

# BEWARE RESEARCHERS BEARING GIFTS - A CAUTIONARY TALE



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## Introduction

Most of our HLA typing is performed on local patient and blood donor populations, which are largely of Northern European Caucasoid extraction. However, since it is of paramount importance that we are able to unambiguously detect and differentiate rare, unusual and new phenotypes we court the typing of single and population samples from non-European Caucasoid subjects as a continuing instructive programme.

We recently had the opportunity to HLA type 196 blood samples from a Sri Lankan population.

## Methods

HLA-A, B, DRB1/3/4/5 and DQB1 typing, essentially at the "split specificity" level, was performed by our standard PCR-SSP technique.

HLA phenotypes were analysed using our standard suite of population genetics programmes. This included the calculation of: goodness-of-fit to Hardy-Weinberg equilibrium and expected homozygosity; phenotype and gene frequencies by maximum likelihood and direct counting; haplotype frequencies and various linkage disequilibrium parameters and their significance.

## Results

As expected, phenotype and gene frequencies indicated a population very different from our own. For example, HLA-A33 was present in 40.8% of subjects and there was a low frequency of HLA-A2 (22.4%). These are found with a frequency of 0.72% and 51.3%, respectively in our local blood donor population.

However, the population fit to Hardy-Weinberg equilibrium was poor (Table 1). This was an unusual finding since all population samples we have HLA typed and examined in the past have generally shown a good fit to Hardy-Weinberg equilibrium.

## Explanation

After an exhaustive investigation it was discovered that an error had occurred in the operation of the study protocol in Sri Lanka.

Blood samples had been collected at the same time as replicate urine samples on 57 subjects (28 duplicate and 29 triplicate samples). The 86 replicate phenotypes were removed from the study group and the corrected population sample (n=110) was reanalysed.

The goodness-of-fit to Hardy-Weinberg equilibrium was now good (p-values for all four loci >0.32) – Table 2.

## Reproducibility of replicate samples

Inspection of the phenotypes of the replicate samples revealed three non-concordant HLA phenotypes. All of these were traced to sample labelling problems in the field hospital in Sri Lanka. After correction for these errors 100% correlation was achieved for HLA specificity assignment and 99.13% correlation for the assignment of allele/allele group (the single error being the assignment of A\*2417 in one sample and an A\*24 allele group (containing A\*2417) in its duplicate.

**Table 1. Summary of Hardy-Weinberg analysis on phenotypes from all samples (n=196)**

Locus	Number of "specificities"	Total If	d.f.	p-value
HLA-A	13	40.6	23	0.014
HLA-B	25	69.4	14	<0.001
HLA-DR	12	46.4	18	<0.001
HLA-DQ	7	19.9	12	0.071

**Table 2. Corrected population sample (n=110) - summary of Hardy-Weinberg analysis**

Locus	Number of "specificities"	Total If	d.f.	p-value
HLA-A	13	15.1	14	0.37
HLA-B	25	11.8	12	0.46
HLA-DR	12	8.0	7	0.33
HLA-DQ	7	6.6	13	0.92

## Comment

This episode clearly shows how easily fundamental problems can occur even during the operation of simple study protocols.

The value of performing tests for Hardy-Weinberg equilibrium and the power of specificity level HLA phenotyping as a "biodiversity" check is amply demonstrated.