยอาเจียน ไข้ หายใจลำบาก เสียงแหบ ไม่สูบบุหรี่ มีประวัติชัก ดื่ม 4 วัน ไข้สูง ปวดศีรษะ infected tonsil + Lipo good consciousness liver 1 cm + RCM Acute Rhinopharyngotonsilitis

	HN.	Serum No.	Name	HI P1	P2	P3	P4	CHIK	TE	Age	Day	grade	Hct	H.P.C	N.S	Lym	Mo	Plate
21. OPD	1625165	RS 80 (Ag)	น. ตัวสำคัญ ชะ สุวรรณ	1:2560	1:5120	1:20480	1:20480	<1:20	1:5120	3½	3.		30 วัน 35	11500				
		RS 92 (Sp.2)		1:640	1:1280	1:2560	1:1280	<1:20	1:160				35	11500				
22. ชัยวัฒ	594502	RS 82	น. ผัดชาวัง ตรีชูพงษ์	1:20480	1:20480	1:5120	1:20480	<1:20	1:10240	12	7	III	40%	4500	73%	27		
OPD		RS 90 (Ag 22)	(β-thalassemia Trait. Hindered 30%)	1:2560	1:2560	1:5120	1:20480	<1:20	1:2560									
23. ชัยวัฒ	242343	RS 98 (Sp.23)	ด. สมชัย ฤทธิรักษ์	1:640	1:320	1:320	1:1280	<1:20	1:20	12/9	4	II	45%	6500	77	15	[illegible]	8
"		RS 100 (Sp.30)		1:1280	1:1280	1:5120	1:2560	<1:20	1:40				42%	4900	59	25	[illegible]	8
OPD		RS 106 (Sp.10)		1:20480	1:20480	1:20480	1:20480	<1:20	1:2560				interstitial					
24. ชัยวัฒ	1853326	RS 96 (Sp.23)	ด. ทวม สุวรรณศิริ	1:20	1:40	1:20	1:40	<1:20	<1:20	9/12	4	III	40%	7300	19	28	P1	
"		RS 97 (Sp.25)		1:40	1:160	1:80	1:80	<1:20	<1:20				37%	9300			[illegible]	
OPD		RS 106 (Sp.10)	P-DHF	1:320	1:320	1:640	1:1280	<1:20	1:320									
25. ไชย	1936989	RS 99 (Sp.27)	ด. เกศกล เทพมุนีรักษ์	1:1280	1:160	1:1280	1:2560	<1:20	1:40	1 4/a		IV	46%	9800	41	38	[illegible]	3
OPD		RS 107 (Sp.10)		1:5120	1:5120	1:10240	1:20480	<1:20	1:10240				39%	7,350	36	52	5	5

RT [illegible] C3

[illegible]



8 ม.ค. 2532 liver 4 cm ที่ RCM sharp edge [illegible]
[illegible] 2 d, [illegible] 30 [illegible]
[illegible] liver 2FB Hct 1 PC 10

[illegible] { เกร็ดเลือด DHF
infected Thrombophlebitis
อาหาร Hypothyroid

30,000

[illegible] 1 [illegible], [illegible] 1 [illegible] [illegible] anemia + [illegible]
hemolytic anemia Hct 33-32 % weak, puffy eyelids, BP 90/60
PR 100/min full, RR 20/m, liver 3 cm ↓, Hct 40%
WBC 4200 - BP 90/60 → 100-110/60-70, TT+ [illegible] 110/90
PR 104/min R 30/min [illegible]

10.2
1.8 43,000

29 ก.ค. 2532 [illegible] 3 [illegible] [illegible]
Pr 84, R 40/min BP 110/70 good consciousness not pale, mild
dehydration, Hb 15.8 Gr fluid 320 cc hydrocortisone 150 mg
panacet 325 30 [illegible] PR 96 RR 30 BP 110/70 Hct 42

38,000
39,000
40,000

[illegible] 1990 2531 [illegible] [illegible]
[illegible] 90-100/70 [illegible] [illegible]
[illegible] T 37.6 Hct 35% [illegible] 29 [illegible] Hct [illegible] [illegible] 160 mm
PR [illegible] 60/min [illegible] Hct 29% [illegible] [illegible]
[illegible] 3 [illegible] liver 4 cm ↓ BP [illegible] 110/70

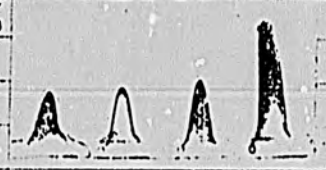
[illegible]
30,000
[illegible] 35,000

[illegible] [illegible] [illegible] [illegible] [illegible] [illegible]
[illegible] ER [illegible] black [illegible] 90/30 37.8°, Pr 152, RR 60
BP 102/62 BW 103 liver 3 cm [illegible] 3
ff-fluid [illegible] liver 2 cm ↓

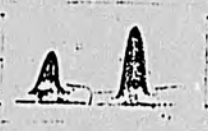
	HN.	Serum No.	Name	HI D1	D2	D3	D4	CHIK	JE	Age	Day	Grade	Hct	HBE	NCE	LYM	ATY	MON
26. เด็กชาย	1939626	RS 102 (Oct 4)	ด. [illegible]	1:2560	1:2560	1:2560	1:2560	1:20	1:640	9	5	II	45% OPD 40%	5100	45%	35%	17%	
		RS 103 (Oct 5)		1:2560	1:2560	1:1280	1:10240	1:20	1:640					5800	52%	29%	10%	
		RS 105 (Oct 6)		1:20480	1:2560	1:2560	1:2560	1:20	1:640					8200	45%	39%	1%	
OPD		RS 112 (Oct 14)		1:20480	1:2560	1:2560	1:2560	1:20	1:2560									
27. เด็กชาย	1816500	RS 109 (Oct 11)	ด. [illegible]	1:1280	1:640	1:640	1:640	1:20	1:640									
OPD		RS 115 (Oct 21)		1:20480	1:2560	1:2560	1:2560	1:20	1:640									
28. เด็กชาย	HCMH 108 1883909	RS 113 (Oct 23)	ด. [illegible]	1:2560	1:2560	1:10240	1:10240	1:20	1:2560	11	7	II	38% 44%	1400	26	71	18	
เด็กชาย	1935621	RS 112 (Oct 20)		1:1280	1:2560	1:10240	1:2560	1:20	[illegible]									
OPD	1935621	RS 118 (Oct 21)		1:10240	1:10240	1:10240	1:10240	1:20	1:10240									
29. เด็กชาย	1947343	RS 119 (Nov 1)	ด. [illegible]	1:1280	1:320	1:640	1:640	1:20	1:320	10	5	IV	45% 50% 48%	3400 4900	34 38	63 46	3 [illegible]	
"		120 (Nov 4)		1:2560	1:1280	1:2560	1:1280	1:20	1:160									
"		121 (Nov 5)		1:20480	1:2560	1:10240	1:2560	1:20	1:2560									
30.																		

137 [illegible] C3

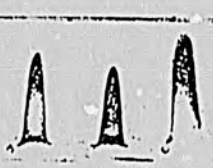
[illegible] (ชั่วโมง)
49,000
41,500
40,000



DHF @ impending shock อาการมาก รพ. มีเลือดออกที่ mucosa, เป็นที่ใช้ยาสูงกว่า ถึงวันที่ 5 เกิดที่ BP ต่ำ 90/50 ร่วมกับ Hct. เพิ่ม 40 → 44 (3) → 48% (4 วัน) platelet ต่ำ 333,000 → 109,000 → 39,000. ที่ตรง BP 90/70 ให้เริ่มให้ coffer ยาแก้ไข CIV. mission Hct 46% ภายหลังจากนี้ BP 100/40 – 90/50 ถึงที่ราคา BP ดีขึ้นเรื่อย Hct 48% ซึ่งเป็นปกติ.

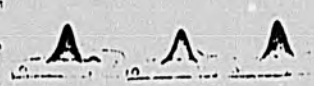


46,500



ผู้ป่วยเด็กชายอายุ 6 วัน (4 วันก่อนมาโรงพยาบาล) ไข้ สูงขึ้น อาเจียน afebrile. P 92/min BP 100/60 R 30/min liver -, spleen - ตรวจพบ Erythematous maculopapular rash + pitting edema ที่ BP แรกรับ เพิ่มขึ้น 150/50 / อุจจาระ ปกติ ยา

35,000
51,000



admit วันที่ 3 ของโรค ไข้ก่อนมา รพ. 4 วัน ซึมลง ไม่ทานอาหาร เอาเป็นปวดท้อง ตลอดเวลา admit ตอนเช้า ภาวะช็อค เข้า กล้ง ICU ด้วยอาการ BT 37.7° P 150/min RR 34/min BP 100/70 skin - no petechiae, Lives 2 cm ↓, spleen - No. ต่อมา Stage of shock BP 90/60 และตรวจพบเลือดออก

51 ปีวิ 1989 * ด. [illegible] (รพ. [illegible])
(จ. [illegible])

1:20 ; 1:40, 1:20, 1:20 < 1:20 , 1:10

	HN.	Serum no	Name	HI D1	D2	D3	D4	GMT	TE	Age	Day	grade	Ht	titer	[illegible]	[illegible]	[illegible]	[illegible]
(1) เด็กห	1852436	RS02 (Apr 4)	✓ด. [illegible]	1:1280	1:640	1:640	1:2560	1:20	1:5120 (3 เม.ย.)	6	8	IV	46%	5160	70	21		
	✓	RS03 (Apr 8)		1:5120	1:5120	1:1280	1:10240	1:20	>1:20480				40%	6,200	48	35		1
		RS012 (Apr 44)		1:10240	1:2560	1:2560	1:2560	1:60	1:640				33%	5,000	46	25		14
(2) เด็กห	1716456	RS05 (Apr 11)	✓ด. [illegible]	1:1280	1:1280	1:2560	1:5120	<1:20	1:640	7	5	FR	49%	8,600	56	21	15%	7
	✓	RS07 (Apr 18)		1:1280	1:2560	1:2560	1:5120	<1:20	1:1280			IV	43%	5600	54%	29%	ATZ 7%	10
		RS013 (Apr 44)		1:5120	1:5120	1:10240	1:5120	<1:20	1:10240				28%	8,100	30	65	7%	31
(3) เด็กห	1993173	RS10 (Apr 11)	✓ด. [illegible]	1:5120	1:10240	1:10240	1:5120	<1:20	1:2560	11	6	II	42%	8,400	21	61	17.3%	
		-11 (Apr 44)		1:2560	1:10240	1:1280	1:2560	<1:20	1:1280								ATZ 15%	
(4) เด็กห	1853394	RS 18 (Apr 28)	✓ด. [illegible]	1:2560	1:10240	1:1280	1:5120	<1:20	1:5120	5	5	III	45%	3,600	27%	50	10%	8
		19 (May 8)	DHF. C [illegible]	>1:20480	>1:20480	1:10240	1:10240	<1:20	1:10240									
(5) OPD	1725386	RS 21 (Jun 21)	✓ด. [illegible]	1:20	<1:20	1:40	<1:20	<1:20	<1:20	3½	6	DF		4,400	36%	51	ATZ 2	14
		44 (1. 22)	S-DHF	<1:20	<1:20	1:40	<1:20	<1:20	<1:20					4,900	35%	58		10
		28 (11. 26)		1:20	1:20	1:80	1:20	<1:20	<1:20									

7/a

145 [illegible] (Dead) 1:320 [illegible] 1:320 [illegible] 1:20 1:320
146. [illegible] <1:20 <1:20 1:320 <1:20 <1:20 1:20
96 [illegible] (Dead) 1:1280 1:640 [illegible] 1:5120 <1:20 1:320 52
47 1:1280 1:640 [illegible] 1:5120 <1:20 1:320

BT Platelets | [illegible]

118,000
49,000
63,000

2 [illegible] 5 [illegible] TT [illegible] no petechiae, mild [illegible] eye lid
[illegible] liver 2 cm. Hct. 57 → 53. [illegible] 100/60-70
Pulse 120-130/min BP [illegible] 90-70. [illegible] R ~30/min
abdomen [illegible] + [illegible] pleural effusion [illegible] shock stage
[illegible] 4 [illegible] BP [illegible] 100/80 [illegible] 17 [illegible]
5. [illegible] BP [illegible] 90/70

DHF c̄ shock
(+ septicemia, pleural effusion + tapping)

50,000
50,000
100,000

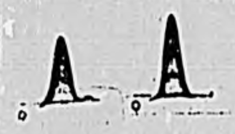
Dx. DHF c̄ shock

[illegible] 11-13 [illegible] 4 [illegible] Hct 44% BP 70/60 [illegible] PR 104 R 40/min wt 19 Kg TT+ [illegible] 1N 5% D/NSS [illegible] BP 90/60 P 104 Hct 40% [illegible] 12 [illegible] Conv.

52,000

[illegible] 16-18 [illegible] 11 [illegible] 3 [illegible] P [illegible]/min [illegible] BP 90/60 [illegible] weak, good conscious, [illegible] 1 cm [illegible] Petechiae [illegible]

14,000
47,000



4 [illegible] BP 80/20 P 120 [illegible] T. 38°C [illegible] 2 cm + [illegible] TT [illegible] Good Conscious, weak not pale no dyspnea [illegible] Petechiae [illegible] BP [illegible] 90/50 - 90/60 [illegible]

260,000
22,000

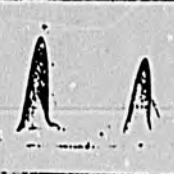
2 [illegible] 2532 [illegible] 4-5 [illegible] Alert, active [illegible] 2 cm [illegible] no petechiae
24 [illegible] BP 90/50

	HN	Serum No	Name	HI D1	D2	D3	D4	CHIK	JE	Mfg. Day	grade	Hct	WBC	PLT	Lym	ATY	Mono
6.	OPD	1871/431	RS 22 (Mon 41) ✓ ก. [illegible]	1:40	1:40	1:40	1:40	1:40	1:40 (1/2)	2	DF	37.7	10,510	48	48	69	
		22 (Jan 20)	P-DHF	1:640	1:1280	1:1280	1:640	1:40	1:640								
		Mos + MKa - dengue (3)															
7)	[illegible]	RS 41 (Jan 42)	ก. [illegible]	1:40	1:40	1:40	1:40	1:40	1:40	5	2Y	32%	8100	30	64	ATY 2	
		29 (n 29)	P-DHF	1:40	1:40	1:40	1:40	1:40	1:40			24-25%	11810	67	53	9	
		30 (1 30)		1:40	1:80	1:80	1:80	1:40	1:40			34%	6,010	37	8	ATY 1	
												25%	9700	52	46		
		Mos + MKa - dengue 2															
8)	[illegible]	RS 23 (Jul 8)	ก. [illegible]	1:1280	1:2560	1:1280	1:2560	1:40	1:1280	13	II						
		34 (Jul 9)	[illegible]	1:2560	1:5120	1:5120	1:10240	1:40	1:320								
	OPD	35 (11 10)		1:1280	1:640	1:640	1:10240	1:40	1:160								
9)	[illegible]	RS 34 (Jul 14)	ก. [illegible]	1:160	1:320	1:320	1:1280	1:40	1:320	5	III	40%	[illegible]	57	35	ATY [illegible]	
		39 (11 13)		1:640	1:1280	1:640	1:5120	1:40	1:640			45%	2440	23	52	[illegible]	
		41 (11 14)		1:320	1:1280	1:2560	1:1280	1:40	1:640			47%				ATY 161	
10)	[illegible]	RS 32 (Jul 10)	ก. [illegible]	1:5120	1:2560	1:5120	1:2560	1:40	1:1280	13	III	55%	[illegible]	57	59	[illegible]	Ahp 165
		38 (1 11)		1:2560	1:2560	1:5120	1:5120	1:40	1:640								
	OPD	50 (1 24)		1:10240	1:10240	1:5120	1:1280	1:40	1:2560								

72a

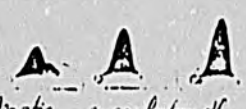
BT | platelets | C3.

211,000



21 [illegible] 2532 [illegible] [illegible] [illegible] [illegible]
26 [illegible] [illegible] [illegible] [illegible] (hemolytic anemia)

43000
48,000
40,000
58,000
54,000

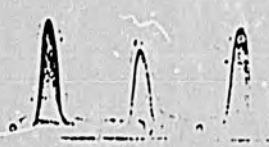


Hepatic encephalopathy
DHF, anemia

25 [illegible] 2532
4 [illegible] [illegible] [illegible] [illegible] [illegible]
T 37.4 BP 93/52 P 182 R 44 BP [illegible] 70-40/cc
PR 200/m [illegible] [illegible] [illegible] [illegible] [illegible]
effect. [illegible] 25 [illegible] - 4 [illegible].

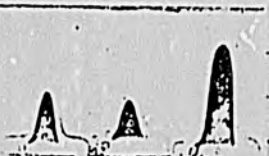
3 [illegible] 2532 - 6 [illegible] 2532
3 [illegible] 2532 [illegible] [illegible] [illegible] BP [illegible]
Hct 49% → [illegible]% BT 38°C P 120 fall R 24 BP [illegible]
[illegible] 1 cm b RCM mild [illegible] Hct [illegible]% WB [illegible] N 57
L 28 [illegible] Hb 17% Plt [illegible] (Dengue shock syndrome)
[illegible] Petechiae [illegible] [illegible] [illegible] [illegible] [illegible]
BP [illegible] 90/60 - 100/70 10 [illegible] [illegible] (Rale) [illegible]
bronchitis.

[illegible]
36000.
[illegible]



4-15 [illegible] 2532, [illegible] 3 [illegible] P 120 BP 110/70 Petechiae
hemorrhage, [illegible] [illegible]
12 [illegible] BP 100/60 [illegible] Hct [illegible] Hct [illegible]
13 [illegible]. [illegible] [illegible] BP 100/60 - 110/70 Pulse 100 [illegible]
Hemoconcentration. [illegible] [illegible] [illegible]
14 [illegible] BP [illegible] 120/70 Hct 44% [illegible] [illegible]
Lung [illegible], [illegible] [illegible] [illegible] [illegible] [illegible]
15 [illegible]. BP OK [illegible] 14 [illegible].

[illegible]
27,000



(9-11 [illegible])
1 [illegible] BP 90/60 [illegible] 6 [illegible] [illegible] [illegible]
[illegible] [illegible] [illegible] P 100/m. RR 18/min [illegible]
anemia/DHF © Shock grade III Hct 58% [illegible] anemia. 10 [illegible] BR [illegible] Plt 45,000
WBC [illegible] L 47 [illegible], [illegible] BP [illegible] [illegible] 9. [illegible] 100
[illegible].

(11) [illegible] [illegible] [illegible] [illegible]

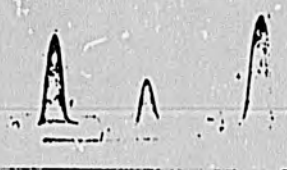
N55

HN.	Serum No	Name	HI D1	D2	D3	D4	CH	JE	Age	Day	Grad	Hct	Hbc	Neu	Lym	Aty	Mono
11)	[illegible]	[illegible]	1:20	<1:20	<1:20	<1:20	<1:20	<1:20 (Clone)	12	4	I-III	46%	9600	58%	43%		2%
	45 (1:20)	(ชุดทดสอบ)	1:20	<1:20	<1:20	<1:20	<1:20	<1:20				41%	8000				
OPD	54 (1:24)		1:320	1:80	1:80	1:160	<1:20	1:40									
12)	[illegible]	[illegible]	1:640	1:640	1:640	1:1280	1:80	1:320	11	5	III	44%46%	3600	53	47	Atk 5%	
	44 (1:20)	[illegible]	1:1280	1:5120	1:10240	1:2560	1:640	1:1280									
OPD	53 (1:24)		71:20480	1:5120	1:1280	1:2560	1:40	1:1280									
13)	[illegible]	[illegible]	1:1280	1:320	1:640	1:640	<1:20	1:160	12	6	III-IV	50%	2730	70	30		
	42 (1:24)		1:2560	1:640	1:1280	1:1280	<1:20	1:320				44%	4400	50	46		3
	48 (1:23)		1:1280	1:640	1:2560	1:1280	<1:20	1:640				44%	6300	55	38	Bo 1%	3
	50 (1:24)		1:5120	1:2560	1:5120	1:1280	<1:20	1:1280				38.5%	8400	55	42	Bo 1%	
14)	[illegible]	[illegible]	1:640	1:640	1:1280	1:640	<1:20	1:320	9	6	IV	55% (เดิม) 46.5 (ใหม่)	1200	56	43		1
	51 (1:48)		1:10240	1:10240	1:5120	1:1280	<1:20	1:2560				(ตาม ค่า interpretation เดิม)					
OPD	61 (1:51)		1:1280	1:2560	1:10240	1:640	<1:20	1:2560									
15)	[illegible]	[illegible]	1:10240	1:10240	1:10240	1:5120	<1:20	1:2560	14	5	II	54 / 47%	5300 / 5300	18 / 18	82 / 82		
OPD	64 (1:97)		1:640	1:1280	1:2560	1:10240	<1:20	1:640									

PT | P1 | C3

2

74,000

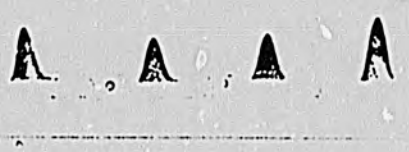


16 กค 2532 มีไข้ 5 วัน ไข้ลดเมื่อ ... ให้ยาลดไข้ ... BP 120/60 ... ER ... 37% ... 120/100 ... 1000 ml drip ... Alum milk ... 20 Hct 40%. BP 120/70 BP ... EV ... ตาขวาตอนนี้รู้สึกเหนื่อย ... มี moving ... 4 ... 7 ... BP ...

