Acoustic technology drives integrated POC diagnostics

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Whilst in many less developed countries there is a paucity of diagnostic testing and appropriate therapies, we in the West are suffering from the ‘modern epidemic’ of over-diagnosis and over-treatment. Today’s highly sensitive biomarker and imaging tests increasingly identify asymptomatic or very mild conditions that if left untreated would not cause symptoms or reduce longevity. A recent report on mammography screening in the UK suggested that 19% of breast cancers were over-diagnosed, and a US task force concluded that PSA-based prostate cancer screening over-diagnosed up to 50% of tumours. Other over-diagnosed and over-treated conditions include thyroid cancers as well as a range of cardiovascular diseases, chronic kidney disease and ADHD. At best treating such subjects is an imprudent use of health service funds; at worst ‘patients’ suffer both psychological and physical harm from their diagnosis and subsequent treatment. Of course effective screening for cancer and other serious conditions is vital, but how can the problem of over-diagnosis be at least alleviated when tests (and cut-off values) must be sensitive enough to detect pathologies that really require treatment?

When diagnostic tests are evaluated for accuracy the average sensitivity and specificity are reported. But of course individuals vary, and diseases have stages of severity. What is needed is the identification of those patients for whom treatment will do more good than harm. Similarly average results in therapeutic trials may be positive, so negative effects in some patient groups are not evident, but again the potential benefit of a treatment should be weighed against possible harm according to disease severity. And subjects being screened should surely be informed about the risk of over-diagnosis. Yet in a recent random sample of 500 Australians, only 10% of the women who had had mammography, and 18% of the men who had had prostate cancer screening reported that they had been told about the limitations of these tests.

There is also an urgent need to scrutinize the panels of medical professionals setting disease definitions. Diagnostic thresholds are frequently lowered without considering the balance between good and harm of treating the additional patient group who have a lower risk or milder symptoms. And although it may sound cynical, panels with three quarters of the members having multiple ties to pharmaceutical companies – some of which will directly benefit from an increased number of patients with the disease under discussion – surely can’t be unbiased! Hopefully appropriate action can be taken before the seemingly inexorable trend towards over-diagnosis makes patients of us all!
One of the major avenues for addressing the rising impact of sexually transmitted infections lies with rapid, early diagnosis to break the cycle of transmission. Here we discuss the potential of a new technology, using the mechanical energy of sound waves to drive integrated point-of-care diagnostics.

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The Ebola Spatial Care Path™: point-of-care lessons learned for stopping outbreaks

Ebola profoundly elevated the impact of point-of-care testing, now recognized worldwide as essential to detect the disease, reduce risk, monitor patients in isolation, achieve recovery, and importantly, contain outbreaks. The goal is to become resilient – a new and possibly more contagious threat might appear. We must stop it where it starts!

by Prof. G. J. Kost, W. Ferguson, A.-T. Truong, D. Prom, J. Hoe, A. Banpavichit and S. Kongpila

Introduction – the essential role of point-of-care testing

Point-of-care testing (POCT) is propelling the convergence, integration and sustainability of global diagnostics. We should not be caught off guard at points of need! Using fever to screen patients for Ebola virus disease (‘Ebola’) occurs too far downstream in the clinical course, casts an excessively wide net confused by other febrile illnesses, defeats rapid epidemiological control of outbreaks and inhibits evidence-based karma essential for compatible point of care culture. In fact, poor focus misleads the public, who, once cognizant of the essential role, importance and comprehensiveness of rapid POC diagnosis, will be receptive to containment and disposed to enter treatment centres, if they are more certain they have Ebola. The Ebola ‘newdemic’ (an unexpected and disruptive problem that affects the health of large numbers of individuals in a crowded world) moved POCT from parochial fiduciaries often stalled by analysis paralysis to action-oriented value generators, that is, inventors and innovators leading the way with next-generation technologies and high stakes strategies, as summarized in this article, which are beneficial for reducing risk and enhancing resilience. It inspired the Ebola Spatial Care Path™ (SCP) and a useful Diagnostic Centre (DC) design equipped with POCT, presented here as well [1].

Rapid evolution of diagnostic tests for Ebola virus disease

Table 1 chronicles the pioneering ongoing efforts of industry, academia and government to produce workable immunoassays and molecular diagnostics for the detection of Ebola. In fact, this research development will spill over to energize POC diagnostics for highly infectious diseases in general. Novel research also is exploring digital detection of Ebola virus and viral load, which is higher in fatal cases and may be related to the development of virus-induced shock. Aside from the logistic challenges of getting assays ready in time, new assays, which might be implemented on instruments like the GenePOC, must be proven to work in clinical studies. As far back as 2006, investigators reviewed laboratory diagnostics for Ebola. Now, nearly a decade later, the FDA is accelerating the ongoing development, validation and approval of new diagnostic tests by issuing emergency use authorizations (EUAs) more or less continuously since autumn 2014 (Table 1).

Ebolavirus-specific challenges for molecular diagnostics include: (a) reduction in initial false negatives (FN), FN = FN(t), as a function of time, to ramp up sensitivity, (TP/[TP + FN(t)] where TP=true positive), to ultrahigh levels in infected patients during the first 72 hours when symptoms may be mild or absent, in order to avoid shunting false negative cases...
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(2a) Emory University Hospital Specialized Isolation Area

<table>
<thead>
<tr>
<th>Manufacturer Website</th>
<th>Instrument</th>
<th>Test(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abaxis</td>
<td>Piccolo Express</td>
<td>Chemistry profiles, Magnesium, Phosphate, liver enzyme assays, others</td>
</tr>
<tr>
<td></td>
<td></td>
<td>available [8]</td>
</tr>
<tr>
<td>Instrumentation Laboratory</td>
<td>GEM Premier 4000</td>
<td>pH, pCO2, pO2, Na+, K+, Ca++, Cl-, Glu, Loc, Ht, Thb, CO2Chemistry, Tbl</td>
</tr>
<tr>
<td>Siemens</td>
<td>CLINITEK Status</td>
<td>Alburnum, Bilirubin, Cr, Glu, Ketone, leukocytes, Nitrue, pH, Protein,</td>
</tr>
<tr>
<td></td>
<td>Automated urinanalysis</td>
<td>Specific Gravity, Unoblitrogen, others available [9]</td>
</tr>
<tr>
<td>Hollmannia Roche</td>
<td>CoaguChek</td>
<td>PT/INR [10]</td>
</tr>
<tr>
<td>Symsesx</td>
<td>posH-100</td>
<td>CBC, WBC (2-part differential), RBC, Hb, Hct, MCV, MCH, MCHC, Platelets</td>
</tr>
<tr>
<td>Alere</td>
<td>BinaxNOW</td>
<td>Malaria</td>
</tr>
<tr>
<td>BioFire Diagnostics</td>
<td>FileArray</td>
<td>Infectious diseases including Ebola [12] (see Table 1)</td>
</tr>
</tbody>
</table>

(2b) University of Nebraska Medical Center Biocontainment BSL-3

<table>
<thead>
<tr>
<th>Manufacturer Website</th>
<th>Instrument/ method</th>
<th>Test(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>i-Stat</td>
<td>GU+ cartridge (pH, pCO2, pO2) &amp; Chemil+ cartridge (Na+, K+, Cl-, TCO2, Ca++, Glu, UN, Cr, Ht)</td>
</tr>
<tr>
<td>International Technidyne Corp.</td>
<td>Hemochron Signature Eline</td>
<td>Citrate prothrombin time (PT), citrate-activated partial thromboplastin time (aPTT)</td>
</tr>
<tr>
<td>Slide Agglutination</td>
<td>Manual</td>
<td>Blood &amp; serum antibody typing (for transfusion)</td>
</tr>
<tr>
<td>Slide Preparation</td>
<td>Manual</td>
<td>Malaria — modified for the slide to be fixed in methanol 15 min before delivering to Core Lab for staining &amp; interpretation</td>
</tr>
<tr>
<td>NS</td>
<td>Rapid manual assay</td>
<td>HIV Ab/Ag</td>
</tr>
<tr>
<td>Urine Dipstick</td>
<td>Manual dipstick</td>
<td>For tests not on strip, specimen transferred with precautions to Core Lab for non-decapped D8800 &amp; DXC8000 [13] analysis</td>
</tr>
<tr>
<td>NS</td>
<td>RPR</td>
<td>Syphilis (card assay)</td>
</tr>
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</table>

(2c) Ebola Holding Units (4), Sierra Leone, West Africa [14]

<table>
<thead>
<tr>
<th>Developer Website</th>
<th>Method</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom’s Defense Science &amp; Technology Laboratory</td>
<td>Rapid diagnostic antigen test</td>
<td>Sensitivity 100%, 95% CI: 78.2–100. Specificity: 96.6%, 95% CI: 91.3–99.1+/-; predictive values: 79.0% (95% CI: 54.4–93.9); 100% (95% CI: 96.7–100).</td>
</tr>
</tbody>
</table>

(2d) ARUP Institute for Clinical and Experimental Pathology

<table>
<thead>
<tr>
<th>Manufacturer Website</th>
<th>Instrument/ method</th>
<th>Tests, evaluation study objectives</th>
</tr>
</thead>
</table>

(2e) Ebola Virus Disease Treatment Unit (EVDTU), Kerry Town, Sierra Leone, West Africa [16]

<table>
<thead>
<tr>
<th>Clinical team</th>
<th>Instrument/ method</th>
<th>Tests, details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wessex Neurological Centre, Southampton &amp; Academic Department of Military Medicine, Royal Centre for Defence Medicine, Medical Directorate, Joint Medical Command, ICT Centre, Birmingham, UK</td>
<td>NS</td>
<td>RTPCR diagnosis, malaria rule out; POC monitoring of ACT, Hb &amp; Hct, transfused blood products given; hemorhage &amp; coagulopathy resolved, negative RTPCR upon discharge</td>
</tr>
</tbody>
</table>

Abbreviations: ACT, activated clotting time; BSL, biosafety level; CBC, complete blood count; Cr, creatinine; EVD, Ebola virus disease; Glu, glucose; Hb, hemoglobin; Hct, hematocrit; Lac, lactate; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; NS, not specified; pCO2, partial pressure of carbon dioxide; pO2, partial pressure of oxygen; PT/INR, prothrombin time/international normalized ratio; RBC, red blood cell; TBL, total bilirubin; TCO2, total carbon dioxide content; THb, total hemoglobin; TP, total protein; UN, urea nitrogen; and WBC, white blood cell.

When performed properly with biohazard precautions in the near-patient testing area of a DC, results will be available much more quickly than sending specimens to a public health laboratory or to the Centers for Disease Control and Prevention (CDC). The gain in time can be substantial, just 1 hour or less needed to obtain an answer (see Table 1), which facilitates rapid screening, focused triage, and effective workflow. Self-contained cartridge/cassette-based rapid molecular tests are available on small portable platforms that test for infectious diseases. Development of POC molecular diagnostics for high-risk infectious diseases forecasts the feasibility of introducing Ebola assays on light-weight platforms, such as the Alere I (see http://www.alere.com/us/en.html), and the tiny light-weight Roche Diagnostics cobas Liat (see https://usdiagnostics.roche.com/en/instrument/cobas-liat.html); both of these nucleic acid testing devices are Clinical laboratory Improvement Amendments (CLIA)-waived, user-friendly and, therefore, good candidates for point-of-need testing.

If tests satisfy certain conditions, they can be ‘waived’. In other words, the tests are cleared by the US Food and Drug Administration (FDA) to be performed in clinics and possibly even at home. Testing is simple to carry out and the instruments are operator-friendly, which make chances of an inaccuracy less likely. Such tests are referred to as a CLIA-waived. We will see facilitated-access, self-testing (FAST) POC solutions emerge as industry moves forward in the chronological evolution of Ebola EUAs in Table 1, some of which will be appearing commercially.
The only glucose meter cleared by the U.S. FDA for use with critically ill patients

In the last several years an unacceptably high number of adverse patient events and more than 16 deaths\(^1\) have been traced to the use of glucose meters in hospitals in the U.S. The FDA has just announced that it now requires hospital meters to be designed for and tested on critically ill patients in order to be cleared for use in these patient populations. To date, only one meter, the Nova StatStrip Glucose Hospital Meter System has been found to be accurate enough to obtain this new FDA clearance.

StatStrip Glucose has been designed specifically to be free of clinical interferences that can be present in critically ill patients. The proof data submitted to the FDA included:

- 1,698 individual critical care patients from five university medical centers had StatStrip Glucose results paired with an IDMS traceable laboratory glucose reference method.

- Data from multiple intensive care settings representing 19 medical condition categories and 257 subcategories as designated by the World Health Organization were included.

- Over 8,000 medications representing 33 parent drug classes and 134 drug subclasses as designated by the United States Pharmacopeia were studied for possible clinical interferences; no clinical interferences were observed.

All other glucose meters currently in use with critically ill patients are now classified as “off-label” by the FDA and become subject to “high complexity testing” requirements under CMS\(^2\). These requirements are so stringent that off label use of glucose meters on critically ill patients is not a practical alternative. Testing would not be performed by nurses, only by individuals degreed in laboratory medical technology.

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1. \(\text{DIABETES CARE, VOLUME 33, Number 4, April 2010.}\)

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as inexpensive, portable, safe, and appropriate for detection of virus in the early stages of clinical illness. True, we are behind on the timeline. However, the good news is that everyone recognizes the need, the problem has been defined, POCT is part of the solution, and the feasibility of immediate testing at points is proven, as summarized in Table 2.

The Ebola Spatial Care Path™

We define a Spatial Care Path (SCP) as the most efficient route taken by the patient when receiving definitive care in a small-world network (SWN). SCP principles include: (a) start diagnosis immediately wherever the patient is located; (b) implement POC technologies according to needs in the home, ambulance, primary care, SWN hubs, and at the bedside in critical care; (c) thereby achieve timely evidence-based decision making based on POC test results as the patient progresses through the SWN of healthcare; (d) coordinate access to the most critical elements and scarce specialists of the SWN to achieve a continuum of care; and (e) optimize the use of medical resources for the problem at hand, especially when the SWN becomes compromised or patients are selectively quarantined.

Spatial in this definition refers to shrewd positioning of POCT, elimination of unnecessary process steps, use of geographic information systems (GISs) to identify effective and efficient routes from population clusters to the nearest medical care, and in the case of Ebola, consolidation of SWN dispersion into one or more community alternative care facilities (ACFs) and DCs in which the useable space and workflow are optimized. Figure 1 illustrates the Ebola SCP with ACF and embedded POCT (on the left) integratively connected to a current expedient solution (on the right) of an individual hospital isolation area with a limited number of beds. A strategic Ebola SCP will deploy the best available molecular diagnostic testing at the point of initial patient contact and eliminate time-consuming steps in the sequence of care, such as transporting high risk Ebola patients from one community to another or sending hazardous samples to reference laboratories in heavily populated cities. Designing SCPs will facilitate prevention, intervention, and resilience in the event of wider presence of Ebola and simultaneously, will fulfill community recommendations of the CDC. We propose that each regional SWN analyse and ready its own SCP with POCT.

The Diagnostic Centre and interpretation of test results

Figure 2 shows the DC designed for Ebola care in Southeast Asia. POCT within the biosafety cabinet (top left) comprises: (a) the Spotchem EZ (Arkay, http://www.arkrayusa.com/) for determination of glucose, total protein, albumin, ALT, AST, alkaline phosphatase, cholesterol, triglycerides, HDL, urea nitrogen, creatinine, calcium, and total bilirubin, or combinations thereof (this instrument has been used for support of patients with viral hemorrhagic fever in Ghana); (b) the Opti CCA-TS2 whole blood analyser (http://www.optimedical.com/products-services/opti-CCA-TS2.html) for measurements of pH, pCO2, pO2, total hemoglobin, oxygen saturation, Na+, K+, Ca++ (ionized or free calcium), Cl−, glucose, urea nitrogen, and lactate, but only eight of these analytes at one time using a directly loading syringe cartridge that minimizes contamination; (c) a hematology instrument (optional), such as the QBC Star (http://www.druckerdiagnostics.com/hematology/qbc-star/qbc-star-centrifugal-hematology-analyzer.html), a dry reagent analyser that produces a nine-component complete blood count [hematocrit, hemoglobin, MCHC (mean corpuscular hemoglobin concentration), platelet count, white blood cell count, granulocyte count and percentage, and lymphocyte/monocyte count and percentage] from a specialized sample tube with stains and float separator inside, or the HemoCue CBC-DIFF (http://www.hemocue.com/en/products/white-blood-cell-count/wbc-diff); and within the isolation confines, (d) a vital signs monitor (e.g. V’Trust TD-2300).

Premonitory POC test results, such as initial leukopenia, suppressed lymphocyte count on the differential, increased percentage of granulocytes and thrombocytopenia help confirm the diagnosis of Ebola. Later, patients have increased white blood cells (WBC), immature granulocytes and atypical lymphocytes. West Africa should
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be replete with POCT and DCs, but is not, thereby handicapping expeditious detection of premonitory signs and evidence-based critical care support in treatment centers. Striking electrolyte changes need monitoring to support repletion. Unfortunately, there is no small FDA-cleared handheld device for monitoring of coagulation (except PT/INR when adjusting warfarin anticoagulant, where PT is prothrombin time and INR is international normalized ratio). Filoviral hemorrhagic fever is accompanied by prolonged PT, activated PTT and bleeding time, potentially progressing to DIC with elevated D-dimer. D-dimer is available on the handheld cobas h232 (Roche Diagnostics, http://www.cobas.com/home/product/point-of-care-testing/cobas-h-232.html) available outside the U.S. As demonstrated by the two recent U.S. Ebola patients, platelets are consumed rapidly early in the course of the infection, and should be trend mapped to see recovery, possibly along with assessment of platelet function. Note that fatally infected patients fail to develop an antibody response. Thus, the detection of virus-specific IgM and IgG is a good prognostic sign. In critically ill Ebola patients, plasma loss and bleeding affect hemoglobin and the hematocrit, both of which should be monitored at the point of care.

Conclusions
POCT is facilitating global health. Now, global health problems are elevating POCT to new levels of importance for accelerating diagnosis and evidence-based decision making during disease outbreaks. Authorities concur that rapid diagnosis has potential to stop disease spread. New technologies offer minimally significant risks for personnel and can be used in conjunction with risk prediction scores for patients. With embedded POCT, strategic SCPs planned by communities fulfill CDC recommendations. POC devices should consolidate multiplex test clusters supporting Ebola patients in isolation. The ultimate future solution is FAST POC. DCs in ACFs and transportable formats also will optimize Ebola SCPs.

Acknowledgements and disclaimer
Spatial Care Path™ is a trademark by William Ferguson and Gerald Kost, Knowledge Optimization®, Davis, CA. Figures and tables were provided courtesy and permission of Knowledge Optimization®, Davis, California, and Visual Logistics, a division of Knowledge Optimization®. Figure 2 was created by Lab Leader Company, Ltd., Bangkok, Thailand. Devices must comply with jurisdictional regulations in specific countries, operator use limitations based on patient conditions, federal and state legal statutes, and hospital accreditation requirements. Not all POC devices presented in this paper are cleared by the FDA for use in the U.S.A. FDA emergency use authorization is limited in scope and term. Please check with manufacturers for the current status of Ebola diagnostics and POC tests within the relevant domain of use.
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References and notes


2. The FDA Emergency Use Authorization (EUA) status can be found at: http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCLegalRegulatoryandPolicyFramework/ucm182568.htm#current.


6. Instrumentation and corporate/academic relationships may have changed. See ‘Letters of Authorization’ on the FDA EUA webpage for details. Contact company and investigator sources for updates.


10. FDA-cleared for warfarin monitoring only.


12. Ebola assay FDA-cleared for emergency use only.

13. Beckman-Coulter, La Brea, California, manufactures the DxI800 and DXC800i.


The authors

Gerald J. Kost* MD, PhD, MS, FACB (emeritus); William Ferguson BS, MS; Anh-Thu Truong; Daisy Prom; Jackie Hoe; Arirat Banpavichit MS, MBA; Surin Kongpila MS

Point-of-Care Center for Teaching and Research (POCT•CTR), School of Medicine, University of California, Davis, CA, USA

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Sound diagnostics: rapid point-of-care nucleic-acid based tests for sexually transmitted infections

One of the major avenues for addressing the rising impact of sexually transmitted infections lies with rapid, early diagnosis to break the cycle of transmission. Here we discuss the potential of a new technology, using the mechanical energy of sound waves, to drive integrated point-of-care diagnostics.

by Dr Julien Reboud, Gaolian Xu and Prof. Jonathan M. Cooper

Point-of-care diagnostics for sexually transmitted infections

Infectious diseases have a huge impact on both health and morbidity – causing more than half of the deaths in low-resource countries. To reduce the impact of these diseases, it is now accepted that early diagnosis is needed in order to break the cycle of infection and transmission. The development of rapid, high performance molecular diagnostic technologies, such as those involved in nucleic acid testing (NAT), has the potential to provide a much-needed step-change in treatment, through the early diagnosis of infection. Importantly, NATs can also be used to identify resistant strains of bacteria, an important step-change in the fight against the evolution of antimicrobial resistance (AMR).

One group of diseases that continues to increase in all areas of the world are the sexually transmitted infections (STIs). For example, chlamydia (caused by *Chlamydia trachomatis*) and gonorrhoea (caused by *Neisseria gonorrhoeae*) remain highly prevalent throughout the world. The WHO/CDC estimate chlamydia to affect 11m in Europe/Central Asia and 5.2m in the US per year; with gonorrhoea affecting 1.1m in Western Europe and >0.7m in the US per annum.

Sexual health clinicians have rated point-of-care (POC) testing as their top priority with their key concern being ‘in-clinic’ latency. Current testing protocols using NATs require an amplification process such as polymerase chain reaction (PCR) or isothermal amplification (e.g. loop-mediated isothermal amplification (LAMP)). When implemented in a laboratory or clinic, the workstream often requires sending samples to an external laboratory, a process that takes several hours. This results in the patient leaving the clinic. Patients then have to be recalled to the clinic for treatment, during which time they remain infectious for others and at risk of developing complications from the infection. Some never return, and remain untreated and a risk to others. The most vulnerable patients from high-risk groups such as the very young or men who have sex with men are less likely to engage with services. About 10% of all those diagnosed in the National Chlamydia Screening Programme in England in 2012 have never been treated. Those patients presenting to clinical services who report recent exposure to chlamydia or gonorrhoea may be treated with antibiotics pending their lab results, even though around half will turn out not to be infected.

Treatment for gonorrhoea now involves parenteral third-generation cephalosporins combined with an oral antibiotic, and there is evidence of increasing drug resistance. Good antibiotic stewardship seeks to limit unnecessary exposure of the population to these agents.

POC testing is a paradigm closely associated with self-diagnosis. Such near-patient devices are easy to use (by untrained people) and are rapid. Other characteristics include the integration of processing steps from sample to answer at a low cost [1]. POC testing of STIs would not only be relevant in developed healthcare systems, but also in the home (bathroom testing) as well as in resource-limited countries (where testing would often be delivered by a healthcare worker within a community) [2]. In all cases, the ability to ‘multiplex’ (testing multiple possible infections) and provide decision support around treatment are desirable. As stated, much evidence already exists that such a test would be desired by both by clinicians [3] and patients [4]. POC testing for chlamydia for example is also likely to be cost-effective. A mathematical model using costings from one of the few commercially available POC tests (Cepheid Xpert CT/NG) was shown to reduce testing costs by up to £16 and save 10 minutes of a healthcare professional’s time per patient [5].

Although there has been significant development in technological research for highly sensitive sensors, along with integrated microfluidic devices, the widespread adoption of POC tests has been limited by appropriately sensitive performance in real patient samples (blood, saliva, urine or feces, for example). Notwithstanding this, the relevance of decentralizing testing has been evidenced in Australia, for example, where a historical systematic review of interventions to prevent HIV and STIs in young people found that testing increased if a non-clinical, non-primary care health-care setting was used [6]. This data confirms what many clinicians are aware of, that in the specific case of sexual health, there is a reticence for individuals to engage formally with healthcare systems.

Acoustic technology for lab-on-a-chip POC diagnostics

Many proposed lab-on-a-chip devices currently rely on a variety of different mechanisms for preparing the sample prior to sensing, such as external pumps and heaters, leading to expensive and complex systems. In addition, microfluidic systems are often constrained by both difficulties associated with the chip interconnection to other instruments, and by difficulties that arise as the sample is moved through the chip (not the least of these being blockages). One outcome is that such diagnostic chips tend to be complex – a fact that increases the cost of the manufacture of the chip and ultimately the cost of the test. We have developed a new technology based on surface acoustic waves to integrate sample manipulation onto low cost disposable
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devices to enable the multiplexed detection of chlamydia and gonorrhea, using isothermal amplification [7].

Acoustic waves contain a mechanical energy that can be used to manipulate fluids. A range of ultrasonic transducers have already been developed, including those using both bulk acoustic waves (BAWs) and surface acoustic wave (SAW) devices [8]. Here we use a widespread configuration where a high frequency electric field is applied to a piezoelectric chip to create an ultrasonic wave, which propagates into the sample. We have now demonstrated a new proprietary technology using the interaction of SAW with fluids and phononic metamaterials [9] that has enabled us to create a tool-box’ of different diagnostic/medical instrumentation functions (including sample processing, cell separation [10], cell lysis [11], PCR [12] and nebulization [13]). Just as in electronics, where discrete components are combined to create a circuit, so we have begun to use different combinations of phononic lattices to create fluidic microcircuits, each of which provides a unique diagnostic function. The approach removes the need for any off-device processing, making sample processing a seamless, simple and fully automated process. Unlike conventional microfluidics, where the sample moves through the chip, our technology simply relies upon controlling the excitation frequency of the acoustic fields within a stationary droplet.

We have recently demonstrated the implementation of isothermal amplification (through LAMP) on our acoustic platform [7], enabling the multiplexed detection of both chlamydia and gonorrhea on a single disposable device, down to a sensitivity of 10 copies. Usually, the acoustic platform results in faster detection, through accelerated mass transfer, which is of paramount importance for a POC platform. We believe that the ease of implementation of both SAW technology and LAMP will have the potential to significantly impact upon near-patient diagnostics.

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References


The authors

Julien Reboud* PhD, Gaolian Xu MSc, Jonathan M. Cooper PhD
Division of Biomedical Engineering, School of Engineering, University of Glasgow, Glasgow, UK

*Corresponding author
E-mail: Julien.reboud@glasgow.ac.uk
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New standards, clinical pathways required to maximize benefits

Point-of-care testing (POCT or POC testing) describes diagnostic tests which are performed at or physically close to a patient. This distinguishes POCT from traditional testing, which involves extracting specimens from a patient and transporting them to a laboratory for analysis. Settings for POC tests range from in-hospital bed sites and primary care offices to patient homes.

