ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Analytical validation and stability studies for basophil activation test to meet IVDR certification

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Introduction: Basophil Activation Tests (BAT) have gained increasing importance in the field of allergy. We hypothesize a thorough assay validation study meeting CLIA requirements is possible and performed analytical and stability studies with Flow CAST BAT assay, to achieve the new EU-IVDR compliance. **Method:** For all studies, four healthy blood donor samples were stimulated with one negative control (stimulation buffer) or one of two positive stimulation controls, anti-FcεRI (PC1) or fMLP (PC2), following CLSI guidelines. The analytical investigated variations in the assay procedure included temperature, incubation time for stimulation, staining and lysis as well as different applied volumes of single kit reagents and blood samples. Kit, components, fresh blood samples and processed blood samples were stored at different temperatures for defined timepoints. **Results:** Repeatability showed a CV of 1.1 – 8.8% and reproducibility a CV of 0.9 – 15.4%. Within-laboratory precision showed highest CV of 1.9 – 21.5%. Robustness included all analytical variations of the protocol and resulted in specific recommendations for each parameter (not shown). Unprocessed EDTA whole blood samples were stable for 48 hours (storage at 2-8 °C) and 24 hours (storage at 28 °C). Processed and fixed basophils remained stable for at least 10 days at 2-8 °C and 48 hours stored at 28 °C. **Conclusion:** All performance criteria for the Flow CAST BAT assay with respect to repeatability, within laboratory precision, reproducibility and stability were successfully achieved to gain world first IVDR approved BAT IVD test. Furthermore, these studies will support harmonization of BAT and promote its use in clinical diagnostic routine.

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