Overview of the ESP Lung and Colon EQA schemes with a focus on Dutch laboratories

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SKML Pathology day
Amersfoort, NL

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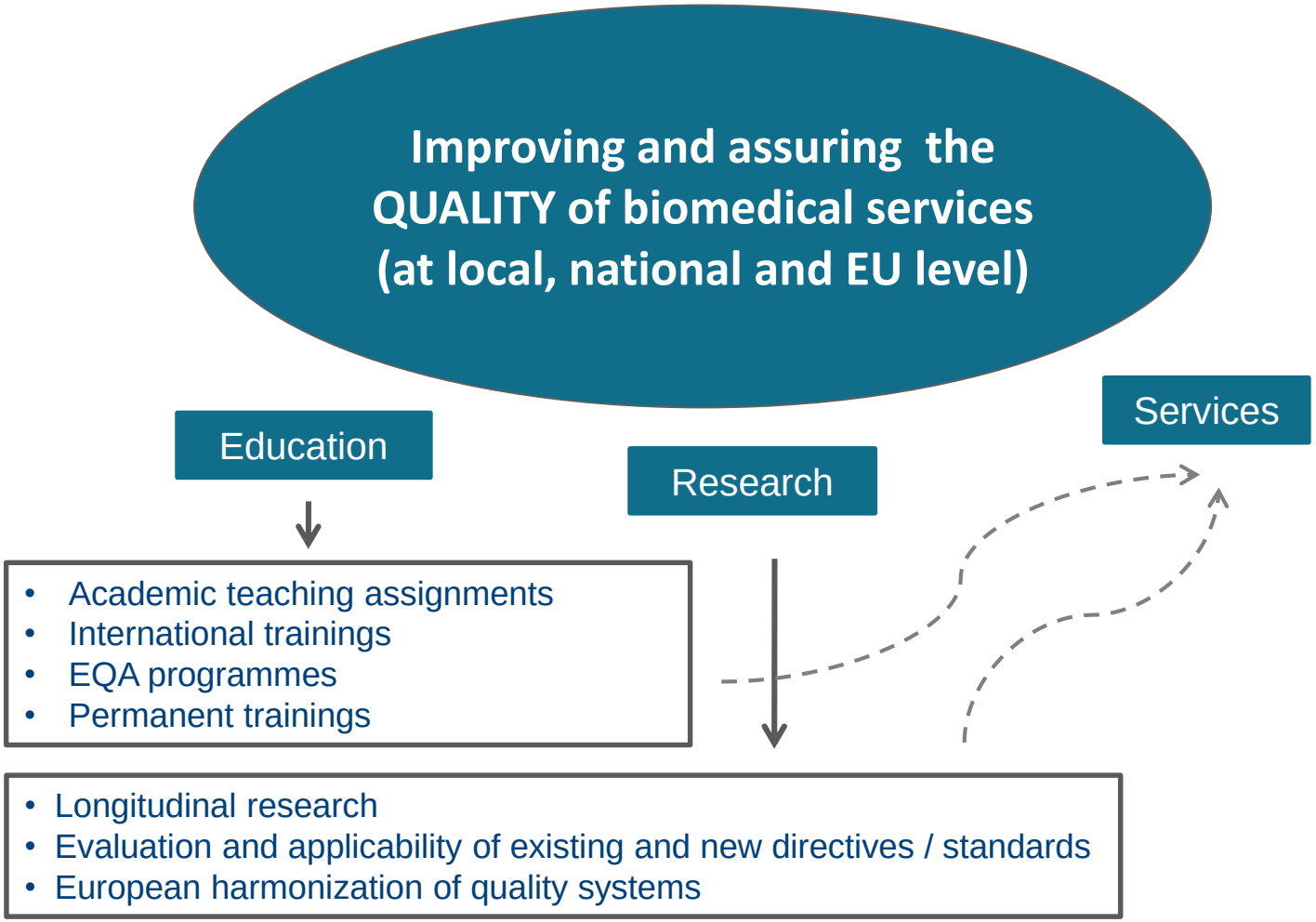
BQA Research Unit – KU Leuven



Introduction: ESP EQA schemes

Set-up of EQA scheme in collaboration with ESP

Assessing biomarker testing

Providing remedial measures

Ensuring uniform performance over time

E. Bellon *et al*, Oncologist, 2011,16 (4), 467-78

E. Dequeker *et al*, Virchows Arch 2011, 459 (2), 155-60

J. Van Krieken, *et al*, Virchows Arch 2013, 462 (1), 27-37

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Introduction: ESP EQA schemes

Group of experts (steering committee) organizing EQA scheme

Medical expert

- Knowledge of the clinical and pathological background
- Pathologists with proven experience in laboratory techniques
- Oncologists with proven experience in evaluating (molecular) alterations

Technical expert

- Expert on laboratory methods / working in the lab
- Experience in methods of (molecular) analysis
- Knowledge of the molecular context and of the technologies used for diagnostic testing

EQA provider

- Organization and management of the EQA program in accordance with ISO 17043
- Experience in quality management
- Solid background in the diagnostic domain of the EQA
- Necessary facilities / team to run such a program

BELAC

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Introduction: ESP EQA schemes

Steering committee is responsible for designing the EQA set up:

- Selection and validation of EQA samples
- Selection of laboratories to prepare EQA samples
- Way of reporting of the results
- Define additional assessors and analysis of results
- Reporting to participants, and regulatory/certifying agencies if required

Colon: Scheme organizers

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Introduction: ESP EQA schemes

Result submission: 14 calendar days

General laboratory characteristics

Used methods

Genotype results

Percentage of neoplastic cells

Written reports for 3 cases

(Stained slides, raw data)

Assessment meeting: Independent scoring by international experts

Appeal phase: 1 month, discussion with medical and technical experts

Online publication of successful laboratories

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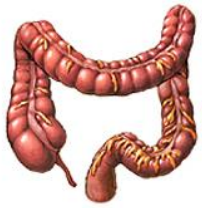
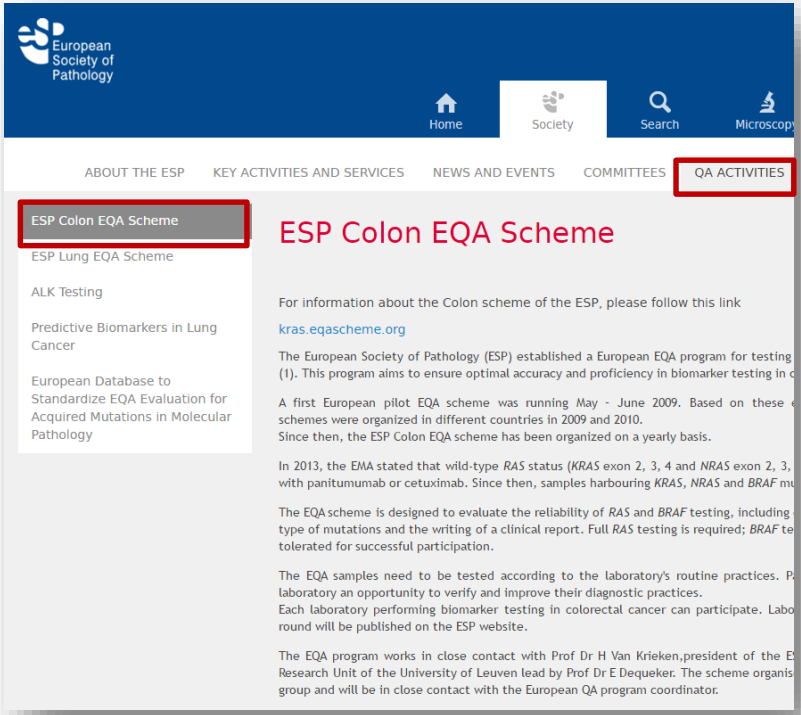
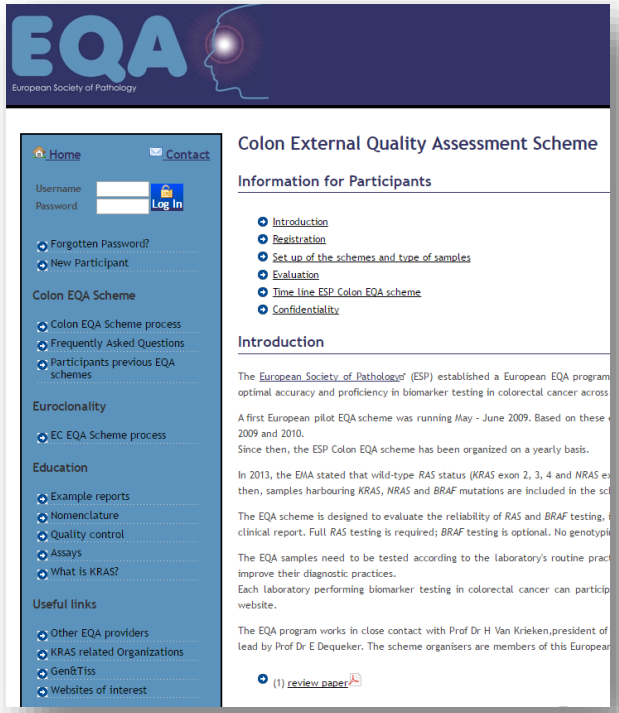


ESP Colon EQA Scheme: organized since 2009

http://www.esp-pathology.org



http://kras.eqascheme.org



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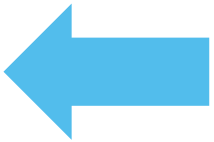
ESP Colon EQA Schemes

