

CHAPTER 7 MOLECULAR AND CYTOGENETIC STUDIES — DIAGNOSTIC APPLICATIONS

7.1 B-cell clonality testing by PCR for diagnostic purposes

7.1.1 Immunoglobulin gene rearrangements

Assessment of IgH gene rearrangements is the principal approach to B-cell clonality testing. Ig light chain (IgL) gene rearrangement assays are also available but are not routinely used. A number of PCR approaches and detection systems may be used for IgH clonality testing. The most commonly used are complementarity determining region 3 (CDR3) strategies, which amplify the CDR3 region where the greatest junctional diversity is generated during gene rearrangement. Typically, degenerate consensus primers annealing to framework region (FR3) of the variable (V) region genes are used in conjunction with consensus primers to the 3' ends of the joining (J) region genes in monoplex, hemi-nested or nested assays. Additional reactions using consensus FR1 or FR2 primers, or use of gene family-specific primers (usually directed at FR1 or leader sequences), can increase the frequency of clonal detection.¹

Qualitative sensitivity of PCR testing for IgH rearrangement

Qualitative sensitivities vary widely, from <50% to virtually 100% of B-cell lymphomas, depending on the assay design, case-mix, primer selection and detection system employed. For example, false negatives are more likely to occur with follicular, marginal zone and diffuse large B-cell lymphomas, owing to V-region somatic hypermutations, particularly in follicular lymphoma, which affects primer annealing in CDR3 assays.²⁻⁷

Using CDR3 assays with consensus primers (typically FR3 region V primers) and conventional gels, the frequency of clonal detection ranges from approximately 60% to 80%. This increases to >90% of cases by using additional assays employing FR2 and/or FR1 or leader region primers, by including assays for IgL gene rearrangements, by adding assays for specific chromosomal translocations, and by using more sensitive gel systems, including CEGS.¹

Analytical (quantitative) sensitivity of PCR testing for IgH rearrangement

Using simple CDR3 strategies, consensus primers and non-denaturing gels, analytical sensitivities are in the range of 1–10% clonal cells in a polyclonal background. Greater sensitivity (0.1–1% clonal cells in a polyclonal background) may be achieved with higher resolution denaturing/sequencing gels with or without automated fluorescent DNA fragment analysis.¹

Specificity and positive predictive value of PCR testing for IgH rearrangement

Specificities of clonal IgH gene rearrangements range from approximately 80% to 100%, and positive predictive values from 70% to 100%.¹

Interlaboratory variability and standardisation

There is a need for interlaboratory standardisation of assays. Significant interlaboratory variations in qualitative sensitivity have been reported using the same lymphoma samples (range 20–90% frequency of clonal detection), particularly in paraffin-embedded tissue.⁸ The BIOMED-2 Concerted Action collaborative study has addressed such deficiencies in PCR clonality testing, and has published standardised primers and protocols for multiplex PCR assays for clonality studies, reporting unprecedentedly high rates of clonal detection.⁹

Guideline — Interpretation of assay results	Level of evidence	Refs
PCR results for IgH clonality testing should: <ul style="list-style-type: none"> (i) be interpreted in the context of a detailed knowledge of the nature of the assay used, its qualitative and analytical sensitivities, and predictive value (ii) recognise that the most commonly employed CDR3 assays using consensus primers may have a significant false negative rate, particularly in follicular, marginal zone and diffuse large B-cell lymphomas. 	IV	2–7

7.2 T-cell clonality testing by PCR for diagnostic purposes

7.2.1 TCR γ gene rearrangements

Because of its simple genomic structure and the requirement for few V γ and J γ primer combinations to detect all possible rearrangements of the gene, the TCR γ gene is the preferred gene for T-cell clonality testing in routine laboratories. As the gene has only four variable region families and five joining segment genes, construction of consensus or gene segment-specific primers is relatively simple. Assays vary in their design and complexity, and a range of different detection systems, including CEGS, may be used, all of which affect the qualitative and analytical sensitivities of the assays.^{7,10–19}