45,000

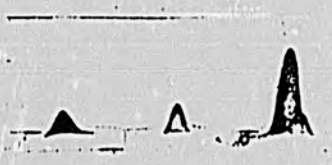
วันวันที่ 14 กค ... มีไข้สูงมาก 4 วัน ... มีไข้ ... Rhchiae ... 48 ชั่วโมง CBB ... 49 ... BP ... 20-21 ... 90 ... 21 ... PR 96 ... BP 100/60 ... DHF @ impending shock. BP ... 100-110/70 mmHg Pulse 90-110 ... 20 BP ... 90/60 - 100/70

45,000
30,000
15,000



21 กค 2532 ... 5 ... BP 100/55 P 120 mmHg Liver ... Hb 15-4 ... EV fluid ... 10 → 20 cc/kg/hr ... BP ... 22 กค 2532 ... BP 100/70 23 กค 2532 ... BP 110/70 ... Size ... pulmonary ...

35,000



เช้าวันที่ 22 กค 32 ... 5 ... PR 110/min BP 16/min ... @ shock ... Hct ...

42,000

24-25 กค 2532 BP 100/70 P 120/min RR 30 Rhchiae ... มีไข้สูงมา 4 วัน ... ตั้งแต่ 24, 25 ... 100/60 ... Rhchiae ... RR 20-24 BP 70-100/60 ...

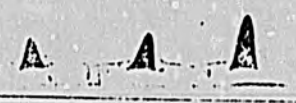
	HN	Serum no	Name	P1	P2	D3	D4	CH	JE	age	day	grade	Net	WBC	N/E	Lym	ATY	Mono
16)	[illegible]	18 [illegible]	NS 52 (31/24)	1:2560	1:640	1:1280	1:640	<1:20	1:320	9	6	III/IV	31% 42%	2700	62	32	12.3	3
			56 (1/22)	1:2560	1:2560	1:2560	1:2560	<1:20	1:640				N:3 1/45%	3100	78	20	ATY (16%)	2%
OPD			62 (Aug 7)	1:2560	1:2560	1:10240	1:20480	<1:20	1:2560									
17)	[illegible]	20 [illegible]	NS 65 (Aug 14)	1:320	1:640	1:1280	1:2560	<1:20	1:640	8	6	III-IV	44.00 45%	5330	55	44		
ICU			66 (Aug 15)	1:320	1:1280	1:1280	1:5120	<1:20	1:640				40-17 / 60:70 34%					2%
"			68 (Aug 18)	1:1280	1:1280	1:5120	1:10240	<1:20	1:2560					2,200	16	77	3%	3%
เด็ก 2			79 (Aug 33)	1:1280	1:2560	1:250	1:20480	<1:20	1:5120				20 lymphocytes neg.	6,350	42	47	[illegible]	3
			MK2 + C6	dengue-2														
18)	[illegible]	[illegible]	NS 69 (31/29)	1:2560	1:640	1:1280	1:2560	1:20	1:1280	12	6	III-IV	46.5%	5100	64	20	ATY 12%	3%
			70 (31/31)	1:10240	1:2560	[illegible]	1:5120	1:80	1:5120									
19)	[illegible]	[illegible]	NS 24 (Aug 21)	1:640	1:640	1:1280	1:1280	<1:20	1:640									
			33 (1/23)	1:10240	1:10240	1:5120	1:20480	<1:20	1:2560									
			34 (1/23)	1:10240	1:10240	1:5120	1:20480	<1:20	1:2560									
20)	[illegible]	[illegible]	NS 72 (Aug 23)	1:640	1:640	1:1280	1:1280	<1:20	1:640	11	6	III	26 41% 45%	11100	75	11	B 8	
			31 (1/28)	1:5120	1:1280	1:2560	1:2560	<1:20	1:1280				11 (Com) 47%					
			32 (1/29)	1:5120	1:5120	1:2560	1:5120	<1:20	1:2560				43				หลังวันนี้	
OPD			90 (Sep 11)	1:5120	1:5120	1:2560	1:5120	<1:20	1:2560				38%					

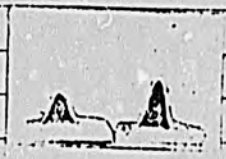
AT	BT	P3.
42,000		วันที่ 26 ก.ค. 2532. มีไข้มา 5 วัน ไข้ลงวันนี้ อาเจียน BP 90/60 P 100/m Hct 3 FB + RCM, no petechiae ตับ ม้าม ปกติ. BP ต่ำมากขณะ 75/75 Hct 44% (14 ชม.) Shock เสียชีวิต Chem. 27 ก.ค. มี marked (macular) เนื้อตาย (ผลตรวจ)
40,000 60,000 70,000 80,000 grade III-IV shock (AT) 160,000		B วัน 2532 เวลา 14.30 น. Pulse 92, BT 37.5 BP 110/60 Resp 24. เป็นไข้มา 4 วันก่อนมา ตรวจ Hepatitis ไข้ไม่ลดไม่ลง อาเจียน ถ่ายเหลว ถ่ายไม่ได้ 12 ชม. ก่อนมา CBC WBC 10,200 N 91 LE B1 Bst OK Hct 31.5% ปรับสารน้ำเส้นเล็กก่อน ถ่ายปัสสาวะได้ 40 cm Hct 42% S/B/T) 1500 SGPT > 1090 BS 87.7% เริ่ม add ที่ Hct ไว้ 45% WBC 4600 N 56 L 12 M 3 plt 62 cm B-4% Hct เพิ่มขึ้น 47% ไม่มี jaundice, ไม่มี petechiae 14 cm Hct 47.5 PR 100/min ตรวจพบ ascite + ve ไม่มี fluid wave BP ตกลง 90/70 PR 100/min เป็น a mild puffy eyelid - Hepatic encephalopathy.
		วันที่ 26 เริ่มมาด้วย increasing shock เริ่มต้นด้วย DHF ด้วย increasing shock Hct 46% plt 60,000 - BP 50/60 วันที่ 29-31 มี BP แกว่งตลอดวัน รับเข้า ER 10 ชม. 12 น. 4 ชั่วโมงแรกได้ไป ให้ไป 500 ml ต่อชั่วโมง 1000 ml ต่อวัน ตกเย็น ให้ไปแล้ว มีปัสสาวะน้อย ต่อมาตกลงที่ ER BP 70/40 P 95/min รู้สึกตัวดี ไม่ซึม ปัสสาวะออกมาก ไม่มี petechiae หรือ bleeding วันที่ 28 20.30 น. BP 80/60 Hct ตกลงมาเหลือ 42%. ให้ไปแล้ว 90-40/40 mm Hg P 95-102/min RR 22 วันที่
60,000		26 ก.ค. 2532. มีไข้มา 4 วัน ไม่ไอ ไม่มีมูก ไม่อาเจียน ถ่ายเหลว แน่นท้องมาก ปัสสาวะ ปกติ เหงือกไม่ออก เลือดไม่ออก BT 37° BP 94/50 P 88 R 20 ที่ ER 90/70 P 112 Hct ขึ้นเป็น 45% มี petechiae ตามตัว TT+

	HN	วันเดือนปี	Name	HI D1	D2	D3	D4	CT	JT	Age	day	grade	Hct	WBC	NUC	Lym	Aty	Mono
21) เด็ก	2039264	65 39 (Aug 21)	ด. กัญญาณ วลีรักษ์	1:320	1:320	1:160	1:160	<1:20	1:640									
	✓	39 (Sep 1)		1:2560	1:2560	1:1280	1:1280	<1:20	1:2560									
OPD		87 (11 6)		1:10240	1:2560	1:5120	1:1280	<1:20	1:10240									
22) เด็ก	2039247	65 40 (Sep 1)	ด. นัทธร หาดเจริญ	1:1280	1:2560	1:360	1:640	<1:20	1:2560									
	✓	91 (Sep 4)		1:10240	1:10240	1:2560	1:5120	<1:20	1:10240									
23) เด็ก	2041149	65 84 (Sep 6)	ด. ภูวรรณ เกิดเกตุ	1:640	1:1280	1:2560	1:2560	<1:20	1:320	$13\frac{3}{12}$	5	IV	56%	9912	35	55	Mono7 Eo2	
		85 (1 7)		1:20480	1:640	1:10240	1:1280	<1:20	1:320									
OPD		91 (1 11)		1:20480	1:640	1:20480	1:1280	<1:20	1:320									
24) เด็ก	6009703	65 95 (Sep 10)	ด. วรรีนัย จันทร์พรกิ	1:20	1:20	1:20	1:20	<1:20	<1:20									
เด็ก3		95 (Sep 13)		1:80	1:40	1:40	1:40	<1:20	<1:20									
OPD		110 (Oct 2)		1:160	1:40	1:40	1:40	<1:20	1:40									
25) เด็ก	2045339	65 97 (Sep 17)	ด. พรพรรณ โชคอนุกูล	1:1280	1:320	1:1280	1:640	<1:20	1:40	7	6	IV	47%	8300	17	68	14	Mono 8
		98 (1 18)	DHF ไข้ เลือดออก shock	1:2560	1:640	1:1280	1:1280	<1:20	1:320			III	41 40 36%	8000	65	30		
	✓	98 (1 19)	Septicemia	1:2560	1:640	1:2560	1:2560	<1:20	1:640					8170	57	37		5
		102 (1 23)	Hospital acquired infection.	1:2560	1:2560	1:2560	1:2560	<1:20	1:640									
		104 (1 24)		1:10240	1:5120	1:20480	1:2560	<1:20	1:640									

754

Bt | Pt | Cs



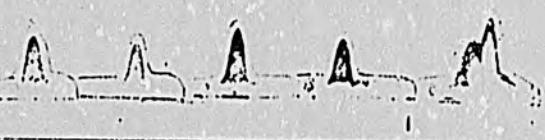


22,000

5-7 พ.ย. 2532
5 พ.ย. 2532 / [illegible]
weak BP 40/50 [illegible] hypotension PR 112-
V พ.ย. 2532 [illegible] PR 72-84
DHF C° shock RR 20 BP 100/60 [illegible] petechiae

50,000

34,000



เดือนที่ 5 อายุครรภ์ [illegible] 17 พ.ย. [illegible] 10 น.
[illegible] ไข้ต่ำ [illegible] P96 R28
BP 68/0 pulse [illegible] ไม่ [illegible] BP
[illegible] 170

03 61

	HN.	Serum NO	Name	HI D1	D2	D3	D4	CH	JE	Age	day	grade	Hct	WBC	PMN	LYM	ATY	Mo
26 [illegible] 3	112033	RS-100 (Sep 19)	[illegible]	1:1720	1:2560	1:1280	1:1280	L1:20	1:640									
OPD		RS-112 (Oct 9)		1:1720	1:2560	1:1640	1:1280	L1:20	1:1280									
27 [illegible]	2049147	FAH RP RS-106 (Sep 26)	[illegible]	1:1280	1:640	1:1280	1:320	L1:20	1:50	4	5	III	54% ↓ 36%	8,280	43	51	1/73	
"		RS-109 (Sep 29)	[illegible] DHF gr III	1:2560	1:1280	1:10240	1:640	L1:20	1:160									
OPD		RS-113 (Oct 9)		1:10240	1:2560	1:20480	1:640	L1:20	1:640									
28 [illegible]	1124636	RS-129 (Oct 16)	[illegible]	1:160	1:640	1:320	1:160	L1:20	1:320	8	5	IV	17 (39% / 18 → 42%	600 / 2,960	74 / 54	26 / 43	NTR	M1.
[illegible] ✓		RS-133 (Oct 18)		1:320	1:320	1:640	1:640	L1:20	1:640									
		RS-134 (Oct 19)		1:320	1:320	1:640	1:640	L1:20	1:640									
		RS-135 (Oct 20)		1:1280	1:2560	1:1280	1:1280	L1:20	1:1280									
				Shock [illegible] 18														
29 [illegible] ✓	1945159	RS-136 (Oct 22)	K. [illegible]	1:640	1:320	1:1280	1:640	L1:20	1:320	5	4	I 22	29 {35% / 31.5% / 24 23% / 25 22%	11,400 / 9,100 / 12800 / 12850	58 / 37 / 19	6 / 55 / 71	28 / 9 / 2	M4 E1 / 2
		RS-141 (Oct 24)	know HbH disease	1:5120	1:1280	1:1280	1:1280	L1:20	1:640									
		RS-143 (Oct 27)		1:5120	1:1280	1:10240	1:1280	L1:20	1:640									
		RS-150 (Nov 6)		1:5120	1:1280	1:10240	1:1280	L1:20	1:640									
30 [illegible]	2054003	RS-146 (Oct 30)	[illegible]	1:320	1:1280	1:1280	1:1280	L1:20	1:320	10	4-5	II / III ?	43% / 39%	2460 / 2010	48 / 12	38 / 85	NTR 20%	
✓		RS-147 (Oct 31)		1:1280	1:1280	1:2560	1:5120	L1:20	1:1280									
		RS-148 (Nov 1)		1:2560	1:1280	1:5120	1:10240	L1:20	1:1280									

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BT	PT	C_3

72,000		admit วันที่ 26.30 เช้า ด้วย ก่อนมา มาด้วย shock BP ไม่วัด N80 load 5% D/N55 ... ml/kg/1 ชม. ได้รับ ถึง ward ไม่มี bleeding ถ้าวัดได้ BP ที่ward 120/60 PR 120 TT+, liver 2cm ↓ ไม่มี ascit

38.8	95,000 53,000	อยู่รพ 17-21 ตค 2532 เด็กเพลงอายุ 8 ปี ป่วย 4 วัน ไข้ตลอดเวลา ซึมเป็นไข้เลือดออก BT 38.8, R 24/m, BP 90/64, petechiae แขนขาบน Liver 3cm 4 วันก่อนเข้าโรงพ. หิวมากขึ้น มีปัสสาวะ 1 ครั้ง ถ่ายเหลว มีไข้สูง TT+ Hct 50% WBC 65 PMN 51% B 5% L 26 M 11% , B 2% plt 216,000 ตอนรพ 1 ปี Hepatomegaly

BP ตั้งแต่ 18 80/60 → 94/90 ย้ายขึ้นบน ward load IV 2 ชั่วโมง BP ตก 90/70
19 94/70 R 80/m → 19 (2.2 AM วันที่ 27)

ไม่มีไข้ 208,000 215,000 208,000	TT(+)	อยู่รพ 22.30 ชั่วโมง เป็นไข้ 3 วัน 10 วันก่อนมาไข้ต่ำ แพทย์ให้ยาลดไข้ 3 วัน หยุดไข้ 3 วัน หลังไข้ลงมาอาหารดี แพทย์ WBC 5 ตัว PMN 1, MONO 4 ตอนรับ Hct 34% Anise pothio ... ไข้ thalassemia trait. RR 32/min BP 100/60 liver 2cm ตับโต 4 cm (Hct ก่อนรับ 31.5) 34 ชั่วโมง มีไข้ต่ำๆ ไม่มี fluid ออก ไม่มี sign of shock ไม่เหนื่อย ไม่ง่วง ... platelet ไม่ต่ำ

84,000 48,000		ไข้สูงมา 4 วัน ก่อน 30 ชั่วโมงก่อนมาพบ หมดสติ อาเจียน เลือดออกทาง ปากก้อนไม่ระบุชัดเจน ถูกตามตัวไม่ทราบ เลือดสีแดงเข้ม มีเลือดน้ำ เล็ก สีแดง no petechiae no active bleeding ตามตัว ตอน admit Hct ต่ำ 33% BP 100/70 วันที่ 31 Glucose VS stable BP 100/70 Hct 39 (17%) แพทย์ให้ shock ให้ IV

ตลอดเวลา ... ไม่มี petechiae

HN	Serum no	Name	HI Di	Da	D3	D4	CH	JE	age	day	grade	Hct	WBC	NUT	LYM	ATY	MON
1622483	RS-101 (Sept 20)	[illegible]	1:320	1:1280	1:80	1:80	<1:20	1:1280	10 ปี	5	IV	56%	5100	40	50	4	3
	RS-103 (Sept 21)		1:2560	1:10240	1:1280	1:640	<1:20	1:5120				52% 21.9%					50 1
		DHF + ALL C shock															
[illegible]	RS-108 (Sept 22)	[illegible]	>1:20480	1:5120	1:2560	1:5120	<1:20	>1:20480	9 ปี 12	5	I	stone 42.6	1900	26	64	MO 2	
"	RS-109 (Sept 23)	[illegible] DHF Gr I	>1:20480	1:10240	1:5120	>1:20480	<1:20	>1:20480									
1314023	RS-109 (Oct 10)	[illegible]	>1:20480	1:10240	1:1280	1:2560	<1:20	1:5120	10	4	III	38%	36	32	E 3	9	
"	RS-110 (Oct 11)	DHF with shock	>1:20480	1:2560	1:1280	1:5120	<1:20	1:5120									
1416836	RS-110 (Oct 11)	[illegible]	1:2560	>1:20480	1:640	1:2560	<1:20	1:5120	7 ปี 10 เดือน	6	I	1992: 40% 1993: 45%	4370	29	37	17	12 Bands
"	RS-114 (Oct 12)	DHF (Mild)	>1:20480	>1:20480	1:2560	1:5120	<1:20	1:10240									
1644349	RS-122 (Oct 18)	[illegible]	1:20	<1:20	1:80	1:20	1:160	1:160	10		IV	(Ca* 15) CR 47%	Hct 47% → 43% → 37%				
	RS-124 (Oct 19)	DHF & shock	1:640	1:40	1:20	1:40	1:160	1:320				47%	5900	52	46	[illegible]	[illegible]

BT	PT	C_3
		2 pl — เด็กชายเป็นไข้มา 5 วัน ไข้สูง ไม่ค่อยกินอาหาร ไม่มีซีด ตาเหลืองเล็กน้อย ตับโต ไม่มาก ม้าม Day 3 มีเส้นเลือดแดงฝอยขึ้นที่ผิวหนัง เริ่มก่อนมีไข้ ตรวจเลือด พบ Known case ALL ตรวจ LBS / → ยกเว้น Petechiae ? ตรวจพบ lymphadenopathy ตับโต liver 2 cm ↓ ตรวจพบมีภาวะ bleeding BP 100/0 Pulse 130/min Platelet ↓ DHF (ไม่ impending shock) ให้ IV fluid 15 cc/kg/hr x 24 hr ปัสสาวะ อันที่ 14 (18 ชม.) เริ่มเจาะ 20 แต่ BP กลับเป็น 100/60 ในวันที่ 20 Hct ตกลง = 42% → 37% อันที่ 21 ให้กลับ เด็กไม่ค่อยดี แต่ BP 100-110/70 มี Petechiae ทั่วไป ตามแขนขา เด็กเสียชีวิตวันที่ 26 เม.ย. 35
378 c	126,000	2 pl — มีไข้มา 5 วัน ไข้สูง ไม่ค่อยกินอาหาร ซึมลงเรื่อยๆ ตรวจร่างกายพบ ตาเหลืองเล็กน้อย ตับโต 1 ซม. ม้าม ไม่โต ไม่มีซีด ไม่มีเส้นเลือดฝอยขึ้นที่ผิวหนัง ตรวจเลือด พบ Hct 41 % Hg ต่ำ ตรวจ ESR ตกลง อาการดีขึ้น 35 วัน มีไข้ขึ้นอีกครั้ง และเสียชีวิตวันที่
90,000-(900)		2 pl — ก่อนมาที่ TT ปีที่ 2 เคยมีเลือดออกจากหลอดอาหารอักเสบ ไม่ได้รับการรักษา มา 3 วัน เริ่มมาเวลา 6.00 วันที่ 9 ตุลาคม ผู้ป่วยซึมลง ตาเหลืองเล็กน้อย ไม่มีเลือดออก TT + good consciousness อันที่ 9 Hct 42 % BP 110/70 ตรวจพบ DHF grade I WBC 6300 N 65 L 31 plt 140 x 10³ เวลา 16.00 น. มี impending shock ปัสสาวะ ได้ 36 ml. BP 100/70 : ตรวจพบ Hct 41% liver ไม่เปลี่ยน DHF 16.00 น. BP 80/60 pulse 108 ไม่มีไข้ BP ↓ ต่อมา ตกลงเป็น PR 100/m BP 90/70 pulse 120 ซึม 23.00 น. เริ่มมีอาเจียน pulse 100, resp 30/min 4.00 แพทย์บอกไว้ ให้ Convalescent ward 10.00 BP 90/60 pulse 100-110/m. BT 37°C ตัวเย็น เด็ก 2
39,000		2 pl — เด็กชายอายุ 10-13 ตุลาคม มีไข้มา 5 วัน BP 90/60 pulse 80/min มี mild dehydration อายุ 23 น. ตุลาคม ไข้ลง 37°C อันที่ 11 ไข้ลงไป P = 100/min R 20/min BP 110/70 ไม่มีอาการ ไม่มี active bleeding แต่ตรวจพบ BP ต่ำ pulse เกือบ 88-116 RR 24-28 เด็กเสียชีวิตวันที่ 14 ไม่ค่อยดี แพทย์ least ไว้ 3 เดือนก่อนเสียชีวิต
68,000		2 pl — ตุลาคม 15-17 ตุลาคม มีไข้ (ระยะวิกฤต) มีไข้ 6 วัน BP 80/60 (BP ต่ำ ตั้งแต่ ESR) P 100/min R 22/min liver 1 cm no edema วันที่ 16 เข้า Convalescent stage ให้ fluid ไม่ได้ BP ต่ำ 80/60 ช่วง ค่ำ 16 BP 80/60 ตายตั้งแต่ตกค่ำ ยังมีชีพ

