The analytical phase

Result interpretation

Test request

Result

Black h

Sampling

ANALYTICAL PHASE

The CASE

Uncle Pete, 67 years old

- Marked abdominal pain
- 8 pm, ED
- Acute abdomen?

Assessment (+ physical exam)

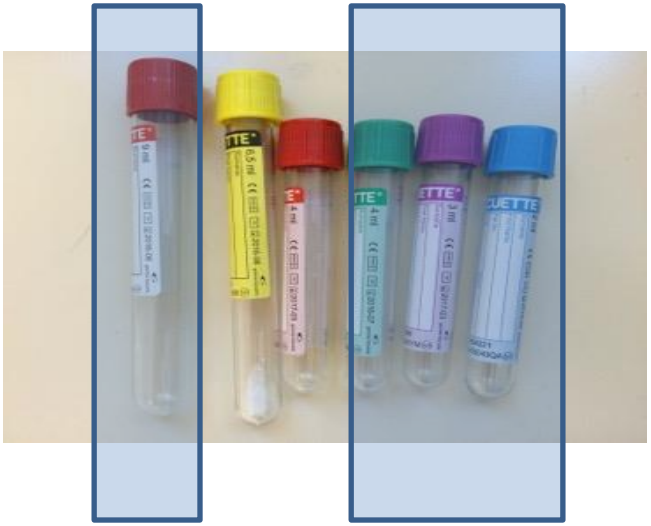
- Sampling for the lab FOR THAT: sending a request
- Radiological tests (US, X-ray)

Requesting a test

- Requesting: via medical informatic system (at the university)
- Sheet for tests (e.g. emergency tests, routine, hemostasis, hormone etc.)
- The software tells the number and type of tubes to be used

Lab tests for Uncle Pete

- **Blood sampling**
- CBC
- PTT, aPTT, TT (hemostasis)
- Chemistry (amilase, ALP, bilirubin, GOT, LDH, GPT)
- troponin
- **Urinary specimen**
- Amilase



Sampling was successful

You remember (don’t you?): Patient’s identification

1. Should be labelled in Uncle Pete’s presence
2. (For unconscious patients: RFID, arm band)
3. Sampling just one patient in a given time-point
4. ID data: name, birth data, ID number
5. Appropriate position of label

Sample – arrived to the lab

1. Registration of sample
   [it is arrived]

2. Processing [centrifuge]

Note: serum vs. plasma

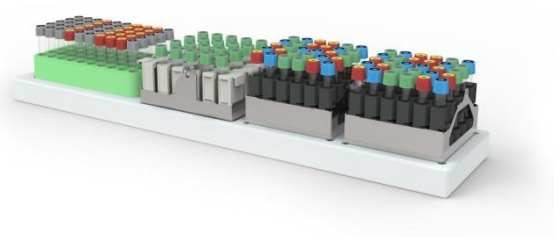
3. Distribution (generation of worklists)

SAMPLE – distribution

urinalysis

Clinica

serotyping



hemostasis

https://www.youtube.com/watch?v=QYVHRt4Qwiw

others

Immune analytics

hematology

SAMPLE – testing

Organization of work within the lab

1. Administrator: encounters samples (registration)
2. Assistants: operation, maintenance of machines
3. Graduated: supervision of workflow, validation of results (comparison with clinical data, preceding results, other test results) (generation of lab findings)

TAT-idő (turn-around-time) = time between arrival of sample to the lab and generation of finding

Emergency: < 1 hour
Routine: <24 [in general 4] hours
Special tests: 1 – 2 weeks, max. 1 month

SAMPLE – testing
that the doctor should know

Tests are done on different analyzers with different reagents.

Characteristics of reagents

1. What is it good for (what analyte on what machine)
2. Measurement range (lower and upper level of detection, LOD)
3. Expiry date (particularly tests applying enzymes and proteins)
4. Number of tests included (collection of specimens…)
5. Lot number
6. Cross-reaction, interference (eg. HAMA, coloric substance etc.)

SAMPLE – testing

Tests are done on different analyzers with different reagents.

