Introduction

Vitamin D (also referred to as “calciferol”) is a fat-soluble vitamin that is naturally present in a few foods, added to endogenously others, and available as a dietary supplement. It is also produced when ultraviolet (UV) rays from sunlight strike the skin and trigger vitamin D synthesis.

Vitamin D obtained from sun exposure, foods, and supplements is biologically inert and must undergo two hydroxylations in the body for activation. The first hydroxylation, which occurs in the liver, converts vitamin D to 25-hydroxyvitamin D [25(OH)D], also known as “calcidiol.” The second hydroxylation occurs primarily in the kidney and forms the physiologically active 1,25-dihydroxyvitamin D [1,25(OH)2D], also known as “calcitriol”.

N Engl J Med 2006;

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal bone mineralization and to prevent hypocalcemic tetany (involuntary contraction of muscles, leading to cramps and spasms). It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts . Without sufficient vitamin D, bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Together with calcium, vitamin D also helps protect older adults from osteoporosis.

Vitamin D has other roles in the body, including reduction of inflammation as well as modulation of such processes as cell growth, neuromuscular and immune function, and glucose metabolism . Many genes encoding proteins that

regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D. Many tissues have vitamin D receptors, and some convert 25(OH)D to 1,25(OH)2D.
BMC Public Health 2017;

In foods and dietary supplements, vitamin D has two main forms, D2 (ergocalciferol) and D3 (cholecalciferol), that differ chemically only in their side-chain structures. Both forms are well absorbed in the small intestine. Absorption occurs by simple passive diffusion and by a mechanism that involves intestinal membrane carrier proteins. The concurrent presence of fat in the gut enhances vitamin D absorption, but some vitamin D is absorbed even without dietary fat. Neither aging nor obesity alters vitamin D absorption from the gut .
Serum concentration of 25(OH)D is currently the main indicator of vitamin D status. It reflects vitamin D produced endogenously and that obtained from foods and supplements . In serum, 25(OH)D has a fairly long circulating half-life of 15 days . Serum concentrations of 25(OH)D are reported in both nanomoles per liter (nmol/L) and nanograms per milliliter (ng/mL). One nmol/L is equal to 0.4 ng/mL, and 1 ng/mL is equal to 2.5 nmol/L.
Lippincott Williams & Wilkins, 2014.(1)

Adequate Intake (AI)

Many other countries around the world and some professional societies have somewhat different guidelines for vitamin D intakes . These differences are a result of an incomplete understanding of the biology and clinical implications of vitamin D, different purposes for the guidelines (e.g., for public health in a healthy population or for clinical practice), and/or the use in some guidelines of

observational studies in addition to randomized clinical trials to establish recommendations . The Endocrine Society states, for example, that to maintain serum 25(OH)D levels above 75 nmol/L (30 ng/mL), adults might need at least 37.5 to 50 mcg (1,500–2,000 IU)/day of supplemental vitamin D, and children and adolescents might need at least 25 mcg (1,000 IU)/day . In contrast, the United Kingdom government recommends intakes of 10 mcg (400 IU)/day for its citizens aged 4 years and older.
national library of medicine(2).

Sources of Vitamin D

Food

Few foods naturally contain vitamin D. The flesh of fatty fish (such as trout, salmon, tuna, and mackerel) and fish liver oils are among the best sources. An animal's diet affects the amount of vitamin D in its tissues. Beef liver, egg yolks, and cheese have small amounts of vitamin D, primarily in the form of vitamin D3 and its metabolite 25(OH)D3. Mushrooms provide variable amounts of vitamin D2 . Some mushrooms available on the market have been treated with UV light to increase their levels of vitamin D2. In addition, the Food and Drug Administration (FDA) has approved UV-treated mushroom powder as a food additive for use as a source of vitamin D2 in food products . Very limited evidence suggests no substantial differences in the bioavailability of vitamin D from various foods .
Nutrients 2018

Animal-based foods typically provide some vitamin D in the form of 25(OH)D in addition to vitamin D3. The impact of this form on vitamin D status is an emerging area of research. Studies show that 25(OH)D appears to be approximately

five times more potent than the parent vitamin for raising serum 25(OH)D concentrations . One study found that when the 25(OH)D content of beef, pork, chicken, turkey, and eggs is taken into account, the total amount of vitamin D in the food is 2 to 18 times higher than the amount in the parent vitamin alone, depending on the food .

Fortified foods provide most of the vitamin D in American diets . For example, almost all of the U.S. milk supply is voluntarily fortified with about 3 mcg/cup (120 IU), usually in the form of vitamin D3 . In Canada, milk must be fortified with 0.88–1.0 mcg/100 mL (35–40 IU), and the required amount for margarine is at least 13.25 mcg/100 g (530 IU). Other dairy products made from milk, such as cheese and ice cream, are not usually fortified in the United States or Canada. Plant milk alternatives (such as beverages made from soy, almond, or oats) are often fortified with similar amounts of vitamin D to those in fortified cow's milk (about 3 mcg [120 IU]/cup); the Nutrition Facts label lists the actual amount . Ready-to-eat breakfast cereals often contain added vitamin D, as do some brands of orange juice, yogurt, margarine, and other food products.

