From the Editor

With the coming of spring, symbolising new beginnings, we at PathCare thought it would be appropriate to launch the new design of our Pathology Forum at the same time! We hope you like the new look and that you will enjoy reading the articles which have been selected to suit our readers' needs.

On page 3, Dr Riaan Writes from our laboratory in Vereeniging, helps you to answer the questions your patients might pose regarding tumour marker testing and on page 5 you can read more about the effects of oral contraceptives on laboratory results. In this article Dr Wessel Meyer, from our laboratory in Bloemfontein, explains what factors should be taken into account when interpreting results of patients on oral contraceptives.

In this issue, you will also find two articles by guest writers from Cape Town's northern suburbs. Have a look at pages 11 and 14! We thank Drs Christo la Grange and Munro Marx for their contributions.

If clinical trials are your field of interest, then the article on page 9 is for you. It gives an overview of the development of our Clinical Trials department and the improvement in the services they offer over the last 20 years.

We have repeated information that has been distributed to you in laboratory update format earlier this year, but due to the importance of the information we felt it necessary to repeat these in article format. These are “The use of troponins in the diagnosis of myocardial infarction” on page 7 and “First trimester screening for Down's syndrome” on page 13, both by Dr Esmé Hitchcock who is based at our reference laboratory at N1 City.

At PathCare it is not always work work work – we also have loads of fun as you will see from our Snippets section at the back!

Lastly, Influenza A H1N1 currently features prominently in the media. Initially we planned to publish an article on this disease in this Forum, but we realise that due to the nature of the disease, information quickly becomes outdated and therefore we aim to keep you informed by publishing information and test statistics on our website at www.pathcare.co.za.
PathCare laboratories
Doctors Dietrich, Voigt, Mia and Partners

Medical diagnostic laboratory
PathCare is a network of pathology laboratories that provide testing of patients’ blood (or other specimens), primarily for medical diagnostic purposes. Drs Dietrich, Voigt, Mia and Partners is a specialist pathology practice owned and led by the partners. The approximately seventy pathologists in PathCare are almost all trained in South Africa through the medical schools and tertiary hospitals.

Specialist practice
As a practice of specialists a patient is referred by a medical colleague for an investigation and expert opinion. The Practice is subject to the ethical rules of the Health Professions Council of South Africa. Within the practice are pathologists, medical technologists, nurses, technicians and other skilled people who work in the core of the operation. Their primary function is to assist the treating doctor in making decisions on the medical management of the patient by providing quality information derived from the analyses of specimens, and specialist interpretation of the results.

A broad range of expertise
We have pathology expertise in a wide variety of areas, including infectious diseases, anatomical pathology, forensic pathology, haematology, endocrinology, chemical pathology and veterinary pathology. We are also able to provide environmental testing, genetic and molecular testing, industrial and occupational health testing, clinical trials laboratory support and testing for life insurance purposes.

Namibia and South Africa
PathCare has over 150 depots in Namibia and South Africa where professional staff are available to take blood and transport it to the closest laboratory. Most hospitals are provided with an on-site emergency laboratory for inpatients who require a rapid turnaround time of testing. Our depots and laboratories and transport vehicles provide a service in Namibia and the greater part of South Africa from Cape Town to Kimberley, Port Elizabeth to Bethlehem, Vereeniging to Jeffrey’s Bay.

Innovative
PathCare has been innovative over the years, being the first to introduce many of the service related improvements presently in the industry, including the first to provide venesection, the first to collect specimens from hospitals and doctors rooms, first to achieve SANAS accreditation to ISO 17025 and ISO 15189 standards, the first radio-immunoassay laboratory in private practice, the first computerised laboratory in Southern Hemisphere, the first robotic automated chemistry immunodiagnostics testing system, the first liquid based cytology (LBC) accredited laboratory in South Africa, the first laboratory to do automated microbiology testing in Africa and the first laboratory with a training academy (over 120 students).

Kenya and Nigeria
PathCare also assisted with the provision of quality services in East and West Africa. PathCare Kenya has now been running for over five years and PathCare Nigeria for over three years; both achieved firsts in being the first internationally accredited medical laboratories in East and West Africa respectively.

Our goal
A visit to have your blood taken is never a favourite outing; our aim is to assist with your medical management by providing a convenient and complete pathology service with the least pain and fuss possible.
Introduction

Clinicians are faced daily with patients asking: “Doctor, please test me for cancer” or “Doctor, we have a certain cancer in our family, are there any tests that could identify this cancer?” An apparent quick answer to the above question is to request a single tumour marker (TM) or a panel of tumour markers (TMs) to confirm or exclude the presence of a malignancy. Far from being an exhaustive overview of specific TMs, the aim of this article is to provide some general guidelines for the rational and cost effective use of TMs. Before a medical doctor requests a TM, they need to be certain as to the specific utility of any TM and the potential role a TM can play in the optimal management of a patient.

Tumour markers are molecules that can be detected in higher or lower than normal amounts in blood, urine or body tissues of some people with certain types of cancer. TMs may be produced by the tumour itself, the surrounding normal tissue in response to the presence of tumour or by metastases, and include: DNA, mRNA, proteins, antigens or hormones measured quantitatively and/or qualitatively. In general practice TMs will mostly be specific soluble glycoproteins detected in serum although molecular markers (DNA and RNA) are also coming to the fore.

The perfect tumour marker

The perfect TM will be:

- absolutely sensitive and specific for a given cancer i.e. no possibility of false positive or false negative results;
- affordable and cost effective; and
- of assistance in one or more of the following aspects surrounding the management of a patient: screening, diagnosis, prognosis, treatment response and disease progression.

