

# Labnews

## Automated Screening of Gynecological Cytology

The implementation of **liquid based collection** for Gynecological Cytology at MDS permits us to introduce a second phase of new technology. FocalPoint™ screening technology will be our primary screening modality for monolayer gynecological cytology. MDS is the first laboratory in Canada to introduce this enhancement which will not affect the way in which you handle Pap Smears in the office. Automated screening together with the additional cytotechnologists we have been able to recruit should enable MDS to reduce turn-around-time. Our aim is to report in the time frame, which we once delivered prior to the shortage of cytotechnologists, during 2002.



There are several advantages to using **automated screening**. The computerized image processing is capable of screening and examining every single cell on a slide and selecting up to 25% of cases as Within Normal Limits for epithelial abnormality and therefore requiring no further review.

This will obviously have a large impact on our turn around time for the equipment will operate 24 hours a day 7 days per week. Another benefit is that our technologists will spend their time working on the population of slides more likely to contain abnormal epithelial cells. As is currently the case abnormalities will be diagnosed and signed out by cytopathologists. MDS reports will clearly identify cases screened as within normal limits by FocalPoint™ as follows:


**Diagnosis**  
**Within Normal Limits (No further review)**  
**Screening performed by automated screening device: FocalPoint**

Validation studies conducted at MDS are consistent with the extensive data submitted to the FDA in the United States to document the effectiveness of the Focal Point screening devices.

As you know, for a variety of reasons related to sampling or interpretation a single sample obtained for screening purposes may not invariably be representative. The vast majority of cases reported as "Within Normal Limits" however are truly normal. For your patients an effective screening programme requires cervical cytology be repeated at intervals of 1 or 2 years following adequate samples reported as normal. At MDS we encourage you to use the tear-off face sheets of the MDS cytopathology requisitions to enable your patients to fully understand these requirements and their responsibility to participate optimally in the screening programme. Provision of adequate clinical information by you is also very important.

The combination of liquid based collection and automation is truly an enhancement to conventional cervical cytology. Liquid based collection improves the quality of the sample and the ease and accuracy of screening. Automation achieves the examination of every single epithelial cell, something which human beings are incapable of achieving when screening manually. We expect that automation will in the future enable skill technologists and pathologists to do only what they do best i.e. to make decisions and diagnoses on cases selected for review by automated screening devices.

By the end of the year, I do believe these technologies together with the additional cytotechnologists we have been able to recruit from training programmes will enable MDS to report gynecological cytology with turn-around-times similar to those we have delivered in the past.

Finally we would like to thank you for your contribution to our smooth transition to monolayer cytology. 

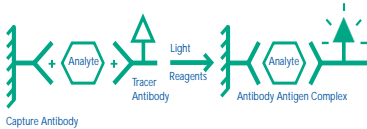
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# False Immunoassay Results due to Interfering Antibodies

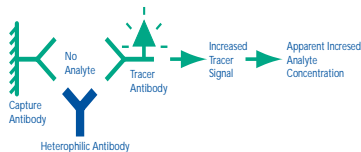
**Figure 1:**  
Immunoassay Reactions  
Mechanism

## A. Normal Reaction



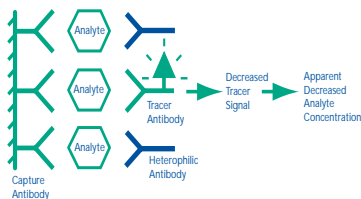
The solid phase capture antibody and tracer antibody binds with the analyte of interest to form an antibody-antigen "sandwich" complex. With the addition of select reagents and a wash step to remove unbound tracer, a chemical change in the tracer occurs to produce a measurable signal proportional to the concentration of analyte in the sample.

## B. False Positive: Reaction Product Measured



A false positive occurs in the absence of the analyte of interest, when the interfering heterophilic antibody (blue) binds with both the capture and tracer antibodies to produce a measurable sandwich complex.

