Molecular diagnostic assays have become routine in the evaluation of lymphoid malignancies. Both Southern transfer and polymerase chain reaction (PCR) technologies are used to assess for B- and T-cell clonality, the presence of rearrangements involving protooncogenes such as \textit{bcl-1} and \textit{bcl-2}, and the monitoring of minimal residual disease. We review the fundamentals of B- and T-cell ontogeny as well as the basic principles of the Southern transfer and PCR assays and their applications to the diagnosis of lymphoid malignancies.

The lymphoid malignancies can be broadly categorized into the malignant lymphomas, which include non-Hodgkin lymphoma and Hodgkin disease, and the acute and chronic lymphoid leukemias [1]. Molecular diagnostic assays for the evaluation of lymphoid malignancies including Southern transfer and PCR have become increasingly popular in the clinical laboratory. Applications include confirmation of diagnosis and complete or partial remission, as an indicator of prognosis, for the monitoring of minimal residual disease and patients after bone marrow transplantation and for the early detection of relapse and distant site involvement. In general, precise diagnosis of hematological malignancies often requires a multiparameter approach that correlates morphological evaluation of traditional hematoxylin- and eosin-stained tissue sections or Wright-stained smears with a variety of special studies. These special studies may include any combination of cytochemical and histochemical stains, immunopathological studies, molecular genetic techniques, and cytogenetic techniques.

The lymphoid malignancies are a heterogeneous group of disorders that occur as a result of neoplastic transformation of B and T lymphocytes at different stages of B- and T-cell development. The wide variety of lymphoid malignancies reflects the various stages of lymphocyte development and the complexity of the immune system. The clinical and pathological characteristics of the lymphoid malignancies are summarized in a comprehensive manner in the recently proposed Revised European American Lymphoma classification [1]. Our understanding of the immune system and ability to diagnose and classify lymphoid malignancies improved substantially in the 1980s because of the development of immunopathological methods utilizing a wide variety of monoclonal antibodies to cell surface antigens [2]. Traditional morphological findings in conjunction with immunopathological studies are now the cornerstone of diagnosis in lymphoid malignancies. In the mid-1980s, the availability of molecular genetic methods further enhanced our ability to diagnose and classify lymphoid malignancies [3].

The major application of molecular genetic methods in the evaluation of lymphoid neoplasms involves the determination of B- and T-cell clonality. These methods are considered to be the gold standard for determining clonality and are utilized primarily when clonality cannot be determined immunopathologically. For B-cell neoplasms, clonality can often be determined immunopathologically by demonstrating the presence of monoclonal surface immunoglobulin [2]. For T-cell malignancies, there is no immunopathological equivalent to monoclonal surface immunoglobulin, although aberrant loss of T-cell antigen expression is considered to be presumptive evidence of T-cell malignancy [2]. Thus, in T-cell malignancies, molecular genetic studies for the determination of clonality are especially important. Other applications of molecular genetic methods to the assessment of lymphoid malignancies include determination of B- or T-cell lineage, detection of chromosomal translocations, detection of minimal residual disease, and detection of viral DNA sequences such as Epstein–Barr virus, which is involved in the pathogenesis of some lymphoid malignancies. Assays for detecting minimal residual disease are becoming increasingly important in evaluating patients before and after bone marrow transplantation [4]. In some cases, the detection of a specific chromosomal translocation may help define a specific type of malignancy. For example, the detection of a clonal \textit{bcl-2} rearrangement indicates the
presence of a chromosomal translocation involving chromosomes 14 and 18, t(14;18), which is commonly associated with non-Hodgkin lymphomas of follicular center cell origin, and the detection of a clonal bcl-1 rearrangement indicates the presence of a t(11;14), which is common to non-Hodgkin lymphomas of mantle cell origin [5].

