

Statistical committee

Information on ISTA STA-GMO
Task Force Work

Speakers: Sylvain Gregoire
Kirk Remund

ISTA has a consistent statistical framework to evaluate and check **accuracy** and **repeatability**

= to be next to the true value

= to find same result if redo check

ISTA management system:

Proficiency Tests

Performance Data Evaluation

Laboratory on site assessment

Tools and assistance

cf GMO session
tomorrow

For each given
question to answer

key points are:

METHOD

TESTING PLAN

SAMPLING

No priority
order, All 3
have to be
considered

System is transparent, understandable, applicable in practice

Chapter 8, need to cope with diversity of situations worldwide

From chapter 8

“8.1.1 Verification of species and variety

8.1.2 Testing for the presence of specified traits

The object is to test for the presence of traits in the submitted sample as specified by the applicant (for examples see 8.2.2) using methods not permissible in a purity test according to Chapter 3.”

read printed version

adventitious presence
of GM seeds in
conventional seed lots,

presence of
conventional seeds in
GM seed lots

...

- Seeds
- Seedlings
- Plants...

Bioassay

ELISA

PCR...

From controlled
conditions

...

to open field
growing

Maize

Cotton

Tomato...

Cope with diversity of methods, units, reporting

From chapter 8

read printed version

“8.2.3 Performance approved methods

Performance approved methods are evaluated, approved and implemented by the testing laboratory according to the principles of the performance based approach as laid down in the ISTA document *Principles and Conditions for Laboratory Accreditation under the Performance Based Approach*. They are restricted to bio-molecular tests and bioassays for the object of testing for the presence of specified traits.

Performance approved methods can only be applied when no standardised method is included in this chapter for the test required »

For bio-molecular trait(s), a number of well established and approved methods can be used

“8.6 Calculation and expression of results

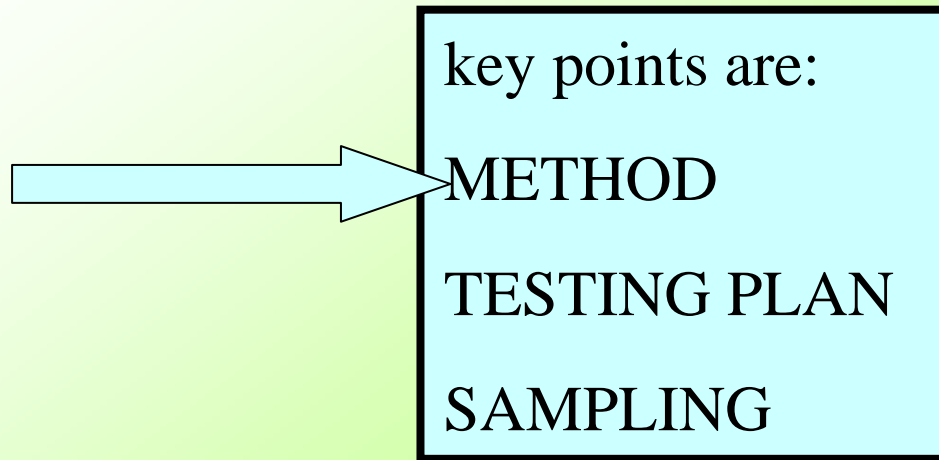
In the case of testing for the presence of specified traits the result shall be expressed as agreed with the applicant by either:

- reporting whether the trait is present or not,
- calculating and expressing the proportion of the trait, or
- calculating and expressing the confidence probability that the true proportion of the trait meets or exceeds a specification on the basis of the test result.

8.7.2.3. *Quantitative measurements of traits in bulk samples*

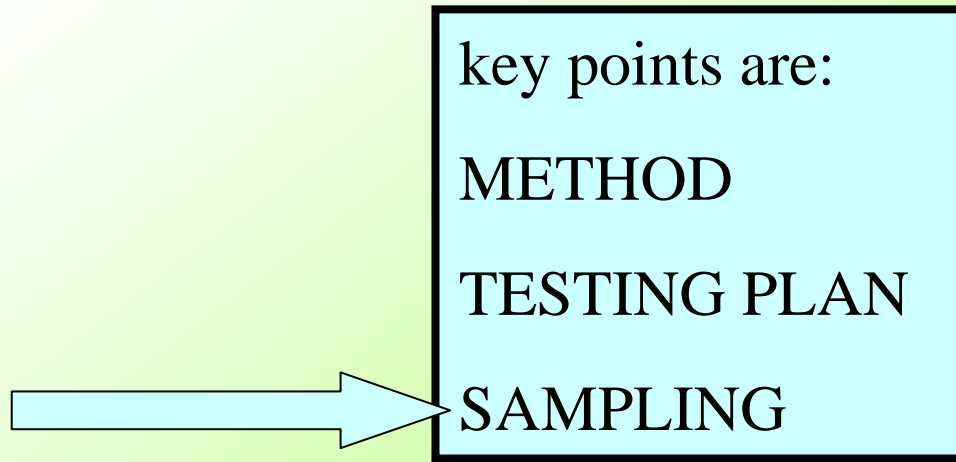
Units may be percent seeds by number, percent seeds by mass, percent by protein, percent by number of DNA copies, or any other determinant by percent”

Different types of results and different units can be used



Method validation and method standardisation
is a core business of ISTA and a number of
other organisations

not covered in this talk



Sampling is recognised as a key feature by ISTA, the importance of sampling is sometimes overlooked by persons who are not aware

At each stage of a testing process, voluntary or involuntary sampling, and/or variability sources are present, they need to be considered

There are variability sources all along the process

Maize seedlot (30 ton)



submitted sample



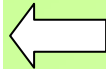
working sample



flour sample extraction



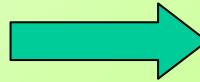
DNA sample for PCR
(200 ng)



