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DIRECTIONS

Guidance for researchers conducting population studies

Focus on monitoring of iodine deficiency disorders (IDD)

Iris Erlund
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Laura Råman
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EUthyroid consortium

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Introduction

This guidance is indented as a practical tool for research professionals planning and conducting population studies. Main focus is on monitoring of iodine deficiency disorders (IDD), but much of it applies to any human study. The guidance may be useful in many ways, for instance, for researchers planning studies, or personnel collecting biosamples or conducting laboratory analysis. It helps when writing the study plan and the study manual and furthermore, it may be used for educational and training purposes.

The guidance was written within the EUthyroid project, which aims to harmonize iodine and thyroid related studies in Europe. This is an important goal, because it improves the overall quality of studies as well as comparability of results. This guidance is one step towards harmonisation.

The guidance is divided into three parts. Part A includes general recommendations and issues to consider when planning a study, including quality issues. Part B offers more detailed instructions and recommendations for specimen collection and sample handling. Part C gives an overview of iodine and thyroid related laboratory analysis. References have been included to suggest useful reading, although the purpose of this document is not to serve as a comprehensive literature review.

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PART A. PLANNING STUDIES

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PART B. SPECIMEN COLLECTION AND SAMPLE HANDLING

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C. LABORATORY ANALYSIS

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PART A. PLANNING STUDIES

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1 The importance of good planning in research projects

Planning is a crucial succes factor in any research project. Planning for even the smallest monitoring studies starts months before participants may be contacted. Large multinational studies take even more time to plan.

Monitoring studies and other observational research studies perfomed by universities and research institutes are not as strictly regulated as studies performed by the pharmaceutical industry, which adheres to Good Clinical Practice (GCP) and Good Laboratory Practice (GLP).

Research projects performed outside pharma require thorough planning also, but there is less published material to guide researchers. Projects may be more variable by nature and the staff may be less experienced. The strict timelines and requirements related to the ethical approval process has pushed researchers to improve planning and quality of all types of human studies. But still, there is room for improvement.

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2 Planning and execution phases do not overlap

Planning of a succesful human study starts with a project plan, which is separate from a scientific research plan. A good project plan helps in optimizing resources. Later, a project manual is needed, serving as an everyday tool when conducting the study. The project manual should be finished well before the field work actually begins.

Detailed planning is important, because multiple factors affecting the ability of staff or participants to follow instructions or collect samples adequately add unaccounted variability into laboratory results and other measurements.

When planning multicenter studies, cultural differences must be considered, because they impact on the communication between investigators, staff and participants. Also, they impact on how much and what kind of information staff and participants can understand. The ability to learn from training, work under tight time-schedules or adopt new technologies or methods vary greatly. In multilingual studies, translations may be needed, so keep in mind that obtaining them and testing them in the right cultural context takes time.

Knowledge about and adherence to relevant regulations, laws and practices relating to clinical studies or material logistics is crucial and vary from country to country.

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3 The project plan optimises resources

A good project plan allows optimisation of resources and timelines, and reduces preanalytical errors. It is less detailed than the project manual. Issues covered:

- Financing and budgets
- Legislatory requirements (ethical approvals etc.)
- Site selection
- Study design
- Recruitment plan (including events for participants)
- Pilot study
- Human resources (staffing and training plan)
- Material needs and logistics
- List of questionnaires
- Time-frame
- Risk management (mitigation and contingency planning = plan B)
- Documentation

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4 The project manual – an execution tool with a practical content

- Background and aims, recruitment criteria, study design, treatments, financing, approvals
- Organizational structure, staffing needs (including substitutes), task descriptions
- Staff training, staff motivation events, staff safety
- Timetables
- Lists of materials (equipment, questionnaires, laboratory materials, sampling materials)
- Logistics
  - Specifications of equipment, sampling materials, questionnaires etc.
  - Sample collection charts (sampling volumes, order of blood collection tubes etc)
  - Sample labelling and identification system
  - Biobanking (sample storage instructions)
- Field work
  - Work flow, process charts
  - Instructions for patient preparation and visits, patient materials
  - Detailed description of tasks, measurements, methods, procedures (incl. waste handling plan)
- Data management instructions, documentation instructions
- Safety of participants, staff and data
- Quality assurance procedures
- Instructions regarding contacts with participants
- Information about dissemination
- Examples of project manuals , see Tolonen et al. 2016 or Lundqvist and Mäki-Opas 2016

Tolonen et al. 2016, Lundqvist and Mäki-Opas 2016.

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5 Laboratory quality

The goals of research laboratories and clinical laboratories overlap partly, but there are significant differences too. Quality issues are important for both, but for different reasons

Clinical laboratories analyzing patient samples are typically accredited and their operations are largely based on automation and a commercial laboratory system. Speed, patient safety and diagnostic accuracy are emphasized. Large numbers of samples can be analyzed with high quality, which is a major strength. However, tailoring of laboratory processes for the needs of research projects is often not possible.

Research laboratories typically work in a state of constant change, tailoring methods and processes, and focusing on scientific progress and development. A high quality level cannot be emphasized enough, because it prevents waste of resources and inaccurate interpretations.

Performing the actual analysis is an important step in obtaining high-quality laboratory results. However, studies have shown that the pre-analytical and post-analytical phases are even more important, because most of the errors occur there.

Plebani et al.2017

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6 Pre-analytical factors – what are they?

Pre-analytics has a major impact on the quality of any human study collecting biological samples. The term covers a wide range of factors affecting the early phase of the laboratory process, such as patient characteristics, choice of sample type and mix-ups during sample collection.

Pre-analytical sources of variability can be divided to four categories, relating to:

- test or biomarker
- patient preparation
- specimen collection
- specimen processing, transport and storage

Simundic et al. 2012

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7 Post-analytical phase – turning data into information

In the final post-analytic phase laboratory results are transferred to clinicians or researchers who interpret them and turn them into information, decisions and recommendations. Handling of the data is an important step, because errors are easily created when data is being transferred. Up-to-date and well-functioning data communication systems prevent losing or unintentionally changing data.

Another type of vulnerability is the interpretation of laboratory results. Inappropriate interpretations is a highly relevant problem in academic research environments, because of the high turn-over of staff and use of student work-force.

Staff competency is a crucial success factor in all phases of laboratory medicine and research. Limited knowledge in laboratory medicine or inexperience in interpreting laboratory data may easily yield incorrect interpretations. When hypotheses are rejected based on inaccurate assumptions or results over-exaggerated, it is a serious problem. Resources may be wasted and scientific efforts taken in the wrong direction.

A functioning dialogue between the laboratory and researchers using the data is crucial to prevent misuse of data and optimal use of resources. Ideally it is set up at the early stages of project planning and kept up until results or recommendations have been published.

Plebani et al. 2017

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8 Obtaining samples for monitoring purposes

Ideally, monitoring is part of national health-examination surveys (HES). Choosing a representative sample of the population, with sufficient sample size, minimises the risk of incorrect interpretation of results due to chance error or selection bias. Unfortunately, samples from vulnerable groups are not always collected in HES. Therefore, less representative samples from smaller research projects are often accepted for monitoring purposes. In these cases too, the information may be useful, although more suggestive by nature.

