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Allegro Evaluation Protocols

Nova Biomedical |Medical & Scientific Affairs

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Preface

Clinical laboratory based method evaluations are a set of studies aimed at characterizing the performance of an assay – a procedure for qualitatively assessing or quantitatively measuring the presence or amount of a target entity, i.e. analyte or measurand. These studies utilize controlled experimental technique to assess the various components of a measurement including the assessment of: method comparison, reproducibility, specificity, and accuracy. Highly accurate and precise measurements are central to the practice of laboratory medicine given that these measurements are used to manage the patient throughout the entire spectrum of care. It is therefore necessary that clinical laboratorians perform their due diligence in the careful selection and subsequent management of laboratory methods. In order to assist the investigator, a collection of evaluation protocols specifically designed to assess the performance of the Allegro has been provided. These protocols are based on the recommendations provided by the Clinical and Laboratory Standards Institute (CLSI), a recognized body that publishes consensus guidelines on the various practice aspects of laboratory medicine.

Introduction

Allegro is a fast, simple, capillary whole blood analyser with a diabetes-focused menu.
The comprehensive test menu available on the Allegro is:

Glycemic Control	**Lipid**	**Kidney Function**
HbA1c	Total Cholesterol	Urine Creatinine
	HDL Cholesterol	Urine Albumin
	Triglyceride	

The Allegro can be used in conjunction with the companion tests below to further extend the diabetes management menu

Companion Tests

Anemia – Hemoglobin and Hematocrit
Glycemic control – Glucose and Ketone

HbA1c, Lipid and Kidney Function tests are provided in their own multi test cartridge so each cartridge measures all analytes within each profile

Scope

The scope of this protocol is to evaluate and assess the analytical performance of the HbA1c, Lipid cartridge and the Kidney Function cartridge.

Analytical performance will be assessed on each profile e.g. Lipid, HbA1c, and Kidney Function, independently and will comprise of:

Within Day precision
Between Day precision
Method Correlation

Supplemental System Specifications:

Operating Environment:

Operating Location:
The analyzer is intended for indoor operation on a stationary, stable, bench.
Operating Temperature and Relative Humidity Range:
The Analyzer shall be able to operate under the following conditions

- Operating Temperature Range: 15 to 32°C (59°F to 90°F)
- Relative Humidity Range: 20 – 85%, non-condensing

Altitude:
The Analyzer shall be able to operate at an altitude of up to 3,660 meters (12,000 feet)

Power Requirements:

Operating Voltage: 90 to 264 VAC
Operating Frequency: 50/60Hz

Contents

1. Urine Creatinine and Albumin

I. Within Day Precision Study Protocol

Protocol based on:

Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Purpose

This protocol is recommended and designed by Nova Biomedical to investigate the within day precision of urine creatinine and urine Albumin measurements on the Allegro system.

Materials Needed

- Nova Biomedical Allegro
- Allegro Kidney Function Cartridges
- Nova Biomedical Allegro Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic tubes for decanting urine samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips
- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: Urine Creatinine and Urine Albumin
Sample Type: Fresh random urine
Sample Size: 25µL
Time per UACR: 7 mins
Linear Range of Operation: 15-500 mg/dL (13.0-44.2 mmol/L)
Linear Range of Operation for urine Albumin: 5-300 mg/L

Expected Normal Values
Urine Creatinine: For a random urine sample, >12 Years Male 20-370 mg/dL (1.8- 32.7 mmol/L), >12 Years Female 20 – 320 mg/dL (1.8-28.3 mmol/L)
Urine Albumin: For a random urine sample, normal values are typically 0 to 30 mg/L

Control Levels:
The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:
Urine Albumin and Creatinine:

Low Level Target:	**Urine Albumin ~50 mg/L**	**Creatinine ~85 mg/dL (~8 mmol/L)**
High Level Target:	**Urine Albumin ~180 mg/L**	**Creatinine ~220 mg/dL (~20 mmol/L)**

Please note that urine creatinine and Albumin are processed at the same time and can be run in both channels.

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. Select low, med and high levels of both urine creatinine and Albumin, ideally using discarded random urine collections or, if these are not available, then urine samples that have been spiked with appropriate concentrations of creatinine spiking solution and/or human Albumin (may need to be tested for homogeneity).

4. Suggested concentrations for low, medium and high samples: -

	Urine Creatinine	Urine Albumin
Low Target	~25-30 mg/dL (~2.2-2.7mmol/L)	~10-15 mg/L
Medium Target	~200 mg/dL (~18 mmol/L)	~40 mg/L
High Target	~ 400-450 mg/dL (~35-40 mmol/L)	~150-200 mg/L

5. Ensure adequate mixing before analysis.

6. Cartridges are stored at 4° each cartridge requires 10-15 mins warm up time so consider this requirement when planning study needs.

7. Run each Level 10 times on Allegro, consider running only in one channel to reduce variability. Record results on Data Collection Sheet.

8. Total Analysis Time: 7 mins x 10 = 70 mins per level. 210 mins for all levels. Approx. 3.5 hrs plus preparation time.

Data Analysis

The within-run precision of the Allegro be assessed by calculating the % coefficient of variation (%CV) from the mean and standard deviation from the replicate measurements. Acceptability criteria are determined by the medical director and/or institution. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [1].

Data Review

This data will be summarized in a final report which will also include an assessment of the accuracy. This report should be reviewed by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of Allegro performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Appendix 1

Within-Day Precision Study Data Collection Sheet

Hospital Name: _____ Date of Study: _____
System Operator(s): _____
Reference Analyzer _____

Level 1 Urine Creatinine and Urine Albumin

Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____

	System Name							
	System Serial #							
	Cartridge Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L					
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Level 2 Urine Creatinine and Urine Albumin

Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____

	System Name							
	System Serial #							
	Cartridge Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L					
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Level 3 Urine Creatinine and Urine Albumin

Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____

	System Name							
	System Serial #							
	Cart Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L					
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

I. Urine Creatinine and Urine Albumin

II. Between-Day Precision Study Protocol

Protocol based on:

Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Purpose

This protocol is recommended and designed by Nova Biomedical to investigate the between-day precision of urine creatinine and urine Albumin measurements on the Allegro system.

