Lab-on-a-chip: Why aren't we all hypochondriacs?

Graham C. Holt

Collegium Basilea (Institute of Advanced Study), Basle, Switzerland

In the field of nanotechnology there has been considerable research into nanometre-size sensors and microfluidics. In medicine, this confluence of technologies has resulted in the "lab-on-a-chip" concept, whereby blood and other body fluid analysis to detect diseases may be conducted at home or at the point-of-care without the need for specialized laboratory equipment. Although the literature is full of research papers in this area, very few devices have made it successfully to market, albeit with some available to specialists in the health services, but practically none available to the general public. Why is this? And why have cheap devices powered by a personal computer or smartphone not become commonplace in the home? This must have been the vision for many a research proposal in justifying funding because of the predicted burgeoning home healthcare market. The following is a review of where we are and the problems involved in realizing the dream of home-based healthcare.

1. Introduction

The UK National Health Service is failing. Ageing population, more diseases treatable, increasingly expensive operations and the cost of pharmaceuticals have all placed such a strain on the free service that successive governments, although claiming to spend more each year, are having to find ever greater savings and efficiency measures. To save money, more and more of the routine work of our hospitals and general practitioners is being outsourced. In America, the Republicans see the Medicare scheme as a precursor to a National Health Service and are fearful that it will eventually bankrupt the USA.

Last year in the UK, the health service took 18% of government expenditure, only slightly less than that of defence and education put together. The £108 billion (1000 million) bill for 2013[1] included nearly £140 million spent on antibiotics[2]—small beer compared to the total pharmaceutical bill but nonetheless significant when a recent study indicates that perhaps as

[1] *Public Expenditure Statistical Analyses 2013*. Presented to Parliament by the Chief Secretary to the Treasury by Command of Her Majesty, July 2013 (Cm 8663).

[2] *PCT Board Prescribing Report* (February 2010). Antibiotic prescribing excluding topical antibiotic preparations—prescribing guidance and discussion points (NHS Business Services Authority).

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much as 15% of prescription antibiotics or £8 million[3] was wasted either because of misdiagnosis or insistence by patients that they be given antibiotics for viral infections. Respiratory tract infections are particularly problematical as their cause can be either bacterial or viral.

This is but one area where savings are being sought, but an area where technology could help. Blood and sputum analysis to confirm diagnosis of a viral or bacterial disease involves shipping samples to a laboratory and typically waiting two to three days for the result. This is a costly process and many have highlighted the benefits of point-of-care accurate diagnosis in terms of both saving money and preventing misdiagnosis and unnecessary drug prescription. One might be forgiven for thinking that here is a market clamouring for lab-on-a-chip devices. The reality is somewhat different. Simple devices, like the laser finger clip that measures blood oxygen level and costs around £30, are becoming ubiquitous at the point-of-care in many general practitioner's surgeries, while many women now purchase home pregnancy tests, which represent a more sophisticated lab-on-a-chip device. However, in research, although only just becoming available on the market, there are many lab-on-a-chip devices. The ability to confirm the presence of a common influenza virus, and in particular the H1N1 'flu virus, is now possible with a cigarette packet-sized lab-on-a-chip connected to a standard personal computer. Development of these devices would seem to be driven not by the needs of health services but by the media and governments in demanding a response to perceived pandemics such as bird 'flu and now Ebola.

Where, then, are these lab-on-a-chip devices?

2. The technologies

Most of the development over the last 10 years has been to produce a smartphone-sized device into which a sample of blood or sputum is dropped; the device then automatically analyses the sample. The device package itself contains all that is required to process the sample and detects the output, which is then fed, typically by USB connexion, to a smartphone or personal computer, which provides both power and the processing to control reactions and analyse the output and present the results. Inside the device package will be a microfluidic device the size of a credit card, channelling the sample through the detection process, and containing any necessary chemicals involved in the reaction as well as the detection system.

