

**9th AEWIEM
Stará Lesná, Slovakia,**

September 27 , 2004

**THE EFFECT OF VARIABILITY OF BLOOD SPOT CARDS
ON DETECTION LIMIT ANALYSIS AND LINEARITY CHECK FOR
GALACTOSE NEWBORN SCREENING KIT**

Tijen Tanyalçin MD,Ph.D.

TANYALÇIN MEDICAL LABORATORY 1359 sok. No: 4/3 35220 ALSANCAK IZMIR/TURKEY,

Fax: +90 232 463 09 10

E-mail tanyalci@med.ege.edu.tr

George J. Reclos Ph.D.

Pharmacist-Immunologist

R&D DIAGNOSTICS LTD

P.O.BOX 66019 – 15510 HOLARGOS– GREECE

Tel: +30-210-6537307& +30-210-6537357 Fax +30-210-6548284

E-mail reklos@otenet.gr or mail@rddiagnostics.com

Website www.rddiagnostics.com

TERMS & DEFINITIONS

DOING THINGS RIGHT

Efficient



DOING THE RIGHT THING

Effective



AIM:

To evaluate the performance of galactose screening kit in order to obtain

The answer

Am I giving accurate high and low values with this kit ?

Am I doing it continuously?

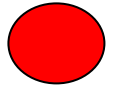
Collaboration

Between end-user and manufacturer

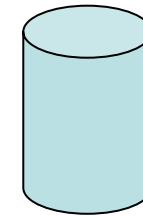
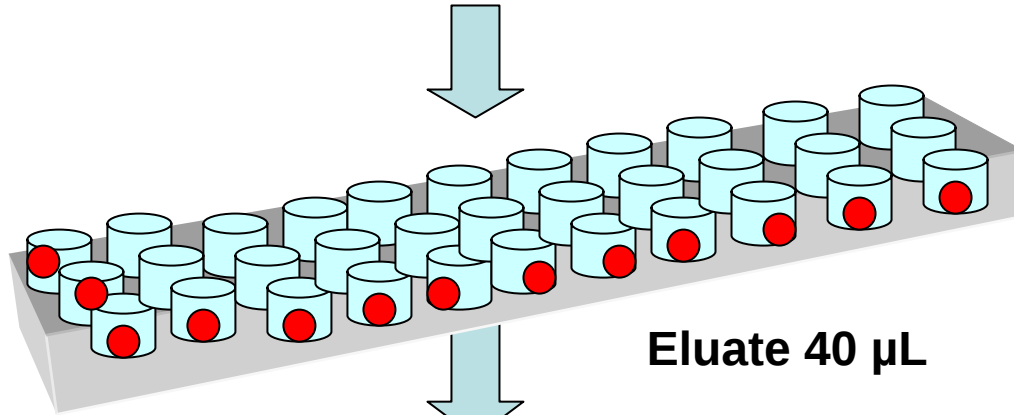
Tijen Tanyalcin (MD, PhD), TANYALÇIN TIP LABORATUVARI/TURKEY

