# Towards Standardization–A New Protocol for Oil drop test (*Neikuri*) in Healthy Subjects

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#### ABSTRACT

**Background:** Oil drop test (*Neikuri*), a method of urine examination used in Siddha Medicine, an Indian system of medicine. In this test, patient urine is collected in a vessel, over which a drop of sesame oil is placed and the behavior of the oil drop on the urine surface is noted. However, due to lack of a standardized protocol for this test and advancement of modern diagnostic facilities, the utility value of this test has decreased to a greater extent. **Objectives:** To develop a new protocol for oil drop test in healthy subjects, so that it can be used as a standard reference to compare with other pathological conditions. **Methods and Material:** Urine samples of 12 healthy volunteers aged between 18 to 25 years were collected. Biochemical analysis was done in the urine samples with urine reagent strip to confirm health status of the subjects. 12  $\mu$ l of sesame oil was dropped over 30 ml of each urine sample and behavior (shape of the oil spread, direction and spread time) was observed. **Results:** Majority of the samples showed circular shape and the most appropriate time to record it after 15 min of the test procedure. **Conclusion:** This test protocol can be taken as a standard technique for Oil drop test. However, further studies must be done in larger population so that it can be used as a non invasive and cost effective diagnostic/prognostic tool.

Key words: Oil drop test, Oil spread shape, Oil spread direction and time, Neikuri, Siddha diagnostic technique.

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#### INTRODUCTION

Oil drop test (Neikuri) is one of the most important diagnostic as well as prognostic test procedures that have been followed Siddha Medicine, an Indian system of medicine for various diseases. History behind the usage of oil drop test (ODT) has been documented in several Siddha as well as Ayurveda text books <sup>[1,2]</sup> and its usage was very familiar till 16th century. In this test protocol, a sesame oil drop is placed over the patient urine sample using a small coconut stick and the behavior (shape of the oil spread, direction and spread time) of oil drop is noted.<sup>[3]</sup> The shape of the oil spread, indicative of good prognosis and found in health subjects is lotus or umbrella or swan. The geometric descriptions for these shapes are circular (lotus), semicircular (umbrella) and triangular shape (swan). In disease state, the oil spread shape is plough, tortoise and so on and their geometric description is irregular.<sup>[4]</sup> Direction of spread towards north, east or west is also an indicative of good prognosis. South, south east, south west, north east and

North West are indicative of bad prognosis.<sup>[4]</sup> However, test protocol is not standardized across the globe. Lack of standardization, advancement of modern instrumentation facilities has caused the use of this test to be lost in the pages of time.

Available literature indicates that characteristic feature of ODT depend on the volume, pH, osmolarity and trace elements in urine, time of urine collection, size and type

Received : 6-07-2015 Revised : 25 -08-2015; Accepted : 17-11- 2015 \*Correspondence : A. Ahamed Basha, Department of Physiology, Chettinad Hospital and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chennai -103, Tamil Nadu, INDIA. E-mail: drahamedbasha@gmail.com Conflict of interest: Nil ; Source of support : Nil Copyright: © 2015 Journal. All rights reserved. DOI : 10.5530/ijpcs.4.4.3 of the vessel used, height of the oil drop from the surface of the urine, duration between urine sample collection and performance of the test.<sup>[5-7]</sup> Another study claims that white variety of sesame (seeds) oil is better when compared to black variety for the ODT.<sup>[3]</sup> Besides different school of thoughts with respect to ODT, urinary excretory metabolites and surface tension determines the quality of ODT.<sup>[3]</sup> Urinary trace elements are influenced by age, sex, physical activities, bowel habits and life style modification. <sup>[8-12]</sup> Moreover it is also documented that environmental factors like seasonal and climate change and geographical demographic location can influence the urinary excretory volume and metabolites.<sup>[13-16]</sup>

Hence, considering all the above mentioned factors and in order to revive the significance of ODT in medical field, this study was done to design a new protocol for the oil drop test (ODT) in healthy subjects so that it can be used as a reference value in future and to standardize the protocol on a later date.

#### METHODOLOGY

## Study subjects

Healthy individual's of either sex (total=12, male=6 and female=6) aged between 18 to 25 years were enrolled for the current study. After getting approval from the institutional human ethical committee, the study was initiated. All the study participants were healthy volunteers without any known ailments and with no history of alcoholism and smoking which could affect the results. Further, the selection of subjects was made in the same demographic areas with more or less similar life style activities.

