Vybrané aspekty cytogenetického vyšetření u mnohočetného myelomu - FISH a externí kontrola kvality -

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Laboratoře molekulární cytogenetiky

Přírodovědecká fakulta MU

Babákův výzkumný institut, Lékařská fakulta MU

- Molekulárně cytogenetická vyšetření pacientů s MM – přehled za rok 2010 v rámci jednotlivých pracovišť

- FISH a externí kontrola kvality 2010

Major cytogenetic prognostic factors

	MGUS (%)	MM (%)	Up-regulated oncogens	Prognosis impact
Del 17p13	<5	11	TP53	Negative
Del 13q14	20	48	RB1 and others	Neutral
t(4;14)(p16.3;q32)	2-10	15	FGFR3 and MMSET	Negative
t(11;14)(q13;q32)	15-20	16-20	Cyclin D1 gene and Myeov	Positive or neutral
t(14;16)(q32;q23)	2-5	5	C-maf and WWOX	Negative
Gain/Amplification 1q21	10-15	42	CKS1B and others	Negative

Adopted from: Fonseca et al., 2004; Avet-Loiseau et al., 2007; Hanamura et al., 2006

Standardizace cytogenetického vyšetření u MM v ČR dotazník EMN – 2004

FISH in Myeloma Questionnaire

Please type your answers in **bold**. Put an **x** after the appropriate statement for the multiple choice questions. Do not worry about maintaining the layout! Please email your reply to fiona.ross@salisbury.nhs.uk **by February 28th**.

Is your work in myeloma associated with plasma cell banking?

Do you also carry out conventional cytogenetic analysis in myeloma?

If so, how do you determine which sample have FISH, which cytogenetics and which have both?

How many myeloma samples do you FISH eacl
- Is this static
  - increasing
  - decreasing?

Do you use
- purified plasma cells
- whole bone marrow
- Other (please state)?

EMN
EMN | MSCNET | BDR-WM TRIAL

Royal Marsden Hospital, London

March 11th 2005

EMN FISH Workshop

Results from Questionnaire

Recommendations for FISH in multiple myeloma

Standardizace cytogenetického vyšetření u MM v Evropě – používané techniky

EMN FISH Workshop, Royal Marsden Hospital, London 2005, 31 laboratoří

XI International Myeloma Workshop, Kos 2007
(Ross et al., European myeloma network recommendation for FISH in myeloma)

Přijatá doporučení:

a) Materiál pro FISH – první odběr KD (purifikace plazmocytů vs. cIg-FISH)
b) Hodnoty cut–off (10% translokace, 20 % delece)
c) Užití kontrolních DNA sond
d) Počty hodnocených buněk (minimum 100)
e) Typy používaných DNA sond (Abbott, Kreatech)
f) Interpretace cytogenetických výsledků (ISCN)

Čejkovice, 2005

Standardizace cytogenetického vyšetření u MM v Evropě – FISH a mezinárodní kontrola kvality I

Květen – září 2007 I. kolo

- účast 21 evropských laboratoří (prof. Hervé AVET-LOISEAU)
- 10 pacientů s MM
- v balíčku zasláno 100 000 plazmatických buněk ve fixáži
- aberace FISH: del 13q14, del p53, IGH, t(11;14), t(4;14), t(14;16)
- ČR: Praha, Olomouc, Brno

Cíl: porovnat kvalitu FISH vyšetření u MM v rámci laboratoří EMN

EMN FISH QC meeting in Nantes, January 22, 2008

Francie, Anglie, Irsko, Holandsko, Belgie, Španělsko, Portugalsko, Řecko, Itálie, Polsko, ČR, Německo, Dánsko, Turecko…

