Blood**GROUPING**

ANTI - A, B & $D_{(IgG+IgM)}$

3 x 10 mL
13601001

Intended Use
These reagents are *in vitro* diagnostic medical device for professional use. The Anti-A reagent is intended for the *in vitro* detection and identification of the human A blood group antigen, Anti-B is intended for the *in vitro* detection and identification of the human B blood group antigen and Anti $D_{(IgG+IgM)}$ reagent is intended for the *in vitro* detection of presence or absence of Rho (D) antigen on human red blood cells by direct agglutination.

Principle & Clinical Significance
The ABO grouping is defined by both, the presence of 'A' and/or 'B' antigens on the surface of the red blood cells and by simultaneous presence of Anti-A and or Anti-B antibodies in the serum. An individual has in his serum the antibodies corresponding to the antigens which are not present on his red blood cells.

Based on the principle of hemagglutination, human red blood cells possessing 'A' and /or 'B' antigen will agglutinate in the presence of corresponding antibody directed towards the antigen.

Human red blood cells are classified as Rh positive (Rh+) or Rh negative (Rh-) depending upon the presence or absence of 'D' antigen on them. Red blood cells possessing D antigen will agglutinate in the presence of reagent containing corresponding antibody.

Reagent Composition
Anti-A **1 x 10 mL**

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatants of in vitro culture of hybridoma of murine origin. The cell line used is 9113 D10. The reagent is a clear blue solution.

Anti-B **1 x 10 mL**

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatant of *in vitro* culture of hybridoma of murine origin. The cell line used is 9621A8. The reagent is a clear yellow solution.

Anti-$D_{(IgG+IgM)}$ **1 x 10 mL**

The reagent is prepared from a monoclonal blend of IgG+IgM antibodies in a storage medium. The monoclonal antibodies are produced from supernatants of *in vitro* culture of hybridoma of human origin. The cell lines used are P3x61, P3x21223B10, P3x35 and P3x290. The reagent is a clear colourless solution.

All the above reagents contain Sodium azide (<0.1%), Sodium arsenite (<0.02%) and Bovine albumin as preservative/stabilizer.

Risk & Safety
Material Safety data sheets (MSDS) will be provided on request.

Storage and Stability
The sealed reagent is stable up to expiry date stated on the vial label, when stored at 2-8°C. Do not use if turbid. Do not dilute. Do not use the reagent beyond the expiry date mentioned on the vial. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses. DO NOT FREEZE.

Sample
Whole blood sample must be examined with in 48 hrs. Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples.

Precautions for Use and Disposal
It is advisable to wear gloves and safety goggles and handle test samples of human origin with caution. Do not use damaged or leaking reagents. This reagent contains <0.1% Sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded to sink flush with a large volume of water to prevent azide build up. As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

Materials Required But Not Provided
Isotonic Saline
Glass Test Tubes
Glass Slides
Pipettes
Optical Aid
Centrifuge
Reagent Red Blood Cells for use in ABO grouping
Timer

Laboratory Procedure
a) Plate Technique at Room Temperature

Bring the reagents to room temperature. Place one drop of Anti-A, Anti -B & Anti-D reagent on a clean slide, and then add one small drop of whole blood. Mix well with a mixing stick uniformly. Rock the slide gently back and forth for about 3 minutes while macroscopically observing the possible appearance of agglutination.

b) Direct Method in a Tube at Room Temperature

Prepare a 5% suspension of red blood cells in isotonic saline solution. Using the vial dropper, place one drop of Anti-A, Anti -B & Anti-D reagent in to labeled test tube. Add a drop of 5 % RBC suspension. Shake to homogenize the mixture, then centrifuge at 1000 RPM for 60 seconds. Read macroscopically for appearance of any agglutination.

Interpretation
With both the above methods, if there is agglutination (the red blood cells form one or several clumps) the reaction is positive and the antigens or at least one of the antigen corresponding to the reagent used is present on the red blood cells tested.

If there is no agglutination (red blood cells form a homogenous suspension), the reaction is negative and the antigen is not present on the red blood cells.

In case of Anti D agglutination, a positive test indicates presence of Rh antigen i.e. Rh Positive. No agglutination or a negative test indicated either presence of a weakerr variant of Rh antigens like Du or absence of Rh antigen. This needs confirmation by carrying out indirect Coomb's test.

Drying at the periphery or fibrin strands should not be misinterpreted as agglutination.