Furthermore it should be simple to collect the required sample and perform the test.

Unfortunately very few TMs come close to fulfilling these requirements.
How to obtain the maximum benefit from an imperfect tool?

The following should always be kept in mind:

1. No serum marker in current use is 100% specific for a malignancy or shows absolute organ specificity.
2. An alarming elevation in the most commonly used TMs could be secondary to a host of benign conditions.
3. Generally, serum marker levels are rarely elevated in patients with early malignancy. With a few exceptions, high levels are usually found only when patients have advanced disease.
4. Apart from possibly hCG in choriocarcinoma, no marker is elevated in 100% of patients with a particular malignancy.
5. Requesting multiple markers (such as CEA and the CA-series of antigens) in an attempt to identify metastases of unknown primary origin is rarely of use.
6. Reference ranges for cancer markers are not well defined and are used for guidance only. Please note that a level below the reference range does not exclude malignancy while concentrations above the reference range does not necessarily indicate the presence of cancer.
7. Changes in levels over time are likely to be more clinically useful than absolute levels at any one point in time.
8. Since many TMs lack agreed international reference preparations, different assay kits may give different results in the same sample.
9. To date no TM has demonstrated a survival benefit in randomized controlled trials of screening in the general population. PSA may be a possible exception to this rule in future.

It is clear that for TMs to play a meaningful role in detecting disease and assessing response to therapy, it should only be applied within the limits of a TM in selected groups of patients:

1. In monitoring patients for disease recurrence, TM levels should be determined only when there is a potential for meaningful treatment.
2. Normalisation of TM marker values may indicate cure despite radiographic evidence of persistent disease. In this situation, the residual tumour is frequently nonviable.
3. Conversely, TM levels may rise after effective treatment (possibly related to cell lysis), but the increase may not indicate treatment failure.
4. However, a consistent increase in tumor marker levels, coupled with lack of clinical improvement, may indicate treatment failure.
5. Residual elevation after definitive treatment usually indicates persistent disease. Following a TM response is particularly useful when other evidence of disease is not readily accessible.

Summary

TM can play a crucial role in detecting disease and assessing response to therapy in selected groups of patients. TMs in general have a limited application and if used inappropriately will expose the patient to unnecessary anguish, costs and a multitude of investigations and interventions (invasive and non-invasive) to confirm or exclude the presence of a malignancy.
Oral contraceptives (OCs) are commonly prescribed medications in women of reproductive age. Several preparations exist and are generally classified as monophasic, biphasic or triphasic, depending on changes in the dosages of constituents during the cycle.

A progesterone-only preparation (minipill) is considered when oestrogen preparations are contra-indicated.

Oral contraceptives have several physiological effects, which can influence routine laboratory investigations. It is important for clinicians to be aware of the more common effects seen in routine clinical practice in order to avoid errors in diagnosis and subsequent management of patients. The more common effects of OCs on tests performed in the routine clinical laboratory are highlighted here. The effects of hormone replacement therapy (HRT) on laboratory tests will be dealt with in a future article.

In general, the effects of OCs on laboratory tests can be influenced by:

- the type of OC used;
- the dosage of the constituents (oestrogen and progestin); and
- the duration of the therapy.

In addition, the changes induced by OCs on laboratory tests can either be due to the physiological effect of the specific OC (such as changes in FSH, LH and E2), or can be indicative of complications associated with OC use.

Hypothalamus-pituitary-gonadal axis

Since the main mechanism of action of OCs is to prevent ovulation by suppressing the cyclical release of FSH and LH from the pituitary, measurement of these analytes in a woman on OC will yield low results. Similarly, since the synthetic oestrogens used in the OC preparations are generally not detected by the highly specific assays used for measurement of oestradiol (E2) in routine clinical practice, measured E2 levels in patients on OCs will be low. This combination of low FSH, low LH and low E2 during routine evaluation of the hypothalamic-pituitary-gonadal axis can lead to a misdiagnosis of secondary hypogonadism if a history of OC use is not obtained at the time of clinical assessment.

Binding proteins

In serum, a wide range of analytes are bound to so called “binding proteins”. This mechanism allows for a physiologically inactive “protein-bound” fraction and a biologically active “free” fraction. Pregnancy and oestrogen therapy (including OCs) tend to increase these binding proteins, and consequently the total levels measured by most current methods used in the routine laboratory.

Changes in cortisol binding globulin (CBG) will result in elevated total cortisol levels measured by most methods in routine use. This may lead to a false diagnosis of hypercortisolism and unnecessary further investigations. Under these circumstances, a 24 hour urine collection for measurement of urinary free cortisol may be more appropriate to exclude a suspicion of hypercortisolism.
Oestrogen increases the circulating levels of thyroxine binding globulin (TBG). This would lead to falsely elevated levels of total thyroid hormone (total T3 and total T4 levels). Current methods for the evaluation of thyroid function measure the free hormones (free T3 and free T4) and thyroid stimulating hormone (TSH). These methods measuring free hormone levels will accurately assess thyroid function in women on OCs. Increased sex hormone binding globulin (SHBG) is seen in women taking OCs. This leads to increased levels of total testosterone, while free levels should be unaffected.

### Carbohydrate metabolism

Oral contraceptives may produce an increase in insulin resistance. These actions seem to be dose dependent, and is probably minimal with low-dose preparations. In lactating women with recent gestational diabetes use of the progestin-only minipill has been associated with a three-fold risk of diabetes mellitus.

