

Tube tests for determination of ABO type of Red cells and serum

- Importance of the ABO system

Most important (clinically significant) Blood Group system for transfusion practice

why?

If ABO antibodies react with antigens in vivo, result is acute hemolysis and possibly death.

- Indications for ABO grouping

- Blood Donors
- Transfusion recipients
- Transplant candidates and donors (ABO antigens are found in other tissues)
- Prenatal patients (cause of HDN)
- Newborns
- Paternity testing

Tube tests for determination of ABO type of Red cells and serum

- What are the following ABO type?

Anti-A	Anti-B	A1 CELL	B CELL
4+	0	2+	4+

Anti-A	Anti-B	A1 CELL	B CELL
4+	0	0	0

Anti-A	Anti-B	A1 CELL	B CELL
4+	4+	4+	4+

Tube tests for determination of ABO type of Red cells and serum

- **KINDS OF DISCREPANCIES**

1. Clerical errors
2. Technical errors
3. Problem with serum testing
4. Problem with red cell testing
5. Problems with both cells and serum



بخش:

کارگاه آموزشی کنترل کیفی بانک خون بیمارستانی آزمایشگاه مرجع سلامت
دانشگاه علوم پزشکی ایران

تاریخ:

نام سوپروایزر:

شماره بیمار	شماره بانک خون	شماره کد بیمار	شماره کد آزمایش	ABO Grouping					ABO - Memory	Rh-Typing			Rh - Memory	Antibody Screen												AB - Screen Memory	DAT		Free wash test	تاریخ	نام	توضیحات																																																																																																																																																																																																																																				
				Cells			Screen/Reagent			Rh (D)	Rh (C)	Rh (E)		Antibody Screen													DAT																																																																																																																																																																																																																																									
				1:1	1:2	1:4	1:2	1:4						1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048			1:4096					1:8192	1:16384	1:32768	1:65536	1:131072	1:262144	1:524288	1:1048576	1:2097152	1:4194304	1:8388608	1:16777216	1:33554432	1:67108864	1:134217728	1:268435456	1:536870912	1:1073741824	1:2147483648	1:4294967296	1:8589934592	1:17179869184	1:34359738368	1:68719476736	1:137438953472	1:274877906944	1:549755813888	1:1099511627776	1:2199023255552	1:4398046511104	1:8796093022208	1:17592186044416	1:35184372088832	1:70368744177664	1:140737488355328	1:281474976710656	1:562949953421312	1:1125899906842624	1:2251799813685248	1:4503599627370496	1:9007199254740992	1:18014398509481984	1:36028797018963968	1:72057594037927936	1:144115188075855872	1:288230376151711744	1:576460752303423488	1:1152921504606846976	1:2305843009213693952	1:4611686018427387904	1:9223372036854775808	1:18446744073709551616	1:36893488147419103232	1:73786976294838206464	1:147573952589676412928	1:295147905179352825856	1:590295810358705651712	1:1180591620717411303424	1:2361183241434822606848	1:4722366482869645213696	1:9444732965739290427392	1:18889465931478580854784	1:37778931862957161709568	1:75557863725914323419136	1:151115727451828646838272	1:302231454903657293676544	1:604462909807314587353088	1:1208925819614629174706176	1:2417851639229258349412352	1:4835703278458516698824704	1:9671406556917033397649408	1:19342813113834066795298816	1:38685626227668133590597632	1:77371252455336267181195264	1:154742504910672534362390528	1:309485009821345068724781056	1:618970019642690137449562112	1:1237940039285380274899124224	1:2475880078570760549798248448	1:4951760157141521099596496896	1:9903520314283042199192993792	1:1980704062856608439838598784	1:3961408125713216879677197568	1:7922816251426433759354395136	1:15845632502852867518708790272	1:31691265005705735037417580544	1:63382530011411470074835161088	1:126765060022822940149670322176	1:253530120045645880299340644352	1:507060240091291760598681288704	1:1014120480182583521197362577408	1:2028240960365167042394725154816	1:4056481920730334084789450309632	1:8112963841460668169578900619264	1:16225927682921336339157801238528	1:32451855365842672678315602477056	1:64903710731685345356631204954112	1:129807421463370690713262409908224	1:259614842926741381426524819816448	1:519229685853482762853049639632896	1:1038459371706965525706099279265792	1:2076918743413931051412198558531584	1:4153837486827862102824397117063168	1:8307674973655724205648794234126336	1:16615349947311448411297588468252672	1:33230699894622896822595176936505344	1:66461399789245793645190353873010688	1:132922799578491587290380707746021376	1:265845599156983174580761415492042752	1:531691198313966349161522830984085504	1:1063382396627932698323045661968171008	1:2126764793255865396646091323936342016	1:4253529586511730793292182647872684032	1:8507059173023461586584365295745368064	1:17014118346046923173168730591490736128	1:34028236692093846346337461182981472256	1:68056473384187692692674922365962944512	1:136112946768375385385349844731925889024	1:272225893536750770770699689463851778048	1:544451787073501541541399378927703556096	1:1088903574147003083082798757854407112192	1:2177807148294006166165597515708814224384	1:4355614296588012332331195031417628448768	1:8711228593176024664662390062835256897536	1:17422457186352049329324780125710513795072	1:34844914372704098658649560251421027590144	1:69689828745408197317299120502842055180288	1:139379657490816394634598241005684110360576	1:278759314981632789269196482011368220721152	1:557518629963265578538392964022736441442304	1:1115037259926531157076785928045472882884608	1:2230074519853062314153571856090945765769216	1:4460149039706124628307143712181891531538432	1:8920298079412249256614287424363783063076864	1:17840596158824498513228574848727566126153728	1:35681192317648997026457149697455132252307456	1:71362384635297994052914299394910264504614912	1:142724769270595988105828598789820529009229824	1:285449538541191976211657197579641058018459648	1:570899077082383952423314395159282116036919296	1:1141798154164767904846628790318564232073838592	1:2283596308329535809693257580637128464147677184	1:4567192616659071619386515161274256928295354368	1:9134385233318143238773030322548513856590708736	1:18268770466636286477546060645097027713181417472	1:36537540933272572955092121290194055426362834944	1:73075081866545145910184242580388110852725669888	1:146150163733090291820368485160776221705451339776	1:292300327466180583640736970321552443410902679552	1:584600654932361167281473940643104886821805359104	1:1169201309864722334562947881286209773643610718208	1:2338402619729444669125895762572419547287221436416	1:4676805239458889338251791525144839094574442872832	1:9353610478917778676503583050289678189148885745664	1:18707220957835557353007166100579356378297771491328	1:37414441915671114706014332201158712756595542982656	1:74828883831342229412028664402317425513191085965312	1:149657767662684458824057328804634851026382171930624	1:299315535325368917648114657609269702052764343861248	1:59863107065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Tube tests for determination of ABO type of Red cells and serum

