Prenatal Screening for Open Neural Tube Defects and Aneuploidies

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Disclosures

- None

Learning Objectives

1) Understand the diseases detected by prenatal screening
2) Identify the analytes, measurements, and methodology used in prenatal screening
3) Interpret the results using multiple of the median and risk analysis

Outline

- Diseases detected by prenatal screening
- Screening tests, analytes, and methodology
- Interpretation of results
- Confirmation tests for positive screens

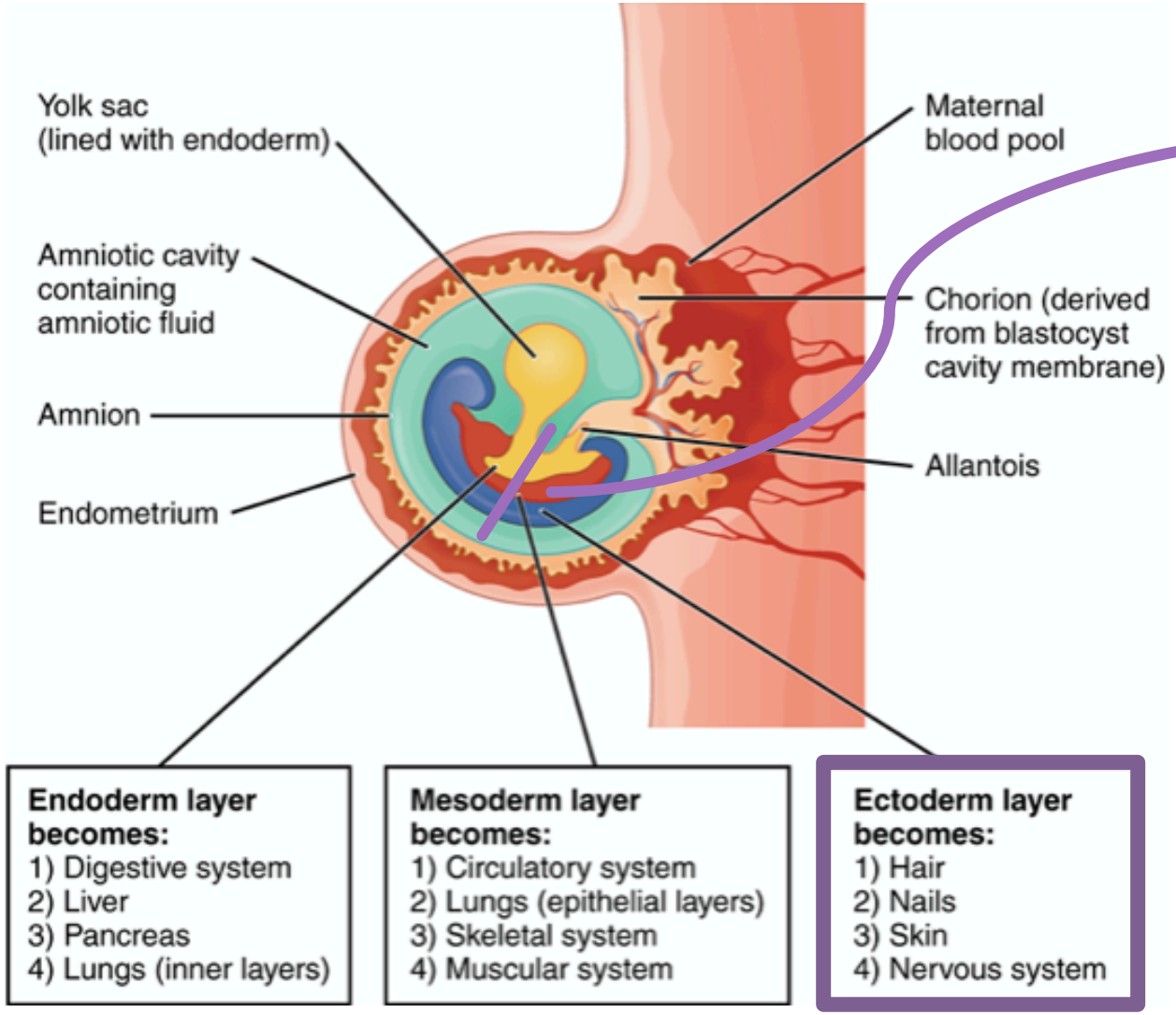
Diseases Detected by Prenatal Screen

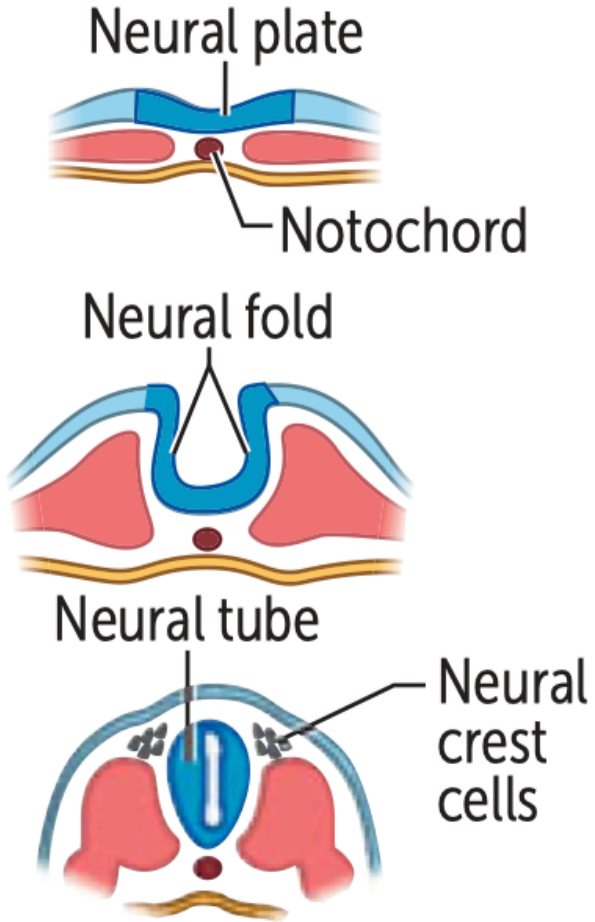
Diseases Detected by Prenatal Screen

- Open neural tube defects (ONTD)
- Trisomy 21 (Down Syndrome)
- Trisomy 18 (Edwards Syndrome)
- Trisomy 13 (Patau Syndrome)

Open Neural Tube Defects

Neural Tube Development





3rd - 4th week gestation

https://opentextbc.ca/anatomyandphysiology/chapter/28-2-embryonic-development/

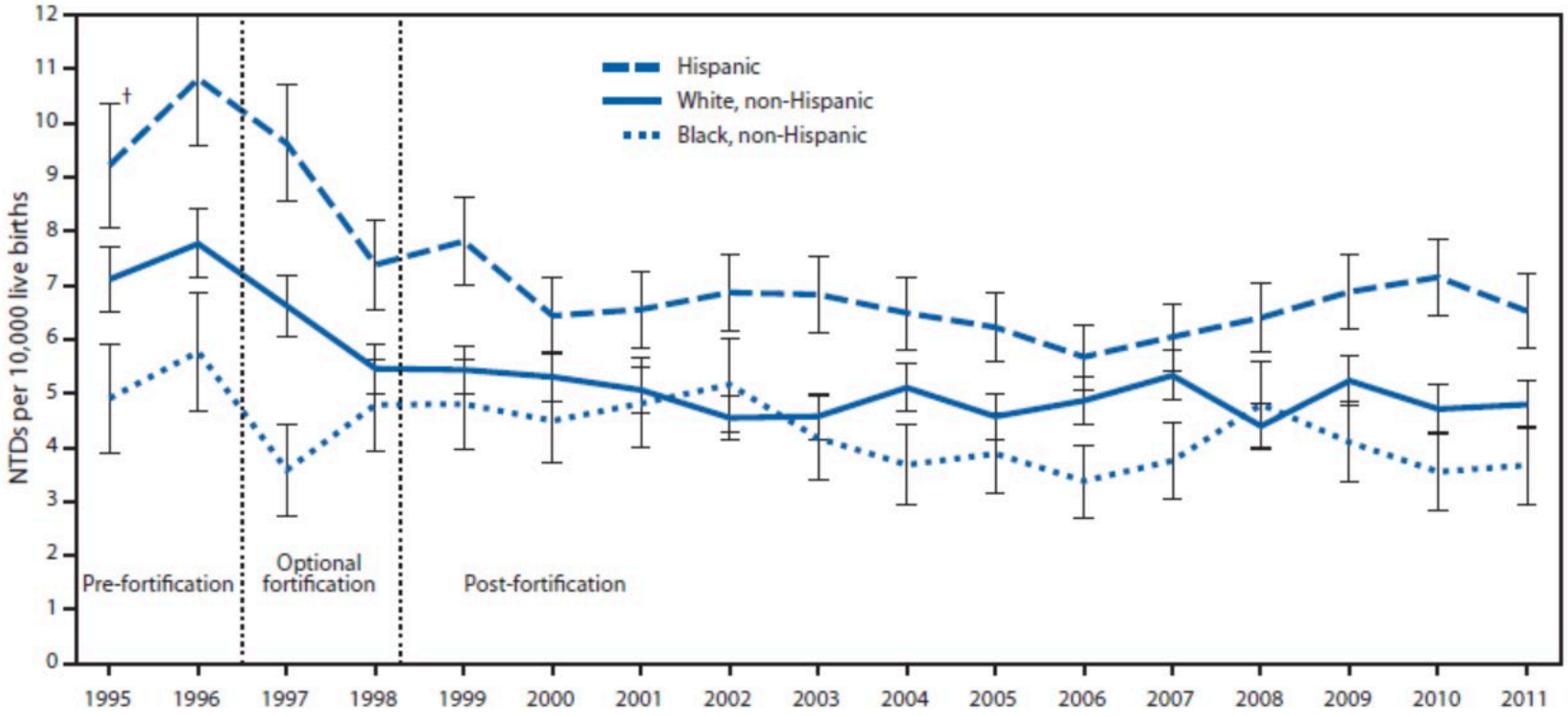
First Aid

Open Neural Tube Defects

- Pathogenesis: Failure of neural tube to close
- More superior = more severe
- Open = Defect exposed or covered by membrane
- Closed = Defect covered by skin

Open Neural Tube Defects

- Incidence: 5.5 per 10,000 births
- Risk factors
  - Folate deficiency
  - Folate antagonists
  - Diabetes
  - Obesity



Williams, Dukhovny, Clarke

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6401a2.htm

https://www.semanticscholar.org/paper/Prevention-of-neural-tube-defects-(NTDs)-in-ethnic-Peake/ccfb8bf874316696951a1ab35156cb1f629291f4/figure/0

Superior ONTD

Anencephaly
Open brain and lack of skull vault

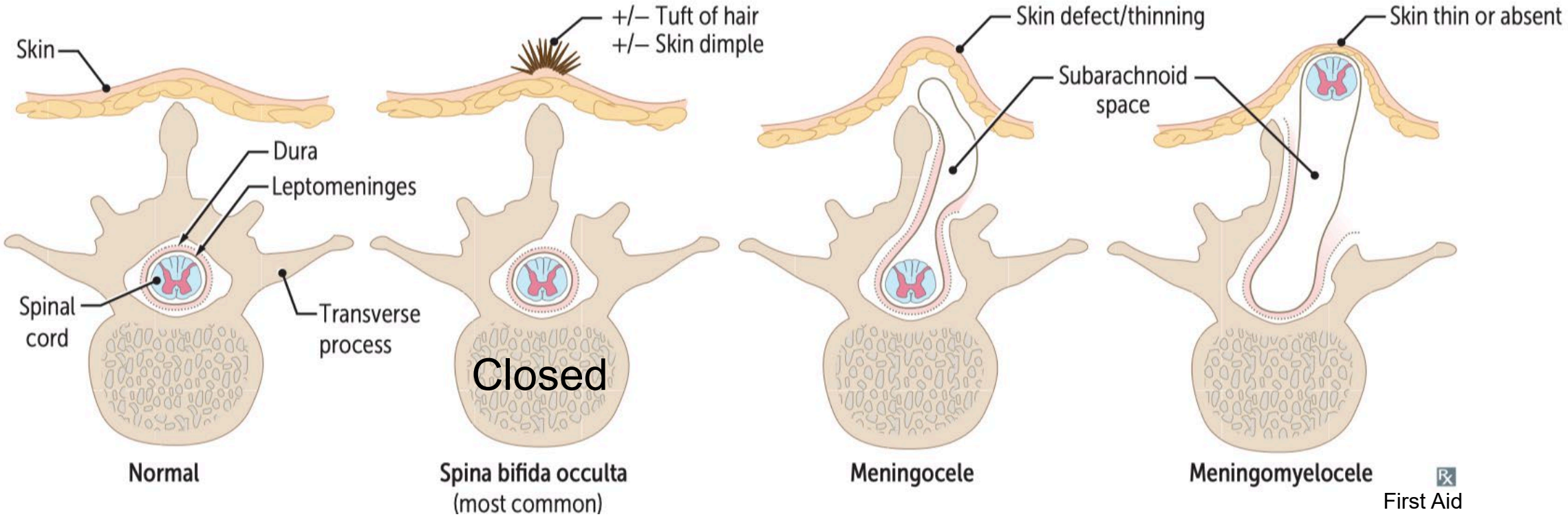
Lethal

Encephalocele
Herniation of the meninges (and brain)

Lethal or severe neurologic damage

Dukhovny

Inferior ONTD



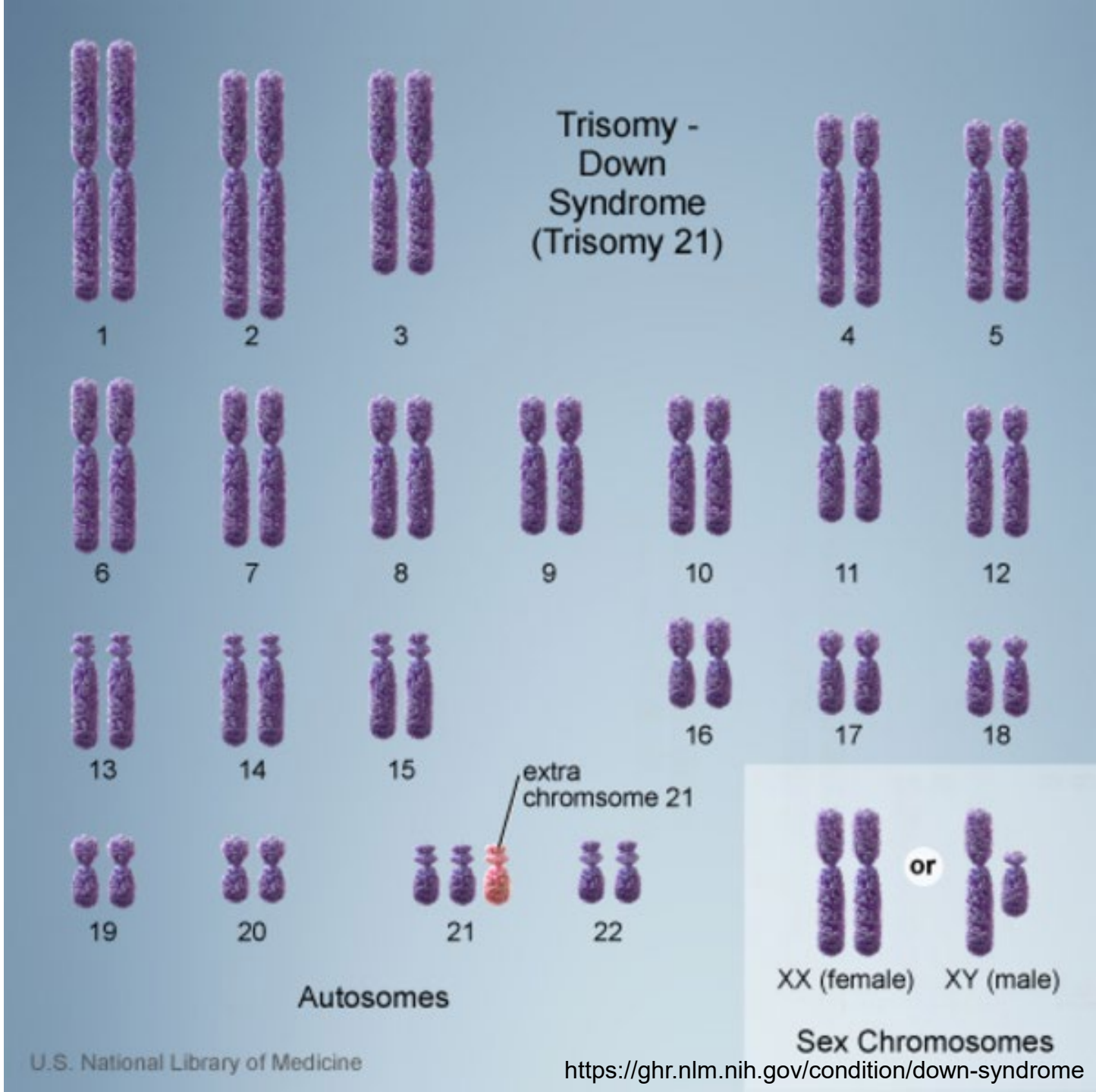
- Prognosis
  - Ranges from healthy to neurologic damage to death
  - More superior = more severe
  - Meningomyelocele most severe (25% death rate by adulthood)

Bowman

Trisomies

Trisomy

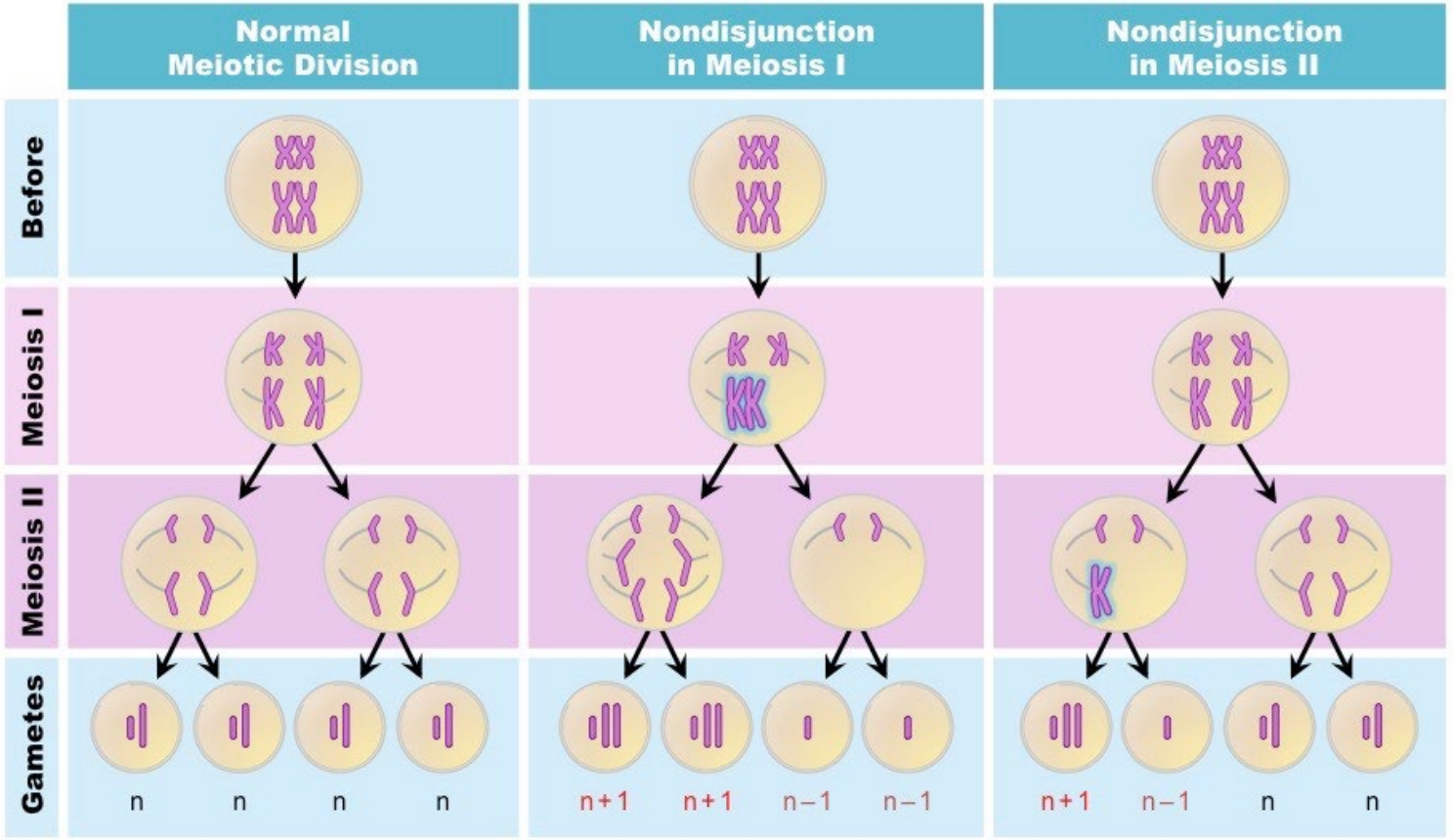
- Human cells usually have 2 copies of each chromosome
- Aneuploidy = Different number of chromosomes
- Trisomy = 3 copies of a chromosome



