WETENSCHAPPELIJK INSTITUUT VOLKSGEZONDHEID
INSTITUT SCIENTIFIQUE DE SANTÉ PUBLIQUE

Scientific institute of public health ï dept. quality of medical laboratories

STABILITY OF TEST MATERIALS A CHALLENGE TO FIND THE BALLANCE

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ITS ALL ABOUT QUALITY

CLINICIANS ï patient care

↓

---------- **QUALITY OF PATIENT CARE** ----------

↗ ↑ ↖

LABORATORIES - IVD (manufacturers) - EQA schemes

QUALITY CONTROL - EDUCATIONAL ROLE - STIMULUS

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EQA PROGRAMS - THE PLAYERS

- *EQAS ORGANIZERS*
- *PARTICIPANTS = LABORATORIES*
- *EQUIPEMENT/REAGENTS = MANUFACTURERS*

➔ ALL PLAYERS AFFECT THE RESULTS OF EQA SURVEYS

EQAS ORGANIZERS

- CHOICE OF CONTROL MATERIAL
- CONCENTRATION RANGES
- FIXATION OF EVALUATION CRITERIA - (U-scores, Z-scores, é)
- PARTITION OF METHODS AND METHOD GROUPS/PEERS
  - MEASURING PRINCIPLE
  - MANUFACTURER
  - é é é é é

LABORATORIES

CONFIDENCE IN :

SCIENTIFIC VALIDITY ➔ material

RELIABILITY ➔ reporting

➔ EQA MATERIAL NEEDS QUALTITY CRITERIA

ñIdeal samplesò for a PT/EQA program would fulfill a range of criteria:

- **Stable** for the conditions under which they will be stored ➔ shipped ➔ stored (labs)
- **Homogeneous** across all the aliquots produced
- Analyte concentrations must include the expected **clinical range**
- Appropriate sample **types** (e.g., urine, whole blood, serum, tissue,é)
- **Available** in sufficient volume
- **Inexpensive** enough for cost not to be an impediment
- Behave in clinical laboratory measurement procedures in the **same manner** as patient samples.

EQA - schemes

ISO/IEC 17043 (4.4.3): Homogeneity and stability

Criteria for suitable homogeneity and stability shall be established and based on their effects on the evaluation of the participantsô performance.

- *Note 1: testing for confirmation of stability and homogeneity*
- *Note 2: not always possible*
- *Note 3: insufficient homogeneous or stable by nature*
- *Note 4: more details in ISO Guide 34, ISO Guide 35, and* ***ISO 13528***
  ñStatistical methods for use in proficiency testing by interlaboratory comparisonsò

EQA ï schemes

Homogeneity and stability:

- ***Procedures*** *shall be documented*
- ***Criteria*** *shall be established*
- ***Stability*** *must be ensured throughout*
  - *the* ***storage*** *period of the material*
  - ***shipping*** *of the material*
  - *the* ***conduct*** *of proficiency testing*

EQA - schemes

Stability of material:

é Criteria shall be establishedé ..
*in function of the **clinical relevance** of the analyte and the evaluation criteria of the EQA scheme.*

quantitative ï semi quantitative ï qualitative - ...)

nature of the analyte/matrix : fresh & unstable, dispersion, liquid, lyophilized,é ..stabilizedé .., tissues

ñIdeal samplesò for a PT/EQA program would fulfill a range of criteria:

- **Stable** for the conditions under which they will be stored ➔ shipped ➔ stored (labs)
- **Homogeneous** across all the aliquots produced
- Analyte concentrations that include the expected **clinical range**
- Appropriate sample **types** (e.g., urine, whole blood, serum, tissue,é)
- **Available** in sufficient volume
- **Inexpensive** enough for cost not to be an impediment
- Behave in clinical laboratory measurement procedures in the **same manner** as patient samples.

In practice, it is not always possible to achieve all these goals and criteria.

➔ for the preparation of EQA materials, some compromises will be necessary.

ASPECTS TO TAKE IN TO CONSIDERATION

It is usual to assess both homogeneity and stability by manufacturing and testing samples additional to those required for distribution to participants. Regardless of the extent of homogeneity and stability testing, it can be expensive, and it uses samples that could be used for other purposes. Expense can be of critical importance if either the sample or the test method is expensive or time intensive, especially if the number of participants is small. The issue is further complicated when the PT provider itself does not have the analytical capability to test the samples and where the laboratories that do have the capability are participants in the PT study. The supply of samples can be critical where access to them is difficult.
There may be situations where the risk of inhomogeneity or instability is acceptably low, and the potential cost associated with this risk is less than the actual cost of performing the necessary homogeneity and stability testing.
It is also important to consider the effectiveness of homogeneity and stability testing. Usually only a small proportion of samples from an entire lot of production are tested. It is therefore possible that the tests will fail to detect problems that exist in a portion of the total number of samples. Thus a proficiency testing sample may actually be unacceptable, even though it has not been demonstrated by the recommended homogeneity and stability testing

Case by case approach ➔ type of survey : qualitative ï quantitative

EQA - Stability

Stability:

Procedures shall be documented ➔

- Type of sample*: fresh - liquid ï lyophilized*
- Type of analyte; *quantitative ï semi quantitative*
- Home made samples: *(long term) storage / no storage*
- Commercial samples*: liquid - lyophilized*

