

Semmelweis University
Department of Laboratory
Medicine

Founded: 2010

- Major profile: diagnostics
 - 4.5 Million tests pro year
 - 450,000 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

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For further info: semmelweis.hu/laboratorium



Central Lab Buda

A map of Budapest, Hungary, showing the Danube River and various districts. A red pin labeled 'A' is located in the center of the city, near the Danube. Several locations are marked with colored circles and labeled with text boxes: 'Central Lab Buda' (light blue circle, top left), 'Endocrine Lab' (light blue circle, middle right), 'STD & serology Lab' (light blue circle, middle), 'Central Lab Pest' (light blue circle, middle right), 'Immune lab' (light blue circle, bottom right), 'Microbiology' (light blue circle, bottom right), and 'Office for education' (light blue circle, bottom right). The map also shows major roads, parks, and landmarks like the Margit Bridge and the Danube River.

Endocrine Lab

STD & serology Lab

Central Lab Pest

Immune lab

Microbiology

Office for education

Some words about this discipline

- Lectures
- Supporting materials

The diagram consists of four overlapping ellipses. A light blue ellipse at the top contains a green box with the text 'Clinical disciplines'. Below it is a large yellow ellipse containing a green box with the text 'LABORATORY MEDICINE'. At the bottom are two overlapping yellow ellipses; the left one contains a green box with the text 'Pathophysiology' and the right one contains a green box with the text 'Microbiology'. The 'LABORATORY MEDICINE' ellipse overlaps with the 'Clinical disciplines' ellipse, and both overlap with the 'Pathophysiology' and 'Microbiology' ellipses.

Clinical disciplines

**LABORATORY
MEDICINE**

Pathophysiology

Microbiology

Exam

- Test exam (single choice)
- See detail on the homepage

Factors having an effect on lab test results

Test request



Result interpretation

Result



Sampling



Black box: the lab



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

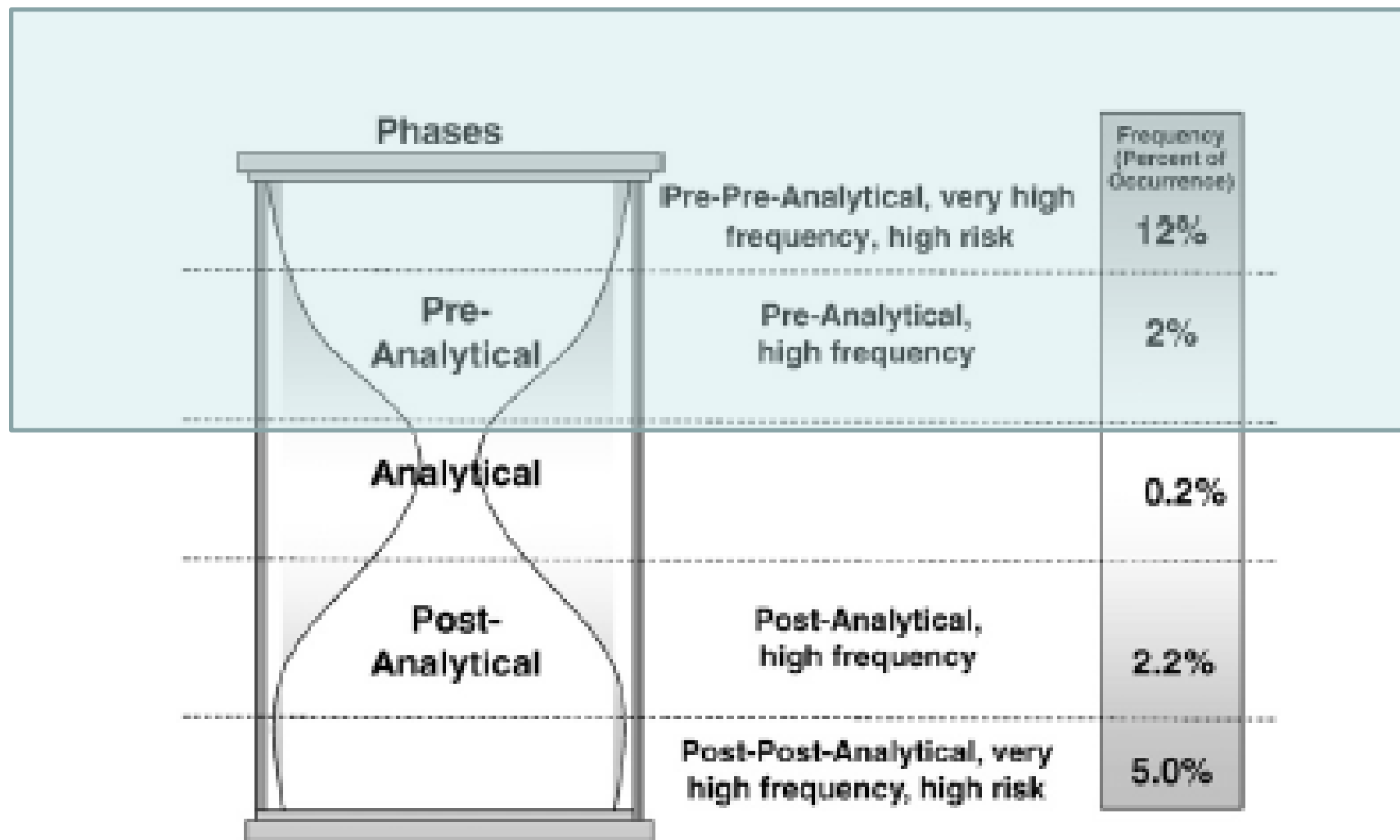
Majority of cases are due to diagnostic error.

