Pitfalls in the development and implementation of NIPT at the regional level: Trisomy Test story

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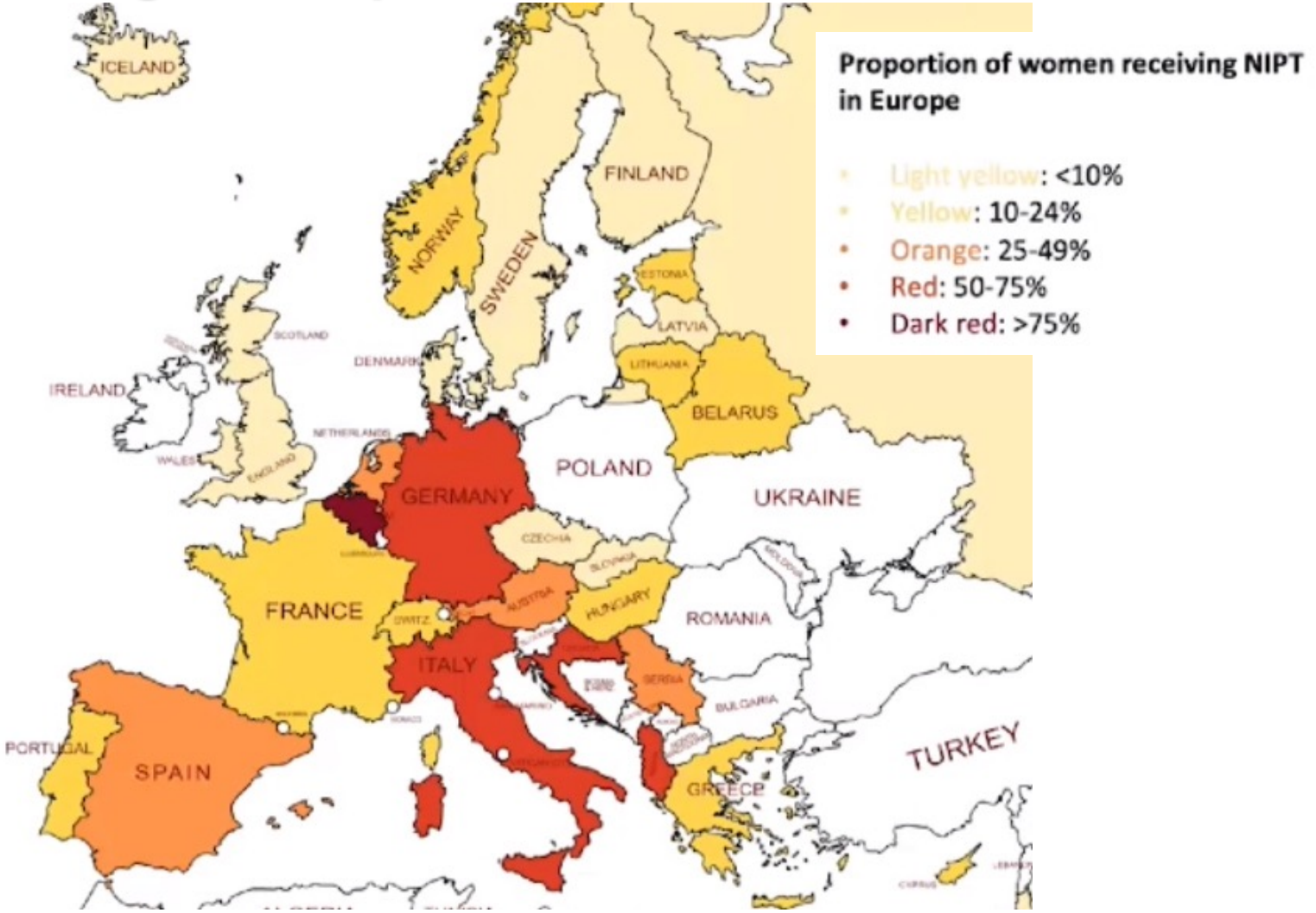
Conflict of Interest Disclosure

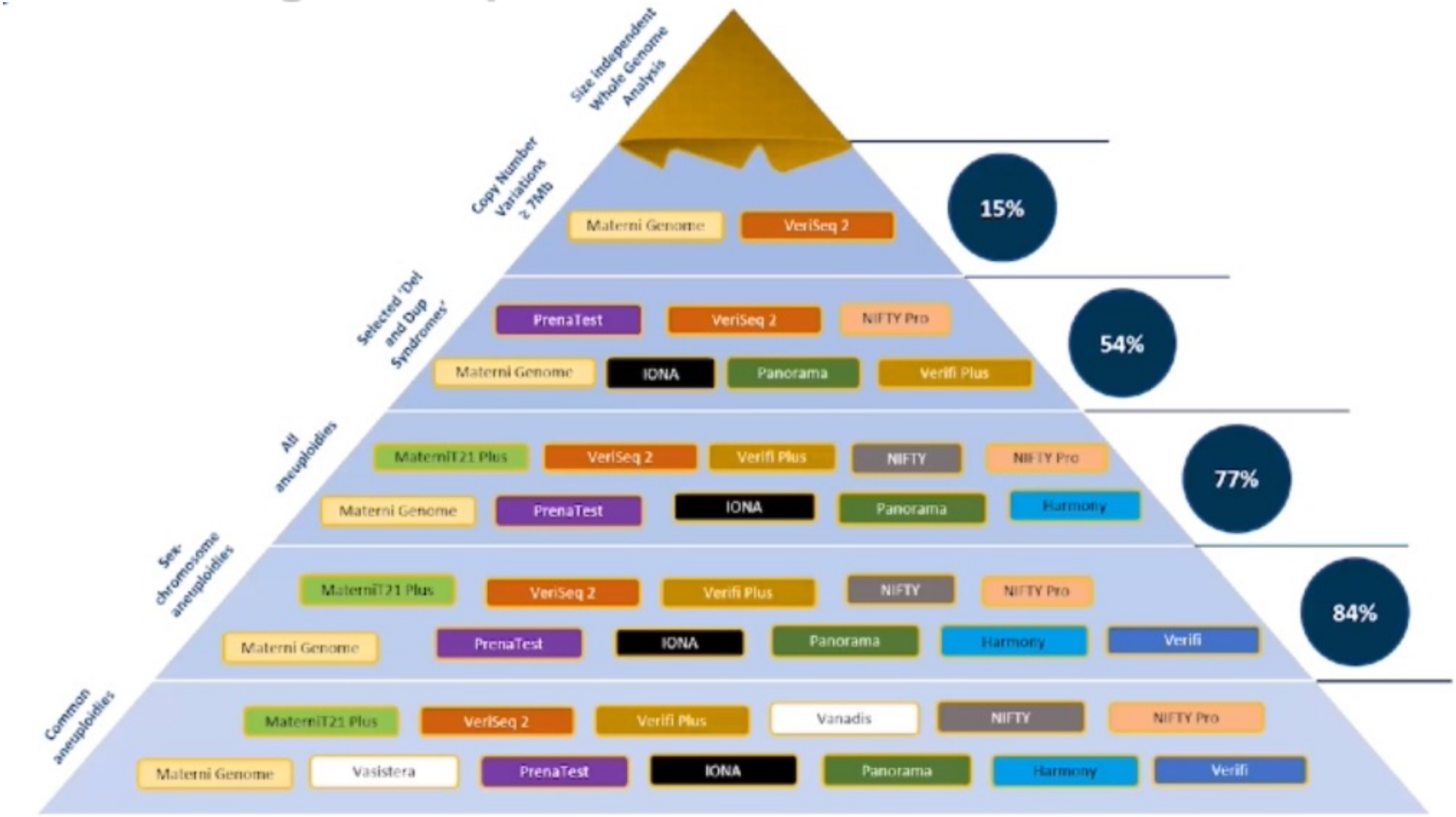
- All authors are employees of Trisomy test Ltd. and Medirex Inc.

NIPT testing in Europe – countries



source: Ida Vogel, Aarhus University, Denmark, COGEN 2023 presentation

NIPT testing in Europe – tests



source: Ripudaman Singh, Arcedi, Denmark, COGEN 2023 presentation

Trisomy & GenomeScreen tests – history

- 2013 **– first NIPT category tests performed in Slovakia** with Illumina MiSeq and IonTorrent PGM platforms validated (small size laboratories scale)
- 2015 (September) – first **TRISOMY tests performed** in Slovakia (*T13, T18 and T21, fetal sex*)
- 2016 (July) – **upgrade to Illumina NextSeq 500/550 platform** (middle size labortories scale)
- 2017 (February) – added **TRISOMY test +** *(+ 5 microdeletion syndromes*)
- 2017 (October) – added **TRISOMY test XY** *(+ sex chromosomes aneuploidies*)
- 2019 (December) – added **TRISOMY test Complete** / **GenomeScreen** (*whole genome resolution & findings interpretation*)
- 2021 (October) – added TTC **+CF** *(CFTR p.F508del mutation)*
- 2022 (May) – added TTC **+SLOS** (6 mutations in *DHCR7* gene)

Trisomy tests – key publications

- Copy Number Variant Detection with Low-Coverage Whole-Genome Sequencing Represents a Viable Alternative to the Conventional Array-CGH.
  - *Diagnostics (Basel). 2021 Apr 15;11(4):708. doi: 10.3390/diagnostics11040708*

- Non-invasive prenatal testing (NIPT) by low coverage genomic sequencing: Detection limits of screened chromosomal microdeletions.
  - *PLoS One. 2020 Aug 26;15(8):e0238245. doi: 10.1371/journal.pone.0238245.*

- Validation of Copy Number Variants Detection from Pregnant Plasma Using Low-Pass Whole-Genome Sequencing in Noninvasive Prenatal Testing-Like Settings.
  - *Diagnostics (Basel). 2020 Aug 8;10(8):569. doi: 10.3390/diagnostics10080569.*

- Result of Prospective Validation of the Trisomy Test® for the Detection of Chromosomal Trisomies.
  - *Diagnostics (Basel). 2019 Oct 2;9(4):138. doi: 10.3390/diagnostics9040138.*

- Combining count- and length-based z-scores leads to improved predictions in non-invasive prenatal testing.
  - *Bioinformatics. 2019 Apr 15;35(8):1284-1291. doi: 10.1093/bioinformatics/bty806.*

- Utilization of Benchtop Next Generation Sequencing Platforms Ion Torrent PGM and MiSeq in Noninvasive Prenatal Testing for Chromosome 21 Trisomy and Testing of Impact of In Silico and Physical Size Selection on Its Analytical Performance.
  - *PLoS One. 2015 Dec 15;10(12):e0144811.*

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Trisomy tests – improper and biased competition