Public Health Nutrition 2020;

Discussion :-

Currently, several observational studies and subsequent systematic review have correlated low serum vitamin D levels to a reduction of natural fertility. Nevertheless, these studies were insufficiently powered to draw definite conclusions. We therefore conducted this stady to firstly evaluated the effectiveness of vitamin D supplementation in infertile women .
Among 4 eligible studies reported clinical pregnancy, 2 studies reported an improved pregnancy, which was consistent with our finding. However, another 2 studies reported inconsistent results that vitamin D supplementation could not improve this outcomes.

Although the data for vitamin D and fertility is not conclusive, several studies have found that vitamin D blood levels of 30 ng/mL or higher are associated with higher pregnancy rates.Two studies found that among populations of mostly Caucasian and non-Hispanic white women, those with a normal vitamin D level were four times more likely to get pregnant through compared to those who had a low vitamin D level.

Significantly, research also shows that up to 42% of the U.S. population is vitamin D deficient . As such, vitamin D levels are often measured during an assessment of female fertility.

Investigations have also shown that pregnant women with higher levels of Vitamin D had higher live birth rates than those with lower levels. So whilst high levels of Vitamin D may not be increasing fertility, the evidence seems to

suggest that deficiencies can be detrimental to fertility and healthy pregnancy.

We therefore doing this stady to firstly evaluated the effectiveness of vitamin D supplementation in infertile women . Based on the results from the present stady , we found that vitamin D supplementation may be have potential ability of improving fertality.

Material and method:

TEST PRINCIPLE
25 OH Quantitative Vitamin D Rapid Test utilizes the principle of Immunochromatography, a unique two-site “Sandwich” immunoassay on a membrane. The test employs a very “Exclusive” pair of anti-25- OH Vitamin D Monoclonal Antibodies; one conjugated with colloidal gold and another one immobilized on the solid phase. This will selectively detect Vitamin D with a high degree of sensitivity and specificity.
As the test sample flows through the membrane assembly within the test device, the colored anti-25-OH Vitamin D-colloidal gold conjugate complexes with 25-OH Vitamin D from the sample. This complex moves further on the membrane by the capillary action to the test region (T) where it is immobilized by another anti-25-OH Vitamin D coated on the membrane, leading to formation of a pink / purple colored band, which confirms a positive test results. The intensity of colored band in the test line region is 25-OH Vitamin D concentration-dependent, higher the concentration of 25-OH Vitamin D in the tested sample, the stronger the colored band is. A control line is present in the test window to work as procedural control. This colored band should always appear on the control line region (C) if the test device is stored in good condition and the test is performed appropriately.
MATERIALS PROVIDED
1. 25 OH Quantitative Vitamin D Rapid Test device (Kit Size: 25 Tests/Box) 2. Sample Buffer (One Bottle of 6.5 ml)
3. UniSamplerTM Device (26 Collection Tubes + 26 Blood Collectors)

4. RFID Card - 1
5. Instructions for use – 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or clock
2. Lancet
3. Alcohol Swab
4. RapiReadTM–Affimedix RR-101(CE marked) to be purchased separately
5. Quality Control- to be purchased separately

STORAGE AND STABILITY

The test device should be stored at 4oC to 30oC and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