The 70% of diagnostic tests are performed in the **lab**.

Prevalence of incorrect results:

1/ 164 - 1/330 measurements

OCCURRENCE OF FAULTS WITH LAB TESTS



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

**THIS LECTURE IS ABOUT
PREANALYTICAL PHASE
(AND ERRORS DONE)**

MAJOR SOURCE OF ERRORS



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 specific 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

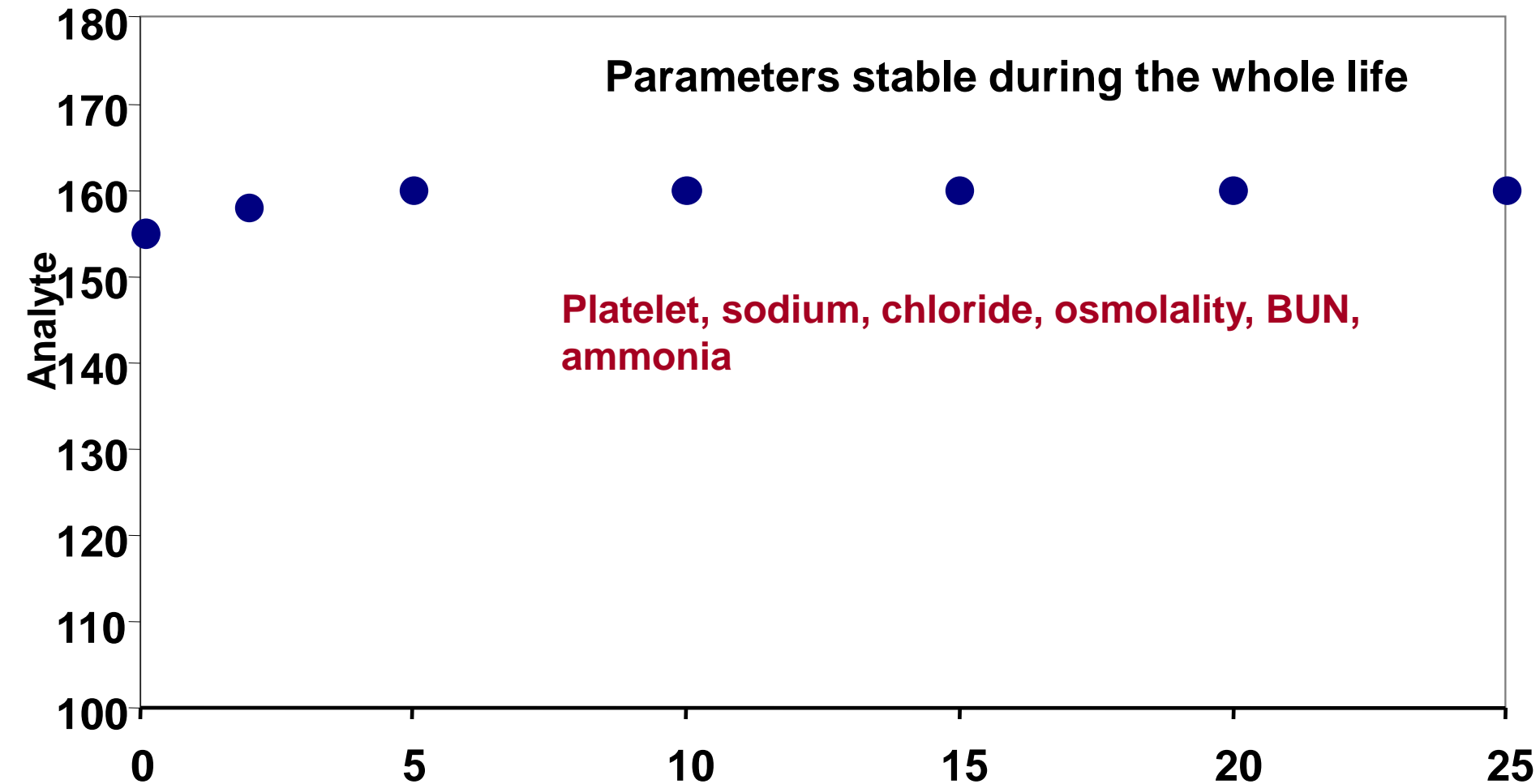
- Request linked to specific conditions
- Restricted lists
- Limitations of repeated requesting
- Education

