

Peer Review File

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Review comments

Reviewer A

1. More statistical processing of the materials is needed. For example X2 analyses.
2. It will be better to analyze the combined phenotypes of ABO and Rh factor. For example O, Rh+; O, Rh-; A, Rh+; A, Rh-; B, Rh+, B, Rh-. AB, Rh+, AB, Rh-.
3. It must be analyze that associated with gender. Especially Rh- donors are females or males.
4. It will be very valuable to calculate the immosensibilization index Based on the materials for the region.

Authors' responses

We acknowledge there might be a need to have statistical analysis but the primary aim of the present study was to report an updated ABO and RhD prevalence among Hong Kong without any intention to compare with historical data or that from other countries. The outcome of the present study is to inform both clinicians and BTS on the actions to follow in managing blood supply and demand. Nevertheless, we regroup the ABO RhD in table 2 as advised in the point 2 and 3 above and also by other reviewers.

Reviewer B

It is a well-structured manuscript about the frequency of ABO and RhD antigens in Hong Kong residents, including Chinese and individuals from other nationalities. The study is retrospective and collects results from 167,091 blood donors. The manuscript is clear and easy to be read. Some suggestions are made to enrich it.

1. Abstract pag 3 line 42: As the frequency of Rh-negative individuals in Caucasians differs greatly from the rest of the ethnic groups, I suggest that this data is highlighted in the abstract.

Authors' responses:

Revised accordingly

2. Page 4 line 52: The beginning of first paragraph of introduction could be improved, with an introductory phrase about transfusion and blood group systems.

Authors' responses:

Revised accordingly

3. Pag 4 line 57: Please write Rhesus with capital R. And put blood groups in the plural.

Authors' responses:

Revised accordingly

4. Pag 4 line 69: The aim of the study is to determine the current frequency of ABO and Rh antigens in Hong Kong blood donors. But it is not clear when the last search was made, and no

references with that information were cited. Is it the first study reporting this data?

Authors' responses:

Historical data was in service statistics and the text was updated

5. Pag 6 line 88: Tube method was used when needed. Please, clarify the situations in which tube method was needed.

Authors' responses:

Revised accordingly

6. Pag 7 line 101-102: As no statistical test was applied, I suggest that the authors do not claim that there was no gender difference in Chinese, Caucasian and Indians. As suggestion: "While Chinese, Caucasian and Indian donors showed similar frequency between genders, female...."

Authors' responses:

Revised accordingly

7. Figure 1. The letters and numbers in the figure are too small

Authors' responses:

Revised accordingly

8. The percentage of D-negative in Chineses is only 1.3%. Several studies report the high prevalence of DEL in this population. Could a part of D-negative individuals be DEL? Has any DEL investigation been carried out? This approach could be discussed.

Authors' responses:

Revised accordingly

9. Page 9 lines 132-134: The phrase "However, an even higher..." is unclear. Do you mean that some studies have shown a higher frequency of A blood group? What do the percentages 43% vs 44% mean?

Authors' responses:

Revised accordingly

10. Page 10 lines 146-149: The phrase "It is because although..." is repeated in the middle of another phrase.

Authors' responses:

Revised accordingly

11. Discussion: I miss a comparison of ABO/Rh frequency of donors and patients. A retrospective survey of blood transfusion to compare ABO/Rh prevalence between blood donors and patients can enrich the study and show whether a specific-ABO type should be recruited.

Authors' responses:

We acknowledge this would be useful. However, as patients data is not available at the BTS nor published, we are unable to compare.

Reviewer C

This manuscript describes the prevalence of ABO RhD types found within blood donors in Hong Kong. Changes between Chinese heritage and non-Chinese individuals is found. This data is valuable to report as with the changing globalisation of the population understanding blood group prevalence allows health services to plan for blood supply and diversify inventories appropriately.

Comments to address are listed below

Generally the manuscript could benefit from rewording by a native English speaker to improve the overall flow especially the discussion section.

