J Forensic Sci, Oct. 1972, Vol. 17, No. 4

A. S. Wiener,[1] *M.D.*

Problems and Pitfalls in Blood Grouping Tests for Non-Parentage IV. Qualifications of Experts

The ready availability of commercial blood typing sera has led to the tacit assumption that any pathologist or laboratory technician is qualified to carry out blood grouping tests in medicolegal cases of disputed paternity, even without any previous experience or contact with problems of this nature. Suspensions of red cells from the putative father, mother, and child are merely mixed in turn with each of a battery of antisera, according to the printed directions of the manufacturer, and after the specified period of incubation, centrifugation or mixing, the reactions are read as positive or negative, depending on the presence or absence of agglutination of the red cells. The reactions for each blood specimen are then listed in tabular form for all the antisera used, and a decision is made whether or not paternity is excluded. However, the tests are by no means as simple as this description indicates; to be fully qualified to carry out such examinations one must have extensive training and experience in the field because the tests are delicate and subject to technical errors. Moreover, when the worker lacks thorough understanding, mistakes in the interpretation of the findings are inevitable, especially when it comes to the important and complex Rh-Hr blood types.

In previous reports [*1,2*] some of the pitfalls of blood grouping tests when applied to problems of disputed paternity were described. The purpose of the present report is to describe two unusual cases, in one of which there were mistakes in blood test reports, and to point out how such errors can be avoided.

Case Reports

Case 1

I was consulted regarding this problem of disputed paternity because the findings reported to a court by two different pathologists did not agree. According to one report paternity was excluded, while according to the other it was not.

As can be seen from Table 1, the two reports agreed with regard to the A-B-O and M-N systems, but differed for the Rh-Hr types. The first pathologist reported the putative father to be rh′ negative (type Rh_2rh) and the child to be hr′ negative (type Rh_zRh_1), on which basis paternity would be excluded. The second pathologist, in contrast, found the two reactions in question both to be positive instead of negative (with both putative

This paper was supported in part by USPHS grant GM-09237. Received for publication 17 April 1972; accepted for publication 7 June 1972.

[1] Department of Forensic Medicine of the New York University School of Medicine and the Serological Laboratory of the Office of the Chief Medical Examiner of the City of New York.

TABLE 1—*Reports by two different pathologists in a case of disputed paternity.*

Report of First Pathologist

Blood of	A-B-O Groups	M-N Types	Rh-Hr Factors Rh_0	rh′	rh″	hr′	hr″	Rh-Hr Type
Putative father	A_1	MN	+	−	+	+	+	Rh_2rh
Mother	O	M	+	+	−	+	+	Rh_1rh
Child	A_1	MN	+	+	+	−	+	Rh_zRh_1

"Because of the reciprocal relationship between the factors **rh′** and **hr′**, a parent who is **rh′** negative cannot have a child who is **hr′** negative ... Paternity is therefore excluded."

Report of Second Pathologist

	A	B	C	c	D	E	e	M	N
Putative father	+	−	+	+	+	+	+	+	+
Mother	−	−	+	+	+	−	+	+	−
Child	+	−	+	+	+	+	+	+	+

"Non-paternity is not established."

father and child in the same Rh-Hr type), so that his results failed to exclude paternity. As will be shown, neither of the two reports was free of error.

Table 2 shows the findings I obtained when I tested the blood specimens, which had been sent to me by mail. The findings for the A-B-O and M-N systems were the same as those reported by the two pathologists but my results for the Rh-Hr types were different from both reports. I found that the putative father was rh′ positive confirming the report of the second pathologist and contrary to that of the first, but I found that the blood of the

TABLE 2—*Author's findings in the case of disputed paternity of Table 1.*

Protocol of Reactions

	A-B-O System			M-N System		Rh-Hr System						
Blood of	**A**	**B**	A_1	**M**	**N**	Rh_0	rh′	rh^w	rh″	hr′	hr″	hr
Putative father	+	−	+	+	+	+	+	−	+	+	+	+
Mother	−	−	−	+	−	+	+	−	−	+	+	+
Child	+	−	+	+	+	+	+	−	+	−	+	−

