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Technical note

Multiplexed labeling of samples with cell tracking dyes facilitates rapid and accurate internally controlled calcium flux measurement by flow cytometry

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ABSTRACT

Calcium flux measurement is a crucial assay in lymphocyte activation. However, with the currently well established flow cytometric methods, it is a tedious procedure that is difficult to control to avoid variation between samples. This leads to unwanted sources of error that can make it problematic to interpret the results. Here we present an improved method that allows different cell populations to be tested in the same sample. Samples are pre-labeled with CFSE or Cy5 then mixed and stimulated to induce calcium flux. This facilitates more rapid and accurate measurement of calcium flux and also dramatically reduces the cost and effort required for this type of assay.

1. Introduction

Calcium flux is one of the hallmark events in the process of lymphocyte activation and is broadly involved in many cellular functions (Feske, 2007). There are many methods available to measure calcium flux by using a diversity of fluorescent Ca^{2+} indicators (Simpson, 2006). Among these indicators, Indo-1 shows a spectral shift in its emission maximum upon Ca^{2+} binding (Nelemans, 2006). By Indo-1 labeling of cells in conjunction with the new generation of digital flow cytometers, calcium flux can be conveniently measured in many cell types. However, although this method greatly facilitates calcium flux measurement, this assay still remains one of the most challenging and tedious techniques in a non-calcium-specialist lab.

Firstly, although this assay is flow cytometry based, one sample usually requires 5–15 min of cytometer time. To obtain consistent and reliable data, a researcher needs to run many samples at a rate of 3–4 samples per hour for several hours. The experimental time lengthens if it is necessary to check several stimulation and treatment conditions. Thus it would be desirable to reduce the number of samples needed without losing statistical confidence. Secondly, in a typical calcium flux assay, it is necessary to frequently add-in reagents and to load and unload samples to the flow cytometer, within a few minutes, and with precise timing. To achieve a satisfactory level of reproducibility, even an experienced flow cytometry user needs to take a lot of time to practice. Under these circumstances, it is easy to introduce errors within a sample or between different samples, mainly in the timing of reagent addition and restart. Unfortunately, when comparison between different samples is crucial, even a very minor mistake is not acceptable and will either completely invalidate the individual sample (no second chance) or skew the data analysis because of even a one second false start or delay in loading or unloading the sample. Thirdly, as a very sensitive method, the calcium flux assay can easily introduce variations between samples due to factors such as dye loading, antibody staining, temperature fluctuation, sample flow rate, adding or mixing of reagents, loading and unloading samples and so on. Some typical errors most likely to occur in the calcium flux assay are depicted in Fig. 1A and B. These hinder the routine use of calcium flux assay in many labs.

Cell tracking dyes (e.g. CFSE and Cy5) have been widely used in many biological function studies. In the new approach

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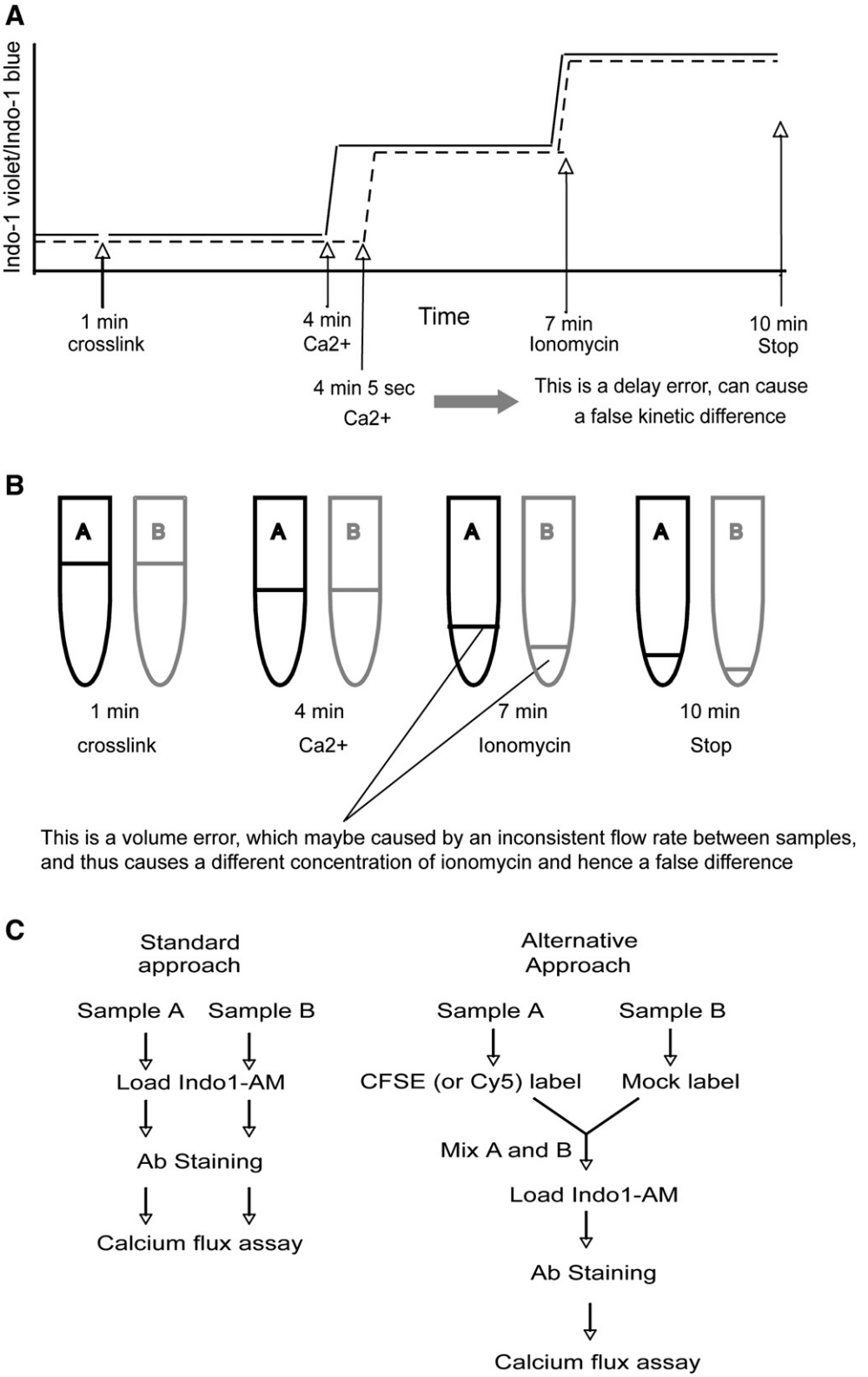

Fig. 1. Typical errors in standard calcium flux assay and the proposed new method. It is easy to introduce variations in calcium flux assay, shown here are two typical errors caused by either inaccurate timing of adding reagents (A), or inconsistent instrumental flow rate between samples (B). These situations prompted us to improve the current standard approach in order to eliminate these variations. The difference between standard and alternative approaches is shown in (C).