Authors' responses:

We have reviewed and revised

I have suggested that with the limited data here that a letter type article may be more suitable.
line 36 change to 'conventional'

Authors' responses:

Revised accordingly

line 38 remove 'of'

Authors' responses:

Revised accordingly

line 42 revise percent values

Authors' responses:

Revised accordingly

line 54 there has been a very recent change to recognised blood group system number so please check again and correct as needed

Authors' responses:

Revised accordingly

line 55 i would say 'some' rather than 'many' as there are a number of reasons for RBC antibody formation

Authors' responses:

Revised accordingly

line 57 - it is not common to refer to the Rh blood group as rhesus anymore

Authors' responses:

Revised accordingly

Please state how you know the donors background - is it asked as part of the donor enrolment process or a demographic questionnaire?

Authors' responses:

It is asked during donor questionnaire and checked again for this study.

Revised accordingly

line 83 i assume you mean that only if there were only more than 100 blood donors were in each category were they used for this analysis?

Authors' responses:

Yes, for those less than 100 donors over the study period, we excluded from analysis. Revised accordingly

i think figure 1 and table 2 are redundant so perhaps keep figure 1 as it is but make table 2 more detailed with the breakdown of O-, O+, A+, A-, B+, B-, AB+ and AB- for each set of donors.

Authors' responses:

Table 2 was updated accordingly

line 125 what do you think makes Hong Kong chinese different to other studies conducted in Chinese individuals? Is there an ethnic subset more likely to reside in Hong Kong?

Authors' responses:

We mean the currently resided population that came for blood donation in Hong Kong. For local Chinese, most are originated from Southern part of China. However, the detailed ethnic subsets were not assessed per BTS questionnaire for blood donation nor for the present study. Nevertheless, we acknowledge this limitation but unless prospective study or detailed requirement are set for this study, it is impossible to get hold of the information. For Filipino and Indonesian, most are coming forward for a period of 2 years or its multiple as domestic helpers.

I am confused by meaning of line 132

Authors' responses:

Revised accordingly

line 146 is not well written and seems to be a repeat of lines 143-145?

Authors' responses:

Revised accordingly

line 150 - 151 not a finished sentence

Authors' responses:

Revised accordingly

line 158 - why italics?

Authors' responses:

Revised accordingly

Can you provide any sense on whether the blood donor population in Hong Kong matches the

general population?

Authors' responses:

We believe the finding of the present study would be close to that of general population. However, without detailed analysis with the population statistics, it is difficult to conclude.

Reviewer D

The study proposed to determine the phenotypic frequency of ABO and RhD Blood groups in Hong Kong blood donors, the objective of the study was achieved, however, there are some minor points which are important to review. It is necessary to explore the introduction topic and bring more information about the importance of blood transfusion therapy and the ABO and RhD Blood Group System, as well as explain the risk of alloimmunization. Furthermore, it would be interesting to carry out the retrospective survey of transfusion performed by the Blood Transfusion Service and to compare the ABO and RhD typing with the ABO/RhD frequency of the donors. This survey could enrich the article. Additionally, the references must comply with the rules of the journal. Please check the rules.

Some suggestions:

Abstract

1-Please rewrite the background according to the introduction.

Authors' responses:

Revised accordingly

Objective

2- In the objectives section you say "the latest distribution of ABO", Are there other similar articles within the Hong Kong population? If so, it is important to discuss and make a comparison of the results found. If not, please remove the word "latest".

Authors' responses:

There were no published paper on this topic but the BTS has all along used the operation data on ABO Rh D distribution on all the blood units collected as reference. Therefore, this paper is the first to describe what we have seen in our blood donors.

Revised accordingly

3- Please, change "among residents in Hong Kong" to "among Hong Kong blood donors"

Authors' responses:

Revised accordingly

Introduction

4-The introduction topic should be further explored. Some suggestions:

• Pag 4: Line 52/53: In the first paragraph describe the importance of blood transfusion therapy.

Authors' responses:

Revised accordingly

- Pag 4: Line 57: Further detail the ABO and Rh blood group systems, the antigens which comprise the systems, the risk of incompatible transfusion and consequently the risk of alloimmunization, in the summary describe the importance of ABO and RhD compatibility in transfusion medicine.

