

METHODICAL STEPS FOR GRAM STAINING OF ORAL CANDIDA (Qualitative)

Steps	Duration
Step 1: Fix the smear by Heating beneath the glass slide	: Few seconds
Step 2: Add drops of Crystal Violet stain to cover the sample	: 1 min
Gentle wash in tap water for few secs	
Step 3: Add drops of Gram's Iodine to cover the sample	: 1 min
Gentle wash in tap water for few secs	
Step 4: De-colored by adding drops of acetone alcohol	: 2-5 mins
Gentle wash in tap water for few secs	
Step 5: Add drops of Carbol Fuschine	: 30 secs
Gentle wash in tap water for few secs	
Step 6 (Final): Air dry the sample and observe in microscope under oil immersion	

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STEP-WISE PROCEDURE

Estimation of Fluoride in HUMAN SERUM

Sample preparation

1. **Draw** whole blood preferably into Vacutainer collection tube containing no anticoagulant, and ion-free.
 2. **Collected whole blood:** Keep upright or slight slanting position at room temperature for nearly 45 minutes and allow clot to set down, and the serum is surfaced to the clot.
 3. **Centrifuge** for 10 minutes at 3000 rpm. To avoid disruption (hemolysis) of formed elements of blood, do **not** use break to stop centrifuge.
 4. **Pipette out** the supernatant (serum) and draw into Cryovials.
Store at 0°C
- NOTE: Discard red-tinged serum, which is due to hemolysis

Fluoride Measurement

1. **Prepare** NaF standard solutions (of three different concentrations ; ie, 1000ppm, 100ppm, 10ppm)
2. **Calibrate** the ISE using 1:1 volume of NaF standard solutions and TISAB II.
3. **Record** the stable voltage reading of the potential after dipping the electrode into the serum added with equal volume of TISAB II.
4. **Print** the result along with the calibration curve.
5. **Rinse** the electrode with de-ionized water before running next sample.

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STEP-WISE

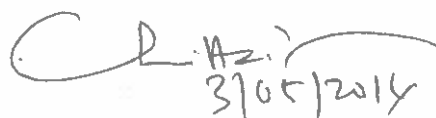
Procedure for Estimation of Fluoride in Soft Drinks, Mineral Waters, Fruit Juice/ Milk Shake, Beverages

Sample preparation

1. **Collect** the fruit juice, mineral waters, soft drinks and beverages
2. **Note** the information given by the manufacturer.
3. **Measure the pH** of each drink.
4. **Place ~10 ml** each carbonated soft drinks and beverages into a plastic vial, which is loosely cover for at least two hours for **de-carbonation**.
5. **Pipette out the 4 ml** of de-carbonated soft drinks/fruit juice/ milk shake/ mineral water/ beverage to a plastic beaker containing equal volume of TISAB II - sample.

Fluoride Measurement

1. **Prepare** the NaF standard solutions (of three different concentrations ; ie, 1000ppm, 100ppm, 10ppm)
2. **Calibrate** the ISE using 1:1 volume of NaF standard solutions and TISAB II.
3. **Record** the stable voltage reading of the potential after dipping the electrode into the sample.
4. **Print** the result along with the calibration curve.
5. **Rinse** the electrode with de-ionized water before the next sample of drinks.


31/05/2014

STEP-WISE

Procedure for Estimation of Fluoride in Soft Drinks, Mineral Waters, Fruit Juice/ Milk Shake, Beverages

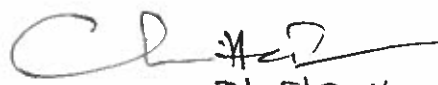
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5. **Rinse** the electrode with de-ionized water before the next sample of drinks.

© Protocol is developed by The Fluoride Research Team of the Department of Oral Biology & Genomic Studies(OBGS), AB Shetty Memorial Institute of Dental Sciences, Nitte University, Deralakatte, Mangalore-575018. Team Leader: Divya Kumari P., Msc, a research scholar for PhD at OBGS. This document has been generated in consultation with published paper and Professor Chitta Ranjan Chowdhury, Head of the Department, Mangalore. May 2014.


3/05/2014

STEP-WISE

Procedure for Estimation of Fluoride in **SALIVA**

1. Give the proper instruction to candidates before collection of saliva from them.
 - Rinse the mouth from water.
 - Chew the fluoride free gum
 - Spit the saliva to the container provided.
(approximately 2 ml)
 - Label the container.
2. Freeze (4° C) the saliva for 24 hours.
3. Weigh the saliva after bring it back to room temperature.
4. Centrifuge it for 15 minutes at 3000 rpm.
5. Separate the supernatant.
6. Add 1ml of TISAB II to 1 ml of supernatant to prepare the sample for estimation of Fluoride.
7. Calibrate the ISE using standard solutions NaF (of 3hree different Conc., ie., 1000, 100 and 10 ppm).
8. Dip the electrode in the sample, and record the reading of the potential (volt) when it is stable.
9. Print the result along with the calibration curve.
10. Rinse the electrode with de-ionized water for next sample.