https://ghr.nlm.nih.gov/condition/down-syndrome

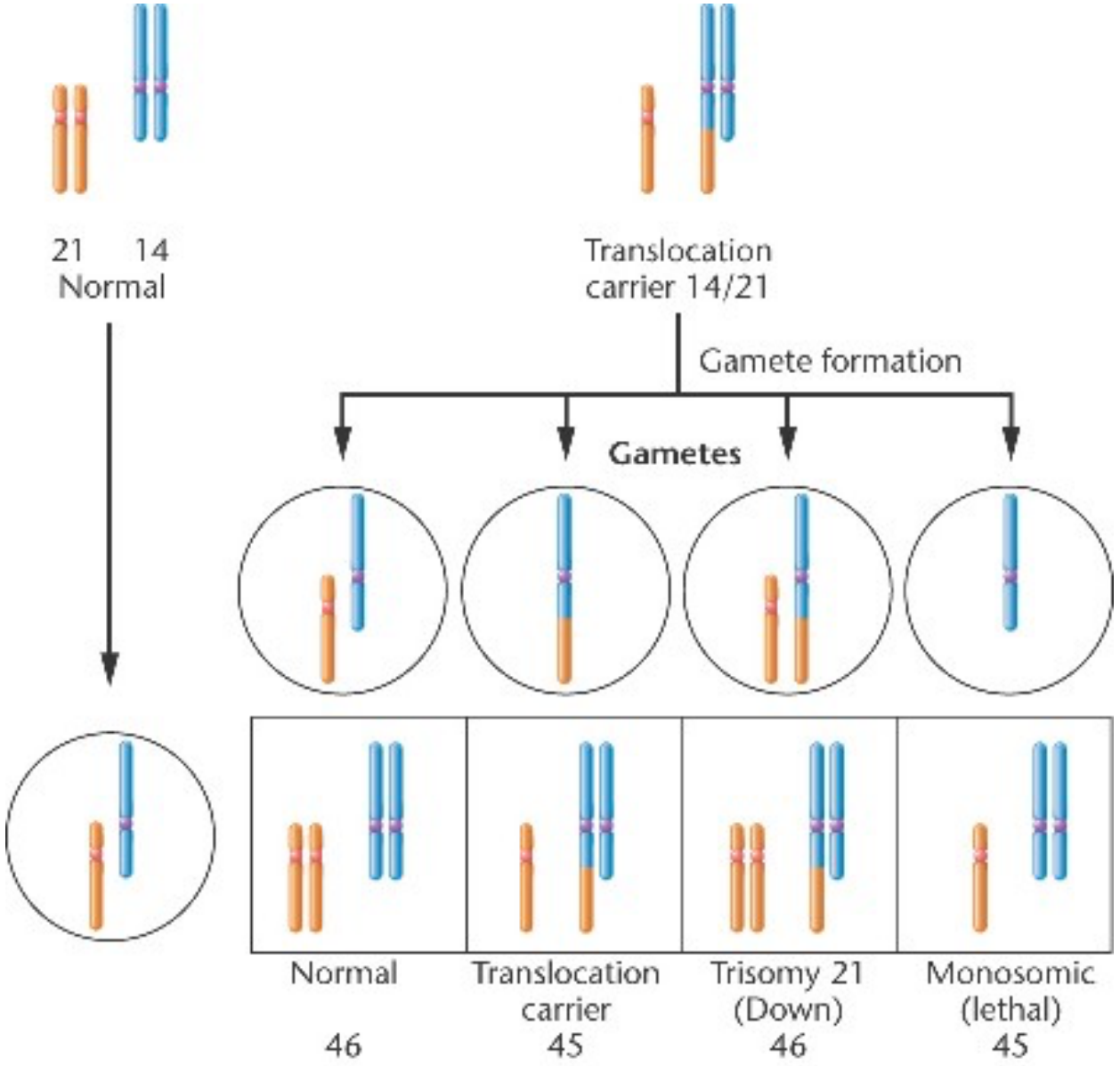
Trisomy Formation

1) Meiotic nondisjunction



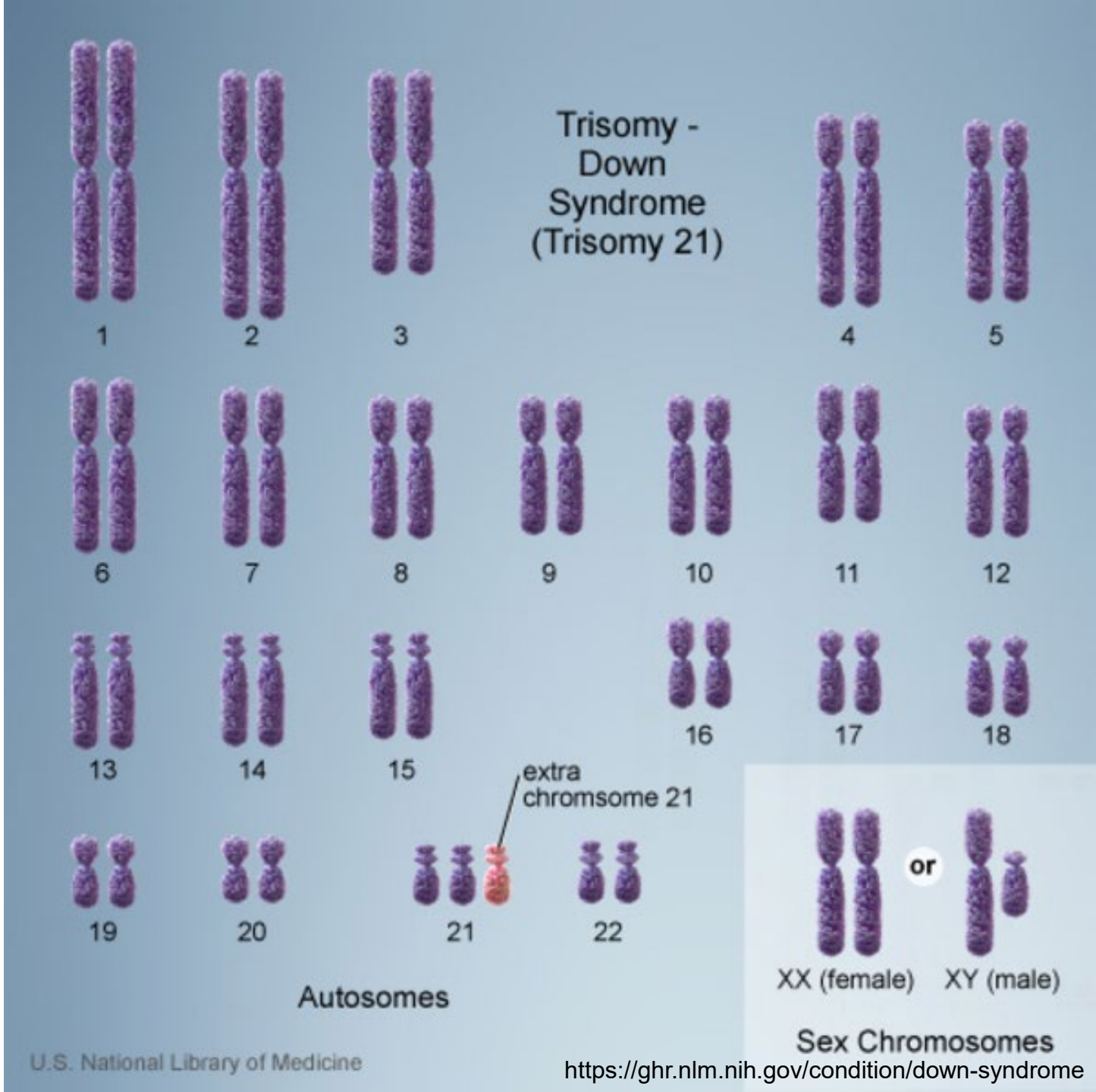
Trisomy Formation

1) Meiotic nondisjunction
2) Robertsonian Translocation



Down Syndrome

- Pathogenesis: Trisomy 21
- Incidence: ~1 in 800 births
  - Most common chromosomal disorder
- Risk factors:
  - Advanced maternal age
  - Prior aneuploidy pregnancy



https://ghr.nlm.nih.gov/condition/down-syndrome

Down Syndrome

- Intellectually disability
- Congenital heart defects
- Intestinal blockage issues
- Thyroid diseases
- Diabetes
- Leukemia
- Male infertility
- Immunodeficiencies

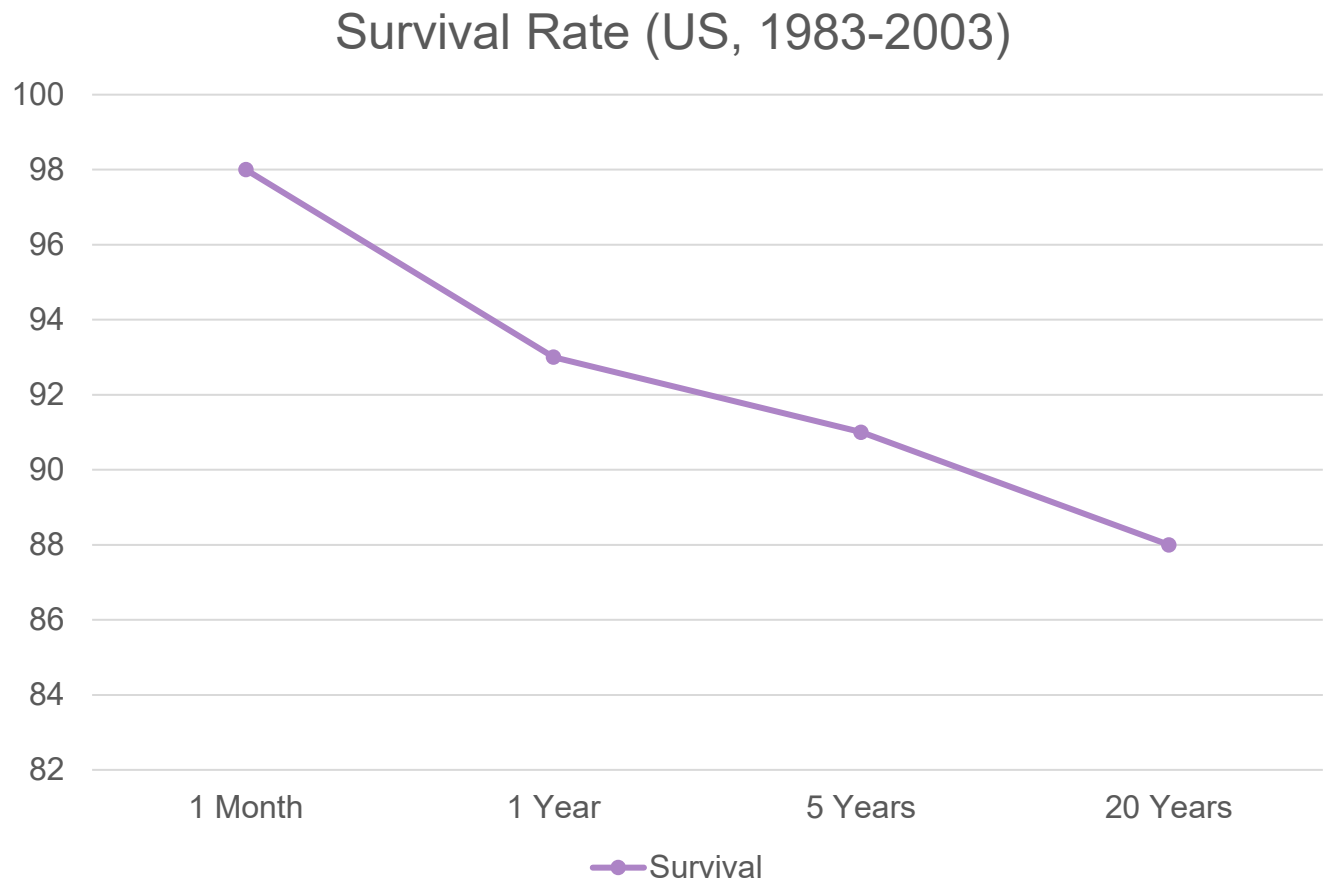
Ostermaier

https://schoolworkhelper.net/down-syndrome-causes-symptoms-treatment/

Down Syndrome

- Prognosis:
  - Shorter life expectancy:

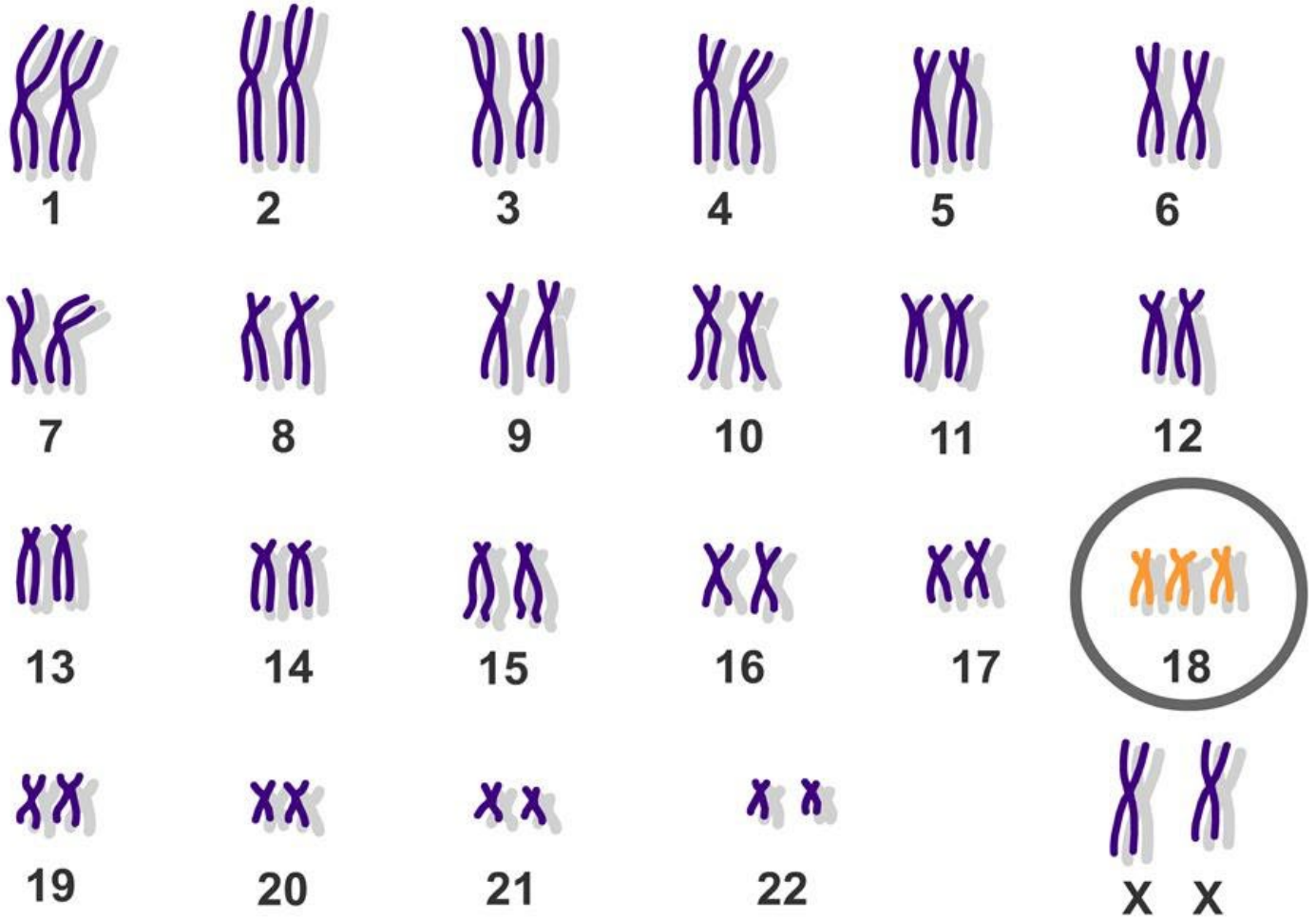
    56.8 years (Sweden)
  - Common causes of death
    - Pneumonia/infections
    - Congenital malformations
    - Circulatory disease
    - Dementia



Ostermaier

Edwards Syndrome

- Pathogenesis: Trisomy 18
- Incidence: 1 in 8000 births
  - 2nd most common trisomy
- Risk factor:
  - Advanced maternal age

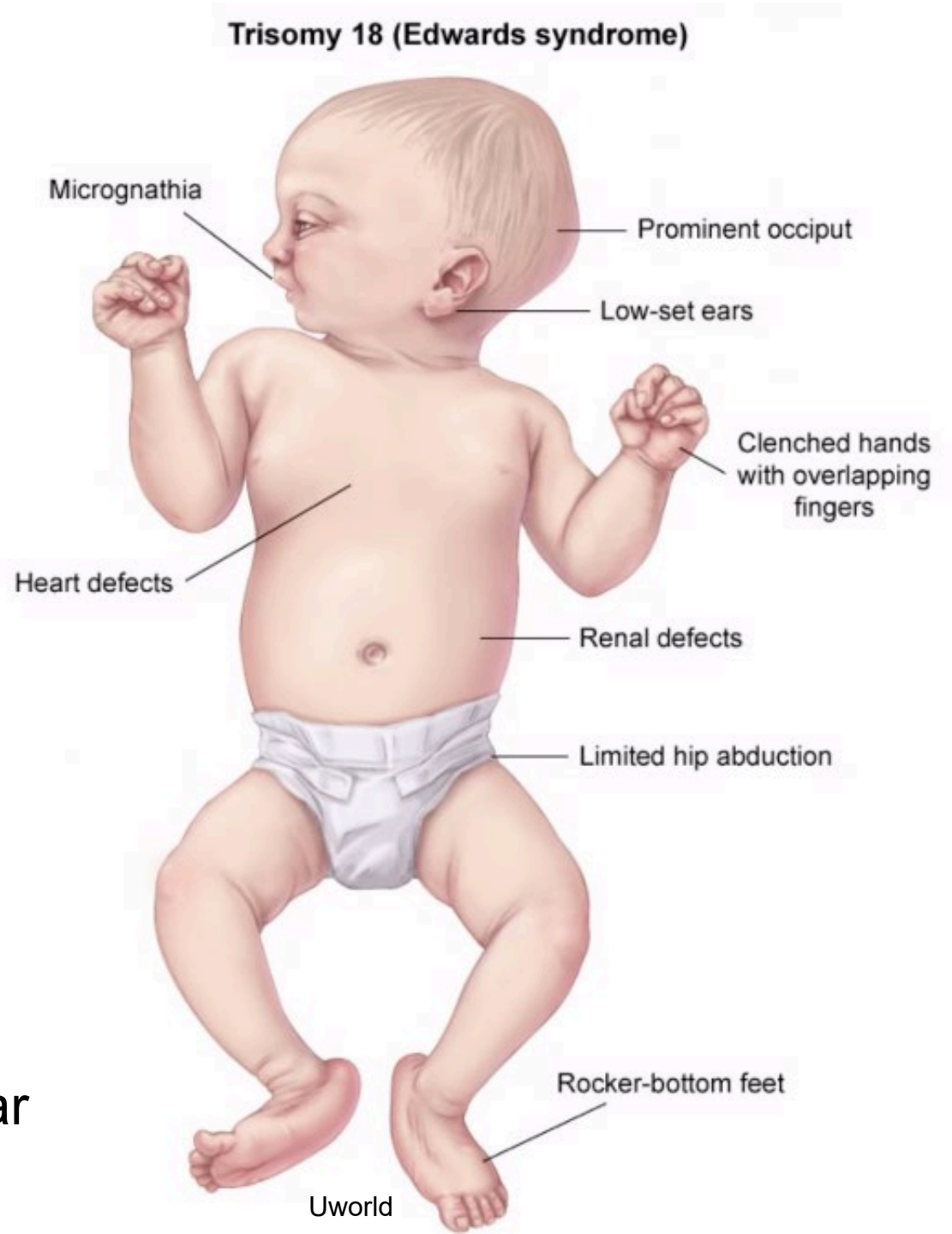


https://www.aboutkidshealth.ca/Article?contentid=875&language=English

Edwards Syndrome

- **P**rominent occiput
- **R**ocker-bottom feet
- **I**ntellectual disability
- **N**ondisjunction
- **C**lenched fists
- **E**ars (low set)

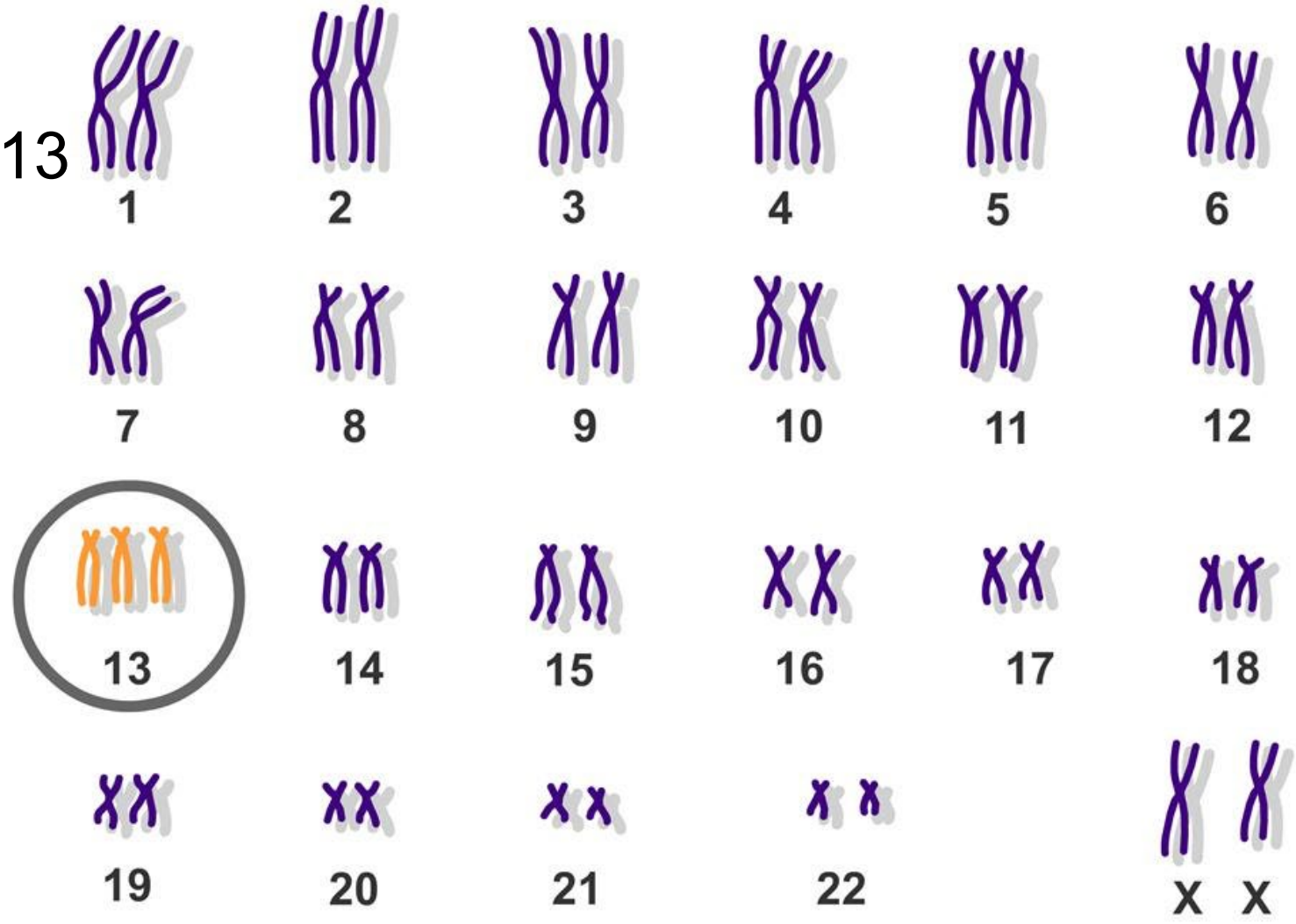
- Prognosis:
  - Majority die in uteuro
  - 50% die in 1-2 weeks, 95% die in first year



