EXPERTISE AND SERVICE PROVISION
QUALITY OF LABORATORIES

CLINICAL BIOLOGY
&
PATHOLOGICAL ANATOMY
COMMISSIONS
COMMITEE OF EXPERTS
WORKING GROUP EQA

EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY
and
PATHOLOGICAL ANATOMY

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A preliminary version of this report was submitted to the experts on the 2020/11/26.

This report was discussed at the meeting of the committee of experts/ working group EQA on the 2020/12/03.

Due to the COVID crisis, a summary of this report could not be presented at the meeting of the Commission of clinical biology.
The results were discussed during the commission of pathological anatomy of the 2020/01/20.

Autorization to release the report: By Bernard China, scheme coordinator, on 2020/12/22

All the reports are also available on our webpage:
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_nl/rapports_annee.htm
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_fr/rapports_annee.htm

TABLE OF CONTENTS

Table of Contents

Introduction

In 2019, 5 surveys were organized by Sciensano for the article 33 bis of the INAMI/RIZIV nomenclature.
Survey 1 (13/05/2019) was dedicated to the detection of JAK2 V617F mutation.
Survey 2 (29/07/2019) was dedicated to the detection of mutations in K-RAS gene.
Survey 3 (09/09/2019) concerned the detection of EGFR gene mutations
Survey 4 (14/12019) was dedicated to the detection by ISH of the Her2 gene amplification.
Survey 5 (11/11/2019) concerned the blood grouping other than ABO Rh, the determination of weak and the determination of D variant.
For some parameters in hemato-oncology, Sciensano support the participation to the UKNeqas panel: http://www.ukneqasli.co.uk/. A reimbursement is organized in 2019 and 2020 for laboratories sharing their results with us.

Results

JAK2

Samples

The samples J-1901 and J-1902 were 20µl genomic-DNA in Tris-EDTA buffer. The concentration was 50ng/µl. The samples are produced by Horizon Discovery Ltd-Waterbeach, United Kingdom and sold by Amplitech, Compiègne, France. Horizon guaranties the presence and the allelic frequencies of the V617F mutation.

Participants

17 laboratories of clinical biology were registered and none of anatomic pathology biology.16 laboratories (94.1%) encoded results. One laboratory encoded two datasets, one qualitative and one quantitative for different methods.

Results

Besides the analysis of the samples, participants were asked to provide their method, the provider and the model of the device used for the analysis and the corresponding allelic frequency of JAK2 mutation if the laboratory was doing a quantitative analysis.

1. Results per sample

Table 1.1. Expected and encoded results

Sample	Expected detected mutations (AF)	Encoded results
J-1901	No mutation	15/17 (88.2%): no mutation
		2/17 (11.8%): JAK2 V617F detected
J-1902	NM_004972.3(JAK2):c.1849G>T (p.Val617Phe) **(10%)**	15/17 (88.2%): JAK2 V617F detected
		2/17 (11.8%): not detected

NB: the total is displayed out of 17 as a laboratory have encoded results for both qualitative and quantitative methods.

On the 34 encoded qualitative results, 30 (88.2%) were correct.

2. Results per laboratory and per method

11 laboratories declared doing a quantitative detection of JAK2 V617F; 15 declared doing a qualitative one. Among those, 10 laboratories are doing both type of detection: 9 of them by using the same technique for both and one laboratory used a different technique.

Table 2.1. Results per lab and per method

Lab.	Method	Quantitative	J1901 (AF)	J1902 (AF)
J1	Digital droplet PCR (BioRad)	Yes	**D (10%)**	**ND**
J2	Denys et al. 2010	Yes	ND	D (11%)
J3	Denys et al. 2010	Yes	ND	D (8.5%)
J4	Locked nuclear acid real-time PCR	Yes	ND	D (9%)
J5	Real-time PCR with LNA lokker in house	Yes	ND	D (6.04%)
J6	TruSight Myeloid NGS Illumina	Yes	ND	D (14%)
J7	TruSight Myeloid NGS Illumina	Yes	ND	D (9.5%)
J8	Home made melting curve	No	ND	D
J8	Pyrosequencing home made	Yes	**D (1%)**	D (9%)
J9	In house allele specific PCR	No	ND	**ND**
J10	Ipsogen JAK2 Mutaquant	Yes	ND	D (20.5%)
J11	Ipsogen JAK2 MutaScreen Kit	No	ND	D
J12	LDT Real-Time PCR	No	ND	D
J13	Real-time PCR, In-House assay	No	ND	D
J14	TruSight custom amplicon	No	ND	D (10%)
J15	In house	No	ND	D
J16	In house	No	ND	D (5.26%)