NOTE:
1. The reactions must be read immediately after centrifuging and re-suspending.
2. Anti $D_{(IgG+IgM)}$ have the special feature of recognizing certain rare antigen motives of type RH33 (RoHar) and may thus yield discordant reactions with polyclonal reagents that recognize them little or not at all.
3. For specific identification of partial D antigen, the use of any 3rd party kit for this purpose is recommended.
4. A false positive reaction may occur if the subject tested has cold agglutinatinins.
5. Anti $D_{(IgG+IgM)}$ cannot ensure the recognition of all weak or variant subjects, due to the variability of antigen motifs.
6. Certain discordances (negative reaction for the direct hemagglutination method and positive reaction for the indirect antiglobulin method) may occur with Anti-$D_{(IgG+IgM)}$. A weak and/or partial D antigen may be suspected.
7. A false –positive reaction may occur, when Anti $D_{(IgG+IgM)}$ is used in the indirect antiglobulin method (IAT), if the RBC from the test subject shows a positive reaction in the direct antiglobulin test (DAT).
8. A negative reaction obtained in an indirect antiglobulin test can be validated with IgG-sensitized red blood cells (not provided with this kit).
9. **For getting high titre use ABD dilution buffer. The ABD dilution buffer will be provided on request.**

Clonal Specificity of Anti-$D_{(IgG+IgM)}$

			D									
			IIIa	D	D	D	D	D	D	D		HMi
		D	IIIb	IVa	IVb	Va	VI	VII	FR	BT	RoHor	
Clones	Type	II	IIIc								(RH33)	
P3x61	IgM	+	+	+	+	+	-	+	+/-	+	+	+
P3x21223B10	IgM	-	+	-	-	+	+	+	+	-	-	+
P3x290	IgG	+	+	+/-	-	+	+/-	+	+	-	-	+
P3x35	IgG	+	+	+	+	-	-	+	-	-	-	+

The table gives the specificity of clones used in Anti-$D_{(IgG+IgM)}$ reagent against variants D Antigen. The intensity of reactions obtained with Anti-$D_{(IgG+IgM)}$ may vary as a function of the number of antigen sites present on the test red blood cells.

SYMBOLS USED ON THE LABELS

SYMBOLS USED ON THE LABELS: IVD IN VITRO DIAGNOSTIC USE | SEE PACKAGE INSERT FOR PROCEDURE | LOT LOT NUMBER | MANUFACTURER'S ADDRESS | MANUFACTURING DATE | EXPIRY DATE | TEMPERATURE LIMIT

BIOSS BioTechnology, GmbH
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ADS/V.02/060114

CE ISO 9001 : 2008
ISO 13485 : 2003

ANTI - A, B & $D_{(IgG+IgM)}$

3 x 10 mL
13601001

Performance Characteristics

In the recommended methods, the reagent comply with the common technical specifications of IVDMD. The assessment demonstrated 100% specificity of the reagent versus expected result. The potency of the reagent has been tested against the following minimum potency reference standard defined by NIB

Anti-A Titre ≥1:256 to 1:512 (Macroscopically) and ≥1:512 (Microscopically)

Anti-B Titre ≥1:256 to 1:512 (Macroscopically) and ≥1:512 (Microscopically)

Anti-$D_{(IgG+IgM)}$ Titre ≥1:64 to 1:128 (Macroscopically) and ≥1:256 (Microscopically)

For getting high titre >1:512 or >1:256 as the case may be, it is recommended to use the **ABD Dilution Buffer** (Product Code: 13601330), which will be available on request.

Limitations

1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. All negative slide tests should be confirmed by tube testing to confirm absence of weak sub groups.
3. Stored blood may give weaker reactions than fresh blood.
4. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
5. False positive or false negative results can occur due to contamination of test material, improper reaction temperature, improper storage materials, omission of test reagents and certain disease states or deviation from recommended technique.

Bibliography

1. Common Technical Specifications (CTs) for the in vitro diagnosis medical devices of annex II, list A, of directive 98/79/EC are notified in the official journal of the European communities under document No.C (2202) 1344, with EEA relevance (2202/364/CE).
2. Mannessier .L Blood transfusion center Lille France. The use of monoclonal antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion –Rome-June 1992.
3. Blood group serology, Boorman, Dodd and Lincoln, Churchill Livingstone, 6th edition.
4. Human blood groups, Geoff Daniels, 1st edition, Blackwell Science, Oxford 1995.

