Resolving ABO Discrepancies

Quick Reference Chart

BIO-RAD

Bio-Rad Laboratories

Clinical Diagnostics Group

Website *www.bio-rad.com/diagnostics* ***Australia*** *+61(2)9914-2800* ***Austria*** *+43-1-877-8901* ***Belgium*** *+32(3)710-53-00* ***Brazil*** *+55(31)3689-6600*
Canada *+1 514 334-4372* ***China*** *+86-21-61698500* ***Czech Republic*** *+420-241-430-532* ***Denmark*** *+45-4452-1000* ***Finland*** *+358-9-804-22-00*
France *+33 1 47 95 60 00* ***Germany*** *+49(0)89-318-840* ***Greece*** *+30-210-7774396* ***Hong Kong*** *+852-2789-3300* ***Hungary*** *+36-1-459-6100*
India *+1-800-180-1224* ***Israel*** *+972-3-9636050* ***Italy*** *+39-02-216091* ***Japan*** *+81-3-6361-7070* ***Korea*** *+82-2-3473-4460* ***Mexico*** *+52(55)5488-7670*
The Netherlands *+31-318-540666* ***New Zealand*** *+64-9-415-2280* ***Norway*** *+47-23-38-41-30* ***Poland*** *+48-22-3319999* ***Portugal*** *+351-21-472-7700*
Russia *+7-495-721-14-04* ***Singapore*** *+65-6415-3170* ***South Africa*** *+27-11-442-85-08* ***Spain*** *+34-91-590-5200* ***Sweden*** *+46-8-555-127-00*
Switzerland *+41 26 674 55 05/06* ***Taiwan*** *+886-2-2578-7189* ***Thailand*** *+662-651-8311* ***United Kingdom*** *+44(0)20-8328-2000*

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Category	Check/Control/Investigate	
1. General Points	▪ Clerical details	
	▪ Previous records	(Transfusion history, medication, age, clinical and obstetric data)
	▪ Sample	(Hemolyzed, spontaneous agglutination, lipemic)
	▪ Functionality of reagents/reagent contamination	
2. Preliminary Investigations	▪ Centrifuge the sample	
	▪ Repeat the test	- cells and serum/plasma - fresh sample - fresh reagents
3. Further Investigations	▪ Patient/donor cells	- wash and repeat
	▪ Patient/donor serum/plasma	- longer incubation time - incubate at 4°C
4. Unresolved Forward (cell) Group Discrepancy	▪ If unrelated to age, disease state or rouleaux formation	
Weak/negative reactions	▪ Possible subgroup	- include anti-A1 and anti-H in extended testing - perform adsorption-elution - perform saliva studies (if secretor) - refer to Reference Laboratory for serum transferase studies and molecular analysis
Mixed-field reactions	▪ If unrelated to transfusion/transplant therapy	
	▪ Possible subgroup (A_3, A_{weak}, B_3, B_{weak})	- see above
	▪ Possible chimera	- separate cell populations and retest each one
Unexpected positive reactions	▪ Polyagglutinable cells	- use monoclonal reagents - use lectins to characterize polyagglutination type
	▪ Direct Antiglobulin Test (DAT) positive cells	- check records (warm washing cells or other method)
	▪ Acquired-B phenotype	- check diagnosis - use monoclonal anti-B known not to react with acquired B
	▪ Spontaneous agglutination (if sample stored at 4°C prior to test)	- cold auto-antibody - retest at 37°C (new sample taken and maintained at 37°C may be necessary)
	▪ Possible B(A) or A(B) phenotype	- use other reagents
5. Unresolved Reverse Group Discrepancy	▪ If unrelated to age (newborn/elderly), immunosuppression, hypogammaglobulinemia or hemolysis of the reagent cells	
Weak/negative reactions	▪ Fresh sample	- retest at 4°C - increase the incubation time - use fresh set of cells and/or additional cells - retest forward group for confirmation
Additional unexpected reactions	▪ Possible allo-antibody	- identify specificity - retest with appropriate reverse grouping cells but negative for the antigen corresponding to the allo-antibody - anti-HI in A_1 samples; confirm with A_1, A_2 and O cell panels, include cord blood as a negative control
	▪ Possible cold auto-antibody	- autologous control - use a pre-warmed technique - autoadsorption and retest
	▪ Possible rouleaux	- saline replacement technique
	▪ A_2 or other A subgroups with anti-A1	- test with other A_1 cells to confirm

Notes

- Recommended laboratory procedures/techniques should be followed for the investigation of any discrepancy e.g. AABB Technical Manual, other practical-based textbook, or in-house established Standard Operating Procedures.
- Where anomalous results persist, family studies can be useful, with serological and molecular biology techniques to establish inheritance pattern and genetic background.
- This chart is not necessarily comprehensive.