MOŽNOSTI TESTU	KLINICKÝ VÝZNAM a další důležité informace	PANORAMA	TRISOMY TEST	PANORAMA	TRISOMY TEST
Testování od ukončeného 9.TT	Časné provedení testu	ANO	NE	ANO	ANO*
Reportuje pohlaví plodu s ≥ 99,9% přesností v publikovaných studiích	Minimalizace diskrepance s UTZ	ANO	NE	ANO	ANO
Screenuje chromozomy 13, 18, 21	Detekce nejčastějších aneuploidií	ANO	ANO	ANO	ANO
Reportuje aneuploidie pohlavních chromozomů	Detekce aneuploidií X a Y	ANO	ANO*	ANO	ANO**
Detekuje triploidii	Riziko abortu, těžkých vrozených defektů plodu, riziko závažných komplikací u matky, unikátní schopnost Panoramy umožněná využitím odlišné technologie	ANO	NE	ANO***	NE
Měří a reportuje fetální frakci (FF)	Základní kvalitativní parametr neinvazivních prenatálních testů. Indikuje dostatečné množství FF pro provedení testu. U Panorama testu se fetální frakce nezbytná pro zhodnocení testu pohybuje nad 2,8% (u ostatních NIPT testů se pohybuje okolo 4%, Prenascan fetální frakci vůbec nestanovuje)	ANO	ANO**	ANO****	ANO****
Screenuje celý genom	Klinický význam minimální, reziduální riziko jiných chromozomálních aberací je u plodů bez UZ nálezu ≤ 1/1000. Testování naopak zvyšuje falešnou pozitivitu testu detekcí aneuploidií přítomných jen v placentě, nikoli u plodu (placentární mozacizmus je přítomen až u 2 % normálních placent)	NE	ANO	NE	ANO*****
Analyzuje fetální genotyp odděleně od mateřského	Abnormality u matky mohou vést k falešně pozitivním výsledkům testu	ANO	NE	ANO	ANO*****
Screenuje mizející dvojče	Častá příčina falešné positivity, pokud není zachyceno	ANO	NE	ANO	NE
Screenuje molární těhotenství	Asociováno se zdravotními komplikacemi a může vést k gestační trofoblastické neoplázii.	ANO	NE	ANO	NE
Validováno v nízko-/vysokorizikové populaci	Schopnost testovat těhotné všech věkových skupin	ANO	NE	ANO	ANO&
Testování dvojčetných těhotenství	NOVĚ! Testování dvojčetných gravidit – velkou výhodou Panorama testu bude schopnost stanovení fetální frakce pro každý plod zvlášť – k dispozici pro naše klienty již od začátku října!!	ANO	ANO	ANO	ANO
Testování těhotenství z darovaných oocytů a surogátních těhotenství	NOVĚ! Doposud velká limitace Panorama testu je již překonána. Od začátku října bude pro naše klienty vyšetření k dispozici!	ANO	ANO	ANO	ANO
Publikované výsledky, odborné publikace	Parametry testu jsou odborně obhájené a dokladované	ANO	NE	ANO	ANO&&
Vnitřní kontrola zpracování vzorku	Detekce případných chyb při odběru, laboratorních chyb, záměn a kontaminací vzorku	ANO	NE	ANO	ANO&&&
CE-IVD certifikace testu včetně bioinformatického zpracování	Test je vysoce standardizovaný, vhodný pro diagnostické účely	ANO	NE	ANO	ANO&&&&
Zpracování vzorku *„in-house"* v ČR/SR	Vzorky jsou zpracovávány v domácí laboratoři	ANO	ANO	NE&&&&&	ANO

NIPT population testing – T21, T18 and T13

- Van Den Bogaert K, et al., Genet Med. 2021 Jun;23(6):1137-1142.
- samples analyzed between 1 July 2017 and 30 June 2019
- N = 153,575
- 0.7% failure rate

Table 1. Performance of noninvasive prenatal screening (NIPS) as a first-tier screening test.

	Incidence	Sensitivity		Specificity		PPV		NPV	
	%	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Trisomy 21	0.32	98.91	97.24–99.58	99.98	99.97–99.99	92.39	89.34–94.61	100.00	99.99–100.00
Trisomy 18	0.07	97.47	91.23–99.30	99.99	99.98–99.99	84.62	75.82–90.61	100.00	100.00–100.00
Trisomy 13	0.06	100.00	90.36–100.00	99.97	99.96–99.98	43.90	33.67–54.68	100.00	100.00–100.00

CI confidence interval, *NPV* negative predictive value, *PPV* positive predictive value.

Trisomy test – T21, T18 and T13

- prospective study
- samples analyzed between 1 July 2016 and 30 June 2021
- N = 20,288
- 0.9% failure rate

Result	Trisomy 21	Trisomy 18	Trisomy 13	All
True positive	**218**	**48**	**25**	**291**
False positive	**2**	**1**	**4**	**7**
True negative	**18938**	**19109**	**19130**	**18859**
False negative	**1**	**1**	**0**	**2**
Sensitivity	**99.54%**	**97.96%**	**100.00%**	**99.32%**
Specificity	**99.99%**	**99.99%**	**99.98%**	**99.96%**
PPV	**99.09%**	**97.96%**	**86.21%**	**97.65%**
NPV	**99.99%**	**99.99%**	**100.00%**	**99.99%**

Metaanalysis results – sex chromosomes aneuploidies

- Bussolaro S, et al., Am J Obstet Gynecol MFM. 2023 Mar;5(3):100844
- N = ~1.5 M samples tested (Bussolaro et al., 2023)
- sensitivity and specificity for SCA were 94% and 99%
- pooled **PPV** for SCA was 49.4% with:

  - **32.0% for monosomy X**
  - **67.6% for XXY**
  - **57.5% for XXX**
  - **70.9% for XYY**

Trisomy test – monosomy X detection

- prospective study
- reported monosomy X detections (all types, maternal and fetal) = 75
    - no follow up: 25 (33%)
        - maternal mosaics: 14 (56%)
    - with follow up: 50 (67%)
        - **FP: 31 (62%)**
        - **TP: 19 (38%)**
        - **PPV: 38%**
            - mosaicism or complex mosaicism: 6 (31.6%)

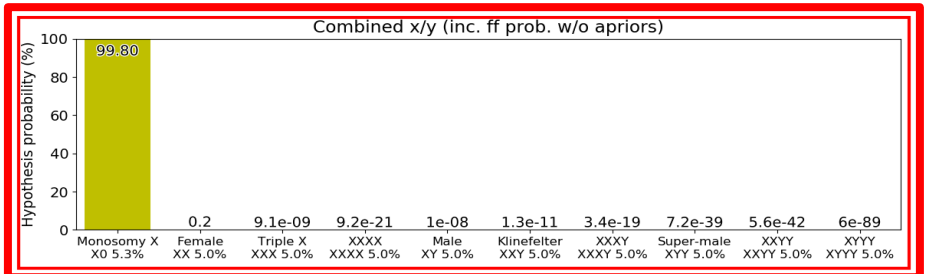
Trisomy test – monosomy X maternal mosaicism

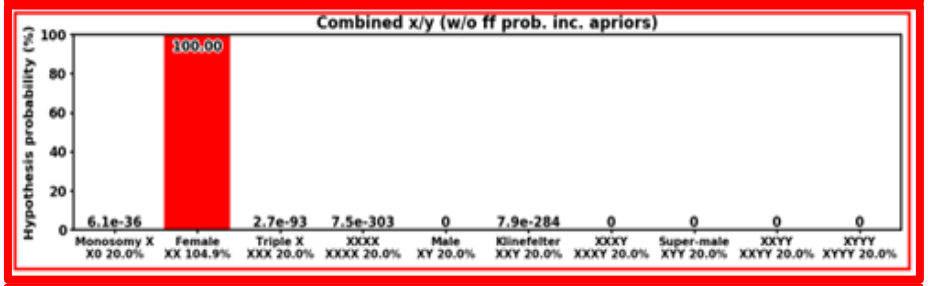
- samples analyzed from 9 September 2019 to 29 November 2021
- N = 5880
- 224 samples with chromosome X with different signal ratio than expected
- 3.8% of tested women are low level mosaics of monosomy X

