

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

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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/(mlxcell) vs. 49.53±13.62 ng/(mlxcell), $p=0.492$), 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.

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.*^{1,5} 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.^{2,6} 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

Sir, we read with interest the article of Renauer *et al.*¹ 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.² 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,^{3,4} and as shown by Renauer *et al.*,¹ this may translate into phenotypic manifestations of SLE. Magnetic microbeads are a cost-efficient means of



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Table 1 Quantitative and qualitative comparison of direct and indirect magnetic bead-based CD4+ T-cell isolation kits with subsequent bead-based DNA isolation

Parameter	CD4 MicroBeads kit (direct)	CD4+T cell isolation kit (indirect)	p Value	Hedges' g (95% CI)
A260/A280	1.82±0.05	1.78±0.03	0.0488	0.88 (−0.03 to 1.80)
c(DNA) (µg/mL)	59.55±27.42	53.00±19.95	0.0924	0.26 (−0.62 to 1.14)
c(DNA) (ng/(mlxcell))	50.97±14.15	49.53±13.62	0.4922	0.10 (−0.78 to 0,98)
Purity CD3+ (%)	93.11±1.47	94.99±1.54	0.1250	−1.1 (−2.46 to 0.21)

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.

using MagCore. Afterwards, DNA purity was estimated by 260/280 ratio and quantified on Nano Photometer P330 (Implen GmbH, München, Germany). Parameters of quality and quantity were compared by Wilcoxon matched-pairs signed-rank test with $p < 0.05$ considered significant. Effect sizes were estimated by Hedges' g.

As can be seen in table 1, DNA purity estimated by A260/A280 ratio was significantly higher, with a strong effect size after direct labelling. Total DNA concentration showed a trend towards higher values after direct isolation as well. This was due to a higher yield in the number of isolated cells, as reflected by a lack of difference after correction for cell number. Finally, purity of isolated T cell was high for both direct and indirect labelling. Hence, direct labelling of CD4+ cells by magnetic beads does not seem to interfere with subsequent magnetic bead-based DNA labelling, as demonstrated by a comparable performance. Moreover, both kits offer a high purity of target cells.

Our experiments show that both direct and indirect magnetic bead-based cellular selection may be used with subsequent bead-based DNA isolation. Direct labelling carries the advantage that flushed cells are free of antibody labelling and can be subjected to further selection processes, for instance to additionally gain B cells. Conversely, indirect labelling would be a better choice, if potential activation of immune cells by the labelling procedures would preclude subsequent experiments.

In conclusion, our experiments show that valuable epigenetic analyses such as those reported by Renauer *et al* may be carried out with greater flexibility (eg, direct or indirect labelling) and the potential for automatization by sequentially using magnetic beads. This in turn may increase validity and allow for higher sample numbers to be analysed in future epigenetic studies.

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