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Documentation for Venous Blood Collection and Assay Protocol

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1. Overview

1.1. Rationale

India, the most populous country in the world with over 1.4 billion people, will soon experience rapid aging of its population. Currently, the roughly 90 million Indians aged 60 and above account for 8% of the population (Government of India, 2010), with elder security and care being predominantly a private, family matter. By 2040, India's 60 and above population is projected to more than double to 245 million (United Nation, 2017). It is estimated that there are already approximately 4.1 million people affected by dementia in India, and this number is projected to double by 2030 and triple by 2050 (Prince et al., 2015). The Harmonized **Diagnostic Assessment of Dementia for the Longitudinal Aging Study in India (LASI-DAD)** is an ambitious project which promises to provide a nationally representative data on elder health issues and shed light on cognitive aging, dementia and cognitive impairment. Previous efforts to study dementia and cognition in India have relied on non-representative samples in geographically restricted areas. For example, a landmark study by Ganguli et al. (1995, 1996) interviewed 374 individuals at age 55 and above at one rural site outside of Delhi in the state of Haryana, and the Indian cohort for the 10/66 study is based in one urban area of Chennai (Prina et al., 2016).[1] ***Longitudinal Aging Study in India (LASI)*** is the first study to examine late-life cognition and dementia in India at the national level. LASI is the most ambitious, nationally representative survey of the physical and cognitive health, economic, and social well-being of the country's aging population, interviewing over 73,408 individuals aged 45 and older (including their spouses, irrespective of age).

1.2. Sample

The Diagnostic Assessment of Dementia for LASI (LASI – DAD) is an in-depth study of late-life cognition and dementia. It draws a sub-sample over 4,000 LASI respondents aged 60 and older, administering in-depth cognitive tests and informant interviews following the Health and Retirement Study (HRS)'s Harmonized Cognitive Aging Project (HCAP) protocol. To ensure a sufficient representation of respondents

[1] The 10/66 study's prevalence estimate of dementia (Prince et al., 2003) was based on a multi-center convenience sample of 760 respondents at age 60+ from six states.

with dementia and mild cognitive impairment (MCI), a stratified random sample design is employed.

Respondents are stratified based on their risk of cognitive impairment, determined by their performance of memory and non-memory domain cognitive tests, overall test performance, refusal or inability to participate in cognitive tests, and proxy interviews conducted in the main LASI study. Approximately half of the LASI-DAD sample is drawn from those at high risk of cognitive impairment. High risk is defined as: (1) overall cognitive test performance in the core LASI was in the bottom tertile; (2) memory score was below the 15th percentile; (3) non-memory cognitive scores were below the 15th percentile; (4) number of missing cognitive tests was above the 85th percentile; or (5) the IQCODE score constructed from proxy report was 3.9 or higher (Jorm & Jacomb, 1989). Within each state, an equal number of respondents at high and low risk of cognitive impairment are randomly selected.

For Wave 2, the study aimed to conduct follow-up interviews with all Wave 1 respondents and recruit refresher samples to account for attrition and improve representativeness. Contact information for both respondents and informants was collected during Wave 1, enabling the team to trace individuals to their new residences if they had moved. For the recruitment of newly age-eligible respondents, the same sampling strategy as Wave 1 was followed. Specifically, the age-eligible LASI sample was stratified by state of residence and cognitive impairment risk, as assessed in the LASI core interview. Within each state, 50% of individuals at high and low risk of cognitive impairment were randomly selected.

Interviews are conducted by 15 participating hospitals [2], which recruited selected LASI respondents and their family members approximately 7 months after the baseline interview. In Wave 1, the HCAP protocol was administered either at the hospital or at the respondent's home, based on their preference. In Wave 2, all surveys were conducted at the respondent's residence. The field team traveled up to 12 hours by automobile to reach respondents in remote villages. Figure 1 illustrates

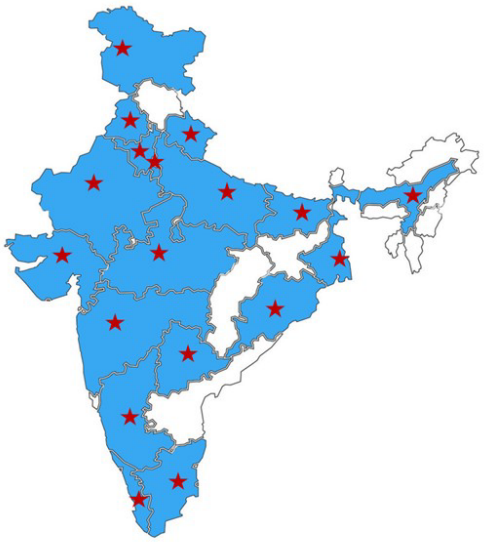
[2] The collaborating hospitals are: the All India Institute of Medical Sciences, New Delhi; Madras Medical College, Chennai; National Institute of Mental Health and Neurosciences, Bangalore; BHU, Varanasi; S.N. Medical College, Jodhpur; TMC, Trivandrum; Grant Medical College, Mumbai; SKIMS, Srinagar, Gauhati Medical College, Guwahati, Assam; Nizam's Institute of Medical Sciences, Hyderabad, All India Institute of Medical Sciences, Bhubaneswar, Odisha; IPGMER, Kolkata; Indira Gandhi Institute of Medical Sciences, Patna, Bihar; All India Institute of Medical Sciences, New Delhi; All India Institute of Medical Sciences, Rishikesh, Uttarakhand; and Government Medical College, Chandigarh, Punjab.

the geographic distribution of the sample across both waves (the Delhi team also recruits and interviews respondents in Haryana).

In Wave 1, the sample was drawn from 18 states and 4 metropolitan cities across the country, all within a 12-hour driving distance from participating hospitals. The states included Assam, Gujarat, Haryana, Jammu & Kashmir, Karnataka, Kerala, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, Bihar, Madhya Pradesh, Uttarakhand, Punjab, and West Bengal. The four metropolitan cities are Chennai, Delhi, Kolkata, and Mumbai. Additionally, in Wave 2 of the study, the sample was expanded to include Andhra Pradesh, Chhattisgarh, Jharkhand, and Puducherry, enhancing the representativeness of the population.

Figure 1. LASI – DAD Sample for Wave 1 and Wave 2

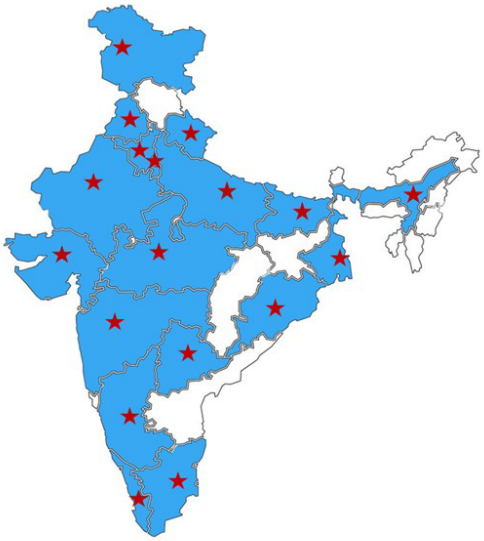
Population Representation For Wave 1



LASI-DAD Study Sites

Phase 1 (Oct 2017 – May 2018)	Phase 2 (Oct 2018 – Apr 2019)	Phase 3 (Oct 2019 – March 2020)
Delhi, Haryana All India Institute of Medical Sciences, New Delhi	**Jammu & Kashmir** Sher-i- Kashmir Institute of Medical Sciences, Srinagar	**Gujarat** Grant Medical College, Mumbai
Rajasthan S. N. Medical College, Jodhpur	**West Bengal** Kolkata Medical College, Kolkata	**Bihar** Indira Gandhi Institute of Medical Sciences, Patna
Uttar Pradesh Banaras Hindu University, Varanasi	**Assam** Guahati Medical College, Guwahati	**Madhya Pradesh** All India Institute of Medical Sciences, New Delhi
Karnataka NIMHANS, Bangalore	**Odisha** AIIMS, Bhubaneshwar	**Uttarakhand** All India Institute of Medical Sciences, Rishikesh
Kerala Trivandrum Medical College, Trivandrum	**Telangana** Nizam's Institute of Medical Sciences, Hyderabad	**Punjab** Government Medical College, Chandigarh
Tamil Nadu Madras Medical College, Chennai	**Maharashtra** Grant Medical College, Mumbai	

Population Representation For Wave 2



LASI-DAD Study Sites

Phase 1 (Dec 2022 – Jun 2023)	Phase 2 (May 2023 – Sep 2023)	Phase 3 (Oct 2023 – Jun 2024)
Delhi, Haryana Venu Geriatric Care Center, New Delhi	**Rajasthan** S.M.S. Medical College, Jaipur	**Chattisgarh** All Insia Institute of Medical Science, Raipur
Assam Gauhati Medical College, Guwahati	**Jammu & Kashmir** Shar-e Kashmir Institute of Medical Science, Srinagar	**Madhya Pradesh** All Insia Institute of Medical Science, Bhopal
West Bengal Medical College, Kolkata	**Kerala** Aster MIMS, Kannur	**Punjab** Government Medical College, Chandigarh
Odisha All Insia Institute of Medical Science, Bhubaneswar	**Tamil Nadu & Puducherry** JIPMER, Puducherry	**Uttarakhand** All Insia Institute of Medical Science, Rishikesh
Maharashtra Grants Medical College & JJ Hospital, Mumbai	**Telangana** All Insia Institute of Medical Science, Bibi Nagar	**Bihar, Jharkhand** All Insia Institute of Medical Science, Patna
Andhra Pradesh All Insia Institute of Medical Science, Mangalgiri		**Gujarat** Grants Medical College, Mumbai
Karnataka NIMHANS, Bangalore		**Uttar Pradesh** Institute of Medical Science, BHU, Varanasi

1.3. Collection & Shipping

The blood collection and shipment were managed by Metropolis, Inc. Metropolis, Inc., a pathology laboratory accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL) in India and the College of American Pathologists (CAP). Metropolis has a wide network of laboratories across study sites to ensure the timely processing of venous blood specimens (VBS) into serum and plasma within 2 hours of its receipt.

In Wave 1, a two-day training was provided to Metropolis phlebotomists regarding the study protocol. Trained phlebotomists visited participating hospitals and respondents' home for blood draw. A panel of experts from the University of Southern California, All India Institute of Medical Sciences (AIIMS) New Delhi, University of California Los Angeles, University of Minnesota, and Metropolis Healthcare Limited Inc. India, developed, reviewed, and revised the protocol and standard operating procedures (SOPs) as necessary. Each State had a team of field investigators, a supervisor, and a phlebotomist assigned by Metropolis, trained to carry out and assist in VBS collection. Initially, multiple phlebotomists from local laboratories closer to the respondents' location were trained. However, the protocol

was later standardized by hiring a single phlebotomist per state to ensure uniformity and minimize bias.

Respondent safety, confidentiality, and adherence to SOPs for sample collection, processing, packaging, shipment, quality check, and reporting adverse events at any step were prioritized.

Phlebotomy service included proper labelling with barcodes, centrifugation, and shipping samples at ambient temperatures (2 to 8°C) to the main Metropolis laboratory in Delhi within 24 hours of collection. Respondents, phlebotomists, and the Metropolis logistics team were notified 48 hours in advance of the scheduled blood draw. Informed consent form was obtained in the presence of a family member, with cognitively impaired participants requires consent from a legal representative Interviews and consent forms were available in12 Indian languages.

Each respondent provided a 17 mL blood sample divided into two serum separation tubes (SSTs) (tubes A and B), two EDTA tubes (tube C & D), and a plasma preparation tube (PPT) (tube E).Table 1 and Table 2 summarize the amount of blood collected per tube. In Wave 1, four out of five tubes (A, B, C & E) were sent to local Metropolis laboratories while tube D was sent to the MedGenome laboratory for Whole Genome Sequencing. The blood sent to local Metropolis laboratories was processed to whole blood, serum, plasma, and buffy coat. Once processed, the blood specimens were sent to the central Metropolis laboratory in Delhi in two shipments:

1) One shipment at 4 °C: containing whole blood and serum sample (Tube A, B & C)
2) One shipment at -20°C: containing plasma sample (Tube E)

Temperature monitoring was conducted using different temperature loggers for each of the shipments. At the central Metropolis laboratory, one five-spot dried blood spot (DBS) card per respondent was created Serum, plasma, buffy coat and DBS samples were also stored at All India Institute of Medical Science (AIIMS), New Delhi.

In Wave 2, all blood samples were shipped to the local Metropolis laboratory for processing. Following an increase in hemolysis rate, the venous blood collection protocol was revised with expert input. Changes included keeping SSTs (tubes A & B) at room temperature for 30 minutes at the respondent's house before transferring them with the other three tubes into the cold chain box. If local lab was over 2 hours from the collection site, the phlebotomists used portable centrifuge machines to process the samples within six hours of sample collection. All tubes were transported

vertically in a tube tray and shipped within a cold chain box (2 to 8°C) using isolation material to prevent physical movement and direct contact with the gel packs. The samples were processed at 3500 rpm for 10 minutes within two hours of reaching the local lab and then shipped to the main or central Metropolis lab in Delhi.