### Lipid metabolism

Oestrogen increases total cholesterol, with an increase in high density lipoprotein (HDL) and a decrease in low density lipoprotein (LDL). In general, progestins antagonise these effects, but the net effect is a function of the type and dose of the progestin as well as the treatment regimen, whether it is monophasic or triphasic.

### Clotting factors

High dose OCs cause an increase in fibrinogen, factor V, factor VIII and factor X. The clinical significance with low dose preparations is less clear.

### Vitamin metabolism

Decreases have been noted in levels of vit. B6 and other B vitamins, as well as decreased levels of vitamin C and folic acid.

### Conclusion

Oral contraceptives are commonly prescribed to women of reproductive age. Since patients often do not regard OCs as “medication”, their use may not be mentioned during routine history-taking. These preparations may affect certain tests in routine use in the clinical laboratory. Some of the more common effects are discussed. Communication between the clinician and the pathologist is essential to avoid errors in interpretation of results and subsequent management in individual patients.

### References


### Effect of oral contraceptives (OCs) on selected routine laboratory tests

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<th>Analyte</th>
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<td>Low density lipoprotein (LDL)</td>
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* High dose preparations. Clinical significance unknown with low dose preparations.
Using the troponins in diagnosis of myocardial infarction - new decision limits

Dr Esmé Hitchcock

In some cardiac patients with normal CK-MB, troponin was raised, indicating that myocardial damage was detected, but the extent of damage was insufficient to raise the CK-MB, or produce ECG patterns that were indicative of acute myocardial infarction (AMI). Retrospective outcome studies confirmed that these patients were at high risk for AMI or cardiac death.

Distinguishing AMI from non-AMI no longer sufficed and the spectrum of clinical presentations caused by acute myocardial ischaemia, ranging from asymptomatic, to unstable angina (UA), to AMI, to sudden cardiac death, became known as the acute coronary syndrome (ACS). Increasing concentrations of troponin are associated with incremental risks in this continuous injury concept.

In 1999 the National Academy of Clinical Biochemistry (NACB) issued their recommendations for the use of 2 decision limits for troponin: a normal upper reference limit, and a higher value that indicates injury to the extent that conforms with a WHO-defined AMI, i.e. a level at which CK-MB would also be raised. Patients with troponin values between these 2 limits, were classified as high risk for adverse cardiac events.

Subsequent NACB guidelines in 2007 recommended a single decision limit, stating that in the presence of a clinical history suggestive of ACS, a troponin level exceeding the 99th percentile of a reference population, during the first 24h after the clinical event, indicates myocardial necrosis consistent with MI. This approach was confirmed by the Joint Task Force from the European Society of Cardiology / American College of Cardiology (ESC/ACC), in the Universal Definition of MI.

Universal Definition of MI
As defined by the joint ESC/ACC/AHA/WHF task force, any one of the following criteria meets the diagnosis for myocardial infarction:

1. Detection of rise and/or fall of cardiac biomarkers (preferably troponin), with at least one value above the 99th percentile upper reference limit (URL) together with evidence of myocardial ischaemia with at least one of the following:
   - Symptoms of ischaemia
   - ECG changes indicative of new ischaemia (new ST-T changes or new left bundle branch block (LBBB)
   - Pathological Q-waves on ECG
   - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
2. Sudden cardiac death, involving cardiac arrest, accompanied by ECG changes or evidence of thrombus on coronary angiography.
3. During percutaneous coronary intervention (PCI):
   - Biomarkers >3 x 99th percentile URL
4. During coronary artery bypass grafting (CABG):
   - Biomarkers >5 x 99th percentile URL, plus:
     - New pathological Q waves or new LBBB, or
     - Angiographically documented occlusion, or
     - Imaging evidence of new loss of viable myocardium
5. Pathological findings of acute MI

Decision limits
Evidence to date indicate that troponin will only be released by irreversibly damaged myocardial cells. The present guidelines therefore reflect the prevailing consensus opinion that any reliably detected elevation of troponin is abnormal and most likely represents necrosis, and if caused by ischaemia, constitutes an AMI.

An increased concentration of cardiac troponin is now defined as exceeding the 99th percentile of a reference control group. Earlier assays lacked sufficient sensitivity to detect troponin in healthy individuals, therefore the 99th percentile would have been undetectable. As the sensitivity improved in later generation assays, low levels of troponin could be detected in healthy individuals and a 99th percentile could be calculated. However, the assay should have sufficient precision to measure the 99th percentile reliably, i.e. with coefficient of variation (CV) less than 10% at this level. As assays continue to improve, the decision limit may change again.

Categorization of Acute Coronary Syndromes (figure 1)
Earlier detection due to lower decision limit
It is well known that troponin levels may be normal during the first few hours after AMI, and other ‘early markers’ such as myoglobin were recommended for analysis in combination with troponin. Using a sensitive assay with a lower decision limit (99th percentile) has shown to improve the early diagnostic performance of troponin, reaching a cumulative sensitivity of 98% at 2 hours.

Role of troponin in the ACS
ACS patients can be categorized according to ECG at presentation: Those with ST-segment elevation, diagnostic of myocardial infarction (STEMI), and those who present with ST-segment depression, T-wave changes, or no ECG abnormalities (non-ST-segment elevation ACS, NSTEACS).

The latter term (NSTEACS) encompasses both unstable angina and non-ST-segment elevation MI (NSTEMI). NSTEMI is distinguished from unstable angina by ischaemia sufficiently severe in intensity and duration to cause irreversible myocardial damage (necrosis), which is recognized clinically by the detection of biomarkers of myocardial injury (see figure 1).

