CSF biochemical markers for Alzheimer’s disease

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Postponing parenthood: why is it a problem?

The British Royal College of Obstetricians and Gynaecologists recently issued a statement warning of the repercussions women may experience by delaying motherhood. There is certainly a significant trend towards older parenthood in most Western countries. In the last twenty years England and Wales have seen births to women aged over 30 years old increase from 28% to 50% of all births, with the greatest percentage increase (about 21% of all births) in women over 35 years old. In Canada during the same period the percentage of first births to women over 35 tripled. Over a quarter of births in Spain, Germany, Italy and Ireland are now to women over 35 years old.

Given the increasing pursuit of higher education by Western women in the last fifty years, leading to more satisfying career choices, greater financial independence and equal status within domestic partnerships, it is inevitable that the trend towards delayed parenthood will continue. So what are the resulting medical problems for both potential older parents and their babies? A major problem is that women’s fertility decreases with age; on average it begins to decline fifteen years before the menopause, and it is estimated that 20% of women aged between 35 and 39 are infertile. Many couples who have postponed starting a family until their late thirties turn to assisted reproductive technology (ART), only to find that this is not the panacea they thought it was.

In the UK the live birth rate per cycle for IVF in 40 year old women is 12.1%, and this plummeted to 1.6% in women of 45. Fertility problems are not confined to the female sex. It is now known that on average men’s fertility also decreases with age. After the age of 25 the volume and motility of sperm gradually reduces so that the ability of the sperm to fertilise an egg is impaired. According to a study published nine years ago (which was not covered by predominantly male tabloid press journalists), the chance of conception within a year of trying to start a family decreases by 3% for every year that a man is over 24.

Quite apart from these fertility issues, pregnancy and childbirth carry more risks for older mothers. Gestational diabetes, placenta praevia and premature birth are all more common, as are spontaneous abortions and stillbirths. There is also an increased risk of maternal mortality. And of course we have been told that older mothers have a higher chance of carrying a baby with a genetic abnormality, though the majority of us are unaware that older men carry an increased risk of fathering a baby with birth defects. Advanced paternal age has been correlated with many such conditions resulting from autosomal dominant mutations, as well as several conditions with less straightforward inheritance patterns. Obviously any solution found for the problem of postponed parenthood will be complex; sociologists, economists, educators and politicians, as well as healthcare workers including ART specialists, should be involved. But wouldn’t it be a start if we were to recognise that this is not exclusively a female problem, but one that affects potential fathers as well as mothers - indeed society as a whole?
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The incidence of Alzheimer’s disease is increasing steadily. AD is currently diagnosed by clinical examination and neuropsychological assessment, as well as by imaging techniques to rule out other causes of dementia; the diagnostic hallmarks of cerebral amyloid plaques and neurofibrillary tangles are only revealed at autopsy. The front cover illustrates a human brain, the source of novel biomarkers that could facilitate early diagnosis and management of AD.

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Lysosomal storage diseases (LSDs) used to be thought of as paediatric disorders, but late onset and adult forms are now recognised. These often show quite different clinical signs and symptoms from the early onset forms. This article focuses on the commoner LSDs in adults and their diagnosis.

by Dr S. Molana and Dr S. Waldek

Lysosomal storage diseases (LSDs) are a group of rare heterogeneous disorders characterised by accumulation of undigested macromolecules within the lysosome as a result of deficiency in an enzyme or activator protein involved in their metabolic pathway. The result is an increase in the size and number of these organelles with disruption of cellular functions and ultimately the production of clinical abnormalities.

Most LSDs are inherited in an autosomal recessive manner. The exceptions are Hunter syndrome (MPSII) that is X-linked recessive; Danon disease that is X-linked dominant; and Fabry disease that is X-linked but can cause disease manifestations in a significant number of female gene carriers. Around 50 LSDs are known at present and are classified according to their metabolic pathway; they include sphingolipidosis, glycoproteinosis, mucolipidosis, mucopolysaccharidosis and oligosaccharidosis [Table 1].

Each LSD has its own unique symptoms and signs, and clinical course. However, one thing that these diseases mostly have in common is that they have a wide range of phenotypes and little genotype-phenotype correlation.

As already mentioned, individual LSDs have a wide variety of manifestations. This review will focus on adult patients presenting to physicians. The main clue to diagnosis is a good history and examination, but this will not be sufficient if storage diseases are not considered in the differential diagnosis. If a disease is not thought of it will not be diagnosed. Once included in the differential diagnosis, most of the conditions (possibly excluding Neiman Pick type C) can be confirmed by a specific enzyme assay. Why should LSDs be diagnosed, some might argue? The answer lies in three facts. Firstly, some are amenable to specific therapy with human recombinant enzyme or substrate reduction therapy while others may be treatable in the future. (This review will not deal with treatment other than to state where it is available for appropriate cases). Secondly, LSDs cause symptoms, either directly or via secondary complications, that can be treated to improve quality and quantity of life. Lastly, the conditions are genetic disorders and appropriate counselling and family screening can be given. The commoner types of LSD in adults will be covered here, with a brief mention given of some of the other, much rarer conditions.

**Anderson-Fabry disease**

This is an X-linked LSD caused by deficiency of alpha-galactosidase resulting in progressive intracellular accumulation of glycosphinogolipids in different tissues. Classical features include angiokeratomas, hypohydrosis, acroparesthesia and pain crisis, cerebrovascular disease, cochlear and vestibular involvement, progressive left ventricular hypertrophy, cardiac conduction defects, coronary artery disease, and progressive renal impairment with proteinuria. Although this disease is X-linked, unlike other X-linked conditions, evidence from natural history studies shows that up to 80% of female “carriers” can have some manifestations of the disease, with a significant number showing symptoms and signs sufficient to affect quality of life.

Evidence from natural history studies and disease registries shows that the time from first symptoms to diagnosis can be over 10 years, but with increasing awareness since the advent of Enzyme Replacement Therapy (ERT), this is improving. Currently, over 50% of new adult cases present through family tracing. However, there are still a few patients who present with classical symptoms or signs such as the classical rash and/or neuropathic pain. A few also come because of incidental classical eye signs found at routine ophthalmologic examination. Most new cases now present because involvement of one of three main organs—heart, kidney or brain—has been detected. Fabry disease should be considered in all cases of left ventricular hypertrophy where there is no obvious cause. Similarly, it should be excluded in cases of Transient Ischaemic Events or completed stroke in younger patients where there are no other evident risk factors. Renal cases can present in several ways. Occasionally, patients can be detected when they present with mild to moderate renal impairment with proteinuria and undergo renal biopsy. Provided the renal biopsy is prepared appropriately (toluene thin section of electron microscopy), the characteristic inclusion bodies will be found. Other cases are detected by screening patients presenting at, or near, end stage renal failure.

As can be seen, screening is a major method of diagnosis. However, a word of explanation is required. In males, the finding of a low enzyme level is diagnostic (there are now laboratories that can measure enzyme using blood spots, see page 8). However, in females the enzyme level is usually normal, and the only way to confirm a diagnosis when the disease is suspected is by doing gene mutation analysis.

Treatment with ERT is available. Two products exist for use outside the USA. These are effective but further details are not included as part of this review. However, it should be mentioned that ERT is not the sole treatment and adjuvant therapy for renal cardiac and cerebro-vascular disease is often needed.

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**Table 1. Classification of lysosomal storage diseases.**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucopolysaccharidoses</td>
<td>MPS I (Hurler), MPS II (Hunter), MPS VI, MPS VII, MSPA, and MPS VII</td>
</tr>
<tr>
<td>Oligosaccharoidoses</td>
<td>Aspartylglucosaminuria, SSID (infantile sialic acid storage disease), Mannosidosis and B, Fucosidosis, Mucolipidosis I (sialidosis), Sialidosis, Gaucher disease, Galectosemia</td>
</tr>
<tr>
<td>Mucolipidoses</td>
<td>GM2-gangliosidosis, GM1-gangliosidosis, Farber disease, Farber disease, Gaucher disease, Tay-Sachs disease, Krabbe disease, Infantile neuronal ceroid lipofuscinosis, Niemann-Pick disease type A and B, Niemann-Pick disease type C, Menke disease, Mucolipidosis II, Mucolipidosis III, Mucolipidosis IV, Mucolipidosis V</td>
</tr>
<tr>
<td>Sphingolipidoses</td>
<td>Fabry disease, Farber disease, Gaucher disease, Tay-Sachs disease, GM1-gangliosidosis, GM2-gangliosidosis, Krabbe disease, Niemann-Pick disease type A and B, Niemann-Pick disease type C</td>
</tr>
<tr>
<td>Other conditions</td>
<td>Glycogen storage disease type II (Pompe) liver and skeletal muscle enzyme deficiency, lsosomal transport defects, Cystinosis, Neuronal ceroid lipofuscinosis, Wolman disease, Danon disease</td>
</tr>
</tbody>
</table>
Gaucher disease
While Gaucher disease is the commonest LSD overall, cases presenting in adult life are not very frequent. However, because of the excellent response to therapy, recognition and diagnosis is important. This is a multisystemic disorder resulting from a mutation in the gene encoding the enzyme glucocerebrosidase. Deficiency leads to the accumulation of its major substrate, glucocerebroside, principally within the lysosomes of macrophages. This in turn results in the characteristic, clinical findings of large liver and spleen, bone marrow infiltration and bone disease. The involvement of liver, spleen and bone marrow results in anaemia and low platelet counts with abnormal clotting, thus predisposing to a bleeding tendency. The principle bone involvement consists of episodes of severe bone pain (crisis) as a result of bone infarcts as well as a very high incidence of avascular necrosis. Decreased bone mineral density is also a feature of Gaucher disease. Other, rarer manifestations are almost never the presenting feature in adults. The disease is divided into three groups: non-neuronopathic form (formerly type 1), and acute and chronic neuropathic forms (type 2 and 3 respectively). Type 2 is confined to infants and is fatal. Type 3 is less severe, but does not present in adults. Gaucher disease is found in all ethnic groups, but is particularly prevalent amongst Ashkenazi Jews.

Presentation of Type 1 in adults is usually as a result of bone involvement. While patients can present with acute pain and avascular necrosis, most adult cases are detected when they present with the consequences of secondary arthritis, often needing joint replacement. The haematological manifestations do also occasionally present in adults, often via the finding of mild abnormalities on requesting a full blood count.

Diagnosis is easily made by enzyme assay once the disease has been considered. The key for those patients presenting with bone and joint problems is accurate interpretation of radiographs. Even when Gaucher disease is only diagnosed in adulthood, ERT is effective although obviously it does not reverse the damage already done.

Pompe disease (acid maltase deficiency)
Glycogen storage disease type II, or acid alpha-glucosidase (acid maltase) deficiency, is an inherited disorder of glycogen metabolism resulting from defective activity of the lysosomal enzyme alpha-glucosidase. In adults the disease is confined to skeletal muscle and the muscle of the diaphragm. Presentation can be at any age with either a progressive symmetrical proximal myopathy or as respiratory failure resulting from diaphragmatic involvement. Many patients have evidence of both conditions in various degrees of severity. Patients can often be misdiagnosed as having muscular dystrophy. The clue to the respiratory involvement is symptoms of nocturnal desaturation, often with positional elements together with significant differences in FEV1 and FVC between the lying and standing positions. There will also be a reduction in FEV1 and FVC between the lying and standing positions. There will also be a reduction in Sniff Nasal Inspiratory Pressure (SNIPs). The diagnosis is confirmed by reduced activity of alpha glucosidase in leukocytes. Enzyme replacement therapy using recombinant human α-glucosidase is now available and can stabilise or even improve patients, thus making it more important than ever to ensure that cases are not missed.

Metachromatic Leukodystrophy (adult types)
Metachromatic leukodystrophy (MLD, sulphatide lipidosis) occurs in 1 in 40,000 births. Two distinct types of adult MLD have been identified. One group has prominent motor disease, with pyramidal and cerebellar signs with dystonia and peripheral neuropathy. The second group presents with behavioural and psychiatric problems (often confused with schizophrenia) followed by dementia and spastic paresis. While there is no curative treatment, enzyme replacement therapy is being considered.

Niemann-Pick disease type C
Caused by a defect in iron cholesterol transport, this is a very rare condition affecting 1 in 120,000 to 150,000. Although a very heterogeneous disease, the condition usually presents before adulthood. However, there are patients with later onset disease who will present with progressive neurological problems of ataxia, dystonia and dysarthria with a variable decline in cognitive function. These patients may not show the splenomegaly or vertical Gaze palsy characteristic of the earlier onset disease. Recently, treatment for NPC with an inhibitor of glycosphingolipid synthesis (Miglustat) has been licensed and can stabilise or slow down the progression of the disease.

Rare conditions in adults: Neimann Pick type B
Though the vast majority of cases of this condition are caused by a deficiency of acid sphingomyelinase present in childhood, there are a few milder phenotypes that can present later in life. Presentation differs little from the younger patients. There is enlargement of liver and spleen with low platelet count and this is the usual presentation. However, there can be associated respiratory symptoms due to accumulation of substrate within the pulmonary alveoli.