The European Health Examination Survey (EHES) Manual provides guidelines and specifies the requirements for implementation of standardized national health examination surveys (HES) in the European countries.
http://www.ehes.info/manuals/EHES_manual/EHES_manual.htm

The WHO STEPS Surveillance Manual: The WHO STEPwise Approach to Chronic Disease Risk Factor Surveillance provides similar guidelines, especially suitable for developing countries.
http://www.who.int/chp/steps/STEPS_Manual.pdf?ua=1

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9 Choosing laboratory

A laboratory with a high quality level should be chosen. Accredited laboratories are recommended, because they comply with international standards for requirements of testing laboratories (e.g. ISO/IEC 17025:2005) and furthermore, they are regularly audited. Research laboratories are typically not accredited, but they also need to show that analyses are performed with sufficient quality. An existing quality system is one proof of quality.

The quality system of the laboratory should cover at least the following topics:

- Management part: management system, documentation/record keeping system, internal/external audits, corrective preventive actions
- Technical part: lab environment, staff competency, equipment, laboratory methods, method validation, quality assurance, proficiency testing, reporting system, known measurement uncertainty

CLSI. 2011.

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10 Choosing biomarker for iodine intake – urinary iodine concentration (UIC)

- Median urinary iodine concentration (UIC) is a well-validated biomarker of iodine intake
- Established recommendations and cut-off values for different levels of deficiency exist. They are issued by WHO and are based on spot urine UIC
- UIC is considered a sensitive indicator of recent iodine intake.
- Inclusion of at least 100 subjects is generally recommended for spot samples to estimate population median. The exact number depends on the distribution of UIC and may vary.
- Between-day variability of UIC is high and therefore about 10 spot samples are required to reliably estimate intake on an individual level.
- Urinary iodine can also be measured in relation to creatinine excretion to correct for differences in urine volume and sample dilution. It is useful in some population groups, however, this approach brings other types of variability into the results, and may be problematic in regions with malnutrition.
- 24-h urine samples are sometimes used to reduce variability, but lack of comparable data as well as reduced compliance or participation may outweigh the benefits.

Knudsen et al. 2000, Rasmussen et al. 2002, WHO 2007, Zimmermann 2009, Karmisholt et al. 2014, Perrine et al. 2014, Pearce and Caldwell 2016.

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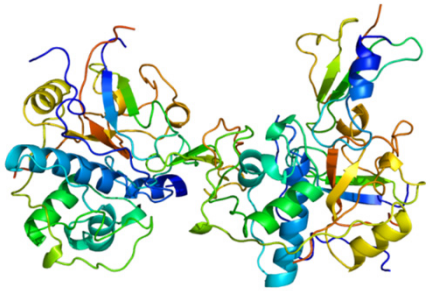
11 Choosing biomarker for iodine intake – thyroglobulin (Tg)



- Tg is a thyroid-specific glycoprotein
- Precursor and storage site for thyroid hormones T3 and T4
- Reflects iodine nutrition over a period of weeks/months
- Elevated in goitre, iodine deficiency/excess, thyroid disorders
- Normal range in adults: 3-40 µg/L
- Serum-Tg is a more sensitive marker of iodine status in children and adults than other thyroid tests (e.g. TSH, FT4)
- Thyroglobulin antibodies (TgAb) are common in adults and cross-react with Tg
- If Tg is measured for adults, TgAb should also be measured. This is less important for children's studies
- Dried blood spots (DBS) are also used because they are easy to collect and transport
- Results are method dependent and not easily standardized, making it difficult to compare results obtained by different methods

Rasmussen et al. 2002, Vejbjerg et al. 2009, Krejberjer et al. 2015

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12 Choosing the right thyroid biomarkers

Thyroid hormone measurements are useful for identifying abnormal thyroid function. Useful thyroid function biomarkers are thyroid-stimulating hormone (TSH), free thyroxine (FT4) and thyroglobulin (Tg). Total T4 can be of additional value in specific cases, such as pregnancy or disorders in binding proteins. Thyroid peroxidase autoantibodies (TPOAb), thyroglobulin autoantibodies (TgAb) and TSH receptor autoantibodies (TRAb) may help to clarify the cause of thyroid dysfunction.

Thyroid hormone and antibody levels are altered by changes in iodine intake. However, the thyroid's ability to adapt to changes in iodine intake varies between individuals. Also, several factors affect thyroid hormone levels (e.g. thyroid related autoimmune diseases, genes, pregnancy). Therefore, thyroid hormone levels alone cannot be used to estimate iodine intake in populations, other measures of iodine intake are also needed.

Bjergved et al. 2012, Ittermann et al. 2015, Pearce and Caldwell 2016, Bliddal et al. 2017, McLachlan and Rapoport 2013

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13 Standardisation of urinary iodine measurements

Standardisation of iodine measurements is possible, because adequate reference materials and an external quality assessment scheme are available.

Ensuring the Quality of Urinary Iodine Procedures Program (EQUIP):

- Organized by the Centers for Disease Control and Prevention (CDC).
- 3 rounds per year, free of charge
- Participation highly recommended
- Results are traceable to NIST standard reference materials with certified mass concentration values (SRM 2670a) and reference mass concentration values (SRM 3668).
- Laboratories may purchase the same reference materials from NIST that EQUIP uses to recalibrate their method (if EQUIP results indicate a need for that). However, it should be kept in mind that such a procedure does not improve repeatability or correct for poor method performance.

In the EUthyroid project, iodine measurements from the national and regional IDD monitoring studies will be standardised by a centralized laboratory at the National Institute for Health and Welfare (THL) in Helsinki, Finland.

Makhmudov and Caldwell 2011

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14 Harmonisation of thyroid related measurements

For TSH, standardisation to a reference measurement procedure is not possible, because a reference material with certified mass-concentration value is not available. Most methods are traceable to WHO International standard (80/558), which is obtained from cadaver pituitary and is non-commutable. However, harmonisation of laboratory methods and procedures is a realistic aim, making the results more agreeable and comparable (although not necessarily more accurate).

External quality assessment schemes (EQAS) are highly recommended. Several programs are available including Labquality, NEQAS, etc. Participation in EQAS shows how results from one laboratory or method relate to others. However, it does not make the results more accurate, partly because EQAS methods suffer from similar standardisation problems as the individual methods.

The need for harmonisation has been widely recognised. The Committee for Standardization of Thyroid Function Test (C-STFT) and equipment manufacturers recently joined their efforts and work towards improved harmonisation of thyroid related measurements.

In the EUthyroid project thyroid biomarkers measured in national and regional IDD monitoring studies are standardised by a centralised laboratory at the National Institute for Health and Welfare (THL) in Helsinki, Finland. This allows standardisation of results and a comparison of thyroid hormone status in Europe.

Greenberg 2014, Faix and Miller 2016.

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15 Typical pitfalls

- Lacking or superficial study manual
- No pilot study performed (difficult steps should be rehersed)
- Poor staff training (insufficient training generally or lack of trained substitutes)
- Data linkage not possible (e.g. errors in the labelling system linking participant with forms and samples)
- Human errors (e.g. mix-ups when collecting, labelling or aliquoting specimens)
- Wrong choice of specimen, specimen not suitable for specific biochemical analysis
- Inadequate participant preparation
- Poor adherence due to unclear instructions (diet, fasting time, medications, smoking, physical activity sampling time)
- Lack of sample rejection criteria
- Unorganised documentation (e.g. lack of sampling documentation or lack of documentation of unexpected events during sample lifespan)
- Inadequate sample storage or shipment temperature
- Lack of laboratory audits
- Poor quality of laboratory measurement
- Post-analytic phase: insufficient measurement of confounders or inadequate adjusting for them

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16 Analyzing costs

Types of costs (examples):

Direct costs

- Intervention, side effects/adverse events, medications, personnel, transportation, materials, equipment, subcontracting

Indirect costs

- Productivity loss, time costs

(Intangible costs)

- Non-monetary costs

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16 Analyzing costs - continues

Steps of a cost-analysis:

Identification of resources/services

- What resources are needed for monitoring activities?
- What services are provided to the population?

