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In vitro diagnostic medical devices — Multiplex molecular testing for nucleic acids —

Part 2: Validation and verification

Dispositifs médicaux de diagnostic in vitro – Tests moléculaires multiplex pour les acides nucléiques —

Partie 2: Validation et vérification

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Foreword

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Introduction

The first generation of in vitro diagnostic (IVD) medical devices for nucleic acid-based molecular tests focused on detection or quantitation of a single nucleic acid sequence (eg, viral RNA, mRNA, and genomic DNA) within a clinical specimen. By comparison, a multiplex molecular test simultaneously measures multiple nucleic acid sequences of interests in a single reaction. The development and clinical use of multiplex IVD medical devices are rapidly expanding with the technological advances and new elucidation of clinical significance of the many biomarkers.

The competition among reactions in multiplex molecular tests can impose more stringent requirements for sample purity, input reagents and platforms to avoid nonspecific reactions and background signal. In comparison to single target analysis, multiplex molecular tests require an increased number of controls, more complex performance evaluation/data analysis algorithms and more complex reporting of results.

Laboratories can develop assays in-house ("home-brew, laboratory-developed, in-house") or use commercially available multiplex assays involving a variety of technologies and instrument platforms. With the increase in the availability and use of multiplex molecular tests, a guideline for the development, validation, verification, control, data analysis, and implementation of multiplex molecular tests is increasingly needed. For a multiplex molecular test to reliably achieve its intended use, there should be control of the process from the acquisition of the sample and preparation of the nucleic acid for testing to the evaluation of the data and the reporting of the results. Multiplex molecular testing provides significant challenges to the laboratory with regards to appropriate validation and verification, acquisition of appropriate control materials, data analysis, and reporting. The complexity of data analysis and reporting of results is increased relative to singleplex assays. Moreover, the availability of sufficient and appropriate control and reference materials (RMs) to properly validate and verify multiplex molecular tests is a major challenge. However, the use of partial or full sequencing techniques can be useful in qualifying control materials. This document describes the recommendations for various aspects of validation and verification of the measurement by multiplex molecular tests in order to ensure reproducible performance of such tests, in developing and implementing multiplex molecular nucleic acid tests for clinical use.