Serology as a Tool for Identification of Dead Bodies.

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INTRODUCTION.

Identification of human remains by the traditional methods of pathology may be impossible or uncertain because of hard damage or decay, and in such cases serologic examinations may provide the necessary evidence for identification. The methods used routinely comprise the ABO blood group antigens, the serumproteins Hp, Gm and Km, the erythrocyte enzymes PGM_1, AcP_1, AK, 6PGD, and ADA. Recently the tissue types, HLA-A,B,C have been added when necessary. While some of these markers degrade soon after the time of death, the HLA antigens have proved remarkably stable, and this rather polymorphic system thus may yield positive evidence for identification.

Methods.

The methods used for blood group, serum protein, and erythrocyte enzyme determinations are all standard techniques as employed for blood stain analysis. The material used is blood remains, bone marrow or muscle tissue.
The microabsorption method used for HLA determination of dead tissue - spleen or muscle - has been described in details elsewhere (Hansen and Gürtler 1981 & 1983). Briefly samples of minced tissue are mixed with 50 µl of HLA antisera selected for the purpose. Absorptions are carried out in duplicate. The recovered serum samples are tested in series of twofold dilutions in the NIH cytotoxic test in order to compare initial and residual antibody activity by means of cells from appropriate testdonors.

Advances in Forensic Haemogenetics 2
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Case 1.

This case concerns the remains of a woman found in the beginning of September after 2-3 weeks in fresh water lakes. The body had been cut into pieces, and the head and the hands were never found. Serologic examinations gave results for ten marker systems, and the materials used were blood remains, bone marrow and muscle tissue, for HLA determinations only muscle tissue. Comparisons were made with the corresponding marker systems found in the presumed family (table 1). The microabsorption method does not allow the distinction between phenotypic HLA-A2 homozygotes and HLA-A2,A28 heterozygotes, hence both possibilities had to be included for the victim in the statistical evaluations. The presumed parents could have a child of the type HLA-A2,B40//A2,B44, in 1/4 of the cases in which no recombination occurs. The statistical evaluations including all the ten marker systems lead to the result that odds are 1186:1 or 99.92% against 0.08% for the victim being a child of this family against the victim being a random Dane. The results of the serologic examinations provided the final proof for the identity of the victim in this case.

Table 1. The Serologic Results in Case 1.

Victim	: B	Hp 2-1	Gm(a-x-b+)	Inv+	PGM_1	1F-1S	AcP_1	B
	AK 1	6PGD A	ADA 1	HLA-A2;B40,B44 or A2,A28;B40,B44				
Father	: O	Hp 2	Gm(a+x-b+)	Inv-	PGM_1	1F-1S	AcP_1	B
	AK 1	6PGD A	ADA 1	HLA-A11,B35//A2,B40				
Mother	: A_2B	Hp 1	Gm(a+x-b+)	Inv+	PGM_1	2F-1S	AcP_1	BC
	AK 1	6PGD AB	ADA 1	HLA-A11,B7//A2,B44				
Brother	: A_2	Hp 2-1	Gm(a+x-b+)	Inv+	PGM_1	1S	AcP_1	BC
	AK 1	6PGD AB	ADA 1	HLA-A11,B35//A11,B7				
Sister	: B	Hp 2-1	Gm(a+x-b+)	Inv-	PGM_1	1S	AcP_1	BC
	AK 1	6PGD AB	ADA 1	HLA-A11,B35//A2,B44				

Case 2.

This case concerns the remains of a female who was found in the harbor of Copenhagen in the end of October after 2-3 weeks in the salt water. The body had been cut into pieces. Seven marker systems could be determined:

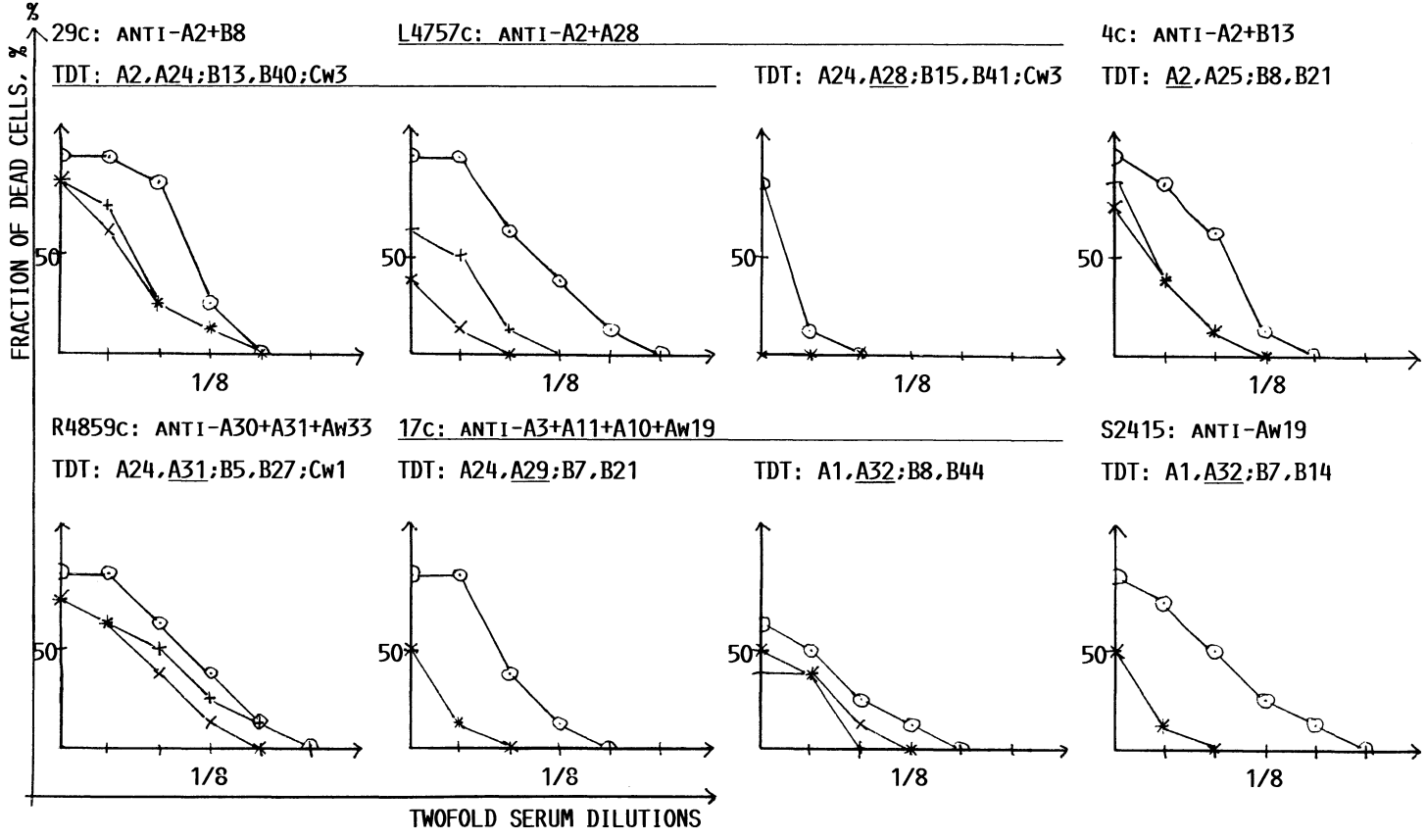
Victim : A PGM_1 1F AcP_1 B AK 1 ADA 1 Hp 2 HLA-A28,Aw19*;B21

For the HLA-B locus reduction by absorption of antibody activity was found only in anti-B21 reagents, however, the antigens HLA-Bw46, Bw48,Bw59,Bw67,Bw70,Bw73 were not tested for.
The presence of HLA-A28 could be established through the reduction of antibody activity in anti-A2+A28 sera, but not in anti-A2 sera, (fig. 1). The presence of an HLA-Aw19 factor other than HLA-A29, A30,A31,A32, or Aw33 could be established through lack of reduction of antibody activity by absorption of reagents of narrow anti-Aw19 specificity, f. ex. serum no. R4859 in fig. 1. Besides reduction by absorption of antibody activity directed against some anti-Aw19 components was found in sera of broad specificity: f. ex. the strong anti-A29, but not the weaker anti-A32 activity was absorbed in serum no. 17c, and in serum no. S2415 the opposite was true (fig. 1). No reduction of antibody activity was found by absorption of anti-A10 reagents.
When people of Oriental origin have been typed in the cytotoxic test using the same reagents, a similar reaction pattern has sometimes been observed, indicating that an HLA-Aw19 factor other than HLA-A29,A30,A31,A32 or Aw33 must be present. This HLA-Aw19* antigen could correspond to the one described by Se Jong Kim et al. in a Korean population (1986).
Because of this HLA-Aw19* antigen, and because the HLA type of the victim was most untypical for a Greenland Eskimo, it was concluded that the victim was of Oriental rather than of Eskimoic origin. She was later identified as being a Japanese.

FIG. 1. HLA-DETERMINATION BY MICROABSORPTION AFTER 2-3 WEEKS IN THE SEA.

EXAMPLES OF COMPARISON OF INITIAL AND RESIDUAL ANTIBODY ACTIVITY IN UNABSORBED AND ABSORBED REAGENTS.

UNABSORBED: 0-0-0-0 DUPLICATE ABSORPTIONS: x-x-x-x & +-+-+-+ TDT: TEST DONOR HLA-TYPE



Case 3.

This case concerns the remains of a male body washed ashore in the beginning of June. The investigations of the pathologists indicated that the remains had been in the sea for 3-6 months. Only most of the sceleton and some tissue from the thighs and the hips remained. The advanced decay allowed determinations of only four genetic marker systems (table 2). Comparisons were with the markers found in two couples of presumed parents neither of whom could have a child of the HLA type of the victim.

Table 2. Serologic Results in Case 3.

	Victim :	O	AcP_1	AB	EsD 1	HLA-A3,A28;B15,B22
Presumed parents:						
1)	Father :	O	AcP_1	B	EsD 1	HLA-A1,A3;B17,B35
	Mother :	B	AcP_1	AB	EsD 1	HLA-A9(24),A11;B15,B35
2)	Father :	A_1	AcP_1	A	EsD 1	HLA-A10(26),A29;B17(w57),B44
	Mother :	O	AcP_1	B	EsD 1	HLA-A1,A11;B5(51),B8

CONCLUDING REMARKS.

In Denmark material is secured at the autopsy for serologic examinations in the case of unidentified bodies or human remains. Determinations of erythrocyte markers are carried out routinely. For HLA determination material may be frozen for investigations when the circumstances of the case makes it necessary.

The examples presented show that serologic examinations may provide an efficient tool for identification, not least when the polymorphic HLA system is included.

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