NORMAL B- AND T-CELL DEVELOPMENT
According to current concepts of the normal humoral immune system, all B lymphocytes arise from pluripotent stem cells in the bone marrow and then subsequently migrate to secondary lymphoid organs such as lymph node follicles and Peyer patches in the gastrointestinal tract. The stages of B-cell differentiation in the bone marrow occur largely independent of the presence of antigen whereas the stages of differentiation in secondary lymphoid organs require the presence of antigen for transformation [6]. The normal stages of B-cell development occur in an orderly fashion beginning with a progenitor B cell, which matures to a terminally differentiated plasma cell (Fig. 1). A variety of recognized changes occur at different maturational stages both at the molecular level and with regard to the presence of specific cellular antigens. At the molecular level, the genes that code for the immunoglobulin heavy and light chain proteins undergo sequential rearrangements early in B-cell development (Fig. 1). Initially, the immunoglobulin \( \mu \) heavy chain located on chromosome 14q32 rearranges and is followed by \( \kappa \) light chain rearrangement on chromosome 2p12 and \( \lambda \) light chain rearrangement on chromosome 22q11 [7]. Subsequent transcription and translation of the \( \mu \) heavy chain gene results in the appearance of cytoplasmic \( \mu \) heavy chain protein, which defines the pre-B-cell stage of development. The immature, mature, and activated B-cell stages are characterized by the presence of an intact surface immunoglobulin receptor, which consists of two heavy and two light chain proteins (Fig. 2A). A variety of cellular antigens can be detected at different stages of B-cell development, and the majority are referred to by CD (cluster designation) numbers (Fig. 1).

The earliest antigens expressed in B cells are terminal deoxynucleotidyltransferase (TdT) within the nucleus and HLA-Dr as a cell surface antigen. Neither of these antigens are B lineage-specific. B-cell-associated antigens CD19, CD20, and CD10 are subsequently expressed. As a B cell matures to a terminally differentiated plasma cell, the majority of B-cell-associated antigens are lost, and the CD38 antigen appears.

The fundamental theory of lymphoid neoplasia is that disorders of lymphoid cells represent cells arrested at various stages in the normal differentiation scheme [8]. For example, pre-B-cell acute lymphocytic leukemia mimics normal pre-B cells showing expression of TdT, HLA-Dr, CD10, CD19, CD20, and cytoplasmic \( \mu \) heavy chains (Fig. 1). Other examples of neoplastic counterparts to normal precursors include chronic lymphocytic leukemia/small lymphocytic lymphoma at the mature B-cell stage, follicular center cell lymphoma at the activated B-cell stage, and multiple myeloma at the plasma cell stage.

T lymphocytes, like their B-cell counterparts, also arise from pluripotent stem cells in the bone marrow. However, in contrast to B-cell development in which the earliest stages of maturation occur in the bone marrow, progenitor T cells migrate from the bone marrow to the thymus, where the early stages of T-cell development occur [9]. Subsequently, mature T cells circulate in the peripheral blood and seed peripheral lymphoid tissues, which include paracortical areas of lymph nodes and periarteriolar sheaths of the spleen. The normal stages of

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1 Nonstandard abbreviations: TdT, terminal deoxynucleotidyltransferase; TCR, T-cell receptor; mbr, major breakpoint cluster region; mcr, minor breakpoint cluster region; MRD, minimal residual disease.
T-cell development in the thymus, analogous to B-cell development, also occur in an orderly fashion (Fig. 3). T lymphocytes possess a surface membrane protein complex referred to as the T-cell receptor (TCR), which is structurally similar to the immunoglobulin receptor [10] (Fig. 2B). The genes that code for the TCR undergo sequential rearrangements early in T-cell development. Four TCR genes (α, β, γ, and δ) code for two types of TCRs that exist as heterodimers—the α-β receptor and the γ-δ receptor. The majority of T cells (98–99%) possess the α-β receptor, with the remaining 1–2% possessing the γ-δ receptor [10]. The α and δ chain genes are located on chromosome 14q11, the β chain gene on chromosome 7q34, and the γ chain gene on chromosome 7p15 [7]. The first TCR gene to rearrange is δ, which is followed sequentially by γ, β, and α genes.

Analogous to developing B cells, a variety of cellular antigens can be detected at different stages of T-cell development (Fig. 3). The earliest antigens expressed are TdT and CD7. The CD3 antigen, which is part of the protein complex associated with the TCR (Fig. 2B), is present early primarily in the cytoplasm and manifests on the cell surface at a later stage. The common thymocyte stage is defined by expression of CD1a, the common thymocyte antigen, and is frequently associated with coexpression of the CD4 (helper/inducer) and CD8 (cytotoxic/suppressor) antigens. As T cells reach the mature stage, they express either CD4 or CD8, but not both. Similar to B-cell neoplasms, T-cell neoplasms occur because of maturation arrest at various stages of T-cell development [8]. For example, lymphoblastic lymphoma frequently mimics normal common thymocytes, showing expression of TdT, CD1a, cytoplasmic CD3, CD7, and coexpression of CD4 and CD8 (Fig. 3). Other examples of neoplastic counterparts to normal precursors include peripheral T-cell lymphoma, cutaneous T-cell lymphoma (mycosis fungoides), and the T-cell type of lymphoproliferative disorder of granular lymphocytes, which are all neoplasms of mature T cells [1].

