seac

9ª SEMANA DE ESTUDOS EM ANÁLISES CLÍNICAS

SEXAGEM FETAL: Avanços no diagnóstico pré-natal não-invasivo

Palestrante: JOSÉ EDUARDO LEVI, INSTITUTO DE MEDICINA TROPICAL DA USP, FUNDAÇÃO PRÓ-SANGUE/HEMOCENTRO DE SÃO PAULO

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28 e 29 de agosto de 2014

Local: Auditório do Bloco H do Centro de Ciências da Saúde da Universidade Federal de Santa Catarina (UFSC)

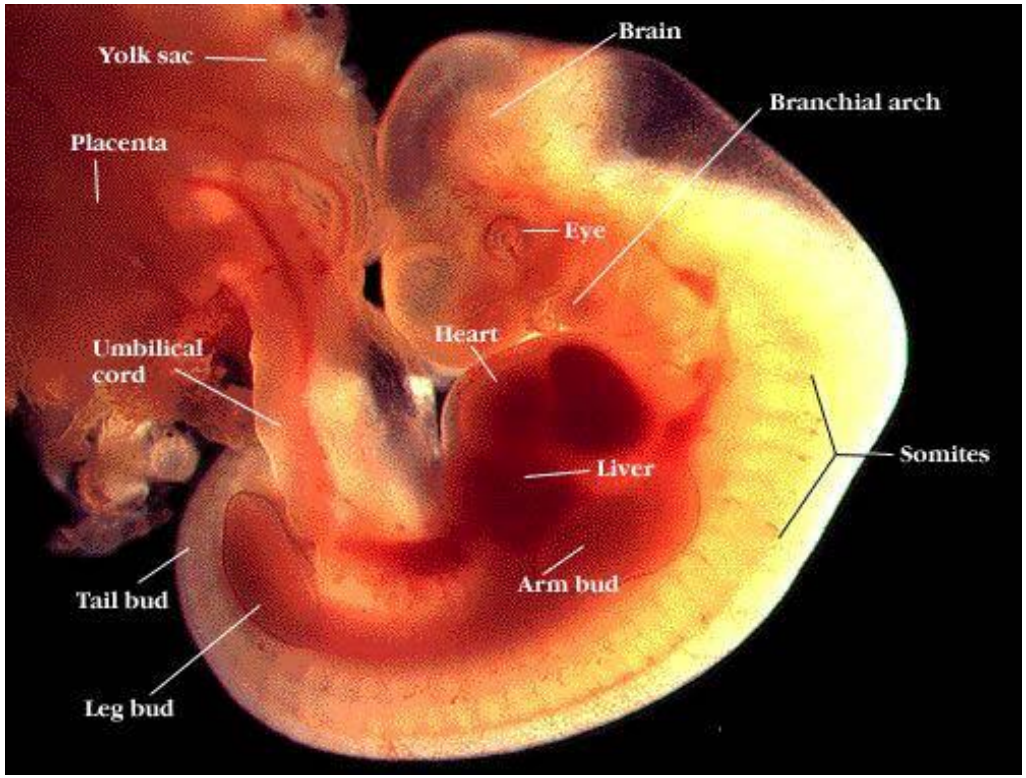
Informações e Inscrições:

www.seac.paginas.ufsc.br

HISTÓRICO

EXISTEM CÉLULAS FETAIS CIRCULANTES NO SANGUE MATERNO EM TODAS AS FASES DA GRAVIDEZ

(taxas de 1:50.000/5.000.000)

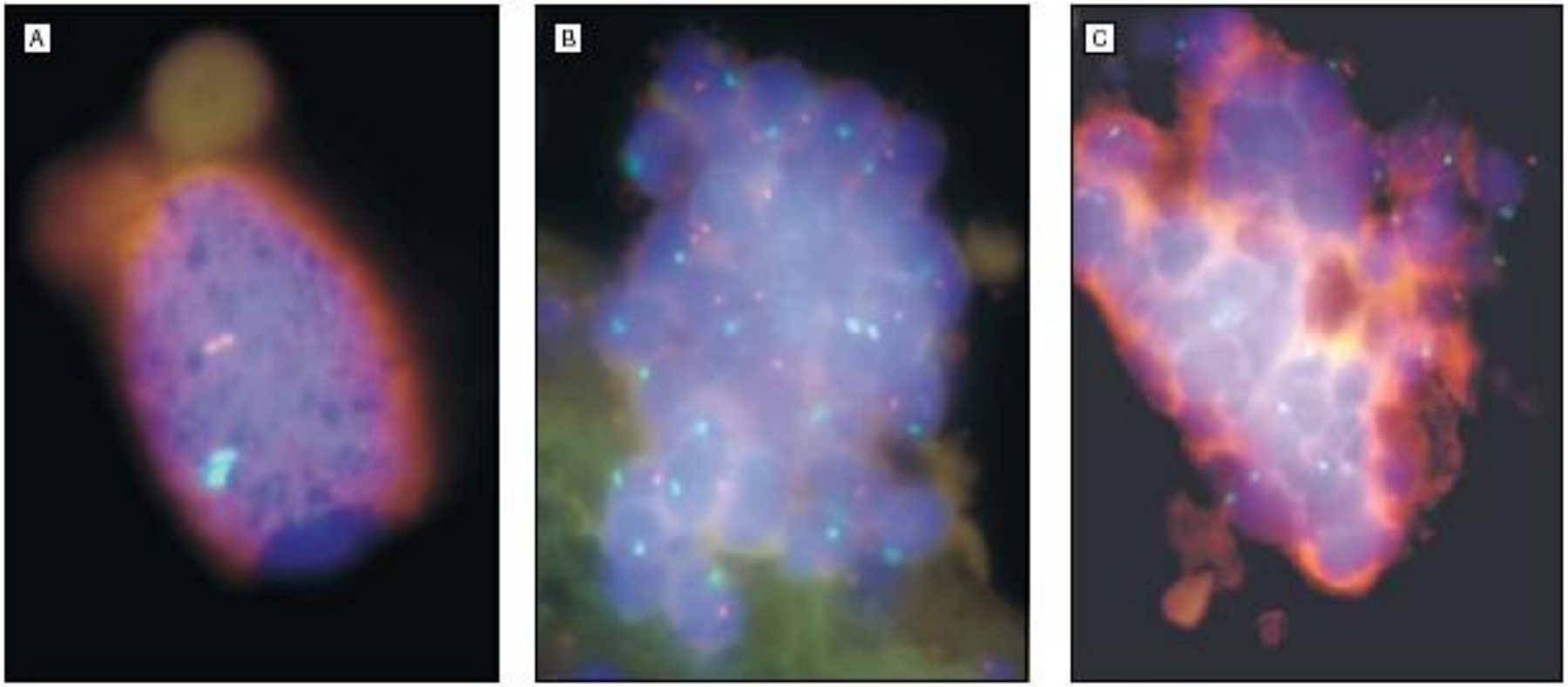


<u>TIPOS CELULARES</u> : TROFOBLASTOS, ERITROBLASTOS, LEUCÓCITOS E *STEM CELLS* CD34+

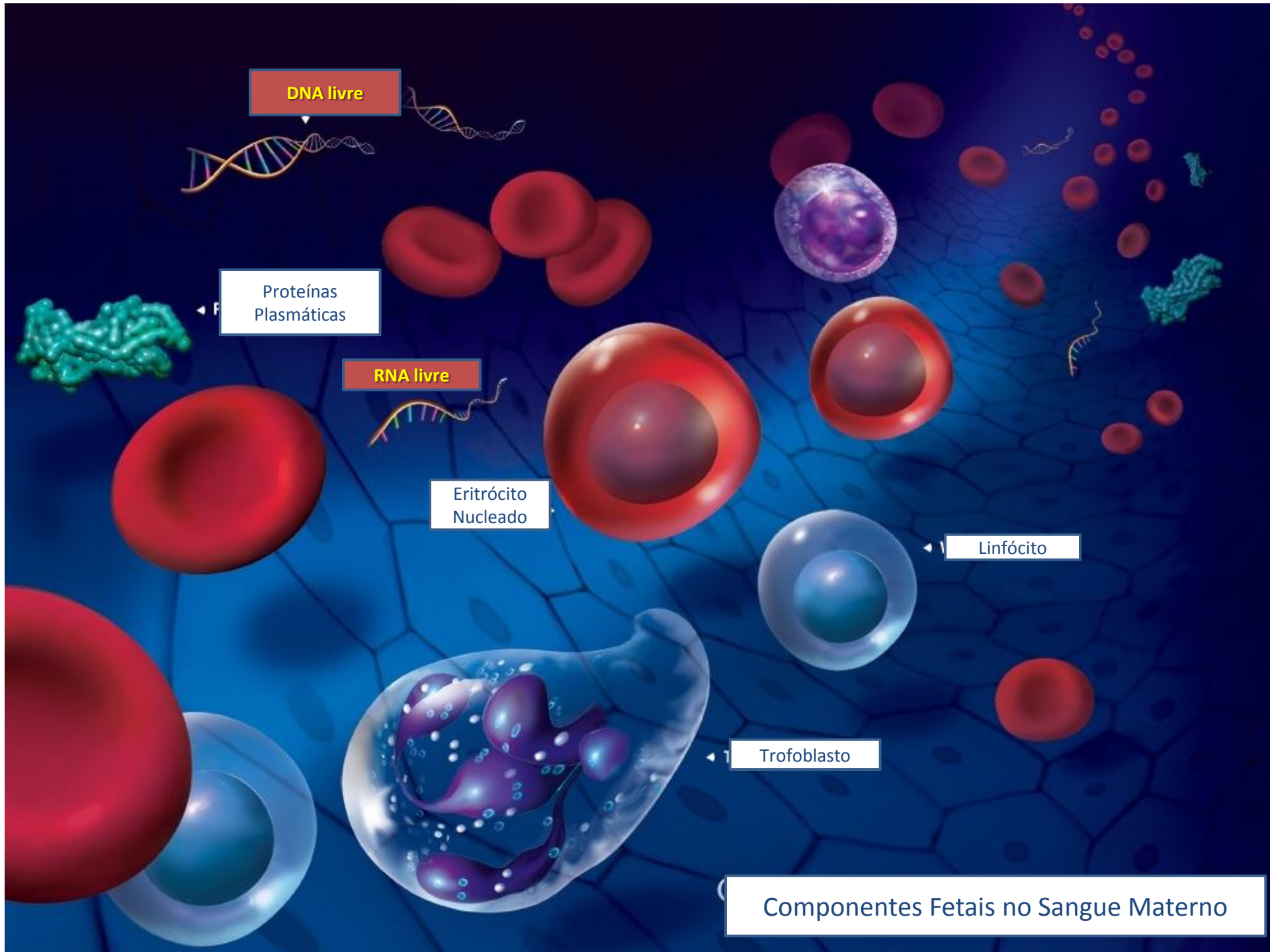
Transfer of Fetal Cells With Multilineage Potential to Maternal Tissue

JAMA, JULY, 2004; 292(1):75-80

Figure 1. Cytokeratin Expression in Microchimeric Cells in Thyroid



Photomicrographs show fluorescence in situ hybridization (FISH) using Cy3-labeled X (orange) and fluorescein isothiocyanate conjugated–labeled Y (green) chromosome probes and immunofluorescence staining for cytokeratin using mouse monoclonal AE1/AE3 anticytokeratin antibody and fluorochrome Texas Red (red). Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (blue). A, Male microchimeric cell with 1 Y chromosome (green), 1 X chromosome (orange), and stained with anticytokeratin antibody (red) (patient A; magnification ×1000). B, Interphase FISH of thyroid tissue showing a group of microchimeric cells identified by the presence of X and Y chromosomes (orange and green, respectively). The X or Y chromosome may not be observed in each nucleus, as they may not be in the same plane of focus (patient C; magnification ×400). This group of cells did not stain positively for cytokeratin. C, Combined FISH and immunofluorescence staining of a group of microchimeric cells with 1 X and 1 Y chromosome. This group of cells expresses cytokeratin (red). The X or Y chromosome may not be observed in each cell, as they may not be in the same plane of focus (patient A; magnification ×400).



Componentes Fetais no Sangue Materno

HISTÓRICO

Detection of single-copy fetal DNA sequence from maternal blood.
Lo Y-M et al, *Lancet* 1990, 33: 1463-1464.

Prenatal determination of fetal RhD status by analysis of peripheral blood of rhesus negative mothers.
Lo Y-M et al, *Lancet* 1993, 341: 1147-1148.

Cancer and mutant DNA in blood plasma.
Mulcahy HE et al, *Lancet* 1996, 348: 628.

Microsatellite alterations in serum DNA of head and neck cancer patients.
Nawroz H et al, *Nature Medicine* 1996, 2: 1035-1037.

HISTÓRICO

Presence of fetal DNA in maternal plasma and serum.

Lo Y-M et al, *Lancet* 1997, 350: 485-487.

Detection of RHD-specific sequences in maternal plasma.

Faas BHW et al, *Lancet* 1998, 352: 1196.

Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma.

Lo Y-M et al., *New Engl J Med* 1998, 339: 1734-1738.

EXISTE DNA FETAL LIVRE NO PLASMA MATERNO EM PROPORÇÕES BEM MAIORES QUE A DE CÉLULAS MATERNAS/FETAIS NO SANGUE PERIFÉRICO.

Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis.
Lo Y-M et al, Am J Hum Genet, 1998, 62: 768-775.

Resultados de 30 grávidas com fetos masculinos

→ **24 plasma, 21 soro, 5 buffy-coat**

25.4 genome equivalents/mL (early pregnancy) - 3,4% do total
292.2 genome equivalents/mL (late pregnancy) - 6,2% do total

O DNA FETAL LIVRE PODE SER DETECTADO JÁ NO PRIMEIRO MÊS DE GRAVIDEZ

Kinetics of SRY gene appearance in maternal serum: detection by real time PCR in early pregnancy after assisted reproductive technique. Guilbert J et al *Human Reproduction*, 18:1733-1736, 2003.

Todas grávidas de meninos. 1 paciente 18 dias, 9 pacientes em até 37 dias após a transferência de embrião.

O DNA FETAL LIVRE NO PLASMA MATERNO É RAPIDAMENTE ELIMINADO NA URINA EM ATÉ 48 HORAS APÓS O PARTO

Rapid clearance of fetal DNA from maternal plasma.

Lo Y-M et al, *Am J Hum Genet* 1999, 64: 218-224.

12 grávidas pré-parto até 1-42h pós-parto.

Vida média do DNA fetal circulante = 16 minutos

QUANTIDADES "ANORMAIS" (ELEVADAS) DE DNA FETAL LIVRE NO PLASMA MATERNO INDICAM SOFRIMENTO PLACENTÁRIO E/OU FETAL

Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. Lo Y-M et al, *Clin Chem* 1999, 45: 184-188.

Cell-free fetal DNA (SRY locus) concentration in maternal plasma is directly correlated to the time elapsed from the onset of preeclampsia to the collection of blood.

Farina et al, *Prenat Diagn* 2004, 2: 293-297.

- **Origem dos ácidos nucleicos fetais em gestantes:**

Trofoblastos da Placenta

- **Detecção de DNA fetal livre em gestações anembrionadas** (Alberry *et al.*, 2007) (Sekizawa *et al.*, 2005)

- **Estudo de caso com placenta increta** (Jimbo *et al.*, 2003)

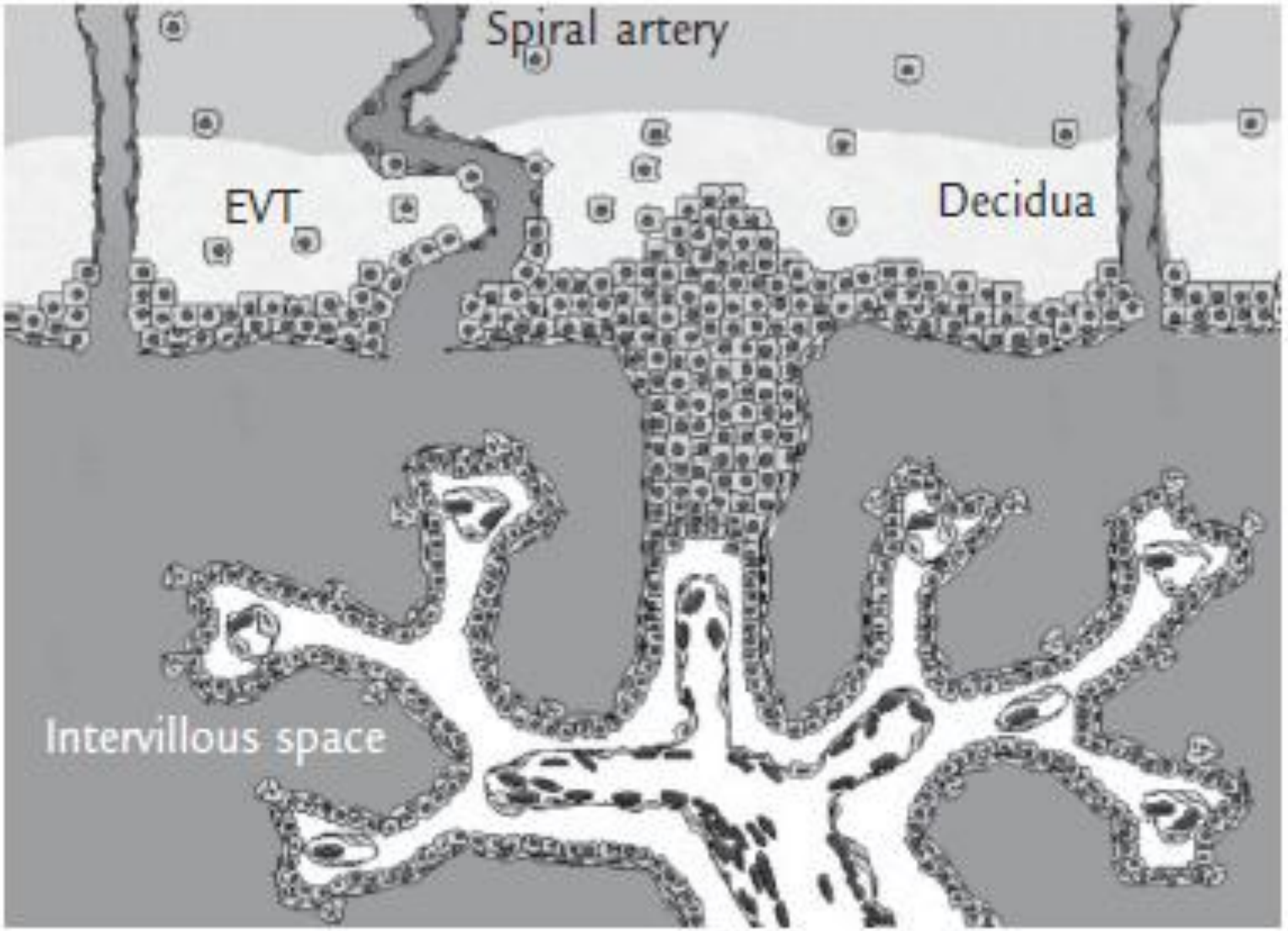


Figure. Structure of the placenta. EVT = extravillous trophoblasts.

