"STEP BY STEP GUIDE FOR BIOMARKER DETECTION"

LPPT UGM 2024

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agenda

01 Sample Preparation

02 Reagent & Material Preparation

03 Procedure

04 Data Analysis

05 Tips & Tricks, Troubleshooting

SAMPLE PREPARATION

01

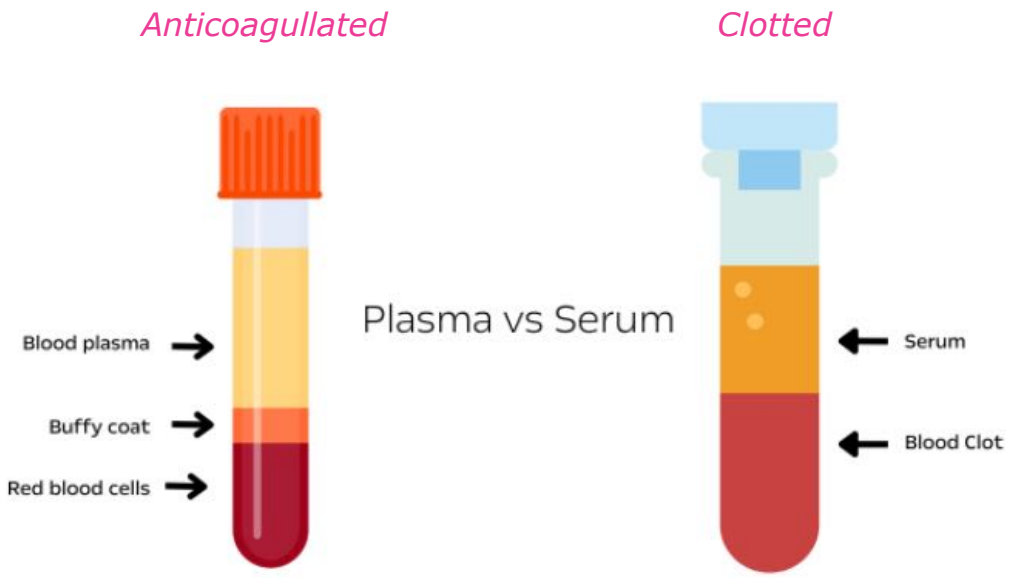
Preparing plasma and serum samples for ELISA

For plasma:

1. Collect whole blood into an EDTA, Citrate or Sodium heparin tube (e.g. BD vacutainer, Cat #8001302 or #16852).
2. Centrifuge 10 minutes at 3,000 rpm
3. Aliquot supernatant into small tubes and store at -80°C until use.

For serum:

1. Collect whole blood into a tube without additives (e.g. BD vacutainer, Cat # 8002527).
2. Keep at room temperature for 20 minutes.
3. Centrifuge 10 minutes at 3,000 rpm.
4. Aliquot supernatant into small tubes and store at -80°C until use.



Plasma =

Cell free part of Blood

Serum =

Plasma – Clotting factors (Fibrinogen)

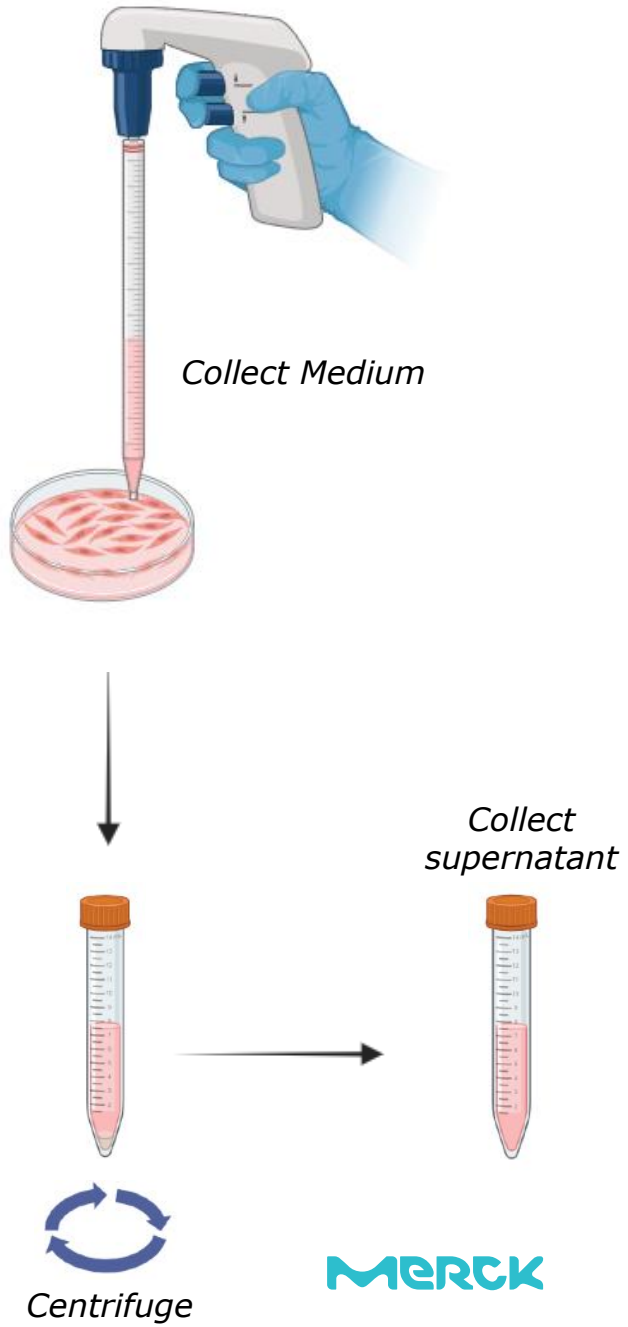
Preparing cell culture media samples for ELISA

