A Comparative, Controlled Study to Evaluate the Clinical Accuracy and Usability Performance of the ACR | U.S. Urine Analysis Test System in the Lay User Hands

Protocol ACR-US-MCU-03

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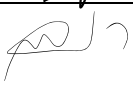
[redacted]	VP Product	December 02, 2021	

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ABBREVIATIONS & DEFINITIONS

ACR – Albumin to Creatinine Ratio

AE – Adverse Event

eSource – Electronic source documents

GCP – Good Clinical Practice

IC – Informed Consent

ICH – International Conference on Harmonization

IEC – Independent Ethics Committee

IEC/IRB – Independent Ethics Committee/Institutional Review Board

IVD – In-Vitro Diagnostic

CKD – Chronic Kidney Disease

Optima – URiSCAN Optima Urine Analyzer

The following protocol is a Clinical Performance Study Plan (CPSP per IVDR 2917/746) and describes the rationale, objectives, design and proposed analysis, methodology, monitoring, conduct and record-keeping of the ACR | U.S. Urine Analysis Test System in the lay user hands.

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1. STUDY SYNOPSIS

Name of Device:	ACR | U.S. Urine Analysis Test System (henceforth ACR | U.S.)
Device Description:	The ACR | U.S. Urine Analysis Test System is an in-vitro diagnostic, home-use device for the semi-quantitative measurement of the Albumin-Creatinine Ratio (ACR). The device consists of a smartphone application, proprietary Color-Board and an ACR reagent strip. The device is available for prescription-use only. Results are intended to be used in conjunction with clinical evaluation as an aid in the assessment of kidney function.
Patient Population:	At least 150 male and female subjects, 18-80 years of age, that represent the intended use population of the ACR | U.S. device, including high-risk subjects with a known medical condition and/or risk factor for kidney damage that can potentially present as an abnormal concentration of urine albumin, such as Diabetes (Type I/Type II, or gestational diabetes), Hypertension, Cardiovascular Disease, Family History of Kidney Disease and History of Acute Kidney Injury (AKI), as well as subjects with other medical conditions and/or risk factors that impact kidney function (e.g. older age, smoking, obesity, dyslipidemia, inherited kidney disease, malignancies, recurrent kidney infections, and more). In addition, subjects with no known significant medical conditions.
Structure:	A comparative, controlled, accuracy and usability study.
Objectives:	To evaluate the accuracy and usability performance of the ACR | U.S. in the hands of the potential lay user.
Primary Endpoint:	The degree of agreement of the ACR | U.S. (tested by a lay user) as compared to the URiSCAN Optima Urine Analyzer (tested by a healthcare professional), for the different concentrations (blocks) reported by the ACR | U.S. device.
Secondary Endpoint:	Evaluation of the ACR | U.S. usability, by potential lay user under actual use conditions of a simulated home environment.
Study Design:	Potential candidates for the study will be recruited at the designated clinical site by the study personnel. Following subject consent on site, the subjects will be evaluated for eligibility based on their health condition and medical history. The ACR | U.S. kit in its original packaging, along with a mobile phone (Google Pixel 3a) with the pre-installed ACR | U.S. application, will be provided to the subject in a simulated home- use environment. Each subject will follow an in-app instructional guide in order to perform the tasks required to complete the test (e.g. operate the ACR | U.S. app, provide a urine sample, use a dipstick, take a scan, etc.). Following each test performed by a lay user, the urine sample will be tested by a professional study staff using the comparator device – the URiSCAN Optima Urine Analyzer. These results will be considered as the "true value". A designated study operator will complete a questionnaire during each test to collect measurable usability information and

	evaluate the critical steps in the study. Each subject will complete a post-test usability questionnaire in order to assess the usability of the ACR | U.S. device.
Data analysis:	Results from the ACR | U.S. Urine Analysis Test System and the comparator device, the URiSCAN Optima Urine Analyzer, will be presented in tables listing the results in each group and the % exact match (percent agreement) and the % ±1 block match (percent agreement). Additionally, measurable usability criteria for specific and critical steps, such as time to completion of task, number of requests for assistance, numerical ratings, etc., will be evaluated by the operator and user questionnaire responses.
Study Sponsor:	Healthy.io Ltd.
Principal Investigators:	

2. INTRODUCTION

2.1. Urinalysis and Albumin-to-Creatinine Ratio Overview

Urinalysis is an array of tests performed on urine, and one of the most common methods of medical diagnosis. The target parameters that can be measured or quantified in a urinalysis test include many substances and cells, as well as other properties, such as specific gravity and pH. Urinalysis can be performed by using urine test strips, in which the test results can be measured on the basis of color changes. Other methods of urinalysis include light microscopy[1].

An Albumin-Creatinine Ratio (ACR) urine test strip is a basic diagnostic tool used to determine pathological changes in a patient's urine in standard urinalysis. A standard ACR urine test strip usually contains two different chemical pads or reagents which react (change color) when immersed in, and then removed from, a urine sample. The test can often be read after 60 seconds after immersion. Routine testing of the urine with ACR urine test strips is the first step in the diagnosis of a wide range of kidney diseases. The analysis includes testing for the Albumin and Creatinine, and then calculating the ratio between them.

Albumin is the single-most important protein that is pathologically present in the urine in most chronic kidney diseases. A properly-functioning kidney will permit extremely small amounts of Albumin to pass into urine, and thus even a minute presence – defined as a quantity greater than 30 mg/L – is a sign of a diseased or distressed kidney. Creatinine is a byproduct of muscle metabolism released into the urine at a constant rate; its level in urine is an indication of urine concentration. The Albumin-to-Creatinine Ratio measurement is a widespread and effective screen for the early stages of CKD[2], in most instances appearing before the reduction in the estimated glomerular filtration rate (or eGFR, which requires a blood test).

ACR urine test strips can be used in many areas of the healthcare chain including screening for routine examinations, treatment monitoring, self-monitoring by patients and/or general preventive medicine. The ACR urine test is an effective measurement to identify patients with early stages of Chronic Kidney Disease (CKD), when patients are often asymptomatic, which can reduce the risks of cardiovascular disease, End-Stage Renal Failure (ESRF), and death that are the hallmarks of a malfunctioning kidney. According to the Henry Ford Health System, only 5% of the general Medicare population undergoes a

[1] Simerville JA, Maxted WC, Pahira JJ. "Urinalysis: a comprehensive review". American Family Physician. 2005; 71(6) 1153-62

[2] "Albumin-to-creatinine ratio (ACR) is the first method of preference to detect elevated protein." See the National Kidney Foundation's website detailing the importance of the ACR test: https://www.kidney.org/kidneydisease/siemens_hcp_acr

screening urinalysis, while only 2% of high-risk subjects screened through a National Kidney Foundation initiative self-reported a history of kidney disease.[3]

Healthy.io has developed the ACR | U.S. Urine Analysis Test System (ACR | U.S.). The ACR | U.S. is a semi-quantitative, prescription-based, in vitro diagnostic (IVD) home use device for the measurement of the ratio between albumin to creatinine in a urine sample using a single-wrapped ACR urine reagent strip (microalbumin/creatinine).

Automatic analysis of urine test strips using automated urine test strip analyzers is a well-established practice in modern day urinalysis. It guarantees rapid, standardized measurement and immediate reliable documentation of the result. Most of these urine analyzers are based on the technology of reflectance photometry while additional technologies such as microscopy or flow cytometry exist (performed directly on the urine specimen).

The basic elements of the reflectance photometry are based on the analysis of the intensity and color of light reflected from the reagent areas on a urinalysis test strip. Most of the automated urine analyzers are specifically intended for lab environments and are operated by professional users. In recent years, following the tremendous development in communication media (especially with mobile media) there is a shift from "in-clinic" testing to the home-use self-testing. This trend enables manufacturers to develop cost effective, reliable, easy-to-use, self-monitoring test methods, including urinalysis methods.

Healthy.io Ltd. employs computer vision technology to turn smartphones into clinical-grade diagnostic devices. Previously, the company developed a smartphone application containing image recognition software that enables users to remotely and independently perform a urinalysis test, the DIP | U.S. (510(k) clearance number: K173327)[4]. The DIP | U.S. device is a prescription, home-use device intended for the analysis of six urine analytes. The company also developed a point of care, prescription-only, professional-use smartphone application to test for microalbuminuria, the ACR | LAB device (510(k) clearance number: K182384)[5]. Building off this experience, Healthy.io developed the ACR | U.S. to test for the Albumin-Creatinine Ratio. Further details on the device components and functionality are described in the device description section below.

[3] Chronic Kidney Disease. *Henry Ford Health System*. Version 7, 2015.
[4] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K173327.pdf
[5] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K182384.pdf

3. DEVICE DESCRIPTION

3.1. General

The ACR | U.S. is a prescription, in-vitro diagnostic, home-use device for the semi-quantitative measurement of the Albumin-Creatinine Ratio. The device comprises a kit with a urine receptacle, an ACR urine test strip, an absorbing pad, a Color-Board and a user manual. The device also consists of an easy-to-use smartphone application and image recognition algorithm.

The ACR | U.S., while also a photometric based device, employs a computer vision technique, executed on various hardware platforms, in an uncontrolled lighting environment, used by an untrained user. The test has no pre-calibration step. The calibration and test are executed simultaneously during each test individually. This is executed via a Color-Board, which is part of the user kit. Performing test calibration eliminates lighting variances & camera disruptions and enable delivery of clinically valid semi-quantitative measurements.

The device consists of seven elements, which have been designed for easy usage in a home setting:

1. Urine receptacle (i.e. urine cup)
2. ACR urine test strip (i.e. reagent strip, dipstick)
3. Absorbing pad (i.e. blotting pad)
4. Color-Board
5. Smartphone application
6. Software algorithms
7. User manual

The kit components are shown in ***Figure 1***. An outline of the main components is described below.

Figure 1: ACR | U.S. Device Components

3.2. Device Components Outline

1. Urine Receptacle

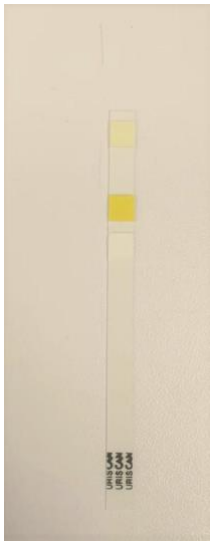
The urine receptacle is comprised of a plastic cup which holds the subject's urine. It is designed to contain the sufficient amount of urine needed for the test and allow easy immersion of the test strip along its entire length. **See *Figure 2.***

Figure 2: Urine Receptacle

2. ACR Urine Test Strip

A single-wrapped ACR urine reagent strip (microalbumin/creatinine). The strip consists of 3 chemical reagents, which change color after being immersed in a urine sample. One patch for albumin, another for creatinine and an additional compensation patch. **See *Figure 3.***

Figure 3: Urine Test Strip



3. Absorbing Pad

A single piece of standard absorbing material. It is designated to assist in absorbing remaining liquids from the strip after it has been immersed in the urine sample.

4. Color-Board

A cardboard-based, high-quality printed Color-Board facilitates the image recognition and calibration processes. Its main features include proper positioning of the urine strip in the frame, neutralization of the surrounding lighting and/or camera interruptions and providing a reference color spectrum for the strip reagent colors for later data algorithm analysis. **See *Figure 4.***

Figure 4: Color-Board

5. Smartphone Application

The smartphone application will be tested using a Google Pixel 3a smartphone (Android) and is designed to enable the following:

- Device interface instructions for the user to ensure proper performance of the urinalysis test, avoiding key human errors. These include interactive video texts and sound instruction to guide the users through the testing procedure.
- The software includes an augmented reality layer on the smartphone camera which guides users while taking the scan. This is a real-time analysis of the scan, which finds and centers the Color-Board within the camera frame. The software will also analyze the scan and evaluate if it meets

the system boundary conditions for urinalysis measurement. If not, the software will instruct the user to take another scan which can be analyzed.

- Presentation and interpretation of the ACR analysis
- Secured transmission of urinalysis measurement result to a clinician for viewing and interpretation.

See *Figure 5* (next page).

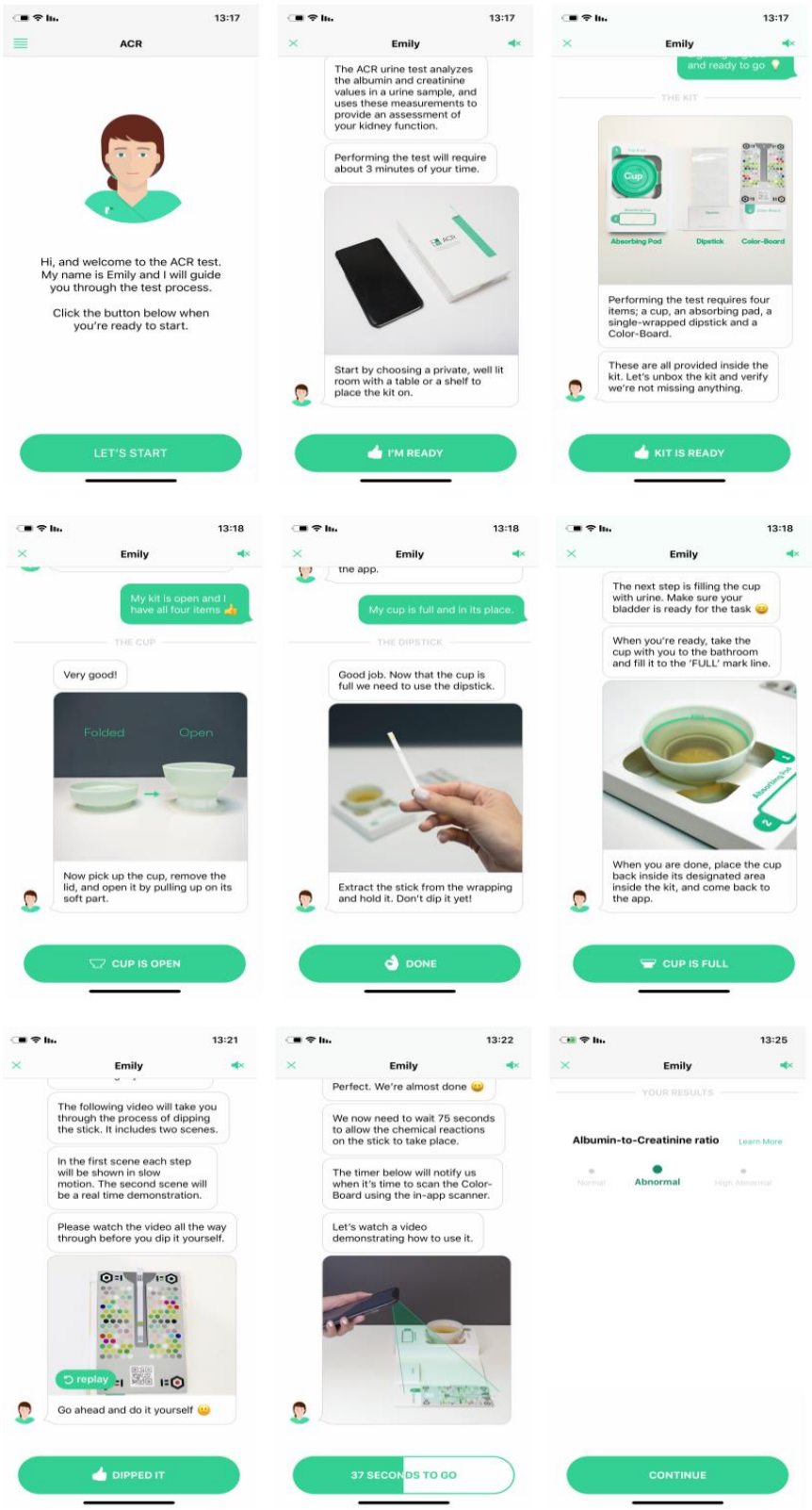
6. Software Algorithms

The ACR | U.S. smartphone algorithm is a cloud-based, image-processing system. Both albumin and creatinine analytes are analyzed based on a separate, unique set of eight target colors and a set of calculations which are completely independent from each other. Once these two measurements are taken, the algorithm on the cloud will use these two numbers to calculate the subsequent Albumin-Creatinine Ratio, which is measured in units of mg/g or mg/mmol.