Interest from many laboratories

mCRC EQA Scheme	Genes	Number of labs	Number of labs with maximum score	Average score
KRAS EQA 2009 - Pilot	KRAS	61	69%	95%
KRAS EQA 2010	KRAS	76	67%	95%
KRAS EQA 2011	KRAS	124	82%	96%
KRAS EQA 2012	KRAS	105	73% (*88%)	94%
Colon EQA 2013	KRAS, NRAS, BRAF	131	73%	full RAS testing 94.78% KRAS exon 2 91.83%
Colon EQA 2014-2015	KRAS, NRAS, BRAF	125	66%**	90.28%
Colon EQA 2016	KRAS, NRAS, BRAF	127	*(ongoing)*	*(ongoing)*



* One challenging sample –> 10 % mutant alleles, without this sample error rate would have been 12.4%
** More stringent criteria: no major genotyping error and a score on technical evaluation of ≥18/20

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ESP Colon EQA Scheme 2013:
Effect of new regulations

July 2013

Panitumumab and cetuximab

Wild-type *RAS* status required

First *RAS* EQA 6 months later

Exons 2, 3, 4

Codons 12, 13, 59, 61, 117, 146

49% of participants implemented new test requirements

71% and 73% of the laboratories tested KRAS and NRAS exon 4 respectively

Higher error rate compared to previous schemes

- Labs have difficulties extending routine testing
- Learning phase ongoing

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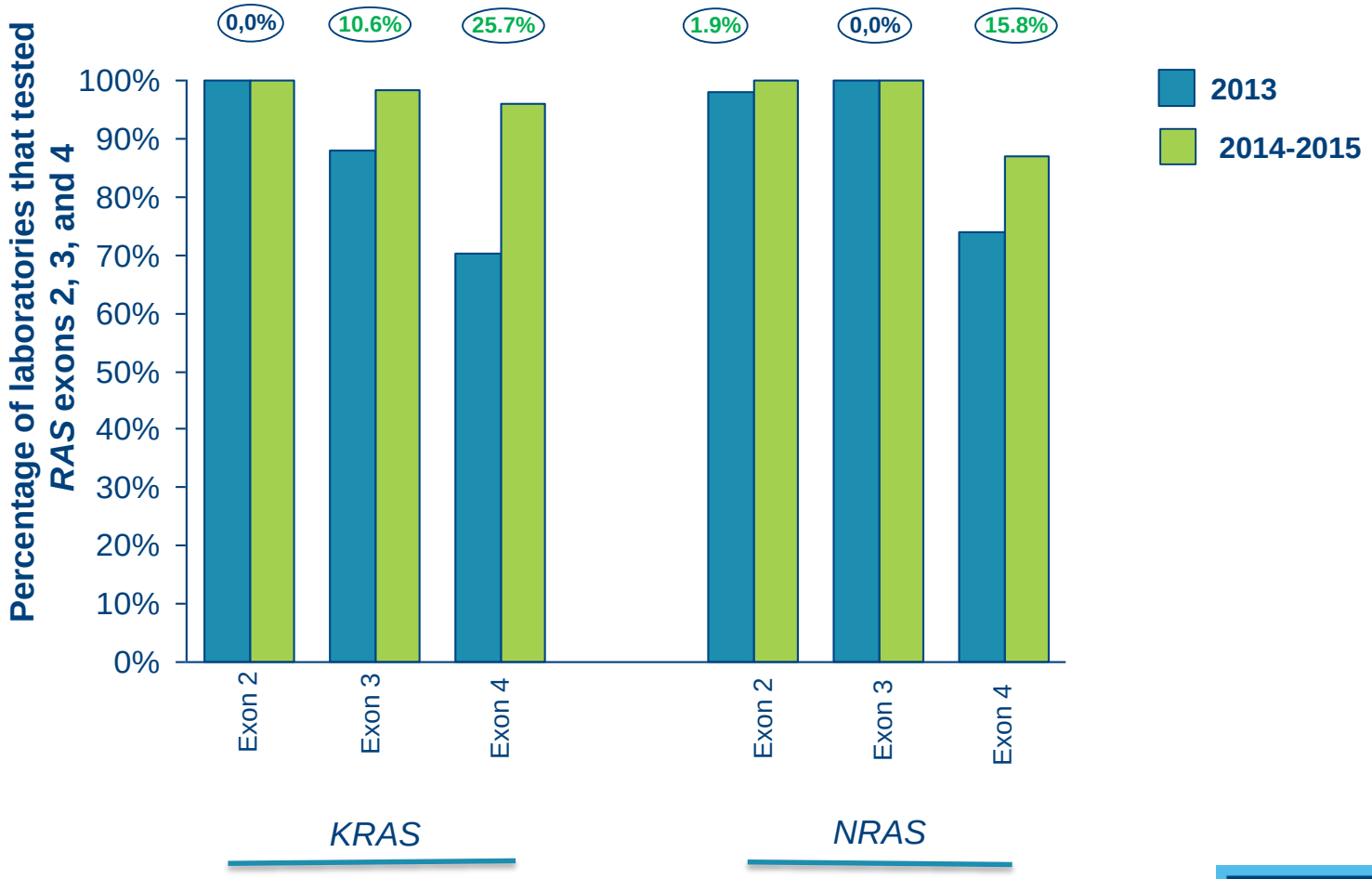
Tack V, Ligtenberg MJ, et al. Oncologist, 2015 Mar;20(3):257-62.

Effect of new regulations:
1 Year Later.....



Tack V, Ligtenberg MJ, et al. Oncologist, 2015 Mar;20(3):257-62.

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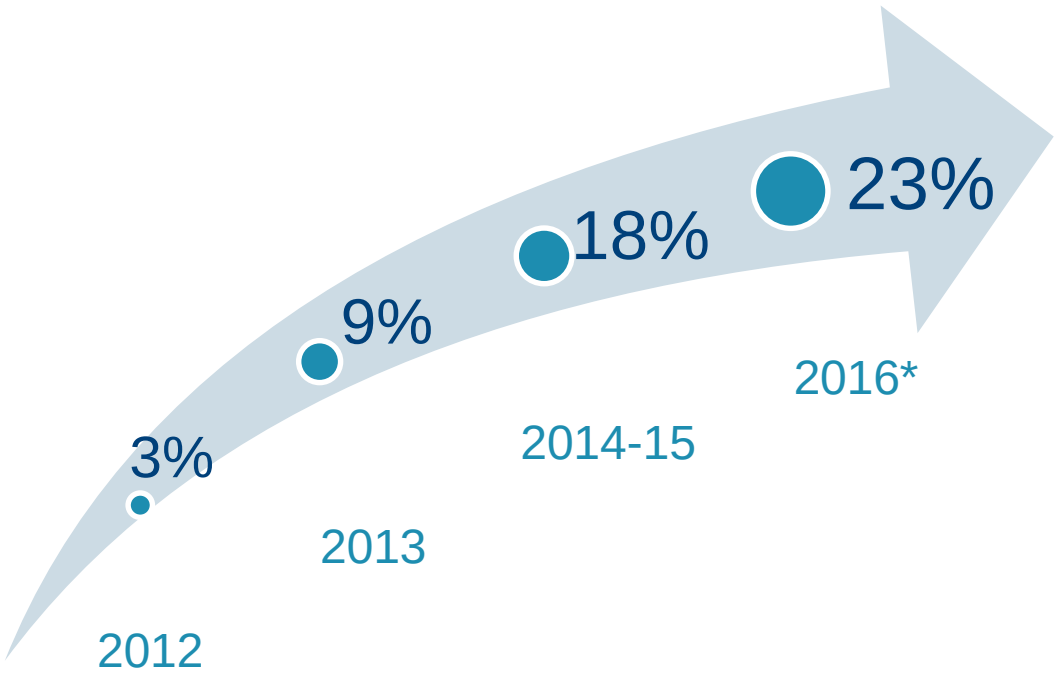
ESP Colon EQA Schemes: overview

Technologal advances: rapid expension of number of NGS users for *RAS* over the past years



