F. Calcitonin (CT) and RET Proto-oncogene

Medullary carcinoma of thethyroid (MTC) arises from a malignant transformation of the parafollicular C cells of the thyroid and accounts for ~5-8% of all cases of thyroid cancer, of which approximately 75% are sporadic in presentation and 25% are hereditary (11,327). In a study of thyroid nodules, the prevalence of MTC is reported as 0.57% (328). The behavior and management of MTC differs from that of well-differentiated follicular-derived thyroid carcinomas (329). The inherited forms of MTC come under the heading of multiple endocrine neoplasia (MEN) types 2A and 2B. These are autosomal dominant inherited multiglandular syndromes with age-related penetrance and variable expression. Familial MTC (FMTC) is characterized by the occurrence of MTC without any associated endocrinopathy. In 1993, genetic mutations in the RET proto-oncogene were discovered (330,331). The gene responsible for these diseases is known to be located on the chromosome sub-band 10q11.2. The phenotypic expression of inherited MEN are summarized in Table 7.

Table 7. MEN Disease Phenotypes

PHENOTYPE	CLINICAL FEATURES	
MEN2A (60%)	Medullary thyroid Carcinoma (MTC) Pheochromocytoma Hyperparathyroidism Notalgia	100% 8-60% 5-20% <5%
MEN2B (5%)	MTC Pheochromocytoma Marfanoid Habitus Mucosal neuromas and ganglionneuromatosis of the gut	100% 50% 100% 100%
FMTC (35%)	MTC	100%

(a) Detection of MTC by Measuring Serum Calcitonin (CT)

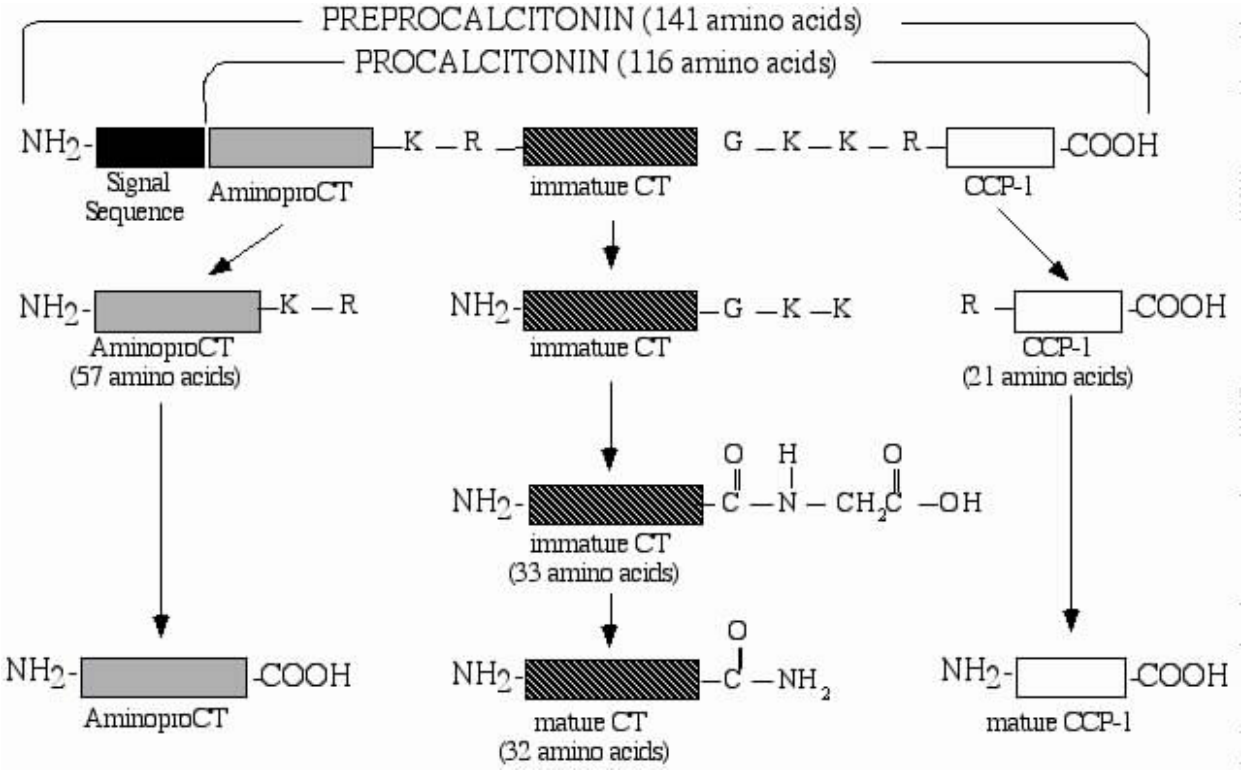
(i) Calcitonin Biosynthesis

The *CALC-1* gene encoding human CT is located on the tip of the short arm of chromosome 11 (11p15.3-15.5). Although the parafollicular C cells of the thyroid gland are the dominant source of circulating mature CT, several other categories of neuroendocrine cells besides thyroid normally contain and can secrete CT.

Mature CT is a 32-amino-acid polypeptide with a disulfide bridge and a carboxyterminal proline amide that play functionally important roles in mature CT (331). As shown in Figure 11, mature CT results from the post-translational modification of a larger 141 amino-acid precursor (preprocalcitonin) within the parafollicular C cells. Preprocalcitonin first undergoes cleavage of a signal peptide to form procalcitonin (proCT), a prohormone consisting of 116 amino-acid residues. At the proCT amino-terminus there is a 57-amino-acid peptide, called aminoproCT (or PAS-57), and at the carboxyl terminus, there is a 21 amino-acid peptide called calcitonin carboxyterminal peptide-1 (CCP-1 or

Katacalcin). The immature CT peptide consisting of 33 aminoacids is located centrally within the ProCT molecule. The mature, active, 32 aminoacid CT (which includes an amidated proline at its carboxyterminus) is produced from immature CT by the enzyme peptidylglycine-amidating mono-oxidase (PAM).

Figure 11. Post Transcriptional Calcitonin Maturation



(ii) Calcitonin (CT) Methods

Until 1988, CT assay methods were primarily based on radioimmunoassay techniques that involved the use of polyclonal antibodies that recognized both the mature CT monomer and other circulating forms (precursors and degradation products). These earlier assays lacked specificity and sensitivity. Since 1988, the advent of new immunometric techniques based on the use of monoclonal antibodies (one of which recognizes the N-terminal region and the other the C-terminal region) has made possible the development of specific and sensitive assays that detect the mature-32 amino-acid monomer of CT. Currently two-site immunometric assays can detect CT in fasting plasma samples in 83% of healthy men and 46% of healthy women (332-334). The CT values produced by different methods can differhowever, leading to difficulties in the interpretation of CT results. It is important for physicians to recognize that inter-method differences do exist and can play a role in the proper interpretation and use of CT for the diagnosis and management of MCT.

(iii) Basal CT Values

Basal CT values in the diagnosis of MTC were found to be a diagnostically useful marker of this malignancy in 1968 (335). Currently two-site IMAs, specific to mature CT, usually report CT levels below 10 ng/L (pg/ml) for all normal healthy controls and 90 % of patients with thyroid abmormalities, other than MTC (328,336-338).

F.1 Guidelines: Calcitonin (CT) Assays

(a) Mature (32 amino acid) CT is the principal tumor marker for MTC.
(b) CT measurements for the diagnosis of MTC and for monitoring purposes should be performed using two-site immunometric assays that are specific to the mature 32 amino acid monomer of CT.
(c) Currently, the lower normal threshold for CT is generally accepted as under 10 pg/ml (ng/L).
(d) As new, more sensitive CT kits become available, the lower CT threshold should be redefined.

Patients with micro or macro forms of MTC (sporadic or familial form) have elevated CT levels that correlate with the tumor mass (339). Hyperplasia of the C cells (HCC) is the earliest histologicfinding prior to the development of a microcarcinoma, when patients present with MEN2. HCC appears soon after birth, and at this stage in the disease, the basal CT levels can be normal. A normal CT result therefore cannot rule out C-cell pathology at its earliest stages.

F.2 Guidelines: Clinical Utility of Serum CT Measurements for MTC Diagnosis

- Calcitonin (CT) measurements are method-dependent. This can impact on the interpretation of CT results.
- Increased levels of calcitonin in the serum can be observed for patients with autoimmune thyroid diseases (Hashimoto's thyroiditis or Graves' disease).
- Hyperplasia of the C cells (HCC) is the earliest histological finding prior to the development of a microcarcinoma. A non-elevated CT may be seen with HCC in the earliest stages of developing MTC.
- A rise in serum CT levels above 10 pg/ml (ng/L) suggests early MTC at the microcarcinoma stage
- There is a positive correlation between CT levels and tumor mass.

(iv) Provocative Calcitonin-Stimulation Tests Used for Diagnosing MTC

In countries where genetic testing is readily accessible, calcium and pentagastrin (Pg) stimulation tests are used less often to help make the diagnosis of MTC. In fact, in the United States, Pentagastrin has become difficult to obtain and in some centers the majority of surgeries are now performed based on genetic testing alone.

Provocative tests are usually employed:

- To confirm the diagnosis of MTC preoperatively when basal CT levels are only mildly elevated (less than 100 pg/ml).
- To detect C-cell disease in *RET*-positive gene carriers
- For pre-surgical monitoring of *RET*-positive children
- For post-operative monitoring for tumor recurrence
- When genetic testing is not readily available

Secretagoges, such as calcium and pentagastrin (Pg) have been used to expose C-cell abnormalities, since they induce an increase in the CT level at all stages of MTC (342-345). One advantage of these tests is that they are able to detect HCC before MTC appears. In countries like the United States where genetic testing is readily available, surgery for gene carriers is based on genetic testing alone and provocative tests are rarely used.

• Pentagastrin (Pg) Stimulation Test

The Pg stimulation test has been widely used for the diagnosis of MTC. This test consists of an I.V. infusion of 0.5μg/kg/body weight of Pg over 5 seconds. Slow administration of pentagastrin reduces transient side effects (nausea, vomiting, substernal tightness, flushing, and tingling of the extremities) and improves patient tolerance of the test. Blood samples are drawn at baseline and 1, 2, 5 and sometimes 10 minutes after the infusion is started.

Table 8. Interpretation of the Pentagastrin (Pg) Test

#	CT ng/L (pg/mL)	Interpretation
1	CT Peak < 10	Normal
2	CT Peak <10	96% of normal adults
3	CT Peak >30 <50	4% of normal adults
4	CT Peak >50 <100	Possible CMT or other thyroid pathologies
5	CT Peak >100	Probable CMT
6	Basal or post Pg CT value > 10 pg/ml	C cell pathology or residual tissue in MEN 2 patients and MTC after surgery

The interpretation of Pg-stimulated CT values is tabulated in Table 8. The Pg-stimulated peak in CT is typically under 10 ng/L (pg/ml) for 80% of healthy adult volunteers, and under 30 ng/L (pg/ml) for 95% of the general population. Normal men exhibit higher values than women. A positive test [CT peak response above 100 ng/L (pg/ml)] suggests the presence of MTC. When patients have the familial mutation responsible for MEN 2, a peak between 30 and 100 ng/L(pg/ml) is seen and suggests HCC or a microcarcinoma. Although a Pg-induced increase in CT of less than 100 ng/L (pg/ml) is known to occur in adults with thyroid abnormalities other than MTC, no such results have ever been obtained in children under 12 years of age bearing the *RET* mutation (346). The absence of an increase in CT in a young individual bearing the *RET* mutation does not rule out the possibility that MTC may occur at an older age.

The best age to test for a C cell pathology in children bearing the RET mutation for MEN 2 with the pentagastrin stimulation test has not yet been established. It varies with the type of mutation and the type of MEN 2 occurring in these families (347,348). Hence, young carriers of the mutation with normal basal levels of CT should have a pentagastrin stimulation test performed as early as possible post-natal for MEN 2B, and at 2 years of age for MEN 2A. However, it should be stressed that high CT levels are normally found in neonates followed by an age-related decline from birth to about1 year of age; no data is yet available on this age-group as far as the pentagastrin test is concerned (349). This test should be repeated at least once a year until it becomes positive, at which time a total thyroidectomy should be performed. Given the prognosis of MTC, the low tolerance to a pentagastrin test, and the psychological implications for the family, some physicians prefer not to repeat the Pg test until it becomes positive and opt to perform a thyroidectomy on all 4 to 5 year old carriers of the *RET* mutation.

