QuIP External Quality Assurance in IHC & Molecular Pathology

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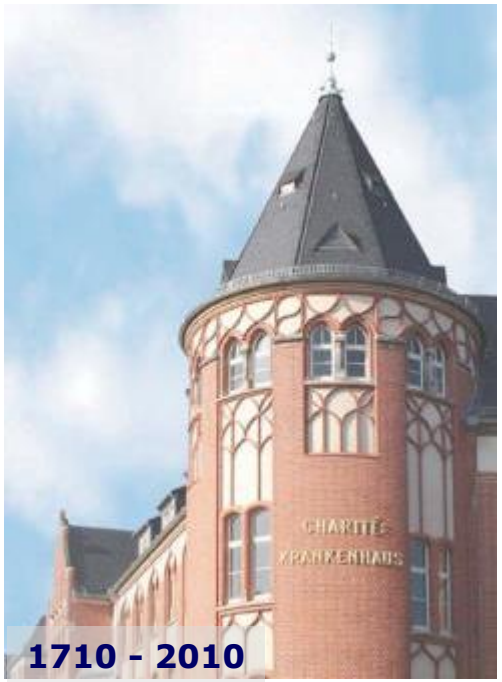

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QuIP® Ltd. → Quality Initiative in Pathology

DEUTSCHE GESELLSCHAFT FÜR PATHOLOGIE E.V.
Seit 1897 - dem Leben verpflichtet

BUNDESVERBAND DEUTSCHER PATHOLOGEN e.V.

Jörg Maas - CEO and DGP Secretary General

Korinna Jöhrens – Medical Advisor

Thomas Pilz – Administrative CEO

Why do we need eQA

The quality of pathology services is central to the definitive diagnosis of many diseases, particularly of cancer and its subsequent classification. However, the pathologists are responsible not only to diagnose cancer but also to report histological, immunohistochemical and molecular features of prognostic and predictive significance, thus helping to ensure that patients are treated appropriately.

To extract from the patients' tissue and blood-born biopsies as many information as possible is the pathologist's challenge of today.

This underlines the need of reliable and reproducible diagnoses, which have to be independent of the institute where the diagnostic work-up is done and the individual pathologist at the microscope or the biologist in the molecular-pathology laboratories. Ideally this should be true around the globe.

Why do we need eQA

However, this claim is by far not reality and in many respective publications a relatively high interobserver and intraobserver variability is stated.

Standardized Quantification in Tumor Pathology

An International Ki67 Reproducibility Study

Mei-Yin C. Polley, Samuel C. Y. Leung, Lisa M. McShane, Dongxia Gao, Judith C. Hugh, Mauro G. Mastropasqua, Giuseppe Viale, Lila A. Zabaglo, Frédérique Penault-Llorca, John M.S. Bartlett, Allen M. Gown, W. Fraser Symmans, Tammy Piper, Erika Mehl, Rebecca A. Enos, Daniel F. Hayes, Mitch Dowsett, Torsten O. Nielsen, on behalf of the International Ki67 in Breast Cancer Working Group of the Breast International Group and North American Breast Cancer Group

Manuscript received April 2, 2013; revised September 3, 2013; accepted September 16, 2013.

Correspondence to: Torsten Nielsen, MD, PhD, FRCPC, University of British Columbia Pathology and Laboratory Medicine, Anatomical Pathology, JP 1401, Vancouver Hospital & Health Sciences Centre, 855 W 12th Ave, Vancouver, BC V5Z 1M9, Canada (e-mail: torsten@mail.ubc.ca).

Background In breast cancer, immunohistochemical assessment of proliferation using the marker Ki67 has potential use in both research and clinical management. However, lack of consistency across laboratories has limited Ki67's value. A working group was assembled to devise a strategy to harmonize Ki67 analysis and increase scoring concordance. Toward that goal, we conducted a Ki67 reproducibility study.

Conclusions Substantial variability in Ki67 scoring was observed among some of the world's most experienced laboratories. Ki67 values and cutoffs for clinical decision-making cannot be transferred between laboratories without standardizing scoring methodology because analytical validity is limited.

