Standardization of column agglutination (CAT) and solid phase red cell adherence (SPRCA) techniques against the conventional tube technique (CTT) for titration of naturally occurring antibodies (anti-A, anti-B) in group O individuals: a pilot study from a tertiary healthcare center

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Introduction

• In O blood group individuals anti-A and anti B antibodies belong to both IgM and IgG class.
• Though, the proportion of IgG is small but they have potential to cause intravascular hemolysis.
• There are reports of hemolysis of recipient’s red cells after transfusion of O group plasma to non-O group patients.
Aim

- Now a days in a tertiary healthcare center, isoagglutinins titration in ABO incompatible solid organ transplantation is a routine investigation.
- By considering the known logistic and objectivity of CAT and SPRCA, we aimed to establish/standardize CAT and SPRCA against CTT for titration reporting.
Materials and methods

• Prospective pilot study
• performed at a tertiary healthcare center in north India between August 2015 to July 2016 (one year)
• Isoagglutinins (anti A and anti B) titer was done in O blood group individuals. Both IgG and IgM titers were done.
• Manufacturer’s instructions and dept. SOP were followed for titration study.
Materials and methods

• IgG titration was done without DTT treatment.
• Dilution for CTT titration was done as per the AABB standards.
• CAT titration for IgG was done on semiautomatic Ortho BioVue using monospecific IgG cassettes and IgM was done using neutral cassettes (ortho clinical diagnostics, USA).
Materials and methods

- SPRCA titration was done using fully automatic NEO (Gamma Immucor, USA). IgM titer was done using hemagglutination method. 96 well microplates were used.
- IgG titer was done using 96 well Capture Select plates. Two different assays were used for low and high titration.
- Automated serial dilution was performed in red cell coated strip.
- The titer endpoint was the reciprocal of the highest dilution yielding 1+ reaction strength.
- The strength of reaction was measured from 1+ to 4+ following AABB technical manual and manufacturer’s instructions.
- CTT was considered gold standard for standardization of CAT and SPRCA for titration.
MATERIALS AND METHODS

• %age median coherence of end titer was calculated at 1+ reaction strength

And

• Considering higher sensitivity of CAT and SPRCA %age coherence was observed at higher strength of reaction against CTT
RESULTS

• There were a total of 570 O group individuals in which titration study was done.
• Majority were males (97%).
• CTT is more time consuming and cumbersome method than CAT and SPRCA (CTT=120 mins, SPRCA=60 mins and CAT=30 mins).
Isoagglutinins titer using three different methods considering 1+ reaction strength as end point titer (N=570)

<table>
<thead>
<tr>
<th>Titer</th>
<th>Anti -A</th>
<th>Anti-B</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgG</td>
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<tr>
<td></td>
<td>CTT</td>
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</tr>
<tr>
<td>&lt;128</td>
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<td>26%</td>
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<td>&gt;128</td>
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<td>25%</td>
<td>46%</td>
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Poor coherence observed

- Considering 1+ reaction as the endpoint titer we could observe a poor cumulative coherence of CAT and SPRCA against CTT
- Anti A+anti B IgG (CAT= 1%, SPRCA=10%).
But......

• **Good coherence was observed** when we chose 2+ reaction as the titer end point reaction in CAT and SPRCA (against the 1+ reaction strength in CTT)

• **Coherence improved significantly** (SPRCA= 88%, CAT=82%).
Considering 1+ reaction as the endpoint titer we could observe a poor coherence of CAT and SPRCA against CTT for both antiA and anti B IgG antibodies (CAT= 1%, SPRCA=10%). But when we chose 2+ reaction as the titer end point reaction in CAT and SPRCA (against the 1+ reaction strength in CTT) the coherence improved significantly for IgG (antiA+anti B).
In case of IgM, 1+ reaction strength showed good coherence with SPRCA method (75%) while CAT showed good coherence at 2+ reaction strength (83%).
CONCLUSION

• CAT and SPRCA are more sensitive than CTT
• CTT and SPRCA less time consuming with requirement of less technical expertise
• We found CAT and SPRCA as a very useful objective method for titration study with good confidence in reporting.
• Adjustment of reaction strength in CAT and SPRCA significantly improved the coherence of end point titer.
THANK YOU