Authors' responses:

Revised accordingly

- Pag 4: Line 66 -69 Move the “Locally, population...of the local population” to line 63 after the work mobility.

Authors' responses:

Revised accordingly

Methods

5-Page 6 line 89: please, describe the monoclonal antibodies and manufacturers used for ABO and RhD phenotyping.

Authors' responses:

As the study covered samples or tests for 10 years, therefore, a number of manufacturers were used. After review, we suggest not to include this in the manuscript. However, details could be retrieved under our quality management system.

Results

6- The DEL phenotype, which appears RhD-negative on routine blood group serology even though it is RhD-positive, is highly prevalent in the Chinese population. Several studies support this information. Has any DEL investigation been carried out? It is important to discuss this.

Authors' responses:

Revised accordingly

7-Figure 1. The letters and numbers in the figure are too small

Authors' responses:

Revised accordingly

Discussion

8- Page 10 line 146-151 Please, check this paragraph as it is duplicated.

Authors' responses:

Revised accordingly

References

9-Please, check the rules, the reference should be referenced according to the Vancouver reference style. In the text, references should be identified using numbers in round brackets. If a report has more than three authors, the first three authors should be listed followed by “et al.”

Authors' responses:

Revised accordingly

Reviewer E

Comments to the Author

This manuscript determined the phenotypic distribution of ABO, Rhesus D (RhD) blood group antigens among different ethnic groups residing in Hong Kong. Chinese and non-Chinese (Caucasians, Indians, Indonesians and Philippines) donors were included for analysis. This is an interesting study that provides update on the frequency of blood groups antigens, however some points described below need improvement.

Major points:

1) The author does not describe the criteria to define the non-Chinese donors. If they are immigrants or if they are descendent from another ethnic group. The authors should better explain the reason to choice NCD with number of blood donors more than 100, we believe that is the most representative NCD population residing in Hong Kong.

Authors' responses:

Revised accordingly

2) Other important information is related to the exclusion of repeat donors. Did the authors certify that donors were considered for analysis only once?

Authors' responses:

Only the first donation entry was retrieved

3) In the methods section it is not described whether the presence of blood subgroups for ABO or weak reaction for RhD was observed. How was the evaluation score defined for positive or negative reaction? Did the authors observe samples with undetermined status? How was the reaction with uncertain agglutination reaction confirmed?

Authors' responses:

As described, it was a retrospective analysis and ABO blood group has been confirmed at the donation testing time.

4) The frequency of ABO subgroups and discrepant or weak reactions to RhD are relevant information that should be considered.

Authors' responses:

As replied to point 3, the results were already concluded in the system.

5) To become the data consistent is necessary to apply a statistical analysis.

Authors' responses:

As described above, we considered that statistics were not required in the frequency distribution report in this study.

6) A more detailed description of the number of donors evaluated per year could be provided to understand the impact of population movement on the phenotypic frequency of blood group antigens. This information is also important to know if the number of donors is kept constant over the years.

Authors' responses:

After review, we considered the number of new donors annually not to include in this manuscript. However, we would like to highlight that the number of new donors were dropping as in many developed countries. Below is a table

	New donors	Previous donors
2011	46,311	123,622
2012	43,033	129,773
2013	41,249	130,229
2014	38,174	133,047
2015	36,182	137,916
2016	35,850	136,960
2017	32,800	129,974
2018	29,549	120,743
2019	30,037	114,489
2020	18,983	92,638

7) The manuscript requires major review of the English grammar.

Authors' responses:

We have reviewed and revised.

8) The authors should better explain if the donors with interest blood group phenotype for blood transfusion are included in a digital platform to be readily available to donate when requested.

Authors' responses:

For those donors with blood groups of special interest e.g. rare phenotype, we invited them for a test and obtained their consent to be available a rare blood group phenotype. However, our objectives of this study is to report on the ABO and Rh (D) distribution in the population we served. The information is useful for the daily operation in managing and matching blood supply to demand. Therefore, we did not have an intention nor have retrieved detailed information for this purpose.

