APPENDICES

APPENDIX-1: ASSESSMENT OF GOITER (WHO, 2007)

The size of the thyroid gland changes inversely in response to alterations in iodine intake, with a lag interval that varies from a few months to several years, depending on many factors. These include the severity and duration of iodine deficiency, the type and effectiveness of iodine supplementation, age, sex, and possible additional goitrogenic factors. The term “goitre” refers to a thyroid gland that is enlarged. The statement that “a thyroid gland each of whose lobes have a volume greater than the terminal phalanges of the thumb of the person examined will be considered goitrous” is empiric, but has been used in most epidemiological studies of endemic goitre and is still recommended. Palpation of the thyroid is particularly useful in assessing goitre prevalence before the introduction of any intervention to control IDD. **Neonates:** It is neither feasible nor practical to assess goitre among neonates, whether by palpation or ultrasound. Performance is poor.

**School-age children (6–12 years):** This is the preferred group, as it is usually easily accessible. However, the highest prevalence of goitre occurs during puberty and childbearing age. Some studies have focused on children 8 to 10 years of age. There is a practical reason for not measuring very young age groups. The smaller the child, the smaller the thyroid, and the more difficult it is to perform palpation. If the proportion of children attending school is low, schoolchildren may not be representative. In these cases, spot surveys should be conducted among those who attend school and those who do not, to ascertain if there is any significant difference between the two. Alternatively, children can be surveyed in households.

**Adults:** Pregnant and lactating women are of particular concern. Pregnant women are a prime target group for IDD control activities because they are especially sensitive to even marginal iodine deficiency. Often they are relatively accessible given their participation in antenatal clinics.

**Adolescent girls:** Adolescent girls and women of childbearing age (15 to 44 years) may be surveyed in households. The specificity and sensitivity of palpation are low in grades 0 and 1 due to a high inter-observer variation. As demonstrated by studies of experienced examiners, misclassification can be high.

**Technique**

The subject to be examined stands in front of the examiner, who looks carefully at the neck for any sign of visible thyroid enlargement. The subject is then asked to look up and thereby to fully extend the neck. This pushes the thyroid forward and makes any enlargement more obvious. Finally, the examiner palpates the thyroid by gently sliding their own thumb along the side of the trachea (wind-pipe) between the cricoid cartilage and the top of the sternum. Both sides of the trachea are checked. The size and consistency of the thyroid gland are carefully noted.
If necessary, the subject is asked to swallow (e.g. some water) when being examined – the thyroid moves up on swallowing. The size of each lobe of the thyroid is compared to the size of the tip (terminal phalanx) of the thumb of the subject being examined.¹

**Simplified Classification of Goiter by Palpation (WHO, 2007)**

<table>
<thead>
<tr>
<th>Grade 0</th>
<th>No palpable or visible goiter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>A goiter that is palpable but not visible when the neck is in the normal position (i.e., the thyroid is not visibly enlarged) Thyroid nodules in a thyroid which is otherwise not enlarged fall into this category</td>
</tr>
<tr>
<td>Grade 2</td>
<td>A swelling in the neck that is clearly visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated</td>
</tr>
</tbody>
</table>

The specificity and sensitivity of palpation are low in grades 0 and 1 due to a high inter-observer variation. As demonstrated by studies of experienced examiners, misclassification can be high.

**Interpretation**

The terms mild, moderate, and severe are relative and should be interpreted in context with information from other indicators. It is recommended that a TGR (number with goitres of grades 1 and 2 divided by total examined) of 5% or more in schoolchildren 6 to 12 years of age be used to signal the presence of a public health problem. This recommendation is based on the observation that in normal, iodine-replete populations, the prevalence of goitre should be quite low. The cut-off point of 5% allows both for some margin of error of goitre assessment, and for goitre that may occur in iodine-replete populations due to other causes such as goitrogens and autoimmune thyroid diseases.

**Epidemiological criteria for assessing the severity of IDD based on the prevalence of goiter in school-age children (WHO, 2007)**

<table>
<thead>
<tr>
<th>Degrees of IDD. Expressed as percentage of the total of the number of children surveyed</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Goiter Rate (TGR)</td>
<td>0.0-4.9%</td>
<td>5.0-19.9%</td>
<td>20.0-29.9%</td>
<td>&gt;30%</td>
</tr>
</tbody>
</table>

*goiter prevalence responds slowly to changes in iodine intake
APPENDIX-2: DETERMINATION OF URINARY IODINE CONCENTRATION
(Dunn et al, 1993)

Methodology for collection
The Casual urine sample was collected from pregnant mothers, school age children and adolescent girl enrolled in the study. The subjects were provided with wide mouthed screw capped plastic bottles for collection of the urine sample. A drop of toluene was added to each sample to prevent bacterial growth and minimize bad odor.

Methodology for Analysis of Urinary Iodine Concentration Level

The Urinary Iodine Concentration (UIC) level was determined by the wet digestion method (Dunn et al 1993). The results were expressed as μg/L.

Principle:
Urine is digested with chloric acid under mild conditions and iodine is determined manually by its catalytic role in the reduction of ceric ammonium sulfate in the presence of arsenious acid. As the reduction proceeds the intensity of color decreases and this can be readily measured in a spectrophotometer at 420 nm. The method is fast and inexpensive, and the digestion is less harsh than some other methods. This method can measure urinary iodine concentrations in the range of 0-150 mcg/liter but can be extended further to cover a wider range of values.

Equipment and Chemicals:

Equipment:
Oven with fan exhaust, Vented fume hood on oven for perchloric acid escape, UV spectrophotometer, Thermometer, Timer (stop watch reliable to 5 second), Test tubes (15 ml), Funnel (56 x 100 mm), Reagent flasks, Bottles, Pipettes, Whatman no 1 filter paper, Laboratory balance.

Chemicals (analytical grade AR /GR)
KCLO₃ (potassium chlorate), HClO₄ (perchloric acid, 70%), As₂O₃ (arsenic trioxide), NaOH (sodium hydroxide), H₂SO₄ (sulfuric acid), Ce(NH₄)₄(SO₄)₂·H₂O (ceric ammonium sulfate), KI0₃ (potassium iodate), HCl (Hydrochloric Acid), Double distilled water (free of iodine and other contaminants)

Preparation of Reagents:

Chloric acid solution:
In a 2000ml Erlenmeyer flask, 500g potassium chlorate was dissolved in 910ml hot double distilled water until the soluble state (normally a little amount remain undissolved). 375 ml of 70% perchloric acid was added dropwise (approx. 15 ml/min) while stirring constantly. This preparation was carried out in a vented fume hood as it produces toxic fumes. Subsequently, the solution was kept in a
APPENDICES

freezer of refrigerator overnight for better separation. The next day it was filtered through a filter paper, (Whatman # 1) and stored in a refrigerator at 40C.

