East West Immunogenetics Conference
Prague, Czech Republic
March 5 – 6, 2009

Practical Aspects and Typing Strategy in Molecular ABO and RH Blood Group Diagnosis

M a r t i n a P r a g e r
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Topics

- General and specific application
- Clinical cases
- Investigation strategy for clarification of unclear serological blood group typing
- Examples of genomic nomenclature (*ABO* and *RHD*)
- Description of the BAGene DNA SSP Kits

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General Application

- Discrepant, weak or unexpected results in blood group serology
- Donor Pass
- Rhesusprophylaxis / Transfusions
- Today a donor, tomorrow a patient and vice versa

Specific Application

- Genotype multi-transfused recipients
- Genotype patients after ABO-incompatible bone marrow transplantation
- Determine *RHD* zygosity of partners of alloimmunized D negative women before pregnancies
- Genotype Rh D negative donors with C or E in order to exclude the presence of the *RHD* gene and thus preventing anti-D alloimmunization of recipients caused by hidden Rh D variants in RBC units
- Identify genotype in case of weakly expressed Rh D (e.g. DEL) in donors
- Confirm weak D genotype in recipients in order to avoid the donation of Rh D negative blood units
- Quality control of serological methods
- External Quality Assurance trials

Molecular Genetic Blood Group Typing

Principle of PolymeraseChainReaction using SequenceSpecificPrimers

Perfect match → Amplification
(specific Allel)

Mismatch → no Amplification
(unspecific Allel)

Maniatis et al.
1989. Molecular Cloning: A Laboratory Manual.
New York: Cold Spring Harbour Laboratory

Beutler E et al.
1990. BioTechniques 9:166

Olerup O, Zetterquist H
1992. Tissue Antigens 39:225-235

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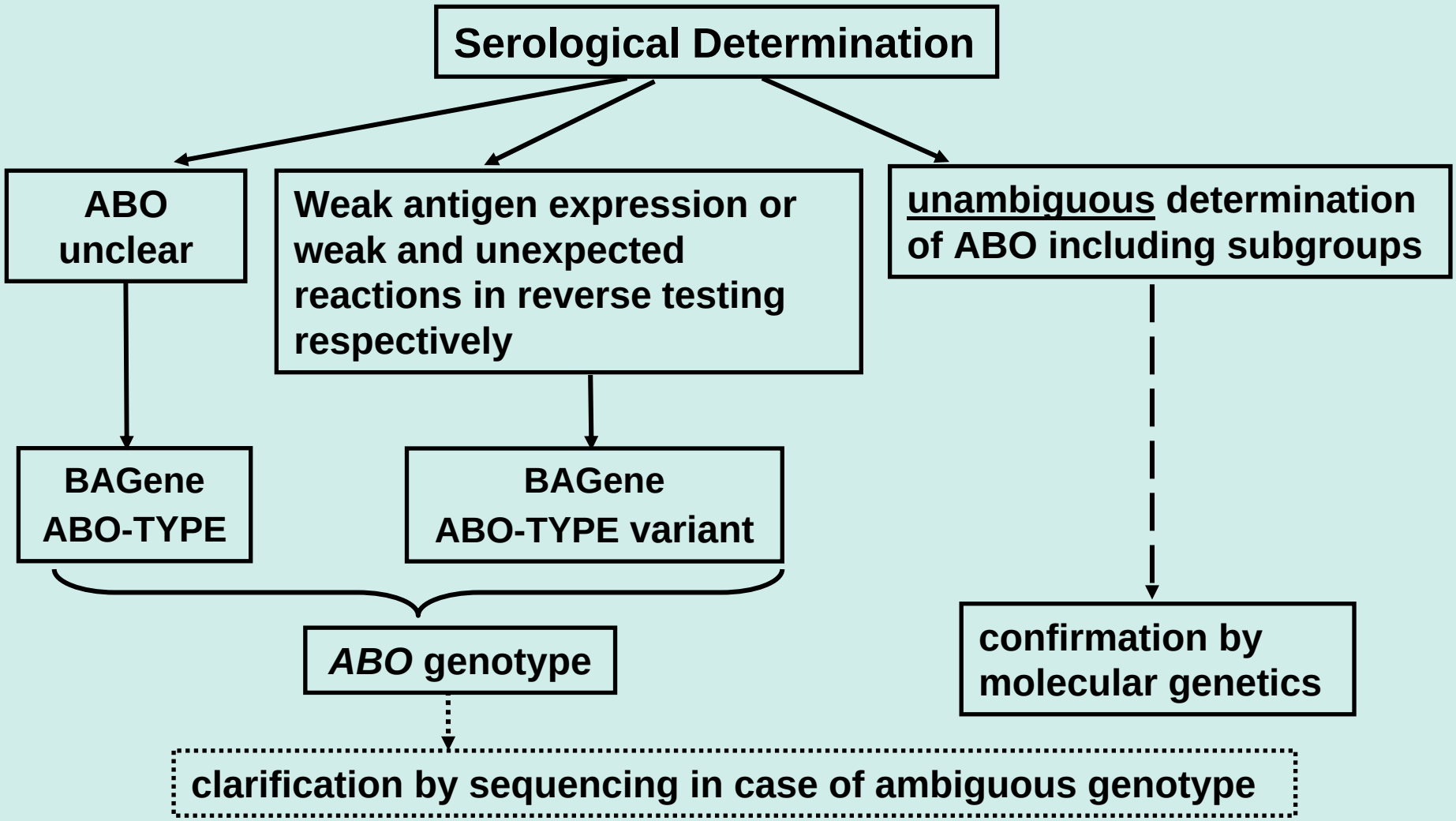
Investigation Strategy