Materials Needed

- Nova Biomedical Allegro
- Allegro Kidney Function Cartridges
- Nova Biomedical Allegro Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic tubes for decanting urine samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips
- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: Urine Creatinine and Urine Albumin
Sample Type: Fresh random urine
Sample Size: 25µL
Time per UABCR: 7 mins
Linear Range of Operation for Urine Creatinine: 15-500 mg/dL (13.0-44.2 mmol/L)
Linear Range of Operation for Urine Albumin: 3-300 mg/L

Expected Normal Values
Urine Creatinine: For a random urine sample, >12 Years Male 20-370 mg/dL (1.8- 32.7 mmol/L), >12 Years Female 20 – 320 mg/dL (1.8-28.3 mmol/L)
Urine Albumin: For a random urine sample, normal values are typically 0 to 30 mg/dL

Control Levels:
The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:
Urine Albumin and Creatinine:

Low Level Target:	**Urine Albumin ~50 mg/L**	**Creatinine ~85 mg/L (~8 mmol/L)**
High Level Target:	**Urine Albumin ~180 mg/L**	**Creatinine ~220 mg/L (~20 mmol/L)**

Please note that urine creatinine and urine Albumin are processed at the same time

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Analyse controls on Allegro every day that testing is carried out, 2 runs per day would be performed one in the morning and the other in the afternoon so QC would need to be performed prior to each run, make sure results are within specification before proceeding. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. Select low, med and high levels of both urine creatinine and Albumin. These can be obtained from one or more of the following: a) samples from random urine collections, b) samples spiked with stock concentrations of Albumin and/or creatinine, or c) samples used for the within day precision test. Forty aliquots must be prepared from each sample and frozen.

4. A freshly thawed aliquot will then be analysed every morning and afternoon in duplicate for 10 consecutive working days

5. Suggested concentrations for low, medium and high samples: -

	Urine Creatinine		**Urine Albumin**
Low Target	~25-30 mg/dL	(~2.2-2.7 mmol/L)	~10-15 mg/L
Medium Target	~200 mg/dL	(~18 mmol/L)	~40 mg/L
High Target	~ 400-450 mg/dL	(~35-40 mmol/L)	~150-200 mg/L

6. Ensure adequate mixing before analysis.

7. Cartridges are stored at 4° each cartridge requires 10-15 mins warm up time so consider this requirement when planning study needs.

8. Run each level in duplicate on the Allegro using the same channel to reduce variability

9. Collect, review and collate data

10. Total Analysis Time**:** 7 mins x 6 = 42 mins in morning, 7 mins x 6 = 42 mins in afternoon plus preparation time

Data Analysis

The between-day precision of the Allegro be assessed by calculating the % coefficient of variation (%CV) from the mean and standard deviation from the replicate measurements. Acceptability criteria are determined by the medical director and/or institution. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [1, 2].

Data Review

This data will be summarized in a final report which will also include an assessment of the accuracy. This report should be reviewed by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of Allegro performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Appendix 2

Between-Day Precision Study Data Collection Sheet

Hospital Name: _____ Date of Study: _____
System Operator(s): _____
Reference Analyzer _____

Level 1 Urine Creatinine and Urine Albumin
Morning
Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____
Afternoon
Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____

	System Name								
	System Serial #								
	Cartridge Lot #								
Low QC	Range:								
High QC	Range:								
		Morning				**Afternoon**			
	Replicate #	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or g/L	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or g/L
	Day 1								
	Day 2								
	Day 3								
	Day 4								
	Day 5								
	Day 6								
	Day 7								
	Day 8								
	Day 9								
	Day 10								

Level 2 Urine Creatinine and Urine Albumin

Morning
Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____
Afternoon
Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____

	System Name								
	System Serial #								
	Cartridge Lot #								
Low QC	Range:								
High QC	Range:								
		Morning				**Afternoon**			
	Replicate #	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or g/L	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or g/L
	Day 1								
	Day 2								
	Day 3								
	Day 4								
	Day 5								
	Day 6								
	Day 7								
	Day 8								
	Day 9								
	Day 10								

Level 3 Urine Creatinine and Urine Albumin

Morning
Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____
Afternoon
Reference Analyzer Result: Urine Creatinine mg/dL or mmol/L _____
Reference Analyzer Result: Urine Albumin mg/L or g/L_____

	System Name								
	System Serial #								
	Cartridge Lot #								
Low QC	Range:								
High QC	Range:								
		Morning				**Afternoon**			
	Replicate #	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or gl/L	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Urine Albumin Result mg/L or gl/L	Urine Albumin Result mg/L or g/L
	Day 1								
	Day 2								
	Day 3								
	Day 4								
	Day 5								
	Day 6								
	Day 7								
	Day 8								
	Day 9								
	Day 10								

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

I. Urine Creatinine and Urine Albumin

III. Method Comparison Study Protocol

Protocol based on the following guidance documents:

Clinical & Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. EP9-A2.*

Purpose

This protocol is designed to investigate how Allegro results compare to a recognized central laboratory reference method. These studies are important in order to establish the relationship between the Allegro System and the reference method with respect to statistical correlation and measurement bias. This protocol is designed and recommended for the Allegro System.

Background

Good practice dictates that the performance of medical devices such as the Allegro System be verified before the conductance of clinical studies. Verification studies include: method comparisons to determine how the results of the Allegro Systems compare to a recognized reference method, studies of imprecision to determine the reproducibility of the various tests available on the Allegro, and to determine the analytical trueness of the Allegro measurements. The protocol below describes how to conduct a method comparison (Allegro vs. Reference Method).

Materials Needed

- Nova Biomedical Allegro
- Allegro Kidney Function Cartridges
- Nova Biomedical Allegro Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic tubes for decanting urine samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips
- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: Urine Creatinine and urine Albumin
Sample Type: Fresh random urine
Sample Size: 25µL
Time per UABCR: 7 mins
Linear Range of Operation: 15-500 mg/dL (13.0-44.2 mmol/L)
Linear Range of Operation for Urine Albumin: 3-300 mg/L

Expected Normal Values
Urine Creatinine: For a random urine sample, >12 Years Male 20-370 mg/dL (1.8- 32.7 mmol/L), >12 Years Female 20 – 320 mg/dL (1.8-28.3 mmol/L)
Urine Albumin: For a random urine sample, normal values are typically 0 to 30 mg/L

Control Levels:

The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:

Urine Albumin and Creatinine:

Low Level Target:	**Urine Albumin ~50 mg/L**	**Creatinine ~85mg/dL (~8 mmol/L)**
High Level Target:	**Urine Albumin ~180 mg/L**	**Creatinine ~220 mg/dL (~20 mmol/L)**

Please note that urine creatinine and urine Albumin are processed at the same time and can be run in both channels

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. 40-50 fresh (within 24hrs) discarded random urine samples should be collected and run in duplicate on Allegro. Again spiking with creatinine spiking solutions and human Albumin may be required to cover the measuring range.