Investigation of quantities

- How often on average are resources/services needed/provided?
- Database for quantities of resources/services

Collection of prices

- Price per resource?
- Database for prices

Determination of costs

- What? How often? Price? Proportion of the population?
- Database for costs

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PART B.
SPECIMEN COLLECTION AND SAMPLE HANDLING

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1 Sample collection - standards and practices

- Different standards and practices related to sample collection are applied in different countries and vary between institutions within a country
- For phlebotomy, WHO's guidelines may be used to establish standard operating procedures.
- A new standard for blood sample collection was recently published by CLSI
- When planning a study and preparing SOP's, it is important to follow local practices. The staff has usually been trained locally and changing old habits is surprisingly difficult.
- All laboratory specimens should be handled as infectious and standard precautions applied
- Detailed written instructions on sample collection and treatment should be at hand for staff performing sample collection. This includes diagrams with order of blood draw, types and number of blood collection tubes, number of aliquots, etc. Priority list for use of samples in the laboratory should also be available to staff.

WHO 2010, CLSI 2017.

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2 Participant preparation in advance

- Clear instructions on dietary restrictions must be given
  - Fasting
  - Intake of liquids (water as usual, no caffeine containing beverages or other drinks)
- Smoking should be refrained from
- Medications – thyroid medications are usually not taken on the morning of sampling
- Physical activity - avoid heavy physical activity prior to sampling
- Timepoint of blood sampling must be given
  - Morning is preferred
  - Circadian rythm plays a role - not always possible to collect sample at ideal time of day
- Timepoint of sampling should be given

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3 Participant reception at study site

- Identify the participant
- Obtain consent
- Record all relevant information about the participant: name, ID, date and time of sampling, fasting time, hours urine has been in bladder, medications
- Explain the sampling procedure to the participant in detail
- Ask if the participant is anemic or whether any condition or impediment hindering sample collection exists

Woodcock et al. 2010

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4 Identification by using a unique indentifier

- Unique identification must be used to link participants to their consent, questionnaires and laboratory samples.
- All forms and sample collection tubes/vials must contain a label with the participants unique identification number.
- Labelling sheets with participants' all labels should be prepared beforehand.
- On the first visit to the study site, the participant receives a random number/labelling sheet, which is used throughout the study.
- Crucial step – any vials or forms lacking unique identifier should be discarded, any potential mixups must be immediately and reliably recorded.
- If labels are printed out when particant arrives to the site, problems with data systems may slow down sample collection and be very annoing for the participant
- Suitability of label materials for storage in deep-freezing temperatures should be tested beforehand.

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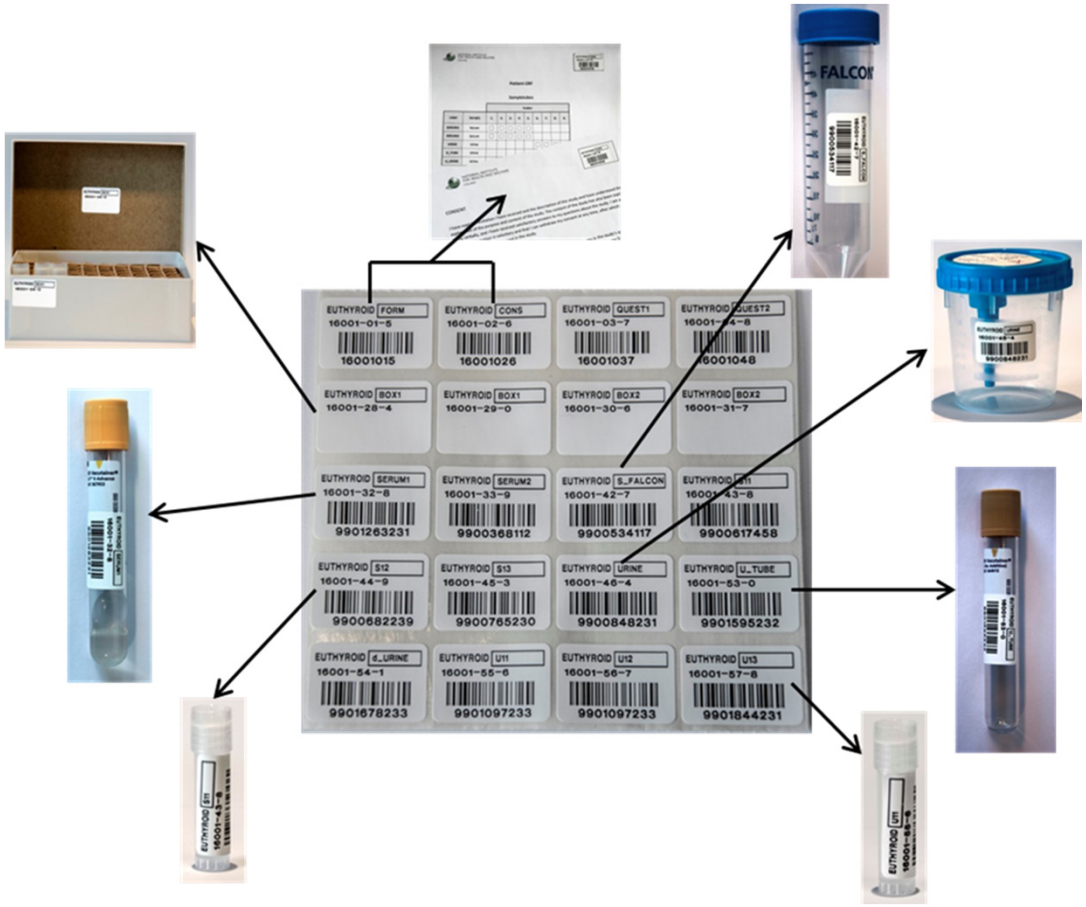
A label with a unique identifier should be attached to all samples and forms obtained from a participant



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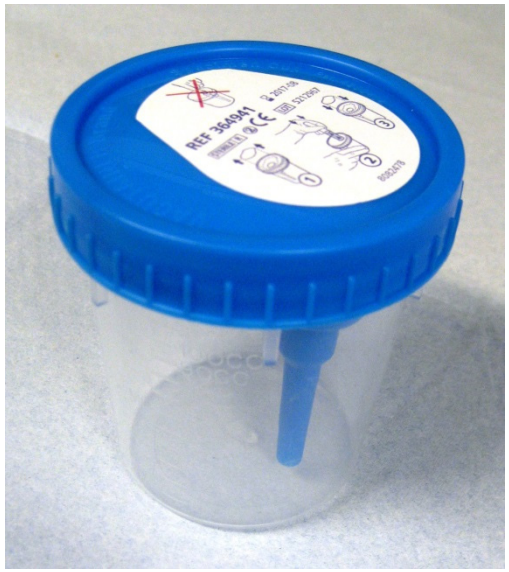
5 Urine sampling

5.1 Workplace preparation

Materials needed:

- Toilet with shower head or towelettes
- 24h sample:
  - Urine collection cup or a specimen collection pan that fits in the toilet
  - Urine storage container (brown or orange container with volume scale is preferred)
- Spot sample:
  - Urine collection cup with integrated transfer device or urine collection cup/bag with transfer straw
- No stabilizers in urine tubes allowed unless suitability tested for iodine measurements.
- If dipstick testing is performed, part of the sample should be poured into a separate tube for that purpose
- Labels

Supplies should only be used until the declared expiry date.