Messerlian, Giersch ABS, First Aid

Patau Syndrome

- Pathogenesis: Trisomy 13
- Incidence: 1 in 15,000
- Risk factor:
  - Advanced maternal age

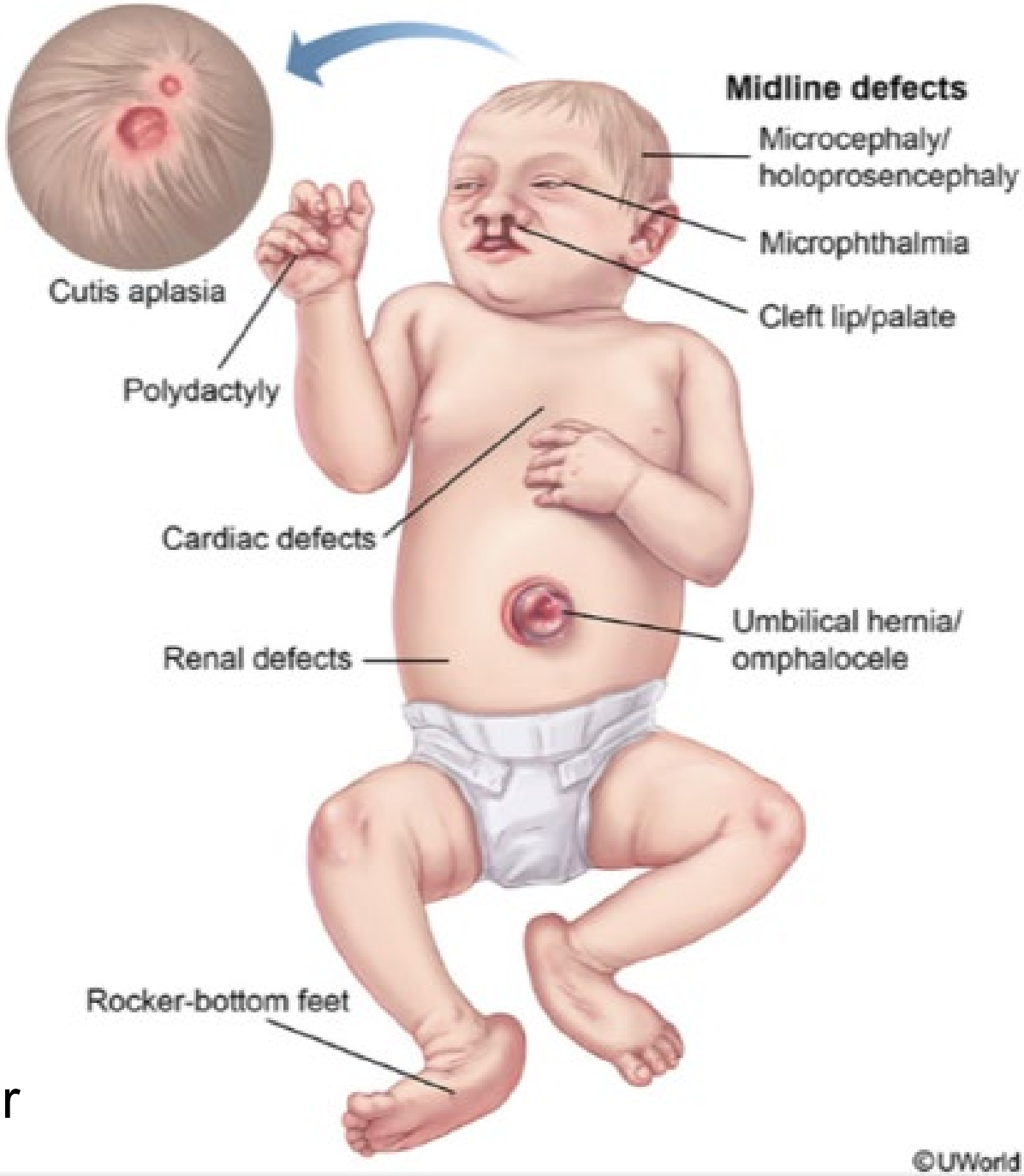


https://www.aboutkidshealth.ca/Article?contentid=876&language=English

Patau Syndrome

- Intellectual disability
- Holoprosencephaly: Failure of left and right hemispheres to separate
- Congenital heart defects
- Intrauterine growth deficiency

- Prognosis:
  - Majority die in uteuro
  - Median survival of liveborn: 7 days
  - 80% die by 1 month, 91% die by 1 year

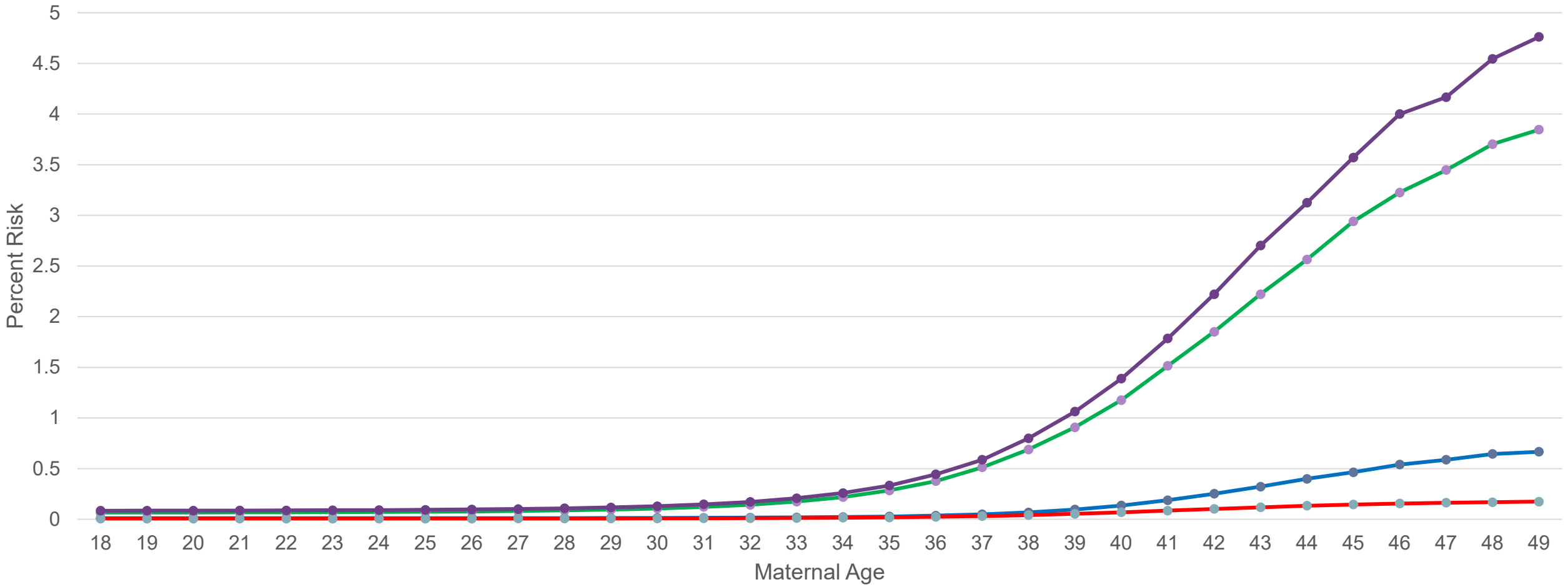


Giersch

Trisomy Risk by Maternal Age

Maternal Age	Trisomy 21 Risk (1:n)	Trisomy 18 Risk (1:n)	Trisomy 13 Risk (1:n)	Combined Trisomy Risk (1:n)
18	1495	9010	13,700	1175
19	1490	8985	13,670	1165
20	1475	8960	13,635	1160
21	1460	8930	13,580	1150
22	1440	8885	13,510	1135
23	1415	8825	13,410	1115
24	1380	8745	13,275	1095
25	1340	8630	13,090	1065
26	1285	8480	12,840	1025
27	1220	8280	12,500	980
28	1140	8010	12,050	920
29	1045	7660	11,470	850
30	935	7215	10,735	770
31	815	6655	9830	675
32	695	5990	8770	580
33	570	5220	7585	480
34	455	4380	6345	385
35	350	3530	5130	300
36	265	2725	4030	225
37	195	2025	3100	170
38	145	1455	2370	125
39	110	1035	1825	94
40	85	735	1430	72
41	66	530	1160	56
42	54	395	970	45
43	45	310	840	37
44	39	250	745	32
45	34	215	685	28
46	31	185	640	25
47	29	170	610	24
48	27	155	590	22
49	26	150	570	21

Trisomy Risk by Maternal Age



Messerlian

Screening Tests and Methodology

What is a screening test?

- A screening test identifies those at increased risk of disease
- It does NOT diagnosis a disease
  - Diagnosis requires confirmation testing
- It will result in many false positives
  - Ensures patients with the disease are identified

Overview of Screening Tests

	1st Trimester Combined Screen	2nd Trimester Quadruple Screen
Timing	9-14 weeks	15-23 weeks
Analytes	▪ Nuchal translucency (NT) ▪ Pregnancy associated plasma protein A (PAPP-A) ▪ Beta human chorionic gonadotropin (β-hCG)	▪ Alpha fetoprotein (AFP) ▪ Beta human chorionic gonadotropin (β-hCG) ▪ Unconjugated estriol (uE_3) ▪ Dimeric inhibin A (DIA)

Clarke, Messerlian

Combining Screening Tests

	Integrated Screen	Contingent Screen
Method	▪ Do 1st and 2nd trimester screens ▪ Don't release results until both are done	▪ Do 1st trimester screen ▪ If positive, offer diagnostic testing ▪ If high to medium risk, offer 2nd trimester screen ▪ If low risk, stop testing
Pros	▪ Most sensitive ▪ Fewer false positives	▪ Fewer false positives ▪ No waiting ▪ Can do chorionic villus sampling
Cons	▪ Waiting ▪ Can't do chorionic villus sampling	▪ Slightly less sensitive than integrated screen

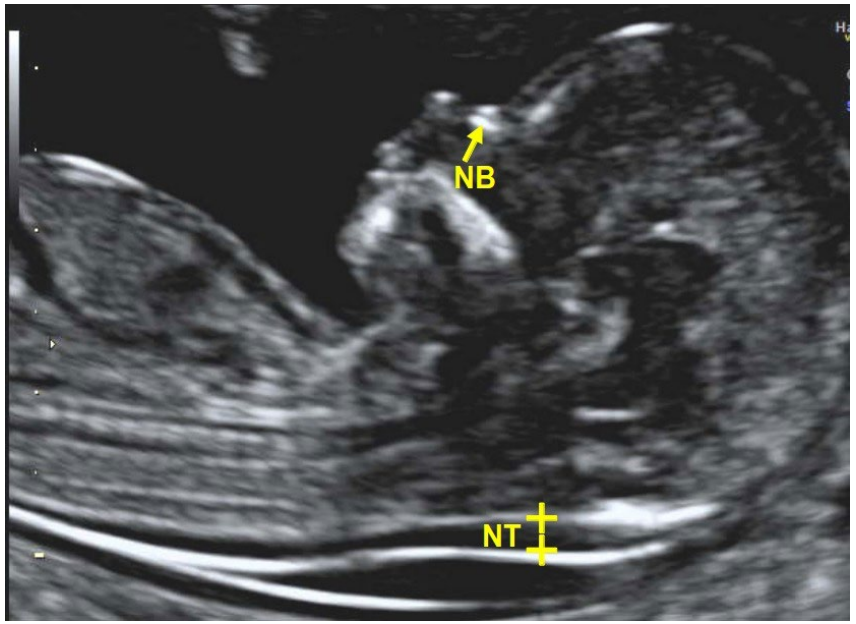
Clarke

Analytes and Measurements

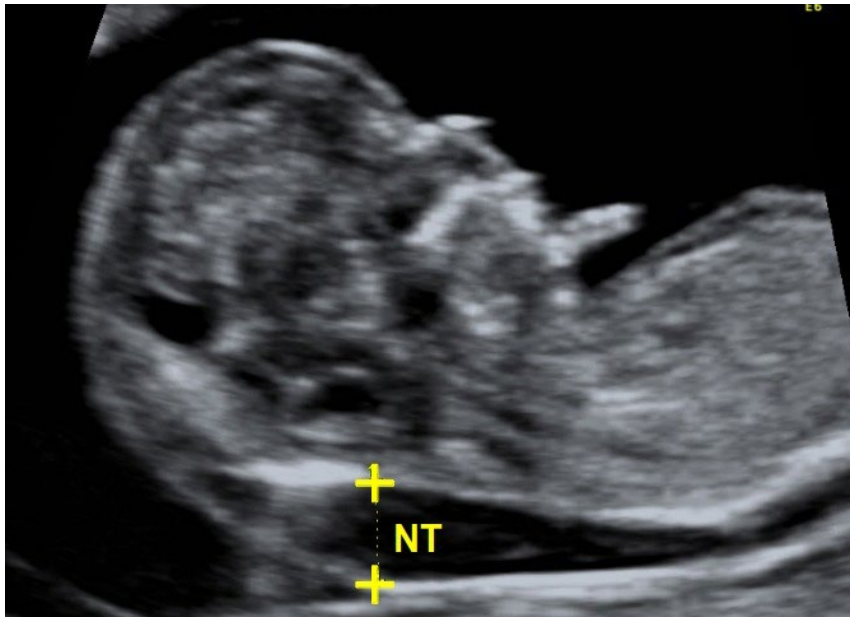
https://fetalmedicine.org/fmf-certification/certificates-of-competence/nuchal-translucency-scan

Nuchal Translucency (NT)

- Timing: 10w3d to 13w6d (ideal 12-13w)
- Must be done a certified sonographer
- Measure the hypoechoic (dark) space in posterior neck by ultrasound
- Increased thickness (≥3.0 mm) = increased aneuploidy risk



Healthy



Affected

Messerlian, Clarke

Pregnancy associated plasma protein A (PAPP-A)

- Synthesized by placenta
- Increases with gestational age
- Pregnancy form: Heterotetrametric complex (htPAPP-A)
  - Two PAPP-A subunits
  - Two subunits of eosinophil major base protein (pro-MBP)
- Function: Insulin-like growth factor (IGF) protease
  - IGF is essential for fetal growth

Clarke, Yarbrough

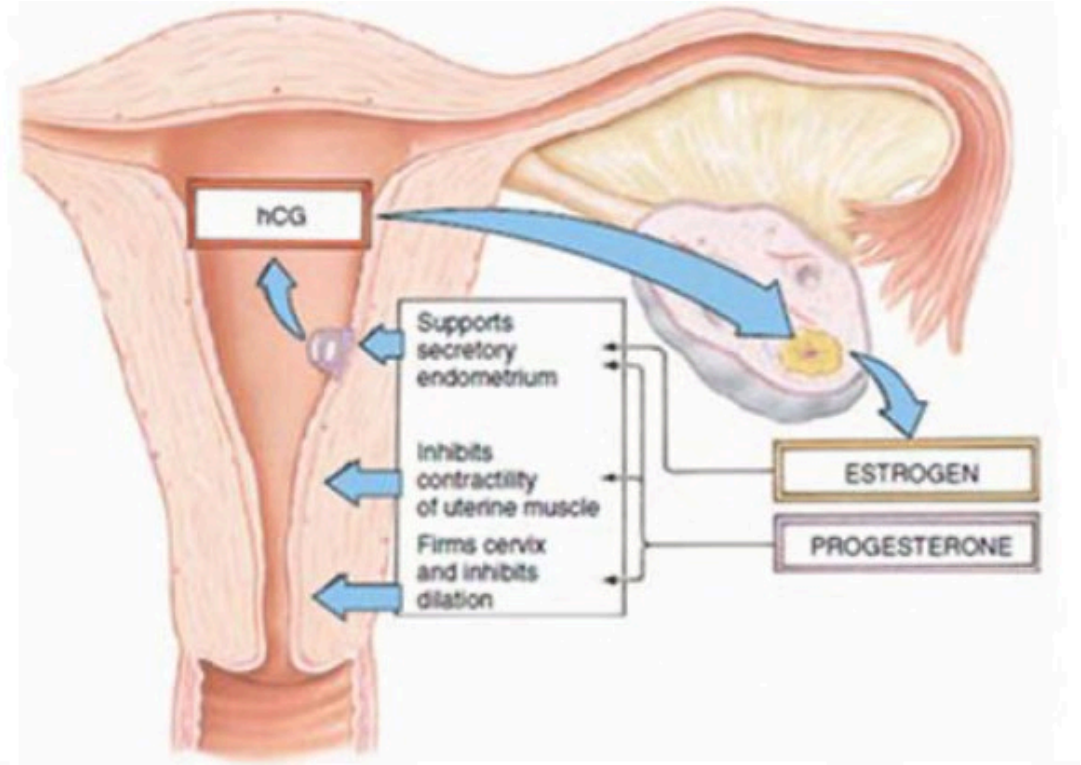
Alpha fetoprotein (AFP)

- Binding protein similar to albumin
- Synthesized by fetal liver and yolk sac
- Peaks at 25w, then gradually declines
- ONTD: AFP in fetal circulation leaks across defect → ↑AFP in amniotic fluid → ↑AFP in maternal serum
- Also ↑AFP in yolk sac tumors, hepatocellular carcinoma, viral hepatitis, and cirrhosis

Clarke, Yarbrough

Beta human chorionic gonadotropin (β-hCG)

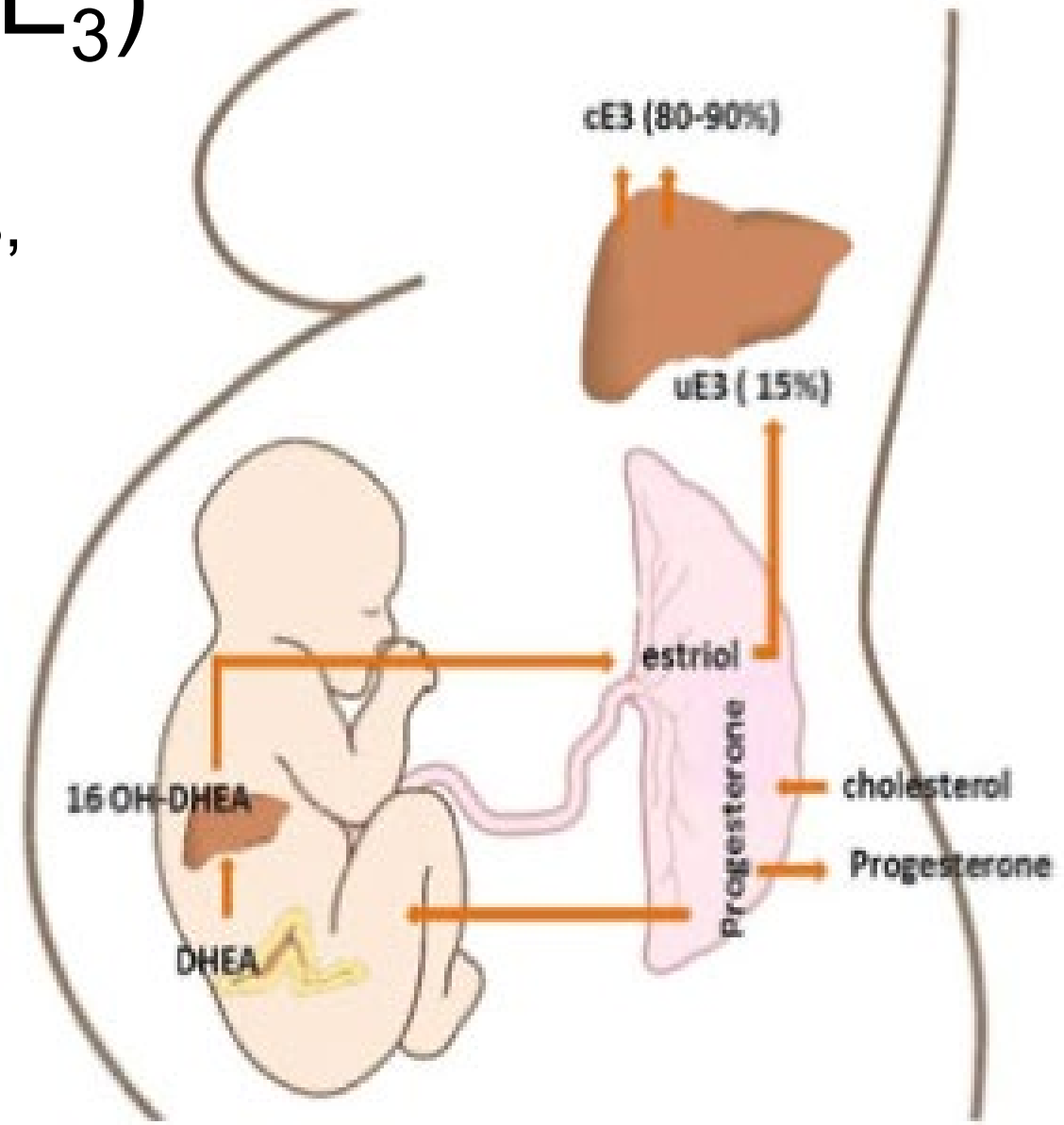
- 2 subunits
  - α: Shared with LH, FSH, TSH, and hCG
  - β: Unique to hCG
- Synthesized by placenta
- Detectable at 1 week, peaks at 8-10 weeks
  - Pregnancy test
- Maintains corpus luteum → Estrogen and progesterone



- Also high with multiple gestations, choriocarcinoma, hydatidiform moles, and dysgerminoma

Unconjugated estriol (uE_3)

- Synthesized by fetal liver, adrenals, and placenta
  - Half life of 20 minutes before maternal liver conjugates it
- Increases with gestational age



