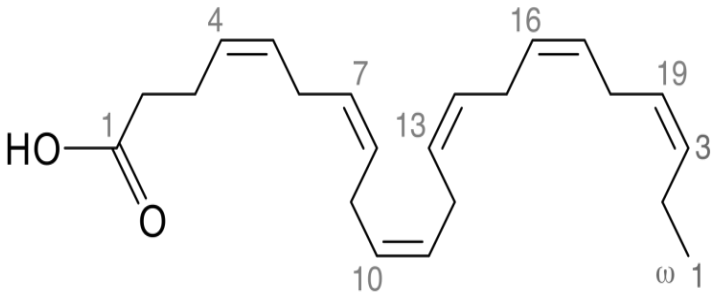

FIG. 1

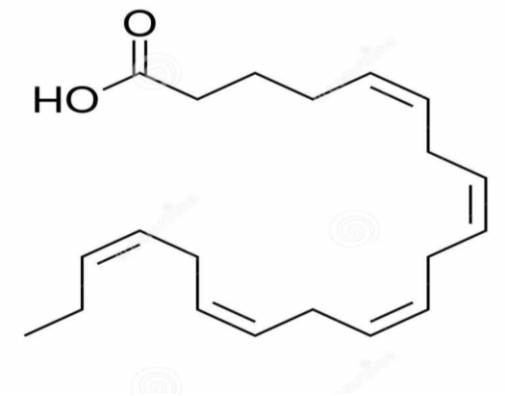

FIG 2.

