



ORTHO™  
workstation

ORTHO BioVue®  
System Handbook



TRANSFUSION MEDICINE

Ortho Clinical Diagnostics

J55888EN

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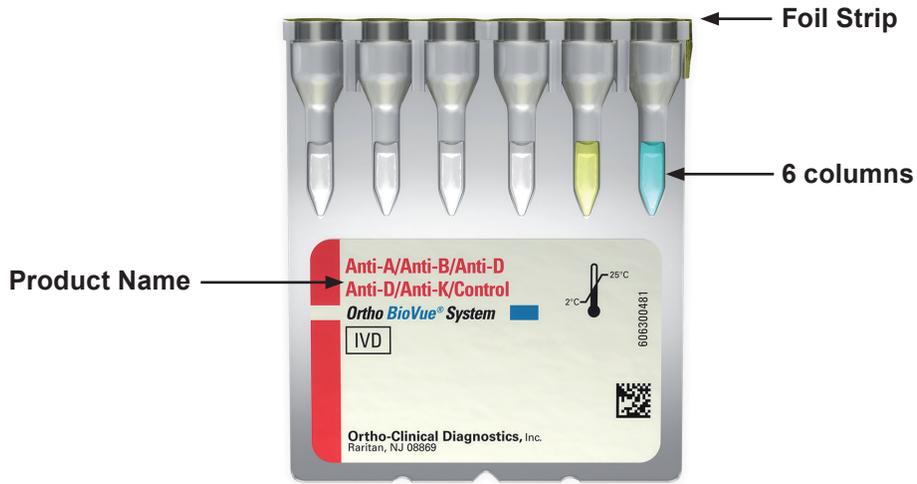
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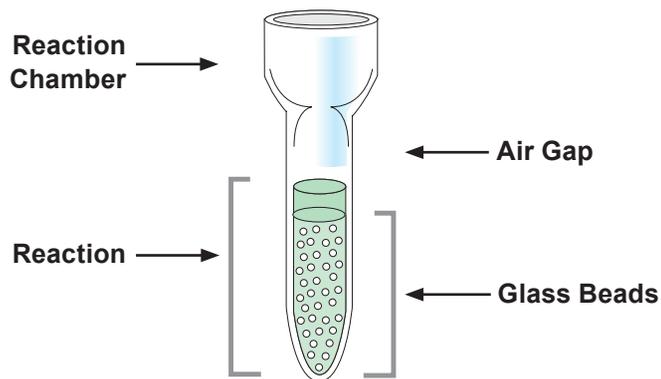
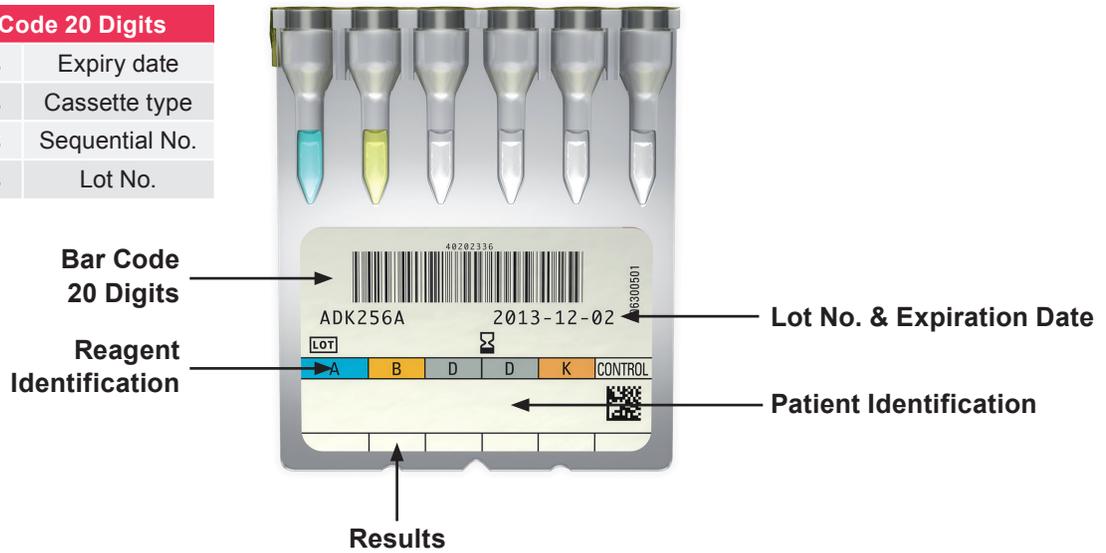
IMPORTANT: All the information included in this hand-book are extracted from the ORTHO BioVue® cassettes Instructions For Use which are the only authorized source for information.

