



ST VINCENT'S  
HEALTH AUSTRALIA



# BioRad Papain: Comparison of two reagents for antibody identification



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# Outline of presentation

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- Why am I comparing two BioRad papain reagents?
- What is papain, how does it work and why is it useful?
- What are the two reagents and how do they compare?
  - Tests per kit and cost.
  - Stability and shipment.
  - Test profiles available on automated analyser.
  - Tests available by manual CAT.
  - Results of patient testing.
- Summary - which reagent is suited to our laboratory.

# Why am I comparing two BioRad papain reagents

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- We have a BioRad IH-1000.
- Use a pre-papainised 11 cell panel, ID-DiaPanel-P.
- Cannot routinely hold panel on-board:

Only 28 reagent positions.

- Screening (3), Reverse A<sub>1</sub> and B (2), Rh Neg subset (3), 11 cell panel (11)
- Only 9 spaces left, cannot hold additional panel

Reagents stored on-board at room temperature.

- Loading and unloading reagents is not ideal.
- ID-Papain requires 2 reagent positions, possible alternative?



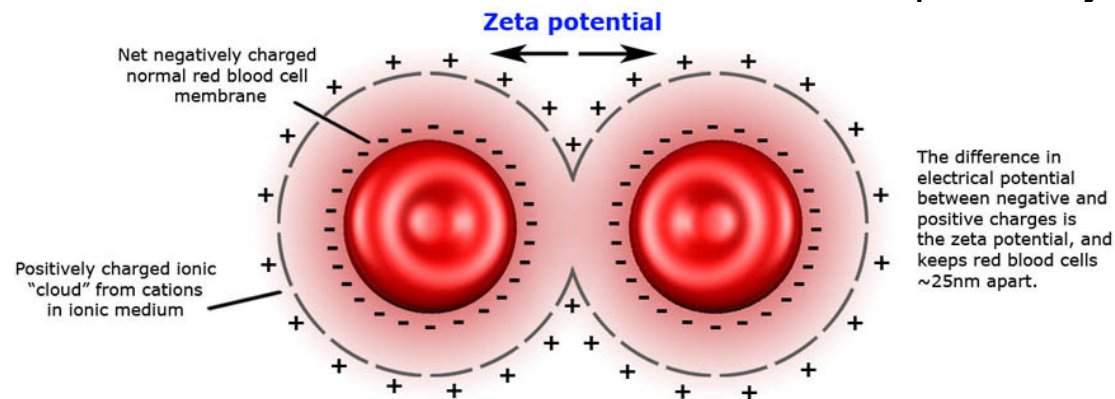
# What is papain?

- Papain is a proteolytic enzyme.
- Extracted from skins of unripe pawpaw (papaya) fruit.



# How does it work?

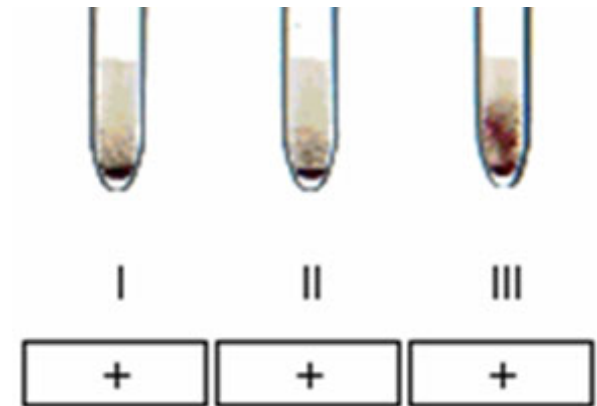
- **Modifies red cell membrane to enhance red cell agglutination:**
  - Removes negatively charged sialic acid molecules from cell membrane, thus zeta potential is reduced, red cells come into closer proximity.



- Removes proteins adjacent to antigens, exposing antigens, thus allowing antibodies better access to antigens.
- **Modifies red cell membrane destroying some antigens:**
  - Cleaves proteins on red cell membrane.

# Why is it useful?

- Antigen-antibody reactions may be:
  - Enhanced (ABO/H, Rh, Kidd, Lewis, I, P1).
  - Decreased (MNS, Duffy).
  - Unaffected (Kell).
- Assists antibody identification when there are:
  - Weak reactions.
  - Mixtures of antibodies.
  - Drugs interfering with the IAT ie daratumumab, isatuximab.
- Ideal for a busy laboratory to have an automated enzyme method.