Characteristics of analyzers

1. What is it used for
2. Test number done per hour
3. Time required to complete a test
4. Sample requirement
5. Requirement for reagent and consumables

SAMPLE – testing

Tests are done on different analyzers with different reagents.

Analyzers + reagent = together a system
(IVD qualified systems)

1. Imprecision [Repeatability and Reproducibility](CV%) – difference between results repeatedly measured within and between run
2. Trueness – approximation of real (target value)
3. Accuracy - depends on the first 2 factors

Cause of imprecision = random error

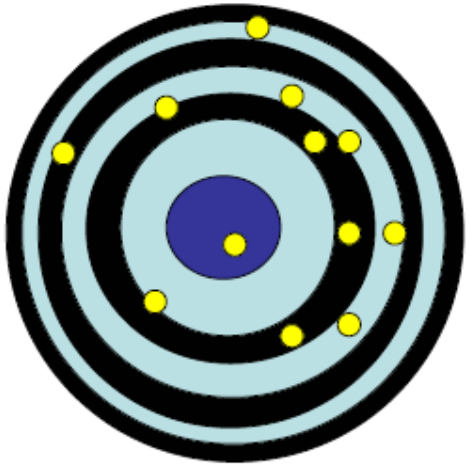
Instrument condition

Temperature

Pipetting

Carry-over

Personal factors



Random error is high

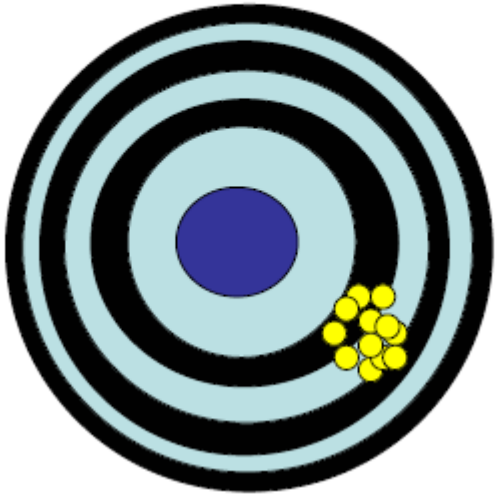
–Rrepeatability: standard deviations of repeated test results done under the same conditions (within-run or intra-run assays)

– Reproducibility): standard deviations of repeated test results done under different conditions (between-run or inter-run assays)

Systemic error (bias)

Basic setting of the device

Calibration errors



An example for biased (but accurate) results: uncle Pete's case [continued]

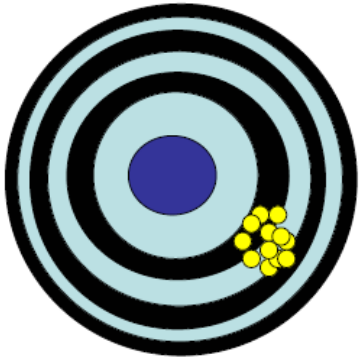
- The doctor requested glucose levels and electorlytes the next day morning.

- Serum glucose: 1.8 mmol/L
  Lactate: 6.9 mmol/L
  LDH: 1500 U/L

  ????

  Lab is documentedly proficient in these tests: 5 different labs measured the same

• Na^+	143	145	145	141	143	mmol/L
• K^+	7,4	7,6	7,7	7,5	7,5	mmol/L
• Glukóz	1,8	2,0	2,0	1,7	1,8	mmol/L
• Laktát	6,9	7,3	6,8	7,1	7,0	mmol/L
• Amiláz	100	97	95	108	90	U/L
• LDH	1500	1650	1700	1490	1600	U/L



Reproducibility is about 5 per cent

Possible explanation:

- Unprocessed sample was present for 8-10 hours in tube; glucose utilization was continuous while lactate increased
- Cell membrane damage; micro-hemolysis leading to an increase in potassium and LDH
- Estimated decrease:
  - Glucose (half / one third)
- Estimated increase:
  - Potassium by 100%
  - LDH by 2-300%
- Conclusion:
  - While lab provided precise results [imprecision was low], they were biased and trueness was low; accuracy was also low

How can I monitor the functioning of the system?