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

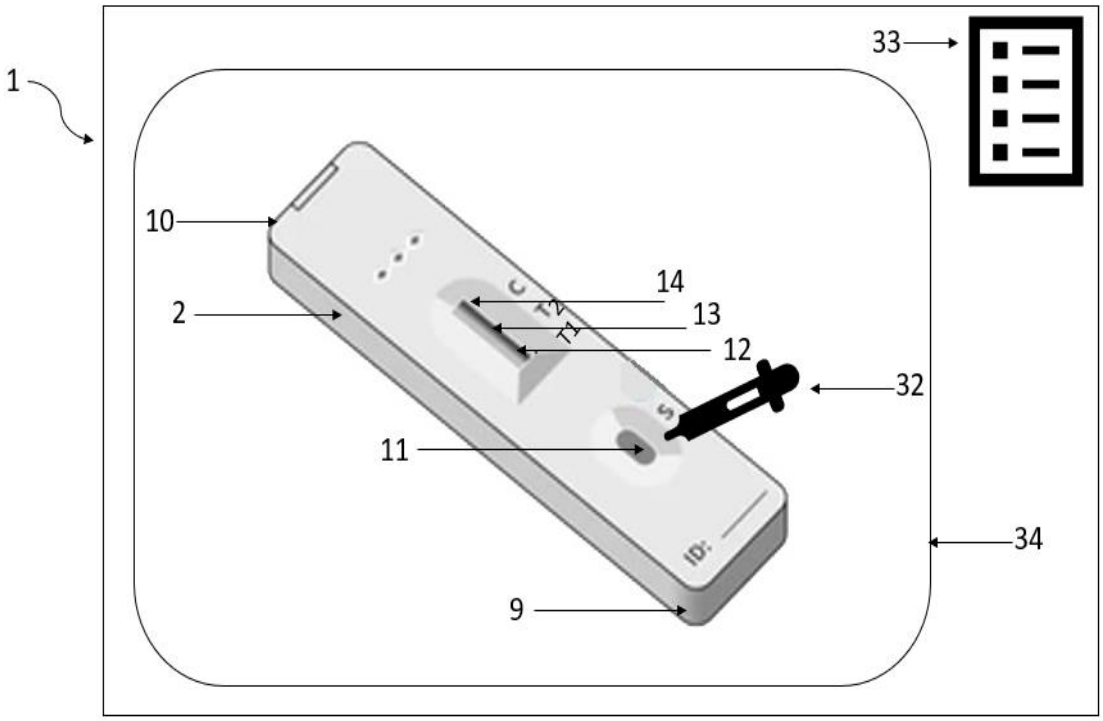

FIG. 3

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

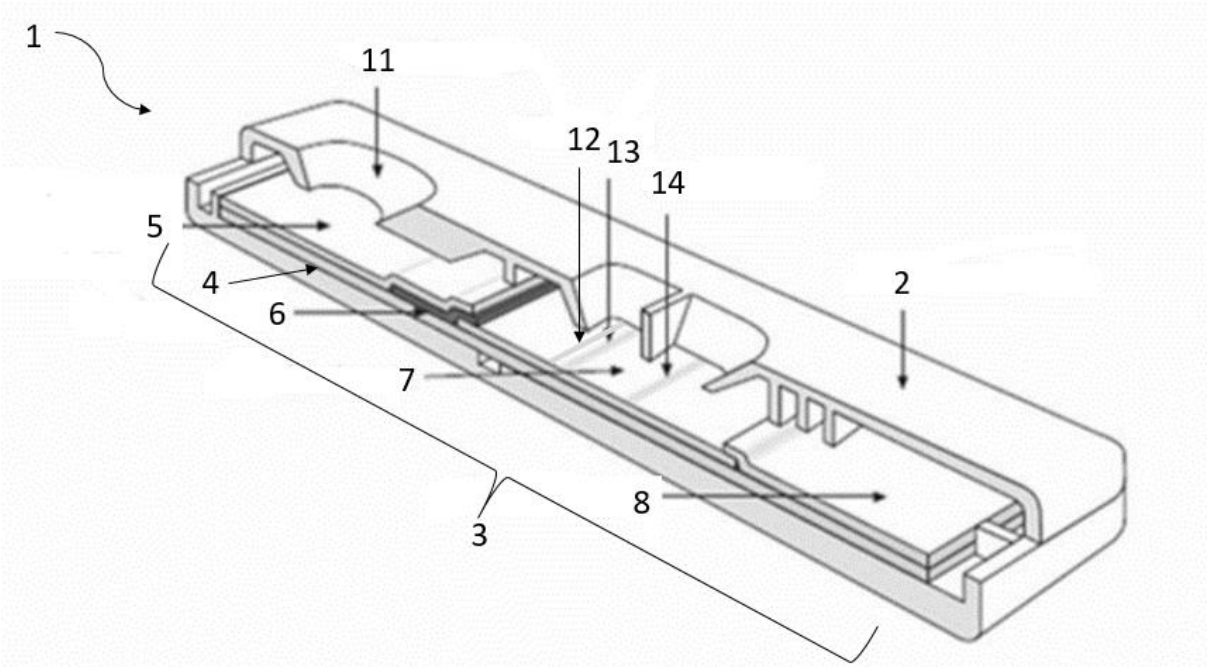

FIG. 4

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

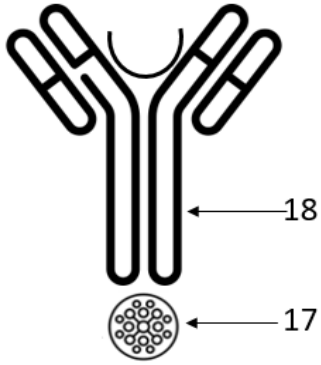

FIG. 5

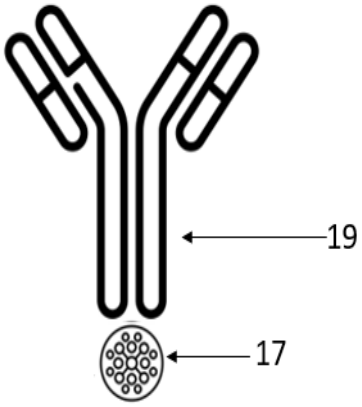

FIG. 6

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

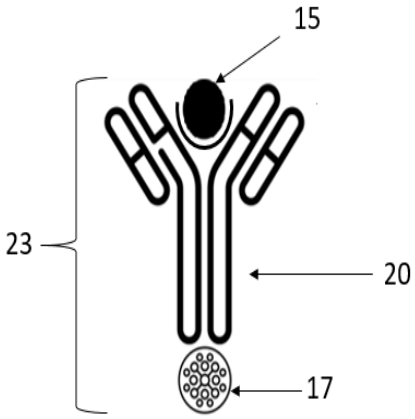

FIG. 7

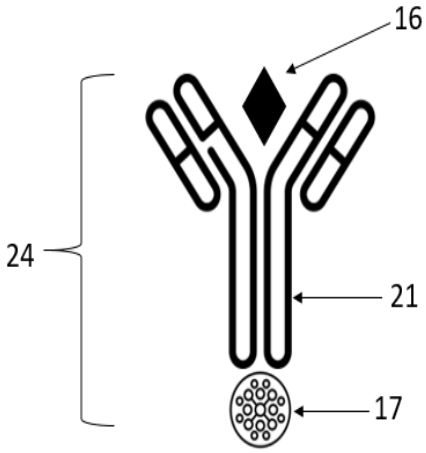

FIG. 8.

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

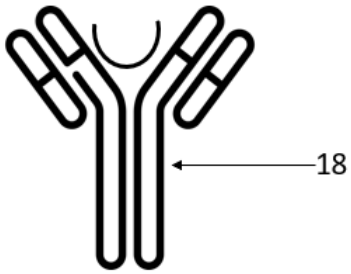

FIG. 9. A.

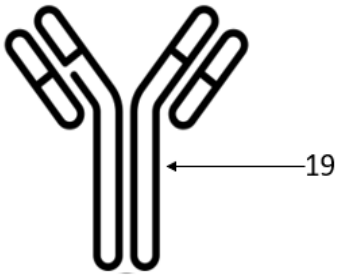

FIG. 9. B.