The microfluidic device, the eponymous "lab-on-a-chip", is key to all of these point-of-care medical aids. Channels are cut in a substrate, using electronic chip fabrication techniques; the channels can vary from a few hundred nanometres (nm) to hundreds of micrometres (μm) in diameter. Fluids in these channels are characterized by a low Reynolds number enabling laminar flow and requiring only a weak force to move the fluid, either by pressure or electrokinetics. A typical arrangement might be a receptacle for receiving the sample of the order of a few microlitres (1 μL or 10^{18} nm^3) in volume (i.e., a small drop), which may then be subdivided into further channels, leading either to a reaction chamber or to a detection system.

To appreciate the scale of the technology, note that a human red blood cell is between 6 and 8 μm in diameter (10^{11} nm^3 in volume), the *E. coli* bacterium is a tube 2 μm long with a diameter

[3] C. Currie et al., Antibiotic treatment failure in four common infections in UK primary care 1991–2012: longitudinal analysis. *BMJ* **349** (2014) g5493.

of 0.5 μm (10^9 nm^3), while the 'flu virus is a sphere between 80 to 120 nm in diameter (10^6 nm^3). On the whole viruses are much smaller than bacteria but there are always exceptions and the *Streptococcus pneumoniae* bacterium is only marginally larger than the 'flu virus, see Figure 1. Typical of body fluids, blood is mostly water but contains about 4% of red blood cells so that 1 μL contains 5 million red blood cells.

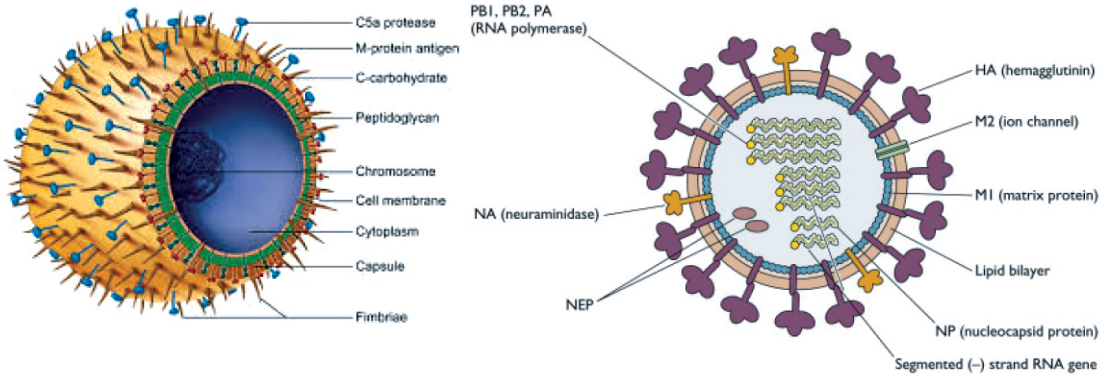

Figure 1. Schematic drawings of the pneumonia bacterium (left) and 'flu virus (right).

The disease detection process depends on, and tends to be very specific to, the given infection to be detected. In some devices almost full DNA analysis is conducted using the polymerase chain reaction (PCR), while others will look for specific antigens on a virus or bacterium by applying the appropriate antibody marked with a molecule detectable typically by fluorescence or surface plasmon resonance. Promising recent research employs nanopore technology whereby DNA strands are pulled through a hole a few nanometres in diameter that reacts to one of the four nucleotide bases.[4] This technology, operating at 100 μs per base, should also be faster than PCR.

For example, the 'flu virus attaches itself to epithelial cells in the respiratory tract using the blood antigens haemagglutinin (H) and neuraminidase (N), which determine blood type, and it is by this means that they are absorbed or fused into the cell to release their RNA. Thus, if a lab-on-a-chip device can detect one infected cell, the size of a red blood cell, then the sensitivity is better than one part per million from a 1 μL drop of sputum or mucus (saliva). Unfortunately, the 'flu virus evolves through antigenic shift so that the blood antibodies for H and N fail to recognize and destroy them. H3N2 was the strain of the Asian 'flu virus of 1968 while H1N1 was the prevalent strain of the 2009 pandemic.