Mucopolysaccharidosis, mucolipidosis and alpha mannosidosis
These conditions are caused by a variety of enzyme defects; all have typical skeletal and morphological manifestations. Presentation is nearly always in infancy or childhood. However, all have attenuated phenotypes that can manifest as skeletal or joint problems. They might also present as complications such as carpal tunnel syndrome or cervical spinal cord compression due to the mechanical effects of substrate accumulation. Although cardiac disease and corneal clouding can also occur in many of these LSDs, presentation with these problems in adult life is exceptionally unusual. MPS I, II and VI are amenable to enzyme replacement therapy.

Gauchidosis (Tay-Sachs disease)
While this condition classically causes a progressive fatal neurological disease in infants and children, there is a rare adult phenotype presenting in early adulthood with a neuro-psychiatric syndrome.

Wolman disease
This condition, caused by a deficiency of acid lipase, is a severe progressive neurological disease in infancy, which can very occasionally present in adults. The cardinal features are massive, often tender, hepatomegaly that can progress to cirrhosis and portal hypertension. It is associated with exceptionally high levels of cholesterol in the plasma but there is no evidence that this gives an increased incidence of cardio-vascular disease, probably because of the small number of patients followed. Anecdotally, treatment with a combination of cholesterol lowering agents may positively influence the progression of the disease.

References

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Diagnosing Pompe disease by measuring enzyme activity in dried blood spots

The measurement of acid alpha-glucosidase in dried blood spots has improved the ability to screen for Pompe disease with diagnostic accuracy similar to the gold standard assay using cultured skin fibroblasts. This article discusses the DBS assay and its advantages compared with other diagnostic methods.

by Dr J. Hamilton

Pompe disease is a lysosomal storage disorder due to deficiency of the enzyme acid alpha-glucosidase (GAA; EC 3.2.1.20) [1]. The condition is also classified as glycogen storage disease Type II.

The enzyme hydrolyses both alpha-1,4- and alpha-1,6-glycosidic linkages at acid pH leading to the complete hydrolysis of glycogen in the lysosome. Deficiency results in accumulation of glycogen particularly in heart and skeletal muscle [2]. Clinical presentation is variable with a number of phenotypes. The early-onset infantile form presents with rapidly progressive muscle disease resulting in cardiomegaly and hypotonia. Death occurs in the first year of life due to cardiac or respiratory failure. Late-onset Pompe disease is more common and presents with muscle weakness and eventual death due to respiratory failure. Onset ranges from the first to sixth decade of life. Inheritance is autosomal recessive with an incidence estimated at 1:40,000 but this may vary in different ethnic populations [3].

Conventional methods for the investigation of Pompe disease rely on the measurement of acid alpha-glucosidase using the fluorimetric substrate 4-methylumbelliferyl-alpha-D-glucopyranoside (4-MUG) or glycogen. Samples used include cultured skin fibroblasts, muscle biopsy, leucocytes and lymphocytes [1]. There are a number of disadvantages with these sample types. Obtaining muscle and skin biopsies are invasive procedures and additionally fibroblasts require two to three weeks for cell culture. Leucocytes and lymphocytes require isolation from whole blood using relatively large volumes of blood (5 to 10 mLs). Enzyme stability in whole blood is a problem requiring transport to the laboratory and sample preparation within one to two days. This can be a major problem for referral laboratories receiving samples from outlying centres. Measurement of protein is also required on each sample with enzyme activity expressed per mg protein.

Measuring enzyme activity in dried blood spots

The measurement of lysosomal enzymes in dried blood spots (DBS) on filter paper was pioneered by Chamois and the method first published in 2001 [4]. The method has been adapted for use in 96-well plates and extended to include up to 12 lysosomal enzymes using fluorimetric, radiometric and tandem mass spectrometry (TMS) methods [5-7]. Samples are prepared by spotting whole blood directly from a finger prick or from lithium heparin or KEDTA samples on to Guthrie cards (Whatman 903 filter paper) and leaving to dry for a minimum of 4 hours. Samples prepared in this way are stable for weeks at room temperature [8] and can be sent to the referral laboratory by post with negligible loss of enzyme activity. This is a major advantage in the investigation of lysosomal storage disorders enabling samples to be transported with ease to specialist centres. Dried blood spot samples can be stored at 4°C or -20°C, preferably double bagged with dessicant, and are stable for months without significant loss of activity [9].

Alpha-glucosidase isoenzymes and the use of acarbose inhibitor

The measurement of lysosomal acid alpha-glucosidase is made difficult by the presence of other forms of acid glucosidase which hydrolyse 4-MUG and result in false negative results in cases of Pompe disease. Maltase glucosidase (MGA) is a form of alpha-glucosidase expressed in neutrophils which interferes with the assay. Traditional methods of overcoming the problem have been the use of fibroblasts or lymphocytes. Both approaches have problems. Fibroblasts are free of MGA and have been the gold standard for diagnosis of Pompe disease [10] but the sample is more difficult to obtain and requires cell culture. Lymphocytes are easier to prepare and do not contain MGA but contamination of the lymphocyte preparation with leucocytes containing MGA may result in false negatives [10].

The use of maltose and acarbose has recently been shown to selectively inhibit MGA and has allowed the reliable measurement of acid alpha-glucosidase in leucocytes and whole blood using the DBS sample [5, 11]. Acarbose has been shown to be a more potent inhibitor of acid alpha-glucosidase than maltose [12] and measurement of acid alpha-glucosidase using acarbose inhibitor with DBS samples has become the method of choice for screening for Pompe disease [10].

Screening for Pompe disease

Our biochemistry lab at Yorkshire Hospital, Glasgow, UK adopted the assay based on the method of Kalwass [13] using DBS samples to measure acid alpha-glucosidase activity at pH 3.8 with 4-MUG substrate and acarbose inhibitor. The assay is also performed simultaneously at pH 7.0 measuring neutral acid alpha-glucosidase activity as a form of reference enzyme to monitor the quality of each sample. Therefore an independent control enzyme (alpha-galactosidase) is only performed on samples with low acid-alpha-glucosidase activity. A repeat sample is requested if either acid alpha-glucosidase activity is low suggesting Pompe disease, or if neutral alpha-glucosidase activity is low suggesting poor sample quality. It is recommended that DBS samples with low acid-alpha-glucosidase activity should be confirmed using a second sample type, preferably cultured fibroblasts [10]. The method is conducted using 96-well plates, which allows batches of samples to be processed rapidly and read using a fluorimetric plate reader.

A reference range was established using DBS samples from 82 normal controls (37 males, 45 females) aged two weeks to 72 years [Table 1]. DBS samples were obtained from 15 known cases of Pompe disease (both infantile-onset and late-onset forms) and two obligate carriers [Table 1 & Figure 1]. Activity in all Pompe cases is clearly below the 2.5th percentile for normals allowing good distinction between normal controls and Pompe disease. Carriers show intermediate levels of activity

<table>
<thead>
<tr>
<th></th>
<th>Normals (n = 82)</th>
<th>Affected cases (n = 15)</th>
<th>Carriers (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid alpha-glucosidase (pH 3.8 with acarbose)</td>
<td>4.8 – 170</td>
<td>0.7 – 2.9</td>
<td>3.5 , 4.0</td>
</tr>
</tbody>
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Table 1. Established reference range: activity expressed as pmol/punch/hour [12].
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Enzyme replacement therapy and newborn screening

Enzyme replacement therapy (ERT) is now available for Pompe disease using recombinant acid α-glucosidase (Myozyme, Genzyme Corp.) from Chinese hamster ovary cells. Clinical studies in the early-onset form of disease have shown good response with heart size decreased, cardiac function improved and improved muscle tone [14]. It is also clear that the best responses are associated with early initiation of ERT. Therefore early diagnosis is of importance in being able to implement ERT at an earlier stage. As a result methods have been developed for newborn screening for Pompe disease using both fluorimetric and TMS based methods. TMS methods also allow multiplexing with simultaneous discrimination of late-onset adult cases giving lower enzyme activity in DBS compared to higher residual enzyme activity in fibroblasts [13]. The majority of samples received in our laboratory are submitted from neurologists investigating adult patients with muscle weakness/myopathy. They have readily adopted the DBS sample in place of skin fibroblasts. We accept both DBS samples and whole blood with some clinicians preferring to send blood which is transferred onto filter paper on receipt of the specimen.

Conclusions

The measurement of acid α-glucosidase in DBS has improved the ability to screen for Pompe disease with diagnostic accuracy similar to the gold standard assay using cultured skin fibroblasts. The assay is rapid, relatively inexpensive and uses a more convenient sample which is stable and non-invasive.

By avoiding the requirement for skin biopsy the method has improved the ability to screen for late-onset Pompe disease in adults by offering a simple and reliable screening test which can be extended to a broader group of patients. The stability of lysosomal enzymes in DBS and development of methods using this sample is a significant advance in the investigation of this group of disorders. The availability of ERT for Pompe disease and the requirement for early diagnosis and treatment have seen the introduction of newborn screening for Pompe disease by this method.

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Figure 1. Alpha-glucosidase activity in Pompe disease patients, carriers and controls.
An improved method for constructing tissue microarrays from prostate needle biopsy specimens.

Prostate cancer diagnosis is routinely made by the histopathological examination of formalin fixed needle biopsy specimens. Frequently this is the only cancer tissue available from the patient for the analysis of diagnostic and prognostic biomarkers. There is, therefore, an urgent need for methods that allow the high-throughput analysis of these biopsy samples using immunohistochemical (IHC) markers and fluorescence in situ hybridisation (FISH) analysis based markers. A method that allows the construction of TMAs from diagnostic prostate needle biopsy cores has previously been reported. However, the technique only allows the production of low-density biopsy TMAs with a maximum of 20 cores per TMA. Here two methods are presented that allow the rapid and uniform production of biopsy TMAs containing between 54 and 72 biopsy cores. IHC and FISH techniques were used to detect biomarker status. Biopsy TMAs were constructed from prostate needle biopsy specimens taken from 102 patients entered into an active surveillance trial and 201 patients in a radiotherapy trial. The detection rate for cancer in slices of these biopsy TMAs was 66% and 79% respectively. Slices of a biopsy TMA prepared from biopsies from active surveillance patients were used to detect multiple IHC markers and to score TMPRSS2-ERG fusion status in a FISH-based assay. An effective method was thus provided for the multiplex analysis of IHC and FISH markers and for their assessment as prognostic biomarkers in the context of clinical trials.


Validation of tissue microarray technology in malignant peripheral nerve sheath tumours.

It has been suggested that the donor tissue cores used in TMAs may not be representative of the whole tissue section. This study aimed to validate the use of TMA technology in the study of malignant peripheral nerve sheath tumours (MPNSTs). A TMA was constructed containing five independent core biopsy samples of 14 formalin-fixed, paraffin-embedded MPNSTs. The immunohistochemical (IHC) results of the five cores from the same tissue block on TMAs were compared with readings from whole sections using two antibodies: anti-Ki-67 and anti-S-100. Digital image analysis was performed to calculate the percentage...
Tissue microarrays are reliable tools for the clinicopathological characterisation of lung cancer tissue.

The advantage of tissue microarray (TMA) technology is its ability to efficiently analyse large numbers of tissue specimens in a methodologically uniform way. The reliability of TMAs, especially with regard to clinicopathological characterisations, when compared to conventional immunohistochemistry (IHC) was evaluated. Seventy-two embedded tissue sections from lung cancer specimens were stained with monoclonal antibodies against the tumour-associated markers TA-MUC1 and Lewis Y. Three representative cores of every tumour were embedded in a paraffin array multiblock. The IHC was evaluated by the immunoreactive score (IRS). The data for the TMA IHC and the conventional IHC were concordant for both markers. Discordance was low, and sensitivity and specificity were above 80% for both markers. In the samples with high positive expression, the concordance increased, discordance disappeared and sensitivity and specificity increased above 90% for both markers. Using Cox regression models, all the clinicopathological dependencies were equivalent for both techniques and both markers. The authors conclude that immunohistochemistry with tissue microarrays is valid and provides results equivalent to conventional immunohistochemistry with respect to expression patterns and clinicopathological characterisations.


The theougth of TRAIL and its death receptors in cervical cancer.

Preclinical data indicate a synergistic effect on apoptosis between irradiation and recombinant human (rh) tumour necrosis factor-related apoptosis inducing ligand (TRAIL), making the TRAIL death receptors (DR) interesting drug targets. The aim of this study was to analyse the expression of DR4, DR5 and TRAIL in cervical cancer and to determine their predictive and prognostic value. TMAs were constructed from tumours of 645 cervical cancer patients treated with surgery and/or (chemo-)radiation between 1980 and 2004. DR4, DR5 and TRAIL expression in the tumour was studied by immunohistochemistry and correlated to clinicopathological variables, response to radiotherapy and disease-specific survival. Cytoplasmatic DR4, DR5 and TRAIL immunostaining were observed in cervical tumours from 99%, 88% and 81% of the patients, respectively. In patients treated primarily with radiotherapy, TRAIL-positive tumours less frequently obtained a pathological complete response compared with TRAIL-negative tumours. DR4, DR5, and TRAIL expression were not prognostic for disease-specific survival. Absence of TRAIL expression was associated with a higher pathological complete response rate to radiotherapy.


Analysis of biological prognostic factors using tissue microarrays in neuroblastic tumours.

