

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

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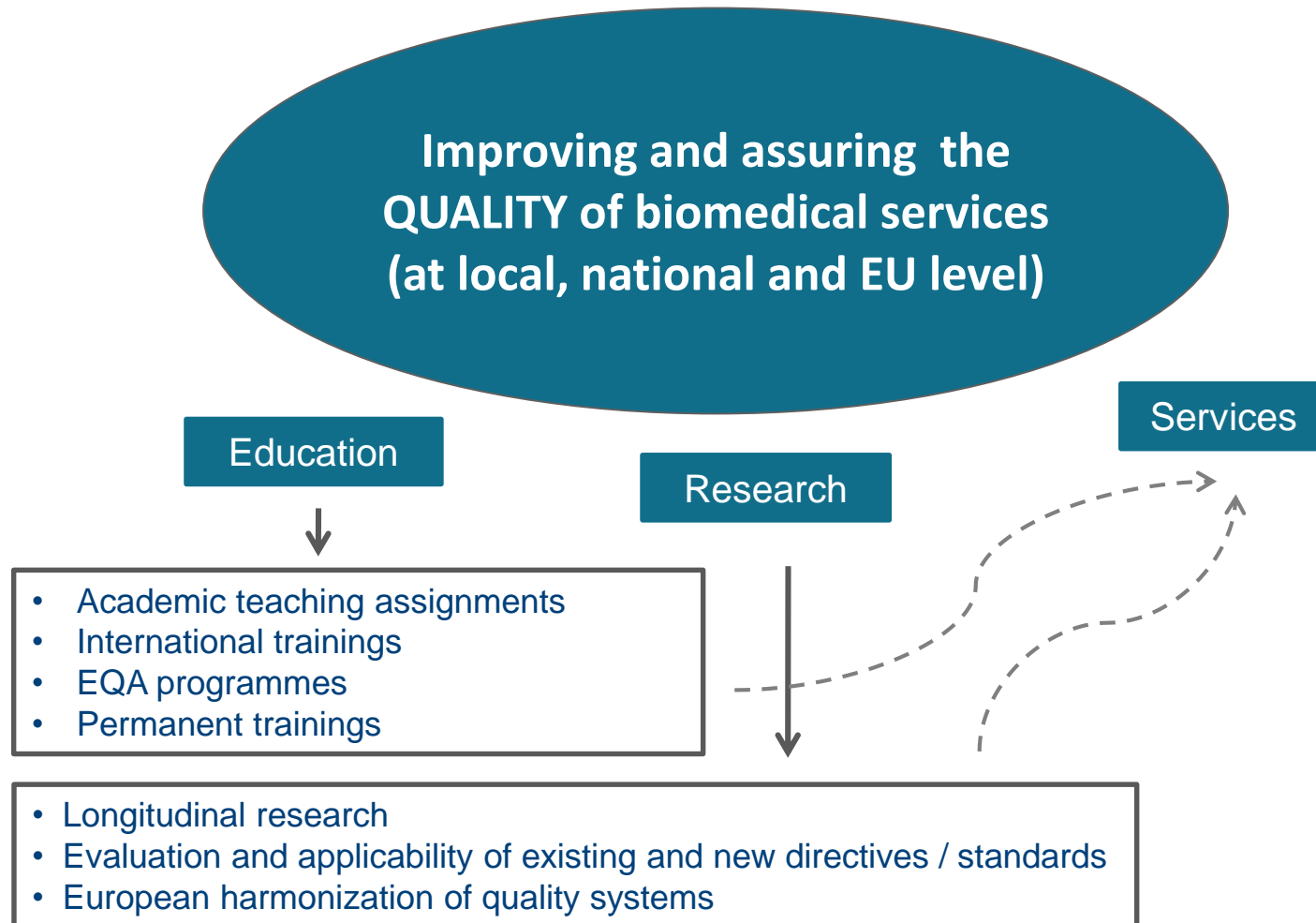


SKML Pathology day
Amersfoort, NL



May 26, 2016

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



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

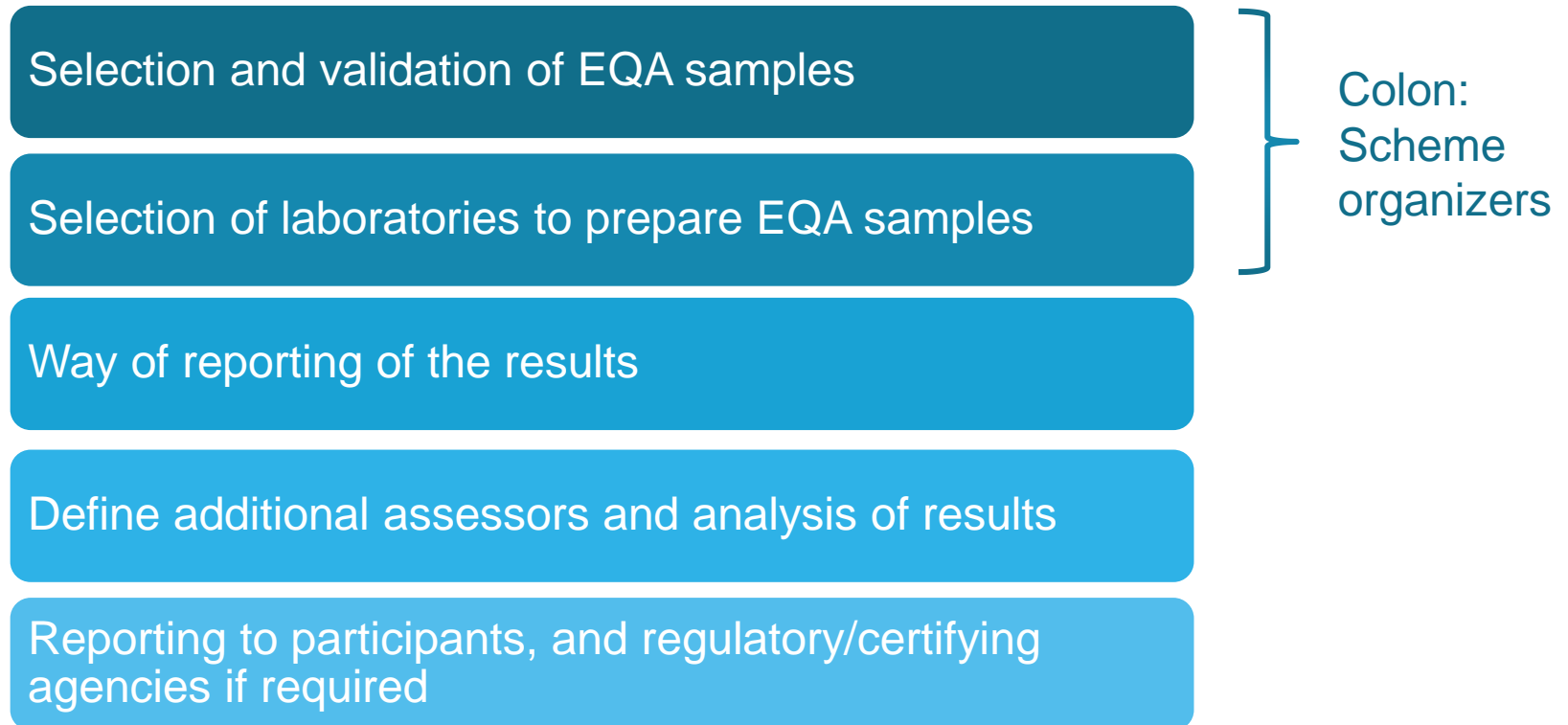
EQA provider

- 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



Introduction: ESP EQA schemes

Steering committee is responsible for designing the EQA set up:



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



ESP Colon EQA Scheme: organized since 2009

<http://www.esp-pathology.org>

European Society of Pathology

Home Society Search Microscopy

ABOUT THE ESP KEY ACTIVITIES AND SERVICES NEWS AND EVENTS COMMITTEES QA ACTIVITIES

ESP Colon EQA Scheme

ESP Lung EQA Scheme

ALK Testing

Predictive Biomarkers in Lung Cancer

European Database to Standardize EQA Evaluation for Acquired Mutations in Molecular Pathology

ESP Colon EQA Scheme

For information about the Colon scheme of the ESP, please follow this link kras.eqascheme.org

The European Society of Pathology (ESP) established a European EQA program for testing (1). This program aims to ensure optimal accuracy and proficiency in biomarker testing in colorectal cancer.

A first European pilot EQA scheme was running May - June 2009. Based on these schemes were organized in different countries in 2009 and 2010. Since then, the ESP Colon EQA scheme has been organized on a yearly basis.

