Performance characteristics of analytical tests

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- Bottlenecks
  - Detection limits, definitions
  - procedures in various countries
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Background

- Laboratories should have procedures for the validation of performance characteristics of the methods used
- Currently there are no CEN / ISO-standards available for the determination of performance characteristics of test methods for soil samples
- Procedures used for establishing performance characteristics differ a lot over several countries
  - ➔ performance characteristics are hardly or not comparable!
- This is especially the case for LOD / LOQ and measurement uncertainty

Performance characteristics(1)

- Repeatability
- Reproducibility
- Trueness, recovery
- Detection limit (LOD), quantification limit (LOQ), reporting limit (RL)
- Measurement uncertainty
- Selectivity, robustness

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Performance characteristics (2)

- International bottlenecks
  - Procedures for establishing
    - LOD, LOQ
    - Measurement uncertainty

Limit of detection

Limit of quantification

Reporting limit

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Basis for LOD/LOQ: repeatability or reproducibility?

- Repeatability
  - Standard deviation in analytical result of a sample analyzed on 1 day by one analist in one batch on 1 instrument
  - ➔ estimation of the "best" variance of the analytical result produced by a lab

- Reproducibility (within lab)
  - Same as repeatability, but sample analyzed by several analists on different days, on several instruments
  - ➔ estimation of the "real life" variance of the analytical result over a longer period by that lab

- Repeatability < Reproducibility
  - Experience: [reproducibility ~ 1.5 – 2.5 X repeatability]

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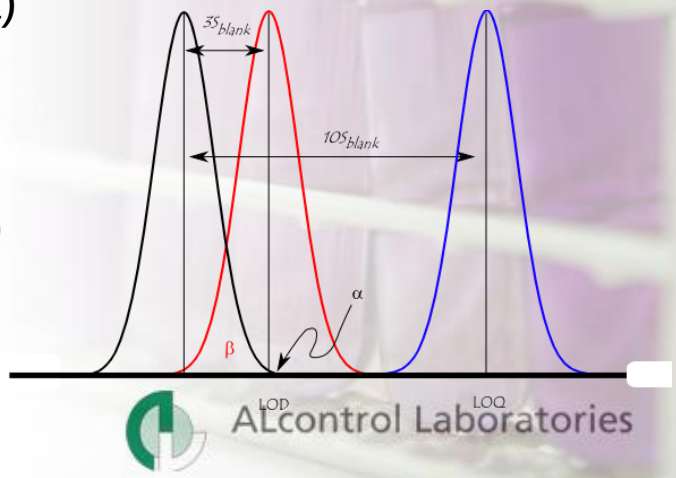
Detection limits, definitions

- LOD (Limit of Detection)
  - lowest quantity of a substance that can be distinguished from the absence of that substance (a *blank value*) within a stated confidence limit (IUPAC)
- IDL (Instrument Detection Limit)
  - The IDL is the analyte concentration that is required to produce a signal greater than three times the standard deviation of the noise level (Wikipedia).
- MDL (Method Detection Limit)
  - Same as LOD but accounting for all steps in the analysis and possible other sources of errors (Wikipedia)
- LOQ (Limit of Quantification)
  - LOQ = 3,3 x LOD
- PQL (Practical Quantification Limit)
  - PQL = 5 x MDL (Wikipedia)



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Detection limits, procedures
(Wikipedia)

- LOD (Limit of Detection)
  - 3x standard deviation of a blank sample
- IDL (Instrument Detection Limit)
  - measurement of 8 standard solutions on expected IDL level: IDL = 3x standard deviation
- MDL (Method Detection Limit)
  - measurement of 7 samples, inclusive all steps like sample pretreatment, extraction, etc., on expected MDL level: MDL = 3x standard deviation
- LOQ (Limit of Quantification)
  - LOQ = 3,3 x LOD
- PQL (Practical Quantification Limit)
  - PQL = 5 x MDL

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Reporting limit (RL)

- Usually: RL ≥ LOQ or PQL

- Example:
  - LOD = 1 ug/l
  - LOQ = 3,3 ug/l
  - RL = 5 ug/l

  (➔ if a compound is not found, the report states: < 5 ug/l)