Neuroblastic tumours (NT) are paediatric neoplasms with a heterogeneous genetic profile. They present genotypic alterations of prognostic value, the study of which is mandatory in designing therapeutic management. Tissue microarrays (TMA) from paraffin material allow the analysis of a large number of cases with minimal costs. The main purpose of this study was to analyse specific genetic markers of neuroblastic tumours included in TMAs and determine their prognostic value. The results obtained by different molecular techniques on different substrates were compared to evaluate the feasibility of the TMA assays. One hundred and thirty-nine samples were included in four different TMAs. FISH assays were performed to determine the status of MYCN gene, 1p36 region and 17q23 arm. The prognostic value of the genetic markers as well as the statistical correlation among clinical variables and outcome were analysed. MYCN amplification was detected in 35.3% of the cases, whereas 1p36 deletion and 17q23 gain was observed in 46.8% and 58.3% of the cases, respectively. An adverse prognosis was noted among these patients. Other adverse factors were age (>18 months) as well as high stage of disease (stage 4). Phenotypic signs of differentiation correlated with good outcome. Retrospective studies using paraffin-embedded tissues assembled in TMA are a useful tool for the analysis of prognostic factors in NT.

New Guidelines recommend that serum free light chain (FLC) assays be used in the initial evaluation of suspected myeloma

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Biomarkers for dementia: putting the focus on microglia

The current diagnosis of Alzheimer’s disease is based on clinical and cognition testing. Investigations are attempting to define the usefulness of biomarkers in the management of patients. The criteria for establishing and validating candidate biomarkers are critical. While CSF biomolecules have been proposed, markers in the serum possibly including antibodies to microglial cells may usher in a paradigm shift in the work-up of patients suspected of dementia.

by Dr G. Davis, Dr N. Baboolal and Prof. A. McRae

Dementia, in particular Alzheimer’s disease (AD), is fast becoming a major health issue in many countries where life expectancy is increasing and chronic non-communicable diseases have replaced infectious disease as the major cause of morbidity and mortality. To date a cure for AD is yet to be found and the best that can be hoped for is to slow down the progression of the disease and improve the quality of life of the individual. These temporising measures would postpone the onset of the final and debilitating stages of the disease. Interventions may be simple but profound. For example, encouraging results have been seen in pre-morbid and affected individuals in whom an active cognitively-stimulating lifestyle has been facilitated. With this background it is easy to appreciate the need for early detection and intervention, justifying the spirited search for biomarkers. Predicting AD is problematic due to the very nature of the disorder as clinical features only become apparent following a period of insidious but substantial cell loss. Therefore there is a need for biomarkers to flag early cell death as this may allow for treatment interventions which could arrest the neuropathological processes.

According to the criteria of the Consensus Report of the Working Group on Molecular and Biochemical Markers of AD an ideal biomarker should be 1) able to detect a fundamental feature of AD neuropathology, 2) validated in neuropathologically confirmed AD cases, 3) precise (able to detect AD early in its course and distinguish it from other dementias), 4) reliable, 5) non-invasive, 6) simple to perform and 7) inexpensive [1]. Using these guide points a description of a potential marker antibody specifically directed towards activated microglia is described below.

1. Able to detect a fundamental feature of AD neuropathology

Indisputably, the major pathological features in AD are senile plaques, containing β-amyloid (Aβ), and neurofibrillary tangles with tau protein substantially altered very early in the disease process of AD. Plaques and neurofibrillary tangles are located in the midst of an arsenal of immunocompetent cells, notably activated microglia [2]. Not only are microglia immunocompetent but these cells play a central role in early events leading to the formation of plaques and tangles [2].

Routine immunocytochemical screening of AD cerebrospinal fluid (CSF) antibodies on fixed sections of the developing rat brain, an enriched source of activated microglia, revealed that some AD CSF contained an IgG species directed against the progression of the disease and improve the quality of life of the individual. These temporising measures would postpone the onset of the final and debilitating stages of the disease. Interventions may be simple but profound. For example, encouraging results have been seen in pre-morbid and affected individuals in whom an active cognitively-stimulating lifestyle has been facilitated. With this background it is easy to appreciate the need for early detection and intervention, justifying the spirited search for biomarkers. Predicting AD is problematic due to the very nature of the disorder as clinical features only become apparent following a period of insidious but substantial cell loss. Therefore there is a need for biomarkers to flag early cell death as this may allow for treatment interventions which could arrest the neuropathological processes.

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microglia [3], [Figure 1]. This antibody would then qualify for further consideration as a biomarker since it detects a fundamental feature of AD neuropathology, that being microglia. These encouraging results constituted our basis to verify whether this antibody fulfilled remaining guidelines for a biomarker.

2) Validated in neuropathologically confirmed AD cases

Following the observation of microglia antibodies in AD CSF of clinically diagnosed patients, an investigation was carried out using CSF from neuropathologically confirmed cases. The study included 38 subjects. Data revealed that even people with a moderate amount of neuropathology of the AD-type at autopsy showed the microglial antibody in their cerebrospinal fluid; none of the controls (those without AD-type neuropathology at autopsy) showed the microglial antibodies. Remarkably these results showed that CSF microglial antibodies are present in a high percentage (71.5%) of neuropathologically-confirmed definite AD cases and, most importantly, the frequency of CSF microglial antibodies was comparable (80%) in the group of cases with moderate AD-type cortical changes. The results add support to the fact that CSF microglial antibodies may be an early marker for an ongoing inflammatory process in AD. The frequency of microglial antibodies showed a good correlation with the severity of AD-type cortical changes. In the groups of patients where a high number of senile plaques accumulated in the cerebral cortex, the percentage of cases with microglial antibodies was also very high. This association was further supported by a similar correlation between the cortical Aβ levels and the frequency of microglial antibodies. Both were very high in the groups with moderate AD-type changes and with definite AD [4].

3) Precise (able to detect AD early in its course and distinguish it from other dementias)

AD is a slowly progressing disorder. Pathological modifications which eventually destroy cognitive function may be active up to 20 years before measurable clinical outcomes. The ability to detect early pathological events would therefore be a definite advantage for a biomarker. This would allow intervention at a point in time when brain function could be rescued. Not only would these interventions improve the quality of life for the patient, they would also significantly reduce the financial and healthcare burden on the patient’s family and the healthcare system. As microglia are considered to participate in early events in AD pathology, our findings that these antibodies are present in the CSF of persons at least two years before clinical signs strongly suggests that these antibodies could be early warnings of ongoing pathology before the onset of measurable cognitive decline [5]. In this regard the presence of microglia antibodies may predict a person’s susceptibility to further develop the disorder.

An examination of CSF samples from a diverse dementia population confirmed their presence at a greater frequency in AD compared to other dementias or other neurodegenerative disorder [5]. Recently investigations conducted on Trinidadian subjects revealed that the antibody is present in the serum of AD and distinguishes this disorder from cognitively healthy age-matched subjects [6]. Patients with vascular dementia did not show a significant presence of serum microglia antibodies when compared to healthy age-matched subjects [6]. These findings strongly suggest that the presence of microglia antibodies not only in the CSF but in the serum has the capacity to differentiate between AD and at least healthy age-matched subjects. Work in progress is aimed at establishing the ability of the serum antibody to differentiate AD from other dementias in the same way as the CSF antibody.

4) Reliable

At present measuring CSF levels of total tau protein, phosphorylated tau at residue 181, and the 42-residue form of beta-amyloid protein are gaining support as a means of predicting which patients with mild cognitive impairment will develop full blown AD [7, 8]. However as reports emerge about the possible success in the use of these markers there is a growing concern about reliability as results are not uniform across participating clinical centres [7,8]. A notable advantage of the antibody in comparison to these approaches is that there is no need to standardise levels of a substance. The presence of the antibody is sufficient to discriminate between a patient with AD from those with other dementias as well as neurodegenerative disorders. In view of this advantage, it is noteworthy that the detection of microglia antibodies has successfully differentiated AD from other patients in Europe, North America, the Caribbean and Asia.

5) Non-invasive

The recent findings of serum antibodies and the encouraging results that this antibody distinguishes between AD and healthy age-matched controls provides impetus to this non-invasive approach. Compared to the use of CSF the detection and use of serum would have world-wide appeal and application.
6) Simple to perform
The current techniques used to detect these antibodies is an immunocytochemical approach. There is a need to develop less labour intensive strategies such as an ELISA based test or automated systems which would facilitate widespread testing.

7) Inexpensive
As tests and technologies improve this would actually impact on the costs.

Additional markers
While a major focus in our laboratory is to evaluate the accuracy and biomarker characteristics of this antibody, additional markers are currently being investigated. These include serum levels of homocysteine (tHcy) and C-reactive protein (CRP). High circulating concentrations of the amino acid homocysteine are an independent risk factor for stroke. Though not fully confirmed, elevated levels of tHcy have been reported to be indicators of cognitive decline. Even more, CRP has a link to cardiovascular disorders and has been investigated in relationship to the development of certain dementia. Testing of these serum markers was carried out on a population consisting of 46 healthy individuals who were members of a seniors group, 19 AD patients and 10 vascular dementia (VaD) patients [6]. Unusual results were obtained with these serum markers. Though tHcy has been suggested to be elevated in AD patients in other populations, our study did not reveal significant differences in tHcy between AD and controls. However, notable differences were found between the VaD patients and controls. Our results suggest that CRP is not a predictor of cognitive status in dementia patients [6].

Ongoing work in our laboratory, which relies on serum assays, demonstrates the potential diagnostic capacity of microglia antibodies for detecting AD-type pathology, and tHcy for detecting VaD.

Conclusion
The gold standard for an AD biomarker is likely to be one that detects the earliest occurrence of underlying pathology [9]. The disease process, which includes accumulation of Aβ plaques, abnormal Tau in tangles and neuron and synaptic loss, probably presents the greatest challenge for a biomarker as these processes may not occur at the same time. However upstream from these processes is the microglia, a single cell considered to conduct this sinister orchestra. For these reasons we consider it a great advantage that a serum antibody towards this cell has fulfilled the majority of biomarker prerequisites. The use of the antibody would reduce the many concerns surrounding most current biochemical markers. Presently a major setback with a recent multicentre study using CSF β-amyloid 1-42 (Aβ42), total tau protein (T-tau), and tau phosphorylated at position threonine 181 (P-tau) has been the intertest assay variability. The authors recognise a need for standardisation of analytical techniques and clinical procedures [7,8]. Due to the facility of testing for serum antibodies, interstae assay variability should be reduced. The advantage here is that a positive signal due to the presence of the antibody would suffice to predict AD or a person’s susceptibility to develop the disorder. Furthermore, standardisation would be easier to establish and this in itself should reduce the need for special clinical procedures. It is anticipated that the serum biomarker discussed in this report would find an application in the most sophisticated clinical settings as well as the most remote ones. In view of the encouraging results obtained to date with the antibody, we are planning to conduct longitudinal studies with a large cohort of mild cognitively impaired subjects and to compare the presence of the antibody with neuroimaging measures. There are a number of neuroimaging candidate markers that are promising, such as hippocampus and entorhinal cortex volumes, basal forebrain nuclei, cortical thickness, deformation-based and voxel-based morphometry, structural and effective connectivity by using diffusion tensor imaging, tractography, and functional magnetic resonance imaging. New technologies have evolved which measure amyloid plaques and neurofibrillary tangles, the neuropathological hallmarks of AD. We are in agreement that one specific biomarker alone may not be useful in the management of a patient with dementia, and a combination of imaging measures and biomarkers may be necessary.

Recently when a group of potential AD markers were investigated using the quantitative surrogate validation level of evidence scheme (QSVLES), none of the biomarkers studied had a strong enough evidence base to be considered as a surrogate end point [10]. The latter report serves as the impetus to continue working with all the biomarkers currently under investigation.

References

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Biochemical markers for Alzheimer’s disease in cerebrospinal fluid

CSF biomarkers Aβ, Tau and p-Tau181 are related to the pathophysiology of Alzheimer’s Disease (AD) and deserve a place in the diagnosis of AD. However, variations in levels are multi-factorial and a cause for concern. While intra-assay variation is adequate, inter-assay variation should be improved. There is also within and between lab variation, exacerbated by emerging assay methods that yield different results. Despite the variation, CSF biomarkers appear to be useful and while introduction into clinical practice is indicated, serious efforts should be made to improve assay standardisation.

by Dr M. A. Blankenstein, Dr N.A. Verwey, Dr C. Mulder, Dr M.I. Kester, Dr W.M. van der Flier, Dr R. Veerhuis, Dr C.E. Teunissen and Dr P. Scheltens

The incidence of Alzheimer’s Disease (AD) is increasing steadily. With an incidence of 24.3 million in 2001 and an estimated doubling time of 20 years, by 2040 the number of people affected worldwide will be over 81 million [1].

The pathological hallmarks of the disease are senile plaques, containing amyloid-beta peptides (Aβ), and neurofibrillary tangles, composed of Tau protein and its phosphorylated forms. Obviously, these diagnostic hallmarks are only revealed at autopsy and that is why currently the diagnosis of AD is still made by clinical examination and neuropsychological assessment [2,3]. Revision of the original diagnostic criteria have been proposed [4].

