Duke University School of Medicine
Immunology Quality Assessment Center

To: Cheryl Jennings at the VQA

From: Raul Louzao at the IQA Laboratory, Duke University-Durham NC

Date: April 20, 2009

Subject: FBS lot to lot comparison study.

The following is the summary from the three proposed new lots of Fetal Bovine Serum (FBS) received by the IQA laboratory on February 2, 2009 (see table 1). The comparison study was measured using a Lymphocyte Proliferation Assay (LPA).

Table 1. FBS Vendor Name and Lot Number.

Materials/ Reagents	Vendor	Lot Number
FBS (control)	Gemini	A61101C
FBS (test)	Sigma Aldrich	088K8413
FBS (test)	Gemini	A61A01X

The LPA panel consisted of a set concentration 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 LPA 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	Vendor	Final Concentration (ug/ml)
Candida Albicans	Greer Laboratory	10
PHA	Sigma Aldrich	5
PWM	Sigma Aldrich	0.2

The samples were run in quadruplicates wells, resulting in net CPMs greater than 1,500 CPM and control wells [cells alone-background] lower than 1000 CPM. The stimulation indices [SI] for each of the antigens and mitogens, were greater than 3, which indicated a positive response. Overall all the cells responded well to the stimulants. Cells alone background well CPMs, for all cells resulted in low background from each of the FBS lots which were tested.

VIROLOGY QUALITY ASSESSMENT PROGRAM

STATISTICAL CENTER

TO: Jim Bremer
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CC: Mike Ussery
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FROM: Donald Brambilla, Suzanne Granger, Brian Harty, Meena Doshi.

DATE: March 25, 2009

SUBJECT: Comparison of new and established lots of FBS

The following is a report on the results from the FBS comparison study using qualitative HIV macrococulture panel CUL023CC. The panel consisted of one specimen from each of three HIV-infected donors and one specimen from an uninfected donor. All four specimens were included in the analysis. Data were obtained from 4 laboratories. Panels A, B and C (see Table 1) were tested using cells cultured and stimulated with medium containing FBS lot A, B and C, respectively, using HIV macrocultures.

Table 1: HIV Culture Panel ID and FBS Vendor Name and Lot Number

Material/Reagent	Panel ID	Vendor	Lot Number
FBS lot A (control)	CUL023CC.01-04A	Gemini	A61101C
FBS lot B (test)	CUL023CC.01-04B	Sigma Aldrich	088K8413
FBS lot C (test)	CUL023CC.01-04C	Gemini	A61A01X

A macroculture consists of a single culture flask, run as described in the ACTG Laboratory Manual. Culture results were classified as positive or negative using the following algorithm. A result from a macroculture is considered positive if one of three criteria is satisfied:

1. Two consecutive p24 values $\geq$ 30 pg/ml are obtained, with the second being at least four times the first or out of range;
2. Two consecutive values that are out of range are obtained; or
3. Three consecutively increasing p24 values are obtained, all of which are $\geq$ 30 pg/ml and the third of which is at least four times the first.

A result is considered negative if it does not satisfy the criteria for a positive culture after 28 days.

Culture results are summarized below and in Table 2:

Specimen 1: positive results were obtained using all three FBS lots in all four laboratories. The median number of days across laboratories to obtain a positive culture was 7 days for lot A, 3 days for lot B, and 3 days for lot C.

Specimen 2: positive results were obtained using all three FBS lots in all four laboratories. The median number of days across laboratories to obtain a positive culture was 7 days for lot A, 3 days for lot B, and 3 days for lot C.

Specimen 3: a negative consensus result was obtained on the culture proficiency testing for this sample. However, two indeterminate results were obtained on this specimen in two laboratories: one laboratory obtained an indeterminate result with a culture using FBS lot A and a second laboratory obtained an indeterminate result with a culture using FBS lot C. The culture was indeterminate in laboratory 3 due to a single p24 value of

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74pg/mL on day 10 (all other p24 values were less than 30gp/mL). The culture was indeterminate in laboratory 4 due to a single p24 result of 44pg/mL on day 10 (all other p24 values were less than 30pg/mL).

Specimen 4: positive results were obtained using all three FBS lots in all four laboratories. The median number of days across laboratories to obtain a positive culture was 7 days for lot A, 7 days for lot B, and 7 days for lot C.

Table 2: Summary of Outcomes by Panel and Laboratory
N: Negative Result
P: Positive Result
I: Indeterminate Result
(n): Days to Terminate Culture as Positive

	CUL023CC.01			CUL023CC.02			CUL023CC.03			CUL023CC.04		
LAB	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
1	P (07)	P (07)	P (07)	P (07)	P (07)	P (07)	N	N	N	P (07)	P (07)	P (14)
2	P (07)	P (03)	P (03)	P (03)	P (03)	P (03)	N	N	N	P (07)	P (07)	P (07)
3	P (07)	P (03)	P (03)	P (07)	P (07)	P (07)	N	N	I	P (10)	P (07)	P (07)
4	P (07)	P (03)	P (03)	P (03)	P (03)	P (07)	I	N	N	P (07)	P (03)	P (03)

The results for this comparison are limited. However, the data suggest that FBS Lots B and C are comparable in performance to Lot A for HIV co-culture.

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