Qualitative sensitivity

There is a wide range in the reported frequency of clonal detection (~60% to virtually 100%), reflecting the effects of the case-mix, nature of the PCR assay employed, primer selection and sensitivity of the detection system. By using multiple primer combinations, which will detect all possible TCR γ gene rearrangements, and routine PAGE, clonal detection rates of 80% to 90% may be achieved. This may be increased to >90% and approaching 100% by employing high-resolution complex gels or automated fluorescent DNA fragment analysis.¹ Additional testing for TCR β gene rearrangements (see below), either in separate assays or by including TCR β primers in multiplex TCR γ and TCR β primer mixes, will increase the clonal detection rate by as much as 20%.¹

Analytical sensitivity

Between 1% and 5% of clonal T cells can be detected in a background of polyclonal TCR γ gene rearrangements in non-denaturing polyacrylamide gels, although inferior sensitivities may result using paraffin-embedded tissue.²⁰ A ten-fold increase in sensitivity (0.1–1%) may be achieved with high-resolution complex gels, or by CEGS, which is fast, accurate, has a high analytical sensitivity at least equal to denaturing gradient electrophoresis DGGE (~0.1–1% in a polyclonal T-cell background), and is able to detect \geq 90% of T-NHL (reviewed in Spagnolo et al.¹).

Test specificity and positive predictive value

As for mature B-NHL, these values range widely and it is difficult to compare data. Both specificities and positive predictive values range from approximately 70% to 100%.¹ In cutaneous B-NHL, the incidence of TCR γ and/or TCR β clonal gene rearrangements may be particularly high²¹ (i.e. relatively low positive predictive value). Similarly, dual genotypes in mature T-NHL are disproportionately higher in cutaneous cases, compared with non-cutaneous cases.²¹ In inflammatory skin disorders in particular, PCR assays should be repeated because of the frequent occurrence of pseudoclonal TCR γ rearrangements, which in a single PCR assay could be misinterpreted as being monoclonal.^{22,23}

7.2.2 TCR β gene rearrangements

Because of its complexity, the TCR β gene is used less often in T-cell clonality testing. It has a large germline repertoire that includes numerous V gene families and J segments, thus restricting the design of sensitive but simple assays based on limited numbers of consensus primers. The large intron separating rearranged VDJ segments from C regions largely precludes DNA-based assays using V and C region primers, which requires RT-PCR, adding to the complexity of the assays. A variety of PCR approaches are published, varying in design complexity, qualitative and quantitative sensitivities.¹

Guideline — Interpretation of assay results	Level of evidence	Refs
PCR analysis of TCR γ gene rearrangements is the recommended first-line approach for T-cell clonality testing. The results should be interpreted in the context of a detailed knowledge of the qualitative and analytical sensitivities, and the predictive value of the assay used.	IV	7, 10–19

7.3 Minimal residual disease detection and monitoring (MRDDM)

PCR assays, and RQ-PCR in particular, are being used increasingly for MRDDM in lymphoma. The critical interpretation and clinical significance of results requires consideration of technical, biological and clinical factors.²⁴ There is a need for greater standardisation of methodology and criteria for interpreting results²⁵, along the lines proposed by the BIOMED-2 Concerted Action^{26,27} and the Europe Against Cancer Program^{28,29}, but there are significant cost implications in this approach.

7.3.1 Molecular targets in MRDDM

These are essentially the same as those used for primary lymphoma diagnosis, specifically AgR gene rearrangements (which may involve using patient-specific oligonucleotide primers or probes), chromosomal translocations (DNA based) or fusion gene transcripts resulting from chromosomal translocations (RT-PCR). Sensitive assays are needed to achieve the required analytical sensitivities of 10^{-4} to 10^{-6} , particularly in AgR gene rearrangement assays where clonal rearrangements need to be distinguished from any background polyclonal rearrangements.¹

7.3.2 RQ-PCR in MRDDM

RQ-PCR assays are now the preferred approach to MRDDM²⁵. The choice of strategy depends on the disease category, the nature of the molecular target, the analytical sensitivity required, and the expertise of the laboratory. Each method has potential advantages and disadvantages.²⁵

