Microfabricated Biosensors and Microanalytical Systems for Blood Analysis

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Introduction

Today, blood testing is predominantly performed in a clinical laboratory. There are about 740 tests currently offered by a typical large clinical laboratory.[1] The vast majority of these analyses are performed on blood. This analytical service is offered by the disciplines of clinical chemistry and hematology, subfields of laboratory medicine. This service is supported by the in vitro diagnostics industry. The chemical analysis of blood has a $50 billion cost worldwide: $17 billion is the market for the manufacturers of in vitro diagnostic equipment and supplies,[2] and $33 billion is the estimated total cost of medical technology/clinical chemistry staff and other associated service costs.

Of the 740 tests currently offered by laboratory medicine, less than 30 tests (*blood gases*, pH, $P(CO_2)$, $P(O_2)$; *electrolytes*, Na, K, Ca, Cl, Mg; *metabolites*, glucose, urea, creatinine, bilirubin, lactate; *enzymes,* alanine transaminase, amylase; *coagulation indices*, prothrombin time, activated partial thromboplastin time, activated clot time; *hematology*, white blood cell count, hematocrit (packed cell volume), hemoglobin, platelets; *immunochemistry*, cardiac markers) are used by physicians to *monitor* a patient during the course of a disease, during the course of a treatment, or during a procedure. The remaining 700 or so tests (and many of the same 30 monitoring tests) are used as *diagnostic* tests; that is, they are used by physicians to assist in the diagnosis of symptomatic patients or as a component of a preventative program when performed during a routine checkup or as part of a mass screening.

The time value of monitoring versus diagnostic testing can be quite different. For the large majority of diagnostic tests the value of the result is little different if the result is provided instantaneously versus hours or even days later. Obvious exceptions are those tests that diagnose a condi-

tion where speedy medical intervention is essential such as cardiac markers for myocardial infarct. For monitoring tests the value of a result rapidly diminishes with time. For certain tests such as those for blood gases, where the physiological chemical concentration rate of change can be fast, close to real time results are often mandatory. Many monitoring tests are preferred rapidly for improved medical treatment. Some monitoring tests are operationally valuable when delivered in a timely manner because the result can be more effectively integrated into other patient data for the clinician to act upon.

A diagnostic test or battery of tests is typically performed only once per patient per medical encounter. A monitoring test is performed on a patient on many occasions per medical encounter. Because tests are run frequently on a given patient when being monitored, it is not surprising that the 30 out of 740 or 4% of the tests represent greater than 70% of the test volume of the laboratory.[1]

By and large laboratory medicine is organized to deliver blood testing services in essentially the same operational modality regardless of whether tests are used in a diagnostic or monitoring application; that is, both monitoring and diagnostic tests are typically provided by instruments located in full service clinical laboratories located centrally within the healthcare facility (with the exception of blood gases that are delivered from near-patient laboratories in many institutions). Further, it has been a tightly held truth within the laboratory medicine community that chemical analysis of blood properly *only* belongs in a laboratory environment for reasons of quality. A view also has developed that centralization of testing through the ability to batch test specimens *necessarily* also leads to a lower cost of service per specimen than noncentralized testing services. In summary there has been a significant reticence to move testing close to the patient in response to the pressure for faster service because there has been a strong belief by the laboratory community that poorer quality and higher cost will result.

A laboratory which provides monitoring and diagnostic testing services with the same operational structure has to compromise on turnaround time, a key performance tradeoff when considering the very different time value of monitoring versus diagnostic tests. A laboratory organized for cost efficiency through batch handling provides poor turnaround time for monitoring tests. A laboratory organized for fast service (called a stat laboratory) is less cost-effective. The best turnaround times are delivered from highly automated laboratories equipped with analyzers designed for fast turnaround time and connected to the end-user departments by pneumatic tubes for rapid blood transportation. Two hour turnaround times for fast turnaround (stat) requisitions and 20–30 min for very fast turnaround (super stat pH, $P(O_2)$, $P(CO_2)$, K, hemoglobin) can be consistently obtained.

The process structure and the associated total process cost of laboratory blood testing services are typical of a

hospital's processes in general. Numerous people are engaged in the process flow, with considerable distances between required people and equipment, complex communication, and coordination, and because of numerous people and process steps, there are numerous potential quality failure points. The total process to deliver a blood test result includes both the processes occurring in the patient-care department, test requisition (nurse), sample acquisition (nurse or phlebotomist), labeling (nurse or phlebotomist), transportation (transporter), and receipt of results (nurse), and those in the laboratory, sample receipt and aliquotting (clerk) and centrifuging, analysis, and results reporting (medical technologist). Total process costs are those associated with analytical equipment and testing supplies, sample acquisition and transport supplies, as well as all the labor costs incurred across all departments associated with the total process. Costs vary widely from institution to institution because of different process flows, different degrees of automation, and differing labor rates.