* Preliminary results

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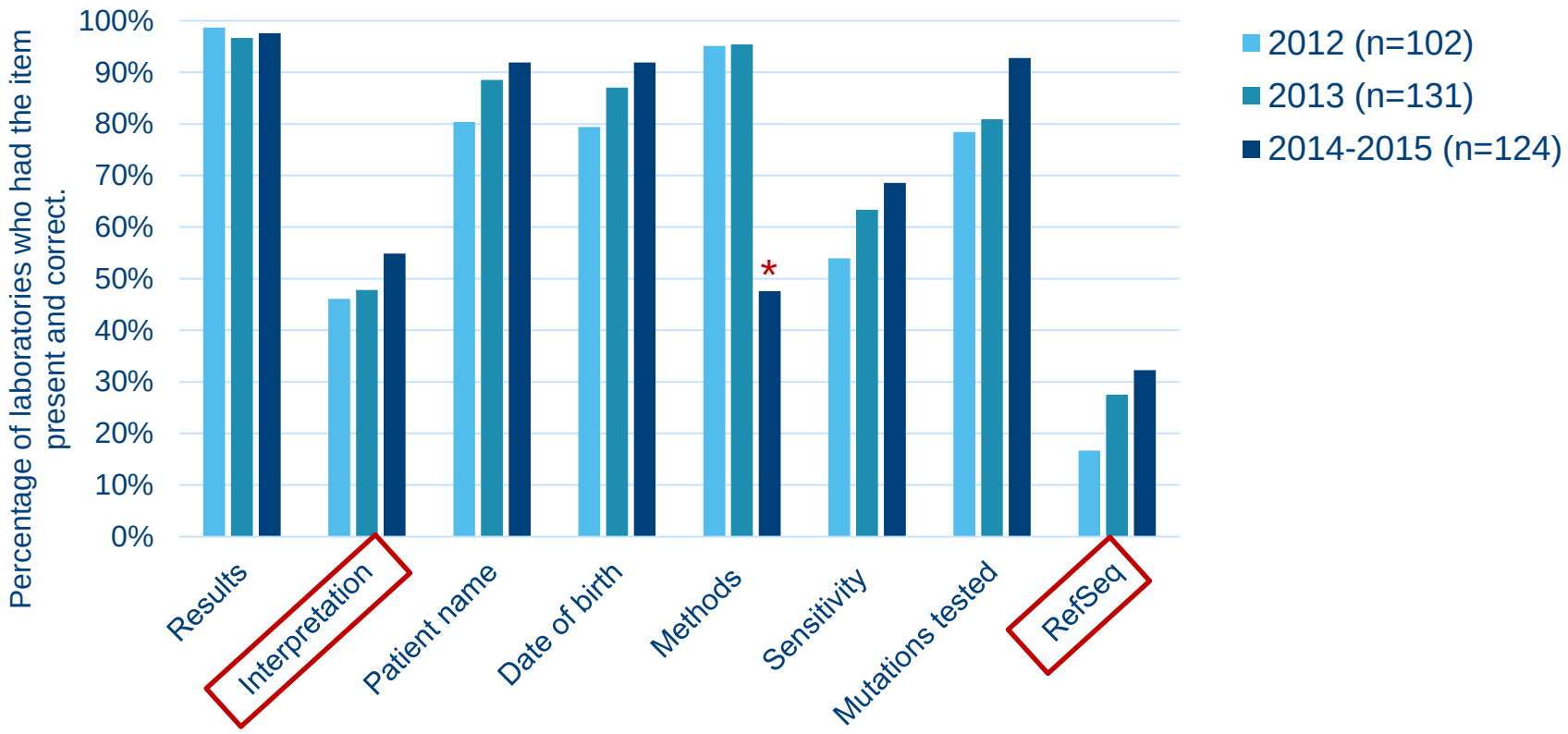
ESP colon EQA scheme: Reporting



*In case of a commercial method, the method should be mentioned in detail, including the **version number**

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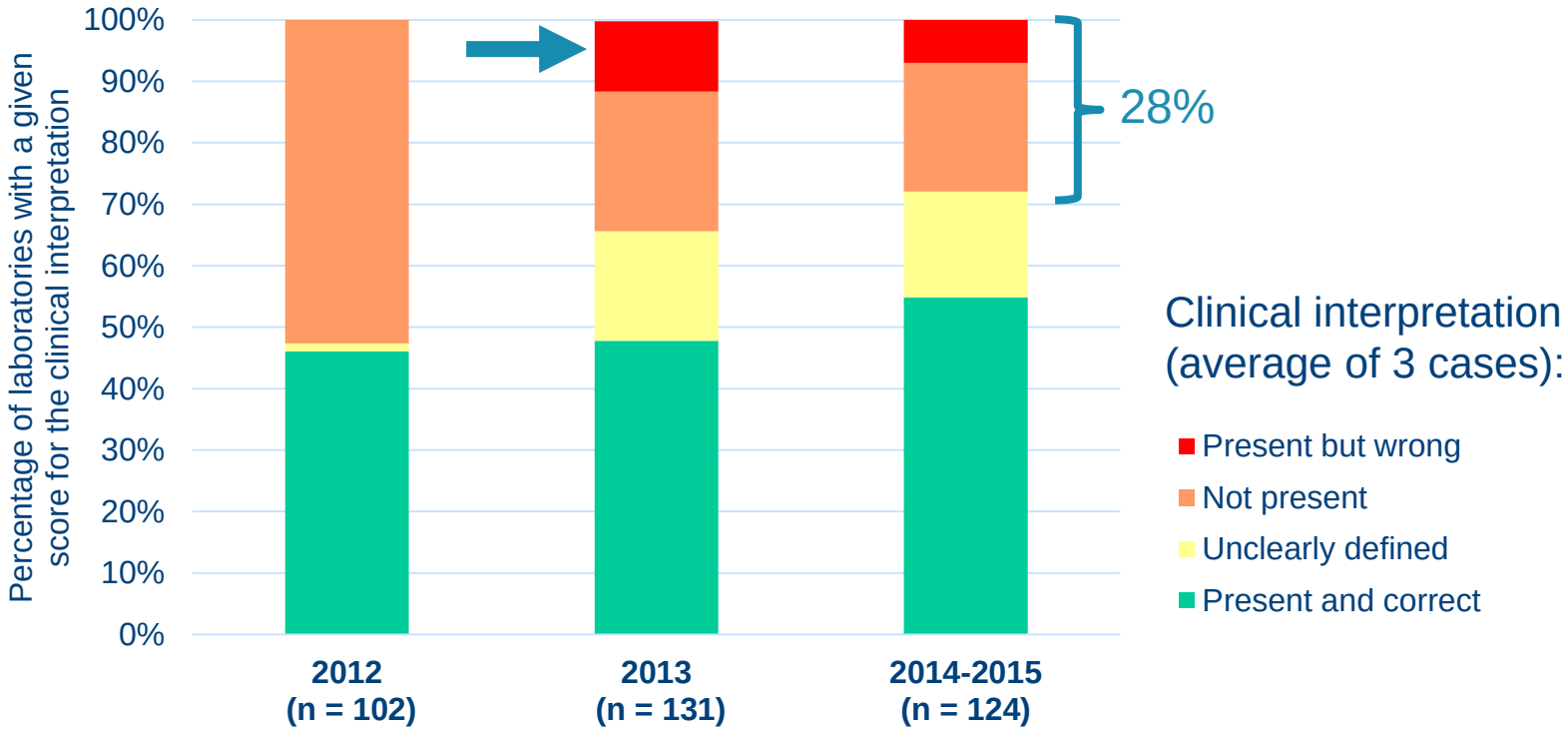
ESP colon EQA scheme: Reporting



New test requirements:
Claiming that a sample is WT without full *RAS* testing is considered wrong!

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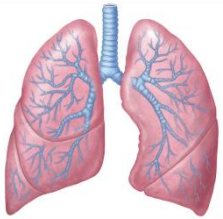

ESP Lung EQA Scheme: organized since 2012

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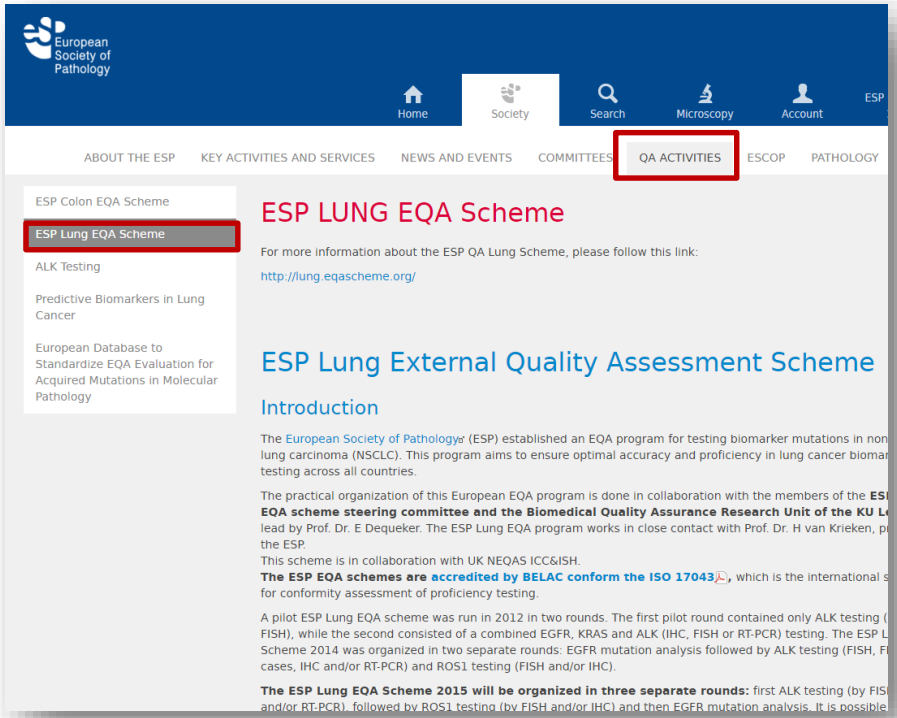
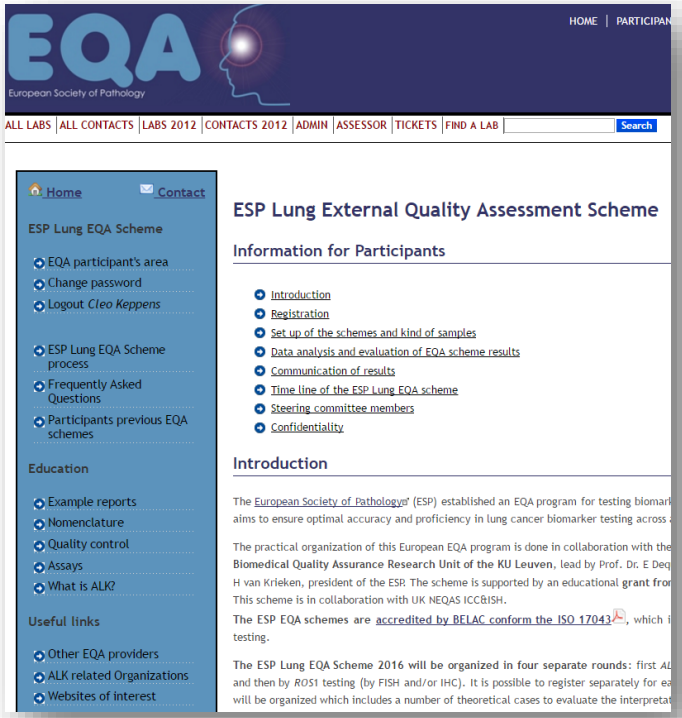


http://lung.eqascheme.org



Evolution in NSCLC

- 2004 Dutch guideline (NVALT 1.0)
  - Pathological evaluation to determine the histological subtype.