3.3. Intended Use

The ACR | U.S. Urine Analysis Test System is an in-vitro diagnostic, home-use device for the semi-quantitative measurement of the Albumin-Creatinine Ratio (ACR). The device consists of a smartphone application, proprietary Color-Board and an ACR reagent strip. The device is available for prescription-use only. Results are intended to be used in conjunction with clinical evaluation as an aid in the assessment of kidney function.

Figure 5: Smartphone Application Screens



4. PERFORMANCE TESTING AND CLINICAL EVALUATION

4.1. ACR | U.S. Performance Tests

Healthy.io has designed a series of bench-testing experiments to verify the performances of the ACR | U.S. These tests have been designed according to the guidance provided by the Clinical and Laboratory Standards Institute (CLSI).

- **Precision:** Comprises two separate tests - Repeatability and Reproducibility. The precision studies are designed in accordance with guidance provided by Clinical and Laboratory Standards Institute document EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
    - Repeatability is defined as the closeness of the agreement between results of successive measurements of the same measure and carried out under the same conditions of measurement.
    - Reproducibility is defined as closeness of the agreement between the results of measurements of the same measure carried out under changed conditions of measurement over the course of multiple days.
- **Interference:** Testing of potential interfering substances with the ACR | U.S. was designed in accordance with guidance provided by the Clinical and Laboratory Standards Institute document EP07 – Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition.
- **Limit of Detection:** Testing of the ACR | U.S. assay's detection was designed in accordance with guidance provided by Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.
- **Linearity:** Testing the linearity of the ACR | U.S. was designed in accordance with guidance provided by the Clinical and Laboratory Standards Institute (CLSI) document *EP06-A – Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*
- **Stability:** The stability experiment is designed to evaluate the environmental conditions under which the ACR | U.S. is suitable for its intended use. This study was designed with the guidance provided by the Clinical and Laboratory Standards Institute (CLSI) document *EP-25A Evaluation of In-Vitro Diagnostics Reagents; Approved Guideline.*

- **Additional Studies**:
    - Illumination Study: Healthy.io designed an experiment to test the ACR | U.S. under multiple lighting conditions.
    - Boundary Study: Healthy.io designed an experiment to test the physical boundaries (such as the phone's distance and angle in relation to the Color-Board) which would prevent the ACR | U.S. from capturing an accurate image that can be evaluated by the algorithm.
    - Multiple Phones and Operating Systems Study: Healthy.io designed an experiment to test the ACR | U.S. with multiple smartphone devices and operating systems versions (iOS and Android).
    - Timing Flex Study: Healthy.io designed an experiment to test the impact of different dipping times, assay-times, and strip wetting times on the ACR | U.S.

4.2. Previous ACR Urinalysis Method Comparison Studies:

The following table (***Table 1.0***) summarizes the method comparison studies conducted on several marketed lab urine analyzers and test strips with similar indications for use as the ACR | U.S.

The key difference being that these studies were operated by lab professionals while the ACR | U.S. is intended for lay users who are operating the device at home.

Table 1.0: Urinalysis analyzers method comparison studies summary:

Study details	Mission U120 Ultra Urine (K142391)[6]	Uritek TC-201 Urine Analyzer (K152835)[7]	URiSCAN Optima Urine Analyzer (K141874)[8]
No. of urine samples	429 samples	402	351
Urine sources	Fresh	Fresh	Fresh

[6] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K142391.pdf
[7] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K152835.pdf
[8] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K141874.pdf

Study details	Mission U120 Ultra Urine (K142391)[6]	Uritek TC-201 Urine Analyzer (K152835)[7]	URiSCAN Optima Urine Analyzer (K141874)[8]
Percentage of Contrived Samples Used	12-13%	9%	0%
Subjects medical history	From 510k summary: "Test results may be used in screening urine specimens for microalbuminuria as an aid in the detection of patients at risk for developing kidney damage." From User Manual: "Patients with the highest risk of developing early kidney damage are those with diabetes and hypertension, followed by patients who have immune disorders or have been exposed to nephrotoxins."	From the 510K summary: "Test results may be used in screening urine specimens for microalbuminuria as an aid in the detection of patients at risk for developing kidney damage."	From the 510K summary: "These measurements are useful in the evaluation of renal, urinary and metabolic disorders."
Compared predicate	Clinitek Status Analyzer (K031947)	Clinitek Status+ Analyzer (K091216)	Clinitek Status (Siemens) (K091216)
No. of strip lots	3	3	3

5. RATIONALE FOR THE STUDY

The rationale for the study is to evaluate the performance of the ACR | U.S. Urine Analysis Test System in the hands of the potential intended-lay-user in a simulated home environment.

The ACR | U.S. Urine Analysis Test System is classified as an in-vitro diagnostic device. According to the regulatory requirements, such devices undergo several analytical performance tests. These tests performed on "ground truth" samples, provide data on the device precision, repeatability, reproducibility, linearity, and effect of substances in the urine, while compared to a known validated reference or predicate test method. These tests were performed as described in the previous section.

In addition to the analytical performance tests described above, method comparison and usability tests are also a crucial step before companies are permitted to market such a device. All marketed urinalysis devices and urine test strips for home use have performed method comparison and usability studies tested by the intended lay user. Normally in these studies, fresh urine samples are collected and tested by a lay user, using the new home use device, and by a device operator or lab professional using the comparison device. The lay user test results are compared to the results obtained by testing the same urine sample on the comparison device. The method comparison and usability studies provide data on the accuracy and usability of the device, including the lay user's ability to understand and implement the user manual instructions and evaluate the ease of use of the device under actual use conditions (home environment).

Furthermore, the study provides valuable data collected from subjects representing the intended use population of the device, which includes subjects with a relevant medical condition and/or risk factor for kidney damage that might be reflected in a pathological urine sample. Thus, it enables the company to collect a wide range of analyte values, cover the entire range of the method, and strengthen the results of the bench analytical test.

The ACR | U.S. method comparison study should include similar elements and be designed similarly to previously tested urinalysis analyzers. The study design is meant to demonstrate that the ACR | U.S. results are in agreement with another legally marketed urine analyzer device (the URiSCAN Optima Urine Analyzer) and may be easily used by a lay person.

6. OBJECTIVES

6.1. General

The aim of the study is to establish the validity and performance of the ACR | U.S. Urine Analysis Test System in the hands of the potential lay user, by evaluating the accuracy and usability performance as compared to the comparator device.

6.2. Primary Objective

The primary objective of the study is to evaluate the % exact match (percent agreement) and the % ±1 block match (percent agreement) of ACR | U.S. (tested by the lay user) compared to the URiSCAN Optima Urine Analyzer (the comparator device, tested by a healthcare professional), for each tested concentration (block).

6.3. Secondary Objective

The secondary objective of the study is to evaluate the ACR | U.S. usability, by potential lay users under actual use conditions (home environment).

7. STUDY POPULATION

7.1. General Considerations

The study population should represent the intended use population of the ACR | U.S. device and include subjects that are likely to, or will derive benefit from assessing their kidney health.

Early identification of CKD by targeted testing based on known risk factors, offers the potential to substantially reduce morbidity and mortality from CKD and its related complications. The results of screening can inform treatment modifications, such as appropriate drug dosing and improved lifestyle changes and can slow or even halt the progression of kidney damage. [9]

The guideline-based testing for kidney disease varies across different populations as it takes into account local policies, resource limitations, payment systems, and care delivery models. [10]

In general, initial efforts for early testing should target high-risk subjects with well-established risk factors for kidney damage, such as those with Diabetes (Type I, Type II, or gestational diabetes), Hypertension, Dyslipidemia, Cardiovascular diseases, Family history of kidney disease, History of acute kidney injury (AKI). [11, 12]

In addition, CKD testing should be implemented in other high-risk groups with other medical conditions that impact kidney function. Risk factors include, but are not limited to: older age (> 60, smoking, obesity, inherited kidney disease (e.g. Polycystic kidney disease), prolonged obstruction of the urinary tract from different conditions (e.g. enlarged prostate, kidney stones), malignancies, recurrent kidney infections, autoimmune diseases (e.g. SLE), genetic risk factors, exposure to nephrotoxins (e.g. environmental, frequent use of certain medications). [11, 12]

Overall, the study population will include at least 150 subjects from any genders, ethnicities, socioeconomic status, and education level, between 18-80 years of age, meeting the eligibility requirements. The subjects will be recruited by the study personnel of the clinical site. Informed consent will be obtained from potential subjects according to local and national IRB requirements. Potential candidates will be screened according to the following inclusion/exclusion criteria.

[9] Chronic kidney disease awareness, screening and prevention: rationale for the design of a public education program, Allan J Collins et al., Nephrology (Carlton) . 2010 Jun;15 Suppl 2:37-42.

[10] See screening guidelines of different associations -

- **NKF** ("reference for kidney disease screening")
- **RPA** ("U.S. Preventative Services Taskforce - Recommendation on Screening for CKD")
- **ISN** and **KDIGO** ("Early Identification & Intervention Toolkit")
- **ADA** (Screening for Kidney Disease in Adults with Diabetes, Diabetes Care 2005 Jul; 28(7): 1813-1816.)

[11] Risk factors for chronic kidney disease: an update, Rumeyza Kazancioğlu, Kidney Int Suppl (2011), 2013 Dec;3(4):368-371.

[12] UpToDate: Early detection of chronic kidney disease

7.2. Inclusion Criteria

- Males and Females 18-80 years of age.
- Subjects with a known, well-established medical condition and/or risk factor for kidney damage, that can potentially present as an abnormal concentration of urine albumin:
    - Diabetes (Type I/Type II, or Gestational Diabetes)
    - High Blood Pressure (i.e., Hypertension)
    - Cardiovascular Diseases
    - Family History of Kidney Disease
    - History of Acute Kidney Injury (AKI)

  Or; subjects with other medical conditions and/or risk factors that impact kidney function:
    - Dyslipidemia
    - Heavy smoking
    - Obesity
    - Inherited kidney disease (e.g. Polycystic kidney disease)
    - Prolonged obstruction of the urinary tract from different conditions (e.g. enlarged prostate, kidney stones)
    - Malignancies
    - Recurrent kidney infections
    - Other relevant conditions

  Or; subjects with no known significant medical conditions
- Subjects who are able to use both hands
- Subjects who are familiar with the use of a smartphone
- Subjects who are capable of comprehending and following instructions in English.

7.3. Exclusion criteria

- Subjects with a major cognitive impairment (e.g.: dementia, memory loss, severe mental disorder)
- Subjects who are not able to collect urine in a receptacle.
- Subjects who are visually impaired (i.e., cannot read the user manual)
- Any additional reason that disqualifies the subject from participating in the study according to the study physician

8. STUDY DESIGN

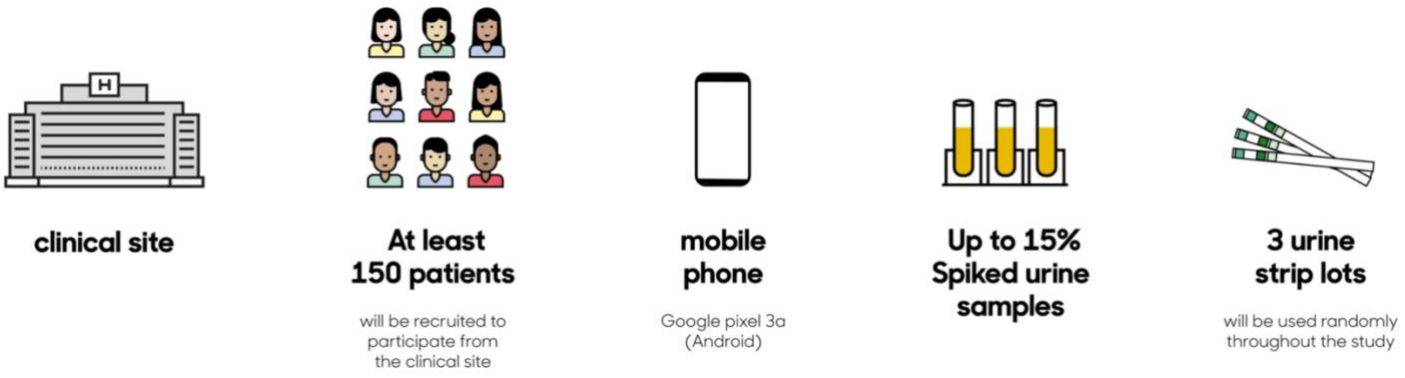
The Study is a Method Comparison clinical study using actual, fresh, human urine samples that will be collected by the lay-users in a lab-based home setting.

The study will recruit at least 150 subjects with a known medical condition and/or risk factor for kidney damage, including patients with a disease that normally represents itself with an abnormal concentration of urine albumin.

8.1. Framework

- At least 150 patients will be recruited to participate
- Urine samples will be collected mainly from subjects with a relevant background condition and/or risk factor for kidney damage, that are potential candidates for kidney screening test.
- Up to 15% spiked samples will be used if there are insufficient native samples to cover all possible reported results by the ACR | U.S device.
- Clinical research site to recruit potential candidates meeting the eligibility requirements.
- Mobile phone: Google Pixel 3a (Android).
- 3 urine strip lots will be used randomly throughout the study.

Figure 5: Study Framework



8.2. Urine Samples

Fresh urine samples will be collected from subjects meeting the eligibility criteria detailed above, so that each analyte concentrations will be distributed over the entire analytical measurement range, to the extent possible to cover all possible reported results. The study might be supplemented with spiked urine samples with the missing concentrations. However, the number of spiked samples will not exceed 15% of the total

samples. Lay users will test their own personal urine and additionally may be asked to test another spiked sample, that will be provided by the study staff as necessary and in accordance to protocol.

8.3. Analyzers

Each urine sample will be tested two times using the two urine analyzer methods: the ACR | U.S. (running on Google Pixel 3a) and the URiSCAN Optima analyzer (the comparator device).

8.4. Reagent Strips

To control for bias among the test strips, three lots of reagent strips will be used during the study. To enable an equal and unbiased distribution of the lots, one lot will be randomly selected by the professional user for the testing of each urine sample. Separate strips will be used in each test: with the ACR | U.S. and the URiSCAN Optima analyzer.

8.5. Testing Methods

Subjects will be recruited for the study based on their ability to provide a urine sample and comfortably complete tasks using a smartphone. Each urine sample will be tested by a lay user on the ACR | U.S. app, and then transferred to a professional user to conduct the test on the URiSCAN Optima device. The professional user will be blinded to the results of the lay users until after they have completed the test using the URiSCAN Optima device.

