Diagnostic Assessment of Dementia for LASI

Documentation – 2019 Wave 1, Early Release Version A

Venous Blood Collection and Assay Protocol

This project is funded by National Institute on Aging/National Institutes of Health (1R01A051125, 1RF1AG055273)

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Contents

List of Tables

List of Figures

1. Overview

1.1. Rationale

India, the second most populous country in the world, will soon experience rapid aging of its population. Currently, the roughly 90 million Indians aged 60+ accounts 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+ 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 LASI-DAD Project (Longitudinal Aging Study in India - Diagnostic Assessment of Dementia) 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+ 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 wellbeing of the country's aging population, interviewing over 70,000 individuals aged 45+ (including their spouses, irrespective of age).

[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.

1.2. Sample

Diagnostic Assessment of Dementia for LASI (LASI – DAD) is an in-depth study of late-life cognition and dementia using hospitals as phenotyping centers. It draws a sub-sample of 4,000 LASI respondents aged 60 and older and administers in-depth cognitive tests and informant interviews, following the Health and Retirement Study (HRS)'s Harmonized Cognitive Aging Project (HCAP) protocol. To guarantee a sufficient number of respondents with dementia and mild cognitive impairment (MCI), we use a stratified random sample design.

We first stratify all respondents' risk of cognitive impairment based on the performance of memory and non-memory domain cognitive tests, as well as overall test performance, refusal or inability to participate in cognitive tests, and proxy interview in the main LASI study. Specifically, we draw about half of the LASI-DAD sample from those at high risk of cognitive impairment. High risk is determined if (1) total cognition score is at the bottom tertile, (2) memory score or non-memory test score is at the bottom 15^{th} percentile, (3) the number of missing cognitive tests are at the top 15^{th} percentile, or (4) the informant report suggests cognitive impairment risk (i.e., IQCODE score is 3.9 or higher) (Jorm & Jacomb, 1989).

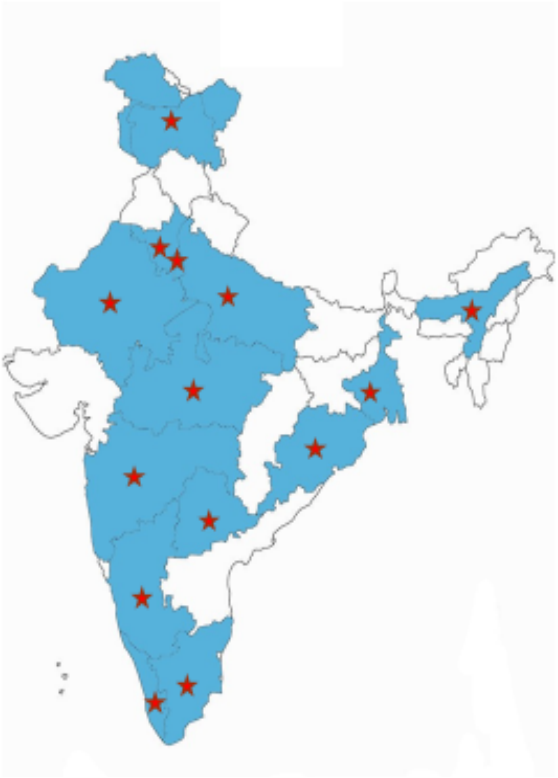
Then, within each state we randomly draw about equal number of respondents at high and low risk of cognitive impairment with a target sample size proportionate to the population size.

On average, about 4-months after the baseline interview, 14 participating hospitals recruited selected LASI respondents and their family members for an interview.[2] Based on respondents' preference, our interview team administered the HCAP protocol either at the

[2] The collaborating hospitals are: the All India Institute of Medical Sciences, Delhi, Madras Medical College, Chennai, National Institute of Mental Health and Neurosciences, Bangalore, BHU, Varanasi, S.N. Medical College, Jodhpur, TMC, Trivandrum, J.J. Hospital, Mumbai, SKIMS, Srinagar, Gauhati Medical College, Guwahati, Nizam's Institute of Medical Sciences, Hyderabad, All India Institute of Medical Sciences, Bhubaneswar, Odisha, and IPGMER, Kolkata.