• Calcium Stimulation Test

This test consists of administering 2.5 mg/kg of calcium gluconate over 30 seconds. Blood samples are drawn at baseline and 1, 2 and 5 minutes after the stimulus. C-cell hyperplasia is suspected if the plasma CT level rises above 100 ng/L. No important adverse effects have been observed with this test, with the exception of mild and transient generalized warmth sensation. Calcium infusions have been reported to be less sensitive than the pentagastrin test for the diagnosis of MTC (350-352). Furthermore, this test has not been evaluated using a CT assay specific for the mature CT monomer, and thus needs to be re-evaluated. It has been reported that calcium infusion combined with Pg test enhances the sensitivity of the Pg test (342).

(v) Basal and Post-Stimulated CT Levels in the Follow-Up of Surgery Patients

After a thyroidectomy, serum CT measurements are the accepted tumor marker for detecting residual thyroid tissue or metastases. A detectable basal or post Pg stimulated CT level constitutes proof that there is some residual tumor tissue present (353,354).

F.3 Guidelines: Postoperative Follow-up of MTC

- Serum CT and CEA should be measured just prior to and 6 months after surgery for MCT. Serum CT levels fall slowly in some patients. The first post-operative CT measurement should not be made until 2 weeks after surgery.
- The presence of residual tissue or a recurrence of MTC can only be ruled out if both basal and post pentagastrin or calcium-stimulated CT levels are undetectable.

In view of the variations in the rate of disappearance of serum calcitonin, the first post-operative control sample should be taken at least 2 weeks after surgery (355). It should be noted that carcino-embryonic antigen (CEA) is also used along with CT to detect the recurrence of MTC. In addition, CEA appears to be a useful marker of MTC de-differentiation, and indicative of a poor prognosis.

(vi) Elevated Calcitonin Levels in Conditions other than MTC

As shown in Table 9, elevated calcitonin levels have also been observed in other pathologies besides MTC, neuroendocrine tumors and thyroid disease. Increased serum calcitonin release occurs with autoimmune thyroid diseases (Hashimoto's thyroiditis or Graves' disease) (356,357). Non-thyroid conditions where elevated CT has been noted include severe renal deficiency, hypercalcemia and hypergastrinemia, acute pulmonary inflammatory conditions and other local or general forms of sepsis (Biermer's disease, iatrogenic disorders, etc.) (358-360).

Since, in some cases the elevated CT levels were detected by polyclonal RIA, these reports require confirmation using the more current assays specific for mature CT. Studies using specific antiserum raised against ProCT, CT and CCP-1, in conjunction with HPLC and gel filtration techniques, have shown that patients with an elevated calcitonin associated with a non-thyroid condition have markedly increased serum levels of intact ProCT, and to a lesser extent the uncleaved form, CT-CCP-1. In contrast, these patients usually have normal or only minimally elevated levels of mature CT. Using epitope-specific antiserum and isolation techniques, it has been shown that tumors other than MTC can secrete large amounts of mature CT and various CT precursors (361). This can be seen in various neuroendocrine tumors, especially small cell lung cancer and bronchial carcinoid. However, only a slight increase in the CT level, if any, is observed after the Pg test when patients with these neuroendocrine tumors are tested (362). C-cell hyperplasia occurs in lymphocytic thyroiditis and some patients with differentiated thyroid cancer (363)-365). This HCC may be responsible for a slightly elevated mature CT level and for the increased CT response to the Pg test.

Table 9. Conditions with Elevated Calcitonin other than MTC

Neuroendocrine tumors	Lung small cell carcinoma, bronchial and intestinal carcinoid, all neuroendocrine tumors
Benign C-cell Hyperplasia (HCC)	Autoimmune thyroid diseases Differentiated thyroid cancer
Other diseases	Kidney disease Hypergastrinemia Hypercalcemia

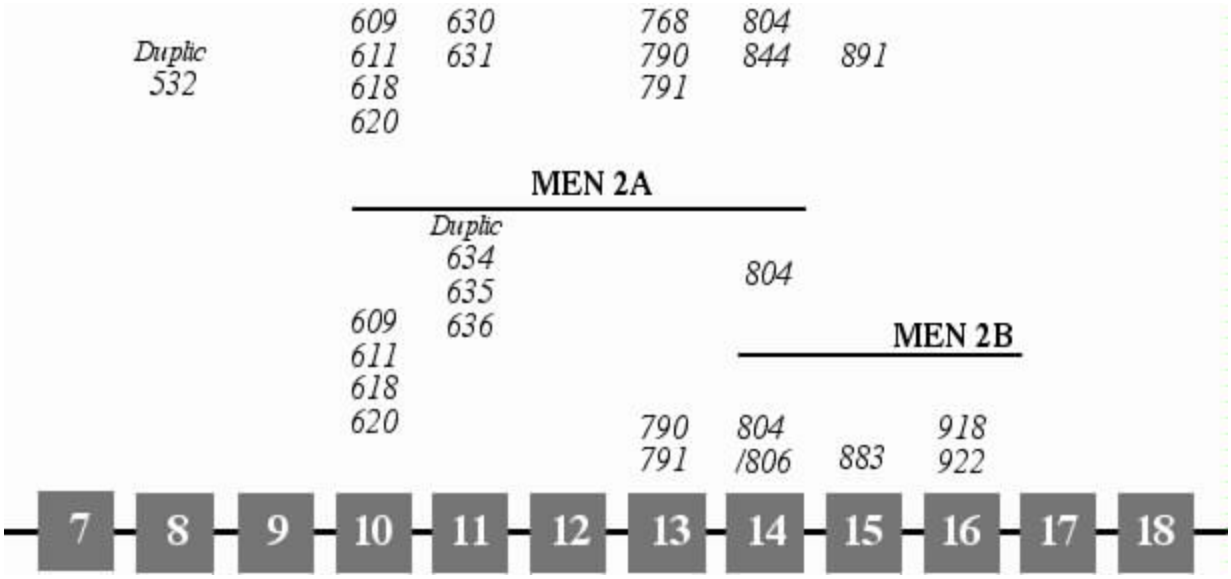
(b) Detection of MTC by Measuring the *RET* Proto-oncogene

Until 1987, the only method available for detecting subjects at risk for MTC was to perform repeated stimulated CT measurements on t family members of MTC patients. The subsequent identification of the locus 10q11.2 responsible for MEN 2 on chromosome 10 then made it possible to detect at-risk subjects by genetic screening (357). It has now been established that several types of mutations on chromosome 10 can activate proto-oncogene RET, which is responsible for MEN 2 (330,331). This now allows physicians to screen for the condition before the first biological signs appear. Currently in many developed countries,

genetic studies are the first line approach for this diagnosis. For accurate disease prediction however, it is necessary that positive genetic screening results be followed with an exhaustive survey of both the healthy and affected members of the family.

The *ret* gene is a 21 exon gene that encodes a membrane tyrosine kinase receptor. This membrane-associated receptor is characterized by a cadherin-like region in the extra-cellular domain, a cysteine-rich region immediately external to the membrane, and an intracellular tyrosine kinase domain. As shown in Figure 12, the mutations described so far in MEN2 are located in exons 8, 10, 11, 13, 14, 15 and 16 (Figure 12) (348,366-370).

Figure 12. Proto-oncogene RET Mutations

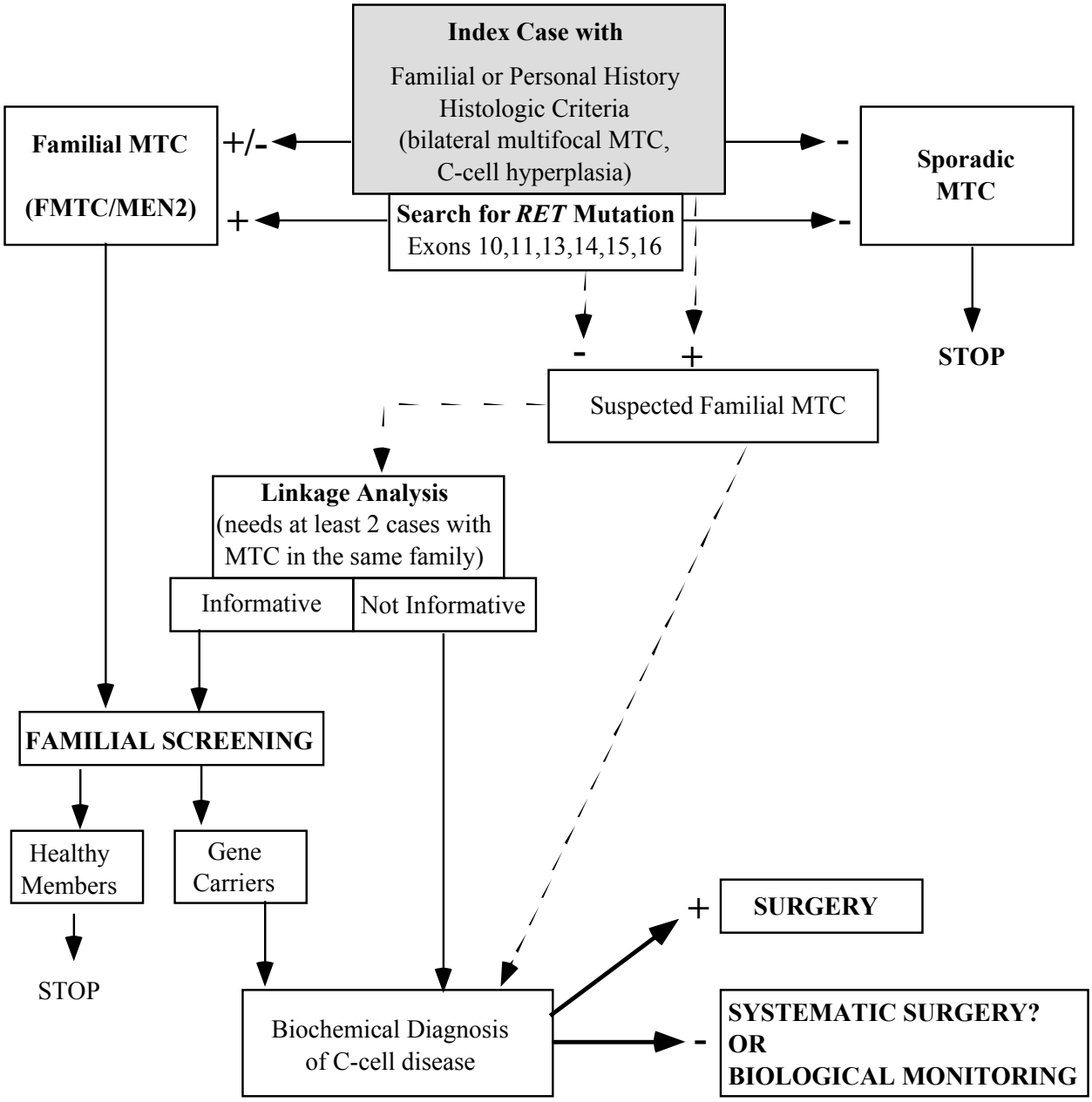


(i) Genetic Screening for MEN 2 Diagnosis

MEN2 is an autosomal, dominant familial disease, caused by the activation of missense mutations in the *RET* proto-oncogene (330). Approximately 75% of all MTCs are sporadic and solitary in origin. In 44% of such tumors a somatic mutation at codon 918 is present (371). Screening must be performed on all collateral family members, ancestors and descendants of the index case, and then all of the descendants of members known to be affected. The screening is based on the identification of a proto-oncogene *RET* genomic mutation using genomic DNA sequence analysis of the index case and on a systematic search for this mutation in all the potentially affected members of the family (Figure 13) (372,373).