In 2013, the EMA stated that wild-type RAS status (KRAS exon 2, 3, 4 and NRAS exon 2, 3, 4) with panitumumab or cetuximab. Since then, samples harbouring KRAS, NRAS and BRAF mutations are included in the scheme.

The EQA scheme is designed to evaluate the reliability of RAS and BRAF testing, including type of mutations and the writing of a clinical report. Full RAS testing is required; BRAF testing is optional for successful participation.

The EQA samples need to be tested according to the laboratory's routine practices. Participants have the opportunity to verify and improve their diagnostic practices. Each laboratory performing biomarker testing in colorectal cancer can participate. Laboratory results will be published on the ESP website.

The EQA program works in close contact with Prof Dr H Van Krieken, president of the ESP Research Unit of the University of Leuven lead by Prof Dr E Dequeker. The scheme organizers are members of this European group and will be in close contact with the European QA program coordinator.

<http://kras.eqascheme.org>

EQA European Society of Pathology

Home Contact

Username Password Log In

Forgotten Password? New Participant

Colon External Quality Assessment Scheme

Information for Participants

- Introduction
- Registration
- Set up of the schemes and type of samples
- Evaluation
- Time line ESP Colon EQA scheme
- Confidentiality

Introduction

The European Society of Pathology (ESP) established a European EQA program for testing (1). This program aims to ensure optimal accuracy and proficiency in biomarker testing in colorectal cancer across Europe.

A first European pilot EQA scheme was running May - June 2009. Based on these schemes were organized in different countries in 2009 and 2010. Since then, the ESP Colon EQA scheme has been organized on a yearly basis.

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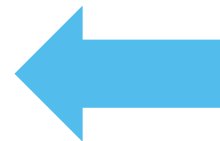
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(1) [review paper](#)

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

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

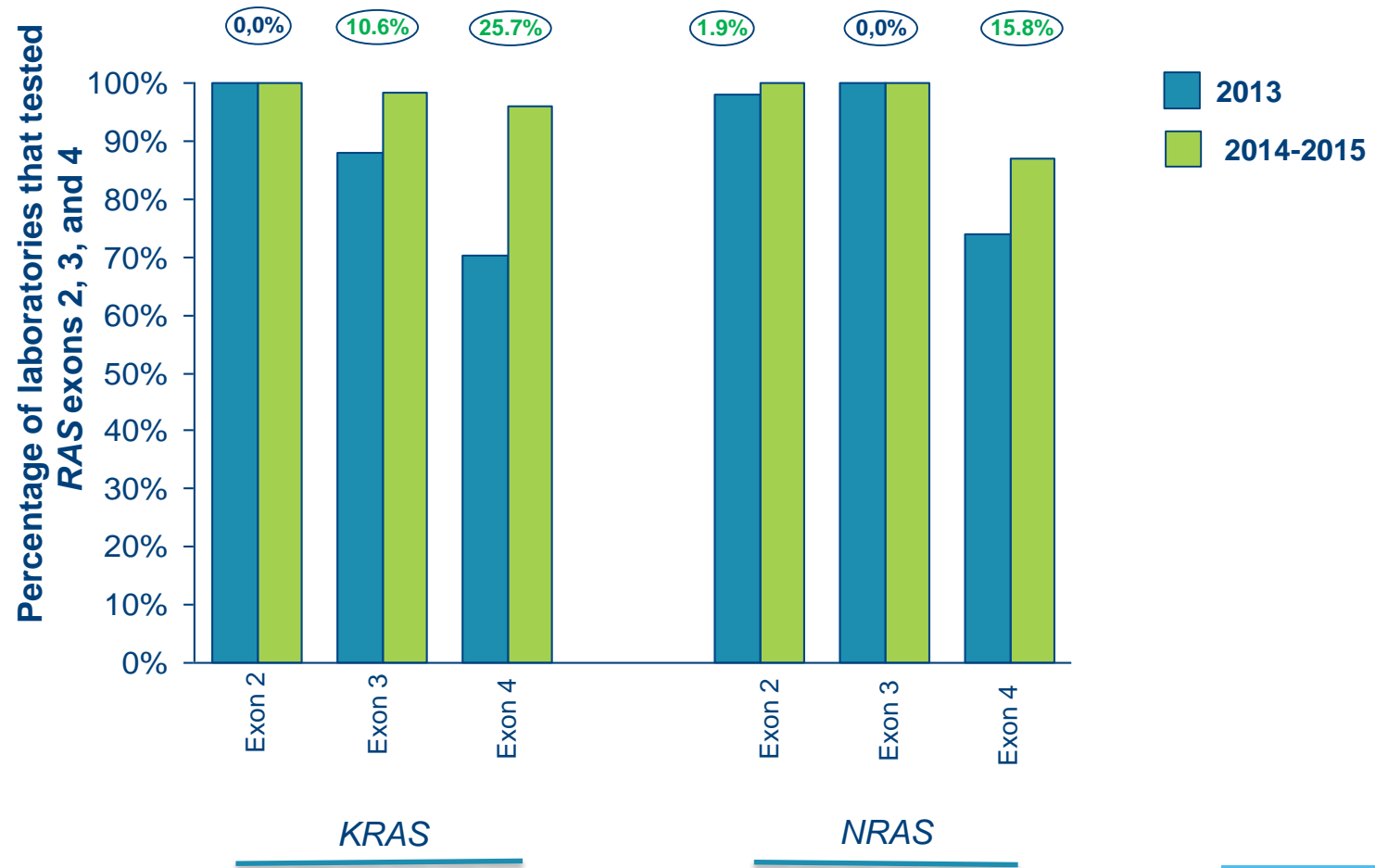
71% and 73% of the laboratories tested KRAS and
NRAS exon 4 respectively



Higher error rate compared to previous schemes

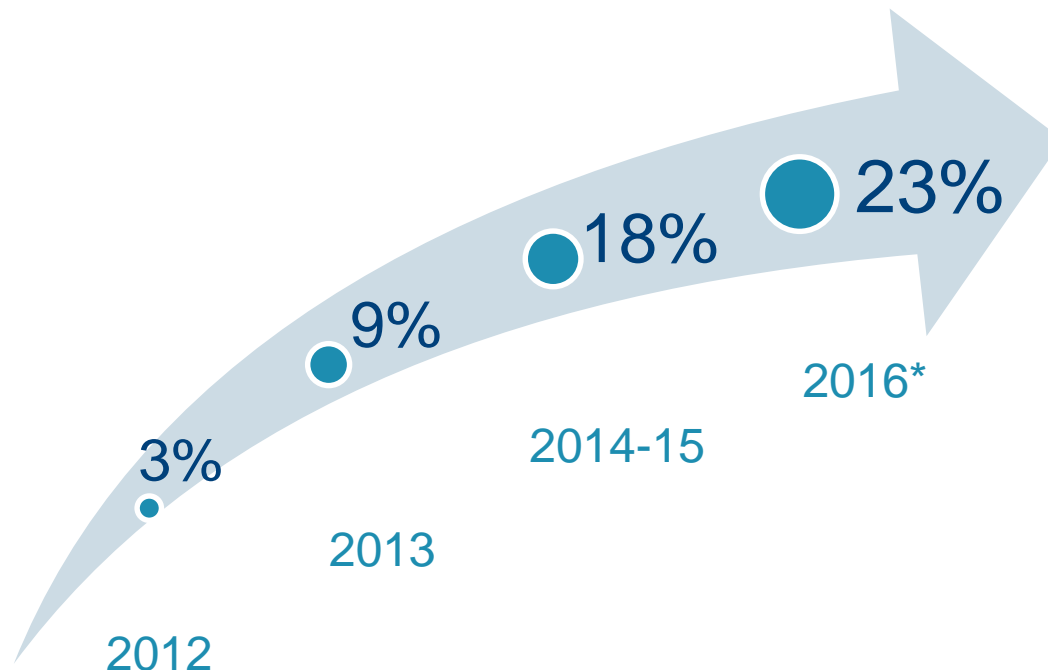
- Labs have difficulties extending routine testing
- Learning phase ongoing

