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Review

Recommendations for the evaluation of specimen stability for flow cytometric testing during drug development

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ABSTRACT

The objective of this manuscript is to present an approach for evaluating specimen stability for flow cytometric methods used during drug development. While this approach specifically addresses stability assessment for assays to be used in clinical trials with centralized testing facilities, the concepts can be applied to any stability assessment for flow cytometric methods. The proposed approach is implemented during assay development and optimization, and includes suggestions for designing a stability assessment plan, data evaluation and acceptance criteria. Given that no single solution will be applicable in all scenarios, this manuscript offers the reader a roadmap for stability assessment and is intended to guide the investigator during both the method development phase and in the experimental design of the validation plan.

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1. Introduction

Multiparametric flow cytometry is the leading technology for the simultaneous characterization of individual cells. When suitable reagents are available, flow cytometry can be used to determine the developmental phenotype and functional status of a cell, including its activation state, developmental stage, cell cycle status, and signal transduction pathway engagement.

Flow cytometry is useful across all phases of drug development. Examples include analyses of drug target occupancy and pharmacodynamics in pre-clinical and clinical studies, as well as determination of patient eligibility and stratification for clinical trials, and assessment of study endpoints (Green et al., 2011; O'Hara et al., 2011). Typically, later stage drug development clinical trials include multiple investigative sites distributed globally, necessitating the need for specimens to be shipped to a centralized testing facility. The advantages of centralized analysis are a significant decrease in the variability associated with differences in sample processing, instrumentation, and data analysis. The primary challenge associated with centralized testing is the delay in testing after specimen collection. Thus, a thorough assessment of specimen stability is critical to successful centralized testing. In this manuscript we propose a process for assessing specimen stability, with an emphasis on the challenges associated with specimen stability in cell-based fluorescence methods.

2. Specimen stability assessment

Key variables that affect stability include specimen type, sample collection methods, and assay design such as monoclonal antibody (mAb) clone selection, and fluorochrome/antigen pairing. Logistical considerations such as transportation temperature and time also impact specimen stability. Considerations regarding specimen stability should be incorporated into the assay development process (Fig. 1). If specimen stability does not meet the requirements for the intended use of the assay, reconfiguring the assay (e.g. new anticoagulant, mAb clones, staining conditions) may result in increased stability.

2.1. Process overview

After initial determination of the assay objective, the flow cytometry panel is designed and the type of specimen and collection procedure established. Next, the assay should be fully optimized with regard to antibody titration, staining conditions (time and temperature), lyse, wash and fixation sequences and buffer selection (Tanqri et al., 2013). Method validation and stability assessment should begin only after the assay has been fully optimized.

Initial stability assessments should be conducted with specimens stored in the laboratory at ambient temperature (18 to 26 °C); later the stability of shipped samples should also be evaluated. When assessing the stability of shipped samples, it is important to consider geographic locations and seasonal temperature fluctuations that may be encountered within the clinical study. If acceptable stability is not achieved with specimens maintained at ambient temperature, storage at 2 to 8 °C should be considered. Refrigeration may preserve specimen stability but may also increase the risk of clotting (CLSI, 2007) and alter surface antigen expression. For temperature sensitive assays, insulated shipping containers and refrigerants such as gel packs may be required to maintain the required temperature during transit. In this case, temperature tracking devices are recommended as they provide additional quality monitoring data.

2.2. Specimen type and collection

For clinical trials, peripheral whole blood is the most frequently collected specimen type for flow cytometric analysis. Bone marrow, cerebral spinal fluid, synovial fluid, and tissue biopsies require more invasive collection techniques and are used less often as a result. Whole blood is typically drawn by venipuncture into vacuum tubes containing anticoagulant, and in some cases, a preservative or stabilization solution. The choice of anticoagulant and blood collection tube is often driven by logistical considerations and the type of assay being performed (e.g., immunophenotyping, and assessments of signal transduction and other intracellular functions). In all cases, a thorough understanding of exactly what will be measured and the intended use of the data, is required for appropriate anticoagulant selection (Narayanan, 2000). Each anticoagulant has potential advantages and limitations as discussed below (Carter et al., 1992; Son et al., 1996; McCarthy, 2007).

2.2.1. EDTA

Ethylenediaminetetraacetic acid (EDTA) is available in several different formulations, has several distinct advantages