EVALUATION REPORT

Testing Blood Types using EldonCards
prepared with Monoclonal Antibodies Anti-A, Anti-B, and Anti-D.

Multi Centre Performance Study on 3000 Blood Samples.

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Summary

The blood typing performance of EldonCards was investigated with a specially designed "Experimental EldonCard" in four European centres of blood typing. The performance was blindly compared to the results of the standard typing procedures of the centres.

An EldonCard is made of plastic upon which dried antibody formulations is placed. Blood typing is achieved by mixing a drop of blood with each of the reagents and observing the presence or absence of agglutinates obtained by direct agglutination. The results obtained with an EldonCard are therefore only comparable with the initial screening of standard blood typing procedures.

The standard blood typing procedure of the centres consists of two steps: An initial screening with directly agglutinating antibodies, and for selected samples – in particular those that were found Rhesus D negative – a second test with the indirect antiglobulin technique.

The designation "weak A" refers to samples that did not give unambiguously agglutination with a directly agglutinating anti-A antibody. In some cases, further and more refined techniques were needed to establish a "weak A".

The designation "weak D" (or "Du") refers to samples that gave no or doubtful positive Rhesus D reaction at the initial screening, but in an indirect antiglobulin test with another anti-D antibody gave a positive reaction. All initially Rhesus D negative donor blood must undergo such a test.

The term "strong D" is used here for the Rhesus D positive samples found by the initial screening.

A total of 3010 samples were tested on 3019 experimental EldonCards during the study.

12 of the samples were specially selected weak D's with known antigenic properties and were tested separately by one of the centres.

The remaining 2998 samples were tested by the three other centres. Of these samples, 8 were special in being weak A's and 39 were special in being weak D's.

The results were:

Of 2990 non-special samples regarding ABO blood types, 2988 concordant results were found at the initial testing (> 99.9 % concordance).

Of the 8 weak A's, 3 were detected by the EldonCards, 4 were doubtfully positive, and 1 was not detected.

Of the 2998 samples, 2373 were found to be strong Rhesus D's by the conventional testing. Of these, 2372 were detected by the experimental EldonCard at the initial testing (> 99.9 % concordance).

Of the 39 weak Rhesus D samples, 7 were detected by the anti-D formulation already in use on commercial EldonCards. The two new, experimental anti-D formulations on the cards were able to detect 15 and 19 of the weak D samples.

The results of the ABO typing as well as the Rh D typing (including the results of the typing of weak D samples) show that an EldonCard is a reliable blood typing device which can be used for blood typing of patients, and for primary screening of blood donors.

An independent part of the study focused on the ability of "laymen" (persons without laboratory experience) to determine their own blood type by the use of two different EldonKits. The results were compared to the results obtained by "professionals" (blood bank technicians) using the same kits and to the blood types as established by the standard blood typing procedures of the centres.

At each of the three continental centres 20 to 21 laymen (donors) tested their own capillary blood using an EldonKit (EldonCard + utensils including a written instruction).

The laymen obtained varying performance results at the different centres ranging from excellent to less satisfactory results. Most discrepancies arose from the Rhesus D antigen determinations.

The professionals obtained correct results in 59 out of 60 tests. Also here, the discrepancy was due to a Rhesus D antigen determination.

Due to the experience obtained in these studies of laymen performances, the user instructions will be re-written and the utensils of the kits will be modified.

A new study on laymen using the improved instruction and improved utensils will be performed soon.

Purpose of the Study

According to the directive 98/79/EU, in vitro diagnostic medical devices must be CE marked to be marketed in the European Union after 2003-12-07. Reagents for blood typing are in a specific category covered by List A in Annex II to the directive, and the approval of such reagents is further described in the "Common Technical Specifications for in vitro diagnostic medical devices" (EU Commission decision of 2002-05-07).

One of the requirements for the reagents to be approved is that their reliability is documented in a clinical study comprising 3000 tests or more. Consequently, a study was made in four European centres (Blood banks). Blood typing was performed on 3000 EldonCards and compared to the standard typing procedures of the various centres. We further took the opportunity to include some new formulations of reagents in two extra anti-D fields.