4. Ensure adequate mixing before analysis.

5. Samples should be analysed promptly on the reference analyser.

7. Cartridges are stored at 4° each cartridge requires 10-15 mins warm up time so consider this requirement when planning study needs.

8. Analyse all samples in duplicate on the Allegro using the same channel to reduce variability

9. Collect, Review and collate data.

10. Total Analysis Time: 7 mins x 80-100 =560 -700 mins. Approx. 9.3 – 11.6 hrs plus preparation time

11. To reduce analysis time consider running samples in singles or perhaps less samples in duplicate whichever best suits site

Data Analysis

As part of this study paired urine creatinine and urine Albumin measurements will be collected. Analysis of accuracy is based on pairing individual results from the Allegro with the corresponding reference analyser measurement and calculating the difference (bias). Scatter plots and linear regression analysis are appropriate tools to assess the relationship between the Allegro results and the reference analyzer. Moreover Bland-Altman plots can be used to assess the bias relative to the range of urine creatinine and urine Albumin concentrations studied. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [1, 2]. The validity of the conclusions is dependent on experimental design, chiefly number of samples, the distribution of results, and the calibration of the reference analyzer.

Data Review

This data will be summarized in a summary report which will also include an assessment of precision. This report should be reviewed and signed off by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of the Allegro System performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. EP9-A2.*

Appendix 4

Method Comparison Data Collection Sheet (Case Report Form)

Study Site: _____ Date of Study: _____
System Operator(s): _____

Specimen Type (Urine) _____
Reference Analyzer _____

	System Name					
	System Serial #					
	Cartridge Lot #					
Low QC	Range:					
High QC	Range:					
Specimen ID	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Reference Analyzer Result Urine Creat mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or g/L	Reference Analyser Result Urine Albumin mg/L or g/L
1						
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7						
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9						
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11						
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25						
26						

27						
28						
29						
30						

Method Comparison Data Collection Sheet (Case Report Form)

	System Name					
	System Serial #					
	Cart Lot #					
Specimen ID	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Reference Analyzer Result Urine Creat mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or g/L	Reference Analyser Result Urine Albumin mg/L or g/L
31						
32						
33						
34						
35						
36						
37						
38						
39						
40						
41						
42						
43						
44						
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46						
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62						
63						
64						
65						

66						
67						
68						
69						
70						
71						
72						

	System Name					
	System Serial #					
	Cart Lot #					
Specimen ID	Urine Creat Result mg/dL or mmol/L	Urine Creat Result mg/dL or mmol/L	Reference Analyzer Result Urine Creat mg/dL or mmol/L	Urine Albumin Result mg/L or g/L	Urine Albumin Result mg/L or g/L	Reference Analyser Result Urine Albumin mg/L or g/L
73						
74						
75						
76						
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79						
80						
81						
82						
83						
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98						
99						
100						

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

1. Urine Creatinine and Urine Albumin

Summary Report Template

Urine Creatinine and Urine Albumin Summary Report

Date:

Report Author:

Study Site:

Study Site Principal Investigator/Department:

Materials and Methods

See Protocols I-IV. *Any deviations from protocol should be noted here.*

Summary of Results

I. Precision Studies

Table 1. **Urine Creatinine Within-Day** Precision for Allegro

	Low Urine Creat	Mid Urine Creat	High Urine Creat
Mean mg/dL or mmol/L			
Standard Deviation			
%CV			
N			
Matrix: Urine			

Table 2. **Urine Albumin Within-Day** Precision for Allegro

	Low Urine Urine Albumin	Mid Urine Urine Albumin	High Urine Urine Albumin
Mean mg/L or g/L			
Standard Deviation			
%CV			
N			
Matrix: Urine			

Table 3. **Urine Creatinine Between-Day** Precision for Allegro

	Low Urine Creat	Mid Urine Creat	High Urine Creat
Mean mg/dL or mmol/L			
Standard Deviation			
%CV			
N			
Matrix: Urine			

Table 4. **Urine Albumin Between-Day** Precision for Allegro

	Low Urine Urine Albumin	Mid Urine Urine Albumin	High Urine Urine Albumin
Mean mg/L or g/L			
Standard Deviation			
%CV			
N			
Matrix: Urine			

II. Method Comparison

Table 7. **Summary of Linear Regression Analysis Results** Urine creatinine and Urine Albumin for the Allegro

	Urine Creat	Urine Albumin
Slope		
y-intercept (mg/dL)		
R^2		
N		
Urine Creat Range mg/dL or mmol/L		
Urine Albumin Range mg/L or g/L		

Table 8. Summary of Bland-Altman Results

	Urine Creat	Urine Albumin

Mean Bias (mg/dL)		
95% Limits of Agreement		
Lower		
Upper		
Urine Creat Range mg/dL or mmol/L		
Urine Albumin Range mg/L or gl/L		

Summary Findings

Allegro demonstrated ….

This report has been reviewed and approved by:

Signature of Study Site Principal Investigator Date

Signature of MASA Director Date

2. HbA1c

I. Within Day Precision Study Protocol

Protocol based on:

Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Purpose

This protocol is recommended and designed by Nova Biomedical to investigate the within-day precision of whole blood HbA1c measurements on the Allegro system.

Materials Needed

- Nova Biomedical Allegro
- Allegro Glycaemic Control Cartridges
- Nova Biomedical Allegro Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic microcentrifuge tubes for decanting EDTA samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips

- Appropriate Spiking stock solutions

Important Factors to Consider

Tests: HbA1c

Sample Type:

- Capillary blood (from finger stick)
- Fresh venous whole blood with anticoagulants ethylenediamine tetra-acetic acid (EDTA); Li heparin; Na heparin

Sample Size: 1.5 µL

Time per HbA1c: 6.5 mins

Linear Range of Operation for HbA1c: 4-15% (20.2 - 140.4 mmol/L)

Potential Interferences

HbA1c measurement is not affected by Hb variants: HbAc, HbAD, HbAE, HbF, HbAG and HbAS

Control Levels:

The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:

HbA1c:

Low Level Target: ~5% (31.1 mmol/mol)

High Level Target: ~10% (85.5 mmol/mol)

Normal Values

HbA1c: - A normal non-diabetic HbA1c is <5.5% (36mmol/mol). In diabetes about **6.5%** (48 mmol/mol) is good.