D=detected
ND=not detected

The wrong results are indicated in red.

3. Comments

- One of the laboratory that have given a wrong has in fact inverted the two samples.
- One laboratory encoded correct results for qualitative results but gave a positive result (1%) for the quantitative result of the negative sample. Nevertheless, two different methods were used for qualitative and quantitative analysis.
- One laboratory encoded a negative result for the positive sample.

4. Methods

According to the participants, the following methods were used for (i) the quantitative detection and (ii) the qualitative detection.

Table 4.1 Methods for the quantitative detection

Method for the qualitative detection	N	%
Denys et al. J Md Diagn 2010	2	12.5
Home made melting curve	1	6.25
In house allele specific PCR*	1	6.25
Ipsogen JAK2 Mutaquant	1	6.25
Ipsogen JAK2 MutaScreen Kit*	1	6.25
LDT	1	6.25
LDT Real-Time PCR*	1	6.25
Locked nuclear acid real-time PCR	1	6.25
Real-time PCR, In-House assay*	1	6.25
Real-time PCR with LNA lokker in house	1	6.25
TruSight Myeloid NGS Illumina	2	12.5
TruSight custom amplicon*	1	6.25

*laboratories doing only a qualitative analysis

Table 4.2 Methods for the qualitative detection

Method for the quantitative detection	N	%
Digital droplet PCR with dd PCR mutation assay from Biorad*	1	6.25
Denys et al. J Md Diagn 2010	2	12.5
Pyrosequengage home made	1	6.25
Ipsogen JAK2 Mutaquant	1	6.25
LDT	1	6.25
Locked nuclear acid real-time PCR	1	6.25
Real-time PCR with LNA lokker in house	1	6.25
TruSight Myeloid NGS Illumina	2	12.5

*laboratories doing only a quantitative analysis

K-RAS

Samples

The samples K-1901 and K-1902 were FFPE slices from continuous cell line. The samples are produced by Horizon Discovery Ltd-Waterbeach, United Kingdom and sold by Amplitech, Compiègne, France. Horizon guaranties the presence and the allelic frequencies of certain KRAS mutations.

Participants

19 participants were registered: 12 laboratories of pathologic anatomy and 7 laboratories of clinical biology. 18 registered participants have encoded results (94.7%).

Results

Participants were asked to analyze the samples and were asked to select the KRAS mutation that they have detected withy the corresponding allelic frequency. The participants were also asked to provide their method, the provider and the model of the device used for the analysis.

1. Results per sample

Table 1.1. Expected and encoded results

Sample	Expected detected Mutations (AF)	Encoded results	N (%)
K-1901	No mutation detected	No mutation detected	18/18 (100)
K-1902	NM_004985.5(KRAS):c.35G>A (p.Gly12Asp) **(5%)**	Mutation detected	13/18 (72.2)
	NM_004985.5(KRAS):c.38G>A (p.Gly13Asp) **(5%)**	Mutation detected	18/18 (100)
	NM_004985.5(KRAS):c.183A>C (p.Gln61His) **(5%)**	Mutation detected	13/18 (72.2)
	NM_004985.5(KRAS):c.436G>A (p.Ala146Thr) **(5%)**	Mutation detected	13/18 (72.2)

One laboratory declared doing no distinction between the following KRAS mutation: 436G>A (A146T); 436G>C (A146P) and 436G>T (A146V) using the Idylla CE-IVD KRAS mutation test.