EldonCards are also delivered in different kits to be used by non-professionals for determining their own blood type ("Home Kits"). "Non-professionals" or "laymen" are defined as persons without any prior training or experience in doing blood typing. In vitro diagnostic kits for self-testing must also be officially approved. Thus it should be proved that laymen are able to carry out the test correctly to a sufficiently high degree.

Thus, the study had three purposes.

1. To demonstrate the reliability of blood typing on EldonCards (dry format blood typing cards), by comparing results obtained blindly using EldonCards with results of conventional blood typing techniques.
2. To evaluate the sensitivity of two new (low ionic strength) anti-D formulations.
3. To verify that laymen are able to determine their own blood type by using EldonKits 2511 or 2521 and capillary blood. These two kits differ in the layout of the EldonCards, in the lancet used, and in the blood application/stirring device (EldonComb or EldonSticks).

The following four distinguished European centres participated in the study:

The Clinical Immunological Department of Skejby University Hospital, Aarhus, Denmark. In the following referred to as "Aarhus".

The German Red Cross Blood Transfusion Centre, Baden-Baden, Germany. In the following referred to as "Baden-Baden".

The Transfusion Centre of the General Regional Hospital, Bolzano, Italy. In the following referred to as "Bolzano".

The International Blood Group Reference Laboratory, Bristol, UK. In the following referred to as "Bristol".

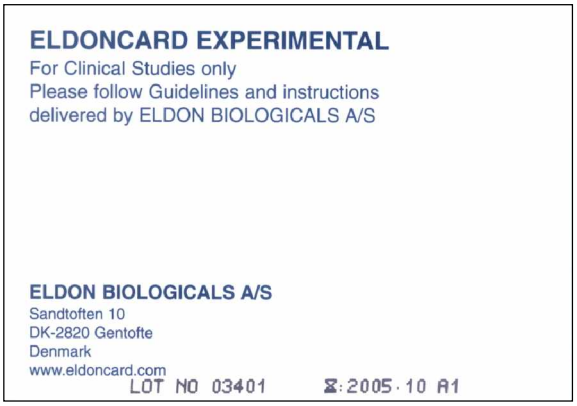
Materials and Methods

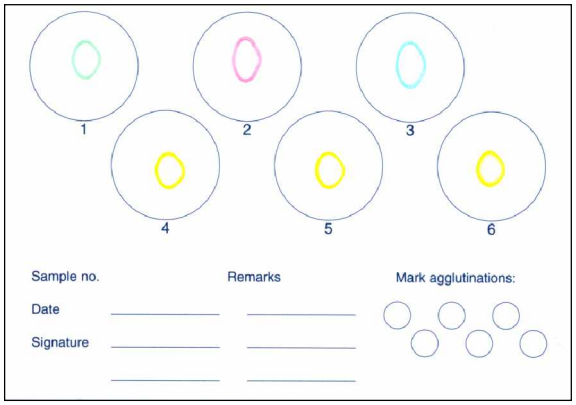
A special EldonCard (EldonCard Experimental) was made to combine the needs posed by parts 1 and 2 of the study. This card has 6 circular reagent fields containing:

Field No.	Antibody	Formulation	Colour
1	Anti-A Birma-1	NISS	Green
2	Anti-B LB-2	NISS	Red
3	None (control)	NISS	Blue
4	Anti-D MS-201	NISS	Yellow
5	Anti-D MS-201	LISS	Yellow
6	Anti-D RUM-1	LISS	Yellow

"NISS" means normal ionic strength solution, while "LISS" means low ionic strength solution. These designations refer to the ionic strength of the formulations applied onto the fields before drying.

The two sides of an experimental EldonCard are shown below in diminished size (real size = 105 x 73 mm):





The cards were individually packed in envelopes of aluminium/plastic foil to preserve them and delivered to the centres as specified in the results section. For stirring, a sufficient number of EldonSticks were supplied. The instruction written for an EldonCard 2521 using iv collected blood was enclosed (id-code 2tosfe).

The testing of samples on the experimental EldonCards and by the conventional methods was done "blindly", i.e. the technician(s) performing the tests on the EldonCards do not know the result of the routine testing of the centre, and vice versa. The results of the EldonCard testing and the routine testing were compared after completion of the separate tests. Following testing, the EldonCards are dried at room temperature, covered with a self adhesive transparent foil and glued onto support sheets. Test results can still be read years after the testing with the exception of very weak agglutinates where, according to experience, test results are best seen immediately after performing the test. On completion of the entire investigation all centres returned their EldonCards and work sheets (enclosure 1 is such a form) to Eldon Biologicals for further study.