STABILITY

ORIGINE	TYPE	STABILITY	VALIDATION
COMMERCIAL	LYOPHILIZED	Evaluated by the manufacturer	Certified by manufact. **??**
	LIQUID/FROZEN	Evaluated by the manufacturer *(cave transport conditions)*	Certified by manufact. **??**
HOME MADE	LIQUID STORED Fresh / Frozen	Process validation ï (validation on historical data)	Historical validation
	LYOPHILIZED	Process validation ï (validation on historical data)	Historical validation
	(LIQUID) FRESH No storage	Testing at start and closing date of the survey.	Cfr. Literature Historical validation
	Other matrixes (e.g. sperm, germs ,é	Evaluation of stability on historical data.	Cfr. Literature Historical validation
THIRD PARTY (ex. Collaborating labs, expertsé ..	Procedures must be approved by the EQAS organizer		

PROCEDURES for STABILITY testing
ex. during conducting period

According to **ISO 13528 (Annex B.4), -** *testing over the time delay cfr. EQA scheme* - validation should be performed on the first day (start of the EQA period), and the closing date.

➔ **send ñgò sets of samples to the laboratory (g Ó3)**

(who also performed homogeneity testing)

ñ2gò*sets of samples should be prepared and analyzed at random under repeatability conditions.*

Which analytes? All ?? Which criteria ??

Economical equilibrium ??

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DIFFERENT SURVEYS ARE DISCUSSED :

TYPE OF THE ANALYTES:

- Å Quantitative results
- Å Qualitative results

NATURE OF THE SAMPLE:

- Å Fresh unstable
- Å Liquid relatively stable

For all surveys, samples are prepared in bulk, aliquoted into vials and shipped to the participants

STABILITY TESTING for ETHANOL

QUANTITATIVE PARAMETER ï RELATIVELY STABLE

Human plasma pool, spiked with alcohol, 5 different concentrations, aliquoted and shipped to the participants

EQA ➔ STARTS AT DAY 1 ï ENDS AT DAY 14

Homogeneity (N=10x2) and stability (N=3x2) are tested conform ISO 17043:10 & ISO 13528:2005

STABILITY TESTING FOR ETHANOL (g/L) ï ISO 13528

| $\bar{X}_s$.,.,. ï $\bar{Y}_h$.,.,. | Ò 0.3 σ sd for proficiency assessment (??)

SAMPLE	STABILITY $\bar{X}$	HOMOGEN. $\bar{Y}$	DIFF $\bar{X} - \bar{Y}$	DIFF/0.3	Lowest - SD survey (σ_L)	Highest - SD survey (σ_H)
E/13029	0.762	0.737	0.025	0.083	0.012	0.030
E/13030	2.683	2.708	-0.024	-0.081	0.037	0.215
E/13032	1.533	1.510	0.023	0.078	0.026	0.096
E/13033	1.347	1.300	0.047	0.157	0.030	0.074
E/13034	0.375	0.367	0.008	0.028	0.007	0.030

N= 6 res./sample

N= 20 res./sample

? ?

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STABILITY TESTING FOR ETHANOL (g/L)

WHICH CRITERIA ??

$\bar{X}$,,. Ò MED ±3 SD°

$\bar{X}$,,. Ò MED ± d_{TAE}

(d = fixed limit = 15%)

SAMPLE	STABILITY $\bar{X}$	SD of Survey for METHOD*	MEDIAN of Survey for METHOD*	Lower Limit CI	Higher Limit CI	MEDIAN – 15%	MEDIAN + 15%
E/13029	0.762	0.026	0.745	0.71	0.78	0.633	0.854
E/13030	2.683	0.215	2.625	2.36	2.89	2.231	3.019
E/13032	1.533	0.096	1.495	1.38	1.61	1.271	1.719
E/13033	1.347	0.067	1.320	1.24	1.40	1.122	1.518
E/13034	0.375	0.030	0.375	0.34	0.41	0.319	0.431