	Patient 6410	Patient 6411	Patient 6412	Patient 6422	Patient 6425	Patient 6426	Patient 6441	Patient 6442	Patient 6444	Patient 6447		
Center A	88%	92%	13%	7%	90%	3%	97%	5%	13%	100%	Abbott-Vysis Rb+D13S319	
Center B	94%	92%	0%	0%	100%	0%	96%	4%	0%	96%	Abbott-Vysis Rb+D13S319	
Center C	96%	92%	2%	4%	97%	6%	93%	2%	3%	87%	Kreatech	
Center D	93%	85%	0%	0%	92%	0%	95%	0%	0%	90%	?	20-40 cells/slide
Center E	86%	95%	8%	9%	90%	5%	91%	5%	8%	93%	Abbott-Vysis	200 cells/slide
Center F	100%	100%	0%	0%	100%	0%	92%	0%	0%	100%	Abbott-Vysis D13S319+13q34	
Center G	95%	100%	5%	3%	100%	1%	97%	1%	0%	98%	Abbott-Vysis Rb	
Center H	97%	97%	0%	5%	98%	3%	96%	4%	4%	95%	Abbott-Vysis D13S319+13q34	
Center I	76%	95%	4%	5%	90%	3%	93%	5%	10%	85%	Abbott-Vysis	200 cells/slide
Center J	95%	98%	0%	0%	94%	0%	99%	0%	0%	99%	Abbott-Vysis D13S319+13q34	
Center K	100%	100%	0%	0%	100%	0%	100%	0%	0%	100%	Home-made	
Center L	100%	97%	4%	7%	100%	55%	100%	8%	5%	99%	Abbott-Vysis D13S319+13q34	
Center M	90%	95%	1%	2%	92%	3%	95%	2%	0%	96%	Abbott-Vysis D13S319+13q34	
Center N	95%	86%	0%	0%	82%	0%	89%	0%	0%	85%	Abbott-Vysis Rb	
Center O	96%	96%	0%	0%	97%	1%	95%	0%	0%	99%	Abbott-Vysis D13S319+ Rb home-made	
Center P	96%	89%	Failure	Failure	99%	6%	89%	0%	1%	92%	Abbott-Vysis D13S319+CEP9	
Center Q	94%	87%	3%	2%	95%	2%	89%	2%	1%	93%	Abbott-Vysis D13S319	
Center R	90%	79%	5%	3%	91%	0%	93%	2%		94%	Abbott-Vysis D13S319+CEP9	
Center S	88%	79%	8%	7%	100%	7%	88%	6%	7%	78%	Abbott-Vysis D13S319+13q34	
Center T	93%	99%	6%	6%	94%	8%	75%	Failure	5%	Failure	Abbott-Vysis Rb	
Center U	95%	97%	3%	2%	96%	2%	94%	4%	3%	96%	Commercial	

Standardizace cytogenetického vyšetření u MM v Evropě – MULTIPLE MYELOMA FISH EXTERNAL QUALITY CONTROL 2010

Září – prosinec 2010 II. kolo

- **STUDY DESIGN:**
- Five plasma cells samples **purified using CD138 coated magnetic beads** were sent to 22 europeans centres.
- These samples were prepared at the haematology laboratory of **Nantes** university hospital with five bones marrows resulting from patients diagnosed with a multiple
- myeloma.
- Aliquots with 100 000 plasma cells with a purity higher than 90% were prepared and sent to different labs, for each samples.
- These samples were treated in the different labs as samples regularly sent to the lab for routine purposes.

STUDY OBJECTIVES:

- The objectives of the external quality assessment were to compare the practices on different labs performing the interphase fluorescence in situ hybridization analysis in **multiple myeloma:**

- **The genomic aberrations screened**
- **The cut off used for each genomic aberrations screened**
- **The results interpreted with respectively cut off for the five samples**

MULTIPLE MYELOMA FISH EXTERNAL QUALITY CONTROL 2010

Nejčastěji vyšetřované aberace u MM v Evropě

RESULTS:

- **Percentage of returned results:**

The samples were sent at 22 centres, 19 returned the results in the deadlines → **86% of participation.**

- **Genomic aberrations screened:**

		IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
Analyzed	N	12	19	14	16	18	19	10	8
	%	63%	100%	74%	84%	95%	100%	53%	42%
Not analyzed	N	7	0	5	3	1	0	9	11
	%	37%	0%	26%	16%	5%	0%	47%	58%

FISH EQC 2010 - cut off

MULTIPLE MYELOMA FISH EXTERNAL QUALITY CONTROL 2010_RESULTS REPORT — **Laboratory N°18**

- Cut off used for each genomic aberrations screened:

Center	IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
1	none	none	none	none	5%	5%	ND	ND
2	ND	5%	5%	5%	5%	5%	5%	5%
3	3%	3%	3%	ND	10%	10%	5%	2%
4	ND	?	?	ND	10%	10%	ND	ND
5	ND	1%	7%	2%	2%	1%	/	ND
6	10%	10%	10%	10%	20%	20%	20%	20%
7	4%	2% / 27%	ND	2% / 18%	20%	20%	ND	ND
8	20%	20%	20%	20%	20%	20%	20%	20%
9	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND
11	ND	10%	10%	10%	20%	20%	ND	20%
12	rearrangement 10% deletion 20%	10%	10%	10%	10%	20%	20%	ND
13	ND	10% DF 20% SF	ND	10% DF 20% SF	20%	20%	ND	ND
14	20%	10%	10%	10%	20%	20%	ND	ND
15	ND	0%	ND	1%	ND	16.5%	ND	ND
16	10%	10-20% *if we see one fusion only instead of two*	10-20% *if we see one fusion only instead of two*	10-20% *if we see one fusion only instead of two*	10-20%	10-20%	ND	ND
17	10%	10%	10%	10%	20%	20%	10%	10%
18	20%	20%	ND	20%	20%	20%	20	20%
19	10%	10%	10%	10%	20%	20%	10%	ND
20	ND	ND	ND	ND	ND	ND	ND	ND
21	rearrangement 10% del IgVH 20%	10%	10%	10%	20%	20%	10%	10%
22	ND	30%	ND	30%	ND	30%	ND	ND

