



# Certified Reference Materials for Prenatal and Neonatal Testing

Korea Research Institute of Standards and Science (KRIS)

Center for Bioanalysis

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2017 JCTLM M&S meeting

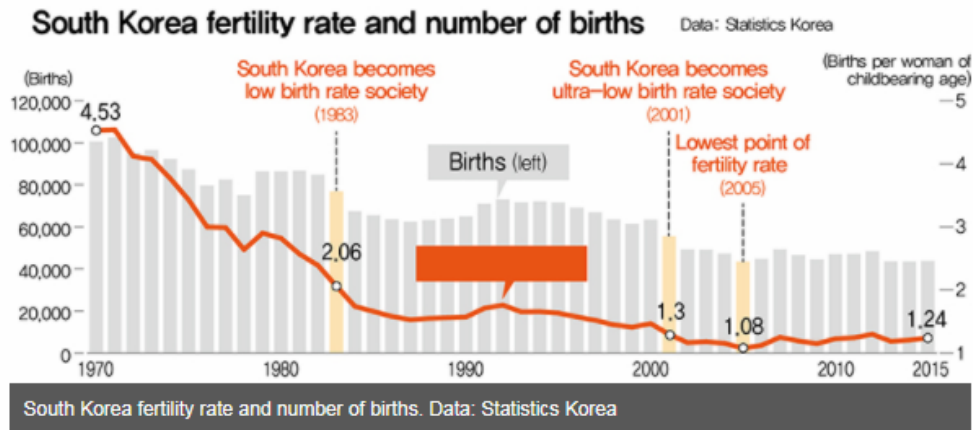


# Background

## Already OECD lowest, South Korea's birthrate getting worse

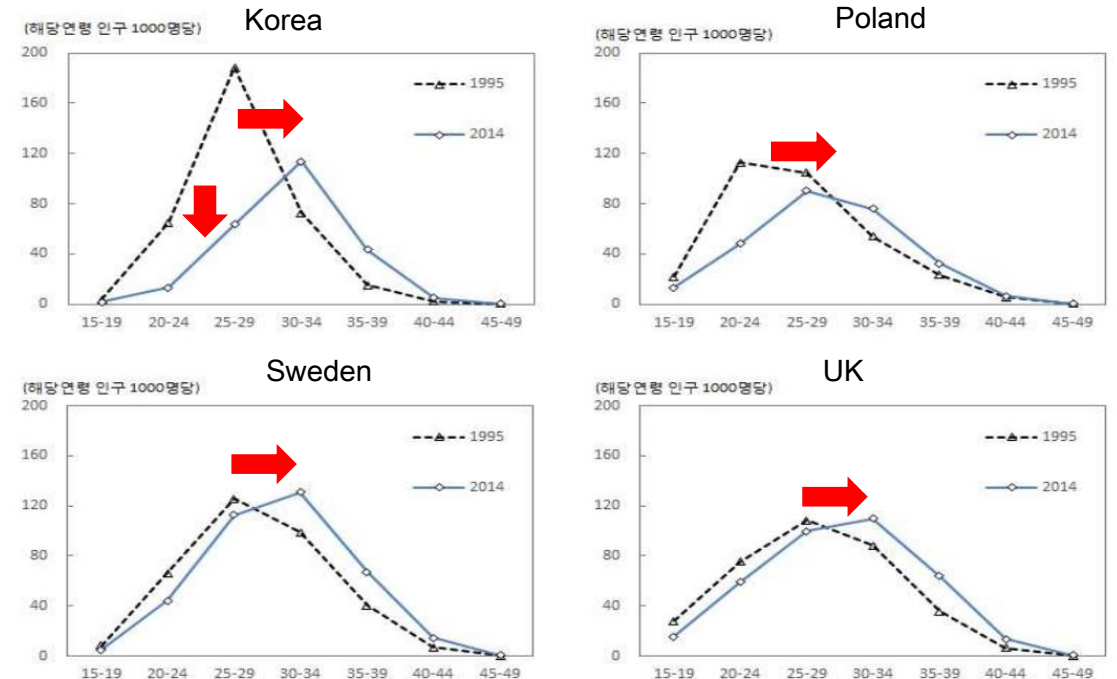
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News from The hankyoreh, 2016-08-28