- Sometimes RL used is much higher than the LOQ
  - E.g. in case the legislative target value is much higher (for example < 50 ug/l, with the figures mentioned above and legislative target value of 100 ug/l)

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LOD, LOQ, procedures in several countries (1)

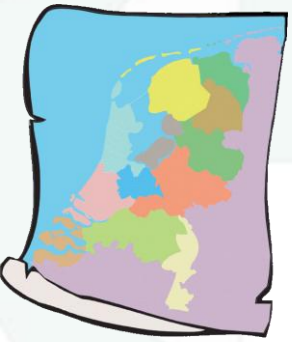
Netherlands (NEN 7777; draft Sept. 2010)

- Prepare a sample at low concentration level (around expected LOD) or use a real sample with low concentration

- Analyze this sample ≥ 8 time under **reproducibility** conditions
  - LOD = 3x standard deviation
  - Reporting limit = LOD

- Alternative: analyze several (real) samples on low concentration level on different days in duplicates (identical formulas)

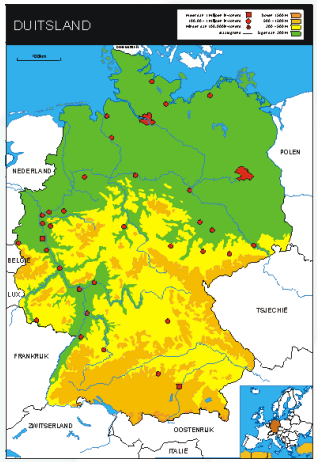
- NB: in the current NEN 7777 version (2003) LOD can also be established under **repeatability** conditions (≥ 8 time). In that case:
  - LOD = 3x standard deviation
  - Reporting limit = 3,3 x LOD



LOD, LOQ, procedures in several countries (2)

Germany

- DIN 32645:1994 (scope: all chemical-analytical tests)
  - Nachweisgrenze = LOD
  - Erfassungsgrenze = Detection Capability ~ 2x LOD
  - Bestimmungsgrenze = LOQ ~ 3x LOD
  - reporting limit = LOQ



- Nachweisgrenze (LOD)
  - Based on <u>blank</u> sample
  - tests under <u>repeatability</u> conditions
  - LOD = 1,2 x factor x standard deviation
    (factor: depends on number of tests and significancy level;
    factor = 2 for 8 tests and significancy level = 0,05)

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LOD, LOQ, procedures in several countries (3)

France

- NF T 90 210:2009 (scope: water tests)
  - standard gives no method for establishing **the** LOD, LOQ etc., but gives procedures for establishing if a proposed LOQ acceptable is, based on test results
  - tests to be carried out for assessing the proposed LOQ:
    - Take one blank matrix sample (or prepare one, if not available)
    - Add compounds on the proposed LOQ level
    - Analyze the compounds on at least 5 days, at least in duplicate
- Use the average and the standard deviation of the average for the assessment
  - ➔ reproducibility and spike at the matrix are the basis!

LOD, LOQ, procedures in several countries (4)

UK

- MCERTS system for soil samples (v3, Maart 2006)
  - use blank matrix sample and blank sand sample
    (if normally distributed results around zero can be produced
    ➔ if not: prepare a matrix sample, spiked on max 5x expected LOD)
  - Analyze the samples (inclusive sample pretreatment, extraction, etc.)
  - **LOD = 2√2.t (*df*,α= 0.05).sw,** with
    - **df** is the number degrees of freedom (minimal 10)
    - **t** is the 1-sided Student's t-test (95% reliability level)
    - **sw** is the within-batch standard deviation of the results of the samples which idealiter contain:
      - A "zero" concentration of the concerning parameter
    - LOD = 2√2.**t x sw** = 5.13 x **sw** (in case of 10 duplicate tests over 10 days)
  - ➔ UK: based on repeatability and either blank or low spike

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LOD, LOQ, procedures in several countries (5)

Sweden

- LOD based on blank samples
- analyze samples with low concentration on assumed LOD level
- use of a statistical model in order to assess if the assumed of the LOD correct is (looks like French model; details missing)

Spain:

- No standardized method available:
  up to the lab to define a procedure and up to ENAC to evaluate this procedure

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LOD, LOQ, procedures in several countries (6)

