

# THE ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES: FIVE DECADES OF COLLABORATIVE MEDICAL RESEARCH

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**Abstract.** The Armed Forces Research Institute of Medical Sciences (AFRIMS) is a 50-year-old joint institute of the US and Royal Thai Army Medical Departments located in Bangkok, Thailand. Investigators from the Institute have carried out research in Thailand and the region, in collaboration with many partners, focused on a large number of tropical infectious diseases. In celebration of the 50<sup>th</sup> anniversary, this paper summarizes highlights of this research, focusing on malaria, Japanese encephalitis, dengue, diarrhea and HIV. In addition, research done in support of the medical problems of refugees and of the health of Thai peace-keeping forces are summarized. The research carried out by AFRIMS and added to the scientific literature has contributed significantly to advancement in multiple areas of tropical infectious disease.

**Keywords:** Army, research, tropical disease, Thailand

## INTRODUCTION

In December 1960, letters of agreement were exchanged between United States Department of State and the Thai Ministry of Foreign Affairs providing the political basis for a joint medical research laboratory in Thailand between the US and Royal Thai Army Medical Departments. This laboratory was known as the 'SEATO' Laboratory up until the late 1970s when the Southeast Asia Treaty

Organization was disestablished. The laboratory was renamed in 1977 as the Armed Forces Research Institute of Medical Sciences or 'AFRIMS'.

As AFRIMS celebrates its 50th anniversary of collaborative research on tropical infectious diseases, this article provides a summary of highlights from articles published during this half century.

## MATERIALS AND METHODS

Research at AFRIMS through these 50 years, originally stimulated by spread of the cholera pandemic to Thailand, has remained focused on tropical infectious diseases. In this paper, research highlights, drawn from published reports, are summarized. Selection of articles was generally based upon their having been cited more than 100 times in subsequent

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publications. In the case of recent papers, publication in high impact (score > 4) journals was considered as an alternate criterion. Though the spectrum of infectious disease research at AFRIMS has been wide, here summaries are limited to infections which have been major and/or continued foci of the Institute's research: malaria, Japanese encephalitis, dengue, diarrhea and HIV. Nearly all the research efforts were done as collaborations, between the two Components of AFRIMS and/or with local or regional partners, that is government ministries of health, military medical departments or health-related NGOs. In addition, a section is included of research and screening done in support of refugee populations and UN peacekeeping forces. These latter efforts were usually initiated in response to requests from the responsible health authority or NGO.

## RESULTS

### Malaria

Malaria has been the leading focus of AFRIMS research through the Institute's 50-year history, and has resulted in more than 500 publications (Fig 1). This emphasis is consistent with repeated assessments that malaria is the leading infectious disease threat to deployments of the US Military (Burnette *et al*, 2008), as well as being an endemic threat to Thailand in many border areas. Citations of the Institute's 88 malaria research publications during the first 15 years are provided in a bibliographic article by the eighth commander of the US Component (Segal, 1978).

The development and evaluation and continued monitoring of drug therapy for falciparum malaria has been a consistent research priority of AFRIMS because of the disease's severity and the repeated

loss in the region of effective drugs as the parasite acquired resistance. In accordance with US FDA standards and in compliance with animal care guidelines, candidate drugs are screened in AFRIMS animal colonies for safety and efficacy. In humans, it was Institute investigators who demonstrated the effectiveness of mefloquine in the late 1970s (Hall *et al*, 1977; Doberstyn *et al*, 1979) and the development of parasite resistance less than 10 years later (Webster *et al*, 1985b). Though efficacy can be preserved thru combination with artesunate (Looareesuwan *et al*, 1992), AFRIMS investigators noted that drug resistance continues to evolve in favor of the parasite (Wongsrichanalai *et al*, 2002) and that artesunate resistance is now a major concern (Noedl *et al*, 2008). Assays to detect and better monitor drug sensitivity have been developed at the Institute (Webster *et al*, 1985a; Noedl *et al*, 2002).