♦ ABO blood group typing



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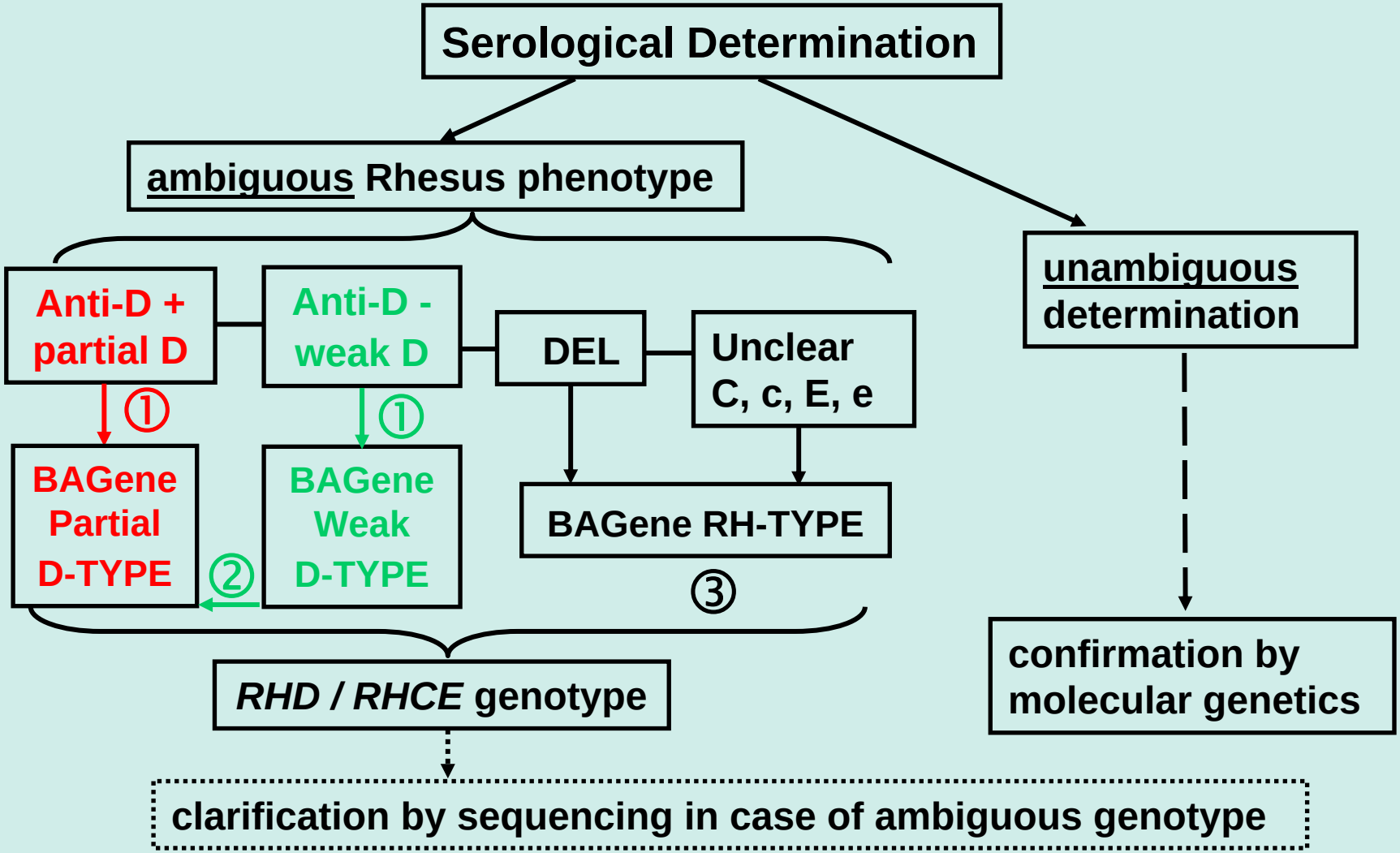
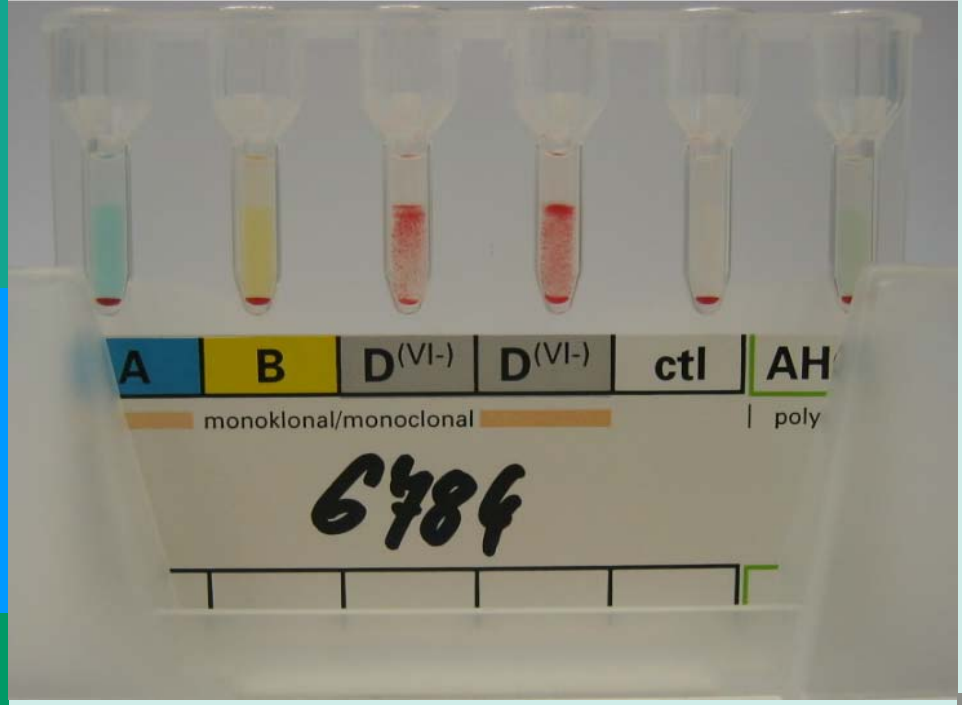
Investigation Strategy

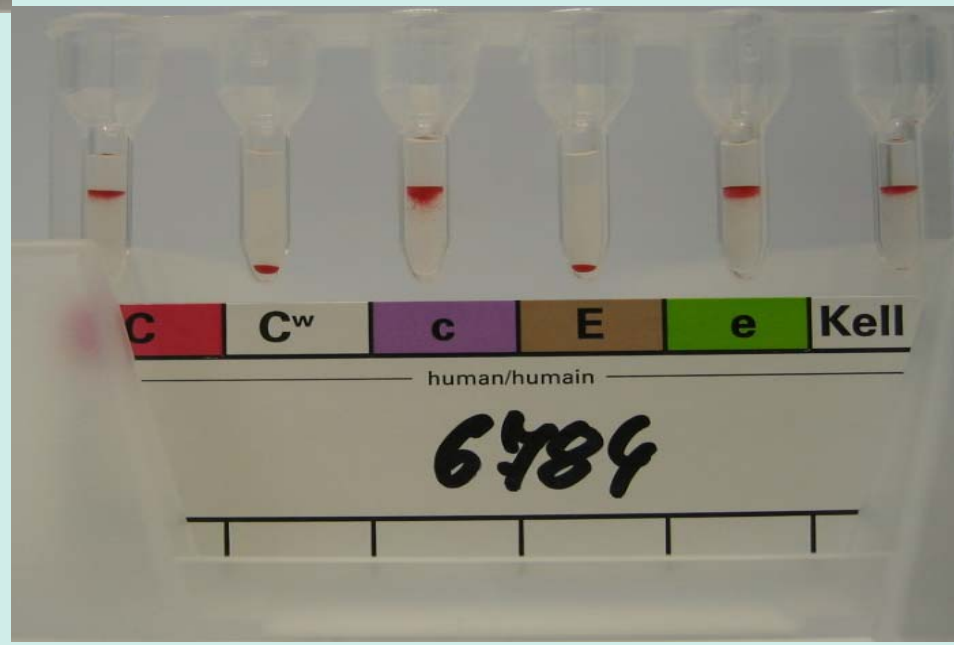
Rhesus typing





Result according to Transfusion guideline

Rhesus D positive
CcD.ee Kell positive



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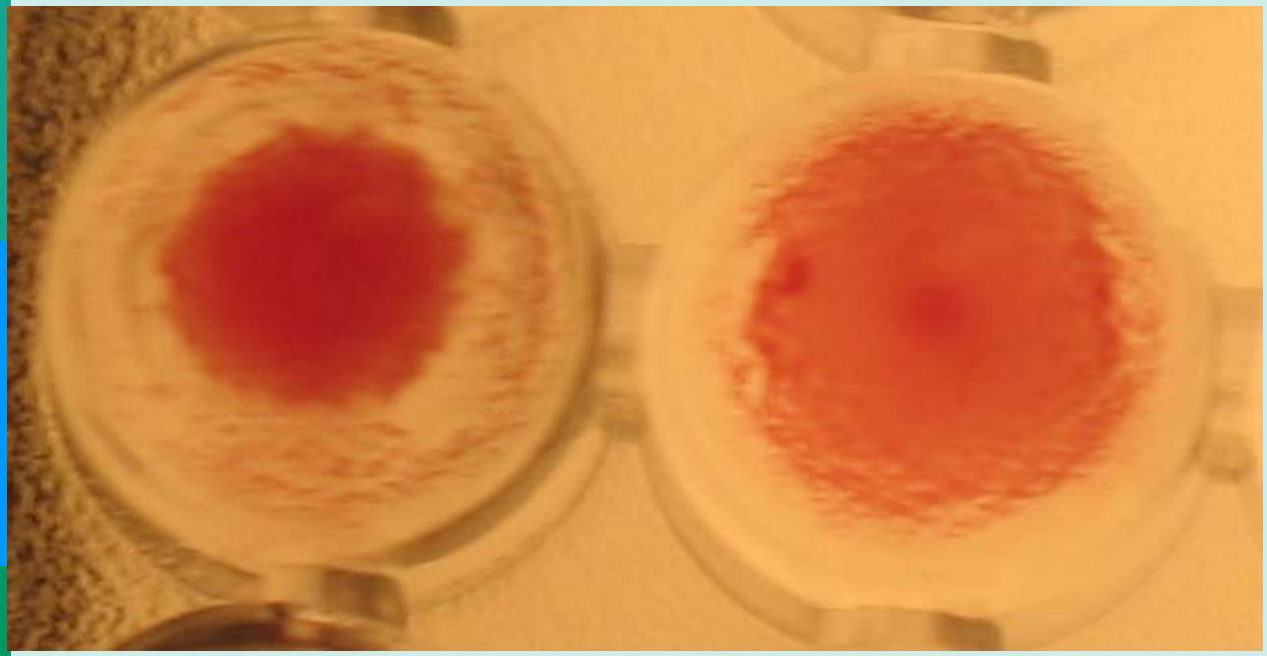
C
c
D
E
e
ctl
human/humain
E000 206 10.95
M 9031-6784



