Fetal *RHD* genotyping in maternal plasma:

from validation to managment of a non invasive prenatal test

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*f*etal *RHD* genotyping on maternal plasma

- ***RHD* gene : many variant forms**
- **analysis of 3 regions of the *RHD* gene**:

-Sensibility +++, specificity +++, Cut off values

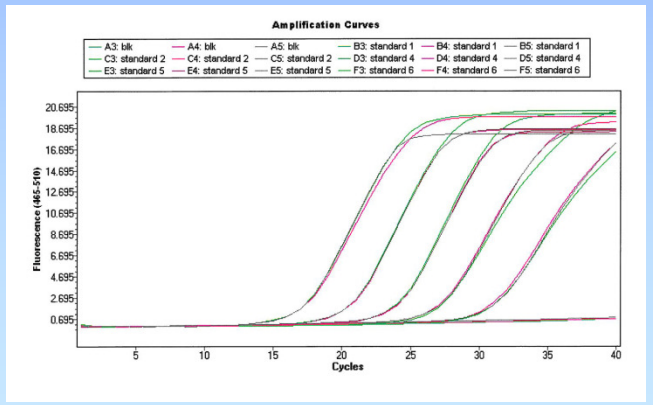
-Validation of the method

⇒ **False negative=0**

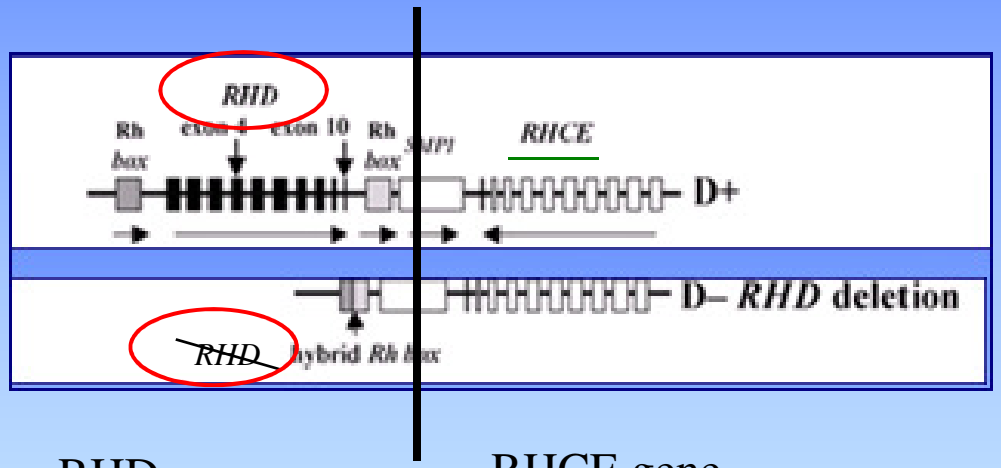
2 steps

-DNA extraction

-real time PCR



RHD gene particularities



D positive

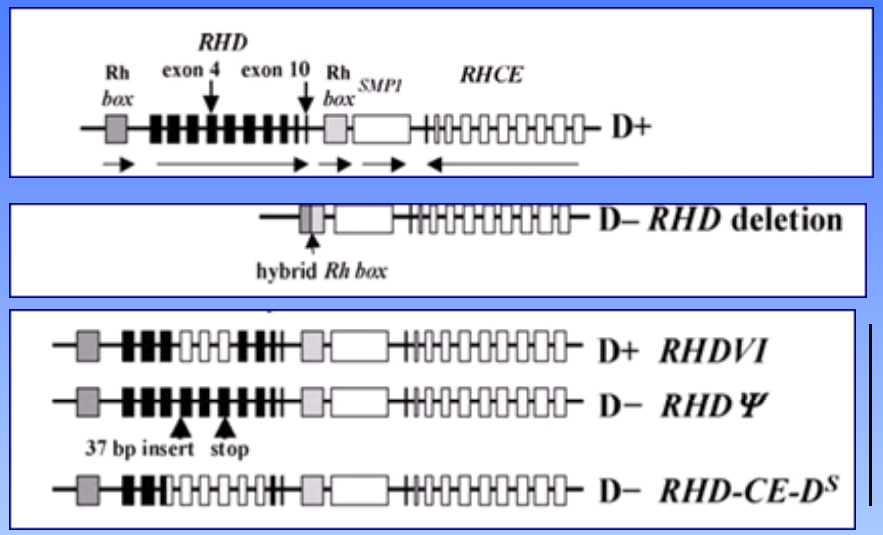
D negative

RHD gene

RHCE gene

Other cases

From gene

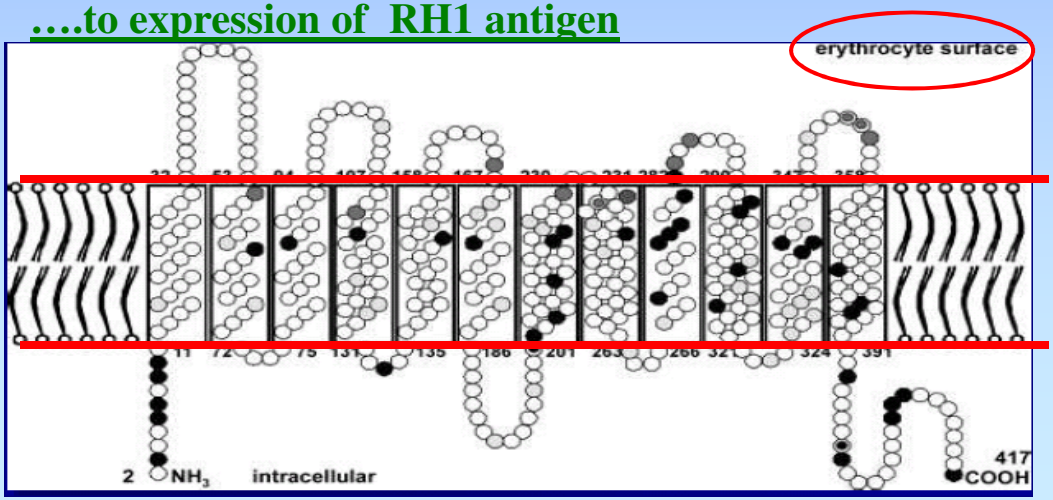


Presence of the RHD gene
Expected phenotype: RH:1 (positive)

Absence of the RHD gene
Expected phenotype: RH:-1 (negative)

Presence of an abnormal RHD gene
