ABO, Rhesus, and Kell Antigens, Alleles, and Haplotypes in West Bengal, India

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Supplemental Material

Supplementary Material and Methods

Immunohematology

Blood group serology was done with conventional tube method, as described previously.[9-11] The study was prospective observational, as the blood donors were tested at the time of their blood donation during the 18 months' study period.

Blood Group Test Reagents

The antisera were anti-A (clone no. 11H5), anti-B (clone no. 6F9), anti-A,B (clones 11H5 and 6F9), anti-D (clone P3×61; all monoclonal antibodies); and anti-H (*Ulex europaeus* lectin, all from Tulip Diagnostics, Goa, India); as well as anti-C (clone MS-24), anti-E (clone MS-260), anti-c (clone MS-33), anti-e (clones MS-16, MS-21, and MS-63), anti-K (clone MS-56; all human monoclonal antibodies), and anti-k (human polyclonal antibody, lot no. 10640.4310 and 10640.4440; all from DiaMed/Bio-Rad, Cressier-sur Murat, Switzerland).

Confirmation of D Negative Phenotype

All samples that tested D negative were confirmed by a weak D test using anti-D (clone MCAD6, IgG; Tulip Diagnostics) and a polyspecific antiglobulin reagent (anti-human globulin and anti-C3d; DiaMed/BioRad) in tube technique. A reaction of 1+ to 4+ agglutination strength was recorded as positive for the serologic weak D phenotype. This approach is widely applied as standard operating procedure in blood banks worldwide.[12] In the absence of macroscopic agglutination in the tube, we checked for a positive reaction microscopically. No sample among the 52 D negative donors was weak D or microscopically positive.
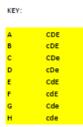
Confirmation of K Negative Phenotype

We assumed a kk phenotype (table 1), if the anti-K reacted negative (no test with anti-k). If a sample reacted positive with the anti-K, we tested with anti-k to distinguish the KK and Kk phenotypes. This approach is widely applied as standard operating procedure in blood banks worldwide.

Supplemental fig. 1. Estimates for *RH* haplotypes. The iteration steps and results are shown for the counting method.

Phenotype	Rh phenotypes observed (n)
CcDee	424
CCDee	749
CcDEe	210
ccDEe	52
ccDEE	11
ccDee	15
CCDEe	11
CcDEE	0
CCDEE	4
ccddee	42
Ccddee	8
CCddee	0
CcddEe	0
CCddEe	0
ccddEe	2
ccddEE	0
CcddEE	0
CCddEE	0
Total	1528

KEY:

A	CDE
B	cDE
C	CDe
D	cDe
E	CdE
F	cdE
G	Cde
H	cde

Priors

pA	pB	pC	PD	pE	pF	pG	pH
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3
0.038613	0.03534	0.457461	0.084751	0.010798	0.014725	0.203534	0.154123
0.020942	0.060209	0.535995	0.082134	0.002618	0.016034	0.150524	0.131545
0.011453	0.073298	0.581806	0.07788	0.000982	0.014398	0.116165	0.124673
0.008508	0.079188	0.608639	0.073953	0.000654	0.012107	0.092605	0.125
0.007199	0.081479	0.626636	0.069699	0.000654	0.010471	0.075589	0.128272
0.007199	0.083115	0.639398	0.065445	0.000654	0.008835	0.062827	0.132526
0.007199	0.084424	0.649215	0.061518	0.000654	0.007853	0.05301	0.13678
0.006872	0.085079	0.656741	0.056937	0.000654	0.006872	0.045157	0.14038
0.006872	0.085733	0.662958	0.053338	0.000654	0.006217	0.039267	0.144306
0.007199	0.086715	0.668521	0.050065	0.000654	0.005563	0.034686	0.147906
0.006872	0.087042	0.67212	0.046793	0.000654	0.005236	0.030432	0.150851
0.006872	0.087042	0.675065	0.043848	0.000654	0.004908	0.026832	0.153469
0.006872	0.087369	0.678338	0.041558	0.000654	0.004908	0.024215	0.156086
0.006872	0.087696	0.679974	0.039594	0.000654	0.004908	0.022251	0.15805
0.006872	0.087369	0.681937	0.037958	0.000654	0.004908	0.020615	0.159686
0.006872	0.087696	0.683246	0.036649	0.000654	0.004908	0.019306	0.161322
0.007199	0.087696	0.684555	0.035668	0.000654	0.004908	0.017997	0.162631
0.007199	0.087369	0.685537	0.034359	0.000654	0.004908	0.017016	0.163613
0.007199	0.087696	0.686191	0.033377	0.000654	0.004908	0.016361	0.164921
0.007199	0.087696	0.686518	0.032395	0.000654	0.004581	0.016034	0.165576
0.007199	0.087369	0.686846	0.031414	0.000654	0.004254	0.01538	0.16623
0.007199	0.087696	0.687173	0.030759	0.000654	0.003927	0.015052	0.166885
0.007199	0.088351	0.688154	0.030105	0.000654	0.003927	0.014398	0.167866
0.007199	0.088351	0.688154	0.02945	0.000654	0.003927	0.014071	0.168194
0.007199	0.088351	0.688809	0.029123	0.000654	0.003927	0.013743	0.168848
0.007199	0.088351	0.688809	0.028796	0.000654	0.003927	0.013743	0.169175
0.007199	0.088351	0.688482	0.028469	0.000654	0.003927	0.013743	0.169175
0.007199	0.088024	0.688809	0.028141	0.000654	0.003927	0.013743	0.169503
0.007199	0.088024	0.688809	0.027814	0.000654	0.003927	0.013743	0.16983
0.007199	0.088024	0.688809	0.027814	0.000654	0.003927	0.013743	0.16983
0.007199	0.088024	0.688809	0.027814	0.000654	0.003927	0.013743	0.16983
0.007199	0.088024	0.688809	0.027814	0.000654	0.003927	0.013743	0.16983
0.007199	0.088024	0.688809	0.027814	0.000654	0.003927	0.013743	0.16983

