



- **Units of measurement in specified trait testing in seeds**
- **influence of different units on the test result**

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**Kirk REMUND (US)**



**Some quality controls/checks are routinely performed by companies and governments without any DNA extraction**



plots

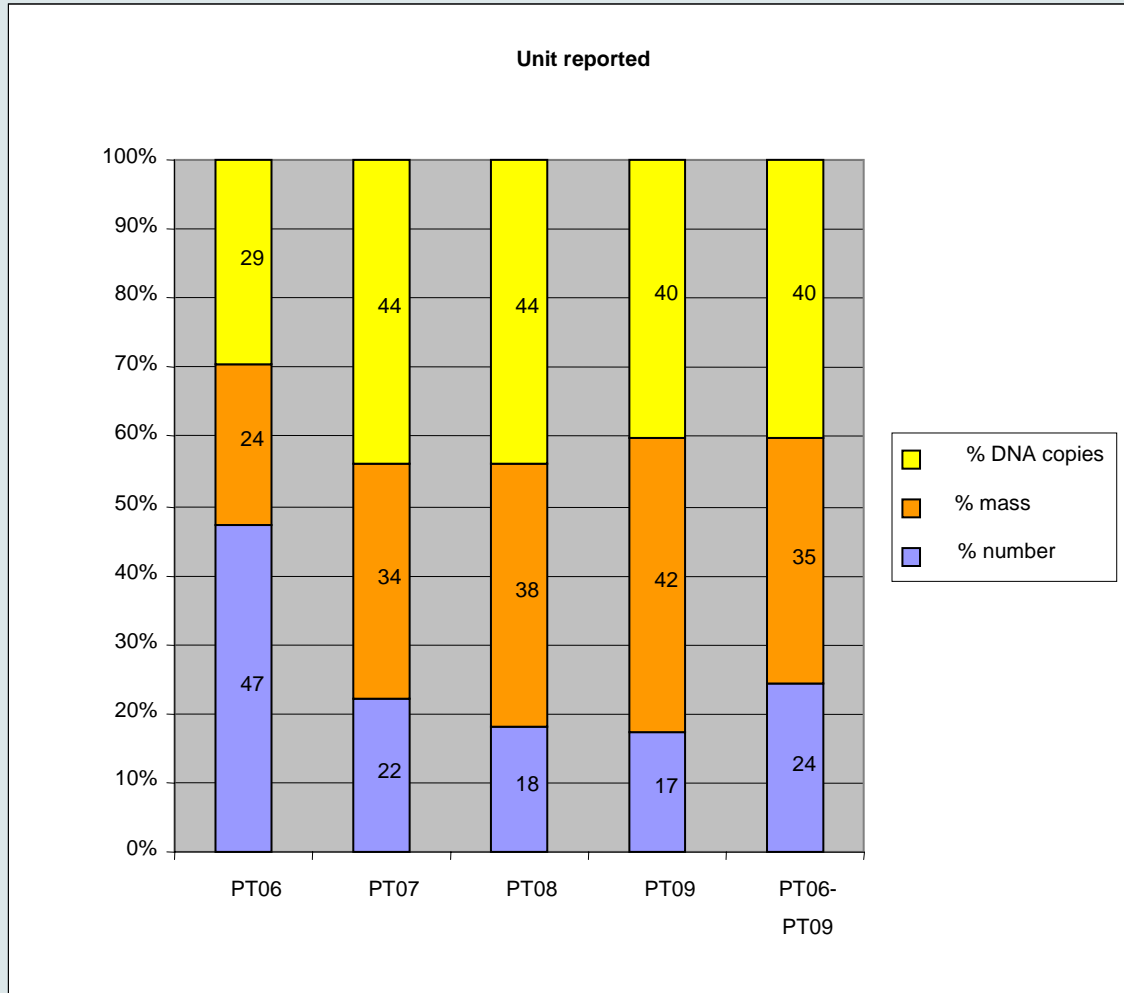


pots

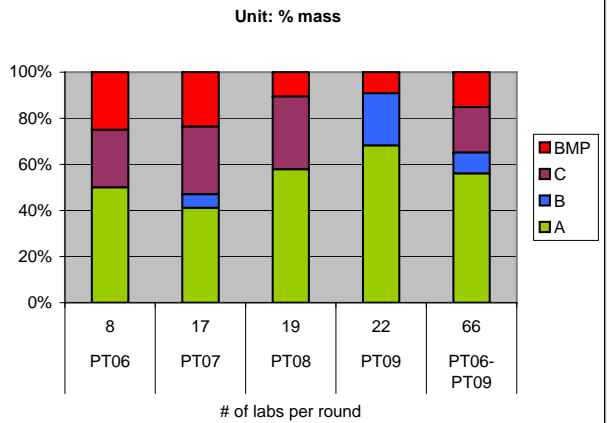
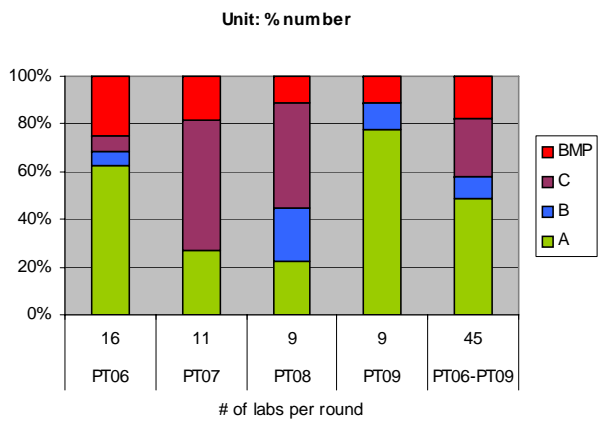
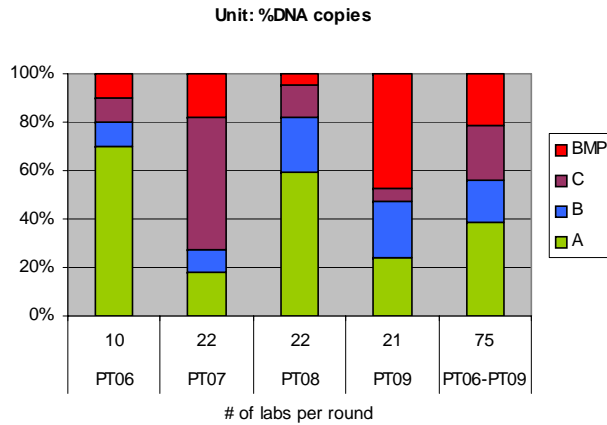
Bio assays and DNA techniques are both in use when the aim is to check the purity of seeds with specified trait(s)



