APPENDICES
MAP OF KERALA WITH SELECTED DISTRICTS FOR THE STUDY OF DENTAL FLUOROSIS.
Details of Fluoride Estimation Technique

Fluoride estimation in drinking water were done by *Potentiometric method* using *Ion Selective Electrode (ISE)*. The essential components of this method are the following.

a. A Sensing electrode
b. A Reference electrode
c. A Sensing electrode with built-in Reference electrode (Combination Electrode System)
d. A read out device (Ion Meter)
e. A sample containing the ion to be measured / standard solution.

Advantages of Ion Selective Electrode method

- It is the most sensitive method for fluoride estimation.
- The values are not affected by colour or turbidity of the sample solution.
- Unlike most other wet chemical methods, ISE measurement is not disturbed by solid particles in the sample solution.
- No health hazard is caused as no organic solvents and other hazardous chemicals are required.
- This is the method of choice for analyzing samples where there is a wide range of ionic concentration without recalibration.
• Once the electrode has been calibrated (standardized), a large number of samples can be analyzed over a short period of time.

**Pre-requisites for fluoride estimation by ion Selective Electrode**

**• Reagents:**

1. Stock standard fluoride solution (100 ppm)
2. Working fluoride standard of varying concentrations viz. 0.01, 0.1, 1.0, 2.5, 5.0, and 10.0 ppm, can be prepared by diluting stock standard solution
3. Total Ionic Strength Adjustment Buffer (TISAB-II or TISAB-III)
4. Electrode filling solution
5. De-ionized water for dilution of standards and washing of electrode
6. Distilled water for washing and rinsing

**• Pipettes and Tips**

1. Trasnferpette (variable pipette) (i) Capacity 0.5-5.0 ml (ii) 0.2-1.0 ml
2. Tips (Plastic) for Transpette (i) capacity 5.0 ml (ii) 1.0 ml

**• Glassware**

1. Volumetric flasks - (i) Capacity 50 ml & (ii) 100 ml
2. Beaker - 250 ml

**• Plastic ware**

1. Volumetric flasks (i) Capacity 50 ml & (ii) 100 ml
2. Graduated conical cups (i) Capacity 15 ml & (ii) 60 ml
3. Bottles for collecting urine / ground water from field: Capacity 100-150 ml
5. Plastic tubes with screw cap for transferring / storing the blood samples
6. Plastic serum collection vials

7. Wash bottle with nozzle: Capacity 500 ml – 2 Nos.

- Miscellaneous items

  1. Glass marking pen
  2. Soft tissue paper roll
  3. Adhesive tape
  4. Blade / Scissors
  5. Plastic tray
  6. Hand gloves

**Collection of samples for fluoride analysis:**

**Type of water samples:**

i) **Grab sample:** One time collection from a source

ii) **Composite sample:** Same source but sample collected at different time intervals (Example: Morning & evening samples)

iii) **Integrated sample:** Samples collected simultaneously from different points (depth and width of different locations-if the source is river or stream)

**How to collect water samples:**

i) Water should be collected in 150ml capacity plastic bottles of double lid.

ii) Water should be collected directly from the source and not from the stored containers in the house.
iii) Water sample is stored at room temperature, no preservative required.

**Labeling** (If it is a hospital based patient)

The bottle should be labeled using plastic tape instead of plastic labels.

The details listed below to be inscribed on the label using a pencil or ball pen and not ink pen to avoid smudging of the writing.

- a) Date of collection
- b) Name & Address of the owner
- c) Location of the source
- d) Type of source (HP /TW/OW/ any other) (to be ticked)

**Preparation of the Electrode for sampling analysis**

- Remove the rubber cap covering the electrode tip.
- Lift the spout of the bottle containing electrode filling solution to a vertical position.
- Insert the spout into the hole in the outer sleeve of the electrode and a small quantity of filling solution to the outer chamber. Rotate the electrode to wet the O-ring which is above the hole on the electrode and then return the electrode to a vertical position.
- Holding the electrode by the barrel with one end, use the thumb to push down the electrode cap, allowing a few drops of filling solution to drain and wet the inner cone.
• Add filling solution through the filling hole. The filling solution level should be at least **one inch above the level of sample / standard solution** in the beaker to ensure a proper flow rate.

• Do not seal the filling hole while making calibration and measurements.

• Add filling solution each day before using electrode.

• Connect the electrode to the Ion Meter

**Activation of electrode:**

• Dip the electrode tip into low concentration of fluoride solution (viz 0.1 ppm) 2 or 3 hours for activation of the electrode to get better results.

• Rinse the electrode thoroughly with distilled water before starting of measurements.

**Checking Electrode Operation (Slope):**

• Switch on the Ion meter into millivolt (mV) mode

• To place 5 ml of the de-ionised water and 5 ml of TISAB – II or 10 ml of de-ionised water and 1 ml of TISAB – iii into 50 ml plastic beaker.

• To rinse the electrode with distilled water and place in the above mentioned solution.

• To pipette 0.1 ml of ppm standard solution into the beaker.

