Assessment of direct versus indirect magnetic bead-based T-cell isolation procedures followed by magnetic bead-based DNA isolation

Anna Rosenbaum, Ellen Bleck, Matthias Schneider, Georg Pongratz, Stefan Vordenbäumen

ABSTRACT

Objective: To compare direct and indirect bead-based T-cell isolation followed by magnetic bead-based DNA isolation.

Methods: T-cells were isolated by direct or indirect selection with magnetic bead-coated antibodies followed by magnetic bead-based automated DNA isolation in 10 healthy subjects. Purity of T-cells, purity of DNA (by A260/A280 ratio measurement) and DNA concentration were assessed.

Results: Direct and indirect labelling resulted in comparable T-cell purity (93.11±1.47% vs. 94.99±1.54%, p=0.125) and DNA concentration per cell (50.97±14.15 ng/(mL×cell) vs. 49.53±13.62 ng/(mL×cell), p=0.492), while DNA purity was significantly higher after direct labelling (1.82±0.05 vs. 1.78±0.03, p=0.0488).

Conclusions: Both direct and indirect magnetic bead-based T-cell selection may be used prior to magnetic bead-based DNA isolation procedures.

Sir, we read with interest the article of Renauer et al. who recently analysed epigenetic modifications in T cells of patients with systemic lupus erythematosus (SLE) and related them to phenotypic manifestations of the disease. Our group is similarly interested in epigenetics of SLE and the construction of biorepositories. We noted that Renauer et al selected CD4+ T cells by magnetic bead-based negative selection and subsequently extracted DNA manually. Indeed, isolation and analysis of DNA from distinct cellular subsets is an increasingly used tool in immunological research. For example, epigenetic DNA modifications such as methylation are often critically responsible for diversity and plasticity of immune cells within the complex human immune network, and as shown by Renauer et al., this may translate into phenotypic manifestations of SLE. Magnetic microbeads are a cost-efficient means of isolating distinct cellular subsets by either direct labelling of target cells or indirect selection following labelling and eradication of non-target immune cells, as employed by Renauer et al. Besides manual extraction of DNA, magnetic bead-based procedures are among the choices for subsequent DNA or RNA isolation with the advantages of potential automation and good quality. However, interactions with the previous magnetic bead-based cellular isolation procedure have not been systematically assessed previously and are a source of concern.

We therefore compared two commercially available magnetic bead-based CD4+ T-cell isolation kits and subsequent magnetic bead-based automatic isolation of DNA from CD4+ cells by the MagCore instrument (MagCore Genomic DNA Whole Blood Kit, RBC Bioscience, New Taipei City, Taiwan) on 10 healthy subjects (5 males, 5 females, 28.5±4.1 years). The following CD4+ T-cell isolation kits were assessed: CD4 MicroBeads human employs direct labelling (direct), and CD4+ T-cell isolation kit human employs indirect labelling of non-CD4+ cells (indirect). Blood was collected in three 5 mL EDTA tubes (3 mL each) and promptly processed. Peripheral mononuclear cells were isolated from 4 mL EDTA blood by density gradient centrifugation with Leucosep (Greiner Bio-One GmbH, Frickenhausen, Germany) and Lymphoprep (AXIS-SHIELD PoC AS, Oslo, Norway) followed by magnetic bead-based separation (Miltenyi Biotec GmbH) according to manufacturer’s instructions. After separations, the cells were counted and their purity assessed with CD3 labelling on MACSQuant Analyzer 10 for five subjects. Data analysis was performed by MACSQuantify software (all Miltenyi Biotec GmbH). DNA was automatically isolated...
Table 1 Quantitative and qualitative comparison of direct and indirect magnetic bead-based CD4+ T-cell isolation kits with subsequent bead-based DNA isolation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CD4 MicroBeads kit (direct)</th>
<th>CD4+T cell isolation kit (indirect)</th>
<th>p Value</th>
<th>Hedges’ g (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A260/A280</td>
<td>1.82±0.05</td>
<td>1.78±0.03</td>
<td>0.0488</td>
<td>0.88 (−0.03 to 1.80)</td>
</tr>
<tr>
<td>c(DNA) (µg/mL)</td>
<td>59.55±27.42</td>
<td>53.00±19.95</td>
<td>0.0924</td>
<td>0.26 (−0.62 to 1.14)</td>
</tr>
<tr>
<td>c(DNA) (ng/10^6 cells)</td>
<td>50.97±14.15</td>
<td>49.53±13.62</td>
<td>0.4922</td>
<td>0.10 (−0.78 to 0.98)</td>
</tr>
<tr>
<td>Purity CD3+ (%)</td>
<td>93.11±1.47</td>
<td>94.99±1.54</td>
<td>0.1250</td>
<td>−1.1 (−2.46 to 0.21)</td>
</tr>
</tbody>
</table>

p-value according to Wilcoxon matched-pairs signed-rank test (significant results with p<0.05 printed in bold), effect size estimated by Hedges’ g with 95% CI.

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Contributors AR: data acquisition, data analysis, manuscript draft. EB: data acquisition. MS: conceived the study. GP: data acquisition. SV: conceived study, data acquisition, data analysis, manuscript draft. All authors: data interpretation, final approval.

Competing interests None declared.

Ethics approval Ethics committee of the Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany.

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