Correspondence

PCR Methods for Determining B Cell Clonality

To the Editor:

We read with interest the article by Elenitoba-Johnson et al,¹ in which the authors demonstrated potential false positive results obtained using a polymerase chain reaction (PCR)-based method to determine B cell clonality. This is an important observation, and one must be cognizant of the potential for detecting clones of uncertain clinical significance in specimens with very few B cells.

However, we would like to make the observation that the source of this problem is the hemi-nested PCR method used by the authors, which is compounded when only small amounts of DNA are used in the assay. When a standard single-step PCR method is used in specimens with very few B cells, one would see essentially no PCR product, leading one to conclude that either there are too few B cells to detect with the PCR method, or the DNA is of insufficient quality or quantity for PCR analysis. The latter case can be ruled out using the proper controls.

In our laboratory, we are frequently asked to detect B cell clones in the peripheral blood or bone marrow of patients who have been treated with anti-B cell antibodies for B cell lymphoma. These patients routinely have virtually no B cells detectable by flow cytometry, and essentially no PCR product is seen with a single-step method using VH Framework 3/JH primers and capillary electrophoresis to detect fluorescently labeled PCR products. Occasionally we see several very faint PCR products, but the intensity of the products is not sufficient for us to make a diagnosis of clonality. The adequacy of the DNA is confirmed using primers for a control gene. In this situation, we inform the ordering physician that no PCR products were obtained, consistent with an absence of B cells in the specimen.

Admittedly, it is unsatisfying to report such inconclusive results. However, we feel that by using a single-step PCR method, we can provide useful diagnostic information when enough B cells are present to make a diagnosis. In the absence of B cells, we do not have to contend with possible false positive results and the potential clinical consequences thereof.

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References

1. Elenitoba-Johnson KSJ, Mitchell RS, Bohling SD, Brown MS, Roboto-rye RS: PCR analysis of the immunoglobulin heavy chain gene in polyclonal processes can yield pseudoclonal bands as an artifact of low B cell number. *J Mol Diag* 2000, 2:92-96

Authors' Reply:

We appreciate the interest of Drs. Sabath, Wood, and Kussick in our recent article.¹ Our study highlighted the potential for incorrectly assigning monoclonal status to samples containing polyclonal or oligoclonal B cell populations. In our study, we compared hemi-nested and non-nested single-step PCR approaches followed by polyacrylamide gel electrophoresis. While we state that "the hemi-nested assay is more prone to the generation of pseudo-monoclonal bands" in agreement with their experience, we also demonstrate that this phenomenon may occur, albeit less frequently, in non-nested assays.¹ Recognition of this phenomenon is particularly important when the DNA source is fixed paraffin-embedded tissue, where the DNA quality may be suboptimal. This technically diminishes the DNA target that is available for amplification.

Whereas an amplification control such as a housekeeping gene would be expected to yield a distinct PCR product generated from all of the cells within the sample, including lymphocytes and stromal cells, the products for IgH PCR are restricted to B cells, which constitute only a small proportion of some biopsy samples. Furthermore, each B cell carries a unique rearrangement that renders its product size different from that of other B cells. These factors explain the occurrence of faint or absent IgH PCR bands when amplification of housekeeping gene controls yields robust and bright bands. Although the performance of immunohistochemical analyses may give an indication of the abundance of B cells in the specimen, it does not provide a good assessment of the quality of DNA in paraffin-embedded sections with abundant B cell numbers.

Hemi-nested assays clearly improve the sensitivity of IgH PCR. Our study brings into focus the propensity for increased rates of false positive results with the increased sensitivity provided by such assays. As stated

earlier, we have observed false positives due to low B cell number using a single-step method. We also agree with Sabbath et al that their practice of informing the ordering physician that no PCR products are obtained when faint PCR products are observed is suboptimal.

The key recommendation emanating from our study is that diagnostic accuracy in clonality assessment may be improved by evaluating duplicate or triplicate aliquots of a sample in parallel, as has been documented by other investigators.² Our conclusions have broader implications for the reporting of the results of PCR-based clonality studies of the IgH²⁻⁴ and T cell receptor genes^{5,6} involving a variety of amplification and gel separation methods, including capillary electrophoresis.

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References

1. Elenitoba-Johnson KSJ, Mitchell RS, Bohling SD, Brown MS, Robetorye RS: PCR analysis of the immunoglobulin heavy chain gene in polyclonal processes can yield pseudoclonal bands as an artifact of low B cell number. *J Mol Diag* 2000, 2:92-96
2. Taylor JME, Spagnolo DV, Kay PH: B cell target DNA quantity is a critical factor in the interpretation of B-cell clonality by PCR. *Pathology* 1997, 29:309-312
3. Wan JF, Sykes PJ, Orell SR, Morley AA: Rapid method for detecting monoclonality in B cell lymphoma in lymph node aspirates using the polymerase chain reaction. *J Clin Pathol* 1992, 45:420-423
4. Nihal M, Mikkola D, Wood GS: Detection of clonally restricted immunoglobulin heavy chain gene rearrangements in normal and lesional analysis of the B cell component of the skin-associated lymphoid tissue and implication for the molecular diagnostic of cutaneous B cell lymphomas. *J Mol Diag* 2000, 2:5-10
5. Segal GH, Hussey CE, Wittwer CT: PCR for T-cell rearrangements. *Diagn Mol Pathol* 1996, 5:297-298
6. Lee S-C, Berg KD, Racke FK, Griffin CA, Eshleman JR: Pseudospikes are common in histologically benign lymphoid tissues. *J Mol Diag* 2000, 2:145-152