**Fig. 2. Schematic diagram of immunoglobulin and TCRs.**

- **A**, the immunoglobulin protein is a heterodimer composed of two heavy and two light chains, each of which has V and C regions. **B**, the TCR is also a heterodimer composed of either one α and one β chain or one γ and one δ chain. Each of the TCR proteins has V and C regions. CD3 is a complex of 5 proteins associated with the TCR.

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**B-CELL IMMUNOGLOBULIN AND TCR GENE REARRANGEMENTS**

The B-cell immunoglobulin and TCRs are involved in the process of antigen recognition by normal B and T lymphocytes. These receptors are structurally similar, being heterodimer proteins linked by disulfide bonds, and are composed of both variable (V) and constant (C) regions [7] (Fig. 2). The variable regions of these proteins are similarly involved in antigen recognition. The constant region of the immunoglobulin heavy chain protein defines the nine immunoglobulin classes (IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE) [6]. The genes that code for the B- and T-cell receptors are also structurally similar and consist of a large number of exons, referred to as a supergene family, that undergo a similar process of DNA recombination leading eventually to the formation of functional receptor proteins [3, 6, 7, 10, 11].

A general scheme of B-cell immunoglobulin and TCR gene rearrangements is shown in Fig. 4. The germline configuration refers to non-rearranged DNA. The exons that code for the variable regions of the immunoglobulin and TCRs are referred to as variable (V), diversity (D), and joining (J) segments, and those that code for the constant regions are referred to as C segments. The process of gene rearrangement first involves the selective apposition of one D segment with one J segment by deletion of the intervening coding and noncoding DNA sequences, resulting in a DJ rearrangement. By a similar process of rearrangement, a V segment, located in the 5’ direction, becomes apposed to D and J to form a VDJ rearrangement. Transcription to mRNA then occurs, even though the VDJ segments are not yet directly apposed to C segments, which are remotely located in the 3’ direction. Subsequent splicing of the mRNA with deletion of non-coding sequences results in apposition of VDJ with C to form a VDJC mRNA, which can then be translated into an immunoglobulin or TCR protein. The genes coding for the immunoglobulin heavy chain protein and TCRβ and -δ proteins include V, D, J, and C segments. The genes
coding for the κ and λ light chain proteins and the TCRα and -γ proteins include only V, J, and C segments without D segments [3, 7, 11].

The complex process of DNA recombination or rearrangement allows tremendous diversity of both the humoral and cell-mediated immune systems and the ability to detect a wide array of antigens [3, 6, 7, 10, 11]. The large number of V, D, J, and C segments results in many combinations, which can be transcribed and translated to millions of different antigen receptors. A detailed diagram of the B-cell heavy chain and the TCRβ chain superfamilies is shown in Fig. 5. The immunoglobulin heavy chain gene consists of at least 100 V segments, ~30 D segments, 6 J segments, and 9 C segments. The TCRβ chain gene includes 75–100 V segments and two tandem DJC complexes referred to as D1J1C1 and D2J2C2. Each DJC complex contains one D segment and one C segment. The first DJC complex contains six J segments (Jβ1 group) and the second DJC complex contains seven J segments (Jβ2 group) [3, 7, 11].

DETERMINATION OF B- AND T-CELL CLONALITY BY SOUTHERN TRANSFER ANALYSIS

To establish a diagnosis of B- or T-cell malignancy, the ability to prove that a neoplastic population of B- or T-cells is monoclonal in origin is of central importance. A monoclonal, or simply clonal, cell population refers to a population of cells that share similar characteristics and are all derived from a single precursor cell. In lymphoid malignancies clonality can be defined in several different ways. Clonality may be suggested on the basis of traditional morphology if a monomorphous cell population is present, immunopathologically by showing the presence of monoclonal surface immunoglobulin (in the case of B-cell neoplasms), cytogenetically by demonstrating a recurrent chromosomal alteration such as recurrent translocation, and by molecular genetics by demonstrating the presence of a clonal B- or T-cell gene rearrangement. In B- and T-cell neoplasms, the primary application of molecular genetics is to prove clonality in cases that are not morphologically malignant and in which clonality cannot be proven immunopathologically. Southern transfer analysis is a very sensitive and specific method for determining clonality and may detect a monoclonal population as little as 1–5% of the total cell population [3, 7].

