



Measuring the other side of immunity

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# QuantiFERON<sup>®</sup>-TB Gold

## Procedures & Guidance

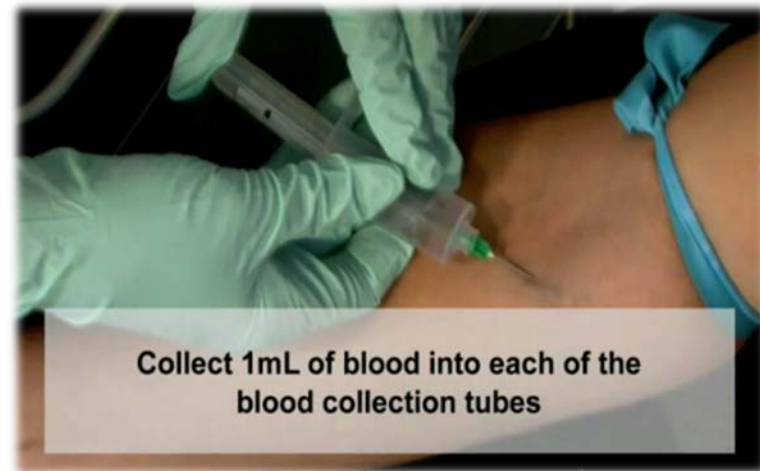
- Blood Collection
- Laboratory ELISA
- Data Analysis



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# QFT Procedure – Clinic and Lab

- Procedures in Clinic
  - Blood Collection
  - Shaking of Tubes
  - Blood Incubation
  - Plasma Separation
- Procedures in Lab
  - ELISA
  - Data Analysis



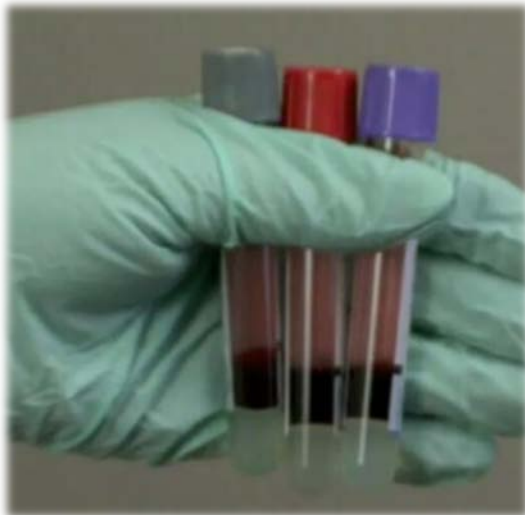
# Blood Collection

- Set of three collection tubes: Nil, TB-Antigen, Mitogen
- Draw 1 mL of blood into each of the 3 tubes
- Black side marking on the tube indicates the 1mL fill line



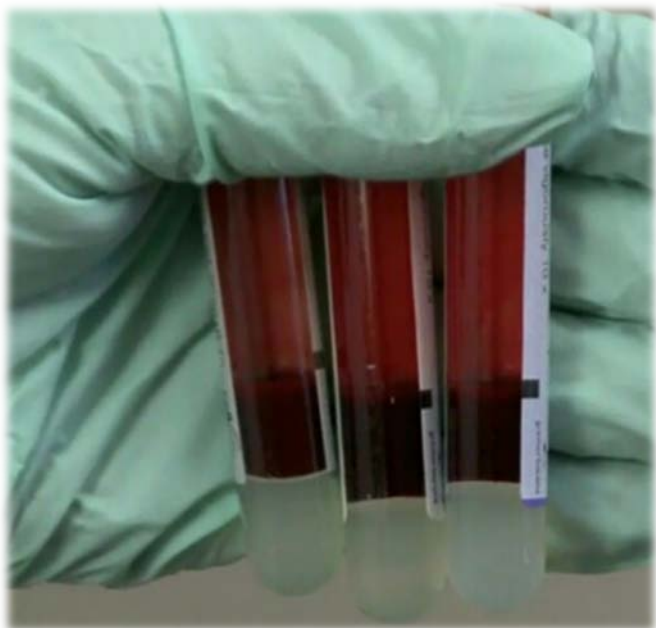
# Shaking of Tubes

- Tubes are mixed by shaking for 5 seconds (~10x)
- After shaking, the entire inner surface of each tube should be coated with blood
- Proper shaking will lead to some frothing of the blood



Frothing of blood should be expected

# After Blood Collection and Shaking



Option 1:

Tubes can be held at **Room Temperature** for up to **16 hours**

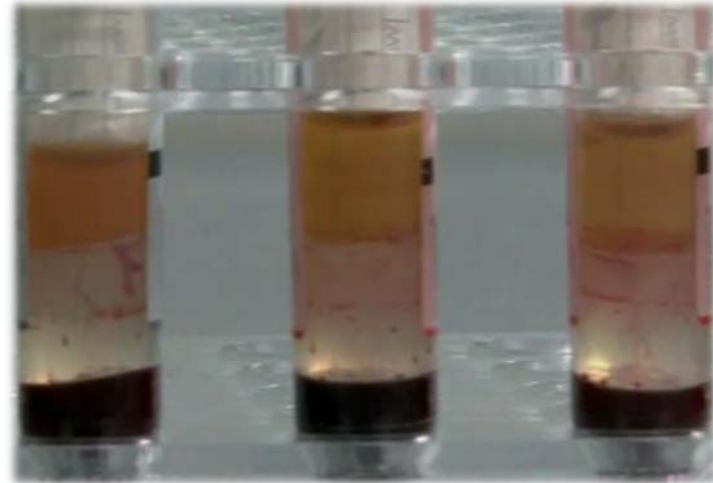
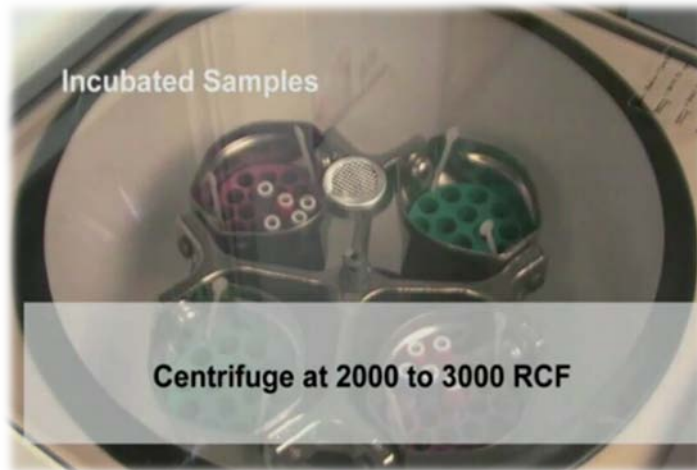
Option 2:

Within **16 hours** of collection/shaking, tubes must be incubated at **37°C** for **16-24 hours**

Following incubation, tubes have **up to 3 days** for transfer to **lab** for QFT ELISA

# Plasma Separation

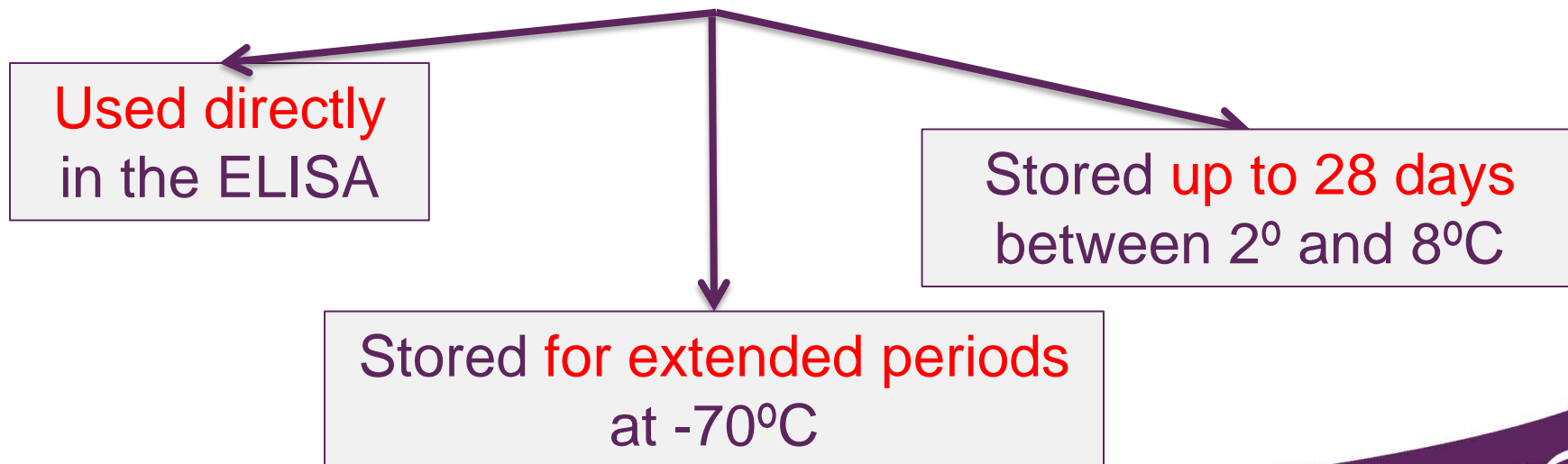
- Centrifuge tubes for 15 minutes at 2000-3000 RCF
- This separates the plasma from the cell layer
- The plasma is used for the subsequent ELISA step



# Handling Options after Plasma Separation



Plasma can be:





# ELISA in the Laboratory

- The ELISA measures interferon-gamma (IFN- $\gamma$ ) produced by sensitized T-cells stimulated by TB-specific antigens
- Standard lab instrumentation required: incubator, centrifuge, plate shaker, washer, reader



450nm filter and a reference filter of between 620 and 650nm

# Data Analysis and Results

- Results are reported as:
  - Positive
  - Negative
  - Indeterminate
- Indeterminate
  - Low mitogen
  - High Nil

**cellestis** **QuantIFERON®-TB Gold In-Tube Results**

Run Date: Montag 20 Oktober 2008  
 Operator: CS  
 Run Number: sample run  
 Kit Batch Number: sample run

Version 2.50.3

Valid ELISA test run.

