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Amplification-Based DNA Fingerprinting: from Artifactual to Definitive Typing and In Between

During past years, several hundred publications have appeared describing the successful application of arbitrarily primed PCR or randomly amplified polymorphic DNA analysis. From a screening of MedLine for articles on this PCR-based DNA fingerprinting method, it appears that in 56% of all publications the subject concerns microbiological typing. Recently, two extensive reviews focusing on the technical aspects of PCR-based DNA fingerprinting of clinically relevant bacteria appeared in peer-reviewed journals (9, 11). It is striking to observe the rather extreme differences in the points of view formulated by the different authors. Although it is clear that the PCR-mediated methodology is currently far from optimal with respect to experimental reproducibility, especially when intercenter studies are concerned (8, 13), the contrast between the conclusions reached requires additional discussion.

Power (9) foresees that PCR-mediated fingerprinting, although presently considered to be a comparative typing procedure at best, will eventually develop into a speedy, flexible, and cost-effective definitive typing strategy. This proposition may be considered somewhat optimistic in light of the current state of affairs, although in view of modern developments in the field of DNA chip technology (2) optimization of the interpretation and standardization of PCR fingerprinting may be feasible in the not-too-distant future. Precisely this novel revolutionary and miniaturized DNA-probing methodology will most probably replace all of the current typing procedures in the long run.

In clear contrast with Power's recommendations (9), Tyler et al. (11) suggest that PCR-mediated typing should be performed "for the sole purpose of satisfying some immediate internal goal or curiosity" and consider larger comparative studies a waste of time and effort. This type of reasoning is completely opposite to that of the previously mentioned author, but is it less reasonable? Several omissions are evident in the paper by Tyler et al. The authors do not discuss the pivotal role that laboratory organization and highly qualified laboratory personnel play in developing an adequate PCR-mediated typing infrastructure. In addition, the authors seem to neglect the vast amount of data on the qualitative comparison between PCR-mediated typing and other (microbial) typing procedures. The validity of the relationship between PCR typing and molecular epidemiology and genome evolution is questioned without a clear reason and, once more, without regard to data in the current literature. Furthermore, Tyler et al. forget to mention that reproducible, multicentered microbial typing studies using DNA technology in general have only been successfully performed for *Mycobacterium tuberculosis* (5). Finally, emphasizing background staining in control lanes while at the same time giving the solution to this problem seems peculiar to me. Furthermore, PCR typing can be used successfully for the isolation of genetic markers on a wide spectrum of phylogenetic levels and thus to link typing directly to diagnostics (4, 14).

In my opinion, Tyler et al. correctly question several of the technical aspects of the PCR-mediated approaches. This, however, has been done in detail before (1, 6, 7, 10, 12), and, to my knowledge, experimental pitfalls (3) have never been highlighted as explicitly for other molecular typing procedures.

Finally, the choice of a certain typing system depends on a number of professional and personal criteria. Obvious factors are speed, resolution, reproducibility, cost-effectiveness, and technical feasibility. The number of typing assays that are to be performed on an annual basis and whether or not the typing technology can be implemented in molecular research and diagnostics are important secondary aspects. The procedure of choice should appeal to users and customers, and PCR typing meets many of the aforementioned requirements. In this respect, it may be worth emphasizing that a Ferrari Testarossa was never meant for ploughing farmland.

REFERENCES

1. **Caetano-Anolles, G.** 1993. Amplifying DNA with arbitrary oligonucleotide primers. PCR Methods Applic. **3:**85–94.
2. **Chee, M., R. Yang, E. Hubbell, A. Berno, X. C. Huang, D. Stern, J. Winkler, D. J. Lockhart, M. S. Morris, and S. P. A. Fodor.** 1996. Accessing genetic information with high-density DNA arrays. Science **274:**610–614.
3. **Cookson, B. D., P. Aparicio, A. Deplano, M. Struelens, R. Goering, and R. Marples.** 1996. Inter-centre comparison of pulsed-field gel electrophoresis for the typing of methicillin resistant *Staphylococcus aureus*. J. Med. Microbiol. **44:**179–184.
4. **Giessendorf, B. A. J., W. G. V. Quint, P. Vandamme, and A. van Belkum.** 1996. Generation of DNA probes for detection of microorganisms by PCR. Zentralbl. Bakteriol. **283:**417–430.
5. **Hermans, P. W. M., F. Massadi, H. Guebrexahber, D. van Soolingen, P. E. W. de Haas, H. Heersma, H. de Neeling, A. Ayoub, F. Portaels, D. Frommel, M. Zribi, and J. D. A. van Embden.** 1995. Analysis of the population structure of *Mycobacterium tuberculosis* in Ethiopia, Tunisia and the Netherlands: usefulness of DNA typing for global tuberculosis epidemiology. J. Infect. Dis. **171:**1504–1513.
6. **Lamboy, W. F.** 1994. Computing genetic similarity coefficients from RAPD data: the effects of PCR artifacts. PCR Methods Applic. **4:**31–37.
7. **Meunier, J. R., and P. A. D. Grimont.** 1993. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res. Microbiol. **144:** 373–379.
8. **Penner, G. A., A. Bush, R. Wise, L. Domier, K. Kasha, A. Laroche, G. Scoles, S. J. Molnar, and G. Fedak.** 1993. Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Applic. **2:**341–345.
9. **Power, E. G. M.** 1996. RAPD typing in microbiology; a technical review. J. Hosp. Infect. **34:**247–265.
10. **Schierwater, B., and A. Ender.** 1993. Different thermostable DNA polymerases may amplify different RAPD products. Nucleic Acids Res. **21:**4647–4648.
11. **Tyler, K. D., G. Wang, S. D. Tyler, and W. M. Johnson.** 1997. Factors affecting reliability and reproducibility of amplification-based DNA fingerprinting of representative bacterial pathogens. J. Clin. Microbiol. **35:**339–346.
12. **Van Belkum, A.** 1994. DNA fingerprinting of medically important microorganisms by use of PCR. Clin. Microbiol. Rev. **7:**174–184.
13. **Van Belkum, A., J. Kluytmans, W. van Leeuwen, R. Bax, W. Quint, E. Peters, A. Fluit, C. Vandenbroucke-Grauls, A. van den Brule, H. Koeleman, W. Melchers, J. Meis, A. Elaichouni, M. Vaneechoutte, F. Moonens, N. Maes, M. Struelens, F. Tenover, and H. A. Verbrugh.** 1995. Multicenter evaluation of arbitrarily primed PCR for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. **33:**1537–1547.
14. **Van Leeuwen, W., M. Sijmons, J. Sluijs, H. A. Verbrugh, and A. van Belkum.** 1996. On the nature and use of randomly amplified DNA from *Staphylococcus aureus*. J. Clin. Microbiol. **34:**2770–2777.

