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External quality assessment (EQA) in molecular immunohematology: the INSTAND proficiency test program

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Abstract

Background—Genotyping for red blood cell (RBC), platelet and granulocyte antigens is a new tool for clinical pathology, transfusion medicine services and blood banks. Proficiency in laboratory tests can be established by external quality assessments (EQAs), which are required for clinical application in many health care systems. There are few EQAs for molecular immunohematology.

Methods—We analyzed the participation and pass rates in an EQA for RBC, platelet and granulocyte antigens. This EQA was distributed by INSTAND, a large non-profit provider of proficiency tests, twice per year since fall 2006 as EQA no. 235 Immunohematology A (molecular diagnostic). The coordinators defined at the outset which alleles are mandatory for detection.

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Statement of Disclaimer: The views expressed do not necessarily represent the view of the National Institutes of Health, the Department of Health and Human Services, or the U.S. Federal Government.

None of the molecular immunohematology kits and platforms that had been discussed in this forum have been cleared or approved by the Food and Drug Administration (FDA).

Web resources

1. Sektion Immunhämatologie und Immungenetik. Deutsche Gesellschaft für Transfusionsmedizin (DGTI). <http://www.dgti.de/indexL2.jsp?neid=20037>
2. INSTAND e.V., Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V. (formerly Hämometerprüfstelle). <http://www.instandev.de/en.html>
3. Bundesärztekammer. Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (Rili-BÄK). <http://www.aerzteblatt.de/pdf.asp?id=139417>
4. U.S. Food and Drug Administration. Establishment of Reference Panels for Blood Group Genotyping Tests (Dr Zhugong Liu). <http://www.fda.gov/BiologicsBloodVaccines/ScienceResearch/BiologicsResearchAreas/ucm352425.htm>
5. RCPA Quality Assurance Programs Pty Limited. <http://www.rcpaqap.com.au/>

Conflict of interest disclosure: WAF, UJS and GB were coordinating this proficiency test program without having received any funding from INSTAND. WAF receives royalties and holds intellectual property rights for *RHD* genotyping. IC declares no competing interests relevant to this article.

Supporting Information

Additional Supporting Information can be found in the online version of this article:

1. **Allele lists, INSTAND forms and** supplementary tables S4 to S6. Original list of 2003 for the mandatory alleles and minimal characterization (Table S1); Current package insert (Table S2); Example for the report forms (Table S3); Profile of participation (Table S4); and Performance rates (Tables S5 and S6).

Results—The number of participants steadily increased from 51 to 73 per proficiency by fall 2012. More than 60 institutions utilized this EQA at least once a year. Approximately 80% of them participated in RBC, 68% in platelet and 22% in granulocyte systems. With the exceptions of *RHD* (82%) and granulocytes (85%), pass rates exceeded 93%. While the pass rate increased for granulocyte and decreased for the ABO system, the pass rates for the other systems changed little over 6 ½ years.

Conclusions—The INSTAND proficiency test program was regularly used for EQA by many institutions, particularly in Central Europe. While the technical standards and pass rates in the participating laboratories were high, there has been little improvement in pass rates since 2006.

Introduction

Transfusion medicine laboratories have utilized different blood group serology methods for decades. Molecular immunohematology have a number of complementary and potential advantages and many laboratories already embrace their clinical and diagnostic opportunities.^{1–5} Reliable results, even with a recent history of transfusion, were recognized early as one of the major advantages offered by red cell genotyping.^{6,7} External quality assessment (EQA) can be helpful for the introduction of the new technology in a laboratory and is typically required in many health care systems for clinical application and to maintain accreditation. Soon after the genes were identified in the 1990s, genotyping workshops for red cells,⁸ platelets^{9,10} and granulocytes^{10,11} were organized by professional societies as an adjunct to serology workshops at international conferences.

