Clinical Investigation Report

""Ligence Heart 2.1" software as a medical device verification"

(CIV-ID: CIV-LT-21-02-035769)

Version: 1.0; 2021-04-09

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Investigational Device: Ligence Heart, version 2.1

FOR QUALIFIED INVESTIGATORS, STUDY STAFF, AND THEIR ETHICS COMMITTEE(S) ONLY

CONFIDENTIALITY STATEMENT

Information in this RESEARCH STUDY PROTOCOL is for investigators, site personnel involved with the study, ethics committee(s), and/or their authorized representative(s) except as required to obtain consent from study participants or as otherwise required by law. Once signed, the terms of the protocol are binding for all parties.

The Sponsor and Investigator have approved this protocol version, and I confirm hereby to conduct the study according to the protocol and in accordance with applicable principles of the World Medical Association Declaration of Helsinki and Good Clinical Practice (GCP) guidelines as per ISO 14155:2011, any conditions of approval imposed by the reviewing EC or governing regulatory body, and applicable laws and regulations. The investigator and sponsor should not deviate from this protocol except for emergency use. I have read and understood and agree to abide by all the conditions and instructions contained in this protocol.

Site Name, Department, Address: Šiauliai Republican Hospital, Cardiology department, V. Kudirkos str. 99, 76231 Šiauliai, Lithuania

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Contents

1 Document Control

This section records all changes made to the protocol for a specific study. In the table below, record every relevant change by indicating what changes were made.

Revision	Date	Revision Author	Comments/Changes
1.0	2021-04-01	Arnas Karužas	The original version of the document is created.

2 List of Abbreviations and Terms

2D TTE 2-dimensional transthoracic echocardiography

AE	Adverse Event
ADE	Adverse Device Effect
ALARP	As Low as Reasonably Possible
CA	Competent Authority
CRF	Case Report Form
DCF	Data Clarification Form
EC	Ethics Committee
EU	European Union
GCP	Good Clinical Practice (see ISO 14155:2011)
ICF	Informed Consent Form
ISO	International Standards Organization
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
USADE	Unexpected Serious Adverse Device Effect
A2CH	Apical 2 Chamber
A4CH	Apical 4 Chamber
AAo	Ascending Aorta Diameter
AcT	Acceleration Time
AoA	Aortic Annulus
AoS	Aortic Sinus Diameter
AV Vmax	Aortic Peak Velocity
AV VTI	Aortic Valve Velocity Time Integral
Dec	Transmitral E Velocity Deceleration Time
E	Transmitral E Velocity
IVSd	Interventricular Septum (diastole)
LAD	Left Aclinical investigation Diameter (PLA view)
LAV	Left Aclinical investigation Volume
LVEDD	Left Ventricle End-Diastolic Diameter
LVEDV	LV End Diastolic Volume
LVESD	Left Ventricle End-Systolic Diameter
LVESV	LV End Systolic Volume
LVPWd	Left Ventricle Posterior Wall (Diastole)
MD	Medical Device
MDC	Minimal Detectable Change
PLA	Parasternal long axis
RAA	Right Aclinical investigation Area
RAMAD	Right Aclinical investigation Minor Axis Dimension (a4ch)
RVB	Right Ventricular Basal Diameter
RVEDA	Right Ventricle End Diastolic Area
RVESA	Right Ventricle End Systolic Area

RVM Right Ventricular Middle Diameter

RVOTPD Right Ventricular Outflow Tract Proximal Diameter (PLA)

S’ RV S' right ventricle lateral wall

SEM Standard Error of Measurement

STJ Sinotubular Junction

TR Vmax Tricuspid Regurgitation Peak Velocity

TR VTI Tricuspid Regurgitation - Velocity Time Integral

VM View Mode

INVESTIGATION SYNOPSIS	
Sponsor:	UAB Ligence
Research center:	Republican Šiauliai Hospital (Respublikinė Šiaulių Ligoninė) Address: V. Kudirkos str. 99, 76231 Šiauliai, Lithuania Tel: (8 41) 524 257 e-mail: info@siauliuligonine.lt Title of the department (s): Cardiology department
Research Type:	Single center retrospective, randomized, quantitative clinical investigation that will use the DICOM images of retrospectively enrolled subjects for the evaluation of medical device software. The study will be conducted in two parts, analyzing the manually performed measurements by the software (part 1) and analyzing the automatically performed measurements (part 2).
Regulatory Status:	This is a pre-market clinical evaluation study for the Ligence Heart product.

Procedures/ Methods:	No direct clinical procedures will be performed on the enrolled subjects. The clinical procedure that will take place is the analysis of the 2D TTE examination DICOM images that were included in this study. Once enough subjects are chosen and enrolled in the study, the following parts take place: During Part 1 of the study, investigators will analyze the same images using Ligence Heart software, Intellispace Cardiovascular 5.1 software, and EchoPAC version 112 software. The purpose of part 1 is to calculate the accuracy of the manually made measurements on the Ligence Heart software with other CE marked state-of-the-art software. During part 2 of the study, investigators will analyze 2D TTE images and the results will be compared to the results of the analysis of Ligence Heart automatic functionalities. The purpose of part 2 is to compare Ligence Heart automatic measurements accuracy and reliability with human physicians in real clinical setting. All members of the study staff will be qualified based on experience and ensured to have the required expertise in echocardiography prior to participation. Patients who are 18 years of age and underwent transthoracic 2D TTE during the time period of 2010-08-01 to 2020-08-01 and have their 2D TTE exam images saved in the investigational site database are the group to be enrolled in the investigation. The ultrasound machines that were used to perform the 2D TTE examinations that are included in the clinical investigation: • GE Vivid E9 Ultrasound Machine; • GE Vivid S5 Ultrasound Machine; • Philips Affiniti 70 Ultrasound system; • Philips EPIQ 7 Ultrasound system; • Philips Sparq ultrasound system. After both, part 1 and part 2 of the study are finished, the analysis of the generated data will take place. The statistical conclusions formulated from the data will be used to fulfill the clinical investigation objectives and to either accept or reject the hypotheses.
Objectives:	Data from Part 1 and Part 2 may be pooled or analyzed as separate subgroups, as determined necessary by the sponsor. Objectives: 1. Manual measurement analysis: To calculate the reliability of Ligence Heart manual functions that are used to perform echocardiographic measurements comparing it with the manual measurements performed with other CE marked state-of-the-art medical image viewers. 2. Automatic measurement analysis: To compare Ligence Heart automatic measurements accuracy, variance, and error rate with human physicians in real clinical setting. 3. Time used comparison: To compare Ligence Heart automatic measurement tool execution time with the time it takes a physician to perform the measurements manually. The **safety objective** is to collect safety information, including type and number of AEs, SAEs, and other device issues.

<table>
<tr><td>Endpoints:</td><td colspan="2">Primary endpoints:
1. Echocardiographic measurements performed manually with Ligence Heart and comparator software. The inter-software reliability between manual measurements performed with Ligence Heart and other CE marked state-of-the-art medical image viewers will be calculated (Part 1 of the investigation);
2. Echocardiographic measurements performed automatically by Ligence Heart software and echocardiographic measurements performed manually by physicians. The accuracy of automatic functions of Ligence Heart will be compared with the accuracy of physicians performing measurements manually (Part 2 of the investigation);
3. Time used to perform echocardiographic measurements manually and time used to perform the measurements automatically.
The safety endpoint is the type and number of AEs, SAEs, and device issues.</td></tr>
<tr><td>Eligibility criteria:</td><td>Inclusion criteria:
All included subjects will be:
1. Aged 18 years at the time when the 2D TTE examination was performed;
2. 2D TTE images are saved in the database of investigational site;
3. Examination was performed during the time period of 2010-08-01 to 2020-08-01;
4. 2D TTE images contain the following view modes that are necessary to perform the analysis of the images according to this clinical investigation plan:
a. apical 4 chamber view;
b. apical 2 chamber view;
c. parasternal long axis view.</td><td>Exclusion criteria:
Subjects will be excluded that:
1. Have anatomical or functional cardiac characteristics that prevent appropriate completion of 2D TTE image analysis using the study device - a list of such characteristics can be found in the section 4.6.2 Contraindications.</td></tr>
<tr><td>Sample size and Sites:</td><td colspan="2">Required sample size was established with the help of statistical power calculation tool „G*Power 3.1.9.7", which estimates the minimum sample size required to find and statistically prove if there is an effect to be found. In the analyses that were carried out the sample size was calculated for Repeated measures, within-between interaction ANOVA. Obtained minimal sample size was established to be 54 for both part 1 and part 2 of the clinical investigation.

Sites of investigation: 1 (one) site. Research center:
Respublikinė Šiaulių Ligoninė / Republican Šiauliai Hospital
Title of the department (s): Cardiology department</td></tr>
</table>

Study duration:	The study is expected to last approximately 1 month. Estimated start date: 2021-03-10 Estimated end date: 2021-04-10

ADMINISTRATIVE STRUCTURE OF INVESTIGATION		
Chief Technical Officer (Sponsor Contact):	Karolis Šablauskas *Tel*: +37067707580 *e-mail*: k.sablauskas@ligence.io	UAB Ligence
Chief Medical Officer and clinical manager:	Arnas Karužas *Tel*: +37067926921 *e-mail*: a.karuzas@ligence.io	UAB Ligence

3 Background and Justification

Cardiovascular diseases (CVD) or diseases of the heart and circulatory system account for 37% of all deaths in the EU and 45% of all deaths in geographical Europe. Each year, there are over 6.1 million new cases of CVD in the EU and the estimated cost of CVD to the EU economy is €210 billion a year (1).

The most versatile and cost-effective CVD diagnostic method currently available is the ultrasound of the heart, also known as echocardiography and the commonly used type of echocardiography - two-dimensional transthoracic echocardiography (2D TTE) (2). It is routinely used in different stages of CVD management including diagnosis, patient follow-up as well as pre- and post-operative care. It produces a wealth of information that guides the management and the treatment of a wide range of disorders including but not limited to heart failure, myocardial infarction, cardiomyopathies, or differential diagnosis of chest pain (3).

The increasing geriatric population as well as increasing incidence of cardiovascular diseases increases the need of medical imaging (4). The medical imaging market has been growing with the continuous annual growth of 5.5% and its value may reach USD 33.5 billion cap in 2024 (5). Moreover, the trends in the global medical staff market are also worth noting. According to the World Health Organization there will be a shortage of 12.9 million healthcare workers across the globe in 2035 (6). This lack of medical staff and automation in current healthcare will only worsen the patient care. Therefore, solutions for healthcare that help understaffed hospitals will be at very large demand. Nevertheless, despite the high demand for early diagnostics of CVDs, there are factors contributing to the suboptimal efficiency of echocardiography and thus resulting in reduced access for patients with wait times of 5 weeks in most EU countries (7). One of the main limitations of echocardiography is that it is highly dependent on the person performing the examination (the observer); studies have shown that inaccuracies can be found in up to 30% of transthoracic echocardiography reports (8). Moreover, analyzing echocardiographic test data requires manual work as even using the most advanced software, around 90% of the required heart parameters have to be evaluated manually. During our in-house study we found that an experienced cardiologist spends 50-85% of the average total test time (30-90 min) on performing manual measurements. Therefore, the need of an automated heart ultrasound system is as ever in demand as it has been.

To this date, there have been quite a few attempts to prove the feasibility of creating an automated heart ultrasound analysis system. The growth of artificial intelligence (AI) use in echocardiography over the past

years has been exponential, offering new paths to increase the performance and efficiency of 2D TTE examinations. There are quite a few commercially available and widely used software applications which aid the physician in analysis of 2D TTE data (e.g. EchoPAC by GE healthcare, QLAB by Philips etc.). These programs already perform a variety of tasks - segmentation and detection of anatomic landmarks, blood tracking. However, the result of the software analysis is completely dependent on the operator's ability to acquire high quality data and annotate it (9). For the procedure to become automated, different components of the echocardiography must be targeted e.g. recognition of image view, heart cycle detection and anatomical landmark segmentation. The research showed that a deep learning model was able to classify among 12 video views with 97.8% overall test accuracy without overfitting. Even on single low-resolution images, accuracy among 15 views was 91.7% vs. 70.2–84.0% for board-certified echocardiographers (10). In addition, deep learning applications were shown to be as effective as physicians (sometimes even with less variability compared to physicians) in many different echocardiography tasks: quantification of heart chamber size, ejection/filling parameters, identify left ventricular territory of regional wall motion abnormality on parasternal short-axis views, distinguish hypertrophic cardiomyopathy, cardiac amyloidosis, and pulmonary arterial hypertension from controls (11–13). Artificial intelligence tools for echocardiography data interpretation have been proving themselves useful in automation of the heart ultrasound examination.

In summary, Artificial Intelligence applications in echocardiography show promising results for the automation of manual and repetitive tasks. Further developments in this field might reduce the clinicians inter-observer variability, cognitive errors, and greatly increase efficiency (13,14). Ligence Heart is an example of such previously described Artificial Intelligence tools and this study would allow to evaluate its safety and performance compared to the current state-of-the-art alternatives in clinical care of cardiology patients.

3.1 Justification for the choice of investigational site

The investigation site has been selected for participation in the clinical study based on the following criteria:

- Adequate staff (lead principal investigator) that is accessible and has sufficient time and management competences to manage the clinical investigation and data reporting requirements;
- Site personnel has demonstrated experience with conducting clinical investigations, are familiar with ISO 14155:2011;
- Site has sufficient technical possibilities to support the usage of Ligence Heart software according to all requirements;
- Ability to securely store data and use medical software Ligence Heart according to the User manual (Instructions for use);
- Ability to perform required clinical investigation on site (has all the required resources, staff expertise in performing 2D TTE examinations).

3.2 Controls and Minimization of Bias

The following bias control methods will be implemented during this study: data samples are randomly selected from a larger sample database of subjects undergone 2D TTE examination to prevent judgement or systematic biases. All information of the subjects that would allow investigators to identify them was removed prior to the analysis of the 2D TTE images to prevent performance bias.

4 Device description

Name: Ligence Heart

Manufacturer: UAB Ligence

Software version: version 2.1

Regulatory Status: Pre-market

4.1 Intended purpose

Ligence Heart is a software used to detect, measure, and calculate various specifications of structure and function of the heart and great vessels by analyzing echocardiographic images.

The device is intended to be used, when the patient is not in a life-threatening state of health, time is not critical for medical decisions and no major therapeutic interventions are required.

4.2 Benefits and expected performance

Benefits

The use of Ligence Heart software brings a modern, quicker and accurate way for understanding visual echocardiography data needed for the management of cardiology patients. In addition to manual analysis of echocardiography images, Ligence Heart allows the user to automatically perform parts of the echocardiography image evaluation with non-inferior accuracy compared to cardiologists, reducing the variability of measurements, and reducing the time needed for analysis.

Expected performance

Expected performance of manual functionalities:

- The manual functionalities of Ligence Heart are expected to provide equally accurate and reliable tools for echocardiography evaluation compared to other state of the art CE marked medical software.

