Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(11):62-68
(http://derpharmachemica.com/archive.html)

Analysis of differences in *viral load* results of DBS, DPS between the refrigerator and storage at a temperature of 37°C with plasma: A study in the blood of patients with HIV/AIDS in Jayapura, Indonesia

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ABSTRACT

The existence of a variety of advances in medicine and pharmaceuticals as well as primary prevention and secondary, such as several other countries, Indonesia has shifted policy focus on monitoring the viral load (VL) patients Anti Retroviral Therapy (ART) where previously the CD4 count is the main tool to monitor the effectiveness of treatment (CD4 count remains the criteria for determining the start of antiretroviral therapy in HIV-infected patients). Rationally this change is the accumulation of evidence of treatment failure where it is clinical or immunological criteria that cause problems delay the identification of viral failure that leads to the formation of resistant mutations. This type of research used in this research is the study sectional using data analysis analytically to describe the relationship the results of viral load / calculation of the amount of the HIV virus - RNA drops of dried blood/DBS (Dried Blood Spots) and DPS (Dried Plasma Spots) against Plasma. From this research we can conclude that there are differences in viral load results 37 ºC DBS, DBS Refrigerator, 37 ºC DPS, DPS Refrigerator and plasma viral load results. Based on the comparison of the average (mean) of the viral load results DBS 37 ºC, DBS Refrigerator, DPS 37 ºC, DPS Refrigerator and viral load results Plasma, then the result of the average value of viral load refrigerator is higher than the average value of viral load results 37 ºC, the average value of viral load results DPS more higher than the average value of viral load results DBS. While the average value of the results of plasma viral load higher than the average value of viral load results DPS. The results of this study open the cooperative research field of molecular biology and microbiology clinic to find the best way of sample storage.

Keywords: Results of *viral load*, DBS-DPS, refrigerator, HIV/AIDS, Jayapura- Indonesia

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INTRODUCTION

Infection with the *Human Immunodeficiency Virus* (HIV) is a problem all over the world, including Indonesia. UNAIDS report in 2010 stated that despite the global infection with HIV has dropped 19%, but the number of people living with HIV/AIDS continues to increase as the number of deaths down where in 2010, an estimated 33.3 million people are living with HIV/AIDS. Total AIDS cases all over the Indonesia cumulatively amounted to 26,483.

HIV infection and AIDS disease is still a health problem in the world as well as in Indonesia. A growing problem in connection with this disease is due to morbidity and mortality are still high, this is caused partly because of the delay to get the Anti Retroviral treatment.

Advances in medicine and pharmaceuticals as well as primary prevention and secondary, like several other countries, Indonesia has shifted policy focus on monitoring the viral load (VL) patients Anti Retroviral Therapy (ART) where previously the CD4 count is the main tool for monitor the effectiveness of treatment (CD4 count remains the criteria for determining the start of antiretroviral therapy in HIV-infected patients). Rationally this change is the accumulation of evidence of treatment failure where it is clinical or immunological criteria that cause problems delay the identification of viral failure that leads to the formation of resistant mutations. Use of *dried blood spots* (DBS) was proposed because its collection easier and more likely to be moved in the room temperature to the laboratory, Calculation of Human Immunodeficiency Virus-Ribonunleic acid (HIV-RNA) from filter paper that has been evaluated by a number of studies with different technique [1].

The results of these studies varied such as: the amount of virus that is relatively high (> 5,000 copies/mL), while samples below this figure gives inadequate results. However, a number of recent studies using specimen storage at -20 °C before being sent to the laboratory for analysis. It is not allowed in the province of Papua (at least in the near future). A number of studies have examined whether DBS samples stored at 2-8 °C and the temperature of the room has enough integrity to be used in monitoring the amount of virus, with varying results [2-4].