For the PCR lab-on-a-chip, a sample is fed to a micro reaction chamber, which is then supplied with the relevant polymerases and subjected to cycles of heating and cooling to denature and anneal the DNA. Electrophoresis of the resulting oligonucleotides is undertaken in the subsequent channel fitted with nanometre-sized sensors such as carbon nanotube resistance detectors to indicate the presence of specific nucleotides (Figure 2). The DNA-copying chain reaction driven by the heating and cooling cycles takes about 15 minutes and this essentially

[4] P. Krishnakumar et al., Slowing DNA translocation through a nanopore using a functionalized electrode. *ACS Nano* **7** (2013) 10319–10326.

determines the analysis time. For this level of analysis, a precursor microreactor is required to separate the DNA or RNA from the cell, often using benzyl chloride to destroy the cell wall. This type of lab-on-a-chip is perhaps the most complicated, having many parts that will have to be disposable in order to prevent cross-contamination from reuse. In effect, the microfluid chip is a disposable item, processed as medical waste.

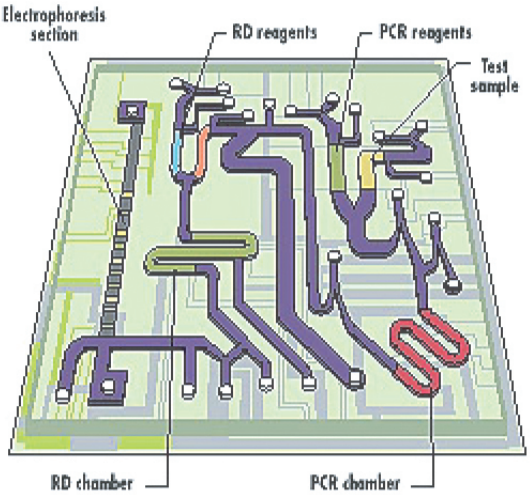

Figure 2. PCR microfluidic device (schematic of the University of Michigan "Genotyper").

The less reductionist approaches to analysis rely on the effect of the antibody–antigen reaction as the fluid sample emerges from a reaction chamber containing the appropriate antibodies. Techniques include: a fluorescent molecule attached to an antibody, which can be detected when illuminated (enzyme-linked immunosorbent assay, ELISA); surface plasmon resonance of a gold nanoparticle similarly attached, where resonance may be detected with laser illumination; and, more directly, absorption changes, refraction changes (optical waveguide lightmode spectroscopy OWLS) or electrical resistance changes due to the reaction. These latter methods employ detection in the reaction chamber with the antibodies anchored to the substrate.

In order to make these devices commercially viable, all components need to be available at very low cost since, as mentioned, the microfluid chip is a single-use device.

3. Market availability

A recent paper attempted to review the lab-on-a-chip market in America. A snapshot of some of the major manufacturers with the technologies employed for detection is shown in Table 1. There is evidently no shortage of devices and the range of diseases diagnosable at point-of-care is listed in Table 2. The authors found that for these devices to be useful in the poorer countries, where often pandemics begin (such as HIV in Africa), the disposable element had to cost no more than a few dollars to be viable. The other major problem is that of reliability of diagnosis. A false-positive result is almost worse than a false-negative, and this is an area still in its infancy. Furthermore, many of these devices can avoid full clinical trials prior to market launch and thus regulation (or lack of it) is a serious issue.

Table 1. US companies involved in lab-on-a-chip manufacture and the technology employed. From C.D. Chin et al., Commercialization of microfluidic point-of-care diagnostic devices, *Lab. Chip* **12** (2012) 2118–2134.