# Units reported for quantitative results in four most recent ISTA proficiency tests



% mass  
% number  
%DNA copies  
are all in use



# Rating of laboratories is similar for the 3 units

For each of the 3 units, we see very good laboratories

accurate and repeatable

For each of the 3 units we see problems in quantification for some laboratories

*Below Minimum Performance :*

*more than half of results are less than 1/2 of true value, more than double of true value*



## Detailed analysis of ISTA proficiency tests show that results reported in :

% mass fraction,

% seed number,

% number of haploid genome copies,

**have the same capability to be:**

**accurate, reliable, repeatable, reproducible**

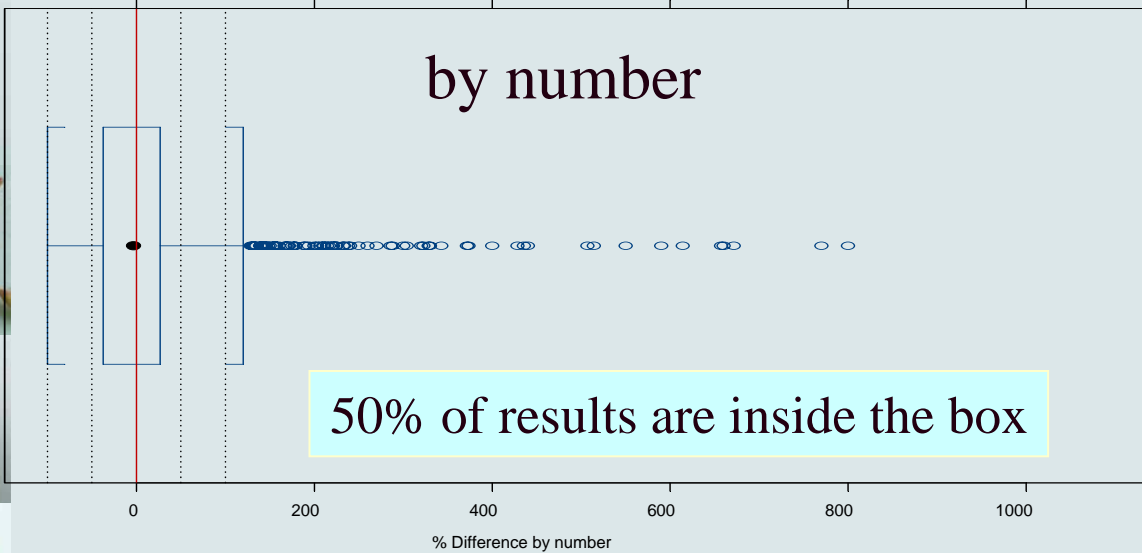
***With all 3 units we see the same problems:***

*important under and over estimation occurs with all units in some laboratories*

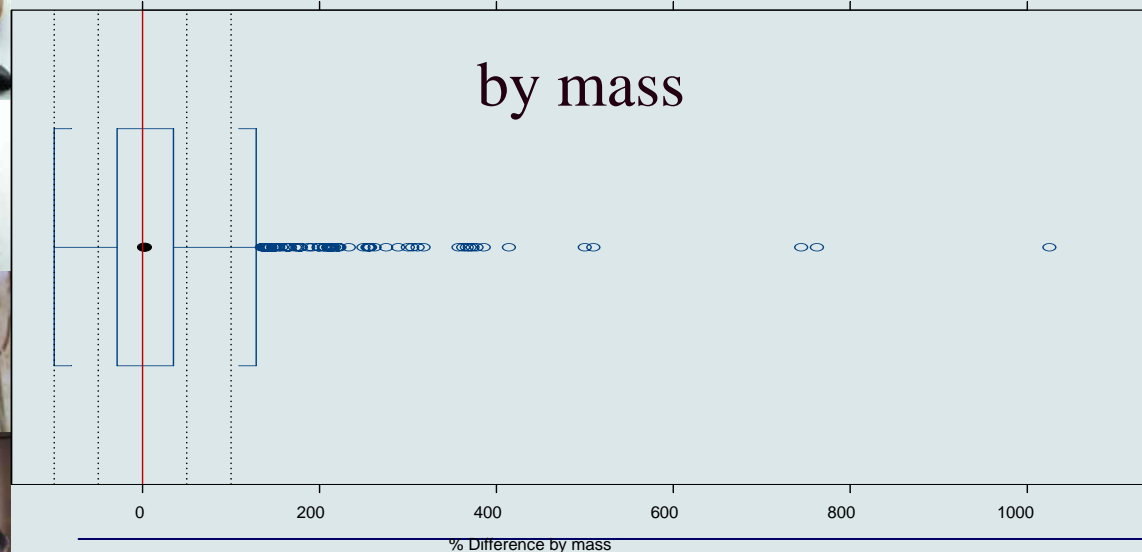
*there are still some discrepancies between laboratories when unit is haploid genome copy number, we try to solve this*



Median is close to target (median is on red line)



