

## Subtypes and phylogenetic analysis of human immunodeficiency virus-1 in Jayapura

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### ABSTRACT

#### BACKGROUND

Human immunodeficiency virus (HIV) is a retrovirus that infects human immune system. HIV is genetically differentiated into HIV-1 and HIV-2. HIV-1 is classified into 3 groups : M,N and O. M Group is divided into 9 subtypes, namely : A, B, C, D, F, G, H, J and K. HIV subtype mapping would become a good reference to study the epidemiology of HIV-1. HIV phylogenetic analysis required to understand virus origin, geographical distribution and tracking parameter of HIV transmission process in sample area. The objective of this study was to determine HIV-1 subtype distribution and the relationship between HIV-1 subtypes from with genBank HIV isolate based on envelope gene fragment.

#### METHODS

A cross sectional design was conducted involving 36 subjects in Jayapura between January to March 2013. Blood samples were taken and analyzed using reverse transcription polymerase chain reaction (RT-PCR), nested PCR and confirmed with sequencing. Phylogenetic analysis performed along 400 bp of envelope gene fragment. The data were analyzed with Bioedit ver.7 and MEGA 5.1. software.

#### RESULT

Two types of HIV-1 subtype were found in Jayapura Regency. CRF01\_AE was confirmed in 31 patients (86.1%) and subtype B in 5 patients (13.9%). Phylogenetic analysis showed phylogenetic relationship between CRF01\_AE from jayapura with subtype from Singapore and Thailand, while subtype B from Jayapura were closely related with subtype from United States and United Kingdom

#### CONCLUSION

This study reflect the independent introduction of multiple HIV-1 strains into Papua, with the rapid spread in the majority of infected patients tested of single strain of HIV-1 (CRF01\_AE).

**Keywords:** HIV-1 subtype, phylogenetic analysis, CRF01\_AE, Subtype B, Papua

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## **Analisis subtipe dan filogenetik human immunodeficiency virus-1 di Jayapura**

### **ABSTRAK**

#### **LATAR BELAKANG**

Human immunodeficiency virus (HIV) adalah retrovirus yang menginfeksi sistem kekebalan tubuh manusia. Secara genetik HIV dibedakan menjadi HIV-1 dan HIV-2. HIV-1 diklasifikasikan kedalam 3 grup yaitu : M, N, dan O. Grup M dibagi menjadi 9 subtipe yaitu: A, B, C, D, F, G, H, J dan K. Pemetaan subtipe dapat menjadi rujukan dalam perkembangan epidemi HIV-1. Analisis filogenetik juga diperlukan untuk mengetahui asal-usul virus, distribusi geografis dan parameter pelacakan proses transmisi HIV pada area sampel. Penelitian ini bertujuan untuk menentukan distribusi subtipe HIV-1 dan hubungan kekerabatan isolat HIV-1 Jayapura dengan isolat HIV GenBank berdasarkan fragmen gen envelope

#### **METODE**

Sebuah rancangan potong lintang (cross sectional) dilakukan pada 36 subjek di Jayapura antara bulan Januari-Maret 2013. Sampel darah dianalisis menggunakan teknik reverse transcription polymerase chain reaction (RT-PCR), nested PCR dan dikonfirmasi dengan sekuensing. Analisis filogenetik dilakukan berdasarkan fragmen gen sampul (envelope) sepanjang 400 pasangan basa (bp). Hasil dianalisis dengan program Bioedit dan MEGA 5.1.

#### **HASIL**

Dua subtipe ditemukan di Kabupaten Jayapura. 31 pasien (86.1%) CRF01\_AE dan 5 pasien (13.9%) subtippe B. Analisis filogenetik menunjukkan hubungan antara CRF01\_AE Jayapura dengan Singapura dan Thailand, sedangkan subtippe B dari Jayapura memiliki hubungan dengan isolat dari Amerika dan Inggris.

#### **KESIMPULAN**

Penelitian ini merefleksikan introduksi independen beberapa strain HIV-1 ke Papua, melalui penyebaran cepat dengan mayoritas pasien yang terinfeksi strain HIV-1 adalah CRF01\_AE.

