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REVIEW

Potential of next-generation sequencing to match blood group antigens for transfusion

Agnieszka Orzinska
Katarzyna Guz
Ewa Brojer

Institute of Hematology and Transfusion Medicine, Department of Hematological and Transfusion Immunology, Warsaw, Poland

Abstract: A review of the advances in applying next-generation sequencing (NGS) to transfusion medicine for the purpose of genotyping alleles encoding clinically important red blood cell and platelet antigens. NGS data from published studies confirm the possibility of antigen prediction based on sequencing of the whole genome, exome or targeted regions. What remains a challenge, to provide highly accurate NGS genotyping, is the further improvement of bioinformatic solutions for automated interpretation based on publicly accessible and improved reference databases appropriate for NGS methods as well as validation of a method based on the examination of a large number of individuals. There is no doubt, however, as to the future of NGS as a supplementary test used to provide highly compatible blood as well as to reduce the risk of patient's alloimmunization. This is part of personalized medicine.

Keywords: alloimmunization, transfusion medicine, whole genome, exome, allele

Prevention of alloimmunization against blood group antigens

Transfusion is the procedure of introducing donor material with unknown blood cell antigens into the recipient's circulatory system. The recipient's immune system recognizes foreign antigens, produces specific antibodies and sensitization (alloimmunization) occurs.[1,2] To date, more than 300 red blood cell (RBC) and 33 human platelet antigens (HPA) have been described.[3,4] Antibodies against these antigens may have varying clinical significance and cause acute hemolytic reactions, delayed hemolytic reactions, hemolytic disease of the fetus/ newborn or ineffective platelet transfusion in the case of sensitization to platelet antigens.[5,6] Some alloantibodies are clinically irrelevant. According to the Serious Hazard of Transfusion data, hemolytic transfusion reactions were responsible for 8% of deaths attributed to the presence of antibodies acting against donor RBC antigens.[7]

The percentage of patients immunized as a result of blood transfusion varies from 5% to 50%.[8–10] The underlying disease and ethnic origin of the donor as well as the recipient are also important factors in the risk of alloimmunization.[11] Transfusion-dependent patients (with thalassemia or hemoglobinopathies such as sickle cell disease [SCD]) are particularly prone to sensitization. The prevalence of alloantibody detection in this group is up to 50% as compared to patients with histories of a single transfusion.[12] It seems that the number of the recipient's exposures to donor antigens may be significant in triggering the immunological

Correspondence: Agnieszka Orzinska
Department of Hematological and Transfusion Immunology, Institute of Hematology and Transfusion Medicine, 14 Gandhi Street, Warsaw 02-776, Poland
Email aorzinska@ihit.waw.pl

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Box 1 Advantages of NGS overcoming the limitations of routinely used BG antigen genotyping platforms

- Revealing of the direct sequence of the entire gene, all exons, the promotors, intronic regions, the associated genes having impact on with an BG antigen expression or whole genome in one test
- Revealing any nucleotide variants in the tested sequences
- Quantification of sequences for estimation of copy number variation, revealing of chimerism or mosaic phenotype and solving structural variants such as hybrid genes
- Genome mapping of haplotype and cis/trans location
- Different depth of reading useful for testing different type of materials
- Cost-effective capacity of NGS experiment useful for high-throughput testing

Abbreviations: NGS, next-generation sequencing; BG, blood group.

development of tools for reading DNA sequences and for collecting and analyzing the data obtained, and as a result, the development of the high-throughput solution known as NGS. The currently available technologies allow various levels of sequencing; either the whole genome (WGS), coding regions – exons (WES) or only selected genes or regions of interest. NGS technology significantly reduces the cost of testing. It has been successfully implemented in transplantation medicine for testing donors' genotypes of HLA antigens in high-throughput mode.[48] Over 9000 HLA alleles for over 500 individuals can be identified per run. Such a huge capacity is a green light for blood group and platelet gene screening in blood donors as well as for the setting up of a database with compatible blood units dedicated for transfusion.