To date, five mutations of the *RET* gene are present in 97% of all cases of MEN2 (Figure 12). Mutations responsible for MEN 2A mainly affect the cysteine-rich extracellular domain, each converting a cysteine into another amino acid. Mutations in the cysteine codons 609, 611, 618 and 620 of exon 10 and cysteine codon 634 (348,357). Familial Medullary Thyroid Carcinoma (FMTC) is most frequently associated with mutations in the cysteine codons in exon 10 as well as codons 768 and 804 in exons 13 and 14 (348). Most (87 %) of the mutations in codon 634 in exon 11 are associated with the multiple organ manifestations of MEN2A (MTC, pheochromocytoma, and hyperparathyroidism) (11,357).

Figure 13. Diagnosis/Treatment Algorithm for MTC



MEN2B-associated tumors are caused by mutations in the intracellular TK2 domain. Most (97%) MEN2B cases involve amino acid 918 in exon 16 converting a methionine into a threonine. These often occur as new (de novo) germline mutations (374). A minority (5%) of MEN2B mutations affect amino acids 883 in exon 15 or 922 (357,373). A correlations between phenotype and genotype suggests that in FMTC patients with non-cysteine *RET* mutations, the onset of C-cell disease is delayed to later in life compared to patients with classical *RET* mutations in exon 10 (348,375).

F.4 Guideline: Genetic Risk

- In MEN 2 kindred 50% of the family members are potentially affected by the disease.
- Almost all patients bearing *RET* mutations will develop MTC. (Note: inactivating mutations of the *ret* gene also cause Hirschsprung's disease).

- 5-10% of sporadic MTC have been found to carry germline *RET* mutations. Therefore *RET* analysis is justified in all patients with apparently sporadic MTC.

When a mutation has been identified in a family, one can be certain that family members and their descendants not bearing the mutation are free of the pathology. Conversely, the subjects bearing the mutation have the pathology and will require surgical treatment to manage, or prevent the development of disease (Figure 13). If no genomic mutation is identified in the index case, as is the case in less than 3% of MEN 2A and 5% of FMTC, linkage analysis can be used to predict the risk level for the family members. If no predictions of this kind are possible because of the genealogy of the family, the detection of disease will have to be carried out by repeated appropriate clinical studies and specific biological tests at appropriate intervals.

G. Urinary Iodine Measurement

An adequate dietary intake of iodine is necessary for normal thyroid gland hormone production to maintain a euthyroid state. It follows therefore that the measurement of iodine intake from foodstuffs or medications has clinical relevance. In the clinical laboratory, iodine measurements are used primarily for epidemiological studies or for research (3). To date, the major application of iodine analysis is to assess the dietary iodine intake of a given population (3,376,377). This is an issue of considerable importance, since it has been estimated that iodine deficiency disorders (IDD) potentially affect 2.2 billion people throughout the world. Even in developed countries such as the USA and Australia, a decline in dietary iodine intake has been demonstrated, while borderline dietary intake has long been characteristic in much of Europe (377,378).

As the majority of ingested iodine is excreted in the urine, themeasurement of urinary iodine excretion (UI) provides an accurate approximation of dietary iodine intake (378). In most circumstances the determination of UI provides little useful information on the long-term iodine status of an individual, since the results obtained merely reflect recent dietary iodine intake. However, measuring UI in a representative cohort of individuals from a specific population provides a useful index of the iodine level endemic to that region (378,379). Besides estimating the UI concentration in people, other applications of iodine measurements include determining iodine in milk, food products and drinking water (380,381). Iodine measurement in thyroid or breast tissue has been performed as part of research studies (382). Since low inorganic iodine concentrations in serum (~ 1pg/dL) are associated with relatively abundant hormonal iodine, the measurement of plasma inorganic iodide (PII) has been restricted to research studies in pregnancy (383).

(a) Urinary Iodine (UI) excretion

The UI-excretion level from a population study can provide a relatively accurate estimation of the iodine dietary status of that population (378,379). Iodine intake is best determined from a 24-hour urine sample, but logistics make it impractical to use such measurements for epidemiological studies. Differences in the dilution of spot urine specimens can be compensated for by expressing results normalized to urine creatinine as µg of iodine excreted/g of creatinine (384). The diurnal and seasonal cycles of iodide and creatinine urinary excretion are different. Therefore the ratio of iodide/creatinine can vary during the day or the time of year. In addition, there is no ideal substitute for the accuracy of a 24-hour urine collection, which can be difficult to obtain. However, the UI estimation of iodine intake is most important in developing countries where the iodine/creatinine index may be less satisfactory and where there is a lower creatinine excretion rate secondary to varying degrees of malnourishment. (385). It has also been shown that urinary iodine excretion can be variable even in healthy, well nourished subjects. For these reasons, and to avoid errors introduced in the performance of different creatinine assays, the World Health Organization (WHO) has recommended that for epidemiological studies, the excretion of UI may be expressed as µg of iodine per volume (pg/dL or µg/l) of urine. Differences in urine dilution states inherent in obtaining a spot urine sample can be partially compensated for by using a large number (~50) of subjects in each study population. Recent reports suggest that the use of age and sex adjusted (UI/Cr) ratios in a fasting morning specimen comes close to the true (24 hour) iodine excretion if nutrition is generally adequate (379,386). Temporal factors both time of day and time of year can influence the interpretation of

UI results. Although seasonal variations may not be as important in warmer climates, they do affect the results in Northern Europe where dairy milk is a major source of dietary iodine. In such populations, the practice of indoor feeding of cattle with mineral rich supplements results in higher UI excretion during the winter months. More recently it has been suggested that UI also has a diurnal variation, with values reaching a median in early morning or 8-12 hours after the last meal suggesting that samples should be collected at these times (387).

(b) Dietary Iodine

In many countries, adequate dietary iodine intake is achieved through the iodization of salt but the availability of iodized salt is only mandatory in some developed countries and voluntary in many others. There is also evidence of a decline in iodine consumption in some of the industrial countries (378). Diminished iodine intake can occur with vegetarian diets, particularly in areas where the fruits and vegetables are grown in iodine deficient soil (388).

(c) Units of UI Measurement

For epidemiological studies, iodine excretion is normally expressed as µg of iodine excreted. Conversion to equivalent SI units:
(e) µg/dL = 0.07874 µM/L
(f) 1.0pM/L = 12.7 pg/dL.

(d) Applications of Iodine Measurement

The major application of iodine measurements is for epidemiological surveys. The recommended daily iodine intake is: - 90 µg/day for children, 150 µg/day for adults and 200 µg/day for pregnant or lactating mothers. The suggested norms for UI excretion as an index of the severity of iodine deficiency are shown in Table 10 (377).

Table 10.

Iodine Deficiency	**None**	**Mild**	**Moderate**	**Severe**	**Median**
UI µg/L	>100	50-99	20-49		<20
Goiter prevalence<5%		5.0-19.9%	2~29.9%	>30%	

*IDD Newsletter Aug 1999 15: 33-48

(e) Pregnancy and the Neonate

Fortunately, the occurrence of severe iodine deficiency that leads to endemic cretinism has been reduced as a result of dietary iodine supplementation programs. However, iodine deficiency still persists in large areas of the globe. The situation where dietary iodine deficiency may have more serious consequences is in the pregnant woman, where maternal iodine deficiency can compromise the thyroid status of the fetus and newborn child (389,390). Reports on the variation in UI excretion during pregnancy differ. Some studies have reported a decline or no change, while others have shown an increase (40,391-393). These differences may reflect variations in the dietary iodine supply (394). However, the use of urinary iodine to estimate iodine sufficiency during pregnancy can be misleading, since pregnancy causes an increase in the iodine excretion rate. This results in a relative increase in the urinary iodine concentration, thus giving a false sense of adequacy of iodine nutrition (395). It has been shown when dietary iodine intake is inadequate during pregnancy, there is evidence of thyroidal stress, an increase in both thyroid volume and serum Tg and a relative decrease in FT4 (40). Administration of iodide to pregnant mother's results in increased UI excretion and a reversal of the observed iodine deficient thyroidal changes. The importance in avoiding any compromise with thyroid function during pregnancy was recently emphasized by the report that children of

even mildly hypothyroid mothers can develop defects in neuropsychological development (61,62). This finding is consistent with earlier reports that plasma inorganic iodide (PII) declines during pregnancy. Early methods of measuring (PII) were based on the administration of a tracer dose of ^{131}-I to patients and measuring the specific activity of radioisotope in serum and urine (384). Other methods depended on the ratio of iodide to creatinine in serum and urine (384,396). A recent study using perchlorate digestion and the formula PII = Total Serum Iodine - Protein Bound Iodide concluded that, at least in iodine sufficient areas, there was no trend for PII values to be depressed during pregnancy (383).

(f) Excessive Iodine Intake

It is well established that excessive iodine intake may, in susceptible individuals, lead to the inhibition of thyroid hormone synthesis (the Wolff Chaikhoff effect) and can be of iatrogenic origin (397,398). A similar excess of iodine intake by previously iodide deficient individuals with thyroid autonomy may result in hyperthyroidism (the Jod Basedow effect) (377,399-401). Population based dietary iodine intake programs can influence the form of thyroid disease that presents. This is particularly true for hyperthyroidism, with toxic nodular goiter being more prominent when iodine intake is low, and Graves' disease more prominent when iodine intake is high. However, it has been shown that a program of controlled dietary iodine intake can, after a transient increase in hyperthyroidism in the first year, cause a decrease in both toxic nodular goiter and Graves Disease if followed over a period of time (402). Differences in disease presentation can also alter the epidemiological profile of thyroid cancer with a relative increase in papillary thyroid carcinoma together with an improved prognosis when the iodine supply is increased (403).

Fear of the side effects of excess iodine has impeded the introduction of programs of iodine prophylaxis or even the possibility of administering iodide following accidental release of radioactive iodine. There is however, general agreement that the benefits of iodine administration far exceed therisks from excessive iodine exposure (377). Thus the requirement for iodine measurements in the assessment of iodine excess states may exceed that for iodine deficiency. Iodine excess can result from the use of iodine rich medications such as thecommonly prescribed cardiac antiarrythymic drug amiodarone or antiseptics containing iodine (Guideline 2.5) (81,398,400-402,404). The thyroidal consequences of amiodarone therapy may depend on the underlying dietary iodine status of the area where the patient resides. Hypothyroidism is more frequent where dietary iodine intake is high, such as in the USA, and hyperthyroidism is more frequent where the intake is low, such as in parts of Europe (405).

Excess dietary iodine intake has also been implicated in the increased prevalence of autoimmune thyroiditis or increase in thyroglobulin antibody positivity following iodine prophylaxis. This may be due to increased antigenicity of more highly iodinated forms of thyroglobulin (406,407). The assessment of iodine excess is usually made with a 24hr urine collection. It should beunderstood that organic iodide present in radiological contrast material can be taken up into body fat. Theslow release of iodine from body fat stores has been associated with a high UI excretion rate that can persist for several months following the administration of this contrast material. (408).

(g) Iodide Methods

Methods that measure iodine content in biological specimens, have traditionally relied on the conversion of organic iodinated compounds to inorganic iodide and the removal of potential interfering substances (eg. thiocyanate) that can interfere with the colorimetric measurement of the inorganic iodide. (409). The procedure involves a preliminary digestion step followed by the colorimetric estimation of iodide through its catalytic action in the Sandell-Kolthoff (SK) reaction. In this reaction, Ce^{4+} (ceric ions) are reduced to Ce^{3+} (cerous ions) in the presence of As^{3+} (arsenious ions) which are then oxidized to As^{5+} (arsenic ions) producing a change in color from yellow to colorless. Following a short incubation period, this color change can be determined colorimetrically. As this reaction is time dependent, some reports suggest stopping the reaction with the addition of ferrous ammonium sulfate and performing the colorimetric

readings at a later time. Further modifications of the SK reaction can produce a kinetic assay by altering the ratio of Ce/As ions. This kinetic method approach can increasethe sensitivity of the assay (410). The problems associated with the removal of interfering substances such as thiocyanate in the SK reaction have been previously mentioned, and a report comparing 6 methods for iodine analysis attributed much of these interferences with the SK reaction to inadequate digestion procedures (409). Two major methods of sample digestion, dry ashing and wet ashing are routinely employed.

