Emerging technologies for point-of-care genetic testing

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In the coming years, genetic test results will be increasingly used as indicators that influence medical decision making. Novel instrumentation that is able to detect relevant mutations in a point-of-care setting is being developed to facilitate this increase, frequently as a spin-off from recent research in the area of biothreat monitoring. This market review will describe the current generation of instrumentation that is most suitable for use in a point-of-care setting; it will also try to identify some of the technologies that will make-up the next generation of instrumentation currently being prepared for the market.

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Pharmacogenetics is the study of the genetic basis of differing response of individuals to drugs. The first examples of drugs that displayed pharmacogenetic variability were found in the 1950s. For example, it was noticed that individuals with an inherited deficiency of the enzyme butyrylcholinesterase are at risk of an adverse reaction to the drug succinylcholine. In the decades since this and other similar discoveries, the link between genotype and phenotype was strengthened by the application of novel genetic, biochemical and molecular technologies, which ultimately resulted in the determination of the molecular basis of the link [1,2].

It is expected that genetic markers will be increasingly used for medical decision making. Anticipated new uses include their use as an indication for (or against) treatment with a particular drug [3–5]. A specific example is that of warfarin, where the amount of the drug prescribed to patients at the extremes of the dosing range varies over 120-fold [6]. An estimated 25% of this variability can be attributed to ten polymorphisms of a single gene [7]. Hemorrhagic complications occur in approximately 1.1–2.8 patients per 100 patient-years [8].

One of the major outcomes hoped for from pharmacogenetic testing is an increased ability to predict the starting dose of drugs, such as warfarin; thereby reducing the number of adverse events. This is expected to increase the quality of patient care whilst also decreasing the cost of drug discovery through a reduction in the number of potentially useful drugs not pursued as a result of adverse reactions in a subset of patients. The advantages of pharmacogenetic testing may be best realized if testing can be performed at the point of care, and thus an increase in the number of hospital visits associated with the introduction of pharmacogenetic testing could be avoided.

A number of problems must be overcome in order that routine pharmacogenetic testing may become practical. The most important is the establishment of robust evidence of clinical utility [201]. Such data are already available for some markers and more will emerge in increasing numbers in coming years. In addition, all the issues that exist when genetic testing is performed in dedicated laboratories exist for testing in a point-of-care situation; the most notable are the interpretation of results and test performance. In a dedicated laboratory, these problems are addressed by expertise, experimental quality control and a carefully constructed framework of quality management. It will be necessary to develop methods of achieving the same quality in a point-of-care setting, although the means by which this may be achieved is beyond the scope of this review.

A further problem that must be addressed is the development of suitable technologies able to perform pharmacogenetic testing at a reasonable

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price. The progress in this aspect of the point-of care genetic testing field will be examined in this review. The intention is to identify the instruments that are most suitable in their current form and also to identify technologies that may, in the future, be useful as part of such an instrument. Several of the instruments discussed are available for purchase at the time of writing, although the majority are being prepared for release or are available in very limited numbers.

For an instrument to be useful for point-of-care genetic testing, it must have certain attributes. It is important that genotypes, are determined from patient samples, without user intervention, in order to both minimize the expertise required to perform the assay and, it is hoped, to increase the reproducibility of the assay. A suitable instrument must be fast; approximately 1 h would be considered a good time for an entire assay, although faster is better. The accuracy of results is of critical importance; it is likely that performance levels similar to those achieved in a traditional laboratory will be expected of point-of-care instruments, although this will ultimately be determined by the clinical use to which a test result is put [9,10].

In addition to these prerequisites, other aspects of the design of an instrument are important. Ideally, an instrument should be able to determine a number of different genotypes from a single sample; this would be important for situations such as warfarin, where the phenotype is determined by additive effect of a number of polymorphisms. Finally, instruments must be designed and manufactured to meet the exacting standards required by regulators the of point-of-care diagnostic devices.

Biotage/454 Life Sciences pyrosequencers

The instruments manufactured by Biotage and 454 Corp. both use the pyrosequencing process [11]. The process involves the cyclical addition of individual nucleotides to an otherwise complete polymerase reaction. When a nucleotide is added to the nucleic acid (NA) chain by polymerase, an enzyme cascade results in the production of light, which is detected and combined with knowledge of the nucleotide added to produce the sequence.

Biotage PyroMark MD

Biotage's PyroMark MD System is designed for rapid, short-length sequencing of PCR products and is intended for the laboratory analysis of methylation and single nucleotide polymorphisms (SNPs). The instrument is based on a 96-well plate format and contains a robotic arm to enable the sequencing of ten plates without user intervention.

Prior to NA sequencing, preparation, PCR and PCR clean-up are necessary; however, the assay itself is fast; able to sequence 96 samples in (a claimed) 10 min. Multiplexing is possible to a limited extent, but since pyrosequencing is currently a single-channel process, this is more complex than multiplex real-time PCR assays [11,12].

454 Life Sciences

454 Life Sciences uses a massively parallel pyrosequencing method that enables the sequencing of an entire genome from a single sample. The process involves the selection then PCR of single 200–400 base sections of genomic DNA in microreactors formed in an emulsion of oil. The products, which are immobilized on the surface of beads, are packed, one to a 44-µm well, on a 1.6 million-well PicoTiterPlate™ of which approximately 35% are in use during a typical assay [13]. The most recent systems are claimed to achieve average sequence lengths of 200–300 bp [202].

The reagents required for pyrosequencing are passed over the surface of the PicoTiterPlate and the resulting light is captured by an integral charged coupled device (CCD) camera. The sequence of the original sample is reconstructed bioinformatically [13].