For Southern transfer analysis, DNA is first extracted and purified from the cells that are to be analyzed. Fresh or frozen specimens are most suitable for Southern transfer analysis of hematological disorders and include cell suspensions prepared from peripheral blood, bone marrow aspirates, body fluids, and cell suspensions or cryostat sections prepared from tissues such as lymph nodes or extranodal masses. Separate samples of purified DNA are then digested with various restriction enzymes, which cleave DNA at specific sites by recognizing specific base pair sequences [12, 13]. The digested DNA fragments are then separated according to fragment size by agarose gel electrophoresis. The DNA fragments are then transferred to a nylon membrane and hybridized with a specific DNA probe. A monoclonal population will result in a discrete band where the hybridized DNA is enriched for the DNA sequence corresponding to the rearranged gene of the clonal cell population. The presence of a discrete band demonstrates the clonal origin of the cell population.

Fig. 3. Normal stages of T-cell development. cCD3, cytoplasmic CD3; TCR, TCR rearrangements; ALL, acute lymphocytic leukemia; PTCL, peripheral T-cell lymphoma; LGL, lymphoproliferative disorder of granular lymphocytes; CTCL, cutaneous T-cell lymphoma.
DNA probe detection systems include radioactive labeling with $^{32}$P, chemiluminescence, and colorimetry [13, 14]. DNA probes that are commonly used for detection of monoclonal B-cell populations recognize the heavy chain joining ($J_{H}$) segments and the $\kappa$ light chain joining ($J_{K}$) segments (Fig. 5). DNA probes that are commonly used for detection of monoclonal T-cell populations recognize the two groups of $\beta$ chain joining ($J$) segments and the two $\beta$ chain constant (C) segments (Fig. 5).

The Southern transfer approach for detecting B-cell gene rearrangements is shown schematically in Fig. 6. In reactive or polyclonal lymphocyte populations, the primary band identified with a $J_{H}$ probe is the germline band (lane A). Thousands of different rearrangements are actually present in this lane, but individually, the rearrangements are too small to be detected. In a monoclonal B-cell population, all B cells are derived from a single precursor cell and have identical gene rearrangements that will be detected by Southern transfers as a novel band. If the monoclonal B-cell population has a DJ rearrangement, numerous intervening coding and noncoding DNA sequences are deleted, resulting in a smaller fragment of DNA detected by the $J_{H}$ probe (lane B). If the monoclonal B-cell population has a VDJ rearrangement, a restriction enzyme cleavage site is also deleted, resulting in a larger fragment of DNA detected by the $J_{H}$ probe (lane C).

The presence of clonal B-cell gene rearrangements detected with a $J_{H}$ probe (IGH J6, Dako) in a case of B-cell non-Hodgkin lymphoma is shown in Fig. 7 (lanes 3, 6, and 9). Fig. 8 (lanes 2 and 5) shows the presence of clonal T-cell gene rearrangements that were detected with a TCR $\beta$ chain probe (TCRBC, Dako) in a case of T-cell non-Hodgkin lymphoma. In each set of blots, a marker lane (lane M) consisting of predigested fragments of $\lambda$ phage DNA is present to establish restriction fragment sizes. Separate DNA samples were digested with three restriction enzymes for both B- and T-cell probes. Each probe was independently labeled with digoxigenin-dUTP for chemiluminescent detection (Genius 1 Kit, Boehringer Mannheim). With each enzyme digest, a control lane consisting of normal placental DNA is run to identify the germline configuration (Figs. 7 and 8, lanes 1, 4, and 7). A novel band refers to any band occurring in a lane other than a germline band; a cross-hybridization band, which occurs because of hybridization of the probe to partially homologous DNA sequences in other areas of the genome; or a partial digest band, which occurs because of incomplete digestion of DNA by a restriction enzyme. A diagnosis of a clonal B- or T-cell rearrangement is established according to the guidelines set forth by Cossman et al. [12], which require the identification of at least two novel bands present either in two separate enzyme digests or in the same enzyme digest.
Determination of Clonality by in Vitro Amplification