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## ORTHO BioVue® Cassette Description



Bar Code 20 Digits	
6 digits	Expiry date
2 digits	Cassette type
6 digits	Sequential No.
6 digits	Lot No.

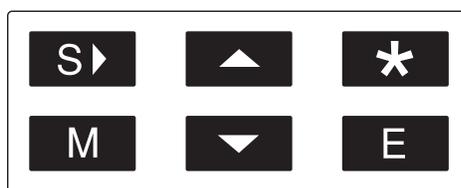
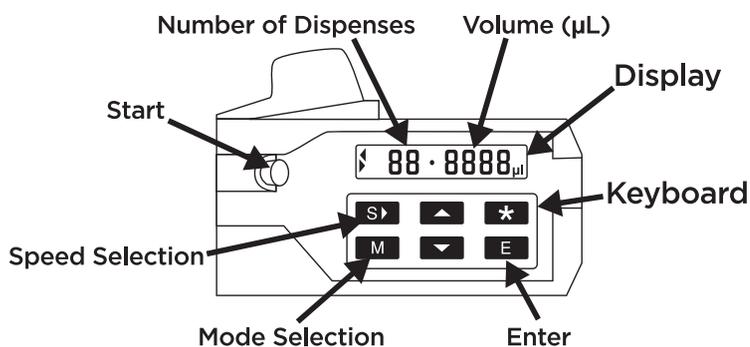
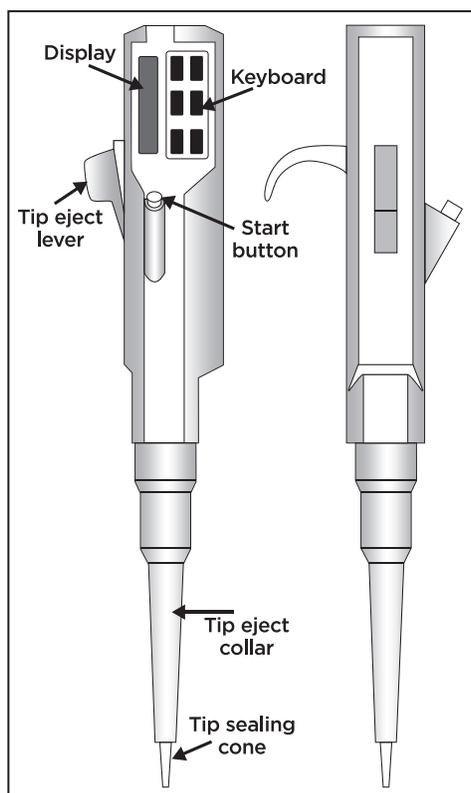


## ORTHO™ Workstation Description



CENTRIFUGE	
Cycle Speed	Phase 1 – 793 rpm $\pm$ 10 rpm Phase 2 – 1509 rpm $\pm$ 10 rpm
Speed Indicator	4 digits
Timing Indicator	4 digits
Cycle Timing	Phase 1 – two minutes $\pm$ 10 sec. (including ramp-up time) Phase 2 – three minutes $\pm$ 10 sec. (including ramp-up time)
Centrifuge Cassette Capacity	10 cassettes
INCUBATOR	
Incubation Temperature	37°C $\pm$ 2°C
Incubator Cassette Capacity	20 cassettes (two sections of 10)

## ORTHO BioVue® Pipette Description



To change the program, **press M**  
 When the desired program appears, **press E**  
 Choose the number of dispenses  
 To confirm, **press E** again

PROGRAM	NUMBER	TAKEN	DELIVERED
P1	1 → 6x	40 → 240 µl	40 µl
P2	1 → 24x	10 → 240 µl	10 µl
P3	1 → 6X	50 → 300 µl	50 µl
P4	1 → 6X	60 → 360 µl	60 µl
P5		190µl + 10 µl	200 µl

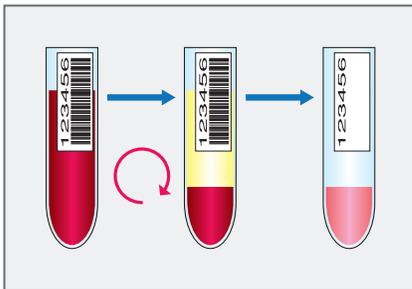
IMPORTANT: In the specific procedures sheets, a symbol like P1 refers to the electronic pipette program to use.

