Chronic Condition Management and the Basic Metabolic Panel

Chronic Kidney disease (CKD) afflicts nearly 1 out every 7 Americans. Many CKD patients do not feel ill or even notice that they are symptomatic, and as a result need to be tested regularly for kidney function. Regular testing of kidney function for these patients is through the Basic Metabolic Panel (BMP). A BMP test is the best standard of care to date, and the frequency of this test could be as often as weekly for advanced patients. BMP testing is an outpatient service that normally requires a scheduled visit to a health care provider which may be challenging for the suffering patient. Studies have shown that this leads to reduced patient compliance and delays in diagnosis and treatment of End Stage Renal Disease (ESRD). This is even more difficult for remote rural patients that require frequent CKD monitoring. A 2019 review of Medicare costs found that an average CKD patient costs $24.5K/yr for a total annual Medicare cost of $87.2 Billion, while an ESRD patient costs $86.4K/yr for a total annual Medicare cost of $37.2 Billion, representing 7% of all Medicare claims[1]. Delayed or missed diagnosis of CKD progression and ESRD have negative impacts in patient outcomes and there is thus an unmet medical need in this large patient population. GattaCo has produced a candidate solution that will overcome the hurdles of outpatient testing and challenges with remote blood collection in a cost effective and patent friendly approach.

GattaCo has developed a finger-stick collection device that isolates and separates collected plasma from other destabilizing blood components, thereby preserving the plasma's quality for testing. Our proprietary technology that passively isolates liquid plasma from whole blood is our key advantage over current lab blood draw methods that rely on venipuncture blood collection tubes or capillary blood collection, without separation of plasma from cellular components. BMP samples can spend hours or days in transit prior to processing and these delays cause aging of the blood and clinically significant changes in biomarker levels (Table 1). We have shown that GattaCo's device separates plasma and preserves biomarker levels during transit equivalent to freshly collected and centrifuged samples (Table 2). The first generation GattaCo device, the A-PON® Plasma Separator, registered with the USFDA as a Class-I Medical Device, is shown in Figure 1.

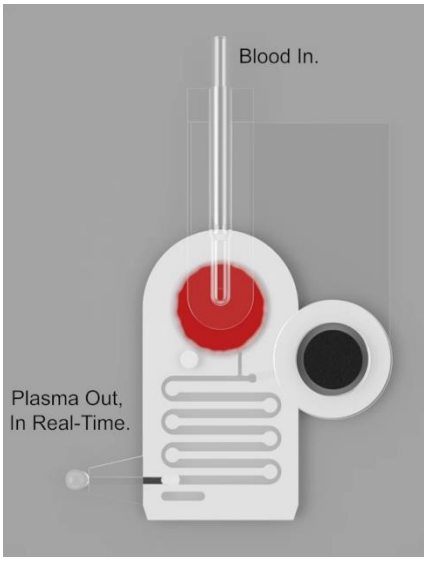


Fig. 1: *The A-PON® Plasma Separator.*

1. *JAMA Intern Med.* 2020;180(6):852-860. doi:10.1001/jamainternmed.2020.0562

Comparison with Dried Blood Spots (DBS)

Although not applicable to BMP testing, Dried Blood Spot (DBS) devices are commonly used for the detection of biomarkers for general health and wellness and newborn screening, employing multiple sample analysis methods including ELISAs, Mass-Spec, NGS, and others.

However, the challenges of DBS are multi-fold, including a sample rejection rate of up to 20% due to improper collection methods by lay persons[2], and extensive sample recovery processes by skilled laboratorians, that may also involve the use of expensive automation equipment for large screening applications, sometimes lasting multiple hours depending on the biomarkers attempting to be recovered. Many labs, particularly those involved in high-throughput clinical chemistry, avoid DBS due to the challenges of high sample rejection rate and the increase in manual labor or capital equipment costs[3].