(Sekizawa *et al.*, 2005)

ESTUDO

Determinação do sexo fetal pela análise do plasma materno

PACIENTES:

- Voluntárias
- Qualquer idade gestacional

Termo de consentimento livre e esclarecido

Coleta de 5 mL de sangue periférico

Local : Banco de Sangue Hosp. Sírio Libanês, São Paulo - SP

ESTUDO

Determinação do sexo fetal pela análise do plasma materno

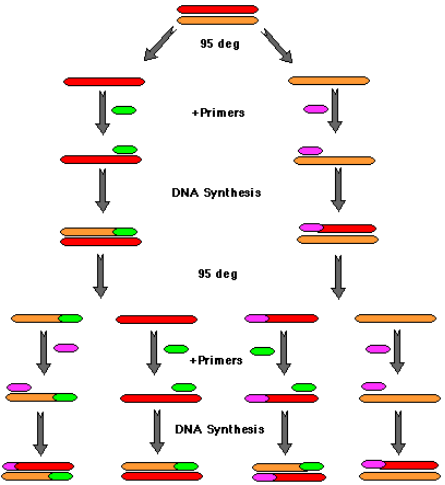
MÉTODO:
Extração de DNA e PCR de região do Cromossomo Y
DYS14 – gene expresso no testículo

RESULTADO:
Checado pela informação obtida das mães pelo ultra-som ou nascimento.
Período : Dez/2001 - Julho 2003
N= 200

MÉTODO DE PCR

O sangue total é centrifugado e o plasma separado em até 2 horas da coleta.

O DNA é isolado em duplicatas de 140 µL de plasma por um método comercial de extração baseado em microcolunas de sílica.

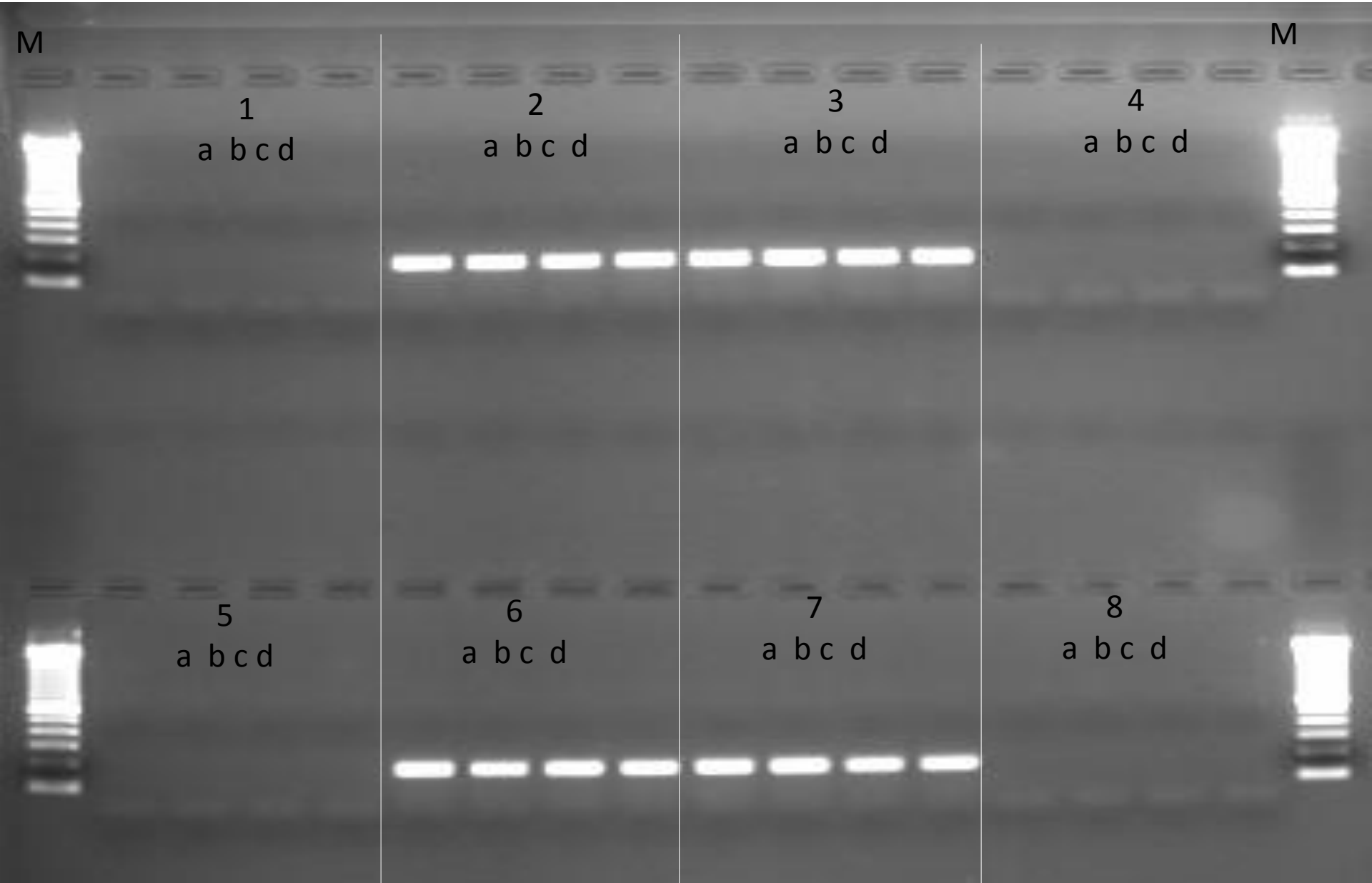


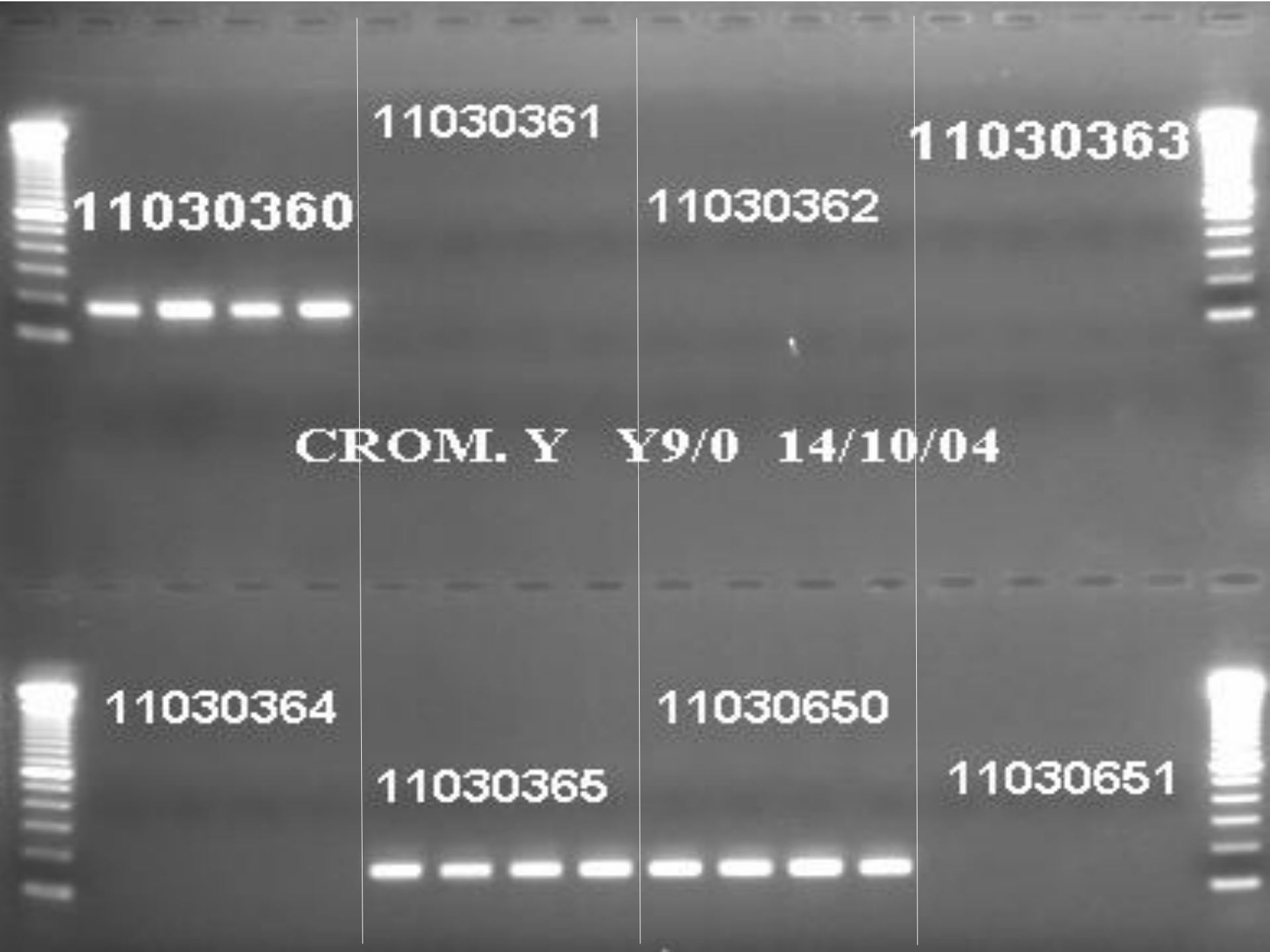
Multiplying a DNA template with PCR

Uma fração do DNA isolado, correspondendo á 30 µL de plasma é submetido a reação de PCR com primers do gene DYS14, amplificando um fragmento de 198 pares de bases.

DETERMINAÇÃO DO SEXO FETAL

LEVI et al., *Rev. Bras. Ginecol. Obstet.*, 2003, vol.25, no.9, p.687-690




11030360
11030361
11030362
11030363
CROM. Y Y9/0 14/10/04
11030364
11030365
11030650
11030651


11030742
11030744
11030741
11030743
CROM. Y Y9/0 08/12/04
11031376
11031378
11030745
11031377

RESULTADOS

CASUÍSTICA C/ CONFIRMAÇÃO
1.026 PACIENTES
sobre um total de 6.000 exames realizados
(Agosto 2008)

	N=
ATÉ 8 SEMANAS	48
8-10	489
11-12	197
>=13	292
TOTAL	1026

RESULTADOS

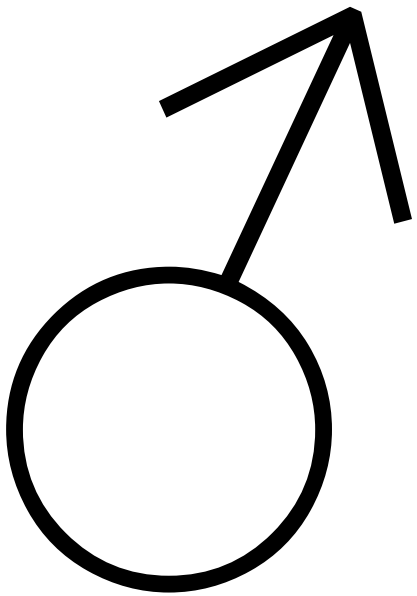
CASUÍSTICA C/ CONFIRMAÇÃO
1.026 PACIENTES

Índice de acerto	MENINA	MENINO	GERAL
ATÉ 8 SEMANAS	77,27%	100,00%	89,58%
8-10	98,82%	100,00%	99,38%
11-12	98,99%	100,00%	99,49%
>=13	99,38%	100,00%	99,66%
GERAL	98,14%	100,00%	99,02%

RESULTADOS

DETECÇÃO MAIS PRECOCE

5 SEMANAS



RESULTADOS

ERROS

SOMA GERAL ATÉ AGOSTO 2008							
	N=	ERROS	A/O	O/A	RES A	RES O	TOTAL
ATÉ 8 SEMANAS	48	5	5	0	22	26	48
8-10	489	3	3	0	254	233	487
11-12	197	1	1	0	99	98	197
>=13	292	1	1	0	162	130	292
TOTAL	1026	10	10	0	537	487	1024

CONCLUSÕES

- **O método apresentou uma excelente especificidade.**

- **A sensibilidade é maior para fetos do sexo masculino, principalmente no começo da gestação (< 8 semanas).**

- **No caso de resultado de sexo feminino nas primeiras 8 semanas sugere-se a repetição do teste com idade gestacional mais avançada.**

RBGO 25 (9): 687-690, 2003

Determinação Pré-natal do Sexo Fetal por meio da Análise de DNA no Plasma Materno

Prenatal Fetal Gender Determination by Analysis of DNA from Maternal Pasma

José Eduardo Levi[1], Silvano Wendel[1,2], Deise Tihe Takaoka[2]

DIAGNÓSTICO PRÉ-NATAL DA DOENÇA DE HUNTINGTON

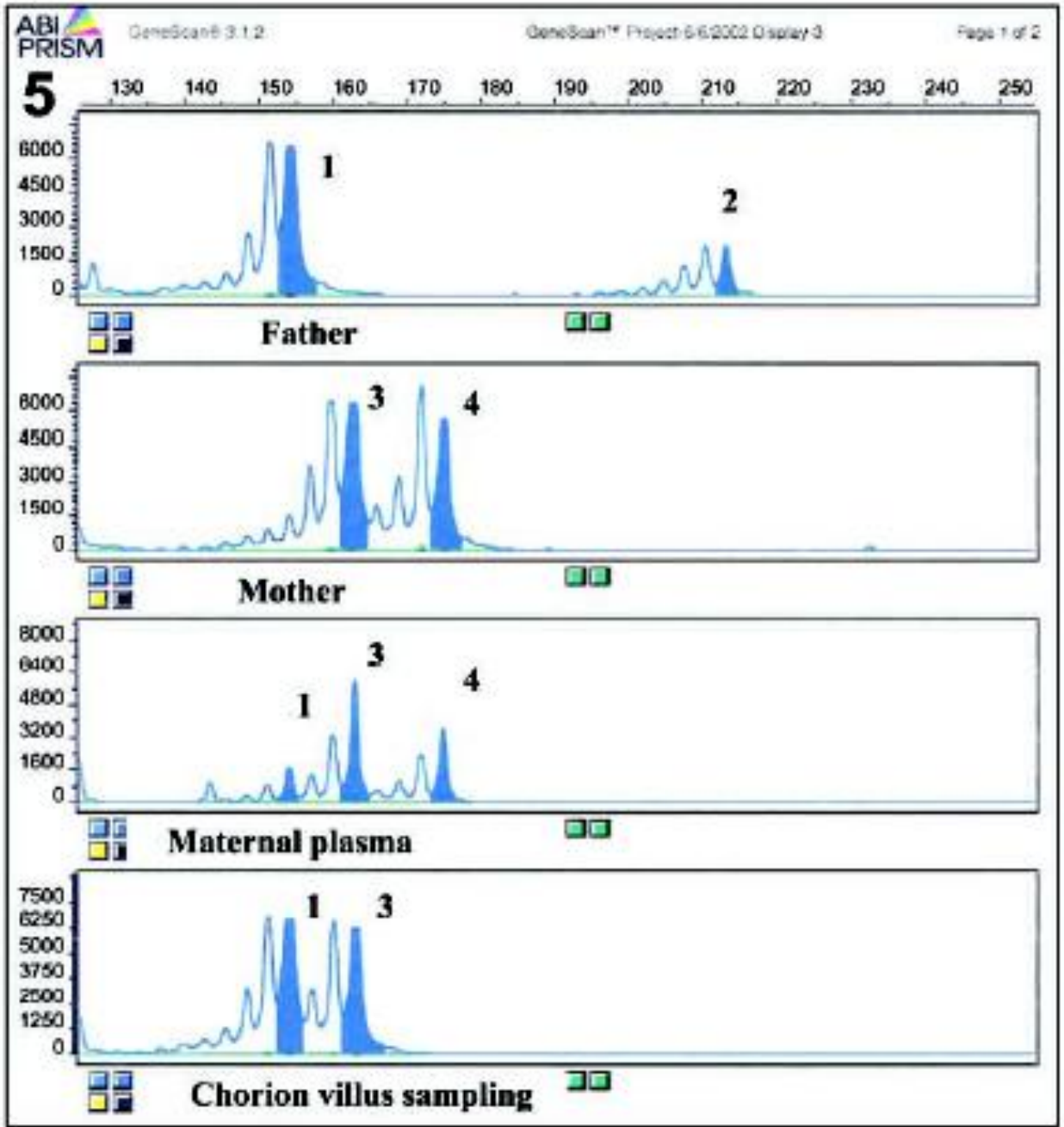


Figure 5 HD study of a healthy fetus in maternal plasma. (From González-González et al. Prenat Diagn 2003;23:232–234. Copyright John Wiley & Sons, Ltd. Reproduced with permission.)