Urine collection cup with an integrated transfer device

ECLM 2000, CLSI 2009

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5.2 Urine sample collection

5.2.1 Cleansing

- Aim for a clean catch specimen to avoid bacterial growth in sample
- Wash your hands with soap and warm water
- Female:
  - with a towelette or hand shower, cleanse the urethral meatus and surrounding area from the front to the back
- Male:
  - withdraw the penis foreskin and with a towelett or shower, cleanse the glans and urethral opening from the front to the back
- Infants:
  - cleanse the entire pubic and perineal areas and dry them

ECLM 2000, CLSI 2009

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5.2.2 24h-sample

A 24h sample collection may start at any time during the day, but usually it begins in the morning.

- Empty bladder into the toilet (this urine is not collected). Write down the starting time.
- During the following 24 hours, collect all urine passed into a collection cup.
- Pour the urine into a storage container each time you urinate. Keep the storage container cold and protected from light. The sample must not freeze.
- 24 hours after the start time, urinate again at the same time, to finish the collection process. Write down the time.
- Document unexpected events (e.g. losing part of the sample, strong sweating because of sauna, physical stress etc).
- Document the use of medications during sample collection.

Once the urine collection has been completed, the storage container should be taken to the lab (ASAP).

- Total sample volume and the quality of sample must be documented. Ensure that the catch is homogeneous.

ECLM 2000, CLSI 2009

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5.2.3 Spot sample

Collect the mid-stream urine specimen in a urine collection cup or bag.

- Hold your urethral opening open
- Allow the first urinary flow to escape, collect the mid-stream urine and finish urinating into the toilet bowl
- Do not touch the inside of the collection cup

Infants

- Place an adhesive urine collection bag over the infants' genital area and wait until it is filled.

  OR
- Collect the sample in a urine collection cup by placing the cup into the front of a potty.

  OR
- Collect the urine in a pad placed inside a diaper (only pads tested for this purpose may be used).

ECLM 2000, CLSI 2009, Dorey and Zimmermann 2008

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5.2.4 Sample transfer to the collection tube

- Performed by patient or staff, depending on study
- Follow separate instructions from your laboratory.
- Generally:
  - Label cups and tubes as instructed
  - After collection of the urine sample, close the urine collection cup tightly
  - Transfer part of the sample into the urine tube that will be taken to the laboratory. Mix the urine sample before transferring it (do not shake)
  - Store the tube as instructed
  - Dispose surplus sampling materials

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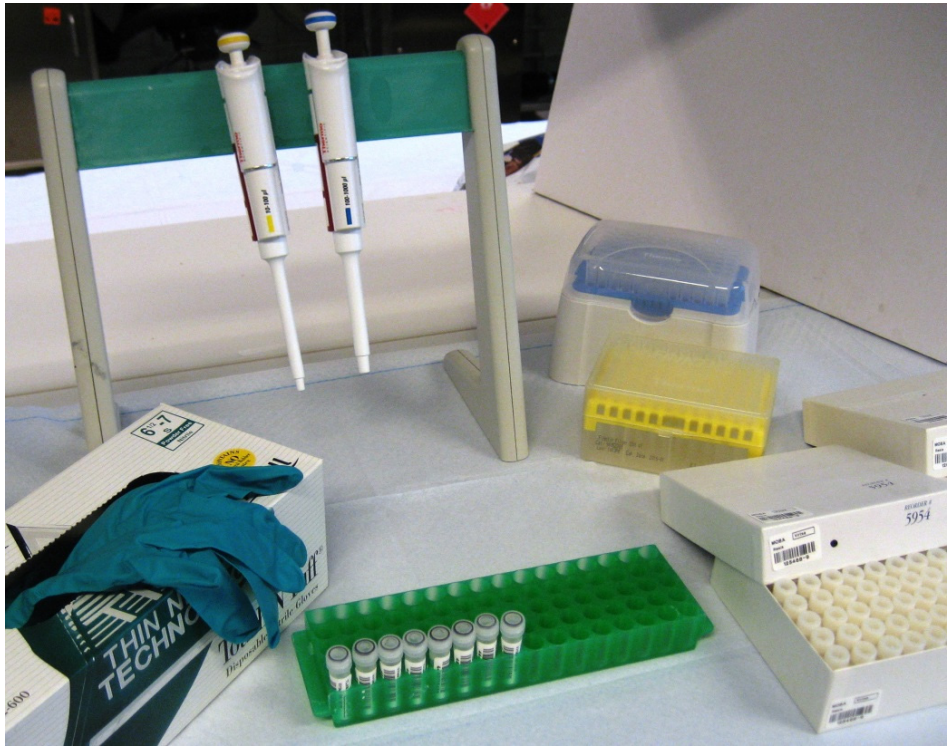
6 Urine sample handling

6.1 Workplace preparation

Materials

- Pipette
- Storage tubes
- Storage boxes
- Disposable gloves



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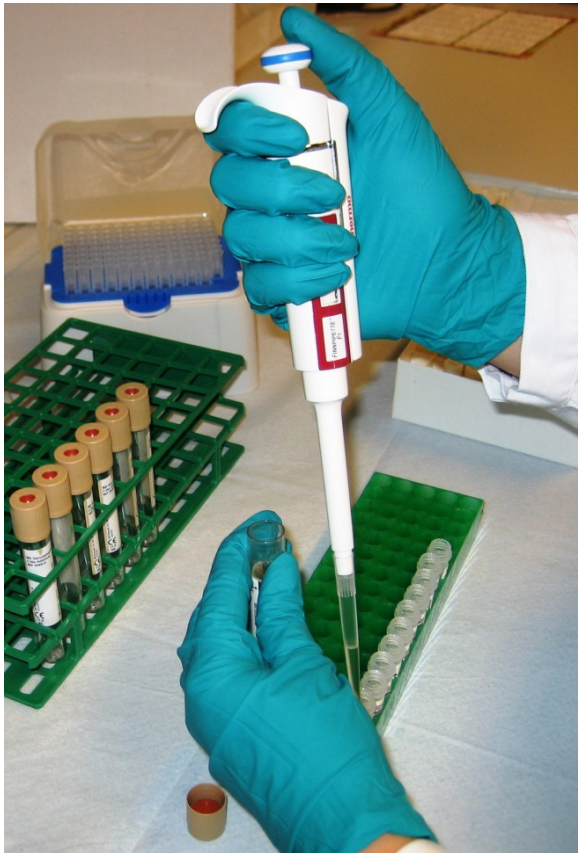
6.2 Sample transfer to the storage tubes

- Label storage vials
- Pipette aliquots into storage vials, close caps
- Place the vials into the storage boxes, label boxes and place them into the freezer
- Temperature -20 °C or colder



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7 Blood sampling

7.1 Workplace preparation

Materials:

- Disinfection solution (>80 % alcohol)
- Blood collection tubes
- Needle with integrated needle holder (new standard)

  OR
- Needle + needle holder
- Tourniquets
- Swabs, gauze pads, skin tape, bandages
- Disposable gloves

Supplies should only be used until the expiration date.

