Molecular testing for Chlamydia and gonorrhoea

Pg. 30

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Substantial progress has been made in the prevention, diagnosis, monitoring and treatment of HIV/AIDS in recent years. Thanks particularly to the introduction of effective antiretroviral therapy (ART), HIV/AIDS in developed countries is now no longer considered an inevitably fatal disease but rather a chronic condition, albeit one which involves careful and continuous monitoring. Unfortunately, the majority of HIV/AIDS patients are found in resource-poor settings where fewer than half of HIV-infected people who need ART have access to it. This problem is suitably reflected in the theme ‘Universal access and human rights’ that was adopted for the 22nd World AIDS Day, which took place on December 1st. While access to appropriate healthcare is a basic human right, there are however other violations of human rights which not only seriously impact the quality of life of HIV-positive people, but can significantly fuel the spread of AIDS.

Approximately 60% of those infected with HIV in sub-Saharan Africa are women, with young women being three times more likely to become infected from a heterosexual encounter with an HIV-positive partner than their male peers. In many of these countries that have been subject to political unrest and military conflict, over a third of women report that their first sexual experience was forced: in many areas the rape of women has become so normal that it goes unpunished. In addition, in cultures where women do not have the right to refuse sex with husbands who may have other wives or sexual partners, it is highly unlikely that married women could insist on the use of condoms. Women are thus powerless to practise safe sex, and monogamy on their part does not necessarily prevent them from becoming infected. This situation could be alleviated if recently developed female condoms and microbicides become more widely available. While the difference in access to appropriate medication between developed and less developed countries is huge, the stigma of being HIV positive is unfortunately more evenly spread throughout the world. Around the globe people with HIV can still face discrimination from potential employers or insurance providers. Some countries do not even allow HIV-positive foreigners past their borders; until the beginning of this year the USA was one such country. However HIV-positive women from resource-poor countries are particularly subject to stigma, not only by their communities but, tragically, often by their families as well. While the valiant efforts to try to make ART drugs accessible in less-developed countries are to be applauded, full relief will only come when basic, cultural injustices to women are rectified.

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Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) are common sexually-transmitted infections worldwide, both of which can be asymptomatic and can have serious long-term consequences if left untreated. Numerous molecular platforms, which use PCR, SDA or TMA methodologies, are available for CT and/or NG testing. However, in-house assays are essential in order for some laboratories to provide an effective diagnostic service. The front cover image is a scanning electron micrograph of Neisseria gonorrhoeae diplococci.

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Ecarin Chromogenic Assay
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  - Levels of coagulation factors
  - VKA or Heparins
  - Lupus anticoagulant / antiphospholipid syndrome
- Automated Chromogenic assay
Determining the exact concentration of direct thrombin inhibitors (DTI) in plasma

There is a growing interest in direct thrombin inhibitors (DTI) as anticoagulants. However, currently dosing is monitored using tests that lack specificity and do not provide a clear dose response relationship, or monitoring is not recommended at all. In some patients, this results in either drug accumulation or inadequate blood levels. The ecarin chromogenic assay (ECA) is not influenced by any changes in coagulation factors, lupus anticoagulants, heparin or vitamin K antagonist therapy. It allows the rapid automated determination of DTI levels, and could make therapy with DTIs safer and more efficient. The ECA can rapidly rule out or confirm DTI effects in cases with unexplained pathological coagulation test results.

by Dr Hans-Jürgen Kolde

Thrombin: a key enzyme in haemostasis

Thrombin formation leads to activation of platelets, thereby providing a surface for coagulation activation that amplifies additional thrombin generation and leads to fibrin formation [Figure 1, Table 1]. Thrombin is thus a natural target for therapeutic anticoagulation. In vivo, thrombin generation is tightly regulated. Antithrombin (AT III) is an important regulator, but it requires glycosaminoglycans such as infused heparin as cofactor [1].

Direct Thrombin Inhibitors

Direct thrombin inhibitors (DTI) are anticoagulants which inhibit thrombin in the absence of any cofactor [Table 2]. They block the action of thrombin by binding to specific domains: the active site (AS) and one of two exosites. Exosite 1, a binding site for substrates such as fibrinogen, is located close to the AS and directs the scissile peptide bonds into the AS. Hirudin forms a very stable 1:1 complex with thrombin. Bivalirudin binds to exosite 1 and the AS and is slowly cleaved, which restores the AS. The inhibition is only transient. The competitive inhibitors argatroban and dabigatran leave a small amount of free thrombin available which may lower the risk of bleeding. In contrast to heparin/ATIII, DTIs also inhibit fibrin bound thrombin [3].

Clinical use of DTIs

DTIs are used for various indications [4]. An important area is in heparin-induced thrombocytopenia (HIT), a severe adverse drug reaction mediated by the immune system [5]. Antibodies directed against a complex formed by heparin and platelet factor 4 activate platelets, enhance thrombin generation and may cause venous and arterial thrombosis. Discontinuation of heparin is not enough, because these patients have an extremely high risk of thromboembolic events. Alternative anticoagulants such as DTIs are required. However, their use is associated with a higher rate of bleeding. Antibody development can often influence the half life of hirudin in both directions [6].

Another important area for DTIs is acute coronary syndrome (ACS). Such patients remain at risk for recurrent myocardial ischaemia, despite treatment with
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antiplatelet drugs and heparin. DTIs are probably still underused in this setting. Compared with heparin, serious bleeding occurred less frequently among patients receiving DTIs though there is heterogeneity for this outcome. DTIs have been successfully used for patients on haemodialysis and for thrombosis prevention in orthopaedic surgery. In the coming years novel oral DTIs, such as dabigatran, which do not require general monitoring, may often replace vitamin K antagonists (VKA) for long term protection against thromboembolism.

### Dose adjustment of DTIs

There is no antidote for rapidly reversing the effect of DTIs. Monitoring and dose adjustment of those DTIs which cannot simply be prescribed at fixed doses is important for patients with a high risk of bleeding, especially patients with renal or multiple organ dysfunction syndrome. Renal function must be considered while deciding on the initial and maintenance dose of those DTIs which are primarily eliminated by the kidney [Table 2]. The elderly represent the largest patient group requiring cardiovascular and orthopaedic surgery. Kidney function declines with age and may further deteriorate during critical illness. A high proportion of patients in intensive units or after cardio surgery suffer from an acute kidney injury. Creatinine, a slow reacting marker for renal function, only increases when there is already a persisting massive kidney injury; this biomarker can even remain in the normal range. Simply taking a single serum creatinine value for the assessment of renal function may result in overdosing with those DTIs which are eliminated via the kidney. The use of anti-platelet drugs is a further cause of increased risk of bleeding in cardiac patients. Reduced liver function impairs the clearance of those DTIs which are primarily eliminated by the liver (eg. argatroban) and may result in low levels of coagulation factors and fibrinogen split products which impair clotting. This can also contribute to an increased risk of bleeding during treatment with DTIs.

#### Laboratory tests for monitoring and dose adjustment of DTIs

Probably the clinical history of DTI use, specifically hirudin, would have been different if more reliable automated tests had been available. Difficulties in dosing with these drugs using global tests such as ACT, aPTT and PT, and especially problems of bleeding prompted the development of more specific tests. The dosing generally recommended for hirudin might be inappropriate for critically ill patients with at least one dysfunctional organ; bleeding rates of up to 19% have been reported [7]. Table 3 summarises the current range of automated methods for DTI monitoring.

#### Global assays: ACT, PT and aPTT

These tests were introduced long before any DTIs were developed, and were never designed for the monitoring of such drugs. The effect of DTIs on global tests is highly reagent-dependent; a certain aPTT clotting time can reflect quite different levels of a DTI [8]. The limitations of global tests are obvious: they are highly dependent on the level of coagulation factors and partially dependent on physiological protease inhibitors in the sample. Except in cases of genetic variability, e.g. the FII G20210A variant, these levels can be greatly altered in patients with consumption or dilution coagulopathy, in those with liver disease, or during inflammation with increase of FVIII and fibrinogen. Fibrinogen split products (FSP) may prolong clotting. A reliable assessment of risk of bleeding or inadequate anticoagulation is hardly possible via global tests. Major inter-individual differences in response to hirudin on aPTT

### Drug Structure

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Route of administration</th>
<th>Usage of administration</th>
<th>Toxicity</th>
<th>Potential difficulties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirudin (lepirudin, desirudin)</td>
<td>Binds to exosite I and active site of thrombin. It forms a very stable complex and leaves only minimum free thrombin accessible.</td>
<td>Intravenous, subcutaneous</td>
<td>Kidney</td>
<td>Child: 1 h, Adult: 1 h</td>
<td>Accumulation in renal disease possible.</td>
</tr>
<tr>
<td>Argatroban</td>
<td>Binds to exosite I and active site of thrombin. Is slowly cleaved.</td>
<td>Intravenous, subcutaneous</td>
<td>Kidney</td>
<td>25 min</td>
<td>Prolongation of half life with accumulation in patients with impaired creatinine clearance possible.</td>
</tr>
<tr>
<td>Dabigatran</td>
<td>Competitive inhibitor</td>
<td>Intravenous, subcutaneous</td>
<td>Oral</td>
<td>45 min</td>
<td>May require dosage adjustment in liver disease.</td>
</tr>
</tbody>
</table>

### Table 2. Currently used direct thrombin inhibitors.

<table>
<thead>
<tr>
<th>Test</th>
<th>Principle</th>
<th>Drug response, measuring range for DTIs</th>
<th>Specificity</th>
<th>Interference by other anticoagulant drugs</th>
<th>Dependency from FVII, FX, FII and fibrinogen</th>
<th>Interference by lupus anticoagulant a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated partial thromboplastin time (aPTT)</td>
<td>Clotting assay: Activation of coagulation via contact phase</td>
<td>Clotting assay-dependent</td>
<td>Non-linear, prolongation is dependent on serum fibrinogen levels</td>
<td>Low or elevated levels of all coagulation factors</td>
<td>Moderate, dose dependent</td>
<td>Yes, but strongly reagent dependent</td>
</tr>
<tr>
<td>Prothrombinase induced clotting time (FICBT)</td>
<td>Clotting assay: Activation of coagulation with thrombin</td>
<td>Clear dose response (drug-dependent)</td>
<td>Vitamin K antagonists, direct FXa inhibitors, high concentrations of heparin or heparin-like drugs</td>
<td>Low or elevated levels of aPTT clotting time, high concentrations of FII, V, VII, and of fibrinogen.</td>
<td>Moderate, dose dependent</td>
<td>Yes, but strongly reagent dependent</td>
</tr>
<tr>
<td>Activated coagulation time (ACT)</td>
<td>Clotting assay: Activation of coagulation via FXa and a TF activating make venom enzyme</td>
<td>No</td>
<td>Dependency from FVII, FX and fibrinogen</td>
<td>Yes, but strongly reagent dependent</td>
<td>Moderate, dose dependent</td>
<td>Yes, but strongly reagent dependent</td>
</tr>
<tr>
<td>Chromogenic thrombin inhibition assay</td>
<td>Clear dose response, wide measuring range</td>
<td>No</td>
<td>Dependency from FII and fibrinogen.</td>
<td>No, No</td>
<td>No, No</td>
<td>No, No</td>
</tr>
<tr>
<td>Chromogenic assay (ECA)</td>
<td>Linear dose response for DTIs, wide measuring range</td>
<td>No</td>
<td>No</td>
<td>No, No</td>
<td>No, No</td>
<td>No, No</td>
</tr>
</tbody>
</table>

### Table 3. Characteristics of automated laboratory tests for DTIs.
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BS-800 M2
1600T/H (photometer), 2400T/H (with ISE)

BS-800 M3
2400T/H (photometer), 3600T/H (with ISE)

BS-800 M4
3200T/H (photometer), 4800T/H (with ISE)
were noted. The relationship between the aPTT and hirudin concentration is nonlinear, shows a plateau at higher levels, and it is not possible to discriminate between therapeutic concentrations and a dangerous overdose which may cause bleeding [9]. Switching from heparin to a DTI, followed by a VKA prior to discharge, as for HIT, creates a problem with the aPTT. In the case of argatroban, the dose response in plasma from patients differs considerably from that of normal subjects [10]. A significant drawback of the aPTT is the potential influence of lupus anticoagulants (LAC) which prolong the clotting time in a reagent-dependent manner. LACs are a frequent cause of thromboembolic disease, hence many patients on treatment with a DTI could also have or will develop a LAC [11]. It was demonstrated that aPTT both under- and overestimated [11]. It was demonstrated that aPTT both under- and overestimated [11].