7.3.3 Analytical sensitivity of RQ-PCR, controls and quantitation

Using fusion gene mRNA transcripts as PCR targets, sensitivities of 10^{-4} to 10^{-6} can be achieved, with little risk of false positivity from detection of low-level fusion transcripts present in normal cells. With AgR gene rearrangements as targets, sensitivities between 10^{-3} to 10^{-5} are achieved.^{25,30} Control genes must be included in the assays to correct for DNA or RNA/cDNA quality, as this affects product quantitation.^{25,31}

7.4 Testing for chromosomal translocations

Recurring chromosomal abnormalities (see Table 7.1) characterise certain non-Hodgkin lymphomas (NHLs) and are used for both diagnostic purposes and MRDDM. They may be detected by a variety of techniques, including SB, DNA-PCR, RT-PCR, classical cytogenetics and FISH. The method of

choice depends on the particular translocation being assessed. Among the most frequently assessed translocations in lymphoma diagnosis are the t(14;18)(q32;q21) in follicular lymphoma, the t(11;14)(q13;q32) of mantle cell lymphoma, and the t(2;5)(p23;q35) of systemic anaplastic large-cell lymphoma (ALCL).

7.4.1 t(14;18)(q32;q21)

This is the most common non-random chromosomal translocation occurring in NHL. It is detected by cytogenetics in 80% to 90% of follicular lymphomas and in 20% to 30% of diffuse large B-cell lymphomas. Although genomic PCR may be used for detection, other modalities are more sensitive, namely SB (but PCR is more cost effective), conventional cytogenetics, FISH and fibre-FISH (in increasing order of sensitivity).^{32–39}

PCR diagnostic assays for t(14;18)(q32;q21)

Comparable qualitative sensitivities may be achieved in frozen or paraffin-embedded tissue approaching that of conventional cytogenetics, and optimised assays may achieve analytical sensitivities of 1 in 10⁵ cells.¹ Employing two sets of primers specific for both the major breakpoint region and minor cluster region, the translocation will be detected in 60% to 80% of cases. The presence of small numbers of translocation-positive cells in normal individuals and in hyperplastic nodes¹ argue against the use of very sensitive, nested assays, or RQ-PCR assays designed for MRD detection. These potential false positives are avoided by using standard single-primer set diagnostic assays.

t(14;18) PCR for MRDDM

Sensitive nested t(14;18) PCR assays with sensitivities of 1 in 10⁵–10⁶ are used for MRDDM, and for assessing the efficacy of marrow purging prior to autologous transplantation. Where available, RQ-PCR assays are now the preferred method of testing, using TaqMan and LightCycler systems that are at least as sensitive as conventional nested assays.¹ Their high analytical sensitivity mandates caution in interpreting ‘molecular relapse’ in treated patients, as translocation-positive cells in normal individuals can be detected at levels as high as 1 in 10⁴ cells.⁴⁰

Interlaboratory variability

Multi-institutional studies have reported a wide interlaboratory variability in *bcl-2* testing methodology, with a large proportion of laboratories not knowing the analytical sensitivity of their system (i.e. the lower limit of detection), and having significant false-positive rates and low sensitivities.^{38,41} These indicate the need for greater interlaboratory standardisation for these assays, especially in the setting of MRD detection.

Guideline	Level of evidence	Refs
FISH or PCR assays are the methods of choice for detecting the t(14;18)(q32;q21).	IV	32, 34–38

7.4.2 t(11;14)(q13;q32)

This translocation between the *CCND1/BCL-1* and *IgH* genes, which characterises mantle cell lymphoma (MCL) and is rarely found in other lymphomas, results in deregulated cyclin D1 expression. In decreasing order of qualitative sensitivity, the modalities for detecting aberrations of the *bcl-1* gene are DNA fibre-FISH (~100%), conventional FISH including interphase FISH (>95%), *in situ* mRNA hybridisation (>80%), immunohistochemical staining for cyclin D1 protein (range 70% to >90%), conventional cytogenetics (60–70%), SB (~70%), and genomic PCR (most studies <50%).^{33,39,42} Almost all translocation-positive MCL will be detected by the various FISH techniques

available^{36,37,43–46}. Genomic PCR, including real-time quantitative PCR assays, is of limited sensitivity (40–50%), as only translocations involving the major translocation cluster of *bcl-1* will be detected with standard assays. Immunohistochemical demonstration of nuclear cyclin D1 protein expression is the most cost-effective ancillary diagnostic test for MCL, with sensitivities ranging between 70% and >95%.^{45–49}