## Charts on delayed motherhood (1995 and 2014)



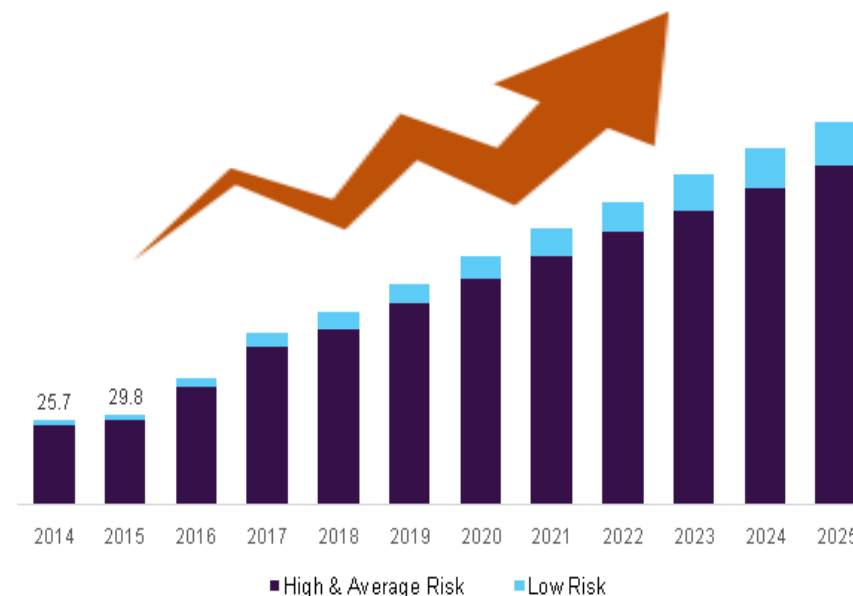
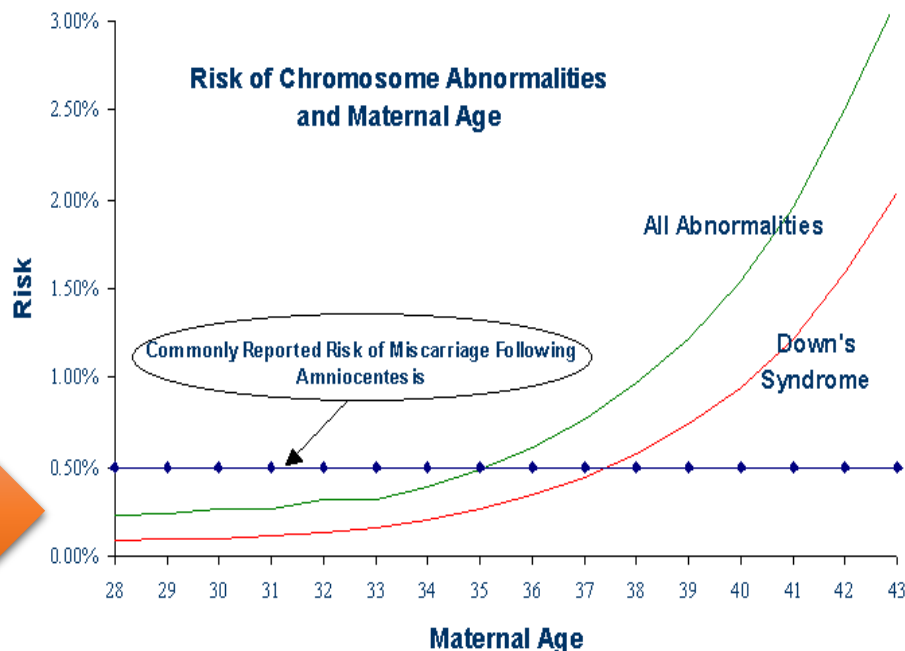
자료: OECD(2016), Family database.