All results were computerised to facilitate further analysis. Enclosures 2, 3 and 4 are the results from "Aarhus", "Baden-Baden" and "Bolzano", respectively.

By far the most samples showed identical findings whether EldonCards or conventional blood typing techniques were used.

The few (46 out of 2998) samples where some difference of findings occurred are described in detail in enclosure 5.

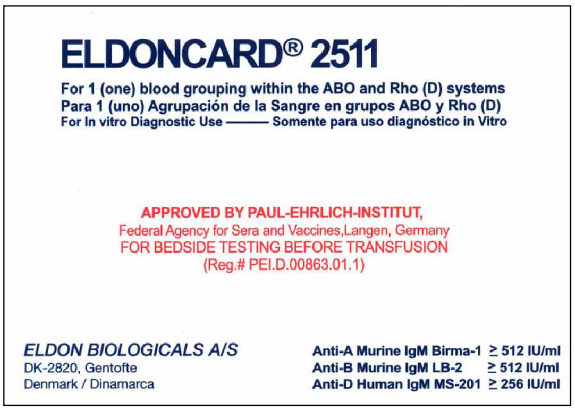
The third part of the study included two different EldonKits, EldonKit 2511-1 and 2521-1.

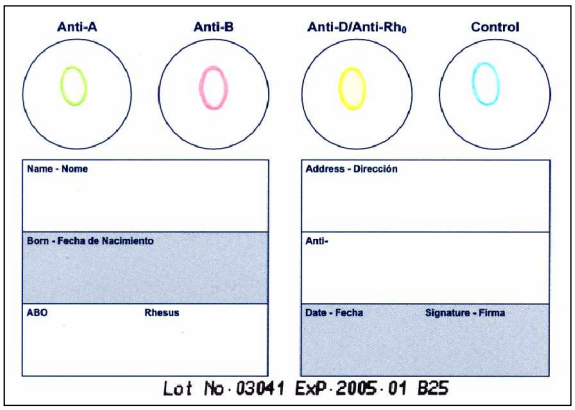
The EldonKit 2511-1 contained one EldonCard 2511 in an aluminium/plastic foil envelope, one plastic dropper (Fine Tip Ultra Micro Pastette, Alpha Labs, UK), one skin cleansing swab (Medi-Swab, Seton Healthcare Group plc, UK) , one automatic lancet (Unistik® 2, Owens Mumford Ltd., UK), one EldonComb, one piece of EldonFoil, and one instruction (id-code 1cukfe).

The EldonKit 2521-1 contained one EldonCard 2521 (a double card for two blood type determinations) in an aluminium/plastic foil envelope, one plastic dropper, one skin cleansing swab, one standard lancet (Assistent No. 366, Karl Hecht GmbH, Germany), four EldonSticks, one piece of EldonFoil divisible, and one instruction (id-code 2cssfe).

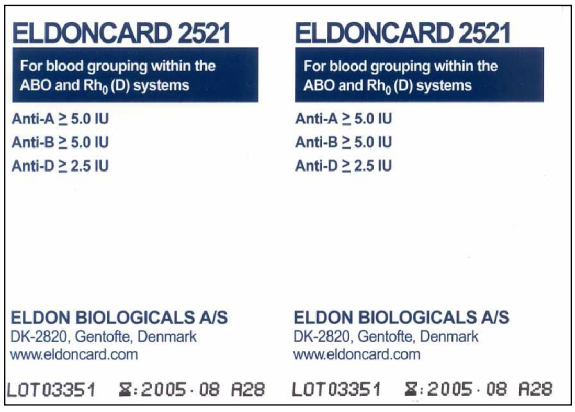
The two EldonCards used in the study are depicted below at diminished size (real size = 105 x 73 mm):

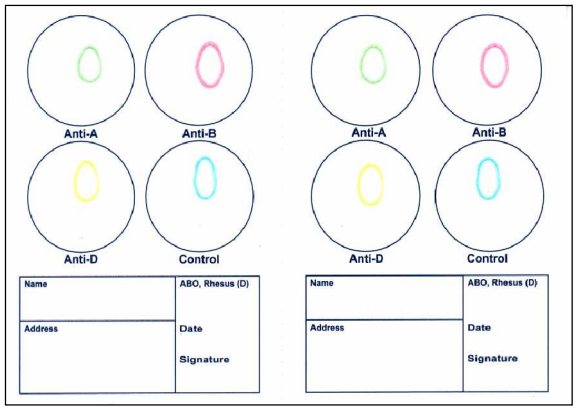
EldonCard 2511:





EldonCard 2521:





We refer to the study protocol for a complete description of the study.