(i) Dry Ashing

The dry-ashing technique was first introduced in 1944 and subsequently modified. The method involves the preliminary drying of specimens in an oven at 100°C. The dried residue is then incinerated in the presence of strong alkali (KOH/K_2CO_3) for approximately 3 hours at 600°C. The ash is then reconstituted in distilled H_20 and the iodide content is measured colorimetrically as described above. This is a somewhat time consuming and expensive method requiring thick-walled pyrex test tubes to withstand the high temperatures and a muffel furnace, ideally equipped with microchip temperature control. However, it does yield excellent results not only in urine samples but is also suitable for measuring the iodine content of foodstuffs and tissue samples that require complete digestion. Strict temperature control is particularly useful in preventing iodine loss should the temperature drift above 600°C or if the time of incineration is extended (410,411). It is also important that the iodine standards be subjected to incineration, as the added KOH is known to reduce the sensitivity of the SK reaction based assay. These methods were developed for the determination of protein bound iodine (PBI) used to measure thyroid hormones before the advent of specific radioimmunoassays for T4 and T3. As samples are incinerated together in a muffle furnace, the dry-ashing procedure is particularly susceptible to cross-contamination by a high iodine-containing specimen. To overcome this possibility some investigators have suggested prior screening of samples to detect such specimens. The problem of cross-contamination is particularly problematical with the dry-ashing procedure but has the potential to affect all iodine quantitation methods. It is therefore desirable that the iodine measurement area be isolated and kept as far away from other laboratory activities, particularly those that might involve use of iodine-containing reagents. The aesthetics of handling and volatilizing large volumes of urine for epidemiological studies also makes the isolated laboratory desirable.

(ii) Wet Ashing

The most widely used method of digestion is the wet-ashing technique first proposed in 1951, although this approach is controversial. In this method the urine specimens are digested using perchloric acid. This method has been automated using a Technicon autoanalyser. While the autoanalyser method has found widespread use, it does depend on the use of acid digestion and a dialysis module. The latter has been shown to be prone to significant interference by substances such as thiocyanate (409). Several variations of the wet ashing method for iodine measurements have been developed. These are primarily aimed at simplifying the methods, reducing the labor cost and rendering the method more suitable for on site epidemiological studies. Various methods have been described which yield similar results to established methods (413). In one such method, the authors indicate that a single technician can perform 150 tests per day at a cost of less than $0.50 each (413). More recently, even simpler methods using either acid digestion or UV irradiation of samples have been described (414). The wet-ashing technique has drawbacks in that perchloric acid and potassium chlorate are potentially explosive and their use requires the use of a dedicated and expensive fume hood. For this reason a less hazardous method of digesting urine samples using ammonium persulfate as the oxidizing agent was proposed. However, the use of ammonium persulfate was shown not to be a very efficient means for mineralizing iodinated compounds such as T3, T4, amiodarone etc. A further modification involving the incorporation of the digestion and reaction process into amicroplate technology has been reported (415). More recently an assay was developed in kit form that allows for a more rapid quantitative measurement of UI after charcoal purification. (Urojod, Merck KGaA, Darmstadt, Germany). This method appears simple to perform and has the potential to be used in the field for epidemiological studies or for occasional use in the assessment of excess iodide ingestion (416).

(iii) Sensitivity and Specificity of Iodine Methods

Assays using the SK reaction yield sensitivities between 10 and 40 mg/L that is more than adequate for UI measurement. Greater sensitivity has been reported using the kinetic assay (0.01pg/L) (410). Reported sensitivities using the inductively coupled plasma mass spectrometry (ICP-MS) technique is in the area of 2 µg/L (393,416). Providing the initial digestion is complete, the SK assay is very specific for iodide. However incomplete digestion can lead to interference by substances such as iodine containing medications, thiocyanates, ascorbic acid or heavy metals such as Hg or Ag (410). In expert hands the SK reaction yields excellent intra- and inter-assay precision with CV's < 5% routinely achieved. This is provided the digestion is adequately controlled so that the recovery of the iodide standard is 90 to 100% (410,411,414).

(iv) Non Incineration Assays

In addition to methods based on alkaline and acid digestion, other published methods for iodine determination include the use of bromine in acid conditions as a digesting agent or the use of ultraviolet radiation (411). Iodide selective electrodes have been used to measure iodine in various fluids including urine (418). In this case the iodide activity that is measured approximates the iodine concentration. A drawback to this method is that the electrodes become coated and requires frequent polishing and other ions such as sulphite interfere. This approach is therefore not ideally suited for measurements in urine but can be used to measure iodide in other fluids and extracts of foodstuffs. Although not suitable for routine UI measurement, the technique can be applied to the assessment of iodide overload in urine in patients treated with amiodarone or other iodine rich compounds (418). As the electrode only responds to iodide and not to iodinated compounds, it can be a useful means of specifically measuring iodide in the presence of other iodinated compounds. Many other techniques that are clearly unsuitable for routine clinical use include nuclear activation analysis, or HPLC. One method that has been widely reported is the use of (ICP-MS) (414,419). This method has been shown to have good agreement with conventional digestion techniques using SK quantitation (414,415). However, the required equipment is expensive and not readily available. Isotope dilution analysis has been applied to the analysis of both urine and drinking water (381). In vivo measurement of intrathyroidal iodine content has been achieved using X-ray fluorescence which can have relevance to the assessment of patients with amiodarone induced hyperthyroidism (400).

(h) Summary

Measurement of iodine in tissues and biological fluids is unlikely to play a key role in routine clinical biochemistry laboratories in the immediate future. However in view of the large number of individuals with IDD worldwide (2.2 billion affected) and recent reports that dietary iodine intake is declining in the United States and Australia, the assessment of UI as part of epidemiological studies will continue to be of considerable interest and importance. Reference laboratories will no doubt continue to use dry- or wet-ashing techniques, depending on availability of equipment and space. Recent recommendations that laboratories " have several different methods available to allow the user to select the one best suited to specific needs" would seem a prudent course for centers specializing in iodine measurements.

G.1 Guidelines for Urinary Iodine Measurement

- The Technicon Autoanalyser is generally no longer commercially available, with the result that laboratories seeking to commence iodine measurement will need to develop manual in-house methods.
- Mass spectrometry is a simple and reproducible method which can be recommended if such equipment is already on site.
- Many simplified digestion methods incorporating SK colorimetry have been descri bed.
- Wet-ashing reagents perchloric acid and potassium chlorate are potentially explosive and their use requires the availability of an expensive fume hood. A less hazardous system using ammonium persulfate may be preferable

- Measurement of iodine in samples other than urine (e.g. tissues, foodstuffs) may still require the more conventional dry or wet-ashing techniques.
- Inter and intra assay CV should be < 10% and recovery of added iodide should be between 90 and 100%.
- In industrialized countries, clinical laboratories are most frequently requested to perform urinary iodine measurements to investigate iodine overload. One of the simplified methods outlined above, or a semi-quantitative kit is the method of choice.
- To facilitate uniformity in concentration units used to report urinary iodine excretion, UI should be expressed as µg Iodine /L of urine (µg/L).

H. Thyroid Fine Needle Aspiration (FNA) and Cytology

The prevalence of palpable thyroid nodules in adults increases with age (average 4 -7% for the United States population) with thyroid nodules being more common in women than men (420-422). In adults, 95% of these nodules are benign. In contrast, although rare (0.22% to 1.8%), patients with thyroid nodules who are under 21 years of age have a higher incidence of malignancy (33% versus 5%, children versus adults, respectively) (423-426). The methods currently used for assessing thyroid nodules include, fine needle aspiration (FNA), thyroid scan and ultrasound. Practice guidelines suggest that an initial FNA is more diagnostically useful and cost effective than other forms of investigation (427). Despite such guidelines, a recent United States study reported that in 1996, FNA was only used as the initial procedure in 53% of thyroid nodule cases (428). This is in part because, although isotopically "cold" thyroid nodules are considered suspicious for carcinoma, most benign thyroid nodules (cysts, colloid nodules, benign follicular lesions, hyperplastic nodules and nodules of Hashimoto's thyroiditis) also present as "cold" nodules. In addition, "warm" or isofunctioning nodules that do not result in a completely suppressed TSH and thus surrounding normal thyroid tissue is not suppressed, can be malignant. Logistic regression analysis indicates that adequate cytologic material significantly increases with the size of the nodule (429). Although ultrasound can be used to detect non-palpable nodules, ultrasound cannot differentiate between benign and malignant lesions. In general, ultrasound is typically used for evaluating complex cystic masses and nodules that are difficult to palpate (430). Ultrasound is also used to determine the size of nodules and to monitor nodule growth, as well as verify the presence of non-palpable nodules that have been incidentally detected by other imaging procedures. Ultrasound-guided FNA should be used for hypoechoic nodules and when aspiration cytology fails to yield adequate cellular material (207,431,432).

(a) Indications for FNA

All solitary or dominant nodules ≥ 1cm in diameter should be evaluated by FNA. FNA is preferred to thyroid scanning or ultrasonography as the initial diagnostic test for evaluating patients with thyroid nodules (433). Since FNA became popular in the 1970s, the number of thyroid surgical procedures has decreased by 50% whereas the percent yield of cancers for patients undergoing surgery for thyroid nodules has increased from 10-15% to 20-50% (434). The frequency of false negative FNA reports is related to the skill of the operator and the experience of the cytopathologist (435). False negative rates appear to be less than 2 % (436).

H.1 Guideline: For Physicians

(g) In general, FNA is recommended for all palpable solitary or dominant nodules, independent of size.
(h) FNA is preferred over thyroid scan or ultrasonography as the initial diagnostic test for thyroid nodules. However, a previous ultrasound may aid the physician performing the aspiration.
(i) When patients are thyrotoxic, or the TSH is suppressed a nuclear scan is indicated, although the result of the scan should not exclude the necessity for FNA.

(b) Risk Factors for Thyroid Cancer

A number of factors are associated with a higher risk for thyroid carcinoma (437-439).

These are:

- Age, < 20 or > 40 years
- Nodule size > 2cm diameter
- Regional adenopathy
- Presence of distant metastases
- Prior head or neck irradiation
- Rapidly growing lesion
- Development of hoarseness, progressive dysphagia, or shortness of breath
- Family history of papillary thyroid cancer
- Family history of medullary cancer or MEN Type 2

Some of these risk factors are included in tumor risk-assessment protocols. The TNM classification protocol (tumor size, presence of lymph nodes, distant metastases) and age is the general tumor risk assessment algorithm. A number of thyroid-specific staging protocols have been developed (12). These protocols are used to provide objective information necessary for establishing an appropriate treatment plan for the projected outcome. Although theTNM classification protocol is in general use, it can be misleading when applied to thyroid tumors. Specifically, with non-thyroid cancers, the presence of lymph node metastases is a heavily weighted factor that negatively impacts on mortality. In contrast, differentiated thyroid cancers often arise in young patients in whom the presence of lymph node metastases may or may not have a minimal effect on mortality, but increases the risk of recurrence.

H.2 Guideline: For Physicians

- It is important that the endocrinologist, surgeon, nuclear medicine physician and cytopathologist act in concert to integrate the staging information into a long-term treatment plan and thereby ensure continuity of care.
- Preferably, the physicians responsible for the long-term management of the patient should review the slides with the cytopathologist and understand the cytopathologic interpretation to establish meaningful treatment strategies for the patient.

(c) Factors Suggesting a Low Risk for Thyroid Cancer

FNA may be deferred in low-risk patients with the following characteristics:

- Autonomous “hot” nodules (serum TSH < 0.1 mIU/L).
- Incidental nodules < 1 cm, detected by ultrasound.
- Pregnant patients presenting with a solitary nodule. FNA of nodules detected during pregnancy can be deferred until after delivery without increasing the risk of morbidity from DTC (440). If it is necessary to surgically remove a nodule during pregnancy, surgery during the 2nd. Trimester minimizes the risk to the fetus.
- Multinodular thyroid glands with nodules < 1 cm.
- Fluctuating or soft nodules.
- Hashimotos Thyroiditis. Indications include firm, “rubbery” gland on physical examination without dominent nodules and an associated elevation in TPOAb.