Expected performance of automated functionalities:

- Ligence Heart performs automated measurements with non-inferior accuracy compared to a cardiologist;
- Automatic functionalities perform echocardiographic measurements with lower intra-rater variability than a cardiologist;
- On average automatic functionalities perform the evaluation of echocardiographic images faster than a cardiologist.

4.3 General description

In order to better understand the method of working of the software, it is convenient to separate the process of echocardiography exam into two steps:

1. Data acquisition. During the first step, the operator of an ultrasound machine manipulates a probe interacting with the patient in order to produce the echocardiographic images of the heart. The images are then saved and stored digitally in DICOM format.
2. Data analysis. Using medical image viewing software the acquired echocardiography images are opened, annotated, measured and clinical conclusions are drawn based on the generated data.

Having established these steps, it is important to identify how the process of echocardiography exam takes place in the specific case of using Ligence Heart. The first step (data acquisition) remains the same as a regular echocardiography exam in accordance with standard clinical setting and is in no way affected using Ligence Heart software. Ligence Heart software is used to perform the entirety of the second step. To fulfill its intended purpose, the software Ligence Heart is used as a post-processing tool that is accessible via the sonographer’s/physician’s workstation in the office or any other dedicated area for patient’s clinical data analysis.

Ligence Heart is used as a post-processing tool to perform the second step (data analysis), during which the following processes take place:

1. Echocardiography images are loaded to the Ligence Heart software via integration to PACS or manual study DICOM files upload.
2. The analysis phase:
   a) **Manual functionalities** - user of the software manually identifies the relevant structures or features, annotates them, measurement results are displayed in the viewer.
   b) **Automatic functionalities** - the software has algorithms implemented, that allow automatic identification, annotation, and calculation of a number of relevant heart parameters. These algorithms are based on deep learning technology. The results provided by these algorithms must be reviewed and approved by a clinician.
3. A report is generated. The results of the whole study analysis process are summarized in a report. The report document provides a table of measurements performed, a summary template, an illustrations list of measurements made.
   The automatically generated measurements and the finalized report must be approved by a medical professional who is certified and eligible to conduct echocardiography examinations and formulate a report without the use of Ligence Heart automatic functions. The automatically generated and physician approved report of echocardiogram analysis serves only as a decision support tool. The conclusion of diagnosis must be always taken by the physician.

4.4 User groups

There are 2 groups of users that can work with Ligence Heart:

1. **Sonographer/physician**. Ligence Heart can be used by medical professionals that are certified and eligible by local legislation to conduct regular echocardiography examinations in a clinical setting. The automatically generated measurements and the finalized report have to be approved by a medical professional who is certified and eligible by local legislation to conduct echocardiography examinations and formulate a report.
2. **Administrator**. Ligence Heart can be used by client's system administrators that are not medical practitioners for the purpose of system administration, but not for clinical purposes.

4.5 Device identification for traceability and risk class

Product Identification	
Product name (as listed on label)	Ligence Heart
Product name (using nomenclature of basic UDI-DI)	HRT1
basic UDI-DI code	477905160HRT1JQ
Classification of product and classification rule resulting in highest risk class	
Device Class	IIb
Classification Rule including subclause according to MDR Annex VIII	Rule 11

4.6 Intended patient population and medical conditions

4.6.1 Indications

The software is intended to be used in analysis of echocardiography images acquired from patients that are of any gender and race in accordance with the latest guidelines for echocardiography examination.

4.6.2 Contraindications

The automatic functionalities should not be used to analyze echocardiography images of patients younger than 18 years old. Also, automatic functionalities should not be used to analyze images of patients with

heart diseases/procedures done that significantly alter heart anatomy or geometry that significantly distort the echocardiography images. A list of contraindications is provided in the table below:

Contraindications for automatic functionalities
Isolated congenital aortic valve disease and bicuspid aortic disease
Isolated congenital mitral valve disease (except parachute valve, cleft leaflet)
Mild isolated pulmonary stenosis (infundibular, valvular, supravalvular)
Isolated ventricular septal defect, or patent ductus arteriosus
Sinus venosus defect, ventricular septal defect, or patent ductus arteriosus without residuae or sequellae, such as chamber enlargement, ventricular dysfunction, or elevated pulmonary artery pressure.
Anomalous pulmonary venous connection (partial or total)
Anomalous coronary artery arising from the pulmonary artery
Anomalous coronary artery arising from the opposite sinus
Congenital aortic stenosis - subvalvular or supravalvular
Atrioventricular septal defect, partial or complete, including primum aclinical investigation septal defect (excluding pulmonary vascular disease)
Coarctation of the aorta
Double chambered right ventricle
Ebstein anomaly
Marfan syndrome and related heritable thoracic aortic disease, Turner Syndrome, congenital connective tissue disorders with heart or major vessel involvement
Patent ductus arteriosus, moderate or large unrepaired (excluding pulmonary vascular disease)
Peripheral pulmonary stenosis
Pulmonary stenosis (infundibular, valvular, supravalvular), moderate or severe
Sinus of Valsalva aneurysm/fistula
Sinus venosus defect
Tetralogy of Fallot repaired
Transposition of the great arteries after arterial switch operation
Ventricular septal defect with associated abnormalities (excluding pulmonary vascular disease) and/or moderate or greater shunt.
Any Congenital Heart Disease (repaired or unrepaired) associated with pulmonary vascular disease (including Eisenmenger syndrome)
Any cyanotic Congenital Heart Disease (unoperated or palliated)
Double-outlet ventricle
Fontan circulation
Interrupted aortic arch
Pulmonary atresia (all forms)
Transposition of the great arteries (except for patients with arterial switch operation)

Univentricular heart (including double inlet left/right ventricle, tricuspid/mitral atresia, hypoplastic left heart syndrome, any other anatomic abnormality with a functionally single ventricle)
Truncus arteriosus
Other complex abnormalities of atrioventricular and ventriculoarterial connection (i.e. crisscross heart, heterotaxy syndromes, ventricular inversion).
Congenital tricuspid and pulmonary valve disease
Situs inversus or dextrocardia
Heart tumors
Prosthetic valves, post-operative heart valves, cardiac geometry changing cardiothoracic surgeries
Implanted dual-chamber or ventricular pacemaker
Bi-ventricular resynchronizator
cardioverter-defibrillator
Letf ventricle assisting device, Right ventricle assisting device

4.7 Principles of operation of the device

4.7.1 Manual functionalities

The device visualizes echocardiography imaging data in a web browser and allows inspecting the imaging data and performing measurements by drawing annotations superimposed on the visualized data. The annotations are then used to calculate the relevant geometric and functional heart parameters.

4.7.2 Automatic functionalities

The device performs a series of steps that involve automated recognition of the echocardiography imaging data, recognition of echocardiographic probe position and detecting a set of anatomical (e.g. heart chamber borders, landmarks). The automated functionalities rely on the metadata obtained from the DICOM format (e.g. imaging mode) as well as predictions made by deep neural networks from the echocardiographic images (e.g. echocardiographic probe position recognition, heart chamber border, landmark detection).

To summarize, the input to the device is an echocardiography DICOM image. After the image is analyzed using automated and/or manual functionalities, a finalized report is generated, which is the output of the software.

4.8 Qualification of the product as a medical device

Definition of medical device according to EU MDR 2017/745

‘Medical device’ means any instrument, apparatus, appliance, software, implant, reagent, material, or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes:

- diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease;
- diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability;
- investigation, replacement or modification of the anatomy or of a physiological or pathological process or state;
- providing information by means of in vitro examination of specimens derived from the human body, including organ, blood and tissue donations;

- and which does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means.

The following products shall also be deemed to be medical devices:

- devices for the control or support of conception;
- products specifically intended for the cleaning, disinfection or sterilization of devices as referred to in Article 1(4) (EU MDR 2017/745) and of those referred to in the first paragraph of this point.

Ligence Heart meets requirements which are described in definition of medical device which is placed in Medical Device Regulation 2017/745, based on the definition of a medical device:

medical device - software, intended by the manufacturer for use in humans for the purpose of:

- diagnosis, prevention, prediction, prognosis, treatment or alleviation of the disease.
- investigation, replacement or modification of the anatomy or of a physiological or pathological process or state.

4.9 Explanation of any novel features

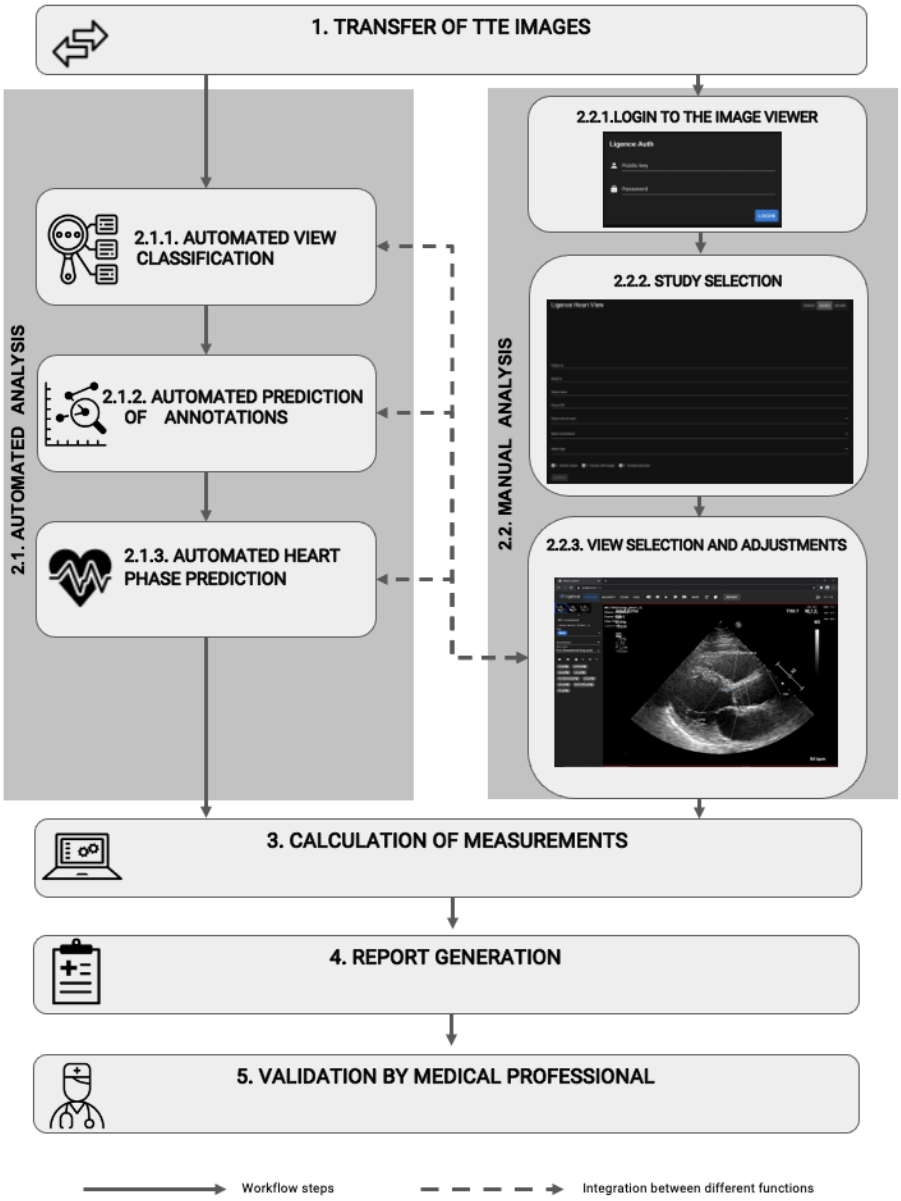
Ligence Heart offers novel functionality that allows automatic analysis of a number of heart structure and function parameters. Therefore, the parameters that are analyzed themselves are not novel, but the automation of some of these measurements is novel (none of the manual functionalities are novel). The automatic functionalities are based on Deep Learning technologies (more details in the System detailed design and specification in the Technical File). These automatic functionalities offer the ability to automate activities that are usually performed manually during regular echocardiography image analysis.

4.10 Description of all configurations/variants of the product

There is a possibility, on the request of the customer, to have different functionalities of Ligence Heart turned on/off for each customer via the manufacturers control mechanisms. The product basic package will always allow to manually annotate images and receive calculations of measurements. The algorithms to automatically perform some of these manual tasks will be turned on/off depending on the customer needs and sale agreement.

4.11 General description of key functional elements

Functional elements scheme.



Explanation of functional elements

Key function	Description
1. Transfer of echocardiography images	Personal data is removed from echocardiography images (if needed) and the images are transferred from hospital PACS to Ligence Heart software
2. Analysis	Echocardiography images analysis step using automated or manual analysis
2.1. Automated analysis	
2.1.1. Automated view classification	Automated system determines the view mode of echocardiography image. This step is needed for further analysis of images
2.1.2. Automated prediction of annotations	Automated system predicts annotations that are used to measure heart anatomy based on the view mode of echocardiography DICOM image

2.1.3. Automated heart phase prediction	Automated system tracks cardiac cycle and identifies the frames that are crucial for the analysis of echocardiography images, e.g. end-systolic and end-diastolic
2.2. Manual analysis	
2.2.1. Login to the image viewer	Authentication/authorization to the software step needed to be able to access studies
2.2.2. Study selection	Selection of accessible study by filtering/searching step
2.2.3. View selection and adjustments	Study analysis step, physician sets the view mode of echocardiography image, performs annotations, or adjusts measurements already made by automated analysis
3. Calculation of measurements	Calculation of measurements based on the annotations on echocardiography image performed by the combination of manual and automatic functions
4. Report generation	Study analysis report, which consists of all annotations, measurements made along with automated suggested diagnosis text, is generated for review and approval of physician
5. Validation by medical professional	Medical professional validates all annotations and measurements made and adjusts the annotations if needed, updating the report respectively

4.12 State of the Art and Comparator/Reference Standard

4.12.1 State of the Art

The current state of the art of echocardiographic examination is thoroughly defined in the guidelines of American Society of Echocardiography (ASE), European Association of Cardiovascular Imaging (EACVI), European Association of Echocardiography (EAE) (15–22).

Based on the reviewed scientific literature, clinical guidelines, analyzed products on the market, the current state of the art is as follows:

A physician or a technician acquires echocardiography images using an up-to-date certified modern ultrasound machine. The acquired images are then analyzed either on the ultrasound machine or in post-processing using a workstation after the exam has been performed. The analysis is performed with different certified medical image analysis software (e.g. EchoPAC by GE or Intellispace Cardiovascular by Philips, which were chosen as comparator/reference standards). The heart view mode selection, choice of frames to analyze, and annotations are all performed manually by the physician according to the guidelines that are mentioned previously. To finalize, a report that includes all relevant findings is generated.

The analysis of echocardiography images is subjective and dependent on the physician's skill and ability to perform the mentioned manual tasks and therefore is prone to errors. The literature on reproducibility of echocardiographic measurements shows that intra and inter-observer variability is depended on the measurements in question and can be as high as 40% (mean error) for some measurements (23,24).

