



केन्द्रीय आयुर्वेद अनुसंधान संस्थान  
Central Ayurveda Research Institute  
(Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India)  
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Ref: No. 6-82/2019/CARI/BNG/IMR(AMRA)/ 628.

Date. 16-07-2022

### सूचना/NOTICE

इस संस्थान की आम्र परियोजना के लिए प्रयोगशाला परीक्षण सेवाएं प्रदान करने के लिए इस संस्थान की निविदा संख्या 6-82/2019/CARI/BNG/IMR (AMRA)/542, दिनांक 01-07-2022 के संदर्भ में, अनुबंध-II में उल्लिखित अतिरिक्त प्रयोगशाला सेवा आवश्यकताओं को सम्मिलित करते हुए निविदा/उद्धरण प्राप्त करने की अंतिम दिनांक को 30-07-2022 तक बढ़ा दिया गया है।

With reference to this Institute's tender No.6-82/2019/CARI/BNG/IMR(AMRA)/542, dated 01-07-2022 for providing laboratory investigation services for AMRA project of this institute, the last date for receiving the tender/quotation has been extended to 30-07-2022 with inclusion of additional laboratory service requirements mentioned in Annexure – II. A.

प्रभारी सहायक निदेशक (आयु.)  
प्रभारी सहायक निदेशक (आयु.)  
दिनांक 16/7/2022.

Additional Laboratory Investigation Service requirements in respect of the project AMRA study

**Serum and Plasma collection for interleukins**

Blood samples will be collected from patients using the following blood collection tubes; normal clotting tube (SST II Advance, BD Biosciences) for serum and sodium heparin (NH), EDTA, or sodium citrate (NC) tubes for collecting plasma (all BD Biosciences) during the visit. All samples will be kept on room temperature and will be spun within 30 minutes at  $700 \times g$  in cold centrifuge. Cell free plasma or serum will be aliquoted and stored at  $-80^{\circ}\text{C}$  until analysis.

Before analysis, all thawed samples will be centrifuged through a polypropylene centrifuge tube containing a  $0.22 \mu\text{m}$  nylon membrane (Spin-X column; Corning, Corning, NY, USA) to remove debris.

Non-specific heterophilic antibodies, such as natural polyspecific antibodies, idiotypic antibodies and natural rheumatoid factors, will be pre-absorbed, without loss of cytokine concentration, from all samples with protein-L pre coated ELISA plates (Pierce, Rockford, IL, USA)

$100 \mu\text{l}$  of sample well will be incubated for 1 hour at room temperature under continuous shaking. As this incubation step will remove 60-80% of the total immunoglobulin fraction without depleting natural occurring cytokines samples will be then diluted with 10% v/v normal rat and mouse serum (1:1 ratio; Rockland, Gilbertsville PA, USA) and incubated for 10 additional minutes at room temperature to block any residual interfering proteins which will be able to bind (a) specifically to rat or mouse immunoglobulin's. Animal serum batches will be tested upfront, and only used when they did not show any cross-reactivity within the assay.

All samples obtained will be approved for collection by the medical ethical committee after Informed consent be obtained.

Note- Samples will be preserved in the labs at Central Ayurveda Research Institute, Bengaluru, Arya Vaidya Pharmacy Coimbatore and RRAP – CARL, Mumbai in  $-86$  degree refrigerator . The samples would be collected by the approved lab once in 6 months and the tests would be conducted at the end of the study period of 2 years .

Lab Investigations	Test Method	1 <sup>st</sup> Screening	2 <sup>nd</sup> Baseline	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>	11 <sup>th</sup>
Interleukin - 1, 4, 6, 10, 17	CLIA		*			*			*			*
Interleukin - 6	ECLIA		*			*			*			*
D - Dimer	Quantitative (Immunoturbidimetry)		*			*			*			*
IFN gamma	CLIA		*			*			*			*
TNF $\alpha$ (Tumor Necrosis Factor) Alpha	CLIA		*			*			*			*

*Dr*  
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