Factors responsible for preanalytical errors - PATIENT

Biological variability and environmental conditions

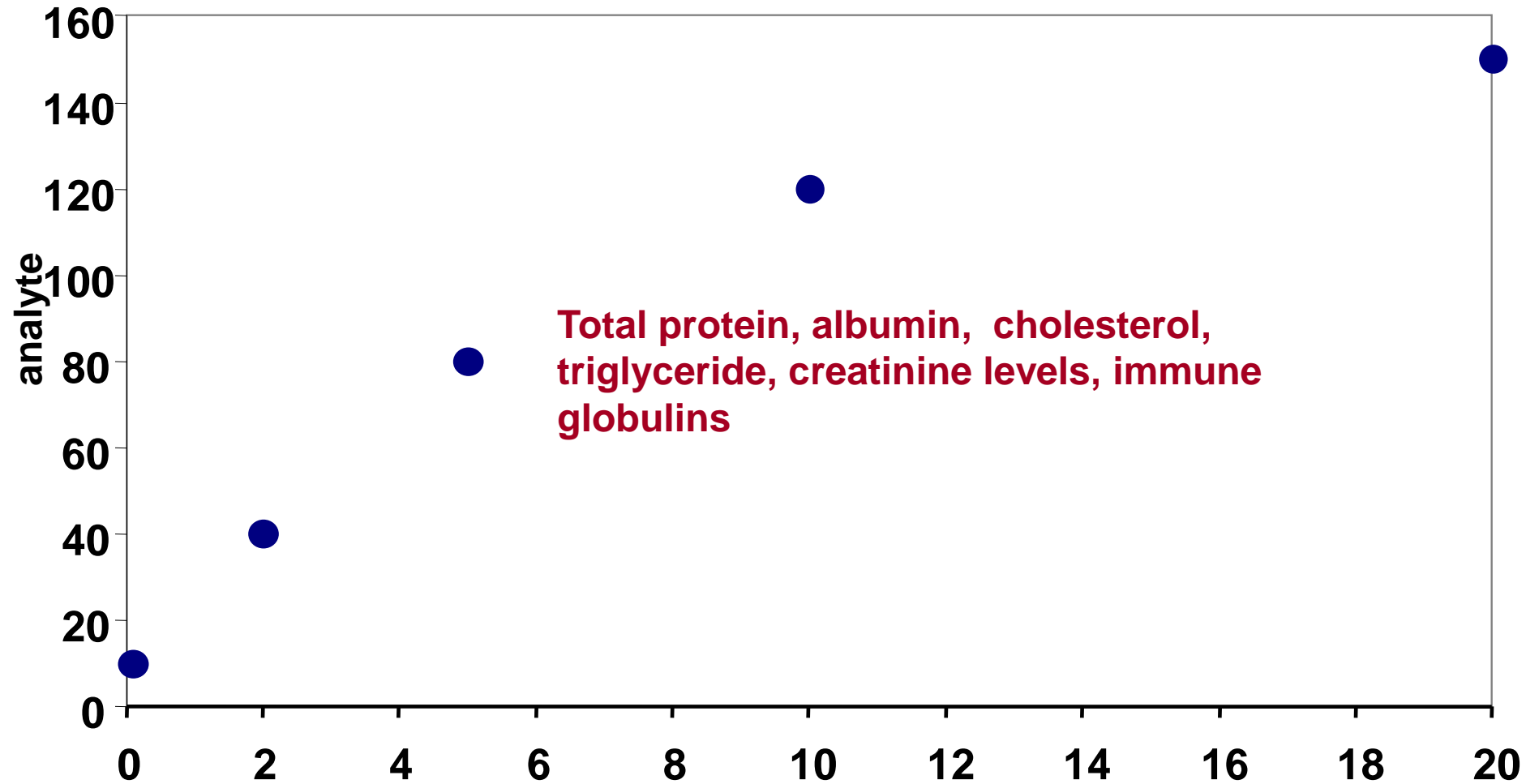
- Gender, period in fertile women
- Age
- Circadian / seasonal fluctuation
- Posture and prior exercise
- Diet