## Procedure to be Applied to ORTHO BioVue® Cassettes

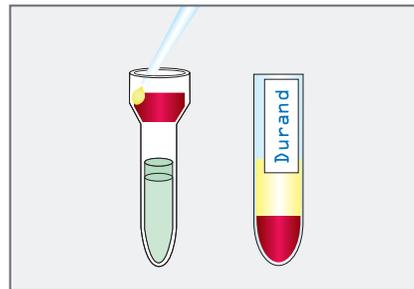
### 1. Visual control of the ORTHO BioVue® cassettes

- + Do not use reagents beyond their labeled expiration date.
- + Do not use cassettes that appear damaged (i.e., break in foil seal or a break, crack or bubble in the column) or exhibit drying (i.e., liquid level is at or below the top of the glass beads) or exhibit discoloration (due to bacterial contamination which can cause false reactions).

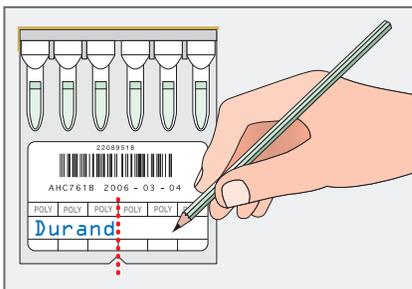
### 2. Sample identification and preparation



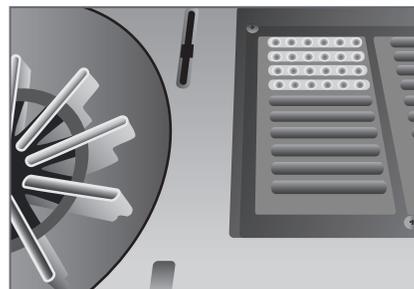
### 5. Sample pipetting



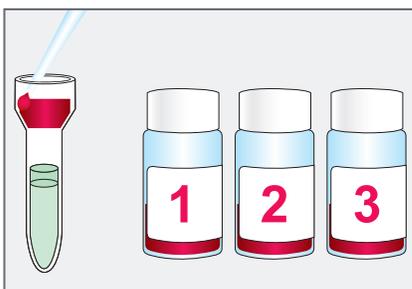
### 3. Cassettes identification and preparation