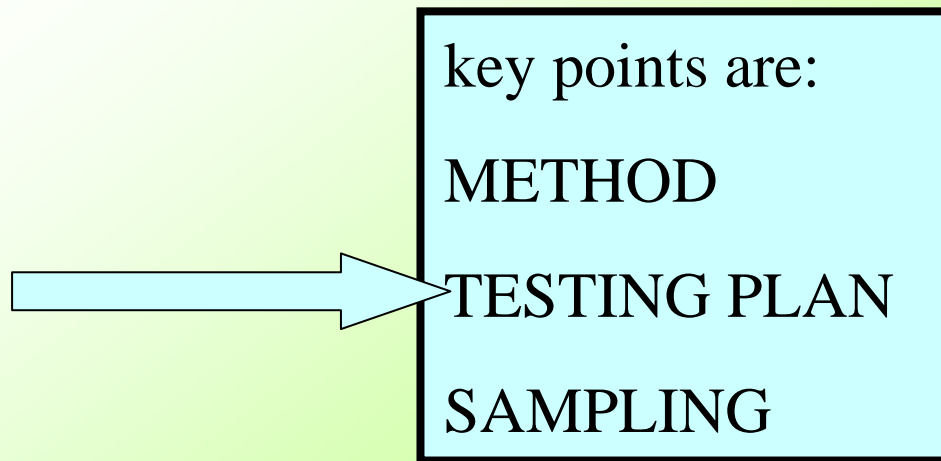
Measurements

Seed lots are processed to be homogeneous,

if each step is carefully managed, tests are precise and reliable



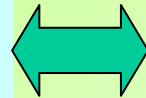
Maize seed lot	$3 \cdot 10^4$ kg	$2 \cdot 10^{-10}$ kg	Sample in machine
Mass of mankind	$50 \text{ kg} * 6 \cdot 10^9 \text{ humans} = 3 \cdot 10^{11} \text{ kg}$	$2 \cdot 10^{-3} \text{ kg} = 2 \text{ g}$	Zn (oligo element) content of a 70kg human
Everest mountain	8800 m	$7 \cdot 10^{-10} \text{ m}$	7 times the diameter of an atom
A life time	70 years * 31558149 s	$1.4 \cdot 10^{-5} \text{ s}$	100 times faster than nervous transmission through myelin-axone
2005 Gross National Product Worldwide	$32\,000 \cdot 10^9 \text{ US\$}$	0.16 US\$	not much money



Seedcalc can be used to derive a testing plan and calculate estimates/confidence intervals/... for a number of various methods, units, questions

Check/ find a testing plan which is effective and appropriate

Compute an estimate to report with confidence limits...



End point PCR pos/neg on seed pools

Real time PCR on seed pool(s)

...other methods

Statistical theory

+ you can enter your own data
and/or explore a range of possible data

of Seed Pools

of Seeds per Pool

Total Seeds Tested

Deviants Pools

Computed % in sample

Measured property on seed pools

Desired Confidence Level

Upper Bound of True % Impurity
(95% confident that the lot impurity is below 0,32%.)

2-sided CI for True % Impurity to

Lower Bound of True % Purity
(95% confident that the lot purity is above 99,68%.)

2-sided CI for True % Purity to

End point PCR and real time PCR
can lead to very similar results

From
seedcalc
freely
available on
ISTA
website

Seeds per Pool (m)

Confidence Level (%)

Flour Sub-samples

b-Factor

Measurements per Sample

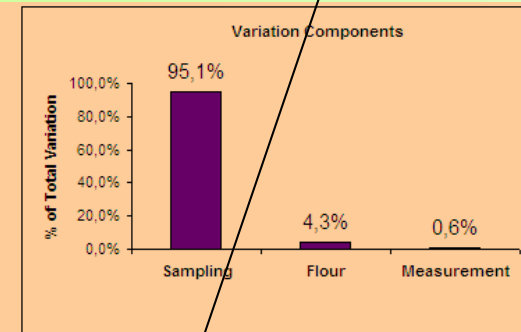
Total Seeds Tested

Pools

Measurement CV (%)

Flour Std. Dev.

Measurement #	Flour Sub-sample #	
	#1	#2
1	0.1400%	0.0900%
2	0.1200%	0.1020%
3	0.1300%	0.0960%



Estimated % Impurity

Display confidence intervals without sample variability

Upper Bound of True Lot % Impurity
(95% confident that the lot impurity is below 0,24%.)

2-sided CI for True Lot % Impurity to

Upper Bound of True Sample % Impurity
(without sampling variability)

2-sided CI for True Sample % Impurity to
(without sampling variability)

SAMPLING TYPE

SINGLE
 DOUBLE

Find Plan

Analyze

Seeds per Pool false_pos_rate 0,0%
false_neg_rate 0,0%

1 Stage

of Pools (N1)

= 3000 seeds

% impurity LQL 0,90% AQL 0,20%

Accept Lot if # deviants does not exceed C1

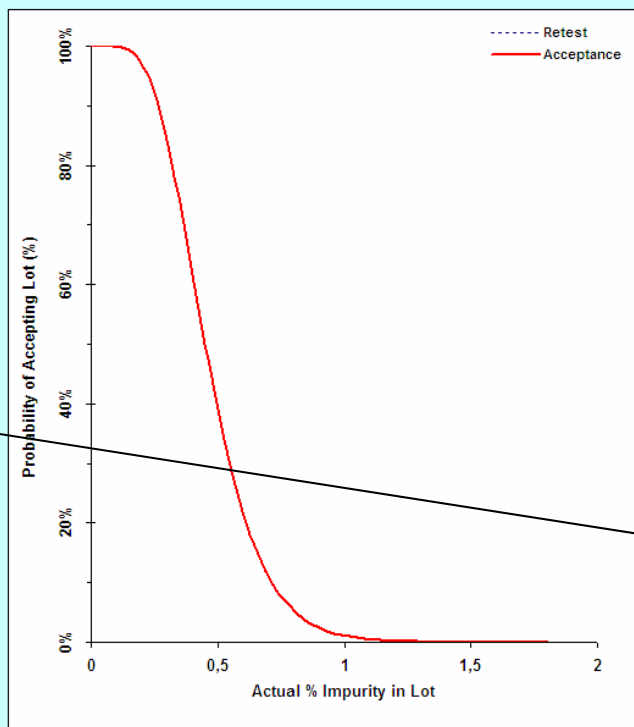
Consumer (beta) Risk 2,47%
Producer (alpha) Risk 2,80%

Confidence Level (%) 97,53% 97,20%

Target Consumer Confidence Level

Target Producer Confidence Level

Max # Seeds Tested



For given levels to check (AQL and LQL)