hospital or at the respondent's home setting. The field team traveled up to 12 hours by automobile to reach out to respondents residing in remote villages. See Figure 1 for the geographic distribution of our sample (Delhi team also recruits and interviews respondents in Haryana). We draw the sample from 14 states and 4 metropolitan cities across the country, within 6 hours of driving distance from participating hospitals. The states we draw the LASI-DAD sample from include: Gujarat, Haryana, Jammu & Kashmir, Karnataka, Kerala, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, and West Bengal, and the four metropolitan cities are: Chennai, Delhi, Kolkata, and Mumbai.

Figure 1. LASI – DAD Sample



Collaborating Institutions across 14 states

Phase 1	Phase 2
Delhi All India Institute of Medical Sciences, Delhi	Jammu & Kashmir Sher-i- Kashmir institute of Medical sciences, Srinagar
Rajasthan S. N. Medical College, Jodhpur	West Bengal Kolkata Medical College
Uttar Pradesh Banaras Hindu University	Assam Guwahati Medical College
Karnataka NIMHANS, Bangalore	Odisha AIIMS, Bhubaneshwar
Kerala Trivandrum Medical College, Trivandrum	Telangana Nizam's Institute of Medical Sciences, Hyderabad
Tamil Nadu Madras Medical College, Chennai	Maharashtra Grant Medical college, Mumbai
Haryana All India Institute of Medical Sciences, Delhi	Madhya Pradesh Gwalior Medical College, Gwalior

1.3. Collection & Shipping

The blood collection and shipment was managed by Metropolis, Inc. Metropolis, Inc. is a pathology laboratory accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL) in India. It has a wide network of laboratories at all the study sites to ensure the processing of venous blood to serum and plasma within 2 hours of its receipt.

We provided 2-day training to Metropolis phlebotomists regarding our study protocol, and the trained phlebotomists then visited participating hospitals and respondents' home for blood draw. The phlebotomy service also included proper labelling of tubes, as described below, as well as shipping samples at required temperatures to the main Metropolis laboratory in Delhi. The transportation of samples was done on daily basis.

In total, we collected 17 ml venous blood from respondents. The venous blood sample was collected by the trained phlebotomist from the local Metropolis laboratory at each site. The sample was collected as the first part of the interview and preferably fasting blood sample which was noted in the questionnaire.

Table 1 shows the amount of blood collected in each of five tubes. Four out of five tubes (A, B, C & E) were sent to local Metropolis laboratories while tube D was sent to MedGenome laboratory for whole Genome sequencing. The blood sent to local Metropolis laboratories was processed to serum and plasma. Once processed, the blood specimens were sent to the central Metropolis laboratory in Delhi.

The processed blood was shipped from the local to the central laboratory at Delhi in two shipments

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

For temperature monitoring, we used different temperature loggers for each of the shipments.

Table 1. Blood Collection and Shipping Protocol

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

In addition to assays at the laboratory in Delhi, we stored the serum, plasma, buffy coat and dried blood spot card (DBS) from each respondent at All India Institute of Medical Science (AIIMS), New Delhi.

1.4. Participation

As the baseline LASI fieldwork was carried out in phases, the fieldwork for the LASI-DAD was also carried out in two phases. The first phase fieldwork was carried out from October 2017 to June 2018, 2 to 6 months after the baseline LASI interview. From the total eligible respondents of 3,891, 3,224 respondents participated in cognitive interview, and 3,191 informants completed informant interview. The response rates for cognitive test and informant reports were 82.9% and 82.0%, respectively. Among them, we were able to obtain the venous blood specimens (VBS) from 2,254 participants (a success rate of 57.9%). Table 2 presents the number of completed interviews, VBS assay completed.