Nat J Inst., preprint November 2013

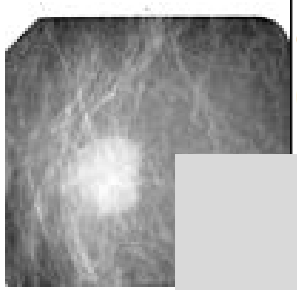
The strong reputation of pathology is becoming weaker .....

January 4, 2008, 8:09 am

THE WALL STREET JOURNAL.

Breast Cancer Test Errors Cause Faulty Treatment

Posted by Jacob Goldstein



Health

The Cancer That Shouldn't Be

Claire Cain Miller 01.28.08, 12:00 AM ET

This situation has to be changed by continuous and transparent eQA activities.

doctors still cling to

Genetech's

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"If we tried t

the market," says A

the WSJ. "It means

iation."I was devastated, and incredibly pissed at my doctor's office. If they'd found the tumor three years earlier, I could have kept my uterus and had a child," says Baze, now 39 years old and executive director of the Yellow Umbrella, a cervical cancer prevention group she founded in 2002.

A study published

performed Her-2 tes

Herceptin. It found

negative. Of tho

It borders on the scandalous that cervical cancer, among the few cancers that are preventable, kills 310,000 women a year worldwide. In 2007, 11,150 women in the U.S. were diagnosed with it. Half of them had not had a recent Pap test. Another third did get tested but got false negatives from the 65-year-old Papanicolaou biopsy. The Pap test is valuable, having cut the rate of cervical cancer by 70%, but it is archaic. It calls on a lab technician or machine to peer at a daub of cervical cells under a microscope to spot the abnormal precancerous ones. This

Why do we need eQA

The necessity to introduce transparent external QA/QC systems is obvious and should be pushed forward by

national and international societies of pathology.

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The necessity to introduce transparent external QA/QC systems is obvious and should be pushed forward by

national and international societies of pathology.

European Activities in EQC of Anatomic Pathology
Distribution of EQA/C Providers in Europe

Qualitätssicherungs-Initiative Pathologie GmbH

RCPA
The Royal College of Pathologists of Australasia

Royal College of Pathologists of Australasia (RCPAQAP)

Harmonization of Quality Control in Anatomic Pathology

Qualitätssicherungs-Initiative Pathologie GmbH

The European Society of Pathology and QuIP

agreed to mutually accept the certifications since organization, structure and evaluation of both Round Robin tests are very similar and almost exchangeable.

Problems

Qualitätssicherungs-Initiative Pathologie GmbH

The basic intention of the QuIP-EQA initiative can be summarized by the motto **“Quality Assurance from Pathologists for Pathologists**”.

A nice idea but reality is something else, because

- the willingness of institutes of pathology to perform RT is decreasing
- the number of RT is increasing
- the workload per RT is increasing
- the costs for the institutes are tremendous since up-to-now there was no adequate reimbursement and
- the scientific value is limited, in particular for young colleagues

So we were thinking about a new structure.

From QuIP to QuIP® Ltd.

- QuIP®´s legal status was changed from a civil law association towards a private limited company (Ltd.).
- QuIP belongs formally and in equal parts to the DGP and to the German Ass. of Pathology.
- Co-operation with other QC organizations will continue and will be intensified, e.g. RfB (Referenzinst. f. Bioanalytik), multiblock, provitro, IQNPath, EQA-ESP, NordiQC etc.
- The conducting institutes of pathology will be reimbursed for their efforts.
- For repeated RT the institutes have to pay adequately for participation and certification.
- As in the past, co-operation with industry will continue and will be facilitated by the B to B structure.

What has to be controlled by eQA

To approach this goal the following points have to be fulfilled:

- Documentation of **pre-analytic** steps, identification of the specimen
- **standardized** grossing and reporting
- definition and control of **standard operating procedures** (SOPs) of the whole technical process including staining protocols, antibody application, extraction techniques, sequencing protocol, bio-informatics etc.,
- clearly defined **work-flow** and **distribution of duties** in the histological, immunohistochemical and molecular laboratories,
- continuous participation in **External Quality Assurance (EQA) programs**, also known as round Robin tests or proficiency testing, e.g. QuIP,
- **continuous education** and training of pathologists to ensure competent application of current guidelines.