° MED - 3(sd/ √6)*

° MED + 3(sd/ √6)*

**Method-group used by the laboratory who tested for stability and homogeneity - Gas Chromatography*

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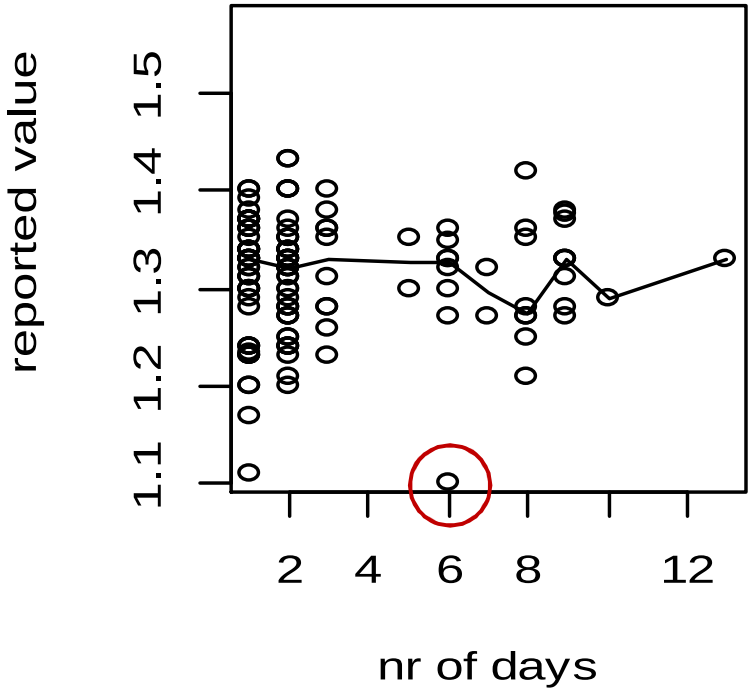
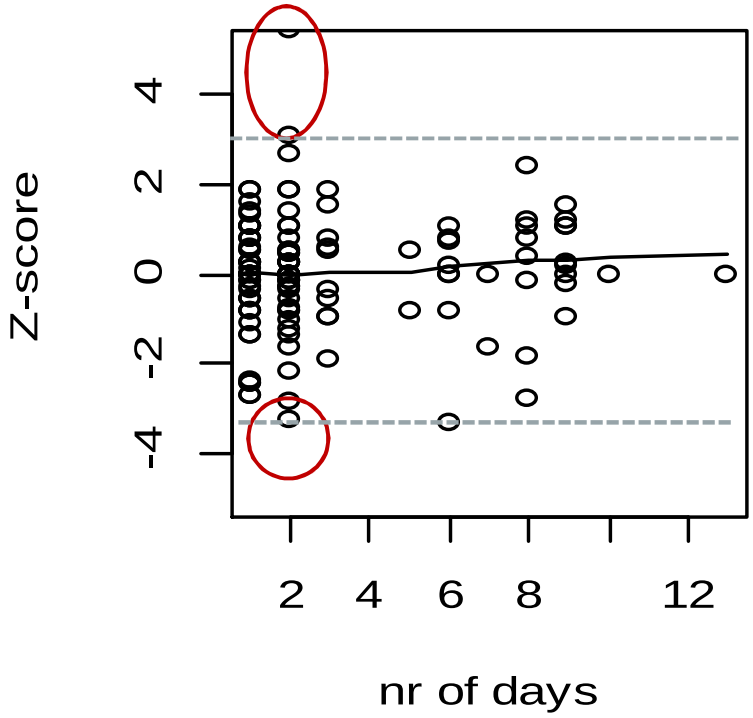
ETHANOL SAMPLE E/13033

ALL METHODS / ALL RESULTS





EVALUATION OF Z-FLAGS OVER THE CONDUCTION PERIOD

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13
N	46	43	10	0	2	8	2	8	9	1	0	0	1
Median	1.33	1.32	1.33		1.32	1.32	1.29	1.27	1.33	1.29			1.33
SD	0.06	0.05	0.06		0.02	0.03	0.02	0.06	0.04	0			0
Median Z-score	0	-0.03	0.09		-0.17	0.07	-0.82	0.54	0.25	0			-0.03
Frequency of Z-flags	2.17	11.63	0		0	12.5	0	0	0	0			0
Total of Z-flags	1	5	0		0	1	0	0	0	0			0

STABILITY VALIDATED ??

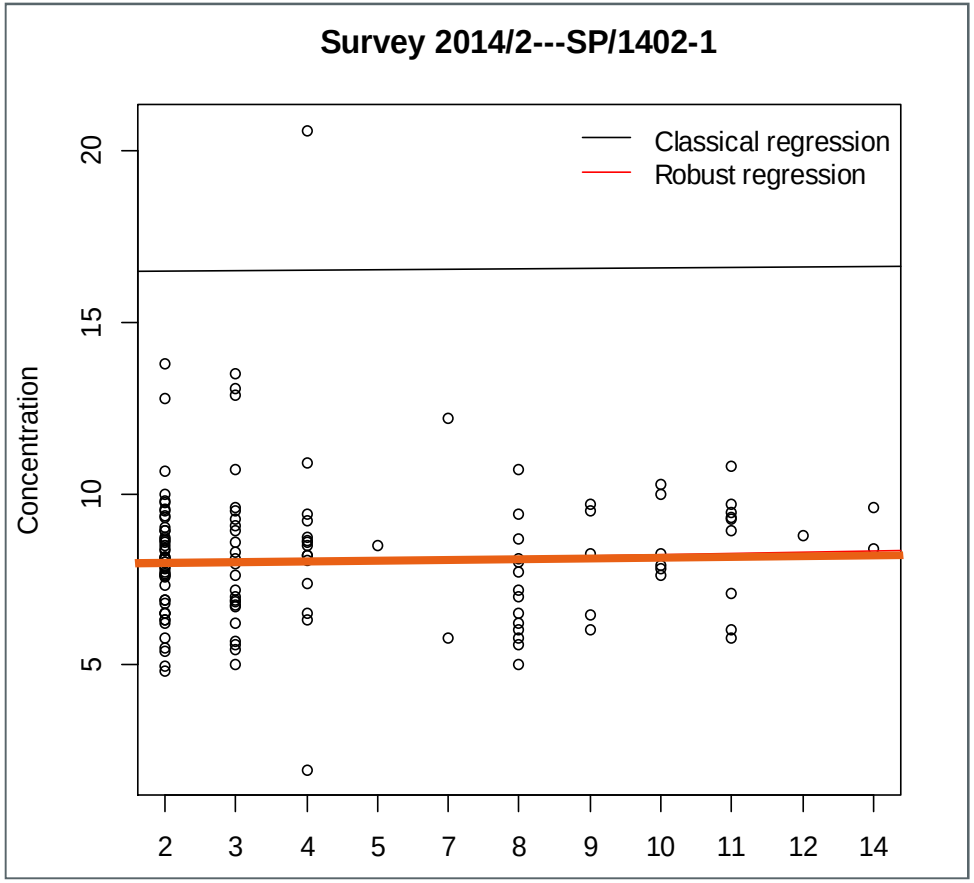
STABILITY TESTING
for
SPERM ANALYSIS
Morphology and Counting