Table 2. Number of Participants Contacted and Response Rates by Urbanicity

Coverscreen Interview			**Total**	**Urban**	**Rural**
A	1	Contacted	3947	2171	1776
	2	Unable to contact	162	50	112
	3	Deceased	56	28	28
	4	Refused	201	51	150
	5	Eligible	3528	2042	1486

Cognitive Interview			Total	Urban	Rural
B	1	Total Eligible (A2 + A4 + A5)	3891	2143	1748
	2	Completed Interview	3224	1916	1308
	3	Response Rate (%) = B2 / B1	82.9	89.4	74.8

Informant Interview			Total	Urban	Rural
C	1	Total Eligible (B1)	3891	2143	1748
	2	Completed Interview	3191	1898	1293
	3	Response Rate (%) = C2 / C1	82.0	88.6	74.0

Blood collection			Total	Urban	Rural
D	1	Total Eligible (B1)	3891	2143	1748
	2	Completed Interview	2254	1400	854
	3	Response Rate (%) = D2 / D1	57.9	65.3	48.9

1.5. Bioassays

After extensive consultations with Advisory Committees and Regional Geriatrics Centers in India, LASI-DAD team selected a battery of venous blood based tests based on following principles: 1) focusing on potential causes and risk factors for dementia, 2) assay feasibility in India, and 3) harmonization with other studies within Health and Retirement Study (HRS) family. Specific tests performed are summarized in Table 4.

Table 3. List of Venous Blood Based Tests Performed at Metropolis Laboratory

Whole blood based assays
Complete blood cell counts (CBC), including white blood cell counts, hemoglobin, and platelet counts
Glycosylated hemoglobin (HbA1c)
Serum based assays
Glucose
Lipid panel (total, LDL-, HDL-cholesterol, and triglyceride)
Lipoprotein (a)
Pro N-terminal B-type natriuretic peptide (proBNP)
High-sensitivity C-reactive protein (hsCRP)
Metabolic panel, including renal and liver functions
Cystatin C
Thyroid-stimulating hormone (TSH), Total Triiodothyronine (T3), Total Thyroxine (T4)
Vitamin B12
Folic acid
Homocysteine
25-hydroxy-vitamin D

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 has performed consistently well in all external quality control programs.

2.2. Assay Methodology and Reference Ranges

The assay methods are summarized in Table 5, and the descriptions of each marker, measurement units, and reference ranges are summarized in Table 6.

Table 4. Assay Methodology Used at Metropolis Laboratory

Test	Equipment	Assay Methodology
Whole blood based assays		
Hemoglobin	Beckman Coulter LH780	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	
Red Blood Cell Count	Beckman Coulter LH780	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 106 cells/µLν Uncorrected White Blood Cell (UWBC) • Measure directly, multiplied by the calibration factor. • UWBC = N x 103 cells/µLν
White Cell Count	Beckman Coulter LH780	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 103 cells/µLν
Differential Leucocyte Count (DLC)		
Glycosylated haemoglobin (HbA1c)	Bio -Rad D-10	The test is based on chromatographic separation of the analyte by ion exchange HPLC.
Serum based tests		
Glucose	Architect ci8200	The glucose method is an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method, presented as a general clinical laboratory method by Kunst, et al. This method is more specific than general reducing methods and will give results lower than those obtained by such reducing methods. Hexokinase (HK) catalyzes the phosphorylation of glucose by adenosine-5'-triphosphate (ATP) to glucose-6-phosphate which is oxidized to 6-phosphogluconolactone by glucose-6-phosphate dehydrogenase (G-6-PDH) with simultaneous reduction of nicotinamide-adenine dinucleotide phosphate

		(NADP). One mole of NADP is reduced to one mole of NADPH for each mole of glucose present. The absorbance due to NADPH (and thus the glucose concentration) is determined using a bichromatic (340 and 383 nm) endpoint technique.
Lipid Profile		
Cholesterol (total)	Architect ci8200	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	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 chromagenic coupler to develop color for the quantitative determination of HDL cholesterol. Methodology: Accelerator Selective Detergent
LDL Cholesterol	Architect ci8200	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. Methodology: Measured, Liquid Selective Detergent
Triglycerides	Architect ci8200	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	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	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. Methodology: Biuret
Albumin	Architect ci8200	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	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	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	Architect ci8200	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	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	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 carbondioxide. 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	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.
Uric acid	Architect ci8200	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 H2O2 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	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.
Thyroid Function Tests		
Total Thyroxine (T4)	Architect ci8200	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.
Total Triiodothyronine (T3)	Architect ci8200	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.
TSH (Ultrasensitive)	Architect ci8200	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.
Other tests		
Vitamin B12	Architect ci8200	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.
Folic acid	Architect ci8200	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.
25 Hydroxy, Vitamin D	Architect ci8200	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.
Homocysteine	Architect ci8200	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	The assay principle combines a one-step immunoassay sandwich method with a final fluorescent detection.
High-sensitivity C-Reactive Protein (hsCRP)	BNProSpec	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	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.