NGS in revealing unknown blood group variations

NGS is particularly effective for finding unknown variations responsible for different phenotypes in patients with antibodies of unknown specificity because it enables screening of the whole genome, exome or particular genes and finding a "needle in a haystack" – an unknown or rare variant. Recent studies have confirmed NGS effectiveness in resolving the molecular background of orphan antigens with an as yet unknown genetic basis.[49–59] The WES strategy based on genomic DNA from patients with detected antibodies and their relatives combined with appropriate variant filtering have revealed one SNV in the *GYPA* gene.[49] This discovery allowed the SARA antigen to join the MNS blood group system. The WES solution was applied for identification of a 17-bp deletion in

the *SMIM1* gene in Vel negative individuals.[50] The WES strategy also helped to define the molecular background of the AnWj antigen negativity through indication of homozygous SNV in *SMYD1* gene, which encodes a transcription factor not directly associated with blood group genes.[15] NGS technology was recently used for resolving specificity of unknown antibodies acting against the AUG1 antigen and revealed a novel allele, encoding the AUG3 antigen.[52,53] The WES approach was also used for recent identification of the missense variation in the *PRNP* gene responsible for the KANNO antigen negativity in the Japanese population.[54] Another successful example was the use of NGS for solving the Xg blood group molecular background as reported by Yeh et al.[55] The study revealed a variation in a GATA motif located beyond the *XG* gene but with an impact on its transcription.

Schoeman et al described the molecular background of seven unexplained cases with complex serological results.[56] The WES method with the TruSightOne panel and Illumina technology revealed rare and novel variants in the Rh, Duffy, Lan, Cromer blood group systems and *KLF1* from the transcription factor family. Similarly, with the use of customized panel targeting exons encoding 15 blood group systems and Ion Torrent sequencing platform, Jakobsen et al detected rare single nucleotide variations that may correspond to a variant or weakly expressed antigens.[57] Wen et al tested *RHAG* and *ANK1* sequences for explaining weaker Rh expression.[58] The NGS analysis with a custom-made primer panel for exons of genes involved in RBC membrane disorders identified a homozygous variant of the *RHAG* gene which changes interactions of the RhAG-RhD complex. In a group of RhD negative individuals, El Wafi et al revealed five novel intronic *RHD* variants that probably affected the splicing of D protein.[59]

Moller et al analyzed the genomic sequences of genes encoding 36 blood group systems from over 2500 individuals enrolled in the 1000 Genome Projects.[32] The study revealed 1241 non-synonymous variants. Their clinical role is unknown and 80% of them were absent in reference databases. Analysis of these sequencing data also led to the discovery of a huge deletion of 5–7 exons in the *ABO* gene described as the third common variation in an African population with O blood group phenotype.[60]

Montemayor-Garcia et al presented a new geographical distribution of allelic blood group variants, the description of which was based on genomic sequences from the same project.[61] The report presented a novel distribution of known variants and 12 novel variants, probably antigenic

in 4 cases, from the Kell, Kidd, Scianna and Cromer systems due to the predicted amino acid substitution.

All the above findings highlight the usefulness of NGS for detecting novel, unsuspected genetic variants. Each new discovery expands our knowledge and if they are submitted and introduced into the appropriate reference databases they will contribute to the better identification of patient/donor genotypes and phenotypes and more effective donor–recipient antigen matching. However, currently, the problem hindering the possibilities of studies is the lack of an accurately developed and verified, publicly accessible database of reference sequences in the form of a program adapted to work with the data obtained by NGS.

NGS in blood group screening

NGS is also effective in reducing the risk of post-transfusion alloimmunization since the huge capacity of one experiment enables the immediate and cost-effective determination of all RBC and platelet antigen genotypes. Study results support extended profiling of donors and patients for the best prophylactic antigen matching to prevent alloimmunization.[16,22–24]

Literature on the use of NGS for studying blood group antigens shows a picture of rapidly developing technology which may prove highly reliable. The technology is now being validated and upgraded especially in terms of antigens with a complex genetic background or with highly homological regions.[62–67] Once the bioinformatics and economic solutions have been worked out, NGS will undoubtedly supersede other genetic methods used for predicting blood group phenotypes based on genotyping with predefined oligos. To date, difficulties in the sequencing of alleles encoding blood cell antigens have only partially been solved. Targeted panels for sequencing the coding regions of blood cell antigens have been designed. Some difficulties related to the high homology of the *RHD/RHCE* and *GYA/GYPB/GYPE* genes are beginning to be overcome, the database of blood group and platelet reference allele sequences is currently being improved for correct alignment, and special algorithms are being designed and amended for correct phasing and haplotype reconstruction from short-read sequences. The first software for automatic blood group phenotype interpretation based on WSG data has been developed. Automation of the NGS process required for high-throughput donor testing has been performed. The main task now is to improve the accuracy of NGS tests by creating an expanded database of allelic sequences found in different populations

with related serological data. It will then be possible to develop a standardized panel for diagnostic tests for proper analysis of donor or recipient data. Numerous NGS reports on blood grouping reveal the presence of SNVs of unknown serological characteristics and unknown clinical significance that are absent in reference databases. The crucial step now is to supplement the reference database with hundreds of thousands of genomic sequences, paired with individual serological and genomic backgrounds obtained with other molecular techniques. Once the task has been performed NGS can be implemented into routine diagnostics to replace serological antigen determinations.