Alternative Method for Calculation of Allele Frequencies

ABO Phenotypes and Allele Frequencies
We observed the ABO antigens as B = 33.77%, O = 32.07%, A = 25.13% and AB = 9.03%.

Using an iterative method to determine maximum-likelihood estimates, the *ABO* allele frequencies were calculated as $O = 0.566$, $A = 0.189$, $B = 0.244$ (Table 1).

Alternatively, the allele frequencies are often calculated by the following method:[16]

The ABO system is determined by 3 alleles (*A*, *B* and *O*) of a single gene. *A* and *B* are co-dominant, and both are dominant over O. The alleles in the population are in a Hardy-Weinberg equilibrium.

Frequency of *A* allele (F_A) = X
Frequency of *B* allele (F_B) = Y
Frequency of *O* allele (F_O) = Z
Calculation of *O* allele frequency (Z)
Z^2 = Prevalence O phenotype = 0.3207
$Z = \sqrt{Z} = \sqrt{0.3207}$
$Z = 0.567$
Calculation of *A* allele frequency (X)
$X^2 + 2ZX + Z^2 = (Z+X)^2$
$Z+X = \sqrt{(X^2 + 2XZ + Z^2)}$
$X = \sqrt{(X^2 + 2XZ + Z^2)} - Z$
Assuming the alleles are in a Hardy-Weinberg equilibrium, we may conclude:
$AA = X^2$ $AO = 2ZX$ $OO = Z^2$
Prevalence of A antigen = $X^2 + 2ZX = 0.2513$
$X = \sqrt{(0.2513 + 0.3207)} - 0.5663$
$X = \sqrt{0.572} - 0.5663$
$X = 0.7563 - 0.5663$
$X = 0.19$
Calculation of *B* allele frequency (Y)
$X+Y+Z = 1$
$Y = 1 - (X+Z)$
$Y = 1 - (0.19 + 0.5663)$
$Y = 1 - 0.7563$
$Y = 0.2437$
Then, the genotype distribution in the study population is calculated:
$AA = X^2 = 0.04$ $AO = 2XZ = 0.22$ $BB = Y^2 = 0.06$
$BO = 2YZ = 0.28$ $AB = 2XY = 0.09$ $OO = Z^2 = 0.32$

Kell Phenotypes and Allele Frequencies
We observed the KEL antigens as K = 0.79% (n = 12) and k = 99.21%.

Using an iterative method to determine maximum-likelihood estimates, the *KEL* allele frequencies were calculated as $K = 0.004$ and $k = 0.996$ (Table 1).

Alternatively, the allele frequency are often calculated by the following method:[16]

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The KEL system is determined by 2 alleles (K and k) of a single gene. K and k are co-dominant. The alleles in the population are in a Hardy-Weinberg equilibrium.

Frequency of K allele = A
Frequency of k allele = B
Frequency of KK genotype = A^2
Frequency of Kk genotype = 2AB
Frequency of kk genotype = B^2
Assuming the alleles are in a Hardy-Weinberg equilibrium, we may conclude:
K = 0.79% and A^2 + 2AB = 0.0079
Because A^2 + 2AB + B^2 = 1, it follows that B^2 = 1 - (A^2 + 2AB)
B^2 = 1 - 0.0079 = 0.9921
B = 0.996
Because A + B = 1, it follows that A = 1 - 0.996 = 0.004
Hence, the frequency of the K allele is 0.004 and the k allele is 0.996.
Then, the genotype distribution in the study population is calculated:
KK = A^2 = 0.000016 Kk = 2AB = 0.008 kk = B^2 = 0.992

Rhesus Phenotypes and Haplotype Frequencies

In the RH blood group system, there is no straightforward way to calculate haplotypes[16] from phenotype data (table 2), because a large number of antigens and concatenated *RHCE* and *RHD* alleles (haplotypes) is involved.

Using the counting method of Ceppellini et al.,[13] allowed to determine maximum-likelihood estimates (table 3) in an iterative process.