Restructuring to patient-side processes for blood testing is typical of other so-called patient-focused initiatives which seek to provide better care at lower cost by simplification of the processes of care or service delivery. Cost savings come from a reduction of all of the labor costs applied to the multistep laboratory process versus the one-step patient-side alternative. Specific to blood testing at the patient side, the principle is that there is no more work done by the nurse to extract a blood sample into a receptacle than for the nurse to perform the multiple tasks of the patient-care department's piece of the laboratory process *so long as the patient-side device is designed appropriately*. Also there is significant increased value when results can be obtained in a couple of minutes at the patient-side without the care-giver ever having to leave the patient, as compared with the significantly longer turnaround times obtained with even the most automated laboratories connected with pneumatic tube sample transport systems, or some of the newer semiautomated near-patient workstations.

The remainder of this Account shows how technology can provide the appropriate design solutions for decentralization of laboratory medicine. I will describe the design vision and technology development approach that i-STAT has taken to realize general-purpose tools for blood analysis that allow testing to be performed reliably and accurately at the patient-side *and*, therefore, importantly lead to an overall significant reduction in the cost of the testing process.

Analytical Instrumentation for Patient-Side Blood Testing

Tests for which there is a high time value will be performed at the patient-side. Assuming availability of technology, such testing devices will be optimally patient-connected (in vivo, ex vivo in line with automated sampling, noninvasive or minimally invasive) when (1) continuous, real time measurements are required or

numerous tests are needed at a frequency of testing per patient higher than once per about 10 min and (2) the access to the patient sample is difficult or time-consuming. In all other patient-side testing situations, which is the vast majority of cases, a discrete sample and test technology is optimal.

i-STAT's vision is of a general-purpose blood testing tool which can be used at every patient-side location in an acute care setting in a hospital or by a healthcare professional at the patient-side in any alternate site setting. Our design challenge was to realize this general-purpose tool in such a way that its operation is simple and robust enough to deliver laboratory accuracy and reliability in patient-side locations which are far less well controlled than the laboratory. The system design uses a discrete sample receptacle containing one or multiple microfabricated electrochemical electrodes on a chip (biosensors), and a low-cost general-purpose electromechanical read-out device. The design calls for only a single use for the biosensors, which is the same design principle employed in unit-use paper strips for glucose monitoring of diabetics as well as unitized reagents or slides used in some "mainframe" central laboratory, high-volume, random access analyzers. There is considerable simplification of the analytical system and freedom from complex maintenance, calibration, and quality control procedures when a unit-use design approach is employed. Also, a much broader range of clinical chemistry test methods is accessible with a unit-use format compared to a reusable biosensor approach; for blood gases, electrolytes, and some substrates different manufacturers have used both unit-use and reusable formats. The vast majority of all other test methods are configured as unit-use because either they employ dry reagents which are used in the test and need replenishment each test time or biosensors and the specific biochemical reactions that form the basis of the test method are irreversible and components or materials need to be replenished each test time (immunoassay, DNA probe, coagulation, enzyme assay). A further design requirement was to deliver laboratory grade performance with the unit-use approach. Thus, we designed to obtain a $1-3\%$ coefficient of variation specification rather than the $5-8\%$ coefficient of variation that is typically obtained with paper strip technologies. To achieve this higher level of performance, and to obtain robustness in operation, we designed to automate all of the manual steps of the analytical process typical of paper strip technologies. In our system we achieve this automation by design of a microanalytical system around the biosensors.

Our design vision also anticipated, for maximal user convenience and utility, that the entire useful set of blood tests (the 30 monitoring tests), arranged in groupings according to medical need, will be delivered by a single tool. That is, a single device design will be required to accomplish blood gas analysis, electrolyte analysis, enzyme substrate analysis, enzyme activity assay, clot detection and coagulation assays, cell counting and analysis, and immunoassay.

The final important design requirement was that our biosensor array and its microanalytical system be manufactured at volume at a cost per analyte equivalent to that of paper strip technology.

Microanalytical Systems

To realize the low product cost specification, we started with the simplest structure: a microfabricated biosensor array contained in a plastic housing, the plastic housing serving also as the sample receptacle. The housing is a sandwich formed by sealing the biosensor chip between a lower and upper plastic component. A lower and upper plastic component and sealing means are also the minimum necessary components of a chip package used to house an electronic semiconductor device. We have designed and engineered into this de-minima package all of the necessary fluidics (chambers, pumps, valves, conduits, mixers, separators, solid reagents, liquid reagents, gaseous reagents, calibrators, washes, ...) so that the packaged biosensor chip now can function as a total microanalytical system. Therefore, the microanalytical system was achieved at essentially the same cost as just the chip contained in a sample receptacle configured as a de-minima package. This design is described below in more detail.

