ADVANCES IN THE USE OF TROPHOBLASTIC CELLS FOR PRENATAL NON-INVASIVE DIAGNOSTICS OF GENETIC DISORDERS

15th INTERNATIONAL CONFERENCE
ON PREIMPLANTATION GENETIC DIAGNOSIS
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Instituts thématiques Inserm Institut national de la santé et de la recherche médicale
PARIS DESCARTES
Rarecells
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS NECKER-ENFANTS MALADES

Disclosure

Inventor/coinventor of ISET patents
Founder ans scientific advisor of Rarecells
Academic tasks:
Teaching (University Paris Descartes)
Developing and implementing new tests (Hôpital Necker)
Research activity (INSERM Unit)

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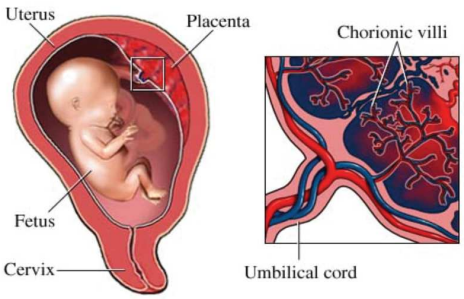

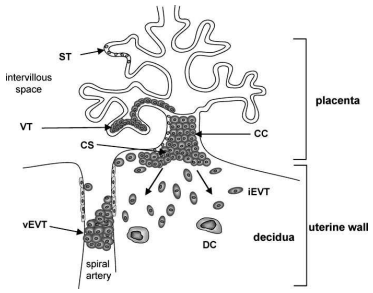

Circulating Fetal Trophoblastic Cells (CFTC)

CFTC do not have to cross the placenta

Endovascular trophoblasts invade the lumen of spiral arteries coming in contact with maternal blood

Invasion follows two waves: middle of 1T and end of 1T

- Isolation by density gradient, MACS, from 20 to 50 ml of blood, followed by Y PCR or FISH: very inconsistent results (Oudejans C, 2003)
- Trophoblastic markers proven to be not specific: HLA-G, NeuroD2, placenta GF (Tioa ML, 2007)
- 30 ml blood, CD105, CD141, CK: Hatt L et al, 2013; Shlutter et al 2014; Shlutter et al, 2015:

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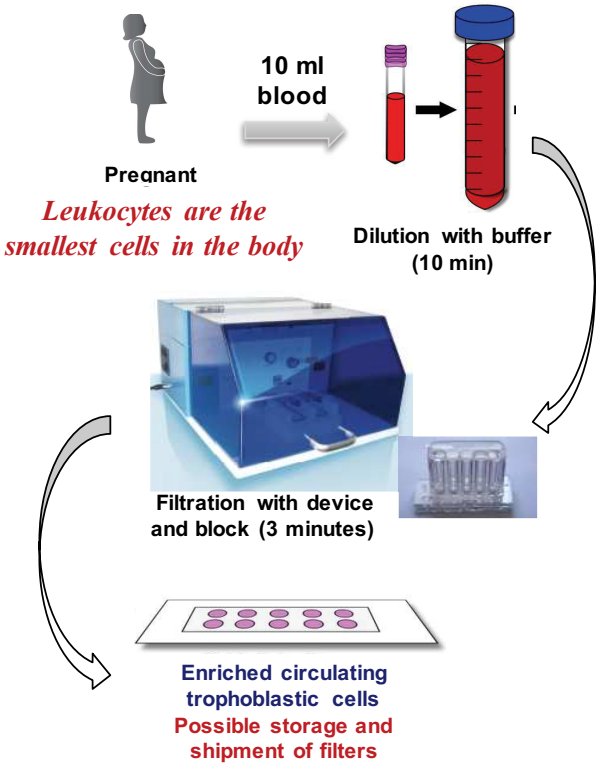
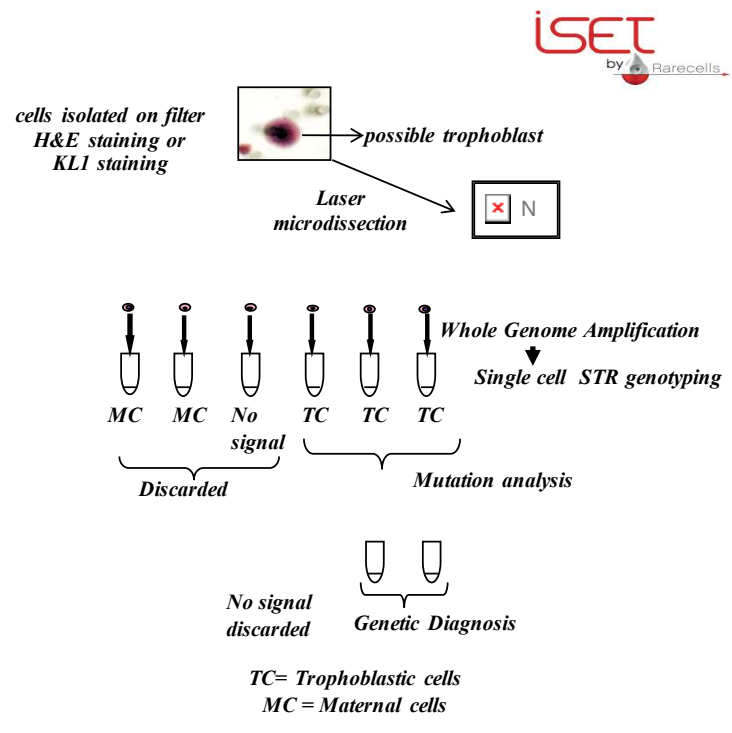
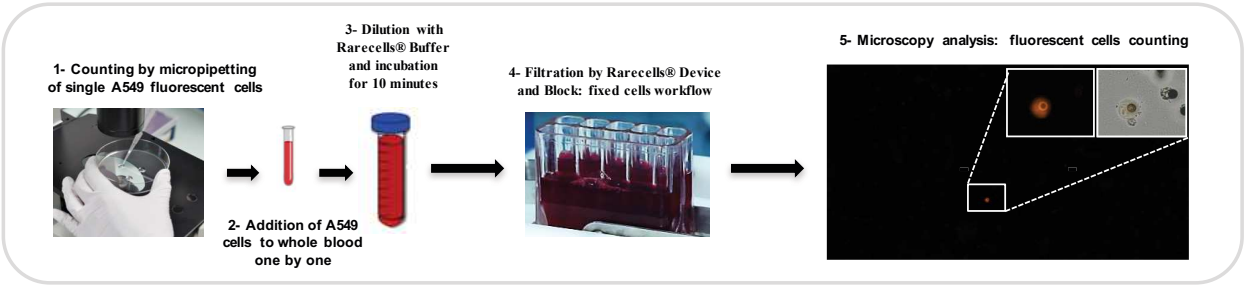
ISET® TECHNOLOGY for CIRCULATING FETAL TROPHOBLASTIC CELLS





ISET by Rarecells: Patented combination of parameters allowing very sensitive and rapid isolation of CFC

Workflow for ISET® *in vitro* assay of sensitivity and reproducibility with fixed cells counted by micropipetting



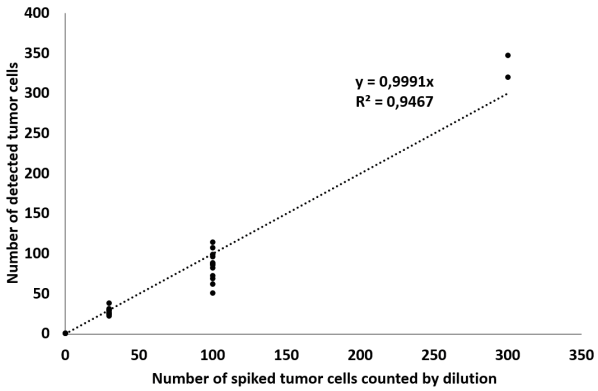


Table 2: Sensitivity and repeatability of the ISET® system (fixed cells)

mL of blood processed	1 mL	5 mL	10 mL
Number of spiked tumor cells	2	2	2
Number of tumor cells detected by ISET®	1	1	2
	2	2	2
	2	2	2
	1	2	2
	2	2	2
	2	1	2
total found /spiked	10/12	10/12	12/12
Recovery success rate	83,3%	83,3%	100%

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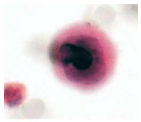
Results



G. VONA, …….PATERLINI-BRÉCHOT. Enrichment and genetic analyses of fetal cells circulating in the maternal blood by the ISET technique and single cell microdissection : a non-invasive tool for early prenatal diagnosis. Am J Pathol, 160 : 51-58, 2002.

