HMCL223
Clinical Diagnostic Techniques

Session 5:
Micronutrient Assessment
Session Objectives

**Micronutrient Assessment**
- Serum and Red Cell Tests
- Plasma tests
- Micronutrient Assessments
- Pyrrole testing
Micronutrient Assessment
Clinical Assessment Methods

- Clinical assessment of malnutrition is the first method of clinical assessment. This includes a full medical history and physical examination including observations and symptoms associated with malnutrition.

- Signs and symptoms can be non specific and may only develop in advanced stages of nutritional depletion.

- Therefore, laboratory methods of assessment should be considered. (Gibson, 2015)
Micronutrient Assessment

- Commercial laboratories offer panels of tests evaluating intracellular levels of micronutrients (essential vitamins and minerals).

- Other ‘in clinic’ tests are available for micronutrient assessment.

- Potential uses of these tests include screening for nutritional deficiencies in healthy people or those with chronic disease and aiding in the diagnosis of disease in patients with nonspecific symptoms.
Micronutrient Assessment

- The term “micronutrients” refers to essential vitamins and minerals necessary in trace amounts for health.

- Clinical deficiency states (states occurring after prolonged consumption of a diet lacking the nutrient that is treated by adding the nutrient to the diet) are not uncommon for vitamins A, B1, B12, C, and D, selenium, iodine, zinc and other micronutrients.
Micronutrient Assessment

- Laboratory tests are available for individual micronutrients and are generally used to confirm suspected micronutrient deficiencies.

- Testing is performed by serum analysis using standardized values for defining normal and deficient states.

- Commercial laboratories offer panels of vitamin and mineral testing that also use serum analysis.
Micronutrient Assessment

- Intracellular micronutrient analysis, micronutrient testing, or functional intracellular analysis, is sometimes claimed to be superior to serum testing.

- This is because intracellular levels may reflect more stable micronutrient levels over longer time periods than serum levels.

- Intracellular levels are not influenced by recent nutrition intake.
Micronutrient Assessment

- However, the relation between serum and intracellular levels of micronutrients is complex.

- The balance of intra and extracellular levels depends on several factors such as the:
  - physiology of cellular transport mechanisms
  - individual cell type
Micronutrient Assessment

- Several other in clinic collection assessments are available to identify micronutrient deficiencies. These are readily available for practitioner use and can be used as an adjunct to other naturopathic assessment tools
  - Oral
  - Urine
  - Topical application
Micronutrient Assessment
Serum and Red Cell Tests
Serum Testing
Water Soluble Vitamins
Vitamin B
Clinical Presentations

- A client may present with the following likely presenting complaints:
  - Fatigue
  - Anxiety and nervous system excitability
  - Sleep disorders
  - Fluid retention
  - Female reproductive disorders

(Pagana, 2014)
Symptoms of Vitamin B Deficiency

- Common deficiency symptoms include:
  - a rash
  - dermatitis
  - inflamed tongue
  - numbness
  - tingling or burning in the hands or feet
  - anaemia
  - fatigue
  - mental state changes

(Pagana, 2014)
B Vitamins and Testing

- The B vitamins are nutrients that the body requires in small amounts (micronutrient) for:
  - metabolism
  - energy production
  - cell, skin, bone, muscle, organ, nervous system

- B vitamin tests measure specific compounds in the blood or urine to help evaluate a person's nutritional status.

(Pagana, 2014)
Vitamin B Deficiencies

- Deficiencies can occur when:
  - There is an inadequate supply of B vitamins
  - There is poor absorbency or utilization one or more of the vitamins
  - Foods consumption inhibits the action of a vitamin
  - A deficiency in another vitamin or mineral prevents its uptake or utilization
  - Medication may inhibit utilisation
  - The need for the vitamin is increased

(Pagana, 2014)
Activated B’s vitamins Vs Standard B Vitamins

There are two different types of B vitamins available in the market place:

- Activated simply means that the B vitamins are in their active form.
- Most vitamin B supplement products contain only the inactive forms.
- Inactive B vitamins require conversion by the liver so they can be absorbed and utilized.
Activated B Vitamins v’s Standard B Vitamins

- Exciting new current research by a past Endeavour Honours student compares the effectiveness of activated Bs against standard B vitamins for clinical presentations such as:
  - Stress
  - Anxiety
  - Fatigue

- Outcomes of research such as this have an essential in patient-centred evidenced based naturopathic practice (Davenport, 2018).
Serum & Red Cell Tests