Table 1. Blood Collection and Shipping Protocol for Wave 1

Tube No.	Quantity of Blood	Color of tube	Send to	Processed to
A	3.5 ml	Yellow Top: SST (Serum Separation tubes)	Metropolis	Serum and used for various assays
B	3.5 ml	Yellow Top: SST (Serum Separation tubes)	Metropolis	Serum
C	3 ml	Lavender Top	Metropolis	CBC and HbA1c
D	2 ml	Lavender Top	MedGenome	Whole genome sequencing
E	5 mL	White top (Plasma Preparation tube)	Metropolis	Plasma and buffy coat

Table 2. Blood Collection and Shipping Protocol for Wave 2

Tube No.	Quantity of Blood	Color of tube	Send to	Processed to
A	3.5 ml	Yellow Top: SST (Serum Separation tube)	Metropolis	Serum and used for various assays
B	3.5 ml	Yellow Top: SST (Serum Separation tube)	Metropolis	Serum
C	2 ml	Lavender Top: EDTA tube	Metropolis then MedGenome	Whole genome sequencing
D	3 ml	Lavender Top: EDTA Tube	Metropolis	CBC and HbA1c
E	5 mL	White top: PPT (Plasma Preparation tube)	Metropolis then AIIMS	Plasma and AD Biomarker Assays

1.4. Participation

As the baseline LASI fieldwork was carried out in phases, the LASI-DAD survey followed a similar approach. Wave 1 was conducted in three phases from 2017-2019, while Wave 2 took place in three phases from 2022-2024. Across both waves, the total eligible respondents increased from 5,074 in Wave 1 to 6,128 in Wave 2. In Wave 1, 4,096 respondents completed the cognitive interview, compared to 4,562 in Wave 2. The informant interviews were completed by 4,047 respondents in Wave 1 and 4,490 in Wave 2. Blood specimen collection was successful for 2,892 participants in Wave 1 and 3,252 in Wave 2. Table 3 provides a detailed breakdown of the number of participants contacted and response rates by urbanicity.

Table 3. Number of Participants Contacted and Response Rates by Urbanicity

	Wave 1			Wave 2		
Coverscreen Interview	**Total**	**Urban**	**Rural**	**Total**	**Urban**	**Rural**
Contacted	5179	3001	2178	8365	2989	5376
Unable to contact	268	112	156	755	443	312
Deceased	105	62	43	2237	786	1451
Refused	304	108	196	735	360	375
Eligible	4502	2719	1783	4638	1400	3238

	Total	**Urban**	**Rural**	**Total**	**Urban**	**Rural**
Cognitive Interview	4096	2539	1557	4562	1379	3183
Informant Interview	4047	2513	1534	4490	1354	3136
Blood collection	2892	1875	1017	3252	970	2282

2. Laboratory

2.1. Overview

Metropolis laboratory is the leading independent pathology laboratory in India that offers a comprehensive menu of over 4,500 tests in clinical chemistry, clinical microbiology, cytogenetics, hematology, molecular diagnostics, and surgical pathology. Metropolis delivers over 30 million tests a year, catering to more than 10,000 hospitals, nursing homes, and other laboratories. It is accredited by NABL in India and CAP and has performed consistently well in all external quality control programs.

A unique barcode ID was assigned to each respondent and added to sample acquisition form, the vacutainers and the study database using a Computer-Assisted Personal Interview (CAPI) device. Assigning a unique barcode ID ensured accurate identification while maintaining confidentiality during subsequent sample processing steps. The trained staff followed good phlebotomy practices during blood collection to prevent the likelihood of sample contamination and transmission of infectious disease agents. In the event of a residual scar, pain, edema, abscess, cellulitis, adverse events in the past during VBS collection, or any other discomfort in both arms, VBS collection was not done. Moreover, in some rare cases where it was difficult to stabilize the vein, the blood flow was slow or an insufficient quantity was

collected, a respondent-consented second attempt was made to collect the remaining blood sample.

As per the manufacturer's instructions for use, the SSTs were gently inverted five times and kept vertical for 30 minutes at room temperature before shipment or on-site processing. It allowed the blood to clot and prevented the suspension of cellular components in the serum that could affect the analyte concentration. The EDTA tubes and the PPTs were gently inverted 10 times to mix the blood with the additives. After mixing, the tubes were immediately transferred to the cold chain box. As soon as VBS was collected from the first respondent, a single-use Appresys temperature logger was placed with the samples to record the temperature of the cold chain at a logging interval of 5 minutes until it reached the central laboratory in Delhi. The details of VBS collection such as date, time, and number of tubes collected corresponding to each unique barcode, temperature logger ID, collection location, and shipment destination were entered in the sample acquisition form and the CAPI.

All the vacutainers were placed vertically in a tube tray in an insulated Styrofoam box. Gel packs were added to maintain an ambient cold chain temperature of 2-8°C. Air-filled polyethylene packaging films were placed to stabilize the tubes and prevent their direct contact with the gel packs. The EDTA tubes were placed in the center and the SSTs and PPTs at the periphery to prevent whole blood from freezing.

2.2. Assay Methodology and Reference Ranges

The assay methods for Wave 1 are summarized in table 5, and the descriptions of each marker, measurement units, and reference ranges are summarized in table 6. For Wave 2, the updated assay methodology and equipment is given in table 7.

Table 4. Assay Methodology Used at Metropolis Laboratory during Waves 1 and 2

Test	W1 Equipment	W2 Equipment	Assay Methodology (For Wave 1 and Wave 2)
Whole Blood-based Assays			
Hemoglobin	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** The Coulter method is used for complete blood cell count and hemoglobin. Hemoglobin or Hemoglobin Concentration

			• Transmittance of light at 525 nm through a lysed WBC solution in the hemoglobin cuvette, compared to the transmittance of the same light through a reagent blank. The system converts this ratio to the hemoglobin value using a calibration factor. • Weight (mass) of hemoglobin determined from the degree of absorbance found through photo current transmittance expressed in g/dL. • Corrected for WBC interference. • Hemoglobin (g/dL) = [constant X $\log^{10}$ (Reference %T/Sample %T)].
Platelet Count	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** The Coulter method is used for complete blood cell count and hemoglobin. The Coulter Principle is based on the detection and measurement of changes in electrical resistance produced by a particle or cell suspended in a conductive liquid (diluent) traversing through a small aperture. When particles or cells are suspended in a conductive liquid, they function as discrete insulators. • The number of Platelets derived from platelet histogram, multiply by a calibration factor. • Plt =N x 10^3 Cells/ µL
Red Blood Cell Count	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** Red Blood Cell Count or Erythrocyte Count • Measure directly, multiplied by the calibration factor • Corrected for very high white count if necessary. RBC = N X 10^6 cells/µLν Uncorrected White Blood Cell (UWBC) • Measure directly, multiplied by the calibration factor. • UWBC = N x 10^3 cells/µLν
White Cell Count	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** White Blood Cell Count or Leukocyte Count • Measure directly, multiplied by the calibration factor. • Corrected for interference if necessary.

			If no correction required, then WBC = UWBC. WBC = N x 10^3 cells/µLv
Basophils (DLC-BO)	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** Analyzed by VCS Technology - Volume, Conductivity, Scatter, Opacity and RLS measurements are taken. Each cell is then assigned an X, Y and Z coordinate in a 3-Dimensional array based respectively on its RLS, Volume and Opacity. • Basophil Percent: [(BA event/(NE+LY+MO+EO+BA events)] x100 • Expressed as percentage (%)
Eosinophils (DLC-EO)	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** Analyzed by VCS Technology - Volume, Conductivity, Scatter, Opacity and RLS measurements are taken. Each cell is then assigned an X, Y and Z coordinate in a 3-Dimensional array based respectively on it's RLS, Volume and Opacity. • Eosinophil Percent: [(EO event/(NE+LY+MO+EO+BA events)] x100 • Expressed as percentage(%)
Lymphocytes (DLC-LY)	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** Analyzed by VCS Technology - Volume, Conductivity, Scatter, Opacity and RLS measurements are taken. Each cell is then assigned an X, Y and Z coordinate in a 3-Dimensional array based respectively on its RLS, Volume and Opacity. • Lymphocyte Percent: [(LY event/ (NE+LY+MO+EO+BA events)] x100 • Expressed as percentage (%)
Monocytes (DLC-MO)	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** Analyzed by VCS Technology - Volume, Conductivity, Scatter, Opacity and RLS measurements are taken. Each cell is then assigned an X, Y and Z coordinate in a 3-Dimensional array based respectively on it's RLS, Volume and Opacity. • Monocyte Percent: [(MO event/(NE+LY+MO+EO+BA events)] x100 • Expressed as percentage(%)
Neutrophils (DLC-NE)	Beckman Coulter LH780	Beckman Coulter UniCel DxH 800	**For Wave 1 & Wave 2: Same** Analyzed by VCS Technology - Volume, Conductivity, Scatter, Opacity and RLS measurements are taken. Each cell is then assigned an X, Y and Z coordinate in a 3-Dimensional array based respectively on it's RLS, Volume and Opacity. • Neutrophil Percent:

			[(NE event/(NE+LY+MO+EO+BA events)] x100 • Expressed as percentage(%)
Glycosylated Haemoglobin (HbA1c)	Bio -Rad D-10	Tosoh G8	**For Wave 1 & Wave 2: Same** The test is based on chromatographic separation of the analyte by ion exchange HPLC.
Serum-based Tests			
Glucose (Estimated, Reported along with HbA1C)	Calculated with HbA1C-Estimated	Calculated with HbA1C-Estimated	Not Applicable
Lipid Profile			
Cholesterol (total)	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Enzymatic, colorimetric method:** Total cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration.
HDL Cholesterol	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Homogeneous enzymatic colorimetric test.** The Ultra HDL assay is a homogeneous method for directly measuring HDL cholesterol concentrations in serum or plasma without the need for off-line pretreatment or centrifugation steps. The method uses a two-reagent format and depends on the properties of a unique detergent. In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction yielding a colorless product. The second reagent consists of a detergent (capable of solubilizing HDL cholesterol), cholesterol esterase (CE), and chromogenic coupler to develop color for the quantitative determination of HDL cholesterol. Methodology: Accelerator Selective Detergent
LDL Cholesterol	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Homogeneous enzymatic colorimetric assay** The MULTIGENT Direct LDL assay is a homogeneous method for directly measuring LDL levels in serum or

			plasma, without the need for off-line pretreatment or centrifugation steps. The method is in a two-reagent format and depends on the properties of a unique detergent. This detergent, R1, solubilizes only the non-LDL particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color-forming reaction. A second detergent, R2, solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.
Triglycerides	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Enzymatic colorimetric test:** Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H2O2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm.
Metabolic Panel			
Bilirubin (Total and Direct)	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Colorimetric diazo method:** Bilirubin determination is generally based on the reaction of bilirubin with a diazotized sulfanilic acid, described by Ehrlich. In this method, direct (conjugated fractions) bilirubin couples with a diazonium salt in the presence of sulfamic acid to form the colored compound azobilirubin. The increase in absorbance at 548 nm due to azobilirubin is proportional to the direct bilirubin concentration. Methodology: Diazo Reaction
Total Protein	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Colorimetric assay: Biuret** Polypeptides containing at least two peptide bonds react with biuret reagent. In alkaline solution, cupric ion forms a coordination complex with protein nitrogen with very little difference between albumin and globulin on a protein-nitrogen basis.
Albumin	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Colorimetric assay: Bromcresol green (BCG)** The Albumin BCG procedure is based on the binding of bromcresol green with albumin to produce a colored complex. The absorbance of the complex at 628 nm is directly proportional to the albumin concentration in the sample.

Alanine Aminotransferase (ALT)	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Methodology: NADH without P5P** ALT present in the sample catalyzes the transfer of the amino group from L-alanine to -ketoglutarate forming pyruvate and L-glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase (LD), is reduced to L-lactate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD.
Aspartate Aminotransferase (AST)	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Methodology: NADH without P5P** AST present in the sample catalyzes the transfer of the amino group from L-alanine to -ketoglutarate, forming oxaloacetate and L-glutamate. Oxaloacetate in the presence of NADH and malate dehydrogenase (MDH) is reduced to L- malate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD.
Alkaline Phosphatase (ALP)	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Colorimetric assay: Para-Nitrophenyl-phosphate** Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless p-nitrophenyl phosphate (p-NNP) to give p-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the p-nitrophenol is in the yellow phenoxide form. The rate of absorbance increases at 404 nm and is directly proportional to the alkaline phosphatase in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.
Gamma-Glutamyl Transferase (GGT)	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Enzymatic colorimetric assay: L-Gamma-glutamyl-3-carboxy -4 - nitroanalyte** GGT catalyzes the transfer of the gamma-glutamyl group from the donor substrate (3-carboxy-4-nitroanilide) to the glycylglycine acceptor to yield 3-carboxy-4-nitroliniline. The rate of the absorbance increase at 412 nm is directly proportional to the GGT in the sample.

Blood Urea Nitrogen (BUN)	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Kinetic test with urease and glutamate dehydrogenase:** The Urea Nitrogen assay is a modification of a totally enzymatic procedure first described by Talke and Schubert (1965). The test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited period of time. Urea in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction, catalyzed by glutamate dehydrogenase (GLD) converts ammonia and á -ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide hydroxide (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH are oxidized for each mole of urea present. The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample. Methodology: Urease method
Creatinine	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **kinetic colorimetric assay: Jaffé method** At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample.
Cystatin C	BNProSpec	Atellica NEPH 630	**For Wave 1 & Wave 2: Same** **Methodology: Nephlometry** Polystyrene particles coated with specific antibodies to human cystatin Care aggregated when mixed with samples containing human cystatin C. These aggregates scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the respective protein in the sample. The result is evaluated by comparison with a standard of known concentration.
Uric acid	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Enzymatic colorimetric test: Uricase** The Uric Acid assay is based on the methods of Trivedi and Kabasakalian. Uric acid is oxidized to allantoin by uricase with the production of hydrogen peroxide (H_2O_2). The H_2O_2 reacts with 4-aminoantipyrine (4-AAP) and 2,4,6-tribromo-3-hydroxy benzoic acid (TBHB) in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to the uric acid concentration in the sample.