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ADS/V.02/060114

CE ISO 9001 : 2008
ISO 13485 : 2003

BloodGROUPING

BIOSS
See Life More Clearly

ANTI - A

1 x 10 mL
13601010

Anti - A (Murine Monoclonal IgM)

Intended Use
This reagent is *in vitro* diagnostic medical device for professional use. The Anti-A reagent is intended for the *in vitro* detection and identification of the human A blood group antigen by direct agglutination.

Principle
The manual technique employed, on a plate or in a tube utilizes the principle of hemagglutination. When used by the recommended technique, this reagent will cause agglutination (clumping) of red blood cells carrying the A antigen. Lack of agglutination demonstrates the absence of the A antigen.

Reagent Composition
Anti-A **1 x 10 mL**

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatants of in vitro culture of hybridoma of murine origin. The cell line used is 9113 D10. This reagent contains sodium azide (<0.01%), Sodium arsenite (<0.02%) and Bovine albumin. The reagent is a clear blue solution.

Risk & Safety
Material Safety data sheets (MSDS) will be provided on request.

Storage and Stability
The sealed reagent is stable up to expiry date stated on the vial label, when stored at 2-8°C. Do not use if turbid. Do not dilute. Do not use the reagent beyond the expiry date mentioned on the vial. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses. DO NOT FREEZE.

Sample
Whole blood sample must be examined with in 48 hrs. Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples.

Precautions for Use and Disposal
It is advisable to wear gloves and safety goggles and handle test samples of human origin with caution. Do not use damaged or leaking reagents. This reagent contains <0.1% Sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded to sink flush with a large volume of water to prevent azide build up. As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

Materials Required But Not Provided
Isotonic Saline
Reagent Red Blood Cells for use in ABO grouping
Glass Test Tubes
Glass Slides
Pipettes
Optical Aid
Centrifuge
Timer

Laboratory Procedure
a) Plate Technique at Room Temperature

Bring the reagents to room temperature. Place one drop of Anti-A reagent on a clean slide, and then add one small drop(40-50 µL) of whole blood. Mix well with a mixing stick uniformly. Rock the slide gently back and forth for about 3 minutes while macroscopically observing the possible appearance of agglutination.

b) Direct Method in a Tube at Room Temperature

Prepare a 5% suspension of red blood cells in isotonic saline solution. Using the vial dropper, place one drop of Anti-A reagent in to labeled test tube. Add a drop of 5 % RBC suspension. Shake to homogenize the mixture, then centrifuge at 1000 RPM for 60 seconds. Read macroscopically while gently shaking the tubes so as to detach the red blood cell pellets. Note the appearance of any agglutinates.

Interpretation
If there is agglutination (i.e clumping of red blood cells) the test result indicates the presence of A antigen.
If there is no visible agglutination (i.e clumping is absent), the test result indicates the absence of A antigen.

Note:
For getting high titre use ABD dilution buffer. The ABD dilution buffer will be provided on request.

Performance Characteristics
In the recommended methods, the reagent comply with the common technical specifications of IVDMD. The assessment demonstrated 100% specificity of the reagent versus expected result. The potency of the reagent has been tested against the following minimum potency reference standard defined by NIB
Anti-A Titre ≥1:256 to 1:512 (Macroscopically) and ≥1:512 (Microscopically)
For getting high titre>1:512 as the case may be, it is recommended to use the **ABD Dilution Buffer** (Product Code: 13601330), which will be available on request.

Limitations
1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. All negative slide tests should be confirmed by tube testing to confirm absence of weak sub groups.
3. Stored blood may give weaker reactions than fresh blood.
4. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
5. False positive or false negative results can occur due to contamination of test material, improper reaction temperature, improper storage materials, omission of test reagents and certain disease states or deviation from recommended technique.

Bibliography

1. Mannessier, L.; Blood transfusion center Lille France. The use of monoclonal antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion –Rome-June 1992.
2. Blood group serology, Boorman, Dodd and LincolChurchil Livingstone 6th edition.
3. Human blood groups, Geoff Daniela, 1st edition Blackwell Science, Oxford 1995.

SYMBOLS USED ON THE LABELS

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ADS/V.02/060114

CE ISO 9001 : 2008
ISO 13485 : 2003

BloodGROUPING

BIOSS
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ANTI - B

1 x 10 mL
13601009

Anti - B (Murine Monoclonal IgM)

Intended Use
This reagent is *in vitro* diagnostic medical device for professional use. The Anti-B reagent is intended for the *in vitro* detection and identification of the human B blood group antigen by direct agglutination.