Vitamin B Testing

- Random blood sample drawn from a vein in the arm
- Vitamin B complex (thiamine or thiamin (B1); riboflavin (B2); niacin (B3); pantothenic acid (B5); pyridoxal phosphate (B6); biotin (B7)
- Used to screen for and detect moderate to severe vitamin B deficiencies

(Principles of Nutritional Assessment, Gibson 2005)
Serum and Red Cell Tests

- Tested when presenting symptoms may be due to a B vitamin deficiency, the client is at risk for a deficiency, or there is a condition associated with malabsorption

- Clinical presentations may include:
  - Alcoholism
  - Celiac Disease
  - Malnutrition
  - Malabsorption

(Principles of Nutritional Assessment, Gibson 2005)
Discussion

Consider how you may interpret these results and how they may influence your management of:

- diet
- lifestyle
- nutraceutical recommendations?
- herbal recommendations
- other therapeutic recommendations?
- how may these tests be distorted?

What other clinical presentations would you look for in a patient to determine further investigative testing?
Folate
(Folic Acid, Folinic Acid, methylated Folate)
Folate: Interpreting the Results

Reference range (Adult)
Plasma folate: 11-57 mmol/L
Red cell folate: 317-1422nmol/L

<table>
<thead>
<tr>
<th>HIGH</th>
<th>LOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pernicious anaemia</td>
<td>Malnutrition</td>
</tr>
<tr>
<td>Vegetarianism / veganism</td>
<td>Malabsorption (e.g. coeliac disease)</td>
</tr>
<tr>
<td>Recent blood transfusion (e.g. following surgery)</td>
<td>Pregnancy</td>
</tr>
<tr>
<td></td>
<td>Megaloblastic anaemia</td>
</tr>
<tr>
<td></td>
<td>Haemolytic anaemia</td>
</tr>
<tr>
<td></td>
<td>Malignancy</td>
</tr>
<tr>
<td></td>
<td>Liver disease</td>
</tr>
<tr>
<td></td>
<td>Alcoholism</td>
</tr>
<tr>
<td></td>
<td>Chronic renal disease</td>
</tr>
</tbody>
</table>
Folate Assessment Evaluation

- It is important to consider relevant evidence for measuring folic acid concentration.

- A recent research clinical trial completed by 5 Endeavour Honours students investigated the bioavailability of three different forms of folate over time (24 hours) (folic acid, folinic acid and methylated folate).

(Bayes, 2018)
Folate Assessment Evaluation

- Following supplementation the researchers found that serum concentration increased equally for all three forms.

- At later time points the researchers found that folic acid and methylated folate went down but folinic acid remained consistently concentrated in the blood.

(Bayes, 2018)
Folate Assessment Evaluation

These findings suggest three things

1. That blood concentrations of three forms of folic acid are associated recent oral intake of supplements;

2. That cellular uptake may influence serum concentration

3. That cellular uptake of folinic acid is not good (and most of the therapeutic effects occur within the nucleus of cells).

(Bayes, 2018)
Vitamin B1 – Thiamine (Vitamin F)

Reference Range (Adult):
RBC transketolase <15% increase
Whole blood thiamine (HPLC) >16ng/mL

- Test collection is a blood sample

- B1 is a coenzyme. It helps the body produce energy, is involved in glucose, amino acid, and alcohol metabolism, and is required for the proper functioning of the nervous system, heart, and muscles.

- Deficiency found primarily with chronic alcoholism.

- Can cause cardiovascular, nervous system disorders and mental state changes

(Pagana, 2014)
B6 – Pyridoxal Phosphate (PLP) Clinical Presentation

- The clinical presentation of B6 deficiencies or excess may include:
  - decreased immunity
  - smoking
  - chronic alcoholism
  - malabsorption eg: IBS and other digestive disorders
  - convulsions
  - peripheral neuropathy.
  - fluid retention
  - Pre Menstrual Syndrome

(Pagana, 2014)
Vitamin B6 – Pyridoxal Phosphate (PLP)

Adult reference Ranges:
- Serum: >50ng/mL
- Tryptophan load: <35mg/24hr xanthurenic acid
- AST: <1.5 (ratio) long term status
- ALT: <1.25 long term status
- Plasma P5P: >30nmol/L (borderline deficient)
- Urinary 4-PA: >3.0mol/d useful for recent intakes
- Serum homocysteine: <10µmol/L raised indicates B6 deficiency

- Test name: Pyridoxal phosphate (PLP) Urine 4-pyridoxic acid, urine xanthurenic acid
- Three main forms: Pyridoxine, pyridoxamine, pyridoxal
- A coenzyme involved in amino acid metabolism, hemoglobin synthesis, nervous and immune system function.