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

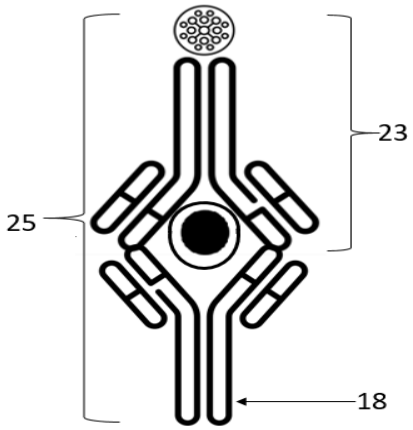

FIG. 10.

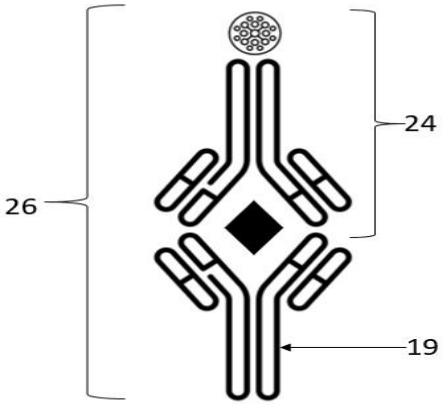

FIG. 11.

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

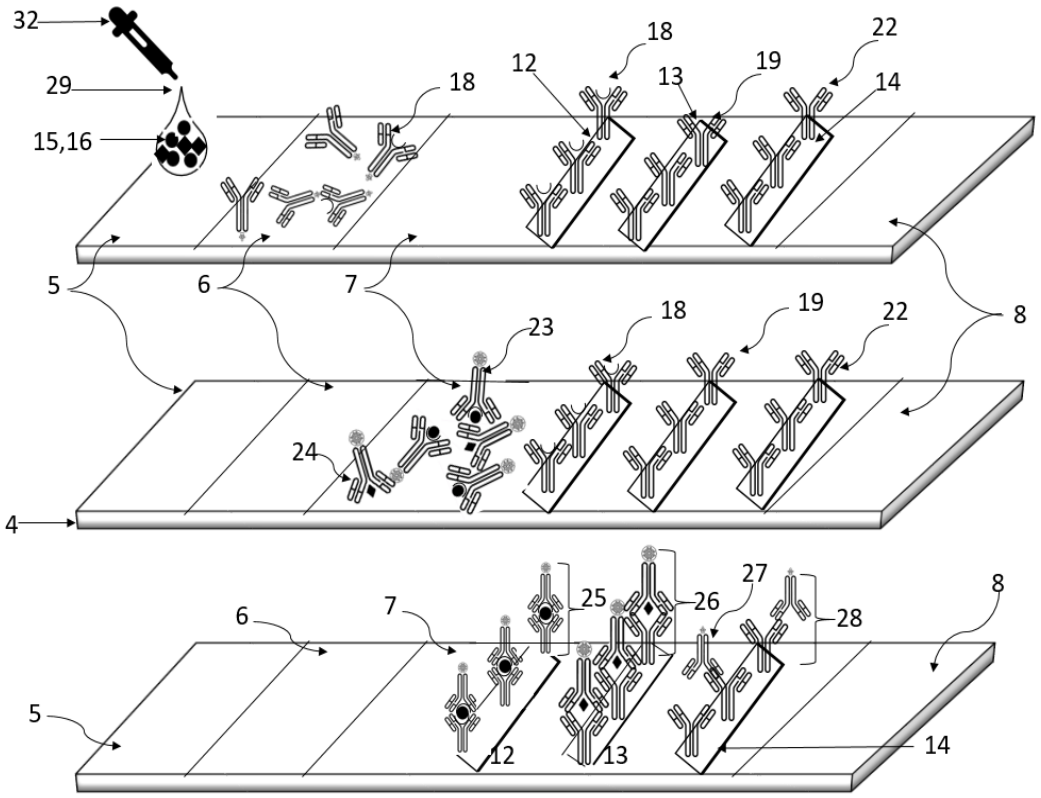

FIG. 12.

