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New molecular technology in laboratory animal health monitoring

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Since the introduction of immunochemical techniques the basis of daily bench routine in a microbiology laboratory has been little changed. Nevertheless, turn-out times have been much improved and computers speed up the output of the results. However, during the last several years there has been a lot of excitement (1) generated in the clinical microbiology community over the prospect of using DNA probes to detect and identify infectious microorganisms.

The basic principles of molecular biology and several approaches towards the development of specific DNA probes for infectious agents will be reviewed in this article; comments will also be addressed to the limitations and pitfalls of this methodology.

Basic principles

Deoxyribonucleic Acid (DNA) is a double stranded helical molecule made up of small units, the nucleotides, each of which consist of a nitrogenous base (Adenine, Cytosine, Guanine, Thymine) linked to a sugar (pentose) which is in turn linked to a phosphate group. A DNA duplex is formed when two single strands are aligned. The whole molecule resembles a ladder with the base molecules linked together by hydrogen bonds. Alignment is always very precise with base pairing between adenine and thymidine and between guanine and cytosine (2). The two strands may be denatured by heating (in excess of 70°C) or by raising the pH or lowering the ionic strenghts of the DNA solution (3). Related to DNA, but differing slightly in structure, is ribonucleic acid (RNA). RNA usually exists as a single stranded molecule which contains uracil instead of thymidine and a different sugar moiety (4).

A fundamental property of single stranded DNA (and RNA) is that it will hybridize (form a duplex by hydrogen bonding) with another single strand which has a completely base sequence. It is this property which can be so usefully exploited for diagnosis using nucleic acid probes.

Molecular hybridization technology

Probes

A probe in the chemical or biological sense is a molecule which has a strong interaction with a specific target after which it can then be detected. Examples of such interactions are: antibody-antigen, avidin – biotin, receptor – ligand and complementary nucleic acid hybridization. Protein probes interact with their target at only a few specific sites through a mixture of forces: hydrophobic, ionic and hydrogen bonding. By contrast nucleic acid probes interact with their complementary structure primarily through hydrogen bonding at tens, hundreds or thousands of sites, depending on the length of the hybrid.

A nucleic acid probe is constructed from a unique base sequence taken from the genetic material (DNA or RNA) of a disease causing agent. The probe may then be applied to suitably treated pathological specimens or cell cultures. Probe bonding will only occur when complementary nucleic acid sequences are present in the specimen, these are called target sequences. After binding there must be method of differentiating between the bound and unbound probe.

Targets

Theoretically a probe (single stranded DNA or RNA) will hybridise to its complement-

ary target sequence in a single stranded nucleic acid template, for most practical applications the template is provided by the genomic DNA or RNA of an infectious organism, so the preparation of the pathological specimen is of great importance. Purified extracts of nucleic acids are ideal for most hybridization procedures although probing methods for many crude samples (swabs, thin sections, etc.) have been established (5, 8–10).

Hybridization formats and detection procedures

Various formats can be used for the detection of specific hybridization. In solution formats (11) both the reacting and non reacting nucleic acids remain free in solution and although these methods have the fastest hybridization rates the problem of separating the hybrid from the free probe remains.

Methods in which one of the reacting nucleic acids is immobilized on a solid support while the other is free in solution are however more commonly used (5, 12–14). In this case unhybridized nucleic acid is washed away leaving only that which is hybridised to the nucleic acid bound to the solid support e.g. nitrocellulose or nylon filters, latex, magnetic beads or microtiter plates. Once immobilized, nucleic acids can be detected by hybridization.

The most common solid support hybridisation procedures are:

The Spot or Dot Blot (15–17): This is the simplest hybridisation procedure in which crude or purified mixtures of nucleic acids are spotted onto the surface of a filter. This is a rapid, semi-quantitative procedure which can be used to detect the presence of specific nucleic acids in many samples simultaneously on the same filter.

The *Southern blot* hybridization (18, 19, 5) is a more sophisticated technique in which the DNA is separated by agarose gel-electrophoresis before transfer onto a filter by capillary action. The method in which RNA is transferred from gel to membranes is called *Northern blotting* (20, 5), while the transfer of proteins onto membranes is called *Western blotting* (21, 22). Once immobilized on a filter all nucleic acid sequences are hybridized in similar fashion. Hybridization buffer containing the labelled probe is incubated with the filters to allow hybridization of the probe to the target sequence. Following hybridization, unhybridized probe is removed by a series of washes. Sensitivity can be controlled by the stringency of these washes by altering salt and temperature conditions. Factors which must also be optimized for the specific probes used. The signal-to-noise ratio can also be affected by probe length, purity, concentration, sequence and by target contamination (5).