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STEP-WISE

Procedure for Estimation of Fluoride in **URINE**

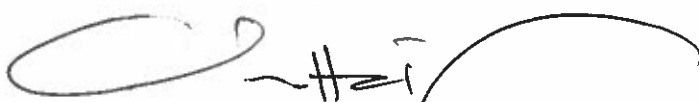
Sample preparation:

1. **Collect** the fasting Urine sample (approximately 2-3 ml).
2. **Add 1 ml** of urine aliquot to equal volume of TISAB II.

Fluoride Measurement:

1. **Calibrate** the ISE using NaF standard solutions (of three different conc., i.e., 1000, 100 and 10 ppm).
3. **Dip the electrode in the sample, and record** the reading of the potential (volt) when it is stable.
4. **Print** the result along with the calibration curve.
5. **Rinse** the electrode with de-ionized water for the next sample.

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22/02/2014

Fluoride mouth rinses

Instructional Leaflet [Feb 2015, Updatable]

Dental decay is one of the common oral diseases affects the population throughout the globe at a varied severity. If this ailment is not detected early and prevented appropriately, a loss of tooth may happen eventually, and that impacts on quality of life (QoL) and economy as well ^[1,2]. The prevalence of dental caries is more in non-fluoridated areas. Judicial use of fluoride helps decline the prevalence rate of dental caries and stop its sequel. Common means of fluoride delivery are fluoridated drinking water, fluoridated milk, salt, F-supplemented dentifrices, tablets, varnishes etc.

One of the fluoride delivery systems is Fluoride mouth rinse. Fluoride mouth rinse is a formulated concentration of Fluoride used for daily or weekly basis. And Sodium fluoride (NaF) is the superior solution comparing to other preparations³. The strength of 0.05% NaF solution contains 230 ppm fluoride is recommended for daily use (once a day), on the other hand , 0.2% NaF is used once in a week or two weeks. The later contains 0.2% NaF ie., 900 ppm fluoride ^[2,3]. Supervised daily use of NaF has got better effect in preventing dental caries comparing to weekly or fort-nightly regimen³.

HOW WE FORMULATE THEM

Fluoride mouth rinses (NaF) were prepared in the fluoride research division of the Department of Oral Biology, AB Shetty Memorial Institute of Dental Sciences of Nitte University Deralakatte, Mangalore, India. Before preparation of the product, the 1000 ml volumetric flask, weighing glasses, spatula and plastic storage bottles and de-ionized water were autoclaved. The pH 7 was recorded by using pH meter.

Mouth rinse / pH	Preparation	Fluoride in ppm	Fluoride in ppm detected by ISE ¹
0.2 % NaF / pH 7	2 grams of NaF was dissolved in 1000 ml of de-ionized water.	900 – 920	907
0.05% NaF / pH 7	0.25 grams of NaF was dissolved in 500 ml of de-ionized water.	225 – 230	221.8

¹ F-Ion-selective Electrode is available at the Fluoride Research Division of the Department of Oral Biology.

P.T.O.

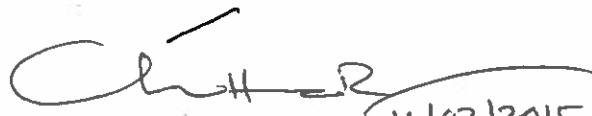
References

1. MPH, Elmer L. Green, DDS, MPH, and Valerie B. Haley, BS. Am J Public Health. 1998 Dec;88(12):Changes in Dental Fluorosis and Dental Caries in Newburgh and Kingston, New York Jayanth V Kumar, DDS, MPH, Philip A. Swango, DDS, MPH, Lloyd L. Lininger, PhD, Gary S. Leske, DDS, 1866-70
2. Effect of mouthrinsing with a 0.2 per cent neutral NaF solution on the deciduous dentition of first to third grade school children Louis W. Ripa, DDS, MS Gary S. Leske, DDS, MS, MPH Andre Varma, MD, MS, The American Academy of Pedodontics/Vol. 6 No. 2.
3. Delivering Better Oral Health – An evidence-based toolkit for prevention (<http://www.dh.gov.uk>).

