Antibody Identification & RESOLVIGEN

Ortho Clinical Diagnostics

PART OF THE Johnson & Johnson FAMILY OF COMPANIES

Agenda

- ✓ Antibody identification
- ✓ The OCD's solution: Resolvigen
- ✓ Resolvigen – History and Overview
- ✓ Resolvigen – How does it work?
- ✓ Resolvigen - Advantages
- ✓ Resolvigen – Applications
- ✓ Resolvigen – Clinical data & Validation
- ✓ Resolvigen – Summary

Antibody Identification

Antibody Identification in BB/TS

If an irregular antibody to erythrocytes is suspected because of a positive antibody screen or a positive crossmatch or extra positivities in ABO reverse typing or in the case of an unexplained hemolytic transfusion reaction, additional investigations are required in order to detect and identify the possible antibody.

The test method used are the same a those employed for antibody screening. However, it may be necessary to expend the test spectrum, to optimize the test procedure, and, e.g. in case of suspected cold antibodies, to adjust the temperature.

All of this is included in the “Antibody identification” testing.

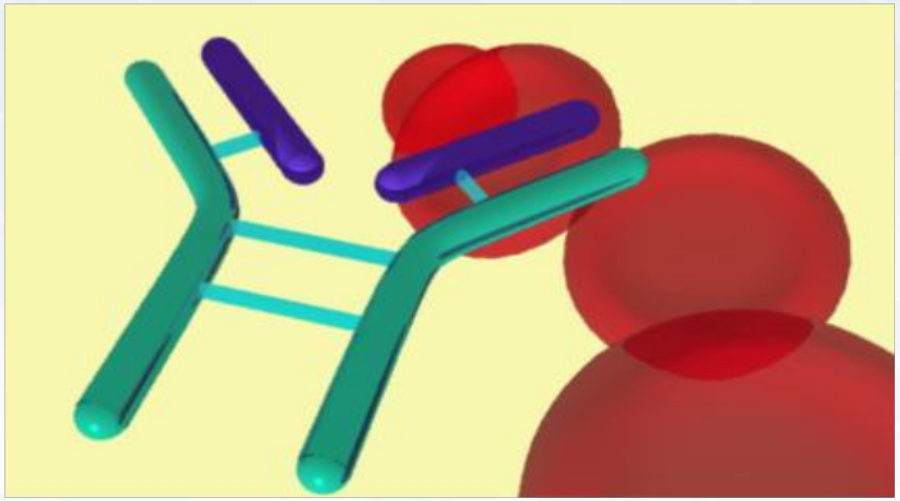
Antibody Identification in BB/TS

- The antibody identification is one of the most complex method in IH
- Skill and deep knowledge of the serological behavior of all clinically significant antibodies are required
- Quick availability of immunohematology technical and scientific information is important
- Difficulties in Antibody identification can delay the transfusion to the patient
- The antibodu identification is often done manually and customers are required a traceability of the results as well as a process control

Resolvigen: the OCD's solution

RESOLVIGEN

A “Virtual” Immunohematologist Expert



Ortho Clinical Diagnostics

PART OF THE Johnson & Johnson FAMILY OF COMPANIES

Resolvigen

Resolvigen is an expert software developed *to assist* user in the interpretation of antibody identification results and to provide suggestions for additional tests

- The software *will suggest* antibody specificity based upon a reaction pattern and information on the antigen expression of the reagent red cells used in the test.

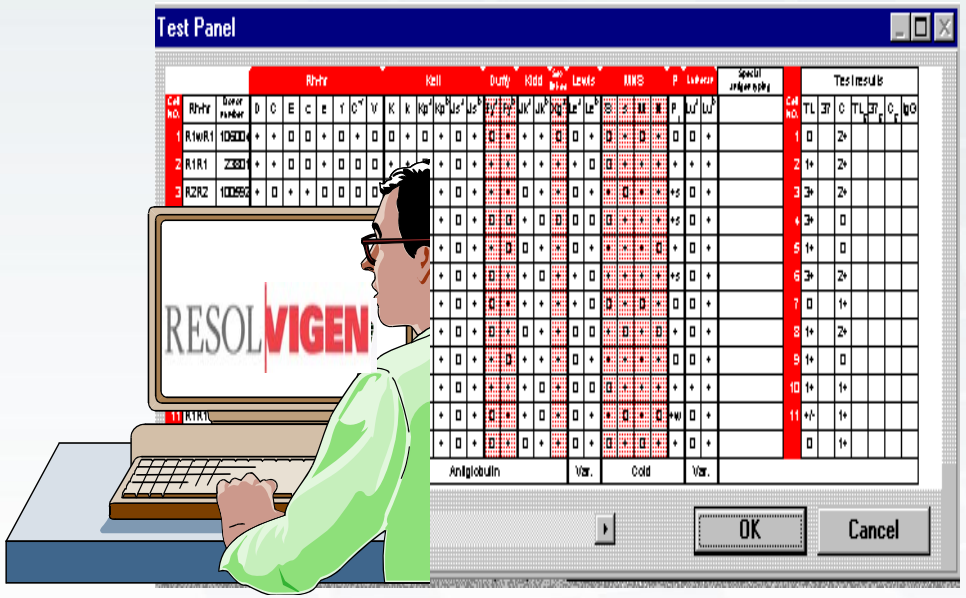
Resolvigen

- Allow you to create your own Rare Blood Cell Archive :
  An archive with public and private antigens that can be searched by different criteria and used to solve complex identification

Resolvigen: History and Overview

Resolvigen – History and Overview

- Resolvigen 2.0 was launched in 2000 – Installed & used in more then 200 transfusion centers
- Has been developped by Technosoft (Milan) in 1999 with OCD Italy collaboration



- Software-CD ROM with USB Key
- Printed user manual

Resolvigen – History and Overview

Resolvigen is a stand alone software that can run on:

- Windows 95/98/Millenium
- Windows NT/XP
- Linux SuSE 8.08.1

Can be installed on any PC with following characteristics **(e.g. Innova PC)**

Hardware description	**Minimum**	**Recommended**
Microprocessor	Celeron 300	Pentium 400
memory	64 MB	128 MB
video resolution	800x600	1024x768

To use Resolvigen , the hardware specific protection key which is delivered with the software has to be installed!