Table 5. Venous blood based markers with reference range

Test	Variable Descriptions	Unit	Reference range
Whole blood based assays			
Hemoglobin	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+: 12.0 to 16.0 g/dl, mean 14.0 g/dl; Male, ages 18+: 13.0 to 16.0 g/dl, mean 14.5 g/dl
Platelet Count	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/cumm	4.7 to 6.0
Differential Leucocyte Count (DLC)	Differential leucocyte counts (DLC): The white blood cells take important part in the body's immune system. They are divided into five types of cells: neutrophils, basophils, eosinophils, lymphocytes and monocytes. The percentages of these cells in the total white blood cell count are called WBC differential counts. This is important for diagnosing different diseases like infections, blood malignancies etc.	(%)	
Mean Corpuscular Hemoglobin (MCH)	Mean corpuscular hemoglobin (MCH) It 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	28 to 32
Mean Corpuscular Volume (MCV)	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	80 to 100
Packed Cell Volume (PCV)	Packed cell volume (PCV): The hematocrit (Ht or HCT hematocrit), also known as packed cell volume (PCV) or erythrocyte volume fraction	%	Male-47% ±5% Female-42% ±5%

	(EVF), is the volume percentage (%) of red blood cells in blood.		
Glycosylated hemoglobin (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
Serum based tests			
Glucose	Glucose: 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	80-99
Lipid Profile			
Cholesterol (total)	Cholesterol and its derivatives are important constituents of cell membranes and precursors of 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.	mg/dL	<200mg/dl
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	> 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	< 190; target goal is lower for patients with known cardiovascular disease or cardiovascular risk factors
VLDL Cholesterol	VLDL is one of the four major lipoprotein particles i.e. HDL, LDL, VLDL, and chylomicrons. It transports the maximum amount of triglycerides. High levels are a risk factor for coronary artery disease.	mg/dL	< 30
LDL/HDL RATIO	LDL/HDL RATIO: A high LDL/HDL ratio is a risk factor for coronary artery disease		2.5 to 3.5
Chol/HDL Ratio	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.		< 5:1 (ideally below 3.5:1)
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: <150 mg/dL;Borderline high:- 150-199 mg/dL; High: 200-499 mg/dL; Very High: > 500 mg/dL.
Liver Function Tests			
Bilirubin (Total and Direct)	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 glucuronyltransferase. Total bilirubin is the sum of the unconjugated and conjugated fractions. Bilirubin is elevated in		Direct- 0 to 0.4 mg/dL Total(direct+indirect) 0.2 to 1.2 mg/dL

	conditions causing obstruction of the bile duct, hepatitis, cirrhosis, hemolytic disorders, and several inherited enzyme deficiencies.		
Bilirubin Indirect		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	6.4 to 8.3
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 kidney disease, liver disease, malabsorption, malnutrition, severe burns, infections, and cancer.	g/dL	3.5 to 5.2
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	2.6 to 4.6
A/G Ratio	A/G Ratio is the ratio of albumin to globulin in serum.		0.8 to 2.0

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	20 to 60
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	10 to 40
Alkaline Phosphatase	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 133
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	12 to 48 (males, all ages); 6 to 29 (females, all ages)
Renal Function Tests and Electrolytes			
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, 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	mg/dL	7 to 20

	postrenal (obstructions of the urinary tract) hyperuremia.		
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	0.72 to 1.25
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	3.5-7.2
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	8.5 to 10.3
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	5.0 to 12.5
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	70 to 204

TSH (Ultrasensitive)	TSH (Ultrasensitive): TSH assay is used as an aid in the assessment of thyroid status, diagnosis of thyroid disease and treatment of thyroid disease.	µIU/mL	0.45 to 4.5
Other tests			
Vitamin B12	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	110 to 1,500
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 to 17
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	20 to 50
Homocysteine	Homocysteine s 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	pg/mL	< 450; cut-off points often vary by age.

