Evaluation of VACUETTE® K_2EDTA Gel Tubes for Molecular Diagnostics

Background:

Greiner Bio-One, Austria has sold plastic evacuated tubes (VACUETTE®) for venous blood collection since 1986.

The interior of the VACUETTE® K_2EDTA Gel tubes is coated with K_2EDTA. EDTA binds calcium ions and blocks the coagulation cascade. These tubes, which upon centrifugation separate undiluted plasma, are used for testing plasma in molecular diagnostics and viral load detection. The VACUETTE® K_2EDTA Gel tube combines a spray-dried anticoagulant and a gel material, which separates erythrocytes, granulocytes, lymphocytes and monocytes from the supernatant.

Samples can be collected, processed and transported in the primary tube which reduces exposure to blood borne pathogens, e.g. HIV, HCV, at the collection and sample processing sites.

Study Objective:

A clinical evaluation was conducted to compare the performance of the Greiner Bio-One VACUETTE® K_2EDTA Gel tube to the Becton Dickinson Vacutainer® PPT™ Gel tube. The following studies were performed:

a) Lower Detection Limit/ Recovery Study

b) Equivalency Study

c) Delay in Plasma Separation Study

d) Multiple Freeze-Thaw Cycles Study

Study design:

The study design was based on recommendations made by reviewers from the FDA Center for Biologics Evaluation and Research, Division of Blood Applications (CBER).

The following tube types were used in this study:

Sample ID	Manufacturer	Description
A	Greiner Bio-One	VACUETTE® 6 mL K_2EDTA Gel tube
B	BD	Vacutainer® 6 mL PPT™ Plasma Preparation tube

Venous blood was 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 patient. Samples were quantitated for HIV and HCV RNA using the Roche Diagnostics COBAS AMPLICOR MONITOR® tests.

a) **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 VACUETTE® tube was evaluated for its impact on the LDL of HIV and HCV copies in plasma relative to the BD Vacutainer® PPT™ tube. For each virus type (HIV and HCV), six whole blood samples were collected from three healthy participants into three Greiner VACUETTE® and three BD Vacutainer® 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.

b) Equivalency Study

This study evaluated the equivalence of HIV or HCV PCR assay test results using plasma collected in the Greiner VACUETTE® K_2EDTA Gel and BD Vacutainer® PPT™ tubes.

For each virus type, whole blood samples were drawn in the Greiner VACUETTE® and BD Vacutainer® 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 Roche Diagnostics COBAS AMPLICOR HIV or HCV MONITOR® Tests.

c) Delay in Plasma Separation Study

This study used three participants for each virus type for a total of six participants. Three Greiner VACUETTE® and three BD Vacutainer® tubes were collected from each participant. Each tube type per participant was spiked with HIV or HCV virus. Tubes were centrifuged 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 Roche Diagnostics COBAS AMPLICOR HIV or HCV MONITOR® Tests.

d) Multiple Freeze-Thaw Cycles Study

These studies used forty participants per virus type for a total of eighty participants. Three Greiner VACUETTE® tubes and three BD Vacutainer® 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 Roche Diagnostics COBAS AMPLICOR HIV or HCV MONITOR® Tests.

Conclusions:

a) Lower Detection Limit/ Recovery Study

The Greiner VACUETTE® K_2EDTA Gel and BD Vacutainer® PPT™ 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.

Equivalency Study

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

Delay in Plasma Separation Study

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® K_2EDTA Gel tube was subject to delays in centrifugation up to 24 hours.

b) Multiple Freeze-Thaw Cycles Study

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.

Results/Discussion:

a) Lower Detection Limit/ Recovery Study

The results for the HIV testing are summarized in Table 1 (see Annex). Both the Greiner and BD tubes showed similar results at the lower detection limit (ANOVA, p>0.05). Although the 10x LDL (4000 IU/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 (see Annex). Although both levels (10000 and 1000 IU/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 (1000 IU/mL).

Tables 3 and 4 (see Annex) 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 that the Mean virus value for the 1 x LDL sample will be 10% of the 10 x 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.

b) Equivalency Study

Results of the study for HIV are presented in Table 5 (see Annex). 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 (see Annex). ANOVA calculations performed on the results showed no statistically significant differences between Greiner and BD tubes (p>0.05).

c) Delay in plasma separation study

The results of the HIV and HCV studies are presented in Tables 7 and 8 (see Annex), 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 K_2EDTA and BD PPT™ tubes (p>0.05).

d) Multiple Freeze-Thaw Cycles Study

The results of the study for HIV are summarized in Table 9 (see Annex). There were no statistically significant differences between fresh, 1x frozen and 5x frozen plasma results for HIV using the Greiner VACUETTE® K_2EDTA Gel tube (p>0.05). There were also no statistically significant differences in the 1x or 5x frozen samples between Greiner VACUETTE® and BD Vacutainer® tubes (p>0.05).