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

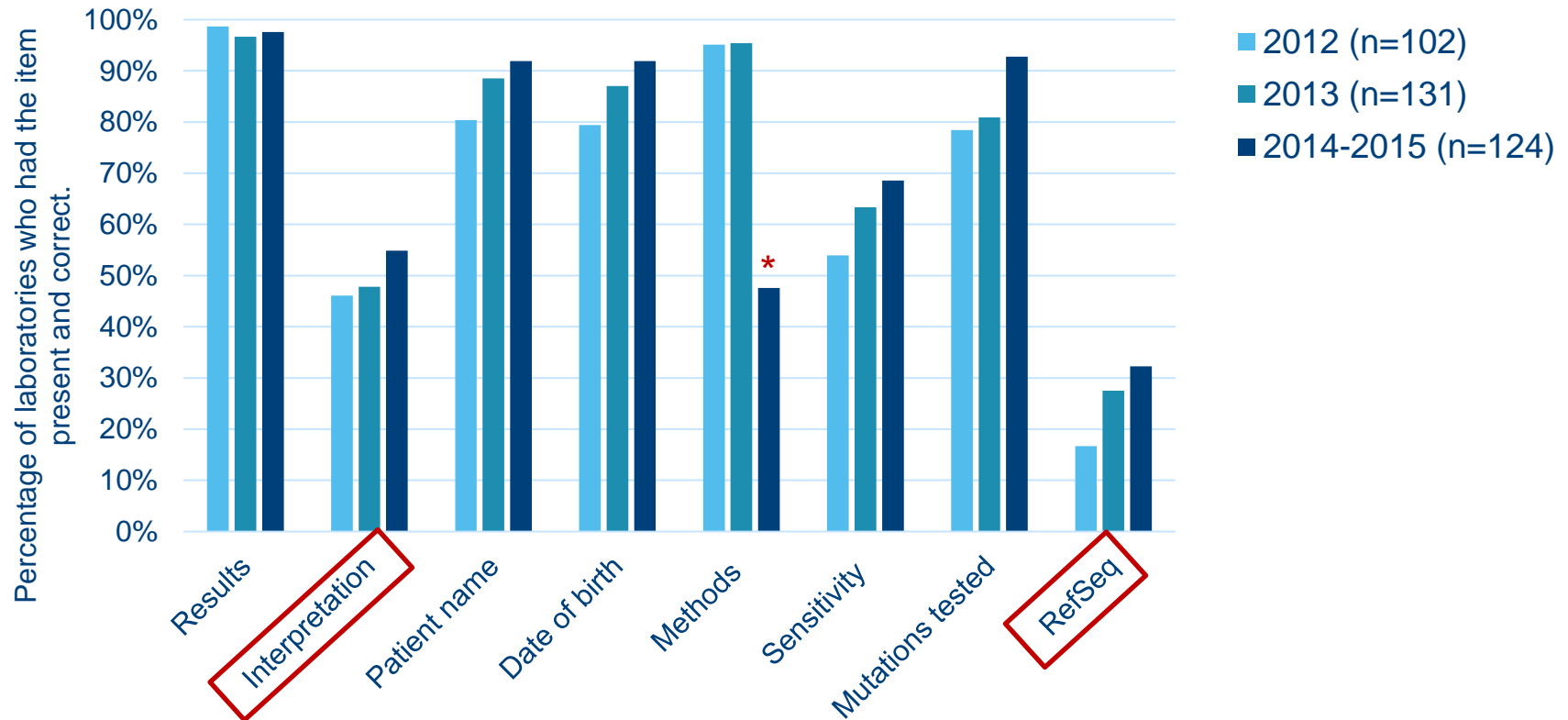


ESP Colon EQA Schemes: overview

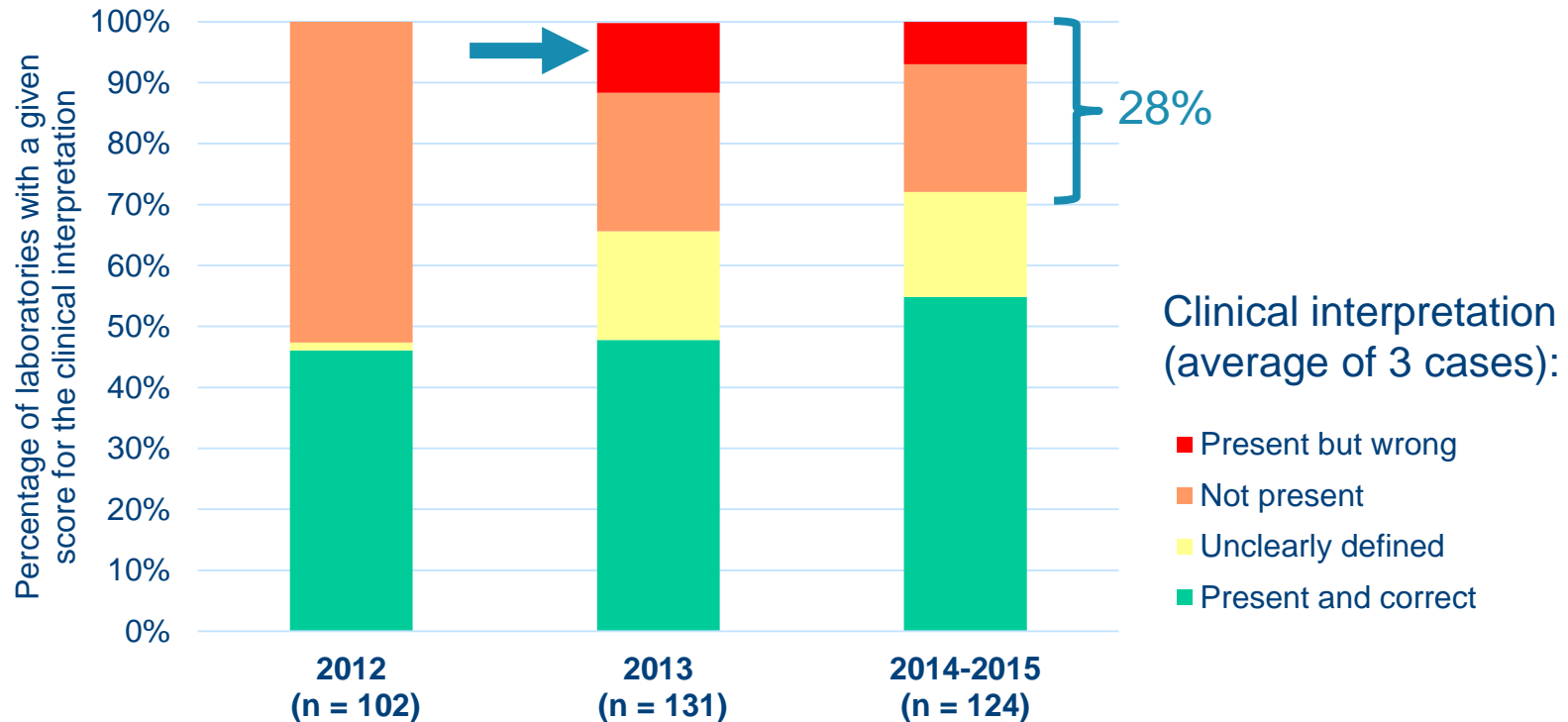
Technological advances: rapid expansion of number of NGS users for *RAS* over the past years



ESP colon EQA scheme: Reporting



ESP colon EQA scheme: Reporting



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



ESP Lung EQA Scheme: organized since 2012

<http://www.esp-pathology.org>

European Society of Pathology

Home Society Search Microscopy Account ESP

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ESP Lung EQA Scheme

ALK Testing

Predictive Biomarkers in Lung Cancer

European Database to Standardize EQA Evaluation for Acquired Mutations in Molecular Pathology

ESP LUNG EQA Scheme

For more information about the ESP QA Lung Scheme, please follow this link:
<http://lung.eqascheme.org/>

ESP Lung External Quality Assessment Scheme

Introduction

The [European Society of Pathology](#) (ESP) established an EQA program for testing biomarker mutations in non lung carcinoma (NSCLC). This program aims to ensure optimal accuracy and proficiency in lung cancer biomarker testing across all countries.

The practical organization of this European EQA program is done in collaboration with the members of the **ESP Lung EQA scheme steering committee and the Biomedical Quality Assurance Research Unit of the KU Leuven**, lead by Prof. Dr. E Dequeker. The ESP Lung EQA program works in close contact with Prof. Dr. H van Krieken, president of the ESP.

This scheme is in collaboration with UK NEQAS ICC&ISH.

The ESP EQA schemes are accredited by BELAC conform the ISO 17043, which is the international standard for conformity assessment of proficiency testing.

A pilot ESP Lung EQA scheme was run in 2012 in two rounds. The first pilot round contained only ALK testing (by FISH), while the second consisted of a combined EGFR, KRAS and ALK (IHC, FISH or RT-PCR) testing. The ESP Lung EQA Scheme 2014 was organized in two separate rounds: EGFR mutation analysis followed by ALK testing (FISH, FISH cases, IHC and/or RT-PCR) and ROS1 testing (FISH and/or IHC).