نمونه Specimen

1. حداقل ۵-۲ میلی لیتر خون بیمار در لوله حاوی ضد انعقاد EDTA / خون لخته قابل قبول است.
2. ثبت نام و نام خانوادگی بیمار و شماره منحصر به فرد شناسایی کننده بر روی لوله حاوی خون بیمار الزامی است.
3. ثبت نام خونگیر و تاریخ خونگیری بر روی لوله حاوی خون بیمار است.
4. نمونه نوزادان کمتر از ۴ ماه معمولاً حاوی Anti-A یا Anti-B نمی باشد ، بنابراین فقط آزمایش گلبول قرمز جهت این بیماران انجام شود.
5. زمانی که سابقه انجام آزمایش ABO بیمار در بانک خون موجود نیست در صورت نیاز به تزریق خون انجام یکی از بندهای ذیل با رعایت الویت الزامی است:
 - از بیمار نمونه گیری مجدد شده و به بانک خون جهت تکرار و تأیید نتیجه آزمایش ABO اولیه ارسال شود.
 - آزمایش ABO مجدداً بر روی نمونه توسط کارشناس دیگری تکرار و تأیید گردد.
 - کارشناس انجام دهنده آزمایش اولیه ABO مجدداً آزمایش ABO را تکرار، تأیید و مستند سازی نماید.
6. از نمونه هائی که ظاهر همولیز یا لیپیمی (hemolyzed –lipemic) دارند، استفاده نکنید. نمونه گیری مجدد انجام شود.