Phenotype can not be determined

….to expression of RH1 antigen

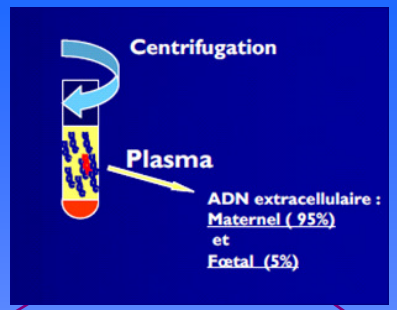


Erythrocyte's surface

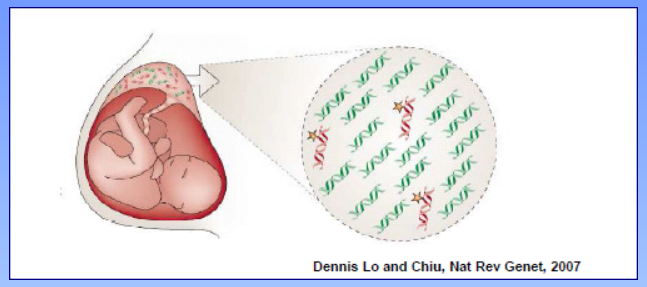
Known RHD gene variant forms tested by the method used in 2010-2011 in our laboratory

(poster, congress SFBC, Lyon 2011)

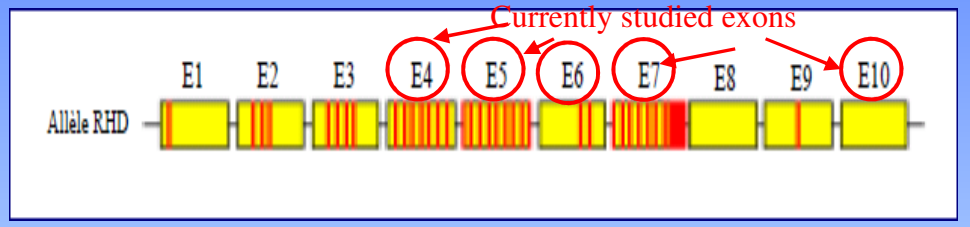
EXON4	EXON5	EXON10	CARACTERISTICS	DENOMINATION
-	-	+	P	RH1 partial DVI type2
-	-	+	P	D variant type VI type?
-	-	+	P	African origin pseudo gene ψ or r's?
-	+	-	P	DHAR (*)
-	-	+	P immunogenicity+++	DVI type 3
-	-	+	P?	D partial III type4+ deletion exons 4 to 7
+	+	+	P	DNB(*)
+	+	+	F	D weak type10
+	+	+	F	D weak
+	+	+	F	D weak
+	+	+	F	Initially known as D- then D weak type 1
+	+	+	F	D weak type 5 without allo-immunisation
+	+	+	F	RH1 weak type11
+	+	+	F	D weak type?
+	+	+	F	D weak type?