Calcium	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Colorimetric assay :5-nitro-5'-methyl-BAPTA (NM-BAPTA):** Arsenazo-III dye reacts with calcium in an acid solution to form a blue-purple complex. The color developed is measured at 660 nm and is proportional to the calcium concentration in the sample.
Phosphorous	Architect ci8200	Roche Cobas 8000	**For Wave 1 & Wave 2: Same** **Colorimetric assay: Molybdate UV** Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula $(NH_4)_3[PO_4(MoO_3)_{12}]$ with ammonium molybdate in the presence of sulfuric acid. The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photometrically.
Thyroid Function Tests			
Total Thyroxine (T4)	Architect ci8200	Roche Cobas 8000	**For Wave 1: Architect ci8200** **Methodology: Chemiluminescent Microparticle Immunoassay (CMIA)** The Total T4 assay is a two-step immunoassay to determine the presence of thyroxine (Total T4) in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample and anti-T4 coated paramagnetic microparticles are combined. Bound T4 is removed from the binding sites on thyroxine binding globulin, prealbumin and albumin. T4 present in the sample binds to the anti-T4 coated microparticles. After washing, T3 acridinium-labeled conjugate is added in the second step. Pre-Trigger and Trigger solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of Total T4 in the sample and the RLUs detected by the Architect optical system. **For Wave 2: Roche Cobas 8000** **Methodology: Electrochemiluminescence Immunoassay (ECLIA)** Competition principle: ▪ 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by 8-anilino-1-naphthalene sulfonic acid (ANS).

			▪ 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T4, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. ▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. ▪ Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
Total Triiodothyronine (T3)	Architect ci8200	Roche Cobas 8000	**For Wave 1: Architect ci8200** **Methodology: Chemiluminescent Microparticle Immunoassay (CMIA)** The Total T3 assay is a two-step immunoassay to determine the presence of Triiodothyronine (Total T3) in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex, as for total T4 levels. **For Wave 2: Roche Cobas 8000** **Methodology: Electrochemiluminescence Immunoassay (ECLIA)** Competition principle: ▪ 1st incubation: 30 µL of sample and a T3-specific antibody labeled with a ruthenium complex; bound T3 is released from the binding proteins in the sample by 8-anilino-1-naphthalene sulfonic acid (ANS). ▪ 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T3, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. ▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

			▪ Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
Thyroid-stimulating Hormone (TSH) (Ultrasensitive)	Architect ci8200	Roche Cobas 8000	**For Wave 1: Architect ci8200** **Methodology: Chemiluminescent Microparticle Immunoassay (CMIA)** The TSH assay is two-step immunoassay to determine the presence of TSH in human serum using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols. **For Wave 2: Roche Cobas 8000** **Methodology: Electrochemiluminescence Immunoassay (ECLIA)** Sandwich principle: ▪ 1st incubation: 50 µL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex. ▪ 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. ▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. ▪ Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
Other Tests			

Vitamin B12	Architect ci8200	Roche Cobas 8000	**For Wave 1: Architect ci8200** **Methodology: Chemiluminescent Microparticle Immunoassay (CMIA)** The B12 assay is two-step assay with an automated sample pretreatment, for determining the presence of vitamin B12 in human serum & plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. **For Wave 2: Roche Cobas 8000** **Methodology: Electrochemiluminescence Immunoassay (ECLIA)** Competition principle. ▪ 1st incubation: By incubating the sample (15 µL) with the vitamin B12 pretreatment 1 and pretreatment 2, bound vitamin B12 is released. ▪ 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample. ▪ 3rd incubation: After addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor-vitamin B12 biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. ▪ Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
Folic acid	Architect ci8200	Roche Cobas 8000	**For Wave 1: Architect ci8200** **Methodology: Chemiluminescent Microparticle Immunoassay (CMIA)** The Architect folate assay is a two-step assay for the quantitative determination of folate in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology with flexible assay protocols. **For Wave 2 : Roche Cobas 8000**

			Methodology: Electrochemiluminescence Immunoassay (ECLIA) Competition principle: ▪ 1st incubation: By incubating 25 µL of sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins. ▪ 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample. ▪ 3rd incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. ▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. ▪ Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
25 Hydroxy, Vitamin D	Architect ci8200	Roche Cobas 8000	**For Wave 1: Architect ci8200** **Methodology: Chemiluminescent Microparticle Immunoassay (CMIA)** The 25-OH VITAMIN D assay is a delayed one-step immunoassay including a sample pretreatment for the quantitative determination of the presence of 25-OH VITAMIN D in human serum & plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. **For Wave 2: Roche Cobas 8000** **Methodology: Electrochemiluminescence Immunoassay (ECLIA)** Competition principle: ▪ 1st incubation: By incubating the sample (9 µL) with pretreatment reagent 1 and 2, bound 25-hydroxyvitamin D is released from the vitamin D binding protein (VDBP).

			▪ 2nd incubation: By incubating the pretreated sample with the ruthenium labeled VDBP, a complex between the 25-hydroxyvitamin D and the ruthenylated VDBP is formed. A specific unlabeled antibody binds to 24,25-dihydroxyvitamin D present in the sample and inhibits cross-reactivity to this vitamin D metabolite. ▪ 3rd incubation: After addition of streptavidin-coated microparticles and 25-hydroxyvitamin D labeled with biotin, unbound ruthenylated labeled VDBP become occupied. A complex consisting of the ruthenylated VDBP and the biotinylated 25-hydroxyvitamin D is formed and becomes bound to the solid phase via interaction of biotin and streptavidin. ▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. ▪ Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the cobas link.
Homocysteine	Architect ci8200	Architect i2000	**For Wave 1 & Wave 2: Same** **Methodology: Chemiluminescent Microparticle Immunoassay (CMIA)** The homocysteine assay is a two-step immunoassay to determine the presence of homocysteine in human serum & plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex.
NT pro BNP	MiniVidas	Roche Cobas 8000	**For Wave 1: MiniVidas** **Methodology: Enzyme-Linked Fluorescent Assay (ELFA)** The assay principle combines a one-step immunoassay sandwich method with a final fluorescent detection. **For Wave 2: Roche Cobas 8000** **Methodology: Electrochemiluminescence Immunoassay (ECLIA)** Sandwich principle: ▪ 1st incubation: Antigen in the sample (9 µL), a biotinylated monoclonal NT-proBNP-specific antibody, and a monoclonal NT-proBNP-specific

			antibody labeled with a ruthenium complex form a sandwich complex. ▪ 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. Total duration of assay: 9 minutes. ▪ During a 9minute incubation, antigen in the sample (9 µL), a biotinylated monoclonal NT-proBNP-specific antibody, a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase. ▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. ▪ Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the cobas link.
High-sensitivity C- Reactive Protein (hsCRP)	BNProSpec	Atellica NEPH 630	**For Wave 1 & Wave 2: Same** **Methodology: Nephlometry** In an immunochemical reaction, the proteins contained in the human serum form immune complexes with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.
Lipoprotein (a)	BNProSpec	Roche Cobas 8000	**For Wave1: BNProSpec** **Methodology: Nephlometry** In an immunochemical reaction, the proteins contained in the human serum form immune complexes with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration. **For Wave 2: Roche Cobas 8000** **Particle enhanced immunoturbidimetric assay:** Human lipoprotein (a) agglutinates with latex particles coated with anti-Lp(a) antibodies. The precipitate is determined turbidimetrically at 800 / 660 nm.

Wave 2 Only			
Iron	NA	Roche Cobas 8000	**For Wave 2: Roche Cobas 8000** **Colorimetric assay:** **Condition:** pH < 2.0 **Reaction 1:** Transferrin-Fe-complex → apotransferrin + Fe^{3+} **Reaction 2:** Fe^{3+} + ascorbate → Fe^{2+} **Reaction 3:** Fe^{2+} + FerroZine → colored complex **Mechanism:** Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe3+ ions to Fe2+ ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.
Unsaturated Iron Binding Capacity (UIBC)	NA	Roche Cobas 8000	**For Wave 2 : Roche Cobas 8000** **Methodology: Direct determination with FerroZine** **Reaction 1:** Fe(II) + transferrin → Transferrin-Fe(III) + Fe(II) (excess) *(in the presence of alkaline buffer)* **Reaction 2:** Fe(II) (excess) + 3 FerroZine → Fe(II)-(FerroZine) **Mechanism:** The color intensity is directly proportional to the unbound excess iron concentration and indirectly proportional to the unsaturated iron binding capacity. It is determined by measuring the increase in absorbance photometrically.
Ferritin	NA	Roche Cobas 8000	**For Wave 2: Roche Cobas 8000** **Methodology: Electrochemiluminescence Immunoassay (ECLIA)** **Sandwich principle** A biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complexa) form a sandwich complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

			The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Table 5. Venous blood-based markers descriptions with reference range for Waves 1 and 2

Test	Variable Descriptions	Unit	Reference Range
Whole Blood-based Assays			
Hemoglobin	Hemoglobin is a complex protein found in red blood cells that contains an iron molecule. The main function of hemoglobin is to carry oxygen from the lungs to the body tissues, and to exchange the oxygen for carbon dioxide, and then carry the carbon dioxide back to the lungs where it is exchanged for oxygen.	g/dL	Female, ages 18+: 11.7 to 15.7 g/dl, mean 14.0 g/dl; Male, ages 18+: 13.3 to 17.7 g/dl, mean 14.5 g/dl
Platelet Count	Platelets are factors present in blood important for blood clotting. Having more than 450,000 platelets is a condition called thrombocytosis while having less than 150,000 is known as thrombocytopenia.	Platelets per microliter of blood	150,000 to 450,000
Red Blood Cell Count	Red blood cells are the most common type of blood cells and the main cells which carry oxygen in the body. The RBCs contain hemoglobin in their cytoplasm which helps in carrying oxygen.	Mill/cum m	4.7 to 6.0
White Cell Count	White blood cells are a part of your immune system that protects your body from infection. These cells circulate through your bloodstream and tissues to respond to injury or illness by attacking any unknown organisms that enter your body.	cells/cu. mm	4300-10300
Basophils (DLC)	Basophils are a type of white blood cell in your immune system that help defend your body from allergens, pathogens and parasites. Basophils release histamine to improve blood flow to damaged tissue and heparin to prevent unwanted blood clots.	%	0-2

	Abnormal basophil levels may indicate various conditions, from allergic reactions to cancer.		
Eosinophils (DLC)	Eosinophilia happens when your body produces an unusually high number of eosinophils. Eosinophils are one of several white blood cells that support your immune system. Sometimes, certain medical conditions and medications cause high eosinophil levels.	%	1-6
Lymphocytes (DLC)	Lymphocytes are a type of white blood cell. They help your body's immune system fight cancer and foreign viruses and bacteria. Your lymphocyte count can be taken during a normal blood test at your healthcare provider's office. Lymphocyte levels vary depending on your age, race, sex, altitude and lifestyle.	%	20-40
Monocytes (DLC)	Monocytes are a type of white blood cell in your immune system. Monocytes turn into macrophage or dendritic cells when a germ enters your body. The cells either kill the invader or alert other blood cells to help destroy it and prevent infection. High or low monocyte counts may be a sign of a condition that a healthcare provider needs to diagnose.	%	2-10
Neutrophils (DLC)	Neutrophils help your immune system fight infections and heal injuries. Neutrophils are the most common type of white blood cell in your body. An absolute neutrophil count identifies whether your body has enough neutrophils or if your count is above or below a healthy range.	%	40-80
Glycosylated Haemoglobin (HbA1c)	HbA1c is a measure of mean blood glucose level over a period of 8-10 weeks and it remains unaffected by the short term fluctuation in blood glucose levels. The measurement of glycosylated hemoglobin has therefore been accepted for the diagnosis and clinical management of diabetes mellitus. It predicts risk of progression of diabetes complications. This test is also used to monitor patient's compliance with therapeutic regimen.	%	4 to 5.6
Mean Corpuscular Hemoglobin	MCHC (mean corpuscular hemoglobin concentration) measures the average amount of hemoglobin per red blood cell. It's one value on a complete blood count (CBC) that provides information about the health of	gm/dL	32-36