Minor points:

A) The words and numbers present in Figure 1 should be larger to facilitate the reading and understanding of the manuscript.

Authors' responses:

Revised accordingly

B) A comparative table containing previous studies on the prevalence of ABO and RHD blood

groups could be included in the manuscript.

Authors' responses:

We have only service provision data on the blood group distribution of all the donation received and therefore, we do not intend to put up such a table.

C) In the results section, Line 97 "Of these, there were 78,100 men" the number of donors could be represented as a percentage.

Authors' responses:

Revised accordingly

D) In the results section, Line 99 "93,093 (56.7%) were minors", the beginning of the paragraph could be modified to read "The majority (56.7%) were"

Authors' responses:

Revised accordingly

E) The acronym BTS was not described.

Authors' responses:

Revised accordingly

F) In the Discussion section, the sentence (Line 146-149) "So those RhDIt..." is a repetitive sentence.

Authors' responses:

Revised accordingly

G) A statistical analysis containing the p value should be added in the manuscript.

Authors' responses:

See the responses to Reviewer A.

Reviewer F

General

The study was of interest as it was conducted over a 10 year period and it gave a nice overview of frequencies of ABO and Rh blood groups among blood donors at one center in Hong Kong. While the concept of a very low prevalence of Rh negative individuals in the Chinese population is not novel, the manuscript would benefit from the blood center authors stating specifically what was done with donor recruitment to ensure the number of ABO and Rh compatible donors were sufficient to meet the transfusion needs of the population. As the manuscript discusses the movement of the population a table showing the yearly changes in ABO and Rh from 2011 through 2022 would benefit the manuscript as this would show the reader if the blood groups had changed with changes in demographics of the donor population as the manuscript discusses. Please review the manuscript for English grammar as there were some concerns throughout the manuscript.

Specific

Abstract

(Page 3, line 42)- change 968% to 96.8%.

Authors' responses:

Revised accordingly

Methods

(Page 6, line 88) ...microplate technique and conventional tube method, if needed- please clarify under what circumstances the conventional tube test would be needed and if available, how many (number and percent) of the ABO and Rh results required tube testing.

Authors' responses:

Revised accordingly

As table 1 shows donors <20 and > 60 years of age, please add the minimum and maximum age of donors allowed at this blood center in the methods section.

Authors' responses:

The eligible age for blood donation in Hong Kong is from 16 to 65 for first time donation.

Maximal eligible age for donation for previous or repeated donors is up to 76.

Results

(Page 7, lines 105-109)- It is stated: Locally, the most commonly observed ABO blood group was group O ranging from 35.4% to 45.0% with 42.8 and 45.0% in Chinese and Caucasian donors respectively. It was followed by group A with a range of 23.4% to 34.1%; group B from 15.6% to 30.7% and lastly AB from 5.3% to 7.1%. As this section is covered nicely in table 2, consider removing from the manuscript.

Authors' responses:

Revised accordingly

(Page 7, lines 112-113)- It is stated- being 98.7% in Chinese, 96.8% in Indonesian and 93.6% for those come from Philippines. As this section is covered nicely in the table 2, consider removing from the manuscript.

Authors' responses:

Table 2 was revised

Discussion

(Page 11, lines 163-167) – It is stated- Last but not the least, with more population migration and changes, the BTS should continue to work the prevalence of other blood groups, e.g. the prevalence of CcEe antigens in the population and patients so that appropriate strategies be implemented to ensure the availability of suitably matched blood for clinical transfusion therapy. This section appears out of place as it does not address ABO and Rh, suggest to remove from the manuscript.

References

References 12 and 13 are identical, please remove reference 13 and renumber in the manuscript.

Authors' responses:
Revised accordingly

Reviewer G

The manuscript by Ng et al, entitled "Frequencies of ABO and RhD blood groups among blood donors in Hong Kong" describes the current distribution of ABO and RhD blood groups among residents in Hong Kong. The intent of the manuscript is to inform blood transfusion services of the changing population of Hong Kong due to recent population drift. The information is informative to readers and transfusion services within Hong Kong and could encourage other populations that were at one time homogenous to reevaluate their ABO and RhD blood group distributions as areas become intermixed and diverse. The purpose of the assessment is to ensure blood transfusion services have the ability to supply the varied population with appropriate blood for transfusion.