George Reclos (PhD) R&D DIAGNOSTICS LTD/ GREECE

TOTAL GALACTOSE METHOD

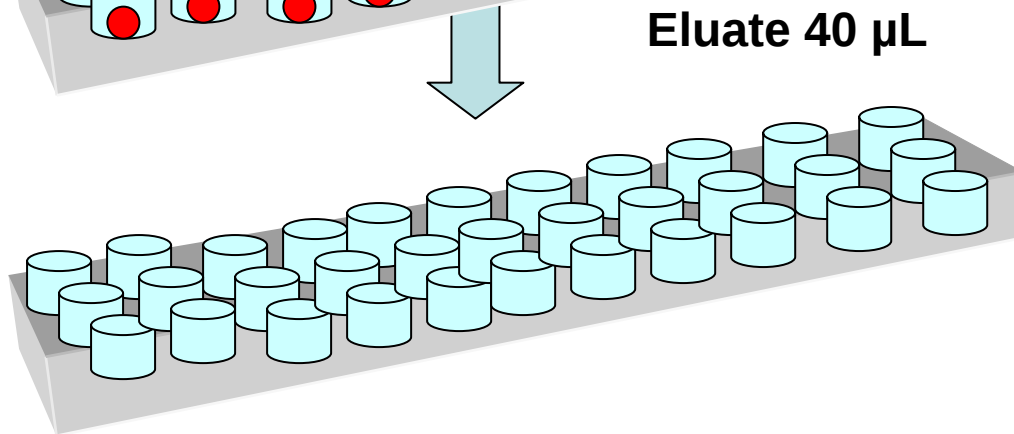


S&S903
3mm puncher

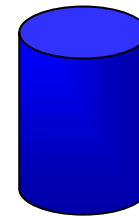


TCA 3 % 60 µL

Shaker 30 min

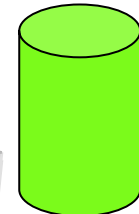


Eluate 40 µL



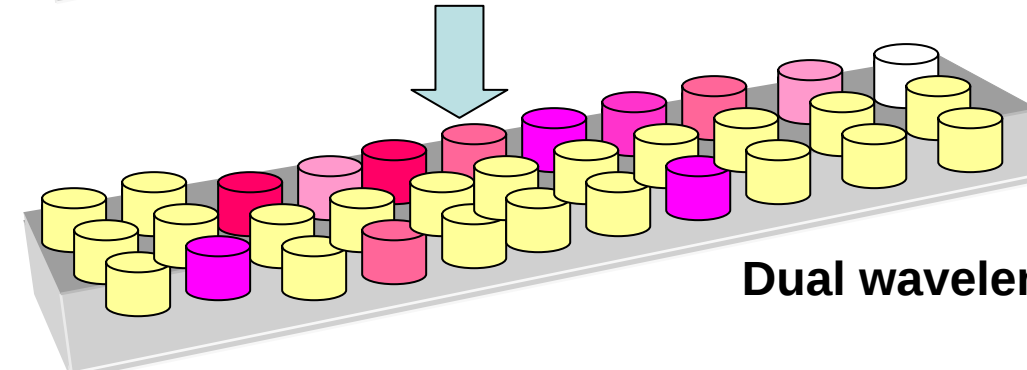
Reaction mixture
Alkaline Phosphatase
Galactose Dehydrogenase
NAD
100 µL

Shaker 30 min



NBT
Color reagent 80 µL

10 minutes after



Dual wavelength 570/690 nm measurement

Method Validation

- 1.Linearity
- 2.Repeatibility (within run, intra assay)
- 3.Reproducibility (between run , inter assay)
- 4.Detection limit analysis
- 5.Recovery
- 6.Interference study
- 7.Method comparison
- 8.
- 9.



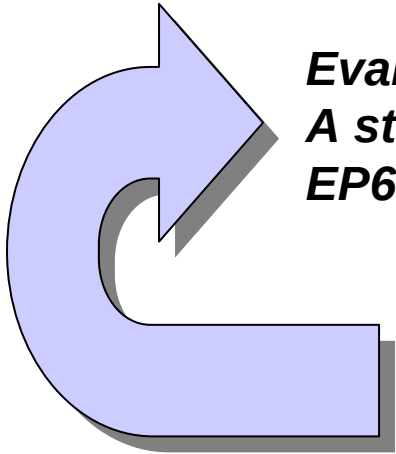
YOU make our own rules ,
Try to be realistic with the real life samples
Make **YOUR own cut off** and **YOUR own reference values**
Accurate interpretation of lab results (less false positives and **NO!!!** false negatives)

LINEARITY CHECK

NCCLS Document

***Evaluation of the linearity of Quantitative Measurement Procedures:
A statistical Approach ; Approved Guideline
EP6-A 2003***

***Daniel W. Tholen
Clin Lab New ,January 2004***



LINEARITY

The ability to provide results that are directly proportional to the concentration of the analyte in the test sample

5 dilutions should be performed

1

Very low (1)

Intermediate (3)

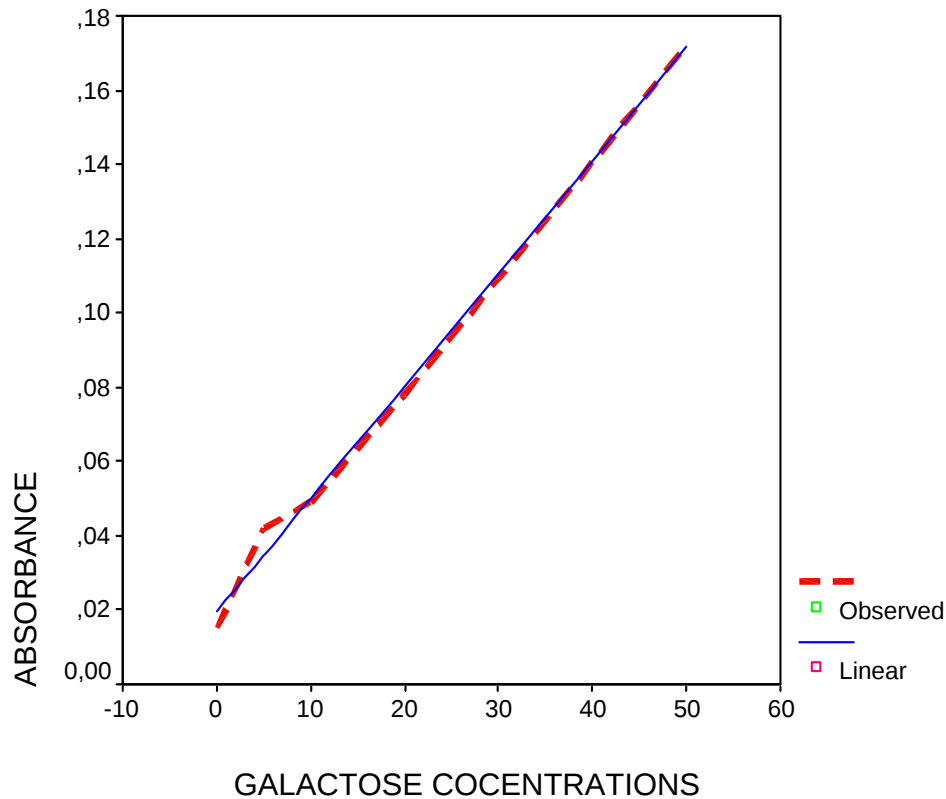
Very high (1)