microwell plate
antisera
dilution 1:5

agglutination
strength (1) -1

tube test
immediate spin

questionable
agglutination
with monoclonal
Anti-D

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৭০৩১-১৭৭৭

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BAGene

CE LOT 0902 WD

IVD 2010-08

Weak D-TYPE REF 6647

Worksheet und Auswertetabelle / *Worksheet and Evaluation diagram*

Reaktions-Nr. / *Reaction No.*	1	2	3	4	5	6	7	8
PCR-Produkt (Größe in bp) *PCR product (size in bp)*	150	126	165	101	130 83	112	198 83	153
weak D Allele / *weak D alleles*								
weak D type 1	+	–	–	–	–	–	–	–
weak D type 2	–	+	–	–	–	–	–	–
⇨ weak D type 3	–	–	+	–	–	–	–	–
weak D type 4.0, 4.1	–	–	–	+	–	–	–	–
weak D type 4.2, DAR	–	–	–	+	130	–	–	–
weak D type 5	–	–	–	–	–	+	–	–
weak D type 11 (haplotype cDe)	–	–	–	–	–	–	198	–
RHD(M295I) (haplotype $CD_{el}e$)	–	–	–	–	–	–	198	–
⇨ weak D type 15	–	–	–	–	–	–	–	+
weak D type 17	–	–	–	–	83	–	83	–
weak D type 4.2, 17	–	–	–	+	130 | 83	–	83	–
Weak D type 11 / *RHD*(M295I), 17	–	–	–	–	83	–	198 | 83	–
RHD pos. oder / or *RHD* neg.	–	–	–	–	–	–	–	–

Ergebnis *Result*	1	2	3	4	5	6	7	8	Genotyp *Genotype*

Proben-ID / *Sample-ID:* 11 9031 6784

Name:

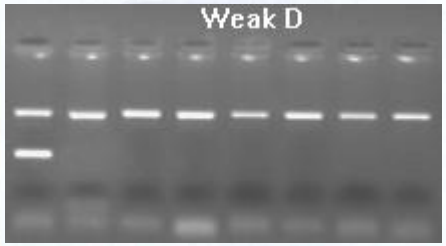
Geb.-Datum / *Birthdate:*

Ergebnis / *Result:* DW typ 1

Datum / *Date:* 22.10.04

Unterschrift / *Signature:* Lorimer P.

Gelbild / *Gel Picture*



Final result weak D type 1

Rhesus prophylaxis not required

Transfusion of D positive red blood cells is possible

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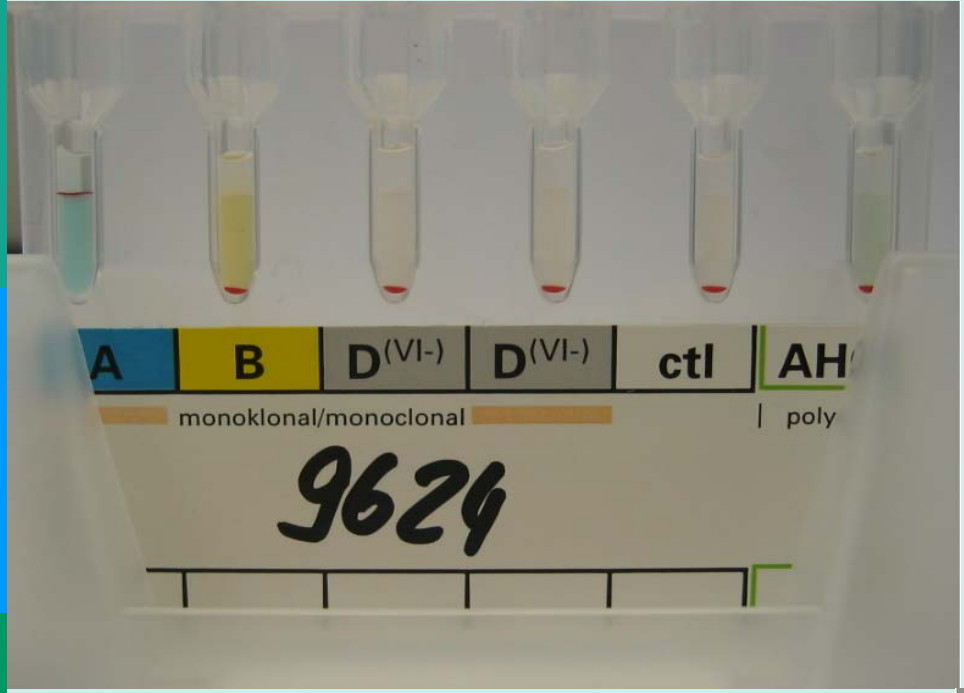
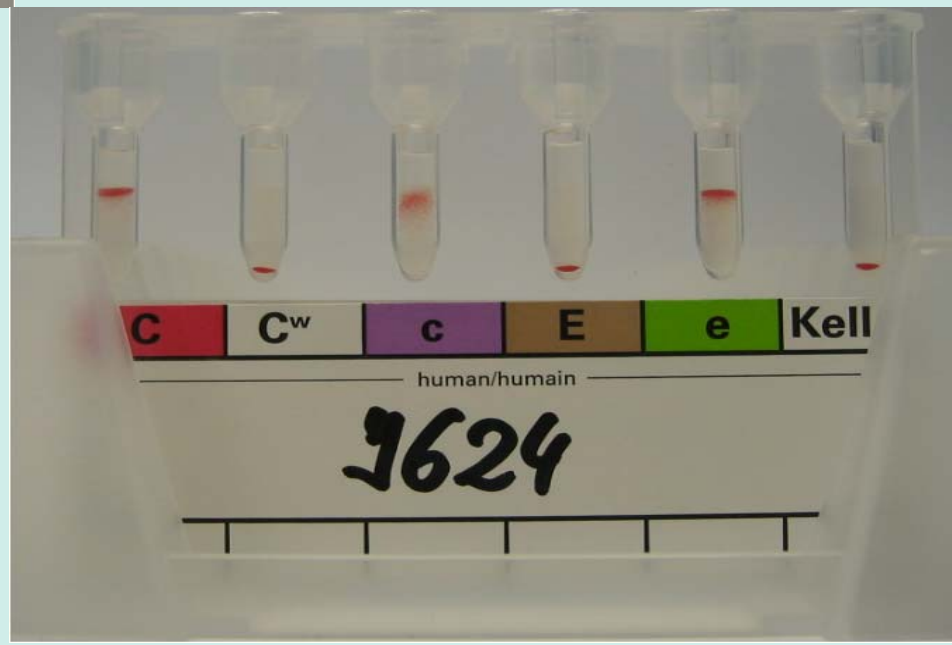