Factors responsible for preanalytical variations - PATIENT



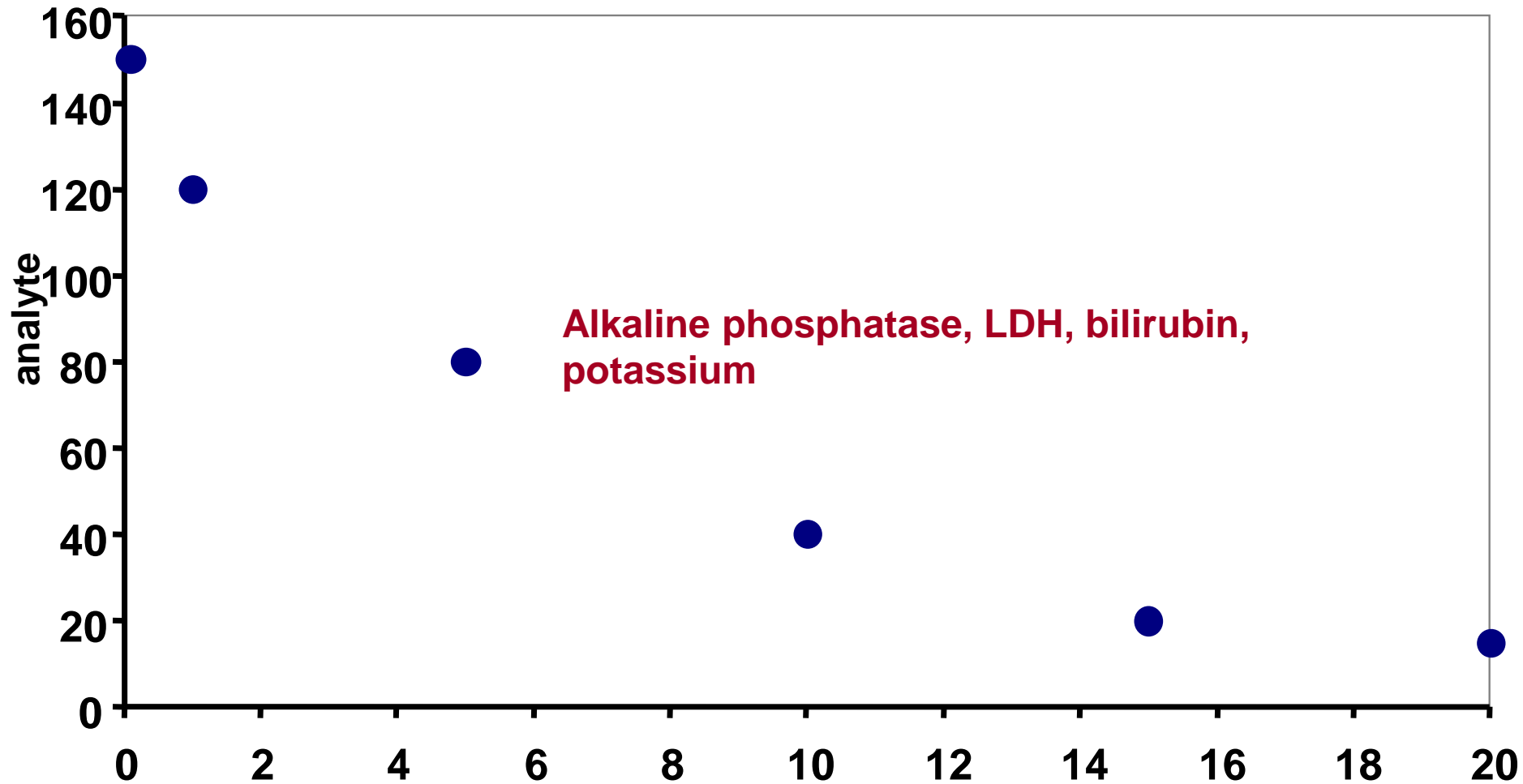
Factors responsible for preanalytical variations - PATIENT

