Appendix 1

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:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR</td>
<td></td>
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<tr>
<td>HLA-DQ</td>
<td></td>
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<tr>
<td>Anti-TPO antibody</td>
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<tr>
<td>Anti-Tg antibody</td>
<td></td>
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<tr>
<td>TSH</td>
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<td>T₄</td>
<td></td>
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<tr>
<td>T₃</td>
<td></td>
</tr>
</tbody>
</table>
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)

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

- **Typing Trays**
  The typing trays contain oligonucleotide primers for the amplification of HLA alleles and the human β-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 -20º C.

- **D-mix**
  The D-mix tube contains the PCR-buffer, dNTPs and MgCl2.

  - **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

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**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 µL 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.
<table>
<thead>
<tr>
<th>Serologically or DNA-defined HLA-DRB alleles</th>
<th>HLA-DR locus</th>
<th>Alleles</th>
<th>Serological specificity</th>
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<tr>
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<td><strong>Serological specificity</strong></td>
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<td><strong>DRB1</strong></td>
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<td>0103</td>
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<td>1501 – 1504</td>
<td>DR103</td>
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<td>1101 – 1113</td>
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<td>DR53</td>
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   If yes:   First degree ( ); Second degree ( )

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