2

Specimens should be free of known interferences

STEPS FOR LINEARITY CHECK

- ✓ First make your dilutions,
- ✓ Then from the average absorbance values, calculate the concentration from the linear regression
- ✓ Then take the +5% and -5% values to make the limits
- ✓ Then, calculate your concentration again from quadratic equation,
- ✓ Define again your +5% and -5% values to make the limits.
- ✓ When you take 5% of those values, check if they are inside the limits obtained from linear equation
- ✓ If they are, then your method can be accepted as linear.



MODEL: MOD
Dependent variable.. ABS

Method.. LINEAR

Multiple R ,99728
R Square ,99457
Adjusted R Square ,99276
Standard Error ,00516

Analysis of Variance:

	DF	Sum of Squares	Mean Square
Regression	1	,01463093	,01463093
Residuals	3	,00007987	,00002662

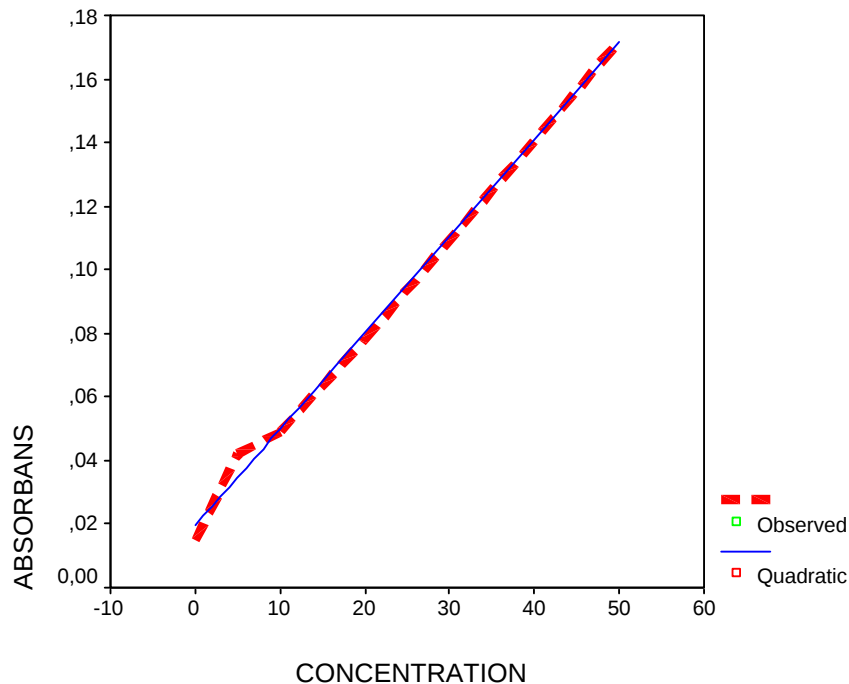
F = 549,52926 Signif F = ,0002

----- Variables in the Equation -----

Variable	B	SE B	Beta	T	Sig T
CON	,003043	,000130	,997282	23,442	,0002
(Constant)	,019468	,003193		6,097	,0089

$$Y = ax + b$$

$$Y = 0,0030 x + 0,019$$



MODEL: MOD_2.

Dependent variable.. ABS

Method.. QUADRATIC

Listwise Deletion of Missing Data

Multiple R ,99728
R Square ,99457
Adjusted R Square ,98915
Standard Error ,00632

Analysis of Variance:

	DF	Sum of Squares	Mean Square
Regression	2	,01463097	,00731549
Residuals	2	,00007983	,00003991

F = 183,28452 Signif F = ,0054

----- Variables in the Equation -----

Variable	B	SE B	Beta	T	Sig T
CON	,003021	,000665	,990025	4,539	,0453
CON**2	4,23011844E-07	1,2347E-05	,007472	,034	,9758
(Constant)	,019589	,005258		3,725	,0651

