I. Screening for Congenital Hypothyroidism

The prevalence of congenital primary hypothyroidism (CH) (approximately 1:3500 births) is greater than that of central hypothyroidism (hypothalamic or pituitary) CH (approximately l:100,000). The prevalence is higher in some ethnic groups and increased in iodine deficient regions of the world *(476,477)*. 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 *(478-480)*.

Participating testing laboratories may be from the private sector or run by State governments, but must have in place an acceptable quality assurance program and participate in proficiency testing.

Thyroid dysgenesis resulting from aplasia, hypoplasia or an ectopic thyroid gland is the most common cause of congenital hypothyroidism 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 TSH isoforms with enhanced bioactivity in syndromes of thyroid hormone resistance [Section-3 C4(g)ii] *(244)*. 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.

Guideline 64. Laboratories Performing Neonatal Screening for Congenital Hypothyroidism

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

The proper interpretation of newborn thyroid function requires some understanding of the interaction between mother and fetus. Iodine, thyrotropin releasing hormone, antithyroid medications and IgG antibodies readily cross the placenta. There is no trans-placental passage of TSH or triiodothyronine. In contrast, contrary to previous thought it is now recognized that thyroxine crosses the placenta in sufficient quantities to protect the hypothyroid fetus from the consequences of thyroxine deficiency until detection by neonatal screening programs after birth *(481)*. Immediately following delivery there is a surge of TSH in the neonate during the first 24 hours, presumably in response to cooling. In the term newborn, circulating thyroxine increases 2 to 3-fold higher than adult levels during the first 48 hours then stabilizes and returns to cord levels by 5-6 days. The response in the premature infant is less marked and inversely related to immaturity. Both circulating T4 and TSH concentrations remain above adult levels throughout infancy and decrease during childhood to reach adult concentrations after puberty (Table 3) *(42)*.

1. Criteria needed for CH screening laboratories

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 maternity wards 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. Laboratories should follow up and tightly control the rates of false negative and false positive results. A referral pediatric endocrinologist should be available for follow up testing to ensure that the correct diagnosis and treatment is achieved.

2. Screening Strategies

Screening methods should have low costs and be easy to perform.
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 approaches 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 3).

Guideline 65. For Laboratories Performing Thyroid Testing of Neonates and Infants

- Thyroid test results in neonates must be reported with gestation and age-specific reference intervals, respectively.
- Each Laboratory should establish its own cut off levels according to the method used.

(a) Primary TT4 with reflex TSH measurement

Most North American screening programs use an initial TT4 measurement, with 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, test kits for TT4 were more reliable, the screening was performed earlier in the neonatal 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 *(482)*. The TT4-first screening approach has some advantages, particularly in programs where samples need to be collected early in the neonatal period. TT4 is also less influenced by the TSH surge that follows the cutting of the umbilical cord and lasts for the first 24 hours. Both of these factors suggest that TT4 screening will result in fewer false-positives when early (< 24 hour) testing is necessary. 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 rise in TSH. 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 *(483-485)*. TBG deficiency has no clinical consequence such that the treatment of this condition 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 *(482)*.

(b) 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 these infants have an increased frequency of high blood spot TSH levels. TSH screening makes it possible to monitor the iodine supply in the newborn population, especially since many European countries are still iodine deficient *(486)*. Additionally, there is now little difference in cost between TSH and TT4 test reagents.

The TSH cutoff level used for recall varies between programs. In one program a two-tiered approach was adopted *(487)*. 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 *(482)*. 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.

Guideline 66. Pre-term and Early Discharge of Neonates

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 rise in TSH, 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.

3. 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 increase the whole blood units by 30-50% to approximate the serum units.

Guideline 67. Countries with Iodine Deficiency

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

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-2 C]. 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.

Other automated systems that do not use a microtitre plate format however, can be successfully used for blood spot TSH assays. These usually require 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 within 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 unique patient 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.

Guideline 68. Performance Criteria for Blood Spot TSH Screening of Newborns

- Functional sensitivity of the TSH assay should be at least 1.0 mIU/L.
- Between run coefficient of variation should ideally be <10% and not more than 20%.
- Internal quality control samples should cover the reportable range and must be included in every run.
- At least one of the quality controls materials should be supplied by a different manufacturer from the TSH reagent manufacturer.
- Standards should be made in blood, i.e. be identical to specimens tested.
- Use the same filter paper for the samples, standards and controls.
- Participation in National and/or International external quality control programs is essential (see Appendix B).