Studies of malaria prophylaxis at AFRIMS have involved both drugs and vaccines. Doxycycline, an already licensed drug, was shown to be effective for prophylaxis when taken daily (Pang *et al*, 1987); primaquine and mefloquine were each found to be effective when used at less demanding weekly intervals (Fryauff *et al*, 1995; Ohrt *et al*, 1997). Preventive malaria vaccine trials were carried out in populations living or working in falciparum malaria endemic areas along the eastern or western borders of Thailand (Brown *et al*, 1994; Nosten *et al*, 1996). The eastern border trial was a military-to-military collaboration and the first field trial of a malaria vaccine in Asia. Neither was found to be efficacious.

Malaria vector and disease epidemiology were additional areas where research has yielded significant insights. Rosenberg and colleagues (1990) described the

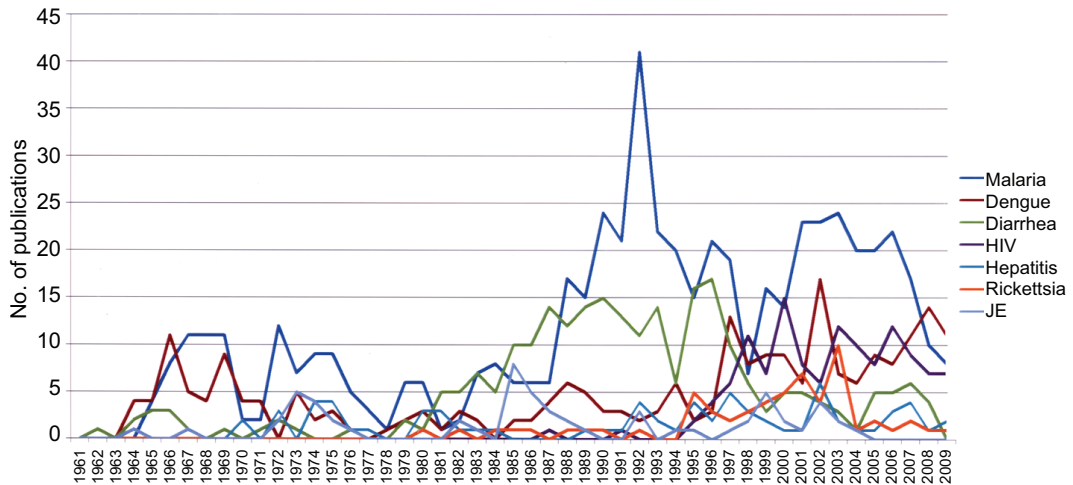


Fig 1—The number of AFRIMS publications each year, 1961-2009, for seven infectious disease areas.

restoration of efficient falciparum malaria transmission in formerly deforested areas after introduction of rubber trees (Rosenberg *et al*, 1990). *Plasmodium vivax* was shown to have two variants of sporozoite surface protein and assays were developed to distinguish these (Rosenberg *et al*, 1989; Wirtz *et al*, 1992). A taxonomic tour de force representing decades of work was completed by Dr Rampa Rattanaarithikul who led a team to write and illustrate a uniquely comprehensive and useful series of keys of the mosquitoes of Thailand (Rattanaarithikul *et al*, 2006).

Immunologic studies at AFRIMS have helped to dissect the humoral and cellular immune responses to malaria (Webster *et al*, 1987; Ho and Webster, 1989; Hiseada *et al*, 2000; Pichyangkul *et al*, 2004), as well the immune suppression that is caused by acute falciparum malaria infections (Wells *et al*, 1980; Ho *et al*, 1995).

This interdisciplinary research on malaria continues with foci on mosquito biology and identification of new drug prophylaxis regimens; modeled on the Walter Reed Army Institute of Research

(WRAIR) falciparum malaria challenge model, a human challenge model for vivax malaria is planned jointly by the US and RTA Components to open a new dimension to the evaluation of anti-PV drugs and vaccines.

### Japanese encephalitis

In 1969, Thailand (Chiang Mai) had an outbreak of encephalitis with more than 600 reported cases, mostly in children, of which nearly one quarter died (Endy and Nisalak, 2002). An AFRIMS team responded and confirmed that the diseases were caused by the Japanese encephalitis (JE) virus, characterized the clinical infections and serologically determined that there were large numbers of inapparent infections (Grossman *et al*, 1973a,b). Because isolation of this virus from blood is difficult, serologic assays are particularly important to the clinician but are complicated by the presence of antibodies to dengue virus (another flavivirus). AFRIMS researchers developed an assay to detect JE-specific IgM during acute infections (Burke *et al*, 1982) and showed that the presence of this IgM in

spinal fluid reflected infection of the brain (Burke *et al*, 1985a). Serodiagnostics were complemented by descriptions of the immunopathology caused by JE virus in the brains of humans (Johnson *et al*, 1985).

