TVA™: Imaging-based, non-radioactive detection of NK cell activity provides results comparable to classic Chromium Release Assays using fewer cells and with less labor

Srividya Sundararaman, Kinga Karacsony, Diana Roen, and Paul V. Lehmann

Cellular Technology Limited • R&D Department • 20521 Chagrin Boulevard • Shaker Heights, OH USA

INTRODUCTION: Natural Killer (NK) cell activity and Antibody Dependent Cell-mediated Cytotoxicity (ADCC) has traditionally been assessed by detecting the lysis of tumor cells in the Chromium Release Assay (CRA). This classic assay relies on radioactive label, is laborious, and requires substantial quantities of patient blood. We have developed a Target cell Visualization Assay (TVA™) that measures cytolytic activity by high-throughput imaging of fluorescence-labeled target cells. We also developed a miniaturized version of the TVA™ to measure NK/ADCC activity with a fraction of the blood needed for a classic CRA, and with less labor.

METHODS: Similar to a classic CRA, in the TVA™ a fixed number of labeled target cells and serially diluted effector cells are co-cultured. Instead of radioactive chromium, target cells are labeled with a fluorescent dye. After 4 hours, the remaining viable individual target cells were detected and counted using an ImmunoSpot® Analyzer (S6ULT-00-9000). This assay was performed using both 96-well and Terasaki plate formats. The percentage of lysis is computed by determining the difference in counts between test wells and control wells.

RESULTS: Only viable target cells retain the fluorescent dye; it is lost upon cell death *(Figure 1)*. When effector cells and target cells are mixed in various ratios, the number of viable cells is inversely proportional to the number of effector cells *(Figures 2 and 3)* with high inter-assay repeatability and intermediate precision *(Figure 4)*. CRA and TVA™ in a 96-well format provide equivalent results *(Figure 5)*. TVA™ assays were performed in Terasaki plate formats which reproduced the results from 96-well plate assays, however, it allowed the number of required effector cells to be reduced by tenfold *(Figure 6)*.

CONCLUSIONS: We have demonstrated the feasibility of assessing NK function in a high-throughput capable, non-radioactive system involving less labor. High repeatability, intermediate precision, and audit trails make the assay suitable for regulated immune monitoring. Miniaturization of the assay in Terasaki format is of particular value when access to PBMC is limited, such as in pediatric, geriatric, and immune-deficient populations.

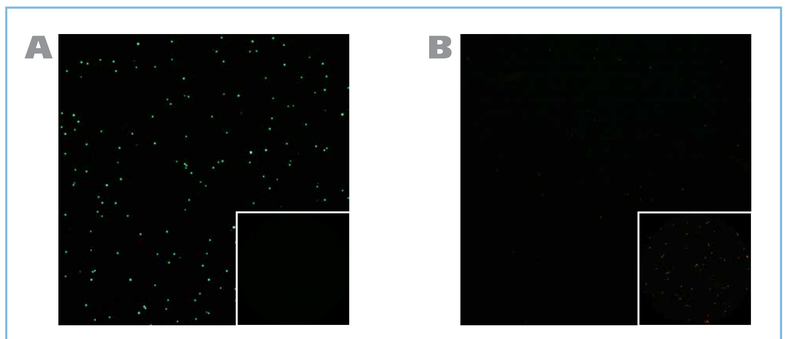

Figure 1: **Fluorescently-stained K562 tumor cells lose their dye after lysis.** K562 tumor cells were fluorescently-labeled and either cultured in the presence of culture media alone (A), or in the presence of 95% ethanol (B) for one hour prior to imaging. Inset images show same cells stained with a fluorescent dye that detects dead cells.

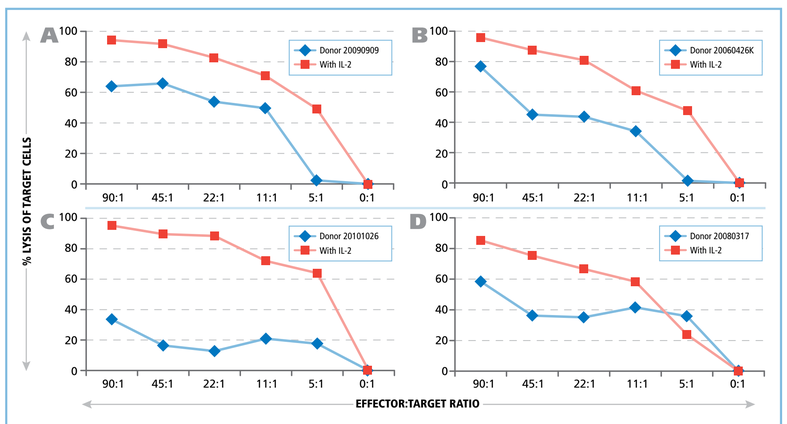

Figure 3: **PBMC-mediated NK activity against K562 tumor cells.** Cryopreserved PBMC of 4 donors (A-D) were tested for their cytolytic function against K562 target cells. The PBMC were either cultured with medium alone, or with 1000U/ml of IL-2 overnight prior to adding them to the target cells at the specified E:T ratios. After 4 hours of co-culture, the viable cells were counted and the % lysis was calculated.