The probe may be labelled in a variety of ways to enable detection, either by *radiolabelling* (e.g. ^{14}C, ^{35}S, ^{32}P) (5) or with *non-radioactive* compounds (colorimetric and chemiluminescent).

The radiolabelled nucleic acids are conveniently detected by autoradiography on film.

Non-radioactive DNA probes can be detected using:

Colorimetric substrate systems: 1) In this system the probe is labelled with a hapten, then the probes are detected by incubation with a primary anti-hapten antibody followed by a species specific, anti-IgG secondary antibody conjugated to alkaline phosphatase, or horseradish peroxidase, enzymes which generate a colour change in a substrate solution indicating the presence of the probe (23). 2) Detection of biotin labelled probes is accomplished with streptavidin, this is easier and faster than hapten detection because it usually requires only one ten-minute binding step in contrast to two 30 or 60 minute antibody binding steps. This procedure can give high background signals so it is very convenient to add an extra step using streptavidin and biotinylated phosphatase instead of a direct conjugate (24).

Chemiluminescent substrate systems: These systems are available for the visualization of

peroxidase and alkaline phosphatase. The signal of both systems can be recorded on an X-ray film (25).

IN SITU hybridization

This method allows the examination of specific sequences within the cells of slide mounted tissue samples, which are pretated to facilitate specific probe binding and better specimen fixation. Following hybridization and development, the slides are examined by light microscopy. The microscopic examination of the specimen allows detection of small amounts of hybridization in a well defined area and by localizing specific sequences within cells *in situ* hybridization can identify the *inter*cellular distribution of these sequences. Estimating the percentage of infected cells and the relative number of sequences per cell can contribute to understanding of the organisms pathogenesis. A variety of radioactive and non-radioactive probes are suitable for use with *in situ* hybridization, the choice depends on the specific application. Following hybridization, slides can be stained to allow visualization of the cells. Haematoxylin Eosin is very effective and widely used (26).

Research continues to find better hybridization techniques with potential commercial applications which are more sensitive and specific. Recent developments have popularized solution hybridization formats such as: *Affinity capture* (27–30), *Sandwich hybridization* (31–37) and *Homogenous solution hybridization assays* (38–44).

Probe and target amplification systems

The future of nucleic acid hybridization as a diagnostic tool and as a useful commercial process lies in the ability of certain techniques to amplify targets or probes. Any of these amplification systems can increase the amount of the target or probe in a sample 10^6 times or more. Since there is currently no system for amplifying protein probes or targets to this degree, nucleic acid amplification assays are by far the most sensitive means of direct pathogen detection and are in fact the most sensitive means of detecting biological molecules.

Target amplification

Polymerase Chain Reaction (45–49). A pair of specific oligomer primers delineate the region of amplification and are used to initiate DNA synthesis by primer extension in combination with a heat-stable Taq DNA polymerase. Following this first round of primer extension at 70°C the mixture is heated to about 93°C to separate the product from its template and then cooled to 37–55°C to allow annealing of the primer molecules to the original template DNA and the newly synthesized DNA fragments. Primer extension is then resumed at 70°C.

By repeating these cycles of denaturation, annealing and replication the original target DNA can be exponentially amplified 2^n times where n is the number of cycles. 25 PCR cycles would result in a 3.9×10^7 fold amplification. However, since the efficiency of each cycle is less than 100 %, the actual amplification after 25 cycles is about $1–3 \times 10^6$ fold.

The size of the amplified region is generally 100–400 base pairs, although stretches of up to 2kb can be efficiently amplified. Nucleic acids either in their crude form or phenol/chloroform purified can be used as target. The amplified DNA can be detected simply by gel electrophoresis with ethidium bromide staining or by DOT Blot Hybridization. More sensitive detection using traditional Southern blotting is possible provided conditions are optimised for short DNA fragments. Sandwich hybridization is another simple and specific method of detecting PCR products.