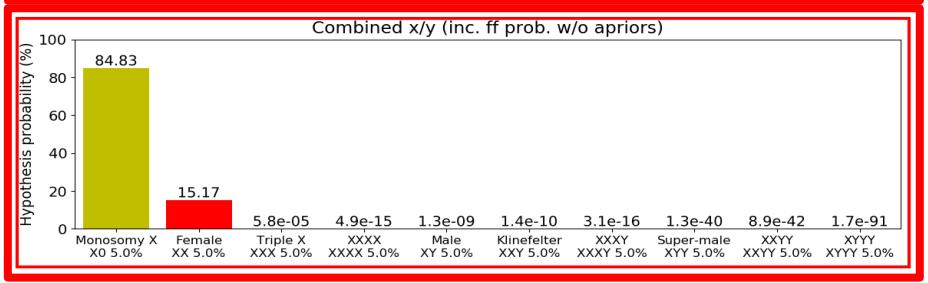
plasma in pregnancy

placenta

plasma after delivery

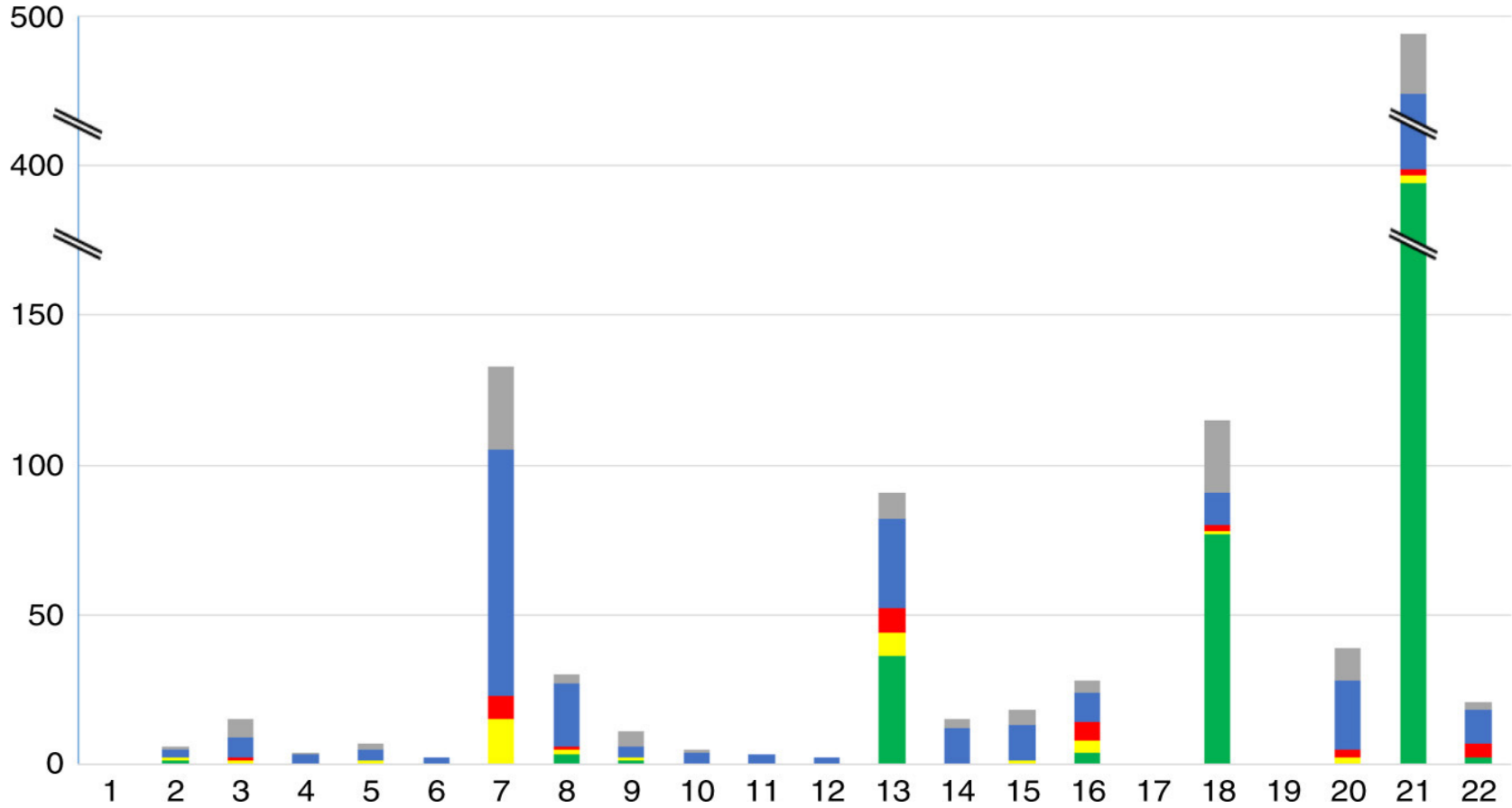






NIPT – rare autosomal trisomies

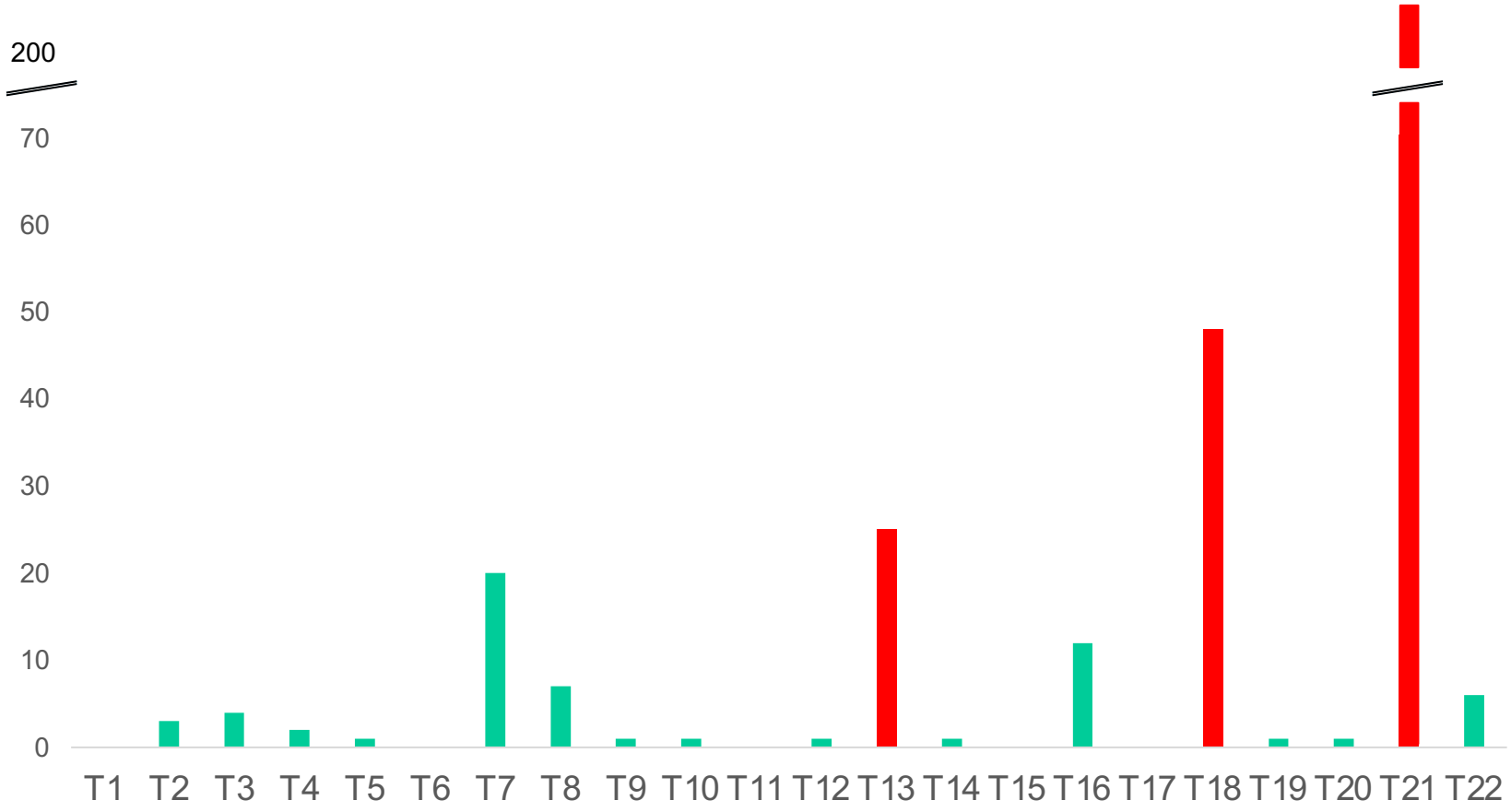
- Van Den Bogaert K, et al., Genet Med. 2021 Jun;23(6):1137-1142.



Incidence of rare autosomal trisomies. Reported autosomal trisomies subdivided by outcome of the follow-up: true positive (green), confined placental mosaicism (yellow), negative on both amniotic fluid and placenta biopsy (red), negative on amniotic fluid but no placenta biopsy available (blue), no invasive follow-up (gray).

Trisomy test – rare autosomal trisomies

- prospective study



NIPT – subchromosomal aberrations

- Xue H, et al. Sci Rep. 2022 Nov 17;12(1):19750.
- prospective study
- N = 31,256
- evaluation of subchromosomal aberations (CNVs)
    - **reported CNVs: 221 (0.7%)**
        - no follow up: 18 (8.1%)
        - with follow up: 203 (91.9%)
            - **FP: 125 (61.6%)**
            - **TP: 78 (38.4%)**
            - **PPVs were 75% for DiGeorge syndrome (DGS), 80% for 22q11.22 microduplication, 50% for Prader–Willi syndrome, and 50% for cri-du-chat, other aberrations combined PPVs were 46.5% (CNVs > 10 Mb) and 28.57% (CNVs ≤ 10 Mb)**

Trisomy test – subchromosomal aberrations

- prospective study
- N = 6402
- evaluation of subchromosomal aberations (CNVs) from 3 Mb
    - reported CNV detection (all types, maternal and fetal): 48 (0.8%)
        - **reported fetal CNVs: 30 (0.5%)**
            - no follow up: 6 (20%)
            - with follow up: 24 (80%)
                - **FP: 6 (25%)**
                - **TP: 18 (75%)**
                - **combined PPV: 75%**

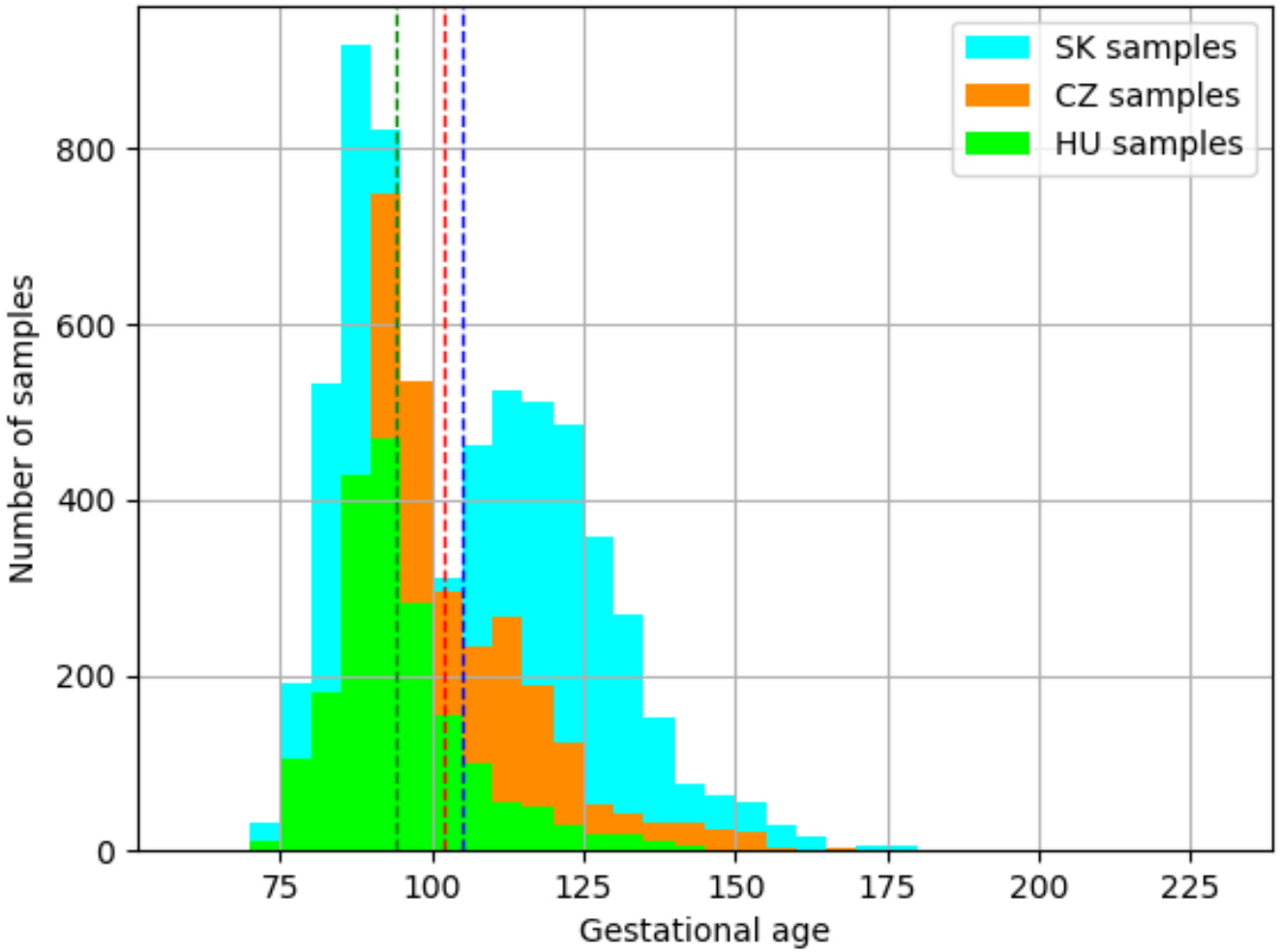
Trisomy test – country specific sample cohorts

