# Evaluation of VACUETTE® $K_3$EDTA and $K_2$EDTA Evacuated Blood Collection Tubes for Viral Marker Testing

**Background:**

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

VACUETTE® Tubes, Holders and Needles are used together as a system for the collection of venous blood. VACUETTE® tubes are used to collect, transport and process blood for testing serum, plasma or whole blood in the clinical laboratory. The tube contains spray-dried EDTA, yielding a ratio of 1.8mg/mL of blood when the evacuated tube is filled correctly to its fill volume. The EDTA binds calcium ions which blocks the coagulation cascade. [1,2]

VACUETTE® EDTA tubes are used for testing whole blood in the clinical laboratory and may be used for viral marker testing.

**Study Objective:**

A clinical evaluation was carried out to compare the performance of the Greiner Bio-One VACUETTE® $K_2$EDTA and VACUETTE® $K_3$EDTA tubes to the Becton Dickinson Vacutainer® Glass No Additive, Non-Siliconized and No Additive Non-Coated Interior Tubes for viral marker testing.

**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 No. | Description |
|---|---|
| 1 | Greiner VACUETTE® $K_2$EDTA, 6 mL, 13x100mm tube |
| 2 | Greiner VACUETTE® $K_3$EDTA, 6 mL, 13x100mm tube |
| 3 | Becton Dickinson Vacutainer™ Glass No Additive, Non-Siliconized, 7 ml, 13x100mm tube, (comparator device) |
| 4 | Becton Dickinson Vacutainer™ Glass No Additive, Non-Coated Interior, 7 ml, 13x100mm tube (comparator device) |

Blood specimens were obtained using each site's standard phlebotomy techniques, referencing Standard Operating Procedures and OSHA's safety requirements for blood collection. The order of draw was randomized.

The following tubes were drawn from each donor at the two Donor Centers:

1) one Greiner VACUETTE® $K_2$EDTA, 6 mL, 13x100mm tube

2) one Greiner VACUETTE® K3EDTA, 6 mL, 13x100mm tube and

3) one Becton Dickinson Vacutainer™ Glass No Additive, Non-Coated Interior, 7 mL, 13x100mm tube

In addition, two Greiner 13x100 VACUETTE® $K_2$EDTA 6 mL half evacuated tubes (3mL) to simulate partial draw and two Greiner 13x100 VACUETTE® $K_3$EDTA 6 mL half evacuated to simulate partial draw, were collected from 10 healthy donors and 6 positive patients at Donor Center #2.

The following three tubes were drawn from each individual at the Reference Laboratories:

1) one Greiner VACUETTE® $K_2$EDTA, 6 mL, 13x100mm tube

2) one Greiner VACUETTE® $K_3$EDTA, 6 mL, 13x100mm tube and

3) one Becton Dickinson Vacutainer™ Glass No Additive, Non-Siliconized, 7 mL, 13x100mm tube

A. Donor Center - #1:

1) 50 apparently healthy donors

B. Donor Center - Site #2:

1) 50 apparently healthy donors (full draw tubes)

2) Subset: 10 healthy donors (full and partial draw) for delayed testing (Day 0 and Day 7)

3) 6 patients positive for one viral marker (full and partial draw) for delayed testing (Day 0 and Day 7)

4) 6 patients positive for one viral marker (full and partial draw) for delayed tube mixing (Day 0 and Day 7)

C. Reference Laboratories – Site #3:

1) 50 known positive patients for HBV, HCV and/or HIV

The tubes were gently mixed using 8-10 complete inversions immediately following blood collection. Tubes were centrifuged using the laboratory's standard procedure to separate cellular elements completely from the plasma.

The used instrumentation, assays and tests can be found in the Annex (see Table # 1).