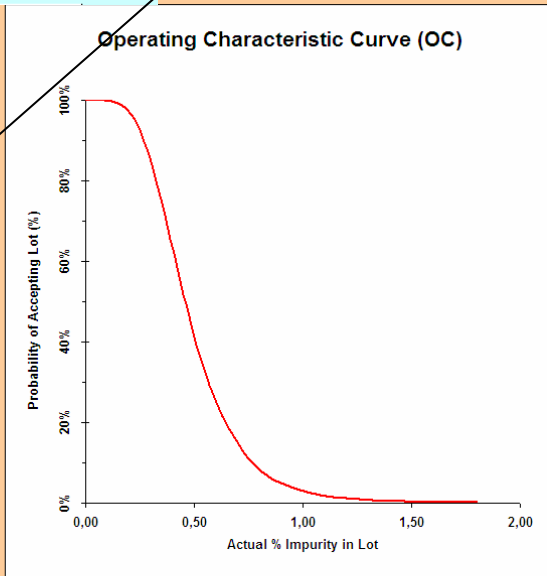
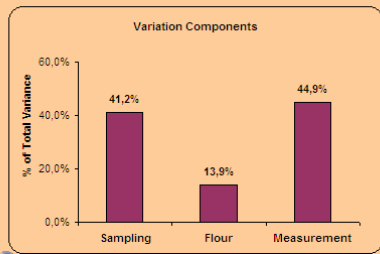
testing plans can be found for

end point and/or real time PCR

Expected Testing Costs

Same efficiency, same confidence, when aim is to check limits

of Pools Find Plan
Seeds per Pool = 3000 seeds
Flour Samples per Pool
Measurements / Flour Sample
Acceptance Limit
Measurement CV
Flour Sub-sample Std Dev
b-Factor
Impurity LQL 0,90% AQL 0,20%
Consumer (beta) Risk 4,99% Producer (alpha) Risk 2,80%
Confidence Level 95,01% 97,20%
Target Consumer Confidence Level
Target Producer Confidence Level



Transfer Next Plan # 3 Plan Name Quant Test Plan 3 Clear All Plans

Do we have any evidence from actual experience in ISTA work that some methods, some units, ... are less reliable or less precise than others to test seeds?

PT2 – PT6 Data Quantification Evaluation

Performance Categories

- Accuracy
- Relative variability
- Repeatability... (not covered)

Comparisons

- Overall
- Units
- Method Type
- Methods

Metric for Comparisons

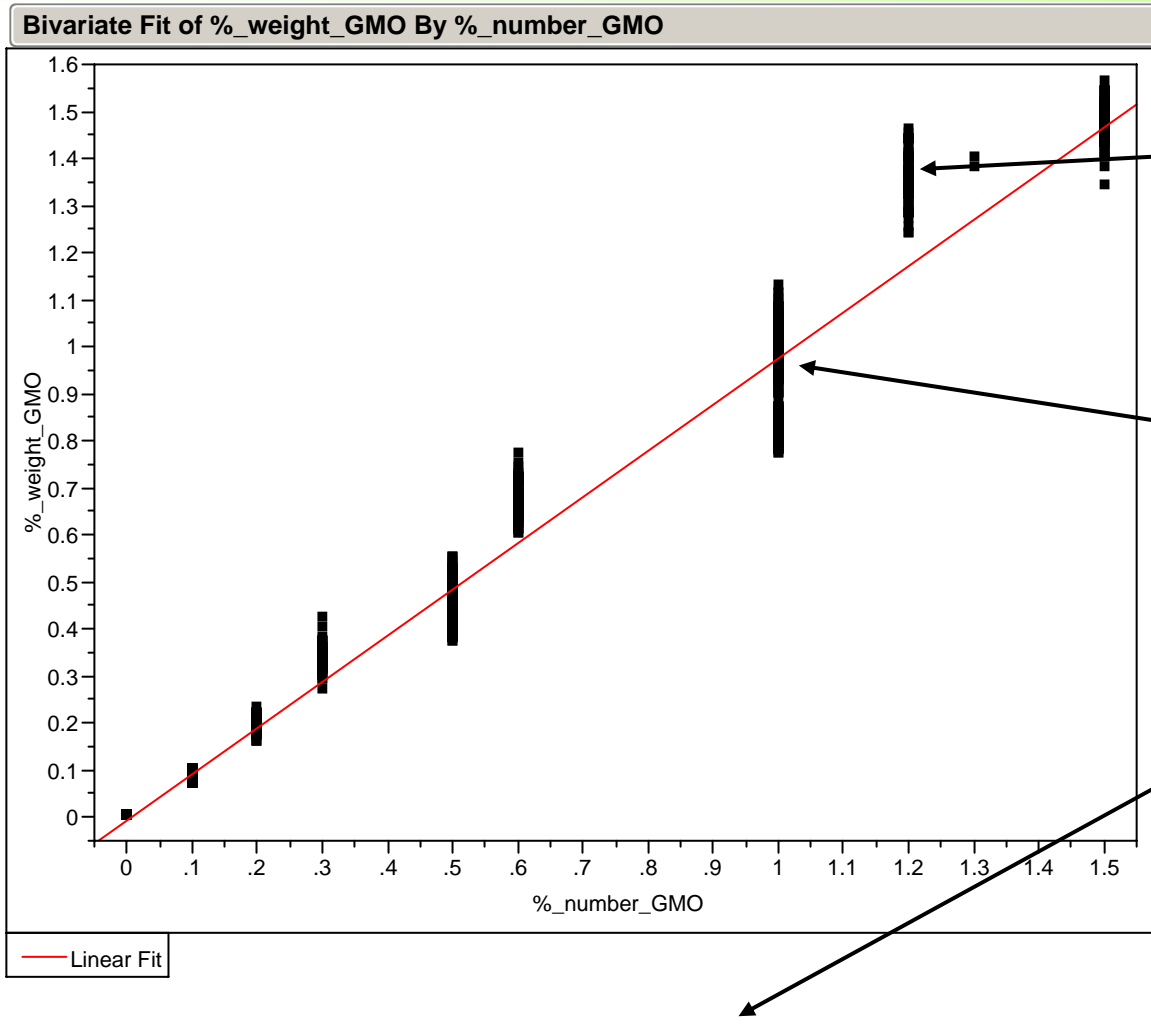
% Difference = (observed – true)/true

true = %GM by number OR

true = %GM by mass

Caution: No attempt was made to separate labs with differing levels of experience

Agreement between % number and % mass



% in number and % in seeds are different, due to differences on individual seed weights