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7.2 Blood sample collection

7.2.1 Preparing for blood sampling

- Ask participant to remove tight clothes that might constrict the upper arm
- Participant should be sitting in a comfortable chair with arms to provide support in case the patient faints
- If possible, the participant should remain in sitting position five to ten minutes prior to blood sampling
- Participant's position: hand downward, palm upwards
- Use gloves or disinfect hands
- Phlebotomy may be performed by qualified personnel only

Alfthan et al. 2016

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7.2.2 Blood sampling

Blood sampling instructions with the vacuum technique:

- Apply tourniquet (not necessary if vein is easy to find)
- Select vein
  - The median cubital vein is the first choice for blood collection. This vein is usually large, visible, well anchored and does not bruise easily.
  - The cephalic vein is the second choice for blood collection. It is not as well anchored and may be more difficult to find.
  - The basilic vein is the third choise for blood collection. This vein tends to roll away and bruise more easily.
- Clean the site with disinfectant, let dry
- Venipuncture and start filling the tubes in the specified order
- Loosen the torniquet immediately when the bloodstream runs
- Fill the tube
- When the tube is filled and the bloodstream stops, take the tube out of the holder
- Check that the tube is filled to the adequate volume
- If several types of blood samples are collected, the order of draw is citrate → serum → heparin → EDTA. Do not take the needle out from the vein when changing tube.

Alfthan et al. 2016, CLSI 2017

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7.2.3 After blood sampling

- Immediately after removing the holder, mix the serum tube by inverting it top-down 5-10 times (depeding on the manufacturers's instructions)
- Store the tubes in a vertical position until sample handling
- Apply the gauze and remove the needle from the vein
- Apply pressure on the punctured site with the gauze until bleeding stops and bandage the arm
- Dispose all surplus sampling materials according to your facility's policy
- Label the tubes in the presence of the participant
- Document the number of sample tubes, time of sampling and unexpected events during sample collection
- Examples of unexpeted events:
  - Stasis not opened: Prolonged venous occlusion can cause changes in the concentration of blood constituents.
  - Participant lying down during sampling: The position of the subject can influence some of the measured values.

Alfthan et al. 2016

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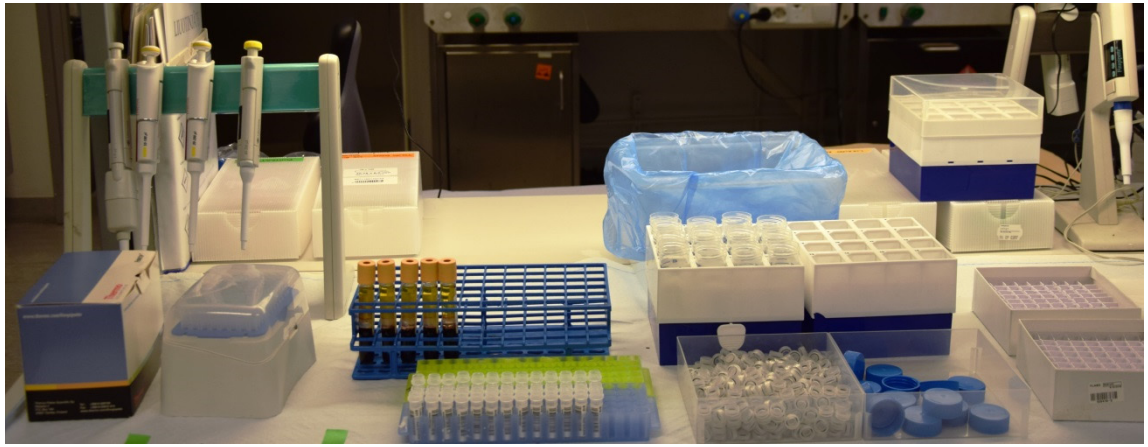
8 Blood sample handling

8.1 Workplace preparation

Materials

- Centrifuge
- Pipette
- (Pooling tube)
- Storage tubes
- Storage boxes
- Disposable gloves



Separate serum or plasma according to manufacturers' instructions

Serum should be separated within 60 minutes (at the most)

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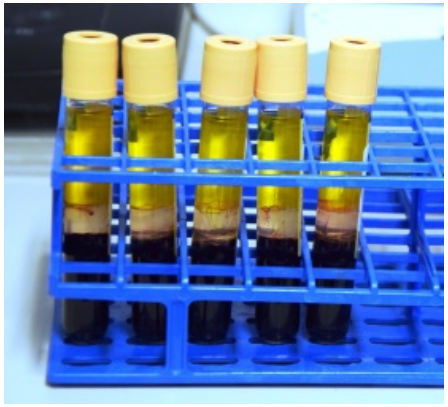
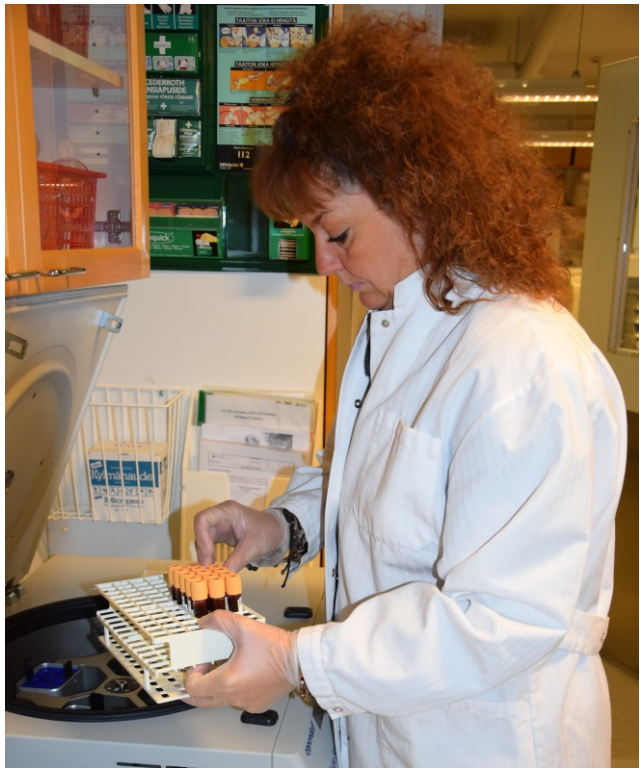
8.2 Centrifugation

- Place the sample tubes in the centrifuge
- Check that all tubes are resting on the bottom of the centrifuge rack and that the centrifuge rotor and tubes are in balance
- Centrifuge the tubes according to manufacturer's instructions (typically at about 1000 g at room temperature)
- After the centrifugation check that serum has been separated properly