Guideline	Level of evidence	Refs
Immunostaining for cyclin D1 protein is the recommended modality for confirming a diagnosis of mantle cell lymphoma.	IV	45–49
FISH techniques, if available, are the most sensitive means of demonstrating the t(11;14)(q13;q32).	IV	36, 37, 43–46

7.4.3 t(2;5)(p23;q35)

This translocation between the novel ALK and the NPM genes, which characterises most ALCL of T/null cell phenotype, generates a fusion gene — ALK/NPM — resulting in dysregulated ALK protein expression in nucleus and cytoplasm. At least 20% of ALCL harbour variant ALK translocations involving a translocation partner other than NPM, but still resulting in dysregulated ALK protein expression restricted to the cytoplasm and/or cell membrane.¹

The t(2;5)(p23;q35) translocation may be detected by a variety of methods. The most sensitive and practical is ALK protein immunostaining^{50,51}, which correlates well with other detection methods.⁵² Virtually all translocation-positive cases can be detected by RT-PCR, but variant 2p23 anomalies will not be detected, false positives from contamination will be missed owing to the constant size of the PCR product, and low-level transcripts present in normal individuals may be over interpreted. Long-range genomic DNA-PCR is the preferred PCR modality to avoid these potential pitfalls, but requires high-quality undegraded DNA.¹ FISH, including interphase FISH, can also detect the t(2;5)(p23;q35) and variant 2p23 anomalies.¹

Guideline — Immunostaining — anaplastic large-cell lymphoma	Level of evidence	Refs
Immunostaining for ALK protein expression is the recommended test for detecting ALK and anaplastic large-cell lymphoma of T/null cell type	IV	50–52

Table 7.1 Common chromosome translocations in non-Hodgkin lymphomas

Chromosome aberration	Lymphoma	Genes involved
t(14;18)(q32;q21)	Follicular Diffuse large B-cell	<i>BCL-2, IgH</i>
t(8;14)(q24;q32)	Burkitt lymphoma	<i>C-MYC, IgH</i>
t(8;22)(q24;q11)	Burkitt lymphoma	<i>C-MYC, IgL</i>
t(2;8)(p11;q24)	Burkitt lymphoma	<i>C-MYC, IgK</i>
t(11;14)(q13;q32)	Mantle cell B-CLL, small subset	<i>CCND1 (cyclin D1; BCL-1), IgH</i>
t(11;18)(q21;q21)	Marginal zone/extranodal MALT	<i>API2, MALT1</i>
t(14;18)(q32;q21)	Marginal zone/extranodal MALT	<i>MALT, IgH</i>
t(1;14)(p22;q21)	Marginal zone/extranodal MALT	<i>BCL-10, IgH</i>
t(1;2)(p22;p12)	Marginal zone/extranodal MALT	<i>BCL-10, IgK</i>
t(2;18)(p11;q21)	CLL/SLL (5%)	<i>BCL-2, Igκ</i>
t(18;22)(q21;q11)	CLL/SLL (5%)	<i>BCL-2, Igλ</i>
t(14;19)(q32;q13)	CLL/SLL (<5%)	<i>BCL-3, IgH</i>
t(9;14)(p13;q32)	lymphoplasmacytoid lymphoma	<i>PAX5, IgH</i>
t(3;14)(q27;q32)*	<i>de novo</i> diffuse large B-cell	<i>BCL-6, IgH</i>
t(3;22)(q27;q11)	<i>de novo</i> diffuse large B-cell	<i>BCL-6, Igλ</i>
t(2;3)(p12;q27)	<i>de novo</i> diffuse large B-cell	<i>BCL-6, Igκ</i>
2p13–15 amplification	diffuse large B-cell, extranodal	<i>REL amplification</i> (<i>NFKB</i> family member)
t(2;5)(p23;q35)**	Anaplastic large cell, T/null	<i>ALK, NPM</i>