(Pagana, 2014)
Vitamin B12

Vitamin $B_{12}$ and folate should be measured when:

- a FBC indicates the presence of large red cells (macrocytic cells).

- Mental or behavioral changes: irritability, confusion, depression and/or paranoia. Elderly are of high risk.

- Physical symptoms: dizziness, weakness, fatigue, tingling or numbness in extremities, or a sore mouth or tongue.
Vitamin – $B_{12}$ Cyanocobalamin
Clinical Presentations

Adult Reference Range: Vitamin $B_{12}$ – 118-701 pmol/L

There are a number of reasons a client may wish to check their Vitamin B12 levels including:

- concern about a thyroid disorder
- experiencing unexplained fatigue
- experiencing recurrent miscarriages
- trying to conceive (avoid foetal abnormalities)
- megaloblastic anaemia

Forms of B12

- Vitamin B12 can take two forms in the body:
  - Active B12 (or ‘holotranscobalamin,’)
  - Inactive B12

- The active form of B12 can be used by the body, where the inactive form cannot.

- Therefore it’s the levels of **active B12** we really need to review if there are concerns for thyroid issues, fatigue or other symptoms associated with a B12 deficiency.

(Pizzorno, 2013, p. 179; NHMRC & MoH, 2015; RCPA Manual, 2015, Pagana,
Active B12

- Three carrier proteins are involved in the transport of Vitamin B12 around the body
  - Intrinsic Factor (IF)
  - Transcobalamin (TC)
  - Haptocorrin (HC)

- Health conditions involving intestinal malabsorption, inflammation and abnormal disorders of the stomach, pancreatic weakness and inadequate stomach acid may contribute to carrier protein problems

Testing For B12

- The most widely used B12 test checks total levels of B12, including the inactive form of it.

- Therefore, B12 levels might show as normal but in reality there may be low levels of active B12 - the version the body can actually use.

- Active B12 makes up 10% - 30% of total body B12, so someone sitting in the “normal,” range of total B12, might actually be B12 deficient.

- Therefore, this sort of testing could fail to diagnose a deficiency accurately.

# B12: Interpreting the Results

<table>
<thead>
<tr>
<th>HIGH</th>
<th>LOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>Pernicious anaemia</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>Malabsorption (IBD, Crohn Dx)</td>
</tr>
<tr>
<td>Severe liver dysfunction</td>
<td>Intestinal worm infestation</td>
</tr>
<tr>
<td>Myeloproliferative disease</td>
<td>Atrophic gastritis</td>
</tr>
<tr>
<td>(false elevation due to ↑ transcobalamin)</td>
<td>Zollinger-Ellison syndrome</td>
</tr>
<tr>
<td></td>
<td>Proximal gastrectomy</td>
</tr>
<tr>
<td></td>
<td>Resection of Ileum</td>
</tr>
<tr>
<td></td>
<td>Achlorhydria</td>
</tr>
<tr>
<td></td>
<td>Pregnancy</td>
</tr>
<tr>
<td></td>
<td>Vitamin C deficiency</td>
</tr>
<tr>
<td></td>
<td>Folic Acid deficiency</td>
</tr>
</tbody>
</table>
Evaluating Pernicious Anemia

- Vitamin B12 deficiency, secondary to an intrinsic factor deficiency is the major cause of megaloblastic anemia.

- Intrinsic factor deficiency occurs mostly because of an auto-immune process that either destroys the parietal cells that produce IF, or directly destroys the IF itself.

- Surgical gastrectomy may also result in IF deficiency.

- If pernicious anemia is suspected, anti-parietal cell antibodies and anti-intrinsic factor antibodies should be performed to determine autoimmune pernicious anemia.
Discussion

Consider how you may interpret these results and how they may influence your management of:

- diet
- lifestyle
- nutraceutical recommendations?
- herbal recommendations
- other therapeutic recommendations?

