J Hematopathol (2012) 5:99–103
DOI 10.1007/s12308-011-0120-x

ORIGINAL ARTICLE

The EuroClonality website: information, education and support on clonality testing

Paul D. M. Rombout · Tim C. Diss · Elizabeth Hodges · Michael Hummel · Jacques J. M. van Dongen · Anton W. Langerak · Patricia J. T. A. Groenen

Received: 31 August 2011 / Accepted: 7 November 2011 / Published online: 30 November 2011

Abstract Clonality assessment is an established tool in the diagnosis of malignant lymphoma; however, the evaluation of immunoglobulin/T-cell receptor (Ig/TCR) gene rearrangement profiles is not always straightforward. Therefore, the EuroClonality group supports and advises diagnostic laboratories in their clonality assessments on samples from patients suspicious for lymphoma, who are seen in the routine diagnostic setting. The support for clonality assessment is provided via the EuroClonality website: http://www.euroclonality.org. The features and procedures of the website are presented in this paper.

P. D. M. Rombout (✉) · P. J. T. A. Groenen
Department of Pathology, Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands
e-mail: p.rombout@pathol.umcn.nl

P. D. M. Rombout
e-mail: info@euroclonality.org

T. C. Diss
Department of Histopathology, University College London Medical School,
London, UK

E. Hodges
Molecular Pathology, Southampton University Hospitals,
Southampton, UK

M. Hummel
Institute of Pathology, Campus Benjamin Franklin, Charité,
Berlin, Germany

J. J. M. van Dongen · A. W. Langerak
Department of Immunology, Erasmus MC,
Rotterdam, The Netherlands

Keywords EuroClonality website · Clonality · Clonality testing

Introduction

The diagnosis of malignant lymphoma is recognized as a difficult area in histopathology. Modern hematopathology includes molecular tests such as clonality assays, since in principle B-cell and T-cell lymphomas are clonal diseases. Between 5 and 15% of hematopathology cases may benefit from clonality testing due to uncertainties based on histological and immunophenotypical evaluation. The development of new primer sets for clonality testing by the BIOMED-2 consortium [1] has resulted in standardization and significantly improved detection of clonality of malignant B-cell lymphomas/leukemias with the Ig-primer sets [2] and of malignant T-cell lymphomas/leukemias with the TCR primer sets [3]. Because of their high detection rates (99% for both B-cell and T-cell malignancies) and the complementarity of the primer sets, these assays are now widely used for detection of clonality in lymphoproliferative disorders.

The original BIOMED-2 study group has continued collaboration and is now known as the EuroClonality consortium. EuroClonality focuses on educational activities for Ig/TCR clonality testing, on quality control and development of guidelines for performance and evaluation of diagnostic clonality testing. The EuroClonality members meet twice a year to discuss technical and interpretation problems on clinical cases, to study specific lymphoma subtypes, to develop new applications and methodologies and to promote further developments in the field of hemato-oncology including a common nomenclature for the description and reporting of results derived from clonality testing.

From questions by others during the last few years, it is clear that clonality testing, especially the evaluation of results for patient diagnosis, can be difficult. After design and evaluation of the BIOMED-2/EuroClonality assays, we have therefore taken the responsibility to further educate the users of the clonality tests by organizing workshops, by reporting educational and practical examples of clonality testing [4–6] and by offering clonality support via the EuroClonality website. The public part is accessible to the wider clonality testing community and can be used to obtain information, education or support about clonality testing in routine diagnostic practice. In addition, the website functions as a communication medium for the EuroClonality group itself (the non-public part that is password protected).

EuroClonality public website: information, workshop and FAQs

On the website, general information about the EuroClonality consortium and the main goals of the group are documented. In addition, there is information on the workshop: "Clonality Assessment in Pathology", which is organized for laboratories that have introduced clonality testing using the BIOMED-2/EuroClonality primer sets. Currently many laboratories throughout the world are still introducing the technology, resulting in questions about the technology and interpretation of the results in routine practice. In addition, these laboratories wish to further improve the quality and reliability of their clonality assessment. For these laboratories, the EuroClonality consortium organizes annual workshops. Since the vision of the EuroClonality consortium is that clonality testing can only be performed in a reliable way when a close interaction exists between the (clinical) molecular biologist and the hematopathologist, these workshops are organized for such teams of specialists. Participants can download a registration form and send it to the workshop secretariat (workshop@euroclonality.org). Besides educational sessions, there are sessions in which individual cases are discussed. The participants can bring their own cases, which are difficult from the perspective of pathology and/or from the molecular results (GeneScan or gel based heteroduplex analysis of clonality results). All cases are discussed within the group in 20 headed microscope sessions, and knowledge and opinions are shared (Fig. 1). These workshops have proven to be very fruitful learning experiences for all participants. In the future we will consider organization of similar workshops for hematologists and immunologists.