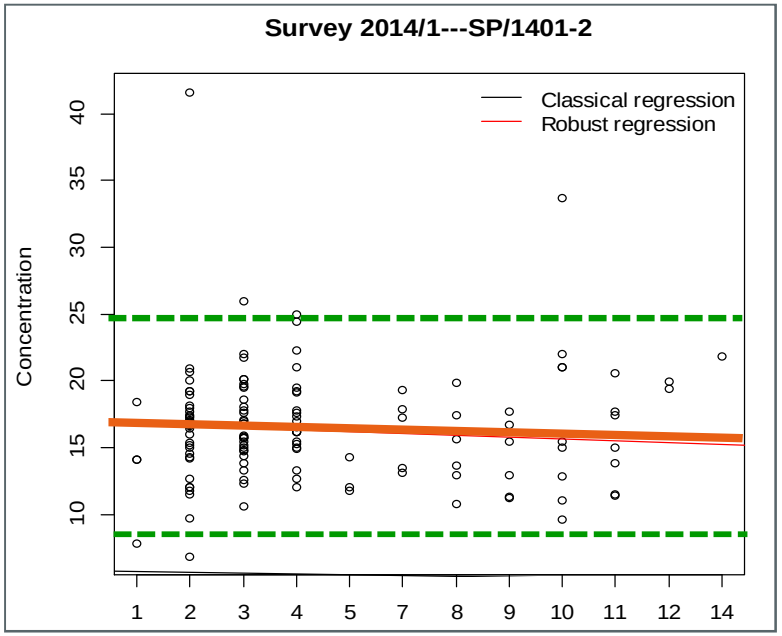
HISTORICAL PROCESS VALIDATION

By means of « Robust Regression Analysis » of survey results

EQA scheme (N~130)

Collection of samples ➔ Aliquoting ➔ Shipping
EQA starts at Day 1 ï ends at Day 14


Survey 2014/2---SP/1402-1
Classical regression
Robust regression
Concentration
5
10
15
20
2
3
4
5
7
8
9
10
11
12
14


Survey 2014/1---SP/1401-2
Classical regression
Robust regression
Concentration
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25
30
35
40
1
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4
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7
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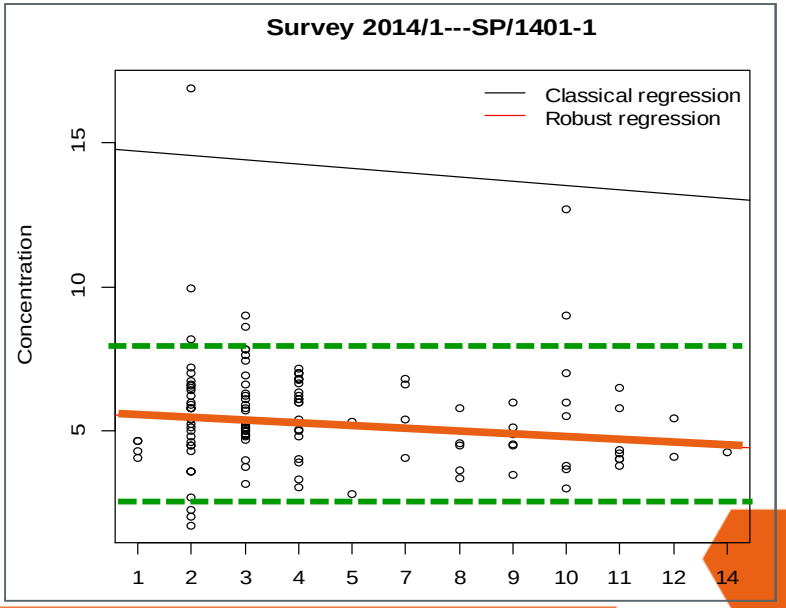
11
12
14


Survey 2014/1---SP/1401-1
Classical regression
Robust regression
Concentration
5
10
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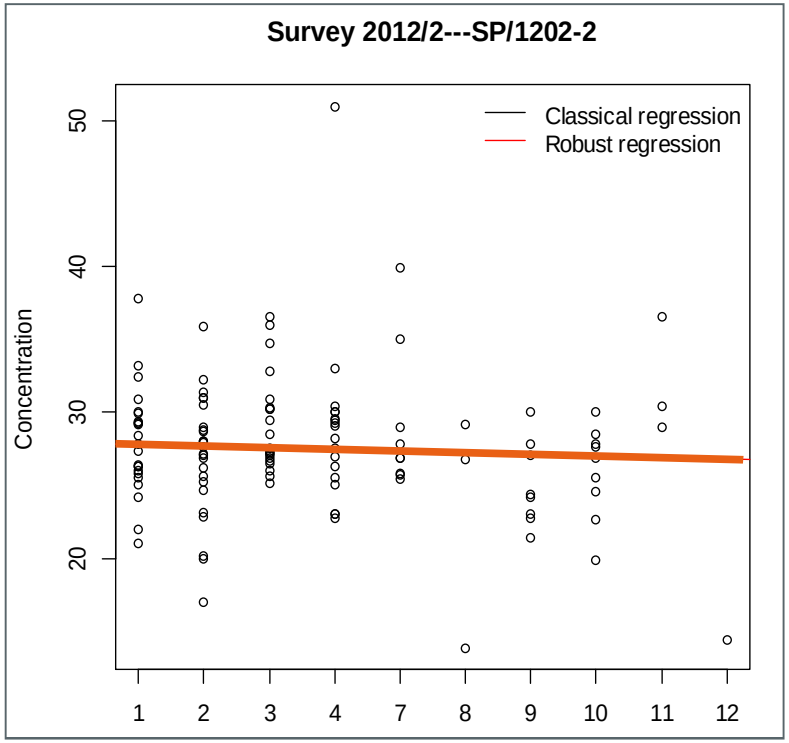
11
12
14