# Urine container and ODT oil

Traces of lipid molecules or any other surface active molecules on the container can affect the ODT to a great extent. Hence, S line glass borosil Petri dishes (100 mm outer diameter \* 15 mm height circular) were purchased, autoclaved and used as a urine sample container while performing ODT. Reason behind the usage of this Petri dish is due to the very close flatness tolerance of its inner bottom as high accuracy for many biological assays depend on this great clarity. This Petri dish also has free from air bubbles and made up of soda lime glass so that inner surface is absolutely flat. Secondly, glass container can be easily washed, sterilised, reused and is cheaper too. Selection of oil<sup>[3]</sup> (extracted from white variety of sesame seeds) for ODT was done based on the previously described methods. Sesame oil was purchased from Idhayam V.R.Muthu and Brothers, Idhayam group of companies, Chennai–81, India.

# Urine collection and biochemical analysis

All the 12 study volunteers were asked to collect the single time mid stream urine sample (at least of 100 ml) around 9.00 am on the day of the test. This time point is different from the methodologies described in literature. This time was selected as it is easier for patients to collect the same in hospital at 9.00 am and follow the rest of the procedure as described below. After collection, from each collected (stock) urine samples, 30 ml were taken and routine urine parameters like urine colour and leucocytes, urobilinogen, pH, blood, specific gravity, glucose, ketones, bilirubin, protein and nitrite were analysed by using urine reagent strips (Uro dip 10 A - ERBA diagnostics, Germany). Dip-stick test reactions were observed and recorded at the time intervals specified as mentioned by the package instruction manual. Samples of 12 healthy volunteers were tested for the following parameters: 1.Biochemical analysis of urine: colour and dip-stick test reactions for leucocytes, urobilinogen, pH, blood, specific gravity, glucose, ketones, bilirubin and protein nitrite.

## Procedure for ODT

30 ml of each urine sample was poured into one Petri dish and it was covered with a lid. All the urine samples were kept aside at normal room temperature without disturbing for one hour in order to stabilise the surface vibration and surface tension.

After 1 h, 12  $\mu$ <sup>[3]</sup> of sesame oil was taken with the help of micropipette and dropped into each urine sample at a single press till second stop of the plunger. Consistent speed, pressure and smoothness of the plunger were maintained almost similar for all samples. Height of the micro tip of pipette was fixed at 1 cm<sup>[17]</sup> from the urine level of the Petri dish to maintain uniformity using a stand (Figure 1). Photographs were taken under proper illumination and behavior of the oil (shape, direction of spread and area) were observed. Shape of the oil on the urine surface was observed immediately, after 5 min, after 15 min, after 1 h, after 2 h and after 3 h. The The diameter of the oil spread was calculated using Image J software. Direction of the spread of oil on urine surface was also observed immediately, after 5 min, after 15 min, after 1 h, after 2 h and after 3 h. Direction of spread of the oil was observed with relation to the centre of the Petridish. A magnetic compass was used to observe the direction of spread. The results were tabulated with their percentages.



Figure 1: Oil drop test procedure

Figure shows ODT test procedure, Label 1–Micropipette stand, 2–Micropipette volume indicator showing 12  $\mu$ l of oil, 3–Micro tip, 4–Oil drop (dropped from a height of 1 cm), 5–S line glass borosil Petri dish, 6-Petridish stand.

#### RESULTS

Of the 12 urine sample observed, 33% (4), 25% (3), 25%(3) and 17% (2) were amber, pale, dark and transparent in colour respectively. 25% (3) samples were positive for leucocytes (++125) and 8% (1) for haematuria. Urobilinogen, specific gravity and pH, glucose, ketones, bilirubin, protein and nitrite were found to be within normal limits in all the samples (Table 1).

Immediately after the oil drop, shape of the oil spread was small circle (Figure 2a, diameter=1 to 15 mm) in all 12 samples. However, after 5 minutes, oil drop transformed its shape to a medium sized circle (Figure 2b, diameter=15 to 30 mm) in 42% (5), large sized circle (Figure 2c, diameter = 30 to 45 mm) in 42% (5), scattered (irregular shape) in 1 (8%) (Figure 2d) and maintained its small circular shape in 1 (8%) sample. Further, after 15 minutes oil drop again transformed its shape to oval in 8% (1) (Figure 2e), pearl in 8% (1) (Figure 2f), elliptical in 8% (1) (Figure 2g) and sieve in 8% (1) (Figure 2h) large circular with two small circles within in 17% (2) (Figure 2i), medium sized circle 25% (3) and large sized circle 25% (3) samples. Moreover, after 1, 2 and 3 hour, oil drop were maintained almost in medium sized circle shape in 92% (11) and scattered in 1 (8%) sample (Table 2a).