The PCR technique is becoming an increasingly popular method for evaluating the presence or absence of B- and T-cell clonality in lymphoid neoplasms [15–19]. This method of DNA analysis allows for the evaluation of minute quantities of DNA by in vitro amplification [20]. Analogous to Southern transfer methods, the application of PCR to detect B- and T-cell clonality involves evaluation of gene rearrangements in those segments of DNA that code for the variable regions of the immunoglobulin and TCR genes. Each V segment of DNA has unique DNA sequences that contribute to the great diversity of the immunoglobulin and TCR antigen recognition sites. In addition, short sequences of DNA are shared by nearly all of the V segments that can be recognized by a primer referred to as a consensus V region primer. In a similar fashion, short sequences of DNA shared by nearly all of the J segments can be recognized by a consensus J region primer [15–19]. For T-cell neoplasms, because TCRα genes are highly complex and TCRβ genes are often deleted in mature T cells, TCRβ and TCRγ provide the most efficient targets for PCR amplification [23]. Some authors have reported the use of primers specific for the TCRβ gene, which includes primers directed to D segments, whereas others prefer analysis of the TCRγ gene, which lacks D segments and is thus an easier and less complex target for amplification [21–23]. TCRγ amplification protocols appear to be easier to optimize, more sensitive in detecting monoclonal T-cell populations, and provide more robust PCR reactions [23].

A diagram illustrating the application of PCR to detect B-cell heavy chain gene rearrangements with VH and JH consensus primers is shown in Figs. 9 and 10. Similar approaches with consensus sequences as primer target sites for PCR detection of TCR gene rearrangements are also used. An ethidium bromide-stained PCR gel is shown in Figs. 11 for B-cell and T-cell gene rearrangements, respectively. The primers must recognize DNA sequences within a short segment of DNA to successfully amplify a segment of DNA by PCR. In the germline configuration, because primer target sequences in the V and J segments are widely separated, no substantial DNA product is obtained after amplification by PCR (Fig. 9, lane A). If a VDJ rearrangement occurs, the proximity of the V and J segments allows for the synthesis of an amplified DNA product. A polyclonal B- or T-cell population has a large number of rearrangements that differ in size, resulting in a smear pattern (Fig. 9, lane B, and Fig. 11, bottom, lanes 4 and 5). In contrast, monoclonal B- or T-cell populations contain identical rearrangements that result in the formation of a distinct band (Fig. 9, lane C, and Fig. 11, top, lanes 3, 4, 7, and 8, and bottom, lanes 3 and 6). Because of the many advantages of the PCR over
Southern transfer, assessment for B- or T-cell gene rearrangements in our laboratory first involves PCR approaches. For the evaluation of B-cell neoplasms, two consensus VJ primer sets are used that will detect B-cell clonality in 50–60% of B-cell neoplasms. Primer sets consist of one primer directed to the framework III region of the V segments and one consensus JH primer (Fig. 10). For the evaluation of T-cell neoplasms, a single multiplex PCR consisting of seven primers specific for V and J segments of the TCRγ gene complex is used. This reaction will detect T-cell clonality in 60–70% of T-cell neoplasms (unpublished data). If analysis for B- or T-cell clonality is negative by PCR, Southern transfer analysis is performed, which will detect clonal B- or T-cell gene rearrangements in 80–90% of cases.

Although the Southern transfer method has been the gold standard for demonstrating clonality in lymphoid neoplasms, PCR offers distinct advantages [15, 17] (Table 1). Southern transfer is costly and labor-intensive, requiring 7–10 days to obtain a result; PCR can be performed at a lower cost in just 1–2 days. In addition, Southern transfer requires a relatively large amount of high-quality intact DNA and must be obtained from fresh or frozen tissue samples. In contrast, because the amplification of DNA by PCR requires only short segments of DNA, PCR analysis can be performed on small samples of DNA and on DNA that is of low quality or only partially intact (such as DNA extracted from paraffin-embedded tissues). Finally, whereas Southern transfer may detect a 1–5% clonal lymphoid population, PCR may detect as little as a 0.1% clonal lymphoid population [18]. Despite the many advantages of PCR in evaluating for B- and T-cell clonality, the technique is associated with a higher percentage of false-negative results than Southern transfer. This high false-negative rate likely occurs because of the inability of consensus V primers to recognize complementary DNA sequences in all of the V segments and because of the inability of V and J primers to recognize genetic alterations such as partial rearrangements (DJ rearrangements) and chromosomal translocations and somatic mutations involving the antigen receptor gene loci [14, 18].