Turnaround time key to POCT appeal
The principal objective of POCT testing is to reduce turnaround time (TAT) - a reference to the duration between a test and the obtaining of results which aid in making clinical decisions. In the past, such a process was unavoidable because of the sophistication and size of equipment required for the vast majority of medical diagnostic tests. However, technology developments have since made it possible to perform a growing number of tests outside of the laboratory.

Product miniaturization
Since the late 1980s, one of the key drivers of POCT has been product miniaturization with dedicated onboard integrated circuits. As described in a recent book on biomedical engineering, increasingly sophisticated microdevices have made it feasible to diagnose disease at point-of-care. These include “microfilters, microchannels, microarrays, micropumps, microvalves and microelectronics”, with their mechanical and electrical components “integrated onto chips to analyse and control biological objects at the microscale.” The authors list the key advantages offered by miniaturizing diagnostic tests as compared to centralized laboratory testing: portability, small size and low power consumption, simpler operation, smaller reagent volumes, faster analysis, parallel analysis, and functional integration of multiple devices.

Healthcare reforms drive POCT
Healthcare reforms have also driven POCT demand. Spending controls and hospital mergers have led to shorter stays and faster patient turnaround. There have been growing demand for tests in outpatient clinics and patient homes. Test results have been needed quickly, not only for reasons of clinical urgency but also to ease patient waiting lists and reduce backlogs in emergency departments. Accompanying this has been the closure of several large central laboratories, which have further enhanced demand for POCT.

Making a case
The case for POCT has grown with time. In 2004, it was associated with a significant reduction in the time to treatment initiation and a shorter length of stay. More recently, a POCT cardiac marker screening stage at six UK hospitals led to a marked increase in the percentage of successful home discharges. Such breakthroughs will increase as POCT use grows further, and as the tests become more sophisticated.

Early POC tests were based on the simple transfer of traditional methods from a central laboratory, accompanied by their downscaling to smaller platforms. Subsequently, unique and innovative assays were designed specifically for POCT (such as the rapid streptococcal antigen test). Wide arrays of POCT-specific analytic methods have also been developed, ranging from simple (such as pH paper for assessing amniotic fluid) to the ultra-sophisticated (for example, thromboelastogram for intraoperative coagulation assessment).

Contemporary POCT systems are usually based on test kits and portable, often handheld, instruments. Many tests are realized as easy-to-use membrane-based strips, often enclosed by a plastic cassette. This requires only a single drop of whole blood, urine or
saliva, and they can be performed and interpreted by any general physician within minutes.

**Hospital emergency departments**

Given its time-sensitive relevance, one of the fastest growing users of POCT have been hospital emergency departments (EDs).

In 2008, a study in ‘Academic Emergency Medicine’ simulated the impact of reduced turnaround times and established grounds for a “compelling improvement in ED efficiency.” Though its authors concluded that specific outcomes such as the length of stay and throughput in the emergency department warranted further investigation, they categorically recommended POCTs as a means to improve turnaround time.

Over recent years, favourable perspectives on POC tests in the ED have strengthened. At the end of last year, a study in ‘Critical Care’ found POCT increased the number of patients discharged in a timely manner, expedited triage of urgent but non-emergency patients, and decrease delays to treatment initiation. The study quantitatively assessed several conditions such as acute coronary syndrome, venous thromboembolic disease, severe sepsis and stroke, and concluded that POCT, when used effectively, “may alleviate the negative impacts of overcrowding on the safety, effectiveness, and person-centeredness of care in the ED.”

Other POCT users include ICUs as well as endocrinology, cardiology, gastroenterology and hematology.

**Primary care remains principal user**

The bulk of POC tests are however conducted by primary care physicians.

In 2014, the ‘British Medical Journal’ published the findings of the first-ever survey of POCT use by primary care physicians in five countries (Australia, Belgium, the Netherlands, the UK and the USA). The study found that blood glucose, urine pregnancy and urine leukocytes or nitrite were the most frequently used POC tests. Overall, more respondents in the UK and the USA reported using POC tests than respondents in the other countries. The widest gap in use of POC test was for fecal occult blood, used by 83% of US doctors against only 2–18% of primary care clinicians in the other countries. One of the key findings of the ‘British Medical Journal’ study, however, was that there was an unmet need for new POC tests. Included here were tests for D-dimer, troponin, chlamydia, gonorrhea, B-type natriuretic peptide, CRP, glycated hemoglobin, white cell count and hemoglobin, which were desired by more than half of respondents across all the five countries.

**Fast growing market**

Over the past two-and-a-half decades, the availability and use of POCT has steadily increased. By 2012, nearly 100 companies worldwide were developing, manufacturing or marketing POC tests. One study, cited by the National Institutes of Health in the US, places POCT sales in 2011 at about $15 billion (€13.5 billion). Of this figure, the US accounted for a share of 55%, Europe for 30% and Asia for 12%. The market is projected to show compound annual growth of 4% to reach $18 billion (€16.2 billion) by 2016.

Further growth in the use of POCT is expected to be driven by increases in accuracy, reliability and convenience. Alongside, one of the biggest catalysts for increased POCT use may consist of quality standards.

**The quality challenge**

Issues about POCT quality continue to vex experts. Variability in the interpretation of POC test results is a widespread concern, given differences in the education and experience of staff who conduct the

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tests. In addition, POCT results may also not be comparable across sites (e.g. when patients travel) and differences in specimen types (serum, plasma or whole blood) can impact on results – as compared to those from a traditional central laboratory.

In a laboratory setting, analytical quality is usually assessed by QC (quality control) and QA (quality assurance) procedures. Their aim is “to monitor the stability of the analytical measurement system and to alert the operator to a change in stability”...“that may lead to a medically important error.” While these processes serve a laboratory well, it is unclear whether these processes are relevant, transferable and practical for monitoring quality on POCT devices.

Regulators and POCT in the US and the EU

Future developments are expected to be driven by regulatory bodies. In the US, CLIA88 (Clinical Laboratory Improvement Amendments of 1988) provided a major impetus for growth in POCT. The rules, published in 1992, expanded the definition of ‘laboratory’ to include any site where a clinical laboratory test occurred (including a patient’s bedside or clinic) and specified quality standards for personnel, patient test management and quality. One of CLIA88’s biggest contributions to POCT growth was to define tests by complexity (waived, moderate complexity and high complexity control), with minimal quality assurance for the waived category. CLIA88 has been followed by US federal and state regulations, along with accreditation standards developed by the College of American Pathologists and The Joint Commission. These have established POCT performance guidelines and provided strong incentives to ensure the quality of testing.

In Europe, POCT devices are regulated under the 1998 European Directive 98/79/EC on in vitro diagnostic medical devices, although the term itself is not specifically mentioned. There have since been several amendments, most recently in 2011 (2011/100/EU), as well as standards based on the Directive’s framework. However, at the European level, specific coverage of POCT is referred to by international standard ISO 22870:2006, used in conjunction with ISO 15189 which covers competence and quality in medical laboratories. It is important to note that patient self-testing in a home or community setting is not covered by ISO standards.

The role of ICT

The role of ICT in driving the growth of POCT is also likely to become crucial. In the late 1990s, there were concerns that POCT implementation, especially in the real-time critical care context, was accompanied by little understanding of its information technology requirements. However, the situation has since changed dramatically, especially as ICT is seen as the only appropriate interface between POC test results and computerized patient records – seen as the means to restructure clinical care pathways. ICT is also accepted as the best means to standardize care protocols. In 2012, a study found that the impact of point-of-care panel assessment on successful discharge and costs varied markedly from one hospital to another and that outcomes depended on local protocols, staff practices and available facilities. In effect, the study highlighted the importance of optimizing clinical pathways to derive maximum benefit from the reduced turnaround times provided by POCT.
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| Precision Intra-Assay (%) | 2.60 |
| Precision Inter-Assay (%) | 3.50 |
| Linearity (R²) | 0.999 |

25-OH Vitamin D3

| LOD (ng/L) | 1.48 |
| LOQ (ng/L) | 4.90 |
| Accuracy (%) | 98.50 |
| Precision Intra-Assay (%) | 4.60 |
| Precision Inter-Assay (%) | 5.40 |
| Linearity (R²) | 0.988 |

The test results have been validated with an AB Sciex API 4000 LC-MS/MS instrument in Nov. 2014.

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In spite of increased publicity in the Western world about malaria and drives to provide mosquito nets, the disease is still endemic in a large part of the world. This article discusses different methods of malaria diagnosis and the role that point-of-care tests can play in the ultimate goal of malaria elimination.

by Dr Jackie Cook

Finding the balance: over- and under-diagnosing malaria
Malaria remains a huge burden in many parts of the world, particularly in sub-Saharan Africa. Despite increased availability of effective treatments and interventions, malaria elimination is still out of reach for many countries. Whilst availability of effective interventions that reduce contact with infected mosquitoes, such as insecticide treated bed-nets or indoor spraying with insecticide are key to reducing malaria prevalence, case management also plays a key role. Many who need treatment are unable to get it, either through lack of access to healthcare, or because infections remain undiagnosed. Conversely, some studies suggest that many patients are receiving anti-malarials unnecessarily due to a tendency to diagnose based solely on clinical symptoms, many of which are similar to other infections, rather than using a diagnostic. In under-resourced settings, this can result in any child presenting with a fever being prescribed malaria drugs. This simultaneously means non-malaria fevers remain undiagnosed and untreated, as well as a large proportion of unnecessary prescriptions for malaria drugs, which increases healthcare costs and the risk of drug resistance, a very potent threat. In reality, the sensitivity of the test depends greatly on the microscopist. In areas where malaria transmission is declining, microscopists can go months without seeing a positive slide, and as such, skills may begin to decline. In addition, the need for well-maintained microscopes and access to slides and stain can mean microscopy is not always available.

Rapid diagnostic tests
The first malaria rapid diagnostic test (RDT) was developed in 1993 and in the decades since many variations have proliferated on the market. RDTs are typically immunochromatographic tests that use monoclonal antibodies to detect the presence of plasmodium antigens (proteins produced by the parasite) which are present in the blood of infected, or recently infected, individuals. They are generally stable at a range of temperatures and do not require special storage conditions. RDTs require significantly less training for use than microscopy and a positive infection is easy to identify by visualization of a ‘positive’ line, meaning the results are much less subjective. Most RDTs require 15–20 minutes for development, meaning treatment can be given while patients wait at health facilities.

However, there are a few downsides to the use of RDTs. The presence of parasite antigen doesn't always equate with a current infection, but can signify a recently cleared infection from within the previous two weeks. In addition, several studies have reported the deletion of certain antigens detected by RDTs in plasmodium parasites, meaning false-negative results may be obtained in areas using these types of RDTs [1]. The World Health Organization (WHO), in collaboration with the Foundation for Innovative New Diagnostics (FIND), has set up an RDT product testing programme, an essential quality assurance component considering the huge influx of RDT brands that have popped up in the past 20 years [2]. The reports from the programme make worrying reading with very low sensitivity for some brands, differences between batches of RDT and a general lower sensitivity for non-falciparum infections for nearly all brands.

The hidden reservoir: asymptomatic, low-density infections
In general, the limited sensitivity of both microscopy and RDT (unreliable detection in infections with a parasite density less than 100 parasites per microlitre) is not an issue for symptomatic malaria infections, the majority of which will consist of high parasite densities. However, asymptomatic infections are numerous, in high and low transmission settings. These asymptomatic infections pose a problem for control programmes. The carriers do not feel unwell so have no reason to present to a health facility for testing and yet, they may be infectious to mosquitoes, meaning they pose a risk for onward transmission. In order to detect and treat these asymptomatic infections, malaria programmes are now taking their diagnostics into the community in a strategy termed Mass Screening and Treatment (MSAT). This involves testing everyone within a community regardless of whether they have symptoms. Many of these infections are asymptomatic and therefore also likely to be low-density; hence which test you use can mean the difference between detecting 10 infections or 100 infections. Whilst RDT is ideal for field conditions, studies have shown that they can miss a large proportion of infections that are present [3].

Molecular tests
Polymerase chain reaction
More sensitive diagnostics are available in the terms of molecular tests. The most commonly used is polymerase chain reaction (PCR). Numerous PCR assays have been developed, many based on amplifying the 18S ribosomal RNA (18S rRNA), first published by Snounou and colleagues in 1993 [4]. PCR detects parasite nucleic acids and can detect much lower parasite...
Point-of-care testing for AMI and Heart Failure

Point-of-care testing has been one of the most rapidly growing areas in clinical diagnostics. More and more traditional testing is moving to patient side testing. This is most evident in high acuity settings, e.g. emergency rooms, intensive and critical care units, and operating rooms where the patient’s condition changes rapidly and care decisions must be made quickly.

Point-of-care testing has numerous advantages over traditional lab-based testing, such as:

- **Immediacy of test results near the site of patient care to expedite decision making, thus improving patient health outcomes.**
- **Portable equipment that requires minimal or zero maintenance, allowing tests to be carried out in a variety of locations to meet a diversity of medical needs.**
- **Small sample volumes, which means less blood loss and anemia for patients requiring frequent testing (especially for neonatal and pediatric patients).**
- **Tests can be performed with different type of specimens: serum, plasma, or whole blood.**
- **Efficiency: Point-of-care testing has significantly fewer moves by eliminating steps such as transporting samples to a central laboratory and then processing and communicating results back to the physician.**

To increase market acceptance and to enable end users to further realize the benefits, EDAN’s point of care devices continue to focus on several key areas: expansion of test menu, improvement of product functionality, and cost reduction of consumables.

