REVIEW ARTICLE

Polymerase chain reaction in cancer diagnosis

K. E. GUNTHER, R. J. COHN, B. V. MENDELOW

Since it was first described,[1,2] the polymerase chain reaction (PCR) has had an enormous impact on the field of molecular biology. It is rapidly becoming an indispensable research tool and its introduction into the clinical laboratory has the potential significantly to affect aspects of routine health care.

Many of the molecular techniques previously shown to be of value in DNA-based diagnostics have been too expensive and laborious and too limited in application for routine use, particularly in the South African context where huge demands are already placed on an overstretched health budget. This has led to the idea that molecular techniques are not really applicable locally, except in a few specialised research laboratories. PCR has the potential to be different.

Basic principles

In essence PCR provides a means of exponentially amplifying selected 'target' sequences of nucleic acids. Numerous modifications have been described to extend its utility but in its simplest form it operates as follows: short DNA segments complementary to sequences flanking the target region are used as 'primers' to initiate the enzymatic production of new DNA strands complementary to both the sense and antisense strands of the target sequence. These new strands can themselves act as templates for the same primers. During repeated cycles of *heat denaturation*, the DNA is unravelled into single strands; *annealing* follows, during which the oligonucleotide primers hybridise specifically to their complementary sequences. *Extension* by a DNA polymerase in the presence of an excess of nucleotides will thus result in exponential amplification of the DNA bounded by the primers. This constitutes the PCR (Fig. 1).[3]

Applications of PCR to oncology

The applications of PCR appear to be limited only by the imagination of those using it. In clinical medicine, PCR is probably used most commonly in the detection of microbial pathogens. Its use in the diagnosis of tuberculosis has already been commented on,[4] as has its role in prenatal and carrier diagnosis of genetic diseases such as cystic fibrosis.[5-7]

This review will therefore concentrate on the role of PCR in the clinical oncology laboratory. In this context the use of PCR depends largely on the sensitive detection of pathological sequences not normally present in the genome of the patient. Such sequences arise as a result of gene insertion (as in the case of insertion of viral DNA in human T-cell leukaemia virus I infection[8]), mutation (e.g. in the p53 tumour suppressor gene associated with numerous malignancies[9-11]), translocation which results in juxtaposition of sequences not normally contiguous (e.g. the t(14;18) in follicular lymphoma,[12] the Philadelphia chromosome in chronic myeloid leukaemia[13]) or deletion (e.g. within the retinoblastoma gene[14]).

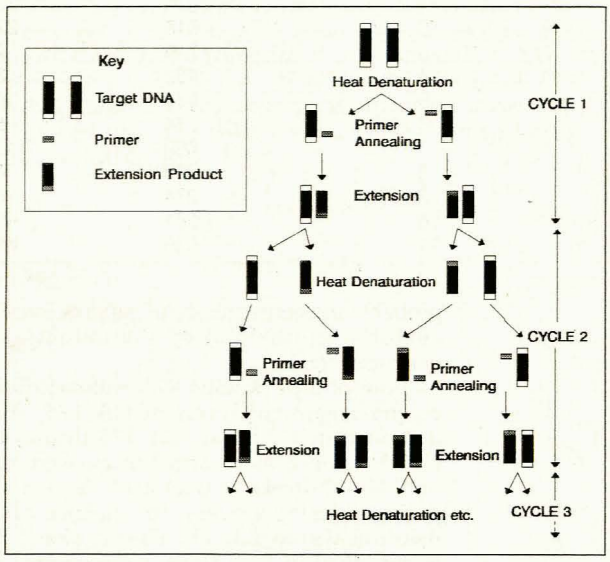

FIG. 1.
Principle of the PCR reaction.

The increasing recognition of various non-random chromosomal aberrations associated mainly with haematological malignancies, but also with some solid tumours, makes detection of such changes useful in primary diagnosis, in differentiation between reactive and malignant states, in the detection of 'minimal residual disease' and in the follow-up of patients on therapy or after bone marrow transplantation.[12,13,15,16]

An interesting but useful alternative to the detection of pathological sequences is the detection of 'physiological' sequences resulting from rearrangement of immunoglobulin and T-cell receptor genes. For example, immunoglobulin gene rearrangement which occurs early during B-cell ontogeny is necessary for immunoglobulin production.[17] Such rearrangement brings together sequences of the genome which are otherwise too far apart to support PCR amplification by means of a single set of primers (in a manner analogous to a translocation). In addition, the length of the rearranged gene varies with each immunoglobulin gene rearrangement. As a result, PCR amplification of these genes is not only useful in the determination of lineage (non-lymphoid cells will have DNA in the germline configuration which will not support amplification) but also in the detection of monoclonality, as a polyclonal B-cell population will give rise to amplified fragments of different lengths while a monoclonal population will give rise to fragments of a single length (Fig. 2).[18,19]

Feasibility of PCR as a diagnostic tool

Whether PCR is feasible for routine use in the South African context will depend on the ease with which the technique can be implemented, the speed with which results can be obtained, the specificity, sensitivity and

Departments of Haematology and Paediatrics, University of the Witwatersrand and the South African Institute for Medical Research, Johannesburg
K. E. GUNTHER, M.B. CH.B.
R. J. COHN, M.B. B.CH., D.C.H. (S.A.), F.C.P. (S.A.) (PAED.)
B. V. MENDELOW, M.B. B.CH., PH.D. (MED.)