Result according to Transfusion guideline

Rhesus D negative,
Ccddee Kell negative



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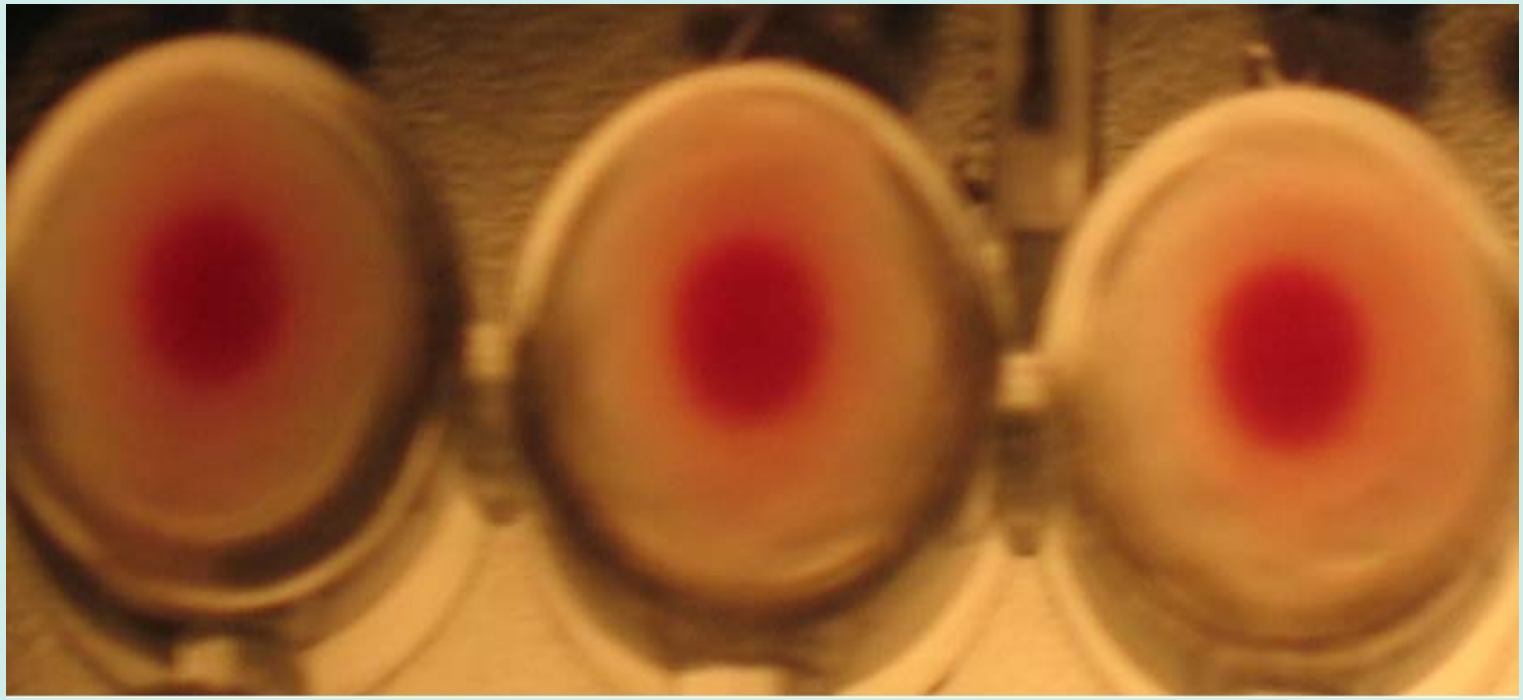
C
c
D
E
e
ctl
human/humain
E000 206 10.95
627-9624



microwell plate
antisera dilution 1:5

negative reaction

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96 24

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BAGene

CE LOT 0902 WD

IVD 2010-08

REF 6647

Weak D-TYPE

Worksheet und Auswertetabelle / *Worksheet and Evaluation diagram*

Reaktions-Nr. / *Reaction No.*	1	2	3	4	5	6	7	8
PCR-Produkt (Größe in bp) *PCR product (size in bp)*	150	126	165	101	130 83	112	198 83	153
weak D Allele / *weak D alleles*								
weak D type 1	+	–	–	–	–	–	–	–
weak D type 2	–	+	–	–	–	–	–	–
⇨ weak D type 3	–	–	+	–	–	–	–	–
weak D type 4.0, 4.1	–	–	–	+	–	–	–	–
weak D type 4.2, DAR	–	–	–	+	130	–	–	–
weak D type 5	–	–	–	–	–	+	–	–
weak D type 11 (haplotype cDe)	–	–	–	–	–	–	198	–
RHD(M295I) (haplotype $CD_{el}e$)	–	–	–	–	–	–	198	–
⇨ weak D type 15	–	–	–	–	–	–	–	+
weak D type 17	–	–	–	–	83	–	83	–
weak D type 4.2, 17	–	–	–	+	130 | 83	–	83	–
Weak D type 11 / *RHD*(M295I), 17	–	–	–	–	83	–	198 | 83	–
RHD pos. oder / or *RHD* neg.	–	–	–	–	–	–	–	–

Ergebnis *Result*	1	2	3	4	5	6	7	8	Genotyp *Genotype*

Proben-ID / *Sample-ID:* 0627 9624

Name:

Geb.-Datum / *Birthdate:*

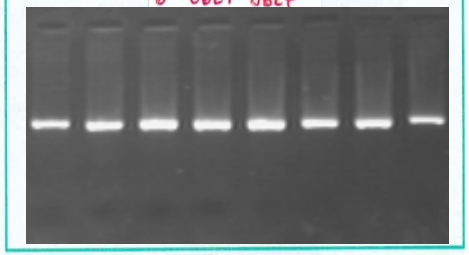
Ergebnis / *Result:* => D. partial

Datum / *Date:* 25. 10. 04

Unterschrift / *Signature:* Krämer

Gelbild / *Gel Picture*

DW 0627 9624



BAGene

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Partial D-TYPE

♦ Eine fehlende Bande in Reaktion Nr. 4 weist auf DFR (serologisch schwach D-positiv) oder *RHD* psi (hemi- bzw. homozygot, serologisch D-negativ) hin. Bei fehlender Information zur Serologie kann *RHD* psi mit **BAGene** RH-TYPE bestätigt oder ausgeschlossen werden.

♦ *A missing band in reaction no. 4 may indicate DFR (weak positive with anti-D) or RHD psi (hemi- or homozygous, D-negative in serology). If serological information is lacking, confirmation or exclusion of RHD psi can be obtained using* **BAGene** RH-TYPE.

CE LOT 0812 PD

IVD 2010-06

REF 6646

Worksheet und Auswertetabelle / *Worksheet and Evaluation diagram*

Reaktions-Nr. / *Reaction No.*		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PCR-Produkt (Größe in bp) *PCR product (size in bp)*		134	146	118	135	132	132	120	673	184	132	166	107	117	129	140
RHD Exons * Mix 8: Intron 7/Exon 8	Haplotyp *Haplotype*	D_1	D_2 C	D_3	D_4	D_5	D_6	D_7	$D_{7/8}$*	D_9	D_{10}	D_2	D_6	D_7	D_7	D_8
Spezifität / *Specificity*		**Standard *RHD***														
⇨ D-positive		+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
		***RHD* negative**														
D-negative, cc		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D-negative, Cc oder / or CC		–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
		***RHD* Varianten** / *RHD Variants*														
⇨ *D cat. II* n.t.	CDe	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–
D cat.IIIa, IIIc, III type 4 n.t.	cDe, CDe, CDe	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–
D cat. IIIb n.t.	cDe	+	–	+	+	+	+	+	+	+	+	–	–	–	–	–
D cat. IVa n.t.	cDe	+	+	–	+	+	+	–	–	+	+	–	–	–	–	–
D cat. IVb, IVb(J) n.t.	CDe	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–
D cat. IV type 3	CDe	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–
D cat. IV type 4	CDe	+	+	+	+	+	+	–	+	+	+	–	–	–	+	–
D cat. Va, Va-like, Va-associated, DBS	CDe	+	+	+	+	–	+	+	+	+	+	–	–	–	–	–
D cat. VI type 1	cDE	+	+	+	–	–	+	+	+	+	+	–	–	–	–	–
⇨ D cat. VI type 2	CDe	+	+	+	–	–	–	+	+	+	+	–	–	–	–	–
D cat. VI type 3	CDe	+	+	–	–	–	–	+	+	+	+	–	–	–	–	–
D cat. VI type 4 [*DHMii*] n.t.	CDe, CDE	+	+	–	–	–	+	+	+	+	+	–	–	–	–	–
D cat. VII	CDe	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–
DAR (weak D-Type 4.2)	cDe	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–
DAU	cDe	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+
DBT type 1		+	+	+	+	–	–	–	–	+	+	–	–	–	–	–
DBT type 2 n.t.	CDe	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–
DFR ♦ oder / *or RHD* psi		+	+	+	–	+	+	+	+	+	+	–	–	–	–	–
DHMi	cDE	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–
⇨ DNB	CDe	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–
RHCE-D(5)-CE (DHAR (Rh33))	cDe	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
RHCE(1-9)-D(10)	cdE	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
RHD-CE(2-9)-D	Cde	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–
RHD-CE(3-7)-D *d(C)ce*[s]	Cde	+	+	–	–	–	–	+	+	+	+	–	–	–	–	–
RHD-CE(4-7)-D	cdE	+	+	+	–	–	–	–	–	+	+	–	–	–	–	–
RHD-CE(8-9)-D	Cde	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–
RHD(delEx9)	CDe	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–
RHD(Q41X) n.t.	Cde	–	+	+	+	+	+	+	+	+	+	–	–	–	–	–
DCS, [DFW, DHR, DIM, DNU] n.t.	diverse / various	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–

n.t. = not tested currently

Ergebnis *Result*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Phänotyp *Phenotype*	Genotyp *Genotype*

Proben-ID / *Sample-ID:* 0627 9624

Name:

Geb.-Datum / *Birthdate:*

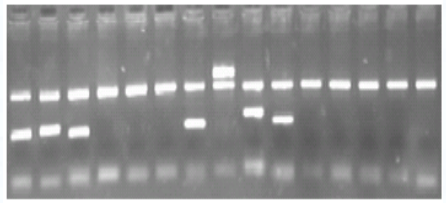
Ergebnis / *Result:* D. VI

Datum / *Date:* 26. 10. 04

Unterschrift / *Signature:* Krämer

Gelbild / *Gel Picture*

D.part. 0627 9624



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BAGene

CE LOT 0902 WD

Weak D-

Worksheet

Reaktions-Nr. / Re								
PCR-Produkt (Grö								
PCR product (size in								
weak D type 1								
weak D type 2								
⇨ weak D type 3								
weak D type 4.0, 4.1								
weak D type 4.2, DAR								
weak D type 5								
weak D type 11 (hapl								
RHD(M295I) (haploty								
⇨ weak D type 15								
weak D type 17								
weak D type 4.2, 17								
Weak D type 11 / *RHD*(M295I), 17	–	–	–	–	83	–	198 83	–
RHD pos. oder / or *RHD* neg.	–	–	–	–	–	–	–	–

Ergebnis *Result*	1	2	3	4	5	6	7	8	Genotyp *Genotype*

Proben-ID / *Sample-ID:* 0627 9624

Name:

Geb.-Datum / *Birthdate:*

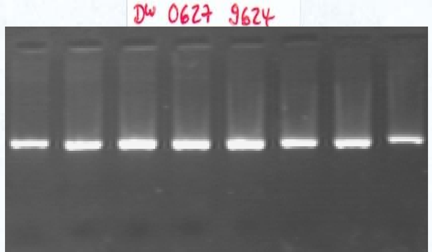
Ergebnis / *Result:* => D. partial

Datum / *Date:* 25. 10. 04

Unterschrift / *Signature:* [signature]

Gelbild / *Gel Picture*

DW 0627 9624



Final result: D category VI type II

Rhesus prophylaxis required

Transfusion of D negative red blood cells is required

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BAGene

Partial D-TYPE

♦ Eine fehlende Bande in Reaktion Nr. 4 weist auf DFR (serologisch schwach D-positiv) oder *RHD* psi (hemi- bzw. homozygot, serologisch D-negativ) hin. Bei fehlender Information zur Serologie kann *RHD* psi mit BAGene RH-TYPE bestätigt oder ausgeschlossen werden.

♦ *A missing band in reaction no. 4 may indicate DFR (weak positive with anti-D) or RHD psi (hemi- or homo-*

CE IVD LOT 0812 PD 2010-06 REF 6646

diagram

		13	14	15
		117	129	140
		D_7	D_7	D_8

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
⇨ DNB	CDe	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–
RHCE-D(5)-CE (DHAR (Rh33))	cDe	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
RHCE(1-9)-D(10)	cdE	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
RHD-CE(2-9)-D	Cde	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–
RHD-CE(3-7)-D *d(C)ce*[s]	Cde	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–
RHD-CE(4-7)-D	cdE	+	+	+	–	–	–	–	–	+	+	–	–	–	–	–
RHD-CE(8-9)-D	Cde	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–
RHD(delEx9)	CDe	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–
RHD(Q41X) [n.t.]	Cde	–	+	+	+	+	+	+	+	+	+	–	–	–	–	–
DCS, [DFW, DHR, DIM, DNU] [n.t.]	diverse / various	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–

n.t. = not tested currently

Ergebnis *Result*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Phänotyp *Phenotype*	Genotyp *Genotype*

Proben-ID / *Sample-ID:* 0627 9624

Name:

Geb.-Datum / *Birthdate:*

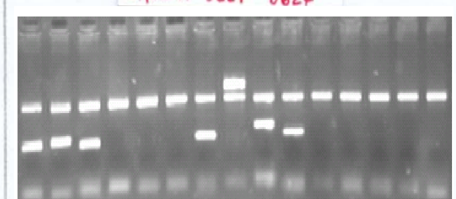
Ergebnis / *Result:* D. VI

Datum / *Date:* 26. 10. 04

Unterschrift / *Signature:* [signature]

Gelbild / *Gel Picture*

D part. 0627 9624



Genomic Nomenclature - *ABO* blood groups

* The Blood Group Antigen Gene Mutation Database

http://www.ncbi.nlm.nih.gov/projects/gv/mhc/xslcgi.cgi?cmd=bgmut/systems_alleles&system=abo

ABO Phenotype	*ABO Genotype*	
	„old“	* BGMUT
A_1	A^1	*ABO*A101*
A_2	A^2	*ABO*A201*
A_3	A^3	*ABO*A301*
A_{el}	A^{el}	*ABO*Ael01*
A_w	A^w	*ABO*Aw04, 06, 07, 11*
A_x	A^x	*ABO*Ax01, 02, 03, 04, 05, 06*
B	B^1	*ABO*B101*
B_3	B^3	*ABO*B302*
B_w	B^w	*ABO*Bw09*
B_x	B^x	*ABO*Bx01*
O	O^1	*ABO*O01*
O	O^{1v}	*ABO*O02*
O	O^2	*ABO*O03*

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Genomic Nomenclature – Rhesus D

The Rhesus Site (*RHD* Mutation Database)

http://www.uni-ulm.de/%7Efwagner/RH/RB/

RhD Phenotype	*RHD Genotype (Allele)*	Mutation
D positive	*RHD*	./.
D negative	*RHD deletion*	Deletion of *RHD*
partial D, DVI	*DVI*	Hybrid
partial D, DFR	*DFR*	Hybrid
partial D, DBT	*DBT*	Hybrid
partial D, Rh33	*DHAR*	Hybrid
D negative, (C)cde^{s}	*RHD-CE(3-7)-D*	Hybrid
partial D, DVII	*DVII*	Single nucleotide exchange
weak D	*e.g. weak D type 1, 2, 3*	Single nucleotide exchange
partial D	*DHMi*	Single nucleotide exchange
partial D, DNB	*DNB*	Single nucleotide exchange
weak D	*weak D type 4.0/4.1, DAR/weak D type 4.2*	Multiple nucleotide exchange
partial D, DAU	*DAU*	Multiple nucleotide exchange
D_{el}	*DEL types RHD(K409K), RHD(M295I)*	Splice site
D negative	*RHD(W16X)*	Stop codon
D negative	*RHDψ*	Multiple nucleotide exchange, deletion and insertion

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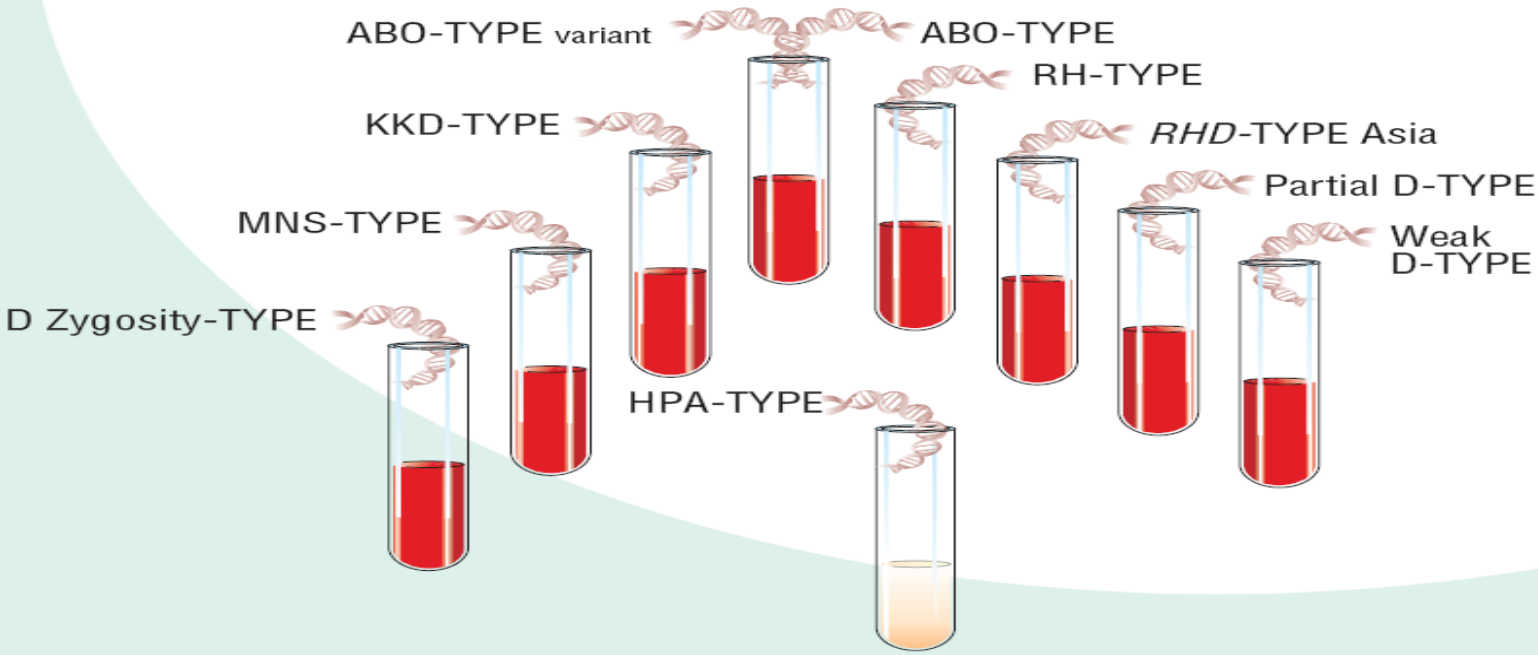
BAGene