## Prenatal Testing



### Why NIPT?



U.S. Non-Invasive prenatal testing market, 2014 - 2025 (USD Million)

- Possibility of fetus abnormalities ↑ = Prenatal testing ↑
- High false-positive rate in Quad screening vs. **Higher** risk of miscarriage after amniocentesis
- **Demand of prenatal testing with high accuracy !**
- The global Non-Invasive Prenatal Testing (NIPT) market size was valued at USD 1.1 billion in 2016. Korean NIPT market size was developed 1000% for 1-year (2016).

## Prenatal Testing



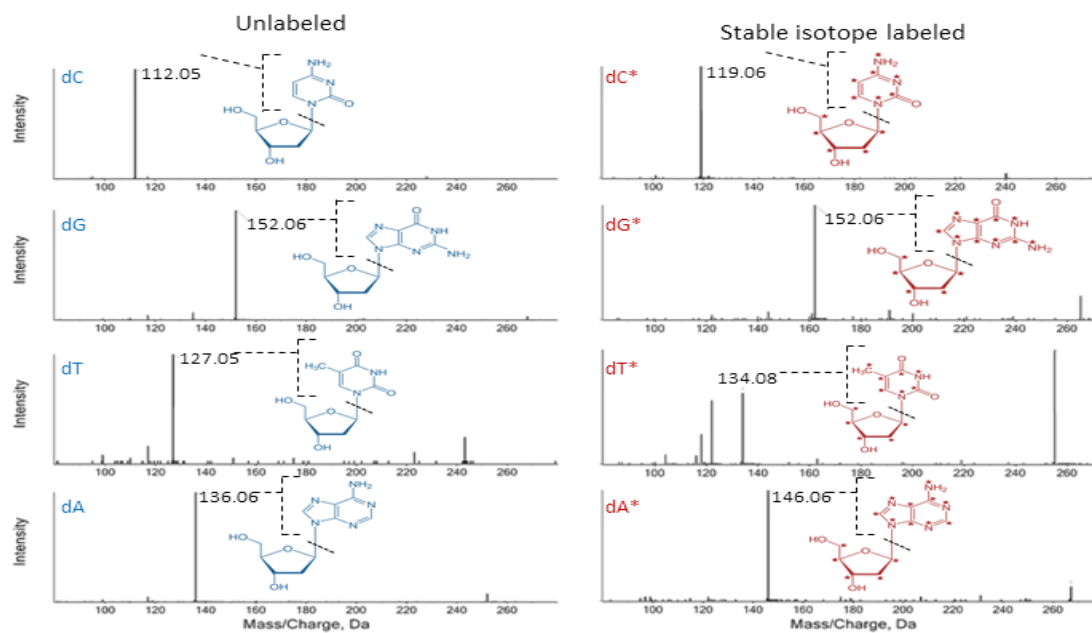
## The strategy of KRIS CRM for NIPT

Subject	Target	Technical issue
Matrix matched CRM	Serum	<ul style="list-style-type: none"> <li>- Inactivation of DNase</li> <li>- Elimination of endogenous cfDNA</li> <li>- Nucleosomal DNA</li> <li>** Exact matching to cfDNA in human serum</li> </ul>
Full ID-MS of DNA quantification	Trace level	<ul style="list-style-type: none"> <li>- SIL-DNA production for compensation of sample loss or digestion imperfection</li> <li>- High-sensitivity detection method</li> <li>- Traceability of DNA quantity for CRM preparation</li> </ul>

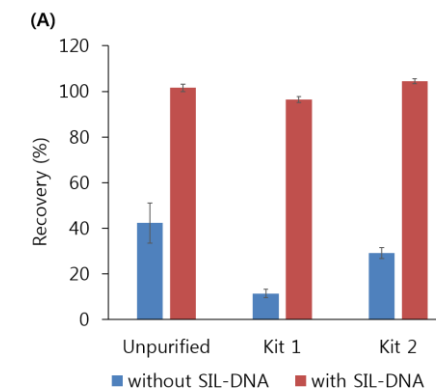


# Development of an LC-MS/MS Method using an Isotopically Labeled DNA as an Internal Standard for the Quantification of Cell Free DNA