$$Y = ax^2 + bx + c =$$

$$Y = 0,00000042301184 x^2 + 0,003021x + 0,019589$$

Number of dilutions

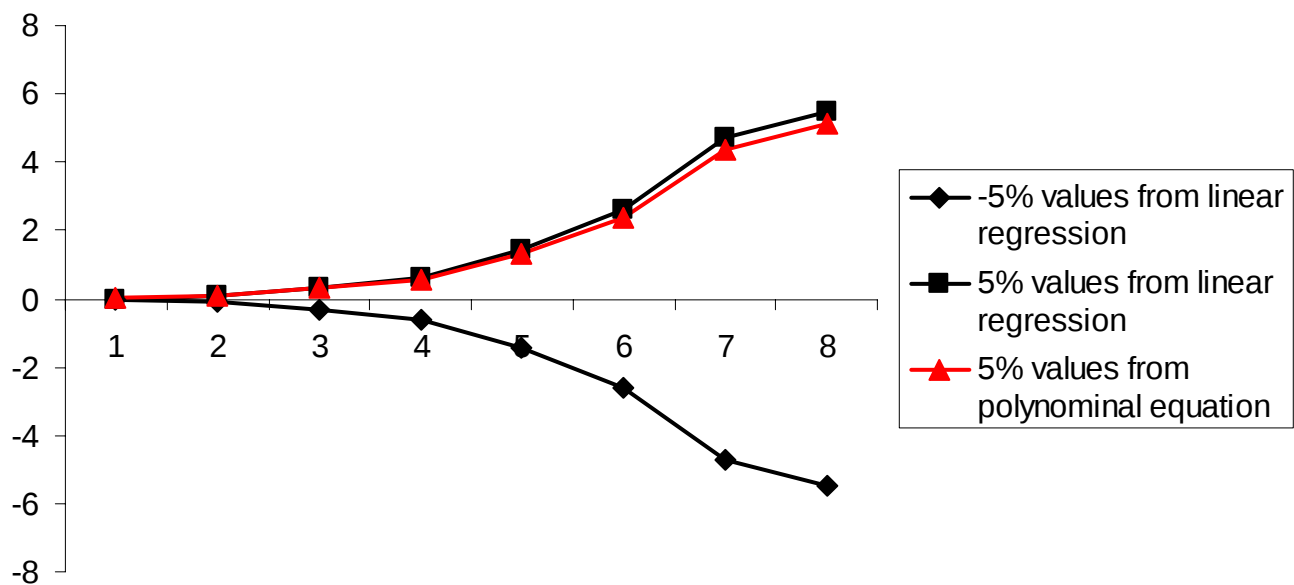
Galactose
concentrations

lin value	5% neg	5 % pos	resultsfrom quadratic	eq	%5 pos
109,1	-5,46	5,46	102,05		5,1
93,74	-4,69	4,69	87,71		4,38
51,91	-2,6	2,6	48,66		1,33
28,37	-1,42	1,42	26,71		0,58
12,36	-0,62	0,62	11,78		0,31
6,48	-0,32	0,32	6,29		2,4
0,1	-0,1	0,1	2,03		0,1
-0,06	0	0	0,2		0,01