Kroll et al.¹² reported on a genotyping workshop for platelet, granulocyte and red cell alloantigens, which was independent of serology workshops and conferences. Initial support came from the German Federal Ministry of Health (BMG) as part of a program for quality assurance in molecular diagnostics. 33 institutions from Germany, Austria and Switzerland submitted results before its December 18, 1998 deadline. The authors documented the reliability of several molecular techniques and high standard of the participating laboratories.¹² The first workshop of this series was held in 1997. It initiated an emerging EQA program organized by the German Society for Transfusion Medicine and Immunohematology (DGTI)¹³ and established a proficiency test program for molecular immunohematology that continued annually until 2006,^{13–16} when administration and specimen shipment functions were transitioned to a non-profit provider. The Immunohematology and Immunogenetics Section¹⁷ defined clinical indications for red cell and platelet genotyping in April 2000¹⁸ and in September 2003 established a list of required alleles (Table S1).

The College of American Pathologists (CAP)¹⁹ has been offering a proficiency testing panel for genes associated with diseases, including *RHD*, for over 10 years (CAP/ACMG Molecular Genetics MGL2). Each panel contains at least three DNA specimens and is shipped twice per year. Eleven laboratories participated in the most recent panel in 2012. Molecular EQA for RhCE and other blood group systems has not been offered.

The International Society of Blood Transfusion (ISBT) has organized a workshop^{20–23} for EQA every other year since 2004.²⁰ Participation has increased from 30²⁰ to 50 institutions.²³ The ISBT workshop organizers concluded that there is room for improvement^{22,23} and, with greater care and attention to detail, very high standards could be set for red cell genotyping.^{21–23} They defined appropriate controls for the molecular assays.²¹

The Consortium for Blood Group Genes (CBGG) was also initiated in 2004.²⁴ There are red blood cell (RBC) and platelet exchange participants from approximately 32 institutions,

mostly in both Americas.^{25,26} This working party has offered a practical, low-cost proficiency program shipping twice per year and a forum for DNA testing verification and result analysis²⁴ with regularly published reports.^{25–28}

INSTAND is a non-profit provider of proficiency test programs in all fields of clinical diagnostics since 1968, with 9,047 participating institutions and 148,000 sets of proficiency testing with 2 to 12 specimens each in 2012.²⁹ The arguable oldest source of regular proficiency workshops for molecular immunohematology^{12,16} became a full-fledged EQA program when the annual workshops were transitioned to professional non-profit management. Starting in fall of 2006, the distribution list, report collection and certification were handled by INSTAND.

We report on the results of the molecular immunohematology proficiency test program by INSTAND. From 2006 until fall of 2012, this EQA offered 13 proficiency sets with 4 specimens and up to 73 participants per set.

Methods

An EQA for molecular immunohematology has been offered since fall 2006. One proficiency set was sent each April and October to participants. All samples were distributed and results analyzed by the Giessen laboratory. Each set is always comprised of 4 specimens, typically two specimens for RBC and one each for platelet and granulocyte, in random order. RBC specimens were provided by Ulm, Germany and platelet and granulocyte specimens by Giessen, Germany. Participants were testing all 4 specimens, even if only a single proficiency certificate was of interest. Hence, the platelet/granulocyte specimens served as normal controls for the RBC proficiency and vice versa. There was no intention to change the degree of specimen difficulty, such as an increased molecular complexity, during the study period.

This EQA is labeled INSTAND no. 235 Immunohematology A (molecular diagnostic) and “INSTAND Ringversuch Nr. 235.” The Bundesärztekammer (German Physician Board) recently granted INSTAND deemed status in Germany for offering molecular genetics proficiencies in laboratory medicine (see web resources).³⁰

Criteria for proficiency certification

The complete list of mandatory alleles was published well before any proficiency set was sent out (Tables 1 and S1). A DGTI working group^{12,18} defined the list for the RBC systems, while the program coordinators defined the list for the platelet and granulocyte systems. Mandatory alleles contained in the proficiency set had to be detected and reported according to a defined minimal characterization. If any other allele, even a clinically relevant one, occurred in a proficiency set, detection was not required to obtain the proficiency certificate. This approach allowed maximum transparency.

Changes in mandatory alleles occurred as new clinically relevant genes or alleles were recognized, and were announced with sufficient lead time to allow technical adjustments by the participating laboratories. For RBC and platelet proficiencies, systems and mandatory alleles remained unchanged since inception of this EQA.

