Contamination Management of Broad-Range Ribosomal DNA PCR: Where Is the Evidence?

We have read with great interest the minireview of Millar and colleagues concerning the risk of contamination of broad-range ribosomal DNA (rDNA) PCR (3). We completely agree with the authors that the high sensitivity of broad-range rDNA PCR could lead to false-positive results. As stated by Millar and colleagues, a critical analysis of the work flow and the obtained results is warranted, as is the introduction of sufficient and appropriate negative and positive controls.

We take issue, however, with the statement that strict segregation of the laboratory work flow is a fundamental requirement for successful broad-range rDNA PCR. The introduction of a segregated work flow, PCR setup cabinets, HEPA filters, and class II safety cabinets greatly increases the PCR costs. The use of different sets of pipettes, the exclusive use of filtered tips, the requirement of sterile gloves, and frequent UV irradiation of the rooms and materials further increase the costs for molecular diagnostics. Moreover, sterilization of materials, the skin, and the PCR room does not necessarily eliminate DNA from dead bacteria. All these measures are surely beneficial for the order books of biomedical supply companies, but the advantages of many of these measures in eliminating contamination of broad-range ribosomal PCRs remain to be proven. Since evidence-based medicine is the ultimate goal in clinical medicine, why not in laboratory medicine (2)?

The magnitude of the problem of contamination of rDNA PCRs is highly variable, and important contamination may come from an unexpected quarter. On the one hand, some authors, such as Corless and colleagues (1), reported major contamination of the samples with 16S rDNA. All kinds of strategies aimed to reduce the number of false positives also affected the yield of the quantitative PCR. On the other hand, in our experience contamination of 16S rDNA TaqMan quantitative PCR was low-level, with an average surplus of only 55 copies and a maximum of 150 surplus copies in a large series of samples (4, 5). In vivo samples, the amount of 16S rDNA recovered was many times higher than that for the highest negative control (5). The water used was critical: Milli-Cure water generated three times more contamination than double-distilled water. All samples were prepared in the routine laboratory with the utmost “good laboratory practice” care, and quantitative PCR was performed in a separate room. The many other precautions proposed in the review of Millar and colleagues, however, were lacking. In our experience, a critical analysis of the work flow combined with an appropriate cutoff were enough to overcome the very low-level contamination of the samples.

We completely agree with the authors that an appropriate quality control of rDNA PCR is indispensable. However, the publication of these contamination management recommendations in your journal may have a great impact on the organization of molecular diagnostics in many hospitals and research laboratories, amplifying the costs many times. Molecular diagnostics almost becomes a ritual driven by fear of contamination. In our opinion, much more experimental evidence on the cost-benefit relation of all these measures to reduce rDNA contamination is warranted before they can be propagated as the new standard of “good molecular diagnostics practice.”

REFERENCES


Stefaan J. Vandecasteele*
Department of Internal Medicine and Infectious Diseases
University Hospital Leuven
Herestraat 49
B-3000 Leuven, Belgium

Johan Frans
Medical Microbiology
Sint-Imelda Ziekenhuis
Imeldalaan 9
B-2820 Bonheiden, Belgium

Marc Van Ranst
Department of Virology
University Hospital Leuven
Herestraat 49
B-3000 Leuven, Belgium

*Phone: 32.16.34.62.74
Fax: 32.16.34.62.75
E-mail: stefaan.vandecasteele@uz.kuleuven.ac.be

Authors’ Reply

We read with interest the letter of Vandecasteele et al. commenting on our recent minireview on contamination management of broad-range ribosomal DNA PCR (3). In their letter, these authors question the need for strict segregation of the laboratory work flow, as well as several other guidelines that we suggested for the minimization of DNA contamination, including the use of PCR and class II safety cabinets, dedicated sets of pipettes, filtered tips, sterile gloves, and UV irradiation as a decontaminating agent. Vandecasteele et al. believe these guidelines to be unproven, as well as an expensive and unnecessary outlay when organizing a broad-range molecular diagnostic laboratory.

We disagree with these comments. Such laboratory design, work flow, and guidelines as outlined in the minireview are crucial in the quality control of all molecular applications associated with broad-range rDNA PCR, and these control measures are common practice in most PCR laboratories involved in specific PCR and/or broad-range PCR. This is evident both by the publication of such details by numerous laboratories.
which perform molecular assays on a routine basis (1, 2, 4) and from our laboratory experience over several years.

Broad-range rDNA PCR requires more stringent quality controls than specific PCR. The ultimate aim of the molecular diagnostic laboratory is to achieve zero levels of contamination and not low levels of contamination, and it is important that laboratories strive towards this goal. Additionally, it has been our experience that when stringency measures to avoid contamination are minimized, e.g., the use of nonfiltered pipette tips, etc., albeit there is a financial savings, it indeed represents a false economy, as more false positives are generated, all of which require relatively expensive downstream sequence analysis. Where low levels of contamination do persist, we believe it is a dangerous practice to establish arbitrary cutoff values for such contamination, as advocated by Vandecasteele et al., as it is possible for low copy numbers to be present in certain clinical scenarios, e.g., low-grade bacteremia associated with infective endocarditis, with potential medical-legal consequences. We therefore advocate adoption of highly stringent controls of contamination, which will result in a lower rate of false positivity; however, we strongly recommend sequence analysis of all positives, to avoid pathogens with low copy numbers from being missed.

Contamination is indeed the demon of broad-range rDNA PCR, and complacency in trying to minimize sources of contamination has the potential of making a useful technique moribund.

REFERENCES

Beverley C. Millar
Xu Jiru
John E. Moore
Northern Ireland Public Health Laboratory
Department of Bacteriology
Belfast City Hospital
Lisburn Road
Belfast BT9 7AD, Northern Ireland