This study will examine whether monitoring the amount of virus with DBS stored on (1) a temperature of 37 °C and (2) 2-8 °C before being checked in the lab still can check. With limited medical infrastructure in Papua province, DBS specimens ability to track the number of virus patients without storage at a temperature of -30 °C and can be moved at room temperature may be "modifier inspection methods". Nevertheless, limited transportation and logistics infrastructure in most parts of the Land of Papua province of Indonesia, will continue to lead the movement of people, and laboratory specimens is a major challenge in the future. The technological advances that can respond to the constraints of the system will help accelerate the pace of expansion of ART-quality services in the province.
To support the treatment needs examination regular viral load, the amount of viral load testing as many as 654 samples with results of 52 samples (8%) are not eligible to be examined, possibly because the sample lipemik, lysis and broken at the time of delivery (Health Laboratory of Jayapura, Papua province). That requires the method of taking and sending samples much simpler, because the examination viral load current must use plasma samples in sufficient quantities with storage at a temperature of minus 20 °C. It is of course difficult to reach in Papua, given the geographic Papua a relatively difficult, the researchers determined to make research a new breakthrough in the field of molecular biology laboratories so that areas that are difficult to monitor the success of treatment of HIV/AIDS.

MATERIALS AND METHODS

This research was conducted in Papua Provincial Health Laboratory, in February-April 2016. The type of research used in this study is a cross-sectional study using data analytically to describe the relationship the results of viral load/calculation of the amount of HIV virus-RNA DBS and DPS on Plasma [5-9]. The population in this study were all patients ART in Jayapura Hospital on VCT clinic. The sample in this study is a part of the population is taken using purposive sampling as many as 23 patients. Sampling consists of: taking venous blood; making procedures and drying drops of dried blood specimens; how the EDTA plasma processing; how the packaging sample specimen drops of dried blood; sample storage means drops of dried blood specimens and plasma; method of calculating the amount of HIV-RNA, which include: the plasma samples for HIV *viral load*; dry blood spot (DBS) [10-12].

Examination of viral load will be using a *Reverse Transcription-Polymerase Chain Reaction* (RT-PCR), which is a variation of PCR in generating viral quantification of HIV-1 RNA. Data of HIV-1 RNA will be transformed into $\log^{10}$ prior to analysis. As was mentioned earlier, this study wanted to see if the drops of blood drip dry or dry plasma can replace blood plasma viral load in the examination. By looking at whether there are significant differences among the three methods used (blood plasma, dried blood drops room temperature and dried blood drops temperature of 2-8 °C). Data analysis will compare the proportion of test results of each method [13-15].

RESULTS AND DISCUSSION

The results of the examination of samples obtained from VCT clinics, Hospital Dok II Jayapura, which examined HIV *viral load* by instrument RT-PCR in Jayapura Health Laboratory, with the following results:

Table 1. Viral load test results of DBS, DPS, and plasma

VIRAL LOAD LOG (copies/ml)						
No.	**Sample code**	**dried blood spot**		**dried plasma spot**		**plasma**
		37 ℃	**refrigerator**	**37 ℃**	**refrigerator**	
1.	MGL	0	0	0	0	< 1,60
2.	TRM	2,74	3,17	2,45	3.21	5,55
3.	RDL	2,46	2,39	1,95	3.07	5,2
4.	LDP	1,60	2.43	1,89	2,37	4,15
5.	LSM	2,52	2,85	2,92	2,81	5,20
6.	NRJ	2,11	2,75	2,77	3.25	5,32
7.	RSN	3,21	3,64	3,17	3.64	5,97
8.	JWY	2,40	2.73	2,72	3.17	5,24
9.	SLV	3,33	3,79	2,99	3.16	5,98
10.	ALM	<1,60	2,26	3,41	3.79	4,71
11.	WDO	0	0	0	0	< 1,60
12.	LMT	2,95	3,58	2,85	4.12	6,10
13.	FRL	0	0	0	0	2,00
14.	YNM	4,20	4,92	4,77	5,13	> 7,00
15.	AWO	< 1,60	2,43	< 1,60	2,21	3,99
16.	RGN	2,40	3,05	2,78	2.93	5,15
17.	SSI	1,75	2,37	2.43	2,54	4,84
18.	KRS	3,70	3,98	4,00	4,12	6,25
19.	HLN	1,89	2,73	2,62	2,78	4,94
20.	DEN	2,39	3,14	2,86	3,07	5,46
21.	JAM	2,74	3,25	2,84	3,09	5,34
22.	SAL	2,65	3,16	2,87	3.32	5,61
23.	CIN	2,60	2,87	2,93	3.58	5,32