**Arsenious Acid Solution:**

0.986 g arsenic trioxide was taken in a 1000 ml volumetric flask and was dissolved in 10 ml of 0.5 N hot sodium hydroxide. This solution was transferred into 750 ml chilled double distilled water. Then 20 ml concentrated HCL and 39.6 ml conc. Sulphuric acid (98%) was added dropwise with constant mixing. The solution was stored in amber color bottle at room temperature. (The solution is stable for months).

**Sulphuric Acid Solution (3.5N H2SO4):**

97 ml concentrated sulfuric acid (98%) was added dropwise into 800 ml chilled double distilled water (carefully as this generates heat) and final volume was made up to 1 liter with double distilled water.

**Ceric ammonium sulfate solution:**

48 g Ceric ammonium sulfate was dissolved in 1 liter of 3.5N H2SO4. This was stored in a amber color bottle at room temperature. (The solution is stable for months).

**Stock Iodine Standard (1mg/ml):**

168.5 mg KIO3 was dissolved in double distilled water to make a final volume of 100 ml. This was stored in a amber color bottle (This solution is stable for months).

**Dilute Iodine Standard (1µg/ml):**

Take 100 ul of Stock Iodine Standard and make a volume to 100 ml with double distilled water.

**Working Iodine Standard:**

Make the following serial dilutions from diluted Iodine Standard (1µg/ml) into volumetric flasks (10 ml) with double distilled water (diluent). These dilutions are made freshly.

<table>
<thead>
<tr>
<th>ug/dl</th>
<th>Dilution factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 µg</td>
<td>0.5 ml of 1 µg/ml standard + 9.5 ml diluent</td>
</tr>
<tr>
<td>10 µg</td>
<td>1.0 ml of 1 µg/ml standard + 9.0 ml diluent</td>
</tr>
<tr>
<td>15 µg</td>
<td>1.5 ml of 1 µg/ml standard + 8.5 ml diluent</td>
</tr>
<tr>
<td>20 µg</td>
<td>2.0 ml of 1 µg/ml standard + 8.0 ml diluent</td>
</tr>
</tbody>
</table>

**Procedure:**

**Step I:** The urine sample was shaken to evenly suspend any sediment. 250 µl of each urine sample was pipette into a 15x100 mm test tube. Iodine standards were prepared from the 1 µg/ml stock iodine solution. The iodine standards corresponding to 0/5/10/15 and 20 µg/dl were prepared.
Step II: 50 micro liter of chloric acid solution was added to each tube (samples, blank, internal quality control sample, standards) and mixed gently. All tubes were placed in the oven at 1100C-1200C for 75 minutes (with a fume hood for the trapping of perchloric acid). There will be very little volume change during heating. Some samples may be faintly yellow. All the tubes were cooled at room temperature for 15 minutes. Then, the decreased volume was adjusted with double distilled water to their original volume (1.0 ml) and vortexed.

Step III: 3.5 ml of Arsenious Acid was added to each test tube and after mixing all test tubes were kept for 15 minutes at room temperature.

Step IV: 350 microliter of ceric ammonium sulfate solution was added at a fixed interval of time to each tube and quickly mixed with help of a vortex. A stopwatch was used to keep a constant interval between additions to successive tubes, (30 seconds was a convenient interval). Exactly 20 minutes after addition of ceric ammonium sulfate to the first tube, the reduction was read spectrophotometrically at 420 nm against the reagent blank at the same interval. (Successive tubes were arranged in such a manner that the interval between the time of addition of ceric ammonium sulfate and the time of the reading was the exactly 20 minutes for all samples, standards and blanks).

Calculation of results:
The exact value of urine sample's iodine was calculated as follows:

a) The average absorbance value for each set of reference standard, control and samples was calculated.

b) A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration mcg/dl on linear graph paper, with absorbance on the vertical (Y) axis and concentration (micro g / dl) on the horizontal (X) axis.

Precautions:
i. Since the digestion procedure has no specific end point, it is essential to run blanks and Iodine standards with each assay to allow for variations in heating time, etc.

ii. The exact temperature, heating time and cooling time can vary. However, within each assay, the interval between the time of addition of ceric ammonium sulfate and the time of the reading must be the same for all samples, standards, and blanks.

iii. In this procedure it is convenient to run 60 sample’s tubes per assay of which 5 are standards (at concentrations of 0/5/10/15and 20 mcg/dl).

iv. Perchloric acid fumes can be toxic and the complex generated may be harmful, particularly if allowed to dry in a ventilation system. The recommended method releases much less perchloric acid than other digestion methods.

v. The exact time and temperature is not critical as long as all tubes are heated the same way.

vi. 1.68mg KIO3 contains 1 mg iodine KIO3 is preferred over KI because it is more stable.

vii. Test tubes can be reused if they are carefully washed to eliminate any iodine contamination.
viii. Separate pipettes should be used for all the test tubes and also pipettes used for preparation of each standard solution should be kept separately and not be mixed with the general pool of glasswares. They should be kept separately for all times to avoid contamination.

**Methodology for Internal Quality Control adopted**

The Internal Quality Control (IQC) methodology was adopted during UIC analysis. A pooled urine sample was prepared. This was considered the IQC sample, and it was stored in a refrigerator. It was analyzed twenty-five times with standards and blank in duplicate. The mean UIC and standard deviation of this pooled sample were calculated. The 95% confidence interval for the mean UIC of the IQC sample was then calculated. This was used as the operating control range. The methodology adopted was as follows:

**Sample mean (X) ±2SD**

\[ X \pm 2SD = \text{the lower confidence limit or lower concentration value (LCV)} \]

\[ X + 2SD = \text{the upper confidence limit or upper concentration value (UCV)} \]

The operating control range for the IQC sample was between LCV and UCV. A regular linear graph paper was utilized to prepare Levey–Jennings plots. The mean UIC of the IQC sample was plotted as a continuous horizontal line on the y-axis. The LCV was plotted below the mean line on the y-axis scale and the UCV was plotted above the mean line on the y-axis scale. The x-axis was used to plot the date on which the IQC sample was analyzed. This chart was used to plot the date-specific analysis. The pooled urine sample was analyzed with every batch of samples submitted for UIC estimation. The UIC was obtained for the IQC sample analyzed with each batch. If the UIC value of the IQC sample was between the two limit lines of LCV and UCV, then the UIC test was deemed in control and all results were accepted. If any value of the IQC sample was plotted outside the two limit lines of LCV and UCV, then the test was considered as out of control and the entire batch was repeated.