- 2011 Dutch guideline (NVALT 2.0)
  - Histological subtyping (P63, TTF-1, CK7, Mucin)
  - Adenocarcinoma: EGFR

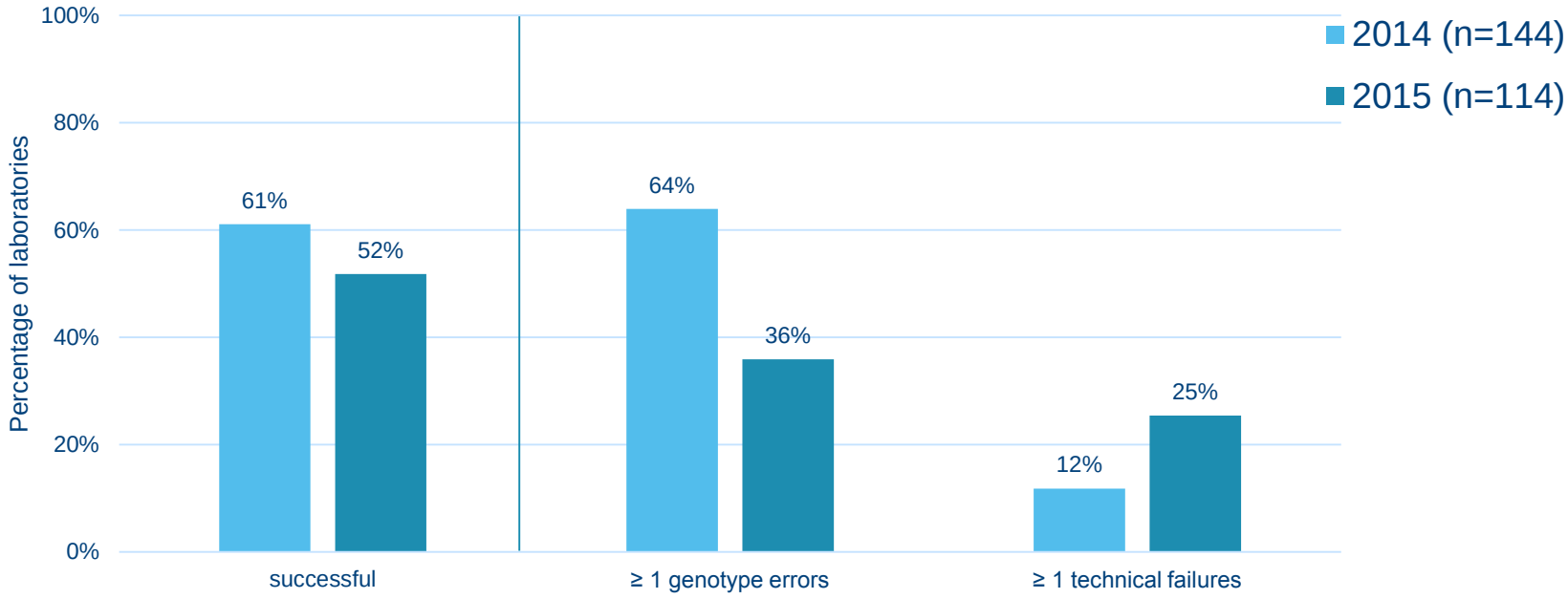
- 2015 Dutch guideline (NVALT 2.2)
  - Histological subtyping (P40/P63, TTF-1, CK7, Mucin)
  - Adenocarcinoma: EGFR, ALK, ROS, RET, Her2, BRAF

ESP Lung EQA Schemes: *EGFR*

European situation

NSCLC EQA Scheme	Number of labs	% of labs successful	Average genotyping score
Lung EQA 2012 b - Pilot	107	educational	73%
Lung EQA 2014, part I	144	61%	88%
Lung EQA 2015, Part III	114	52%	88%

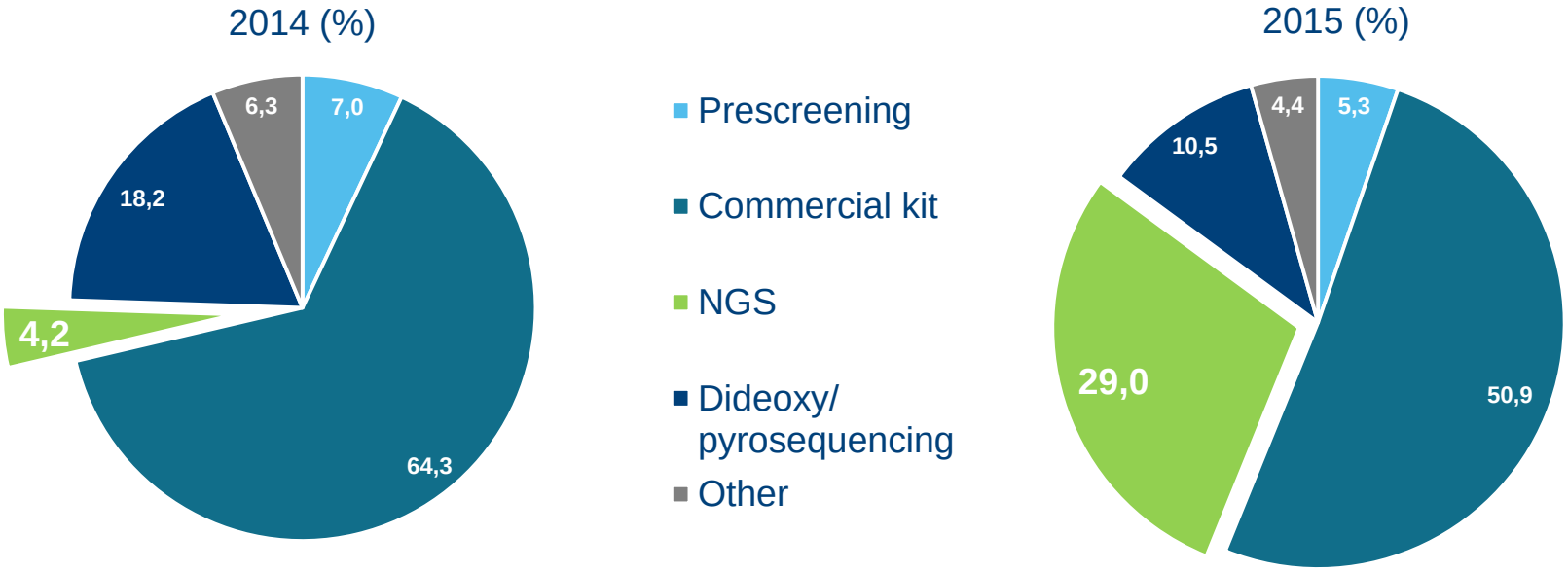
ESP Lung EQA Scheme 2014 - 2015



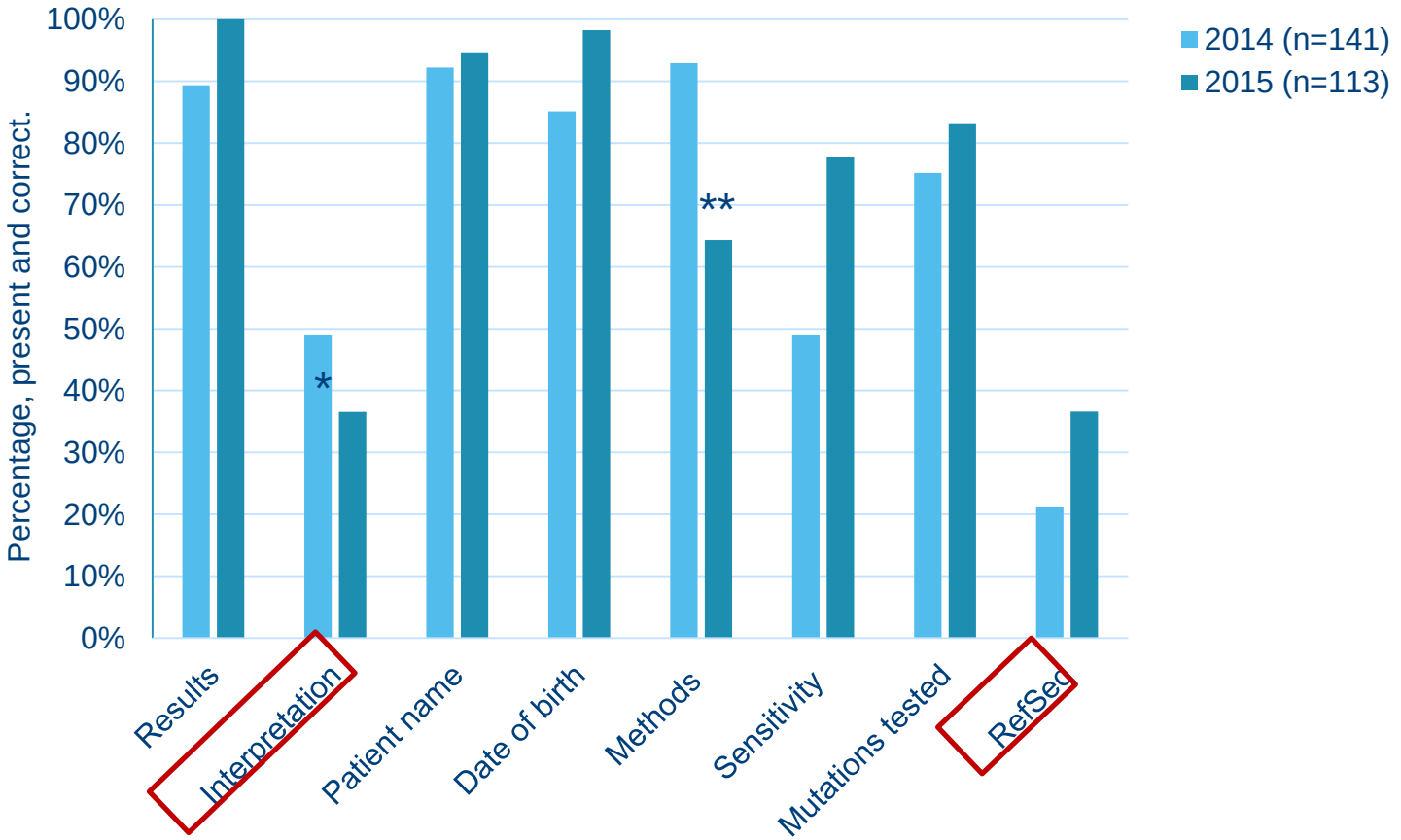
- 8/114 (7%) laboratories reported an additional SNP as a mutation (not calling it a SNP) or an additional mutation (1 point deducted)