Partial BMP	Control	GattaCo	48 hrs
Glucose (Glu)	90.00	98.00	54.00
Potassium (K)	4.00	4.60	>14.0
Creatinine (Crea)	0.85	0.92	0.84
Urea (BUN)	13.80	14.40	14.50
Sodium (Na)	142.00	146*	132.00

***Table 1**- Degradation of important BMP biomarkers over time when stored in whole blood.*

Control plasma separated by centrifugation immediately after collection

GattaCo plasma separated immediately from whole blood by a version of the A-PON device

48 hrs plasma centrifuged 48 hours after collection

Values measured using Ortho-Clinical microslides by GattaCo partner, Cedars Sinai Medical Center

* measured separately

Full BMP	Control	GattaCo
Na	141	144
K	3.7	4.5
Cl	103	110
iCa	1.25	1.06
TCO2	26	25
Glu	81	81
BUN	13	17
Crea	0.8	0.8

***Table 2**- At-Home BMP using fresh and GattaCo separated samples.*

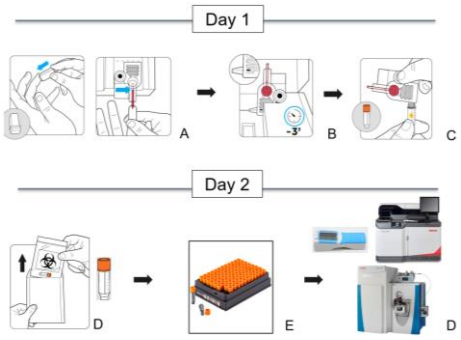
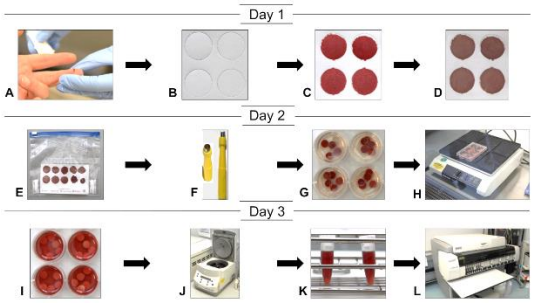
Control measurements taken with whole blood immediately after collection

GattaCo measurements taken using plasma separated immediately from whole blood by a version of the A-PON device

i-STAT Chem-8 cartridge used for data, accepts whole blood or plasma.

A typical DBS collection involves the need for multiple drops of blood, each in a separate circular region of a collection card, with each drop ranging between 50-75µL of blood[3]. Depending on the test(s) being performed, a typical collection card may have between 3 to 12 circular regions that need to be filled. Despite the extensive sample reconstitution process, only roughly 2-3µL worth of viable equivalent sample volume is recovered from each spot. Please see Workflow illustration below.

Alternatively, the first generation commercially available A-PON requires a similar 75µL of capillary blood to fill, but produces almost 15µL of high-purity, diagnostic-test-ready liquid plasma. A second-generation device will produce 100µL of diagnostic-test-ready plasma from an input of 300µL of capillary blood. This

	GattaCo Liquid Plasma Sampling	Dry Blood Spot Sampling [4]
Workflow		
Sample Quality	High quality liquid plasma	Hemolyzed dried blood
Usability Workflow	Precisely controlled collection process	Long drying time and high rate of rejection due to low volume collection mistakes
Time-to-Result	Can be tested immediately	Minimum of 2 – 3 days after collection
Clinical Relevance	Represent the status of the patient at time of collection	Low clinical relevance due to collection process and type of sample
Application Spectrum	Compatible with all central lab liquid plasma tests	Limited to biomarkers that unaffected by hemolysis and aged whole blood
Quantitation	Controlled blood in and plasma out with higher sensitivity	Uncontrolled volumes and diluted samples with low sensitivity
Cost Effectiveness	50% < Conventional methods, and 30% < DBS (Low cost due to minimum sample preparation steps)	30% < Conventional methods

of over 5-fold higher, automatically produced by the device. High-purity liquid plasma is the sample type commonly used by nearly all clinical labs, allowing the potential of straightforward and wide-spread adoption by any lab interested in expanding its testing market, due to the seamless integration of the GattaCo prepared sample.