**Kata kunci :** Subtipe HIV-1, Filogenetik, CRF01\_AE, Subtipe B, Papua

## **INTRODUCTION**

Acquired immunodeficiency syndrome (AIDS) is a collection of symptoms caused by infection with the human immunodeficiency virus (HIV). This retrovirus attacks and destroys T-helper ( $CD4^+$ ) lymphocytes, causing the immune system of patient to weaken and become more susceptible to various infections and malignancies.<sup>(1)</sup> There are two types of HIV that are genetically differentiated, HIV-1 and HIV-2.<sup>(2)</sup> HIV-1 is a common virus found in all parts

of the world (Africa, America, Europe and Asia).<sup>(3)</sup> HIV-1 is classified into three major phylogenetic groups: M, N and O.<sup>(4-6)</sup> Group M is subdivided phylogenetically into nine subtypes: A, B, C, D, F, G, H, J and K.<sup>(7-8)</sup> In addition, 48 new subtypes of hybrid virus called circulating recombinant forms (CRF) have been found in the world, including CRF01\_AE, CRF02\_AG and CRF03\_AB.<sup>(9)</sup> The CRF01\_AE subtype is distributed widely in Southeast Asia.<sup>(10)</sup>

HIV-1 subtype variation could be a potential problem for public health in terms of the

transmission and prevention strategies of HIV/AIDS. HIV subtype is an important molecular marker to monitor the changes in the HIV epidemic in the world.<sup>(11)</sup> HIV-1 subtypes have different distributions between one region to another, where geographical factors can affect the subtypes differences.

In Indonesia, the number of cases of HIV/AIDS continually increases every year. A total of 103,759 of HIV and 43,347 of AIDS cases were recorded at the end of March 2013.<sup>(12)</sup> Papua is the third highest-ranking province for HIV/AIDS, with 10,881 HIV and 7795 AIDS cases being found.<sup>(13)</sup> Research in Jakarta and East Java showed that CRF01\_AE subtype transmission is limited to syringe sharing among drug users and promiscuous sexual intercourse of the heterosexual population.<sup>(14)</sup>

Papua is one of the provinces with the highest number of HIV/AIDS cases compared with other regions in Indonesia. The cases may be increasing, since the regional expansion in Papua allows mobilization of the people in great numbers. This study was conducted in Papua because there were no research data related to HIV subtype distribution and only limited information regarding genetic characteristics of the virus.

Jayapura is one of the regencies in Papua with HIV/AIDS cases. In Jayapura regency there were reportedly 936 people consisting of 537 women and 399 men who were HIV/AIDS positive, and among these there were 150 deaths. There have been no reports on HIV-1 subtype distribution and its relationship with demographic characteristics and risk factors in Jayapura. Therefore, it was considered important to determine the subtypes of HIV-1 and their relationship with demographic characteristics and risk factors in Jayapura regency in order to obtain basic data in HIV-1 subtype mapping, as a reference standard in HIV epidemiology and clinical applications. HIV-1 subtype is associated with clinical manifestations and antiretroviral resistance. Identifying the association between HIV-1 subtype and clinical therapy can assist

clinicians in improving treatment strategies for HIV infection.<sup>(15)</sup> Furthermore, HIV phylogenetic analysis is also needed for determining the origin of the virus and its geographical distribution, and for tracking process parameters of HIV transmission in the sample area. The objective of the present study was to determine HIV-1 subtype distribution and the relationship between HIV-1 subtypes and GenBank HIV isolates based on envelope gene fragment, to obtain basic data in HIV-1 subtype mapping as a reference standard in HIV epidemiology and clinical application.

## METHODS

### Research design

An analytic cross-sectional study was conducted on HIV/AIDS patient undergoing routine care at Yowari Hospital VCT unit, from January to March 2013.

### Research subjects

A total of 36 HIV/AIDS patients were selected based on consecutive sampling among those patients meeting the inclusion criteria. Included in the study were men and women with HIV/AIDS diagnosed by 2 laboratory tests (rapid test and ELISA), after the subjects agreed to participate in the study with signed informed consent.

