Greiner Bio-One VACUETTE®
EDTA K2 Gel Tube for Molecular Preanalytics Evaluation

Device Name
Greiner VACUETTE® EDTA K2 Gel tube, 6.0mL, 13x100mm, Product Listing #456011.

Comparator Device
Becton Dickinson (BD) Vacutainer® PPT™ Plasma Preparation tube, 6.0mL, 13x100mm, Product Listing #362795.

Intended Use
The VACUETTE® EDTA K2 Gel tube provides a means of collecting and transporting an undiluted plasma specimen in a closed evacuated system. The tube contains a gel barrier material and spray-dried EDTA, yielding a ratio of 1.8mg/ml of blood when the evacuated tube is filled correctly to its fill volume.

Product Description
The VACUETTE® EDTA K2 Gel tube is used for plasma preparation and is made of plastic for the collection of venous blood, which upon centrifugation separates undiluted plasma for use in molecular diagnostic test methods (such as but not limited to PCR – Polymerase Chain Reaction), or other procedures where an undiluted plasma specimen is required as determined by the laboratory.

Sample Population
Samples were collected from 92 healthy females and males between 20 and 50 years old. These samples were "spiked" with plasma from 2 HIV or 2 HCV positive patients. Samples were quantitated for HIV and HCV RNA using the Cobas Amplicor Monitor® Kits (Roche – investigational use only in the US).

Study Design
The study design was based on recommendations made by the Office of Blood Research and Review, Division of Blood Applications, CBER.

Lower Detection Limit/Recovery Study
In this study, the HIV and HCV PCR assays' Lower Detection Limits (LDL) were studied using the appropriate WHO standard. The Greiner tube was evaluated for its impact on

Table# 1: Lower Detection Limit Study HIV Results – Summary

WHO	Tube	Overall Mean	Overall SD	Overall %CV	Mean ± 2SD
4,000 IU/mL	G	10207	2484	24.3%	5240 - 15174
	BD	6747	758	11.2%	5232 - 8263
400 IU/mL	G	645	121	18.7%	403 - 887
	BD	715	129	18.1%	456 - 973

Table# 2: Lower Detection Limit Study HCV Results – Summary

WHO	Tube	Overall Mean	Overall SD	Overall %CV	Mean ± 2SD
10,000 IU/mL	G	35060	4774	13.6%	25512 - 44608
	BD	30581	5906	19.3%	18768 - 42393
1,000 IU/mL	G	3889	406	10.4%	3078 - 4701
	BD	3295	544	16.5%	2207 - 4382

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the LDL of HIV and HCV copies in plasma relative to the BD tube. For each virus type (HIV and HCV), six whole blood samples were collected from three healthy participants into three Greiner and three BD tubes and identically spiked with the WHO HIV or HCV standards to yield three levels of virus concentration. The three levels of virus represented ten times the LDL (as claimed by the manufacturer in the assay), at the LDL, and at one-tenth the LDL. The samples were tested in five detection runs for each tube.

LDL Results: The results for the HIV testing are summarized in Table 1. Both the Greiner and BD tubes showed similar results at the lower detection limit (ANOVA, $p>0.05$). Although the 10x LDL (4,000 mIU/mL) showed a statistical difference between the two tube types ($p=1.79E-05$), the 95% limits around the Means (Mean ± 2 SD) showed significant overlap (see Table 1). NOTE: Results for 40 IU/mL sample (0.1 x LDL) were not evaluated because they were below the reportable range of the assay (400 IU/mL).

The results for the HCV testing are summarized in Table 2. Although both levels (10,000 and 1,000 mIU/mL) showed a statistical difference between the two tube types (ANOVA, $p=0.002$ and 0.030, respectively), the 95% limits around the Means showed significant overlap at both levels (see Table 2). NOTE: Results for 100 IU/mL sample (0.1 x LDL) were not evaluated because they were below the reportable range of the assay (1,000 IU/mL).

Lower Detection Limit Conclusion: The Greiner and BD tubes are substantially equivalent in lower detectable limits for the HIV and HCV assays. There were no clinically significant differences in test results with either tube type.

