Semmelweis University Department of Laboratory Medicine

Founded: 2010

- Major profile: diagnostics
  - 6 Million tests pro year
  - 550,000 patients
- Up to 65 per cent of lab investigations performed at Semmelweis University + national centre for special tests
- Education: one semester, 14 lectures started in 2010

Informations on the Institute

Office: **Nagyvárad square, Floor 14.**

Head: prof. Barna Vasarhelyi, DSc

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For further info: semmelweis.hu\laboratorium

Lab at Transplantation Clinic

Central Lab Pest

Microbiology

Immune lab

Office for education

Exam

- Test exam (single choice)
  - 50 questions from a pool of 300 questions
  - in an electronic way
- See details on homepage
  - Circular emails in case of any extra notification

Medical care

The cumulative number of fatalities caused by
- Accidents
- Breast cancer
- HIV infection

is LOWER, than those caused by maltreatment
(US figure).

Estimated number: 50 – 100 thousand per year
Majority of cases are due to diagnostic error.
The 70% of diagnostic tests are performed in the lab.
Prevalence of incorrect results:
1/ 164 - 1/330 measurements

Test request

Result interpretation

Result

Black box: the lab

Sampling

Test request

Result interpretation

Result

Sampling

Specific problem: point of care testing (POCT)
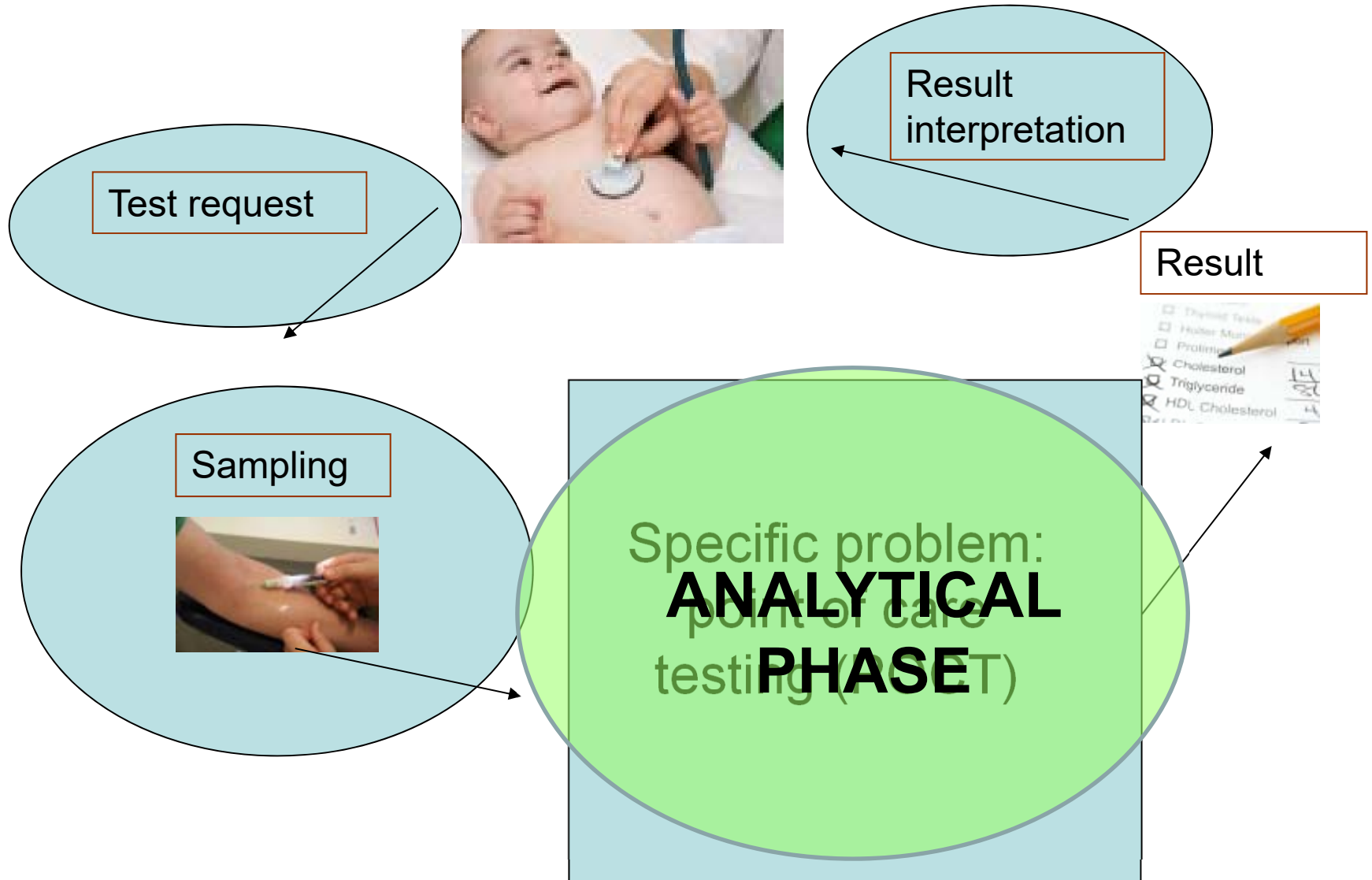
PREANALYTICS

Test request

Sampling

Result interpretation

Result

Specific problem: point of care testing (POCT)

Test request

Result
interpretation

Result

Sampling

Specific problem:
point of care
testing (POCT)

ANALYTICAL PHASE

POSTANALYTICAL PHASE

Result interpretation

Result

Test request

Sampling

Specific problem:
point of care
testing (POCT)

POSTANALYTICAL PHASE

Result interpretation

Test request

PREAN...

Result

Sampling

Specific problem: point of care testing (POCT)

ANALYTICAL PHASE

The preanalytical phase is responsible for up to 84.5 per cent of errors.