### Phylogenetic analysis

Blood serum samples of the 36 patients were collected after written informed consent. Laboratory tests included RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), nested PCR, electrophoresis, and the results were confirmed by sequencing. HIV RNA was extracted from serum and amplified using two pairs of primer. The primers used in RT-PCR were: TCTTAGGAGCAGCAGGAAGCA CTATGGG as gp 40 forward primer, AACGA CAAAGGTGAGTATCCCTGCCTAA as gp41 reverse primer: ACAATTATTGTTGG TATAGT GCAACCA as gp46 forward primer, and

TTAACCTATCAAGCTCCTACTATCATA as gp47 reverse primer.<sup>(14)</sup> To determine HIV-1 subtype, the HIV-1 gene region encoding glycoprotein 41 (gp41) on the viral envelope was amplified. Region 41 is a reliable marker for HIV-1 subtype grouping.<sup>(16)</sup> Nested PCR products were confirmed by electrophoresis and sequencing. Sequencing results were compared with the HIV-1 sequences available in GenBank and analyzed with BLAST. Phylogenetic relationships between HIV-1 isolates were analyzed with Molecular Evolutionary Genetic Analysis (MEGA) version 5.1. software and Bioedit Ver.7. The results were then presented in a phylogenetic tree. RNA extraction, RT-PCR and sequencing were performed at the National Institute of Health Research and Development, Ministry of Health. Sequencing results were analyzed using Bioedit software. The initial stages performed during the analysis process were trimming (cutting) unreadable sequencing results, and combining readable forward and reverse primer sequences for confirmation. The next stage was the Basic Local Alignment Search Tool (BLAST) process, of which the goal is to obtain the HIV virus genotypes and subtypes. BLAST was performed by using the internet at two Genbank sites for confirmation, i.e. National Center for Biotechnology Information (NCBI) BLAST at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) and HIV sequence database at [www.hiv.lanl.gov](http://www.hiv.lanl.gov).

### Data analysis

The results were analyzed descriptively. Nucleotide sequences of HIV sequencing results were then compared with the HIV virus isolates from the United States, Canada, Thailand, China, Vietnam, Singapore, accessible via GeneBank. Phylogenetic analysis was performed to determine the phylogenetic relationship between HIV isolates from Jayapura district and isolates from GenBank by the joining neighbor analysis method using the MEGA version 5.1. program. The phylogenetic relationship was then visualized with the NJPlot program.

### Ethical clearance

Ethical approval was obtained from Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University.

## RESULTS

HIV-1 subtype distribution at Yowari Hospital is shown in Table 1. Among the 36 subjects in this study, two subtypes were found, namely subtype AE in 31 patients (86.1%) and subtype B in 5 patients (13.9%). The phylogenetic relationship diagram between subtype B isolates from Jayapura and references isolates from GenBank is shown in Figure 1. The phylogenetic tree of Jayapura subtype CRF01\_AE isolates and Genbank isolates can be seen in Figure 2.

## DISCUSSION

The preponderance of subtype CRF01\_AE in Jayapura in comparison with subtype B is similar to findings of previous studies in Jakarta and East Java.<sup>(14)</sup> In addition, the study conducted by Foley et al.<sup>(17)</sup> in Timika (Papua) on 16 HIV

Table 1. Subtype and demographic variable at Yowari's Hospital

Variable	Subtype		Total
	AE	B	
Age			
<35 year	24	5	29
>35 year	7	0	7
Sex			
Male	17	1	18
Female	14	4	18
Tribe			
Papua	29	5	34
Non-Papua	2	0	2
Occupation			
Unemployed	12	0	12
Goverment officer	5	0	5
Farmer	9	2	11
Student	2	2	4
Labour	3	1	4