We recommend preparing serum-free or low-serum medium samples, as serum tends to contain cytokines which may produce significant background signals. If it is necessary to test serum containing medium, we recommend also running an uncultured media blank to assess baseline signals. This baseline can then be subtracted from the cultured media sample data.

1. On day 0, seed ~1 million cells in 100 mm tissue culture plate with complete medium.*
2. On day 3, remove medium and replace medium with 6-8 ml of serum-free or low serum containing medium (e.g. medium containing 0.2% calf serum).
3. On day 5, collect medium into 15 ml tube. Centrifuge at 2,000 rpm in centrifuge at 4 °C for 10 minutes. Save the supernatant. Transfer the supernatant into 1.5 ml microtubes. Store supernatant at -80 °C until experiment. Most samples can be stored this way for at least a year.

*The optimal number of seeded cells varies from one cell type to another and may need to be empirically determined.

Note: *In case follow-up experiments are needed, it is strongly recommended to sub-aliquot all samples after preparation to minimize cytokine degradation from multiple freeze-thaw cycles.*



Preparing cell or tissue lysates for ELISA

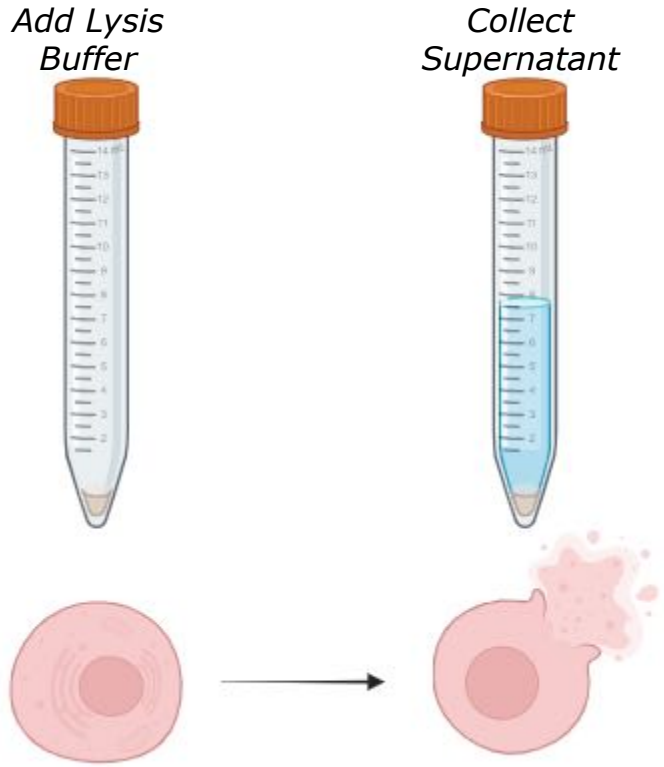
Cell or tissue lysates for use with the kits can be prepared using most conventional methods, e.g. homogenization of cell or tissue in Lysis Buffer. You may also use your own lysis buffer, such as **RIPA** or other formulations optimized for immunoprecipitation.