**LEVY JENNING PLOT**

<table>
<thead>
<tr>
<th>Date</th>
<th>16</th>
<th>17</th>
<th>20</th>
<th>22</th>
</tr>
</thead>
</table>

**Epidemiological criteria for assessing iodine nutrition based on median urinary iodine concentrations of school-age children (6-12 years) and adolescent girls**

<table>
<thead>
<tr>
<th>Median Urinary Iodine Concentration (µg/L)</th>
<th>Iodine Intake</th>
<th>Iodine Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>Insufficient</td>
<td>Severe iodine deficiency</td>
</tr>
<tr>
<td>Population group</td>
<td>Median Urinary Iodine Concentration (g/l)</td>
<td>Iodine intake</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>&lt;150</td>
<td>Insufficient</td>
</tr>
<tr>
<td></td>
<td>150-249</td>
<td>Adequate</td>
</tr>
<tr>
<td></td>
<td>250-499</td>
<td>Above requirements</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>Excessive</td>
</tr>
</tbody>
</table>

Table: Epidemiological criteria for assessing iodine nutrition based on the median or range in urinary iodine concentrations of pregnant women (WHO, 2007)
APPENDIX- 3: DETERMINATION OF THYROID STIMULATING HORMONE (Slazyk and Hannon, 1993; Westgard and Klee, 1999)

Thyroid stimulating hormone is utilized as an indicator of iodine deficiency in neonates. Consent of each subject will be taken on prescribed form. A proforma for general information was administered to each family and subject.

Two blood constituents, TSH and Tg, can serve as surveillance indicators. In a population survey, blood spots on filter paper or serum samples can be used to measure TSH and/or Tg.

Determining serum concentrations of the thyroid hormones, thyroxin (T4) and triiodothyronine (T3), is usually not recommended for monitoring iodine nutrition, because these tests are more cumbersome, more expensive, and less sensitive indicators. In iodine deficiency, the serum T4 is typically lower and the serum T3 higher than in normal populations. However, the overlap is large enough to make these tests impractical for ordinary epidemiological purposes.

**Thyroid stimulating hormone (TSH)**

**Biological features**

The pituitary secretes TSH in response to circulating levels of T4. Serum TSH rises when serum T4 concentrations are low, and falls when they are high. Iodine deficiency lowers circulating T4 and raises the serum TSH, so iodine-deficient populations generally have higher serum TSH concentrations than do iodine-sufficient groups. However, the difference is not great and much overlap occurs between individual TSH values. Therefore, the blood TSH concentration in school-age children and adults is not a practical marker for iodine deficiency, and its routine use in school-based surveys is not recommended. In contrast, TSH in neonates is a valuable indicator for iodine deficiency. The neonatal thyroid has a low iodine content compared to that of the adult, and hence iodine turnover is much higher. This high turnover, which is exaggerated in iodine deficiency, requires increased stimulation by TSH. Hence, TSH levels are increased in iodine-deficient populations for the first few weeks of life – this phenomenon is called transient hyperthyrotopinemia. The prevalence of neonates with elevated TSH levels is therefore a valuable indicator of the severity of iodine deficiency in a given population. It has the additional advantage of highlighting the fact that iodine deficiency directly affects the developing brain. In iodine-sufficient populations, about one in 4000 neonates has congenital hypothyroidism, usually because of thyroid dysplasia. Prompt correction with thyroid hormone is essential to avoid permanent mental retardation. Thyroid hormone affects proper development of the central nervous system, particularly its myelination; a process that is very active in the perinatal period. To detect congenital hypothyroidism and initiate rapid treatment, most developed countries conduct universal screening of neonates with bloodspot TSH taken on filter papers, or occasionally with blood spot T4 followed by TSH. While screening in developed countries is directed at detecting neonates with TSH elevations which are 20mIU/l whole blood or higher, the availability of TSH assays sensitive to 5mIU/l permits detection of mild elevations above normal. This permits detection of transient hyperthyrotopinemia. To be broadly
applicable in a population, the screening must be universal, and not omit children born in remote or impoverished areas. For countries and regions that already have a system of universal neonatal screening with a sensitive TSH assay in place, the data can be examined and transient iodine deficiency recognized, usually without further surveying.

**Feasibility**

Serum TSH is widely used in the field of thyroidology as a sensitive marker for both hypothyroidism and hyperthyroidism. Methods for determining TSH concentrations, from either dried whole blood spots on filter paper or from serum, are well established and widely available. Typically, a few drops of whole blood are collected on filter paper from the cord or by prick of the heel or other site. It is essential that sterile equipment be used, either lancets for blood spot collection or needles and syringes for collecting whole blood from which the serum is separated. Standard procedures for handling blood products or objects contaminated with blood should be followed. The risk of contracting HIV or hepatitis infection from dried blood spots is extremely low. Some experimental data suggest normal values for cord blood are higher than those for heel prick blood. Blood spots, once dried, are stable. They can be stored in a plastic bag and transported even through normal postal systems and are usually stable for up to six weeks.

It must be emphasized that the primary purpose of screening programmes is to detect congenital hypothyroidism, and its use as an indicator of iodine nutrition will be a spin-off. Hence, the only additional cost will be for data analysis. It is not recommended that a neonatal screening programme be set up solely to assess community iodine deficiency. Less expensive means for obtaining this information exist. TSH screening is inappropriate for developing countries where health budgets are low. In such countries, mortality among children under five is high due to nutritional deficiencies and infectious diseases, and screening programmes for congenital hypothyroidism are not cost effective. Performance A variety of kits for measuring TSH are available commercially in developed countries. Most have been carefully standardized, and perform adequately. Assays that utilize monoclonal antibodies, which can detect TSH as low as 5 MIU/l in whole blood spots, are more useful for recognizing iodine deficiency.

**Interpretation**

Permanent sporadic congenital hypothyroidism, with extremely elevated neonatal TSH, occurs in approximately one of 4000 births in iodine-sufficient countries. Other than infrequent cases of goitrogen exposure, iodine deficiency is the only significant factor to increase this incidence. The increase in the number of neonates with moderately elevated TSH concentrations (above 5 MIU/l whole blood) is proportional to the degree of iodine deficiency during pregnancy. It may be higher than 40% in severe endemic areas. When a sensitive TSH assay is used on samples collected three to four days after birth, a <3% frequency of TSH values >5 mIU/l indicates iodine sufficiency in a population. Interpretation is complicated when antiseptics containing beta-iodine, such as povidone iodine (Betadine TM), are used for cleaning the perineum prior to delivery or even the umbilical area.
of the baby. Beta-iodine increases TSH levels in the neonate in both cord blood and heel prick specimens.

