ORIGINAL ARTICLE



World human neutrophil antigens investigation survey

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Abstract

Background and Objectives: Human neutrophil antigens (HNAs) are categorized into five systems: HNA-1 to HNA-5. Given the importance of neutrophils in immunity, we sought to create awareness of the role of HNA diagnostic services in managing immune neutropenia and transfusion-related acute lung injury. To provide health communities all around the world with access to these services, we conducted a survey to create a directory of these HNA diagnostic services.

Materials and Methods: An Excel table-based survey was created to capture information on the laboratory's location and was emailed to 55 individuals with known or possible HNA investigation activity. The collected data were then summarized and analysed.

Results: Of contacted laboratories, the surveys were returned from 23 (38.2%) laboratories; 17 have already established HNA diagnostic (of them 12 were regular participants of the International Granulocyte Immunobiology Workshop [ISBT-IGIW]), 4 laboratories were in the process of establishing their HNA investigation and the remaining 2 responder laboratories, did not conduct HNA investigations. In established laboratories, investigation for autoimmune neutropenia (infancies and adults) was the most frequently requested, and antibodies against HNA-1a and HNA-1b were the most commonly detected.

Conclusion: The directory of survey respondents provides a resource for health professionals wanting to access HNA diagnostic services. The present study offers a comprehensive picture of HNA diagnostics (typing and serology), identifying weak points and areas for improvement for the first time. Identifying more laboratories involved in HNA diagnostics with limited access to international societies in the field will globally improve HNA diagnostics.

Keywords

alloantibody, autoantibody, autoimmune, HNA, neutropenia, neutrophil, TRALI

Highlights

- Investigation for autoimmune neutropenia (infancies and adults) is the most frequently requested analysis for neutrophil serology worldwide.
- Antibodies against human neutrophil antigen (HNA)-1a and HNA-1b are the most commonly detected antibodies involved in autoimmune neutropenia.
- Although neutrophil serology services are distributed across the globe, the number of services available to African, Middle Eastern and Western Pacific populations is limited.

INTRODUCTION

Neutrophils are the major subtype of granulocytes and have a pivotal role in innate and adaptive immunity. Human neutrophil antigens (HNAs) are distributed on five different glycoproteins on the surface of neutrophils and have been designated as HNA-1 to HNA-5 [1]. Eleven HNA alleles have been described so far [2]. HNA incompatibilities during pregnancy or transfusion may induce the production of HNA antibodies [3]. HNA antibodies have been implicated in the mechanism of neonatal alloimmune neutropenia (NAIN), autoimmune

neutropenia (AIN), transfusion-related acute lung injury (TRALI), immune neutropenia after bone marrow transplant and febrile non-haemolytic transfusion reactions [2, 4].

Neutropenia may arise from a defect in neutrophil production in bone marrow or increased neutrophil destruction for various reasons [5]. Immune neutropenia involves the attachment of neutrophil reactive antibodies to neutrophil antigens, leading to their clearance from circulation [6]. Depending on the cause, treatment for neutropenia varies. It is therefore important to distinguish immune neutropenia from other causes. Therefore, the availability of services to detect **TABLE 1A** Directory of participating established laboratories.

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anti-neutrophil antibodies enables the physician to confirm immune neutropenia thus avoiding the invasive bone marrow biopsy [7]. This is particularly useful in cases of infant and childhood neutropenia. The implication of HNA antibodies, particularly anti-HNA-3a in clinically severe TRALI has also significantly emphasized the importance of HNA antibody detection.

The International Society of Blood Transfusion (ISBT)-Granulocyte Immunology Working Party (GIWP) organizes an annual quality assessment workshop to assess a laboratory's ability to carry out neutrophil serology and genotyping investigations. There are currently only 18 reference laboratories participating in this quality assessment workshop, but we expect that there are other laboratories performing similar investigations around the world as well. Given the significant role of neutrophils in immunity, we conducted a survey to identify and catalogue laboratories around the globe performing neutrophil serology and genotyping investigations. The goal is to create a worldwide directory of granulocyte investigating laboratories conducting neutrophil serology and genotyping investigations to increase awareness of these diagnostic services and to make these specialized services more accessible to health communities all around the world.

