Verification of qualitative methods

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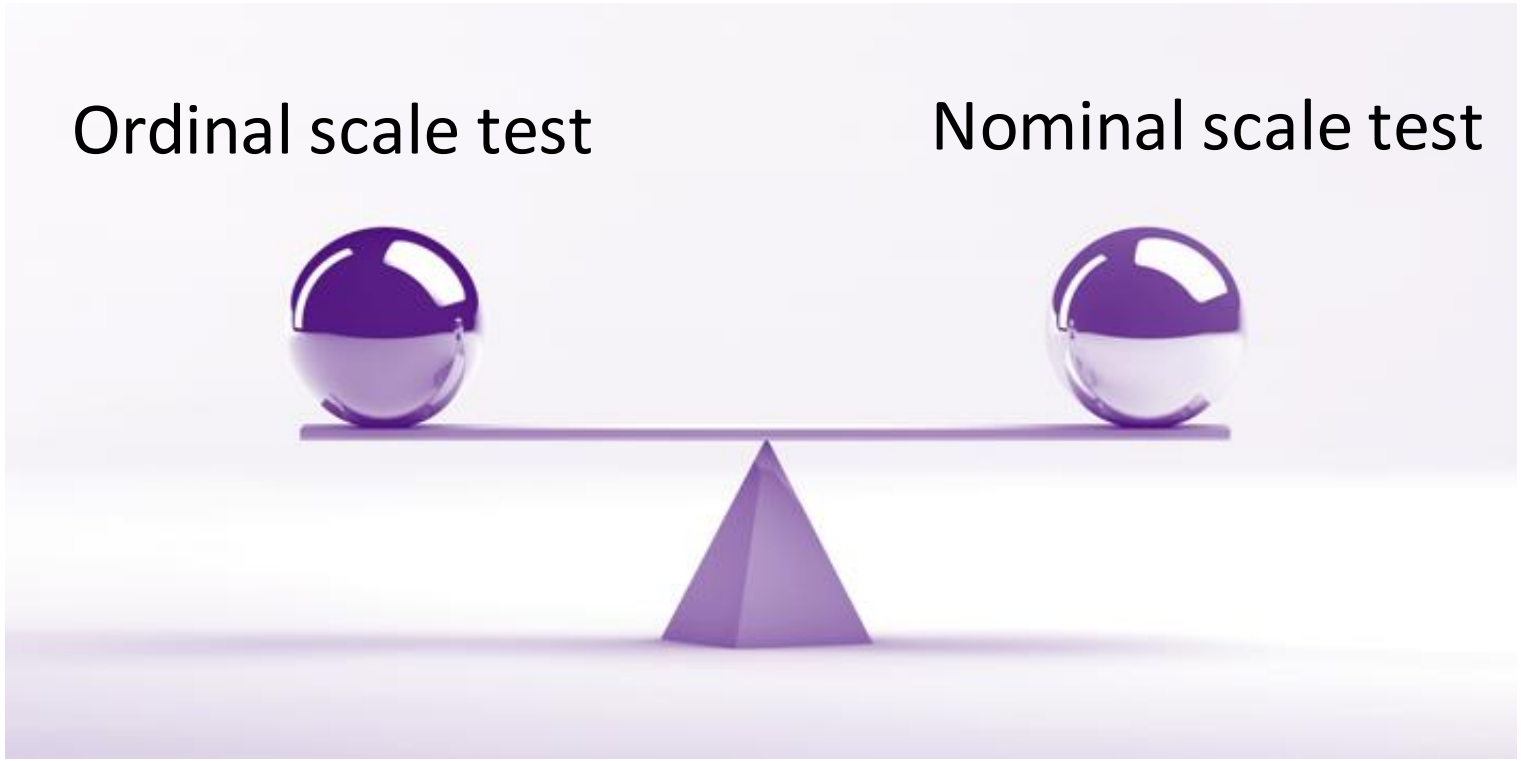
Qualitative methods

- *„…test methods that provide only two categorical responses (i.e., positive/negative or yes/no)…”*

CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI Document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