**Ecarin clotting time (ECT)**

Ecarin, a protease from *Echis carinatus* venom, cleaves prothrombin exclusively into meizothrombin (MZT). MZT cleaves fibrinogen much more slowly than thrombin. In the ECT, MZT generated from the patient's prothrombin immediately binds the DTI present in the sample. The remaining free MZT induces clot formation. ECT shows a linear correlation with wide ranges of DTI levels and there is no interference from heparin or LAC [13]. However, ECT is partially responsive to fibrinogen levels and FII deficiency, e.g. induced by VKA or by haemodialysis, which can falsely elevate the ECT result [14,15]. ECT is more suitable than aPTT or ACT for monitoring DTIs, particularly when higher doses are prescribed, such as in cardiac surgery patients or during haemodialysis. However, no CE-marked or FDA-cleared standardised commercial versions of ECT are available.

**Ecarin chromogenic assay (ECA)**

ECA, an enhancement of the ECT, overcomes many of the limitations of other tests for DTIs [16]. The test retains the advantages of ECT, such as high specificity, with no interference from heparin, VKA treatment or LAC. The test [Figure 2] uses an excess of FII and a chromogenic substrate, making ECA completely independent from variations in the FII and fibrinogen level in the sample. ECA is applicable for all clinically prescribed DTIs, and shows excellent precision and reagent stability. The calibration curve is stable for several weeks. The test is adapted to automated coagulometers (e.g. STA series) and can be offered 24h per day. The procedure is as rapid as an aPTT. Calibrators and controls for several clinically-prescribed DTIs are available and allow the specific concentration of the drug to be reported. This allows clinicians to adjust DTI treatment based on the patient’s exact plasma DTI level, instead of relying on less specific clotting tests influenced by multiple variables. The risks of over- or under-treatment with DTIs can thus be minimised. Studies have confirmed the specificity of ECA and demonstrated clear advantages compared with aPTT [17]. ECA allows the dosage of DTIs to be adjusted based on exact concentrations instead of adjusting doses according to clotting times obtained with tests which are influenced by numerous variables.

**Other tests**

Thrombin-based clotting assays are usually not very precise, have only a narrow measuring range and are poorly standardised. Their specificity for DTIs is limited because they are dependent on the quality and quantity of the fibrinogen of the sample, and are strongly influenced by heparin when no inhibitors are added, or an excess of normal plasma is added. However, thrombin-based chromogenic assays have been successfully used for monitoring DTIs [18]. Limitations, at least for some DTIs, is their precision and the fact that they are not applicable for all types of DTIs, specifically not for argatroban. PICT, a clotting test originally developed for monitoring LMWH, has also been used for the measurement of DTIs [19], but interference by LAC, variability in the level of fibrinogen, FV, and FII in the sample and VKA effects could impair the specificity of the method [19,20]. The similar Heptest also shows a dose response effect with DTIs.

**Future perspective**

The increasing use of oral DTIs such as dabigatran will result in cases in which the interpretation of clotting tests is difficult or impossible. In elderly patients, an oral DTI or direct factor Xa antagonist (DXaI) may become as commonly prescribed as warfarin is today. The consequence will be that results of coagulation tests carried out when such patients arrive at hospital will often be abnormal. A specific confirmation that PT or aPTT prolongation is induced by a DTI and not by VKA is a significant advantage. The short half life of DTIs may allow testing to be delayed until the drug is metabolised and there is no anticoagulant effect, whereas recovery of normal clotting in VKA therapy takes much longer and may require specific therapy. With ECA, reliably ruling out or confirming DTI effects is possible within minutes.

**References**


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Harmonisation and consensus in the use of cardiac biomarkers

High sensitive troponins and the natriuretic peptides are significant additions to the armoury for the diagnosis and assessment of acute chest pain and heart failure respectively. Whilst evidence on their use is growing at a significant rate, there is a risk that different local interpretations of the evidence may result in variations in the diagnoses. There is a need to harmonise not only the measurement of these cardiac biomarkers but also to harmonise the interpretation of the results.

by Dr Martin Myers and Rebecca Allcock

The use of cardiac markers in the assessment of acute chest pain and chronic heart failure has taken a giant leap in the last few years. The two main markers available are high sensitive troponins for use in the investigation of acute chest pain, and natriuretic peptides for use in the investigation of heart failure. Both of these markers are rapidly becoming central to key decision points in the patient pathway. However variations in what exactly is being measured and differences in their interpretation are of concern. There are two main types of troponin in use, Troponin I (TnI) and Troponin T (TnT). Whilst TnT is relatively standardised, there are two types of assay available: the 4th generation TnT and the high sensitive TnT (hsTnT). The situation is more complicated with TnI. There are numerous TnI assays with differences in epitope recognition and the use of different standards. In addition there are the older, less sensitive, TnI assays and new high sensitive TnI assays. With the natriuretic peptides there are different assays such as B-type natriuretic peptide (BNP) and the amino terminal of the pro hormone NTProBNP. In addition to variations in what is actually being measured there is lack of consensus on how to interpret the results. Thus there is a risk of causing confusion to the clinical users as well as a risk of patients being either diagnosed differently depending where they present, or misdiagnosed. This is a recipe for global confusion.

There is an urgent need to harmonise the use of these tests and attempts to harmonise them have started, especially for the measurement of high sensitive troponin T and NTProBNP.

High sensitive troponins

Two important guidelines relevant to the biochemical investigation of patients with acute chest pain have recently been published, namely the Universal Definition of Myocardial Infarction (UDMI) [1] and the UK National Institute for Health and Clinical Excellence (NICE) Clinical Guideline 95, Chest Pain of Recent Onset [2]. The UDMI requires a rise and/or fall of cardiac troponin in patients with evidence of cardiac ischaemia or ECG changes, with at least one value above the 99th percentile of the upper reference limit. The assay should have a coefficient of variation (CV) of <10% at this level, as recommended by the joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Indeed the Task Force recommended that assays that do not meet this specification should not be used for the assessment of acute chest pain. The currently available high sensitive troponins are Roche Elecsys TnT hs, Siemens Centaur TnI Ultra (2nd), Siemens VISTA (2nd), and Ortho Vitros ECI (2nd). These (and other emerging high sensitive troponin assays) will replace those assays that fail to meet the UDMI recommendations.

Whilst the UDMI and NICE guidelines are relatively straightforward, there are differences in certain key elements, and with both sets of guidelines there is potential for different local practices to be adopted. Whilst other guidance is becoming available [3, 4, 5] it is recommended that users of the new high sensitive troponins reach a consensus on how they use these assays, and the first group to undertake this task were users of the high sensitive Troponin T in the UK. In June 2010 a meeting was arranged in Manchester between Clinical Biochemists and Representatives from Roche to discuss the use of hsTnT. The Roche Elecsys high sensitive troponin T (hsTnT) assay meets the new performance criteria (10% CV is at 13ng/L, the 99th percentile is 14 ng/L), Dr Evangelos Giannitsis, Professor of Cardiology at the University Hospital, Heidelberg, Germany gave a comprehensive overview on the current evidence-based use of hsTnT. After reviewing the evidence the group reached a consensus on the practical use of hsTnT - the Manchester Consensus [6].

Manchester consensus

1. It was agreed that the reporting units for hsTnT should be ng/L. This would allow results to be reported in whole numbers, avoiding any confusion caused by units such as ng/mL that require 3 decimal places.
2. It was agreed that results should be reported down to the limit of the blank (3 ng/L) to enable assessment of changes between consecutive samples even when the initial result is below the 10% CV.
3. It was agreed that two measurements of hsTnT are required for the assessment of patients with chest pain. Whilst it is acknowledged that some hospitals may find it difficult to fund two troponin measurements, such hospitals would find that they are at odds with NICE and may be vulnerable to making wrong diagnoses if only one troponin is measured. A single Troponin level can be elevated due to causes other than myocardial infarction [3] and there is a risk of misdiagnosis of patients with acute chest pain if only single measurements are made.
4. NICE and the UDMI differ in their recommendations of when the two Troponins should be measured. Both agree that the first measurement should be at presentation. However, UDMI recommends that the second sample should be measured 6-9 hours after presentation, and NICE recommends that the second sample be measured 10-12 hours after the onset of symptoms. The group agreed that the timings recommended by the UDMI are more defined and that relating the second sample to onset of symptoms (as in NICE) may cause confusion due to large variations in the time elapsed between the acute event and presentation. In addition it is not valid to use a variable time interval between samples when the diagnosis depends on a defined incremental rise. The group therefore agreed to recommend the UDMI guidance on the timing for the second sample. This means that rule-in and rule-out can occur at 6-9 hours post-presentation. However if the second sample does not show an incremental rise yet, clinical suspicion remains and a further sample should be taken 12 hours after presentation.
5. There was significant debate regarding what constitutes a significant increase in the second sample. NICE refer to the UDMI but these guidelines are unclear about the rise expected. UDMI used the analytical imprecision of the assay to conclude that a significant change is 20% in reinfarction. However Professor Giannitsis outlined the impact of biological variation in hsTnT and stated that taking into account analytical and biological variability, a 100% change should be considered as significant. Using a 20% increase will give greater sensitivity but will result in reduced specificity; whereas a 100% increase will reduce the sensitivity but increase the specificity. The balance between the sensitivity and specificity of a test is a common issue, and in this instance the decision may be related to what the local clinical pathway is. Many cardiologists may prefer the 20% change (high sensitivity) and would then investigate patients with small changes in hsTnT further. However there would be a concern if further investigations were not undertaken and if the 20% rise was seen as diagnostic, as false positives would then occur. It is important to understand how hsTnT is being used. As a rule-in marker there is evidence that a 100% rise three hours after presentation equal to or greater than the 99th percentile is associated with a positive predictive value of 88%. Thus using hsTnT in this way could not be used as a rule-out at three hours; later samples would be required, and evidence shows that all patients with non ST elevation myocardial infarction (NSTEMI) had hsTnT equal to or greater than the 99th percentile at six hours post-presentation [7]. Whilst evidence is becoming available for what constitutes a significant rise, evidence on what constitutes a significant fall (included in the UDMI) is unclear, as the rate of fall in hsTnT may be less than the rise in hsTnT. At a follow-up meeting in Cardiff, this dilemma was debated further and the group agreed that there should be three decision categories:

Category a. Less than 20% change: not consistent with an acute event.

Category b. 20-100% change: significant rise in hsTnT, suggest further evaluation to distinguish between acute causes and chronic elevation in hsTnT.