**DETECTING CHROMOSOMAL TRANSLOCATIONS IN NON-HODGKIN LYMPHOMA**

Several specific, nonrandom chromosomal translocations have been described in association with different subtypes of non-Hodgkin lymphoma. These translocations can be demonstrated by traditional cytogenetic methods as well as by molecular genetic methods that include Southern transfer, PCR, and fluorescence in situ hybridization. Because the demonstration of cytogenetic abnormalities in lymphoid neoplasms with traditional cytogenetic methods is technically difficult, especially in low-grade neoplasms that are associated with a low mitotic rate, molecular approaches currently are the methods of choice. The majority of cases of non-Hodgkin lymphoma can be accurately classified based primarily on morphological and immunopathological characteristics;

| Table 1. Comparison of Southern transfer and PCR technologies. |
|-----------------|-----------------|-----------------|
|                 | Southern transfer | PCR             |
| Labor           | More             | Less            |
| Cost            | More             | Less            |
| Sensitivity     | 1–5% clone       | 0.1% clone      |
| DNA quantity    | Large            | Small           |
| DNA quality     | High             | High/low        |
| Tissues         | Fresh            | Fresh/paraffin  |
| False negatives | Low              | High            |
however, in select cases the demonstration of a specific chromosomal translocation may help confirm a diagnosis. For example, the demonstration of a t(8;14) in a lymphoma that is morphologically and immunopathologically suspicious for Burkitt lymphoma would confirm this diagnosis [5]. More importantly, the ability to detect specific chromosomal translocations in lymphomas by highly sensitive methods such as PCR provides a means to potentially monitor patient therapy and to follow patients for evidence of minimal residual disease.

Chromosomal translocations in both leukemia and lymphoma often involve the transposition of a protooncogene from one chromosome to another. Protooncogenes are defined as normal cellular genes that are involved in the regulation of cellular processes such as growth and proliferation and have the potential to contribute to neoplastic transformation when they are structurally or functionally altered, as occurs with a chromosomal translocation [24, 25]. Two examples of protooncogenes known to be involved in lymphomagenesis will be discussed further: bcl-2, which is involved in the pathogenesis of follicular lymphoma [24–26], and bcl-1, which is involved in the pathogenesis of mantle cell lymphoma [24, 27].

Apoptosis or programmed cell death is part of normal homeostasis and is the body’s way of maintaining a delicate balance between cell proliferation and cell death. The protooncogene bcl-2 normally resides on chromosome 18 and is involved in blocking apoptosis [25]. In healthy adults expression of bcl-2 is limited to long-lived cells that include some subsets of normal T and B lymphocytes. In follicular lymphoma, bcl-2 becomes overexpressed after being translocated from chromosome 18 to the heavy chain locus on chromosome 14. The overexpression of bcl-2 is likely one step in the process of lymphomagenesis with increased amounts of bcl-2 extending the lifespan of neoplastic cells [25]. The t(14;18) has been reported in up to 80–90% of cases of follicular lymphoma and less frequently in other types of hematopoietic and nonhematopoietic malignancies [26].

The reciprocal translocation involving the bcl-2 locus on chromosome 18q21 and the immunoglobulin heavy chain locus (IgH) on chromosome 14q32 is shown schematically in Fig. 12. The bcl-2 gene contains 3 exons, including exon 1, a noncoding exon. The majority of chromosomal breaks occur in two regions: the major breakpoint cluster region (mbr), where 50–75% of the breaks occur, and the minor breakpoint cluster region (mcr), where 20–40% of the breaks occur [25, 26]. The mbr is located in exon 3, and the mcr is located downstream from exon 3. The breakpoints in the heavy chain locus involve the JH segments. The t(14;18) results in a bcl-2/IgH fusion gene. Analysis for the presence of bcl-2/IgH gene rearrangements can be performed by both Southern transfer and PCR. PCR is especially suited for analyzing bcl-2/IgH rearrangements because the bcl-2 and JH breakpoints are located within a short segment of DNA [28]. Analysis by PCR is performed with two separate primer combinations to analyze for breaks at both the major and minor breakpoint cluster regions—a combination of mbr and JH primers and a combination of mcr and JH primers (Fig. 13). Like other PCR assays for gene rearrangements, a single amplified band is observed by gel electrophoresis if the translocation is present. If, however, a t(14;18) and hence a bcl-2/IgH rearrangement has not occurred, no PCR product will be detected after amplification.

