IQA Immunology Quality Assessment Program An NIH, NIAID, DAIDS Program

Duke Human Vaccine Institute

To: RePORT India

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Subject: FBS lot to lot comparison study.

The following is the summary on the testing of proposed new lot(s) of Fetal Bovine Serum (FBS) received by the IQA laboratory (see table 1) on 13Oct2025. The comparison study was measured using a traditional enzyme linked immunospot (ELISpot) assay, which includes PBMC assessments of Viability and Viable Recovery, plus functionality and background.

Table 1. FBS Vendor Name, Lot Numbers

FBS Vendor/Cat #	FBS Lot Number	Exp. Date	Outcome
Sigma	24E201	7/31/29	Acceptable
Sigma	22L538	11/30/27	Current FBS Lot

The ELISpot test panel consisted of a set of mitogens and antigens (see table 2 below), plus wells for PBMCs and media alone.

Table 2.

CMV
PHA
CEF

The PBMCs used for the assay, were obtained from one HIV sero Negative or Positive donors. PBMCs were isolated and harvested using Ficoll gradient process. The PBMCs were adjusted to 20x10^6cell/mL and were cryo preserved with freshly made cryo preservation media of 10% DMSO and 90% FBS of the particular lot#. The different FBS lots were used along with a current lot used at the IQA laboratory.

All PBMC samples were thawed and used in this FBS lot to lot test round, resulted in at least 85% viability with greater than 70% viable cell recovery. The PBMC samples were run in triplicate wells.

CONCLUSION: FBS Lot# Sigma 24E201 was selected based on its net (spot forming cells) SFC greater than 700 SFC for PHA, greater than 90 SFC for CMV and less than 2 SFC for CEF, cells alone-background/control wells was lower than 2 SFC per well and the media alone background wells had 3 SFC, resulted in low background.

The results obtain for FBS Lot# Sigma 24E201 demonstrate the best choice, from each of the FBS lots which were tested on 11/21/25.