Parameters increasing with age



Factors responsible for preanalytical variations - PATIENT

Parameters decreasing with age



Factors responsible for preanalytical variations - PATIENT

Daily fluctuation

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%

+ cortisol-regulated cytokines & bone markers

Factors responsible for preanalytical variations - PATIENT

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



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

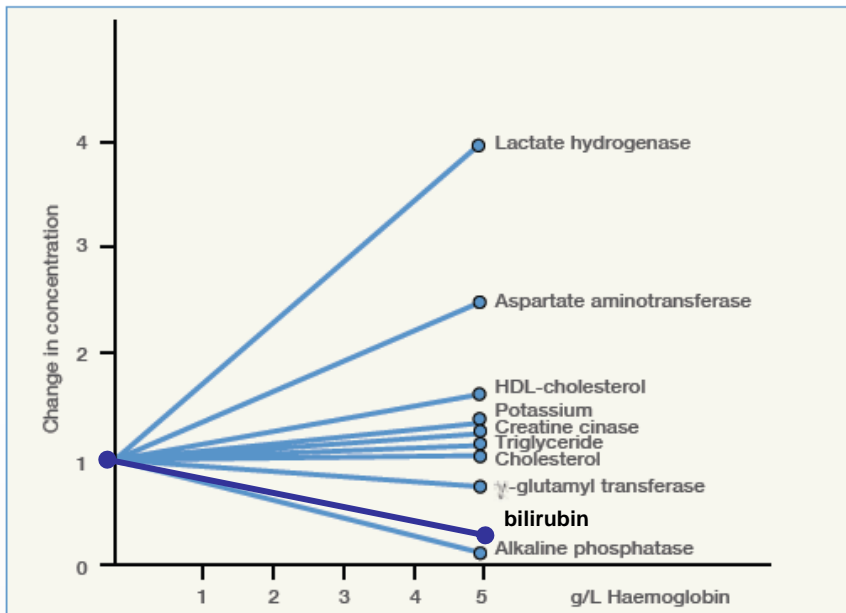
Ambulatory patients	0,1%
Dialysis	1,5%
Intensive care	5,4%
Pediatric ward	8,6%
Emergency department	8,8%