Prior to collecting of the cord blood the first step would be to identify the subject and recording of particulars.

**Precautions**

1. Prior to blood sampling it should be ascertained whether the filter paper is properly marked with personal details such as Name, Age, Date of collection i.e. Name of the mother, Village, Sub center or PHC etc.
2. Interpretation of TSH levels is difficult when iodine-containing antiseptics are used during delivery. Hence, it was ensured that no iodine containing antiseptics are applied prior to cutting of the cord.
3. Samples should be collected carefully and handle all blood or serum specimens as if capable of transmitting hepatitis virus and/or HIV. Disposable gloves must be wearing while running the test.
4. There should be a homogenous spread of the blood spot.
5. A dry clot formation should be avoided on filter paper stip.
6. Use ball point pen to write down address for identification.
7. Filter paper should be kept in a dry place and in dirt free environment.
8. The filter paper and envelop should be kept in a dry place at room temperature preferably in a polythene envelop to avoid dust. The Identification of data of newborns whose blood samples have been collected should be noted in a register with the health functionary/institution.

**Method and Principle of TSH estimation**

The cord was squeezed gently immediately after it is cut so as to obtain a large drop of whole blood. The blood drop was wiped off and a second similar whole blood drop was obtained. This drop was then be applied to the center of one of the circles marked on the special filter supplied by the nodal laboratory. Note: Each drop was applied to the back of the paper so as to permeate through to the front side of the paper. The blood spots are stable for a period up to 6 weeks but was transported soon. The filter paper is 11 cm long and 7 cm broad. A red line on filter paper divides it in to two parts i.e. i). Filter Paper Part A of 2.5 cm and ii). Filter Paper Part B of 8.5 cm. The identification data of child i.e. name of the mother, village, sub-centre, PHC and date of collection was written on part A of the filter paper. The blood spot was collected on part B. The filter paper and envelop was kept in a dry place at room temperature preferably in a polythene envelop to avoid dust.

The filter paper was then allowed to air dry at room temperature for 4 hours. The dried card was then be placed in a paper envelope and placed in another 2nd envelope for transportation.

The samples collected on the filter paper was sent to the nodal laboratory New Delhi for estimation of TSH. The services of this laboratory have been chosen because it carried out TSH estimation by filter paper method in New Delhi over 10 years and it is NABL accredited. The methodology of TSH
estimation at the nodal laboratory is based on the principle of Micro ELISA. The identification of data of newborn whose blood was collected was noted in register with the health functionaries/institutions.

1. Collection Time

Cord blood samples was collected after the completion of fetal delivery and preferably before the delivery of placenta.

2. Collection Method

a. About 2-3 drops of umbilical cord blood are made to fall freely on the filter paper.
b. Blood spot was spread over an area more than of one rupee coin size i.e. 3 cm in diameter.

3. Drying of blood spot on filter paper

a. It was dried in shadow at room temperature-away from direct sunlight and heat
b. No. Side of the wet blood spot should touch any other surface.
c. Filter paper was placed tilted so that the body of paper remains free and paper rest or its edges.
d. It takes about 15 minutes to 1 hour to dry depending on weather conditions.

Data was also collected on gender, birth weight and gestational age of the neonates.

Principle:

The Neonatal TSH Screening Assay is a based on sandwich ELISA method. Dry blood spot eluted in anti-TSH antibodies coated micro wells and incubated with peroxidase labeled anti-TSH monoclonal antibodies. After washing, the unbound antibodies are washed off and bound conjugate remains in micro well. These bound conjugates further react with substrate 3,3',5,5' Tetramethylbenzidine (TMB) and produces a colour product. Concentration of TSH is directly proportional to colour produced. Absorbance is read at 450 nm and a value of TSH is expressed in the units μIU/ml of blood.

In order to measure the concentration of TSH in the test sample, the calibration standards and controls are used. The calibration standards and controls are assayed for producing a standard curve of TSH by O.D versus TSH concentration (μ IU/mL). Therefore, by comparing the O.D of the test samples to this standard curve, the concentration of the TSH can be determined.

Reagents:

1. Monoclonal anti-TSH antibody coated microplate wells
2. Enzyme Conjugate reagent
3. TSH blood spot standards cards
4. TSH blood spot control cards
5. Substrate (TMB)
6. Stop Solution
7. Wash Solution

**Sample acceptance criteria**

1. Blood samples collected on Guthrie card in pre-marked area.

**Sample rejection criteria**

1. Blood samples received on other filter papers rather than Guthrie cards.
2. Repeatedly taking of blood drops on same point on pre-marked circle on Guthrie card.
3. Cards showing presence of dust particles/ moisture on blood drops collected in pre-marked circle.
4. Specimen showing evidence of pooling, clotting, or incomplete saturation.
5. Specimen showing fungal growth.
6. Blood collected within three weeks of blood transfusion done to the baby.

**Procedure**

1. Punch (one) 1/8-inch diameter disk from each Standard, Control or specimen into an antibody coated microplate from kit. Add 100µL of Conjugate Reagent in all wells containing Standards, Controls and patient specimens.
2. Place the microplate on shaker and shake at room temperature (18-25°C) for 30 minutes at 600 rpm followed by 3 hours at 350 rpm. Ensure all punched discs are immersed in the Conjugate Reagent throughout.
3. After incubation, decant the contents of the wells, including the punched discs into a suitable waste container.
4. Wash the microplate 5 times by using a plate washer or manually, using a minimum of 300µL wash solution per well. After the last wash, firmly tap the plate on absorbent paper to remove any residual wash. Add 100µL of Substrate reagent (TMB) to all wells. Seal the wells and incubate the plate at room temperature (18-25°C) for 30 minutes in the dark. Add 100µL of Stop Solution to all wells. Gently mix the plate. After addition of Stop Solution read absorbance at 450nm within 30 minutes.
Picture 7: Steps in Cord Blood Sample Collection (For Iodine Status in neonates)

<table>
<thead>
<tr>
<th>Steps</th>
<th>Pictorial Representation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clamping of cord</td>
<td>![Clamping of cord]</td>
</tr>
<tr>
<td>Cleaning of cord</td>
<td>![Cleaning of cord]</td>
</tr>
<tr>
<td>Withdraw Blood from Umbilical Cord</td>
<td>![Withdraw Blood]</td>
</tr>
<tr>
<td>Putting Drop of Blood on Filter Paper</td>
<td>![Putting Drop]</td>
</tr>
<tr>
<td>Drying of Blood Sample</td>
<td>![Drying of Blood]</td>
</tr>
<tr>
<td>Taking identification details of the patient</td>
<td>![Taking ID]</td>
</tr>
<tr>
<td>After 4 hours of Drying, Place Filter Paper in paper envelop</td>
<td>![After Drying]</td>
</tr>
<tr>
<td>Place the Paper Envelop in Auto Seal LDPE Pouch</td>
<td>![Place Envelop]</td>
</tr>
</tbody>
</table>
### Steps in Collection of Blood Sample on Filter Paper by Heel Prick Method (For Neonatal Hypothyroidism)