Category c. Greater than 100% change: consistent with myocardial infarction.

6. It was emphasised that high levels of hsTnT can be used for risk stratification in both acute coronary syndrome (ACS) and non-ACS patients. Patients with chronically elevated hsTnT (>14ng/L) are at risk of future cardiac events and should be followed up.

Natriuretic peptides

The use of natriuretic peptide measurements to act as an initial rule-out test for chronic heart failure in primary care, prior to referral for echocardiography, has been recommended by the European Society of Cardiology and NICE [8, 9]. B-type natriuretic peptide (BNP) and an inactive product released during post-translational modification, the N-terminal portion of the pro-hormone (NTproBNP), have both been used for this purpose but the superior stability of NTproBNP makes this the peptide of choice. Whilst a consensus meeting similar to that of the Manchester consensus for hsTnT has not yet taken place, the case for a consensus was...
Cardiac markers

Figure 1. Algorithm for the assessment of acute chest pain using high sensitive Troponin T.

NTproBNP >4000 ng/L (Normal levels):
- Suggest specialist assessment and echo within 6 weeks.
- The level does not differentiate between heart failure due to left ventricular systolic dysfunction and heart failure with preserved ejection fraction.
- Please note that elevated levels can have causes other than heart failure (left ventricular hypertrophy, ischaemia, tachycardia, right ventricular overload, hypoxaemia [including pulmonary embolism], eGFR <60 mL/minute, sepsis, COPD, diabetes, age >70 and liver cirrhosis).

NTproBNP >2000 ng/L (High levels):
- Suggest specialist assessment and echo within 2 weeks.
- The level does not differentiate between heart failure due to left ventricular systolic dysfunction and heart failure with preserved ejection fraction.
- Please note that elevated levels can have causes other than heart failure (left ventricular hypertrophy, ischaemia, tachycardia, right ventricular overload, hypoxaemia [including pulmonary embolism], eGFR <60 mL/minute, sepsis, COPD, diabetes, age >70 and liver cirrhosis).

NTproBNP <4000 ng/L (Raised levels):
- Suggest specialist assessment and echo within 6 weeks.
- If clinical suspicion remains high consider further investigation. Please note that NTproBNP levels <50 ng/L (<50 years), <75 ng/L (50-75 years), and <250 ng/L (>75 years) make heart failure highly unlikely.

NTproBNP 400-2000 ng/L (Raised levels):
- Suggest specialist assessment and echo within 6 weeks.
- The level does not differentiate between heart failure due to left ventricular systolic dysfunction and heart failure with preserved ejection fraction.
- Please note that elevated levels can have causes other than heart failure (left ventricular hypertrophy, ischaemia, tachycardia, right ventricular overload, hypoxaemia [including pulmonary embolism], eGFR <60 mL/minute, sepsis, COPD, diabetes, age >70 and liver cirrhosis).

NTproBNP <400 ng/L (Normal levels):
- Please note obesity, diuretics, ACE inhibitors, beta-blockers, ARBs and aldosterone antagonists can reduce levels.
- If clinical suspicion remains high consider further investigation. Please note that NTproBNP levels <50 ng/L (<50 years), <75 ng/L (50-75 years), and <250 ng/L (>75 years) make heart failure highly unlikely.

Conclusion

Laboratory medicine and in vitro diagnostics will have key roles in the investigation of acute chest pain (using high sensitive troponins) and chronic heart failure (using natriuretic peptides). The use of high sensitive troponins will lead to better diagnosis with earlier rule-in or rule-out of patients presenting with acute chest pain. The natriuretic peptides will be used in the first instance to rule-out or rule-in patients with suspected heart failure. These simple and relatively cheap tests will not only improve diagnosis but will improve the patient journey and save on healthcare costs.

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presented to users of NTProBNP at a meeting in Birmingham in October 2010.

NICE has published an algorithm on the use of NTproBNP [Figure 2] with suggested cut-offs for each category [9]. However, NICE cut-offs are higher than in other published articles and do not include age-related reference ranges [10]. Whilst this improves specificity, there is a loss of sensitivity which has raised concern that the NICE “normal” cut-off may miss up to 25% of patients with chronic heart failure [10]. It should be noted that NTproBNP can be elevated in conditions other than heart failure and that certain drugs can reduce levels [9]. Thus NICE cut-off levels should be used initially for the interpretation of NTproBNP, but drug therapy, age, other conditions and clinical suspicion should be taken into account.

The following comments, based on NICE recommendations and taking into account the concerns of others [10] could form the basis of a proposed consensus:

NTproBNP <400 ng/L (Normal levels):
- Please note obesity, diuretics, ACE inhibitors, beta-blockers, ARBs and aldosterone antagonists can reduce levels.
- If clinical suspicion remains high consider further investigation. Please note that NTproBNP levels <50 ng/L (<50 years), <75 ng/L (50-75 years), and <250 ng/L (>75 years) make heart failure highly unlikely.

NTproBNP 400-2000 ng/L (Raised levels):
- Suggest specialist assessment and echo within 6 weeks.
- The level does not differentiate between heart failure due to left ventricular systolic dysfunction and heart failure with preserved ejection fraction.
- Please note that elevated levels can have causes other than heart failure (left ventricular hypertrophy, ischaemia, tachycardia, right ventricular overload, hypoxaemia [including pulmonary embolism], eGFR <60 mL/minute, sepsis, COPD, diabetes, age >70 and liver cirrhosis).

NTproBNP >2000 ng/L (High levels):
- Suggest specialist assessment and echo within 2 weeks.
- The level does not differentiate between heart failure due to left ventricular systolic dysfunction and heart failure with preserved ejection fraction.
- Please note that elevated levels can have causes other than heart failure (left ventricular hypertrophy, ischaemia, tachycardia, right ventricular overload, hypoxaemia [including pulmonary embolism], eGFR <60 mL/minute, sepsis, COPD, diabetes, age >70 and liver cirrhosis).

Figure 1. Algorithm summarising recommendations for the diagnosis of heart failure [9].
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The clinical importance of urinary sediment analysis

Urinary sediment analysis has not become less important as diagnostic procedures in medicine develop. In recent decades, because time-consuming manual methods were used, the majority of urinary sediment analyses were only carried out routinely if such an analysis was requested by the nephrologist and urologist. Nowadays, as well as fully automated chemical examination, automated urinary sediment analysis is available [Figure 1]. However, it is important to be aware that the general appearance of urine sediment, the presence of certain particles in the urine and the morphology of individual particles all provide a broader spectrum of information. This short overview of urinary sediment analysis should be useful for all healthcare workers involved in disease diagnosis.

by Dr Tibor Kovács, Dr Zsuzsanna Rékási and Prof. István Wittmann

Urinary examination was already part of the Hippocratic procedure more than 2500 years ago, but the examination was carried out by the naked eye at that time. [1]. After the construction of the first simple microscope in the 16th century, there were an increasing number of descriptions concerning the constituents of urinary sediment. In the early 20th century, Thomas Addis explored the connections between constituents of urinary sediment and different renal diseases using routine microscopic examination [2]. In recent years not only has the routine chemical examination of urine become automated, but an increasing number of clinical laboratories can provide detailed, electronically-transformed information on urinary sediment. Because fewer microscopic examinations are carried out by experts, the value of these examinations may decrease. This article summarises some important key points of urinary sediment examination in which the data are crucial in diagnosis. Due to space restrictions, small parts of a whole viewfield (HPF-like) microscopic image [Figure 2] generated by the UriSed Fully Automated Urine Sediment Analyzer (77Elektronika, Hungary) will be used to illustrate points.

Red blood cells – haematuria

Dipstick or chemical examinations are very sensitive for detecting red blood cells (RBC) in urine, but these methods are unable to differentiate between different forms of RBCs, or between RBCs and haemoglobin. On the basis of the different morphology of the RBCs, they can be categorised into three groups. Normal RBCs in urinary sediment are characteristic of non-immunological renal and urinary tract diseases, e.g., pyelonephritis, cystitis, calculi, tumours, as well as of extra-renal diseases, e.g., bleeding tendency, acute febrile episodes [Figure 3]. Glomerular type RBCs - dysmorphic or distorted erythrocytes, (acanthocytes) are characteristic of glomerulonephritis [Figure 4]. The membrane blebs seen on these RBCs may be caused by oxidative and carbonyl stress occurring while the RBCs are passing through the tubules [3]. The examination of urine sediment for dysmorphic RBCs is a first simple step towards making a diagnosis of glomerulonephritis. If more than 5% of RBCs in the urine sediment are
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dysmorphic, the specificity and sensitivity for glomerular disease is 98% and 52% respectively [4]. Crenated RBCs—due to a longer period that RBCs have remained in hyperosmolaric urine—is a sign of supravital damage and these RBCs should be differentiated from glomerular type RBCs [Figure 5]. The characteristics of these RBCs may help clinicians to refer patients with haematuria to the appropriate clinic (e.g. urology or nephrology).

**White blood cells - leucocytes**

Polymorphonuclear neutrophils are characteristic of bacterial infections include pyelonephritis, cystitis, prostatitis and urethritis [Figure 6], but these can also be observed in all renal diseases. Typically they appear as round granular cells about 12 µm in diameter. On the basis of large numbers of WBCs in the sediment, it can be assumed that a urinary tract infection is present and empirical antibiotic treatment can be started before bacterial culture results, which need at least two days to generate, are available. The efficacy of treatment can also be monitored by counting the number of WBCs per high power microscope field during the period of treatment.

Other constituents of the urinary sediment can only be observed by careful sediment analysis, whereas data on the presence of RBCs and WBCs can also be obtained from routine chemical examination of the urine.

**Epithelial cells**

In general, three different types of epithelial cells are found in urine sediment: renal tubular cells, transitional epithelial cells and squamous epithelial cells [Figure 7].

Increased numbers of renal tubular epithelial cells are found in the urine as a result of acute ischaemic or toxic renal tubular diseases (e.g. acute tubular necrosis), or associated with drug toxicity (non-steroid anti-inflammatory drugs, NSAIDs, aminoglycosides), heavy metals, immunosuppressants and mushroom poisoning. Few transitional epithelial cells are present in the urine sediment from healthy individuals; increased numbers are often present in urine in cases of urinary tract infection. Squamous epithelial cells are the largest cells in the urine sediment. They have no diagnostic significance and are more common in urine from women.

Atypical or a large number of epithelial cells recognised by urine sediment analysis may indicate that further cytological examination is necessary.

**Casts**

Urinary casts, with a core matrix of Tamm-Horsfall protein, are formed in the distal and collecting renal tubules. Any nephron component, whether a chemical or a formed component, can be found incorporated into a cast (e.g. RBCs, WBCs, pigments, fat globules). Degradation processes occurring within the casts can transform leukocytes or tubular cell casts into coarse granular casts [Figure 8]. Hyalin and finely granular casts can also be observed in the urine of healthy individuals [Figure 9]. However, different cells (e.g. RBCs, WBCs, epithelial cells, bacteria), pigments (e.g. bilirubin, haemoglobin) and other inclusions (e.g. crystals, granular structures) in the casts may indicate glomerular or tubulointerstitial diseases. The presence of RBC casts is diagnostic for glomerular bleeding in proliferative/necrotising glomerulonephritis. Leukocyte casts can be observed in acute interstitial nephritis, acute pyelonephritis and proliferative glomerulonephritis. Fatty casts may indicate marked proteinuria and nephrotic syndrome.