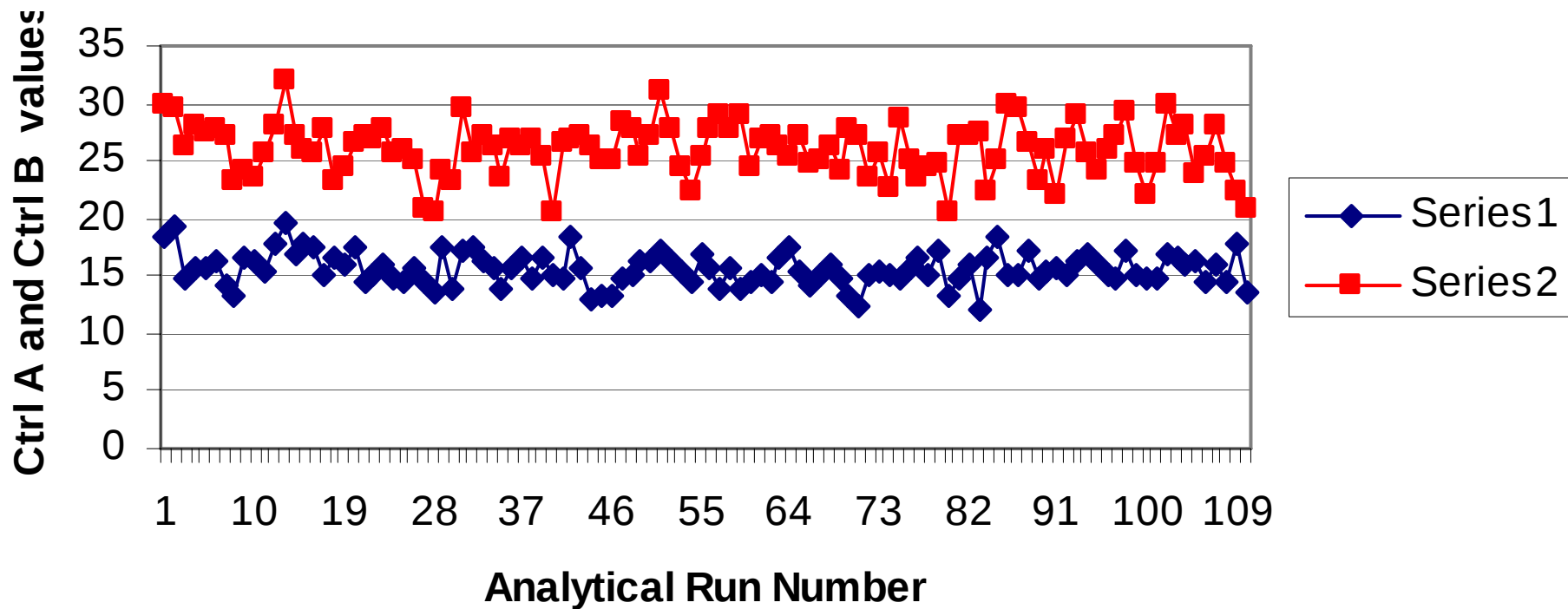


1	120
2	60
3	30
4	15
5	7,5
6	3,75
7	1,875
8	0,9375

LINEARITY CHECK GRAPH FOR TOTAL GALACTOSE

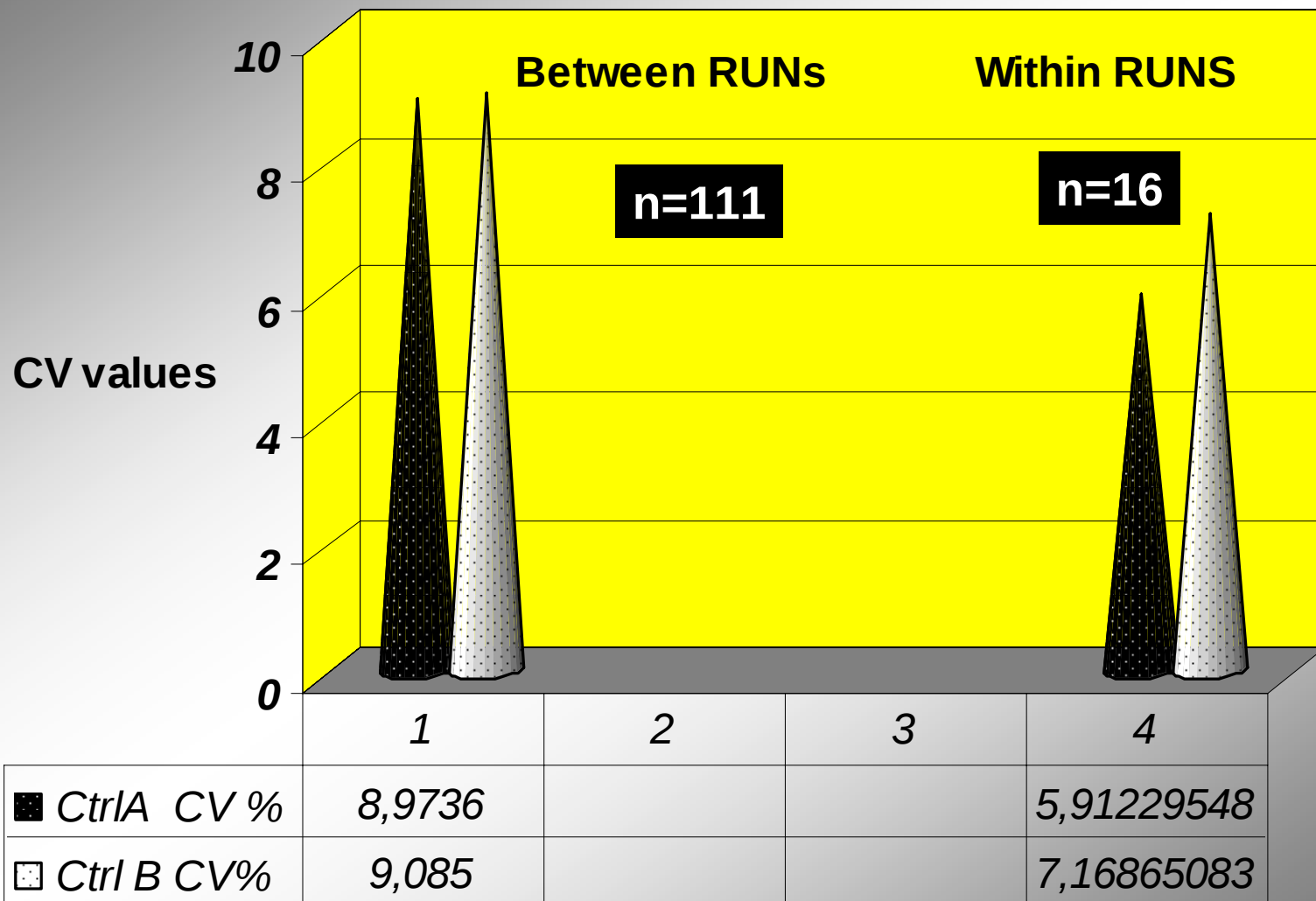


Long Term Value Of Controls



CTRL A 15 (12-18) mg/dL blood
CRTL B 25 (21,3-28,8) mg/dL blood

Repeatability / reproducibility (Within RUN / Between RUN assays)



