

# Appendix 1

QUESTIONNAIRE ON SUDANESE PATIENTS WITH THYROID DISEASES

## Questionnaire on Sudanese Patients with Thyroid Diseases

(A) General information:

- Name ----- No.(    )
- Age (year) -----
- Sex: male (    ); female (    )
- Tribe :-----
- Residence :-----
- Occupation:-----

(B) family history of thyroid disorder: yes(    ); No(    )  
If yes: First degree (    ); Second degree (    )

(C) duration of disease:-----year(s) -----month(s)

(D) diagnosis:-----  
-----

(E) drugs:-----  
-----  
-----

(F) Laboratory investigations:

Test	Result
HLA-DR	
HLA-DQ	
Anti-TPO antibody	
Anti-Tg antibody	
TSH	
T <sub>4</sub>	
T <sub>3</sub>	

## **Appendix 3**

### **Materials for TSH test by Immulite**

**Kit components** (supplied separately)

TSH sample diluent (LTSZ)

LSUBX: Chemiluminescent substrate

LPWS2: Probe wash module

LKPM: Probe cleaning kit

LCHx-y: Sample cup holders (barcoded)

LSCP: Sample cups (disposable)

LSCC: Sample cup caps (optional)

CON6: Tri-level, multi-constituent control

LTGCM: Third generation TSH control (single-level, low control)

## **Appendix 4**

### **Materials for FT4 test by Immulite**

**Kit components** (supplied separately)

LSUBX: Chemiluminescent substrate

LPWS2: Probe wash module

LKPM: Probe cleaning kit

LCHx-y: Sample cup holders (barcoded)

LSCP: Sample cups (disposable)

LSCC: Sample cup caps (optional)

CON6: Tri-level, multi-constituent control

## **Appendix 5**

### **Materials for FT3 test by Immulite**

**Kit components** (supplied separately)

LSUBX: Chemiluminescent substrate

LPWS2: Probe wash module

LKPM: Probe cleaning kit

LCHx-y: Sample cup holders (barcoded)

LSCP: Sample cups (disposable)

LSCC: Sample cup caps (optional)

CON6: Tri-level, multi-constituent control

## **Appendix 6**

### **Materials for thyroid antibodies test by ELISA**

Stat Fax 2100 washer

Stat Fax 2100 reader

Washing buffer

Sample diluent

Positive control

Negative control

Conjugate

Substrate

Stop solution

Microplate

## Appendix 7

### Reagents and equipment of the PCR-SSP technique

- **Typing Kit**

Micro SSP HLA DNA typing kits from One Lambda Inc., USA contain the following: (See table below)

Cat. No. of the Micro SSP DNA Typing Trays (One Lambda)	Alleles	No. of tests	No. of D-mix tubes	No. of tray seals	No. of PCR reactions per test.	D-mix vol. per test (µl)	DNA volume per test (µl)	***Taq polymerase volume per test (µl)
SSP2L	ClassII; Generic	30	30	10	32	360	39	2

- **Typing Trays**

The typing trays contain oligonucleotide primers for the amplification of HLA alleles and the human  $\beta$ -globin gene (internal control), dried in different wells of 0.2 ml thin – walled tube trays. The negative control reaction tube (H1) detects the presence of the internal control PCR product, which is the most likely contaminating PCR product due to its amplification in every well.

The amount of each primer is adjusted for optimal amplification of 100 ng of sample DNA when used in conjunction with the D-mix, the Taq-polymerase and the prescribed PCR program. The trays should be stored at -20° C.

- **D-mix**

The D-mix tube contains the PCR-buffer, dNTPs and MgCl<sub>2</sub>.

- **Taq Polymerase** from Applied Biosystems is used at a concentration of 5 U/µl.
- **DNA Sample** at a concentration of 50-200 µg/ml and a A260/A280 ratio of 1.65-1.9.
- **Distilled Water**
- **Adjustable Pipettes** (10 µl, 200 µl, 1000 µl ) and disposable pipette tips.
- **Vortex Mixer.**
- **Micro Centrifuge**
- **PCR Tray Holder**
- **Electronic Multiple Volume Dispenser Pipette** (10 x 10 µl) (Eppendorf)
- **Pressure Pad** for tray seal

- **Thermocycler** for PCR with 96-well format and tray /retainer for 0.2 ml thin-walled reaction tubes

## **Appendix 8**

### **Reagents and equipment for gel electrophoresis:**

- Agarose (Electrophoresis grade) from Sigma
- 1X Tris Borate EDTA (TBE) buffer (89 mM Tris-borate; 2 mM disodium EDTA, pH 8.0).
- Distilled water
- Ethidium Bromide (0.5 µg/ml in final concentration) (Gibco BRL)
- Weighing balance (Sartorius CE-0616)
- Micro SSP Gel System
- Power Pack (150 volts)
- Microwave oven
- Measuring flask (250 ml)
- Measuring cylinders (50 & 100ml)
- Spatula and wax paper for weighing

Bottle dispensers for 100ml and 10ml volumes

## **Appendix 9**

### **10% TBE Buffer**

108 gm Tris base

55 gm Boric acid

750 mL distilled water

### **2.5% Agarose Gel**

To 2.5 gm agarose, in a flask add 100 mL of 1X TBE buffer. Heat in a microwave to dissolve, and wait for a while before adding 3  $\mu$ L ethidium bromide.

### **Micro SSP Gel System**

#### 1. Components:

Well combs x 12

Electrode combs x 2

Base

Gel Box

Comb holder

Gel box cover

**To set up the gel apparatus:**

- Slide the locking pin on the base to open position
  - Insert the gel box into the base matching the color-coded sides to assure proper orientation
  - Lock the gel box into the base by sliding the locking pin into the locked position.
  - Use the leveling bubble and the three height adjustable legs to level the base.
2. Orient and fully insert the 14 gel combs into the gel comb holder.
  3. To 100ml of 1x TBE buffer add 2.5 g electrophoresis grade agarose. Microwave for approximately 2½ min. until a clear, homogenous solution is formed. Add 3 µL of ethidium bromide solution and quickly swirl.
  4. Measure 30 ml of the gel solution in a cylinder and add to the gel box. Make sure the agarose covers the entire surface evenly by tilting the gel box back and forth immediately after adding the gel solution. Quickly place the gel comb holder on the filled gel box by matching the color coding. Allow it to set for 15 minutes.
  5. Remove the gel combs by lifting the gel comb holder while holding the base.

Add 10-12 ml of 1x TBE containing 0.5µg/ml ethidium bromide evenly across the gel to fill every well



**Serologically or DNA-defined HLA-DRB alleles**

<b>HLA-DR locus</b>	<b>Alleles</b>	<b>Serological specificity</b>
DRB1	0101/0102/0104	DR1
	0103	DR103
	1501 – 1504	DR15 (2)
	1601 – 1606	DR16 (2)
	0301	DR17 (3)
	0302 – 302	DR18 (3)
	0401 – 0419	DR4
	1101 – 1113	DR11 (5)
	1201 – 1203	DR12 (5)
	1301 – 1313	DR13 (6)
	1401 – 1417	DR14 (6)
	0701	DR7
	0801 – 0811	DR8
	0901	DR9
	1001	DR10
	DRB3	0101
0201 – 0202		DR52b
0301		DR52c
DRB4	0101 – 0103	DR53
DRB5	0101 – 0102	DR51
	0201 – 0203	

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(L) Occupation:-----

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5. diagnosis:-----

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6. drugs:-----

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7. Laboratory investigations:

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Anti-Tg antibody	
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