CHIMIA 2000, 54, No. 1/2

Chimia 54 (2000) 21–24

ISSN 0009–4293

Focal Point: Analytical Chemistry

ILMAC Congress: October 14, 1999

Trends in Clinical Chemistry*

Analytical chemistry and clinical chemistry have come closer together over the past thirty years and increasingly profit from each other. Advances in elucidating the human genome and the genomes of viruses and microorganisms have made it possible to track down a disease to the level of the nucleic acids and the genetic code, and to identify a disease on the molecular level. Examples are given of the diagnosis of a complex of inherited diseases, the porphyrias, on a molecular basis and of the identification of a virus infection referred to as *Bordetella Pertussis*. In addition, the trend to on-site analysis, which can provide immediate information about the state of a patient, is contrasted with high-throughput analysis in central laboratory units. The second contribution deals with the logistics of point-of-care testing (POCT) and its technological prerequisites in Sweden.

In clinical chemistry, novel technologies are not primarily rated according to their originality but rather according to logistic considerations. These include economic factors, the time-elapse to information, the ease of quality assessment and the consequences of a positive result, *i.e.* of a result which exceeds the decision limit separating sick patients from the population of healthy individuals. During the past 30 years, since 1968, the technical equipment used in clinical chemistry has been revolutionized and it has become much more widespread. Currently major advances are being made in Point-of-Care Testing (POCT) and Home Care Testing where Laboratory Information Technology (LIMS) and global exchanges *via* the Ethernet and Internet have become important links in the information chain. Automation has been driven forward so that it is now possible to automate the separation steps, the sample pretreatment and sample distribution steps at high throughput. When performing special analyses where the analytical procedure cannot be implemented using high-throughput instruments, it has been beneficial to apply advances in analytical chemistry to clinical chemistry. Such advances include analytical techniques such as mass spectrometry, gas chromatography, and HPLC. In some special domains where analytical technologies are applied to provide specific information based on, for example, the separation and identification of peptides and proteins *via*, for instance, two-dimensional electrophoresis, blotting techniques or PCR-relied analyses, clinical chemistry is even more advanced than analytical chemistry. However, in clinical chemistry, using an analytical technique is only justified if a positive result can lead to a useful medical treatment and to an improvement in the state of health of the patient.

At the same time, the work that aims to describe the entire human genome by the year 2003 [1] has stimulated, and continues to stimulate, practices and techniques that allow gains in information to be tracked down to the source of a disease. It has also become possible in some cases to predict the probability of being affected by an 'illness', which is based on a genetic predisposition, before symptoms break out. This means that prevention can be more effective, so that people liable to be affected by a hereditary disease can be trained to adjust their everyday behavior and to try to prevent the illness. In microbiology, it is now possible with PCR and electrophoresis to identify microorganisms in a timeframe of single days instead of the days and weeks needed to cultivate microorganisms.

In the following, the authors provide insights into the organization of point-of-care testing (POCT) and describe some specific techniques which should solve some problems currently facing clinical chemistry.

Keywords: *Bordetella pertussis* · Quality assessment · PCR · POCT · Porphyria · TaqMan · Virus infection

[1] F.S. Collins, A. Patrinos, E. Jordan, A. Chakravarti, R. Gesteland, R. Walters, members of the DOE and NIH planning groups, *Science* **1998**, *282*, 682.

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Porphyria and Analysis at the Genome Level

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The advancement of clinical laboratory science by molecular biology was illustrated by three examples:

A) the increased reliability of diagnosis, enabling prevention of disease,

B) the improved prediction of prognosis allowing measures to be taken to avoid complications,
C) the elucidation of apparently 'non-classical' inheritance to support genetic counseling in the future.

To this aim a group of inherited diseases called 'the porphyrias' are used as a model. The common biochemical basis is that they all are enzyme defects of haem biosynthesis [1]. There are six distinctive enzymes catalyzing the sequential steps to produce haem. For all of them, inborn defects associated with specific clinical diseases are known. Two of the enzymes and their corresponding diseases will be considered more closely as some fascinating novel facts have been elucidated.

