

**Non-conventional
adsorption agents
for AIHA investigations**

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Transfusion Problem in AIHA

- Warm autoantibody (autoAb) is present in about 41-70% of AIHA patients.
- RBC autoAbs react with random RBCs. No compatible blood.
- RBC autoAbs mask presence of RBC alloAb
⇒ risk of alloimmune hemolytic transfusion reaction.
- Rate of alloAb is 14-40%, compared to 2-3% in general patients.

Serologic Resolutions

Removal of RBC autoAbs by adsorption.

Conventional adsorption methods -

Use of ZZAP- or protease-treated RBCs in

- autologous adsorption
- allogeneic adsorption
 - homologous adsorption
 - differential adsorption

Autologous adsorption

Limitations

- Patient not transfused within past 3 months.
- Inadequate volume of RBCs in severely anemic situation.

Homologous/Differential adsorption

Limitations

- Extensive source of pre-phenotyped RBCs for selection.
- Availability of sufficiently large amount of the selected RBCs.
- ‘Ad hoc’ phenotyping is labour consuming.

Ideal adsorption agent

- Simple to use
- Easily available
- Long storage life
- Re-usable
- Efficient in removing the RBC autoAbs
- No non-specific adsorption of RBC alloAbs

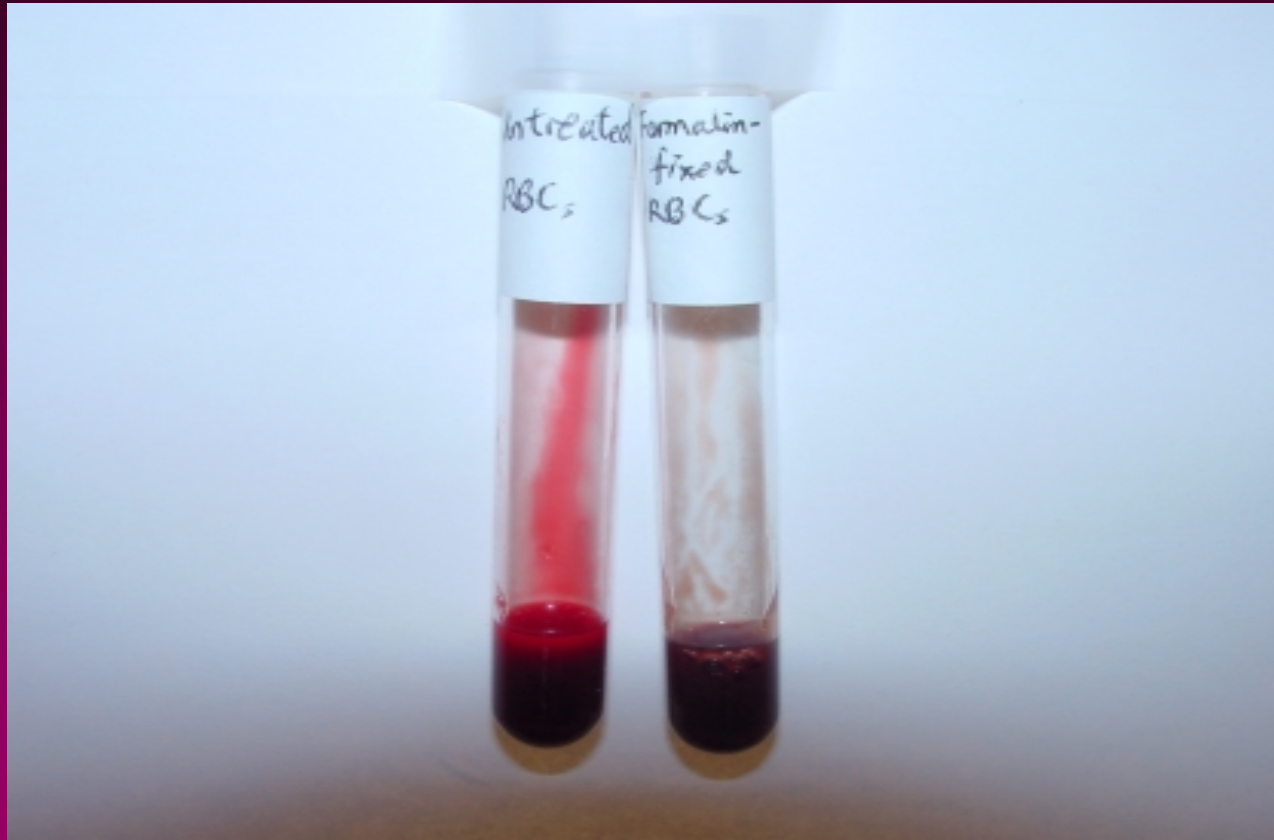
Non-conventional agents

Are there any non-conventional adsorption agents which fit the criteria of an ideal adsorption agent?