MATERIALS AND METHODS

The survey consisted of an Excel table that captured information on the laboratory's location, the range of neutrophil investigations (e.g., AIN, TRALI, neutrophil reactive antibodies in transplants and convalescent plasma of COVID-19 patients) conducted, list of techniques used for serology and molecular investigations, and whether they participated in a quality assessment programme(s) in the 12-month period from January 2019 to January 2020. The survey was emailed to 55 individuals with known or possible HNA investigation activity, and recipients were encouraged to forward the survey to any other laboratories that may conduct HNA investigations. A literature search (keywords: HNA frequency, human neutrophil antigens, neutrophils, neutrophil antigen 'country') was conducted to gather reports on HNA frequencies from different populations.

RESULTS

A total of 23 surveys were returned equating to a return rate of 41.8%. From the responses received, 17 established laboratories regularly conducted HNA investigations, 3 (17.6%) are in the Americas, 10 (58.8%) in Europe, 1 in South-East Asia (5.9%) and 3 (17.6%) in the Western Pacific area (Table 1A). Of these 17 laboratories, 15 (88.2%) participated in a granulocyte quality assessment program, the most common (n = 11) being the International Society of Blood Transfusion-International Granulocyte Immunobiology Workshop (ISBT-IGIW) and the other being the INSTAND External Quality Assessment (EQA) programme (Table 1A). Four institutes (National Blood Centre Thailand, South Africa, Iran and South Korea) are in the process of establishing their HNA investigation services and have

been clustered as 'Laboratories in development' (Table 1B). The remaining two responses did not conduct HNA investigations: the New Zealand Blood Transfusion Service referred their investigations to Australia, and Dr Olnaiyi Olanrewaju from the Irrua Specialist Teaching Hospital, Irrua/Ambrose Alli University, Ekpoma, Edo State Nigeria reported that they had cases of immune neutropenia but did not have a laboratory to refer the samples to.

Serological testing was conducted by 16 of 17 established laboratories and all 4 laboratories in development (Table 2) but the range of techniques varied. All 16 laboratories from first group and 1 from second group conducted granulocyte immunofluorescence tests (GIFT) and used a typed panel of granulocytes. All these laboratories complimented the GIFT with the granulocyte agglutination test (GAT) or another technique, except in Aalborg and Créteil. LabScreen Multi was used by 6 established laboratories and 3 of 4 laboratories in development (Table 2) [8]. Monoclonal antibody immobilization of granulocyte antigen (MAIGA) [9] was conducted by 12 established laboratories, with Sao Paulo and Tehran in the process of optimizing the assay. All laboratories investigating CD16 used two monoclonal antibodies (mAb) except for USA. Versiti, MEM-166 was the most common CD177 mAb and Bear 1 for CD11b. Sixteen established laboratories and two laboratories in development conducted genotyping for HNA-1, HNA-3, HNA-4 and HNA-5, but only five from first group also genotyped for HNA-2 (Table 3).

NAIN investigations were conducted by 15 established laboratories (Table 4). Of the samples tested in the survey period, 88 samples were positive, and the most common antibodies detected were anti-HNA-1a and anti-HNA-1b. Samples were referred by physicians from

TABLE 1B	Directory of participating laboratories in
development.	

1	Africa	Dr Derrick Nelson, email: derrick.nelson@ sanbs.org.za
		Specialized Laboratory Services, South African National Blood Service, Johannesburg, South Africa.
2	Eastern Mediterranean	Dr Esmaeili Behnaz, email: esmaeili. behnaz@yahoo.com
		Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran.
3	W.Pac	Dr Hyungsuk Kim, email: hyungsuk. kim79@gmail.com
		Seoul National University Hospital, Seoul, Korea.
4	South East Asia	Dr Pawinee Kupatawintu, email: pawinee. k@redcross.or.th
		Dr Atthapol Srisuddee, email: atthapol.s@ redcross.or.th
		National Blood Centre, Thai Red Cross Society, Bangkok, Thailand.

Abbreviations: EQA, External Quality Assessment; HNA, human neutrophil antigens; ISBT-IGIW, International Society of Blood Transfusion-International Granulocyte Immunobiology Workshop.