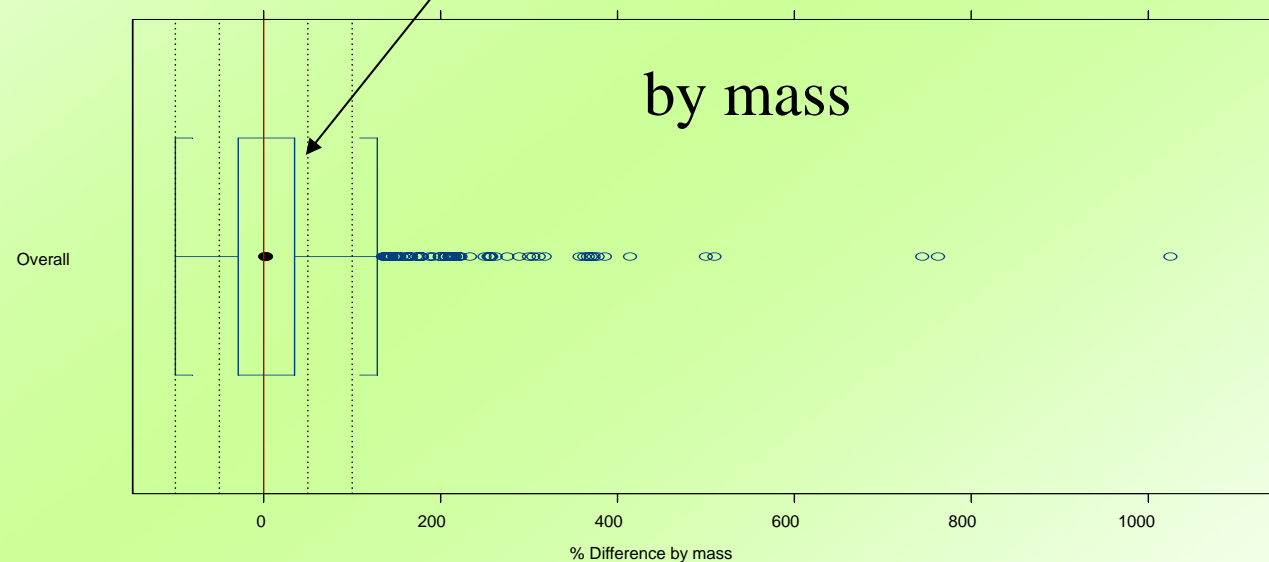
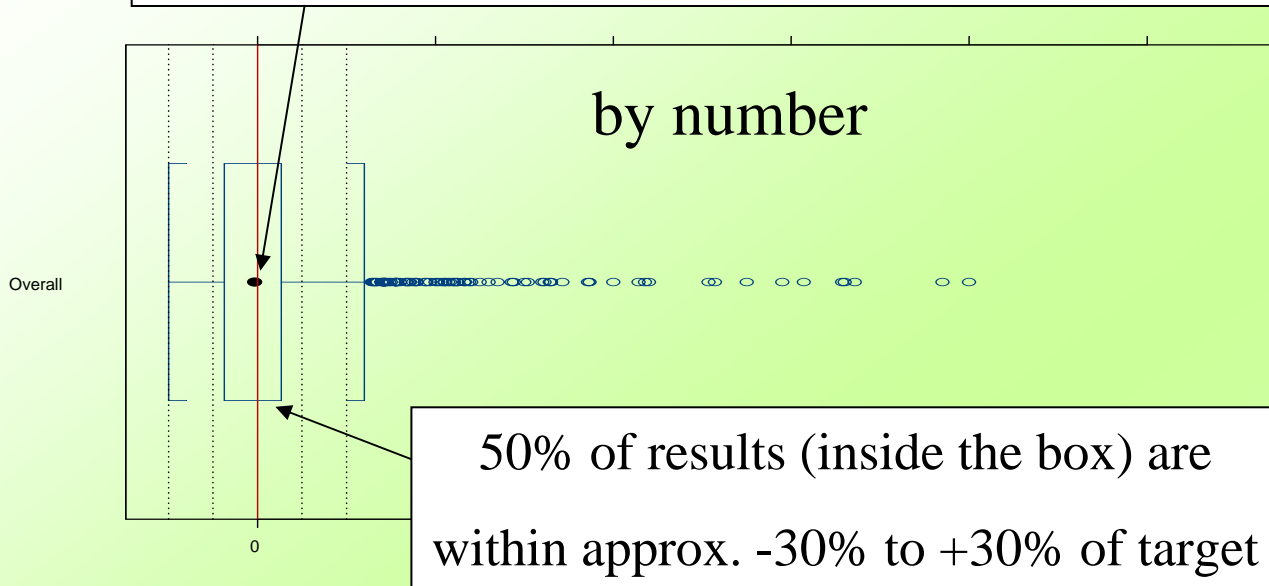
There is variability in results

The slope is approx. 1 and intercept is approx. 0 which indicates a global agreement between units.

