Test request

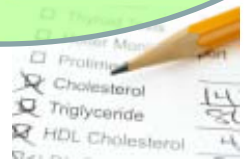
Sampling

Black box: the lab

POSTANALYTICAL PHASE

Result interpretation

Result



And then the findings are created

- Sent via the informatics system
- Printed form

Inherent elements of a lab report

Report Date	**17-Jun-2008**		LabDriver LIMS System
Subject Id	**XY0242**		180 Acre Road, Kingston upon Thames
Subject No			Surrey KT2 6EU, UK
Sex	**M**		Tel +44(0)208 255 0356
Lab No	**0804502**		Fax +44(0) 871 2473055

Study	**Study_101**	Ref No	
Visit	**SCREEN**	Screen ID	**0002920**
Investigator	**Dr Ian Doctor**	Centre ID	**0002**

QC Sign	**FGR**	Doctor		Date
Centre: London Hospital CR				Page 1 of 2

Laboratory Report (Final)

CHEMISTRY ESig: SLR Received: 07-May-2008 Sample: 07-May-2008 12:59

	Result	**Flag**	**Units**	**Reference**	**Range**
SGPT(ALAT)	26		IU/L	12	44
Albumin	43		g/L	38	47
Alkaline Phosphatase	60		IU/L	39	100
SGOT(ASAT)	28		IU/L	17	37
Total Bilirubin	11.8		umol/L	6.0	21.0
Total Protein	70		g/L	64	80
Creatinine	95		umol/L	65	105
Urea	**7.9**	**H**	**mmol/L**	**3.5**	**7.5**
Uric Acid	0.329		mmol/L	0.197	0.422
Creatine Kinase	194		IU/L	53	320
Gamma GT	19		IU/L	13	50
Potassium	4.56		mmol/L	3.90	5.20
Sodium	140		mmol/L	135	143
Calcium	2.30		mmol/L	2.14	2.55
Magnesium	0.84		mmol/L	0.70	1.10
Inorganic Phosphate	0.97		mmol/L	0.82	1.32

VIROLOGY ESig: SK Received: 07-May-2008 Sample: 07-May-2008 12:59

	Result	**Flag**	**Units**	**Reference**	**Range**
Hepatitis B surface Antigen	Negative				
HCV	Negative				

HAEMATOLOGY ESig: SLR Received: 07-May-2008 Sample: 07-May-2008 12:59

	Result	**Flag**	**Units**	**Reference**	**Range**
Haemoglobin	13.9		g/dL	12.8	16.8
Haematocrit	0.40		L/L	0.37	0.49
Red Cell Count	4.5		10*12/L	4.1	5.5
White Cell Count	6.4		10*9/L	3.9	10.8
Lymphocytes	1.9		10*9/L	0.9	3.1
Neutrophils	3.9		10*9/L	2.2	6.9
Monocytes	0.5		10*9/L	0.3	0.9
Basophils	0.03		10*9/L	0.00	0.10
Eosinophils	0.12		10*9/L	0.00	0.40
Lymphocytes (%)	30		%	17	42
Neutrophils (%)	60		%	48	75
Monocytes (%)	7		%	5	13
Basophils (%)	0.4		%	0.0	1.0
Eosinophils (%)	1.8		%	0.0	5.0
Platelets	238		10*9/L	132	321

Data	NOTE
Patient's name and identifier	Items required depends on lab. Finding without name / identifier cannot be used for clinical decision
Name of the analyte measured	There are several analytes on one finding; these are listed consequtively
Result	Most often a number, rarely a note (positive, negative)
Parameter (unit)	Result without parameter cannot be used
Healthy reference range	or 'normal value'.
Comment	Any comment on test or test specimen (eg. hemolyzed, or few amount), any interpretation of result
Lab performing the test	Private or state
Name and identifier of validating staff	In general a doctor or clinical biochemist

The question of units

- The units are not universal
- There are conventional and SI units
- UK, AUS, NZL, CAN, HU, NL – SI units (mmol/L)
- USA, D, ISR, JA – conventional units (mg/dL)
- The value is meanless without the unit.

Some examples: the electrolytes

analyte	SI	conventional
se Na, se K, se Cl, se HCO3-	1 mmol/l	1 maeq/L
se Ca	1 mmol/L	4 mg/dL or 2 maeq/L
se Mg	1 mmol/L	2.4 mg/dL
se P	1.2 mmol/L	3 mg/dL

A characteristic example: the calcium

Let’s say: serum calcium is 3.05.

Is it high, normal or low?