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

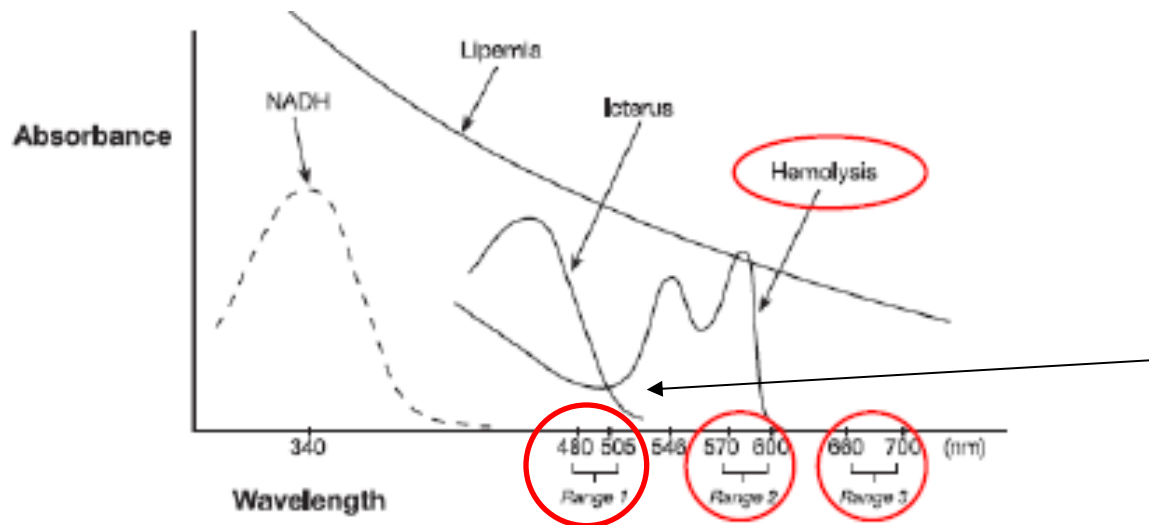
1. Inspection of sample (56%)
2. Measurement of hemolysis index (43%)
3. Potassium levels

Often invisible

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**

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!!!	

● Blood cells

● Protein molecules

● Calcium

● Protein bound substance

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.



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.



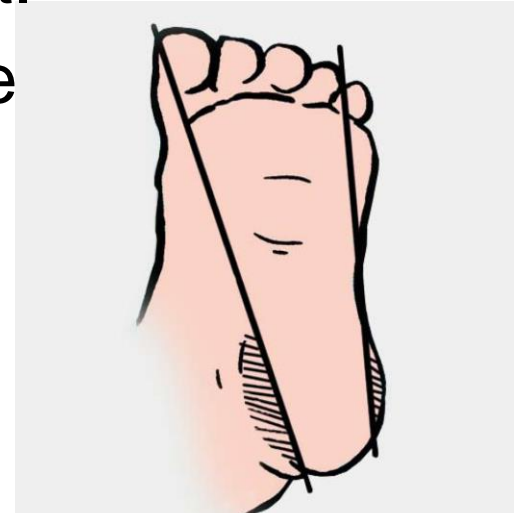
CAPILLARY SAMPLING II.