**Crystals**

Urinary sediment contains many types of crystals resulting from the precipitation of urinary solutes out of solution. Several factors influence crystal formation including the concentration of the solute in the urine, its pH, the flow of the primary urine through the tubules and urine storage. Examination of the urine crystals is informative in assessing individuals with kidney stone disease, in some rare inherited metabolic disorders...
and with suspected drug nephrotoxicity. Most of the different crystals are formed in acidic urine (e.g. amorphous urate, uric acid, calcium oxalate [Figure 10], bilirubin, cystine [Figure 11], and crystals from medications including ampicillin and sulphonamides. Amorphous phosphate, triple phosphate [Figure 12] and calcium carbonate are typically formed in alkaline urine.

**Bacteria & Yeast**
Detecting bacteria in the sediment of freshly voided urine suggests a type of urinary tract infection before the result of bacterial culture is available. [Figure 13]. During urinary tract infections, bacteruria are accompanied by WBCs (pyuria) in the urine sediment. Both rod-shaped (bacilli) and coccoid forms may be identified, although those most commonly encountered are gram-negative rods.

Yeast are ovoid, colourless cells often present with budding forms [Figure 14]. Yeast in the urine sediment often results from a vaginal infection, more usually in immuno-compromised patients (e.g. treated immune disease, diabetes mellitus); yeasts may also indicate urinary tract infection.

**Conclusion**
On the basis of this brief summary on the importance of urine sediment analysis, we can conclude that careful and accurate urine sediment examination should provide more information about the patient’s state of health than simple chemical or urine test strip analysis. The automated analysis of the urine sediment by digital imaging software can provide rapid, accurate results, and evaluation can be carried out on screen any time after the examination; there is no need for a manual microscopic investigation.

**References**

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Lower occurrence of atopic dermatitis if exposure to farm animals and cats

Atopic dermatitis (also known as atopic eczema) is a chronic and extremely painful inflammation of the skin that frequently occurs in early childhood, generally already in infancy. Up to 20 percent of all children in industrialised countries are affected, making it one of the most common childhood skin diseases. Atopic dermatitis is an allergic condition and thus results from complex interactions of genetic and environmental factors on the immune system. Earlier research has already indicated that allergies are less common in children who grow up on farms and whose mothers live on farms during their pregnancy. Exposure to farm animals and bacteria frequently found in farms as well as drinking milk from the dairy offer the immune system protection. However, proof of this protective effect in connection with atopic dermatitis has remained elusive.

Now a study at the University of Zurich, Switzerland, has analysed how prenatal environmental factors and genetic mechanisms influence the development of atopic dermatitis during the first two years of life. Children in rural areas of five European countries: Austria, Finland, France, Germany and Switzerland were examined. Of the 1,063 children taking part in the study, 508 were from families that lived on farms, and 555 were not farm children. It was demonstrated that women who spend their pregnancy in the proximity of farm animals and cats have children with a reduced risk of developing atopic dermatitis in their first two years of life. Two genes in these children were also identified that are of vital importance for innate immunity; the expression of these genes was linked to a lower likelihood of a physician’s diagnosis of an allergic condition.

http://tinyurl.com/382nog7

Smoking may thin the cerebral cortex

Many brain imaging studies have reported that tobacco smoking is associated with large-scale and widespread structural brain abnormalities. The cerebral cortex is a specific area of the brain responsible for many important higher-order functions, including language, information processing and memory. Reduced cortical thickness has been associated with normal ageing, reduced intelligence and impaired cognition.

Researchers at Yale University, USA have now compared cortical thickness in volunteers, both smokers and never-smokers, who were without medical or psychiatric illnesses. Smokers exhibited cortical thinning in the left medial orbitofrontal cortex. In addition, their cortical thickness measures negatively correlated with the amount of cigarettes smoked per day and the magnitude of lifetime exposure to tobacco smoke. In other words, heavier smoking was associated with more pronounced thinning of cortical tissue. The orbitofrontal cortex has frequently been implicated in drug addiction. The current findings suggest that smoking-related cortical thinning may increase the risk for addictions, including smoking. This concerning finding highlights the importance of targeting young smokers for antismoking interventions.

www.solp.org/journal

Genetic mutations associated with increased PSA and prostate cancer

Austrian researchers from Innsbruck Medical University have uncovered mutations throughout the mitochondrial genome that are associated with prostate cancer. An exciting aspect of the study is the association of tRNA mutations with elevated levels of prostate-specific antigen (PSA) in Austrian men diagnosed with various stages of prostate cancer.

Prostate cancer is among the most prevalent cancers diagnosed in the United States and Europe. The most common and noninvasive way to detect prostate cancer is to check PSA levels. This is a routine part of men’s health checks starting around the age of 50. Elevated PSA levels indicate the possibility of prostate cancer. Prostate biopsies are used for verification of PSA results and cancer diagnosis. Treatment may include surgery, radiation, or chemotherapy. Recognising the important role mtDNA mutations have been found to play in development and progression of many types of cancer, the researchers set out to sequence the entire mitochondrial genome in 30 prostate cancer patients. The group used a high-quality sequencing approach to detect differences in mtDNA sequence between cancerous and noncancerous tissue from the same 30 men.

By examining both the frequency and types of somatic mtDNA mutations in prostate cancer patients, several genetic changes having clinical significance were identified. They suggest that sequencing of selected mitochondrial regions will probably result in a mutation spectrum useful for prognosis. Perhaps the most striking finding of the study is the association between somatic tRNA mutations and PSA levels at diagnosis.

http://www.uibk.ac.at/forschung/

Faulty gene linked to disorders of sexual development

Scientists have discovered that the alteration of a single gene could cause some male embryos to develop as females. The breakthrough will improve diagnosis and clinical management of patients with disorders of sex development (DSD). These conditions occur when the testis or ovary does not develop properly in the embryo, causing genital abnormalities in one in 4500 babies.

An international team including University of Melbourne researchers at the Murdoch Childrens Research Institute, Australia identified the gene alteration in a group of patients including two families with several affected members. The alteration occurred in the MAP3K1 gene, which plays a role in switching on genes that direct the gonad to become a testis. The researchers found that the alteration of the MAP3K1 gene disrupted the normal process of testis development, resulting in a male embryo developing female characteristics including female genitalia and an overall feminine appearance. The discovery, showing the underlying cause of testis failure in these patients, should help provide a diagnosis and guide clinical management of cases in the future.

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HPV-related disease and vaccination programmes

Cervical cancer is an important public health concern and is the second most clinically important cancer after breast cancer in women aged 15–44 years. Until recently, the focus was on screening for cervical cancer. However, as today’s adolescents become sexually active at a much younger age, the focus is now on the use of vaccination as an effective measure to prevent progression of HPV infection to cancer. Primary prevention through vaccination is now possible in Europe using either the quadrivalent HPV vaccine, Gardasil (Sanofi Pasteur MSD), or the bivalent HPV vaccine, Cervarix (GSK), which are both highly immunogenic.

by Dr José Ramet

Recently, two human papillomavirus (HPV) vaccines have been introduced: a bivalent vaccine containing HPV-16 and HPV-18 pseudovirions and a quadrivalent vaccine containing HPV-16, HPV-18, HPV-6 and HPV-11 pseudo-virions. HPV-16 and HPV-18 are the two major HPV types involved in the development of cervical cancer, and HPV-6 and HPV-11 cause anogenital warts. As well as being the first gynaecological cancer that can be prevented by vaccination, cervical cancer has been transformed from a disease that is diagnosed and treated by gynaecologists to one that can be prevented by a programme to vaccinate adolescents that also involves paediatricians.

Epidemiology of HPV disease

More than 100 HPV types have been identified. Of these, approximately 40 infect the anogenital region and may be categorised as low or high risk, according to their oncogenic potential and association with cervical cancer. To date 15 HPV types have been identified as high risk, 12 types have been identified as low risk and a further three types are classified as probable high risk. It is estimated that approximately 70% of sexually active adults will be infected with HPV during their lifetime. However, the majority of these infections are asymptomatic and transient.

Although it is necessary to have a persistent infection with a carcinogenic HPV type (such as HPV-16 or HPV-18) for the development of cervical cancer, most women with such infections do not develop cancer and other factors are necessary. Increasing age and immunosuppression have been reported to be associated with persistent infections, and infections with multiple HPV types seem to act synergistically in cervical carcinogenesis. Use of oral contraceptives for longer than five years, multiparity, smoking and co-infection with human immunodeficiency virus or other sexually transmitted infections appear to increase the risk of cervical cancer. The 2002 GLOBOCAN survey found that, worldwide, there are almost 500,000 new cases of cervical cancer annually. Consistent with the patterns for prevalence of HPV infection, the highest incidences of cervical cancer were seen in sub-Saharan Africa and Central and South America. Across Europe, the lowest rates of cervical cancer were seen in Finland, Sweden, the Netherlands, Ireland, Spain, Italy and Greece, while the highest incidences were seen in Central and Eastern Europe. Studies have also found evidence of a trend towards an increasing incidence among younger women. The case–control studies of squamous cell carcinoma also demonstrated that a large number of different HPV types are associated with cervical cancer, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. Consequently, these have been designated high-risk types.

HPV and other cancers

Although less extensively studied than cervical cancer, there is evidence that HPV infection is involved in the aetiology of a number of other epithelial cancers. These include cancers of the anus, vagina, vulva and penis. HPV-16 is the most common type identified in all anogenital tumours, followed by HPV-18, HPV-31 and HPV-33.

HPV and genital warts

Approximately 90% of cases of genital warts are associated with HPV-6 and HPV-11 [Table 1], with the highest incidence of these lesions being seen in young adults.

Respiratory papillomatosis

Another manifestation of low-risk HPV infection is laryngeal papillomatosis in young children. This condition generally presents as stridor and dysphonia with onset between 3 and 5 years of age.

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**Table 1. Summary of HPV-related diseases:**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Evidence for HPV involvement</th>
<th>HPV types involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical cancer</td>
<td>HPV DNA detected in 99.7% of tumours*</td>
<td>HPV-16, -18, -45, -31, -33, -52, -58 and -35* (HPV-16 and HPV-18 associated with ~70% of cases)*</td>
</tr>
<tr>
<td>Cancer of vagina</td>
<td>HPV DNA detected in 64–91% of tumours*</td>
<td>HPV-16, -18, -31, and -33*</td>
</tr>
<tr>
<td>Cancer of vulva (basaloid)</td>
<td>HPV DNA detected in 60–90% of tumours*</td>
<td>HPV-16, -18, -31, and -33*</td>
</tr>
<tr>
<td>Cancer of anus</td>
<td>HPV DNA detected in 88–94% of tumours*</td>
<td>HPV-16, -18, -31, -33*</td>
</tr>
<tr>
<td>Cancer of penis (basaloid)</td>
<td>HPV DNA detected in 60–90% of tumours*</td>
<td>HPV-16 and -18*</td>
</tr>
<tr>
<td>Cancer of oropharynx (squamous cell)</td>
<td>HPV DNA detected in 35.6% of tumours (range 11–100%)*</td>
<td>HPV-16 and -18*</td>
</tr>
<tr>
<td>Cancer of oral cavity and larynx (squamous cell)</td>
<td>HPV DNA detected in 23% of tumours (range 4–80%)*</td>
<td>HPV-16 and -18*</td>
</tr>
<tr>
<td>Genital warts</td>
<td>HPV DNA detected in approximately 90% of cases.</td>
<td>HPV-6 and -11*</td>
</tr>
<tr>
<td>Respiratory papillomatosis</td>
<td>7/1000 births in women with a history of genital warts resulted in disease in children, 231.4 (95% CI, 135.3, 395.9) times higher risk versus births without a maternal history of genital warts*</td>
<td>HPV-6 and -11</td>
</tr>
</tbody>
</table>

* [49]; † [11]; ‡ [66].
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It is believed to be due to transmission of HPV-6 and HPV-11 in the birth canal, as the incidence is significantly higher among children whose mothers have a history of genital warts during pregnancy. Treatment of respiratory papillomatosis requires the surgical ablation of obstructing lesions from the airway. The disease is often recurrent, and children may require several treatments annually.