Survey 2012/2---SP/1202-2
Classical regression
Robust regression
Concentration
20
30
40
50
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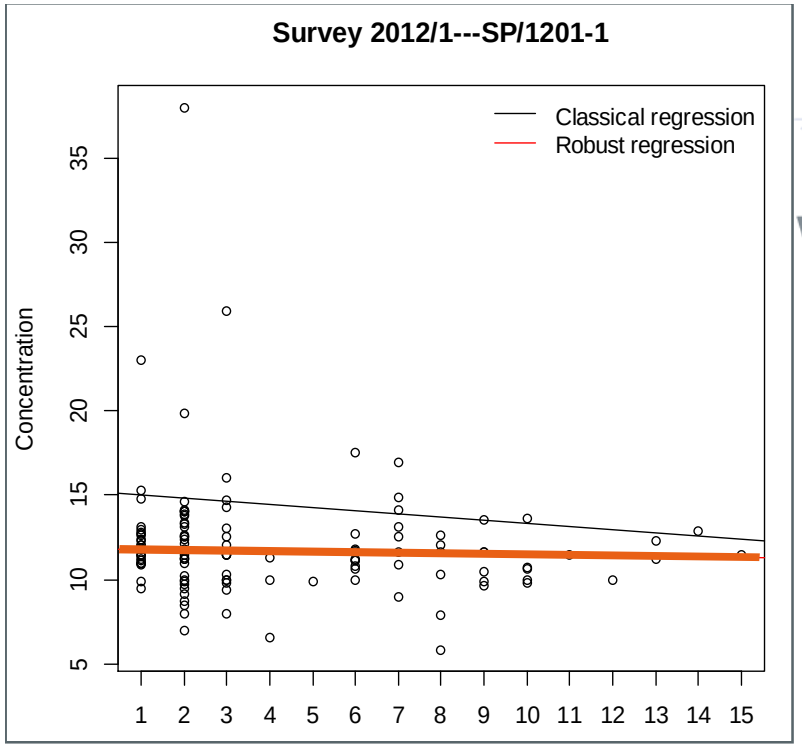
11
12


Survey 2012/1---SP/1201-1
Classical regression
Robust regression
Concentration
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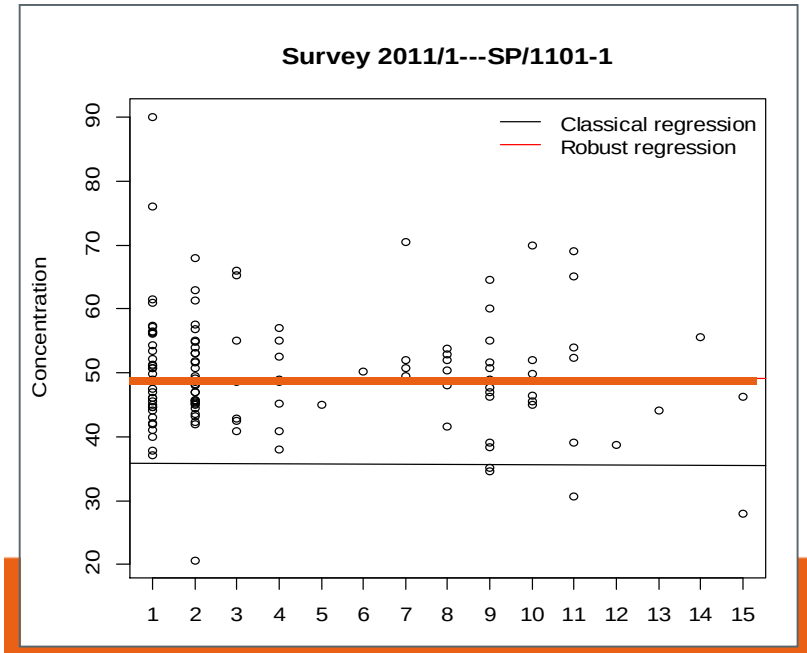
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Classical regression
Robust regression
Concentration
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60
70
80
90
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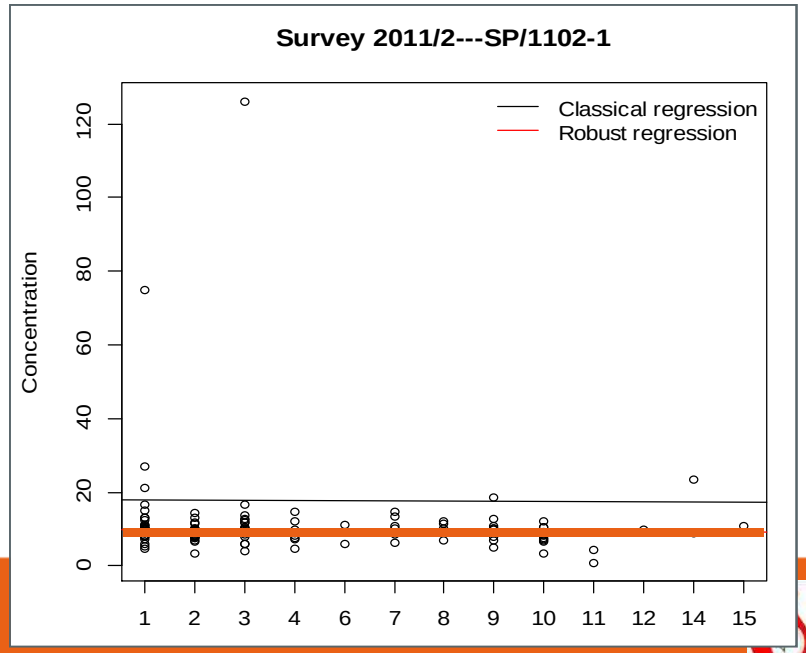
13
14
15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Classical regression
Robust regression
Concentration
0
20
40
60
80
100
120
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12
14
15