**Expansion of test menu:** in addition to further improving test accuracy and reliability for biomarkers such as cTnl, Myo, CK-MB, NT-proBNP. EDAN is developing and introducing to market new tests including D-dimer, CRP, PCT, NGAL and IL-6 etc. In addition, EDAN is also developing tests for infectious disease such as Strep B/B, legionella, HBsAg test, mononucleosis, malaria, respiratory viruses, influenza, meningitis, filariasis, adenovirus, tuberculosis, Epsteinbarr virus, measles, and mumps, rubella, chlamydia, gonorrhea, HIV and MRSA etc.

**Zero maintenance** - EDAN’s portable equipment utilizes single-use disposable cartridge format and has a very unique advantage over traditional analyzers: it is maintenance-free and always ready for patient analysis. Thus it permits analysis in facilities previously not possible - such as community and rural clinics, ambulances, medical helicopters, and medical transport aircraft.

**Network connectivity** – wired (LAN) or wireless (WIFI) transmission of information directly into LIS/HIS. Automatic aspiration of samples - makes sampling easy and eliminates handling errors. Colour graphic display and touch screen with intuitive user interface.
densities than RDT or microscopy, with tests reportedly able to detect down to 1 parasite per microlitre of blood, as well as being able to accurately distinguish between plasmodium species. However, the number of assays available has resulted in calls for a standardized test so results can be compared across the world. PCR tests are generally performed on blood collected on filter paper but the equipment required for PCR and the expense of maintaining a sterile lab environment precludes PCR from being available in many health facilities. This means that samples need to be sent away, with an often long wait for results. Although more field-friendly PCR methods are in the pipeline, currently, PCR is not generally considered suitable for a point-of-care test, although it’s use in epidemiological studies is undisputed.

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) was first developed in 2000, with the aim to amplify DNA in a sensitive, specific and speedy manner (Figs 1, 2). One of the main advantages is the fact it can be performed under isothermal conditions, and thus averting the need for a thermocycler. LAMP can be thought of as a ‘rough-and-ready’ PCR, as it is also less sensitive to inhibitors present in biological samples, and therefore allows the use of simple and cheap DNA extraction methods. The fast time-to-results and the minimal equipment required make LAMP an attractive option for field diagnosis. In order to make this a viable option, FIND and partners Eiken Chemical Ltd, Japan, and the Hospital for Tropical Diseases (HTD), London, UK, have developed a field-stable kit with all reagents freeze-dried into the lid of the reaction tube, which means minimal processing is required. Although still in the development and testing stage, current results of the use of the kit are promising, with strong agreement with PCR results and a considerably higher sensitivity than RDT [5-8]. Whilst seemingly the most sensitive of the point-of-care tests available, there are some downsides to LAMP. Results still take considerably longer than RDT, requiring patients to wait at clinics for 2 hours for results, or leaving the health facility staff with the complicated task of contacting and following up any positive patients. In addition, electricity is required for the processing of samples, making it not practical for many places.

Future for point-of-care diagnostics for malaria

These advances in molecular diagnostics mean infections that would previously have remained undetected can now be confirmed, treated and cleared. Identifying and treating all infections becomes a greater priority as transmission reduces and the possibility of elimination comes into focus. This is occurring in areas around the world such as Swaziland and Zanzibar in Africa and in South East Asia, where the need to eliminate has become even more important with the emergence of drug-resistant parasites. In these areas, identification of every last parasite is the aim and development of a quick, sensitive and reliable diagnostic is key to that. As more studies reveal the extent of the low-density parasite reservoir, there is a sense of ‘the more we look the more malaria we will find’. But do we need to find all these infections in order to eliminate malaria? It should be noted that these ‘super-sensitive’ tests are a relatively recent phenomena and that countries have succeeded in malaria elimination without them. The role these low-density parasites play in transmission is not fully understood but for now the aim remains to clear the last parasite standing.

References


The author

Jackie Cook PhD
London School of Hygiene and Tropical Medicine, London, UK
E-mail: Jackie.cook@lshtm.ac.uk
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Tumour markers for oropharyngeal cancers

Head and neck cancers (HNC) are a globally prevalent malignancy. Despite the efforts of reducing several known etiological factors such as smoking and drinking to lower the incidence of HNC at the population level, the incidence of oropharyngeal cancers (OPC) is on the rise. OPC is caused by human papillomavirus (HPV) and most prevalent in a younger age group. This review critically examines the epidemiology, biology and laboratory detection of OPC and provides future insights into combating this debilitating disease.

by X. C. Sun, P. Tran and Dr C. Punyadeera

Introduction

Head and neck cancers (HNC) are the sixth most prevalent neoplasm in the world with approximately 650,000 cases diagnosed each year [1–5]. Oral and oropharyngeal squamous cell carcinomas (OSCC & OPSCC) together constitute 90% of malignancies in the head and neck region. Several known traditional etiological factors such as tobacco and alcohol use are recognized in the development of these cancers. More recently, human papillomavirus (HPV) infection is recognized as an additional risk factor for the development of a subset of HNCs, mainly OPSCC [6].

In recent decades, the overall incidence of HNC caused by smoking and alcohol is on the decline. In contrast, HPV⁺ve OPSCC is on the rise. In developed countries such as the United States of America, the incidence of HPV⁺ve OPSCC is escalating, with predictions that more than 50% of patients will be HPV⁺ve by 2030 [7]. Interestingly, patients who are HPV⁺ve OPSCC are relatively younger than HPV⁻ve HNC patients and are therefore less likely to have any history of chronic or excessive alcohol or tobacco use but are more likely to engage in social habits that increase the likelihood of HPV transmission (oral sex). The clear distinction between HPV⁺ve OPSCC and HPV⁻ve cases provides multiple downstream inputs that can be applied into clinical treatment modalities. Conversely, it provides an exciting opportunity for the development of early diagnostic and screening methods to combat HNC at a population level through prevention strategies.

HPV⁺ve OPSCC are both clinically and biologically distinct tumour entities compared with HPV⁻ve counterparts. Classically, HPV⁺ve OPSCC patients present with a molecular profile that includes retinoblastoma (pRB) pathway inactivation, p53 degradation and p16 upregulation. Clinically, HPV⁺ve OPSCC patients often present with smaller primary tumours but more advanced nodal disease, similar rates of metastasis and differing patterns of metastasis [8, 9]. In addition, patients with HPV⁺ve tumours have better prognosis with 5-year survival at 75% (c.f. 25% for HPV⁻ve patients).

There are a number of techniques for the diagnosis and detection of HPV⁺ve OPSCC, including histopathology, polymerase chain reaction (PCR) and immunohistochemistry (IHC).

Biology

Upon integration of HPV DNA into the host genome, E6 and E7 viral oncoproteins activate a number of pathways within the host cell. The primary molecular target of E7 is the Rb protein and the E7 viral oncoprotein reprogrammes terminally differentiated epithelial cells to re-enter the cell cycle. E7 disrupts the Rb–E2F complex leading to the release of E2F, subsequently resulting in cyclin A and E activation and entry of the cell into S phase. As a consequence p16 is over-expressed [10, 11]. The E6-associated protein (E6-AP) is a specific ubiquitin-ligase that binds to the viral E6 oncoprotein, resulting in p53 degradation. E6 and E7 have also been shown to interfere with growth inhibitory cytokines [such as tumour necrosis factor-α (TNFα)] and to disrupt the mitochondrial apoptotic pathway by interfering with pro-apoptotic BAK and BAX [10]. E6 and E7 alone are insufficient to cause malignant cell transformation; however, due to their interference with proliferation checkpoints and apoptotic pathways, it is certain that the accumulation and damaged

Figure 1. A schematic representation of human papilloma virus oncoproteins E6 and E7 integration leading to malignant cellular transformations. (Adapted from Salazar et al., Expert Reviews in Molecular Medicine, 2014 [18]).
DNA, mitotic defects and integration of foreign DNA substantially increase the risk of malignant progression [10].

**Detection methods**

A number of diagnostic methods are currently available to evaluate whether a tumour is HPV+. These methods include both indirect as well as the direct methods; i.e. the latter includes the detection of HPV genomic DNA (gDNA). Besides clinical examination, current methods for the diagnosis of HPV status include tissue biopsy staining for p16 (indirect method). Biopsies may fail when tumours are too small to access or when they are located in hidden anatomical sites [10]. Other methods include the detection of HPV gDNA using PCR and in situ hybridization (ISH) as well as the detection of HPV viral transcripts E6 and E7 by PCR.

p16 detection by IHC is widely used in cervical cancer cases for the detection of HPV and it is being studied extensively in the field of HNC [12]. During immortalization of host cells, the E7 protein binds to Rb, resulting in the compensatory overexpression of the tumour suppressor gene p16 in HPV-infected tumour cells. Therefore, IHC detection of p16 is considered as an indirect surrogate marker to determine the presence of HPV [11]. However, there are pitfalls associated with p16 IHC detection. A number of studies have shown suboptimal specificity of IHC [10, 11]. As a consequence of the extreme anatomical and biological heterogeneity in HNC, elevation of p16 by non-viral materials may contribute to a considerable false positive rate [11]. Although it has been reported that p16-positive patients have a better prognosis and increased radiosensitivity, it has been advised that p16 detection by IHC alone cannot accurately identify HPV infection in HNC [12].

Detection of HPV gDNA is a widely used method because of its high sensitivity and cost-effectiveness. Common primers (MY09/MY11 and GPS/GP6) that target the L1 open reading frame are used to detect wide-spectrum HPV genotypes [11]. However, standard PCR primers do not allow detection of specific HPV genotypes [10]. In addition, the target L1 region could also be deleted upon viral integration, which may affect sensitivity of the test [10, 11]. Although, specific E6 and E7 primers have been designed and used to overcome L1 deletion, this method still lacks the ability to distinguish stromal/tumour and episomal/integrated DNA materials and is prone to contamination interference, which undermines the clinical usefulness [11].

The HPV DNA ISH method is unique because of its high specificity and the ability to be evaluated microscopically. The visible hybridization signals that precipitate within the nuclei help distinguish integrated and episomal DNA [11]. It is noteworthy that the presence of HPV DNA detected by ISH significantly correlates with p16 detection by IHC. [10]. However, ISH methods carry lower sensitivity compared to its excellent specificity [11]. The detection of HPV-16 viral transcripts E6 and E7 can highlight whether a patient is suffering from persistent infection – information that is clinically more valuable [12]. However, because of the fragile nature of mRNA, formalin-fixed paraffin-embedded (FFPE) specimens are often not ideal for RNA analysis and frozen fresh specimen are required [12].

The detection of HPV-specific IgG in serum is a useful biomarker to determine previous and current HPV infection status [13]. Serological biomarkers are not site-specific, and can arise due to HPV infections at sites other than the oral cavity, hence potentially affecting the specificity of the assay.

**The effect of Gardasil™**

From treatment and management of HPV-related diseases, the paradigm of HPV care has shifted to a preventative approach since the breakthrough introduction of the HPV vaccine, Gardasil™ (Merck & Co.). The biologic basis of HPV vaccines relies on the mechanism of neutralizing antibodies generated against virus-like particles (VLP), which consist of the major capsid protein HPV L1 [10]. The quadrivalent HPV 6/11/16/18 vaccine Gardasil™ was licensed by the FDA to prevent cervical, vaginal and vulvar infections in women in 2006 and genital warts in men in 2009, followed by the licensing of bivalent HPV 16/18 vaccine Cervarix™ (GlaxoSmithKline), in women in 2009 [14].

The benefits of HPV vaccination for the oral cavity include not only the biologically-plausible direct effect on oral infections, but also the sequential oral infection reduction following genital infection reduction, due to the sexually transmitted nature of HPV. To date, there are only a few studies examining the effect of Gardasil on oral infection; however, the results showed a promising outlook with high vaccine efficacy (as high as 93% in 4 years time, as recorded by randomized controlled trial in Costa Rica) [15] and reduced viral prevalence (oral prevalence dropped to 1.4% from 9.3% in the 15–23 age group in youth clinics in Sweden) [16].

**Future outlook**

As previously mentioned, HPV-related OPSCC involve a new segment of the population, which is distinctively different from the traditional HNC patient cohort caused by excessive smoking and drinking. This requires clinicians to conduct thorough cancer screening of at-risk groups. Such screening programs should pay particular attention to cervical lymph nodes as some subtypes of HNC, especially OPSCC, involve hard-to-examine areas for clinical visual examination.

As a result of the sexually transmitted nature of HPV, some studies have advocated routine sexual behaviour education in clinical practice. However, this practice carries inherent controversies of sexual harassment and confidentiality [17]. Public awareness campaigns have been argued to be a more efficient preventative means in altering patients’ behaviour. Studies have shown that many oral health practitioners have limited knowledge with regards to HPV-related HNC and HPV vaccinations [17]. Professional bodies and health authorities are required to address this knowledge gap by establishing new clinical guidelines and using continuing educational methods, in order to effectively control the rising trend of HPV-related HNC.

**Conclusion**

All current diagnostic methods require excision of tumour tissue and this can be challenging when they are located in hidden sites. Efforts have been made globally to develop a less invasive, more cost-effective and clinically-relevant test. Serology tests that detect HPV-specific IgG have been shown to indicate viral presence and are linked with prognostication; however, this method inherently lacks site specificity [10]. Oral specimens, more specifically oral rinse, have shown promise in this field. Oral rinse
samples not only are non-invasive and cost-effective, the proximity of collection to the area of interest ensures the localized sampling field. It is also important to note that shedding of normal cells into the oral cavity/oral pharynx may interfere with and/or decrease the HPV detection level [10]. OraRisk* HPV test, uses oral rinses for HPV detection [10]. Translational collaborations between scientists and clinicians have resulted in an assortment of tumour markers and diagnostic techniques for OPSCC. However, these need to be tested in clinical trials to determine the cost-effectiveness.

