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Are patients with anti-M being managed appropriately?

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M antigen (MNS system)

Clinical significance of allo-anti-M

Transfusion reaction	Only in extremely rare cases
HDN	Only in extremely rare cases

In vitro characteristics of allo-anti-M

Immunoglobulin class	IgG (cold reactive;many agglutinating) - 78%; IgM - 22% (Smith & Beck. *Transfusion* 1979;**19**:472)
Reactive by	4°C; RT; (rarely also reactive by IAT when it is likely "carryover")
Complement binding	No

Experts concur that anti-M, not reactive at strict 37°C, is not clinically significant.

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Anti-M

- Naturally occuring, cold reactive
- More common in children than adults, and in patients with bacterial infections. Not uncommon for pregnant M- women to produce anti-M but to give birth to an M- baby.
- **Exception** to classical serological reactivity.

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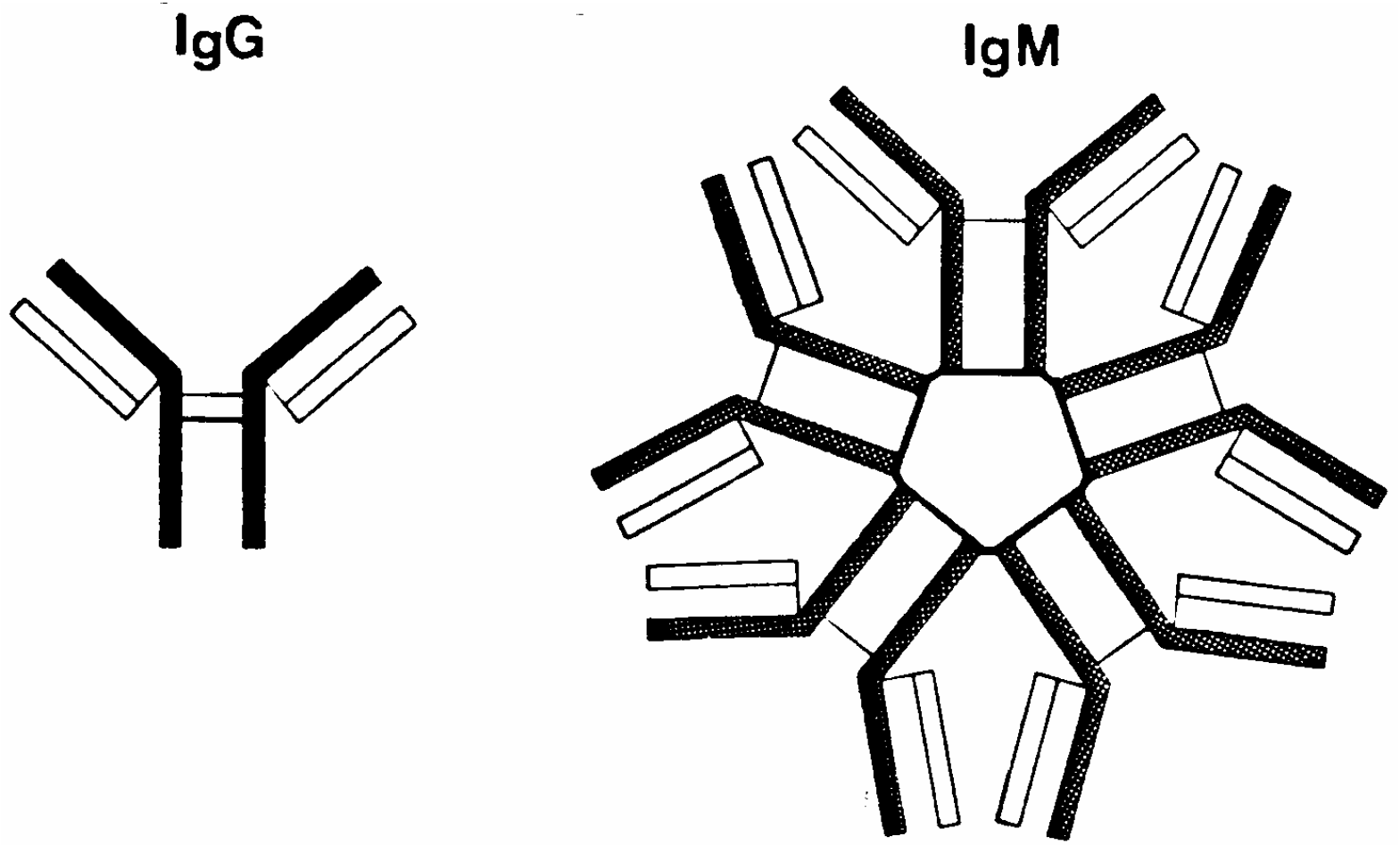
Immunoglobulin Size