To document all these points is a prerequisite to get a **clinical cancer tumor board** certified by the **German Society of Cancer.**

Key characteristics

Qualitätssicherungs-Initiative Pathologie GmbH

To have a clear and transparent organizational structure of QuIP-RTs some key characteristics have been defined by the board:

- All German and non-German institutes of anatomic pathology, which are active in **diagnostic pathology** and are headed by a specialist of pathology, can participate (limited only by number),
- almost all ring trials are **tissue-based** using (unstained) slides to simulate reality as close as possible,
- **liquid biopsies** are recently included (e.g. for T790M),
- all RTs are exclusively **result orientated** independent of the methods applied,
- the **evaluation** of the individual results is done by experienced pathologists and
- if ever possible an **educational** approach is supported.

General Rules and Regulations of QuIP: Establishment of a new Ring Trial

Phase I: Preparation

- The key panel institutes perform selection of tumor material (n=10) followed by
- blinded testing between the panel institutes, for EGFR e.g. B/Hd/Jena

Phase II: Internal ring trial

- distribution of slides of 10 cases to 6-8 institutes selected due to their experience in molecular pathology (2nd level panel institutes)
- discussion of results

Phase III: Roll-out

- General roll-out in Germany and Switzerland (Inst. of Israel/Sweden/Greece/ Ireland)
- Publication of the results on the home page (xx % of the participants failed)

This scheme is applied to IHC as well as molecular RT.

Results of the ring trials on KRAS molecular tests

Quality in Pathology

QuIP

2006 => 15 % of 45 participants failed

2007 => 4,5 % of 55 participants faile

2010 => 2,8 % of 69 participants faile

Today 106 Institutes of Pathology pass

the certificate.

Qualitätssicherungs-Initiative Pathologie

QuIP

Ringversuche Immunhistochemie und Molekularpathologie

Teilnahmezertifikat
Ringversuch
Molekularpathologischer
KRAS Mutationstest beim Kolonkarzinom

2011

Prof. Dr. Manfred Dietel
Pathologisches Institut der Charité, Berlin

hat am Ringversuch mit Erfolg teilgenommen.

Leiter des Ringversuchs:
PD Dr. rer. nat. A. Jung und Prof. Dr. med. T. Kirchner

Hannover / Regensburg, 14.04.2011

Prof. Dr. med. Hans H. Kreipe

Prof. Dr. med. Ferdinand Hofstädter

CHARITÉ

Final report for the 2. ring-trial for the molecular-pathological detection of RAS mutations in human metastatic colorectal cancer

Andreas Jung, Inst. of Pathology, LMU Munic

	all		passed		
	#	%	#	%	%%
Austria	2	3.0	2	3.4	100
Germany	63	95.5	56	94.9	88.9
Switzerland	1	1.5	1	1.7	100
All participants	66		59		89.4

Tab. 1: Participant of the 2. ringtrial for pathological detection of mutations in t
all – all participants, passed – particpants successfully given both in numbers (#) a (%). The rate of particpants passing in th (%%).