SENSIBILITY: detection of fetal DNA (5%)



SPECIFICITY: detection of *RHD* gene

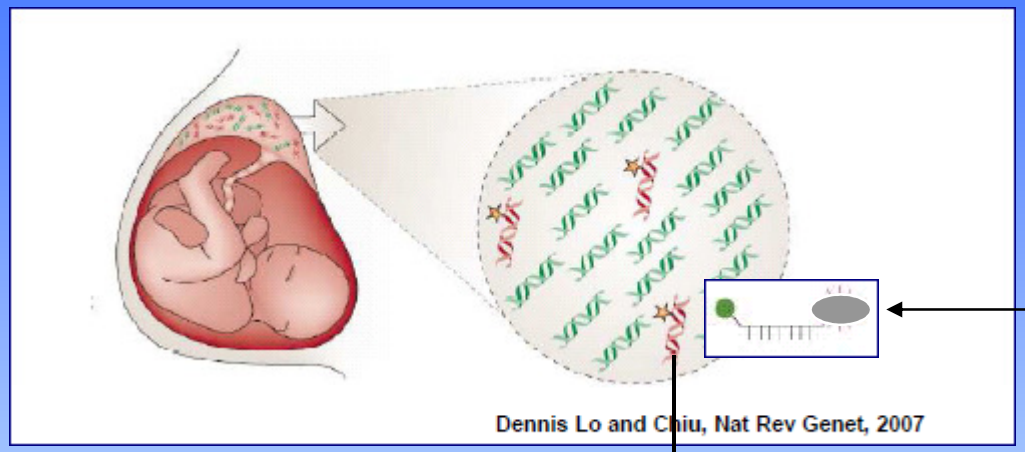


RHD gene structure

fetal RHD genotyping

3 levels of specificity

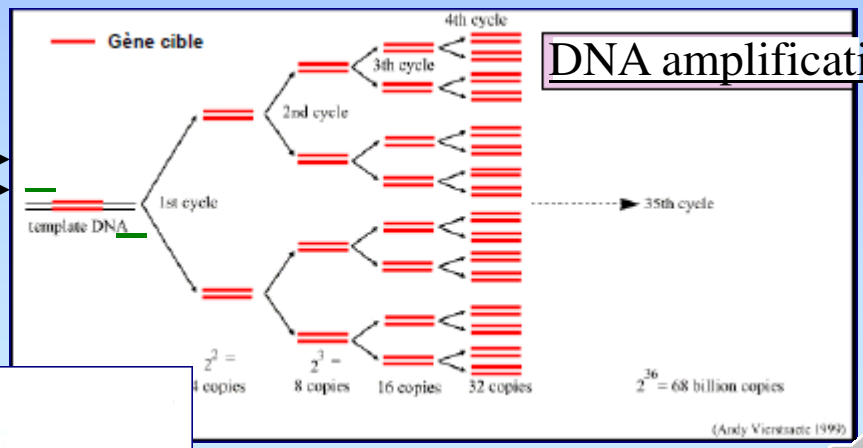
RHD gene detection



1) SPECIFIC probes

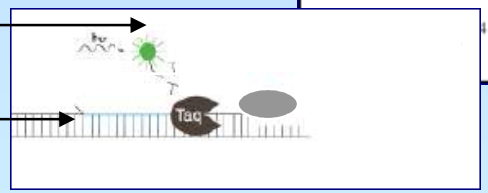
2) SPECIFIC primers