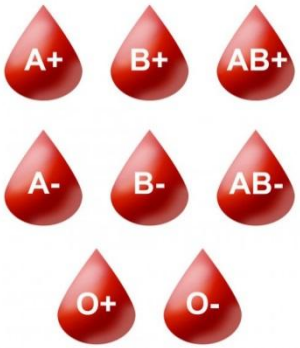
Qualitative methods



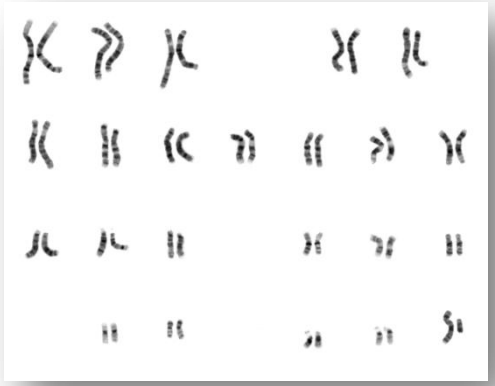
Nordin G. Before defining performance criteria we must agree on what a "qualitative test procedure" is. Clin Chem Lab Med 2015; 53(6): 939–41.

Nominal scale test

- blood types
- molecular/genetic tests



wt/wt wt/mut mut/mut

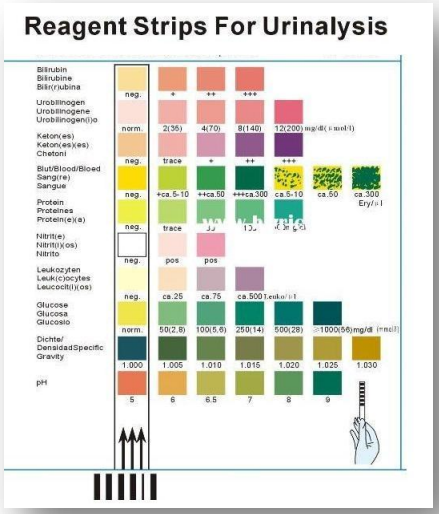


Ordinal scale test

- grading test results
- positive/negative

> or <

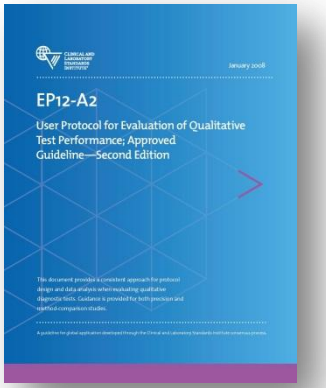
- urine test strip
- pregnancy test
- immunology screening tests



Verification of qualitative (ordinal scale) methods

- ISO 15189:2012
- CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance

- verification of all types of methods!
- defined by the laboratory

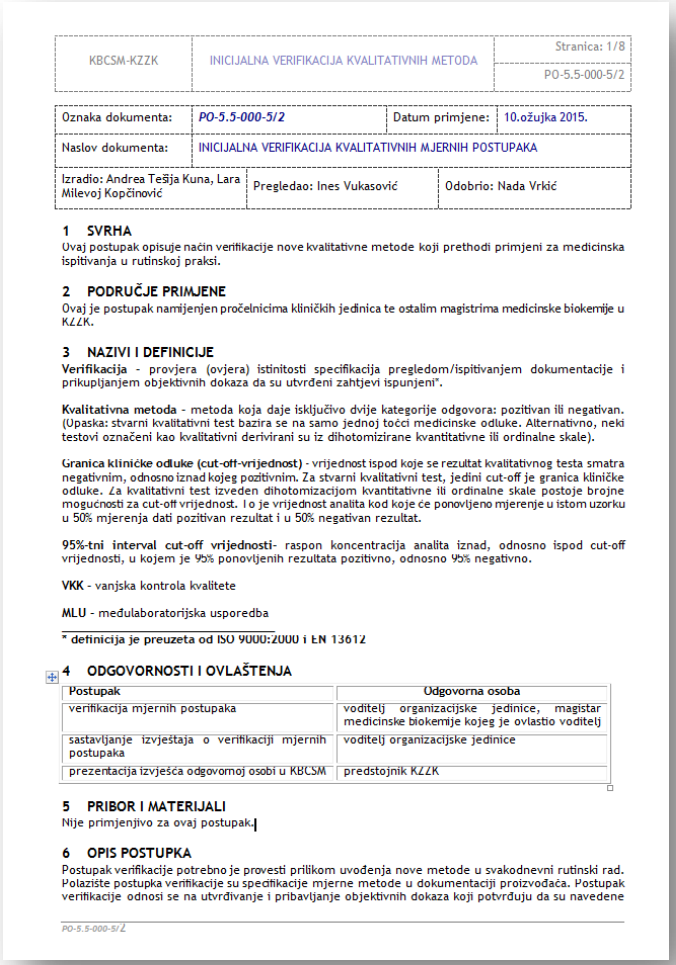


Verification protocol by University Department of Chemistry Sestre Milosrdnice University Hospital Center

