04 September 2014

Daniel B. Goodman, MD

Vice President and Medical Director BioTelemetry Research

Polina Voloshko, MD

Chief Medical Officer BioTelemetry Research





V WY MARCH

TABLE OF CONTENTS

Abstract	4
Introduction	5
Protocol Information	5
ECHO Parameters Analyzed	6
ECHO Methodology	7
LVEDV and LVESV	7
LVEF	7
LAESV	7
LV Mass	7
Mitral Inflow Velocities (E-point, A-point and E/A ratio)	7
Statistical Methods	8
Results	8
Findings	9
Inter-reader Variability	9
Intra-reader Variability	9
Discussion	10
Conclusion	10
	Abstract

TABLES

Table 1:	Tabulation of ECHO Readings by Reader	5
Table 2:	Numbers of ECHO Recordings by Protocol	5
Table 3:	ECHO Parameters Studied	6
Table 4:	Inter-reader Results	11
Table 5:	Intra-reader Results	11
Table 6:	Inter-reader Summary Statistics	12
Table 7:	Intra-reader Summary Statistics	13
Table 8:	Inter-reader Power Statistics	14
Table 9:	Intra-reader Power Statistics	14



V WY MARCH

TABLE OF CONTENTS

FIGURES

Figure 1:	Inter-reader LVEDV	15
Figure 2:	Inter-reader LVESV	15
Figure 3:	Inter-reader LVEF	16
Figure 4:	Inter-reader LAESV	16
Figure 5:	Inter-reader LV Mass	17
Figure 6:	Inter-reader MV Peak E	17
Figure 7:	Inter-reader MV Peak A	18
Figure 8:	Inter-reader MV E/A Ratio	18
Figure 9:	Intra-reader LVEDV	19
Figure 10:	Intra-reader LVESV	19
Figure 11:	Intra-reader LVEF	20
Figure 12:	Intra-reader LAESV	20
Figure 13:	Intra-reader LV Mass	21
Figure 14:	Intra-reader MV Peak E	21
Figure 15:	Intra-reader MV Peak A	22
Figure 16:	Intra-reader MV Peak E/A Ratio	22



ABSTRACT

This report describes the inter-reader and intra-reader variability in a central reference laboratory for 8 ECHO Cardiogram (ECHO) parameters.

PURPOSE: To define the variability in repeat readings between readers (interreader) and for a single reader on repeat assessment (intra-reader). Particular attention is given to determination of Left Ventricular Ejection Fraction during repeat determination by a single reader.

METHODS: 58 ECHO studies were read by 2 physicians and 29 ECHOs re-read by the same reader, in a blinded manner and as part of regular workflow. Selection of the recordings was random and without regard to the original results. Parameters studied were: left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular ejection fraction (LVEF), left atrial end-systolic volume (LAESV), left ventricular mass (LV Mass), mitral inflow velocities peak at the E-point (Peak E), mitral inflow velocities peak at the A-point (Peak A), and the ratio of the mitral inflow velocities peaks at the E-point and the A-point (Peak E/A ratio).

STATISTICAL ANALYSIS: Differences (Diff) and the absolute values of the difference (Abs Diff) between the two readings for each parameter, and the absolute difference as a percent of the mean of the two readings (Percent Diff), were calculated. Also tabulated was the number and percent of Abs Diff and Percent Diff values exceeding, respectively, the smallest clinically important difference and the

expected variability, based on published standards.

FINDINGS: Inter-reader findings showed high level of agreement between readers with the percent of values of Abs Diff exceeding the smallest clinically important difference ranging from 3.8% (Peak E) to 44.8% (LVESV), and the percent of values of Percent Diff exceeding the expected variability from 0% (LV mass) to 50% (LVEF). Intra-reader agreement between the 2 readings was exceptionally high with no values of Abs Diff exceeding the smallest clinically important difference for any parameters except 11.8% for LAESV, and no values of Percent Diff exceeding the expected variability except 10.3% for LVEF and 17.6% for LAESV. For LVEF, the mean intra-reader Abs Diff was 1.83 (absolute)% and the mean Percent Diff for LVEF was 2.88%. No values of LVEF Abs Diff exceeded the published standard for the smallest clinically important difference of 35 (absolute)%, and only 10.3% of values of Percent Diff exceeded the published standard of 5.71%.