Company	Material and manufacturing	Reagent storage	Sample types	Sample pre-treatment	Fluid actuation	Fluid control	Fluid mixing	Signal detection
Abaxis	Plastic disc	Dry reagents on disc	Whole blood	On disc	Centrifugal and capillary	Passive	Passive	Absorbance
Advanced Liquid Logic	Glass, insulated electrodes	On- and off-chip reservoirs	Whole blood	On chip	Electrokinetic (electrowetting)	Electrokinetic	Electrokinetic	Fluorescence, chemiluminescence
Alere (formerly Inverness Medical)	Plastic and elastically deformable materials	On cartridge	Whole blood (capillary)	On cartridge	Mechanical (peristaltic), capillary	Passive	Passive	Fluorescence
Biosite (Alere)	Strip with textured microstructures	On test strip	Whole blood (venous), plasma	Centrifugation	Capillary	Passive	Passive	Fluroescence
Cepheid	Disposable plastic cartridge	On test cartridge	Whole blood, sputum	Sample dilution buffer, shaking	Pneumatic (syringe plunger)	Rotary valves	Passive, manual	Fluorescence (with molecular beacons)
Claros Diagnostics (Opko)	Plastic cassette	On cassette	Whole blood, urine	On cassette	Pneumatic	Passive	Passive	Absorbance
Daktari Diagnostics	Plastic cartridge	On cartridge blister packs	Whole blood	On cartridge	Pneumatic	Passive	Passive	Electrochemical (impedance spectroscopy)
Diagnostics For All	Paper	Dry reagents on paper	Whole blood, urine	On stamp	Capillary	Passive	Passive	Colorimetric
Epocal (Alere)	Film, epoxy laminates	Dry reagents	Whole blood	On chip	Capillary, pneumatic, electrokinetic	Electrokinetic	Electrokinetic	Electrochemical, chemiluminescence
Focus Dx (Quest)	Plastic (polypropylene)	PCR reagent prep needed	Nasal & pharyngeal swabs	Nucleic acid extraction	Centrifugal	Passive	Passive	Fluroescence
HandyLab (BD)	Disposable cartridges	Dry reagents	Vaginal, rectal, nasal swabs	Broth enrichment, cell lysis (MRSA)	Electrokinetic, thermal pneumatics	Valves	Passive	Fluorescence (with molecular beacons)
i-STAT Corp (Abbott)	Plastic cartridge with silicon microchip	On cartridge	Whole blood, urine	On cartridge	Pneumatic (air bladder), capillary	Passive	Passive	Electrochemical (potentiometry, amperometry, conductivity)
Micronics (Sony)	Plastic, laminates, paper	On cartridge	Whole blood, stool	On cartridge	Pneumatic, capillary	Passive, valves	Passive	Absorbance, colorimetry
MBio Diagnostics, Inc.	Plastic, transparent planar components	On chip	Whole blood	On chip	Pneumatic	Passive	Passive	Fluorescence (with planar waveguides)
Philips	Plastic cartridge	Dry reagents on cartridge	Whole blood, saliva	On cartridge	Pneumatic	Passive	Passive	Optical (frustrated total internal reflectance)
TearLab	Polycarbonate	None	Tear	On chip	Capillary	Passive	Passive	Electrochemical
Zyomyx	Glass, plastic	Dry reagents	Whole blood	Bead-conjugated Ab incubation	Gravitational	Passive	Passive	Colorimetric (cell stacking)

Table 2. Diagnostic capabilities of US FDA-approved lab-on-a-chip devices.

Blood chemistries (e.g. metabolites, lipid, electrolytes, gases)
HIV/AIDS, clotting time
Respiratory infections (bacterial and viral), cancer
Urological maladies, infectious diseases
Influenza, intestinal pathogens
Bacterial infections and drug susceptibility testing
Upper respiratory tract infections (viral), bioterrorism agents
Malaria, shiga toxin-producing *E. coli*, ABO blood typing

The US Food and Drug Administration (FDA) has approved or waived approval for many devices capable of diagnosing of the diseases in Table 2. It is noteworthy that the waiver applicable to clinical laboratory improvement amendments (CLIA) requires the following of any approved device:[5]

- Self-contained and fully-automated test, and use of unprocessed specimen. In case of blood markers, this requirement bars the user from centrifuging blood to produce serum or plasma. Hence, the test must either use whole blood as the sample or include an automated blood separation step without user intervention.

[5] *Recommendations: Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices*. Silver Spring, Maryland: FDA (2008).

- No specialized training. Any user shall be able to operate the test without technical training, based on reading instructions written in English.
- Easily interpreted results. The readout should be directly usable for a clinical decision, without the need for additional calculation or calibration.
- Robust method. The result must be reliable when the device is operated under real-world conditions that account for user-based variations (such as sample collection, timing of user actions and storage conditions).