Concentration (MCHC)	your red blood cells. Considered alongside other test results, it can help your healthcare provider diagnose anemia and determine what's likely causing it.		
Mean Corpuscular Hemoglobin (MCH)	Mean corpuscular hemoglobin (MCH) is an estimate of the amount of hemoglobin in an average erythrocyte, derived from the ratio between the amount of hemoglobin and the number of erythrocytes present.	pg	27 to 31
Mean Corpuscular Volume (MCV)	The reference range for mean corpuscular volume, or MCV, in a blood test is 80 to 100 femtoliter per red cell. This reference range may differ slightly depending upon the particular laboratory and the patient's age.	fL	78 to 100
Mean Platelet Volume (MPV)	An MPV blood test measures the average size of your platelets, the blood cells that help your blood clot. When considered alongside other test results on a complete blood count (CBC), an MPV test can help your healthcare provider diagnose blood disorders and other conditions.	fL	6-9.5
Packed Cell Volume (PCV)	The hematocrit (Ht or HCT hematocrit), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood.	%	Male: 47% ±5%; Female: 42% ±5%
Serum-based Tests			
Glucose (Test reported at Metropolis both in Wave 1 & 2 is Estimated Glucose, Calculated analyte in profile along with HbA1C) –Fluoride sample was never part of the project.	The blood glucose test is ordered to measure the amount of glucose in the blood right at the time of sample collection. It is used to detect both hyperglycemia and hypoglycemia, to help diagnose diabetes, and to monitor glucose levels in persons with diabetes.	mg/dL	80 to 99
Lipid Profile			
Cholesterol (total)	Cholesterol and its derivatives are important constituents of cell membranes and precursors of	mg/dL	Less than 200mg/dl

	other steroid compounds. Measurement of serum cholesterol levels can serve as an indicator of liver function, biliary function, intestinal absorption, propensity toward coronary artery disease, and thyroid function. Cholesterol levels are important in the diagnosis and classification of hyperlipoproteinemia. Elevated levels of cholesterol increase the risk for coronary heart disease (CHD). Cholesterol is measured to help assess the patient's risk status and to follow the progress of patient's treatment to lower serum cholesterol concentrations.		
HDL Cholesterol	Low HDL cholesterol levels are strongly associated with an increased risk of coronary heart disease. Hence, the determination of serum HDL cholesterol is a useful tool in identifying high-risk patients. Coronary risk increases markedly as the HDL concentration decreases from 40- to 30 mg/dL. HDL-cholesterol values are also used in the calculation of LDL-cholesterol (see LDL section below).	mg/dL	Larger than 40
LDL Cholesterol	LDL is the main cholesterol-containing particle in plasma. When present in excessive amounts, LDL-C can be deposited in the arterial wall resulting in atherosclerosis. LDL-cholesterol is measured to assess risk for coronary heart disease and to follow the progress of patients being treated to lower LDL-cholesterol concentrations.	mg/dL	Optimal: less than 100; Near Optimal: 100 to 129; Borderline high: 130 to 159; High: 160 to 189; Very High: larger or equal to 190
Triglycerides	Triglycerides are a family of lipids absorbed from the diet and produced endogenously from carbohydrates and fatty acids. High levels of serum triglycerides help mark conditions that are associated with increased risk for coronary heart disease and peripheral atherosclerosis. High triglycerides are associated with increased risk for coronary artery disease in patients with other risk factors, such as low HDL-cholesterol, some patient groups with elevated apolipoprotein B concentrations, and patients with forms of LDL that may be particularly atherogenic.	mg/dL	Desirable fasting triglyceride levels: less than 150; Borderline high: 150 to 199; High: 200 to 499; Very High: larger than 500
VLDL Cholesterol	VLDL is one of the four major lipoprotein particles i.e. HDL, LDL, VLDL, and chylomicrons. It transports the	mg/dL	Less than 30

	maximum amount of triglycerides. High levels are a risk factor for coronary artery disease.		
LDL/HDL ratio	A high LDL/HDL ratio is a risk factor for coronary artery disease.	NA	2.5 to 3.5
Chol/HDL ratio	The total cholesterol to HDL cholesterol ratio is also referred to as the cholesterol ratio. The goal is to keep this ratio below 5:1, with the ideal being below 3.5:1.	NA	Less than 5:1 (ideally below 3.5:1)
Metabolic Panel			
Bilirubin (Total and Direct)	Once formed from the reticuloendothelial cells, bilirubin is transported to the liver bound to albumin as it is water insoluble. This fraction of bilirubin is referred to as indirect or unconjugated bilirubin. In the liver, bilirubin is conjugated to glucuronic acid (mono- and di-glucuronides) to form conjugated bilirubin by the enzyme uridyl diphosphate glucanosyltransferase. Total bilirubin is the sum of the unconjugated and conjugated fractions. Bilirubin is elevated in conditions causing obstruction of the bile duct, hepatitis, cirrhosis, hemolytic disorders, and several inherited enzyme deficiencies.	mg/dL	Direct: 0 to 0.5; Total (direct + indirect): 0.2 to 1.2
Bilirubin (Indirect)	Bilirubin is elevated in conditions causing obstruction of the bile duct, hepatitis, cirrhosis, hemolytic disorders, and several inherited enzyme deficiencies.	mg/dL	0.1 to 1.0
Total Protein	Plasma proteins derive primarily from synthesis in the liver, plasma cells, lymph nodes, spleen, and bone marrow. In disease states, both the total plasma protein level and the ratio of the individual fractions may be dramatically altered from their normal values. The total protein test measures the total amount of two classes of proteins found in the fluid portion of your blood. These are albumin and globulin. Proteins are important parts of all cells and tissues. Albumin helps prevent fluid from leaking out of blood vessels. Globulins are an important part of your immune system. The A/G ratio has commonly been used as an index of the distribution between the albumin and globulin fractions. This ratio can be significantly altered in such conditions as cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosis, and in some acute and chronic infections.	g/dL	6.2 to 8.1
Albumin	Albumin is the major serum protein in normal individuals. Elevated serum albumin levels are usually the result of dehydration. Decreased albumin levels are found in a wide variety of conditions, including	g/dL	3.2 to 4.6

	kidney disease, liver disease, malabsorption, malnutrition, severe burns, infections, and cancer.		
Globulin	Globulin is made in the liver by the immune system. Globulins play an important role in liver function, blood clotting, and fighting infection. There are four main types of globulins. They are called alpha 1, alpha 2, beta, and gamma. Serum globulin tests are done to find out the following: liver damage or disease, kidney disease, nutritional problems, autoimmune disorders, and certain types of cancer like multiple myeloma.	g/dL	1.8 to 3.6
A/G ratio	A/G Ratio is the ratio of albumin to globulin in serum.	NA	1.1 to 2.2
Alanine Aminotransferase (ALT)	Alanine aminotransferase (ALT) is an enzyme involved in amino acid metabolism. It is found in many tissues, but the highest levels are found in liver and kidney tissues. Tissue destruction leads to the release of the intracellular enzyme into the circulating blood. ALT is increased with liver damage and is used to screen for and/or monitor liver disease.	IU/L	0 to 45
Aspartate Aminotransferase (AST)	Aspartate aminotransferase (AST) is generally localized to the liver and heart, whose activity is measured in a lab test to check for damage to these organs. It is also commonly known as aspartate transaminase.	U/L	0 to 35
Alkaline Phosphatase (ALP)	Serum alkaline phosphatase is found in liver, bone, intestine and placenta. This test is used to help detect two groups of diseases -hepatobiliary and bone disease. High ALP levels in liver indicate cirrhosis, hepatitis, blockage in bile duct.	IU/L	41 to 129
Gamma-Glutamyl Transferase (GGT)	Gamma-glutamyl transferase (GGT) is an enzyme produces in liver, kidneys and pancreas. It is currently the most sensitive enzymatic indicator of liver disease, mostly of obstructive nature like obstructive jaundice. It also serves as a screening test for occult alcoholism.	U/L	Male, all ages: 12 to 64; Females, all ages: 6 to 29
Blood Urea Nitrogen (BUN)	A BUN test measures the amount of urea nitrogen that's in the blood. The main causes of an increase in BUN are: high protein diet, decrease in glomerular filtration rate (GFR) (suggestive of renal failure) and in blood volume (hypovolemia), congestive heart failure,	mg/dL	8 to 23

	gastrointestinal hemorrhage, and increased catabolism. The determination of serum urea nitrogen is a widely used test for the evaluation of kidney function. The test is frequently requested in conjunction with the serum creatinine test for the differential diagnosis of prerenal (cardiac decompensation, water depletion, increased protein catabolism), renal (glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, tubular necrosis), and postrenal (obstructions of the urinary tract) hyperuricemia.		
Creatinine	Creatinine is a waste product formed in muscles from the high energy storage compound, creatine phosphate. The amount of creatinine produced is fairly constant (unlike Urea) and is primarily a function of muscle mass. It is not greatly affected by diet, age, sex or exercise. Creatinine is removed from plasma by glomerular filtration and then excreted in urine without any appreciable resorption by the tubules. Creatinine is used to assess renal function; however, serum creatinine levels do not start to rise until renal function has decreased by at least 50%.	mg/dL	Female: 0.40 to1.10; Male: 0.50 to1.20
Cystatin C	Cystatin C is a cysteine proteinase inhibitor with a relative molecular weight of 13.250 Da and is formed by all nucleated cells investigated. Since it is formed at a constant rate and freely filtered by the healthy kidney, this protein is a good marker of renal function. Serum concentrations of cystatin C are almost totally dependent on the glomerular filtration rate. A reduction in the glomerular filtration rate (GFR) causes a rise in the concentration of cystatin C. Cystatin C has been shown to be not affected by factors such as muscle mass or nutrition, factors which have been demonstrated to affect, e.g. creatinine values. In addition, a rise in creatinine does not become evident until the GFR has fallen by approximately 50 %Cystatin C can be used for calculation of eGFR (estimated glomerular filtration rate), a measure that is used in combination with albumin excretion in urine for diagnosis and staging of kidney disease. Evidence exists that increased levels of cystatin C in serum are associated with an increased risk of all cause and cardiovascular mortality in the general populations. Notably, a linear correlation between serum cystatin C levels and cardiovascular mortality is observed.	mg/L.	0.62 to 1.11

Uric acid	Uric acid is a product of the metabolic breakdown of purine nucleotides. Acute uric acid nephropathy can cause acute renal failure due to uric acid precipitation within tubules. This is most commonly seen in patients with hematologic malignancies.	mg/dL	3.5 to 7.2
Calcium	Calcium is the most abundant and one of the most important minerals in the body. It is essential for cell signaling and the proper functioning of muscles, nerves, and the heart. Calcium is needed for blood clotting and is crucial for the formation, density, and maintenance of bones and teeth.	mg/dL	8.4 to 10.2
Phosphorous	Phosphorus is an important element that's vital to several of the body's physiological processes. It helps with bone growth, energy storage, and nerve and muscle production. Many foods — especially meat and dairy products — contain phosphorus, so it's usually easy to get enough of this mineral in your diet. The ratio of phosphate to calcium in the blood is approximately 6:10. An increase in the level of phosphorus causes a decrease in the calcium level. The mechanism is influenced by interactions between parathormone and vitamin D. Hypoparathyroidism, vitamin D intoxication and renal failure with decreased glomerular phosphate filtration give rise to hyperphosphatemia	mg/dL	2.3-4.7
Thyroid Function Tests			
Total Thyroxine (T4)	Clinically, T4 measurements have long been recognized as an aid in the assessment & diagnosis of thyroid status. Elevated T4 values are characteristically seen in patients with overt hyperthyroidism, while T4 levels are generally depressed in patients with overt hypothyroidism.	ng/dL	5.1 to 14.1
Total Triiodothyronine (T3)	Clinically measurements of serum T3 concentration are especially valuable in diagnosing hyperthyroidism. T3 plays an important role in the maintenance of the euthyroid state. Serum T3 measurement can be a valuable component of a thyroid screening panel in diagnosing certain disorders of thyroid function as well as conditions caused by iodine deficiency.	ng/dL	84.6 to 201.8
Thyroid-stimulating	TSH assay is used as an aid in the assessment of thyroid status, diagnosis of thyroid disease and treatment of thyroid disease.	µIU/mL	0.54 to 5.3

Hormone (TSH) (Ultrasensitive)			
Other Tests			
Vitamin B12	Adequate intakes of vitamin B12 are necessary for the production of red blood cells and to prevent anemia. Vitamin B12 is also important for neurological function and the synthesis of our DNA. A vitamin B12 blood test is most often performed to test for deficiencies.	pg/mL	197 to 771
Folic acid	Folic acid deficiency can be caused by low dietary intake, malabsorption due to gastrointestinal diseases, inadequate utilization due to enzyme deficiencies or folate antagonist therapy, such as alcohol and oral contraceptives, and excessive folate demand, such as during pregnancy. Deficiencies of both vitamin B12 and folate can lead to megaloblastic (macrocytic) anemia.	ng/mL	3.1 to 17.5
25 Hydroxy, Vitamin D	Vitamin D helps our body absorb calcium and maintain strong bones throughout your entire life. It can be an important indicator of osteoporosis (bone weakness) and rickets (bone malformation). Vitamin D (25-OH VITAMIN D) is a cause of secondary parathyroidism and diseases related to impaired bone metabolism (like rickets, osteoporosis, osteomalacia). Reduced vitamin D concentrations in blood (vitamin D insufficiency) have been associated with an increasing risk of many chronic illnesses, including common cancers, autoimmune or infectious disease or cardiovascular problems.	ng/ml	Deficiency: less than 10; Insufficiency: 10 to 30; Sufficiency: 30 to100; Hypervitaminosis: larger than 100
Homocysteine	Homocysteine is metabolized to either cystein or methionine, mainly by the folate and cobalamin dependent enzyme methionine synthase. Hyperhomocysteinemia is caused by nutritional and genetic deficiencies. The majority of elevated homocysteine cases in the general population are due to deficiency of folic acid, vitamin B6 and vitamin B12.	µmol/L	5.46 to 16.2

NT pro BNP	B-type natriuretic peptide, N-terminal pro (NT-proBNP): BNP is a hormone produced by the heart. N-terminal (NT)-pro hormone BNP (NT-proBNP) is a non-active prohormone that is released from the same molecule that produces BNP. Both BNP and NT-proBNPare released in response to changes in pressure inside the heart. These changes can be related to heart failure and other cardiac problems. A test for B-type natriuretic peptide (BNP) or N-terminal pro b-type natriuretic peptide (NT-proBNP) is primarily used to help diagnose and evaluate the severity of heart failure.	pg/mL	Less than 450; cut-off points often vary by age
High-sensitivity C-Reactive Protein (hsCRP)	C-reactive protein (CRP) is a substance produced by the liver in response to inflammation and/or infections. High CRP levels have been associated with increased risk of cardiovascular events and mortality, and metabolic syndrome.	mg/dL	less than 3
Lipoprotein (a)	The individual concentration of Lp(a) in the blood depends on genetic factors; the range of variation in a population is relatively large. Elevated concentrations of Lp(a) are a risk factor for coronary heart disease. Determination of Lp(a) may be useful to guide management of individuals with a family history of or with existing coronary heart disease.	mg/dL	0 to 30
Wave 2 Only			
Iron	Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissue of the two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. Iron determinations are performed for the diagnosis and monitoring of microcytic anemia (e.g. due to iron metabolism disorders and hemoglobinopathy), macrocytic anemia (e.g. due to vitamin B12 deficiency, folic acid deficiency and drug-induced metabolic disorders of unknown origin) as well as normocytic anemias such as renal anemia (erythropoetin deficiency), hemolytic anemia, hemoglobinopathy, bone marrow disease and toxic bone marrow damage.	µg/dL	33-193