2. Zakaria, Rosita and Greaves, Ronda F.. "The re-emergence of dried blood spot sampling – are we ready?" Clinical Chemistry and Laboratory Medicine (CCLM), vol. 57, no. 12, 2019, pp. 1805-1807. https://doi.org/10.1515/cclm-2019-1062
3. https://www.myadlm.org/cln/articles/2022/september/dried-blood-spots-and-beyond`
4. Grüner, N., Stambouli, O., Ross, R. S. Dried Blood Spots - Preparing and Processing for Use in Immunoassays and in Molecular Techniques. J. Vis. Exp. (97), e52619, doi:10.3791/52619 (2015).

Biomarkers Beyond the BMP

The validation of GattaCo's technology for BMP testing was performed with our clinical partner, Cedars Sinai Medical Center, in Los Angeles, California. Another clinical partner, Mitsubishi Tanabe Pharma Corp, a part of the Mitsubishi Chemical Group, performed additional testing on a larger set of biomarkers, including blood chemistry markers, PK/PD markers, nucleic acids and small to medium molecules, using LC-MS, commercial blood chemistry analyzers, and other methods. A listing of an expanded set of biomarkers analyzed, that were measured within 10% of centrifuged sample values, is shown below.

Select Clinical Chemistry Biomarkers
(±10% of centrifuged plasma using Gen-2 device)

alkaline phosphatase (ALP)	indirect bilirubin
aspartate aminotransferase (AST)	urea nitrogen
alanine aminotransferase (ALT)	uric acid
gamma-glutamyl transferase (GGT)	total cholesterol
potassium (K)	triglyceride
sodium (Na)	HDL-cholesterol
chloride (Cl)	LDL-cholesterol
calcium (Ca)	total protein (TP)
inorganic phosphorus	creatine kinase (CK)
A/G Ratio	creatinine
Glucose	LDH
total bilirubin	cholinesterase

The convenience of home or remote collection of a clinical-quality plasma sample, has attracted the interest of cancer researchers involved in clinical trials. Much cancer research involving liquid biopsies, requires large volumes of sample analyzed for cell-free DNA or circulating tumor cells. However, some labs study miRNA as indicators of cancer, which are available in abundance in small volumes. Dr. Dave Hoon, the Director of Saint Johns Cancer Institute Translational Research, Translational Molecular Medicine & Genome Sequencing Center of the Providence Health Service, was able to identify over 2,800 miRNAs in 20µL of plasma separated by using two A-PON devices per patient. Figures 2 and 3 show the results of comparing the miRNAs in GattaCo separated plasma with centrifuged derived plasma, demonstrating a high correlation between the two sample types, using NGS techniques.

Clinical trials, whether in oncology or for other conditions, struggle with patient recruitment and patient retention. Dr. Hoon identified the value of the A-PON, in addition to producing a high-quality liquid plasma sample, was the added convenience of the potential of at-home sample collection. During normal trials, a patient may need to regularly go to a clinic or blood collection center to donate blood via venipuncture. When that is not possible, a phlebotomist must be sent to the patient's home, which may lead to scheduling challenges and privacy issues. The convenience of at-home collection, of a clinical quality plasma sample, will ultimately reduce the cost of clinical trials due to increased patient recruitment and retention.

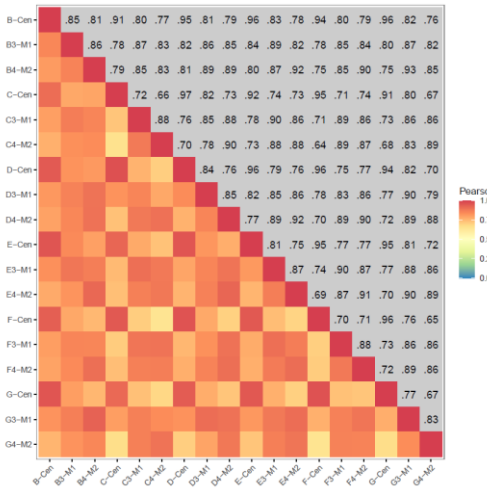


Fig. 2- *miRNA Whole Transcriptomic Analysis showed high correlation values (>0.65) across all plasma samples (12 GattaCo, 6 centrifuge).*