The ESP Lung EQA Scheme 2015 will be organized in three separate rounds: first ALK testing (by FISH and/or RT-PCR), followed by ROS1 testing (by FISH and/or IHC) and then EGFR mutation analysis. It is possible

<http://lung.eqascheme.org>

HOME | PARTICIPANTS

ALL LABS | ALL CONTACTS | LABS 2012 | CONTACTS 2012 | ADMIN | ASSESSOR | TICKETS | FIND A LAB | Search

ESP Lung External Quality Assessment Scheme

Information for Participants

- Introduction
- Registration
- Set up of the schemes and kind of samples
- Data analysis and evaluation of EQA scheme results
- Communication of results
- Time line of the ESP Lung EQA scheme
- Steering committee members
- Confidentiality

Introduction

The [European Society of Pathology](#) (ESP) established an EQA program for testing biomarker mutations in non lung carcinoma (NSCLC). This program aims to ensure optimal accuracy and proficiency in lung cancer biomarker testing across all countries.

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This scheme is in collaboration with UK NEQAS ICC&ISH.

The ESP EQA schemes are accredited by BELAC conform the ISO 17043, which is the international standard for conformity assessment of proficiency testing.

The ESP Lung EQA Scheme 2016 will be organized in four separate rounds: first ALK testing (by FISH and/or RT-PCR), followed by ROS1 testing (by FISH and/or IHC). It is possible to register separately for each round. The second round will be organized which includes a number of theoretical cases to evaluate the interpretation of results.

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



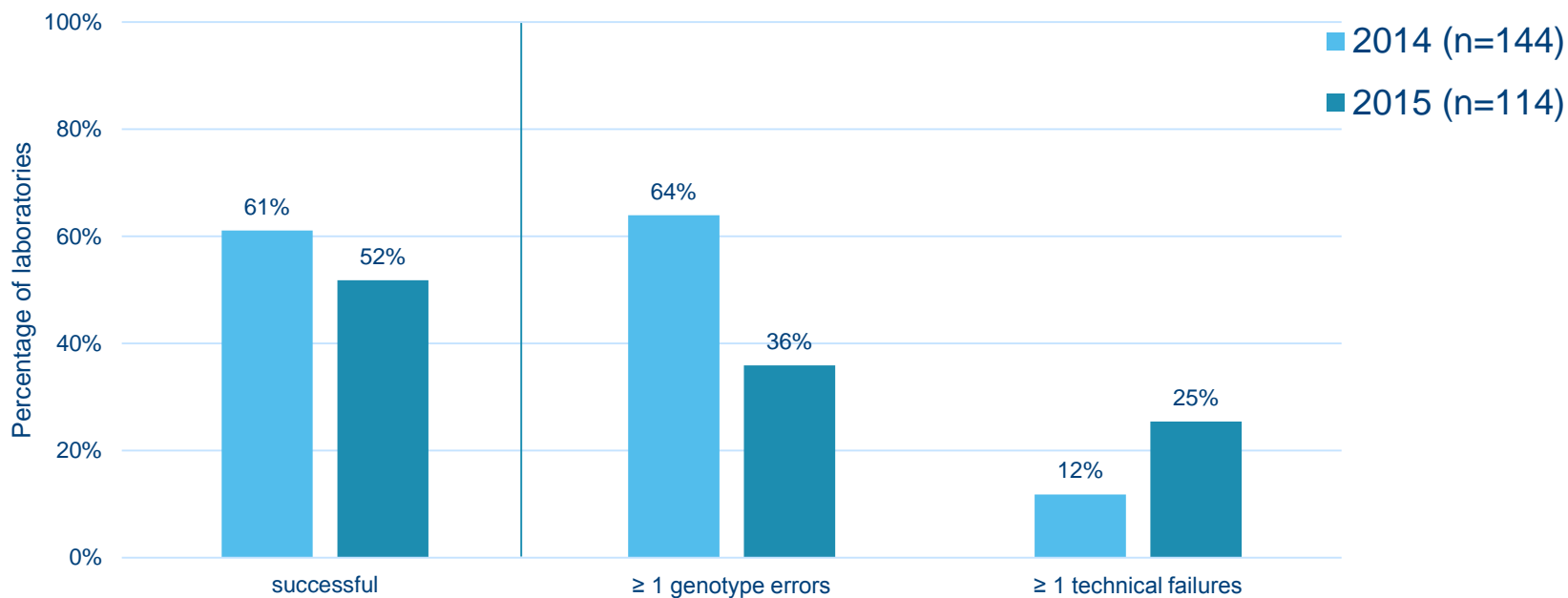
ESP Lung EQA Schemes: *EGFR*

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



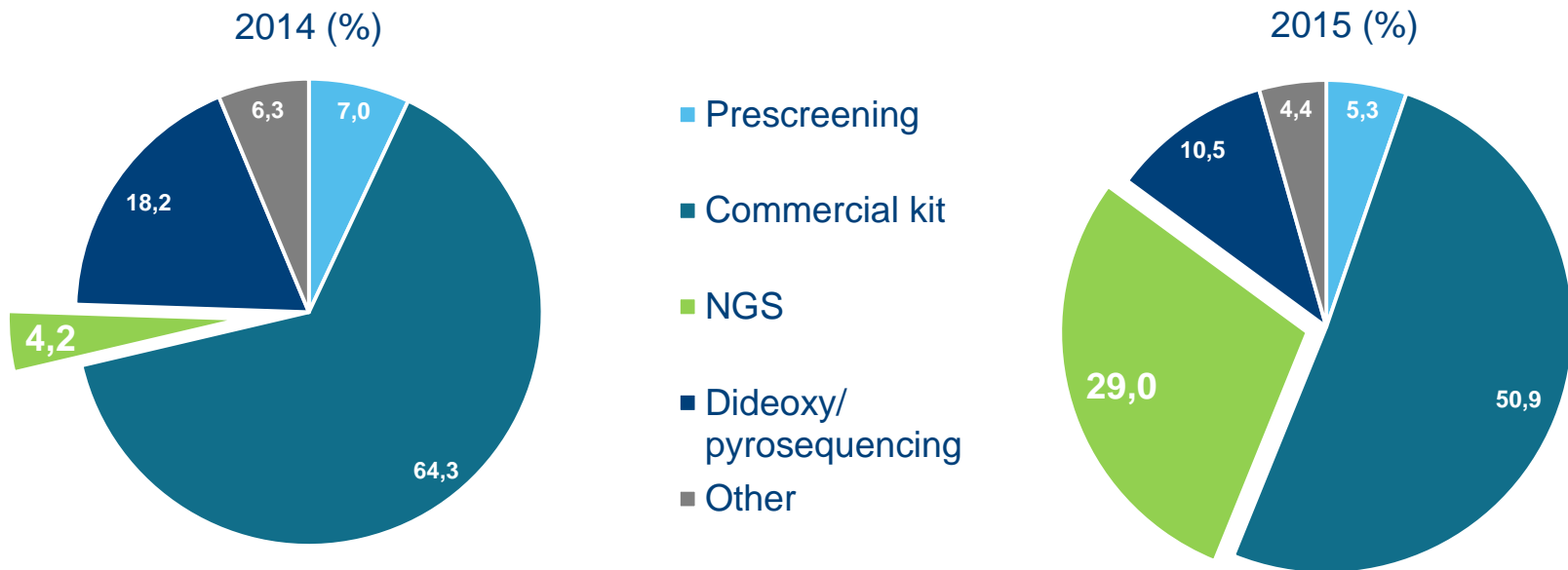
- 8/114 (7%) laboratories reported an additional SNP as a mutation (not calling it a SNP) or an additional mutation (1 point deducted)