Structural proteins (troponins, myoglobin) and other intracellular macromolecules (CK-MB, AST, LD) are released from irreversibly damaged myocardial cells and serve as biomarkers of myocardial necrosis. Due to non-specificity, total CK, AST and LD are no longer recommended for use. The preferred biomarker for myocardial necrosis is troponin (I or T), which has nearly absolute myocardial tissue specificity as well as high clinical sensitivity.

It should be noted that troponin is specific for myocardial tissue, but not for the mechanism of injury. In the absence of evidence for myocardial ischaemia, other etiologies should be considered (table 2).

The demonstration of a rising and/or falling pattern is also needed to distinguish background elevated troponin levels from elevations in the same patient at presentation and at 6 - 9 hours later.

Troponin I vs. troponin T
Which troponin test should be used? How do these two cardiac markers differ?

- Structural differences exist. Troponin T is found primarily as free troponin T, but also as complexes with troponin I and troponin C (T-I-C), and some smaller immunoreactive forms. Troponin I also exists in the T-I-C complexed form, but the major circulating form is dimeric with troponin C (I-C). Differences in phosphorylation, reduction and oxidation also occur.
- Due to different calibrators, different cut-off levels are used.
- Different epitope/antibody recognition sites will result in differences in the detection of complexes and degradation products.
- Release kinetics of cardiac troponins after AMI are similar, but troponin I returns to normal slightly earlier than troponin T.

It follows that in certain situations, troponin I and troponin T results may differ, and therefore these two assays cannot be used interchangeably. After cardiac surgery, significantly different results can be expected from these two assays, with troponin I reaching much higher levels than troponin T.

In end-stage renal disease (ESRD) patients, a greater number of patients have increased baseline troponin T levels than troponin I. Although the exact reason for this difference is unknown, it is likely related to the mechanism by which troponins are released, degraded and/or cleared from the circulation. However, both troponin I and T are approved for use in determining risk in ESRD patients. When ESRD patients present with possible ACS, a dynamic change in troponin T or troponin I of 20% should be used to diagnose AMI. Troponin I and T show similar diagnostic and prognostic value in CVD. All current international guidelines indicate that troponin is the cardiac marker of choice, with no preference for either troponin I or troponin T.

In summary
- The troponins are the cardiac markers of choice.
- A single cut-off at the 99th percentile is recommended by international guidelines.
- A troponin value above the 99th percentile URL, together with evidence of myocardial ischaemia, meets the diagnosis of MI.
- In the absence of evidence for ischaemia, other etiologies of raised troponin should be sought.
- A dynamic rising and/or falling pattern of troponin levels will help distinguish background elevated levels (e.g. chronic renal failure) from AMI.
- Troponin I and troponin T have equal diagnostic performance in ACS.

References
Eur Heart J 2007(28): 2525-2538
Clin Chem 2007(53): 552-574
Am Heart J 2004(148): 574-581
Clin Chem 2006(52): 1104-1121

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TABLE 2
Elevated troponin in absence of ischaemic heart disease
- Cardiac contusion, surgery, ablation, pacing
- Congestive heart failure (acute and chronic)
- Aortic dissection
- Aortic valve disease
- Hypertrophic cardiomyopathy
- Tachy- or bradyarrhythmias, or heart block
- Apical ballooning syndrome (acute dilated cardiomyopathy)
- Rhabdomyolysis with cardiac injury
- Pulmonary embolism
- Severe pulmonary hypertension
- Renal failure
- Acute neurological disease, including stroke, subarachnoid haemorrhage
- Infiltrative diseases, e.g. amyloidosis, haemochromatosis, sarcoidosis, scleroderma
- Inflammatory diseases, e.g. myocarditis, extension of endo-/periendocardium
- Drug toxicity or toxins
- Critically ill patients, esp. with respiratory failure, or sepsis
- Burns, especially if >30%
- Extreme exertion
Introduction

In 1989 the Mulligan and Partners practice was asked to participate in an otitis media study. This was the beginning of Pathcare Clinical Trials. There have been numerous changes and advances in the past twenty years including the setting up of a dedicated laboratory, IT section and project management departments.

Laboratory

The need for a dedicated clinical trials laboratory became apparent early in 2000, and with the amalgamation with Drs Penman, Kock and Partners, an opportunity presented itself to develop such a dedicated laboratory. The focus of the laboratory was to deliver a service for clinical trial work, in compliance with ICH GCP. The laboratory was inspected by SANAS at the end of 2000 and obtained ISO 17025 accreditation. In recent years, this was replaced with ISO 15189 accreditation status.

During 2001 the laboratory was established as a central laboratory for a number of international clients, as well as to South African based pharmaceutical companies. This allowed for standardised laboratory instrumentation and procedures. The laboratory instruments were upgraded, and from a humble Hitachi 902 and Technicon H1, the laboratory has gone through the full Roche Hitachi ranges of 911 and 917 and today has 2 modular systems. The haematology system was upgraded to Advia 120.

Specialized instruments such as the Bio-Rad Variant II were introduced in 2001 for the analysis of HbA1c. The laboratory obtained a NGSP level 1 certification for the first time in 2002 and to today still enrols in this certification program. In 2007 the Bio-Rad D10 replaced the Variant II for HbA1c analysis. This instrument also holds a NGSP Level 1 certification.

