

MICRONUTRIENT RESEARCH LABORATORY,
DIVISION OF BASIC MEDICAL SCIENCES,
SCHOOL OF MEDICINE AND HEALTH SCIENCES,
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The Micronutrient Research Laboratory (MNL) in the School of Medicine and Health Sciences (SMHS) University of Papua New Guinea (UPNG) was set up in 2002. The Iodine Research Unit (IRU) was set up in the MNL-SMHS UPNG in 2003 for monitoring the implementation of the universal salt iodization (USI) strategy in PNG. We were informed about the EQUIP program in March 2004. We received further briefing and an official invitation to join EQUIP via an email dated 18th June 2004. We received an email with the attached EQUIP membership application forms on the 20th June 2004. We completed the EQUIP membership application forms and returned them via email attachment on the 24th June 2004. The response from EQUIP, which we consider to be the official date of registration, was sent to us in an email by Dr. Tracy L Dearth-Wesley. Tracy's email dated 29th June 2004 reads thus:

*Dear Victor, Amir Makhmudov sent EQUIP samples to your lab via FedEx today. The tracking number for the shipment is 7901-9026-9030. This EQUIP round consists of twelve vials, 1.8 mL per vial-three each of four natural urine pools for iodine analysis. The vial labelled "Round Robin Level 3" is included and to be used for calibration verification. There is no need to report back results for this pool. The target value for "Round Robin Level 3" is 19.1ug/L (acceptable range is 13.4 – 24.8). The other three pools results need to be reported by August 14, 2004. We would encourage you to report these results electronically, which are described in detail below. You may also e-mail them to Kathy Caldwell (KCaldwell@cdc.gov). Your lab ID is 48. A reporting system for the EQUIP program has been developed by CDC, which includes an electronic data submissions form. This data submissions form is a part of a web site that has been specifically created for EQUIP program participants. You can access the site at www2a.cdc.gov/nceh/dls/itndatasubmission/login; The EQUIP web site is password protected. The user login information for your lab is as follows:
* E-mail: templevj@upng.ac.pg; * Password (case-sensitive): hYwShI0K.
The EQUIP web site includes links to announcements,.....
Thanks, Tracy*

Thus, in June 2004 our MNL was enrolled in the EQUIP program.

Our major focus as a micronutrient research laboratory is to strive to obtain accurate appropriate research data that can be used to assist in formulating public health policies aimed at improving maternal and child micronutrient status in PNG. Our major impetus for joining EQUIP was the desire to ensure that all urinary iodine (UI) results produced in our MNL-SMHS are viable and accurate. Thus, the need to participate in effective internal and external quality assurance programs cannot be overemphasized. Quality assurance (QA) in UI laboratory testing is particularly relevant in resource limited countries like PNG. EQUIP provides unquantifiable support for UI laboratories in these countries.

There were no substantive bench quality control (QC) and QA programs in the IRU in the MNL-SMHS before June 2004. The assay of UI was by the spectrophotometric method (420nm) of Sandell-Kolthoff reaction, after digesting the urine with Ammonium Persulfate in water bath at 100°C. This is the "Method A" recommended by the ICCIDD/UNICEF/ WHO expert committee. We used and continue to use the procedure

detailed in “A guide for program managers” Annex 3 [1]. Before joining EQUIP in June 2004, an Iodine standard curve was prepared for each set of UI assay carried out. Thus we could only do UI assay of four samples in duplicate { 7 test tubes for the Iodine standard curve and 4 (duplicates) UI samples}. The reason for this slow procedure was because the absorbance (Ab at 420nm) reduces significantly for each set of assays. The absorbance of the blanks also significantly reduces for each set of assay. After excluding all possible forms of errors, including contamination, we then searched the literature on UI assay extensively for an answer or explanation for the rapid reduction in the absorbance of the blanks. No mention was made about such changes; they were not reported in any of the several references on IDD that we had checked.