$$\%_weight_GMO = -0.006 + 0.982 \%_number_GMO$$

Overall % Difference Results for PT2 – PT6

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



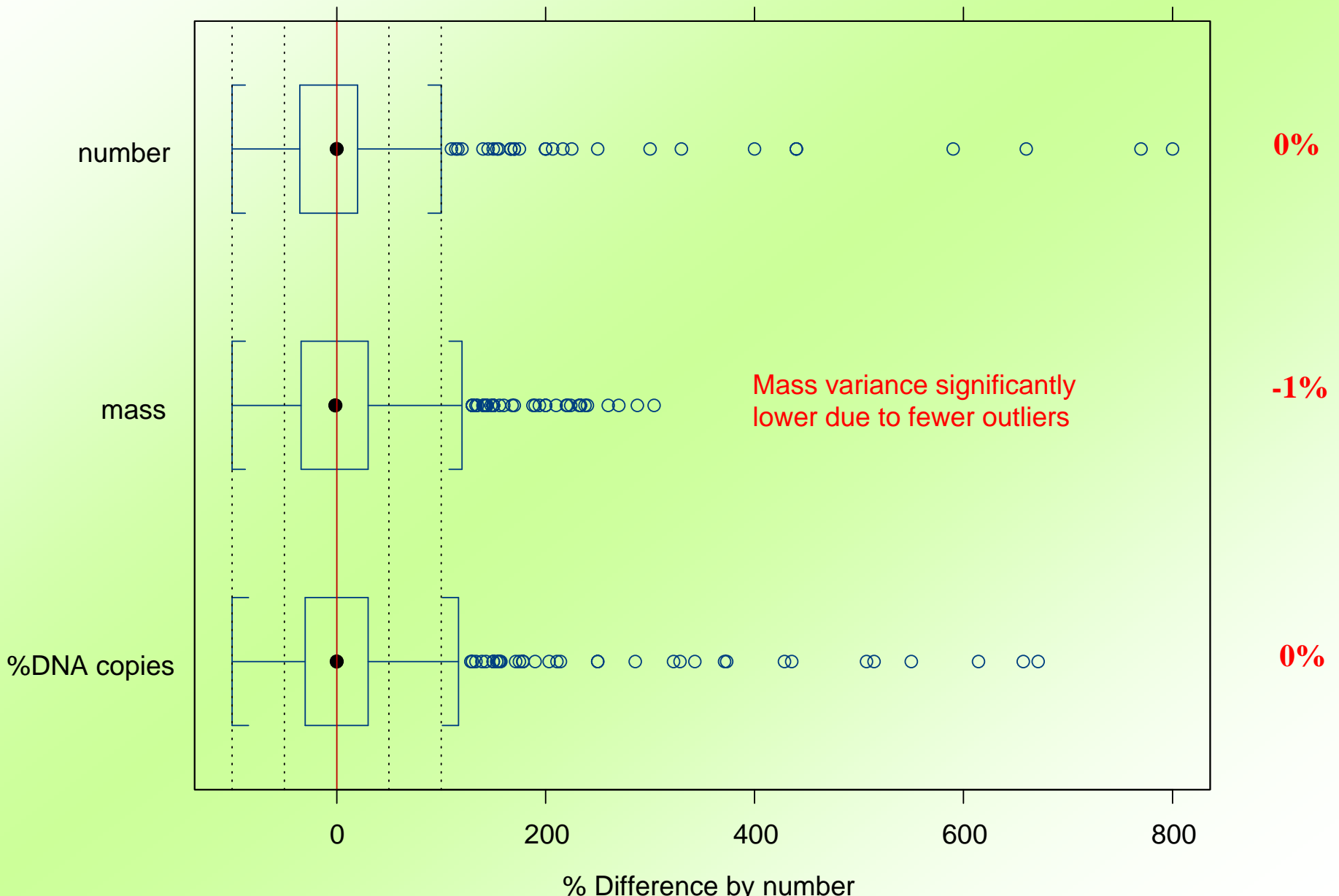
- More extreme results above target
- 90% of results within approx. [-100% to 150%] of target

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

Unit (true=number)

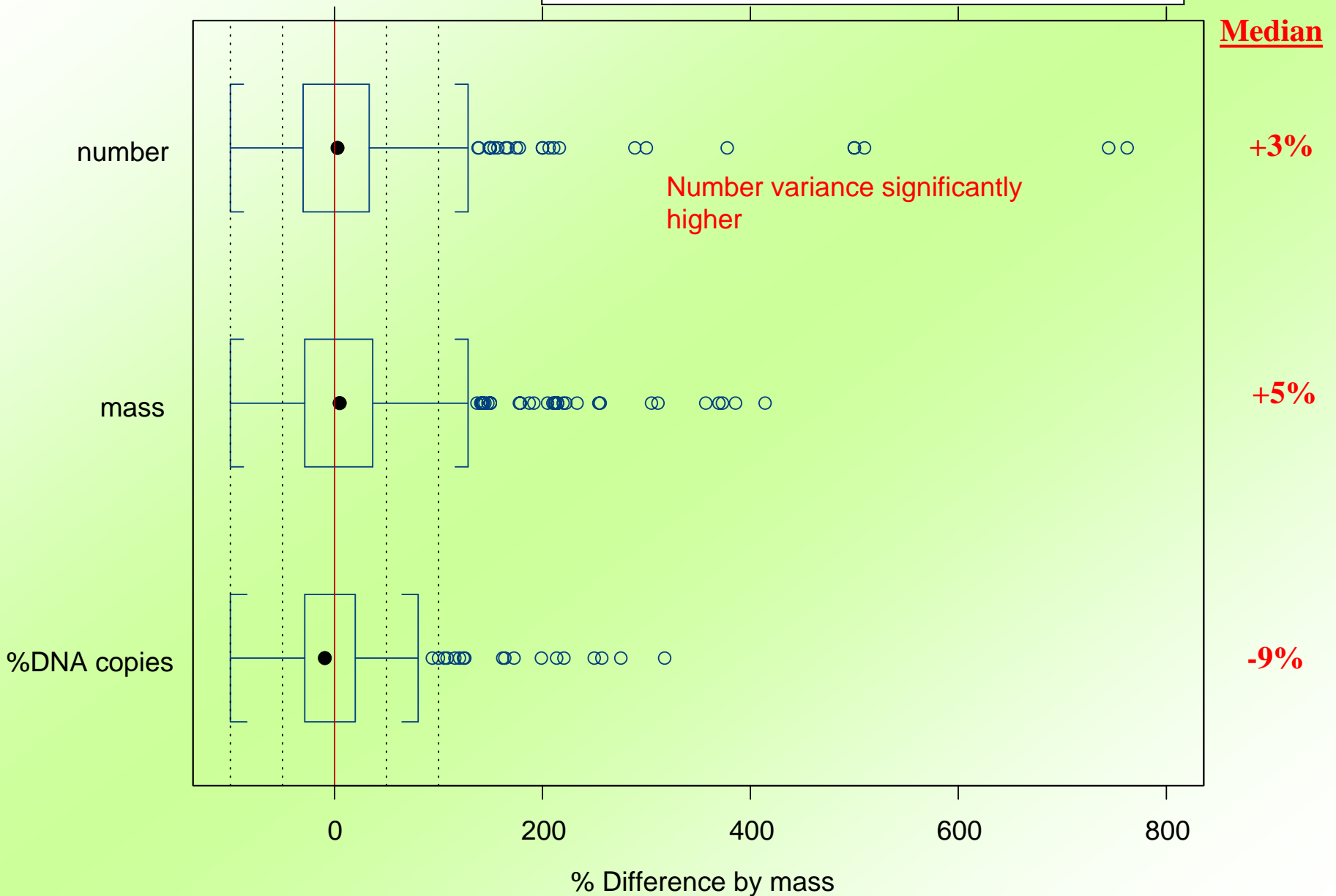
Median and middle 50% of data are virtually the same

