



ACCU-CHEK[®] Systems Evaluation Protocol

Hematocrit Variable Study Protocol for Glucose Meter Evaluations

Purpose of Protocol

This protocol is a guidance tool for performing studies that demonstrate the effect of hematocrit on blood glucose meter results and is recommended for use with the Roche ACCU-CHEK[®] blood glucose monitoring systems.¹ If you wish to include other manufacturers' systems in your studies you should contact each vendor for recommended evaluation protocols.²

Introduction

Extremes in hematocrit may occur in patients who are being tested using blood glucose monitoring systems; therefore it is important to understand how hematocrit variability impacts results from blood glucose monitoring systems.³ This procedure guides you in preparing a series of samples at various hematocrit and glucose levels to demonstrate the effect of hematocrit on blood glucose monitoring system results. This procedure contains instructions for preparing three sets of samples with glucose levels of approximately 40, 250 and 400 mg/dL and hematocrit values of approximately 20, 45 and 60% at each glucose level.

Sample Requirements

This study utilizes heparinized venous whole blood. Always refer to the ACCU-CHEK test strip package insert for specific information on what types of blood samples (venous, arterial, capillary or neonate) and anticoagulants may be used with each type of ACCU-CHEK test strip. This procedure cannot be used to evaluate systems that are not cleared for use with venous blood.

Safety Considerations

Follow all of your internally established biohazard / bloodborne pathogen safety procedures when handling and disposing of biological materials and sharps.

Pre-Study Preparation

1. On the afternoon of the day before you plan to conduct your study, collect 6 tubes (30 mL) of heparinized venous whole blood from a single healthy donor. Be sure the donor is well hydrated so that the sample hematocrit is normal. Dehydration can cause an elevated hematocrit in which case the sample may not provide an adequate volume of plasma for the study.
2. Allow the tubes to lie on their side at room temperature overnight to glycolyze.

Materials

Gather the following materials in preparation for your study along with the whole blood sample collected according to the Pre-Study Preparation instructions above:

- ACCU-CHEK blood glucose meters (1-2 meters are recommended)
- ACCU-CHEK test strips compatible with the meter system
- ACCU-CHEK Quality Control Solutions Level 1 (Low) and Control Level 2 (High) (Level 1 and Level 2) compatible with the meter system
- Hematology analyzer or supplies for microhematocrit testing
- Three – 15 mL plastic graduated conical tubes with leak proof caps
- Twelve – 5 mL plastic aliquot tubes with leak proof caps
- Three – 10 mL serological pipettes
- One – 10 microliter pipettor with tips
- Pipettors capable of delivering 300, 450, and 550 microliters
- Transfer pipettes
- Countertop disinfectant
- Biohazard container
- Forms for recording results (see **Hematocrit Variable Study Data Log Form Appendix** at the end of this procedure for sample forms).

Study Procedure

Preparation:

1. Calibrate each meter for the test strip lot you will be using for your study. Perform quality control testing on all meters using all levels of the appropriate Roche ACCU-CHEK Quality Control Solutions (Level 1 and Level 2). Proceed with your study only when control results are within range.
2. Gather supplies listed above and arrange your meters and supplies on a countertop for efficient testing.
3. Gently mix all of the samples for a few minutes and then perform a meter test on one of the tubes to determine the baseline glucose level. The result must be ≤ 60 mg/dL to proceed.
4. Measure the hematocrit of one of the tubes to be sure that it is between 40% and 45%.
5. Label the three 15 mL conical tubes, (Low, Mid, and High), to correspond to the 3 glucose levels you will be trying to achieve.
6. Transfer the contents of the 6 heparinized tubes to the 3 tubes labeled “Low, Mid, and High”. (Two heparinized tubes per conical tube)

Sample Spiking Procedure: (refer to the [Roche Blood Glucose Spiking Protocol for Blood Glucose Meter Evaluations](#) for detailed instructions on preparation of spiking solution and sample spiking)

1. Using the 20% spiking solution, adjust the glucose in the “Low” tube to 20-60 mg/dL
2. Adjust the glucose in the “Mid” tube to 200-275 mg/dL
3. Adjust the glucose in the “High” tube to 375-450 mg/dL
4. Allow the blood in all three tubes to equilibrate for at least 30 minutes with occasional mixing. (You may put them on a rocker if one is available.)

Hematocrit Adjustment Procedure:

1. Using the conical tube labeled “Low” and a serological pipette, dispense four 2 mL blood samples into four 5mL tubes labeled 1, 2, 3, and XL. Pipette #2 last.
2. Spin the tubes labeled 1, 3, and XL in a centrifuge for 5-7 minutes. **Do not centrifuge tube #2!**
3. Using a micro-pipette, carefully place the pipette tip into the bottom of tube #3 and remove 300 uL of the packed red cells and discard. **Note: Be sure to leave the pipette tip in the packed red cells for a few additional seconds due to the increased viscosity of the red cells.**
4. Using a micro-pipette with a clean tip, transfer 550 uL of plasma from tube #1 into tube #3.
5. Using a micro-pipette with a clean tip, transfer 450 uL of plasma from the tube labeled XL into tube #3.
6. Discard the remainder of tube XL.
7. The final hematocrits of tubes 1, 2 and 3 should be approximately 60%, 45%, and 20%, respectively.
8. Place the three tubes on a rocker for 10 minutes to fully re-suspend the red cells.

Testing Procedure:

1. Meter testing should then proceed starting with tube #1, followed by #2 and then tube #3.
2. Perform a total of 10 replicate tests. The 10 replicates can all be performed on a single meter or divided between 2 meters. Record your results on the data collection form found at the end of this procedure.
3. Perform hematocrits on all three tubes.
4. Spin the tubes down and transfer the plasma to a reference analyzer cup for glucose testing.
5. Repeat steps 1-8 of the **Hematocrit Adjustment Procedure** using the “Mid” tube and labeling your 5 mL tubes 4, 5, 6, and XM, remembering not to centrifuge tube #5.
6. Repeat steps 1-8 of the **Hematocrit Adjustment Procedure** using the “High” tube and labeling your 5 mL tubes 7, 8, 9, and XH, remembering not to centrifuge tube #8.

Analysis of Results

1. Calculate the mean for each sample aliquot by adding all test results for each aliquot and dividing the total by the number of tests. The formula for calculating the mean is as follows:

$$Mean = \frac{\sum(X_i)}{N}$$

X_i = the value of each test
N = the total number of tests

2. Calculate mean meter bias from your Chemistry reference analyzer for each sample aliquot as follows ^{4,5}:

For reference analyzer glucose values less than 75 mg/dL, subtract the reference value from the mean meter value using the equation:

$$Mean\ Meter\ value\ (mg/dL) - Reference\ value\ (mg/dL) = Difference\ (mg/dL)$$

For reference analyzer glucose values greater than or equal to 75 mg/dL, subtract the reference value from the blood glucose meter value. Divide the difference by the reference value. Multiply the result by 100 to obtain the % difference using the equation:

$$\left(\frac{MeterValue(mg / dL) - ReferenceValue(mg / dL)}{ReferenceValue(mg / dL)} \right) \times 100 = \% Difference$$

3. Various computer software programs can be used to plot the meter biases for a visual analysis of the effect of hematocrit.
4. Evaluate the results for acceptability according to your internally established acceptability criteria.

Procedure Notes

1. Call ACCU-CHEK Customer Care line at 1-800-440-3638 if you observe any unexpected results.
2. A common recommendation is to evaluate glucose meter accuracy in terms of mg/dL difference from reference values below 75 mg/dL and percent differences from reference values greater than or equal to 75 mg/dL. This eliminates the mathematical exaggeration of percentages at low glucose levels.^{4,5}

Appendix

Hematocrit Variable Study Data Log

Hospital Name: _____
Date of Study: _____

Meter Type: _____

Replicate Test	Low Glucose Sample			Mid Glucose Sample			High Glucose Sample		
	Tube #1	Tube #2	Tube #3	Tube #4	Tube #5	Tube #6	Tube #7	Tube #8	Tube #9
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
Mean Meter Glucose, mg/dL									
Lab Analyzer Glucose, mg/dL									
Mean Meter Bias*									

Test Strip Lot: _____

Meter SN _____

Test Strip Exp: _____

Meter SN _____

Performed by: _____

*Bias is calculated as mg/dL difference from lab results <75 mg/dL and as % difference from lab results >75 mg/dL.

References

- ¹ All ACCU-CHEK products must be used according to their labeling. All evaluation protocols are supplemental to proper use as described in each system's packaging.
- ² CLSI C30A-2; Point of Care Glucose Testing in Acute and Chronic Care Facilities; Approved Guideline-Second Edition; 2002; Item 6.3.2.1.2; Page 7.
- ³ CLSI C30A-2; Point of Care Glucose Testing in Acute and Chronic Care Facilities; Approved Guideline-Second Edition; 2002; Item 6.3.1; Page 6.
- ⁴ CLSI C30A-2; Point of Care Glucose Testing in Acute and Chronic Care Facilities; Approved Guideline-Second Edition; 2002; Item 6.3.2.4; Page 7.
- ⁵ ISO 15197, *In Vitro* diagnostic test systems – Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus, 2003, Sections 7.4.1; Page 23.

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