- accredited according to ISO 15189:2012
- all methods (quantitative and qualitative) are verified before implementation in routine work

- verified qualitative methods:
  - urine test strip
  - indirect immunofluorescence tests (IIF): ANA, AMA, ASMA, LKM, ANCA
  - fecal occult blood test
  - …

Verification protocol by University Department of Chemistry



KBCSM-KZZK	INICIJALNA VERIFIKACIJA KVALITATIVNIH METODA	Stranica: 1/8
		PO-5.5-000-5/2

Oznaka dokumenta:	PO-5.5-000-5/2	Datum primjene:	10.ožujka 2015.
Naslov dokumenta:	INICIJALNA VERIFIKACIJA KVALITATIVNIH MJERNIH POSTUPAKA		
Izradio: Andrea Tešija Kuna, Lara Milevoj Kopčinović	Pregledao: Ines Vukasović	Odobrio: Nada Vrkić	

1 SVRHA

Ovaj postupak opisuje način verifikacije nove kvalitativne metode koji prethodi primjeni za medicinska ispitivanja u rutinskoj praksi.

2 PODRUČJE PRIMJENE

Ovaj je postupak namijenjen pročelnicima kliničkih jedinica te ostalim magistrima medicinske biokemije u KZZK.

3 NAZIVI I DEFINICIJE

Verifikacija - provjera (ovjera) istinitosti specifikacija pregledom/ispitivanjem dokumentacije i prikupljanjem objektivnih dokaza da su utvrđeni zahtjevi ispunjeni*.

Kvalitativna metoda - metoda koja daje isključivo dvije kategorije odgovora: pozitivan ili negativan. (Upaska: stvarni kvalitativni test bazira se na samo jednoj točci medicinske odluke. Alternativno, neki testovi označeni kao kvalitativni derivirani su iz dihotomizirane kvantitativne ili ordinalne skale).

Granica kliničke odluke (cut-off-vrijednost) - vrijednost ispod koje se rezultat kvalitativnog testa smatra negativnim, odnosno iznad kojeg pozitivnim. Za stvarni kvalitativni test, jedini cut-off je granica kliničke odluke. Za kvalitativni test izveden dihotomizacijom kvantitativne ili ordinalne skale postoje brojne mogućnosti za cut-off vrijednost. I o je vrijednost analita kod koje će ponovljeno mjerenje u istom uzorku u 50% mjerenja dati pozitivan rezultat i u 50% negativan rezultat.

95%-tni interval cut-off vrijednosti- raspon koncentracija analita iznad, odnosno ispod cut-off vrijednosti, u kojem je 95% ponovljenih rezultata pozitivno, odnosno 95% negativno.

VKK - vanjska kontrola kvalitete

MLU - međulaboratorijska usporedba

* definicija je preuzeta od ISO 9000:2000 i EN 13612

4 ODGOVORNOSTI I OVLAŠTENJA

Postupak	Odgovorna osoba
verifikacija mjernih postupaka	voditelj organizacijske jedinice, magistar medicinske biokemije kojeg je ovlastio voditelj
sastavljanje izvještaja o verifikaciji mjernih postupaka	voditelj organizacijske jedinice
prezentacija izvješća odgovornoj osobi u KBCSM	predstojnik KZZK

5 PRIBOR I MATERIJALI

Nije primjenjivo za ovaj postupak.

6 OPIS POSTUPKA

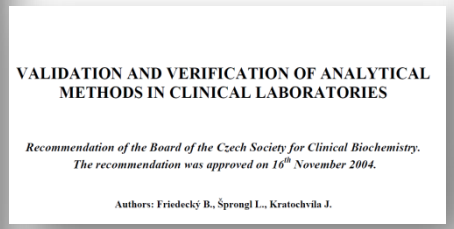
Postupak verifikacije potrebno je provesti prilikom uvođenja nove metode u svakodnevni rutinski rad. Polazište postupka verifikacije su specifikacije mjerne metode u dokumentaciji proizvođača. Postupak verifikacije odnosi se na utvrđivanje i pribavljanje objektivnih dokaza koji potvrđuju da su navedene

PO-5.5-000-5/Z

Standard Operating Procedure:

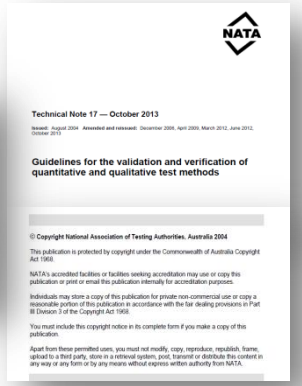
Initial verification of qualitative measurement procedures

Based on:


VALIDATION AND VERIFICATION OF ANALYTICAL METHODS IN CLINICAL LABORATORIES

Recommendation of the Board of the Czech Society for Clinical Biochemistry. The recommendation was approved on 16th November 2004.

