Before use, read this Package Insert.

TNF-α - IRMA

An immunoradiometric assay kit for human tumour necrosis factor Alpha.
For Research Use only

I GENERAL INFORMATION

A. Proprietary Name : DIAsource TNF-alpha IRMA kit

B. Catalogue Number : KIC1751 : 96 determinations

C. Manufactured by : DIAsource ImmunoAssays S.A.
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II APPLICATION AND INTENDED USE

Human Tumour Necrosis Factor Alpha (TNF-α), also named cachectin, is a 157 A.A. – unglycosylated polypeptide cytokine mainly produced by activated macrophages (monocytes).
Lipopolysaccharide (LPS), the cell-wall component of gram-negative bacteria (endoxin), is a potent stimulus for TNF-α production by macrophages and TNF-α is an important mediator of the well-known in vivo effects of LPS such as tumour hemorrhagic necrosis, fever, shock and activation of neutrophils. The various biological activities of TNF-α may be classified as :

1. antitumoral and growth regulatory activities : TNF displays a selective toxicity for tumoral and virus-infected cells. Conversely, it is angiogenic and stimulates the growth of cultured fibroblasts;
2. immunomodulatory and pro inflammatory activities : TNF activates macrophages, neutrophils and eosinophils, as well as endothelial cells (which display procoagulant activity). It regulates the production of antibodies by B cells and stimulates cytotoxic T cells. It induces the production of several other inflammatory mediators such as IL-1, IL-6, colony stimulating factors, prostaglandins, platelet-activating factor (PAF), collagenases, etc;
3. metabolic activities : TNF-α strongly inhibits lipoprotein lipase and adipocyte gene expression.

TNF-α has a major pathogenic role : in cachexia associated with chronic infectious or cancerous diseases; in spectic shock where the neutralization of TNF protects against the associated acute lethality; in graft rejection and graft-versus-host disease; and in parasitic infections where TNF may provide some protection but also favors more severe forms of the disease (e.g. the cerebral form of malaria). TNF-α, often in combination with other cytokines, has also been involved in several autoimmune diseases and even in the pathogenesis of arterioscleriosis.
Abnormal production and serum TNF detection only begin to be evaluated in human diseases.
Abnormally high levels of serum TNF-α have already been described in septic shock, graft rejection, parasitic infections, cancer, post hemofiltrations products, during in vivo cytoline (IL-2) therapy, etc. Besides an insight into pathogenesis, these determinations might provide an aid in diagnosis (e.g. in graft rejection) and have prognostic value (e.g. in systemic infections) (fig. 1).

III PRINCIPLES OF THE DIAsource TNF-α IRMA ASSAY

The DIAsource TNF-α IRMA is an immunoradiometric assay based on coated-tube separation. $Mabs_1$-the capture antibodies are attached to the lower and inner surface of the plastic tube. Standards or samples added to the tubes will at first show low affinity for antibodies. The signal antibody labelled with ^{125}I, will trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration.

IV REAGENTS PROVIDED

1. Reagents

Reagents	Quantity	Colour Code	Reconstitution
Anti-TNF-α coated tubes	2 x 48	green	**Ready** for use
Anti-TNF-α ^{125}I in phosphate buffer with bovine serum albumin, azide and inert red dye	1 vial 5.5 ml (350 kBq = 9.5 µCI)	red	**Ready** for use
Standard 0 pg/ml in human serum with antimicrobial agents	1 vial lyophilized	yellow	**Add** 8 ml distilled water
Standards 1-6 (see exact value on the vials labels) in human serum with antimicrobial agents	6 vials lyophilized	Yellow	**Add** 2 ml distilled water
Washing solution	1 vial 10 ml	brown	**Dilute** the contents in 700 ml distilled water (use a magnetic stirrer)
Controls 1 and 2 in human serum	2 vials lyophilised	silver	**Add** 1 ml distilled water

1 pg of our reference preparation is equivalent to 32 mlU MRC 87/650.

2. Storage

Store all kit reagents until the expiry date and any residual solution up to 8 days after reconstitution at 2 to 8°C. For prolonged storage until the expiry date, the standards must be kept at -20°C

V MATERIAL REQUIRED BUT NOT PROVIDED

1. Distilled water.
2. Pipettes : 50 µl, 200 µl, 1 ml, 2 ml (the use of accurate micropipettes with disposable plastic tips is recommended).
3. 5 ml automatic syringe (Cornwall type) for washing.
4. Water aspiration system (optional).
5. Gamma counter (^{125}I).
6. Vortex mixer and magnetic stirrer.

