

AN EVALUATION OF ROUTINE EPSTEIN-BARR VIRUS TRANSFORMATION OF B-LYMPHOCYTES



WELSH BLOOD SERVICE
GWASANAETH GWAED CYMRU

WELSH TRANSPLANTATION AND
IMMUNOGENETICS LABORATORY



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H BASS, M G GUTTRIDGE AND
C DARKE

Introduction

Epstein-Barr virus transformed B-lymphoblastoid cell lines (BCLs) have been routinely produced in this laboratory for over five years. These provide reference material for DNA-based typing, cells for antibody screening by flow cytometry, and archival material for our collection of new and rare alleles. Novel, rare and unusual HLA phenotypes are highlighted during our routine clinical testing and from typing volunteer bone marrow donors for the Welsh Bone Marrow Donor Registry.

Methods

Sample preparation

A total of 152 samples (147 peripheral blood lymphocyte; 5 spleen cell) were transformed. Of these, 83 were obtained specifically for BCLs (between 1995-2000), taken into ACD, separated and frozen within 36 hours, under sterile conditions. A further 69 EDTA samples, had been processed under non-sterile laboratory conditions (between 1989-1997). Periodically, 20 frozen lymphocyte samples were selected for their allele of interest from the two batches, thawed and transformed.

Transformation

Both the 'in-house' production of Epstein-Barr virus supernatant and the lymphocyte transformation was carried out according to the methods outlined in: Cell and Tissue Culture: Laboratory Procedures, Section 26G, Doyle A. and Newell D.G. (Eds) John Wiley & Sons (Chichester) 1996.

Quality control

Each cell line was coded and tested for bacterial, fungal and mycoplasmal contamination using Hoechst 33258 (bis-benzimide tri-hydrochloride) in a DNA staining method with Vero cells as the indicator cell line (Cell and Tissue Culture: Laboratory Procedures, Section 7A: 1.4).

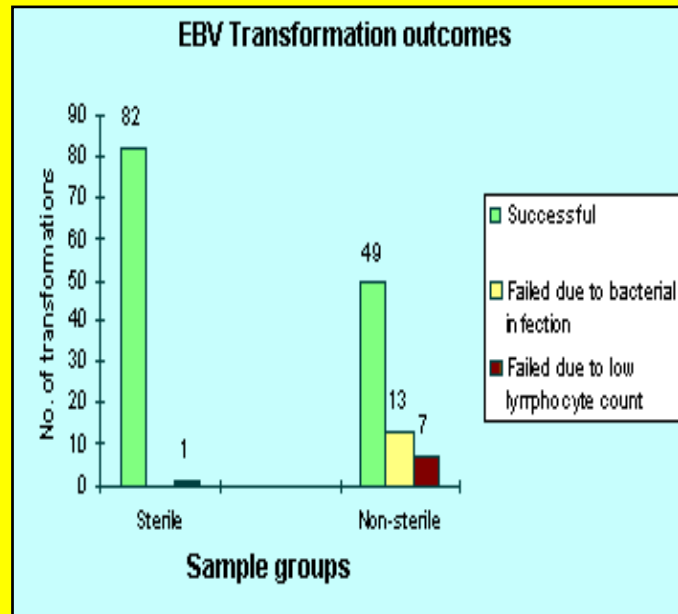
A 'bio-identity' check was performed on each BCL by confirming HLA-A, B, DR, and DQ concordance, by PCR-SSP, before and after transformation and after each expansion.

Results

Transformation

Overall, successful B-cell transformation was achieved for 131 (86.2%) samples. The 83 sterile samples produced 82 BCLs (98.8% success) while the 69 non-sterile samples produced 49 lines (71.0%). Two of the successful non-sterile samples had been frozen for 11 years prior to transformation.

Of the 20 failures in the non-sterile group, 13 were due to bacterial infection and 7 were probably due to the initial low lymphocyte yield, as was the single failure in the sterile-sample group. This latter sample and 9 of the non-sterile sample failures have since been transformed successfully with repeat sterile material.



Quality Control

Hoechst staining of the 131 BCLs showed that all of the lines were bacteria, fungi and mycoplasma free. All the 'bio-identity' checks revealed total concordance before and after transformation and expansion.

Comments

The high overall success rate of transformation (86.2%) may be attributed to the benefits of using a single operator (HB), in a dedicated facility, providing strict control and continuity of the process. Indeed, transformation success can be virtually guaranteed (98.8%) if good quality blood samples are processed in a sterile environment.

Strict quality control procedures, including 'bio-identity' checks, are absolutely essential when providing BCLs for use as laboratory reagents.