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. Using aliquoted venous EDTA whole blood identify three levels low, medium and high.

4. Suggested concentrations for low, medium and high samples: -

	HbA1c
Low Target	~5% (31.1 mmol/mol)
Medium Target	~7% (53 mmol/mol)

High Target ~10% (85.8 mmol/mol)

5. Ensure adequate mixing before analysis.

6. Cartridges are stored at 4° each cartridge the HbA1c cartridge can be used cold

7. Run each Level 10 times on Allegro, the HbA1c cartridge can only be run in the 600nm channel which is on the right hand side of the analyser.

8. Collect, review and collate data.

Total Analysis Time: 6.25 mins x 10 = 62.5 mins per level. 187.5 mins for all levels. Approx. 3.15 hrs plus preparation time

Data Analysis

The within-run precision of the Allegro be assessed by calculating the % coefficient of variation (%CV) from the mean and standard deviation from the replicate measurements. Acceptability criteria are determined by the medical director and/or institution. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [4].

Data Review

This data will be summarized in a final report which will also include an assessment of the accuracy. This report should be reviewed by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of Allegro performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Appendix 1

Within-Day Precision Study Data Collection Sheet

Hospital Name: _____ Date of Study: _____
System Operator(s): _____
Reference Analyzer _____

Level 1 HbA1c

Reference Analyzer Result % mmol/mol_____

	System Name							
	System Serial #							
	Cartridge Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	HbA1c						

		% or mmol/Mol						
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Level 2 HbA1c

Reference Analyzer Result % or mmol/mol _____

	System Name							
	System Serial #							
	Cartridge Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	HbA1c % or mmol/mol						
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							

	9							
	10							

Level 3 HbA1c

Reference Analyzer Result % or mmol/mol_____

	System Name							
	System Serial #							
	Cartridge Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	HbA1c % or mmol/mol						
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

2. HbA1c

II. Between-Day Precision Study Protocol

Protocol based on:

Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Purpose

This protocol is recommended and designed by Nova Biomedical to investigate the between-day precision of whole blood HbA1c measurements on the Allegro system.

Materials Needed

- Nova Biomedical Allegro
- Allegro Glycemic Control Cartridges
- Nova Biomedical Allegro Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic microcentrifuge tubes for decanting EDTA samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips

- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: HbA1c

Sample Type:

- Capillary blood (from finger stick)
- Fresh venous whole blood with anticoagulants ethylenediamine tetra-acetic acid (EDTA); Li heparin; Na heparin

Sample Size: 1.5 µL

Time per HbA1c: 6.5 mins

Linear Range of Operation for HbA1c: 4-15% (20.2 - 140.4 mmol/L)

Potential Interferences

HbA1c measurement is not affected by Hb variants: HbAc, HbAD, HbAE, HbF, HbAG and HbAS

Control Levels:

The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:

HbA1c:

Low Level Target: ~5% (~31.1 mmol/mol)

High Level Target: ~10% (~85.8 mmol/mol)

Normal Values

HbA1c:- A normal non-diabetic HbA1c is <5.5% (36mmol/mol). In diabetes about **6.5%** (48 mmol/mol) is good.

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Analyse controls on Allegro every day that testing is carried out, 2 runs per day would be performed one in the morning and the other in the afternoon so QC would need to be performed prior to each run, make sure results are within specification before proceeding. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. Because of the logistical difficulty of using whole blood over a 2-week period the suggestion here is to use the Control Solutions, these provide only 2 levels, but these should be aliquoted into 40 x 50µL aliquots and frozen to reduce variables.

4. A freshly aliquot will be thawed, mixed and analysed every morning and afternoon in duplicate for 10 consecutive working days.

6. Cartridges are stored at 4° each cartridge the HbA1c cartridge can be used cold

7. Run each Level in duplicate on Allegro, the HbA1c cartridge can only be run in the 600nm channel which is on the right hand side of the analyser.

8. Collect, review and collate data

Total Analysis Time: 6.25 mins x 4 =25 mins in morning, 6.25 mins x 4 = 25 mins in afternoon. Approx. 50 mins plus preparation time

Data Analysis

The between-day precision of the Allegro be assessed by calculating the % coefficient of variation (%CV) from the mean and standard deviation from the replicate measurements. Acceptability criteria are determined by the medical director and/or institution. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [1].

Data Review

This data will be summarized in a final report which will also include an assessment of the accuracy. This report should be reviewed by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of Allegro performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Appendix 2

Between-Day Precision Study Data Collection Sheet

Hospital Name: _____ Date of Study: _____
System Operator(s): _____
Reference Analyzer _____

Level 1 HbA1c

Morning
Reference Analyzer Result % or mmol/mol _____
Afternoon

Reference Analyzer Result % or mmol/mol _____

	System Name				
	System Serial #				
	Cartridge Lot #				
Low QC	Range:				
High QC	Range:				
		Morning		**Afternoon**	
	Replicate #	HbA1c Result % or mmol/mol	HbA1c Result % or mmol/mol	HbA1c Result % or mmol/mol	HbA1c Result % or mmol/mol
	Day 1				
	Day 2				
	Day 3				
	Day 4				
	Day 5				
	Day 6				
	Day 7				
	Day 8				
	Day 9				
	Day 10				

Level 2 HbA1c

Morning
Reference Analyzer Result % or mmol/mol _____
Afternoon
Reference Analyzer Result % or mmol/mol _____

	System Name				
	System Serial #				
	Cartridge Lot #				
Low QC	Range:				
High QC	Range:				
		Morning		**Afternoon**	

	Replicate #	HbA1c Result % or mmol/mol	HbA1c Result % or mmol/mol	HbA1c Result % or mmol/mol	HbA1c Result % or mmol/mol
	Day 1				
	Day 2				
	Day 3				
	Day 4				
	Day 5				
	Day 6				
	Day 7				
	Day 8				
	Day 9				
	Day 10				

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

2. HbA1c

III. Method Comparison Study Protocol

Protocol based on the following guidance documents:

Clinical & Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. EP9-A2.*

Purpose

This protocol is designed to investigate how Allegro results compare to a recognized central laboratory reference method. These studies are important in order to establish the relationship between the Allegro System and the reference method with respect to statistical correlation and measurement bias. This protocol is designed and recommended for the Allegro System.