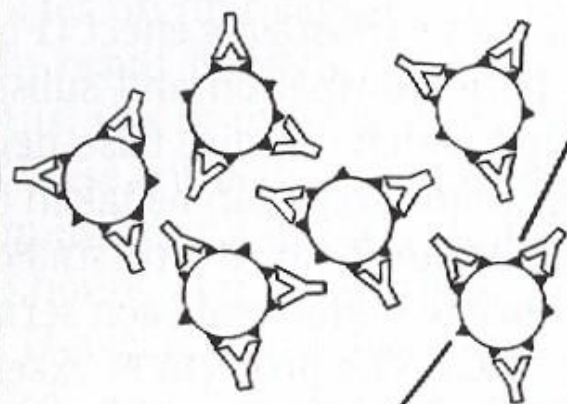
7. شرایط نگهداری نمونه Storage

- به دستورالعمل تولید کننده معرف ها جهت محدودیت و شرایط ذخیره سازی نمونه ها رجوع شود.
- نمونه های خون دردمای یخچال ۲-۸ درجه سانتیگراد و به مدت حداقل ۷ روز جهت هرگونه آزمایش بعدی ذخیره شود.
- جهت پیشگیری از وقوع هرگونه خطای انسانی ، جداسازی سرم یا پلاسما از گلبول قرمز خون نمونه بیمار و ذخیره سازی در لوله های تفکیک شده توصیه نمی شود.

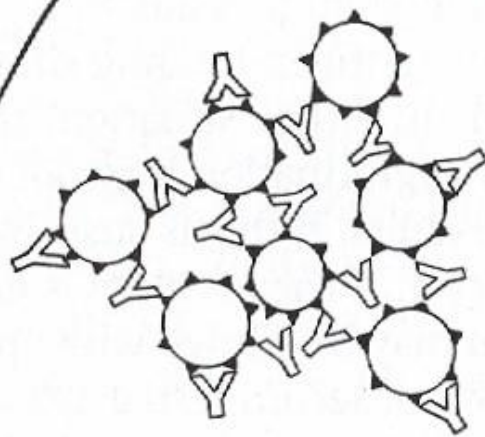
Preparation of A1 cells , B cells

-3% Red cell Suspension SOP

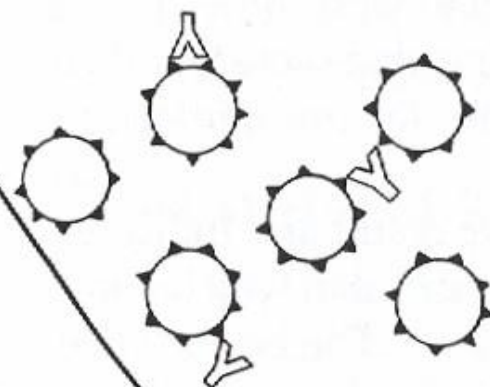
- 3% red cell suspension is a common reagent in many serologic procedures
- 2-5% suspension can achieve the appropriate antigen – antibody ratio
- Zone of equivalence
- Pro zone (excess of unbound immunoglobulin)
- Post zone (surplus of antigen)



Antibody Excess
(Prozone)