Please note the following guidelines on lysis buffer composition:

1. Avoid using >0.1% SDS or other strongly denaturing detergents. In general, non-ionic detergents such as Triton X-100 or NP-40 are best, although zwitterionic detergents such as CHAPS, or mild ionic detergents such as sodium deoxycholate will work.
2. Use no more than 2% v/v total detergent
3. Avoid the use of sodium azide
4. Avoid using >10 mM reducing agents, such as dithiothreitol or mercaptoethanols
5. After homogenization, centrifuge the lysates to remove cell/tissue debris (5 min @10,000 x g or 10 min @5,000 x g) and save the supernatant. Unless testing fresh, lysates should be frozen as soon as possible and stored at -20°C (or -80°C, if possible).

**We strongly recommend adding a protease inhibitor cocktail to the lysis buffer prior to homogenization. determine the protein concentration of your lysates using a total protein assay not inhibited by detergents, normalize the volume of each sample used to deliver the same amount of total protein*

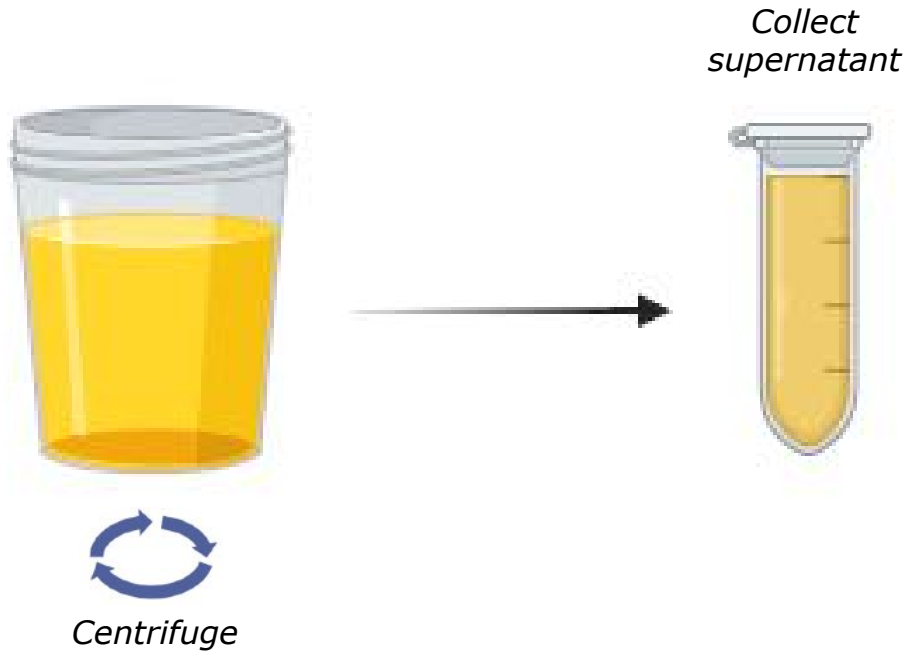
*** we suggest using 500 µL of lysis buffer per 1x106 cells or 10 mg tissue*



Preparing urine samples for ELISA

For Urine:

1. Collect urine without adding stabilizers.
2. Centrifuge the samples hard (eg. 10,000 x g for 1 min or 5,000 x g for 2 min).
3. Aliquot supernatant, quick freeze in dry ice, and store at -80°C until use.



REAGENT & MATERIAL PREPARATION

02

REAGENT & MATERIAL REQUIRED :

ELISA Kit based on Sample (Species Reactivity)

ELISA Reader with 450 nm Absorbance

ELISA Washer 8 Manifold

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REAGENT & MATERIAL REQUIRED :

Duran Schoot
500 & 1000 mL

Tube
1.5, 15 & 50 mL

Spin Down

Plate Shaker
4° to 65°C range temp.

Reagent reservoir

Tube Rack

Seal Plate 96 Well

Single &
Multichannel
Pippete

Vortex

MiliQ Water
or Distilled
Water

PROCEDURE

03

PLATE MAP :

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	U1	U9	U17	U25	U33	U41	U49	U57	U65	U73
B	S2	S2	U2	U10	U18	U26	U34	U42	U50	U58	U66	U74
C	S3	S3	U3	U11	U19	U27	U35	U43	U51	U59	U67	U75
D	S4	S4	U4	U12	U20	U28	U36	U44	U52	U60	U68	U76
E	S5	S5	U5	U13	U21	U29	U37	U45	U53	U61	U69	U77
F	S6	S6	U6	U14	U22	U30	U38	U46	U54	U62	U70	U78
G	S7	S7	U7	U15	U23	U31	U39	U47	U55	U63	U71	U79
H	BL	BL	U8	U16	U24	U32	U40	U48	U56	U64	U72	U80