SSP kits for determination of

- **ABO blood groups**
- **RH types**
- **Kell, Kidd, Duffy systems**
- **MNS system**
- **HPA specificities**

on a molecular genetic basis



Contents of our BAGene SSP kits

- PCR plates or strips with prealiquoted, dried and colored reaction mixes containing allele specific primers, internal control primers (specific for the HGH gene) and nucleotides.
- 10 x PCR buffer
- PCR strip caps
- Worksheets and Evaluation Diagrams
- Instructions for Use

BAG HEALTH CARE


RHD-TYPE Asia
REF 6649
Kit zur molekulargenetischen RHD-Bestimmung einschließlich schwacher und selt
RHD-Varianten (Weak D Typ 15, 17 , RHD(K409K))
Kit for determination of RHD including weak and rare RHD variants (weak
RHD(K409K)) on a molecular genet sis
PCR-Streifen
CR strips (Mix 1-7)
t / ready to use, prealiqu
uffer
caps
ns for use
iagrams
Instructions for use
BAGene DNA-SSP Kits
Test kits for determination of ABO blood groups, Rh types Kell, Kidd and Duffy systems HPA specificities on a molecular genetic basis
IVD
10/20 typings
ready to use prealiquoted
REF 6640: BAGene ABO-TYPE
REF 6641: BAGene ABO-TYPE variant
REF 6645: BAGene RH-TYPE
REF 6646: BAGene Partial D-TYPE
REF 6647: BAGene Weak D-TYPE
REF 6648: BAGene D Zygosity-TYPE
REF 6649: BAGene RHD-TYPE Asia
REF 6655: BAGene KKD-TYPE
REF 6666: BAGene HPA-TYPE
(8 * mixes, red)
(16 * mixes red)
(12 * mixes, purple)
(16 * mixes, yellow)
(8 * mixes, white)
(2 * mixes, blue)
(7 * mixes, yellow)
(8 * mixes, green)
(12 * mixes, blue)
* No. of mixes conditionally
Contents

Evaluation of Results - *RHD-RHCE* genotyping

BAGene

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RH-TYPE

CE VD

LOT 0806 RH | 2009-12 | REF 6645

Worksheet und Auswertetabelle / *Worksheet and Evaluation diagram*

Reaktions-Nr. / *Reaction No.*	1	2	3	4	5	6	7	8	9	10	11	12	13
PCR-Produkt/e / *PCR product/s* (Größe in bp/*size in bp*)	224 123	390 248 154	140	113 *sw*	113	198	143	215	162	145	157	155	181
Reaktionsmuster / *Reaction pattern*	$D_{4/4}$ D_7	D_7 *RHD(W16X)* *RHD Ψ*	D_s *RHD Ψ* neg.	DEL *RHD* (K409K) neg.	DEL *RHD* (K409K)	DEL *RHD* (M295I)	DEL *RHD* (IVS3+1G>A)	Cde^s	C C^w	c	E	e	C^w
Phänotyp / *Phenotype* / Genotyp / *Genotype*													
Standard *RHD/RHCE* Allele/s													
D-positive	+ +	+	+	+	+	−	−	−	−				
D-negative	−	−	−	−	−	−	−	−	−				
C									+	−	−	−	−
C^w									+	−	−	−	+
c									−	+	−	−	−
E									−	−	+	−	−
e									−	−	−	+	−
RHD-Varianten / *RHD variants*													
D-positive	*RHD / RHD*(K409K)	+ +	+	+	+	+	−	−	−				
D_{el}	*RHD*(K409K)	+ +	+	+	−	+	−	−	−				
D-positive	*RHD / RHD*(M295I)	+ +	+	+	+	−	+	−	−				
D_{el}	*RHD*(M295I)	+ +	+	+	+	−	+	−	−				
D-positive	*RHD/ RHD*(IVS3+1G>A)	+ +	+	+	+	−	−	+	−				
D_{el}	*RHD*(IVS3+1G>A)	+ +	+	+	+	−	−	+	−				
D-positive	*RHD / RHD Ψ*	+ +	+ +	+	+	−	−	−	−				
D-negative	*RHD Ψ*	+ +	+ +	−	+	−	−	−	−				
D-negative	*RHD-CE(8-9)-D*	+ +	−	+	−	−	−	−	−				
D-negative	*RHD*(W16X)	+ +	+ +	+	+	−	−	−	−				
D-positive	*RHD* / Cde^s	+ +	+	+	+	−	−	−	+				
D-negative	Cde^s / Cde^s; Cde^s/d	−	+	−	+	−	−	−	+				
Beispiele / *Examples*													
ccddee		−	−	−	−	−	−	−	−	−	+	−	+	−
Ccddee		−	−	−	−	−	−	−	−	+	+	−	+	−
CcD.Ee		+ +	+	+	+	−	−	−	−	+	+	+	+	−
ccD.EE		+ +	+	+	+	−	−	−	−	−	+	+	−	−
CCD.ee		+ +	+	+	+	−	−	−	−	+	−	−	+	−
$CC^wD.Ee$		+ +	+	+	+	−	−	−	−	+	−	+	+	+
D cat. VI		+	+	−	+	−	−	−	−					
D cat. IV type III		+	−	+	−	−	−	−	−					
DFR		+ +	+	−	+	−	−	−	−					

Reaktion Nr. 4 sw - manchmal schwach

Reaction no. 4 sw - sometimes weak

Größe der internen PCR-Kontrolle in Reaktion Nr. 2: 659 bp, in allen anderen Reaktionen: 434 bp (HGH)

Size of internal PCR control in reaction no. 2: 659 bp, in all the other reactions: 434 bp (HGH)

Fehlende Banden in den Reaktionen Nr. 1,2,3 oder 4 können auf *RHD* psi oder auf Partial D-Gene hinweisen. In Gegenwart von Cde^s kommt es zum Ausfall der Reaktion Nr. 9.

Missing bands in reaction no. 1,2,3 or 4 may either indicate RHD psi or partial D genes. In presence of Cde^s reaction no. 9 is missing.

Ergebnis / *Result*	1	2	3	4	5	6	7	8	9	10	11	12	13	Phänotyp / *Phenotype*	Genotyp / *Genotype*

Proben-ID / *Sample-ID:*

Probenname / *Name:*

Geb.-Datum / *Born:*

Ergebnis / *Result:*

Datum / *Date:*

Unterschrift / *Signature:*

Gelbild / *Gel Picture*

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Kurzanleitung / *Short Instructions*

1. Die gewünschte Anzahl BAGene-Platten/-Streifen aus dem Gefrierschrank (-20…-80°C) nehmen und den 10 x PCR-Puffer bei Raumtemperatur auftauen.
   Remove the required number of BAGene-plates/-strips from -20…-80°C and thaw the 10 x PCR-buffer.
2. Der erste Reaktionsmix ist durch Aufdruck der Lot-Nr. markiert.
   The first reaction mix is marked by the printed lot number.
3. Den Mastermix, bestehend aus 10 x PCR-Puffer, DNA-Lösung, Taq-Polymerase und Aqua dest. zusammenpipettieren und gründlich vortexen. Die verschiedenen BAGene DNA-SSP Kits werden mit dem gleichen Mastermix angesetzt und sind daher miteinander kombinierbar.
   Pipet the mastermix, consisting of 10 x PCR-buffer, DNA-solution, Taq-Polymerase and aqua dest. and mix well. The different BAGene DNA-SSP Kits do all work with the same mastermix and can therefore be combined.