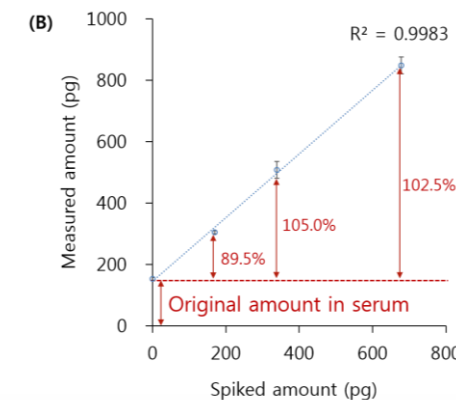
## Prenatal Testing



MRM scans of dNMP and SIL-dNMP



Compensation of extraction loss



Linearity

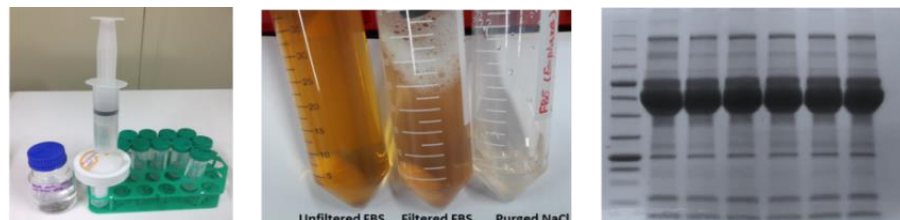
## Serum CRMs for NIPT

- **Preparation of DNA-free serum**
  - Comparable with blank (water) after treatment
  - Similar properties but only 'DNA-free' after treatment
- **First batch of KRISS CRM for NIPT:** it used for test materials for **proficiency test** by Korean Institute of Genetic Testing Evaluation.

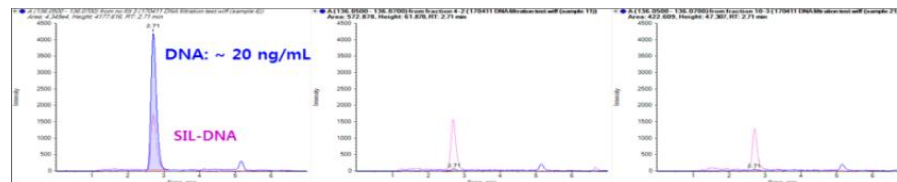
## Prenatal Testing



### Preparation of DNA-free serum



### Confirmation of DNA-clearance in serum



문서번호 : 유정원 2017-19호 <직인생략>  
시행일자 : 2017. 5. 15.  
수 신 : 한국표준과학원 원장  
참 조 : 바이오임상센터장  
제 목 : 비침습산전기형아검사(NIPT)의 외부정도관리 평가용 유전자(DNA) 제조 요청

혈중 태아 DNA 분율 (average %)		13.79(6개 기관)				
결과 (기관 수)	9번 삼염색체증		13번 삼염색체증	6/6 음성	16번 삼염색체증	1/1 음성
	18번 삼염색체증	6/6 음성	21번 삼염색체증	6/6 양성	22번 삼염색체증	1/1 음성
	( )번 삼염색체증		(X) 성염색체증	5/5 음성	(XXY) 성염색체증	5/5 음성
	(XXX) 성염색체증	5/5 음성	(XYY) 성염색체증	2/2 음성	( ) 성염색체증	

### Proficiency tests for NIPT





# Neonatal/Newborn screening

## Neonatal Testing



## Dried blood spot (DBS)

- ✓ Simple !
- ✓ Less-invasive!
- ✓ Easy!
- ✓ Economic !