VI SPECIMEN COLLECTION AND PREPARATION

TNF-α may be assayed in serum, plasma, other biological fluids, or supernatants from in vivo cultured cells.
For serum or plasma TNF-α assay, very strict precautions have to be taken during sampling, to avoid impurities contained in sampling materials that would stimulate TNF production by blood cells and thus falsely increase plasma TNF values (e.g. various commercial heparinised tubes contain endotoxins that stimulate TNF production).
Therefore it is recommended :

- either to utilize serum collected on sterile, clean, dry tubes, and rapidly separated after coagulation,
- either to utilize plasma rapidly separated from sample collected in EDTA sterile tube.
  Heparin and other substances non-tested in tubes should be avoided, as well as prolonged delay before cellular phase separation. Samples must be kept frozen (-20°C) if assaying is not done immediately.

After macrophage activation by LPS, the cell culture medium can contain high concentration of TNF-α. To obtain reliable results, cell culture medium must be diluted at least ¼ with standard 0 prior to the assay.

VII ASSAY PROCEDURE

Adherence to the instructions are mandatory to obtain reliable results. Do not mix coated tubes from different lots.

1. Preparation of reagents : first reconstitute standards with 2 ml distilled water and controls with 1 ml distilled water; then prepare washing solution : dilute the contents of the washing solution in 700 ml distilled water.
   Use a magnetic stirrer until complete homogeneity is obtained.
2. Label coated tubes in duplicate for each standard and sample. For determination of total counts, label 2 uncoated tubes.
3. Vortex mix, briefly, standards, samples and dispense 200 µl of each into respective tubes.
4. Dispense 50 µl of tracer into each tube.
5. Shake the rack containing the tubes gently by hand.
6. Incubate for 16 to 20 hours at room temperature.
7. Aspirate (or decant) the complete contents of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
8. Wash tubes with 2 ml washing solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the washing solution.
9. In order to increase the reproducibility of the assay, leave the tubes on the table for two minutes and aspirate the remaining drop of liquid.
10. Count tubes in a gamma counter for 60 seconds.
11. The standard curve is prepared by plotting cpm or B/T (%) on the ordinate against the standard concentration on the abscissa using either linear-linear or semi-log graph paper. Draw the best curve rejecting obvious outlyers. Examples of standard curves are included (see table : Example of a typical reference curve). The data are presented as an example only and should not be used instead of the standard curve prepared by each analyst during each new assay.
12. Determine TNF-α concentrations of samples by interpolation of cpm or B/T (%) values. If computer assisted method is used, a polynomial function usually gives the best results.

SUMMARY OF ASSAY PROCEDURE

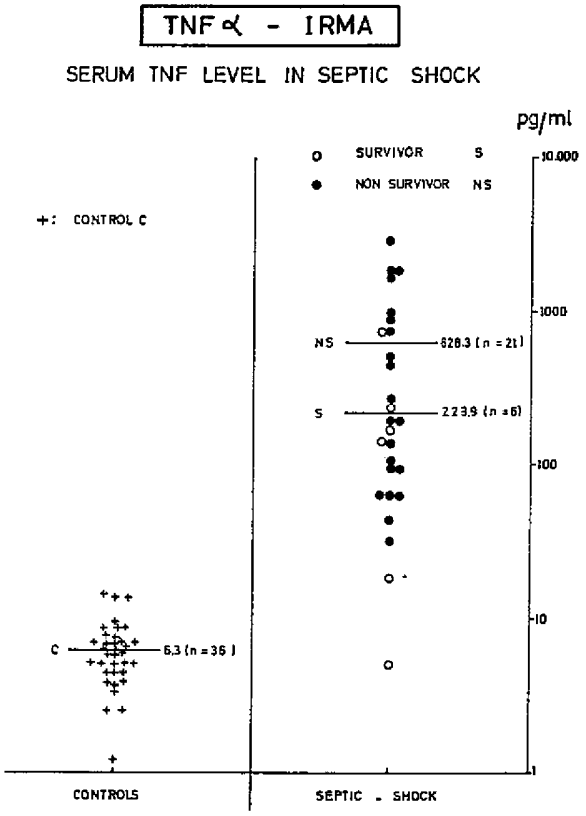
	Total Counts ml	Standards ml	Sample(s) ml
Standards (0-6)	-	0.20	-
Samples	-	-	0.20
Tracer	0.05	0.05	0.05
Incubation	16 to 20 hours at R.T.		
Separation	-	aspirate (or decant)	
Washing solution	-	2.0	2.0
Separation	-	aspirate (or decant)	
Separation	-	aspirate (or decant)	
Counting	Count tubes for 60 seconds		

EXAMPLE OF TYPICAL REFERENCE CURVE

		cpm	B/T x 100
Total count		148939	100.0
Std :	0 pg/ml	399	0.3
	15	905	0.6
	50	1990	1.3
	150	4613	3.1
	500	13735	9.2
	1500	34275	23.0
	5000	78033	52.4

VIII EXPECTED VALUES

The figures show serum TNF-α levels in normal controls and in patients with septic shock. Patients display a much higher serum TNF level and non survivors have significantly higher levels than survivors (indicating the prognostic value of serum TNF at the time of diagnosis).