Furthermore, a comprehensive TTE examination on average lasts from 30 to 60 min but can take up to 2 hours in more complicated cases (25,26).

4.12.2 Comparator/Reference standards

The comparator/reference standards used for comparison with the investigational product Ligence Heart software are separated into two groups, which are based on the functionalities that are being compared:

1. Manual functionalities: Ligence Heart software's manual functionalities that are used to analyze echocardiographic images are equivalent to the ones offered by other state-of-the-art software on the market. The manual functionalities will be compared to the commercially available CE marked state-of-the-art medical image viewers Intellispace Cardiovascular 5.1 by PHILIPS, and EchoPAC version 112 software by GE. To elaborate, Ligence Heart software's ability to calculate various measurements based on the user's annotations will be compared to other state-of-the-art technologies.
2. Automatic functionalities: The automated measurements performed by Ligence Heart software do not yet have equivalents on the market and are considered novel features. The current state-of-the-art for echocardiographic image analysis is the standard manual procedure - the physician that performs the examination must perform the annotations of images manually by identifying various structures of the heart himself. Therefore, the performance of the physician is the reference standard against which the performance of automatic functionalities of Ligence Heart will be compared.

4.13 Accountability

Accurate and adequate records will be maintained for the use of the medical devices as required by applicable laws and regulations.

The sponsor is responsible, working together with the IT staff of the investigational site, for the installation of and access to the Ligence Heart software. The comparator software that will be used during the clinical investigation is already installed and managed by the IT staff at the investigational site and used with an official approval for the clinical investigation from the administrators of the investigational site. The sponsor's procedures for verification and documentation of device safety, traceability of software versions, device labeling, and device disposition will be followed. The Principal Investigator and the IT manager will be ultimately responsible for the security and integrity of research devices at the site during the study.

4.14 Issuance

Device will be provided by the Sponsor to the site. There is no additional calibration or maintenance of study devices planned after the initial installation. The Sponsor may provide maintenance and monitoring of devices as necessary to maintain the integrity of study data. If any issues regarding the device arise, the investigators shall contact the Sponsor's staff (contacts and a more in-depth explanation provided during clinical investigation training).

4.15 Disposition

The medical device software Ligence Heart will no longer be accessible from the investigational site for the investigation purposes after the clinical investigation has ended, in accordance with applicable laws and regulations. No personal information will be retained in the device after disposition, as all the data that will be included in the study will be pseudonymized with all personal information removed.

4.16 Anticipated Risks

The device under study has undergone risk assessment, in accordance with International Standards Organization (ISO) 14971, and risks have been mitigated to levels as low as reasonably possible (ALARP).

Medical software does not have direct contact with subjects and no additional direct clinical procedures will be conducted for the clinical investigation. Therefore, no harm to the subjects' health is possible during the clinical investigation.

The personal data of the subjects will be processed and stored in the same way as other personal medical information: the researchers undertake to provide the client only with depersonalized (anonymous) information collected during the biomedical research.

Risk management for the medical software Ligence Heart is performed in accordance with ISO 14971:2012.

The following risks that have been identified as relevant for the clinical investigation:

- Cybersecurity breach (R-1);

The risks that have been identified as irrelevant for the clinical investigation, because they are associated with direct management and decision making of the patient's care during regular clinical practice, and during clinical investigation no direct decision making on the further subject clinical management will take place:

- Bug in the code (R-2)
- Software failure during the login and/or study selection process (R-3)
- Failure of the software to save the work progress (R-4)
- Errors of the functions that allow manipulation of the images (such as contrast changing, zooming, adding (annotations etc.) due to bugs (R-5)
- Automatic analysis of inadequate quality echocardiography images (R-6)
- Failure of 3rd party software components (R-6)
- The cloud server is unreachable (R-7)
- Incorrectly configured user's rights (R-8)
- Hardware does not have sufficient resources to run the software adequately (R-9)
- User runs Ligence Heart on incompatible software/operating system (R-10)
- Errors in the communication of the software with the hospital's information system (R-11)
- Erroneous performance of annotations/other aspects of echocardiography exam and approval of the final results by the user (R-12)
- Poor user interface design (R-13)
- Newly released software update is not validated correctly (R-14)

4.17 Established Risk Control Measures

To control and minimize the relevant identified risks, risk control measures have been established.

Cybersecurity threat mitigation measures are implemented based on Eichelberg2020a article from literature review which proposes security measures to be used in healthcare IT infrastructure. Article references security related publications NIST Special publication 1800-24 “Securing Picture Archiving and Communication System (PACS)” and various European Union Agency for Network and Information Security (ENISA) articles. Main measures implemented are:

- Firewalls and network segmentation
- Whitelisting of application
- User authentication and access rights
- Encryption
- Audit trail/logging
- Ensuring secure configurations
- Client certificates
- Penetration testing
- Image de-Identification
- Transport Security

Moreover, healthcare facilities security mitigation measures are described for clients to implement according to Eichelberg2020 article from literature review which describes main attack vectors against hospitals. Security measures for hospitals:

- Physically secure location of workstations connected to the software
- Regular updates
- Antimalware software
- Communication filtering

A more extensive risk control measure analysis on the other previously mentioned risks (which are not directly applicable to the clinical investigation, however, relevant to the application of the software in a real clinical setting) can be found in the Risk management file.

4.18 Risk-to-benefit rationale of the investigation

In the context of this clinical investigation, most of the risks that are identified to be associated with the use of Ligence Heart software are irrelevant, as no clinical decisions will be made based on diagnostic procedure that will take place during the investigation. The relevant risks, that have been previously described in sections 4.16 and 4.17 are strictly controlled as provided in the risk control measures description. Due to the design of the investigation, no harm to the subjects’ health is possible. Therefore, a conclusion can be made that the potential benefits of the future application of Ligence Heart software in real clinical practice and the benefits of the clinical investigation that is due to take place greatly outweigh the risks posed by this investigation.

5 Study Objectives and Endpoints

5.1 Purpose of the Study

The purpose of this study is to evaluate the safety and performance of the manual and automatic functionalities of medical device software Ligence Heart comparing them to the state-of-the-art alternatives.

5.2 Objectives

5.2.1 Primary Objectives

4. Manual measurement analysis: To calculate the reliability of Ligence Heart manual functions that are used to perform echocardiographic measurements comparing it with the manual measurements performed with other CE marked state-of-the-art medical image viewers.
5. Automatic measurement analysis: To compare Ligence Heart automatic measurements accuracy, variance, and error rate with human physicians in real clinical setting.
6. Time used comparison: To compare Ligence Heart automatic measurement tool execution time with the time it takes a physician to perform the measurements manually.

5.2.2 Safety Objective(s)

To collect safety information, including type and number of AEs, SAEs, and device issues.

5.3 Study Endpoints

5.3.1 Primary Endpoints

The end points respectively to the objective number:

4. Echocardiographic measurements performed manually with Ligence Heart and comparator software. The inter-software reliability between manual measurements performed with Ligence Heart and other CE marked state-of-the-art medical image viewers will be calculated (Part 1 of the investigation);
5. Echocardiographic measurements performed automatically by Ligence Heart software and echocardiographic measurements performed manually by physicians. The accuracy of automatic functions of Ligence Heart will be compared with the accuracy of physicians performing measurements manually (Part 2 of the investigation);
6. Time used to perform echocardiographic measurements manually and time used to perform the measurements automatically.

5.3.2 Safety Endpoints(s)

Type and number of AEs, SAEs, and device issues.

6 Study Design

6.1 Summary of Study Design

Single center retrospective, randomized, quantitative clinical investigation, that will enroll subjects from the adult (aged >18 years) population that have undergone 2D TTE examination and have their study results saved in the investigation site's database during the period from 2010-08-01 to 2020-08-01. The study will be conducted in two stages, beginning with the Part 1, in which data will be generated for the assessment of the manual functionalities of Ligence Heart, followed by Part 2, in which data will be generated for the assessment of the automatic functionalities of Ligence Heart.

After both Part 1 and Part 2 of the study are finished, the analysis of the generated data will take place. The statistical conclusions formulated from the data will be used to fulfill the clinical investigation objectives and to either accept or reject the hypotheses of the clinical investigation.

The study will be conducted at the Republican Šiauliai Hospital (Respublikinė Šiaulių Ligoninė).

6.2 Study Population

Adult (aged >18 years) population that have undergone 2D TTE examination having various indications due to various cardiac pathologies and have their study results saved in the investigation site's database during the time period from 2010-08-01 to 2020-08-01.

The ultrasound machines that were used to perform the 2D TTE examinations that are included in the clinical investigation:

- GE Vivid E9 Ultrasound Machine;
- GE Vivid S5 Ultrasound Machine;
- Philips Affiniti 70 Ultrasound system;
- Philips EPIQ 7 Ultrasound system;
- Philips Sparq ultrasound system.

6.3 Number of Subjects

Required sample size was established with the help of statistical power calculation tool „G*Power 3.1.9.7", which estimates the minimum sample size required to find and statistically prove if there is an effect to be found. In the analyses that were carried out the sample size was calculated for Repeated measures, within-between interaction ANOVA. Obtained minimal sample size was established to be 54 for both Part 1 and Part 2 of the clinical investigation (Variables used in calculations are provided in the table below).

Coefficient	Value
Effect Size	0.25
α	0.05
Power (1 - β)	0.95
Number of groups	3
Number of measurements	2
Correlation among repeated measures	0.6

6.4 Protection of Vulnerable Subjects

The Sponsor shall avoid improper influence on, or inducement of, the subject, any investigator(s), or other parties participating in, or contributing to, the clinical investigation.

All investigators shall avoid improper influence on, or inducement of, the subject, Sponsor, other investigator(s), or other parties participating in, or contributing to, the clinical investigation.

6.5 Eligibility Criteria

6.5.1 Inclusion Criteria

All included subjects will meet the following criteria:

- Aged 18 years at the time when the 2D TTE examination was performed;
- 2D TTE images are saved in the database of investigational site;
- Examination was performed during the time period of 2010-08-01 to 2020-08-01;
- 2D TTE images contain the following view modes that are necessary to perform the analysis of the images according to this clinical investigation plan:
    - apical 4 chamber view;
    - apical 2 chamber view;
    - parasternal long axis view.

6.5.2 Exclusion Criteria

Subjects will be excluded that:

- Have anatomical or functional cardiac characteristics that prevent appropriate completion of 2D TTE image analysis using the study device - a list of such characteristics can be found in the section 4.6.2 Contraindications;

6.6 Recruiting and Screening

Studies will be randomly selected from time period 2010.08.01 – 2020.08.01 from the 2D TTE image database in Republican Šiauliai Hospital. The steps for including studies into the clinical investigation:

1. During the time period 2010.08.01 – 2020.08.01 Republican Šiauliai Hospital have stored 17410 2D TTE studies into their internal database, from which random studies are extracted as potential studies to be included in the clinical investigation.
2. Using a random number generator different numbers are generated (between 1 and 17410), each of which corresponds to one random number from the complete list of potential studies to be included.
3. IT specialist of the Investigational site extracts the chosen studies and provides the studies to the Principal Investigator.
4. Principal investigator assesses the chosen studies based on the inclusion/exclusion criteria. If the chosen study is found to be unsuitable based on the criteria, the Principal investigator requests a new randomly chosen study to be extracted by the IT specialist.
5. Once all required studies have been included in the clinical investigation, the Principal investigator extracts all required data from the subjects' files.
6. IT specialist assigns each of the studies a specific ID number which is then associated with the original study ID in the Investigational Site's database. Then all personal details from the studies are removed (including metadata, labels on the images themselves, etc.). This step completes the inclusion of subjects into the clinical investigation and the pseudonymized studies without any personal details are ready to be used for the Step 1 and Step 2 of the clinical investigation.

6.7 Criteria for Withdrawal/Discontinuation

A subject may withdraw from study participation at any time, for any reason. The study staff may withdraw a subject at any time, for any reason. The reasons for withdrawal and discontinuation for any subject shall be recorded to the Sponsor-provided case report form (CRF). These will be reported to the Sponsor.

7 Study Procedures

7.1 Study Parts

No direct clinical procedures will be performed on the enrolled subjects. The procedure that will take place is the analysis of the 2D TTE examination DICOM images that were included in this study. Once enough subjects are chosen and enrolled in the study, the following parts take place:

During Part 1 of the study, investigators will analyze the same images using Ligence Heart software, Intellispace Cardiovascular 5.1 software, and EchoPAC version 112 software. The purpose of part 1 is to calculate the accuracy of the manually made measurements on the Ligence Heart software with other CE marked state-of-the-art software.

During part 2 of the study, investigators will analyze 2D TTE images and the results will be compared to the results of the analysis that automatic functionalities of Ligence Heart allow. The purpose of part 2 is to compare Ligence Heart automatic measurements precision and reliability with human physicians in real clinical setting.

Study Parts

Study Part	Functionalities tested	Comparator
Part 1	Manual	Other state-of-the-art CE marked medical images analysis software: Intellispace Cardiovascular 5.1 by PHILIPS, and EchoPAC version 112 software by GE
Part 2	Automatic	Cardiologists with expertise in echocardiology

7.2 Subject Procedure

Part 1

The purpose of the procedure in the Part 1 is to calculate the accuracy of Ligence Heart manual functions that are used to perform echocardiographic measurements comparing it with the accuracy of manual measurements performed with other CE marked state-of-the-art medical image viewers. Measurements made in Ligence Heart software will be compared with CE marked software “IntelliSpace Cardiovascular 5.1” (Phillips) and “EchoPAC” version 112 from GE Healthcare.

One cardiologist will perform 2D TTE measurements of each patient on each system 2 times. Time difference between repeated measures must be at least two days.

Part 2

The purpose of the procedure in the Part 2 is to compare Ligence Heart automatic measurements accuracy and reliability with human cardiologists in real clinical setting.

Four physicians will make 2D TTE measurements of each subject study 2 times. Physicians cannot share thoughts or results between themselves. Time difference between repeated measures must be at least two days. Measurements of three physicians will be used in modeling and results of the 4th will be used as a validation with automated system.

During both parts, time used for the analysis of the 2D TTE images will be recorded. During manual 2D TTE image analysis, time it takes for physician to make measurements will be registered. Stopwatch starts when physician opens study in Ligence Heart and stops when all listed measurements are made. In the use case including automatic measurements, the time it takes for automatic system to make measurements and physician to correct/validate the results is registered.

