Abstract

Purpose: Many commercial immunoassays are available for quantifying biomarkers. Comparing data across platforms requires accurate calibration to international reference standards. Most NIBSC/WHO standards are commercially available as lyophilized reference standards. However, limited information is available on vial-to-vial variability of freshly prepared standards.

Methods: Vial-to-vial variability of freshly prepared NIBSC/WHO calibrators was evaluated by reconstituting and analyzing 2 vials of each standard by one operator on the same day. Vial-to-vial variability of freshly prepared Proinflammatory Panel 1 (human) calibrators was evaluated by reconstituting and prepared samples. Data from stimulated samples is expressed as percent of freshly thawed samples. As shown, NIBSC/WHO standards demonstrate a standard, which is partially purified from supernatants of lectin activated human leukocytes, remains unclear. Alternate NIBSC/WHO recombinant IFN-β, specifically, we compared to using aliquots that were freshly reconstituted from lyophilized reference standards, calibration using frozen aliquots created the largest variability. NIBSC/WHO product inserts generally do not advise against freezing reconstituted material and in some cases recommend freezing aliquots. However, after a single freeze–thaw cycle, recovery relative to freshly prepared aliquots was <80% for most analytes. Similarly, we observed degradation of internal reference controls using freshly reconstituted standards or lyophilized aliquots. However, these controls were stable after a single freeze–thaw cycle. The process of calibrating immunoassays to international reference standards is impacted by numerous parameters affecting storage stability of reconstituted reference material as well as data collection parameters.

Conclusion: The process of calibrating immunoassays to international reference standards is impacted by numerous parameters affecting storage stability of reconstituted reference material as well as data collection parameters. NIBSC/WHO NIBSC/WHO standards are reported in International Units (IU). Concentration ratios were transformed into a conversion factor (pg/IU) based on NIBSC/WHO assigned analyte activity (pg/IU) divided by the conversion factor to achieve NIBSC/WHO standards values. In all cases, recoveries 1 ± 2%.

Results: Calibration of MSD Assays to NIBSC/WHO standards

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Methods

Lyophilized NIBSC/WHO standards were reconstituted and aliquots were prepared according to instructions provided by the vendor. Standards were used to create 2 vials for each standard, which was then reconstituted in separate wells. Data from stimulated samples is expressed as percent of freshly thawed samples. As shown, NIBSC/WHO standards demonstrate a standard, which is partially purified from supernatants of lectin activated human leukocytes, remains unclear. Alternate NIBSC/WHO recombinant IFN-β, specifically, we compared to using aliquots that were freshly reconstituted from lyophilized reference standards, calibration using frozen aliquots created the largest variability. NIBSC/WHO product inserts generally do not advise against freezing reconstituted material and in some cases recommend freezing aliquots. However, after a single freeze–thaw cycle, recovery relative to freshly prepared aliquots was <80% for most analytes. Similarly, we observed degradation of internal reference controls using freshly reconstituted standards or lyophilized aliquots. However, these controls were stable after a single freeze–thaw cycle. The process of calibrating immunoassays to international reference standards is impacted by numerous parameters affecting storage stability of reconstituted reference material as well as data collection parameters.

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