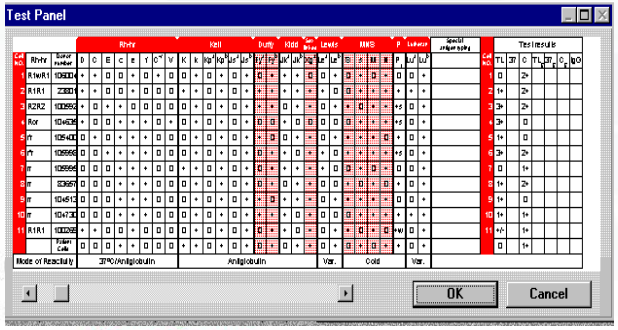
© Ortho-Clinical Diagnostics, Inc

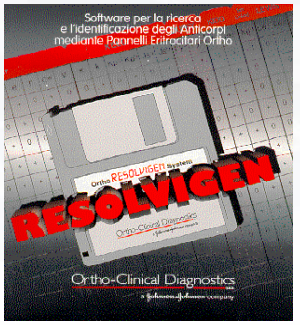
Resolvigen – History and Overview

- Resolvigen 3.0 is designed for use during antibody identification testing to resolve the often complex algorithm of results that is obtained with an identification panel.

  These panels, Ortho Resolve A, B or C may contain as many as 22 cell, each with a distinct antigen profile.

- RBCs Antigram antigen profiles are delivered to the laboratory in three different ways:
  - Floppy disk shipped together with Ortho Resolve Panel
  - E-mail distribution
  - Downloaded from e-antigram library



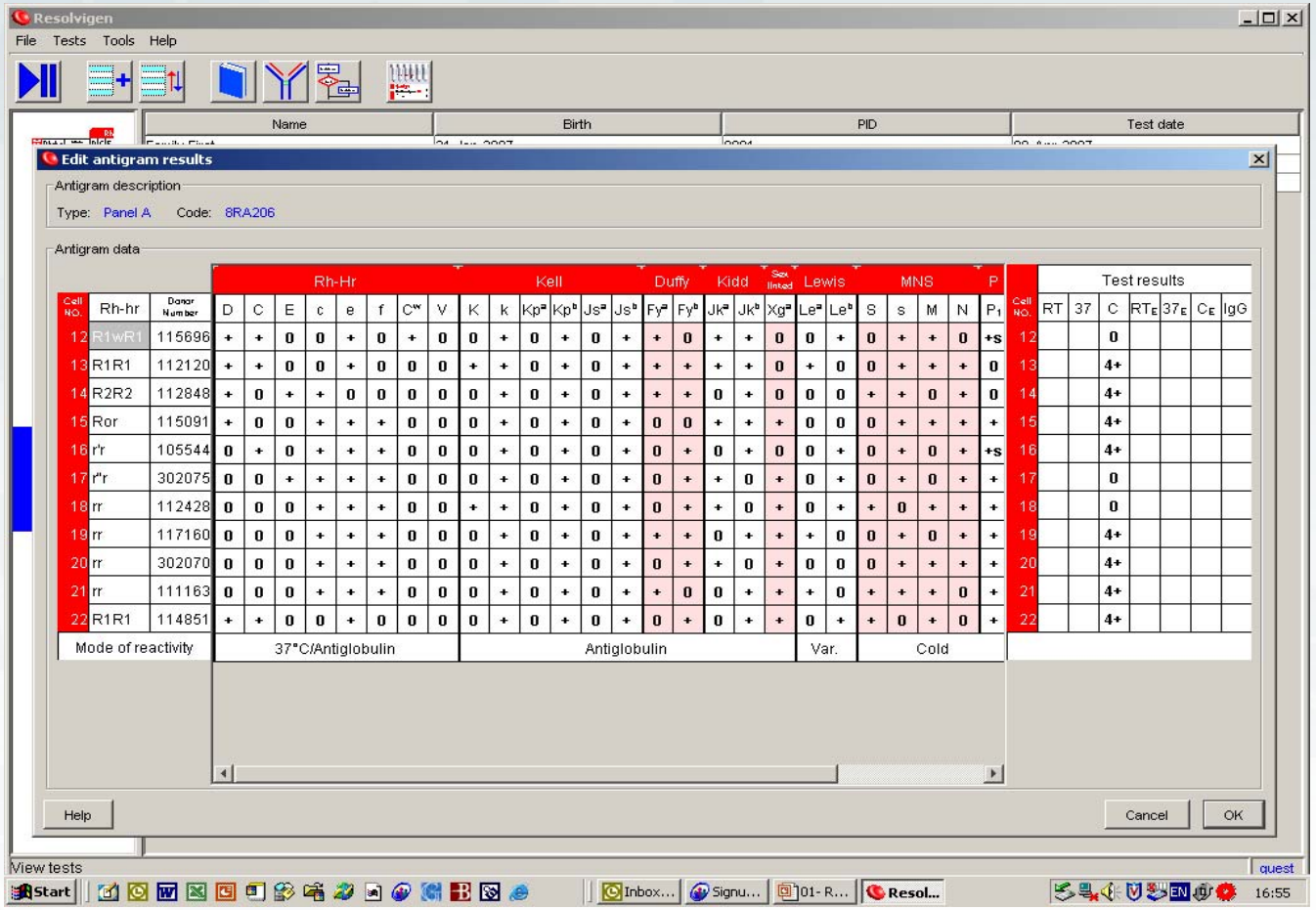


Resolvigen: How Does it Work?