Relevance to point-of-care market

Current pyrosequencing systems are not suitable for point-of-care use as they tend to be large and require expert operators. Sequencing does have advantages for point-of-care use; the ability to genotype almost all types of mutations other than long runs of a single base and the related fact that sequencing is regarded as the gold standard method of genetic analysis may aid clinical uptake. It should be possible to miniaturize pyrosequencing instruments using microfluidics and several groups are working in this area. However, to date, only a single publication has described such a system [14].

Cepheid GeneXpert®

The Cepheid GeneXpert® is a fully integrated DNA isolation and real-time PCR system that is able to genotype blood samples in 30–40 min, without user intervention other than sample insertion. The system uses a self-contained cartridge that is claimed to be able to isolate DNA from any type of human liquid sample or swab, including blood and emulsified tissue, in approximately 5 min [15].

DNA preparation inside the cartridge consists of an initial filtration in order to concentrate cells, which are then mechanically disrupted using ultrasonic agitation of glass beads. The DNA is purified by binding it to a solid phase then eluting it after washing; purified DNA is combined with lyophilized reagents and subjected to PCR.

PCR thermocycling and real-time detection of products is performed in a reaction chamber with a high surface area to volume ratio, which protrudes into the air-mediated heating and optical module. The four-channel optical detection system uses light-emitting diodes (LEDs) and photodiodes for excitation and detection, all of which have appropriate filters. Thermocycling and detection takes approximately 30 min. Results are automatically called using a custom software system [15,203]. The company currently has 28 US patents, the majority of which are related to aspects of the GeneXpert cartridge.

Analytes available

Cepheid currently has a BCR-ABL kit available for the GeneXpert, which is labeled for research use only. The company recently gained FDA 510(k) approval for their Group B *Streptococcus* assay, and is expecting similar approval for their MRSA and enterovirus assays soon [204].

Relevance to point-of-care market

The GeneXpert system is currently being targeted at the microbial identification market rather than that of genetic testing. The instrument is very fast (~30min) and is probably the only sample-in-answer-out system currently available for purchase. The instrument is not capable of high levels of multiplexing and the only way of increasing the number of genotypes called is to run more assays. This limits the ability of the instrument to type more than two genotypes per assay as only four channels are available and one channel each is likely to be required for positive and negative controls.

CombiMatrix Corp.

CustomArray™

The core CombiMatrix Corp. technology is an oligonucleotide microarray platform called CustomArray™; the latest version of this is the CustomArray 12K which has 12,544 44 µm electrodes, each of which is individually addressable. CustomArrays can be designed by the user and are then manufactured using electronically controlled phosphoramidite chemistry on the dedicated CustomArray Synthesizer. Electrical detection of hybridization is possible using the ElectraSense™ system.

Integrated microfluidic array

The company has released several papers that describe a "fully integrated microfluidic array device" [16–18]. This device has no on-chip DNA preparation or PCR capabilities. However, hybridization, washing with buffers of several stringencies and labeling are performed by the self contained system prior to fluorescent detection on a microarray scanner. The on-board microfluidics of the device consist of five electrochemical pumps, five chambers for buffer storage, six check valves and waste and hybridization chambers. The device has been used for resequencing and gene expression assays, with both types of assay claimed to take approximately 2 h (excluding NA preparation and PCR) [16].

ElectraSense™

The ElectraSense microarray reader is an electrochemical microarray detection system. It is powered by an external electricity supply, weighs 4.5 kg (excluding laptop) and can electrochemically read a 12K CustomArray in 25–45 s.

The instrument detects biotin-labeled target DNA using a streptavidin–horseradish peroxidase labeling enzyme conjugate which is used to oxidize a tetramethylbenzidine $(TMB)_{red}$ substrate to TMB_{ox}. The subsequent reduction of the TMB_{ox} back to TMB_{red} at the electrode surface is measured electrically [19]. According to an ElectraSense manual, the total assay requires 5 h after RNA preparation, with the fluidic manipulations involved in hybridization and labeling being performed manually [205].

Relevance to point-of-care market

CombiMatrix Corp. are currently targeting the clinical diagnostics market, specifically the market associated with infectious diseases, rather than the point-of-care pharmacogenetic market. Their microarray system has the advantage of an easy means of custom array manufacture with minimal user intervention. The ElectraSense detection system is compact and fast. With the addition of NA preparation and amplification systems, the system could be adapted for point-of-care use.

Directif Diagnostic Solutions

Directif Diagnostic Solutions was spun out from the German company November AG in 2002; their Lab on a Chip instrument, which is still in development, is a fully integrated, desktop system that is claimed to be able to prepare an RNA or DNA sample automatically, perform PCR, then hybridize and electrically detect up to 99 targets without user intervention in approximately 2 h. The system is intended for medical diagnostics, environmental analysis and food testing [206].

The system consists of the analyzer and an injection-molded disposable microfluidic cartridge, inside which all reagents are stored and all processing takes place. In order to initiate a test, a sample is injected into the cartridge, which is then placed into the analyzer. NA preparation, PCR and detection on a 114-position array occurs automatically. The cartridge is produced by a subcontractor, Wilden AG, and is claimed to be production-ready. The precise means of operation has not been described, but some clues can be gained from the literature.

Staff from November AG have described the development of a genetic testing assay based on a conductive plastic electrode made of carbon fibers embedded in a polycarbonate matrix [101]. The electrodes were used to enrich DNA by hybridization to a solid-phase bound complementary NA; the rate of hybridization is increased by the application of an electrical field. In the abstract to Hassmann *et al.*'s paper, they stated that "This combination of experimental methods is the basis for a molecular diagnosis system, including a disposable NA-modified working electrode for specific enrichment, detection and quantification, and an optional capillary PCR module for fast amplification" [20].