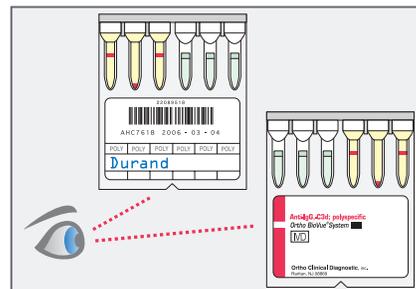
### 6. Incubation – Centrifugation



### 4. Reagent pipetting



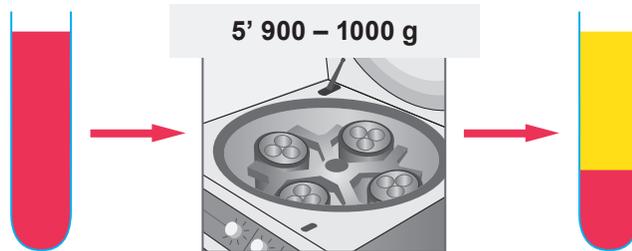
### 7. Reading



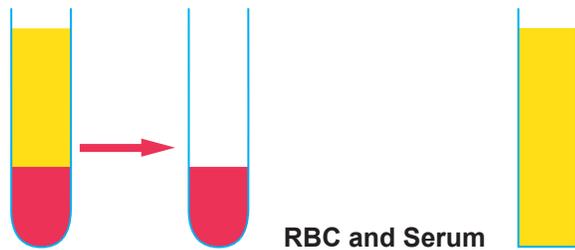
## Sample Preparation

### 1. Sample identification

### 2. Sample centrifugation



### 3. Sample preparation

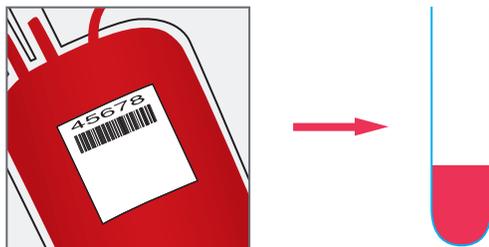


### 4. RBC suspension preparation - A6 or A7

#### MAXIMUM STORAGE TIME AT 2 - 8°C

Anticoagulant	EDTA	Oxalate	Heparin	Citrate	No Anticoagulant
Days	7	2	2	7	7

### Donor RBC Preparation



### 1. Optional: Wash red blood cells one time in isotonic saline.

### 2. Donor RBC suspension preparation - A6 or A7

## A6

### Preparation of 3-5% Suspensions

**1. ORTHO BioVue® electronic pipette - Program P5**

190 µl NaCl + 10 µl packed red blood cells = 200 µl 3-5% red blood cells suspension

**2. Manual preparation**

1 ml NaCl + 50 µl packed red blood cells

## A7

### Preparation of 0.8% Suspensions

**1. In Saline:**

1 ml Saline + 10 µl packed red blood cells

**2. In 0.8% ORTHO® Red Cell Diluent – for 0.8% Cross-Match:**

1 ml 0.8% ORTHO® Red Cell Diluent + 10 µl packed donor red blood cells

**MAXIMUM STORAGE TIME AT 2 - 8°C: 24 HOURS**

## ABO/D Typing

### 1. Cassettes

ORTHO BioVue® ABO-Rh (A, B, AB, D, CDE, Control) or  
 ORTHO BioVue® ABODD (A, B, AB, D, D, Control) or  
 ORTHO BioVue® ABDD/K (A, B, D, D, Kell, Control) or  
 ORTHO BioVue® ABD Confirmation (A, B, D, A, B, D)

**IMPORTANT: Cassettes to be used with the liner!**

### 2. Sample

3-5% patient red blood cells suspensions in saline (A6) or  
 0.8% patient red blood cells suspensions in saline (A7)

### 3. 3-5% Procedure

- + Identify the cassette
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)

### 4. 0.8% Procedure

- + Identify the cassette
- + Add 50 µl 0.8% patient red blood cells suspensions - **P3**
- + 5 minutes centrifugation
- + Reading (both sides)



## ABO Reverse Typing

### 1. Cassettes

ORTHO BioVue® Reverse Diluent (6 x Reverse)

### 2. Reagents

Affirmagen® 4 (A1, A2, B & O cells) *or*

0.8% Affirmagen® 4 (A1, A2, B & O cells) *or*

Affirmagen® 2 (A1 & B cells) *or*

0.8% Affirmagen® 2 (A1 & B cells) *or*

0.8% Affirmagen® 3 (A1, A2 & B cells)

### 3. Sample

Patient Serum/Plasma

### 4. 3-5% Procedure

- + Identify the cassette
- + Add 10 µl Affirmagen® 2 (A1, A2, B & O cells) - **P2**
- + Add 40 µl patient serum/plasma - **P1**
- + 5 minutes centrifugation
- + Reading (both sides)

### 5. 0.8% Procedure

- + Identify the cassette
- + Add 50 µl 0.8% Affirmagen® 2 (A1, A2, B & O cells) - **P3**
- + Add 40 µl patient serum/plasma - **P1**
- + 5 minutes centrifugation
- + Reading (both sides)



## ABO/D Typing & ABO Reverse Typing

### 1. Cassettes

ORTHO BioVue® ABO-Rh D Combo (A, B, D, Control, Reverse, Reverse)

**IMPORTANT: Cassettes to be used with the liner!**

### 2. Reagents

Affirmagen® 2 (A1 & B cells) *or*  
0.8% Affirmagen® 2 (A1 & B cells)

### 3. Sample

3-5% patient or donor red blood cells suspensions (A6) *or*  
0.8% patient or donor red blood cells suspensions (A7) *and*  
Patient Serum/Plasma

### 4. 3-5% Procedure

- + Identify the cassette
- + Add 10 µl Affirmagen® 2 (A1 & B cells) in the reverse columns - **P2**
- + Add 40 µl patient serum/plasma in the reverse columns - **P1**
- + Add 10 µl 3-5% patient red blood cells suspensions in the A, B, D & Control columns - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)