The greatest drawback to PCR is the likelihood of PCR products contaminating negative samples and the surrounding environment generating false positive results (50). This may be prevented by dedicating two separate laboratories to PCR work; one in which sample preparation and amplification

takes place and another to process the amplified DNA. Well planned negative controls minimise the possibility of contamination. If any of these controls is positive the entire experiment is discarded. Positive controls are essential to ensure that both sample preparation methods and assay system are functional and to confirm the lack of any PCR inhibitors in the DNA preparations.

Transcription based amplification system (TAS) (51). Uses transcription to amplify an RNA target rather than using DNA replication to amplify a DNA target. Oligonucleotide primers define the end points of the amplified region, enzymes and temperature cycles are used to sequentially amplify the original target. The TAS cycles are complex and require repeated additions of two different enzymes because enzyme activity is destroyed during the denaturation step. TAS can only be used for RNA amplification and has no advantage over PCR.

Probe amplification

These systems amplify the probe nucleic acid or the probe signal after hybridization.

Q-Beta replicase system (52). This system is based on the ability of RNA dependent RNA polymerase from bacteriophage QB to synthesise large amounts of RNA from a small amount of template. The reaction is exponential because each product strand can serve as a template strand following synthesis. RNA synthesis continues at an exponential rate until the number of RNA molecules equals the number of polymerase molecules.

Probe networks (53, 54). These are solid phase sandwich hybridization assays. A system of three probe types is used to achieve signal amplification. Firstly, multiple primary probes bind to the target sequence. Secondly, an amplification multimer binds to each primary probe, and thirdly, multiple enzyme-labelled probes bind to each amplification multimer. The degree of signal amplification achieved is at least a 100 fold.

The effectiveness and usefullness of DNA probes in laboratory animal health monitoring

The microbiology laboratory assists the veterinarian as a routine health monitor and in the diagnosis of infectious diseases in a variety of ways; for example, collecting specimens or giving information on how to obtain them, or by the isolation and identification of the pathogens present in the specimen by any of the common methods, culture, virology or microscopy.

Molecular biological techniques (Southern blot, dot blot, Northern blot and *in situ* hybridization) have been used for many years in the study of the pathogenesis and tropism of laboratory animal pathogens, but it was not until the appearance of DNA amplification systems that the utilization of molecular biology in a diagnostic capacity became possible. Some research groups have already developed PCR primers for the detection of certain pathogens for example, rodent coronaviruses, rodent parvoviruses and *Mycoplasma pulmonis* (55–58).

Some of the pathogens that affect laboratory animals are too fastidious to grow in culture. To detect the presence of Mycoplasma Spp. from a sample a minimum of 15 days is required and antibodies do not appear until 3 months after the infection.

There is a possibility that DNA probes will shorten turn-around times for the identification of these fastidious pathogens and will confirm doubtful serological results.

Current DNA probe tests require between 1 to 30 hours to complete, but this is fast when compared with traditional methods requiring 2 weeks. For example, DNA probes offer identification of *M. pulmonis* within 6 hours of specimen receipt. Compared to the period of time it would take to isolate and identify these organisms using traditional methods, the DNA probe test is indeed rapid. When we compare these DNA tests with ELISA tests there is a clear 3 hours disadvantage, but, DNA probe tests can be