	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		
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 1
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

3. Quality Control

3.1. Shipping

Once blood was collected, it would be shipped to the central Delhi Metropolis laboratory and MedGenome in Bengaluru. The recording and monitoring of the shipping times and transport temperatures are 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 laboratory, depending on 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 phase 2: All blood would be transported to the local Metropolis laboratory and the

courier would come to the local laboratory to collect the blood for MedGenome. The supervisor at the local hospital was responsible for the recording of the shipment times in the online Blood Management system. The shipment to the Metropolis laboratory was monitored by temperature loggers that stayed with the sample the whole journey from the local hospital to the central Metropolis laboratory on to the storage location at AIIMS. The ID numbers of the temperature loggers were recorded by the supervisor as well. Once received the local Metropolis laboratory, the blood shipped to the central Metropolis was divided into a 4 degree Celsius and -20 degree Celsius shipment. At every step, the time the blood was received and shipped out again was recorded. At AIIMS, the temperature logger data were uploaded to the server. Because all the shipment times were recorded online in real time, we could track the location of a specific tube of blood at all times.

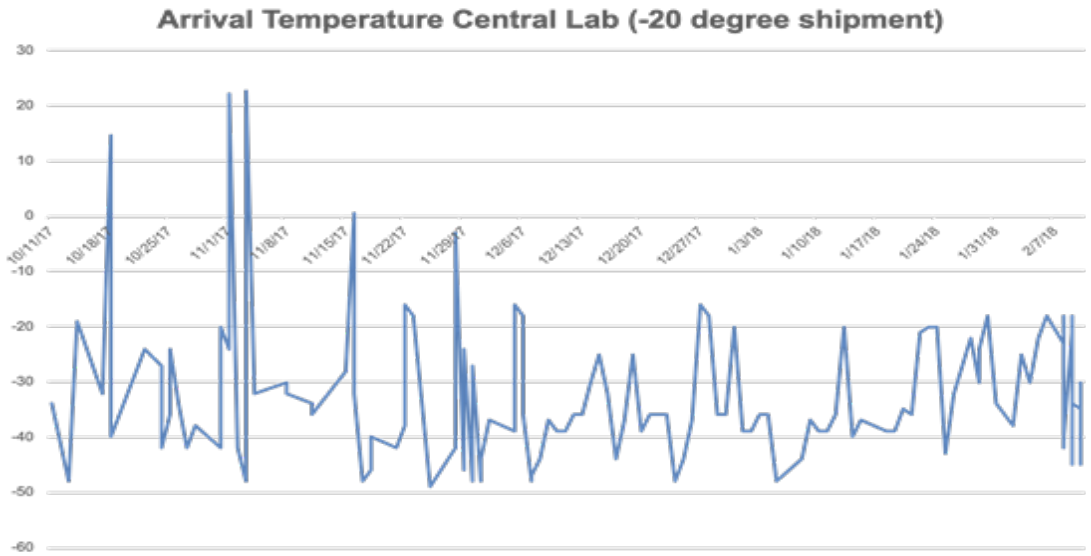
Table 6. Overview table of transit times and temperatures

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

An automated procedure in the Blood Management System was in place that checked these transit times to make sure they were not exceeded. If a tube was in transit too long, an automated email would be sent to the fieldwork supervisor who could follow up with the laboratories and the couriers. The temperature logs were checked on a weekly basis to ensure temperatures had stayed below the above-mentioned shipment temperatures.

Figure 2 shows the temperature observed at arrival. As shown in Figure 2, the arrival temperature in the beginning of the fieldwork were often too high. But after notifying Metropolis of our findings, they became compliant to our protocol, keeping the arrival temperatures as targeted.

Figure 2. Mean temperature observed at arrival



3.2. Laboratory Procedures

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. Whole blood from tube C was used to create a DBS card, using Whatman 903 protein saver card. 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. Remaining serum, plasma, and buffy coat were transferred to cryovials and transferred to AIIMS, together with DBS cards.