The development of specific serologic assays made possible the diagnosis of subclinical infections and therefore the testing and evaluation of preventive vaccines. In collaboration with the Thai Ministry of Public Health, AFRIMS investigators designed and carried out a large clinical trial to evaluate the safety and efficacy of an inactivated JE vaccine used in northeast Asia but unlicensed in the US and Thailand. The trial involved 40,000 school children in Kamphaeng Phet Province and showed the vaccine to be safe and 91% efficacious (Hoke *et al*, 1988). These results were key to the vaccine's licensure in the US and Thailand, and its subsequent inclusion in the Thai Expanded Program of Immunization (EPI). The relationship between AFRIMS and health authorities in Kamphaeng Phet Province which was built during this vaccine trial has continued and evolved into a permanent field laboratory and the site of subsequent hepatitis A (Innis *et al*, 1994) and dengue epidemiology and vaccine studies (Gibbons, manuscript in preparation).

The study of this zoonotic disease documented that pigs have high rates of new infection even when there are no human cases in the same locale, that pigs are preferred over humans by the vector (Burke *et al*, 1985b). Thus, even as human cases have become rare among Thais, the risk to visiting military and travelers remains. The pathogenesis of JE virus in the prominent vector mosquito in Thailand, *Culex tritaeniorhynchus*, was documented (Leake and Johnson, 1987). Non-human primate models were established and an

intranasal challenge model for vaccine testing developed (Angsubhakorn *et al*, 1986; Myint *et al*, 1999).

Regionally, AFRIMS has assisted the Ministry of Health of Nepal to diagnosis JE cases (Zimmerman *et al*, 1997) and has provided its reference lab with diagnostic reagents. In summary, AFRIMS research on JE has spanned epidemiologic description, vector characterization, development of diagnostics, establishment of animal models and field trial of a preventive vaccine.

### Dengue

Dengue, another endemic disease in Thailand and among the top three infectious disease threats to the US Military (Burnette *et al*, 2008), has been studied at AFRIMS throughout its 50-year history. Of the more than 200 dengue publications (Fig 1) by AFRIMS investigators and colleagues, 18 have been cited more than 100 times. This subset of widely referenced papers is used to highlight the Institute's research.

Russell, Nisalak and colleagues published a pair of papers in 1967 which together have been cited some 400 times. They described an efficient, practical and accurate technique to detect and measure neutralizing antibody to dengue virus (Russell *et al*, 1967). The pair of papers showed that this assay could identify and classify dengue viruses of the four serotypes (Russell and Nisalak, 1967). Further diagnostic challenges were addressed in the 1980s: distinguishing between anti-dengue and anti-JE (both flaviviruses) antibody responses, and between primary and secondary dengue infections. Innis and colleagues (1989) described a solution to both with an ELISA which quantitated IgM and IgG antibodies to dengue and to JE viruses.

As dengue can be fatal, the determinants of severity have been studied and elucidated. Analyses of children hospitalized in the 1960s with hemorrhagic fever showed that dengue, but not chikungunya, cases could manifest shock (Nimmannitya *et al*, 1969), a finding once again relevant as chikungunya re-emerges in Thailand. Halstead and colleagues (1970) treated and studied more than 500 children over three years with dengue hemorrhagic fever (DHF) and found that shock correlated with secondary-type antibody response to the virus. Their theory of immune enhancement derives from these studies. A prospective study of Bangkok schoolchildren in 1980-1981 added evidence by showing that pre-existent dengue immunity placed children at increased risk of DHF when next infected (Burke *et al*, 1988). Clinical studies in the 1990s identified simple clinical and laboratory parameters to help identify children with dengue fever (DF) or DHF (Kalayanarooj *et al*, 1997), and showed viremia correlated with fever and that all four serotypes can cause DHF (Vaughn *et al*, 1997). Disease severity correlates continued to be identified and proposed mechanisms refined. Potential correlates include virus burden, with serotype 2 carrying extra risk, and immune response, characteristically a secondary infection with immune activation of type-1 cytokines (Vaughn *et al*, 2000; Librarty *et al*, 2002).