8.6. Timing

Ideally, simultaneous sampling of each urine specimen with both methods would be performed, but since the usability of the ACR | U.S. device is tested as well, such a design is not feasible. Therefore, each urine sample will be tested sequentially with no more than 15 minutes elapsing between methods (i.e. between the end of one test and the beginning of the next). Real time differences across the two methods are not anticipated, as a time difference constituted of minutes is unlikely to affect the value obtained from a sample. Moreover, regarding specimen acceptability, the CLSI guidelines recommend urinalysis within two hours from sampling. The specimen should not be centrifuged or refrigerated in between tests.[13]

[13] Clinical Laboratory Standards Institute (CLSI). Urinalysis; approved guideline -third Edition. CLSI document GP16-A3. Wayne, PA, USA: CLSI, 2009.

8.7. Requirements

The Method Comparison Study must be conducted in accordance with Good Clinical Practice (GCP), according to ISO 20916:2019, 21 CFR Part 50, 54, 56, 812, and relevant local and national regulations (IRB or Helsinki EC approved). The Method Comparison Study shall comply with CLSI EP09c: Method Comparison and Bias Estimation Using Patient Samples; Approved Guidelines – Third Edition – In addition, it shall comply with EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices. Since the ACR | U.S. is classified as a urinalysis analyzer intended for lay users to test their urine in a home environment, the design of the device clinical comparative study should include parameters from both lab urinalysis analyzers and urine strip method comparison studies. The study design is mainly based on the above-mentioned method comparison studies in section 3.2 when used by a lay user and FDA recognized consensus standards CLSI EP09c and for usability testing of medical devices - ANSI/AAMI/IEC 62366-1:2015 Medical devices – Part 1: Application of usability engineering to medical devices.

9. STUDY PROCEDURES

9.1. General

The study consists of 6 stages as follows:

- Familiarization Period
- Screening
- Pre-testing
- Testing
- Monitoring
- Post-testing

9.2. Stage I – Familiarization Period

As outlined in the EP9 standard, evaluating an analytical method requires sufficient time for the operators to become familiar with the study protocol and device operation, therefore the standard recommends a study familiarization period. During the familiarization period, the study operators will become familiar with all aspects of set-up, operation, maintenance, quality control, and troubleshooting of both methods. This period will precede the study evaluation process and will coincide with the manufacturer's training performed during the Site Initiation Visit (SIV). In order to familiarize the study staff with the study flow and procedures, up to 15 subjects will be serve as a training period at the beginning of the site and will not be included in the final study results and statistical analysis. After the SIV and familiarization period, the study will begin.

9.3. Stage II – Screening

The screening of potential candidates will be performed by the clinical site. Potential candidates will receive complete information describing the study and their role and will be encouraged to ask any questions regarding the study. The risks and requirements of this clinical research trial will be explained to each potential subject. Those volunteering to take part will read and sign the Informed Consent Form for participation in the clinical research trial before any study-related procedures are performed. The Informed consent signing process will be in front of a study personnel, and the principal investigator (PI) will review and sign all source documents, informed consents, and results once a week. Upon obtaining the signed informed consent from each subject, inclusion and exclusion criteria will be reviewed to verify the subjects' eligibility.

9.4. Stage III – Pre-testing

Subjects who were found eligible to participate in the study and signed informed consent will be enrolled into the study and assigned a subject ID number, which will be used along with their initials for study record identification.

Designated eSource forms will be completed with the subject's general details, including demographics, medical history, diagnosis, and concomitant medications.

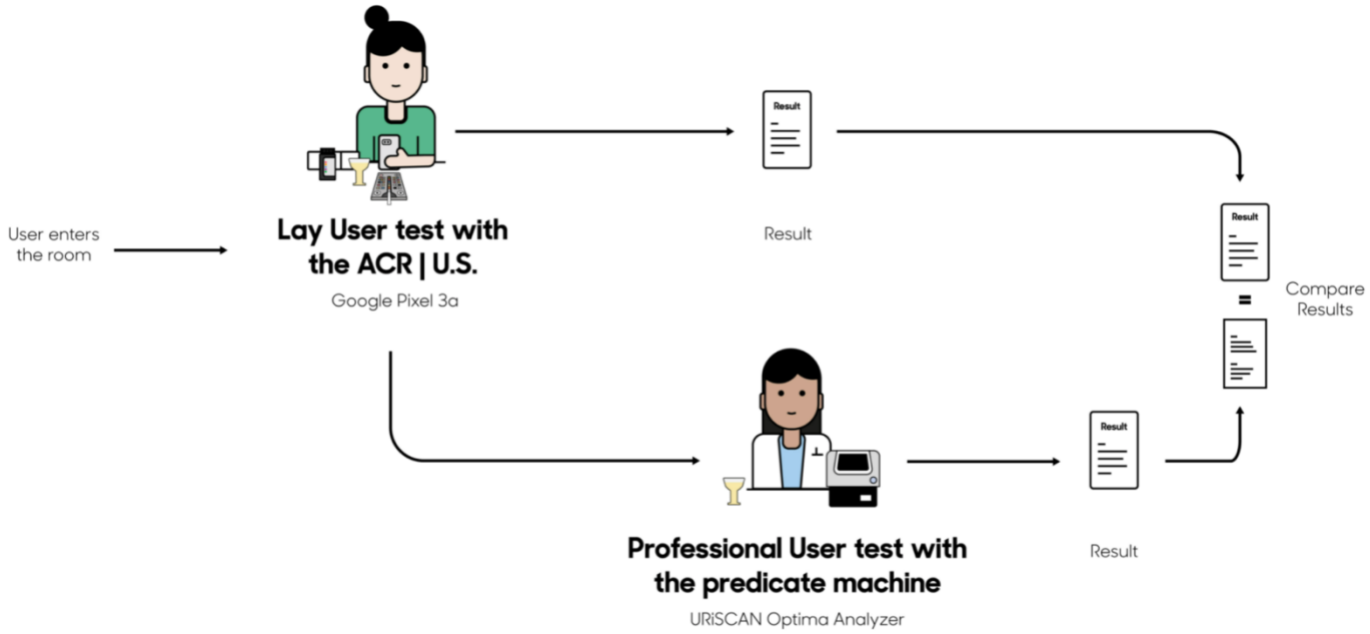
9.5. Stage IV – Testing

1) Each subject will be placed in the private room, with restroom accessibility and internet connectivity, to perform the urinalysis test and complete the task list found below. The clinic will be set up so that the self-testing will allow the subject to perform the urinalysis test without outside influence and to simulate the home-use testing of the device.
2) No training, assistance, feedback, or supplemental instructional materials, other than those described, will be provided to participants. The healthcare professional or other site staff will not be allowed to intervene or answer questions from the subjects during testing, although a 1-800 or clinical assistance phone number, which will be provided to the subject in actual use, will be made available to the subject in case contact is attempted by the subject.
3) The room will be prepared with a desk and chair. The ACR | U.S., in its original packaging, will be placed on the desk. The device packaging contains the kit components and the user manual. An in-app instructional guide containing the information most critical to safety and effectiveness is also provided in the form of a readable and understandable, visual, and textual guide on the device App. The patient labeling will be in the format intended for distribution to allow for a realistic evaluation of the labeling effectiveness.
4) The subjects will be asked to follow the device in-app instructional guide and/or user manual and perform the following tasks:
    - Access the ACR | U.S. App on the smartphone (Google Pixel 3a).
    - Provide a urine sample in the urine receptacle (to the level marked on the cup) that is included in the ACR | U.S. kit.
    - Immerse the urine strip (provided in the ACR | U.S. kit) in the collected urine specimen for about one second.
    - Absorb any excess amount of urine from the test strip on the absorbing pad (i.e. blotting pad).

- Place the urine strip on the designated area on the Color-Board and wait at least 75 seconds, but not more than 180 seconds.
- Scan the urine strip and Color-Board with the smartphone App.
- Send the test results to the clinician using the smartphone App.

5) During each test, a study operator will observe the subject throughout the entire flow and during that time fill-out a dedicated post-test questionnaire, designed to document important key points in the process.
6) At the end of the test, each subject will be requested to complete a lay user questionnaire, designed to collect data relevant to user experience.
7) Upon completion of the usability study tasks, the study operator will use the urine sample for further testing on the comparator device, the URiSCAN Optima Analyzer. The study operator will be blinded to the results of the ACR | U.S. test, until the test with the comparison device is completed.
8) The results of both the ACR | U.S. and the URiSCAN Optima analyzer will be used for study purposes only and not for any purposes of patient management including diagnostic or therapeutic. In the case that urinalysis results are required for patient management, urine specimens from the subject will be sent independently to the hospital/clinic laboratory for analysis and only these results will be used for patient care and management. The patient will provide only one urine sample for use in the study and any subsequent clinical urinalysis testing.

Figure 6: Method Comparison Study Flow



9.6. Stage V – Monitoring

The objectives of the study monitoring are to confirm the quality and integrity of the data, to ensure that critical elements such as written ICF are reviewed for accuracy and compliance and to train or re-train the personnel on site as needed. The monitoring process will start after first patient is screened for the study and will include regular monitoring visits to the site. The monitoring visit will include review of adverse events, confirmation of protocol adherence, ICF review, eCRF review and source document verification, queries and error correction and review of lab samples. On each monitoring visit, a monitoring visit log will be recorded with the date of the activity, the individual(s) conducting and participating in the activity, a summary of the data or activities reviewed, a description of any noncompliance, potential noncompliance, data irregularities, or other deficiencies identified, and a description of any actions taken or recommended. The monitoring frequency as well as the monitoring type (i.e., onsite, remote, or central) will be determined by the risk rating allocated. Monitoring visits may be revised depending on subject enrolment rate, quality issues, site compliance or other study issues.

9.7. Stage VI – Post-testing

Statistical analyses comparing the results from the ACR | U.S. and the Optima will be performed using an appropriate statistical program and methodology.

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eSource and all source documents that support the data collected from each patient, as well as all study documents as specified in ICH/GCP Section 8, Essential, Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

A clinical performance study report, signed by a medical practitioner or any other authorized person responsible, will be written to include information on the clinical performance study protocol plan, results and conclusions of the clinical performance study, including negative findings. The results and conclusions will be transparent, free of bias and clinically relevant. The report will contain sufficient information to enable it to be understood by an independent party without reference to other documents. The report shall also include as appropriate any protocol amendments or deviations, and data exclusions with the appropriate rationale.

10. STUDY EVALUATION

10.1. Accuracy Evaluation

The ACR | U.S. test results will be compared to the URiSCAN Optima Analyzer test results. The data will be presented descriptively and graphically to compare the percent exact match (percent agreement) and the percent ±1 block match (percent agreement) between the ACR | U.S. (tested by the lay user) and the comparator device (tested by a healthcare professional), for the different tested concentrations (blocks).

10.2. Usability Evaluation

The usability of the ACR | U.S. will be determined by evaluating the percentage of study subjects able to complete the device related tasks, including operating the ACR | U.S. with minimal attempts to ask for assistance. The following measurable usability criteria for specific, critical steps, based on both observer and user questionnaire responses, will be assessed through analysis of the data:

1) The **lay-user post-test questionnaire** will be used to evaluate the participants' experience of using the ACR | U.S. Information regarding the ease of use of the ACR | U.S. test system at key stages of the test as well as information about the usefulness of the device and it's added value for the participants, will be collected through the questionnaire.
2) The **observer test questionnaire** will be used to assess the participants' success in performing the key tasks of the study (e.g., to successfully access the App on the smartphone, to properly immerse the urine strip in the urine sample, to scan the urine strip using the App, etc.), the number of requests for assistance during the test and detailed description of each request if occurred, and the time-to-completion of the test.
3) Qualitative information regarding specific user errors and inefficiencies will be collected through both observer evaluation and user post-test questionnaire responses.

The device usability information will be compared to identified device risks. Device and user related hazards were identified and documented in the ACR | U.S. Risk Analysis file. These specific device and user related risks will be evaluated in the usability study, according to the following steps:

1) Identify device and user related hazards (Risk Analysis)
2) Identify critical steps to be completed (Task List)
3) Compare device's use with identified risks (Observer Evaluation and User Questionnaire)
4) Apply Criteria - percentage of permissible failures
5) Link assessment to the overall risk analysis (study conclusions)

In addition, key system errors will be evaluated in some subjects. Examples of key errors may include the following:

1) Interruption during the test by an incoming phone call.
2) Interruption during the test by an incoming message.
3) No Internet connection.

The subject will be required to continue device operation according to the user manual.

11. SUBJECT COMPLETION / WITHDRAWAL

11.1. Completion

A subject will be considered to have completed the study if the urine sample has been tested.

A subject will be considered to have passed the usability test if he/she was able to scan the Color-Board successfully.

In case a subject fails his/her first attempt but identifies the problem alone and requests a second kit, he/she will be provided with another ACR | U.S. kit, and if the second test is completed successfully, the subject will be considered to have passed the usability test.

11.2. Withdrawal from the study

A subject will be withdrawn from the study for any of the following reasons:

1) Patient cannot provide a sufficient amount of urine for the test.
2) Withdrawal of consent.
3) The study is prematurely stopped or halted (e.g. clinical halt).
4) The investigator believes that for safety reasons (e.g. an adverse event) it is in the best interest of the patient to stop the test.

If a patient withdrawal occurs before the study is completed, the reason for withdrawal will be documented in the eSource.

12. STATISTICAL CONSIDERATIONS

12.1. Study Design and Objectives

The study is designed as a comparative, controlled, trial to evaluate the accuracy and usability performance of ACR | U.S. Urine Analyzer Test System in the hands of a potential lay user.

12.2. Study Endpoints

1. Primary Efficacy Endpoint

The primary endpoint of the study is the degree of agreement of the ACR | U.S. Urine Analyzer Test System (tested by the lay user) as compared to the URiSCAN Optima Urine Analyzer (tested by a healthcare professional), for the different concentrations (blocks) reported by the ACR | U.S. device.

2. Secondary Efficacy Endpoint

The secondary endpoint of the study is to evaluate the ACR | U.S. usability, by potential lay users under actual use conditions (home environment).

12.3. Sample Size Estimation

Sample size estimation was determined according to previous method comparison studies performed using other urine analyzers with similar Indications for Use (see *table 1.0*), and based on the EP9-A2 standard and recommendations.

Furthermore, based on our experience in a previous, similar study, at least 150 subjects should represent a sufficient sample size to cover all concentrations and determine the agreement between the ACR | U.S. and the URiSCAN Optima Analyzer.

12.4. Subject Tracking

Throughout the study, tracking will be performed to evaluate whether the data collected covers the entire range of concentrations measured by the device. If the data is insufficient, spiked urine samples will be used to supplement the missing data. The number of spiked samples will not exceed 15% of the total number of samples (all blocks combined).

12.5. Data Analysis Sets

1. Efficacy Analysis Set

The Efficacy analysis set will consist of all subjects without major protocol deviations. Protocol deviations will be defined and classified as minor or major.

2. Statistical Analysis of Analysis Sets

The Efficacy analysis set will serve as the main analysis set for the method comparison and usability assessments.

12.6. Statistical Analysis

1. General Considerations

Statistical analyses will be performed using an appropriate statistical program and methodology.

Baseline demographic and safety analyses will be performed on all enrolled subjects.

2. Demographic and Other Baseline Variables

Demographic and baseline condition related characteristics will be tabulated. Continuous variables will be summarized by a mean, standard deviation, minimum, median and maximum, and categorical variables by a count and percentage.

3. Disposition of Subjects

The number and percent of subjects who fail to complete the study and the number and percent of subjects who fail to complete the study because of Adverse Events will be presented.