The first example: Patients with acute-intermittent porphyria (AIP) suffer from recurrent attacks of severe abdominal pain that are precipitated by the intake of otherwise innocuous drugs, such as painkillers or sleeping pills. Thus, attacks can be prevented by avoiding these drugs. As AIP is autosomal dominant, fifty percent of direct relatives are affected. Because of the possibility of prevention it is recommended that the direct relatives of any patient are examined. Two conventional laboratory tests are utilized *i.e.* determination of abnormal compounds in urine and assay of enzyme activity in red blood cells, but both have limited reliability. In asymptomatic periods no abnormal compounds are excreted in urine and in about 15% of patients enzyme activity is not conclusive or even misleading. From our study in known Swiss AIP patients the sensitivity of mutation detection has been determined to be >95% and it reaches nearly 100% reliability in family members of a patient with a known mutation [2].

The second example shows that molecular techniques provide the means for an improved prediction of prognosis. Erythropoietic protoporphyria (EPP) is characterized by acute photosensitivity (burning pain after a few minutes of sunlight exposure). This light sensitivity is caused by accumulation of the metabolite protoporphyrin. A minority of 5–10% of the patients develop protoporphyrin-induced fatal liver failure. From a total of 100 patients examined for their mutations [3–6] all 16 with complicating liver disease have a 'null allele' mutation (*i.e.* a mutation causing a shortened gene product), but none of the 13 patients with a missense mutation shows complicating liver damage ($p<0.05$) [7]. The conclusion of this genotype–phenotype correlation is that patients with missense mutations require a less stringent control of their liver function than those with 'null allele' mutations.

The final example: Family studies have elucidated that EPP does not exactly follow classical Mendelian genetics. Based on an exhaustive family study, Prof. Went proposed that there must be three alleles of the enzyme ferrochelatase: one being the normal or so-called wild type, which is by far the most abundant in the general population [8]. The second allele, present in all EPP patients and in their family members with reduced enzyme activity was the mutated allele. A hypothetical third allele present in the population is totally innocent by itself. But if it combines by chance with a mutated allele it causes disease.

The allele-specific inheritance in EPP families was studied by haplotype analysis [9–12]. It became evident that one specific haplotype, if combined with a mutated allele, causes the disease. This finding proved that the three allele hypothesis was correct on a molecular level and has elucidated why there is an incomplete penetrance in this inherited disorder.

With this new insight into inheritance pattern, genetic counseling in EPP becomes more reliable.

These three examples illustrate that molecular biology will make matters more complex, and much new information on diseases will become available. This broadened knowledge base will enable us to understand disorders more precisely and will – hopefully – ultimately improve our therapeutic strategies in order to reduce suffering.

Supported by: SNF No. 31-53799.98, Stiftung für wissenschaftliche Forschung der Universität Zürich and the Roche Research Foundation.