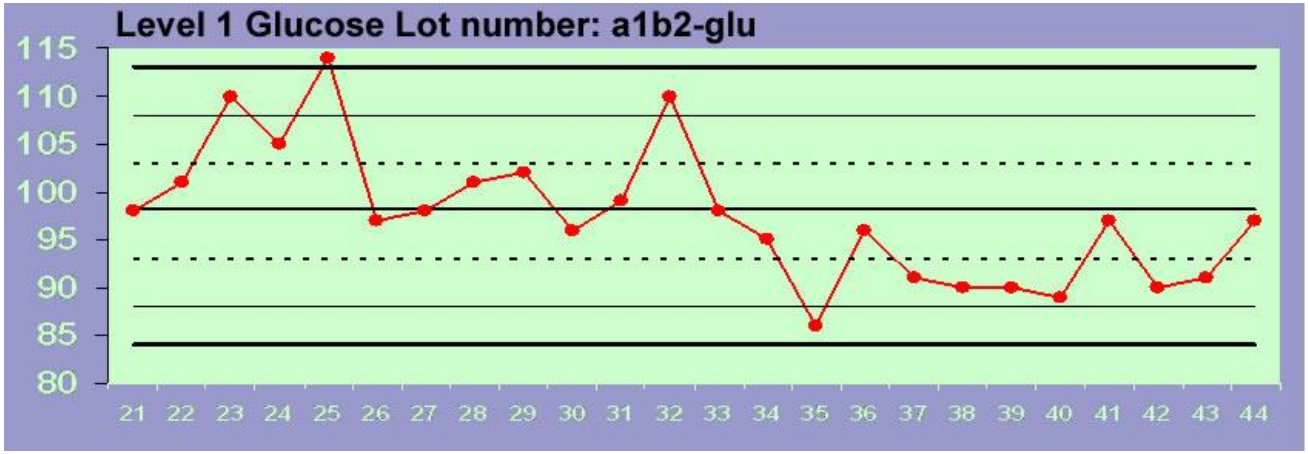
Answer: quality control
(not cheap: may be up to 10-30% of costs – depending on lab load)

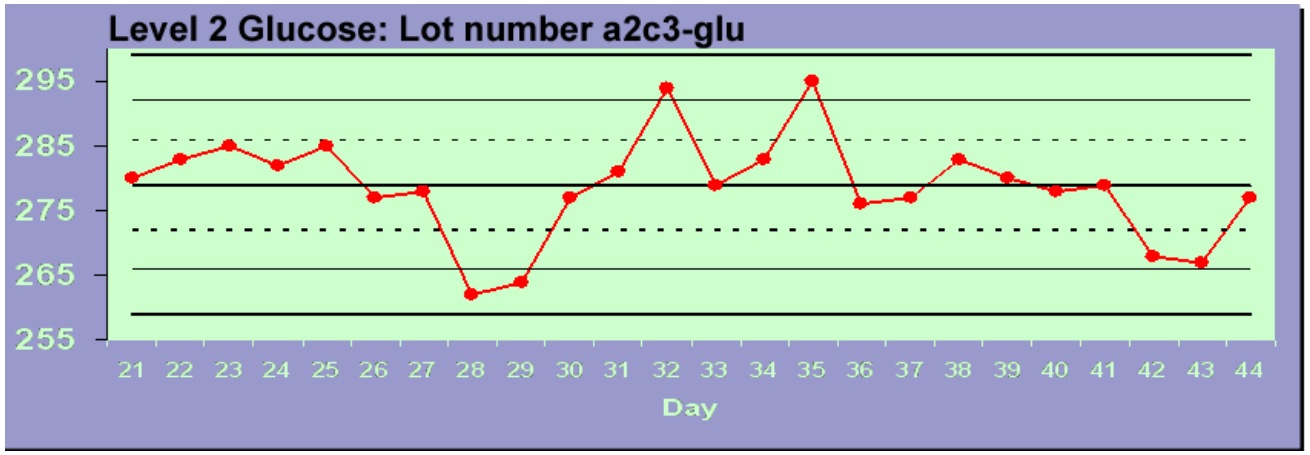
Quality control / QC

Internal QC: daily / regular measurement of control samples of known composition and concentration.
[Control charts]

