

DIAGNOSTICS

UPDATE .COM

NEWSLETTER
Issue 1 February 2004

Editorial

It is now back to the future with Diagnosticupdate.com newsletter. Focusing mainly on the understanding and diagnosis of disease, this newsletter aims to contribute to the knowledge of diagnostic medicine.

As we struggle to keep up with changes in diagnostic methods and technology, we hope the newsletter plays a pivotal and important role to our own society as it strives to keep you informed and up to date with the most recent and relevant diagnostic methods. Relevancy of diagnostic methods has become a worldwide concern since pathology has struggled to define and implement changes to improve test-requesting practices. As long as society is not well informed of recent events, some tests will still be under utilized which could otherwise help in diagnosis and cut the costs on the patients.

Apart from the medical personnel the newsletter also targets the ordinary person in the street giving information on the conditions commonly taken for granted but dangerous if left unattended.

In our quest to be the first with technology and information, a website is in the making where the newsletter will be accessible. Letters to the editor will be most welcome. Contributions from medical personnel and interested parties are invited for the next issue which is expected in May 2004 and can be channeled through to our email: lab@diagnofirm.co.bw

Many thanks to Diagnofirm Medical Laboratories for sponsoring the newsletter.

Till the next issue, stay informed!

Munyaradi Mangwendza
mmangwendza@yahoo.co.uk

Inside this Issue

	Page
Diagnofirm medical laboratories	1
Facts on HIV Monitoring	2
The Haematology Department@ DML	3
Apolipoproteins and Cardio-Vascular Disease	4-5
Candidiasis - A women' Scourge!	6
Natriuretic Peptides and Congestive Heart Failure	7
The Allergic State, the Hygiene Hypothesis and Allergy Prevention	8



Doctors and Pharmacists from Nyangabwe Hospital, Tati River Clinic, Legae Clinic and Delta Clinic in Gaborone



DIAGNOFIRM MEDICAL LABORATORIES

Recent Events.

- Diagnofirm has gone on the world wide web. (www.diagnofirm.co.bw). This will enable our clients (Doctors & Patients) to view results online confidentially.
- Diagnofirm Medical Laboratories in association with Roche diagnostics sponsored a lecture on " Management of Heart Failure" Roche's pro-BNP initiative the novel uses of pro BNP which was presented by Prof.K.Bhagat and M.Y.Sommi. This lecture was first presented on the 19th October 2003 at Gaborone Sun Hotel and then on 5th December 2003 at Cresta Thapama Hotel in Fransistown.
- DML also sponsored a presentation on "Pan luco gating - CD 4" New method of testing CD4 which was

presented by A.Gowans of Beckman Coulter diagnostics. The presentation was part of the 34th General Meeting and Scientific congress of the Medical and Dental association of Botswana at the Grand Palm Hotel on the 16th October 2003.

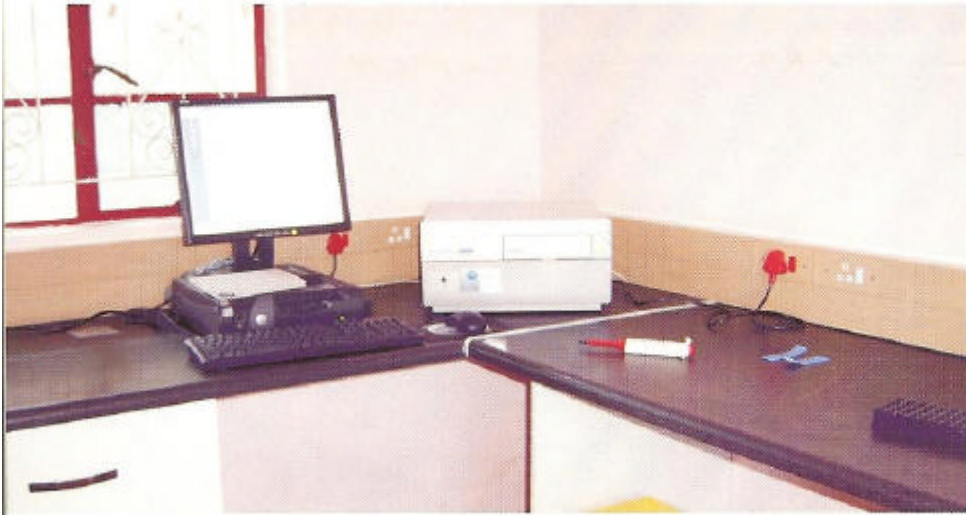
Community Services:

- November 15 was declared by the World Health Organization (WHO) as "World Diabetes Day" and Diagnofirm in association with Medical Chambers (Family Medical Centre, Cardiac Clinic, Paediatric Care) and some Dieticians, sponsored free blood glucose testing at the westgate mall for volunteers from all walks of life. The response to the exercise was very impressive as 400 plus people got their blood glucose tested on the day. Observers among them journalists

Continue on page 8

Tel: (267) 395 0007 Fax: (267) 395 7980 Cell: (267) 713 02499
Private Bag 283 Gaborone Botswana

FACTS ON HIV MONITORING



Nuclisens Easy Q Analyser

There is a lot of effort being put towards curbing the spread of HIV/AIDS in the general population. However, there is already a significant percentage of the population that has been affected by the virus, hence the need to monitor the infected population. The determination of T-cell subsets and Viral load are the primary tests for staging HIV disease, providing guidelines for differential diagnosis of patient complaints, making therapeutic decisions regarding antiretroviral therapy and instituting prophylaxis against opportunistic infections.

The idea of determining the viral load is meant to keep the number of viral copies in the system of an individual as low as possible so as to spare the CD4 cells from being depleted by the viruses. The factors that increase the viral load include failing antiretroviral therapy, active concomitant infections especially TB, immunisation (which results in the temporary elevation of the viral load) and progressive disease. Any effective antiretroviral therapy has to reduce the viral load by at least one log within 6-8 weeks of initiating therapy, and an absolute viral load of <50 RNA copies/ml by 6 months after starting therapy. It is the

changes of >0.6 log that are considered significant in individuals who are on antiretrovirals. The failure to meet the above criteria is an indication to change the patient's antiretroviral regimen. It is important that the decision to change therapy be based on two viral loads at least one week apart.

There are several factors that, however, affect the determination of the viral load

- i) The PCR method of determining the free viral RNA copies only detects free viruses in plasma and does not include those within the red cells. (Replication of viruses take place within the red cells). In this case haemolysed samples tend to give higher loads.
- ii) Technically small volumes of the sample complicate the test as there would be very little of the plasma to be used for the test.
- iii) Some Practitioners tend to use the viral load and not the viral log to monitor the patients.

Recently it has been realised that the

changes in the T-cell subsets could be a better marker in monitoring HIV patients (SF AIDS FOUNDATION). The expected ranges of the T-cells are, CD4+ cells (500-1500/ul), CD8+ (500-1000/ul), and total T-cell (750-2700/ul). In adults an absolute CD4 cell count of <350/ul, or a CD4 cell count between 350-500/ul that falls by >80 cells per year highlights the need for antiretroviral therapy. An absolute CD4 cell count of <200/ul indicates the need for prophylaxis. The CD4 count is one indicator of how much damage HIV has caused to the immune system. Raised CD8s are observed during acute retroviral syndrome and a sustained elevation is associated with a more favourable prognosis in patients with viral loads of <50 RNA copies/mL. A sudden and persistent reduction in the CD8s count to below normal heralds profound functional immuno-depletion. CD8s produce a chemical called cytokine which blocks HIV replication in infected cells. CD3s usually remain constant for several years and their decline is associated with a high risk of clinical disease progression during the ensuing 12-18 months.

Like any other test the determination of T-cell subsets is affected by a number of factors

- i) Samples from far places may be refrigerated and this gives reduced CD4 levels.
- ii) Transportation has a similar effect over long distances as temperatures tend to vary greatly.
- iii) The period from sample collection to processing must not exceed 24 hours.

Ultimately, although the advent of antiretrovirals has brought relief to practitioners, the ability of viruses to mutate poses a great threat to the success of antiretrovirals ♦

THE HAEMATOLOGY DEPARTMENT @ DML

The Haematology Department at Diagnofirm Medical Laboratories is one of the highly equipped and well operated section of the laboratory. It covers all the haematological tests and the coagulation tests. Of great interest in this department is the assistance it offers in the monitoring of HIV patients. As the logo of the laboratory clearly states it "Pathology you can trust", this department is trustworthy in all it does hence the changes it always implement in its set-up.