**Conclusion:**

The Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA tubes demonstrated substantial equivalence to the Becton Dickinson Vacutainer™ Glass No Additive, Non-Coated or No Additive Non-Siliconized tubes in terms of agreement for the viral marker testing results with blood donors and antibody positive individuals. In addition, the tubes demonstrated similar results when delayed testing was performed (Day 7) and compared to initial testing (Day 0), when testing was performed on partially filled tubes and compared to fully drawn tubes, and when testing was performed on tubes that were subjected to delayed mixing.

**Results/Discussion:**

**Anti- HBs (detects HBsAg)**

Testing for HBsAg was performed on samples from 100 apparently healthy adults [AHA] (Sites #1 and 2) and 50 patients (Site #3), using the Abbott AUSZYME® MONOCLONAL Enzyme Immunoassay (Sites #1 and 3) or the ORTHO® Antibody to HBsAg ELISA Test System 2 (Site #2). There were 100 non-reactive AHA samples and 4 initially reactive patient samples. The initially reactive results were repeated in duplicate. All results for the Greiner tubes were 100% concordant with the BD tubes.

**HBcAg (detects total anti-HBc)**

Testing for total anti-HBc was performed on samples from 100 apparently healthy adults [AHA] (Sites #1 and 2) and 55 patients (Sites #2 and 3), using the Abbott CORZYME® Enzyme Immunoassay (Sites #1 and 3) or the ORTHO® HBc ELISA Test System (Site #2). There were 94 non-reactive AHA samples and 31 initially reactive patient samples. The initially reactive results were repeated in duplicate. All results for the Greiner tubes were 100% concordant with the BD tubes.

**HCV (detects anti-HCV)**

Testing for anti-HCV was performed on samples from 100 apparently healthy adults [AHA] (Sites #1 and 2) and 54 patients (Sites #2 and 3), using the Abbott HCV EIA 2.0 Enzyme Immunoassay (Sites #1 and 3) or the ORTHO® HCV Version 3.0 ELISA Test System (Site #2). There were 100 non-reactive AHA samples and 31 initially reactive patient samples. The initially reactive results were repeated in duplicate and were 100% concordant between the Greiner and BD tubes.

**HIV 1/2 (detects anti-HIV 1/2)**

Testing for anti-HIV 1/2 was performed on samples from 100 apparently healthy adults [AHA] (Sites #1 and 2) and 50 patients (Site #3), using the Abbott HIVAB™ HIV-1/HIV-2 Enzyme Immunoassay (Sites #1 and 3) or the BIO-RAD Genetic Systems™ HIV-1/HIV-2 Peptide EIA (Site #2). There were 100 non-reactive AHA samples and 30 initially reactive patient samples. The initially reactive results were repeated in duplicate. All results for the Greiner tubes were 100% concordant with the BD tubes.

**HTLV I/II (detects anti-HTLV I/II)**

Testing for anti-HTLV I/II was performed on samples from 100 apparently healthy adults [AHA] (Sites #1 and 2) and 50 patients (Site #3), using the Abbott HTLV-I/HTLV-II Enzyme Immunoassay (Sites #1 and 3) or the Organon Teknika HTLV-I/II Vironostika® Microelisa System (Site #2). There were 100 non-reactive AHA samples and 3 initially reactive patient samples. The initially reactive results were repeated in duplicate. All results for the Greiner tubes were 100% concordant with the BD tubes.

**Anti-CMV (detects antibodies to CMV)**

Testing for anti-CMV was performed on samples from 100 apparently healthy adults [AHA] (Sites #1 and 2) and 50 patients (Site #3).

The Olympus® PK™ CMV-PA System was used at Site #1, with the Immucor Capture-CMV® Microplate Assay for some sample testing. Site #2 and Site #3 used the Abbott Commander® System.

There were 37 negative AHA samples and 41 positive patient samples. All results from the patient population and all but one from the AHA population showed 100% concordance between the Greiner tubes and the BD tubes. One AHA sample was negative by the Greiner $K_3$EDTA tube and the BD tube, but was positive by the Greiner $K_2$EDTA tube. The Olympus PK™ result for the Greiner $K_2$EDTA tube printed out as a positive result with question marks, indicating that this questionable result could be caused by a dirty well or old plate. The laboratory takes a conservative position on these types of results and reports them as positive with no repeat testing.