An exploded cross-sectional view of an i-STAT blood gas and electrolyte cartridge is shown in Figure 1. A top view of the cartridge is shown in Figure 2, including a fluidic circuit diagram of the microanalytical system's components as overlay. This design and variations on its theme are described in ref 3. Figure 1 shows that there is a sealed foil pack containing aqueous fluid and an elastomer gasket in addition to the labeled de-minima package of upper and lower plastic components plus sealant. Chambers and fluid conduits are molded into the lower ABS base and the upper polycarbonate cover. In the assembly process fluidic features are precision aligned to sensor structures, and this subassembly is then aligned with openings in a precision laser machined tape gasket at tolerances of about 1 mil, and the overall structure is pressure laminated to seal the chips. The tape gasket also serves to form the diaphragm of two diaphragm pumping mechanisms designed into the cartridge. In operation, blood is collected into the sample-entry well by the user. The cartridge is closed by flipping the hinged sample-entry gasket over the sample-entry well. The cartridge is inserted into the read-out device, and the remainder of the analytical procedure is conducted under automatic instrument control.

To understand the analytical process in detail, refer to the fluidic circuit schematic of Figure 2.

(1) When blood is introduced into the sample-entry well, it is drawn into the cartridge by capillary pumping action. A capillary break serves as a pressure sensitive valve and halts the blood movement at the fill-to line.

(2) The hinged sample-entry well closure with its gasket acts as a three-way valve, at once closing the sample-entry well conduit and connecting the sample diaphragm pump
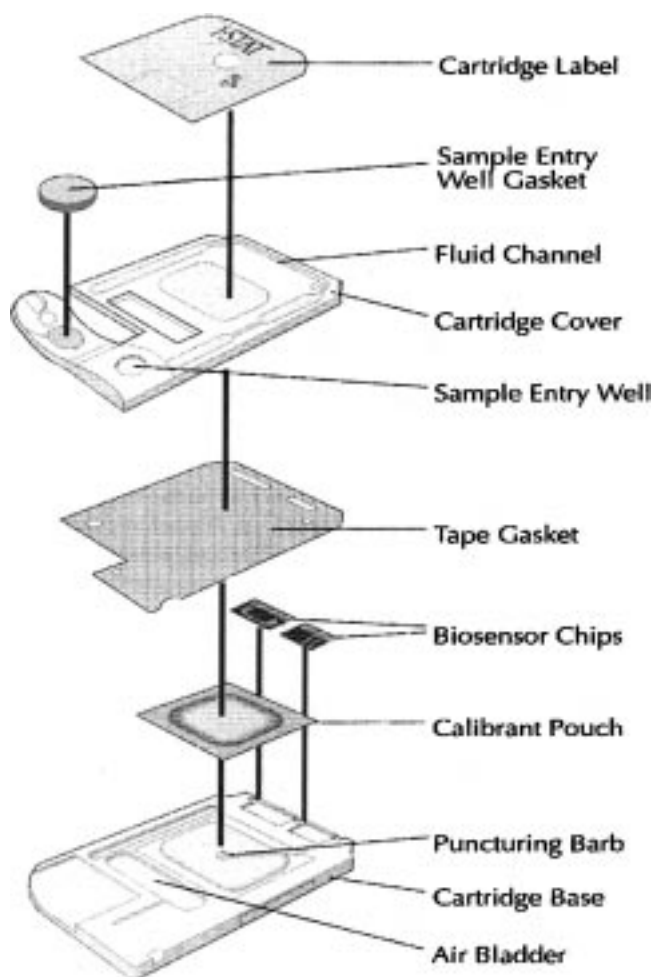

FIGURE 1. Exploded cross-sectional view of the i-STAT cartridge microanalytical system.

conduit. Sample remains in the sample conduit where it can be pretreated with one or more reagents before proceeding through the analytical process.

(3) When the cartridge is inserted into the electromechanical read-out device, the analytical process continues under automated instrument control.

(4) A z-action connector array is brought vertically down to contact the array of electrode pads on one end of the biosensor chips. This connector is designed to ensure static-free contact and to recognize different cartridge types containing different test combinations.[4]

(5) A z-action thermal probe and electrical connectors are brought vertically up to contact a thin film heater on the underside of the biosensor chips.