Principle
The manual technique employed, on a plate or in a tube utilizes the principle of hemagglutination. When used by the recommended technique, this reagent will cause agglutination (clumping) of red blood cells carrying the B antigen. Lack of agglutination demonstrates the absence of the B antigen.

Reagent Composition
Anti-B **1 x 10 mL**

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatant of *in vitro* culture of hybridoma of murine origin. The cell line used is 9621A8. The reagent contains Sodium azide (<0.1%), Sodium arsenite (<0.02%) and Bovine albumin. The reagent is a clear yellow solution.

Risk & Safety
Material Safety data sheets (MSDS) will be provided on request.

Storage and Stability
The sealed reagent is stable up to expiry date stated on the vial label, when stored at 2-8^{o}C. Do not use if turbid. Do not dilute. Do not use the reagent beyond the expiry date mentioned on the vial. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses. DO NOT FREEZE.

Sample
Whole blood sample must be examined with in 48 hrs. Samples should be stored at 2-8^{o}C, if not tested immediately. Do not use haemolysed samples.

Precautions for Use and Disposal
It is advisable to wear gloves and safety goggles and handle test samples of human origin with caution. Do not use damaged or leaking reagents.This reagent contains <0.1% Sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded to sink flush with a large volume of water to prevent azide build up. As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

Materials Required But Not Provided
Isotonic Saline
Reagent Red Blood Cells for use in ABO grouping
Glass Test Tubes
Glass Slides
Pipettes
Optical Aid
Centrifuge
Timer

Laboratory Procedure
a) Plate Technique at Room Temperature

Bring the reagents to room temperature. Place one drop of Anti-B reagent on a clean slide, and then add one small drop(40-50 µL) of whole blood. Mix well with a mixing stick uniformly. Rock the slide gently back and forth for about 3 minutes while macroscopically observing the possible appearance of agglutination.

b) Direct Method in a Tube at Room Temperature

Prepare a 5% suspension of red blood cells in isotonic saline solution. Using the vial dropper, place one drop of Anti-B reagent in to labeled test tube. Add a drop of 5 % RBC suspension. Shake to homogenize the mixture, then centrifuge at 1000 RPM for 60 seconds. Read macroscopically while gently shaking the tubes so as to detach the red blood cell pellets. Note the appearance of any agglutinates.

Interpretation
If there is agglutination (i.e clumping of red blood cells) the test result indicates the presence of B antigen.
If there is no visible agglutination (i.e clumping is absent), the test result indicates the absence of B antigen.

Note:
For getting high titre use ABD dilution buffer. The ABD dilution buffer will be provided on request.

Performance Characteristics
In the recommended methods, the reagent comply with the common technical specifications of IVDMD. The assessment demonstrated 100% specificity of the reagent versus expected result. The potency of the reagent has been tested against the following minimum potency reference standard defined by NIB
Anti-B Titre ≥1:256 to 1:512 (Macroscopically) and ≥ 1:512 (Microscopically)
For getting high titre>1:512 as the case may be, it is recommended to use the **ABD Dilution Buffer** (Product Code: 13601330), which will be available on request.

Limitations
1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. All negative slide tests should be confirmed by tube testing to confirm absence of weak sub groups.
3. Stored blood may give weaker reactions than fresh blood.
4. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
5. False positive or false negative results can occur due to contamination of test material, improper reaction temperature, improper storage materials, omission of test reagents and certain disease states or deviation from recommended technique.

Bibliography

1. Mannessier, L.; Blood transfusion center Lille France. The use of monoclonal antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion –Rome-June 1992.
2. Blood group serology, Boorman, Dodd and LincolChurchil Livingstone 6th edition.
3. Human blood groups, Geoff Daniela, 1st edition Blackwell Science, Oxford 1995.

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ADS/V.02/060114

CE ISO 9001 : 2008
ISO 13485 : 2003

Blood**GROUPING**

BIOSS
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ANTI - $D_{(IgG+IgM)}$

1 x 10 mL
13601003

Anti - D(IgG+IgM) Monoconal

Intended Use
This reagent is *in vitro* diagnostic medical device for professional use. The Anti D(IgG+IgM) reagent is intended for the *in vitro* detection of presence or absence of Rho (D) antigen on human red blood cells.