Determinação do Rh fetal através de análise do plasma materno

OBJETIVOS :

1- Evitar a triagem sistemática de mães RhD-negativas para a presença de anticorpos anti-D.

2- Evitar procedimentos invasivos de monitoração de DHPN.

3- Evitar o uso da "vacina" anti-D (imunoprofilaxia) para :
- Grávidas submetidas á procedimentos invasivos como amniocentese e biópsia de vilosidade coriônica, além de hemorragias.

IMUNOPROFILAXIA ANTI-D :

Imunobiológico de origem humana. Normalmente administrado em 2 doses, uma na 28ª-30ª semana de gestação, outra logo após o parto.

INCONVENIENTES:

- **Transmissão de agentes infecciosos**

- **Custo de cada dose é de aproximadamente R$ 210,00**

- **Baixa oferta**

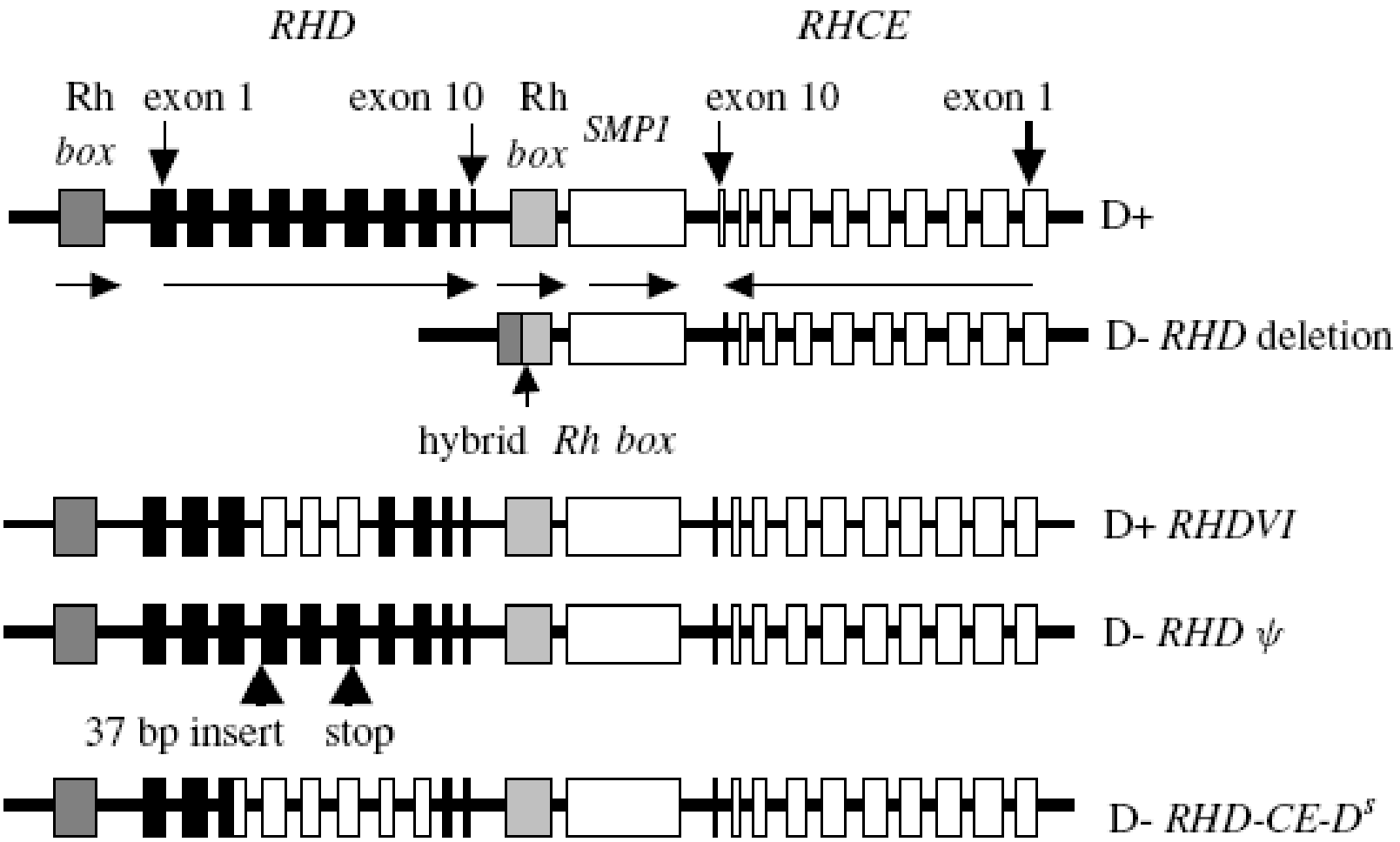


Figure 1—Diagram of the Rh genes, the *Rh boxes* flanking *RHD*, and *SMP1* between *RHD* and *RHCE*, in five haplotypes, two producing D (D+) and three producing no D (D−)

A clinical service in the UK to predict fetal Rh (Rhesus) D blood group using free fetal DNA in maternal plasma. Finning K, Martin P and Daniels G. Annals of the New York Academy of Sciences June 2004, 1022:119-123.

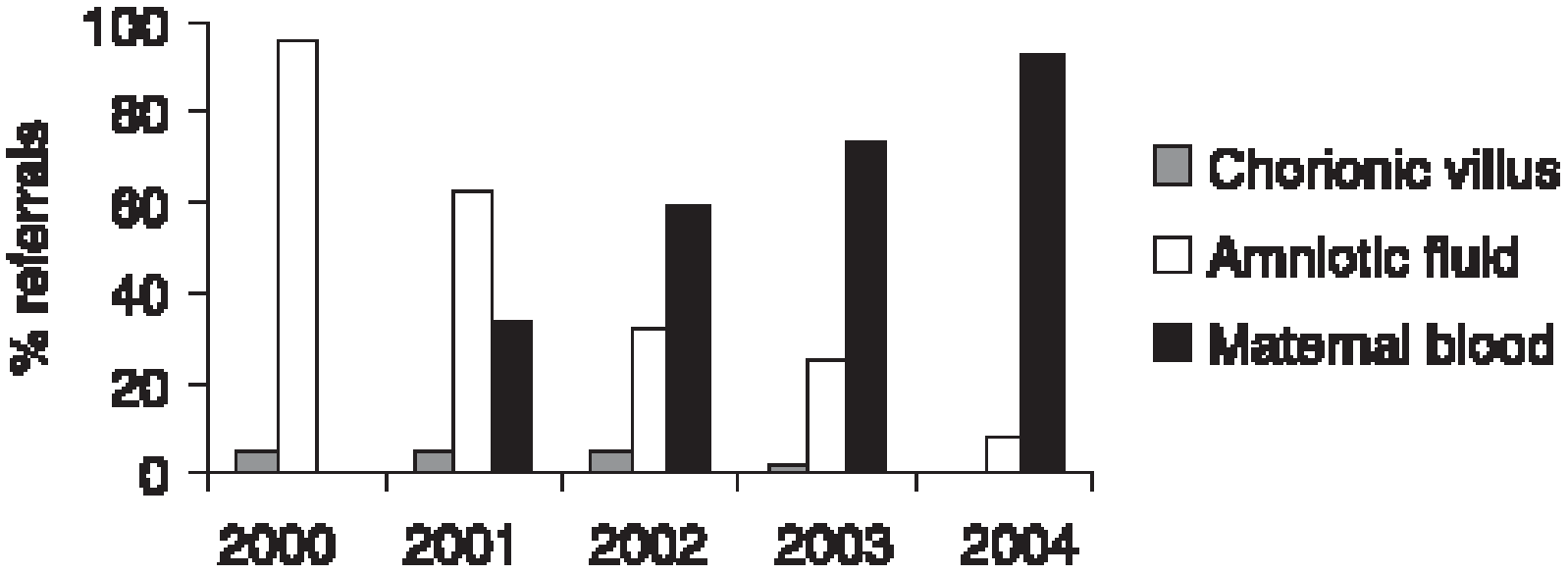


Fig. 1 Proportional changes in the source of fetal DNA in samples referred to the International Blood Group Reference Laboratory (IBGRL), Bristol, for fetal *RHD* genotyping between 2000 and 2004.

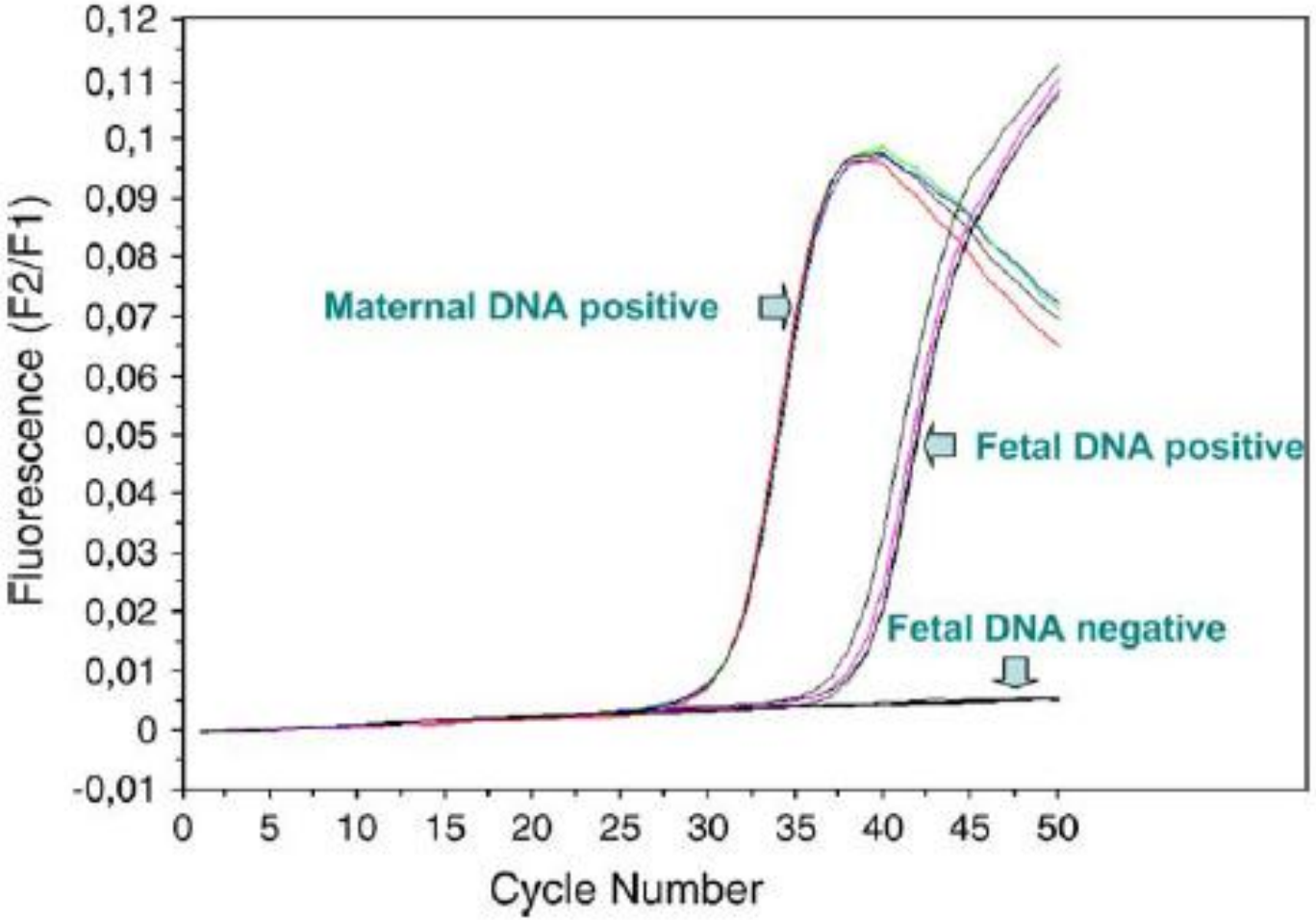


Figure Detection of the RHD gene in maternal serum using real-time PCR.Typical results observed from patient samples: one giving a positive result for fetal DNA (1), the second a negative one (2), and the third one corresponding to an RhD-negative woman but carrying the RHD gene in her genome (3).

Effect of high throughput *RHD* typing of fetal DNA in maternal plasma on use of anti-RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study

BMJ 2008;336;816-818;

Results of testing 1869 DNA samples from plasma of RhD negative pregnant women for fetal *RHD* and comparison with serologically determined phenotype of her baby's cord sample*

Predicted phenotype from fetal DNA	Serological phenotype of cord sample	No (%)	Conclusion
RhD postive	RhD postive	1118 (59.8)	Correct
RhD negative	RhD negative	670 (35.9)	Correct
RhD positive	RhD negative	14 (0.8)	False positive
RhD negative	RhD postive	3 (0.2)	False negative
RHD variant	4 RhD postive/4 RhD negative	8 (0.4)	Inconclusive
Inconclusive	13 RhD postive†/18 RhD negative	31 (1.7)	Inconclusive
Inconclusive‡	18 RhD postive/7 RhD negative	25 (1.3)	Inconclusive

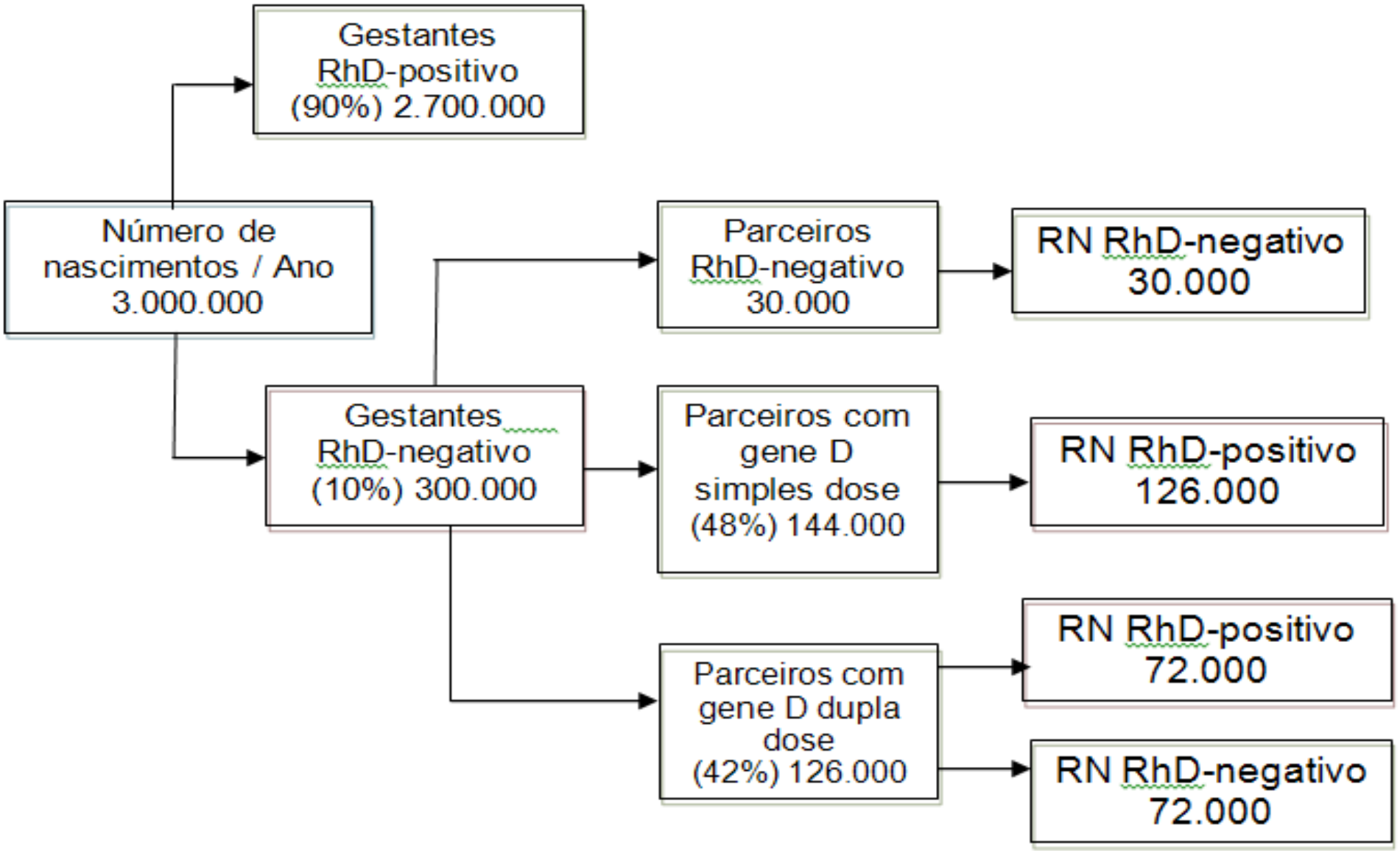
*If anti-RhD is given only when predicted phenotype from DNA is RhD positive, sensitivity of test is 96.7% (95% CI 95.5% to 97.6%) and specificity is 98% (96.7% to 98.8%). If anti-RhD is given when predicted phenotype from DNA is RhD positive, *RHD* variant, or inconclusive, sensitivity is 99.7% (99.2% to 99.9%) and specificity is 94% (92.0% to 95.5%).