* many other *bcl-6* translocation partners are also described

** >20% of ALCL harbour variant 2p23 rearrangements involving genes other than *NPM* as a translocation partner (e.g. *TPM3, TFG, ATIC, MSN, CLTCL*).¹

7.5 Virus detection by *in situ* hybridisation

A number of viruses are implicated in the development of human lymphomas. EBV is the best known. EBV genomic material may be detected in almost 50% of classical Hodgkin disease, in nearly all endemic Burkitt lymphomas, in nasal-type natural killer/T cell lymphoma, angiocentric B-cell lymphoma (lymphomatoid granulomatosis), post-transplant lymphoproliferative disorders, AIDS-associated lymphomas, and primary effusion lymphomas.⁵³ The presence of EBV can be demonstrated in a number of ways, including SB and PCR, but the method of choice is by EBV

EBER *in situ* hybridisation⁵⁴⁻⁵⁷, which is easily applied in paraffin sections with high sensitivity, and is not expensive.

7.6 Standardisation of molecular tests

There is a relative lack of interlaboratory standardisation in molecular testing⁵⁸, which complicates the comparison of data. Few multicentre studies have addressed this issue. Significant interlaboratory variations in assay methodology and clonality detection rates have been found in TCR gene testing²⁰, IgH gene testing⁸ and t(14;18) detection.^{38,41}

Recent multicentre European collaborative studies have been instituted to optimise and standardise PCR assays for the purposes of clonality studies in lymphoma (BIOMED-2 Concerted Action)^{9,59}, leukaemia diagnosis, and MRD detection (Europe Against Cancer Program).^{28,29} While this approach to standardisation and improved clonal detection is to be lauded, the complexity and costs involved are major drawbacks in a routine laboratory setting. The need for standardisation and guidelines for assignment of monoclonality becomes even more critical with the increasing use of new and sensitive detection methods, such as CEGS, in order to avoid false positive results.

7.7 References

1. Spagnolo D, Ellis D, Juneja S, et al. The role of molecular studies in lymphoma diagnosis: a review. *Pathology* 2004; 36: 19–44.
2. Diss TC, Peng H, Wotherspoon AC, Isaacson PG, Pan L. Detection of monoclonality in low-grade B-cell lymphomas using the polymerase chain reaction is dependent on primer selection and lymphoma type. *J Pathol* 1993; 169: 291–5.
3. Segal GH, Jorgensen T, Masih AS, Braylan RC. Optimal primer selection for clonality assessment by polymerase chain reaction analysis: I. Low grade B-cell lymphoproliferative disorders of nonfollicular center cell type. *Hum Pathol* 1994; 25: 1269–75.
4. Segal GH, Jorgensen T, Scott M, Braylan RC. Optimal primer selection for clonality assessment by polymerase chain reaction analysis: II. Follicular lymphomas. *Hum Pathol* 1994; 25: 1276–82.
5. Lombardo JF, Hwang TS, Maiese RL, Millson A, Segal GH. Optimal primer selection for clonality assessment by polymerase chain reaction analysis. III. Intermediate and high-grade B-cell neoplasms. *Hum Pathol* 1996; 27: 373–80.
6. Derksen PW, Langerak AW, Kerkhof E, et al. Comparison of different polymerase chain reaction-based approaches for clonality assessment of immunoglobulin heavy-chain gene rearrangements in B-cell neoplasia. *Mod Pathol* 1999; 12: 794–805.
7. Thériault C, Galoin S, Valmary S, et al. PCR analysis of immunoglobulin heavy chain (IgH) and TcR- γ chain gene rearrangements in the diagnosis of lymphoproliferative disorders: results of a study of 525 cases. *Mod Pathol* 2000; 13: 1269–79.
8. Bagg A, Brazier RM, Arber DA, Bijwaard KE, Chu AY. Immunoglobulin heavy chain gene analysis in lymphomas: a multi-center study demonstrating the heterogeneity of performance of polymerase chain reaction assays. *J Mol Diagn* 2002; 4: 81–9.
9. van Dongen JJ, Langerak AW, Bruggemann M, et al. 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* 2003; 17: 2257–317.