The aim of the department is to carry out required tests using top of the class technology giving high quality results with a short turn-around time. Recently this has been made much more easier by the purchasing of the FAST TEST 1 machine which performs Erythrocyte Sedimentation Rate (ESR) in only 3minutes 20 seconds. This means that clients need not wait for long for their results when they are in a hurry. The Full Blood Count (FBC) is carried out using the machine that also carries out the differential count. All samples which do not have abnormalities on the machine-run are quickly processed as the results are transferred to the computer and capturing of the data is thus made easier and faster.

The question of efficiency is also true about all the Coagulation tests. The most common tests that is the International Normalised Ratio (INR) and the Activated Partial Prothrombin Time (APTT) are done using the latest machine called the Coag Check Pro-DM which gives results in just 45seconds at most and uses only a drop of blood from a finger prick. This means less invasive techniques on the clients, fast and accurate results in less time than the other tests commonly used.

A great improvement has been made in HIV monitoring as samples are now being processed locally and results made



Dez, Bozo, Karen and West during training

available with 24 hrs of sample collection.

The department has however experienced a few discrepancies in some of its results because of:

1. Patients tend to get varying results within a short time because of the effect of normal biological variation" as they do not keep a consistent time of sample collection eg. if they have tests in the morning they do not keep coming in the morning each time they need to have the test done.
2. Some samples from other places have continuously given rather unpleasant results because at times the Doctors collect very little samples. Viral load counts are currently one using plasma hence the need to collect as much blood as possible for all the samples for CD4 and Viral load counts.
3. Samples collected over the weekend are sometimes refridgerated before transportation and this procedure gives false low T-cell counts.

In all that happens in Haematology Department, quality, efficiency, short turn-around time, and reliability of results remain the fundamental aspects of the Department ♦



LEFT TO RIGHT: Stanley Mapiki, Dr Sayana, Margaret Mapiki and Sofia S.M. Chitimbe



Guest Speaker: Professor Kiran Bhagat of the Cardiac Clinic in Gaborone



APOLIPOPROTEIN

Apolipoproteins are particles of protein that are mostly formed in the liver and intestine. They play an important role in the production and transport of cholesterol around the body. They transport excess cellular cholesterol from extrahepatic tissue and peripheral cells to the liver. There are at least nine types of apolipoprotein including the medically important apoA-1, apoB and apoE.

ApoB is the major apolipoprotein of LDL (Low Density lipoprotein) and it plays an important role in the secretion of VLDL (Very low density lipoprotein) and chylomicrons. It is recognized by the LDL receptor in the hepatic and peripheral tissues and allows LDL receptor mediated internalization of LDL. Apo B levels are



FROM RIGHT TO LEFT: Keletso Thaga, David Matema, Tshopo Kabelo and Sefetogi all from Nyangabwe



LEFT TO RIGHT: Symposium organiser and sponsor, Mohammed Iqbal Chand, Tshidi Kgosidialwa, Prof. Kiran Bhagat, Dr. Chothia and Cytologist Thaga

IS AND CARDIO-VASCULAR DISEASE.

increased in pregnancy, hypercholesterolaemia, LDL receptor defect, bile obstruction, hyperlipaemia type 2 and in nephrotic syndrome. Levels may be decreased in liver disease, beta lipoproteinaemia, sepsis and under estrogen administration.

ApoA1 and ApoA2 constitute about 90% of total HDL (High density lipoprotein) protein. ApoA1 is also a cofactor for LCAT (Lecithin cholesterol acyltransferase), the enzyme responsible for forming cholesterol esters in plasma thereby enhancing the lipid carrying capacity of the lipoproteins. ApoA 1 levels are decreased in inherited hypo-alpha-lipoproteinaemia (e.g Tangier's disease), cholestasis, sepsis and ATHEROS-CLEROSIS)

In the early 1970's Alaupovic suggested that apolipoproteins should be considered when evaluating the contribution of lipids and lipoproteins to the development of ATHERO-SCLEROTIC disease. Several studies demonstrated that in people with coronary heart disease (CHD) changes in the serum concentration of apo A1 and apo B 100 are similar to those of HDL and LDL respectively. APO B 100 increases and apo A1 decrease in people with CHD compared to those without disease. In most studies apo A1 and apo B 100 were somewhat better discriminators of people with CHD than the cholesterol concentration of the corresponding lipoprotein. Further more these two apolipoproteins were shown to correlate better with the degree

By Desire Mhiabi
of coronary stenosis than LDL and HDL cholesterol.