†One resulting from DNA extraction failure.

‡*RHD* detected in maternal DNA.

PROJEÇÃO BRASIL – Tese Mestrado

Shirley Lopes de Castilho , Instituto Fernandes Figueira
FIOCRUZ, RJ



The controversy about controls for fetal blood group genotyping by cell-free fetal DNA in maternal plasma

Peter G. Scheffer[a,b], Masja de Haas[a,c] and C. Ellen van der Schoot[a]

Table 1 Published studies on fetal blood group genotyping in maternal plasma (January 2009–June 2011)

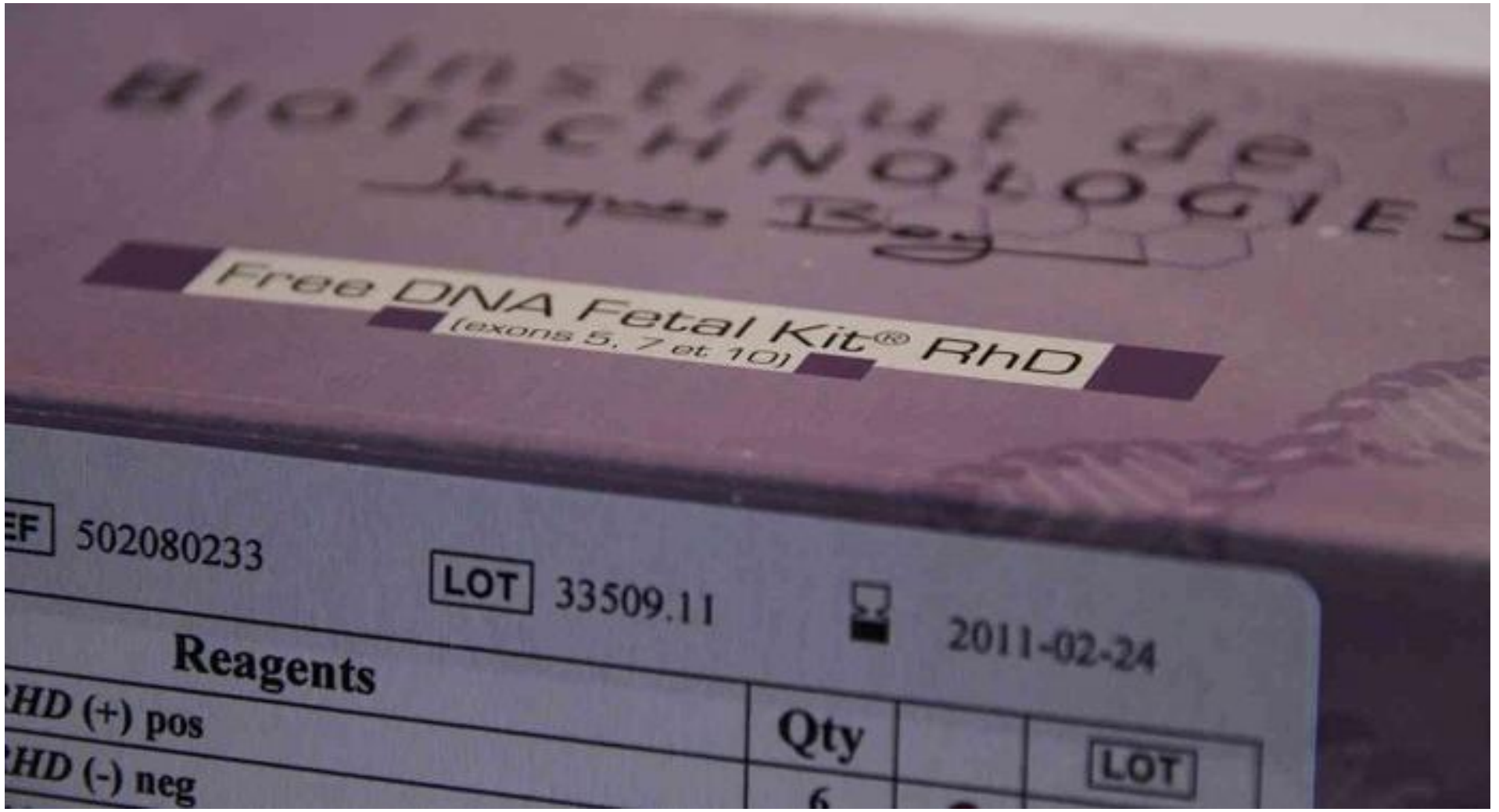
Author	Technique	No. samples tested[a]	Gestation (weeks)	Tested plasma equivalent (ml) per replicate[c]	No. replicates	Target gene/allele	Total cell-free DNA control	Fetal cell-free DNA control	Sensitivity (%)	False-negative results
Akolekar *et al.* [11•]	Real-time PCR	591	11–14	N/A	3	*RHD*	*CCR5*	None	98.2	6
Bombard *et al.* [12]	Mass spectrometry	236	11–13	0.33	1	*RHD*	*TGIF*	*DBY*, *SRY*, *TTTY2*[b]	97.2	4
Scheffer *et al.* [13•]	Real-time PCR	212	7–38	0.33	3	*RHD*, *c*, *E*, *K*	*albumin*	*SRY*, biallelic polymorphisms	100	0
Gutensohn *et al.* [14]	Real-time PCR	181[d]	12–28	N/A	2	*C*, *c*, *E*	*β-globin*	None	100	0
Grill *et al.* [15]	Mass spectrometry	178	N/A	0.04	2	*RHD*	None	None	96.1	5
Tynan *et al.* [16]	Mass spectrometry	150	<32	0.36	1	*RHD*	*albumin*, *AMG*	*SRY*[b]	100	0
Hyland *et al.* [17]	Real-time PCR	140	12–40	0.07	4	*RHD*	*CCR5*	*SRY*, *RASSF1A*	100	0
Achargui *et al.* [18]	Conventional PCR	120	10–40	0.13	1	*RHD*	None	None	98.8	1
Chinen *et al.* [19]	Real-time PCR	102	7–36	0.08	3	*RHD*	*β-globin*	None	100	0
Cardo *et al.* [20]	Real-time PCR	100	9–13	N/A	3	*RHD*	*β-globin*	None	100	0
Clausen *et al.* [21]	Real-time PCR	97[e]	6–37	0.04	3	*RHD*	*GAPDH*	None	98.7	1
Sedrak *et al.* [22]	Real-time PCR	90	7–24	N/A	2	*RHD*	*β-globin*	None	96.7	2
Amaral *et al.* [23]	Real-time PCR	88	11–39	0.07	3	*RHD*	*CCR5*	*SRY*[b]	100	0
Tounta *et al.* [24•]	Multiplex fluorescent PCR	84	7–24	0.16	2	*RHD*	None	*SRY*, *RASSF1A*	100	0
Wang *et al.* [25]	Real-time PCR	78	14–40	0.16	3	*RHD*	*β-globin*	*SRY*, STR's	100	0
Mohammed *et al.* [26]	Real-time PCR	21	20–39	0.08–0.17	2	*RHD*	*β-globin*	None	92.3	1

Current Opinion in Hematology 2011, 18:467–473

Free *DNA Fetal Kit* ® RhD

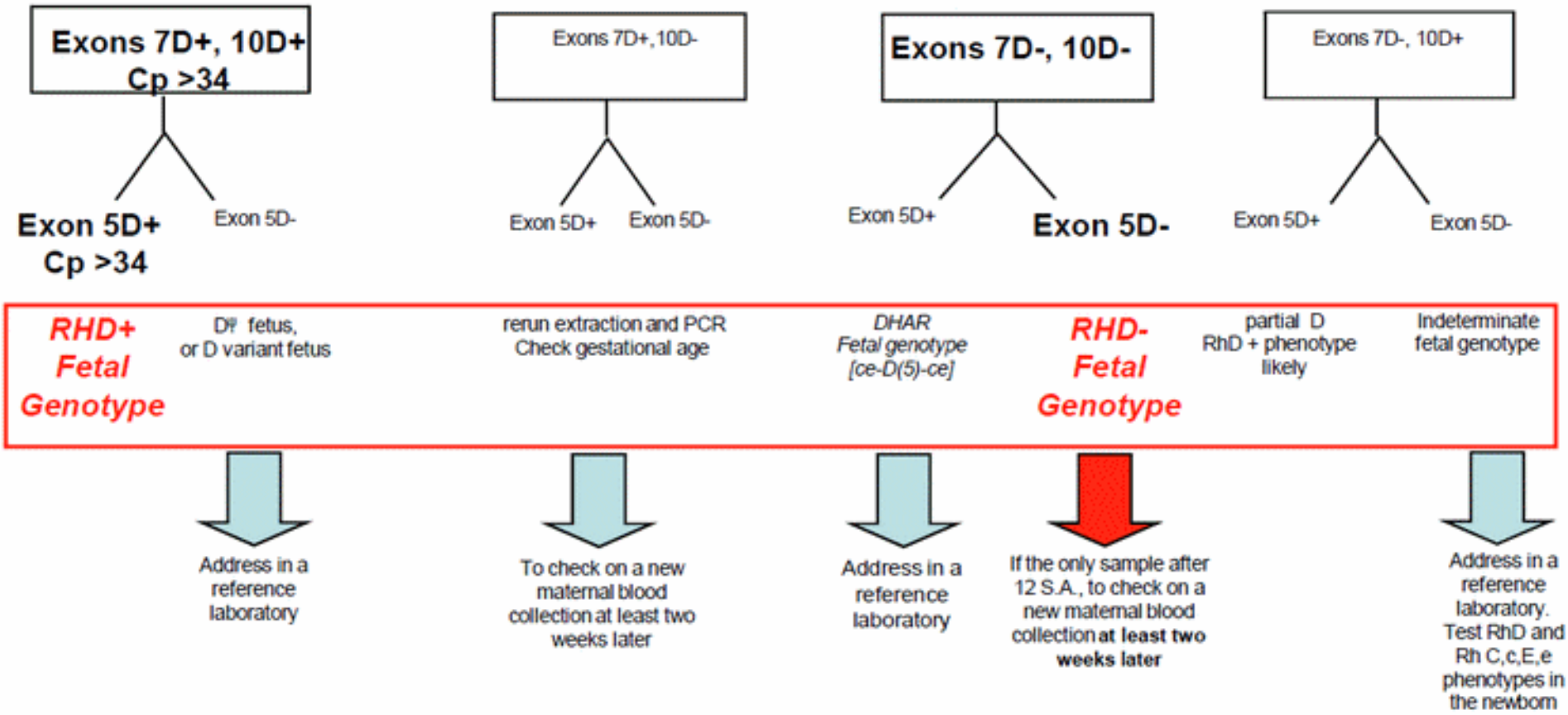
Non invasive fetal *RHD* genotyping in RhD-Negative pregnant women plasma DNA (Real-Time PCR)

Ref.: 502080233



Interpretation guide - *RHD* Fetal genotyping on maternal blood

In all cases, the Maize DNA Control must be positive
If Maize Control Cp >36 : rerun the test



Institut de BIOTECHNOLOGIES Jacques Boy

Version 03.05.2010

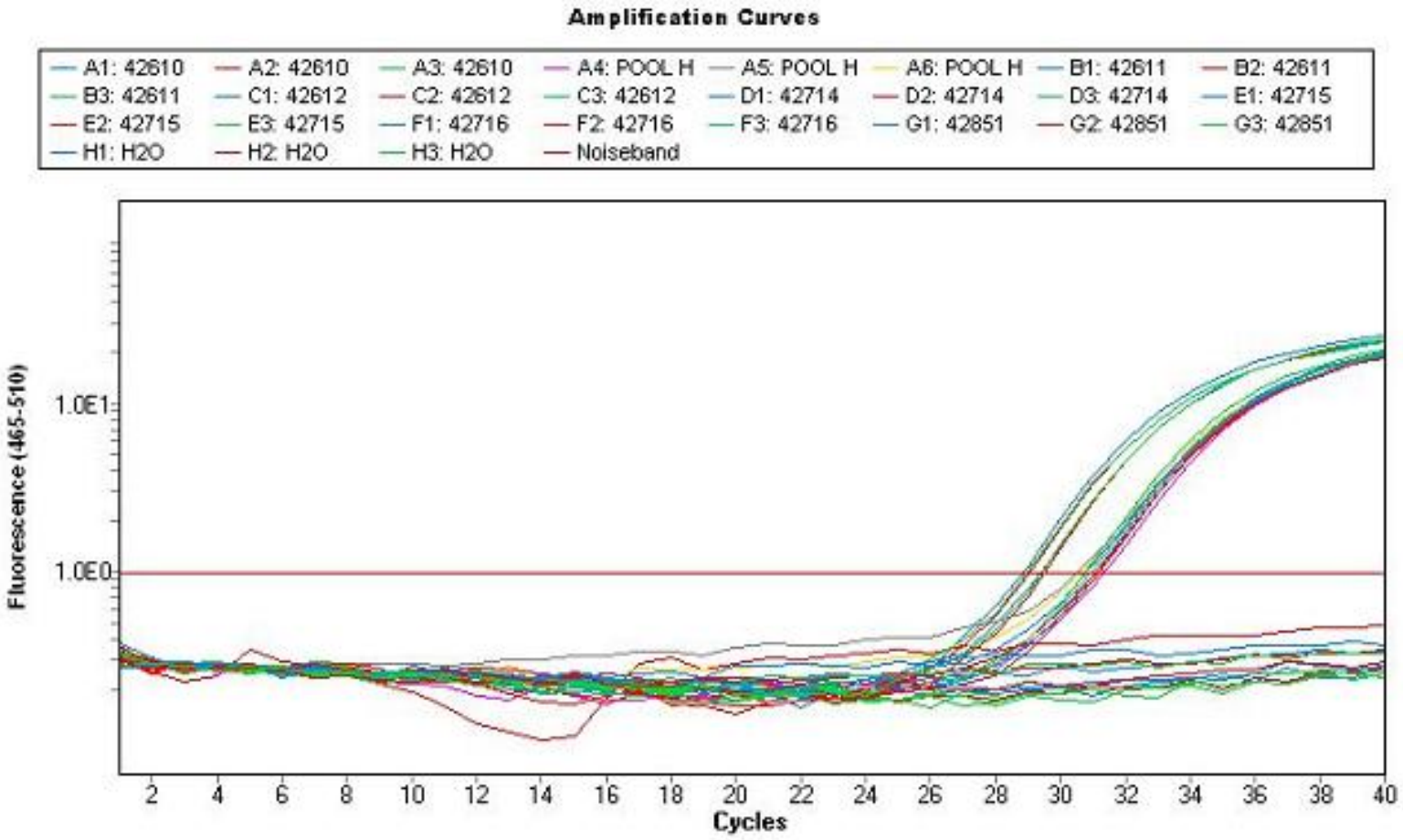
Table S2. Scoring model for fetal *RHD* exon 5, *RHD* exon 7, *c*, *E* and *K* polymerase chain reaction

	Isolation 1			Isolation 2				
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**Test result**	**Further proceedings**
	+	+	+	+	+	+	Positive	Report positive fetal blood group antigen
	+	+	+	+	+	-	Positive	Report positive fetal blood group antigen
	+	+	-	+	+	-	Positive	Report positive fetal blood group antigen
	+	+	+	+	-	-	Inconclusive	Repeat test with same sample
	+	+	+	-	-	-	Inconclusive	Repeat test with same sample
	+	+	-	-	-	-	Inconclusive	Repeat test with same sample
	+	+	-	+	-	-	Inconclusive	Repeat test with new sample
	+	-	-	+	-	-	Inconclusive	Repeat test with same sample
	+	-	-	-	-	-	Negative	Confirm presence of fetal DNA; report negative fetal blood group antigen
	-	-	-	-	-	-	Negative	Confirm presence of fetal DNA; report negative fetal blood group antigen

PCR, polymerase chain reaction; Ct, cycle threshold value.