### 5. 0.8% Procedure

- + Identify the cassette
- + Add 50 µl 0.8% Affirmagen® 2 (A1 & B cells) in the reverse columns - **P3**
- + Add 40 µl patient serum/plasma in the reverse columns - **P1**
- + Add 50 µl 0.8% patient red blood cells suspensions in the A, B, D & Control columns - **P3**
- + 5 minutes centrifugation
- + Reading (both sides)



## ABO/D + Direct Coombs - Newborn

### 1. Cassettes

ORTHO BioVue® Newborn (A, B, AB, D, Control, AHG Anti-IgG)

**IMPORTANT: Cassettes to be used with the liner!**

### 2. Sample

3-5% patient red blood cells suspensions in saline (A6) or

0.8% patient red blood cells suspensions in saline (A7)

### 3. 3-5% Procedure

- + Identify the cassette
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)

### 4. 0.8% Procedure

- + Identify the cassette
- + Add 50 µl 0.8% patient red blood cells suspensions - **P3**
- + 5 minutes centrifugation
- + Reading (both sides)



## Rh/K Phenotyping

### 1. Cassettes

- ORTHO BioVue® Rh/K (C, E, c, e, K, Control)
- ORTHO BioVue® Rh-hr (D, C, E, c, e, Control)
- ORTHO BioVue® Kell ( 6 x K)
- ORTHO BioVue® Kell/Control (K, Control, K, Control, K, Control)

**IMPORTANT: Cassettes to be used with the liner!**

### 2. Sample

- 3-5% patient red blood cells suspensions in saline (A6) *or*
- 0.8% patient red blood cells suspensions in saline (A7)

### 3. 3-5% Procedure

- + Identify the cassette
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)

### 4. 0.8% Procedure

- + Identify the cassette
- + Add 50 µl 0.8% patient red blood cells suspensions - **P3**
- + 5 minutes centrifugation
- + Reading (both sides)



## Direct Coombs

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific *or*  
ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Sample

3-5% patient red blood cells suspensions in saline (A6)

### 3. Procedure

- + Identify the cassette
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)



## Direct Coombs Specific

### 1. Cassettes

ORTHO BioVue® DAT/IDAT (AHG Anti-IgG, Anti-C3b & -C3d, Control)

### 2. Sample

3-5% patient red blood cells suspensions in saline (A6)

### 3. Procedure

- + Identify the cassette
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)



## 3-5% Antibody Screening - Indirect Coombs Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific *or*  
ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Reagents

Selectogen® (2 cells) *or* Surgiscreen® (3 cells) *and* Ortho® BLISS

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 50 µl Ortho® BLISS - **P3**
- + Add 10 µl 3-5% Test Red Blood Cells - **P2**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 0.8% Antibody Screening - Indirect Coombs Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific *or*  
ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Reagents

0.8% Selectogen® (2 cells) *or* 0.8% Surgiscreen® (3 cells)

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 50 µl 0.8% Test Red Blood Cells - **P3**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 3-5% Antibody Screening - IAT & Enzyme Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific/Neutral

### 2. Reagents

ORTHO BioVue® Screen Ficin (3 untreated & 3 ficin-treated cells) *and*  
Ortho® BLISS

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 50 µl Ortho® BLISS in the 3 Polyspecific Columns - **P3**
- + Add 10 µl 3-5% untreated Test Red Blood Cells in the  
3 Polyspecific Columns - **P2**
- + Add 10 µl 3-5% ficin-treated Test Red Blood Cells in the  
3 neutral Columns - **P2**
- + Add 40 µl patient serum/plasma in the 6 columns - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 0.8% Antibody Screening - IAT & Enzyme Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific/Neutral

### 2. Reagents

0.8% ORTHO BioVue® Screen Ficin (3 untreated & 3 ficin-treated cells)

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 50 µl 0.8% untreated Test Red Blood Cells in the 3 Polyspecific Columns - **P3**
- + Add 50 µl 0.8% ficin-treated Test Red Blood Cells in the 3 neutral Columns - **P3**
- + Add 40 µl patient serum/plasma in the 6 columns - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 3-5% Cross-Match - Indirect Coombs Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific *or*  
ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Reagents

Ortho® BLISS

### 3. Sample

Patient Serum/Plasma

3-5% donor red blood cells suspensions in Saline (A6)

### 4. Procedure

- + Identify the cassette
- + Add 50 µl Ortho® BLISS in the 3 Polyspecific Columns - **P3**
- + Add 10 µl 3-5% donor red blood cells - **P2**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 0.8% Cross-Match - Indirect Coombs Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific *or*  
 ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Reagent