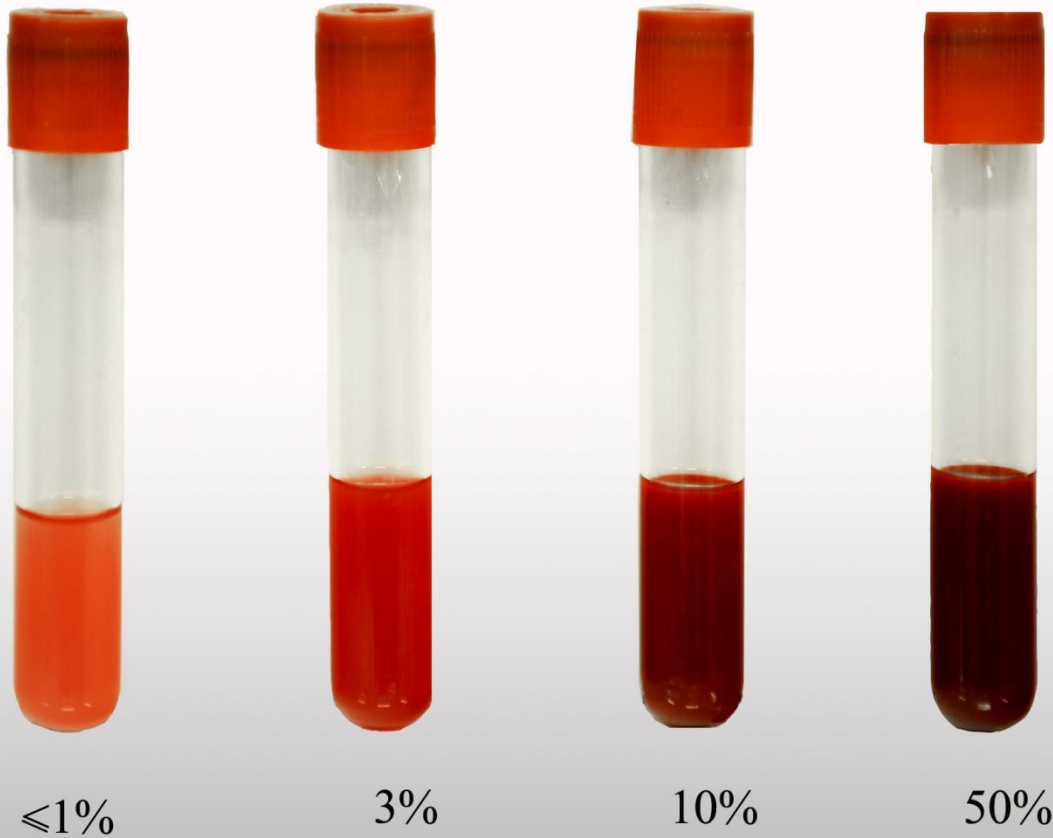


Equivalence
(Optimum Proportions of
Antigen and Antibody)



Antigen Excess
(Postzone)

3% Suspension Color and Density Standard



Comparison Evaluation

Grading Test Results SOP

- Is to allow comparison of reaction strengths
- Can be used to detect and identify ABO subgroups
- Is beneficial in detecting multiple antibody specificities and antibodies exhibiting dosage
- Grading should be standardized among all members of the laboratory staff
- Use a light source and an agglutination viewer as an aid

جدول A

تفسير واكنش و درجه بندى الكوتيناسيون Interpretation of Agglutination Reactions	
Macrosopically Observed Findings	Designation
One Solid agglutinate	4+
Several large agglutinates	3+
Medium-size agglutinates, clear background	2+
Small agglutinates, turbid background	1+
Very Small agglutinates, turbid background	1 ^w
Barely visible agglutination ,turbid back ground	W ⁺
No agglutination	O
Mixtures of agglutinated and unagglutinated red cells (mixed field)	mf
Complete hemolysis	H
Partial hemoysis , some red cells remain	pH

Grading Test Results

0

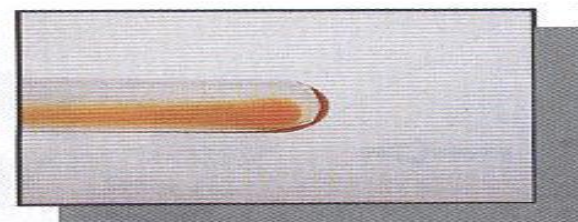
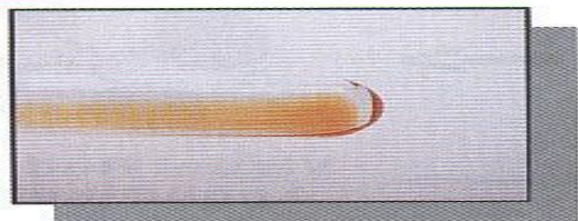
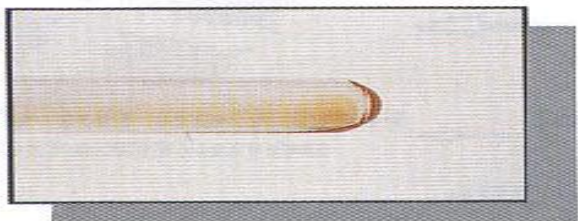
W+