Clarke, Yarbrough

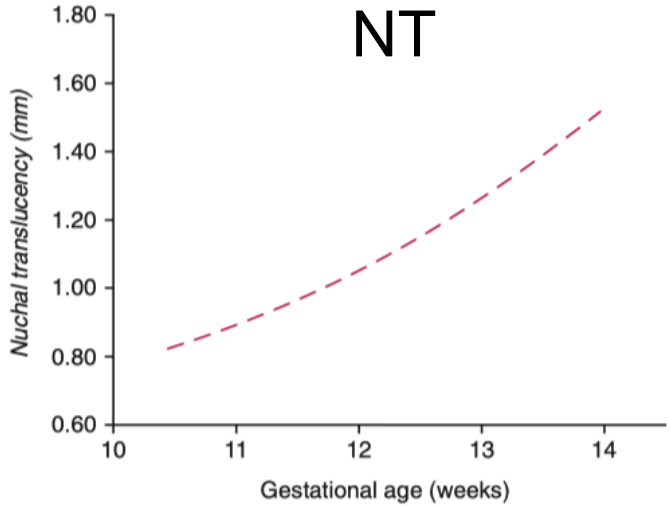
Dimeric inhibin A (DIA)

- Dimer
- Synthesized by placenta in pregnancy
- Increases with gestational age
- Inhibits follicle stimulating hormone
- Also increased in ovarian granulosa cell cancer

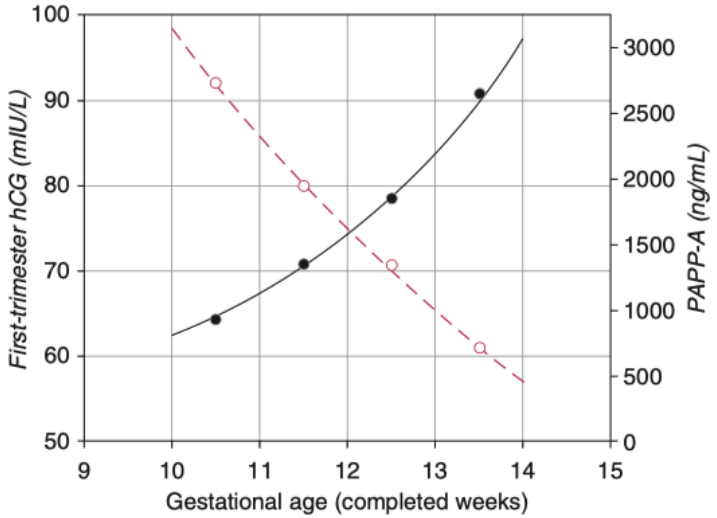
Clarke, Yarbrough

Analyte Concentrations & Measurments

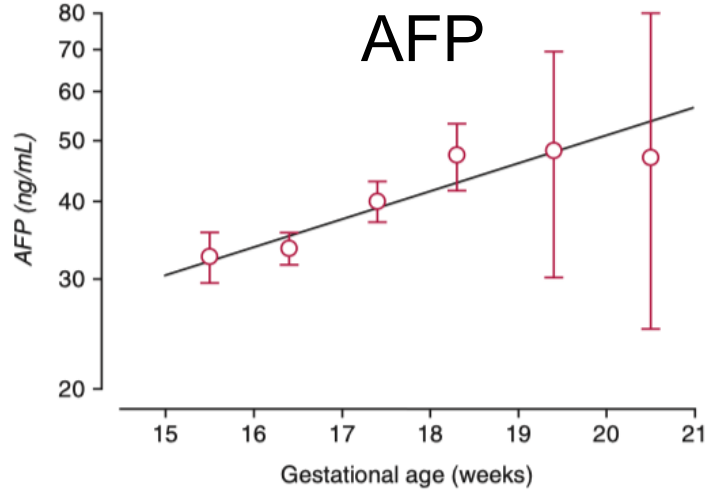
NT



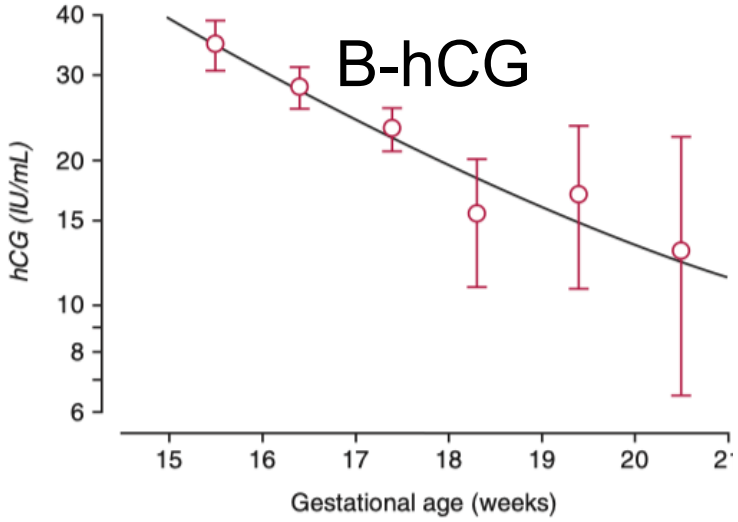
PAPP-A



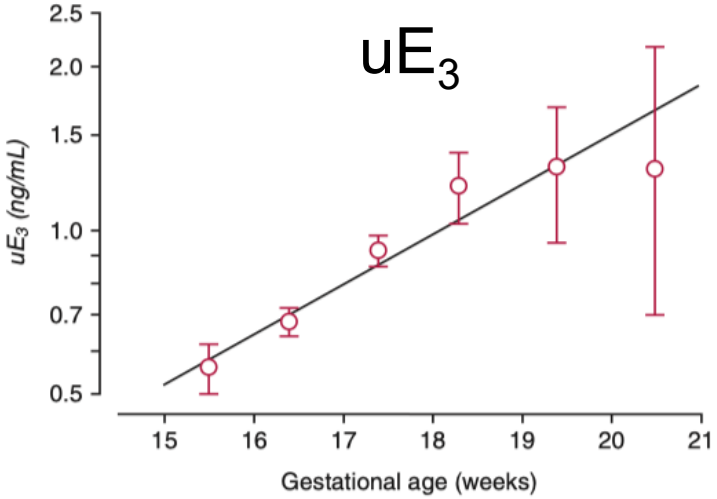
AFP



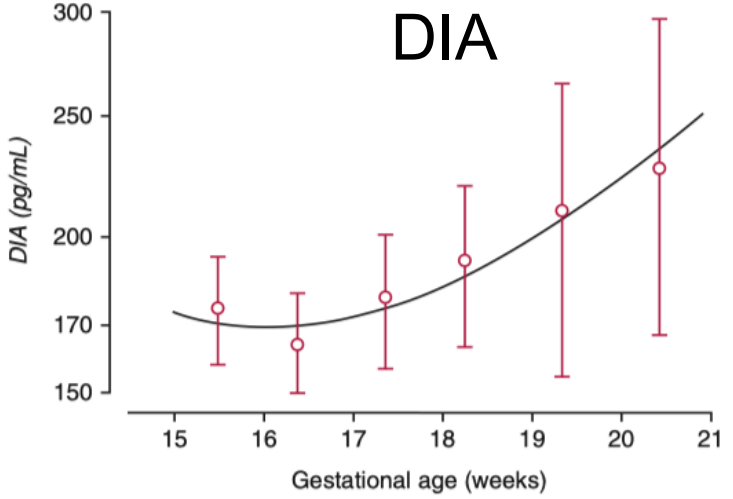
B-hCG



uE_3



DIA

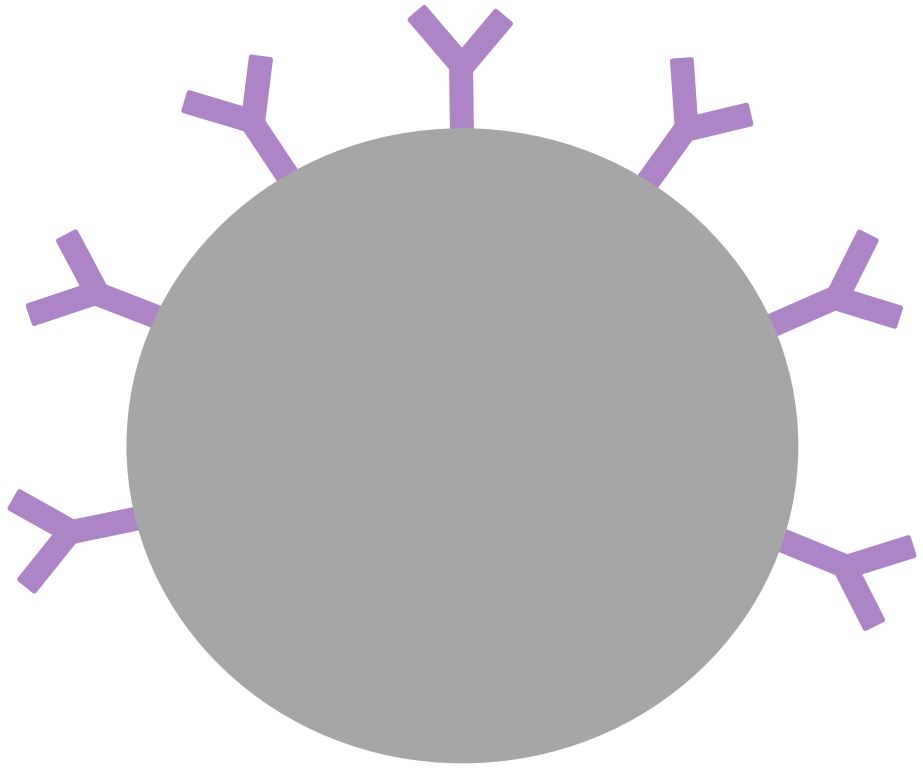


Yarbrough

Sandwich IA (Immunoassay)

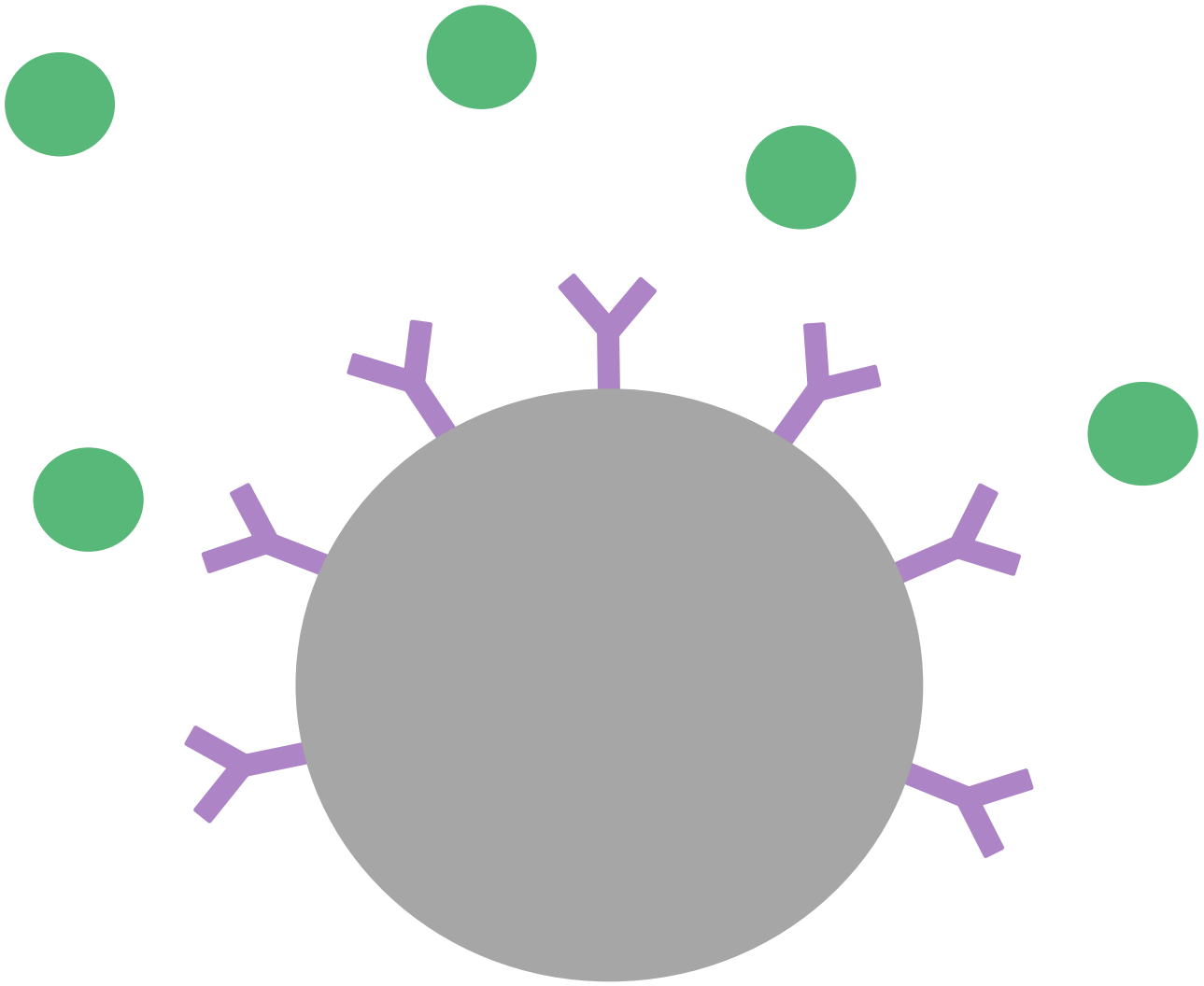
- PAPP-A
- AFP
- β-hCG
- DIA

Sandwich IA for PAPP-A, AFP, β-hCG, DIA



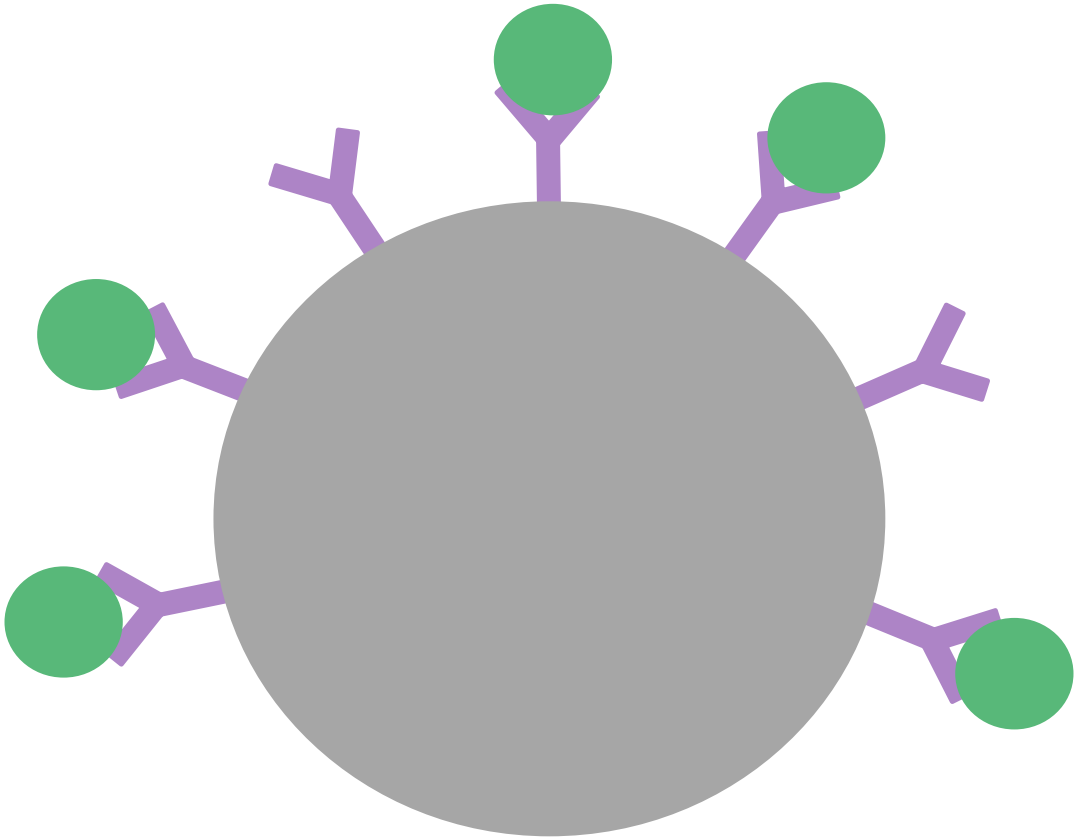
1) Incubate:
   - Paramagnetic particles coated with anti-PAPP-A antibodies

Sandwich IA for PAPP-A, AFP, β-hCG, DIA



1) Incubate:
   - Paramagnetic particles coated with anti-PAPP-A antibodies
   - Maternal serum (contains PAPP-A)

Sandwich IA for PAPP-A, AFP, β-hCG, DIA



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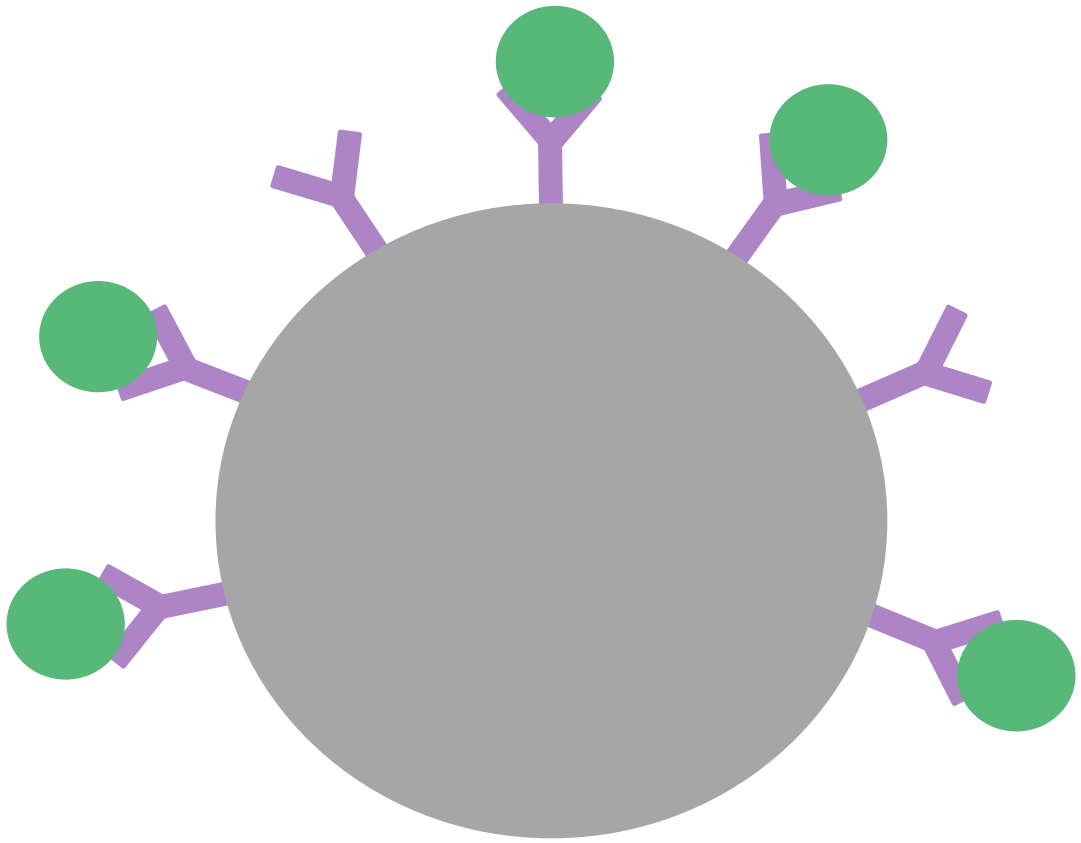
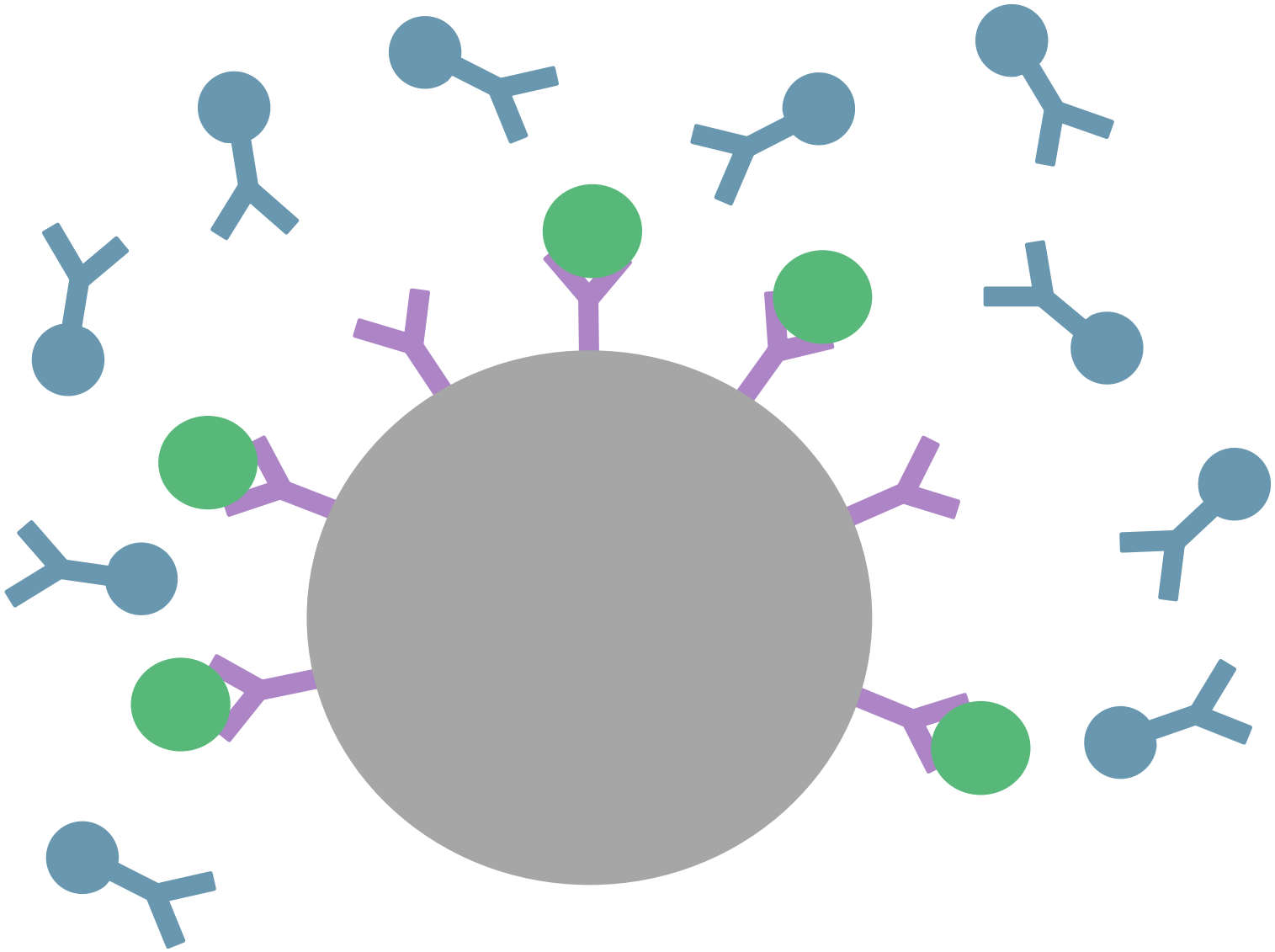
   - Maternal serum (contains PAPP-A)