Background

Good practice dictates that the performance of medical devices such as the Allegro System be verified before the conductance of clinical studies. Verification studies include: method comparisons to determine how the results of the Allegro Systems compare to a recognized reference method, studies of imprecision to determine the reproducibility of the various tests available on the Allegro to determine the analytical trueness of the Allegro measurements. The protocol below describes how to conduct a method comparison (Allegro vs. Reference Analyser).

Materials Needed

- Nova Biomedical Allegro
- Allegro Glycaemic Control Cartridges
- Nova Biomedical Allegro Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic microcentrifuge tubes for decanting EDTA samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips
- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: HbA1c

Sample Type:

- Capillary blood (from finger stick)
- Fresh venous whole blood with anticoagulants ethylenediamine tetra-acetic acid (EDTA); Li heparin; Na heparin

Sample Size: 1.5 µL

Time per HbA1c: 6.25 mins

Linear Range of Operation for HbA1c: 4-15% (20.2 - 140.4 mmol/L)

Potential Interferences

HbA1c measurement is not affected by Hb variants: HbAc, HbAD, HbAE, HbF, HbAG and HbAS

Control Levels:

The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:

HbA1c:

Low Level Target: ~5% (~31.1 mmol/mol)

High Level Target: ~10% (~85.5 mmol/mol)

Normal Values

HbA1c:- A normal non-diabetic HbA1c is <5.5% (36mmol/mol). In diabetes about **6.5%** (48 mmol/mol) is good.

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. Select 40-50 fresh discarded HbA1c EDTA samples that cover the measuring range if possible.

4. Ensure adequate mixing before analysis.

5. Cartridges are stored at 4° each cartridge the HbA1c cartridge can be used cold

6. Run all samples in duplicate on Allegro following analysis on the reference analyser. Cartridges Allegro, the HbA1c cartridge can only be run in the 600nm channel which is on the right hand side of the analyser.

7. Collect, Review and collate data.

8. **Total Analysis Time:** 6.25 mins x 80 - 100 = 500 - 625 mins. Approx. 8.3 – 10.4 hrs plus preparation time

9. To reduce analysis time consider running samples in singles or perhaps less samples in duplicate whichever best suits site

Data Analysis

As part of this study paired HbA1c measurements will be collected. Analysis of accuracy is based on pairing individual results from the Allegro with the corresponding reference analyser measurement and calculating the difference (bias). Scatter plots and linear regression analysis are appropriate tools to assess the relationship between the Allegro results and the reference analyzer. Moreover, Bland-Altman plots can be used to assess the bias relative to the range studied. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [2]. The validity of the conclusions is dependent on experimental design, chiefly number of samples, the distribution of results, and the calibration of the reference analyzer.

Data Review

This data will be summarized in a summary report which will also include an assessment of precision. This report should be reviewed and signed off by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of the Allegro System performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. EP9-A2.*

Appendix 4

Method Comparison Data Collection Sheet (Case Report Form)

Study Site: _____ Date of Study: _____
System Operator(s): _____

Specimen Type (Venous EDTA Whole Blood) _____
Reference Analyzer _____

	System Name		
	System Serial #		
	Cartridge Lot #		
Low QC	Range:		
High QC	Range:		
Specimen ID	Reference Analyzer Result % or mmol/mol	HbA1c result % or mmol/mol	HbA1c result % or mmol/mol
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			

	System Name		
	System Serial #		
	Cart Lot #		
Specimen ID	Reference Analyzer Result % or mmol/mol	HbA1c result % or mmol/mol	HbA1c result % or mmol/mol
31			
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			
47			
48			
48			
49			
50			
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			
61			
62			
63			
64			
65			
66			
67			
68			
69			
70			
71			
72			
73			

	System Name		
	System Serial #		
	Cart Lot #		
Specimen ID	Reference Analyzer Result % or mmol/mol	HbA1c result % or mmol/mol	HbA1c result % or mmol/mol
74			
75			
76			
77			
78			
79			
80			
81			
82			
83			
84			
85			
86			
87			
88			
89			
90			
91			
92			
93			
94			
95			
96			
97			
98			
99			
100			

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

2. HbA1c

Summary Report Template

HbA1c Summary Report

Date:

Report Author:

Study Site:

Study Site Principal Investigator/Department:

Materials and Methods

See Protocols I-IV. *Any deviations from protocol should be noted here.*

Summary of Results

I. Precision Studies

Table 1. **HbA1c Within-Day** Precision for Allegro

	Low HbA1c	Mid HbA1c	High HbA1c
Mean % or mol/mol			
Standard Deviation			
%CV			
N			
Matrix			

Table 2. **HbA1c Between-Day** Precision for Allegro

	Low HbA1c	Mid HbA1c	High HbA1c
Mean % or mol/mol			
Standard Deviation			
%CV			
N			
Matrix			

II. Method Comparison

Table 5. **Summary of Linear Regression Analysis Results** HbA1C for Allegro

	HbA1c
Slope	
y-intercept (% or mmol/mol))	
R^2	
N	
HbA1c Range % or mmol/mol	

Table 6. Summary of Bland-Altman Results

	HbA1c
Mean Bias (% or mmol/mol)	
95% Limits of Agreement	
Lower	
Upper	
HbA1c (% or mmol/mol)	

Summary Findings

Allegro demonstrated ….

This report has been reviewed and approved by:

Signature of Study Site Principal Investigator Date

Signature of MASA Director Date

3. Total Cholesterol, Triglyceride and HDL Cholesterol

I. Within Day Precision Study Protocol

Protocol based on:

Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Purpose

This protocol is recommended and designed by Nova Biomedical to investigate the within day precision of whole blood total cholesterol, triglycerides and HDL cholesterol measurements on the Allegro system.

Materials Needed

- Nova Biomedical Allegro
- Allegro Lipid Risk Cartridges
- Nova Biomedical Allegro Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic microcentrifuge tubes for decanting Na Heparin or Li Heparin samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips
- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: Total Cholesterol, Triglycerides, HDL Cholesterol

Sample Type: Subject to change but to date**:**

- Capillary whole blood (from finger stick)
- Venous whole blood with anticoagulants Na heparin; Li heparin
- Venous plasma with anticoagulants Na heparin; Li heparin
- Serum

Sample Size: 5 µL

Time per Lipid: 10 mins

Linear Range of Operation for Total Cholesterol: 100-450 mg/dL (2.59-11.64 mmol/L)

Linear Range of Operation for Triglyceride: 50-600 mg/dL (0.56-6.76 mmol/L)

Linear Range of Operation for HDL Cholesterol: 20-100 mg/dL (0.52- 2.59 mmol/L)

Please note that all parameters are processed at the same time.

