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Iodine and creatinine testing in urine dried on filter paper

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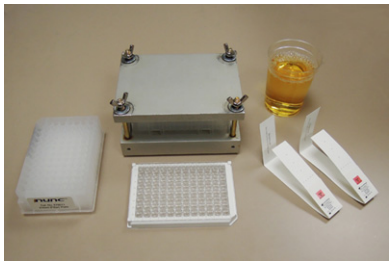
HIGHLIGHTS

- Dried urine iodine and creatinine extract quantitatively correlates well with liquid urine.
- Filter paper strips can be easily shipped and stored.
- Urine iodine and creatinine are stable at ambient temperature when dried on filter paper.
- Dried urine iodine and creatinine are run using a 96-well format.

GRAPHICAL ABSTRACT



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ABSTRACT

Iodine deficiency is a world-wide health problem. A simple, convenient, and inexpensive method to monitor urine iodine levels would have enormous benefit in determining an individual's recent iodine intake or in identifying populations at risk for iodine deficiency or excess. Current methods used to monitor iodine levels require collection of a large volume of urine and its transport to a testing laboratory, both of which are inconvenient and impractical in parts of the world lacking refrigerated storage and transportation. To circumvent these limitations we developed and validated methods to collect and measure iodine and creatinine in urine dried on filter paper strips. We tested liquid urine and liquid-extracted dried urine for iodine and creatinine in a 96-well format using Sandell–Kolthoff and Jaffe reactions, respectively. Our modified dried urine iodine and creatinine assays correlated well with established liquid urine methods (iodine: $R^2 = 0.9483$; creatinine: $R^2 = 0.9782$). Results demonstrate that the dried urine iodine and creatinine assays are ideal for testing the iodine status of individuals and for wide scale application in iodine screening programs.

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1. Introduction

Iodine deficiency affects an estimated 1.88 billion individuals worldwide [1], representing a major public health concern and creating a need for a convenient, practical, simple, and cost-effective test to monitor urine iodine levels in population groups or individuals. Iodine is an essential component of the thyroid hormones thyroxine and triiodothyronine, which regulate growth, development and metabolism. Serious disorders can result from iodine deficiency, including irreversible cretinism, pregnancy complications, goiter, compromised thyroid hormone production, mental impairment and decreased cognitive function [2]. A meta-analysis released in 1994 concluded that thyroid dysfunction caused by iodine deficiency resulted in populations with an average IQ of 13.5 points lower than iodine sufficient controls [3]. While iodine deficiency can occur at any age, the most devastating consequences occur in the developing fetus and early childhood. It is estimated that over 240 million school aged children are not getting enough dietary iodine [1]. Iodine deficiency during pregnancy and lactation is the leading cause of mental retardation worldwide due to inadequate thyroid hormone delivery to the developing brain of the fetus or newborn [4].

Since human beings eliminate over 90% of dietary iodine in their urine, it is possible to accurately measure recent iodine intake using urinary analysis [5–7]. Despite great efforts to eliminate

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iodine deficiency world-wide, recent reports indicate that iodine deficiency persists even in the Western world. Vegans and pregnant women in the United States, along with teenage girls living in the United Kingdom, areas believed to have sufficient iodine intake, are susceptible to iodine deficiency [4,8–10]. When a population tests as iodine sufficient, subgroups and individuals may still be at risk, mostly due to their dietary habits [1]. Over 60% of countries still do not have regular iodine screening programs [8,11]. Because iodine sufficiency is critical to the health and well-being of present and future generations, monitoring of the iodine status of all countries, whether previously identified as iodine sufficient or not, is essential to reduce the prevalence of iodine deficiency disorders (IDD).

To our knowledge, no method exists for iodine analysis using urine collected on filter paper. The primary goal of our research was to develop a more convenient, simple, and cost-effective method for collection, storage, transport, and testing of urinary iodine and creatinine for large-scale population studies and for individuals at risk for iodine deficiency. The dried urine iodine and creatinine assays described here represent an innovative testing solution for this purpose, which most laboratories can accommodate at minimal cost.

2. Experimental

2.1. Urine samples

Three hundred liquid urine samples provided by ZRT laboratory employees were collected from September 2009 to October 2011 and stored at −80 °C. All participants were healthy individuals consuming a normal Western diet. None of the participants were using nutritional supplements containing iodine. Each sample was coded to protect the donor's identity and there were no inclusion or exclusion criteria for the donors. In several cases multiple samples were collected by a single person. There was no inclusion or exclusion criterion for the samples. Procedures were in accordance with the current revision of the Helsinki Declaration and signed informed consent was obtained from each individual before the collection of urine samples. A 2.5 cm × 5.0 cm filter paper strip (Ahlstrom grade 226; ID Biological Systems) with an external protective cover was dipped into the thawed and mixed urine sample, and then hung to dry off the edge of a counter from its protective cover using tape. Strips were left indoors in the open air at 22 °C and 50% humidity to dry for at least 3 h (See Section 3.8). Strips were labeled with indelible ink on the external protective cover. The remainder of the urine was reserved for liquid urine testing.