[1] A. Kappas, S. Sassa, R.A. Galbraith, Y. Nordmann, 'The porphyrias', in 'The Metabolic and Molecular Basis of Inherited Disease', 7th ed., Eds. C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle, McGraw-Hill, New York, **1995**, p. 2139.
[2] X. Schneider-Yin, C. Bogard, U. Rüfenacht, H. Puy, Y. Nordmann, E.I. Minder, *Human Hered.* in press.
[3] U. Rüfenacht, L. Gouya, X. Schneider-Yin, H. Puy, B. Schäfer B, Y. Nordmann, E.I. Minder, J.C. Deybach, *Am. J. Hum. Genet.* **1998**, *62*, 1341.
[4] U. Rüfenacht, X. Schneider-Yin, B. Schäfer, S. Taketani, J.C. Deybach, E.I. Minder, *Clin. Chem. Lab. Med.* **1998**, *36*, 763.
[5] L. Gouya, X. Schneider-Yin, U. Rüfenacht, C. Herrero, M. Lecha, J. Mascaro, Y. Nordmann, J.C. Deybach, E.I. Minder, *J. Invest. Dermatol.* **1998**, *11*, 406.
[6] J. Bloomer, C. Bruzzone, L. Zhu, Y. Scarlett, S. Magness, D. Brenner, *J. Clin. Invest.* **1998**, *102*, 107.
[7] X. Schneider-Yin, L. Gouya, A. Meier-Weinand, J.C. Deybach, E.I. Minder, *Eur. J. Pediatr.* submitted.
[8] L.N. Went, E.C. Klasen, *Ann. J. Hum. Gen.* **1984**, *48*, 105.
[9] J. Frank, J. Nelson, X. Wang, L. Yang, W. Ahmad, H. Lam, F. Jugert, K. Kalka, M. Poh-Fitzpatrick, G. Goerz, H. Merk, A. Christiano, *J. Investig. Med.* **1999**, *47*, 278.
[10] L. Gouya, J.C. Deybach, J. Lamoril, V. Da Silva, C. Beaumont, B. Grandchamp, Y. Nordmann, *Am. J. Hum. Genet.* **1996**, *58*, 292.
[11] L. Gouya, H. Puy, L. Lamoril, V. Da Silva, B. Grandchamp, Y. Nordmann, J.C. Deybach, *Blood* **1999**, *93*, 2105.
[12] M. Henriksson, K. Timonen, P. Mustajoki, H. Pihlaja, R. Tenhunen, L. Peltonen, R. Kauppinen, *J. Invest. Dermatol.* **1996**, *106*, 346.

Real-Time PCR for the Quantitation of Viruses

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What is the state of the art in the detection of pathogens? The need for pathogen detection in a clinical laboratory is increasing and detection is expected to be very fast and precise. Experience from the last three years and a large list of publications show that real-time Polymerase Chain Reaction (PCR) is an excellent tool for the detection and even quantitation of pathogens [1–7].

Real-time PCR is based on a conventional PCR system where a fluorogenic probe, also called TaqMan® probe, is added (Fig. 1). The sequence of the TaqMan® probe is designed to hybridize to the target sequence between the two primers. The TaqMan® probe is labeled with two fluorescent dyes, a reporter dye R (on the 5' end) and a quencher dye Q (on the 3' end). No emission from the reporter dye can be measured while the TaqMan® probe is intact because of an energy transfer from the reporter to the quencher. As a result of 5' nuclease activity of the *Taq* polymerase, the TaqMan® probe is cleaved during the polymerization of the PCR product. Cleavage leads to a dislocation of the fluorescent dyes and therefore to an increase of the reporter fluorescence. The levels of emission of all fluorescent dyes in a reaction are measured automatically and continuously during the PCR run by an optical system (Fig. 2).