HN.	SERUM NO	NAME	HI D-1	D-2	D-3	D-4	CHK	JE	AGE	DAY	GRADE	Hct	WBC	Neu	Lym	Aty	HM
2556(ด)	2074018	RS-123 (Oct 16) ด.ด.ชิตา โกสลพิพัฒ	1:2560	1:10240	1:10240	1:2560	<1:20	1:2560	6	5	15 วัน III	52%	10,800	37	63	7	
		RS-124 (Oct 17)	>1:20480	>1:20480	1:320	>1:20480	<1:20	1:10240				41%					
2554(ด)	2004102	RS-128 (Oct 17) ด.ด.เด็กชาย ศักดิ์ศรี	1:1280	>1:20480	>1:20480	1:640	<1:20	1:10240	4½ ปี	6	III	41%	2740	52	48	AT 102	
		RS-132 (Oct 18)	>1:20480	>1:20480	1:10240	1:10240	<1:20	1:10240									
2560(ด)	1246636	RS-129 (Oct 18) ด.ด.กฤษฎา ประดิษฐอาจินตนา	1:160	1:640	1:320	1:160	<1:20	1:320	8	5	III	42%	7,900	51	43	2	
(ด)		RS-133 (Oct 19)	1:320	1:320	1:640	1:640	<1:20	1:640				39%	7,900	54	43	3	
		RS-134 (Oct 19)	1:320	1:320	1:640	1:640	<1:20	1:640				49%					
		RS-135 (Oct 20)										43%					
2560(ด)	1496369	RS-142 (Oct 21) ด.ด.นนท์ ศิริเจือ	1:640	>1:20480	1:640	1:10240	<1:20	1:10240	5	6	III-IV	55%	11,800	44	37	M 3	
		RS-144 (Oct 22) DHF C shock	1:10240	>1:20480	1:5120	>1:20480	1:20	>1:20480				35%	7,54	24	69	ATL 162 M 2 ATP 18	
2561(ด)	2060552	RS-149 (Nov 6) ด.ด.นพ.ศักดิ์ ศิริอัมพร							4ปี 7วัน	5	III-IV	44%	5200	30	63	AL 27	
		RS-151 (Nov 7) DHF C hemoconc C shock	1:640	1:640	1:10240			1:320									
		GI bleeding (ดำ) Grade IV -															

BT pt C_3

36,000

2 μl

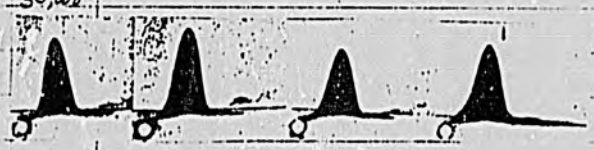
แข็งตัวดี ตรวจ 4 ครั้งแล้ว เช้าวัน 15 ธ.ค. เวลา 15.51
เลือดไหลดี ตัวเย็น อาเจียนเล็กน้อย ไม่ clinic ทางเพศ เคยเป็นที่
เรื่องตรวจ ดีไม่ได้ BP เดิมไว้ 80. P. ตก look sick.
Petechial hemorrhage. Liver 3 cm ↓ RCM Hct 52%
ตั้งแต่ 16. ปวดท้องไม่มีไข้ เดินไม่ได้ ตับโต 2 cm ↓ RCM ไม่มี active
bleeding Hct. 4 → 37 - 38 - 37.5 ก่อนให้ BP ดีขึ้น
90/60 - 100/70 pulse 100 - 100 / นาที RR เร็ว 26-36 ซี่ rash

36.6 5,000

2 μl

เช้า 15-19 ตุลาคม. มีไข้ 5 วัน ไม่ได้ไม่มีปัญหา P 100/— RR 20/—
BP 80/60 ตอนเช้ามี impending shock เช้า Hct เช้า Hct
ขึ้นตลอด 44 → 43.5 ตอน 21.00 น. ปวดท้องบริเวณสีข้างขวา TT+
มีปัสสาวะเป็น Oliguria

53,000



2 μl

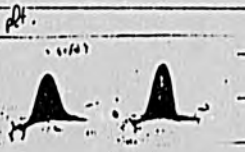
มีไข้ตั้งแต่ 4 เช้า ไม่ได้ ไม่มีปัญหา ดีขึ้นอาการเจ็บ อาเจียนมากขึ้น ไม่มีท้องเสีย
ไม่มีก้อนเลือด ไม่ถ่ายดำ ไม่มีอาเจียน BP 115/60 P132 เช้า
RR 84 liver 2 FB ↓ RCM ท้องไม่ โตเล็ก TT−
เช้า รพ 17 ตุลาคม 18 ธ.ค. BP 120/70 pulse 100/นาที
liver congestion. liver 2 FB ↓ RCM C ปัสสาวะ
ใน รพ 17 - 21 ตค ผลเลือด วันที่ (18) มี Hepatomegaly
เช้า BP 80/60 → 90/70 ตายวันที่ 19.ค. ตั้งแต่อยู่ที่ (19)
อยู่ รพ ที่ เวลา 20

36.6 135,000
47,000

2 μl

x ปัสสาวะ 6 วัน ไม่ได้ ไม่มีปัญหา R 32/ BP 110/80 P 110/m.
Liver 1 cm. CBL Hct 55% BP ตกลงมา BP ตกไม่ได้ RR 90/60 m Hg ในวันที่
26. BP แตกอยู่ในเกณฑ์ 90/70 ตอนเช้า รพ ดีขึ้น ไม่มี

ย้ายเข้า Pt.
66,000
42,000



2 μl

อยู่วัน 6 พ.ย.-เดือน เวลา 1:36 เลือดก้าวเดินตลอด 1 วัน
มา admit มี hepatomegaly แล้ว epistaxis ก่อนตลอดเวลา
แล้ว Shock ไป เดาเอา เช่น ที่ Pt conc, RBC, plasma ดีขึ้น อยู่
ใน ICU 2 วัน Hct 44 % ดีขึ้นไม่ปวดท้อง อาเจียนเป็น
เรื่องมาก ตอนกลับบ้าน Hct 22 %

	HN.	SERUM NO	NAME	HI D-1	D-2	D-3	D-4	OHIK	JE	AGE	DAY	GRADE	Hct	WBC	NUT	LYM	ATY	MONO
141(OPD)	2060849	RS-152(Nov4)	ช ชื่อ [illegible]	1:640	1:320	1:1280			1:320	6	4	III	6 mo 42%	5,600		Atm 7 Am	23%	
"		RS-154(Nov5)	Aedes tonsilis R/O DHF	1:1280	1:640	1:1280			1:640				38% 46%					
										Lab Rama			Hct 45%	WBC 4233	Na 39	Lym 67%		
										Aty 23%			Atym 1+	Mico 1+	84%			
142(OPD)	1569208	RS-153(Nov4)	ช ชื่อ [illegible]	1:640	1:5120	1:2560			1:1280	8½ 10		III	35% 46%	4120	27			
		RS-159(Nov8)	DHF + shock [illegible]	1:1280	1:10240	1:2560			1:640									
143(OPD)	2062723	RS-159(Nov12)	ช. [illegible]	1:2560	1:2560	1:10240			1:1280	5	5 6	III	42% 35%	8400 5600	78 62	23 35	1 81	2 3
		RS-160(Nov13)		1:2560	1:5120	1:1280			1:2560				39%	8320	52	88	4 7	4
		RS-162(Nov14)		1:5120	1:5120	1:10240			1:2560									
144(OPD)	2074133	RS-174(Nov28)	ช อยู่ [illegible]							Hct 26.	4 6	III	45.4	35.4 2330	75	14	2	
"		Nov 29-Dec 3 RS-179(Jan4)																

DT	PT	C3.
56,000	2 μC	เดินได้ 3 วัน เจ็บรน ปวด 253 เวร 1:04 ไข้ต่ำ มีปวดท้อง ไม่ถ่ายอุจจาระ ท้องแน่นท้อง รับประทานได้ เป็นไข้เรื้อรัง ตรวจร่างกายเจ็บ liver 3 cm. TT + 8 70. ไม่มี edema ตั้งแต่วันที่ 7 ก.พ. 7 ก.พ. ซีดมาก แผนการรักษา ให้อะไรไม่ได้ ไม่ถ่าย 4 วัน Hct 46 เวร 2:04 BP ตกลง 80 R 140 เหนื่อย ใจสั่น และ dry lip tachycardic liver 3 cm. fluid ตั้งแต่ 9 ตอนบ่าย รพ.
310,000 (3 พ.ย.) 50,000	2 μC	อุ่นรพ. 3-9 พ.ย. เริ่มที่ 3 มีไข้ 1 วัน ปวดท้อง ตอนเช้า ซึมก่อน ส่งรพ. ความดันต่ำ BP ↓ ในวันที่ 5 BP ตกลง 110/70 pulse 120/นาที แทงเส้นใหญ่ I. DHF Day 5 ไข้ลง 39°C BP 00 ↓ Hct 48 plt 45000 PMN 38 L 61 M 1 liver ราบ spleen negative 6 พ.ย. ตอนเช้า 39°C Control เรื่องอาหาร 7 ก.พ. ให้ nute out DHF ไข้ 37.2 TT + วันที่ 8 สังเกต BP ~110/70 ไม่มี IV ตอนเช้า
36.1 70,000 53,000 4000	2 μC	อุ่นรพ 11-14 ก.พ. มีไข้ 4 วัน เลือดกำเดาไหล เริ่มก่อนมา ตอนเช้า เดินช่องท้องตึง Pulse ช้า 140 ต่อนาที RR 30 BP 90/60 mild dehydration TT + วันที่ 12 BC แดง 00/00 P108 ตั้งให้ fluid มีจ้ำ Petechiae ขึ้นเยอะ แกล้ง ไม่มี bleeding ที่อื่น วันที่ 12 ปัสสาวะ ออก Control fluid ทั้งวัน
54,000	2 μC	อุ่นรพ. 26 ต.ค. เวร 8:33 มีไข้สูง มา 3 วัน กระสับกระส่าย ห้ามอยู่กับที่ ใช้สูงถึงมากเกิน. ตรวจร่างกายเจ็บ. ไม่มีเลือดออก ท้องแน่น Pulse 120/นาที BP 90/60. look ให้ fluid admit ward ใช้ตอน 36.8 กระสับกระส่าย เช็ด BP ได้ 90/70 fluid ต่อ ~~วันที่ 18~~

142 B L SP. → MM.
25
21
13. B L SP
16

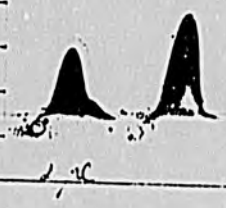
69

ปีเก่า

HN.	SERUM NO	NAME	HI D-1	D-2	D-3	DH	CHIK	JE	Age	day	grade	Hct	WBC	NUT	LYMP.	ALY	M
1(ก)	2026072	RS(ครั้งแรก) ก.พ.ปีนี้ เดือนละ 2 ครั้ง	<20	20	<20	<20		<20	5เดือน	6	II-III	44 33%	4000 7600	25 6	70 82	4% 8%	1 4 8
CPP		RS-005(Jan 15)	<20	20	20	20		<20				27%					
		dengue type 2															
1(ก)	2017748	+RS-008 (Feb 14) → L/H 182 ก.พ.ปีนี้ แต่ปีที่	<20	<20	<20	<20		<20	11	4	III	50%	6440	44	50		
		RS-009 (Feb 20)	<20	40	20	20		<20				(60%) 45.9%	4190	40	44	At 5	1
		? RS-010 (Feb 21)	160	320	320	320		160									
3(ก)	1552516	? RS-014 (Mar 4) ก.ย.ปีก่อน แต่ปีก่อน	1:40	1:40	1:80	1:80	L1:80	1:40	เดือน 7เดือน	6	IV	6เดือน 46% 44.8	4400 4580	38 16	61 57	18 20	6 4
		RS-015 (Mar 8) เพราะ DSS grade III - IV	1:160	1:160	1:320	1:320	L1:80	1:160									
		? RS-022 (Mar 14)	1:640	1:320	1:1280	1:640	L1:80	1:160									
4(ก)	2019066	+ DH ? RS-016 (Mar 13) ก.พ.ปีก่อน กรณีระบาด	1:80	1:160	1:160	1:160	L1:80	1:160	เดือน	5	III	52% 39%	6500 9600	63 80	20 44	6 6	1 1
		? RS-019 (Mar 14) DHF/DSS impending	1:160 shock	1:640	1:640	1:320	L1:80	1:160				37.4	8300	59	37	3	3
		RS-020 (Mar 15)	1:160	1:640	1:320	1:320	1:80	1:640				34%	9300	81	26	3	
5(ก)	2019425	R RS-021 (Mar 20) ก.พ.ปีก่อน กรณีระบาด	1:40	1:160	1:40	1:80	L1:80	1:80	23 30 ปีเดือน	6	III	49% 47%	6000 8400	60 39	26% 53	13% 6	1 8
		– RS-023 (Mar 24) impending shock	1:40	1:160	1:80	1:80	L1:80	1:80				40	10410	48	47	5	
		– RS-024 (Mar 27)	1:80	1:160	1:40	1:40	L1:80	1:80				35	8590	65	24	5	5

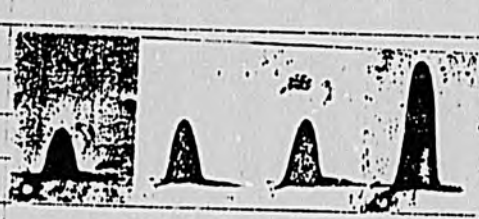
80

BT	Pt	Cl_2
39,600		2 μC
16,000		



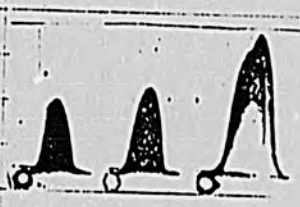
อุ่นๆ 3-5 วัน มีผื่นๆ ตามตัว 76 ชั่วโมง liver 3cm. pulse 130 bm. BP 102/82 4 วัน ไข้ลง VS stable

22,000		2 μC
19,000		



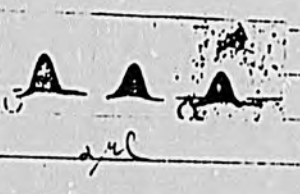
18 ตค อ่อนเพลีย มีไข้ ปวดท้อง ขึ้นตามตัว
19 ตค มา ER มี Acute pharyngotonsillitis R/O DHF.
ต่อมา F.U. 17 ตค 19 ตค ก่อนหลับตอนกลางคืน ปวดท้อง ไข้ลงเรื่อย
มีอาเจียน BP ตกไม่ได้ Hct 50 % (ตอน 8 เช้าวัน
เป็น DHF ช่วง ต่อมาเริ่ม ดูแลก็อาไป ซึมๆ ไม่ค่อยรู้สึก ตัวเย็น
มี petechiae ตามๆ ตีขึ้นเป็นจุดแดงๆ ก้อนแรงเป็น ไม่มี hepatomegaly
วันที่ 20 BP 90/60 → 100/80 pulse 1 ครั้ง ต้องให้ Soft อาเจียน 1 ครั้ง ก่อนหลับ 22 กค. ตัวเย็นๆ
ตื่นขึ้นมาตอน BP 110/70 23 กค ไม่มีไข้ ซึมๆ ปกติ

58,000		2 μC
47,000		



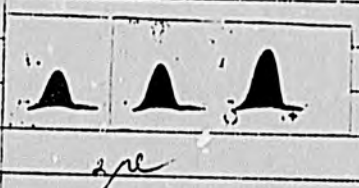
เช้าวัน 6-8 เป็นต้น ผื่นขึ้นเป็นจุดใหญ่ มีไข้ขึ้นสูง 4 วัน 1 ชั่วโมง
3 วันก่อนหลับตื่นเช้า ปวดท้องบริเวณสีข้าง BP 50/0 severe dehydrate
hi fluid BP ตกไป 110/70 มี Petechiae ที่แขนขาและที่ TT liver
3cm ↓

16,000		2 μC
27,000		
29,000		
26,000		



เช้าวัน 12 ชั่วโมง - 16 ชั่วโมง ดูแล อาเจียร 4 ครั้ง ต่อมา อาเจียรเป็น
สีดำเลือด (coffee) bleeding ใน oral cavity เป็นๆ
liver 2cm ↓ 13 ชั่วโมง BP 110/70 PR 88 RR 24
มี Stage of shock 19.00 น. BP 110/70 pulse fade 80-90

130,000		2 μC



อุ่นๆ 19 ชั่วโมงเศษ 16.29 น. มีไข้ขึ้นมา 1 วัน. หลัง ปัสสาวะ 110/70
หายใจไม่ออก ช่วงเวลา ไม่อาเจียร ปวดท้อง อาเจียร TT+ BP 86/150
P 120 1 ครั้ง. ปวดท้อง ใน fluid ลดลง วันที่ 20 BP 110/
Pulse 100 เศษ. วันที่ 21 ท้องตึง ตัวเย็น ไม่มี rectal bleeding
ซึมๆ ทุกอย่าง liver 3cm ↓ ไม่มี epistaxis.

H.N.	Serum No.	NAME	HI D-1	HI D-2	HI D-3	HI D4	HI OHK	HI JE	Age	day	grade	Hct	WBC	NUE	LYM	ATY	Mon
6(a) 2111795	RS-027(May 9) D3?	ด.ญ.ศิริลักษณ์ โอฬารกานนท์	1:640	1:640	1:1560	1:640	1:80	1:1960	7	6	II	33% 38%					
a2.	RS-028(May 9)		1:1960	1:640	1:1560	1:640	1:80	1:1960				36%					
	10 mo.								36			36%	1500	69	31		
	14 mo.								32			32%	6280	45	50		
	19 mo.								24			24%	10100	68	38		
	22 mo.								28.3			28.2%	8600	42	39		
7(a2) 716640	RS-029 (May 10)	ด.ด.ชาตาย เหมืองสิงห์	1:640	1:320	1:640	1:640	L1:80	1:1280	12	4	III	48%		17	72		8
a2	RS-030 (May 16)		1:640	1:320	1:640	1:1280	L1:80	1:640									B1
8(a2) 1233200	RS-032(Jun 1)	ด.ชาญพล แก่งวรรณ	1:320	1:640	1:640	1:640	1:40	1:640	7	7	IV	(31) 0.03) 55%	6100	56	31	5%	7
a2.	RS-033(Jun 2)		1:1280	1:640	1:5120	1:5120	L1:80	1:1280				(1 ไม่ชัด CBC) 37	4900	18	68	9%	5
OPD	RS-034(Jun 11)		1:640	1:640	1:5120	1:2560	L1:80	1:5120									
9(a2) 885452	RS-035(Jun 20)	ด.ดวงพิมพ์ แซ่ลิ้มกุลกรณ	1:40	1:40	1:40	L1:80	L1:40	1:40	11 ปี 3	3	I						
a2	RS-036(Jun 21)	dengue - 1.	1:80	1:320	1:160	1:160	L1:40	1:160									
10(a2) 651987	RS-036(Jun 21)	ด.ด.อธิชาติ ศิตาทิมีกนกา	1:320	1:160	1:320	1:160	L1:40	1:320	15 yr	6	IV	35% 47%	4050 3880	65	32	2%	
a2	RS-037(Jun 22)	อุ่นๆ. (20-22 ชั่ว)	1:640	1:640	1:640	1:1280	L1:80	1:1280									
OPD	RS-040(Jun 25)	DHF @ shock	1:1280	1:320	1:1280	1:1280	L1:80	1:1280									

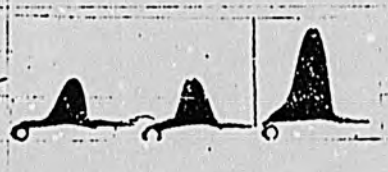
8/a

วัน	Pt.	
	74,000 – adj.	
(วันที่ 6. Hct 27% WBC 8800 N70 L28 M2 Bo PLT 74,000 (รวมกัน) (ตั้งแต่ 7 ม.ค.) BP 110/80 (86; ยาก plt+)		

G6PD def. + DHF มีไข้สูง ตัวร้อน มีเลือดกำเดาไหล ผื่นตามตัว 6 วัน เลือดข้นขึ้น ซึมเซา เริ่มมีอาการตาเหลืองเล็กน้อย ซึมลงเรื่อยๆ ปัสสาวะสีเข้มขึ้น แพทย์ให้รับตัวเด็กไว้ในโรงพยาบาล อยู่ๆ ก็อ้วก เลือดออกตามไรฟัน ตับโต เริ่มมี jaundice. ปัสสาวะสีชา อาเจียนเป็นเลือดเก่า วันที่ 7. เกร็ด ไม่ค่อยดูดนม หายใจลำบาก มีเลือดไหลซึมตามที่ต่างๆ มี petechiae ปรากฏ Hepato-splenomegaly

35,000		

4 วัน มีไข้สูง ตัวแดง ผื่นขึ้น คลื่นไส้อาเจียน ซึม ปากเขียวแล้ว BP 100/80 มีอาการเขียว มี petechiae ตามแขนขา วันที่ 16 BP 90/60 ตัวเย็น ชีพจรเต้นอยู่ถึง 17 ก.