Depends on the unit used:

Total calcium, reference range:

2.3 – 2.6 mmol/L H

4.6 – 5.2 maeq/L L

9.2 – 10.4 mg/dL LL

Some examples: chemicstry & haematology

SI (System International)	Conventional units
Blood Hgb 148 g/L	14.8 g/dL
Total protein 66 g/L	6.6 g/dL
Se Glucose 4.3 mmol/L	77 mg/dL
BUN 25 mmol/L	70 mg/dL
Se Chol. 5.5 mmol/L	212 mg/dL
Se TG 3.3 mmol/L	292 mg/dL
Creatinine 88 µmol/L	0,99 mg/dL

Some examples: chemicstry & haematology

SI (System International)	Conventional units
Se Bilirubin 50 µmol/L	2,9 mg/dL
Lactate 2 mmol/L	18 mg/dL
Ammonia 40 µmol/L	68 µg/dL
Se T4 total 113 nmol/L	8,8 µg/dL
Se T4 free 12 pmol/L	0,9 ng/dL
Bacterial CFU 10^9/L	10^6/mL

Data	NOTE
Patient's name and identifier	Items required depends on lab. Finding without name / identifier cannot be used for clinical decision
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Interpretation of test results

Common interpretations:

- ‘negative’
- ‘Bad / good’
- ‘Normals’
- XY is increased

Problem:

- Healthy reference value? (may be age, labor and population specific!)
- Preanalitical problems are not considered (‘the labor works ugly’)
- The meaning of the test is not taken in account

First question: compare to with

- Matched age?
- Same gender?
- Documentedly healthy?
- Not affected from the investigated condition (but still unhealthy)
- What conditions are allowed to have a control subject?
- Size of control population?

(answers depend on a number of factors)

Interpretation of test result

Important terms:

SENSITIVITY =

Positive / Total number of patients

$$\text{sensitivity} = \frac{\text{number of True Positives}}{\text{number of True Positives} + \text{number of False Negatives}}$$

SPECIFICITY =

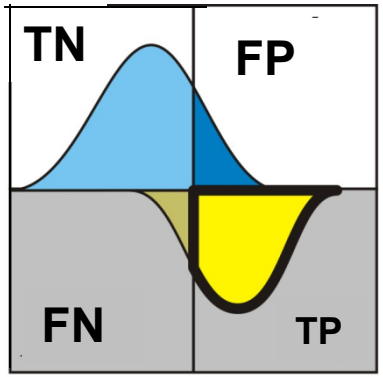
Negative / Total number of healthy

$$\text{specificity} = \frac{\text{number of True Negatives}}{\text{number of True Negatives} + \text{number of False Positives}}$$

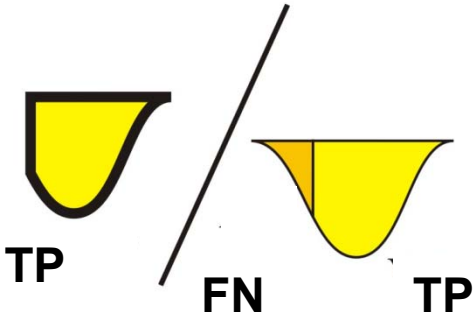
sensitivity

Rate of patients with true positive result

(se)



