TECHNICAL NOTE

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Evaluation of HLA in Detection of Non-Parentage Among Known False Trios

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ABSTRACT: This paternity study was performed with trios in which the putative father was not the biological father (NBF), in order to evaluate the effectiveness of HLA-A and -B phenotyping for routine testing.

All 372 generated trios had ABO, Rh, MNS, Kell, Duffy, Kidd and HLA (A and B) systems tested.

HLA-A and -B phenotyping directly excluded 81.73% of NBF. Red blood cell markers excluded 8.82% of NBF missed by HLA; only 0.81% of NBF were not identified by the markers used.

Each laboratory engaged in disputed paternity testing should evaluate its actual performance in detecting non-biological fathers and its exclusion rates in real trios. These data together can be used as a guide of mother's reliability.

KEYWORDS: pathology and biology, HLA, parentage, blood, red blood cell systems

Nowadays, in Brazil, cases of disputed parentage are increasing in numbers and the resulting requests for tests are also growing.

Although DNA typing can be used for parentage analysis, its use for routine analysis (at least 3000 trios/year) is not possible yet in Brazil. Population studies required, including recombination/mutation frequencies [*1,2*], are not available at the moment.

Red blood cell (RBC) genetic marker systems (ABO, Rh, MNS, Kell, Duffy, Kidd) and Human Leukocyte Antigens (HLA) however, can provide the suggested Cumulative Probability of Exclusion (CPE) of at least 95% [2]. HLA, with its extensive polymorphism, is the major contributor to the CPE; but the results may not be as reliable as suggested. If few other genetic markers are tested, an incompatibility may be missed and the false probability of paternity then calculated can be influenced by a single polymorphic system [*3*].

The purposes of this study were: 1) to analyze the exclusions obtained by HLA genetic markers in generated trios in order to evaluate the value of sequential testing in paternity

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cases; 2) to suggest some considerations to be included in the report that could help the court.

Material and Methods

Generated trios were created by moving the alleged father (AF) in one trio to the next trio. Trios were selected so as to maintain the same racial classification of the non-biological father (NBF) and mother and child. Cases selected were in chronological order, over an 18 months period. All tests had been performed upon judicial request.

Tests for RBC antigens included anti-A, -B, -O; -C^W, -C, -c, -D, -E, -e; -M, -N, -S, -s; -K, -k; -Fy^a, -Fy^b; -Jk^a, -Jk^b sera. HLA specificities recognized were: A1, A2, A3, A9, A10, A11, A28, A19, B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, B22, B27, B35, B37, B40, as well as Bw4 and Bw6. HLA-A and -B sera available to identify splits varied during the study.

All sera were from commercial sources: Biotest S.A. Ind. e Com. (São Paulo—Brazil), Biotest A.G. (Frankfurt—Germany) and Immucor Inc. (Georgia—USA) for RBC phenotyping; Biotest A. G. (Frankfurt—Germany), Pel-Freez Clinical (Wisconsin—USA), C-Six Diagnostics, Inc. (Wisconsin—USA) for HLA specificities. RBC antigens were identified by the usual tube agglutination techniques following the manufacturer's instructions. HLA specificities were identified by the microlymphocytotoxicity method.

RBC systems exclusions were grouped as direct (D) and indirect (I) [*3*]. HLA system exclusions were considered direct when at least one obligatory paternal specificity was absent in markers identified in the NBF. All incompatibilities based on blanks in either the NBF or child (C), were considered indirect exclusions.

Failure to identify the NBF were grouped as absolute (no HLA exclusion at all) or relative (HLA I class exclusion). These two groups were then classified according to RBC marker results as not excluded (NE) or as direct (D) or indirect exclusions (I).

Likelihood of paternity (W) [*3*] was determined when a NBF was not excluded. Gene frequencies used for ABO, Rh, MNS and Kell systems were obtained in the same laboratory where this study was conducted [*4*]. Other Caucasoid gene frequencies were used for the Duffy, Kidd [*5*] and HLA [*6*] systems.

Results

Table 1 shows the results of exclusion and failure to exclude according to the racial classification of the generated trios.

TABLE 1—*Interpretation of test results from seven genetic systems on 372 generated trios classified according to racial background.*

	Racial group of generated trio		
Results of tests	White	Mulatto	White + Mulatto
Direct exclusion (RBC &/or HLA)	151[a] (89.9)[b]	68 (93.1)	117 (89.3)
Indirect exclusion (RBC &/or HLA)	15 (8.9)	5 (6.9)	13 (9.9)
Not excluded	2 (1.2)	0 (0)	1 (0.8)
Total	168	73	131

[a]Number of trios.
[b]% of trios of similar racial background.

TABLE 2—*Red cell marker test results on 68 trios where HLA failed to directly exclude the non biologic father.*

	HLA test results	
RBC antigen result	Not excluded	Indirect exclusion
Direct exclusion	6[a] (1.61)[b]	26[a] (6.99)[b]
Indirect exclusion	5 (1.34)	13 (3.49)
Not excluded	3 (0.81)	15 (4.03)
Total	14 (3.76)	54 (14.51)

[a]Number of trios.
[b]% of 372 generated trios.

HLA absolute and relative failures according to RBCs test results in 372 generated trios are presented in Table 2. It was observed that HLA test results did not directly exclude 18.27% (68/372) of NBF in the generated trios and RBC test results directly excluded 8.82% (6/68) of NBF in HLA missed trios. In Table 3, probabilities of paternity of three unexcluded NBF are presented. Values obtained for two of them were greater than 95%.

Discussion

The extensive polymorphism of HLA allows detection of 97.15% NBF [*7*]. This value depends heavily upon the number of antisera available and the applicability to the tested population. The criteria used for exclusion in this study were: a) child has antigens absent from AF's and M's phenotypes; b) child lacks both alleles of one of the AF's locus [*3*]. This second exclusion type allows inclusion of incompatibilities such as C with blank / AF with both specificities detectable and vice-versa. The HLA tests done in this study were not performed with ideal batteries of sera; hence, all exclusions involving a blank, either in AF or C, were considered as indirect exclusions.

In routine cases, isolated indirect paternity exclusions require further investigations in order to achieve a direct exclusion, or to determine if a rare allele is causing the apparent incompatibility. According to the results observed with 372 generated trios, 81.73% of NBF were excluded directly by HLA and 14.51% of them would require more studies. Only 3.76% of NBFs were not identified by HLA. Combined RBC test results allowed identification of 78.6% (11/14) of these NBFs. Thus only 3 (0.81%) of NBFs were not detected by either HLA or RBC tests. Table 3 shows that, in two of these trios, the observed probabilities of paternity (W) were greater than 95%. The present data suggest that HLA phenotyping could be used as a screening test for compatibility between AF and C and that, besides the RBC markers used, others should be added to detect all NBFs.

TABLE 3—*Probability of paternity in three cases with no exclusion.*

Trio[a]	Probability of paternity (W)[b]
1	72.77
2	96.08
3	99.15

[a]1 & 3 White, 2 White + Mulatto.
[b]Prior probability 0.5.

As there are practical limitations on the number of genetic marker systems that can be investigated, each laboratory should clearly state in the report, its actual exclusion probability. On the other hand, calculation of the laboratory's exclusion proportions in real trios, considering its (99.19%) performance in identifying NBF, could provide a guide to the mother's reliability in naming the biologic father.

Although not biologically conclusive, both sets of data, properly presented to the court, can help in considering the testing results appropriately in reaching a decision.

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