- **Slovakia**
  - >>> physicians over the country
  - 2 types of blood collection tubes – >>EDTA, <<Streck
  - 2 central collection points
  - 1 central laboratory

- **Czech republic**
  - >> physicians over the country + 2 local prenatal care clinics (*only 1 included in statistics)
  - 2 types of blood collection tubes – EDTA, Streck
  - 1 central collection point + 2 local collection points
  - 1 central laboratory + 2 local laboratories (*only 1 included in statistics)

- **Hungary**
  - >> physicians over the country
  - 1 type of blood collection tubes – Streck
  - 1 central collection point with plasma separation
  - transported and processed in central laboratory in Slovakia

Trisomy test – gestational age between countries



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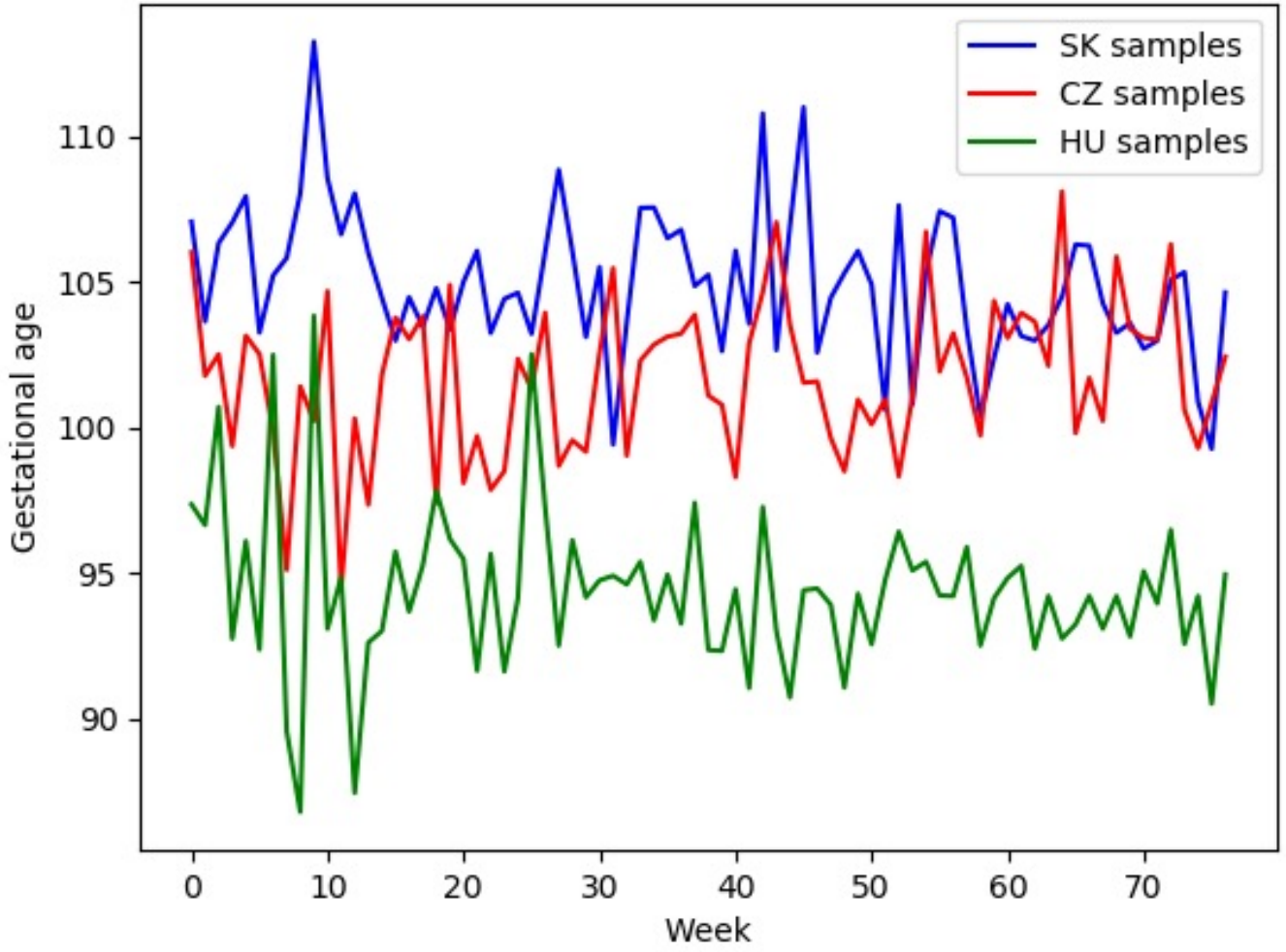
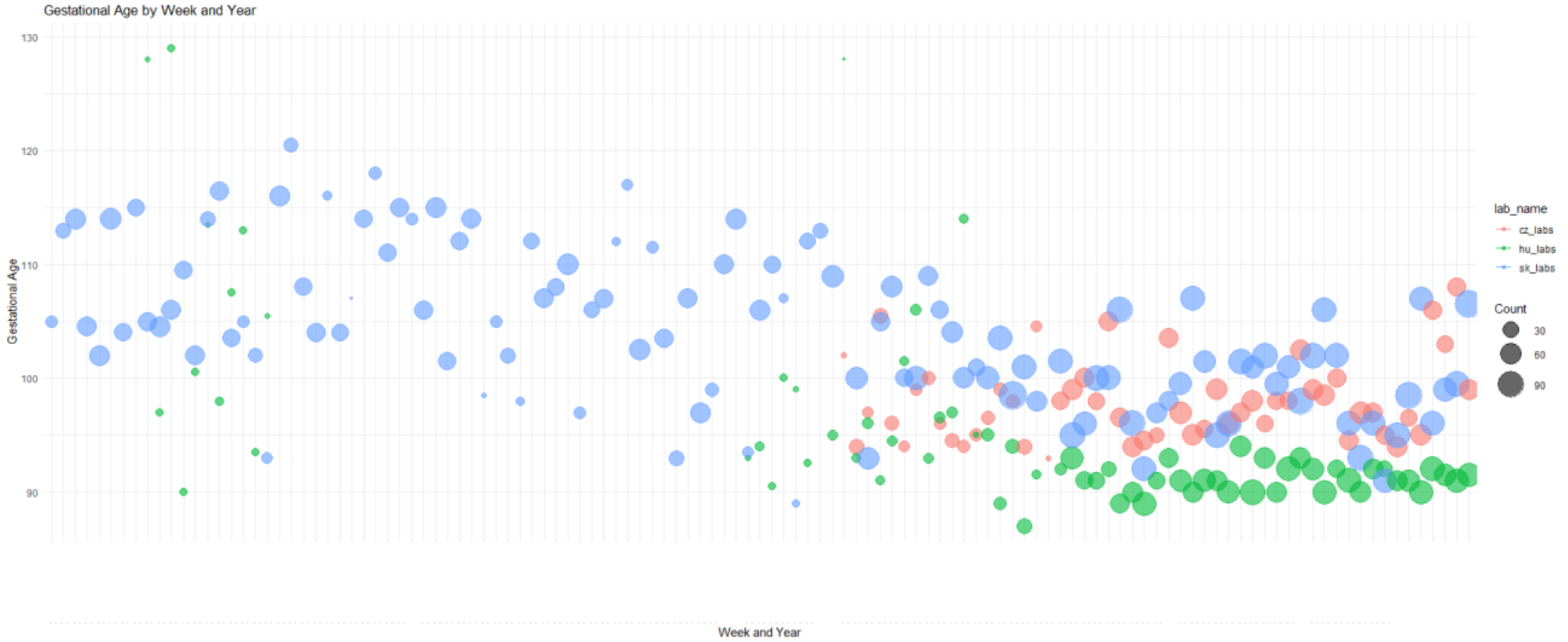
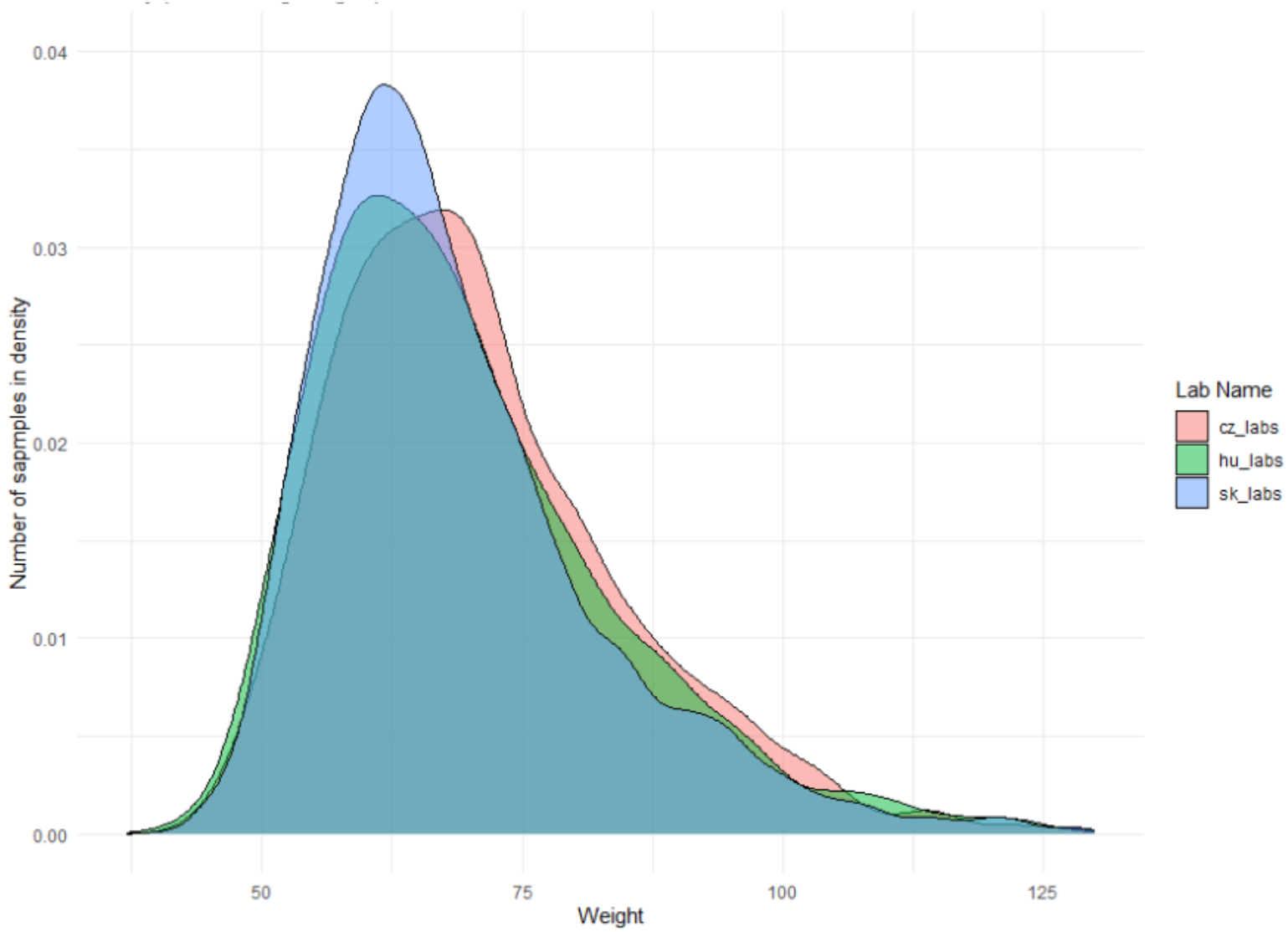
Trisomy test – gestational age between countries



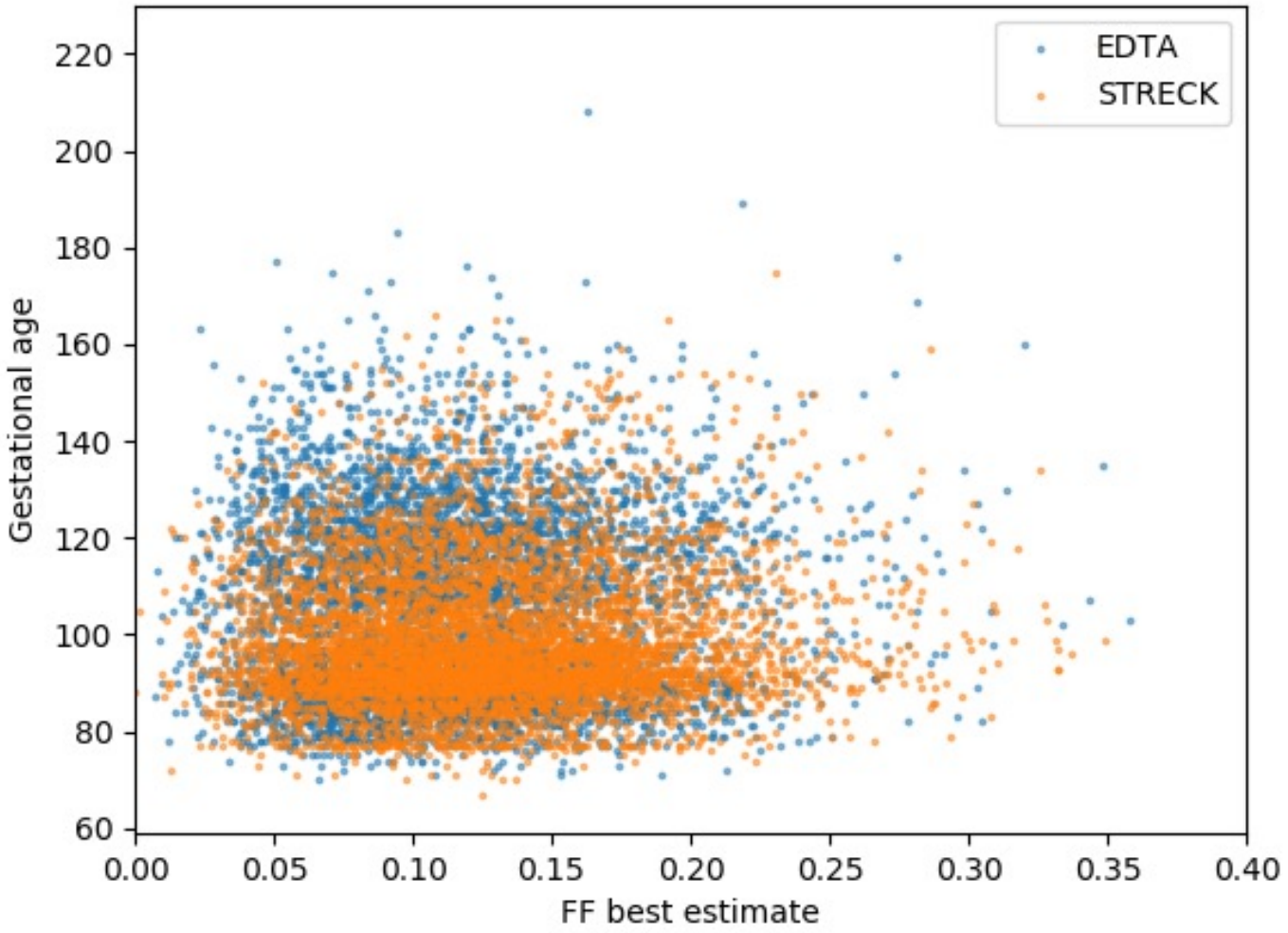
Trisomy test – gestational age



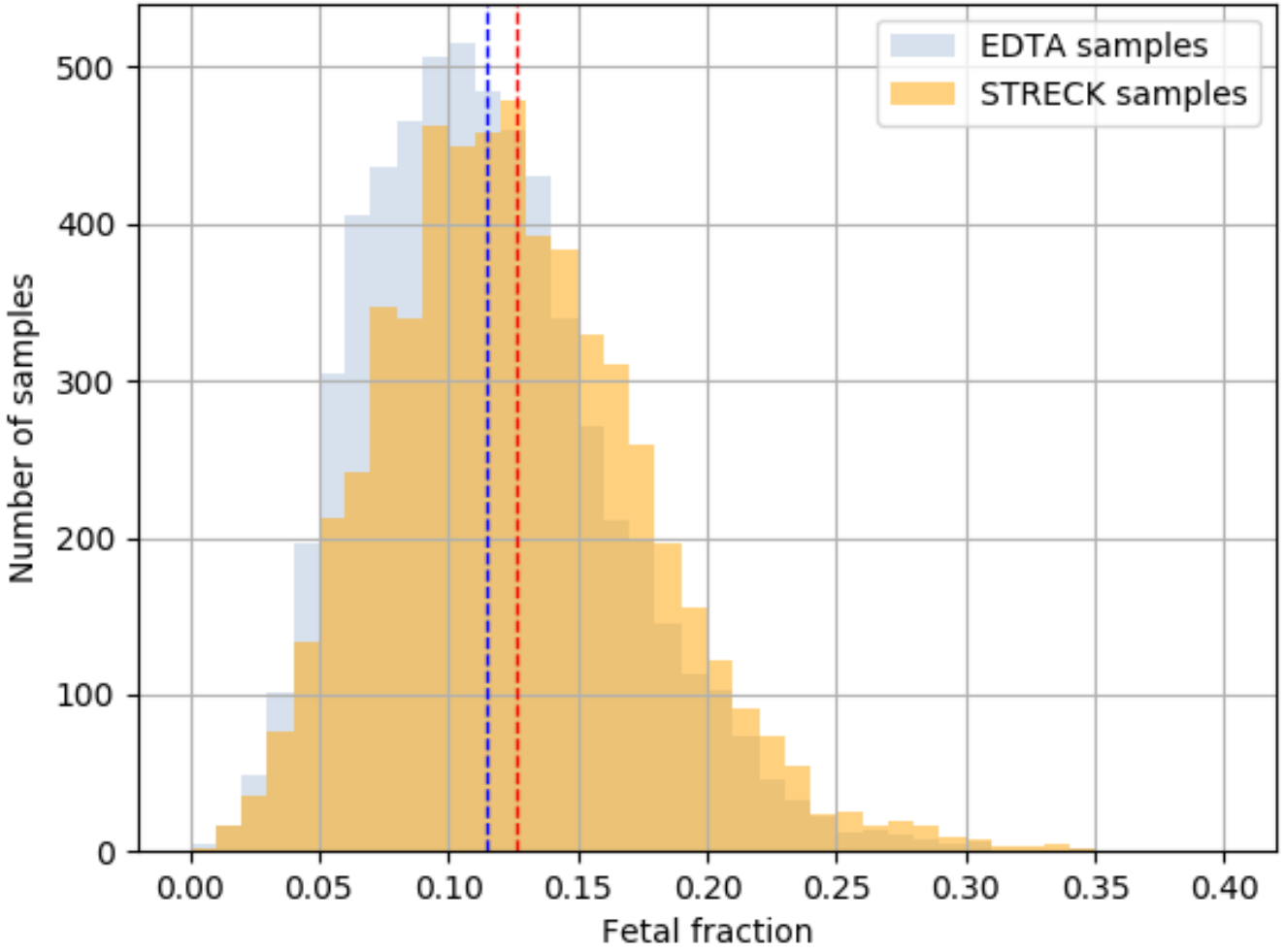
Trisomy test – weight of pregnant women



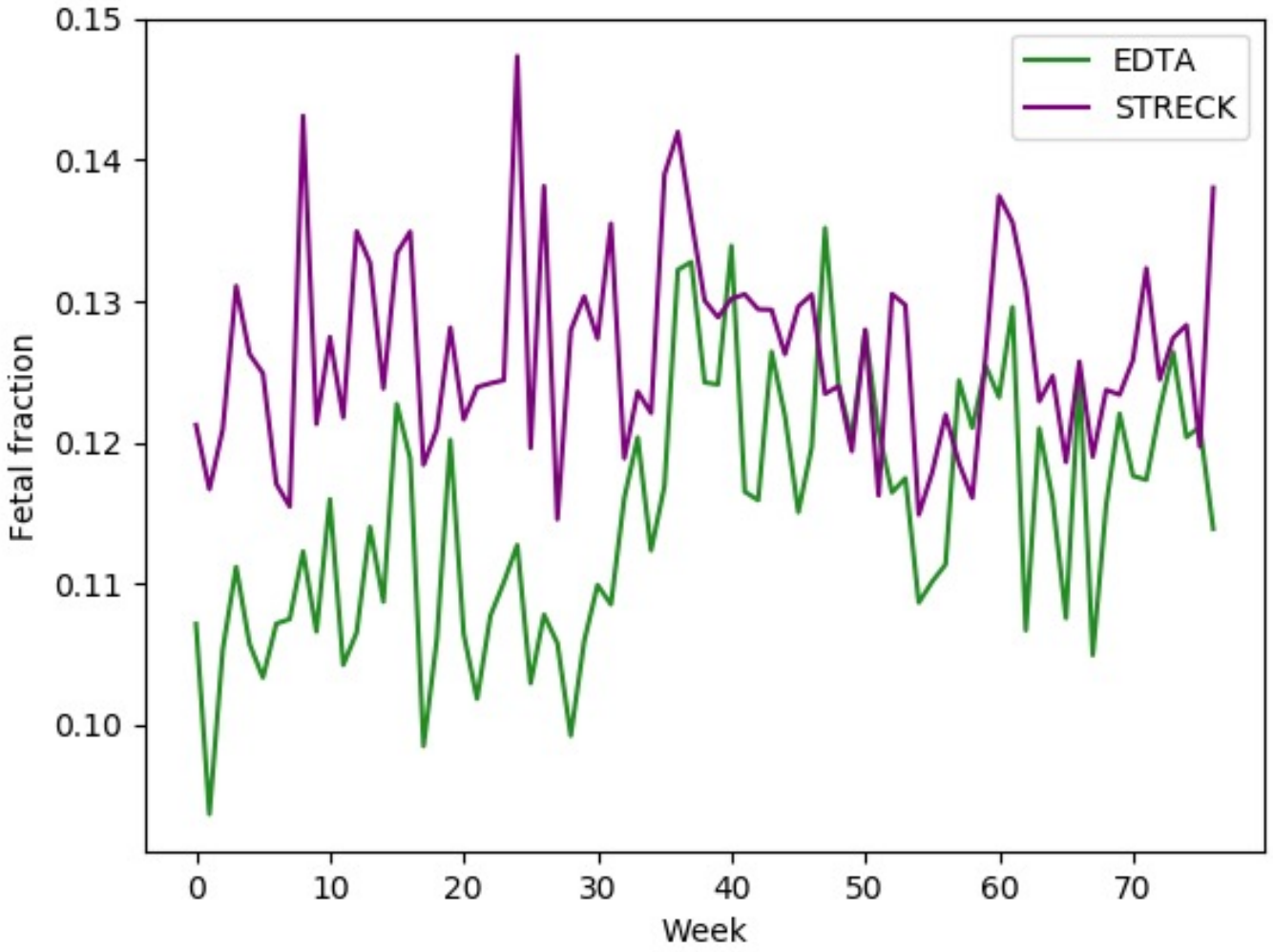
Trisomy test – gestational age and tube types



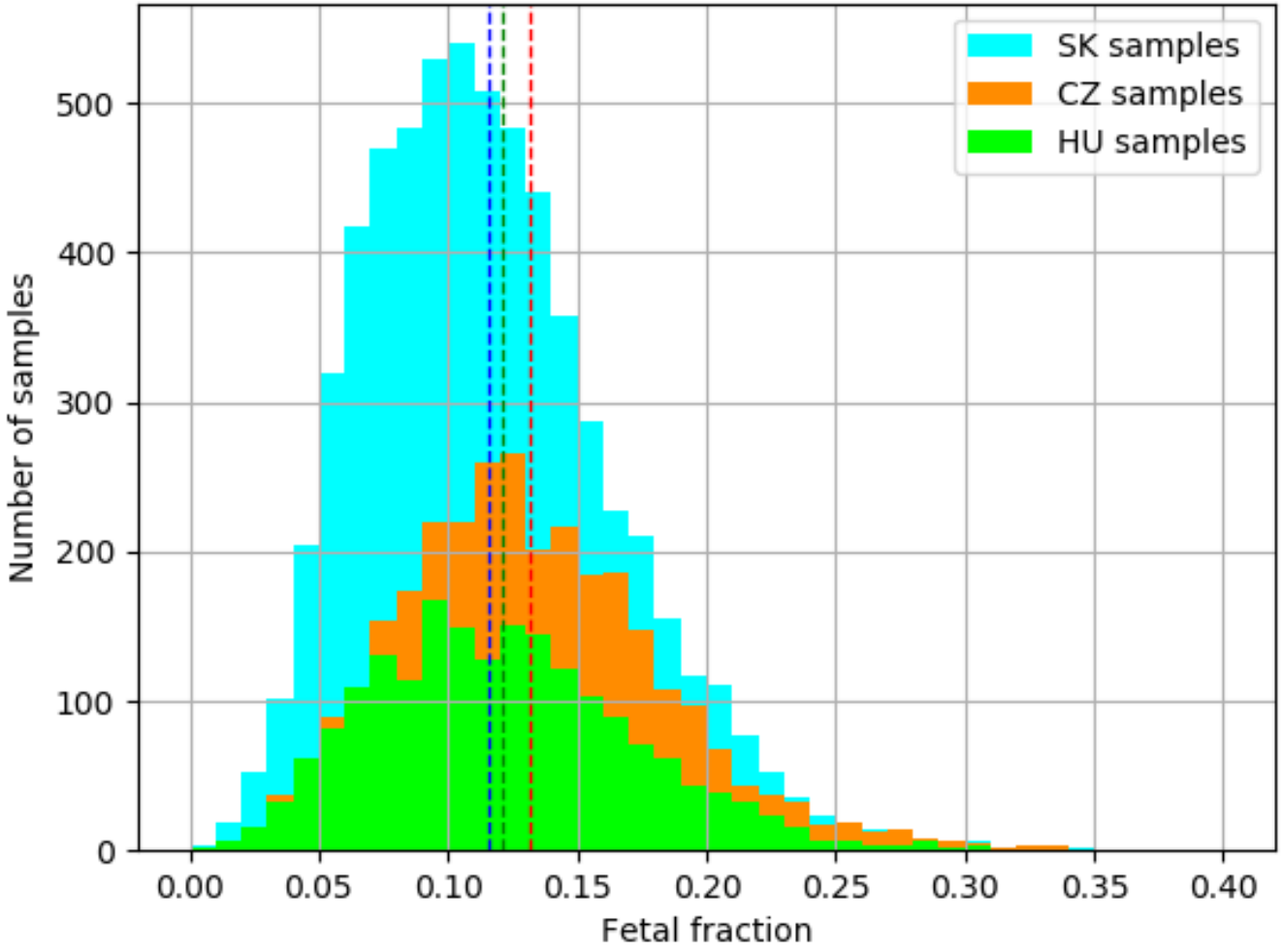
Trisomy test – fetal fraction and tube types