Authors: Friedecký B., Šprongl L., Kratochvíla J.



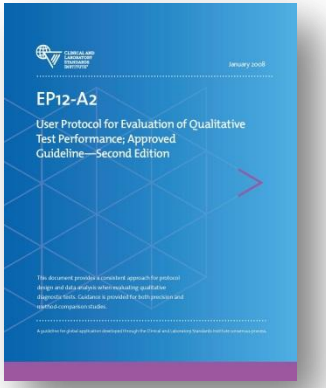
Verification procedures in our laboratory

- precision (repeatability, reproducibility)
- accuracy
- method comparison
- verification of cut-off value (clinical decision limit)
  – reference interval

Precision

- *„…closeness of the agreement between the results of measurements of the same measurand…”*

- repeatability
- reproducibility



CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI Document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

Precision – qualitative tests

1. repeatability
   - one sample
   - one facility
   - short period of time
   - same equipment
   - constant conditions
2. reproducibility
   - series of measurement
   - different facilities
   - different times/days
   - different equipment
   - variable conditions

Qualitative test verification

- positive and negative control sample
- patients samples

- predefined acceptable criteria

Repeatability

- urine test strip
- patient samples (normal and pathological)
- 20 repeats
- consecutively
- short period of time

Reproducibility

- urine test strip
- commercially available control samples
  - level 1 and level 2
- during 10 days in duplicate

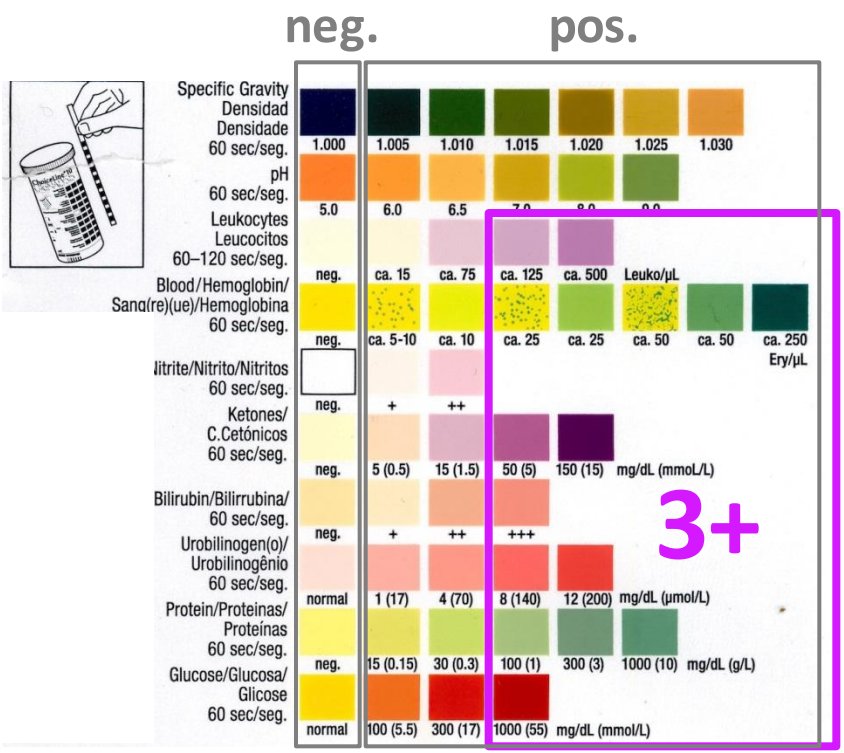
Acceptable criteria for precision

- acceptable agreement 90%

  or

- based on clinically acceptable criteria:
  - specific gravity: ±0.005
  - pH: ±1
  - leukocytes, hemoglobin, ketones, protein (categories 4+, 5+, …)