There are eight points in the procedure in Annex 3 [1]. The last point stated “Allow to sit at room temperature. Exactly 30 minutes after addition of Ceric Ammonium Sulfate to the first tube, read its absorbance at 420nm. Read successive tubes at the same interval as when adding the Ceric Ammonium Sulfate”. The procedure (section on notes) made no mention of the effect of temperature on the Absorbance to be read at 420nm.

After our registration with EQUIP the first shipment that we received contained the following information *The vial labelled “Round Robin Level 3” is included and to be used for calibration verification. There is no need to report back results for this pool. The target value for “Round Robin Level 3” is 19.1ug/L (acceptable range is 13.4 – 24.8).*

The “Round Robin Level 3” enabled us to find the solution to the problem, because of the acceptable range that was provided. We found that when the UI assay was carried out in the morning, the result obtained was within the acceptable range, but in the afternoon the result was outside the range. The only difference that we noted was the temperature in the laboratory (room temperature), which was about 24 – 26°C in the mornings and about 30 – 34°C in the afternoon.

Thus, we decided to check the effect of temperature on the assay procedure. The procedure from 1 – 5 as indicated in Annex 3 [1] was followed, but the procedure from 6 – 8 was carried out in a water bath at a different temperature. The results obtained for the standard curves at various temperatures are presented in the table below. As usual, to obtain the standard curve, the Log Ab was plotted against the concentration (ug/L) of the Iodine standard solution. The data clearly indicate significant changes in Absorbance at 430nm at different temperature ranges. The higher the temperature, the flatter the standard curves.

After obtaining this data, we then searched the literature for any similar work that had been published. Dung et al [2] reported a similar finding for the assay of UI by the spectrophotometric method (405nm) of Sandell-Kolthoff reaction, after digestion with Chloric Acid. According to Dung et al, the low absorbance reading was due to the rapid catalytic conversion of Ceric to Cerous ions caused by the high ambient room temperature [2]. Our findings support the idea proposed by these authors.

Effect of Temperature on Absorbance (Abs) at 420nm for Potassium Iodate Standards

Iodine Std (ug/L)	Temperature °C				
	21 – 23 °C	24 – 26 °C	27 – 30 °C	31 – 34 °C	35 – 40 °C
	Abs	Abs	Abs	Abs	Abs
0	1.7	1.59	1.46	1.21	0.93
20.0	1.505	1.39	1.165	0.99	0.74
40.0	1.38	1.285	1.04	0.85	0.635
80.0	1.125	1.0	0.795	0.625	0.405
120.0	0.97	0.8	0.615	0.44	0.25
160.0	0.80	0.63	0.465	0.33	0.175
200.0	0.67	0.545	0.34	0.215	0.14

Thus, because of lack of adequate resources and problems in maintaining effective air conditioning in our MNL, all UI assays are carried out in a water bath maintained at 22 – 23°C by addition of ice cubes. A similar set up can be proposed for laboratories in the other resource limited countries in the tropics. Solving this problem resulted in significant increase in the rate of assay of UI samples in our MNL, because we were able to assay six UI samples (in duplicate) and two QC samples per assay set of 15 tubes. Instead of preparing Iodine standard curve for a set of UI assay, we prepare and use a standard curve (mean of five curves) for the set of reagents for about 1000 UI assays.

The EQUIP program provided the UI samples used to set up the internal quality control (QC) program in the IRU in 2004. The internal QC in the IRU is the Levy Jennings Chart and Westgard Rules. Routine participation in EQUIP is the major QA program in our IRU.

Development in the IRU MNL-SMHS has been progressive, with the IRU playing a leading role in obtaining research grants. Our regular and continuous participation in the EQUIP program assures the high quality of data produced in the IRU. It strengthened our resolve to maintain high internal QC through effective participation in the EQUIP program.

Our enrolment and performance in the EQUIP program elevated the status of the IRU MNL-SMHS, proving beyond doubt that we are capable of carrying out analyses of the UI at the highest level of competence. The IRU has contributed immensely to the

assessment and reassessment of progress in the control and elimination of iodine deficiency disorders (IDD) in PNG.