The results of the study for HCV are summarized in Table 10 (see Annex). There were no statistically significant differences between fresh and 1x frozen plasma results for HCV using the Greiner VACUETTE® K_2EDTA 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 VACUETTE® K_2EDTA Gel and BD Vacutainer® PPT™ tubes in the 1x or 5x frozen samples (p>0.05).

References:

(1) Greiner Bio-One, Greiner VACUETTE® K2EDTA Gel Tube 510 (k) Summary. Monroe, NC.June 2001.

(2) Greiner Bio-One, Evacuated Blood Collection System For In Vitro Diagnostic Use. Product Insert. Kremsmünster, Austria (2001).

Annex/ Results in detail:

Table 1: LDL Study, HIV Results - Summary

WHO	Tube	Overall Mean	Overall SD	Overall %CV	Mean +/- 2SD
4000 IU/mL	Greiner	10207	2484	24.3	5240 - 15174
	BD	6747	758	11.2	5232 - 8263
400 IU/mL	Greiner	645	121	18.7	403 - 887
	BD	715	129	18.1	456 - 973

Table 2: LDL Study, HCV Results - Summary

WHO	Tube	Overall Mean	Overall SD	Overall %CV	Mean +/- 2SD
10000 IU/mL	Greiner	35060	4774	13.6	25512 - 44608
	BD	30581	5906	19.3	18768 - 42393
1000 IU/mL	Greiner	3889	406	10.4	3078 - 4701
	BD	3295	544	16.5	2207 - 4382

Table 3: Recovery Study, HIV Results - Summary

Patient	Tube	4000 IU/mL (10x)	Mean (10x)	400 IU/mL (1x)	Mean (1x)	%Recovery (%1x/10x)	Mean %Recovery (%1x/10x)
1	Greiner	11934	10207	716	638	6.0	6.3
2	Greiner	8050		544		6.8	
3	Greiner	10638		655		6.2	
1	BD	6421	6747	801	683	12.5	10.2
2	BD	6758		608		9.0	
3	BD	7062		639		9.0	

Table 4: Recovery Study, HCV Results - Summary

Patient	Tube	10000 IU/mL (10x)	Mean (10x)	1000 IU/mL (1x)	Mean (1x)	%Recovery (%1x/10x)	Mean %Recovery (%1x/10x)
1	Greiner	32366	35060	3792	3889	11.7	11.1
2	Greiner	36448		3714		10.2	
3	Greiner	36366		4162		11.4	
1	BD	32986	30581	3406	3295	10.3	11.4
2	BD	35190		2660		7.6	
3	BD	23566		3818		16.2	

Table 5: Equivalency Study, HIV Results [IU/mL] – Greiner K_2 EDTA vs. BD PPT^{TM} 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

Table 5: Equivalency Study, HIV Results [IU/mL] – Greiner K_2 EDTA vs. BD PPT™ Tubes

Patient	Greiner	BD		Patient	Greiner	BD
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.

Table 6: Equivalency Study, HCV Results [IU/mL] – Greiner K_2 EDTA vs. BD PPT™ 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 centifugation	Patient	Tube	Mean Result (IU/mL)	Std Dev (IU/mL)	%CV	Mean Across Times	%CV Across Times
30 min.	1	Greiner	1167	310	26.6	1616	24.3
2 h	1	Greiner	2234	807	36.1		
24 h	1	Greiner	1898	802	42.3		
30 min.	1	BD	2033	316	15.5	1911	14.1
2 h	1	BD	2099	975	46.5		
24 h	1	BD	1602	461	28.8		
30 min.	2	Greiner	783	264	33.7	1066	27.0
2 h	2	Greiner	1058	330	31.1		
24 h	2	Greiner	1358	130	9.5		
30 min.	2	BD	1615	113	7.0	1293	21.6
2 h	2	BD	1120	94	8.4		
24 h	2	BD	1143	467	40.9		
30 min.	3	Greiner	1881	282	15.0	1452	51.6
2 h	3	Greiner	1887	838	44.4		
24 h	3	Greiner	587	79	13.5		
30 min.	3	BD	1486	298	20.0	1107	43.2
2 h	3	BD	1265	99	7.8		
24 h	3	BD	569	119	20.9		

Table 8: Delay in Plasma Separation Study, HCV Results - Summary

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

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

Tube		Fresh	1x 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

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

Tube		Fresh	1x 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