

EuroClonality EQA scheme for IG/TR clonality testing in suspected Lymphoproliferations/Lymphoma

Patricia Groenen, PhD

Clinical scientist in molecular pathology

Participants

Country	EuroClonality labs		New labs			Total
	Both IG/TR	IG alone	Both IG/TR	IG alone	TR alone	
Belgium	1		8			9
Denmark	1		3			4
Finland			1			1
France	2					2
Germany	3		1			4
Ireland			1			1
Israel			1			1
Italy	2		3			5
Netherlands	3	1	4	4	1	13
Norway			2			2
Portugal	1					1
Spain	3		3		1	7
Sweden	1		2		1	4
Switzerland			1			1
United Kingdom	8		3			11
Total	25	1	33	4	3	66

57 laboratories submitted results for both the IG and TR schemes, 5 laboratories for the IG scheme only and 3 for the TR scheme only.

Set-up of EQA scheme

- Paper – based: 5 IG and 5 TR
- Participants scored the presented data:
 - Technical scoring of the PCR tubes
 - Final molecular interpretation
- Performance of each laboratory: based by scoring the final molecular interpretation

REVIEW

Leukemia 2012, 10:2159-71

EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations

AW Langerak¹, PJTA Groenen², M Brüggemann³, K Beldjord⁴, C Bellan⁵, L Bonello⁶, E Boone⁷, GI Carter⁸, M Catherwood⁹, F Davi¹⁰, M-H Delfau-Larue¹¹, T Diss¹², PAS Evans¹³, P Gameiro¹⁴, R Garcia Sanz¹⁵, D Gonzalez¹⁶, D Grand¹⁷, Å Håkansson¹⁸, M Hummel¹⁹, H Liu²⁰, L Lombardia²¹, EA Macintyre²², BJ Milner²³, S Montes-Moreno²⁴, E Schuurin²⁵, M Spaargaren²⁶, E Hodges²⁷ and JJM van Dongen¹

Overall technical description for all Ig or TCR targets

Molecular interpretation / conclusion

Optional: a more detailed molecular interpretation to describe additional aspects

-no (specific) product, poor DNA quality

-not evaluable, due to poor DNA quality

-no (specific) product

-no rearrangement in Ig/ TCR targets detected

-clonal (... nt) ^f

-clonality detected

-clonality detected (biallelic products)
 -clonality detected (biclinality)
 -clonality detected (minor clonal product)
 -clonality detected (isolated rearrangement)
 -clonality detected (with caution, plus advice for follow-up analysis / new sample)
 -clonality detected in addition to background of B / T cells

-pseudoclonal (one or more non-reproducible products)

-no clonality detected, suggestive of low template amount

-multiple reproducible products ($n \geq 3$) ^b

-oligoclonality / multiple clones detected

-dominant clones in oligo/polyclonal background

-polyclonal (not clonal ^c)

-polyclonality detected (no clonality detected ^c)

-polyclonality detected plus minor clone of unknown significance ^d

-not evaluable

-not evaluable ^e

Consensus molecular interpretation / conclusion

Clinical Case + target gene	Molecular interpretation/conclusion	% correct results (# labs/total)
Case 1 IG	Clonality detected	98% (61/62)
Case 2 IG	Clonality detected	100% (62/62)
Case 3 IG	Polyclonality detected (no clonality detected)	97% (60/62)
Case 4 IG	Clonality detected	95% (59/62)
Case 5 IG	Clonality detected	100% (62/62)
Case 1 TR	Clonality detected	97% (58/60)
Case 2 TR	Clonality detected	97% (58/60)
Case 3 TR	Polyclonality detected (no clonality detected)	97% (58/60)
Case 4 TR	Clonality detected	100% (60/60)
Case 5 TR	Clonality detected	97% (58/60)