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



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

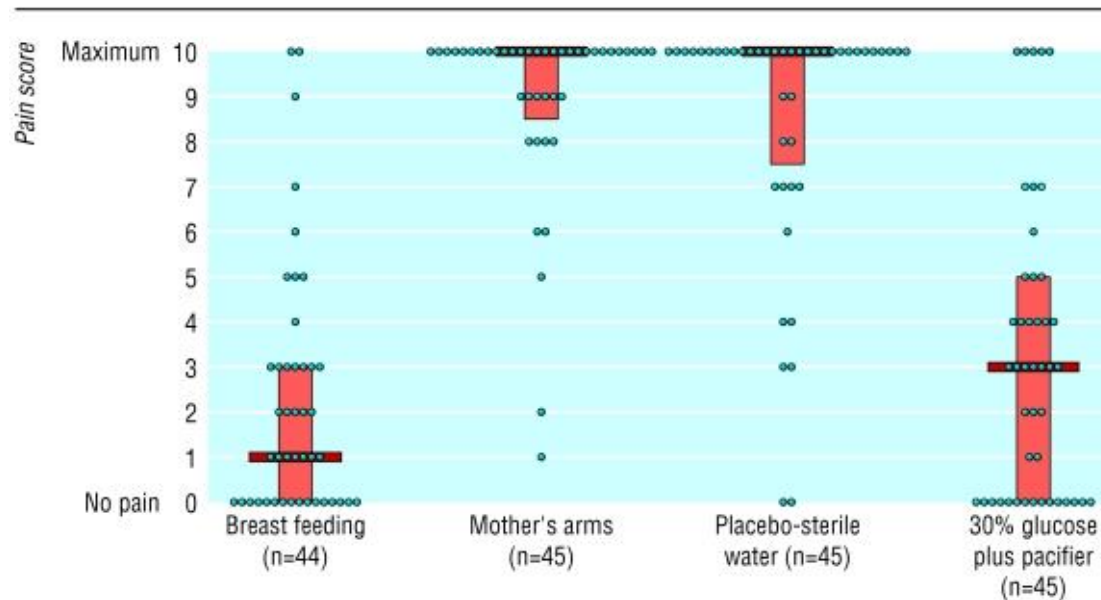
- Preparation, calming (anxiety is a barrier for sampling)

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.

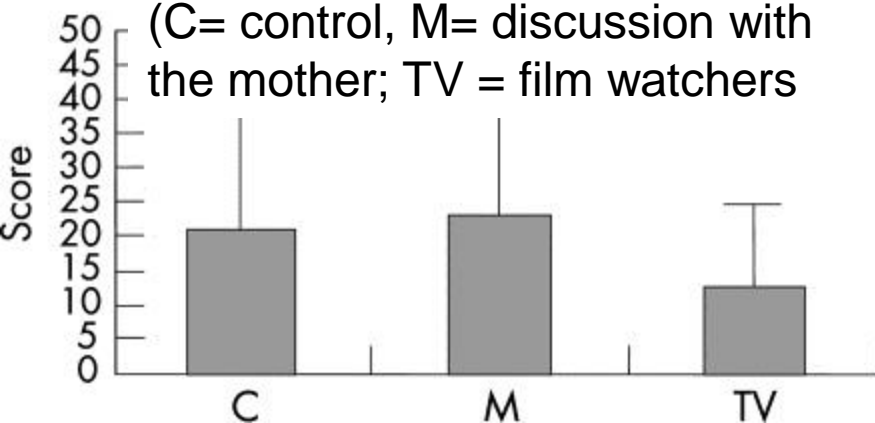
After 4 years of age

The role of communication increases. Details should be explained (presentation of the needle, explanation of sampling equipment etc.)

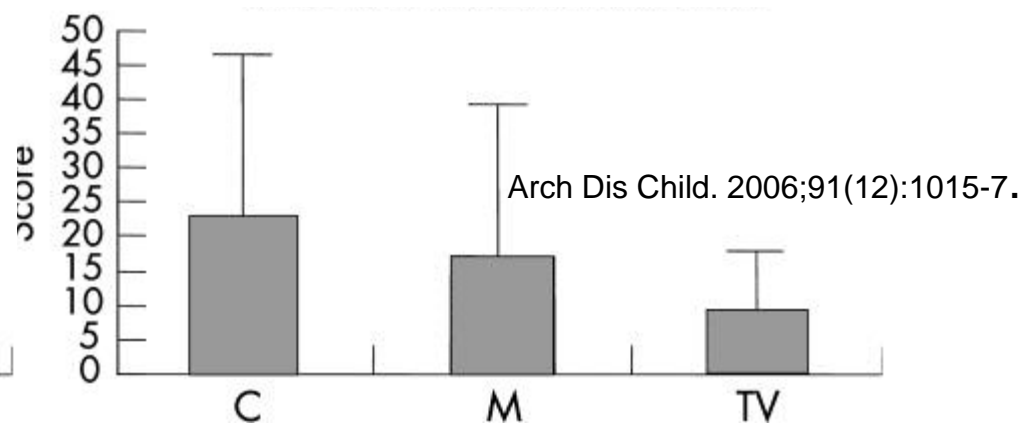
Film [TV] watching during waiting

Pain sensed by the mothers

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



Pain sensed by the infants



Arch Dis Child. 2006;91(12):1015-7.