Replicates are scored positive (+) if Ct is 34 or more and negative (–) if no amplification is observed. Ct's under 34 in the *RHD* exon 5 and/or *RHD* exon 7 PCR suggest amplification of maternal DNA and are scored as inconclusive. Ct's over 43 in the *K* PCR are scored as inconclusive because of potential non-specific amplification of the maternal *k* allele.

RESULTADOS TESE MESTRADO ALUNA KAREN CHINOCA, DEPTO. DE GINECOLOGIA E OBSTETRÍCA DA FMUSP



RESULTADOS TESE MESTRADO ALUNA KAREN CHINOCA, DEPTO. DE GINECOLOGIA E OBSTETRÍCA DA FMUSP

Tabela 5b : Resultados do DNA Plasmático x Fenotipagem do RN após a repetição dos Inconclusivos

	RESULTADO RN	
DNA PLASMÁTICO	Rh pos (n=81)	Rh neg (n=32)
Positivo	81 (100%)	0 (0%)
Inconclusivo	0	4 (12,5%)
Negativo	0	28 (87,5%)

Noninvasive fetal blood group genotyping of rhesus D, c, E and of K in alloimmunised pregnant women: evaluation of a 7-year clinical experience

BJOG 2011;118:1340–1348.

Table 2. Results for maternal and paternal *RHD* analysis performed because of atypical fetal *RHD* exon 5 and/or *RHD* exon 7 polymerase chain reaction results

Case	*RHD* PCR plasma		Maternal *RHD* analysis (genotype)	Paternal *RHD* analysis (genotype)	Conclusion fetal RhD status
	Exon 5 Ct	Exon 7 Ct			
1	37	32	*RHDΨ/d*	N/A	D positive
2	39	29	*RHDΨ/d*	N/A	D positive
3	35	30	*RHDΨ/d*	*RHD/DAU*	D positive
4	38	31	*RHDΨ/d*	N/A	D positive
5	38	32	*RHDΨ/d*	*RHD/RHD*	D positive
6	37	30	*RHDΨ/RHD-CE-D^s*	*RHD/DIII type 5*	D positive
7	38	Und	*d/d*	*DIVa/d*	D positive
8	Und	36	*d/d*	*RHD/DAU5*	D positive
9	35	39	*d/d*	*RHD/DNU*	D positive
10	Und	31	*RHDΨ/d*	*RHDΨ/DAU*	D negative
11	Und	31	*RHDΨ/RHD-CE-D^s*	N/A	D negative
12	30	30	*RHD(343delC)/d*	N/A	inconclusive
13	31	31	*RHD(IVS1+1G>A)/d*	*d/d*	inconclusive

Ct, cycle threshold value; *d*, deletion/complete absence of *RHD* gene; N/A, not available; *RHD*, normal *RHD* gene; *RHDΨ*, *RHD* pseudogene; Und, undetermined (no amplification).

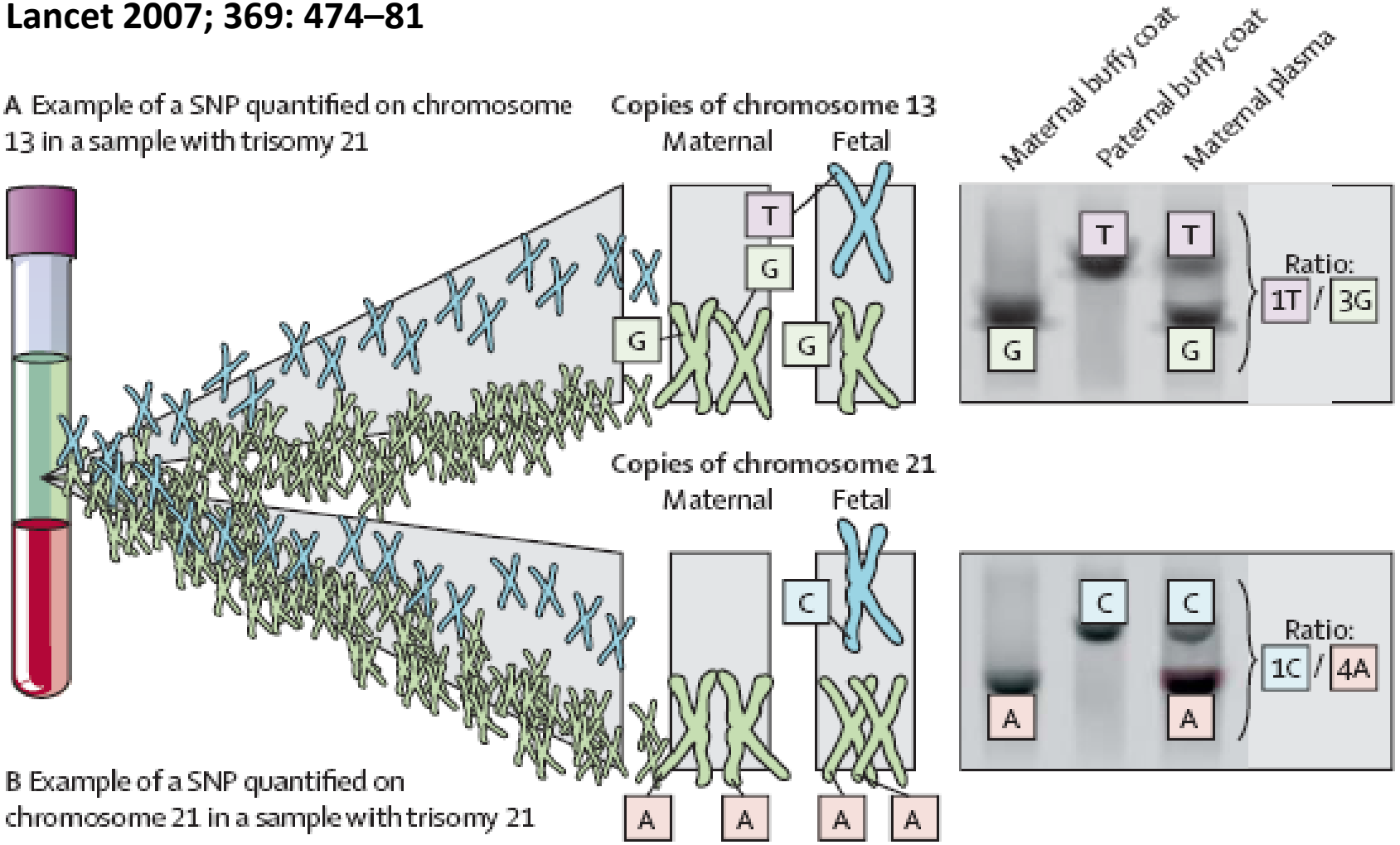
RHDΨ, *RHD-CE-D^s*, *RHD(343delC)* and *RHD(IVS1+1G>A)*: *RHD* variant genes leading to a D-negative phenotype.

DAU, DIII type 5, DIVa, DAU5 and DNU: *RHD* variant genes leading to a D-positive phenotype.

A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study

Ravinder Dhallan, Xin Guo, Sarah Emche, Marian Damewood, Philip Bayliss, Michael Cronin, Julie Barry, Jordan Betz, Kara Franz, Katie Gold, Brett Vallecillo, John Varney

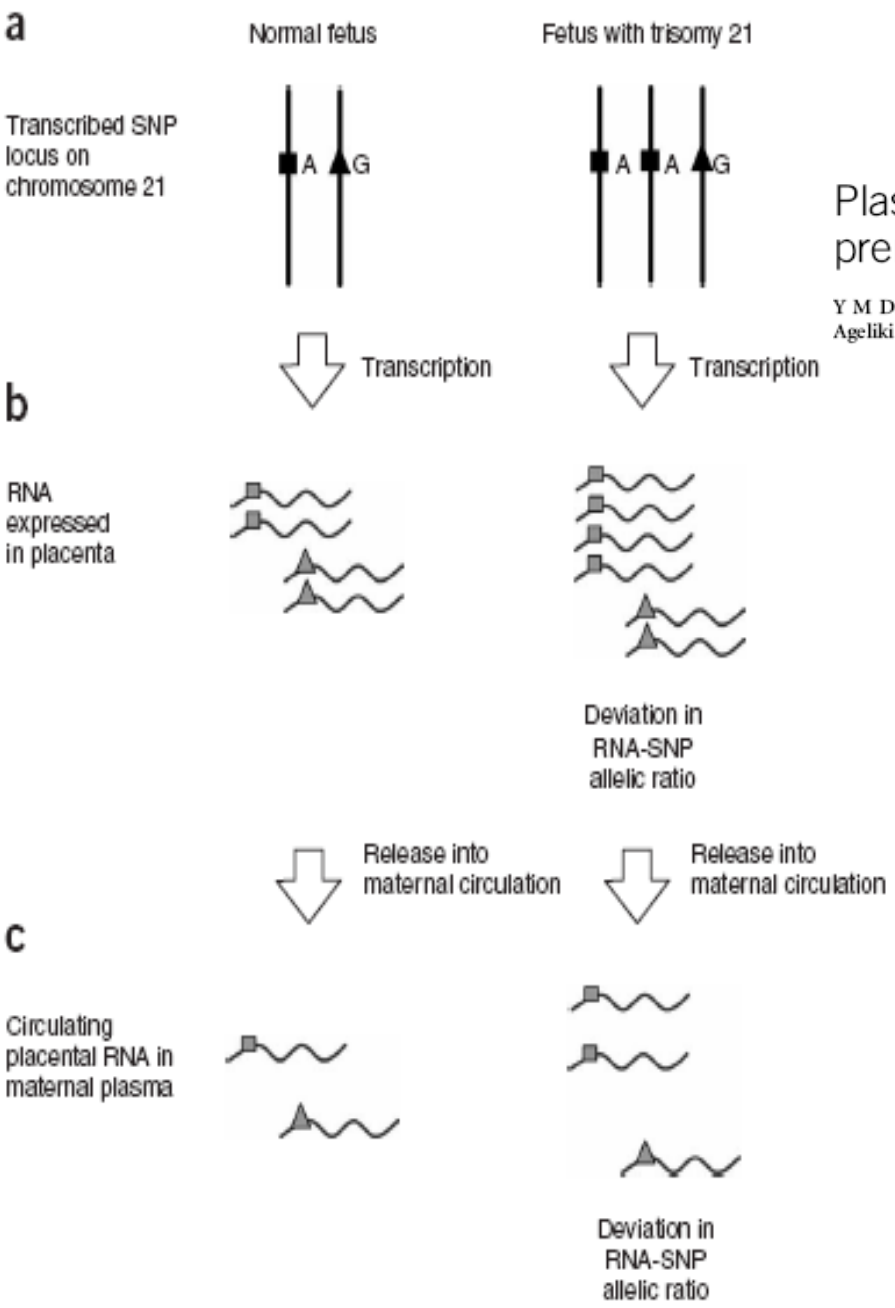
Lancet 2007; 369: 474–81



nature medicine

Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection

Y M Dennis Lo[1,2,7], Nancy B Y Tsui[2,7], Rossa W K Chiu[1,2,7], Tze K Lau[3], Tse N Leung[3], Macy M S Heung[2], Ageliki Gerovassili[4], Yongjie Jin[5], Kypros H Nicolaides[4], Charles R Cantor[6] & Chunming Ding[1,5,7]



Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood

20458–20463 | PNAS | December 23, 2008 |

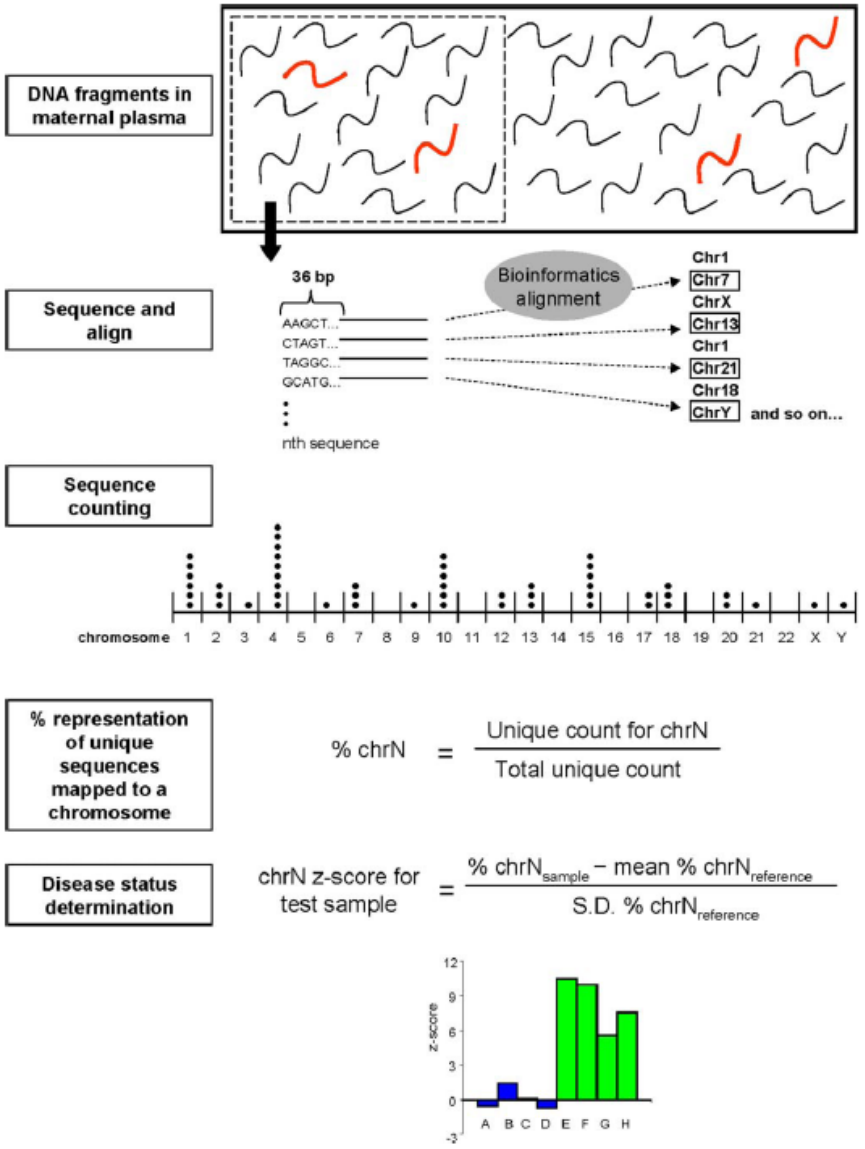
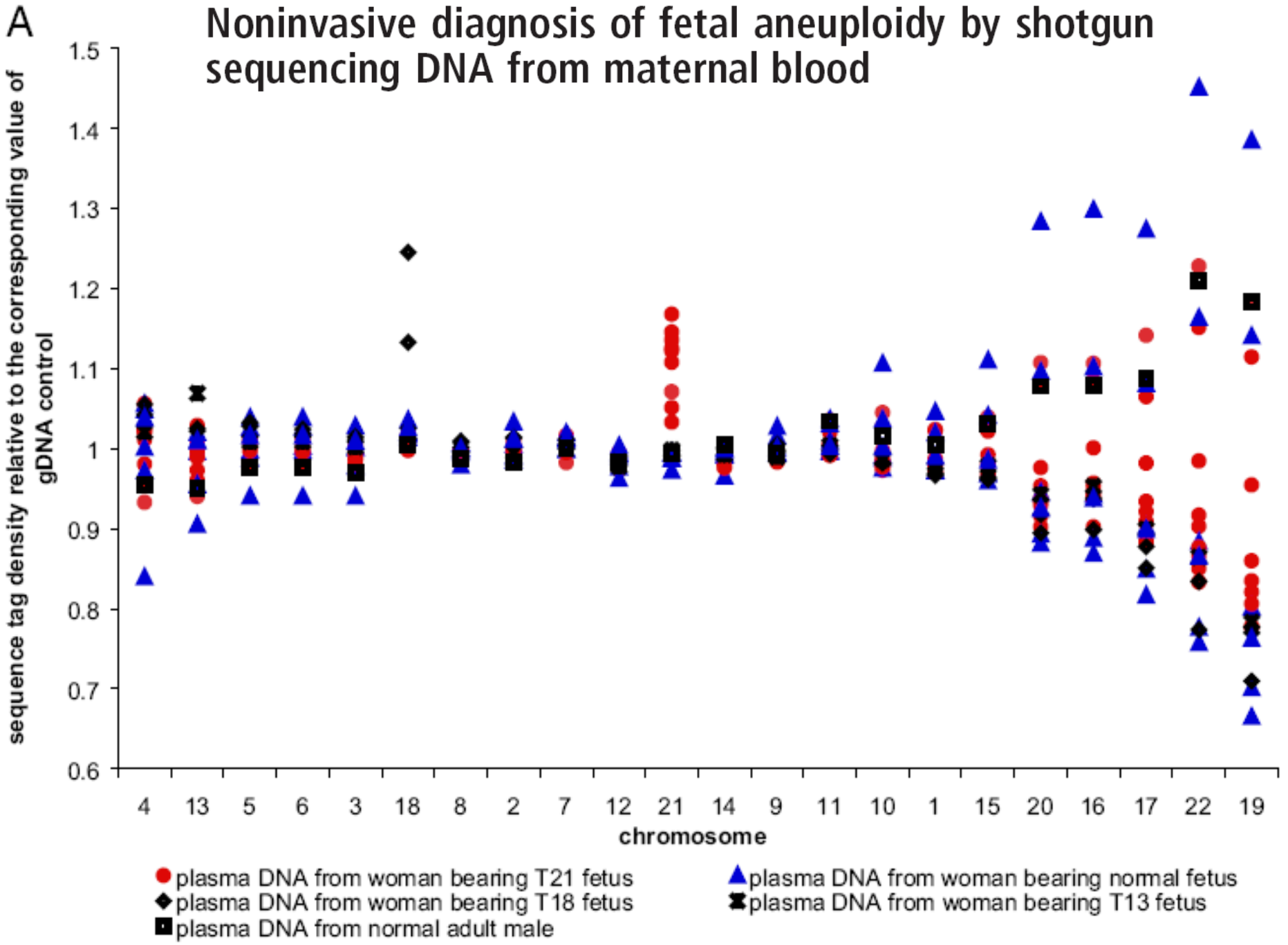