Biomarkers are needed for the various stages in the aetiology of the disease, i.e. for diagnosis, for determination of the extent of the disease, for prediction of treatment, and may be even more necessary for prediction of resistance to proposed treatments. Finally, in the absence of clinical parameters, biomarkers could be used as surrogate endpoint to monitor the course of disease in clinical trials and during application of established therapies. Unfortunately, such versatile biomarkers have not yet been identified and that is why the search is going on. The first generation of biochemical markers for AD consists of the components of the plaques and the tangles.

Plaques are considered to originate from fibrils which are made of Aβ, a small (4 kD) splice product of a transmembrane protein, the amyloid precursor protein (APP). Because of the Aβ aggregation and deposition in the brain, it is believed that the drainage to the cerebrospinal fluid (CSF) is impaired, and as a consequence its concentration in the CSF is decreased.

Tangles reflect the damage inflicted upon the microtubule of the neuronal cells. Under normal conditions the tau protein binds to and stabilises the microtubule. Phosphorylation of tau at certain sites interferes with the binding and results in tau aggregation and tangle formation. Release of tau and phosphorylated tau at position 181 (p-Tau181) from affected and dying neuronal cells as well as end-stage tangles then leads to an increase in the CSF concentration of Tau and p-Tau181.

Thus, the concentrations of Amyloid-beta(1-42) (Aβ42), Tau and p-Tau181 in CSF can be used as markers for AD as reviewed by Blennow and Hampel [5]. This paper describes some of our experience in this fascinating area.

Assay robustness

Several laboratories are active in this field and a major challenge is to translate the technology from the laboratory to clinical use. This means that the technique should be robust, and that results obtained in different centres should be comparable to the highest possible degree. This is especially true in multi-centre clinical trials where intra- and inter-assay variation may be exceeded by inter-laboratory variation [6].

Our group at the Alzheimer Centre in the VU University Medical Centre, Amsterdam embarked on measuring these CSF biomarkers for AD in 2000, and has been providing the testing for external healthcare providers since 2003 [Figure 1]. Like other laboratories, we have used the reagents available from Innogenetics (Belgium). We have found that intra-assay variation, as assessed by differences in duplicates in eight randomly selected series, is within that specified by the manufacturer, i.e. around 6%. The p-Tau181 assay performed considerably better with an intra-assay CV of approximately 3%. Inter-assay variation is monitored with the use of pools prepared from surplus CSF specimens and varied more, as expected. In the period 2003-2007, multiple pool specimens with various concentrations that were included in 7-18 runs each have been used for this purpose. The inter-assay coefficients of variation (mean ± SD) were 11.3±4.9% for Aβ42; 9.3±1.5% for Tau and 9.4±2.5% for p-Tau181.

![Figure 1. Number of CSF specimens analysed for AD Biomarker in VUmc since 2000 and for external health care providers since 2003.](image-url)
Cut-off values
In our memory clinic, CSF biomarker levels are not (yet) taken into consideration in the establishment of the diagnosis and this has enabled us to evaluate the long term performance of these markers in relation to the independently established diagnosis [7,8]. We have observed that sensitivity and specificity may vary over time. In a cohort of 248 patients with AD and 131 patients with subjective memory complaints, studied over a six years period from 2001-2007, overall cut-off values to separate AD patients from patients with subjective memory complaints with an 85% sensitivity were 550, 375 and 52 ng/L respectively for Aβ42, Tau and p-Tau181. Corresponding specificities were 83, 78 and 68%. When all three markers were combined in a logistic regression model, sensitivity was 93.5% and specificity was 82.7%. Even though this is not yet ideal, these figures demonstrate the potential of CSF biomarkers and present a huge challenge for the discovery of new markers.

Of note is the variability in the cut-off values required to obtain 85% sensitivity that we observed when the six year period was divided into four 18 months periods. The cut-off value for of Aβ42 varied from 531-570 ng/L and that for Tau and p-Tau181 from 325-405 and 48-56 ng/L respectively. Variation was higher in the first two periods than in the last two. Despite a thorough search no single explanation for this observation was identified [9]. Lot-to-lot variation in reagent kits and increased experience with the assays, among other things, may contribute. Since the number of patients studied increased with time [Figure 1] we were led to conclude that apparently both clinic and laboratory have reached a certain level of experience in sample acquisition, pre-analytical treatment and analytical performance of the CSF biomarker tests. This illustrates that the introduction of CSF biomarker assays into a memory clinic is not as simple as one might infer from the literature.

CSF biomarker tests in different laboratories
As with other laboratory tests, comparison of results between different institutions and in multi-centre clinical trial is crucially dependent on the performance of the biomarker tests in the various institutions. External quality assessment schemes have been designed in attempts to harmonise results between laboratories for a large number of analytes, but for CSF biomarker assays, no such scheme was available and that is why we took the initiative in 2004 to send samples to a number of laboratories involved in CSF biomarker assays. Preliminary results were presented at the Euromedlab meeting in 2005 and a second round of specimens was distributed in early 2008. The results have recently been published [10] and revealed, as anticipated, serious differences in the results reported by the different laboratories. Overall results for Tau were slightly better in 2008 than in 2004 (mean CV 21% in 2004 and 16% in 2008). For p-Tau181 the mean CV increased slightly, i.e. from 13 in 2004 to 15 in 2008. The largest overall change was seen for Aβ42, where the between laboratory variation increased from 31% to 37%. Variation among laboratories that used the Innogenics improved from 30% in 2004 to 22% in 2008, indicating that standardisation of the assay procedure may contribute significantly to the reduction of between-laboratory variation. Three laboratories used a new test, i.e. the AlzBio3 test (Innogenics) and results of these laboratories showed a 20% variation. Although the variation in these two methods was comparable, the numerical results differed considerably. Samples giving results of on average 278, 417 and 674 ng/L in the Innogenics ELISA, gave results of 144, 160 and 252 ng/L in the AlzBio3 test. Large differences were also seen for the Tau and p-Tau181 levels measured with the two methods. These findings call for a serious effort in standardisation of both assay technology and assay calibration. In addition, efforts should be made to standardise the acquisition and the pre-analytical treatment of the specimens. Special attention should be paid to the receptacles for the CSF, because standard CSF collection kits for AD biomarker tests do not always include polypropylene tubes, and tubes other than these may seriously affect the measured concentration due to adsorption of the CSF proteins.

Progression and biomarkers
There is also interest in the contribution that the assessment of CSF biomarker levels could make in monitoring the course of the disease, by repeated sampling. In our lab, the variation in the biomarker (Aβ1-42, tau and p-Tau181) levels in CSF over time did not exceed experimental variation [7,11]. This means that the change in biomarker levels has already occurred before the first assessment and that further pathological changes are not reflected in CSF. The latter conclusion has important implications since CSF does not appear to be useful in monitoring progression of disease.

Advances in this area could be made if biomarkers could also be measured in body fluids that are more readily available. Plasma would be the ideal source in this respect. We have briefly evaluated new tests for the assessment of Aβ40 and Aβ42 in plasma in a small group of patients with a clear cut AD biomarker profile in CSF, but the plasma tests were unable to distinguish these patients from a control group [12]. Other researchers have also attempted this with conflicting results [13,14].

Future directions
First and foremost efforts to standardise current assays and to harmonise the results have priority. Apart from that, the quest for new biochemical markers for Alzheimer’s disease is not expected to end soon, because of the limitations of the current markers described here. Developments in proteomics will provide new leads, and the proteins identified will have to compete with or complement the current markers in order to improve significantly the 93.5% sensitivity and 82.7% specificity that can (maximally, under ideal circumstances) be achieved today. Other modalities, especially imaging, may complement or even supersede biochemical markers [15].

Alternatively, improved understanding of the biochemical pathways involved in the pathophysiology of Alzheimer’s disease may identify new candidate markers, involved in either the development of senile plaques and/or neurofibrillary tangles or the inflammatory processes thought to be associated with AD [16]. Any new marker should undergo a thorough process of analytical, as well as clinical validation, before translation from the laboratory to clinical practice will have a significant chance of success.

Summary
• CSF biomarkers Aβ, Tau and p-Tau181 are related to pathophysiology of AD and deserve a place in the diagnosis of Alzheimer’s disease
• Variations in CSF biomarker levels are multifactorial and a cause for concern
• Intra-assay variation is adequate
• Inter-assay variation should be improved
• Within and between laboratory variation is poor, especially in view of the emergence of new methods that give different results
• Despite the variation, CSF biomarkers appear to be useful and introduction in clinical practice is indicated.
• Laboratories should provide the best results to the clinician and serious efforts to improve assay standardisation are indicated.

References

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Ovarian reserve testing in the assessment of age-related infertility: the role of AMH

Women are born with a predetermined number of ovarian follicles (approximately two million) and these are subsequently reduced by apoptosis and ovulation. However, most women are unaware of this and how it can affect their fertility. In Europe we are facing an epidemic of infertility as women delay motherhood, confident that they can resort to successful in vitro fertilisation techniques whatever their age. If women understood the concept of ‘ovarian reserve’, they would be able to make informed decisions about when to time motherhood. This is where there is an increasing role for the relatively unknown anti-Müllerian hormone (AMH) assay. Its ability to measure ovarian reserve may provide a vital indication of remaining fertility and therefore help predict the probable success of in vitro fertilisation.

by Dr G. M. Lockwood

In a single generation, the average age of European women using maternity services for the first time has increased from 23 to 29 years [1]. Lifestyle changes, extended education and greater equality has led to women wanting to delay motherhood.

The message that this is possible without the risk of infertility is reinforced by stories of celebrities giving birth successfully in their 40s and 50s, and even in their 60s, becoming pregnant after IVF treatment with donor eggs. However, the reality is somewhat different. Instead, many women find they have become another statistic in what the medical profession recognises as an age-related infertility epidemic now spreading across Europe.

According to one UK study, almost 50% of women who expressed a desire to have children at 30 had not achieved that ambition by the age of 40 [2]. Many such women then turn to in vitro fertilisation (IVF) but find that this is also unsuccessful because their fertility has declined too far.

Why fertility decreases with age

As women age, there is a progressive decline in both the quantity and quality of follicles in their ovaries (ovarian reserve) and this reduces their fertility. A young woman with diminished ovarian reserve will still have a reasonable chance of pregnancy, despite having a limited number of eggs, whereas reduced egg quality is likely to limit the success of becoming pregnant in women over 40, no matter how many eggs they still have available [3].

In the UK, for instance, one in six couples now seeks medical help from their general practitioner (GP) because of fertility problems. Apart for chronic health conditions, difficulty conceiving and related fertility issues have become the most common reasons for women in their thirties to consult their doctor [7].

Reliance on assisted reproductive technology

While many women are aware that by delaying pregnancy it may take longer to conceive, they believe they can always turn successfully to assisted reproductive technology (ART). There is an increasing trend throughout Europe of older women consulting IVF clinics. In Germany, for example, the average age increased from 32.5 to 34.5 years between 1997 and 2006; women aged 36 or older now account for 42% of IVF patients compared to 35% in 1995 [6].

The chances of success decrease substantially as women age. A French study showed that 11% of healthy women under 31 years old using donor sperm became pregnant per normal cycle, however, this dropped to 9% for those aged 31-35 years and to 6.5% for those over 35 years [8].

Data from the UK Human Fertility and Embryology Agency shows that for every cycle of IVF, the percentage of live births already falls to just 12% at 40 years, and decreases to less than 2% at 45 (Table 1) [9]. Even if a woman in her late 30s or 40s does conceive, the risk of birth defects and miscarriage also increase as women age [10].

Assessing fertility status (ovarian reserve)

Successful conception, either naturally or through IVF, is primarily dependent on the

<table>
<thead>
<tr>
<th>Age</th>
<th>Live Birth Rate (per cycle of IVF)</th>
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<tbody>
<tr>
<td>40</td>
<td>12.1%</td>
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<tr>
<td>41</td>
<td>10.3%</td>
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<tr>
<td>42</td>
<td>7.6%</td>
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<td>43</td>
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<tr>
<td>44</td>
<td>2.6%</td>
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<tr>
<td>45</td>
<td>1.6%</td>
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Table 1. UK live birth rates (per cycle of IVF) (Source: Human Fertilisation & Embryology Authority, 2006).
fertility status or ovarian reserve of the woman, and for spontaneous conception, tubal status and sperm function of the partner are also significant factors. It is therefore important to be able to determine ovarian reserve for women who want children, particularly if they are over 35. It is then easy to estimate the likelihood of them becoming pregnant, either naturally or through IVF [11]. Such assessments of likely success rates also enable decisions to be made on eligibility for state-funded IVF treatment [6].

Even women who are not contemplating motherhood in the immediate future can benefit from a fertility assessment. Knowing the extent of their ovarian reserve will help them to decide how long they can delay maternity, and whether options for extending fertility should be considered.

There are currently two options for extending fertility. These are cryopreservation of ovarian tissue and cryopreservation of individual oocytes. Ovarian tissue needs to be transplanted back into the ovaries following thawing, but to date the tissue is only functional for a limited period following transplantation. As a result egg freezing by vitrification (in which mature eggs are harvested as in an IVF procedure following ovarian stimulation with gonadotrophins) is usually the best approach [12].