0.8% Ortho® Red Cell Diluent

### 3. Sample

Patient Serum/Plasma

0.8% donor red blood cells suspensions in 0.8% Ortho® Red Cell Diluent (A7)

### 4. Procedure

- + Identify the cassette
- + Add 50 µl 0.8% donor red blood cells - **P3**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 3-5% Cross-Match - Enzyme Technique

### 1. Cassettes

ORTHO BioVue® Neutral

### 2. Reagents

Enzyme Solution

### 3. Sample

Patient Serum/Plasma

3-5% donor red blood cells suspensions in Saline (A6)

### 4. Procedure

- + Identify the cassette
- + Add 40 µl Enzyme - **P1**
- + Add 10 µl 3-5% donor red blood cells - **P2**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation
- + 5 minutes centrifugation
- + Reading (both sides)



## 3-5% Antibody Identification - Indirect Coombs Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific *or*  
ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Reagents

Resolve® Panel A (11 cells), Resolve® Panel B (11 cells) *or*  
Resolve® Panel (R) C (11 cells untreated) *and* Ortho® BLISS

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 50 µl Ortho® BLISS - **P3**
- + Add 10 µl 3-5% Test Red Blood Cells - **P2**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 0.8% Antibody Identification - Indirect Coombs Technique

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Polyspecific *or*  
 ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Reagents

0.8% Resolve® Panel A (11 cells), 0.8% Resolve® Panel B (11 cells) *or*  
 0.8% Resolve® Panel (R) C (11 cells untreated)

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 50 µl 0.8% Test Red Blood Cells - **P3**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 3-5% Antibody Identification - Enzyme Technique

### 1. Cassettes

ORTHO BioVue® Neutral

### 2. Reagents

Resolve® Panel C (the 11 ficin-treated cells)

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 10 µl ficin-treated Test Red Blood Cells - **P2**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## 0.8% Antibody Identification - Enzyme Technique

### 1. Cassettes

ORTHO BioVue® Neutral

### 2. Reagents

0.8% Resolve® Panel C (the 11 ficin-treated cells)

### 3. Sample

Patient Serum/Plasma

### 4. Procedure

- + Identify the cassette
- + Add 50 µl ficin-treated Test Red Blood Cells - **P3**
- + Add 40 µl patient serum/plasma - **P1**
- + 10 – 15 minutes incubation (Max. 30 minutes)
- + 5 minutes centrifugation
- + Reading (both sides)



## Extended Phenotyping – Anti-Fy<sup>a</sup>, Anti-Fy<sup>b</sup>, Anti-S, Anti-s, Anti-D (IAT)

### 1. Cassettes

ORTHO BioVue® Anti-Human Globulin Anti-IgG

### 2. Reagents

ORTHO™ Sera Anti-Fy<sup>a</sup> (Anti-FY1)

ORTHO™ Sera Anti-Fy<sup>b</sup> (Anti-FY2)

ORTHO™ Sera Anti-S (Anti-MNS3)

ORTHO™ Sera Anti-s (Anti-MNS4)

ORTHO™ Sera Anti-D (IAT) (Anti-RH1)

### 3. Sample

3-5% patient red blood cells suspensions in saline (A6) or

0.8% patient red blood cells suspensions in saline (A7)

### 4. 3-5% Procedure

- + Identify the cassette
- + Add 40 µl ORTHO Sera - **P1**
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 15 minutes incubation
- + 5 minutes centrifugation
- + Reading (both sides)

### 5. 0.8% Procedure

- + Identify the cassette
- + Add 40 µl ORTHO Sera - **P1**
- + Add 50 µl 0.8% patient red blood cells suspensions - **P3**
- + 15 minutes incubation
- + 5 minutes centrifugation
- + Reading (both sides)



## Extended Phenotyping – Anti-Jk<sup>a</sup>, Anti-Jk<sup>b</sup>, Anti-K, Anti-D (DVI), Anti-P1, Anti- Le<sup>a</sup>, Anti-Le<sup>b</sup>

### 1. Cassettes

ORTHO BioVue® Reverse Diluent (6 x Reverse)