Issues

- **Multiple clones – clonal**
- Over interpretation of results

Difficulties scoring case 1 IG

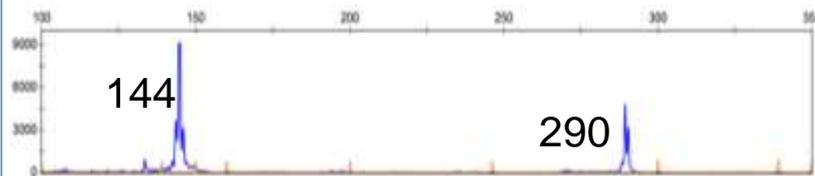
- Bone marrow mononuclear cells, showed typical hairy lymphoid cells
- Final interpretation **Clonal (61 labs)**
Oligoclonality/ multiple clones (1)
- Due to difficulty in technical scoring of multiple “clonal” peaks
Case 1 IGH C/C, IGK-VJ C/C and IGK-DE C/C
- Diagnosis: hairy cell leukemia

Case 1: IGK

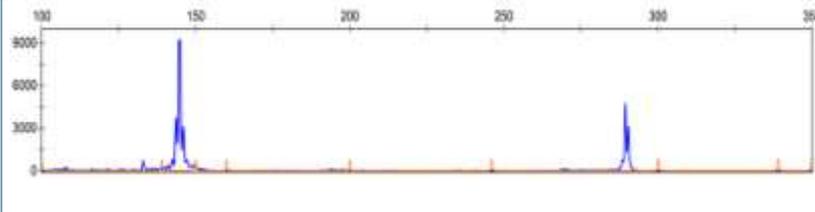
IGK- tube A

IGK- tube B

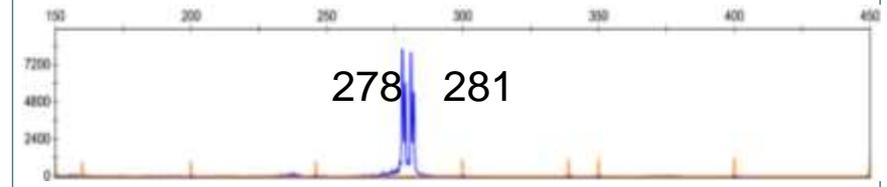
IGK tube A DETAIL – Case 1



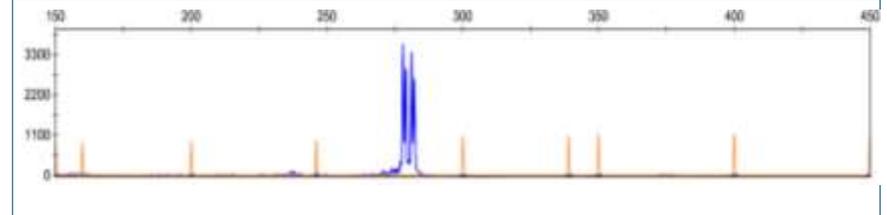
IGK tube A DETAIL – Case 1



IGK tube B DETAIL – Case 1



IGK tube B DETAIL – Case 1



Clonal

Clonal

Vkf1/6/Vk7- Jk

Vkf2/Vk4/Vk5- Jk

intronRSS- Kde
intronRSS-Kde

- 4 clonal peaks consistent with one clone: V-J / intron-Kde and V-J / intron-Kde
- **THM: Kappa can have up to 4R that can belong to one clone**