Non-conventional agents

- Formalin-fixed RBCs
- Formalin-fixed platelets
- Digitonin RBC stroma
- Phospholipids bound on activated agarose column, including:
 - 3-Phosphoglyceric acid (PGA), &
 - Phosphocholine chloride (PC)

Formalin-fixed RBCs



Formalin-fixed RBCs

- formerly applied in removal of anti-A & -B in production of anti-D

(Lockyer JW, Darke C, Webb J. (1967) Absorption of unwanted anti-A -B agglutinins from anti-Rh D grouping sera using time-expired formalised heat-treated A₁B Rh D positive red cells. Vox Sang 12, 75-7.)

- aldehyde-fixed RBCs was once reported in removal of RBC autoAbs in 1982. However, only 3 anti-E, 1 anti-D, 1 anti-S, 1 anti-Jk^a, 1 anti-Mi^a, 1 anti-Wr^a were used in study of the non-specific uptake of RBC alloAbs

(Reid ME, Ellisor SS. (1982) Absorption of warm autoantibodies using glutaraldehyde-treated human red cells. Am J Med Technol 48, 679-684.)

Formalin-fixed platelets

- No RBC antigens of the major blood group systems, except minute amount of A or B antigens.
- RBC autoAbs were found to be anti-phospholipids in some WAIHA cases. Platelets were rich in phospholipids and were fixed to remove RBC autoAbs in 1994.

(Fitzgerald M, Butz RE, Procter JL and Davey RJ. (1994) Adsorption of warm-reactive RBC autoantibodies using formalin-fixed platelets (abstract). Transfusion 34 (suppl), 78S.)

Digitonin RBC stroma

- digitonin is a non-ionic detergent which increases the permeability of RBC membrane & releases the cytoplasmic contents.
- digitonin allows Abs to traverse across the RBC membrane, may then increase the accessible antigen sites for adsorption.

Phospholipids on agarose column

- PGA (anionic) & PC (cationic) were bound to activated agarose column. Unbound sites were blocked by bovine serum albumin.
- application in removal of RBC autoAbs was once reported in 1995, with success rate of 76.9%. Three out 13 cases failed.

(Butz RE, Bowman C, Wannebo C, Procter JL and Read EJ. (1995) Adsorption of RBC autoantibodies using phospholipid and phosphodiester ligands on an activated matrix (abstract). Transfusion 35 (suppl), 21S.)

Evaluation Protocol

Non-specific or preferential uptake of RBC alloAbs

- Formalin-fixed RBCs & digitonin RBC stroma were prepared from antigen-negative RBCs
- Platelets & Phospholipids - insignificant or no RBC antigens
- Pre- and Post-adsorption scores of the RBC alloAbs were compared
- Reduced score after adsorption \Rightarrow non-specific uptake

Evaluation Protocol

‘ Matuhasi-Ogata’ uptake (Non-specific uptake of Ab along with pre-formed Ag-Ab complex)

- Anti-E mixed with RBC autoAb or Anti-C, -D & -e.
- Formalin-fixed RBCs & digitonin RBC stroma were prepared from CCDee RBCs
- Pre- & post-adsorption scores of anti-E were compared.
- Reduced score after adsorption \Rightarrow non-specific uptake

Evaluation Protocol

Efficiency in removal of RBC autoAbs

- 21 samples of RBC autoAbs were adsorbed for 4 changes by each agent. 10 samples out of which are failed to be completely removed by conventional method.
- Post-adsorption score per change of adsorption was obtained
- Post-adsorption scores from each sample were summed and compared among the various methods
- Smaller sum \Rightarrow more efficient

Evaluation Protocol

Comparison of 'fresh' & 're-used' fixed RBCs & Platelets

- Digitonin RBC stroma & phospholipid not tested (no capacity in re-usability)
- Fixed agent can be recovered by washing with 60°C D.W. after use.
- 'Fresh', '2nd use', '3rd use', '4th use' fixed reagents were tested
- Same sample of RBC autoAb & alloAb used in each batch of comparison
- Elevated post-adsorption score \Rightarrow reduced efficiency

Evaluation protocol

Comparison with respect to different storage periods

- Phospholipids not tested as periodic maintenance was time consuming
- Formalin-fixed agents & digitonin RBC stroma was evaluated when freshly prepared, stored for 2 weeks, 1 month & 2 months.
- Elevated post-adsorption score \Rightarrow reduced efficiency

Results

Non-specific or preferential uptake of RBC alloAbs

- Formalin-fixed RBCs showed **slight but significant non-specific uptake**. Av. reduction in score was 0.5.
- Formalin-fixed platelets showed **remarkable and significant non-specific uptake**. Av. reduction in score was 1.8. Hence, abandoned from later studies.
- Digitonin RBC stroma, PGA & PC gave **no non-specific uptake**.