S : Standard
U : Unknown Sample
BL : Blank

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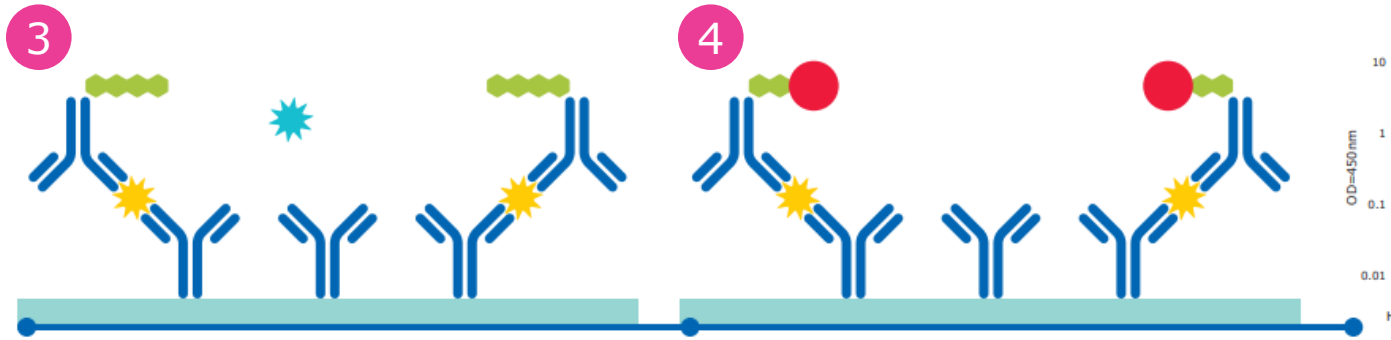
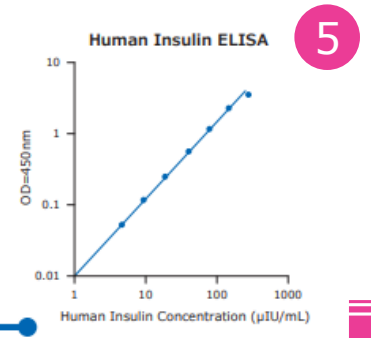
More than 1000 ELISAs, 11 Species

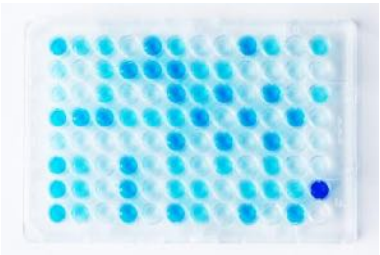
Sandwich ELISA

Quantitative, sandwich ELISA for detection of soluble factors in a variety of species.

WASH







ANALYSIS

- **Wide Range** : Merck and Sigma Elisa Kit
- **High Sensitivity** : Conferma® ELISA

