Resolving Rh Discrepancies

Quick Reference Chart

BIO-RAD

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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	- wash cells - fresh sample - fresh reagents - additional sera of same specificity
3. Unresolved Problems		
Unexpected positive reactions	▪ Check for rouleaux formation related to disease state (rouleaux not associated with the ID-System)	
Previously tested negative or autocontrol also positive	▪ Direct Antiglobulin Test (DAT) positive cells	- use monoclonal reagents - remove *in vivo* coating by warm-washing cells or other approved method
	▪ Confirm positive result	- adsorption and elution
	▪ Antibody to low-frequency antigen present in human polyclonal reagent	- use monoclonal reagents
	▪ Polyagglutinable cells	- use monoclonal reagents - use lectins to characterize polyagglutination type
	▪ Sample mix up this time/previous time tested	- check all previous records; confirm identity and repeat from a second sample
Antisera of apparently the same specificity showing discrepant results	▪ Possible antigen variant – most commonly associated with D antigen	- test with a panel of monoclonal anti-D's to characterize the variant D type - test with antisera to known Rh low-frequency antigens associated with partial D types - perform family studies - refer to Reference Laboratory for molecular analysis
	▪ Possible weak D	- characterize and differentiate from a partial D type as above
Unexpected weak or negative reactions	▪ Weak positive may be due to any of the above reasons. Check and control	
	▪ Rare phenotypes	- Rh_{null}/Rh_{mod} - deletions e.g. D-- - suppressed antigenic complexes e.g. (C)D(e)
	▪ Compound antisera	- human polyclonal anti-C is often predominantly anti-Ce +C, weak or negative reactions with certain phenotypes
	▪ Sample mix up this time/previous time tested	- check all previous records; confirm identity and repeat from a second sample
Mixed-field reactions	▪ Post-transfusion	- check records
	▪ Post-transplantation therapy (bone-marrow/stem cells)	- check records
	▪ Rh mosaicism due to myeloproliferative disorder	- monitor Rh type beyond remission
	▪ Chimerism (twin or dispermic)	- separate cell populations and retest - full blood group phenotyping to check for chimerism in other blood group systems - cytogenetic studies - tissue culture e.g. analysis of fibroblasts

Notes

- Generally a sample confirmed as a weak D and/or variant D must be treated as RhD positive for donor purposes. Transfusion recipients/antenatal patients should only be treated as RhD positive when clear-cut reactions have been obtained with suitable anti-D reagents in accordance with local/national guidelines.
- The DVI phenotype is the most important variant to consider.
  Anti-D known to react with DVI should be used for RhD typing of donor bloods, and weak D types should be detected.
  Anti-D known NOT to react with DVI bloods should be used for RhD typing of transfusion recipients/antenatal patients.
- Human Anti-D sera most often require a potentiating medium and will give positive reactions with variant D as well as weak D types.
  Care should be exercised not to misclassify a DVI patient sample as RhD positive, particularly in the case of pre-menopausal females.
- 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.