ESP Lung EQA Scheme 2014 - 2015

Technologal advancement:
Rapid expansion of NGS users (%) for *EGFR* in favor of other methods

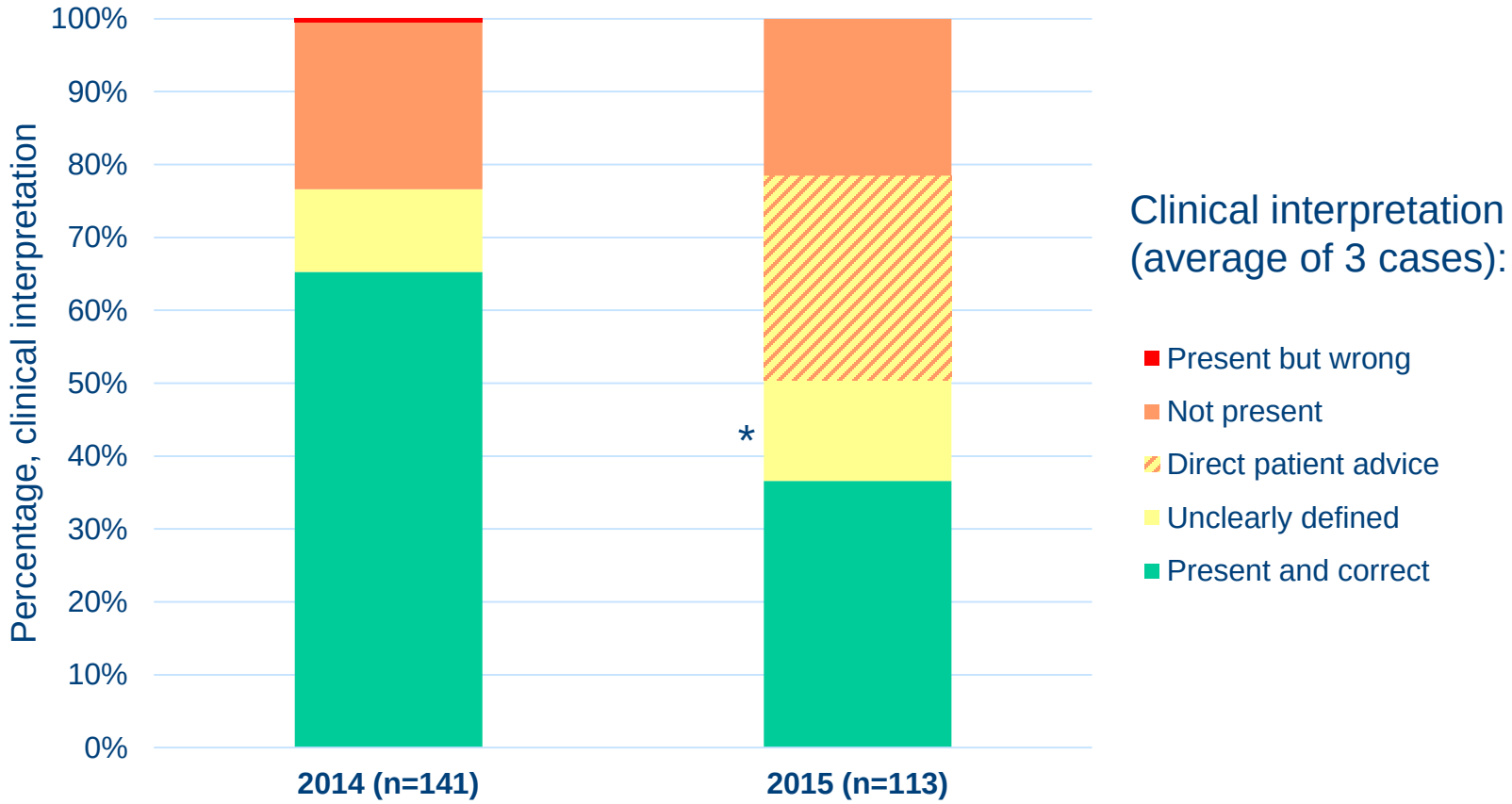


ESP Lung EQA Scheme 2014-2015: Reporting



*One case with combined p.(Thr790Met) and p.(Leu858Arg) was considered incorrect if therapy was recommended without knowledge of clones/allelic frequencies, except if the advise of a tumor molecular board wass recommended.
**Full traceability in the future needs to be warranted

ESP lung EQA scheme: Reporting



* One case with combined p.(Thr790Met) and p.(Leu858Arg)

ESP Lung EQA Schemes: *EGFR*
Dutch situation

NSCLC EQA Scheme	Number of participants Total/NL	% of labs successful Total/NL	Average genotyping score Total/nl
Lung EQA 2014, part I	144 / 23	61 / 69,6%	88 / 89,6%
Lung EQA 2015, Part III	114 / 20	51 / 30,0%	88 / 83,8%

Incorrect use of HGVS nomenclature:

- **39%** and **45%** of NL laboratories in 2014-2015 resp.

- 18 laboratories participated to 2014 ánd 2015:
- 6 laboratories: no HGVS error
- 4 laboratories: improved nomencl.
- 4 laboratories: kept error between 14-15
- 4 laboratories: made error since 2015

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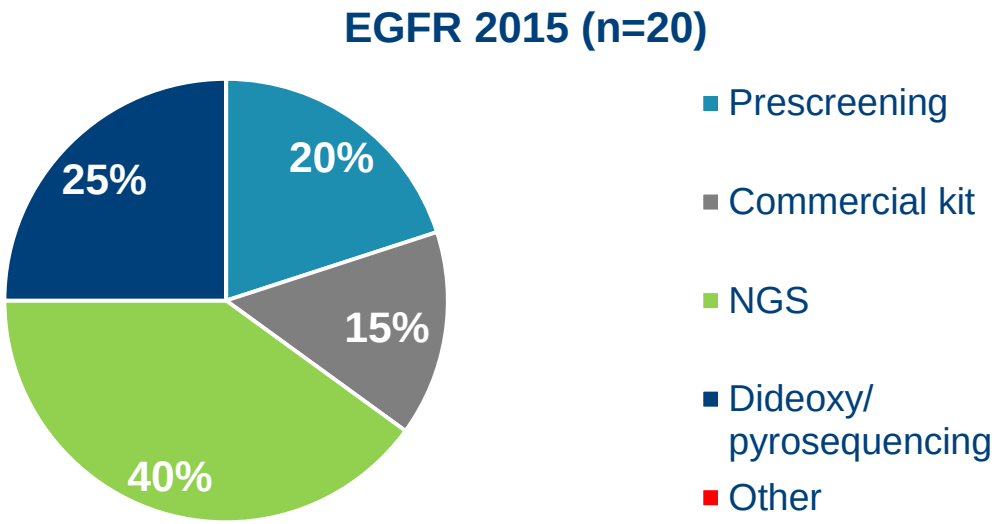
ESP Lung EQA Schemes: Methods (NL)



Panel	EGFR 2015
Ampliseq Custom panel Regions selected by the laboratory (Life technologies)	**62,5%**
Ion AmpliSeq Colon and Lung Cancer Panel (Life technologies)	25,0%
Ion Ampliseq hotspot cancer panel v2 (Life technologies)	12,5%

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What's happening in Europe?

NSCLC EQA Scheme	Number of participants Total/NL	% of labs successful Total/NL	Average genotyping score Total/nl
Lung EQA 2014, part I	144 / 23	61 / 69,6%	88 / 89,6%
Lung EQA 2015, Part III	114 / 20	51 / 80,0%	88 / 83,8%

	% successful	% of labs with technical errors	% of labs with ≥2 technical errors
NGS-lab (n=33)	51,5%	30,3%	9,1%
Non-NGS-lab (n=81)	51,8%	23,5%	9,9%

- Slightly different numbers in technical errors, in favour of non-NGS-labs.
- No explanation found when experience is taken into account.
- No explanation found by comparing cases (1 case was excluded).

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What's happening in Europe?

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What's happening in The Netherlands?