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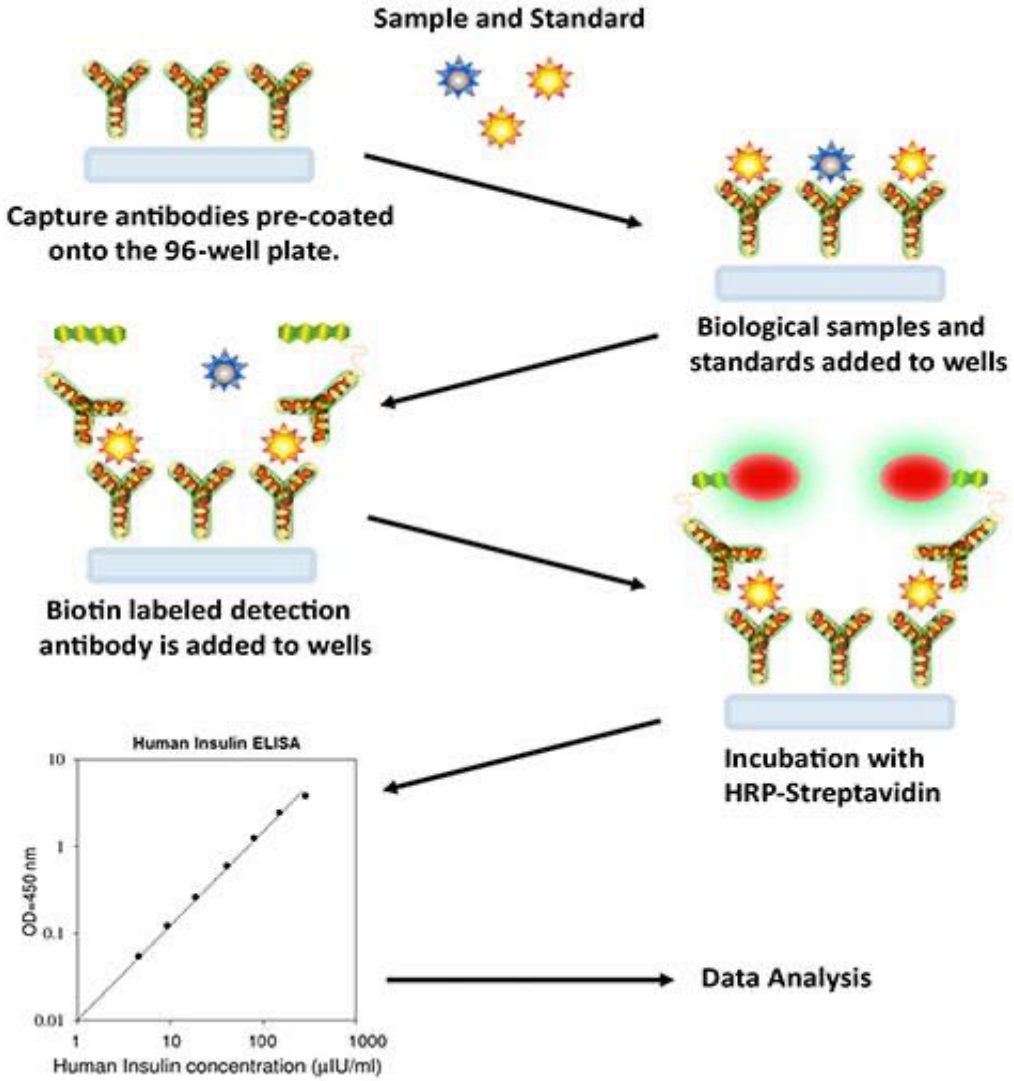
MERCK: ELISA



Sandwich Assay Procedure :

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended,all standards and samples be run at least in duplicate.

2. Add 100 µl of each standard and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. After the last wash, remove any remaining Wash Buffer by aspirating or decanting, invert the plate,blot it against clean paper towels.

4. Add 100 µl of 1x prepared Detection Antibody to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash procedure as in step 3.

6. Add 100 µl of prepared Streptavidin solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Cover wells and incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µl of Stop Solution (Item I) to each well. Read absorbance at 450 nm immediately.