Clinical Trials’ IT support

- Pre-1996 - Clinical Trials were set up utilizing the existing routine laboratory computer system, with very limited adaptation.
- 1996 - Meditech computer system introduced. Tests were adapted to be more trial specific, but this was still limited due to the Clinical Trials department not having dedicated IT staff.
- 2002 - A qualified medical technologist with extensive Meditech experience joined the Clinical Trials team. Now the department was able to offer study-specific group test codes, reporting names and units, calculations and computer generated reflexes (triggered at the required values) to add tests or comments. Study set-up was now thoroughly validated through the use of test patients. Data management issues could now be addressed by one person, who could also liaise with the IT department to set up encrypted e-mail results and to program electronic data transfers.
- 2003 - Access to web-based results was introduced.
- 2006 - A continuously increasing workload resulted in another medical technologist joining the Clinical Trials IT team.
- 2007 - Ownership and maintenance of the Clinical Trials reference range document was transferred to the Clinical Trials IT team with version 4. This is updated annually and addendums are provided between updates, where required.
2008/9 - PathProvider system replaces web-based results. PathProvider offers cumulative reporting in both tabular and graphic form - this allows for reviewing trends in patient results.

PathCare's 21 CFR (Code of Federal Regulations) part 11 compliance plan is ongoing, with program modifications requested and applied as required.

Project management
In 1989 clinical trial support consisted of blue specimen stickers and the preparation of hand written forms. Later special plastic specimen bags and pre-printed forms were introduced. The department consisted of two part time staff members.

The services developed over the years to now include the following:

- Comprehensive study setup and management
- Electronic data transfer set up to client specification
- Resulting per fax, hard copy, e-mail and PathProvider
- Specialized tests referred to laboratories in the same facility (PCR, microbiology, histology and cytology)
- 24hr specimen reception
- Dedicated microbiology trial unit
- Data management
- Storage facilities
- Archive facilities
- Tracking system used for kit production and distribution, expiry date notification as well as tracking of returned samples and frozen sample management.
- Quality assurance: A dedicated QA officer ensures that the quality standards are maintained.


**Studies:**

- Then: One microbiology study
- Now: More than 100 active studies including oncology, cardiac, rheumatology, psychiatry, coagulation, metabolic, respiratory and microbiology studies.

**Staff:**

- Then: Staff consisting of 2 part-time members
- Now: Permanent staff employed is 20

**Request forms:**

- Then: Handwritten forms.
- Now: Customised request forms according to protocol requirements

**Kits (tubes and specimen collection materials):**

- Then: Kits consisted of handwritten forms, tubes and a plastic ziplock bag
- Now: Customised kits produced and distributed in approved IATA containers with full tracking capabilities.

The inputs of Dr C de Goveia, Sulette Mostert and Wendy Steytler are gratefully acknowledged.
Herewith the revised recommendations from the CDC (Centre for Disease Control) and the American College of Obstetricians and Gynecologists to limit the early onset of GBS in the newborn (NB).

**Background**
The alimentary canal is the natural reservoir of GBS and colonises the vagina and rectum from there. Ten to 30% of all pregnant patients are colonised. Of this group there will be transmission to the foetus during labour in up to 70% of cases. Of these NBs 1-2% will develop the symptoms of GBS. This is very serious and the mortality is 50%.

Culture of GBS and consequent treatment was 50% more successful in limiting infection in NBs as could be achieved with risk factors alone.

**Recommendation**
1. All pregnant patients must have a GBS culture between 35 - 37 weeks (the result is of no value if obtained more than five weeks previously, except if GBS was cultured earlier in the urine).
2. Treatment should be withheld until these patients are in labour or the membranes have ruptured (this implies that the patient must be informed of her positive culture and carry an identification with her to show the midwife on admission).
3. Treatment should be commenced as soon as possible after admission and it should be attempted to administer at least 2 doses to the mother, otherwise prophylaxis will be incomplete.
4. Treatment is only by the intravenous route. Oral treatment is useless.
5. The pediatrician must be informed so that he/she can continue with the treatment.
6. After delivery there is no need to further treat the mother.
7. A patient who has been booked for an elective caesarean and presents with ruptured membranes, must receive her first dose of antibiotic immediately.
8. Doctors who do not consider it necessary to screen for GBS, must realise that it must be an informed decision by the patient.

**Specimen collection**
A vaginal and a rectal swab are taken between 35 - 37 weeks and sent to the laboratory in a gel medium. It is important to take the swab from the lower vagina and NOT of the cervix. The rectal swab is taken from the area just inside of the anal sphincter.

(Only one swab could be taken, where first the vaginal and then the rectal specimen is obtained because the treatment does not differ in respect of where the colony GBS is found).
Who are treated?
1. All with a history of a previous NB with a GBS infection.
2. All who during the pregnancy (at any gestation) have had a GBS culture of the urine (even if she was treated).
3. All who during this pregnancy have had a positive culture on vaginal or rectal swabs that were taken between 35 and 37 weeks (except at elective caesarean section without rupture of membranes).
4. All with unknown or no GBS result plus any of the following:
   a. Labour before 37 weeks gestation
   b. Ruptured membranes for more than 18 hours
   c. Intrapartum pyrexia of 38°C or more

Who are not treated?
1. Previous pregnancy with a positive GBS culture but baby was not affected and in the current pregnancy culture for GBS is negative.
2. Elective caesarian section with a positive GBS culture and where the patient is not in labour or where the membranes have not ruptured.
3. Negative GBS culture of the vagina and rectum between 35 and 37 weeks in this pregnancy.