STABILITY TESTING IN

HAEMATOLOGY-COUNTING & IMMUNO-HAEMATOLOGY

Freshly prepared samples ï single donations

- QUALITATIVE RESULTS (Immuno-haematology)[1]
- QUANTITATIVE RESULTS (Haematology-Counting)[2]
- N ~ 200
- SPECIAL DELIVERY *(TAXI POST - within 24h delivery)*

EQA ➔ [1] STARTS AT DAY 1 - ENDS AT DAY 14
[2] STARTS AT DAY 1 - ENDS AT DAY 2

STABILITY TESTING IN IMMUNO-HAEMATOLOGY

➔ Blood group typing and cross matching (Qualitative survey)

MATERIAL: 3 bulk preparations of freshly prepared serum
2 bulk preparations of freshly prepared RBC

➔ single donations

For stability testing, all the requested parameters are tested <u>before shipping</u> and <u>after closing</u> date of the survey.

CRITERIA ➔ Results must be <u>in agreement with original data</u>

STABILITY TESTING IMMUNO-HEMATOLOGY
BLOOD GROUP TYPING EQA 2014

RBC *SAMPLE*	KNOWN (patient) RESULTS		RESULTS			
			on shipping date *17-03-2014*		on closing date *31-03-2014*	
	RBC 1 *I /1402*	RBC 2 *I /1404*	RBC 1 *I /1402*	RBC 2 *I /1404*	RBC1 *I /1402*	RBC 2 *I /1404*
BLOOD TYPE	0	0	**0**	0	**0**	0
D-FACTOR	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE
RHESUS SUBTYPES	CcEe K-	CCee K+	CcEe K-	CCee K+	CcEe K-	CCee K+
RAGT	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
OTHER BLOOD TYPES: Fy^a	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE

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STABILITY TESTING IMMUNO-HEMATOLOGY ANTIBODY INDENTIFICATION EQA 2014

SAMPLES	RESULTS for antibody identification / TITRATION		
	TECHNIQUE	**Before shipping date** *14-03-2014*	**On closing date** *31-03-2014*
Serum 1 I / 1401	Albumin ï AGT 37° C	Anti-K (16) + Anti-Fy^{a} (16)	Anti-K (16) + Anti-Fy^{a} (8)
	LISS-Coombs phase 37° C Bio-Rad	Anti-K (16) + Anti-Fy^{a} (32)	Anti-K (16) + Anti-Fy^{a} (32)
Serum 2 I / 1403	Albumin ï AGT 37° C	**NEGATIVE**	**NEGATIVE**
	LISS-Coombs phase 37° C Bio-Rad	**NEGATIVE**	**NEGATIVE**
Serum 3 I / 1405	Albumin ï AGT 37° C	**Anti-D (4)**	**Anti-D (4)**
	LISS-Coombs phase 37° C Bio-Rad	**Anti-D (8)**	**Anti-D (16)**

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STABILITY TESTING IMMUNO-HEMATOLOGY CROSSMATCHING EQA 2014

CROSSMATCH	TECHNIQUE	RESULTS	
		on shipping date *17-03-2014*	**on closing date** *31-03-2014*
Serum 1 + RBC 1 (I / 1401 + I / 1402)	20° C Salt phase	**NEGATIVE**	**NEGATIVE**
	Albumin ï AGT 37° C	**POSITIVE**	**POSITIVE**
	LISS-Coombsôphase 37° C Bio-Rad	**POSITIVE**	**POSITIVE**
Serum 1 + RBC 2 (I / 1401 + I / 1404)	20° C Salt phase	**NEGATIVE**	**NEGATIVE**
	Albumin ï AGT 37° C	**POSITIVE**	**POSITIVE**
	LISS-Coombsôphase 37° C Bio-Rad	**POSITIVE**	**POSITIVE**

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STABILITY TESTING IMMUNO-HEMATOLOGY CROSSMATCHING EQA 2014

CROSSMATCH	TECHNIQUE	RESULTS	
		on shipping date *17-03-2014*	**on closing date** *31-03-2014*
Serum 2 + RBC 1 (I / 1403 + I / 1402)	20° C Salt phase	**NEGATIVE**	**NEGATIVE**
	Albumin ï AGT 37° C	**NEGATIVE**	**NEGATIVE**
	LISS-Coombsôphase 37° C Bio-Rad	**NEGATIVE**	**NEGATIVE**
Serum 2 + RBC 2 (I / 1403 + I / 1404)	20° C Salt phase	**NEGATIVE**	**NEGATIVE**
	Albumin ï AGT 37° C	**NEGATIVE**	**NEGATIVE**
	LISS-Coombsôphase 37° C Bio-Rad	**NEGATIVE**	**NEGATIVE**

STABILITY TESTING IN HAEMATOLOGY - COUNTING

➔ **Quantitative survey ï evaluation of Z- and U-scores, based on consensus results of the participants by method**

MATERIAL: 2 bulk preparations of fresh prepared EDTA-blood, aliquoted and shipped on the same day

➔ **Fresh single donations**

To avoid deterioration of the material (fresh blood), samples must be analyzed by the participants within 2 days after arrival.

