## Assessing limits of detection

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## Overview

- Detection limits
  - concepts
- Statistical basis of limits
- Determination of detection limits
- Using and reporting sub-LOD data
- Some unresolved issues

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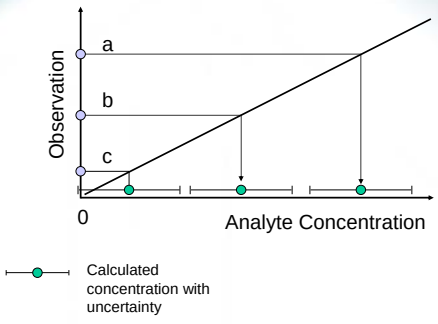
## Detection limits




Calculated concentration with uncertainty

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## Concepts

- Critical value
  - instrument response used to trigger action
- Detection limit
  - amount of substance leading to action
- Quantitation limit
  - lowest level at which uncertainty is acceptable

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## Purpose of detection limits

- Indication of lower operating limit
  - approximate values required
- Setting rigorous decision and control limits
  - statistical inference required

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## Types of error

- False positive: (Type I error) Wrongly declaring a substance to be present
  - observation high by chance (due to random variation in measurement)
  - incurs clean-up or control cost
- False negative: (Type II error) Wrongly declaring a substance absent
  - may incur health or safety hazard

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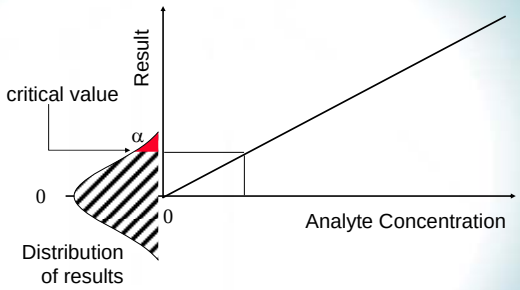
## Statistical basis of limits

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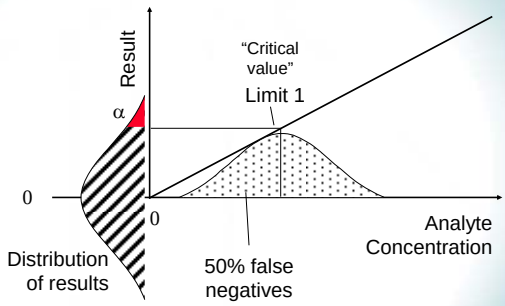
## Setting limits to control error

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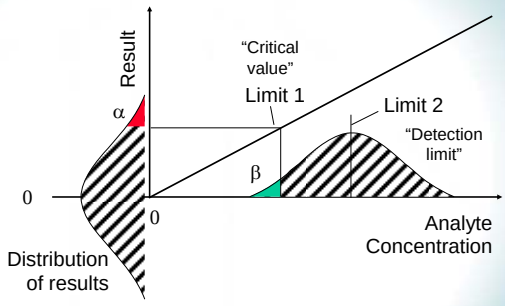
## Setting limits to control error

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## Typical experiments

- Standard deviation of blank response
  - typically, 6-10 replicates of the whole method are necessary
- Standard deviation of lowest spike
  - 6-10 replicates required
- Successive dilution
  - dilute until approximately 50% of results indicate no analyte present

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## Approximate detection limits

- LOD study repeats full method including any blank/baseline correction:
  - $3.3s$ + (baseline or blank response)
  - sometimes approximated to $3s$ + (baseline or blank response)
- LOD study does not replicate corrections used in practice:
  - $4.65s$ + (baseline or blank response)

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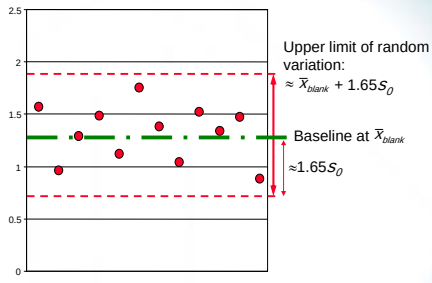
## Lower limits - basic principle

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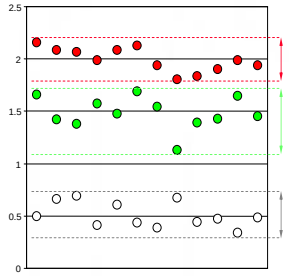
## Independent corrections