Std	Conc	Mean	% CV	QC Result
S1	4,00	0,607	0,8	PASS
S2	1,00	0,173	9,8	PASS
S3	0,23	0,100	N/A	PASS
S4	0,00	0,096	N/A	PASS

Intercept: -1,3190 Slope: 0,6501 Correlation Coefficient: 0,98 (PASS)

Raw Data (OD)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,610	0,506	0,506	4,090	0,640	0,582	0,000	0,000	0,000	0,000	0,000	0,000
B	0,161	0,559	1,559	2,989	0,909	0,579	0,000	0,000	0,000	0,000	0,000	0,000
C	0,029	0,396	0,424	0,060	0,663	0,599	0,000	0,000	0,000	0,000	0,000	0,000
D	0,092	2,411	0,353	4,221	2,433	0,960	0,000	0,000	0,000	0,000	0,000	0,000
E	0,693	3,920	1,303	3,943	0,471	3,124	0,000	0,000	0,000	0,000	0,000	0,000
F	0,185	0,478	0,620	0,330	0,436	0,952	0,000	0,000	0,000	0,000	0,000	0,000
G	0,102	0,553	0,428	4,444	0,205	0,799	0,000	0,000	0,000	0,000	0,000	0,000
H	0,110	0,506	0,506	0,506	3,389	4,544	0,000	0,000	0,000	0,000	0,000	0,000

QuantIFERON®-TB Gold In-Tube results are interpreted as follows:

Nil <sup>1</sup> (IU/mL)	TB Antigen minus Nil (IU/mL)	Mitogen minus Nil (IU/mL)	QuantIFERON®-TB Gold IT Result	Report/Interpretation
<= 0,35	<= 0,35	>= 0,5	Negative	M.tuberculosis infection NOT likely
>= 0,35 and <= 25% of Nil value	>= 0,35 and >= 25% of Nil value	Any	Positive	M.tuberculosis infection likely
<= 0,35	< 0,35	< 0,5	Indeterminate	Result are indeterminate for TB Antigen responsiveness
> 0,0	>= 0,35 and <= 25% of Nil value	< 0,5	Any	

<sup>1</sup>Nil control must be <= 0,5 IU/mL and Mitogen - Nil must be >= 0,5 IU/mL. OR TB Antigen - Nil must be >= 0,30 IU/mL for a subject to have a valid QuantIFERON®-TB Gold In-Tube result.

The Mitogen control generally elicits the greatest IFN-gamma response of the 3 samples from each subject. In some cases, the Mitogen control OD value will be above the limit of the microplate reader, this has no impact on the test interpretation. The IFN-gamma level of the Nil control is considered background and is subtracted from the TB Antigen and Mitogen results for that blood specimen. In clinical studies, less than 0,25% of subjects had IFN-gamma levels of > 0,0 IU/mL for the Nil control.

The cut-off for the QuantIFERON®-TB Gold In-Tube test is 0,30 IU/mL above the Nil control (and TB Antigen minus Nil is >= 25% of the Nil control) for the TB Antigen stimulated plasma sample. Individuals displaying a response to the TB Antigen above this cut-off are likely to be infected with M. tuberculosis.

The magnitude of the measured IFN-gamma level cannot be correlated with stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease. A positive QuantIFERON®-TB Gold In-Tube result does not necessarily indicate the presence of active tuberculosis disease. Other diagnostic procedures, such as X-ray examination of the chest and microbiological examination of sputum, should be used when TB disease is suspected.

More detailed information can be found in the "Interpretation of Results" section of the QuantIFERON®-TB Gold In-Tube Package Insert.

Valid ELISA test run.

Results (IU/mL)						
Subject ID	Nil	TB Ag	Mitogen	TB Ag- Nil	Mitogen- Nil	Result
Negative (3G)	3,63	3,63	90,30	0,00	86,67	NEGATIVE
Positive (3G)	4,23	21,09	53,15	16,86	48,92	POSITIVE
IND Low Mitogen (3G)	2,49	2,76	0,14	0,27	-2,35	INDETERMINATE
IND High Nil (3G)	40,05	2,36	94,78	-37,69	54,73	INDETERMINATE

The logo for cellestis features the word "cellestis" in a lowercase, sans-serif font. The letters "e", "l", "e", and "s" are a dark purple, while "l", "e", "s", "t", and "i" are a lighter purple. Two curved, brushstroke-like elements in the same colors are positioned above and below the text, framing it.

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