Normal Values:

Total Cholesterol: Desirable is below 200 mg/dL (<5.17 mmol/L)

Triglycerides: Desirable is below 150 mg/dL (<1.69 mmol/L)

HDL Cholesterol: Desirable is 60 mg/dL and above (> 1.55 mg/dL)

Control Levels:

The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:

	Total Cholesterol	**Triglycerides**	**HDL Cholesterol**
Low Level Target:	~ 170 mg/dL (~4.40 mmol/L)	~150 mg/dL (~1.69 mmol/L)	~40 mg/dL (~1.04 mmol/L)
High Level Target:	~ 240 mg/dL (~6.21 mmol/L)	~300 mg/dL (~3.39 mmol/L)	~60 mg/dL (1.55 mmol/L) (~1.55 mmol/L)

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. If possible using fresh aliquoted venous Na or Lithium heparin whole blood samples from qualified patients identify levels low, medium and high Total Cholesterol, Triglycerides and HDL Cholesterol levels.

4. Suggested concentrations for low, medium and high samples: -

	Total Cholesterol	**Triglycerides**	**HDL Cholesterol**
Low Level Target:	~ 150 mg/dL (~3.88 mmol/L)	~100 mg/dL (~1.13mmol/L)	~40 mg/dL (~1.03 mmol/L)
Medium Level Target:	~ 250 mg/dL (~6.47 mmol/L)	~200 mg/dL (~2.26 mmol/L)	~60 mg/dL (~1.55 mmol/L)
High Level Target:	~ 400 mg/dL (~10.35 mmol/L)	~300 mg/dL ~3.39 mmol/L)	~80 mg/dL (~2.07 mmol/L)

2. Cartridges are stored at 4° each cartridge the HbA1c cartridge can be used cold

3. Cartridges are stored at 4° each cartridge the lipids cartridge is only stable at RT for 1 hour

4. Run each Level 10 times on Allegro, the Lipids cartridge can only be run in the 660nm channel which is on the left hand side of the analyser.

6. Collect, review and collate data

Total Analysis time: 10 x 10 mins=100 mins per level. 300 mins for all levels this is just run time. Approx. 5 hrs. plus preparation time

Data Analysis

The within-run precision of the Allegro be assessed by calculating the % coefficient of variation (%CV) from the mean and standard deviation from the replicate measurements. Acceptability criteria are determined by the medical director and/or institution. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [1, 2].

Data Review

This data will be summarized in a final report which will also include an assessment of the accuracy. This report should be reviewed by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of Allegro performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Appendix 1

Within-Day Precision Study Data Collection Sheet

Hospital Name: _____ Date of Study: _____
System Operator(s): _____
Reference Analyzer _____

Level 1 Total Cholesterol, Triglycerides and HDL Cholesterol

Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____
Reference Analyzer Result mg/dL or mmol/L Triglycerides _____
Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____

	System Name							
	System Serial #							
	Cartridge Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	Total Cholesterol mg/dL or mmol/L	Triglycerides mg/dL or mmol/L	HDL Cholesterol mg/dL or mmol/L				
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Level 2 Total Cholesterol, Triglycerides and HDL Cholesterol

Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____
Reference Analyzer Result mg/dL or mmol/L Triglycerides _____

Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____

	System Name							
	System Serial #							
	Cartridge Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	Total Cholesterol mg/dL or mmol/L	Triglycerides mg/dL or mmol/L	HDL Cholesterol mg/dL or mmol/L				
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Level 3 Total Cholesterol, Triglycerides and HDL Cholesterol

Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____
Reference Analyzer Result mg/dL or mmol/L Triglycerides _____
Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____

	System Name							
	System Serial #							
	Cart Lot #							
Low QC	Range:							
High QC	Range:							
	Replicate #	Total Cholesterol mg/dL or mmol/L	Triglycerides mg/dL or mmol/L	HDL Cholesterol mg/dL or mmol/L				
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

3. Total Cholesterol, Triglyceride and HDL Cholesterol

II. Between-Day Precision Study Protocol

Protocol based on:

Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Purpose

This protocol is recommended and designed by Nova Biomedical to investigate the between-day precision of urine creatinine and urine albumin measurements on the Allegro system.

Materials Needed

- Nova Biomedical Allegro
- Allegro Lipid Cartridges
- Nova Biomedical Allegro Lipid Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic microcentrifuge tubes for decanting Na Heparin or Li Heparin samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips
- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: Total Cholesterol, Triglycerides, HDL Cholesterol

Sample Type: Subject to change but to date**:**

- Capillary whole blood (from finger stick)
- Venous whole blood with anticoagulants Na heparin; Li heparin
- Venous plasma with anticoagulants Na heparin; Li heparin
- Serum

Sample Size: 5 µL

Time per Lipid: 10 mins

Linear Range of Operation for Total Cholesterol: 100-450 mg/dL (2.59-11.64 mmol/L)

Linear Range of Operation for Triglyceride: 50-600 mg/dL (0.56-6.76 mmol/L)

Linear Range of Operation for HDL Cholesterol: 20-100 mg/dL (0.52- 2.59 mmol/L)

Please note that all parameters are processed at the same time.

Normal Values:

Total Cholesterol: Desirable is below 200 mg/dL (<5.17 mmol/L)

Triglycerides: Desirable is below 150 mg/dL (<1.69 mmol/L)

HDL Cholesterol: Desirable is 60 mg/dL and above (> 1.55 mg/dL)

Accuracy Expectations

Total Cholesterol: Target value $\pm$ 3%

Triglycerides: Target value $\pm$ 5%

HDL Cholesterol: Target value $\pm$ 5%

Control Levels:

The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:

	Total Cholesterol	**Triglycerides**	**HDL Cholesterol**
Low Level Target:	~ 170 mg/dL (~4.40 mmol/L)	~150 mg/dL (~1.69 mmol/L)	~40 mg/dL (~1.04 mmol/L)
High Level Target:	~ 240mg/dL (~6.21 mmol/L)	~300 mg/dL (~3.39 mmol/L)	~60 mg/dL (1.55 mmol/L) (~1.55 mmol/L)

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Analyse controls on Allegro every day that testing is carried out, 2 runs per day would be performed one in the morning and the other in the afternoon so QC would need to be performed prior to each run, make sure results are within specification before proceeding. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. Because of the logistical difficulty of using whole blood over a 2-week period the suggestion here is to use the Control Solutions, these provide only 2 levels, but these should be aliquoted into 40 x 50µL aliquots and frozen to reduce variables.