Sandwich IA for PAPP-A, AFP, β-hCG, DIA



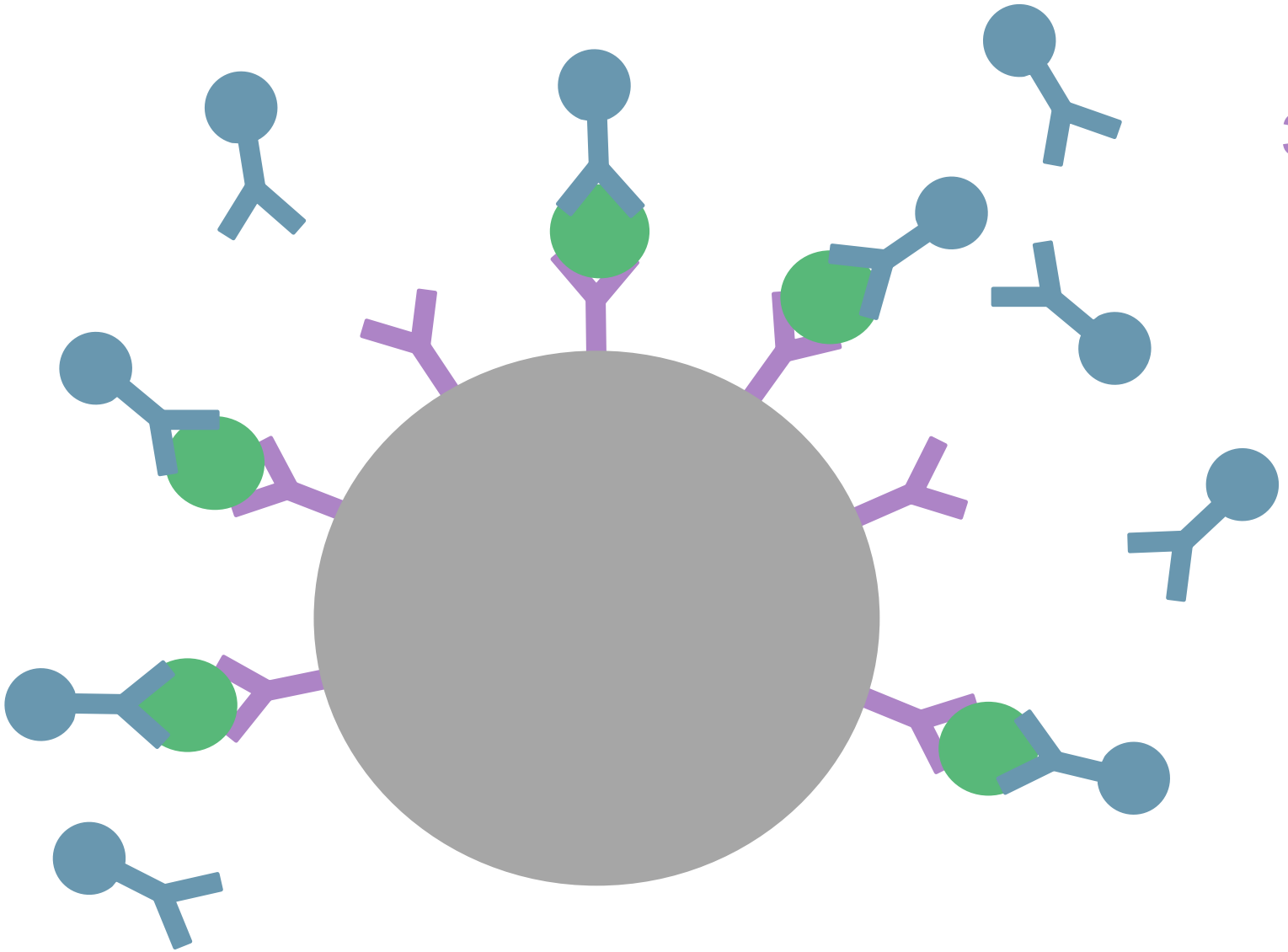
2) Wash; particles held by magnetic field

Sandwich IA for PAPP-A, AFP, β-hCG, DIA



3) Add anti-PAPP-A monoclonal antibody – alkaline phosphatase conjugate

Sandwich IA for PAPP-A, AFP, β-hCG, DIA



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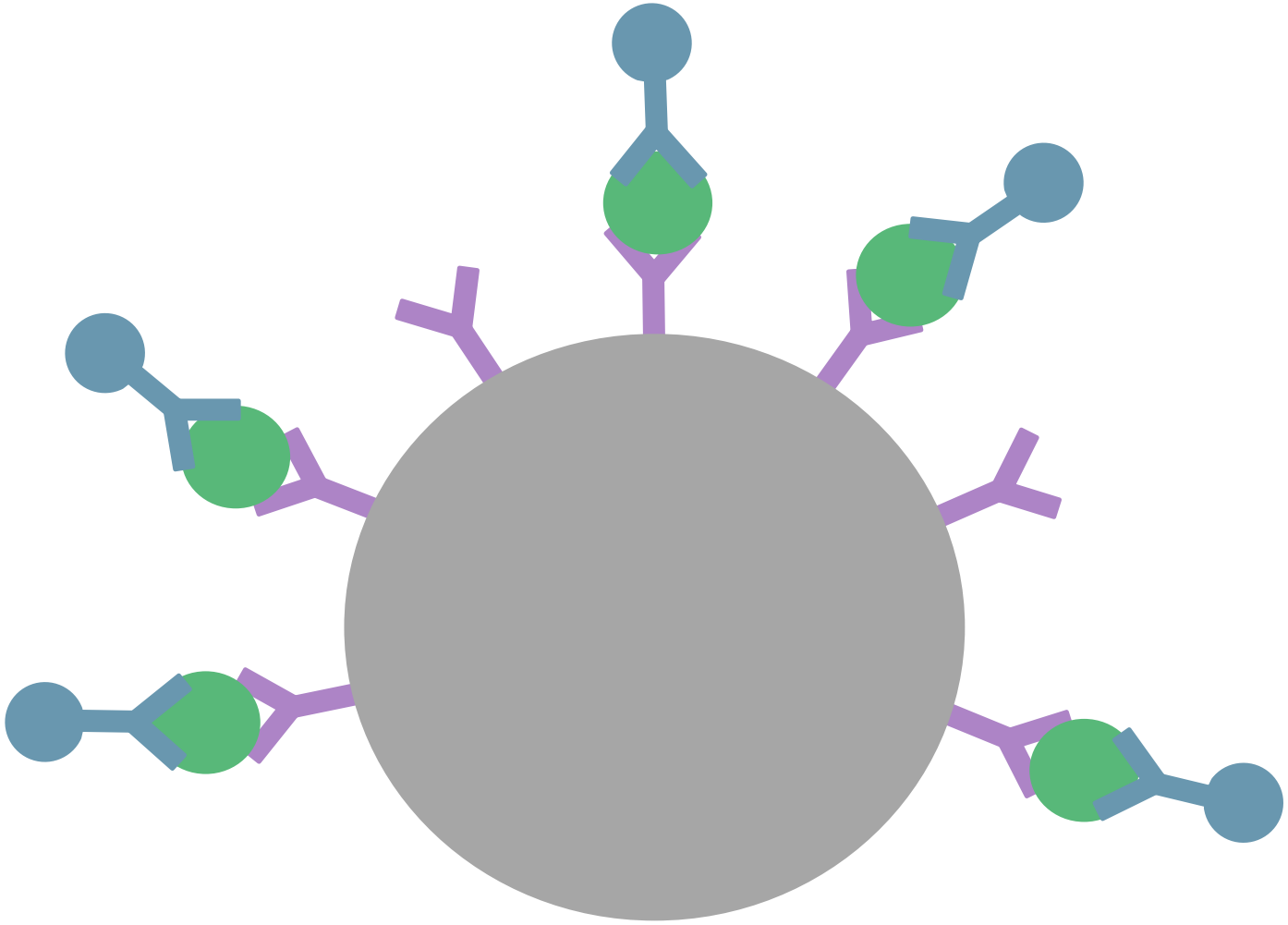
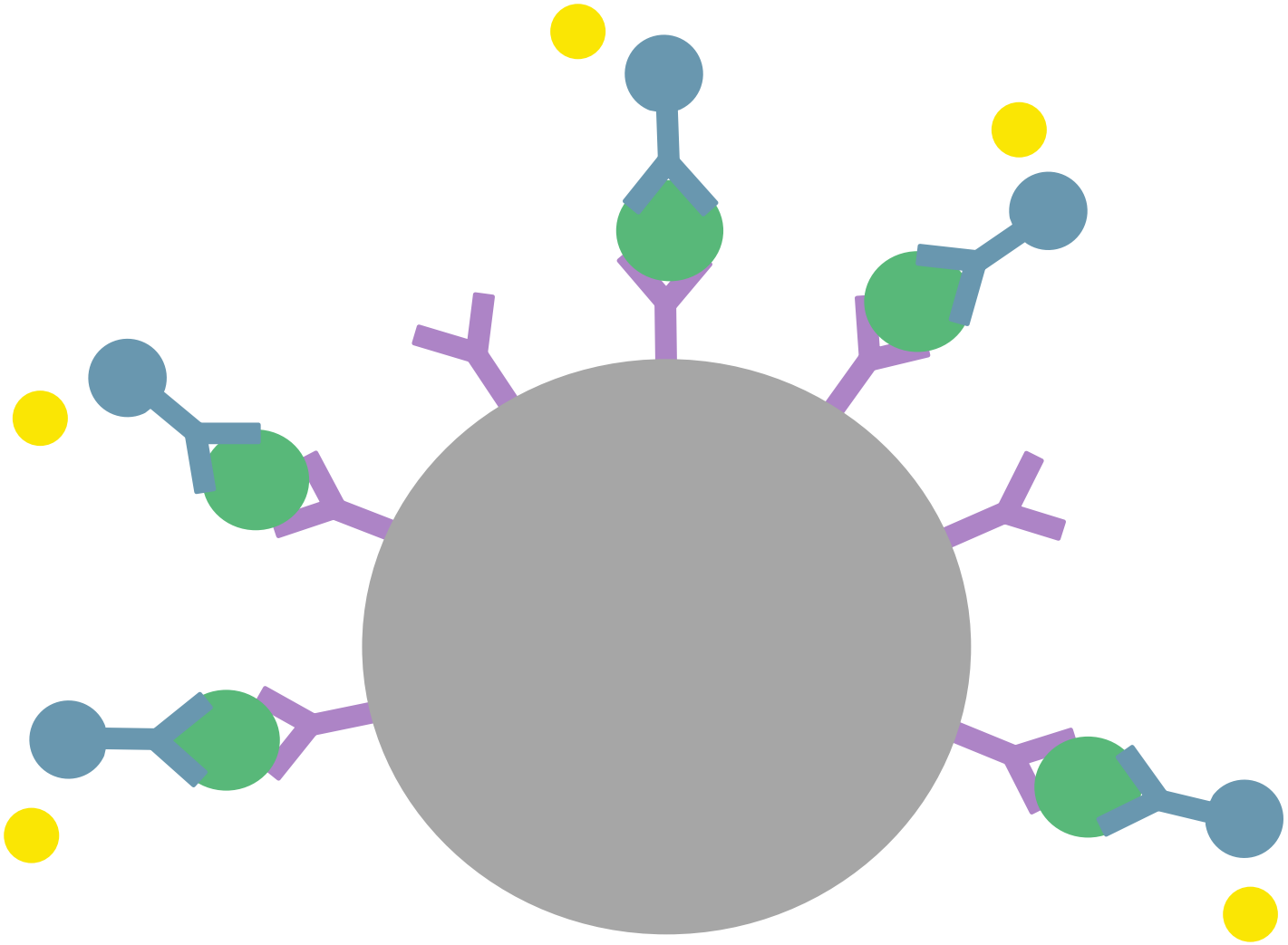
Sandwich IA for PAPP-A, AFP, β-hCG, DIA



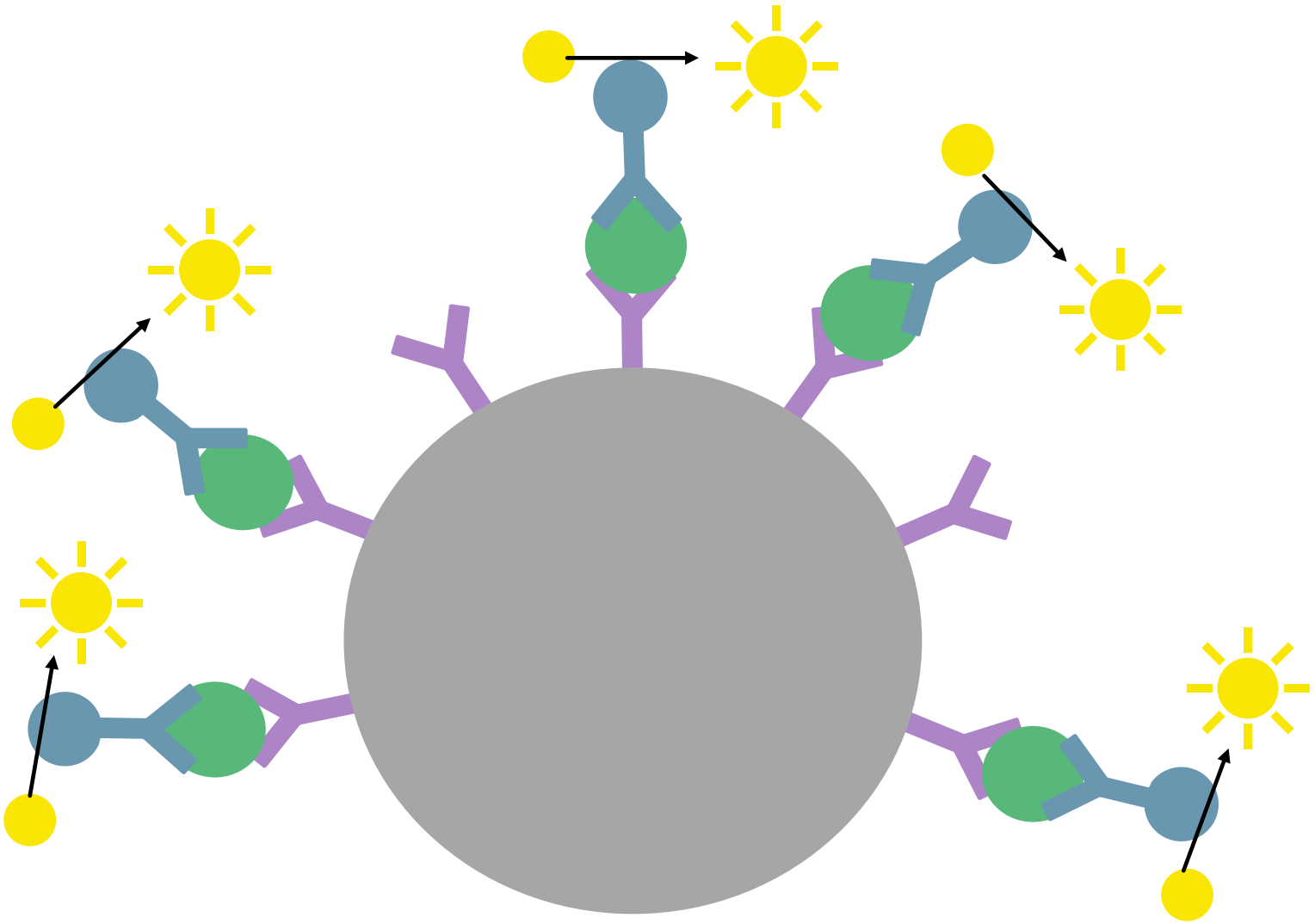
4) Wash

Sandwich IA for PAPP-A, AFP, β-hCG, DIA



5) Add Lumi-Phos 530

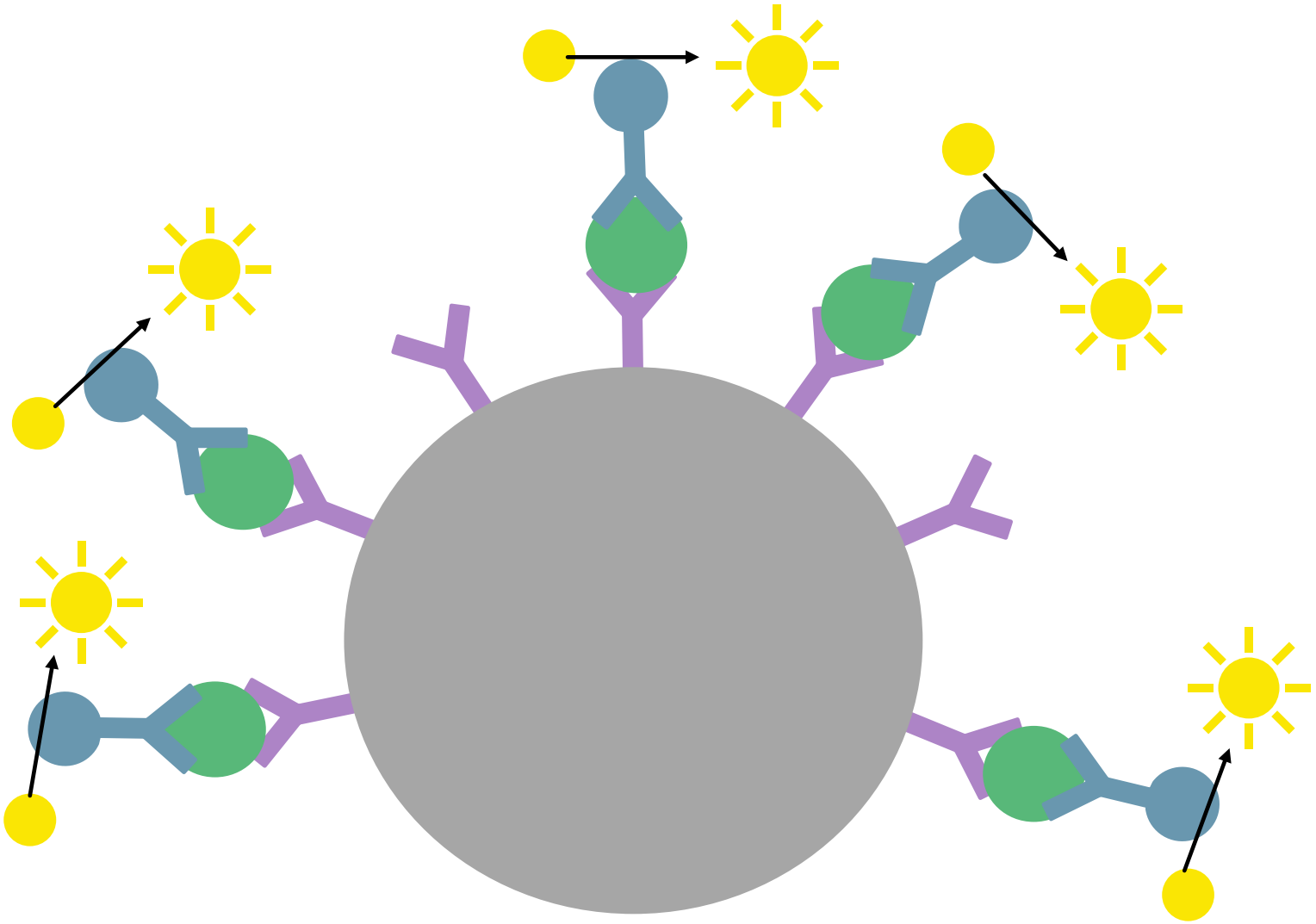
Sandwich IA for PAPP-A, AFP, β-hCG, DIA



5) Add Lumi-Phos 530

6) Measure luminescence with luminometer

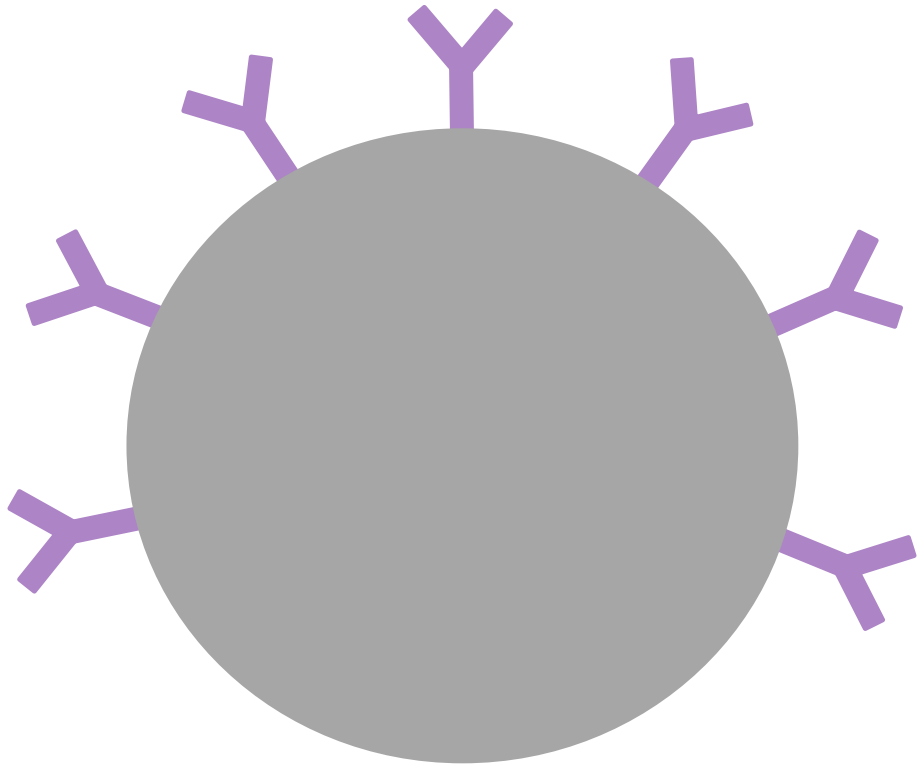
Sandwich IA for PAPP-A, AFP, β-hCG, DIA



- Direct correlation:
  ↑ luminescence = ↑PAPP-A
- Quantitate by comparing to calibration curve