What other clinical presentations would you look for in a patient to determine further investigative testing? What tests would you suggest and why?
Serum Testing

Copper
Serum Copper

Reference Range (Adult)
Serum copper  13-22 µmol/L.
Copper Urine  < 1.2 µmol/24h
Wilson’s disease  > 1.6 µmol/24h

- Measures the amount of copper in the blood
- Tested when presenting symptoms of Wilson’s disease are evident (diagnose or monitor disease progression)
- May be used to identify copper deficiency or excess
- Used at intervals to manage copper levels when client is treated for a copper-related condition
Clinical Presentation

- Copper deficiency or excess may present with the following clinical presentations:
  - jaundice
  - fatigue
  - abdominal pain
  - behavioural changes
  - tremors
  - Other symptoms linked to Wilson disease
Serum Copper

- Low blood copper concentrations along with increased urine copper levels, low ceruloplasmin levels, and increased hepatic copper are seen with Wilson disease.
- Clients treated for Wilson disease/copper toxicity copper binding drugs eg: chelators, may need to be tested several hours away from taking the drug as it may distort the reading.
- If the clients ceruloplasmin and total copper concentrations begin to rise, then the condition is likely responding to the treatment.
Serum Copper

- Copper test results must be evaluated in context and are usually compared to ceruloplasmin levels.
- Abnormal copper results do not diagnose a specific condition but reflect the need for additional investigation.
- Interpretation can be complicated by the presence of inflammation or severe infection.
- During pregnancy and the use of estrogen and oral contraceptive use levels of ceruloplasmin and copper are increase.
Discussion

Consider how you may interpret these results and how they may influence your management of:

- age related degenerative disease
- mood & cognitive related conditions
- fatigue
- digestive complaints
- reproductive disorders?

What other clinical presentations would you look for in a patient to determine further investigative testing? What tests would you suggest and why?
Serum Testing
Magnesium
Serum Magnesium

References ranges (Adult)
Magnesium  1.3-2.1 mEq/L
Critical Values:  <1 or >9 mEq/L
Magnesium Urine  2.5-8.0 mmol/24 h (related to daily intake)

- Magnesium is the major intracellular cation and is involved in almost every metabolic & neuromuscular function in the body.
- This test is used to identify magnesium deficiency or overload.

# Mg Interpreting the Results

<table>
<thead>
<tr>
<th><strong>HIGH</strong></th>
<th><strong>LOW</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>Malnutrition</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>Diarrhoea</td>
</tr>
<tr>
<td>Addison's disease</td>
<td>Malabsorption</td>
</tr>
<tr>
<td>Ingestion of magnesium-containing antacids &amp; / or magnesium salts</td>
<td>Hypoparathyroidism</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>Alcoholism</td>
</tr>
<tr>
<td><strong>Critical High - &gt;9mEq/L</strong></td>
<td>Chronic renal tubular disease</td>
</tr>
<tr>
<td>Retardation of neuromuscular conduction</td>
<td>Diabetic acidosis</td>
</tr>
<tr>
<td>Slowing of cardiac conduction</td>
<td>Heavy metal burden</td>
</tr>
<tr>
<td>Respiratory depression</td>
<td>B6 deficiency</td>
</tr>
<tr>
<td>Lethargy</td>
<td><strong>Critical Low - &lt;1mEq/L</strong></td>
</tr>
<tr>
<td>Nausea &amp; vomiting</td>
<td>Cardiac irritability and arrhythmia</td>
</tr>
<tr>
<td>Slurred speech</td>
<td>Muscular weakness</td>
</tr>
<tr>
<td></td>
<td>Mental irritability and delirium convulsions</td>
</tr>
</tbody>
</table>

Mg: Interfering/Risk Factors

- Haemolysis of the collected blood sample should be considered in patients with a significantly elevated serum magnesium, who are otherwise asymptomatic.

- Drugs that reduce kidney excretion increase Mg\(^{2+}\) levels include thyroid medication, antacids, laxatives, calcium-containing medication, lithium, loop diuretics, and aminoglycoside antibiotics.

- Drugs that increase kidney excretion decrease Mg\(^{2+}\) levels include diuretics, some antibiotics and insulin.

Mg & Ca

- Magnesium is closely related to calcium: is required for calcium absorption from the intestines, magnesium deficiency may cause calcium resorption from the bones.

- Serum magnesium & calcium are bound to albumin so albumin level checks are also important to make sure that albumin is not decreased reducing the blood carrying capacity of the Mg and Calcium.