DETECTION LIMIT

The limit defined as the smallest value that can be distinguished from zero with a defined degree of confidence

We may define the confidence by taking the

Mean (zero abs value) ± 1 SD (68,2 %) or

Mean (zero abs value) ± 2 SD ((95,5 %) or

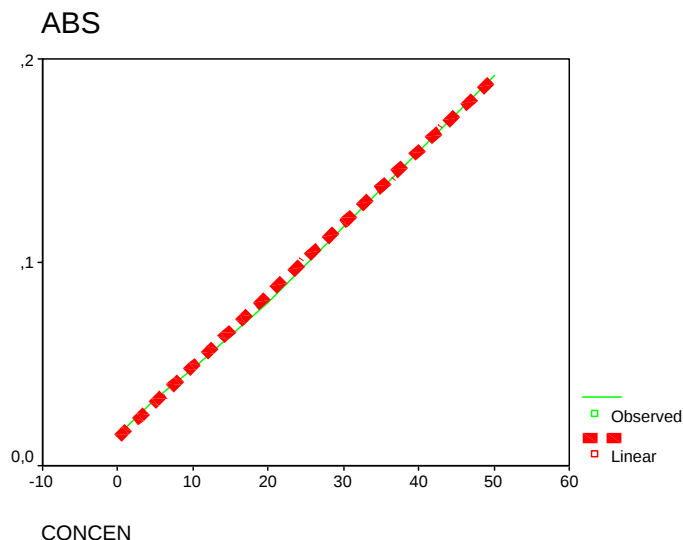
Mean (zero abs value) ± 3 SD (99,7 %)

You can define the detection limit by extrapolating the imprecision measured at a very low concentration to zero

Detection limit determination

- 1. Punch 20 times a zero concentration (white spots) in one run**
- 2. Calculate the mean abs value from the curve**
- 3. Find the SD of absorbances and multiply by 2 (95,5% of distribution)**
- 4. Calculate the maximum mean abs value**
- 5. Place it at the linear equation and find the concentration**
- 6. Report your lab results according to your value**

DETECTION LIMIT CALCULATION



MODEL: MOD_1.
Dependent variable.. ABS Method.. LINEAR

Multiple R ,99932
R Square ,99864
Analysis of Variance:

----- Variables in the Equation -----

Variable	B	SE B	Beta	T	Sig T
CONCEN	,003537	7,5469E-05	,999318	46,872	,0000
(Constant)	,013465	,001856		7,254	,0054

$Y = 0,003X + 0,013$ *Linear regression equation*

$Y = \text{MEAN abs (empty card)} + 2 \cdot \text{SD}$

$Y = 0,0145 + 0,00492 = 0,019$  **$0,01942 = 0,003537 x + 0,013465$**

$X = 1,68$

Any value lower than 1,68 should be reported as $< 1,68$

Not an actual figure.

DO I FIND ALWAYS THE SAME VALUE ?

NOOOOO!!!!

What affects the variability ??

BLOOD SPOT CARDS ????

YESSSS!!!!

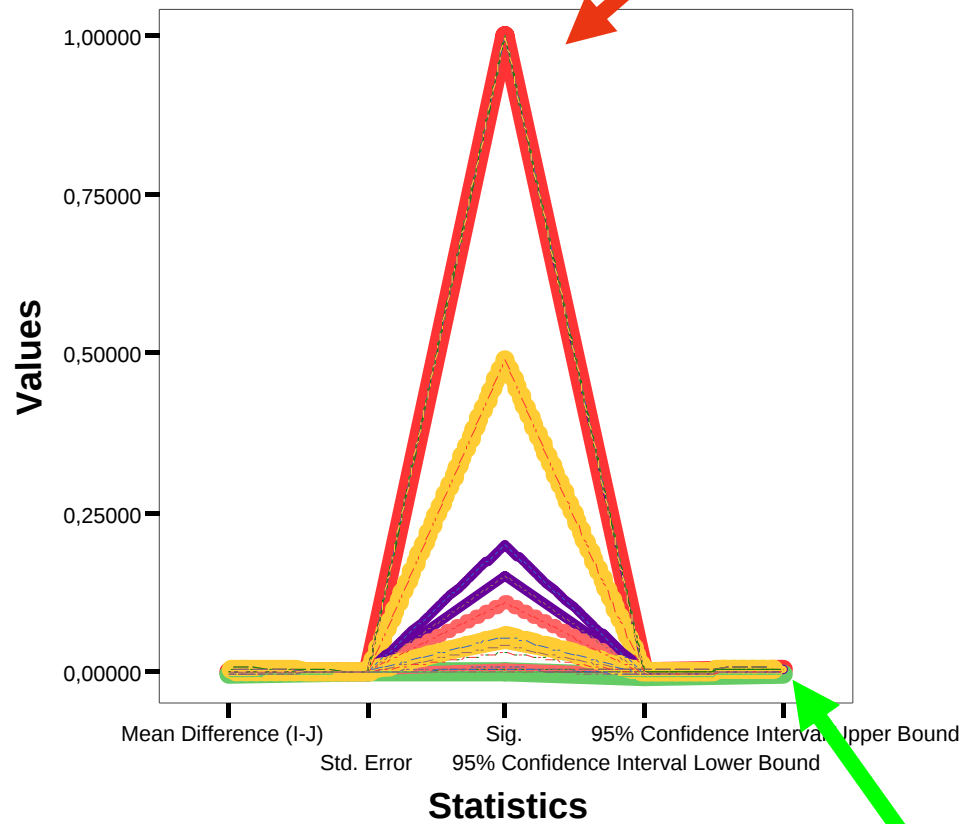
NBSCARD AARM	NBSCARD CDC QA Taiwan Tanyalci 903 R&D DGKC 2992 DGKC 903 EWS2003	NBSCARD R&D	NBSCARD AARM CDC QA Taiwan Tanyalci
CDC QA	AARM Taiwan Tanyalci 903 R&D DGKC 2992 DGKC 903 EWS2003	DGKC 2992	DGKC S&S2992 DGKC S&S 903 EWS2003 AARM CDC QA Taiwan Tanyalci 903 R&D DGKC 903 EWS2003
Taiwan	AARM CDC QA Tanyalci 903 R&D DGKC 2992 DGKC 903 EWS 2003	DGKC 903	AARM CDC QA Taiwan Tanyalci 903 R&D DGKC 2992 EWS2003
Tanyalci	AARM CDC QA Taiwan R&D DGKC 2992 DGKC 903 EWS2003	EWS2003	AARM CDC QA Taiwan Tanyalci 903 R&D DGKC S&S2992 DGKC S&S 903

