Peer Review File
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The study proposed a review about serological analysis of Rh antigens, which is quite important, since Rh antigens are highly immunogenic and are involved in transfusion reactions. Some considerations are pointed to improve the review. It is necessary to explore the introduction topic emphasizing the controversies surrounding the Rh blood group system, the objective of the review needs to be clear in the text and some points of the conclusion should be improved. Follow some considerations:

Abstract

1- Please describe the purpose of the review
Included an additional paragraph in the abstract to give a summary of the purpose of this review: This review serves to highlight the complexities of the Rh blood group antigens and how serological analysis continues to play a crucial role in the typing and investigation of Rh antigens within the blood donor and clinical setting.

2- Line 13 – I suggest to complete the information ¨among systems¨ add the number of blood group systems described so far.
Amended the first line to "The Rh blood group remains the most clinically significant blood group system, next to the ABO system and it is one of the most complex among the 45 currently recognised blood group systems."

3- Line 24 – Please describe gene names all caps and italicized.
Gene names are capitalized and italicised

Introduction

4- Please, nominate the first three paragraphs after abstract (lines 32 to 54) as introduction topic
Paragraph header 'Introduction' has been included as advised

5- The introduction topic should be further explored, follow some suggestions:

- First, I suggest an introductory sentence in the beginning of the introduction.

Added the following paragraph in the introduction.
"The antigens that make-up the Rh blood group system, in particular the D, C, c, E and e antigens are highly immunogenic and can be the source of immunisation and subsequent haemolytic transfusion reactions (HTR) when antigen positive red cells are transfused to a corresponding antigen negative subject. It is also a common contributor to haemolytic disease of foetus and newborn (HDFN)."

- In the first two paragraphs, when mentioning the observations of Landsteiner and Wiener and Levine and Stetson, add the year or decade in which the facts occurred
  1940 has been included in the text as the year the first paper on Rh was published.

- Explore further the ¨controversies¨ surrounding the Rh blood group system
  Added the following paragraph to expand on some of the controversies which are included in the references.
  "While the Fisher- Race terminology was based on the proposal that three closely linked genes C/c, E/e and D conferred the different Rh phenotypes, the Wiener nomenclature took the opposing view that a single gene encoded the different rhesus blood group factors. Both the theories were however found to be incorrect, and it was the proposal by Tippett that the various Rh antigens can be explained by the interaction of just two genes, RHD and RHCE that was subsequently proven to be correct."
- Several nomenclatures have been used to describe antigens, proteins, and genes in the Rh system. Describe the nomenclature recommended by the ISBT, and also the nomenclature used in the article.
  The following paragraph has been included in the text. I think the scope of this review is outside the discussion of specific nomenclature, and therefore I have elected to direct the reader to the specific documents on the ISBT website.
  The nomenclature used in this review article follows the general guidelines for naming of blood group alleles (https://www.isbtweb.org/resource/isbt-guidelines-naming-blood-group-alleles.html) and for the naming of RH alleles (https://www.isbtweb.org/resource/guidelines-naming-rh-alleles--pdf.html) as outlined by the International Society of Blood Transfusion (ISBT).

Antigenic expression and molecular background of Rh antigens

6- I suggest reformulating the subtitle to become more concise: ¨Molecular basis of Rh antigens¨
Subtitle has been amended as suggested.

7- Line 59: Please, describe about exons of the RHD and RHCE genes and their homology
Added the following line to the first paragraph.
"Both genes encode 417 amino acids and show close homology. They occur in inverted orientations, contain 10 exons each and are 97% identical."

D-negative

9- Line 75: please describe the region of the gene where the insertion of a 37-bp insertion
Included the position of the insertion by amending the sentence to:
"Among those of African descent, inactivation of RHD commonly results from a 37-bp internal duplication in exon-4 leading to a premature stop codon at position 210."

10-Line 76: Please, alter the sentence "…occurs from null expression of the D antigen…" by "occurs due to the absence of D antigen"
Sentence has been amended as suggested.

D and CE variants

11- I suggest starting this subject with the last paragraph (lines 100-107) and add the concept of D mosaic

Subtitle has been moved as suggested.

12- Line 96- please, describe other weak D variants besides Del and also describe CE variants

I did not describe in detail on D mosaics, weak D variants and Del as I believe there are articles in this review series that have exhaustively described these variants. I believe it would be a duplication of information and is outside the scope of this article which is focused more on serology rather than molecular details.

Monoclonals antibodies

13 - I suggest describing about the evolution of monoclonal antibodies

Added an additional paragraph on phage display and an additional reference on the history of monoclonal antibody development.

"Recognising the inherent limitations in human B-cell immortalisation and low EBV transformational efficiencies, more advanced techniques such as phage display have been developed to produced monoclonal antibodies to specific epitopes of Rh antigens that previously could not be achieved. "

14- When using monoclonal anti-D, it is important to use RBCs that have been stored appropriately. Please add this information in the paragraph

Information added as suggested.

"Rh proteins are conformation-dependant and are influenced by other proteins and lipids that are present on the red cell membrane. When defining D epitopes using monoclonal antibodies, it is therefore important that the testing is performed at the correct pH, temperature, ionic strength, and antibody concentration as well as to always use red cells that have been stored correctly."

Rh typing of blood donors

16- Line 182 – Accurately DEL identifying is particularly important in Asians. Please, describe frequency in Asian donors

Added the following line into the paragraph.

"It is reported that 23.3% of Chinese of Han ethnicity and 10% of Thai individuals who type as RhD negative are of Del phenotype."

Resolving Rh typing discrepancies

17- Lines 322, 323, 324 - ¨different reactivity patterns, serological testing using tubes and column agglutination technology¨. It is important to clarify which methodology was more efficient (Tube or Column?),

Included an additional sentence stating that:

"Tube tests generally have a higher degree of subjectivity due to the many technical variables in its performance as compared to column agglutination technology."

Issues and pitfalls in serotyping

18- Line 345: Please, replace the word ¨null¨ by “negative”

Change has been made as suggested.

19- Line 347: Please describe gene names in capital letters and in italics

Gene names have been italicized.

Conclusion

20- I suggest in conclusion the importance of Rh phenotypic diversity and which Rh variants can impact routine tests.

Have amended the paragraph to refer to Rh phenotypic diversity. I have however not outlined the Rh variants that can pose a problem in routine testing, as I think this would have been covered in the main text.

“Serological typing of Rh antigens in routine donor and clinical settings is well entrenched and will remain so for many decades to come. The technique is reliable and cost-effective, simple, inexpensive and easily interpreted. It is easily accessible even in regions with limited resources. However, the huge diversity of Rh antigens, and the myriad rearrangements between the RHD and RHCE genes producing the Rh variants, may pose a problem with interpretation of serological typing results. Unusual Rh antigens and variants oftentimes can yield misleading and inconclusive results.”