Clinical experience with HPV vaccines

Two HPV vaccines have been approved for use in Europe. Both vaccines are produced using recombinant DNA technology and consist of the L1 proteins that form the viral capsid, which self-assemble to resemble closely the intact virus particle but are non-infectious. The quadrivalent vaccine (Gardasil, Sanofi Pasteur MSD) containing L1 proteins for HPV types 16, 18, 6 and 11 was the first to be approved. The second vaccine (Cervarix, GlaxoSmithKline Biologicals) contains L1 proteins for HPV types 16 and 18.

Both vaccines have been shown to be highly immunogenic when administered to adolescent girls and young women, with the immune response persisting for at least 5 years after vaccination and with similarly excellent efficacy against infection and disease endpoints. The immunogenicity of both vaccines appears to be greater in younger subjects than in older individuals.

Efficacy against HPV diseases

The efficacy of both vaccines has been demonstrated in clinical trials in healthy young women. The FUTURE I study investigated the incidences of genital warts, vulvar intraepithelial neoplasia (VIN), vaginal intraepithelial neoplasia (VaIN), vaginal or vulvar cancer, CIN, adenocarcinoma in situ (AIS) or cervical cancer associated with HPV types 6, 11, 16 or 18 as co-primary endpoints in women aged between 16 and 24 years who had been vaccinated with the quadrivalent vaccine. The study randomised 5,455 women to receive either vaccine or placebo, with a follow-up of three years after the third dose. In the per-protocol population of women who were seronegative and polymerase chain reaction (PCR) negative for the vaccine-specific HPV types up to one month after administration of the third dose, the vaccine was 100% effective in preventing all of the co-primary endpoints.

Practical strategies for HPV vaccination

Targeting young adolescents before the onset of sexual activity and, therefore, before exposure to the HPV types involved in anogenital lesions is likely to provide the greatest long-term health benefits both for the individuals vaccinated and the general population. In addition, at this age, parents are still responsible for making decisions about healthcare, including vaccinations. Thus, younger adolescents are an easier target for an immunisation programme than older adolescents. Furthermore, young adolescents can be targeted via established school vaccination programmes, where they exist, with the potential opportunity to add HPV vaccination to the current vaccination calendar for this age group.

Paediatricians have an important role to play in the development of HPV vaccination programmes and also in the delivery of the vaccine. They have access to adolescents and their parents, and in many European countries they are already involved in the vaccination of adolescents.

The role of cervical screening programmes

It is clear that the combination of cervical screening together with HPV vaccination in comprehensive cervical cancer prevention programmes will offer the most effective long-term protection against cervical cancer. Even after the introduction of HPV vaccination programmes and the vaccination of a significant number of young women, cervical screening will continue to play an important role in the prevention of cervical cancer. The vaccines have no therapeutic effect against existing HPV infections, and women will therefore need to be monitored for the possible development of lesions due to persistent infections acquired before vaccination. Introduction of HPV vaccination programmes for adolescents and the associated publicity and health education initiatives will increase awareness of HPV diseases among the general population.

HPV vaccination in men

To date, most HPV vaccination strategies have been directed towards vaccinating adolescent girls. However, HPV infections in men also cause genital warts and more serious conditions, such as anal and penile cancers. In addition, transmission of HPV during sexual activity, including non-penetrative genital contact, means that men are an important source of infections in women. To date, immunogenicity data in boys are available only for the quadrivalent vaccine, and there are no published efficacy data for either vaccine in men.

Conclusions

Infection with one or more of the high risk HPV types is the necessary cause of cervical cancer and also appears to contribute to the aetiology of other epithelial cancers of the anogenital region. To obtain maximum benefit from HPV vaccines, vaccination programmes should initially target adolescent girls before the onset of sexual activity with possible catch-up programmes, as already introduced in most European countries. Close collaboration between paediatricians, gynaecologists and other health care professionals involved in the development and delivery of vaccination programmes will be essential.

References


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Over 100,000 Biological Safety Cabinets Located Throughout the World
In-house testing for Chlamydia and gonorrhoea: is running your own molecular service an easy way out?

Our laboratory ran an in-house CT NG quadruplex assay until a commercial supplier could offer an affordable, equivalent combined assay, with good performance [1]. But is an in-house assay right for your laboratory? This article gives a brief overview of some issues to consider before striking out on your own.

by Dr M. Hopkins

CT/NG infection and diagnosis
Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) are common sexually transmitted infections worldwide. Both can be asymptomatic and both can have serious long-term consequences if left untreated. The Health Protection Agency in the UK recorded 217,570 chlamydia diagnoses and 17,385 gonorrhoea infections in 2009. The majority of these infections were in young adults. In women, the highest rates of infection were found in those aged between 16-19 years old whereas the equivalent male group was slightly older, aged 20-24 years. A National Chlamydia Screening Programme (NCSP) was introduced in England in 2003 to provide opportunistic testing of young sexually active adults to enable early detection and treatment of asymptomatic infection. This is additional to the sexual health screens provided for people of any age attending Genitourinary Medicine (GUM) clinics. Some pharmacies also offer private CT NG testing, for a fee, whereby a sample is collected at home and posted to the laboratory for testing. Point-of-care tests that generate a result while the patient is still in clinic are common in countries with a strong “doctor’s office provision” or those where reliable laboratory results are unavailable. Accessibility to testing is only likely to increase as technology advances. A consortium that includes St George’s University of London is already exploring the use of nanotechnology for an STI testing device that plugs into a mobile phone. For the time being however, the European CT/NG testing market is likely to remain predominantly laboratory-based.

Numerous laboratory systems are available for CT and/or NG testing. Abbott, BD, Genprobe and Roche all offer integrated systems with large workload capacities. These are molecular platforms that use PCR, Strand Displacement Amplification (SDA) or Transcripton-Mediated Amplification (TMA) methodologies. A number of laboratories run “laboratory developed”, or “in-house” assays which are sometimes seen as the cheaper, more flexible option. However, this direction is not without its shortfalls and some factors to consider are presented.

Choice of genetic target
Amplification assays are limited in that they only detect what you look for. A major benefit of running in-house assays is that you decide what these targets should be. This allows a high degree of responsiveness in relation to your service user requirements. However, it is your responsibility to validate the performance of your in-house protocol and you will not have the back-up of a commercial provider if something goes wrong. Choosing a target in the same genetic region as other tests allows you to relate to some surveillance updates, but make sure you remain aware of them and that your laboratory is capable of adapting to the new information [2].

Screening and confirmation assays often detect different genes and the flexibility of using an in-house confirmation assay allows a laboratory to tailor a secondary assay to complement the screening targets.

EQA and networks
How well is your assay performing in relation to others? External Quality Assurance (EQA) schemes will often include challenging samples that you might not normally encounter, such as the CT Swedish variant.
It is also a good idea to join a network or society with a forum for open discussion of issues and updates (e.g. UK Clinical Virology Network, or British Association for Sexual Health and HIV).

**Staff and support**
Commercial providers will supply training, documentation and advice for what should hopefully (but not always) be a seamless transition to implementing a new assay. Help and support will also be available from the reagent and instrument manufacturers of your in-house assay component parts, but responsibility for troubleshooting ultimately lies with the laboratory. Experienced staff capable of resolving problems and compiling all appropriate documentation are a necessity for running an in-house molecular service.

**System validation**
The introduction of any new test requires validation, but this is even more true for in-house assays which will not have the support of manufacturers’ datasets [3]. Evaluation data should detail the sensitivity and specificity of your testing protocol and allow calculation of the positive and negative predictive values in your patient population. It might be necessary to include appropriate confirmation tests to increase the specificity of your diagnostic protocol in low prevalence settings.

**Format**
Combined CT/NG assays from several commercial providers allow detection of both organisms from a single sample with little increase in reagent and labour costs for the NG result. In addition, a multiplex that includes more than one target for a particular pathogen (e.g. cryptic plasmid and outer membrane protein in CT) can provide greater confidence in your overall result and could highlight potential genetic variants if one target becomes negative over time. Alternative combinations of pathogens might be of interest to some users. The maximum number of targets that can be distinguished in the same reaction is limited by methodology. Up to six targets can be multiplexed with a real-time PCR machine, for example [4]. Thus, in-house assays can allow the freedom and flexibility to introduce and validate multiplexed targets relatively quickly and maintain a responsive service until the commercial market adapts to your needs.

**Throughput and specimen turn-round-time**
Give careful consideration to the turn round time your users expect for a test result to be issued. Dedicated automation is a major advantage of commercial systems for high throughput applications such as CT and NG testing. The ergonomic design of the Roche, Genprobe or BD integrated platforms gives a single system the capacity to process over 100,000 specimens a year. The often fragmented instrumentation used for in-house assays can make all but the most organised laboratories look inefficient and make laboratory quality systems more complex.

**Regulations and license**
Be aware of the regulatory environment in which you are working. The EU *In Vitro* Diagnostics Directive makes provision (within certain limits) for the use of in-house tests, and as such, laboratories are able to validate their own assays for CT NG testing. However, guidance from other regulatory and accreditation bodies may apply. For example, the UK National Chlamydia Screening Programme (NCSP) stipulates that laboratories are required to use commercial Nucleic Acid Amplification Tests (NAATs) for all specimens received through this programme. Service license might also apply. Roche has several patents covering PCR. The 5'-nuclease licenses expire in 2011 but others continue until 2017. Legislation can be difficult to interpret and it is
always prudent to seek professional advice. Additionally, make sure you have consent to test for each pathogen and that appropriate care pathways are in place to deal with any positive results. Guidance is available from numerous sources and it is worth checking the national guidelines with someone in your area [5-7].

There is no doubt that in-house assays are essential for some laboratories to provide an effective diagnostic service. These tests fill the void when commercial providers deem a pathogen or condition low priority for assay development. In-house tests allow laboratories to be responsive to the ever changing world of infectious disease and genetics, either through what they detect or how they detect it. However, thought needs to be given to the considerable amount of quality and regulatory issues that accompany this route, especially when commercial systems can provide an attractive alternative.

References

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Scientific Literature

Immunity and vaccines against sexually transmitted Chlamydia trachomatis infection.
This article reviews recent findings on immunity and vaccine development in Chlamydia trachomatis. There is increasing knowledge on the interactions between C. trachomatis and infected host cells. During genital infection the organism avoids generating protective immunity but immune responses to a number of chlamydial proteins have been associated with reproductive tract pathology. Various vaccine and adjuvant preparations have been tried experimentally. Information generated by proteomics and complex studies of serological and T-lymphocyte immune responses points to novel vaccine candidates.

To develop rational C. trachomatis vaccines it is necessary to understand the complex lifecycle of the organism, the host immune response to infection and how these relate to disease. Infection does not prevent re-infection and antibiotic treatment prevents antibody production at a population level. It remains unclear what type of immune response would be sufficient to prevent infection and/or re-infection. Although the prevalence and demographics of infection and the severity of disease associations suggest that it would be desirable, there is no vaccine currently available. A number of studies have identified novel vaccine candidates.


