Greiner Bio-One VACUETTE®
EDTA K3 and EDTA K2 Evacuated Blood Collection Tubes Evaluation Using the Olympus® PK7200™

Device Names
Greiner VACUETTE® EDTA K3, 6.0mL, 13x100mm tube, Product Listing #456099

Greiner VACUETTE® EDTA K2, 6.0mL, 13x100mm tube, Product Listing #456023

Comparator Device
Becton Dickinson Vacutainer™ Glass K_3EDTA, 7.0mL, 13x100mm tube, Product Listing #366450

Intended Use
Greiner VACUETTE® EDTA K3 and EDTA K2 tubes provide a means of collecting and transporting an undiluted plasma specimen in a closed evacuated system. The tubes contain 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]

Specimen Collection
Blood specimens were obtained using the 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 three tubes were drawn from each donor: 1) one Greiner VACUETTE® EDTA K3, 6.0mL, 13x100mm tube 2) one Greiner VACUETTE® EDTA K2, 6.0mL, 13x100mm tube and 3) one Becton Dickinson Vacutainer™ Glass K_3EDTA, 7.0mL, 13x100mm tube. In addition, six Greiner VACUETTE® EDTA tubes - one full draw and two half evacuated to simulate half draw, 6.0mL, 13x100mm EDTA K3 tubes and one full draw and two half evacuated to simulate half draw, 6.0mL, 13x100mm EDTA K2, tubes were collected from each of the 10 known red cell antibody positive donors.

A. The following donors were drawn:
1) 50 apparently healthy donors (full draw tubes)
2) Subset: 10 apparently healthy donors for antigen phenotyping
3) Subset: 10 apparently healthy donors for delayed antigen phenotyping (0, 15 or 19 days)
4) 15 known red cell antibody positive blood donors (full draw tubes)
5) Subset: 10 known antibody positive donors (half-draw/half-evacuated tubes)
6) Subset: 10 known antibody positive individuals (full and half-draw/half-evacuated tubes) for delayed testing

Handling Techniques
The tubes were gently mixed using eight complete inversions immediately following blood collection. Tubes were centrifuged using the laboratory's standard procedure, to separate cellular elements completely from the plasma. All but three samples were tested within 24 hours. Testing was delayed for two days for two positive antibody samples and three days for another positive antibody sample.

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).

Instrumentation and Tests
Olympus® PK7200™ Automated Microplate System: ABO, Rh

Standard Manual Tube Method :
1) DAT: Anti-Human Globulin (IgG) Reagent, Immucor®, Inc.
2) Antibody Screening and Identifications: Immucor®, Inc.
3) Antigen Phenotyping: Gamma® Biologicals, Inc., Immucor®, Inc.,

Sample Stability Study/Delay in Testing:
1) Antibody Positive Samples: ABO, Rh, DAT, Antibody Screening and Identification using full and half-draw/half-evacuated tubes
2) Antigen Phenotyping Samples: Antigen Phenotyping using full draw tubes

Discussion
ABO/Rh Testing
ABO/Rh typing was performed on matching tubes of blood from 50 apparently healthy blood donors. The testing was performed using an Olympus® PK7200™, according to the manufacturer's recommended procedure. Fifteen known antibody positive donors had the ABO and Rh typing performed manually. There were no inaccurately reported results with the Greiner VACUETTE® EDTA K3 and EDTA K2 tubes when compared to the BD Vacutainer™ Glass K_3EDTA tubes.

Antigen Phenotyping
Antigen phenotyping was performed on matching tubes of blood from 10 apparently healthy blood donors. The samples were screened for the most common antigens of the Rh (C,

E, c, e), Kell (K), Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), and MNS (M, N, S, s) blood group systems. The distribution of results is summarized in Table #1. [3,4]

Table # 1		
	EDTA K3 (#Pos/#Neg)	EDTA K2 (#Pos/#Neg)
C	6/4	6/4
E	1/9	1/9
c	8/2	8/2
e	9/1	9/1
K	0/10	0/10
k	NT	NT
Fy^a	7/3	7/3
Fy^b	9/1	9/1
Jk^a	8/2	8/2
Jk^b	5/5	5/5
S	7/3	7/3
s	10/0	10/0
M	7/3	7/3
N	8/2	8/2

*NT = Not Tested

Antibody Screening and Identification

Full Draw Tube

Antibody screening was performed on 50 apparently healthy blood donors, 15 known positive blood donors using the full draw Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and the BD Vacutainer™ Glass K_3EDTA tubes. The testing was performed according to the manufacturer's recommended procedures. All positive antibody screening samples were followed up with antibody identification.