The distribution of the value of the average, median, standard deviation, minimum and maximum values of *viral load* test results above are as follows:

Table 2. The results of descriptive statistical analysis of *viral load* of DBS, DPS and Plasma

		DBS37dC	DBSRef	DPS37dC	DPSRef	Plasma
N	Valid	23	23	23	23	23
	Missing	0	0	0	0	0
Mean		2.2104	2.6735	2.4704	2.8417	4.8922
Median		2.4000	2.8500	2.7800	3.0900	5.2400
Std. Deviation		1.09223	1.22322	1.17206	1.28705	1.41017
Minimum		.00	.00	.00	.00	1.60
Maximum		4.20	4.92	4.77	5.13	7.00

Distribution graph of *viral load* results DBS, DPS on the results of *plasma viral load*

The distribution of *viral load* results DBS 37 °C on plasma viral load results, as seen below:

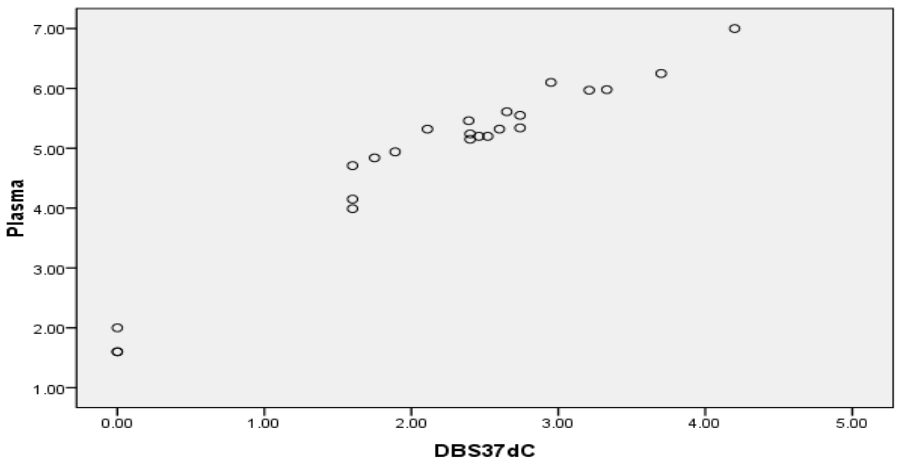

Fig 1. The distribution of *viral load* results DBS 37 °C on plasma viral load results

From the graph 1 above, looks distribution of *viral load* results DBS 37 °C on collected plasma viral load pattern resembles a linear line which can indicate the presence of a unidirectional relationship between *viral load* results DBS temperature of 37 °C with a plasma viral load results.

Distribution of DBS refrigerator *viral load* results against the the results of plasma *viral load*

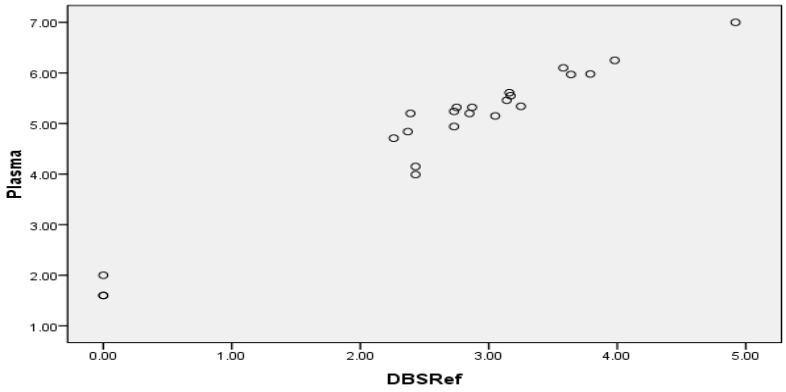

Fig 2. The graph shows the distribution of *viral load* results against the the results of DBS Refrigerator Plasma *viral load*

In the graph 2 above, seemed that distribution of DBS Refrigerator *viral load* results to the results of collected plasma *viral load* pattern resembles a linear line which can indicate the presence of a unidirectional relationship between viral load results DBS Refrigerator with Plasma viral load results.