OCCURRENCE OF FAULTS WITH LAB TESTS

M. Plebani / Clinica Chimica Acta 404 (2009) 16–23
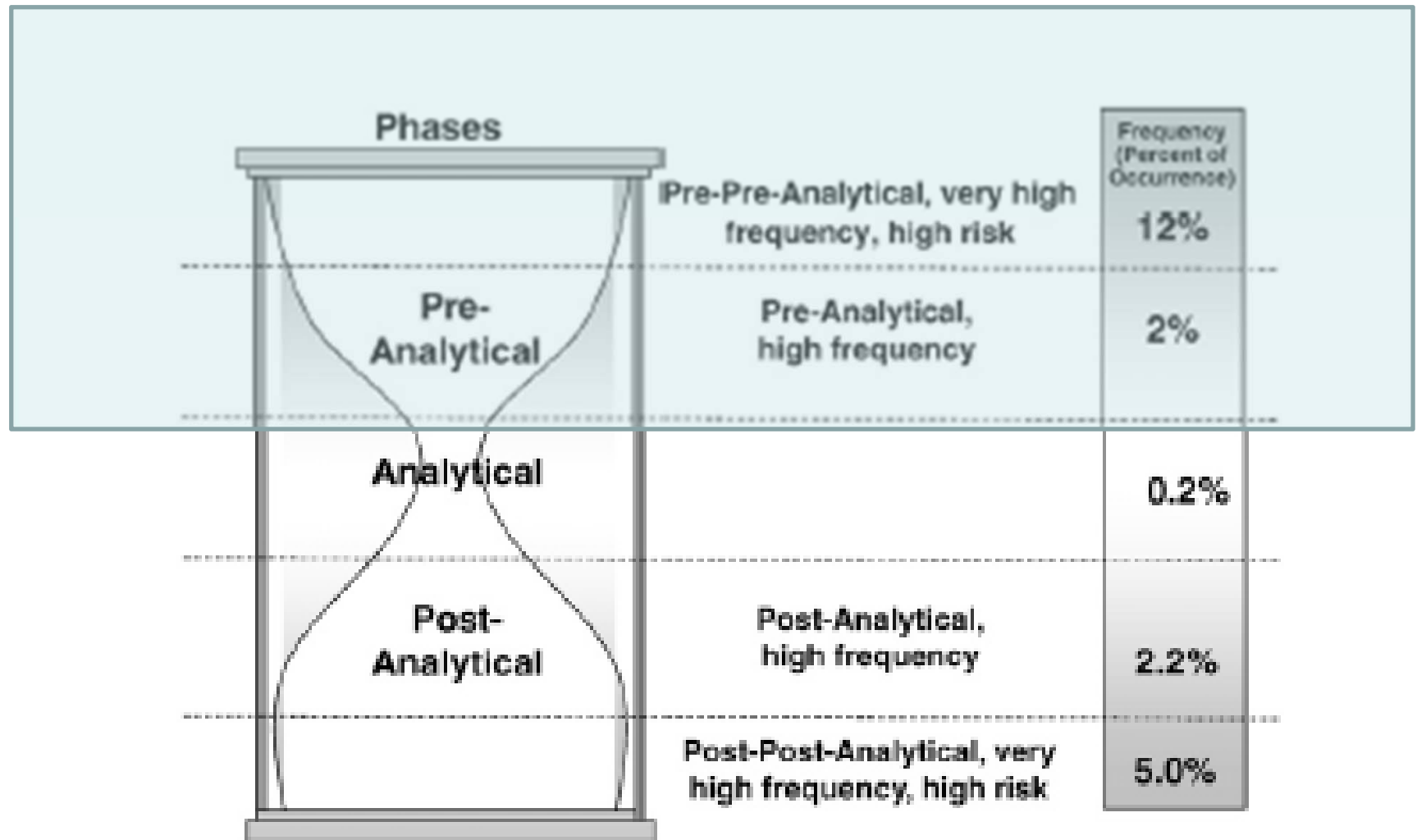
Phase	Percentage of all missed diagnosis
Pre-Analytic	
-Failure to order appropriate diagnostic or laboratory tests	55
-Adequate diagnostic or laboratory tests ordered but not performed	9
Analytic	
-Diagnostic or laboratory test performed incorrectly	8
Post-Analytic	
-Incorrect interpretation of diagnostic or laboratory tests	37
-Responsible provider did not receive diagnostic or laboratory test results.	12

Factors having an effect on lab test results

Major factors responsible for preanalytical variations

DOCTOR:

1. Indication of lab test

PATIENT:

1. Biological variability

2. Environmental factors

SAMPLING:

1. Patient ID

2. Patient preparation

3. Sampling and transport

Factors responsible for preanalytical errors - DOCTOR

Should know:

     What (what kind of test)

     What for (the goal)

     When (or how frequent)

     Source of specimen (blood, urine)

     Destination (lab the sample to be sent)

     Costs (…)

Factors responsible for preanalytical errors - DOCTOR

Why does somebody ask lab test?

- Establish the diagnosis

- Monitoring the therapy / condition

- Screening (exclusion of a disease)

The parameter to be tested depends on the goal [see: lecture on postanalytics]

Factors responsible for preanalytical errors - DOCTOR

When is a lab test justified?

If it adds significant info supporting the clinical decision making

Component	Note	Contraindication (example)
significant	ie: information essential for the decision making	Requesting vitamin D-levels in infection
adds	Ie: data available just by this way or by another more expensive /invasive test or	Cholesterol levels in acute infarction
Decision making	Ie: it is time to decide anything]	Tumor marker levels in terminal phase of malignancy

Factors responsible for preanalytical errors - DOCTOR

What does one expect from a lab test?

- To be as specific and as sensitive as it is possible. Discriminate the disease from the healthy condition or recognize condition requiring intervention

- An appropriate marker should be selected.

Factors responsible for preanalytical errors - DOCTOR

Marker: what does one expect from a biomarker?