Zero means no deviation from the true value and 100% means twice the true value





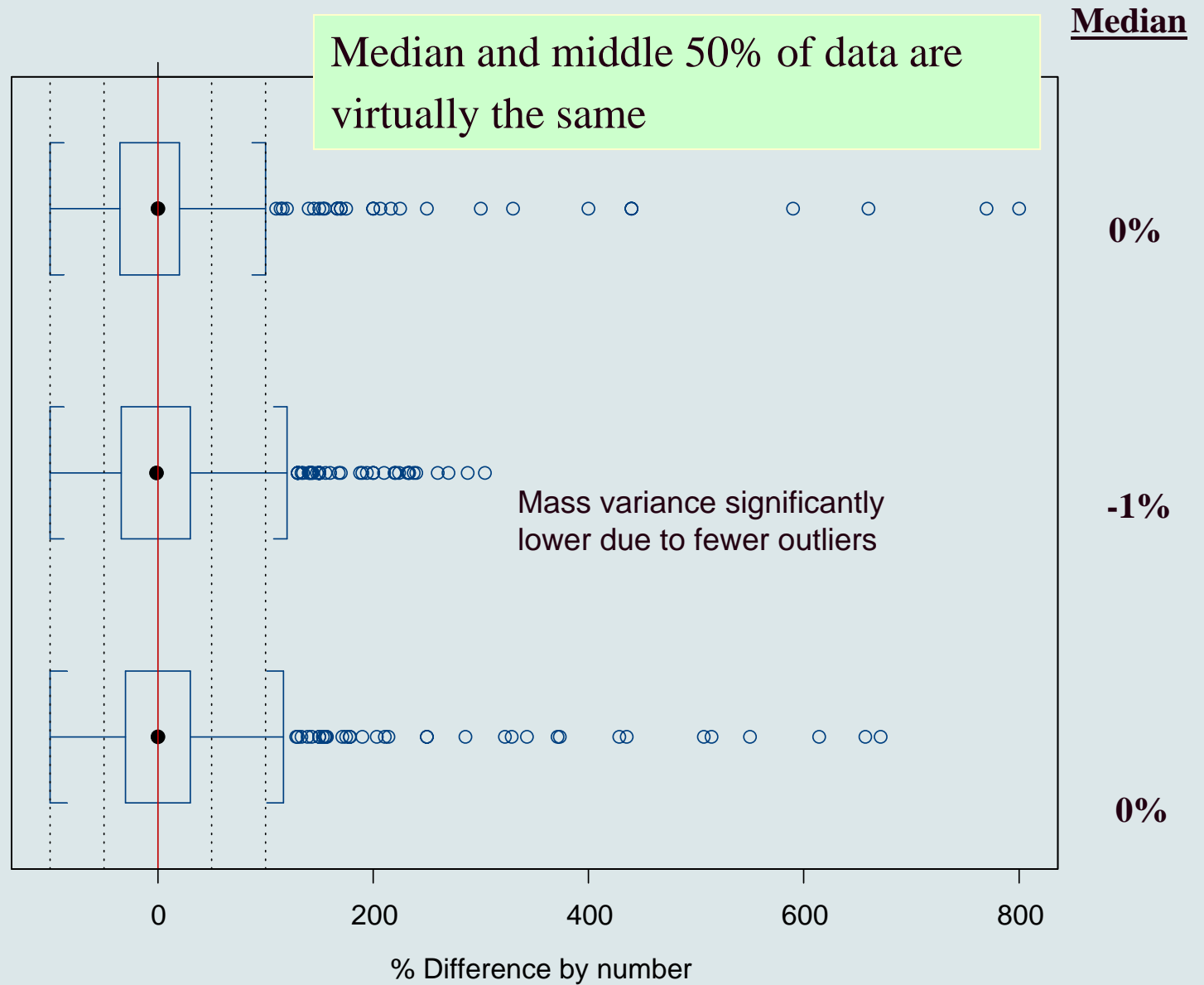
# Unit (true=number)