Median



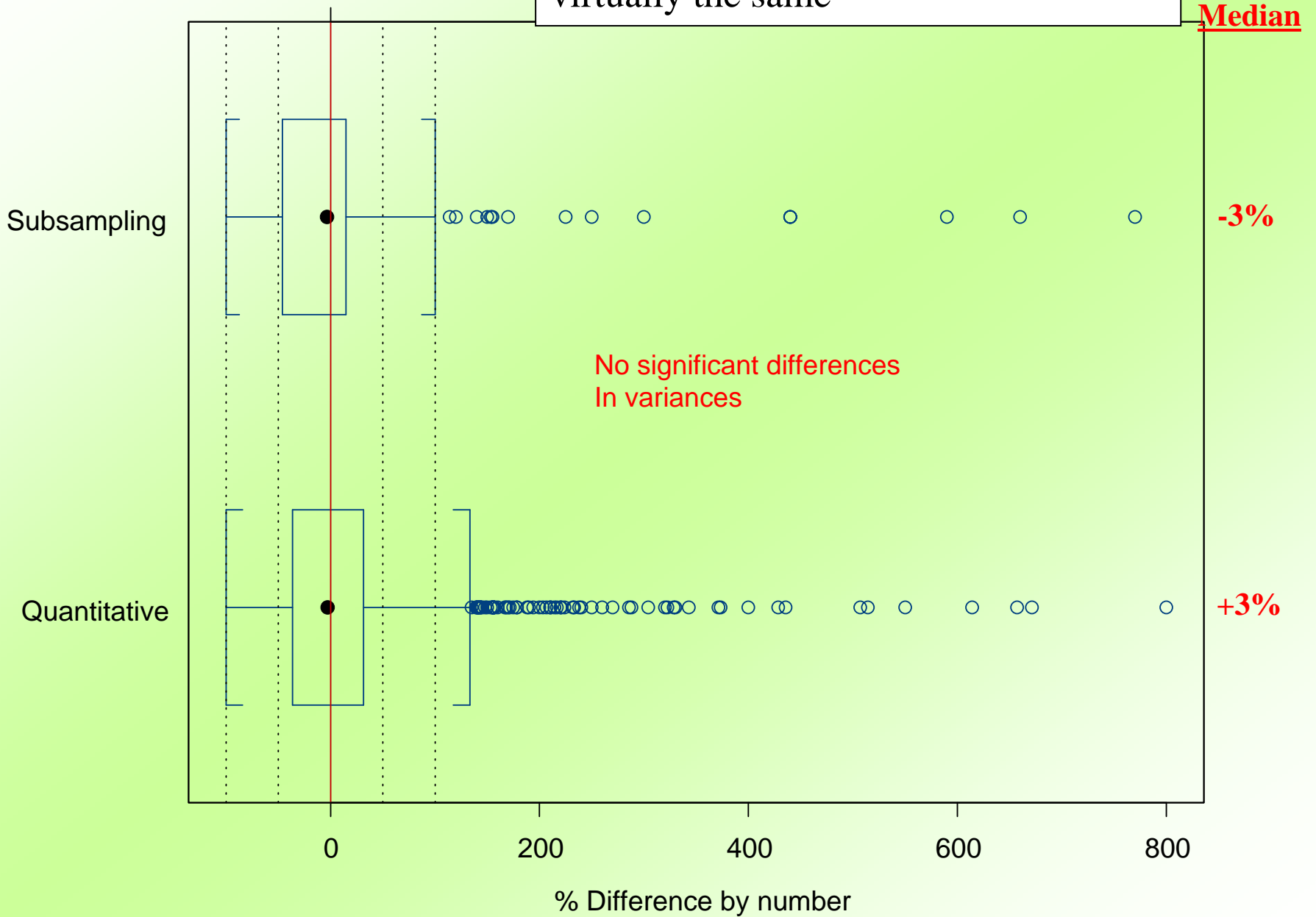
Unit (true=mass)

Median and middle 50% of data are virtually the same



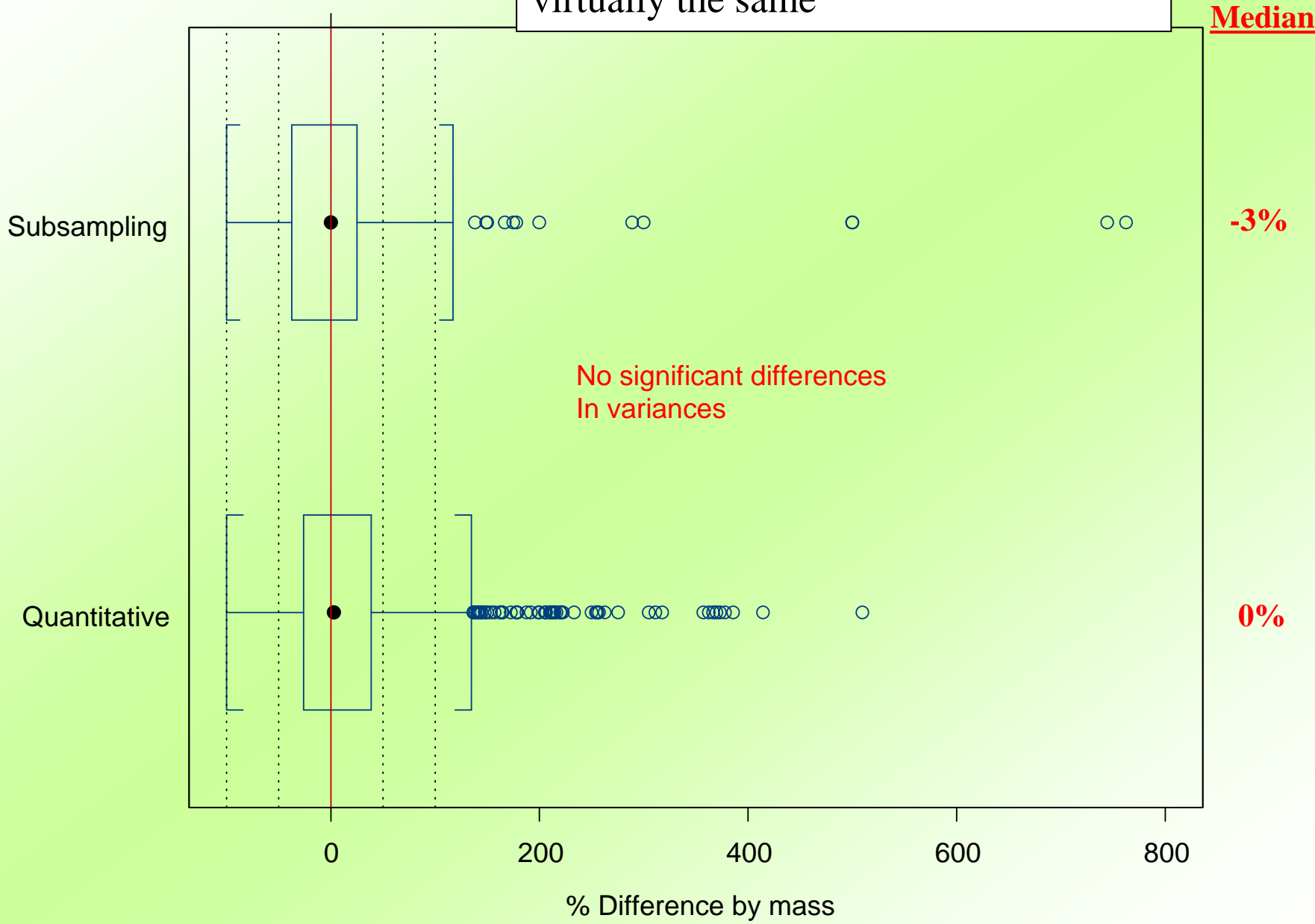
Method Type (true=number)