Not Not <th></th> <th>CD11a CD11a HLA CD177 (LFA-1α) CD11b CD18 class1</th> <th>MEM166 Bear1</th> <th>7D8 IB4</th> <th>7D8</th> <th>MEM- HI111 Bear1 7E4 166</th> <th></th> <th>MEM166</th> <th>MEM- HI111 Bear1 166</th> <th>MEM166 7D8 25.3.1 Bear1 B1G6</th> <th>MEM166 7D8 Bear1 W6/32</th> <th>MEM166 TAG4 IB4</th> <th>MEM166 Bearl 7E4</th> <th>MEM166 7D8 Bear1 7E4</th> <th></th> <th></th> <th>MEM166 25.3.1 Bear1 W6/32</th> <th>29</th> <th></th> <th></th>		CD11a CD11a HLA CD177 (LFA-1α) CD11b CD18 class1	MEM166 Bear1	7D8 IB4	7D8	MEM- HI111 Bear1 7E4 166		MEM166	MEM- HI111 Bear1 166	MEM166 7D8 25.3.1 Bear1 B1G6	MEM166 7D8 Bear1 W6/32	MEM166 TAG4 IB4	MEM166 Bearl 7E4	MEM166 7D8 Bear1 7E4			MEM166 25.3.1 Bear1 W6/32	29		
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patients with neutropenia (absolute neutrophil count [ANC] < 1500 cells/ μ L for adults and ANC < 1000 per microliter). The number of investigations for AIN was higher and the positive rates ranged from 9% to 52.2% (Table 5). Requests for AIN investigations were mainly for patients aged 2–36 months and in adults (Table 5). Only 16 established laboratories conducted TRALI investigations, and the number of samples tested in each laboratory varied considerably (Table 6). The majority of TRALI-associated antibodies were in donor samples and only three established laboratories detected antibodies in the patient.

DISCUSSION

The survey results show that the distribution of HNA investigation capability among laboratories is varied. There is a large cluster of laboratories in Europe (58.8%), three in America (17.6%), three in Western Pacific (17.6%) and one in South East Asia (5.8%; Tables 1A and 1B). There are presently no established laboratories in Africa or Eastern Mediterranean, but there is a laboratory in development in Tehran, Iran.

NAIN is a disorder of the foetus that results from maternal alloimmunization against paternal HNA expressed on fetal neutrophils [4, 10]. NAIN is analogous to haemolytic disease of the foetus/ newborn (HDFN) which affects red blood cells and foetal/neonatal alloimmune thrombocytopenia (FNAIT) which affects platelets [11]. In NAIN, the mother develops alloantibodies against HNAs expressed on foetal neutrophils that may cause elimination of neutrophils leading to neutropenia in foetus [11]. Of the 17 established laboratories that regularly investigate NAIN, the majority (58.8%) are located in Europe, and there are none in Africa or Easter Mediterranean (Tables 1A and 1B). The glycoproteins carrying epitopes for HNA-1 and HNA-2 are the most frequently involved in the NAIN cases in this study. Seven laboratories identified alloantibodies against HNA-2 as the second most frequent alloantibodies involved in NAIN cases. In the reports from four laboratories (Creteil and Nantes in France, Sanquin in the Netherlands and NHS in UK), alloantibodies against HNA-1c were described in the cases of NAIN. Interestingly, only the UK laboratory reported alloantibodies against HNA-3a in cases of NAIN. There have been rare reports on NAIN associated with high-frequency antigens HNA-4a [12] and HNA-5a [13]. As HNA-4b frequency is very low in many populations, this may explain the rare reports of NAIN cases mediated by alloantibodies against this antigen. In addition, the low frequency of antibodies against HNA-4 and HNA-5 can also be correlated to the non-immunogenic structure of CD11b and CD11a where HNA-4 and HNA-5 epitopes are located.

The survey results show that AIN is the most requested HNA investigation, conducted in 15 established laboratories and the laboratories in development in Iran (Table 5). Specifically, primary AIN occurs mainly in infants and children. The AIN autoantibody is elusive because the antibody concentration can change. Hence, AIN investigation often requires testing of multiple patient sera at different times to detect the antibody [14]. The antibody specificity has

TABLE 2 (Continued)

				2	Ö				Monoclonal antibodies used in MAIGA		
		Ľ	Typed typed	rped t	yped						CD11a HLA
Serc	erology	פֿד	ed I ba	anel	GIIS	screen	Other techniques MANGA CD10	MAIGA	CD16		
2	2 Iran, Tehran	7	х х х	2			LIFT	inP	LNK16	MEM166	
ო	3 Korea, Seoul					×		z			
4	4 Thailand, Red Cross					~		z			

Abbreviations: GAT, granulocyte agglutination test; GCLT, granulocyte chemiluminescence test; GIFT, granulocyte immunofluorescence test; HNA, human neutrophil antigen; ICFA, immunocomplex capture luorescence analysis; LIFT, lymphocyte immunofluorescence test; MAIGA, monoclonal antibody immobilization of granulocyte antigen; WIFT, white cell immunofluorescence test. TABLE 3 Human neutrophil antigen (HNA)-genotyping conducted by participants.