These observations and correlations stimulated further study of pathogenesis. Antibody-dependent enhancement of virus growth in cell culture was shown (Kliks *et al*, 1989). T cells were found to be activated *in vivo* during DHF and DF, and both CD4 and CD8 cells are activated to a greater extent in DHF than in DF (Kurane *et al*, 1991; Green *et al*, 1999). Study of

dendritic cells has added further refinement, with activation varying both with infection of these cells and the presence of cytokines in the local micro-environment (Librarty *et al*, 2001).

The mosquito vector of dengue (*Aedes aegypti*) is central to the fluctuations in transmission. Temperature-induced variation in vector efficacy was shown (Watts *et al*, 1987) which may explain the annual cyclic pattern of DF/DHF epidemics in climates like Bangkok. Release-recapture studies of *Ae. aegypti* showed that these mosquitoes disperse relatively short distances, suggesting that people, not mosquitoes, are the main mode of dengue virus dissemination (Harrington *et al*, 2005).

Dengue research efforts continue with studies including viral evolution, human immune responses to both infection and vaccine, and vector dynamics. Top priority is currently the phase 3 assessment of a tetravalent vaccine for prevention of disease, in support of the global effort to reach the goal of a licensed, safe, effective, affordable and practical vaccine.

### Diarrhea

Thailand's cholera epidemic in the late 1950s was the stimulus for establishing the joint medical research institute which has become AFRIMS. Subsequently, diarrheal disease has been a consistent focus of research and more than 250 articles have been published (Fig 1). Bacterial diarrhea is among the top three infectious disease threats to the US Military (Burnette *et al*, 2008) and remains a major problem for children in the developing world and for travelers.

Initial studies at AFRIMS (then SEATO Medical Research Lab) explored the pathogenesis of cholera in a rabbit model and contributed to the character-



ization of procholera A (Finkelstein *et al*, 1966), a protein now designated as the A subunit of cholera toxin. This work contributed to the understanding of secretory diarrheas and to developing approaches for both more effective treatments and preventative vaccines.

The North American soldier and international travelers, when arriving in less developed parts of the world, share a vulnerability to diarrhea. Thus, travelers' diarrhea has been a repeated focus for study by AFRIMS researchers. In 1979, more than 50% of American Peace Corps volunteers developed diarrhea during their first five weeks in Thailand, caused by a variety of organisms, many of which were drug resistant (Echeverria *et al*, 1981). AFRIMS researchers carried out studies to help resolve the question of whether *Aeromonas* and *Plesiomonas* are enteric pathogens. Their findings suggested that the former, but not the latter, is pathogenic in Thais and travelers (Pitarangsi *et al*, 1982).

Studies of travelers and foreign residents in Nepal documented that the infecting organisms varied with length of time abroad and duration of symptoms (Taylor *et al*, 1988). When symptoms exceeded two weeks, *Giardia* was more likely and *Shigella* less so. *Cyclospora* was found to be associated with prolonged diarrhea in Nepal; Hoge and colleagues (1993) provided strong evidence that this newly described organism was indeed a pathogen and most likely waterborne. The team then carried out a placebo-controlled trial, and showed that Co-trimoxazole was curative (Hoge *et al*, 1995). At about the same time, another AFRIMS collaboration showed in a double-blind study of US Military personnel that loperamide was a safe addition to antibiotics for reducing diarrheal symptoms (Petruccioli *et al*,

1992); this product has since become the antimotility drug of choice for control of diarrhea.

Additional highlights of the diarrheal studies were the isolation and genetic sequencing of an enterotoxin (ST Ib) of *E. coli* (Moseley *et al*, 1983) and the description of antibiotic resistance among diarrhea pathogens across a 15-year time span (Hoge *et al*, 1998). More recently, AFRIMS participated in a prospective multi-national study which clarified the disease burden caused by shigellosis in Asia and the continued emergence of antibiotic-resistant strains (von Seidlein *et al*, 2006). To address this continuing public health problem, AFRIMS researchers are assessing candidate vaccines in monkeys and, with colleagues at Mahidol University, in a carefully controlled model of challenge in humans.