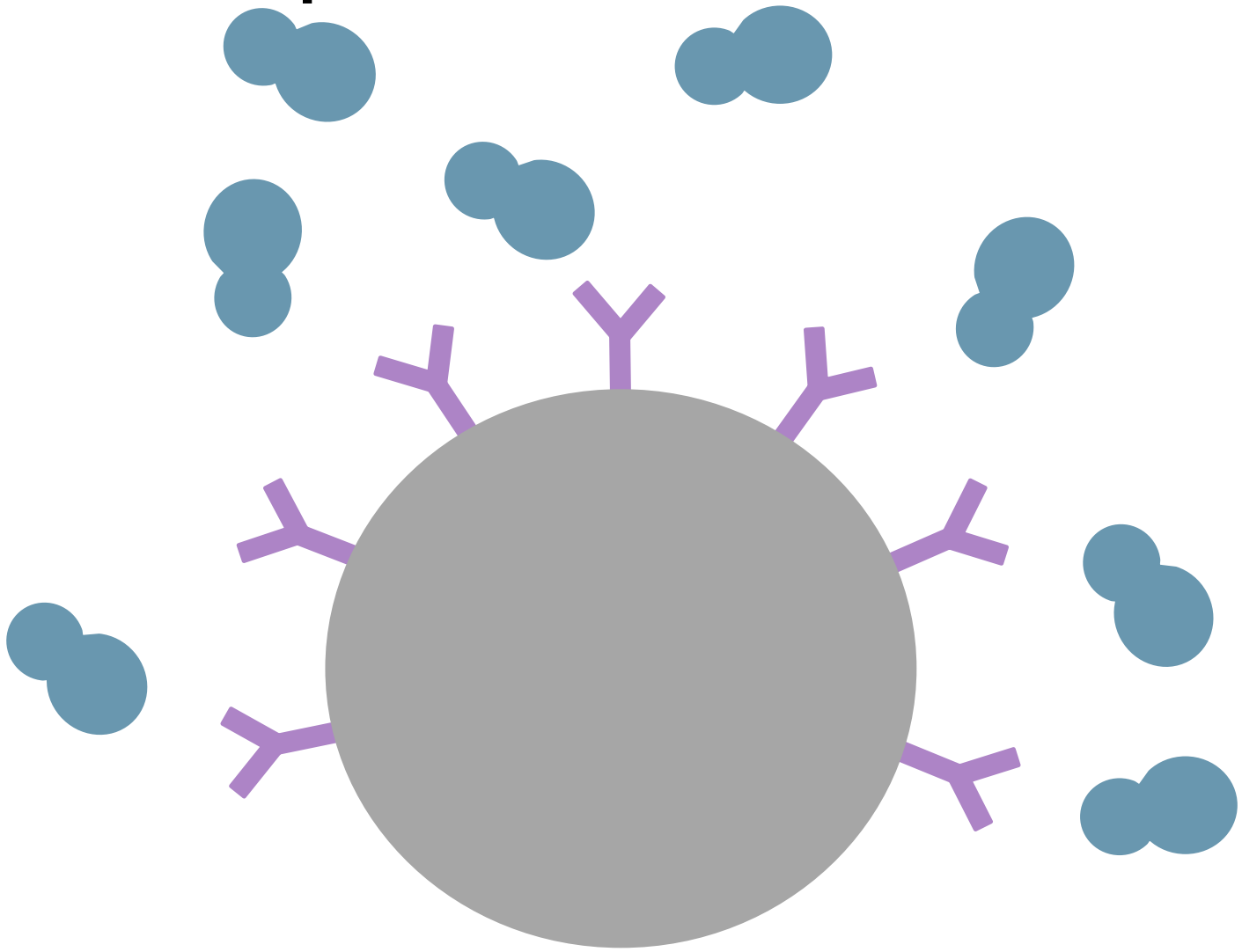
Competitive IA (Immunoassay) - Estriol

Competitive IA for Estriol



1) Incubate:
   - Paramagnetic particles coated with anti-estriol antibodies

Competitive IA for Estriol



1) Incubate:
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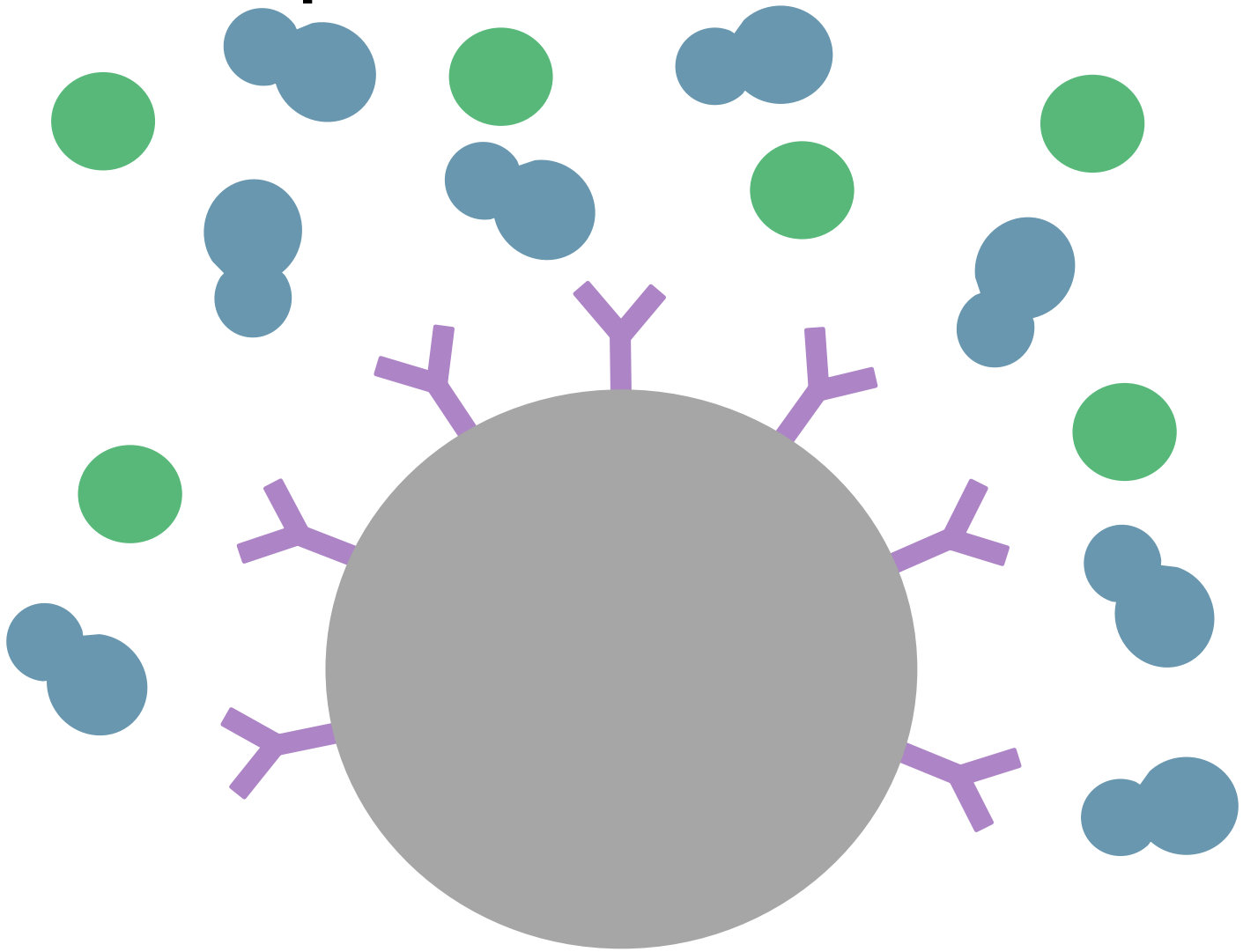
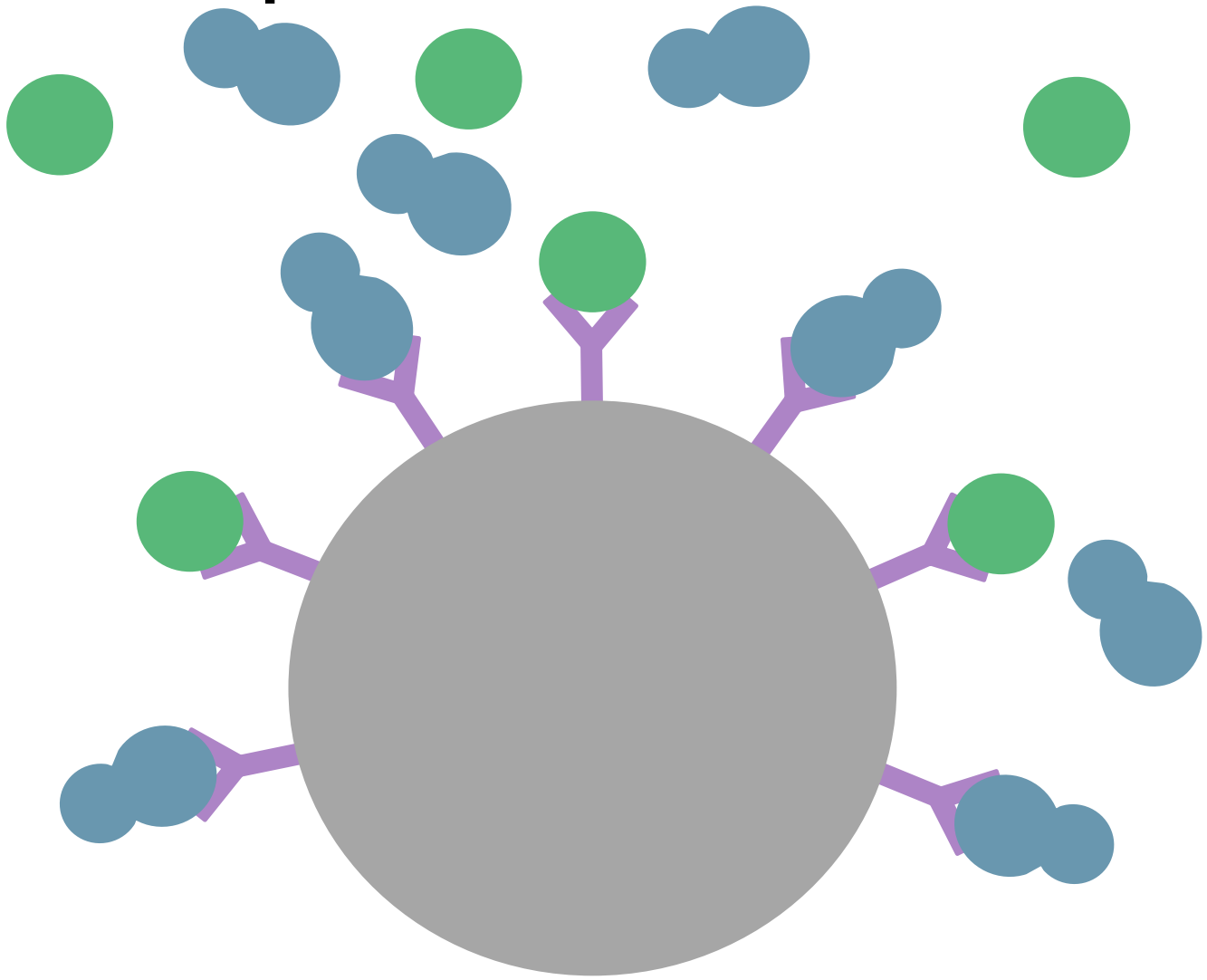
   - Estriol-alkaline phosphatase conjugate

Competitive IA for Estriol



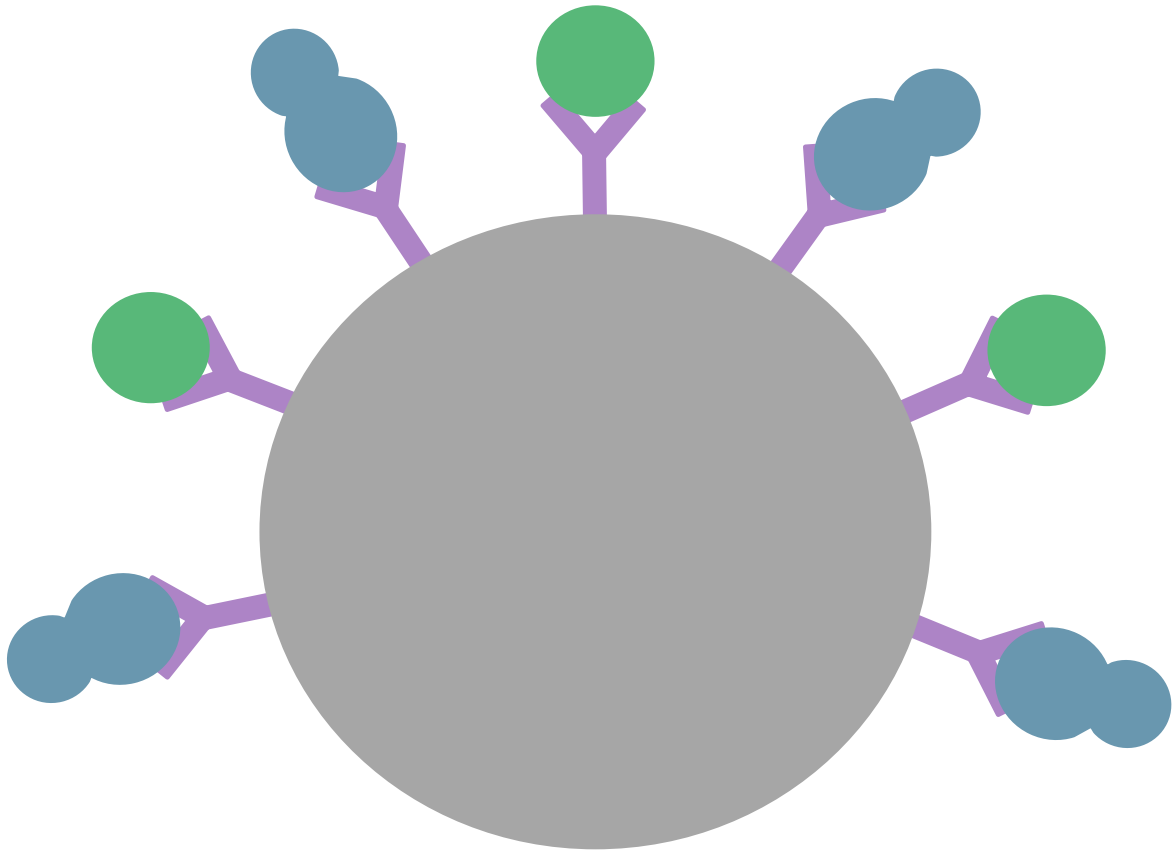
1) Incubate:
   - Paramagnetic particles coated with anti-estriol antibodies
   - Estriol-alkaline phosphatase conjugate
   - Maternal serum (contains estriol)

Competitive IA for Estriol



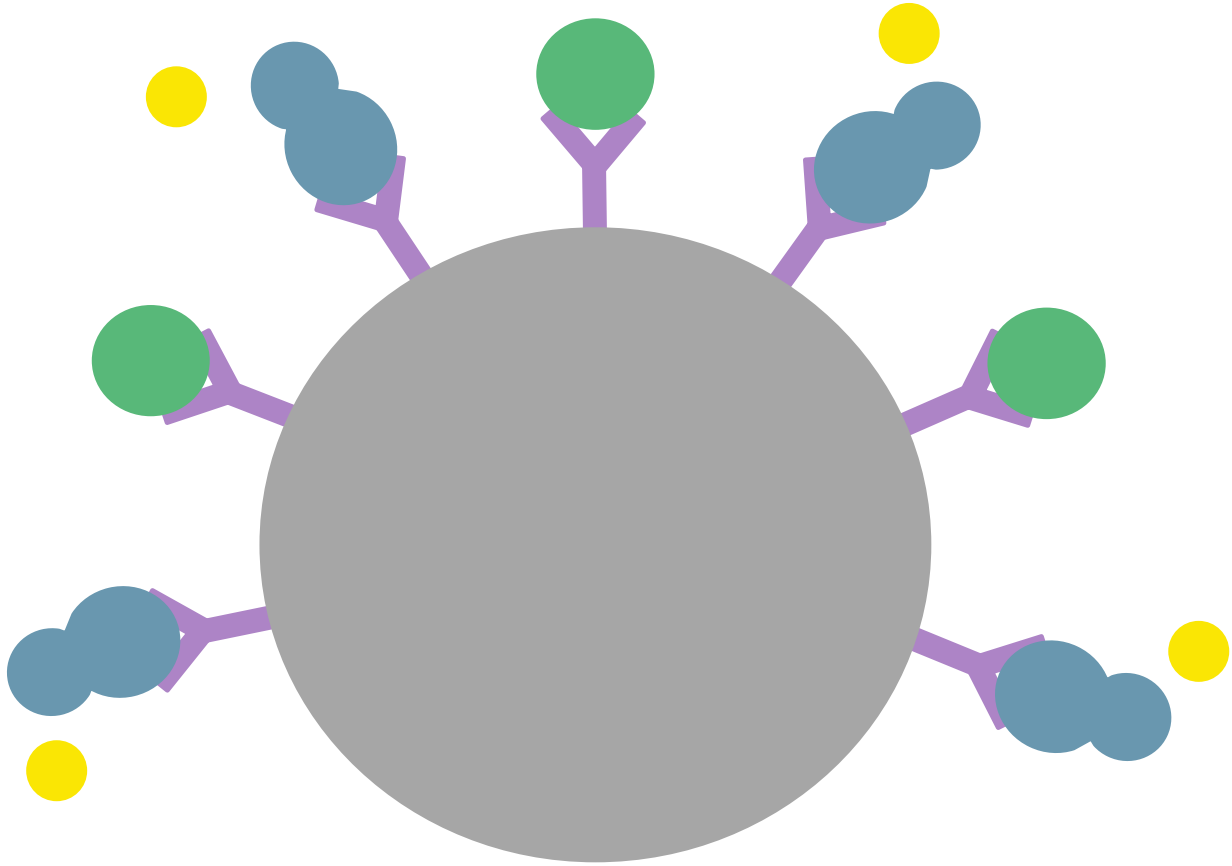
1) Incubate:
   - Paramagnetic particles coated with anti-estriol antibodies
   - Estriol-alkaline phosphatase conjugate
   - Maternal serum (contains estriol)