Concordant results were obtained between the Greiner VACUETTE® EDTA K3 and EDTA K2 tubes when compared to the BD Vacutainer™ Glass K_3EDTA tubes. However, in some of the comparisons, there was a 1+ difference in reaction grade, but none of these results demonstrated a change to a negative reading. This variation is within the expected reproducibility of a subjective grading system.

Half-Draw Tube

In addition, ABO/Rh, DAT, antibody screening and antibody identification were performed on a subset of 10 of the known antibody positive blood donors using half-draw/half-evacuated Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and full draw BD Vacutainer™ Glass K_3EDTA tubes. The testing was performed according to the manufacturer's recommended procedures.

Concordant results were obtained between the half-draw/half-evacuated Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and the full draw BD Vacutainer™ Glass K_3EDTA tubes. However, in some of the comparisons, there was a 1+ difference in reaction grade, but none of these results demonstrated a change to a negative reading. This variation is within the expected reproducibility of a subjective grading system.

DAT

DAT testing was performed on 50 apparently healthy blood donors and 15 known antibody positive blood donors using the Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and the BD Vacutainer™ Glass K_3EDTA tubes. There were no DAT positive results among the 50 blood donors. Concordant results were obtained with the Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and the BD Vacutainer™ Glass K_3EDTA tubes.

In addition, a panel of 5 simulated DAT positive samples was prepared and tested using the Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and the BD Vacutainer™ Glass K_3EDTA tubes. Preparation of the coated red cells followed the procedure for using red cells coated with Anti-Fy^a described in the FDA Center for Biologics Evaluation and Research Guidance Document "Recommended Methods for Anti-Human Globulin Evaluation", issued in March 1992.[5] The dilutions used in this study were selected to represent a range of positive reactivity. The samples were tested on Day 0 (date of preparation) and repeated on Days 7 and 14. Concordant results were obtained between the Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and the BD Vacutainer™ Glass K_3EDTA tubes on Days 0, 7 and 14. In some of the samples, there was a 1+ difference in reaction grade of the results. This variation is within the expected reproducibility of a subjective grading system. [3,4]

Delay in Testing

Ten of the antigen phenotyping samples and 10 of the known antibody positive blood donor samples (full and half - draw/half-evacuated tubes) were stored at 2-8°C following initial testing. Testing was repeated at 15 - 19 days after collection. The antigen phenotyping samples were only repeated for antigen phenotyping testing. These results were concordant at Day 19. The antibody positive blood donor samples were repeated for ABO/Rh typing, DAT, and antibody screening and identification. Concordant results were obtained between the full and half-draw/half-evacuated Greiner VACUETTE® EDTA K3 and EDTA K2 tubes and the full draw BD Vacutainer™ Glass K_3EDTA tubes at Day 14. However, in some of the comparisons, there was a 1+ difference in reaction grade. This variation is within the expected reproducibility of a subjective grading system. A decrease in grading results was observed in some samples between Day 0 and the last day of testing (Day 15 or Day 19). This is also not unexpected, considering the age of the sample.[6]

Conclusion

The Greiner VACUETTTE® EDTA K3 and EDTA K2 tubes (full and half-draw/half-evacuated) demonstrated substantial equivalence to the Becton Dickinson Vacutainer™ Glass

K_3EDTA tubes with various standard assays using donor populations. Antigen and antibody identification did not change over time when samples were stored in the Greiner VACUETTE® EDTA K3 and EDTA K2 tubes, demonstrating that these proteins were not adsorbed onto the plastic walls of the tubes and interfering substances were not leached from the walls of the tubes. [7,8,9,10]

References

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4. Gamma® Biologicals, Inc. Gamma Blood Grouping Reagents, Anti-Fy^a, Anti-Fy^b by Indirect Agglutination Test Product Insert. Houston, Texas. December 2000.

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