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Abstract

We have developed an accurate, precise and stability-indicating þow cytometry (FC) based assay to directly measure antigenicity of H4 protein in a vaccine formulation of H4-IC31, without desorbing the H4 protein from the IC31 adjuvant. This method involves immuno-staining of H4-IC31 complex with anti-H4 monoclonal antibodies (mAbs) followed by FC analysis. The assay is not only able to consistently measure H4 antigenicity levels

in H4-IC31 stored under normal condition at 2-8ÁC, but also able to detect changes in H4 antigenicity after H4-IC31 undergoes heat stress or freeze-thawing. In addition, the FC method is able to characterize particle morphology while measuring antigenicity. The biological relevance of the changes in H4 antigenicity detected by the FC assay was supported by an in vitro cell based functional assay using human PBMCs to measure IFN-gamma (IFN-ɔ) secretion upon re-stimulation with H4-IC31. Our results show that the FC based antigenicity assay can e ciently monitor the biological and physicochemical properties of H4-IC31 and is an indicator for adjuvanted vaccine product stability.