Principle
The manual technique employed, on a plate or in a tube utilizes the principle of hemagglutination. Human erythrocytes having Rho Antigen (D-Ag) will cause agglutination is the presence of Anti-D antibodies present in the reagent. Human red blood cells are classified as Rho(D) positive or Rho (D) negative depending upon the presence or absence of D(Rho) antigen on them. All negative test results should be further confirmed by Coomb's test.

Reagent Composition
Anti-D(IgG+IgM) **1 x 10 mL**

The reagent is prepared from a monoclonal blend of IgG+IgM antibodies in a storage medium. The monoclonal antibodies are produced from supernatants of *in vitro* culture of hybridoma of human origin. The cell lines used are P3x61, P3x21223B10, P3x35 and P3x290. The reagent contains Sodium azide (<0.1%), Sodium arsenite (<0.02%) and Bovine albumin. The reagent is a clear colourless solution.

Risk & Safety
Material Safety data sheets (MSDS) will be provided on request

Storage and Stability
The sealed reagent is stable up to expiry date stated on the vial label, when stored at 2-8°C. Do not use if turbid. Do not dilute. Do not use the reagent beyond the expiry date mentioned on the vial. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses. DO NOT FREEZE.

Sample
Whole blood sample must be examined with in 48 hrs. Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples.

Precautions for Use and Disposal
It is advisable to wear gloves and safety goggles and handle test samples of human origin with caution. Do not use damaged or leaking reagents.This reagent contains <0.1% Sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded to sink flush with a large volume of water to prevent azide build up. As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

Materials Required But Not Provided
Isotonic Saline
Glass Test Tubes
Glass Slides
Pipettes
Optical Aid
Centrifuge
Reagent Red Blood Cells for use in ABO grouping
Timer

Laboratory Procedure
a) Plate Technique at Room Temperature

Bring the reagents to room temperature. Place one drop of Anti-D(IgG+IgM) reagent on a clean slide, and then add one small drop(40-50 µL) of whole blood. Mix well with a mixing stick uniformly. Rock the slide gently back and forth for about 3 minutes while macroscopically observing the possible appearance of agglutination.

b) Direct Method in a Tube at Room Temperature

Prepare a 5% suspension of red blood cells in isotonic saline solution. Using the vial dropper, place one drop of Anti-D(IgG+IgM) reagent in to labeled test tube. Add a drop of 5 % RBC suspension. Shake to homogenize the mixture, then centrifuge at 1000 RPM for 60 seconds. Read macroscopically while gently shaking the tubes so as to detach the red blood cell pellets. Note the appearance of any agglutinates.

Interpretation
If there is agglutination (i.e. clumping red blood cells) while using direct hemagglutination method on a plate or in a tube, the test result indicates the presence of D antigen (Rh positive).

If there is no agglutination, while using direct hemagglutination method on a plate or in a tube, the test result indicates the absence of D antigen (Rh negative).

The presence or absence of weak and / or partial D antigen, should be confirmed by carrying out Indirect Antiglobulin Test (IAT).

Drying at the periphery or fibrin strands should not be misinterpreted as agglutination.

Notes:
1. The reactions must be read immediately after centrifuging and re-suspending.
2. Anti-$D_{(IgG+IgM)}$ have the special feature of recognizing certain rare antigen motives of type RH33 (RoHar) and may thus yield discordant reactions with polyclonal reagents that recognize them little or not at all.
3. For specific identification of partial D antigen, the use of any 3rd party kit for this purpose is recommended.
4. A false positive reaction may occur if the subject tested has cold agglutinatinins.
5. Anti D (IgG+IgM) cannot ensure the recognition of all weak or variant subjects, due to the variability of antigen motifs.
6. Certain discordances (negative reaction for the direct hemagglutination method and positive reaction for the indirect antiglobulin method) may occur with Anti D (IgG+IgM). A weak and/or partial D antigen may be suspected.
7. A false –positive reaction may occur, when Anti D (IgG+IgM) is used in the indirect antiglobulin method (IAT), if the RBC from the test subject shows a positive reaction in the direct antiglobulin test (DAT).
8. A negative reaction obtained in an indirect antiglobulin test can be validated with IgG-sensitized red blood cells (not provided with this kit).
9. **For getting high titre use ABD dilution buffer. The ABD dilution buffer will be provided on request.**(Product Code: 13601330)