NSCLC EQA Scheme	Number of participants Total/NL	% of labs successful Total/NL	Average genotyping score Total/nl
Lung EQA 2014, part I	144 / 23	61 / 69,6%	88 / 89,6%
Lung EQA 2015, Part III	114 / 20	51 / 30,0%	88 / 83,8%

	% successful	% of labs with technical errors	% of labs with ≥2 technical errors
NGS-lab (n=8)	37,5%	50%	13%
Non-NGS-lab (n=12)	25%	67%	50%

Increase in technical errors in 2015 compared to 2014 results:
9% technical errors increased to 60% technical errors

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ESP Lung EQA Scheme: organized since 2012

August 2011

- FDA approves Xalkori (Crizotinib) with Companion Diagnostic for a Type of Late-Stage Lung Cancer

ALK

March 2016

- FDA expands use of Xalkori (Crizotinib) to treat rare form of advanced non-small cell lung cancer

ROS1

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Evolution in NSCLC

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  - Pathological evaluation to determine the histological subtype.

- 2011 Dutch guideline (NVALT 2.0)
  - Histological subtyping (P63, TTF-1, CK7, Mucin)
  - Adenocarcinoma: EGFR

- 2015 Dutch guideline (NVALT 2.2)
  - Histological subtyping (P40/P63, TTF-1, CK7, Mucin)
  - Adenocarcinoma: EGFR, ALK, ROS, RET, Her2, BRAF

ESP Lung EQA Schemes: *ALK/ROS1*
European situation

Scheme	Subscheme	Number of labs	% of labs successful
Lung EQA 2012 a - Pilot	ALK FISH	54	72%
Lung EQA 2012 b - Pilot	ALK FISH	104	68%
Lung EQA 2014, part II	ALK FISH	116	69%
Lung EQA 2015, Part I	ALK FISH	111	79%

Scheme	Subscheme	Number of labs	% of labs successful
Lung EQA 2012 a - Pilot	ALK IHC	29	52%
Lung EQA 2012 b - Pilot	ALK IHC	58	64%
Lung EQA 2014, part II	ALK IHC	96	70%
Lung EQA 2015, Part I	ALK IHC	95	92%

Scheme	Subscheme	Number of labs	% of labs successful
Lung EQA 2014, part II	ROS1 FISH	56	64%
	ROS1 IHC	31	90%
Lung EQA 2015, Part II	ROS1 FISH	68	78%
	ROS1 IHC	31	58%

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Tembuyser L, Tack V, Zwaenepoel K, et al. Plos One 2014;9(11);e112159

ESP Lung EQA Schemes: *ALK/ROS1*
Dutch situation

NSCLC EQA Scheme	Subscheme	Number of Dutch participants	Average score (%)	% of labs successful
Lung EQA 2014, part II	ALK FISH	17	82,6	58,8
	ALK FISH Digital	12	83,3	83,3
	ALK IHC	17	95,2	70,6
	ROS1 FISH	7	73,9	42,9
	ROS1 IHC	3	100,0	100,0
Lung EQA 2015, Part I	ALK FISH (+Digital)	16	95,2	87,5
	ALK IHC	16	100,0	100,0
Lung EQA 2015, Part II	ROS1 FISH	15	92,0	80,0
	ROS1 IHC	6	90,0	**50,0**

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ALK IHC pilot scheme for technical evaluation of immuno-staining

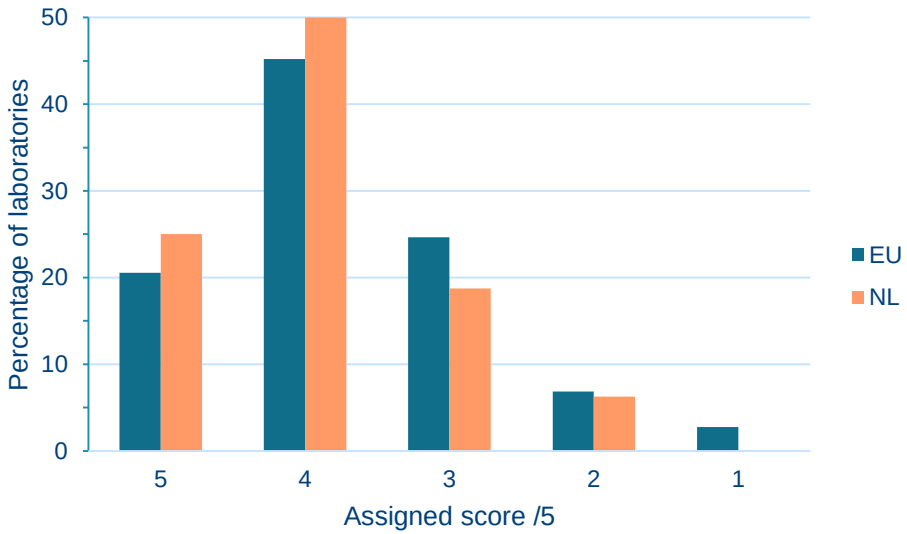
73 EU participants/25 countries

16 NL labs (22%)

Average score

76,6%

80,0%



- 2 independent pathologists
- 5 ALK stained slides
- Individual comments
- Labs with a borderline score of 3 (or less) should re-evaluate their methods
- 34% vs. 25%
- 2016: ROS IHC

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ESP Colon EQA Schemes
Dutch situation

mCRC EQA scheme	Marker	Number of labs Total/NL	Average genotyping score Total/NL	Nr of labs successful Total/NL
2009	KRAS	61 / 14	69 / 98%	95 / 100%
2010	KRAS	76 / 16	67 / 91%	95 / 88%
2011	KRAS	124 / 19	82 / 99%	96 / 95%
2012	KRAS	105 / 22	73 / 95%	94 / 91%
2013	KRAS, NRAS, BRAF	131 / 22	73 / 94%	95 / 91%*
2014-2015	KRAS, NRAS, BRAF	125 / 22	66 / 90%	90 / 77%**
2016***	KRAS, NRAS, BRAF	127 / 22	- / 96%***	- / 86%***

* 17 full *RAS* testing; 3 *KRAS* testing
** More stringent criteria: no major genotyping error and a score on technical evaluation of ≥18/20
***Preliminary results

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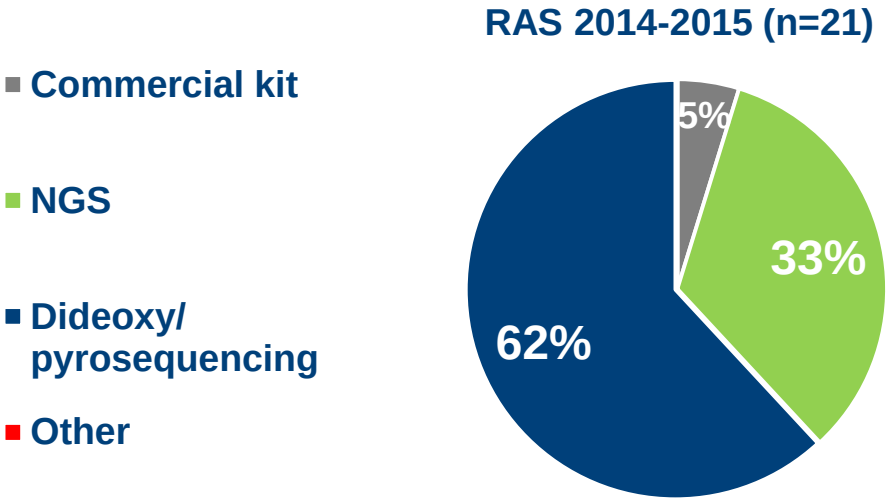
ESP Colon EQA Schemes: Methods (NL)



Panel	RAS 2014-2015
Ampliseq Custom panel Regions selected by the laboratory (Life technologies)	28,6%
Ion AmpliSeq Colon and Lung Cancer Panel (Life technologies)	**42,9%**
Ion Ampliseq hotspot cancer panel v2 (Life technologies)	14,3%
GS Junior (Roche)	14,3%

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ESP Lung/Colon EQA Schemes: Reporting

Lung 2015: *EGFR*

Average NL 88,7% N=20

Europe 87,4% N=113

Colon 2014-2015

Average NL 88,3% N=22

Europe 86,9% N=124

Lung 2015: *ALK*

Average NL 85,3% N=19

Europe 85,6% N=106

Lung 2015: *ROS1*

Average NL 83,8% N=14

Europe 84,8% N=65

Conclusions

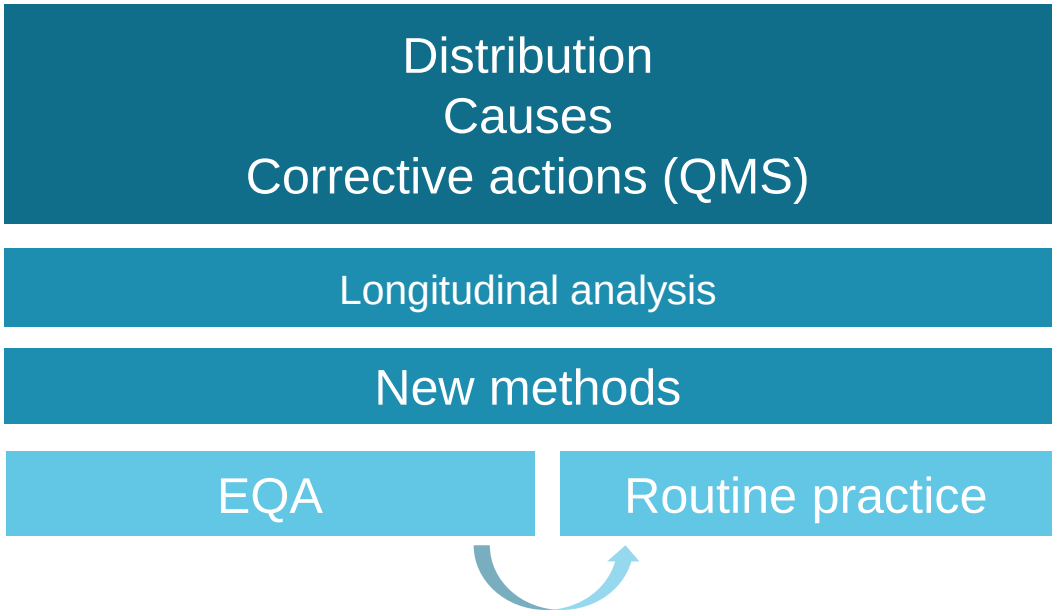
- The ESP EQA schemes **highlight the need for continuing EQA**
- Some labs do not test all required RAS codons still
- EQA scheme assesses not only the laboratory's **ability to obtain accurate, reliable results**, but also **the ability to safely interpret the results** and ensure that the referring clinician has the correct information.
- The **quality of the reports improved**

Research is needed!