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 is that testing of clinical/study samples would not be initiated if value of one QC sample is beyond three standard deviations (S.D.) from the laboratory-established mean or values of two QC samples are beyond two S.D. from the mean.

During the period when LASI-DAD study samples were tested, LASI-DAD team also independently monitored QC sample results on real-time basis. For all assays, QC sample values were within the criteria established by Metropolis laboratory.

4. Results

We provide a table of descriptive results for the bioassays from the first phase sample.

Table 7. Descriptive Results for the First Phase Sample

Test	N	mean	sd	min	max
Complete Blood Cell Counts (CBC)					
Hemoglobin	2219	12.68	1.91	3.8	20.5
Platelet Count	2213	230.25	83.70	33	1368
Red Blood Cell Count	2198	4.44	0.63	1.38	8.9
Red Cell Distribution Width (RDW)	2198	15.22	2.08	11.7	31.5
Total Leucocyte Count	2208	7256.57	2243.47	1600	39500
Mean Corpuscular Haemoglobin Concentration (MCHC)	2198	32.57	1.24	22.8	46
Mean Corpuscular Hemoglobin (MCH)	2198	28.71	3.43	11.2	42.8
Mean Corpuscular Volume (MCV)	2198	87.98	8.95	49.2	125.6
Packed Cell Volume (PCV)	2219	38.84	5.34	15	67.4
Glycosylated haemoglobin (HbA1c)	2217	6.31	1.54	3.8	17.5
Serum Based Tests					
Glucose	2217	134.40	44.21	62.36	455.55
Lipid Profile					
Cholesterol (total)	2247	183.57	42.35	62	409
HDL Cholesterol	2247	44.02	11.75	7	135
LDL Cholesterol	2241	111.51	35.83	12	310.2
VLDL Cholesterol	2147	26.23	11.16	7.4	59.8
LDL/HDL RATIO	2147	2.63	0.99	0.48	7.96
Chol/HDL Ratio	2247	4.38	1.55	1.67	46.71
Triglycerides	2247	144.14	89.38	37	1501
Liver Function Tests					
Bilirubin (Total and Direct)	2245	0.64	0.34	0.11	2.87
Bilirubin Direct	2242	0.24	0.12	0.1	1.3
Bilirubin Indirect	2245	0.40	0.24	0.01	2.16
Total Protein	2245	7.40	0.57	5.16	10.4
Albumin	2245	4.13	0.33	2.2	6.5
Globulin	2245	3.27	0.49	1.23	7.1
A/G Ratio	2245	1.29	0.22	0.42	3.42
Alanine Aminotransferase (ALT)	2245	19.06	14.48	6	303

Aspartate Aminotransferase (AST)	2245	25.96	16.76	8	402
Alkaline Phosphatase	2245	93.99	38.14	5	644
Gamma-Glutamyl Transferase (GGT)	2243	26.60	44.27	4	1288
Renal Function Tests and Electrolytes					
Blood Urea Nitrogen (BUN)	2247	12.70	4.97	3.8	56.6
Creatinine	2247	0.89	0.42	0.38	8.47
Uric acid	2246	4.90	1.39	1.2	12.9
Calcium	2247	9.18	0.50	5.8	11.6
Thyroid Function Tests					
Total Thyroxine (T4)	2247	7.50	1.60	1.26	14.54
Total Triiodothyronine (T3)	2238	90.72	21.90	40.72	479.62
TSH (Ultrasensitive)	2246	3.22	8.80	0.003	221.345
Other tests					
Vitamin B12	2137	396.20	320.29	83	1968
Folic acid	2183	6.29	3.69	1	20
25 Hydroxy, Vitamin D	2238	21.42	12.16	3.1	154.9
Homocysteine	2243	21.13	11.61	4.56	121.28
NT pro BNP	2229	361.63	1156.80	15	22355
High-sensitivity C- Reactive Protein (hsCRP)	2216	4.66	10.06	0.15	140
Lipoprotein (a)	2206	49.08	456.71	2.42	12400

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