SOP 99-01 Generating immortalized macaque B-lymphoblastoid cell lines

Purpose and principle:
Autologous cells are used at targets in assays to detect cytotoxic T cell activity. This SOP describes the generation of immortalized B-LCL from macaque monkey PBMC using a transforming Herpesvirus.

Materials:
- Macaque whole blood
- RPMI 1640 with 12% FCS
- *Herpesvirus papio* containing supernatant (from S594 cell cultures, for monkey B-LCL)
- 15 ml polypropylene tubes
- 15 ml polystyrene tubes
- Pipets
- 96-well plates
- Table-top centrifuge
- 25 cm^2 flasks
- 75 cm^2 flasks

Procedure:
1. Ficoll isolate lymphocytes from monkey blood and lyse any red cells.
2. Plate 10^5 lymphocytes per well in a 96 well plate in 100 μl of culture medium.
3. Add 100 μl of S594 supernatant or 100 μl media for controls.
4. Incubate at 37°C, 5% CO_2, changing 100 μl of the medium at least once a week.
5. Dark foci of immortalized cells should appear within 2-4 weeks.
6. Expand when possible and maintain cell lines at $0.4x10^6$ cells/ml in 12% FCS RPMI in T25 flasks.

Special handling/waste disposal:
Treat *H.papio* as a biohazard and handle at BSL2+.

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Revised: