The American Approach to Prenatal Microarrays

Born in the USA

Main influences on Prenatal Microarray (CMA) Testing

- Primary Influences
  - ACOG recommendations, 2007, 2013
  - Wapner NEJM paper, 2012
  - ACMG recommendations for NIPT, 2013
- Secondary Influences
  - Insurance
  - Improvement in public databases and software analysis tools
  - Laboratories experience with postnatal
    - Private databases of local populations

ACOG Recommendations: 2007

- Early amniocentesis (<15wks) should not be performed
- Amniocentesis and CVS safe (0.33-0.2% loss rate)
- Offer invasive testing if:
  - Previous fetus or child with an autosomal trisomy or sex chromosome abnormality,
  - Current pregnancy with one major or at least two minor fetal structural defects identified by ultrasonography,
  - Either parent with a chromosomal translocation or chromosomal inversion, or parental aneuploidy.
- Make available to all women to rule out aneuploidy, irrespective of *a priori* risk
- CMA not ready for prime time – G-banding remains gold standard

Obstet Gynecol. 2007; 110 (6):1459-67

Wapner *et al.* 2012: Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

- Clinically significant CNV Detection rates
  - In presence of fetal anomalies, +6% DR
  - In otherwise normal pregnancies, +1.7%
- VUS detection (all microarray)
  - 3.4% total
  - 1.8% Likely benign
  - 1.6% Likely pathogenic
- Misses (predictably)
  - Triploidies
  - Balanced rearrangements

N Engl J Med. 2012;367:2175-84

ACOG Recommendations: 2013

- Use CMA
  - For fetuses with abnormal ultrasound findings
  - For women of any age, because the anomalies detected do not correlate to maternal age; but standard karyotype OK for otherwise normal pregnancy.
  - To analyze genetic material in cases of fetal demise or stillbirth.
    - Not to evaluate first- and second-trimester pregnancy loss.
  - Require pretest and post-test genetic counseling
  - Informed consent
    - Documented
      - Must include discussion of findings of uncertain significance, consanguinity, non-paternity, and adult-onset disease.

Obstet Gynecol. 2012; 122 (6):1374-77

ACMG Policy Statement for NIPS (NIPT): 2013

- Generally, ACMG statement is guarded regarding the use of NIPT
  - 50% of cytogenetic abnormalities detectable by amniocentesis or CVS will not be detected if only 13, 18 and 21 are screened
  - In the presence of fetal anomalies, invasive testing with CMA may be the better testing option
  - NIPT positive results must be confirmed by invasive testing
  - Recommendation for registry of PPV and NPV for clinically relevant metrics

Genet Med. 2013:15(5):395–398

NIPT: Promises and Pitfalls

- Clinical utility of NIPT in the era of Prenatal CMA
  - Suited to pregnancies at increased risk for common aneuploidies based upon biochemical markers
    - Leads to more acceptance by patients
  - Fetuses with structural anomalies
    - If NIPT is normal, what is the post-NIPT residual risk for a chromosome abnormality that would be detectable by IT- CMA?
  - If NIPT is abnormal but not confirmed by IT- QF-PCR or karyotyping, where does CMA fit in?

Secondary Influences

- Public Databases
  - CNV databases: ISCA, DGV – curation is improving on an ongoing basis
- Software
  - Array platforms come with vastly improved client databases and analysis tools
- Expanded knowledge base – Postnatal array labs with Private Databases
  - Thousands of CNVs detected, categorized privately
    - Rare, recurrent, benign variants for local population, and platform specific/design associated variation

Secondary Influences

- Availability of Medical Insurance
  - Not universal, despite 2013 practice guidelines from ACOG
  - United Healthcare considers CMA medically necessary for women undergoing invasive testing
    - Effective June 1, 2014
  - Capital Blue considers prenatal CMA still investigational
    - Effective date June 1, 2014

US Platforms and Reporting Practices

- SNP or Oligo/SNP hybrid platforms
  - SNP data is primarily intended for detection of UPD in imprinted chromosomes
  - Otherwise, minimum reportable AOH size is 15-25Mb and minimum reportable IBD is 4%
- Functional resolution is similar irrespective of platform used:~50Kb
  - Reportable VUS size is the same between platforms
    - 1-1.5Mb loss
    - 1-2Mb gain
- Karyotyping is usually an 'extra'

Summary

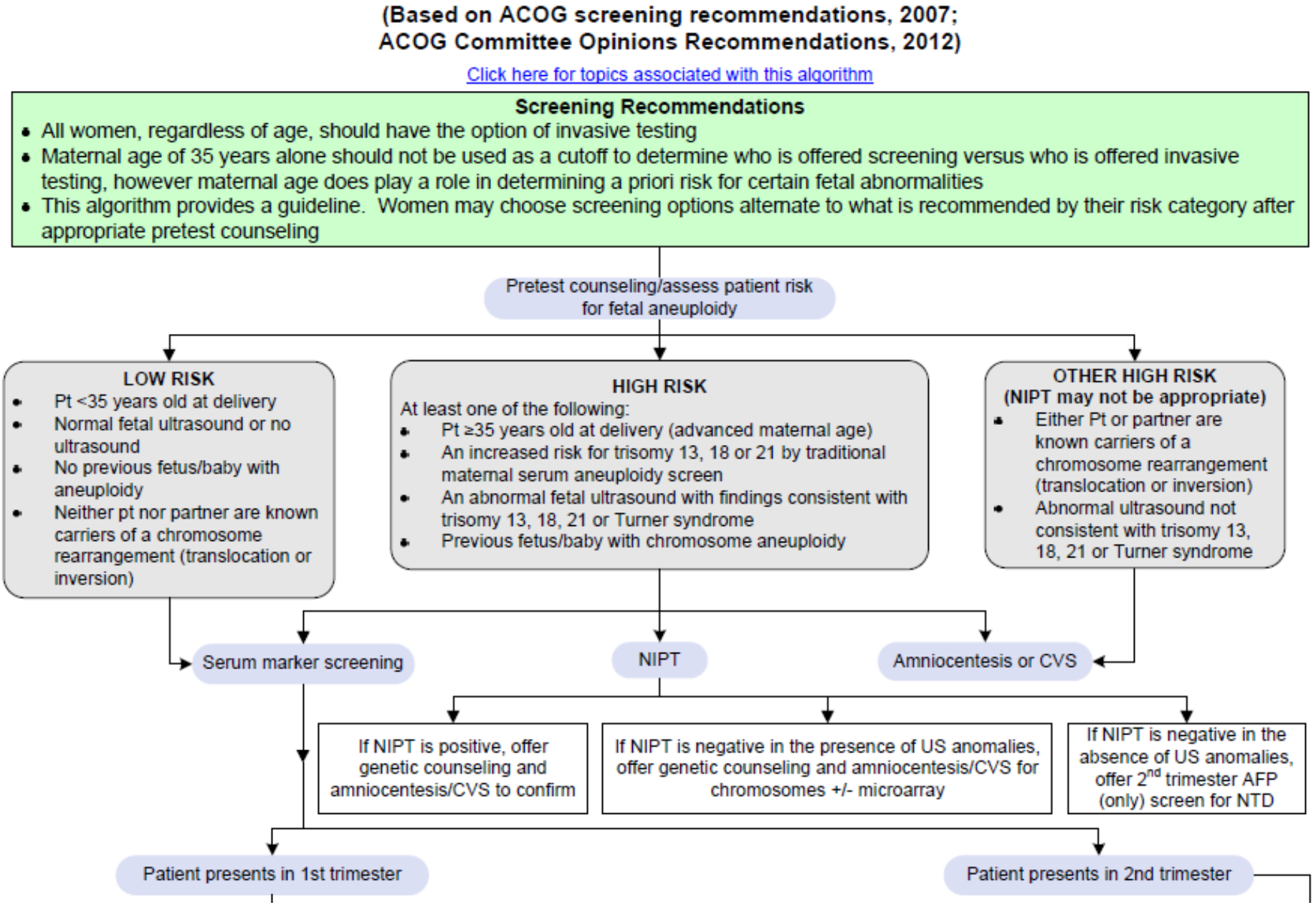
- CMA with invasive testing has become a standard of care in the USA, BUT
  - Private insurance is inconsistent
- Reporting standards are similar, irrespective of platform used
  - ISCA gene targets plus backbone
  - SNP or Oligo + SNP hybrid
  - Avoidance of reporting VUS <1Mb in size
  - Informed consent is required
- NIPT
  - Recommended for aneuploidy screening
  - Not to replace CMA invasive testing when ultrasound anomalies are present

Appendices

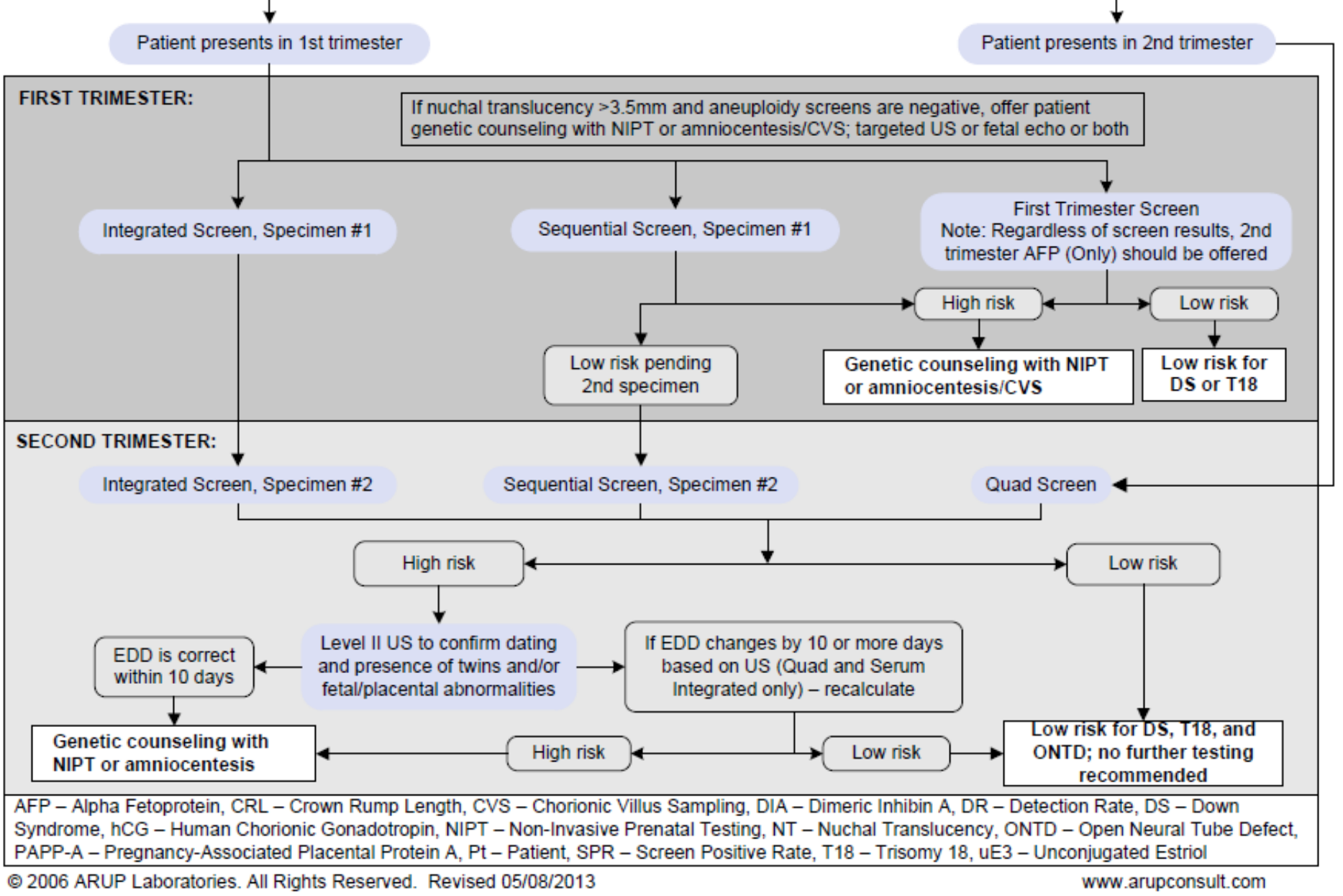
1. Lab platform comparisons
2. Integrated algorithm (from screen to invasive testing) from ARUP National Reference Laboratory
3. NIPT versus Invasive testing comparison

Comparisons	Labcorp	Baylor	Gene Dx	ARUP
Platform	2.6million/SNP	180K Oligo/SNP Combo	180K Oligo/SNP Combo (also a low res alternative)	2.6million/SNP
Minimal Targets	ISCA +	ISCA+	ISCA+	ISCA+
Test requirements	20cc fluid or 20mg villi or 3xT25+4slides	20-25cc fluid or 30-35mg villi	20cc fluid or 2xT25 cultured cells (AF or CVS)	15-20cc fluid or 10-15mg villi or 2xT25 flasks ,
VUS – deletions	>1Mb	>1Mb	1.5Mb	>1Mb
VUS -Duplications	>2Mb	>1Mb	1.5Mb	>2Mb
Claiming to report	50Kb	No info	500bp-100Kb	50Kb
UPD/Consanguinity	Yes – no additional info available	UPD of imprinted chromosomes only	>4% of genome or >25Mb within a chromosome	>10% of genome or >15Mb within a chromosomes
Susceptibility genes	Yes – if clear phenotype known	No info	No info	No info
Karyotyping	Choice – extra	Always	Choice - extra	Choice – extra

Appendix 1.



Appendix 2. Algorithm from ARUP labs



Appendix 2. Algorithm from ARUP labs

NIPT *versus* Invasive Testing (IT)

BEFORE NIPT (2011)

- 638 screen positive patients
  - 47.2% underwent IT
  - 52.8% declined further testing

WITH NIPT (2012-2013)

- 398 screen positive patients
  - 39.2% underwent IT
  - 39.4% had NIPT
  - 21.1% declined further testing

Net result of introduction of NIPT: More follow-up to screen positives but less invasive testing

Appendix 3. Prenat Diagn. 2013 Jun;33(6):542-6.