Another of the company's patent applications describes a system capable of the automatic PCR and subsequent electrochemical detection of NA sequences. A disposable cartridge was used that docked to a Peltier unit in a base unit to enable the thermocycling of an injected sample. Electrochemical detection utilized four working electrodes, four counters and one reference electrode (working electrodes that had been coated with NA probe molecules and were lowered in to the sample after PCR). Detection of NA was via a potentiometric stripping analysis [102].

Another of Directif's patent applications describes a means of detecting DNA using conductivity measurements across two electrodes, between which a single-stranded DNA is stretched [103].

Relevance to point-of-care market

Directif is developing the most advanced point-of-care genotyping system of which we are aware. No other system has the ability to analyze a large number of polymorphisms rapidly and

autonomously from a blood sample. Directif press releases claim the company has an essentially working instrument, although no release date has yet been set.

GeneOhm ePlex

The GeneOhm ePlex is an electronic DNA-detecting instrument that utilizes a patented molecular biology system prior to label-free electrochemical detection of 20 multiplexed NA species in each well of a 96-well plate.

Details of the molecular biology method used for the ePlex have been described [21,104]. Genomic DNA sequences of interest are amplified by multiplex PCR and are then hybridized to a probe, which bridges the ends of the product when they are perfectly complementary. The resulting construct is ligated to form a circular DNA molecule, which is used to generate an RNA transcript using rolling circle amplification (RCA). A crucial advantage of the RCA method is that the RNA transcript will contain multiple RNA copies of the original PCR product.

Specific technical details of the detection system are not known, but a possible detection principle can be gathered from a patent application submitted by the company [105]. The patent describes a system of detection based on ruthenium complexes; these are electrochemical labels that electrostatically associate with the negatively charged sugar-phosphate backbone of a NA. When a NA binds to an electrode-immobilized probe, the cloud of ruthenium complexes can be detected using electrochemistry. In order to maximize the signal achieved on binding, the application suggests that synthetic, uncharged, DNA analogue probes be used to prevent the ruthenium label from associating with the probe molecules. The RCA method described previously effectively complements this form of labeling, because increasing the amount of NA hybridized to the probe would also increase the amount of ruthenium, and therefore signal.

The ePlex system was evaluated and described by Xu *et al.,* who found the system to demonstrate very high concordance with results from Roche's LightCycler® and Nanogen Inc.'s NanoChip® assays [21]. Genotypes took 8–10 h to be determined; approximately 3 h of this was hands-on time. The authors considered the instrument to be reliable and convenient and concluded that, while the turnaround time was longer than that observed for the LightCycler real-time PCR instrument, the hands-on time was shorter as a result of the multiplexing available on the ePlex platform.

The system was intended to be released during 2005 together with an ASR for six thrombophilia polymorphisms; it has yet to make it to market [21]. The company was taken over by Becton Dickinson in February 2006.

Relevance to point-of-care market

Relatively little information about the system currently exists. However, the system not suitable for point-of-care use, owing to the lack of DNA preparation ability and the fact that the assay appears to be relatively slow. The detection system does have potential for point-of-care use as it appears to be easily scalable, compact and cheap to produce.

Idaho Technology Inc.

LightScanner®

The LightScanner® instrument produced by Idaho Technology Inc. measures the melting of a DNA duplex at high resolution using a fluorescent DNA-binding dye. The system is intended for mutation scanning and is able to scan a 96- or 384-well plate of PCR products for heterozygotes in a matter of minutes. The instruments are intended to be used with the proprietary fluorophore LCGreen. LCGreen can be used at a high concentration in a PCR reaction without inhibiting it, allowing the use of concentrations that saturate the DNA strand; this increases the resolution of melting analysis [22,23].

Some work has been performed on the use of high-resolution melting for high-throughput genotyping of SNPs. In this assay, an unlabeled probe that spans the SNP of interest is added to an asymmetric PCR; the probe must be 3′-blocked to prevent extension. After PCR, the genotype can be determined by a melting analysis. The technique is a closed-tube method for genomic analysis that avoids the use of a labeled probe [24].

Relevance to point-of-care market

The LightScanner is not suitable for use in a point-of-care setting as it does not have the ability to prepare DNA and cannot perform PCR. The melting assay itself, however, could have advantages over real-time PCR for genotyping.

Portable pathogen-detection systems

Idaho Technologies produce two systems that are designed for field use in areas such as military hospitals or by first responders. The systems are not available for export from the USA without prior permission as they are controlled under the International Traffic in Arms Regulations.

R.A.P.I.D.® (ruggedized advanced pathogen identification device) is a toughened, portable real-time PCR instrument. The instrument weighs 23 kg, including laptop and centrifuge, and has a 32-sample capacity with three-color optics, but no automated DNA preparation abilities. Assays are designed and tested in the laboratory then may be used by non-specialist field personnel in push-button mode in which the instrument reports yes-or-no results [207]. A comparison of the system to the Roche LightCycler and Cepheid SmartCycler® yielded comparable results for all three systems [25].

RAZOR® is a handheld (4.2 kg), battery-powered, real-time thermocycler with single channel optical detection, which is intended for the field-based typing of biological warfare agents. The external, manual sample preparation is relatively fast (<5 min) and does not require a centrifuge or pipette. PCR is performed in a plastic bag, the reaction is moved physically between temperature zones during thermocycling. The entire process is claimed to take 30 min, including DNA preparation. The instrument utilizes a push-button interface that gives yes-or-no answers. The freeze-dried reagents are stable at room temperature for 6 months [207].

