NWU® | Centre for Human Metabolomics

Centre For Human Metabolomics
North-West University
11 Hoffman Street Potchefstroom
Building F3, Laboratory G19 (Ground Floor)

Telephone number: +27 (0)18 299 2312
e-mail address: pliem@nwu.ac.za
Website: nationalmetabolomicsplatform.co.za

TRIMETHYLAMINURIA DIAGNOSTIC PROTOCOL (FISH ODOUR SYNDROME)

Important: The protocol must commence only under the strict supervision of a Health Care Professional.

The TMA-mixture [1.3 ml TMA [Fluka, ~45% assay; cat #92262 is mixed with 250 ml orange juice by the National Metabolomics Platform Laboratory – for patients 5 years and older] will be (frozen) to the applicable pathology laboratory for the loading test on PRIOR arrangement with our laboratory [pliem@nwu.ac.za]. Arrangements will be co-ordinated with the referring pathology laboratory, referring clinician and patient in order to ensure that the loading protocol does not have to be repeated.

BACKGROUND

Trimethylaminuria (TMAU) or Fish odour syndrome (OMIM 602079) is inherited recessively as a defect in hepatic nitrogen-oxidation of dietary derived trimethylamine. The subsequent conversion of malodorous trimethylamine (TMA) to non-odorous N-trimethylamine oxide (TMAO), by an enzyme known as the flavin- containing mono-oxygenase 3 (FMO3) is partially or fully defective (Wise *et al.*, 2011). FMO3 gene mutations, including polymorphisms, associated with less severe symptoms are the causative element of trimethylaminuria (Dolphin *et al.*, 1997). Clinical symptoms are currently not directly linked to mortality or mobility, but are associated with fish-like to garbage-like odour of urine (trimethylaminuria), sweat (ishthyhidrosis) and in some cases breath (halitosis) consequently leading to psycho-clinical symptoms such as depression (Akerman *et al.*, 1999, Wise *et al.*, 2011).

CHALLENGES IN TMAU DIAGNOSIS:

Investigative procedure for TMAU may be a lengthy process which includes the elimination of conditions which may result in secondary presentation of abnormal body odour. The following routine investigations should be performed **before** undertaking the expensive route of testing for TMAU:

- The assessment of hygiene practises.
- Testing for *Helicobacter pylori* (Done at routine pathology laboratories).
- Determination of the *origin of halitosis*. The condition may be associated with plaque on the posterior dorsal tongue surface and not with TMAU.

DIAGNOSTIC PROTOCOL FOR TMAU

The diagnosis of TMAU remains challenging and requires investigation on metabolite- and genetic level, under supervision of a qualified health care professional. Section A and B below is therefore required for comprehensive assessment to investigate for the presence of TMAU.

A. THE LOADING TEST AND URINE COLLECTION:

Trimethylamine is part of the normal every day diet as it is found in eggs, fish, legumes and other products mentioned in table 1 (Mackay *et al.,* 2011). Larger quantities of trimethylamine can have undesirable side effects but quantities as high as 900 mg can be efficiently metabolised by a healthy adult liver. In the loading test we use a maximum of 600 mg of trimethylamine. Carriers and affected individuals will show a reduced conversion ratio of trimethylamine to trimethylamine oxide and this principle forms the basis of our differentiation analysis between patients, carriers and unaffected individuals. The loading protocol is based on a publication by Mackay *et al.,* 2011.

A.1 ADULTS AND CHILDREN (5 YEARS AND OLDER) ***24 hours prior to loading test:***

- Avoid the foodstuffs listed in table 1 [Broccoli, swedes, brussels sprouts, cauliflower, cabbage, tea, coffee, mayonnaise, fish and seafood, liver, eggs, lecithin containing foods including beans and other legumes as well as L-carnitine, glycine betaine and choline containing supplements].
- At the end of this document, there is a form that needs to be completed as the loading process proceeds: Please note / write down the types of food consumed within the 24 hours leading up to the loading test.
- Preferably use no medication. If any medication is used, please indicate this on the same form [see end of document].
- **No loading test should be performed on liver-disease patients**.

Procedure to follow for the loading test: Important: Please indicate any personal details, information on diet and medication intake as well as symptoms before- and during loading on the form provided at the end of this document.

- Collect a urine sample to be used as control [**BEFORE** the TMA-mixture is administered]. Please mark the urine container clearly as **TIME 0 - baseline. Wrap/cover** the baseline urine with **aluminium foil** and **freeze the sample** for transport with the other urine samples on **dry ice.**
- **After** the baseline urine sample was collected, the **TMA-mixture should be administered orally** and the **total volume of the mixture** must be consumed by the patient for the loading and testing of the samples to be **significant** for a possible diagnosis. The mixture **will be sent frozen** to the pathology laboratory where the loading test is done and it should be **left outside the freezer to thaw** completely, before it is administered. Please **do NOT** defrost the **TMA-mixture by placing it in hot/boiling water or any other means.** If the loading test is going to commence early in the morning, the TMA-mixture may be taken out of the freezer the evening before, so that it can defrost overnight and be ready to use for the purpose of the loading test.
- Collect urine samples **every hour after intake of the TMA-mixture** for the **next four hours** and **note the time on the container** as well as the **form** at the end of this document **[TIME 1** / **TIME 2** / **TIME 3** / **TIME 4** – these times refer to the hour(s) after the TMA-mixture was taken in by the patient].