= the probability of positive test result in a patient

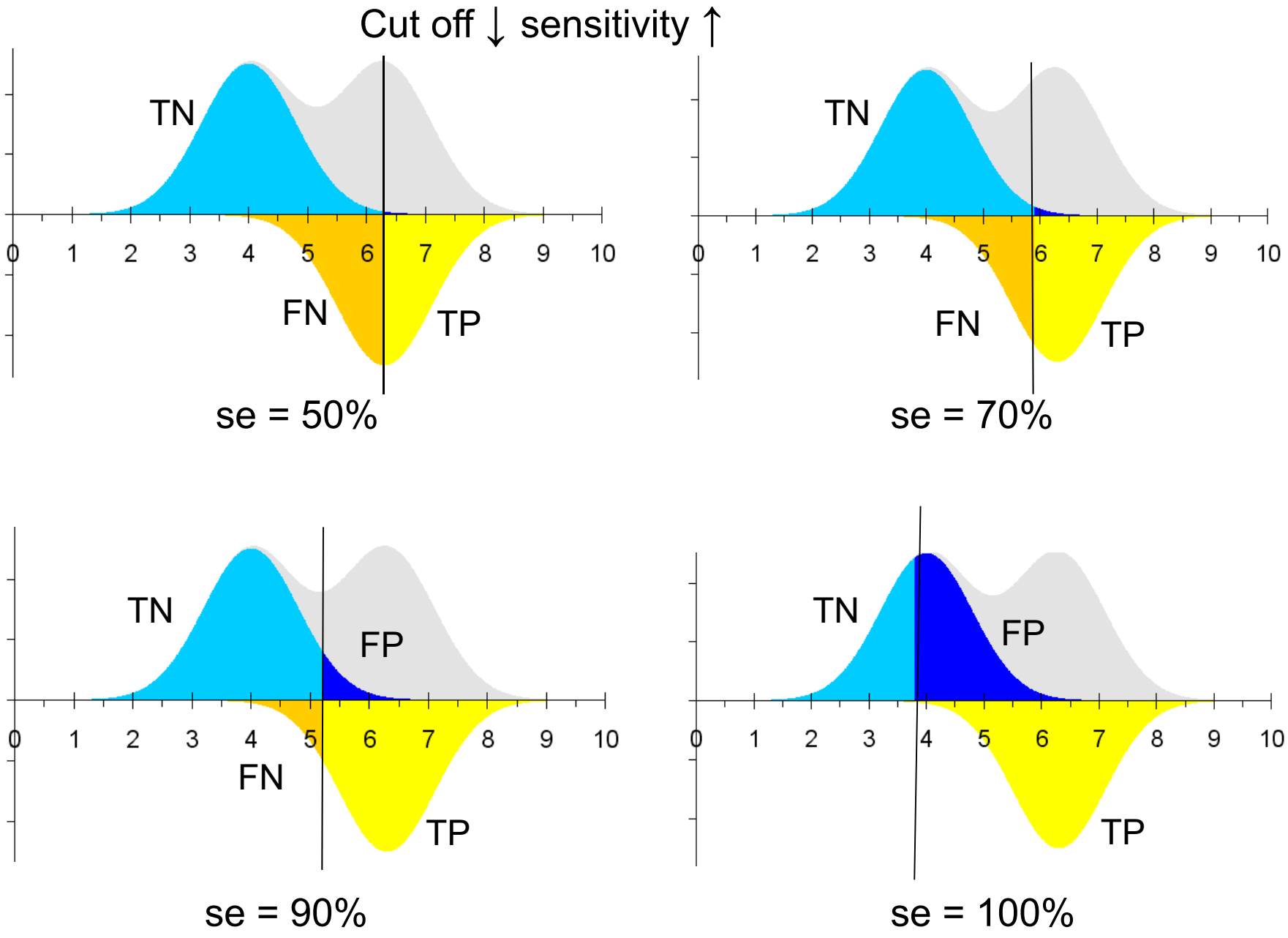


= TP / (TP + FN)

se

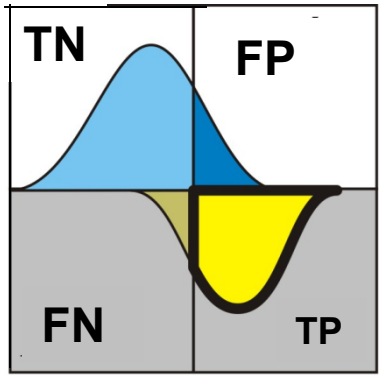
For screening high sensitivity test is required (i.e. to miss just a few patients)

(Sensitivity should be lowered if there is no sufficient resource for treatment of identified cases)

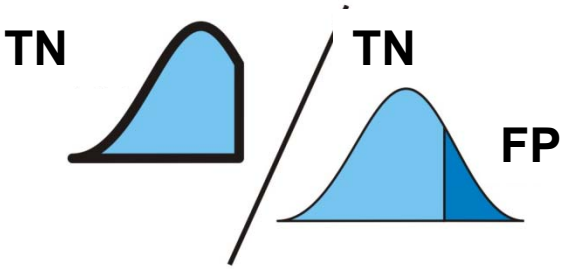

Cut off ↓ sensitivity ↑
TN
FN
TP
0 1 2 3 4 5 6 7 8 9 10
se = 50%
TN
FN
TP
0 1 2 3 4 5 6 7 8 9 10
se = 70%
TN
FP
FN
TP
0 1 2 3 4 5 6 7 8 9 10
se = 90%
TN
FP
TP
0 1 2 3 4 5 6 7 8 9 10
se = 100%