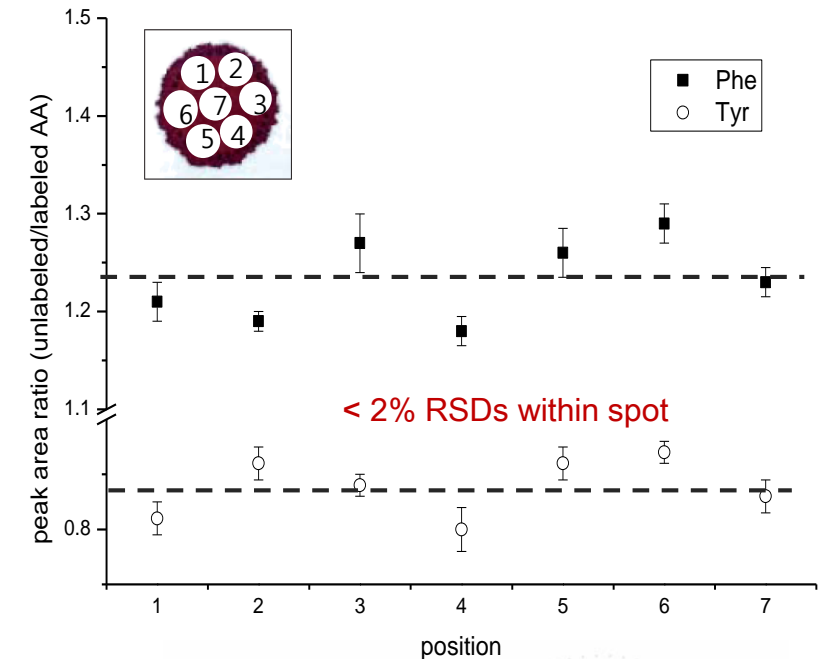
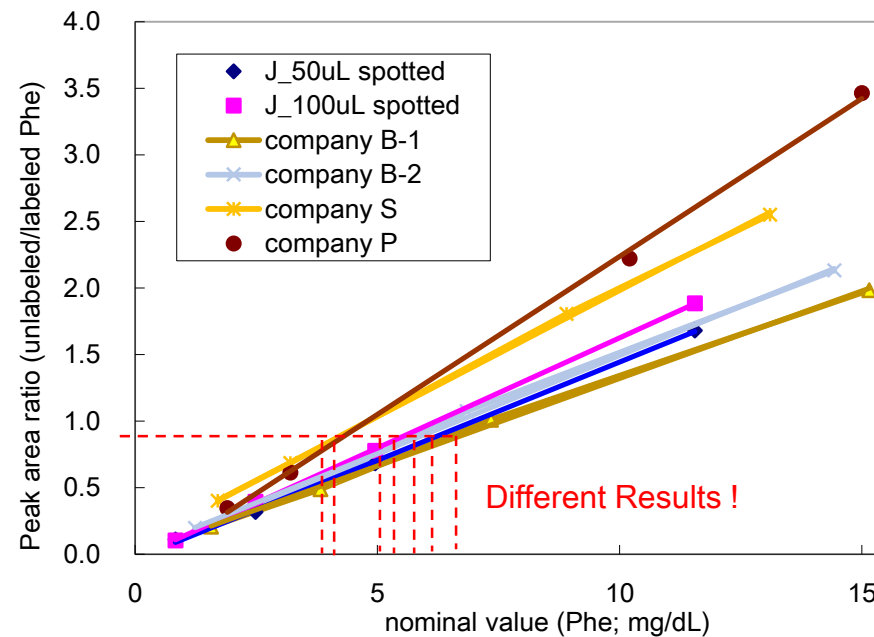


## Neonatal Testing



# Things to think for DBS as CRM

- **Diversity of samples:** sensitive to blood conditions, and manufacturing conditions (Viscosity, Hematocrit, Adsorbed volume, Paper type, etc)
- **Extremely small size sample:** slight differences → different results
- **Sample homogeneity:** within spot and between spot