Some of the achievements of the IRU since our enrolment in EQUIP include providing verifiable scientific data highlighting the iodine nutritional status of vulnerable groups in various regions of PNG. A database of results on monitoring of salt iodine in the households and retail shops, as well as of the iodine nutritional status of women and children in the various regions of PNG is now available in the IRU MNL-SMHS UPNG.

Although our MNL-SMHS is a small not-for-profit research laboratory, we are within our capacity, contributing maximally to improving the micronutrient status of maternal and child health in PNG. Members of our IRU MNL serve as unpaid consultants to various government and non-governmental agencies working towards improving the general nutrition and public health status of Papua New Guineans.

We are involved in a number of programs and activities in PNG as indicated below:

- ❑ Resource person: Iodised salt monitoring workshop: Train the Trainers: Training for quarantine officers and food inspectors. Sponsored by UNICEF and NDOH: 1st to 5th June 2009, Madang Resort Hotel, Madang Province (See attached program)
- ❑ Resource person: Technical meeting on Proposed Diethylcarbamazine (DEC) Fortified Salt as a Strategy for the Control and Elimination of Lymphatic Filariasis (LF) in PNG: The need to protect the iodine content of salt. Sponsored by NDOH: 13th Feb 2008, NDOH Conference Room, Level 2 – Aopi Centre.
- ❑ Resource person: Iodised salt monitoring workshop: Training for quarantine officers and food inspectors. Sponsored by NDOH and UNICEF: 22nd to 26th May 2007, Madang Resort Hotel, Madang Province, PNG.
- ❑ Resource person: Iodised salt monitoring workshop: Training for quarantine officers and food inspectors. Sponsored by NDOH and UNICEF: 22nd to 26th May 2006, Madang Resort Hotel, Madang Province, PNG (See attached program)
- ❑ Chairman, PNG Food Fortification Technical Committee: Sub-committee of PNG Food Sanitation Council, NDOH: September 2007 to date.
- ❑ Member, Food Fortification Inspection and Monitoring Committee: Sept 2007 to date
- ❑ Member, PNG National Technical Working Group (NTWG) NDOH, on the National Program to Eliminate Lymphatic Filariasis (LF). September 2007 to date.

Some of the major projects completed by the IRU include the following:

- ❑ Assessment of UI status in school-age children (6 – 12 years) in Hella region (Tari and Koroba districts) Southern Highland Province, PNG (Joint research project with the Division of Public Health SMHS).
- ❑ Assessment of UI status in non-pregnant, pregnant and lactating women in the National Capital District (NCD), PNG. (Joint research project with Department of Obstetrics & Gynaecology Port Moresby General Hospital (PMGH)).
- ❑ PNG First National Micronutrient Survey (NMS 2005): Project conducted in collaboration with the International Micronutrient Malnutrition Prevention and Control Program (IMMPaCt) National Center for Chronic Disease Prevention and Health Promotion, UNICEF, PNG National Department of Health (NDOH)

and SMHS UPNG. The IRU was responsible for the assays of UI in all urine samples and the iodine content in all salt samples collected from households.

- ❑ Assessment of iodine nutritional status of infants (breast fed and mixed fed) and their lactating mothers resident in the NCD, PNG: (Joint project with the Division of Child Health SMHS).
- ❑ Assessment of the iodine nutritional status of School-age Children (6 – 12 yrs) in Honiara, Solomon Islands: (Joint Project with the Department of Health and Department of Education Honiara, Solomon Islands) – An international project
- ❑ Assessing the iodine nutritional status of infants, age 6 to 24 months, resident in the NCD, PNG (Joint project with the Division of Child Health SMHS)
- ❑ Iodine nutritional status of school-age children (6 – 12years) in Aseki-Menyamy District, Morobe Province, PNG: (Joint project with the Division of Public Health SMHS and the National Department of Health PNG).
- ❑ Using thyroid function tests to assess the impact of successful implementation of universal salt iodization on the iodine nutritional status of mothers and infants in NCD and Central Province PNG (Joint projects with the Division of Child Health and the Department of Obstetrics and Gynaecology PMGH).
 - Using cord blood to assess the thyroid status of neonates.
 - Assessment of thyroid function and iodine nutritional status of pregnant women attending antenatal clinics in NCD and Central Province.
 - Assessment of discretionary intake of salt and the iodine content of salt in households and retail shops in NCD and Central Province.