Date of analysis must be reported.

HAEMOGLOBIN (g/L) ï TL (d) = 4% EQA 2013/3

METHODS	Median	SD	CV(%)	N
055 Abbott Cell-Dyn 3200	*121 125*			2
042 Abbott Cell-Dyn Ruby	119	4	3.7	6
040 Abbott Cell-Dyn Sapphire	125	1	0.9	11
015 ABX Pentra/Octra	*123*			1
150 Beckman Coulter LH 500/750/755/780	120	2	1.9	19
200 Beckman Coulter Unicel DxH 800	120	1	1.2	13
074 Siemens Advia 120/2120/2120i	123	2	1.8	38
064 Sysmex KX 21	*121*			1
073 Sysmex pocH-100i	*120 120 125*			3
067 Sysmex XE 2100(D)/XE-alpha/HST 430/XE 5000	121	1	1.2	46
233 Sysmex XN 1000/XN 2000/XN 3000/XN 9000	122	1	1.2	19
234 Sysmex XP300	*121*			1
231 Sysmex XS 1000i/XS 800i	120	0		9
060 Sysmex XT 2000i/XT 1800i/XT 4000i	121	1	1.2	28

GLOBAL RESULTS ALL METHODS	**121**	**2**	**1.7**	**197**

EQA 2014/1

GLOBAL RESULTS ALL METHODS	**130**	**1**	**1.1**	**200**

EQA 2013/3	Citations Day 1	Non-citations Day 1	% Citations Day 1	Citations Day 2	Non-citations Day 2	% Citations Day 2	Corrected P-value
RBC	7	355	1.9	1	27	3.6	0.992
WBC	13	351	3.6	0	28	0	0.999
HB	13	340	3.7	0	28	0	0.999
HCT	12	352	3.3	0	28	0	1
MCV	18	342	5	4	24	14.3	0.411
THROMB	6	356	1.7	0	28	0	1
RETICUL	5	307	1.6	0	28	0	1
RETICUL NBR	15	295	4.8	1	27	3.6	1

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EQA 2014/4	Citations Day 1	Non-citations Day 1	% Citations Day 1	Citations Day 2	Non-citations Day 2	% Citations Day 2	Corrected P-value
RBC	4	350	1.1	0	38	0	**1**
WBC	5	349	1.4	3	37	7.5	**0.266**
HB	13	341	3.7	0	38	0	**1**
HCT	4	350	1.1	2	38	5	**0.627**
MCV	1	351	0.3	2	36	5.3	**0.19**
THROMB	8	346	2.3	1	37	2.6	**0.999**
RETICUL	9	309	2.8	0	32	0	**1**
RETICUL NBR	12	304	3.8	2	30	6.2	**0.977**

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- DIFFERENT SURVEYS ASK DIFFERENT EVALUATION METHODS AND CRITERIA

- WHENEVER POSSIBLE, STATISTICS SHOULD BE USED, HOWEVER COMMON SENSE MAY AVOID TO INCORRECTLY DISCARD (valuable / expensive) SURVEY RESULTS

- IF POSSIBLE, STABILITY OF SAMPLES (processes) CAN BE VALIDATED BASED ON HISTORICAL DATA, IN ORDER TO MAINTAIN AN ECONOMICAL EQUILIBRIUM BETWEEN BENEFIT AND EXPENSES

URINE	Price (ú)/test	Nbr. tests	Prelimin. results *DRUGS OF ABUSE*			
			DOA_1	DOA_2		Units
Opiates	45,66	6*	negative	morphine	2512,3	µg/L
				oxycodone	139,2	µg/L
Methadone	45,66	6*	negative	methadone	676,2	µg/L
Cocaine	45,66	6*	negative	benzoylecgonine	4112,6	µg/L
Amphetamines	45,66	6*	negative	amphétamine	746,9	µg/L
				methamphetamine	1968,3	µg/L
				MDMA	807,2	µg/L
Cannabis	45,66	6*	negative	THC-COOH	86,48	µg/L
Barbiturates	45,66	6*	negative	secobarbital	0,90	mg/L
Benzodiazepines	45,66	6*	negative	nordiazepam	2,35	mg/L
				oxazepam	0,82	mg/L
Tricyclic anti depressants	45,66	6*	negative	nortriptyline	1,92	mg/L

*: 3x2 tests for stability validation ➔ **ú 2190,00** JUSTIFIED ??

HOW ABOUT COMMERCIAL QC MATERIAL

- WHAT CAN/SHOULD BE EXPECTED FROM THE MANUFACTURERS OF QC-MATERIAL ??

- VALIDATION OF HOMOGENEITY ?

➔ WHAT INFORMATION SHOULD BE PROVIDED TO THE EQA ORANIZERS ?

- VALIDATION OF STABILITY ?

➔ WHAT INFORMATION SHOULD BE PROVIDED TO THE EQA ORANIZERS ?

CERTIFICATE-OF-ANALYSIS

Product Name:

Master Lot Number:

Product Base: Human Serum

Physical Form: Liquid

Date of Manufacture: 2012-08

Description	Catalog Number	Lot Number	Expiration Date
Level 1			2015-08-31
Level 2			2015-08-31
Level 3			2015-08-31

Testing Results:

Each human donor unit used to manufacture this product was tested and found non-reactive at the donor level per current applicable FDA requirements using FDA-accepted methods including testing for HIV-1/HIV-2 antibody, HBsAg, HCV Antibody, HIV-1 and HCV by a nucleic acid test (NAT).