(d) Follow-up of patients with deferred FNA

The follow-up frequency (i.e. every 6 to 24 months) should be appropriate for the degree of diagnostic certainty that the nodule is benign. The efficacy of L-T4 therapy to suppress TSH can be variable. The goal of follow-up is to identify patients with undiagnosed or subsequent malignancy and to specifically recognize any progressive enlargement that could result in local compressive complications and

cosmetic concerns by monitoring nodule size preferably with ultrasound. If ultrasound is not available, a careful physical examination should be made. This can usually be accomplished by:

- Placing a tape over the nodule and outlining the borders with a pen. Placing the tape into the patient's chart.
- Use a ruler to record the nodule diameter in two dimensions
- Palpate for enlarged adjacent lymph nodes
- Diagnose any associated clinical or mild (subclinical) thyroid dysfunction by periodic serum TSH and TPOAb measurements.
- Evaluate patients for signs of undiagnosed or subsequent malignancy such as:
  - Progressive nodule or goiter enlargement
  - A rising serum Tg level.
  - Local compression and invasive symptoms (i.e. dysphagia, dyspnea, cough, pain, hoarseness)
  - Tracheal deviation
  - Regional lymphadenopathy

(e) Guidelines for who should perform FNA

Experience with aspiration cytology is essential. If the cytologist or ultrasonographer performs the FNA, there must be an exchange of appropriate information with the clinician. Physicians performing FNA should be able to request a review of the slides with the cytopathologist and understand the cytology results in order to recommend appropriate therapy based on the tissue diagnosis. Ideally, the physician performing the FNA should also be the physician responsible for the long-term management of the patient in order to assure continuity of care.

H.3 Guidelines: Selection of Physicians to Perform FNA

- Thyroid gland aspirations should be performed by physicians who:
- Are skilled in the technique and perform thyroid aspirations frequently.
- Can understand the interpretation of the cytology results.
- Are able to recommend appropriate therapy depending on the results of the aspiration.

(f) Technical Aspects of FNA

It is recommended that aspirin or other agents that affect coagulation be discontinued before the procedure. FNA is typically performed using 22 to 25 gauge needles and 10 or 20 ml syringes that may, or may not be attached to a "pistol-grip" device. For bloody nodules, a bare 25 or 27 gauge needle should be inserted without applying vacuum. Aspiration should be as minimally traumatic as possible. Some physicians favor administering topical local anesthetic (1% lidocaine) while others do not. It is recommended that a minimum of two passes be made into various portions of the nodule to decrease sampling error. Slides are typically fixed in Papanicolaou's fixative and stained. It is imperative to fix immediately and avoid drying and drying artifacts to preserve nuclear detail. It is also useful to use a rapid stain, such as Diff-Quik and examine the slides at the time of aspiration to assess adequacy of specimen for cytologic evaluation. Other slides may be air-dried for alcohol fixation and subsequent staining (excellent for detecting colloid). Any additional material can be combined with material rinsed from the needle and spun down to form a cell block which can then be embedded in agar. Cell-blocks can provide histologic information and be used for special staining studies. It is important to adequately protect the slides for transport to the laboratory. Slides should be submitted to the cytopathologist with the pertinent clinical details together with the size, location and consistency of the nodule.

Firm nodules are usually suspicious for carcinoma whereas fluctuant or soft nodules suggest a benign process. When cyst fluid is aspirated the volume, color and presence of blood should be recorded together

with a record of any residual mass left after aspiration. If there is a residual mass after cyst aspiration it should be re-aspirated. Clear, colorless fluid suggests a parathyroid cyst, whereas yellow fluid is more typical of a cyst of thyroid follicular origin. After aspiration, local pressure should be applied to the site of the aspiration for 10-15 minutes to minimize the likelihood of swelling. The patient can be discharged with a small bandage over the aspiration site with instructions to apply ice should discomfort occur later.

Often the FNA cytology information can be augmented by submitting the material for flow cytometry or immunoperoxidase staining [Section 3H(h)]. Also, thyroglobulin measurement of the needle washout can detect metastatic cervical lymph nodes even when the cytology is negative. The laboratory should have access to these samples as well as routine cytology. Any thyroid tissue in a lateral neck node is thyroid cancer (99%) unless proven otherwise!

(g) Cytologic Evaluation

Thyroid cytology interpretation can be difficult and challenging. The evaluation should assess:

- The presence or absence of follicles (microfollicles versus variable-sized follicles)
- Cell size (uniform versus variable)
- Staining characteristics of the cells
- Tissue polarity (cell block only)
- Presence of nuclear grooves and/or nuclear clearing
- Presence of nucleoli
- Presence and type of colloid (watery and free versus thick and viscous)
- Monotonous population of either follicular or Hurthle cells
- Presence of lymphocytes
- The amount of tissue contained on the slides (depending on the method of aspiration, ultrasound versus manual)

If a cytopathologist experienced with the thyroid is not available locally, it may be essential that the slides be sent to an outside expert for review. In the future, electronic review of cytopathology specimens will become increasingly available as tele-cytopathology technology develops.

H.4 Guidelines: Selection of the Cytopathologist

- The cytopathologist should have an interest and experience in reading thyroid cytology. If an experienced cytopathologist is not available locally, the slides should be sent for review by a cytopathologist with thyroid expertise outside the institution.
- Cytopathologists should be willing to review the slides with the patient's physician on request.

(h) Special Tissue Stains

Special tissue stains can be helpful in the following situations:

- When there is a mass of questionable malignancy or thyroid origin - Use specific antibody stains for Tg, TPO (MoAb 47) Galectin-3 and CEA (443-448).
- For questionable lymphoma, use B-cell immunotyping
- Undifferentiated/anaplastic thyroid cancer - stains for vimentin, P53, keratin
- Questionable medullary thyroid cancer - stains for calcitonin, neuro-specific enolase, chromogranin and/or somatostatin.

H.5 Guidelines: For Laboratories & Physicians

- In addition to routine cytology, the laboratory should provide access to special immunoperoxidase

staining for CT, Tg, TPO or Galectin-3 for special cases. (Send out to a different laboratory if necessary).

- Laboratories should archive all slides and tissue blocks "in trust" for the patient and make materials available for a second opinion when requested.

- Cytopathology laboratories should use standardized reporting of FNAs. The simplest approach uses four diagnostic categories: (1) Benign, (2) Malignant, (3) Indeterminate/Suspicious, and (4) Unsatisfactory/Inadequate. This should help achieve meaningful comparisons among different laboratories regarding outcomes. Also, it would allow appropriate assessment of the quality of service provided by the laboratory. Cytopathology laboratories should share their analysis of FNA results with clinicians by citing their rates for "True Positive," "True Negative," "False Positive," and "False Negative."

(i) Diagnostic Categories

Some cytopathologists believe that there must be at least six clusters of follicular cells of 10 to 20 cells each on two different slides in order to accurately report a thyroid lesion as benign (427,441). A cytologic diagnosis of malignancy can be made from fewer cells, provided that the characteristic cytologic features of malignancy are present. The classifications described below and their clinical relevance should be easily understood.

(i) Benign Lesions (~ 70% of cases)

• Clinical presentations that suggest a benign condition *(but not necessarily exclude FNA)*

- Sudden onset of pain or tenderness suggests hemorrhage into a benign adenoma or cyst, or subacute granulomatous thyroiditis, respectively. (However, hemorrhage into a cancer can also present with sudden pain).
- Symptoms suggesting hyperthyroidism or autoimmune thyroiditis (Hashimoto's).
- Family history of benign nodular disease, Hashimoto's thyroiditis or other autoimmune disease.
- Smooth, soft easily mobile nodule.
- Multi-nodularity (no dominant nodule).
- Mid-line nodule over hyoid bone that moves up and down with protrusion of tongue is likely to be a thyroglossal duct cyst.

• Cytologic and/or Laboratory Analyses that suggest a Benign Condition *include:*

- presence of abundant watery colloid
- foamy macrophages
- cyst or cyst degeneration of a solid nodule
- hyperplastic nodule
- abnormal serum TSH
- lymphocytes (suggests Hashimoto's thyroiditis or rarely lymphoma)
- high levels of TPOAb (Hashimoto's thyroiditis)

H.6 Guidelines: Follow-up of Patients with Benign Disease

- Some advocate performing a second FNA several months later to confirm the test.
- Others do not recommend a repeat FNA if the first yielded adequate tissue, provided that the nodule was less than 2 cm and had been stable in size during a year of follow up. In this case, follow-up with an annual physical examination and measurement of the nodule size, preferably with ultrasound is recommended. If ultrasound is not available, changes in nodule size may be detected by measurements made by a tape and/or ruler.

- It is recommended that enlarging lesions or any clinically suspicious nodules should be re-aspirated.

Benign Conditions *include, but are not be limited to, the following:*

- simple goiter
- multinodular goiter
- colloid nodule*
- colloid cyst*
- simple cyst*
- degenerating colloid nodule
- Hashimoto's thyroiditis
- hyperplastic nodule
- (often show inadequate cytologic specimen due to lack of follicular cells)

(ii) Malignant Lesions (~ 5-10% of cases)

There are differences of opinion regarding the optimal degree of surgery for thyroid malignancies. In most centers in the United States, near-total or total thyroidectomy, performed by an experienced surgeon, is the favored opinion. In Europe, other opinions exists (442). The risk of complications is lower when a surgeon is selected who performs thyroid operations frequently.

• Papillary Carcinoma (~ 80% of malignancies)

This classification includes mixed papillary and follicular and variants such as the tall cell variant and the sclerosing variant (a histological diagnosis)

Cytologic/Histologic. Two or more of the features below suggest a papillary malignancy:

- nuclear inclusions, "cleared-out", "ground glass" or "orphan annie" nuclei.
- nuclear "grooves" (not just a few)
- overlapping nuclei
- psammoma bodies (rare)
- papillary projections with fibrovascular core
- "ropey" colloid

• Follicular or Hurthle Cell Neoplasms (~20% of malignancies)

Lesions in this diagnostic category display cytologic evidence that may be compatible with malignancy but are not diagnostic (438). Definitive diagnosis requires histologic examination of the nodule to demonstrate the presence of capsular or vascular invasion. Re-aspiration is usually discouraged as it rarely provides useful information. There are currently no genetic, histologic or biochemical tests that are routinely used to differentiate between benign and malignant lesions in this category. Appropriate markers would need to be shown to distinguish between benign and malignant neoplasms in FNA specimens by multiple investigators. A number of studies suggest that TPO expression, measured by the monoclonal antibody MoAb 47, improves the specificity of correctly diagnosing histologically benign lesions over FNAB cytology alone (83 versus 55%, TPOAb immunodetection versus cytology alone, respectively) (443,444). More recently, Galectin-3, a beta-galactoside binding protein has been found to be highly and diffusely expressed in all thyroid malignancies of follicular cell origin (including papillary, follicular, hurthle and anaplastic carcinomas) but minimally in benign conditions (445-448). Most surgeons believe that an intra-operative frozen section offers minimal value in differentiating malignant from benign lesions when patients have follicular or Hurthle cell neoplasms (449). Sometimes a staged lobectomy is performed followed by a completion thyroidectomy within 4

to 12 weeks if capsular or vascular invasion in the histologic specimen indicates malignancy. A recent study found that the prognosis for patients with Hurthle cell carcinoma is predicted by well-defined histomorphologic characteristics (450).

Cytologic/Histologic. Features suggesting a Follicular or Hurthle malignancy include:

(j) minimal amounts of free colloid
(k) high density cell population of either follicular or Hurthle cells
(l) microfollicles

Cytology. These lesions may be reported as:

- "Hurthle cell neoplasm"
- "suspicious for follicular neoplasm"
- "follicular neoplasm/lesion"
- "indeterminate" or "non-diagnostic"

• Medullary Carcinoma (1-5% of thyroid malignancies)

This type of thyroid cancer should be suspected when patients have a family history of medullary cancer or multiple endocrine neoplasia (MEN) Type 2 [Section 3F].

