

TECHNICAL BULLETIN

Whole blood activation method



For *in vitro* diagnostic

CMI-tech-bulletin ver 0312 EN

1. General information

Interferon gamma (IFN-g) is a cytokine secreted by T cells and natural killer cells and actively involved in almost all phases of immune responses, including macrophage activation. Because IFN-g is involved in the immune response, it has been identified as a potential marker for a variety of infectious diseases.

Detection of IFN-g by the sandwich ELISA method is widely used to detect the cellular response to pathogens such as *Mycobacterium avium* subsp *paratuberculosis* (MAP) or *Mycobacterium bovis*

For determination of T-cell antigen-specific response, each whole blood sample must be incubated with different antigens (Figure 1):

- Tested antigen (specific activation): *PPD bovis*
- Non specific antigen: *PPD avium*
- Positive activation control: *mitogen*. IDvet recommends the use of a mitogen in order to determine that the leukocytes are indeed capable of producing interferon gamma
- Negative activation control: *PBS*

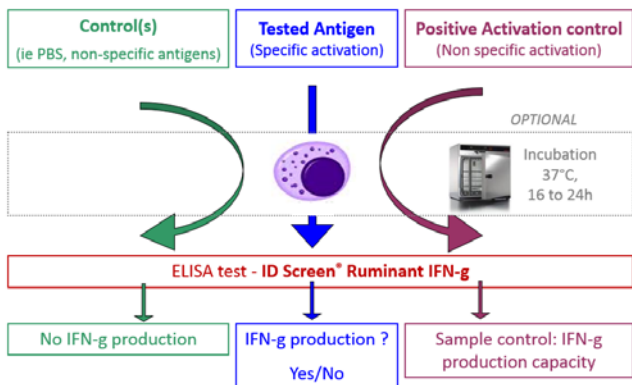


Figure 1: Stimulating antigens for IFN-g production

2. Required materials

- Heparin blood collection tube
- Mono or multi-channel micropipettes capable of delivering volumes of 10 µl, 100µl, and 200µl.
- Sterile tips
- Sterile “Vent” tubes
- Sterile 96-well cell culture plate
- Agitator
- 37°C humid incubator
- Centrifuge

3. Blood Collection

Whole blood is collected into a blood collection tube containing heparin (17 U/ml) as anti-coagulant. EDTA can not be used because it inactivates IFN-g non specific antigens (like pokeweed).

Blood samples are **mixed gently** several times by inverting the tube to dissolve the heparin.

As described by *Robbe-Austermann S. et al* [1], blood samples must be stored and transported between **16°C and 22°C** to maintain the highest optical density levels and used within 16 hours after collection, preferably within **8 to 10 hours**.

4. T cell Activation

The main steps of the sample activation protocol are presented in Figure 2 below:

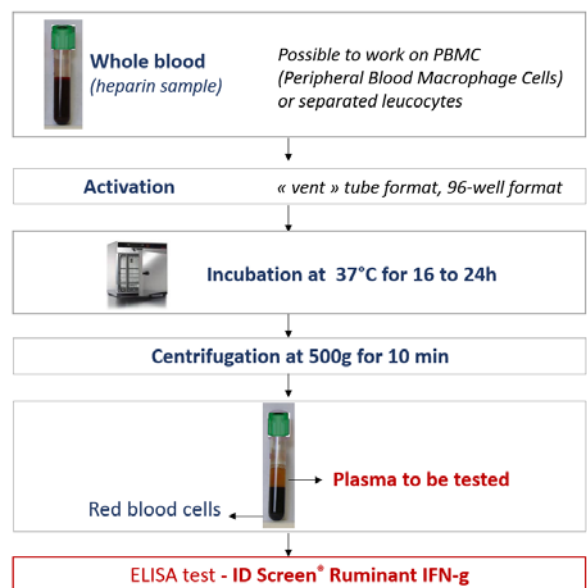


Figure 2: Main steps of sample activation protocol

Whole blood culture:

This step can be performed either in sterile 96-well cell culture plate or in “vent” tubes under sterile conditions.

Note: Sample volumes and stimulating antigens may be adapted. The protocol described hereafter is given as an example. This protocol gave satisfactory results when tested at ID-VET.

- Whole blood sample are mixed several times and separated into aliquots of 0.2 ml, one for each activator.

Addition of stimulation antigens:

- Under sterile conditions, 20 µl of stimulation antigens (specific and non-specific) and controls (PBS and mitogen) are added at the appropriate concentration (ie: 10µg / ml) as described in Figure 3:

	2	4	6	8	10	12	14	16	18	20	22
	PBS	PB S	PB S	PB S	PB S	PB S	PB S	PB S	PB S	PB S	PB S
	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A
	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
	M	M	M	M	M	M	M	M	M	M	M
PBS	PBS	PB S	PB S	PB S	PB S	PB S	PB S	PB S	PB S	PB S	PB S
NSA	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A	NS A
SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
M	M	M	M	M	M	M	M	M	M	M	M
1	3	5	7	9	11	13	15	17	19	21	23

Figure 3: Example of antigens and controls distribution in sterile 96-well plate. **PBS:** negative control; **NSA:** non-specific antigen; **SA:** specific antigen; **M:** mitogen, positive control; **N°1 to 23:** animal identification

- The plate or tubes are **mixed** between **1 and 5 minutes** on an agitator (ie: 500rpm) and **incubated** at 37 ± 2°C in humid incubator.
- After **16 to 24 hours of incubation**, whole bloods are centrifuged 10 minutes at 500g at room temperature.
- Plasma samples are **collected carefully** (do not pipette red blood cells as it can interfere with ELISA results) and transferred into a pre-dilution plate or tubes.
- Samples can be:
 - tested immediately with the ID Screen® Ruminant IFN-g ELISA test
 - stored at 5°C (±3°C) for 72 hours
 - frozen at ≤-16°C up to 3 months.

Note: For a longer storage period, samples must be frozen at ≤-70°C. Samples with low protein level such as culture media should be stored at ≤-70°.

5. Literature

[1] Robbe-Austermann S, Krull AC, Stabel JR, **2006**. Time delay, temperature effects and assessment of positive controls on whole blood for the gamma interferon ELISA to detect Paratuberculosis. J vet Med B Infect Dis Vet Public Health, 53(5):213-7.

Available on line at URL:
phoenix.nal.usda.gov/.../1/IND43812339.pdf

A close-up photograph of a multi-well microplate with several white pipette tips inserted into the wells. The liquid in the wells is a light green color. The image is partially obscured by a white curved graphic element at the bottom of the page.

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