Uniformity of the spread of oil drop was observed in relation to the smoothness of the margins of the oil drop. Immediately after oil drop, direction of the oil spread was at centre. After 5 min, the direction of spread of the oil were uniform from centre in 58% (7), uniform towards south east in 17% (2), south west in 8% (1), north west in 8% (1) and irregularly scattered (no uniform margins) in 8% (1) samples. Further, after 15 min similar pattern of direction of oil spread was observed as recorded after 5 min. Moreover, the direction of spread of oil after 1 h was uniform from centre in 8% (1), uniform towards south east in 25% (3), north in 17% (2), north in 17% (2), south in 8% (1), north east in 8% (1), north west in 8% (1) and irregularly scattered in 8% (1) sample. Likewise, the direction of spread of oil after 2 h was uniform at centre in 8% (1), south west 25% (3), north west in 33% (4), south east in 8% (1), south in 8% (1), divided into two separate circle one (medium) at east and another at south east (small) in 8% (1) and irregularly scattered in 8% (1) sample. No further change in direction was observed at 3 h (Table 2b).

#### DISCUSSION

In Traditional Siddha system of medicine, patient's pathophysiological state is examined under a broad classification called "*envagai thaervu*" (8 types of examinations) which include examination of Pulse, Urine, Stool, Tongue, Voice, Touch, Eye and General appearance.<sup>[4,18]</sup> ODT is one of diagnostic as well as prognostic test under urine examination that has been followed very popularly till medieval period in India<sup>[4]</sup> for various diseases including diabetes mellitus<sup>[19]</sup> and cancer.<sup>[7]</sup> Even though ODT was widely used during ancient period, the interpretation of the test results was made based on the text books and personal experience. Hence, there is a need for the standardization and scientific validation of ODT. By substantiating this age-old method by modern knowledge, its efficacy can be proved and new horizons can be unveiled.

Available literature indicates urine sample for ODT has to be collected 1 h 36 min before the sun rise at early morning.<sup>[3]</sup> The probable reason could be due to rapid fall





Shapes of oil drop: 2A–Small sized circle, 2B–Small sized circle, 2C–Small sized circle, 2D–Small sized circle, 2E–Small sized circle, 2F–Small sized circle, 2G–Small sized circle, 2H–Small sized circle and 2l–Small sized circle, Figure 2D and E has given as grey scale image for better visibility Small, medium and large circle was classified based on the diameter of the circle, small circle [diameter=1 to 15 mm], medium sized circle [diameter=30 to 45 mm].

in the surface tension so that urine could be more stable. In the current study, mid stream urine sample was collected at 9 am due to easy availability of the subjects, without disturbing their sleep because sleep disturbances can lead to stress. Moreover, this time point can be taken as a standard reference time for outpatient as well as inpatient in the health care unit, because majority of the modern diagnostic laboratory tests are being done in the morning hours (except in case of emergency).

ODT is based on the consistency, thickness, density of urine and by observing the behavior of oil drop on the urine surface.<sup>[3,4]</sup> One study explained the physiological basis of the ODT based on the mechanism of oil spill on sea surface.<sup>[3]</sup> The authors explained by three stages. 1. Immediately after the oil spill, the oil slick is thick, so gravity and inertia forces plays a dominant role in spreading process-gravity acts as an accelerating force and inertia act as a retarding force. 2. Later, oil slick become thin and inertia force will be replaced by viscous force. 3. In third stage, interfacial tension and viscous forces play a major role in spreading. When correlating the above mentioned

Table 1: Bioch	emical analy	ysis of urine	sample									
Parameters	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12
Colour	Amber	Transparent	Transparent	Pale	Dark	Pale	Pale	Amber	Dark	Dark	Amber	Amber
Leucocytes	Negative	++125	++125	Negative	Negative	++125	Negative	Negative	Negative	Negative	Negative	Negative
Urobilinogen	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Hd	9	9	9	9	9	9	7	9	9	9	9	9
Blood	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	+++200	Negative	Negative	Negative
Specific gravity	1.030	1.025	1.025	1.025	1.030	1.020	1015	1.030	1.025	1.025	1020	1.025
Glucose												
Ketones												
Bilirubin	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Protein												
Nitrite												
Urine colour and dip-stick	k test reactions of le	eucocytes, urobilino(	gen, pH, blood, speci	ific gravity, glucose	e, ketones, bilirubi	n, protein and nitrit	e of the samples.					