CONCLUSIONS: These results showed high agreement between readers and an especially high level of reproducibility of measurement for repeat interpretations by the same reader. All inter-reader results exceeded or met the expected reproducibility based on published standards. Determination of LVEF from ECHO is highly reproducible if performed by a single reader in a highly structured central reference laboratory.



1. INTRODUCTION

This report describes the inter-reader and intra-reader variability for several ECHO Cardiogram (ECHO) parameters for the central laboratory operations of Cardiocore, Inc. In addition, comparison to reference standard provides an assessment of inter- and intra-reader agreement. To assist in protocol planning, particular attention is given to determination of Left Ventricular Ejection Fraction during repeat determination by a single reader. Interpretation was performed by blinded rereading of 58 ECHO studies by 2 physicians and 29 ECHOs by the same reader. The ECHOs selected were submitted to each reader as part of their regular workflow, and were interpreted using standard procedures. Selection of the recordings was in random manner from among multiple protocols, and without regard to the original results.

TABLE 1: TABULATION OF ECHO READINGS BY READER

Number of ECHOs in Study – Total	87
Number of ECHOs for Inter-reader Variability	58
Number of ECHOs for Intra-reader Variability	29
Number of Readings by MD – Total	174
MD 1	116
MD 2	58

2. PROTOCOL INFORMATION

The ECHOs were selected from 3 clinical research protocols in abnormal subjects. These included patients with stable chronic Congestive Heart Failure, patients with Fabry's Disease (who have a potential for cardiac involvement), and patients with chronic hepatitis C (where cardiac disease due to the primary diagnosis is unlikely). Of note is that the CHF patients were quite ill with NYHA classification II or III, and left ventricular ejection fractions (LVEF) of <35%.

TABLE 2: NUMBERS OF ECHO RECORDINGS BY PROTOCOL

	Number of ECHOs				
	Inter-reader Intra-reade				
Congestive	33	0			
Heart Failure					
Fabry's Disease	7	11			
Chronic	18	18			
Hepatitis C					



3. ECHO PARAMETERS ANALYZED

The ECHO parameters included in the study were:

- left ventricular end-diastolic volume (LVEDV)
- left ventricular end-systolic volume (LVESV)
- left ventricular ejection fraction (LVEF)
- left atrial end-systolic volume (LAESV)
- left ventricular mass (LV Mass)
- mitral inflow velocities peak at the E-point (Peak E) by Doppler
- mitral inflow velocities peak at the A-point (Peak A) by Dopper
- ratio of the mitral inflow velocities peaks at the E-point and the A-point (Peak E/A ratio)

TABLE 3: ECHO PARAMETERS STUDIED

Parameter (units) Smallest Clinically **Expected Difference Expected Difference** Inter-reader Intra-reader **Important Difference** All except LAESV)1 (LAESV)2 All except LAESV)1 (LAESV)2 LVEDV (mL) 22 mL 15.12% 11.63% 12 mL LVESV (mL) 19.23% 19.23% LVEF (%) 5.71% 5.0% 7.14% LAESV (mL) 9 19% 19% 26.79% LV Mass (gm) 35.3 gm 22.32% Doppler Mitral inflow -16.5 cm/sec 15.6% 14.28% Peak E (cm/sec) Doppler Mitral inflow -13.3 cm/sec 20.23% 19.26% Peak A (cm/sec) Doppler Mitral inflow -0.45 15.79% 14.21% Peak E/A ratio

 Kuecherer HF, Kee LL, Modin G, Cheitlin, MD, Schiller NB Echocardiography in Serial Evaluation of Left Ventricular Systolic and Diastolic Function: Importance of Acquisition, Quantitation, and Physiologic Variability in Clinical and Investigational Applications J AM Soc EcHo 1991;4:203-14.