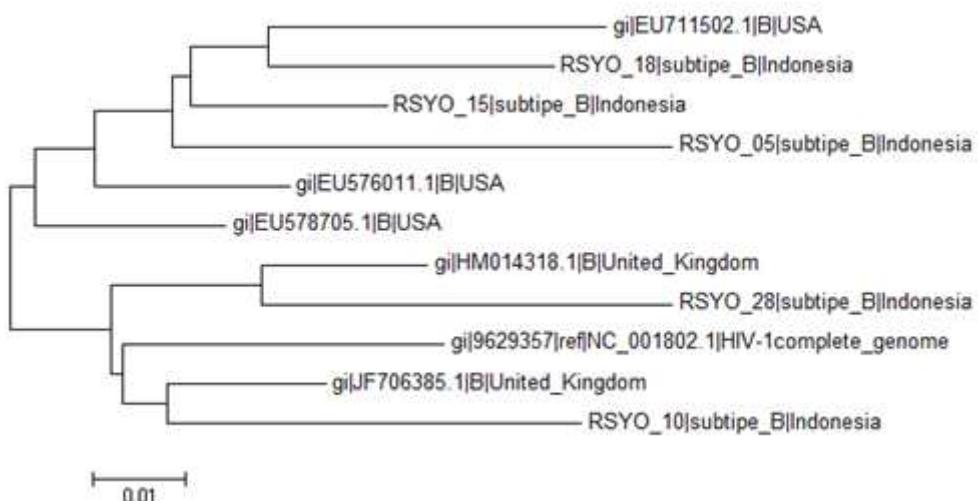


Figure 1. Phylogenetic relationship between Indonesian origin HIV subtype B and HIV subtype B reference isolate from other countries with Neighbour Joining analysis from MEGA 5.1 software

patients also found a higher prevalence of subtype CRF01\_AE (87.5%) in comparison with subtype B (12.5%). CRF01\_AE is predominantly found in Southeast Asia and is widespread in Thailand and Cambodia, where the number of persons with HIV is as high as one million.<sup>(11)</sup> CRF01\_AE has also been reported in China, Vietnam, Malaysia, Myanmar, Laos, Taiwan, Philippines and Singapore.<sup>(19)</sup> Historically, subtype B was generally found in Western Europe, North America, Latin America, Japan, Australia, and New Zealand. The molecular subtype B in this study was included in the American cluster sequences, because of its close genetical relationship to the latter. This had also been found in previous studies on subtype B sequences from Indonesia. The global distribution of HIV-1 subtype is more related to social change, the economy, immigration and international travel compared with differences in the nature or the power transmission of the virus. In addition to the above factors, geographical factors and population mobility also play an important role in the spread and genetic diversity of HIV-1.<sup>(18)</sup> The geographically strategic position of Jayapura has led to a high vulnerability of its residents to HIV infection. The mobility of local residents, non-indigenous

persons, and sex workers from Timika to Jayapura is one factor in the transmission chain, including the possibility of transmission of subtypes CRF01\_AE and B.

Results from BLAST data on subtype B isolates in Jayapura showed a similarity with isolates from the USA and the United Kingdom. Subtype B isolated from Jayapura shares the same roots with both isolates from USA and United Kingdom, as shown in Figure 1. Subtype B isolates from Jayapura and the reference isolates were divided into two clusters. Cluster 1 was occupied by the USA isolates and isolates from Jayapura. Isolate number 18 had the same root as the US isolates, whereas isolates 15 and 5 were in one cluster with US isolates but separate from the reference isolates and formed their own branch of the cluster. Cluster 2 was occupied by the UK isolates and isolates from Jayapura, where isolates 28 and 10 had the same roots as the UK isolates.

The phylogenetic relationship between subtype B isolates from Jayapura and the GenBank reference isolates is shown in the phylogenetic tree analyzed with Neighbour Joining.<sup>(19,20)</sup> Based on data from BLAST, sequenced CRF01\_AE isolates from Jayapura has a similarity with sequenced isolates from