18/20 samples



Repeatability

Sample 1 27/1/1500	SG	pH	Leu	Nit	Pro	Glu	Ket	Ubg	Bil	Ery
1	1.010	6.0	neg	neg	neg	neg	neg	neg	neg	neg
2	1.010	6.0	neg	neg	neg	neg	neg	neg	neg	neg
3	1.015	6.0	neg	neg	neg	neg	neg	neg	neg	neg
...	1.010	6.0	neg	neg	neg	neg	neg	neg	neg	neg
19	1.010	6.0	neg	neg	neg	neg	neg	neg	neg	neg
20	1.010	6.0	neg	neg	neg	neg	neg	neg	neg	neg
Bias	**20/20**	**20/20**	**20/20**	**20/20**	**20/20**	**20/20**	**20/20**	**20/20**	**20/20**	**20/20**

Negative sample

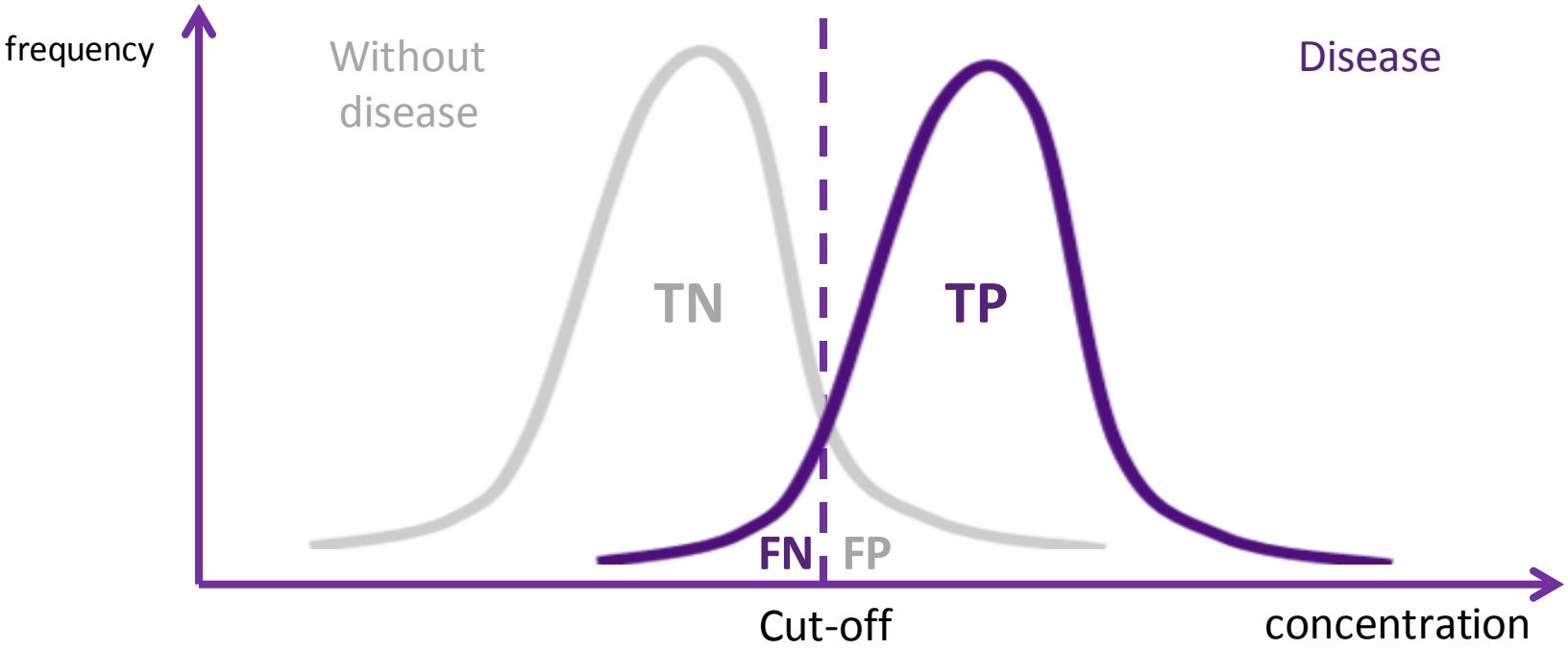
Sample 2 28/1/15	SG	pH	Leu	Nit	Pro	Glu	Ket	Ubg	Bil	Ery
1	1.015	5.0	neg	neg	3+	3+	4+	neg	neg	5+
2	1.015	5.0	1+	neg	3+	3+	4+	neg	neg	5+
3	1.015	5.5	1+	neg	3+	3+	4+	neg	neg	4+
...	1.015	5.0	1+	neg	3+	3+	3+	neg	neg	5+
19	1.015	5.0	1+	neg	3+	3+	3+	neg	neg	5+
20	1.015	5.0	1+	neg	3+	2+	3+	neg	neg	5+
Bias	**20/20**	**20/20**	**18/20**	**20/20**	**20/20**	**19/20**	**20/20**	**20/20**	**20/20**	**20/20**