Table 1 presents a list of original papers considered to be milestones in the development of NGS technology and applications in transfusion medicine. They indicate the difficulties related to genotyping blood group antigens and introducing NGS as a basic tool for the next-generation serologists.

In their study, Fichou et al present NGS in comprehensive blood group typing and report the application of a targeted panel for NGS of 18 genes.[63] In parallel, they explore the coding and intronic sequences encoding 15 blood group systems using four well-characterized DNA samples from the EQC program. To overcome the misalignment of sequenced regions from the homologous genes *RH* and *GYP*, the authors used specific primers for library preparation. In a single run, they obtained sequences that covered 86% of all coding regions, with the exception of the *ABO* antigen coding region. In our NGS study, we also focused on sequencing regions of interest flanking polymorphisms encoding blood group and platelet antigens in 57 blood donors and used primers designed by AmpliSeq software, complemented with *RHD* and *RHCE*C/c* specific primers incorporated into a customized mixture of primers.[64] Our results were consistent with the serological and genotyping outcome, except for *GYPA* and intron 2 of *RHCE*C* with lack of or a poor number of amplicons. The above studies prove the utility of the NGS approach as a screening tool dedicated for testing donors and indicate the main difficulties concerning complex or homological genes.

Donor–recipient compatibility with regard to ABO is of crucial importance, so precise identification of ABO subtypes is a challenge. However, *ABO* gene genotyping is complicated by the complex genetic background of the A and B glycosyltransferases indirectly responsible for the presence of A and B antigens. NGS is the best method for their identification since the large numbers of variants are

Table 1 Published reports on application of NGS to red blood cell and platelet antigen genotyping for donor/recipient antigen matching

References	BG genes	Tested group	NGS mode	Achievements, limitations and conclusions
Fichou et al (2014)[63]	18 blood group genes	4 reference samples	Customized Ampliseq panel/ Ion Torrent	NGS is suitable for genotyping of clinically important blood group antigens in a single run (an exception is *ABO*).
Moller et al (2016)[32]	36 blood groups	2504 individuals from 1000 Genome Project	WGS	NGS analysis revealed 1241 non-synonymous variants. Creation of *Erythrogene* database of the complete coding regions of 43 blood group related genes.
Lane et al (2016)[90]	45 BG genes and 6 HPA genes	1 patient from MedSeqProjet	WGS	Demonstration of the NGS approach for antigen genotyping.
Fox et al (2016)[65]	*ABO*	6432 Americans	WES	5 novel structural *ABO* variants were revealed. Different algorithms for data analysis were applied for correct *ABO* mapping. Confirmation of any novel findings is required.
Fichou et al (2016)[74]	17 blood group genes	48 SCD patients	Customized Ampliseq panel/ Ion Torrent	Phenotypes predicted for 12 blood group systems and partially for Rh and M/N antigens. Predicted phenotypes of S/s, Fy, Jk, K/k antigens matched serological typing in 98.9%.
Lang et al (2016)[68]	*ABO* (exons 6 and 7)	1,693,287 bone marrow donors	Designed panel/Illumina	Demonstration of the use of full capacity of NGS approach as a screening tool for HLA as well as ABO antigens. Highlighted cost-effectiveness of multiplexed sequencing regions of interest. Novel 287 *ABO* variants were found.
Orzinska et al (2017)[64]	ROI from 6 BG genes, and 5 HPA antigens	57 blood donors	Customized Ampliseq panel/ Ion Torrent	Demonstration of the effectiveness of NGS technology in identifying blood group and platelet antigen SNVs on mass scale and determination of natural chimerism in donors.
El Wafi et al (2017)[59]	*RHD*	9 RhD negative Maroccans	True Seq Kit/Illumina	6 novel intronic SNVs in *RHD* variants were revealed probably affecting mRNA splicing.
Schoeman et al (2017)[70]	28 BG genes, *KLF1* and *GATA1*	28 blood donors	TruSight One WES/Illumina	Demonstration of the potential for correct identification of hybrid and homological *RH* and *GYP* genes using manual analysis of copy number variations of exons. Unknown SNVs revealed not annotated with rs number.
Jakcobsen et al (2017)[57]	15 rare BG antigens	72 donors	Customized Ampliseq panel/ Ion Torrent	Revealed rare SNVs in coding and non-coding regions of BG genes, not included in commercial assays. The genotype of Chido/Rogers antigens was not determined due to high homology of both genes.