There are several major edits required for the manuscript, see below;

1. All references to Philippines should be changed to Filipino which refers to the people identified with the country the Philippines, including tables and figures.

Authors' responses:
Revised accordingly

2. All references to Caucasian should be changed to White (or to a more accurate term such as European), including tables and figures. Caucasian is an obsolete racial classification.

Authors' responses:
Revised accordingly

3. Introduction, Page 4, Strongly suggest the author mention why they are interested in studying the frequency of RhD, i.e. provide RhD negative blood to RhD negative women of child-bearing age to prevent alloimmunization to the D antigen which could result in hemolytic disease of the fetus and newborn.

Authors' responses:
Revised accordingly

4. Materials and Methods, Page 6, describe how the ethnicities were determined. Did each donor self-select an ethnicity during the donor history questionnaire?

Authors' responses:
It is asked during donor questionnaire and checked again for this study.
Revised accordingly

5. Materials and Methods, Page 6, under what circumstances was the conventional tube method performed? Can you provide a reference to the conventional tube method (i.e. Technical Method protocol)?

It is asked during donor questionnaire and checked again for this study.
Revised accordingly

6. Results, Page 7, Remove speculation about the predominance of female donors as locally domestic helpers. This statement could be viewed as disrespectful as does not add value to the manuscript without substantiating evidence.

Authors' responses:

Revised accordingly

7. Discussion, Page 9, line 127. Incomplete sentence, needs to be corrected. With at least 18.5% of the world population being Chinese...

Authors' responses:

Revised accordingly

8. Page 10, lines 143-149, correct the repeating statements in reference to RhD

Authors' responses:

Revised accordingly

9. Discussion section; recommend the authors include a statement about the ability to provide group O blood across all blood groups (O RBC compatibility) despite the changing population.

Authors' responses:

Revised accordingly

10. Capitalize all references to the Table and Figure within the manuscript.

There are several grammatical and minor edits required, see below;

1. Page 2, line 36, change convectional to conventional

Authors' responses:

Revised accordingly

2. Page 2, line 40, add the word "groups" before A and B vary

Authors' responses:

Revised accordingly

3. Page 3, line 42, correct percentage, 968%

Authors' responses:

Revised accordingly

4. Page 4, line 56, change showed to shown

Authors' responses:

Revised accordingly

5. Page 4, line 57, capitalize Rhesus

Authors' responses:

Revised accordingly

6. Page 4, line 66, change "has been getting more frequent" to "has increased"
Authors' responses:
Revised accordingly
7. Page 7, line 100, remove "on the other hand" this transitional phrase is not needed.
Authors' responses:
Revised accordingly
8. Page 7, line 113, change the "chance of having" to "number of".
Authors' responses:
Revised accordingly
9. Page 9, line 132, change "closing to" to "closer".
Authors' responses:
Revised accordingly
10. Page 9, line 136, remove the word among
Authors' responses:
Revised accordingly
11. Page 9, line 137, write out HK for Hong Kong so that is referred to consistently within the manuscript.
Authors' responses:
Revised accordingly
12. Page 10, line 144, select another term to refer to the local people, (i.e. local population)
Authors' responses:
Revised accordingly
13. Page 10, line 154, use BTS
Authors' responses:
Revised accordingly
14. Page 10, line 158, remove italics from RhD
Authors' responses:
Revised accordingly
15. Page 11, line 161, use NCD
Authors' responses:
Revised accordingly

Further Review Comments

Reviewer A

I still feel some context about the general HK population is needed to put this data into context. Do more non-Chinese go for blood donation because it is more normal from their cultural background and therefore there is a skewed bias resulting in more RhD-percentage? - you reference population migration data so match it up to your dataset here and explain the proportions of each type of background you are reporting on.
introduction sentence 1 - need reference

Authors' responses

Sentences rewrote to clarify reviewer's concern. As Hong Kong is an international city with many travelers and foreign people studying and working in Hong Kong, reliable information would be available from the government census and statistics who do population survey in every 5 and 10 years. Therefore, authors rewrote the sentences to make it clear there are population changes.