4. Efficacy Analysis

The % exact match (agreement) of the ACR | U.S. compared to the comparator device, for the same Albumin-Creatinine Ratio concentration (block) will be calculated. The % ±1 block match (agreement) of the ACR | U.S. compared to the comparator device, for the same Albumin-Creatinine Ratio concentration (block) will also be calculated.

5. Usability Analysis

The usability of the ACR | U.S. will be determined by evaluating the percentage of study subjects who are able to complete the device-related tasks, including accessing the ACR | U.S. App, providing a urine sample in the urine receptacle, dipping the kit urine strip in the urine specimen, positioning the strip on

the Color-Board and taking a picture using the smartphone App, with minimal attempts to ask for assistance.

Measurable usability criteria for specific, critical steps, such as time-to-completion, number of requests for assistance, numerical ratings, etc., will be evaluated by observer evaluation and user questionnaire responses. Descriptive statistics will be provided for usability criteria.

6. Adverse Event

In general, there are no anticipated adverse events in this study; however, if any adverse events do occur they will be recorded and presented in tables.

13. RISK / BENEFIT ANALYSIS

13.1. Risks

The risks to patients resulting from potential device hazards have been analyzed using the Risk Management Standard - ISO 14971. The different types of hazards were identified and evaluated using risk assessment numerical parameters. Applicable controls for the risks were analyzed. After the implementation of appropriate risk control measures, the level of risk was re-evaluated and found to be acceptable. The risks associated with the device are minimal if any. Below is a summary of the risks to the subject and the applicable control measures.

Category	Identified Risk	Mitigation Plan
Device components	Urine receptacle material may cause irritation if it comes in contact with the skin	The urine receptacle is manufactured from a combination of FDA food grade approved materials: Polypropylene and Thermo Plastic Elastomer (TPE) in over-molding / core injection process. The materials will also be subjected to biocompatibility testing in a certified lab according to the ISO 10993 standard
Design	The urine receptacle is not comfortable for collecting urine or for immersing the urine strip	The urine receptacle has been designed taking into consideration human factors analysis. The design is such that the urine will flow into the cup and avoid splashing. The cup is designed so that the user can immerse the urine strip easily in one quick insertion
Technical	The Color-Board gets wet and damaged	The Color-Board is manufactured from sturdy carton material, and is designed for one-time use
Usability	The user places the urine strip in the wrong position on the Color-Board pad	The device App will instruct the subject to reposition the urine strip

These risks following the mitigation are considered minimal, if not negligible.

13.2. Benefits

There are no immediate benefits to the subject from their participation in the study. The results of the study may provide valuable information and data towards their eventual benefit from the device as a new, home-use urine analyzer device.

14. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies is crucial for the protection of patients, investigators and the sponsor, and are mandated by regulatory agencies worldwide.

14.1. Definitions

Adverse Event Definitions and Classifications

- **Adverse Event**

An adverse event is any untoward medical occurrence in a clinical study patient. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of an investigational product, whether or not it is related to the investigational product. (Definition per International Conference on Harmonization [ICH])

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

- **Serious Adverse Event**

A serious adverse event as defined by ICH is any untoward medical occurrence that meets any of the following conditions:

  - Results in death
  - Is life-threatening
  - Requires inpatient hospitalization
  - Results in persistent or significant disability/incapacity, or
  - Is a congenital anomaly/birth defect

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any adverse event is considered a serious adverse event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

- **Unlisted (Unexpected) Adverse Event**

An unlisted adverse event, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or the package insert/summary of product characteristics for an approved product) (ICH)

- **Associated with the Use of the Device**

An adverse event is considered associated with the use of the device if the attribution is possible, probable, or very likely by the definitions listed below.

Relationship to Investigational Device

For all adverse events, the relationship to the study device and / or procedure will be determined by the investigator, using the following terms:

- **Probably related:**

Follows a reasonable temporal sequence from study device delivery / retrieval, and cannot be reasonably explained by known characteristics of the patient's clinical data or the surgical procedure applied.

- **Possibly related:**

Follows a reasonable temporal sequence from study device delivery / retrieval but could have been produced by the patient's clinical state or by the surgical procedures regardless of the study device.

- **Probably not related:**

Temporal association is such that the study device is not likely to have had any reasonable association with the observed event.

- **Not related:**

No relationship to study device activation is perceived.

14.2. Procedures

All Adverse Events

All adverse events will be reported from the time a signed and dated informed consent form is obtained until completion of the last study-related procedure. Events meeting the definition of serious adverse events must be reported using the Serious Adverse Event Form, including serious adverse events

spontaneously reported to the investigator within 30 days after the patient has completed the study (including post-study follow up).

All events that meet the definition of a serious adverse event will be reported as serious adverse events, regardless of whether they are protocol-specific assessments. All adverse events, regardless of seriousness, severity, or presumed relationship to study treatment, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "Upper respiratory infection"). Investigators must record in the CRF their opinion concerning the relationship of the adverse event to study treatment. All measures required for adverse event management must be recorded in the source document and reported according to sponsor instructions.

The sponsor assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The investigator (or sponsor where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

Serious Adverse Events

All serious adverse events occurring during clinical studies must be reported to the appropriate sponsor contact person by investigational staff within 24 hours of their knowledge of event.

Information regarding serious adverse events will be transmitted to the sponsor using the Serious Adverse Form, which must be signed by a member of the investigational staff. The initial report of a serious adverse event may be made by facsimile (fax), e-mail or telephone. It is preferable that serious adverse events are reported via fax or e-mail. Subsequent to a telephone report of a serious adverse event, a Serious Adverse Event Form must be completed by the investigational staff and transmitted to the sponsor within one working day.

All serious adverse events that have not been resolved by the end of the study, or that have not been resolved upon discontinuation of the patient's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value is available
- The event can be attributed to agents other that the study treatment or to factors unrelated to the study conduct

- When it becomes unlikely any additional information can be obtained (i.e. patient or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

The cause of death in a clinical study, whether or not the event is expected or associated with the investigational agent, is considered a serious adverse event. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a patient's participation in a clinical study must be reported as a serious adverse event, except hospitalization for:

- Pre-planned hospitalizations, i.e. before enrollment into the study and which are not related to the disease itself
- Social reasons in absence of an adverse event
- Surgery or procedure planned before entry into the study (must be documented in the CRF)

15. ETHICAL ASPECTS

15.1. Study-Specific Design Considerations

Only patients who have the capacity to provide informed consent are allowed to enroll in the study. As part of the screening of patients for entry into the study, the investigator will assess each patient's ability to provide informed consent for participation in the study.

The protocol includes strict requirements to ensure adequate protection of all patients participating in the study, including:

- Patients will be carefully screened using medical history before enrollment. Those who are judged to be at a high risk for adverse events will be excluded.
- Patients may withdraw their consent at any time without having to give a reason.
- Patients are fully informed as to the risks of study participation and will be provided with any new information about the study testing that might become available during their participation in the study.
- Informed consent is obtained from patients without undue enticement. Patients will not be coerced in any way to participate in this study. Excessive financial compensation will not be offered to patients or to investigators.

15.2. Regulatory Ethics Compliance

- Investigators Responsibilities

The investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical study data are credible.

- Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator will provide the IEC/IRB with current and complete copies of the following documents:

- Final protocol and if applicable, amendments
- Informed consent form (and any other written materials to be provided to the patients)
- Investigator's Brochure (or equivalent information) and amendments
- Patient recruiting materials, if applicable
- Information on compensation for study-related injuries or payments to patients for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for patients
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after IEC/IRB has given full approval of the final protocol, amendments (if any), and the informed consent form, applicable recruiting materials, and after the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the documents being approved.

During the study, the investigator will send the following documents to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments
- Revision(s) to informed consent form and any other written materials to be provided to patients
- If applicable, new or revised patient recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to patients for participations in the study, if applicable
- Investigator's Brochure amendments or new edition(s)
- Summaries of the status of the study (at least annually or at intervals stipulated in guidelines of the IEC/IRB)
- Reports of any serious adverse events
- New information that may adversely affect the safety of the patients or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the patients
- Report of death of patients under investigator's care
- Notification if new investigator is responsible for the study at the site
- Any other requirements of the IEC/IRB

For protocol amendments that increase patient risk, the amendments and applicable informed consent form revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s)

At least once a year the IEC/IRB will be asked to review and re-approve this clinical study. This request and approval should be documented in writing.

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

- Informed Consent

Each patient must give written consent according to local requirements after the nature of the study has been fully explained.

The consent form must be signed before performance of any study-related activity. The consent form used must be approved by both the sponsor and by the reviewing IEC/IRB. The informed consent should be in accordance with principles set forth in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before entry into the study, the investigator or an authorized member of the investigational staff must explain to potential patients the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort it may entail. Patients will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care they patient will receive for the treatment for his/her disease. Patients will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. Finally, they will be told that the investigator will maintain a patient identification register for the purposes of long-term follow up if needed and that their records may be accessed by health authorities without violating the confidentiality of the patient, to the extent permitted by the applicable law(s) or regulations. By signing the informed consent form the patient is authorizing such access, and agrees to be re-contacted after the study's completion, by health authorities and authorized sponsor staff, for the purpose of obtaining consent for additional safety evaluations if needed.

The patient will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by name of the patient, patient's signature and date of signature. After having obtained the consent, a copy of the informed consent form must be given to the patient.

If the patient or legally acceptable representative is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written explanations) and should personally date and sign the inform consent form after the oral consent of the patient or legally acceptable representative is obtained.

- Privacy of Personal Data

The collection and processing of personal data from patients enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study. This data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

16. ADMINISTRATIVE REQUIREMENTS

16.1. Protocol Modifications

The investigator will not modify this protocol without a formal amendment, if applicable. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the patients in which case the amendment must be promptly submitted to the IEC/IRB and the relevant competent authority. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

The investigator or other physician in attendance will contact the appropriate sponsor representative by fax or telephone regarding any situations requiring a departure from the protocol. If possible, contact will be made before implementing any departure from the protocol. In all cases contact with the sponsor must be made as soon as possible in order to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF will reflect any departure from the protocol.

16.2. Regulatory Documentation

- Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

- Required Pre-study Documentation

The following documents must be available and maintained during the study:

  - Approved Study Protocol and amendment(s)
  - A copy of the dated and written IEC/IRB approval of the protocol, amendments, informed consent form, any recruiting materials and if applicable, patient compensation programs. This approval must clearly identify the specific protocol by title and number.
  - Regulatory authority approval or notification, if applicable
  - Documentation of investigator qualifications (e.g., curriculum vitae)
  - Completed financial disclosure form
  - Signed and dated clinical trial agreement, which includes the financial agreements
  - Other documentation required by local regulations

- Patient Identification Register and Patient Screening Log

The investigator agrees to complete a patient identification register to permit easy identification of each patient during and after the study.

The patient identification register will be treated as confidential. To ensure patient confidentiality, no copy will be made. All reports and communications relating to the study will identify patients by initials and assigned number only.

The investigator will also complete a patient-screening log, which reports all patients who were seen to determine eligibility for inclusion in the study.

- eSource Completion

All data relating to the study will be recorded in electronic source documents. Data will be entered into eSource in English. The eSource are to be completed at the time of the patient's visit, so that they always reflect the latest observations on the patients participating in the study.

The investigator must verify that all data entries in the eSource are accurate and correct.

- Monitoring Plan

Once the clinical site begins enrolling participants for the study, monitoring will be conducted to verify that the study is conducted in accordance with the IVDR, ISO 20916:2019 and any other applicable requirements. During routine monitoring it will be verified, among other issues, that:

- The IVD is used according to CPSP or instructions for use
- The IVD is available and IVD accountability is performed accurately
- Study records are correct, complete, and up to date
- Safety documenting and SAE reporting is done appropriately to country legislation; and:
- Data protection regulations such as the General Data Protection Regulation (GDPR) are respected.

Activities conducted as well as findings and observations will be documented in a monitoring report.

- Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eSource and all source documents that support the data collected from each patient, as well as all study documents as specified in ICH/GCP Section 8, Essential, Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational products. These documents will be retained for a longer period if required by regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator must permit access to such report.

16.3. Device Accountability

All study devices and products will be administered according to the IRB approved protocol to ensure that:

- The device is stored in a secure location with access limited to the essential research personnel
- The device is used only in patients that consented to participate in the trial
- Records related to inventory, lot numbers, quantities in stock, and expiration dates for all study IVD medical devices and products are adequately maintained, including shipping invoices, confirmation or receipt, condition upon receipt
- The storage of investigational device is in a secure

16.4. Study Completion/Termination

- Study Completion

The study is considered completed with the last patient undergoing the study.

- Study Termination

The sponsor reserves the right to close the investigational site or terminate the study at any time. The investigational site will be closed upon study completion. Reasons for the early closure of an investigational site or termination of the study may include but are not limited to:

- Safety concerns
- Sufficient data suggesting lack of efficacy
- Inadequate recruitment of patients by the investigator

17. APPENDICIES

17.1. STUDY PROTOCOL AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study treatment and the conduct of the study.

_____

Investigator's Signature Date (Day Month Year)

_____

Name of Investigator (Typed or Printed)

_____

_____

_____

Institution and Address*

_____

Telephone number*

_____

Sponsor's Representative Signature Date (Day Month Year

_____

Name of Sponsor's Representative (Typed or Printed)

_____

_____

_____

Sponsor Address*

_____

Telephone number*

* If the address or telephone number of the investigator changes during the course of study, written notification will be provided by the investigator to the sponsor and will not require protocol amendment(s).

18. REFERENCES

[1] Simerville JA, Maxted WC, Pahira JJ. "Urinalysis: a comprehensive review". American Family Physician. 2005; 71(6) 1153-62

[2] Albumin-to-creatinine ratio (ACR) is the first method of preference to detect elevated protein, the National Kidney Foundation's (NKF), https://www.kidney.org/kidneydisease/siemens_hcp_acr

[3] Chronic Kidney Disease. Henry Ford Health System. Version 7, 2015.

[4] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K173327.pdf

[5] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K182384.pdf

[6] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K142391.pdf

[7] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K152835.pdf

[8] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K141874.pdf

[9] Chronic kidney disease awareness, screening and prevention: rationale for the design of a public education program, Allan J Collins et al., Nephrology (Carlton) . 2010 Jun;15 Suppl 2:37-42.

[10] See screening guidelines of different associations -

- **NKF** ("reference for kidney disease screening")
- **RPA** ("U.S. Preventative Services Taskforce - Recommendation on Screening for CKD")
- **ISN** and **KDIGO** ("Early Identification & Intervention Toolkit")
- **ADA** (Screening for Kidney Disease in Adults with Diabetes, Diabetes Care 2005 Jul; 28(7): 1813-1816).

[11] Risk factors for chronic kidney disease: an update, Rumeyza Kazancioğlu, Kidney Int Suppl (2011), 2013 Dec;3(4):368-371.