Blood typing methods used by the participating centres in the initial screening

"Aarhus" used microtiter plates and monoclonal antibodies.

"Baden-Baden" used automatic microtube techniques (Olympus) and gel diffusion techniques. Monoclonal antibodies.

"Bolzano" used automatic microtube techniques (Autovue or ABS Precis). Certain patients were typed manually. Umbilical cord samples were typed in Ortho Bio Vue System Cassettes. Monoclonal antibodies (Ortho, Immunocor, Biotest)

"Bristol": Manual tube tests, incubation for 15 minutes at room temperature followed by centrifugation for 1 minute at 100 rcf. Monoclonal antibodies MS-201 and RUM-1.

Results

The number and various types of samples of the various centres can be summarised as follows:

Center:	Aarhus	Baden-Baden	Bolzano	Bristol	Total	Protocol*
Total number of samples	995	1000	1003	12	3010	3000
Patient samples	103	100	190		393	300
Type A, B, AB	580	574	523		1677	> 1200
Newborn (umbilical cord)	21	20	78		119	60
All Rhesus D positive	783	802	827	12	2424	
- hereof weak D's	8	20	11	12	51	> 48
EDTA blood	962	890	980		2832	> 51
CPD blood	33	110	23		166	> 51
Washed cells	20	0	38	12	70	> 51
Weak A's (A3, Ax)	3	5	0		8	
Haemolytic anemia	1	1	1		3	
Direct Coombs positive	7	21	3		31	
Indirect Coombs positive	0	0	1		1	

*: The numbers stated in the protocol are based on the requirements given by the "Common Technical Specifications" referred to earlier.

The task of "Bristol" was specifically to test the different anti-D reagents with a panel of well characterised samples of weak D erythrocytes. The results from this part of the investigation will be discussed later together with the weak D results from the other centres.

A total of 1010 cards were delivered to each of the three continental centres. 1000 of these were meant to be used for the study, and 10 for pre-study training of the staff. The cards were used as follows:

Center:	Primary tests	Repeated tests	Faulty cards	Total
"Aarhus"	995	0	2	997
"Baden-Baden"	1000	2	2	1004
"Bolzano"	1003	7	0	1010
Total	2998	9	4	3011

Cards identified as faulty were discarded before the investigation. They all lacked the three anti-D reagents. Faulty cards will be discussed later.

Results of ABO typing

The results regarding the ABO blood typing can be summarised as follows:

	Total number	Dif	Reaction in control field	Remaining Dif's	Weak A's
"Aarhus"	995	1	1	0	3
"Baden-Baden"	1000	6	0	6	5
"Bolzano"	1003	5	4	1	0

"Dif" stands for notion of discrepancy – independent of the possible significance of this discrepancy.

One difference was found in "Aarhus". A card (no. 994) with strong agglutinations in fields 2, 4, 5 and 6 was read correctly as B positive but showed weak agglutination in fields 1 (A) and 3 (control), indicating an unspecific reaction. The result of the conventional testing was also B positive. This underlines the importance of the control field when using EldonCard for blood typing. The control field should always be negative. The 3 weak A samples – see below.

Of the 6 differences in "Baden-Baden", 5 were due to very weak A's (A3 and Ax) which passed undetected – see below. The last difference (no. 80) was a (weak) reaction in the B-field with a type O positive blood sample which was interpreted as B positive. When retested (no. 1001) no reactions were (correctly) seen in the A-, B- or control fields. The false (but weak) reaction may be due to a tiny splash of reagent from one of the D-fields. This may occur when the EldonStick is abruptly lifted out of the reaction mixture and the liquid contact is broken.