Relevance to point-of-care market

R.A.P.I.D. is simply a toughened real-time PCR machine that offers little advantage over a standard real-time PCR instrument for routine point-of-care use. RAZOR is interesting in that it is a very fast system. However, the instrument has a single-channel optical system that is not ideally suited for SNP detection and, in addition, the system requires manual sample preparation.

Integrated Nano-Technologies

Integrated Nano-Technologies are developing the BioDetect analyzer, a self-contained system for DNA preparation and analysis that does not require PCR. The system is reportedly available for military pathogen detection use, but the company is also targeting the pharmacogenetic market [208].

The company's core technology, exclusively licensed from research performed at the Israel Institute of Technology (Haifa, Israel), is the ability to metallize strands of DNA. This metallization process is used to reduce the resistance of a DNA bridge formed over a gap between two interdigitated electrodes. Recently published proof-of-concept measurements detected, without PCR, a 5.6-kb plasmid insert over a 1-µm electrode gap by means of two 27-mer capture oligonucleotides. Hybridization of as little as 200-ng of DNA was reported to take 20 min and was conducted in a volume of 60 µl; the time required for metallization was not stated. The reported system, an array of 200 pairs of electrodes, was operated manually on the bench rather than as an automated system [106,209].

The process, which is intended to be followed in a final automated device, is described on the company website as follows: a solid or liquid sample is premixed with a buffer prior to injection to the device, cell lysis occurs in the device by chemical or ultrasonic means. The lysate is filtered to remove everything except DNA, which is sheared and incubated on the detector array for hybridization. After hybridization and washing, the remaining DNA is metallized and measured electrically [208]. It may be intended to separate DNA electrically, using a method described in a patent application; a pair of electrodes between which there is a DC bias can be used to concentrate DNA in a moving fluid [107].

The company has some technology related to the bottom-up manufacturing of nanoscale electrodes based on the immobilization and subsequent metallization of immobilized DNA. These arrays could be used to create nanoscale electrode arrays that would be able to detect DNA fragments shorter than the 5.6-kb fragment previously described [108,209]. A miniaturization of the electrode arrays could have beneficial effects on device sensitivity because the chance of a bridging DNA molecule forming over a smaller electrode gap is increased.

Relevance to point-of-care market

If the system can be adapted to detect SNPs without a compromise, such as the need for PCR, Integrated Nano-Technologies have a powerful and cost-effective detection technology. It is not clear how close the instrument is to market.

Ionian Technologies Inc./HISSS

Ionian Technologies Inc. have developed an isothermal amplification method, illustrated schematically in FIGURE 1, which utilizes oligonucleotides containing recognition sequences for a DNA nicking enzyme. The nicking enzyme generates a single-strand break in the DNA duplex. This creates one long fragment and one shorter fragment that is only approximately 12 bases long. This shorter segment melts from the DNA strand, thereby enabling DNA polymerase to extend the remaining longer primer segment. A subtly different arrangement of primers may be used for exponential amplification [26]. A patent application for the technique is under consideration [109].

Ionian Technologies and Northrop Grumman Corp. are collaborating to produce a handheld isothermal silver standard sensor (HISSS), which is a DNA detection instrument intended for the identification of biological warfare agents [210].

Relevance to point-of-care market

The isothermal amplification method could be an attractive means of amplification for handheld mutation-detecting devices if a robust method of SNP detection can be invented.

IQuum, Inc.

IQuum, Inc. is commercializing the handheld single sample Liat™ Analyzer, which is based on the company's lab-in-a-tube (Liat) technology. The instrument is claimed to have the capability to perform sample-in-answer-out genotyping in 30–60 min using a real-time PCR based assay.

The Liat technology is based on a flexible plastic tube that is segmented with peelable seals to contain reagents (FIGURE 2). Sample processing occurs by the use of a series of actuators and clamps that initiate sample movement, mixing and the breaking

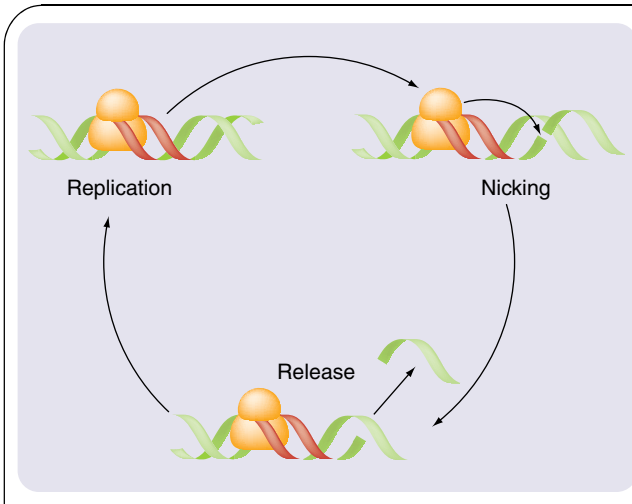

Figure 1. Ionian technologies amplification method. A DNA template is sequentially nicked then extended, thus enabling single-temperature amplification. This example illustrates the linear amplification method. An exponential method has also been demonstrated.
Adapted from an image kindly provided by Ionian Technologies Inc.

of the seals. Patient samples are collected directly into Liat tubes, which are then placed into the instrument. All subsequent preparation and PCR of the DNA is performed automatically. NAs are prepared using magnetic bead chemistry, thermocycling relies on the movement of samples between constant temperature zones, and the real-time PCR system has four channels for detection. Full descriptions of the instrument can be found in an article written by the IQuum Chief Executive Officer [211], and the granted patents, several of which cover the technologies [110,111].