Median and middle 50% of data are virtually the same

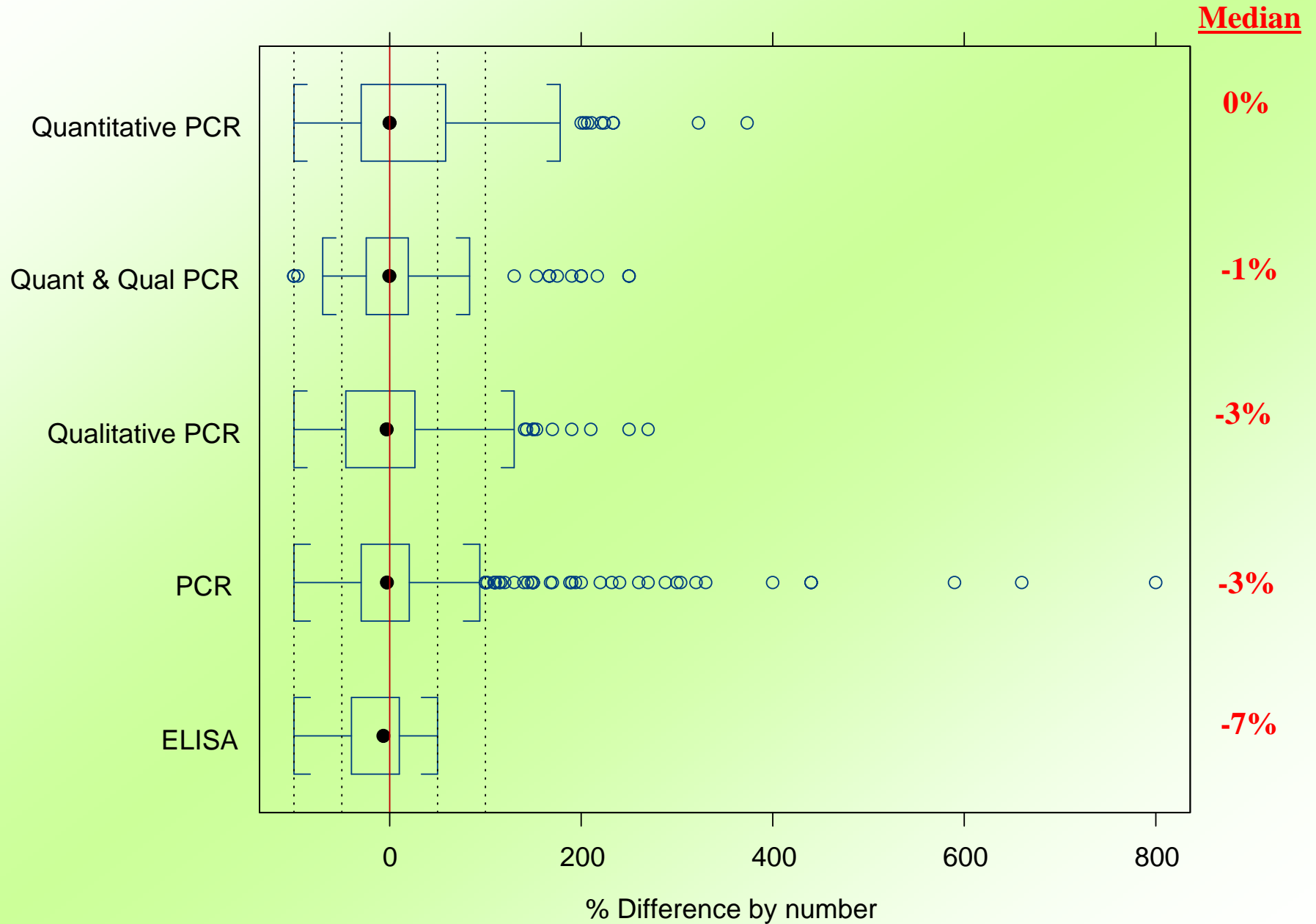


Method Type (true=mass)