specificity

Rate of healthy subjects presenting true negative results



= probability of having a negative test result in a healthy subject

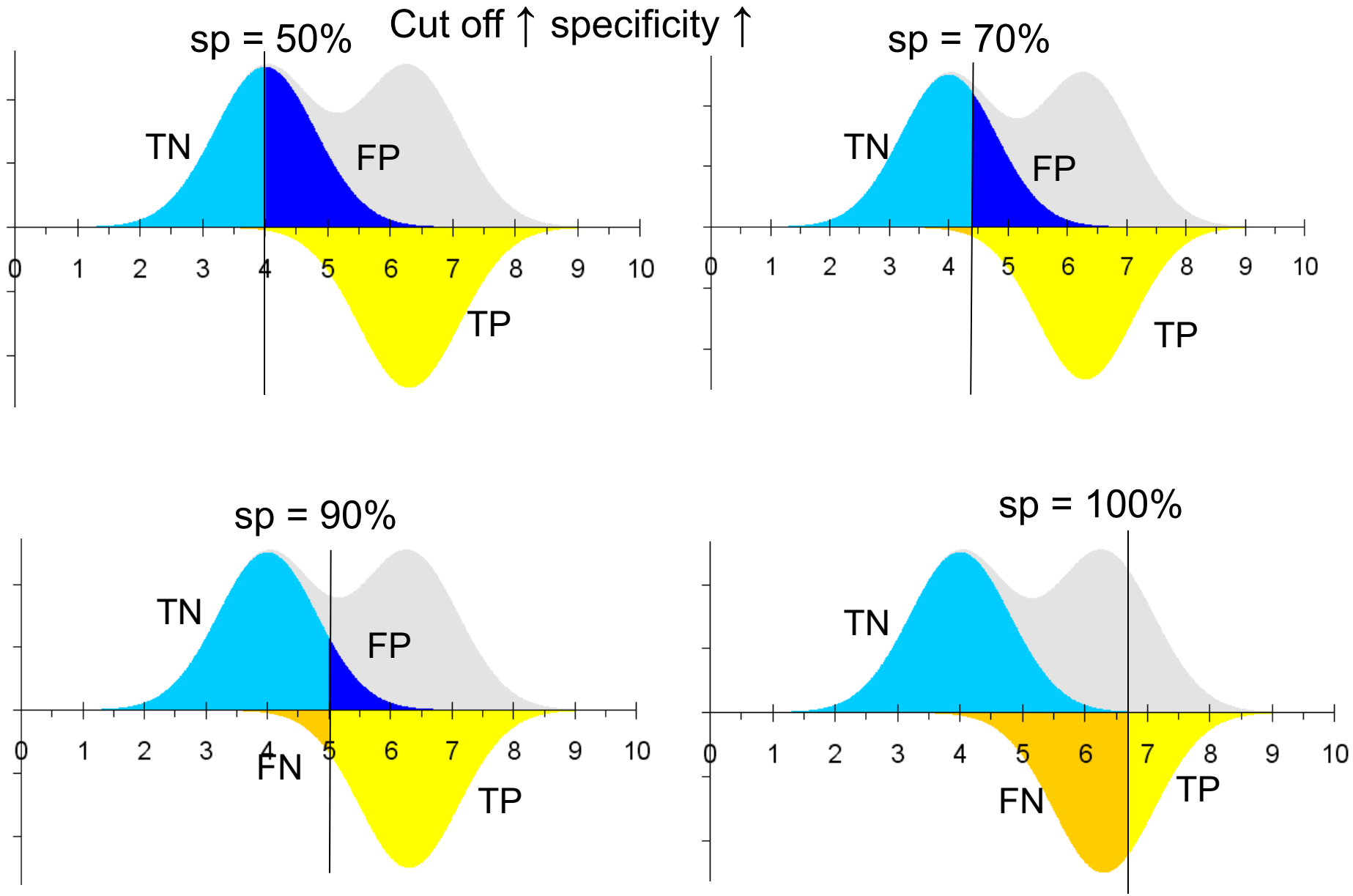


= TN / (TN + FP)

sp

High specificity is required when false positive results should be disclosed.

(Usually required for confirmatory tests)


Cut off ↑ specificity ↑
sp = 50%
TN
FP
TP
0 1 2 3 4 5 6 7 8 9 10
sp = 70%
TN
FP
TP
0 1 2 3 4 5 6 7 8 9 10
sp = 90%
TN
FP
FN
TP
0 1 2 3 4 5 6 7 8 9 10
sp = 100%
TN
FN
TP
0 1 2 3 4 5 6 7 8 9 10

Predictive values

Prevalence of predictive value of a lab test depends on prevalence of disease in population.

It indicates the probability that a positive test result confers to disease (positive predictive value), a negative test result confers to healthy condition (negative predictive value).

- **positive predictive value: if test result is positive, then what is the probability that the tested subject is affected?**

  positive predictive value (PPV): TP/(TP+FP)

- **Negatív predictive value: if test result is negative, then what is the probability that the tested subject is not affected?**

  negatív predictive value (NPV): TN/(TN+FN)

Depends on prevalence in population

Predictive values

Prevalence of predictive value of a lab test depends on prevalence of disease in population.

It indicates th…ease (positive …lthy condition

- **positive p…hat is the proba…**

  positive …

- **Negatív p…what is the probability that the tested subject is not affected?**

  negatív predictive value (NPV): TN/(TN+FN)

Analogies

Screening for severe congenital metabolic disorders

Searching for bombs at the airport

Depends on prevalence in population

Predictive value

- Importance of PREVALENCE
- The rate of healthy and diseased subjects have an impact on test PPV and NPV values.
- That means: the information provided by a given test depends on characteristics of tested population
- If I use even a very high sensitivity test (but there is no patient in the tested population), then I will get false positive results only

An example

- HIV-test: both specificity and sensitivity are around 0,99
- If 20000 HIV infected patients are in Hungary, the prevalence of HIV infection is 0.002.
- Let’s suppose that prevalence of HIV positivity among blood donators is the same (in fact it is lower)
- Question: what will be the rate of false positive results amon blood donators?
- SP:0,99, SN:0,99, PR:0,0020,

The PPV

$$\frac{0{,}99*0{,}0020}{(0{,}99*0{,}0020)+(0{,}01*0{,}9980)}=\frac{0{,}00198}{0{,}01196}=0{,}165$$

That means that out of 100 positive (reactive) samples just 17 confers to HIV seropositive blood donators, and 83 results are false positive (In fact this rate is much more higher).

The real NPV

$$\frac{0.99*0.998}{(0.99*0{,}002)+(0.99*0{,}998)}=\frac{0.988}{0.99}=0{,}998$$

Therefore, the risk of obtaining a false negative result is very-very low.

The PPV

$$\frac{0,99*0,0020}{(0,9} \qquad \frac{0,00198}{} = 0,165$$

That m … es just
17 con … 33
results … ore
higher …

Appropriate response

Other methods to be used (instead of repeated measurements)

Risk analyzis (identification of populations with higher prevalence of disease)

Bayes analysis (estimation of predictive value based on a priori data)

$$\frac{}{(0.99}$$

Population-dependent testing strategies (nation-specific approaches)

Therefore, the risk of obtaining a false negative result is very-very low.

Another example

- PSA-test
- >8 ng/ml : suggestive for prostate cancer
- Z.Z patient, aged 68 ys is presenting with PSA = 13 ng/ml
- What is the PPV value if the patient is

Male?

Female?

Receiver Operating Characteristics (ROC) curve

In case of dichotomic results the cut-off value is clear (usually).
In case of continuous results the cut-off calue should be established carefully.