External QC: independent organization provides samples of unknown analyte levels 4 -6 times per year; central evaluation is based on difference from target values

Levey-Jenning charts: presentation of internal control values by time

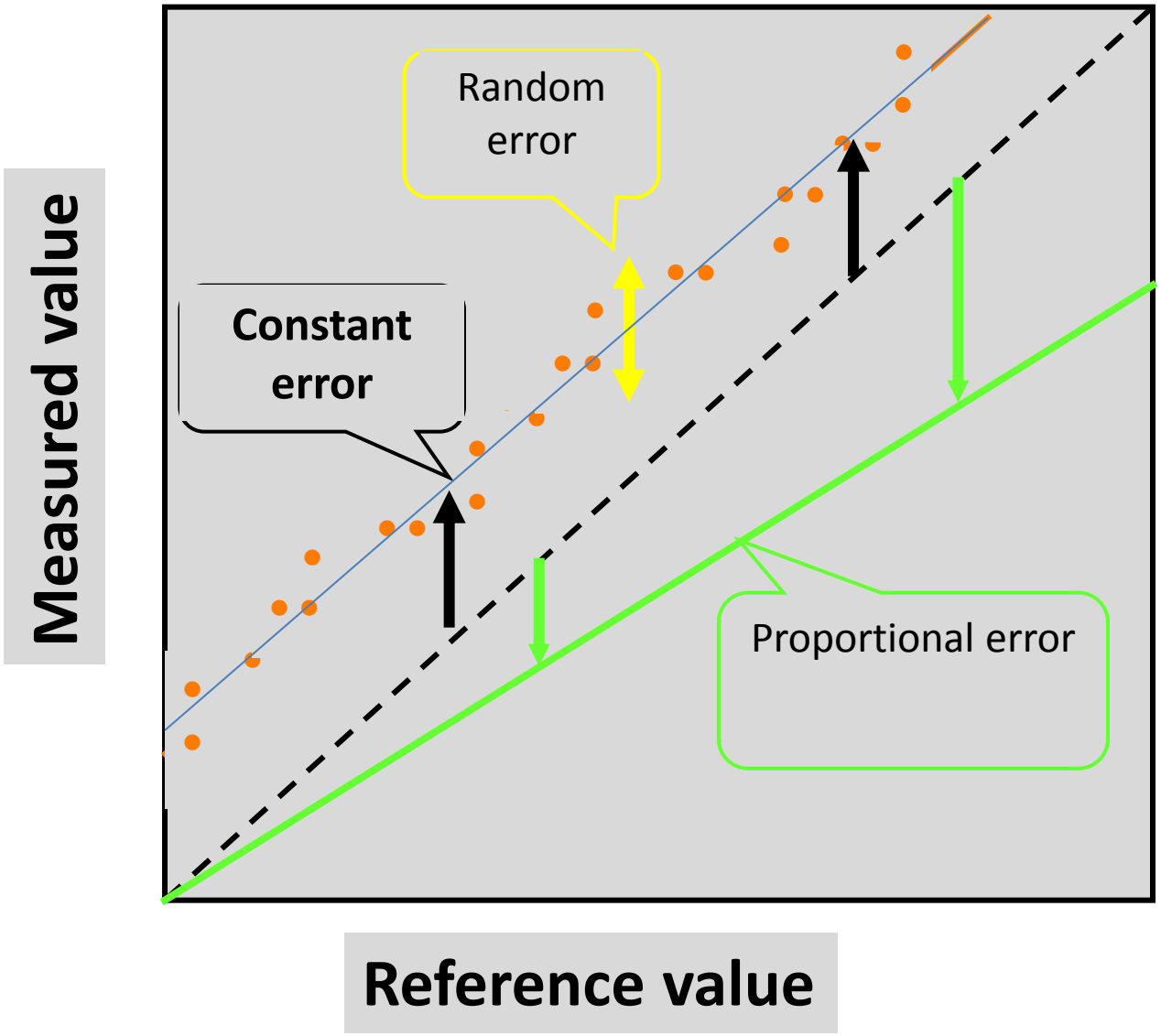




NOTE:

There is NO diagnostic test without control measurements (including POCT assays)

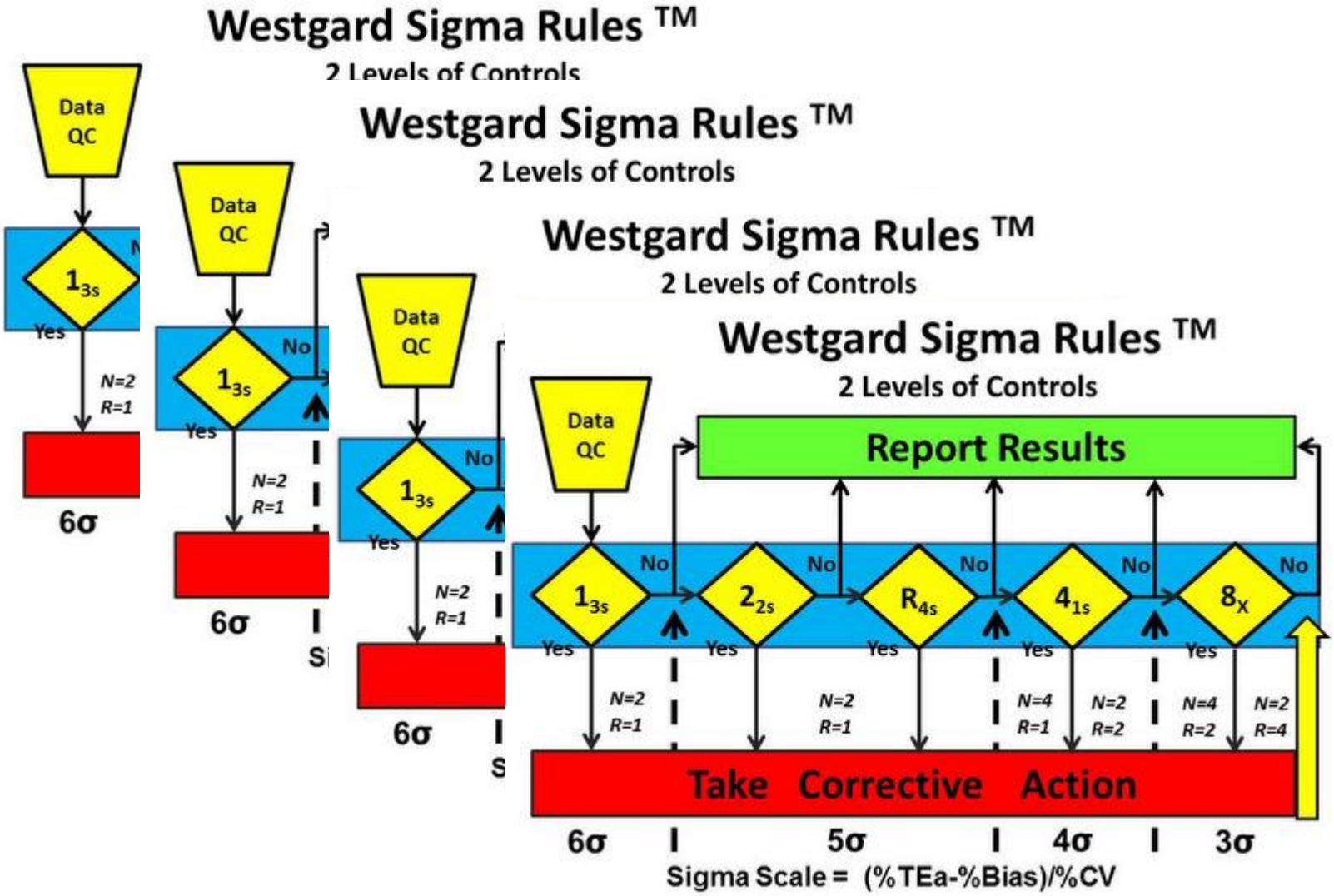
Systemic and random errors can be detected



Westgard rules

- Principles that are used to decide based on control results whether daily measurements can be done
- E.g. deviation from target values based on 2SD limits; presence of systemic error
- Appropriate interventions (e.g. change of reagent, new calibration etc.)