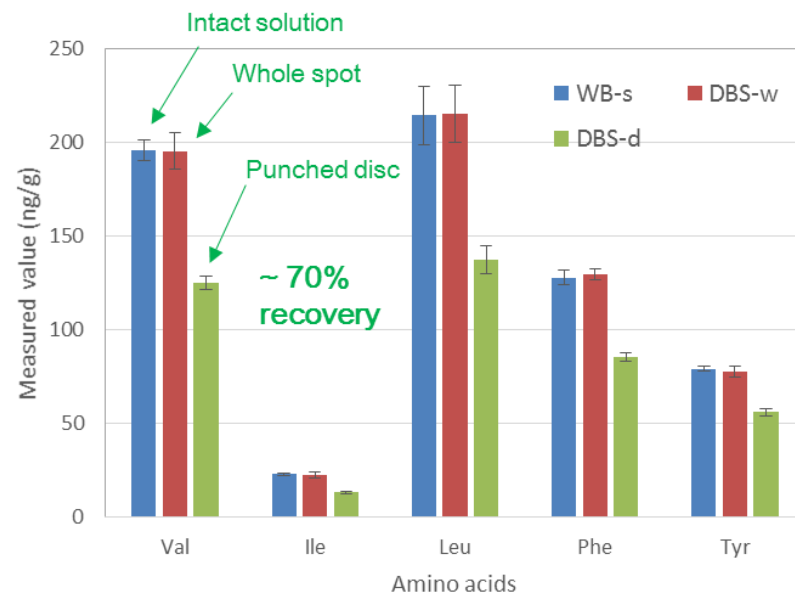




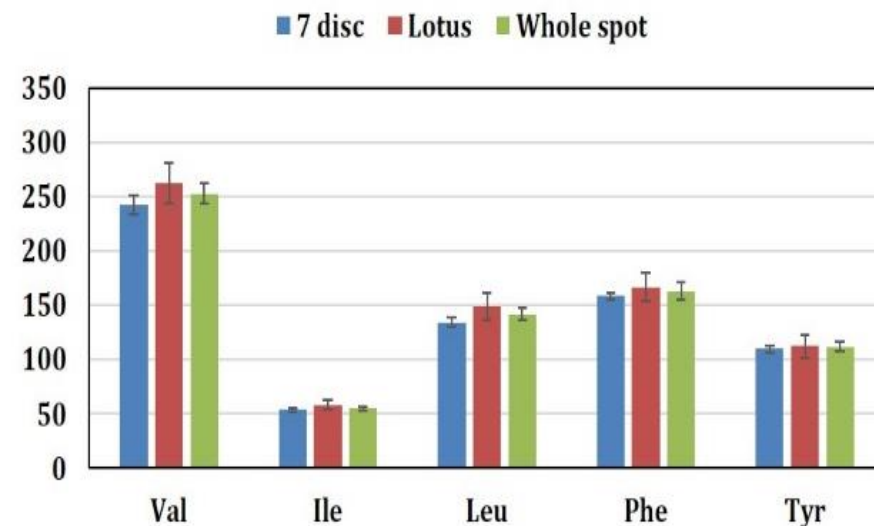
# Sampling bias of punched disc

- **Whole spot vs. partial spot** (area = sample volume)
- No extraction or measurement issue
- Revise calculation of disc area : underestimation of sampling volume
- **Whole spot sampling for certification**