Changes in granulocyte systems

Once the *HNA-3* gene was identified,³¹ this clinically important granulocyte system was promptly added as a separate test in 2010(1) and mandatory for the Granulocyte I certificate since 2010(2). Because of its limited clinical relevance,³² HNA-5 has been offered as a separate Granulocyte II certificate since 2008(1).³³ HNA-2 (CD177, formerly NB1) is not

among the granulocyte systems as there are neither antithetical alleles³² nor a prevalent cause for the HNA-2 null phenotypes.³⁴

Required information for result reporting

Each proficiency set had a package insert (Table S2) with the complete listing of the mandatory alleles and the minimal characterization that was required to pass the proficiency (Table 1). The results were manually entered into report forms and either faxed or mailed (Table 3). Recently, online results submission has become available.

Statistics

Trends were calculated for participation and pass rates using the Pearson product-moment correlation coefficient r (Tables 2 to 4). We interpreted the trend as none ($|r| < 0.1$), small ($-0.3 < r < -0.1$ or $0.1 < r < 0.3$), medium ($-0.5 < r < -0.3$ or $0.3 < r < 0.5$), and strong ($|r| > 0.5$). The ordinal two-tailed Mann-Whitney U rank-sum test (Table S5) and the nominal two-tailed Fisher's exact test for 2×2 contingency tables (Table S6) were applied; $P = 0.05$ was considered statistically significant. In Tables 3 and 4, the mean was calculated per proficiency for n proficiency sets ($n = 13$, except granulocyte II with $n = 10$); the mean of these n means was shown for participation and pass rates. The mean for all tests over all proficiency sets was also calculated (Table S6).

Nomenclature

The newer nomenclature of the internet-based RBC allele terminology that was drafted by an ISBT subcommittee³⁵ was not necessarily reflected in the listing of mandatory alleles (Table 1) and report forms (Tables S1 to S3), which were established in 2006 and before. The RhesusBase was used for *RHD* allele terminology.³⁶ The human platelet³⁷ and granulocyte (neutrophil) terminologies^{38,39} available at that time were also considered.⁴⁰

Results

Since inception of this external quality assurance (EQA) program in October 2006, the number of participants has been steadily increasing (Table 2). Most accrual of additional laboratories occurred in Germany and the other European countries. Participation by laboratories from non-European countries was sporadic. 52 specimens of genomic DNA were used in the 13 proficiencies; 42 specimens came from blood donor samples, including all for the red cell systems, and 10 from B-lymphoblastoid cell lines,⁴¹ while no synthetic specimens were utilized. Overall 109 different laboratories participated (Fig. 1), to whom 785 proficiency sets were distributed representing 3140 aliquots of genomic DNA.

Participation by testing groups

A large number of laboratories have consistently participated in the EQA for RBC and platelet (Table S4, group 1), when comparing the first and last proficiencies of this study, which were representative for the trend. For RBC (group 2) and RBC/platelet/granulocyte (group 3), the participation more than doubled. Few laboratories participated in platelet/granulocyte only (groups 4 and 5).

Participation by systems

Most RBC systems had participation rates exceeding 77%, which changed little or not at all (Table 3). Exceptions were *RHD* zygosity with a stable, but low, participation rate averaging 53%, and *MNS* with a medium positive trend for the participation rate. The platelet testing showed a strong negative trend, despite its absolute increase from 38 to 46 participants

(Table S4). Strong positive trends occurred for both granulocyte I and II proficiencies (Table 3), although they showed the lowest absolute participation even in the latest proficiency.

Pass rates

On average, more than 93% of the participants passed the proficiency tests for most systems (Table 4). If these rates changed at all, most showed small negative trends. Exceptions were RHD with 82% (no change in the pass rate throughout the 13 proficiencies) and granulocyte I with 85% (strong positive trend). However, the ABO system showed medium negative trend during the 6 ½ years of this EQA program.

Experience of participants and their performance

The passing rates did not correlate with the experience of the participants with this EQA (Table S5). Failures to pass occurred equally among frequent participants (8 or more participations) and less frequent participants (6 or less). When testing the latest granulocyte proficiency of sufficient power to allow statistical significance, we observed a marginally significant correlation favoring the frequently participating institutions ($P = 0.02$). The performance of the institutions with < 3 and ≥ 3 participations were also calculated (Table S6). Although the pass rates for institutions with < 3 participations were generally lower, the MNS system was the only one with a statistically significant difference.