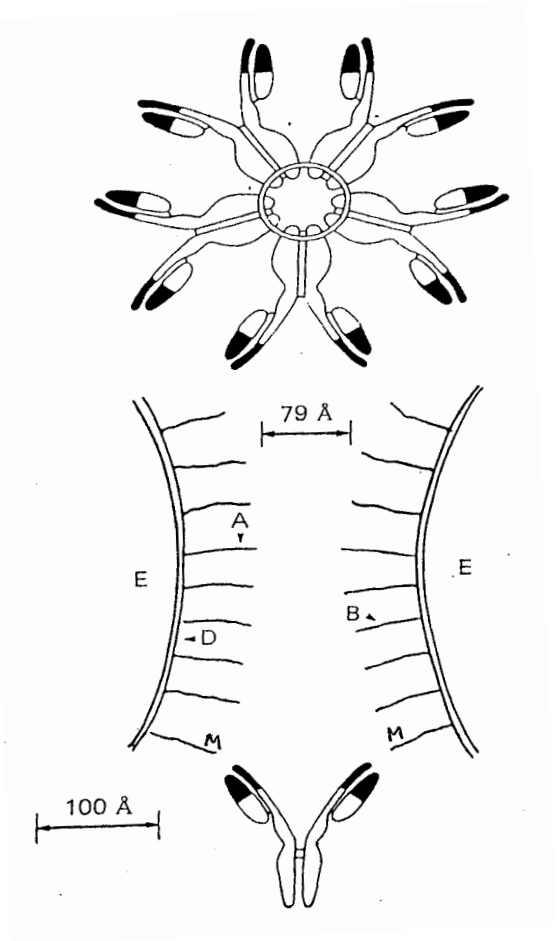
14nm

30nm

Accessibility of Antigens

- The RhD antigen protudes very little above the lipid bilayer and therefore requires IgM (30nm) to bridge the gap.

- The gap between M antigens on different cells is approx. 13.9nm (7.9nm + 3nm + 3nm*), which is just within the range of IgG agglutinating span of 14nm.