- Easily accessible (e.g. present in blood)
- Easily measured (does not require specifif machine)
- Stable and easy to repeat
- Influencing factors are known
- Reference range is known
- Cheap

Factors responsible for preanalytical errors - DOCTOR

- Currently: app. 400 parameters measured routinely
- Semmelweis University: 6 million test per year
- Incidence of unnecessary test: estimated 10 – 40%

Factors responsible for preanalytical errors - DOCTOR

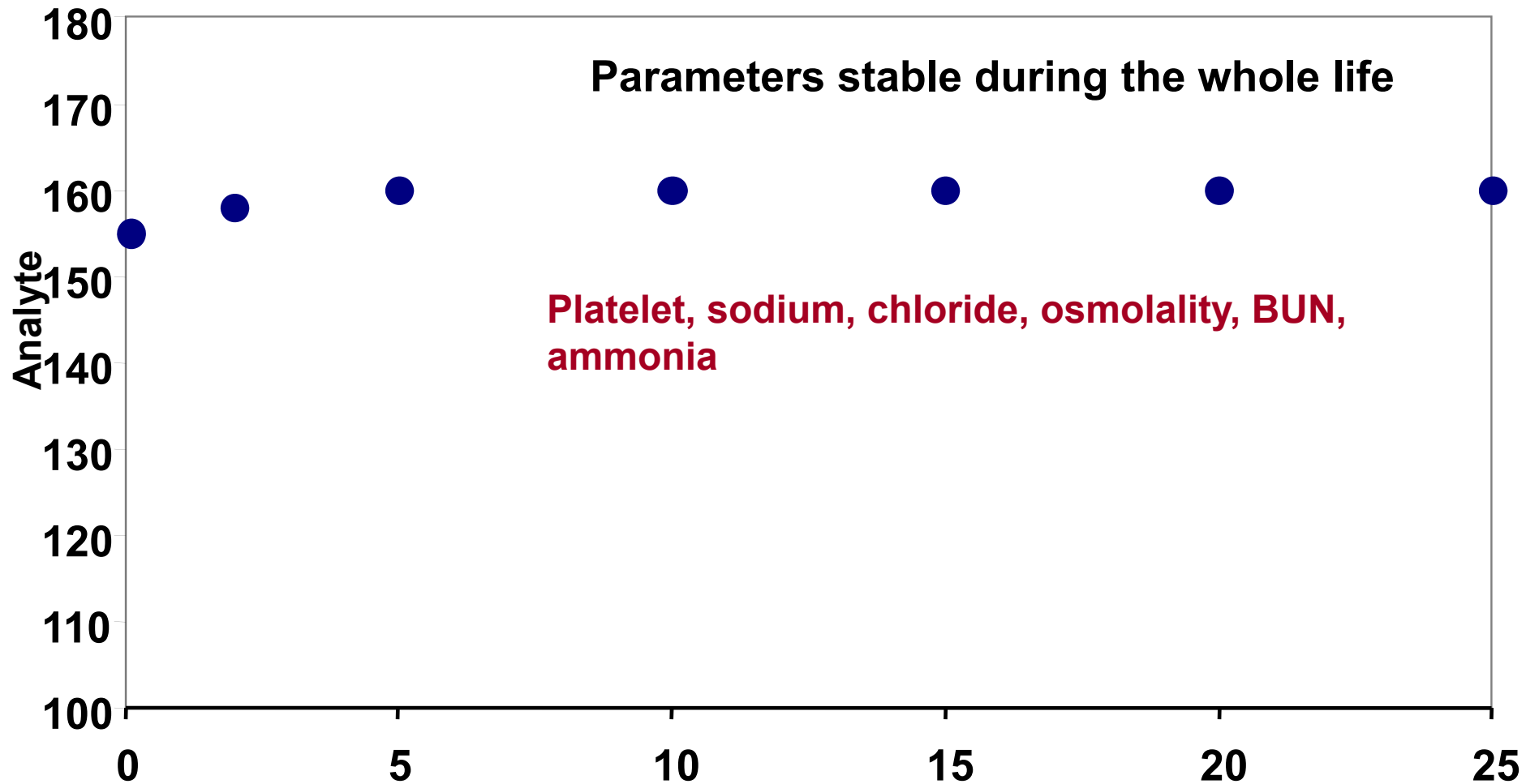**Efforts to decrease numbers of unnecessary tests**

- Request linked to specific conditions
- Restricted lists
- Limitations of repeated requesting
- Education

Factors responsible for preanalytical errors - PATIENT

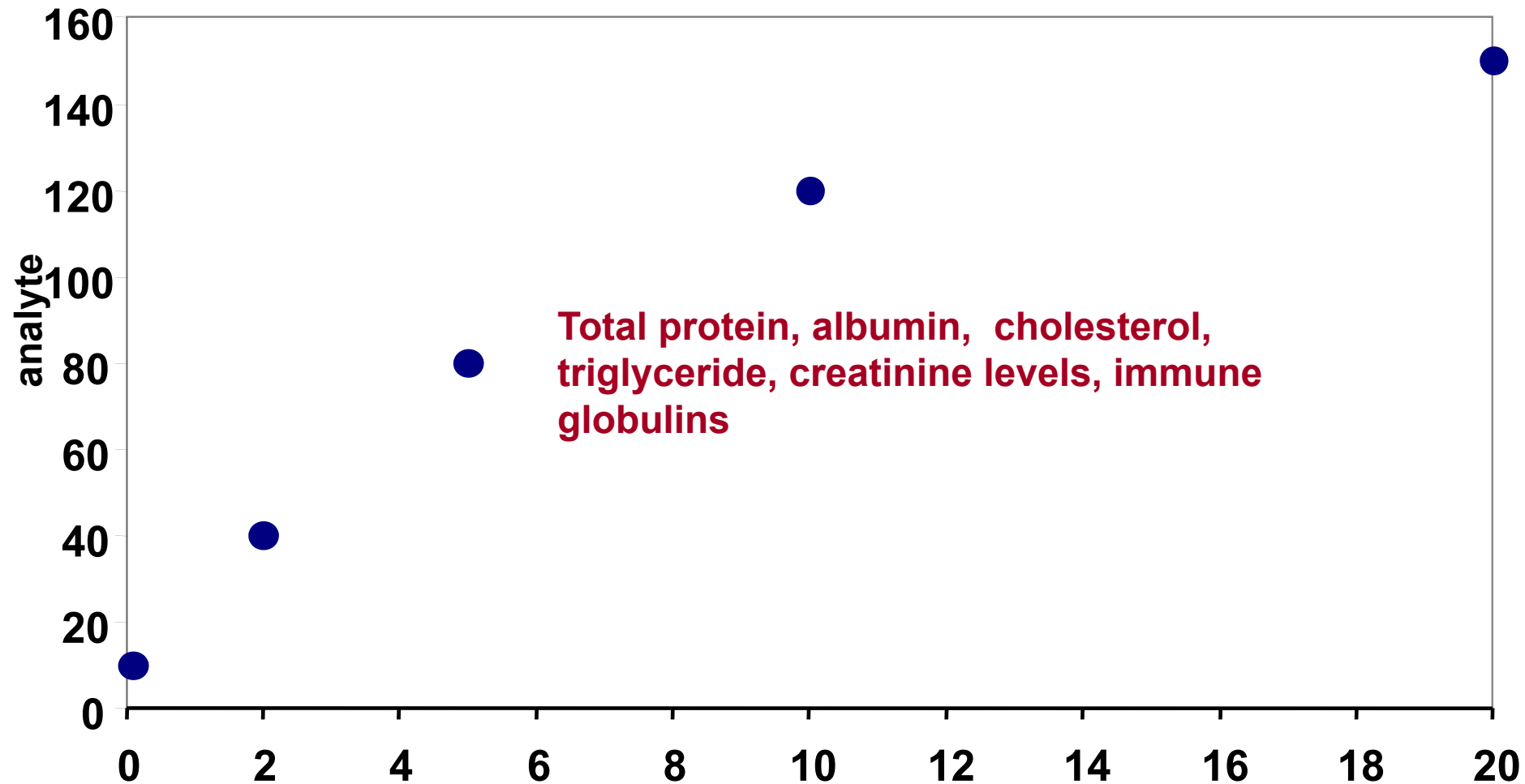Biological variability and environmental conditions