### 2. Reagents

ORTHO™ Sera Anti-Jk<sup>a</sup> (Anti-JK1)  
 ORTHO™ Sera Anti-Jk<sup>b</sup> (Anti-JK2)  
 ORTHO™ Sera Anti-K (Anti-KEL1)  
 ORTHO™ Sera Anti-D (DVI) (Anti-RH1)  
 ORTHO™ Sera Anti-P1 (Anti-P1PK1)  
 ORTHO™ Sera Anti-Le<sup>a</sup> (Anti-LE1)  
 ORTHO™ Sera Anti-Le<sup>b</sup> (Anti-LE2)

### 3. Sample

3-5% patient red blood cells suspensions in saline (A6) *or*  
 0.8% patient red blood cells suspensions in saline (A7)

### 4. 3-5% Procedure

- + Identify the cassette
- + Add 40 µl ORTHO Sera - **P1**
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)

### 5. 0.8% Procedure

- + Identify the cassette
- + Add 40 µl ORTHO Sera - **P1**
- + Add 50 µl 0.8% patient red blood cells suspensions - **P3**
- + 5 minutes centrifugation
- + Reading (both sides)



## Extended Phenotyping – Anti-M, Anti-N

### 1. Cassettes

ORTHO BioVue® Neutral

### 2. Reagents

ORTHO™ Sera Anti-M (Anti-MNS1)

ORTHO™ Sera Anti-N (Anti-MNS2)

### 3. Sample

3-5% patient red blood cells suspensions in saline (A6) *or*

0.8% patient red blood cells suspensions in saline (A7)

### 4. 3-5% Procedure

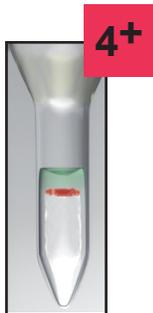
- + Identify the cassette
- + Add 40 µl ORTHO Sera - **P1**
- + Add 10 µl 3-5% patient red blood cells suspensions - **P2**
- + 5 minutes centrifugation
- + Reading (both sides)

### 5. 0.8% Procedure

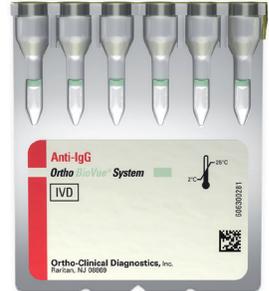
- + Identify the cassette
- + Add 40 µl ORTHO Sera - **P1**
- + Add 50 µl 0.8% patient red blood cells suspensions - **P3**
- + 5 minutes centrifugation
- + Reading (both sides)



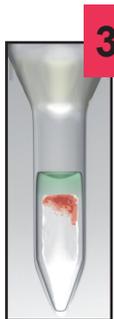
## Agglutination Grading Chart

**4+**

Agglutinated cells form a band at the top of the bead column.



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**3+**

Most agglutinated cells remain in the upper half of the bead column.

**2+**

Agglutinated cells observed throughout the length of the bead column. A small button of cells may also be visible at the bottom of the bead column.

**1+**

Most agglutinated cells remain in the lower half of the bead column. A button of cells will also be visible at the bottom of the bead column.

**Neg**

All cells pass through the beads and form a button at the bottom of the bead column.

## Difficulties Solving/Troubleshooting

PROBLEM	POSSIBLE EFFECT	CAUSE	SOLUTION(S)
<b>Bubble in the glass beads column</b>	False positive results	Transport and/or storage	<ul style="list-style-type: none"> <li>If RBCs “hangs up” on the bubble the test should be repeated</li> <li>Cassettes with breaks or bubbles in the glass beads column should not be used</li> </ul>
<b>Before testing, liquid level above glass beads is low (&lt;1 mm)</b>	False positive results “J” cell button	<ol style="list-style-type: none"> <li>Expired cassette</li> <li>Dried/drying column</li> </ol>	<ol style="list-style-type: none"> <li>Test has to be repeated with a non-expired cassette</li> <li>Cassettes should not be stored near a heat source. Optimal storage temperature: 2 - 25°C. Do not store the cassettes in a self-defrosting refrigerator/freezer.</li> </ol>
<b>Cold antibodies</b>	All tests and auto control are positive	Because of the principle of non-washing AHG test and presence of macromolecular potentiators in the column, warm and cold antibodies with lower affinity could react	Test(s) should be repeated, Serum/Plasma and RBC should be warmed up to 37°C, incubation time = 30 minutes
<b>Centrifugation not horizontal</b>	All columns showed disrupted cell buttons	<ol style="list-style-type: none"> <li>Cassette carrier is blocked</li> <li>Under/over rotation of cassettes in the centrifuge</li> </ol>	<ul style="list-style-type: none"> <li>Cassette carrier should be checked, should move free and in to the right</li> <li>Balance of cassettes should be checked before starting centrifugation</li> <li>Centrifuge should be installed on a horizontal support</li> </ul>
<b>Weak antibodies</b>	Nonspecific reactions “J” cell button	Antibodies with lower titer and/or lower affinity in Serum/Plasma	<ul style="list-style-type: none"> <li>Increase the incubation time to 30 minutes</li> <li>Antibody should be identified</li> <li>If needed, use of enzyme treatment of RBC</li> </ul>
<b>Wrong centrifugation speed</b>	<ol style="list-style-type: none"> <li>False positive results</li> <li>Reactions not clear</li> <li>False negative results</li> </ol>	<ol style="list-style-type: none"> <li>Centrifugation speed too low: The non agglutinated erythrocytes stay in the glass beads column (reaction like a weak positive or indeterminate reaction)</li> <li>Centrifugation speed too high: Weak agglutinated cells are pressed to the bottom of the column and give a negative reaction</li> </ol>	<ul style="list-style-type: none"> <li>Centrifugation speed has to be checked regularly</li> <li>Centrifuge outside of the tolerance limits should not be used</li> </ul>