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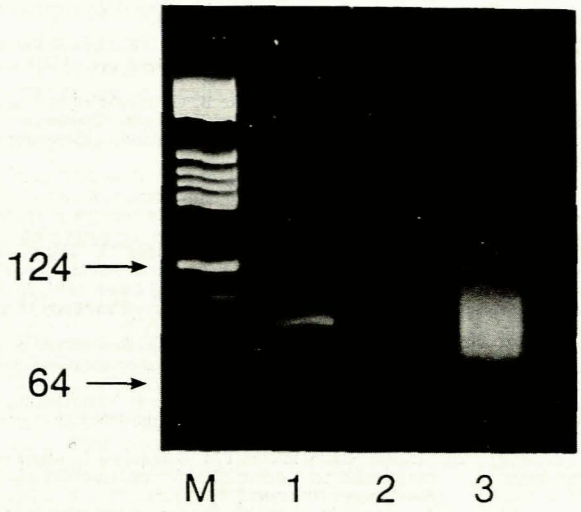

FIG. 2.
PCR amplification of immunoglobulin gene rearrangements from patients with common ALL (lane 1), ANLL (lane 2), and normal peripheral blood lymphocytes (lane 3). M is DNA molecular weight marker V (Boehringer Mannheim). Primers FR3A, LJH and VLJH as described by Trainor *et al.*,[18] were used. The reaction mixture (50 µl volume) contained 0,5 µg DNA; 25 picomoles of each primer; each of the four deoxyribonucleotides at a final concentration of 150 µM; 1,25 units of Taq DNA polymerase (Promega enzyme — SA Scientific); 5 µl of the reaction buffer supplied with the above enzyme and $MgCl_2$ at a final concentration of 1,5 mM. Thirty cycles of the PCR (2 minutes each at 94°, 52° and 72°) were performed, first with primers FR3A and LJH and then with 2 µl of the amplified product from this reaction as substrate for a second semi-nested PCR reaction with primers FR3A and VLJH. Reaction products were visualised under ultraviolet light after electrophoresis through a 4% agarose gel stained with ethidium bromide.

reliability of the technique in our hands, the costs involved and the potential demand for such investigations.

There can be little doubt about the demand for PCR in the context of HIV and tuberculosis, for which a quick and reliable method of diagnosis is essential. However, even some of the more specialised applications, such as diagnosis of haematological malignancies, would be well utilised in the central laboratory of a referral centre (Table I).

TABLE I.
Marrow aspirates suitable for PCR (Johannesburg Hospital, May 1992)

Disease	No. of cases (new cases)	PCR detectable rearrangement
ALL	34 (5)	Ig gene, TCR, t(9:22), t(1:19)
CML	5 (4)	t(9:22)
APL	2 (1)	t(15:17)
CLL	2 (1)	Ig gene
Other	4	Ig gene, t(14:18)

Once the reaction conditions for a given set of primers have been determined, PCR does not require high levels of technical expertise and an understanding of the underlying principles is certainly not a prerequisite for its use. The existence of customised 'kits' for some of the more common applications makes the routine laboratory use of PCR still simpler. These contain all that is required for processing of the specimen and interpretation of the results.

Results of PCR can be obtained extremely rapidly and it would definitely be possible to process a specimen within 1 day. Extraction of DNA from samples before setting up of the PCR reaction has until recently been a rate-limiting step but new methods of sample preparation have now been described which will be far more rapid and less laborious.[20,21]

The sensitivity, specificity and reliability of the technique will vary with different primers used, but one of the major problems is the risk of false positives resulting from contamination. Guidelines on practical ways of limiting this have been published.[22] The recently developed technique of substituting uracil for thymine in the reaction mixture effectively prevents any risk of contamination of DNA samples by the millions of copies of the DNA produced in a previous PCR reaction.[23]

Problems are also experienced with false negatives, but the use of an 'internal control' (amplification of an unrelated but constant part of the genome) with each sample and appropriate positive and negative controls with each batch of samples, should prevent misinterpretation of negative results.

As with any laboratory test, before PCR can safely become a routine laboratory tool, the issue of quality control must be addressed. Luckily, since for most PCR applications the 'correct answer' can be unequivocally defined, this should be fairly easy to implement.

The deciding factor as to the routine use of PCR in South African laboratories will, however, be cost and fortunately this is surprisingly low. The cost of consumables for a single unreplicated test visualised by agarose gel electrophoresis, assuming optimally efficient use of the cheapest available reagents and batch processing, is approximately R7,80 per test (Table II). This compares very favourably, for example, with a routine full blood count. The figure does not include the cost of research and development in optimising reaction conditions, which can be extremely high and is in part responsible for the higher prices gazetted by Roche for their customised PCR diagnostic kits (approximately R60 - R70 per test in the public sector).

TABLE II.
Cost of consumables required per test*

Stage	Cost
DNA extraction Reagents: triton X, saline, phenol, chloroform, isopropranol Disposables: pasteur pipettes, polypropylene tubes, etc.	R0,80
PCR reaction Reagents: Taq, buffer, primers, etc. Disposables: pipette tips, etc.	R5,20
Agarose gel electrophoresis Agarose, buffer, DNA size markers, etc.	R1,80
Total	R7,80

* Figure calculated for 100 µl reaction volume. Reaction volumes as low as 25 µl can be used. Simpler methods of sample preparation may be used.

Conclusion

There can be little doubt that marked changes in allocation of health care funding will take place in the new South Africa. However, PCR provides an excellent example of how progress and research can lead to the development of molecular techniques which, far from being a First-World privilege, may well become a necessity in order to maintain a reasonable standard of health care in developing countries or those such as South Africa facing economic crisis and social upheaval.

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