Italy

- prepare a blank sample
- analyze the blank sample (whole procedure, inclusive sample pretreatment)
- LOD = average + 3x standard deviation
- LOQ = average + 10x standard deviation

- unknown:
  - number of tests
  - repeatability or reproducibility conditions

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LOD, LOQ, procedures in several countries (7)

Europe, development

- CEN/TC 230 (Water)
  - Proposal by France for developing a standard for establishing performance characteristics
  - Aim: procedure also suitable for soil, air and biota

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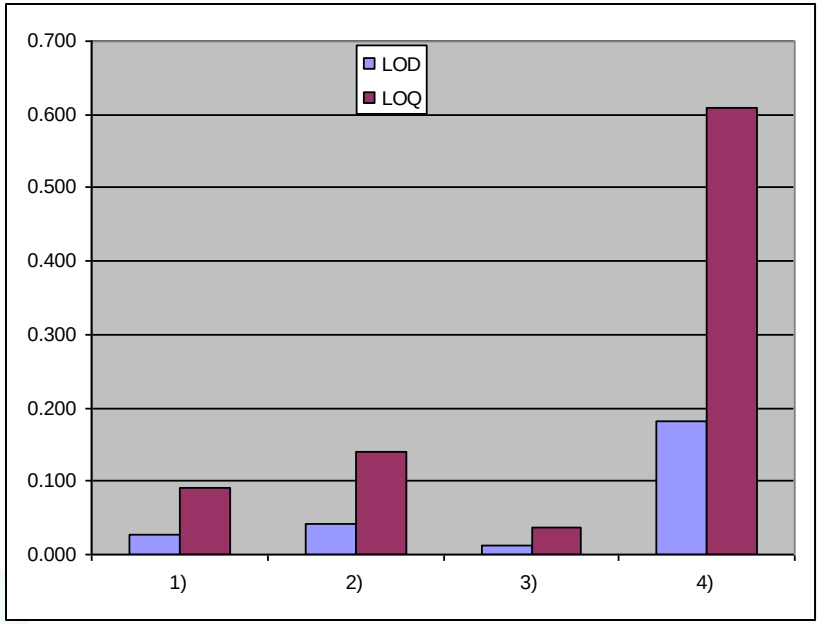
Examples of LOD's, LOQ's (1)



Cadmium Cd				
	Blank Aqua Regia not digested	Blank Aqua Regia digested	Soil	Sediment
	1)	2)	3)	4)
	0.0097	0.0046	0.171	0.741
	0.0148	0.0256	0.179	0.813
	0.0133	0.0146	0.176	0.775
	-0.0176	-0.0278	0.174	0.639
	0.0036	0.0137	0.174	0.703
	-0.0028	-0.0067	0.182	0.671
	0.0005	-0.0043	0.175	0.667
	-0.0067	-0.0020	0.181	0.71
	0.0078	0.0024	0.181	0.636
	-0.0057	-0.0063	0.179	
	0.0016	0.0059	0.182	
	0.0033	-0.0126	0.180	
stdev	0.0092	0.0140	0.0038	0.0608
LOD	0.028	0.042	0.011	0.182
LOQ	0.092	0.140	0.038	0.608

1. blank Aqua regia: direct measurement, reproducibility conditions
2. blank Aqua Regia destruct: blank samples whole procedure, reproducibility conditions
3. soil: low concentration spiked on sand (organic matter< 2%); repeatability conditions
4. Sediment: low concentration spiked on sediment (organic matter ~ 12%); reproducibility conditions