In the UK (and similarly in much of Europe), regulation is set by the Medicines and Healthcare Products Regulatory Agency, which requires clinical trials according to perceived risk (highest last):[6]

- general *in vitro* devices (IVDs); i.e., for self-testing such as blood gas analysers, therapeutic monitoring reagents and tissue processors;
- IVDs for self-testing (a device intended by the manufacturer to be able to be used by lay persons in a home environment);
- IVDs that, amongst others, include reagents and products for rubella, toxoplasmosis and phenylketonuria as well as devices for self-testing for blood sugar;
- IVDs that include reagents and products for HIV I and II, Hepatitis B, C and D, and reagents and products for determining ABO type and anti-Kell including those used to test donated blood plus tests for screening vCJD.

Obviously, carrying out clinical trials and gaining CE marking are among the factors slowing the progress of research and development—result to demonstrator to market—and this hurdle is linked to the (un)reliability of the diagnosis.[7]

4. Reliability and cost

Currently available on the market are blood test analysers using a disposable microfluidic smartcard into which is placed some tens of microlitres of blood. One particular cigarette packet-sized device is WiFi-connected to a host computer to run the analysis; the results are then displayed on the device (into which the card is placed). Such blood analysers seem to be reliable but are expensive, costing £500 for the main device and a few £10s for a set of microfluidic cards. PCR analysis devices are only just coming onto the market with a disposable microfluidic device fed by a cartridge system containing the polymerase enzyme and accessory reagents. This is even more expensive but, because it is looking for specific nucleotides, it is presumably more reliable. However, the nanopore device mentioned above should, when developed, hopefully sell for less than $1000.

The situation for virus detection in particular is best exemplified by the advice given for the use of rapid detection devices. The US Centers for Disease Control and Prevention[7] suggest the following for 'flu virus detection; when 'flu prevalence in the community is low, false-positive

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[6] *Guidance on the In Vitro Diagnostic Medical Devices Directive 98/79/EC*, London: MHRA (August 2013).

[7] Rapid Diagnostic Testing for Influenza: Information for Clinical Laboratory Directors from the Centers for Disease Control and Prevention, 2014 (http://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm).

detection can be as high as 50%; when 'flu prevalence is high, the problem is false-negatives as high as 30%. In both cases, the advice, if the diagnosis is critical, is that it should be confirmed by PCR or viral culture.

5. Conclusions

To return to the title, one might be forgiven for thinking that the proliferation of rapid diagnosis lab-on-a-chip devices at the point-of-care and at home might make us all amateur medical doctors. This may not be a bad thing—individuals become more responsible for their own health, possibly lessening the load on the overstretched UK National Health Service. Inevitably, the proliferation of readily available diagnostic devices will see an expansion of public use and could promote within the population many hypochondriacs demanding drugs for perceived diseases. The cynical might regard this as good for GDP as presumably the drugs will be brought privately.

From the foregoing, it is apparent that this scenario is not likely to become a reality for at least ten years, the gestation time for full clinical trials for many of these devices, although some are likely to make the market sooner through the CLIA waiver, probably requiring only laboratory analysis confirmation before treatment is initiated. Despite the plethora of devices, their current cost puts most of them out of reach of the general public. Moreover, it is likely that the health authorities will not approve many devices simply because of their unreliability and, as it is likely that the health services are the largest market for these devices, economies of scale of manufacturing will not then be available to bring the price down to affordable levels in the near future. This is particularly true of lab-on-a-chip devices that rely heavily on a one-off software application, often the most expensive part of initial development, which in some way must be added to the device cost even if the disposable elements are very inexpensive.

In general, the market is uncertain; early 'flu virus detection is beneficial if it can be used to isolate those infected and help prevent contagion, but the 'flu virus itself is typically one step ahead of any lab-on-a-chip device due, as mentioned, to antigenic shift, which makes the devices unreliable; health authorities are likely to consider the diagnosis of a qualified general practitioner more effective.

Blood testing and PCR analysis devices are more reliable but, again, their current high cost makes them unattractive in general practice. The scenario where real savings may be made to the nation's health care is perhaps in the accurate diagnosis of respiratory tract diseases, which can save money both by returning patients to health sooner and avoiding the use of ineffective antibiotics. Rapid self-detection of the plethora of respiratory infections using a lab-on-a-chip device might still be some way off but is surely a worthy aim for current research.