Summary of the clinical investigation parts

#	Analysis	Purpose	TTE Examination Protocol	Measurements
Part 1	Manual Measurement Analysis	To calculate the accuracy of Ligence Heart manual functions that are used to perform echocardiographic measurements comparing it with the accuracy of manual measurements performed with other CE marked state-of-the-art medical image viewers.	One physician will make 2D TTE measurements of each patient on each system 2 times. Time difference between repeated measures must be at least two days.	• AoS (PLA) • RVB (A4CH) • LVEDV (A4CH) • LVEDV (A2CH) • RVEDA (A4CH) • RAA (A4CH) • AV-Vmax

		Measurements made in Ligence Heart software will be compared with CE marked software “Tomtec“ ("IntelliSpace Cardiovascular 5.1") (Phillips) and „EchoPAC“ version 112 from GE Healthcare.		• TR-Vmax • E • S'RV • DEC • ACT • AV-VTI • TR-VTI • LVEDV (A2CH + A4CH)
Part 2	Automatic Measurement Analysis	To compare Ligence Heart automatic measurements precision and reliability with human physicians in real clinical setting.	Four physicians will make 2D TTE measurements of each patient 2 times. Physicians cannot share thoughts or results between themselves. Time difference between repeated measures must be at least two days. Measurements of three physicians will be used in modeling and results of the 4th will be used as a validation with automated system.	• RAMAD (A4CH) • AoA • AoS • STJ • AAo • LAD (PLA) • LAV • IVSd • LVPWd • LVEDD • LVESD • LVEDV (A4CH) • LVESV (A4CH) • LVEDV (A2CH) • LVESV (A2CH) • RAA • RVB • RVM • RVOTPD • RVEDA • RVESA
	Time used Comparison	To compare Ligence Heart automatic measurement tool execution time with time it takes a physician to perform measurements manually.	During manual TTE analysis, time it takes for physician to make measurements will be registered. Stopwatch starts when physician opens study in Ligence Heart and stops when all listed measurements are	Time to perform measurements with automatic functions vs. time to perform measurements without automatic functions.

			made. In the use case including automatic measurements, the time it takes for automatic system to make measurements and physician to correct/validate the results is registered.	

8 Study Data Collection and Assessments

8.1 Primary Assessment

For the primary objectives of the study the following measurement data will be collected:

Measurements performed on Ligence Heart and comparator software manually (Part 1):

- AoS (PLA)
- RVB (A4CH)
- LVEDV (A4CH)
- LVEDV (A2CH)
- RVEDA (A4CH)
- RAA (A4CH)
- AV-Vmax
- TR-Vmax
- E
- S'RV
- DEC
- ACT
- AV-VTI
- TR-VTI
- LVEDV (A2CH + A4CH)

Measurements performed by automatic functionalities of Ligence Heart and the comparator – the same measurements performed by cardiologists (Part 2):

- RAMAD (A4CH)
- AoA
- AoS
- STJ
- AAo
- LAD (PLA)
- LAV
- IVSd
- LVPWd
- LVEDD
- LVESD
- LVEDV (A4CH)
- LVESV (A4CH)
- LVEDV (A2CH)
- LVESV (A2CH)

- RAA
- RVB
- RVM
- RVOTPD
- RVEDARVESA

8.2 Exploratory Assessments

The Principal Investigator collects descriptive statistics data from the enrolled subjects that have been enrolled before they are sent to the IT staff for pseudonymization. The following descriptive statistics data will be collected:

- Gender
- Age (years)
- Weight (kg)
- Height (cm)
- Ethnicity
- Ultrasound machine model
- Total number of DICOM files
- History of heart failure
- History of myocardial infarction
- History of percutaneous coronary intervention (PCI)
- History of open cardiac surgery
- Atrial fibrillation
- History of arterial hypertension
- History of valvular heart disease
- History of congenital heart disease
- History of cardiomyopathy
- History of implanted devices/closures/prostheses

8.3 Safety Assessments

The description, severity, and device relatedness of any AE or SAE during the study will be recorded. In the event of any device issues, the event will be recorded. Safety reporting will be conducted as described in this protocol.

9 Qualification and Training Plan

9.1 Staff Qualifications

The investigators are included in the clinical study if compliant with the following requirements:

- Investigators are appropriately qualified cardiologists and experienced in the performance of 2D TTE exams by using medical software with the same intended purpose Ligence Heart;
- Investigators have adequate time to comply to participate in the study and provide results that are up to the required quality and safety standards;
- Investigators are willing to comply with the clinical investigation plan;
- Investigators are willing to sign the appropriate clinical investigation agreement;
- Investigators have past experience with conducting clinical studies or appropriate training;
- Investigators are familiar with ISO 14155:2011 requirements.

9.2 Training Plan for the Protocol and Research Device/Product

Before starting the study, the study staff will be trained based on their role in the study on the clinical investigation requirements set forth in this study protocol according to the training plan, as follows:

Protocol Training – All study staff will be trained on the clinical investigation plan and, as applicable, on devices. Documentation of such training will be retained and provided together with the clinical investigation report.

Training logistics – CMO of UAB Ligence is responsible for conduct of the training.

Target audience – All site personnel involved with the conduct of the study will be trained on the protocol and, as necessary, on device use.

Device Training – All users will be trained on the Ligence Heart feature per the user manual.

IT staff of the investigational site directly operating or maintaining the research device will be trained based by the Sponsor during the training.

The Principal Investigator will be ultimately responsible for execution of this study in accordance with the protocol and for device/product use in this study by members of the study staff.

10 Safety

The description, severity, and study device relatedness of any AE or SAE during the study will be recorded. In the event of any study device issues, the issues will be recorded. Safety reporting will be conducted as described in this protocol.

10.1 Anticipated Adverse Events

No foreseen risks that could be considered adverse events could results from this study, as no direct contact with the patients will be held and no additional clinical procedures will be performed.

There is always a chance of unforeseen risks. Throughout the study, the Sponsor will evaluate and update safety information in study documents.

10.2 Adverse Event Definitions

Adverse Event (AE): any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device [ISO 14155:2011 3.2]. This includes events related to the investigational device or the comparator and to the procedures involved. For users or other persons, this is restricted to events related to the investigational medical device.

Serious Adverse Event (SAE): an adverse event that led to death; led to a serious deterioration in the health of the subject, that either resulted in a life-threatening illness or injury, a permanent impairment of a body structure or a body function, or in-patient or prolonged hospitalization, or medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to body structure or a body function; or led to fetal distress, fetal death or a congenital abnormality or birth defect. Planned hospitalization for a pre-existing condition, or a procedure required by the protocol without serious deterioration in health, is not considered a SAE [ISO 14155:2011 3.37].

Adverse Device Effect (ADE): an adverse event related to the use of an investigational medical device [ISO 14155:2011 3.1]. This includes any adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device. This includes any event that is a result of a user error or intentional misuse of the investigational device [ISO 14155:2011 3.43].

Serious Adverse Device Effect (SADE): an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event [ISO 14155:2011 3.36].

Device deficiency: an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance, such as malfunctions, use errors, and inadequate labelling [ISO 14155:2011 3.15].

Unanticipated serious adverse device effect (USADE): a serious adverse device effect, which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report [ISO 14155:2011 3.42]. In the United States, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the study documents, will be reported in accordance with 21 CFR §812.3 and applicable laws and regulations.

10.3 Recording and reporting of Adverse Events

Adverse Event (AE) information will be collected throughout the study and reported to the Sponsor on the Adverse Event CRF. All Adverse Events, regardless of relatedness or outcome, must be reported. The investigator is responsible for reporting all AE to the Sponsor.

See the Adverse Event CRF for the information to be reported for each Adverse Event.

For Adverse Events that require immediate reporting, initial reporting may be done by phone, e-mail (contact the designated contact person for this clinical investigation), or on the CRF with as much information as is available.

The Adverse Event case report form:

MEDDEV 2.7/3 SAE Report Table- V1															
EUDAMED - ID:															
Title of Clinical Investigation:															
CIP Number:															
Contact person (Name, Address, E-Mail, Telephone Number)								Device type:							
MS+NCA Reference Numbers for all participating Countries:								Reference Member State:							
No. of Patients enrolled to date (date of report):								No. of Invest. Devices used to date							
Date of Report:															
Status: a, m, u	Date Sponsor received Report of SAE	Country	Study Center	Patient ID Code	Date of Procedure/ First Use	Date of Event Onset	Event: Organ System	Description of event	action/ treatment/ patient outcome	Assessment of Relationship to Procedure: Yes No Possibly	Assessment of Relationship to Investigational Device: Yes No Possibly	Unanticipated SADE yes/ No	Treatment Arm: Investigational Device/ Control Group/ blinded/ n.a	Event Status: Resolved / Resolved with Sequelae / Ongoing / Death	Date of Event Resolution

10.4 Adverse Event and Device Deficiency review process

All Adverse Events and Device Deficiencies will be reviewed by the Sponsor's Investigation Management and/ or designee. This review will include the determination whether the Adverse Event/Device Deficiency meets regulatory reporting requirements. The sponsor will ensure timely Adverse Event/Device Deficiency reporting to meet global and country specific regulatory requirements.

Table Adverse Event Reporting Requirements

Serious Adverse Device Effects (SADE), including Unanticipated Serious Adverse Device Effect (USADE):	
Investigators submit to:	
Sponsor	As soon as possible, but in no case later than 3 calendar days after the clinical site study team first learns of the event or of new information in relation with an already reported event.
Regulatory Authority	Reporting timeframe as per local reporting requirement.
Sponsors submit to:	
Regulatory Authority	Reporting timeframe as per local reporting requirement.
Bioethics Committee	Reporting timeframe as per local reporting requirement.
Serious Adverse Events (SAE)	
Investigators submit to:	
Sponsor	As soon as possible, but in no case later than 3 calendar days after the clinical site study team first learns of the event or of new information in relation with an already reported event.
Regulatory Authority	Reporting timeframe as per local reporting requirement.
Sponsors submit to:	
Regulatory Authority	Reporting timeframe as per local reporting requirement.
Bioethics Committee	Reporting timeframe as per local reporting requirement.
Adverse Device Effects (ADE)	
Investigators submit to:	
Sponsor	As soon as possible, but in no case later than 3 calendar days after the clinical site study team first learns of the event.
Regulatory Authority	Reporting timeframe as per local reporting requirement.
Sponsors submit to:	
Regulatory Authority	Reporting timeframe as per local reporting requirement.
Bioethics Committee	Reporting timeframe as per local reporting requirement.
Device Deficiency with SADE potential	
Investigators submit to:	
Sponsor	As soon as possible, but in no case later than 3 calendar days after the clinical site study team first learns of the event or of new information in relation with an already reported event.
Regulatory Authority	Reporting timeframe as per local reporting requirement.
Sponsors submit to:	
Regulatory Authority	Reporting timeframe as per local reporting requirement.

Bioethics Committee	Reporting timeframe as per local reporting requirement.
All other Device Deficiencies	
Investigators submit to:	
Sponsor	Submit in a timely manner after the clinical site study team first learns of the deficiency.
Regulatory Authority	Reporting timeframe as per local reporting requirement.

10.5 Device Deficiencies/Complaints

Device deficiencies/complaints should be reported to the study Sponsor contact identified on the cover page of this protocol. All device deficiencies/complaints are to be collected, fully investigated, and documented together with the case report forms and summarized in the clinical investigation report. The Principal Investigator is responsible for notifying the Sponsor if there are any device issues that could potentially lead to a SAE.

11 Ethical Conduct of the Study

The study will be carried out in accordance with the protocol and with principles enunciated in the current version of the Declaration of Helsinki; the guidelines of Good Clinical Practice (GCP) for medical devices, as set forth by ISO 14155:2011 and ISO 14971:2010.

The study will be conducted and reported in accordance with applicable policies of the governing Ethics Committee (EC) and governing regulatory authorities.

If national or regional EC requirements are less strict than the requirements of GCP, such as ISO 14155:2011 for medical devices, the Sponsor shall make attempts to apply the requirements of this International Standard to the greatest extent possible, irrespective of any lesser requirements, and shall record such efforts.

11.1 Ethics Committee

The responsible Principal Investigator at each site will ensure that approval from an appropriately constituted EC is attained for the clinical study prior to enrolling subjects, and Principal Investigator and the Sponsor will ensure that documentation of approval is maintained for the duration of the study.

The Principal Investigator will ensure that the Sponsor is notified of any withdrawal of EC approval within 5 working days of such occurrence. If approval is terminated or suspended, the Principal Investigator will promptly notify the Sponsor and provide written explanation.

Documents, which are provided to the responsible Bioethics Committee for review:

- Application for biomedical research;
- Reasoning for disbandment of Informed Consent forms;
- Application for a permission for clinical investigation;
- Research protocol;
- Documentation on the medical device.

Bioethics Committee approval of the biomedical clinical investigation must be received before commencement of the biomedical clinical investigation at the investigation site. The approval letter must contain enough information to identify the version or date of the documents approved.

11.2 Regulatory Agencies and Competent Authority(ies)

All the previously mentioned documents provided to the EC will be reviewed by the State Health Care Accreditation Agency prior to the final decision of the EC.

11.3 Management of Protocol Modifications and Amendments

Substantial amendments will only be implemented after approval of the EC.

A deviation is any instance(s) of failure to follow, intentionally or unintentionally, the requirements of the protocol. Under emergency circumstances, deviations from the protocol to protect the rights, safety, and wellbeing of human subjects may proceed without prior approval of the Sponsor and the EC/competent authority (CA). Such deviations shall be documented and reported to the Sponsor and the EC as soon as possible. Deviations will be reported as:

Critical Deviations: Deviations that significantly affect the safety, efficacy, integrity, or conduct of the study. These deviations must be reported to the Sponsor no later than 5 working days from awareness of occurrence and reported to the EC per the deviation reporting policy.

Non-Critical Deviations: Protocol deviations that do not significantly affect the safety, efficacy, integrity, or conduct of the clinical investigation. These deviations must be documented on the CRF Protocol Deviation page and will be reviewed by the sponsor.

Non-substantial modifications may be made during the normal course of device optimization, maintenance, and feasibility testing. Non-substantial modifications will be communicated to the CA as soon as possible, if applicable, and to the EC per their policy.

11.4 Participant Information and Informed Consent

The bioethics committee was provided with and has approved the reasoning and documentation regarding the disbandment of informed consent forms during this clinical investigation. The reasons that were provided and were found to be sufficient for the disbandment are as follows:

- The informed consent forms of subjects who had had a 2D TTE exam performed and the resulting DICOM study saved in the investigation site's database during the time period from 2010-08-01 to 2020-08-01 would be inconsequential, because before the commencement of the analysis of the data all personal information from the DICOM studies will be removed by the IT specialists in the investigation's site. Therefore, the probability of identification of the subjects during analysis is close to zero and such consequences of the unlawful and damaging identification of subjects would require irrational amount of resources.
- The retrospective analysis of DICOM images will not affect the subjects for whom the 2D TTE examination was performed in any way as no new medical procedures would be performed, no side effects are possible, and no personal information would be accessed by either the researchers, or the sponsor.
- Often it would require unreasonable resources or would be impossible to trace back and contact the subjects for whom the 2D TTE examinations were performed due to difficult current health or social status, unreachable/unknown/incomplete contact information.

11.5 Early Termination of the Study

The Sponsor may terminate the study prematurely according to certain circumstances. Examples of such circumstances include ethical concerns, insufficient participant recruitment, participant safety concerns, alterations in accepted clinical practice that make the continuation of a clinical investigation unwise, early evidence of benefit or harm of the research product, or for any other reason.