ND : Not Done

- Results for the five patients

The following tables synthesise the results for five samples.

Globally, the results appear very homogeneous. The rare discrepancies are highlighted in yellow.

FISH EQC 2010 – vzorek 1

MULTIPLE MYELOMA FISH EXTERNAL QUALITY CONTROL 2010_RESULTS REPORT — **Laboratory N°18**

Results for sample 1_N°10311

Center	IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
1	No	No	3x11	3x16	No	No	ND	ND
2	ND	No	3x11 (79%)	3x16 (37%)	No	No	2	ND
3	No	No	No	ND	No	No	2	Trisomy 9, trisomy 15
4	ND	No	No	ND	No	Yes (18%)	ND	ND
5	ND	No	No	No	No	No	2	ND
6	No	No	3x11	No	No	No	2	2x9, trisomy 15
7	No	ND	ND	No	No	No	ND	ND
8	No	No	No	No	No	No	2	Trisomy 5, trisomy 15
9	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND
11	ND	No	No	No	No	No	ND	Trisomy 5, trisomy 15
12	ND	ND	ND	ND	ND	ND	ND	ND
13	ND	No	ND	No	No	No	ND	ND
14	No	No	No	No	No	No	ND	ND
15	ND	No	ND	No	ND	No	ND	ND
16	No	No	3x11 (85%)	No	No	No	ND	ND
17	No	ND	No	No	No	No	ND	Trisomy 9, trisomy 11
18	No	ND	ND	ND	No	No	2	Yes (62%)
19	No	No	No	No	No	No	2	ND
20	ND	ND	ND	ND	ND	ND	ND	ND
21	No	ND	ND	ND	No	No	2	Trisomy 9, trisomy 15
22	ND	No	ND	No	No	No	ND	ND

Summary table:

	IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
Number	22	22	22	22	22	22	22	22
Not done	11	8	10	8	5	4	14	15
Negative	11	14	12	14	17	17	8	0
% *	100%	100%	100%	100%	100%	94%	100%	0%
Positive	0	0	0	0	0	1	0	7
% *	0%	0%	0%	0%	0%	6%	0%	100%
Fail	0	0	0	0	0	0	0	0
% *	0%	0%	0%	0%	0%	0%	0%	0%

** Only on the centres which realized the analysis*

FISH EQC – vzorek 2

MULTIPLE MYELOMA FISH EXTERNAL QUALITY CONTROL 2010_RESULTS REPORT — **Laboratory N°18**

Results for sample 2_N°10315

Center	IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
1	No	No	3x11	No	No	3x17	ND	ND
2	ND	No	4x11	No	No	No	Fail	ND
3	No	No	No	ND	No	3x17	ND	Trisomy 9 (65%), trisomy 15 (35%)
4	ND	No	No	ND	No	ND	ND	ND
5	ND	ND	No	No	No	No	Yes (65%)	ND
6	No	No	4x11	No	No	3x17	Yes (86%)	Trisomy 9 (89%)
7	No	ND	ND	ND	Fail	Fail	ND	ND
8	No	No	No	No	No	No	Yes	Tetrasomy 5, tetrasomy 9, tetrasomy 15
9	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND
11	ND	No	No	No	No	No	ND	Trisomy 5, trisomy 9, trisomy 15
12	No	ND	ND	ND	No	No	ND	ND
13	ND	No	ND	No	No	No	ND	ND
14	No	No	No	No	No	3x17	ND	ND
15	ND	No	ND	No	ND	No	ND	ND
16	No	No	4x11	No	No	3x17	ND	ND
17	ND	No	No	No	No	No	Yes (99%)	Trisomy 9, tetrasomy 11
18	No	ND	ND	ND	No	No	Yes	Yes (70%)
19	No	No	No	No	No	3x17	Yes (87%)	ND
20	ND	ND	ND	ND	ND	ND	ND	ND
21	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail
22	ND	No	ND	No	ND	Fail	ND	ND

Summary table:

	IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
Number	22	22	22	22	22	22	22	22
Not done	11	7	9	8	5	4	14	15
Négative	10	14	12	13	15	15	0	0
% *	91%	93%	92%	93%	88%	83%	0%	0%
Positive	0	0	0	0	0	0	6	6
% *	0%	0%	0%	0%	0%	0%	75%	86%
Fail	1	1	1	1	2	3	2	1
% *	9%	7%	8%	7%	12%	17%	25%	14%

** Only on the centres which realized the analysis*

FISH EQC 2010 – vzorek 3

MULTIPLE MYELOMA FISH EXTERNAL QUALITY CONTROL 2010_RESULTS REPORT — **Laboratory N°18**

Results for sample 3_N°10321

Center	IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
1	No	3x4	No	No	Yes	?	ND	ND
2	ND	3x4	No	No	Yes (88%)	Yes (90%)	2	Tetrasomy 11
3	No	No	No	ND	Yes (66%)	Yes (93%)	2	Trisomy 9 (70%), trisomy 15 (76%)
4	ND	No	No	ND	Yes(89%)	Yes(96%)	ND	ND
5	ND	No	No	No	Yes(63%)	Yes(68%)	2	ND
6	No	3x4	No	No	Yes (93%)	Yes (97%)	2	Trisomy 9 (77%), trisomy 15 (19%)
7	No	No	ND	No	Yes (96%)	Yes (96%)	ND	ND
8	No	No	No	No	Yes (99%)	No	2	Trisomy 5, trisomy 9, trisomy 15
9	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND
11	ND	No	No	No	Yes (94%)	Yes (97%)	ND	Trisomy 5, trisomy 9, trisomy 15
12	ND	ND	ND	ND	ND	ND	ND	ND
13	ND	No	ND	No	Yes (85%)	Yes (82%)	ND	ND
14	No	No	No	No	Yes (94%)	Yes (87%)	ND	ND
15	ND	No	ND	No	ND	Yes (88%)	ND	ND
16	No	3x4	4x11	No	Yes (90%)	Yes (92%)	ND	ND
17	ND	No	No	No	Yes (90%)	Yes (95%)	ND	Trisomy 9, tetrasomy 11
18	No	No	ND	No	Yes (82%)	Yes (83%)	2	Yes
19	No	No	No	No	Yes (91%)	Yes (90%)	No	ND
20	ND	ND	ND	ND	ND	ND	ND	ND
21	No	ND	ND	ND	Yes (78%)	Yes (88%)	No	Yes
22	ND	No	ND	No	ND	Yes (70%)	ND	ND

Summary table:

	IGH	t(4;14)	t(11;14)	t(14;16)	del(13)	del(17p)	1q21	Hyperdiploidy
Number	22	22	22	22	22	22	22	22
Not done	12	5	10	7	6	4	14	14
Négative	10	17	12	15	0	1	8	0
% *	100%	100%	100%	100%	0%	6%	100%	0%
Positive	0	0	0	0	16	16	0	8
% *	0%	0%	0%	0%	100%	89%	0%	100%
Fail	0	0	0	0	0	0	0	0
% *	0%	0%	0%	0%	0%	0%	0%	0%

* *Only on the centres which realized the analysis*

probes	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5		
%	B	O	P	B	O	P	B	O	P	B	O	P	B	O	P
IGH	**11**	**12**	**x**	**8**	**x**	**x**	**19**	**x**	**x**	**17**	**15**	**x**	**84**	**87**	**ano**
t(4;14)	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**80**	**84**	**92**
t(11;14)	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**0**
t(14;16)	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**0**
del (13)	**2**	**x**	**x**	**5**	**x**	**x**	**82**	**78**	**x**	**28**	**29**	**x**	**96**	**89**	**95**
del 17p	**4**	**<20**	**x**	**8**	**x**	**x**	**83**	**80**	**x**	**12**	**<20**	**x**	**10**	**<20**	**0**
1q21	**2**	**<10**	**x**	**6**	**x**	**x**	**2**	**<10**	**x**	**3**	**<10**	**x**	**3**	**<10**	**31**
hyperd	**62**	**ano**	**x**	**70**	**x**	**x**	**74**	**ano**	**x**	**27**	**ano**	**x**	**0**	**0**	**x**
CEP 7	**x**	**0**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**+7**	**x**	**x**	**x**	**x**
CEP 9	**x**	**+9**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**+9**	**x**	**x**	**x**	**x**
CEP 11	**x**	**0**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**+11**	**x**	**x**	**x**	**x**
CEP 15	**x**	**+15**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**+15**	**x**	**x**	**x**	**x**

Děkuji za pozornost