number

mass

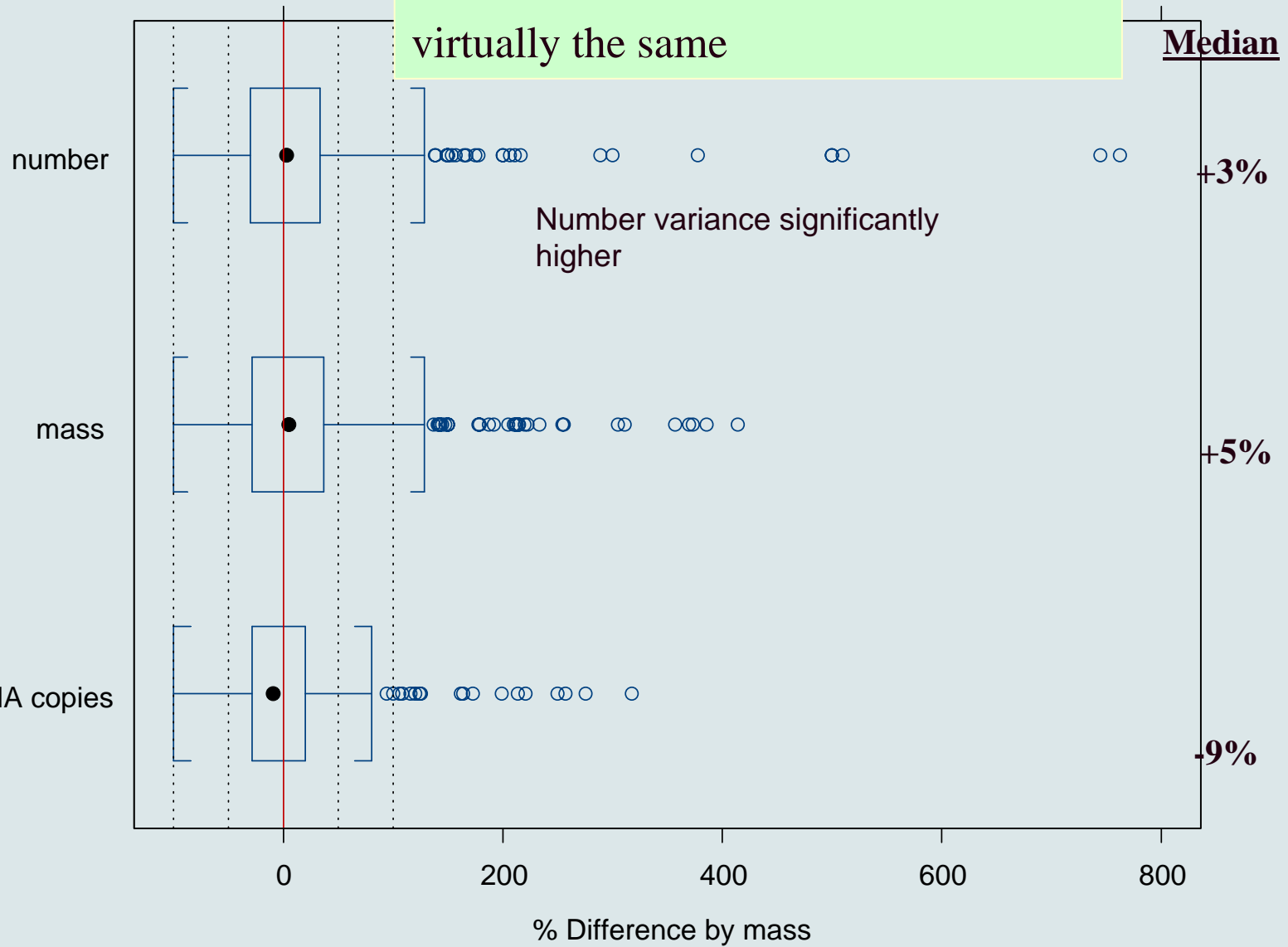
%DNA copies





# Unit (true=mass)

Median and middle 50% of data are virtually the same





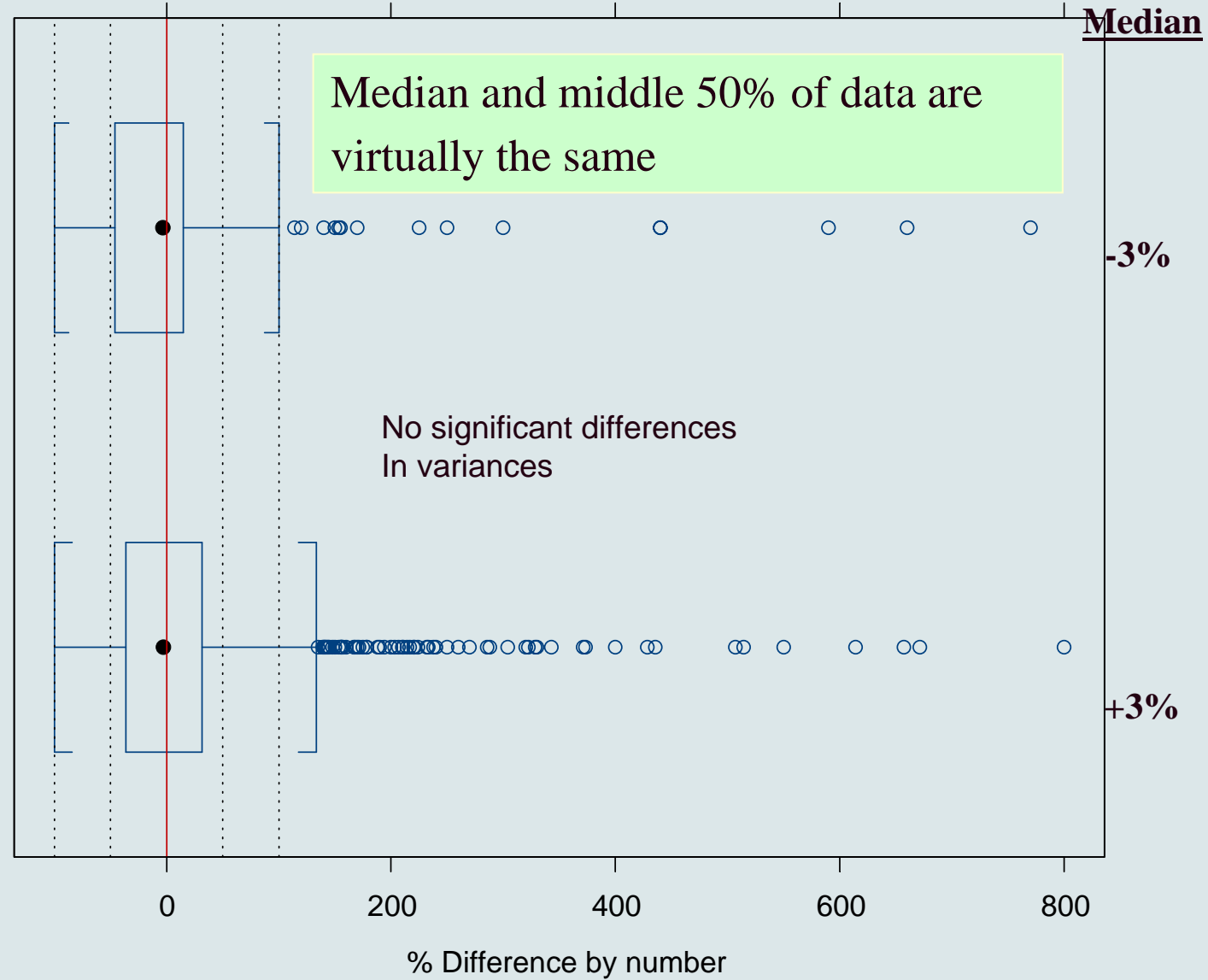
# Method Type (true=number)