Zusammensetzung des Mastermixes in Abhängigkeit von der Anzahl der Reaktionsmixe
Composition of the Mastermix depending on the number of reaction mixes

No. of mixes	Aqua dest.	10 x PCR-buffer	DNA-sol. (50-100 ng/µl)	Taq-Polymerase (5 U/µl)	total volume app.	
1	8	1	1	0,08	10	µl
2	16	2	2	0,2	20	µl
6✶	50	7	7	0,5	65	µl
7	70	9	9	0,7	90	µl
8	80	10	10	0,8	100	µl
9	88	11	11	0,9	110	µl
10	96	12	12	1,0	120	µl
11	104	13	13	1,0	130	µl
12	112	14	14	1,1	140	µl
13	128	16	16	1,3	160	µl
14	136	17	17	1,4	170	µl
15	144	18	18	1,4	180	µl
16	152	19	19	1,5	190	µl

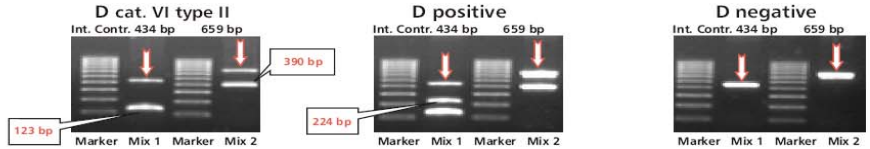
⇨ Bei abweichenden DNA-Konzentrationen sind die Mengen von DNA und Wasser entsprechend zu variieren.
For different DNA concentrations, the quantities of DNA and water must be varied accordingly.

✶ Mastermix für 6 Reaktionsmixe wird wegen des geringen Volumens Taq-Polymerase als Mindestansatz empfohlen.
Minimum preparation of mastermix for 6 reaction mixes is recommended due to the small volume of Taq-Polymerase.

4. Nach dem Vortexen werden von diesem Gemisch umgehend je **10 µl** zu den angetrockneten Reaktionsmixen pipettiert.
   *After vortexing add **10 µl** of this mixture immediately to the dried reaction mixes.*
5. Die Reaktionsgefäße werden mit den dafür vorgesehenen Deckeln **dicht** verschlossen. Die Platten/Streifen werden leicht bewegt, um das Pellet auf dem Gefäßboden etwas anzulösen. Die gesamte Reaktionslösung soll sich im Gefäßboden befinden.
   ***Tightly** close the tubes with the respective strip caps. Slightly move the plates/strips to dissolve the pellet at the bottom of the tube. All PCR-solution should settle on the bottom of the tube.*
6. Nach der PCR und Auftrennung der Amplifikate im Gel erfolgt die Auswertung.
   After PCR and separation of the amplicons in the gel, the evaluation can be carried out.
7. Alle BAGene Reagenzien sind nach Gebrauch wieder bei -20…-80°C zu lagern.
   Return all BAGene reagents to -20…-80°C after use.

⇨ Ablesen der Ergebnisse in Pfeilrichtung / *Read the results in direction of the arrow*

Beispiel: Gelbilder der Reaktionen 1 und 2
Example: gel pictures of reaction 1 and 2



Test Procedure - Workflow

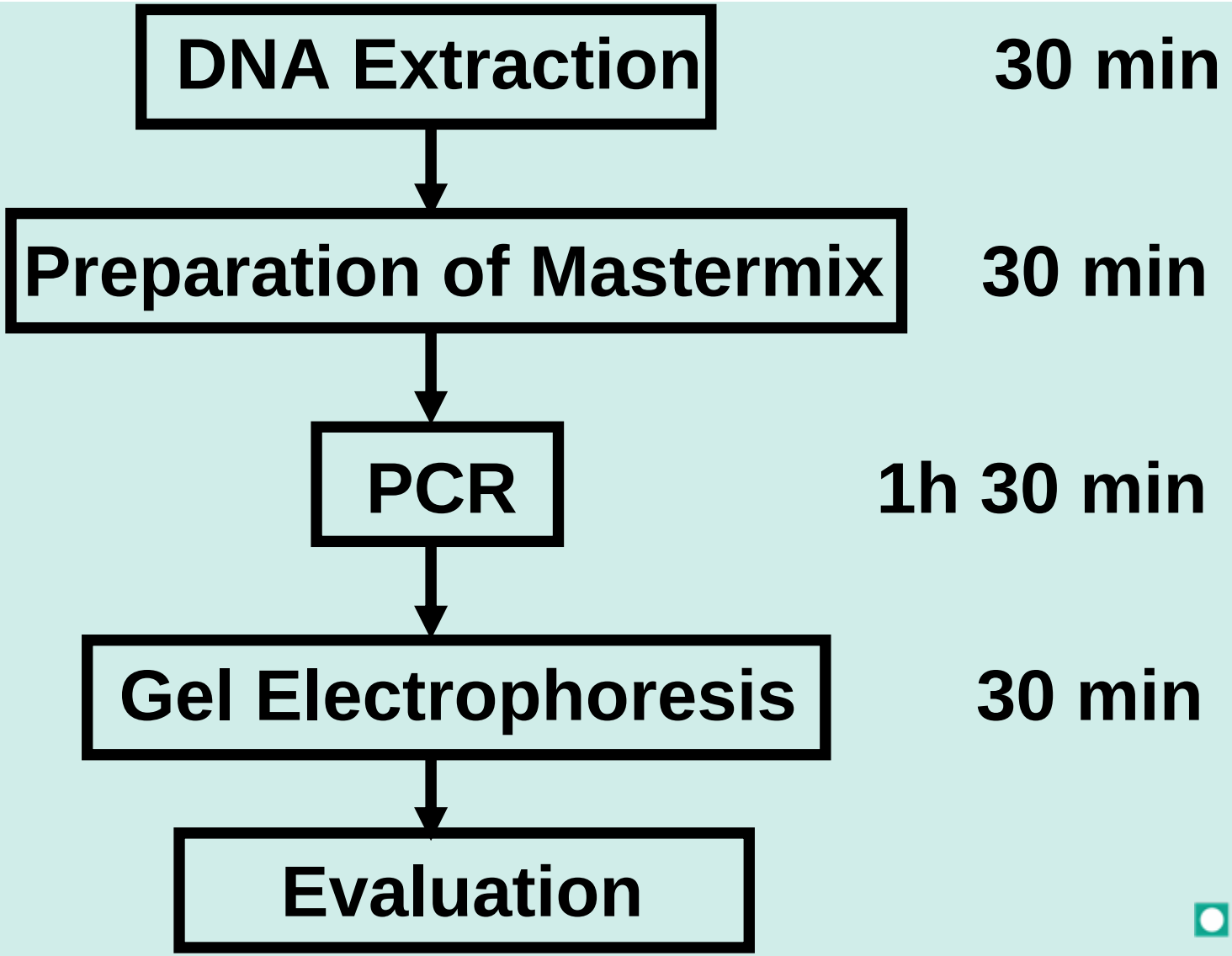


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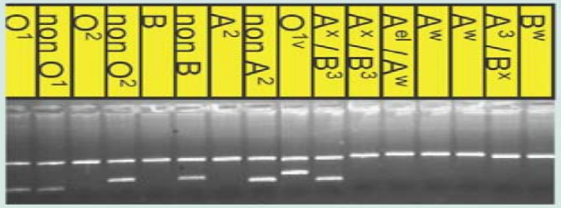
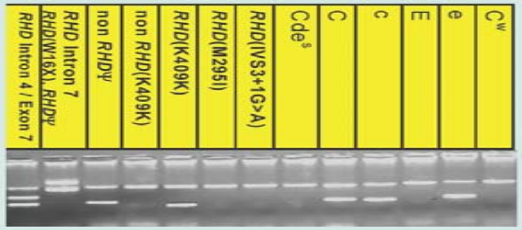
Figure 1