Table 2a: Sha	pe of an oil	on urine su	rface									
Shape of the oil spread	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12
Immediately	Small circle	Small circle	Small circle	Small circle	Small circle	Small circle	Small circle	Small circle	Small circle	Small circle	Small circle	Small circle
After 5 min	Small circle	Medium circle	Medium circle	Medium circle	Large circle	Medium circle	scattered	Large circle	Medium circle	Large circle	Large circle	Large circle
After 15 min	Pearl shape	Medium circle	Medium circle	Elliptical shape	Circular with two small circle within interiorly	Medium circle	Sieve shape	Large circle	Large circle	Circular with two small circle within interiorly	Oval shape	Large circle
After 1 h	Medium circle	Medium circle	Medium circle	Medium circle	Medium circle	Medium circle	Scattered	Medium circle	Medium circle	Medium circle	Medium circle	Medium circle
After 2 and 3 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	Similar to 1 h	One small and another medium circle	Similar to 1 h

Table 2b: L	<b>Direction of</b>	spread of a	nn oil on urin	e surface								
Direction of oil spread	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12
Immediately	Centre	Centre	Centre	Centre	Centre	Centre	Centre	Centre	Centre	Centre	Centre	Centre
After 5 min	Uniform from Centre	Uniform from Centre	Uniform from Centre	Uniform towards south east (10*)	Uniform from Centre	Uniform from Centre	Irregularly scattered	Uniform from Centre	Uniform towards North west (4*)	Uniform towards south west distally (2*)	Uniform towards south east proximally (10 *)	Uniform from Centre
After 15 min	Uniform from Centre	Uniform from Centre	Uniform from Centre	Uniform towards south east (10*)	Uniform from Centre	Uniform from Centre	Irregularly scattered	Uniform from Centre	Uniform towards North west (4*)	Uniform towards south west distally (2*)	Uniform towards south east proximally (10*)	Uniform from Centre
After 1 h	Uniform at East (9*)	Uniform at South east (11*)	Uniform at Centre	Uniform at South east (11*)	Uniform at North (6*)	Uniform at North (6*)	Irregularly scattered	Uniform at North west (5*)	Uniform at South (12*)	Uniform at North east (8*)	Uniform at East (9*)	Uniform at South east (11*)
After 2 h	Uniform at South west (1*)	Uniform at Centre	Uniform at North west (5*)	Uniform at South east (11*)	Uniform at North west (5*)	Uniform at South west (2*)	Irregularly scattered	Uniform at North west (5*)	Uniform at North west (4*)	Uniform at South west (10*)	Uniform – one at East and another at South east (9* and 10*)	Uniform at South (12*)
After 3 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h	Similar to 2 h
Direction of spread * Represents clock	of an oil on urine (	surface-recorded i	immediately, after 5	min, 15min, 1, 2 anc	d 3 h							

hypothesis with ODT, the gravity interference plays a negligible role because the oil volume used in the current study is only  $12 \mu$ l. Hence, role of interfacial tension and viscous forces plays a major role in ODT and determines different shape and speed of the oil spread.

Pattern of the oil spread is determined by the surface active molecules and metabolites that are present in trace amount.<sup>[20]</sup> The direction of spread of oil is due paramagnetic molecules that arrange in a line with respect to earth electromagnetic field.<sup>[3]</sup> In the current study, biochemical analysis of the urine showed results within the normal limits and ODT in majority of subjects showed circular in shape with uniformity in spread irrespective of direction which is in agreement with the earlier study.<sup>[21]</sup> However, ODT showed significant changes only after 15 min of the test procedure. Hence, in ODT, timing of observation has to be considered as significant factor. Additionally, if ODT is done in any pathological state it has to be compared on the same day with the control subjects in order to avoid demographical, climatic and ultradian errors.

The present study was a preliminary effort to develop a new protocol for oil drop test (*neikuri*) in healthy subjects. In future, it has to be further standardized with higher number of healthy subjects.

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