



To: ACTG/IMPAACT

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Subject: FBS lot to lot comparison study.

The following is the summary from four proposed new lots of Fetal Bovine Serum (FBS) received by the IQA laboratory (see table 1). The comparison study was measured using a traditional ELISPOT Assay.

Table 1. FBS Vendor Name, Lot Numbers

Materials/ Reagents	Vendor	Lot Number
FBS (test)	SIGMA	20E043
FBS (test)	SIGMA	20H250

The ELISPOT panel consisted of a set of mitogens and antigens (see table 2).

PBMCs used for the assay were obtained from two HIV sero negative donors. PBMC vials were cryo preserved with 10% DMSO and 90% FBS. The different FBS lots were used along with a current lot used at the IQA laboratory. The PBMCs were frozen at a concentration of 10 million per ml, and thawed at a later date for the ELISPOT assay use. All PBMC samples thawed resulted in at least 90% viability and had at greater than 80% viable cell recovery.

Table 2.

Materials/ Reagents
CMV
PHA
CEF

The samples were run in triplicate wells, resulting in net SFC greater than 700 SFC for PHA, greater than 500 for CMV and greater than 500 SFC for CEF. Control wells [cells alone-background] lower than 2 SFC per well. Cells alone background wells SFC, resulted in low background from each of the FBS lots which were tested.