12 Statistical Methods

12.1 Statistical Hypotheses

The following hypotheses will be tested with the statistical methods:

1. The manual functionalities of Ligence Heart provide equally accurate tools for echocardiography evaluation compared to other state of the art CE marked medical software.
2. Automatic functionalities of Ligence Heart software perform echocardiography image analysis with non-inferior accuracy compared to a cardiologist.
3. Automatic functionalities of Ligence Heart software perform echocardiography image analysis with lower intra-rater variability compared to a cardiologist.
4. Automatic functionalities produce measurement values faster than a cardiologist.

12.2 Sample Size Determination

Required sample size was established with the help of statistical power calculation tool „G*Power 3.1.9.7", which estimates the minimum sample size required to find and statistically prove if there is an effect to be found. In the analyses that were carried out the sample size was calculated for Repeated measures, within-between interaction ANOVA. Obtained minimal sample size was established to be 54 for both Part 1 and Part 2 of the clinical investigation (Variables used in calculations are provided in the table below).

Sample size determination variables

Coefficient	Value
Effect Size	0.25
α	0.05
Power (1 - β)	0.95
Number of groups	3
Number of measurements	2
Correlation among repeated measures	0.6

12.3 Statistical Analysis

Data analysis will be carried out with Python >= 3.6 with statistical package pingouin >= 0.3.10. Pingouin will be used to calculate ANOVA results (calculations provided in https://pingouin-stats.org/generated/pingouin.anova.html#pingouin.anova). All other calculations will be done according to formulas provided in this section.

12.4 Manual Functionalities Analysis (Part 1)

12.4.1 Statistical Methods Values and Characteristics

Two-way ANOVA – test used to detect any overall differences between related means.

ANOVA characteristics

Source of variation	Degrees of Freedom	Mean Square
Patient	n - 1	MSS
Software	t - 1	MSR

Error (Patient x Software)	(n - 1)(t - 1)	MSSR
Error (Software)	nt(m - 1)	MSE

ANOVA variance components

Estimates of Variance Components
$\widehat{\sigma}_S^2 = \frac{MSS - MSE}{mt}$
$\widehat{\sigma}_R^2 = \frac{MSR - MSSR}{mn}$
$\widehat{\sigma}_{SR}^2 = \frac{MSSR - MSE}{m}$
$\widehat{\sigma}_e^2 = \mathbf{MSE}$

Interrater reliability coefficient characterizes reliability of two different raters to measure subjects similarly. Coefficient calculation formulas are given below:

$$\rho = \frac{\hat{\sigma}_S^2}{\hat{\sigma}_S^2 + \hat{\sigma}_R^2 + \hat{\sigma}_{SR}^2 + \hat{\sigma}_e^2}$$

12.4.2 Data analysis

Two-way ANOVA will be employed to test for significant differences between measurements made in different medical imaging software. Interrater reliability coefficient and Pearson correlation coefficient will be calculated to check the level of interchangeability between software.

Interrater reliability coefficient interpretation

Interpretation	Value
Slight	0.00-0.20
Fair	0.21-0.40
Moderate	0.41-0.60
Substantial	0.61-0.80
Almost perfect	0.81-1.00

12.5 Automatic Measurements Analysis (Part 2)

12.5.1 Statistical Methods Values and Characteristics

Two-way ANOVA – test used to detect any overall differences between related means.

ANOVA characteristics

Source of variation	Degrees of Freedom	Mean Square
Patient	n - 1	MSS
Cardiologist	t - 1	MSR
Error (Patient x Cardiologist)	(n - 1)(t - 1)	MSSR
Error (Cardiologist)	nt(m - 1)	MSE

ANOVA variance components

Estimates of Variance Components
$\widehat{\sigma}_S^2 = \frac{\boldsymbol{MSS - MSE}}{\boldsymbol{mt}}$
$\widehat{\sigma}_R^2 = \frac{\boldsymbol{MSR - MSSR}}{\boldsymbol{mn}}$
$\widehat{\sigma}_{SR}^2 = \frac{\boldsymbol{MSSR - MSE}}{\boldsymbol{m}}$
$\widehat{\sigma}_e^2 = \mathbf{MSE}$

Standard Error of Measurement – a measure of how much same measurements made by different physicians are spread around a "true" score. SEM in Two-way repeated measures ANOVA includes both the variability among raters' measurements and within raters' measurements.

$$SEM = \sqrt{\frac{MSR + (n-1)MSSR + n(m-1)MSE}{mn}}$$

Minimal Detectable Change – minimal amount of change that is required to distinguish a true change from a change due to variability of measurement or random error. MDC is derived from SEM and calculated as confidence interval. In this analysis 95% confidence interval will be used.

$$MDC_{95} = 1.96 * \sqrt{2}\text{SEM}$$

12.5.2 Data analysis

Two-way ANOVA will be employed in this analysis to prepare for further investigation. Inter-rater reliability and SEM will be calculated from the results of ANOVA. SEM will be used to get minimal detectable change. Absolute difference between the automated system measurement and mean of the raters' measurements will be compared with MDC as the primary endpoint of the analysis to check if there is a significant difference between physicians and automated system. The same difference will be compared with SEM as the secondary endpoint. Same analysis will be conducted with measurements of the 4th rater. Results will allow to compare the accuracy of automated systems measurements and physicians' measurements.

13 Quality Assurance and Control

13.1 Data Management

Data management processes for handling study data will be maintained by the Sponsor.

13.2 Completion of Case Report Forms (CRFs)

The data reported on the CRFs shall be derived from source documents and be consistent with these source documents. Electronic for of CRFs will be used. The Sponsor will provide CRFs and train study staff on completion of CRFs during the training on clinical investigation plan.

CRFs are considered to be the reports generated while using Ligence Heart software. CRFs are completed using the software. CRFs certified by signing in and approving the report with the accounts individually assigned to each investigator. The Principal Investigator is ultimately responsible for ensuring completion of CRFs.

If discrepancies are discovered on paper CRFs during monitoring, the Sponsor's CMO will ensure that the study staff makes necessary corrections directly to the CRF(s) prior to collection.

If a site discovers discrepancies after CRF collection, the site may notify the Sponsor and request data modification.

13.3 Data Handling and Record Keeping

All documents and data shall be produced and maintained in a manner that assures control and traceability.

13.4 Source Data and Documents

Source data includes information in original records, certified copies of original records of clinical findings, observations, or other activities for the study. Source documents for each subject must be retained throughout the investigation, including printed or electronic documents containing source data.

The Principal Investigator or institution shall provide direct access to source data during and after the clinical investigation for monitoring, audits, EC review, and regulatory authority inspections.

13.5 Archiving

All study data must be archived for a minimum of 3 years after study termination (or as required by local law) or premature termination of the clinical investigation. No source documents or study records will be destroyed without Sponsor notification and approval.

14 Monitoring Plan

In collaboration with the site, the Sponsor will ensure proper monitoring of the study to confirm that all the research requirements are met. The Sponsor will oversee the progress of a clinical investigation and ensure that it is conducted, recorded, and reported in accordance with the protocol, written procedures, Good Clinical Practice (GCP) ISO 14155:2011, and the applicable regulatory requirements.

14.1 Confidentiality and Data Protection

The investigator affirms and upholds the principle of the subject's right to privacy, and the investigator shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing data in scientific journals.

Individual subject medical information obtained as a result of this study will be considered confidential, and disclosure to third parties will be prohibited. Subject confidentiality will be further ensured by utilizing subject identification code numbers, that is, all data provided to the Sponsor will be pseudonymized and will not contain any personal information. For data verification purposes a competent authority (CA), or an

ethics committee (EC) may require direct access to parts of the medical records relevant to the study, including subject medical history.

14.2 Storage of Images and Associated Health Data

Research images and associated data will be collected and disclosed to the Sponsor as part of this study. Fully de-identified data, which has had all personal identifying information removed, may be stored and used by the Sponsor indefinitely. The Sponsor and/or its authorized representatives may use any de-identified data collected in this study for future technology and engineering development, marketing purposes, education, regulatory claims, or other possible uses.

14.3 Publication Policy

The results of this study may be used in future publications, at the discretion of the Sponsor. The conditions of publication are described in a separate contractual agreement. The investigator should in good faith make the Sponsor aware of any possible public scientific contributions, such as publications or presentations. The Sponsor may request modifications or to delay presentations at its discretion.

15 Investigation Results

15.1 Demographic data

A total of 58 persons from a single center in Lithuania referred to 2D transthoracic echocardiography (TTE) in non-emergency setting were enrolled in the study retrospectively. Among the participants, 100% were Caucasian, 50.0% were female. The mean LVEF was 50.81 ± 9.77%, with 13.79% of the participants having LVEF < 50%. The median age was 62.0 years. All participants had sinus rhythm at the time of performing TTE (Table 1a and 1b).

Table 1a. Main parameters of the study cohort.

	Mean	**± SD**
Age (years)	62.05	14.92
Weight (kg)	83.17	14.25
Height (cm)	172.57	10.36
Body surface area	1.99	0.21
Ejection Fraction (%)	50.81	9.85

Table 1b. Main descriptive statistics.

	N	**(%)**
Females	29	50.00
History of Heart Failure	29	50.00
History of Myocardial Infarction	11	18.97
History of Percutaneous Coronary Intervention	14	24.14
History of Coronary Artery Bypass Graft	4	6.90

History of Arterial Hypertension	1	1.72
History of Valvular Heart Disease	43	74.14
History of Coronary Heart Disease	14	24.14
History of Cardiomyopathy	3	5.17
Implantable Device (Pacemaker or Defibrillator)	3	5.17

15.1 Manual functionalities (Part 1)

All measurements performed manually using Ligence Heart were equally accurate and reliable when compared to state-of-the-art certified medical image analysis software. Median inter-software reliability was 95.52% (IQR 94.31 - 97.17) with the lowest value observed for RAA (89.30%) and the highest for APV (99.26%). All tested measurements had inter-software reliability above 0.8, corresponding to high level of agreement between different platforms **(Table 2)** and there was no significant difference in the measurements produced by Ligence Heart in comparison to other CE marked state of the art medical device software.

Table 2. Inter-software reliability for Ligence Heart compared to EchoPAC and Intellispace Cardiovascular.

Measurement (abb.)	**Measurement (full)**	**Inter-software reliability**
AoS	Aortic sinus diameter	97.81%
RVB	Right ventricle basal diameter	93.28%
LVEDV4A	Left ventricle end diastolic volume (A4Ch)	97.12%
LVEDV2A	Left ventricle end diastolic volume (A2Ch)	93.95%
RVEDA	Right ventricle end diastolic area	92.17%
RAA	Right atrium area	89.30%
APV	Aortic peak velocity	99.26%
PTRV	Peak tricuspid regurgitation velocity	97.18%
E	Transmitral E velocity	99.21%
SP	S prime right ventricle lateral wall	97.16%
Dec	Transmitral E velocity deceleration time	95.41%
ACT	Acceleration time	95.52%
AVVTI	Aortic valve maximum velocity time integral	98.21%
TR VTI	Tricuspid regurgitation velocity time integral	94.66%
LVEDV	Left ventricle end diastolic volume (biplane)	95.52%

15.2 Accuracy of automatic measurements (Part 2)

In order to establish the baseline inter- and intra-observer variability, the original rater group (ORG) consisting of three board certified cardiologists analyzed 58 2D TTE studies, repeating the measurements twice. This generated six values for each measurement in each study. The new rater group (NRG) consisting of a fourth cardiologist (FC) and Ligence Heart performed measurements in the same 58 studies. NRG performance was evaluated by calculating the number of measurements of each type that were in the limits of variation. FC was used as the performance benchmark for Ligence Heart.

There was no significant difference in variation between Ligence Heart and FC ($p > 0.05$) and Ligence Heart had non-inferior accuracy to FC for all automatic measurements **(Table 3)**. The lowest number of studies in agreement with ORG was 93.1% in RAA and 94.55% in RVM for Ligence Heart and FC, respectively. For AoS, STJ, IVSd, LVEDD, LVESD, LVESV4A and RVOTPD both FC and Ligence Heart were in agreement with ORG for 100% of studies. Ligence Heart discarded 2-4 studies in RAMAD, LAV4A, LVEDV4A, LVESV4A, LVEDV2A, LVESV2A, RVEDA and RVESA due to insufficient quality scores of automatic predictions **(Table 3)**. Ligence Heart confidence interval for the number of studies in agreement with ORG intersected FC for all automatic measurements **(Figure 1)** and comparing each measurement between FC and Ligence Heart yielded a median Pearson correlation R 0.74 (IQR 0.59-0.83) **(Figure 2).**

Comparison of correlation between all raters and NRG measurement relation to ORG measurements is visualized in **Supplementary figures 1 and 2**, respectively.

Table 3. Comparison of Ligence Heart and cardiologist agreement with original rater group.