Positive sample

Accuracy

- Analytical accuracy
  - comparison with true concentration (quantitative test results)
- Diagnostic accuracy
  - comparison with known clinical diagnosis

Accuracy



$$\text{Sensitivity} = 100 \times \left[\frac{TP}{(TP+FN)}\right]$$

$$\text{Specificity} = 100 \times \left[\frac{TN}{(TN+FP)}\right]$$

TP – true positive
FP – false positive
TN – true negative
FN – false negative

Analytical accuracy

- quantitative method („gold standard”)
  - verified
  - acceptable EQA or interlaboratory comparison results
  - test results important for clinical decision:
    - urine dipstick: protein, glucose
    - pregnancy test: hCG
    - drug screening test: GC/MS confirmation

Analytical accuracy: urinary protein

	Ordinal scale method		Quantitative method
Manufacturer	Roche		Abbott
Analyzer	Cobas u411		Architect c8000
Reagent	Combur 10 urine dipstick		Urine/CSF Protein
Accredited	✓		✓
Method	color change		turbidimetric
Sensitivity	0.1 g/L		0.07 g/L
Declared categories	neg	<0.25 g/L	
	1+	0.25-0.75 g/L	
	2+	0.75-1.5g/L	
	3+	>1.5 g/L	

Analytical accuracy: urinary protein

Sample	Cobas u411	Expected values	Architect c8000 (g/L)
1	1+	1+	0.48
2	1+	1+	0.58
3	1+	neg	0.23
4	3+	3+	1.78
5	1+	1+	0.38
6	1+	1+	0.32
7	3+	3+	2.72
8	2+	2+	0.84
9	neg	neg	0.21
10	1+	neg	0.61
11	1+	1+	0.32
12	2+	2+	0.76
13	neg	1+	0.27
14	neg	neg	0.15
15	neg	neg	0.10
16	1+	neg	0.44
17	1+	1+	0.72
18	neg	neg	0.13
19	3+	3+	1.56
20	neg	neg	0.12

True positive

False positive

False negative

True negative

Cat.	Conc.
neg	<0.25 g/L
1+	0.25-0.75 g/L
2+	0.75-1.5g/L
3+	>1.5 g/L

Analytical accuracy: urinary protein

Test method	Quantitative test		Total
	POSITIVE	NEGATIVE	
POSITIVE	TP	FP	TP + FP
NEGATIVE	FN	TN	FN + TN
TOTAL	**TP + FN**	**FP + TN**	**N**

$$\text{Sensitivity} = 100 \times \left[\frac{TP}{(TP+FN)}\right]$$

$$\text{Specificity} = 100 \times \left[\frac{TN}{(TN+FP)}\right]$$

Cobas u411	Architect c8000		Total
	POSITIVE	NEGATIVE	
POSITIVE	11	3	14
NEGATIVE	1	5	6
TOTAL	**12**	**8**	**20**

$$\text{Sensitivity} = 100 \times \left[\frac{11}{(11+1)}\right] = 91.7\%$$

$$\text{Specificity} = 100 \times \left[\frac{5}{(5+3)}\right] = 62.5\%$$

Clinical accuracy

- known diagnosis
  - clinicians
  - immunology tests (IIF: ANA, AMA, ASMA, LKM, ANCA)

Clinical specificity

Subjects without known autoimmune diseases

	AMA/AGLM/LKM IIF titer 1:80	AMA/ASMA/LKM IIF titer 1:100
1	neg	neg
2	Weak pos. ASMA	neg
3	neg	neg
4	neg	neg
5	neg	neg
6	neg	neg
7	neg	neg
8	neg	neg
9	Weak pos. ASMA	neg
10	neg	neg
11	neg	neg
12	neg	neg
13	Weak pos. ASMA	neg
14	neg	neg
15	neg	neg
16	neg	neg
17	neg	neg
18	Weak pos. ASMA	neg
19	neg	neg
20	neg	neg
21	neg	neg
22	neg	neg
23	Pos. ASMA	Weak pos. ASMA
24	neg	neg
25	Pos. ASMA	Weak pos. ASMA
26	Pos. ASMA	Pos. ASMA
27	neg	neg
28	neg	neg
29	neg	neg
30	neg	neg