(Weatherby & Ferguson, 2002, p. 116)
Discussion

Consider how you may interpret these results and how they may influence your management of:

- CVD
- age related degenerative disease
- mood & cognitive related conditions
- fatigue & pain management
- digestive complaints
- reproductive disorders?

What other clinical presentations would you look for in a patient to determine further investigative testing? What tests would you suggest and why?
Other Micronutrient Testing
Calcium Assessment

- The total calcium test is the test most frequently ordered to evaluate calcium status.

- In most cases it is a good reflection of the amount of free calcium involved in metabolism since the balance between free and bound is usually stable and predictable.

Calcium - Serum

- Plasma calcium is tested to screen for, diagnosis, and monitor a range of conditions relating to the bones, heart, nerves, kidneys and teeth.

- Plasma calcium levels do not directly tell how much calcium is in the bones, but rather, how much calcium is circulating in the blood.

Calcium - Serum

- A total calcium level is often measured as part of health screening. It is sometimes included in the E/LFT panel of tests but is often asked for together with plasma phosphate and albumin.

- When an abnormal total calcium result is obtained it is viewed as an indicator of some kind of underlying problem.

- Measuring calcium and Para Thyroid Hormone (PTH) together can help determine whether the parathyroid gland is functioning normally.

Calcium - Urine

- Measuring urine calcium can help determine whether the kidneys are excreting the proper amount of calcium.

- Testing for vitamin D, phosphate, and/or magnesium along side calcium can help determine whether other deficiencies or excesses exist.

- Frequently the balance among these different substances, and the changes in them, are just as important as the concentrations.

Calcium – Combined testing

- To help diagnose the underlying problem, additional tests are often done to measure ionised calcium, urine calcium, phosphate, magnesium, vitamin D and parathyroid hormone (PTH).

- PTH and vitamin D are responsible for maintaining calcium concentrations in the blood within a narrow range of values.

Calcium – Combined Testing

- Some path labs report ‘corrected calcium’ or Ca (corr)

- This is a total calcium adjusted for abnormally high or low levels of albumin in the blood which can cause the total calcium level to appear falsely high or low

- In some patients, the balance between bound and free calcium is disturbed. This means both total calcium and/or corrected calcium does not reflect calcium status. In these circumstances, measurement of ionised calcium is necessary

Calcium Bone Mineral Density Test

- A bone mineral density (BMD) test measures how much calcium and other types of minerals are in an area of bone.

- This test helps detect osteoporosis and predict risk for bone fractures.

- The most common and accurate way uses a dual-energy x-ray absorptiometry (DEXA) scan. DEXA uses low-dose x-rays. (You receive more radiation from a chest x-ray.)

Ionised Calcium

- Some conditions where ionised calcium should be the test of choice include:
  - critically ill patients
  - patients receiving transfusions or IV fluids
  - patients undergoing major surgery
  - patients with blood protein abnormalities

- Monitoring of ionised is important as unstable levels can cause cardiovascular problems, muscle spasm (tetany), confusion or even coma.

Serum Coenzyme Q10

- This blood test measures levels of the antioxidant Coenzyme Q10 (CoQ10).

- Coenzyme Q10 (CoQ10) is an antioxidant found in every cell of the body that helps protect cells from damage caused by free radicals and create energy needed for cell growth and repair.

- CoQ10 is often said to be linked to anti-aging and is used to monitor conditions like Parkinson's Disease, Alzheimer's, cancer and heart disease.

Iodine Testing

- There are numerous ways to test for an iodine deficiency and they all provide valuable information as to the iodine status of a patient. The four most popular are:

  - One sample iodine urine test
  - Iodine serum blood test
  - Iodine patch test
  - Iodine loading test

(Weatherby & Ferguson, 2002; NHMRC & MoH, 2015; RCPA Manual, 2015; Pagana, 2014)
Clinical Presentation of Iodine Deficiency

- There are several clinical presentations of iodine deficiency including:
  - Patient suspects thyroid problems
  - Fatigue
  - Hair loss
  - Low vitality
  - Cognitive decline
  - Reproductive problems

(Weatherby & Ferguson, 2002; NHMRC & MoH, 2015; RCPA Manual, 2015; Pagana 2014)
One Sample Iodine Urine Test

- This is the urine test typically performed by most medical doctors to determine the levels of iodine and provides a biological indicator of iodine deficiency disorders.

- The measurement of urinary iodine (IU) may provide an accurate approximation of dietary iodine intake.