April 2014

59 of 66 participants passed → 89,4%

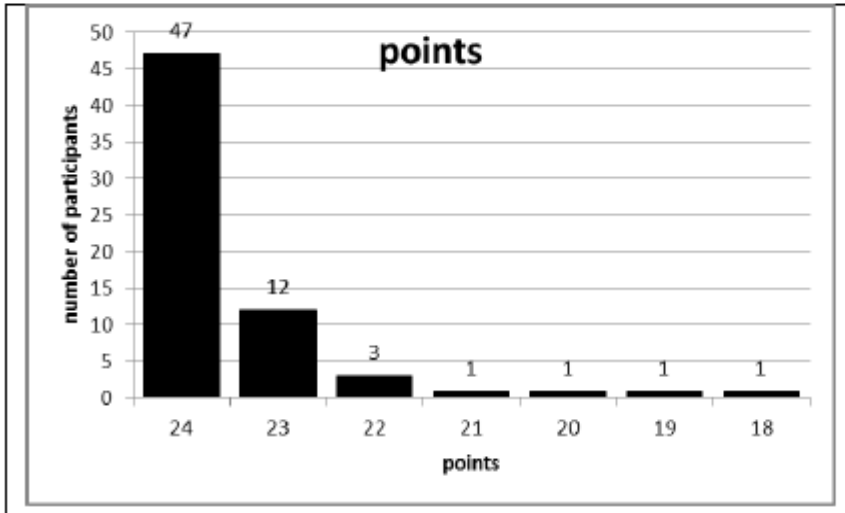


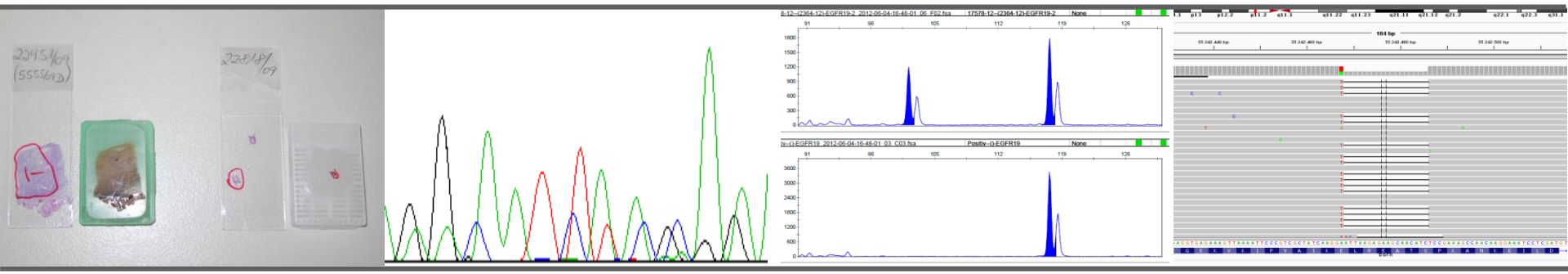
Fig. 2: 59 of all participants of the 2. RAS ring-trial passed the test. Of the 66 participants 59 passed (89.4%) the ring trial as they hat 23 or more points. Of these 56 came from Germany.

Quality in Pathology*

QuIP

UNIKLINIK KÖLN

CIO Centrum für Integrierte Onkologie Köln Bonn



Ring Trial EGFR T790M
Tissue and Blood

26.01.2016

Sabine Merkelbach-Bruse

Institut für Pathologie – Charité Berlin

CHARITÉ

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Schedule of T790M ring trial

09/2015	- 1. Panel meeting, information and planning
10-11/2015	- selection of the tissue specimen (all panel centres)
	- pre-testing of blood samples (Cologne)
12/2015	- definition of the trial conditions
	- testing of tissue specimen around the panel
01/2016	- testing of blood specimen around the panel
01/2016	- evaluation and 2nd meeting of the panellists
02/2016	- announcement of the open ring trial
04/2016	- public activation of the open ring trial

T790M from Tissue – Design of the RT

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10 cases:	5x	*EGFR* Ex 20 T790M
	3x	*EGFR* Ex 20 WT,
		EGFR Ex 19 od. 21 mut
	2x	*EGFR* Ex 19, 20, 21 WT

Material sent around: 2 x 10 µm slides, 1 slide for HE staining

Transportation: 09.12.15 byTNT

arrival: 4 centres 10.12.

1 centre 11.12.

Report of results: until 23.12.15

T790M from tissue → specimen

Blöcke mit T790M	Allelfrequenz T790M	Tumorzellgehalt	Auswahl f. Ringversuch
C15.18595 A	61,76%	90%	Fall 9
C15 18595 D	45,27%	60%	Fall 4
C15.18595 B	63,22%	60%	Fall 5
C15.31873 1CRC	6,84%	20%	Nicht ausgewählt
C15.31873 1ARC	12,3%	15%	Fall 2
C11.26061	80%	80%	Fall 7
C14.11820 1	9,8%	35%	Nicht ausgewählt
Block ext1	18%	35%	Test in Köln
Block ext3	58%	80%	Test in Köln
Block ext4	10%	60%	Test in Köln
Andere *EGFR* Mut			
C11.16744 1	-	70%	Fall 3
C11.16744 2	-	60%	Fall 6
C14.7653 2B	-	80%	Fall 10
WT			
C15.37161 A	-	60%	Fall 1
C15.38321 2D	-	80%	Fall 8