Of the 5 differences in "Bolzano", 4 (nos. 122, 168, 169 and 985) were due to weak reactions in field A and/or field B (all of them were positive in the D-fields). However, all of these cards show a weak reaction in the control field as well, indicating an unspecific reaction. One of the samples (B positive) was retested (no. 985 repeated as no. 999) following washing/dilution after which no reactions were seen neither in the A- nor in the control field. Nevertheless, the blood types read from these cards were correctly interpreted in agreement with the standard typing of the blood bank. The 5th difference (no. 175) was a weak reaction in the B-field with a type A positive blood sample. When the test was repeated (no. 1008) the reaction in the B-field had disappeared. The explanation may again be a tiny splash of reaction mixture from the A-field or one of the D-fields.

Weak A's

The designation "weak A" is used to describe a sample that gives an ambiguous agglutination with a directly agglutinating anti-A antibody. In some cases, further and more refined techniques are needed to establish a "weak A" and its class. Weak A classes are A3 (characterised by the so-called "mixed field" agglutination) and Ax (very very weak).

All three weak A (A3) samples in "Aarhus" were detected by the EldonCards.

Of the five weak A samples (3 A3 and 2 Ax) in "Baden-Baden", 4 (nos. 942, 943, 944, and 945) were read on the EldonCards as "very doubtfully positive", and 1 (no. 941) was not at all detected. However, these five samples from "Baden-Baden" were not part of the routine testing of the centre, but were selected specifically from their collection of frozen, rare specimens. The samples had such weak A-antigens that they could hardly be found by the centre's routine testing but were detected as A positive only during further work up. This explains the findings with the EldonCards.

Conclusions regarding ABO typing

Excluding the 8 weak A's, there were 2990 samples. Of these, 2988 were ABO typed at the initial testing in agreement with the typing results of the centres, giving an agreement of more than 99.9 %.

The two disagreements were due to two false positive B's. Re-testing gave no reaction in the B-fields. The explanation for these false positive reactions may be that a small amount of reagent from other fields with positive reactions was transferred.

In 5 cases, weak positive reactions were seen in the control field, indicating an unspecific reaction as rouleax formation. In principle, these tests should have been repeated with washed erythrocytes or diluted blood. This was done in one of the cases, and the reaction in the control field disappeared. In all cases, the initial results of the reading of the cards were in agreement with the results of the conventional testing. The tests were performed by very experienced technicians and the weak reactions in the control fields did obviously not mislead them to wrong conclusions.

Results of Rhesus D typing

We will divide this part into two, the first one dealing with the results obtained for the strong D's (the samples found D positive at the primary screening in the centres), and the second one dealing with the results for the weak D's (the D negative samples from the primary screening that were found D positive by the indirect antiglobulin test).

The results of the strong D typing

The strong D results can be summarised as follows:

	Total number of samples	Strong D's (conventional)	D positive at first test on EldonCard
Aarhus	995	775	775
Baden-Baden	1000	782	781
Bolzano	1003	816	816
Total	2998	2373	2372

In "Aarhus", all the 775 strong D's was detected by all three D-fields on the EldonCard. With one exception: On card no. 144, field 5 failed to give agglutination although both fields 4 and 6 did. The reason was probably lacking reagent in that field. The sample was not re-tested on another card.

In "Baden-Baden", 781 out of 782 strong D's were detected by all three anti-D fields. The 782nd (card no. 117) gave no reactions at all in any of the 3 anti-D fields. At re-testing (as no. 1002), all the anti-D fields gave agglutinations. The first card probably lacked all three anti-D reagents.

In "Bolzano", all of the 812 normal D's were detected by all three D-fields on the EldonCard.

The results obtained with the weak D's

The purpose of this part of the investigation is to see if it is possible to detect the weak D's by direct agglutination on an EldonCard, using low ionic strength formulations and another anti-D antibody.

This is the reason for using experimental EldonCards containing three different antibody formulations. Field 4 holds the same reagent as is used on the commercial EldonCards (MS-201, NISS), while fields 5 and 6 are the experimental fields holding MS-201 and RUM-1 in a LISS formulation.