to

1+

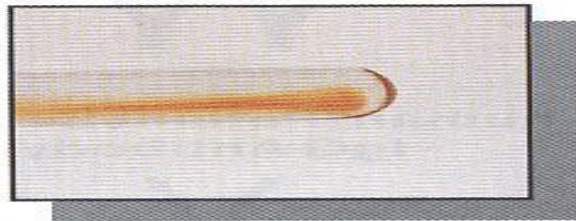
2+

**NO AGGLUTINATION OR
HEMOLYSIS**



NEGATIVE

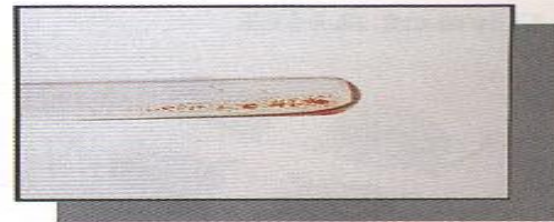
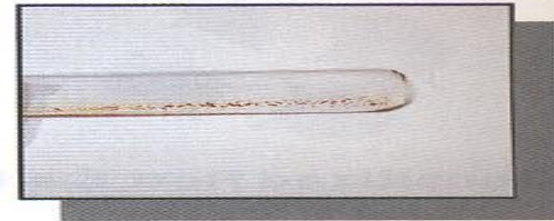
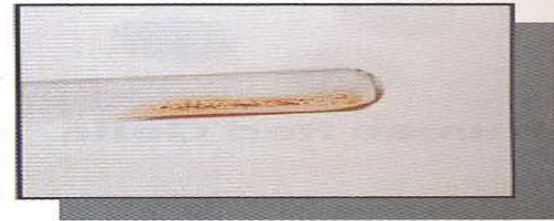
**TINY
AGGLUTINATES
TURBID
BACKGROUND**



POSITIVE

**SMALL
AGGLUTINATES
TURBID
BACKGROUND**

**MEDIUM-SIZED
AGGLUTINATES-CLEAR
BACKGROUND**

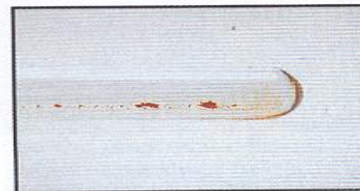
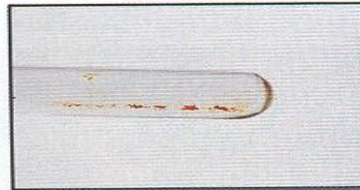
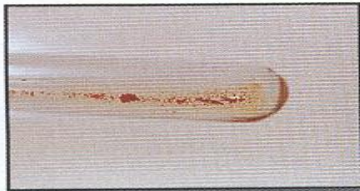


MICROSCOPIC

Grading Test Results

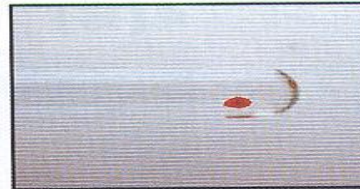
3+

**SEVERAL LARGE
AGGLUTINATES-CLEAR
BACKGROUND**

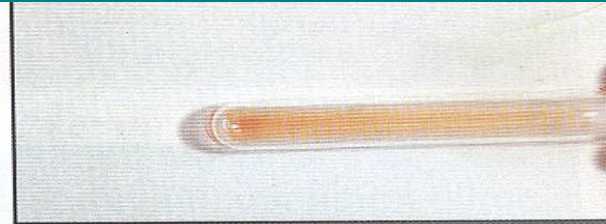


4+

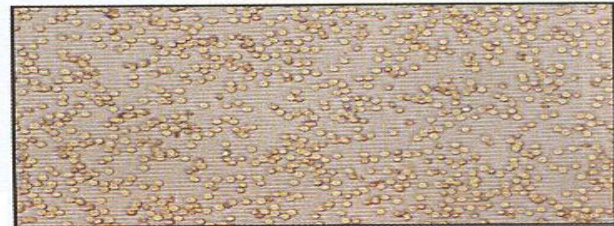
**ONE SOLID
AGGLUTINATE**



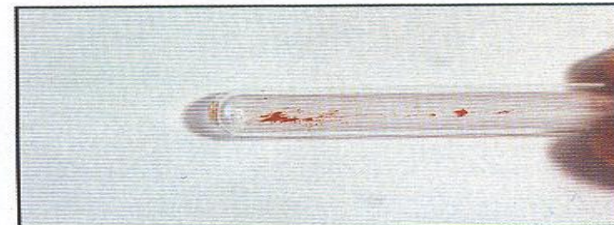
NOTE:
Partial or Complete
Hemolysis is a
Positive Reaction