Fig. 1. Schematic illustration of the procedural framework for using mas-


A
Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood
sequence tag density relative to the corresponding value of gDNA control
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
4 13 5 6 3 18 8 2 7 12 21 14 9 11 10 1 15 20 16 17 22 19
chromosome
plasma DNA from woman bearing T21 fetus
plasma DNA from woman bearing normal fetus
plasma DNA from woman bearing T18 fetus
plasma DNA from woman bearing T13 fetus
plasma DNA from normal adult male

Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Open

DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study

Glenn E. Palomaki, PhD[1], Cosmin Deciu, MS[2], Edward M. Kloza, MS[1], Geralyn M. Lambert-Messerlian, PhD[1], James E. Haddow, MD[1], Louis M. Neveux, BA[1], Mathias Ehrich, MD[3], Dirk van den Boom, PhD[3], Allan T. Bombard MD, MBA[2–4], Wayne W. Grody, MD, PhD[5–7], Stanley F. Nelson, MD[5,7,8] and Jacob A. Canick, PhD[1]

certified university laboratory. **Results:** Down syndrome detection rate was 98.6% (209/212), the false-positive rate was 0.20% (3/1471), and the testing failed in 13 pregnancies (0.8%); all were euploid. Before

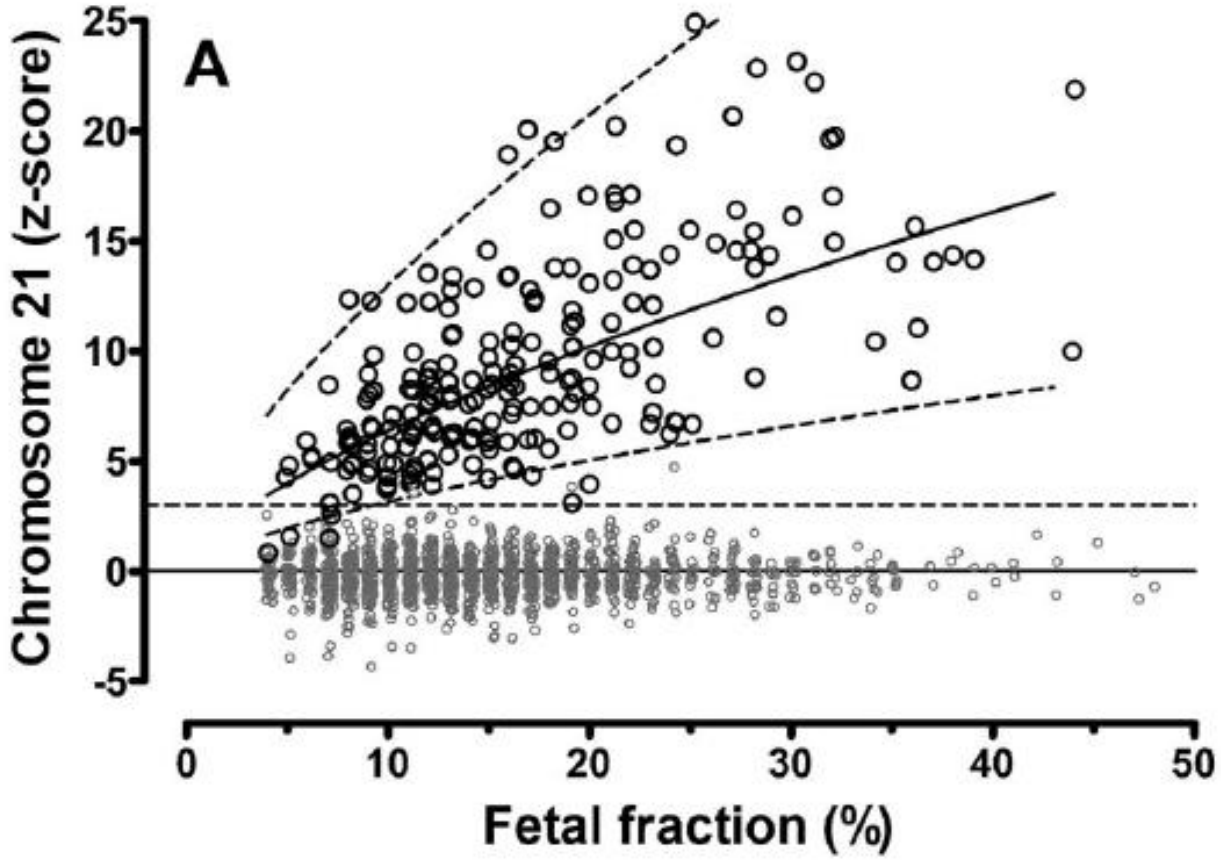
Table 1 Clinical sites enrolled in the study, along with related enrollment and outcome information

Enrollment site	Location	Clinical investigator	Singleton pregnancy		Other	Patients enrolled
			Down syndrome	Normal karyotype		
North York General Hospital	Toronto, Canada	Wendy S. Meschino, MD	41	651	86	778
Istituto G. Gaslini	Genoa, Italy	Pierangela De Biasio, MD	27	492	35	554
Hospital Clinic Barcelona	Barcelona, Spain	Antoni Borrell, MD, PhD	24	291	44	359
Centrum Lekarske Genetiky	Ceske Budejovice, Czech Republic	David Cutka, MD	14	362	19	395
Hospital Italiano	Buenos Aires, Argentina	Lucas Otaño, MD, PhD	13	68	14	95
Dalhousie University	Halifax, Canada	Michiel Van den Hof, MD	12	115	18	145
Rotunda Hospital	Dublin, Ireland	Fergal Malone, MD	12	70	12	94
Semmelweis University	Budapest, Hungary	Csaba Papp, MD, PhD	10	64	9	83
IMALAB s.r.o. Medical Laboratories	Zlin, Czech Republic	Jaroslav Loucky, RNDr	9	238	8	255
CEMIC	Buenos Aires, Argentina	Maria Laura Igarzabal, MD	8	224	49	281
University of Iowa	Iowa City, IA	Kristi Borowski, MD	8	135	30	173
Women & Infants Hospital	Providence, RI	Barbara O'Brien, MD	6	99	21	126
University of Pécs	Pécs, Hungary	Béla Veszprémi, MD, PhD	4	172	31	207
University of Alabama at Birmingham	Birmingham, AL	Joseph Biggio, MD	4	169	20	193
Rambam Medical Center	Haifa, Israel	Zeev Weiner, MD	4	133	10	147
Cedars Sinai PDC	Los Angeles, CA	John Williams, MD	3	192	28	223
Northwestern University	Chicago, IL	Jeffrey Dungan, MD	3	88	11	102
Henry Ford Hospital	Detroit, MI	Jacquelyn Roberson, MD	3	74	14	91
University of Virginia	Charlottesville, VA	Devereux N. Saller, Jr, MD	3	21	8	32
University of British Columbia	Vancouver, Canada	Sylvie Langlois, MD	2	67	14	83
Intermountain Healthcare	Salt Lake City, UT	Nancy Rose, MD	2	67	9	78
Brigham and Women's Hospital	Boston, MA	Louise Wilkins-Haug, MD	2	21	8	31
Baylor College of Medicine	Houston, TX	Anthony Johnson, DO	2	20	0	22
Yale University	New Haven, CT	Maurice J. Mahoney, MD, JD	1	31	9	41
New Beginnings Perinatal Consultants	Providence, RI	Marshall Carpenter, MD	1	7	4	12
University of Calgary	Calgary, Canada	Jo-Ann Johnson, MD	0	52	5	57
Royal North Shore Hospital	Sydney, Australia	Vitomir Tasevski, PhD	0	7	0	7
All			218	3,930	516	4,664

Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Open

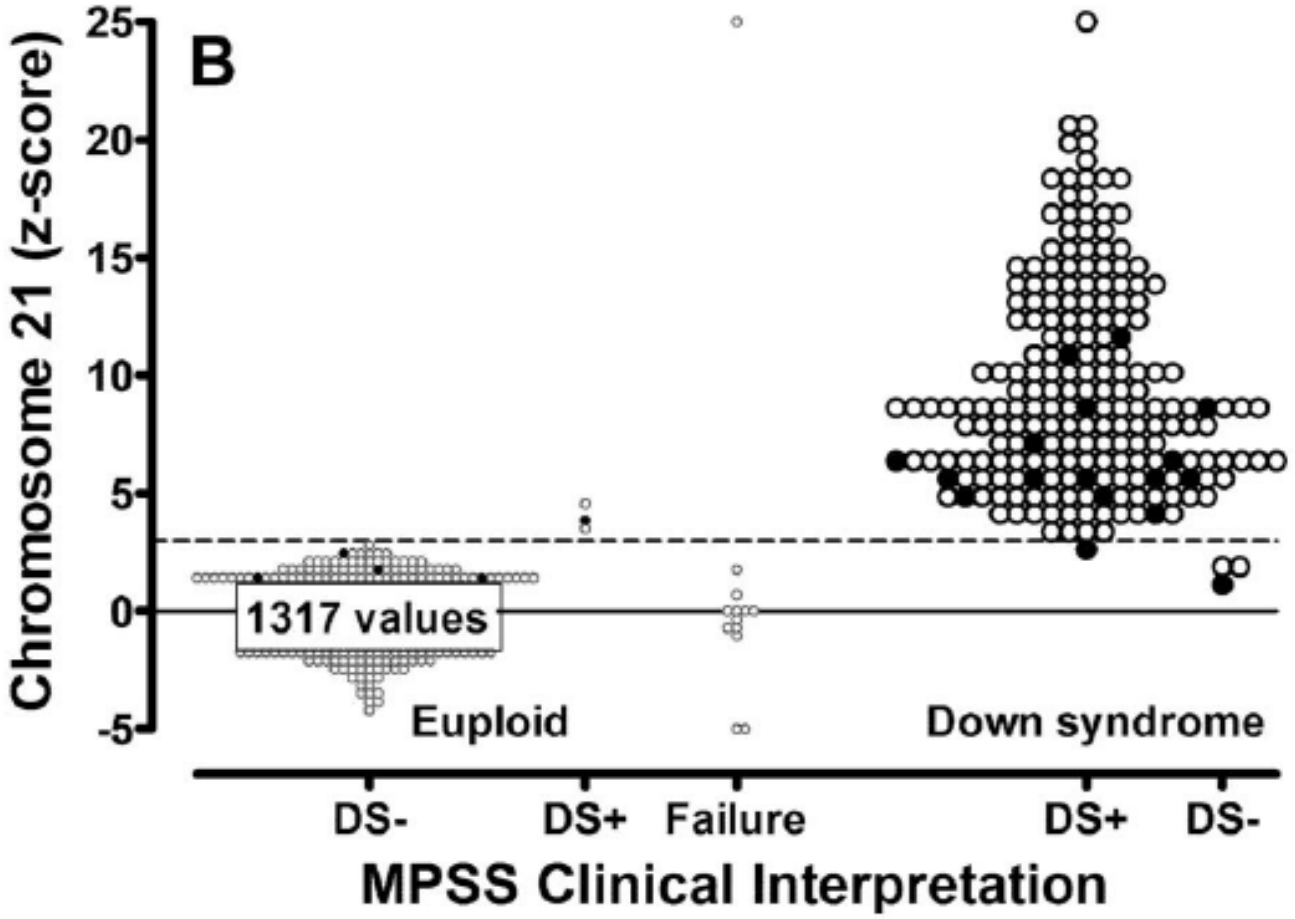
DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study



Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Open

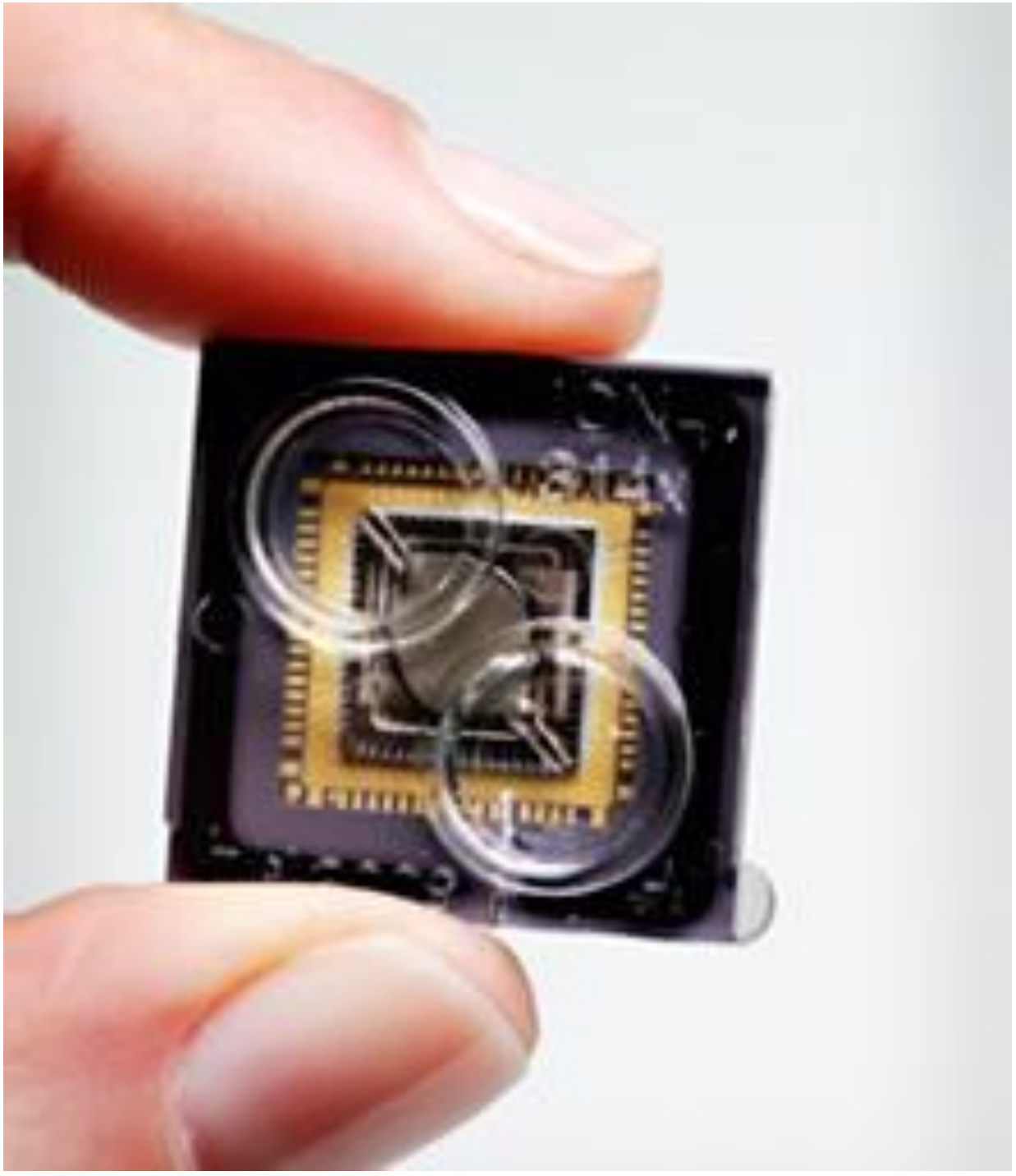
DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study