Results

‘Matuhasi-Ogata’ uptake of RBC alloAbs

‘Matuhasi-Ogata’ uptake of RBC alloAbs was shown by formalin-fixed platelets.

Results

Efficiency in removal of RBC autoAbs

- From sum of post-adsorption score:
Digitonin RBC stroma > Formalin-fixed
RBC > PGA, PC

Results

Success rate

- Digitonin RBC stroma (86%) > Formalin-fixed RBC (72%) > PGA (62%) > PC (57%)
- Digitonin RBC stroma
 - used to complete adsorption at 1-2 cycles
 - only 3 out of 21 cases failed (also failed with conventional method)

Results

Success rate

- Formalin-fixed RBCs
 - 6 out of 21 cases required 3-4 cycles to complete
 - 6 cases failed

Results

Success rate

- **PGA & PC**
 - Usually required 3-4 cycles to complete (except 1 case).
 - equivalent performance except 1 case in which PC failed
 - high failure rate: 8 out of 21 cases & 9 out of 21 cases respectively

Results

Re-usability of Formalin-fixed RBCs

No significant reduction in adsorption efficiency was shown among 'fresh', '2nd use', '3rd use' & '4th use' preparations.

Results

Storage effect

No significant reduction in adsorption efficiency of:

- Formalin-fixed RBC stored at 1-6°C for up to 2 months
- Digitonin RBC stroma stored at 1-6°C or < -20°C for up to 2 months

Discussion

Advantages of formalin-fixed RBCs

- higher success rate than conventional method (72% vs 52%)
- easy to pack tight, minimal dilution effect
- long storage life (not less than 2 months)
- obviate use of RBC preservative for storage
- re-usable for at least 4 times, or probably longer
- easy and simple processing on retrieval for use
- allows easy transport

Discussion

Disadvantages of formalin-fixed RBCs

- slight non-specific uptake of RBC alloAbs. Effect might be augmented upon multiple adsorption steps.
- too sticky, difficult to resuspend.
- cause brownish discoloration of the adsorbed serum.

Discussion

Advantages of formalin-fixed platelets

- obviate the consideration of RBC phenotypes
- readily available. Expired platelets could be used.
- easy to pack tight, minimal dilution effect.

Disadvantage of formalin-fixed platelets

- Remarkable non-specific uptake of RBC alloAb
- Abandoned from later part of the study

Discussion

Advantages of digitonin RBC stroma

- no non-specific or 'Matuhasi-Ogata' uptake of RBC alloAbs
- highly efficient, mostly complete with 2 cycles
- high success rate (86%)
- long storage life (not less than 2 months)
- easy to store (at 1-6°C or <-20°C) and transport
- pack to very small volume, minimal dilution effect.

Discussion

Disadvantages of digitonin RBC stroma

- Residual digitonin causes hemolysis in later steps. Thorough washing of stroma and use of pre-warmed digitonin were required.
- Discoloration of the white stroma was observed on storage at 1-6°C. Better preservation by freezing.
- Still limited by the availability of phenotyped RBCs.

Discussion

Advantages of PGA & PC

- no non-specific or 'Matuhasi-Ogata' uptake of RBC alloAbs
- obviate the consideration of RBC phenotypes
- readily available. Use of standard chemicals.

Discussion

Disadvantages of PGA & PC

- Inferior adsorption efficiency. Mostly complete with 3-4 cycles.
- Less satisfactory success rate (62% & 57% respectively)
- Time consuming preparation steps

Discussion

Limitations of the study

- Reliability of results could be improved with use of greater size of fresh samples.

Discussion

Potential developments

- Use of unfixed platelets might obviate the problem of non-specific adsorption
- Extend the study to longer storage period
- Adsorption efficiency may be improved if:
 - RBCs are ZZAP- or protease-treated prior to formalin-fixation or digitonin-treatment

Conclusion

- **Digitonin RBC stroma** was a superior adsorption agent for removal of RBC autoantibodies for being highly efficient & it caused no non-specific uptake of RBC alloAbs
- **Formalin-fixed RBCs and platelets** were not satisfactory because they caused non-specific uptake of RBC alloAbs

Conclusion

- Phosphoglyceric acid & phosphocholine chloride bound to activated agarose column were not satisfactory because they were inefficient and time consuming in preparation.