The results can be summarised as follows:

	Total number of samples	Weak D's (conv.)	Number of weak D's detected by:		
			Field 4	Field 5	Field 6
Aarhus	995	8	1	1	1
Baden-Baden	1000	20	6	10	13
Bolzano	1003	11	0	4	5
Total	2998	39	7	15	19

In "Aarhus", one out of 8 weak D's was detected by the EldonCard, seven were not.

Amongst the 20 weak D samples in "Baden-Baden", 6 were detected by all D-fields on the EldonCard, 10 were detected by fields 5 and 6, and 13 were detected by field 6 only.

Of the 11 weak D samples in "Bolzano", none was detected by field 4, 4 were detected by fields 5 and 6, and 5 were detected by field 6 only.

Of the 39 weak D's in total, 7 were detected by field 4, 15 by field 5, and 19 by field 6.

The investigation in "Bristol" went into much more detail about the sensitivity of the anti-D fields on the experimental EldonCard. A number of well characterised D variants were tested on the Experimental EldonCard and compared with the findings of a sensitive tube technique. The Rhesus D reactions found in "Bristol" in the tube tests and on the Experimental EldonCard can be summarised as follows:

D variant	ISBT No.	**Tube tests**		**EldonCards**		
		% Reactivity		Field 4	Field 5	Field 6
		RUM-1	MS-201	MS-201 NISS	MS-201 LISS	RUM-1 LISS
D III b	9	73	56	0	0	0
D IV b	10	100	92	strong	strong	strong
D IV b	33	105	100	strong	strong	strong
D V a	37	100	100	strong	strong	strong
D V b	11	0	0	0	0	0
D VI type 1	44	0	0	0	0	0
D VI type 2	35	0	0	0	0	0
D VII	31	96	86	weak	medium	strong
D FR	41	56	25	0	0	0
Strong weak D (3000 spc*)		82	70	weak	medium	medium
Medium weak D (1350 spc)		52	41	0	0	0
Weak weak D (< 1000 spc)		21	18	0	0	0

Strong, medium and weak are ratings of the strength of observed agglutinations. *: Spc = antigenic sites per cell.

The EldonCard technique is a "slide technique" and the possibility of improving the sensitivity of the antibodies ise limited. There is more room for optimising tube techniques. Two important parameters for promoting agglutination, incubation time and temperature, can be freely selected. The tube testing used by "Bristol" is such an optimised and refined technique and it allows for the rating of agglutinations in % reactivity.

Weak D's are weak for one of two reasons and sometimes both. Either they are partial D's meaning that they miss part(s) of the complete D antigen, or the number of complete D antigens per

erythrocyte is lower than normal. Sometimes, the number of partial D antigens may also be reduced, making the detection even more difficult.

The above results show that the EldonCard is less sensitive than the optimised tube test used in Bristol. At 70 % reactivity in the tube test (with a strong weak D and MS-201 antibody), the rating on the EldonCard is "weak". That seems to be the detection limit regarding number of antigenic sites per cell.

The partial D-variants IV b, V a, and VII can be detected by the anti-D fields on the experimental EldonCard, but to a different degree. In particular, field 6 with the RUM-1 antibody in LISS formulation gives a stronger agglutination with the D VII variant. This may explain why this field detected more weak D's than field 5.

The fact that field 5 detected 15 weak D's while field 4 (with the same antibody), only detected 7, indicates that it is the LISS formulation which causes the major part of the difference.

The partial D variants III b, V b, VI (both types 1 and 2), and FR are not at all detected by the experimental EldonCard. The variants V b and VI (1 + 2) were also not detected by the tube test, meaning that they lack the antigenic determinant which the two antibodies are directed against. The variants III b and FR gave in the tube test a so low reactivity, that it should be beyond expectation to see anything on the EldonCard.

Conclusions regarding D typing

Out of 2373 **strong D** samples, 2372 were detected in the first testing. This gives a concordance of more than 99.9 %. The sample which escaped detection was tested on a card without D reagents. Upon re-testing, it was positive in all three anti-D fields.

Out of the 39 samples registered as **weak D**'s – as initial screening gave no agglutinations or doubtful agglutinations – 7 were detected as D positive by field 4, 15 by field 5, and 19 by field 6.

The results indicate the sensitivity of the Anti-D formulations upon the Experimental EldonCard being equal to or better than the sensitivity of the in vitro diagnostics used in the initial screenings.