Total Iron Binding Capacity (TIBC)	The sum of the serum iron and UIBC represents total iron-binding capacity (TIBC). TIBC is a measurement for the maximum iron concentration that transferrin can bind. The serum TIBC varies in disorders of iron metabolism. In iron-deficiency anemia the TIBC is elevated, and the transferrin saturation is lowered to 15 % or less. Low serum iron associated with low TIBC is characteristic of the anemia of chronic disorders, malignant tumors, and infections.	µg/dL	250-450
Transferrin Saturation	**Transferrin saturation** (TS), measured as a percentage, is a medical laboratory value. It is the value of serum iron divided by the total iron-binding capacity of the available transferrin, the main protein that binds iron in the blood, this value tells a clinician how much serum iron is bound. For instance, a value of 15% means that 15% of iron-binding sites of transferrin are being occupied by iron. The three results are usually reported together. A low transferrin saturation is a common indicator of iron deficiency anemia whereas a high transferrin saturation may indicate iron overload or hemochromatosis.	%	14-50
Unsaturated Iron Binding Capacity (UIBC)	The total iron content of the body is about 3 to 3.5 g. Of this amount about 2.5 g is contained in erythrocytes or their precursors in the bone marrow. Plasma contains only about 2.5 mg of iron. Iron is transported as Fe(III) bound to the plasma protein apotransferrin. The apotransferrin-Fe(III) complex is called transferrin. Normally only about one third of the iron-binding sites of transferrin are occupied by Fe(III). The additional amount of iron that can be bound is the unsaturated (or latent) iron-binding capacity (UIBC).	µg/dL	Male: 125-345 Female: 135-392

Ferritin	Clinically, a threshold value of 20 µg/L (ng/mL) has proved useful in the detection of prelatent iron deficiency. This value provides a reliable indication of exhaustion of the iron reserves that can be mobilized for hemoglobin synthesis. Latent iron deficiency is defined as a fall below the 12 µg/L (ng/mL) ferritin threshold. These two values necessitate no further laboratory elucidation, even when the blood picture is still morphologically normal. If the depressed ferritin level is accompanied by hypochromic, microcytal anemia, then manifest iron deficiency is present. When the ferritin level is elevated and the possibility of a distribution disorder can be ruled out, this is a manifestation of iron overloading in the body. 400 µg/L (ng/mL) ferritin is used as the threshold value. Elevated ferritin values are also encountered with the following tumors: acute leukemia, Hodgkin's disease and carcinoma of the lung, colon, liver and prostate. The determination of ferritin has proved to be of value in liver metastasis.	ng/mL	Men, 20-60 years: 30-400 ng/mL Women, 17-60 years: 13-150 ng/mL

3. Quality Control

3.1. Shipping

Once blood was collected, it was shipped to the central Delhi Metropolis laboratory and MedGenome in Bengaluru in Wave 1. The recording and monitoring of the shipping times and transport temperatures were essential to guarantee the quality of the samples. Literature has shown that both shipping duration and temperature could potentially either increase or decrease the values reported by the laboratory, depending on the analytes being measured. Four tubes per respondent (A, B, C, and E) were transported to the local Metropolis laboratory for processing and further shipment to the Delhi central Metropolis laboratory. One tube (D) per respondent stayed at the local hospital in a tabletop fridge until it was picked up later in the day by a courier to be shipped to MedGenome in Bengaluru. This procedure was changed in Wave 1 phase 2: All blood would be transported to the local Metropolis laboratory, and the courier collected the blood for MedGenome from there. The supervisor at the local hospital/Metropolis laboratory was responsible for recording the shipment times in the online Blood Management System (BMS). Once received by the local Metropolis laboratory, the blood shipped to the central Metropolis was divided into a 4°C and -20°Cshipment.

For Wave 2, within four hours of collection, all samples were shipped to a local Metropolis Healthcare laboratory for centrifugation or processed on-site if required. The sample collection and receiving details were entered into the BMS by trained staff at the local laboratory. Each state had unique login credentials to record these details. Within two hours of reaching the local laboratory, SSTs and PPTs were centrifuged at 3500 revolutions per minute (rpm) for 10 minutes. The time of sample processing was entered, and images of the centrifuged samples were taken and uploaded. The images were used to record and compare the hemolysis status of samples at the local laboratories and after they were received at the central laboratory in Delhi. The EDTA tubes were stored unprocessed in the cold chain. The samples were packed per the protocol described in the previous section and shipped to the central Metropolis Healthcare laboratory in Delhi within six hours of receiving samples at the local laboratory. The time of shipment was also entered into the BMS to track the samples in real time.

The shipment to the Metropolis laboratory was monitored by single-use Appresys temperature loggers that recorded the temperature of the cold chain at 5-minute intervals from the time of collection to the central Metropolis laboratory. The median receiving temperature was 6.1°C (IQR: 4.1 degree Celsius to 8.1 °C). The ID numbers of the temperature loggers were recorded by the supervisor. At every step, the time the blood was received and shipped out again was recorded. In Wave 1, the temperature logger data were uploaded to the server at AIIMS. For Wave 2, the temperature logger data was uploaded by the USC research staff in India.

Table 6. Overview table of transit times and temperatures for Wave 1

From	To	Purpose	Temperature	Max transit time
Local hospital	MedGenome	DNA	4°C	24 hours
Local hospital	Local Metropolis	Centrifuge	4°C	4 hours
Local Metropolis	Central Metropolis	Assay	4°C / -20°C	24 hours
Central Metropolis	AIIMS	Storage	4°C	4 hours

Table 7. Overview table of transit times and temperatures for Wave 2

From	To	Purpose	Temperature	Max transit time
Collection site	Local Metropolis	Shipment	4°C	4 hours
Local Metropolis	Local Metropolis	Sample receipt and centrifugation	4°C	2 hours
Local Metropolis	Local Metropolis	Shipment	4°C	6 hours
Local Metropolis	Central Metropolis	Sample receipt and assay	4°C	24 hours

Central Metropolis	Central Metropolis	Assay result upload	-	30 hours

An automated procedure in the BMS checked transit times to ensure they were not exceeded. If a tube was in transit for too long, an automated email was sent to the relevant personnel, who could follow up with the laboratories and the couriers. The temperature logs were checked weekly to ensure temperatures had stayed within the required range (2 – 8 °C). The overall hemolysis rate was another indicator of sample quality. A subjective but standardized scale was used to check for hemolysis in the samples. For resolving discrepancies, a consensus was reached through discussion between the Metropolis staff and the research team. Metropolis staff recorded and uploaded pictures of centrifuged samples on the BMS server at the local and the central laboratory. The sample pictures were recorded such that the sample as well as the barcode was visible to correctly identify the sample in question. The status of sample hemolysis was recorded at three stages: after centrifugation at the local lab, at the central lab, and when the cryovials were received at AIIMS. The hemolysis rate was closely monitored and reviewed fortnightly for each state. The overall hemolysis rate was 6.7%, with 6.2% for SSTs and 1.2% for PPTs.

3.2. Laboratory Procedures

For Wave 1, after Central Metropolis laboratory in Delhi received the shipped specimens, serum separation tubes (tubes A and B) and plasma preparation tube (E) were centrifuged again at 3500 rpm for 10 minutes. Whole blood from tube C was used to create a 5-spot dried blood spot (DBS) card, using Whatman 903 protein saver card. The DBS cards were dried for at least four hours or overnight. Part of the serum and whole blood specimens were used for the planned assays at the Metropolis laboratory. The remaining serum, plasma, and buffy coat were transferred in cryovials to AIIMS, along with the DBS cards. Plasma cryovials were stored at -80°C for subsequent neurodegenerative biomarker assays. Similar to Wave 1, in Wave 2, serum and plasma tubes were centrifuged again at the central Metropolis laboratory at 3500 rpm for 10 minutes. After running the serum- and whole-blood-based assays, the remaining serum, whole blood for MedGenome, and plasma were stored in cryovials at -80°C. Plasma cryovials were later shipped to AIIMS for running the neurodegenerative biomarker assays.

For assay quality control (QC), Delhi Metropolis laboratory runs QC samples every morning before testing clinical or LASI-DAD study samples. The number of QC samples measured varies from two (low or high levels) to three (low, mid, or high levels), depending on the assay. The laboratory protocol stipulates that testing of clinical/study samples will not be initiated if value of one QC sample is beyond three standard deviations (S.D.) from the mean During the period when LASI-DAD study samples were tested, the LASI-DAD team independently monitored QC sample results in real-time. For all assays, QC sample values were within the criteria established by Metropolis laboratory.

4. Results

Here, we provide a table of descriptive results for the bioassays. The sample size of the bioassays varies slightly due to insufficient serum quantity for some tests or unreliable values resulting from questionable quality of whole blood specimens.

Table 8. Descriptive Results for Wave 1 and Wave 2

Test		N	mean	sd	min	max
Complete Blood Cell Counts (CBC)						
Hemoglobin	W 1	2833	12.68	1.91	3.8	20.5
	W 2	3196	12.27	1.882	3.3	19.5
Platelet Count	W 1	2826	232.18	83.68	33	1368
	W 2	3196	232.36	82.87	50	1016
Red Blood Cell Count	W 1	2812	4.43	0.64	1.38	8.9
	W 2	3196	4.37	0.62	1.54	7.87
Red Cell Distribution Width (RDW)	W 1	2812	15.27	2.09	11.7	31.5
	W 2	3196	15.48	2.05	12.1	40
Total Leucocyte Count	W 1	2822	7293.20	2255.58	1600	39500
	W 2	3196	7165.83	2265.37	1600	28700
Mean Corpuscular Haemoglobin Concentration (MCHC)	W 1	2812	32.62	1.26	22.8	46
	W 2	3196	32.39	1.43	23.1	49.2
Mean Corpuscular Hemoglobin (MCH)	W 1	2812	28.79	3.61	11.2	44
	W 2	3196	28.28	3.66	14.1	51.5
Mean Corpuscular Volume (MCV)	W 1	2812	88.09	9.34	49.2	130.8
	W 2	3196	87.16	9.54	51.6	146.8
Packed Cell Volume (PCV)	W 1	2780	38.79	5.33	15	67.4
	W 2	3196	37.86	5.15	13.1	63.3

Glycosylated haemoglobin (HbA1c)	W 1	2831	6.29	1.56	3.8	20.7
	W 2	3169	6.25	1.46	3.8	15.1
Serum-based Tests						
Glucose	W 1	2831	133.80	44.11	62.36	455.55
	W 2	3169	132.60	41.82	62.36	386.67
Lipid Profile						
Cholesterol (total)	W 1	2892	183.86	42.13	62	409
	W 2	3241	179.40	42.90	65.9	427.9
HDL Cholesterol	W 1	2892	44.49	11.90	7	135
	W 2	3241	45.82	12.08	7.1	137.9
LDL Cholesterol	W 1	2883	111.36	35.45	12	310.2
	W 2	3242	104.70	35.72	13.4	331.5
VLDL Cholesterol	W 1	2761	26.32	11.22	7.4	59.8
	W 2	3080	27.07	11.01	3.16	103.32
LDL/HDL RATIO	W 1	2883	2.63	0.98	0.48	7.96
	W 2	3241	2.41	0.99	0.4	14.98
Chol/HDL Ratio	W 1	2892	4.35	1.48	1.67	46.71
	W 2	3241	4.11	1.28	1.53	18.63
Triglycerides	W 1	2892	144.83	89.31	37	1501
	W 2	3242	148.95	85.43	15.8	1242.1
Liver Function Tests						
Bilirubin (Total and Direct)	W 1	2889	0.62	0.33	0.11	2.87
	W 2	3236	0.51	0.29	0.1	5.51
Bilirubin Direct	W 1	2886	0.23	0.11	0.1	1.3
	W 2	3212	0.17	0.89	0.1	2.35
Bilirubin Indirect	W 1	2889	0.39	0.23	0.01	2.16
	W 2	3225	0.35	0.21	0.02	3.16
Total Protein	W 1	2889	7.42	0.57	5.16	10.4
	W 2	3237	7.39	0.54	4.39	10.35
Albumin	W 1	2889	4.14	0.33	2.2	6.5
	W 2	3236	4.21	0.34	2.32	5.29
Globulin	W 1	2889	3.28	0.49	1.23	7.1
	W 2	3237	3.18	0.49	1.41	6.93
A/G Ratio	W 1	2889	1.29	0.22	0.42	3.42
	W 2	3237	1.36	0.25	0	2.88
Alanine Aminotransferase (ALT)	W 1	2889	19.04	13.68	6	303
	W 2	3236	17.99	21.58	6	867