Discussion

The current program originated in 1998 and represents one of the largest external quality assessments (EQA) in molecular immunohematology. 72 institutions participated in 6 or more proficiencies since 2006 (Fig. 1), most of them at least once a year as required by many health care systems. When adjusted for population size, the number of participants in Austria and Switzerland was similar to Germany (Table 2). This may indicate that the current need for this proficiency has been met in these countries. However, 30 institutions participated only once or twice and did not return after the initial experiences.

Almost all institutions participated in the RBC, 60% in the platelet and 23% in the granulocyte proficiencies (Tables 3 and S4). The pass rates were lower than for serologic immunohematology proficiencies which had typical pass rates of $> 95\%$. This may be caused by the limited experience of the laboratories; however, there was neither improvement in the pass rates over the years nor a correlation with experience in this EQA (Tables 4, S5 and S6). Some test kits may not be easy to use in non-specialized settings. ABO and RHD systems seem to pose challenges for genotyping, which has been observed before^{12,20} and was also documented in this study. The two systems each have a large number of alleles caused by recombination, which in case of RHD is facilitated by the two linked *RH* genes with their tail ends facing each other.⁴² Hence, biological features may explain that some systems are recalcitrant to genotyping and pose challenges to genetic description in various populations and nomenclature. We conclude that intrinsically more robust technologies, such as automated platforms for assay and evaluation,^{43–45} may be needed for genotyping such systems, unless the data are used for screening purposes only.

The clinical applications of molecular immunohematology are expanding,^{18,46–50} as is the number of blood group serologists who apply this new technology in the diagnostics of transfusion recipients, pregnant women and blood donors.⁵ EQAs participation may facilitate the implementation of clinical applications and improve the quality of patient care; successful participation once per year may be required for reimbursement.^{5,28} EQAs organized by external non-profit organizations, such as INSTAND or CAP, open a venue for professional and independent management, especially when it comes to summary reports.⁵

This is an advantage over peer-group organized^{20,25} or vendor-based EQAs.⁵ We expect a wider implementation of molecular immunohematology will increase the demand for EQAs.

Several of the devices, genotyping platform or test kits, used by participants have had the Conformité Européenne (CE)-label for many years, implying that it is technically and legally possible, within the specifications of the CE-certification process for in vitro diagnostic devices in the European Union (EU), to replace several blood group serology tasks with genotyping.⁴⁹ However, none of these molecular immunohematology devices have been cleared or approved by the FDA. Resolving regulatory issues may facilitate the implementation of the technology to improve patient care at a level that cannot be reached by any available serology alone.⁵

DNA is very stable and usually available in large quantities from blood donors. However, the preferred source of reference DNA is B-lymphoblastoid cell lines,^{41,51} which should be established for all common alleles, while we may have to be content with homogeneous synthetic controls for many of the less common alleles. Use of reference reagents as quality control material, currently developed by the FDA (see Web resource) and ISBT,⁵¹ will be widely applied in the future. Similar to monoclonal antibodies, such reagents are easily available, and, once established, pose no consent issues. Their supply can hardly be exhausted, and experience accumulates while being used.

Red cell genotyping was offered based on individual systems. In difference, all relevant platelet and granulocyte systems, if indicated, should be tested in groups of systems and were offered as groups. Some clinically relevant antibodies to platelets cannot be detected with common assays.⁵² Screening for genotypes of all involved platelet systems was hence recommended. The clinical relevance of low-avidity antibodies to platelets that may escape serologic detection was recently shown in pregnancies⁵² and in 2 mouse models.^{53,54} The same rationale was applied to genotyping of granulocyte I as a group.

When designing EQAs, the coordinators need to decide what the EQA is supposed to validate. An EQA does not have to cover all aspects of the typical laboratory workflow. Aspects that are critical in many assays, but not assessed in the current EQA, such as the quality of the sample and the DNA extraction, may be assured by validations involving separate EQAs. The preferred sample material and DNA source also vary as technologies develop: the use of amniotic fluid and chorionic cells¹² waned over the past decade, while that of buccal swaps may increase. The current EQA reflects a donor setting well, where molecular screening is performed independent of prior serologic results. The clinical application is often not closely reflected, however, because red cell, platelet and granulocyte genotyping of a clinical patient sample is typically guided by serologic results and questions, not posed by most EQAs.