- Iodine results account for urine that is very concentrated or dilute. The majority of iodine that is ingested (90%) is excreted via the urine if the body does not need to uptake it. It may not be as accurate as the iodine loading test.

(Weatherby & Ferguson, 2002; NHMRC & MoH, 2015; RCPA Manual, 2015; Pagana, 2014)
Dried Urine Iodine Collection

- Dual collections of urine are placed directly on a filter strip, upon awakening and just before bed

- Dried urine iodine collections may be more convenient and be more accurate than a 24 hr urine collection

- Iodine and creatinine in dried urine may be stable for several weeks at room temperature. This provides flexibility in collection, shipment, testing, and storage

- Iodine results account for variations in urine concentrations.

(Weatherby & Ferguson, 2002; NHMRC & MoH, 2015; RCPA Manual, 2015; Pagana, 2014)
Iodine Serum Test

- This may be an accurate way to test the iodine levels, however it’s not offered by most labs and is more testing thyroid function.

- Blood iodine almost entirely resides on the thyroid hormones thyroxine (T4) and triiodothyronine (T3), so blood iodine measures thyroid function more than iodine status.

- Extreme iodine deficiency may cause hypothyroidism, but this is not usually the clinical question, which would be answered by thyroid hormone testing anyway.
Iodine Patch Test

- This is a general test which may help determine whether someone is deficient in iodine. It involves drawing a 2 x 2 patch on your forearm using a 2% tincture of iodine.

- For someone who isn’t iodine deficient, the patch shouldn’t begin to fade until after 24 hours.

- Someone who is deficient in iodine will see the patch disappear in a shorter amount of time. If there is a severe iodine deficiency, the patch will begin to fade or disappear completely in 12 hours or less.
Iodine Patch Test

- Once again, this may not be the most accurate test

- Even though it isn’t accurate, it can help to give a general idea as to whether someone is deficient in iodine, and needing supplementation

- While it’s acceptable to start off with this test, eventually it is recommended that they receive an iodine loading test to get a more specific reading
Iodine Loading Test

- This test measures the excretion of iodine over a 24-hour period.

- It admittedly isn’t the most convenient test, as you need to collect EVERY urine sample within a 24-hour period.

- Before this test you need to take a 50 mg tablet of iodine.
Iodine Loading Test

- This usually includes people with Hashimoto’s Thyroiditis and those with Hashimoto’s Thyroiditis are cautious about taking this test due to the ingestion of iodine.

- Ideally someone who has a sufficient amount of iodine should excrete at least 90% of the iodine over a 24-hour period.

- If it is less than this then the person may have an iodine deficiency. The lower the excretion rate, the greater the iodine deficiency.
Urine Iodine Testing Evaluation

- Urine iodine testing is used because it is convenient and non-invasive but can be inaccurate.

- The concentration of most analytes in urine, including iodine, depend on the amount of water also excreted into urine.

- Therefore if a patient is dehydrated they appear high, if they drink water before the test, they may appear low.
Urine Iodine Testing Evaluation

- The usual way of correcting for the water content of urine is to correct the concentration of the analyte in question by comparing to the urine creatinine level.

- While iodine/creatinine ratios may be more useful when assessing individuals there are two problems:
  
  (i) there are undefined research validated thresholds for defining deficiency and
  (ii) creatinine levels are higher in men than women. This means men have lower iodine/creatinine ratios than women.
Urine Iodine Testing Evaluation

- In pregnant women, where there is increased fluid filtration by the kidneys, a distortion can occur in daytime creatine levels.

- This may lead to low daytime creatinine levels and low urine iodine levels in pregnant women may reflect fluid status far more than iodine status.
Discussion

○ Is there error in assuming that 90% of a 50 mg dose of iodine can be cleared from the body within 24 hours?

○ Although it’s true that 90% of dietary iodine is eventually cleared from the body via the urine, according to the loading dose theory, if your body doesn’t clear at least 45 of the 50 mg dose of iodine within 24 hours, you’re flagged as having whole body iodine insufficiency and needing iodine supplements? What are your thoughts about this?

○ Does this generalisation over estimate the need for iodine supplementation?
Discussion

Consider how you may interpret these results and how they may influence your management of:

- age related degenerative disease
- mood & cognitive related conditions
- fatigue
- digestive complaints
- reproductive disorders?

What other clinical presentations would you look for in a patient to determine further investigative testing? What tests would you suggest and why?
Zinc Testing

- Zinc is a intracellular cation so serum and urine don’t accurately reflect tissue levels.