Genotyping	Y/N	HNA-1	HNA-2	HNA-3	HNA-4	HNA-5
1. Brazil, Sao Paulo	Υ	HNA-1		HNA-3	HNA-4	HNA-5
2. USA, ARC	Y	HNA-1	HNA-2	HNA-3	HNA-4	HNA-5
3. USA, Versiti	Y	HNA-1		HNA-3	HNA-4	HNA-5
4. Austria, Vienna	Y	HNA-1	HNA-2	HNA-3	HNA-4	HNA-5
5. Denmark, Aalborg	Υ	HNA-1	HNA-2	HNA-3	HNA-4	HNA-5
6. France, Créteil	Υ	HNA-1		HNA-3	HNA-4	HNA-5
7. France, Nantes	Υ	HNA-1		HNA-3	HNA-4	HNA-5
8. Germany, Dessau	Υ	HNA-1		HNA-3	HNA-4	HNA-5
9. Germany, Giessen	Υ	HNA-1		HNA-3	HNA-4	HNA-5
10. The Netherlands, Sanquin	Υ	HNA-1		HNA-3	HNA-4	HNA-5
11. Poland, Warsaw	Υ	HNA-1	HNA-2	HNA-3	HNA-4	HNA-5
12. United Kingdom, NHS	Υ	HNA-1		HNA-3	HNA-4	HNA-5
13. Slovenia, Ljubljana	Υ	HNA-1		HNA-3	HNA-4	HNA-5
14. Thailand, Thammasat University	Y	HNA-1		HNA-3	HNA-4	HNA-5
15. Australia, Lifeblood	Υ	HNA-1		HNA-3	HNA-4	HNA-5
16. Hong Kong, Queen Mary	Υ	HNA-1	HNA-2	HNA-3	HNA-4	HNA-5
17. Japan, JRC	Υ	HNA-1		HNA-3		
Laboratories in development						
1. South Africa, SANBS	Ν					
2. Iran, Tehran	Υ	HNA-1		HNA-3	HNA-4	HNA-5
3. Korea, Seoul	Υ	HNA-1		HNA-3	HNA-4	HNA-5
4. Thailand, Red Cross	Ν					

TABLE 4 Human neutrophil antigen (HNA) antibody specificities detected in neonatal alloimmune neutropenia investigations.

				Antibody	specificity	(%)						
	natal alloimmune neutropenia stigations	Samples tested (n)	Samples positive (n)	HNA-1a	HNA-1b	HNA-1c	HNA-2	HNA-3a	HNA-4a	HLA- class II	HLA- class I	CD16 Iso Ab
1	Brazil, Sao Paulo	2	1	0	100	0	0	0	0	0	0	0
2	USA, ARC	6	2	0	100	0	0	0	0	0	0	0
3	USA, Versiti	40	3	50	25	0	25	0	0	0	0	0
4	Austria, Vienna	5	3+	0	33.3	0	33.3	0	0	0	0	33.3
5	Denmark, Aalborg	7	1	0	100	0	0	0	0	0	0	0
6	France, Créteil	37	8	62	25	13	0	0	0	0	0	0
7	France, Nantes	24	6	0	33	17	17	0	0		0	33
8	Germany, Dessau	3	0	0	0	0	0	0	0	0	0	0
9	Germany, Giessen	38	8	25	25	0	25	0	0	0	12.5	12.5
10	The Netherlands, Sanquin	12	5	20	0	20	20	0	0	0	0	40
11	Warsaw, Poland		380 ^a	42	13	0	0	0	2	6		25
12	United Kingdom, NHS	55	7	43	0	14	29	14	0	0	0	0
13	Slovenia, Ljubljana	6	1	50	30	0	20	0	0	0	0	0
14	Australia, Lifeblood	4	1	0	100	0	0	0	0	0	0	0
15	Hong Kong, Queen Mary	36	10	100	0	0	0	0	0	0	0	0

^aTotal number of diagnosed neutropenia (auto- or alloimmune).