If documentation of proficiency is required every year, the organizers of the ISBT workshop encouraged participants in the off years to take part in the EQA scheme offered by INSTAND.²¹ Institutions in Japan and Australia are often using EQA organized by the Royal College of Pathologists of Australasia (RCPA). The RCPA Transfusion Quality Assurance Program (QAP) has commenced a molecular genetics program including a range of technical and disease specific modules; however, there is no molecular blood group module yet established. Molecular viral proficiency programs are offered by the National Serology Reference Laboratory (NRL) in Australia, whose scope does not cover molecular immunohematology. Hence, the gap for red cell genotyping is often filled by participation in international programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Friedrich Schunter contributed to the early implementation into the routine as chairman of the Immunohematology and Immunogenetics Section by organizing annual meetings in Oldenburg on August 28-30, 1996 and September 3, 1997.^{46,55} This process was also promoted by the subsequent and current chairmen, Eduard K Petershofen and Franz F Wagner, until the EQA became institutionalized in 2006.

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Figure 1.

The number of participating institutions and their participation in the 13 proficiencies offered between 2006 and 2012. Overall 109 different institutions received proficiency sets; 19 participated only once and 19 in all 13 proficiencies.

Table 1

Mandatory alleles and minimal characterization *

Allele	Minimal characterization	Allele	Minimal characterization
Red cells		Red cells	
<i>ABO</i>		<i>RHCE</i>	
A1	A	C	C
A2	A2	c	c
B	B	Cw	Cw
01	01	E	E
02	02	e	e
<i>RHD</i>		<i>KEL</i>	
D	D positive	KEL1	KEL1 (K, Kell)
weak D type 1	weak D type 1	KEL2	KEL2 (cellano)
weak D type 2	weak D type 2		
weak D type 3	weak D type 3	<i>JK</i>	
weak D type 4.0/4.1	weak D type 4.0/4.1	JK * A	JK * A
weak D type 4.2	weak D type 4.2	JK * B	JK * B
weak D type 5	weak D type 5		
weak D type 11	weak D type 11	<i>FY</i>	
RHD(M295I)	RHD(M295I)	FY * A	FY * A
weak D type 15	weak D type 15	FY * B	FY * B
RHD(K409K)	RHD (K409K)	FY * null	FY * null
RHD(IVS3+1G>A)	RHD(IVS3+1G>A)	FY * X	FY * X
D category IIIa	D category III		
D category IIIb	D category III	<i>MNSs</i>	
D category IIIc	D category III	M	M
D category IVa	D category IV D category IV	N	N
D category IVb	D category V	S	S
D category Va (Kou.)	D category V	s	s
D category Va (Hus.)	D category VI		
D category VI type I	D category VI	Platelets	
D category VI type II	D category VI	ITGB3 *196T	HPA-1a (PIA1, Zwa)
D category VI type III	D category VI	ITGB3 *196C	HPA-1b (PIA2, Zwb)
D category VI type IV	D category VII	GP1BA *524C	HPA-2a (Kob)
D category VII	DBT	GP1BA *524T	HPA-2b (Koa)
DBT type I	DBT	ITGA2B *2622T	HPA-3a (Baka)
DBT type II	DHMi	ITGA2B *2622G	HPA-3b (Bakb)
DHMi	DNB	ITGA2 *1648G	HPA-5a (Brb)
DNB	DFR	ITGA2 *1648A	HPA-5b (Bra)
DFR	RHCE-D(5)-CE (ROHAR, Rh33)	CD109 *2108C	HPA-15a (Govb)
RHCE-D(5)-CE (ROHAR)	RHCE(1-9)-D(10)		
RHCE(1-9)-D(10)	RHD-CE(2-9)-D		
RHD-CE(2-9)-D	RHD-CE(2-7)-D		
RHD-CE(2-7)-D	RHD-CE(3-7)-D		
RHD-CE(3-7)-D	RHD-CE(4-7)-D		
RHD-CE(4-7)-D	RHD-CE(8-9)-D		
RHD-CE(8-9)-D	RHD(delEx9)		
RHD(delEx9)	d(C)ces		
d(C)ces	RHD psi (RHD)		
RHD psi (RHD)	D negative		
dd			