Competitive IA for Estriol



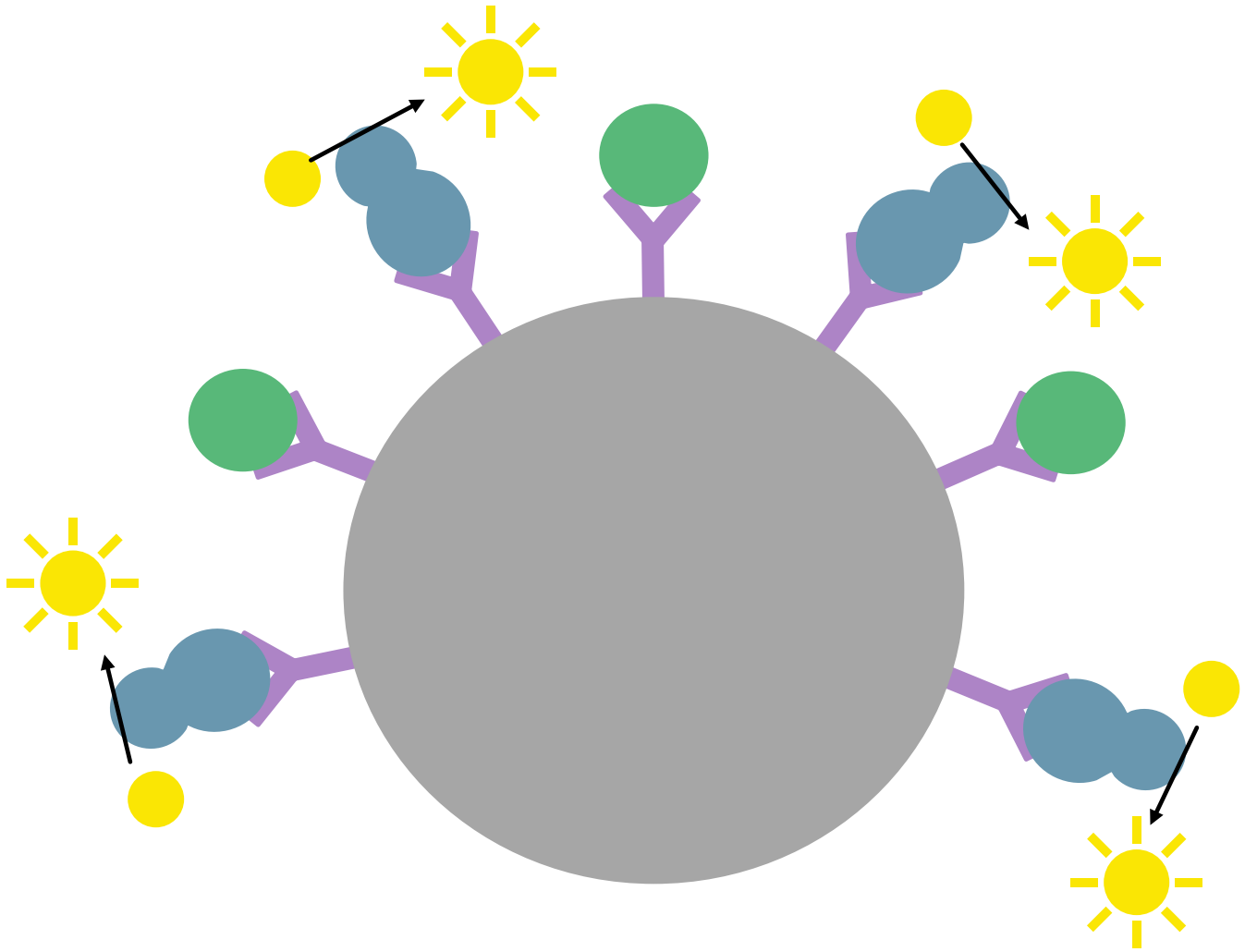
2) Wash

Competitive IA for Estriol



3) Add Lumi-Phos 530

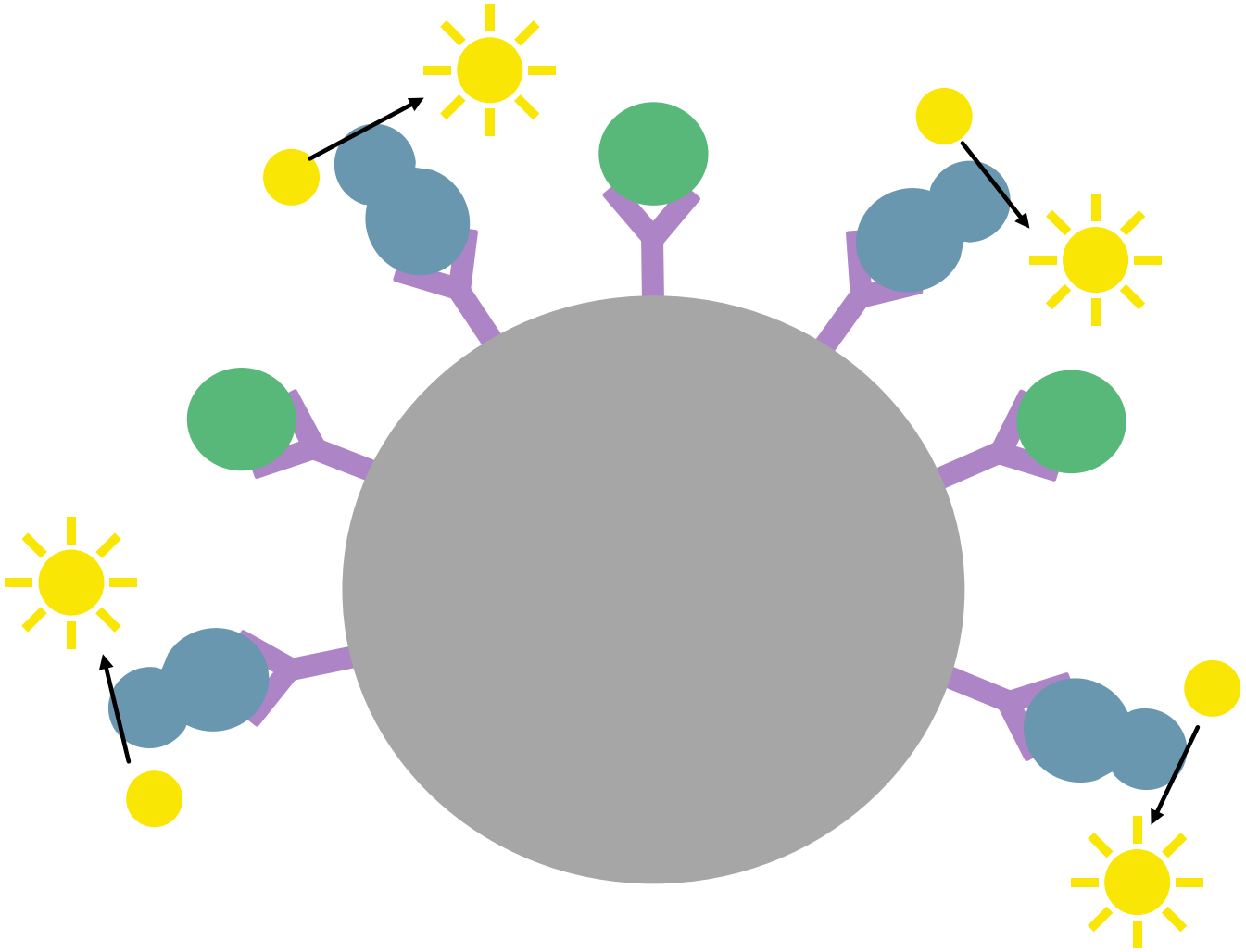
Competitive IA for Estriol



3) Add Lumi-Phos 530

4) Measure luminescence with luminometer

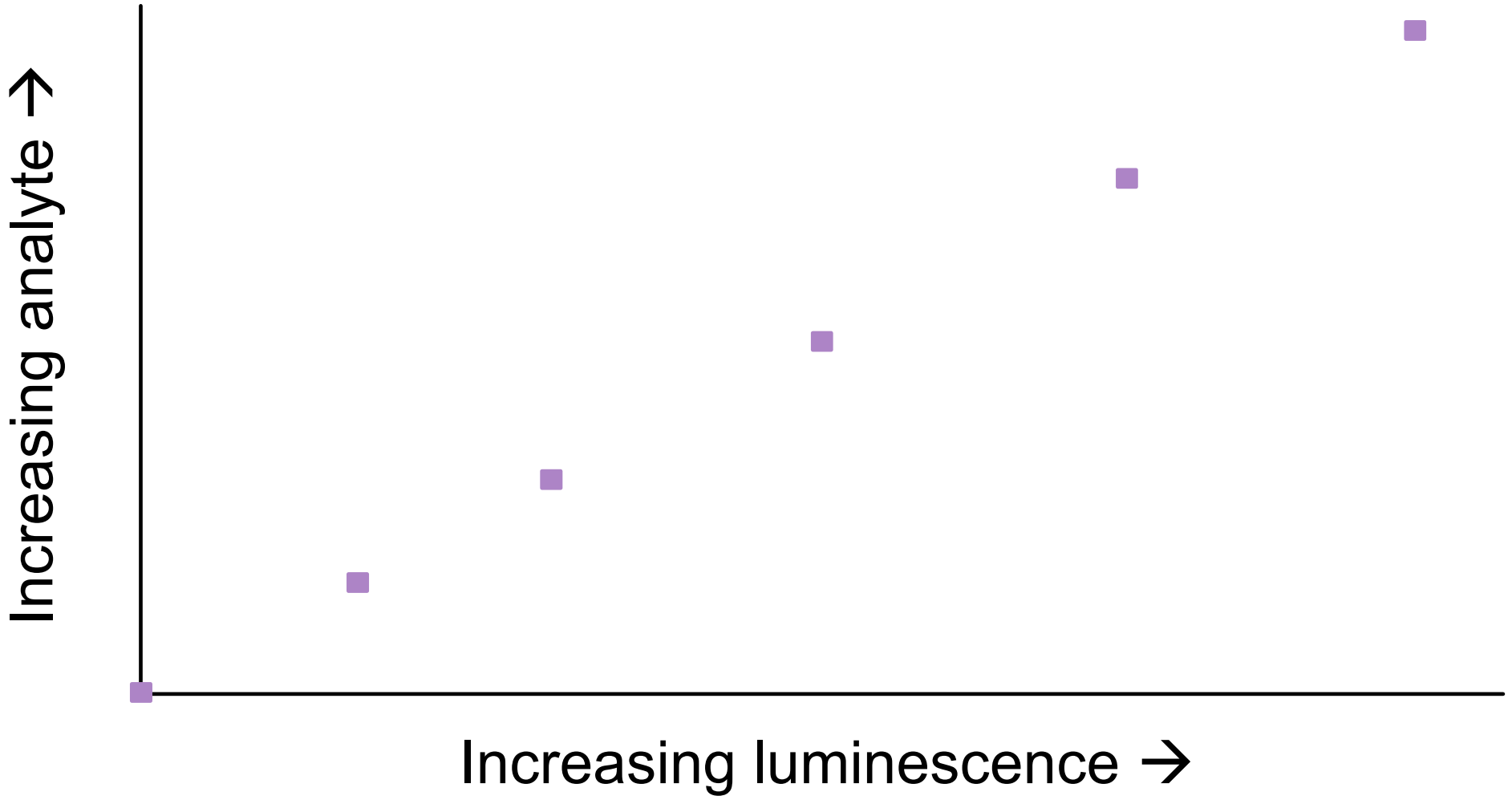
Competitive IA for Estriol



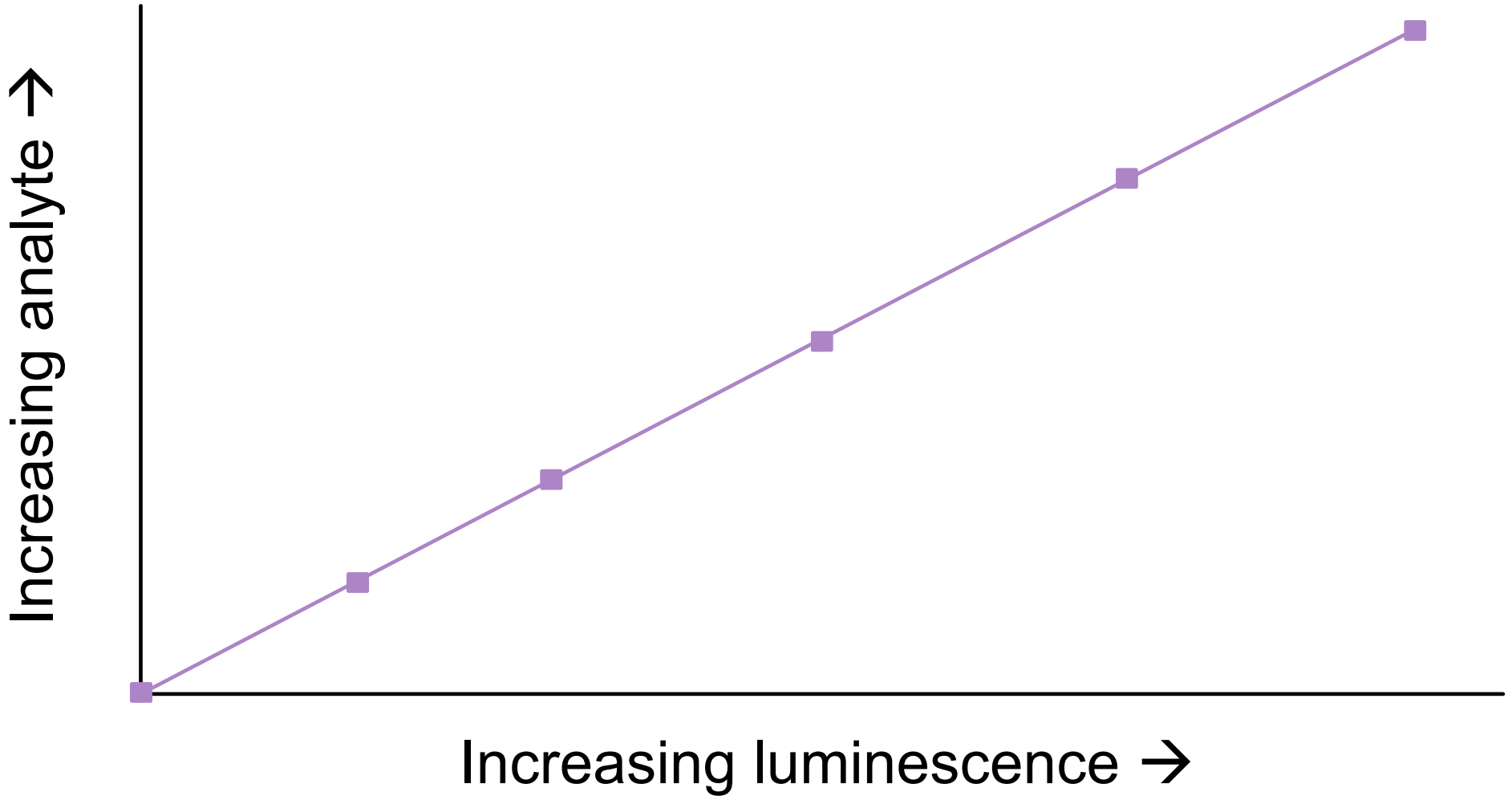
- Indirect correlation:
  ↑ luminescence = ↓ Estriol
- Quantitate by comparing to calibration curve

Calibration Curves

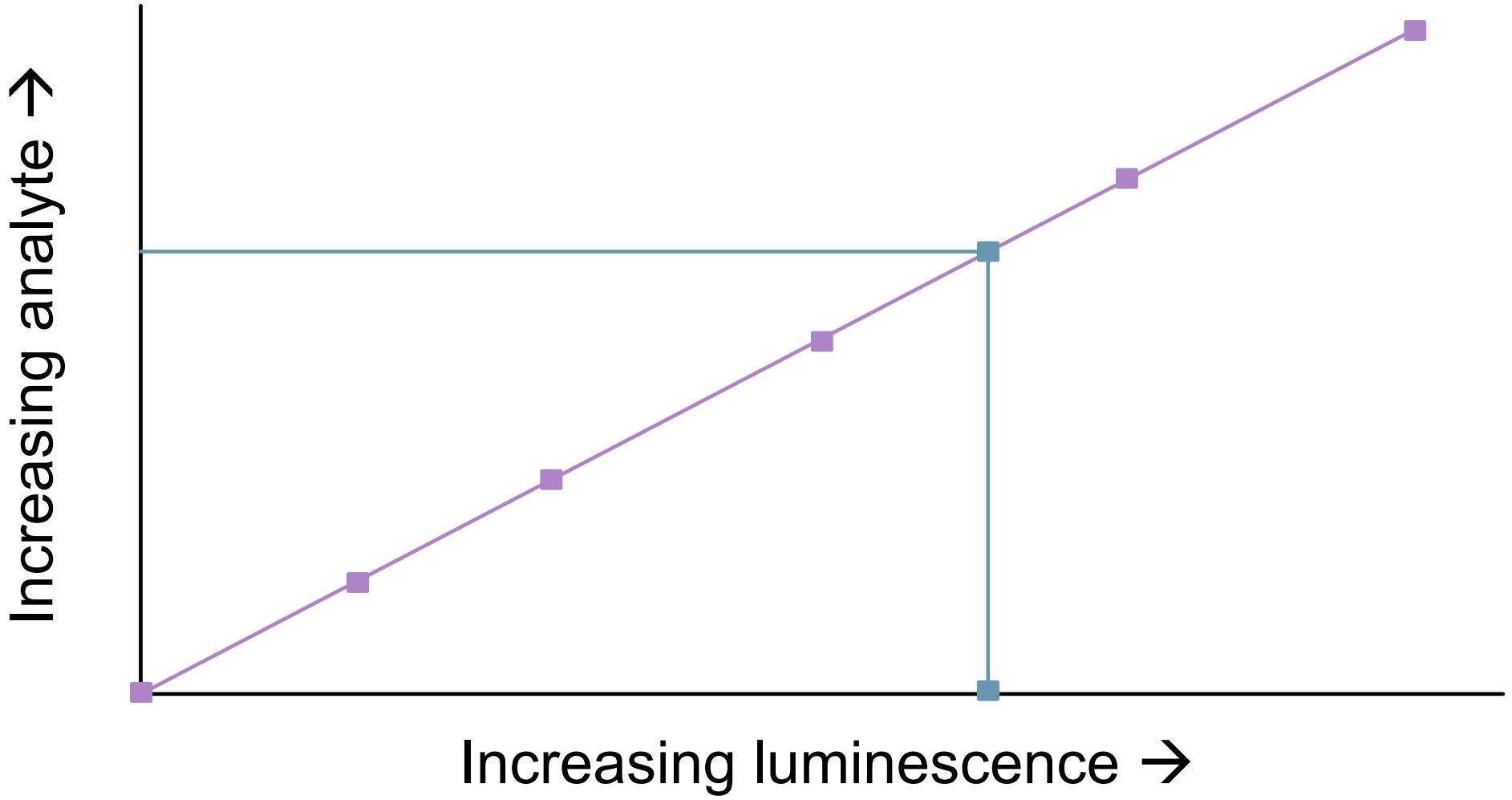
Calibration Curve - Direct



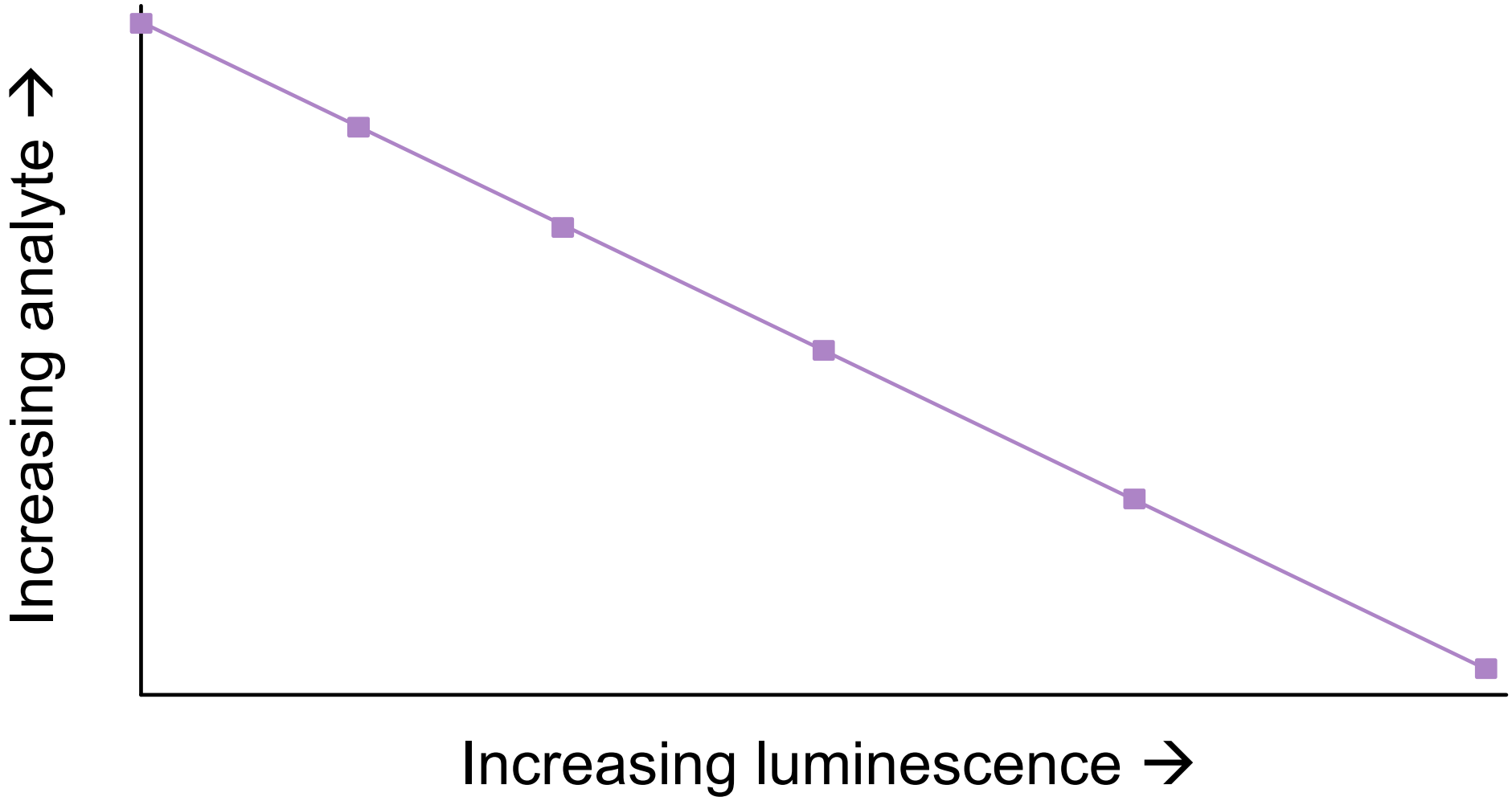
Calibration Curve - Direct



Calibration Curve - Direct



Calibration Curve - Indirect



Interpretation of Results

Multiple of the Median

Multiple of the Median (MoM)

- Analytes differ based on:
  - Gestational age (GA) } Included in MoM calculation
  - Maternal weight
  - Race
  - Diabetes status
  - Number of fetuses

} Adjustment factor after MoM calculation

Multiple of the Median (MoM)

- MoM standardizes results by gestational age

$$\text{MoM} = \frac{\text{Patient's analyte concentration}}{\text{Median analyte concentration for GA}}$$

MoM Examples

Patient's analyte concentration	Median analyte concentration	MoM
60	100	

MoM Examples

Patient's analyte concentration	Median analyte concentration	MoM
60	100	$\frac{60}{100} = 0.6$

MoM Examples

Patient's analyte concentration	Median analyte concentration	MoM
60	100	$\frac{60}{100} = 0.6$
110	100	

MoM Examples

Patient's analyte concentration	Median analyte concentration	MoM
60	100	$\frac{60}{100} = 0.6$
110	100	$\frac{110}{100} = 1.1$

MoM Examples

Patient's analyte concentration	Median analyte concentration	MoM
60	100	$\frac{60}{100} = 0.6$
110	100	$\frac{110}{100} = 1.1$
110	200	

MoM Examples

Patient's analyte concentration	Median analyte concentration	MoM
60	100	$\frac{60}{100} = 0.6$
110	100	$\frac{110}{100} = 1.1$
110	200	$\frac{110}{200} = 0.55$

Wrong Gestational Age?

- An incorrect gestational age will throw off MoM calculations

Gestational Age	Patient's analyte concentration	Median analyte concentration	MoM
15w0d	100	100	1

Wrong Gestational Age?