- Gender, period in fertile women
- Age
- Circadian / seasonal fluctuation
- Posture and prior exercise
- Diet

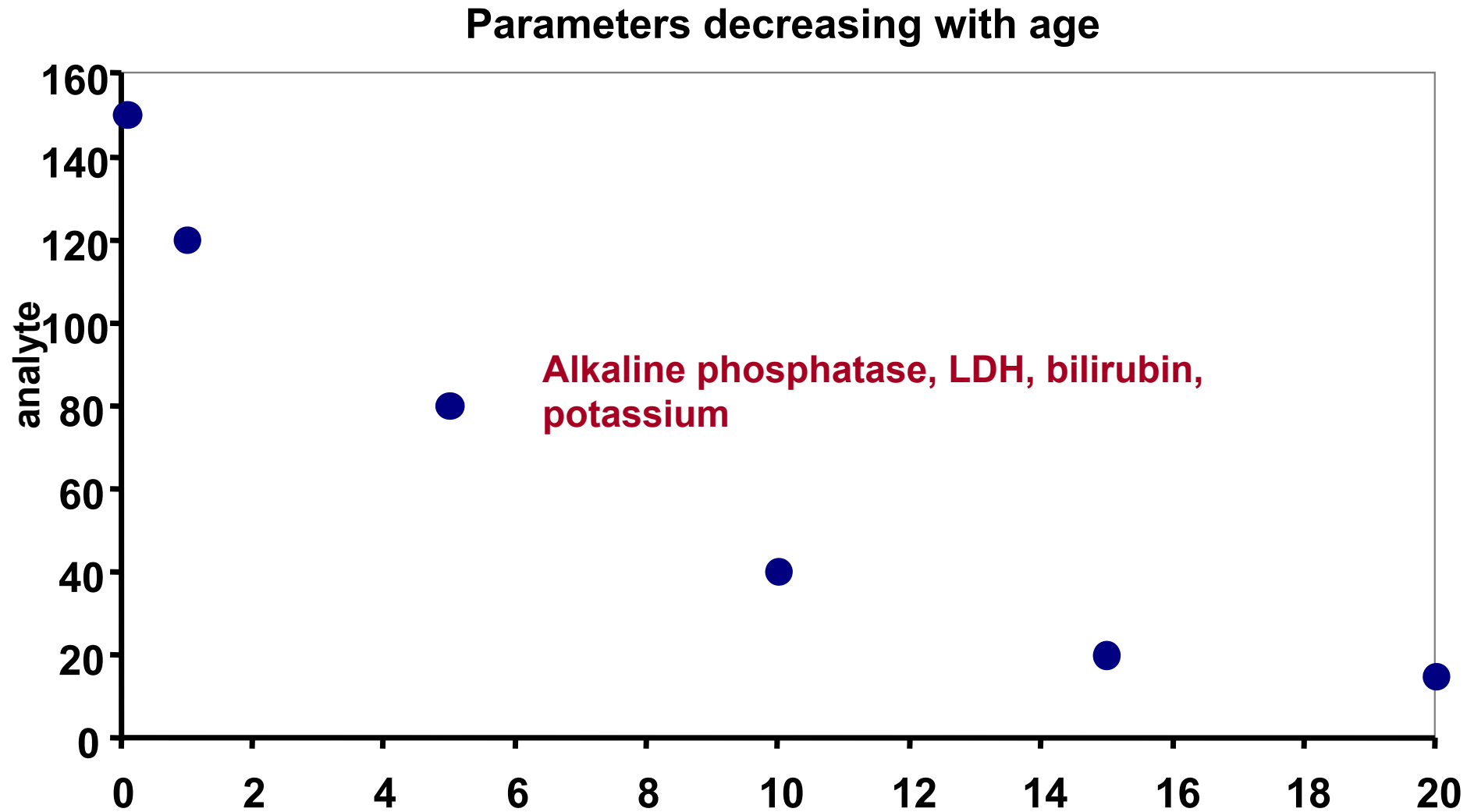# Factors responsible for preanalytical variations - PATIENT

Parameters stable during the whole life

Platelet, sodium, chloride, osmolality, BUN, ammonia

Analyte (y-axis: 100 to 180)

x-axis: 0 to 25

Factors responsible for preanalytical variations - PATIENT

Parameters increasing with age

Total protein, albumin, cholesterol, triglyceride, creatinine levels, immune globulins

Factors responsible for preanalytical variations - PATIENT

Parameters decreasing with age

Alkaline phosphatase, LDH, bilirubin, potassium

Factors responsible for preanalytical variations - PATIENT Daily fluctuation

+ cortisol-regulated cytokines & bone markers

Maximum in the morning			
ACTH	200%	Epinephrine	20%
Renin	140%	Hemoglobin	20%
Norepinephrine	120%	Hematocrit	20%
Prolactin	100%	Leukocyte count	20%
Aldosterone	80%	Protein	20%
Cortisol and related parameters	50%	Tyroxine (T4)	20%
Testosterone	50%	Bilirubin	20%
Maximum at noon			
Iron	100%	Potassium	15%
Eosinophil granulocytes	30%		
Maximum in the evening			
Uric acid	50%	Acid phosphatase	200%
TSH	50%	CK	100%

Posture

In sitting position the levels of the majority of cellular and large molecules (eg. albumin) increase by 5 – 15%; free drug levels and small molecule levels remain unaltered.