10. McCarthy KP, Sloane JP, Kabarowski JH, Matutes E, Wiedemann LM. A simplified method of detection of clonal rearrangements of the T-cell receptor- γ chain gene. *Diagn Mol Pathol* 1992; 1: 173–9.
11. Griesser H. Gene rearrangements and chromosomal translocations in T cell lymphoma — diagnostic applications and their limits. *Virchows Arch* 1995; 426: 323–38.
12. Lorenzen J, Jux G, Zhao-Hohn M, Klockner A, Fischer R, Hansmann ML. Detection of T-cell clonality in paraffin-embedded tissues. *Diagn Mol Pathol* 1994; 3: 93–9.
13. Greiner TC, Raffeld M, Lutz C, Dick F, Jaffe ES. Analysis of T cell receptor- γ gene rearrangements by denaturing gradient gel electrophoresis of GC-clamped polymerase chain reaction products. Correlation with tumor-specific sequences. *Am J Pathol* 1995; 146: 46–55.
14. Födinger M, Winkler K, Mannhalter C, Chott A. Combined polymerase chain reaction approach for clonality detection in lymphoid neoplasms. *Diagn Mol Pathol* 1999; 8: 80–91.
15. Sprouse JT, Werling R, Hanke D, et al. T-cell clonality determination using polymerase chain reaction (PCR) amplification of the T-cell receptor gamma-chain gene and capillary electrophoresis of fluorescently labeled PCR products. *Am J Clin Pathol* 2000; 113: 838–50.
16. Luo V, Lessin SR, Wilson RB, et al. Detection of clonal T-cell receptor γ gene rearrangements using fluorescent-based PCR and automated high-resolution capillary electrophoresis. *Mol Diagn* 2001; 6: 169–79.
17. Cairns SM, Taylor JM, Gould PR, Spagnolo DV. Comparative evaluation of PCR-based methods for the assessment of T cell clonality in the diagnosis of T cell lymphoma. *Pathology* 2002; 34: 320–5.
18. Greiner TC, Rubocki RJ. Effectiveness of capillary electrophoresis using fluorescent-labeled primers in detecting T-cell receptor γ gene rearrangements. *J Mol Diagn* 2002; 4: 137–43.
19. Lukowsky A. Clonality analysis by T-cell receptor γ PCR and high-resolution electrophoresis in the diagnosis of cutaneous T-cell lymphoma (CTCL). *Methods Mol Biol* 2003; 218:303–20.
20. Arber DA, Brazier RM, Bagg A, Bijwaard KE. Evaluation of T cell receptor testing in lymphoid neoplasms: results of a multicenter study of 29 extracted DNA and paraffin-embedded samples. *J Mol Diagn* 2001; 3: 133–40.
21. Krafft AE, Taubenberger JK, Sheng ZM, et al. Enhanced sensitivity with a novel TCR γ PCR assay for clonality studies in 569 formalin-fixed, paraffin-embedded (FFPE) cases. *Mol Diagn* 1999; 4: 119–33.
22. Dippel E, Assaf C, Hummel M, et al. Clonal T-cell receptor γ -chain gene rearrangement by PCR-based GeneScan analysis in advanced cutaneous T-cell lymphoma: a critical evaluation. *J Pathol* 1999; 188: 146–54.
23. Assaf C, Hummel M, Dippel E, et al. High detection rate of T-cell receptor beta chain rearrangements in T-cell lymphoproliferations by family specific polymerase chain reaction in combination with the GeneScan technique and DNA sequencing. *Blood* 2000; 96: 640–6.
24. Bagg A. Commentary: minimal residual disease: how low do we go? *Mol Diagn* 2001; 6: 155–60.