   Note: If Allegro can analyse plasma then the samples used for within day precision can be centrifuged, aliquoted into 40 x 50µL microcentrifuge tubes, and frozen.

4. A freshly thawed aliquot will then be analysed every morning and afternoon in duplicate for 10 consecutive working days

5. Ensure adequate mixing before analysis.

5. Cartridges are stored at 4° each cartridge the lipids cartridge is only stable at RT for 1 hour

6. Run each Level in duplicate on the Allegro, the Lipids cartridge can only be run in the 660nm channel which is on the left hand side of the analyser.

   6. Collect, review and collate data

   Total Analysis Time: 10 mins x 4 = 40 mins in morning 10 mins x 4 = 40 mins in afternoon plus preparation time

Data Analysis

The between-day precision of the Allegro be assessed by calculating the % coefficient of variation (%CV) from the mean and standard deviation from the replicate measurements. Acceptability criteria are determined by the medical director and/or institution. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [1, 2].

Data Review

This data will be summarized in a final report which will also include an assessment of the accuracy. This report should be reviewed by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of Allegro performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* EP5-A2.

Appendix 2

Between-Day Precision Study Data Collection Sheet

Hospital Name: _____ Date of Study: _____
System Operator(s): _____
Reference Analyzer _____

Level 1 Total Cholesterol, Triglycerides and HDL Cholesterol

Morning
Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____
Reference Analyzer Result mg/dL or mmol/L Triglycerides _____
Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____
Afternoon
Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____
Reference Analyzer Result mg/dL or mmol/L Triglycerides _____
Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____

	System Name												
	System Serial #												
	Cartridge Lot #												
Low QC	Range:												
High QC	Range:												
		Morning						**Afternoon**					
	Replicate #	Total Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L)	Trigs mg/dL or mmol/L	Trigs mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L)	Trigs mg/dL or mmol/L	Trigs mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	HDL Chol mg/dL or mmol/L
	Day 1												
	Day 2												
	Day 3												
	Day 4												
	Day 5												
	Day 6												
	Day 7												
	Day 8												
	Day 9												
	Day 10												

Level 2 Total Cholesterol, Triglycerides and HDL Cholesterol

Morning
Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____

Reference Analyzer Result mg/dL or mmol/L Triglycerides _____
Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____

Afternoon

Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____
Reference Analyzer Result mg/dL or mmol/L Triglycerides _____
Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____

	System Name												
	System Serial #												
	Cartridge Lot #												
Low QC	Range:												
High QC	Range:												
		Morning						**Afternoon**					
	Replicate #	Total Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L)	Trigs mg/dL or mmol/L	Trigs mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L)	Trigs mg/dL or mmol/L	Trigs mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	HDL Chol mg/dL or mmol/L
	Day 1												
	Day 2												
	Day 3												
	Day 4												
	Day 5												
	Day 6												
	Day 7												
	Day 8												
	Day 9												
	Day 10												

3. Total Cholesterol, Triglyceride and HDL Cholesterol

III. Method Comparison Study Protocol

Protocol based on the following guidance documents:

Clinical & Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. EP9-A2.*

Purpose

This protocol is designed to investigate how Allegro results compare to a recognized central laboratory reference method. These studies are important in order to establish the relationship between the Allegro System and the reference method with respect to statistical correlation and measurement bias. This protocol is designed and recommended for the Allegro System.

Background

Good practice dictates that the performance of medical devices such as the Allegro System be verified before the conductance of clinical studies. Verification studies include: method comparisons to determine how the results of the Allegro Systems compare to a recognized reference method, studies of imprecision to determine the reproducibility of the various tests available on the Allegro studies to determine the analytical trueness of the Allegro measurements. The protocol below describes how to conduct a method comparison (Allegro vs. Reference Method).

Materials Needed

- Nova Biomedical Allegro
- Allegro Lipid Cartridges
- Nova Biomedical Allegro Lipid Quality Control (QC) Solution
- Reference Analyzer and reagents
- 2mL plastic microcentrifuge tubes for decanting Na heparin or Li heparin samples
- Plastic 2mL disposable pipettes
- Full range of adjustable pipettes and tips
- If required appropriate Spiking stock solutions

Important Factors to Consider

Tests: Total Cholesterol, Triglycerides, HDL Cholesterol

Sample Type: Subject to change but to date**:**

- Capillary whole blood (from finger stick)
- Venous whole blood with anticoagulants Na heparin; Li heparin
- Venous plasma with anticoagulants Na heparin; Li heparin
- Serum

Sample Size: 5 µL

Time per Lipid: 10 mins

Linear Range of Operation for Total Cholesterol: 100-450 mg/dL (2.59-11.64 mmol/L)

Linear Range of Operation for Triglyceride: 50-600 mg/dL (0.56-6.76 mmol/L)

Linear Range of Operation for HDL Cholesterol: 20-100 mg/dL (0.52- 2.59 mmol/L)

Please note that all parameters are processed at the same time.

Normal Values:
Total Cholesterol: Desirable is below 200 mg/dL (<5.17 mmol/L)
Triglycerides: Desirable is below 150 mg/dL (<1.69 mmol/L)
HDL Cholesterol: Desirable is 60 mg/dL and above (> 1.55 mg/dL)

Control Levels:
The Allegro supports two levels of controls. The following are suggested ranges for each parameter and level:

	Total Cholesterol	**Triglycerides**	**HDL Cholesterol**
Low Level Target:	~ 170 mg/dL (~4.40 mmol/L)	~150 mg/dL (~1.69 mmol/L)	~40 mg/dL (~1.04 mmol/L)
High Level Target:	~ 240 mg/dL (~6.21 mmol/L)	~300 mg/dL (~3.39 mmol/L)	~60 mg/dL (1.55 mmol/L) (~1.55 mmol/L)

Procedure

1. Fill out the requested information (Allegro serial numbers, cartridge lot numbers, QC lot numbers, QC ranges) on the provided Data Sheets (see Appendix).

2. Perform quality control runs on the Allegro being evaluated and the reference analyzer. Record the results for all systems on the Data Sheet and make sure the results are within the manufacturer's control range. **Proceed with testing only if all control results are within range.**

3. 40-50 freshly collected/discarded whole blood venous, Na or Lithium heparin samples should be selected, that if possible, cover the analytical range across all analytes. Again spiking with appropriate spiking solutions may be required to completely cover the analytical range.