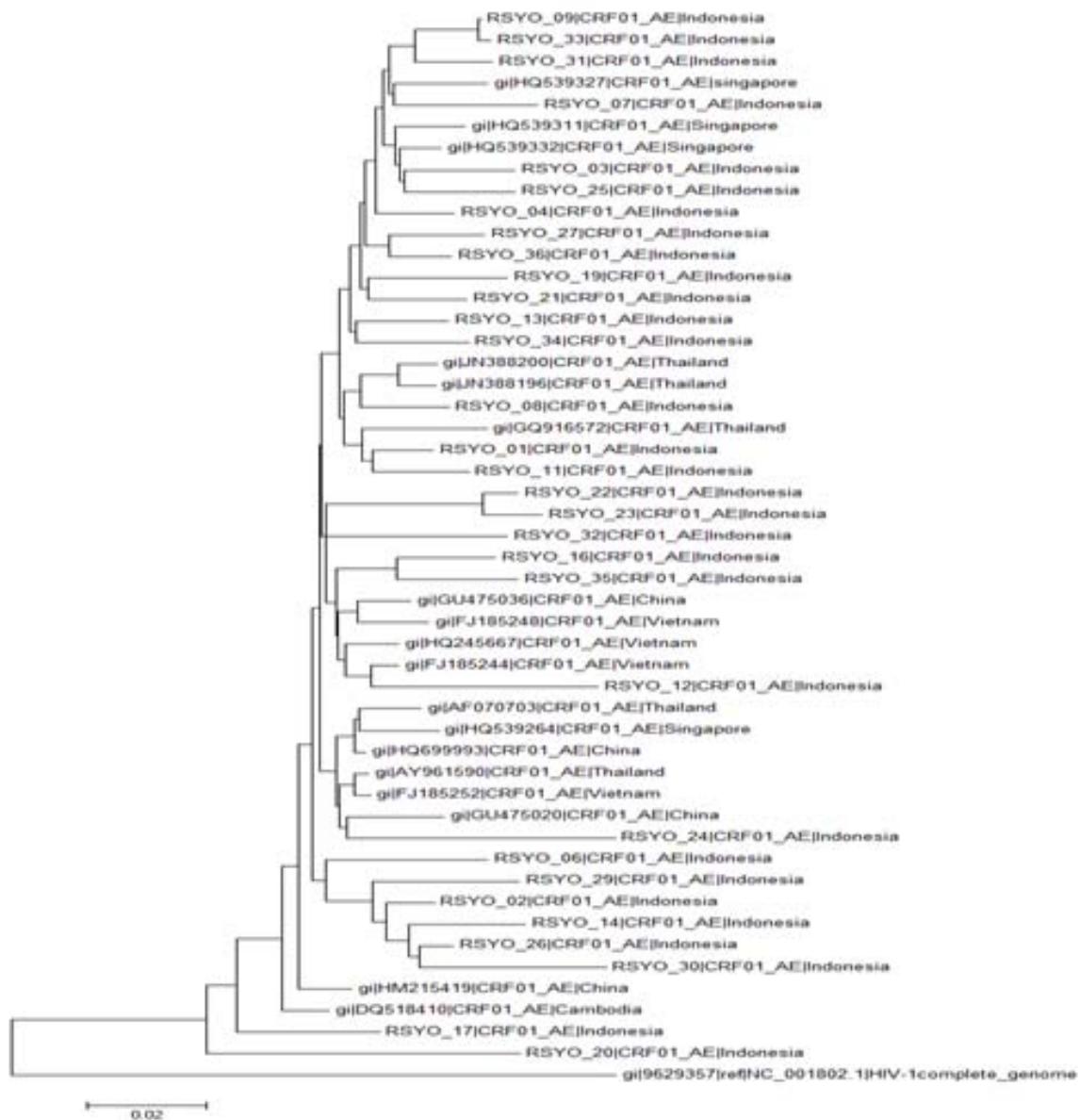


Figure 2. Phylogenetic relationship between Indonesian origin HIV subtype CRF01\_AE and HIV subtype CRF01\_AE reference isolate from other countries with Neighbour Joining analysis from MEGA 5.1 software

Thailand (GenBank access code: JN388200, AF0070703, AY961590, GQ916570 and JN388190), China (GenBank access code: GU475036, HM215419, HQ69993, GU475020), Vietnam (GenBank access code: FJ185244, FJ185248, FJ185252, HQ245652), Singapore (GenBank access code: HQ539264, HQ539327, HQ539332, HQ539311) and Cambodia (GenBank access code: DQ518410). Sequenced isolates from these five countries were subsequently used as reference isolate.

From the phylogenetic tree (Figure 2) it is apparent that the HIV-1 subtypes AE are divided into several clusters, with the Indonesian subtype AE originating from Jayapura occupying the same roots as isolates from Singapore, Thailand, Vietnam and China.