Examples for Westgard rules



What should / can be done in case of significant error

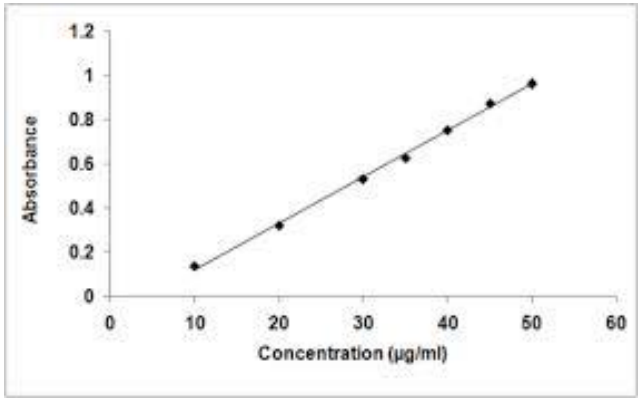
- Should not measure
- System's status should be tested (e.g. reagents, consumables, clarity, electrode, cuvette etc.)
- In case of necessity: calibration

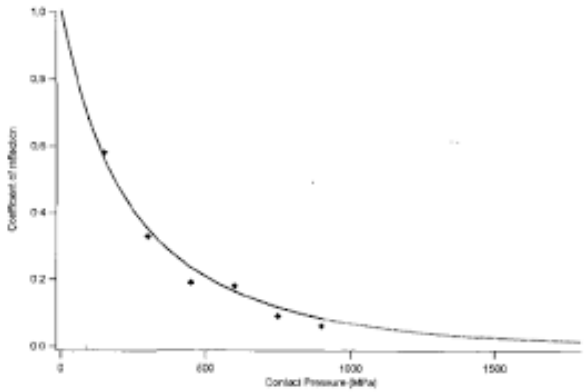
Calibration:

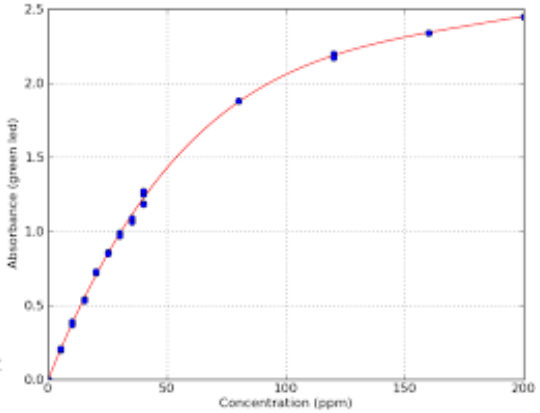
- One should teach the device what results should be associated to a given measured signal

Calibration

- Use of reference materials
- Number of plots (calibration curves)







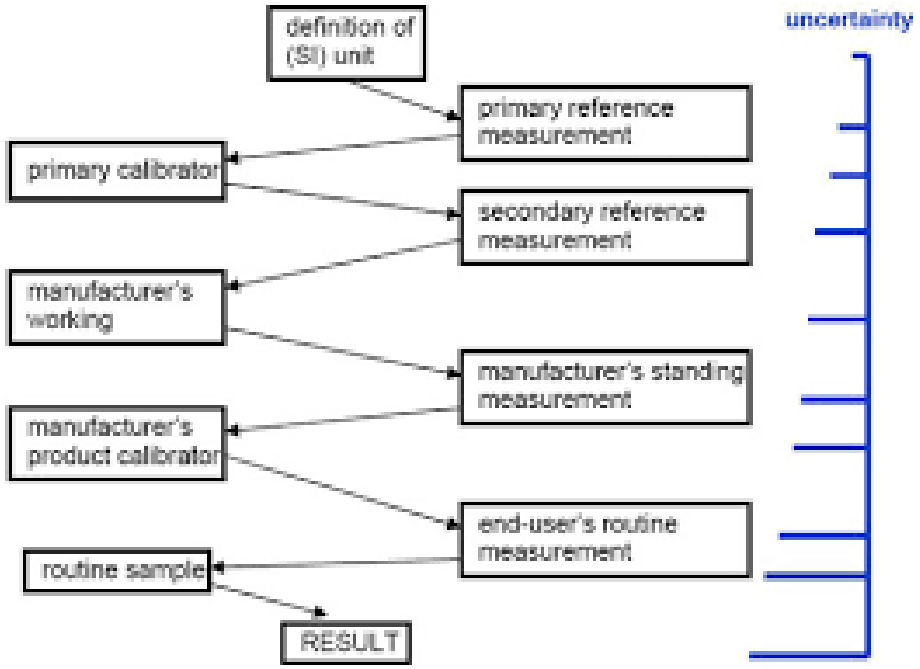
Reference materials: a particular science