Apolipoproteins may also be good predictors of the possibility of future CHD as defects in the synthesis of especially apo A1 has been found to be associated with increased risk of developing premature CHD. Apolipoproteins can therefore be used in combination with other laboratory tests like High sensitive CRP (C-reactive protein), Pro BNP, Homocysteine, Cardiac troponins, Creatine kinase (total CK and CK-MB) in the prediction, diagnosis and management of coronary heart disease and other related conditions ♦

CANDIDIASIS - A WOMAN' SCOURGE !

By West, Bozo & David (Microbiology)

Yeast infections of the genital tract are becoming increasingly common in women. Candidiasis, or vulvovaginal candidiasis (VVC) is caused by yeasts known as Candida. Candida albicans is the most common species causing this infection. This is not a sexually transmitted disease, but it is due to an overgrowth of yeast (Candida) that already exists in the person's body. Candida species grow well in moist, warm areas of the body especially in mucosal areas such as the mouth or genitalia. The yeasts are also commonly found in small amounts on such sites as the groin, skin, armpits, beneath the breasts, skin folds of obese individuals and in the gut without causing any symptoms. However, due to some predisposing factors, these organisms tend to overgrow and cause symptoms to appear. The predisposing factors commonly associated with yeast overgrowth are

- (a) use of broad spectrum antibiotics,
- (B) use of oral contraceptives,
- (c) pregnancy,
- (d) menstruation,
- (e) diabetes mellitus,
- (f) constrictive undergarments and
- (G) immuno-suppression.

What are the symptoms of vaginal candidiasis ?. The disease presents as vulval irritation, a burning sensation when urinating and a "Cottage cheese-

like" discharge. Sometimes the vulva may be inflamed with excoriation and fissures. The discharge may be absent, but the irritation might be severe. If left untreated, sexual transmission might be possible.

To diagnose this disease, the physician takes a cotton swab or a smear from the affected area and this is examined in the laboratory by Gram stain and culture, which enables the yeasts to be identified.

The table below illustrates the incidence of Candida and other vaginal infectious agents isolated at Diagnofirm medical laboratory, microbiology department during the months of July, August, September and October 2003.

This table shows that during the period under review, Candida species were the most frequent isolates accounting for 36% of all the women tested. Of these 82% were Candida Albicans. A significant number of Candida species (8.4%) were isolated alongside other UTI (Urinary tract infection) causative agents, especially Escherichia Coli and group B streptococci. The group B streptococci, which may also exist in the vagina as normal flora if present in small amounts, with 16.9% of total, were the second most

isolated, followed by E.coli (11.3%). Other bacteria such as Klebsiella Pneumoniae, Proteus species, group D streptococci, Citrobacter and Staphylococcus Aureus were occasionally encountered (5.9% combined) Though figures were not established, this study also revealed that most patients had revisited their physicians for review, having been earlier diagnosed with Candidiasis. Of these, a greater number appears to have responded to treatment while a few remained untreated or had recurrent infections.

What is the treatment for VVC? Genital Candidiasis is treated successfully with anti-fungal drugs which may be taken orally, applied directly to the affected area, or intravaginally. However, infections that do not respond to treatment are becoming more common due to weakened immune systems. Prolonged and frequent use of these antifungal drugs can lessen their effectiveness. Over The Counter Treatment (OCT) for vaginal Candidiasis is also becoming more available. The problem arises however, when women think they have Candidiasis and start treating themselves with OCTs without any laboratory tests to confirm the presence of Candida, in most cases they misdiagnose themselves and apply treatment which is not appropriate, as their discomfort might be due to UTI agents other than Candida. This might then lead to resistant infections. Women are therefore advised to see their clinicians every time they feel an irritation or discomfort. This will ensure that proper diagnosis and treatment are effected ♦

Organisms	Actual figures	% of Total
Total Candida species isolated	220	36.00%
Candida albicans	180	29.50%
Candida not albicans	40	6.60%
Candida species isolated along other microbes	51	8.40%
Streptococcus group B	103	16.90%
Escherichia coli	69	11.30%
Other bacteria	36	5.90%
growth or no significant growth	214	35.10%
Total Number of Swabs tested 610 (High vaginal, vulval & cervical)	610	-

NATRIURETIC PEPTIDES AND CONGESTIVE HEART FAILURE

Kiran Bhagat Cardiac Clinic Gaborone

Heat failure is a major public health problem. Statistics for Africa are scant and unreliable. Nonetheless, if one looks at data that has been published, currently, there are more than 15 million patients with heart failure in North America and Europe, with almost 1.5 million new cases every year. Heart failure is the most frequent cause of hospitalization in patients older than 65 years, and these hospitalizations contribute significantly to the enormous cost of the disease.