ESP Lung EQA Scheme 2014 - 2015

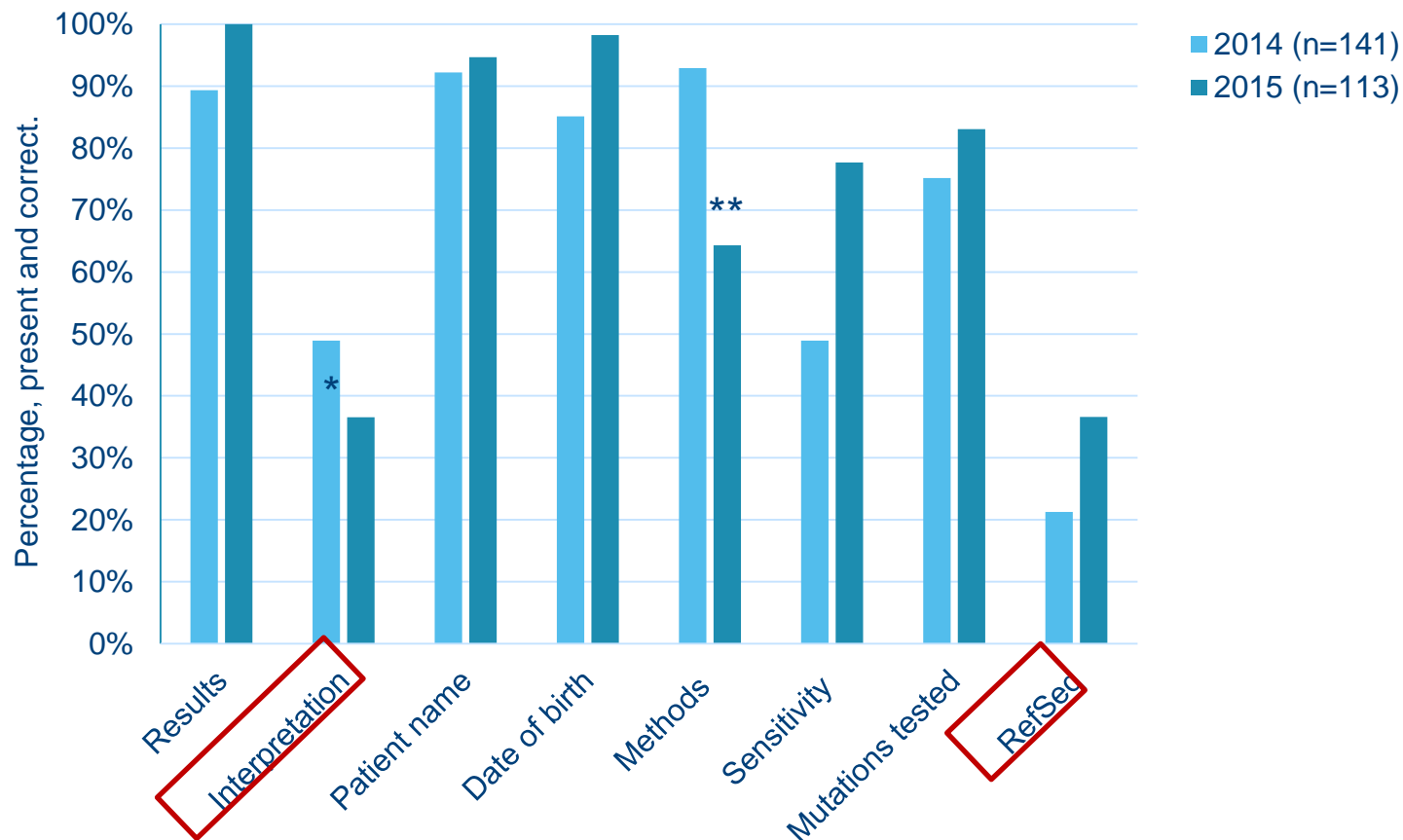
Technological advancement:

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





ESP Lung EQA Scheme 2014-2015: Reporting

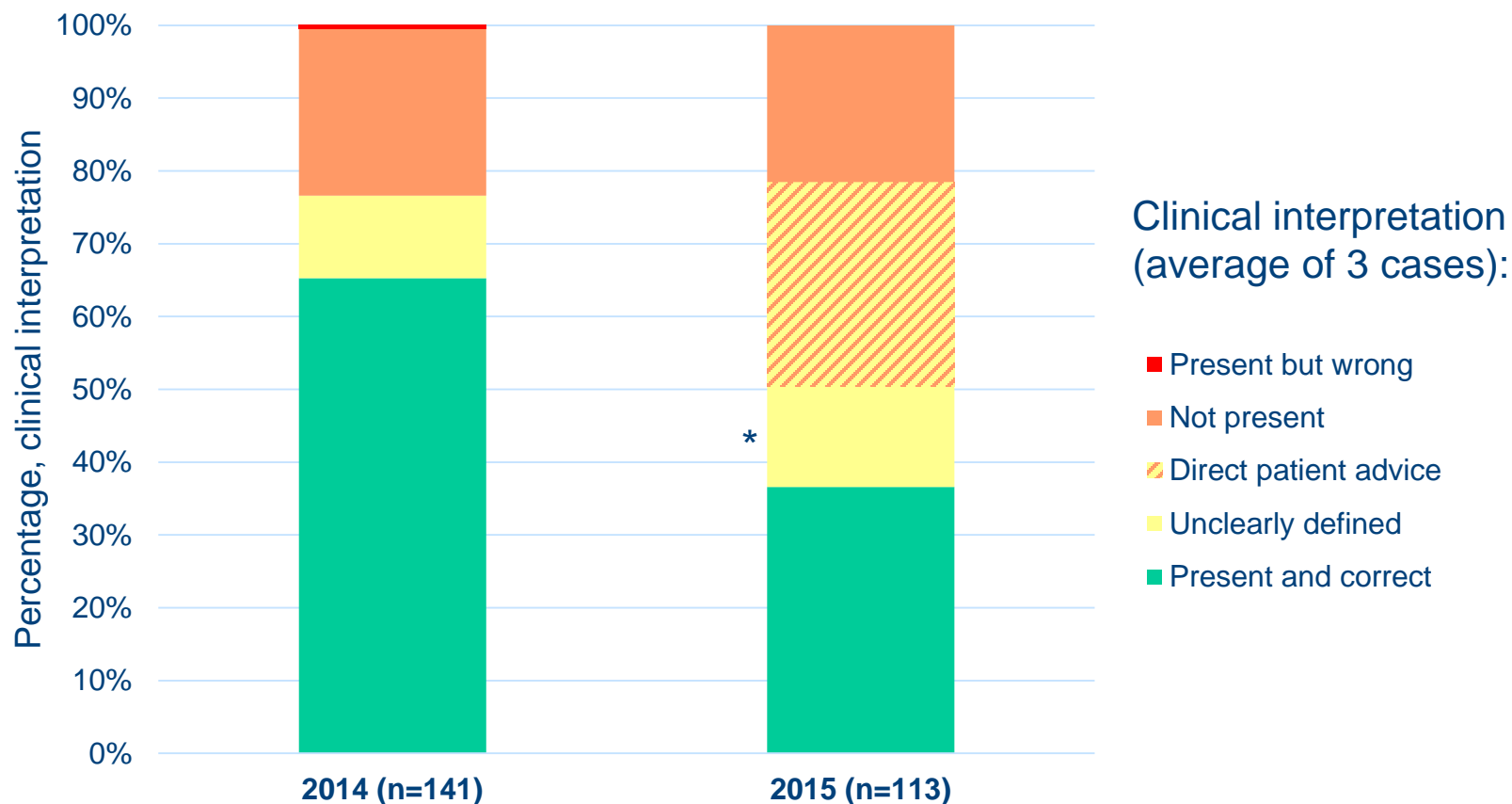


*One case with combined p.(Thr790Met) and p.(Leu858Arg) was considered incorrect if therapy was recommended without knowledge of clones/allelic frequencies, except if the advise of a tumor molecular board wass recommended.

**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%

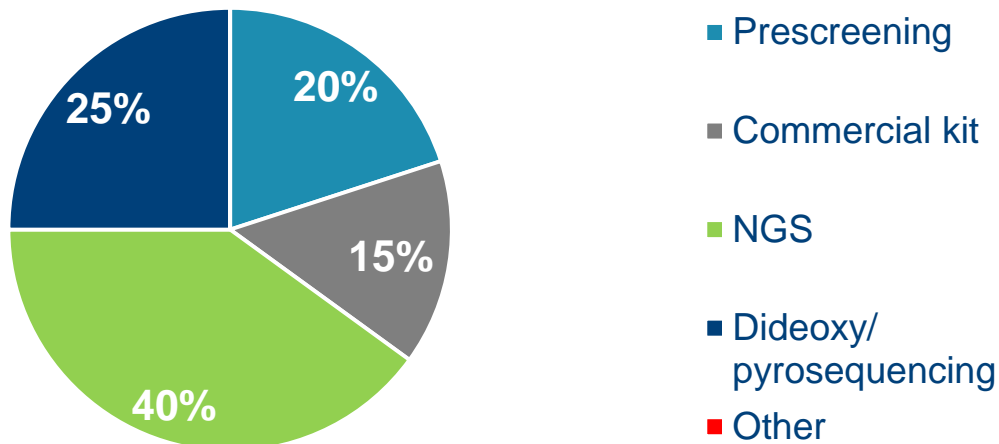
Incorrect use of HGVS nomenclature:

- **39%** and **45%** of NL laboratories in 2014-2015 resp.
- 18 laboratories participated to 2014 and 2015:
- 6 laboratories: no HGVS error
- 4 laboratories: improved nomencl.
- 4 laboratories: kept error between 14-15
- 4 laboratories: made error since 2015

ESP Lung EQA Schemes: Methods (NL)



EGFR 2015 (n=20)



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%

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).