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.



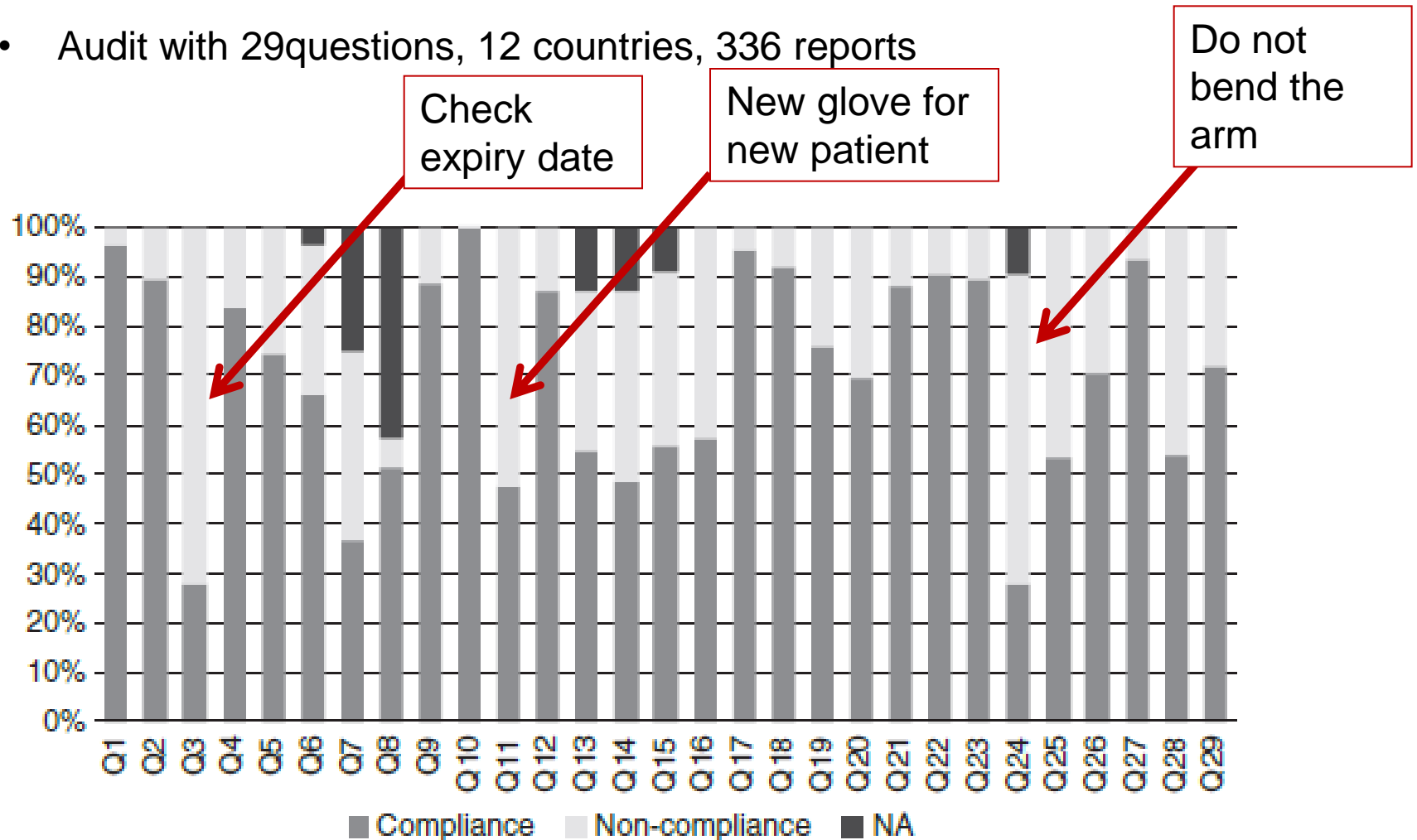
Preanalytical errors – Patient ID errors

- 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

[Ann Emerg Med. 2010;55:503-509.]

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





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