<table>
<thead>
<tr>
<th>Steps</th>
<th>Pictorial Representation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatched/Lined area indicate safe area for prick site</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>Cleansing of prick site with sterile alcohol swab</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Pricking of Heel with Sterile Lancet</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Gently touch filter paper to large blood drop</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Drying of Filter Paper in Paper Rack</td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td>After 4 hours of Drying, Place Filter Paper in paper envelop</td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Place the Paper Envelop in Auto Seal LDPE Pouch</td>
<td><img src="image7.png" alt="Image" /></td>
</tr>
</tbody>
</table>
APPENDIX -4: IODINE CONTENT OF THE SALT (Karmakar et al, 1986)

Determination of iodine from Iodized Salt
The iodine content of salt was estimated by the iodometric titration method (Karmarker et al 1986). Salt samples with iodine content less than 15 ppm were considered as having inadequate iodine concentration.

Principle
The iodine content of salt is estimated by titration procedure known as iodometric titration. Free iodine reacts with sodium thiosulphate solution to give a light yellow colour complex. This colour complex combines with soluble chemical starch which indicates the presence of sodium iodide.

Equipment and Chemicals

Equipment
i Laboratory balance
ii Gas burner or kerosene stove
iii Reagent bottles with stoppers - 250ml, 500 ml and 1000 ml
iv Measuring cylinder with stopper - 50 ml
v Wash bottle - 500ml
vi Glass stirring rod
vii Conical flask with stopper - 100ml
viii Glass or plastic funnel
ix Burette - 10 ml
x Burette stand
xi Clock or watch
xii A closed box, cupboard or drawer to keep the conical flask

Chemicals
i Sodium thiosulphate, (Na2S2O3 5H2O)
ii Concentrated sulphuric acid,(H2SO4)
iii Potassium iodide,(KI)
iv Soluble chemical starch

All the chemicals used were analytical grade and double distilled water was used which was free of iodine and other contaminants.

Preparation of reagents

a) Sodium thiosulphate (0.005 M): 1.24 g sodium thiosulphate was dissolved in 1 litre hot double distilled water. This volume was sufficient for testing 200 salt samples. The solution was stored in a cool, dark place.

b) Sulphuric acid (2N H2SO4) : 5.56 ml concentrated sulfuric acid was added drop wise into 90 ml of chilled double distilled water and the final volume was made upto 100 ml with double distilled water. This volume was sufficient for testing 100 salt samples.
CAUTION: To avoid violent and dangerous reaction always add the acid to water, never add water to acid.

c) Potassium iodide (KI): 100 g potassium iodide was dissolved in 1000 ml double distilled water. This volume was sufficient for testing 200 salt samples. This was stored in a refrigerator.

d) Saturated Salt Solution: 100 ml double distilled water was taken in a conical flask and sodium chloride was added until the salt was insoluble. This solution was heated till the NaCl crystals formed on the sides of the vessels. After cooling down the saturated salt solution at room temperature the supernatant was transferred in a clean bottle.

e) Soluble Chemical Starch: 10g soluble chemical starch was dissolved in 100 ml hot double distilled water. Then the volume was adjusted upto 1000 ml with saturated sodium chloride solution. This was sufficient for testing 200 salt samples. The starch solution was prepared fresh every day.

Procedure

10 g of salt was dissolved in 50 ml double distilled water. Then 1.0 ml of 2 N sulphuric acid and 5.0 ml of 10% potassium iodide was added. On shaking, the solution turned a yellow colour. This was incubated at room temperature in the dark for 10 minutes. During the incubation period sodium thiosulphate solution was poured into the burette and the level of solution was adjusted to zero. The samples were removed from the dark and titrated against the sodium thiosulphate solution until it turned into a very light yellow colour (pale yellow). Subsequently, a few drops (1-5ml) of 1% starch solution were added. The solution turned a deep purple colour. Finally, it was titrated until the solution became colorless and the final reading was observed.

Calculation of Results

From table given below, the iodine content of the sample in parts per million was calculated accordingly. The results were recorded in a register, under the following parameters

- Date of collection
- Date of testing
- Sample number
- The level of iodine in the sample.

Precautions

i Adding sulphuric acid to a solution of iodated salt liberates iodine, which is titrated with sodium thiosulphate. Potassium iodide (KI) is used because of the low solubility of iodine and more iodine is dissolved in order to maintain the equilibrium.

ii Potassium iodide solution is added to keep the iodine in the dissolved state.
iii The reaction mixture should be kept in the dark for 10 minutes before titration because light accelerates a side reaction in which iodide ions are oxidized to iodine by atmospheric oxygen.

iv Starch solution must be prepared freshly.

v Starch is used as an external indicator.

vi The starch solution must be added near the end of the titration, when very little amount of iodine is left and the solution has a faint-yellow colour. If starch is added earlier, the iodine-starch complex becomes very strong and reacts too slowly with sodium thiosulphate, resulting in false high readings.

vii The titration should be done in a comfortably cool room because iodine is volatile and the sensitivity of the starch indicator diminishes as the temperature rises.

**Method for Internal Quality Control adopted**

A known positive iodized salt sample was obtained and by performing multiple analyses on this positive salt sample, a concentration range was established and used for internal quality control purposes. Once a sufficient number of these test results were obtained, the samples mean concentration (X) in ppm and standard deviation (SD) was also calculated. The 95% confidence interval was then calculated and used as the operating control range, as follows:

Sample Mean (X) ± 2(SD)

The X - 2(SD) = the lower confidence limit (L)

X + 2(SD) = the upper confidence limit (U)

The operating control range is between L and U.

This internal quality control sample was run with every batch of the test samples and the internal quality control was successful when the results of this sample were between the lower and upper limits (i.e., the L-U range), for 95% of test results. If the results were outside the established range they were considered potentially suspicious and the whole batch was repeated.