[12] UpToDate: Early detection of chronic kidney disease, https://www.uptodate.com/contents/early-detection-of-chronic-kidney-disease

[13] Clinical Laboratory Standards Institute (CLSI). Urinalysis; approved guideline -third Edition. CLSI document GP16-A3. Wayne, PA, USA: CLSI, 20

A Comparative, Controlled Study to Evaluate the Clinical Accuracy and Usability Performance of the ACR | U.S. Urine Analysis Test System in the Lay User Hands

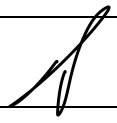
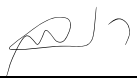
Protocol ACR-US-MCU-04

Issue day: January 18, 2022

Document No.: ACR-US-MCU-04

Version No.: 1.0

APPROVALS:

Name	Position	Date	Signature
[redacted]	Clinical Product Lead	January 18, 2022	
[redacted]	VP Product	January 18, 2022	

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ABBREVIATIONS & DEFINITIONS

ACR – Albumin to Creatinine Ratio

AE – Adverse Event

eSource – Electronic source documents

GCP – Good Clinical Practice

IC – Informed Consent

ICH – International Conference on Harmonization

IEC – Independent Ethics Committee

IEC/IRB – Independent Ethics Committee/Institutional Review Board

IVD – In-Vitro Diagnostic

CKD – Chronic Kidney Disease

Optima – URiSCAN Optima Urine Analyzer

The following protocol is a Clinical Performance Study Plan (CPSP per IVDR 2917/746) and describes the rationale, objectives, design and proposed analysis, methodology, monitoring, conduct and record-keeping of the ACR | U.S. Urine Analysis Test System in the lay user hands.

TABLE OF CONTENTS

1. STUDY SYNOPSIS

Name of Device:	ACR | U.S. Urine Analysis Test System (henceforth ACR | U.S.)
Device Description:	The ACR | U.S. Urine Analysis Test System is an in-vitro diagnostic, home-use device for the semi-quantitative measurement of the Albumin-Creatinine Ratio (ACR). The device consists of a smartphone application, proprietary Color-Board and an ACR reagent strip. The device is available for prescription-use only. Results are intended to be used in conjunction with clinical evaluation as an aid in the assessment of kidney function.
Patient Population:	At least 50 male and female subjects, 18-80 years of age, that represent the intended use population of the ACR | U.S. device, including high-risk subjects with a known medical condition and/or risk factor for kidney damage that can potentially present as an abnormal concentration of urine albumin, such as Diabetes (Type I/Type II, or gestational diabetes), Hypertension, Cardiovascular Disease, Family History of Kidney Disease and History of Acute Kidney Injury (AKI), as well as subjects with other medical conditions and/or risk factors that impact kidney function (e.g. older age, smoking, obesity, dyslipidemia, inherited kidney disease, malignancies, recurrent kidney infections, and more). In addition, subjects with no known significant medical conditions.
Structure:	A comparative, controlled, accuracy and usability study.
Objectives:	To evaluate the accuracy and usability performance of the ACR | U.S. in the hands of the potential lay user.
Primary Endpoint:	The degree of agreement of the ACR | U.S. (tested by a lay user) as compared to the URiSCAN Optima Urine Analyzer (tested by a healthcare professional), for the different concentrations (blocks) reported by the ACR | U.S. device.
Secondary Endpoint:	Evaluation of the ACR | U.S. usability, by potential lay user under actual use conditions of a simulated home environment.
Study Design:	Potential candidates for the study will be recruited at the designated clinical site by the study personnel. Following subject consent on site, the subjects will be evaluated for eligibility based on their health condition and medical history. The ACR | U.S. kit in its original packaging, along with a mobile phone (iPhone 11) with the pre-installed ACR | U.S. application, will be provided to the subject in a simulated home- use environment. Each subject will follow an in-app instructional guide in order to perform the tasks required to complete the test (e.g. operate the ACR | U.S. app, provide a urine sample, use a dipstick, take a scan, etc.). Following each test performed by a lay user, the urine sample will be tested by a professional study staff using the comparator device – the URiSCAN Optima Urine Analyzer. These results will be considered as the "true value". A designated study operator will complete a questionnaire during each test to collect measurable usability information and

	evaluate the critical steps in the study. Each subject will complete a post-test usability questionnaire in order to assess the usability of the ACR | U.S. device.
Data analysis:	Results from the ACR | U.S. Urine Analysis Test System and the comparator device, the URiSCAN Optima Urine Analyzer, will be presented in tables listing the results in each group and the % exact match (percent agreement) and the % ±1 block match (percent agreement). Additionally, measurable usability criteria for specific and critical steps, such as time to completion of task, number of requests for assistance, numerical ratings, etc., will be evaluated by the operator and user questionnaire responses.
Study Sponsor:	Healthy.io Ltd.
Principal Investigators:	

2. INTRODUCTION

2.1. Urinalysis and Albumin-to-Creatinine Ratio Overview

Urinalysis is an array of tests performed on urine, and one of the most common methods of medical diagnosis. The target parameters that can be measured or quantified in a urinalysis test include many substances and cells, as well as other properties, such as specific gravity and pH. Urinalysis can be performed by using urine test strips, in which the test results can be measured on the basis of color changes. Other methods of urinalysis include light microscopy[1].

An Albumin-Creatinine Ratio (ACR) urine test strip is a basic diagnostic tool used to determine pathological changes in a patient's urine in standard urinalysis. A standard ACR urine test strip usually contains two different chemical pads or reagents which react (change color) when immersed in, and then removed from, a urine sample. The test can often be read after 60 seconds after immersion. Routine testing of the urine with ACR urine test strips is the first step in the diagnosis of a wide range of kidney diseases. The analysis includes testing for the Albumin and Creatinine, and then calculating the ratio between them.

Albumin is the single-most important protein that is pathologically present in the urine in most chronic kidney diseases. A properly-functioning kidney will permit extremely small amounts of Albumin to pass into urine, and thus even a minute presence – defined as a quantity greater than 30 mg/L – is a sign of a diseased or distressed kidney. Creatinine is a byproduct of muscle metabolism released into the urine at a constant rate; its level in urine is an indication of urine concentration. The Albumin-to-Creatinine Ratio measurement is a widespread and effective screen for the early stages of CKD[2], in most instances appearing before the reduction in the estimated glomerular filtration rate (or eGFR, which requires a blood test).

ACR urine test strips can be used in many areas of the healthcare chain including screening for routine examinations, treatment monitoring, self-monitoring by patients and/or general preventive medicine. The ACR urine test is an effective measurement to identify patients with early stages of Chronic Kidney Disease (CKD), when patients are often asymptomatic, which can reduce the risks of cardiovascular disease, End-Stage Renal Failure (ESRF), and death that are the hallmarks of a malfunctioning kidney. According to the Henry Ford Health System, only 5% of the general Medicare population undergoes a

[1] Simerville JA, Maxted WC, Pahira JJ. "Urinalysis: a comprehensive review". American Family Physician. 2005; 71(6) 1153-62

[2] "Albumin-to-creatinine ratio (ACR) is the first method of preference to detect elevated protein." See the National Kidney Foundation's website detailing the importance of the ACR test: https://www.kidney.org/kidneydisease/siemens_hcp_acr

screening urinalysis, while only 2% of high-risk subjects screened through a National Kidney Foundation initiative self-reported a history of kidney disease.[3]

Healthy.io has developed the ACR | U.S. Urine Analysis Test System (ACR | U.S.). The ACR | U.S. is a semi-quantitative, prescription-based, in vitro diagnostic (IVD) home use device for the measurement of the ratio between albumin to creatinine in a urine sample using a single-wrapped ACR urine reagent strip (microalbumin/creatinine).

Automatic analysis of urine test strips using automated urine test strip analyzers is a well-established practice in modern day urinalysis. It guarantees rapid, standardized measurement and immediate reliable documentation of the result. Most of these urine analyzers are based on the technology of reflectance photometry while additional technologies such as microscopy or flow cytometry exist (performed directly on the urine specimen).

The basic elements of the reflectance photometry are based on the analysis of the intensity and color of light reflected from the reagent areas on a urinalysis test strip. Most of the automated urine analyzers are specifically intended for lab environments and are operated by professional users. In recent years, following the tremendous development in communication media (especially with mobile media) there is a shift from "in-clinic" testing to the home-use self-testing. This trend enables manufacturers to develop cost effective, reliable, easy-to-use, self-monitoring test methods, including urinalysis methods.

Healthy.io Ltd. employs computer vision technology to turn smartphones into clinical-grade diagnostic devices. Previously, the company developed a smartphone application containing image recognition software that enables users to remotely and independently perform a urinalysis test, the DIP | U.S. (510(k) clearance number: K173327)[4]. The DIP | U.S. device is a prescription, home-use device intended for the analysis of six urine analytes. The company also developed a point of care, prescription-only, professional-use smartphone application to test for microalbuminuria, the ACR | LAB device (510(k) clearance number: K182384)[5]. Building off this experience, Healthy.io developed the ACR | U.S. to test for the Albumin-Creatinine Ratio. Further details on the device components and functionality are described in the device description section below.

[3] Chronic Kidney Disease. *Henry Ford Health System*. Version 7, 2015.
[4] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K173327.pdf
[5] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K182384.pdf

3. DEVICE DESCRIPTION

3.1. General

The ACR | U.S. is a prescription, in-vitro diagnostic, home-use device for the semi-quantitative measurement of the Albumin-Creatinine Ratio. The device comprises a kit with a urine receptacle, an ACR urine test strip, an absorbing pad, a Color-Board and a user manual. The device also consists of an easy-to-use smartphone application and image recognition algorithm.

The ACR | U.S., while also a photometric based device, employs a computer vision technique, executed on various hardware platforms, in an uncontrolled lighting environment, used by an untrained user. The test has no pre-calibration step. The calibration and test are executed simultaneously during each test individually. This is executed via a Color-Board, which is part of the user kit. Performing test calibration eliminates lighting variances & camera disruptions and enable delivery of clinically valid semi-quantitative measurements.

The device consists of seven elements, which have been designed for easy usage in a home setting:

1. Urine receptacle (i.e. urine cup)
2. ACR urine test strip (i.e. reagent strip, dipstick)
3. Absorbing pad (i.e. blotting pad)
4. Color-Board
5. Smartphone application
6. Software algorithms
7. User manual

The kit components are shown in ***Figure 1***. An outline of the main components is described below.

Figure 1: ACR | U.S. Device Components

3.2. Device Components Outline

1. Urine Receptacle

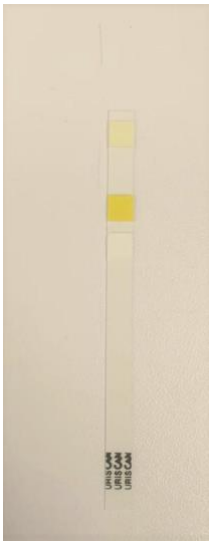
The urine receptacle is comprised of a plastic cup which holds the subject's urine. It is designed to contain the sufficient amount of urine needed for the test and allow easy immersion of the test strip along its entire length. **See *Figure 2.***

Figure 2: Urine Receptacle

2. ACR Urine Test Strip

A single-wrapped ACR urine reagent strip (microalbumin/creatinine). The strip consists of 3 chemical reagents, which change color after being immersed in a urine sample. One patch for albumin, another for creatinine and an additional compensation patch. **See *Figure 3.***

Figure 3: Urine Test Strip



3. Absorbing Pad

A single piece of standard absorbing material. It is designated to assist in absorbing remaining liquids from the strip after it has been immersed in the urine sample.

4. Color-Board

A cardboard-based, high-quality printed Color-Board facilitates the image recognition and calibration processes. Its main features include proper positioning of the urine strip in the frame, neutralization of the surrounding lighting and/or camera interruptions and providing a reference color spectrum for the strip reagent colors for later data algorithm analysis. **See *Figure 4.***

Figure 4: Color-Board

5. Smartphone Application

The smartphone application will be tested using a iPhone 11 smartphone (iOS) and is designed to enable the following:

- Device interface instructions for the user to ensure proper performance of the urinalysis test, avoiding key human errors. These include interactive video texts and sound instruction to guide the users through the testing procedure.
- The software includes an augmented reality layer on the smartphone camera which guides users while taking the scan. This is a real-time analysis of the scan, which finds and centers the Color-Board within the camera frame. The software will also analyze the scan and evaluate if it meets

the system boundary conditions for urinalysis measurement. If not, the software will instruct the user to take another scan which can be analyzed.

- Presentation and interpretation of the ACR analysis
- Secured transmission of urinalysis measurement result to a clinician for viewing and interpretation.

See *Figure 5* (next page).

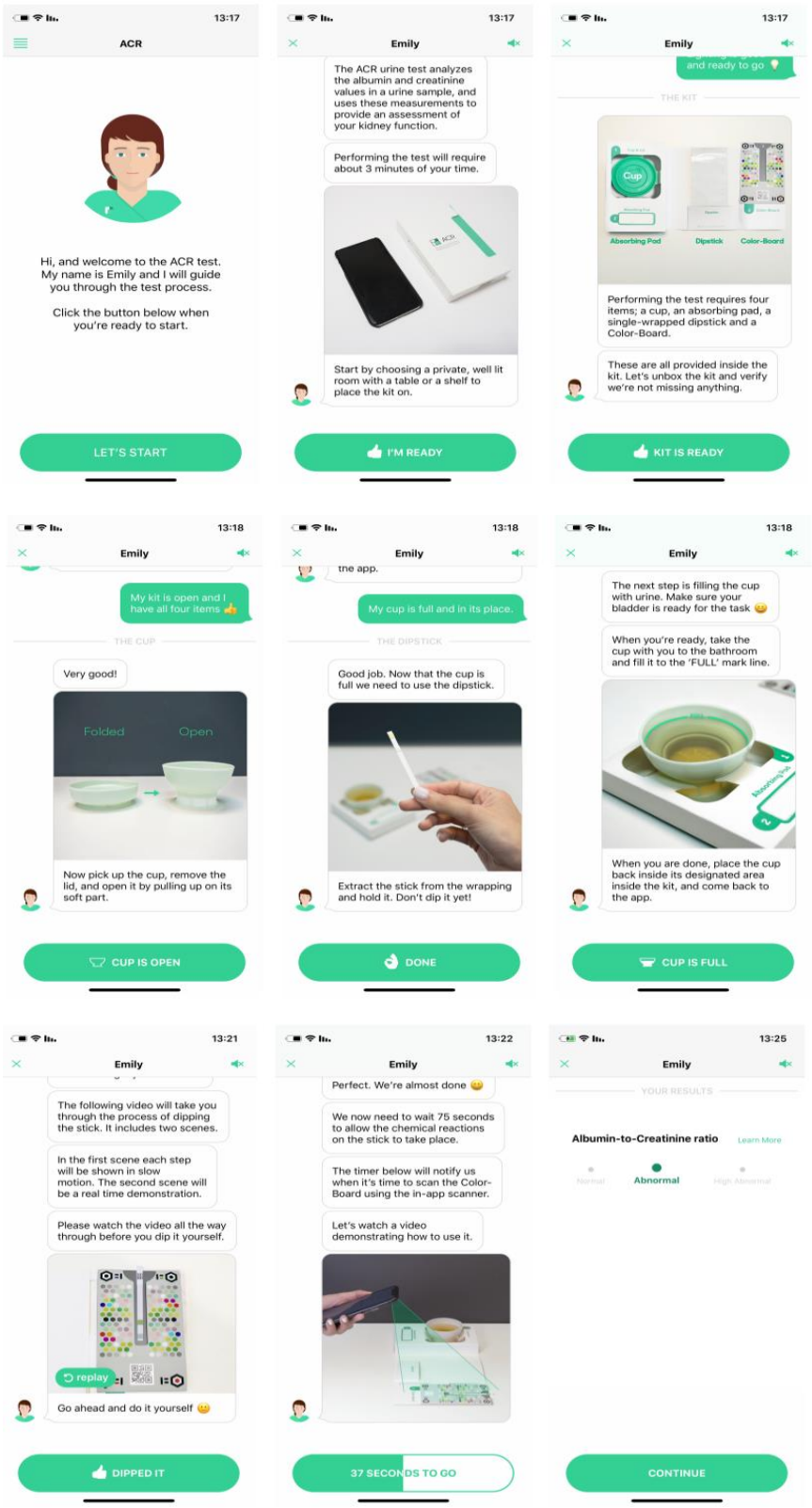
6. Software Algorithms

The ACR | U.S. smartphone algorithm is a cloud-based, image-processing system. Both albumin and creatinine analytes are analyzed based on a separate, unique set of eight target colors and a set of calculations which are completely independent from each other. Once these two measurements are taken, the algorithm on the cloud will use these two numbers to calculate the subsequent Albumin-Creatinine Ratio, which is measured in units of mg/g or mg/mmol.