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The authors
Xiaohang Charles Sun1, Peter Tran1 and Chamindie Punyadeera2 MSc, PhD
1School of Dentistry, The University of Queensland, Brisbane, Australia
2The Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

*Corresponding author
E-mail: chamindie.punyadeera@qut.edu.au

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INNOVATIVE SOLUTIONS FOR EFFECTIVE DIAGNOSTICS
Detection of von Hippel–Lindau (VHL) gene copy number variation

Detection of copy number variations (CNV) in the VHL gene is part of the genetic workup of VHL-related tumours. Current methods for CNV determination have complex workflows and limitations. Digital droplet PCR is a promising methodology that could be used for CNV determination. Its advantages include shorter turnaround time, decreased DNA input and superior precision.

by Dragana Milosevic, Dr Stefan K. Grebe, Dr Alicia Algeciras-Schimnich

Background
Von Hippel-Lindau (VHL) disease is an autosomal dominant cancer syndrome with an incidence of approximately 1 in 36,000 live births. It predisposes affected individuals to the development of five main types of neoplasms: retinal angioma (>90% penetrance), cerebellar hemangioblastoma (>80% penetrance), clear-cell renal cell carcinoma (~75% penetrance), spinal hemangioblastoma (~50% penetrance), and pheochromocytoma (~30% penetrance). The disease is caused by mutations or large deletions in the VHL tumour suppressor gene (VHL). The VHL gene is located on chromosome 3p25-26 and encodes a protein that is involved in ubiquitination and degradation of a variety of proteins, most notably hypoxia-inducible factor (HIF) [1]. HIF induces expression of genes that promote cell survival and angiogenesis under conditions of hypoxia. It is believed that diminished HIF degradation due to inactivation of the VHL protein causes the tumours in VHL disease. Tumours form when the remaining intact copy of the VHL gene is somatically inactivated in target tissues.

VHL patients are subdivided in two groups, based on the genotype/phenotype correlations; those at low risk of developing pheochromocytoma are designated type I, whereas those with a high risk of pheochromocytoma (with or without renal cell carcinoma) are classified as type II. Deletions in the VHL gene are more common in type II VHL syndrome [2, 3]. To date, there have been more than 300 germline mutations and large deletions identified in the VHL gene that cause loss of function [2]. Germline loss-of-function point mutations and small deletions or insertions accounts for approximately 70–80% of cases; whereas large germline deletions of one copy of the VHL gene accounts for approximately 20–30% of cases.

VHL genetic testing
The clinical diagnosis of VHL disease is suspected in individuals who present with one or several of the characteristic tumours described above. Molecular genetic testing of VHL is performed to confirm the clinical diagnosis. The genetic testing includes sequencing of the three exons of VHL gene and evaluation of copy number variations (CNV) to assess deletions of large regions of the gene. Historically, detection of these large deletions was done by Southern blot. Today, most clinical laboratories offering CNV determination use multiplex ligation probe amplification (MLPA)-based assays. MLPA is a method based on sequence specific probe hybridization, ligation and PCR amplification and detection of multiple targets with a single set of universal primers. CNVs are detected by comparison of the signal from each target region to control genes and normal control samples.

Although MLPA-based assays are of superior quality and more robust than previous technologies, technical success of MLPA assays is dependent on input of high quantities (at least 400ng of germline DNA) of high quality DNA. Although less labour intensive than Southern blotting, the MLPA work flow is still more complex than PCR-based assays and typically takes two days until completion. Finally, MLPA does not allow for absolute quantification and cannot distinguish copy numbers greater than three with high accuracy.

Digital droplet PCR
Digital droplet PCR (ddPCR) is a methodology that has gained favour as a robust alternative with improved precision to quantitative real-time PCR (qPCR) for DNA quantification. DdPCR also lends itself to exact CNV determination, detection of rare variants, translocations, and/or point mutations (SNP genotyping).

DdPCR is based on traditional PCR amplification and fluorescent probe-based detection methods, but partitions each reaction into 15 000–20 000 nanodroplets. Provided that the starting DNA concentration is not too high, some of these reactions will contain one or more target DNA molecules, whereas others will not contain any. Those with at least one target DNA molecule will yield an amplification product, while those without won’t. Quantification is based on counting the proportion of droplets that show amplification, using a microfluidic counting device. The proportion of reactions with and without amplification obeys Poisson statistics and allows back-calculation of the starting concentration based on the distribution function. When enough droplets are used, copy number ascertainment is of unprecedented accuracy and reproducibility (CVs of 2–10%) [4]. Compared to standard qPCR methods, ddPCR eliminates the need for standard curves and measures both target and reference DNA within the same well. Applications where ddPCR has been used include: rare allele detection in heterogeneous tumours, assessment of tumour burden by analysis of peripheral body fluids (mainly blood), non-invasive prenatal diagnostics, viral load detection, CNV, assays with limited sample material such as single cell gene expression and archival formalin-fixed paraffin-embedded (FFPE) samples, DNA quality control tests before sequencing, and validation of low frequency mutations identified by sequencing.
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Recently, ddPCR has become commercially available in a format that allows for rapid microfluidic analysis of thousands of droplets per sample making it practical for routine use in clinical laboratories. A recent study on the analytical performance of ddPCR has shown greater precision (CVs decreased by 37–86%) and improved day-to-day reproducibility and comparable sensitivity to real-time PCR for absolute quantification of microRNAs [5]. A study that evaluated the use of ddPCR to detect BCR-ABL1 fusion transcripts demonstrated that ddPCR is able to achieve lower limit of detection and quantification than currently used in quantitative PCR methods [6].

Our group has evaluated ddPCR for VHL CNV and shown improved performance compared to MLPA [7]. The method showed 100% concordance with the MLPA method and 100% self-concordance within and between runs. The method showed reproducible results with DNA inputs as low as 10 ng, a 40-fold DNA-input reduction compared with MLPA. Because of this advantage, difficult specimen types, such as archival FFPE specimens, are now capable of being characterized for VHL CNVs, a feat previously impossible by MPLA, because of the often poor DNA quality of such samples (Fig. 1). Additionally, same-day results are available with the ddPCR method, reducing the total run-time from 48 hours for the MLPA method to 3 hours.

One limitation of current ddPCR platforms is the limited ability for multiplexing. For example the Bio-Rad’s ddPCR system can detect only two colours (FAM and HEX), limiting the number of genes that could be evaluated simultaneously in a single reaction. Development of platforms that allow greater multiplexing should, therefore, further facilitate the adaptation of this technology in clinical laboratories.

Figure 1. Comparison between MLPA (left panels) and digital droplet PCR (right panels) methods for VHL gene deletion detection in germline and paraffin-embedded tissue DNA samples.

In the MLPA assay a ratio between 0.8 and 1.2 (green lines) represents 2 copies of a gene, a ratio below 0.8 represents 1 copy and ratio above 1.2 represents >2 copies (note: VHL exons 1–3 were tested in duplicate).

In digital droplet PCR, copy numbers are represented numerically (red line, 1 copy; blue line, 2 copies).

(a) Copy number variation (CNV) in two high concentration and quality germline DNA samples; one with two copies of the VHL gene (top panel) and one with a single copy deletion of the VHL gene.

(b) CNV in two DNA samples obtained from formalin-fixed paraffin-embedded (FFPE) tissue blocks; one with two copies of VHL gene (top panel) and one with a single copy deletion of the VHL gene.
Conclusions

Improvement of current methods for VHL CNV testing is desired to obtain accurate and cost-effective results in clinical laboratories. Currently used methods are still labour intensive and not suitable for rapid turnaround time. DdPCR is an elegant adaptation of the current quantitative PCR format and has the potential to be applied widely in clinical laboratories. For VHL CNV, ddPCR provides a greatly improved turnaround time and requires only minimal nucleic acid input that does not have to be of the highest quality. With no need for standard curves or controls, ddPCR overcomes the issues associated with traditional qPCR, while increasing both robustness (superior sensitivity, specificity, and precision) and utility in other specimen types such as paraffin-embedded tissue, circulating cell-free DNA, circulating tumour cells, and microRNA detection [8–10].

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The authors

Dragana Milosevic MS; Stefan K. Grebe MD, PhD; Alicia Algeciras-Schimnich* PhD
Department of Laboratory Medicine and Pathology, Rochester, MN, USA
*Corresponding author
E-mail: Algeciras.Alicia@mayo.edu

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Diagnostically challenging cases: distinguishing primary from secondary ovarian tumours

Tumours found in the ovaries can be either from primary ovarian tumour processes or metastases (secondary tumours) foremost from colorectal cancer (CRC), appendiceal tumours or stomach cancer. Correctly distinguishing between these tumour subsets using hematoxylin-eosin staining in combination with immunohistochemistry can be problematic [1–3], but is crucial for correct treatment choice. Mutation profiles, generated in a fast and cost-effective way by (targeted) Next Generation Sequencing (NGS), can assist in correctly diagnosing ovarian tumours.

by Stijn Crobach and Prof. Hans Morreau

Background
The ovaries are a preferential location for metastases from, among others, colon, stomach, appendiceal, breast and endometrium carcinomas. The percentage of secondary ovarian tumours (metastases), varies in several reports ranging from 8–30% [4, 5]. Several reasons can be given to explain why the range of percentages is so broad. First, studies are different by design. Some studies are based on autopsy findings, others on prophylactic oophorectomies. Second, differences in incidence of primary tumours can cause a variance in patterns of metastases. For example, stomach cancer has a higher incidence in Japan than in many other countries; therefore, metastases of stomach cancer to the ovaries are expected to be more common in Japan. In general, however, the gastrointestinal tract (GIT) seems to be the main source of ovarian metastases [5].

Macroscopic and histologic approaches
A gross distinction between primary and secondary ovarian tumours can be made taking tumour size and unilaterality versus bilaterality into account [6]. Following the decision tree depicted in Figure 1, it is possible to estimate whether an ovarian tumour is a primary tumour or a metastasis. A unilateral ovarian tumour with a diameter larger than 10 cm is probably a primary tumour. All bilateral and unilateral tumours smaller than 10 cm are much more likely to be metastases.

The histologic characteristics of metastatic GIT ovarian tumours can resemble primary endometrioid and mucinous ovarian tumours, but not serous papillary or clear cell tumours. Thus, based on histology a subset of primary ovarian tumours does not cause diagnostic doubt about the origin of the malignancy. Furthermore, other histologic findings can assist in defining the malignancy. For example, on the one hand, surface involvement by malignant epithelial cells is much more often seen in metastases than in primary ovarian tumours. On the other hand, however, an expansive growth pattern is more often seen in primary ovarian tumours. So, with the help of histopathological findings the characterization of a primary origin or a metastatic process becomes more achievable.

Immunohistochemical approaches
The logical next step in differentiating primary ovarian tumours from metastases is with the use of immunohistochemistry. For example, primary ovarian tumours are classically positive for keratin 7 and negative for keratin 20, whereas colorectal tumours show the opposite staining pattern (keratin 7 negative, keratin 20 positive) [7]. Other markers can also be used, not only to rule out an ovarian origin of the tumour but also to get an idea about the location of the primary tumour. Positivity of intestinal markers [such as carcinoembryonic antigen (CEA) and caudal type homeobox 2 (CDX-2)] can be an argument for an intestinal origin of the tumour cells [8].

Furthermore, when a colon carcinoma is already diagnosed before the ovarian tumour is discovered, the staining profile of the metastasis can be compared with the primary tumour. However, in up to 38% of cases the detection of ovarian metastases precedes the detection of the primary tumours. Also, secondary primary ovarian tumours can occur in patients that anamnestically suffered from another malignancy, complicating the diagnostic procedures. In practice,

Figure 1. Decision tree based on size and unilaterality versus bilaterality used to determine whether the ovarian tumour is a primary tumour or a metastasis.
immunohistochemistry is frequently not fully discriminating. As mentioned, primary ovarian tumours tend to have a Ker7+/Ker20− immunoprofile and colonic metastases a Ker7−/Ker20+ immunoprofile. Nevertheless, keratin 7 positivity can be seen in proximal located GIT tumours, and keratin 20 positivity can also be seen in primary ovarian malignancies. In Figure 2, a guided immunohistochemical decision scheme is shown for complex cases.

**Molecular diagnostic approaches**

With the combined use of clinical information, histologic features and immunohistochemical staining patterns, differentiating primary tumours from metastases is possible in a substantial subset of cases. With a history of a colorectal tumour and the presentation of a large ovarian mass a few years later showing a similar immunoprofile, it is not difficult to decide that this tumour is a metastasis. Nevertheless, there are cases that are not as clear-cut. In those cases tumour size, unilateral-ity versus bilaterality and the histologic findings are not discriminating enough to solve the challenge. New approaches using massive parallel DNA sequencing (Next Generation Sequencing; NGS) have emerged in recent years.

"It is possible to differentiate primary tumours from metastases in a substantial subset of cases using combined clinical information, histologic features and immunohistochemical staining patterns..."

Cancer driver genes (oncogenes and tumour suppressor genes) can be screened for DNA mutations in different tumour types. In the Catalogue Of Somatic Mutations In Cancer (COSMIC; http://cancer.sanger.ac.uk/cosmic), literature on these profiles has been compiled [9]. It was hoped that comparing mutational profiles of primary ovarian tumours versus metastases from different organs would reveal specific mutation patterns and/or mutation types in different tumour types.