Treatment of Group B Streptococcal infection in the mother
1. **Penicillin** (benzylpenicillin): 5 million units every 4 hours intravenously (over 10 minutes) until labour (where there is no history of penicillin allergy).

   Penicillin B is stil regarded as the treatment of choice for GBS. It has a narrow spectrum of action and will not evoke resistance in other organisms.

2. **Ampicillin**: 2 grams immediately, followed by 1 gram intravenously every 4 hours until labour, can be used as an alternative, but it has a broader spectrum and will easily evoke resistance in other organisms (can also only be used where there is no penicillin allergy present).

   Penicillin allergy can be classified in two main groups:
   a. Immediate reaction (anaphylaxis / angio-oedema / asthma)
   b. Delayed reaction (skin eruption / pruritis)

3. **Cefazolin**: 2 grams immediately and then 1 gram every 4 hours intravenously until labour, can be administered with type b) penicillin allergy.

With penicillin allergy type a) use:
4. **Clindamycin**: 900mg intravenously 8 hourly until labour, or
5. **Erythromycin**: 500mg intravenously 6 hourly until labour (follow the sensitivity report from the laboratory).

Inform the pediatrician of your treatment thus far.

Reasons for the decision to mainly use PathCare’s facilities:
PathCare will give priority attention to these swabs in order to obtain a result as quickly as possible. Only GBS will be identified and a sensitivity test will only be performed if you indicate that this patient is allergic to penicillin. PathCare has made and distributed special stickers for all obstetricians to affix to the form. PathCare will maintain a record of positive cases and will report back after a year. If you should require swabs or labels, merely phone PathCare and ask for Dr Ossie van Rensburg 021 917 8000.

The inputs of Dr Ossie Van Rensburg and Prof Gert Kirsten are gratefully acknowledged.
First trimester screening for Down's syndrome

Biochemistry at 8-10 weeks
First trimester screening for Down's syndrome is usually performed between 11w - 13w6d gestation. Several studies have shown that when biochemistry is done at 8-10 weeks, the detection rate is improved, mainly due to the stronger discriminatory power of PAPP-A at earlier gestation.

To take advantage of this, the Combined First Trimester Down's Screen can be done in a consecutive fashion, with bloods taken for biochemistry at 8-10 weeks and NT measurement done at 11-13 weeks. The new PathCare request forms include a “1st Trimester Biochemistry Only” section at the bottom, for this purpose.

Suggested protocol:
1. At gestation 8w0d - 10w6d:
   a.) Take bloods and request “1st Trimester Biochemistry Only” PathCare will analyse PAPP-A and Free -bHCG. This is done on a Kryptor instrument, accredited with the FMF.
   b.) Please supply maternal weight - it influences the serum biochemistry values.
   c.) If the patient is referred to a sonographer for the risk calculation, please indicate the name of the sonographer on the form, in order to receive a copy of the biochemistry results.

2. At gestation 11 - 13w, preferably 12w:
   a.) Perform ultrasound
   b.) Please complete the “Maternal & Gestational data” and “1st Trimester sonar data” (sections A & B) on the new request form and supply the usual information for risk calculation:
      i. Maternal age
      ii. Ethnic origin
      iii. Obstetric history (gravida, para)
      iv. Smoking habit
      v. Number of foetuses
      vi. Previous chromosomal anomaly
      vii. Date of ultrasound
      viii. Crown-rump length
      ix. Nuchal translucency
      x. Nasal bone
      xi. Sonographer
   c.) Please supply the laboratory number of the biochemistry results. PathCare will combine the biochemistry results, ultrasound measurements and other data to calculate the risk.

There is no extra charge for performing the Combined First Trimester Down's Screening in two stages.

To order the new PathCare custom-made request forms for gynaecologists, please contact your client services officer.
**Genetic evaluation of abortus material**

Fetal loss (FL) and recurrent miscarriage syndrome are often associated with genetic/ chromosomal abnormalities, endocrine abnormalities, anatomic anomalies and blood coagulation defects. The prevalence of each of these conditions in inducing fetal loss remains unclear. If the clinician vigorously evaluates these patients, the causative defects may be diagnosed and treated, often making a normal term pregnancy possible. Chromosome anomalies are the single most common cause of spontaneous abortion. The majority of chromosomal anomalies (95%) are numerical of which 60% are ascribed to trisomies. About 20% are monosomies and 15% have polyploidy, especially triploidy. In the case of numerical chromosomal anomalies, couples have a 1% increased risk for a recurrence of a numerical anomaly and prenatal diagnosis may be considered for any future pregnancies.

In about 5% of couples with at least 2 spontaneous abortions, one partner carries a balanced chromosome rearrangement and chromosome analysis of the couple is prudent. When a parent carries a balanced chromosome rearrangement, the chance of having a live birth with an unbalanced chromosome complement is usually about 1% to 15%, depending on the specific chromosome involved, size of the fragments involved and mode of ascertainment.

Two diagnostic procedures may be followed for genetic evaluation of abortus material, viz. full chromosome analysis after cell culture and direct FISH analysis. Full chromosome analysis evaluates numerical and structural anomalies of each chromosome while FISH analysis evaluates numerical anomalies of chromosomes 13, 16, 18, 21, the X and Y as well as polyploidy. Each procedure has merit with specific advantages and shortcomings (see table below).

Over the past 3½ years, Unistel Medical Laboratories evaluated each of these procedures in 163 spontaneous abortus specimens received. A further 71 abortus tissues were analyzed using only FISH.