The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable : ABSNBS

Test : Bonferroni



(J) NBSCARD

- CDC QA
- Taiwan
- Tanyalcin S&S 903
- R&D
- DGKC S&S2992
- DGKC S&S 903
- EWS2003
- AARM



(I) NBSCARD

- AARM
- CDC QA
- Taiwan
- Tanyalcin S&S 903
- R&D
- DGKC S&S2992
- DGKC S&S 903
- EWS2003

CONCLUSION

We can give confidently the results now

But still, what about the long term acceptability of the performance of the test ?

Currently, using reference range based goals :

CV should not exceed 1/8 of the width of reference range
expressed as a percentage of the mean of that range

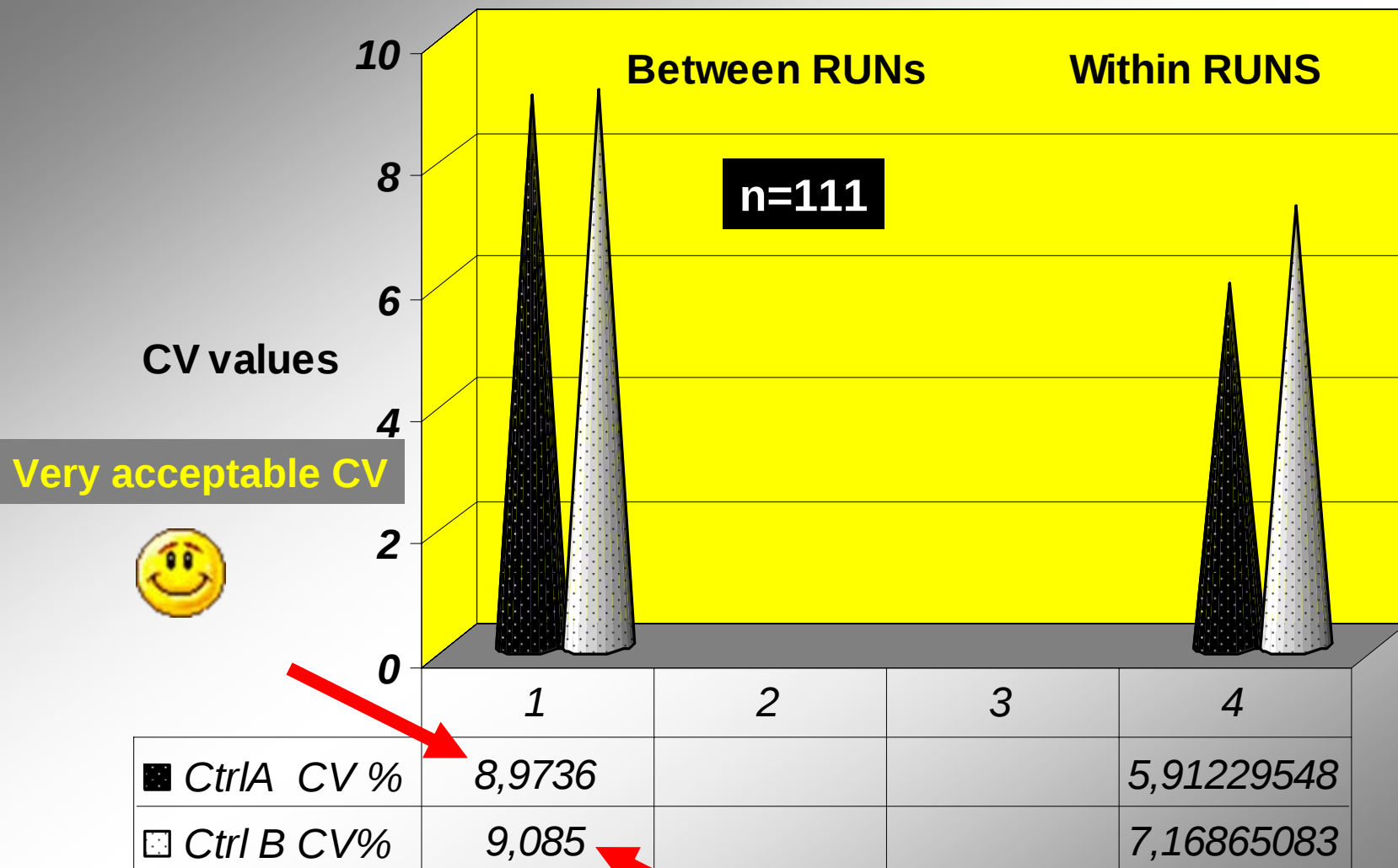
Example: For galactose 1,68 – 10 mg/dL blood