The bovine source material(s) of this product is collected in USDA licensed establishments. These animals received ante and post mortem inspections at the abattoir by a US veterinary service inspectors.

This product has been manufactured under applicable guidelines/standards and meets all established requirements

Homogeneity Claim:

ñTesting has been conducted to verify sufficient homogeneity in accordance with established requirements

Mr Abc — 10/23/2012

(QC Supervisor/Manager)

Mrs Xyz — 3/14/13

(QA Supervisor/Manager)

QUALITY ASSURANCE TJ DOCUMENT CONTROL

Effective Date: March 21, 2012 / Supersedes: Doc # 330-63.26 Rev. # 4

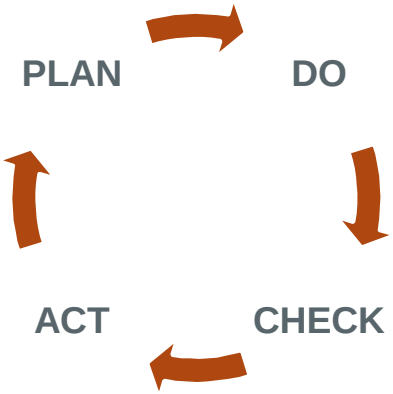
DIFFERENT APPROACHES/PROCEDURES ARE NECESSARY

NO ñONE SIZE FITS ALLò EXISTS

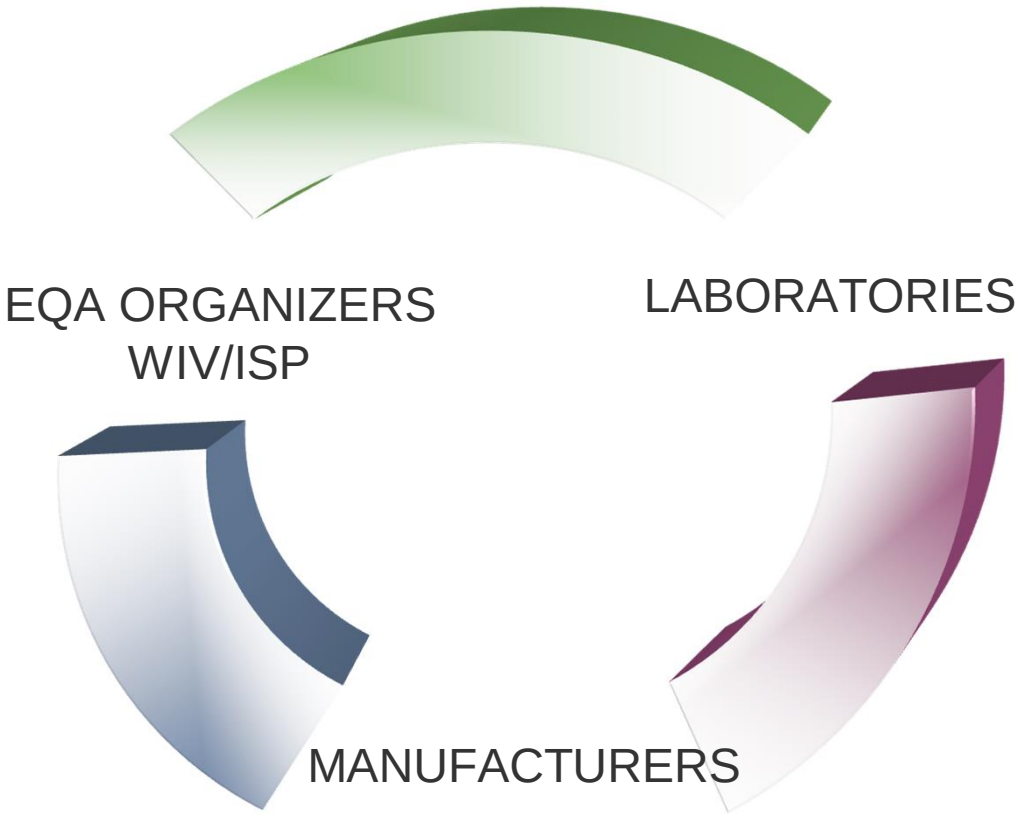
STATISTICS ARE NECESSARY BUT EVEN SO IS COMMON SENSE

KEEP IN CONTACT WITH MANUFACTURERS TO GET THEM ON THE SAME (ISO) LEVEL TO PROVIDE THE EQA ORGANIZER THE NECESSARY INFORMATION TO ENSURE SCIENTIFIC VALIDITY OF THE MATERIAL

REMEMBER DEMING :



EQA is not just statistics !



Evaluation & Communication ➔ Amelioration of quality for all parties concerned ➔ OPTIMAL PATIENT CARE !

LABORATORIES & EQAS

Evaluation & Communication ➔ Amelioration - Education - Amelioration

CONFIDENCE IN :

SCIENTIFIC VALIDITY ➔ material

RELIABILITY ➔ reporting

➔ EQA MATERIAL NEEDS QUALTITY CRITERIA

Having confidence is good, to check your confidence is betteré

Thanks to:

- China Bernard (EQA – Andrology)
- Coucke Wim (EQA – Ethanol - Statistics)
- Soumali Rida (Statistics)
- Van Blerk Marjan (EQA – Haematology-Immuno-Haematology)

- EQALM SYMPOSIUM 2014 Organizers