NGS enables the screening of a large number of genes in a fast and cost-effective way. Previously, Sanger DNA sequencing was used to detect mutations in clinically relevant genes. However, screening complete genes and multiple genes in this way is a time-consuming process. Now, with the introduction of the disruptive NGS technology, it is possible to sequence multiple genes at the same time. NGS will become a standard technique in diagnostics for identifying gene mutations, chromosomal rearrangements and RNA expression/mRNA patterns [10]. One would expect that large scale screening of molecular alterations will result in very specific profiles per tumour type. Each tumour type could be defined by subsets of mutated genes. However, recent studies show that the mutation profiles do not differ so much between tumour types [11]. A few well-known so-called cancer driver genes seem to be important in many malignancies. Other (passenger) mutations, which are also needed in tumorigenesis, seem to be interchangeable. Apparently, there is wide overlap in mutation profiles. Looking at mutations described in the COSMIC database or The Cancer Genome...
Atlas (TCGA) at the current time, similar mutations can be seen in both primary ovarian tumours and metastases, although with different frequencies. The latter would suggest that the applicability of such tests is limited. However, a more select approach shows that certain genes can be discriminatory.

For example, CTNNB1 mutations are found in primary endometrioid carcinoma of the ovary. CTNNB1 mutations are also found in colon tumours, but only in mismatch repair deficient colon tumours, that do not tend to metastasize to the ovary. This reasoning could also be followed for APC, which is frequently mutated in colon carcinomas but not typically in mucinous and endometrioid primary ovarian carcinomas. However, genes such as these, which show such a ‘black-and-white’ phenomenon, are sparse. Therefore, mutation profiles that are used to guide clinical decision taking will probably be based on combining information from multiple genes. Most of these genes will not provide significant differences on their own, but a combination of odds-ratios will make one diagnosis more probable than the other.

Along with solutions at a mutational level, characterizing the transcriptome, methylation patterns and copy numbers of a tumour could also provide useful information. This field of ‘omics’ has developed rapidly in recent years. In diagnostically challenging cases from unknown primary tumours (UPT) or alternatively named carcinoma of unknown primary (CUP), expression array based assays were developed in order to identify the primary tumours. Genomics will also probably become effective in determining the origin of the tumour. Furthermore, in depth comparison of molecular features of synchronously presenting tumours at different sites might reveal whether the tumours have arisen independently or are clonally related. The readout of these tests can be seen in the context of increased odds-ratios. The use of such tests is still in a premature phase, and not used routinely in clinical practice.

Summary

In conclusion, a combination of the various molecular features will hopefully reveal specific molecular profiles that can be used to correctly identify the origin of malignancies in problematic cases. These techniques are applicable on ovarian tumours, to determine whether tumours are primary ovarian in origin or metastases to the ovaries [12].

References


The authors

Stijn Crobach BSc; Hans Morreau MD, PhD
Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands

*Corresponding author
E-mail: j.morreau@lumc.nl
Familial Hypercholesterolemia

Familial Hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism. It is a common autosomal dominant, or inherited, disease which affects the plasma clearance of LDL-cholesterol (LDL-C), resulting in premature onset of cardiovascular disease (CVD) and a higher mortality risk.

Early diagnosis of FH is very advantageous as by the time heterozygous FH sufferers enter early adulthood they will have accumulated years of continuous build-up of fatty or lipid masses in arterial walls and are at one hundred-fold greater risk of a heart attack than their non-FH peers. If left untreated, men and women with heterozygous FH with total cholesterol levels of 8–15 mmol/L typically develop coronary heart disease (CHD) before age 55 and 60, while homozygotes with total cholesterol levels of 12–30 mmol/L typically develop CHD very early in life and if untreated die before age 20.

Clinical diagnosis of FH relies on five criteria: family history, clinical history of premature CHD, physical examination for xanthomas and corneal arcus, very high LDL cholesterol on repeated measurements, and / or a causative mutation detected by molecular genetics. To formally quantify this, a number of sets of statistically and genetically validated criteria have been devised; namely the Dutch Lipid Clinic Network Criteria and the Simon Broome Criteria. These classify suspected cases into definite, possible and probable diagnoses of FH. In the absence of definitive diagnosis through detection of a causative mutation using molecular genetics, clinical diagnosis could miss a considerable proportion of FH patients, particularly those with a mild phenotype and the pediatric population in whom the phenotype has not appeared yet.

The UK, US and international guidelines now recommend that probable or possible FH patients undergo a DNA test to confirm the diagnosis of FH. Recommendations also advocate that once an activating mutation has been found in one family member (the index case), cascade screening of that mutation in first degree relatives of the index case should proceed. Cascade screening using a molecular assay can thus identify index family members who may otherwise be asymptomatic.

The good news is that if detected early, FH can be treated successfully with lipid lowering therapy and lifestyle changes. In comparison to other hyperlipidemias, FH therapy tends to be more aggressive, so definitive diagnosis has additional benefits in determining care packages. Statin drug therapy significantly reduces the morbidity and mortality from premature coronary disease in FH, particularly if affected individuals are identified and treated in childhood or early adulthood. Accurate and early diagnosis of specific mutations can result in a...
better overall outcome for patients through the prescribing of tailored treatments to reduce morbidity and mortality from premature cardiovascular disease. Different mutations can dictate different directions of management, such as the poorer response to lipid-lowering therapy with certain LDLR mutations. The identity of the gene involved can potentially aid the clinician to decide on how aggressive the treatment strategy will be.

Mutation diagnosis also provides clarity, and can help with an individual’s understanding and acceptance of their condition. Also a greater compliance with cholesterol lowering medication is observed with those who have been genetically diagnosed with FH.

**Mutational profiling of FH**

Currently, ~1200 mutations have been documented worldwide in LDLR; these affect all functional domains of the LDL receptor protein and include single-nucleotide mutations, copy number variations, and splicing mutations throughout the LDLR gene. A single mutation, Arg3500Gln, is the only common FH-related mutation in APOB, while c.1120G>T mutation is predominately detected in PCSK9. Heterozygous LDLR, APOB, and PCSK9 mutations are found in >90%, ~5%, and ~1%, respectively, of heterozygous FH subjects with a causative mutation. Prevalence varies geographically.

The abundance of different FH mutations can make genetic testing labour-intensive and costly, with many laboratories defaulting to performing expensive and lengthy Next Generation Sequencing (NGS) tests in an effort to ensure a comprehensive mutational screen. However, as our understanding of the genetic drivers of FH, as well as common population-specific mutations, increases, novel assays and techniques are being developed to meet the needs facing clinical genetics laboratories, including cost, throughput and time to result.

Randox Laboratories have developed The Familial Hypercholesterolaemia (FH) Arrays I and II that are rapid, simple and accurate diagnostic tests which enable simultaneous detection of 40 FH-causing mutations (20 mutations per array) within the LDLR, ApoB and PCSK9 genes. The assay is based on multiplex PCR followed by biochip array hybridization. Using mutation rate data from a study of 500 UK and Ireland families with genetically-confirmed FH, the Randox FH Arrays are capable of detecting approximately 71% of activating mutations in this population. The mutations will also be detected in other geographical regions.

The assay can be completed from extracted DNA to an easy-to-interpret result report in 3 hours, with the requirement for only 20ng of genomic DNA per array. The system can be used to detect small base changes, insertions and deletions within the same multiplex PCR, allowing addition of new FH mutational targets if required. The arrays are designed for use on the Evidence Investigator (Randox Laboratories Limited, Crumlin, UK). This instrument has been developed alongside Randox’s proprietary Biochip Array Technology (BAT), a multiplex testing platform founded on ELISA principles that currently has application within clinical immunoassays, drug development R&D, clinical research, forensic and clinical toxicology, veterinary drug residues and molecular diagnostics.

**FH Array I and II workflow**

Randox’s multiplex assays, such as FH Array I and II, have been specifically designed to detect the most common mutations, provide a cost-effective and clinically relevant alternative to NGS testing. Targeting the most commonly detected mutations in a given population enables diagnosis within hours rather than months. Where a mutation is identified in an index patient, cascade testing of family members only requires the mutation in question to be targeted; therefore negating the use of broad profiling approaches such as NGS in this setting.

**Conclusion**

FH is a common yet underdiagnosed condition that poses a significant risk to public health worldwide. In 2008, cardiovascular diseases were the leading cause of non-communicable deaths worldwide, with an estimated mortality rate of 17 million people. Raised cholesterol was attributed to 2.6 million deaths. Understanding a person’s genetic predisposition to cardiovascular disease through genetic testing will allow patients to receive appropriate therapeutic and interventional treatment to reduce morbidity and mortality associated with cardiovascular disease. Pioneering multiplex diagnostic assays, tailored to incorporate the relevant FH-causing mutations, provide a promising future for both genetic laboratories, where a rapid, cost-effective approach to determine mutational status in cases of suspected FH is enabled, and the patient, whose treatment and care pathway is managed effectively.

**The author**

Martin Crockard, PhD
Randox Laboratories Ltd.
55 Diamond Road, Crumlin, Co. Antrim U.K.
Human papillomaviruses
Human papillomaviruses are uncoated double-stranded DNA viruses which infect epithelial cells of the skin and mucous membranes. They are transmitted by sexual contact. Infection is assumed to occur via tiny lesions in the basal cells of the epithelium. Thus, the most frequent place of infection is the transformation zone of the cervix, where dividing basal cells lie near to the surface. The size of the cells, their histology and the duration of the lesion can influence the number of cells infected. The course and outcome of the infection depends on the HPV type, the anatomy of the infection site and the differentiation status of the host cells. Infections with HPV are always local and are not accompanied by viremia. Following infection, the viral DNA is replicated in the host cell nuclei. Viral proteins produced in the infected cells can trigger uncontrolled tumour-like growth of the cells. This is, depending on the infecting HPV subtype, mostly benign, leading to warts at the site of infection. However, some HPV types can induce malignant changes, particularly cervical cancer. A significant proportion of vaginal, penile, anal and head and neck carcinomas are also assumed to be caused by HPV infection.

HPV subtypes
Around 130 types of HPV have so far been described of which 30 infect exclusively the skin and mucous membranes in the anogenital area. HPV are divided into two groups according to their oncogenic potential. High-risk HPV cause cervical carcinoma. Low-risk HPV alone do not induce tumours, but cause non-malignant tissue changes. Concurrent infections with multiple HPV subtypes are common and known to increase the risk of malignant cell transformations. Of the high-risk anogenital types, HPV 16 and HPV 18 are responsible for around 70% of cervical carcinomas. HPV 16 is found in 50 to 60% of cases and HPV 18 in 10 to 20%. Other types classified as high-risk by the WHO are 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66. Types 26, 53, 68, 73 and 82 have also been detected in cervical carcinoma and should be considered as high-risk types.

Of the low-risk types, HPV 6 and 11 are the main causative agents of genital warts (condylomata acuminata, fig warts). Further low-risk types are 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89.

Cervical carcinoma
HPV infection is a prerequisite for the development of cervical carcinoma. However, HPV infection does not necessarily lead to cancer. Most infected women eliminate the virus within two years. If the virus remains detectable for longer than 18 months, the infection is considered to be persistent. A persistent infection, in particular with a high-risk HPV subtype, increases the risk of developing cervical carcinoma by around 300-fold. HPV infections are often asymptomatic and tend to remain unnoticed. The initial stages of cervical carcinoma also proceed without pain, and the only symptom may be light bleeding. With increased tumour
size, the cancer manifests with a blood-tinged, sweet smelling discharge.

Around 528,000 new cases of cervical carcinoma occur annually worldwide, making it the fourth most frequent cancer in women after breast, colorectal and lung cancers. It is also the fourth most common cause of cancer mortality, causing approximately 266,000 deaths in 2012 (International Agency for Research on Cancer).

In the early stages, treatment involves removal of the altered tissue by conisation. In later stages of the disease, the uterus and surrounding tissue must be removed.

Role of HPV detection and typing

Along with the current diagnostic gold standard, the Papanicolaou (Pap) test, HPV direct detection plays an important role in the early diagnosis of cervical carcinoma. In contrast to the Pap test, which is used to investigate cervical cells for pathological changes, PCR-based methods detect viral nucleic acids directly, and can thus identify an HPV infection at a very early stage before morphological cell changes have even occurred. Moreover, while the Pap test is based on subjective evaluation, HPV detection represents an objective as well as extremely sensitive test method.

In HPV screening it is crucial to differentiate between high- and low-risk types and also to discriminate between different high-risk viruses. A positive result for high-risk HPV indicates an increased risk for cervical carcinoma, which can then be minimized by more frequent follow-up examinations to detect morphological cell changes at an early stage. A positive result for low-risk HPV can help to clarify uncomfortable and embarrassing symptoms for patients. Since low-risk HPV can also cause mild dysplasia, HPV subtyping is also useful for excluding a high-risk HPV infection and a corresponding risk of cervical cancer in these cases. Women who are HPV negative can forgo Pap smears for a longer time interval, based on the recommendations of the respective professional societies.

The PCR detection strategy is a critical aspect of direct HPV analysis. Tests with primer or probe systems based on conserved genes like L1 may yield false negative results in some cases due to loss of these genes during integration of the viral DNA into the host DNA. The highest possible detection sensitivity is achieved using the viral oncoproteins E6/E7. Detection of variable sequences in these genes enables differentiation of the different HPV subtypes.