In lying position there is a dilution effect: eg. cholesterol, triglíceride, decrease (10-12%).

The effect of position depends on plasma volumes (water compartments change quickly in children).

Posture

The level is increased when the lying patient stands up	
<10%	hemoglobin, leukocyte count, total calcium, ASAT, ALAT, tyroxine, IgG, IgA, albumin, total protein, cholesterol, triglycerides
10 – 20%	hematocrit, red cell count, apolipoproteins
>50%	epinephrine, renin

Factors responsible for preanalytical variations - SAMPLE

HOW TO TAKE BLOOD SPECIMEN? – see video

https://www.youtube.com/watch?v=_8ZsqXFqvQM

STANDARDIZING SAMPLING

- Most common complication: bruises
- Should press swab for a few minutes
- DO NOT BEND the arm (keep extended)

Sampling error

The majority of preanalytical errors (60%) occurring with blood samples are due to sampling problems:

Insufficient quality / quantity of blood

Haemolysed or lipaemic sample	54%
Insufficient volume	21%
Inappropriate tube	13%
Clots	5%
Misidentification	1-2%
Other	4-5%

Sampling in fasting state: WHY is it important?

Adults: 12 hour fasting state is recommended:

Alkaline phosphatase
Cholesterol
Dopamine
Iron
Glucose
Uric acid
Insulin
Potassium
Cortisol
CRH-stimulation test
Inorganic phosphate
Triglyceride

IF sampling is done after a meal:
lipemic serum that interferes the tests

Hemolysed samples

Cca 3.3% of clinical samples are haemolyzed	
64%	<0.5 g/l Hb
31%	0.5 – 3 g/l Hb
5%	> 3 g/l Hb

About 40-70% of sample inappropriate for measurements are haemolysed.

Distribution of test ordering departments of 8440 haemolysed samples

Ambulatory patients	0,1%
Dialysis	1,5%
Intensive care	5,4%
Pediatric ward	8,6%
Emergency department	8,8%

Clin Chem Lab Med. 2009;47(5):616-8

HEMOLYSED SAMPLE – DEFINITION

Free hemoglobin, upper limit	0.02 g/l (plasma)
	0.05 g/l (serum)
Visible haemolysis: (when 0.5% of red cells is haemolysed)	> 0.30-0.50 g/l

HEMOLYSED SAMPLES
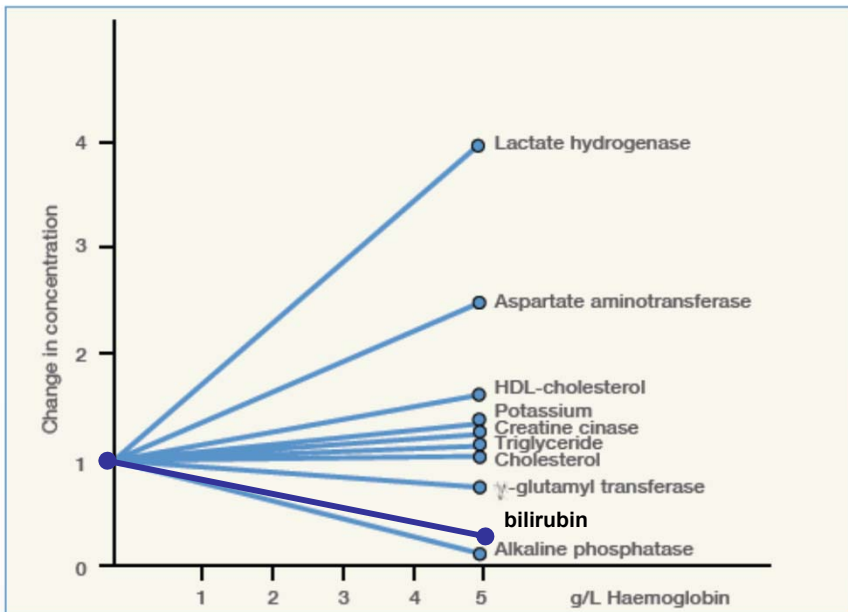
Change of parameters with free haemoglobin