⇨ *13 pregnant women*
11-12 WG
Before CVS
CFTC in all the mothers, Y and STR genotyping

C. BÉROUD, ……..PATERLINI-BRÉCHOT. Prenatal diagnosis of Spinal Muscular Atrophy (SMA) by genetic analysis of circulating fetal cells. The Lancet, 361 :1013-4, 2003.

⇨ *12 mothers at risk for baby with SMA*
10-12 WG before CVS
Blind analysis vs CVS
CFTC in all the mothers, correct diagnosis

A. SAKER,……., P. PATERLINI-BRÉCHOT.
Genetic characterization of circulating fetal cells allows Non-Invasive prenatal diagnosis of cystic fibrosis. Prenat Diagn, 26 : 906-16, 2006.

⇨ *12 mothers at risk for baby with CF*
10-12 WG
Blind analysis vs CVS
CFTC in all the mothers, correct diagnosis

H. MOUAWIA, …… P. PATERLINI-BRECHOT. Trophoblasts enriched from maternal blood provide definitive genetic diagnosis in 63 consecutive fetuses at risk for Cystic Fibrosis or Spinal Muscular Atrophy, Reproductive Biomedicine Online, 2012

⇨ *63 mothers at risk for baby with SMA or CF*
10-12 WG, before CVS
Blind analysis vs CVS
CFTC in all the mothers, correct diagnosis

PFEIFER I, ……P. PATERLINI-BRECHOT. Cevical Trophoblasts for non-invasive single cell genotyping and prenatal diagnosis, Placenta, 2015

⇨ *21 pregnant women*
6 before CVS, 3 CF, 3 SMA, correct diagnosis
15 before TOP
PAP like sampling 8-12 WG
2 to 12 trophoblasts in 2 out of 10 ml
TC in all the mothers, correct diagnosis

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ISET Clinical validation study
NI-PND of Cystic Fibrosis and Spinal Muscular Atrophy
NECKER HOSPITAL BLIND PROTOCOL:
Pr Benachi (obstetrician); Dr Bonnefont (geneticist); JP Jais (statistician)

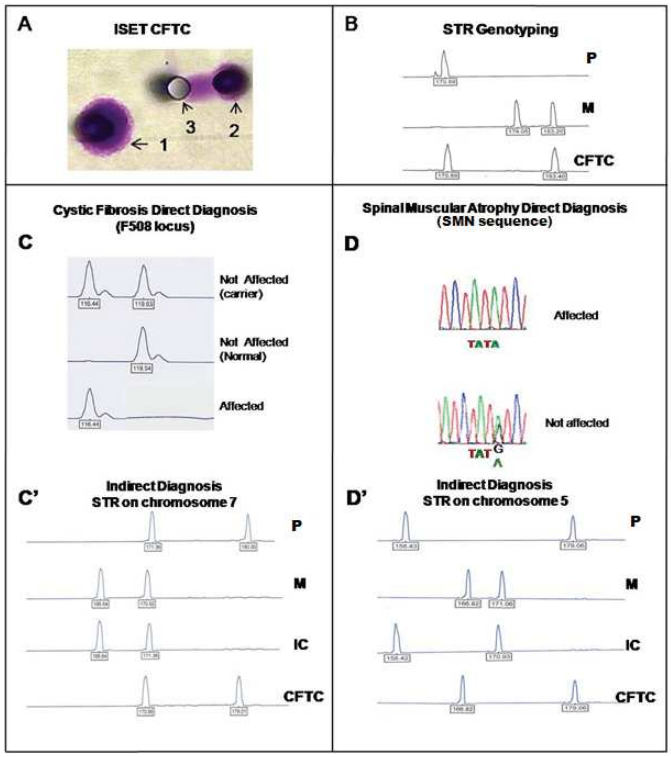
Table 2 Non invasive-prenatal diagnosis for spinal muscular atrophy.