Resolvigen - How Does it Work?

Like an Immunohematologist…..

- Checks all not reactive RBC sample rows on the Panel
- Excludes all antibodies marked + on any of those rows
- Checks the combination that cover all reacting RBCs
- Tries to exclude the remaining antibodies using additional RBCs



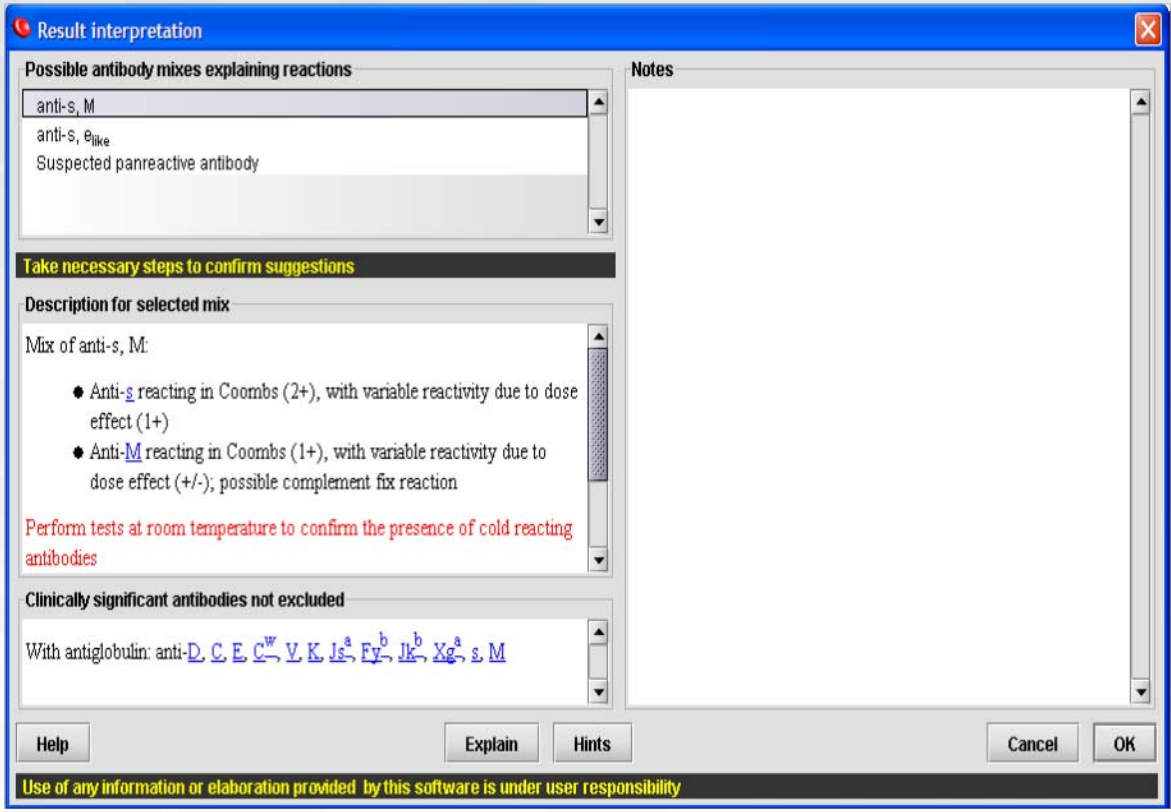
Ortho Clinical Diagnostics

PART OF THE Johnson&Johnson FAMILY OF COMPANIES

Resolvigen - How Does it Work?

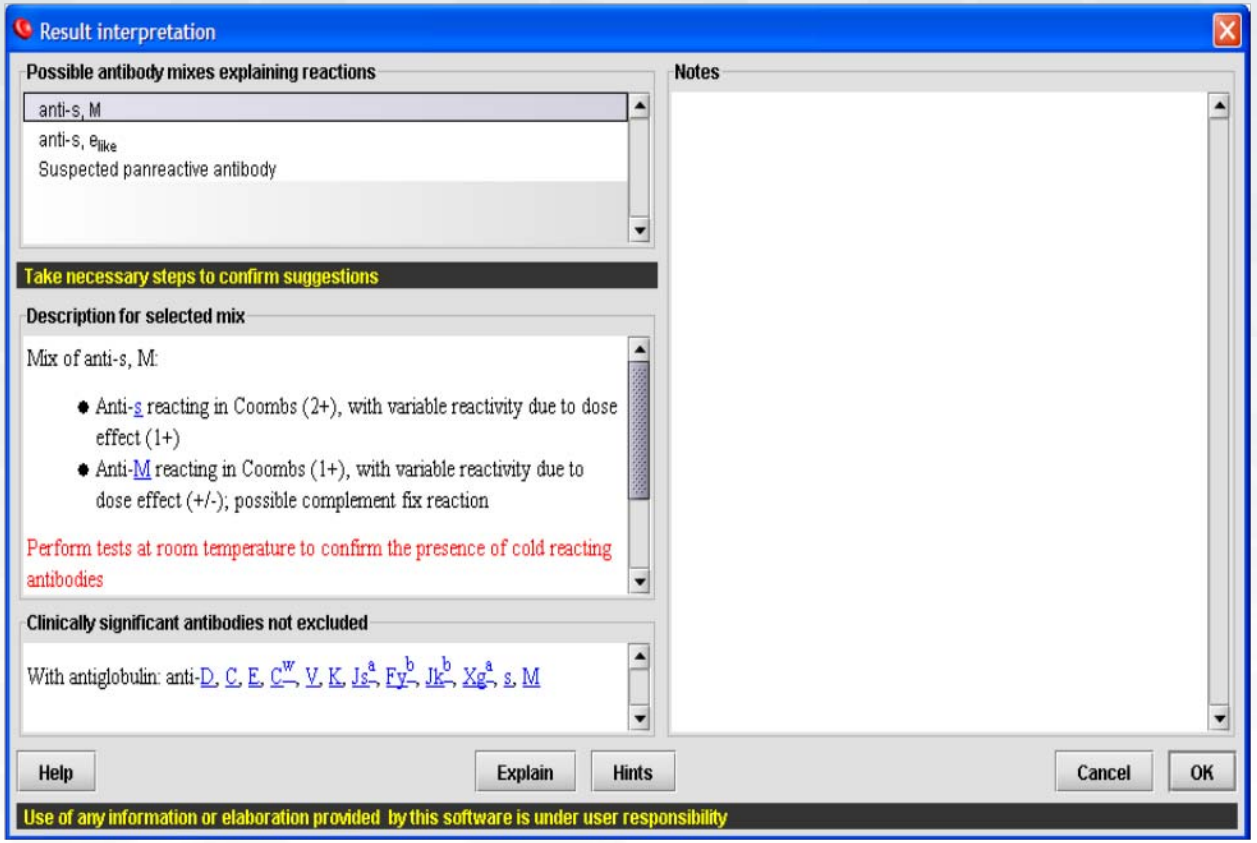
Resolvigen3 takes in account also information as:

- Dose effects
- Effects due to variable antigen expression
- Reactions due to Complement fixation
- Effect of enzymes on antibody reaction
- Behaviour in different phases RT, 37°C, Coombs (IgG+C3d), Coombs (IgG), Enzymes



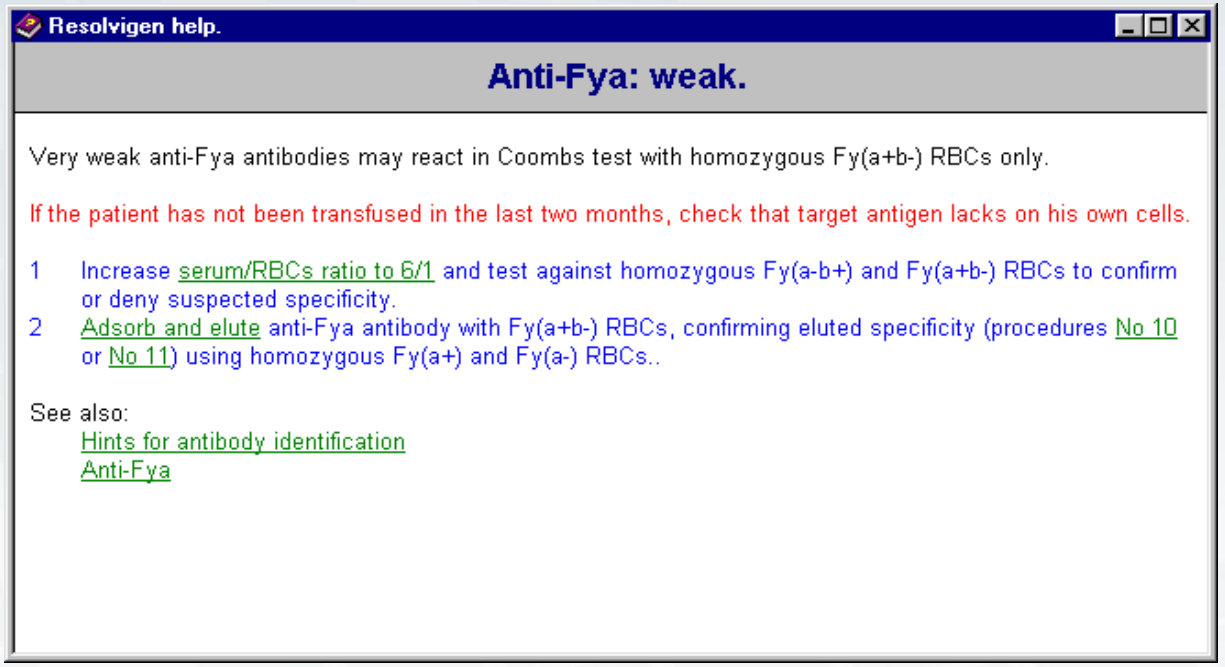
Resolvigen - How Does it Work?

- Identification of antibodies related to antigens on panels
- Gives possible antibody mixes explaining reactions