Frequently asked questions (FAQs) can also be found on the website. There is already a list of questions from "detection rate of clonality tests" to "software used in clonality analysis" which have been answered by an expert team. Those who perform clonality analysis can use this information as an educational resource. The FAQs as they are currently available via the website are documented in Table 1.

EuroClonality public website: online support service

EuroClonality offers an online support service for questions on cases that are not answered by the frequently asked questions section. With this service, scientists that perform

Fig. 1 Photographs taken during an educational session (**a**, **b**) and during the case presentations and discussions (**c**, **d**)

Table 1 Frequently asked questions in clonality testing currently available via the website

1	What is the clonality detection rate of the BIOMED-2/EuroClonality primers?
2	How can I learn clonality testing?
3	Which Taq polymerase should I use?
4	Can agarose gels be used as read-out for clonality-testing?
5	DNA extraction from formalin-fixed-paraffin-embedded tissues (FFPE)
6	Does the use of unbuffered or buffered formalin affect molecular clonality testing?
7	Is the 3730 DNA analyzer (Applied Biosystems) suitable for clonality testing?
8	Which software can be used?
9	Protocols and literature about clonality
10	Control samples
11	Why can skin biopsies be problematic for clonality testing?
12	Commercially available BIOMED-2 EuroClonality primers (Invivoscribe) versus own primer mixes
13	What are the non-specific products in the EuroClonality/BIOMED-2 PCRs?

clonality testing can submit a problematic clonality case by filling in an online support form with details of the case and results obtained. A review board of experts is available to discuss technical and interpretation problems on clonality assessment and will reply to the queries within 10 working days.