(6) A mechanism in the read-out device activates the delivery of an aqueous fluid by applying pressure to a reservoir created by a cavity in the lower housing and the tape gasket diaphragm. Fluid, which is hermetically sealed into the reservoir in a sealed metal-coated pack, is released by the controlled rupture of the pack as it is pressurized through the diaphragm against a sharp piercing barb molded into the plastic of the lower cartridge housing. Fluid is pumped over the biosensor array. The
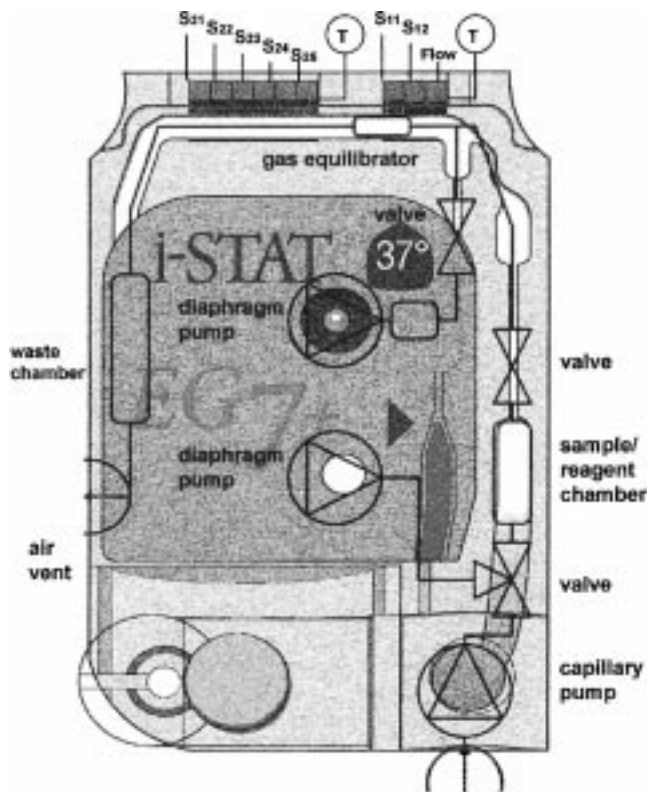
FIGURE 2. Unit-use microanalytical system for blood gases, electrolytes, enzyme substrates, and hematocrit. Fluidic circuit diagram overlay.

fluid achieves a known oxygen concentration by interaction with a gas reservoir engineered into the upper plastic housing.[5]

(7) A conductivity sensor located on one of the biosensor chips monitors the flow of fluid.

(8) Chips are heated to 37 °C and thermostated to ±0.06 °C (1 standard deviation).

(9) A battery of measurements is made by the biosensor chip to achieve the following: (a) To assess the initial conformance to specification of each individual biosensor. This is a vital step in ensuring reliable performance. Quality predictive initial measurements are one of the foundations of our automated quality system. Unit-use devices have factory ascertained means and ranges for certain quality predictive parameters against which the actual values in the field can be compared to make decisions on conformance. Multiuse devices, in contrast, cannot use such measurements in the same way, because the parameter value depends on all device history which includes the factory as well as its in-use history. For these devices a traditional in-line statistical process control methodology using pseudosamples is more appropriate. (b) To assess the conformance of the fluid flow processes. (c) To calibrate the biosensors.

(10) Sample is then brought into contact with the biosensors when the read-out device's mechanism actuates a second diaphragm pumping action.

(11) A battery of measurements is made by the biosensor chip to (a) asses the continued conformance to specification of biosensors and (b) asses the conformance

to specification of the collected blood sample and its delivery to the biosensor chip: for example, the flow sensor scrutinizes the sample for adequacy of fill, sufficient collected volume, sample bubbles, or segmentation.

(12) Measurements are performed on the concentrations of the analytes in the blood sample.

(13) Assuming that quality predictor values are within acceptable conformance ranges, results are displayed on the screen of the read-out device; otherwise results are not reported, but an indication of the category of nonconformance is displayed instead.

A useful characteristic of plastic molding technology is that any configuration of conduits, pumps, and valves is simply and cost-effectively rendered by modification of the mold design only. Thus, a broad range of different measurement types and different microfluidic arrangements can be realized within the same general high-volume manufacturing process. For example, coagulation testing can be accomplished by arrangement of conduits and pumps so as to effect a measurement of clotting through viscometry.[6]

Our current commercial devices use conduits whose typical cross-section is 0.5 mm × 1 mm. A 0.6 μL reaction cell volume is created by a 1.2 mm long conduit over a single biosensor. Current mechanisms which achieve linear displacement accuracy of 0.02 mm and resolution of about 0.005 mm act upon diaphragms with a 50 mm² cross-section, allowing controlled flow of 0.25 μL. Conduits with a 0.2 mm × 0.2 mm cross-section are routinely achievable in high-volume plastic molding technology, and much smaller dimensions are accessible by supplemental laser machining of the plastic components. These conduit downsizings will accompany the biosensor downsizing program of our production volume ramp, as described in the next section. For example, we will achieve 0.2 mm × 0.2 mm cross-section conduits per 0.5 mm long biosensors for 20 nL per biosensor. Diaphragm cross-sections, when downsized to 2 mm², will achieve 25 nL flow control.