Going from Anti-D/ MS-201 to Anti-D/RUM-1 and going from a NISS to a LISS added to the sensitivity of the formulation.

Other special types of blood

Samples from patients with autoimmune diseases, for instance autoimmune haemolytic anaemia, are known to contain "interfering substances" such as immunoglobulins. In total, 3 samples were from patients with haemolytic anaemia. This condition did not disturb blood type determination on EldonCards.

In total, 31 samples were from patients with a positive direct Coombs test and suspected of an auto-immune diagnosis. This condition did not disturb blood type determination on EldonCards.

One single sample was positive in an indirect Coombs test. This condition also did not disturb blood type determination on EldonCards.

Due to the commendable initiative of "Bolzano" the study of umbilical cord blood samples was expanded at this centre. The presence of a gelatinous substance (Whartons jelly) in umbilical cord blood constitute a potential interfering substance in blood typing from this source. A total of 60 "Bolzano" samples were typed with the standard method (Ortho micro cassette system) as well as

on Eldon Cards. Of these 60 samples 22 were tested directly on EldonCards, 20 were tested as washed erythrocytes in 5% suspension and 18 were tested directly as well as diluted with PBS to a 10 % suspension.

Whether umbilical cord blood was typed directly, as washed erythrocytes, or as PBS diluted blood on EldonCards no discrepancies from the standard method were observed. Findings at "Aarhus" and "Baden-Baden" with 21 and 20 umbilical cord blood samples, respectively, also showed total agreement with standard blood typing techniques.

Anticoagulants

By far the most samples (2832) tested in this study had EDTA as an anticoagulant. 166 samples used CPD as anticoagulant. No difference concerning blood typing results on EldonCards was seen between these two anticoagulants.

Faulty cards.

Cards lacking reagents.

Four cards were excluded from the investigation because lack of reagents was observed in all the anti D fields. These cards were returned to Eldon Biologicals together with the used cards.

One card (Baden-Baden) gave no reactions in any of the anti D-fields with a D positive sample. Repeated testing on another card gave clear reactions in all of the anti D fields. Most likely no reagents were present in the anti D fields on the first card.

One card in Aarhus gave no reaction in field 5 with a D positive sample. The most probable reason is that the reagent lacked in only that field.

Cards with additional reagents.

Three cards that were used during the investigation contained an extra row of anti-A, anti-B, and Control reagents to the left of the three anti-D fields. The extra reagent spots did not affect the blood type determination on these particular cards.

Explanation of lacking and additional reagents on the Experimental EldonCards.

The experimental EldonCards were produced in two rounds on Eldon Biologicals' new dispensing machine. The machine is built with four pumps to simultaneously dispense the four reagents used on the standard, commercial cards. Placing 6 different reagents on the experimental EldonCards required two passages through the dispensing machine and the drying unit. At the first passage, reagents were dispensed in the first row (fields 1, 2 and 3 i.e. anti-A, anti-B, and Control), and at the second passage, reagents were dispensed in the second row (fields 4, 5, and 6 i.e. the three different anti-D's). A small number of production errors have been observed during the production of 3000 experimental EldonCards used in the present study. Two cards – one on top of the other – might pass through the dispensing machine causing A) one (bottom) card lacking reagents, B) one (top) card with additional, displaced reagents – these reagents being intended for the "following" card which erroneously is hidden by the card on top.

The explanation for the missing reagent in just one field (in Aarhus) is probably that an air bubble had built up in the reagent tubing and was dispensed instead of reagent.

Minor rebuilding and readjustment of the new dispensing machine seem to have eliminated these errors. Very careful, manual scrutinizing of 15078 EldonCards produced later by the new dispensing machine has neither revealed cards lacking reagents nor cards with additional reagents.

Eldon Kits.

In an independent part of the investigation, Eldon kits were tested to se whether persons without any laboratory training ("laymen") can determine their own blood type, using capillary blood, EldonKits 2511 or 2521 and the enclosed instructions only. As many people may be hesitant to pierce their own skin with a manual lancet (EldonKit 2521), assistance from a helper was offered.

Control experiments were performed by blood bank technicians of the various continental centres ("professionals") testing either their own capillary blood or blood from donors. The investigation comprises the following:

1) A professional performs the test using EldonKit 2511 and reads the result (10 tests).