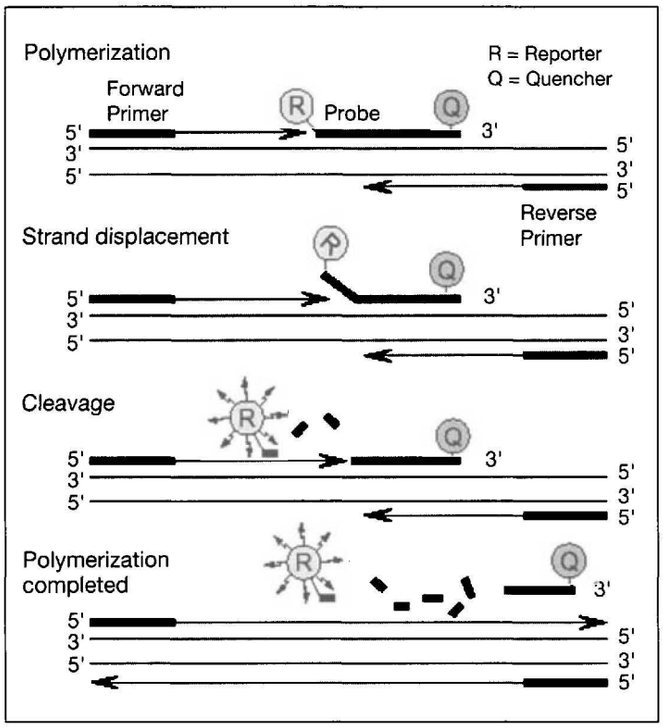

Fig. 1. The fork-like structure-dependent, polymerization-associated 5'–3' nuclease activity of AmpliTaq Gold DNA polymerase during PCR.

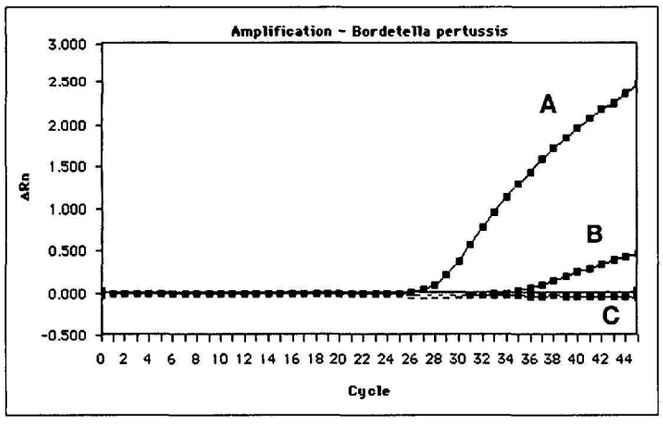

Fig. 2. Real-time PCR amplification of two samples (A and B) containing *Bordetella pertussis* and one sample (C) without *B. pertussis* (x-axis: PCR cycles, y-axis: normalized reporter dye signal).

These systems, either the ABI Prism® 7700 Sequence Detection System or the GeneAmp® 5700 Sequence Detection System from PE Biosystems, allow the detection and quantitation of viruses (RNA and DNA), bacteria, and parasites. Experience shows that the high sensitivity of this technology allows the detection of even a few copies of a pathogen in a clinical sample. Our former work with feline coronaviruses (FcoV) in the clinical laboratory of Prof. Hans Lutz (Department of Internal Veterinary Medicine, University of Zürich) showed a 10 to 100 times higher sensitivity of the TaqMan® one-step reverse transcription-PCR system compared to the conventional nested reverse transcription PCR [6]. Other characteristics are a nearly absent risk of cross-over contamination (because the PCR progress is measured during PCR and the tubes stay closed after the PCR run) and a very high reproducibility in detection and quantitation. The reasons for this impressive reproducibility are both high precision optics as well as an internal fluorescence normalization system in the buffer.

As a result of detection during the PCR, post amplification steps (such as gel electrophoresis) are no longer necessary. The systems are completely flexible in terms of the chemistry that can be used or the assay conditions of all existing PCR assays. If needed, universal cycling conditions and universal reaction conditions can be used to run all assays with the same conditions. The 96-well microtiter format permits a flexible sample throughput and the analysis of up to 96 samples in less than 3 h (including pipetting of all 96 samples and data analysis), during which time the instrument runs automatically for 1.5 h.

The integrated systems ABI Prism® 7700 Sequence Detection System and GeneAmp® 5700 Sequence Detection System from PE Biosystems allow a fast, reliable, and highly reproducible detection and quantitation of pathogens but are also a tool for the quantitation of gene expression, the automated detection of point mutations (genotyping), and the detection and quantitation of translocations.