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also been reported to change with time [15]. Diagnosis of primary AIN is especially useful as it supports exclusion of other causes of neutropenia [16].

Although in many cases of adult AIN no antigen specificity is described, antibodies against HNA-1a and HNA-1b have been detected as the most common target antigens for autoantibodies involved in cases of AIN in adults [17]. In contrast to primary AIN in

infancy, AIN in adults is not a self-limiting condition and manifests a higher frequency in females (up to 70% of cases) [6].

Transfusion of blood products containing HNA alloantibodies to recipients with the cognate antigen has been known to induce reactions such as TRALI [18]. The role of neutrophils in TRALI is well established [19]. However, some alloantibodies such as alloantibodies against HNA-3a have been implicated in severe and some fatal TRALI

TABLE 5 Patient age distribution and positivity rate in neonatal autoimmune neutropenia investigations.

		Sample	Positive	results	Patient age	distribution (%)		
Autoimn	nune neutropenia investigations	tested (n)	n	%	Newborn	2-36 months	Primary	Adult
1	Brazil, Sao Paulo	33	11	33.3	0.0	36.4	18.2	45.5
2	USA, ARC	95	32	33.7	0.0	43.2	0.0	56.8
3	USA, Versiti	1981	526	26.6	0.1	47.0	18.2	34.8
4	Austria, Vienna	75	15	20.0	21.4	64.3	0.0	14.3
5	Denmark, Aalborg	90	20	22.2	0.0	54.1	18.9	27.0
6	France, Créteil	1169	105	9.0	0.0	30.1	0.0	69.9
7	France, Nantes	774	87	11.2	1.2	26.2	0.0	72.6
8	Germany, Dessau	63	9	14.3	0.0	36.4	36.4	27.3
9	Germany, Giessen	990	255	25.8	0.0	17.9	13.5	68.6
10	The Netherlands, Sanquin	270	79	29.3	57.0			43.0
11	Warsaw, Poland	380			0.0	91.5	0.0	8.5
12	United Kingdom, NHS	2004	488	24.4	0.6	58.5	30.4	10.5
13	Slovenia, Ljubljana	30	8	26.7	0.0	43.2	18.9	37.8
14	Australia, Lifeblood	141	58	41.1	0.0	2.1	1.4	96.5
15	Hong Kong, Queen Mary	11	0	0.0	0.0	18.2	36.4	45.5
LiD	Iran, Tehran	23	12	52.2	0.0	40.0	40.0	20.0

TABLE 6 Transfusion-related acute lung injury (TRALI) investigation findings.

		Samples	Patient antibody	/	Donor and	tibody
TRALI inve	stigations	tested (n)	n	%	n	%
1	USA, ARC	60	0	0	6	10.0
2	USA, Versiti	73			1	1.4
3	Austria, Vienna	3	0	0	2	66.7
4	Denmark, Aalborg	9			1	11.1
5	Germany (Dessau)	276	1	0.3	1	0.3
6	Germany (Giessen)	37	0	0	3	8.1
7	France, Nantes	29	0	0	0	0.0
8	The Netherlands, Sanquin	2	0	0	0	0.0
9	Poland, Warsaw	22			1	4.5
10	United Kingdom, NHS	78	Not tested		4	20.0
11	Slovenia, Ljubljana	3			1	33.3
12	Thailand Red Cross	7	1	14.3	1	14.3
13	Thailand, Thammasat University	2	0	0	2	100.0
14	Australia, Lifeblood	2	0	0	1	50.0
15	Hong Kong	0	0	0	0	0
16	Japan, JRC	72	9	12.5	28	38.9