Table 1.2. Proficiency per sample

Sample	Mutations detected	N	%
K-1901	0/0	18	100
K-1902	4/4	13	72.2
	1 /4	5	27.8

2. Results per laboratory and per method

Table 2.1. Results per laboratory and per method

		K1901	K1902 (AF)			
Lab.	**Method**	NMD*	NM_004985.5(KRAS):c.35G>A (p.Gly12Asp)	NM_004985.5(KRAS):c.38G>A (p.Gly13Asp)	NM_004985.5(KRAS):c.183A>C (p.Gln61His)	NM_004985.5(KRAS):c.436G>A (p.Ala146Thr)
K1	PCR Idylla	NMD	**ND**	D	**ND**	**ND**
K2	PCR Idylla	NMD	**ND**	D	**ND**	**ND**
K3	PCR Idylla	NMD	**ND**	D	**ND**	**ND**
K4	PCR Idylla	NMD	**ND**	D	**ND**	**ND**
K5	PCR Idylla	NMD	**ND**	D	**ND**	**ND**
K6	PCR Idylla	NMD	D	D	D	D
K7	NGS MISEQ	NMD	D (4.2%)	D (5.3%)	D (4.8%)	D (7%)
K8	NGS MISEQ	NMD	D (4%)	D (5%)	D (5%)	D (5%)
K9	NGS MISEQ	NMD	D (3.55%)	D (4.48%)	D (4.13%)	D(3.4%)
K10	NGS MISEQ	NMD	D (3.6%)	D (8.1%)	D (8.4%)	D (13.6%)
K11	NGS MISEQ	NMD	D (13%)	D (15%)	D (26%)	D (37%)
K12	NGS MISEQ	NMD	D (4.5%)	D (6.1%)	D (4.9%)	D (5.7%)
K13	NGS MISEQ	NMD	D (4%)	D (5%)	D (5%)	D (5%)
K14	NGS MISEQ	NMD	D (4%)	D (5%)	D (5%)	D (5%)
K15	NGS MISEQ	NMD	D (5.54%)	D (4.31%)	D(4.76%)	D (5.64%)
K16	NGS NextSeq	NMD	D (5%)	D (4%)	D (4%)	D (4%)
K17	NGS Ion Studio	NMD	D (5%)	D (5%)	D (4%)	D (4%)
K18	NGM Ion PGM	NMD	D (5%)	D (7%)	D (3%)	D (4%)

NMD= no mutation detected
ND= not detected
D= detected

3. Comments

- The five laboratories that have detected only one KRAS mutation have detected the same mutation: p.Gly13Asp (G13D) using the Idylla KRAS mutation test (Biocartis). The LOD of the method is ≤ 5% according to the manufacturer of the test and all the other mutations can be detected via this test also according to the manufacturer. Among these five laboratories 4 have registered for the cyclus 2020. Their results with the same method were good but the mutation to be detected was not the same as in 2019 and also the allelic frequencies.

4. Methods

According to participant's answer, the following methods were used for the detection of KRAS mutations.

Table 4.1 Methods

Method	N	%
PCR Idylla KRAS mutation test	6	33.3
NGS	12	66.7
Miseq	9	50.0
Nextseq	1	5.6
Ion gene studio	1	5.6
Ion PGM	1	5.6

EGFR

Samples

The samples E-1901, E-1902 and E-1903 were FFPE slices from continuous cell line. The samples are produced by Horizon Discovery Ltd-Waterbeach, United Kingdom and sold by Amplitech, Compiègne, France. Horizon guaranties the presence and the allelic frequencies of certain EGFR mutations.

Participants

19 participants were registered: 12 laboratories of anatomic pathology and 7 laboratories of clinical biology. 16 registered participants have encoded results (84.2%).

Results

Participants were asked to analyze the samples and were asked to select the EGFR mutation that they have detected with the corresponding allelic frequency. The participants were also asked to provide their method, the provider and the model of the device used for the analysis.