# What are the two reagents?

1. ID-DiaPanel-P, an 11 cell 0.8% pre-papainised panel.
  - For use with Column Agglutination Technology (CAT).
  - Utilises two stage technique.
    - Cells are pre-treated with papain and the enzyme washed away.
    - 50uL of treated cells, 25uL of test plasma.



# What are the two reagents?

## 2. ID-Papain, a 10mL reagent of papain.

- For use with CAT.
- Utilises one-stage technique on analyser:
  - 50uL of cells, 25uL of test plasma, 25uL of papain.
  - Uses existing untreated cells.
  - Requires additional reagent, “SET-UP Clean” diluted 20% .
- Can use for two-stage technique and pre-treat cells manually.





# Reagent comparison



	ID-DiaPanel-P	ID-Papain
<b>Technique</b>	Two Stage	One Stage
<b>Spaces required on IH-1000 reagent rack</b>	11 (11 x 4mL vials)	2 (Papain & diluted SET-UP clean)
<b>Stability</b>	7 week	7 week
<b>Shipment</b>	Standing order every 4 weeks	Standing order every 4 weeks
<b>Max number panels per kit</b>	80	36
<b>Cost</b>	\$177 <sup>#</sup>	~ \$50 <sup>*</sup>
<b>Cost of reagent per panel</b>	~ \$2.20	~ \$1.40
<b>Funded by NBA<sup>##</sup></b>	Yes	No
<b>Tests available on auto analyser</b>	11 cell IAT panel (LISS/Coombs) 11 cell panel (NaCl card)	11 cell panel + auto cntl (NaCl card) Auto
<b>Additional tests that can be performed by manual CAT</b>	Nil	11 cell panel (NaCl card) 3 cell screen +/- auto 11 cell IAT panel +/- auto

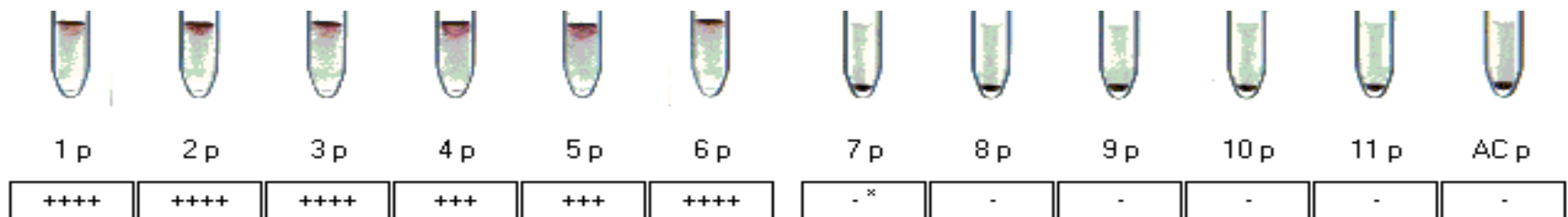
<sup>#</sup> As listed on the current National Blood Authority (NBA) standing offer for diagnostic reagent red cells.

<sup>##</sup> For many public laboratories these reagents are funded by the NBA.

<sup>\*</sup> As determined by contract with supplier, cost does not include 11 cell panel or Set-up clean (~\$300/500mL bottle (~1yr expiry) & ~\$35 on-board bottles.

# Advantages of ID-Papain




































- Cheaper.
- Less reagent vials.
- Can perform an auto control with panel.
  - Useful if all cells are positive, is it due to an auto-antibody or antibody to a high incidence antigen?



- Can perform a 3 cell screen by manual CAT to save \$\$.

# Advantages of ID-Papain

- Uses existing cells, allows for comparison with IAT results, unless already using BioRad cells. ie. Anti-K using Immulab cells.