(Not including duplicate tests or controls)

Phenotypes

	A-B-O Groups	M-N Types	Rh-Hr Types
Putative father	A_1	MN	Rh_2rh
Mother	O	M	Rh_1rh
Child	A_1	MN	Rh_2Rh_1

Genotypes

	A-B-O System	M-N System	Rh-Hr System
Putative father	A^1A^1, A^1A^2 or A^1O	*MN*	R^zr R^zR^0 or R^0r^y
Mother	*OO*	*MM*	R^1r, R^1R^0 or R^0r'
Child	A^1O	*MN*	R^zR^1, R^zr' or R^1r^y

child was hr′ negative, this time confirming the report of the first pathologist and not that of the second.

Though the reactions reported by the second pathologist were not all correct, his conclusion was correct that paternity was not excluded. Still, of the two reports, that of this pathologist was by far the less sophisticated one. His report consisted entirely of a simple table listing the reactions, plus or minus, of the three blood specimens with the various antisera, and he made no attempt to interpret the reactions in terms of the appropriate phenotype symbols; nor did he separate the reactions according to blood group systems. Such a naive report could have been prepared by any laboratory technician with no, or hardly any, experience with blood grouping, merely by copying the symbols on the labels of the blood grouping sera purchased for the tests.

The report of the first pathologist, despite his false exclusion of paternity, was far more intelligent in that it did not merely consist of a protocol of reactions, but also interpreted his findings in terms of the correct phenotype symbols, arranged according to blood group system. To this pathologist's credit, moreover, was his avoidance of the naive and fallacious C-D-E symbols for the Rh-Hr blood types. Unfortunately, however, he was not sufficiently impressed by his own finding that the child was type Rh_zRh_1 and therefore belonged to one of the three rare genotypes, R^zR^1, R^zr', or R^1r^y. Thus, the child had to be a carrier of one or the other of the very rare genes R^z or r^y, which his father must also have had since his mother lacked the gene. If the first pathologist had paid more attention to the child's unusual blood type, he might have suspected the possibility of an error in the Rh-Hr typing of the putative father's blood, and would then have repeated the test and could have found the error unless the anti-**rh′** serum he was using was not actually of that specificity.

It is evidently not universally appreciated that many of the commercially available sera labelled as anti-**rh′** are not really of that specificity, but are anti-$\mathbf{rh_i}$ sera instead.[2] Anti-**rh′** and anti-$\mathbf{rh_i}$ sera give parallel reactions except with certain rare blood specimens, and that is why the two kinds of reagents are so readily confused. Anti-**rh′** serum reacts with red cells from individuals who carry any of the four genes, r', r^y, R^1 or R^z, while anti-$\mathbf{rh_i}$ reacts with red cells of only those who carry gene r' or R^1. Since genes $\mathbf{r^y}$ and R^z are quite rare, the two reagents will give the same reactions except in the case of red cells of individuals of one of the rare phenotypes, rh_yrh, rh_yrh'', Rh_zrh and Rh_zRh_2, since such red cells will be agglutinated by anti-**rh′** serum but not by anti-$\mathbf{rh_i}$ serum. Thus, if anti-$\mathbf{rh_i}$ serum is used erroneously in place of anti-**rh′** serum, blood from an individual of type Rh_zrh would be incorrectly typed as Rh_2rh, and this probably explains the unfortunate error made by the first pathologist in his otherwise excellent report.

Paradoxically, as has been indicated above, the second pathologist, even though he correctly reported that paternity was not excluded, was far less qualified to carry out blood grouping tests. This is shown not only by the naive nature of his report, but also by his gross error in typing the child's blood, which he reported to be like that of the putative father as C+c+D+E+e+ or Rh_zRh_0, instead its correct type Rh_zRh_1. He, therefore, of course, missed the main point that the child and putative father both had the rare gene R^z (or r^y) which the mother lacked, so that the tests not merely failed to exclude paternity, but, on the contrary, provided very strong circumstantial evidence that the accused man actually was the father. To establish whether the accused man was a carrier of the gene R^z (or r^y) special tests with the very rare anti-**hr** serum had to be carried out

[2] To avoid ambiguity, symbols for blood factors and their corresponding antibodies are printed in **boldface** type, symbols for genes and genotypes are printed in *italics*, and symbols for agglutinogens, phenotypes, and blood group systems are printed in regular type.