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Authors' Reply

In his letter, Dr. Alex van Belkum states that there are "...rather extreme differences in the points of view formulated by the different authors," with specific reference to two recent articles reviewing PCR-based DNA fingerprinting (2, 3). However, we feel that there are more similarities than differences between these two articles. Both articles examine a number of technical challenges inherent in PCR typing techniques and offer suggestions for optimization of such techniques. Although these reviews document some of the current problems and limitations associated with PCR-based DNA fingerprinting, it is strictly speculative and possibly inappropriate to presume that future advancements, such as DNA chip technology, will permit further refinement of these techniques. We agree unequivocally with Dr. Power's concluding remarks: "It remains imperative that the power of the technique should not be misused by those who perceive it as a quick and 'easy' method to type their strains. Much work is needed before RAPD typing can take a place alongside recognized definitive typing techniques...."

As Dr. van Belkum has stated, there are an increasing number of publications appearing in peer-reviewed journals which describe the application of these techniques, and in view of the current limitations in this technology it is beneficial to the uninitiated to be aware of these problems. Dr. van Belkum also contends that PCR-mediated methodologies are far from optimal with respect to experimental reproducibility, especially with interlaboratory data comparisons. With specific reference to Dr. van Belkum's comments on typing of *M. tuberculosis*, it is worth noting that the report cited refers to a study involving a technique based on Southern hybridization targeting the IS*6110* insertion element, a technique which is not subject to the extreme variabilities seen with PCR-based approaches. In addition, the Southern method has been established as an international standard, thereby permitting meaningful interlaboratory comparisons.

Although we agree that technical skill and experience are key factors when any molecular methodology is put to use, our prime concerns relate to lack of reproducibility with some PCR-based typing methods and the possibility of artifacts which can appear from exogenous DNA present in different preparations of *Taq* polymerase. If bands can appear or disappear or appear at different intensities within the same sample, it becomes difficult to interpret the significance of subtle differences in banding patterns. We acknowledge that when gross patterns are examined they may correlate favorably with results obtained by other typing procedures and epidemiological data. However, subtle differences must be viewed with extreme caution as they may be entirely artifactual in nature.

In conclusion, we would like to point out that there are a large number of phenotypic and genotypic bacterial typing techniques, and choosing the most appropriate one depends on the nature and biological complexity of the organism being investigated. It is tempting to be swayed by the glamour and enticement of a "high-tech" procedure, but, as we have learned from personal experience (1), sometimes the tried-and-true methods are the most reliable and definitive. Dr. van Belkum lists a number of factors which should be considered when deciding on a typing method, all of which are important. We maintain that the most significant criterion in this list is reproducibility. It matters little if a technique is fast, inexpensive, and easy to perform if the results are variable and difficult to interpret. With this in mind, Dr. van Belkum's comment that "...a Ferrari Testarossa was never meant for ploughing farmlands" may not be the best analogy. In fact, we have found that this "high-performance" technique is highly suited to "ploughing" through large numbers of samples in order to identify those that warrant further investigation by classical methods.

REFERENCES

1. **Martin, I. E., S. D. Tyler, K. D. Tyler, R. Khakhria, and W. M. Johnson.** 1996. Evaluation of ribotyping as epidemiologic tool for typing *Escherichia coli* serogroup O157 isolates. J. Clin. Microbiol. **34:**720–723.
2. **Power, E. G. M.** 1996. RAPD typing in microbiology; a technical review. J. Hosp. Infect. **34:**247–265.
3. **Tyler, K. D., G. Wang, S. D. Tyler, and W. M. Johnson.** 1997. Factors affecting reliability and reproducibility of amplification-based DNA fingerprinting of representative bacterial pathogens. J. Clin. Microbiol. **35:**339–346.

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