Indonesian isolates and GenBank original isolates were divided into several clusters. Cluster 1 was occupied by isolates from Indonesia, Singapore and Thailand. The first branch was occupied by Indonesian and

Singaporean isolates, three Indonesian isolates (number 9, 33 and 31) were grouped into one single root, while number 7 isolate was in the same root as reference isolates from Singapore. The second branch was occupied by Singaporean and Indonesian isolates, with isolates number 3 and 25 being in the same roots as isolates from Singapore. The third, fourth, fifth and sixth branches were occupied by isolates from Indonesia (number 4, 27, 36, 19, 21, 13, and 34), which were separated from the reference isolates and formed new branches. The seventh branch was occupied by Thai and Indonesian isolates, with isolates 8, 1, and 11 occupying the same roots as the Thai isolates. The eighth branch was occupied by isolates from Indonesia (number 22, 23, 32) which were separate from the reference isolates and formed their own branch in the cluster.

Cluster 2 was occupied by isolates from Indonesia, Vietnam and China. The first branch was occupied by Indonesian isolate number 16 and 35, with both isolates were separate from the reference isolates. The second branch was occupied by isolates from China and Vietnam, while the third branch was occupied by Vietnamese and Indonesian isolates, where isolate number 12 was in one root with the isolates from Vietnam.

Cluster 3 was occupied by isolates from Thailand, Singapore, Vietnam, China and Indonesia. Indonesian isolate number 24 was in one root with the isolates from China. Cluster 4 was occupied by isolates from Indonesia, where isolates number 6, 29, 2, 14, 26 and 30 were separate from the reference isolates and formed a new cluster. The next clusters were occupied by isolates from China, Cambodia and Indonesia, which were individually separate in their own cluster. Furthermore, all isolates (originating from Indonesia, Thailand, China, Singapore, Vietnam and Cambodia) showed significant genetic distances against HIV-1 nc\_001802 complete genome isolate. Thus, these results might explain the possibility that HIV-1 nc\_001802 isolate is not the ancestor of the

isolates from Indonesia and other Southeast Asian countries.

NCBI BLAST molecular analysis showed a close relationship between the sequences of CRF01\_AE from Jayapura regency with sequences from Southeast Asian countries (Thailand, Vietnam, Cambodia and Singapore). From this point of view it is suggested that subtype CRF01\_AE in Jayapura came from Southeast Asia countries (Singapore and or other surrounding countries). The transmission may have been the result of intensive interactions between peoples in Southeast Asia.<sup>(14)</sup> The introduction of the virus into high-risk groups could quickly spread the disease to the surrounding areas. Phylogenetic analysis studies in Timika (Papua) showed that subtype CRF01\_AE from Timika has a close genetic relationship with viruses from Thailand, and that subtype B from Timika has a close relationship with American and European isolates.<sup>(17)</sup>

Phylogenetic analysis showed that HIV-1 isolates in Jayapura regency were both locally transmitted viruses and viruses imported from other regions. HIV-1 viral isolates from Jayapura were not spread uniformly, which means that the viral isolates derived from imported as well as local viruses. Imported viruses would be randomly distributed among the reference isolates. However, in the phylogenetic tree a larger monophyletic group of local transmission was seen to appear, suggesting a more local transmission from a single source of infection, so that the subtypes obtained from the samples were almost entirely CRF01\_AE. Due to the dynamic nature of HIV epidemics, it is suggested to conduct periodic HIV subtype studies, so as to update the information for program planning in education, prevention, medication, and vaccine manufacture.

## CONCLUSIONS

This study found that there were two HIV-1 subtypes in Jayapura, subtype CRF01\_AE in the majority of samples and subtype B in a

minority. Phylogenetic analysis showed a phylogenetic relationship between subtype CRF01\_AE from Jayapura and subtypes from Singapore and Thailand, while subtype B from Jayapura was closely related to subtypes from the United States and the United Kingdom. Cultural norms for sexual behavior within Papuan society also need to be explored in order to understand which educational programs promoting behavior change are most warranted.

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