- Whole blood analysis assesses zinc present in serum, intracellularly & in the cell membranes of red & white blood cells.
Zinc Status & Assessment

- Zinc status is assessed by measurement of zinc in:
  - Plasma (Erythrocytes neutrophils, lymphocytes)
  - Hair
  - Metabolic balance studies
  - Urinary zinc excretion
  - Cu:Zn ratio
  - Zinc tolerance test
  - Measurement of activities of zinc-dependent enzymes in suitable biological samples
Zinc Status & Assessment

- Available data indicate that zinc in neutrophils and the assay of activity of alkaline phosphatase in neutrophils may be the best tools for the diagnosis of zinc deficiency.

- Measurement of zinc in the plasma is simple and readily available in many laboratories.

- Plasma zinc is useful provided conditions, such as infections, acute stress, myocardial infarction and intravascular hemolysis, are ruled out.
Zinc Status & Assessment

- Hair, urine and fingernail assessments may provide varying results

- Hair and erythrocytes turn over slowly, their zinc levels do not reflect recent changes with respect to zinc status.

- Both hair samples and urine samples may provide old results

- It is possible to use semen analyses and plasma concentration as samples
Zinc Status & Assessment

- Diminished taste acuity (hypogeusia) has been linked to zinc deficiency in humans. This forms the basis of the Zinc Taste Test (ZTT).

- Although depletion of zinc leads to decreased taste acuity, it does not explain all cases of hypogeusia.

- Various other influences on taste perception should be considered in relation to the validity of the ZTT.

- Although helpful, its validity has not yet been firmly established (Gruner, 2012).
Serum Selenium

Reference Range (Adult)
Plasma: 0.75-1.35 µmol/L
Blood: 1.1-2.5 µmol/L

While red blood cell selenium is thought of as the ideal marker, it is important to note that ideal levels of selenium for glutathione peroxidase activity, Graves disease and thyroid cancer have been formulated on serum selenium levels.

Selenium is distributed in the tissues and therefore test samples may not accurately equate to serum levels. Therefore, whole blood is a more accurate assessment of selenium status.

Selenium

Selenium provides both a protective & corrective role in the body. It assists with:

- Buffering oxidative stress associated with TPO antibody elevation
- Assists with lowering antibodies
- Preventing or minimising orbitopathy associated with Grave’s
- Assists in maintaining a better level of T3 in euthyroid individuals

Selenium – Clinical Presentations

- Both selenium depletion and toxicity is evident within society and clinical presentations may include:

  Depletion
  - Fatigue
  - Sudden weight gain or weight loss
  - Mental health changes

  Excess
  - Autism, attention deficit disorder, Alzheimer's

Discussion

Consider how you may interpret these zinc and selenium results and how they may influence your management of:

- age related degenerative disease
- mood & cognitive related conditions
- fatigue
- digestive complaints
- reproductive disorders?

What other clinical presentations would you look for in a patient to determine further investigative testing? What tests would you suggest and why?
Serum Testing
Fat Soluble Vitamins
Fat Soluble Vitamins

Vitamin D

**Vitamin D:** serum 25-OH vitamin D is the primary circulating form

**Reference Ranges (Adult)**

- Vitamin D adequacy: $\geq 50$ nmol/L at the end of winter (level may need to be $10–20$ nmol/L higher at the end of summer, to allow for seasonal decrease)
- Mild vitamin D deficiency: $30–49$ nmol/L
- Moderate vitamin D deficiency: $12.5–29$ nmol/L
- Severe vitamin D deficiency: $< 12.5$ nmol/L

**Vitamin D Nutritional Reference Range**

Deficiency: cancer development, autoimmune diseases, maternal intake childhood diseases (due to maternal intake). Increases: serum APT, parathyroid hormone & low phosphorus. Test: limited sun exposure, low intake, impaired absorption (kidney, digestive diseases), excessive animal protein, calcium intake can lower blood levels. Urinary markers of bone collagen by-products will be elevated

Pyrrole Testing
# Pyrrole Testing

## Pyrrole Testing: Mauve Factor

<table>
<thead>
<tr>
<th>Urine</th>
<th>Urinary KryptopyrroleTesting for presence of hydroxyhemopyrrolin-2-one (HPL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Ranges (Adult)</td>
<td>Normal Range of HPL</td>
</tr>
<tr>
<td></td>
<td>&lt; 10ug/dL</td>
</tr>
</tbody>
</table>

Normalized HPL result is corrected for patient hydration using specific gravity. Porphyrin (serum/ urine/ faeces) can be used but is not as specific.