## Neonatal Testing



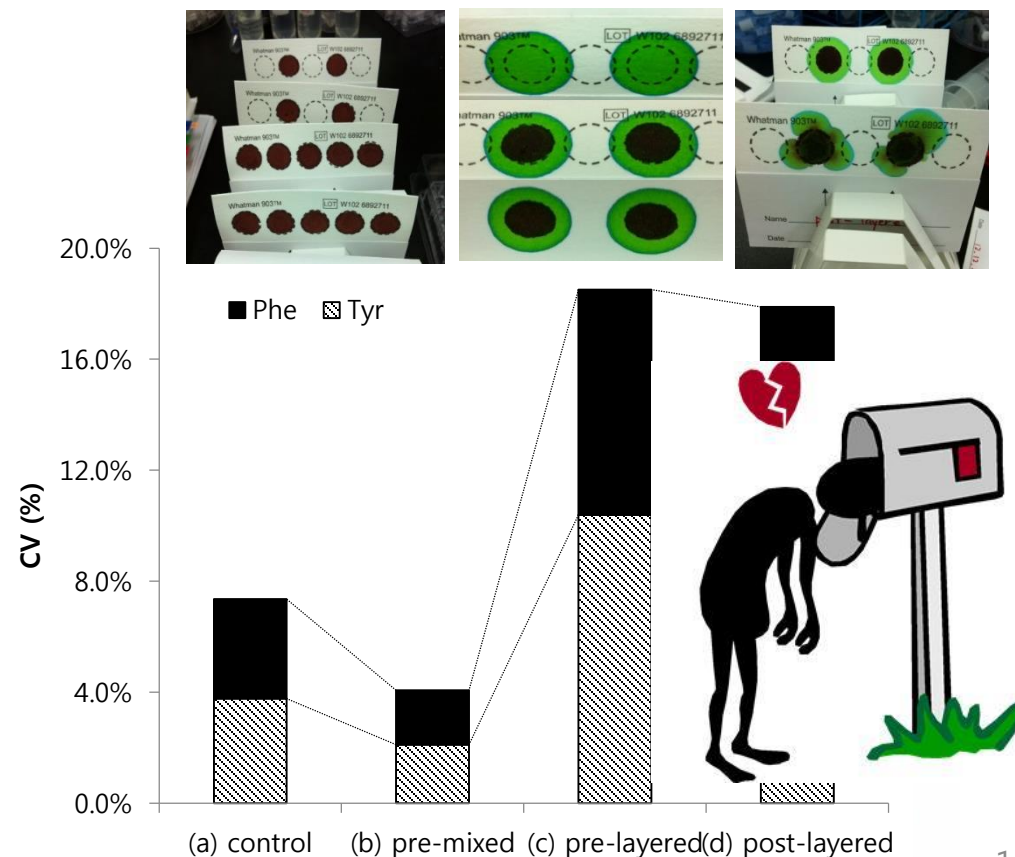
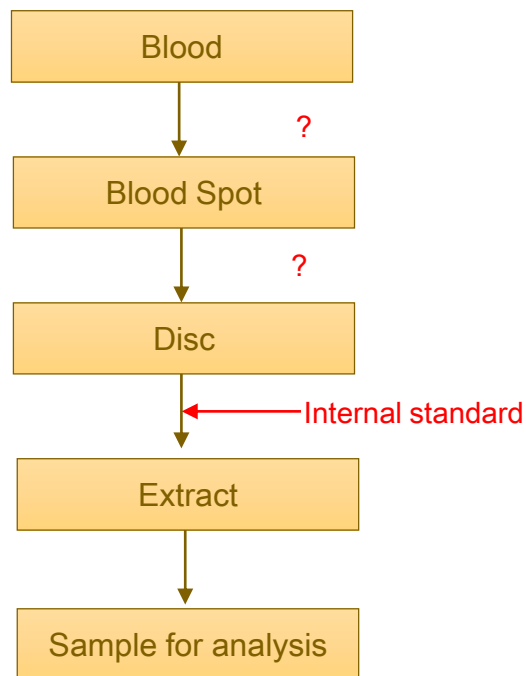
After adjustment of disc area



# Challenge to establish traceability to SI unit

- **Black box in sampling step:** Lack of traceability chain to final stage
- Metrological challenge: KRISS is developing **SI-traceable DBS CRMs** for neonatal screening