- An incorrect gestational age will throw off MoM calculations

Gestational Age	Patient's analyte concentration	Median analyte concentration	MoM
15w0d	100	100	1
18w0d	100	300	0.33

Disease Interpretation

Disease Interpretation

1st Trimester	NT	PAPP-A	β-hCG
Trisomy 21	↑	↓	↑
Trisomy 18	↑	↓	↓
Trisomy 13	↑	↓	↓

2nd Trimester	AFP	β-hCG	Estriol	Inhibin A
ONTD	↑	-	-	-
Trisomy 21	↓	↑	↓	↑
Trisomy 18	↓	↓	↓	- / ↓*

Clarke, Messerlian, Baldwin

*Not included in risk calculation

Relative Risk for Trisomies

Relative Risk for Trisomies

- Report a relative risk for each trisomy
- Calculated using population statistics

Relative Risk for Trisomies

1) Pre-test odds (maternal age chart)

Trisomy Risk by Maternal Age

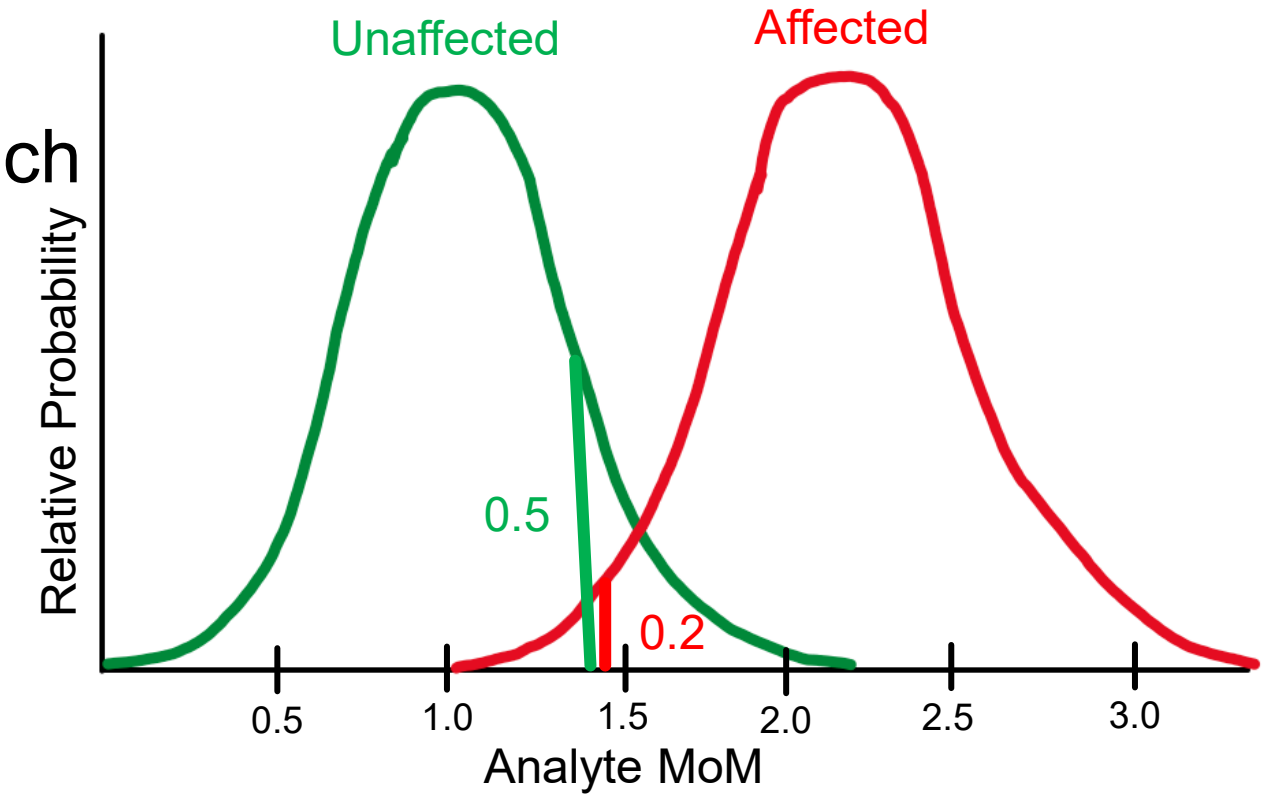
Maternal Age	Trisomy 21 Risk (1:n)	Trisomy 18 Risk (1:n)	Trisomy 13 Risk (1:n)	Combined Trisomy Risk (1:n)
18	1495	9010	13,700	1175
19	1490	8985	13,670	1165
20	1475	8960	13,635	1160
21	1460	8930	13,580	1150
22	1440	8885	13,510	1135
23	1415	8825	13,410	1115
24	1380	8745	13,275	1095
25	1340	8630	13,090	1065
26	1285	8480	12,840	1025
27	1220	8280	12,500	980
28	1140	8010	12,050	920
29	1045	7660	11,470	850
30	935	7215	10,735	770
31	815	6655	9830	675
32	695	5990	8770	580
33	570	5220	7585	480
34	455	4380	6345	385
35	350	3530	5130	300
36	265	2725	4030	225
37	195	2025	3100	170
38	145	1455	2370	125
39	110	1035	1825	94
40	85	735	1430	72
41	66	530	1160	56
42	54	395	970	45
43	45	310	840	37
44	39	250	745	32
45	34	215	685	28
46	31	185	640	25
47	29	170	610	24
48	27	155	590	22
49	26	150	570	21

Relative Risk Percentage for Trisomies

1) Pre-test odds (1 in 935)
2) Calculate MoM (1.4)

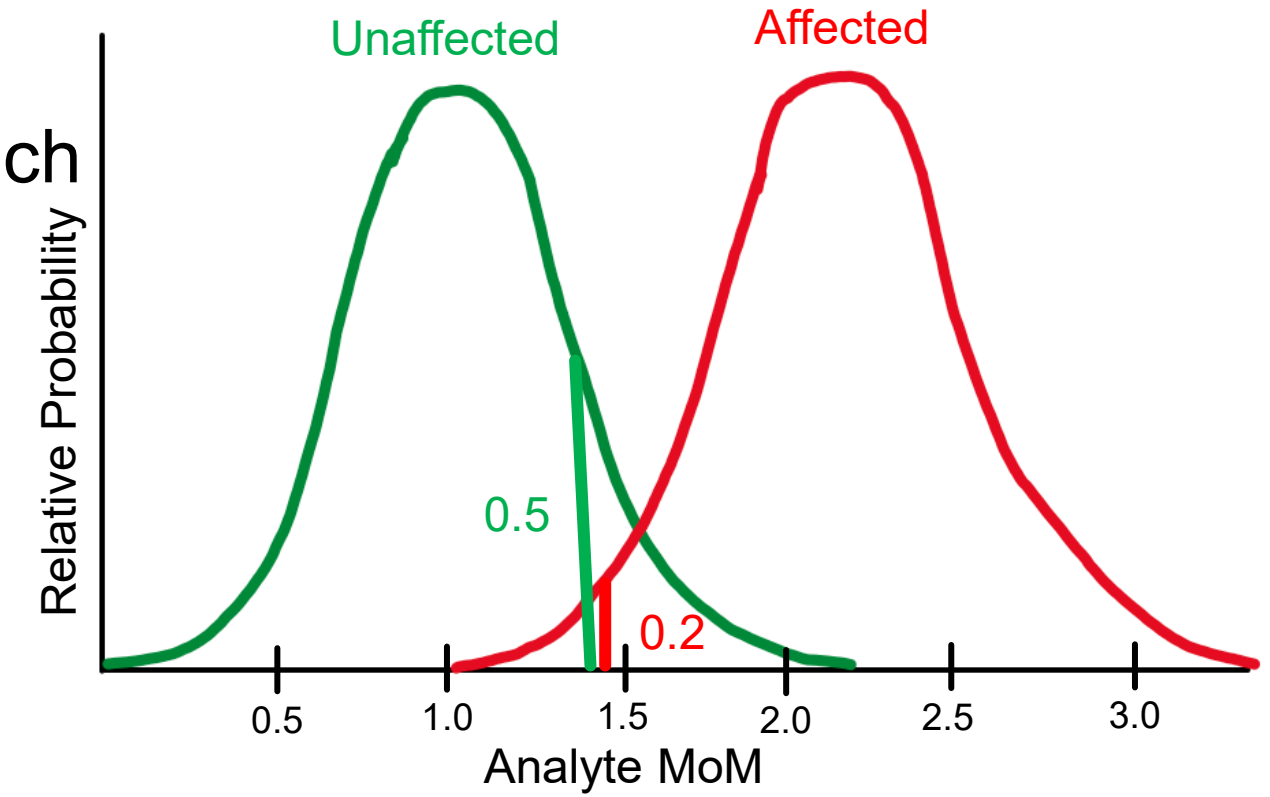
Relative Risk Percentage for Trisomies

1) Pre-test odds (1 in 935)
2) Calculate MoM (1.4)
3) Calculate likelihood ratio for each analyte using the MoM
   - $LR = \frac{\text{Probility of affected}}{\text{Probability of unaffected}}$
   - $LR = \frac{0.2}{0.5} = 0.4$



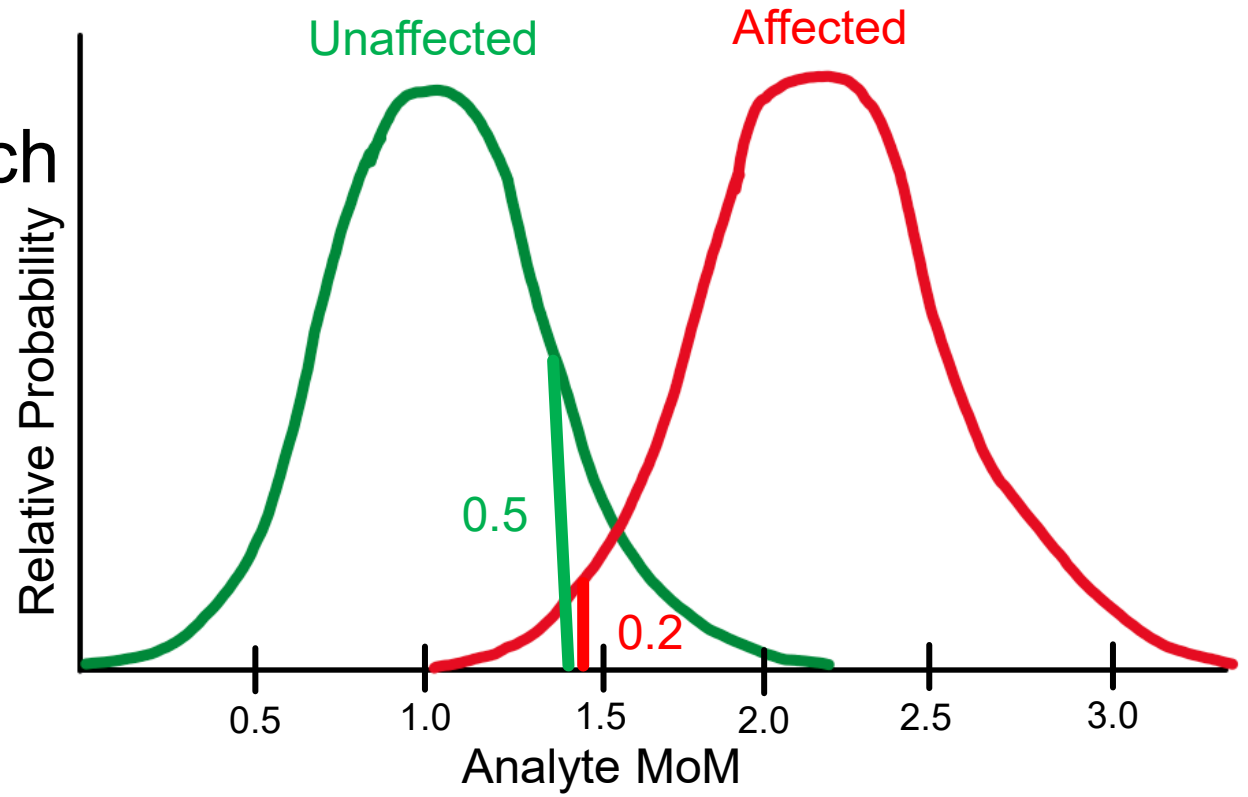
Relative Risk Percentage for Trisomies

1) Pre-test odds (1 in 935)
2) Calculate MoM (1.4)
3) Calculate likelihood ratio for each analyte using the MoM
   - For multiple analytes, multiply the LR of each together
   - Ex: 0.4 x 1.9 x 2.3 x 3.5 = 6.1



Relative Risk Percentage for Trisomies

1) Pre-test odds (1 in 935)
2) Calculate MoM (1.4)
3) Calculate likelihood ratio for each analyte using the MoM (6.1)
4) RR = Pre-test odds x LR
   - RR = 1/935 x 6.1 = 1 in 153 = 0.6%



Relative Risk Cutoff

- At what point is the relative risk “positive?”
- Often the risk of Down Syndrome for a 35-year-old is used as the cutoff (1:270)
- May raise or lower the cutoff to customize testing

Test Performance

Detection Rates for ONTD

Testing for	Sensitivity
ONTD	95%
Anencephaly	97%
Open spina bifida	99%
Abdominal wall defects	40-79%

Detection Rates for Down Syndrome

Test	Detection Rate	False Positive Rate	T21 Cutoff
Combined (1st)	85%	6%	1/230
Quad (2nd)	81%	4-5%	1/150
Integrated	87%	1.0%	1/110
Contingent	63% (1st) 23% (2nd) 86% (Total)	0.6% (1st) 1.0% (2nd) 1.6% (Total)	1/25 (1st) 1/110 (2nd)

Baldwin

Positive Predictive Value (Down Syndrome)

- Positive predictive values at 85% detection rate
- Good screening test

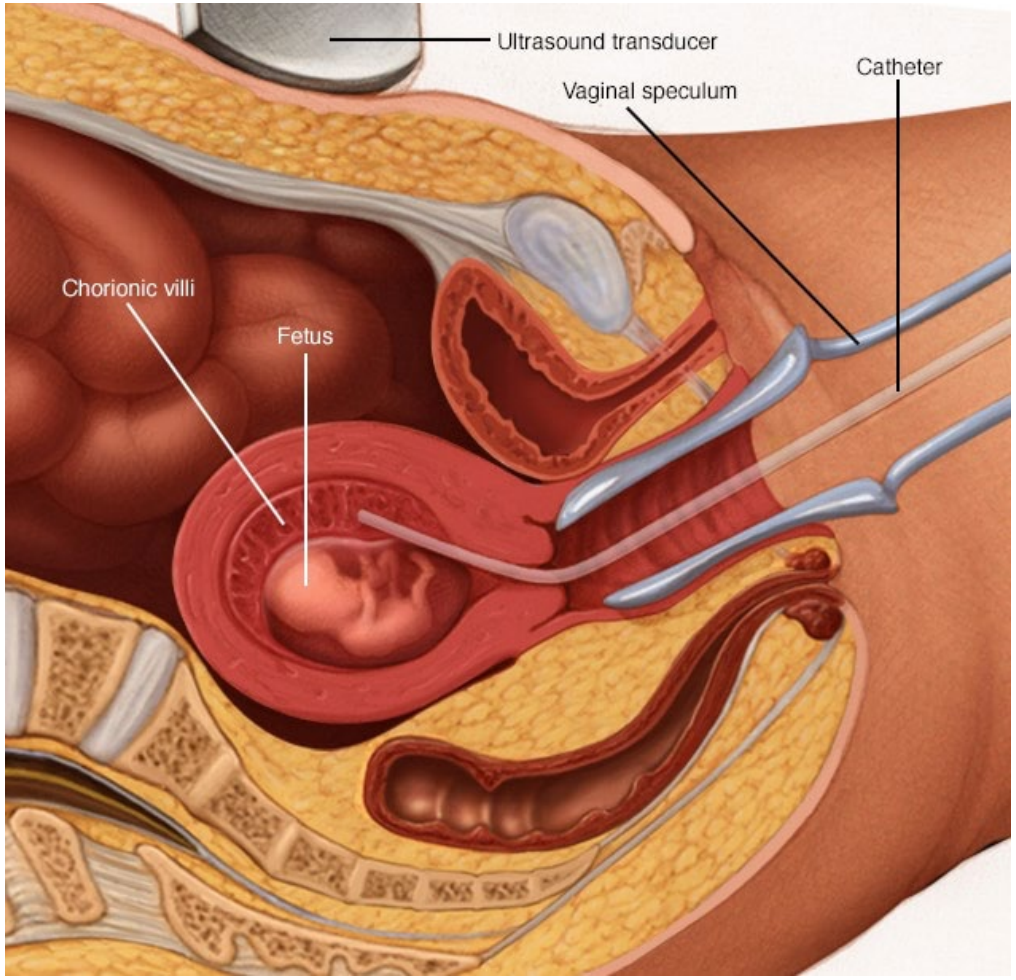
Integrated	17%
Combined (1st)	3%
Quad (2nd)	3%

Wald

What Next?

Trisomy Confirmation

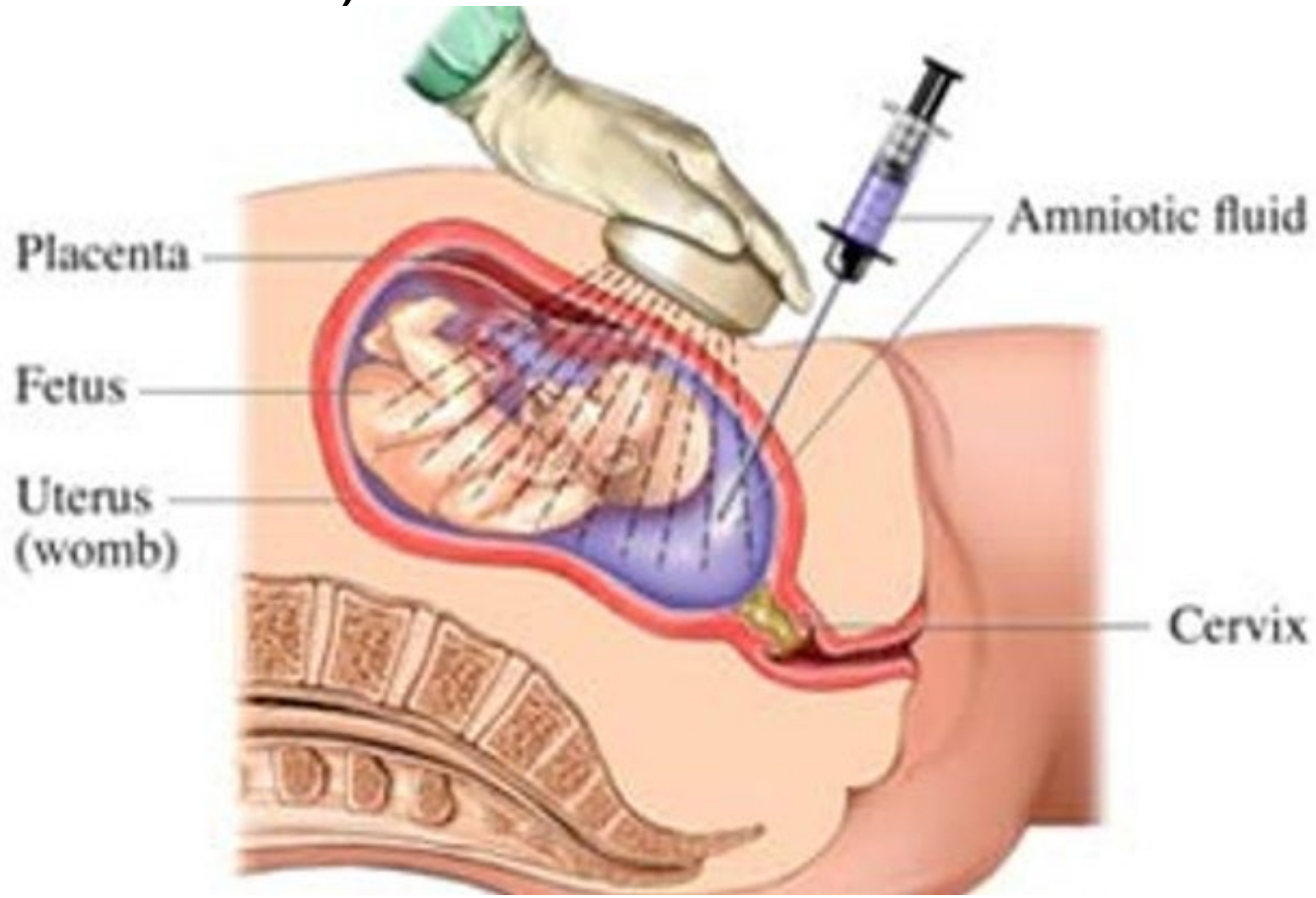
- Chorionic villus sampling (10-14 weeks)
  - Chromosomal analysis
  - Fetal loss rate up to 2%



https://www.mayoclinic.org/tests-procedures/chorionic-villus-sampling/about/pac-20393533

Trisomy Confirmation

- Chorionic villus sampling (10-14 weeks)
  - Chromosomal analysis
  - Fetal loss rate up to 2%
- Amniocentesis (>15 weeks)
  - Chromosomal analysis
  - Fetal loss rate up to 1%



https://www.madeformums.com/pregnancy/what-is-amniocentesis/

ONTD Confirmation

- Ultrasound to confirm GA and look for abnormalities
- Amniocentesis
  - Measure AFP
    - If high, also measure acetylcholinesterase (AChE)
      - If high, ONTD confirmed
  - Chromosomal analysis
    - Increased risk of chromosomal abnormalities

One More Option

Non-invasive Prenatal Testing (NIPT)

- Introduced in 2011
- Offered to all pregnant women, not just high-risk patients
  - Option for those who screen positive on 1st or 2nd trimester screen, but don't want to do invasive testing
- Detects fetal cell free DNA (cfDNA) in maternal blood
  - Rises with gestational age
  - ~11-13% of cfDNA is fetal at 1st to 2nd trimester transition
- Each chromosome makes up a certain percentage of cfDNA
  - Chromosome percentage increased = trisomy

Palomaki, Grenache, Norton

Non-invasive Prenatal Testing (NIPT)

	Detection Rate	False Positive Rate	Positive Predictive Value	Negative Predictive Value
Trisomy 21	99.5%	0.05%	85%	>99.9%
Trisomy 18	97.7%	0.04%	69%	>99.9%
Trisomy 13	96.1%	0.06%	33%	>99.9%

- High detection rate
- Low false positive rate
- Decent positive predictive value = screening test only
- High negative predictive value

Palomaki, Grenache

Non-invasive Prenatal Testing (NIPT)

- Why not replace serum screening with NIPT?
  - Cost
  - Insurance coverage
  - Availability
  - Can’t detect ONTD
- Ultimately, which (if any) screening test to do is a personal decision

Conclusion

Conclusion

- Prenatal screening detects:
  - Open neural tube defects
  - Trisomy 21
  - Trisomy 18
  - Trisomy 13
- Screening tests:
  - Combined screen (1^{st}): NT, PAPP-A, β-hCG
  - Quad screen (2^{nd}): AFP, β-hCG, uE_3, DIA
  - Non-invasive prenatal testing

Conclusion

- Result reporting
  - Multiple of the Median (MoM)
  - Relative risk
- Confirmation testing
  - Chorionic villus sampling (10-14w)
  - Amniocentesis (>15w)

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