Clonal Specificity of Anti-$D_{(IgG + IgM)}$

			D									
			IIIa	D	D	D	D	D	D	D		HMi
		D	IIIb	IVa	IVb	Va	VI	VII	FR	BT	RoHor	
Clones	Type	II	IIIc								(RH33)	
P3x61	IgM	+	+	+	+	+	-	+	+/-	+	+	+
P3x21223B10	IgM	-	+	-	-	+	+	+	+	-	-	+
P3x290	IgG	+	+	+/-	-	+	+/-	+	+	-	-	+
P3x35	IgG	+	+	+	+	-	-	+	-	-	-	+

The table gives the specificity of clones used in Anti D (IgG+IgM) reagent against variants D Antigen. The intensity of reactions obtained with Anti-$D_{(IgG+IgM)}$ may vary as a function of the number of antigen sites present on the test red blood cells.

Performance Characteristics
In the recommended methods, the reagent comply with the common technical specifications of IVDMD. The assessment demonstrated 100% specificity of the reagent versus expected result. The potency of the reagent has been tested against the following minimum potency reference standard defined by NIB.

Anti-$D_{(IgG+IgM)}$ Titre≥ 1:64 to 1:128 (Macroscopically) and ≥ 1:256 (Microscopically)

For getting high titre>1:256, it is recommended to use the **ABD Dilution Buffer** (Product Code: 13601330), which will be available on request.

Limitations
1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. All negative slide tests should be confirmed by tube testing to confirm absence of weak sub groups.
3. Stored blood may give weaker reactions than fresh blood.

SYMBOLS USED ON THE LABELS

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ADS/V.02/060114

CE ISO 9001 : 2008
ISO 13485 : 2003

4. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
5. False positive or false negative results can occur due to contamination of test material, improper reaction temperature, improper storage materials, omission of test reagents and certain disease states or deviation from recommended technique.

Bibliography

1. Mannessier, L.; Blood transfusion center Lille France. The use of monoclonal antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion –Rome-June 1992.
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Blood**GROUPING**

BIOSS
See Life More Clearly

ANTI - $D_{(IgM)}$

1 x 10 mL
13601015

Anti - D(IgM) Monoclonal

Intended Use
This reagent is *in vitro* diagnostic medical device for professional use. This Anti D(IgM) reagent is intended for the *in vitro* detection of presence or absence of Rho (D) antigen on human red blood cells.

Principle
The manual technique employed, on a plate or in a tube utilizes the principle of hemagglutination. The test procedure recommended for the use of this antisera are based upon the agglutination (clumping) of red blood cells carrying the D antigen in the presence of an IgM Anti-D antibody. All negative test results should be further confirmed by Du test.

Reagent Composition
Anti-$D_{(IgM)}$ 1 x 10 mL
The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatants of in vitro culture of hybridoma of human origin. The cell line used is P3x61. The reagent contains Sodium azide (<0.1%), Sodium arsenite (<0.02%) and Bovine al bumin. The reagent is a clear colourless solution.

Risk & Safety
Material Safety data sheets (MSDS) will be provided on request

Reagent Storage and Stability
The sealed reagents are stable up to expiry date stated on the label when stored at 2-8°C. Do not use if turbid. Do not dilute. Do not use the reagent beyond the expiry date mentioned on the vial. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses. DO NOT FREEZE

Sample
Whole blood samples must be examined within 48 hrs. Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples.

Precautions for Use and Disposal
It is advisable to wear gloves and safety goggles and handle test samples of human origin with caution. Do not use damaged or leaking reagents. This reagent contains <0.1% Sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded to sink flush with a large volume of water to prevent azide build up. As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

Materials Required But Not Provided
Isotonic Saline
Reagent Red Blood Cells for use in ABO grouping
Glass Test Tubes
Glass Slides
Pipettes
Optical Aid
Centrifuge
Timer

Laboratory Procedure

a) Plate Technique at Room Temperature
Bring the reagents to room temperature. Place one drop of Anti-$D_{(IgM)}$ reagent on a clean slide, then add one small drop (40-50 µL) of whole blood. Mix well with a mixing stick uniformly. Rock the slide gently back and forth for about 3 minutes while macroscopically observing the possible appearance of agglutination.

b) Direct Method in a Tube at Room Temperature
Prepare a 5% suspension of red blood cells in isotonic saline solution. Using the vial dropper, transfer a drop reagent Anti- $D_{(IgM)}$ reagent in to a labelled test tube. Add a drop of red blood cell suspension. Shake to homogenize the mixture, then centrifuge at 1000 rpm for one minute. Read macroscopically while gently shaking the tubes so as to detach the RBC pellets. Note the appearance of any agglutination.