2. Himelman RB1, Cassidy MM, Landzberg JS, Schiller NB **Reproducibility of quantitative two dimensional echocardiography** Am Heart J. 1988 115:425-31.

These parameters are detailed in Table 3 with the reference values for the smallest difference of clinical importance, and published values of expected average variability (as percent difference of the mean value) for the inter- and intra-reader differences.



4. ECHO METHODOLOGY

All readings were performed in the highly structured environment of a central reference laboratory. Standardized reading methodologies for each of the parameters were used, as follows:

4.1. LVEDV AND LVESV

Left ventricular end-diastolic volume and end-systolic volume were calculated using the paired orthogonal long axis apical views biplane (known as Simpson's volumetric method). The LV endocardial border was traced from one side of the mitral annulus to the other side excluding the papillary muscles at end-diastole (frame before mitral valve close or maximum LV cavity) and endsystole (frame before mitral valve opens or minimum LV cavity) in the 2 and 4 chamber views.

4.2. LVEF

Left ventricular ejection fraction was measured from apical 2 and apical 4 chamber views using by the biplane Simpson's volumetric method, as described above. LVEF was determined from LV volumes using the formula: LVEF = 100 * (LVEDV - LVESV)/LVEDV

4.3. LAESV

LA volume was measured from the apical 2 and apical 4 chamber view using the biplane Method of Disks. The LA cavity area was traced at ventricular end systole (maximum LA size or frame before mitral valve opens).

4.4. LV MASS

Left ventricular mass was measured from the 2-dimension parasternal short axis view using truncated ellipsoid method by tracing the epicardial and endocardial margins of the left ventricular at the mid papillary short axis view, measurement of the long axis of the LV from a 2 or 4 chamber view, and indication of the point of the long axis at the level of the widest minor axis.

4.5. MITRAL INFLOW VELOCITIES (E-POINT, A-POINT AND E/A RATIO)

Velocities were measured from the baseline to the peak of the signal from a pulsedwave Doppler, using linear measurements of the peak E-point and A-point.



5. STATISTICAL METHODS

Differences (Diff) and the absolute values of the difference (Abs Diff) between the two readings were computed as were the absolute difference as a percent of the mean of the two readings (Percent Diff). The ECHO results were tabulated for the number and percent of studies where the absolute difference exceeded the smallest clinically important difference and where the percentage difference exceeded the expected variability. Also calculated, to assess measurement variation for power calculations in future clinical trials, were the ratio of the mean of each parameter to the standard deviations of the differences, and the ratio of the smallest clinically important difference to the standard deviations of the differences.

Bland-Altman plots were constructed showing, on the X-axis, the difference between the 2 readings, and on the Y axis, the mean of the 2 readings. Reference lines in the plots were added to indicate the mean difference and 3 twice the standard deviation of the differences.

6. RESULTS

Table 4 lists the findings for inter-reader variability and Table 5 lists the values for intra-reader variability. Summary statistics are presented in Table 6 for inter-reader variability and Table 7 for intra-reader variability.

Ratios of the mean value of the various parameters to the standard deviations of the differences and of the smallest clinical difference to the standard deviations of the differences are provided in Table 8 and Table 9. Bland-Altman plots are presented in Figures 1 through Figure 7 for inter-reader variability and in Figure 8 though Figure 14 for intrareader variability.



7. FINDINGS

7.1. INTER-READER VARIABILITY

Mean differences were close to 0 for all parameters except LVEDV which had a mean difference of 6.89 mL. Mean absolute differences were variable and the differences of these as a percent of the mean parameter value ranged from 5.72% for Doppler Mitral inflow – Peak E to 11.06% for LVESV.

The percent of values exceeding the smallest clinical difference ranged from 3.8% for the Peak E to 44.8% for LVESV. The percent of values exceeding the expected percent difference, which should be about 50% if variability matched the published results, ranged from 0% for LV mass to 50.0% for LVEF. Of the 8 parameters, 4 had less than 20% of values in excess of the expected percent difference.

Ratios of the various parameters mean value to the standard deviation of the differences ranged from 3.29 to 14.14 The ratio of the smallest clinically important difference to the standard deviation ranged from 0.97 to 2.42.