The callenge: to identify cut-off value to discriminate positive and negative with the best specificity and sensitivity (ROC curve)

ROC curve:

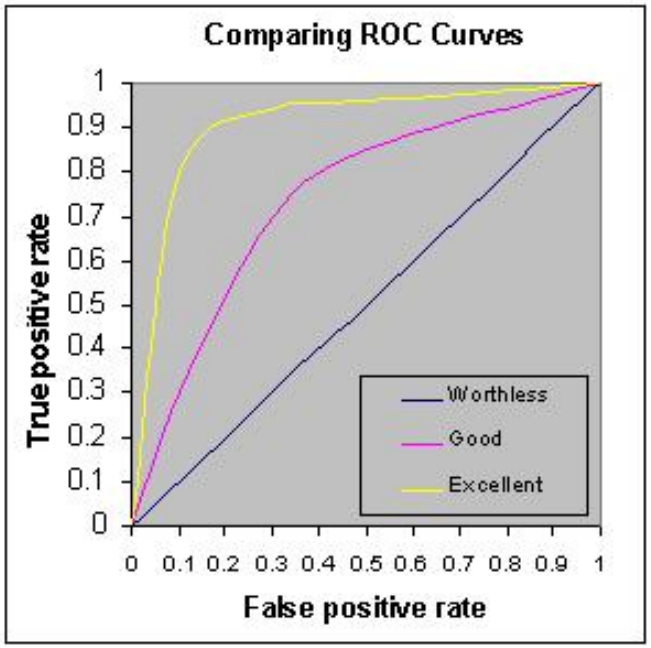
- Indicates the true positive rate and the false-positive rate at difference cut-off values

(i.e.: compares sensitivity to 1- specificity values)

- Appropriate to assess clinical utility of a given test
- Supports the selection of the most appropriate cut-off (reference) value

ROC curve

sensitivity



1-specificity

Area under the curve
0,9-0,99= excellent
0,8-0,9 = good
0,7-0,8 = moderate
0,6-0,7 = fair
<0,6 = failed

The test is more accurate whe the curve tends to follow left and up border.
The clinical utility of test is lower if the curve's slope is nearer to 45°.

Test combination

- Let’s think a bit:

Test with low specificity and high sensitivity (large number of high positive results)

Convenient for screening

The screened positive patients should be subjected to highly specific and low sensitive tests.)

Combined / hierarchic assays

Example: thyroid hormone tests

Finally: I have a reference range

- The referencia range has too (lower and upper) limits.
- In some cases data are normally distributed
- In other cases they are skewed to the left or the right
- Abnormal = any value outside the range.

Examples when abnormal is below the ref range:

- Vitamine levels
- Protein levels (non-acute phase)
- CBC

Examples when abnormal is above ref. range:

- Tumor markers
- Markers of tissue necrosis
- Some hormones
- CBC

Some specific reference ranges

- Pediatric values
- Gender-specific values
- Cycle-dependent ranges
- Diurnal variations
- In some cases reference values are changing dramatically and just relative values can be given