2) A professional performs the test using EldonKit 2521 and reads the result (10 tests).

3) A layman performs the test using EldonKit 2511 and reads the result. Also, a professional reads the result (10 tests).

4) A layman performs the test using EldonKit 2521 and reads the result. Also, a professional reads the result (10 tests).

All results are compared with the result of the standard blood type determination in the laboratory. "Baden-Baden" had the commendable initiative to use the same donors for laymen testing as well as for professional testing. Thus all laymen testing were double-controlled by professionals making capillary blood typing on Eldon Cards as well as laboratory standard typing.

The experiments can be summarised as follows:

Number of tests:

	Part 1 Prof + 2511	Part 2 Prof + 2521	Part 3 Lay + 2511	Part 4 Lay + 2521
Aarhus	10	10	10	12
Baden-Baden	10	10	10	11
Bolzano	10	10	10	10
Total	30	30	30	33

Results from part 1:

	ABO agreement	D agreement
Aarhus (10)	10	10
Baden-Baden (10)	10	10
Bolzano (10)	10	10
All 30:	30	30

Results from part 2:

	ABO agreement	D agreement
Aarhus (10)	10	9
Baden-Baden (10)	10	10
Bolzano (10)	10	10
Total (30)	30	29

Results from part 3:

	ABO agreement		D agreement	
	layman reading	prof. reading	layman reading	prof. reading
Aarhus (10)	8	8	7	6
Baden-Baden (10)	10	10	7	7
Bolzano (10)	10	10	9	9
Total (30):	28	28	23	22

Results from part 4:

	ABO agreement		D agreement	
	layman reading	prof. reading	layman reading	prof. reading
Aarhus (12)	11	10	9	10
Baden-Baden (11)	11	11	10	10
Bolzano (10)	10	10	10	10
Total (33)	32	32	29	30

The difference between EldonCards 2511 and 2521 is in the layout (see pictures in the Materials and Methods section):

On the 2511 card, the reagent fields are arranged in one row.

The EldonCard 2521 has half the size and the reagent fields are arranged in two rows. The difference between the EldonKits 2511 and 2521 used in this study is – apart from the cards – in the accessories.

In the EldonKit 2511, an automatic lancet is used to pierce the skin, and a comb with four teeth is used to collect, apply onto the fields, and stir the blood.

In the EldonKit 2521, a standard lancet is used to pierce the skin, and EldonSticks are used to collect, apply, and stir the blood.

Comparison of parts 1 and 3:

The professionals succeeded to determine the right blood type in all cases. The success rate of laymen was lower, in particular regarding the D- typing.

Comparison of parts 2 and 4:

The professionals succeeded to determine the right blood type in all cases except for one D-typing. Again, the success rate of laymen was lower, in particular regarding the D-typing.

Comparison of parts 3 and 4:

The success rate is higher with 2521 kits than with 2511 kits. This may be due to the fact that it is easier (with the 2521 kit) to collect one blood drop at a time and to apply and control the stirring in one field at a time. It is more difficult (with the 2511 kit) to collect four drops of blood on the teeth of the comb, apply and stir in four fields at a time.

General observations:

Valuable comments were given by Bolzano on the design and practical use of EldonCards, sticks, pipettes etc.

These comments will be given due consideration in the future design of Eldon utensils.

It is noteworthy that the rate of agreement of tests performed by laymen was considerably lower in "Aarhus" than in "Baden-Baden" and "Bolzano". The "Bolzano" results show clearly that laymen may well perform blood typing on EldonCards in excellent agreement with standard tests. However, not all laymen everywhere will be able to perform satisfactorily under all circumstances. Similarly noteworthy is the fact that the success rate for determination of the D type is markedly lower than for determination of the ABO type. This may be caused by the rate of agglutination in the Rhesus D field being slower than in the A- and B- fields. If laymen observe agglutination in the A or B fields they may believe that the test is finished thus overlooking a D-agglutination still under development.

The instruction will be rewritten emphasising the use of sufficient stirring time (10 seconds) and sufficient time for tilting the card (4 x 10 seconds) to obtain valid results. Furthermore, the EldonComb will be discontinued and replaced by EldonSticks for stirring.