7.2. INTRA-READER VARIABILITY

Mean differences were close to 0 for all parameters and the mean absolute percent differences ranged from 2.29% for Peak E to 10.67% LV mass.

The percent of values exceeding the smallest clinical difference were 0 for all parameters except LAESV which was 11.8%. The percent of values exceeding the expected percent difference was 0 for all except 10.3% for LVEF and 17.6% for LAESV.

Ratios of the various parameters mean value to the standard deviation of the differences ranged from 9.07 to 39.64 The ratio of the smallest clinically important difference to the standard deviation ranged from 2.45 to 8.12.



8. DISCUSSION

The very sick CHF population, comprising 33 of the 58 of the subjects for the interreader data, increased the intra-subject and inter-subject variability of the parameters themselves and considerably increased the challenge in obtaining optimal quantitation. This was a particularly important contributor to increased inter-reader variability. Single outlier values for each the interreader differences for the three Mitral inflow parameters, Peak A, Peak E and Peak E/A ratio were noted. It is possible that these were the result of a transposition of the values for Peak A and Peak E in one of the readings. No further investigation was performed. This contributed to the relatively lower level agreement for these parameters.

9. CONCLUSION

These results show a high degree of agreement between readers and, an especially high reproducibility for repeat interpretations by the same reader. The agreement between 2 readers less than the value considered the smallest clinically important difference was shown in a very high percentage of readings. The parameter with the highest percent of differences exceeding that threshold was 44.8% for the LVESV inter-reader values. All inter-reader results exceeded or met the expected percentage difference based on published standards. For intra-reader agreement between the 2 determination, no values of mean absolute value of differences exceeded the smallest clinically important difference for any parameters except 11.8% for LAESV, and no values of percent difference exceeded the expected variability except 10.3% for LVEF and 17.6% for LAESV.

For LVEF, the intra-reader mean Abs Diff between readings was 1.83 (absolute)% and the mean Percent Diff was 2.88%. No values of Abs Diff exceeded the published standard for the smallest clinically important difference of 35 (absolute)%, and only 10.3% of values of Percent Diff exceeded the published standard of 5.71%.

Anticipating future clinical trials, measurement variation would add only slightly to sample sizes required to account for biologic variation intrinsic to repeated testing of individual subjects and the population variation across subjects. Particularly, these findings show that determination of LVEF from ECHO is highly reproducible if performed by a single reader in a highly structured central reference laboratory.



V WY MARCH

TABLE 4: INTER-READER RESULTS

Parameter (units)	Mean Reader 1	Mean Reader 2	Mean Diff	Mean Abs Diff	Mean Percent Diff	Number (%) > Smallest Clinical Difference	Number (%) > Expected Percent Difference
LVEDV (mL)	140.86	148.03	6.89	10.70	8.64%	3 (10.3)	11 (37.9)
LVESV (mL)	86.62	88.01	1.39	8.09	11.06%	13 (44.8)	9 (31.0)
LVEF (%)	44.06	45.50	1.44	3.56	9.85%	17 (29.3)	29 (50.0)
LAESV (mL)	63.06	60.97	-1.37	4.94	8.49%	6 (28.6)	3 (14.3)
LV mass (gm)	213.77	198.09	-4.87	12.71	6.49%	1(5.6)	0 (0)
Doppler Mitral inflow - Peak E (cm/sec)	78.71	78.51	-0.20	4.23	5.72%	1 (3.8)	1 (3.8)
Doppler Mitral inflow - Peak A (cm/sec)	58.91	59.24	0.44	5.10	8.61%	3 (5.7)	3 (5.7)
Doppler Mitral inflow - Peak E/A ratio	1.53	1.53	-0.01	0.16	9.26%	1(4.0)	12 (48.0)