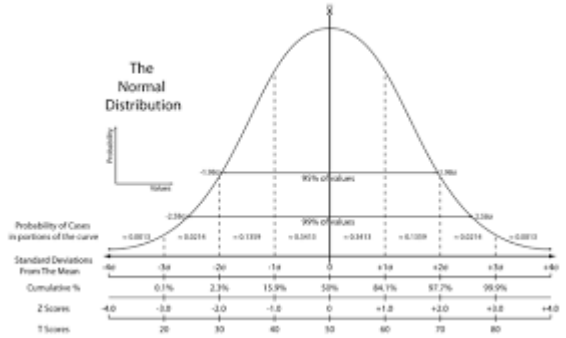
Percentile

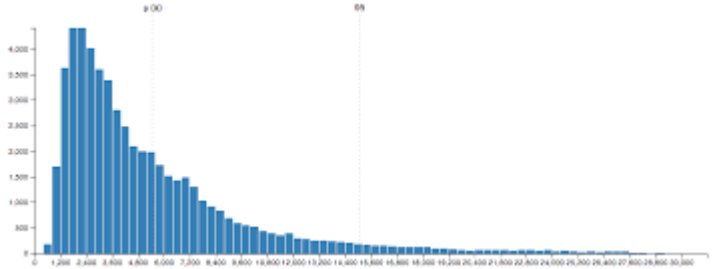
MoM

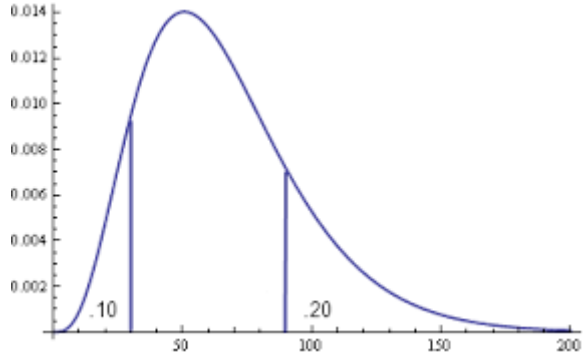
T-score és Z-score

Special reference ranges

Percentile

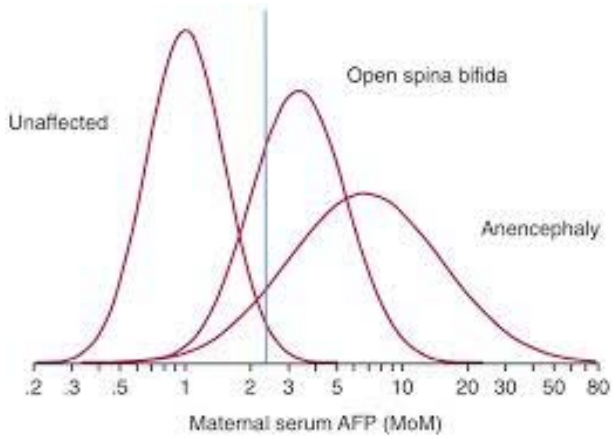


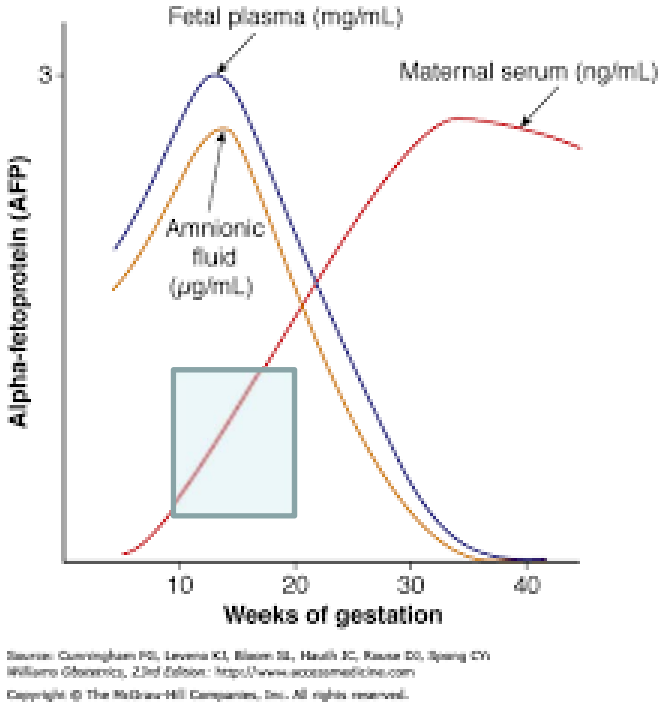




Special reference ranges

MoM



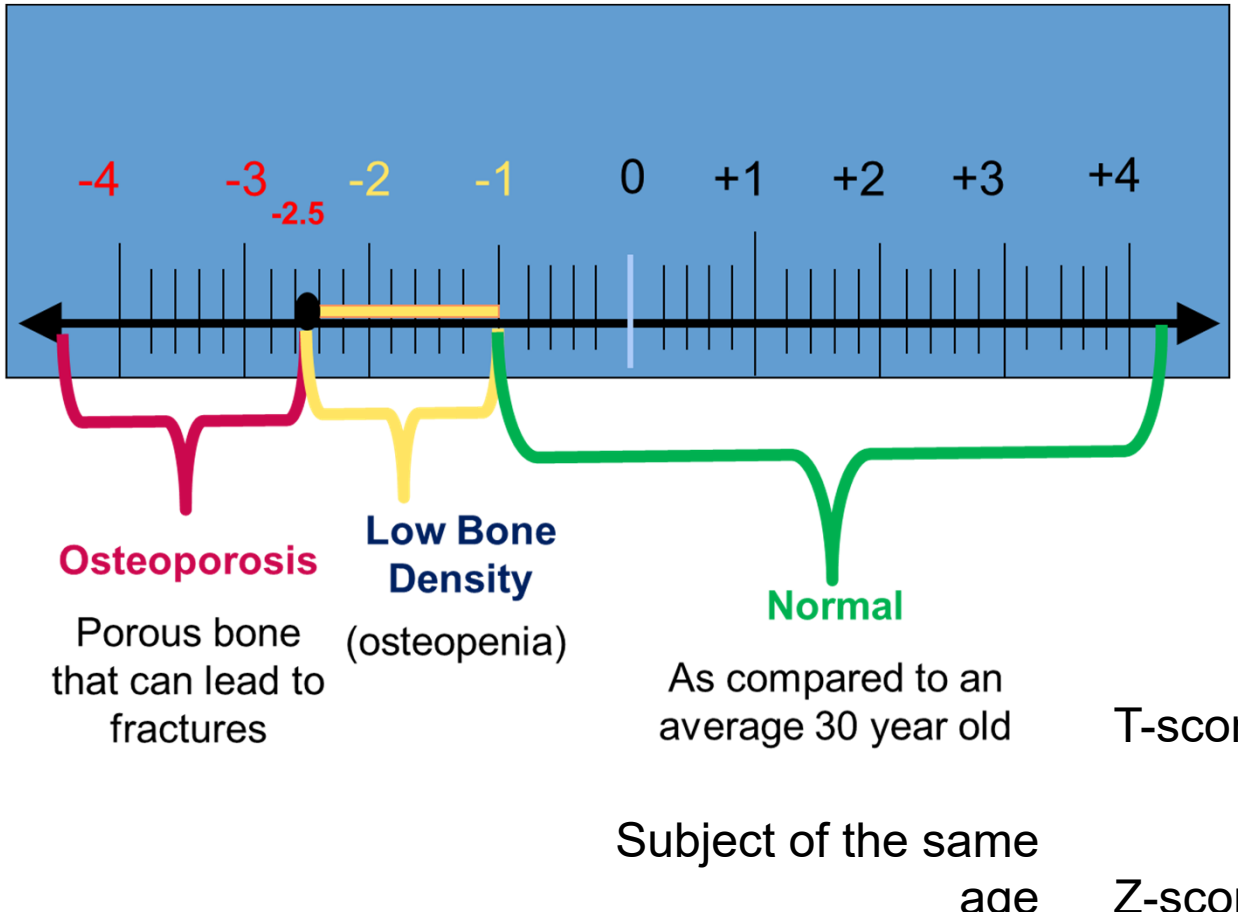


MoM = multiples of median

For those cases when reference range changes very quickly in time (e.g pregnancy or cancer)