Subsampling



Quantitative





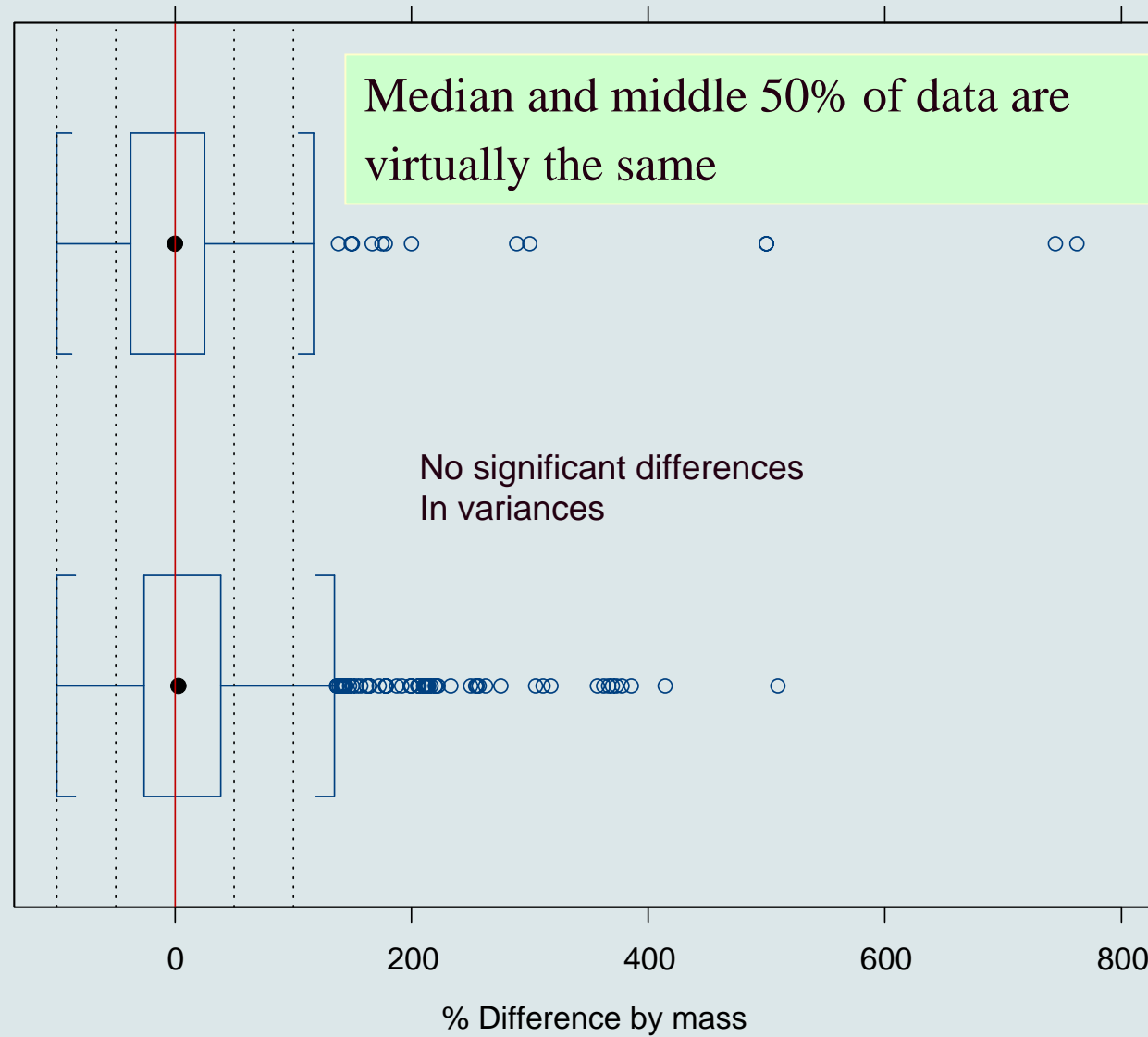
# Method Type (true=mass)