25. van der Velden VHJ, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia* 2003; 17: 1013–34.
26. Pongers-Willemse MJ, Seriu T, Stolz F, et al. Primers and protocols for standardized detection of minimal residual disease in acute lymphoblastic leukemia using immunoglobulin and T cell receptor gene rearrangements and TAL1 deletions as PCR targets: report of the BIOMED-1 CONCERTED ACTION: investigation of minimal residual disease in acute leukemia. *Leukemia* 1999; 13: 110–8.
27. van Dongen JJ, Macintyre EA, Gabert JA, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia* 1999; 13: 1901–28.
28. Beillard E, Pallisgaard N, van der Velden VH et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using ‘real-time’ quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) — a Europe Against Cancer program. *Leukemia* 2003; 17: 2474–86.
29. Gabert J, Beillard E, van der Velden VHJ, et al. Standardization and quality control of ‘real-time’ quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia — a Europe against cancer program. *Leukemia* 2003; 17: 2318–57.
30. Szczepanski T, Flohr T, van der Velden VH, Bartram CR, van Dongen JJ. Molecular monitoring of residual disease using antigen receptor genes in childhood acute lymphoblastic leukaemia. *Best Pract Res Clin Haematol* 2002; 15: 37–57.
31. Lossos IS, Czerwinski DK, Wechser MA, Levy R. Optimization of quantitative real-time RT-PCR parameters for the study of lymphoid malignancies. *Leukemia* 2003; 17: 789–95.
32. Horsman DE, Gascoyne RD, Coupland RW, Coldman AJ, Adomat SA. Comparison of cytogenetic analysis, southern analysis, and polymerase chain reaction for the detection of t(14;18) in follicular lymphoma. *Am J Clin Pathol* 1995; 103: 472–8.
33. Medeiros LJ, Carr J. Overview of the role of molecular methods in the diagnosis of malignant lymphomas. *Arch Pathol Lab Med* 1999; 123: 1189–207.
34. Estalilla OC, Medeiros LJ, Manning JT, Jr., Luthra R. 5’→3’ exonuclease-based real-time PCR assays for detecting the t(14;18)(q32;21): a survey of 162 malignant lymphomas and reactive specimens. *Mod Pathol* 2000; 13: 661–6.
35. Vaandrager JW, Schuurin E, Raap T, Philippo K, Kleiverda K, Kluin P. Interphase FISH detection of BCL2 rearrangement in follicular lymphoma using breakpoint-flanking probes. *Genes Chromosomes Cancer* 2000; 27: 85–94.
36. Frater JL, Tsiftsakis EK, Hsi ED, Pettay J, Tubbs RR. Use of novel t(11;14) and t(14;18) dual-fusion fluorescence in situ hybridization probes in the differential diagnosis of lymphomas of small lymphocytes. *Diagn Mol Pathol* 2001; 10: 214–22.
37. Haralambieva E, Kleiverda K, Mason DY, Schuurin E, Kluin PM. Detection of three common translocation breakpoints in non-Hodgkin’s lymphomas by fluorescence in situ hybridization on routine paraffin-embedded tissue sections. *J Pathol* 2002; 198: 163–70.