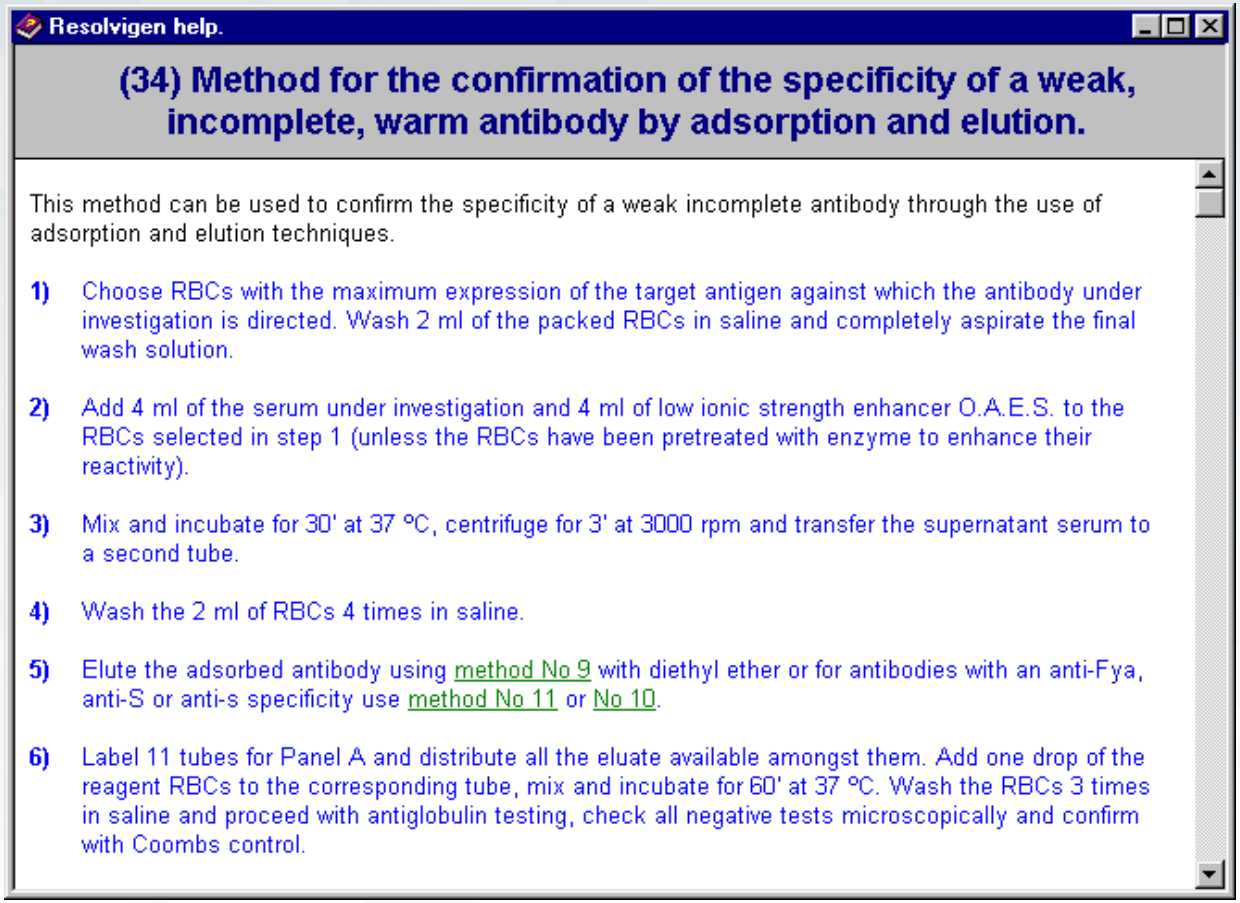
Resolvigen - How Does it Work?

- Provides on line reference for antibodies with immunohaematological and clinical data
- Suggests hints for antibody identification with complex mixes or weak antibodies



Resolvigen - How Does it Work?

- Describes 40 laboratory procedures

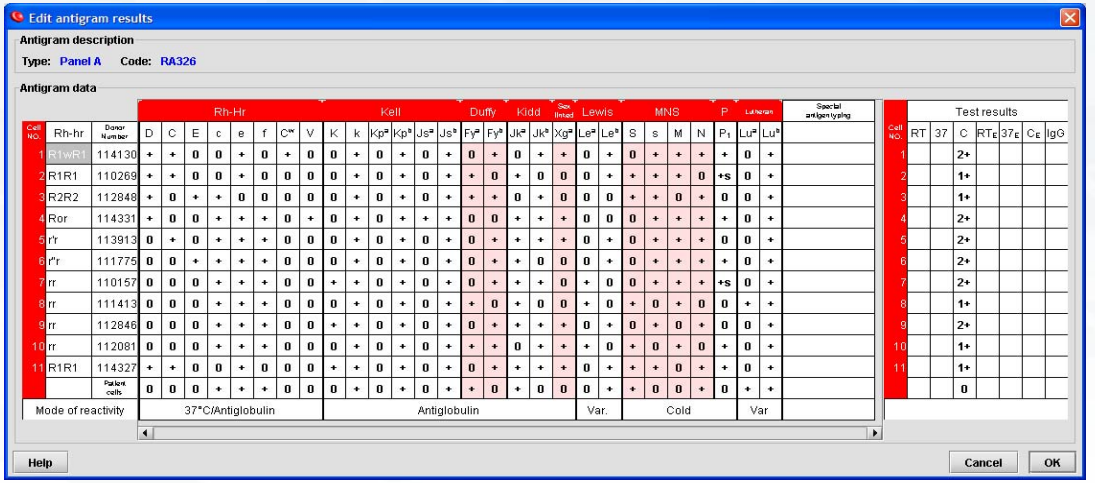


Resolvigen: Advantages

Resolvigen - Advantages

Greatly improves:

- Identification of antibodies mixes that give a **complete covering of panels reactions**
- Reading of **individual scores** and **images** (1+,2+,3+,4+) and accordingly interpretation of results
- Identification of **very weak partially reacting antibodies**