4. Ensure adequate mixing before analysis.

5. Analyse all samples in duplicate on the Allegro.

6. Samples should be spun down promptly (plasma) and then be analysed on the reference analyser.

7. Cartridges are stored at 4° each cartridge the lipids cartridge is only stable at RT for 1 hour

8. Run all samples in duplicate Allegro, the Lipids cartridge can only be run in the 660nm channel which is on the left hand side of the analyser.

9. Collect, Review and collate data.

10. Total Analysis Time: 10 mins x 80-100 = 800-1000 mins. Approx. 13.3 – 16.6 hrs plus preparation time

11. To reduce analysis time consider running samples in singles or perhaps less samples in duplicate whichever best suits site

Data Analysis

As part of this study paired total cholesterol, triglycerides and HDL cholesterol measurements will be collected. Analysis of accuracy is based on pairing individual results from the Allegro with the corresponding reference analyser measurement and calculating the difference (bias). Scatter plots and linear regression analysis are appropriate tools to assess the relationship between the Allegro results and the reference analyzer. Moreover, Bland-Altman plots can be used to assess the bias relative to the range of urine creatinine and urine Albumin concentrations studied. Investigators are referred to organizations such as CLSI and the International Standards Organization (ISO) which offer published acceptance criteria [1, 2]. The validity of the conclusions is dependent on experimental design, chiefly number of samples, the distribution of results, and the calibration of the reference analyzer.

Data Review

This data will be summarized in a summary report which will also include an assessment of precision. This report should be reviewed and signed off by the study site principal investigator. Results of this laboratory evaluation can be used to document verification of the Allegro System performance prior to the conductance of clinical studies.

References

1. Clinical & Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. EP9-A2.*

Appendix 4

Method Comparison Data Collection Sheet (Case Report Form)

Study Site: _____ Date of Study: _____
System Operator(s): _____

Specimen Type (Na or Lithium heparin venous blood) _____
Reference Analyzer Result mg/dL or mmol/L Total Cholesterol _____
Reference Analyzer Result mg/dL or mmol/L Triglycerides _____
Reference Analyzer Result mg/dL or mmol/L HDL Cholesterol _____

	System Name								
	System Serial #								
	Cartridge Lot #								
Low QC	Range:								
High QC	Range:								
Specimen ID	Total Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L	Reference Analyzer Result Total Chol (mg/dL or mmol/L)	Trigs mg/dL or mmol/L	Trigs mg/dL/ or mmol/L	Reference Analyzer Result Trig (mg/dL or mmol/L)	HDL Chol mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	Reference Analyzer Result HDL Chol(mg/dL or mmol/L)
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	System Name								
	System Serial #								
	Cart Lot #								
Specimen ID	Total Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L	Reference Analyzer Result Total Chol (mg/dL or mmol/L)	Trigs mg/dL or mmol/L	Trigs mg/dL/ or mmol/L	Reference Analyzer Result Trig (mg/dL or mmol/L)	HDL Chol mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	Reference Analyzer Result HDL Chol(mg/dL or mmol/L)
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	System Name								
	System Serial #								
	Cart Lot #								
Specimen ID	Total Chol mg/dL or mmol/L	Total Chol mg/dL or mmol/L	Reference Analyzer Result Total Chol (mg/dL or mmol/L)	Trigs mg/dL or mmol/L	Trigs mg/dL/ or mmol/L	Reference Analyzer Result Trig (mg/dL or mmol/L)	HDL Chol mg/dL or mmol/L	HDL Chol mg/dL or mmol/L	Reference Analyzer Result HDL Chol(mg/dL or mmol/L)
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100									

Name of Person Collecting Data: _____
Signature of Person Collecting Data: _____
Date: _____

3. Total Cholesterol, Triglyceride and HDL Cholesterol

Summary Report Template

Total Cholesterol, Triglyceride and HDL Cholesterol Summary Report

Date:

Report Author:

Study Site:

Study Site Principal Investigator/Department:

Materials and Methods

See Protocols I-IV. *Any deviations from protocol should be noted here.*

Summary of Results

I. Precision Studies

Table 1. **Total Cholesterol Within-Day** Precision for Allegro

	Low Cholesterol	Mid Cholesterol	High Cholesterol
Mean mg/dL or mmol/L			
Standard Deviation			
%CV			
N			
Matrix			

Table 2. **Triglyceride Within-Day** Precision for Allegro

	Low Triglyceride	Mid Triglyceride	High Triglyceride
Mean mg/dL or mmol/L			
Standard Deviation			
%CV			
N			
Matrix			

Table 3. **HDL Cholesterol Within-Day** Precision for Allegro

	Low Cholesterol	Mid Cholesterol	High Cholesterol
Mean (mg/dL)			
Standard Deviation			
%CV			
N			
Matrix			

Table 4. **Total Cholesterol Between-Day** Precision for Allegro

	Low Cholesterol	Mid Cholesterol	High Cholesterol
Mean (mg/dL)			
Standard Deviation			
%CV			
N			
Matrix			

Table 5. **Triglyceride Between-Day** Precision for Allegro

	Low Triglyceride	Mid Triglyceride	High Triglyceride
Mean (mg/dL)			
Standard Deviation			
%CV			
N			
Matrix			

Table 6. **HDL Cholesterol Between-Day** Precision for Allegro

	Low HDL Chol	Mid HDL Chol	High HDL Chol
Mean (mg/dL)			
Standard Deviation			
%CV			
N			
Matrix			

III. Method Comparison

Table 11. **Summary of Linear Regression Analysis Result**s: Total Cholesterol, Triglycerides and HDL Cholesterol for Allegro

	Total Cholesterol	Triglyceride	HDL Cholesterol
Slope			
y-intercept (mg/dL)			
R^2			
N			
Total Cholesterol Range mg/dL or mmol/L			
Triglyceride Range mg/dL or mmol/L			
HDL Cholesterol mg/dL or mmol/L			

Table 12. Summary of Bland-Altman Results

	Total Cholesterol	Triglyceride	HDL Cholesterol
Mean Bias (mg/dL)			
95% Limits of Agreement			
Lower			
Upper			
Total Cholesterol Range mg/dL or mmol/L			
Triglyceride Range mg/dL or mmol/L			
HDL Cholesterol mg/dL or mmol/L			

Summary Findings

Allegro demonstrated ….

This report has been reviewed and approved by:

Signature of Study Site Principal Investigator Date

Signature of MASA Director Date