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m Thai 0.619 Thai 0.548 n Thai 0.677 stern Thai 0.677 stern Thai 0.677 stern Thai 0.677 stern Thai 0.667 stern Thai 0.667 stern Thai 0.667 stern Thai 0.667 e(Han, Guangzhou) 0.667 e(Han, Zhejiang) 0.613 e(Total) 0.613 r 0.613 e 0.623 e 0.623 e 0.605 n 0.725 r 0.736 n 0.738 n 0.348 (St. Petersburg) 0.348 n 0.326 n 0.326 n 0.326 n 0.326 n 0.326 n 0.328 n 0.326 n 0.326 n 0.328 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>										
Thai 0.548 m Thai 0.677 n Thai 0.677 astern Thai 0.677 astern Thai 0.676 astern Thai 0.677 astern Thai 0.677 astern Thai 0.677 astern Thai 0.677 astern Thai 0.678 e (Han, Guangzhou) 0.667 e (Han, Zhejiang) 0.613 b (Han, Zhejiang) 0.613 c (Han, Zhejiang) 0.613 e (Total) 0.725 n 0.725 n 0.726 n 0.706 n 0.726 n 0.726 n 0.706 n 0.706 n 0.706 n 0.706 n 0.706 n 0.726 n 0.738 n	55 0.012			0.808	0.192	0.973	0.027	0.656	0.344	Intharanut et al., 2019 [42]
m Thai 0.677 astern Thai 0.696 astern Thai 0.696 e (Han, Guangzhou) 0.667 e (Han, Zhejiang) 0.613 e (Han, Zhejiang) 0.613 e (Total) 0.613 e (Lan, Zhejiang) 0.613 e (Han, Zhejiang) 0.613 e (Lan, Zhejiang) 0.613 e (Total) 0.623 e 0.623 i 0.725 i 0.725 i 0.726 i 0.348 i 0.348 i 0.384 i 0.385 i 0.395 i 0.395 <td>52 0.004</td> <td></td> <td></td> <td>0.718</td> <td>0.282</td> <td>0.975</td> <td>0.025</td> <td>0.771</td> <td>0.229</td> <td>Intharanut et al., 2019 [42]</td>	52 0.004			0.718	0.282	0.975	0.025	0.771	0.229	Intharanut et al., 2019 [42]
stern Thai 0.696 (Han, Guangzhou) 0.667 (Hong Kong) 0.678 (Hong Kong) 0.613 (Han, Zhejiang) 0.613 e 0.605 e 0.605 (Total) 0.706 (Total) 0.706 (Caucasoid) 0.348 (Caucasoid) 0.348 (St. Petersburg) 0.395 n 0.395 n 0.395 States (Black population) 0.59	23 0			0.775	0.225	0.965	0.035	0.748	0.252	Intharanut et al., 2019 [42]
e (Han, Guangzhou) 0.667 e (Hong Kong) 0.678 e (Han, Zhejiang) 0.613 e (Han, Zhejiang) 0.613 e 0.623 e 0.623 e 0.623 e 0.623 e 0.338 (Caucasoid) 0.318 (Caucasoid) 0.318 (Caucasoid) 0.318 (St. Petersburg) 0.395 n 0.375 (St. Petersburg) 0.395 States (Black population) 0.59	10 0			0.785	0.215	0.972	0.028	0.676	0.324	Intharanut et al., 2019 [42]
e (Han, Guangzhou) 0.657 e (Hong Kong) 0.678 e (Han, Zhejiang) 0.613 e (Han, Zhejiang) 0.613 e (Total) 0.623 e 0.623 fordal) 0.725 n 0.725 n 0.725 n 0.726 n 0.726 n 0.726 n 0.348 (St. Petersburg) 0.384 n 0.326 n 0.326 n 0.326 1(St. Petersburg) 0.326 n 0.326		0.995	0.005							Nathalang et al., 2018 [41]
e (Hong Kong) 0.678 e (Han, Zhejiang) 0.613 e (Han, Zhejiang) 0.613 e 0.613 e 0.605 e 0.605 (Total) 0.706 n 0.348 n 0.348 (Caucasoid) 0.348 n 0.348 n 0.395 n 0.359 States (Black population) 0.59	33 0	1	0	0.738	0.262	0.996	0.004	0.854	0.146	Xia et al., 2011 [<mark>33</mark>]
e (Han, Zhejiang) 0.613 e 0.433 e 0.623 e 0.623 e 0.625 (Total) 0.725 (Total) 0.706 0.348 (Caucasoid) 0.318 (St. Petersburg) 0.384 n 0.395 n 0.375 (St. Petersburg) 0.384 (St. Petersburg) 0.59 States (Black population) 0.59	5 0	0.983	0.017	0.71	0.29	0.995	0.005	0.852	0.148	Tam et al., 2018 [<mark>35</mark>]