1. Results per sample

Table 1.1. Expected and encoded results

Sample	Expected detected mutations (AF)	Encoded results	N (%)
E-1901	NM_005228.5(EGFR):c.2369C>T (p.Thr790Met) (20 %)	Mutation detected	15/16 (93.75)
E-1902	NM_005228.5(EGFR):c.2155G>A (p.Gly719Ser) (50 %)	Mutation detected	15/16 (93.75)
E-1903	No mutation (WT)	No mutation detected (WT)	15/16 (93.75)

2. Results par laboratory and per method

Table 3.3. results per laboratory and per method

		E-1901 (AF)	E-1902 (AF)	E-1903
Lab.	**Method**	NM_005228.5(EGFR):c.2369 C>T (p.Thr790Met)	NM_005228.5(EGFR):c.2155 G>A (p.Gly719Ser)	NMD
E1	Cobas EGFR	D	D	NMD
E2	PCR Idylla	D	D	NMD
E3	PCR Idylla	D	D	NMD
E4	PCR Idylla	D	D	NMD
E5	PCR Idylla	D	D	NMD
E6	PCR Idylla	D	D	NMD
E7	PCR Idylla	D	D	NMD
E8	NGS Miseq	D (15%)	D (50%)	NMD
E9	NGS Miseq	D	**Not Done**	**Not Done**
E10	NGS Miseq	D (21%)	D (50%)	NMD
E11	NGS Miseq	D	D	NMD
E12	NGS Miseq	D	D	NMD
E13	NGS Nextseq	D	D	NMD
E14	NGS PGM318	**Not Done**	D	**Not Done**
E15	NGS Ion Studio	D	D	NMD
E16	NGS Ion Studio	D (21%)	D (47%)	NMD

NMD= No mutation detected
D=Detected

3. Comments

- The laboratory that was not able to perform the analysis for the samples E-1902 and E-1903 declared a failure in the library preparation.
- The laboratory that was not able to perform the analysis for the samples E-1901 and E-1903 simply declared a technical problem.

4. Methods

Table 4.1. Methods

Method	N	%
Cobas EGFR mutation test V2	1	6.25
Idylla EGFR mutation assay	6	37.5
Illumina Miseq	5	31.25
Illumina Nextseq	1	6.25
Ion Torrent PGM 318	1	6.25
Ion Torrent S5 gene studio	2	12.5

Her 2

Samples

The samples H-1901 and H-1902 were made of FFPE slices from continuous cell lines mounted on glass slides. The samples were produced by Horizon and distributed by Amplitech. Horizon guaranties the amplification or not of the HER2 gene.

Participants

24 laboratories were registered: 21 laboratories of pathologic anatomy and 3 laboratories of clinical biology. 24 laboratories (100%) encoded results.

Results

Participants were asked to analyze the slides provided and to interpret them according to the guidelines that they are using for the detection of Her2 amplification. Participants were asked to cite the guidelines to which they are referring besides the method and the kit that they used.

1. Results par sample

Table 4.1. Expected and encoded results

Samples	Expected results	Encoded results
H-1901	Not amplified	23 (95.8%): Not amplified
		1 (4.2 %): no interpretation possible (due to an artefact)
H-1902	Amplified	23 (95.8%): Amplified
		1 (4.17 %): no interpretation possible (due to an artefact)

NB: An error state in the preliminary report where we indicated that 1 laboratory has answered amplified for the sample H-1901.

2. Guidelines

Table 2.1. Used guidelines

guidelines	N	%
ASCO-CAP 2018	20	83.3
ASCO/ CAP 2013	0	0
Belgian Guidelines 2014	1	4.2
ASCO/ CAP 2018 + Belgian Guidelines 2014	3	12.5

3. Comments

- The laboratory that was not able to interpret the slides due to an artefact used the SISH INFORM (Ventana) technique.

4. Methods

According to participant's answer, the following methods were used for the detection of the amplification of the HER2 gene.

Table 4.1. Methods

Method	N	%
SISH INFORM Ventana	14	58.3
HER2 dual ISH probe Ventana	2	8.3
PathVysion Her2-DNA probe kit, Abbott	4	16.6
Her 2 IQ FISH pharmDX, DAKO	4	16.6

MOLECULAR HEMATOLOGY

Samples

The samples MH1901 was human DNA extracted from the blood of a healthy donor (20 µg/mL). The laboratories received two vials of 100 µl each.