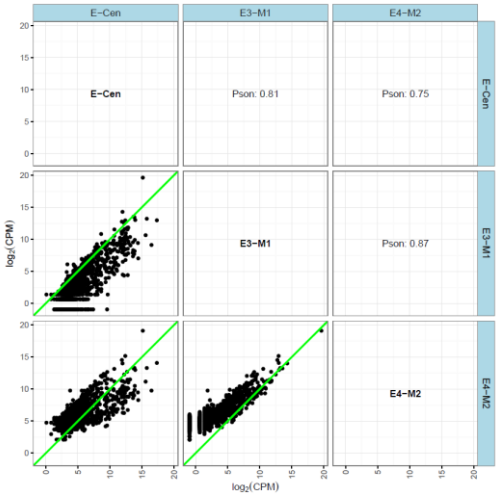


Fig. 3- *100% assay markers detected: >2800 miRNA/sample (Donor-E data shown. GattaCo:M1,M2; Centrifuge: C) 20ul plasma per sample used in Direct NGS WTA assay. All samples passed QC.*

The A-PON and Next Generation GattaCo Product Usability

The A-PON device is intended for use in a healthcare setting by a trained user. Dozens of evaluations of the A-PON have been performed by groups world-wide. In many cases they have benefited from an in-person demo or demo by video call, where a few nuanced characteristics of the use of the A-PON are explained. Generally, after the training or demo, no further guidance is needed and the trained individual, either a skilled laboratorian or lay person, is able to perform subsequent collections without difficulty. However, the current FDA registration of the A-PON is for use in a healthcare setting, whether that is for collecting a sample that is to be analyzed in a lab later, or, in some cases, the sample may be dispensed immediately into a point of care test, thus eliminating the need for venipuncture and centrifugation of a blood tube to obtain a precise volume of plasma for quantitative rapid testing.

While GattaCo has performed multiple studies demonstrating consistent and repeatable performance of the A-PON, improvements in design for greater ease of use are being developed for new versions of the product. These improvements will allow for a lay person to successfully perform a collection without supervision simply by referring to a Quick Reference Guide for instructions.

Broad Array of Use Cases for Screening and Health Management

GattaCo's proprietary technology that allows for the passive separation and collection of plasma from whole blood, is scalable from very small sample volumes, such as 5µL of plasma from a very small drop of finger-stick blood, to mL volumes of plasma separated from blood collected by venipuncture. The plasma volume produced by our Gen-1 device, the A-PON (approx. 15µL), is sufficient for single ELISA tests performed manually, a panel of test performed using mass-spec, or a panel of tests performed using specialized protein arrays, such as those developed by Olink, Quanterix or Luminex. Our Gen-2 device, which produces 100µL of plasma, is applicable for a few select clinical chemistry panels, such as the BMP, Liver function panel, or cardiometabolic panel, using clinical chemistry analyzers that have nominal sample dead-volumes.

The A-PON, or a larger volume prototype version of our Gen-2 design, are available for labs to validate the quality of the plasma collected for use with the sample collection and testing methodology the lab is considering. Once our Gen-2 product is available, we intend to validate its use with the Basic Metabolic Panel and register it as a Class-II device with the FDA. Numerous other use cases are possible with Gen-1, Gen-2 or a future sample collection device that is developed. The hardware for performing the testing is already available, in clinical labs.

Integrated Technology for Point of Care (POC) Testing

While most laboratory analysis systems are designed to work with larger volumes of sample, many new single-use, disposable biosensor devices, intended for Point of Care (POC) testing, can work with small samples, including 5µL or less. These new biosensors commonly exploit the latest semiconductor design and manufacturing methods, combined with new biochemical surface binding techniques, to develop highly sensitive and specific sensors capable of label-free, low concentration detection of single or multiple biomarkers. In almost every case, however, this high sensitivity and specificity is only possible if plasma is used, because the cells of whole blood can easily overwhelm the sensors with non-specific