* M determinants are approx 3nm below the sialoglycoprotein surface

Adapted from: Van Oss. *Vox Sang* 1983;**44**:183

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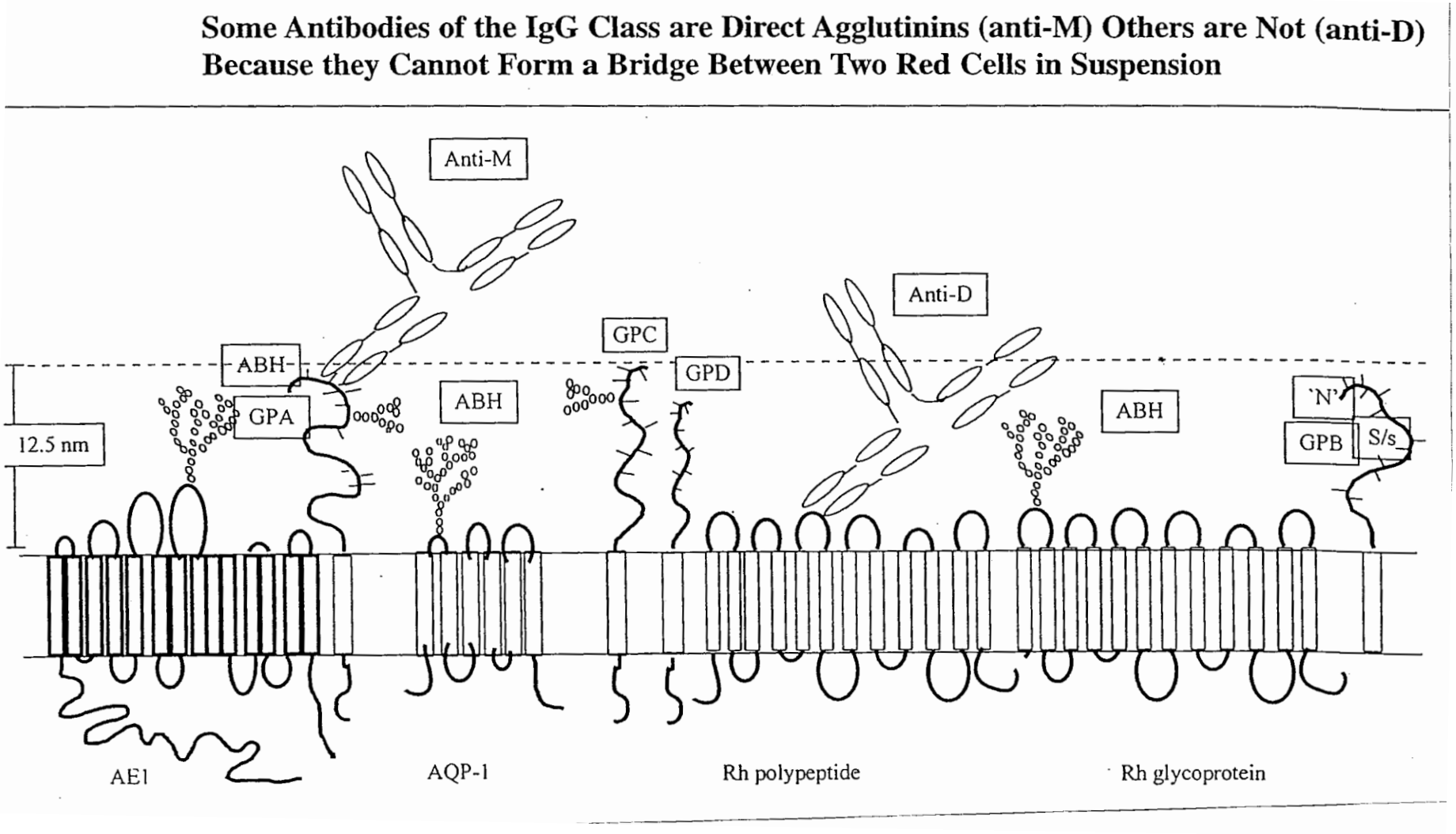
Red Cell Membrane/Antigens



Adapted from Issitt & Anstee. Applied Blood Group Serology. 4th Ed. 1998

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Study Results

- 32 anti-M patients were initially tested by standard BioRad IAT and tube-saline 18°C, samples positive at 18°C were categorised:
- Cat1 - Those ***negative by IAT*** were then tested at 25-32°C.
- Cat2 - Those ***reactive by IAT*** were then tested by tube-IAT performed strictly at 37°C.
- Strict 37°C tube-IAT was performed by pre-warming all reagents and hardware to 37°C (except centrifuge)

Review of Cases

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Cases Studied	32			
Antenatal	21		Non A/N	11
Strict 37°C	***3***			***2***
DTT Studies	11			4
IgG	4	***(1)***		3
IgM	7	***(1)***		1 ***(1)***
No antenatal cases reported where problems were encountered with baby. Where IgG anti-M reported baby was DAT negative +/- M-				

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Modified DTT Test

- Normally PBS- and DTT-treated plasmas titrated in IAT/IgG
- Additional titrations performed in tube LISS at 18°C (as anti-M whether IgG or IgM is generally cold-agglutinating).

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Non-Antenatal

- N = 11
- 5 had underlying sepsis
- 1 donor (strong auto-anti-M blocking filters, Ig class could not be determined)
- 2 with additional antibodies
- 2 appear to have anti-M truly reactive at 37°C – although unable to complete investigations due to lack of sample.

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Is Anti-M active at 37°C?