**Syphilis Screening**

The STS screening test for Syphilis was performed on samples from 100 apparently health adults [AHA] (Site #1 and 2) and 50 patients (Site #3). Sites #1 and 2 used the Olympus PK™TP System, a fully automated hemagglutinaltion assay in which the instrument reads the cell patterns. Site #3 used the BioKit Sure-Vue™ RPR Assay, a manual charcoal agglutination assay in which the laboratorian reads the aggregate patterns.

There were 99 negative AHA samples and 6 positive patient samples. The initially reactive result from the AHA population was confirmed reactive by TPA testing. In the AHA population, all results for the Greiner tubes were 100% concordant with the BD tube. In the patient population, there were 2 samples (GR07 and GR08) which were discordant: GR07 was non reactive in the BD tube but reactive in the Greiner $K_2$EDTA and $K_3$EDTA tubes; GR08 was non reactive in the BD tube and the Greiner $K_3$EDTA tube but was reactive in the Greiner $K_2$EDTA tube. Testing was not repeated on these samples. However, testing of new samples drawn from the two patients showed negative results which were 100% concordant between the Greiner and BD tubes.

**ALT Testing**

Testing for alanine aminotransferase (ALT) was performed on samples from 100 apparently healthy adults [AHA] (Sites #1 and 2) and 50 patients (Site #3). The Abbott AEROSET® Clinical Chemistry System was used at Site #1, the Olympus® AU640e™ Chemistry Immuno Analyzer (ALT Slides) was used at Site #2, and the Ortho-Clinical Diagnostics VITROS® 950 Chemistry System was used at Site #3.

Samples were considered negative if the ALT concentration was within the manufacturers' published expected ranges. The expected ranges were ≤40 U/mL for the Abbott assay, 7-52 U/mL for the Olympus assay, and 11-66 U/L for the Ortho assay. There were 96 negative AHA samples and 13 positive patient samples. All results for the Greiner tubes were 100% concordant with the BD tubes.

**Full and Partial-Draw Study**

A study was conducted for informational purposes only to evaluate the performance of the viral marker tests in samples simulating partially drawn tubes. The testing was performed on a subset of 10 AHAs and 6 patients at Site #2 using the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA tubes at partial draw and full draw and the BD Vacutainer® Glass No Additive Non-Coated tube at full draw.

Samples from five of the AHAs were tested for detection of HBsAg, total anti-HBc, anti-HCV, anti-CMV, and ALT. The results are summarized in Table #2 (see Annex). Of the 5 AHAs tested, all 5 were negative for HBsAg, anti-HCV, and ALT, one was repeatedly reactive for total anti-HBc, and all 5 were positive for anti-CMV. There was 100% concordance between results obtained with the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA partial draw tubes as compared to the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA full draw tubes and the BD full draw tubes.

Samples from an additional five AHAs were tested for detection of HIV 1/2, HTLV I/II, and STS. The results are summarized in Table #3 (see Annex). Of the 5 AHAs tested, all 5 were negative for anti-HIV 1/2, HTLV I/II, and STS. There was 100% concordance between results obtained with the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA partial draw tubes as compared to the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA full draw tubes and the BD full draw tubes.

The samples from five of the six patients were tested for detection of total anti-HBc and the samples from four of the six patients were tested for detection of anti-HCV. The results are summarized in Table #4 (see Annex). Of the five patients tested for total anti-HBc, all 5 were repeatedly reactive. Of the four patients tested for anti-HCV, 2 were repeatedly reactive.