Microarray for complete HPV typing

A standardized microarray based on PCR detection of E6/E7 has been developed for complete HPV typing in routine diagnosis. Using an extensive panel of specific primers and probes, the EUROArray HPV detects all thirty genetically relevant HPV subtypes in one test, distinguishing eighteen high-risk subtypes that may trigger cancer (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and twelve low-risk subtypes that cause benign warts (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89). Multiple infections are reliably identified, and primary and persistent infections can be differentiated.

Simple procedure with automated evaluation

The EUROArray procedure (Figure 1) is extremely easy to perform and does not require any in-depth molecular biology knowledge. DNA prepared from patient cervical smear samples is first amplified by a single multiplex polymerase chain reaction (PCR). The fluorescently-labelled PCR products are then incubated with biochip microarray slides (Figure 2) containing immobilized complementary DNA probes. Specific binding (hybridization) of the PCR products to their corresponding microarray spots is detected using a specialized microarray scanner.

In contrast to manually evaluated tests, the results are evaluated (Figure 3) and interpreted fully automatically by user-friendly software (EUROArrayScan). A detailed result report (Figure 4) is produced for each patient and all data is documented and archived. Meticulously designed primers and probes, ready-to-use PCR components and integrated controls all contribute to the reliability of the analysis. The entire EUROArray process from sample arrival to report release is IVD validated and CE registered, supporting quality management in diagnostic laboratories.

Conclusion

As evidence mounts about the efficacy of HPV testing for primary cervical cancer screening, multiplex microarrays are poised to become a major tool in prevention programmes worldwide. The EUROArray HPV, in particular, is ideally positioned for high-throughput HPV screening, providing fast and sensitive detection of all high- and low-risk anogenital HPV types combined with fully automated data analysis.

The author

Jacqueline Gosink, PhD
EUROIMMUN AG
Seekamp 31
23560 Luebeck
Germany
E-mail: j.gosink@euroimmun.de
A new vaccine against HPV infections has the potential to prevent 90 per cent of all of the conditions triggered by the human papillomavirus. These are the findings of a randomized, controlled, international study involving a new, 9-component vaccine against HPV used on more than 14,000 young women aged between 16 and 26 years. The study was led by Elmar Joura from the University Department of Gynecology at the MedUni Vienna. The study has now been published in the “New England Journal of Medicine”.

Nine sub-types of the human papillomavirus are responsible for 85 per cent of pre-cancerous cells of the cervix. The new, highly effective vaccine now means that these can largely be prevented. The new vaccine is 20 per cent more effective against cervical cancer than the previous 4-component vaccine, up to 30 per cent more effective against the early stages of cervical cancer and up to five to 15 per cent more effective against other types of cancer (such as vaginal or anal carcinoma).

Human papillomaviruses (HPV) infect epithelial cells in the skin and mucosal tissue and can cause tumour-like growth. Some of these viruses also develop malignant tumours, especially cervical cancer in women. Men too can develop cancer caused by HPV infections, however. Over a hundred HPV sub-types have now been identified.

In Austria, up to 400 women a year develop invasive cervical cancer. In more than 90 per cent of the cases human papillomaviruses are responsible. According to Statistik Austria, around 150 to 180 women die from the condition. In Austria, around 6,000 women are admitted to hospital every year for treatment of the early stages of cervical cancer.

The paper has also been featured in the New England Journal of Medicine’s editorial, which is a major honour. “This issue of the journal reports on a milestone in research into cancers associated with the human papillomavirus (HPV),” it says.

There has been a quadruple HPV vaccine since 2006 which protects against the most dangerous oncogenic HPV strains that cause cervical cancer and other types of cancer in the genital and throat area, but which also cause genital warts. The MedUni Vienna takes its responsibility in this area very seriously, and has not only initiated an HPV action day but has also provided a reasonably priced vaccination campaign for employees and students.

Introducing the DxN VERIS molecular diagnostics system from Beckman Coulter, Inc.

Delegates at ECCMID 2015, held in Copenhagen from 25th to 28th April 2015, attended a symposium introducing Beckman Coulter’s new DxN VERIS Molecular Diagnostics System.* DxN VERIS provides a fully automated sample to result platform with true single sample random access, integrating sample introduction, nucleic acid extraction, reaction setup, real-time PCR amplification, detection and results interpretation into a single system that is set to revolutionize laboratory workflows.

Speakers from four of the 10 DxN VERIS beta study sites shared their experiences and results from comparative evaluations of this new system.

Meeting molecular diagnostic needs
By way of introduction, Hervé Fleury described the molecular diagnostic needs in Europe, where laboratories are becoming fewer and larger, both in the public and private sectors. The number of molecular scientists available for routine tasks is also decreasing, he said, and there is a need for the level of automation, from preanalytics to analytical, that the DxN VERIS will bring.

He then described the DxN VERIS technology, which is able to provide results in approximately 75 minutes for DNA targets and in around 110 minutes for RNA targets, performing in excess of 150 and 100 results in 8 hours for DNA and RNA targets respectively. CE marked DxN VERIS assays for human cytomegalovirus (CMV) and hepatitis B virus (HBV) are already available, in addition to assays for hepatitis C virus (HCV) and human immunodeficiency virus (HIV). DxN VERIS products in the pipeline include assays for Chlamydia trachomatis and Neisseria gonorrhoea (CT/NG), MRSA (screen), Clostridium difficile, respiratory virus multiplex and human papilloma virus (HPV).

Excellent performance criteria
All four speakers at the ECCMID Symposium described excellent analytical and clinical performance criteria for the VERIS assays evaluated.

Jacques Izopet reported very good analytical performance results for all four VERIS assays that are currently available (table 1). In addition, these assays demonstrated good agreement with an alternative method (Cobas® Ampliprep/ Cobas® TaqMan™). Significantly, in a patient monitoring setting, the VERIS CMV assay demonstrated overlapping patterns compared to this alternative for plasma samples and compared to a whole blood reference method (figure 1).

Rafael Delgado then went on to present the results from his evaluation of the VERIS CMV and HBV assays. At his laboratory, both assays were extremely sensitive and specific, exhibited a high linearity and repeatability, and showed good correlation with an alternative method (Cobas Ampliprep/ Cobas TaqMan*) (figure 2). In addition, the system demonstrated no carry over when known high positive samples were interspersed among known negative samples.

Rafael Delgado concluded that the overall performance and easy to use design of the DxN VERIS platform facilitated the introduction of this technology in the laboratory and that the DxN VERIS CMV and HBV viral load assays are helpful new solutions for patient management.

Table 1. Analytical performance of VERIS assays at Toulouse University Hospital.

<table>
<thead>
<tr>
<th>VERIS Assay</th>
<th>Intra-assay reproducibility (SD log IU/mL)</th>
<th>Inter-assay reproducibility (SD log IU/mL)</th>
<th>Limit of detection (IU/mL)</th>
<th>Linearity (log IU/mL)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>≤ 0.21</td>
<td>≤ 0.12</td>
<td>14.6</td>
<td>2.3 – 5.6</td>
<td>98.9</td>
</tr>
<tr>
<td>HBV</td>
<td>≤ 0.15</td>
<td>≤ 0.15</td>
<td>5.4</td>
<td>0.93 – 8.23</td>
<td>100</td>
</tr>
<tr>
<td>HCV</td>
<td>≤ 0.12</td>
<td>≤ 0.12</td>
<td>13.9</td>
<td>1.47 – 6.35</td>
<td>100</td>
</tr>
<tr>
<td>HIV</td>
<td>≤ 0.11</td>
<td>≤ 0.11</td>
<td>24.3</td>
<td>2.44 – 6.91</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Comparative precision at lower levels of HBV DNA.

<table>
<thead>
<tr>
<th></th>
<th>Beckman</th>
<th>Qiaogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log1</td>
<td>0.12</td>
<td>0.26</td>
</tr>
<tr>
<td>CV</td>
<td>9.45%</td>
<td>18.20%</td>
</tr>
<tr>
<td>Log2</td>
<td>0.002</td>
<td>0.13</td>
</tr>
<tr>
<td>CV</td>
<td>3.64%</td>
<td>10.97%</td>
</tr>
<tr>
<td>Log3</td>
<td>0.073</td>
<td>0.026</td>
</tr>
<tr>
<td>CV</td>
<td>2.31%</td>
<td>4.80%</td>
</tr>
</tbody>
</table>

Figure 1. Comparative patient monitoring results at Toulouse University Hospital.
In his evaluation of the DxN VERIS HBV assay, Duncan Whittaker observed excellent precision (within and between run), with a standard deviation of ≤ 0.12, and a limit of detection of 7.99 IU/mL, which is less than the manufacturer's claim of 10 IU/mL. He described the existing method at the Sheffield laboratory as very manual (with separate extraction and amplification systems) which was adequate when they received just 10-12 HBV samples every two weeks but which struggles to cope now that they are receiving up to 80 samples per week.

Duncan Whittaker reported that the quantitative results from the VERIS assay were similar to their existing method (Qiagen) (figure 3) with improved precision at lower levels (table 2). He was also able to demonstrate excellent performance and reproducibility across HBV genotypes. In conclusion, he stated that the DxN VERIS Molecular Diagnostics System offers significant improvements in laboratory workflow and time.

Finally, Giovanni Gesu also shared his results from the evaluation of the DxN VERIS HBV assay. At the Niguarda ca' Granda Hospital in Milan, DxN VERIS HBV demonstrated excellent within and between run precision (SD ≤ 0.156), linearity (1.63 – 8.82 log IU/mL) and sensitivity (limit of detection 6.82 IU/mL), and performed well compared to an alternative HBV real time method (Abbott m2000).

In order to demonstrate the potential workflow and throughput efficiencies that the DxN VERIS platform could achieve, Giovanni Gesu applied the throughput capabilities of this new system to a typical day in his laboratory, in which 33 CMV, 17 HBV, 26 HCV and 21 HIV samples were received. With samples arriving at two hour intervals throughout the day between 10am and 4pm, the true single sample random access capability of the DxN VERIS platform combined with assay runtimes of around 70 minutes for DNA tests and around 110 minutes for RNA tests, would mean that samples would not need to be batched and that all results could be reported by 6pm on the same day (figure 4).

Conclusions
In conclusion, each of the speakers at the ECCMID Symposium agreed that the analytical performance of the DxN VERIS assays evaluated was excellent, and they compared well to other molecular diagnostic assays currently available. In addition, the sample-to-result automation and true single sample random access of the DxN VERIS Molecular Diagnostics System offer workflow improvements and laboratory efficiencies.

Figure 2. Comparative evaluation of VERIS CMV (a) and VERIS HBV (b) at Hospital Universitario 12 de Octubre, Madrid.

Figure 3. Comparative evaluation of the VERIS HBV assay at Sheffield University Hospitals NHS Foundation Trust.

Figure 4. Potential changes to laboratory workflow at Niguarda ca' Granda Hospital, Milan.

For further information about the DxN VERIS Molecular Diagnostics System and the DxN VERIS assays currently available, please contact: Tiffany Page, Senior Pan European Marketing Manager Molecular Diagnostics, Email: info@beckmannmolecular.com

*Not for sale or distribution in the U.S.; not available in all markets.
** TaqMan® is a registered trademark of Roche Molecular Systems, Inc. Used under permission and license.

Beckman Coulter, the stylized logo, DxN and VERIS are trademarks of Beckman Coulter, Inc. Beckman Coulter and the stylized logo are registered in the USPTO.
Immunochromatographic strip for STD pathogen detection

Operon has developed a new molecular diagnostic line based on the detection of amplified PCR products by immunochromatographic strip: the OligoGen products. This technology detects DNA from several pathogens associated with sexually transmitted diseases (STD) such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Herpes simplex*, *Mycoplasma genitalium* and more. The general procedure has three steps: DNA extraction, amplification and hybridization/detection. During this hybridization step, the specific DNA fragments bind to probes covalently bonded to latex colored particles (colloid). Subsequently, during immunochromatography, these DNA complex + colloid are detected by specific antibodies that recognize labels added during PCR, resulting in the appearance of a colored band pattern. The main advantages of these products include ready to use reagents provided in the kits, minimal equipment requirements and a hybridization step carried out in a tube, which increases the specificity and reproducibility of the process.

THE BINDING SITE
AACC, Atlanta, booth #3505
i www.cli-online.com & search 27012

Biochemistry urine control

Urine analysis is an integral part of laboratory routine and constitutes a first window to assess the individual physiological and metabolic state. All kinds of parameters have been progressively included in urine analysis, many of them present both in serum and in urine. Problems may arise when assessing the accuracy of results, because the user often only has serum matrix controls. So matrix commutability (analysis results independently from matrix type) is not guaranteed. Currently, general recommendations advocate the use of control material with identical (or similar) matrix to the one of the analysed sample whenever possible. Aware of this situation, Biosystems S.A. has developed a Multiparametric Urine Control specific for biochemistry tests (Albumin, alpha Amylase, alpha Amylase Pancreatic, Calcium, Chloride, Citrate, Creatinine, Glucose, Magnesium, Phosphorus, Potassium, Protein Urine, Sodium, Urea and Uric Acid). Values have been assigned using the recommended International Reference standards. This ensures traceability of results and comparison between laboratories. The urine control is lyophilized to guarantee maximum stability (expiration date of 24 months, 1 month at -20ºC after reconstitution) without matrix interferents. Along with this Biochemistry Urine Control, Biosystems also offers a specific Urine Control for the analysis of metabolites (5-ALA, 5-HIIA, 17-OH, 17-KETO, VMA, Metanephrines), which completes its range for routine urine analysis in all kind of laboratories.