The following results were obtained:

**Culture vs. FISH**

Number of abnormalities identified with chromosome analysis but NOT with FISH: 10 = 5%. Number of abnormalities identified with FISH on unsuccessful cultures: 17 = 32%. Maternal contamination cells only cultured: 9 (normal female).

**Most recent statistics after the inclusion of chromosome 16**

<table>
<thead>
<tr>
<th>Summary</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal samples</td>
<td>152</td>
<td>22.79</td>
</tr>
<tr>
<td>Normal samples</td>
<td>515</td>
<td>77.21</td>
</tr>
<tr>
<td>Total samples</td>
<td>667</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Discussion**

Chromosome anomalies are known to be the single most common cause of spontaneous fetal loss. It is thought that 50% of expelled abortusses are chromosomally abnormal. In the case of numeric abnormalities the parental chromosomes are usually normal and parental karyotyping is not indicated.

In the light of the most recent statistics, we offer only FISH analysis for abortus tissues as the success rate with culturing is extremely low, mostly as a result of the tissue being necrotic and/or severely infected. Culturing will be offered in specific cases where familial chromosome abnormalities have previously been identified.

Analysis of expelled abortus tissue forms an important part of the management of a patient who has experienced a miscarriage. If the expelled fetus is chromosomally abnormal the process of healing for the parents is made so much easier. In the light of this, genetic analysis of expelled fetal material is justified.

**Tissue Requirements**

Sample of abortus tissue in saline.

If possible, a small biopsy of fetal skin in saline.

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**Advantages of FISH**

1. Almost 100% success rate.
2. Very accurate and especially suited to define chimeric and mosaic results.
3. Results available within 72 hours after receipt of specimen.
4. Only about 5% of chromosome anomalies not detected.
5. Polyploidy chromosome complement accurately detected.

**Disadvantages of FISH**

1. Only numerical abnormalities of chromosomes 13, 16, 18, 21, X and Y identified.
2. Translocations and deletions not detected.

**Advantages of full chromosome analysis**

1. Full chromosome complement can be analysed.
2. Translocations and deletions can be identified.
3. Mosaics are identified.

**Disadvantages of full chromosome analysis**

1. High failure rate as a result of difficult growth and infections.
2. Long turnaround time.
3. Maternal cells dominate clonal growth
4. Mosaic and chimeric chromosome complement often missed.

Contact: Dr Munro Marx or Ms Leonora Theart, Unistel Medical Laboratories, Tel. 021 9389213 / 4 / 5.
The annual 2009 Physician's Refresher Course took place on Friday and Saturday, 24 - 25 April 2009 at The Lord Charles Hotel & Conference Centre in Somerset West.

PathCare had an exhibition stand at the congress and activities at the stand included promoting web-based results (PathProvider), as well as PathCare Mobile (a new pathology results delivery system designed for doctors to access pathology results via a website enabled cell phone).

The congress was attended by 164 delegates. In the picture are two of the PathCare representatives at this event: Johlene Coetzee from Worcester and Elsje van der Spuy from Somerset West. Lynne Heyns from Paarl also represented PathCare at this congress, but was not present at the time the picture was taken.

**Fifth International Congress of the African Association of Blood Transfusion**

Dr Teresa Nel, a haematology pathologist from the PathCare laboratory at the Medi-Clinic in Bloemfontein, attended the 5th International Congress of the African Association of Blood Transfusion on 25 to 27 June 2009 in Nairobi, Kenya.

At the congress she presented two talks of which the one was on the Postgraduate Diploma in Transfusion Medicine at the University of the Free State in which she is also involved as affiliated lecturer.

This is the only programme of its type in Africa and therefore plays a critical role in the educating of doctors on the correct use of blood products.

**CPC QualiCare Open Day**

20 June 2009

The annual CPC QualiCare Open Day took place on Saturday, 20 June 2009 in Cape Town. PathCare had an exhibition stand at the day and Dr Johan Kock, a chemical pathologist at the reference laboratory at N1 City in Cape Town, spoke on the interpretation of pathology results.

The open day was attended by 216 delegates and a wide variety of topics such as HIV, depression, reimbursement, angina, NSAIDs and drug dependences to mention a few, was spoken on by experts in their respective fields.

In the picture are the two PathCare representatives at this event: Annabel Botha and Marietjie Pretorius.
Bone Marrow Congress, Genevé, Switzerland
7 - 9 May 2009

A Bone Marrow Congress was held in Genevé, Switzerland on 7 - 9 May 2009. The congress was attended by 195 pathologists from 35 countries from all five continents. Six haematology pathologists and clinical pathologists from South Africa attended the congress.

On the picture from l.t.r Drs Riaan Writes (PathCare, Vereeniging); Debbie Jafta (NHLS, Universitas Hospital, Bloemfontein); Erina Pretorius (PathCare, Port Elizabeth); Engela le Roux (PathCare, Bloemfontein) en Zoran Djordjevic (Ampath, Johannesburg).

Relocation of the Cormed Laboratory in Vanderbijlpark

Pathcare moved its Cormed laboratory in Vanderbijlpark to new enlarged premises next to Cormed hospital on 29 May 2009. Above and beyond the previous list of urgent and routine tests offered, Cormed laboratory will now also be performing some endocrine testing and drug monitoring on site, which will improve the turnaroundtime (TAT) for these tests.

The laboratory is open 24 hours a day, 7 days a week and performs urgent as well as routine testing for the Emfuleni Medi-Clinic, Cormed as well as Vaalpark hospitals. Designated phlebotomy sisters are assigned to the above hospitals 24 hours a day. Contact numbers for the laboratory are 016 981 9898 or 016 981 6710.