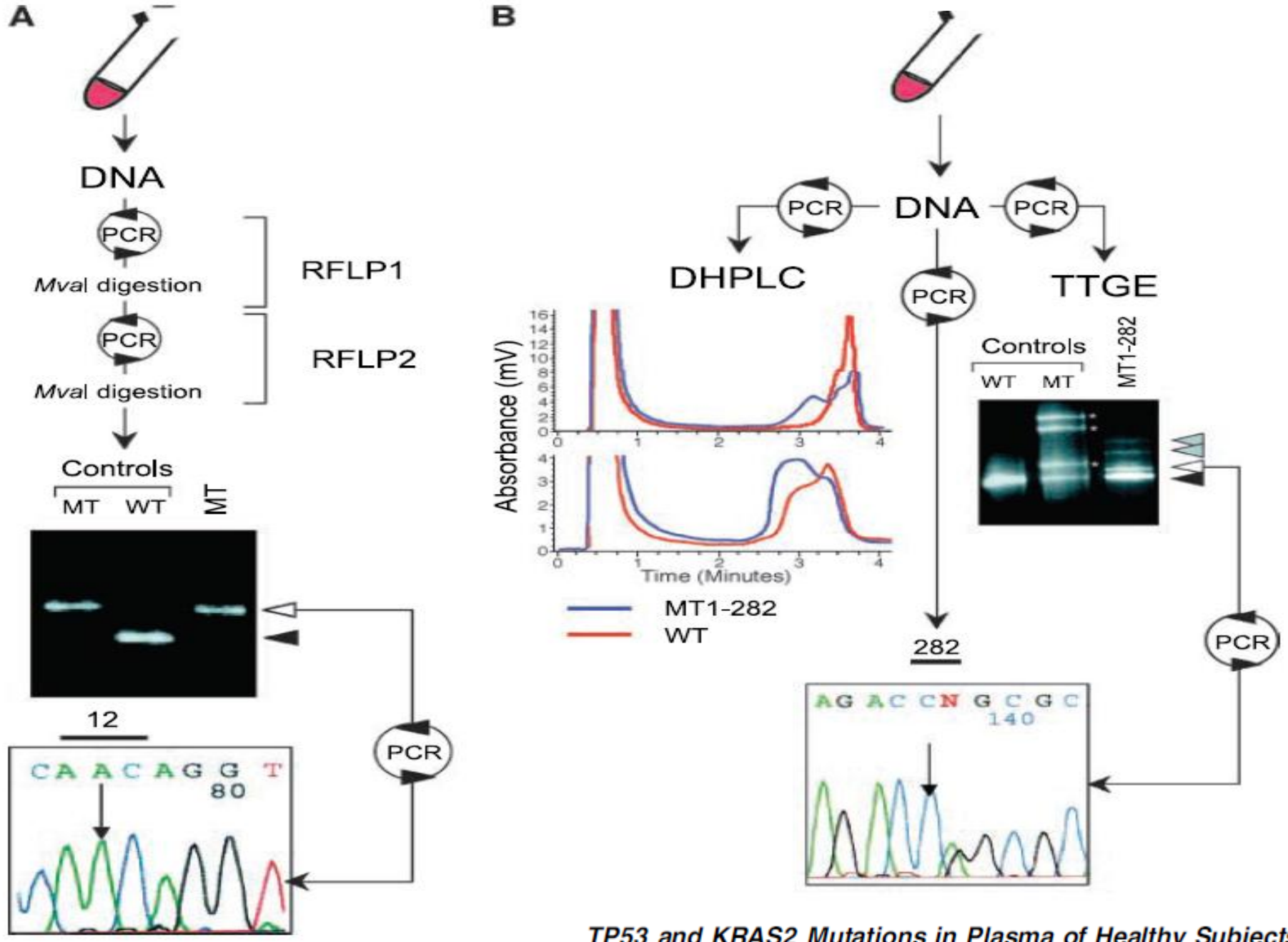
- **Abordagem SNP/mRNA = simples, barata, adaptável a rotina de laboratórios pequenos (sequenciamento de pequenos fragmentos, e não do genoma completo)**

- **IMT-USP (Ion Torrent – Life Technologies)**

  - Escala
  - Simplicidade
  - ↑ Acurácia
  - ↑ Rendimento
  - ↓ Tempo
  - ↓ Preço



Cancer Res 2006; 66: (13). July 1, 2006



TP53 and KRAS2 Mutations in Plasma of Healthy Subjects

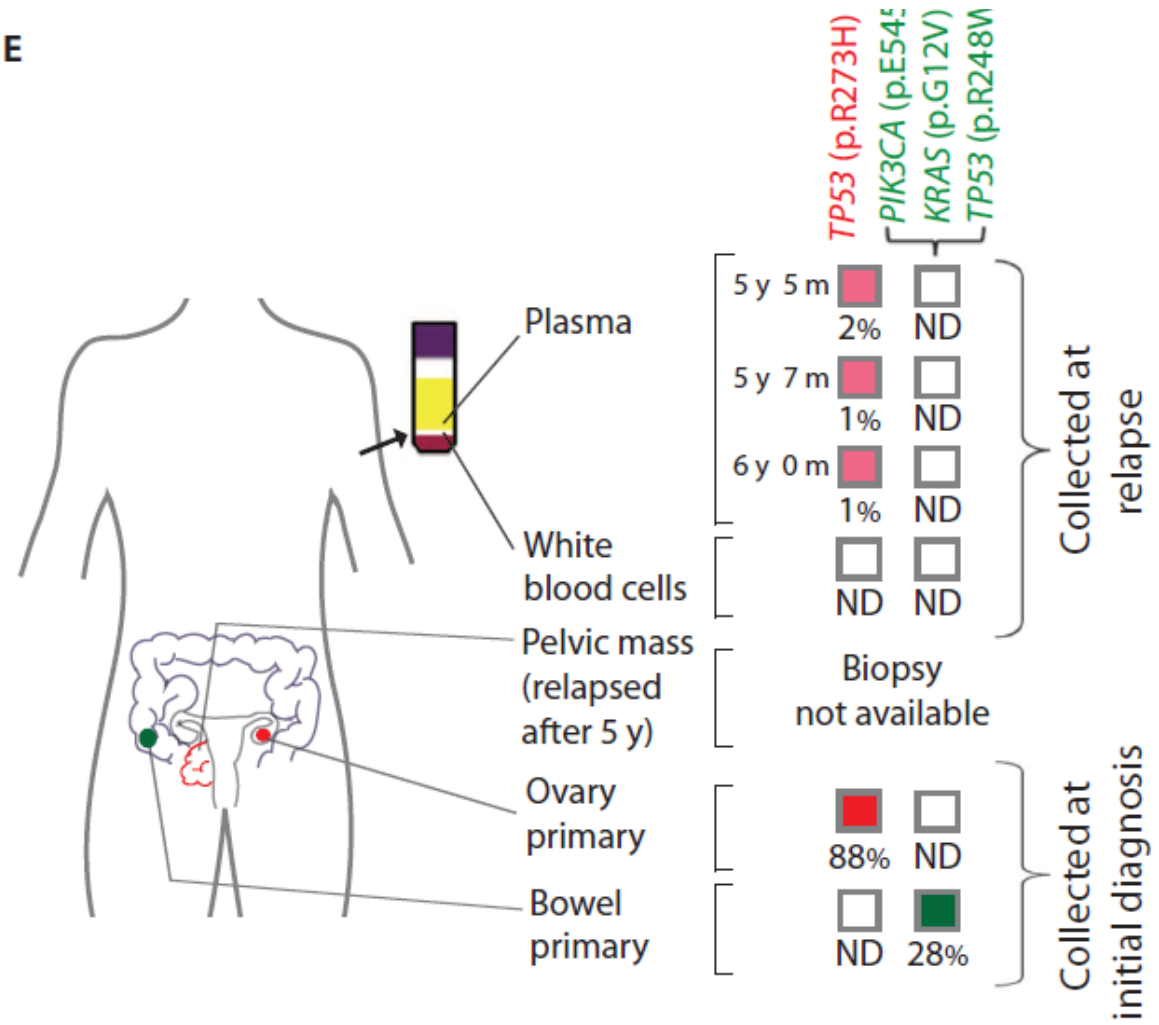
Table 2.

Codon	Mutation classification*	Mutation	Amino acid change	Cancer site	Tim
(A) Mutations detected in CFDNA of subjects who subsequently developed cancer					
KRAS2					
12		GGT>GTT	Gly>Val	Bladder	
12		GGT>GTT	Gly>Val	Bladder	
12		GGT>GTT	Gly>Val	Bladder	
12		GGT>GTT	Gly>Val	Bladder	
12		GGT>GAT	Gly>Asp	Bladder	
12		GGT>GTT	Gly>Val	UADT	
TP53					
175	MT1	CGC>TGC	Arg>Cys	Lung	
179	MT2	CAT>CCT	His>Pro	Bladder	
181	MT1	CGC>CAC	Arg>His	Lung	
196	MT2	CGA>CAA	Arg>Gln	Leukemia	
207 †	MT2	GAT>GGT	Asp>Gly	Bladder	
216 †	MT3	GTG>GTA	Val>Val		
262	MT2	GGT>AGT	Gly>Ser	Bladder	
271	MT2	GAG>GGG	Glu>Gly	Bladder	
282	MT1	CGG>CAG	Arg>Glu	Bladder	
324	MT2	GAT>GGT	Asp>Gly	Bladder	
IN4 (13051)	MT3	G>C	None	Bladder	
(B) Mutations detected in CFDNA of control subjects					
KRAS2					
12		GGT>GTT	Gly>Val		
12		GGT>GTT	Gly>Val		
12		GGT>GTT	Gly>Val		
12		GGT>AGT	Gly>Ser		
12		GGT>GTT	Gly>Val		

Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

www.ScienceTranslationalMedicine.org 30 May 2012 Vol 4 Issue 136 136ra68

E



Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

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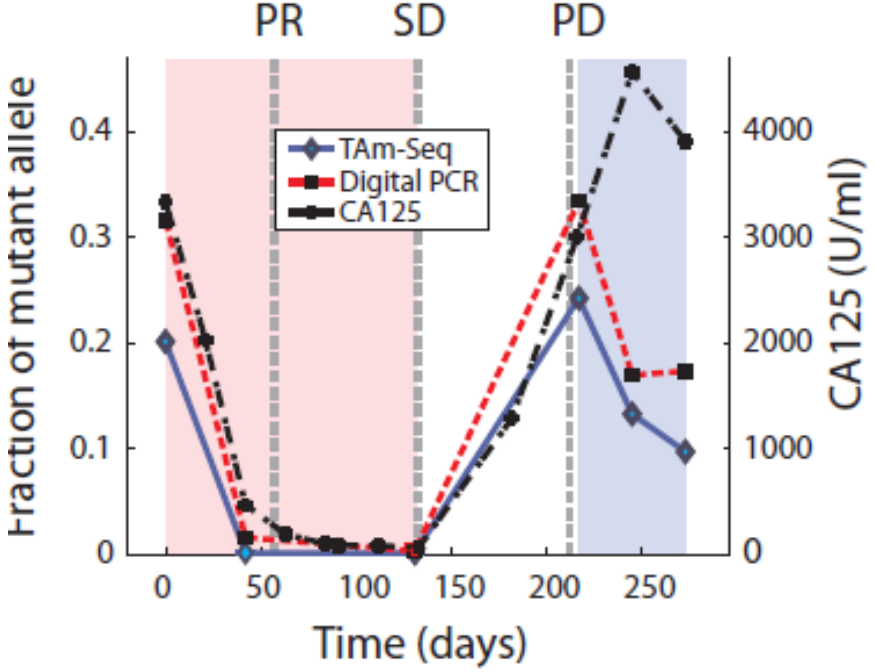
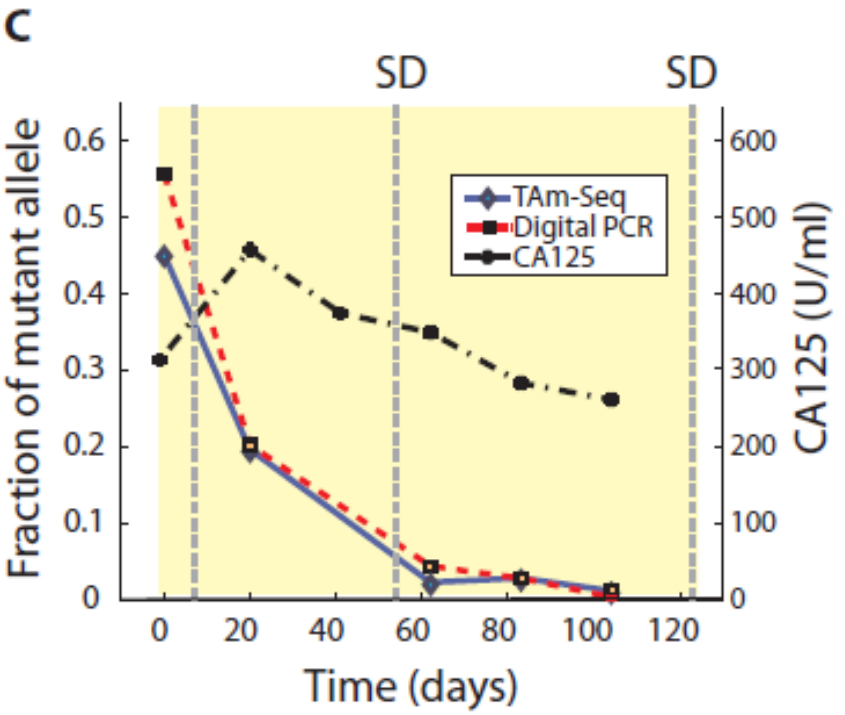




; SD, stable disease; PD, progressive disease.

Science Translational Medicine
AAAS

Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies

Chetan Bettegowda *et al.*

Sci Transl Med **6**, 224ra24 (2014);
DOI: 10.1126/scitranslmed.3007094

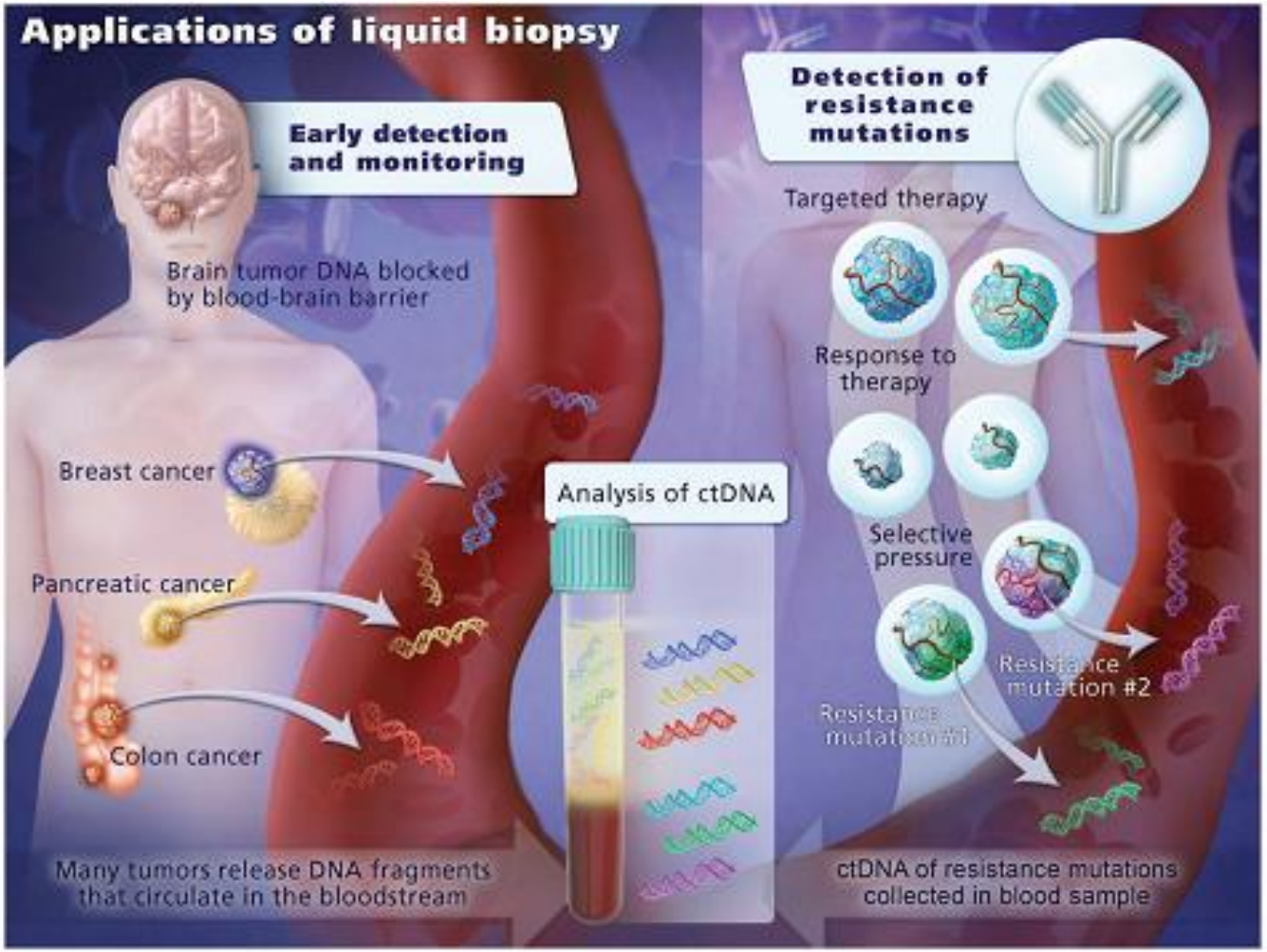
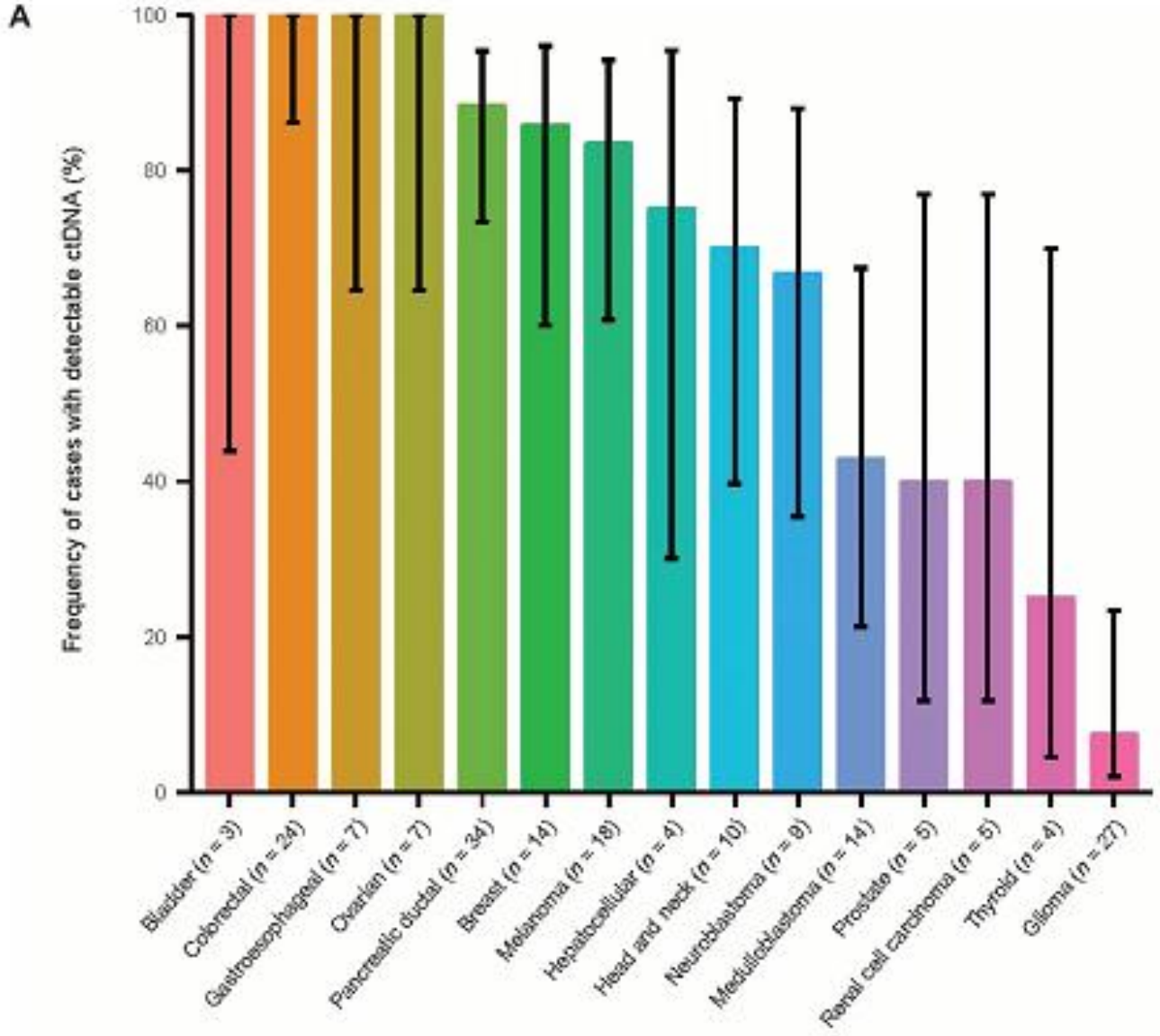