Well-separated serum samples



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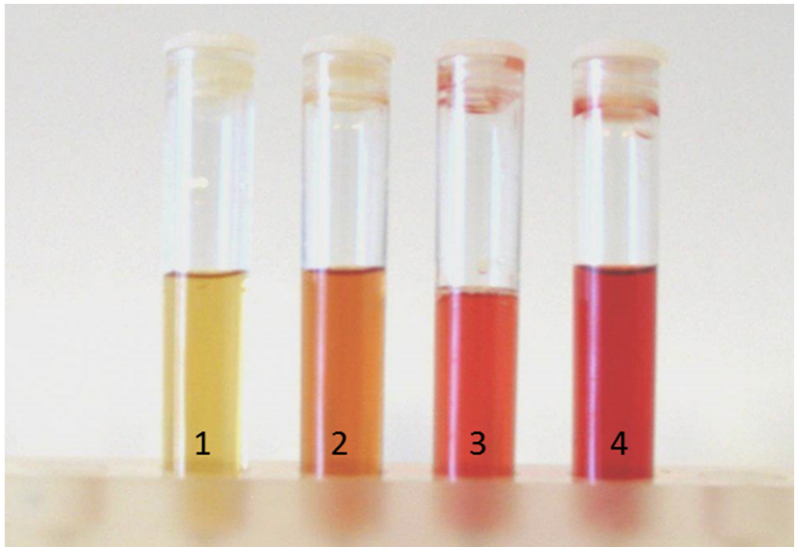
8.3 Hemolysis

- Hemolysis means the destruction of red blood cells and the release of hemoglobin into the surrounding fluid.
- If the classification of hemolysis is based on visual inspection, a photograph of different degrees of hemolysis should be kept close by



1 Not hemolysed
2 Slight hemolysis
3 Moderate hemolysis
4 Gross hemolysis

Alfthan et al. 2016

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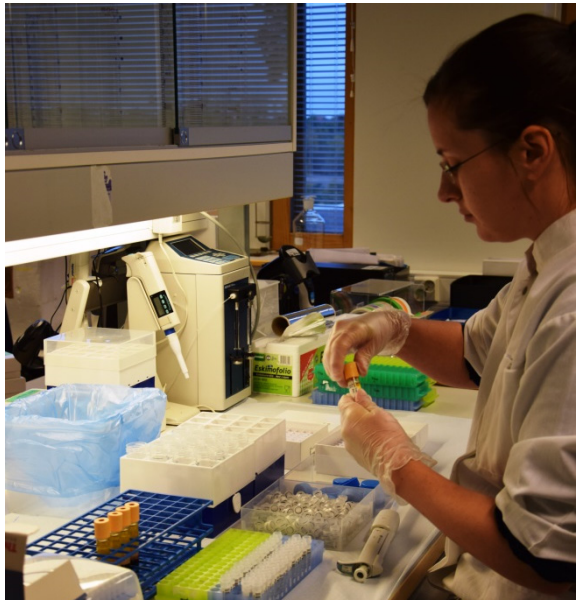
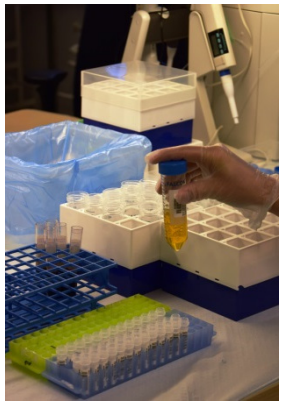
8.4 Sample transfer to the storage tubes

- If you have more than one sample tube, separate the serum from the sample tubes into a pooling tube and mix gently
  - If one of the samples is hemolysed, do not mix it with the others
- Document the quality of samples (hemolysed, lipemic, icteric), the abnormal sample amount and which storage vials contain abnormal sample
- Label storage vials – use labels which have been tested for your process
- Pipette aliquots into storage vials, close caps
- Place the vials into the storage boxes, label boxes and place them without delay into the freezer (-70 °C)





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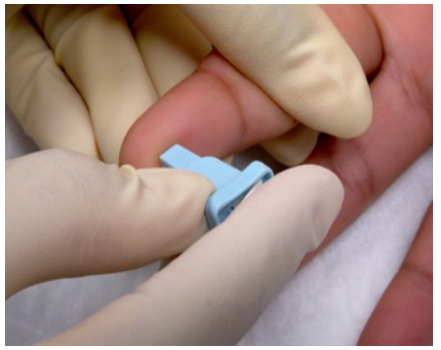
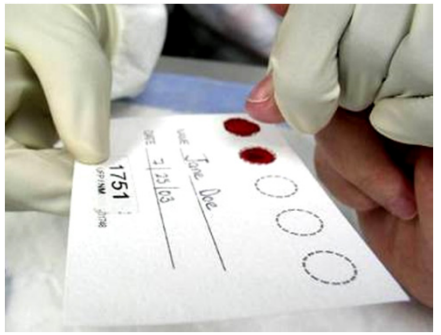
9 Dried blood spot (DBS) sampling

- Position hand palm-side up, choose least calloused finger
- Clean fingertip with alcohol, let air dry
- Hold the finger, place sterile lancet off-center to the fingertip, firmly press the lancet into the tissue
- Wipe away first drop of blood
- Invert the finger, allow a large drop of blood to form on the tip of the finger, touch it to circle on filter card
- Apply 1 drop per circle, the circle has to be completely filled, blood has to soak through the paper
- Label the card, air-dry for 24h, store at -20°C in sealed plastic bag (several cards per bag)





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10 Common mistakes when taking DBS and tips to improve spot size and quality

- Blood drop too small → circle on card not completely filled or paper not soaked through
- Several blood drops applied to 1 circle
- Finger was touched to the filter card, blood smeared
- Squeezing/milking the finger too hard therefore ”diluting” the blood
- Card did not dry sufficiently

- Warm up finger by massage, kneading it / rotating arm
- Press lancet down firmly, use at least2x1.5mm lancet
- Hold finger below height of heart while waiting for drop to form
- if blood flow stopped, wipe the finger, maybe it was already clotted
- Softly (!) press the finger to increase blood flow
- Find which finger works best (index finger is not recommended)
- Maybe ask for a second prick on another finger

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11 Documentation

Clear documentation instructions for all procedures must be prepared beforehand. Deviations from instructions should be documented in an organized way. Comments from entire sample lifespan should be collected. A coding system should be developed beforehand and piloted. After that it is ideally kept unchanged throughout the project. Space for free-text comments should be given, but should be exceptions, because they are more difficult to use later.

Typical neglections:

- Inappropriate coding system or poor adherence to it by staff
- Events during specimen collection (e.g. patient position, prolonged tourniquet application) not documented
- Time of specimen collection and fasting time not documented
- Quality of sample not documented
- Sample comments not available from entire sample lifespan
- Sample rejection criteria lacking
- Lacking visual inspection of sample
- Documentation of lipemia or degree of hemolysis lacking
- Number of freeze-thaw cycles not reliably documented

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12 Sample shipment

- Plan the sample shipments in advance
- Make sure that appropriate shipment conditions will be retained during the whole shipment (e.g. frozen samples)
- Use reliable courier
- The shipment has to be packed according to IATA (International Air Transport Association) regulations
- Packing should be done according to relevant packing instruction
- Regulations should be followed when using dry ice

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C. LABORATORY ANALYSIS

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1 Urinary iodine concentration (UIC)

- Urinary iodine concentration (UIC) is used to define the iodine status of populations.
- The most commonly used methods are based on the Sandell-Kolthoff reaction. It is a relatively economical option and the method can be set up in any laboratory. However, the staff needs to be relatively skilled, because the method is not among the simplest ones.
- The gold standard method is inductively coupled plasma - mass spectrometry (ICP-MS) . This method requires a suitable laboratory environment with more expensive equipment. The staff should be highly skilled and experienced, with special know-how in performing mass-spectrometric analyses and maintaining a suitable laboratory environment.
- In the EUthyroid project, UIC from various European studied are compared after a standardisation based on reanalysis of a subset of samples from each study. The analyses are performed by ICP-MS at the National Institution for Health and Welfare (THL).