(Continued)

Table 1 (Continued).

References	BG genes	Tested group	NGS mode	Achievements, limitations and conclusions
Dezan et al (2017)[76]	RHD RHCE	35 SCD Brazilian patients with anti-Rh	Designed panels for separate RHD/RHCE libraries/Ion Torrent	The NGS assay with RHD, RHCE gene-specific primers was accurate in identifying RH variants, clarified patients' phenotypes and improved transfusion support. In 62% of patients serological methods inaccurately classified Rh blood group (73% were clinically relevant).
Chou et al (2017)[71]	RHD RHCE RHAG CD47 LW GYPB	27 SCD children/27 control	WES/Illumina	The study proved the utility of WES for RH genotyping, but intronic variants (insertions in RHDpsi and RHCE*C) were not covered. Data analysis of RH required some modifications for correct assignment NGS results for RH improved red blood cell matching.
Tousin et al (2018)[73]	RHD RHAG	69 donors with serological data	LP-PCR and panel/Ion Torrent	NGS analysis identified intronic SNP pattern link to DcE or DCe, DCE, Dce haplotypes. Two reference sequences of the whole RHD were described.
Wu et al (2018)[67]	ABO	24 individuals with discrepant serology	True Seq Kit with targeted capture probes/Illumina	Demonstration of the potential of NGS in identifying novel ABO variants or structural variants. 3 novel variants were found, 5% chimerism was detected.
Schoeman et al (2018)[78]	36 BG genes KLF1 and GATA1	72 indogenous Australians	WES	Determination of complete BG profile of this ethnic cohort. 152 novel variants identified in genes encoding ABO, Rh, Kn, In, MNS, CD59 BG antigens.
Moller et al (2018)[60]	ABO	2504 individuals from 1000 Genome Project	WGS	Novel ABO deletion encompassing exons 5–7 was identified among individuals of African origin. False prediction of another deletion indicates the need for confirmation of NGS discoveries.
Wheeler et al (2018)[72]	RH	1135 Asian and native Americans+4 WHO samples+1715 African Americans	Targeted panel captured 41 BG relevant genes or WGS/Illumina	Demonstration of NGS potential for identifying RH structural variants with 99% concordance for D and C antigen versus serology. Structural variants at the RH locus found in 61% of African Americans. Description of RHCE and RHD alleles not reported in ISBT
Montemayor-Garcia et al (2018)[61]	42 BGs related genes	2504 individuals from 1000 Genome Project	WGS	Demonstration of a new blood group gene distribution in populations. 1198 non-synomous variations identified which required further study. 12 novel variations identified probably with impact on antigen expression.
Boccoz et al (2018)[75]	9 BGs	95 blood donors	Designed two panels/Illumina	Demonstration of fast and complete antigen screening in order to identify rare blood units.

(*Continued*)

Table I (Continued).

References	BG genes	Tested group	NGS mode	Achievements, limitations and conclusions
Lane et al (2018)[66]	38 BGs and 22 HPA antigens	200 patients and 220 individuals from INTERVAL Study	WGS	Improvement of reference database. Development of a new algorithm for NGS data interpretation – bloodTyper. The approach was validated for 21 BG antigens encoded by 14 genes achieving 99.2% accuracy close to serological and DNA array level.
Lane et al (2019)[91]	P1 XG	113 individuals from MedSeqProject	WGS	NGS analysis revealed rs311103 correlated with Xg(a-)phenotype and 4 SNVs related to P1- negative phenotype. Improvement of bloodTyper to predict Xg and P1 phenotypes from WGS data.