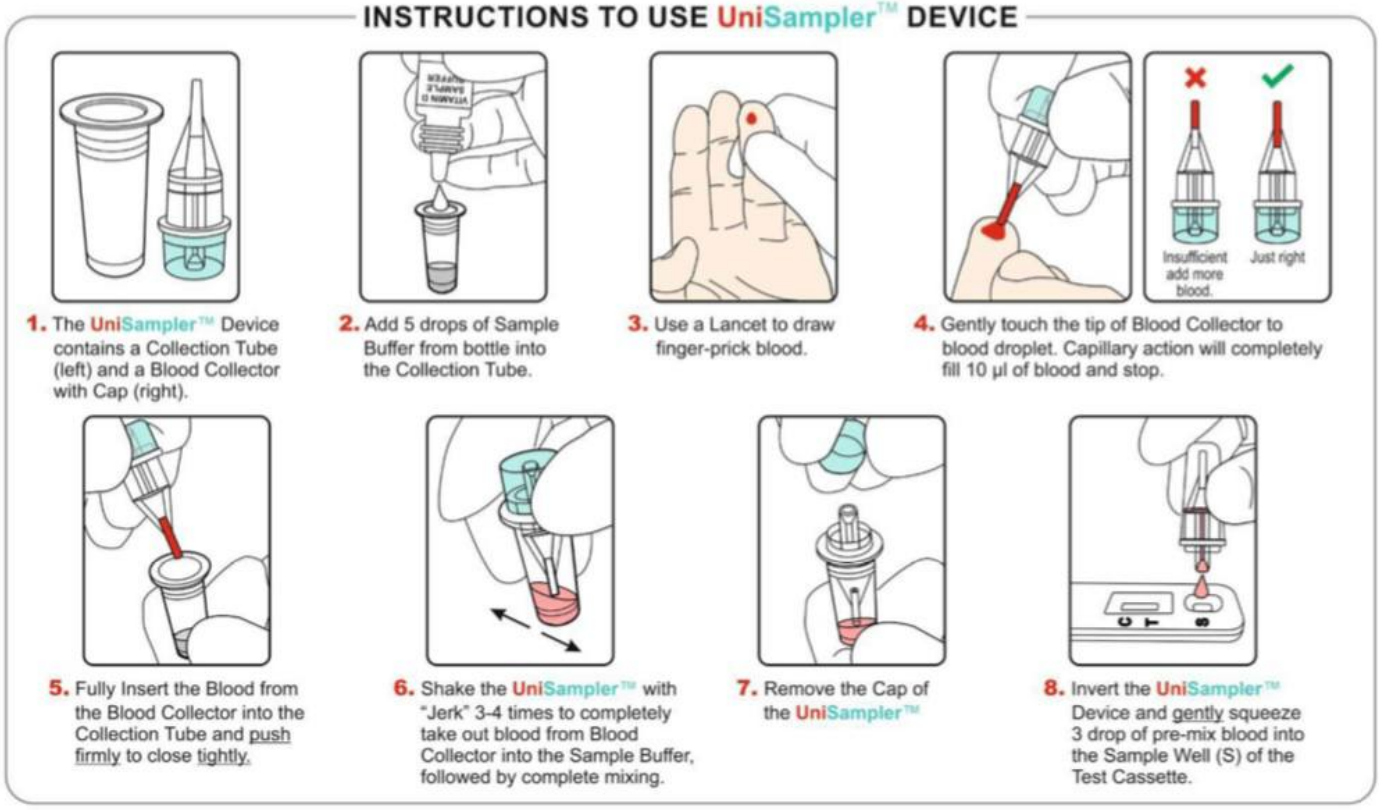
PRECAUTIONS

1. For in vitro diagnostic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.

QUALITY CONTROL

Good Laboratory Practice recommends the frequent use of control materials to validate the reliability of 25 OH Quantitative Vitamin D Rapid Test device. If control values do not fall within established range, assay results are invalid. A set of two “LC-MS/MS confirmed” Vitamin D Controls is provided with the kit (optional).

The provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of Vitamin D. If the control line does not appear, the test device should be discarded and the obtained result is



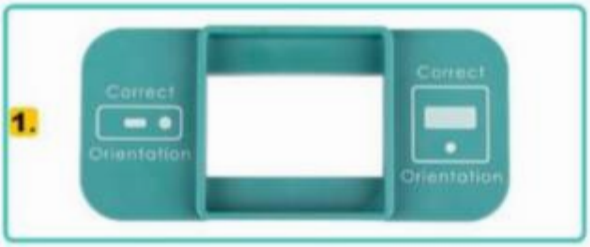
invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

25 OH Quantitative Vitamin D Rapid Test has been designed for “Decision-Point” Finger-prick Blood (or Serum) samples ONLY. NO Anticoagulated Blood or Plasma samples should be used for testing 25 OH Quantitative Vitamin D Rapid Test as Anticoagulants will impact the test results.

SPECIMEN COLLECTION AND PREPARATION

1. Wash your hand thoroughly and dry completely.
2. Rub and Wipe your ring or middle finger of non-dominant hand.
3. Using safety lancet puncture the side of your finger.
4. Collect 10 µl blood using Blood Collector (See instructions below) and perform testing immediately.

QUANTITATIVE DETECTION USING *RapiRead*™ CUBE READER



1. Check the "Correct Orientation" shown on the Adaptor for the Test Device and *RapiRead*™ CUBE Reader.

2. Place the Adaptor on top of the Test Device correctly.

3. Place the *RapiRead*™ CUBE Reader on top of the Adaptor correctly:

- For Non-Timer Protocol: After 15 Minutes of testing.
- For Timer Protocol: After adding 3 drops of pre-mix blood into the Sample Well (S) of the Test Cassette.

NON-TIMER PROTOCOL

4. Turn-on the *RapiRead*™ by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. To perform a reading, press the black button again once for 1 second.

5. The display will show "RFID".

6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*™. This will upload Vitamin D test specific Calibration data from RFID Card to *RapiRead*™.