Long Service Awards CONGRATULATIONS!
The local laboratory in Klerksdorp handed long service awards to staff members who have been employed for 10 years or more. Twenty-one persons who have together served for 428 years, received awards!! The photo shows some of the recipients. The years appear in brackets behind the name.

1. Jacob Rathlogo (17 years)
2. Tina Rothman (23 years)
3. Johannes Senoge (18 years)
4. Force Gaji (21 years)
5. Lenie Nell (17 years)
6. John Peper (25 years)
7. Mara-Louise Myburgh (11 years)
8. Betty Seithlolo (26 years)
9. Abel Wesenyane (33 years)
10. Pamela Mokgothu (18 years)
11. Marilyn Atkins (10 years)
12. Magdaleen Knoesen (29 years)
13. Johanna Grobler (15 years)
14. Celeste Diedericks (18 years)
15. Joseph Dick (18 years)
16. Kobie van der Schyff (20 years)

The following employees were not present at the time the picture was taken:

17. Ida Hattingh (13 years)
18. Martha Sibanda (25 years)
19. Charlotte Schoeman (25 years)
20. Niki Boshoff (19 years)
21. Magda Smit (27 years)
Metropolitan Bellville’s marketing employees were the first official insurance clients to visit PathCare’s newly built reference laboratory and business centre in 2005. Mr Egshaan Jattiem, the then acting regional manager of the Bellville branch was quoted in the Metropolitan Today newsletter: “We, as a region, enjoyed the occasion tremendously. We want to say a big thank you to PathCare for their service excellence. You will always be our number one choice.” Four years later, in July 2009, Mr Egshaan Jattiem, now regional manager of the Bellville branch and his team still with some of familiar faces, were invited for a laboratory tour of the facility.

Mr Jattiem is true to his word, PathCare remains their number one choice: “Please convey our heartiest thanks to all at PathCare for a most pleasant learning experience and hospitality, we all enjoyed this occasion thoroughly, this was service excellence at its best, therefore PathCare will always be our first choice. We wish you all the best for the future”.

**PathCare offers the following services to insurance clients:**
- Dedicated help desk (021 596 3700)
- 24 - 28 hour turnaround time
- Mobile phlebotomy service (at client’s workplace)
- Personalized insurance request forms
- Electronic request forms
- E-mail and SMS confirmation service of the client’s visit
- Countrywide branches with extended hours
- Insurance medical examination service
- Personal pre-test counseling (by appointment)
- Broker information package (CD)
- ASISA Accredited Laboratory

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PathCare Snippets continues...
PathCare Bethlehem
Sanlam/CANSA golf day

The annual Sanlam/CANSA golf day in Bethlehem is sponsored by Sanlam and all proceeds go towards CANSA with whom PathCare in Bethlehem and the Eastern Free State have built a long-lasting relationship over the years. Our supporters who take part in this day get spoilt by PathCare by means of a golf cart that drives behind the players, filled with refreshments and snacks to ensure the tummies stay full and the throats wet! Dr Riaan van Lill, a pediatrician in Bethlehem says it is the one event in a year that he and Dr Neil Basson, a gynaecologist, do not miss out on. The PathCare brand is prominently displayed at the first hole and club house and this year was the first year we had sunny weather and a windless day. Thank you very much to all our supporters who help us to make this day a success together with Sanlam and CANSA.

NAMPO SHOW 2009

In the picture is Ansie Roets, veterinary technologist at the PathCare Vetlab in Bloemfontein.

At the annual Nampo Show in Bothaville, Ansie gave demonstrations to the public and students on bull sheathwashes (fertility examination) and identification of parasites.

The PathCare stand in the Nampo Hall provides information to farmers, breeders and veterinary surgeons on current herd diseases and diagnostic procedures offered by the Vetlab.

New PathCare laboratory at Kathu

PathCare is proud to announce the opening of a new laboratory at the Kathu Medi-Clinic. The laboratory was officially opened on Thursday 30 July 2009 by Dr John Douglass, CEO of PathCare. Also present at the opening were Dr Rudi Botha (histopathologist at the laboratory in Kimberley), Mr Gerrit du Toit (Director Pathology Operations), local PathCare staff and supporters.

A full laboratory service is offered at this facility. The laboratory is open during conventional office hours (see below) and also provides a telephonic call service after-hours.

Hours: 08h00 - 16h30 (Mon - Fri)
Closed on Sat & Sun except for call out service

Tel: 053 723 1520
PathCare is at the forefront of technological developments in the testing and resulting products we offer.

We acknowledge the complexities that go hand in hand with disease management and are continuously looking for ways that will improve the treatment of your patients by adding diagnostic value.

We offer

### Testing
- **Respiratory Bacterial PCR**
  - *Legionella pneumophila*
  - *Mycoplasma pneumoniae*
  - *Haemophilus influenzae*
  - *Streptococcus pneumoniae*
  - *Bordetella pertussis*
  - *Chlamydophila pneumoniae*
- **Respiratory Viral PCR**
  - *Influenzavirus A and B*
  - *Parainfluenzavirus 1, 2 or 3*
  - *Metapneumovirus*
  - *Respiratory Syncititial Virus*
  - *Adenovirus*
  - *Rhinovirus*

### Results
- Cumulative results
- Electronic results (encrypted results via email)
- PathProvider (web-based results)
- PathCare Mobile (results via web-enabled cell phone)

### Additional Value Services
- SANAS accredited laboratory
- State of the art automated technology
- 24/7 help desk facility
- Quick turnaround time

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