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
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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

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

Evolution in NSCLC



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 - 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%

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



ALK IHC pilot scheme for technical evaluation of immuno-staining



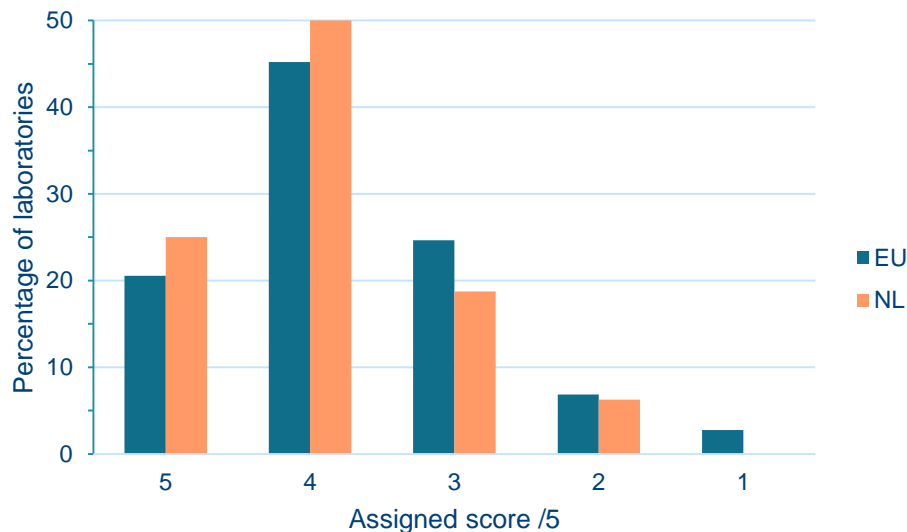
73 EU participants/25 countries

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

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%***

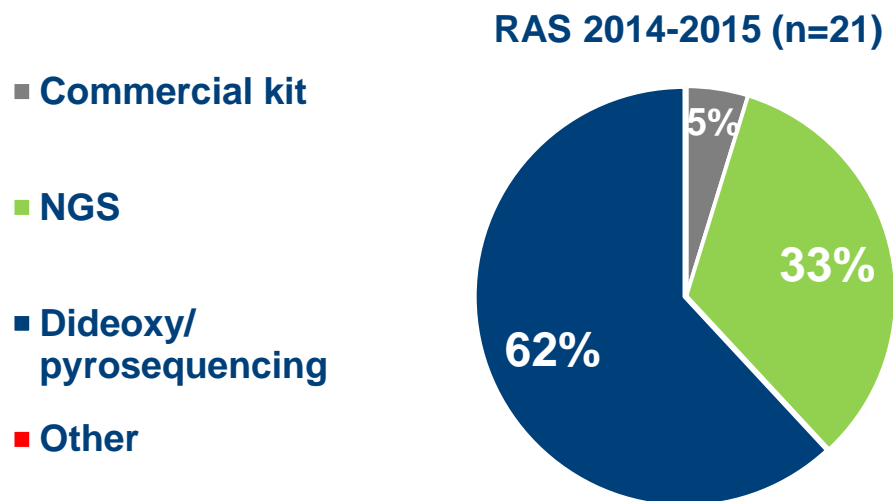
* 17 full *RAS* testing; 3 *KRAS* testing

** More stringent criteria: no major genotyping error and a score on technical evaluation of $\geq 18/20$

*** Preliminary results



ESP Colon EQA Schemes: Methods (NL)



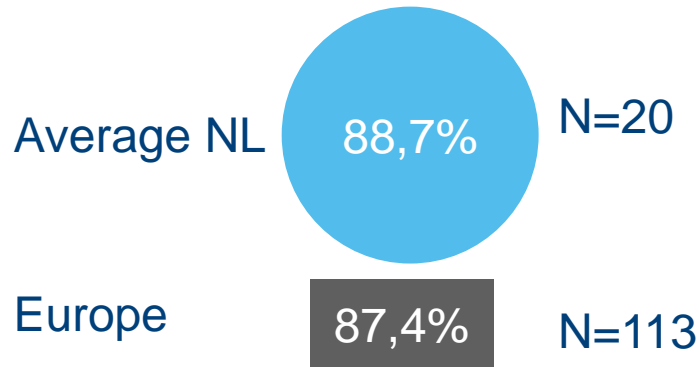
Panel	RAS 2014-2015
Ampliseq Custom panel Regions selected by the laboratory (Life technologies)	28,6%
Ion AmpliSeq Colon and Lung Cancer Panel (Life technologies)	42,9%
Ion Ampliseq hotspot cancer panel v2 (Life technologies)	14,3%
GS Junior (Roche)	14,3%



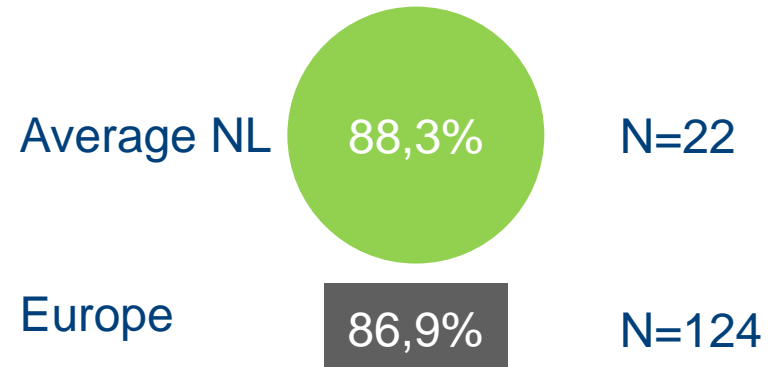
ESP Lung/Colon EQA Schemes: Reporting



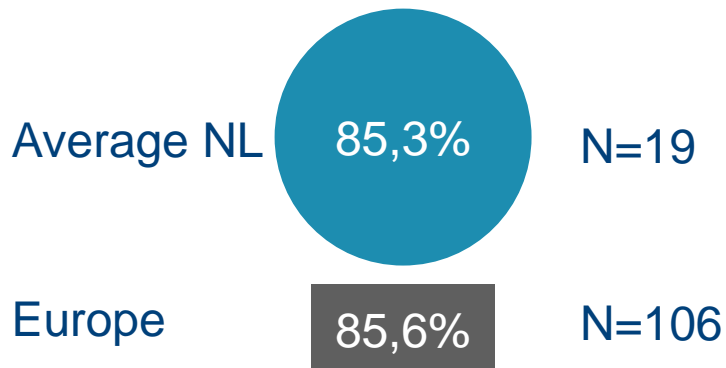
Lung 2015: *EGFR*



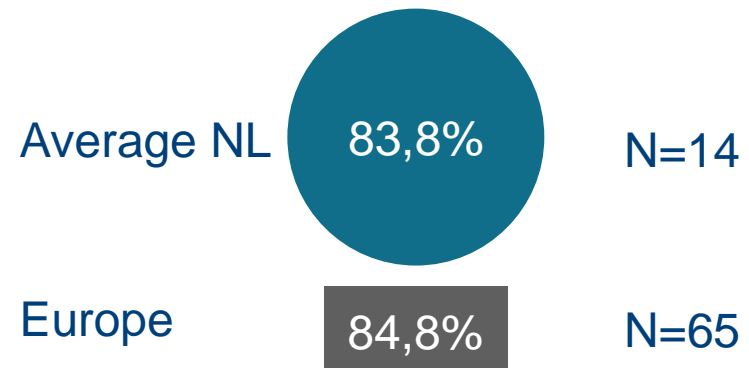
Colon 2014-2015



Lung 2015: *ALK*



Lung 2015: *ROS1*



Conclusions

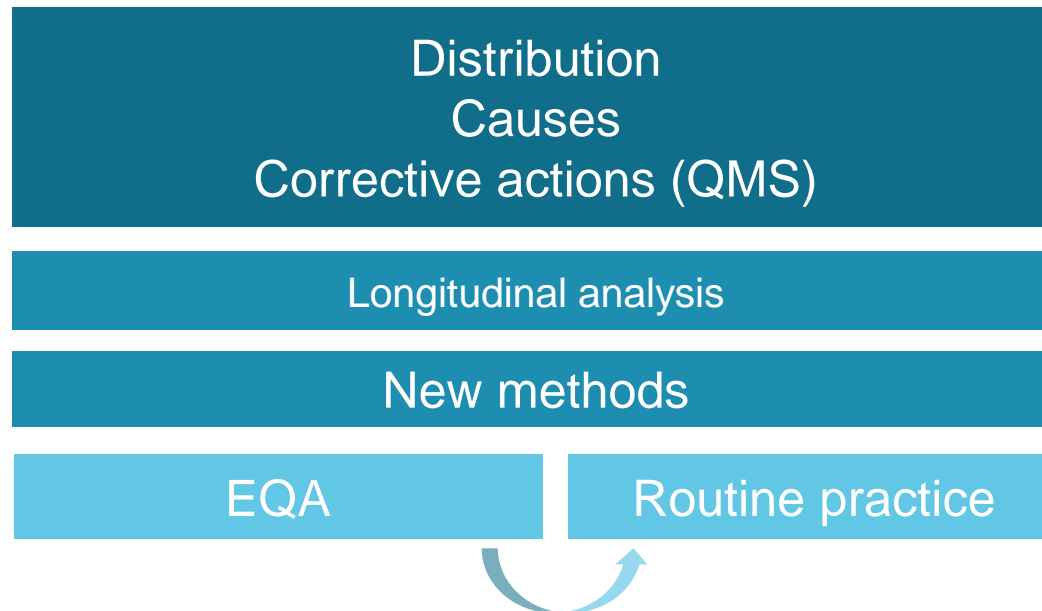
- The ESP EQA schemes **highlight the need for continuing EQA**
- Some labs do not test all required RAS codons still
- EQA scheme assesses not only the laboratory's **ability to obtain accurate, reliable results**, but also **the ability to safely interpret the results** and ensure that the referring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