The reference basis is of outmost importance
– if it is inappropriate, the functioning of the whole system is unreliable.

Reference materials should be traceable

Qualified reference materials

Qualifications of different level



External QC programs

- Independent organization
- Distributes samples regularly to labs
- Based on results' relationship to targeted values (within 2SD): indication whether lab's work is acceptable
- Theoretically: if a lab fails, it should not perform the implicated test

In Vitro Diagnosztikai Minőségellenőrzési Kht.
6720 Szeged, Somogyi u. 6.: 6701 Szeged, Pf.: 910
Tel./Fax: 62/543-015, Tel.: 62/543-016
E-mail: mail@qualicont.com
Web: www.qualicont.com

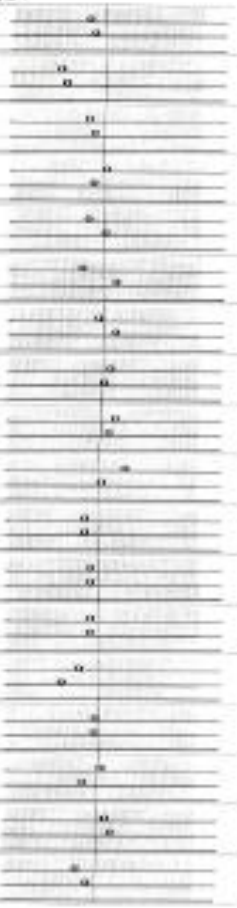
Egyedi kiértékelés

SE ÁOK I. Gyermekklinika LABORATÓRIUM

QC kód: 041 **Kör:** 2007.IV. **Kiküldés dátuma:** 07. aug. 06. **Beérkezés dátuma:** 07. aug. 21.

