

Biotechnology and microbiology – have we reached the end of the line?

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To predict what further benefits biotechnology and molecular biology may offer to public health microbiology, it is helpful to consider what their most significant contributions have been so far.

Many techniques in the fields of immunology and molecular biology may be proposed as the most significant recent developments, including enzyme immunoassays (ELISAs) with monoclonal antibodies, molecular cloning and other genetic engineering, DNA sequencing, and the polymerase chain reaction (PCR). All of these have had an impact on diagnostic microbiology. It is worth inquiring when these technologies were first described: one then learns that they stem from discoveries made as long ago as the 1970s and early 1980s.

Kohler and Milstein's first paper describing monoclonal antibodies was published in 1975¹, and soon after ELISAs using such monoclonal antibodies were constructed². Cohen, Boyer and colleagues developed molecular cloning, and Sanger and colleagues DNA sequencing around the same time³⁻⁵. About 10 years later PCR was devised⁶. The most important molecular technologies used for the detection of microbes were all therefore in place by the end of the 1980s. Has anything of importance happened since then?

The answer is a qualified 'yes'. During the 1990s, various modifications and extensions of standard molecular biology methods were used to differentiate between different isolates of the same bacterial or viral species, and phylogenetic and bioinformatic tools were developed to store and interpret the data generated by them. These tools have not had as

much impact as either ELISA, cloning, sequencing, or PCR, but they have yielded clinically useful information and clarified relations between bacterial taxons and among virus isolates.

With PCR it is possible both to detect a very few copies of a bacterial or viral genome in a clinical specimen and, more recently, estimate how much is present. Hence the conversion by diagnostic laboratories of most of their qualitative PCR assays to ones with a quantitative or real-time format, so that the 'load' of a genome (e.g. that of HIV-1) can be measured, and measured quickly. This allows more effective treatments to be applied, and a better and more rapid assessment to be made of the seriousness of an outbreak of infection.

PCR amplifies RNA or DNA, mimicking what happens in the living cell. With notable exceptions (e.g. prions), proteins cannot be amplified without the DNA or RNA template that codes for them. For this reason, ELISAs, while still useful, are much less sensitive than PCR. The most sensitive enhanced chemiluminescence protocol, for example, can detect about 10⁻¹²g of an antigen⁷. This is equivalent to about 200,000 molecules of a 30,000 dalton protein. It could therefore be said that ELISAs are some five orders of magnitude less sensitive than PCRs. Increasing the sensitivity of ELISAs to that of PCR would indeed be of great potential benefit to diagnostic microbiology, and there have been attempts to do this by coupling PCR amplification to antigen-antibody binding⁸. With very few exceptions⁹⁻¹¹, however, this immuno-PCR approach has not

proved to be sufficiently reliable, robust, or contamination resistant to be universally applicable. Immuno-PCR is technically complex, and a positive signal can be lost in high background noise when complex specimens are investigated. The invention of a method that is able to detect specific proteins present in low amounts, and which can be relatively easily implemented, is therefore a prize still waiting to be claimed.

Methods that are able to produce biologically active molecules such as antibodies without the need to use experimental animals would obviously be of great benefit too. One promising technology of the past few years that might be able to do this is that of *in vitro* generation of molecular diversity by phage display or the synthesis of random aptamers¹²⁻¹⁶. Unfortunately, although limited success has been attained, particularly in combinatorial chemistry for drug design, neither phage display nor *in vitro* selection have yet entered the mainstream of applied microbiological research.

Much attention has been given to microarrays of synthetic oligonucleotides or PCR amplicons as a means of DNA sequencing, investigating RNA expression, and identifying and typing microorganisms. Their greatest contribution to date has, however, been in revealing the complexity of RNA transcription in various developmental and pathological states, and it is still not clear to what extent they can contribute to routine viral or bacterial diagnosis and strain differentiation^{17,18}. A cynic might argue that arrays are merely old fashioned hybridisation, albeit on a large scale. It is just such high

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throughput methods, however, that may contribute most to clinical microbiology in the future, if they can be put into practice reliably.

Instrumentation that is able to automate the high throughput processing of samples allows molecular analyses to be carried out routinely on clinical specimens. The result is that many more data are available for epidemiological interpretation. In particular, manual nucleic extraction, whether from faeces, throat swabs, blood, food, or environmental samples, is arduous, time consuming, and susceptible to operator error. Automation can transform these procedures. PCR amplification is similarly difficult to do accurately for large numbers specimens without the aid of some form of robotic sample handling. Fortunately, several devices have been built for nucleic extraction and other parts of the PCR and sequencing processes, and these devices point the way towards what is a black-box approach to diagnostic molecular microbiology. The sample will be fed in at one end, DNA and RNA in the sample will be amplified and sequenced, and the machine will supply an interpretation of what the sequences mean. To what extent this is possible, or even desirable, remains to be determined over the next few years.

In short, in the years immediately before and after the

turn of the century a consolidation has taken place of the discoveries made in the 1970s and 1980s, with benefits feeding through to clinical medicine. The next great leaps forward – for example *in vitro* amplification of proteins are eagerly awaited.

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