Subsampling



Quantitative





# Method (true=number)



Quantitative PCR



Quant & Qual PCR



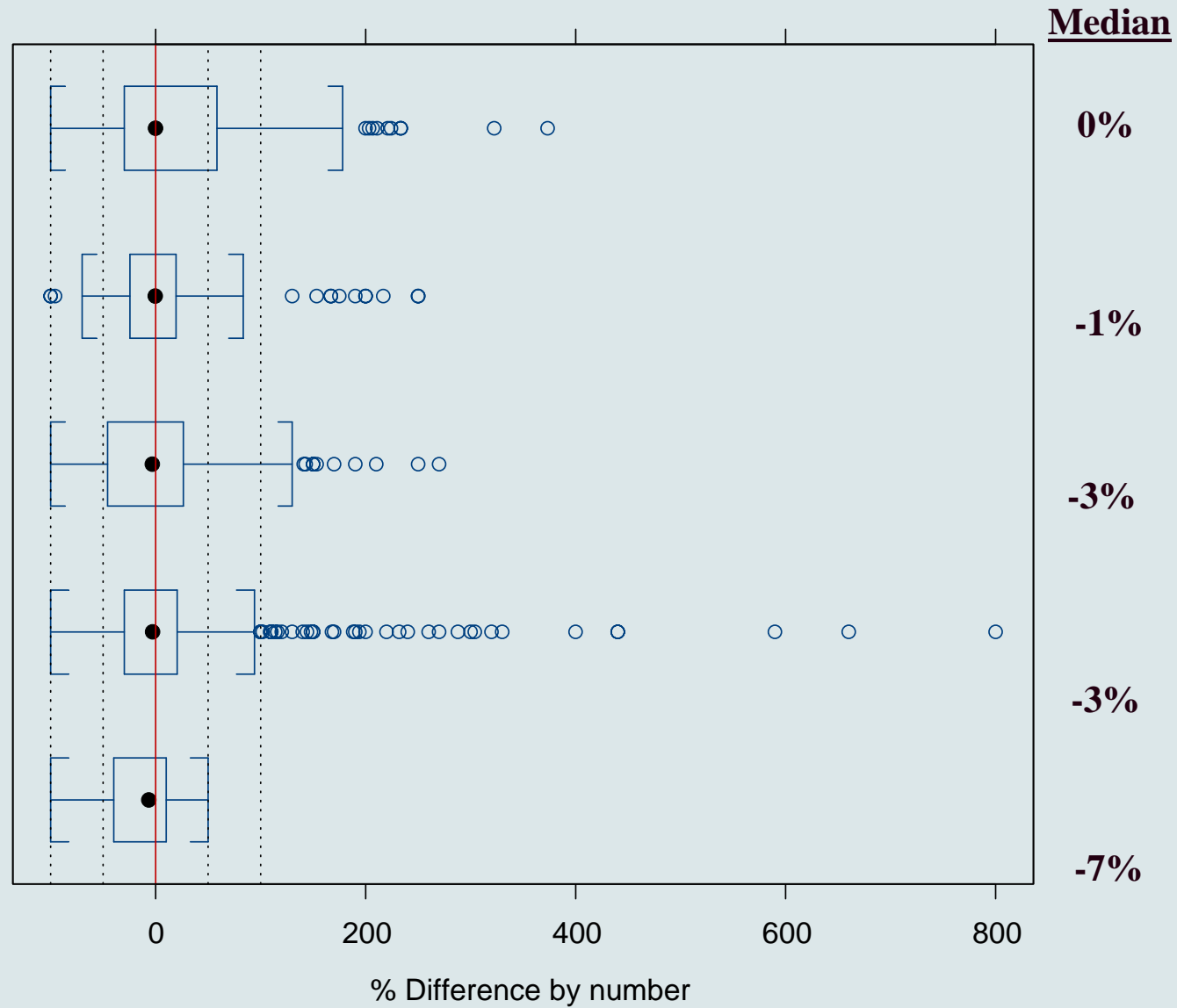
Qualitative PCR



PCR

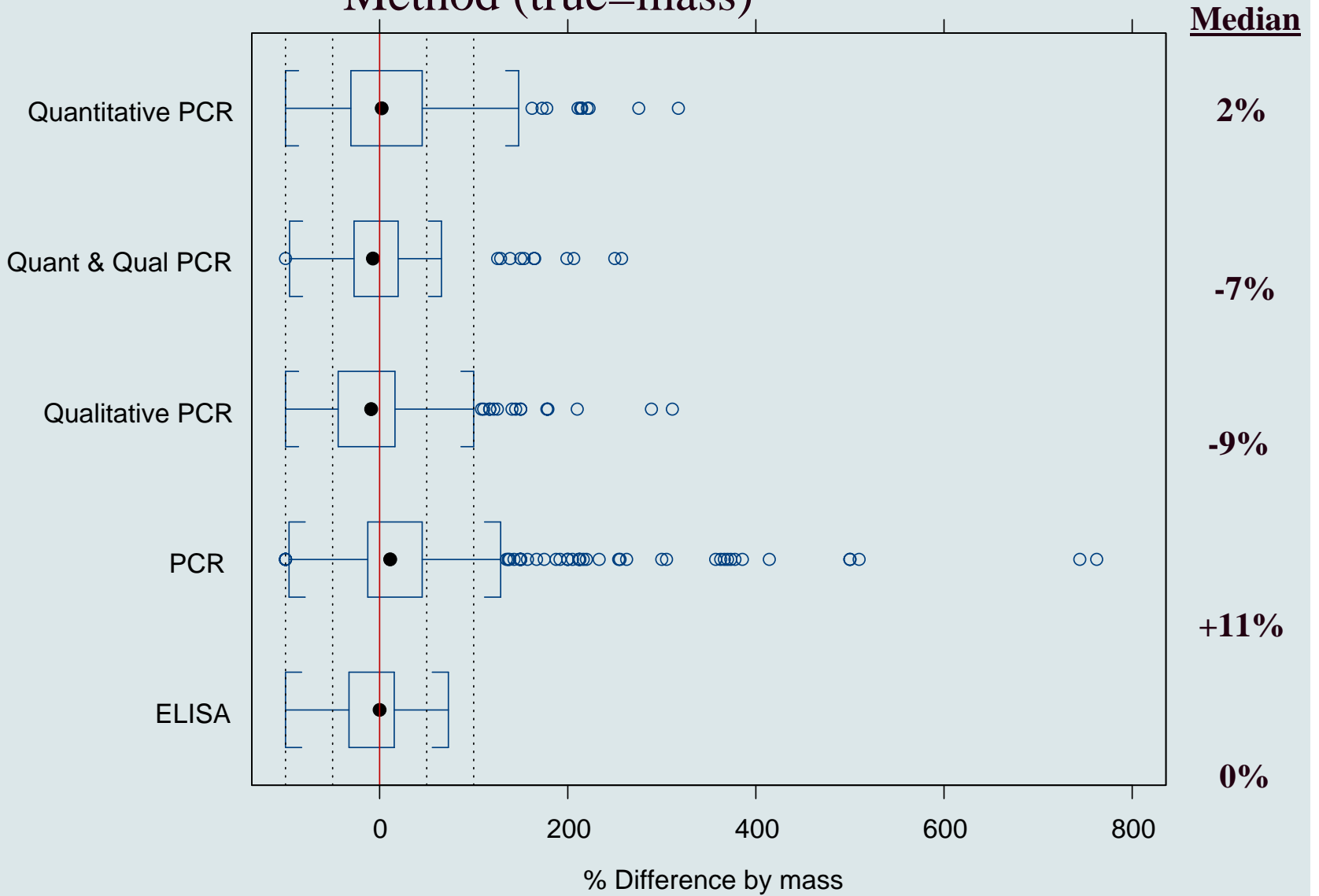


ELISA





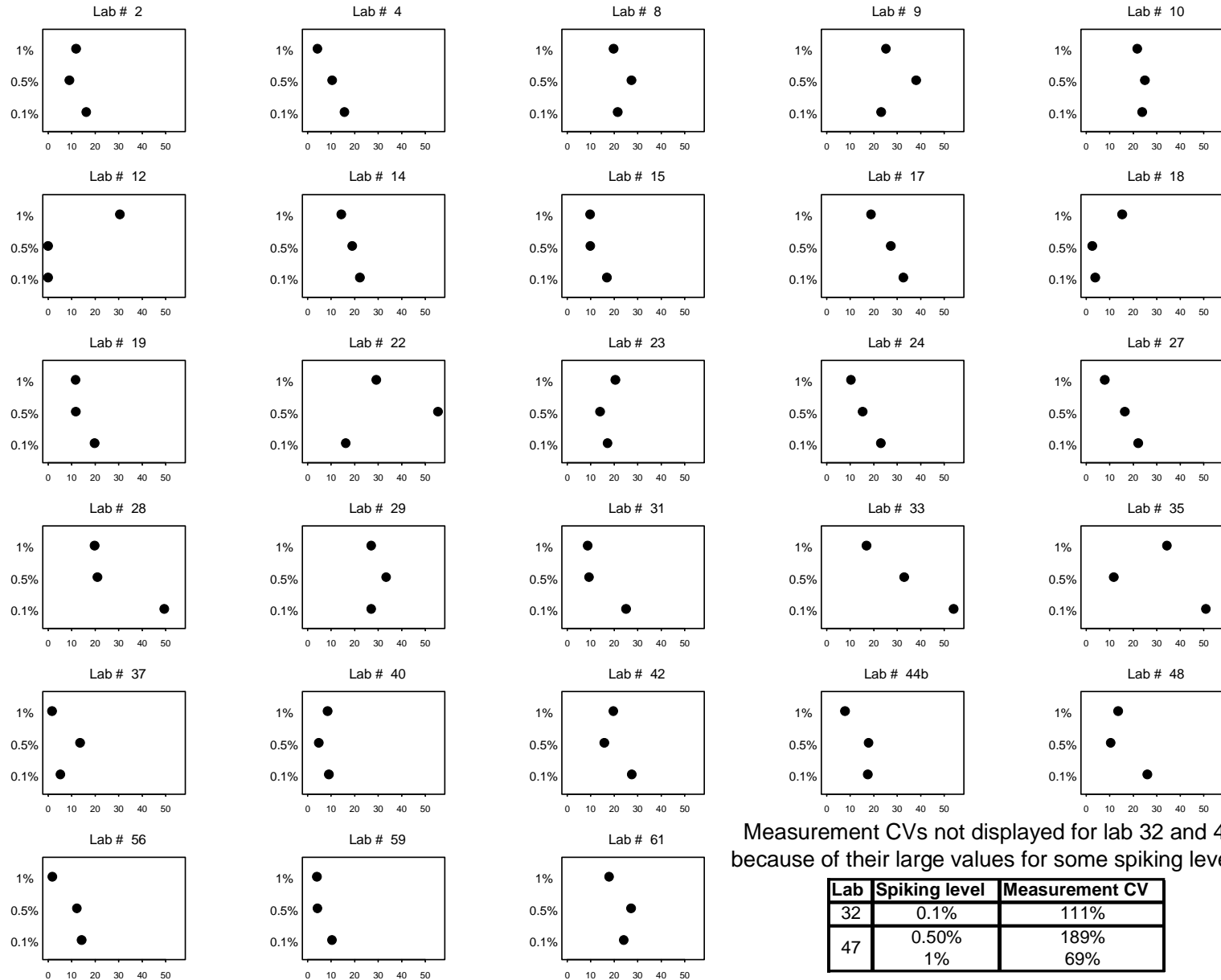
## Method (true=mass)