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

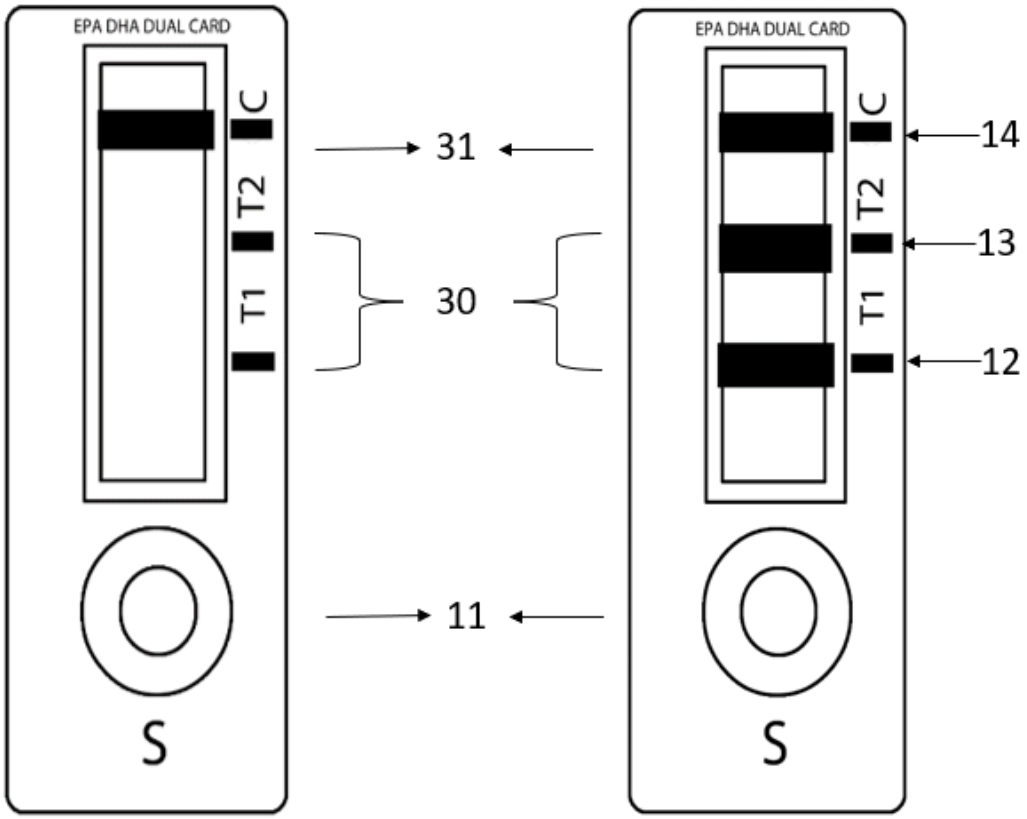

FIG. 13 A.

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

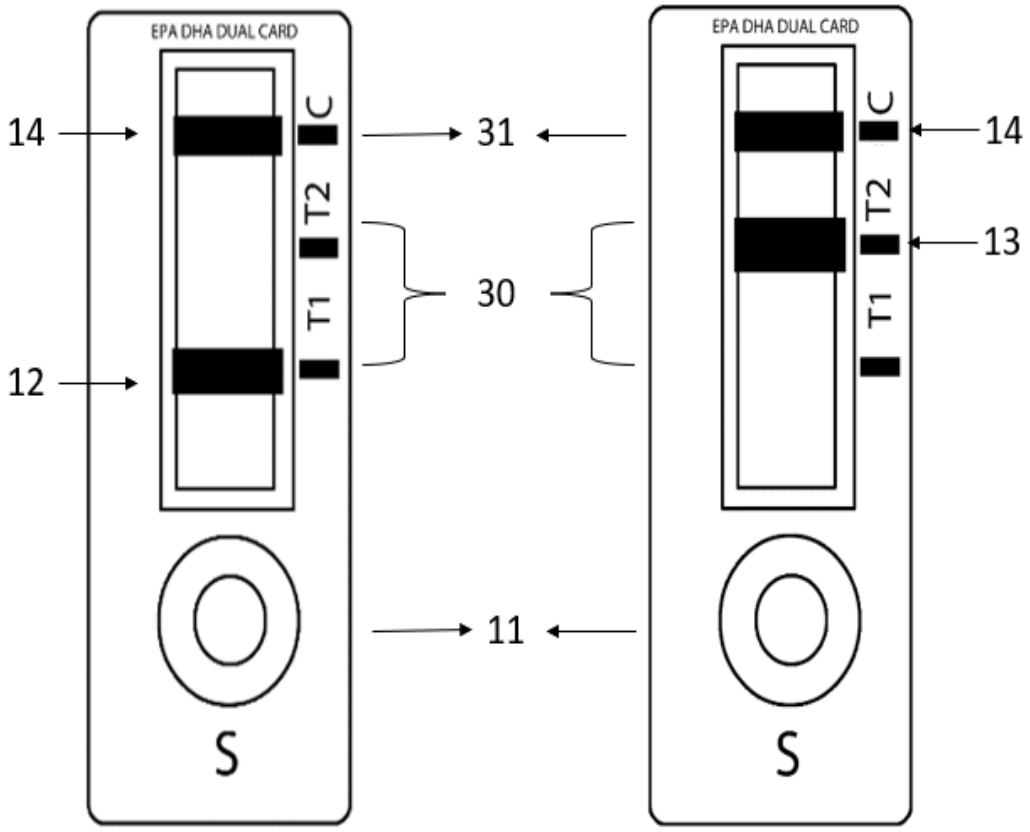

FIG. 13 B.

For applicants,

Mrs. Allison Katariya
Agent of the applicant -IN/PA-2190

FORM 2

THE PATENTS ACT, 1970

(39 OF 1970)

AND

THE PATENTS RULES, 2003

COMPLETE SPECIFICATION

(See Section 10; rule 13)

A RAPID DUAL TEST KIT FOR DHA AND EPA DETECTION AND METHOD OF PREPARATION THEREOF

Applicants:

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&

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The following specification particularly describes the invention and the manner in which it is to be performed.