True negative

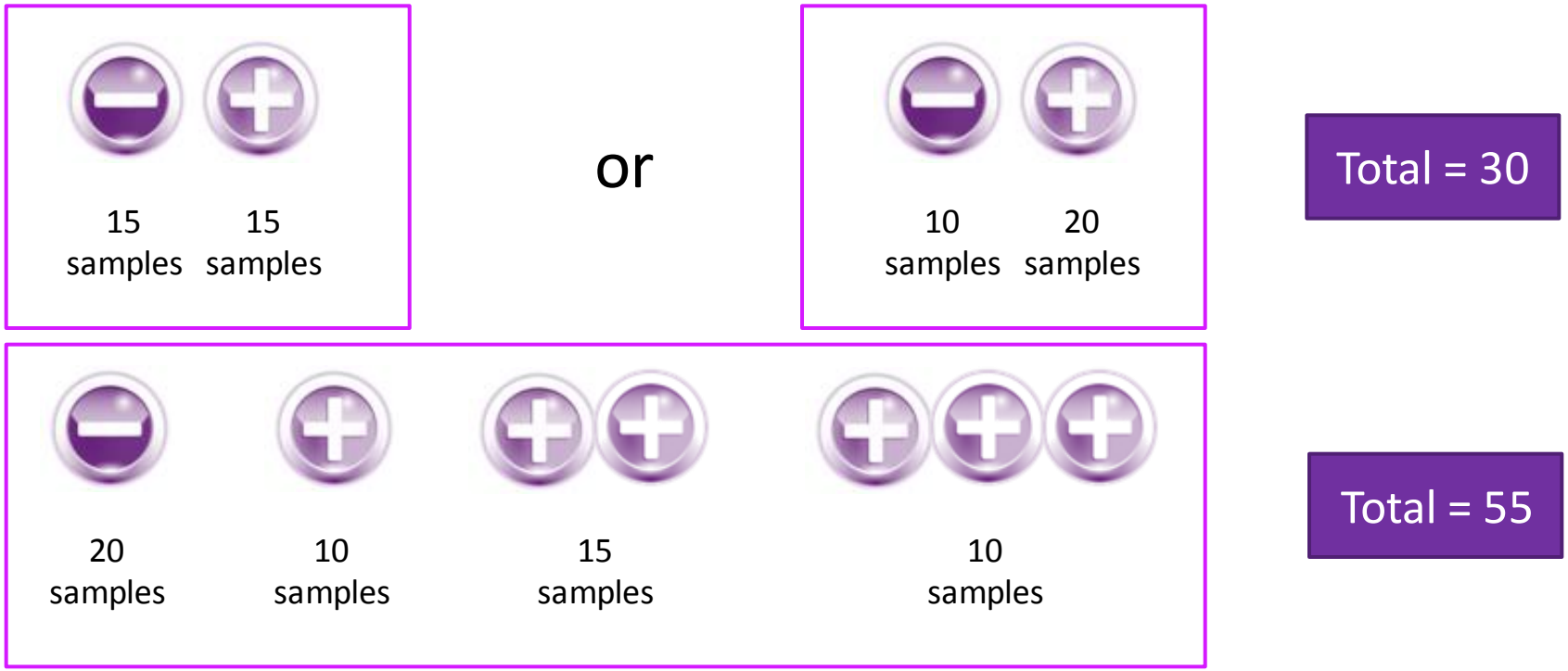
False positive

Titer 1:80 (%) =
(TN / TN + FP) x 100
=(23/23+7) x 100=**76.6%**

Titer 1:100 (%) =
(TN / TN + FP) x 100
=(27/27+3) x 100=**90%**

Method comparison

- daily (min. 10 days)
- min. 10 samples per category (available result)
- for 2 categories min. total of 30 samples



Method comparison analysis

- Agreement between data (κ coefficient)

Lessons in biostatistics

Interrater reliability: the kappa statistic

Mary L. McHugh

Department of Nursing, National University, Aero Court, San Diego, California

Corresponding author: mchugh8688@gmail.com

McHugh ML. Interrater reliability: the kappa statistic. Biochem Med 2012;22(3):276-82

κ coefficient

- Interrater reliability – multiple data collectors (person or analyzer), one measurement each

- Intrarater reliability – single data collector (person or analyzer), several measurements

- subjective
- influence of many variables

Interpretation of kappa coefficient

Value of Kappa	Level of Agreement	% of Data that are Reliable
0-.20	None	0-4%
.21-.39	Minimal	4-15%
.40-.59	Weak	15-35%
.60-.79	Moderate	35-63%
.80-.90	Strong	64-81%
Above .90	Almost Perfect	82-100%

Acceptable

Expressed with 95% CI!