used at the onset of the infection before antibodies are produced.
There are commercially available DNA probe reagents for the early detection (24h culture) of bacteria such as *Mycobacterium tuberculosis, M. avium, M. intercellulare, Campylobacter jejuni* (59, 60). The formats being solution hybridization and filter hybridization. Their use has been restricted to the human microbiology laboratory but they can prove to be very useful for the quick screening of suspected bacteria e.g. determining the presence or absence of salmonella in a suspected colony in only 24h instead of the customary 72h or more. Though costs are higher than with more conventional tests the accurate and rapid procurement of results may be highly desirable, even essential, in specific cases. However, there are still no commercially available tests for the direct detection of most of these organisms from specimens (without culture), but they will be available soon.
Specimens obtained from an animal, such as biopsies and necropsy samples, can be directly tested with DNA probes for the detection of infectious agents. These tests can either be run on the day the samples are obtained, or later when a batch is prosessed; they also have the advantage of allowing tissue samples fixed in 10 % normal buffered formalin to be tested.
The application of this technology is very versatile and may be adapted to suit the circumstances.
These nucleic acid tests will allow the elimination of tests such as mouse and rat antibody tests (MAP, RAP). Tumours, cell lines, ascites or in fact almost any kind of animal product can be tested cost effectively both financially and in terms of animal lives. A battery of probes eliminate or confirm the presence of any of the possible contaminants within hours.
Probes only detect viable organisms, a property which may be utilized in establishing whether an antibody titer in a vaccinated animal is due to an infection or the effect of vaccination. They may also be used to demonstrate the elimination or continued presence of a pathogen after treatment.
One of the greatest problems faced by the microbiology laboratory is the possibility of interspecies infection. The only way of determining whether an antibody titer is specific is virus isolation which is very time consuming and extremely expensive (e.g. serum containing anti-mouse coronavirus antibodies can crossreact with rat or coronaviruses from other species). The appropriate probes will be able to answer these questions in a matter of hours.
Because probes can be used on a target that has been fixed, the study of achieved and historical tissues has become possible as has the study of tissues from toxicological investigations, ensuring the absence of any complicating factors such as pathogenic organisms.
New problems will arise with these novel techniques: Is the presence of virus in a damaged tissue the cause of the damage or is its presence merely incidental? Will the detection of a virus result in pathological symptoms? If so, what will the nature of these symptoms be? These questions are currently being addressed.
Nowadays immunodeficient animals are in important tool for research. The health monitoring of such atypical animals, nude and severe combined immunodeficiency (SCID) mice, is based on the use of immunocompetent animals as sentinels, but their is an exiguous availability of guaranteed very clean or gnotobiotic animals. In these particular cases study of the virological status of these colonies and study of any health problem the use of DNA probes will be a great break through avoiding the necessity for viral isolation in tissue culture or live animals.
The use of DNA probes as a diagnostic tool has to be approached carefully. In order to be able to interpret the significance of the results obtained a good knowledge of pathogenesis and molecular biology is essential.
Will DNA probes make antibody-based test

and agar – based bacterial cultures disappear from the clinical laboratory?

Probes make a reasonable alternative provided there is adequate training of technical personnel, sufficient quality control to ensure accuracy of results, periodic monitoring of test results and quality control of records by qualified personnel. However, the probability of probes totally replacing culture in the next few years is low. The antibiotic bacterial susceptibility or serotype of one organism can only be obtained if the organism is isolated in pure culture, so the culture of samples determined to be positive will still be necessary. Moreover, as is well known by any microbiologist the presence of microorganisms in a given sample can not be predicted. The laboratory should be able to determine the presence of unexpected or unusual organisms. If culture techniques are eliminated and replaced by a set of probes this ability as well as that of finding common organisms in unusual sites will be diminished. In cases where a selective elimination of certain microorganisms is sufficient, such as breeding institutions, DNA probe batteries could be routinely used to demonstrate the absence of those microorganisms. Where positive results occur the organism may then be cultured to obtain either serotype or antibiotic susceptibility data if required. The same rationale could be used to detect the presence of previously established groups of viruses.

Transient virus (those which cause an acute disease and are then eliminated by the animal) cannot be detected by the probes once they are gone so the sampling procedure is of extreme importance, choosing the wrong animal of the wrong age or the wrong sample site could produce false results. The sample site and sample processing are very important and have to be properly standardized beforehand.

DNA probes mark a revolutionary breakthrough in the rapid and accurate identification of pathogenic organisms actually present in animal samples, for this particular type of analysis probes are unsurpassed. However, this does not mean that traditional diagnostic methods can be replaced in the foreseeable future.

To bridge the gap between laboratory research and practical applications (clinical assays), DNA probe tests must be simple, rapid and inexpensive, competing favourably with existing antibody based assays. High sensitivity would be required to provide the incentive to purchase and become familiar with the new assay. Nonetheless, the rapid evolution of new molecular techniques ensures that nucleic acid probes will soon be incorporated into the modern health monitoring laboratory.