e 0.433 e 0.433 e 0.623 e 0.625 (Total) 0.706 0.725 (Total) 0.706 (Caucasoid) 0.348 (Caucasoid) 0.348 (Caucasoid) 0.348 (St. Petersburg) 0.384 0.42 n 0.395 n 0.395 States (Black population) 0.59	37 0			0.654	0.346	1	0	0.896	0.104	He and Zhang, 2014 [34]
e 0.433 e 0.623 e 0.605 (Total) 0.706 n 0.725 (Caucasoid) 0.706 n 0.348 (Caucasoid) 0.348 (St. Petersburg) 0.348 n 0.395 n 0.395 n 0.59 States (Black population) 0.59				0.695	0.305	0.986	0.014	0.959	0.041	Han and Han, 2015 [38]; Han and Han, 2006 [39]
e 0.623 e 0.605 (Total) 0.706) 0.725 (O.326 0.348 (Caucasoid) 0.348 (Caucasoid) 0.318 (St. Petersburg) 0.384 n 0.395 n 0.395 States (Black population) 0.59	14 0.086	0.9927	0.012	0.812	0.188	0.955	0.045	0.237	0.763	Gogri et al., 2022 [<mark>36</mark>]
e 0.605 (Total) 0.706 0.725 0.706 0.348 (Caucasoid) 0.348 (St. Petersburg) 0.318 (St. Petersburg) 0.328 0.42 n 0.395 n 0.395 States (Black population) 0.59	7 0	0.987	0.013	0.654	0.346	1	0	0.84	0.16	Matsuhashi et al., 2012 [37]
Total)0.725n0.706n0.706n0.36n0.348(Caucasoid)0.318(St. Petersburg)0.384n0.336n0.336States (Black population)0.59States (African American)0.59	0.031			0.747	0.253	0.971	0.029	0.559	0.441	Simtonget al., 2018 [32]
(Total) 0.706 0.306 (Caucasoid) 0.348 (Caucasoid) 0.318 (St. Petersburg) 0.384 0.42 n 0.395 n 0.395 States (Black population) 0.59	,5 0			0.845	0.155	0.956	0.044	0.693	0.307	Simtong et al., 2018 [<mark>32</mark>]
n 0.36 0.348 (Caucasoid) 0.318 (St. Petersburg) 0.318 n 0.42 0.42 0.42 0.42 0.42 0.55 n 0.59 States (Black population) 0.59	0.037			0.758	0.242	0.977	0.023	0.708	0.292	Manaf et al., 2015 [40]
n 0.36 0.348 (Caucasoid) 0.318 (St. Petersburg) 0.384 0.42 n 0.395 n 0.395 States (Black population) 0.59										
0.348 (Caucasoid) 0.318 (St. Petersburg) 0.384 0.42 0.42 n 0.395 n 0.395 States (Black population) 0.59	31 0.019			0.801	0.199	0.889	0.111	0.665	0.335	Grabowski et al., 2019 [44]
(Caucasoid) 0.318 (St. Petersburg) 0.384 0.42 0.42 n 0.395 States (Black population) 0.59	23 0.029			0.814	0.186	0.881	0.119	0.724	0.276	Nielsen et al., 2012 [<mark>26</mark>]
(St. Petersburg) 0.384 0.42 0.42 0.395 0.395 States (Black population) 0.59 States (African American)	68 0.014			0.768	0.232	0.882	0.118	0.736	0.264	Cardoso et al., 2013 [43]
0.42 n 0.395 States (Black population) 0.59 States (African American)	34 0.032			0.804	0.196	0.898	0.102	0.708	0.292	Krobinets et al., 2020 [45]
n 0.395 States (Black population) 0.59 States (African American)	64 0.03			0.737	0.263	0.881	0.119	0.754	0.246	Hauck et al., 2011 [46]
n 0.395 States (Black population) 0.59 States (African American)										
States (Black population) 0.59 States (African American)	l5 0.25			0.975	0.025	0.895	0.105	0.5	0.5	Nielsen et al., 2012 [<mark>26</mark>]
0.59										
I Inited States (African American)	0.23									Kissel et al., 2000 [31]
סווורכת סומורכי לי אוויכמון ל אוויכוויכמון				0.929	0.071					Bowens et al., 2012 [30]
United States (Hispanic/Latino)				0.826	0.174					Bowens et al., 2012 [30]
United States (Native)				0.946	0.054					Bowens et al., 2012 [30]
Brazilian 0.315 0.637	37 0.048			0.81	0.19	0.822	0.178	0.711	0.289	Santos et al., 2016 [27]; Lopes et al., 2014 [28]; Cardone et al., 2006 [29]
										(Continues)