Measurement	Ligence Heart studies in agreement with ORG (95% CI)	FC studies in agreement with ORG (95% CI)	P-value*	N studies	N studies not passing confidence filter in Ligence Heart	Pass
RAMAD	96.3 ± 5.03	98.15 ± 3.59	0.56	54	4	Yes
AoA	98.28 ± 3.35	98.28 ± 3.35	1	58	0	Yes
AoS	100.0 ± 0.0	100.0 ± 0.0		58	0	Yes
STJ	100.0 ± 0.0	100.0 ± 0.0		58	0	Yes
AAo	98.28 ± 3.35	100.0 ± 0.0	0.32	58	0	Yes
LAD	94.83 ± 5.7	100.0 ± 0.0	0.08	58	0	Yes
LAV4A	98.21 ± 3.47	94.64 ± 5.9	0.31	56	2	Yes
IVSd	100.0 ± 0.0	100.0 ± 0.0		58	0	Yes
LVPWd	96.55 ± 4.7	100.0 ± 0.0	0.16	58	0	Yes
LVEDD	100.0 ± 0.0	100.0 ± 0.0		58	0	Yes
LVESD	100.0 ± 0.0	100.0 ± 0.0		58	0	Yes
LVEDV4A	98.21 ± 3.47	100.0 ± 0.0	0.32	56	2	Yes
LVESV4A	100.0 ± 0.0	100.0 ± 0.0		56	2	Yes
LVEDV2A	96.3 ± 5.03	100.0 ± 0.0	0.16	54	4	Yes

LVESV2A	96.3 ± 5.03	100.0 ± 0.0	0.16	54	4	Yes
RAA	94.44 ± 6.11	98.15 ± 3.59	0.31	54	4	Yes
RVB	98.18 ± 3.53	98.18 ± 3.53	1	55	3	Yes
RVM	100.0 ± 0.0	94.55 ± 6.0	0.08	55	3	Yes
RVOTPD	100.0 ± 0.0	100.0 ± 0.0		58	0	Yes
RVEDA	98.18 ± 3.53	100.0 ± 0.0	0.32	55	3	Yes
RVESA	98.18 ± 3.53	100.0 ± 0.0	0.32	55	3	Yes

*ORG - original rater group consisting of three board certified cardiologists; FC – fourth board certified cardiologist. *P values for measurements that fall 100% in agreement in both groups are undefined. The "Pass" column specifies whether measurement accuracy is considered to be non-inferior. In order for the automated measurements to pass, a P value of > 0.05 or undefined is required which means that there is no significant difference in variation between Ligence Heart and FC measurements.*

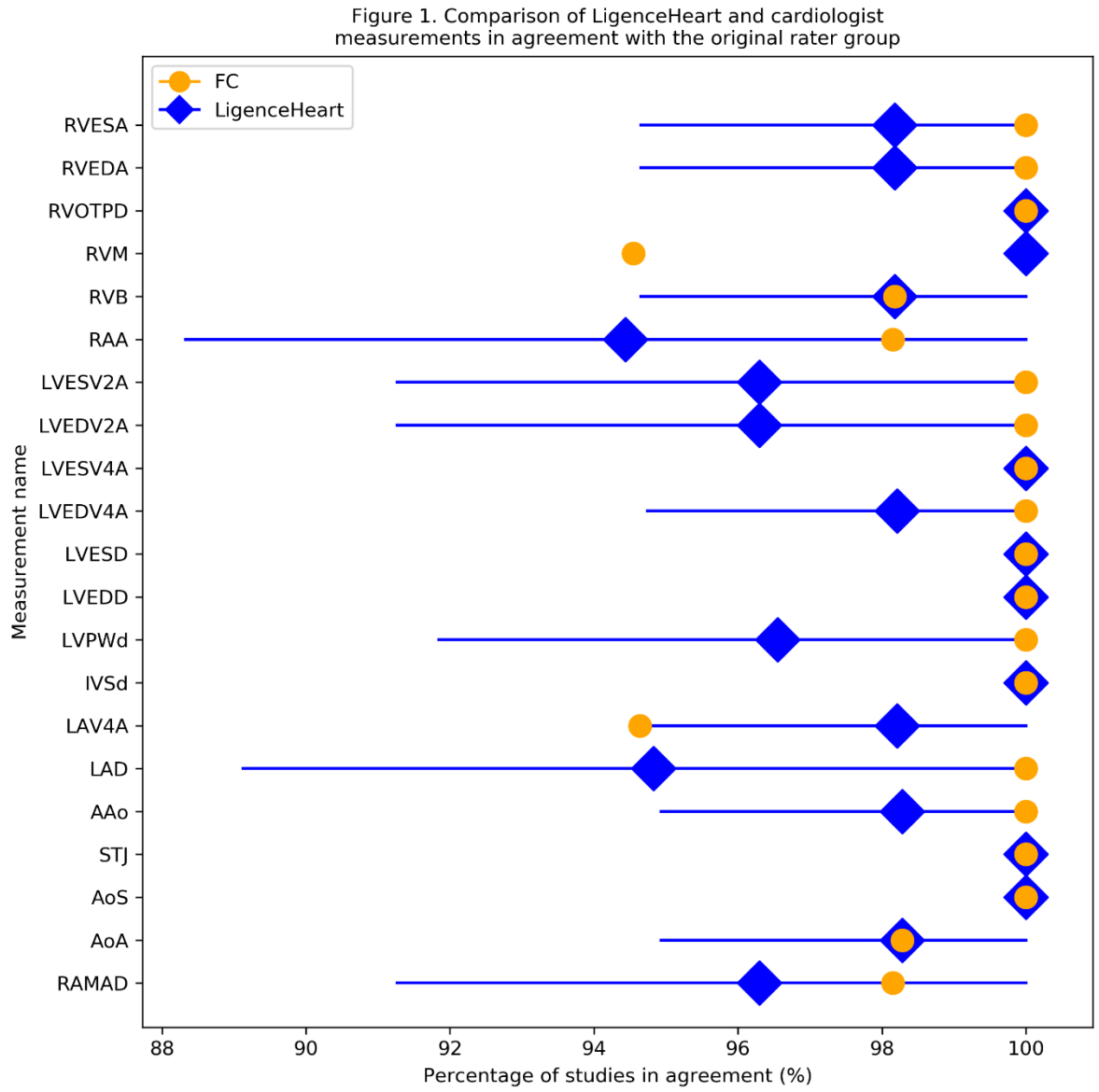
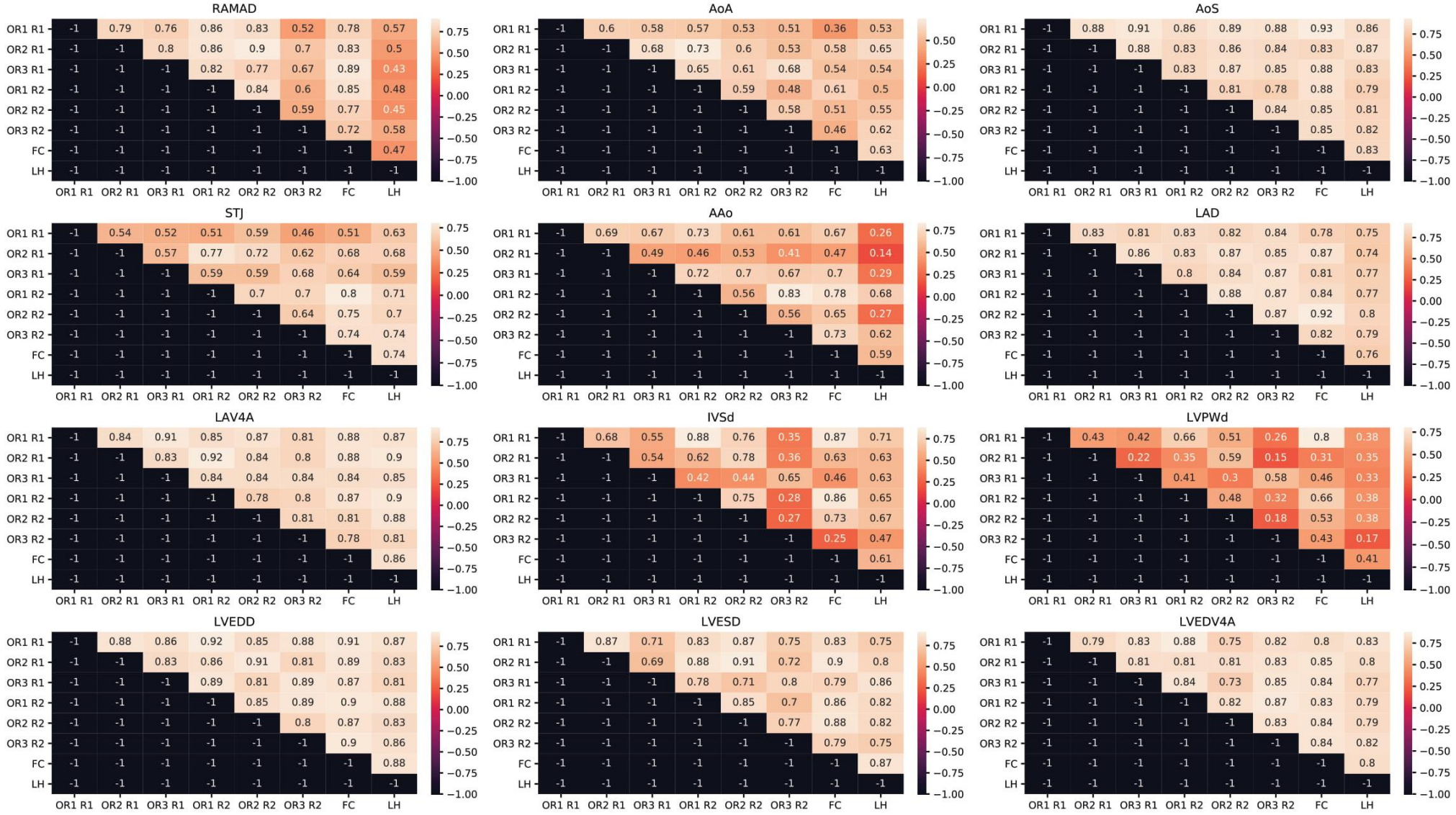
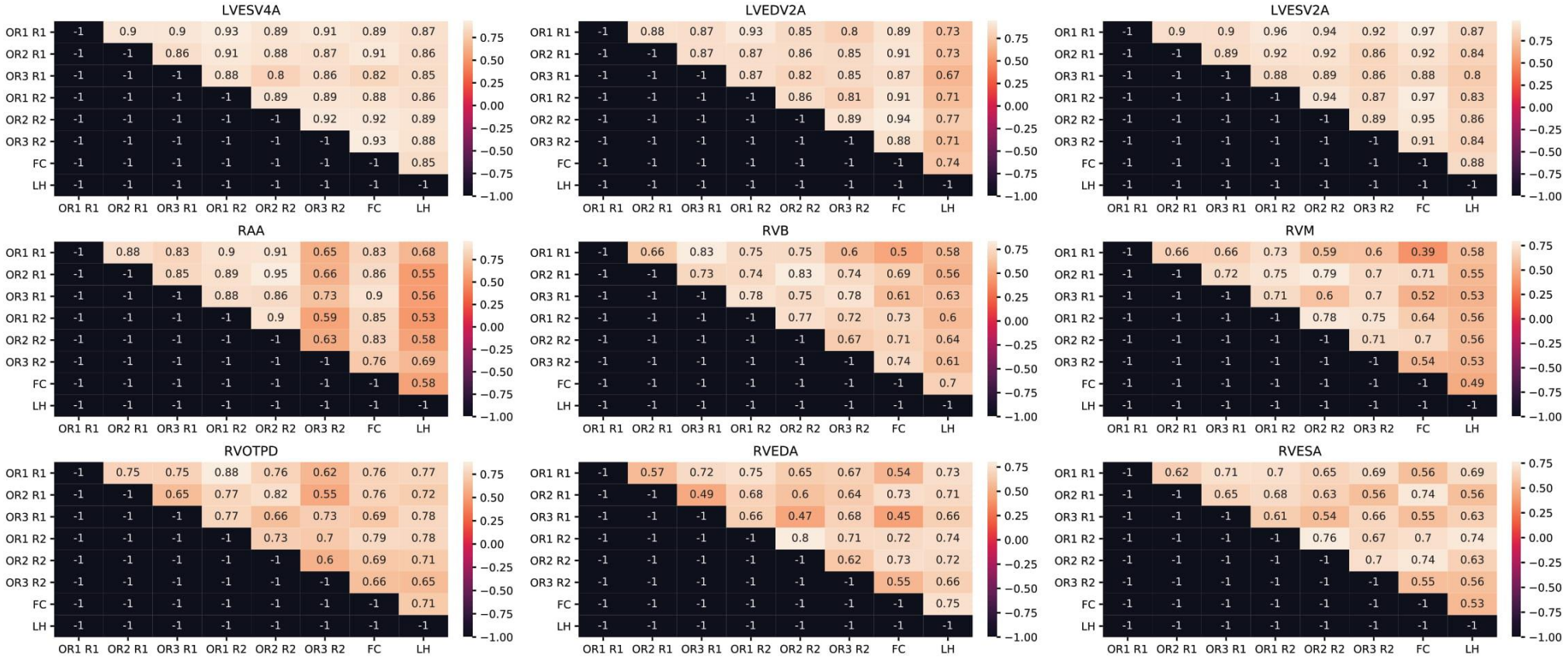


Figure 1. *The X axis represents the number of studies that were measured in agreement with ORG by Ligence Heart or FC. Each Y axis position represents a different measurement. 95% CIs are shown for Ligence Heart only. Ligence Heart 95% CI lower bound is at or above FC performance. ORG - original rater group. FC - fourth cardiologist.*

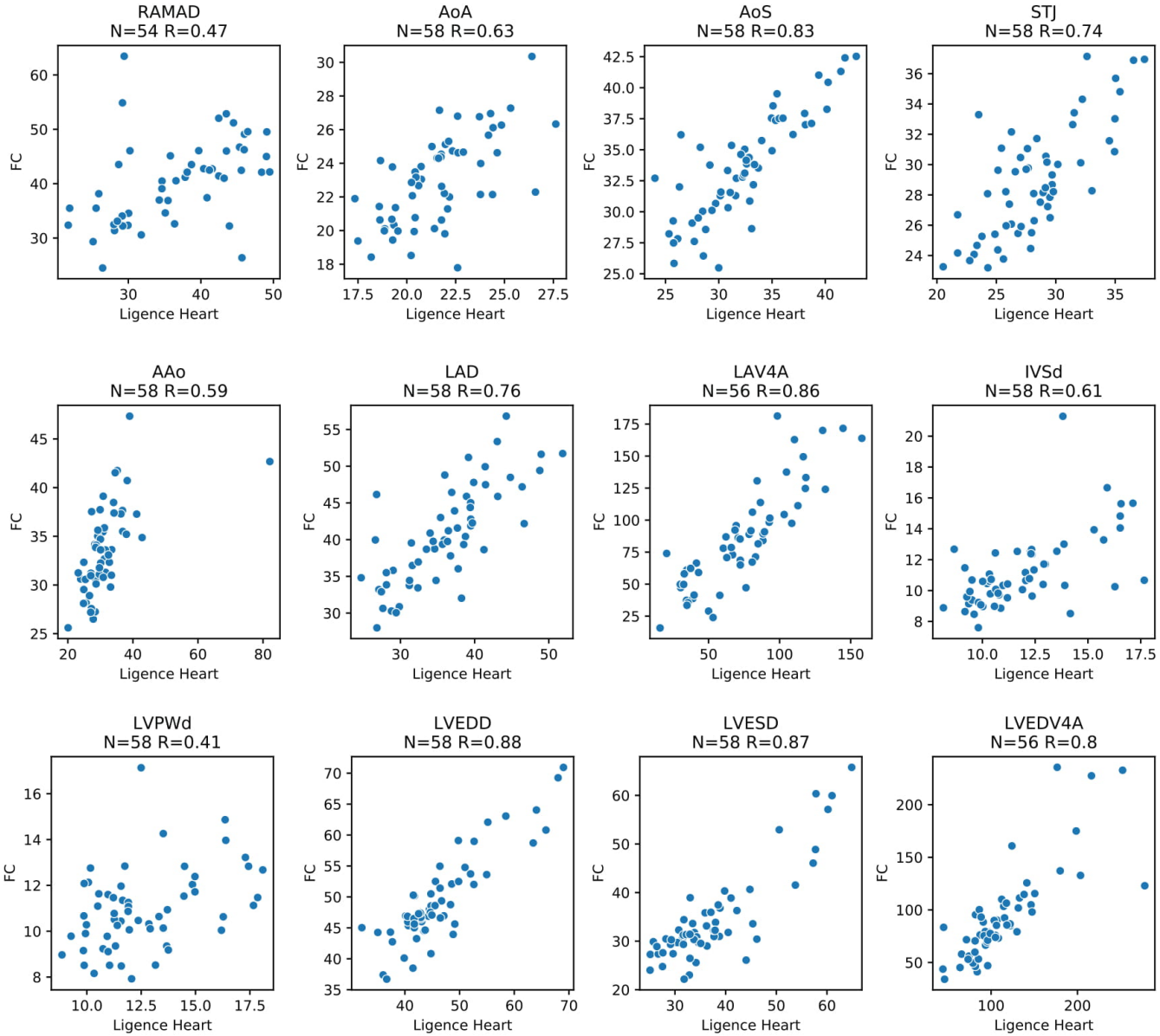
Supplementary figure 1. Correlation of measurements between all raters