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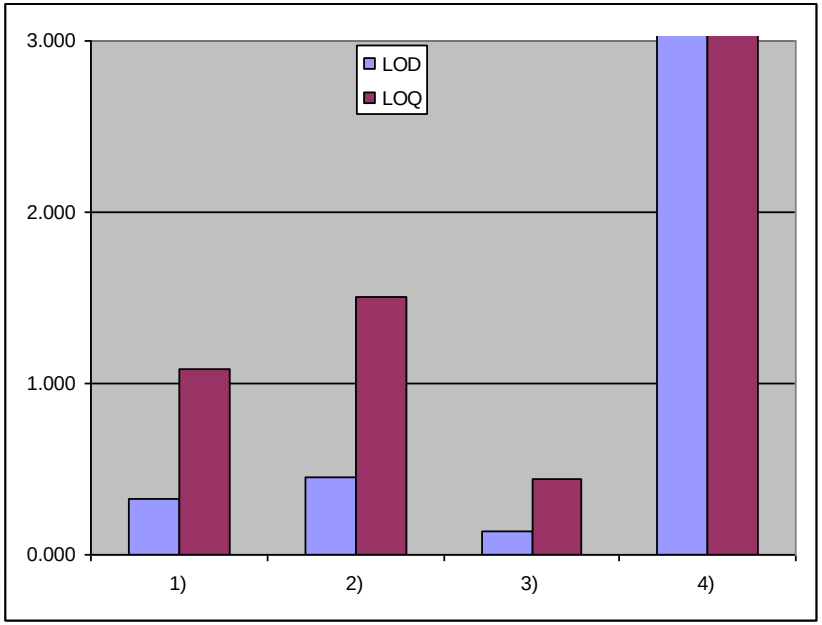
Examples of LOD's, LOQ's (2)



	Lead Pb			
	Blank Aqua Regia not digested	Blank Aqua Regia digested	Soil	Sediment
	1)	2)	3)	4)
	0.1594	0.1457	3.739	10.5
	0.1610	0.5257	3.701	10.5
	0.1077	0.1636	3.719	13.6
	-0.0238	0.1254	3.681	21.2
	-0.1494	0.0703	3.690	12.3
	-0.0176	-0.1381	3.790	12.6
	0.0053	0.1100	3.687	12.4
	-0.0264	0.1849	3.762	10.8
	-0.0324	0.0714	3.762	12.8
	-0.1943	0.1642	3.769	
	-0.0270	0.0832	3.770	
	-0.0480	0.0473	3.649	
stdev	0.1079	0.1509	0.0447	3.2783
LOD	0.324	0.453	0.134	9.835
LOQ	1.079	1.509	0.447	32.783

1. blank Aqua regia: direct measurement, reproducibility conditions
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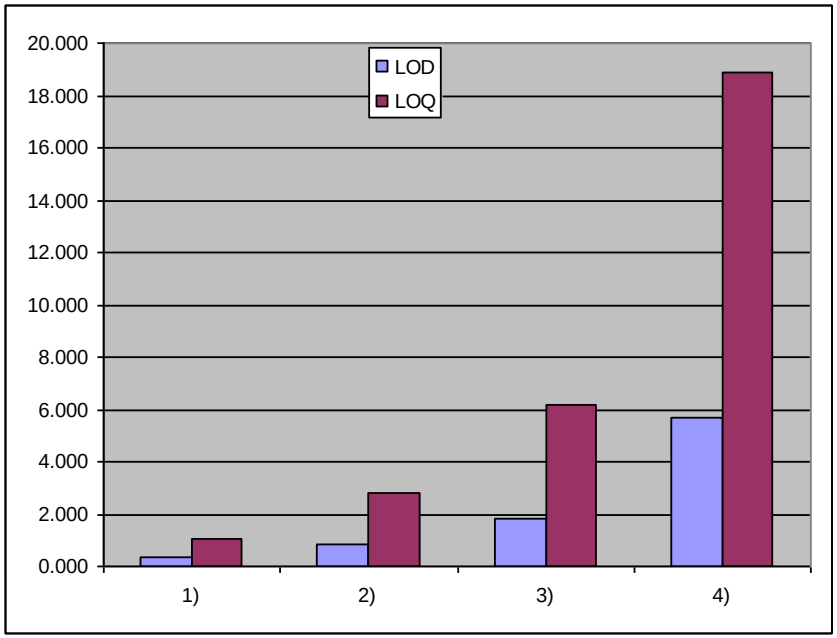
Examples of LOD's, LOQ's (3)