	HNA-1			HNA-2		HNA-3		HNA-4		HNA-5		References
Population	1a	1b	1c	Positive	Negative	3a 3b	Зb	4a 4b	4b	5a	5b	Author, year
Middle Eastern												
Iranian	0.34	0.63	0.03			0.63	0.37	0.85	0.15	0.72	0.28	Esmaeili, 2022 [3]
Syrian	0.375	0.58	0.04			0.742	0.258	0.86	0.14 0.66	0.66	0.34	Hauck-Dlimi et al., 2018 [47]

(Continued)

TABLE 7

cases. This may be due to the fact that HNA-3a is expressed not only on neutrophils but also on other cells such as endothelial cells, monocytes and platelets [18, 20]. Conscious of the role of HNA antibodies in TRALI, the ISBT Working Party on Granulocyte Immunobiology published recommendations for leukocyte antibody investigations in TRALI [21]. Data from this survey confirm that in the majority of cases the antibodies are in the transfused blood product (Table 6). As twothirds of TRALI cases are associated with alloantibodies; both HLA Class I and II, and HNA [22], these investigations provide a vital way to identify and manage donors with the culprit antibodies.

During COVID-19 pandemic, transfusion of plasma obtained from COVID-19-immunized healthy donors containing neutralizing antibodies against COVID-19 antigen to COVID-19 patients (known as COVID-19 convalescent plasma [CCP]) was considered as promising therapy. It is worth noting that previous reports indicate the development of TRALI in COVID-19-infected patients after transfusion with CCPs [23, 24]. Therefore, analysis of convalescent plasma for detection of anti-HNAs and HLAs antibodies was conducted. Among participants of this current survey, four laboratories have tested CCPs. However, the frequency of anti-HNAs positive samples among investigated CCPs in these laboratories is not yet reported.

Investigation for the presence of HNA antibodies usually involves screening test, most commonly GIFT and GAT [25, 26]. Use of HNA-typed panel cells at the screening stage may facilitate identification of antibody specificity. The MAIGA provides another way to confirm antibody specificity but is liable to false negatives. Among this survey's participants, 16 established laboratories and 1 laboratory in development conducted GIFT, 11 of 17 established laboratories and one laboratory in development conduct GAT and 12 established laboratories conduct MAIGA with two laboratories optimizing the MAIGA (Table 2). Six established laboratories and three laboratories in development use the LabScreen Multi test kit [21]. The combination of GIFT, GAT and MAIGA assays is considered as the 'gold standard' for HNA testing.

It is interesting to note that although there are only five established laboratories in the Asia Pacific, since 2012, there have been at least 12 publications of HNA frequencies from that region (Table 7). Genotyping has made this feasible. There is a gap in our knowledge on HNA frequencies in African, the Middle Eastern and Western Pacific populations (Table 7). HNAs allele frequency analysis among different populations indicated a different pattern for HNA-1 alleles between Asian and Western populations; in many Asian populations, no HNA-1c allele has been detected. Currently, we do not know if the deviation in allele frequency is due to genotyping techniques applied in the region or if there is a real absence of HNA-1c in some Asian populations.

To evaluate the accuracy and effectiveness of a laboratory's ability to detect HNA antibodies, the ISBT-GIWP conducts the International Granulocyte Immunobiology Working Party (IGIWP) every year. In 2022, each of the 18 participating laboratories received four blinded samples (four DNA and four serum samples) to analyse. Of the 18 participating laboratories, only 13 responded to this survey. It is hoped that this survey will encourage other laboratories to participate in IGIWP workshop. In addition to IGIWP, there is the INSTAND EQA for Granulocyte Immunobiology. This is an intradisciplinary non-profit, scientific-medical association designated by the German Medical Association to promote quality assurance. For each evaluation, INSTAND distributes blinded samples for HNAs genotyping and detection of anti-HNA-alloantibodies.

This is the first study to provide a comprehensive synopsis of HNA typing and serology conducted in diagnostic laboratories across the whole world. The number of samples received and analysed provides a picture of HNA-related diseases such as adults and infants AIN, immunization frequencies against HNA and is indication of the population-related HNA involved in alloimmune neutropenia. The data provide a summary of investigation techniques employed that may help interested health services develop HNA investigation capability and improve the activities of established laboratories. Importantly, the list of participants (Tables 1A and 1B) provides a useful contact list for physicians and also regional hospitals wanting to contact HNA-diagnostic services.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts identified.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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