**Anti-Müllerian hormone**

Biochemical tests of ovarian reserve are good predictors of a woman’s capacity to produce egg [13]. Hormones that can be measured include luteinising hormone (LH), follicle stimulating hormone (FSH), Inhibin B and AMH. Transvaginal ultrasound is the gold standard for assessing the antral follicle count (AFC) but this is uncomfortable for the patient, not always conveniently available and its accuracy is operator-dependent. Different tests for ovarian reserve are shown in Table 2.

The most appropriate serum marker is one that reflects the number of follicles that have made the transition from the primordial pool into the growing follicle pool, during the gonadotrophin-independent phase prior to follicular recruitment. At present, AMH seems to fulfil this requirement completely and it has the advantage of being cycle independent.

AMH measurement is a relatively new and sensitive parameter and, unlike the other endocrine parameters, measurement is not influenced by the menstrual cycle [15]. The hormone is secreted by the sertoli cells of the testis in men, and by ovarian granulosa cells of the small antral and preantral follicles in women.

AMH is a member of the transforming growth factor-beta (TGF-β) family, and controls sex differentiation in the embryo and foetus. In the embryo it is essential for the development of the male reproductive tract and causes regression of the Müllerian ducts. In the absence of AMH the Müllerian ducts of both sexes develop into the uterus, fallopian tubes and upper vagina [16].

The production of AMH continues at a high level during the foetal and postnatal period, and then decreases dramatically. At puberty levels rise to around 2-5 ng/mL [17], during which time the hormone regulates follicular recruitment and maturation of eggs in women [18].

The average level of AMH for a woman aged 35 is 2.0 ng/mL. AMH level will continue to decline and by the menopause AMH is no longer detectable by immunoassay (<0.025 ng/mL) [Figure 2]. A woman with a low AMH level for her age could expect to go through an earlier menopause, while a woman with a high level for her age could expect to go through a later menopause [6].

A low AMH (less than 1.0 ng/mL) indicates a decreased ovarian reserve, particularly when it is associated with a low AFC (less than 8-10). On average, women who are obese in their late reproductive years have 65% lower AMH levels [19].

At the Midland Fertility Services personnel have been routinely measuring AMH to assess ovarian reserve for more than five years. Out of 833 women, 32% of the women assessed had an AMH level below 0.8 ng/mL, 12% of the women assessed were over 40 years old, and 6% of the women assessed had an AMH level below 0.8 ng/mL and were also over 40 years old. The overall pregnancy rate of the group was 22%, but for women with a low AMH and aged over 40 it was just 3% [20].

**Table 2. How to test ovarian reserve [14].**

<table>
<thead>
<tr>
<th>Biochemical</th>
<th>Basal FSH</th>
<th>Declining pregnancy rates when FSH &gt; 10 IU/L</th>
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<tr>
<td>FSH:LH ratio</td>
<td>An exaggerated FSH: LH ratio even when FSH is normal suggests diminished ovarian reserve</td>
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<tr>
<td>Where LH:FSH suggests a high ovarian response</td>
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<tr>
<td>Inhibin B</td>
<td>A reduction in the early follicular phase suggesting reduced ovarian reserve may occur before an increase in FSH is observed</td>
<td></td>
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<tr>
<td>Anti-Müllerian Hormone (AMH)</td>
<td>A reduction in AMH suggests diminished ovarian reserve. Its advantages are that it is independent of the menstrual cycle and shows low intra individual variation</td>
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<tr>
<th>Ultrasound</th>
<th>Antral follicle count</th>
<th>Both require high quality ultrasound and interpretation is subjective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian volume</td>
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<tr>
<th>Dynamic tests</th>
<th>EFORC</th>
<th>Exogenous FSH ovarian reserve test</th>
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<tbody>
<tr>
<td>GAST</td>
<td>GnRH agonist stimulation test</td>
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<tr>
<td>CCCT</td>
<td>Clomiphene citrate challenge test</td>
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</table>

Addressing the epidemic of infertility

The remaining period in which a woman will be fertile can be estimated by assessing her ovarian reserve. AMH is the best biomarker for assessing fertility because it is the first marker the levels of which change with age, and it is convenient to measure as its level is unaffected by the stage of the ovarian cycle.
If a woman is given an indication of the extent of her ovarian reserve, she would be better able to make an informed choice about when to try to conceive, and have an idea of the likelihood of success, and whether IVF is an option. It may also help her to decide whether she should consider egg freezing. Laboratories, therefore, need to be aware of the probable increased demand for AMH testing and the unique role such testing can play in accurately measuring ovarian reserve.

References

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D.Phil MRCOG
Medical Director
Midland Fertility Services
UK

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www.cli-online.com/comment/GDM

CURRENT MANUAL ASSAYS

AMH is currently available as a microplate based ELISA assay. There are two versions of the AMH ELISA assay commercially available: the DSL kit (DSL10-14400) and the Immunotech (IOT) kit (A16507) and both are supplied by Beckman Coulter. These kits are standardised differently and, as such give different results.

Beckman Coulter will replace the current DSL AMH kit with a new generation assay calibrated to IOT.

BECKMAN COULTER
Nyon, Switzerland
www.cli-online.com & search 24770
Advancements in sample handling and data processing for POCT

Point of Care Testing (POCT) has been rapidly growing since the 1980s, encompassing the performance of clinical analysis in a variety of settings including hospital wards, community health centres, emergency units and physicians’ offices. This large growth in POCT has been driven by the need of physicians to have immediate clinical analysis at or near the patient, as well as by the rapid advancements in technology, which have brought about compact, high speed and more sophisticated devices that can provide accurate and reliable results. In particular, sample handling or delivery systems and analytical processing of signal data and results are critical steps in POCT for limiting sources of error and ensuring good performance. Examples of sample handling and data processing techniques are discussed [Table 1] as a means of illustrating how technology-driven consumer demand is providing new possibilities for POCT devices.

by Dr Kirstin Kriz, Dr Lars-Olof Hansson and Dr Dario Kriz

Advancements in POCT
Convenience and clinical need have been the main driving forces behind developments in POCT, especially for clinical tests performed at high frequency or where the result is urgently required. Additionally, POCT has also provided economical incentives for healthcare professionals by reducing the turn-around time of the clinical decision making and action process, and thus lowering the costs associated with treatment and disease. Furthermore, increased public awareness of health and well being has led to patients taking a more active interest in their diagnostic results, as well as providing them with some general knowledge concerning the presence, absence or risk of certain diseases [1]. All of these factors have contributed to the development of the POCT devices that are used today.

However, advancements in technology have also played a crucial role in POCT. Improvements in technology have enabled the miniaturisation of the large clinical automated analysers used in central hospital testing laboratories, to provide small-bench top and even hand-held portable instruments without compromising system performance. Developments in microprocessor technology and network communication have increased the sensitivity of the assays, decreased the analysis time and allowed for in-built quality control procedures for increased accuracy and precision. New systems for fluid handling within the devices have automated the testing procedure by reducing operator handling of reagents and samples. Furthermore, developments in manufacturing techniques have made such technologies cheap to produce in large-volume, thus providing rapid and reliable analysis near the patient at an affordable price.

Sample handling devices
Operator usage is the predominant source of error in POCT systems. Thus, in order to limit procedural and accidental user errors, sample collection and loading should be the only handling procedures required of the user in an ideal POCT system. Requirement of operator training can limit the use of a POCT device since many clinical settings require devices to be CLIA (Clinical Laboratory Improvement Amendments) waived and operate accurately with error-free use. Thus, various sample handling technologies have been developed to reduce user error and to provide reliable results.

Most POCT devices today use an optical/chemical (signal generation) system which is coupled to an immunological

Leading the way in Point Of Care haematology
Table 1. Sampling handling and data processing techniques used in POCT.

<table>
<thead>
<tr>
<th>POCT System</th>
<th>Sample Handling</th>
<th>Data Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triage® by Biosite</td>
<td>Cartridge/Plastic syringe/Venous blood</td>
<td>Electronic coding chip</td>
</tr>
<tr>
<td>i-STAT® by Abbott</td>
<td>Cartridge/Plastic syringe/Venous blood</td>
<td>Bar code</td>
</tr>
<tr>
<td>Roche Cardiac Reader</td>
<td>Cartridge/Plastic syringe/Venous blood</td>
<td>Electronic coding chip/Bar code</td>
</tr>
<tr>
<td>Afinion™ by Axis Shield</td>
<td>Cartridge/Glass capillary/Capillary blood</td>
<td>Bar code</td>
</tr>
<tr>
<td>Smart by EUROLyser</td>
<td>Cuvette/Pipette/Capillary blood</td>
<td>Radio frequency identification card</td>
</tr>
<tr>
<td>QuickRead® by Orion Diagnostica</td>
<td>Cuvette/Pipette/Capillary blood</td>
<td>Magnetic card</td>
</tr>
<tr>
<td>LifeAssays®</td>
<td>Vial/Glass capillary/Capillary blood</td>
<td>Disposable algorithm chip</td>
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recognition (antibody-antigen binding) or enzymatic degradation (such as consumption of glucose by glucose oxidase). Fluid handling systems are used to automate the analysis procedure and eliminate the need for multiple handling steps such as mixing and pipetting of reagents, washing and timed incubations. In POCT devices, fluid handling systems are intended for single-use and are usually disposable. Most systems use a cartridge of moulded plastic, which holds the reagent required for analysis in liquid form by compartmentalisation, or in dried form using layering technology. Each cartridge can be inserted directly into the POCT device and has a separate compartment for the patient sample. The cartridge is therefore an integral piece of the POCT system connecting the patient sample to the device by moving the sample fluid through the reagent compartments or layers, usually via lateral flow or capillary action, to the detector or device for signal generation and result computation. Examples of such cartridge technologies include the Abbott i-STAT cardiac troponin I assay [2], the Roche cardiac TROPT assay [3], and the Triage BNP test from Biosite [4]. All three POCT systems use anti-coagulated venous whole blood which is loaded onto the cartridge using a plastic syringe as a sample handling device. Effects or errors arising from sample handling and loading are minimised in these systems, since the cartridge allows for sample overfill and gives an error message for underfills.

The Afinion system from Axis Shield [5] uses a cartridge with an integrated sampling device. Whole blood from a finger-prick specimen is collected using an integrated glass capillary located in the removable cap of the cartridge. The glass capillary is inserted into the loading zone of the cartridge and closes the cartridge with the cap of the sampling device. Excess sample found on the outer walls of the capillary is removed automatically by layers present in the cartridge, minimising sampling errors. The cartridge is loaded into the device for automatic measurement and presentation of results.

Examples of less automated sample handling devices used in some POCT settings include the smart CRP test by EUROLyser Diagnostica [6] and QuickRead CRP by Orion Diagnostica [7], both of which use a cuvette technology. In these systems, user procedural issues exist since the patient sample and the reagent system required for analysis are added manually to the plastic cuvette by the user.

Data processing technologies

The analytical processing of each measurement performed by the POCT device into a quantitative result is critical for accurate system performance. The operating systems of POCT devices are typically low-level programmes containing algorithms for conducting three distinct system processes. The first process is to communicate with, and control, the internal hardware of the POCT device, which is specific for the detection technology used (optical or electrochemical measurements). The second process is to mathematically transform the data collected from the first process into a quantitative result using algorithms which contain parameters that are usually downloaded to, or read by, the POCT device using external technologies. A data management system is the third process that allows the result to be displayed, directed, stored or recalled. Setting key parameters such as calibration information, clinical set-points, dynamic range or lot variation affects the accuracy of the final quantitative result. Furthermore, it allows the POCT system to be calibrated and controlled by the manufacturer and not the user, while attempting to account for as many sources of analytical variation as possible. Data processing technologies used for parameter setting in POCT are based on electronic coding chips, bar codes, radio frequency identification and magnetic cards, and examples of each are discussed below.

Electronic coding chips are usually provided by the manufacturer as a component of each test kit, and contain information regarding the test parameters, lot-specific data and calibration data required for device calculation of the quantitative result. Before a new lot of test kits may be used for analysis, the information on the electronic coding chip must be downloaded onto the POCT device. The Roche Cardiac Reader [3] and the Triage Meter from Biosite [4] both use coding chip technology for parameter setting. Additionally, in the Roche system, each disposable test cartridge is labelled with a bar code. To ensure that the operator uses the correct test cartridge with the correct coding chip, the information on the inserted test cartridge is automatically checked against that

Figure 1. LifeAssay’s disposable test vial with integrated sampling device for liquid handling.
manufacturing, detection techniques and data processing are made available. Convenience and error-free handling will remain key requirements of POCT, with a strong emphasis on quantitative and highly accurate result computation.

References

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Conclusion
POCT will continue to evolve as technological advancements in microprocessors, materials, contained in the coding chip. If a match does not appear an error message is given. Bar codes may also be used for transferring vital calibration information and parameters to a device, as used in the Abbott i-STAT cardiac troponin I assay [2] and in the Afinion system by Axis Shield [5]. In the i-STAT system, parameter settings are sent to the device with each measurement by manually scanning the bar code on the test cartridge. Radio-frequency identification (RFID) tags normally used as tracking devices have also been used in POCT for parameter setting. The smart system by EUROlyser Diagnostica [6] provides a RFID card with each test kit and information regarding the calibration curve, test procedure, expiry date and lot number is read by the device when the RFID card is inserted. Another technology used for parameter setting is shown in the QuickRead system by Orion Diagnostica [7] which uses a magnetic card, similar to a credit card, which is swiped and read by the device before each measurement.