The graph shows the distribution of *viral load* results DPS 37 °C against the plasma *viral load*

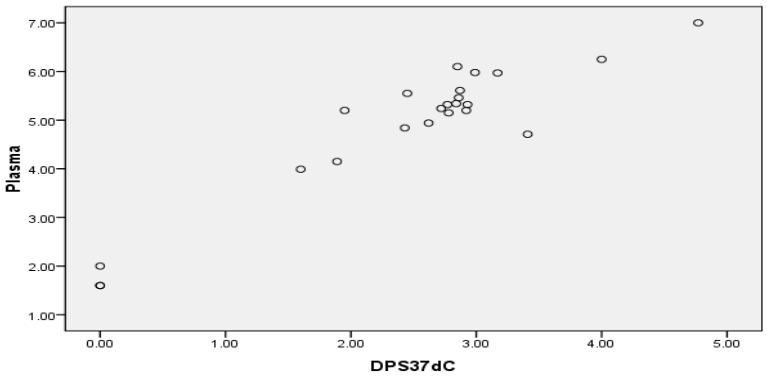

Fig 3. The graph shows the distribution of *viral load* results DPS 37 °C against the plasma *viral load*

From the graph in Figure 3 above, we see Distribution of the viral load results DPS 37 °C against the accumulated Plasma *viral load* results resemble linear line patterns that may indicate the presence of a unidirectional relationship between *viral load* results DPS 37 °C with Plasma *viral load* results.

The distribution of *viral load* results against the DPS Refrigerator Plasma *viral load* results

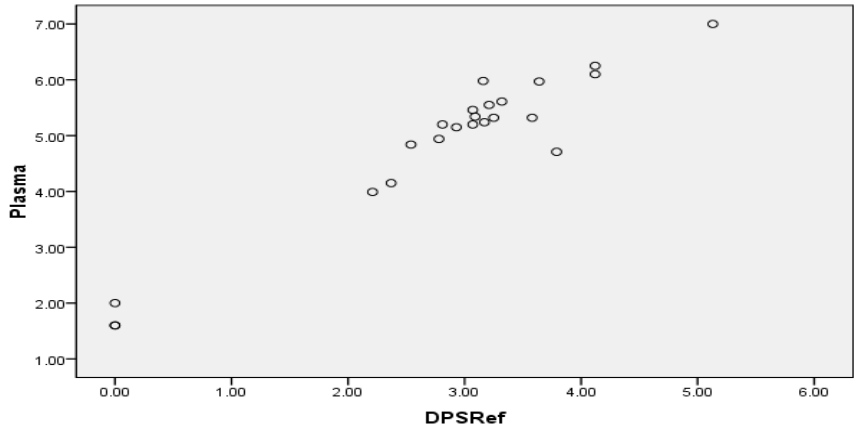

Fig 4. The resulting graph analysis of distribution of *viral load* results against the DPS Refrigerator Plasma *viral load* results

From the graph 4 above seemed distribution of DPS Refrigerator *viral load* results against the the results of the collected plasma *viral load* pattern resembles a linear line which can indicate the presence of a unidirectional relationship between *viral load* results DBS Refrigerator with Plasma *viral load* results.

Differences in *viral load* test results DBS, DPS and Plasma.

From the results of the examination of 33 samples of known differences in viral load test results DBS 37 °C, DBS Refrigerator, 37 °C DPS, DPS Refrigerator and Plasma. The differences seen in the results of viral load was higher in Refrigeration (*cold temp.*) compared with a temperature of 37 °C (*room temp.*), examination DBS and DPS.