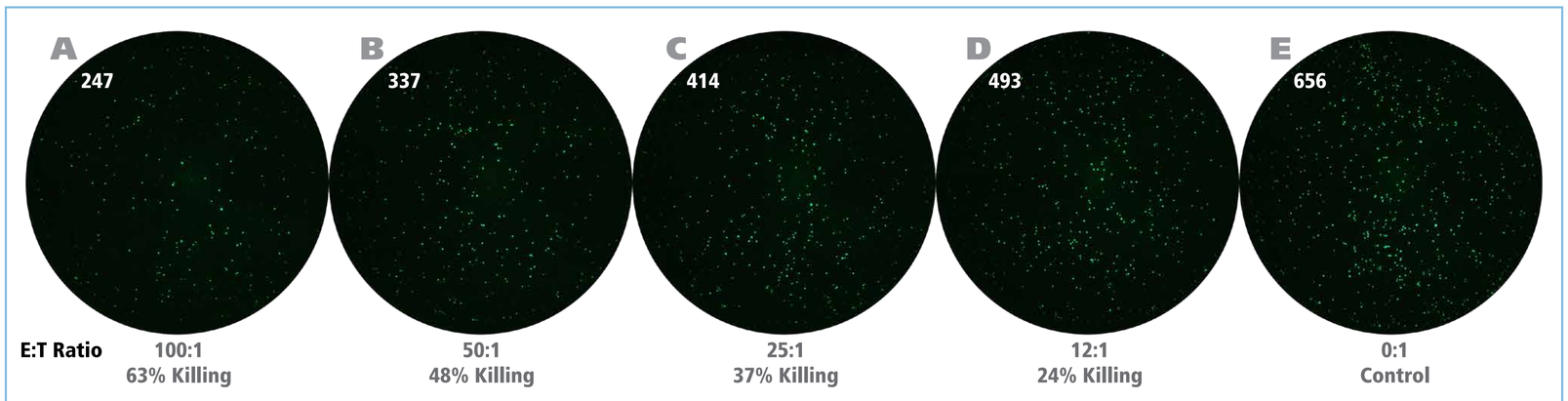

Figure 2: **Representative images of viable target cells after incubation with PBMC at different E:T ratios in a 96-well plate.** A fixed number of fluorescently-labeled K562 tumor cells were incubated with effector cells (PBMC) at the above specified Effector:Target (E:T) ratios in a round-bottom, 96-well plate for 4 hours. Subsequently, 50µl of cell solution was transferred into a flat bottom plate and imaged. Viable target cells were visualized and counted using an automated plate reader. (A-E). The numbers of viable cells counted per well are shown on the images. Control wells do not contain effector cells and thus establish the zero killing value. The difference in viable cell count is expressed as % lysis and is a direct reflection of killing.

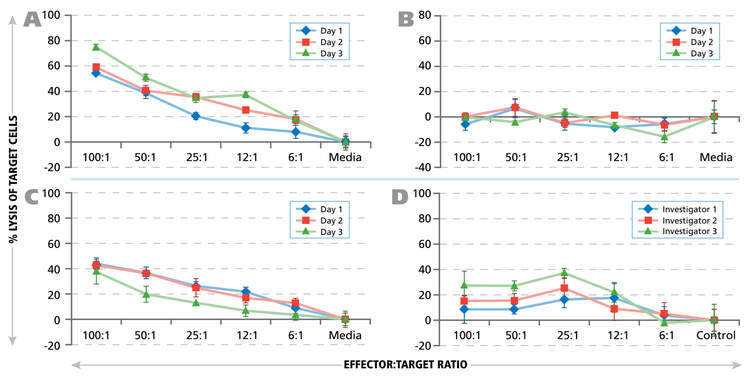

Figure 4: **Precision — Repeatability of the assay.** To test inter-assay repeatability, cryopreserved PBMC of three donors with high (A), intermediate (B), and low (C) NK activity were tested on three different days by the same investigator. To evaluate intermediate precision, the PBMC were tested in parallel by three investigators on the same day (D).