- Error types and causes
- Quality indicators
  - Accreditation, experience, sample flow, lab setting
- Methods
  - Does switching methods lead to errors?
  - Are certain methods performing worse than others?
  - Does technological advance (NGS, liquid biopsies) provoke errors?
- Remedial measures
- Non-EQA participating laboratories

PhD project: EQA and QMS, tools for quality improvement?

- **Electronic questionnaire on follow-up of EQA results**



- **Recommendations for error reduction**

⇒ **Enhance laboratory performance**

⇒ **Improving patient safety**

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Results of the 2015 ESP ALK/ROS1 EQA scheme

- 62 laboratories/24 countries
- ≥ 1 genotype error in FISH and/or IHC
- Technical errors or educational cases not included
- December 2015
- Average TAT: 21 days
- 23/62 (37,1%) of 11 different countries responded
- 6/10 Dutch laboratories responded (26% of total)

→ **31 errors analyzed**

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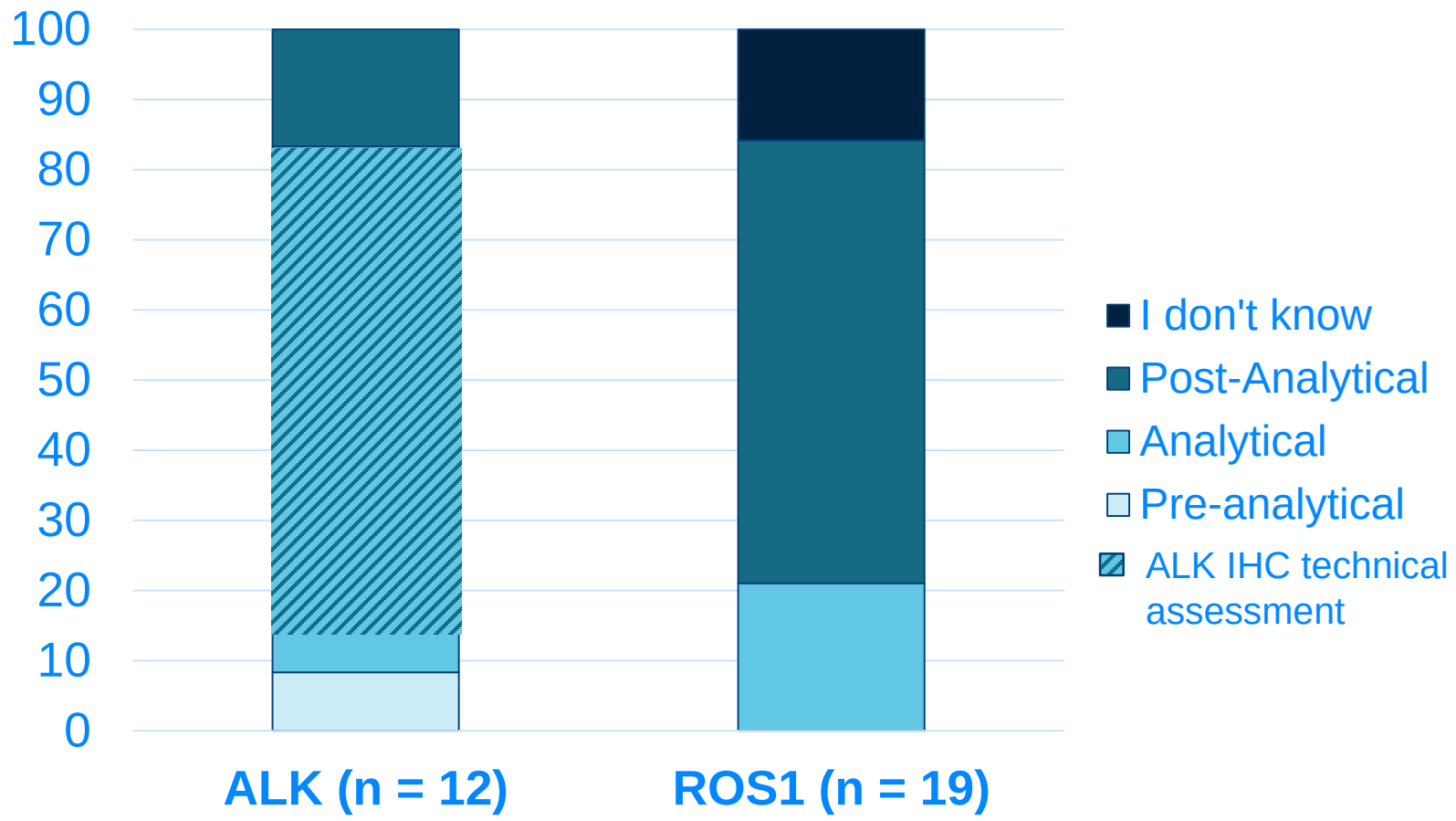
Percentage of errors in the pre-, post- or analytical phase



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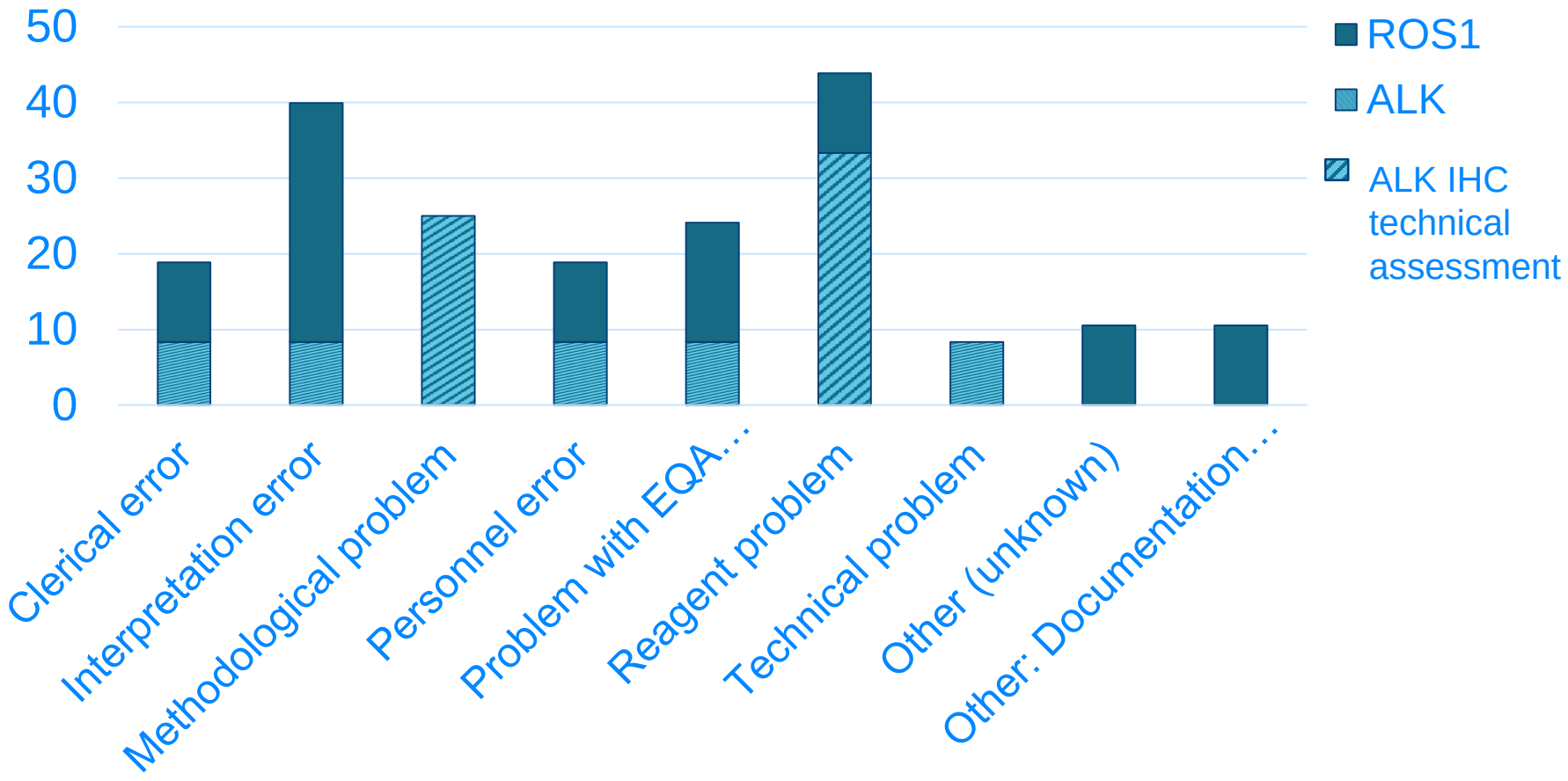
Percentage of error causes per EQA scheme