The two anti-HCV repeatedly reactive samples were confirmed by HCV RIBA. One result was confirmed positive by all tubes and the second result was confirmed positive in the Greiner VACUETTE® $K_2$EDTA partial draw tube and indeterminate in the Greiner VACUETTE® $K_3$EDTA partial draw tube. The results were indeterminate for this sample in the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA full draw tubes. This may have been due to a difference in timing of the HCV RIBA confirmatory testing for these samples. The confirmatory testing was performed four days after the screening for the first sample and seven days after the screening for the second sample. In addition, the difference between an indeterminate and positive result was due to the grading of the c33c band, meaning 1+ or P/N. There was no trend in the result differences and no change in result interpretation (i.e., positive to negative or negative to positive). Therefore, it can be concluded that the differences observed were due to the inherent variability in the HCV RIBA methodology and the subjective nature of the band intensity grading.

**Delay in Testing**

The Full Draw/ Partial Draw Study was repeated after storage of the samples on the red cells at 2-8°C for 7 days from date of collection. This study was performed for information purposes only, to evaluate the performance of the viral marker tests on samples in which the plasma was not separated from the red cells. This is not a recommended procedure. The viral marker assay manufacturers' package inserts state to remove the plasma from the red cells as soon as possible. The results were concordant between Day 0 and Day 7, with the exception of the HCV RIBA, results on one of the repeatedly reactive patients (Patient #726), which is summarized in Table #5 (see Annex). The BD tube, as well as the Greiner tubes, was inconsistent in the results. There was no trend in the result differences and no change in result interpretation (i.e., positive to negative or negative to positive). Therefore, it can be concluded that the differences observed were due to the inherent variability in the HCV RIBA methodology and the subjective nature of the band intensity grading.

**Delay in Mixing**

A study was conducted at Site #2 to evaluate the effect on viral marker results of delayed tube mixing after collection. The participants in the study were the 6 antibody positive patients. Two Greiner full draw tubes for each tube type were collected from each patient. One tube was mixed immediately after collection, per Greiner instructions. The second tube was laid on the table for 10 minutes immediately after collection and then mixed ("delayed mix"). The samples were tested for total anti-HBc or anti-HCV, depending on the known antibody present, on Day 0 and after storage at 2-8°C for 7 days from date of collection. The results are summarized in Table #6 (see Annex). One total anti-HBc repeatedly reactive sample was negative in the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA delayed mix tubes on Day 0 but repeatedly reactive in those tubes on Day 7. This sample had a low reactive absorbance reading (near the cutoff).

In the anti-HCV screening test, there was 100% concordance between results obtained with the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA mixed and delayed mix tubes as compared to the BD mixed tubes. There were two initially reactive and repeatedly reactive samples, which were concordant in all tubes.

The two anti-HCV repeatedly reactive samples were confirmatory tested by HCV RIBA. On Day 0, the first sample was confirmed positive by all tubes (mixed and delayed mix). The second sample (Patient #726) was confirmed positive in the BD tube (mixed) and indeterminate in the Greiner VACUETTE® $K_2$EDTA and $K_3$EDTA tubes (mixed and delayed mix).

Testing on these mixed and delayed mix tubes for the two anti-HCV confirmed positive patients was repeated on Day 7. On the first sample, the mixed and delayed mixed samples were all confirmed positive.

The mixed and delayed mix samples from the second sample (Patient #726) were positive in the Greiner VACUETTE® $K_3$EDTA tube and indeterminate in the Greiner VACUETTE® $K_2$EDTA tube at Day 7. The results are summarized in Table #7 (see Annex). The Greiner tubes and the BD tube were inconsistent in the results. There was no trend in the result differences and no change in result interpretation (i.e., positive to negative or negative to positive). Therefore, it can be concluded that the differences observed were due to the inherent variability in the HCV RIBA methodology and the subjective nature of the band intensity grading.