T790M from tissue - results

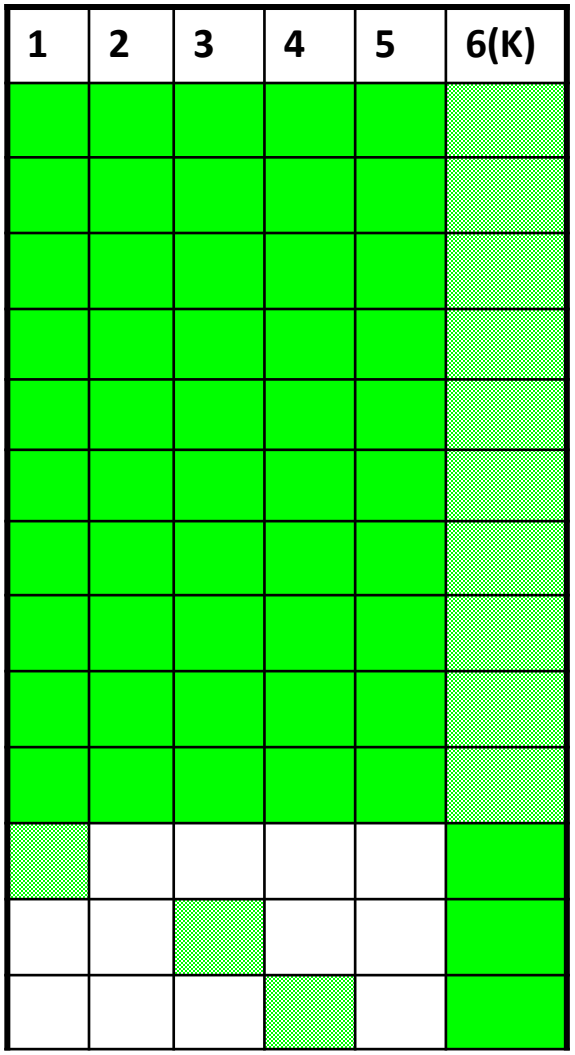
Panel Centres and techniques applied

Zentrum	Technologie	Plattform	Reagenzien
Zentrum 1	Parallelsequenzierung	PGM/Ion Torrent	Colon Lung Panel v2, LifeTech
Zentrum 2	Pyrosequenzierung Real-Time PCR	Pyromark Q24 Rotorgene Q	EGFR Pyro Kit 24, V1, Qiagen Therascreen EGFR RGQ PCR Kit, V2, Qiagen
Zentrum 3	Parallelsequenzierung	PGM/Ion Torrent	Colon Lung Panel, LifeTech
Zentrum 4	Parallelsequenzierung	PGM/Ion Torrent	LCPv2 Custom Panel, LifeTech
Zentrum 5*	Parallelsequenzierung Pyrosequenzierung Real-Time PCR Sanger-Sequenzierung	PGM/Ion Torrent Pyromark Q24 Rotorgene Q ABI3130	Custom Panel, Qiagen Home-brew, T790M Therascreen EGFR RGQ PCR Kit, V2, Qiagen Home-brew
Zentrum 6	Parallelsequenzierung	MiSeq	Custom Panel, Qiagen

*nur die Ergebnisse der Parallelsequenzierung gehen in die Bewertung ein

T790M from tissue - results

Fall	Status T790M	Sonstige Mutation in *EGFR*
1	WT	WT
2	c.2369C>T p.T790M	p.E746_A750del
3	WT	p.E746_A750del
4	c.2369C>T p.T790M	c.2573T>G p.L858R
5	c.2369C>T p.T790M	c.2573T>G p.L858R
6	WT	p.E746_A750del
7	c.2369C>T p.T790M	p.E746_A750del
8	WT	WT
9	c.2369C>T p.T790M	c.2573T>G p.L858R
10	WT	p.E746_A750del
Block ext1	c.2369C>T p.T790M	p.L747_P753delinsS
Block ext3	c.2369C>T p.T790M	WT
Block ext4	c.2369C>T p.T790M	p.E746_A750del