• To mix it well & stir the beaker manually

• To wait for a stable reading displayed

• To record the electrode potential in millivolts

• To pipette 1.0 ml of 100 ppm standard solution into the same beaker
To follow the above mentioned steps again to record the electrode potential in millivolts.

If the difference between the 1st and 2nd potential reading is in the range of 54-60 mV, then it can be ascertained that the electrode is operating well.

**Calibration of Ion Meter:**

Calibration is the process of relating the value indicated on the scale of an analytical device / equipment to the quantity of the chemical (ie; Fluoride in this case) of a substance (ie. Standard) required to be measured.

**Standard solution**

A solution which has a known concentration of a chemical

For any analytical procedure, calibration (ie. setting the machine ready) is important. For calibrating the equipment, a standard solution is required.

**Standard solutions are of 2 types:**

(i) **Stock standard solution**-highest concentration

(ii) **Working standard solution**-lower concentrations

Stock standard fluoride solution is available in the market. Strength of the standard may be expressed as follows:

a) **Molar solution (M):** contains a gram-molecular weight of solute/substance in a litre of solution. Gram-molecular weight / gram mole or mole of a substance is its molecular weight expressed in gram.

b) **Parts per million (ppm):** One mg of solute / substance dissolved in 1 litre of solution (1 ppm= 1 mg / L)
**Procedure for preparation of fluoride working standard solution:**

- Stock standard fluoride solution = 100 ppm (mg/L)
- Pipette out 10 ml of 100 ppm fluoride standard solution in a 100 ml volumetric flask (or 5 ml of 100 ppm fluoride standard solution in a 50 ml volumetric flask). Then add de-ionized water up to the mark. Shake well and store in a plastic container. Label container with the following information.
  
  (I) Concentration: .................mg/L  
  (II) Date of preparation.................  
  (III) Prepared by........................

- Preparation for further dilutions of the working standard solutions are as follows:

<table>
<thead>
<tr>
<th>To make Concentrations of</th>
<th>Volume to be taken</th>
<th>Volume of de-ionized water to be added</th>
<th>Volume to be make up</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0 ppm</td>
<td>10 ml of 100 ppm</td>
<td>90 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td></td>
<td>5 ml of 100 ppm</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>5.0 ppm</td>
<td>50 ml of 10 ppm</td>
<td>50 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td></td>
<td>25 ml of 10 ppm</td>
<td>25 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>2.5 ppm</td>
<td>50 ml of 5 ppm</td>
<td>50 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td></td>
<td>25 ml of 5 ppm</td>
<td>25 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>1.0 ppm</td>
<td>10 ml of 10 ppm</td>
<td>90 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td></td>
<td>5 ml of 10 ppm</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>10 ml of 1 ppm</td>
<td>90 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td></td>
<td>5 ml of 1 ppm</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>0.01 ppm</td>
<td>10 ml of 0.1 ppm</td>
<td>90 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td></td>
<td>5 ml of 0.1 ppm</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
</tbody>
</table>
Precautions to be taken during preparation of dilutions:

- It is important to prepare working standard solutions by serial dilution to minimize the error in concentration.
- Pipetting should be done accurately.
- During preparation of standard solutions, last drop of the solution should be emptied thoroughly from the pipette tip.
- All dilutions of standard solutions must be done using de-ionized water.
- To prevent contamination of the stock solution:
  1. Bottle should be kept closed when not in use.
  2. Pipette tips should not be inserted into the stock bottle.
  3. Standard solution once taken out should not be returned to the stock bottle.
  4. Stock solution should be stored in cool and dark place.

Store fluoride standard solution/ water / in plastic bottle only (but not in glass bottle) because Glass contains silica which has affinity to combine (react) with fluoride in standard solution / sample. Thus, we may not get the actual value of fluoride contents in the sample.

Total Ionic Strength Adjustment Buffer (TISAB) is necessary for fluoride estimation by Ion Meter. Total Ionic Strength Adjustment Buffer (TISAB) is added to all fluoride standards and samples so that the (i) background ionic strength is high, (ii) fluoride de-complex and (iii) the pH of the solution is adjusted.
De-ionized water is compulsory for fluoride analysis

Water generally contains lot of ions in it. During de-ionization all the positively and negatively charged ions in the water are eliminated to the minimum possible level. That is why de-ionized water is a must to avoid a background of ions including fluoride ions. All dilutions of standard solution preparation and washing of electrode while changing samples during fluoride estimation should be done with de-ionized water.

Slope value (checking electrode operation) Slope is defined as the change in millivolts (mV) observed when the concentration changes by a factor of ten (ten fold dilution). Slope value in the range of 54-60 mV per ten fold changes in concentration provided the best means for checking electrode operation.

Preparation of the Electrode for sampling analysis

- Remove the rubber cap covering the electrode tip.
- Lift the spout of the bottle containing electrode filling solution to a vertical position.
- Insert the spout into the hole in the outer sleeve of the electrode and a small quantity of filling solution to the outer chamber. Rotate the electrode to wet the O-ring which is above the hole on the electrode and then return the electrode to a vertical position.
- Holding the electrode by the barrel with one end, use the thumb to push down the electrode cap, allowing a few drops of filling solution to drain and wet the inner cone.
• Add filling solution through the filling hole. The filling solution level should be at least **one inch above the level of sample / standard solution** in the beaker to ensure a proper flow rate.

