Journal of Immunological Methods xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Reproducibility studies for experimental epitope detection in macrophages (EDIM)

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 19 March 2014
Accepted 20 March 2014
Available online xxxx

Keywords:
Flowcytometry
Reproducibility
Epitope detection in macrophages
Colorectal Cancer
Pre-analytic
Analytic

ABSTRACT

Introduction: We have recently described epitope detection in macrophages (EDIM) by flow cytometry. This is a promising tool for the diagnosis and follow-up of malignancies. However, biological and technical validation is warranted before clinical applicability can be explored.
Methods: The pre-analytic and analytic phases were investigated. Five different aspects were assessed: blood sample stability, intra-individual variability in healthy persons, intra-assay variation, inter-assay variation and assay transferability. The post-analytic phase was already partly standardized and described in an earlier study.
Results: The outcomes in the pre-analytic phase showed that samples are stable for 24 h after venipuncture. Biological variation over time was similar to that of serum tumor marker assays; each patient has a baseline value. Intra-assay variation showed good reproducibility, while inter-assay variation showed reproducibility similar to that of to established serum tumor marker assays. Furthermore, the assay showed excellent transferability between analyzers.
Conclusion: Under optimal analytic conditions the EDIM method is technically stable, reproducible and transferable. Biological variation over time needs further assessment in future work.

Abbreviations: APC, allophycocyanin; CD, cluster of differentiation; CEA, carcinoembryonic antigen; CEA-IM, CEA in macrophages; CK, cytokeratin; CK-IM, CK in macrophages; CRC, colorectal cancer; EDIM, epitope detection in macrophages; EDTA, ethylenediaminetetraacetic acid; FI, fluorescence intensity; FITC, fluorescein isothyocyanate; FMO, fluorescence-minus-one; FSC, forward scatter; ICC, intra-class correlation coefficient; M30, M30 neo-epitope; M30-IM, M30 in macrophages; mFMO, modified fluorescence-minus-one; nm, nanometer; PE, phycoerythrin; RT, room temperature; SD, standard deviation; SOP, standard operating procedure; SSC, side scatter; TM, tumormarkers; TKTL1, transketolase-like 1; TKTL1-IM, TKTL1 in macrophages.

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http://dx.doi.org/10.1016/j.jim.2014.03.018
0022-1759/

1. Introduction

Current diagnostic serum assays for assessing tumor markers lack sensitivity. Their use is mostly confined to follow-up strategies, and they are always applied in combination with other diagnostic techniques (Kievit, 2002).

Recently, both Herwig et al. and our own group explored the possibility of using macrophages from peripheral blood samples to detect tumor markers in patients with proven cancer of the prostate or colon (Herwig et al., 2005; Japink et al., 2009). This flow cytometry-based technique enables assessment of internalized tumor-related material in activated tissue associated macrophages, using multi-color fluorochrome labelling. Both the sensitivity and specificity of this approach were better than those of regular serum assays (Japink et al., 2009; Leers et al., 2008). Technical validation of

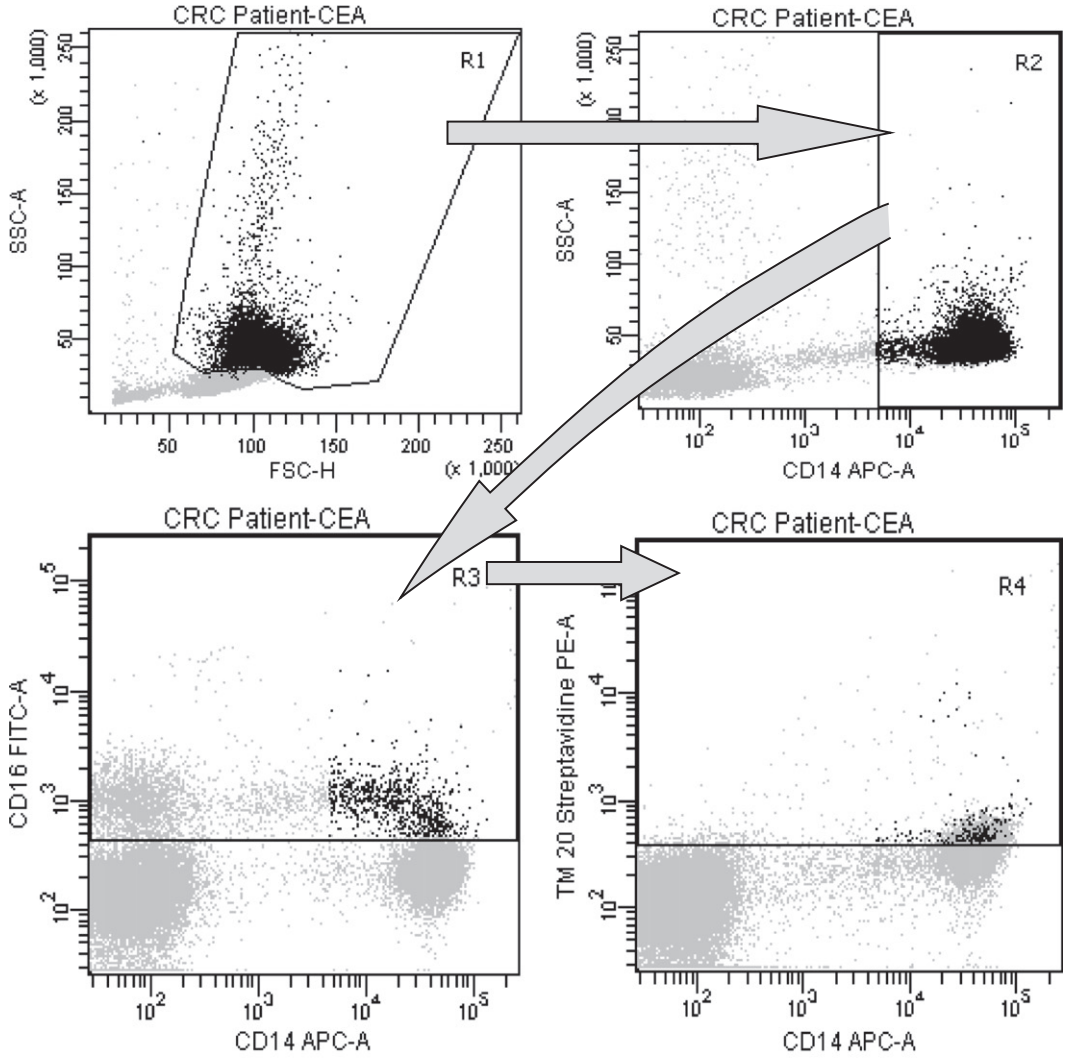

Fig. 1. R1–R4 gating steps including hierarchy.

Please cite this article as: Japink, D., et al., Reproducibility studies for experimental epitope detection in macrophages (EDIM), J. Immunol. Methods (2014), http://dx.doi.org/10.1016/j.jim.2014.03.018