Abbreviations: BG, blood group; NGS, next-generation sequencing; HPA, human platelet antigens; SCD, sickle cell disease; ROI, regions of interest; SNVs, single nucleotide variants; WGS, whole genome sequencing; WES, whole exom sequencing.

associated with SNVs in exons 6 and 7. A recent report on the testing of these regions was performed in parallel with HLA testing in bone marrow donors and proved the NGS capacity for *ABO* screening as well as its cost-effectiveness.[48] Fox et al suggested another approach based on extraction of *ABO* data from WES of individuals of African origin revealing an unknown deletion.[65] Their report demonstrates the importance of manual data inspection, despite applied algorithms, and the need to confirm with other molecular techniques in the case of novel variants. Lane et al report an accurate prediction of *ABO* from WGS data.[66] Automated typing with a slightly modified algorithm called bloodTyper rendered 98% concordance with the serology. The work highlighted the necessity of further validation of the performance of software designed to interpret blood groups from data acquired from sequencing of known subtypes or hybrid *ABO* variants. Wu et al demonstrated the state of art and limitations in the NGS solution of the heterogeneity of the *ABO* molecular background in serologically discrepant samples.[67] With the new technique of customized capture probes, the authors sequenced the entire *ABO* gene and successfully identified both known and novel alleles, microchimerism and cis/ trans association. With this approach, however, it was impossible to phase the haplotypes encompassing exons 6 and 7 in all the samples. A longer sequencing platform (PacBio or Oxford Nanopore) seems to be the best solution for correct phasing of complex genes, as Lang et al have already demonstrated for confirmation of novel *ABO* allelic sequences identified in bone marrow donors.[68]

A current review of NGS technology applied to the prediction of Rh blood group antigens reveals a similar pattern of solutions and limitations as in the case of *ABO* described above. For Rh, the sequencing is even more complicated due to the presence of a high variety of alleles and mixed *RHD/RHCE* hybrid variants and a complex membrane structure. Interpretation of results from short NGS reads is extremely difficult because correct alignment to the reference common allele is often unclear. To overcome the similarity of *RHD* and *RHCE*, gene-specific primers were applied in targeted customized panels and separate libraries developed for both homological genes with different barcodes.[69] The tools for data analysis and algorithms were modified for correct assignment. Schoeman et al demonstrated the need for manual interpretation of copy number variation for correct identification of *RH* rearrangements from the data of 28 individuals with a targeted exon panel.[70] With the purpose of correct

mapping and localization of *RHD*/*RHCE* genes, Chou et al calculated read depth proportion for each of the 54 patients with SCD tested in their study. Misalignment of fragments of *RH* genes was identified as the main source of WES errors which gave approximately 91.7% concordance with other genotyping methods.[71] Lane et al proposed the bloodTyper tool for automated copy number analysis of *RH* from WGS data with a final 100% concordance for RhD typing and 99.5% accuracy at RhC typing.[66] Recently, Wheeler et al have reported the application of a customized panel for specific *RH* detection.[72] It makes use of information from flanking intronic regions for the correct mapping of short NGS reads of highly homological sequences including the problematic exons 1–2 and 8 misaligned to the opposite *RH* gene by other researchers. With this approach, the authors successfully identified *RH* structural variations in a large multi-ethnic group and correctly predicted RhC expression in 99.2% of hybrid cases. On the other hand, Tounsi et al used long-range PCR amplicons of the complete *RHD* and performed WGS of 69 individuals with known Rh serology in order to establish a reference *RHD* allele for NGS analyses.[73] They found multiple intronic SNVs among them, 23 specific to DcE haplotype and 15 linked to DCe, DCE and Dce haplotypes, giving two references *RHD* sequences deposited to GenBank.

NGS prediction of other clinically relevant and rare blood group antigens is highly accurate with the exception of the highly homological genes *GYP* and *CH/RG* encoding the MNS and Chido–Rogers blood group systems.[63,70,75] Study reports on the application of WGS, WES or a customized panel strategy demonstrate that for screening blood donor antigens and patient antigen profiling it is desirable to acquire an individual genotype of common and rare alleles in one step (see Table 1).

Applications of NGS in transfusion medicine

The application of NGS technology for blood typing contributes to the following aspects of patient care:

1) Prevention of alloimmunization in SCD and other transfusion-dependent patients.