34,000 90,000 –		

เริ่ม ก. 31 พ.ค. 2533 (15.20 น.) เดือนนี้ ปี 2530 อาเจียน ไข้ petechiae liver 2 cm, spleen ตรวจพบ อาเจียนเป็นเลือด 20 ซีซี. pulse เต้น 108/นาที BP (80) DHF @ shock and GI bleeding.

* เปิดครั้งแรก ปี ค. 2530. 4 ½ เดือน ปี ๓๓+ Hct 25.5%. grade II. platelet 174,000, Hct 38.4. PMN 54, L41., atyp. 10%. B.S.

		อาเจียนเลือดออก มะเร็งเม็ดเลือดขาว เม็ดเลือดขาวมาก ซึม ต่อมน้ำเหลือง BP ลงต่ำ Hct 38 ปัญญาอ่อน แรง เกร็ง

ปีใหม่ ปี 2, 000 2		

อ. ปีย. มีไข้มา 4 วัน. ใช้ยา ใช้น้ำลด เริ่มวันที่ 4 เริ่มอาเจียน ไม่มีท้องเสีย ซึม. ซาแล้ว เดินไม่ได้ เดือนมีนาคม เดือน 3. ซึม BP 80/- not pale, no jaundice, liver palpable, no petechiae T- hi ... BP ครั้ง แรก 150/80 - 90/70 ปากเขียว ตายแล้ว BP เดิม 100/80 อนุสรณ์ครั้งที่ 21.

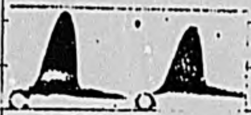
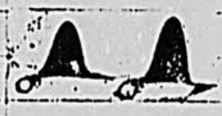
	H.N.	Serum No.	NAME	HI D-1	D-2	D-3	D-4	CHIK	JE	Age	day	grade	HCT	WBC	NUE	Lym	ATM	Mono
11(91)	2426150	RS-041(31/2)	ช. ศักดิ์ชาย [illegible]	1:80	1:160	1:80	1:80	1:160	1:40	13	4		40					
91		RS-042(31/3)		1:80	1:160	1:80	1:80	1:160	1:40									
91		RS-044(31/4)		1:80	1:160	1:80	1:80	1:160	1:40									
91		RS-046(31/5)		1:80	1:80	1:80	1:320	1:320	1:80									
92		RS-050(31/6)		1:80	1:80	1:80	1:640	1:320	1:80									
		~~RS-0[illegible]~~		1:320	1:320	1:320	1:640	1:80	1:320									
12(ICU)	1809147	RS-049(31/6)	ญ. [illegible]	1:160	1:320	1:320	1:640	1:80	1:160	4	5	IV	38.5%					
ICU		RS-054(31/9)		1:1280	1:2560	1:1280	1:2560	1:80	1:1280									
		dx = DHF c̄ shock c̄ pneumonia																
13(OPD)	713211	RS-035(31/11)	ช. [illegible]	1:160	1:160	1:160	1:160	1:80	1:160	14 10/12	5	III	41.6	1400	50	38	5	6
92		RS-036(31/12)	+ [illegible] identify	1:320	1:320	1:160	1:640	1:80	1:640									
92		RS-037(31/16)	dengue type 3	1:1280	1:1280	1:1280	1:1280	1:80	1:1280	40.5 (130)								
14(OPD)	2132287	RS-060(31/19)	ช. [illegible]	1:1280	1:1280	1:1280	1:1280	1:40	1:1280	14	5	I	44.9					
OM		RS-061(31/20)		1:1280	1:1280	1:1280	1:1280	1:40	1:1280				37.1					
15(9)	714340	RS-062(31/20)	ช. [illegible]	1:160	1:160	1:160	1:320	1:80	1:160	13	4	I	37.1	2600	60	40		52
92		RS-066(31/26)	+ [illegible] identify.	1:320	1:320	1:320	1:320	1:80	1:320									
92		RS-068(31/27)	dengue type 1	1:640	1:320	1:640	1:1280	1:80	1:640									

BT	Pt	
	120,000	Viral Infection มีไข้ต่ำ 5 วัน ปวดตึงๆ ไม่มีอาการเจ็บ ท้องเดิน 2 ครั้ง. คิดว่าเป็น typhoid fever มีไข้สูง แล้วต่อด้วย ลดลง 40 → 38 → 37 → 32. BP 90-100 / 60-70
	049 054	วันที่ adm ICU ไข้สูง ซึม เหนื่อย Hepatomegaly + bleeding pulse เร็ว BP 90/50 Hct 45 WBC 800 N17 L12 M8 E 60 Atys PLT 4,500 + 46,000 ตรวจเลือดซ้ำอาการเดิม. ต่อมาวันที่ 4 shock มี bleeding ออกมากทางไรฟัน เป็นที่ 5 BP ตกต่ำ แต่ไข้ 39.2 bleeding มาก. Hct 36 ให้ PRC Hct → 38. / WB 12,700 N75 L10 M1 E3 Atys PLT. 7,000 - มีอาการเลือดออกทั่วไป BP 100-120/70-70 ตายเวลา 20 นาฬิกา.
	88,000	ไข้สูงมาก 5 วัน คุมไข้ไม่ได้ BP 90/60 11-12 กลางคืนมีไข้ BP ต่ำ 70/50 แน่ epistaxis ทั้งวัน 12 กค 80/50 มีไข้ต่ำ. เหงื่อ. ตายเช้า.
	68,000	เช้า 19 กค ไข้ เหนื่อย ตัวร้อนมาก ท้องเสียมาก ท้องอืดมาก เสียชีวิต BP 110/60 ปวดหัวมาก อาเจียนมาก เหงื่อออก. เดี๋ยวนี้ไข้เพิ่มขึ้นถึง 7 ชั่วโมง.
39.5	68,000	ตาย adm เมื่อ 15 กค 2533 ด้วยเรื่อง Encephalitis อาการนำส่งมาด้วย Sinusitis 24 กค ส่งต่อ PTA มีไข้สูง หายใจไม่ทันกัน Hemorrhage อาเจียน 3 ครั้ง. ER Ciprofloxacin ให้ยาลดไข้ BP 38 c Hct 36.6 WBC 1600 plt 191,000. N 82 L7 BP 110/70

25 กค. มีไข้สูงถึง diclyclate ฉีดไม่ทันกัน BP 100/60 มี petechiae ตามตัว ไม่มี bleeding
26 กค BP ต่ำ 90/60 → 80/70 อาการทั่วไปดี.

	H.N.	Serum No	NAME	D-1	D-2	D-3	D-4	CHIK	JE	Age	day	grade	Hct	WBC	NUE	LYM	BFM	[illegible]
16 (Q2)	1474960	RS-007 (July 26)	ช อิ กาน ไกรนนท์	1:640	1:560	1:640	1:160	1:320	1:560									
Q2		RS-061 (July 26)		1:1560	1:560	1:560	1:160	1:320	1:560									
OPD		RS-068 (10 A.M.)		1:1560	1:640	1:1560	1:640	1:320	1:160									
17 Q2	2096142	RS-019 (May 3)	ด. ทรงอาด ศุภโอ	1:40	1:160	1:80	1:160	1:320	1:40	11	5	II-III	50%	2900	25	71	Alg 2	[illegible]
Q2.		RS-218 (May 14)		1:160	1:160	1:160	1:160	1:320	1:160				45%					
18 OPD	2156650	RS-076 (Jun 1)	ศรีวิลาวรรณ เชื้อนปราการ	1:40	1:80	1:320	1:160	1:40	1:160	10	5	III	43.8	2850	70.6	22	3	3.5
Q.2		RS-079 (Jun 1)	dengue type 1	1:640	1:640	1:1280	1:1280	1:40	1:640				41.1	2810	25	67	8	3
OPD		RS-087 (18 A.M.)		1:1560	1:640	1:1280	1:640	1:80	1:5120									[illegible]
19 OPD	2154366	RS-098 (Jun 8)	ฟ ฟ้า วิทย์ ไชยะ: ห่าง	1:80	1:80	1:160	1:80	1:320	1:80									
OPD		RS-098 (Jun 8)	dengue type 2	1:1280	1:640	1:1280	1:1280	1:320	1:2560									
20 Q6	1688266	RS-025 (Jan 8)	ช. นก สว่างดี (อย.)	1:640	1:320	1:640	1:320	1:160	1:640	8	3	II	7 วัน 41.3 / 39%	22000	73	18		9
OPD		RS-093 (Jun 7)	ป่วย 7 - 9 มิ.ย. 33	1:640	1:1280	1:640	1:160	1:320	1:640									

DHF @ bleeding tendency - epistaxis

Bt	Pt	
	74,000	 13 มิ.ย. 5 วัน PTA มีไข้สูงตลอดเวลา ไม่ไอ ไม่มีน้ำมูก ไม่ถ่ายเหลว [illegible] ปวดศีรษะ ปวดท้อง [illegible] TT+ [illegible] ER > BP [illegible] 90/80 - 120/100 [illegible] ไม่มี bleeding มี petechiae [illegible] วันที่ 14 มิ.ย. [illegible] petechiae [illegible] ไม่มี bleeding, Hct 45% BP [illegible] 90/60 อยู่ รพ. 15 มิ.ย. อาการดีขึ้น.
	100,000 158,000	6 [illegible] มีไข้สูง [illegible]. [illegible] ไม่ [illegible] (ไม่มี [illegible].) rash⁻, edema⁻, tonsil enlarge 1+, · 1° malnutrition วันที่ 7 BP 90/68 liver 3 cm ↓ PR [illegible].
		
	146,000 (7 [illegible])	วันที่ 7 [illegible] [illegible] อุณหภูมิ BT 39.8 [illegible] [illegible] [illegible] [illegible] 13 [illegible] 2533 [illegible] ไม่มี petechiae. [illegible] DHF..

ปี 77

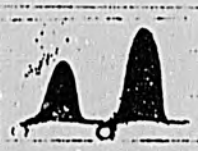
	H.N	Serum No.	NAME	D-1	D-2	D-3	D-4	CHIK	JE	Age	day	grade	Hct	WBC	Neu	Lym	Atm	Mono
21 ก.ค.	1060680	RS-090(10 มค)	ด.ญ. [illegible]	1:640	1:320	1:640	1:320	1:160	1:320	9	4	13	40%	1200	60	38	2	
OWI		RS-091(17 มค)	dengue type 1	1:640	1:320	1:640	1:320	1:160	1:320									
OPD		RS-094(10 มค)		1:1280	1:320	1:1280	1:1280	1:160	1:320									
		30/	(13 มค - 16 มค)															
SEPOD	1913565	RS-096(11 มค)	ด.ญ. [illegible]	1:80	1:320	1:160	1:320	1:160	1:40									
OWI		RS-097(10 มค)		1:320	1:40	1:80	1:320	1:160	1:80									
23																		
ทดร	2136556	RS-098(18 มค)	ด.ญ. [illegible]	1:40	1:320	1:1024	1:1280	1:80	1:320	3	5	III	58%	11200	33	46	14%	7
OPD.	2136358	RS-099(18 มค)		1:1280	1:320	1:5120	1:1280	1:160	1:640				37%	9400	27	60	7%	3
24																		
OPD	1939253	RS-98(19 มค)	ด. [illegible]															
OPD		RS-103(20 มค)																
25	1190271																	
OPD	~~[illegible]~~	RS-99(18 มค)	ด. [illegible]															
OWI		RS-100(21 มค)	dengue type 1															

51 Pt

ailegnal [illegible]

มีไข้สูงมาประมาณ 1 เดือนก่อนมาโรงพยาบาล ไม่มีอาการ petechiae 14 วัน เป็นไข้ต่อ 5 วัน ปวดท้องมาก 2 วัน
Fever c abdominal pain. Hct. 40% (13) → 39% (15)
platelet [illegible] (13) → 95,000 (15) สงสัย enteric fever
หรือ DHF

62,000
52,000



3 วันก่อนมาโรงพยาบาล วันที่ admit (2 พค) มาเจ็บ look weak cold clammy skin, dry lip + mucosa, liver send, spleen T 36.8° P 110/min RR 36/— BP ตกลงมาที่ systolic 70/ วันที่ 3 ไม่มีอาการปัสสาวะ + ถ่ายอุจจาระ ไม่มีเลย T. 36.5° P 100-110 BP 9-10/60-70. RR 24. แต่ดูไม่ค่อยวันที่ 2.

	H.N	Serum no.	NAME	HI D-1	D-2	D-3	D-4	CHIK	JE	Age	Sex	grade	HCT	W.BC	NEU	Lym	ATM	remarks
IPD	2148842	RS-105 (1400)	ด.ญ. ศิริพร ศิลปอัชกอง							10	6	III	42%	4800	76	20	7%	
"	"	RS-106 (900)	dengue type 2										42	2400	76	20	4	
"	"	RS-107C																
"	"	RS-108 (1000)																
"	"	RS-112 (1100)																
													M 44 F 40 M 44	1700 1200	95	4	601	
IPD	2147491	RS-109 (1000)	ด.ญ. เกื้อจิต อาวุโส							5	4	II						
"	"	RS-111 (1100)																
OPD	2149015	RS-90-114 (1300)	N. ศรีนารถ (สิงหรักษา) ใหญ่ทอง.							2 8/12	4	I	39.2	4400	31	58		5
OPD	"	RS-119 (1700)																
OPD	2149758	RS-90-123 (N.O.33)	O.C. พริกแก้ว หน่ำพิโรจน์.							7 4/12	4	I	39.2	3900	27	48	25%	5
OPD	"	RS-90-121 (1400)																
OPD	2149789	RS-90-150 (1400)	O.C. แก้ว หมั่นเจริญ							11	7	II	(2300)	43.7	4400	25	70	10%
OPD	"	RS-90-124 (1800)																

H	PT.	
	53,000	ตรวจพบครั้ง ตุลาคม 9 กค - 2 กค ซีกขวา ., petechiae, epistaxis -, bleeding per gum, liver + FB, tachycardia PR 120/min Tigum ร่วม ตาเหลืองมาก 3-4 วัน.
		9 กค ตาเหลืองเรื่อยๆ ซึมลง BP 90/60 > 70/40, bleeding per gum, liver 2 FB, spleen -, ± GI bleeding BP 80/60 ปวดท้อง ท้องอืดมากขึ้น วันที่ 10 BP 90/70 P 120 เต้นแรง Hct. 15
	69,000 46,000	Puffy eyelid 11 ซึม
		7 กค ให้ยา 4 เม็ด มาตรวจพบว่า BP ไม่ตกลงไป เกร็งเล็บ เลือดไม่ค่อยหยุด (เลยเป็น DHF adm เพื่อระงับอาการเลือด 1 วันก่อน) นัด PU ทุกวัน TT +ve R/O DHF vs ICH
		8 กค ซึมลงไป BP 100/70 DHF gr II-III ē Epitaxis
		9 กค ไข้ T 38.6 มีอาเจียน BP 100/50 → 90/60
		Hold dehydrate มี petechiae เพิ่มขึ้น ตกเหลือ BP 80/50, 70/60 มีภาวะช็อคไม่ได้ มีเลือดกลับเข้าในตัว ให้ FFP gr IV ē VGIB ē Shock
		10 กค ท้องเสียมาก ซึมลงมาก เพิ่มขึ้นมาก petechiae เพิ่มขึ้นมาก ปวดท้อง BP 80/50 → 70/50 มีเลือดออก bleeding ทั่วตัว ท้องบวม ตาเหลือง ตาเหลืองมาก
		11 กค ซึมลงไป ท้องเสียมาก ซึมลงไป เกิด bleeding มี petechiae มากขึ้น แขนขา
		เช้าวัน 12 กค 3 ถ่ายเหลวสีชา ซึม อาเจียน TT+ > 10 ต่อ ถ่ายอุจจาระเป็นสีดำ (17) ไม่อาเจียน ซึมลงไป ตาเหลือง
		24 กค ตา ตรวจ พบกลิ่น ไม่อาเจียนถ่ายเหลวมาก ตับ 2 cm (sharp edge) ส่งเลือด R/O DHF.
36°C	109,000	เช้า 19 กค ซึมลง BP 100/70 T 36°C liver-spleen TT + test > 10 ดูด/ml²
	83,000	เช้า วัน 23 กค - 24 กค. วันที่ 23 เวลา 16.45 น. 5 ถ่ายเหลวสีชา 2 ถ่ายเหลวมีปนเลือด อาเจียน. BP 100/70.

ward	H.N	Serum No.	Name	HI D-1	D-2	D-3	D-4	ch.b	Jp	Bgl	dy	grade	Hct	WBC	HLE	Lym	OLA	MO
OPD	2755675	15 ก.พ. 33 RS-90-116	ด.ญ. ดู๊ด อินทร์							ศิริ	6		40.8	4800	22	59	11	8
OPD	"	15 ก.พ. 33 RS-90-134	dengue type 3 *เลือดออก															
ศิริ5	16/2.553	15 ก.พ. 33 RS90-135	ดก. ตรวจหาว เชื้อไข้รากสาด							4 11/12 ปี	5	III	39.9	4560	72		4	
"	"	15 ก.พ. 33 RS-90-136	เพื่อวินิจฉัย อาจเป็น SHF										38.4		80	19	5	
ศิริ1	"	15 ก.พ. 33 RS-90-137	อาจเป็น para typhoid. หรือไข้ Salmonella typhi A.															
OPD	1289364	14 ก.พ. 33 RS-90-140.	ด.ช. พร้อม พันธวนกุล							8	5		39.1	2300	61	29	2	1
OPD	"	14 ก.พ. 33 RS-90-145											34	2500	45	44	4	6
OPD.	2162900	5 ก.พ. 33 RS-90-147	ด.ช. พวงรัตน์ พรรณรัตนาพงศ															
ศิริ2	"	5 ก.พ. 33 RS-90-148.																
OPD	"	13 ก.พ. 33 RS-90-150.																
[illegible]	"	[illegible]																
OPD.	1974654	7 ก.พ. 33 RS-90-149	ดญ. พาเพาเพ ใกล้เคียงวงศ์.															
"	"	7 ก.พ. 33 RS-90-150																

Dt	Pt	
32.2	39,000 (2700)	8 กค 2533 liver 1 FB + dark + petechiae (body + extremity) BP 100/70, P132 RR 28 มีอ่อนเพลีย ซีด ปัสสาวะ R/O DHF BP ไม่แกว่ง 100/70 dark มาก ปัสสาวะแดงทั่วไป ไม่มี active bleeding
	160,000 101,000	admit 16 กค 2533 มีไข้ก่อนมา 3 วัน ไข้สูงเรื้อง ปวดท้อง ไม่ได้ถ่ายอุจจาระ 1730 ผู้ป่วยเริ่มเข้า shock stage P 144/min Hct 43% liver ++
	(28/10/33) 108,000 (28/10/33)	admit 1 ตุลา 33 - 25 กค 33 เจ็บคอ ได้รับ Vinal infusion นน.ว. มีไข้ก่อน 2 วัน ตรวจร่างกายปกติ ไม่เจอ CBC Hb 12.6 WBC 5700, N 62, M 12, Aty 0.1%, 9 วันก่อนมาไข้ขึ้น 2 วันแล้วหาย ตกเลือดก่อนมา ซึมลง ปัสสาวะ ปัสสาวะ ผู้ป่วยซีด นน. 90 กรม เกือบช่วงต่ำ

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Ward	H.N.	Section no.	Name	[illegible]	Age	[illegible]	[illegible]	Hct	WBC	Neu	Lym	Mono	[illegible]
[illegible]	1483490	9 [illegible] RS-90-134	[illegible]		([illegible])	4	III	41%	3700	54	25	1%	20
OPD.	"	13 [illegible] RS-90-147			([illegible])				2450	33	58	4	5
[illegible]	4/60500	12 [illegible] 33 RS-90-156	[illegible]										
"	"	10 [illegible] 33 RS-90-156											
"	"	13 [illegible] 33 RS-90-157											
"	"	13 [illegible] 33 RS-90-162											
[illegible]	3166690	15 [illegible] 33 RS-90-163	[illegible]										
"	"	17 [illegible] 33 RS-90-164											
OPD	"	17 [illegible] 33 RS-90-165											
OPD	3166250	22 [illegible] 33 RS-90-164	[illegible]										
"	"	7 [illegible] 33 RS-90-181											
[illegible]	3169001	24 [illegible] 33 RS-90-170	[illegible]										
"	"	26 [illegible] 33 RS-90-171											
OPD	"	30 [illegible] 33 RS-90-183											

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11	pt	
		12 mo. ER 8 mo 2533 impending shock BP 90/70 PMO
38c	51,000	RR 32/min ฟัง lives 2 Fd/ ตาขึ้นเขียวเล็ก thermo
	48,000	ห้องคลอดไม่ได้ 9 mo (dg 5) Hct 30 BP 100/60
		10 mo impending shock Hct → 38% ส่งแผนก 7 ธ.ค.