A genetic test from a blood sample is reported to take 30 min and an infectious agent can be analyzed from plasma in 1 h. No data describing an evaluation of the systems have yet been published.

Relevance to point-of-care market

The IQuum system is very impressive and when it comes to market, the Liat Analyzer may represent the first, handheld, point-of-care instrument for genetic diagnostics. The sample processing system uses an elegant method to enable the use of proven molecular biology in an automatic application. The biggest shortcoming of the instrument is that only four channels are available for detection, limiting the instrument to the detection of two polymorphisms per assay.

Nanogen Inc.'s NanoChip® NC400

The Nanogen NC400 is a 400 test site microarray system, employing electronically guided nucleotide patterning, electronically assisted hybridization, automated labeling and two-channel fluorescent detection.

Biotin labeled NA can be addressed to one or more test sites of the microarray by the directed application of a positive bias to the site that is to be functionalized, whilst the other pads are maintained at a neutral potential [27, PERS. COMM. NANOGEN REPRESENTATIVE]. The NA binds to streptavidin, which is embedded in the permeation layer, a 1–10-µm gel layer that is coated on the sensor surface [27,112].

Active hybridization of DNA reduces the time required for hybridization to about 3 min [113]. Stringency control is achieved using standard methods through increasing the concentration of NA over one or more test sites [27]. Nanogen researchers have described methods that allow electronic control over stringency though these methods are not employed on the NC400 [28,29].

Assay design

Any biotin-labeled NA samples can be immobilized on the chip. In most of the literature, Nanogen describe the immobilization of patient-derived PCR products that are then interrogated using fluorescently labeled base-stacking probes, for which Nanogen holds the patent [114]. This amplicon-down assay is very economical as, typically, only a single pad is used per patient. The pad can be stripped and re-probed multiple times to determine multiple genotypes [27]. Nanogen also describes the capture-down sandwich assay, in which capture probes are immobilized on the array. This type of assay is claimed to have benefits in terms of multiplexing, as capture probes for multiple amplicons may be immobilized on a single test site [30].

The assay time required is entirely dependent on the assay being performed. An entire assay of addressing and then probing 400 different samples takes over 14 h, the vast majority of which is accounted for by the addressing of NA to the array [27]. Since it is unlikely that all 400 test sites would be addressed simultaneously in a point-of-care setting, and thus, such a use would result in a much faster assay time, especially since hybridization itself is very fast (<3 min).

The use of re-probing protocols can maximize the amount of information gained for a given chip [27]. Genotypes can be automatically called using the ratio of wild-type and mutant fluorescence intensities [31].

Relevance to point-of-care market

The NC400 has the advantages of high-speed hybridization, is a much more flexible instrument than most other microarray systems and appears to be easily scalable, but does not curently

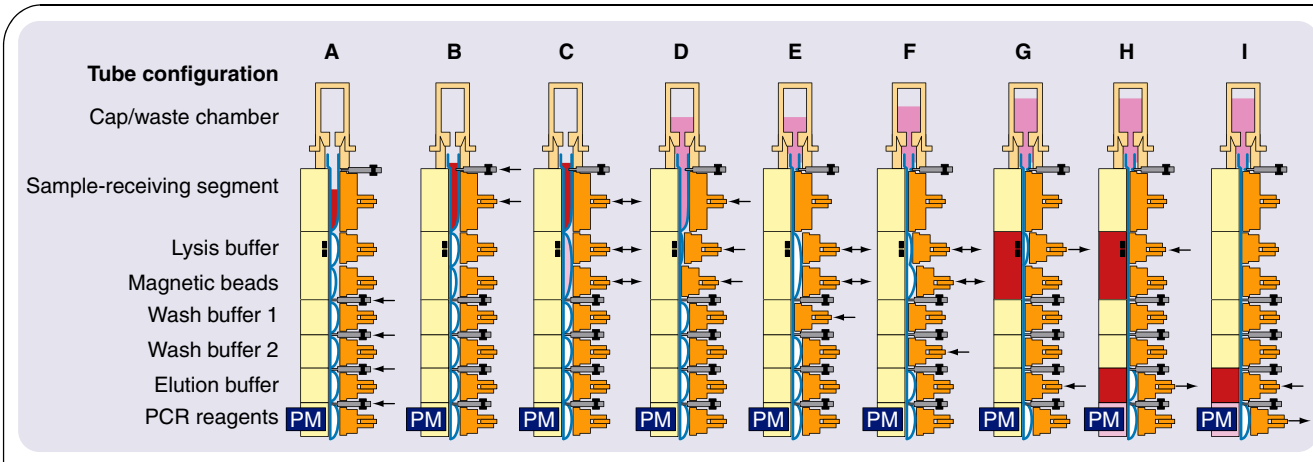

Figure 2. Means of operation of the Liat™ Analyzer. The Liat Tube is shown in light blue and contains a sample (red), reagents (white) and waste (pink). Actuators (orange) and clamps (gray) compress tube segments while blocks (yellow) are heated (red) to incubate the sample. Magnets, used for the separation of nucleic acids (black squares) and fluorescence detection optics are also shown (blue). Reproduced with permission from © IQuum, Inc. (2006).

have the ability to perform DNA preparation or PCR. During review, a Nanogen representative stated that the NanoChip platform "is in no way intended for use at the point-of-care". Rather, the NC400 is intended "for the small-to-medium volume high-complexity diagnostics laboratory" [27]. However, given that the company has several point-of-care assays available for protein markers, that the company has invested in Pharmacogenetics Diagnostic Laboratory, LLC and the advantages of the NC400 the company is well placed for a move into the point-of-care genetics market [212].