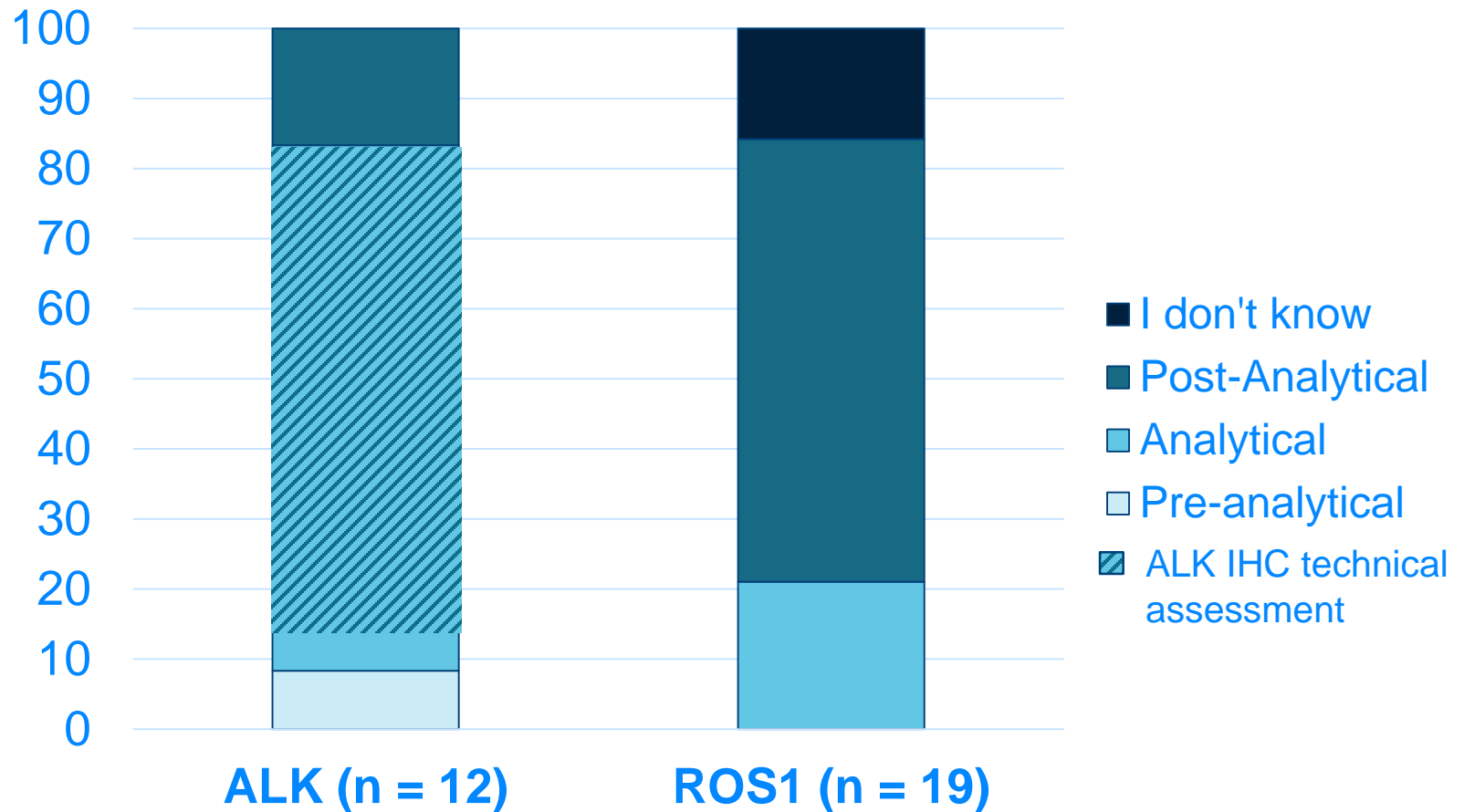
Results of the 2015 ESP ALK/ROS1 EQA scheme

- 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)

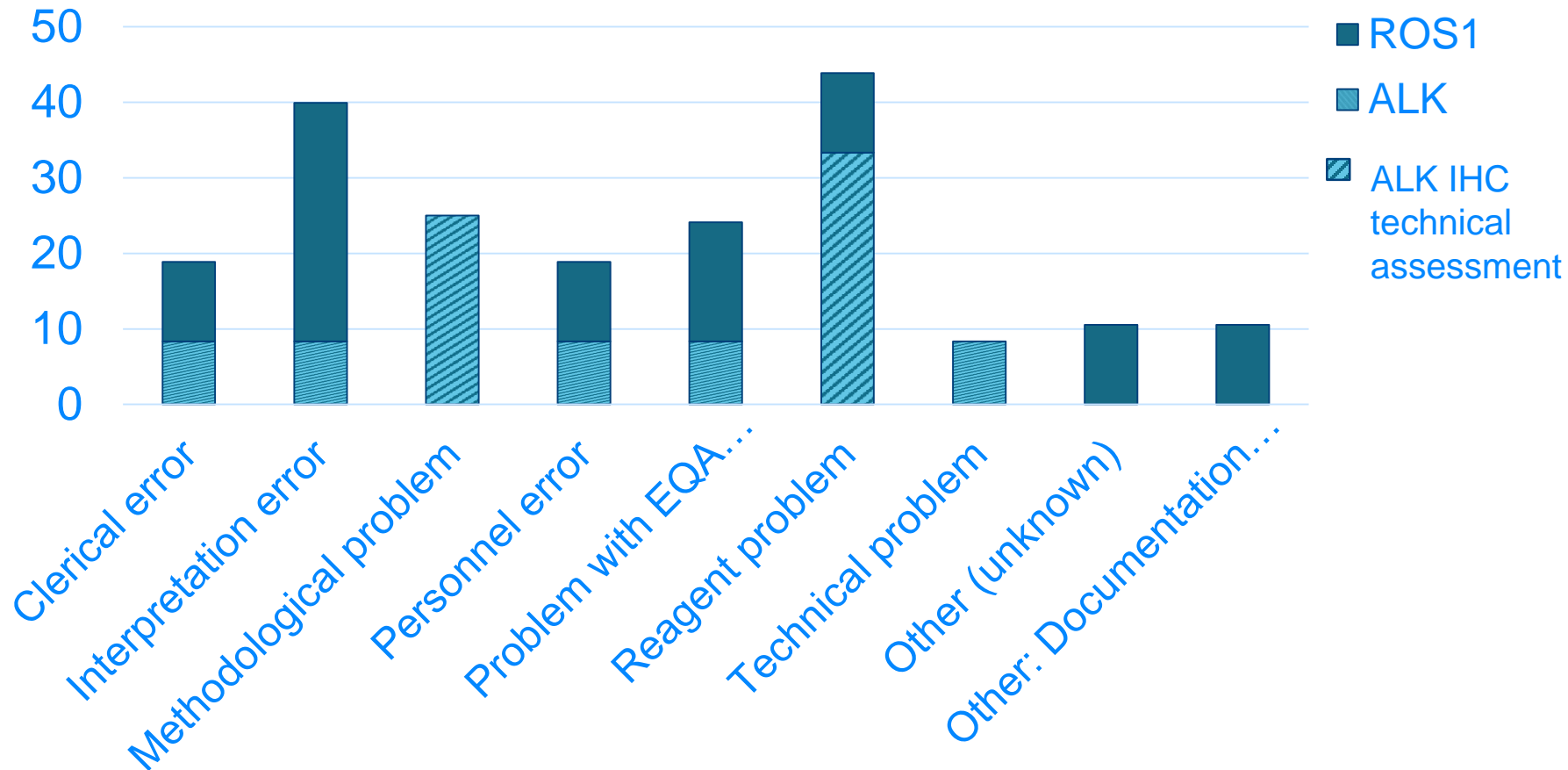


31 errors analyzed

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

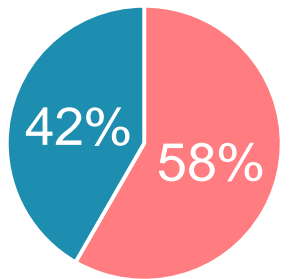


Percentage of error causes per EQA scheme

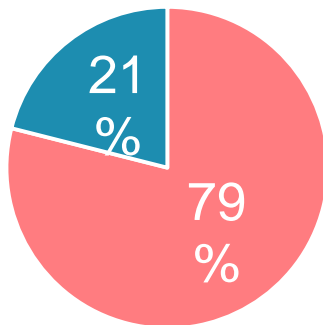


ALK versus ROS1

Do you think about changing your method next year?