Biosensor Arrays: Microfabrication Technology and Manufacturing Cost

i-STAT's core competence is its high-volume planar microfabrication processes for biosensors. Biosensors are manufactured with wafer-scale, planar, thin film, microfabrication processes (chip manufacturing processes). The guiding principle for our design strategy is lowest cost manufacture for devices that should operate with optimal performance specification, *but only once.* Planar microfabrication as a process platform is advantageous for this design strategy because of the following.

(1) Precision manufacturing of materials with small dimensions on wafer-scale processes leads to cost per function (in our case cost per biosensor electrode) that goes down in relation to the surface area of the device. Progressive downsizing of feature dimensions leads ultimately to lowest cost per function compared to any other manufacturing technology, so long as downsizing is

compatible with functional performance requirements. This remarkable property of microfabrication has seen the number of electronic components (transistor gates) per processed wafer increase geometrically over the last few decades as described by the well-known Moore's law. The cost per functional gate has dropped from about $3 per gate to 3×10^{-5} per gate today, with several more orders of magnitude reduction still available in the future. Certainly the largest growth of density of transistors per wafer has resulted from the growth of functional complexity of integrated circuits, but cost reduction also is impressive for discrete components where the density of transistors per wafer also has grown significantly as dimensional design rules have shrunk. Start-up costs to be in the chip business are high, but the leverage in cost reduction with time and volume is tremendous.

(2) Thin film planar microfabrication technology allows both thin film membrane processing and thick film membrane postprocessing, making available the full range of material thicknesses encountered in biosensors or dry reagent chemistry devices in general. In contrast, a thick film technology platform is limited to thick film only, unless nonstandard processes can be developed. We have therefore been able to achieve the necessary breadth of materials fabrication specifications to address all of our planned test menu requirements with the same technology platform. This allows us to achieve our program of menu expansion within the same biosensor factory. Therefore, the cost of production scales with the volume of all products added together, and lower volume specialty tests can be manufactured cost-effectively along with high-volume commodity tests.

(3) Blood testing devices, whether on chips or in other formats, often use dry reagents because chemicals should be stored dry for extended shelf life. Chemicals are wetted-up preceding use. Scaling-down of features to the dimensions of microfabricated thin films helps rapid wet-up of reagents as well as improves the response speed of biosensors.

Our strategy for design has been to use as much as possible existing biosensor device principles developed by the in vitro diagnostics industry for use in making macrosensors, inventing new designs only as a route to process simplification and hence lower cost, or as dimensional scaling laws demand consideration of new device operating principles. Further, our process development strategy has been to use as much as possible existing electronic chip microfabrication principles developed by the chip industry.[7]

All biosensor processes start by thermal oxidation of the top surface of a silicon wafer and backside metalization with a thin titanium–tungsten film to form a resistance heater. Figure 3 shows the side view, simplified schematic of electrochemical base sensors. Other sensors are fabricated by addition of layers over the base sensors as described below.

Potentiometric devices use silver metal electrodes whose sensor end is converted to silver chloride by electrodeless anodization in an oxidizing chloride bath.
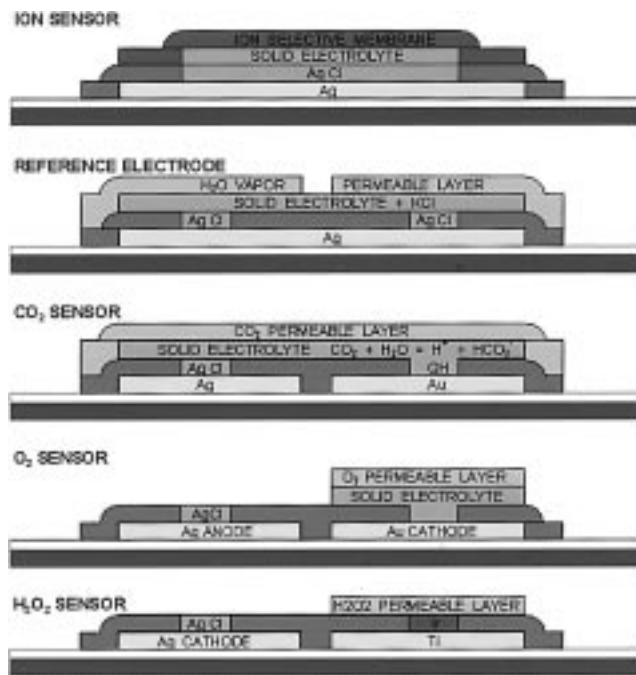

FIGURE 3. Simplified side view schematics of electrochemical base sensors.