The quality control chart was prepared to ensure the validity of the results obtained. A regular linear graph paper was used to prepare these plots. The mean salt iodine concentration (in ppm) of the internal quality control sample was plotted as a continuous horizontal line on the Y-axis. The lower concentration value (L) was plotted below the mean line on the Y-axis scale and the upper concentration value (U) was plotted above the mean line on the Y-axis scale. The X-axis was used to plot the date on which the internal quality control sample was analyzed. This chart was used to plot the specific analysis date, and salt iodine concentration obtained for the control every time it was tested. If
the value of the internal quality control sample was between the two limit lines, then the test was deemed in control, and all results were accepted. Any internal quality control value that was plotted outside the two limit lines then the test was considered as out-of-control, and the entire batch was repeated.

\[
\begin{array}{c|cccc}
& +2SD(U) & \text{Mean (X)} & -2SD(L) \\
\hline
\text{Date} & 16 & 17 & 20 & 22 \\
\end{array}
\]
The iodine content in water samples was measured calorimetrically. Briefly ceric ammonium sulphate gets converted into cerrous ammonium sulphate in presence of arsenoic acid which gets converted into arsenic acid. Inorganic iodide acts as a catalyst for this reaction. Thus the change of ceric ions to cerrous ions depends on the amount of inorganic iodide present. Ce (ic) ions were yellow in color while Ce (ous) ions was colorless. This reaction was measured calorimetrically.

Methodology for collection

The water samples were collected from the shallow wells, tube wells from the villages.

Methodology for Analysis of Water level

The iodine content in water was determined by the wet digestion method (Dunn et al 1993). The results were expressed as µg/L.

Principle:

Water is digested with chloric acid under mild conditions and iodine is determined manually by its catalytic role in the reduction of ceric ammonium sulfate in the presence of arsenious acid. As the reduction proceeds the intensity of color decreases and this can be readily measured in a spectrophotometer at 420 nm. The method is fast and inexpensive, and the digestion is less harsh than some other methods.

Equipment and Chemicals:

Equipment:

Oven with fan exhaust, Vented fume hood on oven for perchloric acid escape, UV spectrophotometer, Thermometer, Timer (stop watch reliable to 5 second), Test tubes (15 ml), Funnel (56 x 100 mm), Reagent flasks, Bottles, Pipettes, Whatman no 1 filter paper, Laboratory balance.

Chemicals (analytical grade AR /GR)

1. KClO3 (potassium chlorate),
2. HClO4 (perchloric acid, 70%)
3. As2O3 (arsenic trioxide),
4. NaOH (sodium hydroxide),
5. H2SO4 (sulfuric acid)
6. Ce(NH4)4 (SO4)4 2H2O (ceric ammonium sulfate),
7. KIO3 (potassium iodate),
8. HCL (Hydrochloric Acid)
9. Double distilled water (free of iodine and other contaminants)
Preparation of Reagents:

i) Chloric acid solution:

In a 2000ml Erlenmeyer flask, 500g potassium chlorate was dissolved in 910ml hot double distilled water until the soluble state (normally a little amount remain undissolved). 375 ml of 70% perchloric acid was added dropwise (approx. 15 ml/min) while stirring constantly. This preparation was carried out in a vented fume hood as it produces toxic fumes. Subsequently, the solution was kept in a freezer or refrigerator overnight for better separation. The next day it was filtered through a filter paper, (Whatman # 1) and stored in a refrigerator at 40C.

ii) Arsenious Acid Solution:

0.986 g arsenic trioxide was taken in a 1000 ml volumetric flask and was dissolved in 10 ml of 0.5 N hot sodium hydroxide. This solution was transferred into 750 ml chilled double distilled water. Then 20 ml concentrated HCL and 39.6 ml conc. Sulphuric acid (98%) was added dropwise with constant mixing. The solution was stored in an amber color bottle at room temperature. (The solution is stable for months).

iii) Sulphuric Acid Solution (3.5N H2SO4):

97 ml concentrated sulfuric acid (98%) was added drop wise into 800 ml chilled double distilled water (carefully as this generates heat) and final volume was made up to 1 liter with double distilled water.

iv) Ceric ammonium sulfate solution:

48 g Ceric ammonium sulfate was dissolved in 1 liter of 3.5N H2SO4. This was stored in a amber color bottle at room temperature. (The solution is stable for months).

v) Stock Iodine Standard (1mg/ml):

168.5 mg KIO3 was dissolved in double distilled water to make a final volume of 100 ml. This was stored in a amber color bottled (This solution is stable for months).

vi) Dilute Iodine Standard (1µg/ml):

Take 100 ul of Stock Iodine Standard and make a volume to 100 ml with double distilled water.

vii) Working Iodine Standard:

Make the following serial dilutions from diluted Iodine Standard (1ug/ml) into volumetric flasks (10 ml) with double distilled water (diluent). These dilutions are made freshly.
ug/dl | Dilution factors
--- | ---
5 µg | 0.5 ml of 1 µg/ml standard + 9.5 ml diluent
10 µg | 1.0 ml of 1 µg/ml standard + 9.0 ml diluent
15 µg | 1.5 ml of 1 µg/ml standard + 8.5 ml diluent
20 µg | 2.0 ml of 1 µg/ml standard + 8.0 ml diluent

Procedure:

**Step I:** The water sample was shaken to evenly suspend any sediment. 250 µl of each water sample was pipette into a 15x100 mm test tube. Iodine standards were prepared from the 1 µg/ml stock iodine solution. The iodine standards corresponding to 0/5/10/15 and 20 µg/dl were prepared.

**Step II:** 50 micro liter of chloric acid solution was added to each tube (samples, blank, internal quality control sample, standards) and mixed gently. All tubes were placed in the oven at 1100C-1200C for 75 minutes (with a fume hood for the trapping of perchloric acid). There will be very little volume change during heating. All the tubes were cooled at room temperature for 15 minutes. Then, the decreased volume was adjusted with double distilled water to their original volume (1.0 ml) and vortexed.

**Step III:** 3.5 ml of Arsenious Acid was added to each test tube and after mixing all test tubes were kept for 15 minutes at room temperature.

**Step IV:** 350 microliter of ceric ammonium sulfate solution was added at a fixed interval of time to each tube and quickly mixed with help of a vortex. A stopwatch was used to keep a constant interval between additions to successive tubes, (30 seconds was a convenient interval). Exactly 20 minutes after addition of ceric ammonium sulfate to the first tube, the reduction was read spectrophotometrically at 420 nm against the reagent blank at the same interval. (Successive tubes were arranged in a such a manner that the interval between the time of addition of ceric ammonium sulfate and the time of the reading was the exactly 20 minutes for all samples, standards and blanks).

Calculation of results:

The exact value of iodine content in water was calculated as follows:

- The average absorbance value for each set of reference standard, control and samples was calculated.
A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration mcg/dl on linear graph paper, with absorbance on the vertical (Y) axis and concentration (micro g / dl) on the horizontal (X) axis.