Nanomix Inc.'s Sensation technology

Nanomix Inc. is commercializing sensors that exploit the semiconducting properties of single-walled carbon nanotubes (SWCN) [213]. The company is aiming the sensors at two markets: analysis of patient breath samples and the analysis of biological samples (particularly DNA, proteins, viruses and glucose).

A version of the DNA sensor has been described in a recent paper and patent application [32,115]. The sensor comprised a pair of interdigitated gold electrodes between which random arrays of SWCN are formed by chemical vapor deposition. DNA oligonucleotide probes are directly immobilized onto nanotubes; binding of a complementary strand to the probe changes the distribution of charge around the DNA strand, and thus also changes the charge and the effective doping characteristics of the semiconductor. The electronic characteristics of the nanotubes can be analyzed by monitoring their conductance in response to an applied gate voltage during DNA hybridization. The prototype sensor was used to differentiate the alleles of a model SNP system comprising short oligonucleotides [32].

Relevance to point-of-care market

This work has the potential to create inexpensive arrays of DNA sensors that could be interrogated with comparatively inexpensive handheld equipment. Commercialization is ongoing; no release date has been set for a DNA detection device at the time of writing.

Nanosphere, Inc.: Verigene® System

The Verigene® System is claimed to be able to assay both DNA and protein in approximately 100 min (without PCR) using the same instrumentation. After introduction of a sample and buffer, all processing occurs automatically in a disposable test cartridge.

DNA assay

For DNA analysis, a prepared sample of genomic DNA is ultrasonically fragmented to an average length of 300–500 bp. Fragmented DNA is hybridized with a capture oligonucleotide probe for the gene of interest, which is bound to the surface of a glass microarray slide. After washing to remove unbound genomic DNA, a secondary hybridization is carried out with an oligonucleotide–15-nm gold nanoparticle conjugate reporter. Silver is deposited on the gold nanoparticles to increase their ability to scatter light, thus amplifying their signal [33].

The two-stage hybridization method avoids the need for PCR. The initial hybridization will bind many unrelated sequences in addition to the desired ones, a result of the high complexity of the human genome. The wash after the initial hybridization vastly decreases the complexity of the sample, allowing the second hybridization with gold reporter conjugates to discriminate sequences of interest from unrelated sequences [33,34]. An alternative protocol, intended for high levels of multiplexing, uses a gold-labeled oligonucleotide with a universal sequence, rather than a gene-specific one. This is hybridized to the immobilized genomic DNA using a bridging mediator oligonucleotide [PERS. COMM. NANOSPHERE REPRESENTATIVE].

The time required for a typical analysis is claimed to be approximately 100 min from the input of a prepared DNA or protein sample [214]. A 2004 paper used a 2-h incubation without ultrasonication when assaying RNA, and a 2005 paper used a 60-min hybridization for the detection of SNPs from patient DNA [33,35].

In addition to the system described, the company has published details of other methods of detection. A nanoparticle probe-labeling system able to provide two-color detection capabilities was described in 2001, although a lower sensitivity than the silver-amplified array system was achieved [36]. Also in 2001, the company described a means of electrically detecting DNA by the selective deposition of silver onto DNA nanoparticle probes immobilized, by hybridization, in the gap between two electrodes [37].

Protein assay technology

Protein detection is accomplished on the same system using bio-barcodes. Bio-barcodes are short sequences of DNA that are hybridized to complementary strands linked to an antibody for the protein of interest. The protein is captured on a solid phase with a second antibody and is labeled with the reporter antibody carrying the bio-barcodes; these are subsequently released and are detected using the silver amplification procedure [38].

Instrumentation

The Verigene system uses glass slides as the array substrate; these can be printed using a standard microarray spotter. The system requires two instruments: the Verigene Processor and the Verigene Reader scanner. The Processor system automates the sonication, hybridization and labeling process. Details of a prototype scanner were published in 2003 [39]; the system described in the paper projects an image of the microscope slide onto a CCD camera in a single image using the slide itself as a waveguide with 75-W halogen lamp or red LED illumination [35].

Relevance to point-of-care market

The Verigene System appears to be impressively fast and the fact that it can assay protein and DNA biomarkers from the same sample is likely to be an attractive feature to the intended laboratory-based customers. The major disadvantages with a point-of-care system are the large size of the system (a Processor and Reader combination occupies about 1.2 meters of bench space) and the lack of integrated DNA preparation capability.

Osmetech eSensor®

Osmetech market the eSensor®, a microarray instrument capable of two-channel electrical detection of labeled DNA probes on a 36-position array. Osmetech purchased the company Clinical Micro Sensors, Inc., the inventors of the eSensor, from Motorola, Inc. in 2005, and launched the instrument in April 2006. The company recently gained 510(k) clearance, from the US FDA, for an eSensor-based cystic fibrosis carrier test [215].

eSensor® assay principle

Preparation of target DNA and PCR is performed off-chip prior to a sandwich-type assay. An initial 2-h hybridization immobilizes target DNA to solid-phase immobilized capture probes; the array then undergoes a second hybridization with ferrocene-labeled DNA-reporter probes. The ferrocene labels are detected using AC cyclic voltammetry only when brought in close proximity to the sensor by hybridization; this eliminates the need for a wash step to remove unbound label.

The ability to differentiate between bound and unbound probes is due to the three-part self-assembly monolayer (SAM) illustrated schematically in FIGURE 3. The SAM consists of three components, the target-specific DNA probes, molecular wires (thiol-terminated oligophenylethynyl molecules) that facilitate electron transfer between the ferrocene labels and the gold electrode, and insulator molecules (polyethylene glycol molecules immobilized with alkanethiols) that prevent the approach of unbound ferrocene molecules to the sensor surface [40].