Interpretation
If there is agglutination (ie, clumping red blood cells) with Anti-$D_{(IgM)}$ antigen D is present.
If there is no agglutination (ie, clumping is absent) it is possible to use Anti D (IgG) in a coomb's test to rule out the presence of weak and partial D antigens.

NOTES
1. The reactions must be read immediately after centrifuging and resuspending.
2. A false positive reaction may occur if the subject tested has cold agglutinins.
3. Anti $D_{(IgM)}$ cannot ensure the recognition of weak or variant subjects, due to the variability of antigen motifs.
4. **For getting high titre use ABD dilution buffer** (Product Code: 13601330) . **The ABD dilution buffer will be provided on request.**

Performance Characteristics
In the recommended methods, the reagent comply with the common technical specifications of IVDMD. The assessment demonstrated 100% specificity of the reagent versus expected result. The potency of the reagent has been tested against the following minimum potency reference standard defined by NIB.
Anti - D(IgM) Titre≥ 1:64 to 1:128 (Macroscopically) and ≥ 1:256 (Microscopically)
For getting high titre>1:256, it is recommended to use the **ABD Dilution Buffer** (Product Code: 13601330), which will be available on request.

Limitations
1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. All negative slide tests should be confirmed by tube testing to confirm absence of weak sub groups.
3. Stored blood may give weaker reactions than fresh blood.
4. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
5. False positive or false negative results can occur due to contamination of test material, improper reaction temperature, improper storage materials, omission of test reagents and certain disease states or deviation from recommended technique.

Bibliography

1. Betermieux, C., Beolet, M. , Keyser, L.; A new strategy for D phenotyping with TOTEM multimonoclonal ANTI –D reagent, XX111rd I.S.B.T. Congress, July 1994.
2. Aramburu, E., Rabasa, P. , Esquiroz, R., Galarretat, B. Valoracion de un antisureo anti-D IgM-IgG monoclonal (DIAGAST) endonantes de sangre concentration expresividaddedli del anti geno. Hematology congress, Madrid, October 1990.
3. Mannessier, L. Blood transfusion center, Lille, France. The use of monoclonal antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion ,Rome,June 199

SYMBOLS USED ON THE LABELS

SYMBOLS USED ON THE LABELS: IVD IN VITRO DIAGNOSTIC USE | SEE PACKAGE INSERT FOR PROCEDURE | LOT LOT NUMBER | MANUFACTURER'S ADDRESS | MANUFACTURING DATE | EXPIRY DATE | TEMPERATURE LIMIT

BIOSS BioTechnology, GmbH
Boekhulter Weg 1a, 47638 Straelen, Germany
E-mail: sales@biossbiotech.de, support@biossbiotech.de
EC REP Arthrex GmbH
Erwin-Hleischer-Strasse 9, 81249 Munchen, Germany

ADS/V.02/060114

CE ISO 9001 : 2008
ISO 13485 : 2003

Blood**GROUPING**

BIOSS
See Life More Clearly

ANTI - A, B & $D_{(IgM)}$

3 x 10 mL
13601013

Intended Use
These reagents are *in vitro* diagnostic medical device for professional use. The Anti-A reagent is intended for the *in vitro* detection and identification of the human A blood group antigen, Anti-B is intended for the *in vitro* detection and identification of the human B blood group antigen and Anti-$D_{(IgM)}$ reagent is intended for the *in vitro* detection of presence or absence of Rho (D) antigen on human red blood cells by direct agglutination.

Principle & Clinical Significance
The ABO grouping is defined by both, the presence of 'A' and/or 'B' antigens on the surface of the red blood cells, and by simultaneous presence of Anti-A and or Anti-B antibodies in the serum. An individual has in his serum the antibodies corresponding to the antigens which are not present on his red blood cells.

Based on the principle of hemagglutination, human red blood cells possessing 'A' and /or 'B' antigen will agglutinate in the presence of corresponding antibody directed towards the antigen.

Human red blood cells are classified as Rh positive (Rh+) or Rh negative (Rh-) depending upon the presence or absence of 'D' antigen on them. Red blood cells possessing D antigen will agglutinate in the presence of reagent containing corresponding antibody.

Reagent Composition
Anti-A **1 x 10 mL**

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatants of in vitro culture of hybridoma of murine origin. The cell line used is 9113 D10. The reagent is a clear blue solution.