McHugh ML. Interrater reliability: the kappa statistic. Biochem Med 2012;22(3):276-82

Kappa coefficient for two analyzers

Interrater kappa coefficient:

Compared analyzers:
1. Miditron Junior II
2. Cobas u411

Parameter	Weighted kappa coefficient (95% CI)
U-SG	0.708 (0.603-0.812)
U-pH	0.777 (0.683-0.870)
U-protein	0.883 (0.810-0.955)
U-glucose	0.952 (0.893-1.000)
U-ketone	0.918 (0.837-0.999)
U-urobilinogen	0.787 (0.607-0.982)
U-bilirubin	0.370 (0.112-0.628)
U-nitrite	0.927 (0.785-1.000)
U-erythrocyte	0.784 (0.720-0.840)
U-leucocyte	0.860 (0.793-0.926)

Example: calculation of kappa (bilirubin)

Inter-rater agreement (kappa)

Observer A	Bil_M
Observer B	Bil_u411

Observer B	Observer A 0	1	2	3	
0	71	0	0	0	71 (81,6%)
1	13	1	0	0	14 (16,1%)
2	0	0	0	0	0 (0,0%)
3	0	0	2	0	2 (2,3%)
	84 (96,6%)	1 (1,1%)	2 (2,3%)	0 (0,0%)	87

Weighted Kappa	0,370
Standard error	0,131
95% CI	0,112 to 0,628

Frequency chart

minimum 10 samples/category

Kappa coefficient reliability

- rare categories (rarely positive antibodies)
- categories with < 10 samples

Cut-off value

- *„…analyte concentration at which repeated tests on same sample yield”*

50% samples

50% samples

CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI Document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

Verification of cut-off value

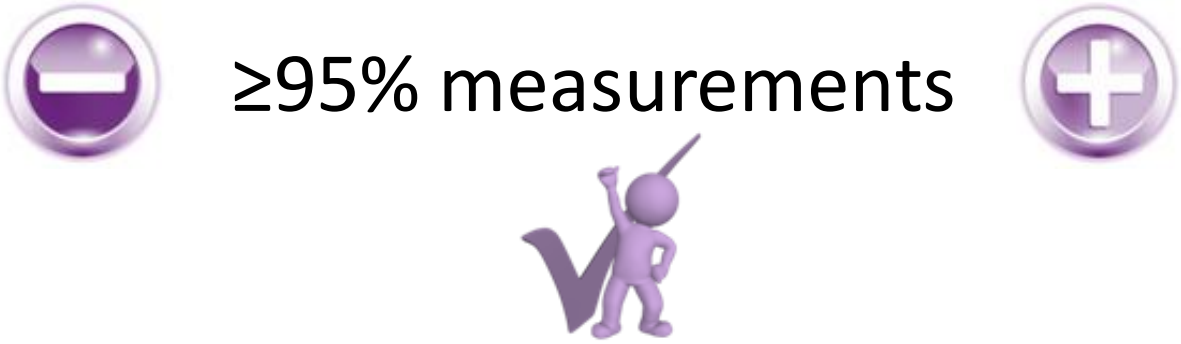
- 3 concentration levels
  - cut-off value
  - 20% above cut-off (+20%)
  - 20% below cut-off (-20%)

- 20 repeats/level
- determine % of positive and negative results

Verification of cut-off value

- Samples with concentration above/below cut-off value

-20% sample +20% sample



≥95% measurements

±20% concentration range in 95% interval

Example: tetrahydrocannabinol (THC) on test strip

- Cut–off value = 50 ng/mL

- -20% (below cut-off)= 40 ng/mL
- +20% (above cut-off) = 60 ng/mL

- 19/20 (95%) samples – negative at 40 ng/mL
- 19/20 (95%) samples – positive at 60 ng/mL

- at concentration range 40 – 60 ng/ml → reliable results

Conclusion

- verify all methods
- define procedure for verification
- define own criteria – analytical, clinical
- use appropriate statistics

- reliable and accurate results

Take a home massage

Verification of qualitative methods

- Diagnostic sensitivity is proportion of true positive subjects with the disease in the group of all subjects with disease (TP/TP+FN).
- Cut-off value in a qualitative test method is the analyte concentration at which repeated tests on the same sample yield positive results 50% of the time and negative results for the other 50%.
- Kappa coefficient for method was 0.66. This result means that 56% of results may be different in the compared methods.
- The result: 18/20 for qualitative analytical method has acceptable repeatability according to predefined criteria.