4. Sample Collection

The technique for collecting blood samples by heel stick on filter-paper is of the utmost importance. Only filter-paper that meets NCCLS standards should be used [“Blood on Filter Paper For Neonatal Screening Programs” Approved Standard – Third Edition. LA4-A3, Vol 17 Nº 16, October 1997. National Committee for Clinical Laboratory Standards] *(488)*. This requires a continuous training program, well-written protocols and establishing criteria for adequacy of specimen collection.

Guideline 69. TSH Cut-off 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 the test on a second blood spot
- Initial blood spot TSH >20 mIU/L whole blood units – recall infant for evaluation by pediatric endocrinologist

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 practice. In many of the 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 the time the umbilical cord is cut. In the majority of infants the increase in TSH returns to normal within 24 hr, but in some infants, TSH 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 rise in TSH, perhaps due to

immaturity of the pituitary-thyroid feedback mechanism *(489)*.

5. Confirmation Testing

Measurements performed on filter paper eluates 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) *(301,490)*.

Guideline 70. 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.

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).

The aim of CH screening programs is to detect CH and expedite thyroid hormone replacement therapy as early as possible (within 14 days). However, additional tests to determine the etiology of CH should also be carried in order to determine whether the condition is transient, permanent or due to genetic causes (needed for genetic counseling) (Table 11). 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 indicated since the presence of blocking antibody in the neonate inhibits the actions of TSH resulting in a lowered FT4 concentration *(301,491)*. 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 *(492)*.

In many cases, at the time of the diagnosis of CH, it is impossible to determine whether the hypothyroidism is permanent or transient. Clues that are associated with transient conditions include a TSH level below 100 mIU/L, male sex, pseudohypoparathyroidism, prematurity, iodine exposure, or dopamine administration *(484)*. In such instances it is best to manage the patient as if he/she has permanent hypothyroidism *(493)*. If the diagnosis has not become apparent by the age of 2 years, L-T4 therapy should be discontinued for one month and the infant monitored with serial determinations of FT4 and TSH.

Guideline 71. 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 only the infant and in some cases the thyroid status of the mother is also investigated using serum FT4, TSH and TPOAb testing.
- Check the mother for TSH receptor blocking antibodies.
- Use method and age-specific reference intervals for TT4 and TSH testing of neonates.

6. 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 a pediatric endocrinologist 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 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 via trans-placental passage *(301,491)*.

A perchlorate discharge test response of >15% suggests an inborn error of metabolism. Specialist centers offer tests that include urinary iodine measurement, tests for a specific gene mutation such as the sodium/iodine symporter, TPO or thyroglobulin *(494)*. More commonly, defects in the oxidation and organification of 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.

Guideline 72. Detection of Transient Congenital Hypothyroidism (CH)

Since CH may be transient as a 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.

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)

7. Long-term Monitoring of Congenital Hypothyroid Patients

Most CH infants and children have normal pituitary-thyroid negative feedback control although T4 and TSH thresholds are set higher (Table 3) *(43)*. 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 (Table 3) *(40)*. In the United States, the L-T4 replacement dose is adjusted to bring the TSH below 20 mIU/L and produce a circulating T4 level in the upper half of the reference range (>10 µg/dl/129 nmol/L) within the first two weeks after starting treatment. Infants are usually maintained on a dose of 10-15 µg L-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 over-treatment *(493)*.

A minority of infants treated for CH 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 *(43)*. In rare cases, transient hypothyroidism may result from the transplacental passage of TSH-receptor blocking antibodies *(282,301)*. It is recommended that the diagnosis of CH be re-evaluated in all cases after 2 years of age. Specifically, after a basal FT4/TSH measurement is made, L-T4 treatment is discontinued and FT4/TSH re-tested after 2 weeks and again after 3 weeks. Almost 100% of children with true CH have clearly elevated TSH after 2 weeks off treatment.

8. 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.

Guideline 73. 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 (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, it is important to 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.

9. 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 programs, 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 maintenance and service
  - internal quality control of filter paper results
  - national and international external quality control participation
- *Post-analytical*
  - co-ordination of follow-up of abnormal tests
  - confirmatory testing where applicable
  - appropriate storage and archiving of specimens for later testing

10. Annual Reporting

This should include items identified by audit and be a comprehensive report of CH screening over the previous twelve months. The report should monitor the distribution of increased blood spot TSH concentrations, and there should be a system to report all cases of true CH and record cases of transient elevations in TSH. The system could also provide information on any missed cases. An efficient screening program depends on a close collaboration between the screening laboratory, pediatricians, endocrinologists and all concerned in the screening process.

Guideline 74. For Physicians

- Repeat tests when the clinical picture conflicts with the laboratory test results!
- Potential pitfalls in screening are ubiquitous and no laboratory is immune!
- Maintain a high degree of vigilance. Despite all safeguards and automated systems, screening programs will 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.