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References

1. *Engleberg NC, Eisenstein BL:* The impact of new cloning techniques on the diagnosis and treatment of infectious diseases. N. Engl. J. Med. 1989, *311*, 892–896.
2. *Watson JD, Crick FHC:* Molecular structure of nucleic acids. A structure for deoxyribose nucleic acid. Nature 1953, *171*, 737–738.
3. *Hamaguchi K, Geiduschek EP:* The effect of electrolytes on the stability of the deoxyribonucleate helix. J. Amer. Chem. Soc. 1962, *84*, 1329–1338.
4. *Brenner S, Jacob F, Meselson M:* An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature 1961, *190*, 576–581.
5. *Sambrook J, Fritsch EF, Maniatis T:* Molecular cloning. A laboratory manual. Book 2. Second edition. Cold Spring Harbor Laboratory Press 1989.
6. *Wallace RB, Shaffer J, Murphy RF, Bonner J, Hirose T, Itakura K:* Hybridization of synthetic oligunucleotides to OX174 DNA. The effect of single base pair mismatchs. Nucleic Acid Res. 1979, *6*, 3343–3347.
7. *Bryan RN, Ruth JL, Smith RD, LeBon JM:* Diagnosis of clinical samples with synthetic oligonucleotide hybridization probes. In: Microbiology. Edt. Schlessinger D. American Society for Microbiology. Washington DC 1986.
8. *Blobel G, Potter V:* Nuclei from rat liver. isolation method that combines purity with high yield. Science 1966, *154*, 1662–1665.

9. *Brawerman G, Mendecki J, Lee S:* A procedure for the isolation of mammalian messenger ribonucleic acid. Biochem. 1972, *11*, 637–640.
10. *Dyall-Smith M, Dyall-Smith D:* Recovering DNA from pathology specimens. A new life for old tissues. Molecular Biology Reports. (Bio – Rad, Labs) 1988, *6:1*, –2.
11. *Hall BD, Spiegelman S:* Sequence complementary of T2 – DNA and T2 – specific RNA. Proc. Natl. Acad. Sci. Wash. 1961, *47*, 137–146.
12. *Bolton ET, McCarthy BJ:* A general method for the isolation of RNA complementary to DNA. Proc. Natl. Acad. Sci. Wash. 1962, *48*, 1390–1397.
13. *McCarthy BJ, Bolton ET:* An approach to the measurement of genetic relatedness among organisms. Proc. Natl. Acad. Sci. USA 1963, *60*, 156–164.
14. *Britten RJ, Graham DE, Neufeld BR:* Analysis of repeating DNA sequences by reassociation. Methods in Enzymology 1974, *29*, 363–418.
15. *Kafatos FC, Jones CW, Efstratiadis A:* Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acid. Res. 1979, *7*, 1541–1552.
16. *Thomas P:* Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA 1980, *77*, 5201–5205.
17. *Grunstein M, Hogness DS:* Colony Hybridization: A method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA 1975, *72*, 3961–3965.
18. *Southern EM:* Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 1975, *98*, 503–517.
19. *Reed KC, Mann DA:* Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acid Res. 1985, *13*, 7207–7221.
20. *Lehrach H, Diamond D, Wozney G, Boedtker H:* RNA molecular weight determinations by gel electrophoresis under denaturing conditions: a critical reexamination. Biochem. 1977, *16*, 4743–4751.