A second translocation, t(11;14) (q13;q32), is associated with mantle cell lymphoma and results in the juxtaposition of the bcl-1 protooncogene from chromosome 11 to the joining segment of the IgH gene on chromosome 14 [29]. The bcl-1 locus includes the PRAD-1 cyclin genes, which is normally located on chromosome 11 and is involved in the regulation of cell cycle progression [30]. This translocation is found in up to 75% of cases of mantle cell lymphoma and thus, analogous to bcl-2 in follicular

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**Fig. 12.** Schematic diagram showing the translocation of bcl-2 from chromosome 18 to the heavy chain gene (IgH) on chromosome 14, resulting in a bcl-2/IgH fusion gene.

For bcl-2, most breaks occur in either the mbr or mcr regions. Breakpoints in the heavy chain gene involve JH segments (arrowhead). The translocation may involve mbr and JH breakpoints (middle panel) or mcr and JH breakpoints (lower panel).
center cell lymphoma, serves as a molecular marker for mantle cell lymphoma. The majority of breakpoints within the bcl-1 gene occur within a rather small segment of the gene referred to as the major translocation cluster region. Because of this, rapid detection by PCR can be obtained when primers directed to the major translocation cluster region are used in combination with consensus JH primers [31–35].

**DETECTION OF MINIMAL RESIDUAL DISEASE**

Combinations of chemotherapy, radiation therapy, and bone marrow transplantation are potentially curative for several hematologic malignancies [36–43]. However, in some patients, occult tumor cells exist and are thought to increase the patient’s risk of relapse. Minimal residual disease (MRD) refers to the presence of a residual clone of malignant cells in a patient that cannot be detected by practiced pathological and radiological staging approaches and may eventually result in disease relapse [37, 40, 41]. For example, at presentation, patients with acute leukemia have a tumor burden consisting of 10^{12} leukemic cells, which is readily detectable by microscopic examination of the bone marrow. After induction chemotherapy, the tumor burden is reduced by several orders of magnitude, resulting in clinical remission (defined as <5% bone marrow blasts); however, an undetectable residual tumor burden of 10^6 or 10^5 leukemic cells may still remain [36–43]. Traditional morphological assessment of the bone marrow cannot distinguish a patient with MRD of 10^5 leukemic cells from a patient with no leukemic cells.

The presence of MRD in patients with leukemia and lymphoma has been assessed by a variety of approaches, including traditional morphology, immunophenotypic analysis by flow cytometry, cell culture methods, conventional cytogenetics, and molecular methods, e.g., fluorescence in situ hybridization, Southern transfer, and PCR [36–43]. Each of these methods has advantages and disadvantages. Except for PCR, the approaches listed lack low detection limits and are capable of detecting an ~1% malignant cell population. In contrast, PCR has substantially lower detection limits and is capable of detecting one malignant cell among 10^6–10^9 nondiseased cells [36–43]. The malignant cell must have a unique set of DNA sequences, distinct from nondiseased cells, to evaluate for the presence of MRD by PCR. Evaluation for the presence of chromosomal translocations such as t(14;18) (q32;q21) in follicular, non-Hodgkin lymphoma and t(11;14) (q13; q32) in mantle cell lymphoma is ideal for detecting MRD. Another approach in B- and T-cell malignancies involves detection of immunoglobulin and TCR gene rearrangements. A variety of PCR-based strategies have been devised on the basis of the premise that each clone of malignant B- or T cells has a unique VDJ rearrangement that can be used as a molecular marker to probe for the presence of MRD [4, 38]. Our ability to detect MRD more precisely would be expected to improve clinical management by optimizing treatment intensity in patients at high risk of relapse and reducing potentially harmful therapies in low-risk patients [36–43].

Molecular genetic applications have greatly enhanced our ability to precisely diagnose and classify the lymphoid malignancies. Both Southern transfer and PCR methods are used in select cases, usually the most diagnostically challenging cases. The most frequent application of these methods is for the determination of B- or T-cell clonality. In addition, the detection of chromosomal translocations involving protooncogenes (such as bcl-1 and bcl-2) may help define a specific subtype of non-Hodgkin lymphoma. In general, PCR methods are more rapid and cost-effective than Southern transfer; however, Southern transfers have a lower false-negative rate. Thus, ideally Southern transfers should be performed in cases that are PCR-negative. Molecular genetic applications provide a cost-effective means of confirming a diagnosis and monitoring patients with lymphomas.

**References**


**Further Suggested Readings**