Revisiting the IFN-γ release assay: Whole blood or PBMC cultures? - And other factors of influence - DTU Orbit (08/11/2017)

Revisiting the IFN-γ release assay: Whole blood or PBMC cultures? - And other factors of influence

The interferon-γ release assay (IGRA) is a widely used test for the presence of a cell-mediated immune (CMI) response in vitro. This measure is used to test for infection with intracellular pathogens or for validating vaccine efficacy, and it is a widely used test for both human as well as cattle. However, there is no consensus whether to use whole blood cultures or purified PBMCs for the assay, and both cell populations are being used and results compared. Therefore the aim of this study was to compare different culture settings using immune cells from previously vaccinated calves, and to shed light on external factors that could influence the read out in terms of IFN-γ levels. It was found that optimal culture conditions varied between individual animals; when polyclonal activated, cells from whole blood cultures were most responsive, but when activated specifically, the optimal cell concentration/population varied with whole blood, 10 × 106 cells/ml PBMC and 5 × 106 cells/ml PBMC being the highest performing conditions. A further investigation of the distribution of cell populations in PBMCs compared to whole blood was conducted, and a significant ($p < 0.001$) decrease in the percentage of CD3+ T lymphocytes within the PBMCs was found. More specifically, this reduction was due to a significant ($p < 0.01$) decrease in the percentage of γδ+ T lymphocytes. Thus measuring immune responses on purified PBMCs might not give a physiologically relevant output. Additionally, it was tested if the choice of incubation plate would interfere with the level of secreted IFN-γ in whole blood cultures from five calves. Six plates (a–f) were tested and no significant difference in absolute levels of IFN-γ was detected in the six plates when cells were polyclonal and specifically activated. However, we observed a significant ($p < 0.05$) higher background level in a flat-bottom plate from Corning® (cat# 3595) (plate d) compared to two different flat-bottom plates from Corning® (cat# 3596) (plate b) and Nunc™ (cat# 167008) (plate a). Furthermore 4 out of 5 calves had maximum specific IFN-γ expression on plate b, and the relative-to-maximum level on this plate was significant ($p < 0.05$) compared to plate a. Altogether these findings highlight the potential weaknesses of the IFN-γ release assay in terms of the many variables that can influence the results, including the cell culture population, the concentration of cells being cultured, and the plastic ware used for the in vitro culture. These findings stress the importance of documenting the precise assay conditions when publishing results of in vitro IFN-γ release assays.

General information

State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Department of Micro- and Nanotechnology, Bioanalytics, BioLabChip
Authors: Hartmann, S. B. (Intern), Emnéus, J. (Intern), Wolff, A. (Intern), Jungersen, G. (Intern)
Pages: 24-31
Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information

Journal: Journal of Immunological Methods
Volume: 434
ISSN (Print): 0022-1759
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.061 SNIP 0.655 CiteScore 1.92
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.068 SNIP 0.76 CiteScore 2.07
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.013 SNIP 0.832 CiteScore 1.99
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.08 SNIP 0.837 CiteScore 2.31
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.172 SNIP 0.94 CiteScore 2.49
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.168 SNIP 0.886 CiteScore 2.4
ISI indexed (2011): ISI indexed yes

Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.085 SNIP 0.866
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.182 SNIP 0.892
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.021 SNIP 0.778
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.032 SNIP 0.876
Scopus rating (2006): SJR 1.287 SNIP 0.952
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.307 SNIP 0.972
Scopus rating (2004): SJR 1.167 SNIP 0.981
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.283 SNIP 1
Scopus rating (2002): SJR 1.275 SNIP 0.955
Scopus rating (2001): SJR 0.974 SNIP 0.772
Scopus rating (2000): SJR 1.058 SNIP 0.989
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.012 SNIP 0.911
Original language: English
Immunology, Immunology and Allergy, Culture conditions, Interferon-gamma release assay, PBMC, Whole blood
DOIs:
10.1016/j.jim.2016.04.003
Source: FindIt
Source-ID: 2303440181
Publication: Research - peer-review › Journal article – Annual report year: 2016