- **Wrap/cover** all the urine samples (time 0, time 1, time 2, time 3, time 4) in **aluminium foil** and **freeze the samples** immediately **after each collection**. Courier the samples to the National Metabolomics Platform on **dry ice**, within 72 hours after completion of the loading test.

A.2 CHILDREN (UNDER 5 YEARS)

24 hours prior to loading test:

- Use the same protocol as for adults

Procedure to follow for the loading test:

Important: Please indicate any personal details, information on diet and medication intake as well as symptoms before- and during loading on the form provided at the end of this document.

- Collect a **urine sample** to be used as control [**BEFORE** choline is administered. TIME 0 - baseline].
- After collection of the baseline urine sample, **1.2 g choline** must be administered orally (equivalent to 600 mg TMA). This will be provided by the National Metabolomics Platform.
- Collect urine samples **EACH TIME the child urinates in the next four hours after choline**
- **administration and note the time on the urine containers as well as on the form** at the end of this document.
- **Cover the urine containers** (including TIME 0] with **aluminium foil**. Store the urine samples immediately in a freezer after collection for transport to the National Metabolomics Platform on **dry ice.**
- Courier the samples to the National Metabolomics Platform **on dry ice, within 72 hours** after completion of the loading test.

Table 1

Choline containing foodstuff that may influence the loading test
Broccoli, swedes, chocolates, brussels sprouts, cauliflower, cabbage, tea, coffee, mayonnaise, fish and seafood, liver, eggs, lecithin containing foods including beans and other legumes as well as L-carnitine, glycine, betaine and choline containing supplements

Pay special attention to the following:

- Please avoid drinking too much fluid during the loading. The urine sample may become too diluted and interfere with the analyses. The patient may eat during the loading test, following the same diet as 24 hours prior to the loading test day.
- Females performing the TMA loading who are menstruating, should consider performing the test just before the onset of menses or just after their period.
- Do not take any antibiotics for 2 weeks before- and during the sample collection.
- In addition, discontinue the therapeutic option for malodour including the intake of activated charcoal tablets or any chlorophyl related products, containing material known as “chlorophyllin complex” or “copper chlorophyllin complex”, 2 weeks prior to testing. The compounds may lead to false negative results.

B. BLOOD COLLECTION FOR GENETIC INVESTIGATION [ALL AGES]

The blood collection for genetic testing of TMAU may proceed after the loading protocol:

- Two tubes of **EDTA blood (purple top tube)** should be collected and sent to the National Metabolomics Platform at ROOM TEMPERATURE. *Do **NOT** spin the sample down*.
- Note: Genetic testing may take up to 6 months for completion and the TAT for the final report is therefore 6 months after the samples were received at the National Metabolomics Platform.
- Important: Please sign the attached consent form for genetic testing of TMAU. The patient should also indicate at the bottom of the page if he/she wants her/his samples to be discarded after analysis or if DNA may be used for future research on TMAU.

Should you have any questions regarding the procedure, do not hesitate to contact us at pliem@nwu.ac.za or +27 (0)18 299 2312

REFERENCES:

1. Akerman, B *et al.* 1999. Two novel mutations of the FMO3 gene in a proband with trimethylaminuria. *Human Mutation,* 13: 376-379.
2. Dolphin, C.T *et al.* 1997. Structural organisation of the human flavin-containing monooxygenase 3 gene (FMO3), the favoured candidate for fish odour syndrome, determined directly from genomic DNA. *Genomics,* 46: 260-267.
3. Mackay, R.J. *et al.* 2001 Trimethylaminuria: Causes and diagnosis of a socially distressing condition, *Clin Biochem Rev,* 31: 33-43
4. Miller *et al.* 2014 Massive Trimethylaminuria Associated with Food Protein–Induced Enterocolitis Syndrome. *JIMD Reports,* 12: 11–15.
5. Wise, P.M *et al.* 2011. Individual reporting idiopathic malodour production: Demographics and incidence of trimethylaminuria. *American journal of medicine*, 124: 1058-1063

PERSONAL ASSESSMENT BEFORE- AND DURING TMAU LOADING:

Send this completed form with the samples or via email to pliem@nwu.ac.za

Name and Surname:			
Date of Birth:			
Gender (Male/Female):			
Diet: 24 hours leading up to TMAU loading			
Medication intake (including dosage) prior and during TMA loading			
Sample Information	**Time of urine collection**	**Rate odour observed (0 being no odour to 5 being extreme odour)**	**Description of odour (3 words)**
Time 0 (before loading)			
Time 1h after TMA intake			
Time 2h after TMA intake			
Time 3h after TMA intake			
Time 4h after TMA intake			