Precautions:

i. Since the digestion procedure has no specific end point, it is essential to run blanks and Iodine standards with each assay to allow for variations in heating time, etc.

ii. The exact temperature, heating time and cooling time can vary. However, within each assay, the interval between the time of addition of ceric ammonium sulfate and the time of the reading must be the same for all samples, standards, and blanks.

iii. In this procedure it is convenient to run 60 sample’s tubes per assay of which 5 are standards (at concentrations of 0/5/10/15 and 20 mcg/dl).

iv. Perchloric acid fumes can be toxic and the complex generated may be harmful, particularly if allowed to dry in a ventilation system. The recommended method releases much less perchloric acid than other digestion methods.

v. The exact time and temperature is not critical as long as all tubes are heated the same way.

vi. 1.68mg KIO₃ contains 1 mg iodine KIO₃ is preferred over KI because it is more stable.

vii. Test tubes can be reused if they are carefully washed to eliminate any iodine contamination.

viii. Separate pipettes should be used for all the test tubes and also pipettes used for preparation of each standard solution should be kept separately and not be mixed with the general pool of glasswares. They should be kept separately for all times to avoid contamination.

Methodology for Internal Quality Control adopted

Same as that of Urine samples
APPENDIX- 6: DETERMINATION OF IODINE CONTENT IN FOOD SAMPLE (Fecher et al, 1998)

Quantification of Iodine by inductively coupled plasma mass spectrometry (ICP-MS)

**Aim:** To determine iodine content in food samples.

**Principle:** The sample solution is pumped at 1mL/min (usually with a peristaltic pump) into a nebulizer, where it is converted into a fine aerosol with argon gas at about 1 L/min. The fine droplets of the aerosol, which represent only 1 - 2% of the sample, are transported into the plasma torch via a sample injector. Once the ions are produced in the plasma, that is energized (ionized) by inductively heating the gas with an electrical coil, and contains a sufficient concentration of ions and electrons to make the gas electrically conductive. Even partially ionized gas in which as little as 1% of the particles are ionized can have the characteristics of a plasma (i.e., response to magnetic fields and high electrical conductivity) are directed through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration. The concentration of a sample can be determined through calibration with certified reference material such as single or multi-element reference standards.

**Instrument:** PERKIN ELMER – ELAN 9000

<table>
<thead>
<tr>
<th><strong>ICP-MS Instrument</strong> –</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf Power</td>
<td>1000-1100 W</td>
</tr>
<tr>
<td>Sampler and skimmer cones</td>
<td>Nickel</td>
</tr>
<tr>
<td>Argon flow rates:</td>
<td></td>
</tr>
<tr>
<td>Outer</td>
<td>15 l min⁻¹</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>0.46 l min⁻¹ (variable)</td>
</tr>
<tr>
<td>Lens Voltage</td>
<td>11.00 volts</td>
</tr>
<tr>
<td>Mass-to-charge ration detected</td>
<td>m/z 127</td>
</tr>
<tr>
<td>Quantitative mode:</td>
<td></td>
</tr>
<tr>
<td>Dwell time per mass</td>
<td>1000 ms</td>
</tr>
<tr>
<td>Sweeps per reading</td>
<td>40</td>
</tr>
<tr>
<td>Readings per replicate</td>
<td>1</td>
</tr>
<tr>
<td>Number of replicates</td>
<td>2</td>
</tr>
<tr>
<td>Scanning mode</td>
<td>Peak hop</td>
</tr>
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</table>

**Sampling system –**

<table>
<thead>
<tr>
<th>Manual</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash time between samples</td>
<td>120 s</td>
</tr>
<tr>
<td>Read delay</td>
<td>80 s</td>
</tr>
<tr>
<td>Peristaltic pump speed</td>
<td>1 ml min⁻¹</td>
</tr>
<tr>
<td>Spray chamber and nebulizer assemblies</td>
<td>(a) Ryton cross flow nebulizer (b) Scott-spray chamber</td>
</tr>
</tbody>
</table>

**Calibration –**

<table>
<thead>
<tr>
<th>Type</th>
<th>External Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main Stock Solution</td>
<td>1ppm potassium iodide</td>
</tr>
<tr>
<td>Calibration Standards</td>
<td>0.1ppb, 1ppb, 10ppb, 20ppb</td>
</tr>
</tbody>
</table>

**Apparatus Required:**

i. Standard volumetric flasks – 10ml, 25ml, 50ml, 100ml

ii. Beakers –100ml, 500ml, 1000ml.
iii. Funnels
iv. Plastic reagent bottles – for Standards – 100ml
v. Test tubes with test tube rack
vi. Micropipette with tips – 100 – 1000µl ,1- 5ml
vii. Head spaced vials (20 ml) (Agilent Cat-no;5182/0837 )
viii. Hdspc Al crmpcap,PTFE/Si sep ,20mm,100PK (Agilent-51834477)

Chemicals Required:
i. TMAH (Tetra methyl ammonium hydroxide)  (SIGMA-1001283483)
ii. KI (Potassium iodide)  (MERCK- 1.05043.0250 )
iii. MilliQ water – 18.2 M Ω

Standards /CRM/RM:
CRM’s – 1549 (Non- fat milk powder)

Procedure:
i. Weigh 0.5 to 1.0g of homogenous powdered sample / CRM / RM in duplicates into 20 mL Head spaced vials.
ii. Add 5ml of Milli Q water and add 1 ml of 25 % TMAH.(SIGMA-1001283483)
iii. Air tight the Head spaced vials (20 ml) (Agilent Cat-no;5182/0837 ) with Hdspc Al crmpcap,PTFE/Si sep ,20mm,100PK (Agilent-51834477) by Crimper (Agilent -93010720) and were placed in a drying oven at 90 °C for 3 hours.
iv. After cooling, Milli-Q water was added to make up the final volume of 50 mL maintaining 10 ppb Rh/Te as Internal Standard.in Blanks,Standards& Samples.(Internal std. was prepared from 1000 ppm solution obtained from MERCK )
v. These solutions were then centrifuged at 8000 rpm for 10 minutes
vi. If any visible particulates remained after centrifuging, the samples were then filtered.
vii. The resulting solutions can then be analyzed directly or with an extra dilution if high matrix concentrations are present.

Preparation of Calibration Standards:

<table>
<thead>
<tr>
<th>Standard/Blank</th>
<th>Conc. (µg/litre)</th>
<th>Stock Std. (1ppm KI ) (ml)</th>
<th>25 % TMAH(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard 1</td>
<td>1</td>
<td>0.05</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard 2</td>
<td>10</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard 3</td>
<td>20</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard 4</td>
<td>50</td>
<td>2.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>
1. All calibration standards and blank are made up to 50 ml with MilliQ water. The instrument is set up for analysis. By using the DPC (Daily performance check solution) the instrument is optimized to give the reported counts.