Supplementary figure 1. *Pearson correlation coefficients between all raters is shown. A value of -1 is assigned whenever the correlation is not calculated, either because it would be a duplicate or because it would compare measurements with the same rater and run. OR - original rater, R1 and R2 - run 1 and run 2, FC - fourth cardiologist, LH - Ligence Heart. Run 1 and run 2 refers to different repeats of the study by the same rater.*

Figure 2. Correlation of measurements between FC and Ligence Heart



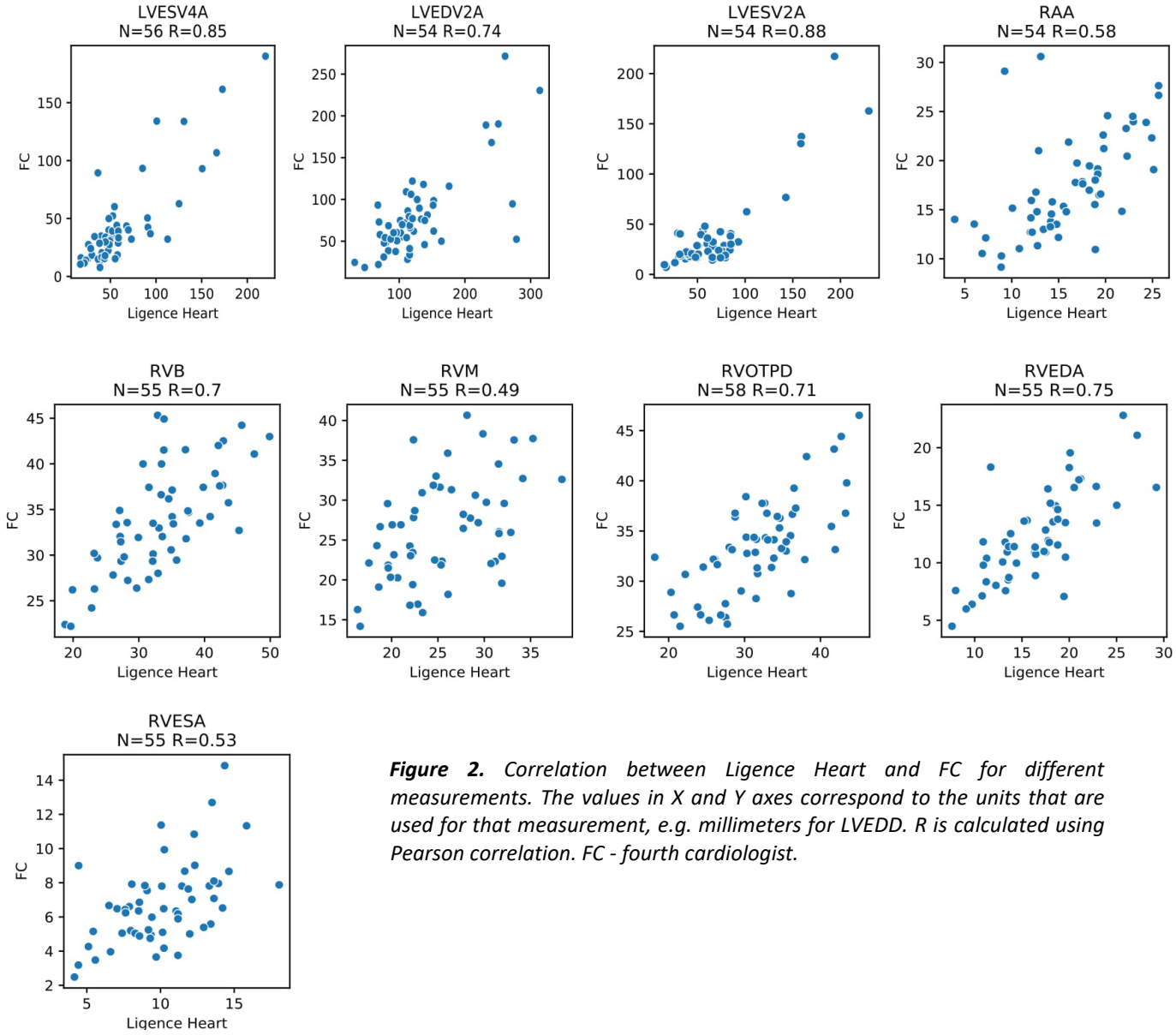
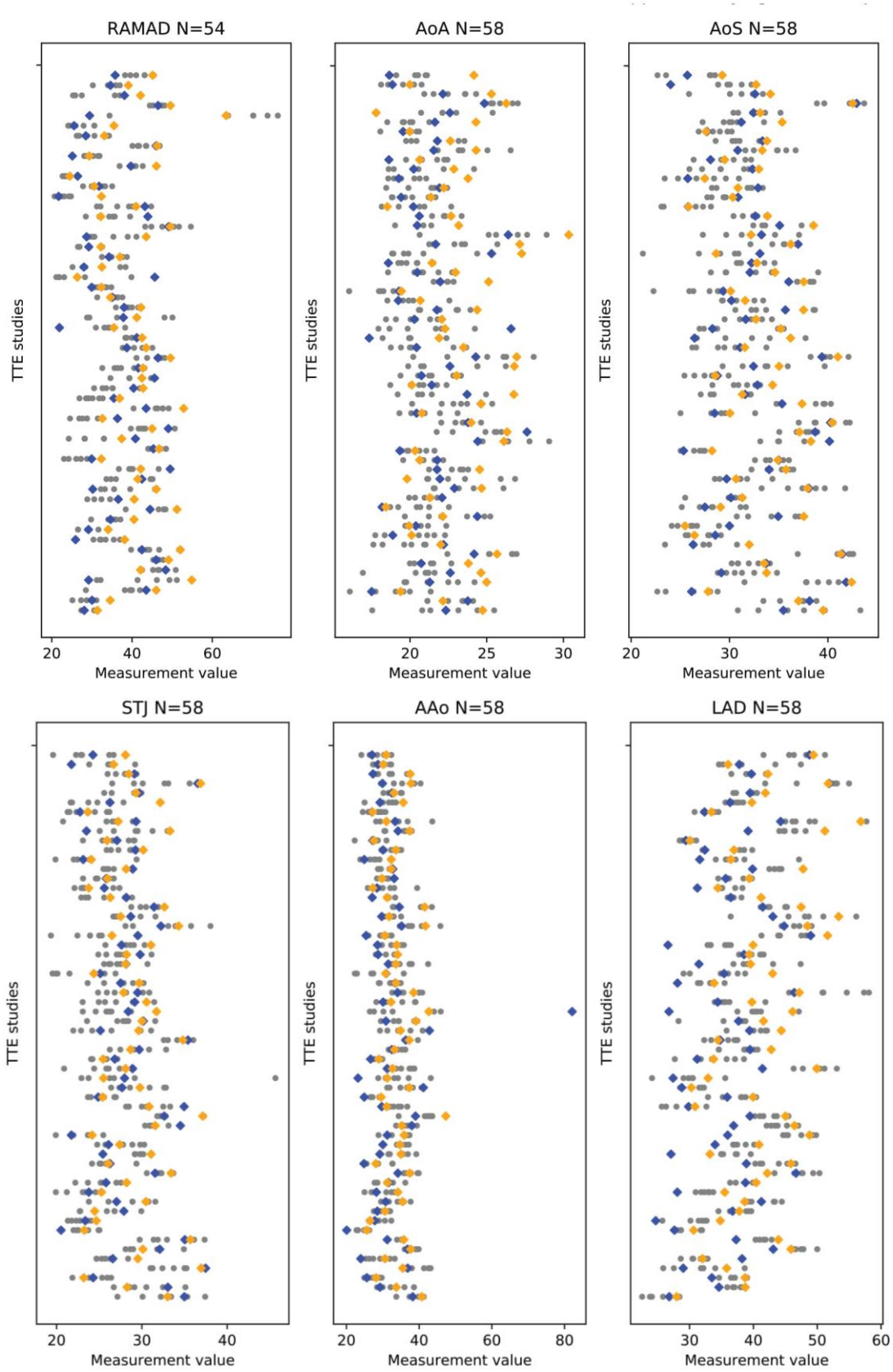
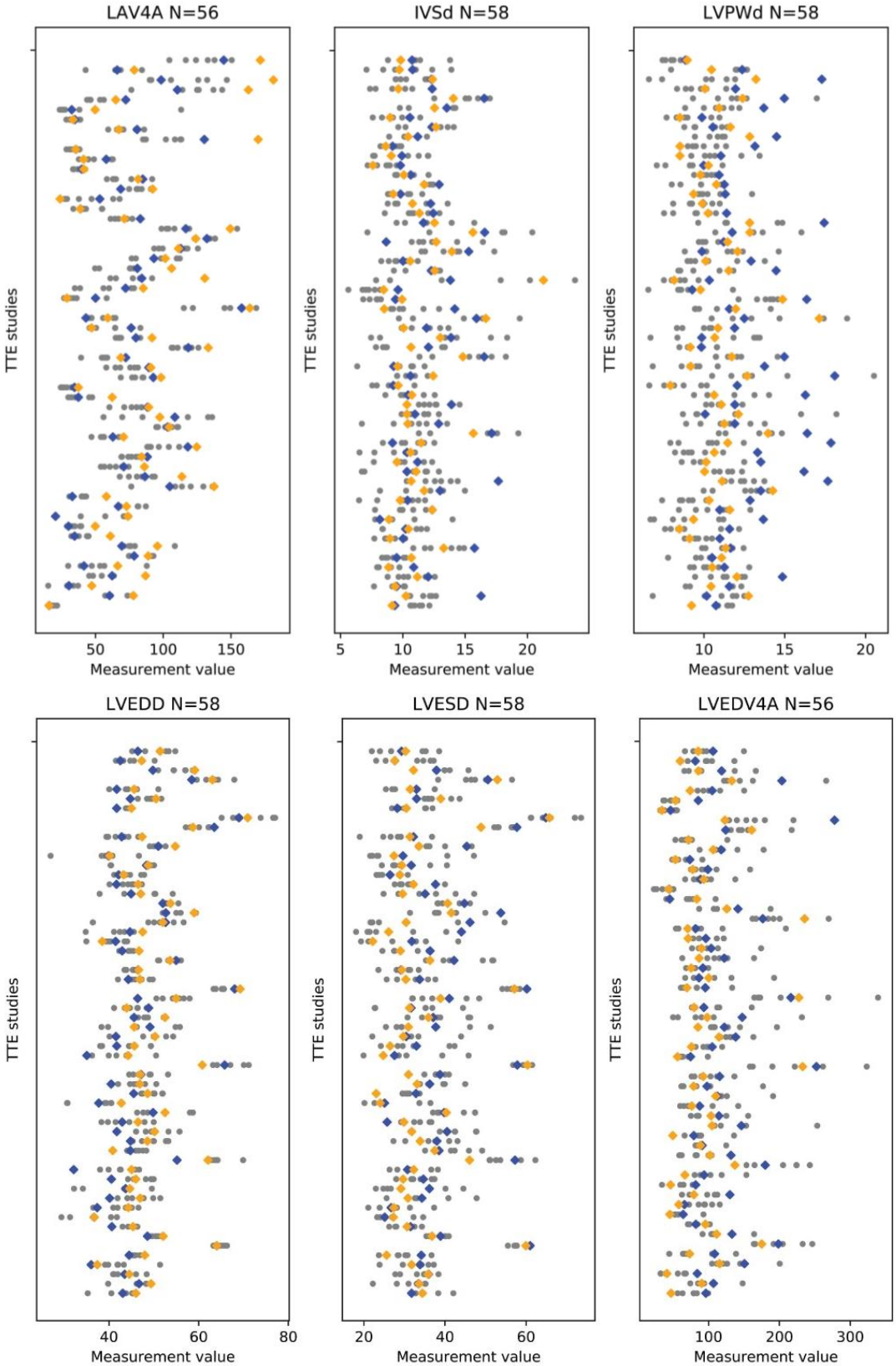
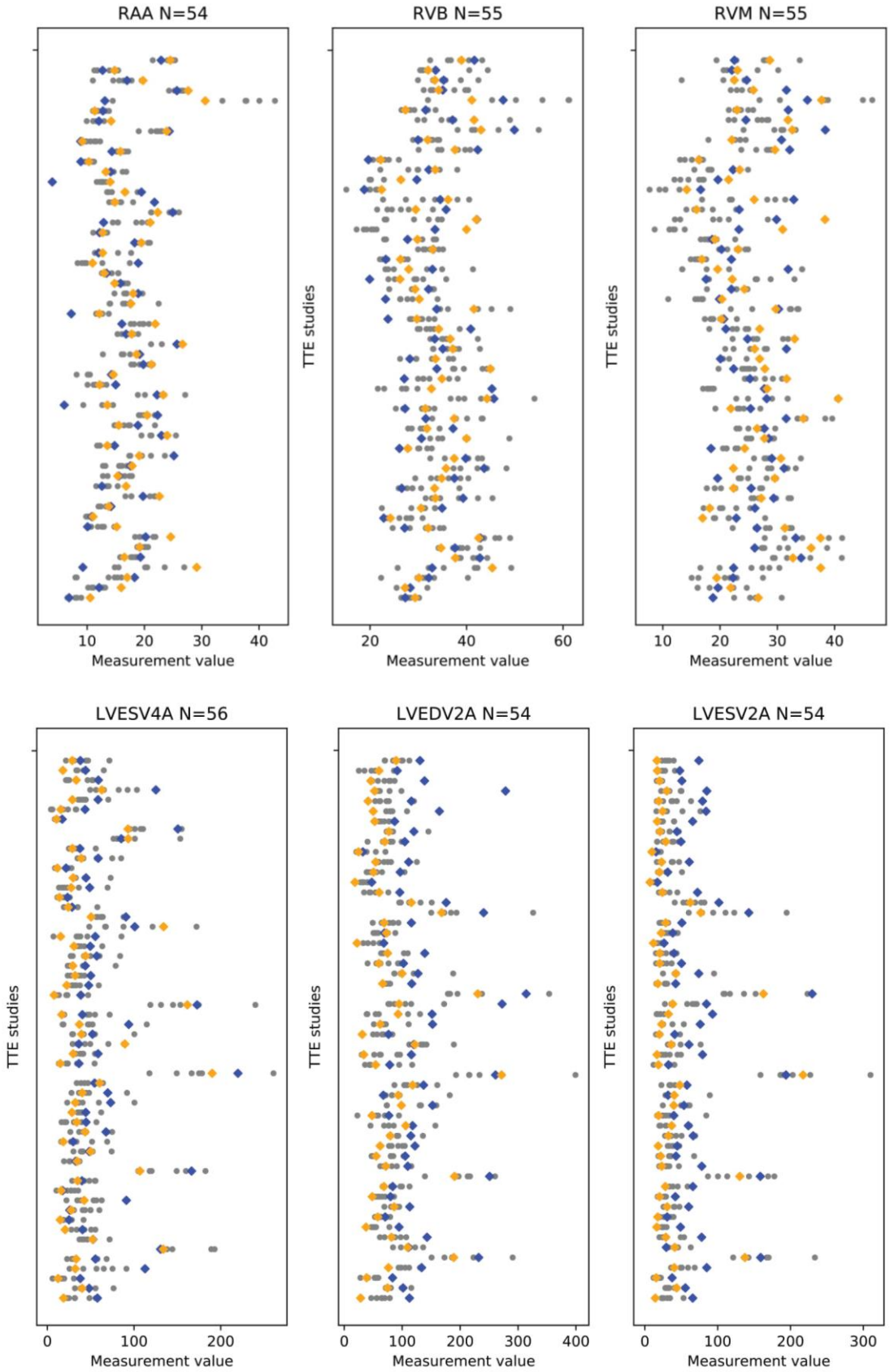