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**Annex / Results in detail:**

| Table #1 | | | |
|---|---|---|---|
| **Instrumentation, Assays, Tests** | | | |
| **Tests** | **Site #1 Donor Center** | **Site #2 Donor Center** | **Site #3 Donor Center** |
| Anti-HBs (detects HBsAg) | Abbott AUSZYME® MONOCLONAL Enzyme Immunoassay Abbott Commander® System | ORTHO® Antibody to HBsAg ELISA Test System 2 Ortho® Summit™ Processor | Abbott AUSZYME® MONOCLONAL Enzyme Immunoassay Abbott Commander® System |
| HbcAg (detects total anti-HBc) | Abbott CORZYME® Enzyme Immunoassay Abbott Commander® System | ORTHO® HBc ELISA Test System Ortho® Summit™ Processor | Abbott CORZYME® Enzyme Immunoassay Abbott Commander® System |
| HCV (detects anti-HCV) | Abbott HCV EIA 2.0 Enzyme Immunoassay Abbott Commander® System | ORTHO® HCV Version 3.0 ELISA Test System Ortho® Summit™ Processor | Abbott HCV EIA 2.0 Enzyme Immunoassay Abbott Commander® System |
| HCV RIBA (Confirmatory) | | Chiron™ RIBA™ HCV 3.0 SIA Ortho® Summit™ Processor | |
| HIV 1/2 (detects anti-HIV 1/2) | Abbott HIV AB™ HIV-1/ HIV-2 (rDNA) EIA Enzyme Immunoassay Abbott Commander® System | BIO-RAD Genetic Systems™ HIV-1 / HIV-2 Peptide EIA Ortho® Summit™ Processor | Abbott HIV AB™ HIV-1/ HIV-2 (rDNA) EIA Enzyme Immunoassay Abbott Commander® System |
| HTLV I/II (detects anti-HTLV I/II) | Abbott HTLV-I/HTLV-II Enzyme Immunoassay Abbott Commander® System | Organon Teknika Vironstika® HTLV I/II Microelisa System Ortho® Summit™ Processor | Abbott HTLV-I/HTLV-II Enzyme Immunoassay Abbott Commander® System |
| Anti-CMV (detects antibodies to CMV, total) | Olympus® PK™TP CMV-PA System Olympus® PK7200™ Automated Microplate System Immucor Capture CMV® Microplate Assay | Abbott CMV Total AB EIA (List#6163) Abbott Commander® System | Abbott CMV Total AB EIA Abbott Commander® System |
| Syphilis Screen | Olympus® PK™ TP System Olympus® PK7200™ Automated Microplate System | Olympus® PK™ TP System Olympus® PK7200™ Automated Microplate System | Biokit Sure-Vue™ RPR |
| RPR/TPA (Confirmatory) | | Fujirebio Diagnostics Serodia® TP*PA | |
| ALT | Abbott AEROSET® Clinical Chemistry System | Olympus® AU640e™ Chemistry Immuno Analyzer | Ortho-Clinical Diagnostics VITROS® 950 Chemistry System |

| Table #2 | | | | | | |
|---|---|---|---|---|---|---|
| **Results from First Five AHAs** | | | | | | |
| AHA | Tube | HBsAg | Total Anti-HBc | Anti-HCV | Anti-CMV | ALT |
| n = 5 | BD full | neg | 1(RR[e]) | neg | pos | neg |
| | Gr $K_2$ full[a] | neg | 1(RR[e]) | neg | pos | neg |
| | Gr $K_2$ half[b] | neg | 1(RR[e]) | neg | pos | neg |
| | Gr $K_3$ full[c] | neg | 1(RR[e]) | neg | pos | neg |
| | Gr $K_3$ half[d] | neg | 1(RR[e]) | neg | pos | neg |

[a] Gr $K_2$ full = Greiner $K_2$EDTA full draw tube
[b] Gr $K_2$ half = Greiner $K_2$EDTA partial draw/ half evacuated tube
[c] Gr $K_3$ full = Greiner $K_3$EDTA full draw tube
[d] Gr $K_3$ half = Greiner $K_3$EDTA partial draw/ half evacuated tube
[e] RR = repeatedly reactive

| Table #3 | | | | |
|---|---|---|---|---|
| **Results from Second Five AHAs** | | | | |
| AHA | Tube | HIV 1/2 | HTLV I/II | STS |
| n = 5 | BD full | neg | neg | neg |
| | Gr $K_2$ full[a] | neg | neg | neg |
| | Gr $K_2$ half[b] | neg | neg | neg |
| | Gr $K_3$ full[c] | neg | neg | neg |
| | Gr $K_3$ half[d] | neg | neg | neg |