Regularly transfused patients are susceptible to sensitization against foreign donor antigens. The different ethnic origins of the donor/recipient, frequently of African origin and carrying Rh variants, are the main cause of alloimmunization. For such patients, precise NGS genotyping as described by Dezan et al has been proved extremely useful and cost-effective.[76] NGS gene-specific assay for typing of 35 SCD patients with unexplained Rh antibodies revealed false serological Rh classification in 62% cases and misinterpretation of anti-Rhe alloantibodies as autoantibodies. According to NGS data, 10% of patients were reclassified as RhD positive which made it possible to save the already scarce/limited supplies of RhD negative blood products as well as to reduce the risk of alloimmunization against Rhc and Rhe antigens associated with RhD negative units from Caucasian donors.

2) Faster and cheaper diagnostics in the case of patients with unexplained, complex serological results.

Patients from specific populations or those with unexplained complex serological results will particularly benefit from NGS analysis used as a first-line test instead of relying on extended phenotyping, multistep antibody identification and numerous single or screening genetic tests.[77–79] In 2014, Mazonson et al estimated the costs of pre-transfusion tests at about 195 USD per patient but for patients with autoimmune hemolytic anemia the cost increased to 1490 USD as compared to 65 USD for six blood genes when using the NGS approach.[80]

3) The huge capacity of the NGS experiment makes this technology an ideal tool for mass screening of blood donors for all clinically important antigens and also to detect individuals with rare blood group antigens in various ethnic groups.[78,79] This facilitates access to compatible donors for alloimmunised patients.[81]

Patients with antibodies to high-frequency antigens and antibodies to numerous antigens will benefit when tested with the NGS approach which provides the recipient's profile of antigens in one step. Moreover, if the donor is genotype-matched further alloantibody formation is limited.[82] It is worth mentioning that there is a growing number of patients with WES or WGS performed for diagnostic reasons and their NGS blood group data is available for transfusion purposes. This also concerns immunized pregnant women who may take advantage of non-invasive prenatal testing to predict feto-maternal compatibility in fetal blood group or platelet antigens. The fetal and maternal blood group and platelet antigen

genotypes are determined with a targeted panel for sequencing and in parallel the antigen profile is provided for potential transfusions in the fetus and the mother.[83]

The issue also concerns an important group of special donors relied upon for production of RBC panel reagents used for antibody screening and identification in all immunohematological laboratories worldwide. NGS typing of all blood group genes in these "products" will upgrade the interpretation of serological results and antigen matching.[84]

Summary

In the future, the NGS technique will contribute to an extension of the donor registry for immunized patients increasing transfusion safety and reducing the risk of delayed post-transfusion reactions. Well-characterized blood products will become more available. NGS provides a deeper insight into the sequence of genes and regions of interest, it determines SNVs and indels together with the quantitative measurement of copy number variations in one experiment with a large number of samples. An important advantage of this technology as compared to serological tests and other high-throughput genetic applications is the ability to identify variations of clinically significant antigens in a single sample. Other authors have also demonstrated NGS to be the correct technology for achieving significant progress in blood transfusion medicine. Implemented on a mass scale, this approach would facilitate access to donors with a known, extended phenotype, and donors with rare and atypical phenotypes. Establishing a digital database of results of NGS blood group testing is of the utmost importance. This sort of professional registry of both donors and recipients should be linked to open access serological data for national blood services. Before applying NGS technology to routine blood group diagnostics, there are some challenges to be faced.[85,86] One of them is the analysis of NGS data which involves determination of the human reference sequences, validation and standardization of algorithms for interpretation and classification of genetic variants with their haplotype localization and for translation of final NGS results to the predicted phenotype. The current accuracy of NGS of blood group antigens is about 95% and should be validated in a large group after the development of a final study protocol together with an accessible program comparing with a reference sequence database. This step depends on data of sequenced individuals with paired serological data collected in a large-scale database. The economic aspect related to the implementation of NGS in routine diagnostics of blood donors as well

as targeted groups of patients remains an open issue. The technology is undoubtedly cheaper but still too expensive. At the level of NGS laboratories, the preparation of CE-IVD marked products and external quality control programs are a challenge as well as the logistics of NGS testing in a centralized and cost-effective way.[87] Another challenge are the huge amounts of NGS data that have to be collected, protected and used for other clinical purposes. Finally, the ethics related to NGS need to be adapted and supplemented with informed consent from both donors and patients. According to a round table discussion at the AABB Meeting, NGS typing of blood donors requires proper ethical guidelines concerning incidental genetic findings such as pathology or clinical phenotype known to be associated with the detected variations.[88,89]

Disclosure

The authors report no conflicts of interest in this work.

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