BROAD ARRAY OF POTENTIAL USE CASES ENABLED BY GATTACO

Application	Biomarkers *Risks Removed & Benefits Added*	Clinical Need	Plasma Sample Required	Annual Market Opportunity-US
Basic Metabolic Panel (BMP)	• K, Na, Cl, Glucose, BUN Creatinine, CO2, Ca • ***Hemolysis, metabolism*** • ***↑Compliance***	Multiple diseases (e.g., kidney, diabetes, high blood pressure)	60µL[4]	**> 50M tests**[6]
Liver Function Panel	• ALT, AST, ALP, TBIL, T-Protein, Alb, GGT, LDH • ***Hemolysis, short half-life*** • ***Easier workflow, ↑Compliance***	Hepatitis, Chronic Liver Disease, response to medications	50µL[4]	**> 20M tests**[6]
Cardiovascular & Cardiometabolic Monitoring	• BNP, NT-ProBNP, hs-Troponin • Lipid Panel, Gly-Alb, Vit-D • ***Hemolysis; short half-life*** • ***Easier workflow; ↑Compliance***	31% of HF patients readmitted to hospital within 3 months[1,2]	30µL[5]	**> 10.5M tests**[3]
Biochemical Genetic Testing	• Glc_4, Lyso-Gb1,-Gb3, -SPM, α-N acetyl, GNS, PPCS, etc. • **↑Quantitation, Easier workflow**	Enzymatic profile screening for Lysosomal Storage Diseases and Monogenic Disorders	10-50µL[9]	**> 4M tests**[10]
Oncology Screening	• 2K+ miRNA • ***Short half-life*** • ***↑Compliance***	Clinical trials, treatment monitoring, recurrence screening	20µL[11]	**> 20M tests**[12]

1. Santaguida, P.L., Don-Wauchope, A.C., Oremus, M. *et al*. Heart Fail Rev 19, 453–470 (2014).
2. Muhammad Shahzeb Khan, Circulation: Heart Failure. 2021;14:e00833
3. 875K HF hospitalizations *12 tests over 6 months
4.Volume estimate based on OrthoClinical Vitros and XT Vitros Microslides plus 10uL DV
5. Typical ELISA sample volume requirement
6. Estimates based on typical CLIA lab annual test reporting
7. Yan Gong, et. Al., JCLA, V33, I7 2019
8. Hirano T. Int Immunol. 2021;33(3):127-148.
9. Typical LC-MS sample volume of diluted plasma and *Int. J. Mol. Sci*. 2020, 21, 2704; doi:10.3390/ijms21082704
10. Number of US newborn screening tests done in 2008, https://www.cdc.gov/nbslabbulletin/pdf/nslb_bulletin.pdf
11. Plasma sample volume of clinical partner screening over 2,800 miRNA,
12. Est. # of Phase III oncology clinical trials (https://www.globaldata.com/data-insights/healthcare/number-of-ongoing-clinical-trials-for-drugs-involving-oncology-by-phase-505160/) x 600 patients per trial x 4 samples per year

signals, rendering them ineffective. Prior to GattaCo, plasma was only available by centrifugation of blood tubes filled using venipuncture, thus largely eliminating the commercial opportunity of all but a few POC products. The point of care products that have been commercialized are mostly limited to those relying on semi-permeable membranes for performing conductivity measurements, or qualitative (yes or no) diagnostics, such as are common for the detection of infectious diseases.

GattaCo has and continues to work with companies developing the latest biosensor concepts including bioFETS, graphene-based systems, optical-based sensing, dried reagent systems, and other modalities, for the development and ultimate commercialization of a new generation of at-home or point of care testing products, which depend on high-purity, consistent, liquid plasma. Many of these biosensors are designed for quantitative and multiplexed applications, allowing for the testing of a panel of important biomarkers from a single, small drop of blood. Some of the tests include applications such as screening for high-risk pregnancy, neurological health, new biomarkers for the detection and control of diabetes (e.g. glycated albumin), Vitamin-D levels, and others. In many cases these new point of care products can be used for actual diagnosis and not just for screening applications, and thus be able to secure a higher insurance reimbursement level compared to many current POC tests.

Summary

The development of easy-to-use self-collection devices that produce clinically relevant, high-purity liquid plasma, will enable increased compliance and patient satisfaction and more frequent and cost-effective health screening and management of chronic conditions. The same technology, integrated into a sample to answer diagnostic cartridge, will enable a new generation of sensitive and specific POC testing products. GattaCo's solution will improve patients' lives and medical outcomes and also reduce the cost of healthcare for both patients and institutions.