38. Hsi ED, Tubbs RR, Lovell MA, Braziel RM, Gulley ML. Detection of bcl-2/J(H) translocation by polymerase chain reaction: a summary of the experience of the Molecular Oncology Survey of the College of American Pathologist. *Arch Pathol Lab Med* 2002; 126: 902–8.
39. Vega F, Medeiros LJ. Chromosomal translocations involved in non-Hodgkin lymphomas. *Arch Pathol Lab Med* 2003; 127: 1148–60.
40. Summers KE, Goff LK, Wilson AG, Gupta RK, Lister TA, Fitzgibbon J. Frequency of the Bcl-2/IgH rearrangement in normal individuals: implications for the monitoring of disease in patients with follicular lymphoma. *J Clin Oncol* 2001; 19: 420–4.
41. Johnson PW, Swinbank K, MacLennan S, et al. Variability of polymerase chain reaction detection of the bcl-2-IgH translocation in an international multicentre study. *Ann Oncol* 1999; 10: 1349–54.
42. Athanasiou E, Kotoula V, Hytiroglou P, Kouidou S, Kaloutsi V, Papadimitriou CS. In situ hybridization and reverse transcription-polymerase chain reaction for cyclin D1 mRNA in the diagnosis of mantle cell lymphoma in paraffin-embedded tissues. *Mod Pathol* 2001; 14: 62–71.
43. de Boer CJ, Vaandrager JW, van Krieken JH, Holmes Z, Kluin PM, Schuurung E. Visualization of mono-allelic chromosomal aberrations 3' and 5' of the cyclin D1 gene in mantle cell lymphoma using DNA fiber fluorescence in situ hybridization. *Oncogene* 1997; 15: 1599–603.
44. Li JY, Gaillard F, Moreau A, et al. Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization. *Am J Pathol* 1999; 154: 1449–52.
45. Belaud-Rotureau MA, Parrens M, Dubus P, Garroste JC, de Mascarel A, Merlio JP. A comparative analysis of FISH, RT-PCR, PCR, and immunohistochemistry for the diagnosis of mantle cell lymphomas. *Mod Pathol* 2002; 15: 517–25.
46. Kodet R, Mrhalova M, Krskova L, et al. Mantle cell lymphoma: improved diagnostics using a combined approach of immunohistochemistry and identification of t(11;14)(q13;q32) by polymerase chain reaction and fluorescence in situ hybridization. *Virchows Arch* 2003; 442: 538–47.
47. Swerdlow SH, Yang WI, Zukerberg LR, Harris NL, Arnold A, Williams ME. Expression of cyclin D1 protein in centrocytic/mantle cell lymphomas with and without rearrangement of the BCL1/cyclin D1 gene. *Hum Pathol* 1995; 26: 999–1004.
48. Korin HW, Schwartz MR, Chirala M, Younes M. Optimized cyclin D1 immunoperoxidase staining in mantle cell lymphoma. *Appl Immunohistochem Mol Morphol* 2000; 8: 57–60.
49. Miranda RN, Briggs RC, Kinney MC, Veno PA, Hammer RD, Cousar JB. Immunohistochemical detection of cyclin D1 using optimized conditions is highly specific for mantle cell lymphoma and hairy cell leukemia. *Mod Pathol* 2000; 13: 1308–14.
50. Pulford K, Lamant L, Morris SW, et al. Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)-ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood* 1997; 89: 1394–404.
51. Falini B. Anaplastic large cell lymphoma: pathological, molecular and clinical features. *Br J Haematol* 2001; 114: 741–60.

52. Cataldo KA, Jalal SM, Law ME, et al. Detection of t(2;5) in anaplastic large cell lymphoma: comparison of immunohistochemical studies, FISH, and RT-PCR in paraffin-embedded tissue. *Am J Surg Pathol* 1999; 23: 1386–92.
53. Arber DA. Molecular diagnostic approach to non-Hodgkin's lymphoma. *J Mol Diagn* 2000; 2: 178–90.
54. Ambinder RF, Mann RB. Epstein-Barr-encoded RNA in situ hybridization: diagnostic applications. *Hum Pathol* 1994; 25: 602–5.
55. Gaal K, Sun NC, Hernandez AM, Arber DA. Sinonasal NK/T-cell lymphomas in the United States. *Am J Surg Pathol* 2000; 24: 1511–7.
56. Gulley ML. Molecular diagnosis of Epstein-Barr virus-related diseases. *J Mol Diagn* 2001; 3: 1–10.
57. Gulley ML, Glaser SL, Craig FE, et al. Guidelines for interpreting EBER in situ hybridization and LMP1 immunohistochemical tests for detecting Epstein-Barr virus in Hodgkin lymphoma. *Am J Clin Pathol* 2002; 117: 259–67.
58. Bagg A, Kallakury BV. Molecular pathology of leukemia and lymphoma. *Am J Clin Pathol* 1999; 112: S76–S92.
59. Sandberg Y, Heule F, Lam K, et al. Molecular immunoglobulin/T-cell receptor clonality analysis in cutaneous lymphoproliferations. Experience with the BIOMED-2 standardized polymerase chain reaction protocol. *Haematologica* 2003; 88: 659–70.