Difficulties scoring case 1 TR

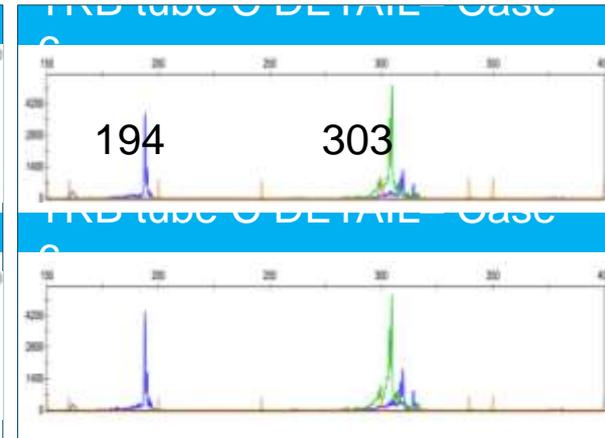
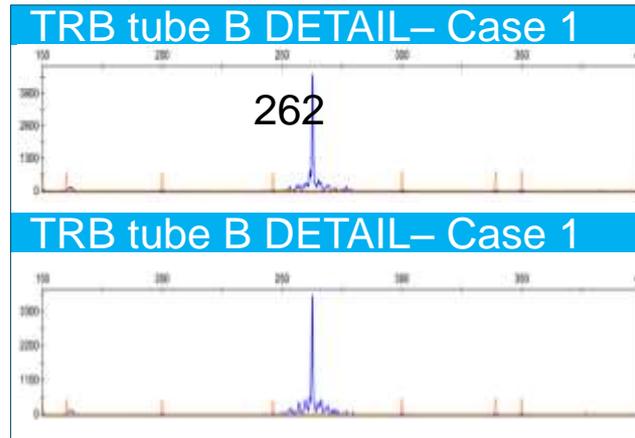
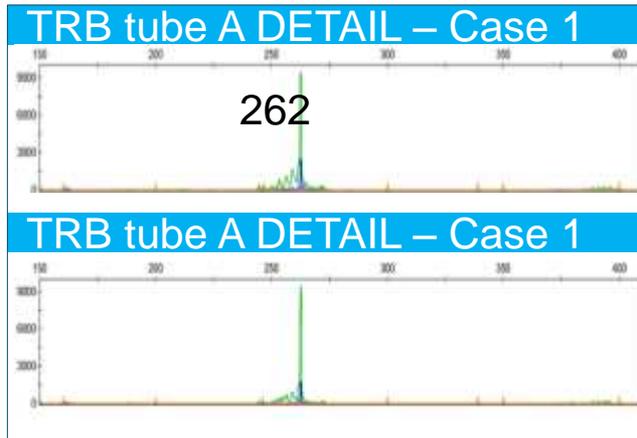
- Fresh frozen skin biopsy
- **Final interpretation:**
 - Clonality detected (58 labs)**
 - Oligoclonality/multiple clones (1 lab)**
 - Not evaluable (1 lab)**
- Due to difficulty in scoring TRB > clonal (bi-clonality)
- Diagnosis: Pt with extra-cutaneous dissemination of mycosis fungoides

Case 1: TRB

TRB-tube A

TRB-tube B

TRB-tube C



Clonal (VB-JB1),

Clonal (VB-JB2),

DB2-JB2 DB1-JB1

Clonal

vs

Multiple products,
Pseudoclonal,
Polyclonal

Pre-testing results (4 labs): These 4 clonal rearrangements, exceed the maximum number of these type of rearrangements, on 2 alleles: biconality suspected.

THM: Multiple clones is 3 or more

Radboudumc

Difficulties scoring case 2 TR

- Fresh frozen skin biopsy
- **Final interpretation:**
 - Clonality detected (58 labs)**
 - Oligoclonality/multiple clones (2 labs)**
- Due to misunderstood definition of multiple clones (3 or more)
- Pt with mycosis fungoides

Issues

- Multiple clones – clonal
- **Over interpretation of results**

Difficulties scoring case 4 IG

- Fresh frozen skin biopsy, suspicion of cutaneous lymphoma
- **Final interpretation:**
 - no rearrangement detected (1 lab)**
 - Clonality detected (59 labs)**
 - Polyclonality detected (2 labs)**
- Technical scoring
 - IGH: polyclonal (60 labs)
 - clonal (2 labs) > *overinterpretation*
 - IGK-VK clonal (C) (60 labs) vs multiple products (1)
 - IGK-DE clonal (C) (60 labs) vs pseudoclonal (1)
- Diagnosis: Pt with reactive leukocytosis