DATA ANALYSIS

04

Comparison of Sepsis vs. Normal Serum/Plasma Samples

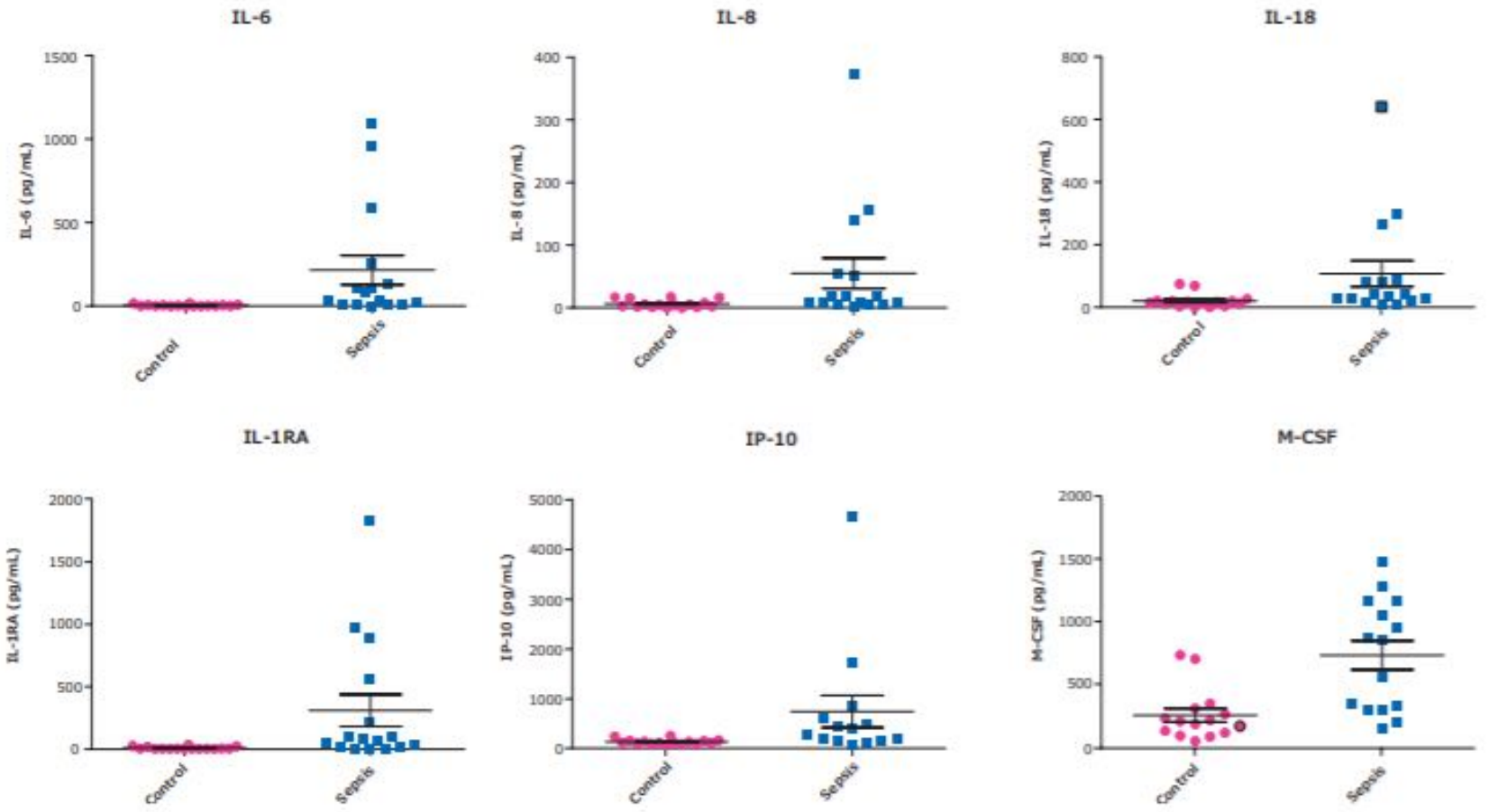


Figure 1. Healthy control (n=20) and sepsis patient (n=16) serum/plasma samples (obtained from BioIVT, Discovery, and BioChemed) were tested neat (25 µL/well) in the HCYTA-60K panel. Shown here are a subset of the analytes which have been mentioned in recent publications to be increased in SARS-CoV-2 cytokine release syndrome (CRS).

05

TIPS & TRICKS, TROUBLESHOOTING

Problem	Possible Cause	Solution
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps. Use an automated plate washer, if available.
	Plates stacked during incubations	Keep plates separated if not rotating plates.
	Pipetting error, poor dilution series	Check pipetting technique and calculations.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
	Poor or variable adsorption of reagents to plate	Check choice of coating buffer, usually PBS, pH 7.4 or carbonate-bicarbonate buffer, pH 9.6. Try extending incubating time. Consider different plates. Check homogeneity of samples.
	Capture antibody did not bind to plate	Use proper ELISA plate; dilute in PBS without other proteins
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.
	Inappropriate ELISA plate used	If using fluorescence detection, appropriate plates must be used.

Problem	Possible Cause	Solution
Poor duplicates	Uneven plate coating	Use proper ELISA plate; check coating and blocking volumes
	Insufficient or not uniform washing	Follow uniform washing procedures; check for any obstructions in washing ports.
	Variation in incubation temperature	Follow proper procedures; avoid incubation near any heat source.
Uneven color development	Incomplete washing of wells	Ensure all wells are filling with wash buffer and are being aspirated completely. Use an automated plate washer, if available.
High background	Cross-Reactivity	Detection antibody cross-reacting with coating antibody. Run appropriate controls.
	Incubation time too long	Reduce incubation time
	Non-specific binding of antibodies	Use appropriate blocking buffer.
	Concentration of conjugated second antibody too high	Perform dilutions to determine optimal working concentration.
	Incorrect assay temperature	Check that the incubation temperature did not exceed 37 °C.
	Buffers contaminated	Use fresh buffers

THANK YOU..

For more information, please contact:

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