# For each lab and each spiking level, measurement CVs



Measurement CVs not displayed for lab 32 and 47 because of their large values for some spiking levels:

Lab	Spiking level	Measurement CV
32	0.1%	111%
47	0.50%	189%
	1%	69%

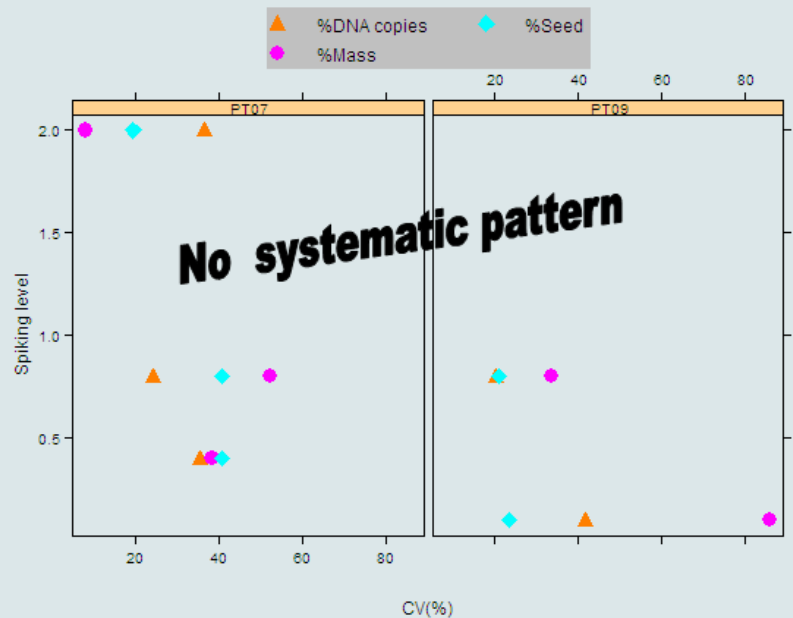


# Mixed-effect model analysis of ISTA GMO Proficiency Tests

ISTA GMO TF – ISTA Statistics Committee

Jean-Louis Laffont

Analysis of laboratory submitted results  
*Residual variation expressed as  $\hat{\sigma}_i / (\hat{\mu} + \hat{\alpha}_i)$*



*Will be presented during this ISTA annual meeting*

“The mixed-effect model analysis of ISTA GMO PTs did not reveal differences in precision when different methods or units of measure are used for estimating GMO%.”





The way to express results in %DNA copies per haploid genome, and the relation with %mass is not understood in the same way in all laboratories worldwide ( $\frac{1}{2}$  ratio when only one gene in maize)

ISTA Online

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TCOM / Info Platform

**Information Platform for GM Seed**

Transgenic Event Descriptions | Analytical Methods | Statistical Tools | Reference Materials | Literature | Accreditation for Specified Trait(s) | GMO Task Force | [Clarification document 'Units'](#)

ISTA helps to clarify and reach a common understanding



INTERNATIONAL SEED TESTING ASSOCIATION  
ASSOCIATION INTERNATIONALE D'ESSAIS DE SEMENCES  
INTERNATIONALE VEREINIGUNG FÜR SAATGUTPRÜFUNG

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This document was written in response to the feedback from ISTA GMO Proficiency Test (PT) participants. It is intended to provide guidance with respect to the set up of calibration curves, quantification units, and the relationship between them:

**Real-time PCR quantitative results of ISTA GMO Proficiency Tests: Standards and Units.**



## 3 units are available

### 3. ISTA's position

ISTA is of the opinion that any obligation to the use of a unique technique could impair the capability to detect and quantify seeds with specified trait(s). The use of methods that have been proved to be fit for the purpose, repeatable and reproducible, shall be accepted. ISTA is of the opinion that in order to cope with the different aims and situations where quantification of seeds with specified trait(s) is required, not only one unit should be accepted for reporting quantitative results of seeds with specified traits in conventional seed lots. Three units are available and shall be accepted :

% DNA copies

% mass fraction

% number of seeds

3 units are worldwide used in practice,  
depending of the question





- Units of measurement in specified trait testing in seed
- influence of different units on the test result
  - Stacked genes
  - Biological factors
  - Supply chain