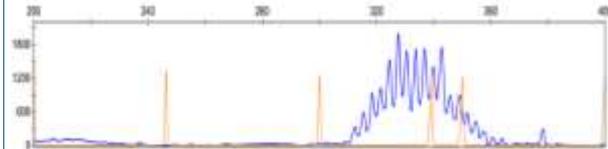
Case 4: IGH

IGH- tube A

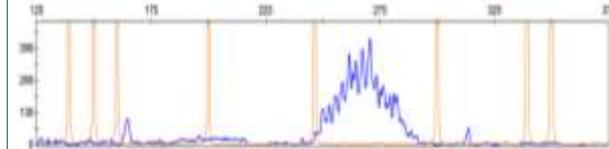
IGH- tube B

IGH- tube C

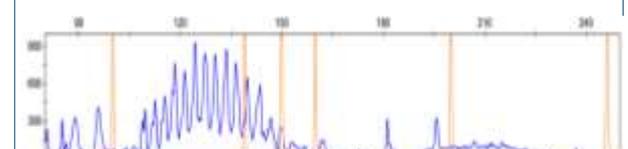
IGH tube A DETAIL – Case 4



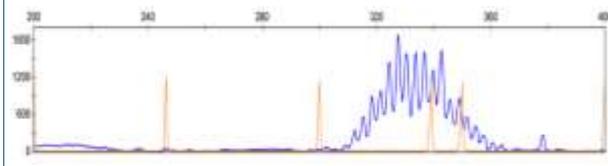
IGH tube B DETAIL – Case 4



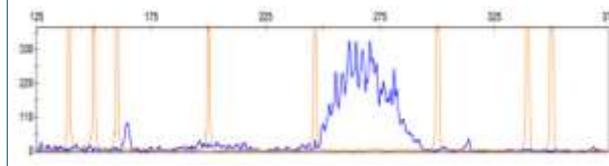
IGH tube C DETAIL – Case 4



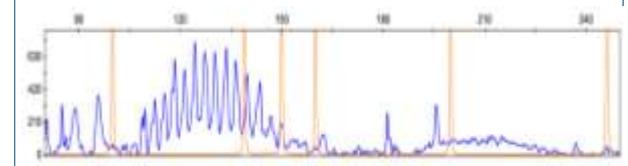
IGH tube A DETAIL – Case 4



IGH tube B DETAIL – Case 4



IGH tube C DETAIL – Case 4



Polyclonal

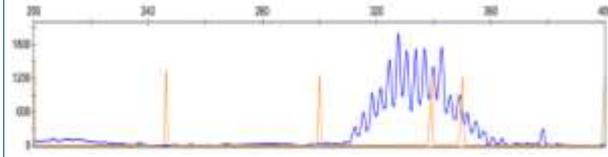
Case 4: IGH

IGH- tube A

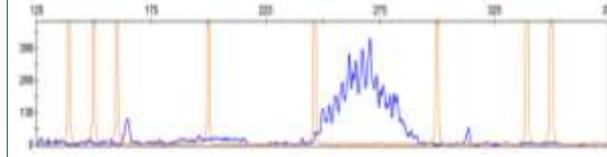
IGH- tube B

IGH- tube C

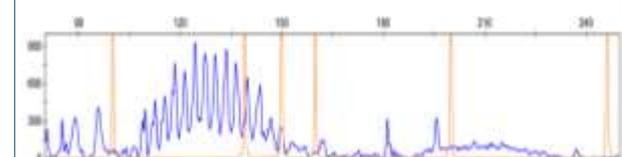
IGH tube A DETAIL – Case 4



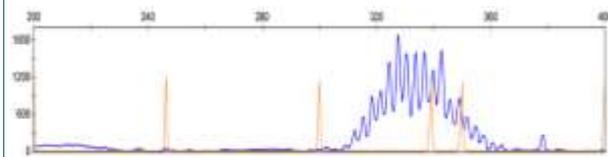
IGH tube B DETAIL – Case 4



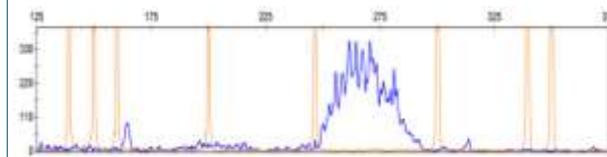