FIELD OF INVENTION:

The present invention relates to the field of healthcare. Particularly, the present invention relates to a rapid dual test kit for detection of the Omega-3 fatty acid molecule, a combination of both, Decosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA), wherein the said rapid dual test kit is based on the principle of lateral flow immunoassay; and enables qualitative detection of DHA and EPA using a color indication test.

BACKGROUND:

Omega-3 fatty acids are a family of essentially important polyunsaturated fatty acids that must be obtained from the diet as they are not produced by the body itself. They play an important role in the body and are also associated with various health benefits. Common foods rich in Omega-3 fatty acids include fatty fish, fish oils, flax seeds, chia seeds, flaxseed oil and walnuts. Omega-3 supplements such as fish oil or algal oil is also recommended. Omega-3 fatty acids are divided mainly into three types- ALA (Alpha- linolenic acid), DHA (Decosahexaenoic acid) and EPA (Eicosapentaenoic acid); providing different health benefits.

DHA is the most important omega-3 fatty acid in human body being the structural component of the brain, the retina of eyes, and numerous other body parts. DHA is more particularly important for pregnant and breastfeeding women to get ample amount of DHA as it may affect the health and intelligence of the baby. Furthermore, fortifying baby formula with DHA leads to improved vision in infants, is vital for brain and nervous system development and functioning in childhood as well as in adults. It can also boost heart health by reducing blood triglycerides and amount of LDL (bad) cholesterol particles.

However, an early-life DHA deficiency is associated with certain problems in late stages of life; such as learning disabilities, attention deficit hyperactivity disorder (ADHD), and aggressive hostility. A decrease in DHA in subsequent years is also linked to impaired brain function and the onset of Alzheimer's disease. DHA also provides positive effects to conditions, such as arthritis, high blood pressure, type 2

diabetes, and some cancers. Therefore, the detection of omega-3 levels in general and especially of DHA becomes evident.

EPA (eicosapentaenoic acid) is a "marine omega-3" as it is found mostly in fatty fish and fish oil. EPA concentrations are highest in herring, salmon, eel, shrimp, and sturgeon. Grass-fed animal products like dairy and meats, also contain some EPA. Some microalgae may also contain EPA. EPA is responsible for various physiological activities and reduce inflammation by using EPA to produce signaling molecules called eicosanoids. A chronic, low-level inflammation is known to drive several common diseases; and may also reduce symptoms of depression. It also prevents the blood from clotting easily, reduces triglyceride levels in blood thus subsiding pain and swelling. Extended use of EPA includes its use as a US FDA-approved prescription drug for reducing triglyceride levels, a supplement for heart disease, preventing heart attack, treating depression and for chemotherapy related side effects, diabetes, recovery after surgery, and many other purposes.

Consequently, the reduction in levels or concentration of EPA fatty acids from blood may have adverse effects on body such as hypertriglyceridemia, atherosclerosis, increase in LDL (bad) cholesterol, increase risk of cardiovascular diseases, arrhythmia, blood clotting, Alzheimer's disease, dementia or age-related macular degeneration. Thus, it is imperative to monitor the omega-3 levels in the human body..

The most conventional way to detect the Omega 3 levels in the body is by doing the blood test whereby the EPA and DHA levels can be identified. This blood test can take more than 4-5 days for providing the results and are expensive (cost ranging between Rs. 6000 to 8000 per test). Alternatively, various omega-3 collection kits are available in the market. These kits allow the user to provide blood samples and send it to the lab for detection. Once the lab receives the sample, it take the samples for ELISA tests; which again takes at least 4-5 working days. Thus, these collection kits also take equally longer duration to provide precise results. and adds to the already expensive lab test cost; thereby making such kits inconvenient and expensive. Moreover, non of the above methods provide rapid test results which is the need of the hour.

The currently available detection kits are expensive and takes longer time for detection and provide an accurate result. To overcome the drawbacks of the prior arts, the present

invention provides a more convenient, cost-effective and rapid detection dual test kit for omega-3; enabling the user to detect the DHA and EPA simultaneously through a single test.

OBJECTS OF THE INVENTION:

An object of the present invention is to provide a rapid dual test kit for determining the levels of Omega-3 fatty acid molecules such as docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) using whole blood, serum or plasma samples.

Another object of the present invention is to provide a method for preparation of said DHA and EPA rapid dual test kit.

Yet another object of the present invention is to provide a DHA and EPA rapid dual test kit which detects the sufficiency levels of DHA and EPA in a sample respectively.

Yet another object of the present invention is to provide a DHA and EPA rapid dual test kit which allows to determine whether a given blood, serum or plasma sample has sufficient or deficient levels of DHA and EPA respectively.

Yet another object of the present invention is to provide a DHA and EPA rapid dual test kit which yields results in 1 min.