Recovery Study Results: Tables 3 and 4 summarize the HIV and HCV Mean values, respectively, for the five detection runs per participant per tube type at the 10 x LDL and 1 x LDL. Results for 0.1 x LDL are not presented in these tables because they were below the detection limits for the assays. It is expected

Table# 3: Recovery Study HIV Results – Summary

HIV Patient	Tube	4,000 IU/mL (10x)	400 IU/mL (1x)	Mean 10x	Mean 1x	%Recovery (%1x/10x)	Mean% Recovery (%1x/10x)
1	G	11934	716			6.0%	
2	G	8050	544			6.8%	
3	G	10638	655	10207	638	6.2%	6.3%
1	BD	6421	801			12.5%	
2	BD	6758	608			9.0%	
3	BD	7062	639	6747	683	9.0%	10.2%

Table# 4: Recovery Study HCV Results – Summary

HCV Patient	Tube	10,000 IU/mL (10x)	1,000 IU/mL (1x)	Mean 10x	Mean 1x	%Recovery (%1x/10x)	Mean% Recovery (%1x/10x)
1	G	32366	3792			11.7%	
2	G	36448	3714			10.2%	
3	G	36366	4162	35060	3889	11.4%	11.1%
1	BD	32986	3406			10.3%	
2	BD	35190	2660			7.6%	
3	BD	23566	3818	30581	3295	16.2%	11.4%

Table# 5: Equivalency Study: HIV Results (IU/mL) Greiner vs. BD EDTA Gel Tubes

Patient	Greiner	BD	Patient	Greiner	BD
1	7470	5903	21	1260	2150
2	2590	5948	22	1860	2690
3	6220	3800	23	1440	1150
4	2270	1510	24	2900	2370
5	1390	4780	25	1950	3410
6	5300	3970	26	1110	883
7	3810	1755	27	2830	1670
8	3908	1350	28	2790	2520
9	1120	1150	29	3820	5910
10	1973	1673	30	5450	6120
11	1210	1720	31	4180	2570
12	668	2850	32	1710	2600
13	4110	4600	33	4930	5110
14	2880	1480	34	2970	2560
15	2000	1780	35	2350	1320
16	933	1310	36	2800	3030
17	434	954	37	1510	1230
18	1580	921	38	1110	1410
19	1110	1410	39	2070	[0]*
20	2440	1600	40	[0]*	[0]*

*[ ]: Outlier result. Value not included in calculations.

that the Mean virus value for the 1 x LDL sample will be 10% of the 10x LDL sample. As can be seen in the tables, samples from the Greiner and BD tubes showed similar recoveries in the assays – 6.3% vs. 10.2% for HIV and 11.1% vs. 11.4% for HCV.

Recovery Study Conclusion: There was no difference in the quantitative recovery for HIV and HCV when using either tube.

Equivalency Study

This study evaluated the equivalence of HIV or HCV PCR assay test results using plasma collected in the Greiner and BD EDTA Gel tubes. For each virus type, whole blood samples were drawn in the Greiner and BD tubes from each of forty participants. Each sample set was spiked with different concentrations of HIV or HCV. All samples were centrifuged at 1500 x g for ten minutes within thirty minutes of collection and the "spiking" procedure. After centrifugation, the plasma from the two tube types was immediately subjected to HIV or HCV virus isolation and detection. Viral RNA detection was performed using the Cobas Amplicor HIV Monitor™ Test or HCV Monitor™ Test.

Equivalency Study Results: Results of the study for HIV are presented in Table 5. ANOVA calculations performed on the results showed no statistically significant difference between Greiner and BD tubes ($p>0.05$).

Results of the study for HCV are presented in Table 6. ANOVA calculations performed on the results showed no statistically significant difference between Greiner and BD tubes ($p>0.05$).

Equivalency Study Conclusion: Plasma collected in the two tubes produced substantially equivalent HIV and HCV PCR quantitative results.