Special reference ranges

- T-score and Z-score



Reference values

Cut-off value

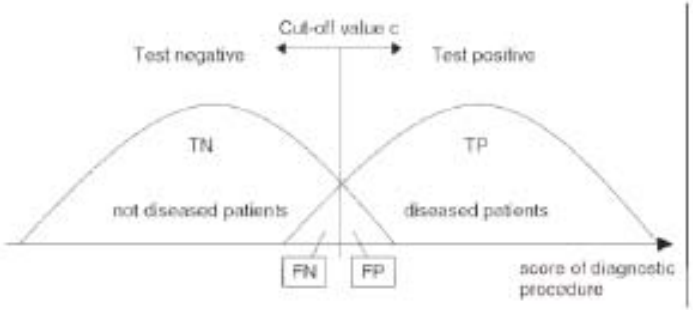
General principle: measured values in 95 (90 – 99)% of healthy subjects should be within the healthy reference range.

The probability is 95% that any analyte is within the healthy reference range.

If 20 analytes are measured in a (healthy) subject, just $0.95^{20} = 0.36$ → 36% is the probability that each analyte is normal.

If the specificity 99% for each, just 81% is the probability that each analyte is normal.

(i.e. the principle 'how to make a diseased person from a healthy one')



And now, let’s see what is happening in real life

- Emergency lab tests
- Alarm values

Some remarks on emergency lab tests

Sürgős lapon feltüntetendő:

Beteg adatai (név, szül. dátum, TAJ szám):
Diagnózis: BNO-kód:

Vizsgálatkérő intézet		Vizsgálatkérő osztály	
Vizsgálatkérő orvos:		Telefonszám (ha nincs, nem kerül az krízis eredmény bemondásra):	

Mintavétel dátum & időpont (óra, perc):
Vizsgálati minta típusa:

Natív vér		EDTA-s vér		Vizelet	
NaF-os vér		Citrátos vér		egyéb	

Glükóz		T.bilirubin		iCa **		VIZELETVIZSGÁLAT TESZTCSÍKKAL	
Na, K, Cl		D.Bilirubin		Ammónia*			
Kalcium		ALP		Laktát*		ÁLTALÁNOS VÉRKÉP	
Foszfát		GOT		Béta-HCG**			
Összfehérje		GPT		Troponin T		VÉRCSOPORT	
Albumin		GGT					
Kreatinin		LDH		haptoglobin**		VÉRGÁZ*	
Karbamid		Szérum amiláz				SZÉKLET VÉR	
Húgysav		Vizelet amiláz					
Szérum ozmolalitás		CRP				LIQUOR fehérje, glükóz, sejtszám	
Vizelet ozmolalitás		Prokalcitonin					

GYÓGYSZERSZINTEK SZÉRUMBAN

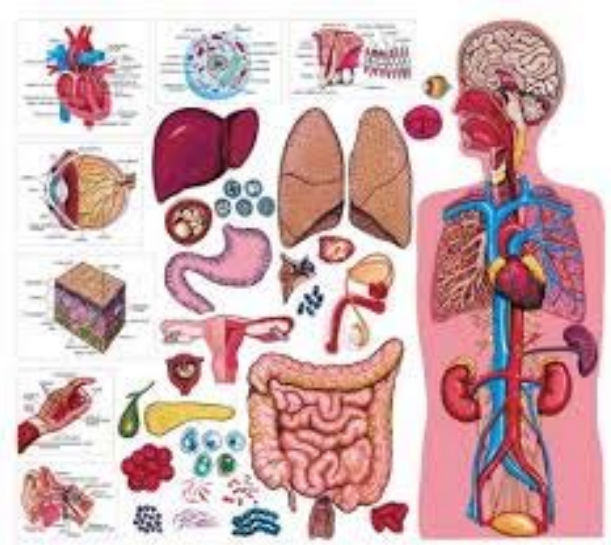
Metotrexát		Digoxin		Fenobarbitál		Valproát		Lítium	
Vankomicin		Karbamazepin		Fenitoin		Drog-teszt**			

HEMOSZTÁZIS VIZSGÁLATOK
Kitöltendő, hogy milyen specifikus kezelést kap a beteg

K-vitamin antagonista:		Nem frakcionált heparin:		Klopidogrel:		Direkt trombingátló:	
LMWH		Aszpirin:		Fibrinolízis:		Rivaroxaban:	
Egyéb kezelés, azaz:							

Protrombin idő		Trombin idő		D-dimer		Heparinszint***	
APTI		Fibrinogén		Antithrombin III.		Rivaroxaban***	