Yet another object of the present invention is to provide a DHA and EPA rapid dual test kit which allows the user to himself visually detect the DHA and EPA levels respectively, without the need of any technical device and personnel for detection and analysis.

Yet another object of the present invention is to provide a DHA and EPA rapid dual test kit which is easy to use, fast and cost-effective.

Yet another object of the present invention is to provide a DHA and EPA rapid dual test kit which eliminates the need of sending samples to a laboratory for detection and analysis.

SUMMARY OF THE INVENTION:

Before the present invention is described, it is to be understood that present invention is not limited to particular methodologies and materials described, as these may vary as per the person skilled in the art. It is also to be understood that the terminology used in the description is for the purpose of describing the particular embodiments only, and is not intended to limit the scope of the present invention.

The present invention relates to a rapid dual test kit for detection DHA and EPA, within 0-20 minutes, preferably one minute, wherein the kit is based on the principle of lateral flow immunoassay. The rapid dual test kit of the present invention comprises of an immuno-chromatographic strip encased in a cassette. The strip further comprises of a sample release pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad arranged in a sequential overlapping manner.

In one aspect, the sample release pad is placed exactly below the sample port such that when a sample is loaded in the sample port, it directly travels and comes in contact with the sample release pad so as to allow the lateral flow of the sample from the sample release pad to the absorbent pad via capillary action.

In one aspect, the lateral flow of sample includes loading a sample in the sample port that comes in contact with the sample release pad, allowing lateral flow to absorbent pad via capillary action, binding of gold nanoparticle conjugated detector antibody complex to DHA and EPA molecules respectively to form a conjugate-antigen-antibody complexes I and II, that moves from the conjugate pad to the nitrocellulose membrane, binding of anti-DHA and anti EPA capture antibodies to the respective DHA or EPA molecules bound to the gold nanoparticle-Detector antibody complexes, capturing all the DHA and EPA bound gold nanoparticle-Detector antibody complexes on the test line, such that the DHA or EPA molecules are sandwiched between the gold nanoparticle-detector antibody complex and anti-DHA or anti EPA capture antibodies respectively, capturing unbound conjugated detector antibodies by anti-Mouse IgG

antibodies to form IgG- IgG complex at the control line, viewing color indication at the control line that indicates the travelling of the sample across the nitrocellulose membrane, collecting the excess sample and gold nanoparticles conjugated detector antibodies at the absorbent pad.

In another aspect, the method of preparation of rapid detection kit include the steps of; cutting of sample release pad; cutting of blood separation pad; cutting of conjugate pad; cutting of nitrocellulose membrane; cutting of absorbent pad, coating of sample release pad, preparation of test and control antibody, preparation of gold conjugated detector antibodies, coating of conjugate pad, coating of nitrocellulose membrane, lamination of plastic pad, cutting of laminated plastic pad, assembly of cut laminates in cassettes, packing of aluminium foil pouch, sealing packed aluminium foil pouch, preparing test diluent, dispensing test diluent into dropper bottle, packing sealed pouches and diluent bottles in boxes.

In another aspect, the rapid dual test kit can detect the DHA and EPA levels upto a minimum detection level ranging between 12.5 to 15 pg/ml ; through a color indication is visible (positive test) when the sample contains Omega-3 fatty acid (including DHA or EPA) molecules up to the said threshold level; and no indication (negative test) at test lines T1 and/or T2 below threshold level thereby confirming the deficiency of DHA and/or EPA and ultimately Omega-3 levels within the sample.

BRIEF DESCRIPTION OF DRAWINGS:

FIG. 1 illustrates the chemical structure of DHA.

FIG. 2 illustrates the chemical structure of EPA.

FIG. 3 illustrates the external view of the rapid dual test kit of the present invention.

FIG. 4 illustrates the 3D cross sectional schematic of the rapid dual test kit of the present invention.

FIG. 5 illustrates schematic diagram of the gold conjugated detector antibodies for DHA.

FIG. 6. illustrates schematic diagram of the gold conjugated detector antibodies for EPA.

FIG. 7. illustrates schematic diagram of the gold conjugated detector antibody-DHA complex.

FIG. 8. illustrates schematic diagram of the gold conjugated detector antibody-EPA complex.

FIG. 9. A. illustrates the schematic diagram of the capture antibodies- for DHA.

FIG. 9. B. illustrates the schematic diagram of the capture antibodies- for EPA.

FIG. 10. illustrates the schematic diagram of the DHA molecule sandwiched between the gold conjugated detector antibody and the capture antibody (Antigen-Antibody Complex I).

FIG. 11. illustrates the schematic diagram of the EPA molecule sandwiched between the gold conjugated detector antibody and the capture antibody (Antigen-Antibody Complex II).

FIG. 12. illustrates the overall schematic of the principle of lateral flow immunoassay.

FIG. 13. A. illustrates the positive and negative results obtained using the rapid dual test kit of the present invention.

FIG. 13. B. illustrate the presence of at least one omega-3 molecule in the sample using the rapid dual test kit of the present invention.