Samples with varying degree of haemolysis

1. Serum levels of intracellular analytes increase

2. Haemoglobin interferes with photometry

3. Hemolysis interferes with chemical reactions
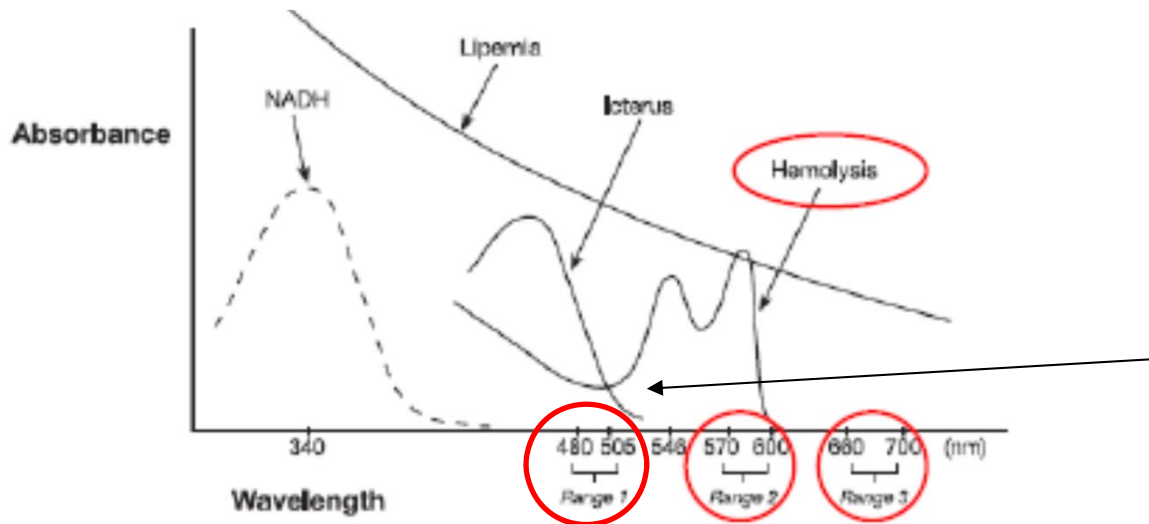
RECOGNISING THE HAEMOLYSIS

1. Inspection of sample (56%) ←————————
2. Measurement of hemolysis index (43%)
3. Potassium levels

Often unvisible

Interobserver variability (40%)

Jaundice may interfere

Over- or underestimated in serum or plasma

Specific absorbance spectrum should be obtained before measurements (option with clinical chemistry analysers) – haemolysis and lipemic indexes

PREVENTION OF HEMOLYSED SAMPLES: STANDARD CONDITIONS FOR SAMPLING

Planning for sampling:

1. Volume   ←   Micro vs. standard volumes

2. Tube   ←   Type

3. How   ←   Preparation of the patient

4. Where from   ←   Venous or capillary

VOLUME

- Volume of sample is particularly important for preterm newborns (blood volume: 80 ml/kgbw)

- Required volume is small, just a few millilitres

- Special care is required to spare sample.

TUBE TYPES

SITE of SAMPLING

- **Venous sample**: hand, cubital vein.
- To be avoided: infusion tubing.
- If tubes are used, this fact should be **documented**.
- Single tests in infants: capillary samples

STANDARDIZING SAMPLING CONDITIONS

IMPORTANT FACTORS at venous sampling

- **appropriate needle size.**
- **strangulation: up to 1 min, 3 – 4 cm distance from sampling site**

- Blood cells
- Protein molecules
- Calcium
- Protein bound substance
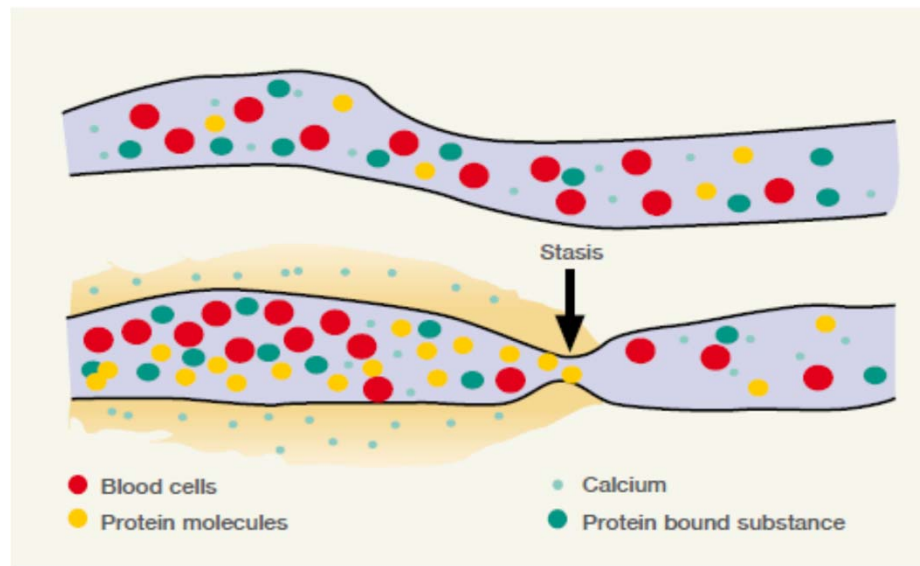
STANDARDIZING SAMPLING CONDITIONS

IMPORTANT FACTORS at venous sampling

- appropriate needle size.
- strangulation: up to 1 min, 3 – 4 cm distance from sampling site

Increase by 6-12%	Decrease by 4%
AST, CK, bilirubin, LDH, albumin, gammGT, ALP, TP, K, Mg	glucose, inorganic phosphates, leukocytes, BUN, creatinine, chloride
HEMOLYSIS!!!	

CAPILLARY SAMPLING

- Capillary sampling:

Beneficial: POCT, anxious patients, 'bad' veins

Pricking: for monitoring, single tests (e.g. blood glucose)

Incision: less pain, quicker, less traumatic. Appropriate for simultaneous tests.

Important: glucose levels are higher, potassium, total protein levels are smaller than those in serum.

www.gbo.com/preanalytics

CAPILLARY SAMPLING I.

1. First drop should be sweapt (high tissue fluid content)

2. Capillary should be in contact with blood drop, not with the skin.

3. Keep the capillary horizontally

4. Forced massage should be avoided (haemolysis, tissue fluid contamination)

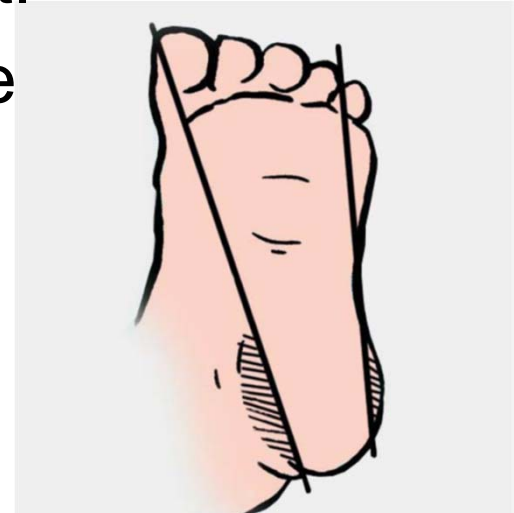5. Tube containing anticoagulants: turning several times.

www.gbo.com/preanalytics

CAPILLARY SAMPLING II.

Under 1 year of age: side of the plant.