Research project in progress:

- ❑ “Does successful universal salt iodization guarantee optimal iodine nutrition in mother and infant?” (Joint project with Divisions of Public Health, Child Health and the Obstetrics and Gynaecology)

Some publications from projects completed by IRU MNL-SMHS:

- ❑ Temple VJ. “Iodine Deficiency Disorders (IDD): Focus on the process and significance of monitoring in PNG” *Med Sci. Bull*, 2003, Vol. 1, 28 – 32.
- ❑ Mapira, P, Temple VJ. & Adeniyi, K. O. “Assessing the status of iodine nutriture in children 6 – 12 years in Hella Region Southern Highland Province, PNG”. *Med Sci Bulletin*, 2003, Vol. 1, 3 – 4.
- ❑ Haindapa B, Temple VJ, Turare R, Masta A. and Amoa AB. “Assessment of Urinary Iodine Levels in Pregnant Women in NCD, PNG”. *Med Sci Bull*, 2004, Vol. 2, 8 – 11.
- ❑ Temple VJ, Mapira P, Adeniyi KO and Sims P. “Iodine Deficiency in Papua New Guinea (Sub-clinical iodine deficiency and salt iodization in the highlands of Papua New Guinea)” *Journal of Public Health* 2005, 27 (1): 45 – 48.

- ❑ Temple VJ. “Progress towards elimination of IDD in PNG” International Council for Control of Iodine Deficiency Disorders. IDD Newsletter, Vol. 22, No 4, Nov. 2006; 11 – 13.
- ❑ Temple VJ, Haindapa B, Turare R, Masta A, Amoa AB, Ripa P. “Status of Iodine Nutrition in Pregnant and Lactating Women in National Capital District, Papua New Guinea. Asia Pac J Clin Nutr: 2006; Vol. 15 (4): 533 – 537.
- ❑ Temple VJ, Oge R, Daphne I, Vince JD, Ripa P, Delange F and Eastman CJ. “Salt Iodization and Iodine Status among Infants and Lactating Mothers in Papua New Guinea” AJFAND, Vol 9, No. 9, Dec 2009, 1807 – 1823.
- ❑ Hapa RZ & Temple VJ. “Status of Iodine Nutrition among School-age Children (6 – 12 years) in Honiara, Solomon Islands” Pac. J. Med. Sci. Vol 7, No. 1, 2010, 21 – 36.
- ❑ Pamu J, Temple VJ, Amoa AB & Grant S. “Using Thyroid Stimulating Hormone (TSH) Level in Cord Blood to Assess the Thyroid Status of Neonates”. Pacific J. Med. Sci. Vol 7, No. 1, 2010, 52 – 62.

Research data produced by the IRU MNL-SMHS in the last eight years allow us to conclude that the current status of the salt iodization program in PNG can be characterized as “existent but needing strengthening.” Further progress requires periodic reviews of the program, to ensure that its tempo is maintained. Consolidation of the current monitoring systems for salt iodization is necessary to achieve the ultimate objective of eliminating IDD in PNG.

Highest Regards,

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References:

1. World Health Organization / United Nations Children’s Fund / International Council for Control of Iodine Deficiency Disorders. Assessment of Iodine Deficiency Disorders and monitoring their elimination: A guide for program managers, 2nd Ed, WHO Pub, Geneva, WHO/NHD/01.1, 2001: 73 - 75.
2. N. T. Dung and M. L. Wellby, Effect of high room temperature on urinary iodine assay. Clinical Chemistry 43, No. 6, 1997, 1084 – 1085.