NEGATIVE: NO AGGREGATES



NEGATIVE: NO AGGREGATES (Microscopic)



**PSEUDOAGGLUTINATION OR STRONG
ROULEAUX (2+)**



**ROULEAUX:Microscopic (original magnification
x10; enlarged 240%) NOTE: The "stack of coins"
appearance of the agglutinates**

Tube tests for determination of ABO type of Red cells and serum

- Quality control to be performed daily
- Reagents
 - All reagents should be certified & approved by MOH reference laboratory
 - Use reagents accordance with manufacture's instructions

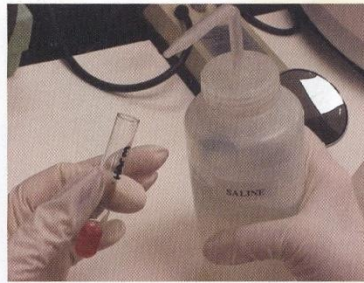
Tube tests for determination of ABO type of Red cells and serum

- Follow S.O.P to perform the test
- Interpretation
 - Hemolysis or agglutination constitute positive results
 - Any discrepancy between results of tests on serum and cells should be resolved before an interpretation is recorded of the donor's ABO

If RBCs react		And Serum/ Plasma react		Then ABO type is
Anti-A	Anti-B	A ₁ RBCs	B RBCs	
0	0	$\geq 3^+$	$\geq 3^+$	0
$\geq 3^+$	0	0	$\geq 3^+$	A
0	$\geq 3^+$	$\geq 3^+$	0	B
$\geq 3^+$	$\geq 3^+$	0	0	AB



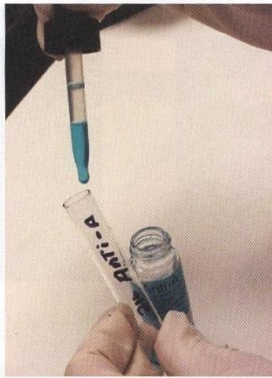
Step 1: Label test tubes.



Step 2: Make a 2-5% patient red cell suspension.



Step 3: Add reagent antisera* (approximately 2 drops).



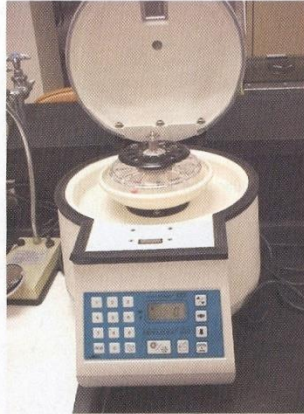
Step 3A: Add reagent Anti-A antiserum* (approximately 2 drops).



Step 3B: Add Anti-B reagent antiserum* (approximately 2 drops).

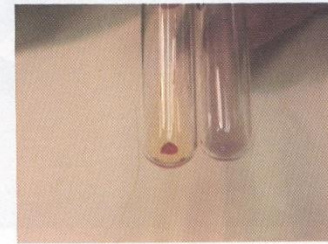


Step 4: Add one drop of 2-5% suspension of patient red cells to each tube.



Step 5: Mix and centrifuge (approximately 20 seconds).

Step 6: Read and record agglutination reactions.