The normal heart secretes Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP) from the atria, which counteract cardiac overload by stimulating renal natriuresis and lowering systemic blood pressure. When the function of the left ventricle deteriorates, the ventricular myocytes also begin to express the ANP and BNP genes, and progressively become the major site of peptide secretion.

This endocrine feature of ventricular peptide expression and secretion has now proven its diagnostic and prognostic value in congestive heart failure patients. Cardiac conditions with impaired function of the left ventricle can be detected by measurement of the circulating concentrations of natriuretic peptides and their precursors. BNP levels have been found to be significantly higher in patients with heart failure as compared with patients whose shortness of breath is due to other causes. Finding a low BNP level in a patient with shortness of breath renders heart failure unlikely and thus helps the doctor to focus on alternative causes. Conversely, a high BNP level strongly suggests heart failure. Accordingly, heart failure medication can be initiated immediately.

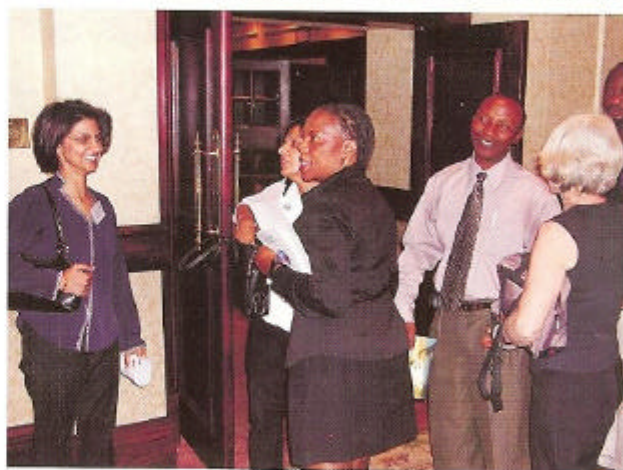
I attended the recent European Society of Cardiology meeting (Vienna, Austria, September 2003) where Dr Christian Müller reported the results of

the BASEL (Brain Natriuretic Peptide for Acute Shortness of Breath Evaluation) study. This study demonstrated that the addition of BNP testing to clinical assessment resulted in a significant reduction in the need for hospital admission (75% versus 85%); a significant, 23% reduction in time to discharge (10.5 days versus 13.7); and a significant, 26% reduction in the total cost of treatment for these patients.

These findings have significant implications. Thirty billion dollars were spent on the hospitalization of suspected heart failure patients in the US in 2000 alone, a cost that could have been reduced by \$7 billion through the use of this simple blood test. It is interesting to note that the European Society of Cardiology has recently included plasma measurement of cardiac natriuretic peptides as diagnostic markers of congestive heart failure.

With the marked ventricular upregulation, especially of the BNP gene, and subsequent high concentration of proBNP-derived peptides in plasma, it seems reasonable to expect increased natriuresis. Despite this fact, congested heart failure patients with highly increased plasma concentrations of potent natriuretic hormones do not show increased renal excretion of sodium and water. Although congestive heart failure is a complex condition, with activation and inhibition of both nervous and many hormonal systems, the paradoxical lack of effect of ANP and BNP is still compelling.

One explanation for this endocrine



Dr. Shweta, Nagpal, Tshidi, Duncan and Dr. D.B. Dickson

paradox may just lie in the heart itself. Both ANP and BNP are synthesized as prohormones and require endoproteolytic maturation by cleavage to release the biologically active peptides. Such cleavage is mainly a feature of atrial peptide expression. As ventricular myocytes have no secretory activity, and their storage capacity for maturing peptides is low, ventricular myocytes may not effectively process the natriuretic peptide precursors into biologically active end products. In agreement with this possibility, congestive heart failure patients have increased plasma concentrations of immunoreactive pro-BNP, and this immunoreactivity seems to consist of a polypeptide corresponding to the unprocessed precursor.