Aspartate Aminotransferase (AST)	W 1	2889	26.19	15.75	8	402
	W 2	3236	25.01	14.92	4.2	556.5
Alkaline Phosphatase	W 1	2889	94.22	36.07	5	644
	W 2	3236	103.03	35.33	34	609
Gamma-Glutamyl Transferase (GGT)	W 1	2887	26.07	40.33	4	1288
	W 2	3216	21.22	28.71	4	748
Renal Function Tests and Electrolytes						
Blood Urea Nitrogen (BUN)	W 1	2892	12.75	5.33	3.8	100.7
	W 2	3243	12.53	5.08	3.8	76
Creatinine	W 1	2892	0.89	0.45	0.38	10.36
	W 2	3243	0.91	0.41	0.21	9.36
Uric acid	W 1	2891	4.93	1.39	1.2	13.1
	W 2	3241	4.97	1.42	1.1	13.3
Calcium	W 1	2892	9.17	0.50	5.8	11.9
	W 2	3242	9.14	0.45	6.61	11.96
Thyroid Function Tests						
Total Thyroxine (T4)	W 1	2880	7.56	1.61	1.26	16.42
	W 2	3242	7.52	1.68	0.60	20.9
Total Triiodothyronine (T3)	W 1	2868	90.70	21.01	40.72	479.62
	W 2	3239	111.49	25.13	23.4	362
TSH (Ultrasensitive)	W 1	2890	3.40	12.85	0.003	453.224
	W 2	3243	4.36	14.12	0.05	452
Other Tests						
Vitamin B12	W 1	2709	379.95	311.61	83	1970
	W 2	3243	488.60	454.18	100	2000
Folic acid	W 1	2799	6.33	3.71	1	20
	W 2	3241	7.58	5.10	0.91	20
25 Hydroxy, Vitamin D	W 1	2880	20.86	11.86	3.1	154.9
	W 2	3238	24.36	11.86	3.11	110
Homocysteine	W 1	2879	22.03	14.08	4.56	211.26
	W 2	3221	19.83	13.72	2.54	180.35
NT pro BNP	W 1	2873	368.70	1251.99	5	32642
	W 2	3226	339.07	909.39	6.23	17788
High-sensitivity C-Reactive Protein (hsCRP)	W 1	2848	4.88	11.26	0.15	158.9
	W 2	3238	4.69	13.02	0	311.75
Lipoprotein (a)	W 1	2813	45.80	404.72	2.42	12400
	W 2	3242	34.50	35.84	0.3	356.51

5. Acknowledgement

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6. References

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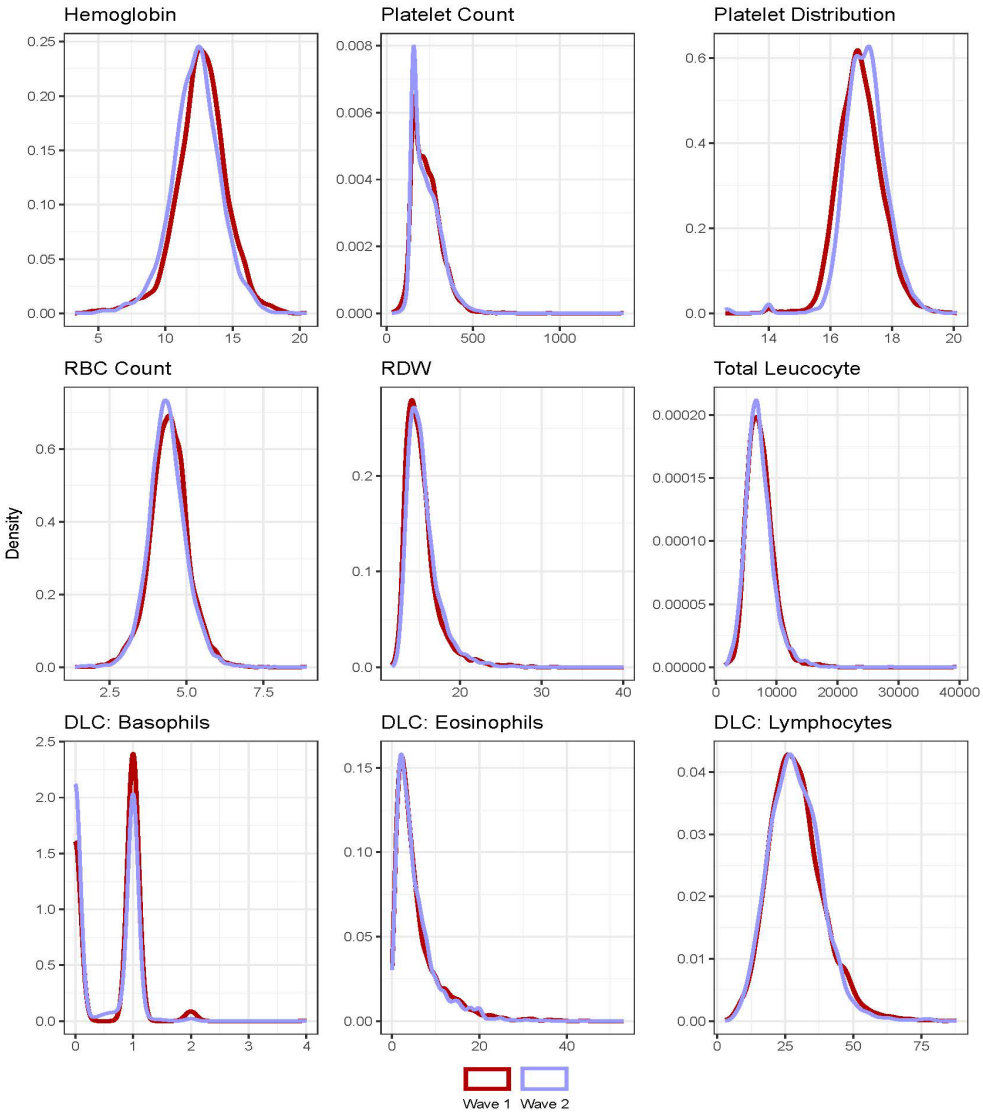
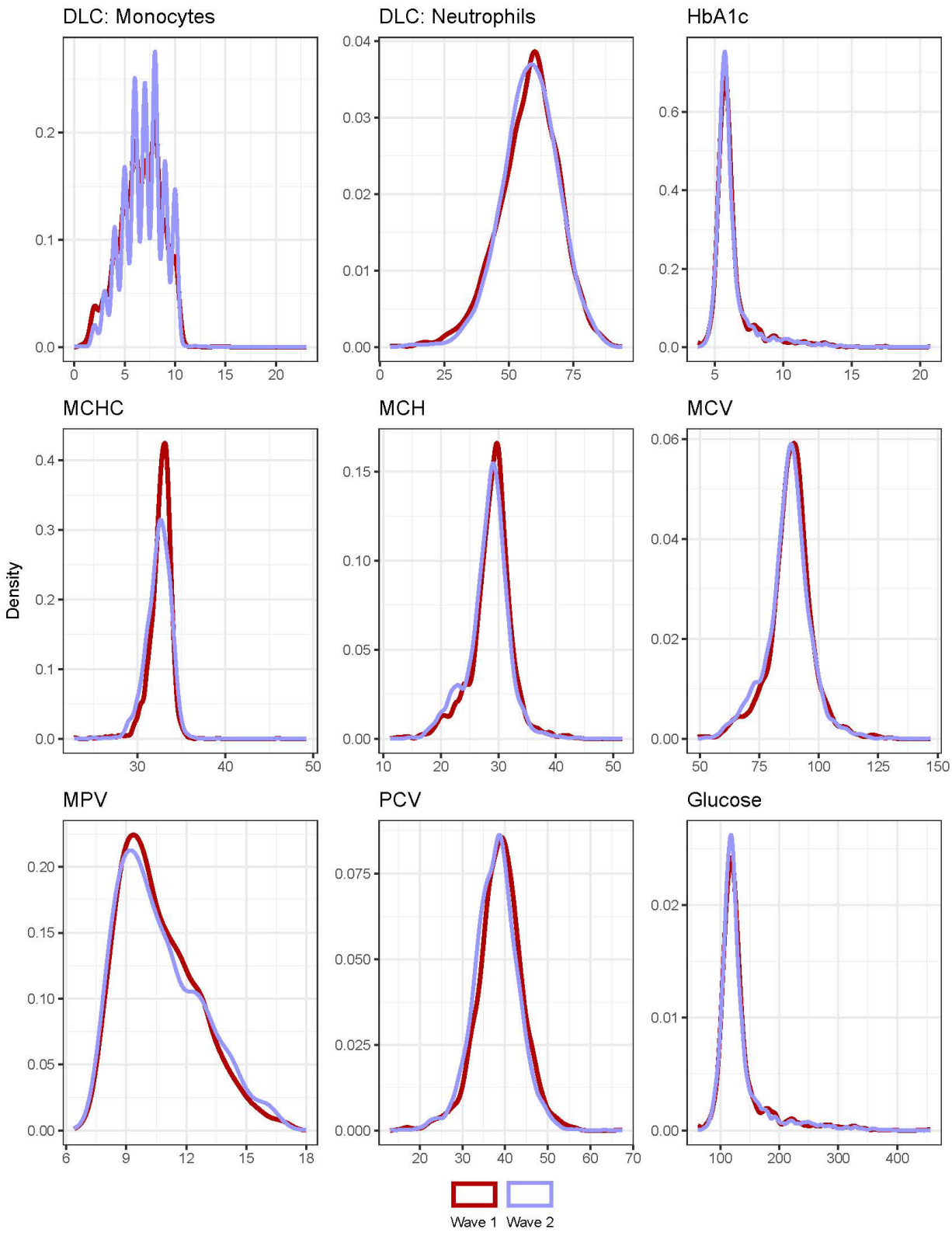
7. Appendix