Cytologic/Histologic Features suggesting this type of malignancy include:

- spindle-type cells with eccentric nuclei
- positive calcitonin stain
- presence of amyloid
- intranuclear inclusions (common)

• Anaplastic Carcinoma (< 1% of thyroid malignancies)

This type of thyroid cancer usually only occurs in elderly patients who present with a rapidly growing thyroid mass. Such patients may have had a previous indolent thyroid mass present for many years. It is necessary to differentiate between anaplastic carcinoma for which there is very limited therapy and thyroid lymphoma for which treatments are available.

Cytologic/Histologic Features that suggest this malignancy include:

- extreme cellular pleomorphism
- multinucleated cells
- giant cells

• Thyroid Lymphoma (rare)

Suggested by rapid growth of a mass in an elderly patient, often with Hashimoto's thyroiditis.

Cytologic/Histologic Features suggesting this malignancy include:

- monomorphic pattern of lymphoid cells
- positive B-cell immunotyping

(iii) Inadequate/Nondiagnostic FNA (~ 5 to 15 %)

A cytologic diagnosis cannot be reached if there is poor specimen handling and preparation or if inadequate cellular material was obtained at the time of FNA. The principle reasons for insufficient material for diagnosis may be inexperience on the part of the physician performing the procedure, insufficient number of aspirations done during the procedure, the size of the mass, or the presence of a cystic lesion. Adequate FNA specimens are defined as containing six groups of follicular cells of 10 to 20 cells each on two different slides (441). When small nodules are of concern, the repeat FNA should be done with ultrasound guidance. FNA using ultrasound guidance reduces the incidence of inadequate specimens from 15-20% down to 3-4% in such patients (207,431,432,451,452). Ultrasound guided FNA is also indicated for nodules <1.5 cm, cystic (complex) nodules to assure sampling of the solid component, posterior or high substernal nodules or any nodule difficult to palpate, especially in the obese, muscular or large frame patient (207,431,432). FNA should be made on dominant nodules within a multinodular goiter using ultrasound guidance in order to focus the procedure on the more clinically suspicious nodule(s).

H.7 Guidelines for Patients with Inadequate or Non-diagnostic FNA

- Repeat FNA for small nodules often yields adequate cellular material for a diagnosis. Preferably, the repeat FNA should be done with ultrasound guidance. FNA using ultrasound guidance reduces the incidence of inadequate specimens from 15-20% down to 3-4%.
- Ultrasound guided FNA is also indicated for nodules <1.5 cm, cystic (complex) nodules to assure sampling of the solid component, posterior or high substernal nodules or any nodule greater than 1.0 cm that is difficult to palpate, especially in the obese, muscular or large frame patient. The principal (i.e dominant) nodule(s) in a multinodular goiter should be biopsied using ultrasound guidance.

I. Screening for Congenital Hypothyroidism

The prevalence of primary congenital hypothyroidism (CH) (approximately 1:3500 births) is greater than that of central CH (approximately l:100,000) and is even higher in iodine deficient regions of the world. Over the last 25 years, screening for CH has been performed on whole blood spotted on filter paper, using either TT4 or TSH as the primary screening test. Such testing has become established practice in the developed world as part of screening programs for a variety of genetic conditions. In order to maximize efficiency, screening programs are frequently centralized or regionalized and operated according to strict guidelines and licensure requirements. Guidelines for CH screening have been published by the American Academy of pediatrics in 1993, by the European Society for Pediatric Endocrinology in 1993, and updated in 1999 (453-455). Participating testing laboratories may either be from the private sector or run by State governments, but must have in place an acceptable quality assurance program and participate in proficiency survey performance testing.

Thyroid dysgenesis resulting from either aplasia, hypoplasia or an ectopic thyroid gland is the most common cause of congenital hypothyrodism and accounts for approximately 85% of presenting cases (12). Inactivating mutations in the TSH receptor have been reported from a number of screening centers, but the prevalence is still unknown. The phenotype associated with TSH resistance is variable but appears to be of two types, partial or severe. Those with a TSH elevation due to partial TSH resistance are euthyroid, have a normal TT4 and may not require L-T4 replacement therapy. There is some evidence for the secretion of a thyrotropin isoform with enhanced bioactivity in syndromes of thyroid hormone resistance [Section 3C(d)vii](238). Another rare cause of CH (six patients) is a mutation of one of the genes encoding for the thyroid transcription factors, TTF-1, TTF-2 and PAX-8. These factors play a key role in controlling thyroid gland morphogenesis, differentiation and the normal development of the thyroid gland in the fetus. They bind Tg and TPO promoters to regulate thyroid hormone production.

<u>(a) Criteria needed for CH Screening Laboratories</u>

Only laboratories with experience in automated immunoassay procedures, information technology and

computer back-up with appropriately trained staff should undertake screening for CH. Neonatal screening programs rely on large numbers of samples coming from a relatively wide area. The logistics of sample transport, i.e. postal transit time, delays in posting at the maternity ward and delays in taking action after the result is produced, are more significant time limiting factors in identifying infants at risk for CH than the speed of analytic testing. Screening should take place on a daily basis so that the results can be immediately available and acted upon. Treatment should begin as soon as possible, preferably within the first two weeks of life. The minimum number of newborns that should be screened per year is debatable and relies on the fact that analytical proficiency is best accomplished when reasonable numbers of positive cases are encountered and cost efficiency is realized with higher volumes of testing. The screening program should ensure that follow-up testing is done on infants with positive screening results and that access to experienced diagnostic expertise is available. A referral pediatric endocrinologist should be available l for follow up testing to ensure that the correct diagnosis and treatment is achieved.

I.1 Recommendation: For Laboratories Performing for Blood Spot Testing:

- Only laboratories with experience in automated immunoassay procedures, information technology and have computer back-up, with appropriately trained staff, should undertake screening for Congenital Hypothyroidism.

(b) Screening methods

Most screening programs for congenital hypothyroidism rely on tests that elute blood from filter paper spots, collected from infants by heel stick. The analytical reagents for measuring thyroid hormone in the filter paper eluates usually require some modification to run on the different automated immunoassay platforms used for this testing. Two different appraoches for thyroid hormone screening of blood spot specimens have evolved –either measuring TT4 or TSH levels. In either case results should be interpreted using age-adjusted reference ranges (see Table 3 and Guideline 2.3).

I. 2 Guideline: For Laboratories Performing Thyroid Testing of Neonates and Infants

Thyroid test results in neonates and infants must be reported with gestation and age-specific reference intervals, respectively (Table 3 and Guideline 2.3).

(i) Primary TT4 with reflex TSH measurement

Most North American screening programs use an initial TT4 measurement, with mandated reflex TSH testing of specimens with low TT4 levels (usually less than the 10^{th} percentile). Historically, this approach was adopted because the turnaround time of the earlier TT4 assays was much shorter than for TSH, the test kits for TT4 were more reliable, the screening was performed earlier in the neontal period (usually at 1-2 days of age), and the cost for TT4 testing was less than that for TSH. Although the measurement of FT4 in serum is readily available, FT4 methods are not usually employed for screening because of sensitivity limitations due to the small sample taken from filter paper blood spots and the high dilution that results from the elution of the specimen (456). The TT4-first screening approach has some advantages, particularly in programs where samples need to be collected earlier in theneonatal period. TT4 is also less influenced by the TSH surge that follows the cutting of the umbilical cord that lasts for the first 24 hours thus TT4 screening results in fewer false-positives in the early time period. Furthermore, the TT4-first approach can detect the rare case of central hypothyroidism that would be missed with a TSH-first approach.

The disadvantages of TT4-first screening relates to the difficulties in setting the TT4 cut-off value low enough to minimize false-positives, but high enough to detect CH in infants with ectopic thyroid glands who may have TT4 concentrations above the 10^{th} percentile. In addition, a low TT4 and normal TSH can be encountered in a number of other conditions: (a) hypothalamic-pituitary hypothyroidism (b) thyroxine binding globulin (TBG) deficiency (c) prematurity (d) illness or (e) a delayed TSH rise. In programs where the follow up of infants with secondary or tertiary hypothyroidism has been carried

out, only 8 of 19 cases were detected by TT4 screening, seven were diagnosed clinically before screening and four, although having low TT4 concentrations on screening, were not followed up (457-459). TBG deficiency has no clinical consequence such that the detection and treatment of such conditions is contraindicated. TT4 screening may also be useful in the very low birth weight infants (< 1500g) in whom TSH is normal at the usual time of screening, and only begins to rise weeks later. However, significantly lower TT4 values are typically seen in pre-term versus full-term infants (456).

(ii) Primary TSH Measurement

Europe and much of the rest of the world have adopted TSH as the primary CH screening assay. Primary TSH screening has advantages over TT4 screening in areas of iodine deficiency, since neonates are more susceptible to the effects of iodine deficiency than adults and such infants have an increased frequency of high blood spot TSH concentrations. TSH screening makes it possible to monitor the iodine supply in the newborn population, especially since many European countries are still iodine deficient (460). Additionally, there is now little difference in cost between TSH and TT4 test reagents.

I.3 Guideline: Pre-term and Early Discharge of Infants

The TSH surge that follows the cutting of the umbilical cord and lasts for the first 24 hours may be delayed in pre-term infants and may lead to more false-positive TSH results when infants are tested within 24 hours of birth.

- When using TSH to screen pre-term infants, a second sample collected 2 to 4 weeks after birth is recommended, since in some cases there is a delayed TSH rise, perhaps due to immaturity of the pituitary-thyroid feedback mechanism.
- The TT4 –first approach may offer advantages for very low birth weight infants or when screening can only be performed within 24 hours of birth.

The TSH cutoff level used for recall varies between programs. In one program a two-tiered approach was adopted (461). Specifically, if the infant is more than 48 hours old and the initial blood spot TSH result is <10 mIU/L whole blood units, no further follow up is done. If the TSH is between 10 and 20 mIU/L whole blood units, a second blood spot is collected from the infant. TSH is normal in most of these repeat specimens. However, if the TSH is >20 mIU/L whole blood units the infant is recalled to be evaluated by a consultant pediatrician and other thyroid function tests are performed on the serum sample. For specimens drawn earlier than 48 hours, appropriate cut off values should be used (456). This approach ensures that the mildest forms of hypothyroidism characterized by only a modest increase in TSH are followed up, although it produces a higher number of false positives that must be followed through the system. Although most results above 20 mIU/L are due to CH, it is important to rule out maternal ingestion of antithyroid drugs or the use of iodine antiseptic solutions at delivery as a cause of transient TSH elevations.

I.4 Guideline: For Countries with Iodine Deficiency

- Primary TSH testing is recommended in deference to primary TT4 with reflex TSH in countries that have mild or moderate iodine deficiency.

(c) Blood Spot Assays for TSH

TSH measurements made on blood spot specimens are either reported in serum units, by relating the whole blood calibrators to serum values as in North American programs, or are reported in whole blood units, as in European programs. The absolute TSH values are significantly lower with the latter approach, because part of the volume of the spot is occupied by red cells. This difference in reporting has created confusion in the past and is still not resolved. It is necessary to t increase the whole blood units by 30-50% to

approximate the serum units.

Screening assays for CH require TSH to be measured in blood spots as small as 3-4 mm in diameter. The new "third generation" TSH IMAs with functional sensitivities down to 0.02 mIU/L are well suited for this purpose [Section 2C]. However, not all manufacturers have developed blood spot TSH assays since it is considered a specialized and limited market. Microtitre-plate assays using non-isotopic signals, such as time resolved fluorescence, are well suited for blood spot specimens and are in widespread use. An advantage of these systems is that as elution of the blood spot is carried out in the microtitre plate well, all of the TSH in the sample is available for binding to the monoclonal antibody on the wall of the microtitre plate well.

I .5 Guidelines: Performance Criteria for Blood Spot TSH Screening of Newborns

- Functional sensitivity of the TSH assayshould be at least 1.0 mIU/L.
- Between run CV's ideally <10% and not more than 15%.
- Internal quality control samples included in every run.
- Participation in National and/or International external quality control programs (see Appendix B).