Screening methods for Chlamydia trachomatis and Neisseria gonorrhoeae in sexu-
ally transmitted infection clinics: what do patients prefer?
To meet the need for services at sexually transmitted infection (STI) clinics, self-
obtained vaginal (SOV) swabs or first-catch urine (FCU) samples collected at a clinic visit have been proposed as an alternative approach for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) screening. The purpose of this clinic-based survey was to determine if non-invasive clinic-based SOV swabs and FCU samples for CT and GC screening are acceptable replacements for a traditional provider visit. Methods Patients seen at STI clinics in three US cities completed a self-administered survey of preferences for methods of CT and GC screening under hypothetical circumstances. A total of 2887 participants completed a self-administered questionnaire that contained multiple-choice questions about their preference. If there were a hypothetical long clinic wait, 58% of the survey participants preferred to wait to see a doctor. If the clinic had to turn patients away, 41% of patients preferred to come back the next business day and 46% preferred to self-collect a sample. The percentages were similar across site, demographic and clinical groups. The findings indicate that more detailed information about self-collection practices must be provided for patients to adopt this new approach.


Older and swinging; need to identify hidden and emerging risk groups at STI clinics.
Identification of STI risk groups is essential for optimal prevention and medical care. Until now, swingers—that is, heterosexual couples who are practising mate-swapping, group sex and who visit sex clubs for couples, are not considered as a specific risk group for STI in healthcare services and prevention. At the STI clinic, South Limburg, the Netherlands, whether an attendee is a swinger has been systematically registered since 2007. STI clinic surveillance data were analysed to assess the swingers’ share of consultations and STI diagnoses—here Chlamydia trachomatis (CT) and/or Neisseria gonorrhoea (NG).

Of 8971 consultations, 12% comprised swingers (median age 43 years, IQR 38-48). Overall, STI prevalence was highest in youth, men who have sex with men (MSM) and swingers. Older swingers had a CT prevalence of 10% and an NG prevalence of 4%. The share in STI diagnoses in the older age group (>45 years) comprised 55% for swingers and 31% for MSM. Swingers, like other groups with risk behaviours, need to be identified and treated as a risk group in STI prevention and care.

The Handbook of Biomarkers
Edited by Kewal K. Jain, published by Humana Press, 2010, 462pp, £135.00
Of the thousands of biomarkers that are currently being discovered, relatively few are being validated for further applications, and the potential of a biomarker can be quite difficult to evaluate. To aid in this imperative research, this book provides a thorough description of many different types of biomarkers and their discovery using various ‘-omics’ technologies, such as proteomics and metabolomics, along with the background information needed for the evaluation of biomarkers as well as the essential procedures for their validation and use in clinical trials. With biomarkers described first according to technologies and then according to various diseases, this detailed book features the key correlations between diseases and classifications of biomarkers, which provides the reader with a guide on current and future biomarkers. Comprehensive and cutting-edge, The Handbook of Biomarkers serves as a vital guide to furthering our understanding of biomarkers, which, by facilitating the combination of therapeutics with diagnostics, promise to play an important role in the development of personalised medicine, one of the most important emerging trends in healthcare today.

Clinical Aspects and Laboratory. Iron Metabolism, Anemias. Concepts in the anemias of malignancies and renal and rheumatoid diseases
Control of iron metabolism and maintenance of iron haemostasis is a crucial part of many aspects of human health and disease. Iron deficiency anaemia is one of the most common diseases worldwide, but there are also anaemias associated with chronic diseases, and other acquired or hereditary defects. Understanding the control of iron metabolism is furthermore important for understanding diseases of iron overload, such as haemochromatosis. This booklet is designed for physicians, clinical lab personnel and medical students. It gives an overview about the principles of regulation of iron metabolism and erythropoiesis. In addition, the various disturbances of iron metabolism and the associated clinical findings are described. A focus on the differential diagnosis of the disorders is provided, as well as therapeutic approaches. Finally, a comprehensive schedule of tests available for the determination of iron metabolism-related parameters in serum/plasma and blood is included, with applied methodologies and appropriate reference ranges.
The 6th International Symposium on clinical applications of serum free light chain analysis (plus Hevylite): meeting highlights

In September 2010, 500 international delegates from the diverse disciplines of biochemistry, haematology and nephrology attended the 6th International Symposium on clinical applications of serum free light chain (plus Hevylite) in the picturesque Bath Assembly Rooms. The main focus of the two day meeting was the clinical utility of these assays in the management of patients with monoclonal gammapathies. In addition, the theory and preliminary data behind Combylite, a novel assay which measures summated polyclonal free light chains (FLCs) was introduced. In this meeting report, we summarise the key highlights with specific focus on topics relevant to the readers of Oncology.

by Ellen Suggate, Alison Levoguer, Josie Evans, Alex Legg and Richard Hughes

Guidelines for the use of serum free light chain (sFLC) assays

Professor Kyle (Mayo Clinic, USA) opened the conference with an overview of the discovery of Bence Jones protein (monoclonal FLC) and how the techniques used to measure this important tumour marker have evolved over the past 150 years from cumbersome electrophoretic tests (using apparatus that were over 6 metres long and 1.5 metres high) to a quantitative serum immunoassay. With the scene set, Dr Durie (Cedars Sinai, USA) spoke on behalf of the International Myeloma Working Group (IMWG) and summarised the key recommendations for the routine use of sFLC assays [1]. These recommendations included: (i) screening for plasma cell dyscrasias (discussed below); (ii) monitoring patients with oligosecretory multiple myeloma and AL amyloidosis; (iii) definition of a stringent response to treatment in all myeloma patients; (iv) early detection of light chain escape and (v) risk stratification of patients with monoclonal gammapathy of undetermined significance (MGUS), smouldering multiple myeloma and other related conditions. Although published in 2009, Dr Durie emphasised the need to update the IMWG guidelines in-line with recently published data. One such potential update was discussed by Dr Katzmann (Head of Dept. Laboratory Medicine and Pathology, Mayo Clinic, USA) who presented data on the diagnostic sensitivity of the sFLC assays and how recommendations on their incorporation in routine laboratory paraprotein screening panels may be altered. Current IMWG guidelines recommend a primary myeloma screen consisting of serum protein electrophoresis (SPE), serum immunofixation (sIFE) and sFLC tests. However, SPE in combination with sFLC tests (with sIFE used only as a reflex test) still achieved 100% sensitivity for multiple myeloma and Waldenström’s macroglobulinaemia [2]. This screening panel has been shown to have a reduced diagnostic sensitivity for MGUS, although Dr Katzmann stated that the missed individuals are likely to be amongst the lowest risk patients [Table 1; discussed below]. He therefore raised the possibility of incorporating this screening algorithm into future IMWG guidelines.

**FLCs in MGUS management**

Management strategies for MGUS patients were also reviewed by Dr Katzmann. Once identified, this premalignant condition has a 1% risk per year of progression to a malignant disease so MGUS patients are regularly followed up. Three independent risk factors for MGUS progression (serum monoclonal protein size ≥15g/L, non-IgG monoclonal protein isotype and abnormal sFLC ratio) have been incorporated into a risk-stratification model to separate MGUS patients into low to high risk groups, which can then be used to tailor subsequent clinical management [Table 1], [3]. Dr Katzmann postulated that reduced follow-up for low-risk MGUS patients (∼40% of all patients) will lessen medical costs as well as patient anxiety.

Dr Landgren (Bethesda, USA) also discussed the role of sFLC analyses in MGUS patient management. He reviewed SPE, sIFE and sFLC data from samples collected from 71 multiple myeloma patients prior to diagnosis [4]. This work identified several key findings: (i) Multiple myeloma is consistently preceded by an MGUS phase. (ii) An abnormal FLC ratio may be the only abnormal finding prior to the diagnosis of multiple myeloma, representing a precursor condition termed ‘FLC-MGUS’. (iii) Approximately half the study patients demonstrated a year-by-year increase in monoclonal protein concentration and FLC ratio prior to myeloma diagnosis. Dr Landgren concluded that there is a role for both SPE and sFLC analyses in routine MGUS follow-up.

**Technical considerations**

Whilst sFLC assays are a valuable clinical tool, certain technical limitations, including

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**Table 1. MGUS risk-stratification model.** Risk factors are serum monoclonal protein ≥15g/L, non-IgG subtype and an abnormal sFLC ratio. *accounting for death as a competing risk.*

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Number of patients (%)</th>
<th>Relative risk</th>
<th>20 year risk of progression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>449 (39)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Low-intermediate</td>
<td>420 (37)</td>
<td>5.4</td>
<td>10</td>
</tr>
<tr>
<td>High-intermediate</td>
<td>226 (20)</td>
<td>10.1</td>
<td>18</td>
</tr>
<tr>
<td>High</td>
<td>53 (5)</td>
<td>20.8</td>
<td>27</td>
</tr>
</tbody>
</table>

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antigen excess (false under-read) and FLC polymerisation (false over-read), were highlighted. Dr Carr-Smith, (production director at Binding Site, UK), explained that antigen excess is inevitable when measuring levels of an analyte with a >10,000-fold range. Nonetheless, this is a rare occurrence with a recent screening study demonstrating an incidence of 0.12% [5]. These limitations are being addressed through several strategies, including the implementation of automated antigen excess detection protocols (currently available on SPAPLUS and Roche Integra platforms). In addition, early development work was described on alternative FLC assay formats, which would not be susceptible to antigen excess or over-reading of polymers. The development of a novel screening assay system that utilises an enzyme immunoassay format was also highlighted. The system would allow for the simultaneous measurement of numerous parameters, including κ and λ sFLCs, the six Heavy/Light chain (HLC) analytes (discussed later) and other key prognostic markers for multiple myeloma.

Polyclonal FLC measurements

Another focus for the speakers was preliminary data on a novel, prognostic marker: summated FLCs. Dr Mead (Binding Site, UK) introduced the concept of Combylite, a single assay format for the simultaneous measurement of both κ and λ sFLCs (free κ + free λ). For patients with monoclonal gammopathies, a key feature of sFLC analyses is the derivation of a FLC κ/λ ratio to provide a sensitive measure of clonality. The Combylite assay does not derive a FLC κ/λ ratio and is not intended to replace the individual FLC assays for the management of monoclonal gammopathies. Dr Mead highlighted its potential clinical utility in any disorder that may affect polyclonal FLC levels.

The prognostic value of summated FLCs in specific disease groups was of particular interest to a number of speakers. Dr Pratt (Heartlands Hospital, Birmingham, UK), Professor Pinto (Instituto Nazionale Tumori, Italy) and Dr Maurer (Mayo Clinic, USA) observed that increased baseline summated FLC values were linked to inferior outcome in patients with chronic lymphocytic leukaemia, classic Hodgkin’s lymphoma and diffuse large B cell lymphoma, respectively. Dr Landgren and Professor Terrier (Groupe Hospitalier Pitié-Salpêtrière, France) also reported that, in the setting of HIV and Hepatitis C infection, raised polyclonal FLCs may act as early biomarkers for identifying lymphoma development [6,7]. Dr Stringer (Queen Elizabeth Hospital, Birmingham, UK) presented data which demonstrated that summated polyclonal FLCs independently predicted survival in patients with chronic kidney disease (CKD), regardless of disease stage, and concluded that summated FLC may play a role in the detection of early renal damage.