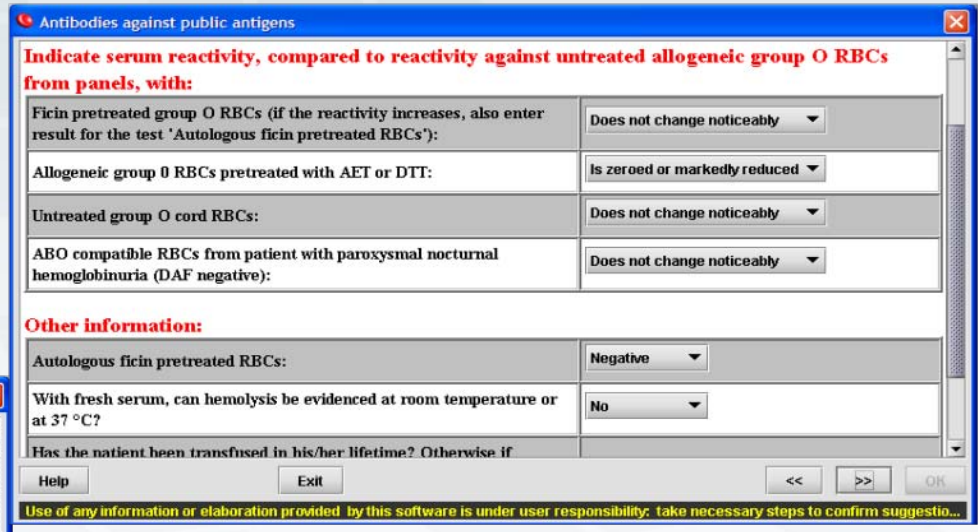
Ortho Clinical Diagnostics

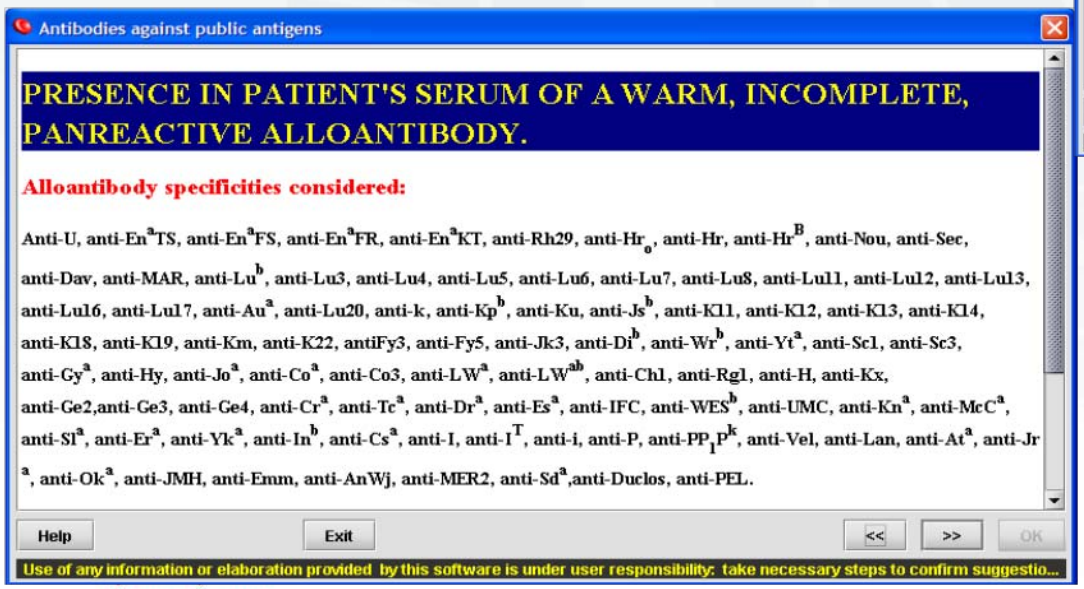
PART OF THE Johnson & Johnson FAMILY OF COMPANIES

Resolvigen - Advantages

Allows:

- Identification of antibodies directed against **public antigens** with a step by step procedure

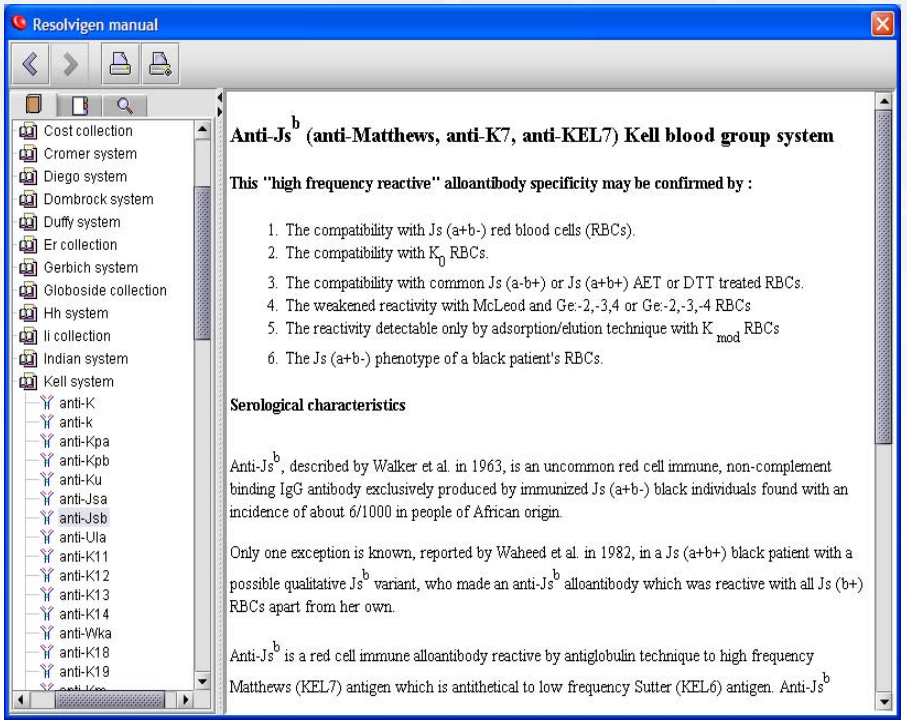




Resolvigen - Advantages

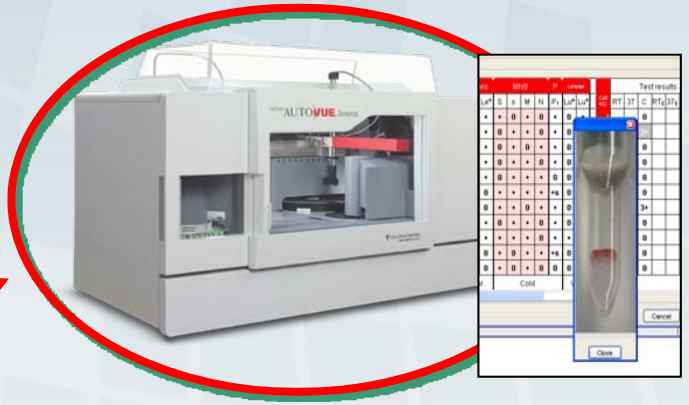
Gives:

- **rare blood archive** extended to public (very common) and private (very rare) antigens (searched by different criteria)
- **on line documentation including over 200 antibodies** with serological and clinical features (enhance communication between lab and clinicians)



Resolvigen: Application

Applications



ORTHO AUTOVUE Innova

- Automatic antibody identification
- Automatic reaction score transfer
- Image transfer

RESOLVIGEN

ORTHOSCAN™ System

- Automatic antibody identification
- Automatic reaction score transfer

- Manual input of reaction' scores

Resolvigen: Clinical Data – Validation

Ortho Clinical Diagnostics

PART OF THE Johnson & Johnson FAMILY OF COMPANIES

Clinical Data - Validation

A multi-center study on second level automation

Aim:

To evaluate the performance of AutoVue Innova/Resolvigen System in difficult conditions

- The Efficacy of the system was evaluated with a comparison among AutoVue Innova/Resolvigen3 antibody interpretation and manual method.
- The Efficiency of the system was evaluated with a comparison between time requested in fully automated process and manual method.