Group B
4+ Agglutination with Anti-B
0 Agglutination with Anti-A



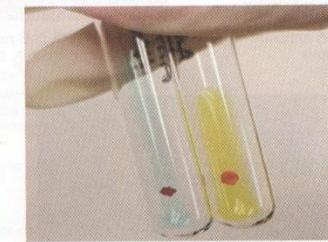
Group B
4+ Agglutination with Anti-B
0 Agglutination with Anti-A



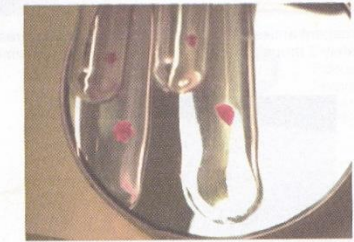
Group A
4+ Agglutination with Anti-A
0 Agglutination with Anti-B



Group A
4+ Agglutination with Anti-A
0 Agglutination with Anti-B



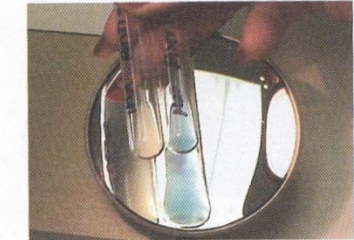
Group AB
4+ Agglutination with Anti-A and Anti-B



Group AB
4+ Agglutination with Anti-A and Anti-B



Group O
No Agglutination with Anti-A or Anti-B



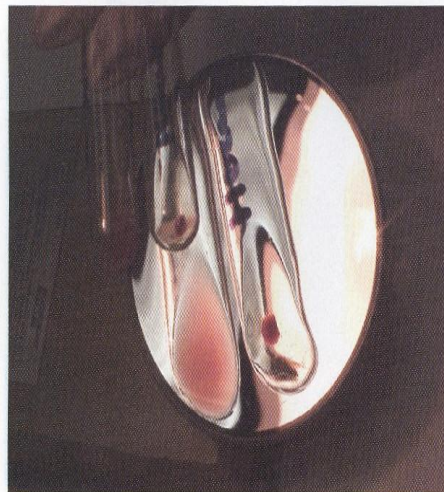
Group O
No Agglutination with Anti-A and Anti-B

* Consult manufacturer's package insert for specifics.

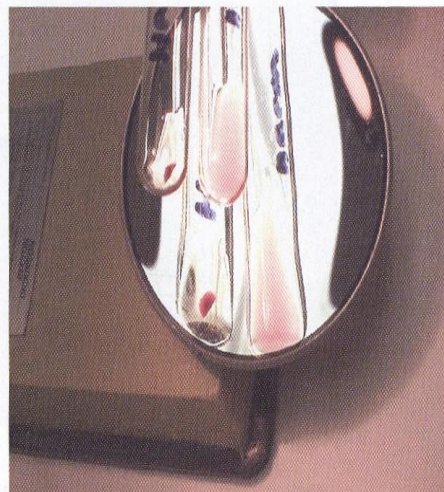
FIGURE 6-1 Procedure for forward grouping. Principle: Detection of antigens on the patient's RBCs with known commercial antisera. (Continued on the following page)

FIGURE 6-1 (continued) Procedure for forward grouping. Principle: Detection of antigens on the patient's RBCs with known commercial antisera.

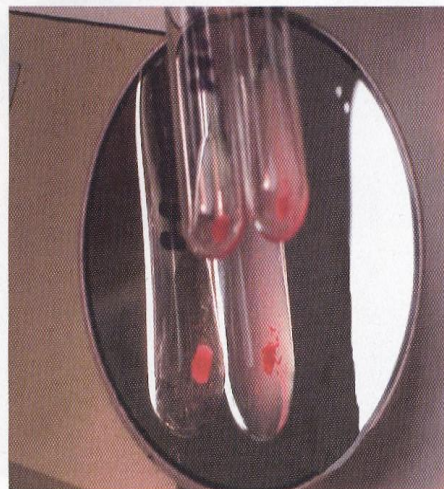
Step 5: Resuspend cells button; interpret and record results.



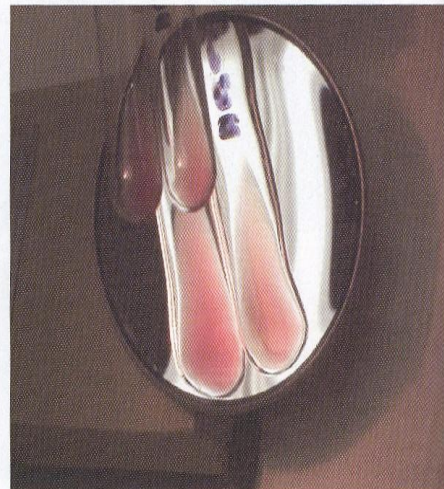
Group A
4+ Agglutination with B Cells
0 Agglutination with A₁ Cells