New possibilities in sample handling and data processing technologies
LifeAssays POCT system has provided convenient solutions for existing operational and procedural issues relating to sample handling and data processing. This system is based on the use of a new patented disposable test vial made out of moulded plastic for sample handling [Figure 1], [8]. The reagent required for analysis is enclosed in a small cup by a penetrable plastic membrane. Whole blood from a finger- prick specimen is collected using an integrated glass capillary attached to the vial cap. The sample is inserted into the vial by penetrating the membrane with the glass capillary and closing the vial with the cap. The glass capillary sampling device cannot be overfilled and the system is not limited to venous blood samples. Data processing is performed using a patented disposable algorithm chip which is provided as a component of the LifeAssays test kit [Figure 2], [9]. A small microprocessor is located in the algorithm chip, which contains all the algorithms, parameter settings and calibration information required for result calculation. In the LifeAssays system, data processing is not performed in the POCT device, but rather the detection signal is sent from the device to the algorithm chip for result calculation, and the final quantitative result is sent back to the device for display or data management sending. The algorithm chip technology offers improved manufacturer calibration control and eliminates the need for software upgrades for devices already placed with users.

Figure 2. The disposable algorithm chip is a component of the LifeAssays test kit and is used for calibration-controlled quantitative result calculation.

Point-of-care test for long term prognosis of patients with chronic liver disease
Researchers at the Hadassah Hebrew University Medical Centre in Israel have developed an effective new tool for assessing the prognosis of patients with chronic liver disease, which could have important implications in determining which patients are the most appropriate candidates for liver transplantation. Previously, prognosis in patients with chronic liver disease has been determined by using a combination of blood tests. Studying 575 patients with varying types and degrees of liver disease, the investigators showed that a rapid, non-invasive 13C-Methacetin breath test could predict which patients would develop complications that would affect their prognosis. The test can also be used in acute liver disease to determine liver function on a daily basis and determine how well therapy is working. Researchers believe that the accuracy of the test, and its capacity to assess liver function, makes the breath test a potentially powerful new tool in predicting prognosis of liver related complications, prioritising patients for organ transplantation and predicting their ability to survive surgery.

www3.imperial.ac.uk

Researchers believe that the accuracy of the test, and its capacity to assess liver function, makes the breath test a potentially powerful new tool in predicting prognosis of liver related complications, prioritising patients for organ transplantation and predicting their ability to survive surgery.

www.hadassah.org.il/english

Point-of-care nanosensors for HIV diagnosis and monitoring to be developed
The London Centre for Nanotechnology will develop a new device to enable people living with HIV to monitor their own health and the effectiveness of their treatments, thanks to a £2 million EPSRC (Engineering and Physical Sciences Research Council) grant. The device will act as an early warning system to alert patients of the need to seek medical help if the virus is resisting anti-retroviral treatments. It will use nano-cantilever arrays to measure HIV and other protein markers that can indicate a rise in the level of the virus and the body’s response to it. Messages will be displayed on a built-in screen, giving patients access to a clear, immediate advice. It could also be of real benefit to doctors in developing countries who urgently need rapid and affordable ways to diagnose and monitor their patients.

www3.imperial.ac.uk
MicroRNAs circulating in blood show promise as biomarkers to detect pancreatic cancer

A blood test for small molecules abnormally expressed in pancreatic cancer may be a promising route to early detection of the disease, according to a team led by Dr Subrata Sen, associate professor in M. D. Anderson’s Department of Molecular Pathology at The University of Texas M. D. Anderson Cancer Center, USA. The team’s analysis of four microRNAs found in the blood plasma of pancreatic cancer patients is proof of principle to further develop a blood test for this evasive disease. Marker miRNAs used in the study were miR-21, miR-210, miR-155 and miR-196a.

There is no accurate, noninvasive way to detect pancreatic cancer, the fourth-leading cause of cancer-related deaths in the United States. Fewer than five percent of patients survive to five years. The four targeted microRNAs had previously been associated in varied ways with pancreatic cancer or with precancerous lesions. Expression of the four markers was analysed in 28 patients with pancreatic cancer and 19 healthy people. The four combined markers accurately identified 64 percent of the pancreatic cancer cases and correctly identified 89 percent of those without disease. This degree of sensitivity and specificity is good for a pilot study but is not high enough for use in the clinic; more circulating microRNAs need to be investigated in a larger sample, including different stages of the disease as well as healthy controls. The study’s small sample size, which compared only the extremes of pancreatic cancer or the complete absence of the disease, is a limitation, but the results justify continued development of this strategy.

http://www.mdanderson.org/

Gene that causes resistance to insulin found

A breakthrough by an international team of researchers in Canada, France, the UK and Denmark has uncovered a new gene that could lead to better treatment of type 2 diabetes, as well as a better understanding of how this widespread disease develops. Unlike most of the genes that have been shown to affect diabetes risk, which reduce the function of the pancreas and specifically the insulin-secreting beta cells, this gene, namely Insulin Receptor Substrate 1 (IRS1), doesn’t affect how insulin is created in the pancreas, but rather how the body responds to insulin already circulating in the blood.

This study, which used genetic material drawn from more than 6,000 French participants divided into two separate groups, represents the final step in a series of collaborations between the international researchers that has redrawn the understanding of diabetes genetics. In this instance, not only did the researchers pinpoint a new diabetes-linked gene, they also found that a SNP about half-a-million base-pairs away was causing a 40 percent reduction in the activity of the IRS1 gene.

The team hopes that this discovery may lead to new therapeutic lines of attack in the future. It is possible that in diabetic patients the signal to turn this gene on and off might be impaired, and an alternative pathway may be able to turn it on.

http://www.medicine.wustl.edu/

Two additional genetic risk factors for Alzheimer’s disease found

A research group led by scientists from the School of Medicine at Cardiff, UK and including scientists from Washington University School of Medicine, St. Louis, USA has completed the largest genome-wide association study ever involving patients with Alzheimer’s disease. The study pooled DNA samples from more than 19,000 older European and U.S. residents. Seven thousand had Alzheimer’s disease, and the others had no clinical symptoms of the disorder.

Prior to this study, only four genes had been definitively associated with Alzheimer’s disease. Three genetic mutations have been identified as causes of rare, inherited forms of early-onset Alzheimer’s. The fourth gene, APOE4, is the only one previously linked to the more common late-onset form of the disease.

By looking at more than 600,000 common DNA markers, researchers on the current study were able to identify two new genes that appeared to be involved in an elevated risk for Alzheimer’s and confirmed the importance of APOE4. The new genes identified in this study are APOJ, also called clustrin, on chromosome 8, and PICALM on chromosome 11.

Levels of clustrin tend to rise when brain tissue is injured or becomes inflamed, and some researchers have noted increased clustrin levels in the brain and cerebrospinal fluid of Alzheimer’s patients. The other gene, PICALM, appears to be involved in the breakdown of synapses between neurons. Some scientists also hypothesise that the gene may be involved in the development of amyloid deposits. This study also identified 13 more gene variants worthy of further investigation.

http://www.medicine.wustl.edu/

Can troponin T level predict outcome of bypass surgery?

Levels of a biomarker used in the diagnosis of Myocardial Infarction (MI) are almost universally elevated in patients who have undergone coronary-artery bypass grafting (CABG) and, when markedly elevated, are highly prognostic, a team of researchers from the Massachusetts General Hospital (MGH) Heart Center, USA has found. Their report implies that, while measurement of cardiac troponin T (cTnT) can help determine patient prognosis, current consensus recommendations regarding the use of cTnT to diagnosis post-CABG MI probably should be reconsidered. Although postoperative concentrations of cTnT were strongly predictive of the risk of complications and death after CABG, the team found that the currently recommended cut-off levels for diagnosing MI are far too low. However the use of cTnT to predict overall postoperative risk does look very promising.

Patients recovering from bypass surgery are at risk for a number of postoperative complications, including MI. Current standards for the diagnosis of post-operative MI include consideration of symptoms such as chest pain, electrocardiogram changes and the results of biomarker tests. However, since patients recovering from cardiac surgery inevitably experience chest pain and the results of postoperative electrocardiograms are often unclear, clinicians may rely heavily on biomarkers like cTnT to diagnose post-CABG MI.

The current study was designed to specifically evaluate the usefulness of cTnT in the diagnosis of post-CABG MI and to examine factors...
Bacteria that manufacture hydroxyapatite (HA) could be used to make stronger, more durable bone implants according to a team led by Professor Lynne Macaskie from the University of Birmingham, UK.

Using *Serratia* bacteria, the research showed that the bacterial cells adhered tightly to surfaces such as titanium alloy, polypropylene, porous glass and polyurethane foam by forming a biofilm layer containing biopolymers that acted as a strong adhesive. The HA coating then built up over the surface. For practical use, the HA layer must stick tightly, then the material is dried and heated to destroy the bacteria. A micro-manipulation technique was used to measure the force needed to overcome the bioglue adhesion, which is dense and more tightly than fresh biofilm. When coated with HA, the adhesion was several times more tight again. Slightly roughening the surface made the bioglue much more effective.

Currently bone implant materials are made by spraying on hydroxyapatite. This does not have good mechanical strength and the spraying only reaches visible areas. This biocoating method reaches all the hidden surfaces as the bacteria can move into hidden nooks and crannies. Bacterial HA also has better properties than chemically synthesised HA as the nanocrystals of HA produced by the bacteria are much smaller than HA crystals produced chemically, giving them a higher mechanical strength.

[[http://www.mgh.harvard.edu/](http://www.mgh.harvard.edu/)]

**Bacteria could facilitate bone replacement**

A new study by researchers at Wake Forest University School of Medicine, USA led by Dr K. Bridget Brosnihan reveals a key component in the development of pre-eclampsia in pregnant women, a condition that can result in miscarriage and maternal death. Pre-eclampsia is a disorder that occurs only during pregnancy and the postpartum period. It is a rapidly progressive condition that impacts multiple body systems, causing high blood pressure, decreased liver function and, in the most severe cases, affecting the activity of the brain, resulting in seizures. Swelling, sudden weight gain, headaches and changes in vision are among the symptoms; however, some women with rapidly advancing disease report few symptoms. The study focused on identifying the differences in the uteri of pregnant women with and without pre-eclampsia and how the mother's tissues vary from the immediately adjacent foetus' tissue in pre-eclamptic women.

Despite numerous research studies, the specific causes of pre-eclampsia remain obscure. One possible pathway that has been identified is the renin-angiotensin system (RAS), which regulates blood pressure and fluid retention. The RAS, when operating normally, results in angiotensin II production. This potent vasoconstrictor binds to angiotensin II receptors throughout the body, including the maternal uterus and the foetal placenta, and causes the muscular walls of blood vessels to contract, narrowing the diameter of the vessels and increasing blood pressure. In normal pregnancy, the uterus has lower RAS activity, producing less angiotensin II, which results in the blood vessels remaining dilated. This further results in lower blood pressure and allows more oxygen and nutrients to pass from the mother's uterus to the placenta and foetus. In pre-eclamptic women, however, the activity of the RAS is increased in the uterus, yet the mother's vessels remain dilated and the foetus' vessels constrict more than normal. Brosnihan and colleagues focused on uncovering the reason for this in the current study.

This study showed that the angiotensin II receptors are not detectable in the uteri of pregnant or pre-eclamptic women. In normal pregnancy, this does not present a problem because there is less angiotensin II being produced, making the receptors less important. In pre-eclamptic women, however, where uterine angiotensin II is high, the hormone does not bind to its receptors in the uterus as it should, but instead passes through to the vessels of the foetal placenta and constricts the foetus' vessels, limiting oxygen and nutrient intake and often causing low birth weight. The study provides some insight into maternal factors that may augment the disease. However inhibitors of the RAS are known to have bad effects on the foetus, so controlling this system in pre-eclamptic women is difficult.

**Insomnia increases blood pressure**

An investigation that measured the 24-hour blood pressure of insomniacs compared to sound sleepers was conducted by researchers from the Université de Montréal, its affiliated Hôpital du Sacré-Cœur de Montréal Sleep Disorders Centre and the Université Laval, Canada.

Over many years, chronic insomnia can have negative effects on the hearts of otherwise healthy individuals. Whereas blood pressure decreases in regular sleepers, insomnia provokes higher night-time blood pressure that can cause long-term cardiovascular risks and damage the heart. Since blood pressure is heightened among insomniacs, those with overt cardiac disease are particularly at risk for progression of the disease. The findings are important given that insomnia, which is a chronic difficulty in falling or staying asleep, affects up to 48 percent of the population at some point in their lives. As part of the study, the scientific team recruited 13 otherwise healthy chronic insomniacs and 13 good sleepers. Subjects spent 40 hours in the sleep laboratory: two nights for adaptation and one for monitoring followed by the intervening day.