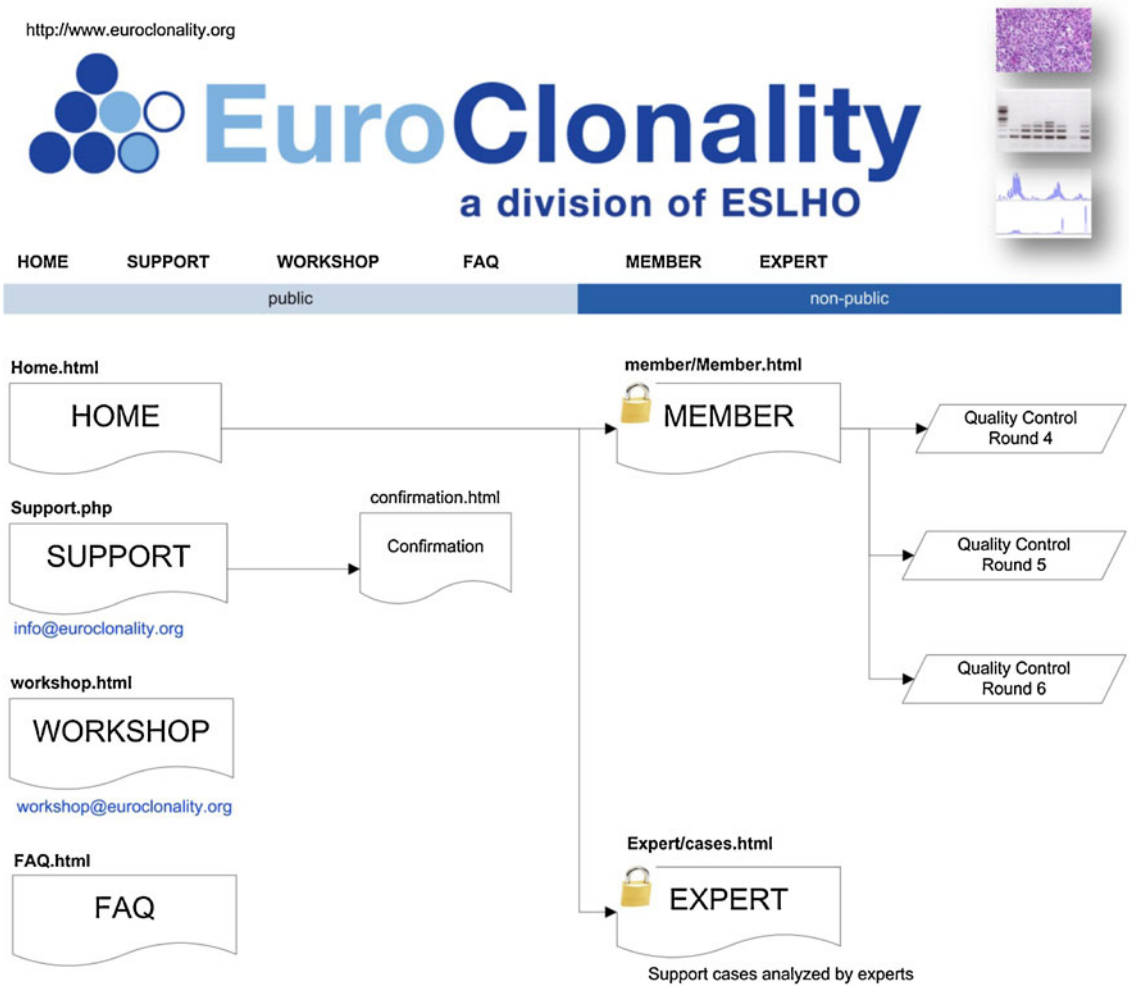

Fig. 2 Sitemap of the EuroClonality website

Logistics for the online support service

The submission of difficult cases occurs online via the EuroClonality web page. For this purpose, a short description of the differential diagnostic question and a compilation of the previous results can be uploaded together with representative images. After completion of the upload process, the expert panel will be notified that there is a new case submitted for review. Each case receives a unique ID number, to distinguish the different cases.

The expert on duty analyzes the support question and reviews the submitted data. Based on his or her opinion, an initial reply proposal is made to the other experts. This proposal is discussed within the expert team by e-mail communication. The members of the expert team respond as fast as possible with comments and additions. The expert on duty incorporates all of the expert opinions and makes a final answer which is sent to the support coordinator. The support coordinator sends an e-mail with the final answer to the case submitter on behalf of the expert team. This final answer is also published on the password-protected expert part of the website and can be used as a basis for other online support requests or for educational purposes within the EuroClonality group.

Most questions are related to interpretation problems involving IGH and IGL rearrangement results followed by questions regarding TCRG and TCRB PCR. A very typical question concerns the importance of dominant PCR products in isolated targets for the final interpretation of a given case. There is a significant degree of uncertainty whether such solitary PCR products are sufficient to conclude that the sample is monoclonal. A second important field of topics is related to more general technical questions such as suitable positive and negative controls, DNA amounts, DNA quality and non-specific bands. According to the experience of the expert panels, recommendations include additional analysis, because the use of insufficient controls, the application of a limited primer set and absence of duplicate results are the major obstacles observed. Most request submissions are derived from European countries, but some are from the USA and Canada.

In addition to the service for pathology laboratories, EuroClonality has now also started a support service for cases that are submitted from an immunological/hematological perspective. To this end experts with a background in immunology and/or hematology are available to answer questions and provide advice on further action. Scientists can upload their cases in the same way as for pathologically problematic cases and can provide details on PCR assay and flow-cytometry results. As this service has only recently been started, it has not been very actively consulted so far. However, it is expected that this will change in the near future.

EuroClonality non-public part for members and expert teams

There is also a private part of the website, which is password protected. This non-public part is available for EuroClonality members and is used for communication purposes. For example, documents and files are available with practical information from the meetings, such as minutes, guidelines and concepts or preview publications. Most of the non-public part is used for the web-based Quality Control rounds (QCs). These QCs are currently organized for members only, with the aim of designing new guidelines and a universal scoring system to improve clonality test reporting. In the near future, the possibility to widen the activities of the group, especially the QCs and to make it accessible to a broader public, will be considered.

Finally, there is a private part of the website for the review board experts only. Experts can download support cases together with the clonality result files. A graphical overview of the design of the website is presented in Fig. 2. The website is hosted by one.com and written in html and php.

Summary

The EuroClonality website offers several tools, such as the EuroClonality workshops, the Frequently Asked Questions (FAQ) and the online support service for questions on clonality assessment in lymphoma diagnostics. The website is open to every pathologist, clinical molecular biologist, hematologist or immunologist interested in the field of lymphoproliferations. By offering educational tools and support, EuroClonality continues to contribute to the improvement of molecular (clonality) diagnosis of malignant lymphomas and thereby to the better management of our patients.

Acknowledgements The authors thank Bas Delfos for his technical support of the website, Annemiek van Raaij as supply webmaster and Anke Vargas for her continuous and excellent administrative support.

References

1. van Dongen JJ, Langerak AW, Brüggemann M et al (2003) Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 17:2257–317

2. Evans PA, Pott Ch, Groenen PJ et al (2007) Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia 21:207–14
3. Brüggemann M, White H, Gaulard P et al (2007) Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies. Report of the BIOMED-2 Concerted Action BHM4 CT98-3936. Leukemia 21:215–21
4. Langerak AW, Groenen PJTA, van Krieken JHJM, van Dongen JJM (2007) Immunoglobulin/T-cell receptor clonality diagnostics. Expert Opin Med Diagn 1:451–61
5. Groenen PJ, Langerak AW, van Dongen JJ, van Krieken JH (2008) Pitfalls in TCR gene clonality testing: teaching cases. J Hematop 1:97–109
6. Langerak AW (2008) Undersized, oversized? It is not one-size-fits-all in lymphoid clonality detection. Leuk Res 322:203–204