Shelor and Dasgupta 2011, Pearce et al. 2016 Völzke et al. 2016

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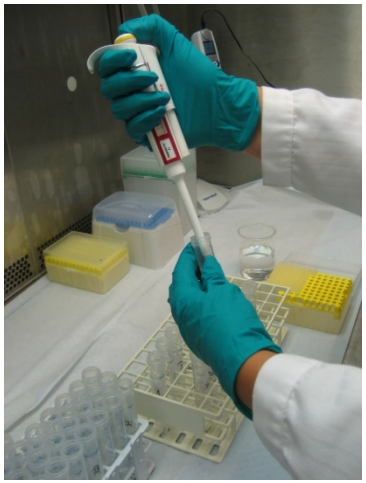
1.1 ICP-MS method

Inductively coupled plasma - mass spectrometry (ICP-MS) ionizes the sample with inductively coupled plasma and then uses a mass spectrometer to separate and quantify specific ions. ICP-MS is capable of detecting metals and several non-metals at very low concentrations.

Urinary iodine is usually measured by preparing the samples in basic solution (using TMAH or NH_4OH).



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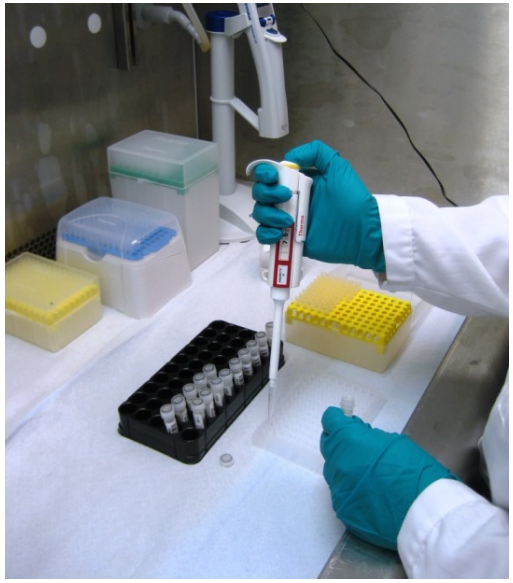
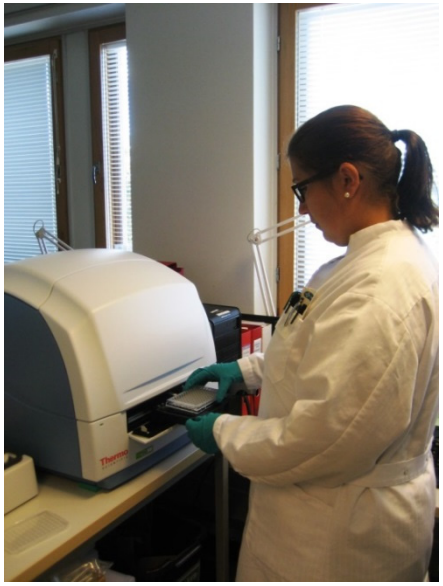
1.2 Sandell-Kolthoff Reaction

In the Sandell–Kolthoff reaction, iodide catalyzes the reduction of yellow ceric ion to the colorless cerous form in the presence of arsenious acid. Impurities present in urine may hamper the analysis. Usually, the sample is pretreated by alkaline ashing or by acidic or basic digestion.

When using a method based on the Sandell-Kolthoff reaction, it is important to use calibrators and controls in the same concentration range that your samples exhibit. Within the same country, some population groups can show high UIC values while others, especially vegans, may have very low UIC values.





$$Ce^{4+}(\text{yellow}) + As^{3+} \xrightarrow{I^-} Ce^{3+}(\text{colorless}) + As^{4+}$$

Elorinne et al. 2016

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1.3 Other methods

Less common methods include:

- Gas chromatography mass spectrometry (GC-MS)
- Inductively coupled plasma atomic emission spectrometry (ICP-AES)
- Atomic absorption spectrometry (AAS)
- Capillary electrophoresis (CE)
- Flow injection analysis (FIA)
- Instrumental neutron activation analysis (INAA)

Khazan et al. 2013

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1.4 Effect of preanalytical factors on UIC

- Fasting status – lower values obtained for samples collected after fasting - record status and hours of fasting
- Within-day variation – mainly caused by variation in intake during the day and possibly circadian rythm
- Lower UIC expected in the morning compared to the afternoon
- Iodine is stable during long-term storage in the freezer
- Shipping or storage in non-freezing temperature presents a problem to quality of urine sample, rather than stability of iodine
- Repeated freeze-thaw cycles is not a problem
- Use of iodine containing drugs may affect iodine concentrations – rare problem
- Stabilizers in urine collection tube may interfere with analysis - avoid stabilizers and if used, record it
- Suitability of all materials and reagents used for urine collection as well as analysis of iodine should be tested to avoid contamination with iodine. Suppliers declare if materials are trace element free and suitable for ICP-MS analysis
- Dipstick contamination - Urine samples collected at antenatal clinics are often tested for glycosuria and proteinuria by using dipsticks. This may contaminate the sample, therefore dipstick testing should be performed from a separate vial

Pearce et al. 2009 and 2016, Perrine et al. 2014, Mirjanic-Azaric et al. 2015

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2 Thyroglobulin (Tg) measurements

- Serum and dried blood spot Tg are used to define the iodine status of populations
- Suitable especially for children, because Tg autoantibodies are less prevalent compared to adults
- Radio-immunoassay (RIA) and various immunoassays with varying functional sensitivity can be used to determine Tg
- Recently, a new low-cost serum and DBS-Tg sandwich enzyme-linked immunosorbent assay was developed

Pearce et al. 2016

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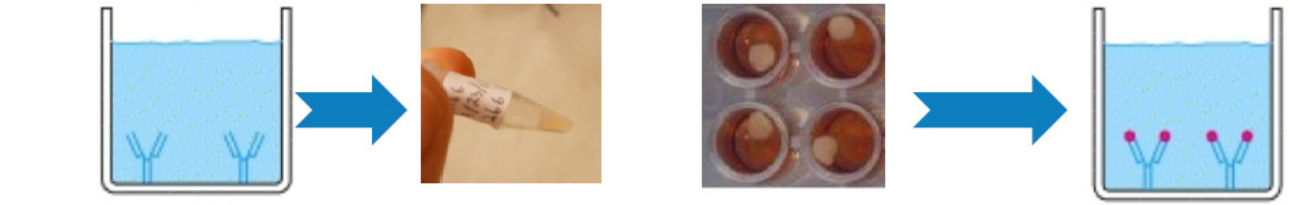
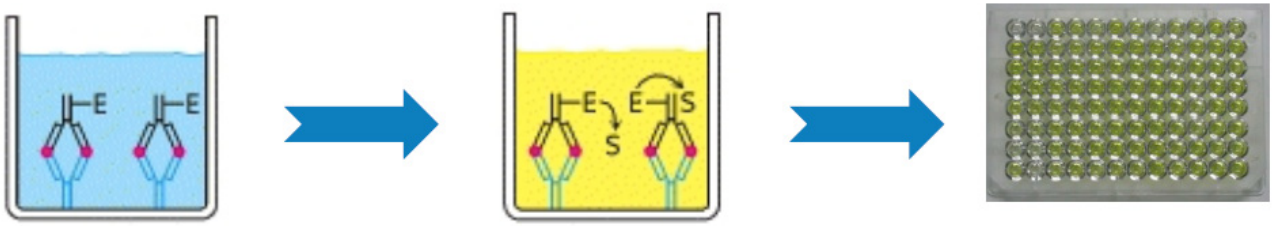
2.1 Principle of sandwich ELISA method