Massimo Ripamonti – Santa Chiara Hospital Trento, Italy
ISBT 2006

Participants and Number of Sera tested

- 5 Transfusion Centers:
  - Trento, A. P. S. S. Chiara Hospital
  - Sondrio, A. O. Valtellina Valchiavenna
  - Verona, A. O. Verona
  - Lucca, A. USL 2 Lucca
  - Ragusa, A. O. Civile e M. P. Arezzo Ragusa
- 250 samples
- 50 samples studied by each site
- Ortho BioVue cassettes and RBC used:
  - IgG, C3D Poly cassette and 0,8% Resolve Panel B and C
  - Neutral Cassette and 0,8% ficin treated Resolve Panel C

Antibodies characteristics

- Warm, incomplete (IgG), single, partially reacting in CAT
- Cold with weak incomplete components
- Mixes, from two to four warm, incomplete alloantibodies
  - **With or without cold-complete (IgM) components,**
  - **Some of them partially reacting with Anti-IgG,-C3d cassette**
- Panreactive
  - **Single warm, incomplete,**
  - **Weakly reactive with variable reaction score**
  - **Strong complete, incomplete, haemolytic**

Antibodies specificities in the samples studied

single antibodies		multiple antibodies	
Anti- Fya (w)	Anti- K + Kpa	Anti- E + S	Anti- D + c + E
Anti- M (vw)	Anti- M(omo)+ D	Anti- c + E	Anti- D + e + K
Anti- P1 (variable)	Anti- Cw + Fya (w)	Anti- Jkb + K	Anti- D + C + E
Anti- Fyb	Anti- D + Fya	Anti- k + E	Anti- c + E + s
Anti- Xga (vw)	Anti- Fya(w) + M	Anti- D + P1	Anti- M + K + C
	Anti- c + Fya	Anti- c + E	Anti- Cw + K + Fya
panreactive antibodies	Anti- D + c	Anti- e + K	Anti- D + C + E + K
Anti- H (h)	Anti- D + Jka	Anti- C + P1	Anti- D + C + E + M
Anti- Vel (vw)	Anti- s + C	Anti- Cw + K	Anti- D + C + E + Fya
Anti- Rh17(Hr°)	Anti- Fyb + E	Anti- K + s	Anti- D + C + E + N
Anti- Cha (variable)	Anti- C + K	Anti- D + K	
Anti- P (h)	Anti- Cw + P1	Anti- Fya + C	
Anti- Yta (variale)	Anti- Fyb + Jkb	Anti- k + D	
Anti- Sc1	Anti-N + S	Anti- E + K	

w: weak

vw: very weak

h: haemolytic

- 50 different samples x 5 Sites
- Total = 250 samples

Concordant results among different sites

140 samples, 28 antibodies mixes

	Expected results	Site 1		Site 2		Site 3		Site 4		Site 5		TOT
		Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res
1	Fya	1	1	1	1	1	1	1	1	1	1	5
2	D + Fya	1	1	1	1	1	1	1	1	1	1	5
3	c + Fya	1	1	1	1	1	0	1	1	1	1	5
4	D + c	1	1	1	1	1	0	1	1	1	1	5
5	D + Jka	1	1	1	1	1	0	1	0	1	1	5
6	s + C	1	1	1	1	1	1	1	1	1	1	5
7	Fyb + E	1	1	1	1	1	1	1	1	1	1	5
8	C + K	1	1	1	1	1	1	1	1	1	1	5
9	P1	1	1	1	1	1	1	1	1	1	1	5
10	D + C + E + K	1	1	1	1	1	1	1	1	1	1	5
11	D + C + E + M	1	1	1	0	1	1	1	1	1	1	5
12	D + C + E + Fya	1	1	1	0	1	1	1	1	1	1	5
13	Fyb + Jkb	1	1	1	1	1	1	1	1	1	1	5
14	E + S	1	1	1	1	1	1	1	1	1	1	5
15	c + E	1	1	1	1	1	1	1	1	1	1	5
16	Jkb + K	1	1	1	1	1	1	1	1	1	1	5
17	k + E	1	1	1	0	1	1	1	1	1	1	5
18	c + E	1	1	1	1	1	1	1	1	1	1	5
19	Cw + K	1	1	1	0	1	1	1	1	1	0	5
20	D + c + E	1	1	1	1	1	1	1	1	1	1	5
21	K + s	1	1	1	1	1	1	1	1	1	1	5
22	D + C + E	1	1	1	0	1	1	1	1	1	1	5
23	D + K	1	1	1	1	1	1	1	1	1	1	5
24	Fyb	1	1	1	1	1	1	1	1	1	1	5
25	c + E + s	1	1	1	1	1	0	1	1	1	1	5
26	Fya + C	1	1	1	1	1	1	1	1	1	1	5
27	k + D	1	1	1	0	1	1	1	1	1	1	5
28	E + K	1	1	1	1	1	1	1	1	1	1	5
	Total n° of concordant samples											140

- 140 samples (100%) were identified by AVI/Resolvigen as expected
- 12 of these samples (8,6%) were correctly identified by AVI/Resolvigen while the manual method didn't identify or partially did it.