7. Following an audible beep signal, "TEST" is displayed. Press the black button, the Reader displays "RUN".

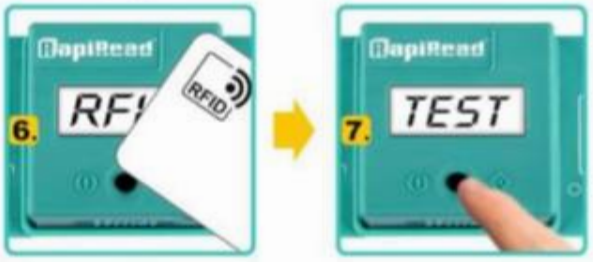
8. After successful data transmission the measurement will start.

9. Vitamin D (Vit D) concentration is displayed in ng/ml with an audible beep signal.

TIMER PROTOCOL

4. Turn-on the *RapiRead*™ by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. Keep pressing black button till display shows RFID.

5. The display will show "RFID"



6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*™. This will upload Vitamin D test specific Calibration data from RFID Card to *RapiRead*™.

7. Following an audible beep signal, "TEST" is displayed. Press the black button, the countdown timer will start.

8. Countdown timer display will start.

9. After 15 minutes Vitamin D (Vit D) concentration is displayed automatically in ng/ml with an audible beep signal.

PROCEDURE:

1. Bring all materials and specimens to room temperature (between 21°C – 24°C).
2. Remove the test card from the sealed foil pouch and place it on a hard flat surface.
3. Follow Instructions to use UniSamplerTM Device.
4. After applying 3 drops of pre-mix blood into the sample well (S), read and record the results at 15 Minutes by RapiReadTM CUBE Reader.

VITAMIN D CONTROL / SERUM PROTOCOL:

25 OH Quantitative Vitamin D Rapid Test has been designed for human finger-prick blood. However, Vitamin D Control or Serum sample can be used for testing. Instead of taking finger prick blood with blood collector, apply 5µl of Vitamin D Control or Serum into the Collection Tube using Micropipette (not provided with the Kit) and follow “Instructions to Use UniSamplerTM Device”.

Important Note: Result after 15 minutes may not be accurate.

Recommendation:

You can get vitamin D from the sun, but it's not so easy. Many people call vitamin D the "sunshine vitamin." And it's true – your skin can synthesize vitamin D from sunlight. But it's not as easy as it sounds.

People with darker pigmented skin may require longer sun exposure than those with lighter pigmented skin to synthesize the same amount of vitamin D.

Geographic location also plays a huge role in how much vitamin D you can get from the sun. For example, people who live in northern latitudes may have difficulty synthesizing vitamin D from the sun during the winter months. So, check out the UV index in your area – the lower the UV index, the lower your ability to make vitamin D from sunlight.

Consider a high-quality vitamin D supplement.

While you can get vitamin D from a few foods, like fatty fish, egg yolks, liver, and fortified milk, many people struggle to get enough from diet alone. And the sun is powerful, but you can't always rely on it for vitamin D synthesis, either. That's where a high-quality

vitamin D supplement can step in.

Unfortunately, not all vitamin D supplements are created equal. Vitamin D3, or cholecalciferol, is the type of vitamin D your body produces after exposure to sunlight, and it's the type that you metabolize most effectively, so it makes sense to choose a vitamin D3 supplement

Vitamin D is a fat-soluble vitamin, so be sure to take your vitamin D supplement with a meal that contains healthy fats to maximize absorption. For example, if you take your supplement with breakfast, the fat from an egg (including

the yolk), nuts, nut butter, seeds, or cheese will help your body absorb vitamin D.
Healthy vitamin D levels are essential in your fertility journey. If you're curious about your own vitamin D level, or if you're concerned that you may not be getting enough vitamin D on a daily basis, talk with your healthcare provider for individualized recommendations.

Is vitamin D also important during pregnancy?

Not only does achieving a normal vitamin D level seem to positively impact fertility, it may also improve the odds of having a healthy pregnancy. Studies have linked vitamin D deficiency during pregnancy with an increased risk of preterm birth, gestational diabetes, preeclampsia (very high blood pressure during pregnancy),
So, continuing a vitamin D supplement once pregnant is good for both mom and baby. Research has shown that taking 2,000 – 4,000 IU of vitamin D is safe and effective in achieving a normal vitamin D level for pregnant women and preventing vitamin D deficiency in newborns.

"Our opinion is that given the simplicity and safety of treatment with vitamin D, the low cost associated with it, we should err on the side of having patients use vitamin D as they go through fertility treatment and enter pregnancy. For any medical intervention, you need to weigh the potential benefits against the risks and costs. The risks and costs are so low for vitamin D that any potential benefit is worth considering," explains Dr. Widra.

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