Bio-Plex® suspension array system

tech note 6029

Normal Physiological Levels of Human Cytokines Using Bio-Plex Pro™ Cytokine Assays

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Introduction

Cytokines function as indicators of inflammation or disease progression and provide a means of manipulating cellular responses *in vivo* and *in vitro*. In healthy individuals, cytokines are expected to have low or undetectable circulating levels, whereas they have been shown to be elevated in a number of disease states. As a result, the ability to detect these factors has become increasingly important to researchers and clinicians. The objective of this study was to determine the range of cytokines in serum collected from apparently normal donors that could be used as reference in clinical studies and to describe the preparation of samples for optimal use with the Bio-Plex® suspension array system. In previous published studies, difficulty in obtaining accurate values has been reported due to variations in sample collection, processing, storage, and day-to-day operation. Therefore, these ranges should be used only as a guideline.

Methods

Human serum samples from 66 normal donors of all ages and ethnicities were obtained from Bioreclamation, Inc. and from the Bio-Rad Laboratories, Inc., Redmond, Washington facility. Two different vendors were chosen to avoid bias in calculating concentration ranges contributed by variations in blood collection, processing, and storage conditions. Samples were tested with human group I and group II Bio-Plex Pro cytokine assays. The samples were measured in duplicate at a low PMT setting using directions provided in the instruction manual. The assays were performed using the Bio-Plex Pro II wash station with the magnetic plate carrier to minimize operator-related variations. A 10-point extended broad range standard curve was used in order to maximize sensitivity for samples containing very low levels of analytes. High-end saturation points were removed from the standard curves for determining sample concentrations.

Results

Most of the cytokines had mean values <100 pg/ml with few exceptions. IL-1β, G-CSF, and β-NGF were not detected in most of the samples tested due to low circulating levels under normal physiological conditions (<1.5 pg/ml). PDGF-BB, RANTES, and SCGF-β had high endogenous levels and may require higher dilution of samples from diseased states. In general, group II cytokines have slightly higher endogenous levels compared to group I cytokines.

Table 1 shows the concentration in range, observed concentration range, and median and mean concentrations. The concentration in range column provides only observed concentration values that fall within the range of valid standards that can be estimated accurately (with 70–130% recovery). The observed concentrations correspond to positive extrapolated data or data above or below the fluorescence intensity of standards and these values do not necessarily fall within the assay working range. Calculated sample values below the standard curve range are included to demonstrate sample sensitivity of the assay. However, these values should not be considered reliable as they fall outside the standard curve. The mean and median correspond to the mean and median of concentration in range values for the 66 samples tested and includes any samples with undetectable levels. All values take into account the 4-fold dilution factor used when screening the samples.

Table 1. Normal physiological levels of group I and II human cytokines.

Analyte	pg/ml			
	Concentrations in Range	Observed Concentration	Median of Concentrations in Range (n=66)	Mean of Concentrations in Range (n=66)
Group I Cytokine Assays				
Basic FGF	4.00–55.00	1.30–55.00	7.54	9.00
Eotaxin	2.00–39.00	1.20–39.00	0.00	3.80
G-CSF	<1.50	<1.50	0.00	0.02
GM-CSF	3.00–122.00	0.80–122.00	6.78	12.47
IFN-γ	7.00–124.00	0.60–124.00	8.68	13.43
IL-1β	<0.70	0.02–0.70	0.00	0.01
IL-1ra	6.00–665.00	0.20–665.00	23.94	42.01
IL-2	2.00–90.00	0.03–90.00	1.24	6.46
IL-4	0.06–3.00	0.01–3.00	0.00	0.10
IL-5	1.00–7.00	0.01–7.00	0.00	0.15
IL-6	0.50–9.00	0.02–9.00	0.00	0.73
IL-7	0.60–13.00	0.01–14.00	0.00	0.27
IL-8	0.40–116.00	0.08–116.00	0.00	7.21
IL-9	2.00–500.00	0.38–500.00	19.40	37.50
IL-10	0.40–2.00	0.10–2.00	0.00	0.13
IL-12(p70)	3.00–6.00	0.10–6.00	0.00	0.14
IL-13	0.80–9.00	0.01–9.00	0.00	0.33
IL-15	2.00–5.00	0.06–5.00	0.00	0.31
IL-17	2.00–31.00	0.22–31.00	0.00	2.30
IP-10	6.00–637.00	5.90–637.00	32.24	93.61
MCP-1 (MCAF)	2.00–48.00	2.00–48.00	17.95	18.24
MIP-1α	<2.00	0.01–2.00	0.00	0.15
MIP-1β	5.00–47.00	1.70–47.00	11.24	14.75
PDGF–BB	6.00–3,667.00	6.00–3,667.00	180.10	394.87
RANTES	100.00–2,282.00	100.00–2,282.00	0.00	203.64
TNF-α	6.00–98.00	0.10–98.00	0.00	5.92
VEGF	0.50–9.00	0.01–9.00	0.00	0.43
Group II Cytokine Assays				
CTACK	1.00–1,086.00	1.00–1,086.00	196.00	246.51
GRO-α	9.00–365.00	9.00–365.00	22.35	36.33
HGF	63.00–1,868.00	63.00–1,868.00	195.20	255.24
IFN-α2	14.00–79.00	3.30–63.00	0.00	16.07
IL-1α	0.50–1.40	0.40–1.40	0.00	0.12
IL-2Rα	28.00–594.00	28.00–594.00	102.66	116.85
IL-3	13.00–170.00	13.00–170.00	41.06	44.54
IL-12(p40)	36.00–646.00	36.00–646.00	0.00	60.19
IL-16	10.00–1,270.00	10.00–1,270.00	77.50	94.79
IL-18	9.00–812.00	9.00–812.00	68.05	75.71
LIF	4.00–55.00	4.00–55.00	14.85	17.20
MCP-3	1.00–78.00	1.00–78.00	2.25	3.28
M-CSF	6.00–208.00	6.00–208.00	29.64	48.68
MIF	6.00–2,003.00	6.00–2,003.00	72.40	170.29
MIG	86.00–7,911.00	86.00–7,911.00	289.00	617.05
β-NGF	<1.10	<1.10	0.00	0.04
SCF	16.00–837.00	16.00–837.00	167.57	172.88
SCGF-β	6,054.00–130,932.00	6,054.00–13,0932.00	47,870.00	48,312.25
SDF-1α	8.00–92.00	8.00–92.00	0.00	13.79
TNF-β	1.00–13.00	0.71–13.00	0.00	0.31
TRAIL	8.00–272.00	8.00–272.00	66.61	65.91

Conclusion

No significant differences were observed in sample analyte measurements from different vendors. Cytokines such as IL-8, IL-15, and IL-17 have previously shown variability in concentrations obtained from samples collected by different vendors. As mentioned above, this variability could be due to differences in blood collection method, storage conditions, and freeze-thaw cycles of samples. Therefore, it is advisable to use these values as a reference. It is recommended that the reference samples and the samples under study be collected and stored under identical conditions. Refer to suggested reading for additional information.

Suggested Reading

Blood collection, storage, and processing:
Aziz N et al. (1999). Variables that affect assays for plasma cytokines and soluble activation markers. Clin and Diag Lab Immun 6, 89–95.

Normal cytokine ranges in plasma:
Kokkonen H et al. (2010). Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis & Rheumatism 62, 383–391.

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