Pyrrole Disorder

- Genetic, metabolic disorder of haem biosynthesis whereby a by-product called hydrohemopyrrolin-2-one (HPL) or pyrrole is produced. Also called Mauve factor.

- High levels of pyrrole are implicated in the presence of mood and learning disorders. May also be asymptomatic.

- Supplementation with therapeutic levels of vitamin B3, vitamin B6 and zinc have shown symptom improvement in those with elevated HPL.

(McGinnis et al. 2008)
<table>
<thead>
<tr>
<th>Current Diagnosis</th>
<th>High HPL in Testing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute intermittent porphyria (AIP)</td>
<td>100</td>
</tr>
<tr>
<td>Latent AIP</td>
<td>70</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>71</td>
</tr>
<tr>
<td>Schizophrenia, acute and chronic</td>
<td>40-80</td>
</tr>
<tr>
<td>Criminal behaviour</td>
<td></td>
</tr>
<tr>
<td>- Adults, sudden deviance</td>
<td>71</td>
</tr>
<tr>
<td>- Youths, violent offenders</td>
<td>31</td>
</tr>
<tr>
<td>Manic depression</td>
<td>47-50</td>
</tr>
<tr>
<td>Depression, non-schizophrenic</td>
<td>12-46</td>
</tr>
<tr>
<td>Autism</td>
<td>46-48</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>44</td>
</tr>
<tr>
<td>Learning disability and ADHD</td>
<td>40-47</td>
</tr>
<tr>
<td>Neuroses</td>
<td>20</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>20-84</td>
</tr>
</tbody>
</table>

(Mc Ginnis et al. 2008)
Discussion
Consider how you may interpret these results and how they may influence your management of:

- diet
- lifestyle
- nutraceutical recommendations?
- herbal recommendations
- other therapeutic recommendations?

What other clinical presentations would you look for in a patient to determine further investigative testing? What tests would you suggest and why?
Case Study

- Describe clinical characteristics that correlate with findings of micronutrient investigations.

- Discuss strengths and limitations of testing techniques.

- Appraise the evidence for the in clinic assessment of zinc.
References

- Australian Biologics Testing Services Sample Live Blood Analysis Report
- Australian National Health and Medical Research Council (NHMRC) & the New Zealand Ministry of Health (MoH), 2015, The Nutrient Reference Values (NRVs)
References

- Lord, RS & Bralley, JA (eds) 2012, Laboratory evaluations for integrative and functional medicine. 2nd edn, Metametrix institute, Duluth, Georgia
References


- Pagana KD & Pagana, TJ (eds) 2014, Mosby’s manual of Diagnostic and Laboratory tests, 5\textsuperscript{th} Edition, Elsevier, Missouri, USA

- Pizzorno, J E Ch 21: Laboratory tests for the determination of vitamin status cited in Pizzorno, JE & Murray, MT (eds) 2013, Textbook of natural medicine, 4\textsuperscript{th} edn, Elsevier Churchill Livingston, Missouri, USA


- Weatherby, D & Ferguson, S 2002, Blood chemistry and CBC analysis, Bear Mountain Publishing, Jacksonville, USA.
Recommended Readings

- Dr Bill Walsh on Pyroluria and Chemical imbalances – Outreach 2010 Bio-balance Health [2 mins] https://www.youtube.com/watch?v=_g3wuTrienM
- Video: HemaView Live Blood Analysis - Wet Blood Analysis https://www.youtube.com/watch?v=gZrxkj_DMnQ
- Video: Live Blood Cell Analysis Calgary: Multiple Vitamin and Mineral Deficiencies https://www.youtube.com/watch?v=bhHe4aS86As
COMMONWEALTH OF AUSTRALIA

Copyright Regulations 1969

WARNING

This material has been reproduced and communicated to you by or on behalf of the Australian College of Natural Medicine Pty Ltd (ACNM) trading as Endeavour College of Natural Health, FIAFitnation, College of Natural Beauty, Wellnation - Pursuant Part VB of the Copyright Act 1968 (the Act).

The material in this communication may be subject to copyright under the Act. Any further reproduction or communication of this material by you may be the subject of copyright protection under the Act.

Do not remove this notice.