DETAILED DESCRIPTION:

Before the present invention is described, it is to be understood that this invention is not limited to methodologies described, as these may vary as per the person skilled in the art. It is also to be understood that the terminology used in the description is for the purpose of describing the particular embodiments only and is not intended to limit the scope of the present invention. Throughout this specification, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. The use of the expression "at least" or "at least one" suggests the use of one or more elements or ingredients or quantities, as the use may be in the embodiment of the invention to achieve one or more of the desired objects or results. Various embodiments of the present invention are described below. It is, however noted that the present invention is not limited to these embodiments, but rather the intention is that modifications those are apparent are also included.

Terminologies:

"Sample" refers to human/animal body fluid such as but not limited to blood, serum and plasma.

"DHA" refers an Omega-3 fatty acid molecule named Docosahexaenoic acid, having the chemical formula as depicted in FIG. 1.

"EPA" refers an Omega-3 fatty acid molecule named eicosapentaenoic acid, having the chemical formula as depicted in FIG. 2.

"Antibody" refers to a molecule that specifically binds an antigenic determinant. It can refer to any whole antibody or functional fragment of an antibody comprising or consisting of at least one antigenic combination site making it possible for said antibody to bind to at least one antigenic determinant of an antigenic compound. It can refer to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. This term encompasses

polyclonal antibodies, monoclonal antibodies, and fragments thereof, as well as molecules engineered from immunoglobulin gene sequences. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Examples of antigen binding molecules or antibodies are immunoglobulins and derivatives, e.g. fragments, thereof such as scFv (single chain variable fragment) chains, etc. These functional fragments can in particular be obtained by genetic engineering.

"Capture antibody" refers to an antibody or a part of an antibody, preferably attached to a solid phase, which is capable of retaining an antigen, for example one or more DHA or EPA molecules, present in a sample, by affinity binding.

"Detector antibody" refers to an antibody or a part of an antibody which is labeled, for example conjugation with gold nanoparticles, is capable of binding to the captured antigen through affinity binding, by recognizing an epitope site which is different from that recognized by the capture antibody or identical due a repeat motif in the capsid.

The present invention relates to a rapid dual test kit (1) for detection and indexing of the Omega-3 fatty acid which has a combination of DHA and EPA molecules as illustrated in Fig. 3. The rapid dual test kit (1) is based on the principle of lateral flow immunoassay. The rapid dual test kit (1) of the present invention comprises of a test device (cassette) (2), a test diluent (sample) (29), a dropper (32), a pack insert (instruction sheet) (33) and a silica gel (34) to maintain ideal storage condition of the cassette (2); wherein the cassette (2) comprises of an immuno-chromatographic strip (hereinafter referred to as 'strip') (3) encased in a test device referred to as a cassette (2). The strip (3) further comprises of a sample release pad (5), a conjugate pad (6), a nitrocellulose membrane (7), and an absorbent pad (8) arranged in a sequential manner.

In a preferred embodiment, the cassette (2) is in the form of a hollow rectangular structure. The strip (3) is encased withing the cassette (2). The cassette (2) consists of three open areas namely a sample port (11), test line viewing area (30) wherefrom test

line T1 (12) and test line T2 (13) are visible and control line viewing area (31). The sample port (11) is the place where the sample is loaded using a dropper (32). The strip (3) is encased such that the proximal end (9) of the strip (3) is towards the sample port (11). The sample release pad (5) is placed exactly below the sample port (11) such that when a sample (29) is loaded in the sample port (11), it directly travels and comes in contact with the sample release pad (5). The cassette (2) is preferably made of plastic. The length of the cassette (2) is 70mm, the breadth is 20 mm and the height is 5mm.

In a preferred embodiment, the components of the strip (3) as illustrated in Fig. 4, – the sample release pad (5), the conjugate pad (6), the nitrocellulose membrane (7), and the absorbent pad (8) are arranged in such a manner that the adjacent pads are connected at an overlapping junction. The sequence of the pads from the proximal end (9) of the strip (3) is as follows – sample release pad (5), followed by conjugate pad (6), followed by the nitrocellulose membrane (7), followed by the absorbent pad (8). Thus, the sample release pad (5) is placed towards the proximal end (9) of the strip (3) while the absorbent pad (8) is placed at the distal end (10) of the strip (3). All the above components of the strip (3) are assembled on a backing material (4) such as but not limited to polystyrene or any other plastic material coated with a medium to high tack adhesive. All the components are laminated to the backing material (4) to provide rigidity and easy handling of the strip (3). The backing material (4) is coated with a pressure-sensitive adhesive to hold the various components in place.