Couple no. (weeks of gestation)	STR markers	Microdissected cells (n)	CFTC (n)	CFTC result (n)		Test results	
				With gene deletion	Without gene deletion	NI-PND	Invasive PND
1 (11)	D16S539, D16S3018, D21S1435			0	5	NA	NA
2 (11)[a]	D75480, D16S3018, D5S465	13	6	5	0	A	A
3 (10)	D75486, D75490, D75523	11	5	0	5	NA	NA
4 (10)	D75486, D21S1435, D21S1437	15	7	0	5	NA	NA
5 (9)[a]	D16S539, C272, D21S1435, D17S800	17	8	5	0	A	A
6 (9)	D16S539, D5S816, D1S1171	15	8	0	5	NA	NA
7 (9)	D21S1437, D17S800, D5S816	13	6	0	5	NA	NA
8 (11)	D16S539, D5S816, D5S1360	14	6	0	5	NA	NA
9 (11)	D16S539, D5S615, D21S1435	12	7	0	5	NA	NA
10 (10)	D75523, D16S539, D5S681	11	5	0	5	NA	NA
11 (9)	D75486, D16S539, D21S1435	19	9	5	0	A	A
12 (11)	D75486, D16S3018, D21S1437	16	7	0	5	NA	NA
13 (10)	D5S637, D16S539, D5S816	14	6	5	0	A	A
14 (11)[a]	D16S539, D5S1360, C272	11	5	0	5	NA	NA
15 (11)	D16S539, D21S1437, D5S465	12	7	0	5	NA	NA
16 (9)[a]	D16S539, D5S615, D5S816	25	13	0	10	NA	NA
17 (11)[a]	D16S539, D5S346, D21S1435	24	11	0	10	NA	NA
18 (10)[a]	D75523, D16S539, D5S681, D17S800	21	10	0	10	NA	NA
19 (10)[a]	D16S3018, D5S615, D17S800	25	12	0	10	NA	NA
20 (11)[a]	D16S539, D5S1360, D75523	21	10	10	0	A	A
21 (11)	D16S539, D21S1435, D1S1171	27	12	0	10	NA	NA
22 (10)[a]	D16S539, D21S1437, D5S465	22	10	0	10	NA	NA
23 (10)[a]	D16S539, D5S681, D21S1435, D17S800	27	13	10	0	A	A
24 (10)[a]	D16S539, D5S816, D5S1360	27	12	10	0	A	A
25 (11)[a]	D16S539, D5S681, D5S637	23	12	0	10	NA	NA
26 (9)[a]	D21S1437, D17S800, C272	23	11	0	10	NA	NA
27 (11)	D16S539, D21S1437, D21S1435	22	10	0	10	NA	NA
28 (10)	D16S539, D21S1435, D1S1171, D75486	21	10	0	10	NA	NA
29 (11)	D21S1435, D75486, D17S800	24	10	0	10	NA	NA
30 (11)	D16S539, D21S1435, D16S3018	22	10	0	10	NA	NA
31 (11)	D21S1435, D1S1171, D75486	21	10	0	10	NA	NA
Total SMA		586	276	50	185		

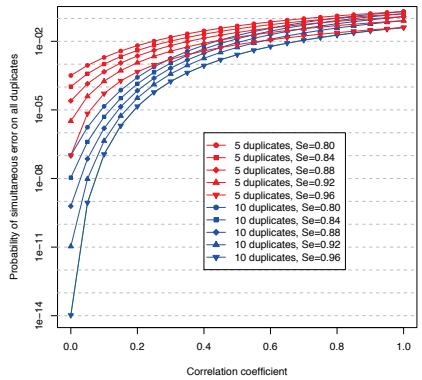
A = affected; C = carrier; CFTC = circulating fetal trophoblastic cells; CVS = chorionic-villous sampling; N = normal; NA = not affected; NI-PND = non-invasive prenatal diagnosis; STR = short tandem repeat.
[a]Cases redundantly tested by indirect diagnosis.