[[http://www.wfubmc.edu](http://www.wfubmc.edu)]

**Bacteria could facilitate bone replacement**

**Key contributor to pre-eclampsia identified**

http://www.sgm.ac.uk/

**Insomnia increases blood pressure**

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**Insomnia increases blood pressure**

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Sliding microtome

Offering outstanding precision, safety and ergonomics, the Leica SM2010 R sliding microtome is designed to be maintenance-free. The microtome is ideally suited for a variety of applications including sectioning paraffin blocks for routine histopathology as well as fixed frozen tissue specimens, especially for neuroscience applications. Enhanced safety and precision are offered by the new SE disposable blade and SN knife holder. Both holders significantly reduce the risk of operator injury via conveniently located, red safety guards. They also provide improved section quality by allowing simple, precise adjustments and movements. The SE holder, recommended for biopsies and soft routine specimens, further reduces the risk of injury with its safer blade insertion and removal system. As an added benefit, precise lateral movement of the SE holder maximises blade usage by permitting access to the full cutting edge of the blade. A range of features ensures maximum productivity. The extremely smooth sledge movement reduces sectioning effort. Precision orientation allows rapid block setup and alignment so that optimal sectioning conditions are easy to achieve. In addition, the large volume anti-static waste tray makes disposal of section waste a simple and infrequent task. The ergonomic design ensures maximum productivity and efficiency, and the close proximity of the object head and an overall lower working height allow a comfortable working position. Operators can quickly customise the microtome to suit their individual needs. For example, users have a selection of three optional ErgoGrip handles for moving the specimen sledge. They can also select the direction of movement for the manual object feed by pushing or pulling the coarse feed lever, and choose whether to rotate the coarse feed hand wheel in a clockwise or counter-clockwise direction.

LEICA MICROSYSTEMS GMBH
Nussloch, Germany
# www.cli-online.com & search 24758

Amplified assays to improve detection of Chlamydia trachomatis and Neisseria gonorrhoeae

The World Health Organisation estimates that 92 million new cases of Chlamydia and 62 million new cases of gonorrhea are diagnosed each year. If left untreated, these infections in women can lead to pelvic inflammatory disease, infertility, ectopic pregnancy and chronic pelvic pain. The ProbeTec Chlamydia trachomatis (CT) Qx amplified DNA assay and the ProbeTec Neisseria gonorrhoeae (GC) Qx amplified DNA assay are for use on the BD Viper System. This next-generation system with XTR technology enables laboratories to process a higher volume of tests automatically from swabs or urine samples, with significantly less labour and more reliable test results. The fully automated system processes up to 736 patient samples in a single work shift. It offers the least hands-on time for setup, sample extraction, workflow and maintenance. One operator can fully execute all of the functions required to report results with several BD Viper Systems operating concurrently. This improvement in process efficiency facilitates a higher number of accurate diagnoses and more appropriate patient care for the two most common sexually transmitted infections.

BD DIAGNOSTICS
Franklin Lakes, NJ, USA
# www.cli-online.com & search 24759

Anti-VZV Glycoprotein ELISA

When a primary infection with the highly contagious varicella zoster virus (VZV) occurs during pregnancy it can cause rare but severe complications, such as congenital varicella syndrome and neonatal varicella infection. Reliable serological diagnosis in pregnant women who have had varicella contact is therefore indispensable. Conventional ELISAs for detection of anti-VZV antibodies use a lysate from VZV-infected cells as the target structure. However, it is inevitable that these reagents also target non-viral antigens, which...
leads to unspecific or ambiguous results. The new Anti-VZV Glycoprotein ELISA for detection of IgM antibodies against VZV utilises the main viral target antigens in the form of highly purified glycoproteins as the test substrate. This minimises false-positive results and cross reactions with other herpes viruses, while maintaining sensitivity. In a clinical study this ELISA proved its unrivalled specificity, particularly in a panel of pregnant women. While 3% of sera from healthy pregnant women were positive with the conventional ELISA, none reacted with the new ELISA. This increased reliability means that some patients may be spared unnecessary treatment and worry.

Taking only 75 minutes to perform, in line with the short incubation times of all the company’s other ELISAs for infectious serology, the procedure can be fully automated on microplate processing systems such as the Analyser I. As an additional aid for differentiating acute and reactivated infections, the antibody avidity can also be determined using a special version of the Anti-VZV ELISA (IgG).

EUROIMMUN AG
Luebeck, Germany
www.cli-online.com & search 24776

Universal test kit for ELISA users

Instruments and equipment are an essential part of microplate ELISA-based test procedures. Test performance must be reproducible within known parameters between maintenance periods and after repair, or after obtaining irregular results, using easy and convenient methods. The Pathozyme ElisaSure kit provides the ability to test all microtitre plate washers, microplate readers and manual and automated pipetting devices. Regular use of the test kit will produce documented records evidencing equipment performance and will assist in the early detection of deficiencies, as well as in troubleshooting, by incorporating four separate equipment checks. Regular monitoring will, in the event of encountering unexpected results during sample testing, provide a means of comparison between current and prior performance and, by incorporation into the laboratory’s maintenance schedule, provide confidence in the performance of equipment.

Clinical analyser for medium size labs

A complete system that integrates biochemistry and turbidimetry, the A25 is designed to achieve the highest performance in clinical testing. The stylish but robust design provides state-of-the-art technology for any kind of environmental conditions and workload. Consumption of reagents and water is optimised and maintenance costs are minimised. Refrigerated racks with up to 30 positions for reagents are provided. The high accuracy dispensing systems allow samples of 3µL, and minimum cuvette volumes of 200 µL. The automated fluid management system and independent power supply ensure optimal performance at any time. The intuitive software allows the automatic or pre-programmed assignment of reagents to racks. The software also allows LIMS integration, STAT and Internal Quality Control Management.

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One litre general purpose centrifuge series

Allowing unparalleled productivity and throughput, the Heraeus one-litre general purpose centrifuge series combines a maximum capacity of 4 x 400 mL with a compact design for optimising workspace. These centrifuges feature multilingual user interfaces and control technology to maximise run performance and reproducibility. Both the Heraeus Megafuge 16 centrifuge for routine sample preparation, and the Heraeus Multifuge X1 centrifuge for high processing versatility, incorporate a range of innovative technologies. The tool-free Auto-Lock III rotor system enables 3-second rotor installation and removal, for quick switch between applications, and easy chamber access for cleaning convenience. The biocontainment certified ClickSeal bucket sealing system employs glove friendly, one-handed snap-on covers, replacing complicated screw caps and clips. In addition, the advanced rotor management SMARTspin system optimises acceleration, braking, temperature control and residual load imbalance to maximise safety and improve separation and pelleting efficiency.

For unmatched versatility, the extensive rotor selection accommodates a variety of tubes, bottles and accessories, bringing an array of routine and high speed applications directly to the benchtop. Fiberlite rotors allow higher G-force and faster speeds, and ensure exceptional security and efficiency with a lightweight, robust and corrosion-free design.

THERMO FISHER SCIENTIFIC
Langenselbold, Germany
www.cli-online.com & search 24775

Biomarker tool for pre-clinical research into kidney function

The PlexMark 3 Renal Biomarker Panel assay is a non-invasive and cost-effective research tool for performing post-transplantation kidney function studies rapidly and easily. The introduction of this tool is expected to help in the development of studies which may lead to more effective methods for monitoring kidney health in transplant research. This new assay provides researchers with an alternative to invasive and expensive procedures, such as biopsies, that are used to obtain kidney tissue samples for post-transplantation kidney function research. The assay measures levels of cytokines, chemokines and receptor levels in urine, which give researchers a better understanding of immune function and response. This may lead to the development of new, non-invasive tests to monitor kidney function and health following transplant procedures. The assay uses Luminex XMAP multiplexing technology for bioassay analysis, in a standard immunoassay format to offer ease-of-use, sensitivity and rapid, reproducible results. The biomarkers in the panel are licensed from Renovar Inc, a pioneer in the development of new tools for assessing kidney inflammation for numerous clinical indications.

LIFE TECHNOLOGIES LTD
Paisley, Renf, UK
www.cli-online.com & search 24744

OMEGA DIAGNOSTICS LIMITED
Alva, Clac, UK
www.cli-online.com & search 24737
Screening test for plague

During the last 15 years more than 20 countries have reported cases of plague to the WHO. This flea-born rodent disease, occasionally transmitted to man, is still endemic in parts of Africa, Asia and the Americas. The causative organism is Yersinia pestis, a Gram negative, non-motile, non-spore-forming coccobacillus. Laboratory diagnosis of plague is based on bacteriological and/or serological tests. Direct staining techniques can provide supportive but not confirmatory evidence of infection. Other methods to diagnose plague, including culture, ELISA and PCR are available, but these are time-consuming, costly and difficult to apply in resource-poor countries. However, successful intervention and containment of plague outbreaks largely depends on early detection. Crystal Ypes is an in vitro qualitative screening dipstick test to diagnose plague, using human serum, plasma, bubo aspirates or sputum swab samples, or samples suspended in Cary Blair medium. This lateral flow immunochromatographic test detects the F1 antigen of Y. Pestis using monoclonal antibodies specific for this antigen. The test allows rapid diagnosis of plague in endemic areas.

SPAN DIAGNOSTICS LTD
Sachin, India

Real-time PCR assays to quantify proteins in human cells

A new line of TaqMan real-time PCR assays that allows researchers to rapidly detect and quantify proteins in human cell samples is available. The TaqMan Protein Expression assays enable researchers to correlate relative levels of specific proteins with cell functions and behaviours, such as different disease conditions, states of pluripotency or differentiation in stem cells. The initial release of these molecular tools includes assays that enable relative quantification of protein markers for pluripotency from limited quantities of cultured human embryonic stem cells. The new molecular tools offer researchers a more quantitative, simpler, and more standardised approach to protein analysis of various cell types, especially stem cells, compared to other more complex methods that also require large amounts of cell sample. The new assays detect and quantify proteins by an innovative technology that combines an antibody-oligonucleotide-tagged immunoassay with a TaqMan assay to generate real-time PCR data for specific proteins present in as little as 10-250 cells. The initial release consists of six pre-designed protein expression assays. Four of the assays target stem cell pluripotency markers, and two target more commonly expressed proteins in a variety of cell types. When combined with the company’s TaqMan assays for microRNA and messenger RNA, and run on one of its family of real-time PCR systems, they allow a quantitative protein analysis method that enables researchers to make comparisons of protein and RNA molecular markers identified on the same platform with the same starting samples.

APPLIED BIOSYSTEMS LTD
Warrington, Cheshire, UK

Adenovirus assay

Adenoviruses are recognised as significant viral pathogens that result in high morbidity and mortality among immunocompromised patients. The ARGENE kit is designed to monitor the viral load of adenovirus for the diagnosis of early disseminated infections as well as for the management of patient therapy. The assay detects and quantifies all 52 HAdV serotypes. It is optimised and clinically validated for all samples that are routinely used in virology laboratories, including plasma, whole blood, stool, nasopharyngeal swabs, CSF, BAL and urine. The assay is compatible with the major extraction systems and commonly available real-time PCR platforms. All controls are provided with the kit in order to monitor the entire process from extraction to amplification. A full range of kits that follow the same protocol allows the simultaneous analysis of other viruses (CMV, EVB, VZV, HHV6, HSV1, HSV2) of relevance in transplantation patients.

ARGENE SA
Verniolle, France

In vitro detection of malaria antigens

A rapid diagnostic solution for the in vitro detection of malarial antigens in whole blood is available in two different formats. Clearview Malaria P.f. is a qualitative test for detecting the most common, virulent and deadly malaria-causing parasite, Plasmodium falciparum. Clearview Malaria Combo detects and differentiates between Plasmodium falciparum, P. vivax, P. malariae and P. ovale antigens. Both of these rapid tests detect antigens from capillary or venous whole blood and provide easy to interpret results in just 15 minutes. Ideal for remote health centres, the rapid test range is manufactured to meet the harsh conditions of malaria endemic regions. The tests can withstand high temperatures, no refrigeration, and little training is required for accurate use. As such, these rapid diagnostic tests play a pivotal role in the Roll Back Malaria Partnership’s Global Malaria Action Plan, where quick diagnosis of the correct malarial strain will enable fast initiation of the correct treatment. Furthermore, the rapid spread of anti-malarial drug resistance will be minimised, as treatment can be highly targeted and controlled.

INVERNESS MEDICAL INNOVATIONS
Bedford, UK

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Troponin T high sensitive assay

The availability of the assay will allow laboratories to consolidate their cardiac assays on a single integrated clinical chemistry and immunoassay platform. Roche offers 185 different assays on such an automated Serum Work Area platform.

ROCHE DIAGNOSTICS
Basel, Switzerland
www.roche.fr/search/24757

Imмуnoassay panel for monitoring of B cell dyscrasias

A novel immunoassay panel designed for analysis of immunoglobulin heavy chain/light chain pairs, Hevylite will be used in monitoring patients with multiple myeloma and other B cell dyscrasias. This panel of immunoassays uses polyclonal antibodies targeted at the unique junctional epitopes between the heavy chain and light chain constant regions of intact immunoglobulins. The immunoassays can identify the different light chain types of each immunoglobulin class separately, i.e. IgAκ, IgAλ, IgGκ, IgGλ, IgMκ and IgMλ. These molecules are then measured in pairs, e.g. IgAκ/IgAλ, to produce ratios of involved immunoglobulin/uninvolved immunoglobulin concentrations, in the same manner as serum free light chain κ/λ ratios (Freelite). The first of the assays for measurement of IgAκ/IgAλ is now available.