## C. False Negative Reaction Product Measured



A false negative occurs when the interfering heterophilic antibody (blue) binds to the analyte forming a non-detectable complex which blocks the binding of the capture antibody (green) and analyte. The apparent concentration of the analyte of interest will therefore be decreased, or negative depending on the relative concentration and binding affinity of the interfering antibody compared to the tracer antibody.

Interference with immunoassays for determination of analytes such as hCG, TSH and PSA due to heterophilic or human anti-animal antibodies (HAAA) is a recognized phenomenon. Other analytes that may be affected by this type of interference are listed in Table I.<sup>1-3</sup>

### Definitions

Heterophilic antibodies are produced against diverse antigens and have multispecific activities. These "weak" antibodies may be generated in patients who suffer from autoimmune diseases, allergies, viral infections such as Epstein-Barr or influenza or after a vaccination.<sup>4</sup>

In contrast, HAAA antibodies have strong avidity for well-defined antigens and may be present as a result of receiving therapeutic treatments containing animal derived monoclonal antibodies. Interestingly, animal handlers may produce antibodies through coincidental immunization that exhibit the characteristics of either HAAA or heterophilic antibodies.<sup>4</sup>

The prevalence of heterophilic/HAAA antibodies is therefore quite common, affecting 20% of the population.

### Effect on assay results


During analysis, these interferents may result in either false positive or false negative results. The mechanism of action is illustrated in Figure I.

Fortunately, most analytical systems successfully neutralize interference from these antibodies with little or no impact to accuracy of the assay. Large concentrations of the interferent or antibodies with high binding avidity can, however, overwhelm the analytical system leading to false results.<sup>5,6</sup>

### Follow-up actions

MDS uses several techniques to verify the accuracy of reported results, however, if a patient result inconsistent with clinical presentation is observed, the clinical evidence should not be discarded. Physicians are urged to request repeat analysis using an alternative technology or specimen type. For example, discordant hCG results may be ruled out using (a) an alternative immunoassay (b) dilution analysis or (c) testing both serum and urine hCG.

As the interfering antibody activity may be non-specific in nature, documentation of known analyte interference(s) should be included in the patient chart and all future immunoassay analyses reviewed and interpreted with caution.

For further assistance please telephone MDS Clinical Biochemist at 416-675-6777, Ext. 2296. 

**Table I:**

Tests that may be affected by HAAA or Heterophilic Antibodies<sup>1-3</sup>

ACTH	CEA	Gastrin	Somatomedin-C
AFP	CK-MB	LH	Thyroglobulin
$\beta_2$ -Microglobulin	Cortisol	Myoglobin	TSH
Calcitonin	C-Peptide	Prolactin	TG
CA 15-3	FSH	PSA	PTH
CA 19-9	FT4	Free PSA	HCG
CA 125	CRP	Renin	

### References

1. Kricka LJ. *Clin Chem.* 2000; 46: 1037-1038.
2. Covinsky M, Laterzo O, *et al.* *Clin Chem.* 2000; 46: 1157-1161.
3. Scantibodies Laboratories Inc, Santee, CA.
4. Kaplan IV and Levinson, SS. *Clin Chem.* 1999; 45: 615-618.
5. Ismail AAA and Barth, JH. *BMJ* 2001; 323: 705-706.
6. Ward G, McKinnon BT and Hickman PE. *Am J Clin Pathol.* 1997; 108: 417-421.

### Spironolactone Interference to Digoxin Assays

The manufacturer of our digoxin assay has informed us of a potential interference to the measurement of digoxin in patients who are receiving combination therapy of digoxin and spironolactone, hydrocortisone or prednisolone. Recent investigations have illustrated a significant decrease (42%) in apparent digoxin concentrations in the presence of 100-600 mg/d doses of Spironolactone.<sup>1</sup> Limited inhibition (<11 %) may be expected at low doses (12-50 mg/d) of spironolactone.<sup>1</sup>

High dosages of steroids such as hydrocortisone and prednisolone may also cause falsely lowered digoxin concentrations (19-28% decrease).