(Table 2). If the accused man had been of the usual phenotype Rh_1Rh_2, which comprises the genotypes R^1R^2, R^1r'' and R^2r', the **hr** reaction would have been negative; but, since I found the **hr** reaction to be positive (Table 2), this proved that his phenotype was Rh_zrh instead, comprising the possible genotypes R^zr, R^zR^0 and R^0r^y. (Since anti-**hr** serum is rare and not commercially available, it is not used routinely for these blood tests, but is reserved only for special problem cases involving the genes R^z and r^y, like the one described here.)

Case 2

I was consulted about this case which was unusual in that the complainant charged a man with the paternity of two of her children. The findings obtained, in terms of the appropriate phenotype symbols, are given in Table 3. The possible corresponding genotypes to the phenotypes of Table 3 are shown in Table 4, and when taken at apparent face value, the findings fail to exclude paternity for either of the two children. The results of the Rh-Hr tests are unusual, however. Since the first child belongs to type rh″rh, of the three possible genotypes corresponding to the mother's phenotype Rh_2rh, genotype R^2R^0 is excluded. The putative father is of type Rh_1rh, and, as can be seen from Table 4, such individuals can belong to any of three possible genotypes and give rise to four kinds of sperm cells, r, r', R^0 or R^1. Similarly, since the mother could be genotype R^2r or R^0r'', she could produce ova carrying any of the four genes, r, r'', R^0 or R^2. The mating of two such individuals can give rise to the zygotes shown in the following checkerboard.

		Ova			
		r	r''	R^0	R^2
Sperm	r	rr	$r''r$	R^0r	R^2r
	r'	$r'r$	$r'r''$	R^0r'	R^2r'
	R^0	R^0r	R^0r''	R^0R^0	R^2R^0
	R^1	R^1r	R^1r''	R^1R^0	R^1R^2

Thus, any of the following 15 genotypes could result from the mating: rr, $r'r$, $r''r$, $r'r''$, R^0r, R^0R^0, R^1r, R^1R^0, R^0r', R^2r, R^2R^0, R^0r'', R^1R^2, R^1r'', and R^2r'. On this basis, the accused man could be the father of the first child, genotype $r''r$, as well as the second child, genotype R^2r, R^2R^0, or R^0r''.

However, this analysis takes into account all possible matings between putative parents of types Rh_1rh and Rh_2rh, and fails to allow for the fact that in any single family no more than four genotypes can occur among the children, since each parent's genotype comprises only two genes. Therefore, in cases involving more than one child it is necessary to analyze each of the possible matings separately. Since, in the present case, the putative father could

TABLE 3—*Results of blood grouping tests in a case of disputed paternity involving two children.*

Blood of	A-B-O Groups	M-N Types	Kell Types	Rh-Hr Types
Putative father	O	N	k	Rh_1rh
Mother	A_2B	MN	k	Rh_2rh
1st child	A_2	MN	k	rh″rh
2nd child	A_2	N	k	Rh_2rh

TABLE 4—*Possible genotypes of the four individuals of Table 3.*

	A-B-O System	M-N System	Kell System	Rh-Hr System
Putative father	*OO*	*NN*	*kk*	R^1r, R^1R^0 or R^0r'
Mother	A^2B	*MN*	*kk*	R^2r or R^0r''
1st child	A^2A^2 or A^2O	*MN*	*kk*	$r''r$
2nd child	A^2A^2 or A^2O	*NN*	*kk*	R^2r, R^2R^0 or R^0r''

NOTE—The mother cannot be genotype R^2R^0 because the first child is genotype $r''r$.

belong to any of three possible genotypes and the mother to two (see Table 5), there are six possible matings to be considered. Only one of these six matings, $R^1r \times R^0r''$, can give use to a child of type rh″rh, but this mating cannot produce a type Rh_2rh child. All of the other five matings can produce a type Rh_2rh child, but not one of type rh″rh. It follows, therefore, that if the accused man were the father of the first child he could not be the father of the second, and vice versa. Therefore, the accused man is shown not to be the father of at least one of the two children, although the results of the tests do not specify which is not his.