Couple no. (weeks of gestation)	STR markers	Microdissected cells (n)	CFTC (n)	CFTC result for F508del (n)			Test results	
				Homozygous	Heterozygous	Without F508del	NI-PND	Invasive PND
1 (11)[a]	D75486, D16S539, D21S1435	14	7	0	5	0	C	C
2 (9)[a]	D75486, D16S3018, D21S1437	12	6	0	5	0	C	C
3 (11)	D16S539, D21S1435, D21S1437	16	7	0	0	5	N	N
4 (10)	D16S3018, D21S1437, D21S1435	15	8	0	5	0	C	C
5 (10)	D16S539, D16S3018, D21S1435	14	6	0	0	5	N	N
6 (9)[a]	D75480, D16S539, D16S3018	13	6	0	0	5	N	N
7 (11)	D16S539, D21S1437, D21S1435	19	9	0	5	0	C	C
8 (11)	D16S539, D16S3018, D21S1435	15	7	0	5	0	C	C
9 (10)[a]	D75480, D16S3018, D21S1435	12	6	5	0	0	A	A
10 (9)[a]	D75486, D75490, D75523	11	5	0	5	0	C	C
11 (10)[a]	D75486, D21S1435, D21S1437	13	6	0	5	0	C	C
12 (10)[a]	D75480, D75486, D75523	17	8	0	0	5	N	N
13 (11)[a]	D75486, D21S1435, D16S3018	13	7	0	5	0	C	C
14 (11)[a]	D75480, D21S1435, D21S1435	13	6	5	0	0	A	A
15 (11)	D75486, D16S539, D21S1435	11	5	0	5	0	C	C
16 (10)[a]	D75486, D16S539, D16S3018	15	7	5	0	0	A	A
17 (10)[a]	D16S539, D21S1437, D75486	23	11	0	0	10	N	N
18 (11)	D16S3018, D21S1435, D1S1171	27	12	0	10	0	C	C
19 (10)[a]	D16S3018, D75486, D5S615	24	11	0	10	0	C	C
20 (9)[a]	D16S3018, D5S816, D75480	26	12	0	0	10	N	N
21 (11)	D16S539, D16S3018, D21S1435	21	10	0	10	0	C	C
22 (11)	D16S539, D21S1437, D5S637	24	11	0	10	0	C	C
23 (11)	D16S539, D21S1435, C272	21	10	0	10	0	C	C
24 (11)[a]	D75480, D16S3018, D16S539	22	10	0	0	10	N	N
25 (11)	D16S539, D5S816, D21S1437	21	11	0	0	10	N	N
26 (10)[b]	D75486, D16S539, D5S816	30	13	10	0	0	A	A
27 (9)[b]	D75486, D75490, D21S1437	23	11	0	0	10	N	N
28 (10)[b]	D75523, D21S1435, D16S539	22	11	0	0	10	N	N
29 (11)[a]	D75486, D16S539, D5S816	25	12	10	0	0	A	A
30 (11)	D21S1435, D75490, D5S681	21	10	0	10	0	C	C
31 (11)[b]	D75523, D21S1435, D75490	26	12	10	0	0	A	A
32 (10)	D75523, D21S1435, D75490	27	12	10	0	0	A	A
Total CF		606	285	55	105	80		

A = affected; C = carrier; CFTC = circulating fetal trophoblastic cells; CVS = chorionic-villous sampling; N = normal; NI-PND = non-invasive prenatal diagnosis; STR = short tandem repeat.
[a]Cases redundantly tested by indirect diagnosis.
[b]One parent with non-F508del mutation.
[c]Both parents with non-F508del mutation.



Successful clinical validation - Mouawia et al, RBMO 2012



Effect of circulating fetal trophoblastic cell replicated tests on the overall reliability of the diagnostic procedure.