Group B
4+ Agglutination with A₁ Cells
0 Agglutination with B Cells

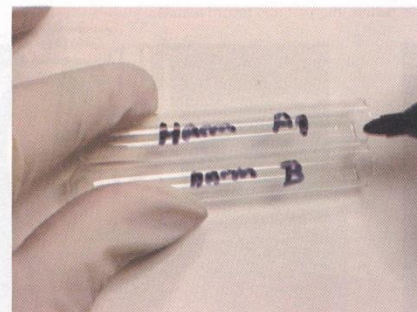


Group O
4+ Agglutination with A₁ Cells
3+ Agglutination with B Cells



Group AB
No Agglutination with A₁ and B Cells

■ **FIGURE 6-2 (continued)** Procedure for reverse grouping. Principle: Detection of antibodies in the patient's serum with known commercial antisera.



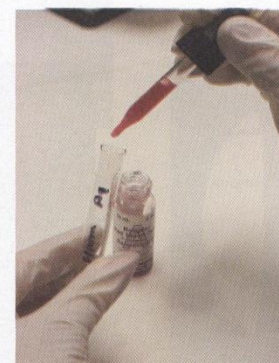
Step 1: Label Test Tubes



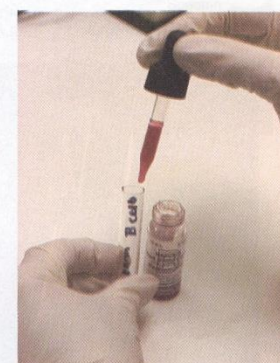
Step 2: Add two drops of patient serum to each tube



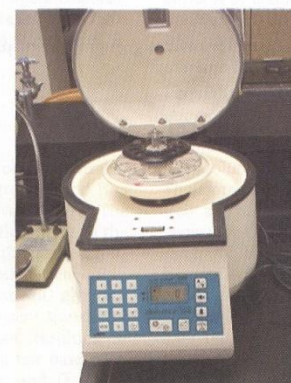
3: Add one drop of reagent cells* to each test tube



Step 3A: Add one drop of Reagent A₁ cells



Step 3B: Add one drop of Reagent B cells



Step 4: Mix and centrifuge (approximately 20 seconds)

* Consult manufacturer's package insert for specifics.

■ **FIGURE 6-2** Procedure for reverse grouping. Principle: Detection of antibodies in the patient's serum with known commercial antisera. (Continued on the following page)

Tube test for Rh testing

- Specimen

- Clotted or anticoagulated blood samples
- Do not use corvac (Gel) tube

- Reagents

- blended IgM/IgG monoclonal/ polyclonal low protein anti-D
- Use reagents accordance with manufacturer's instructions
- ***Use two different anti-D serum for each test***
- ***Rh control reagent to be used for each test***
- AHG(anti-IgG)
- **IgG coated red cells**

Tube test for Rh testing

- Quality control to be performed daily
- Follow S.O.P to perform the Rh(D) test

جدول A

Anti-D	Rh-Control	تفسیر Interpretation
≥ 2	0	نوع Rh بیمار Positive گزارش شود.
< 2	0	از مرحله ۱۰ ادامه دهید.
Positive	Positive	Unresolved

جدول B

Anti-D	Rh-Control	تفسیر Interpretation
≥ 2	0	Rh-Positive
0	0	Rh-Negative
Positive	Positive	Unresolved

Tube test for Rh testing

Weak D (Du)

is a weakly expressed D antigen that will only be demonstrated after incubation at 35-37°C followed with antiglobulin testing.(ie being demonstrated only by coombs technique).

Why do weak D's exist?

There are three explanations for weak D's.

- **Quantitative weak D** there are individuals quantitatively produce fewer D antigen sites.
- **Position Effect weak D** in this case the D weakend by the position of a C on the opposite haplotype which is called the trans position Dce/Ce and DcE/Ce.
- **Partial D antigen (Mosaic D)** in this type of weak D , the individuals lack some of the components of the D antigen and therefore are able to make allanibodies to those specific components if they are transfused with D positive blood.

Many valuable lives depends on your work

