Evaluation of VACUETTE® K_3EDTA and K_2EDTA Evacuated Blood Collection Tubes using the Olympus® PK 7200™

Background:

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

Greiner VACUETTE® K_3EDTA and K_2EDTA 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 1.8 mg/mL of blood when evacuated tube is filled correctly to its fill volume. 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 testing in routine immunohematology i.e. red cell grouping, Rh typing and antibody screens.

Study Objective:

A clinical evaluation was carried out to compare the performance of the Greiner VACUETTE® K_3EDTA and K_2EDTA tubes to the Becton Dickinson Vacutainer® K_3EDTA glass tube using the Olympus® PK 7200™ Automated Microplate System (ABO, Rh).

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	Becton Dickinson Vacutainer® Glass K_3EDTA, 7 mL (13x100 mm) (comparator device)
2	VACUETTE® K_3EDTA, 6 mL (13x100 mm)
3	VACUETTE® K_2EDTA, 6 mL (13x100 mm)

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. In addition, one tube each per Samples No. 1, 2 and 3, six Greiner VACUETTE® EDTA tubes – one full draw and two partial draw/half-evacuated VACUETTE® K_3EDTA and also K_2EDTA tubes were collected from each of the 10 known red cell antibody positive donors. This was carried out to simulate partial draw.

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 9 days)
4) 15 known red cell antibody positive donors (full-draw tubes)
5) Subset: 10 known antibody positive donors (partial draw tubes/ half-evacuated)
6) Subset: 10 known antibody positive donors (full and partial draw/ half-evacuated tubes for delayed testing)

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

Following instrumentations and tests were used:

- Olympus® PK7200™ Automated Microplate System: AB0, Rh
- Standard Manual Tube Method:

  1) DAT: Anti- Human Globulin (lgG) Reagent, Immucor®, Inc.

  2) Antibody Screening and Identifications: Immucor®, Inc.

  3) Antigen Phenotyping: Gamma® Biologicals Inc.
- Sample Stability Study/Delay testing:

  1) Antibody positive Samples. AB0, Rh, DAT, Antibody Screening and Identification using full and a partial draw/ half-evacuated tubes

  2) Antigen Phenotyping Samples: Antigen Phenotyping using full draw tubes

Conclusion:

The Greiner VACUETTE® K_3EDTA and K_2EDTA tubes (full and partial draw/ half-evacuated) demonstrated substantial equivalence to the Becton Dickinson 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® K_3EDTA and K_2EDTA tubes, demonstrating that these proteins were not absorbed onto the plastic walls of the tubes and interfering substances were not leached from the walls of the tubes. [3],[4],[5],[6]

Results/Discussion:

AB0/Rh Testing

AB0/ Rh typing was performed on matching tubes of blood from 50 apparently healthy blood donors. The testing was performed using an Olympus® PK 7200™, according to the manufacturer´s recommended procedure. Fifteen known antibody positive donors had the AB0 and Rh typing performed manually. There were no inaccurately reported results with Greiner VACUETTE K_3EDTA and K_2EDTA tubes when compared to the Vacutainer® Glass K_3EDTA tubes.

AntigenPhenotyping

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. [7],[8]

Table #1

	K_3EDTA (# Pos/ #Neg)	K_2EDTA (# 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 positive known blood donors using the full draw VACUETTE® 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® K_3EDTA and K_2EDTA 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.

Partial draw/ Half-Evacuated Tube

In addition, AB0/ Rh, DAT, antibody screening and antibody identification were performed on a subset of 10 of the known antibody positive blood donors using partial draw/ half-evacuated Greiner VACUETTE® K_3EDTA and K_2EDTA 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 partial draw/ half-evacuated Greiner VACUETTE® K_3EDTA and K_2EDTA 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® K_3EDTA and K_2EDTA 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® K_3EDTA and K_2EDTA 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® K_3EDTA and K_2EDTA tubes and the BD Vacutainer® Glass K_3EDTA tubes.

Preparation of the coated red cells followed the procedure for using red cells coated with 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.[9] 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® K_3EDTA and K_2EDTA tubes and the BD Vacutainer® Glass K_3EDTA tubes on Days 0, 7 and 14. In some samples, there was a 1+ difference in reaction grade of the results. This variation is within the expected reproducibility of a subjective grading system.

Delay in Testing

Ten on the antigen phenotyping samples and 10 of the known antibody positive blood donor samples (full and partial 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 repeated for antigen phenotyping testing. These results were concordant at Day 19. The antibody positive blood donor samples were repeated for AB0/ Rh typing, DAT, and antibody screening and identification. Concordant results were obtained between the full and the partial draw/ half-evacuated Greiner VACUETTE® K_3EDTA and K_2EDTA tubes and the full draw BD Vacutainer® Glass K_3EDTA tubes at Day 14.

However, in some 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.(10)

References:

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

(2) Gruber, H. Greiner Bio-One. Product Manual. Kremsmünster, Austria. July 2002.

(3) Greiner Bio-One 510(k) Submission. Blood Collection Tube-EDTA K3 Pre-Market Notification Addition of Immunohematology Claim. Monroe, December 2002.

(4) Greiner Bio-One 510(k) Submission. Blood Collection Tube-EDTA K2 Pre-Market Notification Addition of Immunohematology Claim. Monroe, April 2003.

(5) Kemper, M. Final Report: Greiner® Evacuated Blood Collection Tubes Blood Bank Study K2EDTA, K3 EDTA vs. BD K3EDTA. SMF-Center For Blood Research. Sacramento, California, March 14, 2003.

(6) Kemper, M. Personal Communication. SMF-Center For Blood Research. Sacramento, California, March 2003

(7) Immunocor®, Inc. Immunocor® Antisera: Anti-K, Anti-k (rev7/99), Anti-Jk^a, Anti-Jk^b (rev 7/99), Anti-C, Anti-E, Anti-c, Anti-e (rev 11/01), Anti-S, Anti-s, (rev 7/01), Anti-Fy^a, Anti-Fy^b (7/99). Product Inserts. Norcross, Georgia.

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

(9) FDA Center for Biologics Evaluation and Research Guidance Document. Recommended Methods for Anti-Human Globulin Evaluation. March 1992.

(10) Sandler, G.S.M.D., Personal Communication. Georgetown University Hospital, July 2003.