Sample. $s_s = 0.11$

Sample - Blank. $s_c = 0.16$

Blank. $s_0 = 0.12$

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## LOD calculation procedure. 1. General Equations

Critical value
Decision criterion, critical level, CCα

$x_0 + k_l s t_{(\nu,\alpha)}$ Close to $1.65s$

Detection Limit
Limit of detection, minimum detectable value... CCβ

$x_0 + k_l s t_{(\nu,\alpha)} + k_l s t_{(\nu,\beta)}$ **Close to 3.3s**

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## Calculation procedure 2: Selecting Values

| **Validation** | **Routine use of method** | |
|---|---|---|
| | Will results be baseline corrected? | |
| $s$ based on: | YES | NO |
| • Observations with independent baseline corrections | $x_0 = 0$<br>$k_l = 1.0$ | *N/A* |
| • Observations without independent baseline corrections | $x_0 = 0$<br>$k_l = \sqrt{1+1/n_B}$ | $x_0 = x_{blank}$<br>$k_l = 1.0$ |

$n_B$ is the number of baseline observations averaged for correction

$x_{blank}$ is either the mean blank response or the observed baseline offset, as appropriate

Usually based on repeatability

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## Quantitation limit (LoQ)

"Level at which the uncertainty becomes unacceptable"

- Common assumptions
  - acceptable uncertainty is 10%
    - leads to $10s_0$
- Other levels used:
  - $5s_0$ , $6s_0$
- Recommendation
  - use $10s_0$ unless otherwise required

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## Alternative procedures

- Instrument response
- ISO 11843-2
  - From calibration data

Usually based on repeatability

- SANCO
  - From multi-level, longer term precision data
  - Uses ISO 11843 procedure

Based on in-house reproducibility

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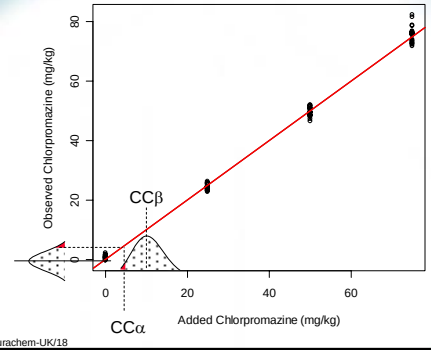
## SANCO/ISO 11843-2




3 Runs
7 replicates per level

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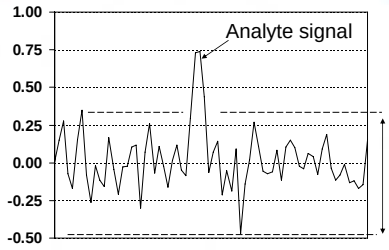
## Setting limits from instrument response




Based on instrument noise

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## Unresolved issues

- Which standard deviation?
- Reporting and subsequent calculation

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## Using sub-LOD data

- "Less than" cannot* be averaged
- "Less than" cannot* be scored in PT
- "Less than" cannot be added
  - except to form a bigger "less than"
- ... or subtracted.
- ... but "-0.2 mg/kg" can...

**Report the raw observation if at all possible**

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*Maximum likelihood and approaches excluded

## Current recommendations

- "Less than LOD" does NOT mean "invalid result"
  - Report the raw result and its uncertainty if you can
- Not all systems provide results below thresholds
  - A case for a different approach?
  - Maximum likelihood estimation...?

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## Which standard deviation?

- Instrument noise?
  - If a signal is visible, there must be some analyte present!
- Repeatability?
  - Takes into account extraction/preparation
- In-house reproducibility?
  - Adds longer-term effects
- Suggestion: Smallest SD for critical value; largest for "LOD" addition.

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## Conclusions

- Detection limits are based on statistical reasoning
- Detection limits determined during validation are indicative
  - for typical in-house validations, approximate values are usually adequate – e.g. 3$s$ for "LOD"
  - decision limits on which action depends should be rigorously checked and monitored regularly
- Report raw values if you can
  - Investigate 'censored data' methods if you can't
- Some more work is needed on which standard deviation to use for critical decisions

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