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Word	H.N.	Serum No.	Name
ICU เด็ก	2169010	[illegible] 33 RS-90-172	ด.ญ. [illegible] [illegible]
"	"	[illegible] 33 RS-90-174	
"	"	[illegible] 33 RS-90-176	
"	"	[illegible] 33 RS-90-177	
"	"	[illegible] 33 RS-90-179	
เด็ก 2	"	[illegible] 33 RS-90-180	
เด็ก 2	1624329	[illegible] 33 RS-90-173	ด.ช. [illegible] แซ่[illegible]
"	"	[illegible] 33 RS-90-175	
เด็ก 2	1501653	30 ม.ค. 33 RS-90-182	ด.ญ. [illegible] [illegible]
"	"	[illegible] 33 RS-90-185	

IMMUNE RESPONSE IN RABBITS TO DENGUE VIRAL PROTEINS

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Abstract. Antisera to all types of dengue and Japanese encephalitis (JE) viruses were raised in rabbits. The first set of rabbits was immunized with crude antigens prepared by a sucrose acetone extraction. The first generation of antisera demonstrated antibody activity towards the group specific flaviviruses, without unwanted antibody activity towards the mouse brain material. Antibody activity towards E, NS3, NS5 and NS1 could be observed by western blot/immunoenzymatic assay. Another set of rabbits was immunized with the precipitin complexes formed between antisera raised against each type of dengue, or JE viruses, and their homologous antigens. Each rabbit serum was screened again by the western blot/immunoenzymatic method, and was absorbed with other types of dengue viruses until the specific activity towards the immunized viral antigen was obtained. The last 2 bands detected on the viral antigen strip were the doublet of protein bands at Mr 67 and 71 kDa, which are the NS3 protein. The specific polyclonal antisera obtained can be used with other tests as well as the monoclonal antibody. Since absorption can lead to type specific antisera, this NS3 protein must be quite unique, it processes a type specific determinant (s) and deserves further study as a target molecule at the polypeptide level as well as at the RNA level.

INTRODUCTION

The flaviviruses are a serologically related group of viruses in the family *Togaviridae*. Cross-reactivity of the immune responses caused difficulty in diagnosing this infection by a hemagglutination-inhibition (HI) test (Westaway, 1968 a; Westaway, 1968 b; Scott *et al*, 1972). Many attempts to produce specific antigens for serological tests have been made (Smith *et al*, 1970; Qureshi and Trent, 1973; Eckels *et al*, 1975). However, in effective serological tests such as the HI (Clarke and Casals, 1958) and enzyme linked immunoabsorbent assay (Burke *et al*, 1985; Innis *et al*, 1989), crude antigens made from sucrose acetone extraction are still used. On the E glycoprotein, the serotypic, flavivirus-group-reactive, and complex-reactive determinants could be identified with polyclonal antisera by a competitive antibody-binding assay (CBA) (Trent, 1977). The hybridoma technology has contributed significantly in the area of rapid identification and the availability of monoclonal antibodies (MAb) to all 4 types of dengue viruses. (Gentry *et al*, 1982; Henchal *et al*, 1982). These MAb have been applied for use with other techniques, such as the indirect immunofluorescence assay, IFA (Henchal *et al*, 1982). The MAb are being routinely used to identify virus agents in mosquitos inoculated with sera from dengue patients in an attempt to detect the etiologic viruses.

In this report, polyclonal antibodies were raised in rabbits. Immunoprecipitin from the first generation of dengue antisera and dengue antigens obtained from counter immunoelectrophoresis (Churdboonchart *et al*, 1979) were used as an immunogen. This short cut was employed before using crossed immunoelectrophoresis precipitates to produce specific herpes simplex virus type 2 antisera (Vestergaard, 1975). The second generation antisera were repeatedly absorbed with other dengue sero-

types to obtain serotypic polyclonal antibodies. The specific antibodies are against the non-structural dengue specified protein NS3. The results suggest the possibility of using them for simple and rapid diagnosis.

MATERIALS AND METHODS

Viruses and viral antigens

Dengue type 1 : Hawaii M 199 (D1); dengue type 2 : New Guinea C (D2); dengue type 3 : H−87 (D3); dengue type 4 : H−241 (D4); and Japanese encephalitis : Nakayama (JE). Viruses were used in this experiment.

Viral antigens were prepared from dengue infected suckling mouse brain. They were extracted by acetone in presence of sucrose (SAE) according to Clarke and Casals (1958). The antigens were titrated by a hemagglutination (HA) test, Viral-antigen-strip/enzymeimmunoassay (VAS/EIA) (Churdboonchart *et al*, 1987), and countercurrent immunoelectrophoresis (CIE) (Churdboonchart *et al*, 1979).

Antisera

Goat anti-Rabbit IgG and rabbit anti-human IgG labeled with peroxidase enzyme were purchased from Sigma. Monoclonal antibodies were raised from hybridoma cell lines (3H5-1) purchased from the American Type Culture Collection (ATCC). Human hyperimmune sera were collected from laboratory confirmed cases of secondary dengue hemorrhagic fever (S-DHF).

Countercurrent immunoelectrophoresis

Countercurrent immunoelectrophoresis (CIE) for forming dengue viral antigen-antibody complexes was carried out as previously described (Churdboonchart *et al*, 1979). Briefly, 18 ml or 0.6% agarose (BDH Chemicals, England) in 0.05 M Veronal buffer, pH 8.2, was layered on a glass slide (3.2 in by 4.0 in). After gelation, 6 rows of 4 mm wells were punched, leaving 3 mm between row 1 and 2, 3 and 4, as well as 5 and 6, in order to form precipitin complexes. The viral antigens, extracted with sucrose acetone, were loaded in the anodal side, or row numbers 1, 3, and 5; and the rabbit anti-dengue antisera were loaded on the cathodal side, or row numbers 2, 4 and 6. The volume of antigens and antibodies loaded into each 4 mm well was 25 µl. Electrophoresis, 20 mA per slide, was done at room temperature for 90 minutes using a 0.05 M Veronal buffer, PH 8.2. Precipitin complexes between viral antigens and antibodies could be seen by the naked eye.

Serology tests

Serology tests for an estimation of anti-dengue activities were done by 2 methods; the micro-modification method of the hemagglutination-inhibition (HI) test (Clarke and Casals, 1958), and the isotype antibody capture enzyme-linked immunosorbent assay for specific dengue virus IgG antibodies (GAG-ELISA) (Burke *et al*, 1985; Innis *et al*, 1989). Sera were extracted by acetone before being subjected to both tests. For the HI test, the antibodies to all 4 types of dengue Japanese encephalitis and chikungunya viruses were titrated. GAG-ELISA was used to detect antibodies towards the dengue group antigen, and Japanese encephalitis viruses.

Viral antigen strips/enzymeimmunoassay (VAS/EIA)

Viral antigen strips (VAS), containing SDS-PAGE separated polypeptides of all 4 types of dengue viruses, and normal mouse brain were prepared as reported previously (Churdboonchart *et al*, 1987). The reducing agent, 2−mercaptoethanol, was excluded from the classical Laemmli protocol (Laemmli, 1970). An immunoenzymatic assay was done using the prepared rabbit anti-dengue antibodies as a primary antibody. Specific rabbit anti-dengue (IgG) antibodies which complexed with dengue viral antigens, were detected by the second antibody, anti-rabbit IgG, labeled with peroxidase enzyme (Sigma). The color intensities of positive bands were graded according to Schupback's criteria (Schupback *et al*, 1985) as follows : +1 for a barely detected or fainted pink color, +2 for a clearly positive seen as a

sharp pink color bands, +3 for a strongly positive (red color), and +4 for a very strongly positive reaction seen with a dark red color.

Polyclonal type specific antisera to dengue viruses.

Polyclonal antisera were raised in rabbits by the inoculation of one type of SAE dengue antigen on each foot pad. One part of the SAE antigen was thoroughly mixed with one part of an incomplete Freund's adjuvant. After 10 days, of 3 immunized doses at weekly intervals, the rabbits were bled and the sera were characterized by CIE, HI, ELISA, and VAS/EIA. The rabbit sera were freed from unwanted antibodies by a liquid phase absorption, using 50% homogenized normal mouse brain in a phosphate buffer for the absorption, until they were devoid of any antibody activity to a normal mouse brain, Usually the antisera against dengue viruses abtained at this step react with type specific as well as dengue group specific antigens.

With CIE, precipitin complexes between dengue viral antigens and rabbit polyclonal antibodies can be obtained. After electrophoresis, the precipitating bands in agarose gel were excised and homogenized with an incomplete Freund's adjuvant at 1 : 1 ratio (V/V). The second set of rabbits were immunized with this precipitin complex using the same immunization schedule. The antibodies obtained at this step usually demonstrated higher antibody activities. The sera were examined by CIE in order to screen for the precipitin bands and were the first to be absorbed with the mouse brain material if any activity to the mouse brain showed up. Then, they were absorbed with other types of dengue viruses. In this experiment, the following programs were employed : D4−SAE antigen to absorb the antisera to D1, D1−SAE antigen to absorb the antisera to D2, D4−SAE antigen to absorb antisera to D3, D1−SAE antigen to absorb the antisera to D4, and D2−SAE antigen to absorb the antisera to JE. After complete absorption, the antisera were screened by VAS/EIA. These antibodies were titrated again by GAG-ELISA to all dengue and JE viruses.

RESULTS

Characterization of the antigens

Antigens that were to be used to detect antisera developed in immunized rabbits were characterized by HA test. Antigens with HA titers higher than 1 : 640 units were characterized by human hyperimmune sera from confirmed DHF cases by HI and by GAG-ELISA. These high titer antibodies to dengue proteins in immune sera bound to viral proteins on the nitrocellulose membrane revealed the kind and quantity of each dengue viral protein. Good batches of dengue antigens usually demonstrated strong color bands to envelope (E) protein (Mr 52−60 kDa), core (C) protein (Mr 16 kDa), NS5 (Mr 91−98 kDa), NS3 (Mr 67 and 71 kDa), and NS1 (Mr 45 kDa). All 4 types of dengue viruses, (D1 to D4), and Japanese encephalitis (JE) antigens were selected after the characterization, in order to be used to produce the viral antigen strips, or VAS for titration of anti-dengue activities in this experiment.

Human immune responses to dengue proteins.

Sera from S-DHF patients, collected during different phases of the illness, were employed for this experiment. Sera from day 4 to 10 after the onset of fever were examined, using the standardized dengue antigen strips. Serological tests of these patients are shown in Table 1, and the antibodies to dengue proteins are demonstrated in Fig 1. Antibody activities to dengue structural proteins : E protein of Mr 59 kDa, with 2 smaller E-proteins of Mr 55 and 50 kDa, and C protein of Mr 16 kDa, could be prominently detected during the convalescent phase. The antibody to M-protein at Mr 8.5 kDa could be seen only in certain cases when the titers to other proteins were already strong. The antibodies to the non-structural proteins : NS 5

Table 1

Serology tests for patient # 343.

Day after onset of fever	HI titers (reciprocal)						ELISA titers			
							Dengue		JE	
	D1	D2	D3	D4	JE	CHIK	IgM	IgG	IgM	IgG
day 4	<20	20	<20	<20	<20	<20	39	21	75	122
day 5	1280	640	640	320	640	<20	ND	ND	ND	Nd
day 6	1280	1280	640	1280	1280	<20	ND	ND	ND	ND
day 10	2560	2560	320	2560	5120	<20	228	362	46	441

ND = not done

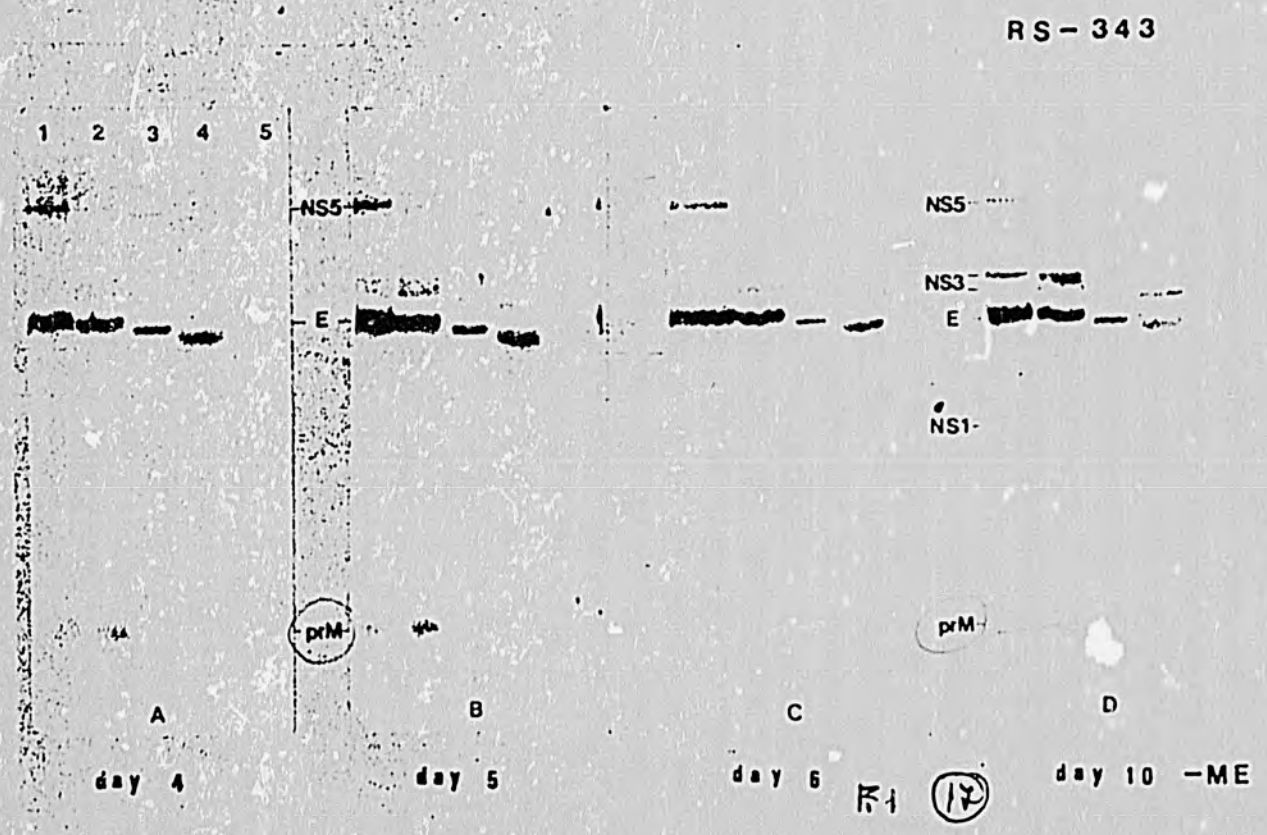


Fig 1–Viral antigen strips A, B, C and D are shown for comparison of the development of dengue antibody responses in children with dengue infection. Each strip has 5 lanes and dengue antigens prepared from dengue viruses type 1 to 4 by loading into lane 1 to lane 4. Normal mouse brain suspension was loaded in lane 5 as the negative control. Antibody responses to E, NS1, NS3, NS5 and prM proteins could be detected during acute phase of illness on day 4 (Strip A) and higher responses could be observed during the convalescent stage on day 10. These standard strips were confirmed to contain enough dengue proteins for detecting the dengue antibodies.

at Mr 92–97 kDa, NS3 at Mr 67 and 71 kDa, and NS1 at Mr 45 kDa, were detected in most S-DHF during the convalescent phase. The first antibody observed was the antibody to E-protein at Mr 59 kDa and the second one was the antibody to NS3 at Mr 67 kDa. The viral antigen strips were comfirmed here to contain enough dengue proteins for detecting dengue antibodies in the serum of an immune person.

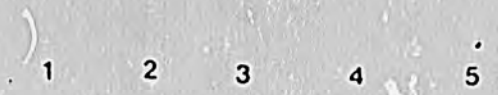
Rabbit immune responses to dengue proteins.

The first generation of rabbit antisera obtained from the immunization of the rabbits with sucrose acetone extracted antigens demonstrated cross-reactivity among dengue serotypes and JE with reference VAS as shown in Fig 2. The antisera were freed from antibodies to mouse brain materials showing an absence of bands to mouse brain antigen. These rabbit antisera to flavivirus antigens were good enough to be used for quantitating the groupspecific antigens prepared from dengue and JE viruses.

A second set of rabbits was immunized with the precipitin complexes formed between the antibodies from the first set of rabbits and their homologous antigens by the CIE method. After absorption, good antisera would show the specific activity toward the inoculated type of antigen as demonstrated in Fig 3 (A, B, and C). It is interesting to note that the bands which indicate the specific reactions toward each type of antigen are against the doublet bands of NS3 at Mr 67 and 71 kDa. The results from 12 rabbits, whose sera demonstrated the type specific antibody activity, are summarized in Table 2.

Application to rapid serotypic identification

The rabbit polyclonal specific antibodies to dengue D2 were applied to identify the type of dengue infection in an infected mouse brain. The results are demonstrated in Fig 4. This antiserum showed specific activity towards D2 (lane 8) in the reference part of the strip. These reference antigens were serially loaded with dengue type 1 to 4, JE, and normal mouse brain



F. 2 (17)

Fig 2–Rabbit serum after immunizing with crude sucrose acetone extracted dengue type 2 antigen and absorbed with homogenized normal mouse brain suspension. Antibodies to all dengue serotypes are shown in lanes 1 to 4 which were loaded with dengue type 1 to type 4. An absence of anti-mouse brain activity can be seen with an absence of stained bands in lane 5 (loaded with normal mouse brain suspension).

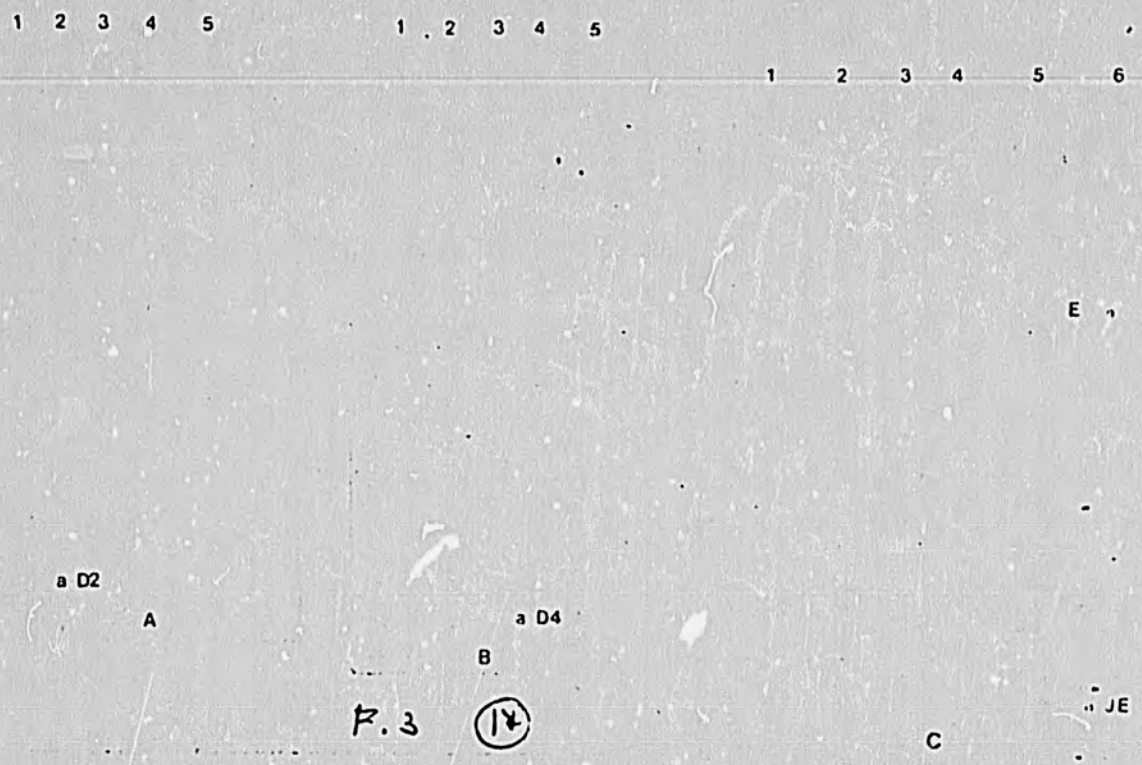
from Lane 7 up to lane 12. The antibody activities towards the bands from patients # 26, # 65, and # 67 were demonstrated at the same position on the nitrocellulose membrane. D2

Table 2

Antibody titers of the polyclonal antibodies.