The validation of a two-channel eSensor utilizing the twin-channel ferrocene labels has been published. The authors demonstrated concordance with results produced by sequencing and restrictive fragment length polymorphism using positive-control cystic fibrosis and hereditary hemochromatosis cell lines [41].

Currently, the eSensor requires external DNA preparation and PCR. However, when Motorola owned Clinical Micro Sensors, Inc., they published papers describing methods of decreasing eSensor hybridization time using a microfluidic mixing system and cavitation microstreaming [42,43]. In addition, the company published details of a fully integrated system that was capable of detecting *Escherichia coli* and SNPs from blood in approximately 2.7 h [44]. The system was machined on a polycarbonate substrate and included magnetic antibody cell separation of *E. coli* cells, thermal cell lysis, on-chip PCR and eSensor detection, as well as acoustic-assisted micromixing and hybridization. Osmetech claims to have acquired this technology from Motorola [PERS. COMM. OSMETECH REPRESENTATIVE].

The claimed advantages of the system are the ability to perform hybridization without PCR clean-up, an undetectable nonspecific signal level and a lack of signal decay. The lack of signal decay is especially useful as it enables kinetic measurements and also allows arrays to be scanned at multiple hybridization temperatures.

A second-generation version of the system, the eSensor XT-8, is expected to be released during 2007 in the USA and during 2008 for the rest of the world. Improvements include an increase in the density of the array, smaller hardware and decrease in hybridization time to 20 min. The company is currently planning an FDA submission for the new system and a CYP450 assay for warfarin [216].

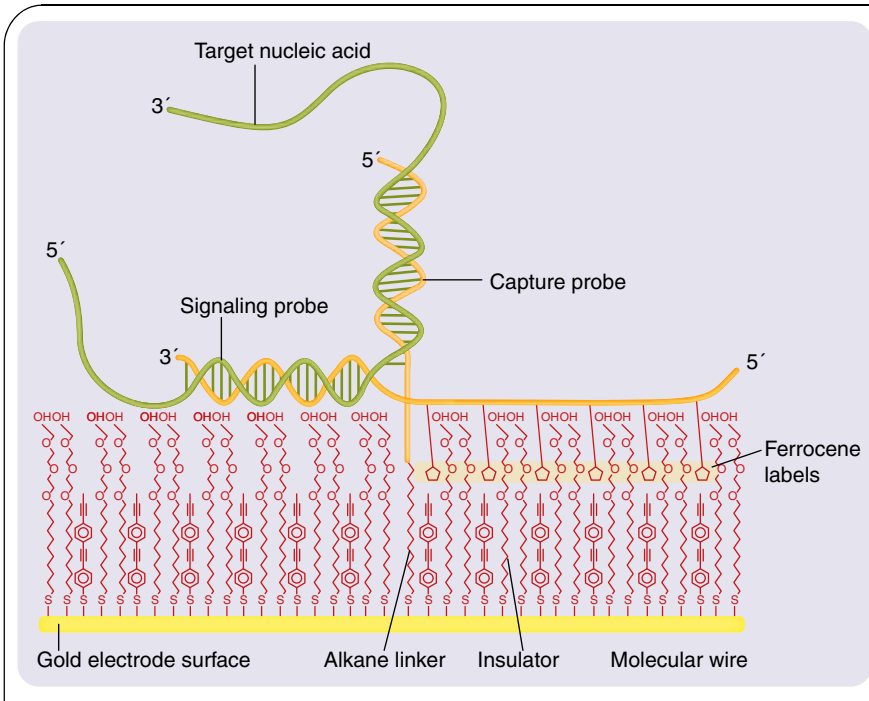

Figure 3. Self-assembly monolayer arrangement used on the eSensor®.
Reproduced with permission from [44] © American Society for Investigative Pathology and the Association for Molecular Pathology.

Relevance to point-of-care market

The eSensor system is currently only available in the USA. It employs an assay procedure that is easily scalable and should be cost-effective to produce. However, the instrument is currently limited to laboratory use as there is no integration of DNA preparation or amplification. It is possible that Osmetech is developing a point-of-care instrument based on technology acquired from Motorola.

The company is well placed to enter the point-of-care market, given their previous experience in selling point-of-care biochemistry instruments, the eSensor platform and the warfarin test currently being developed.

Systems not covered

It is clearly not possible to cover, in a single review, every instrument that may be capable of the point-of-care detection of genetic polymorphisms. Two very interesting systems, by HandyLab, Inc. [217] and Mobidiag [218], had escaped the notice

of the authors until shortly before publication and hence have not been reviewed, but are mentioned here for completeness. Both systems employ microfluidics for preparation and are claimed to analyze clinical samples in 1 and 2.5 h, respectively. The interested reader is directed to the companies' websites for further information [217,218].

Expert commentary

No system currently available for purchase is ideally suited for genetic determination in a point-of-care setting. The Cepheid GeneXpert is the only system available that can perform automated genetic tests, but it is let down only by its inability to perform a maximum of two genotypes per assay. The IQuum Liat analyzer appears to have similar capabilities as the GeneXpert and when it is released will probably be the first handheld genetic analyzer. The Directif lab-on-a-chip system is the first second-generation system to be announced, and is expected be able to perform at least 50 genotypes on a 114-position array.

Unfortunately, very little information exists regarding the most important aspect of these technologies: the accuracy and reproducibility of the genotypes they provide. Therefore, it has not been possible to truly compare the performance of the systems. The systems discussed in this review are listed in the APPENDIX.