BINDING SITE
Birmingham, UK
www.bindingsite.com & search 24773

Media for antibiotic resistance screening

Two new chromogenic media in the Brilliance Resistant Screening agar range are available, namely Brilliance ESBL and Brilliance VRE. These media can be used as screening tests to rapidly identify patients colonised with problematic Extended Spectrum Beta-Lactamase (ESBL) producing organisms and vancomycin resistant enterococci (VRE). Brilliance ESBL agar provides presumptive identification of ESBL-producing E. coli and the Klebsiella, Enterobacter, Serratia and Citrobacter (KESC) group, directly from clinical samples. Supplied in convenient, ready-to-use plates, results are available in just 24 hours. The semi- opaque background of the medium contrasts with the brightly coloured colonies and allows clear and easy differentiation of E. coli (blue colonies) and the KESC group (green colonies). The inclusion of Cefpodoxime, a well recognised marker for ESBL-mediated resistance, developed for use on cobas and modular analytics serum work areas, the Elecsys Troponin T’s assay is available. This highly sensitive diagnostic assay improves the detection of myocardial injury and helps identify those at risk of acute coronary syndromes, detecting Troponin T in the range of 0.003-10 ng/mL of serum or plasma.

Cardiac Troponins (Troponin T and Troponin I) are good indicators of myocardial injury due to their specificity and because levels of these markers remain elevated in blood for an extended period of time. Even a small elevation in cardiac Troponin may indicate damage to the heart and require clinical intervention. New guidelines from the European Society of Cardiology (ESC) and the American College of Cardiology (ACC) recommend a highly sensitive Troponin assay with excellent precision in the low end values (10% or less total variation at the 99th percentile of the reference range). This new assay meets the precision recommendations in the ESC/ACC guidelines. The improved sensitivity is of great diagnostic and prognostic value, not only in patients with acute coronary syndromes, but also in stable patients with a variety of cardiac disorders. The sensitivity and precision will enable more patients with ischaemic symptoms to be identified at an earlier stage, allowing earlier and more targeted treatment.

The sensitivity and precision will enable more patients with ischaemic symptoms to be identified at an earlier stage, allowing earlier and more targeted treatment.

CALENDAR OF EVENTS

October 28-31, 2009
62nd CMEF Autumn 2009
New International Convention & Exhibition Center, Chengdu, China
Reed Sinopharm Exhibitions
Tel. +86 10 6202 8899 ext 3825
Fax +86 20 6235 9314
e-mail: sinopharm@reedexpo.fr
www.escmid.org & search 24775

November 4-6, 2009
Journées Internationales de Biologie (JIB)
Paris, France
Syndicat des Biologistes & Reed Expositions France
Tel. +33 1 47 56 50 79
Fax +33 1 47 56 52 58
e-mail: jib@reedexpo.fr
www.jib-sdbio.fr

November 5-7, 2009
7th Annual World Congress on Insulin Resistance
San Francisco, CA, USA
Tel. +1 818 342 1989
Fax +1 818 342 1538
e-mail: insulinresistance@pacbell.net
www.insulinresistance.us

November 18-20, 2009
ESCMID Conference on Enterococci: from Animals to Man
Barcelona, Spain
European Society of Clinical Microbiology and Infectious Diseases
Tel. +34 61 686 7799
Fax +34 61 686 7798
e-mail: info@escmid.org
www.escmid.org

November 18-21, 2009
MEDICA 2009
Düsseldorf, Germany
Tel. +49 211 541 0011
Fax +49 211 541 0888
e-mail: info@medica.de
www.medica.de

January 16-17, 2010
Melanoma 2010: 20th Annual Cutaneous Malignancy Update
San Diego, CA, USA
Scirpiss Health
Tel. +1 858 652 5400
Fax +1 858 652 5565
e-mail: med educ@scirpisshealth.org
www.scirpiss.org/events/melanoma-annual-cutaneous-malignancy-update

January 25-28, 2010
MEDLAB at Arab Health 2010
Dubai, United Arab Emirates
IR Middle East
Tel. +971 4 3365161
Fax +971 4 3364021
www.arabhealthonline.com/new_MEDLAB.html

February 25-28, 2010
Early Disease Detection and Prevention (EDDP) conference 2010
Munich, Germany
Paragon Conventions
Tel. +49 22 5330 950
Fax +49 22 5802 953
e-mail: eddp2010@paragon-conventions.com
www.paragon-conventions.com/eddp2010/

April 10-13, 2010
ECCMID 2010 – 20th European Congress of Clinical Microbiology & Infectious Disease
Vienna, Austria
Congress Switzerland Ltd
Tel. +41 61 686 77 11
Fax +41 61 686 77 88
e-mail: base@congres.ch
www.congres.ch/eccmid2010/

April 18-21, 2010
63rd CMEF Spring 2010
Shenzhen, China
Reed Sinopharm Exhibitions
Tel. +86 10 6202 8899 ext 3825
Fax +86 20 6235 9314
e-mail: jin.liu2@reedSinopharm.com

May 9-13, 2010
Focus 2010
Glasgow, UK
Association for Clinical Biochemistry
www.focus-abc.org.uk

May 16-22, 2010
International AIDS Conference (AIDS 2010)
Vienna, Austria
www.aids2010.org

July 8-12, 2010
Hospitalar 2010
Sao Paulo, Brazil
Hospitalar Feiras e Congressos
www.hospitalar.com.br

July 18-23, 2010
AIDS 2010
Vienna, Austria
www.aids2010.org

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

For more events see:
www.cli-online.com/events/
infection control measures. Early presumptive identification of ESBL-producing organisms and VRE permits appropriate treatment and infection control procedures to be adopted sooner, improving both treatment outcomes and the effectiveness of infection control measures.

OXOID
Basingstoke, Hants, UK
[www.cli-online.com & search 24761]

Haematology analyser

Combining reliability and accuracy with the benefits of computer-enhanced handling, the Abacus 3 is a heavy duty compact haematology analyser offering an optimal solution for hospitals, clinics and practices requiring high quality and fast CBC results. The analyser offers 18-parameters and a 3 part WBC differential at a throughput of 60 samples per hour. High voltage pulses after each measuring cycle keep the apertures free from clogging. The instrument automatically cleans the sampling tip inside and outside after blood aspiration to minimise the risk of carryover. The sample rotor ensures safe and easy sampling. The high resolution colour LCD display allows monitoring of results.

Multi-level QC, L-J graphs and self-diagnostic functions enable monitoring of performance and accuracy.

DIATRON
Budapest, Hungary
[www.cli-online.com & search 24772]

Kit for the extraction of free circulating NA fragments in human blood

A novel kit, the QIAamp Circulating Nucleic Acid Kit, is available for the extraction of free circulating fragments of tumour- and foetal-derived nucleic acids as well as viral nucleic acids in human blood. These DNA and RNA fragments have great potential for the highly sensitive and non-invasive diagnosis of a wide range of diseases, including congenital disorders, malignancies such as colon and lung cancer, and viral infections.

Free circulating DNA and RNA fragments are found in body fluids such as plasma, serum and urine. It has been demonstrated that plasma, in particular, carries a variety of nucleic acids from viruses and different tissues throughout the body, including from developing foetuses and tumours. In cancer research, it has also been shown that the concentration of tumour DNA fragments is related to the extent of the disease. The analysis of such DNA and RNA fragments thus not only enables new, virtually non-invasive approaches to the early and highly sensitive detection of different malignancies such as colon or lung cancer, but can also help to monitor the progress of the disease and to assess patient outcomes. Likewise, foetal DNA and RNA fragments circulating in maternal blood can be used for the non-invasive molecular detection of congenital disorders in prenatal diagnostics. The new kit enables the isolation and purification of all types and all sizes of nucleic acids from large-scale plasma and serum samples, and thereby enables unprecedented yields of the isolated molecules and the highest sensitivity of downstream applications. The novel kit is currently available for research use only.

QIAGEN GMBH
Hilden, Germany
[www.cli-online.com & search 24774]

TRAIL and Survivin ELISAs

The MaxDiscovery TRAIL and Survivin ELISA test kits include everything needed for the highly sensitive detection and quantitation of the human TRAIL and Survivin cytokines. These kits are colorimetric, sandwich ELISAs that can detect picogram levels of specific analytes in as little as four hours. These high-throughput cytokine diagnostic assays are compatible with serum, plasma, tissue homogenate and cell culture supernatant, making them ideal both for preclinical and basic research analysis. This line of ELISA products contains gene-specific kits for more than 55 different cytokines.

BIO SCIENTIFIC
Austin, TX, USA
[www.cli-online.com & search 24751]

All-in-one microscope systems

The all-in-one FSX100 fluorescence and Fluoview FV10i confocal laser scanning microscope systems enable even the most inexperienced users to create high-end research images. These compact, self-contained microscopes are designed to remove all of the complex steps involved in setting-up and using advanced fluorescence and confocal microscopes, ensuring that users can concentrate on the images and data without any prior expertise in the control of the numerous microscope components involved. By coupling high quality microscopy and imaging hardware with precision automation and advanced software, simplified workflows allow users to obtain high quality images and image series by loading their sample, defining their observation mode and regions of interest (ROI), and then capturing their images - as simple as Set-Select-Capture.

All components are motorised and controlled via software, ensuring that functions such as focusing, exposure, fluorescence wavelength selection and even cover slip thickness correction are automated so the user does not have to touch the microscope at all. As a result, advanced imaging processes such as time-lapses, Z-stacks and multi-position image capture can be carried out with ease and even combined to provide true multi-dimensional imaging. The user-friendly software systems guide users through the whole imaging process.

OLYMPUS LIFE SCIENCE EUROPA GMBH
Hamburg, Germany
[www.cli-online.com & search 24739]
Forensic toxicology screening

A confirmation solution for forensic toxicology based on the Thermo Scientific Exactive benchtop LC/MS platform is available. The solution incorporates the ultra-high resolution LC/MS capabilities of the company’s proprietary Orbitrap technology. The new system expands the current clinical research portfolio of the company’s triple-stage quadrupole and ion trap LC/MS solutions, which include quantitative methodologies for analysing vitamin D, immunosuppressants, testosterone, opiates, benzodiazepines, THC, peptides and other endogenous compounds. This new solution dramatically improves ease of use and sample throughput, while providing high-confidence results. It includes the company’s Transcend TLX UHPLC-based multiplexing system with TurboFlow technology for online sample extraction, and integrates Thermo Scientific ToxID software and reagents.

THERMO FISHER SCIENTIFIC
Langenselbold, Germany
www.cli-online.com & search 24747

Ristocetin cofactor assay

vW Select is a complete assay that measures the functional activity of the von Willebrand factor. A deficiency of von Willebrand factor causes von Willebrand disease, globally the most common bleeding disorder. This all-in-one Ristocetin CoFactor assay system includes all the reagents, control plasma, reference plasma and diluents required to construct a standard reference curve and determine the ristocetin cofactor activity in patient samples. Assay components are formulated, optimised and performance tested as a system to ensure that linear standard reference curves are easy to achieve and test results are consistent, reliable and quickly available. The test addresses the clinical laboratory’s need for a complete, accurate and cost effective test system for the detection, diagnosis and management of von Willebrand disease.

BIO/DATA CORPORATION
Horsham, PA, USA
www.cli-online.com & search 24753

Capillary electrophoresis chip

Developed to separate small quantities of biological molecules by capillary electrophoresis, the glass Mitos Capillary Electrophoresis Chip A measures only 15 x 45 x 2 mm. This electrophoresis chip combines a cross channel design with a 20 µm channel depth and a 30 mm long channel for accurate analysis of the separation. With excellent chemical compatibility, the chip separates species in the interior of the microchannel based on their size to charge ratio. The high surface to volume ratio of the microchannels enable the application of high voltages without overheating the samples. Furthermore, an extremely smooth channel surface, in combination with a wide pressure and temperature range, make the chip ideal for a broad range of applications. Custom designed chips with straight channels up to 150 mm in length, for improved detection sensitivity are also available. Furthermore, the electrophoresis chip can be fabricated with a channel depth varying from 250 nm to 300 µm, allowing accurate analysis of different sample volumes. The thickness of the top and base layers can range from 150 µm to 5 mm, and the entire chip can be fabricated from quartz, if required. With excellent optical transmission and high visibility, this chip is ideal for microscope-based inspection systems.

DOLOMITE
Royston, Herts, UK
www.cli-online.com & search 24750

Safety tube holder

Comfort and safety are the key features of the Vacuette Tipguard. After a routine blood collection procedure, the safety mechanism is activated whilst the needle is still within the vein. By pressing the two blue release triggers on the top of the holder, the spring mechanism automatically withdraws the needle back into the holder. The needle is then safely enclosed inside the holder, ready for disposal with no risk of injury. The tube holder was especially developed for patients with increased risk of infection. To ensure optimal function, the holder should only be used with Vacuette blood collection needles.

GREINER BIO-ONE GMBH
Frickenhausen, Germany
www.cli-online.com & search 24752
Don’t forget to book these dates

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Please visit us at Biotechnica 2009
Hannover, Germany – October 6 – 8
Hall 9 / Booth F41

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