* speciális csőbe veendő ; ** külön telefonos egyeztetés alapján; *** csak abban az esetben, ha összhangban áll a beteg gyógyszerelésével



Some remarks on emergency lab tests

- Vital lab tests
- These include: toxicity, electrolyte levels, metabolic disturbances, tissue necrosis
- TAT : < 1 hour
- Technicians provide them without validation by graduated staff (’intermediate’)
- Restricted lists (depends on institution)
- More expensive and need more efforts

Alarm values

- Critical values that should be communicated to the doctor immediately
- Require immediate clinical intervention
- There is no unique guide, depends on environment (lab & department)

Alarm values – do remember

- Value reported verbally is of no legal power
- The reporter's name and position should be documented
- The basis of clinical decision should be the written findings
- Legal issue: as lab report has just partial impact on clinical decision making, the lab's responsibility is limited

The findings are generated

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

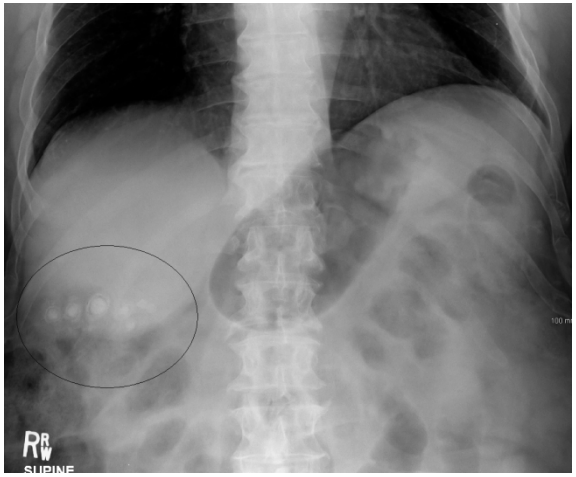
Imaging findings

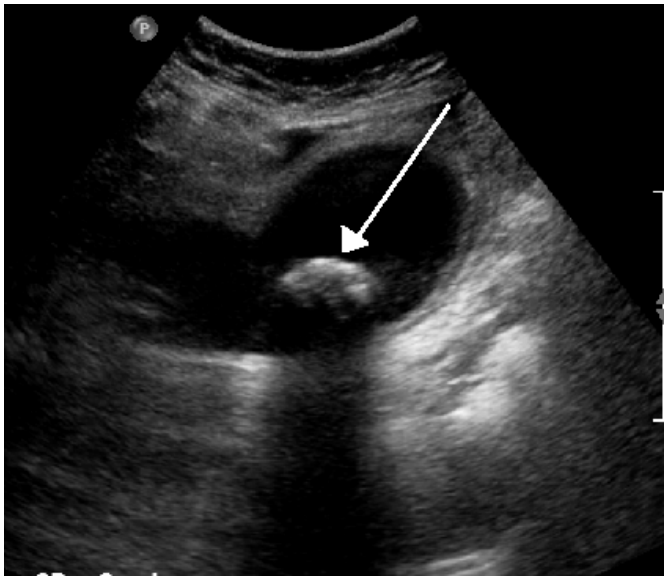
Diagnosis:

Gallstones

(with a still opened pancreas duct)

ERCP





Findings of repeated testing

- ALP: 540 U/L
- Bilirubin: 50 micromol/L
- Direct bilirubin: 24 micromol/L
- Normal: amilase
- WBC: 7.5 G/L

Let’s play a situation game with Pete…

Bonus-tests (as the patient is already there):

- PSA: 5.3 ng/ml (+/0)
- Cholesterol: 6,5 mmol/l (+/0)
- Triglyceride: 2,1 mmol/l
- eGFR: 63 ml/min
- Uric acid: 380 micromol/L
- Na, K, Cl, urea, GOT, GPT, albumin, D-dimer, vitamin-D

Let’s play a situation game with Pete…

- What do prior results trigger?
- Investigation?
- Do not report?
- Monitoring?
- Repeat?

Important: unnecessary result may be harmful either

Data and remnant samples

Issues:

- Ownership (sample and data)
- Who and how could treat the results?
- Issues of data saving
- How to destroy (or store) samples

What can I do with the sample?

- Thumb's rule: diagnostic samples can be used only for treatment purposes
- The use of sample for other purposes requires ethical permission (exception: method development on anonymous samples)
- Biobanks can be established when patients' consent is obtained

A few words about costs

- Lab testing costs money (reagent, salary, consumables, maintenance of lab)
- Costs of lab are calculated using a central list in Hungary
- In general, costs are paid by the physician / hospital
- These may be quite high, but just a small portion of total health care costs (2-4 per cent)
- The most expensive tests are those that are done in an unjustified manner

Summary I

- Postanalytical phase: the phase when the lab test results are used
- Lab tests have their specificity and sensitivity; these are tests' caharacteristics
- The positive and negatív predictive value of the test depends on prevalence either
- The selection of the appropriate reference group is of critical importance
- The higher the number of lab tests the higher the risk of false positivity

Summary II

- Emergency tests are available 24/7; the list is restricted
- Alarm values: depend on environment. This case the lab notifies the health care staff immediately
- One should pay for lab tests
- Most expensive tests are those that are ordered / interpreted improperly