## HIV

In the late 1980s, HIV began to appear in sentinel groups in Thailand. Col Burke, previously of AFRIMS, and LTG Pinyo, RTA Surgeon General, agreed that the RTA Medical Department and the WRAIR would work jointly to strengthen the RTA's HIV testing capacity and establish a vaccine development program. Through the testing program, 50,000 to 60,000 male recruits from across the nation were tested annually. The results documented for the first time that the HIV epidemic could be reversed at a nation level (Mason *et al*, 1995; Torugsa *et al*, 2003). Building on this collaboration, Department of Retrovirology was established within AFRIMS in 1993; its mission was to work jointly with the RTA and other Thai partners toward a preventative vaccine. The first decade of that US-Thai HIV collaborative research program was summarized previously (Brown and Nitayaphan, 2004).

Several manufacturers agreed to

modify their candidate vaccines using the variant strain of HIV (subtype E or CRF01\_A/E) found by WRAIR/AFRIMS investigators to be prevalent in Thailand (McCutchan *et al*, 1992). This was the first attempt by industry to modify candidate HIV vaccines to match subtypes found in the less developed world. AFRIMS facilitated collaborations among in-country scientists from universities, Royal Thai Army and Ministry of Public Health, forming the Thai AIDS Vaccine Evaluation Group (TAVEG). The initial TAVEG trials were with candidate vaccines derived from the virus' envelop glycoprotein (Nitayaphan *et al*, 2000; Pitisuttithum *et al*, 2003). When a product designed to stimulate cellular immunity became available, vaccines were studied as 'prime-boost' combinations in phase I/II trials (Nitayaphan *et al*, 2004, Thongcharoen *et al*, 2007). One combination was advanced to phase III evaluation and was tested in a 16,000-person, 6-year trial which showed for the first time that HIV vaccines can provide protection, though partial (Rergs-Ngarm *et al*, 2009). This finding reinvigorated the field of HIV vaccinology.

In parallel with the clinical trials, AFRIMS carried out related and supportive research. Some highlights follow. There were contributions to HIV taxonomy: the first circulating recombinant form (CRF) was defined (McCutchan *et al*, 1992) and other CRFs were identified (Tovanabuttra *et al*, 2001, 2003; Watanaveeradej *et al*, 2003); strains causing new infections were characterized (Watanaveeradej *et al*, 2006, Arroyo *et al*, 2010). The high level of motivation and willingness to participate in HIV vaccine trials was documented (Jenkins *et al*, 1998, 2000). Evidence of change in sexual behavior associated with the reversal of the heterosexual epidemic was reported (Kitsiripornchai *et al*, 1998). Viral

load assays were assessed across differing viral subtypes (Elbeik *et al*, 2002); the natural history of HIV (subtype E) infections in Thais was determined retrospectively (Rangsin *et al*, 2004).

The AFRIMS retrovirology program is now focused on dissecting the immune response to the vaccine which showed clinical efficacy in order to help guide the design of next generation vaccines. Intense studies of acute HIV infections are also underway, looking closely at the evolution of the virology and immune responses (in both blood and mucosa) during the first weeks of infection.

#### **Refugees, displaced persons and peace-keepers**

During the 1980s and 1990s, Thailand received and hosted refugees on its borders. In the east, Cambodian refugees flowed suddenly into Thailand in 1979 as the Vietnamese routed the Khmer Rouge government. The number of these Indochinese refugees reached about half a million people (Morris *et al*, 1982). AFRIMS physicians were part of the earliest response to this humanitarian challenge (Johnson *et al*, 1981), and then worked with NGOs and the United Nations Border Relief Operation (UNBRO) throughout the next decade to assist in laboratory identification of various infectious diseases causing disease in the refugee camps. Assistance with identification of diarrheal etiologies was also provided at a Transit Center in central Thailand for the approximately 10,000 Cambodians being resettled into the United States (Morris *et al*, 1982) and in a refugee camp along the Laotian border for Hmong refugees, a group which had assisted the US in the Laotian highlands during the Vietnam War.

In response to the 1979 Khmer refugee emergency on the eastern border, a team

of infectious disease specialists from AFRIMS was mobilized and provided clinical care and coordination among the relief agencies (Johnson *et al*, 1981). The Commander of the US Component of AFRIMS was designated as the US Ambassador's advisor on refugee health. In recognition of their contributions, a number of the AFRIMS staff were awarded the Humanitarian Service Award by the US Army.