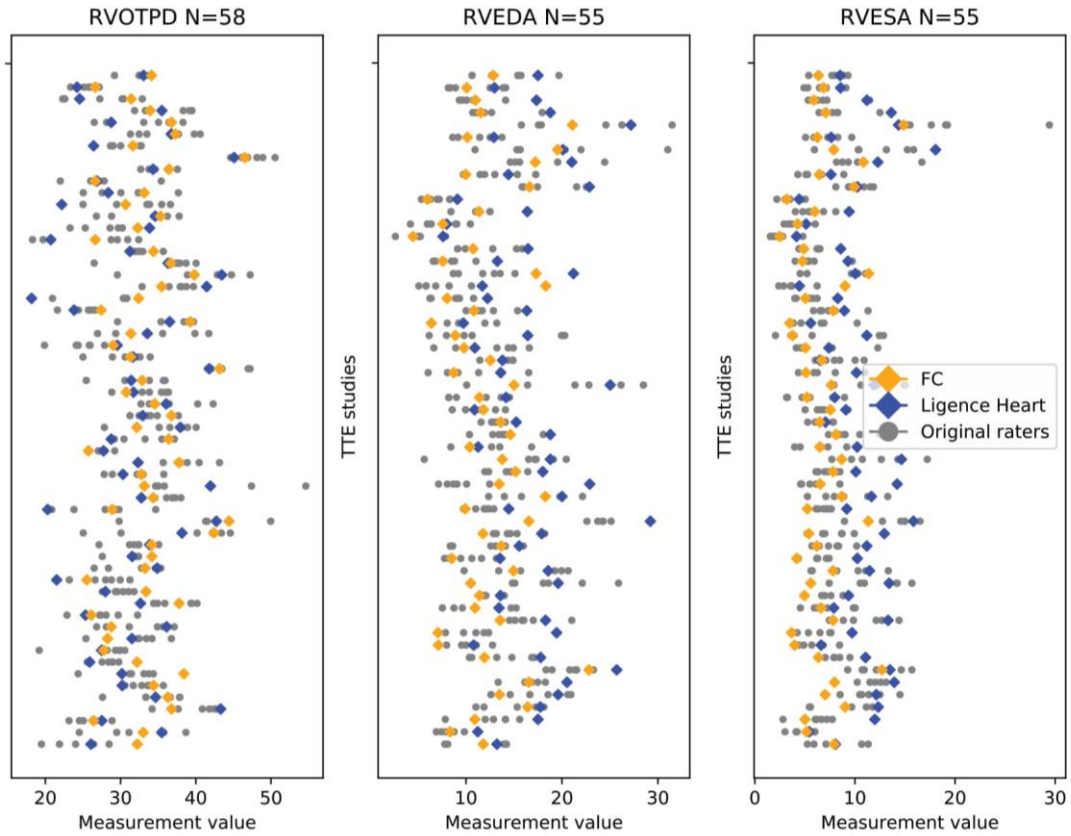
Figure 2. *Correlation between Ligence Heart and FC for different measurements. The values in X and Y axes correspond to the units that are used for that measurement, e.g. millimeters for LVEDD. R is calculated using Pearson correlation. FC - fourth cardiologist.*

Supplementary figure 2. Comparison of measurements between original rater group and new raters









Supplementary figure 2. *All individual measurements from ORG and NRG visualized. Each row represents a different study, grey dots represent values from run 1 and run 2 performed by the ORG. Orange and blue diamonds show the FC and Ligence Heart measurements, respectively. Run 1 and run 2 refers to different repeats of the study by the same rater. ORG - original rater group. NRG - new rater group.*

15.3 Intra-rater variability and time comparison

Comparing measurements for each ORG member between different runs resulted in median R of 0.85 (IQR 0.73 - 0.88), 0.81 (IQR 0.73 - 0.87) and 0.78 (IQR 0.66 - 0.84) for original raters 1, 2 and 3, respectively. Due to Ligence Heart automatic measurements being determined by the input, Ligence Heart had no variation in measurements between different runs and had R of 1.0 for all measurements (Table 4), resulting in significantly lower intra-rater variability ($p < 0.05$).

ORG members on average took 12:58 ± 3:18 minutes to analyze the same measurements in 58 TTE studies while Ligence Heart was significantly faster, taking on average 2:59 ± 1:02 minutes to analyze the same studies ($p < 0.05$).

Table 4. Comparison of correlation coefficients between different runs in original rater group and Ligence Heart.

Label	OR1 Run 1 vs Run 2	OR2 Run 1 vs Run 2	OR3 Run 1 vs Run 2	Ligence Heart Run 1 vs Run 2
RAMAD	0.86	0.86	0.82	1
AoA	0.57	0.73	0.65	1

AoS	0.86	0.83	0.83	1
STJ	0.51	0.77	0.59	1
AAo	0.73	0.46	0.72	1
LAD	0.83	0.83	0.8	1
LAV4A	0.85	0.92	0.84	1
IVSd	0.88	0.62	0.42	1
LVPWd	0.66	0.35	0.41	1
LVEDD	0.92	0.86	0.89	1
LVESD	0.83	0.88	0.78	1
LVEDV4A	0.88	0.81	0.84	1
LVESV4A	0.93	0.91	0.88	1
LVEDV2A	0.93	0.87	0.87	1
LVESV2A	0.96	0.92	0.88	1
RAA	0.9	0.89	0.88	1
RVB	0.75	0.74	0.78	1
RVM	0.73	0.75	0.71	1
RVOTPD	0.88	0.77	0.77	1
RVEDA	0.75	0.68	0.66	1
RVESA	0.7	0.68	0.61	1

Pearson correlation coefficients between different runs for the same rater are shown. Since Ligence Heart performs all measurements automatically and the output is completely determined by input, it has a correlation coefficient of 1 for all measurements. Run 1 and run 2 refers to different repeats of the study by the same rater. OR1 – original rater one; OR2 – original rater two; OR3 – original rater three.

15.4 Summary of Safety

Table 5. Summary of Adverse Events.

Adverse events	**Occurred events**
Serious adverse events (AE): Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device NOTE 1: This definition includes events related to the investigational medical device or the comparator. NOTE 2: This definition includes events related to the procedures involved.	0

NOTE 3: For users or other persons, this definition is restricted to events related to investigational medical devices.	
Adverse device effects (ADE) Adverse event related to the use of an investigational medical device NOTE 1: This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device. NOTE 2: This definition includes any event resulting from use error or from intentional misuse of the investigational medical device	0
Serious Adverse Event (SAE): Adverse event that a) led to death, b) led to serious deterioration in the health of the subject, that either resulted in 1) a life-threatening illness or injury, or 2) a permanent impairment of a body structure or a body function, or 3) in-patient or prolonged hospitalization, or 4) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function, c) led to fetal distress, fetal death or a congenital abnormality or birth defect. NOTE 1: Planned hospitalization for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event.	0
Serious Adverse Device Effect (SADE): Adverse device effect that has resulted in any of the consequences characteristic of a Serious Adverse Event	0
Unanticipated Serious Adverse Device Effect (USADE): Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report NOTE: Anticipated serious adverse device effect (ASADE) is an effect which by its nature, incidence, severity or outcome has been identified in the risk analysis report.	0
Unanticipated Adverse Device Effect (UADE): Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.	0
Device deficiencies Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance NOTE: Device deficiencies include malfunctions, use errors, and inadequate labelling	0

Relevant corrective actions	None Needed

16 Discussion and conclusions

16.1 Manual functionalities (Part 1)

Different types of manual measurements including area, distance and point measurements in B-mode and different spectral Doppler imaging modes have been tested and compared to two other CE marked medical image analysis platforms (EchoPAC by GE and Intellispace Cardiovascular by Philips). The measurement values obtained using the manual Ligence Heart functionalities did not show statistically significant variation when compared with other medical image analysis software. This proves that Ligence Heart can be used as medical image analysis software to analyze echocardiographic imaging data.

16.2 Automatic functionalities (Part 2)

Due to the fact that echocardiography is prone to considerable inter- and intra-observer variability, it was important to perform comparison of the automatic measurements using data from multiple board certified cardiologists. The first three cardiologists established the acceptable intra- and inter-observer variation ranges while the fourth cardiologist and Ligence Heart were used as independent raters with accuracy calculated as the number of times measurements fell within acceptable variation range in the selected studies. Non-inferiority of automatic measurements accuracy to a cardiologist was established by conducting the t-test and assuring p-values are > 0.05 which proves that any differences are non-significant. All of the measurements passed the required performance threshold, and for 7 out of 21 automatic measurements, both Ligence Heart and FC had full agreement with the ORG.

Conversely, Ligence Heart automatic functionalities did not return a value for at least one study in 11 measurements, however, there was a sufficient number of studies for all measurements where automatic analysis value was returned to ensure statistical power. The reason for no value being returned in a particular study has to do with the automatic predictions not passing quality thresholds which are employed in each step of the automatic functionalities (classification, cardiac phase or cycle detection and measurement prediction confidence). This ensures that a lower number of false values is provided to the user of the medical device and serves as a safety mechanism by reducing the number of potential false positive findings.

Supplementary figure 1 provides insight into difficulty of performing different measurements. The measurement that has the highest variability and the lowest correlation coefficients between all raters is LVPWd, most likely due to the fact that the inner border of the left ventricular posterior wall is difficult to visualize precisely in a parasternal long axis view. It is worth mentioning that measuring LVPWd is difficult not only for an automated system but for human operators as well. Looking at individual measurement prediction in **Supplementary figure 2** we can observe that LVESV2A has the highest difference between FC and Ligence Heart predictions, however both operators fall within ranges established by the ORG measurements. It is important that variation arises due to multiple factors as the end result depends on multiple steps: selecting the proper echocardiographic video, selecting the right frame in the cardiac cycle and performing the right annotations. Two different operators might make the same measurement in different echocardiographic videos belonging to the same view and because of that they may end up with different results. Different operators can also choose different cardiac cycles and different frames for making the same measurement. In some cases, there might be no single correct answer due to the factors mentioned before and the only way to establish true values would be to use some other imaging method, which is not always feasible. This explains some of the mechanisms behind the variability observed in echocardiography and further shows the need to use data from multiple raters in establishing a benchmark.

It is worth noting that Ligence Heart is the first and only medical device software to our knowledge that can automatically perform a number of linear measurements: AoA, AoS, STJ, AAo, LAD, RVOTPD, RVB, RVM and RAMAD. Additionally, our results are validated using an end-to-end approach where the automated system has to perform echocardiographic view recognition, cycle detection and measure the relevant structure all

at once. In contrast, machine learning in echocardiography publications often only describe the accuracy of a single isolated task, e.g. classification or segmentation and provide metrics that might not necessarily translate well into clinical practice. The literature review shows that even though several studies were validated in multi-centre clinical trials, nevertheless they were only validated on isolated tasks. For example, ML models were shown to be faster and more repeatable for quantification of LVEF (27–29). ML model for quantification of RVEF showed good agreement with cardiac magnetic resonance imaging (30). Automated left ventricle volume quantification did not significantly deviate from the manual method (31). The clinical practice, however, requires more measurements to be performed, rather than only LVEF, RVEF and left ventricular volumes, for a holistic evaluation of a patient.

The automatic functionalities additionally offer the advantage of reproducibility in re-analysis of the same echocardiographic studies. Even though deep neural networks used in Ligence Heart can be considerably complex, they will produce identical output given the same input. In our analysis, we have shown that there is considerable intra-observer variability which can be reduced by using an automated system. The benefits of automated systems for reduced intra- and inter- observer variability are shown by the research as well. A study by Asch evaluated a ML model for detection of reduced LVEF and showed higher consistency, sensitivity, and specificity for detection of reduced LVEF than human operators did, another study by Myhr found that automated LVEF measurements show smaller intra- and inter-operator variability than manual methods do (27,32).

Finally, the time taken by an automated system to perform the same measurements is generally shorter compared to a human operator. It may vary based on the hardware being used. The literature supports the claim that automated methods perform quicker than manual ones as it was shown by Myhr where acquisition and analysis for calculating LVEF for an automated system was 94 ± 23 seconds whereas for a manual method it was 115 ± 15 seconds (32). In our case we used an upper-mid level consumer grade NVIDIA 2080 RTX GPU, which is not beyond what is reasonably available to a healthcare organization.

Overall, we have shown that 1) the automatic functionalities of Ligence Heart have accuracy that is non-inferior to a cardiologist; 2) Ligence Heart produces the same output across two runs on the same echocardiographic studies and has lower intra-rater variability than human operators; 3) Ligence Heart performs automated measurements faster than a human operator.

16.3 Assessment of risks and clinical benefits

No additional risks have been identified and included in the Risk management file during this Clinical Investigation. As it has been previously summarized in **Table 5** no adverse events occurred during the trial and device deficiencies have been reported. This information further proves the safety of the device under investigation.

Considering that Ligence Heart has been proven to meet all the expected performance requirements (described in section 4.2 of this document) that are based on the definition of the State of the Art (described in section 4.12 of this document), it can be firmly concluded that it provides the defined clinical benefits while minimizing the risks.

16.4 Special considerations and limitations

All patients in our testing cohort had sinus rhythm which means that caution should be used in cases of cardiac arrhythmia. Additionally, all of our validation data comes from a single center in Lithuania and echocardiographic images are obtained from two manufacturers (GE and Philips) ultrasound machines. Our aims for the post-market clinical follow-up are directly related to the aforementioned limitations. We aim to expand our validation study in multiple centers, in order to include participants with cardiac arrhythmias and include studies from a higher variety of ultrasound machines.

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18 Investigation participants list

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