TABLE 5: INTRA-READER RESULTS

Parameter (units)	Mean Reading 1	Mean Reading 2	Mean Diff	Mean Abs Diff	Mean Percent Diff	Number (%) > Smallest Clinical Difference	Number (%) > Expected Percent Difference
LVEDV (mL)	92.65	92.63	1.34	4.24	4.65%	0 (0)	0 (0)
LVESV (mL)	34.73	34.82	0.98	2.04	5.98%	0 (0)	0 (0)
LVEF (%)	63.86	62.96	-0.90	1.83	2.88%	0 (0)	3 (10.3)
LAESV (mL)	38.37	40.76	2.13	3.94	10.67%	2 (11.8)	3 (17.6)
LV Mass (gm)	142.98	135.80	-4.83	7.53	5.43%	0 (0)	0 (0)
Doppler Mitral inflow - Peak E (cm/sec)	80.00	81.17	0.35	1.71	2.29%	0 (0)	0 (0)
Doppler Mitral inflow - Peak A (cm/sec)	58.44	57.78	-0.66	2.05	3.57%	0 (0)	0 (0)
Doppler Mitral inflow - Peak E/A ratio	1.42	1.44	0.02	0.06	4.30%	0 (0)	0 (0)



V WY MARCH

TABLE 6: INTER-READER SUMMARY STATISTICS

		Difference	Abs Difference	% Difference
	count	57	57	57
	mean	6.89	10.70	8.64%
	min	-16.75	0.51	0.43%
LVEDV (ML)	max	27.10	27.10	30.64%
	median	10.31	10.65	6.50%
	std dev	10.37	6.27	6.72%
	count	58	58	58
	mean	1.39	8.09	11.06%
	min	-26.01	0.17	0.67%
LVESV (ML)	max	32.02	32.02	34.73%
	median	3.27	6.57	8.88%
	std dev	10.24	6.35	8.58%
	count	58	58	58
	mean	1.44	3.56	9.85%
	min	-6.44	0.02	0.03%
LVEF (%)	max	16.57	16.57	56.41%
	median	0.57	2.74	7.42%
	std dev	4.48	3.05	10.19%
	count	49	49	49
	mean	-1.37	4.94	8.49%
	min	-9.68	0.24	0.51%
LAESV (ML)	max	14.73	14.73	31.95%
	median	-1.93	4.64	7.92%
	std dev	5.75	3.17	6.17%
	count	42	42	42
	mean	-4.87	12.71	6.49%
LV Mass (am)	min	-35.78	1.02	0.86%
	max	27.32	35.78	18.38%
	median	-5.21	11.42	6.27%
	std dev	14.56	8.41	4.11%
	count	55	55	55
	mean	-0.20	4.23	5.72%
Doppler Mitral inflow - Peak E (cm/sec)	min	-62.28	0.00	0.00%
	max	11.76	62.28	78.52%
	median	0.71	2.32	3.22%
	std dev	9.44	8.43	10.68%
	count	53	53	53
	mean	0.44	5.10	8.61%
Doppler Mitral inflow - Peak A (cm/sec)	min	-18.27	0.19	0.30%
	max	62.92	62.92	102.43%
	median	-0.46	2.90	5.51%
	std dev	10.13	8.73	14.02%
	count	53	53	53
	mean	-0.01	0.16	9.26%
Doppler Mitral inflow - Peak F/A ratio	min	-3.17	0.00	0.08%
	max	0.44	3.17	150.55%
	median	0.01	0.05	5.13%
	std dev	0.46	0.44	20.77%