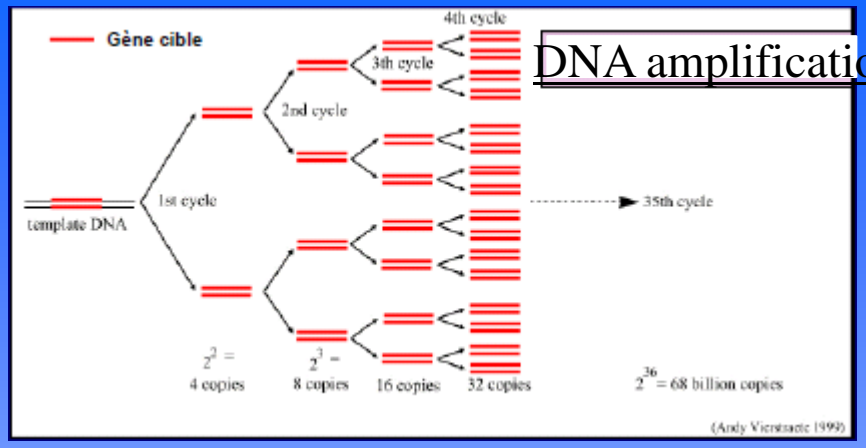
DNA amplification



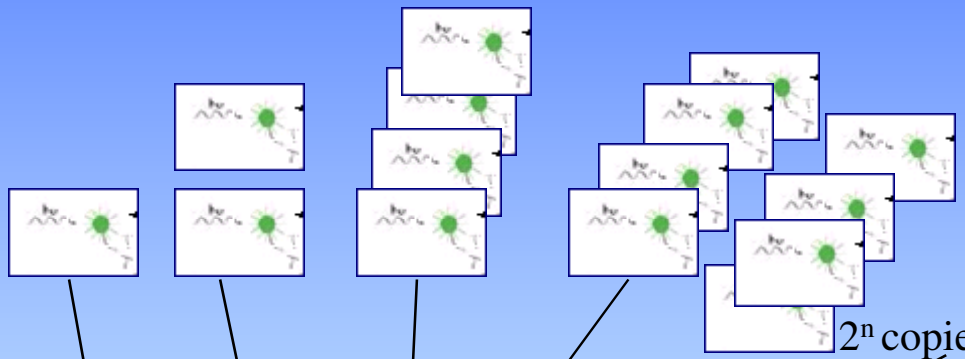
Fluorensence emission

Probe's hydrolysis

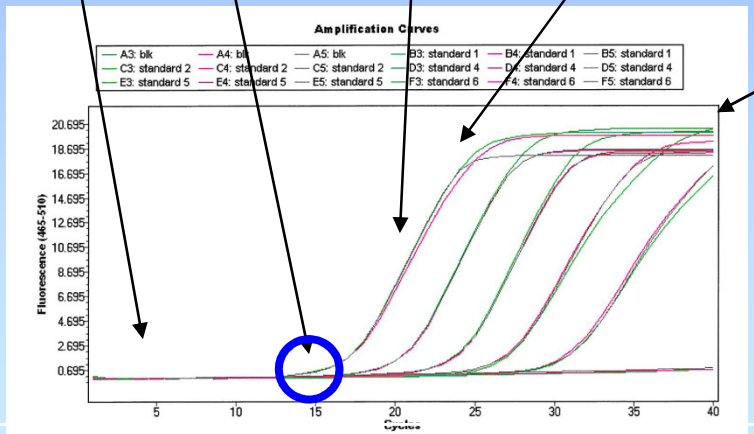




DNA amplification



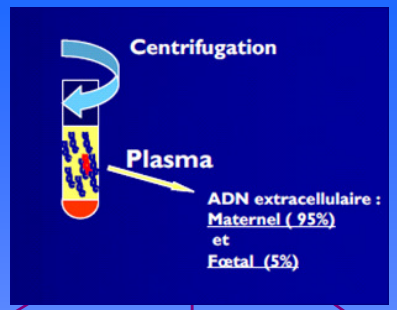
2^n copies of DNA



Fluorescence Intensity

Obtained value= FETAL or VARIANT CARRYING MOTHER?