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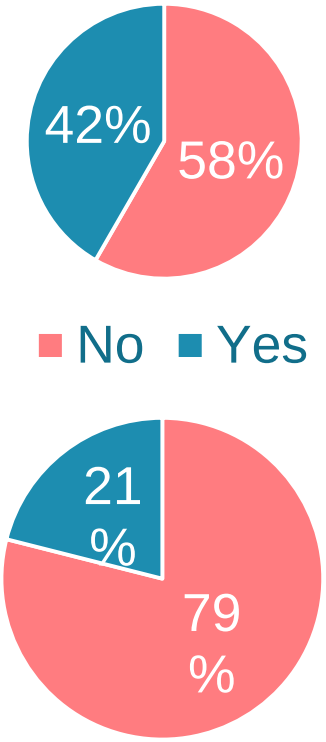
ALK versus ROS1

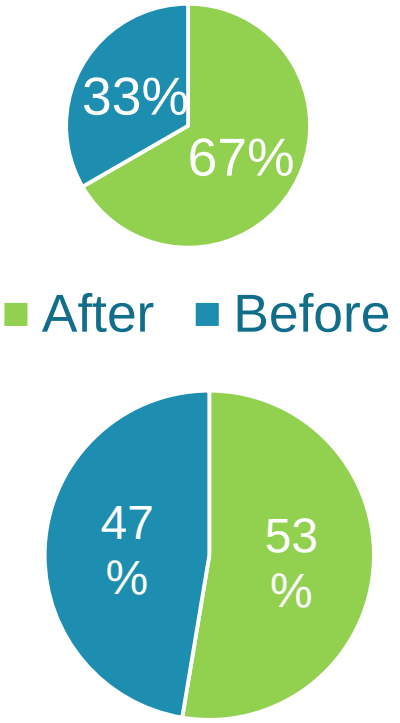
Do you think about changing your method next year?



ALK (n=12)

ROS1 (n=19)

Was this error detected before/after EQA results were released?



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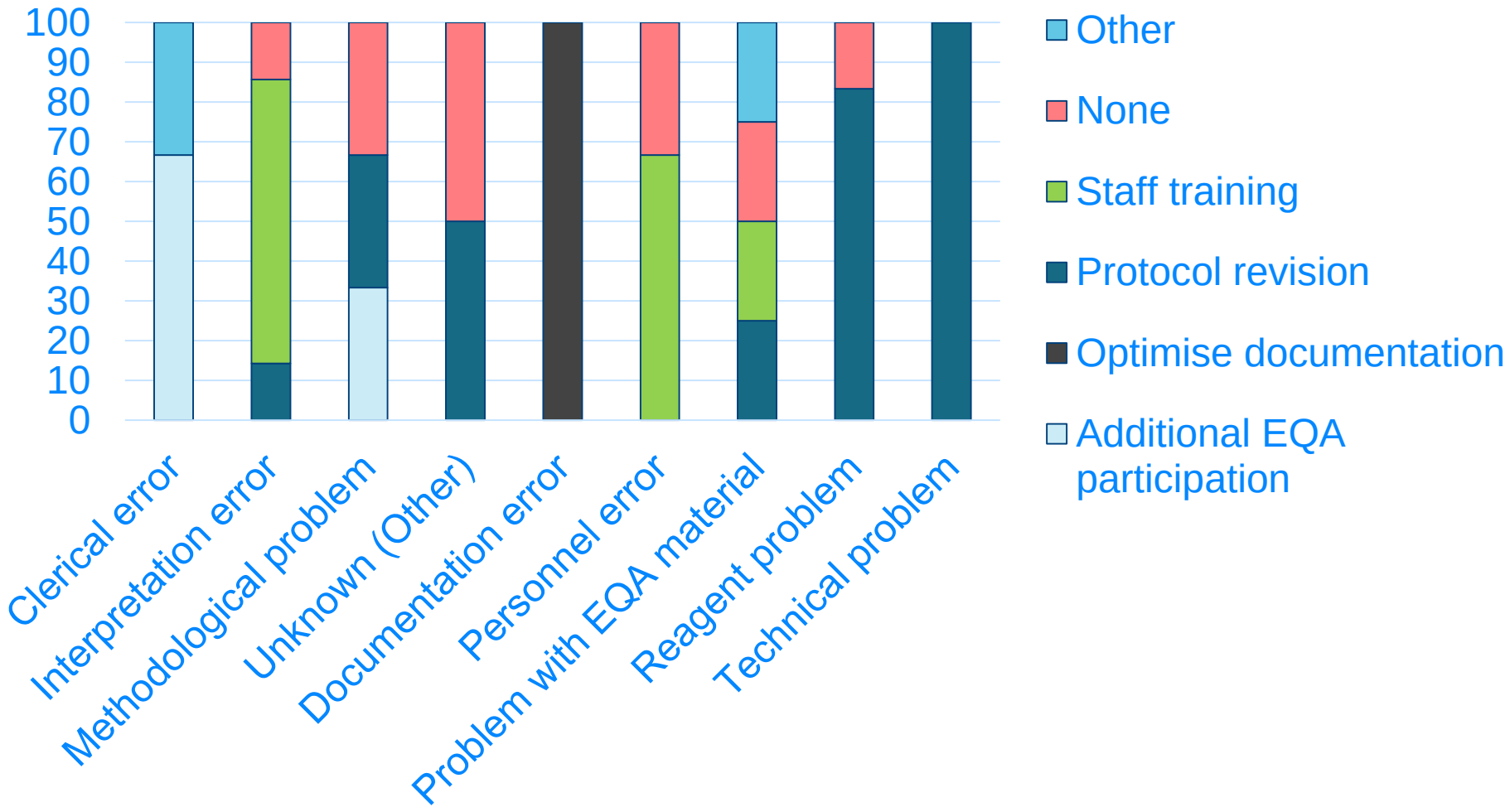
Corrective actions per error type



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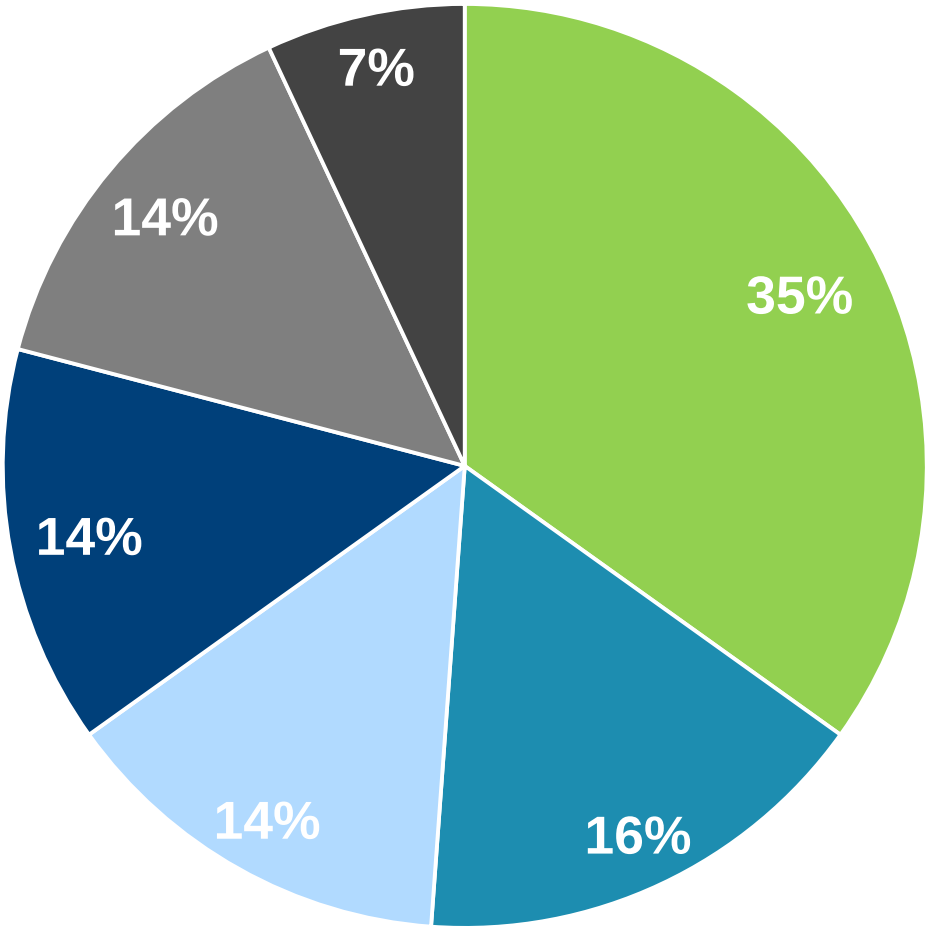
3. Responsible person for corrective actions



- Average:1,5 persons involved
- 46% of the errors required actions by ≥ 2 persons
- Independent of error type

- Pathologist
- Molecular biologist
- Laboratory technician
- Laboratory director
- Lead laboratory technician
- Quality manager

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Conclusions

- More post-analytical for ROS1 (interpretation errors)
- More analytical for ALK (reagent problems)
- Staff training for ROS1, protocol revision for ALK
- Change of method desirable in ALK participants
- IHC more error-prone as compared to FISH?
  - ➡ IHC technical assessment of ESP
- Follow-up mainly by pathologist
- +- 20% of the laboratories does not undertake an action
- No difference ~ accreditation status
- Idem Dutch laboratories

➡ Additional data required:

2015 Gen&Tiss scheme

2015 ESP EGFR scheme

2016 ESP Colon EQA scheme

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Biomedical Quality Assurance Research Unit, KU Leuven

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