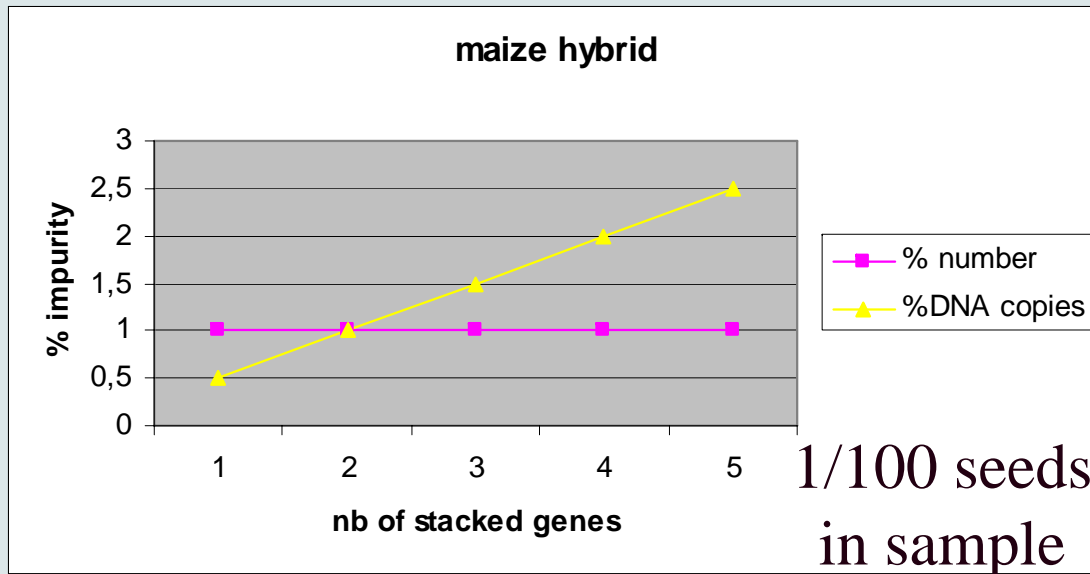


# The 3 units does not measure the same thing

## One shall select the unit which is fit for purpose:

check plants, look for presence of unauthorized events,  
quality control in seed production, food/feed ...

Example : Influence of the number of genes (x) introduced in a variety



% number seeds  
=constant

% copy per haploid  
genome

=1/2x\*genes in corn

=x\*genes in soybean



# A deep look to the relationship between %DNA copies and %mass or %number, show the influence of many biological factors that can interact

variety characteristic,  
parental origin of gene introduction,  
ratio of different tissues,  
industrial processing, ie excluding embryos from grain...  
etc

Y. Trifa and D. Zhang (2004). DNA content in Embryo and Endosperm of Maize Kernel (*Zea mays* L.): Impact on GMO Quantitation. *J. Agric. Food Cem.* 52, 1044-1048

Transgenic Res (2008) 17:393-402  
DOI 10.1007/s11248-007-9114-y

ORIGINAL PAPER

Impact of genetic structures on haploid genome-based quantification of genetically modified DNA: theoretical considerations, experimental data in MON 810 maize kernels (*Zea mays* L.) and some practical applications

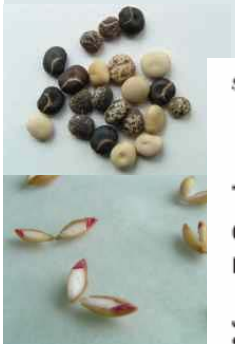
David Zhang · Aurélie Corlet · Stéphane Fouilloux

Work in  
progress  
ENGL  
ISTA

...



# In some circumstances, one can relate one unit to another and account for biological factors

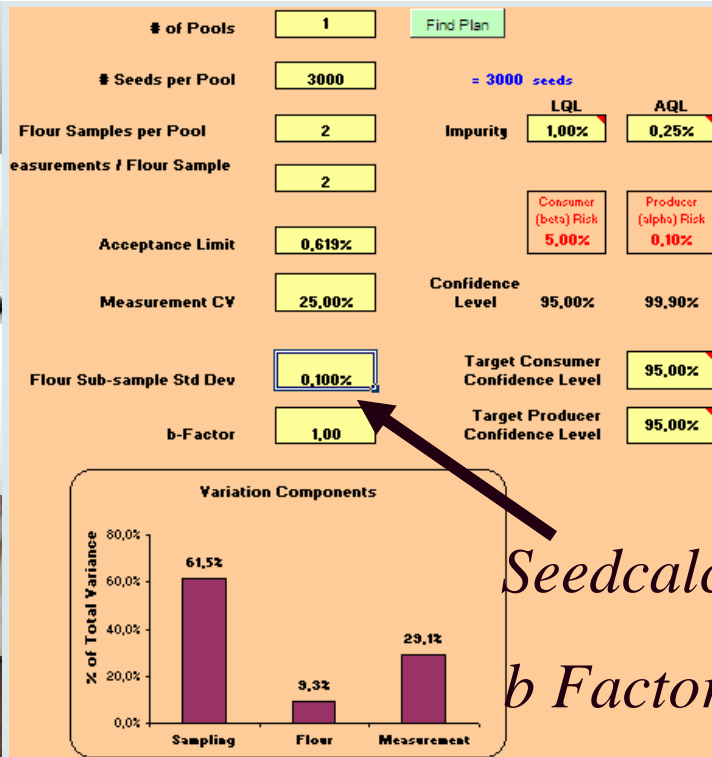


Seed Science Research (2005) 15, 197–204

DOI: 10.1079/SSR2005210

Testing for adventitious presence of transgenic material in conventional seed or grain lots using quantitative laboratory methods: statistical procedures and their implementation

Jean-Louis Laffont<sup>1</sup>, Kirk M. Remund<sup>2\*</sup>, Deanne Wright<sup>3</sup>, Robert D. Simpson<sup>2</sup> and Sylvain Grégoire<sup>4</sup>



BUT, When there are many biological factors involved,

if we do not have knowledge of their relative influence, it is impossible to compute a formula to translate from a given unit to another with enough confidence



# Influence of unit on grain for food/feed truck not well cleaned



TKW corn 300 g  
TKW rape seed 3g

TKW=Thousand Kernel Weight

impurity level in % number	rape seed in corn		corn in rape seed		corn in corn	
	mass	number	mass	number	mass	number
1,000%	0,010%	1,000%	49,749%	1,000%	1,000%	1,000%
0,100%	0,001%	0,100%	9,099%	0,100%	0,100%	0,100%



Laboratories that screen  
for species will detect  
presence of the  
unexpected species