When using 10 CFTC replicates, the protocol of independent diagnostic analysis (correlation = 0), virtually never gives rise to a global (clinical) mistake at an error rate of 10%, i.e. the probability of global diagnostic error rate is 1 in 10 billion. With five CFTC replicates, this extraordinary performance can be reached if the error rate is 1% or less.

SMA + CF: total (31+32) = 63 pregnant women
Consecutive cases
1191 microdissected cells: 475 CFTC

7 + 7 affected fetuses
as compared to CVS
Sensitivity: 100%
Specificity: 100%

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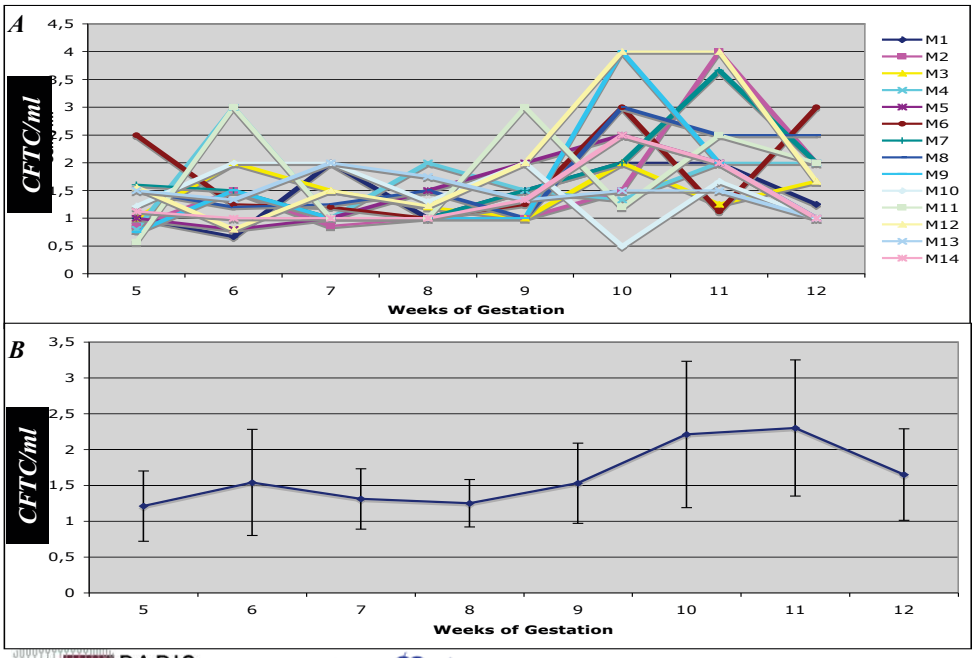
Kinetics of Circulating Fetal Trophoblastic Cells in maternal blood after In Vitro Fertilization (4th to 12th WG) (collab. Prof R Frydman):

CFTC start to circulate at the 5th WG

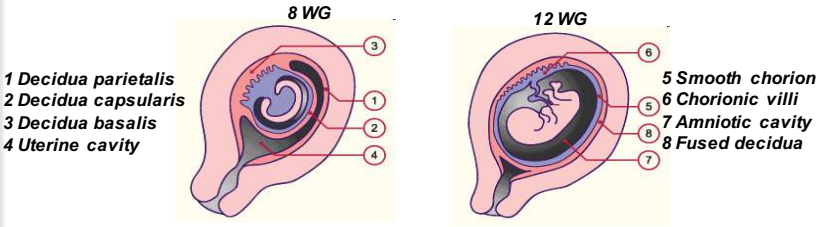
Total 473 CFTC



Mouawia et al, RBMO 2012

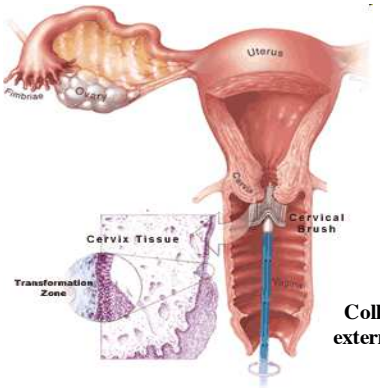
Isolation of trophoblasts from the cervix