2.2. Dried urine iodine and creatinine standards, calibrators and blanks

A standard solution of iodine was prepared by diluting high concentration iodine standard (1000 μg mL^{-1} iodide from ammonium iodide, Inorganic Ventures) in Surine (Surine negative quality control; DTI Innovative Products), a synthetic urine manufactured free of iodine, to a concentration of 500 μg L^{-1}. Filter paper strips were dipped into this solution, and then hung to dry off the edge of a counter from their external protective covers using tape. Strips were left indoors in the open air at 22 °C and 50% humidity to dry for at least 3 h. A creatinine standard solution of 0.2 mg mL^{-1} was prepared and kept in a liquid form by dissolving 20 mg creatinine (Sigma–Aldrich) in 100 mL deionized water. Lyophilized National Institute of Standards and Technology (NIST) reference material (SRM2670a, http://www.nist.gov) and Seronorm (Trace Elements in Urine Level 2, http://www.seronorm.no) were used as iodine calibrators. Bio-Rad Urine Chemistry Controls 397 and 398 were used as creatinine calibrators, and Surine and deionized water were used as blanks for the iodine and creatinine assays, respectively. Filter paper strips were dipped into each calibrator and blank then dried as described above.

2.3. Dried urine iodine and creatinine extraction procedure

Using a dried blood spot puncher (Wallac DBS Puncher; PerkinElmer), six 6.0 mm diameter disks were punched from each of the dried urine samples, the iodine and creatinine calibrators, and the 500 μg L^{-1} iodine standard into individual wells of a 96-well fritted filter plate (1 mL wells with 20 μm frit; Nunc). The filter plate was stacked on top of a deep 96-well plate (2.2 mL wells; VWR) and 300 μL deionized water was added to each well. The two-stacked plates were placed on a plate shaker (Microplate Shaker; VWR) and mixed at 700 rpm for 10 min. The plates were then centrifuged (Beckman GPR centrifuge; Beckman Coulter) at 3000 rpm for 10 min to separate urine extract from the filter paper punches. The deep 96-well plate containing urine extract was placed on a plate shaker for 5 min at 300 rpm to ensure proper mixing.

2.4. Dried urine iodine assay

A modified version of the microplate iodine assay described by Ohashi et al. [12] was used to determine iodine levels in dried urine extract. Extracted 500 μg L^{-1} iodine standard was serially diluted with extracted Surine blank to create a set of six standard solutions with concentrations of 0, 31.25, 62.5, 125, 250, and 500 μg L^{-1}. Fifty microliters of serially diluted iodine standards, extracted calibrators and extracted patient samples were transferred into a fresh, deep 96-well plate, and 100 μL of freshly prepared ammonium persulfate (1.314 mol L^{-1}; Sigma–Aldrich) was added to each well. Two silicone sheets (Silicone cover slips; MSC Industrial Supply) were placed over the plate, which was then tightly sealed in a 96-well deprotection chuck (BioAutomation) and incubated for 60 min at 105 °C. The deprotection chuck and deep 96-well plate were then partially submerged in a cool water bath (15–25 °C) for 30 min to prevent condensation.

Iodine concentration was then determined using the Sandell–Kolthoff reaction in which iodide is the catalyst in the reduction of ceric ammonium sulfate in the presence of arsenious acid. Fifty microliters of digested extract was transferred into a 96-well microtiter plate (Microtiter strip plates; Thermo Scientific), and 100 μL of arsenious acid (0.0253 mol L^{-1}, prepared by dissolving 2.5 g arsenic trioxide and 12.5 g sodium chloride in 100 mL of 2.5 mol L^{-1} sulfuric acid [Arsenic trioxide and sodium chloride; FisherScientific], then adjusting volume to 500 mL with deionized water) was added to each well and mixed by gentle shaking for 10 s. Fifty microliters of ceric ammonium sulfate (0.0158 mol L^{-1}, prepared by adding 2.5 g ceric ammonium sulfate [Ceric ammonium sulfate; Acros Organics] to 250 mL of 1.25 mol L^{-1} sulfuric acid [Sulfuric acid; Fisher Scientific]) was then added, and the plate incubated at room temperature for 45 min before absorbance was read on a plate reader (Wallac Victor2 plate reader; PerkinElmer) at 405 nm.

Unknown sample concentrations were determined from the standard curve by linear regression analysis. The iodine standard curve was created by plotting log of absorbance on the *y*-axis and iodine standard concentration on the *x*-axis. The iodine assay was calibrated with Seronorm and NIST samples that were dried and extracted from filter paper strips in a manner identical to clinical urine specimens. A correction factor was determined and applied to all unknown sample results, calculated by dividing experimental Seronorm and NIST values by their expected values and averaging the two.

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