The Khmer refugee situation stabilized, but diarrhea remained a frequent problem and AFRIMS provided diagnostic support in the identification of cholera (Morris *et al*, 1982), amebiasis (Candler *et al*, 1990), childhood diarrhea (Arthur *et al*, 1992) and the striking phenomenon of enteritis necroticans (Johnson *et al*, 1987). Specific typhus serologic testing at the Army's labs in Malaysia (now closed) and AFRIMS showed that murine typhus is a frequent cause of febrile illness in the refugee camps (Brown *et al*, 1988; Duffy *et al*, 1990), stimulating public health measures to control the rodent carriers. This research and diagnostic support was carried out in collaboration with UNBRO and medical NGOs like Catholic Relief Services and the American Refugee Committee.

On Thailand's western border with Myanmar (then Burma), refugees entered who were displaced from the Karen minority areas by fighting inside Myanmar. In 1984-1985, more than 40,000 took refuge in Thailand and began living in some 10 villages as displaced persons near the border. The border terrain is characterized by forest-covered mountains where *Anopheles dirus* thrives and malaria is endemic. Many refugees have been unable to return home and malaria continues to be a major disease threat.

AFRIMS investigators have been part of an international team which worked to

define and better control malaria among these displaced Burmese people. Investigations were focused on both prevention (drug and vaccine) and treatment, the latter challenged by increasing drug resistance. A large controlled trial made the important finding that a controversial malaria vaccine was indeed not efficacious (Nosten *et al*, 1996). Meanwhile, drug resistance continued to increase. AFRIMS researchers and collaborators from WHO, Doctors without Borders and academia, documented an increase in resistance to mefloquine (Nosten *et al*, 1991), a restoration of this sensitivity when the drug was co-administered with artesunate (Brockman *et al*, 2000), and an association of cardiac toxicity with halofantrine (Nosten *et al*, 1993) which dampened hopes for this new anti-malarial drug.

The most recent refugee initiatives were in Bhutanese refugee camps in Nepal as part of a response to an influenza A outbreak in 2004. A multi-national collaborative team showed that the flu virus (H3N2) circulating was molecularly unique (Daum *et al*, 2005), an observation which led the WHO to modify its recommendation for the 2004-2005 influenza vaccine. While it is uncertain when the next surge of refugees will occur, the Institute remains willing and capable to provide assistance to public health authorities charged with their care.

The Royal Thai Army has been involved in peace-keeping operations with the United Nations since 1999. RTA units have been sent to East Timor, Afghanistan, Iraq, Burundi and Sudan. AFRIMS is responsible for health screening of potential members of these military operations and for studying the epidemiology of infectious diseases in the locale of the assignments. Thus, both pre- and post-deployment blood samples are collected



and screened for markers of selected infectious diseases. More than 8,000 soldiers have been enrolled and tested for these operations. Screening may include serology for HIV, HBV, HCV, HEV, dengue, West Nile, chikungunya, JE viruses, as well as rickettsia, syphilis, malaria, filariasis and leishmaniasis. Infections with West Nile virus have been identified through this program, and the mechanism is in place for both retrospective and prospective analyses of infectious disease exposures during these peace-keeping operations.

### DISCUSSION

This summary of the research that has been carried out at AFRIMS over the past 50 years is not comprehensive, but exemplifies the breadth and quality of tropical disease research which has been a fruit of the unique collaboration which is now AFRIMS. Studies were always done as collaborations, with those in the public health sector, at universities, in other military medical departments or with NGOs working among the under-served. The laboratory was conceived in the context of a pandemic cholera threat and warfare on the Southeast Asian landmass. While these factors have resolved, the Institute which grew from this genesis has contributed to both military and global public health. Such collaborative medical research and the associated local capacity building are core components of what may be called 'medical diplomacy'.

Since medical challenges continue in Southeast Asia, past accomplishments become the stepping stones upon which new researchers address current problems caused by drug-resistant malaria, spreading dengue, the ravages of HIV and other persisting and emerging infectious diseases.

### ACKNOWLEDGEMENTS

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