Zinc Zn				
	Blank Aqua Regia not digested	Blank Aqua Regia digested	Soil	Sediment
	1)	2)	3)	4)
	0.0529	0.3485	7.246	41.5
	0.0472	0.2777	7.586	41.3
	0.0492	0.1832	7.762	44.3
	-0.1625	-0.0891	7.606	42.5
	-0.2360	0.0506	7.615	40.3
	-0.0545	-0.0954	7.766	40.9
	-0.0353	0.2502	6.384	41
	-0.0470	0.4282	6.448	46.2
	-0.0361	0.9224	6.395	42
	-0.2667	0.4192	6.604	
	-0.0325	0.0952	6.488	
	-0.0301	0.0611	6.365	
stdev	0.1059	0.2795	0.6167	1.8900
LOD	0.318	0.838	1.850	5.670
LOQ	1.059	2.795	6.167	18.900

1. blank Aqua regia: direct measurement, reproducibility conditions
2. blank Aqua Regia destruct: blank samples whole procedure, reproducibility conditions
3. soil: low concentration spiked on sand (organic matter< 2%); repeatability conditions
4. Sediment: low concentration spiked on sediment (organic matter ~ 12%); reproducibility conditions

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Measurement uncertainty

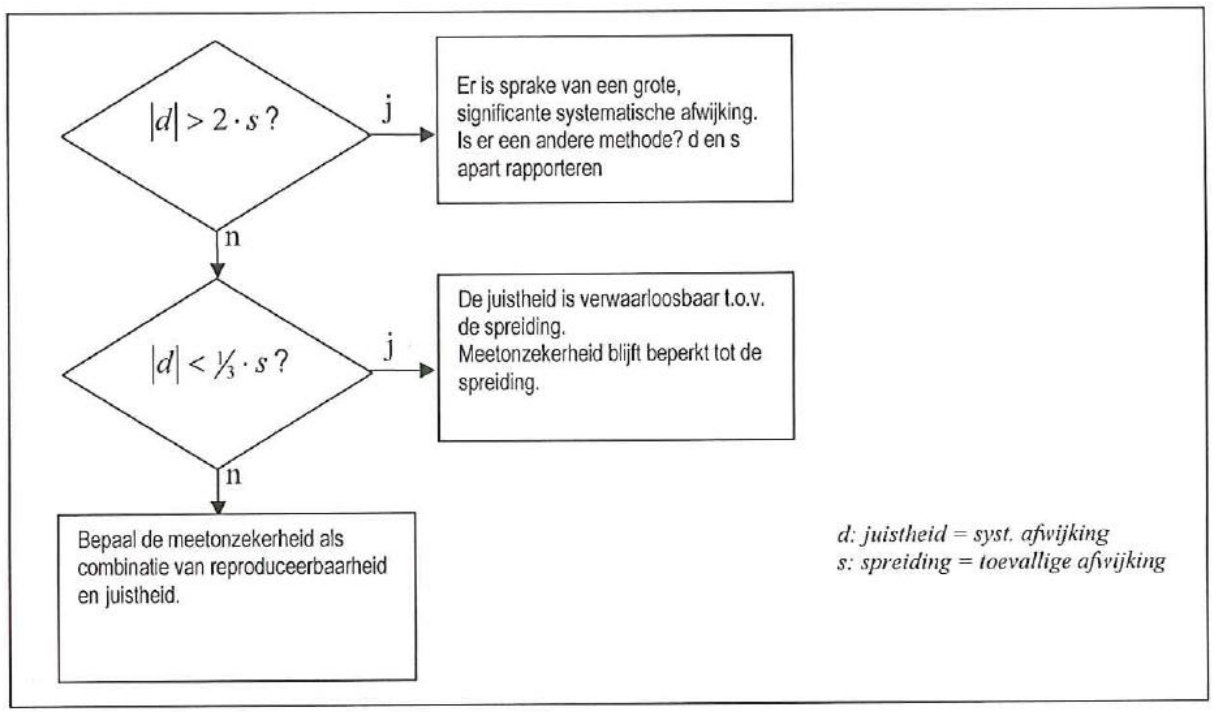
Measurement uncertainty (1)

Is based on:

- reproducibility
- Trueness / Recovery
- Non represented sources
- The whole process is relevant, inclusive sample pretreatment

Measurement uncertainty (2)

NEN 7779 (NL): $U = 2u_c$ $\quad u_c = RMS = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \mu_i)^2} \cong \sqrt{s^2 + \delta^2}$