3.3. Intended Use

The ACR | U.S. Urine Analysis Test System is an in-vitro diagnostic, home-use device for the semi-quantitative measurement of the Albumin-Creatinine Ratio (ACR). The device consists of a smartphone application, proprietary Color-Board and an ACR reagent strip. The device is available for prescription-use only. Results are intended to be used in conjunction with clinical evaluation as an aid in the assessment of kidney function.

Figure 5: Smartphone Application Screens



4. PERFORMANCE TESTING AND CLINICAL EVALUATION

4.1. ACR | U.S. Performance Tests

Healthy.io has designed a series of bench-testing experiments to verify the performances of the ACR | U.S. These tests have been designed according to the guidance provided by the Clinical and Laboratory Standards Institute (CLSI).

- **Precision:** Comprises two separate tests - Repeatability and Reproducibility. The precision studies are designed in accordance with guidance provided by Clinical and Laboratory Standards Institute document EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
    - Repeatability is defined as the closeness of the agreement between results of successive measurements of the same measure and carried out under the same conditions of measurement.
    - Reproducibility is defined as closeness of the agreement between the results of measurements of the same measure carried out under changed conditions of measurement over the course of multiple days.
- **Interference:** Testing of potential interfering substances with the ACR | U.S. was designed in accordance with guidance provided by the Clinical and Laboratory Standards Institute document EP07 – Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition.
- **Limit of Detection:** Testing of the ACR | U.S. assay's detection was designed in accordance with guidance provided by Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.
- **Linearity:** Testing the linearity of the ACR | U.S. was designed in accordance with guidance provided by the Clinical and Laboratory Standards Institute (CLSI) document *EP06-A – Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*
- **Stability:** The stability experiment is designed to evaluate the environmental conditions under which the ACR | U.S. is suitable for its intended use. This study was designed with the guidance provided by the Clinical and Laboratory Standards Institute (CLSI) document *EP-25A Evaluation of In-Vitro Diagnostics Reagents; Approved Guideline.*

- **Additional Studies**:
    - Illumination Study: Healthy.io designed an experiment to test the ACR | U.S. under multiple lighting conditions.
    - Boundary Study: Healthy.io designed an experiment to test the physical boundaries (such as the phone's distance and angle in relation to the Color-Board) which would prevent the ACR | U.S. from capturing an accurate image that can be evaluated by the algorithm.
    - Multiple Phones and Operating Systems Study: Healthy.io designed an experiment to test the ACR | U.S. with multiple smartphone devices and operating systems versions (iOS and Android).
    - Timing Flex Study: Healthy.io designed an experiment to test the impact of different dipping times, assay-times, and strip wetting times on the ACR | U.S.

4.2. Previous ACR Urinalysis Method Comparison Studies:

The following table (***Table 1.0***) summarizes the method comparison studies conducted on several marketed lab urine analyzers and test strips with similar indications for use as the ACR | U.S.

The key difference being that these studies were operated by lab professionals while the ACR | U.S. is intended for lay users who are operating the device at home.

Table 1.0: Urinalysis analyzers method comparison studies summary:

Study details	Mission U120 Ultra Urine (K142391)[6]	Uritek TC-201 Urine Analyzer (K152835)[7]	URiSCAN Optima Urine Analyzer (K141874)[8]
No. of urine samples	429 samples	402	351
Urine sources	Fresh	Fresh	Fresh

[6] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K142391.pdf
[7] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K152835.pdf
[8] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K141874.pdf

Study details	Mission U120 Ultra Urine (K142391)[6]	Uritek TC-201 Urine Analyzer (K152835)[7]	URiSCAN Optima Urine Analyzer (K141874)[8]
Percentage of Contrived Samples Used	12-13%	9%	0%
Subjects medical history	From 510k summary: "Test results may be used in screening urine specimens for microalbuminuria as an aid in the detection of patients at risk for developing kidney damage." From User Manual: "Patients with the highest risk of developing early kidney damage are those with diabetes and hypertension, followed by patients who have immune disorders or have been exposed to nephrotoxins."	From the 510K summary: "Test results may be used in screening urine specimens for microalbuminuria as an aid in the detection of patients at risk for developing kidney damage."	From the 510K summary: "These measurements are useful in the evaluation of renal, urinary and metabolic disorders."
Compared predicate	Clinitek Status Analyzer (K031947)	Clinitek Status+ Analyzer (K091216)	Clinitek Status (Siemens) (K091216)
No. of strip lots	3	3	3

5. RATIONALE FOR THE STUDY

The rationale for the study is to evaluate the performance of the ACR | U.S. Urine Analysis Test System in the hands of the potential intended-lay-user in a simulated home environment.

The ACR | U.S. Urine Analysis Test System is classified as an in-vitro diagnostic device. According to the regulatory requirements, such devices undergo several analytical performance tests. These tests performed on "ground truth" samples, provide data on the device precision, repeatability, reproducibility, linearity, and effect of substances in the urine, while compared to a known validated reference or predicate test method. These tests were performed as described in the previous section.

In addition to the analytical performance tests described above, method comparison and usability tests are also a crucial step before companies are permitted to market such a device. All marketed urinalysis devices and urine test strips for home use have performed method comparison and usability studies tested by the intended lay user. Normally in these studies, fresh urine samples are collected and tested by a lay user, using the new home use device, and by a device operator or lab professional using the comparison device. The lay user test results are compared to the results obtained by testing the same urine sample on the comparison device. The method comparison and usability studies provide data on the accuracy and usability of the device, including the lay user's ability to understand and implement the user manual instructions and evaluate the ease of use of the device under actual use conditions (home environment).

Furthermore, the study provides valuable data collected from subjects representing the intended use population of the device, which includes subjects with a relevant medical condition and/or risk factor for kidney damage that might be reflected in a pathological urine sample. Thus, it enables the company to collect a wide range of analyte values, cover the entire range of the method, and strengthen the results of the bench analytical test.

The ACR | U.S. method comparison study should include similar elements and be designed similarly to previously tested urinalysis analyzers. The study design is meant to demonstrate that the ACR | U.S. results are in agreement with another legally marketed urine analyzer device (the URiSCAN Optima Urine Analyzer) and may be easily used by a lay person.

6. OBJECTIVES

6.1. General

The aim of the study is to establish the validity and performance of the ACR | U.S. Urine Analysis Test System in the hands of the potential lay user, by evaluating the accuracy and usability performance as compared to the comparator device.

6.2. Primary Objective

The primary objective of the study is to evaluate the % exact match (percent agreement) and the % ±1 block match (percent agreement) of ACR | U.S. (tested by the lay user) compared to the URiSCAN Optima Urine Analyzer (the comparator device, tested by a healthcare professional), for each tested concentration (block).

6.3. Secondary Objective

The secondary objective of the study is to evaluate the ACR | U.S. usability, by potential lay users under actual use conditions (home environment).

7. STUDY POPULATION

7.1. General Considerations

The study population should represent the intended use population of the ACR | U.S. device and include subjects that are likely to, or will derive benefit from assessing their kidney health.

Early identification of CKD by targeted testing based on known risk factors, offers the potential to substantially reduce morbidity and mortality from CKD and its related complications. The results of screening can inform treatment modifications, such as appropriate drug dosing and improved lifestyle changes and can slow or even halt the progression of kidney damage. [9]

The guideline-based testing for kidney disease varies across different populations as it takes into account local policies, resource limitations, payment systems, and care delivery models. [10]

In general, initial efforts for early testing should target high-risk subjects with well-established risk factors for kidney damage, such as those with Diabetes (Type I, Type II, or gestational diabetes), Hypertension, Dyslipidemia, Cardiovascular diseases, Family history of kidney disease, History of acute kidney injury (AKI). [11, 12]

In addition, CKD testing should be implemented in other high-risk groups with other medical conditions that impact kidney function. Risk factors include, but are not limited to: older age (> 60, smoking, obesity, inherited kidney disease (e.g. Polycystic kidney disease), prolonged obstruction of the urinary tract from different conditions (e.g. enlarged prostate, kidney stones), malignancies, recurrent kidney infections, autoimmune diseases (e.g. SLE), genetic risk factors, exposure to nephrotoxins (e.g. environmental, frequent use of certain medications). [11, 12]

Overall, the study population will include at least 50 subjects from any genders, ethnicities, socioeconomic status, and education level, between 18-80 years of age, meeting the eligibility requirements. The subjects will be recruited by the study personnel of the clinical site. Informed consent will be obtained from potential subjects according to local and national IRB requirements. Potential candidates will be screened according to the following inclusion/exclusion criteria.

[9] Chronic kidney disease awareness, screening and prevention: rationale for the design of a public education program, Allan J Collins et al., Nephrology (Carlton) . 2010 Jun;15 Suppl 2:37-42.

[10] See screening guidelines of different associations -

- **NKF** ("reference for kidney disease screening")
- **RPA** ("U.S. Preventative Services Taskforce - Recommendation on Screening for CKD")
- **ISN** and **KDIGO** ("Early Identification & Intervention Toolkit")
- **ADA** (Screening for Kidney Disease in Adults with Diabetes, Diabetes Care 2005 Jul; 28(7): 1813-1816.)

[11] Risk factors for chronic kidney disease: an update, Rumeyza Kazancioğlu, Kidney Int Suppl (2011), 2013 Dec;3(4):368-371.

[12] UpToDate: Early detection of chronic kidney disease

7.2. Inclusion Criteria

- Males and Females 18-80 years of age.
- Subjects with a known, well-established medical condition and/or risk factor for kidney damage, that can potentially present as an abnormal concentration of urine albumin:
    - Diabetes (Type I/Type II, or Gestational Diabetes)
    - High Blood Pressure (i.e., Hypertension)
    - Cardiovascular Diseases
    - Family History of Kidney Disease
    - History of Acute Kidney Injury (AKI)

  Or; subjects with other medical conditions and/or risk factors that impact kidney function:
    - Dyslipidemia
    - Heavy smoking
    - Obesity
    - Inherited kidney disease (e.g. Polycystic kidney disease)
    - Prolonged obstruction of the urinary tract from different conditions (e.g. enlarged prostate, kidney stones)
    - Malignancies
    - Recurrent kidney infections
    - Other relevant conditions

  Or; subjects with no known significant medical conditions
- Subjects who are able to use both hands
- Subjects who are familiar with the use of a smartphone
- Subjects who are capable of comprehending and following instructions in English.

7.3. Exclusion criteria

- Subjects with a major cognitive impairment (e.g.: dementia, memory loss, severe mental disorder)
- Subjects who are not able to collect urine in a receptacle.
- Subjects who are visually impaired (i.e., cannot read the user manual)
- Any additional reason that disqualifies the subject from participating in the study according to the study physician

8. STUDY DESIGN

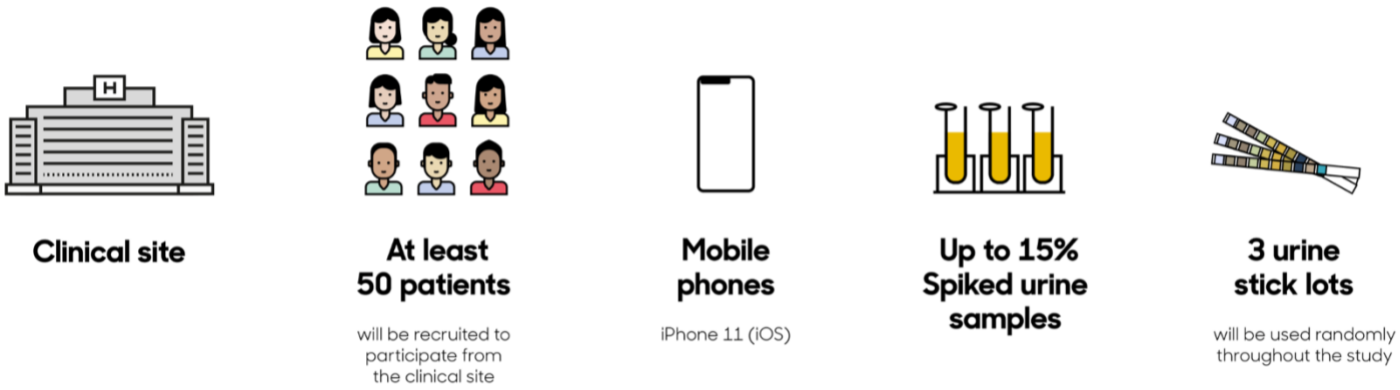
The Study is a Method Comparison clinical study using actual, fresh, human urine samples that will be collected by the lay-users in a lab-based home setting.

The study will recruit at least 50 subjects with a known medical condition and/or risk factor for kidney damage, including patients with a disease that normally represents itself with an abnormal concentration of urine albumin.

8.1. Framework

- At least 50 patients will be recruited to participate
- Urine samples will be collected mainly from subjects with a relevant background condition and/or risk factor for kidney damage, that are potential candidates for kidney screening test.
- Up to 15% spiked samples will be used if there are insufficient native samples to cover all possible reported results by the ACR | U.S device.
- Clinical research site to recruit potential candidates meeting the eligibility requirements.
- Mobile phone: iPhone 11 (iOS).
- 3 urine strip lots will be used randomly throughout the study.

Figure 5: Study Framework



8.2. Urine Samples

Fresh urine samples will be collected from subjects meeting the eligibility criteria detailed above, so that each analyte concentrations will be distributed over the entire analytical measurement range, to the extent possible to cover all possible reported results. The study might be supplemented with spiked urine samples with the missing concentrations. However, the number of spiked samples will not exceed 15% of the total

samples. Lay users will test their own personal urine and additionally may be asked to test another spiked sample, that will be provided by the study staff as necessary and in accordance to protocol.

8.3. Analyzers

Each urine sample will be tested two times using the two urine analyzer methods: the ACR | U.S. (running on iPhone 11) and the URiSCAN Optima analyzer (the comparator device).

8.4. Reagent Strips

To control for bias among the test strips, three lots of reagent strips will be used during the study. To enable an equal and unbiased distribution of the lots, one lot will be randomly selected by the professional user for the testing of each urine sample. Separate strips will be used in each test: with the ACR | U.S. and the URiSCAN Optima analyzer.

8.5. Testing Methods

Subjects will be recruited for the study based on their ability to provide a urine sample and comfortably complete tasks using a smartphone. Each urine sample will be tested by a lay user on the ACR | U.S. app, and then transferred to a professional user to conduct the test on the URiSCAN Optima device. The professional user will be blinded to the results of the lay users until after they have completed the test using the URiSCAN Optima device.