Statistically, these differences seen in the differences, the increase in the value of the average higher than the *viral load* results in the Refrigeration (*cold temp.*) compared with a temperature of 37 °C (*room temp.*). Value or average viral load DBS 37 °C is 2.2104, the average value of viral load in the refrigerator is higher, at 2.6735. Similarly, the DPS, the average value of DPS viral load at 37 °C is 2.4704, the DPS Refrigerator is higher, at 2.8417. Differences in the average value of DBS and DPS *viral load* at 37 °C and in the refrigerator, it is still below the average results of plasma *viral load*, that is 4.8922.

Overall, based on the difference in the average value, the average value of viral load at refrigerator temperature is higher than the value of the average viral load at 37 °C, both on the viral load results DBS and DPS. The average value of viral load at DPS, higher than the average value of viral load DBS. The average value of viral load in plasma inspection higher up than the average value of viral load results DBS and DPS, whether stored at 37 °C and at refrigerator temperature.

The distribution of viral load results DBS and DPS against the a plasma viral load results on a graph.

The distribution of *viral load* results DBS 37 °C to the results of viral load plasma: the the graph Distribution of viral load results DBS 37 °C against the viral load results Plasma is known that the distribution pattern of viral load results that form a straight line or a distribution pattern that is closing in on a line straight. Such distribution pattern is the distribution pattern that can indicate the relationship (correlation) between the viral load results DBS 37 °C to the results of plasma *viral load* [16-20].

Distribution of DBS Refrigerator *viral load* results against the the results of plasma viral load: from distribution of the graph *viral load* results against the the results of DBS Refrigerator Plasma viral load is known that the distribution pattern of viral load results of which form a straight line or the distribution pattern close to a straight line. Such distribution pattern is a distribution pattern that can indicate the relationship (correlation) between the *viral load* results against the the results of DBS Refrigerator plasma viral load.

The distribution of *viral load* results DPS 37 °C to the results of *viral load* plasma: From the graph of Distribution of viral load results DPS 37 °C against the viral load results Plasma is known that the distribution pattern of viral load results that form a straight line or a distribution pattern that is closing in on a line straight. Such distribution pattern is a distribution pattern that can indicate the relationship between viral load results DPS 37 °C against the plasma *viral load* results.

The distribution of *viral load* results DPS refrigerator against the plasma *viral load* results: From the distribution of the graph viral load results against the the results of DPS Refrigerator Plasma viral load is known that the distribution pattern of viral load results of which form a straight line or the distribution pattern close to a straight line. Such distribution pattern is a distribution pattern that can indicate the relationship (correlation) between the viral load results against the DPS *Refrigerator Plasma viral load* results.

The results of the study have been obtained, future research will be conducted on the analysis of the relationship nucleotide mutations in the coding region of the HIV genes to the possibility of drug resistance, such as some previous research in the province of Papua on other diseases [21-29] and other organisms or organelle [26-30].

CONCLUSION

From this research we can conclude that there are differences in *viral load* results DBS 37 °C, *DBS Refrigerator*, DPS 37 °C, *DPS Refrigerator* and plasma viral load results. Based on the comparison of the average of the viral load results DBS 37 °C, DBS Refrigerator, DPS 37 °C, DPS Refrigerator and viral load results Plasma, then the result of the average value of viral load refrigerator is higher than the average value the results of viral load at 37 °C, the average value of viral load results lebioh DPS higher than the average value of viral load results DBS. While the average value of the results of Plasma viral load is higher than the average value of viral load results DPS. Flow continued research focused on the use of more samples and samples from various districts in Papua, so we get a more varied sample. Tests to calculate the viral load results in difficult terrain, which does not have the equipment and personnel competent analysts can use the DBS 37 °C. Tests to calculate the viral load results can use DBS, DPS, either 37 °C or refrigerator, or plasma, should be adjusted to the conditions of the area, where power tools as well as a competent analyst.

Acknowledgements
The authors gratefully acknowledge the support of this work by the Chairperson and colleagues of Health Laboratory of Jayapura, Papua province of Indonesia, and Chairperson of the Magister Program in Biology, University of Cenderawasih that has provided research facilities.

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