Discussion

The cases described here demonstrate how important it is that those who undertake to carry out blood grouping tests in medicolegal cases be fully qualified. Inadequately trained individuals will make errors not only in the performance of the tests but also in the interpretation of the findings. A satisfactory blood test report should consist of at least three sections:

(1) One section should show the identifications of those submitting to the examination in order to avoid substitution. (2) The second section should give a detailed protocol of the reactions obtained (including controls), arranged by glood group system. (3) The third section should interpret the findings in terms of the appropriate phenotype symbols (and where necessary also in terms of possible genotypes), and specify whether or not paternity is excluded by the tests, and explain the basis for this conclusion.

Thus, a satisfactory report is possible only with the aid of appropriate scientific symbols for the phenotypes and genotypes. The principles of blood group nomenclature have been discussed elsewhere [*3,4,5*] in considerable detail and will not be repeated here. Correct nomenclature fosters more thorough understanding and insight, and one must bear in mind that coded designations whether in terms of letters like C-D-E or in terms of numbers like 1-2-3, etc., are not scientific symbols, and therefore have no place in medicolegal reports or scientific publications. Such symbols appeal only to the beginner or uninformed because of their naive simplicity, which entails no knowledge of the subject and actually prevents and discourages one from acquiring such knowledge and understanding, as demonstrated by the second pathologist's report in Case 1 presented here. In fact, the use of the coded symbols, C-D-E or 1-2-3, in a medicolegal report or scientific treatise would be as inappropriate as the use of the Morse code in a published novel. This obvious fact would long ago have eliminated the C-D-E and 1-2-3 symbols from the scientific scene were it not for the failure of the Division of Biologics Standards of the National Institute of Health to modernize the labelling of blood grouping sera.

The DBS regulations regarding Rh-Hr antisera now in effect are 25 years old. These regulations call for duplicate labelling, including not only the correct Rh-Hr symbols but

also, in parentheses, the C-D-E symbols, long ago rendered obsolete by advances in scientific knowledge. Constantly confronted with these naive C-D-E symbols on labels, the unwary technician is encouraged to use them, just as 30 years ago confusion regarding the A-B-O blood groups was fostered by labels of blood grouping sera which carried the Moss/Jansky Roman numerals. The Division of Biologics Standards could easily and quickly solve this problem, since it has both the power and obligation to keep up-to-date the regulations regarding correct labelling of blood grouping tests.

References

[1] Wiener, A. S., "Blood Grouping Tests in Disputed Parentage: Qualifications of Experts. II. An Error Involving the Rare Rh-Hr Blood Types Rh_zRh_1 and Rh_zrh," *Acta Geneticae Medicae et Gemellologiae*, Vol. 13, 1964, pp. 346–347.

[2] Wiener, A. S., "Problems and Pitfalls in Blood Grouping Tests for Non-Parentage. II. The Rh-Hr Blood Group System," *Journal of Forensic Medicine* (South Africa), Vol. 15, 1968, pp. 106–132.

[3] Wiener, A. S., "The Rh-Hr Blood Types; the Anatomy of a Controversy," *Journal of Forensic Medicine* (South Africa), Vol. 15, 1968, pp. 22–40.

[4] Erskine, A. G., "Blood Groups, Transfusion, and Medicolegal Applications," *Gradwohl's Clinical Laboratory Methods and Diagnosis*, S. Frankel, S. Reitman, and A. C. Sonnenworth, Eds., C. V. Mosby, St. Louis, 1970, pp. 699–918.

[5] Wiener, A. S., *Advances in Blood Grouping, Vol. III*, Grune & Stratton, New York, 1970.

64 Rutland Road
Brooklyn, N.Y. 11225