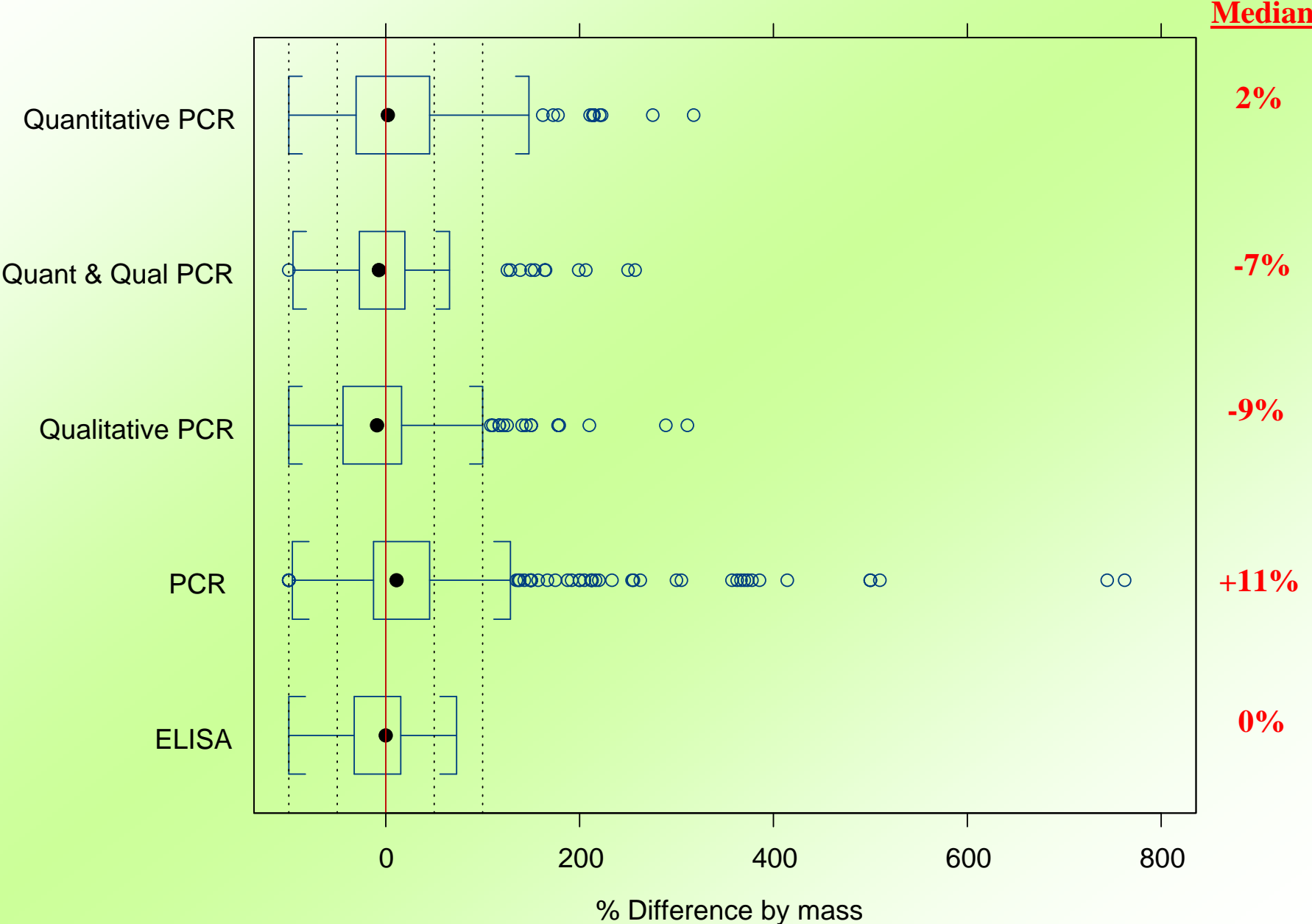
Median and middle 50% of data are virtually the same



Method (true=number)



Method (true=mass)



The previous slides (Seedcalc computations plus actual data from proficiency tests) show no evidence of reduced reliability for any type of method, unit, etc.

Chapter 8

Specified trait(s)

Only approved-reliable methods shall be used

ISTA does not aim to compare methods or units

ISTA is providing seed samples blindly to laboratories, where the true value is known, and computes all data together, to indicate to the lab it's level of performance (proficiency testing)

Ista does not validate a unique method for bio-molecular tests

Different units and level of precision can be managed within ISTA

Conclusions

- ISTA has a consistent statistical framework to evaluate and check accuracy and repeatability for **all situations worldwide** that we are aware
- Independent of the method, method type, unit, etc. results from the six proficiency tests show that **reliable results can be achieved**
- The framework, and the implementation, **can evolve and remain consistent**

The statistical framework and Seedcalc can be, and are effectively, used for many other types of tests, in particular when not only the estimate is needed, but also a testing plan allowing to check versus thresholds with known confidence

Thank you for your attention!

Accuracy = departure from true value

Very pure sources of seeds are used in ISTA PTs to prepare samples.

For each individual sample: the number of both type of seeds is counted for sub-sampling strategy labs, the mass of both source of seeds is measured for all labs

- % in number of seeds ← Is known by test organiser
- % in mass of seeds ← Is known by test organiser
- % DNA copies ← Is estimated, median of reported results in this unit

Could not we know the true value of the sample in % DNA copy at time of sample preparation?

Can we know the true value of the sample in %
DNA copy
at time of sample preparation?

Could ISTA provide certified reference material for the samples to test in Proficiency tests or Performance Data evaluation?

➡ It has been extensively explored, the answer today is no.

Could we know the true % DNA copy value of the GM source by making tests in reference laboratories?

➡ Today there is no such laboratories recognised worldwide

➡ There is a number of issues on what is the true value in specific and easy case

If all GM and non GM seeds of the sample have the same weight, if a CRM at x% has been made from the same source of seeds, and all tissues are diploid, and there is only XXX copy of the gene carried by the mother, then

XXX=1 or 2

% by number = % by mass = % DNA copies
otherwise not

Nowadays we are in the
'otherwise not' situation

Could not we know the true value
on a case by case situation?

A single example on a diploid species ($2n$):

A: there can be 1 or 2 copies of gene

B: 1 gene is usual, but there might be 2 or 3 different genes

=> 1 to 6 copies per seed

If there is 1% GM seeds:

% number of seeds
would remain the
same = 1%
independent of A B

% DNA copies per genome

1%? if seeds have 1 gene copy

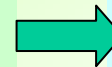
6%? if 3 genes * 2 copies per seed

% DNA copy per haploid genome

0.5% 1 copy

3% if 6 copies

No worldwide consensus yet, it is a difficult matter
to set coefficients from one unit to the other



Choices will have
very strong impacts

If one can uniquely identify+quantify all GM impurities in the sample, one could be able to go from one unit to the other, knowing the construct of all of the GM existing sources, and the coefficient to use (previous slide)

But,

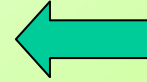
Zhang & Al. have shown in maize embryo(2n) / endosperm(3n) ratio varies depending of varieties

% DNA copies depend on mother/father carrying gene(s)

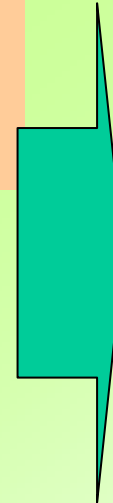
In some maize flour the embryo is excised, not in others (concern for food/feed not for seeds)

Cultivated species are also tetraploids, hexaploid...

What you shall be able to do if you need to check authorised and unauthorised events and check levels



There is still a number of issues under consideration



For more results on units in Proficiency Tests and Performance Data Evaluation, cf GMO session tomorrow