Allele	Minimal characterization	Allele	Minimal characterization
		CD109 *2108A	HPA-15b (Gova)
		Neutrophils	
		FCGR3B *1	HNA-1a (NA1)
		FCGR3B *2	HNA-1b (NA2)
		FCGR3B *3	HNA-1c (SH)
		CTL2 *461G	HNA-3a
		CTL2 *461A	HNA-3b
		ITGAM *230G	HNA-4a
		ITGAM *230A	HNA-4bw

* For a successful participation, the listed alleles must be recognized within the respective category, if such an allele occurs in any of the proficiency specimens.

Table 2

Participants of the molecular immunohematology proficiency test program

Country	Participants (n)														Descriptive statistics *		
	2006		2007		2008		2009		2010		2011		2012				
	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	Mean ± SD	Trend (r)
Germany	41	34	38	42	41	43	44	45	44	47	48	51	50			44 ± 5	+ 0.91
Austria	2	2	3	4	3	4	3	3	4	4	3	4	4			3 ± 1	+ 0.66
Switzerland	3	2	3	3	2	2	3	4	4	2	3	4	4			3 ± 1	+ 0.50
Other European [†]	5	6	9	4	7	10	11	9	11	12	13	12	14			9 ± 3	+ 0.89
Non-European [‡]	0	1	1	1	1	1	1	1	0	2	1	2	1			1 ± 1	+ 0.44
Total	51	45	54	54	54	60	62	62	63	67	68	73	73			60 ± 9	+ 0.97

* n = 13; SD – standard deviation; the trend was calculated by Pearson product-moment correlation coefficient r.

† including Belgium, Croatia, Denmark, France, Finland, Italy, Netherlands, Sweden, UK

‡ including Australia, Brazil, Canada, Israel, USA

Table 3

Participation in RBC, platelet and granulocyte systems

Proficiency Certificate	System	Relative participation (%)			Trend (r) *	Sets offered (n)
		Minimum	Maximum	Mean $\pm$ SD		
RBC	ABO	73	85	82 $\pm$ 3	-0.03	13
	RHD	80	91	85 $\pm$ 3	-0.02	13
	RHD zygosity	43	59	53 $\pm$ 4	+0.08	13
	RHCE	78	93	88 $\pm$ 4	+0.16	13
	KEL	73	94	80 $\pm$ 6	-0.18	13
	FY	61	85	78 $\pm$ 6	+0.27	13
	JK	67	84	77 $\pm$ 5	+0.17	13
	MNS	54	70	64 $\pm$ 5	+0.44	13
Platelet	HPA-1-3,5,15	62	76	68 $\pm$ 5	-0.87	13
Granulocyte I	HNA-1,2,4	16	29	22 $\pm$ 4	+0.62	13
Granulocyte II	HNA-5	9	26	19 $\pm$ 6	+0.81	10 [†]

* Pearson product-moment correlation coefficient r.

[†]This set was offered in 5 years only (n = 10) since the 2008(1) proficiency.

Table 4

Pass rates of the participants

Proficiency Certificate	System	Pass rate (%)			Trend (r) *	Sets offered (n)
		Minimum	Maximum	Mean ± SD		
RBC	ABO	88	100	93 ± 4	−0.44	13
	RHD	63	98	82 ± 10	0.07	13
	RHD zygosity	84	100	93 ± 5	−0.12	13
	RHCE	92	100	95 ± 2	−0.21	13
	KEL	94	100	98 ± 2	−0.22	13
Platelet	FY	82	100	95 ± 6	0.29	13
	JK	96	100	99 ± 1	−0.04	13
	MNS	84	100	94 ± 4	0.00	13
	HPA-1-3,5,15	84	100	93 ± 4	0.01	13
	HNA-1,2,4	58	100	85 ± 15	0.65	13
Granulocyte II	HNA-5	87	100	96 ± 5	−0.36	10 [‡]

* Pearson product-moment correlation coefficient r.

[‡]This set was offered in 5 years only (n = 10) since the 2008(1) proficiency.