This lack of natriuretic effect from ventricular proBNP in congestive heart failure patients could be a novel target for therapeutic intervention.

To this effect Diagnostix Laboratories are currently the only lab in the country offering proBNP measurement locally on their new Elecys 1010 analyzer ♦

THE ALLERGIC STATE, THE HYGIENE HYPOTHESIS AND ALLERGY PREVENTION.

By Dr. John Mullineux Consultant Paediatrician GPH

There has been a marked increase in the incidence of allergic conditions. As this has resulted in a large burden to patients, doctors and society in general, it has stimulated a lot of research to determine its pathogenesis and a means of allergy prevention. Since this increase has occurred relatively recently and over a quite a short period of time, a genetic cause is not likely. It is possible that environmental factors which were until recently unknown or not recognised have had an influence, such as nutritional, environmental or lifestyle patterns. Alternatively, perhaps protective aspects that were associated with traditional "old fashioned" practices common in the past, no longer have as much of an influence.

With our ability to study the details of the immune system, we have been able to clearly identify the dynamics involved in the allergic response. With respect to the production of the IgE antibody, which is directly related to and one of the hallmarks of the allergic state, we know that increases in its concentration result to a large extent from an immune deviation from the TH1 to the TH2-type response and the associated cytokine influences.

It is now thought possible to embark on measures to influence this immune deviation and by exposing young individuals to stimuli which challenge their immune system in a way which results in precursor cell and cytokine activities which don't favour IgE production, prevent the "allergic march".

It is through exposure to and experiencing challenges from the environment such as certain common infections, contact with relatively innocuous allergens and eating foods which promote local bowel antibody

production in a 'favourable' way that one will achieve this goal. In fact, to encourage one's child to spend more time outdoors, "playing in the mud", not over protecting him or her, may be of benefit in this regard! In addition, the avoidance of the over-use of unnecessary antibiotics is sensible, as this will not eradicate an immune challenge which would shift the immune response to a TH1 type. If one rears a child in a relatively sterile and very protected environment, he or she will tend to adopt the TH2 related profile, and probably be an allergic individual. (This concept forms the basis of the so-called 'Hygiene Hypothesis').

One may be pro-active in the case those in whom it is possible to predict and anticipate an atopic state by influencing nutritional intake and feeding a child foods containing pre and probiotics. This can alter the bacterial population in the bowel. By influencing the balance of the indigenous intestinal bacterial microflora and stimulating a TH1-type response and indirectly suppressing TH2 cytokine production, the potential exists to prevent atopy and its related allergic diseases.

A probiotic is a live microbial food supplement that beneficially affects the host. An example is non irradiated natural yoghurt which may contain colonies of bifidobacteria and lactobacilli. A prebiotic is a non-digestible foodstuff which promotes the growth or activities of bacterial colonies. Such natural substances are associated with onions, asparagus, and artichokes. Certain companies, being aware of this hypothesis are presently marketing foodstuffs with the above in mind.

Whilst the concept presented above is thought-provoking, interesting and worth consideration, the Hygiene Hypothesis has not been universally accepted. We will need to "watch this space" ♦

DIAGNOFIRM MEDICAL LABORATORIES

Continued from page 1

from BTV and Monitor Newspaper commended the lab and the doctors for helping raise awareness on the silent killer disease.

- The lab also participated in the National World Aids Day (1 Dec) commemorative activities and distributed T Shirts and Caps carrying the anti-aids campaign Theme 'Live and Let Live' as a way of creating awareness of the deadly disease.

Technology comes to town

- The lab has successfully installed a state of the art Nucli Sens Easy Q analyser. This analyzer is for the quantitative detection of HIV RNA in human plasma (Viral Load) using REAL TIME Molecular Beacon Detection Technology. This machine basically uses 3 technologies, these being
 - i) The boom method for nucleic acid release and isolation
 - ii) NASBA (Nucleic Acid Sequence Based Amplification) Amplification of RNA.
 - iii) Real time detection of Amplicons using fluorescent molecular beacons.The lab is currently installing a BacT ALERT blood culture system which will enhance excellent detection capabilities in blood cultures ♦

Message Corner

HABIT

Even if "H" is taken out of it "A BIT" is there,
Even if "A" is taken out of it "BIT" is there,
Even if "B" is taken out of it "IT" is there,

**AVOID BAD HABITS,
CULTIVATE GOOD HABITS.**