Gesamtpunktzahl 20

erreichte Punktzahl: 20 20 20 20 20

T790M mutation from blood

Preparatory steps

– selection of blood tubes and plasma preparation

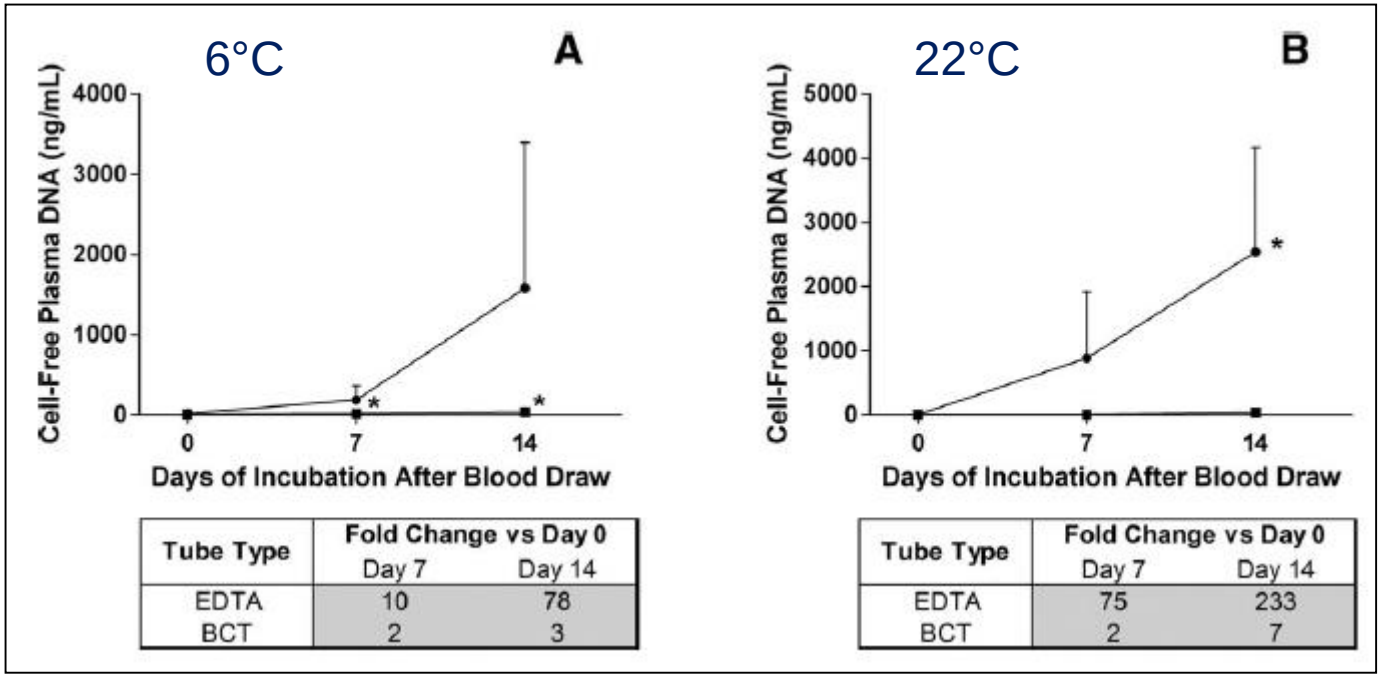
– optimisation of DNA extraction from plasma