8.6. Timing

Ideally, simultaneous sampling of each urine specimen with both methods would be performed, but since the usability of the ACR | U.S. device is tested as well, such a design is not feasible. Therefore, each urine sample will be tested sequentially with no more than 15 minutes elapsing between methods (i.e. between the end of one test and the beginning of the next). Real time differences across the two methods are not anticipated, as a time difference constituted of minutes is unlikely to affect the value obtained from a sample. Moreover, regarding specimen acceptability, the CLSI guidelines recommend urinalysis within two hours from sampling. The specimen should not be centrifuged or refrigerated in between tests.[13]

[13] Clinical Laboratory Standards Institute (CLSI). Urinalysis; approved guideline -third Edition. CLSI document GP16-A3. Wayne, PA, USA: CLSI, 2009.

8.7. Requirements

The Method Comparison Study must be conducted in accordance with Good Clinical Practice (GCP), according to ISO 20916:2019, 21 CFR Part 50, 54, 56, 812, and relevant local and national regulations (IRB or Helsinki EC approved). The Method Comparison Study shall comply with CLSI EP09c: Method Comparison and Bias Estimation Using Patient Samples; Approved Guidelines – Third Edition – In addition, it shall comply with EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices. Since the ACR | U.S. is classified as a urinalysis analyzer intended for lay users to test their urine in a home environment, the design of the device clinical comparative study should include parameters from both lab urinalysis analyzers and urine strip method comparison studies. The study design is mainly based on the above-mentioned method comparison studies in section 3.2 when used by a lay user and FDA recognized consensus standards CLSI EP09c and for usability testing of medical devices - ANSI/AAMI/IEC 62366-1:2015 Medical devices – Part 1: Application of usability engineering to medical devices.

9. STUDY PROCEDURES

9.1. General

The study consists of 6 stages as follows:

- Familiarization Period
- Screening
- Pre-testing
- Testing
- Monitoring
- Post-testing

9.2. Stage I – Familiarization Period

As outlined in the EP9 standard, evaluating an analytical method requires sufficient time for the operators to become familiar with the study protocol and device operation, therefore the standard recommends a study familiarization period. During the familiarization period, the study operators will become familiar with all aspects of set-up, operation, maintenance, quality control, and troubleshooting of both methods. This period will precede the study evaluation process and will coincide with the manufacturer's training performed during the Site Initiation Visit (SIV). In order to familiarize the study staff with the study flow and procedures, up to 15 subjects will be serve as a training period at the beginning of the site and will not be included in the final study results and statistical analysis. After the SIV and familiarization period, the study will begin.

9.3. Stage II – Screening

The screening of potential candidates will be performed by the clinical site. Potential candidates will receive complete information describing the study and their role and will be encouraged to ask any questions regarding the study. The risks and requirements of this clinical research trial will be explained to each potential subject. Those volunteering to take part will read and sign the Informed Consent Form for participation in the clinical research trial before any study-related procedures are performed. The Informed consent signing process will be in front of a study personnel, and the principal investigator (PI) will review and sign all source documents, informed consents, and results once a week. Upon obtaining the signed informed consent from each subject, inclusion and exclusion criteria will be reviewed to verify the subjects' eligibility.

9.4. Stage III – Pre-testing

Subjects who were found eligible to participate in the study and signed informed consent will be enrolled into the study and assigned a subject ID number, which will be used along with their initials for study record identification.

Designated eSource forms will be completed with the subject's general details, including demographics, medical history, diagnosis, and concomitant medications.

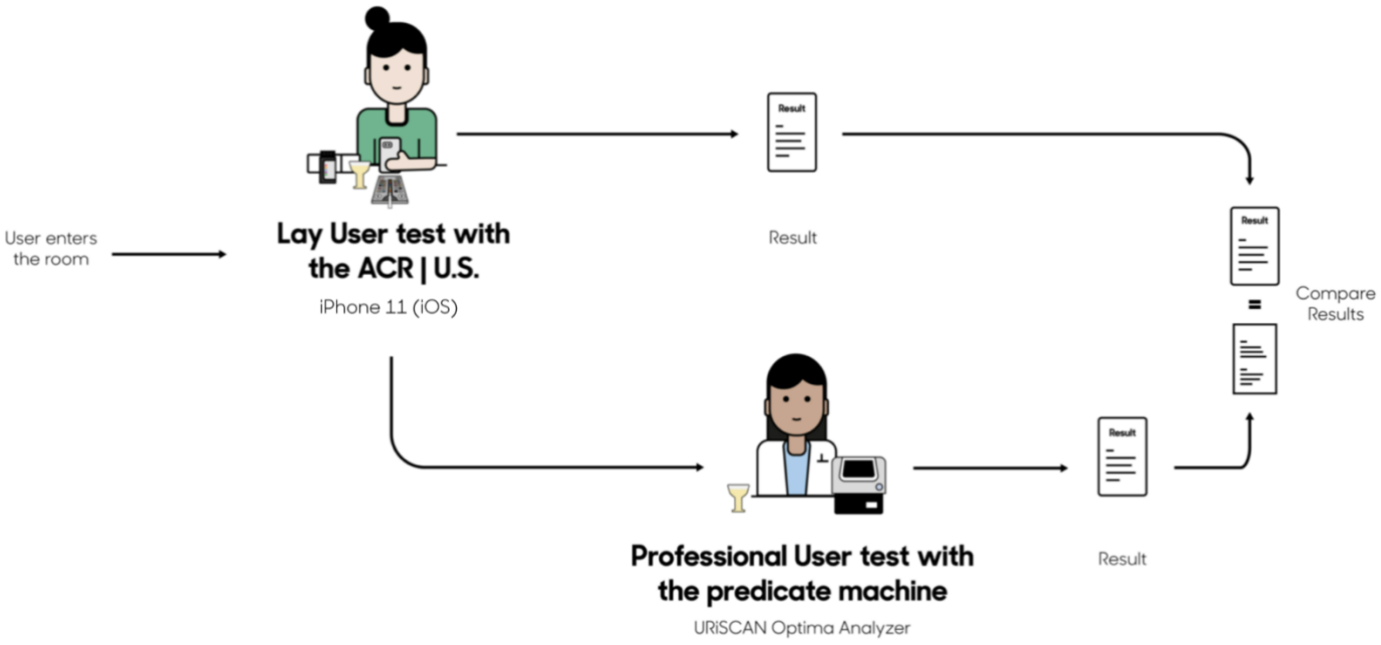
9.5. Stage IV – Testing

1) Each subject will be placed in the private room, with restroom accessibility and internet connectivity, to perform the urinalysis test and complete the task list found below. The clinic will be set up so that the self-testing will allow the subject to perform the urinalysis test without outside influence and to simulate the home-use testing of the device.
2) No training, assistance, feedback, or supplemental instructional materials, other than those described, will be provided to participants. The healthcare professional or other site staff will not be allowed to intervene or answer questions from the subjects during testing, although a 1-800 or clinical assistance phone number, which will be provided to the subject in actual use, will be made available to the subject in case contact is attempted by the subject.
3) The room will be prepared with a desk and chair. The ACR | U.S., in its original packaging, will be placed on the desk. The device packaging contains the kit components and the user manual. An in-app instructional guide containing the information most critical to safety and effectiveness is also provided in the form of a readable and understandable, visual, and textual guide on the device App. The patient labeling will be in the format intended for distribution to allow for a realistic evaluation of the labeling effectiveness.
4) The subjects will be asked to follow the device in-app instructional guide and/or user manual and perform the following tasks:
    - Access the ACR | U.S. App on the smartphone (iPhone 11).
    - Provide a urine sample in the urine receptacle (to the level marked on the cup) that is included in the ACR | U.S. kit.
    - Immerse the urine strip (provided in the ACR | U.S. kit) in the collected urine specimen for about one second.
    - Absorb any excess amount of urine from the test strip on the absorbing pad (i.e. blotting pad).

- Place the urine strip on the designated area on the Color-Board and wait at least 75 seconds, but not more than 180 seconds.
- Scan the urine strip and Color-Board with the smartphone App.
- Send the test results to the clinician using the smartphone App.

5) During each test, a study operator will observe the subject throughout the entire flow and during that time fill-out a dedicated post-test questionnaire, designed to document important key points in the process.
6) At the end of the test, each subject will be requested to complete a lay user questionnaire, designed to collect data relevant to user experience.
7) Upon completion of the usability study tasks, the study operator will use the urine sample for further testing on the comparator device, the URiSCAN Optima Analyzer. The study operator will be blinded to the results of the ACR | U.S. test, until the test with the comparison device is completed.
8) The results of both the ACR | U.S. and the URiSCAN Optima analyzer will be used for study purposes only and not for any purposes of patient management including diagnostic or therapeutic. In the case that urinalysis results are required for patient management, urine specimens from the subject will be sent independently to the hospital/clinic laboratory for analysis and only these results will be used for patient care and management. The patient will provide only one urine sample for use in the study and any subsequent clinical urinalysis testing.

Figure 6: Method Comparison Study Flow



9.6. Stage V – Monitoring

The objectives of the study monitoring are to confirm the quality and integrity of the data, to ensure that critical elements such as written ICF are reviewed for accuracy and compliance and to train or re-train the personnel on site as needed. The monitoring process will start after first patient is screened for the study and will include regular monitoring visits to the site. The monitoring visit will include review of adverse events, confirmation of protocol adherence, ICF review, eCRF review and source document verification, queries and error correction and review of lab samples. On each monitoring visit, a monitoring visit log will be recorded with the date of the activity, the individual(s) conducting and participating in the activity, a summary of the data or activities reviewed, a description of any noncompliance, potential noncompliance, data irregularities, or other deficiencies identified, and a description of any actions taken or recommended. The monitoring frequency as well as the monitoring type (i.e., onsite, remote, or central) will be determined by the risk rating allocated. Monitoring visits may be revised depending on subject enrolment rate, quality issues, site compliance or other study issues.

9.7. Stage VI – Post-testing

Statistical analyses comparing the results from the ACR | U.S. and the Optima will be performed using an appropriate statistical program and methodology.

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eSource and all source documents that support the data collected from each patient, as well as all study documents as specified in ICH/GCP Section 8, Essential, Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

A clinical performance study report, signed by a medical practitioner or any other authorized person responsible, will be written to include information on the clinical performance study protocol plan, results and conclusions of the clinical performance study, including negative findings. The results and conclusions will be transparent, free of bias and clinically relevant. The report will contain sufficient information to enable it to be understood by an independent party without reference to other documents. The report shall also include as appropriate any protocol amendments or deviations, and data exclusions with the appropriate rationale.

10. STUDY EVALUATION

10.1. Accuracy Evaluation

The ACR | U.S. test results will be compared to the URiSCAN Optima Analyzer test results. The data will be presented descriptively and graphically to compare the percent exact match (percent agreement) and the percent ±1 block match (percent agreement) between the ACR | U.S. (tested by the lay user) and the comparator device (tested by a healthcare professional), for the different tested concentrations (blocks).

10.2. Usability Evaluation

The usability of the ACR | U.S. will be determined by evaluating the percentage of study subjects able to complete the device related tasks, including operating the ACR | U.S. with minimal attempts to ask for assistance. The following measurable usability criteria for specific, critical steps, based on both observer and user questionnaire responses, will be assessed through analysis of the data:

1) The **lay-user post-test questionnaire** will be used to evaluate the participants' experience of using the ACR | U.S. Information regarding the ease of use of the ACR | U.S. test system at key stages of the test as well as information about the usefulness of the device and it's added value for the participants, will be collected through the questionnaire.
2) The **observer test questionnaire** will be used to assess the participants' success in performing the key tasks of the study (e.g., to successfully access the App on the smartphone, to properly immerse the urine strip in the urine sample, to scan the urine strip using the App, etc.), the number of requests for assistance during the test and detailed description of each request if occurred, and the time-to-completion of the test.
3) Qualitative information regarding specific user errors and inefficiencies will be collected through both observer evaluation and user post-test questionnaire responses.

The device usability information will be compared to identified device risks. Device and user related hazards were identified and documented in the ACR | U.S. Risk Analysis file. These specific device and user related risks will be evaluated in the usability study, according to the following steps:

1) Identify device and user related hazards (Risk Analysis)
2) Identify critical steps to be completed (Task List)
3) Compare device's use with identified risks (Observer Evaluation and User Questionnaire)
4) Apply Criteria - percentage of permissible failures
5) Link assessment to the overall risk analysis (study conclusions)

In addition, key system errors will be evaluated in some subjects. Examples of key errors may include the following:

1) Interruption during the test by an incoming phone call.
2) Interruption during the test by an incoming message.
3) No Internet connection.

The subject will be required to continue device operation according to the user manual.

11. SUBJECT COMPLETION / WITHDRAWAL

11.1. Completion

A subject will be considered to have completed the study if the urine sample has been tested.

A subject will be considered to have passed the usability test if he/she was able to scan the Color-Board successfully.

In case a subject fails his/her first attempt but identifies the problem alone and requests a second kit, he/she will be provided with another ACR | U.S. kit, and if the second test is completed successfully, the subject will be considered to have passed the usability test.

11.2. Withdrawal from the study

A subject will be withdrawn from the study for any of the following reasons:

1) Patient cannot provide a sufficient amount of urine for the test.
2) Withdrawal of consent.
3) The study is prematurely stopped or halted (e.g. clinical halt).
4) The investigator believes that for safety reasons (e.g. an adverse event) it is in the best interest of the patient to stop the test.

If a patient withdrawal occurs before the study is completed, the reason for withdrawal will be documented in the eSource.

12. STATISTICAL CONSIDERATIONS

12.1. Study Design and Objectives

The study is designed as a comparative, controlled, trial to evaluate the accuracy and usability performance of ACR | U.S. Urine Analyzer Test System in the hands of a potential lay user.

12.2. Study Endpoints

1. Primary Efficacy Endpoint

The primary endpoint of the study is the degree of agreement of the ACR | U.S. Urine Analyzer Test System (tested by the lay user) as compared to the URiSCAN Optima Urine Analyzer (tested by a healthcare professional), for the different concentrations (blocks) reported by the ACR | U.S. device.

2. Secondary Efficacy Endpoint

The secondary endpoint of the study is to evaluate the ACR | U.S. usability, by potential lay users under actual use conditions (home environment).

12.3. Sample Size Estimation

Sample size estimation was determined according to previous method comparison studies performed using other urine analyzers with similar Indications for Use (see *table 1.0*), and based on the EP9-A2 standard and recommendations.

Furthermore, based on our experience in a previous, similar study, at least 50 subjects should represent a sufficient sample size to cover all concentrations and determine the agreement between the ACR | U.S. and the URiSCAN Optima Analyzer.

12.4. Subject Tracking

Throughout the study, tracking will be performed to evaluate whether the data collected covers the entire range of concentrations measured by the device. If the data is insufficient, spiked urine samples will be used to supplement the missing data. The number of spiked samples will not exceed 15% of the total number of samples (all blocks combined).

12.5. Data Analysis Sets

1. Efficacy Analysis Set

The Efficacy analysis set will consist of all subjects without major protocol deviations. Protocol deviations will be defined and classified as minor or major.

2. Statistical Analysis of Analysis Sets

The Efficacy analysis set will serve as the main analysis set for the method comparison and usability assessments.

12.6. Statistical Analysis

1. General Considerations

Statistical analyses will be performed using an appropriate statistical program and methodology.

Baseline demographic and safety analyses will be performed on all enrolled subjects.

2. Demographic and Other Baseline Variables

Demographic and baseline condition related characteristics will be tabulated. Continuous variables will be summarized by a mean, standard deviation, minimum, median and maximum, and categorical variables by a count and percentage.