Electrodes are insulated along their length by a spin-coated and photoformed polyimide film. Ion selective membranes are traditional plasticized poly(vinyl chloride) containing a neutral carrier ionophore or charged ion exchanger: methyl monensin for sodium, valinomycin for potassium, quarternary ammonium chloride for chloride, dioctyl phenyl phosphate for calcium, tridodecylamine for pH, and nonactin for ammonium. The ammonium sensor of the urea electrode is coated with a film of urease contained in a latex carrier. In operation, urease catalyzes the decomposition of urea to ammonia which is measured as ammonium at the underlying electrode at neutral blood pH. Polymer membrane films are deposited from solvent solutions using a nanoliter dispense tool.[8] In this process a planar wafer with controlled surface energy is contacted with a partially formed drop of membrane fluid emerging from a nozzle tip under precise spatial control on an $x-y$ stepper. Membranes with thicknesses in the range 0.1–100 μm and diameters in the range >50 μm can be deposited in this way.

Potentiometric electrode arrays are microfabricated onto chips with integral silver chloride ground and reference electrodes. The reference electrode is a classical design incorporating a silver–silver chloride electrode with potassium chloride salt bridge. In our microfabricated salt bridge reference electrode we have achieved the necessary long path length of the salt bridge by defining the long path of ion transport transversely in a gelatin/KCl film along the planar surface.[9] The gelatin film salt bridge is photoformed to cover the silver chloride and to extend beyond it. The salt bridge film is electrically isolated from the test solution by a thin gas permeable silicone–polycarbonate overlayer which is photodefined to completely cover the salt bridge except at a region remote from the silver chloride where the gelatin film contacts the test
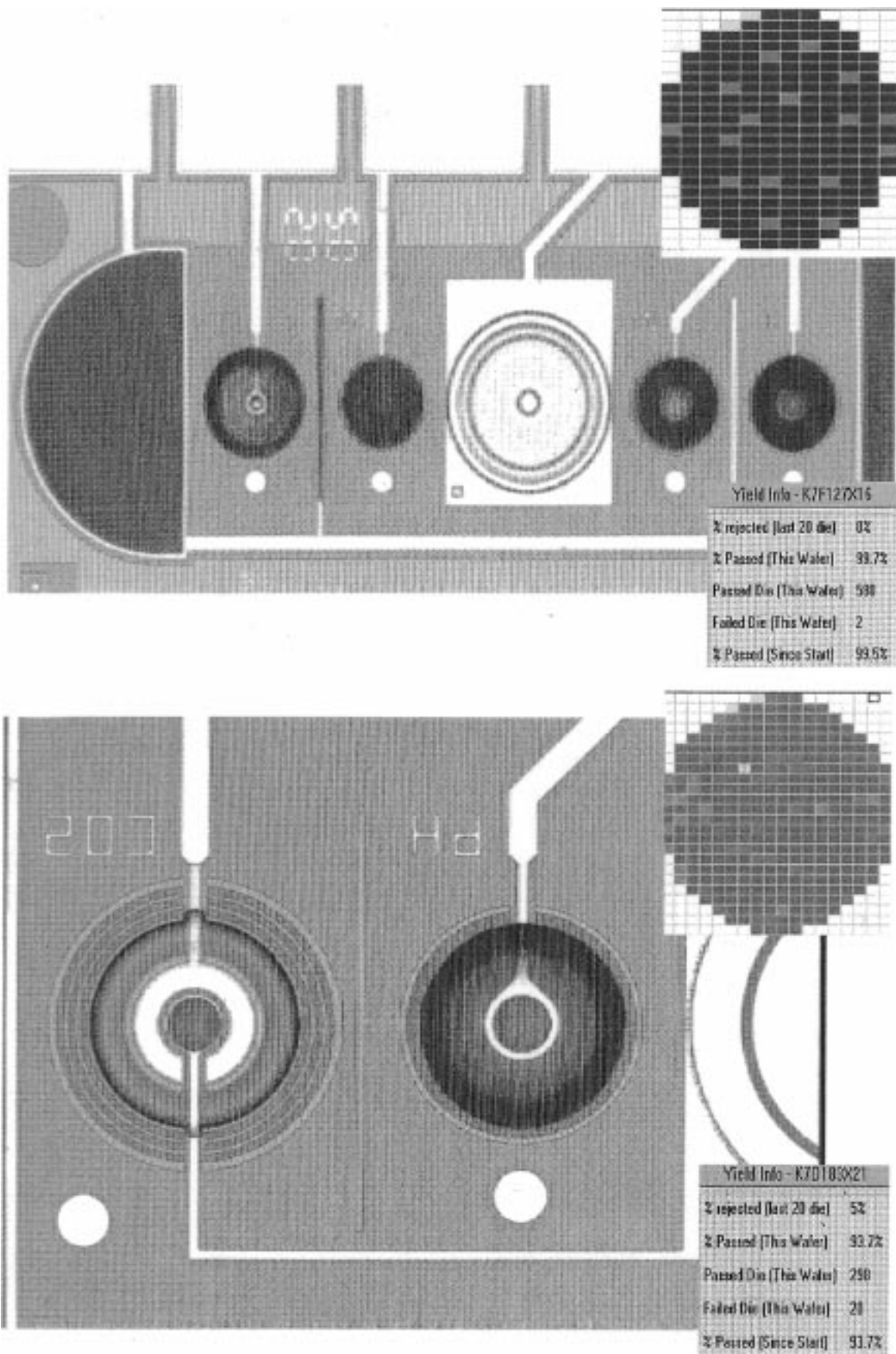
FIGURE 4. Computer screens of automatic visual inspection of potentiometric biosensors: (screen 1, top) eb2 chip (AgCl ground, urea, Cl, salt bridge reference, Na, K); (screen 2, bottom) HPCO2 chip (AgCl ground (partial) CO_2, pH, salt bridge reference (partial)).