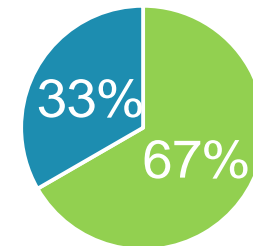


■ No ■ Yes



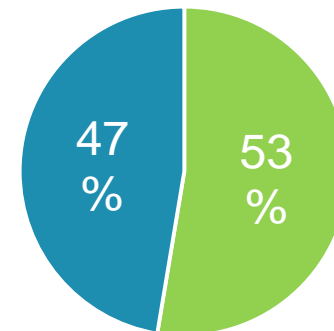
Was this error detected before/after EQA results were released?

ALK
(n=12)

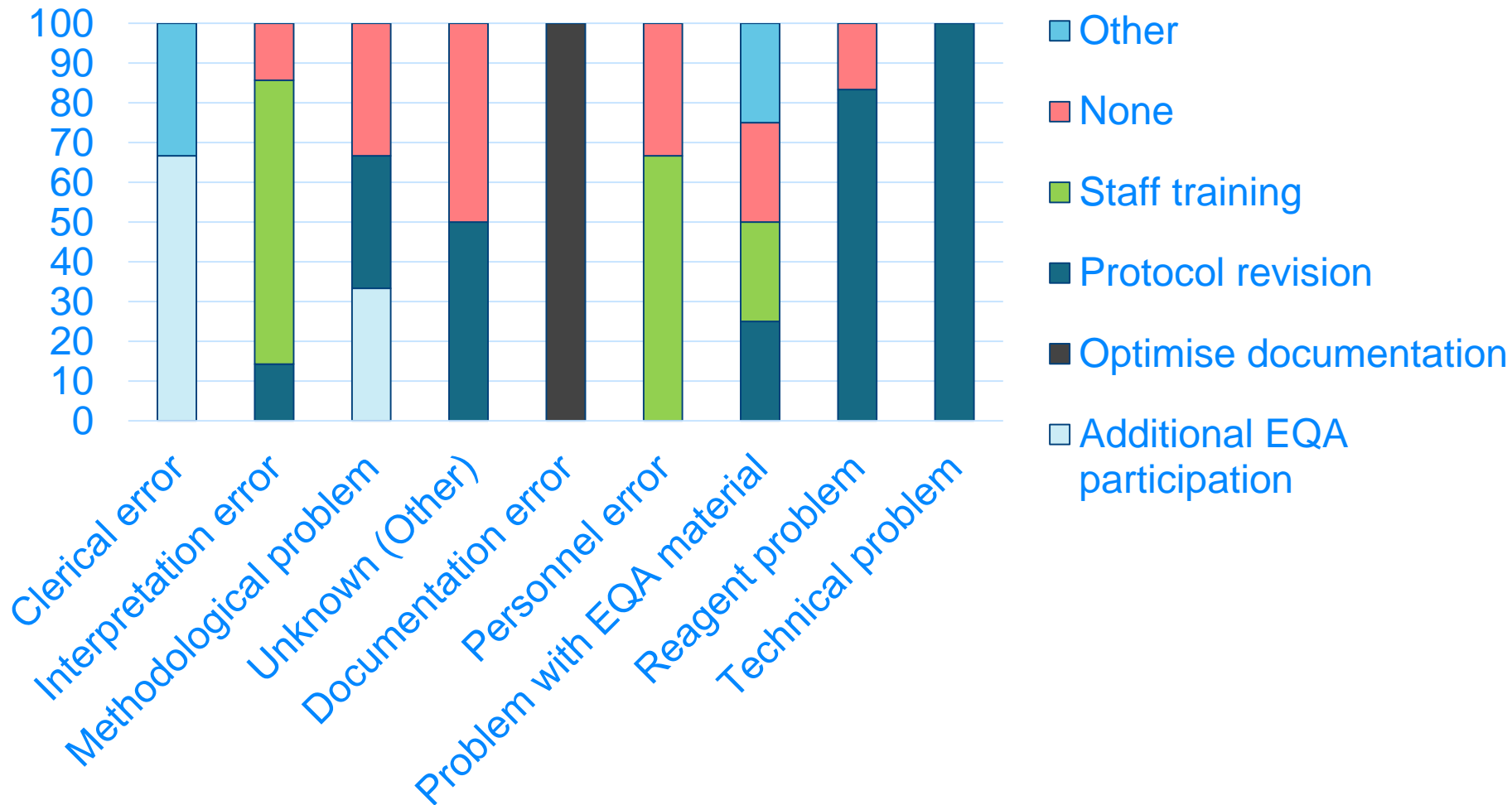


■ After ■ Before

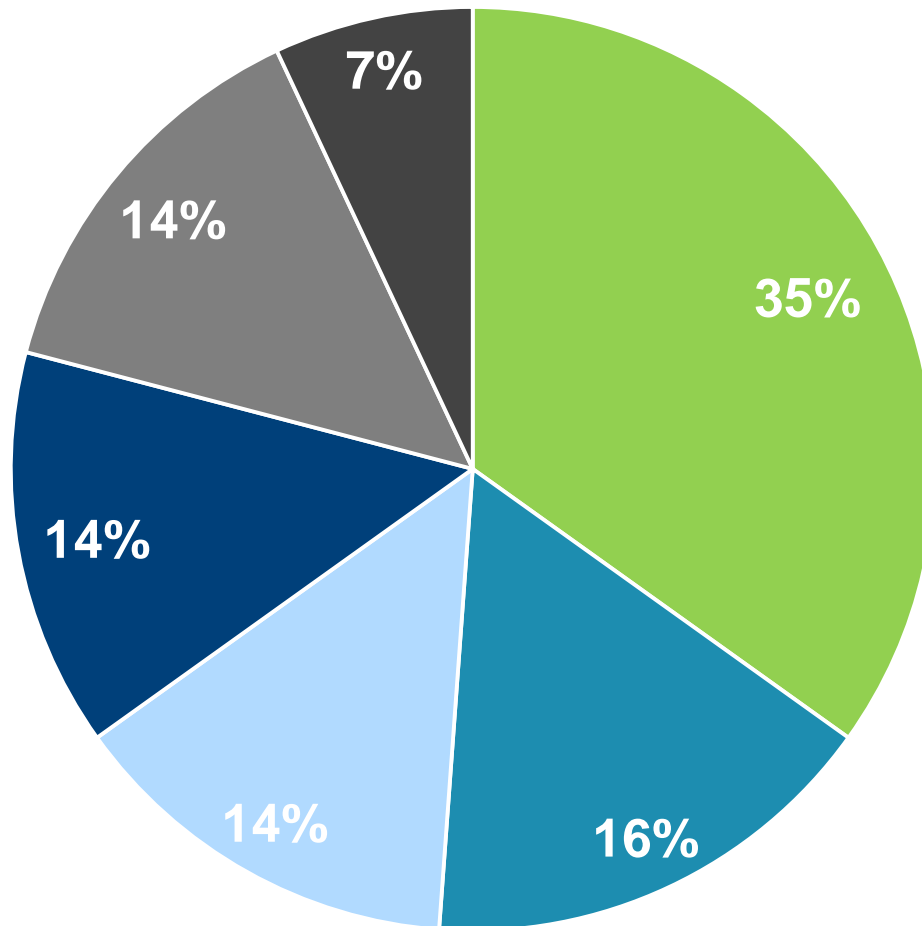
ROS1
(n=19)



Corrective actions per error type



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

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

Acknowledgements

**Biomedical Quality Assurance Research
Unit, KU Leuven**

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