

**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)

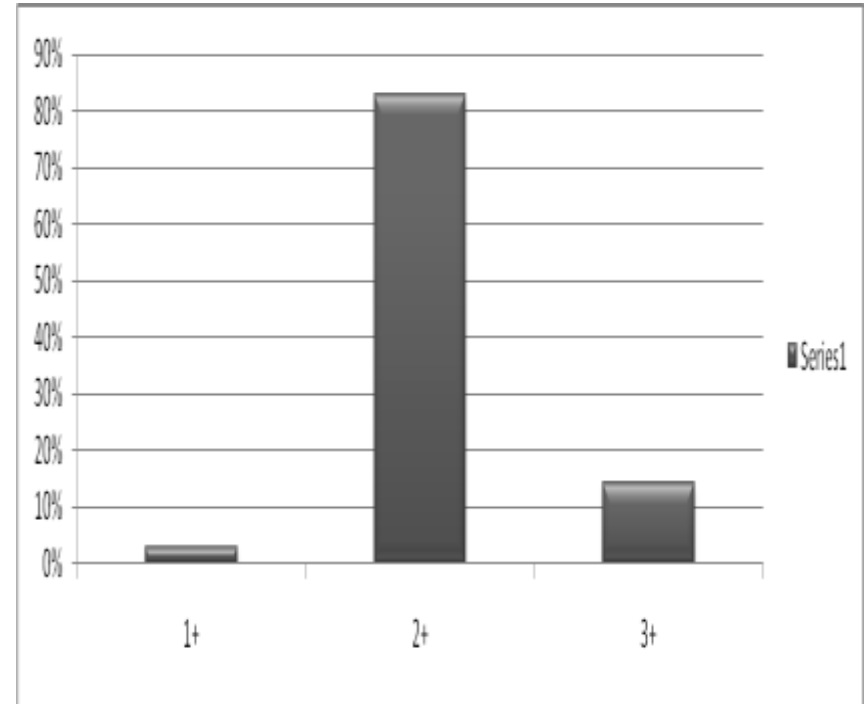
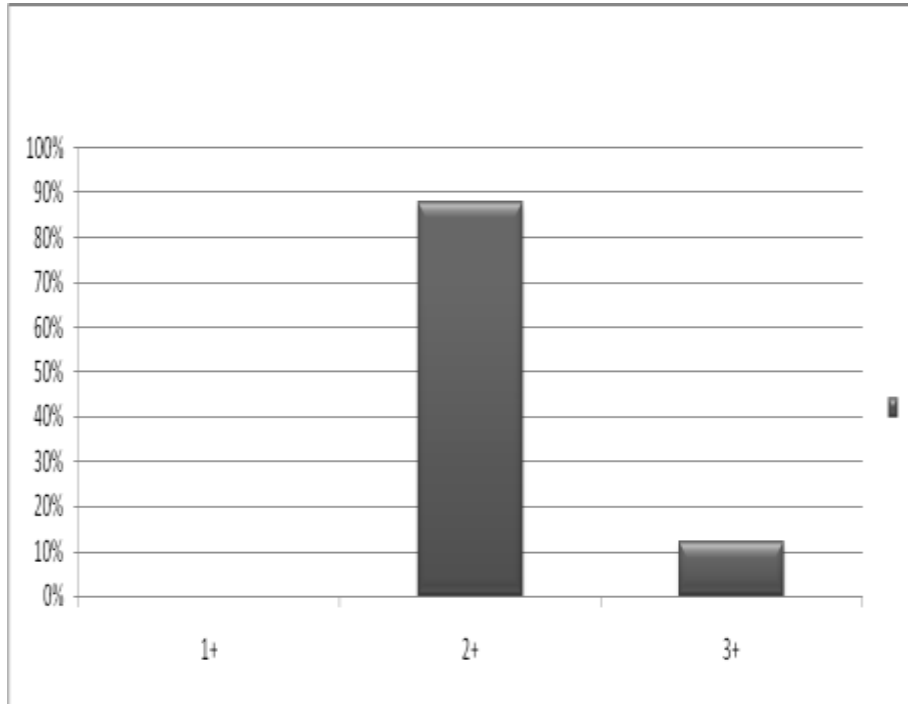
Titer	Anti -A			Anti-B		
	IgG			IgG		
	CTT	CAT	SPRCA	CTT	CAT	SPRCA
<128	50%	26%	30%	58%	21%	21%
128	33%	21%	20%	17%	33%	38%
>128	17%	53%	50%	25%	46%	41%

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%).