Trisomy test – fetal fraction and tube types

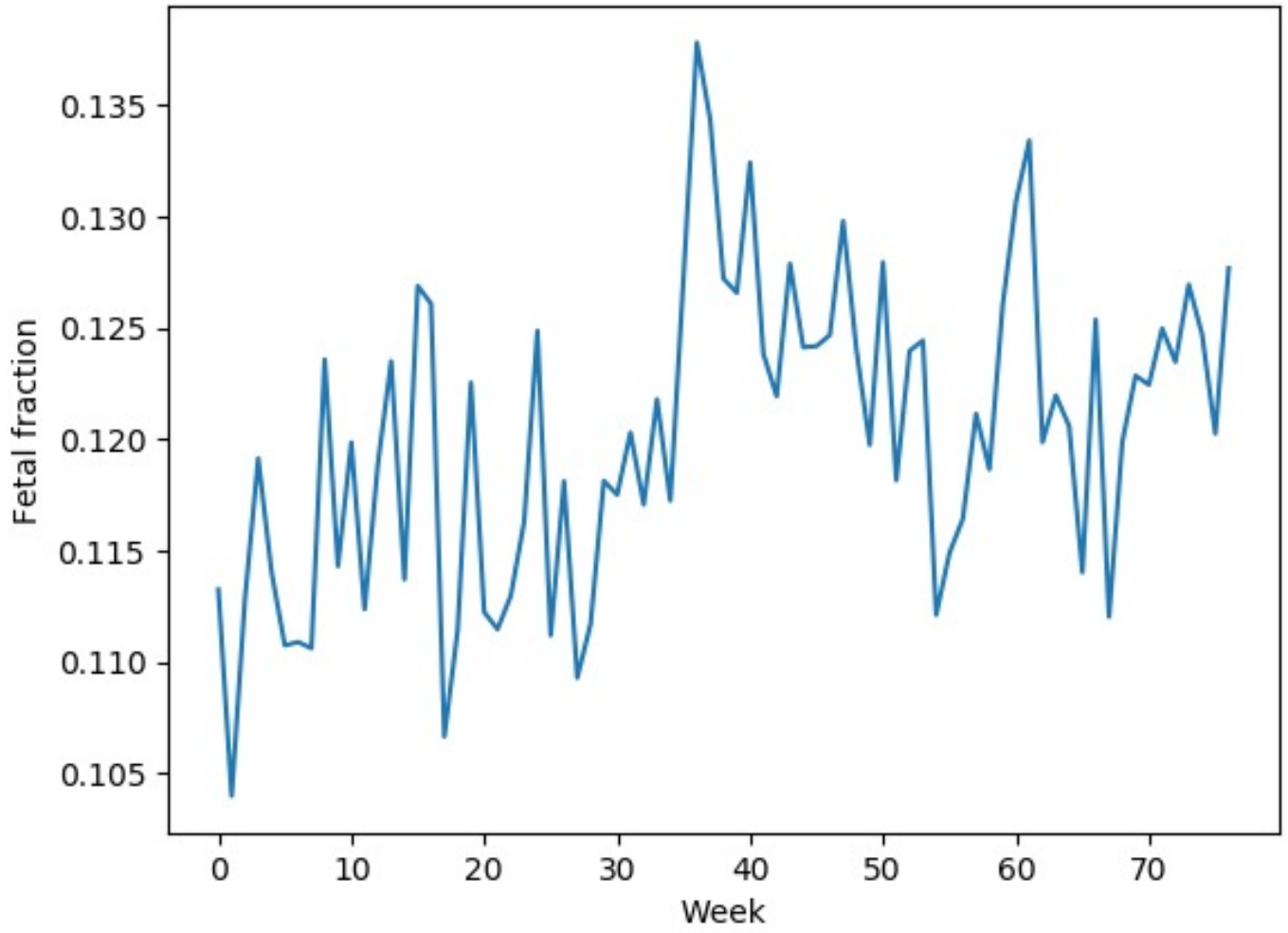


Trisomy test – fetal fractions all samples

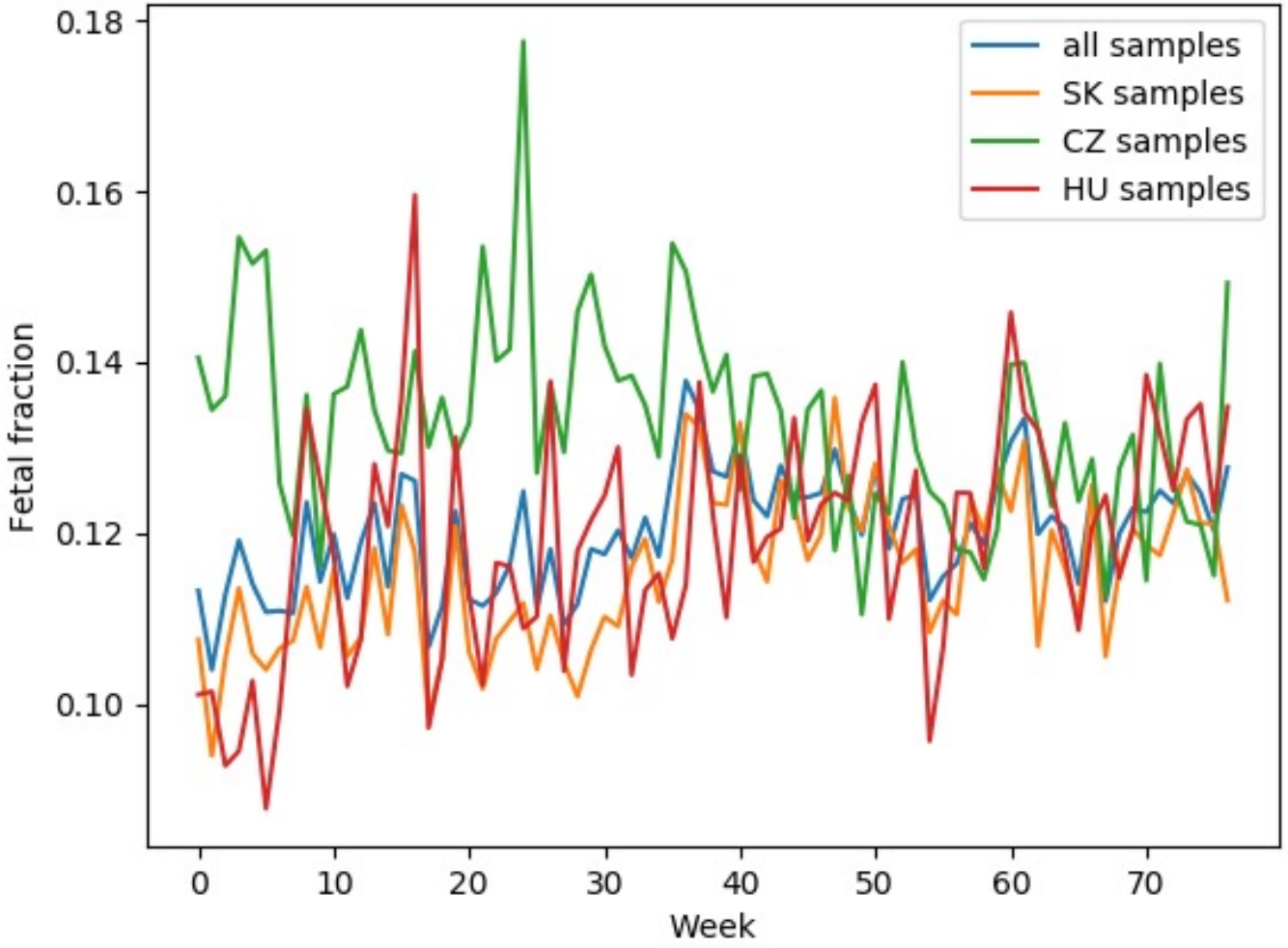


SK samples = 11.8%
HU samples = 12.3%