THE BINDING SITE
AACC, Atlanta, booth #3415
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Special protein analysis

Designed for the special protein laboratory, Optilite is fully optimized to create simplicity from complex analytical processes. It enhances efficiency, saves time and reduces costs with its easy-to-use, intelligent system, making it possible to streamline workload through flexible, unrestricted access to samples, reagents and cuvettes for smart resource management and optimal productivity. Unique features include three methods of antigen excess detection providing unparalleled protection. The custom design ensures the analyser and assays work in harmony and maximizes their potential.

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BÜHLMANN
Automated digital IFA microscope

NOVA View, an automated digital IFA (indirect fluorescent assay) microscope has received FDA de novo clearance. NOVA Lite DAPI ANA Kit, an IFA reagent indicated for use with NOVA View, received 510(k) clearance at the same time. Anti-nuclear antibodies (ANA) is the first line laboratory test for the diagnosis of systemic lupus erythematosus and other systemic autoimmune rheumatic diseases. Early and accurate diagnosis and effective treatment are key to reducing the morbidity associated with these conditions. IFA is the gold standard for ANA testing according to a Position Statement issued by the American College of Rheumatology in 2009. IFA, as it is currently practiced in many laboratories, is time and labour-intensive, subject to interpretation bias and other variables, such as the type of microscope and intensity of the light source. The workflow is manual, and prone to transcription errors. NOVA View addresses many shortcomings of the manual IFA process by reducing hands-on time, providing consistent reading and interpretation conditions, and generating digital images that can be archived. It provides patient sample traceability with positive patient identification and improves assay integrity by using NOVA Lite barcoded IFA slides. The instrument helps standardizing the ANA IFA test and provides clinicians with a reliable test result. In addition, the capability of providing an IFA test result that can be seamlessly uploaded into the patient's electronic medical record will be a remarkable advancement in prompt and accurate reporting. NOVA View Automated Fluorescence Microscope is an automated system consisting of a fluorescence microscope and software that acquires, analyses, stores and displays digital images of stained indirect immunofluorescent slides. It is intended as an aid in the detection and classification of certain antibodies by indirect immunofluorescence technology. The device can only be used with cleared or approved in vitro diagnostic assays that are indicated for use with the device. A trained operator must confirm results generated with the device. NOVA View determines the result (positive or negative), and performs ANA pattern interpretation. After pattern confirmation by the operator, the instrument is able to predict a pattern-specific endpoint titre. Results are recorded electronically in a transcription-free and paperless work environment, and all digital images are archived for future review. NOVA Lite DAPI ANA Kit is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of anti-nuclear antibodies of the IgG isotype in human serum by manual fluorescence microscopy or with NOVA View Automated Fluorescence Microscope. The presence of anti-nuclear antibodies can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of systemic lupus erythematosus and other systemic rheumatic diseases.

INOVA DIAGNOSTICS
AACC, Atlanta, booth #3211
i www.cli-online.com & search 26978
QuikRead go system is an extremely fast point-of-care test system for measuring C-reactive protein (CRP) in whole blood samples: CRP results are available in two minutes. The system simplifies the daily routines of healthcare professionals by bringing reliability and speed to everyday working processes. The intuitive user interface with a large touch screen and a variety of user interface language options add to the easiness of use. The CRP test can be performed near the patient and the CRP result is immediately available to support the diagnosis. The test selection includes CRP, CRP+Hb, hsCRP+Hb, iFOBT and Strep A tests. This year two new products, wrCRP and wrCRP+Hb, will be launched in the product portfolio.

**ORNIO DIAGNOSTICA**

i www.cli-online.com & search 27017

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**Glucose meter offers wireless connectivity**

Nova Biomedical’s StatStrip Glucose Hospital Meter System now offers bidirectional wireless connectivity to the hospital’s HIS or LIS with complete security to protect patient data. The wireless connectivity can transmit patient results directly from the patient bedside, alleviating the need to bring the meter to a fixed location for meter docking and data transmission. Wireless connectivity saves time for the caregiver and allows for faster charting of results and clinical decision making to improve patient care. StatStrip Glucose’s dual-band wireless connectivity provides complete security and encryption to ensure that patient data remains uncompromised. Nova now offers a full range of StatStrip Glucose wireless connectivity capabilities, including wireless meters, wireless carrying cases, and wireless docking stations. All of the wireless devices use industry standard POCT1-A2 data format and are compatible with a choice of middleware partners. StatStrip Glucose is the only glucose meter cleared by the U.S. Food and Drug Administration (FDA) for use with all patients, in all healthcare settings, including critical care. The device received this clearance in 2014 after an extensive, nearly four-year study conducted at five major university medical centres, which included 1,698 critically ill patients with over 257 clinical conditions. Over 8,000 medications were investigated for potential interference to StatStrip Glucose measurement. The device demonstrated excellent agreement compared to central laboratory reference methods and no clinical interferences were found. In addition to the study submitted to the FDA, 138 other independent studies over the last eight years—including 53 critical care studies—have found no clinically significant interferences for StatStrip Glucose.

**NOVA BIOMEDICAL**

AACC, Atlanta, booth #1226

i www.cli-online.com & search 26977

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**Biomarker test for early sepsis identification**

The Stanbio Chemistry Procalcitonin (PCT) LiquiColor Assay enables the quantitative determination of PCT in serum samples, EDTA or lithium heparin plasma samples by latex enhanced immunoturbidimetric methodology. Procalcitonin is a marker for bacterial infection and sepsis and has been recognized as an important adjunct marker in the diagnosis of sepsis. Fast, accurate and convenient, the test provides a precise result, which correlates well with established methodology, within 10 minutes and requires just 20 μL of sample. The reagents may be used on almost any liquid-based chemistry analyser with open-channel capability. In addition, the reagent kit, calibrator and control sets are all available separately. The assay can be performed on a customer’s existing chemistry analyser with the same collection tube used for analysis of other chemistry tests. Therefore, it will provide optimized lab workflow by eliminating the need to split a sample or have a dedicated offline workstation. These features will provide any lab with a cost-effective solution for PCT testing.

**EKF DIAGNOSTICS**

i www.cli-online.com & search 26982
FRONT COVER PRODUCT

Klebsiella pneumoniae carbapenemase detection

To face the tremendous problem of antibiotic-resistant bacteria, Coris BioConcept has developed a new range of tests under the brand name “RESIST”. Carbapenemase-producing Enterobacteriaceae (CPE) represent a major health concern and any diagnostic tool helping their detection in the laboratory could improve patient safety. A first test (OXA-48 K-SeT) has been launched last March for the detection of OXA-48-like-producing Enterobacteriaceae from culture colonies. After this very successful launch supported by two oral presentations during ECCMID in Copenhagen, a second product is now available for the detection of KPC (Klebsiella pneumoniae carbapenemase) belonging to class A carbapenemases. It is the most frequent enzyme of this group and shows the highest spreading activity. This new test, KPC K-SeT, combined with OXA-48 K-SeT will give diagnostic labs the possibility to identify within minutes two of the main carbapenemases observed in Europe.

CORIS BIOCONCEPT
AACC, Atlanta, booth #3731
www.cli-online.com & search 27022

Patient home test kit for calprotectin

IBDoc is the first in-vitro diagnostic test to measure the fecal inflammation marker calprotectin at home. IBDoc just received a CE mark for patient self testing and is a revolutionary new test for gut inflammation aimed at people with inflammatory bowel disease, Crohn’s and ulcerative colitis. IBDoc eliminates the need for patients to bring stool samples to the clinic for analysis. Instead, its simple to use technology combining a stool extraction device CALEX, a calprotectin test strip and the IBDocsmart phone app to read the test result by state-of-the-art image processing, allows IBDoc patients to actively participate in their disease management and communicate with the healthcare providers via the Internet as well. The backbone of this home test system is the IBDoc web portal for patient data management that keeps the IBDoc patient under constant vigilance of the healthcare professional for real time support. The IBDoc portal is designed with the highest security standards to keep confidential patient data safe. Calprotectin measured in stool is a non-invasive biomarker for gastrointestinal inflammation. It correlates well with lesions and with endoscopic scores of disease activity in both, Crohn’s disease and ulcerative colitis. Fecal calprotectin is superior to classically used blood biomarkers, such as C-reactive protein (CRP), erythrocyte sedimentation and others.

BÜHLMANN LABORATORIES
AACC, Atlanta, booth #3854
www.cli-online.com & search 27028

Handheld coagulation analyser

Siemens Healthcare Diagnostics is introducing a first-of-its-kind, handheld portable coagulation analyser. The Xprecia Stride coagulation analyser delivers fast, reliable Prothrombin time testing (PT/INR) for point-of-care monitoring and management of oral anticoagulation therapy (OAT) with warfarin, a vitamin K antagonist. The Xprecia Stride coagulation analyser was specifically designed to meet the growing demand for fast and reliable PT/INR results in physician offices and walk-in clinics to help healthcare professionals make informed decisions about patient care.

SIEMENS HEALTHCARE DIAGNOSTICS
AACC, Atlanta, booth #1409
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25OH Vitamin D Total ELISA (KAP1971)
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- Only 25OH Vitamin D ELISA fully automatable
- FDA approved

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- Unique assay
- The only available direct measurement of free 25OH Vitamin D
- Research Use Only

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- Research Use Only
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- The only coated tube format available on the market

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- The only patented 25OH Vitamin D monodonal antibody and recognizing both 25OH and 1,25(OH)2 in an industry that is moving to monoclonal antibodies
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NEW

THE VITAMIN D EXPERT
Large round chamber autoclave

For laboratories wishing to acquire a high capacity, front loading cylindrical chamber autoclave with an exceptional pack density at a more affordable price, the new 500-litre Q63 laboratory autoclave is now available from Priorclave with a chamber profile taller than that found in most ‘cylindrical’ machines thus making it ideal for larger items. Whilst offering better loading versatility with much easier and safer chamber access due to the lower loading height of just 725 mm, the combination of features such as forced air cooling to reduce cycle times and automatic timed free-steaming for improved air removal add to the overall sterilizing performance of the Q63 autoclave. All operating settings are programmed easily through the Tactrol2 microprocessor controller, which also enables sterilizing performance data to be archived and if necessary forwarded from anywhere in the world direct to Priorclave’s UK Service Centre for technical assistance.

Triple quadrupole mass spectrometer

The LCMS-8060 triple quadrupole mass spectrometer is designed to push the limits of LC/MS/MS quantitation for applications requiring the highest sensitivity and robustness while delivering a meaningful solution for routine LC/MS/MS analyses. It detects substances at ultra-trace level as they occur in complex matrices for example, or in smallest sample concentrations which have to be diluted in order to avoid matrix effects. The LCMS-8060 is the newest member of Shimadzu’s UFMS (Ultra-Fast Mass Spectrometry) family and part of the Shimadzu mass spectrometry platform of MS/MS systems with ultra-fast technologies. The system combines a heated ESI source with all UF technologies including UF sweeper III, a collision cell filled with argon gas. Through its high speed technology, UF sweeper III achieves dwell times of 0.8 ms per MRM. With new UF Qrarr;ay ion guide technology increasing ion production and signal intensity, the LCMS-8060 introduces a new level of sensitivity and makes a real difference to working better and faster. The system features a data acquisition scan speed of 30,000 u/sec and a polarity switching time of 5 msec. The newly developed UF Qrarr;ay boosts ion sensitivity while suppressing noise. By improving the ion sampling device, ion guide and vacuum efficiency, the instrument achieves unprecedented sensitivity in LCMS. Shimadzu’s proprietary technologies allow acquisition of up to 555 MRM channels per second, ultra-fast polarity switching and ultra-fast scanning, all with highest data quality. Robustness of the LCMS-8060 and modified ion optics was assessed by injecting 2,400 samples of femtogram levels of alprazolam spiked into protein-precipitated human plasma extracts over a six day period (over 400 samples were injected each day). RSD of peak area response was 5% over this test period, and use of a deuterated internal standard (alprazolam-d5) led to RSD of 3.5%. As part of the robustness test the vacuum system was vented to model a transient power failure, with however no effect on signal response or baseline noise level.

PRIORITYCLAVE

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CALENDAR OF EVENTS

June 21-25, 2015
IFCC-EFLM EuroMedLab/JIB
Paris, France
www.pari2015.org/

July 26-30, 2015
AACC
Atlanta, GA, USA
www.aacc.org

August 5-7, 2015
FIME 2015
Miami, Fl, USA
www.fimeshow.com

Biochemical & Molecular Basis of Multifactorial Diseases
Maron, Argentina
www.ifcc.org

Sept 1-3, 2015
Analytical & Bioanalytical Techniques
Valencia, Spain
www.analyticalbioanalyticalpharmaconferences.com

Sept 3-5, 2015
Biotech China
Nanjing, China
www.biotechchina-nj.com

Sept 4-6, 2015
British Society for Allergy & Clinical Immunology
Telford, UK
www.bsaci.org

Sept 5-9, 2015
European Congress of Pathology
Belgrade, Serbia
www.esp-congress.org/

Sept 9-12, 2015
18th Annual Meeting of the ESCV (European Society for Clinical Virology)
Edinburgh, Scotland, UK
www.escv2015.com

Oct 18-21, 2015
CMEF Autumn
Wuhan, China
www.cmef.com.cn

Nov 16-19, 2015
Medica
Düsseldorf, Germany
www.medica.de

Jan 25-28, 2016
MEDLAB at Arab Health 2016
Dubai, UAE

For more events see: www.cli-online.com/events/

Dates and descriptions of future events have been obtained from official industrial sources. CLi cannot be held responsible for errors, changes or cancellations.
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