Delay in Plasma Separation Study

This study used three participants for each virus type for a total of six participants. Three Greiner and three BD tubes

were collected from each participant. Each tube type per participant was spiked with HIV or HCV virus. Tubes were lcentrifuged for ten minutes at 1500 x g within 30 minutes, at 2 hours and at 24 hours after blood collection and the spiking procedure. Viral RNA isolation was performed in triplicate on each sample and virus levels were detected using the Cobas HIV Monitor™ Test or HCV Monitor™ Test.

Delay in Plasma Separation Results: Results of the HIV and HCV studies are presented in Tables 7 and 8, respectively. ANOVA was performed on the Greiner tube results to demonstrate equivalence between immediate and 2 hour results, immediate and 24 hour results, and 2 and 24 hour results. No statistically significant differences were seen in HIV or HCV results due to delay time ($p>0.05$). Additional ANOVA was performed to demonstrate equivalent results between Greiner and BD tubes at immediate separation and at 2 hour delay (recommended maximum for BD tubes). No statistically significant differences were seen in HIV or HCV results between Greiner and BD tubes ($p>0.05$).

Delay in Plasma Separation Conclusion: The two manufacturers' tubes were substantially equivalent in response to delay in plasma separation. There was no effect on HIV or HCV results when the Greiner Vacuette® EDTA K2 Gel tube was subject to delays in centrifugation up to 24 hours.

Fresh vs. Frozen/Multiple Freeze – Thaw Studies

These studies used forty participants per virus type for a total of eighty participants. Three Greiner tubes and three BD tubes were collected from each participant. Each tube was "spiked" at a different concentration of either HIV or HCV virus. All samples were centrifuged for ten minutes at 1500 x g within thirty minutes of collection and the "spiking" procedure. After centrifugation, the plasma from the three tubes were subjected to one of the following processes: 1) isolation and detection performed immediately, 2) plasma frozen once at -30°C and thawed at room temperature before isolation and detection, or 3) plasma frozen at -30°C and thawed and refrozen five times before isolation and detection. Viral RNA isolation was performed and virus levels were detected on each sample using the Cobas HIV Monitor™ Test or HCV Monitor™ Test.

Fresh vs. Frozen/Multiple Freeze-Thaw Cycles Study Results: Results of the study for HIV are summarized in Table 9. ANOVA calculations indicate that there were no statistically significant differences between fresh, 1x frozen and 5x frozen plasma results for HIV using the Greiner EDTA K2 Gel tube ($p>0.05$). There were also no statistically significant differences in the 1x or 5x frozen samples between Greiner and BD tubes ($p>0.05$).

Results of the study for HCV are summarized in Table 10. There were no statistically significant differences between fresh and 1x frozen plasma results for HCV using the Greiner EDTA Gel tube ($p>0.05$); there were statistically significant differences between 1x and 5x frozen plasma ($p=0.043$). There were no statistically significant differences between Greiner and BD tubes in the 1x or 5x frozen samples ($p>0.05$).

Fresh vs. Frozen/Multiple Freeze-Thaw Cycles Study Conclusion: There was no difference in HIV or HCV results within or between the two tube types for fresh versus 1x frozen plasma samples or when plasma samples were exposed to five freeze/thaw cycles for HIV or one freeze/thaw cycle for HCV.

References

1. Greiner Bio-One. Greiner VACUETTE® EDTA K2 Gel Tube 510 (k) Summary. Monroe, NC. June 2001.

2. Greiner Bio-One. Greiner VACUETTE® EDTA K2 Gel Tube Product Insert. Kremsmunster, Austria. April 2001.

Table# 6: Equivalency Study: HCV Results (IU/mL) Greiner vs. BD EDTA Gel Tubes

Patient	Greiner	BD	Patient	Greiner	BD
1	83400	104000	21	176400	99400
2	151000	84000	22	61133	67667
3	120000	124000	23	87267	67667
4	103000	91300	24	113400	83067
5	59900	67100	25	48533	73267
6	109000	79500	26	46620	42187
7	91900	63600	27	61133	77933
8	73900	55400	28	101733	164267
9	65700	70100	29	174067	141867
10	49400	64300	30	89133	103000
11	91600	86600	31	86333	81600
12	93600	78900	32	126933	146533
13	118067	147467	33	103600	119000
14	116667	177333	34	63600	66900
15	219800	147933	35	62067	80733
16	169867	134867	36	61600	61600
17	123000	114000	37	91467	55533
18	186667	105933	38	88200	59733
19	115733	63000	39	66733	77933
20	88200	68133	40	67100	55400