cycles of amplification



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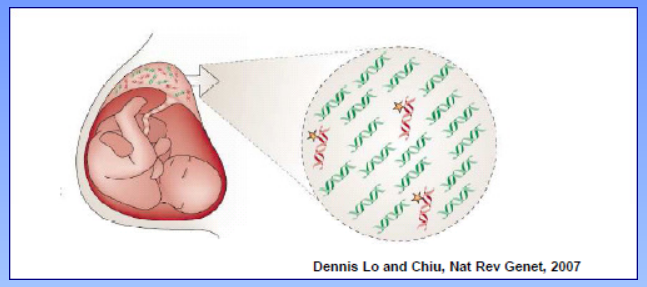
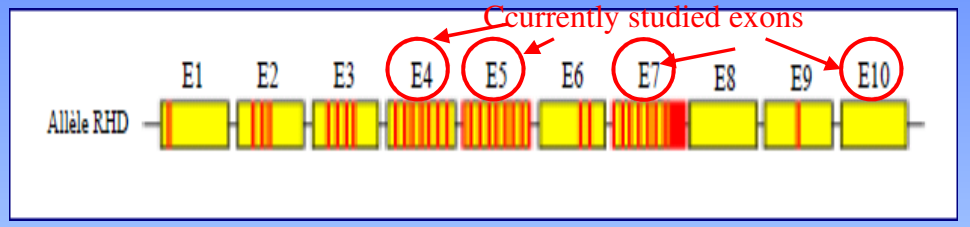


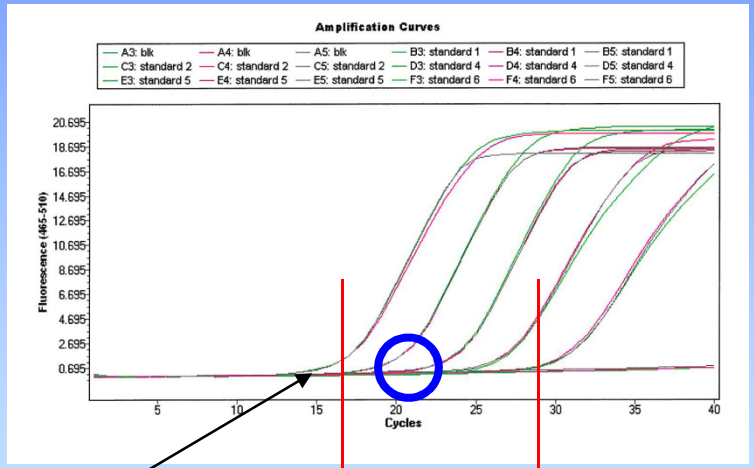
SPECIFICITY: detection of *RHD* gene



RHD gene structure

Detection of fetal RHD gene only?

Necessity to define cut off values to guarantee fetal specificity => 3rd level of specificity



Cut off valuel Ct =FETAL SPECIFICITY
Beetween the two defined values

Earlier amplification = lower Ct value
MATERNAL origin
=> Maternal variant form of RHD gene

Control samples : OK
(known D +, D- samples, blank)

No → New run for all the samples

Yes ↓

Control gene (CCR5) for each patient's sample: OK

No → New run for possible inconclusive sample

Yes ↓

Analysis of each sample's result:

Amplification of each tested exons
In normal range ↓

Presence of the RHD gene

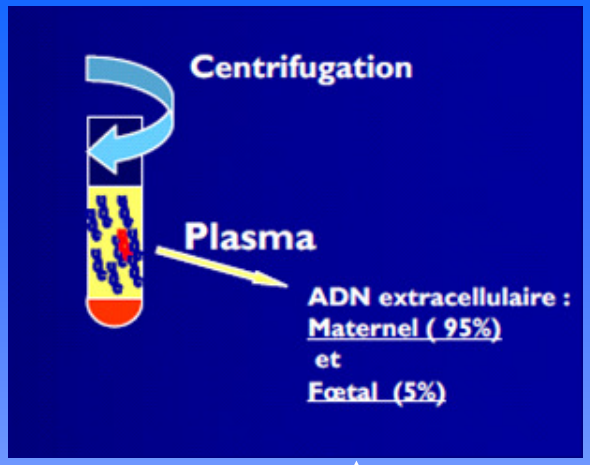
Absence of amplification ↓

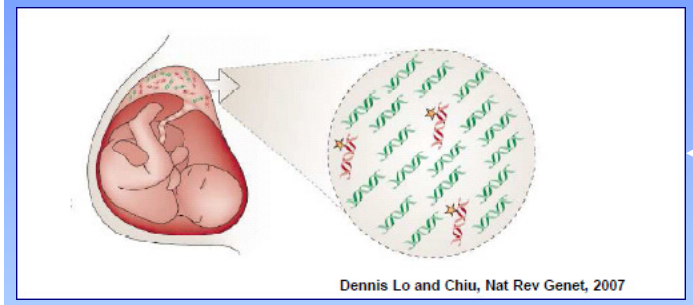
Absence of the RHD gene
(always verified on a second sample)