[a] Gr $K_2$ full = Greiner $K_2$EDTA full draw tube
[b] Gr $K_2$ half = Greiner $K_2$EDTA partial draw/ half evacuated tube
[c] Gr $K_3$ full = Greiner $K_3$EDTA full draw tube
[e] Gr $K_3$ half = Greiner $K_3$EDTA partial draw/ half evacuated tube

| Table #4 | | | |
|---|---|---|---|
| **Full/Partial Draw Study-Patient Results** | | | |
| Patients | Tube | Total anti-HBc n = 5 | Anti-HCV n = 4 |
| n = 6 | BD full | 5 (RR[e]) | 2(RR[e]) |
| | Gr $K_2$ full[a] | 5 (RR[e]) | 2(RR[e]) |
| | Gr $K_2$ half[b] | 5 (RR[e]) | 2(RR[e]) |
| | Gr $K_3$ full[c] | 5 (RR[e]) | 2(RR[e]) |
| | Gr $K_3$ half[d] | 5 (RR[e]) | 2(RR[e]) |

[a] Gr $K_2$ full = Greiner $K_2$EDTA full draw tube
[b] Gr $K_2$ half = Greiner $K_2$EDTA partial draw/ half evacuated tube
[c] Gr $K_3$ full = Greiner $K_3$EDTA full draw tube
[e] Gr $K_3$ half = Greiner $K_3$EDTA partial draw/ half evacuated tube
[e] RR = repeatedly reactive

| Table #5 | | |
|---|---|---|
| **HCV RIBA Results on Patient #726-Delayed Testing** | | |
| Tube | Day 0 Result | Day 7 Result |
| Greiner $K_2$EDTA Full Draw | indeterminate | indeterminate |
| Greiner $K_2$EDTA Half Draw | positive | indeterminate |
| Greiner $K_3$EDTA Full Draw | indeterminate | positive |
| Greiner $K_3$EDTA Half Draw | indeterminate | positive |
| BD full Draw | positive | indeterminate |

| Table #6 | | | | | |
|---|---|---|---|---|---|
| **Mixed/Delayed Mix Study-Patient Results** | | | | | |
| | | Total anti-HBc n = 5 | | Anti-HCV n = 4 | |
| Patients | Tube | Day 0 | Day 7 | Day 0 | Day 7 |
| n = 6 | BD mixed | 5(RR[a]) | 5(RR[a]) | 2(RR[a]) | 2(RR[a]) |
| | Gr $K_2$ mixed | 5(RR[a]) | 5(RR[a]) | 2(RR[a]) | 2(RR[a]) |
| | Gr $K_2$ delayed mix | 4(RR[a]) | 5(RR[a]) | 2(RR[a]) | 2(RR[a]) |
| | Gr $K_3$ mixed | 5(RR[a]) | 5(RR[a]) | 2(RR[a]) | 2(RR[a]) |
| | Gr $K_3$ delayed mix | 4(RR[a]) | 5(RR[a]) | 2(RR[a]) | 2(RR[a]) |

[a]RR = repeatedly reactive

| Table #7 | | | | |
|---|---|---|---|---|
| **HCV RIBA Results on Patient #726- Delayed Mixing** | | | | |
| | Day 0 Result | | Day 7 Result | |
| Tube | Mixed Tube | "Delayed Mix" Tube | Mixed Tube | "Delayed Mix" Tube |
| Greiner $K_2$EDTA Full draw | IND | IND | IND | IND |
| Greiner $K_3$EDTA Full draw | IND | IND | POS | POS |
| BD Full Draw | POS | N/A | IND | N/A |

IND = Indeterminate
POS = Positive
N/A = Not applicable