IX PERFORMANCE CHARACTERISTICS

1. **Sensitivity**
The zero dose standard was measured 20 times. The TNF-α concentration corresponding to the mean cpm + 2 standard deviations was : 5 pg/ml.

2. **Specificity**

Added hormone to a low TNF-α value standard (50 pg/ml		Observed TNF-α values (pg/ml)	Added hormone to a high TNF-α value standard (500 pg/ml)		Observed TNF-α values (pg/ml)
-		50	-		500
TNF-β	100 ng/ml	52.0	TNF-β	100 ng/ml	500
Interleukin-1	1000 ng/ml	52.0	Interleukin-1	1000 ng/ml	510
Interleukin-2	25000 U/ml	45.5	Interleukin-2	25000 U/ml	500
Interferon-α	1000 ng/ml	46.5	Interferon-α	1000 ng/ml	485
Interferon-β	1000 U/ml	56.0	Interferon-β	1000 U/ml	530
Interferon-γ	5000 U/ml	47.5	Interferon-γ	5000 U/ml	525

This demonstrates that the TNF-α IRMA does not cross react with TNF-β, Interleukin-1, Interleukin-2, Interferon-α,β, gamma.

3. **Accuracy**

Sample	Added TNF-α (pg/ml)	Recovered TNF- α (pg/ml)	Recovery	Sample	Dilution	Theoretical concent. (pg/ml)	Measured concent. (pg/ml)
Serum	-	15	-	Serum	1/1	1212	1212
	73	90	103		1/2	606	601
	153	182	109		1/4	303	293
	316	322	97		1/8	152	149
	603	612	99		1/16	76	69
	1371	1396	101		1/32	38	34
	2736	2671	97		1/64	19	16
Plasma	-	17	-	Cell	1/1	881	881
	73	96	108	Culture	1/2	441	432
	153	188	112	medium	1/4	220	216
	316	353	106		1/8	110	105
	603	696	113		1/16	55	52
	1371	1612	116		1/32	28	26
	2736	3107	113				

4. **Precision**

INTRA-ASSAY				INTER-ASSAY			
Serum	**N**	**<X> ± SD (ng/ml)**	**CV (%)**	**Serum**	**N**	**<X> ± SD (pg/ml)**	**CV (%)**
A	20	67.4 ± 4.1	6.0	D	20	65.5 ± 4.6	7.0
B	20	290.0 ± 9.1	3.1	E	20	305.0 ± 17.4	5.7
C	20	1328.0 ± 28.0	2.2	F	20	1398.0 ± 39.0	2.8

5. **Hook-effect**
A sample spiked with TNF-α up to 100.000 pf/ml gives a result higher than the last standard point (5000 pg/ml).

6. **Expiry date**
An expiry date is indicated on the kit label. It refers to the expiry date of the tracer. The expiry date of the other kit components is stated on each vial label.

X WARNINGS

1. FOR LABORATORY RESEARCH USE ONLY : NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. This kit contains ^{125}I (half-life : 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.
3. Human blood components included in this kit have been tested and found non reactive for HBsAg and anti-HIV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/HIH Health Manual :"Biosafety in Microbiological and Biomedical Laboratories" 1984.
4. Certain reagents contain azide as preservative. When dry, azide can be explosive. Therefore it is advisable to rinse the sink with running water whilst decanting solutions which contain azide.
5. Adherence to the basic rules of radiation safety should provide adequate protection. See National Bureau of Standard Handbooks 92, "Safe Handling of Radioactive Materials", March 9, 1964, Washington D.C.
A summary follows :
   - do not eat, drink, smoke or apply cosmetics where radioactive materials are used;
   - do not pipet radioactive solutions by mouth;
   - avoid direct contact with all radioactive materials by using protective clothing such as lab coats and disposable gloves;
   - all radiological work should be done in a designated area away from traffic;
   - radioactive materials should be stored in their original containers in a designated area;
   - a record book for logging receipt and disposal of all radioactive materials should be kept;
   - laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes;
   - any radioactive spills should be taken care of immediately, in accordance with established procedures;
   - all radioactive materials must be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.

XI BIBLIOGRAPHY

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DIAsource Catalogue Nr : KIC1751	P.I. Number : 1700480	Date of issue : 090511/1