21. *Towbin H, Staehelin T, Gordon J:* Electrophoretic transfer of proteins from polyacrilamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA 1979, *76*, 4350.
22. *Burnette WN:* "Western Blotting". Electrophoretic transfer of proteins from sodium dodecyl sulfate – polyacrilamide gels to unmodified nitrocellulose and radiodinated protein A. Anal. Biochem. 1981, *112*, 195–198.
23. *Keller GH, Cunning CU, Huang DP, Manak MM, Ting R:* A chemical method for introducing haptens onto DNA probes. Anal. Biochem. 1988, *170*, 441–450.
24. *Leary JJ, Brigati DJ, Ward DC:* Colorimetric method for visualizing biotin – labeled DNA probes hybridized to DNA or RNA inmobilised on nitrocellulose. Bio-Blots. Proc. Natl. Acad. Sci. USA 1983, *80*, 4045–4049.
25. *Matthews JA, Batki A, Hynds C, Kricka LJ:* Enhanced chemiluminescent method for the detection of DNA dot hybridization assays. Anal. Biochem. 1985, *151*, 205–209.
26. *Pardue M: In situ* hybridization in nucleic acid hybridization. Eds. Hames B, Higgins S. IRL Press. Oxford 1985.
27. *Wilkinson HW, Sampson JS, Plikaytis BB:* Evaluation of a commercial gene probe for identification of *Legionella* cultures. J. Clin. Microbiol. 1986, *23*, 217–220.
28. *Granato PA, Franz MR:* Evaluation of a pathogen of a prototype DNA probe for the noncultural diagnosis of gonorrhoea. J. Clin. Microbiol. 1989, *27*, 632–635.
29. *Yehle CO, Patterson WL, Boguslawski SJ, Albarella JP, Yip KF, Carrico RJ:* A solution hybridization assay for ribosomal RNA from bacteria using biotinilated DNA probes and enzyme-labelled antibody to DNA:RNA. Molecular and Cellular Probes 1987, *1*, 177–193.
30. *Viscidi RP, O'Meara C, Farzadegan H, Yolken R:* Monoclonal antibody solution hybridization assay for detection of human immunodeficiency virus nucleic acids. J. Clin. Microbiol. 1989, *27*, 120–125.
31. *Dunn AR, Hassell JA:* A novel method to map transcripts: evidence for homology between an adenovirus mRNA and discrete multiple regions of the viral genome. Cell. 1977, *12*, 23–26.
32. *Ranki M, Soderlund HE:* Detection of microbial nucleic acids by one-step sandwich hybridization test. US. Patent # 1984, 486, 539.
33. *Ranki M, Palva A, Virtanen M, Laaksonen M, Soderlund HE:* Sandwich hybridization as a convenient method for the detection of nucleic acids in crude samples. Gene. 1983, *21*, 77–85.
34. *Polsky-Cynkin R, Parsons GH, Allerdt L, Landes G, Davis G, Rashtchian A:* Use of DNA inmobilized on plastic and agarose supports to detect DNA by sandwich hybridization. Clin. Chem. 1985, *31*, 1438–1443.
35. *Langdale JA, Malcom ADB:* A rapid method of gene detection using DNA bound to Seohacryl. Gene. 1985, *36*, 201–210.
36. *Dahlen P, Sylvanen AC, Hurskainen P, Kwiatkwski M, Sund C, Ylikoski J, Sunderlund HE, Lovgren T:* Sensitive detection of genes by sandwich hybridization and time – resol-