Transcervical cells (TCC): inner part of the cervix and lower pole of the uterine cavity (Schettles 1971)



TCC sampling methods:

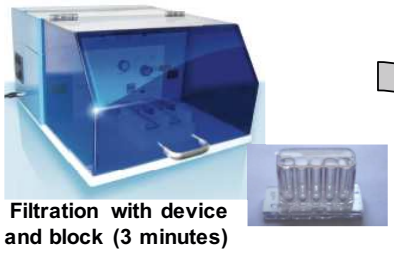
- *Intrauterine lavage*
- *Endocervical lavage*
- *Endocervical mucus aspiration*
- *Endocervical sampling by a cytobrush*



Collection of cells from external part of the cervix

10 ml

1:10 dilution



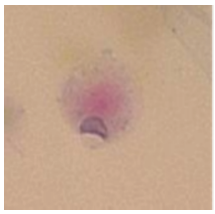
Filtration with device and block (3 minutes)

trophoblastic cells on filter

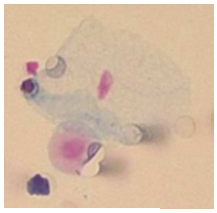
Pfeifer I et al Placenta, 2015

Non-invasive isolation of trophoblasts from the cervix
2ml out of 10 ml analyzed - Pfeifer I 2015

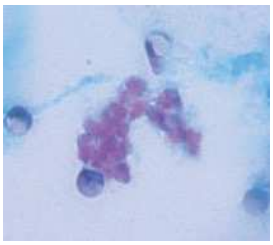
Couple	Term of pregnancy (WG)	Informative STR marker	Cytotropho-blasts/ Syncytio-Trophoblasts* - NIPD**	N° of microdissected cells
1(CF)	12	D7S486/D7S523	4 - carrier	10
2(CF)	12	D7S523	6 - carrier	12
3(CF)	12	D16S539/D7S523	10 - carrier	19
4 (SMA)	12	D5S816/D21S1437	6 – not affected	13
5 (SMA)	12	D21S1435	10 – not affected	21
6 (SMA)	12	D16S539/D7S523	6 – not affected	13
7#	12	D5S816/D21S1437	5	11
8#	12	D16S539	3/2	9
9#	11	D16S539/D5S816	4/2	10
10#	12	D21S1435	10	21
11#	12	D21S1435	6	14
12#	12	D16S3018	7	13
13#	12	D21S1435/D7S523	6	14
14#	12	D16S539/D5S816	2	6
15#	12	D21S11	8/2	16
16#	12	D16S539/D21S1435	12	21
17#	9	D16S3018/D5S615	6	12
18#	9	D5S615/D16S539	4	9
19#	8	D16S539/D5S816	4	10
20#	11	D16S539/D21S11	3	7
21#	8	D5S615/D5S816	3/1	8