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## Difficulties Solving/Troubleshooting

PROBLEM	POSSIBLE EFFECT	CAUSE	SOLUTION(S)
<b>Low liquid level after test is finished</b>	False negative result	No sample has been added	<ul style="list-style-type: none"> <li>• Test has to be repeated</li> <li>• Check that reagent(s) and sample are added</li> </ul>
<b>Top line</b>	<ol style="list-style-type: none"> <li>1) False positive interpretation</li> <li>2) Reaction like mixed field</li> </ol>	<ol style="list-style-type: none"> <li>1) Fibrin or particulates in serum or plasma</li> <li>2) Incompletely washed RBC of donor (XM)</li> <li>3) Red blood cells that stick to the sides of the reaction chamber and fall down later and stay above the glass beads</li> </ol>	<ul style="list-style-type: none"> <li>• Elimination of fibrin or particulates with centrifugation of the sample</li> <li>• Wash red blood cells one time with saline</li> <li>• Result should be read within 1 hour after the centrifugation</li> <li>• Check patient history</li> </ul>
<b>Hemolysis</b>	<ol style="list-style-type: none"> <li>1) Liquid above glass beads is red colored</li> <li>2) No agglutination</li> <li>3) Cells haze above glass beads column</li> </ol>	<ol style="list-style-type: none"> <li>1) Bad quality of the erythrocytes</li> <li>2) Hemolytic serum or plasma has been used for the test</li> <li>3) Serum or plasma contains hemolytic antibodies</li> </ol>	<ul style="list-style-type: none"> <li>• Storage and expiration dates of RBC should be checked. Test should be repeated with a new sample.</li> <li>• Check if sample is already hemolytic</li> <li>• Antibody identification should be done</li> <li>• Test could be repeated with washed cells</li> </ul>
<b>Mixed field</b>	Mixed field reaction = strong positive agglutination above the beads and cell button in the bottom of the column	<ol style="list-style-type: none"> <li>1) Occurs often after transfusion, transplantation, etc</li> <li>2) Could occur when using pooled test cells</li> </ol>	<ol style="list-style-type: none"> <li>1) Check patient history and eventual transfusion</li> <li>2) Antibody identification</li> </ol>
<b>“Old” RBCs</b>	<ol style="list-style-type: none"> <li>1) False positive results</li> <li>2) “Disrupted” reaction</li> <li>3) Not indeterminate reaction haze</li> </ol>	<ol style="list-style-type: none"> <li>1) Some old, clotted samples and samples with anticoagulants could absorb, after long storage, low concentration of complement and IgG. The BioVue system is a very sensitive system and could detect the Ig. Disrupted reactions are often a case of complement binding effect. Such reactions are difficult to reproduce.</li> <li>2) The membranes of erythrocytes changed during the storage, particularly when donor blood and RBCs don't contain additives. Those red blood cells cannot pass the glass beads filter and the reaction is like a pink haze above the beads.</li> </ol>	<ol style="list-style-type: none"> <li>1) Repeat the test(s) with fresh erythrocytes</li> <li>2) Washing and suspension in saline of the erythrocytes</li> </ol>

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