Figure 2. Descriptive graphs for Wave 1and Wave 2

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DLC: Neutrophils
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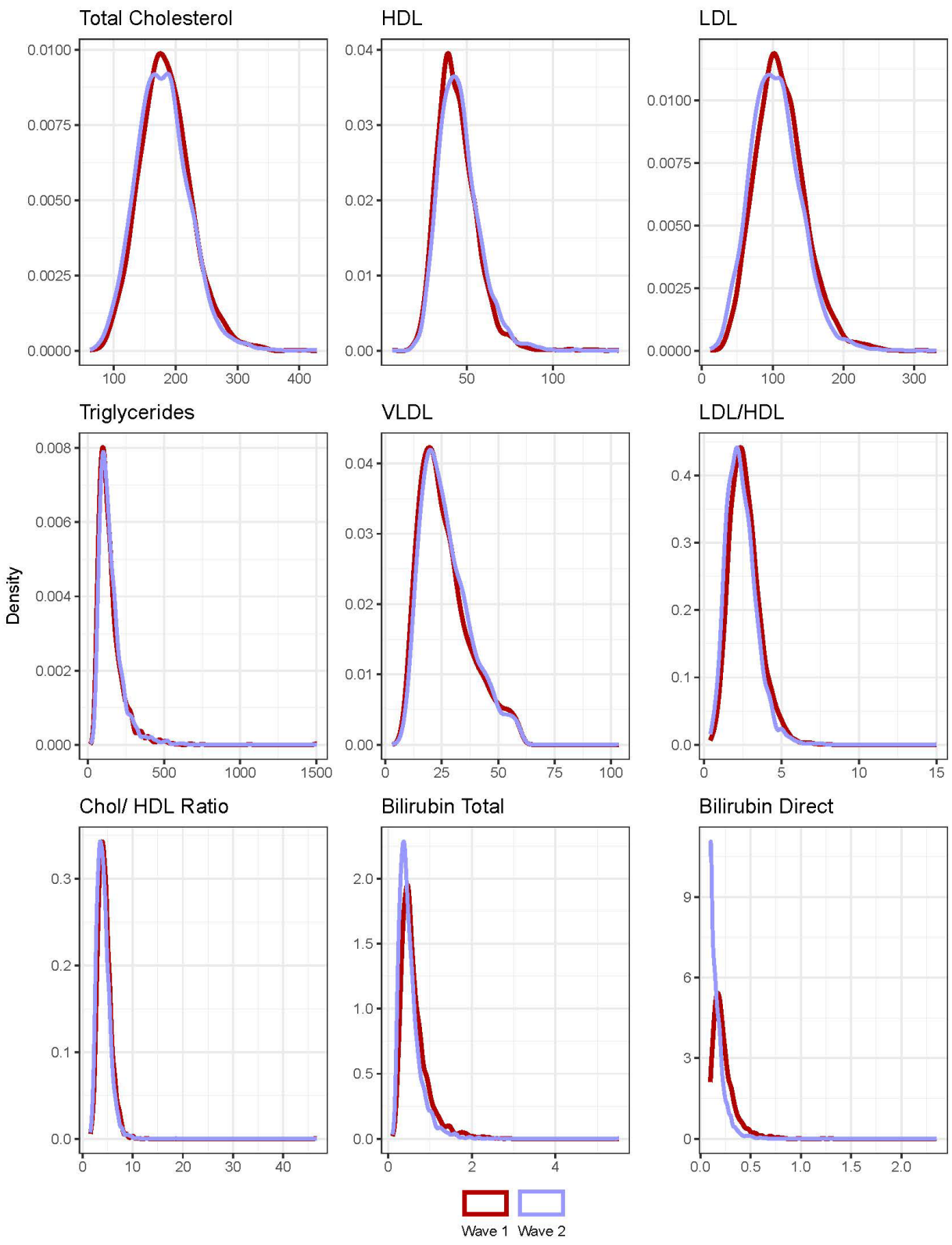
MCHC
MCH
MCV
MPV
PCV
Glucose
Density
Wave 1
Wave 2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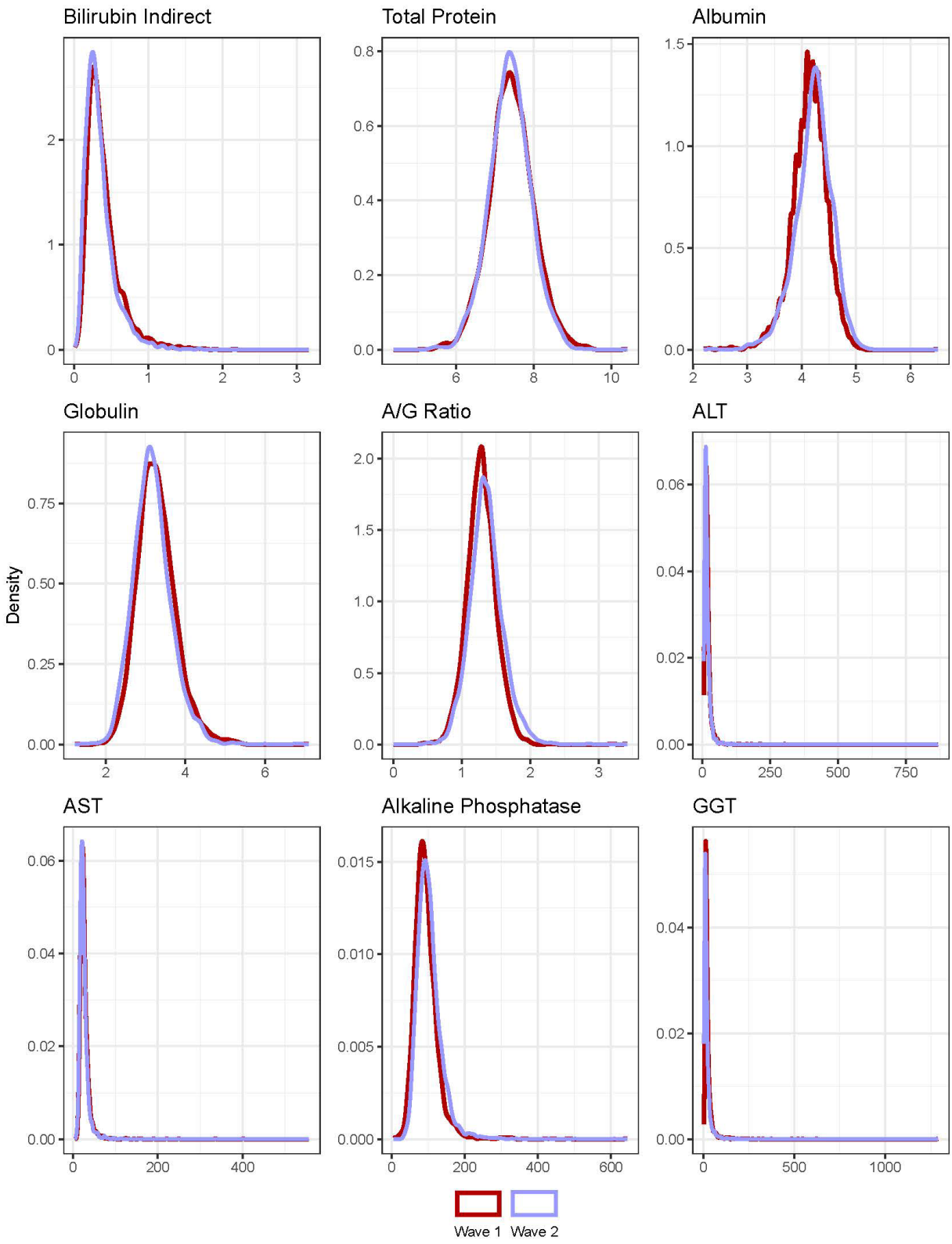
Density
Wave 1
Wave 2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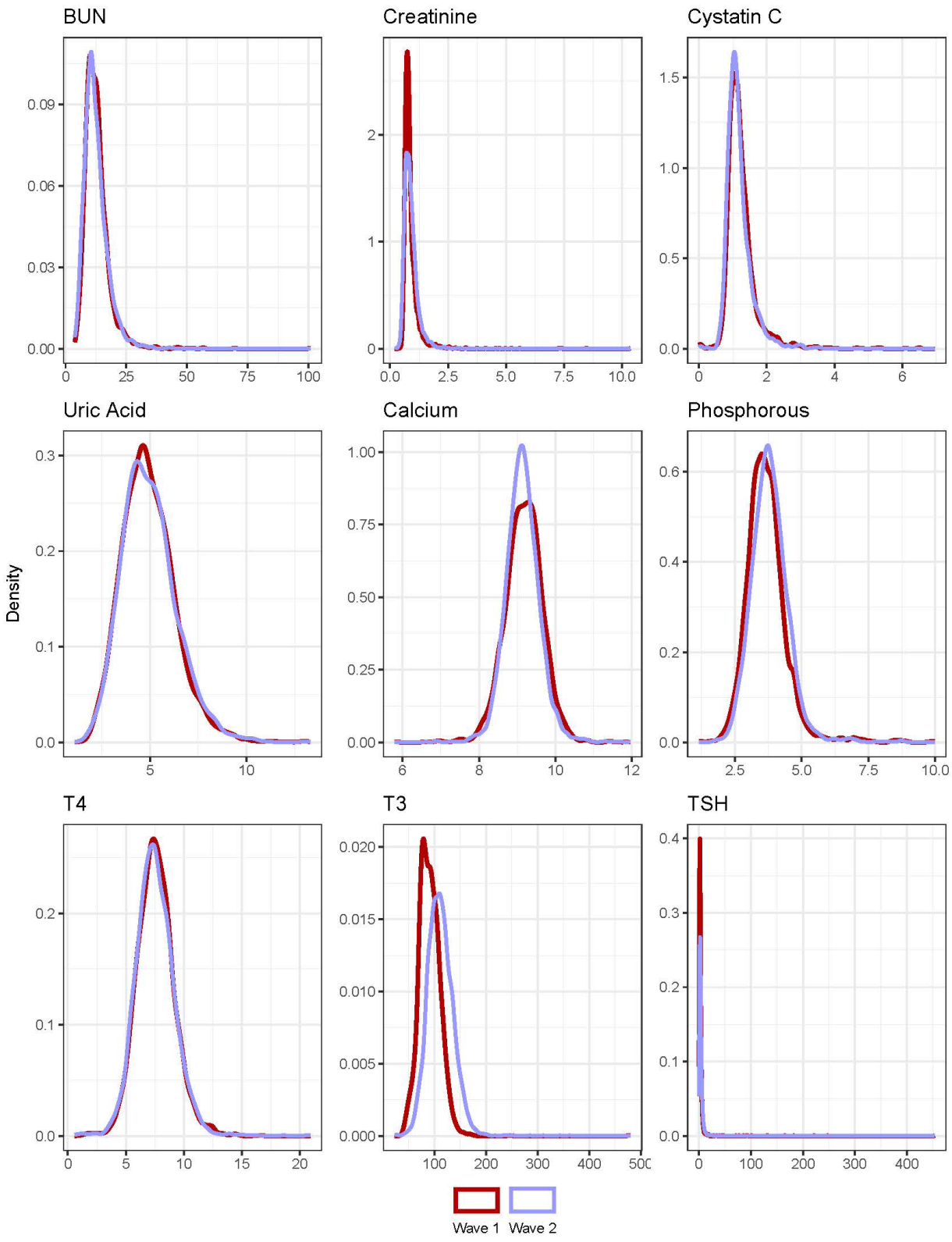
AST
Alkaline Phosphatase
GGT
Density
Wave 1
Wave 2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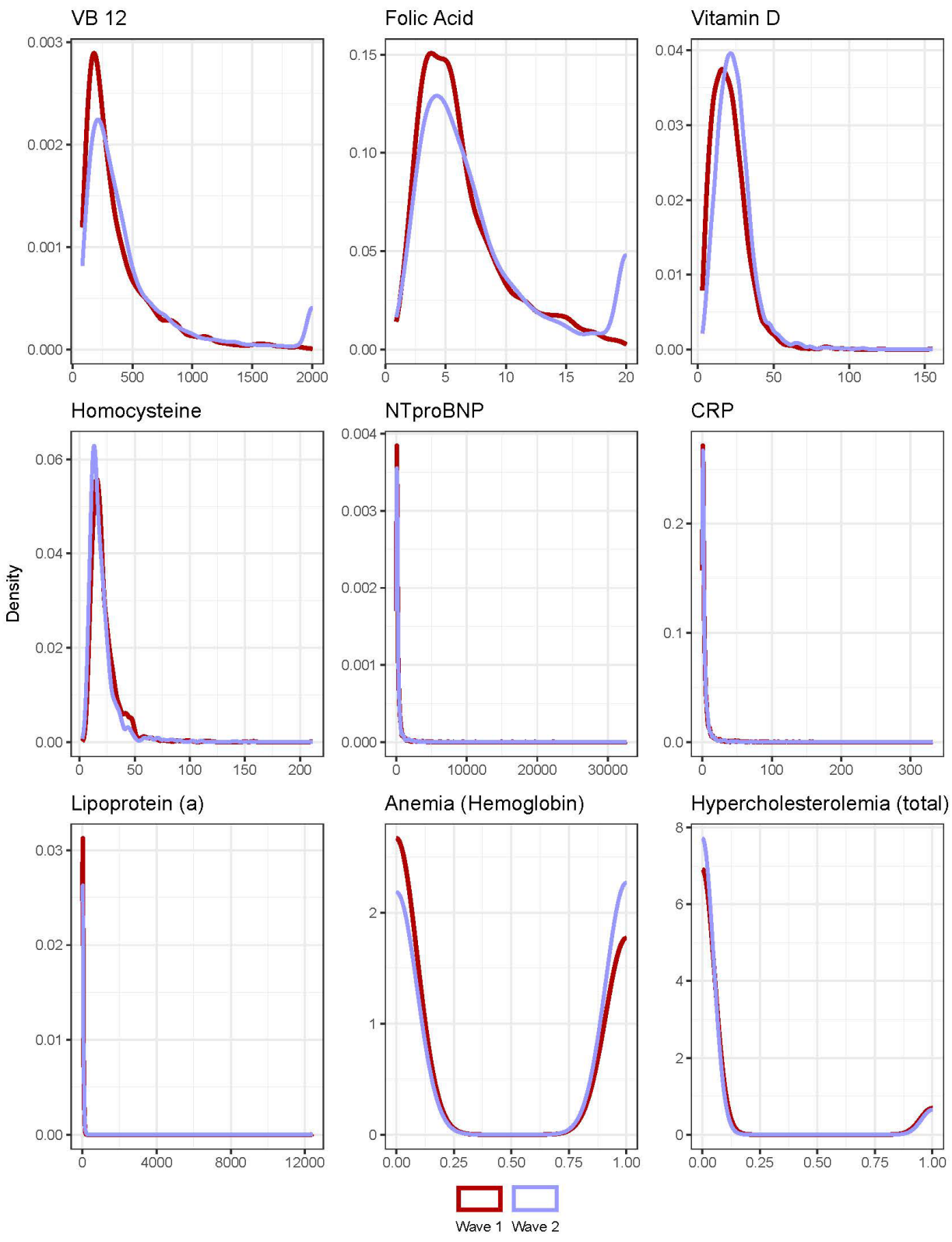
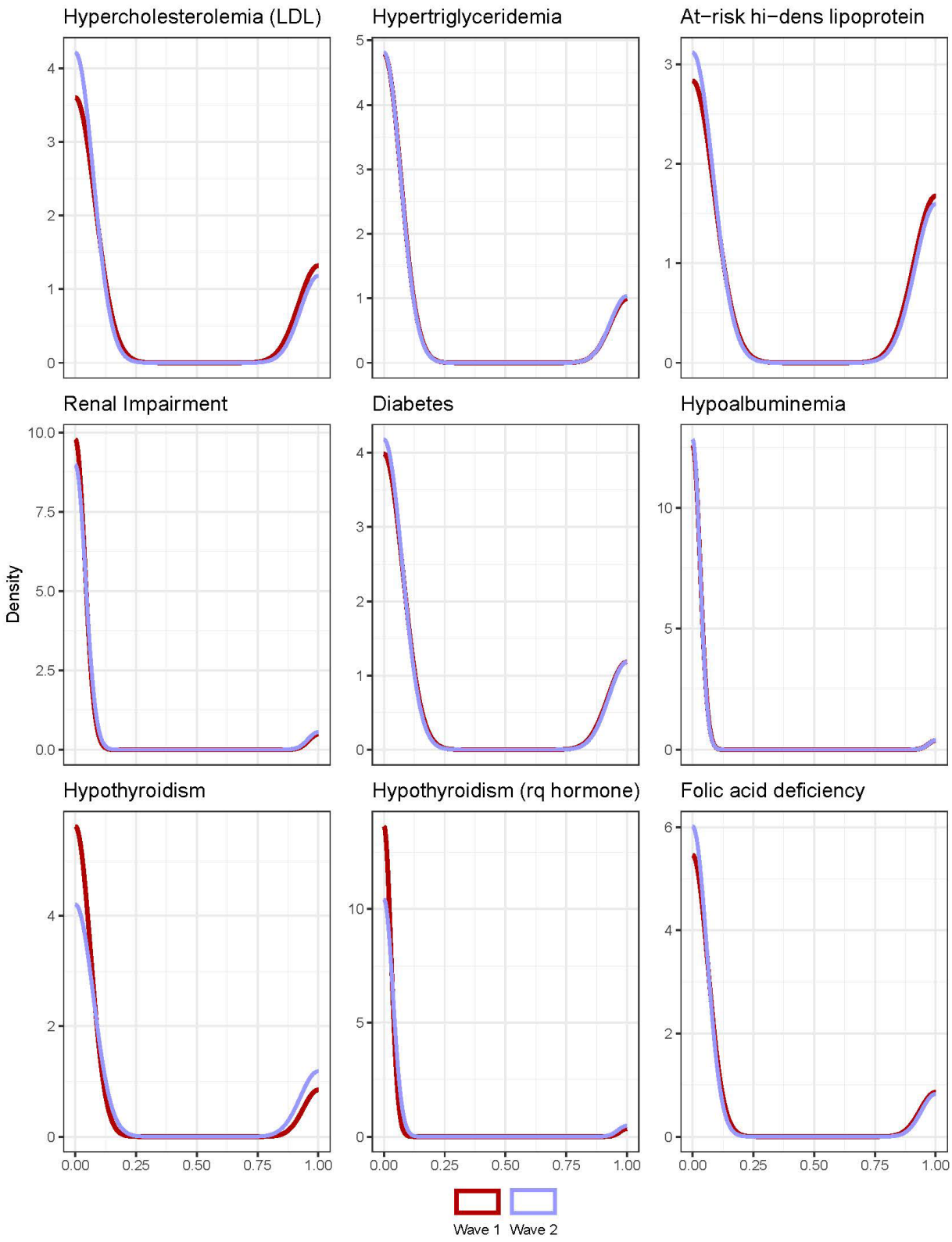
T3
TSH
Density
Wave 1
Wave 2