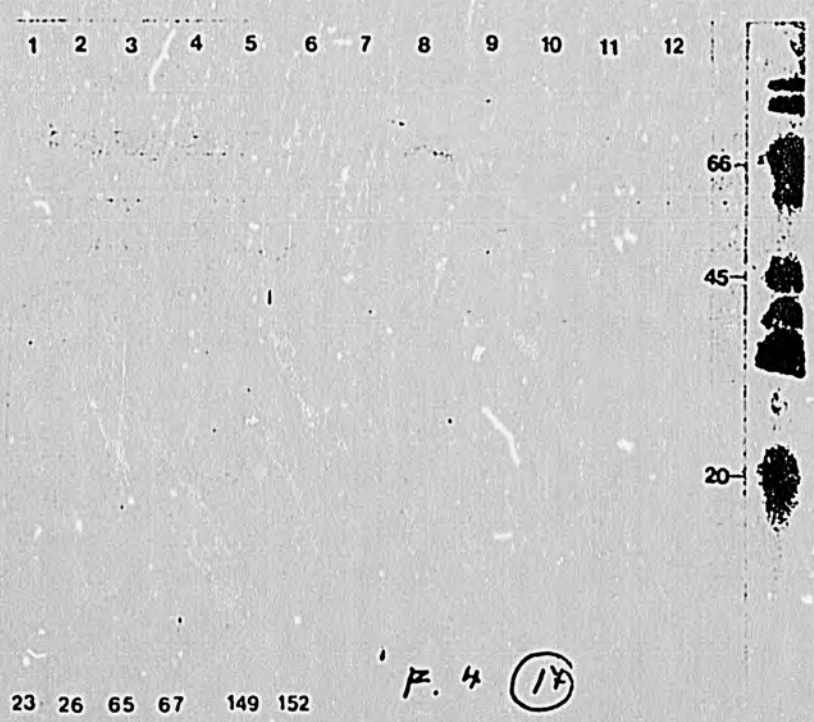
Polyclonal	GAG-ELISA to reference viruses and antigens (OD 492 nm)							Antibody to virion protein	Specificity	Sensitivity to detect dengue antigen (HA unit)	Dilution suitable for ELISA test
	D1	D2	D3	D4	JE	MB	HS				
a-D1 (# 339)	.76	.37	.47	.15	.08	.03	.005	NS 3	D1	2	1:400
a-D1 (# 325)	.91	.33	.61	.19	.06	.45	.43	NS 3	D1	1	1:800
a-D1 (# 369)	1.45	.88	.86	.70	.58	.53	.53	NS 3	D1	2	1:400
a-D2 (# 283)	.43	.85	.39	.41	.31	.31	.34	NS 3	D2	2	1:500
a-D2 (# 202)	.36	.99	.56	.27	.00	.00	.09	NS 3	D2	2	1:100
a-D2 (# 304)	.59	.96	.59	.42	.29	.09	.11	NS 3	D2	2	1.800
a-D2 (# 328)	.60	1.45	.55	.27	.25	.00	.00	NS 3	D2	2	1:1,000
a-D2 (# 324)	.56	.82	.68	.40	.18	.15	.16	NS 3	D2	2	1:400
a-D3 (# 376)	.60	1.17	1.39	1.06	.78	.19	.19	NS 3	D1, 2, 3, 4	8	1:1,500
a-D4 (# 375)	.79	1.2	1.27	1.84	1.18	.31	.31	NS 3	D1, 2, 3, 4	20	1:800
a-Da (# 327)	.66	.00	.66	.78	.21	.18	.18	NS 3	D4	20	1:400
a-JE (# 299)	.12	.16	.12	.17	.74	.04	.05	E, NS 3, NS 1, and prM	JE	4	1:500

MB = mouse brain
HS = healthy human sera



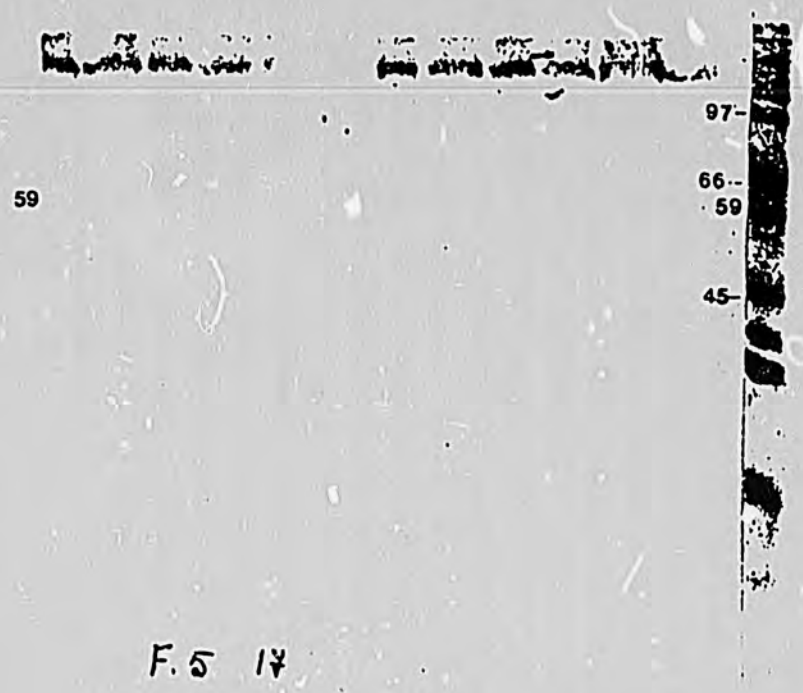
F. 3 (14)

Fig 3–Antibodies to NS3 protein at Mr of 67 and 71 kDa can be seen in Fig 3A (lane 2, to dengue type 2), and in Fig 3B (Lane 4, to dengue type 4). Antibody to JE virus (Fig 3C) after absorption of antibodies to dengue proteins, can be seen toward several Je proteins including at Mr of 50 kDa.



F. 4 (14)

Fig 4–Rabbit specific antibody to dengue type 2 was used to identify the serotype after the infected from 3 confirmed cases in lanes 2, 3 and 4 and detect dengue type 2 reference antigen in lane 8. Reference dengue antigens from types 1, 3 and 4 were loaded in lanes 7, 9 and 10 respectively. Negative control, normal mouse brain, was loaded in lane 11.



F. 5 17

Fig 5—Monoclonal antibody to dengue type 2 was used to detect dengue serotype as comparison. This antibody showed the specific detection of dengue type 2 virus yielding the same result as the rabbit polyclonal antibody (Fig 4).

viruses were isolated from these 3 patients' sera by mosquito and suckling mouse inoculation : the results of identification agreed with the serotype identification by monoclonal antibodies, demonstrated in Fig 5.

DISCUSSION

The first generation of antisera, made by crude sucrose acetone extraction, for each type of virus were employed with the CIE method to quantitate dengue antigens and antibodies (Churdboonchart *et al*, 1979; Churdboonchart *et al*, 1981). These antisera could not differentiate the types of dengue viruses. By using precipitin complexes of dengue antigens and rabbit antisera (which form in agarose get) as an immunogen, the stronger antibody activities can be obtained in rabbit sera after an absorption of the unwanted antibodies, eg the antibody activity to the mouse brain.

After selecting the antisera with CIE bands to be tested with VAS/EIA, the selected antisera were absorbed with other types of dengue viral antigens. After absorption, the last and specific band against each type of dengue virus could be seen at the position of NS3 (Mr 67 and 71 kDa). Besides obtaining monospecific antisera, the antibodies demonstrated specificity toward the NS3 protein. This gave a short cut method by avoiding the purification of this protein, which is not available at present. This protein was described as a hydrophilic protein and carries net 15 positive charges. It was postulated to be involved in RNA replication with an enzymatic role (Rice *et al*, 1985). This protein always shows as a doublet band, of Mr 67 and 71 kDa, with the western blot method as shown in Fig 3 and Fig 4. NS3 proteins must have a type specific determinant. Even though there may be cross reaction among dengue types, this cross reaction can be gotten climinated by a liquid phase absorption with other dengue types. This antibody titer may even be better if the purified NS3 protein was used for immunization. The type specific antibody response created by the NS3 protein indicates the existence of type specific determinants on these proteins. The specific antibody to dengue type 3 is still not

— better

in hand because the antibody response in the first generation was not as strong as the dengue type 2. Dengue type 2 demonstrated itself to be the best immunogen among the dengue serotypes. However, the JE viral antigen gave the best antisera both in the first and second generations. Further application using high titer antibody of anti-NS3 specificity for each type of dengue virus for serotypic identification of dengue viruses deserves further study.

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DENGUE VIRUS SEROTYPIC INDENTIFICATION USING SUCKLING MOUSE AND WESTERN BLOT TECHNIQUE

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Abstract. The suckling mouse which is used in the classical method to detect and propagate dengue viruses was evaluated in conjunction with the western blot and immunoenzymatic methods to detect the infecting strains of dengue viruses. After intracerebral inoculation of patients' sera into the suckling mice for 7 days, the mice were examined for the presence of dengue proteins, even though the mice did not have the neurological symptoms which usually serve as an indicator for the presence of dengue infection in the mouse brain. With a blind study of a set of 12 specimens, the suckling mice could detect the virus with the same frequency as the mosquito system but in shorter time of incubation period. The whole process to identify the type of infection takes 9 days. Another important finding is the demonstration of the virion antigen in the liver. The quantity and quality of viral proteins in liver are comparable to those in the brain suggesting that the virus may replicate in the liver as well as in the brain.

INTRODUCTION

Dengue viruses can be identified after passage of infected human sera to suitable permissive hosts or cells. Originally, the suckling mouse was used to identify dengue viruses from type 1 to type 4 (Sabin, 1950; Sabin, 1952; Hammon *et al*, 1960; Hammon and Sather, 1964). After cell culture systems were developed, several cultured cell types such the LLC-MK2 ; Rhesus monkey kidney cells (Sukhavachna *et al*, 1966) and C6/36 mosquito cells: *Aedes albopictus* (Singh and Paul, 1968) were employed to isolated dengue viruses from patients' specimens. Later, the adult mosquito, *Toxorynchites splendens* (Rosen and Gubler, 1974), was successfully used to isolate the infected virus from patients' sera. With existing systems, viral isolation and identification takes approximately 3 weeks with a recovery rate between 5-40% (Scott *et al*, 1980).

The present report shows that dengue viruses may be identified after a passage in suckling mouse brain which was used in this cases as a biological amplification system. Suckling mouse inoculation together with the western blot technique was used and compared with mosquito inoculation plus indirect immunofluorescence test (IFT). Other mouse organs such as liver, spleen, and also plasma were examined for the presence of the dengue virion antigen by the western blot method.

MATERIALS AND METHODS

Patients

Twelve patients admitted with a clinical diagnosis of dengue infection to the Ramathibodi Hospital, Bangkok, Thailand, in 1989 were randomly selected for this study. Twelve serum samples collected during hospital admission were split and aliguots sent to two laboratories working independently to compare the results. Our laboratory handled the mouse inoculation and western blot technique, while the

Center for Dengue Vaccine Development handled the mosquito inoculation and immunofluorescence assay. The patients were graded according to the severity of disease by the criteria set by WHO (1975).

Viral Antigens

Mouse brain antigens extracted with sucrose acetone (Clarke and Casals, 1958) were prepared from dengue type 1 (D1) : Hawaii M199; dengue type 2 (D2); New Guinea C; dengue type 3 (D3) : H−87; dengue type 4 (D4) : H−241; and Japanese encephalitis (JE), Nakayama strain. They were titrated by hemagglutination (HA) test, and screened by western blot for the presence of the optimum dengue protein bands to be used later as the positive control antigens. (Churdboonchart *et al*, 1987).

Serology

Sera were extracted with acetone and tested by a micromodification of the hemagglutination inhibition (HI) test (Clarke and Casals, 1958). Each case was classified as a primary (P−DHF) or secondary (S−DHF) infection according to the serologic criteria set by WHO (1975).

Mosquito inoculation and immunofluorescence test

Twelve sera were stored at −70°C until used. They were diluted 1 : 5 with RPMI−1640 media (Gibco) before inoculation intrathoracically : 36 ul into 13 adult mosquitos, *Toxorynchites splendens*, aged 1−3 days. After incubation at 32°C for 14 days, the mosquitos were killed and the head parts from 5 out of 13 mosquitos were screened by indirect immunofluorescence assay using human hyperimmune sera to dengue viruses as the primary antibody. Rabbit anti-human-IgG conjugated with fluorescein thiocyanate was used to detect the presence of the bound dengue antibody. If the screening test revealed a positive result, the body parts of the mosquitos were used for serotypic strain identification by indirect immunofluorescence assay using monoclonal antibody to the dengue prototype strain as the primary antibody. Then the rabbit anti-mouse IgG conjugated with fluorescein thiocyanate was used as the second antibody to detect the bound MAb to dengue viruses.

Serotypic identification by suckling mouse and western blot technique

Sera were diluted 1 : 2 with bovine serum albumin (0.75%) in borate buffered saline (BBS) before injecting one drop (.02 ml) intracerebrally to each of the 1−2 days old suckling mice using a tuberculin syringe and 24-gauge needle. Diluted serum from each patient was inoculated into one liter of mice, which is comprises approximately 7−12 suckling mice. Infected mice were killed on day 7 after inoculation and their plasma, brain, liver and spleen were collected and kept separately. These organ specimens were each homogenized after mixing with 0.9% normal saline at approximately an equal ratio (V/V). The mixtures were centrifuged at 10, 000 rpm for 30 minutes at 4°C. The supernatant fluids were solubilized with 1% sodium dodecyl suftate (SDS) in Tris-glycine buffer (25 mM Tris, 192 mM glycine, pH 8.0) at a ratio of 1 : 1 (V/V). They were then electrophoresed in 8% SDS-polyacrylamide gel (Laemmli, 1970) and transferred from gel to nitrocellulose membranes (Towbin *et al*, 1979). The separated viral proteins on nitrocellulose membranes were characterized by an immunoenzymatic method described previously (Churdboonchart *et al*, 1987). Human hyperimmune sera from dengue hemorrhagic fever patients (with HI titers higher than 1 : 20480 and demonstrated +4 score to dengue E protein and +3 score to NS3, NS5, and C proteins by the western blot immunoenzymatic method) were used as the primary antibody using a dilution of 1 : 400. Rabbit anti-human-IgG labeled with peroxidase (Sigma) was used as the secondary antibody with a working dilution of 1 : 800.

RESULTS

Observation of dengue viral proteins in infected suckling mice.

When the mouse brain suspension from each inoculated mouse was electrophoresed under denaturing conditions using 8% SDS−PAGE

and characterized by the immunoenzymatic method, positive bands could be seen as shown as an example in Fig 1. Patient 26 serum was inoculated into a litter composed of 12 suckling mice, and the brain suspension from 5 out of 12 or 41.67% of the mice demonstrated positive bands indicating replication of the infecting virus in the brain. This experiment was a blind study, so the 12 specimens were split for 2 laboratories. Our results agreed with the results from the Center for Dengue Vaccine development. Three dengue type 2 and 2 dengue type 3 were recovered from both systems. The viruses could be recovered from all 3 P–DHF (case # 23, # 26, and # 67) as shown in Table 1. The percentage of viral recovery was 41.67% (5 out of 12). It is surprising that dengue type 3 virus can be recovered from serum collected on day 18 after onset of fever. Sera from the last 2 patients (# 169 and # 171) were collected on days 24 and 22 respectively. According to the regular rule for viral isolation, sera should be collected before day 7 to obtain a high percentage recovery. Patients # 157 and 167 may not in fact represent dengue cases. Only a single specimen was taken from each case, and in normal practice they will not be included. If those 4 cases were taken out, the percentage of virus recovery would be 62.5% (5 out of 8 cases).

Liver suspension gave similar results when compared to the brain. All 5 positive cases revealed dengue antigen in the liver suspension (Fig 2). The position of proteins in liver infected

Fig 1–Analyses of dengue proteins from sera # 26 infected suckling mice. Lane 1 to lane 12 were loaded with 20 ul of homogenized brain material from 12 different suckling mice. Lanes 2, 6, 8, 10 and 12 reacted with dengue hyperimmune sera indicating the presence of dengue proteins which react specifically with the dengue antibody. Lane 2, 8 and 12 showed the highest number of protein bands and the highest color intensity or scores (+4 to Mr 59 kDa E protein, +3 to Mr 71 and 67 kDa NS3 protein, +3 to Mr 16 kDa C protein, +1 to the smaller Mr 55 and 50 kDa E protein, +1 to Mr 45 kDa NS1 protein).

Table 1

Results from the mouse inoculation/compared to and the mosquito inoculation.

Patient number	Clinical history			Diagnosis	Serotypic identification		Reciprocal HI titers
	Age	Grade	Day after onset of fever		Mouse	Mosquito	
23	1yr 3m	DF	2	P-DHF	dengue type 3	dengue type 3	< 20
26	6m	2Y	5	P-DHF	dengue type 2	dengue type 2	< 20
65	8yr	IV	6	S-DHF	dengue type 2	dengue type 2	640
67 (OPD)	10yr	I	3	P-DHF	dengue type 2	dengue type 2	< 20
149	4yr	IV	5	S-DHF	–	–	ND
152	6yr	III	4	S-DHF	–	–	320–1280
157 (OPD)	NA	NA	NA	NA	–	–	20–40
163	12yr	II	5	S-DHF	–	–	320–640
165	13yr	III	18 (CONV)	S-DHF	dengue type 3	dengue type 3	1280
167	9yr	NA	NA	NA	–	–	640–1280
169	6yr	IV	24 (CONV(	S-DHF	–	–	5120– <20480
171	4yr	III	22 (CONV)	S-DHF	–	–	320–1280

Y = with unusual clinical manifestation
CONV = convalescent sera
NA = not available
OPD = sera collected at out patient department

with sera number 26, 65, and 67 demonstrated E-protein at 59 kDa, and other antigens such as NS5, NS3, NS1 and prM could also be observed. The presence of NS3 in liver seemed to be less than in the brain, but the NS1 at Mr 45 kDa showed a stronger band. Liver infected with dengue type 3 (#26) revealed E-protein with lower molecular weight as observed with the infected brain (Mr 56 kDa). The other specimens from dengue infected mice, the plasma and spleen, gave negative results.

Serotypic identification by western blot and immunoenzymatic method

It was observed that the positions of protein bands from different strains of dengue viruses were not at the equivalent position (Fig 1). With the reference dengue prototype strains, the positive brain suspensions were compared as shown in Fig 3 : the serotypic strains of dengue viruses could be identified, using the E-proteins as markers. Dengue type 2 E-protein has Mr 59 kDa and dengue type 3 E-protein is smaller (Mr 56 kDa). The position of the E-proteins from patients numbers 26, 65 and 67 had the same Mr as that of dengue type 2 and the E-proteins from patients numbers 23 and 165 had the same Mr as that of dengue type 3.

DISCUSSION

Detection of dengue virus infection by suckling mice was a classical method which was used to detect the first dengue virus strain forty years ago. Normally, observation of neurological signs of the inoculated mice is used as a sign of virus growth in the mouse brain. When the animals were well, the investigator had to passage the brain to the next liter of mice etc until

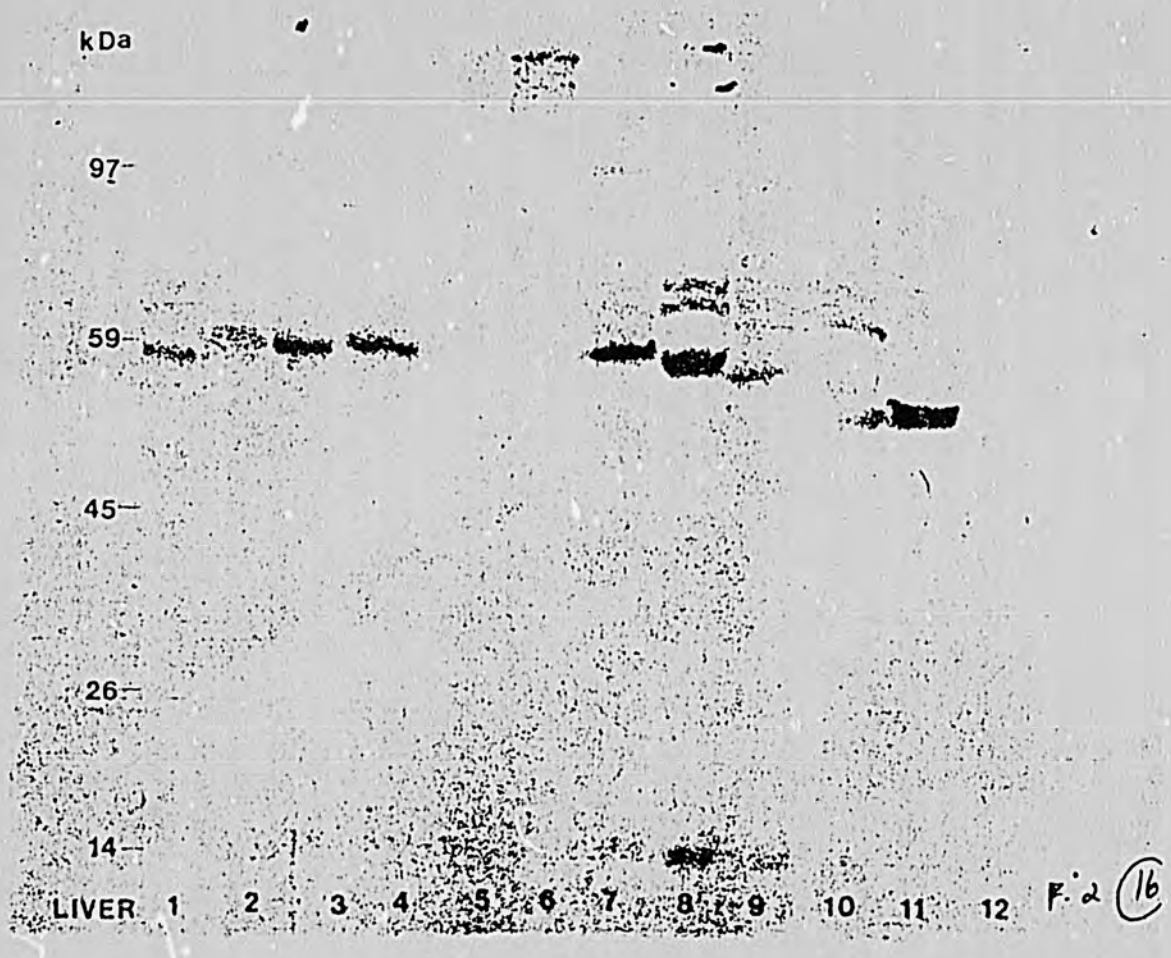

Fig 2–Analyses of dengue proteins from liver inoculated with # 23 (lane 1), # 26 (lane 2), # 65 (lane 3), # 67 (lane 4), # 149 (lane 5), # 152 (lane 6) in comparison with reference dengue viral protein D1 (lane 7), D2 (lane 8), D3 (lane 9), and D4 (lane 10) Japanese encephalitis antigen was loaded into lane 11 and the negative control (normal mouse brain) was in lane 12. The positive liver with serum 23 showed a strong E-protein band (Mr 56), NS3 protein Mr 71 and 67 kDa, NS1 protein at 45 kDa and C-protein Mr 16 kDa.

they observed signs of illness or assumed a negative result.