Participants

9 laboratories participated and have encoded results for this survey.

Results

Participants were asked to determine the presence of other antigens than ABO en Rh with molecular methods. They were also asked to determine the presence or absence of a weak/variant D.

1. Genotyping of blood grouping different than ABO Rh

Table 1.1. Expected results.

Antigenic system	Consensus results	Encoded results	N (%)
MNS	M+N-, S+, š-, U var-	M pos N neg, S Pos, špos, U var neg	2 (22.2)
		M pos N neg, S Pos, špos	5 (55.55)
		S+, š-	1 (11.1)
		M pos S Pos, š pos	1 (11.1)
Lutheran	Lua- Lub+	Lua- Lub+	4 (44.4)
		NVT	5 (55.55)
Kell	K- k+,kpa-, kpb+, Jsa-, Jsb+	K neg k pos, kpa neg, kpb pos, Jsa neg, Jsb pos	3 (33.3)
		K-, k +, kpa -, kpb +	2 (22.2)
		k +	1 (11.1)
		K-, k+	3 3 (33.3)
		kk	1 (11.1)
Duffy	Fya-, fyb+, fy GATA-, fyX-	Fya neg, fyb pos, zwakke fyb neg, fy GATA neg, fyX neg	1 (11.1)
		Fya-, fyb+,	5 (55.55)
		Fya -, fyb +, fyN -, fy X -	1 (11.1)
		fyb pos	1 (11.1)
Kidd	Jka-, Jkb +	Jka-, Jkb +	7 (77.7)
		Jkb +	2 (22.2)
Diégo	Dia-, diab +	Dia-, diab +	4 (44.4)
		NVT	5 (55.55)
Scianna	Sc 1+, 2-	Sc 1+, 2-	1 (11.1)
		NVT	8 (88.9)
Dombrock	Doa +, Dob +	doa + dob +	8 (88.9)
		NVT	1 (11.1)
Colton	Coa +, cob -	Coa +, cob -	4 (44.4)
		NVT	5 (55.55)
Landsteiner-Weiner	LW5, -7	LW5, -7	1 (11.1)
		NVT	8 (88.9)

VEL	VEL +	VEL +	3 (33.3)
		NVT	6 6 (66.7)
RhCE	CcEe	CcEe	4 (44.4)
		e - ċ +	1 (11.1)
		NVT	4 (44.4)
Wright	Wra-, Wrb +	Wra-, Wrb +	2 (22.2)
		NVT	7 (77.8)
Cartwright	Yta +, ytb-	Yta +, ytb-	3 (33.3)
		NVT	6 (66.7)
Knops	Kna + knb -	Kna + knb -	2 (22.2)
		NVT	7 (77.8)

The global results are correct for all antigen. There is only one lab that is not in agreement with the consensus answer for the RhCE antigen using the BAGene PCR SSCP method.

Table 1.2. Methods

Method	N (%)
Bio Array HEA Bead Chips (Immuncor)	1 (11.1)
BAGene PCR SSCP	2 (22.2)
home made	1 (11.1)
RBC FLuoGene Verify (Inno-Train)	1 (11.1)
RBC Ready Gene Verify (Inno-train)	3 (33.3)
RBC verify	1 (11.1)

2. Determination of weak D

7 laboratories encoded results. 1 laboratory declared that the quantity of material provided was not sufficient. 1 laboratory declared that he was not doing the test in routine.

Table 2.1. Expected results.

Samples	Consensus results	Encoded results (N, %)
MH-1901	No weak D phenotype	No weak D phenotype (7, 100 %)

3. Determination of D variant

8 laboratories encoded results. 1 laboratory declared that the quantity of material provided was not sufficient.

Table 3.1. Expected results.

Samples	Consensus results	Encoded results (N, %)
MH-1901	No D variant	No D variant (7, 87.5)
		D positive (1, 12.5)

END