ved fluormetry. Molecular and Cellular Probes 1987, *1*, 159–168.
37. *Keller GH, Huang DP, Manak MM:* A sensitive nonisotopic hybridization assay for HIV – 1 DNA. Anal. Biochem. 1989, *177*, 27–32.
38. *Heller MJ, Morrison LE:* Chemiluminescent and fluorescent probes for DNA hybridization systems. In: Rapid Detection and Identification of Infectious Agents. pp: 245–256. Academic Press. San Diego, CA 1985.
39. *Morrison LE:* Time-resolved detection of energy transfer: Theory and Application to immunoassays. Anal. Biochem. 1988, *174*, 101–120.
40. *Cardullo RA, Agrawal S, Flores C, Zamecnick PC, Wolf DE:* Detection of nucleic acid hybridization by fluorescence resonance energy transfer. Porc. Natl. Acad. Sci. USA 1988, *86*, 8790–8794.
41. *Vary CPH:* A homogenous nucleic acid hybridization assay based on strand displacement. Nucleic Acid Res. 1987, *15*, 6883–6897.
42. *Weeks I, Behesti I, McCapra F, Campbell AK, Woodhead JS:* Acridinium esters as high specificity activity labels in immunoassay. Clin. Chem. 1983, *2*, 1474–1479.
43. *Weeks I, Sturgess M, Brown RC, Woodhead JS:* Immunoassays using Acridinium esters. Methods in Enzymology. 1986, *133*, 366–390.
44. *Nelson NC, Hammond PW, Wise WA, Arnold LJ:* Novel assay formats employing acridinium ester – labelled DNA probes. In Abstracts of the Third San Diego Conference. "Practical Aspects of Molecular Probes". 1988.
45. *Mullis KB, Erlich HA, Arnheim N, Horn GT, Saiki RK, Scharf SJ:* Process for amplifying, detecting and/or cloning nucleic acid sequences. US. Patent 1987, # *4*, 683, 195.
46. *Mullis KB, Faloona FA:* Specific synthesis of DNA *in vitro* via polymerase catalyzed chain reaction. Methods in Enzymology 1987, *155*, 335–350.
47. *Saiki R, Scharf SJ, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N:* Enzymatic amplification of Beta – globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. Science 1985, *230*, 1350–1354.
48. *Mullis KB, Faloona FA, Scharf SJ, Saiki RK, Horn GT, Erlich HA:* Specific enzymatic amplification of DNA *in vitro;* the polymerase chain reaction. Cold Spring Harbour Symp. Quant. Biol. 1986, *51*, 263–273.
49. *Erlich H:* PCR technology: Principles and applications for DNA amplification. Stockton Press. New York 1989.
50. *Kwoh S, Higuchi R:* Avoiding false positives with PCR. Nature 1989, *339*, 237–238.
51. *Kwoh DY, Davis GR, Whitfield KM, Chappelle HL, DiMichelle LJ, Gingeras TR:* Transcription – based amplification system and detection of amplified human immunodeficiency virus type I with a bead – based sandwich hybridization format. Proc. Natl. Acad. Sci. USA 1989, *86*, 1173–1177.
52. *Lizardi PM, Guerra CE, Lomeli H, Tussie-Luna I, Kramer FR:* Exponential amplification of recombinant RNA hybridization probes. Biotechnology 1988, *6*, 1197–1202.
53. *Urdea MS, Running JA, Hoen T, Clyne J, Ku L, Warner BD:* A novel method for the rapid detection of specific nucleotide sequences in crude biological samples without blotting and radioactivity: Application to the analysis of hepatitis B virus in human serum. Gene. 1987, *61*, 253–264.
54. *Sanchez-Pescador R, Stempien MS, Urdea MS:* Rapid chemiluminescent acid assays for the detection of TEM-1 Beta – lactamase – mediated penicillin resistance in *Neisseria gonorrhoeae* and other bacteria. J. Clin. Microbiol. 1988, *26*, 1934–1938.
55. *Besch-Williford C, Purdy G, Riley L, Pintel D:* Detection of rodent Parvoviruses (MVM, H-1 and RV) by Polymerase Chain Reaction. Poster Session. Annual AALAS meeting. 1991.
56. *Homberger FR, Smith AL, Barthold SW:* Detection of rodent Coronaviruses in tissues and cell cultures by using Polymerase Chain Reaction. Journal of Clinical Microbiology 1991, *29 (12):* 2789–2793.
57. *Kunita S, Terada E, Goto K, Kagiyama N:* Polymerase Chain Reaction for detection of Mouse Hepatitis Virus. Poster Session. Annual AALAS meeting. 1991.
58. *Kunita S, Terada E, Goto K, Kagiyama N:* Sensitive detection of *Mycoplasma pulmonis* by using the Polymerase Chain Reaction. Experimental Animals 1990, *29*, 104–107.
59. *Drake TA, Hindler JA, Berling OGW, Bruckner DA:* Rapid identification of *Mycobacterium avium* complex in culture using probes. J. Clin. Microbiol. 1987, *25*, 1442–1444.
60. *Kiehn TE, Edwards F:* Rapid identification using a specific DNA probe of *Mycobacterium avium* complex from patients with acquired immunodeficiency syndrome. J. Clin. Microbiol. 1987, *25*, 1551–1554.

Further reading

1. *Brown TA:* Essential Molecular Biology. Edt. IRL Press, Oxford University Press, Oxford 1991.

2. *Davis LG, Dibner MD, Battley JF:* Basic Methods in Molecular Biology. Elsevier Science Publishers. NY. 1986.
3. DNA probes for infectious diseases. Edt. Tenover F.C. CRC. Press 1989.
4. *Keller GH, Manak MM:* DNA probes. MacMillan Publishers Ltd. Stockton Press 1989.
5. Molecular Diagnostics in Pathology Edts. Fenoglio-Preiser CM, Willman CL. Williams & Wilkins 1991.
6. Nucleic Acid Hybridization. A Practical Approach. Edts. Hames BD & Gigings SJ. IRL Press 1988.
7. PCR Protocols. A Guide to methods and applications. Edts. Innis MA, Gelfand DH, Sininsky JJ, White TJ. Academic Press, INC 1990.
8. *Woodward MJ:* DNA probes for diagnosis: An Introduction. S. Vet J 1989, *43,* 28–71.

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