ABO Genotyping



BAGene ABO-TYPE variant genotype *O¹ᵛAˣ*

RHD, RHCE Genotyping

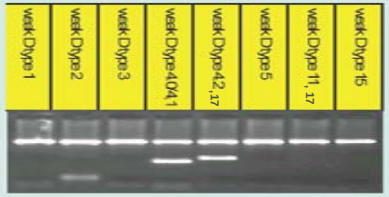


BAGene RH-TYPE genotype *RHD(K409K) Ccee*

Genotyping partial D

BAGene Partial D-TYPE genotype *D cat. VI type II*

Genotyping weak D

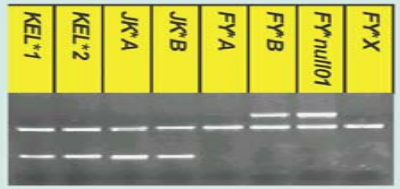


BAGene Weak D-TYPE genotype *weak D type 4.2*

RHD Zygosity

BAGene D Zygosity-TYPE

Genotyping KEL, JK, FY

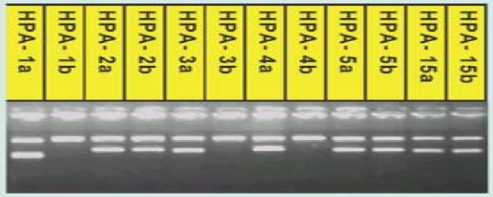


BAGene KKD-TYPE
genotype *KEL*1/KEL*2; JK*A/JK*B; FY*B/FY*null01*

Genotyping MNS

BAGene MNS-TYPE
genotype *NSs*

Genotyping HPA



BAGene HPA-TYPE
genotype *HPA-1a/a; 2a/b; 3a/a; 4a/a; 5a/b; 15 a/b*

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Intended use

- **The molecular determination of blood group antigens using SSP Kits has to be performed after serology**
- **We offer these assays as a supplementary technique to investigate weak or discrepant serological findings. The current assays are not intended to replace serology.**
- **In case of discrepant or unclear genotyping results, transfusion guidelines have to be followed in accordance with serological typings. Final clarification by sequencing analysis is recommended.**

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Conclusion

- SSP technique is helpful to resolve most of the problems caused by discrepant or doubtful serological results
- Easy to handle
- Questionable cases in donor, recipient and patient typing can be examined with acceptable cost.
- Not suitable for high throughput
- Not suitable to replace serology

References

- **Frequency of *DAU*, *RHD psi*, *Cde*^{s} in South African blood donors.**
  Maas JH, Binder E, Smart E, Prager M, Legler T.
  Transfus Med Hemother 2004;31(suppl 3):PS412

- ***RHD* and *RHCE* Genotyping in South African Blood Donors with Prepipetted PCR-SSP Kits.**
  Legler TJ, Binder E, Smart E, Prager M, Maas JH.
  Transfusion 2004;44(suppl):SP285

- **Molecular genetic blood group typing by the use of PCR-SSP technique**
  M Prager, BAG Health Care, Lich, Germany
  Presented at the FDA Workshop on Molecular Methods in Immunohematology Bethesda 25 – 26 Sept 2006
  Transfusion 2007;47:54S-59S.

- ***ABO* genotyping for diagnosis of unusual ABO blood groups: A Comparative Study in German Blood Donor Centers**
  M Prager, EA Scharberg, FF Wagner, J Burkhart, A Seltsam
  Presentation at AABB Annual Meeting 2007, October 20 – 23, Anaheim, California, USA

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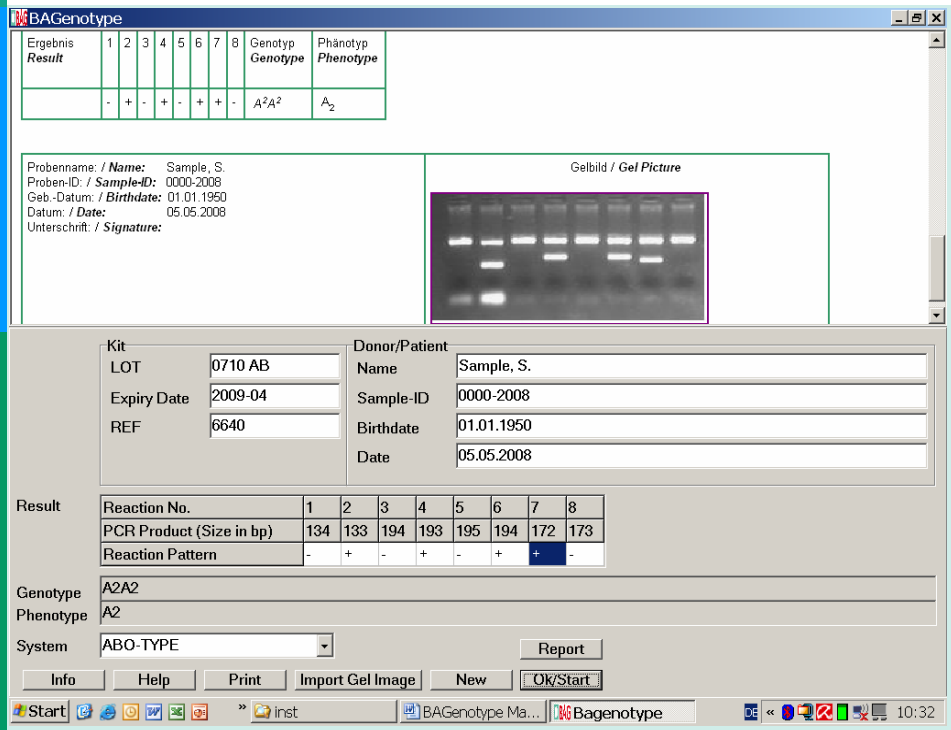
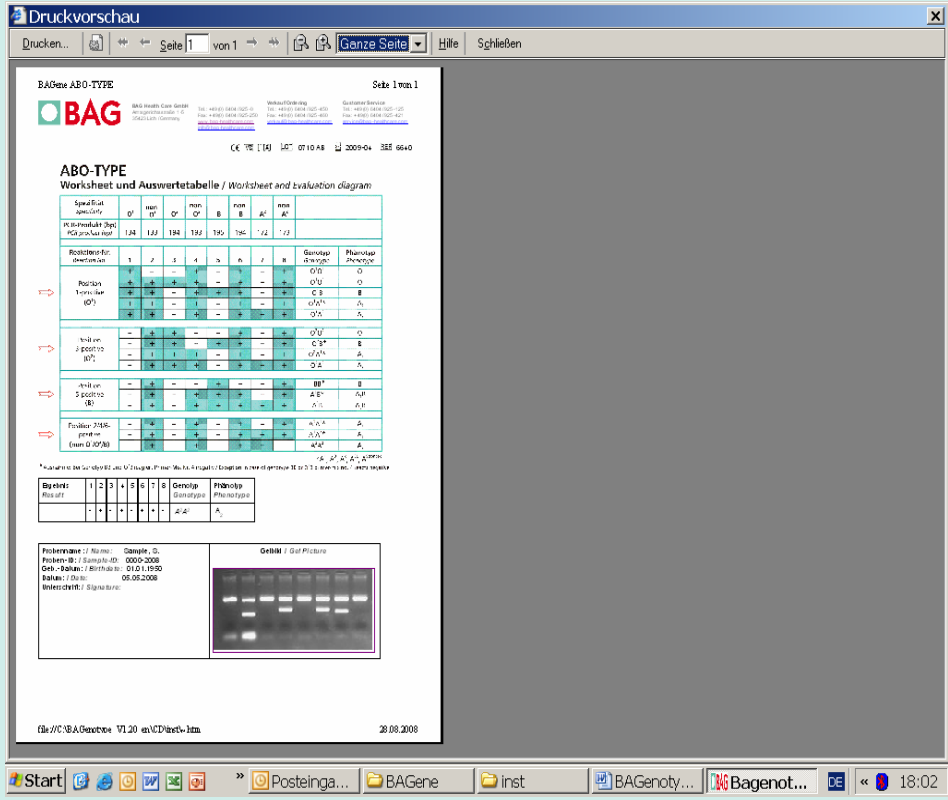
BAGenotype
Novel Evaluation Software





Fast and easy tool for blood group genotyping using

BAGene PCR-SSP test kits

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