100. Program: Klinikai kémia

		Módszer, Reagens	Minta	Eredmény	Célérték	Megfelel	Eltérés
01. Kalcium	(mmol/l)	4 RO	G H	2,2 3,2	2,22 3,21	1,97 - 2,47 2,85 - 3,57	-1 0
02. Klorid	(mmol/l)	7 RO	G H	84 112	87,9 116	79,9 - 95,9 105 - 127	-4 -3
03. Kálium	(mmol/l)	4 RO	G H	3,4 6,3	3,43 6,32	3,12 - 3,74 5,75 - 6,89	-1 0
04. Nátrium	(mmol/l)	4 RO	G H	125 149	124 148	116 - 132 138 - 158	1 0
05. Vas	(µmol/l)	5 RO	G H	20 31	20,4 30,4	17,5 - 23,3 26,1 - 34,7	-2 2
06. TVK	(µmol/l)	99 RO	G H	47 49	49,2 48,9	37,3 - 61,1 38,3 - 57,7	-4 5
10. Magnézium	(mmol/l)	6 RO	G H	1 1,7	0,99 1,63	0,83 - 1,15 1,38 - 1,9	1 4
11. Anorganikus foszfát	(mmol/l)	6 RO	G H	1,2 2	1,16 1,97	0,95 - 1,37 1,61 - 2,33	3 2
12. Összfehérje	(g/l)	2 RO	G H	64 49	62,5 47,9	55,6 - 69,4 42,6 - 53,2	2 2
13. Albumin	(g/l)	1 RO	G H	45 30	41 29,1	31 - 51 22,4 - 35,8	10 3
16. Bilirubin (összes)	(µmol/l)	3 RO	G H	20 76	20,5 77,1	13,7 - 27,3 56,9 - 97,3	-2 -3
17. Bilirubin (direkt)	(µmol/l)	3 RO	G H	12 38	12,3 38,3	7,87 - 16,73 25,1 - 53,5	-2 -1
18. Glükóz	(mmol/l)	3 DC	G H	5,1 13,6	5,11 13,7	4,28 - 5,94 11,6 - 15,9	0 -1
19. Húgysav	(µmol/l)	5 DC	G H	240 632	246 666	211 - 281 573 - 759	-2 -5
20. Karbamid	(mmol/l)	3 DC	G H	6,8 25,8	6,49 26,8	5,06 - 7,92 19,6 - 31,2	0 0
21. Kreatinin	(µmol/l)	5 RO	G H	108 345	97,2 353	76,1 - 119,3 275 - 431	3 -2
22. Triglicerides	(mmol/l)	7 DC	G H	1,4 2,4	1,35 2,29	1,1 - 1,6 1,87 - 3,71	4 5
23. Koleszterin	(mmol/l)	8 DC	G H	2,2 5	2,26 5,05	1,94 - 2,58 4,33 - 5,77	-3 -1



Just some thoughts

- Research vs. Diagnostics:
  these activities should not be mixed

- Difference:
  - QC
  - Quality assurance
  - Organization of workflow
  - Possible conclusions
  - Clinical value
  - Reimbursement
- **Similarity:**
  - Technique
  - Staff

If the system works…

- and the control is OK…
- Work sheets are generated (information system)
- Work sheet: samples (patients) + requests
  - Batch mode: one kind of testing from all the samples
  - Single sample measurement: all the requested tests from one sample are initiated; then it forwards
  - STAT mode: emergency requests are done at first

And finally the test is performed and a result is generated

- Technician inspects, then allows its passing to the laboratory information system
- Graduated staff (doctor, biochemist) checks and relates to other results (delta check: comparison to prior result). If OK, validates
- Validated result = FINDING
  JUST VALIDATED RESULTS CAN BE USED FOR CLINICAL DECISION MAKING.

Some facts about validation

Diagnostic values requiring clinical decision: depends on ANALYTICAL and BIOLOGICAL variability

$$u = 1.96 * \sqrt{(CV_A^2 + CV_I^2)}$$

Autovalidation: an informatical tool to support lab staff that decides automatically whether the result can be passed without intervention into the medical informatical system (may involve about 50-80% of findings)

Support the critical overview of remaining results.

What a lab doctor looks at on a list of results

- Extreme deviation from the normal
- Extreme difference from the prior result (delta check)
- Linkage to other parameters
- Association with clinical parameters
- Trends in analyte levels (may skew during the day)

Some examples for linked parameters

LDH + haptoglobin
Troponin T + CK
GOT, GPT, GGT
High bilirubin and GOT, GPT
Albumin + ionized calcium
Osmolality + Na, glucose and BUN
BUN + creatinine
α-amilase (lipase) + bilirubin
albumin $\leq$ Total protein * 0.7
Conjugated Bilirubin < Total Bilirubin
Total Cholesterol > HDL + LDL

Uncle Pete's findings

Lab findings asked as emergency

Available after 43 min of TAT

- ALP: 1100 U/L
- Bilirubin: 80 micromol/L
- Direct bilirubin: 54 micromol/L
- LDH: 340 U/L
- Not increased: amilase, troponin, GOT, GPT
- WBC: 10.4 G/L
- Urine: Ubg negative, bilirubin positive

Next time you can learn how to use these results

Just to remember from this lecture

- Analytical phase: it is the competency of laboratory
- Complex processes (factory)
- Quality control and assurance are of outmost importance
- Clinical decision making can be done solely on the basis of validated result (finding)