

Compatibility of Procleix® -West Nile Virus (WNV) Assay in Various Anticoagulants

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INTRODUCTION

The Procleix® series of tests are based on the technology of Transcription Mediated Amplification (TMA) for the detection of viral nucleic acids. Currently 80% of the blood banks in US use the Procleix® HIV-1/HCV Assay to detect HCV and HIV-1 RNA. The Procleix® WNV assay has been developed to detect WNV RNA in blood, plasma and organ/tissue donor specimens. Blood screening under an IND began in July 2003. This study was undertaken to determine if the current conditions for HIV-1/HCV specimen collection are acceptable for WNV screening.

Evaluation of potentially interfering substances in specimens analyzed with nucleic acid testing has been explored. Endogenous substances, therapeutic drugs and commonly used anticoagulants for specimen collection can potentially interfere with amplification. Specimen handling, storage conditions and freeze/thaws are also shown to decrease levels of viral RNA. Differences in HIV-1 RNA levels has been shown depending on the type of anticoagulant. Published recommendations and current manufacturer instructions in kit suggest that plasma viral load assays are best performed with EDTA- or ACD-anticoagulated plasma that has been processed with 2h of collection. In this study we have tested the compatibility of the Procleix®-West Nile Virus (WNV) assay with various anticoagulants along with stability and freeze/thaws of collected donor specimens.

METHODS

Blood was collected from 51 donors in various anticoagulant tubes including K₃EDTA glass, K₃EDTA plastic, Sodium Citrate plastic, Sodium Citrate glass, K₂EDTA, ACD plastic, ACD glass, PPT, Heparin (referred to as Group 1), CPDA-1, CPD, CP₂D (referred to as Group 2), Serum was included in both groups. The blood from each donor for each condition collected in multiple tubes because of volume requirements was pooled into one tube and spiked with lineage-1 Vero cell cultured WNV to a final concentration of 300 copies/ml. An aliquot of spiked blood was centrifuged to separate plasma for Day 0 testing and the remaining volume of plasma was frozen. The remaining spiked blood was transferred to original collection tubes and incubated at 30°C for 1 day, followed by incubation at 25°C for the next two days. The whole blood tubes for Group 1 were incubated at 5°C for 5 additional days, and of Group 2 were incubated at 5°C for 10 additional days. All tubes at the end of Day 8 for Group1 and Day 18 for Group 2 were centrifuged to separate plasma and tested. An aliquot of the sample was frozen and thawed three times and also tested the following day. Each sample was tested in duplicate; invalid and non-reactive samples were retested.

RESULTS

The specimen stability for Procleix® -WNV assay in K₃EDTA glass, K₃EDTA plastic, Sodium Citrate plastic, Sodium Citrate glass, K₂EDTA, ACD plastic, ACD glass, PPT, Heparin, CPDA-1, CPD, CP₂D and serum for Day 0, day 8 and Day 18 was acceptable. With the exception of serum the specimen stability was acceptable following three freeze-thaws as well. Where applicable both plastic and glass tubes were acceptable. Decreased signal and occasional non-reactivity was observed following freeze-thaw of stored serum samples.

RESULTS, continued

Validation of Anticoagulants for Use with the Procleix-WNV Assay.

Tube:	Glass	Plastic
Anticoagulant:		
K ₂ EDTA		X
K ₃ EDTA	X	X
PPT		X
ACD	X	X
Sodium citrate	X	X
Heparin		X
CPD		X
CPDA-1		X
CP2D		X
Serum	X	

Anticoagulant	Mean RLU	Day 0	Day 18	Freeze/Thaw
CPDA-1 n=102	Mean RLU	0:00	0:00	0:00
	Analyte RLU %CV	22:04	12:00	2:24
	95% CI for RLU	0:00	0:00	0:00
	% positive	0:00	0:00	0:00
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
CPD n=102	Mean RLU	0:00	0:00	0:00
	Analyte RLU %CV	22:48	16:19	7:12
	95% CI for RLU	0:00	0:00	0:00
	% positive	0:00	0:00	0:00
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
CP2D n=102*, Day 0 n=100+, Days 18 and 19	Mean RLU	0:00	0:00	0:00
	Analyte RLU %CV	22:19	2:24	20:24
	95% CI for RLU	0:00	0:00	0:00
	% positive	0:00	0:00	0:00
	95% CI	96.5 to 100	96.4 to 100	96.4 to 100
Serum (plastic) n = 102	Mean RLU	0:00	0:00	0:00
	Analyte RLU %CV	12:28	21:36	22:04
	95% CI for RLU	0:00	0:00	0:00
	% positive	0:00	0:00	0:00
	95% CI	96.5 to 100	93.1 to 99.8	93.1 to 90.1

Anticoagulant	Mean RLU	Day 0	Day 8	Freeze/Thaw
K ₃ EDTA (glass) n=102	Mean RLU	1531957	1544163	1578172
	Analyte RLU %CV	21.3	17.23	9.01
	95% CI for RLU	64019	54873	28993
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
K ₃ EDTA (plastic) n=102	Mean RLU	1561241	1622438	1615825
	Analyte RLU %CV	16.34	9.91	6.04
	95% CI for RLU	50103	34136	19594
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
SODIUM CITRATE (plastic) n=102	Mean RLU	1487572	1598270	1569160
	Analyte RLU %CV	21.12	9.45	12.25
	95% CI for RLU	62298	32200	41345
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
SODIUM CITRATE (glass) n=102	Mean RLU	1499631	1602833	1596820
	Analyte RLU %CV	24.58	8.22	8.88
	95% CI for RLU	71399	27512	31831
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
K ₂ EDTA (plastic) n=102	Mean RLU	1514092	1582616	1606930
	Analyte RLU %CV	22.68	18.67	13.06
	95% CI for RLU	67515	59043	44408
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
ACD (plastic) n=102	Mean RLU	1476150	1617309	1591017
	Analyte RLU %CV	24.54	4.98	9.6
	95% CI for RLU	70817	16308	32710
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
ACD (glass) n=102	Mean RLU	1510235	1558790	1536278
	Analyte RLU %CV	22.25	12.21	15.34
	95% CI for RLU	66010	39281	48940
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
PPT (plastic) n=102	Mean RLU	1538887	1590727	1588123
	Analyte RLU %CV	19.98	14.92	12.84
	95% CI for RLU	60473	48321	41758
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
Heparin (plastic) n=102	Mean RLU	1528752	1575547	1574851
	Analyte RLU %CV	19.01	11.68	7.96
	95% CI for RLU	57122	37928	24685
	% positive	100	100	100
	95% CI	96.5 to 100	96.5 to 100	96.5 to 100
Serum (plastic) n = 102	Mean RLU	1514112	1477909	1216195
	Analyte RLU %CV	22.52	22.67	39.26
	95% CI for RLU	66770	66104	93118
	% positive	100	100	99
	95% CI	96.5 to 100	96.5 to 100	94.7 to 100

Detection of WNV collected in different anticoagulants and processed according to the package insert conditions using the Procleix WNV Assay.

* 2Replicates of 51 individual donors

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SUMMARY AND CONCLUSIONS

All of the routinely used anticoagulants for specimen collection offer acceptable specimen stability for testing with Procleix®-WNV assay. The specimen is also stable after three freeze-thaws. Specimen stability in serum was acceptable up to 8 days. Decreased specimen stability was observed in frozen and thawed stored serum samples, therefore use of this sample type outside of an investigative clinical protocol is not recommended.