HLC analyses for intact immunoglobulin measurement

On the second afternoon, the focus of the meeting shifted from FLC analyses to methodologies that type and quantify monoclonal intact immunoglobulins. Professor Bradwell (University of Birmingham and Binding Site, UK) discussed the concept of Heyvylite (HLC) and how these automated, nephelometric immunoassays can be used to derive intact immunoglobulin κ/λ ratios, in a manner analogous to sFLC tests. This has only been possible with the recent development of highly specific antisera that recognise epitopes that span both the antibody heavy chain and its associated κ or λ light chain [8]; [see Table 2 for a comparison of κ FLC and IgGκ Heyvylite (HLC) assays].

Particular attention was given to the ability of the HLC assays to eliminate the inaccuracies associated with quantifying low concentration or ‘hidden’ monoclonal proteins by scanning densitometry. This was highlighted in a meeting poster [Figure 1A], [9] which demonstrated that abnormal HLC ratios typed the IgA multiple myeloma samples in concordance with sIFE [Figure 1B] and provided accurate quantitative values for IgA monoclonal proteins that co-migrated with transferrin and other β-region proteins. A second meeting poster demonstrated the ability of the HLC assay to detect and quantify monoclonal proteins in patients with systemic AL amyloidosis [10]. This included a number of AL amyloidosis patients that were negative by SPE and IFE. Professor Ludwig (Centre for Oncology and Haematology, Wilhelminenspital Vienna, Austria) reported that in some multiple myeloma patients, abnormal HLC ratios were more sensitive than IFE in detecting minimal residual disease, and showed earlier relapse in a number of patients than SPE and IFE analyses.

Table 2. Comparison of the protein targets recognised by κ Freelite (FLC) and IgGκ Heyvylite (HLC) assays. The κ Freelite assay comprises sheep polyclonal antisera specific for epitopes on the constant domain of the κ FLC that are exposed on the unbound light chain, but hidden when bound to the heavy chain in an intact immunoglobulin molecule. The IgGκ Heyvylite assay comprises sheep polyclonal antisera specific for epitopes that span constant regions of both the heavy chain and light chain, and will not bind to a FLC or heavy chain alone.
Several lectures and conference abstracts discussed the clinical importance of HLC pair suppression (for example, the suppression of IgGκ in an IgGλ myeloma patient). HLC pair suppression increases the diagnostic sensitivity of the HLC ratio, augments the dynamic range for HLC during monitoring and provides novel, prognostic information. This prognostic value was highlighted by Dr Katzmann, who returned to the lectern to present preliminary HLC data in MGUS. IgG HLC suppression (for example, the suppression of IgGκ in an IgGλ myeloma patient). HLC pair suppression was a strong prognostic value was highlighted by Dr Katzmann, who returned to the lectern to present preliminary HLC data in MGUS. IgG HLC suppression provides novel, prognostic information. This prognostic value was highlighted by Dr Katzmann, who returned to the lectern to present preliminary HLC data in MGUS. IgG HLC suppression provides novel, prognostic information. This prognostic value was highlighted by Dr Katzmann, who returned to the lectern to present preliminary HLC data in MGUS. IgG HLC suppression provides novel, prognostic information. This prognostic value was highlighted by Dr Katzmann, who returned to the lectern to present preliminary HLC data in MGUS. IgG HLC suppression provides novel, prognostic information. This prognostic value was highlighted by Dr Katzmann, who returned to the lectern to present preliminary HLC data in MGUS.

To summarise, this 2010 symposium acted as an important forum to introduce new concepts in the diagnosis and management of B cell disorders. In this largest meeting-to-date, the speakers presented data on a wide breadth of topics and stimulated debate on both established and novel applications for FLC and HLC analyses. Several speakers highlighted the need to update current IMWG guidelines, with further incorporation of routine sFLC testing. Summed FLCs have been shown to have significant prognostic value, and research is actively being conducted to further dissect these observations. Finally, HLC tests have emerged as important assays that can overcome some of the current technical limitations associated with measuring intact immunoglobulins, including quantification of hidden monoclonal bands, and may provide novel prognostic information through the measurement of HLC pair suppression.

Meeting abstracts from the 6th International Symposium on Clinical Applications of Serum Free Light Chain Analysis (plus Hevylite) can be found at: http://www.pagepress.org/journals/index.php/hr/article/view/2062.

References
Ergon Capital Partners signs agreement to become the majority shareholder of ELITech Group

Ergon Capital Partners III SA ("Ergon") announced recently that it has reached an agreement with the shareholders of ELITech Group. Following this agreement, ELITech Group will be acquired by a newly incorporated company controlled by Ergon, alongside the founders (Financière du Bief and Biotech International), the historical financial investors (BNP Paribas Développement, Idia Participations, Naxicap and Synergie Finance), and Management. With six manufacturing sites in France, the USA, the Netherlands and Italy, and 14 subsidiaries, ELITech Group is a leading independent manufacturer and distributor of in vitro diagnostic equipment, tests and reagents focused on small to medium-sized, local and emergency diagnostic laboratories. The Group is currently active in three segments of the diagnostics market: biochemistry, microbiology and molecular diagnostics. ELITech Group also operates as a distributor of third-party products to small- to medium-sized and local laboratories. Pierre Debias, CEO of ELITech Group, said that this was a strategically important step in the development of the Group. Ergon was a reputed investment institution, which would provide ELITech with its expertise in the future transformation of the Group by bringing further professional qualities and capacity to the organisation and by assisting in making add-on acquisitions. He took this opportunity to thank ELITech's historical financial shareholders for their support to date and was looking forward to a continuing partnership with them, together with Ergon.

www.elitechgroup.com

Dako brings BioPorto’s kidney injury test to the world market

From January 2011, Dako will be distributing The NGAL Test – BioPorto’s new test for measuring acute kidney injury. Thea Olesen, CEO of BioPorto, said that Dako was the perfect partner for them, as Dako sold its products in more than 80 countries and they had decades of experience in the launch and sale of diagnostic tests. In addition to ensuring The NGAL Test a quick and direct access to most of the world market, the contract with Dako would also contribute significantly to securing the right level of technical support that was invaluable when launching a completely new type of test. Dako is also pleased with the agreement. Christina Lindved Turner, Vice President of Reagent Partnership Division at Dako, said that the diagnosis of kidney disease was already one of Dako’s core areas in the Reagent Partnership Division, and with access to The NGAL Test the company would now get an opportunity to offer their customers a popular new product, which both they and their customers look forward to. The agreement does not prevent BioPorto from optimising sales channels for The NGAL Test. BioPorto is therefore continuing the discussion on cooperation with the global diagnostics companies that are interested in marketing the kidney injury marker as an important product in the portfolio of tests for their own analytical instruments.

NGAL is a novel biomarker for diagnosing acute kidney injury. The key advantage of NGAL is that it responds earlier than other renal status markers such as serum creatinine and shows a response proportionate to the injury. NGAL therefore provides a new way of identifying patients at risk of developing potentially severe acute kidney injury - up to 48 hours before the problem would otherwise be detected. Using only a few drops of blood or urine, The NGAL Test provides results in just 10 minutes and thus addresses the widespread demand for urgent NGAL determination. The NGAL Test is designed for routine use on chemistry analysers from manufacturers such as Roche, Abbott, Siemens, Olympus etc. effectively giving almost any laboratory immediate access to a fast and easy method to measure NGAL. The test is now offered with research use only claims, for set-up and local evaluation in countries outside the United States, but already from early 2011, The NGAL Test will be available for routine diagnostic use in the EU and other selected countries. Availability in the United States is contingent upon US FDA clearance.

www.bioporto.com

Prokyma scoops innovation in diagnostics award

ProKyma Technologies Ltd won the prestigious ‘Innovation in Diagnostics Project of the Year’ at the UK Northwest Biomedical Awards in November. Presented by Thermo Fisher, the award recognises the significant potential of the company’s innovative technology for the rapid isolation of bacteria or specific target cells in situations where there are high levels of debris such as sputum and blood. Ongoing developments for the technology include the rapid detection of Mycobacterium tuberculosis (TB) in sputum samples and the capture of Circulating Tumour Cells (CTC) in blood. The patented KymaSep system utilises a combination of magnetic and ultrasonic forces (acousto-magnetic) to control magnetic beads in a flow-through chamber. The beads, which are coated to make them highly specific for the target bacteria or cells, can be captured and held on the side of the chamber during aggressive washing and then automatically resuspended and collected. The cells survive the process intact allowing them to be used for further analysis including microscopy and culture. Unaffected by background cells or debris, acousto-magnetic separation is suitable for a wide range of applications that have proved too challenging for traditional rapid methods, particularly those where target cells or bacteria are present in very low numbers. By processing much larger volumes than other techniques, KymaSep captures sufficient cells to produce highly concentrated samples.

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PSP94 (prostatic secretory protein-94), also known as β-microseminoprotein or prostatic inhibin-like protein, is a small, nonglycosylated peptide consisting of 94 amino acids. PSP94 is one of the major secretory proteins of the prostate glands, and together with PSA and PAP, one of the three most abundant proteins in seminal fluid. PSP94 can leak into the bloodstream if benign or malignant prostate epithelial disruption occurs, and can be measured in serum. Several studies have demonstrated a progressive decrease in PSP94 level as prostate cancer progresses from a hormone-dependent to a hormone-independent state, with a complete lack of PSP94 production in highly advanced metastatic prostate cancer. PSP94 could thus be a prognostic clinical marker for prostate cancer, and could help determine which patients have aggressive forms of prostate cancer. The close correlation between the level of PSP94 in serum and in seminal plasma also supports the potential use of PSP94 as a serum marker of prostate secretory function. The Human PSP94 ELISA from BioVendor has enhanced the company’s portfolio of diagnostic kits for oncology applications.

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Modrice, Czech Republic

Tests to detect faecal calprotectin in stool samples

Calprotectin, a protein released by neutrophils, is a non-invasive marker of intestinal inflammation allowing differentiation between organic and functional disorders. The concentration of calprotectin is higher in patients with Inflammatory Bowel Diseases (IBD) than in normal subjects and those with Intestinal Bowel Syndrome (IBS), and normal levels of faecal calprotectin are associated with histological remission of the mucosa. Calprest, a quantitative enzyme immunoassay, and CalFast, a quantitative rapid immune-chromatographic assay, provide a complete panel of tests for the in vitro diagnosis of intestinal inflammation. Both tests are based on calibrators manufactured according to the original standard preparation. Due to their high sensitivity and specificity, these tests are suitable for use with both adult and paediatric patient samples. CalFast is designed for initial screening and the Calprest assay is used to confirm initially positive results. The tests, in association with clinical findings, are ideal for screening, follow-up and monitoring therapy. A positive calprotectin value is highly suggestive of IBD (calprotectin is increased in more than 95% IBD patients), while a negative result allows IBD and IBS to be differentiated. Calprotectin screening might also be used to select patients with inflammation who require further examination.

EUROSPITAL
Trieste, Italy

Test kit for syphilis diagnosis

Syphilis is distributed worldwide but is especially prevalent in less developed countries. It is estimated that twelve million new cases occur annually, and prompt diagnosis is essential for effective treatment. Diagnosis is based on the detection of Treponemal antibodies or the detection of non-Treponemal (Reagin) antibodies, but each of these tests has limitations. The WHO has recommended that a combination of both tests be used for screening and diagnosis, and laboratories worldwide now use a sequential approach, first testing for non-Treponemal antibodies (VDRL test), followed by testing for Treponemal antibodies. While rapid immunochromatographic tests for Treponemal antibodies are available, non-Treponemal antibody tests typically require a well-equipped laboratory, so are not suitable for use in the field. The Signal-Spirolipin, a rapid test which can be used in field conditions, is now available that allows the simultaneous detection of both Treponemal and non-Treponemal antibodies. The flow through spot/immunodot test kit is a qualitative immunoassay that provides visual results in ten minutes. The test incorporates purified recombinant Treponemal antigens, and non-Treponemal antigens that are separately coated on the nitrocellulose membrane. All stages of syphilis except very early primary syphilis can be detected with very high specificity and sensitivity.