Other automated systems that do not use a microtitre plate format however, can be successfully used for blood spot TSH assays. These usually require an off-line elution of the TSH from the blood spot and a sampling of the eluate by the automated immunoassay analyser. Some of these systems have the advantage of results after only 20 minutes and have a high throughput rate of 180 test results per hour. Additionally, these systems incorporate positive identification of the sample, making the identification of an increased blood spot result from the correct patient more secure. An automated punch of the filter paper containing the blood spot has been designed so that bar-coded labels with a unique number, placed on the elution tubes or microtiter plates, are read before punching. The same identification number is then printed on the patient's filter paper card. The automated immunoassay analyzer reads the same bar-coded label on the elution tubes and results are printed or downloaded to the laboratory host computer against the patients unique identification number and demographics if these have been previously entered. For those laboratories without automation, TSH assays utilizing antibody coated tube assays are still suitable, but are not amenable to high throughput automation.

I .6 Guideline: Cut-off TSH values for the Screening of Neonates > 48 hours of age

Reported values should be identified in whole blood or serum units. It is necessary to increase the whole blood units by 30-50% to approximate serum units.

- Initial blood spot TSH < 10 mIU/L whole blood units – no further action
- Initial blood spot TSH 10-20 mIU/L whole blood units – repeat test on second blood spot
- Initial blood spot TSH >20 mIU/L whole blood units – recall infant for evaluation by pediatric endocrinologist

(d) Sample Collection

The technique for taking blood samples by heel stick onto filter paper is of the utmost importance. Only filterpaper that meets NCCLS standards should be used (462). This requires a continuous training program, well-written protocols and establishing criteria for adequacy of specimens.

The decision as to when to obtain the sample is determined by the requirements of other newborn screening protocols and whether the sample is taken in the hospital or at home. In Europe, samples are usually taken between 48 hours and 8 days after birth, depending on local practices. In many screening programs in the United States economic pressures that prompt early discharge dictate that specimens be drawn before 48 hours. Sample collection time impacts on the TSH-first strategy more than TT4-first because a TSH surge

occurs at thetime the umbilical cord is cut. In the majority of infants the increase in TSH returns to normal within 24 hr, but in some can remain elevated for up to 3 days. For pre-term infants, a second sample, collected 2 to 4 weeks after the first sample, is advisable since in some cases there is a delayed TSH rise, perhaps due to immaturity of the pituitary-thyroid feedback mechanism (463).

(e) Confirmation Testing

Measurements performed on filter paper elutes are not diagnostic but are of screening value only and abnormal results must be confirmed with routine quantitative methods! Confirmatory blood samples should be drawn by venipuncture. In some countries a blood sample is also collected from the mother at the same time to check maternal thyroid function. Specifically, TSH receptor blocking antibodies (TBAb/TSBAb) present in mothers carrying a diagnosis of hypothyroidism (even when receiving adequate L-T4 replacement) can cause transient hypothyroidism in the infant (in 1:180,000 neonates) (294). Some programs in Europe advocate follow-up testing with serum FT4, TSH and TPOAb in the mother as well as the infant. It is important to note that serum FT4 and TT4 levels are higher in the neonatal period so that borderline results in infants with mild hypothyroidism should be compared with age-related reference intervals for the particular thyroid test used (Table 3).

While it is the aim of CH screening programs to detect CH and facilitate replacement treatment as early as possible (within 14 days). Additional tests to determine the etiology of CH should also be carried out to evaluate whether the condition is transient, permanent or genetic (needed for genetic counseling) (Table 8). Some of these tests need to be performed before L-T4 replacement treatment begins, while others can be performed during therapy. In the case of transient hypothyroidism due to transplacental passage of TBAb/TSBAb from mother to infant, treatment with L-T4 is indicted since the presence of blocking antibody in the neonate inhibits the actions of TSH resulting in a lowered FT4 concentration (294,464). Once the antibodies have been degraded over a period of three to six months, depending on the amount of antibody present, then L-T4 therapy can be gradually discontinued. The mother's thyroid antibody status should be monitored in any subsequent pregnancies as thyroid antibodies can persist for many years (465).

I .7 Guideline: Filter Paper Eluate Measurements

- Measurements made on filter paper eluates are not diagnostic. Values are at best only semi-quantitative and help identify individuals likely affected by congenital hypothyroidism. Any abnormal newborn screening result must be confirmed with quantitative serum thyroid tests.

In many cases, at the time of the diagnosis of CH, it is impossible to determine whether the hypothyroidism is permanent or transient. Especially, if circumstances render it impractical or impossible to carry out some of the procedures recommended above. Clues that are associated with transient conditions include a TSH below 100 mIU/L, male sex, pseudohypoparathyroidism, prematurity, iodine exposure, or dopamine administration, (458). In such instances it is best to manage the patient as if he/she has permanent hypothyroidism (466). If the diagnosis has not become apparent by the age of 3 or 4 years, L-T4 therapy should be discontinued for one month and the infant monitored with serial determinations of FT4 and TSH.

(f) Tests for the Etiology of Congenital Hypothyroidism

Tests that can be used to establish the diagnosis of CH and investigate its etiology are shown in Table 11. The ordering of such tests is usually the responsibility of the consultant pediatrician and not the screening program. Thyroid scintigraphy is useful to document the presence of any thyroid tissue present and its location. Serum thyroglobulin measurements are more sensitive than scintigraphy for detecting residual functioning thyroid tissue and may be normal or low in cases where scintigraphy shows no uptake. The presence of a thyroid gland is best determined by ultrasonography that can be performed after the start of therapy since ^{123}I scintigraphy is not available everywhere. Many cases show no uptake with scintigraphy and clearly present thyroid tissue by sonography. In these cases, testing should be directed towards determining an inborn error of T4 synthesis (~ 10% of cases) or a transient cause such as acquired TSH receptor blocking antibodies derived trans-placentally (294,464).

A perchlorate discharge test > 15% suggests an inborn error. Specialist centers offer tests that include urinary iodine measurement, tests for a specific gene mutation such as the sodium/iodide symporter, TPO or thyroglobulin (467). More commonly, defects in the oxidation and organification of iodide to iodine and coupling defects resulting from mutation in TPO can occur. Mutations in the thyroglobulin gene give rise to abnormal thyroglobulin synthesis that can result in defective proteolysis and secretion of T4. Deiodinase gene mutations give rise to deiodinase defects as well.

I .8 Guideline: Confirmation Testing for Abnormal Screening Tests (TT4 or TSH)

- Confirmatory blood samples from the neonate should be drawn by venipuncture.
- Some programs in Europe advocate follow-up testing of the infant and in some cases also the mother using serum FT4, TSH and TPOAb.
- Check the mother for TSH receptor blocking antibodies.
- Use method and age-specific reference intervals for TT4 and TSH testing of neonates.

Table 11. Diagnostic Tests in the Evaluation of Congenital Hypothyroidism (CH)

To Establish the Diagnosis:

• **Infant:**	TSH FT4	• **Mother:**	TSH FT4 TPOAb

To Establish Etiology:

• **Infant:**	- Determine size and position of thyroid by either: Ultrasonography (in newborn) Scintigraphy – either ^{99m}Tc or ^{123}I - Functional studies: - ^{123}I uptake - Serum thyroglobulin (Tg) - Inborn error of T4 production is suspected: -^{123}I uptake and perchlorate discharge test - If iodine exposure or deficiency is suspected: -Urinary iodine determination
• **Mother:**	- If autoimmune disease present: -TSH Receptor antibody (TRAb) (also in infant, if present in mother)

(g) Long-term monitoring of CH

Most CH infants and children have normal pituitary-thyroid negative feedback control although T4 and TSH thresholds are set higher (Table 3) (36). Infants and children diagnosed with congenital hypothyroidism should be monitored frequently in the first two years of life using serum TSH as the primary monitoring test with FT4 the secondary parameter employing age-appropriate reference intervals (Table 3) (33). In the United States, the L-T4 replacement dose is adjusted to bring the TSH below 20 mU/L and produce a circulating T4 level in the upper half of the reference range (>10 ug/dl/129 nmol/L) within the first two weeks after starting treatment. Infants are usually maintained on a dose of 10-15 µgL-T4/kg body weight with the monitoring of TSH and T4 every 1-2 months. In Europe, a flat L-T4 dose of 50

µg/day is used with the T4 and TSH measurement made after 2 weeks and monthly thereafter if possible. Experience has shown that with this dosage, the therapy does not need any adjustment for the first 2 years. Frequent dose changes designed to keep a maximal dose per Kg body weight can lead to overtreatment (466). A minority of treated CH infants appear to have variable pituitary-thyroid hormone resistance, with relatively elevated serum TSH levels for their prevailing serum free T4 concentration. This resistance appears to improve with age (36). In rare cases, transient hypothyroidism may result from the transplacental passage of TSH-receptor blocking antibodies (275,294). It is recommended that the diagnosis of CH be re-evaluated in all cases after 3 years of age. Specifically, after a basal FT4/TSH measurement is made, L-T4 treatment is discontinued and FT4/TSH retested after 2 weeks and again after 3 weeks. Almost 100% of children with true CH have clearly elevated TSH after 2 weeks off treatment.

(h) Missed Cases

No biochemical test is 100% diagnostic and technically accurate. One study in which screening checks were made after two-weeks of age revealed that 7% of cases of CH were missed using the TT4-first strategy, and 3% were missed with the TSH-first, approach. Recommendations are needed to address the clinical, financial and legal ramifications of false-negative screening tests and whether mandated retesting at 2 weeks such as practiced in some programs, is desirable and possible.

I .9 Guidelines: Treatment and Follow-up of Infants with Congenital Hypothyroidism

- In Europe, a flat L-T4 dose of 50 µg/day is used to minimize the risk of overtreatment as compared with more frequent dose changes.
- In the USA, treatment is typically initiated with L-T4 at a dose of 10-15 µg/kg/day. The goal is to raise the circulating T4 above 10 µg/dl by the end of the first week.
- During the first year of life, TT4 is usually maintained in the upper half of the normal reference range (therapeutic target 10-16 µg/dl/ 127-203 nmol/L) or if FT4 is used, the therapeutic target is between 1.4 and 2.3 ng/dl (18 and 30 pmol/L) depending on the reference range (Guideline 2.3 and Table 3).
- Infants and children diagnosed with congenital hypothyroidism should be monitored frequently in the first two years of life using serum TSH as the primary monitoring test with FT4 as the secondary parameter, employing age-appropriate reference intervals.
- Monitoring should be every 1-2 months during the first year or life, every 1-3 months during the second and third years and every 3-6 months until growth is complete.
- If circulating T4 levels remain persistently low and the TSH remains high despite progressively larger replacement doses of L-T4, first eliminate the possibility of poor compliance.
- The most frequent reason for failure to respond to replacement therapy has been interference with adsorption by soy-based formulas. L-T4 should not be administered in combination with any soy-based substances or with medications that contain iron.

(i) Quality Assurance

All screening programs should have a continuous system for audit and publish an annual report of the outcome of the audit. By this means, an appraisal can be made of each aspect of the screening procedure against nationally agreed upon quality standards. Although laboratories generally comply with quality standards in that they routinely participate in quality assurance sprograms, the pre-analytical and post-analytical phases of screening typically receive less attention. Quality assurance programs should address each of the following phases:

- *Preanalytical*
  - Training for personnel conducting the sample collection
  - Storage and timely transport of filter papers to the laboratory
  - Linking the identification of the filter paper sample to the analytic result
- *Analytical*

- Equipment maintenanceand service
- Internal QC of filter paper results
- National and International external QC participation

• *Post-analytical*

- Co-ordination of follow-up of abnormal tests
- Confirmatory testing where applicable

I. 10 Guideline: Detection of Transient Congenital Hypothyroidism (CH)

Since CH may be a transient result of transplacental passage of TSH receptor blocking antibodies, it is recommended that the diagnosis be re-evaluated in all cases at 2 years of age.

- At 2 years of age a blood specimen should be obtained for basal serum FT4/TSH measurements. Discontinue L-T4 treatment and retest serum FT4/TSH after 2 weeks and again after 3 weeks. Almost 100% of children with true CH have elevated TSH levels after 2 weeks off of treatment.

(j) Annual Report

This should include items identified under audit and be a comprehensive report of screening for CH over the previous twelve months. The report should monitor the distribution of increased blood spot TSH concentrations, and a system set up to report all cases of true CH and record cases of transient elevations in TSH. This system could also provide information from pediatric colleagues on any missed cases. A close collaboration between the screening laboratory, pediatricians, endocrinologists and all concerned in the screening process needs to be established to maintain an efficient screening program.