Table# 7: Delay in Plasma Separation Study HIV Results – Summary

Time to Centrifugation*	Patient	Tube	Mean Result (IU/mL)	Std Dev (IU/mL)	%CV	Mean Across Times	%CV Across Times
A	1	G	1167	310	26.6%		
B	1	G	2234	807	36.1%		
C	1	G	1898	802	42.3%	1616	24.3%
A	1	BD	2033	316	15.5%		
B	1	BD	2099	975	46.5%		
C	1	BD	1602	461	28.8%	1911	14.1%
A	2	G	783	264	33.7%		
B	2	G	1058	330	31.1%		
C	2	G	1358	130	9.5%	1066	27.0%
A	2	BD	1615	113	7.0%		
B	2	BD	1120	94	8.4%		
C	2	BD	1143	467	40.9%	1293	21.6%
A	3	G	1881	282	15.0%		
B	3	G	1887	838	44.4%		
C	3	G	587	79	13.5%	1452	51.6%
A	3	BD	1486	298	20.0%		
B	3	BD	1265	99	7.8%		
C	3	BD	569	119	20.9%	1107	43.2%

*A=centrifugation within 30 minutes; B=centrifugation at 2 hours; C=centrifugation at 24 hours.

Table# 8: Delay in Plasma Separation Study
HCV Results – Summary

Time to Centrifugation*	Patient	Tube	Mean Result	Std Dev (IU/mL)	%CV (IU/mL)	Mean Across Times	%CV Across Times
A	1	G	93667	9319	9.9%		
B	1	G	130533	21313	16.3%		
C	1	G	96613	27985	29.0%	106938	19.2%
A	1	BD	76333	11566	15.2%		
B	1	BD	101253	7983	7.9%		
C	1	BD	94100	20451	21.7%	90562	14.2%
A	2	G	86500	5756	6.7%		
B	2	G	88693	28168	31.8%		
C	2	G	80444	11864	14.7%	79828	8.8%
A	2	BD	85807	5368	6.3%		
B	2	BD	88700	13610	15.3%		
C	2	BD	101480	9611	9.5%	91996	9.1%
A	3	G	46973	2449	5.2%		
B	3	G	56920	17859	31.4%		
C	3	G	53647	3491	6.5%	49117	8.0%
A	3	BD	66587	16366	24.6%		
B	3	BD	74513	20424	27.4%		
C	3	BD	75550	695	0.9%	72217	6.8%

*A=centrifugation within 30 minutes; B=centrifugation at 2 hours; C=centrifugation at 24 hours.

Table# 9: Fresh vs. Frozen/Multiple Freeze-Thaw Cycles Study
HIV Results – Summary

Tube		Fresh	1 x Frozen	5x Frozen
Greiner	Mean (IU/mL)	2627	2790	2129
	SD (IU/mL)	1617	1587	1206
	CV (%)	61.5%	56.9%	56.6%
	n	39*	40	39*
BD	Mean (IU/mL)	2610	2878	2331
	SD (IU/mL)	1599	1688	1326
	CV (%)	61.3%	58.6%	56.9%
	n	38**	40	40

* One "no result" ** Two "no results"

Table# 10: Fresh vs. Frozen/Multiple Freeze-Thaw Cycles Study
HCV Results – Summary

Tube		Fresh	1 x Frozen	5x Frozen
Greiner	Mean (IU/mL)	100186	90733	79091
	SD (IU/mL)	41045	27384	23189
	CV (%)	41.0%	30.2%	29.3%
	n	40	40	40
BD	Mean (IU/mL)	91319	83791	75563
	SD (IU/mL)	33808	27412	23624
	CV (%)	37.0%	32.7%	31.3%
	n	40	40	40