– *spiking* of blood specimen DNA of cell lines

– planning and logistics

T790M from blood – pre-analyses

Selection of tubes for blood draw



Tube Type	Fold Change vs Day 0	
	Day 7	Day 14
EDTA	10	78
BCT	2	3

Tube Type	Fold Change vs Day 0	
	Day 7	Day 14
EDTA	75	233
BCT	2	7

Norton et al., J Clin Lab Anal 2013

T790M from blood – pre-analyses

UNIKLINIK KÖLN

Selection of devices for blood draw

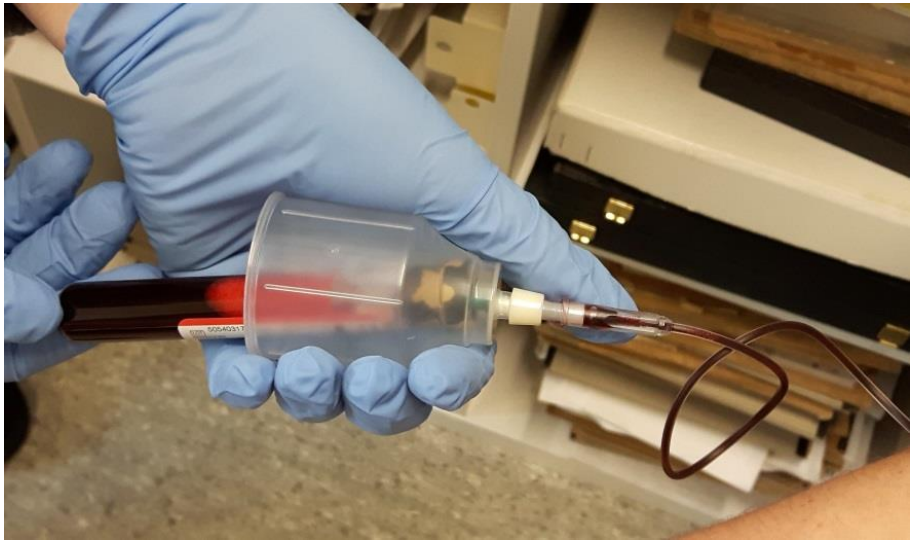
SARSTEDT

Universal-Blutkultur-Adapter

- Der Universal-Blutkultur-Adapter wurde speziell für die Blutentnahme mit der Safety-Multifly®-Kanüle entwickelt. Die besondere Form des Universal-Blutkultur-Adapters ermöglicht die Befüllung der gängigen Blutkulturflaschen mit breitem und schmalem Flaschenhals. Anschließend kann die Blutentnahme mit der S-Monovette® durchgeführt werden.

Bestell-Nr.	Bezeichnung	Verpackung
14.1209	Universal-Blutkultur-Adapter	100/Karton • 20/Innenkarton einzeln steril

Venofix Safety von Braun



CHARITÉ

T790M from blood - results

Fall	Status T790M	Sonstige Mutationen
11	c.2369C>T p.T790M	c.2573T>G p.L858R
12	c.2369C>T p.T790M	c.2573T>G p.L858R
13	WT	*PIK3CA*, E545K
14	c.2369C>T p.T790M	c.2573T>G p.L858R
15	c.2369C>T p.T790M	c.2573T>G p.L858R
16	c.2369C>T p.T790M	c.2573T>G p.L858R
17	c.2369C>T p.T790M	c.2573T>G p.L858R
18	WT	*PIK3CA*, E545K
19	c.2369C>T p.T790M	c.2573T>G p.L858R
20	c.2369C>T p.T790M	c.2573T>G p.L858R

1	2	3	4	5	6
	wt	wt	wt		n.a.
			wt		
		wt	wt		n.a.
			wt		
			wt		
			wt		
			wt		
	wt		wt		

Gesamtpunktzahl 18

erreichte Punktzahl: 18 16 16 4 18 17

T790M - Mutation in Gewebe und Blut 2016 (1) - Auswertung

- Übersichtsauswertung -

Qualitätssicherungs-Initiative Pathologie GmbH

T790M - Gewebe

[Erfolgsquote 19/20 => 95,0 %]

Mutation ja/nein	P 1	P 2	P 3	P 4	P 5
	Bew. / Soll=mut / N	Bew. / Soll=WT / N	Bew. / Soll=WT / N	Bew. / Soll=mut / N	Bew. / Soll=WT / N
WT	0	20	17	0	19
mutiert	20	0	0	20	1
nicht auswertbar	0	0	3	0	0

Mutation ja/nein	P 6	P 7	P 8	P 9	P 10
	Bew. / Soll=WT / N	Bew. / Soll=WT / N	Bew. / Soll=mut / N	Bew. / Soll=mut / N	Bew. / Soll=WT / N
WT	18	20	0	0	19
mutiert	1	0	20	20	0
nicht auswertbar	1	0	0	0	1