[1] H. Kimura, M. Morita, Y. Yabuta, K. Kuzushima, K. Kato, S. Kojima, T. Matsuyama, T. Morishima, *J. Clin. Microbiol.* **1999**, *37*, 132.
[2] C.M. Leutenegger, D. Klein, R. Hofmann-Lehmann, C. Mislin, U. Hummel, J. Boni, F. Boretti, W.H. Guenzburg, H. Lutz, *J. Virol. Methods* **1999**, *78*, 105.
[3] K. Hawrami, J. Breuer, *J. Virol. Methods* **1999**, *79*, 33.
[4] N. Pusterla, J.B. Huder, C.M. Leutenegger, U. Braun, J.E. Madigan, H. Lutz, *J. Clin. Microbiol.* **1999**, *37*, 1329.
[5] A. Pahl, U. Kuhlbrandt, K. Brune, M. Rollinghoff, A. Gessner, *J. Clin. Microbiol.* **1999**, *37*, 1958.
[6] M. Gut, C.M. Leutenegger, J.B. Huder, N.C. Pedersen, H. Lutz, *J. Virol. Methods* **1999**, *77*, 37.
[7] E. Reiss, K. Tanaka, G. Bruker, V. Chazalet, D. Coleman, J.P. Debeaupuis, R. Hanazawa, J.P. Latge, J. Lortholary, K. Makimura, C.J. Morrison, S.Y. Murayama, S. Naoe, S. Paris, J. Sarfati, K. Shibuya, D. Sullivan, K. Uchida, H. Yamaguchi, *Med. Mycol.* **1998**, *36* (Suppl 1), 249.

An Advanced Point-of-Care Testing System (POCT) in Primary Health Care – Difficulties and Possibilities. POCT in Sweden – Some Experiences

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In Sweden the number of hospital beds has been reduced and patients traditionally cared for in hospitals have been transferred to general practitioners (GPs) for primary health care (Fig. 1). Hospitals are about to face new roles as technique and competence centers supporting both home and primary health based care. The total cost (per year) per inhabitant for health care is $ 1600 compared with $ 1400 in Switzerland and $ 3200 in the United States. Sweden has 1 physician per 330 inhabitants and about 4–5% of the total health care cost is used for laboratory services in Sweden, which can be compared with 15–20% in the USA. Financial arrangements for laboratory services vary and in some parts of Sweden, GPs are given money to manage all the care they provide, including laboratory services. Elsewhere central hospital laboratories are given a budget to serve the GPs. Sweden has about 900 primary health care centers with laboratories with different analytical programs. In most decentralized laboratories, only waived tests (B-Hemoglobin, B-Glucose, Sedimentation Ratio (SR), and urinary strip tests) are performed,

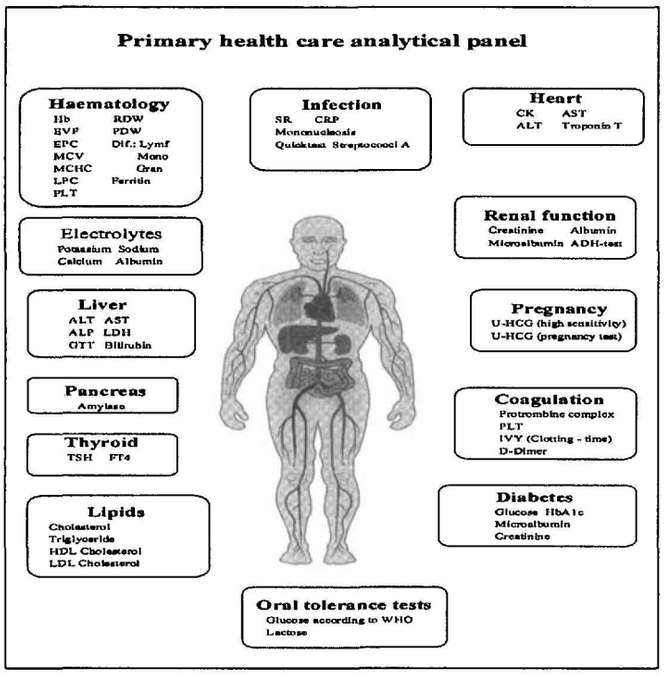

Fig.1. Analytical panel at the Mjolby primary health care center laboratory, which services nine general practitioners. The panel is based on the most common disease conditions in the actual primary health care area and covers >90% of all tests needed by the general practitioners in any out-patient clinic in Sweden.

while in some GPs' laboratories, more than 90% of the tests requested are carried out by medical technologists.