## Neonatal Testing



# Beyond screening!

## Measurement capability

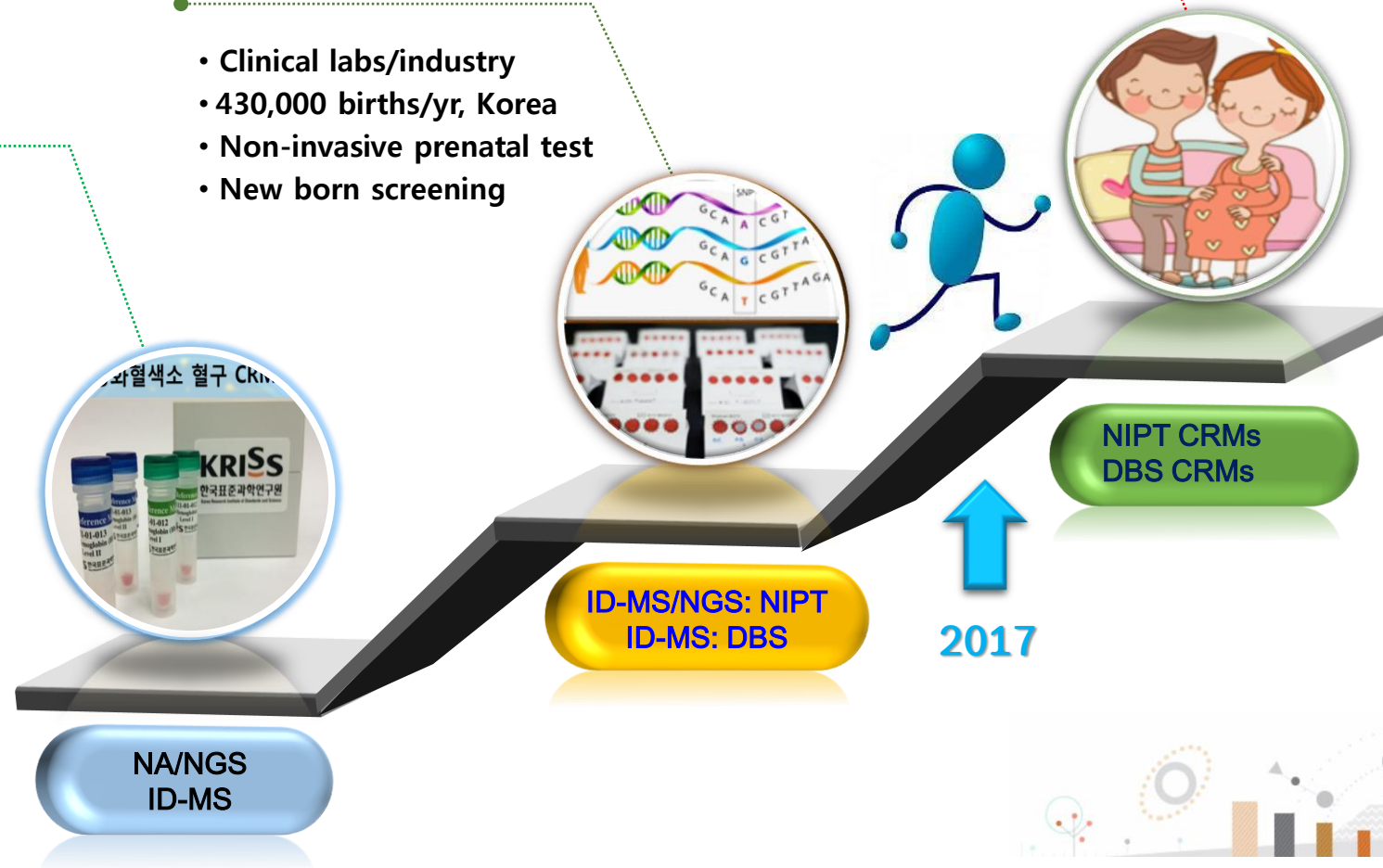
- Nucleic Acids
- NGS
- Clinical analysis
- Serum CRMs

## New CRM needs

- Clinical labs/industry
- 430,000 births/yr, Korea
- Non-invasive prenatal test
- New born screening

## Outcomes

- Matrix matched NIPT CRM
- SI-Traceable DBS CRM
- Expanding applications based on NIPT and DBS





*Better Standards, Better Life!*

Thank you ☺

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