Fig. 1. Potential applications of ctDNA.


A
Frequency of cases with detectable ctDNA (%)
100
80
60
40
20
0
Bladder (n = 3)
Colorectal (n = 24)
Gastroesophageal (n = 7)
Ovarian (n = 7)
Pancreatic ductal (n = 34)
Breast (n = 14)
Melanoma (n = 18)
Hepatocellular (n = 4)
Head and neck (n = 10)
Neuroblastoma (n = 9)
Medulloblastoma (n = 14)
Prostate (n = 5)
Renal cell carcinoma (n = 5)
Thyroid (n = 4)
Glioma (n = 27)

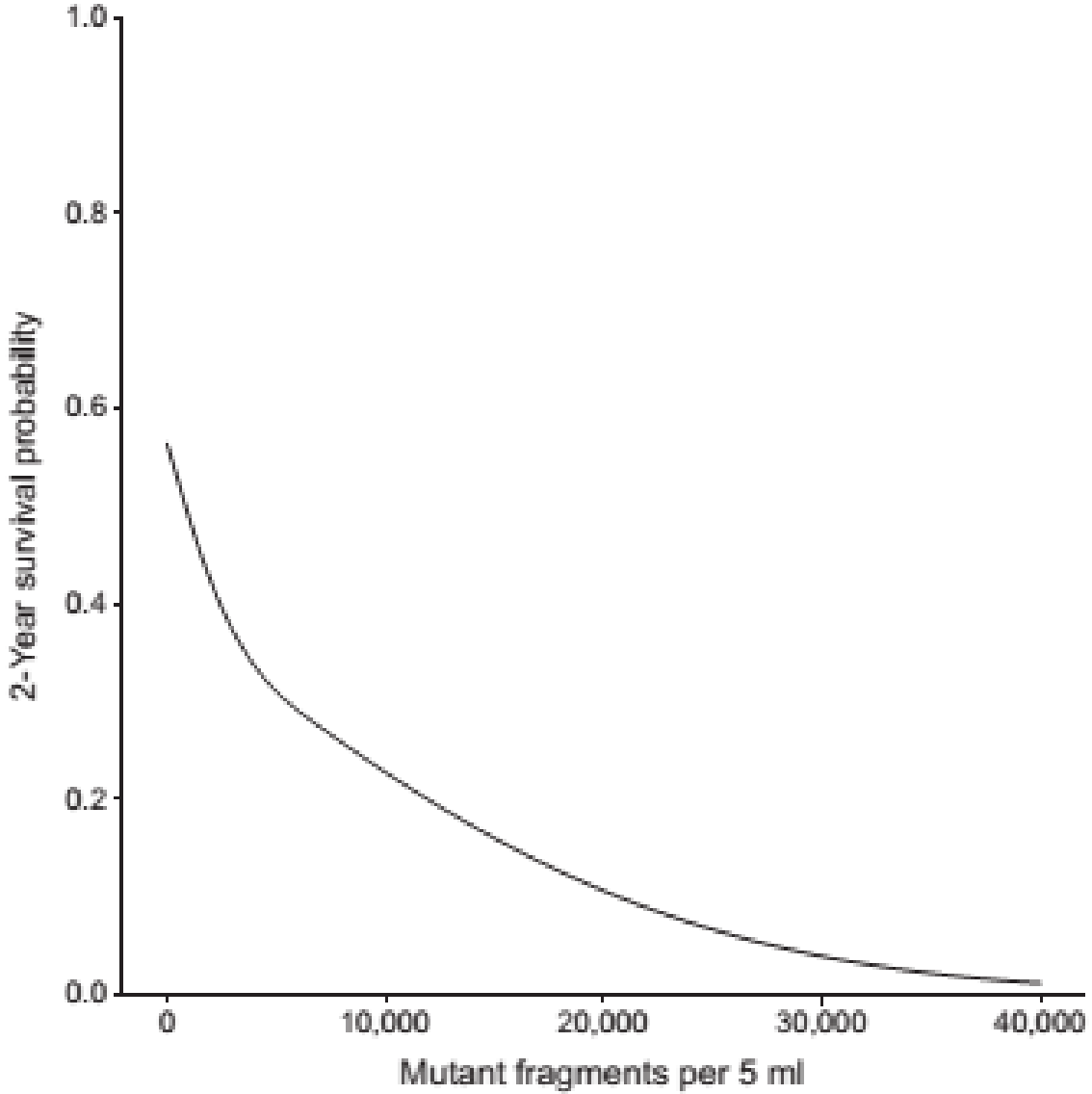


Fig. 5. The relationship between ctDNA concentration (mutant fragments per milliliter) and 2-year survival. The association between surviv-

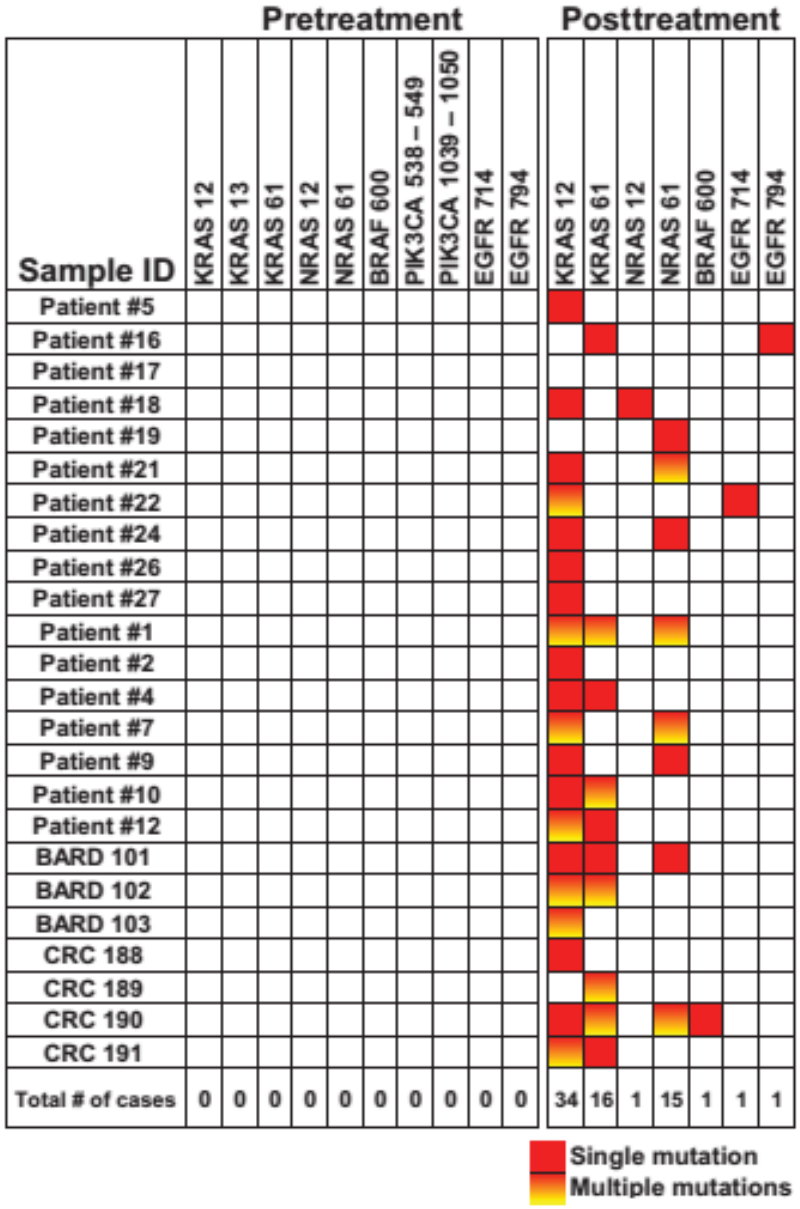


Fig. 6. Heat map of acquired resistance mutations to EGFR blockade in ctDNA from patients with metastatic CRC.

Universal noninvasive detection of solid organ transplant rejection

Thomas M. Snyder[a,b], Kiran K. Khush[c], Hannah A. Valantine[c,1], and Stephen R. Quake[a,b,1]

[a]The Howard Hughes Medical Institute and [b]Departments of Applied Physics and Bioengineering, Stanford University, Stanford, CA 94305; and [c]Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305

PNAS | April 12, 2011 | vol. 108 | no. 15 | 6229–6234

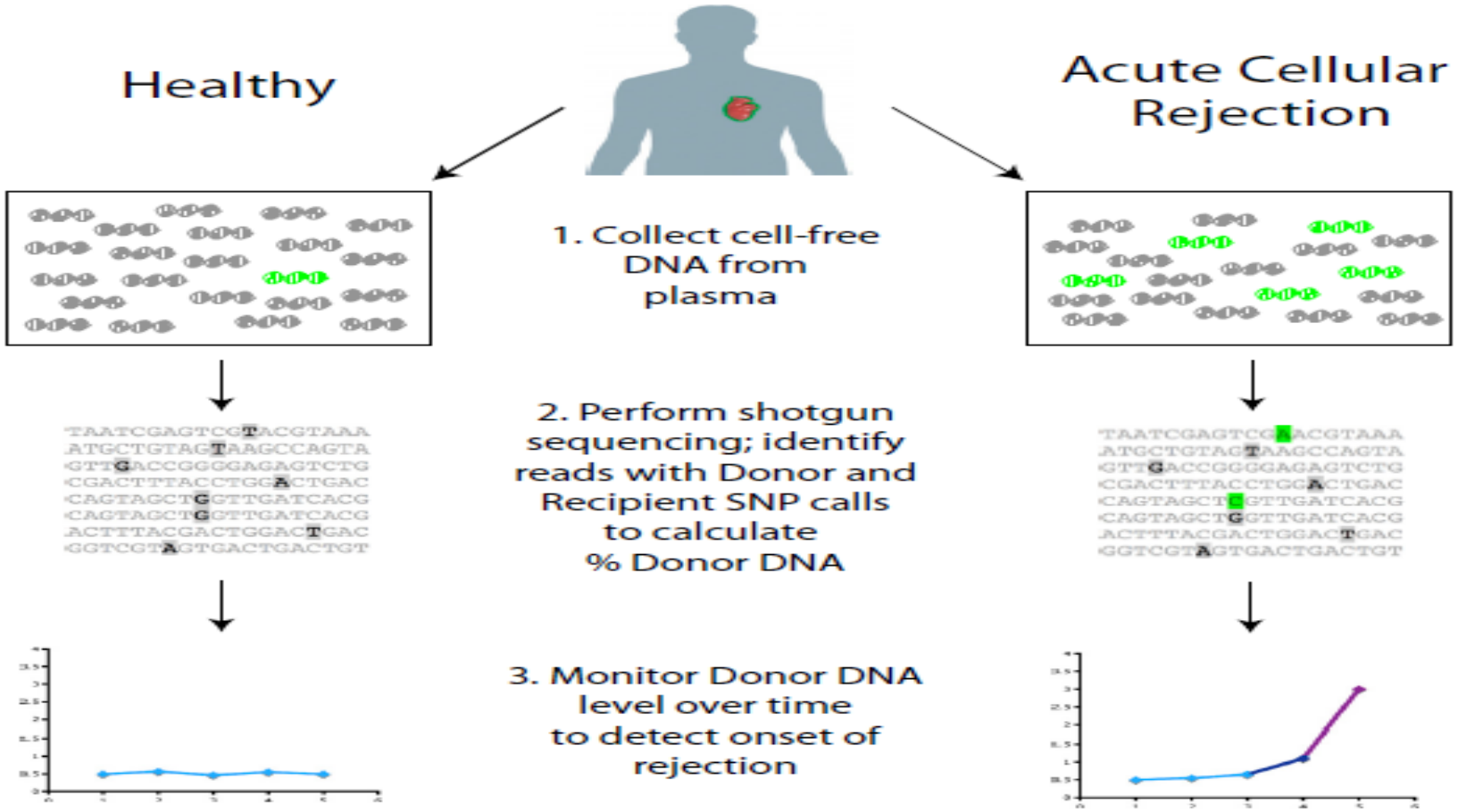


Fig. 1. General scheme for this study. Cell-free DNA collected in plasma contains a majority of molecules from the recipient (in gray) but may also include some from the transplanted organ (green). Due to increased cell death in the organ during a rejection episode, more donor molecules are expected to be present in the blood at these times. Shotgun sequencing of the purified DNA allows for counting recipient versus donor molecules by looking at single nucleotide polymorphisms (SNPs) that vary between donor and recipient. Very high levels of donor DNA, particularly changes from past measurements, will indicate the onset of rejection.

Ácidos Nucleicos Fetais - ANEUPLOIDIAS

Prof. Dr. Mário Burlachini - Departamento de Ginecologia e Obstetrícia da Fac.de Medicina da Universidade de São Paulo.
Renata Romão – Doutoranda, Departamento de Ginecologia e Obstetrícia da Fac. de Medicina da Universidade de São Paulo.

Ácidos Nucleicos Fetais - RhD

Prof. Dr. Adolfo Liao- Departamento de Ginecologia e Obstetrícia da Fac.de Medicina da Universidade de São Paulo.
Karen Chinoca- Mestranda, - Departamento de Ginecologia e Obstetrícia da Fac.de Medicina da Universidade de São Paulo.

DNA Tumoral Circulante

Cristina Mendes de Oliveira, Pós-Doutoranda, Lab. De Virologia do inst. De Medicina Tropical Da Universidade de São Paulo.
Cristiane Centrone , Mestranda , Lab. De Virologia do inst. De Medicina Tropical Da Universidade de São Paulo.

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