Five-year view

In the next 5 years, we expect to see the pharmacogenetic diagnostic market gaining momentum due to greater numbers of polymorphisms with sufficient clinical information available to justify their use. More instruments will be approved by the regulatory authorities for genotyping these polymorphisms and it is possible that instruments capable of operation at the point-of-care will be approved. However, further debate is required in order to clarify precisely, which areas of medical practice personalized genetic testing is appropriate for. The EU and possibly the FDA are likely to issue further regulations in the area of pharmacogenetics which may result in changes to the standards expected of multiplex tests and point-of-care devices.

We would be surprised if genetic testing at the point of care actually becomes commonplace in the next 5 years as the regulatory and quality assurance procedures that must be put in place will probably take longer than this to become fully established. However, it is likely that point-of-care genetic testing will become a reality in this timeframe in a limited number of clinics.

The current, first-generation, systems for molecular diagnostics are based on detection using quantitative-fluorescent PCR; this is proven technology that has been miniaturized, frequently by the use of semiconductor-based illumination and detection. The major limitation of the PCR approach is the number of channels that can be simultaneously used; typically four. This limits the number of genotypes that can be determined from a single assay to two. Over the next few years, we expect more PCR-based systems to be released. Ultimately, however, such systems may be more suited to pathogen detection than pharmacogenetics.

The second generation of molecular diagnostic instruments will be based on microarrays. Many different methods of microarray detection have now been demonstrated, from traditional optical systems to those based on magnetic and electrical detection. All these systems share the advantage of being spatially multiplexed and are therefore practically unlimited in the number of genotypes that can be read from a single sample as one can simply enlarge the available area.

The authors expect to see more encouraging research and proof-of-concept demonstrations of methods for directly reading individual pieces of DNA within the next 5 years. Prototypes are likely to significantly longer.

The Archon X-PRIZE for Genomics has recently been announced. This is a US$10 million prize for the first private company able to sequence 100 diploid human genomes within 10 days for US$1 million [219]. The X-PRIZE is not expected to be won by current technologies; we would be surprised if it is won within the next 5 years. The prize may drive a move to sequencing-based technologies for pharmacogenetics in the coming decade.

Disclaimer

This is not an exhaustive list of the technologies available for genetic analysis. All the information in this review is provided in good faith. However, sections, most notably 'Relevance to point-of-care market' sections, are based on the opinions of the authors; while great efforts have been made to double-check information, some errors and areas of contention may remain. The companies reviewed were invited to comment on a draft of this document. The responses of those that replied were incorporated in the final draft where appropriate; their assistance is very much appreciated.

While this article assesses the technical suitability for point-of-care application for each technology reviewed, the authors are in no way suggesting that any of the manufacturers intend their devices to be used in the point-of-care market or for clinical diagnostics unless this is explicitly stated.

Disclosure

This review was undertaken as part of the EU funded 6th Framework project SNiP2CHIP [220]. The aim of this consortium project is to develop novel technologies for genetic diagnostics.

Key issues

- No currently available system is ideally suited for the point-of-care detection of genetic mutation.
- Instruments that use detection methods based on fluorescent PCR-based techniques have recently been released. These are able to genotype a limited number of polymorphisms per assay (typically two), and can be considered to be the first generation of point-of-care mutation detection instruments.
- Second-generation systems will be able to genotype more than 30 polymorphisms in a single assay; these will be based on array technologies, and these can be expected to be released within the next 5 years.

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Appendix. Summary of systems.

System	Integrated DNA preparation	Integrated PCR	No. of channels	No. of positions on array	Assay time	Assay format	Detection method	Format
PyroMark MD System	No	No	1	NA	55 min*†§	Sequencer	Pyrosequencing	Desktop
454 Genome Sequencer	No	Yes	1	NA	4 h†	Sequencer	Pyrosequencing	Desktop
Cepheid GeneXpert®	Yes	Yes	4	NA	30–40 min	QF-PCR	Fluorescence	Desktop
CombiMatrix Electrasense™	No	No	1	12544	5 h†	Microarray	Electrical	Mid-sized
Directif lab-on-a-chip	Yes	Yes	Unknown	99	2 h	Microarray	Electrical	Desktop
GeneOhm ePlex	No	Unknown	20	20 x 96 *	Unknown	Microarray	Electrical	Desktop
Idaho Technology Lightscanner®	No	No	1	96	20 min*†	Melting	Fluorescence	Desktop
Idaho Technology RAZOR®	No	Yes	1	NA	30 min	QF-PCR	Fluorescence	Handheld
Integrated Nano-Technologies	Yes	Not required	1	Unknown	20 min	Microarray	Electrical	Unknown
Ionian Technologies Inc./HISSS	Unknown	Not required	Unknown	Unknown	Unknown	Unknown	Unknown	Handheld
IQuum Liat Analyzer	Yes	Yes	4	NA	30 min	QF-PCR	Fluorescence	Handheld
Nanogen NC400	No	No	2	400	Variable (see text)*†	Microarray	Fluorescence	Desktop
Nanomix Sensation technology	No	No	1	Unknown	Unknown	Microarray	Electrical	Unknown
Nanosphere Verigene® system	No	Not required	1	Unknown	<35 min†	Microarray	Densitometry	Desktop
Osmetech eSensor®	No	No	>1	36	20 min*†	Microarray	Electrical	Desktop

*Not including PCR; †Not including DNA/RNA preparation; §Sample clean-up accounts for 45 min of this, pyrosequencing takes approximately 10 min.
NA: Not applicable; QF: Quantitative fluorescent.