Anti-B **1 x 10 mL**

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatant of *in vitro* culture of hybridoma of murine origin. The cell line used is 9621A8. The reagent is a clear yellow solution.

Anti-$D_{(IgM)}$ **1 x 10 mL**

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatants of in vitro culture of hybridoma of human origin. The cell line used is P3x61. The reagent contains Sodium azide (<0.1%), Sodium arsenite (<0.02%) and Bovine albumin. The reagent is a clear colourless solution.

All the above reagents contain Sodium azide (<0.1%), Sodium arsenite (<0.02%) and Bovine albumin as preservative/stabilizer.

Risk & Safety
Material Safety data sheets (MSDS) will be provided on request

Reagent Storage and Stability
The sealed reagent is stable up to expiry date stated on the vial label, when stored at 2-8°C. Do not use if turbid. Do not dilute. Do not use the reagent beyond the expiry date mentioned on the vial. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses. DO NOT FREEZE.

Sample
Whole blood sample must be examined with in 48 hrs. Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples.

Precautions for Use and Disposal
It is advisable to wear gloves and safety goggles and handle test samples of human origin with caution. Do not use damaged or leaking reagents. This reagent contains <0.1% Sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded to sink flush with a large volume of water to prevent azide build up. As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

Materials Required But Not Provided
Isotonic Saline
Glass Test Tubes
Glass Slides
Pipettes
Optical Aid
Centrifuge
Reagent Red Blood Cells for use in ABO grouping
Timer

Laboratory Procedure

a) Plate Technique at Room Temperature
Bring the reagents to room temperature. Place one drop of Anti-A,Anti -B & Anti D reagent on a clean slide, and then add one small drop of whole blood. Mix well with a mixing stick uniformly. Rock the slide gently back and forth for about 3 minutes while macroscopically observing the possible appearance of agglutination.

b) Direct Method in a Tube at Room Temperature
Prepare a 5% suspension of red blood cells in isotonic saline solution. Using the vial dropper, place one drop of Anti-A,Anti -B & Anti D reagent in to labeled test tube. Add a drop of 5 % RBC suspension. Shake to homogenize the mixture, then centrifuge at 1000 RPM for 60 seconds. Read macroscopically for appearance of any agglutination.

Interpretation
With both the above methods, if there is agglutination (the red blood cells form one or several clumps) the reaction is positive and the antigen or at least one of the antigens corresponding to the reagent used is present on the red blood cells tested.If there is no agglutination (red blood cells form a homogenous suspension), the reaction is negative and the antigen is not present on the red blood cells.

In case of Anti D agglutination, a positive test indicates presence of Rh antigen i.e. Rh Positive. No agglutination or a negative test indicated either presence of a weakerr variant of Rh antigens like Du or absence of Rh antigen. This needs confirmation by carrying out indirect Coomb's test.

Drying at the periphery or fibrin strands should not be misinterpreted as agglutination.

NOTE

For getting high titre use ABD dilution buffer. The ABD dilution buffer will be provided on request.

Performance Characteristics
In the recommended methods, the reagent comply with the common technical specifications of IVDMD. The assessment demonstrated 100% specificity of the reagent versus expected result. The potency of the reagent has been tested against the following minimum potency reference standard defined by NIB

Anti-A Titre ≥1:256 to 1:512 (Macroscopically) and ≥1:512 (Microscopically)

Anti-B Titre ≥1:256 to 1:512 (Macroscopically) and ≥1:512 (Microscopically)

Anti-$D_{(IgM)}$ Titre ≥1:64 to 1:128 (Macroscopically) and ≥1:256 (Microscopically)

For getting high titre>1:512 or >1:256 as the case may be, it is recommended to use the **ABD Dilution Buffer** (Product Code: 13601330), which will be available on request.

Limitations
1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. All negative slide tests should be confirmed by tube testing to confirm absence of weak sub groups.
3. Stored blood may give weaker reactions than fresh blood.
4. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
5. False positive or false negative results can occur due to contamination of test material, improper reaction temperature, improper storage materials, omission of test reagents and certain disease states or deviation from recommended technique.

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1. Common Technical Specifications (CTs) for the in vitro diagnosis medical devices of annex II, list A, of directive 98/79/EC are notified in the official journal of the European communities under document No.C (2202) 1344, with EEA relevance (2202/364/CE).
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4. Human blood groups, Geoff Daniels, 1st edition, Blackwell Science, Oxford 1995.

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BIOSS BioTechnology, GmbH
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