I .11 Guidelines: For Physicians

- Repeat thyroid tests when the clinical picture conflicts with the laboratory results!
- Potential pitfalls in screening are variable and no laboratory is immune to problems!
- Maintain a high degree of vigilance. Despite all safeguards and the use of automated systems, screening programs occasionally miss infants with congenital hypothyroidism. Do not be lulled into a false sense of security by a laboratory report bearing normal thyroid function values if the clinical suspicion of a thyroid abnormality is present.

Section 4. The Importance of the Laboratory – Physician Interface

Physicians need quality laboratory support for the accurate diagnosis and cost-effective management of patients with thyroid disorders. Laboratories need to offer methods that are both diagnostically accurate and cost effective. These latter qualities are sometimes in conflict. Cost-effectiveness and quality care require that the laboratory serve not only the needs of the majority, but also meet the needs of the minority of patients who have unusual thyroid problems that challenge the diagnostic accuracy of the various thyroid tests performed. Most studies on "cost effectiveness" fail to take into account the human and financial costs resulting from inappropriate management, needless duplication of effort and the unnecessary testing of patients with unusual thyroid disease presentations that test the diagnostic accuracy of the thyroid test methods used. These unusual presentations account for a disproportionately large expenditure of laboratory resources to come up with the correct diagnosis(179). These unusual presentations include binding protein abnormalities that affect the FT4 estimate tests, Tg autoantibodies that interfere with serum Tg measurements, medications that compromise the in vivo and in vitro metabolism of thyroid hormones or severe NTI that have a myriad of effects on thyroid tests.

4.1 Guidelines: For Laboratories and Physicians

- It is essential that clinical laboratory scientists develop an active collaboration with the physicians using their laboratory services in order to select thyroid tests with the most appropriate characteristics to serve the patient population in question.
- An active laboratory-physician interface ensures that high quality, cost-effective assays are used in a logical sequence, to assess abnormal thyroid disease presentations and to investigate discordant thyroid test results.

It is essential that clinical laboratory scientists develop an active collaboration with physicians using their laboratory services and to select thyroid tests with appropriate characteristics to serve the patient population in question. For instance, the effect of nonthyroidal illness (NTI) on the FT4 method is not as important if the laboratory serves primarily an ambulatory patient population. In contrast, it is very important for a hospital laboratory to accurately exclude thyroid dysfunction in hospitalized sick patients. Drugs and other interferences can affect the interpretation of more than 10% of laboratory results in general, and thyroid testing is no exception (70,71,102). It follows that discordant thyroid results are often encountered in clinical practice. These Discordant results need to be interpreted with considerable detail using a collaborative approach between the clinical laboratory scientist who generates the thyroid test result and the physician who manages the patient with suspected or established thyroid disease.

A. What Physicians Should Expect from Their Clinical Laboratory

The physician depends on the laboratory to provide accurate test results and to help investigate discordant results, whether the tests are performed locally or by a reference laboratory. It is particularly important that the laboratory provide readily available data on drug interactions, reference intervals, functional sensitivities and detection limits as well as interferences that affect the methods. The laboratory should avoid frequent or unannounced changes in assay methods and interact closely with physicians before a change in a thyroid test is initiated. The laboratory should also be prepared to collaborate with the physician to develop clinical validation data with theimplementation of any new method, as well as provide data showing a favorable relationship between the old and the proposed new test method as well as provide a conversion factor, if required. The diagnostic value and cost-savings of reflex testing strategies (i.e. adding FT3 when FT4 is high, or FT4 when TSH is abnormal) are usually site-specific (468). Laboratories should only introduce reflex-testing after consultation with the physicians using the laboratory. In the United States, the laboratory cannot perform reflex testing without a written order by the ordering physician because of medicolegal liability issues and government regulations.

Physicians should expect their clinical laboratory to establish a relationship with a reference laboratory and/or another local laboratory that performs thyroid testing using a different manufacturer's methods. Re-

measurement of the specimen by an alternative method is the cornerstone of investigating whether a discordant result is caused by a technical problem, an interfering substance in the specimen or a rare clinical condition [Guideline 2.7 and Table 1].

4.2 Guideline: "Patients Bill of Rights"

- Physicians should have the right to send specimens for testing to non-contracted laboratories when they can show that the contracted laboratory thyroid test results are not diagnostically valid or relevant.
- Physicians should have the right to request their laboratory to send a specimen to another laboratory for testing by a different method if the test results are in disagreement with the clinical presentation.

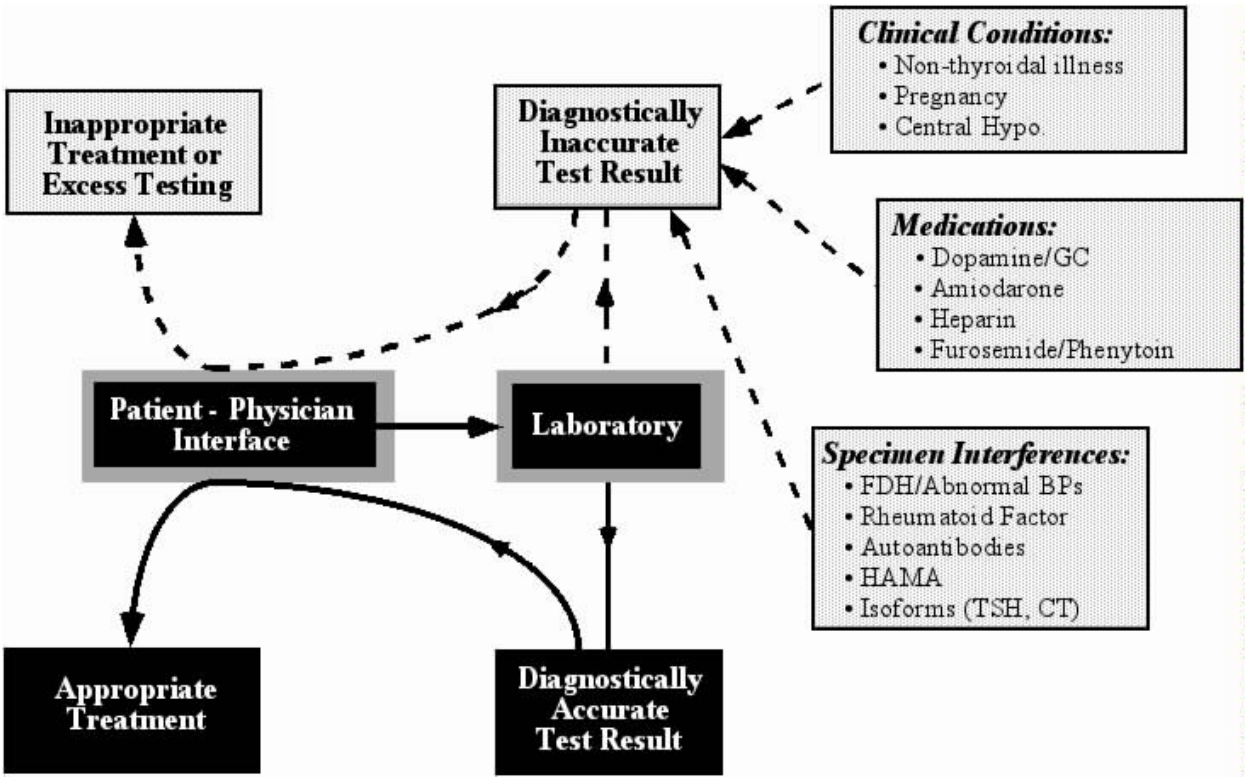
The laboratory should establish and maintain active communication with specialized reference laboratories to ensure the availability of high-quality specialized thyroid tests. These specialized tests may include assays for thyroglobulin (Tg), TPOAb and TSH-receptor antibody (TRAb) tests. In addition, a reference laboratory offering FT4 measurements using a physical separation technique such as equilibrium dialysis should be available. The use of equilibrium dialysis for FT4 testing may be necessary under special circumstances for diagnosing thyroid disease in select patients with thyroid hormone binding protein abnormalities that interfere with the diagnostic accuracy of the routine FT4 estimate test performed in the clinical laboratory. In rare cases, it may be necessary to collaborate with a molecular diagnostic laboratory that has the expertise to identify genetic mutations characteristic of thyroid hormone resistance or medullary thyroid disease.

4.3 Guideline: For Laboratories

- Every clinical laboratory should develop a relationship with another laboratory that uses a different manufacturer's method. Re-measurement of specimens with discordant results by an alternative method is the cornerstone of investigating whether a discordant result is caused by an interfering substance present in the specimen or as a result of "true" disease (Table 1).
- Laboratories should be able to provide physicians with the details of the methodologic principles underpinning the test performance together with functional sensitivity, between-run precision, interferences and any bias relative to the method or other methods, whether the tests are performed locally or sent to a reference laboratory.

As shown in Table 1 and Figure 14, a number of clinical conditions, medications and specimen interferences can give rise to a diagnostically inaccurate test result that has the potential to prompt excess testing, inappropriate treatment, or in the case of central hypothyroidism mask the need for treatment. Some of the misinterpretations that can lead to serious errors are listed in Guideline 4.4.

Figure 14. Consequences of Diagnostically Inaccurate Thyroid Tests



Manufacturers have the responsibility to thoroughly evaluate their methods and cooperate closely with laboratories using their products. Specifically, manufacturers should rapidly inform all users of reagent problems or method interferences and recommend how to minimize any clinical impact of the problem. They should refrain from changing the composition of assay kits, even if the goal is to lessen interference, without informing customers. If the procedure has to be changed this should be indicated on the label of the kit i.e. by a version number.

B. What Laboratories Should Expect of Physicians

Clinical laboratory scientists can reasonably expect that physicians provide relevant clinical information with the submission of the test specimen and have a realistic understanding of the limitations of thyroid tests. For example, in some conditions, the physician should appreciate that the immunologic and biologic activity of TSH may be disconnected when patients have central hypothyroidism. This can result from pituitary dysfunction in which the immunoreactive form of TSH has impaired bioactivity (191,193). The physician should know that anomalous laboratory thyroid test results can occur with certain medications and that the diagnostic accuracy of thyroid tests used for patients with NTI is method dependent. Without clinical feedback, it is not possible for the laboratory to appreciate the consequences of a diagnostic error (179). Misinterpretation, as a result of a transient disequilibrium between serum TSH and FT4 following recent therapy for hypo-or hyperthyroidism may have minor consequences.

4.4 Guidelines: Misinterpretations that lead to Serious Errors:

When physicians or laboratorians are not aware of the limitations of test methods, serious medical errors can result:

- Inappropriate thyroid ablation because high thyroid hormone levels were reported as a result of FDH, the presence of thyroid hormone autoantibodies or thyroid hormone resistance.
- A missed diagnosis of T3-toxicosis in a frail elderly patient with NTI.

- Inappropriate treatment of a hospitalized patient for hypo- or hyperthyroidism on the basis of abnormal thyroid tests caused by NTI or a drug-related interference.
- A missed diagnosis of central hypothyroidism because the immunoreactive TSH level was reported as normal due to the measurement of biologically inactive TSH molecules.
- Failure to recognize recurrent or metastatic disease in a thyroid cancer patient because serum Tg was inappropriately low or undetectable due to TgAb interference or a "hook" effect with an IMA measurement.
- Inappropriate treatment for DTC on the basis of an inappropriately high serum Tg caused by TgAb interference with a Tg RIA method.
- Failure to recognize that neonatal thyrotoxicosis can be masked by transplacental passage of antithyroid drugs given to a mother for Graves' disease.

4.5 Recommendation: For Manufacturers

Manufacturers should cooperate closely with laboratories using their products. Manufacturers should:

- Rapidly inform all users of reagent problems and method interferences and recommend how to minimize their clinical impact.
- The composition of assay kits should not be changed, even if the goal is to reduce interference, without first informing customers. If the procedure has to be changed, the change should be indicated on the label of the kit (i.e. by a version number).

Acknowledgements

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