2. The Blank and Standards are run to plot a linear graph.

**Calculation**

\[
\text{Iodine } \mu g/100g = \frac{\text{Vol.made upto in (50ml) \times Concentration in ppb(from instrument)}}{\text{Wt of sample in gms \times 1000}}
\]

Where: 100 is for 100g of food sample

1000 – for converting ng to µg.

**Accuracy CRM1549: Non-fat milk powder**

<table>
<thead>
<tr>
<th>CRM</th>
<th>IODINE mg/kg</th>
<th>Measured value</th>
<th>Certified value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1549</td>
<td>3.38</td>
<td>3.38±0.02</td>
<td></td>
</tr>
<tr>
<td>1549</td>
<td>3.36</td>
<td>3.38±0.02</td>
<td></td>
</tr>
<tr>
<td>1549</td>
<td>3.34</td>
<td>3.38±0.02</td>
<td></td>
</tr>
<tr>
<td>1549</td>
<td>3.37</td>
<td>3.38±0.02</td>
<td></td>
</tr>
<tr>
<td>1549</td>
<td>3.39</td>
<td>3.38±0.02</td>
<td></td>
</tr>
<tr>
<td>1549</td>
<td>3.35</td>
<td>3.38±0.02</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>3.365</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Quality Control: (Market Rice)**

<table>
<thead>
<tr>
<th>Replicates</th>
<th>IODINE mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0056</td>
</tr>
<tr>
<td>2</td>
<td>0.0058</td>
</tr>
<tr>
<td>3</td>
<td>0.0054</td>
</tr>
<tr>
<td>4</td>
<td>0.0056</td>
</tr>
<tr>
<td>5</td>
<td>0.0054</td>
</tr>
<tr>
<td>6</td>
<td>0.0054</td>
</tr>
<tr>
<td>7</td>
<td>0.0055</td>
</tr>
<tr>
<td>8</td>
<td>0.0055</td>
</tr>
<tr>
<td>9</td>
<td>0.0058</td>
</tr>
<tr>
<td>10</td>
<td>0.0054</td>
</tr>
</tbody>
</table>

APPENDIX-7: PERMISSION LETTER

OFFICE OF THE CHIEF MEDICAL OFFICER,
NAINITAL, UTTARAKHAND

Dated: 9th September 2013

TO WHOM IT MAY CONCERN

This is to inform you, that we will extend the kind co-operation and support for the research work undertaken by Ms. Neha Sareen.

Sahir Sabir
Chimbh Medical Officer
Nainital
Uttarakhand

OFFICE OF THE CHIEF MEDICAL OFFICER,
UDHAM SINGH NAGAR, UTTARAKHAND

Dated: 27th July 2013

TO WHOM IT MAY CONCERN

This is to inform that we will extend the kind support and co-operation for the research work undertaken by Ms. Neha Sareen for her Ph.D.

Dr. Rakesh Singh
Chief Medical Officer
Udhampur Singh Nagar
Uttarakhand

Chief Medical Officer
Udhampur Singh Nagar
APPENDIX-8: QUESTIONNAIRE

भारत में उत्तराखंड के तीन क्षेत्रों में आयोजित की स्थिति
अखिल भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली

नवजात शिशु पर परीक्षण हेतु गर्भवती माताओं के लिए प्रोफार्मा

दिनांक: __________________

यूनिक आईडी: __________

1. संस्थान का नाम: ______________________________________________________________

2. खंड: __________________ जिला: ____________________________________________

3. शिशु की पंजीकरण संख्या: __________________

4. माता का नाम: ______________________________________________________________

5. पिता का नाम: ______________________________________________________________

6. पत्र व्यवहार का पूरा पता: _______________________________________________________

7. दूरभाष नं: __________________

8. गर्भवती की अवधि (सम्पन्त): __________ एल.एम.पी. __________ ई.डी.डी. __________

9. जन्म की तिथि: __________________________________ समय (एम / पीएम): __________

10. लिंग (1=लड़का, 2=लड़की): ______________________________________________

11. जन्म क समय शिशु का वजन (ग्राम): __________________________________________

12. क्या गर्भवती महिला को धार्मिक संस्कार की दौड़ दी जा रही है: _________________ (है / नहीं)

13. बाद ही, तो खुशकी की माता: _________________________________________________

संयहकर्ता/शोधकर्ता का नाम

संयहकर्ता/शोधकर्ता हस्ताक्षर
APPENDICES

APPENDIX-9: PERSONAL INFORMATION SHEET FOR PREGNANT MOTHERS AND MOTHER OF NEWBORNS

[Text in Hindi]

Sareen, Nambiar and Kapil, 2016
APPENDIX-10: PARTICIPANT INFORMED CONSENT FORM (PICF) FROM PREGNANT MOTHERS AND MOTHERS OF NEWBORN

भारत में उत्तराखंड के तीन क्षेत्रों में आयोजित की स्थिति
अखिल भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली

नवजात शिशुओं के माता/पिता/देखभालकर्ता से प्राप्त सहमति पत्र (भ.आई.भी.एफ)

मैं नामित पति/पत्नी जन्म के रूप में शिशु के रक्त परीक्षण की अनुमति देती हूँ। मुझे एक नवजात शिशु के प्रायोगिक चिकित्सक द्वारा प्राप्त परीक्षण पर निर्भरता नहीं है। मैं यह भी जानती हूँ कि यह प्रारंभिक चरण में शिशु के रक्त परीक्षण के लिए उपस्थित होना पड़ सकता है।

इस बात की पूर्वजातीय जानकारी है कि मैं इस शिशु के लिए अपनी इच्छा अनुसार अपने बच्चे को अस्तित्व देने से मना कर सकती हूँ।

इस कार्य में मेरा सहभाग होना पूर्णतः स्वीकृत है।

मैं इस बात से पूर्वजात: सहमत हूँ। मैं सहमत नहीं हूँ कि रक्त की बूंदों को संग्रहित उन्हें भविष्य में शैक्षणिक कार्य हेतु प्रयोग में लिया जाए और इससे संबंधित जानकारी को गायब रखा जाए।

माता/पिता का हस्ताक्षर/बांध अंकूट का निशान

गवाह के हस्ताक्षर/बांध अंकूट का निशान

नाम
पति/पत्नी नाम
पता स्थायी/अस्थायी

फोन नं.:
ई मेल:
दिनांक :

(अनुसंधानकर्ता के हस्ताक्षर)