For this experiment, the mice were killed on day 7 without observation of neurological signs. Some of the mice demonstrated certain signs such as weakness and limping. However, none had the signs usually observed after intracerebral inoculation with 0.02 ml of the prototype strains (2×10^5 plaque-forming units) which are usually employed to propagate dengue viruses. This result indicated that the suckling mice harbored a certain number of viruses even though they did not show the signs of illness. The suckling mouse is still a good biological amplification system to propagate dengue viruses, but one must use a sensitive method to detect the presence of the multiplying virus properly. The detection system used to detect the presence of dengue viruses in the brain material can be the western blot followed by immunoenzymatic assay as reported here or other sensitive test. The whole process took 9 days to complete, a shorter period of time compared with other viral isolation and serotypic identification methods.

The presence of dengue viral antigens in liver tissue correlated well with the brain data. Thus, the presence of viral protein bands such as E, NS3, NS5, and C proteins were similar in liver and brain. At the same time, plasma and spleen specimens were negative. This evidence suggests that liver may be another site of dengue

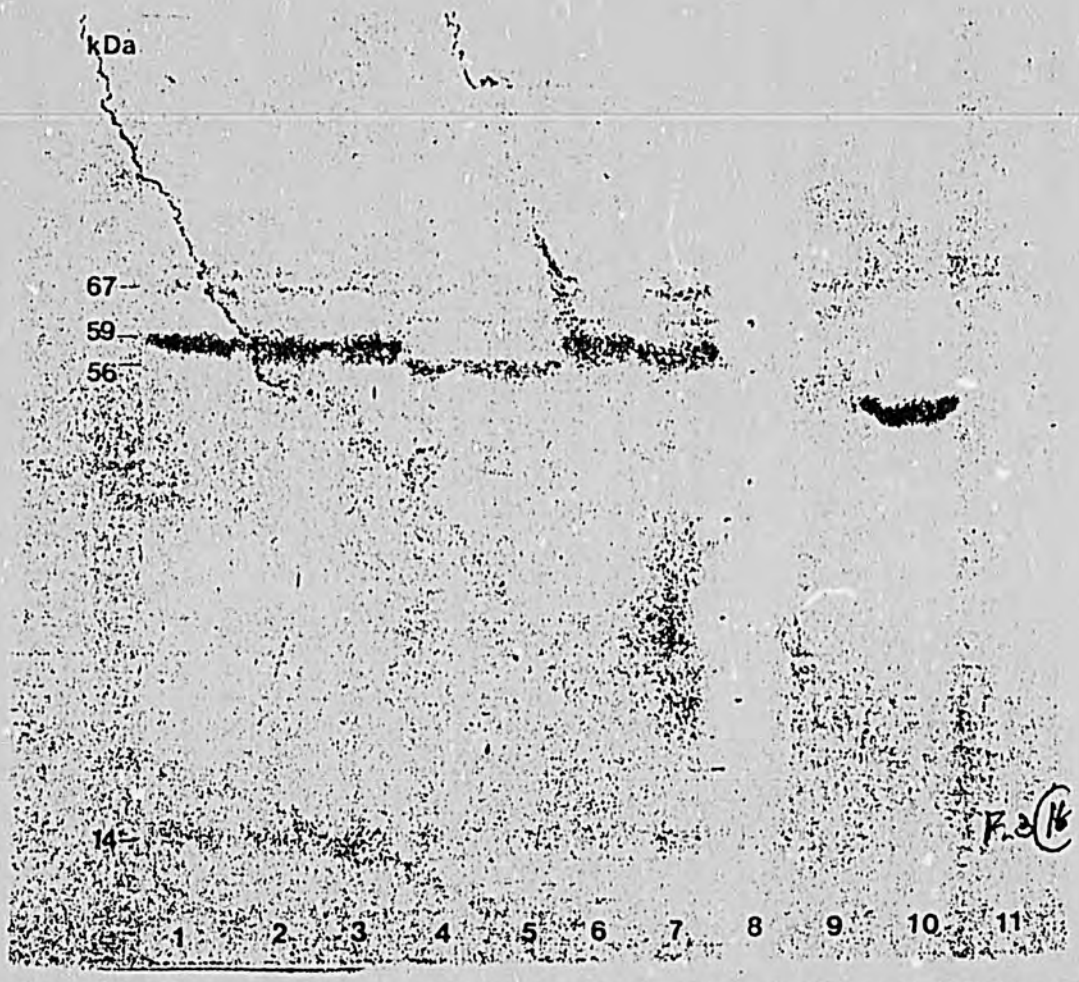

Fig 3–Analyses of dengue proteins from mouses brain inoculated with sera # 26 (lane 1), # 65 (lane 2), # 67 (lane 3), # 23 (lane 4) and # 165 (lane 5). Batteries of sucrose acetone extracted antigens; dengue type 1 (D1) in lane 6, dengue type 2 (D2) in lane 7, dengue type 3 (D3) in lane 8, dengue type 4 (D4) in lane 9 and Japanese encephalitis virus (JE) in lane 10. Normal mouse brain which was used as the negative control was loaded in lane 11. The molecular weight marker was stained with Indian ink and displayed at the right side for comparison. Lane 1, 2 and 3 demonstrated E-protein at Mr 59 kDa, comparable to the E-protein in lane 7 (D2). NS3 (Mr 71 and 67 kDa) from lanes 1, 2, 3, was also similar to NS3 in lane 7. Bands in lanes 4 and 5 demonstrated a similar mobility as the bands in lane 8 which is dengue type 3.

virus replication. In the human, most patients have enlarged livers and hepatomegaly was also found in sick mice. The presence of viral antigen in mouse liver suggests that a similar situation may occur in human infection.

ACKNOWLEDGEMENTS

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ANTIBODIES AGAINST DENGUE VIRAL POLYPEPTIDES IN PRIMARY AND SECONDARY DENGUE INFECTION

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ABSTRACT

Antibodies against dengue viral polypeptides, in dengue infected patients, were demonstrated by western blot and enzymeimmunoassay. Primary dengue cases showed a low titer of IgG class antibodies to envelope (E) proteins and 2 non-structural proteins, NS3 and NS5, in the sera collected during a convalescent phase. Secondary dengue infected patients demonstrated at least an amount of IgG antibodies to E proteins in the sera collected during an acute phase, and high titers of IgG antibodies to many polypeptides, including the NS1, NS3, NS5, and prM proteins in the sera collected during a convalescent phase. An early appearance of antibodies to E, NS3, and NS5 can be detected within 5 days after the onset of fever. These 3 dengue viral proteins may be applicable for further use as an immunodiagnostic reagent.

Keywords : Dengue polypeptides, Dengue viral proteins, Antibodies to Dengue polypeptides, IgG anti-dengue polypeptides, Antibodies to Dengue structural and nonstructural proteins.

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INTRODUCTION

The Flavivirus genus, family Flaviviridae, consists of a group of 70 closely related human or veterinary pathogens including the dengue virus. Infection caused by dengue viruses, type 1 to type 4, can result in a spectrum of ailments ranging from a mild form of illness to a severe one such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). These severe manifestations of dengue infections mostly occur in children and young adults who have a secondary infection, but cases have also been reported those with a primary infection (P-DHF) in infants of less than one year old (1).

The hypothesis for pathogenetic mechanism of this disease involves both the quantitative and qualitative aspects of the immune responses within the individual host. However many tests were employed to quantitate dengue antibodies, none of them could differentiate the antibodies at the polypeptide level.

We have used the application of polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) (2), western blot (3), and enzymeimmunoassay to detect dengue viral polypeptides. This application was called Viral-antigen-strips/enzymeimmunoassay (VAS/EIA) (4). Each viral-antigen-strip could be employed to detect all 4 prototype strains of dengue polypeptides for a comparison. This application covers the use of VAS/EIA to detect IgM and IgG antibodies to dengue viruses in the sera of clinically and serology confirmed dengue infected patients. The immunogenicity of each polypeptide , to elicit immune responses in these 2 groups of patients, are presented.

MATERIALS AND METHODS

Test Sera

Sera from 150 dengue infected patients were collected at Ramathibodi Hospital during the year of 1987. They were then clinically graded according to WHO recommendations (5), and classified as primary (P-DHF) or secondary (S-DHF) dengue infection using Winter's criteria (6). Due to rarity of so many P-DHF cases, all 28 cases were included in these studies, plus another randomly selected 30 cases from the S-DHF group for comparison.

Western Blot and Enzymeimmunoassay for Detection of Dengue Viral Polypeptides.

In order to detect the IgM and IgG antibodies to dengue viral polypeptides, 8% polyacrylamide gel electrophoresis (PAGE) was used to separate dengue viral proteins, then these proteins were transferred to the surface of a nitrocellulose membrane by the Western blot method. Dengue type 1 to type 4, sucrose acetone extracted antigens, and a mouse brain, used as a negative control were included in one strip to compare the pattern of immune responses for each tested sera (Viral-antigen-strips, VAS). After incubated with the specimens to be tested, each VAS were incubated with peroxidase labeled anti-human IgM or IgG. Hydrogenperoxide was used as an enzyme and the 3-amino-9-ethylcarbozole (AEC) was used as a chromogen. The sensitivity and specificity for detecting dengue proteins by these protocols were reported (4). Sera from the P-DHF were diluted to 1:20 and from the S-DHF were diluted to 1:50 before being tested. The scoring of color intensities was done for comparison (7).

RESULTS AND DISCUSSIONS

Antibodies Detected in Primary Dengue Infection (P-DHF)

During the first 6 months of this study (July-December, 1987), endemic outbreaks in Thailand enabled us to collect 28 cases of primary dengue infection from Ramathibodi Hospital. There were 8 severely ill patients with a clinical grade of III and IV. The IgM antibody to dengue is very hard to detect. Only a faint color band (1+), against NS3 and NS5 proteins, could be seen in a few cases. IgG antibodies could be demonstrated in 21 subjects which accounted for 75% of the cases. All 7 negative cases were non-severely ill patients who had a shorter hospital stay, therefore the antibody response might not have been high enough to be detected at the time of sera collection. However from the 21 positive subjects, IgG antibodies could be demonstrated from the sera collected during an acute phase of 3 patients. Two out of these three cases were infants of ages 6 and 10 months old. The first IgG antibodies were formed against the Envelope (E) protein with the highest intensity of a positive color towards the infected dengue type (3+) (Fig 1.) The second antibodies which became visible by this protocol was the NS3 protein. This protein is apparently composed of 2 sizes of molecular weight at 71,000 and 67,000 daltons. The next protein to show itself was the NS5 at a M.W. of 97,000. This antibody could be seen prominently against a dengue type 1 and dengue type 3. Eventhough the isolation was successful in only 7 cases, the immune responses towards the infected type of dengue viruses were higher. The intensity of color was stronger and more bands were seen, indicating a higher immune response towards the homologous antigen. The patterns of antibody development toward each polypeptide were similar in the severely ill as well as the non-severely ill group.

Antibodies Detected in a Secondary Dengue Infection (S-DHF).

As found with other serology tests, antibodies in sera collected from S-DHF varies in levels of antibody responses. Some cases had low titers (1+), but others had remarkably high (4+) titers. When the sera were diluted to 1:50, seroconversion between sera collected during acute and convalescent phases could be clearly observed in all cases. The antibody responses in S-DHF were found against many polypeptides and are listed as follows :

1. Structural proteins : Envelope (E) polypeptides at molecular weight (M.W.) of 59,000 daltons, Pre-matrix (prM) polypeptide at M.W. of 20,000 daltons, and Core (C)protein at a M.W. of 14,000 daltons.

2. The non-structural proteins : NS1 at a M.W. of 45,000 daltons, NS3 at 2 M.W. of 71,000 and 67,000 daltons, NS4a at a M.W. of 30,000 daltons and NS5 at a M.W. of 97,000 daltons. Besides the antibodies to those mentioned polypeptides, there were antibodies found against polypeptides at a M.W. of 55,000 and 50,000 daltons. These 2 proteins may be the envelope proteins with a smaller molecular weight, but the monoclonal antibody against E protein was found to be non-reactive. Therefore, some of these detected proteins remain to be prove in order to confirm the exact type of these polypeptides. The only two confirmed polypeptides are the E-protein at a M.W. of 59.000 daltons and the NS1 at a M.W. of 45,000 daltons, and then by using monoclonal antibodies specific to these 2 proteins (8,9). The example from S-DHF case is illustrated in Fig. 2.

An advantage of the VAS/EIA method is that it can quantitate as well as qualitate the antibody responses towards the viral non-structural proteins as well as the structural proteins. Detection of IgM antibody to dengue proteins revealed only low titers in a few cases. In the S-DHF, a competitive binding of the IgG antibody to the same polypeptide may have occurred and led to negative result. In the P-DHF while a high titer of IgM antibodies can be detected by an IgM captured antibody enzyme linked immunoassay (MAC-ELISA) which was performed simultaneously in our laboratory, the IgM antibodies to dengue polypeptide were hardly detected. Only a low titer to certain non-structural proteins, NS3 and NS5, could be seen. Therefore, the western blot may not be appropriate to detect IgM antibodies. Detection of IgG antibodies to dengue proteins gave more information both quantitatively and qualitatively. Sera collected before the patients left hospital were good enough to use as pair sera to observe seroconversion in S-DHF (2-3 days after hospital admission). For P-DHF, pair sera should be collected at least 10-15 days after hospital admission. In order to observe seroconversion in both P-DHF and S-DHF, pair sera should be collected between 10-15 days after the first blood sampling. This test can then be used for diagnostic purposes, but can only be done by trained technicians at high cost. For research purposes, this test can yield detailed information at the molecular level. Preliminary results indicated an early immune responses toward NS3 and NS5 as well as the E proteins. These 2 non-structural proteins must therefore be good immunogens, while specific antibodies could probably be raised in laboratory animals. During an acute phase, these viral proteins (E, NS3, and NS5) are probably present to elicit an early immune response. We are now preparing a specific antibody using these 2 non-structural proteins, and hope to be able to detect the viral proteins which may circulate within the plasma stream during an acute infection. Antibodies to envelope protein, which appears to be the most immunogenic polypeptide, elicit the first immune response with a long lasting activity. Antibodies to envelope protein was found in all S-DHF sera collected during the admission period and also in every case of the healthy adult population who had the antibody to dengue virus.

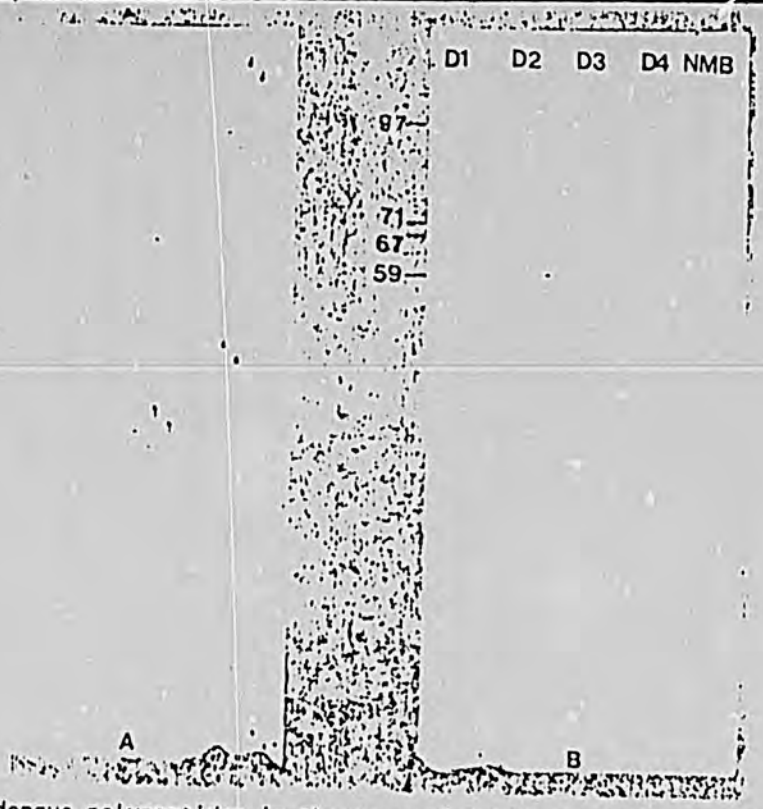

Fig 1. Antibodies to dengue polypeptides in the sera of P-DHF. A. Negative antibodies were found on the third day of the illness (Acute sera). B. Antibodies on day 13 (During the convalescent stage) demonstrated antibodies to E-protein (59kd), NS3 (71, and 67 kd) and NS5 (97 kd). Dengue type 2 virus was isolated from the acute sera.

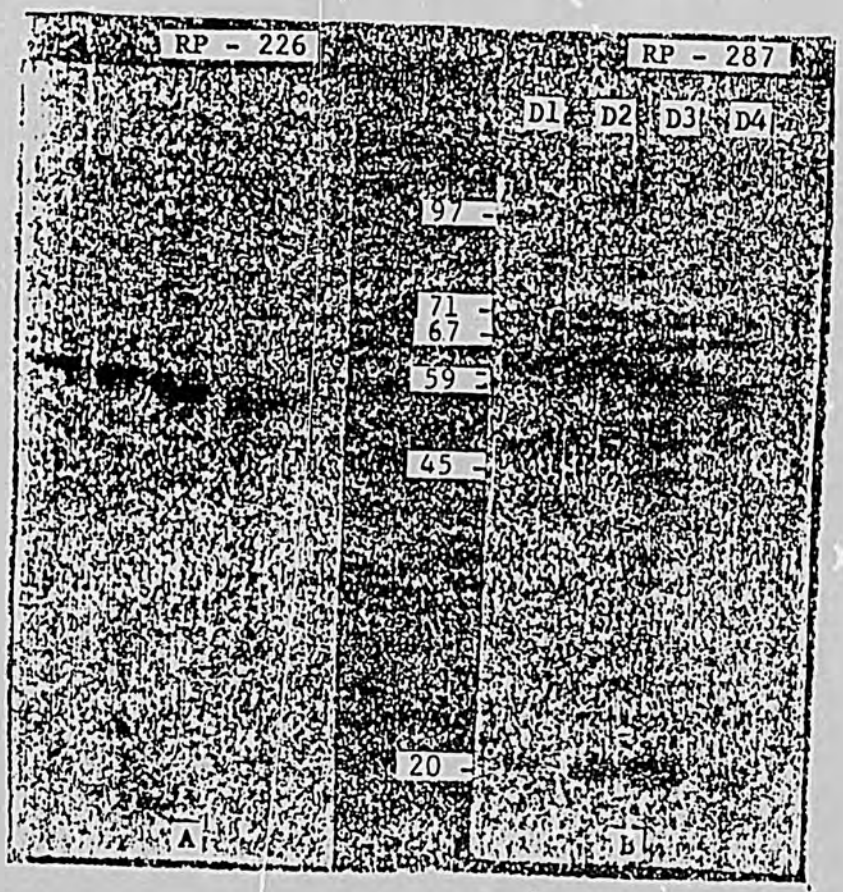

Fig 2. Antibodies to dengue type 1 to type 4 (D1, D2, D3 and D4) in S-DHF. A. In an acute specimen, antibodies were demonstrated against E (59 kd), NS3 (71 and 67kd), NS5 (97 kd), prM (20 kd), and at M.W. of 55 and 50 kd. B. In the convalescent stage, antibodies were found against E, NS3, NS5, prM, at M.W. of 55 kd, 50 kd, and NS1 at M.W. of 45 kd.

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ANTIBODIES AGAINST DENGUE VIRAL PROTEINS IN PRIMARY AND SECONDARY DENGUE INFECTION

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ABSTRACT

Antibodies against dengue viral proteins were demonstrated in sera from dengue infected patients by polyacrylamide gel electrophoresis, western blot, and enzyme immunoassay. Primary dengue cases showed low titers of IgG class antibodies to envelope (E) proteins and 2 non-structural proteins, NS3 and NS5, in sera collected during convalescent phase. Secondary dengue infected patients always demonstrated IgG antibodies to E proteins in sera collected during the acute phase, and high titers of IgG antibodies to many other proteins, including NS1, NS3, NS5, and C proteins in sera collected during the convalescent phase. Appearance of antibodies to E, NS3, and NS5 could be detected within 5 days after the onset of fever. These 3 dengue viral proteins and their corresponding antibodies may be involved in the immunopathologic mechanism underlying this disease. For diagnostic purposes, the non-structural proteins such as NS3 and NS5 may be the best target proteins for early confirmation of the disease by identifying the presence of these 2 proteins.

INTRODUCTION

The Flavivirus genus, family Flaviviridae, consists of a group of 70 closely related human or veterinary pathogens including dengue viruses. Infection caused by dengue viruses, types 1 to 4, can result in a spectrum of disease ranging from a mild illness to the severe patterns of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The latter mostly occur in children and young adults who have a secondary infection, but cases have also been reported in individuals with a primary infection (P-DHF) in infants of less than one year old[1]. Hypotheses of pathogenetic mechanisms underlying this disease involve both quantitative and qualitative aspects of the immune responses within the individual host. Many tests have been employed to quantitate dengue antibodies, however none of them have differentiated the antibody responses at the protein level. We have used a combination of polyacrylamide gel electrophoresis (SDS-PAGE)[2], western blot[3] and enzyme immunoassay to detect dengue viral proteins. This application was called viral-antigen-strips/ enzymeimmunoassay (VAS/EIA)[4]. Each viral-antigen-strip can be employed to detect all 4 prototype strains of dengue proteins for comparison. Here we report the use of VAS/EIA to detect IgM and IgG antibodies to dengue viruses in the sera of clinically and serologically confirmed

dengue infected patients. Data are presented on the immunogenicity of each type of viral protein in eliciting immune responses in these 2 groups of patients.