T790M - Mutation in Gewebe und Blut 2016 (1) - Auswertung

- Übersichtsauswertung -

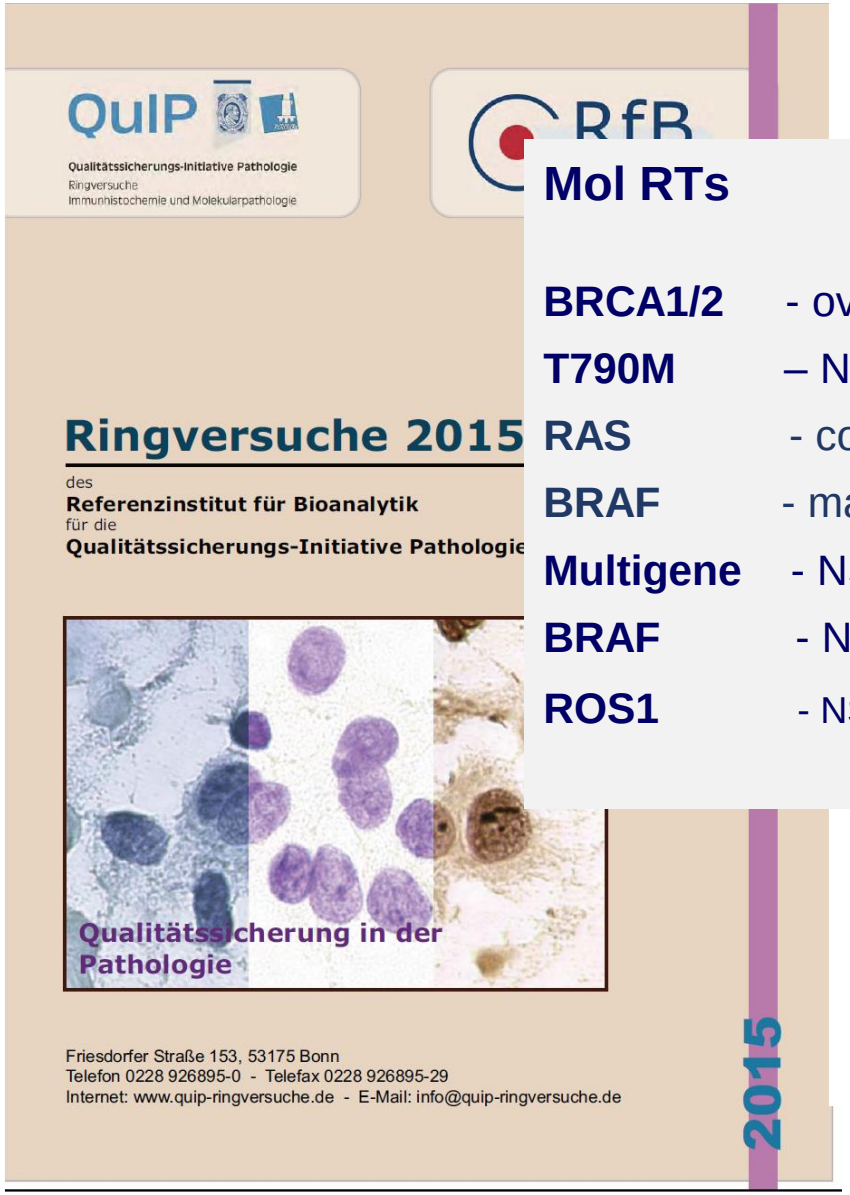
Qualitätssicherungs-Initiative Pathologie GmbH

T790M - Blut

[Erfolgsquote 15/20 => 75,0 %]

Mutation ja/nein	P 1			P 2			P 3			P 4			P 5		
	Bew.	Soll=mut	N	Bew.	Soll=mut	N	Bew.	Soll=mut	N	Bew.	Soll=mut	N	Bew.	Soll=WT	N
WT			1			7			4			2			20
mutiert			19			11			16			17			0
nicht auswertbar			0			2			0			1			0

Mutation ja/nein	P 6			P 7			P 8			P 9			P 10		
	Bew.	Soll=mut	N	Bew.	Soll=mut	N	Bew.	Soll=mut	N	Bew.	Soll=mut	N	Bew.	Soll=WT	N
WT			3			3			1			2			20
mutiert			17			16			19			17			0
nicht auswertbar			0			1			0			1			0



Inhaltsverzeichnis

Mol RTs

BRCA1/2	- ovarian carcinoma
T790M	– NSCLC (tissue and blood)
RAS	- colon
BRAF	- malignant melanoma
Multigene	- NSCLC – planned for 1st. quarter of 2017
BRAF	- NSCLC – 2nd qu. 2017
ROS1	- NSCLC – planned for 2nd half of 2017


Institut für Pathologie,
Rudolf-Virchow-Haus, Charité
Humboldt-Universität zu Berlin
Berliner Medizin-historisches Museum
Alexander Ufer
INSTITUT FÜR PATHOLOGIE
RUDOLF-VIRCHOW-HAUS
CHARITÉ