Better Interpretation

Discrepant results among different sites

75 samples, 15 antibodies mixes

	Expected results	Site 1		Site 2		Site 3		Site 4		Site 5		TOTAL	
		Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	manual
1	M	0	0	0	0	1	1	0	0	1	1	2	2
2	K + Kpa	1	1	0	0	1	1	0	1	0	1	2	4
3	M omo + D	1	1	1	1	0	0	0	0	0	0	2	2
4	Cw + Fya w	1	1	0	0	1	1	0	0	0	1	2	3
5	Fya w + M	1	1	1	1	0	0	1	1	1	0	4	3
6	Cw + K + Fya	1	0	1	0	1	0	0	0	0	0	3	0
7	D + C + E + N	1	1	1	1	1	1	0	0	1	0	4	3
8	Cw + P1	1	1	0	0	0	0	1*	0	0	1	2	2
9	Anti-Xga vw	1	1	1	1	1	1	0	0	1	1	4	4
10	N + S	0	0	0	0	0	0	0	0	0	0	0	0
11	D + P1	1	1	1	0	1	1	0	1	0	0	3	2
12	e + K	1	1	0	0	1	1	0	0	0	0	2	2
13	C + P1	1	1	1	1	1	1	0	1	1	1	4	5
14	D + e + K	1	1	0	0	1	1	0	1	1	0	3	3
15	M +K + C	1	1	1	1	1	1	0	1	1	1	4	5
	Total n°	13	12	8	6	11	10	2	6	7	7	**41**	**40**

Not interpreted as expected by both methods

Better Interpretation

- 41 samples (55%) were identified as expected by AV Innova/Resolvigen
- 26 samples were not interpreted as expected by both methods
  - De-naturated samples
  - Overlapping reactions

Antibodies vs high incidence antigens

35 samples, 7 antibodies specificities

Expected antibodies	Site 1		Site 2		Site 3		Site 4		Site 5		TOTAL	
	Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	Man	Inn/Res	manual
1 Anti-Vel	1	0	0	0	0	1	1	1	0	0	2	2
2 Anti-Rh17(Hro)	1	0	1	0	1	0	1	0	1	0	5	0
3 Anti-Cha	1	0	0	0	0	0	1	1	0	0	2	1
4 Anti-P	1	0	1	0	0	0	1	1	1	1	4	2
5 Anti-Yta	1	0	1	0	1	0	1	1	1	0	5	1
6 Anti-Sc1	1	0	1	0	1	0	1	1	1	1	5	2
7 Anti-H	1	0	1	0	1	0	1	1	1	0	5	1
Total N°	7	0	5	0	4	1	7	6	5	2	**28**	9

Better Interpretation

- 28 samples (80%) were identified as expected from AVInnova/Resolvigen
- In 19 samples (54%) AV Innova/Resolvigen identified better than the manual interpretation

Overall Results

Sample Reactivity	Equal Interpretation	Better Interpretation		Not interpreted as expected by both methods	TOTAL
		Innova/Res	Manual		
Concordant among sites	128	12	0	0	140
Discordant among sites	33	8	8	26	75
Panreactive	8	20	1	6	35
Total	169	40	9	32	250

- 209/250 (83,6%) samples were correctly interpreted by the AutoVue Innova/Resolvigen.
- In 40 cases (16%) the AutoVue Innova/Resolvigen gave the expected diagnosis while manual method didn't.
- The help of AutoVue Innova/Resolvigen is incremental with panreactive antibodies. (right diagnosis was obtained 28/35, 80% of the panreactive samples)
- In 9 cases (3,6%) of the samples tested the ability of the expert technician is still very useful and decisive to complete the antibody identification.

Comparison of Manual and Automated Antibody Identification Processing Times

	AutoVue Innova / Resolvigen	Manual BioVue /Interpretation
Mean Hands-on Operator Time*	5 mins	5 mins
Mean Processing Time (11 cells)	22 mins 42 sec	25 mins 12 sec
Mean Interpretation & Exclusion **	2 mins 9 sec	8 mins 45 sec
Mean Total Time	29 mins 51 sec	38 mins 57 sec

* excluding samples thawing

** excluding panreactive antibodies

Conclusions

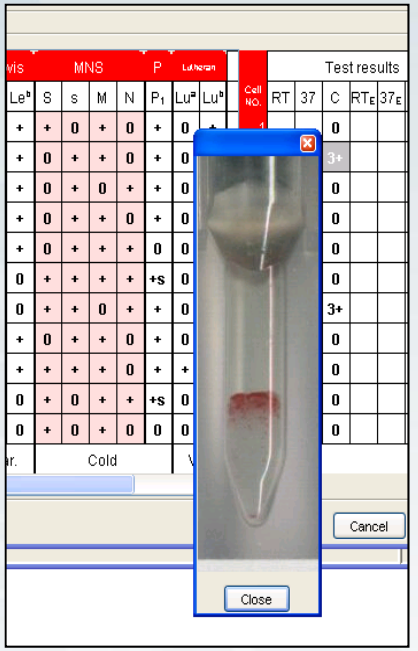
- In simple antibody identification, AutoVue Innova/Resolvigen is useful in providing a fast diagnosis
- Increasing the sample complexity, AutoVue Innova/Resolvigen improves its diagnosis capability and decreases the operator's working time
- In very complex mix and pan-reactive samples Resolvigen is able to give hints and complete the antibody identification even though an immediate diagnosis is not provided

Resolvigen: Summary

Resolvigen - Summary

Automated Antibody identification with AutoVue Innova/Resolvigen provides:

- Assistance in antibody identification in different samples complexity
- Full traceability and standardization of reaction and results interpretation
- reduced risk of errors
- On line information availability of over 40 procedures and 200 antibodies
- Connection with data management system
- Use of digital signature
- Data exchange between different users (Rare Blood Archive)



Thank you