SPAN DIAGNOSTICS LTD
Surat, India

PRODUCT NEWS
A new ELISA based on a designer recombinant fusion protein provides unparalleled sensitivity for detection of autoantibodies against the mitochondrial antigen M2 (AMA-M2) in the diagnosis of primary biliary cirrhosis (PBC), an immune-mediated chronic inflammatory cholestatic liver disease of unknown aetiology. The novel fusion protein combines the immunogenic domains of all three relevant enzyme complexes (3E) of the M2 antigen, presenting them in a defined stoichiometric relationship. This designer antigen is used together with the classical target antigen, native pyruvate dehydrogenase, in the Anti-M2-3E ELISA. The extended antigen spectrum and the resulting increase in diagnostic efficiency make the test a powerful new tool for PBC diagnostics. In a study with 170 sera from clinically characterised PBC patients the new Anti-M2-3E ELISA demonstrated a 14% higher sensitivity than a conventional Anti-M2 ELISA (93% compared to 79% at a specificity of 98%).

Up to 94% of PCB patients exhibit AMA-M2 and these constitute the most important serological markers. The majority of M2-specific autoantibodies are directed against the enzyme pyruvate dehydrogenase, and many commercial ELISAs are based solely on this main antigen. However, 5-10% of patients only exhibit antibodies against the other two main autoantigens of the M2 complex, namely branched-chain 2-oxoacid dehydrogenase and oxoglutarate dehydrogenase, and are therefore not detected with conventional tests. The new ELISA eliminates this shortcoming.

EUROIMMUN AG
Luebeck, Germany
i www.cli-online.com & search 25400

New crimping jaw sets for electric crimper/decapper
The lightweight, semi-automatic, ergonomically designed CRIMPenstein Electric Crimper/Decapper is ideal for laboratories that crimp or decap large volumes of aluminum seals. An adjustable jaw intensity regulates jaw action as required to achieve the perfect crimp. Since the instrument is AC powered, the need for regulated compressed air or batteries which discharge over time is eliminated. Engineered to accommodate 13mm and 20mm flip cap aluminum seals, two new crimping jaw sets are now available for use with the instrument. As with the 8, 11, 13, and 20mm crimper or decapper jaw sets for standard seals, the new flip cap crimping jaws are completely compatible with the instrument’s controller and are interchangeable with the other jaw sets for quickly changing between different sizes and models.

WHEATON SCIENCE PRODUCTS
Minotola, NJ, USA
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Osteoporosis diagnostic test kit

Osteoporosis affects over 75 million people worldwide and in a single year there are more than nine million instances of osteoporotic fractures, making it the fourth most significant non-infectious disease after cardiovascular disorders, cancer and diabetes. It is now known that osteoporosis and the subsequent risk of fracture is under genetic control, with the genes for collagen type 1 (COLIA1) and the vitamin D receptor (VDR) being of significant importance. Genetic testing allows an increased risk of developing osteoporosis to be identified so that preventive treatment can be initiated as soon as possible.

An osteoporosis diagnostic test kit is now available that uses RT-PCR to detect polymorphisms in the COLIA1 and VDR genes and identify defects which result in abnormalities in bone matrix mineralisation. Suitable for clinical applications, the assay is more sensitive and convenient than alternative tests. The kit includes all the necessary primers, enzymes, positive controls and buffers sufficient to perform 100 assays.

ASTRA BIOTECH GMBH
Luckenwalde, Germany
i www.cli-online.com & search 25403

Midkine ELISA

Midkine, also known as neurite growth-promoting factor 2 (NEGF2), is an embryonic cytokine and well-known cancer biomarker that has been found to correlate with cancer progression and malignancy. Midkine has limited expression in healthy adults, but is detectable in high quantities in the blood and urine of patients with a wide variety of cancers. A fully validated ELISA protocol for Midkine detection in blood serum has been developed by BioGenes, and MK ELISA kits are now available through Cellmid (www.cellmid.com.au). The MK ELISA is a highly sensitive and robust assay for the accurate measurement of midkine in serum. The ELISA kit is thus suitable for diagnostic development and the validation of cancer diagnostic and prognostic applications. The test is highly sensitive with a detection limit of 8pg/mL, which is well within the range of healthy serum MK levels of 0 to 300pg/mL. The test is also highly selective and shows no cross-reactivity with the closely related protein pleiotrophin. The assay recognises all major species of midkine including human, mouse, dog and pig.

BIOGENES GMBH
Berlin, Germany
i www.cli-online.com & search 25402

Comprehensive range of laboratory autoclaves

Priorclave’s range of electrically-heated autoclaves includes the PS/MID/C60, a compact, 60L capacity, top-loading autoclave and the PS/QCS/EH150 mid range, 150L capacity, front-loading model with an optional direct steam heating facility. The latest model, the 95L PS/OPL/V95 top-loading unit, from the entry-level OPAL range, has been specifically designed for the smaller laboratory in order to perform cost-effective routine laboratory autoclaving functions, such as growth media preparation and laboratory waste sterilisation. Autoclaves in this range feature automatic free steaming and a thermal safety lock, and are managed by a slimmed-down version of the company’s tried and tested Tactrol microprocessor control system. The autoclaves operate up to 138°C – 2.4 Bar. Models in the standard range feature BioCote anti-microbial coating, clinically proven to be effective against microbial growth on working surfaces, and a valuable second line of defence in the fight against cross-infection in the laboratory.

PRIORCLAVE LTD
London, UK
i www.cli-online.com & search 25406

HER2 test and training

The Leica Bond Oracle HER2 IHC System offers a fully automated, robust and dependable immunohistochemical test for accurate assessment of HER2 status in breast cancer tissue. A new web-based training course in use of the system has been launched. This new E-learning course harnesses advanced training concepts to provide comprehensive training over the web, with convenient access allowing students to study at the time and place that best suits their schedules. The new, interactive E-learning course assists histologists and pathologists considering HER2 testing by covering all aspects of Bond Oracle HER2 IHC staining and interpretation. Students are carefully guided through key concepts such as specimen preparation, performing the test and interpretation of results, with interactive exercises and tests throughout. On completion of the course there is a comprehensive graded test, which includes general questions and an interactive interpretation session that challenges students to correctly interpret and score real cases. Success is recognised by the award of a certificate of completion.

LEICA MICROSYSTEMS GMBH
Wetzlar, Germany
i www.cli-online.com & search 25201
Test system for high-risk HPV types in cervical cancer screening

Virtually all cases of cervical cancer are caused by a persistent infection involving HPV, but not every HPV type carries the same risk for the development of cervical cancer. The new PapilloCheck high-risk test system focuses on parallel analysis of 14 HPV types of the high-risk group (hrHPV) that are recognised as carcinogenic. In a recent clinical study, the test system, based on microarray technology, showed a clinically-relevant sensitivity of 95.8 per cent and a clinical specificity of 96.7 per cent, meeting key requirements for fast and clear results in cervical cancer screening.

GREINER BIO-ONE GMBH
Frickenhausen, Germany
i www.cli-online.com & search 25391

Flow cytometry for validating abnormal haematology samples

Based on Beckman Coulter’s expertise in both haematology and flow cytometry, the HematoFlow solution delivers faster white blood cell (WBC) differential results with greater consistency than manual assessment under the microscope. The company has recently introduced a new five-colour antibody cocktail, the CytoDiff, which uses six monoclonal antibodies to establish an extended flow WBC differential, detecting and quantifying nine cell subsets. It is now possible to replace the microscopic review of all flagged samples by a unique automated flow-based analysis. This decreases turnaround time and reduces manual tasks, enabling the laboratory to handle increasing workloads while providing more objective and reliable results. Research confirms that >95% of cases were successfully diagnosed after running the cocktail. The instruments are LIS-ready, and in conjunction with Beckman Coulter’s haematology analysers, automated preparation system and Remisol Advance middleware, HematoFlow with the CytoDiff reagent can provide a complete sample-in/result-out solution for the haematology laboratory.

The CytoDiff reagent currently runs on the company’s existing FC 500 system and is expected to be extended to other platforms. A new generation of flow cytometers, the Gallios for research use and the Navios for the clinical laboratory, have been released recently. Both can be configured as either a two-laser system providing six- or eight-colour detection, or as a three-laser system offering up to 10-colour analysis.

BECKMAN COULTER
Nyon, Switzerland
i www.cli-online.com & search 25390
Handheld capping/decapping instrument

With its ability to remove and replace screw caps with precision and ease, the 8-Channel Decapper for handheld capping and decapping of multiple tubes in a 96-well microplate footprint, eliminates the time-consuming task of manually capping and decapping, as well as the risk of repetitive strain injury (RSI). This handheld decapper is ideal for any laboratory requiring faster, uniform capping that cannot justify a fully-automated system. In combination with the use of a multichannel pipette, sample processing is effectively streamlined to increase efficiency. The decapper is capable of uncapping a column of eight screw-top tubes in just four seconds. The caps are tightened uniformly and at optimal torque to prevent any spills or leakages, to maintain sample integrity, and to avoid sample loss during cryogenic storage and freeze-thaw cycles. As an ideal midpoint between manual and automated capping, the decapper enables laboratories to decap only a single column as needed and to significantly improve efficiency without investing in capital equipment.

THERMO FISHER SCIENTIFIC
Winchester, VA, USA
www.cli-online.com & search 25181

Rapid NT-proBNP test for the management of heart failure

Heart failure can be confused with other conditions, such as lung disease. BNP and NT-proBNP levels can help differentiate between heart failure and other problems, since the level of natriuretic peptides in the blood increases as chronic heart failure advances. Used in conjunction with clinical assessment, the new Triage NT-proBNP test facilitates the diagnosis and management of patients with heart failure. The new test expands Alere’s point-of-care Triage cardiovascular testing solutions. The test can also aid in assessing risk in patients with heart failure, as well as those with acute coronary syndrome (ACS). The test can also help to assess the increased risk for cardiovascular events and mortality in patients with stable coronary artery disease (CAD) who are also at risk of heart failure.

ALERE
Bedford, UK
www.cli-online.com & search 25333

Urine container for vacuum tubes

A new 120 mL urine container for vacuum tubes is now available. There are several advantages over traditional containers including improved security, hygiene and ease of handling. The closed system not only prevents contamination of the sample, but also protects the user and the work environment from possible splashes. The container also prevents leakage and thus loss of sample, which could necessitate a second sample collection. The process of sample collection is facilitated for both the technician and patient, allowing a clean transfer of the urine from the container directly to the vacuum tube. Designed for use with vacuum collection tubes, the device consists of a transparent container made of polypropylene and a yellow leakproof cap, made of polyethylene, with an internal obturating ring. The cap includes a cannula with an incorporated needle. Operation is simple: when the needle pierces the tube, the sample is collected by vacuum into the tube, and is then ready for analysis. A wide area on the container allows marking for sample identification. The urine containers are available in sterile and non-sterile versions, and are supplied either in bulk or individually wrapped.

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Barcelona, Spain
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