• Do not seal the filling hole while making calibration and measurements.

• Add filling solution each day before using electrode.

• Connect the electrode to the Ion Meter

**Activation of electrode**

• Dip the electrode tip into low concentration of fluoride solution (viz 0.1 ppm) 2 or 3 hours for activation of the electrode to get better results.

• Rinse the electrode thoroughly with distilled water before starting of measurements.

• Measurement of Fluoride Ions from the samples.

(Ref: Adapted from "Handbook on the use, operation and care to be exercised on Ion Meter while estimation of fluoride in drinking water and body fluids"-By Dr. A. K. Susheela, Fluorosis Research & Rural Development Foundation, Delhi- 2007 )
**QUESTIONNAIRE FOR THE STUDY OF DENTAL FLUOROSIS**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Name of the school &amp; place</td>
</tr>
<tr>
<td>2</td>
<td>Age of the student</td>
</tr>
<tr>
<td>3</td>
<td>Address (Panchayath/Municipality/Ward)</td>
</tr>
<tr>
<td>4</td>
<td>Standard in which the student studies with division</td>
</tr>
<tr>
<td>5</td>
<td>Do you reside in this district from birth onwards</td>
</tr>
<tr>
<td>5.1</td>
<td>If not, from which year onwards</td>
</tr>
<tr>
<td>6</td>
<td>Drinking water source</td>
</tr>
<tr>
<td>7</td>
<td>Drinking water</td>
</tr>
<tr>
<td>8</td>
<td>Quantity of water that you drink in a day</td>
</tr>
<tr>
<td>8.1</td>
<td>Water from home/water from school/both</td>
</tr>
<tr>
<td>9</td>
<td>Black tea drinking habit</td>
</tr>
<tr>
<td>10</td>
<td>Sea fish consumption</td>
</tr>
<tr>
<td>11</td>
<td>Dry fish consumption</td>
</tr>
</tbody>
</table>

1. **Yes/No**

2. **Male / Female**

3. **Pipe/Well/Pond/Bore Well/Others**

4. **row water/boiled and cooled water/both**

5. **2-3 glasses/4-6 glasses/ 7 or more glasses**

6. **Occasional/1-2 glasses everyday**

7. **3 or more glasses everyday**

8. **Occasional/once in a week/2-3 days in a week/ all days**

9. **Occasional/once in a week/2-3 days in a week/ all days**
12 Use of Sambar/Rasam with tamarind: Occasional/once in a week/
2-3 days in a week/All days

13 Quantity of milk used in the house per day: ½ lit, occasional/ ½ lit, every day/
1-2 lit, / 2 or more lit, per day/milk powder instead of milk

14 Quantity of milk that the student consumes per day: No consumption/occasionally one
glass/one glass daily/2 glasses or more daily.

1. Consumption of fruits: Occasional/once in a week/ 2 days in a week/3-4 days in a week/all
days

15 Materials used for dental cleaning: Paste/tooth powder/Ayurveda
paste/Ayurveda tooth powder/charcoal/husk charcoal/mango
leaf, others (specify)

1. If paste in used, the brand: Colgate/Close up/Binacca/
Babool/Cibacca/others (specify)

16 Name of the guardian:

1. Total family members:

17 Education of parents: Father: ..................
Mother: ..................

18 Occupation of parents: Father: ..................
Mother: ..................

Date: ....................

19 Dental fluorosis: Yes/No

1. If yes, grade: Questionable, Grade I, Grade II,
Grade III, Grade IV

20 Other dental diseases: ..........................
..........................

Place: ....................
Date: ..................
1. നാവ് കാസ്റ്റ് ചില്ലോഫ്ക

2. വിളയേലത്തിൻ നാസാവേക്കണയാത്രയിൽ ഒരു

3. സോസാമുഖം പോയാടൽ

4. തിരുവിതാംകൂർ പാടാളിക്കുന്നു

5. പോളിന്റെ പാടാളിക്കുന്നു

6. പെട്രോളിയം വാഹനം കായികം

7. പെട്രോളിയം വാഹനം കായികം
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1. സ്ഥാനാർന്നുള്ളന്റെ കാര്യങ്ങൾ

17 ക്രമേണ ലഭിച്ച പ്രാധാന്യം

: സമയം: ---------------

18 ക്രമേണ ലഭിച്ച വിവാഹം

: സമയം: ---------------

19 പരിവർത്തനം

: പരിവർത്തനം: 

1. സ്വതന്ത്രകാലത്തെ

2. സമകാലികൻ അധികാരം

3. അധികാരം II 4. അധികാരം III, 5.അധികാരം IV

20 പ്രവൃത്തി മാംസോത്സവം

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: -----------------------------

ഗവർണ: ----------------------

നിയമം: ----------------------
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