Please be aware of this potential interference. In patients receiving these combination therapies, repeat analysis using an alternative technology is recommended. Digoxin specimens should be collected prior to introduction of an aldosterone inhibitor into the patient treatment plan.

#### References

1. Steiner, W, Muller, C and Eber, B. Clin.Chem. 2002: 48(3); 507-516.

### Diagnosis of Chronic Lymphocytic Leukemia (CLL)

Chronic lymphocytic leukemia is the most frequent form of leukemia accounting for 30% of all leukemias and 1% of all cancers. CLL is a disease of the elderly with over 90% of cases occurring in patients over the age of 50 years.

CLL is diagnosed using the NCI-sponsored CLL Working Group criteria.<sup>1</sup>

1. Sustained peripheral blood lymphocyte count of  $> 5 \times 10^9/L$  with less than 55% of cells appearing atypical.
2. A bone marrow aspirate showing greater than 30% lymphocytes.
3. Peripheral blood lymphocytes identified as monoclonal B cells with the presence of B cell specific differentiation antigens (CD19, CD20 and CD24).

To demonstrate that the lymphocytes are clonal, i.e. all derived from the same clone of cells, it is necessary to carry out "Immunophenotyping by flow cytometry". This involves testing for the presence of unique surface antigens with flores-

cently labeled antibodies in a flow cytometer using a laser detection system. This is essential to establish a clear diagnosis of CLL.

There are two main objectives of immunophenotyping: the first is to prove the clonality by showing cells predominantly possess either kappa or lambda light chains on their surface.

Secondly, to demonstrate the characteristic surface marker profile of CLL cells which is that they are positive for CD5, CD19, CD20, CD23, and negative for FMC7. Also CLL cells have a low density of surface immunoglobulin, which helps to differentiate them from normal lymphocytes. In Figure 2 the cells predominantly demonstrate kappa chains rather than lambda, (Fig. 2A) are positive for CD19 and CD5, and are positive for CD23 (Fig. 2B) but negative for FMC7 (Fig. 2C). These findings are diagnostic of CLL.

When investigating a patient with a sustained lymphocytosis, or one that is  $> 10 \times 10^9/L$ , then one should order "Immunophenotyping for CLL". This test is referred out by MDS to a specialist facility.

#### References

1. Chronic Lymphocytic Leukemia, J.J. Johnson in Wintrobe's Clinical Hematology, 10th Edition Vol 2, 1999, 2405-2427.
2. Prepared with the collaboration of Dr Hong Chang, Hematopathologist, Toronto Medical Laboratories.

### Insulin Testing

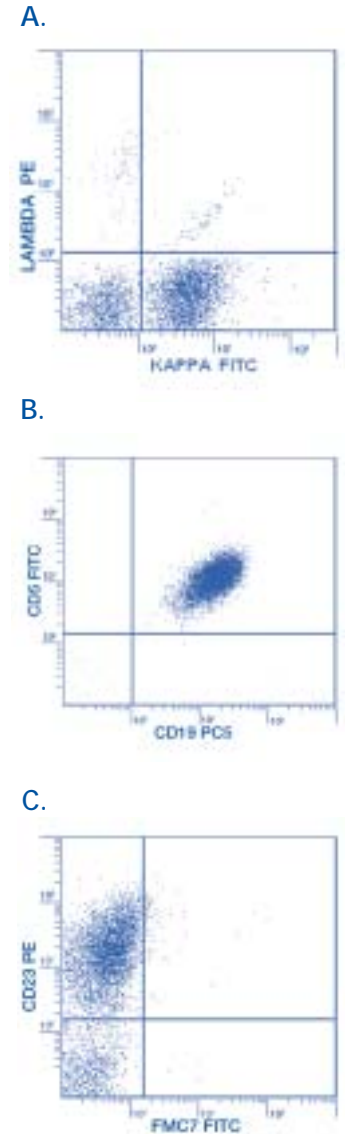
#### Introduction

Measurement of serum insulin is useful in the investigation of hypoglycemia and insulin resistance.<sup>1,2</sup> In keeping with current literature, MDS Ontario has revised its ordering protocols for serum insulin as follows:

1. Fasting insulin (FI).
2. Random insulin (RI).
3. Insulin glucose challenge test (IGCT) including multiple timed insulin and glucose determinations completed prior to and after a 75 g oral glucose load. Before ordering this test, it is strongly recommended that a fasting glucose be done to rule out Diabetes Mellitus.

(Continued on page 4)

Figure 2:  
Flow Cytometry/  
Immunophenotyping  
Histograms



(Insulin Testing- continued from page 3)

**Note:** MDS does not perform an insulin tolerance test (ITT), which is used to assess pituitary-adrenal function in response to insulin-induced hypoglycemia. Trained medical personnel in a hospital environment must complete this test.

#### Clinical Significance

A fasting serum insulin is useful in determining the cause of fasting hypoglycemia. Fasting hypoglycemia with concurrent normal or elevated serum insulin levels can be due to an insulinoma. An elevated C-peptide level at the same time excludes factitious hypoglycemia due to exogenous insulin.

Random insulin measurement can be useful in evaluating insulin resistance, which results in high serum levels in the presence of normoglycemia or hyperglycemia. A low insulin level in the presence of hyperglycemia is consistent with deficient insulin secretion as seen in Type 1 diabetes.

Although, the insulin glucose challenge test is also useful in demonstrating insulin resistance, measurement of random insulin will effectively illustrate this disorder.

This test is not helpful in assessing reactive hypoglycemia and is not recommended for that purpose.

Measurement of insulin is not recommended by the CDA or ADA for diagnosis of Diabetes Mellitus nor is it helpful in diagnosing reactive hypoglycemia.

#### Specimen Requirements

##### Fasting insulin


An early morning specimen is preferred. The patient should not eat any food from midnight the day before, but may drink water. The reference interval for fasting serum insulin is up to 100 pmol/L.

##### Random insulin

Random insulin may be collected at any time of day without reference to the last meal. Expected reference values are not available for

random insulin, however, if random insulin exceeds the upper limit of reference for fasting, further investigation is required.

#### Insulin glucose challenge test

This test requires six specimens collected when fasting and at specified intervals for 4 hours after a 75g oral glucose load. In a normal response, insulin increases to a peak value within one-hour post glucose challenge.<sup>3</sup> Reference intervals for IGCT are method dependent. MDS reference intervals for a normal population are provided in Table II. Values should be used as guidelines only. 

#### References

1. Yeni-Komshian, H., et al. *Diabetes Care*. 2000, 23: 171- 175.
2. Winter, W., E. In *Handbook of Diagnostic Endocrinology*. Ishwarlal, J. et al. (editors), AACC Press, 1999, 49 – 88, 139 – 171.
3. Kraft, J., R. *Laboratory Medicine* 1975, 6: 10 – 22.

Table II. MDS Reference Values for 4 Hr IGCT

	Fasting	0.5 hr	1 hr	2 hr	3 hr	4 hr
Insulin (pmol/L)	Up to 100	110 – 1075	95 – 1285	70 – 770	25 – 235	25 – 235
Glucose (mmol/L)	3.6 – 6.0	6.1 – 9.4	6.7 – 9.4	3.6 – 6.9	3.6 – 6.0	3.6 – 6.0



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suggestions are welcomed.  
Please contact us at:

Medical Support  
Department  
100 International Blvd.,  
Toronto, Ontario  
M9W 6J6

Toll-free: (877) 849-3637  
Fax: (416) 213-4090  
www.mdsdx.com