Table 1. Device Materials and Their Thicknesses

	oxide	metal 1	metal 2	metal 3	insulator	AgCl	memb 1	memb 2	memb 3	memb 4
Na	SiO_2	TiW	TiW/Ag		polyimide	0.8 μm	PVC, methyl monensin			
	1.0 μm	0.03 μm	0.66 μm		1.1 μm		30 μm			
K	SiO_2	TiW	TiW/Ag		polyimide	0.8 μm	PVC, valinomycin			
	1.0 μm	0.03 μm	0.66 μm		1.1 μm	0.8 μm	39 μm			
Cl	SiO_2	TiW	TiW/Ag		polyimide	0.8 μm	PVC, quart. amm. chloride			
	1.0 μm	0.03 μm	0.66 μm		1.1 μm		26 μm			
Ca	SiO_2	TiW	TiW/Ag		polyimide	0.8 μm	PVC,dioctylphenyl phosphate			
	1.0 μm	0.03 μm	0.66 μm		1.1 μm		32 μm			
urea	SiO_2	TiW	TiW/Ag		polyimide	0.8 μm	PVC nonactin	latex, urease		
	1.0 μm	0.03 μm	0.66 μm		1.1 μm		34 μm	33 μm		
pH	SiO_2	TiW	TiW/Ag		polyimide	0.8 μm	PVC tridodecylamine			
	1.0 μm	0.03 μm	0.66 μm		1.1 μm		32 μm			
$P(CO_2)$	SiO_2	TiW	TiW/Ag	TiW/Au	polyimide	0.8 μm	sucrose NaHCO3	dimethylpolysiloxane		
	1.0 μm	0.03 μm	0.66 μm	0.1 μm	1.1 μm		2.0 μm	1.1 μm		
$P(O_2)$	SiO_2	TiW	TiW/Au		polyimide		gelatin	silicone−polycarbonate		
	1.0 μm	0.03 μm	0.1 μm		1.1 μm		0.8 μm	1.5 μm		
Glu	SiO_2	TiW	Ti	Ti/Ir	TiO_2		silane	gelatin, glucose oxidase	silicone polycarbonate	gelatin
	1.0 μm	0.03 μm	0.08 μm	0.06 μm	<5 Å		0.008 μm	0.5 μm	0.08 μm	0.35 μm
Hct	SiO_2	TiW	Ti/Ir							
	1.0 μm	0.03 μm	0.06 μm							
ref	SiO_2	TiW	TiW/Ag		polyimide	0.8 μm	gelatin, KCL	silicone−polycarbonate		gelatin
	1.0 μm	0.03 μm	0.66 μm		1.1 μm		1.7 μm	1.7 μm		1.3 μm

Table 2. Total Imprecision (Standard Deviation, S_{total}) and Coefficient of Variation (CV %) of i-STAT Biosensors at Clinically Relevant Concentrations

test	x_{bar}	S_{total}	CV %
Na	140 mmol/L	0.6 mmol/L	0.5
K	4.5 mmol/L	0.04 mmol/L	1.0
Cl	95 mmol/L	0.6 mmol/L	0.7
Ca	1.2 mmol/L	0.014 mmol/L	1.2
Bun	50 mg/dL	1.4 mg/dL	2.8
pH	7.40	0.007	0.1
$P(CO_2)$	42 mmHg	1.0 mmHg	2.5
$P(O_2)$	70 mmHg	2.1 mmHg	2.9
glucose	120 mg/dL	1.5 mg/dL	1.3
Hct	40	1.2	3.0