VB 12
Folic Acid
Vitamin D
Homocysteine
NTproBNP
CRP
Lipoprotein (a)
Anemia (Hemoglobin)
Hypercholesterolemia (total)
Density
Wave 1
Wave 2


Hypercholesterolemia (LDL)
Hypertriglyceridemia
At−risk hi−dens lipoprotein
Renal Impairment
Diabetes
Hypoalbuminemia
Hypothyroidism
Hypothyroidism (rq hormone)
Folic acid deficiency
Density
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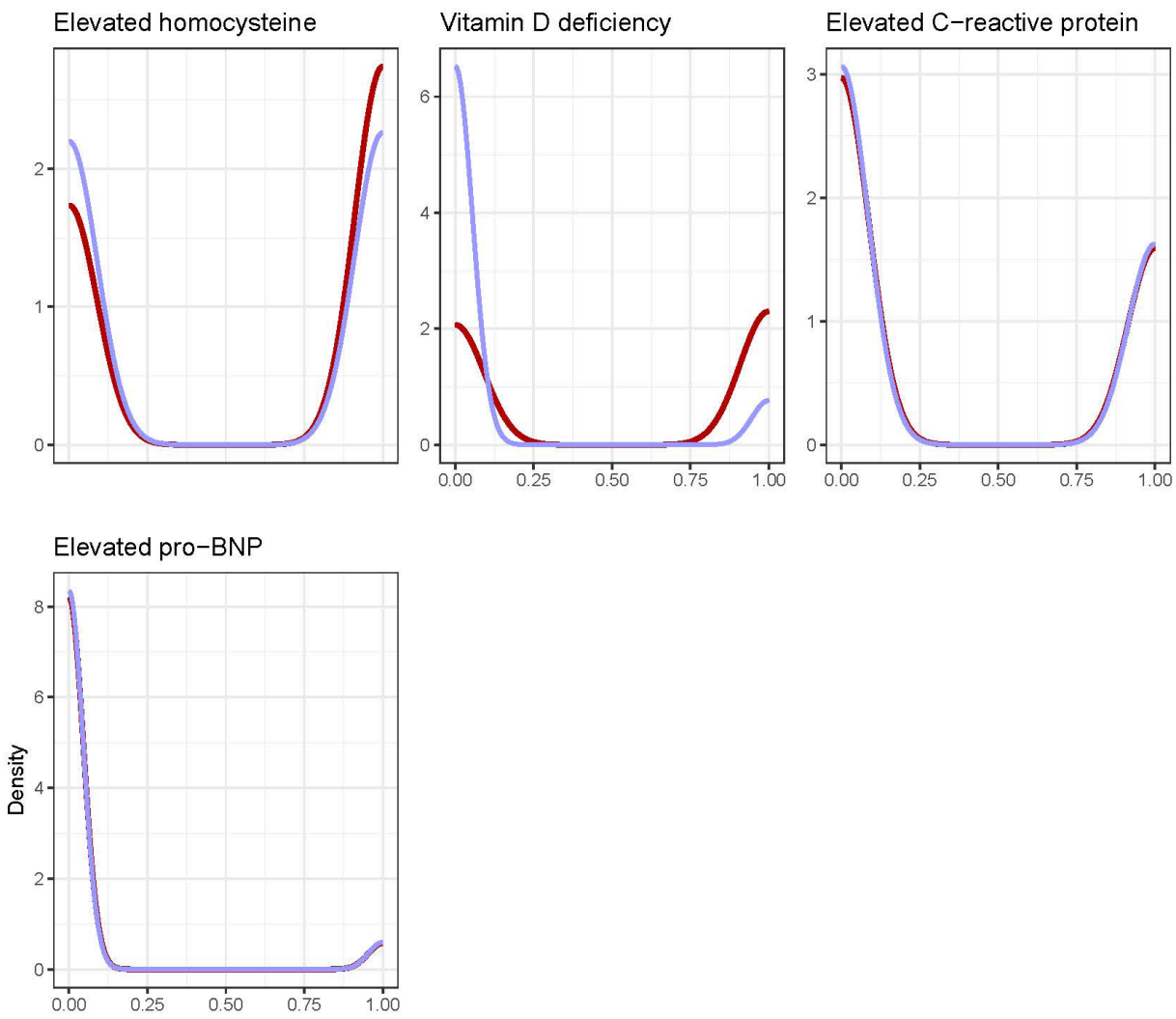
0.50
0.75
1.00
Wave 1
Wave 2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Vitamin D deficiency
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Elevated pro−BNP
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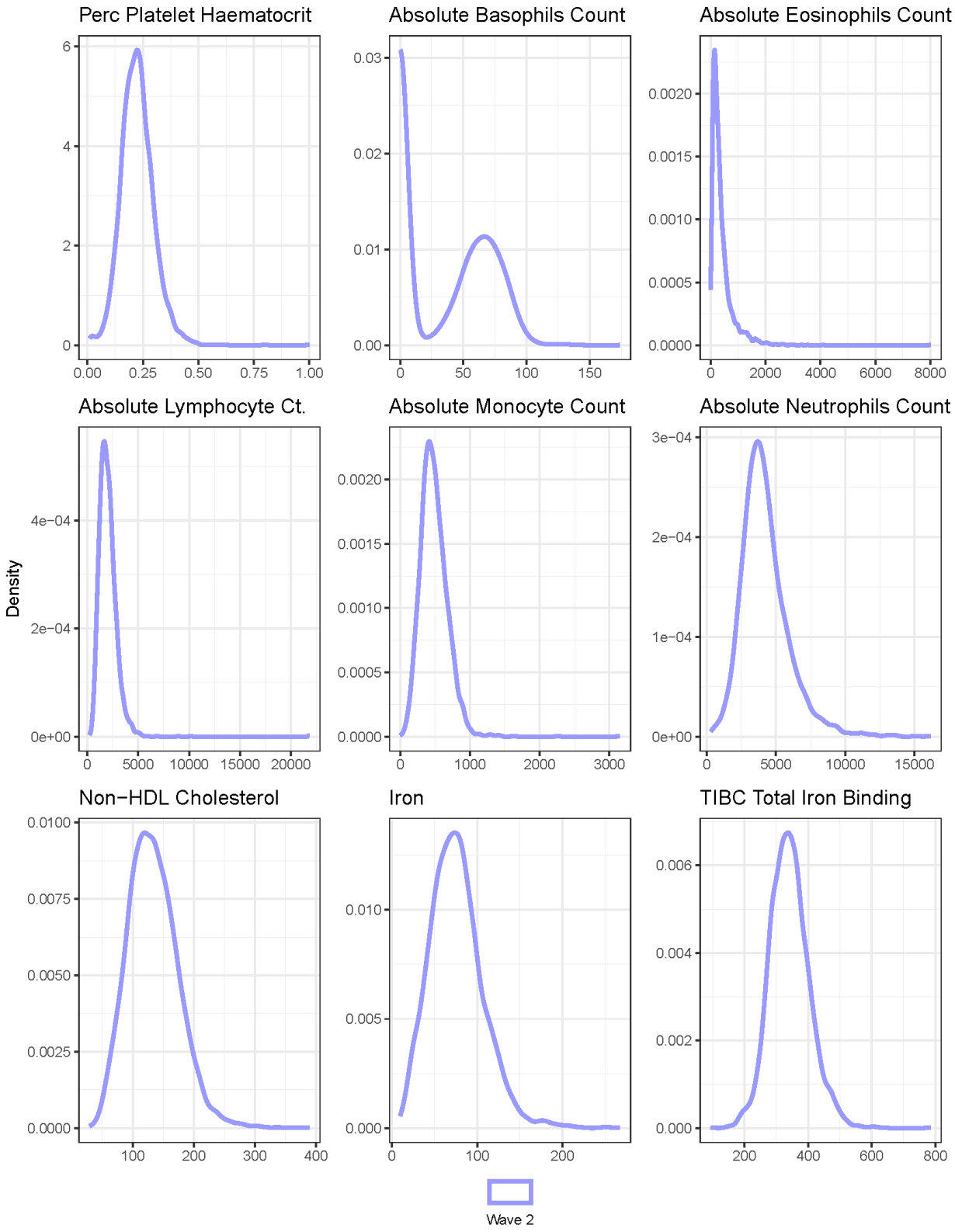
Wave 1
Wave 2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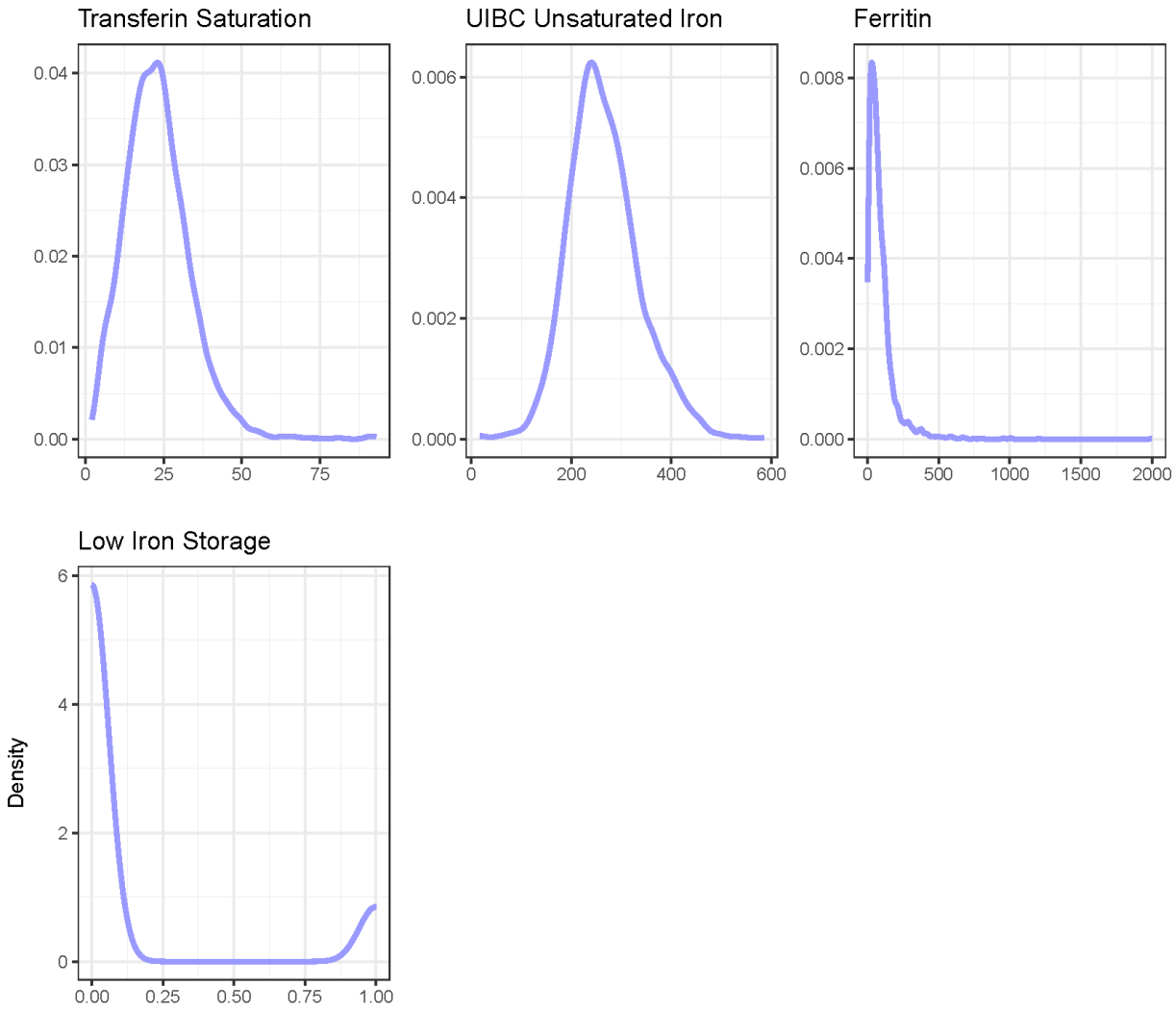
Absolute Monocyte Count
Absolute Neutrophils Count
Non−HDL Cholesterol
Iron
TIBC Total Iron Binding
Density
Wave 2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UIBC Unsaturated Iron
Ferritin
Low Iron Storage
Density
Wave 2