Wells are coated with antibody

Serum or DBS samples are added to the wells

Tg binds to the antibody



A second antibody, linked to an enzyme, binds to Tg

Substrate is added and converted by enzyme into colored product

Absorbances are read at 450 and 620 nm

Stinca et al. 2015

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2.2 Effect of preanalytical factors on Tg

- No effect of repeated freezing and thawing of sample
- No effect of 15-week storage in -20 °C
- Intra-individual variability ~14%
- Smoking increases Tg and must be accounted for
- Age should be accounted for
- TgAb are prevalent in adults and interfere with the Tg measurement

Knudsen et al. 2002, Stinca et al. 2015, Hillebrand et al. 2017

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3 Thyroid function and autoantibody measurements

- Useful laboratory measurements include
  - TSH - Thyroid-Stimulating Hormone (Thyrotropin)
  - FT4 - free Thyroxin
  - FT3 - free Triiodothyronine
  - TPOAb - Thyroid Peroxidase Autoantibodies
  - TgAb - Thyroglobulin Autoantibodies
  - TRAb – THS Receptor Autoantibodies
  - (TT4 - total Thyroxin, rarely used nowadays)

- In EUthyroid, the most commonly used thyroid-related laboratory measurements are included

Pearce et al. 2016,
McLachlan and Rapoport 2013

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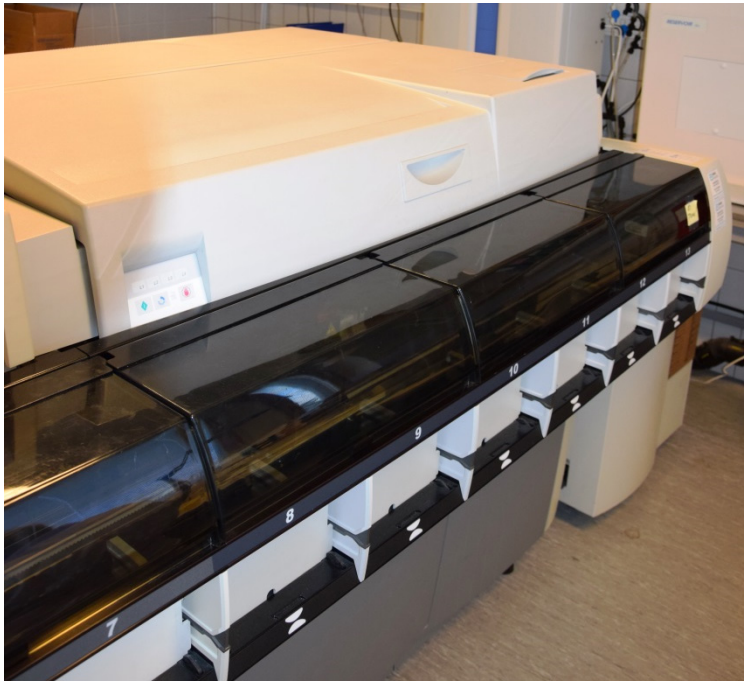
3.1 Clinical chemistry and immunoassay testing

Thyroid-related laboratory measurements are usually performed on automated clinical chemistry and immunoassay platforms. They are available from several providers such as Roche, Siemens, Thermo-Fischer, Abbott etc.

In the EUthyroid project, TSH and thyroid autoantibody results from various European studies are compared after standardisation, which is based on reanalysis of a subset of samples from each study. The analyses are performed at the National Institute for Health and Welfare (THL). The ARCHITECT two-step immunoassays for quantitative determination of human serum (or plasma) using Chemiluminescent Microparticle Immonuassay (CMIA) technology is used.



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3.2 Thyroid stimulating hormone (TSH)

- The most commonly used thyroid-related laboratory measurement
- Used to diagnose thyroid dysfunction and for monitoring of treatment
- Typical additional measurements: free thyroxine (FT4) and thyroid autoantibodies
- Inverse loglinear relationship between TSH and FT4
- TSH measurements are relatively reliable, although results vary by method

Spencer 2017. Faix and Miller 2016

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3.3 Thyroid autoantibodies

- Thyroid peroxidase autoantibody (TPOAb) and thyroglobulin autoantibody (TgAb)
- Primarily recommended as adjunct to other thyroid measurements
- Measured to diagnose autoimmunity-mediated thyroid dysfunction (mainly TPOAb is used)
- TgAb is primarily used in the clinic as a surrogate tumor marker and adjunct to thyroglobulin (Tg) measurement.
- TgAb is also measured to rule out interference on Tg measurement
- Higher iodine intake increases prevalence of TPOAb, but it is unclear whether occurence of low titers is a pathological finding or not.
- Usefulness of antibody measurements is limited by differences in method sensitivity and specificity
- Considerable variability between TPOAb and especially TgAb methods, which hampers comparability of numeric results.

Tozzoli et al. 2016

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3.4 Effect of preanalytical factors on TSH

- A standardized fasting time, similar to what is generally recommended for population studies, will yield more comparable results and allows the use of a single biosample for different analyses. For TSH, non-fasting samples yield somewhat lower TSH results. In the clinic, however, fasting is not usually required.
- Circadian rhythm – lower values obtained for TSH if using other than morning samples (up to 30% difference)
- Intra-individual variation ~20%.
- No need for resting prior to blood sampling
- Effect of storage time is small
- Effect of repeated freeze-thaw cycles small
- Use of certain medications may affect TSH measurement and thyroid function (e.g. glucocorticoid therapy, iodine containing agents)
- Presence of interferring antibodies may distort laboratory results (rare problem)
- Smoking has adverse effects on thyroid function and impacts on TSH levels

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3.5 Preanalytical variables in assessment of thyroid function

Table 1. Classification of preanalytical variables in assessment of thyroid function.

Physiological	*Iatrogenic causes*
Set point for T_4	Prior thyroid disease treatment
Log-linear relation of TSH/FT_4	Surgical
Circadian rhythm	Medical
Seasonal influences	Drug therapy
Environmental	Systemic
Exercise	Topical
Posture and immobilization	Plasmapheresis
Pathophysiological	*Specimen-based variables*
TSH-independent states	Stability and storage
Common	Lipemia
Rare	Stasis
Trophoblastic tumors	Hemolysis
Struma ovarii	Icterus
Generalized resistance	Assay susceptibility
Selective organ resistance	Antibodies
TSH-dependent states	Heterophile
Pituitary adenoma	Autoimmune
Resistance states	Protein binding
Generalized	*Human error*
Pituitary	
Psychiatric states	
Smoking	
Malabsorption	
Compliance	

Keffer 1996.

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