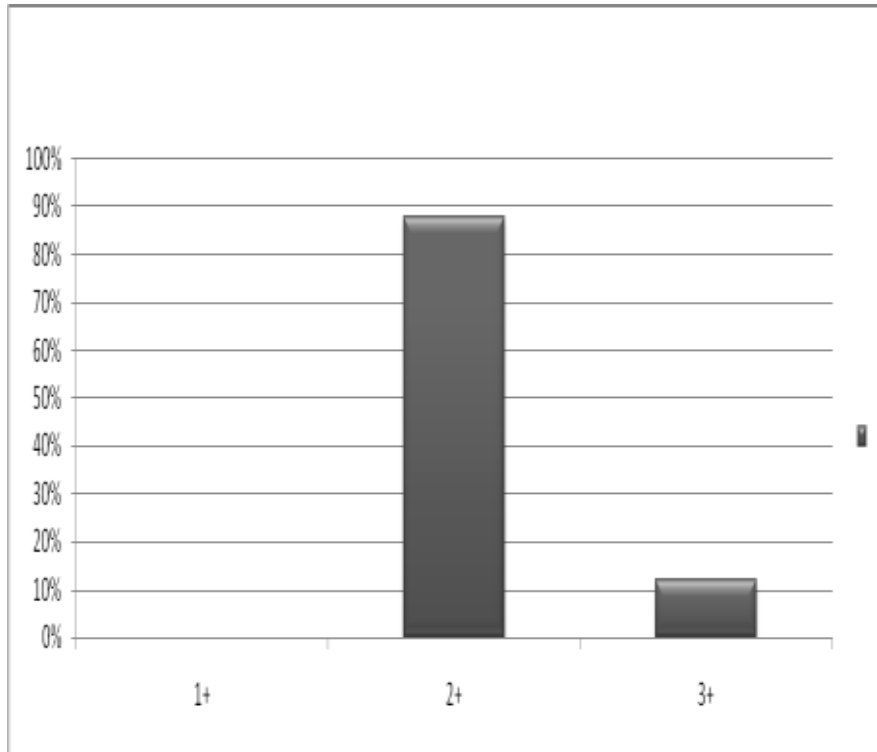


%age coherence of SPRCA IgG(anti A + anti B) considering 2+ reaction as end titer result

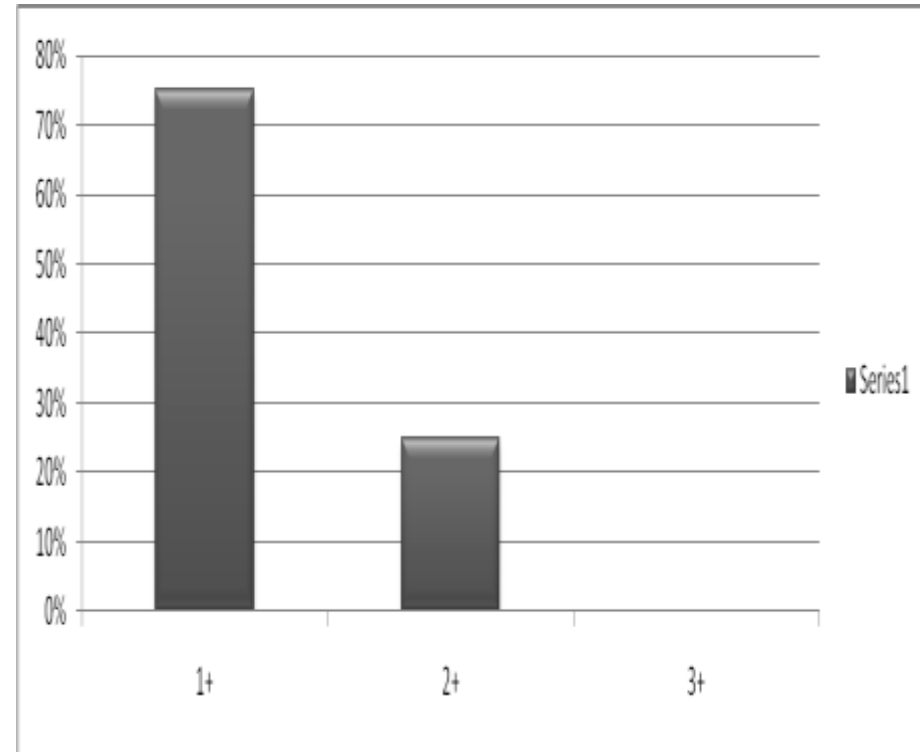
%age coherence of CAT IgG (anti A + anti B) considering 2+ reaction as end titer result

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%).



% age coherence of CAT IgM(anti A + anti B) considering 2+ reaction as end titer result



% age coherence of SPRCA IgM(anti A + anti B) considering 2+ reaction as end titer result

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