Maternal cell



Trophoblastic cell



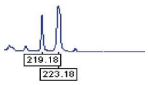
Syncytiotrophoblast

Maternal cell

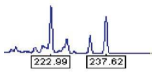
Maternal DNA

Paternal DNA

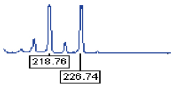
Trophoblastic cell



Syncytiotrophoblast

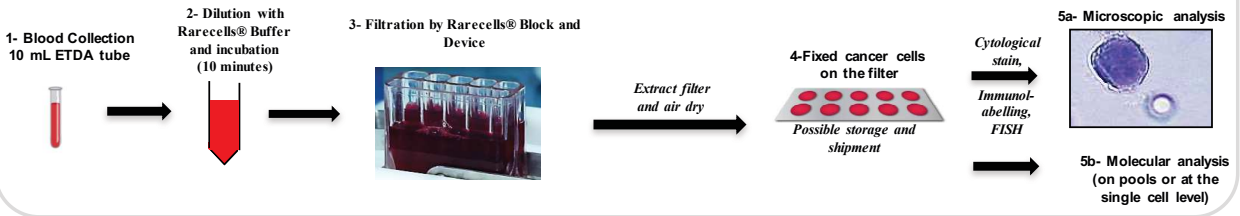


Maternal DNA

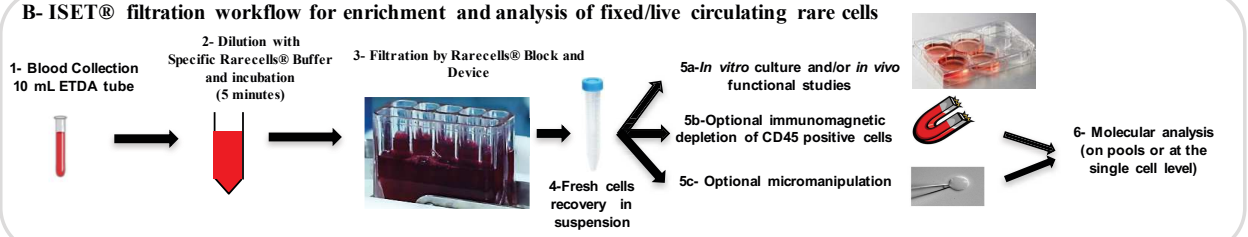


Paternal DNA

A- ISET® filtration workflow for enrichment and analysis of fixed circulating rare cells



B- ISET® filtration workflow for enrichment and analysis of fixed/live circulating rare cells



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- Workflow for ISET® *in vitro* assay of sensitivity and reproducibility with fixed/live cells counted by micropipetting

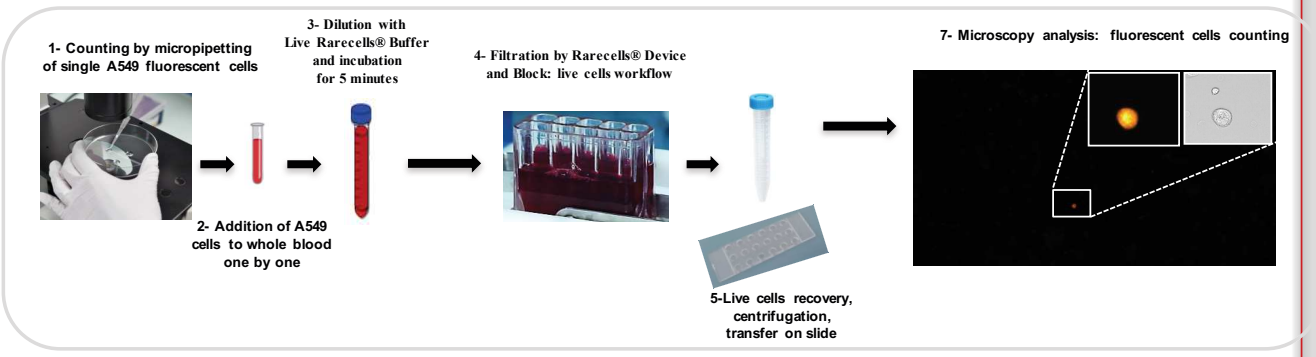

Table 5. Sensitivity and repeatability of the ISET® system for live tumor cells

Cell line	A549				LNCaP	
Number of spiked cells	1	3	5	1	1	5
Number of tumor cells detected by ISET®	1	3	4	9	1	4
	1	3	5	6	1	4
	1	2	4	8	0	5
	1	3	4	9	1	3
	1	2	3	10	1	5
	1	3	4	8		
Total detected cells / spiked cells	6/6	16/18	24/30	50/60	4/5	21/25
Recovery rate (SEM)	100% (0)	88.8% (7)	80% (5.2)	83.3% (5.6)	80% (20)	84% (7.5)

SEM, Standard error of mean

Sensitivity of the ISET® system for dual collection of live rare cells followed by CD45-immunomagnetic depletion

Blood volume (mL)	1
Number of spiked cells	10
Number of rare cells detected by ISET®	4
	3
	3
	4
	3
	3
Total detected cells / spiked cells	21/60
Recovery rate (SEM)	35% (5.2)

SEM, standard error of mean; two independent experiments in triplicates

> 30 CFTC per 10 ml blood

30%= > 10 CFTC WGA genotyping (single cells or pooling) NGS

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