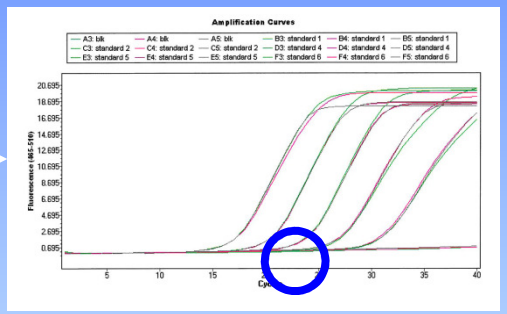
Abnormal amplification ↓

-Amplification of 1 or 2 of the 3 tested exons:
Fetal RHD variant form
-Early amplification of 1, 2 or the 3 tested exons:
Maternal RHD variant form

Inconclusive sample:
RHD gene sequencing







Method with high SENSIBILITY and SPECIFICTY

Fetal SPECIFICITY

Conclusions: validated method
Decision to set up the test routinely

Laboratory

-Pre-PCR area, post-PCR area: are geographically separated

-Access limited to staff

-No manipulation of the post-PCR products

=>in order to LIMIT the risk of DNA contamination

Fetal *RHD* Genotyping in maternal plasma

-Non Invasive Prenatal Diagnosis:

- Tested from maternal plasma sample

- Can be performed starting at 10 weeks of pregnancy

Fetal *RHD* Genotyping in maternal plasma

- Indications of this test:

-anti-D immunised patients: to increase medical supervision during the pregnancy (if the result is positive)

-Prevention of anti-D immunisation

Fetal *RHD* Genotyping in maternal plasma

Continuous improvement and innovation

- Scientific knowledge

-specialized articles in newspapers
- internet (for exemple: http://www.uni-ulm.de)

- Laboratory management : Yearly review

-Performances analysis
-Procedures review
-Key Indicators analysis
-Customers's expectations
-Continuous improvements
-Risk management

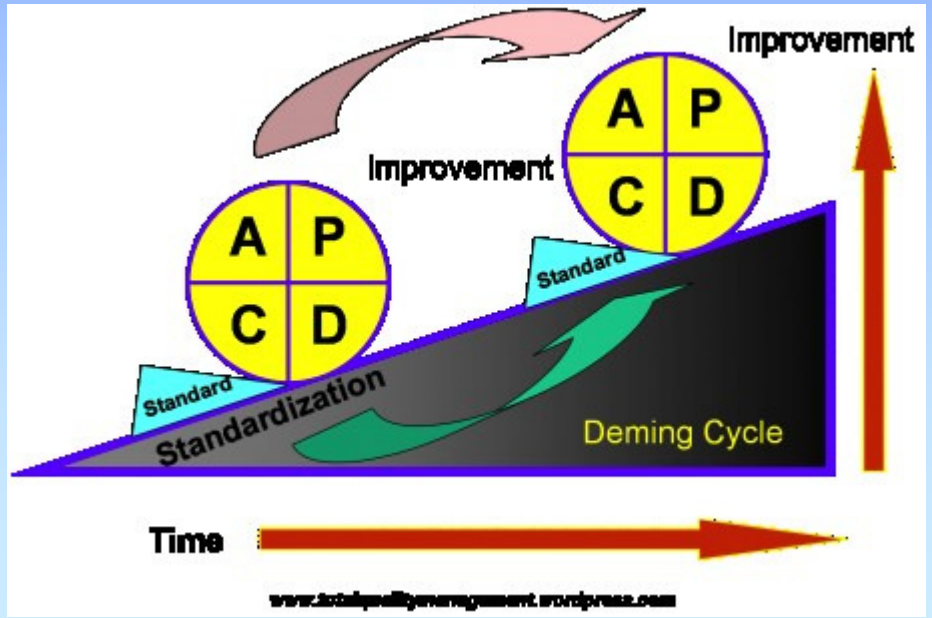
- How to maintain the dynamism of the laboratory?

- Biologists participate in weekly antenatal meetings
-The laboratory is part of a national group

Fetal *RHD* Genotyping in maternal plasma

Continuous improvement and innovation

Quality management : continuous quality improvement is symbolized by the Deming wheel:



Hospital

Cooperation beetween

EFS
ÉTABLISSEMENT FRANÇAIS DU SANG

Laboratory

Physicians

The different healthcare actors

Thank you for your attention!

Any questions?

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Dr Emmanuelle Guinchard