- BCSH guidelines - transfusion protocol for patient's with anti-M
  - active at strict 37°C is M- blood
  - cold-reacting is crossmatch compatible at 37°C

  Irish hospitals classify their anti-M as reacting at 37°C if it reacts by IAT (usually CAT)

- Anti-M samples referred to IBTS are always cold reacting and can come through in IAT either weakly or only reacting with MM cells

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Requests for M- units

- Based on historically identified antibodies (known to be non-reactive by IAT at present).
- Patients transferred from different hospitals with historic anti-M
- Hospital policies based on BCSH Guidelines …query interpretation of strict 37°C!
- Reluctance to refer samples

Phenotypes (% occurrence)

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Phenotype	Caucasians	African Origin
M+N-S+s-	6	2
M+N-S+s+	14	7
M+N-S-s+	8	16
⇨ M+N+S+s-	4	2
⇨ M+N+S+s+	24	13
⇨ M+N+S-s+	22	33
⇨ M-N+S+s-	1	2
⇨ M-N+S+s+	6	5
⇨ M-N+S-s+	15	19
M+N-S-s-	0	0.4
⇨ M+N+S-s-	0	0.4
⇨ M-N+S-s-	0	0.7

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Antigen Screening

- Automated Donor Grouping Laboratory
- Each test approx. €2.20 each (£1.75)
- No. of donations screened 2011 = approx 4500 (cost €9,900 (approx. £8,000))

this does not include the cost of the human resource (time involved), equipment, facilities etc

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Case 1 – Anti-M

- 2 units required (GI bleed, MI, resp tract infection)
- DAT weakly positive (IgG)
- Anti-M detected BioRad IAT and 18°C
- 6 units crossmatched (see table)
- Strong cold anti-M that required pre-warming (tube)

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Unit	M type	BioRad Card		Card pw		Tube 18°C	pw 37 IgG
		RT	IAT	RT	IAT	18°C	IAT
1	M-	0	0				
2	M-	0	0				
3	MM	2+	2+	2+	+s	2+	0
4	MN	+s	1+	0	0	2+	0
5	MM	2+	2+	2+	2+	4+	0
6	MN	+s	2+	+w	1+	0	0

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Case 2

- Antenatal
- Anti-M and Anti-S (pre-warmed tube IAT)
- DTT – IgM
- Baby – M+N+S+s+ (DAT weakly positive IgG)
- Eluate – Anti-S

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Proposed Algorithim

- If you want M negative red cells – send a sample to the RCI lab to determine if required.
- If it is urgent – crossmatch random units from bank (approx. 72% should be negative)
- If it is antenatal case, check at booking, 28 weeks and at delivery. M negative units will only be considered for baby if anti-M active strictly at 37°C (pw tube IAT).

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Testing

- ‘Standard’ IAT / Enzyme panels for antibody ID.
- Tube saline 18°C panel
- Strict 37°C pre-warmed tube LISS-IAT on first sample. If 18°C positive and tube LISS-IAT negative – antibody should be considered cold reactive and transfusion protocol should be for crossmatch compatible units at 37°C.

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What do you do?

- When do you request M negative units?

Immunoglobulin Structure of Human anti-M

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- 78% IgG
- 22% IgM
- M.L. Smith and Beck found no correlation between Ig composition and history of transfusion or pregnancy.

Smith & Beck. *Transfusion* 1979;**19**:472

- **Pathlab talk**
- **MN**
- 8-13-2012, 02:50 AM
- *We see many, many examples of anti-M per year.*

  The only ones that we would suggest require M- blood are those that react by pre-warmed, warm-washed LISS tube IAT at 37oC, otherwise our advice is always to give cross-match compatible blood.

  No patient that has received cross-match compatible blood in these circumstances has had the slightest reaction in the past *12 years whilst I have been working at the Reference Laboratory.*

  The problem is that, when using CAT, the reactants are not mixed at strictly 37oC, and "cold-reacting" antibodies can sensitise red cells expressing the corrresponding antigen extremely quickly, but do not come back off anything like as quickly, so that, by CAT, they appear to react at 37oC.

- **Pathlab talk**
- **SJ**
- 08-12-2012, 03:56 PM
- In my experience we often detect and identify Ant-M be the Gel technique automated or manual. However, my understanding is that the only way to determine the thermal reactivity range of the anti-M is by repeating the tests in tubes at 4°C, RT and 37°C. ***If the anti-M does not react at 37°C (in tubes) then cross-match compatible blood can be issued***. If the anti-M reacts at 37°C then M antigen negative blood should be cross-matched and issued. ***Pre-warming cells, sera, gel cassette, reagents etc. does not work well, if at all with anti-M. The anti-M binds to the M antigen on the cells too strongly to disassociate during the incubation stage of the gel cassette***.

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  In practice we refer all new anti-M samples to our local RCI laboratory for confirmation of the thermal range of the antibody.