ISBT web reference - needs to be annotated as per journal requirements in the reference section

Authors' responses

Updated

reference needed for statement about RhD blood cell inducing anti-D

Authors' responses

Updated with references added

page 4 last sentence on migration - move reference into reference section as per journal requirement.

Authors' responses

Updated

method

sentence 2 - should be '10-year' not '10-years'

Authors' responses

Updated

164,095 is the whole dataset and 2,996 of them are new donors? but then you say no previous donors were included....what does this mean? I gather what you mean is that data from the first donation of 164,095 are Chinese donors and 2,996 non Chinese donors for a total of 167,091 donors was analysed. Blood group results from follow up

donations of those donors were not included.

Authors' responses

Updated

heading on page 6 called 'methods' should be 'blood group method'

Authors' responses

Updated

with the mention of Del testing not being routine does this mean that you did not look for weak D donors. if so you can just say weak expressors were classified as RhD+. first line of results is repeat of methods

Authors' responses

Updated

please put data in on the age range allowed for blood donation in HK

Authors' responses

Updated

please replace 'commonest' with 'most common'

Authors' responses

Updated

liquid stock is not a common term please use something like 'appropriate blood units need to be available'

Authors' responses

Updated

what do you mean by liquid stock? what other stocks of blood do you have that are not liquid?

Authors' responses

It is referred to red cells units in 4 C fridge and rare blood are often stored as frozen red cells unless the donors could be available top donate. In our jargons it is, therefore often referred as liquid stock.

Updated

how often can donors donate whole blood in HK?

Authors' responses

In Hong Kong, male donors are allowed to donate blood once every 75 days and female in 105 days interval. As we only capture results of new donors, this information would not be applicable or affected our analysis

what is RhD italicised on page 10 in the last paragraph?

Authors' responses

Updated

Reviewer B

Methods

As Table 1 shows donors <20 and > 60 years of age, please add the minimum and maximum age of donors allowed at this blood center in the methods section.

Authors' responses

Updated

Please add the ages stated in the reply to the manuscript. This is relevant for readers from other countries where the donation ages vary.

Authors' responses

Updated

Results

- (Page 7, lines 105-109)- It is stated: Locally, the most commonly observed ABO blood group was group O ranging from 35.4% to 45.0%, with 42.8 and 45.0% in Chinese and Caucasian donors, respectively. It was followed by Group A with a range of 23.4% to 34.1%; Group B from 15.6% to 30.7%, and lastly AB from 5.3% to 7.1%. As this section is covered nicely in Table 2, consider removing it from the manuscript.

While it was stated that this was done in the reply, the text remains in the manuscript.

- (Page 7, lines 112-113)- It is stated- being 98.7% in Chinese, 96.8% in Indonesian, and 93.6% for those who come from the Philippines. As this section is covered nicely in table 2, consider removing it from the manuscript.

While it was stated that this was done in the reply, the text remains in the manuscript.

Authors' responses

We consider these sentences should be remained in the manuscript for better clarification.

Discussion

(Page 11, lines 163-167) – It is stated- Last but not least, with more population migration and changes, the BTS should continue to work the prevalence of other blood groups, e.g., the prevalence of CcEe antigens in the population and patients so that appropriate strategies be implemented to ensure the availability of suitably matched blood for clinical transfusion therapy. This section appears out of place as it does not address ABO and Rh, I suggest to remove from the manuscript.

This reviewer provided this recommendation in the original version and it was not addressed in the reply, please consider it.

Authors' responses

While you are correct that a more detailed analysis of the Rh blood group would be useful to address non Chinese patients, however, the present transfusion demand remains mostly at ABO and RhD compatible blood units requested according to the BTS internal data.

Figure 1

From my copy I am unable to tell if the letters and numbers in the figure were made larger. While I can see it on a large computer monitor, I will leave the decision to the journal editor if additional changes are needed prior to publication.

Authors' responses

Updated and also followed up on Editorial office' comments