IMPORTANT NOTE

- **PRESCRIPTION USE ONLY (Consult with dentist before use)** : Both the preparations will be used upon consultation with a qualified dentist, and in ideal situation application will be under direct supervision of a dentist, otherwise, a trained parent / care giver will be acceptable by the dentist. Children below 16 will **NEVER** be allowed to use them unsupervised.
- **CONTRAINDICTION FOR CHILDREN BELOW 7 years-old**: Do not prescribe fluoride mouth rinse to the children below 7 years old.
- **RINSE THE SOLUTION THOROUGHLY AND KEEP THE SOLUTION HOLD FOR 10 MINS**: Take approximately 20 ml of solution inside mouth and rinse thoroughly for 10 mins. Do not drink, please spit it out completely. Immediate after use of this solution do not eat or drink for at least 30 mins. You better wash your teeth with normal tap water after an hour. It helps proper action of Fluoride on your teeth
- **KEEP THE SOLUTION OUT OF REACH OF THE CHILDREN**: Keep the solution out of reach of the children, and shelve them preferably in a cool dark place..
- **REPORT IMMEDIATELY IF AN ADVERSE REACTION HAPPENS FROM THIS RINSE**: It is dangerous if the children drink them, in case you may report it to a qualified dentist or hospital emergency Any unwanted situation and allergy from this product will be reported without delay.
- **SAFE & STERIZED**: The pH of the solution is 7, and the preparation has been autoclaved.
- **WE DO NOT ADD A FLAVOUR** to prevent drinking or over-use of the rinse by the children.

© Developed by The Fluoride Research Team of the Department of Oral Biology & Genomic Studies(OBGS), AB Shetty Memorial Institute of Dental Sciences, Nitte University, Deralakatte, Mangalore-575018. Team: Dr. Shah Nawaz K, MSc, BDS Lecturer; Divya Kumari P., Msc, a PhD researcher at OBGS. This document has been generated in consultation with published papers, updated guideline, and guided by Professor Chitta Ranjan Chowdhury, Head of the Department. February 2015. The document has been circulated to the executive board and expert group of Indian Academy of Oral Biology (IAOB- DKM-S137-2013-14).


16/02/2015

STEP-WISE

Procedure for Estimation Of Fluoride in different Brands of Tea powder

1. Sample preparation

1. Take two (2) grams of tea powder in a plastic beaker.
2. Add 80ml of hot de-ionized water into it.
3. Pipette 3ml of the tea liquor after 10, 15 & 20 min.
4. Add 3ml of TISAB to each samples of tea liquor.

3. Fluoride measurement:

1. Calibrate ISE electrode with standard solution NaF
2. Estimate Fluoride by dipping 2cm to 3cm of sensing part of electrode (ie, base of the electrode) into the sample.
3. Record the stable voltage.
4. Print the result along-with calibration curve

STEP-WISE

Procedure for Estimation Of Fluoride in different Brands of Dentifrices | Mouth Wash |

1. Sample preparation

A. For Tooth paste/ tooth powder

1. Take 0.05 grams of tooth paste / tooth powder in a stainless steel beaker.
2. Add 25ml of TISAB II.
3. Gently boil the mixture on the heating plate provided for two to three minutes.
4. Transfer the mix in 50ml of volumetric flask
5. Make the volume of 50 ml by adding de-ionized water*
6. Pipette 5ml from the mix (of the sample) and transfer to a plastic beaker.

A. For Mouth Wash

1. Take 1ml of sample (mouth wash) in plastic beaker.
2. Add 9ml of de-ionized water and 10ml of TISAB II to it.

A. Fluoride measurement:

1. Calibrate ISE electrode with standard solution NaF
2. Estimate the Fluoride concentration by dipping sensing part of electrode (ei, base of the electrode) into sample.
3. Record the stable voltage
4. Print the result along-with calibration curve

***Check the fluoride concentration of de-ionized water.**

STEP-WISE

Procedure for Estimation Of Fluoride in water samples

1. **Rinse** the Plastic bottle with water sample more than thrice before the collection in it.
2. **Prepare** standard solutions of 1000 ppm, 100 ppm, 10 ppm of NaF in 100 ml volumetric flasks.
3. **Take** equal volume of each of the fluoride solution and of the TISAB buffer into plastic beaker.
4. **Dip** the electrode into the solution and record stable voltage reading of the potential for each solution to calibrate the electrode.
5. **Take** equal volume of water sample and TISAB in a plastic beaker.
6. **Record** the stable voltage reading of the potential after dipping the electrode into the each sample solution.
7. **print** the result along-with calibration curve

Note:

1. **Calibrate** the electrode after 10 samples of water.
2. **Rinse** the system with de-ionized water at the end of the each working session.
3. **Protect** the electrode by replacing the cape provided, whilst it's not in-use.

STEP-WISE

Procedure for Estimation Of Fluoride in different Brands of Milk and Fresh Milk from cow

1. Sample preparation

1. **Store** the collected milk sample at 4 degree Celsius in a refrigerated until fluoride estimation is done.
2. **Allow** the sample to settle at room temperature before analysis.
3. **Add** 3ml of TISAB II to 3ml of milk taken in a plastic beaker.

2. Fluoride measurement:

1. **Calibrate** ISE electrode with standard solution NaF
2. **Estimate** Fluoride by dipping 2cm to 3cm of sensing part of electrode (ei, base of the electrode) into the sample.
3. **Record** the stable voltage.
4. **Print** the result along-with calibration curve