HEEL or FINGER are not appropriate

After 1 year of age: finger III or IV,

distal part.

Should be avoided: ear, thumb,

index

CAPILLARY BLOOD SAMPLING –
Filter paper in the neonate

See video:
 https://www.youtube.com/watch?v=vxshWngJ114

Technical aspects of capillary sampling

Cleansing the area

Isopropil alcohol, 70%. (Ethanol should be avoided as it damages the skin at small infant)

DO NOT blow the area.

Wait until it dries up (risk of haemolysis)

Improving the blood supply

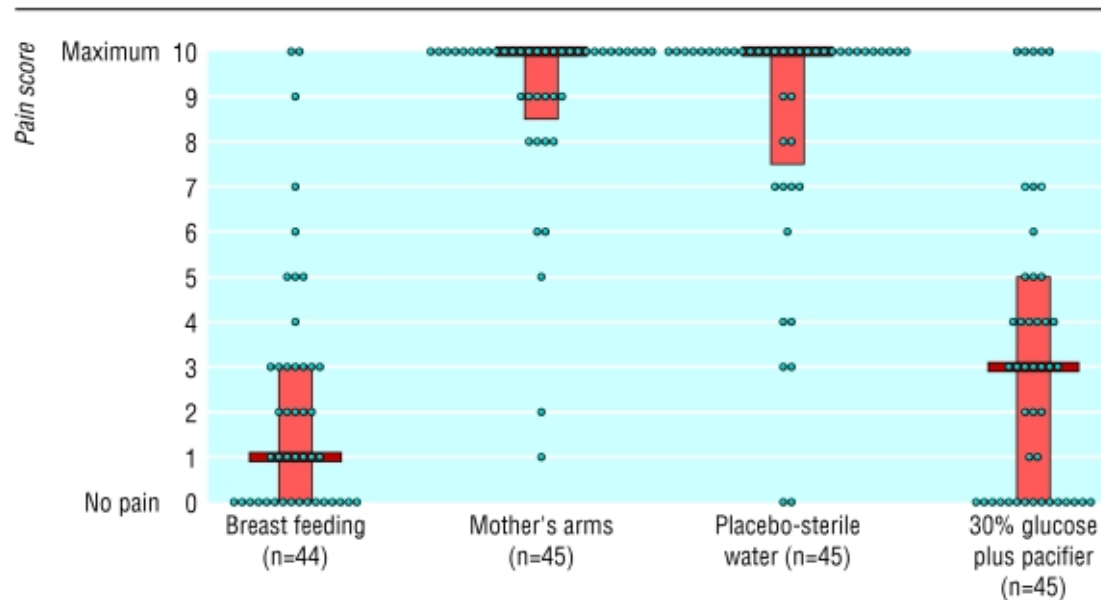Prewarming the area with 38 °C water. DO NOT rub.

SAMPLING CONDITIONS

- ## Preparation, calming (anxiety is a barrier for sampling)

Before 1 year
Calming the infant: oral glucose / saccharose before sampling
[Metaanalysis of 14 trials: pain decreases by 20%; 12 sec shorter crying]
Breast feeding has comparable efficacy

STANDARDISING THE SAMPLING CONDITIONS

Between 1 and 3 years of age
If the parent is appropriate, (s)he should keep the infant.
The phlebotomist should act quickly and reassuringly.
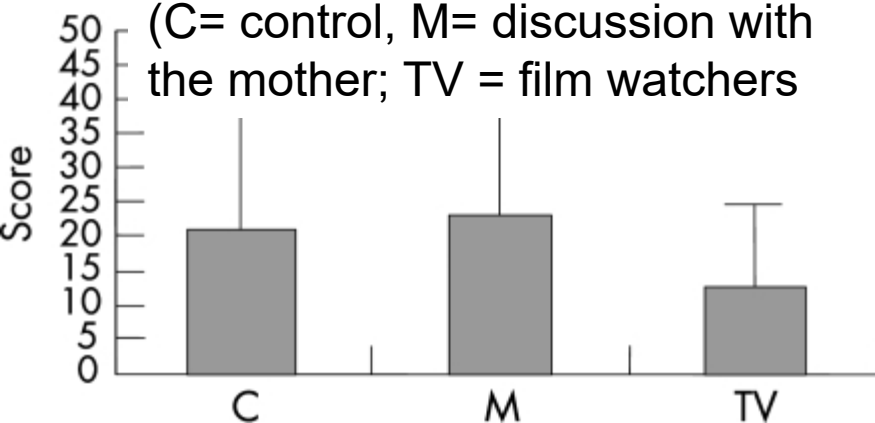Communication is hard.
After 4 years of age
The role of communication increases. Details should be explained
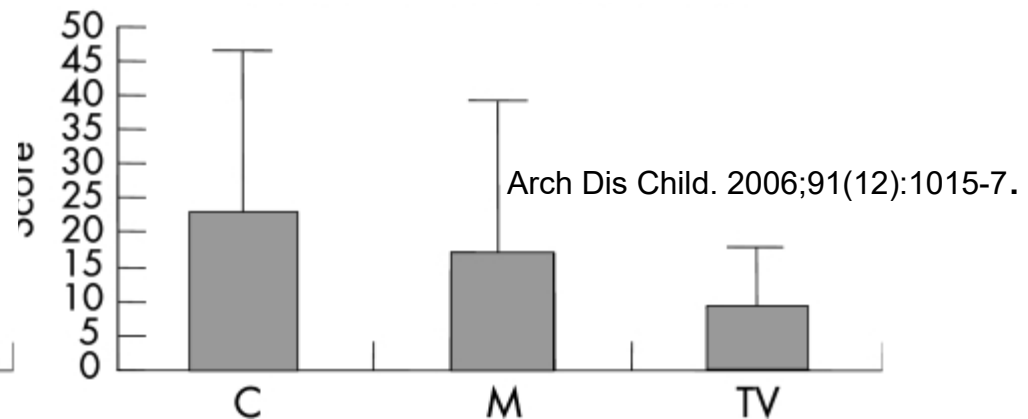(presentation of the needle, explanation of sampling equipment etc.)
Film [TV] watching during waiting