IGH tube C DETAIL – Case 4



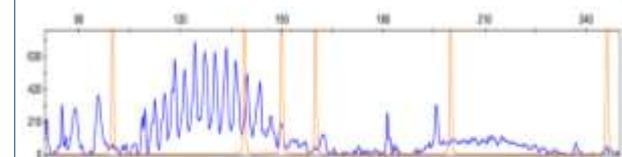
IGH tube A DETAIL – Case 4



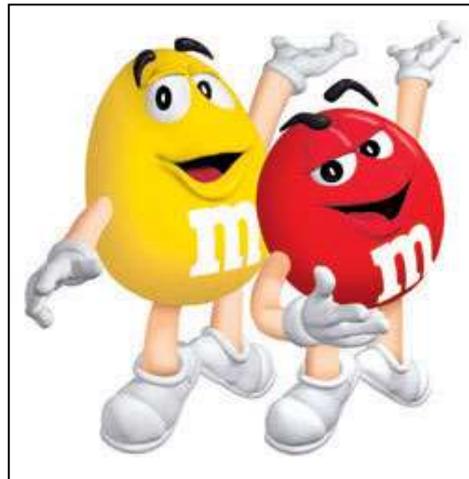
IGH tube B DETAIL – Case 4



IGH tube C DETAIL – Case 4



Polyclonal



Match with histology:

“%B cells is 70% of which most were suspected of being malignant”

Difficulties scoring case 3 TR

- Fresh frozen skin biopsy
- **Final interpretation:**
 - Clonality detected (2 labs)**
 - Polyclonality detected (2 labs)**
- Clonality detected > Due to over-interpretation TRG
- Pt with cutaneous T-cell lymphoma

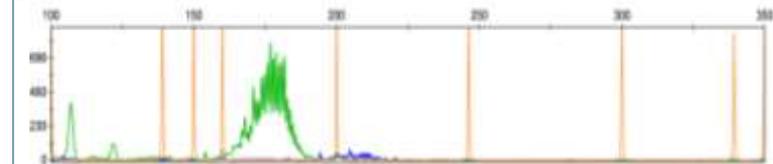
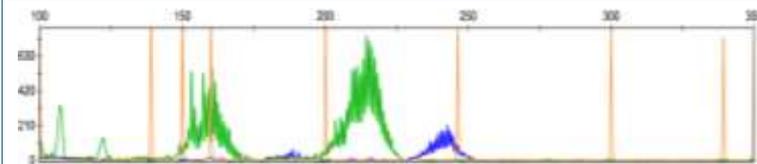
Case 3: TRG

TRG-tube A

TRG-tube B

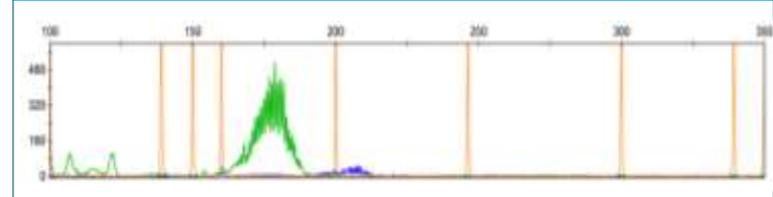
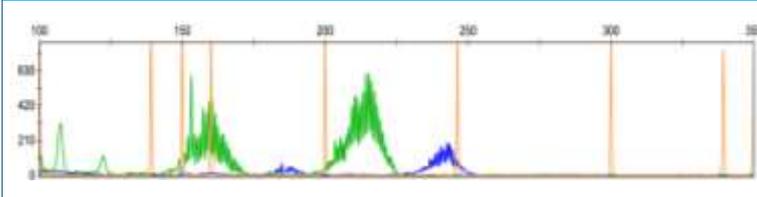
TRG tube A DETAIL– Case 3