CZ1 samples = 14.5%
CZ2 samples = 12.1%

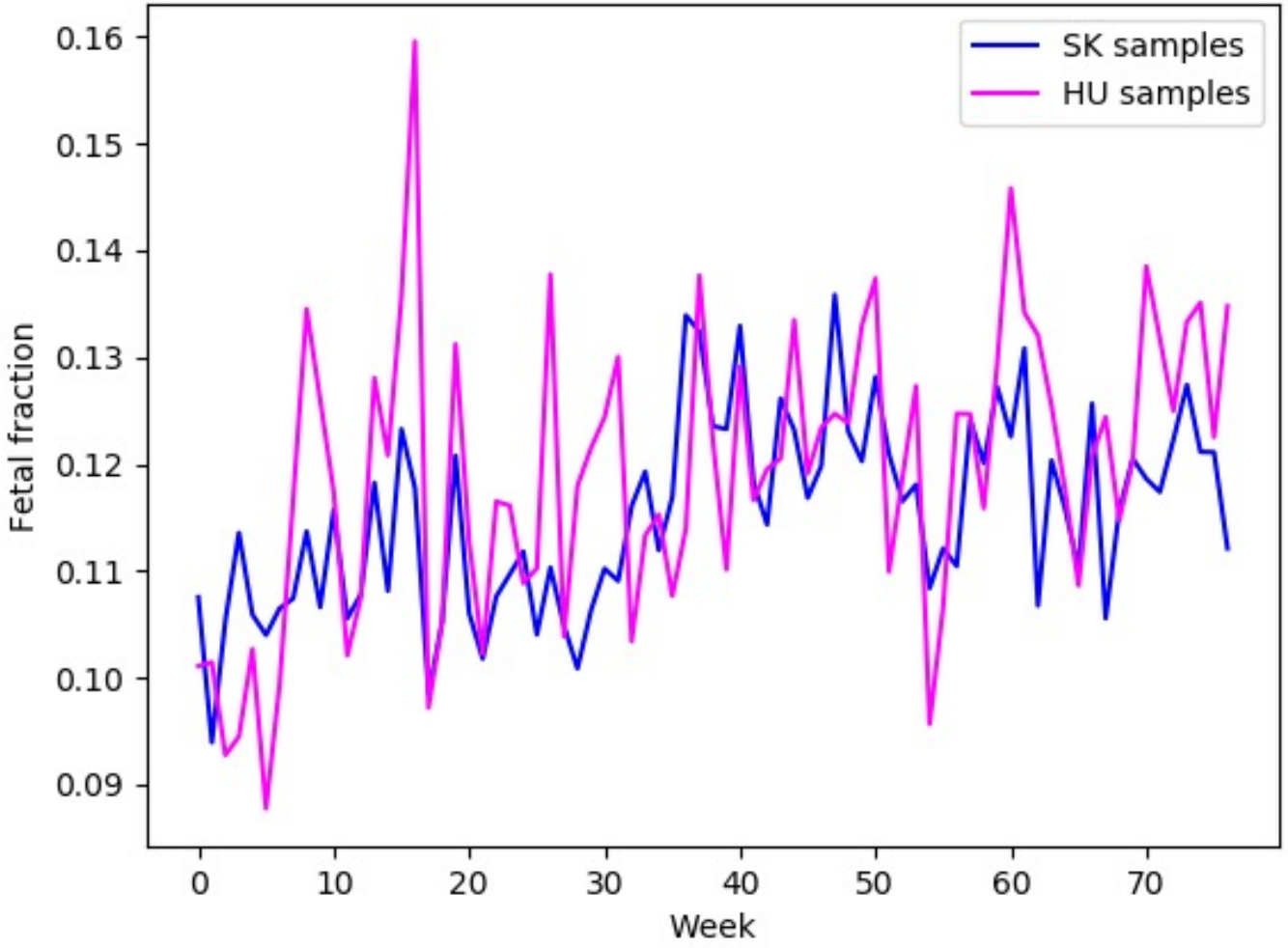
Trisomy test – fetal fraction in all samples



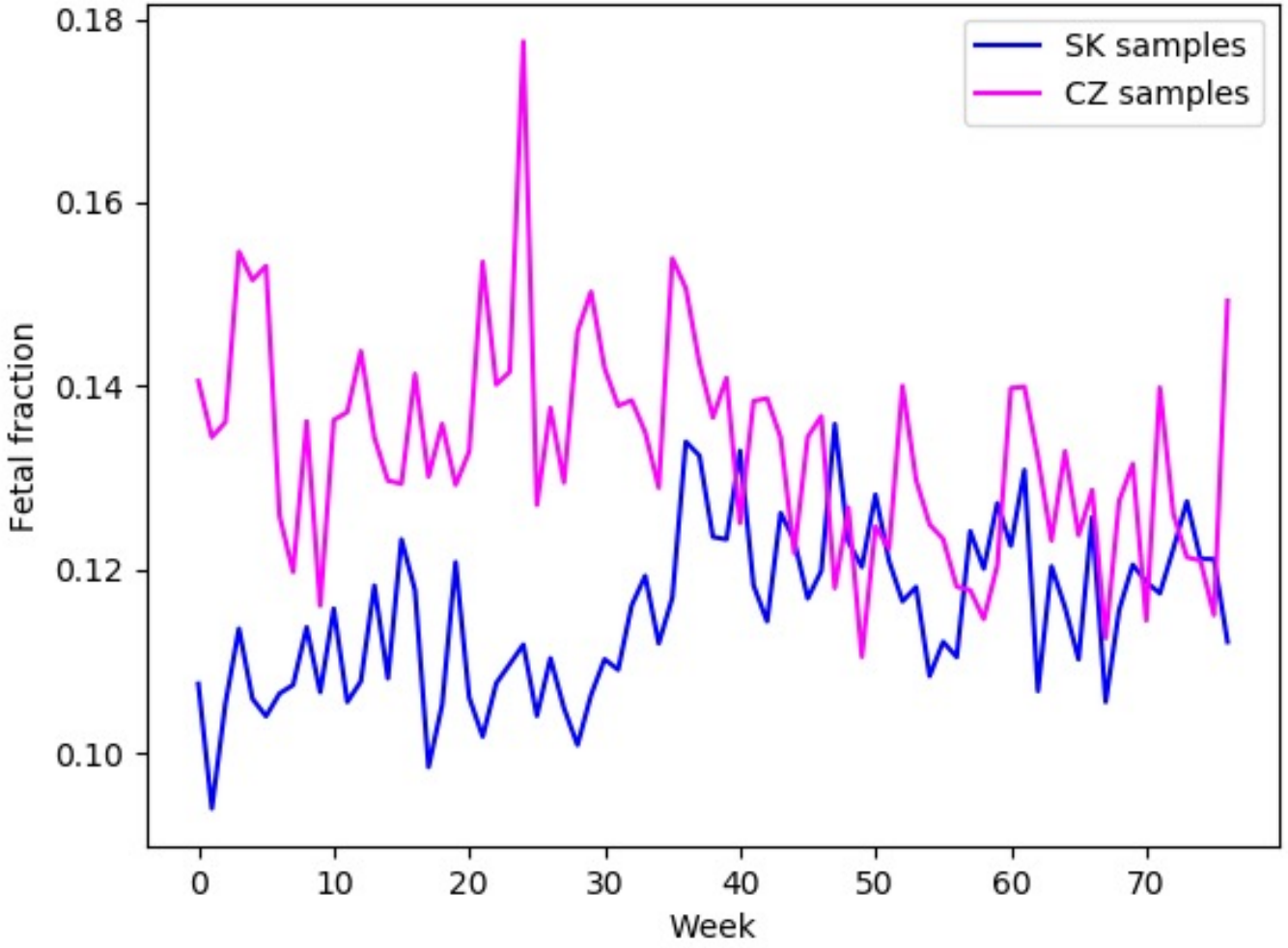
Trisomy test – fetal fraction in all laboratories