The decentralization of former 'esoteric tests' such as HbA1c and TSH, has been possible through accelerated technological development. Another prerequisite for decentralization was a political wish to improve health care for the patients. In our county the GPs defined their need in terms of turn-around time (TAT) for result reports to <30 min. These GPs have identified the advantages of an extensive POCT system (Table). Two major questions concern decentralized testing: Are the Analytical Quality and Costs and Outcomes acceptable? Most decentralized laboratories perform both internal and external quality programs. Most analytical programs in our extensive panel have a running CV% of <8%, which is far below what is requested for correct medical decision making, We also run a harmonization control whereby the same whole blood sample from one patient is analyzed in all central and decentralized laboratory set-ups four times a year. These results indicate that the physicians obtain a result, from any patient, which is comparable wherever the sample is analyzed [1].

Table.

Advantages with POCT in the primary health care sector identified by general practitioners in the county of Ostergotland, Sweden

Reduction of administrative time for patient information
Increased patient convenience
Possibility for direct result discussion with the patient
Reduction in assistance from *e.g.* relatives of elderly people in connection with visits to the physician
Reduction of pre-analytical errors
Increased analytical quality of laboratory results
Reduced sample transportation to the central laboratory
Access to skilled medical technologists for direct consultation on *e.g.* discussion of unexpected results

Few studies have made cost comparisons, but some report considerably higher costs for testing in decentralized settings [2][3]. We performed a cost study on central *versus* decentralized testing after identification of all fixed and variable costs. B-glucose, urine screening, and SR had the same cost, sampling and HDL-cholesterol were more expensive in the hospital laboratories while blood-status, potassium and ALT (Alanine aminotransferase) were more costly in the primary health care laboratories. We concluded that cost calculations are complex and that results are critically dependent on the number of tests performed by a certain staff size [4]. However, today it seems more reasonable to look on this type of economy not by cost per test, but rather as cost per decision or result.

Outcome can be divided into at least four categories: management, medical, service, and economic. With regard to management, the patient saves a lot of time if he gets his result at the same time that he visits his physician. The information compliance is also improved considerably with 'straight-forward face-to-face information' directly on site. The most important gain for the GPs is improved time management and calculations on saved time with POCT indicate that it can be as high as three weeks of working time per year per GP. The medical outcome improvement is most obvious from coagulation tests performed as POCT as fast stabilization of coagulation reduces the risk from thrombosis considerably in critical care [5]. Although more evidence is needed, it seems obvious that POCT, even with somewhat higher cost per test, might turn out to be cost effective if a more global perspective is taken on the overall health economy. It also appears that miniaturization will continue and provide clinical chemistry with hitherto unknown possibilities, the use of which must be planned in close connection with the clinicians.

[1] E. Fremner, B. Kalerud, A.C. Mengel, L. Larsson, *Clin. Chem.* **1997**, *43*, 5.
[2] E. Jacobs, in 'Preparing for Critical Care Analysis in the 21st Century', 16th International Symposium, Ed.: P. Dòrazio, **1996**, p. 126.
[3] W.W. Tsai, D.B. Nash, B. Seamonds, G.J. Weir, *Clin. Therap.* **1994**, *16*, 898.
[4] L. Larsson, E. Fremner, A.C. Mengel, H. Dahlgren, in 'Proceedings of the confluence of critical care analysis and near patient testing', 17th International Symposium, Nice, France, June **1998**, pp. 4–7,
[5] R.C. Becker, J. Cyr, J.M. Corrao, S.P. Ball, *Am. Heart J.* **1994**, *128*, 719

Test Panels in Point-of-Care Testing: The Role of New Technologies

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No abstract received