V WY MARCH

TABLE 7: INTRA-READER SUMMARY STATISTICS

		Difference	Abs Difference	% Difference
	count	18	18	18
	mean	1.34	4.24	4.65%
	min	-11.69	0.50	0.41%
LVEDV (ML)	max	8.67	11.69	10.57%
	median	1.94	3.55	4.11%
	std dev	5.08	2.94	3.10%
	count	18	18	18
	mean	0.98	2.04	5.98%
	min	-5.57	0.00	0.00%
LVESV (ML)	max	4.03	5.57	13.27%
	median	1.60	2.19	5.20%
	std dev	2.39	1.51	4.37%
	count	29	29	29
	mean	-0.90	1.83	2.88%
	min	-4.50	0.03	0.05%
LVEF (%)	max	3.49	4.50	7.25%
	median	-1.01	1.68	2.70%
	std dev	2.04	1.24	1.96%
	count	17	17	17
	mean	2.13	3.94	10.67%
	min	-9.68	0.24	0.58%
LAESV (IIIL)	max	10.07	10.07	31.95%
	median	3.26	3.65	9.76%
	std dev	4.43	2.82	8.52%
	count	27	27	27
	mean	-4.83	7.53	5.43%
LV Mass (am)	min	-22.79	0.84	0.60%
	max	11.20	22.79	14.87%
	median	-4.50	5.93	4.45%
	std dev	8.40	5.99	3.98%
	count	28	28	28
	mean	0.35	1.71	2.29%
Doppler Mitral inflow - Peak E (cm/sec)	min	-4.37	0.13	0.14%
	max	4.07	4.37	8.33%
	median	0.39	1.47	1.93%
	std dev	2.03	1.11	1.73%
	count	28	28	28
	mean	-0.66	2.05	3.57%
Doppler Mitral inflow - Peak A (cm/sec)	min	-6.48	0.15	0.29%
	max	3.51	6.48	9.45%
	median	-0.46	1.70	3.30%
	std dev	2.55	1.60	2.39%
	count	28	28	28
	mean	0.02	0.06	4.30%
Doppler Mitral inflow - Peak F/A ratio	min	-0.18	0.00	0.19%
	max	0.18	0.18	11.65%
	median	0.02	0.05	3.91%
	std dev	0.08	0.05	3.14%



V VENKE AN

TABLE 8: INTER-READER POWER STATISTICS

	Parameter Mean Value	Standard Deviation of Differences	Ratio of the Mean Value of the Parameter to the Standard Deviation of Differences	Ratio of the Smallest Clinically Important Difference to the Standard Deviation of Differences
LVEDV (mL)	144.44	10.37	13.93	2.12
LVESV (mL)	87.31	10.24	8.52	1.17
LVEF (%)	44.78	4.48	9.99	1.12
LAESV (mL)	62.38	5.75	10.84	1.56
LV Mass (gm)	205.93	14.56	14.14	2.42
Doppler Mitral inflow - Peak E (cm/sec)	78.61	9.44	8.32	1.75
Doppler Mitral inflow - Peak A (cm/sec)	59.07	10.13	5.83	1.31
Doppler Mitral inflow - Peak E/A ratio	1.53	0.46	3.29	0.97

TABLE 9: INTRA-READER POWER STATISTICS

	Parameter Mean Value	Standard Deviation of Differences	Ratio of the Mean Value of the Parameter to the Standard Deviation of Differences	Ratio of the Smallest Clinically Important Difference to the Standard Deviation of Differences
LVEDV (mL)	92.64	5.08	18.25	4.33
LVESV (mL)	34.77	2.39	14.57	5.03
LVEF (%)	63.41	2.04	31.07	2.45
LAESV (mL)	39.56	4.36	9.07	2.06
LV Mass (gm)	139.39	8.40	16.59	4.20
Doppler Mitral inflow - Peak E (cm/sec)	80.58	2.03	39.64	8.12
Doppler Mitral inflow - Peak A (cm/sec)	58.11	2.55	22.82	5.22
Doppler Mitral inflow - Peak E/A ratio	1.43	0.08	17.56	5.52







FIGURE 2: INTER-READER LVESV





FIGURE 3: INTER-READER LVEF



FIGURE 4: INTER-READER LAESV







FIGURE 5: INTER-READER LV MASS

FIGURE 6: INTER-READER MV PEAK E







FIGURE 7: INTER-READER MV PEAK A



FIGURE 8: INTER-READER MV E/A RATIO





FIGURE 9: INTRA-READER LVEDV



FIGURE 10: INTRA-READER LVESV





FIGURE 11: INTRA-READER LVEF



FIGURE 12: INTRA-READER LAESV





FIGURE 13: INTRA-READER LV MASS



FIGURE 14: INTRA-READER MV PEAK E





FIGURE 15: INTRA-READER MV PEAK A



FIGURE 16: INTRA-READER MV PEAK E/A RATIO