MATERIALS AND METHODS

Test sera

Sera from 150 clinically confirmed dengue infected patients were collected at Ramathibodi Hospital during the year 1987, clinically graded according to WHO recommendations[5], and classified as primary (P-DHF) or secondary (S-DHF) dengue infection using Winter's criteria[6].

Viruses and viral antigen

The viruses employed in this study were dengue type 1 (Hawaii M199), dengue type 2 (New Guinea C), dengue type 3 (H-87), dengue type 4 (H-241), Japanese encephalitis (Nakayama) and Chikungunya. Dengue viral antigens were raised by virus infection in suckling mouse brain. Sucrose acetone extraction of these antigens was performed as previously described[7]. They were titrated by hemagglutination (HA) test, and were adjusted to have an HA titer of 1:1280 before use.

Antisera

Monoclonal antibodies were raised from hybridoma cell lines purchased from the American Type Culture Collection (ATCC), Washington DC., by conventional

methods[8,9]. These monoclonal antibodies have specific activities toward the E-proteins of their corresponding dengue virus types. Peroxidase labeled antisera to human IgG and IgM were obtained from Sigma

Serology and virology

Serological diagnosis of dengue infection was done by hemagglutination-inhibition test[7] and by isotype antibody capture enzyme-linked immunosorbent assay for specific dengue virus IgM (MAC-ELISA) and IgG (GAC-ELISA) antibodies[10,11]. For the HI test, a micro-modification method was used[10]. Sera were extracted by acetone before testing. Dengue viral antigen for all four prototype strains of dengue type 1 to 4[12], Japanese encephalitis (JE) and chikungunya (CHIK) were prepared by the sucrose acetone method[10].

Viral isolation and the identification of the recovered viruses were done as a courtesy by Dr. Bruce Innis and Dr. Ananda Nisalak of the Armed Forces Research Institute of in Medical Sciences, Bangkok, and Dr. Sutee Yoksan, the WHO Collaborating Center for Dengue Vaccine Development Bangkok. Sera collected on the first hospital day were stored at -70°C until the time of assay. Sera were diluted at the ratio of 1:5 with RPMI-1640 media (Gibco), and inoculated into adult mosquitos,

Toxorynchites splendens[13]. Monoclonal antibodies which had been prepared and analysed[8,9] were employed to identify prototype virus strains and for identification of the envelope protein.

Polyacrylamide gel electrophoresis, western blot and enzymeimmunoassay (EIA) for characterization of antibodies to dengue viral proteins.

In order to detect the IgM and IgG antibodies to dengue viral polypeptides, 8% polyacrylamide gel electrophoresis (PAGE) in presence of sodium dodecyl sulfate (SDS) without any reducing agent was used to separate dengue viral proteins[2], and then these separated proteins were transferred to the surface of a nitrocellulose membrane by the western blot method[3]. Dengue type 1 to 4, sucrose acetone extracted antigens, and a mouse brain as a negative control were included in one strip to compare the pattern of immune responses for each tested serum by EIA. The sensitivity and specificity were previously reported[4] and the same protocol was employed. Sera were diluted with PBS 1:5 (for P-DHF) and 1:20 (for S-DHF) before testing. The scoring of color intensities was done in order to compare antibodies titers[14] as follows : +1 for barely detected or faint pink color, +2 for clearly positive reaction (sharp bands with

pink color), +3 for strongly positive (red), and +4 for very strong positive reaction or dark red color.

Monoclonal antibodies (MAb) to envelope proteins of dengue viruses (hybridomas 3H5-1 for dengue type 2 and 1H10-6 for dengue type 4) were used to locate E proteins on nitrocellulose membranes together with molecular weight markers (MW-SDS-70L and MW-SDS-200 kits, Sigma). Dengue viral protein nomenclature was used according to Rice et al.[15] Briefly, dengue type 2 protein with Mr 59 kDa or molecular weight approximately 59,000 daltons which specifically reacts with MAb to dengue type 2 virus was called the antibody to E or envelope protein. The antibody bound to the Mr 97 kDa protein was called the antibody to the nonstructural protein NS5. The antibodies to a pair of protein bands at Mr 71 and 67 kDa were cited as the antibodies to NS3 protein. The antibody to the Mr 45 kDa protein was called the antibody to NS3 protein, and the antibody to the Mr 16 kDa protein was called as the antibody to C or core protein.

RESULTS

Clinical observations

Out of 150 cases studied, 27 were confirmed as P-DHF. A group of 108 patients were cases of S-DHF but the other 15 cases could not classified be due to insufficient specimens or laboratory data. The 27 primary cases could be separated into 3 groups as follows :

1. DSS (DHF grade III and IV)	8	cases
2. DHF grade I and II	15	cases
3. Classical dengue fever (DF)	4	cases

The 108 secondary cases were graded according to the severity of illness as follows :

1. Grade IV	4	cases
2. Grade III	45	cases
3. Grade II	28	cases
4. Grade I	19	cases
5. DF	8	cases
6. Unable to classify	4	cases

Viral Isolation

From 27 primary cases, 14 plasma samples were sent for viral isolation and the viruses from 7 out of 9 positive cases were identified as dengue type 3. The remaining 2 cases were identified as dengue type 1 and

type 2 respectively. From the 8 severely ill (Grade III and IV) P-DHF, three dengue type 3 and one dengue type 2 viruses were isolated from 5 cases in which virological studies were done (Table 1). The high prevalence of dengue type 3 isolates in this series reflects the dominance of type 3 viruses in the epidemic in the year 1987[16].

From 108 S-DHF cases, 60 sera collected during the acute phase were sent for viral isolation and viruses were isolated from 10 cases. There were 6 cases of dengue type 2 ; 3 cases of dengue type 3, and only one case of dengue type 1 (Table 2).

ELISA and HI test

MAC-ELISA for the detection of IgM antibodies to dengue viruses were positive during the acute phase in all except one DSS/P-DHF case of severely ill P-DHF. Higher MAC-ELISA values were observed in convalescent phase sera (Table 1). On the contrary, GAC-ELISA revealed no IgG antibodies in sera collected during the acute phase but gradual rising antibody titers could be seen towards the convalesent phase.

Most S-DHF cases showed positive GAG-ELISA or HI titers during the acute phase, while higher titers in the convalescent phase could be observed in all cases (Table 2).

Identification of dengue proteins

In order to identify the position of dengue virus envelope proteins, MAb to dengue type 2 and type 4 envelope proteins were used[12,13] (Fig 1). Using the position of the envelope protein and reference molecular weight markers as standards, the Mr of each dengue viral protein recognized by antibody was estimated and the protein identified according to the new nomenclature.[15] The MAb to dengue type 2 revealed that E protein of dengue type 2 had Mr 59-60 kDa, and the MAb to E protein of dengue type 4 indicated that E protein of Dengue type 4 had Mr 52-54 kDa. The differences in Mr of E proteins of the different dengue strains in our study agreed with a previous report.[17]

Antibodies detected in primary dengue infection

During the first 6 months of this study (July-December, 1987), an epidemic outbreak in Thailand enabled us to collect 27 cases of primary dengue infection from Ramathibodi Hospital. IgM antibody to dengue was rarely and inconsistently detected by VAS/EIA: only a faint color band (+1), against NS3 and NS5 proteins, could be seen in a few cases. IgG antibodies could be demonstrated in 21 (75%) of the cases. All 7 negative cases were non-severely ill patients who had a shorter hospital stay, therefore the antibody response might not have been high enough to

be detected at the time of the serum collection. Among the 21 positive subjects, IgG antibodies could be demonstrated in sera collected during the acute phase in 3 patients. Two out of these three cases were infants aged 6 and 10 months respectively. The first IgG antibody was detected on day 3 of illness against the E protein with the +1 score towards the infecting dengue type (Fig 2; case 1 in Table 1). The antibodies which next became visible by this protocol were against the NS3 protein. This protein is apparently composed of a doublet of Mr 71 and 67 kDa (demonstrated by rabbit polyclonal antibodies to this polypeptide, data not shown). The next antibody detected was against NS5 of Mr 97 kDa. This antibody was prominent in D1 and D3 cases. Even though virus isolation was successful in only 7 cases, the immune responses towards the infecting type of dengue virus were higher than cross-reactions with other dengue serotypes. The intensity of color was stronger and a greater number of positive bands could be seen, indicating a higher immune response towards the homologous antigen. The patterns of antibody development toward each polypeptide were similar in the severely ill and the non-severely ill groups.

Antibodies detected in a secondary dengue infection

As found with other serological tests, the levels of antibodies in the sera collected from S-DHF during the acute stage varied (+1 to +4). When the sera were diluted

1:50, higher intensity of positive bands between sera collected during acute and convalescent phases could be clearly observed in all cases. Antibodies to dengue proteins in 10 cases of S-DHF with confirmed type of infecting virus are shown in Table 2 and examples of 2 cases are demonstrated in Fig 3. Antibody responses in S-DHF were found against many proteins and can be summarized as follows :

1. Structural proteins : antibodies to the E protein of Mr 59 kDa, to the core (C) protein of Mr 16 kDa, and to the pre-matrix (prM) protein of Mr 23 kDa could be detected but antibodies to the matrix (M) protein of Mr 8.5 kDa could only be seen in a few cases when high scores of antibodies to other proteins were observed.

2. Non-structural proteins : The antibodies to NS1 of Mr 45 kDa, NS3 of Mr 71 and 67 kDa, and NS5 of Mr 97 kDa could be observed.

Placing all 4 types of dengue viruses in the row in one strip as shown in Fig 3 (Lane 1,2,3, and 4), the molecular weight of E-protein from each dengue type moved differently. E-protein of dengue type 1 was at Mr 60-61 kDa, while the E protein of dengue type 2 was at Mr 59-60, of dengue type 3 was at Mr 54-56, and of dengue type 4 was at Mr 52-54. In addition, antibodies to the smaller molecular weight proteins for example in case of dengue type 2 were found against at Mr 55 and 50 kDa. These two

additional proteins are probably the envelope proteins of smaller molecular weight, but the MAb against E protein did not react with these two bands. Exact identification of some of these detected proteins, especially the C protein of Mr 16 kDa and the prM protein of Mr 23 kDa, remains to be confirmed by specific antibody which was not available at the time of this study. This confirmation is needed because of the molecular weight similarity of NS4a and NS4b to the C and prM proteins. Monoclonal antibody to the NS1 protein used in a separate experiment in our laboratory confirmed that the protein with Mr 45 kDa was NS1.

DISCUSSION

An advantage of the combination of PAGE, western blot, and enzymeimmunoassay for the characterization of antibodies to dengue polypeptides is that it can quantitate as well as qualitate the antibody responses towards the viral non-structural proteins as well as the structural proteins. Detection of IgM antibody to dengue proteins revealed only low titers in a few cases. In S-DHF, competitive binding of the IgG antibody to the same polypeptide may have occurred and led to a negative result. However, in P-DHF while a high titer of IgM antibodies could be detected by MAC-ELISA IgM antibodies to dengue polypeptides were still hardly detected. Only low titers to certain non-structural proteins, NS3 and NS5, could be seen. Therefore, this test may not be appropriate to detect IgM antibodies.

Detection of IgG antibodies to dengue proteins gave more information both quantitatively and qualitatively. Sera collected before the patients left the hospital were good enough to use as paired sera to observe seroconversion in S-DHF (2-3 days after hospital admission). However, another serum sample should be collected at least 10-15 days after hospital admission for laboratory confirmation. This test actually can be used for diagnostic purposes, but can only be done by trained technicians and it is also costly to perform. For research purposes, this test can yield detailed information at the

molecular level, and this information is important concerning possible involvement of antibody to each protein in pathogenesis of the severe syndrome. Preliminary results indicated that immune responses toward NS3 and NS5 could be observed as early as the immune response toward E protein. These 2 non-structural proteins evidently have good immunogenic properties. During the acute phase, these viral proteins (E, NS3, and NS5) must be expressed since they elicit an early immune response. E protein, which appears to be the most immunogenic polypeptide, elicits the first immune response in primary infection with the longest lasting activity. In S-DHF, antibody to E protein was always demonstrated in sera collected during the acute stage of illness. Since dengue shock syndrome has been postulated to be induced by antibody molecules which complex with specific dengue viral protein(s), the quantitation and qualitation of E-protein and its antibody should be done, since it may contribute to the severe form of illness by such a mechanisn. Studies along these lines may lead to a better understanding of the pathogenesis of this disease.

An other important finding is the early and strong immune response to NS3 and NS5 proteins. These 2 proteins may be good as target proteins to be detected by specific antibody for rapid diagnosis, since we observed their corresponding antibodies appeared rapidly in the course of the natural infection. With a sensitive method, the

chance to detect and quantitate these 2 antigens with their specific antibodies appears to be good, and could provide the basis for development of an improved rapid diagnostic assay.

Acknowledgement

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LEGENDS FOR THE ILLUSTRATION

Fig 1. A. Monoclonal antibody from 1 H5-1 hybridoma reacts with E-protein of dengue type 2 in lane 2 (Mr 59 kDa). B. Monoclonal antibody from 1H10-6 hybridoma E-protein of dengue type 4 in lane 4 (Mr 52-54 kDa).

Fig 2. Antibodies to dengue proteins in the sera of P-DHF. A. Antibody (acute sera) to E protein (+1 for D2, and +1 with more pale band for D3) was found on the third day of the illness (Lane 2 and 3). B. Antibodies (convalescent sera) were demonstrated on day 13 against E-protein (Mr 59 kDa), NS3 (Mr 71 and 67 kDa) and NS5 (Mr 97 kDa) (Lane 2). Dengue type 2 virus was isolated from this patient serum collected during the acute phase of illness (patient #1 on Table 1).

Fig 3. The antibody responses in 2 cases of S-DHF. A. Case 1: Low titers of antibodies in acute phase (left) compared to the higher titers in the 2nd specimen (middle) and convalescent phase (far right). B. Case 2: High titers of antibodies were found in the acute phase (left side) and in the convalescent phase (right side). This case is the 7 years old boy listed in Table 2 (fourth patient). Dengue type 2 virus was isolated from sera collected on day 4.

PSTC Research Preproposal FY 1991

1. Project Title : Development and application of a rapid, accurate, and sensitive test for dengue viral infection.
2. PSTC research module
   Biotechnology/Immunology (Module # 1)
3. Investigators :
   1. Vina Churdboonchart, Ph.D. (Pathobiology); Principal investigator
   2. Rungtiva Kongkananda DVM, M.Sc.
   3. Kanchana Sirisidhi, B.Sc.
   4. Amphorn Chaiyotha, B.Sc.
   5. Maliwan Sawatichuto, B.Sc.

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4. Project objective :
   To employ immunological and biochemical technologies to detect dengue viral antigen and genome.
5. Project duration : 4 Years
   Starting date : Jan 1992 or as soon as possible
6. Research rationale and workplan:

6.A. Scientific Antecedents

Dengue infection is caused by 4 distinct types of dengue viruses, type 1 to type 4, which belong to the Togaviridae family, and flavivirus genus (1). It is known that a primary infection of one type will not protect an individual against other types. On top of that, the pre-existing antibody has been considered as a risk factor for the development of hemorrhage and shock syndrome. However, shock syndrome may occur in both primary (P-DHF) and secondary dengue (S-DHF) infection with higher frequency rate in S-DHF (2, 3). Since most of the Thai adult population possesses dengue antibodies without realizing it, dengue infections in certain people may not lead to any noticeable symptoms.

Dengue viruses are enveloped, RNA viruses, in the flavivirus group. Altogether there are 4 different types of dengue-viruses and within each virus type there are sub-type variations which were clearly demonstrated by its nucleic acid sequences (4). Antigenicities of dengue viruses are closely related and display a closer relationship among them than the other viruses in the same sub-group as demonstrated by hemagglutination inhibition test (HI) (5), or other serological tests (6,7,8).

Dengue viruses have at least 3 structural proteins (E, M,and C) and 5 non-structural proteins (NS1, NS2, NS3, NS4, and NS5) (9). With the western blot and emzymeimmunoassay (10), we found that sodium dodecyl sulfate solubilized dengue antigens without a reducing agent can be used to detect circulating antibodies to dengue proteins. Human hyperimmune sera have strong activities toward many viral proteins; E, PrM, C, NS1, NS2, NS3, NS4, and NS5. When 2-mercaptoethanol was used as a reducing agent, the antibodies towards protein at Mr of 85,000 or the dimeric form of NS1 can be demonstrated.

In P-DHF, IgG class antibodies were found reacting to E, NS3, and NS5 in sera collected during the convalescent phase. Secondary infected patients who possessed existing antibodies in acute sera, showed a higher immune responses toward every polypeptides in the convalescent phase (11). E, NS3 and NS5 proteins may serve as a marker proteins to develop a diagnostic test, since the antibodies against these proteins appeared early in natural infections and the antibody to NS3 protein showing type specificity (12).

The nucleotide sequences of dengue type 1 (13), dengue type 2 (14), and dengue type 4 (15) are partly or almost completely available. Nucleic acid probes were developed and employed to detect viral gene in blood sample (16), and later the Polymerase chain reaction (PCR), an in vitro gene amplification, was applied to identify dengue serotypes (17). Detection of dengue viral gene in patients' specimens is promissing by this technique, however field trial should be done in order to search for the replication site, duration of viremia, type and subtype of the viruses associate with the severity of diseases in man.

B. Research rationale

Dengue hemorrhagic fever/dengue shock syndrome as one of a major problem of public health causes epidemics in many parts of the world with findings of new variants and serotypes. Rapid and sensitive characterization of the virus is needed to understand the epidemiology and molecular aspects of the disease. Our laboratory is well equipped to detect viral genome in various type of specimens and capable to use immunochemical methods to detect and characterize dengue antibodies. There is also a possibilily to detect and characterized dengue antigens in our laboratory. All mentioned studies can be done with the same specimens, and the results may lead to be better understanding of the pathogenesis of the severe syndrome occur in certain population, and may be useful for diagnostic as well.

6.B. Workplan

a. Preparation and collection of infected dengue virion
 a.1 infected cell culture e.g. mosquito cell line C6/36, or Monkey kidney cell line (MK2) (duration : 3 months)
 a.2 infected animal e.g. suckling mouse (duration : 3 months)
 a.3 collection of specimens from dengue infected patients
b. Extraction of dengue viral genome (duration 3 months)
 b.1 extraction from aqueous type specimens (sera, plasma)
 b.2 extraction from tissue type specimens (liver, brain, spleen, lymph nodes)
c. Preparation of primers and antisera
 c.1 preparation of primers (3 months)
 c.2 preparation of specific antisera with the viral proteins prepared by genetic engineering (2 years)
d. Detection of dengue genome by polymerase chain reaction (PCR)
 d.1 in infected cell lines (3 months)
 d.2 in infected animals (6 months)
 d.3 in patients specimens (3 year)
e. Detection of circulating dengue viral antigens by rapid, sensitive and laboratory-free technique such as the use of monoclonal antibody coated on red blood cells for rapid detection or other technique available during the next 3 years. (3 years)
f. Evaluation of the presence of viral genomes, viral proteins, and antibodies in patients' sera.

7. Innovation

7.1. The viral isolation technique for dengue viruses are time consuming, and expensive, and the yield is also low. In situ hybridization is not so sensitive and probably due to the minute presence of the viral gene in detected specimens. At the same time, many pyrexia of unknown origin (PUO) patients were overlooked or lack of confirmation. Therefore, the methods for accurate diagnosis using single specimens is needed.

7.2. With the sensitive methods, the presence of dengue viral genomes and antigens may be revealed. Complete study indicate duration, site, quantity and type of virion are needed in order to set an accurate program for laboratory confirmation.

8. The disease has tendency to spread to other countries besides those in the tropical areas of South-east Asia and Western Pacific region. The countries in tropical area in Africa and America were also at risk.

9. Collaborative arrangements can be arranged with
   1. Dr. Leon Rosen, M.D., Dr. P.H., Director, Arbovirus Program, Pacific Biomedical Research Center, University of Hawaii, Leahi Hospital, 3675 Kilauea Ave., Honolulu, Hawaii 96816, U.S.A.
   - Dr. Rosen has a collection of dengue viruses and patients sera which can be shipped for our collaborative study. He is a well known virologist in dengue field.
   2. Dr. Robert E. Shope, Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, 60 Collage street, P.O.Box 3333, New Haven CT 06510-8034
10. Budget (US$144,000.00) 4 years .

Item	Year 1	Year 2	Year 3	Year 4
Salaries	12,000	12,000	12,000	6,000
Equipment/Materials (CO2 incubator, Electrophoretic apparatus Tissue homogenizer, Reagents and materials etc)	41,000	12,000	12,000	6,000
Travel	5,000	5,000	-	-
Training	-	-	-	-
Overhead	-	-	-	-
Other cost (bookeeping, local transportation, postage, secretarial works, photo, copying, text books etc)	6,000	6,000	6,000	3,000
Total	64,000	35,000	30,000	15,000
			Grand total	$144,000

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Dec 6, 1990