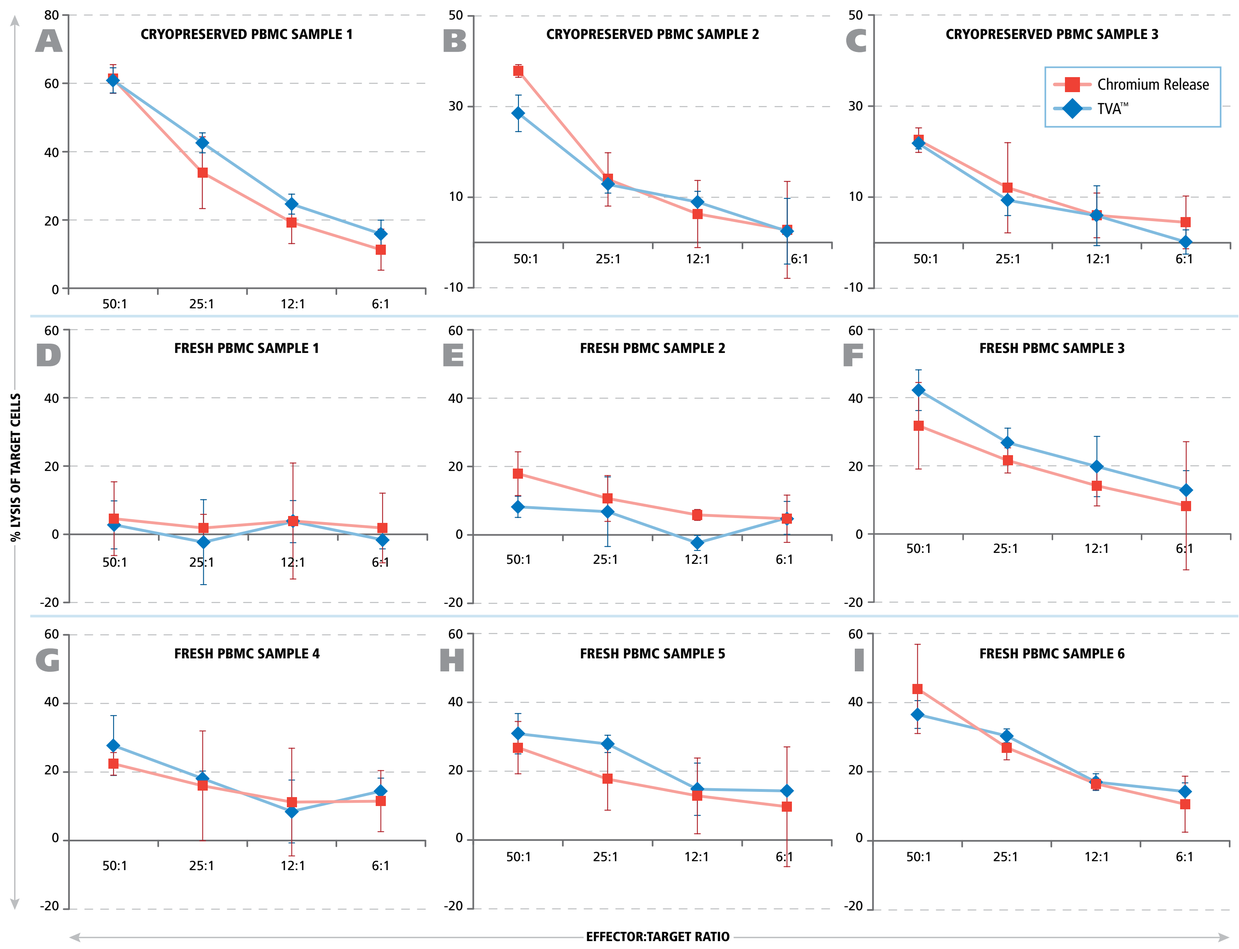

Figure 5: **Accuracy — Comparison with CRA.** Cryopreserved PBMC from three donors (A-C) and freshly-isolated PBMC from six donors (D-I) were tested in the two assays in parallel; the TVA™ and the CRA.The TVA™ was performed as described as in Figure 2. The % lysis was computed according to the formula:

$$\frac{\text{Count in Control Well} - \text{Count in Test Well}}{\text{Count in Control Well}} * 100$$

CRA was performed following standard protocols, measuring radioactivity in supernatants of chromium-51 labeled target cells. The % lysis for CRA was calculated according to the formula:

$$\frac{\text{Test Release} - \text{Spontaneous Release}}{\text{Max Release} - \text{Spontaneous Release}} * 100$$

The results from different donors correlated well between the TVA™ and CRA with no significant differences (p-value<0.05).

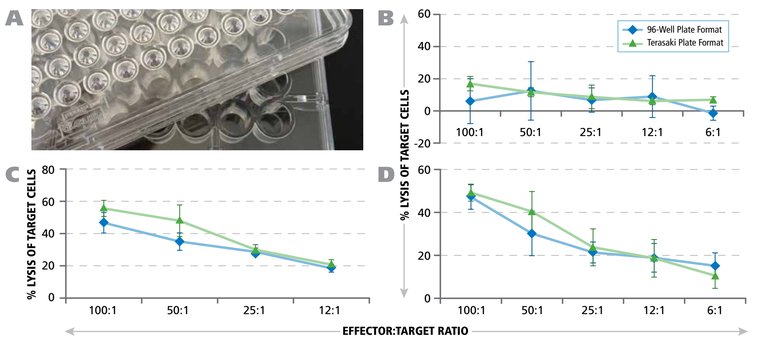

Figure 6: **Miniaturization of TVA™ Assay in Terasaki plate format.** The assay was performed in 96-well and Terasaki plates (A) in parallel. For the Terasaki format, the effector cells from three different donors (B-D) and labeled target cells were premixed before transferring the cells to the Terasaki plate where they were imaged and counted. The Terasaki format uses 1/10th of the number of effector cells required by the 96-well formats and is less laborious because the cells can be directly imaged in the assay wells.

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