3. Disposition of Subjects

The number and percent of subjects who fail to complete the study and the number and percent of subjects who fail to complete the study because of Adverse Events will be presented.

4. Efficacy Analysis

The % exact match (agreement) of the ACR | U.S. compared to the comparator device, for the same Albumin-Creatinine Ratio concentration (block) will be calculated. The % ±1 block match (agreement) of the ACR | U.S. compared to the comparator device, for the same Albumin-Creatinine Ratio concentration (block) will also be calculated.

5. Usability Analysis

The usability of the ACR | U.S. will be determined by evaluating the percentage of study subjects who are able to complete the device-related tasks, including accessing the ACR | U.S. App, providing a urine sample in the urine receptacle, dipping the kit urine strip in the urine specimen, positioning the strip on

the Color-Board and taking a picture using the smartphone App, with minimal attempts to ask for assistance.

Measurable usability criteria for specific, critical steps, such as time-to-completion, number of requests for assistance, numerical ratings, etc., will be evaluated by observer evaluation and user questionnaire responses. Descriptive statistics will be provided for usability criteria.

6. Adverse Event

In general, there are no anticipated adverse events in this study; however, if any adverse events do occur they will be recorded and presented in tables.

13. RISK / BENEFIT ANALYSIS

13.1. Risks

The risks to patients resulting from potential device hazards have been analyzed using the Risk Management Standard - ISO 14971. The different types of hazards were identified and evaluated using risk assessment numerical parameters. Applicable controls for the risks were analyzed. After the implementation of appropriate risk control measures, the level of risk was re-evaluated and found to be acceptable. The risks associated with the device are minimal if any. Below is a summary of the risks to the subject and the applicable control measures.

Category	Identified Risk	Mitigation Plan
Device components	Urine receptacle material may cause irritation if it comes in contact with the skin	The urine receptacle is manufactured from a combination of FDA food grade approved materials: Polypropylene and Thermo Plastic Elastomer (TPE) in over-molding / core injection process. The materials will also be subjected to biocompatibility testing in a certified lab according to the ISO 10993 standard
Design	The urine receptacle is not comfortable for collecting urine or for immersing the urine strip	The urine receptacle has been designed taking into consideration human factors analysis. The design is such that the urine will flow into the cup and avoid splashing. The cup is designed so that the user can immerse the urine strip easily in one quick insertion
Technical	The Color-Board gets wet and damaged	The Color-Board is manufactured from sturdy carton material, and is designed for one-time use
Usability	The user places the urine strip in the wrong position on the Color-Board pad	The device App will instruct the subject to reposition the urine strip

These risks following the mitigation are considered minimal, if not negligible.

13.2. Benefits

There are no immediate benefits to the subject from their participation in the study. The results of the study may provide valuable information and data towards their eventual benefit from the device as a new, home-use urine analyzer device.

14. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies is crucial for the protection of patients, investigators and the sponsor, and are mandated by regulatory agencies worldwide.

14.1. Definitions

Adverse Event Definitions and Classifications

- **Adverse Event**

An adverse event is any untoward medical occurrence in a clinical study patient. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of an investigational product, whether or not it is related to the investigational product. (Definition per International Conference on Harmonization [ICH])

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

- **Serious Adverse Event**

A serious adverse event as defined by ICH is any untoward medical occurrence that meets any of the following conditions:

  - Results in death
  - Is life-threatening
  - Requires inpatient hospitalization
  - Results in persistent or significant disability/incapacity, or
  - Is a congenital anomaly/birth defect

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any adverse event is considered a serious adverse event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

- **Unlisted (Unexpected) Adverse Event**

An unlisted adverse event, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or the package insert/summary of product characteristics for an approved product) (ICH)

- **Associated with the Use of the Device**

An adverse event is considered associated with the use of the device if the attribution is possible, probable, or very likely by the definitions listed below.

Relationship to Investigational Device

For all adverse events, the relationship to the study device and / or procedure will be determined by the investigator, using the following terms:

- **Probably related:**

Follows a reasonable temporal sequence from study device delivery / retrieval, and cannot be reasonably explained by known characteristics of the patient's clinical data or the surgical procedure applied.

- **Possibly related:**

Follows a reasonable temporal sequence from study device delivery / retrieval but could have been produced by the patient's clinical state or by the surgical procedures regardless of the study device.

- **Probably not related:**

Temporal association is such that the study device is not likely to have had any reasonable association with the observed event.

- **Not related:**

No relationship to study device activation is perceived.

14.2. Procedures

All Adverse Events

All adverse events will be reported from the time a signed and dated informed consent form is obtained until completion of the last study-related procedure. Events meeting the definition of serious adverse events must be reported using the Serious Adverse Event Form, including serious adverse events

spontaneously reported to the investigator within 30 days after the patient has completed the study (including post-study follow up).

All events that meet the definition of a serious adverse event will be reported as serious adverse events, regardless of whether they are protocol-specific assessments. All adverse events, regardless of seriousness, severity, or presumed relationship to study treatment, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "Upper respiratory infection"). Investigators must record in the CRF their opinion concerning the relationship of the adverse event to study treatment. All measures required for adverse event management must be recorded in the source document and reported according to sponsor instructions.

The sponsor assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The investigator (or sponsor where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

Serious Adverse Events

All serious adverse events occurring during clinical studies must be reported to the appropriate sponsor contact person by investigational staff within 24 hours of their knowledge of event.

Information regarding serious adverse events will be transmitted to the sponsor using the Serious Adverse Form, which must be signed by a member of the investigational staff. The initial report of a serious adverse event may be made by facsimile (fax), e-mail or telephone. It is preferable that serious adverse events are reported via fax or e-mail. Subsequent to a telephone report of a serious adverse event, a Serious Adverse Event Form must be completed by the investigational staff and transmitted to the sponsor within one working day.

All serious adverse events that have not been resolved by the end of the study, or that have not been resolved upon discontinuation of the patient's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value is available
- The event can be attributed to agents other that the study treatment or to factors unrelated to the study conduct

- When it becomes unlikely any additional information can be obtained (i.e. patient or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

The cause of death in a clinical study, whether or not the event is expected or associated with the investigational agent, is considered a serious adverse event. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a patient's participation in a clinical study must be reported as a serious adverse event, except hospitalization for:

- Pre-planned hospitalizations, i.e. before enrollment into the study and which are not related to the disease itself
- Social reasons in absence of an adverse event
- Surgery or procedure planned before entry into the study (must be documented in the CRF)

15. ETHICAL ASPECTS

15.1. Study-Specific Design Considerations

Only patients who have the capacity to provide informed consent are allowed to enroll in the study. As part of the screening of patients for entry into the study, the investigator will assess each patient's ability to provide informed consent for participation in the study.

The protocol includes strict requirements to ensure adequate protection of all patients participating in the study, including:

- Patients will be carefully screened using medical history before enrollment. Those who are judged to be at a high risk for adverse events will be excluded.
- Patients may withdraw their consent at any time without having to give a reason.
- Patients are fully informed as to the risks of study participation and will be provided with any new information about the study testing that might become available during their participation in the study.
- Informed consent is obtained from patients without undue enticement. Patients will not be coerced in any way to participate in this study. Excessive financial compensation will not be offered to patients or to investigators.

15.2. Regulatory Ethics Compliance

- Investigators Responsibilities

The investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical study data are credible.

- Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator will provide the IEC/IRB with current and complete copies of the following documents:

- Final protocol and if applicable, amendments
- Informed consent form (and any other written materials to be provided to the patients)
- Investigator's Brochure (or equivalent information) and amendments
- Patient recruiting materials, if applicable
- Information on compensation for study-related injuries or payments to patients for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for patients
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after IEC/IRB has given full approval of the final protocol, amendments (if any), and the informed consent form, applicable recruiting materials, and after the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the documents being approved.

During the study, the investigator will send the following documents to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments
- Revision(s) to informed consent form and any other written materials to be provided to patients
- If applicable, new or revised patient recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to patients for participations in the study, if applicable
- Investigator's Brochure amendments or new edition(s)
- Summaries of the status of the study (at least annually or at intervals stipulated in guidelines of the IEC/IRB)
- Reports of any serious adverse events
- New information that may adversely affect the safety of the patients or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the patients
- Report of death of patients under investigator's care
- Notification if new investigator is responsible for the study at the site
- Any other requirements of the IEC/IRB

For protocol amendments that increase patient risk, the amendments and applicable informed consent form revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s)

At least once a year the IEC/IRB will be asked to review and re-approve this clinical study. This request and approval should be documented in writing.

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

- Informed Consent

Each patient must give written consent according to local requirements after the nature of the study has been fully explained.

The consent form must be signed before performance of any study-related activity. The consent form used must be approved by both the sponsor and by the reviewing IEC/IRB. The informed consent should be in accordance with principles set forth in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before entry into the study, the investigator or an authorized member of the investigational staff must explain to potential patients the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort it may entail. Patients will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care they patient will receive for the treatment for his/her disease. Patients will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. Finally, they will be told that the investigator will maintain a patient identification register for the purposes of long-term follow up if needed and that their records may be accessed by health authorities without violating the confidentiality of the patient, to the extent permitted by the applicable law(s) or regulations. By signing the informed consent form the patient is authorizing such access, and agrees to be re-contacted after the study's completion, by health authorities and authorized sponsor staff, for the purpose of obtaining consent for additional safety evaluations if needed.

The patient will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by name of the patient, patient's signature and date of signature. After having obtained the consent, a copy of the informed consent form must be given to the patient.

If the patient or legally acceptable representative is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written explanations) and should personally date and sign the inform consent form after the oral consent of the patient or legally acceptable representative is obtained.

- Privacy of Personal Data

The collection and processing of personal data from patients enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study. This data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

16. ADMINISTRATIVE REQUIREMENTS

16.1. Protocol Modifications

The investigator will not modify this protocol without a formal amendment, if applicable. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the patients in which case the amendment must be promptly submitted to the IEC/IRB and the relevant competent authority. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

The investigator or other physician in attendance will contact the appropriate sponsor representative by fax or telephone regarding any situations requiring a departure from the protocol. If possible, contact will be made before implementing any departure from the protocol. In all cases contact with the sponsor must be made as soon as possible in order to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF will reflect any departure from the protocol.

16.2. Regulatory Documentation

- Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

- Required Pre-study Documentation

The following documents must be available and maintained during the study:

  - Approved Study Protocol and amendment(s)
  - A copy of the dated and written IEC/IRB approval of the protocol, amendments, informed consent form, any recruiting materials and if applicable, patient compensation programs. This approval must clearly identify the specific protocol by title and number.
  - Regulatory authority approval or notification, if applicable
  - Documentation of investigator qualifications (e.g., curriculum vitae)
  - Completed financial disclosure form
  - Signed and dated clinical trial agreement, which includes the financial agreements
  - Other documentation required by local regulations

- Patient Identification Register and Patient Screening Log

The investigator agrees to complete a patient identification register to permit easy identification of each patient during and after the study.

The patient identification register will be treated as confidential. To ensure patient confidentiality, no copy will be made. All reports and communications relating to the study will identify patients by initials and assigned number only.

The investigator will also complete a patient-screening log, which reports all patients who were seen to determine eligibility for inclusion in the study.

- eSource Completion

All data relating to the study will be recorded in electronic source documents. Data will be entered into eSource in English. The eSource are to be completed at the time of the patient's visit, so that they always reflect the latest observations on the patients participating in the study.

The investigator must verify that all data entries in the eSource are accurate and correct.

- Monitoring Plan

Once the clinical site begins enrolling participants for the study, monitoring will be conducted to verify that the study is conducted in accordance with the IVDR, ISO 20916:2019 and any other applicable requirements. During routine monitoring it will be verified, among other issues, that:

- The IVD is used according to CPSP or instructions for use
- The IVD is available and IVD accountability is performed accurately
- Study records are correct, complete, and up to date
- Safety documenting and SAE reporting is done appropriately to country legislation; and:
- Data protection regulations such as the General Data Protection Regulation (GDPR) are respected.

Activities conducted as well as findings and observations will be documented in a monitoring report.

- Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eSource and all source documents that support the data collected from each patient, as well as all study documents as specified in ICH/GCP Section 8, Essential, Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational products. These documents will be retained for a longer period if required by regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator must permit access to such report.

16.3. Device Accountability

All study devices and products will be administered according to the IRB approved protocol to ensure that:

- The device is stored in a secure location with access limited to the essential research personnel
- The device is used only in patients that consented to participate in the trial
- Records related to inventory, lot numbers, quantities in stock, and expiration dates for all study IVD medical devices and products are adequately maintained, including shipping invoices, confirmation or receipt, condition upon receipt
- The storage of investigational device is in a secure

16.4. Study Completion/Termination

- Study Completion

The study is considered completed with the last patient undergoing the study.

- Study Termination

The sponsor reserves the right to close the investigational site or terminate the study at any time. The investigational site will be closed upon study completion. Reasons for the early closure of an investigational site or termination of the study may include but are not limited to:

- Safety concerns
- Sufficient data suggesting lack of efficacy
- Inadequate recruitment of patients by the investigator

17. APPENDICIES

17.1. STUDY PROTOCOL AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study treatment and the conduct of the study.

_____

Investigator's Signature Date (Day Month Year)

_____

Name of Investigator (Typed or Printed)

_____

_____

_____

Institution and Address*

_____

Telephone number*

_____

Sponsor's Representative Signature Date (Day Month Year

_____

Name of Sponsor's Representative (Typed or Printed)

_____

_____

_____

Sponsor Address*

_____

Telephone number*

* If the address or telephone number of the investigator changes during the course of study, written notification will be provided by the investigator to the sponsor and will not require protocol amendment(s).

18. REFERENCES

[1] Simerville JA, Maxted WC, Pahira JJ. "Urinalysis: a comprehensive review". American Family Physician. 2005; 71(6) 1153-62

[2] Albumin-to-creatinine ratio (ACR) is the first method of preference to detect elevated protein, the National Kidney Foundation's (NKF), https://www.kidney.org/kidneydisease/siemens_hcp_acr

[3] Chronic Kidney Disease. Henry Ford Health System. Version 7, 2015.

[4] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K173327.pdf

[5] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K182384.pdf

[6] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K142391.pdf

[7] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K152835.pdf

[8] See the 510(k) Substantial Equivalence Determination Decision Summary: https://www.accessdata.fda.gov/cdrh_docs/reviews/K141874.pdf

[9] Chronic kidney disease awareness, screening and prevention: rationale for the design of a public education program, Allan J Collins et al., Nephrology (Carlton) . 2010 Jun;15 Suppl 2:37-42.

[10] See screening guidelines of different associations -

- **NKF** ("reference for kidney disease screening")
- **RPA** ("U.S. Preventative Services Taskforce - Recommendation on Screening for CKD")
- **ISN** and **KDIGO** ("Early Identification & Intervention Toolkit")
- **ADA** (Screening for Kidney Disease in Adults with Diabetes, Diabetes Care 2005 Jul; 28(7): 1813-1816).

[11] Risk factors for chronic kidney disease: an update, Rumeyza Kazancioğlu, Kidney Int Suppl (2011), 2013 Dec;3(4):368-371.

[12] UpToDate: Early detection of chronic kidney disease, https://www.uptodate.com/contents/early-detection-of-chronic-kidney-disease

[13] Clinical Laboratory Standards Institute (CLSI). Urinalysis; approved guideline -third Edition. CLSI document GP16-A3. Wayne, PA, USA: CLSI, 20