$$X = (10 - 1,68) / 8 = 1,04$$

$$\text{Mean} = 5,84$$

$$(1,04 / 5,84) * 100 = 17,80 \%$$

Repeatability / reproducibility (Within RUN / Between RUN assays)



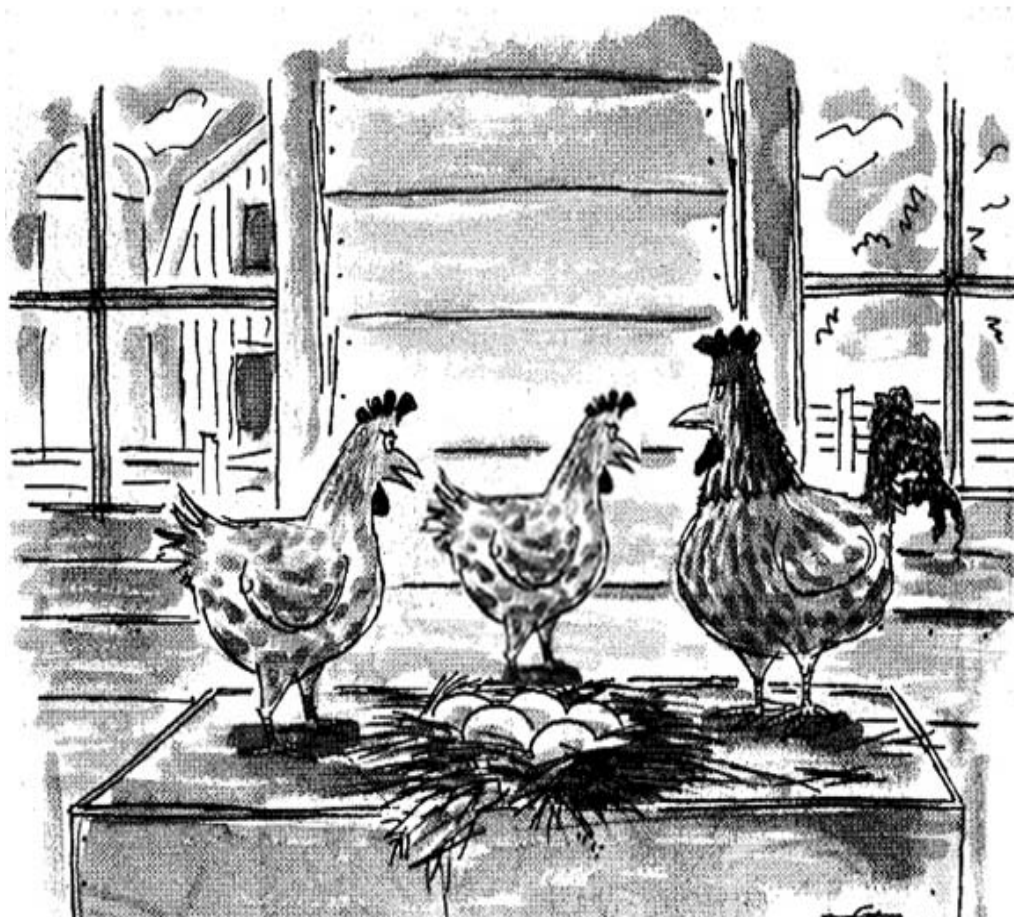
CONCLUSION

We have to know the total allowable error for galactose screening test

**This is also essential for all other dried blood spot screening tests like
PKU, MSUD, as well as enzymatic assays like GALT, G6PD
The total allowable error must be clearly defined**

Biological variations should be determined

The Quality Alliance Working Together



**They're not just our eggs,
they're your eggs too!**



Thank you