29 May 2013

Matt Fierman, Ph.D.
Science Editor
Journal of Visualized Experiments (JoVE)
17 Sellers St., Cambridge, MA 02139

Dear Dr. Fierman:

Enclosed please a manuscript by Lowes et al. entitled "Adaptation of the CellSearch circulating tumor cell (CTC) assay for clinical and preclinical research applications" which we would like to submit for consideration for publication in the *Journal of Visualized Experiments (JoVE).*

Circulating tumor cells have been demonstrated to be of clinical significance in metastatic breast, prostate, and colorectal cancers. The current gold standard in clinical CTC detection and enumeration is the FDA-cleared CellSearch system. This manuscript outlines the standard protocol utilized by this platform as well as two additional adapted protocols that we have developed, including *(1)* the detailed process of user-defined marker optimization for protein characterization of patient CTCs, and *(2)* a comparable protocol for CTC capture in very low volumes of blood for studying *in vivo* pre-clinical mouse models of metastasis. We believe that these protocols will provide a valuable resource for users of this platform interested in pre-clinical and clinical research pertaining to metastasis and CTCs.

This work is particularly suited to publication in *JoVE*'s unique multi-media format for a number of reasons. The clinical importance of CTCs means that all assays developed must be highly quality-controlled and reproducible, not only in the lab(s) where the assays are developed, but also in other labs that implement the assays. Therefore, the ability to have both a detailed step-by-step protocol and a video to help new users establish and trouble-shoot such protocols will be highly valuable, and this is not available through any other journal. In addition, because of the rare nature of CTCs in the bloodstream (estimated to be ~1 CTC per 10^5-10^7 blood mononuclear cells), visualization of these cells is a key component of the assay that allows users to rule out false-positives and false-negatives. The topic of CTC analysis is currently of high interest in the clinical oncology and metastasis biology fields, and we feel that our work helps address a methodology gap in this area.

The authors of this paper are Lori E. Lowes, Benjamin D. Hedley, Michael Keeney, and myself. All authors of this research paper have directly participated in the planning (LEL, BDH, MK, ALA), execution (LEL, BDH), and analysis (LEL, BDH, MK, ALA) of the study and have approved the final version submitted.

We thank Joni Williams and Meredith Wilkes at *JoVE* for their assistance to date in the *JoVE* submission and review process, and we look forward to continuing to work with them throughout the publication process. In addition, we would like to suggest the following reviewers for peer-reviewed consideration of this manuscript:

1. Jaco Kraan (j.kraan@erasmusmc.nl) or Jan Gratama (j.w.gratama@erasmusmc.nl) - Erasmus MC, University Medical Center, Rotterdam, The Netherlands.
2. Kim Chi (kchi@bccancer.bc.ca), BC Cancer Agency, Vancouver BC, Canada.
3. Dario Marchetti (marchett@bcm.edu), Baylor College of Medicine, Houston, TX, USA.

We hope that *JoVE* will consider the findings of this manuscript to be of significant quality and importance for publication, and we look forward to receiving feedback from the *JoVE*'s reviewers and editorial board. If you have any further questions or concerns regarding this manuscript, please do not hesitate to contact me.

Sincerely,

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