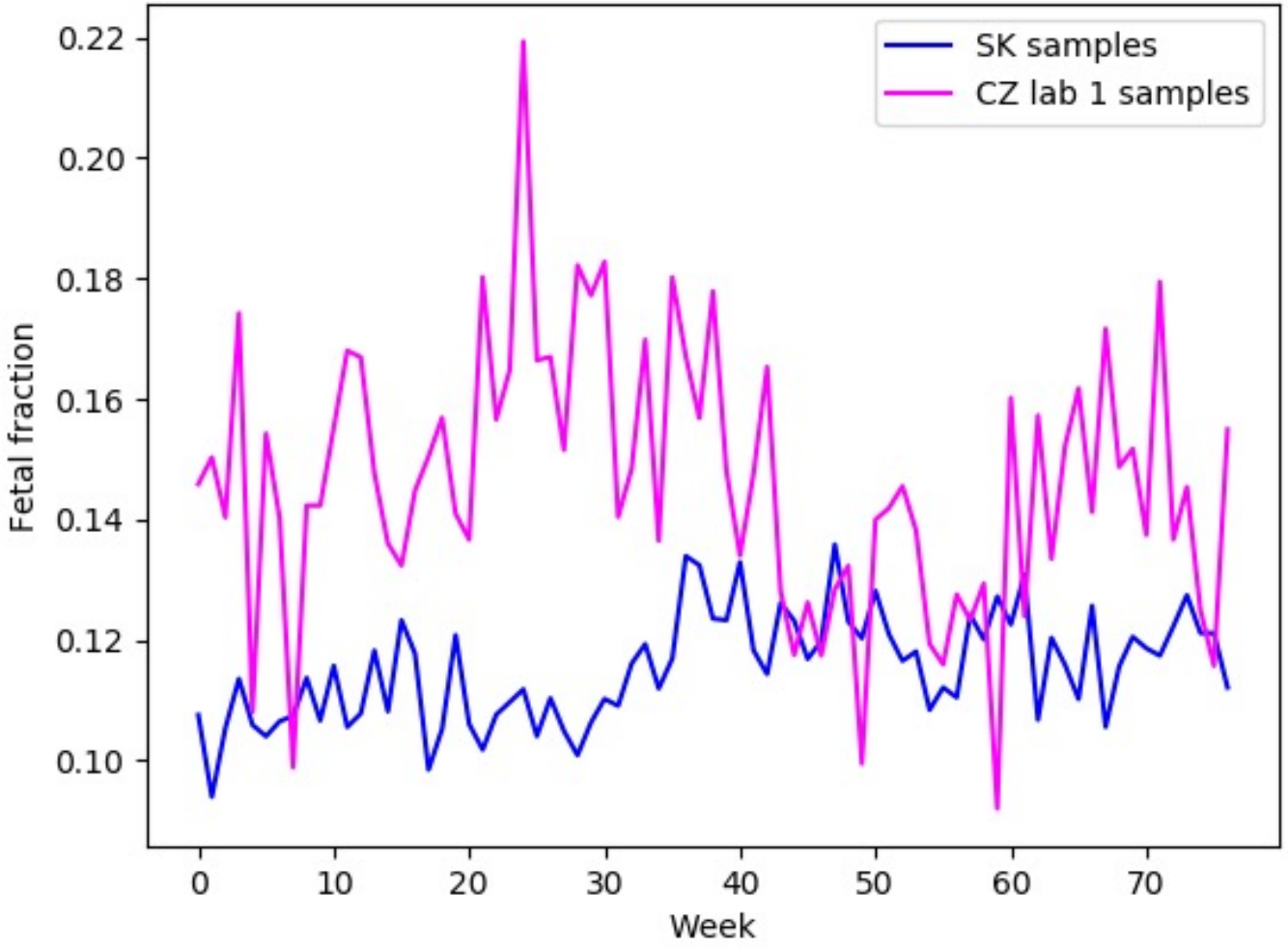
Trisomy test – fetal fraction in laboratories – SK & HU



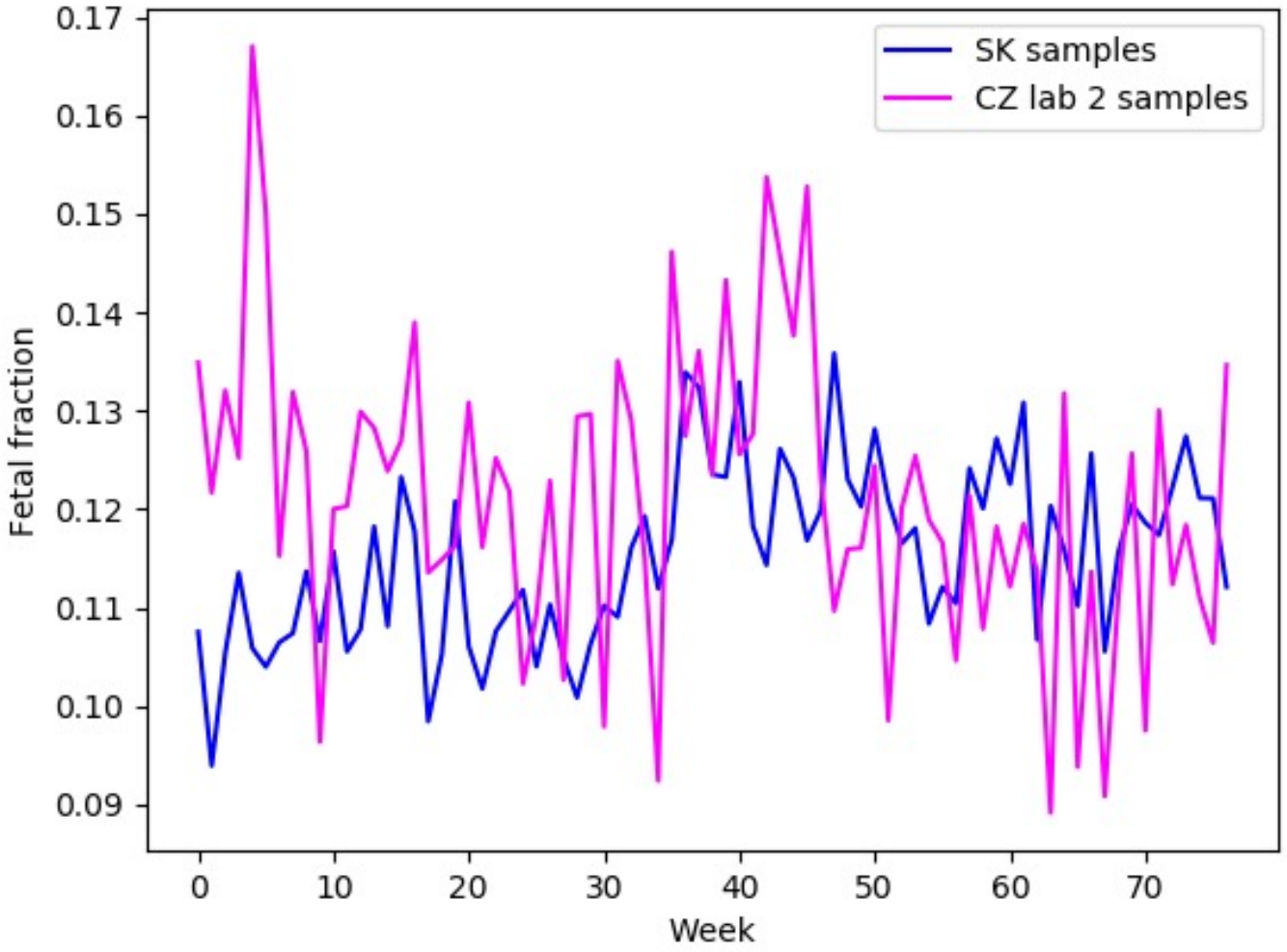
Trisomy test – fetal fraction in laboratories – SK & CZ



Trisomy test – fetal fraction in laboratories – SK & CZ1



Trisomy test – fetal fraction in laboratories – SK & CZ2



NIPT – false, incorrect and uninformative result source

- Low fetal fraction (e.g. high weight of pregnant women) – UNINFORMATIVE
- Maternal aberration – UNINFORMATIVE & FALSE POSITIVES
- Maternal mosaicism (monosomy X) – UNINFORMATIVE & FALSE POSITIVES
- Confined placental mosaicism (RATs) – FALSE POSITIVES
- True fetal mosaicism – FALSE NEGATIVES
- Cancer – UNINFORMATIVE & FALSE POSITIVES
- Vanishing twin syndrome – FALSE POSITIVES
- Blood transfusion – INCORRECT RESULT
- Organ transplantation – INCORRECT RESULT

Summary

- There are many NIPT characteristics that should be in focus when different test types are compared and the selected form and content of comparison should be professionally correct and fair;
- Continual statistical evaluation can detect systematic changes or specific characteristics in preanalytical and analytical phase of the lab processes;
- Experience and routinization of sample collection and processing plays important role in overall test performance;
- Country specificities in organization of prenatal screening can have significant impact on NIPT performance;
- Centralized sample collection and local sample processing is an advantage and improves quantitative characteristics of the analyzed samples;
- For significant proportion of false, incorrect or uninformative results biological reasons are responsible and additional comprehensive testing is needed for their correct evaluation.

Thank you for attention !

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