solution and a liquid junction is formed. In operation, the dry reagent KCl wets-up rapidly by water absorption into the gelatin through the short path of the gas permeable overlayer, but ion transport is along the planar surface transversely along the long path length of the film. Rapid ionic transport is achieved, as required to obtain a stable liquid junction, but the chloride concentration at the silver chloride remains constant through the course of the analytical process because (1) the transverse path length from the liquid junction to the silver chloride electrode is suitably long and (2) the thickness of the silicone−polycarbonate overlayer is chosen so that the rate of water transport is sufficient for fast wet-up, but slow enough so that the change of volume of water absorbed into the gelatin layer in response to osmotic imbalance going from calibrant fluid to whole blood is minimal. This design has been validated to achieve stability to about 50 μV or less when switching from an aqueous calibrant fluid at 100 mmol/L of chloride and 300 mmol/L of osmolarity to whole blood at the extremes of its compositional range. Device designs which feature both transverse and vertical transport paths occur often in our work. The relative transverse to vertical transport path length ratio is about 300 for the salt bridge reference electrode in our current unit-use devices.

The CO_2 sensor is a Severinghaus design.[10] The internal pH electrode is a gold−quinhydrone electrode immersed in an internal solid electolyte containing sodium bicarbonate, sodium chloride, and a sucrose binder. The gas permeable membrane is poly(dimethylsiloxane). We chose gold−quinhydrone in preference to other available thin film pH technologies such as the metal oxides of iridium, palladium, or antimony because there is one less process step and higher yield: a 30% lower process cost in total.

Pictorial examples of microfabricated potentiometric electrodes are shown in Figure 4; these are computer screens of potentiometric chips undergoing automated visual inspection. Note that the central salt bridge reference electrode has silver chloride on the perimeter with the opening to the salt bridge through a central hole.

The O_2 electrode is an amperometric Clark electrode design,[11] except that the internal counter electrode of the standard Clark device is missing. In our modified device[12] a film of gelatin containing electrolytes and buffer is coated and photoformed over a gold cathode. The gold cathode is rf sputtered, patterned by standard photoresist and subtractive etch photoprocessing, and then coated with a passivating polyimide which is photoprocessed to define 10 μm diameter openings which constitute a microelectrode array. A gas permeable silicone−polycarbonate membrane is coated over the solid electrolyte. The perimeter of the electrolyte film extends beyond the gas permeable layer remotely from the cathode. The perimeter of the electrolyte film is in electrical contact with the test fluid. There is, therefore, electrical continuity through a salt bridge formed along the surface of the planar device between the cathode and the counter electrode provided by the main system ground elsewhere on the biosensor array. Thus, the O_2 electrode has only one contact instead of the usual two, resulting in half the chip area and half the cost.

Glucose sensors utilize the enzyme glucose oxidase which catalyzes the oxidation of glucose. This reaction consumes oxygen and produces hydrogen peroxide which is measured amperometrically at a noble metal anode. In our device an iridium film is rf sputtered and patterned

by lift-off. An ultrathin, partially cross-linked aminosilane film is spin-deposited and patterned by standard photoresist and subtractive etch photoprocessing.[13] In operation this layer excludes redox active species larger than hydrogen peroxide from reaching the electrode. A gelatin film containing glucose oxidase is spin-coated and photoformed over the modified electrode. A thin silicone–polycarbonate film is spin-coated and photoprocessed over the enzyme layer. In operation this material attenuates glucose transport but is freely permeable to oxygen. This enables the biosensor to achieve linear response to glucose up to >25 mmol/L, and useful response up to 75 mmol/L.

Hematocrit is measured using ac conductimetry. The conductometric cell is formed by a pair of thin film iridium electrodes.

Table 1 summarizes the major device materials used in our commercially available biosensors.

Table 2 shows data obtained in our testing labs on whole blood and aqueous test materials as well as field verifications at hospitals. The data were aggregated over several years of operation and sample sizes of multiple tens of thousands per each analyte. In summary, and on average, device performance is equivalent to that obtained by large laboratory analyzers.

Independent studies of clinical performance of i-STAT's system are numerous. Published data have verified that performance in the hands of doctors and nurses is equivalent to that obtained by laboratory technologists.[14,15] Process improvements and cost reductions also have been documented.[16,17] Recently, hospital-wide implementations have been described.[18]

Summary

i-STAT devices are now in use in 1000 hospitals in North America, Japan, and Europe. i-STAT blood testing technology has been integrated into other patient-side monitoring tools offered by Hewlett-Packard Co. In about 100 hospitals our blood testing technology has been adopted as an institution-wide, multidepartment program. In the past few years, patient-side testing of critical blood values has become an accepted procedure; indeed it is now a significant growth sector of the in vitro diagnostics industry. For the test menu available for patient-side use (fully developed and performing robustly), the fraction of testing actually restructured to patient-side relative to the total tests amenable to restructuring is still quite small, so that the opportunity for growth of this sector is still enormous. Restructuring to patient-side blood testing activities is no longer limited by availability of technology.

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