TRG tube B DETAIL– Case 3

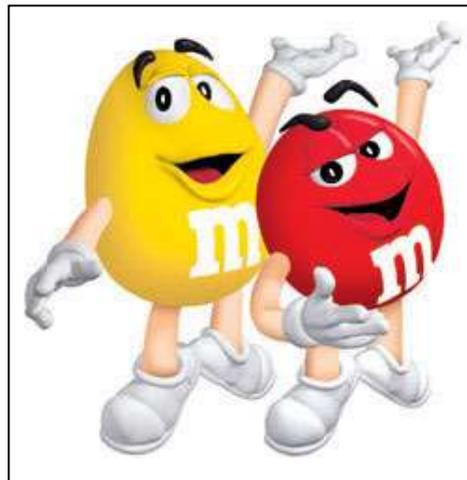


TRG tube A DETAIL – Case 3

TRG tube B DETAIL– Case 3



Polyclonal
vs
Clonal (152bp)



Polyclonal

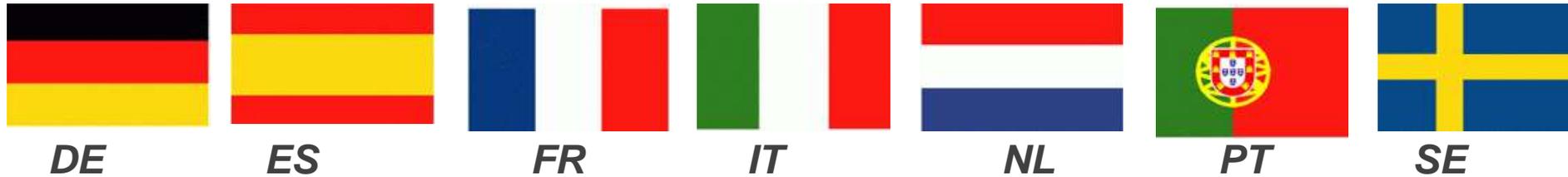
Match with histology:
“Almost all cells are CD2+/CD3+/CD5+/CD4+ and CD7+”.

Summary pitfalls

- IGK, TRB may have up to 4 clonal peaks that *can* belong to one clone
- Definition multiple clones
- Over interpretation PCR results > match with histology

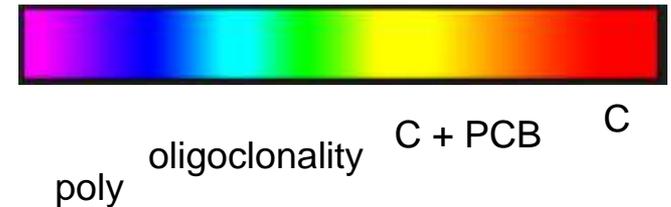
Summary performance rates

- IG scheme:
 - 56 / 62 labs > a 100% performance rate (90.4%)
 - 6 of 62 labs > a 80% performance rate (9.6%)
- TR scheme:
 - 53 / 60 labs > a 100% scoring rate (88.3%)
 - 6 of 60 labs > a 80% scoring rate (10%)
 - 1 of 60 labs > a 60% scoring rate (1.7%)*
- In total 65 laboratories submitted results: either for IG (62 labs) and/or TR (60 labs)
- Per case correct results were obtained by 95% to 100% of the laboratories, depending on the specific case analysed.



Use of the guidelines make the molecular report in clinical pathology

> immunobiology > a continuum



REVIEW

Leukemia 2012, 10:2159-71

EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations

AW Langerak¹, PJTA Groenen², M Brüggemann³, K Beldjord⁴, C Bellan⁵, L Bonello⁶, E Boone⁷, GI Carter⁸, M Catherwood⁹, F Davi¹⁰, M-H Delfau-Larue¹¹, T Diss¹², PAS Evans¹³, P Gameiro¹⁴, R Garcia Sanz¹⁵, D Gonzalez¹⁶, D Grand¹⁷, Å Häkansson¹⁸, M Hummel¹⁹, H Liu²⁰, L Lombardia²¹, EA Macintyre²², BJ Milner²³, S Montes-Moreno²⁴, E Schuurin²⁵, M Spaargaren²⁶, E Hodges²⁷ and JJM van Dongen¹