IAT												
	1	2	3	4	5	6	7	8	9	10	11	AC
	-	++	-	-	-	-	-	++	-	-	-	-
ID-Papain												
	1 p	2 p	3 p	4 p	5 p	6 p	7 p	8 p	9 p	10 p	11 p	AC p
	-	++	-	-	-	-	-	+++	-	-	-	-
ID-DiaPanel-P												
	1P	2P	3P	4P	5P	6P	7P	8P	9P	10P	11P	
	-	++	-	-	-	++	-	-	-	-	-	

# Advantages of ID-DiaPanel-P

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- Can perform a papain IAT panel (LISS/Coombs card) and papain saline panel (NaCl/neutral card) on analyser.
  - LISS/Coombs cheaper.
  - Detection of Bg/HLA often requires the papain IAT method.
  - Anti-CD38 (Dara and Isa) interfere with IAT papain but not saline papain.
- Utilises Two stage technique.
  - **Two-stage papain technique is recognised as being more sensitive for the detection of antibodies than one stage.**

# Patient Testing

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20 patients with antibodies, current and/or historical.

- ID-Papain tested with Immulab Phenocell B 11 cell panel.
- Both methods using BioRad NaCl card.
- Note: No samples were repeated to confirm results.

Results indicate when testing with an NaCl card two-stage technique is more sensitive.

A greater sample size is needed to exclude use of reagent based on the results alone.




































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# Reagent comparison: Patient testing



Antibody	Patient	IAT	ID-Papain	Expected	ID-DiaPanel-P	Expected	Comparable
<b>D</b>	2	2+	4+	Yes			
	4	1+	+/-	No			
	6	2-3+	3-4+	Yes			
	19	1+	Neg	No	2+	Yes	No
<b>C</b>	4	2+	2+	Yes			
	6	2-3+	3-4+	Yes			
<b>E</b>	5	+/-	4+	Yes	4+	Yes	Yes
	7	2+	4+	Yes			
	10	+/-	+/-	Somewhat			
	13	Neg	2+	Yes	1-2+	Yes	Yes
	15	2+	3-4+	Yes	4+	Yes	Yes
	16	1+	3+	Yes			
<b>c</b>	7	1-2+	2-3+	Yes			
<b>K</b>	12	2+	1+	Yes			
	20	2+	2-4+	Yes	3+	Yes	Yes
<b>Jka</b>	3	1-2+	Neg	No	3+	Yes	No
<b>Jkb</b>	14	1-2+	Neg	No	+/-2+	Yes	No
<b>S</b>	2	2+	Neg	Yes			
<b>CD38 (daratumumab)</b>	18	1-2+	Neg	Yes			
<b>I</b>	8	Neg	3-4+	Yes			
<b>Hx αC αKpa</b>	1	Neg	Neg		Neg		
<b>Hx αE</b>	9	Neg	Neg				
<b>Hx αKpa</b>	11	Neg	Neg		Neg		
<b>Hx αE</b>	17	Neg	Neg		Neg		


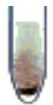










# Anti-Jk(b)

IAT												
	1	2	3	4	5	6	7	8	9	10	11	AC
	-	++	-	++	-	++	-	-	-	+	+	-
ID-Papain												
	1 p	2 p	3 p	4 p	5 p	6 p	7 p	8 p	9 p	10 p	11 p	AC p
	-	-	-	-	-	-	-	-	-	-	-	-
ID-DiaPanel-P												
	1P	2P	3P	4P	5P	6P	7P	8P	9P	10P	11P	
	++ *	- *	+ *	- *	- *	++ *	- *	- *	++ *	++ *	- *	













**Anti-Jk(b) NOT detected by ID-Papain method**

# Anti-D












**IAT**

1	2	3	4	5	6	7	8	9	10	11	AC
											
+	+	+	+	+	-	-	-	-	-	-	-

**ID-Papain**

1 p	2 p	3 p	4 p	5 p	6 p	7 p	8 p	9 p	10 p	11 p	AC p
											
-	-	-	-	-	-	-	-	-	-	-	-

**ID-DiaPanel-P**

1P	2P	3P	4P	5P	6P	7P	8P	9P	10P	11P
										
++	++	++	-	-	-	-	++	-	-	-

**Anti-D NOT detected by ID-Papain method**



## In conclusion

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### ID-Papain:

- Can be accommodated on IH-1000 analyser with routine cells.
- Is a more cost effective product. However as our laboratory receives funded ID-DiaPanel-P this is of no benefit.
- Limited number of tests available on analyser.
- Using a NaCl card failed to identify some clinically significant Ab's.

The laboratories decision is to continue to use ID-DiaPanel-P. May review this and perform more testing if funding is altered or tests available on analyser such as antibody screen and IAT testing is included.

# Questions ?

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