Peer Review File

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Reviewer Comments

Reviewer A

This is a well-written review article summarizing major challenges and limitations of low and medium resolution genotyping. Below are minor comments for author's consideration:

COMMENT 1: Page 3, line 49-55(Methods). I suggest moving "Methods" after "key questions to be addressed" and adding a reference to Table 1.

REPLY 1: Thank you. Both have been done (see Page 4).

COMMENT 2: Page 9, Table 3. As per the ISBT nomenclature, RHCE*01.16 allele is also known as RHCE*ce.16. Please consider adding RHCE*ce.16 to the table under allele alias where it is blank.

REPLY 2: Thank you, the alias has been added.

COMMENT 3: Page 12. Table 5. FY and ACKR1 are both used as gene name. Since FY was used in the text, I suggest using FY in the table instead of ACKR1 for consistency.

REPLY 3: Thank you. FY was added to the table name so that both the official name (ACKR1) and alias (FY) are provided.

COMMENT 4: Page 14, line 255. It seems that the appropriate table to reference is Table 3 and 4 instead of Table 1.

REPLY 4: Thank you for identifying this error; it has been corrected.

Reviewer B

This manuscript describes challenges with assigning RH alleles and predicting phenotypes. The manuscript is very interesting and well-written. The tables and figures are good and informative. The manuscript offers comprehensive information for readers.

Some minor remarks:

COMMENT 1: - the conflict of interest form is for a different, older manuscript
REPLY 1: Thank you for identifying this error; it has been corrected

COMMENT 2: - gene names should be in italics.
REPLY 2: Thank you. Italics have been added as needed.

COMMENT 3: - a reference is missing, line 101
REPLY 3: Thank you for identifying this error; the omission has been corrected

COMMENT 4: - references are not given with the recommended reference style
REPLY 4: Thank you for pointing this out; references have been revised as needed.

COMMENT 5: - Table 4 legend: phenotypes that differ in italics; there is nothing in italics.
REPLY 5: Thank you for pointing out the difficulty in seeing the italics. It has been changed to bold.

COMMENT 6: - line 255, (see Table 1), should be Table 3?
REPLY 6: Thank you for identifying this error; it has been corrected.

COMMENT 7: - line 295, the reference 28 Halls is discordant to the references list where 28 is Justin et al
REPLY 7: Thank you for identifying this error; the last name of the first author has been corrected (i.e., Justin BL Halls)

COMMENT 8: - the narrative review checklist: page and line numbers do not match with the manuscript, but all the topics are covered, ok
REPLY 8: Thank you for identifying this error; a correct narrative review checklist is now included.

Reviewer C
General comments:

COMMENT 1: No discussion is given of the "pros" in the "pros and cons" of commercial systems. They are simpler to use than high-resolution tests and can resolve many patient problems to a sufficient degree. It could be acknowledged that least one manufacturer makes an RHD kit that focuses almost solely on weak D types 1, 2 and 3, under the logic that anything else (setting aside type 4.1) would be managed as D-negative anyway.
REPLY 1: Thank you for this comment; pro has been added to discussion, starting on page 15 line 287.

Specific comments:

COMMENT 2: Table 3 and 4. Table 3 and 4 columns 1 and 2 could be consolidated by moving the aliases to column 1 underneath the allele names (since there are at least 2 lines in each row anyway). Then the third column in Table 3 could be used to list the phenotypes for each allele, as in Table 4.
REPLY 2: Thank you for this suggestion; revisions have been made.

COMMENT 3: Lines 171-178: The terminology of Tiers should be explained.
REPLY 3: Thank you for identifying this omission; detail has been added on page 11 as well as an additional reference.

COMMENT 4: Line 189: reference for MAF.
REPLY 4: Thank you for identifying this omission; the reference has been added.

COMMENT 5: Line 212: Consider deleting "in Papua New Guinea" since it has been seen since then in other locations (Brazil, Sudan).
REPLY 5: Thank you, the sentence has been modified such that it is not the only group in which the allele has been reported.

Reviewer D

In this review, Keller MA addresses the challenges encountered when using commercially available genotyping kit to predict phenotype for the Duffy, but especially the Rh system. This review highlights the strength of the genotyping kits, but also the pitfall and limitation encountered when using those kits. This review will be especially useful for users that are either looking for a genotyping solution or that are new or less familiar RBC genotyping. Advanced user will also benefits from the different examples cited by the author.

The following comments are to be addressed prior to publication:

COMMENT 1: Page 3 Line 53. The allele tables tend to change every 1-2 years, as new alleles and phenotype are added and/or modified, especially for the RH system. The version number of alleles tables and the date on which it was they were consulted should be added.
REPLY 1: Thank you for this suggestion; the allele table versions and dates accessed have been added to Table 1.

COMMENT 2: Page 6 Line 101. The reference is missing.
REPLY 2: Thank you for identifying this error; the omission has been corrected

COMMENT 3: Page 8 Line 155-157. Does the author suggest that for patient with an anti-D, a predict D+ result should always be questioned and taken with caution? Should this approach also be taken for patient needing frequent transfusion or at higher risk of alloimmunization, such as patients with SCD.
REPLY 3: Thank you for pointing out this use case. It has been added to page 11.

COMMENT 4: be explained, as the impact of a tier 1 vs 2 vs 3 is not clear.
REPLY 4: Thank you for identifying this omission; detail has been added on page 11 as well as an additional reference.

COMMENT 5: Page 11 Line 188. The absence of the high prevalence antigen CEAG should also be added.
REPLY 5: If the patient were homozygous for the *RHCE*ce01.06.01* allele the predicted phenotype would be RH:-59 (CEAG-) but in the case described, the patient is heterozygous and therefore they are not predicted the lack the high prevalence antigen CEAG.

COMMENT 6: Page 11-12 Line 195-200. This a very important and pertinent. Does the author suggest that for patients with a RHCE*01.20.03 genotype, a D+ typing should be questioned, and further genetic analysis should always be done? Should a partial D always be suspected?
REPLY 6: Thank you for suggesting this be clarified. Additional information has been added.

COMMENT 7: Page 14 Line 255. The parenthesis should refer to table 3, not 1.聽
REPLY 7: Thank you for identifying this error; it has been corrected.

COMMENT 8: Page 12 Line 265-273. This phasing method using cDNA sequencing and cloning is both clever and very useful. If available, the authors should cite article or reports using this method, as it might be useful for other reference laboratories.
REPLY 8: Thank you for suggesting this. A recent reference has been added.

COMMENT 9: Table 4. The phenotype in italic do not show. There's no text in italic in the table.
REPLY 9: Thank you for pointing out the difficulty in seeing the italics. It has been changed to bold.

COMMENT 10: Table 6. For the RHCE*01.01/RHCE*01.20.01, it is mentioned "some reports of anti-e-like (possible anti-e var). Reference of those reports should be added, if available, as patients with the RHCE*01.01 are usually considered at low risk of alloimmunization.
REPLY 10: Thank you for identifying this omission; reference added.