U	= extended measurement uncertainty
u	= measurement uncertainty
RMS	= Root Mean Square
s	= random error
δ	= systematical error

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Measurement uncertainty (3)

29.4-4´-DDD	Suelo	Código-067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	9,7
32.4-4´-DDT	Suelo	Código-067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	10,7
33.Endosulfan sulfato	Suelo	Código-067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	15,4
50.HCB	Suelo	Código 067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	20,9
51.Fenol	Suelo	Código-067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	8,6
52.2-clorofenol	Suelo	Código-067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	17,9
56.2,4-diclorofenol	Suelo	Código-067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	6,7
59.2,4,5-Triclorofenol	Suelo	Código-067	EPA 8270: 1996	GC/MS	1 - 2500 mg/kg	6,8

An example from one of the European countries …

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Measurement uncertainty (4)

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Reporting Limit | Systematical Error | Random Error | Measurement Uncertainty

Analyse	Monstersoort	LOQ	CAS #	Sytematische Fout (%)	Toevallige Fout (%)	Meetonzekerheid
droge stof	Grond (AS3000)	-		2	2	8%
naftaleen	Grond (AS3000)	0.01	91-20-3	11	26	58%
acenaftyleen	Grond (AS3000)	0.02	208-96-8	12	18	43%
acenafteen	Grond (AS3000)	0.02	83-32-9	10	28	60%
fluoreen	Grond (AS3000)	0.02	86-73-7	10	39	78%
fenantreen	Grond (AS3000)	0.01	85-01-8	12	32	67%
antraceen	Grond (AS3000)	0.01	120-12-7	10	29	62%
fluoranteen	Grond (AS3000)	0.01	206-44-0	11	24	53%
pyreen	Grond (AS3000)	0.02	129-00-0	11	22	49%
benzo(a)antraceen	Grond (AS3000)	0.01	56-55-3	10	22	48%
chryseen	Grond (AS3000)	0.01	218-01-9	7	24	49%
benzo(b)fluoranteen	Grond (AS3000)	0.02	205-99-2	10	24	51%
benzo(k)fluoranteen	Grond (AS3000)	0.01	207-08-9	10	24	51%
benzo(a)pyreen	Grond (AS3000)	0.01	50-32-8	9	24	50%
dibenz(a,h)antraceen	Grond (AS3000)	0.02	53-70-3	11	24	53%
benzo(ghi)peryleen	Grond (AS3000)	0.01	191-24-2	11	25	54%
indeno(1,2,3-cd)pyreen	Grond (AS3000)	0.01	193-39-5	10	33	66%

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Measurement uncertainty (5)

- Fenelab (Dutch Federation of Labs)
  - Appointments made with all labs (June 2010)
  - Advice:
    - Estimate measurement uncertainty based on measurements of duplicate sub-samples (if necessary one or both spiked with analytes) or random real samples under reproducibility conditions over longer periods.
    - Matrix soil: establish the systematical standard deviation preferably with the Dutch reference sample PD152/SETOC 764, if analytes are present.
    - Report the measurement uncertainty as the extended measurement uncertainty (U), (only analysis or analysis plus sampling).
    - Extra information about the systematical and random deviation can be provided at the request of clients.

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Measurement uncertainty (6)

- Appointments needed on
  - Correction for internal standard
  - ➔ can have considerable influence on measurement uncertainty (70% recovery can be changed to 100% recovery, so systematical error decreases from 30 to 0%)

Conclusions

- LOD, LOQ procedures differ from country to country
  - some based on blank samples, some on samples with low spikes
  - some based on spike to blank, some on spike to matrix samples
  - some based on repeatability, others on reproducibility tests
  - some use LOD for reporting limit, others LOQ

  - An international procedure, by which LOD's and LOQ's of all countries can be compared, is lacking!

  - ➔ An international procedure is urgently necessary!

- Measurement uncertainty
  - International agreements are necessary
  - Clear appointment is required for dealing with correction for the recovery of internal standards

Theses

- In Europe there are as many LOD's claimable as there are EU countries
- An international standard for establishing LOD/LOQ is required for:
  - Lab's acting in more than 1 EU country, and/or
  - Any laboratory carrying out tests related to EU legislation

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