Pain sensed by the mothers

(C= control, M= discussion with the mother; TV = film watchers

Pain sensed by the infants

SAMPLING CONDITIONS

Order of tubes:

Venous sampling // until mark

IMPORTANT: avoid tubes over the expiry data

- Blood culture
- Citrated tube for hemostasis tests
- Native tube for serum
- Heparinated blood (plasma)
- EDTA blood for CBC
- NaF tube for blood glucose tests
- Others

Capillary sampling // mark +/- 10%

- EDTA blood
- Tubes with other additives
- Native tube for serum

PATIENT / SAMPLE IDENTIFICATION

1. Label in the presence of patient / relative

2. Name, date, ID number

3. Harsh massage to be avoided (hemolysis, sample tissue contamination)

4. Regular turning of tubes with additive.

5. Label should be positioned appropriately.

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Preanalitical errors – Patient ID errors

- Study: simulated emergency department setting
- 3 volunteers: sampling, medication based on armband
- Each 3rd volunteer was given unmatched band
- 39% of nurses did not noticed this error
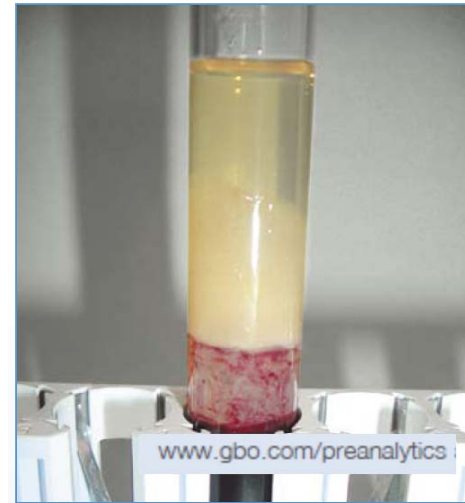
Guideline to standardize sampling processes

- Audit with 29 questions, 12 countries, 336 reports
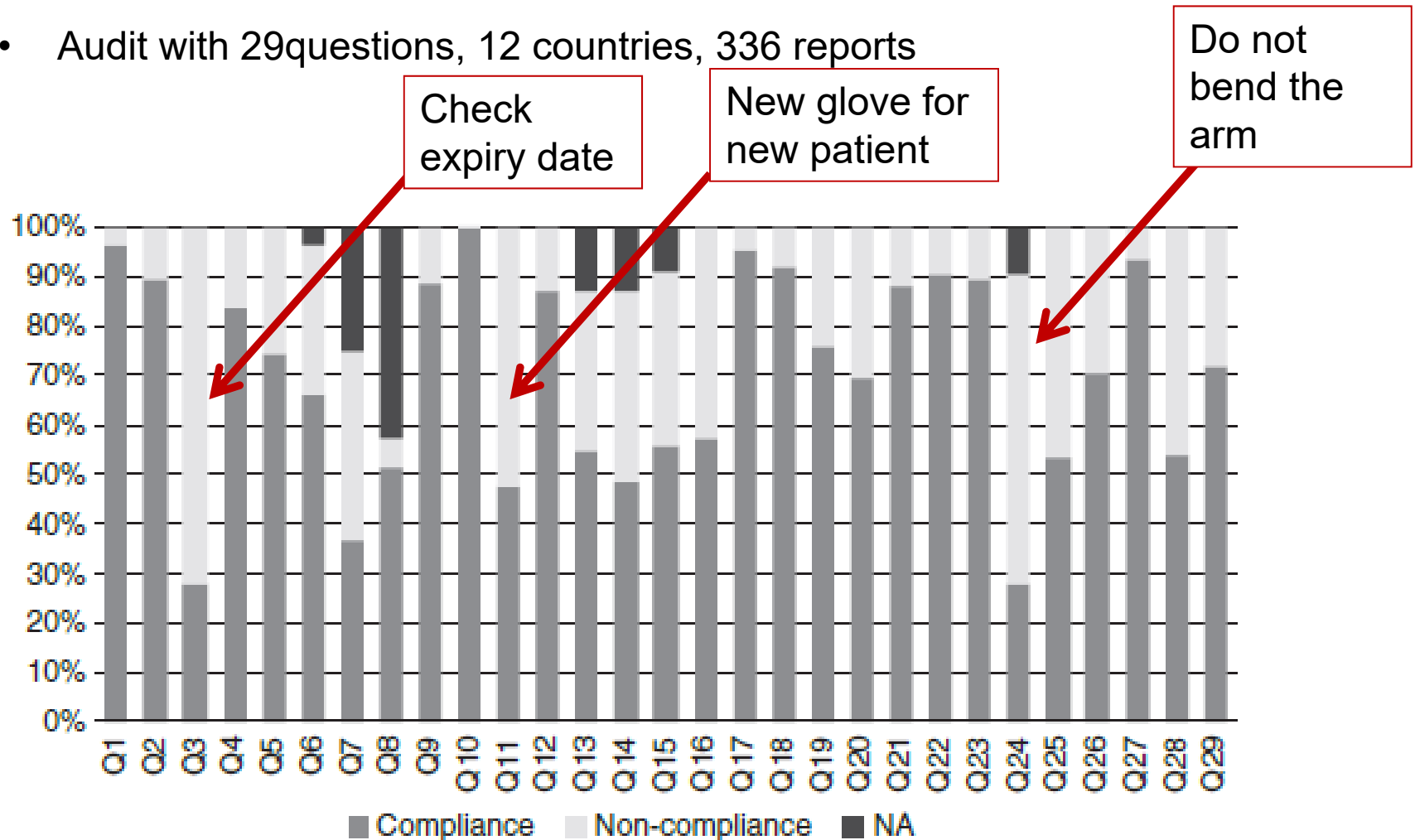
TRANSPORT AND PROCESSING

Some points to be adhered to:

- Transport in vertical position

- Do not expose to sunlight (bilirubin)

- Transferred in 2 hours to the lab

- Temperature

- The way of transport may affect some results

www.gbo.com/preanalytics

SUMMARY

- Several factors have an impact on quality of sample (and obtained results).

- One should remember:
  - justified request
  - identification of the patient
  - volume and sample and way of sampling
  - tube type
  - standardized setting