In an embodiment, the sample release pad (5) is made up of glass fiber which is a hydrophobic material, with a good absorption capacity and is the first pad to come in contact with the sample (29). The sample (29) which can be used to detect the Omega-3 fatty acid, including DHA and EPA levels by means of the kit (1) of the present invention include serum/plasma and whole blood. A drop approximately 10µl of serum or plasma; or two drops approximately 20µl of whole blood samples (29) can be loaded in the sample port (11) of the kit (1). The sample release pad (5) is placed exactly below the sample port (11) such that when a sample (29) is loaded in the sample port (11), it directly travels and comes in contact with the sample release pad (5). All the pads of the strip (3) are made up of such materials and arranged in such a manner so as to allow the lateral flow of the sample (29) from the sample release pad (5) to the absorbent pad (8) via capillary action. The sample release pad (5) essentially serves as receiving

medium for the sample (29) and allows the movement of the sample (29) in a lateral direction towards the conjugate pad (6).

In an embodiment, the conjugate pad (6) is made up of a hydrophobic, synthetic release matrix with good absorption capacity and is the second pad to come in contact with the sample (29). The conjugate pad (6) is coated with detector antibodies for DHA (20) and detector antibodies for EPA (21) conjugated with gold nanoparticles (17) (hereinafter referred to as 'conjugated detector antibodies') (23, 24respectively) of the size 14 nm. The detector antibodies comprise an equal combination of both, anti-DHA polyclonal antibodies (20) or anti-EPA polyclonal antibodies (21) raised in rabbit (IgG); that detects DHA (15) and EPA (16) molecule respectively. The detector antibodies (20,21) can also be raised in horses, sheep, goats, chickens or any other suitable host animals. The antibodies used in the invention may include but are not limited to IgG, IgY, IgA, IgD, IgE and IgM, and/or recombinantly expressed antibodies that may be single chain antibodies, double chain antibodies, or other. The concentration of conjugated detector antibodies (20,21) coated/immobilized on the conjugate pad (6) ranges from 2mg/ml to 6mg/ml with a preferential concentration of 5mg/ml.

The sample (29), including whole blood, serum or plasma, but not limited to it; comprises of various biomolecules including Omega-3 fatty acids such as DHA and EPA. When the sample (29) comes in contact with the conjugate pad (6); the anti-DHA polyclonal conjugated detector antibodies (20) bind to DHA molecules (15) and form a gold nanoparticle-Detector antibody-DHA complex(25); whereas the anti-EPA polyclonal conjugated detector antibodies (21) bind to EPA molecules (16) and form a gold nanoparticle-Detector antibody-EPA complex (26) (hereinafter referred to as 'conjugate-antigen-antibody complex' I (25) and 'conjugate-antigen-antibody complex' II (26)). The conjugate-antigen-antibody complex (25, 26) along with the unbound conjugate-antibodies (27) travel towards the nitrocellulose membrane (7) via capillary movement.

In an embodiment, the nitrocellulose membrane (7) comprises of three indicator lines namely one control line (25), a test line T1 for DHA (24) and a test line T2 for EPA. The control line (31) is coated with anti-mouse IgG antibody (22) raised in mouse at a concentration of 2-5mg/ml. The test line (12) T1 is coated with polyclonal anti-DHA

capture antibodies (18) and test line T2 (13) is coated with anti-EPA capture antibodies (19) raised in rabbit (IgG); so that both the Omega-3 fatty acid molecules can be detected simultaneously. The detector antibodies for DHA and EPA (20,21) can also be raised in horses, sheep, goats, chickens or any other suitable host animals. The antibodies used in the invention may include but are not limited to IgG, IgY, IgA, IgD, IgE and IgM, and/or recombinantly expressed antibodies that may be single chain antibodies or other. The concentration of polyclonal anti-DHA capture antibodies (18) and polyclonal anti-EPA capture antibodies coated/immobilized on the test lines T1 and T2 respectively, range from 1 mg/ml to2 mg/ml with a preferential concentration of 1 mg/ml.

The polyclonal anti-DHA capture antibodies (18) are highly specific and are able to bind specifically to regions of DHA molecules (15); and the polyclonal anti-EPA capture antibodies (19) are highly specific and are able to bind specifically to regions of EPA molecules (16). When the conjugate-antigen-antibody complex (25,26) moves from the conjugate pad (6) to the nitrocellulose membrane (7), the anti-DHA capture antibodies (18) bind to the DHA molecule (15) already bound to the gold nanoparticle-DHA Detector polyclonal antibody complex (23); and the anti-EPA capture antibodies (19) bind to EPA molecule already bound to the gold nanoparticle-EPA detector polyclonal antibody complex (24). In this manner, the test line T1 (12) captures all the DHA bound gold nanoparticle-Detector antibody complexes (23) and test line T2 captures all the EPA bound gold nanoparticle-Detector antibody complexes (24). In this manner, the DHA or EPA molecules (15,16) are sandwiched between the gold nanoparticle-Detector antibody complex (23,24) and anti-DHA or anti-EPA capture antibodies (18,19) respectively, showing a color indication at the test lines T1 and T2 (12,13), that indicates the presence of DHA (15) and EPA (16) molecules respectively in the blood sample (29).

At the same time, the unbound conjugated detector antibodies (27) travel further and are captured at the control line (14) by anti-mouse IgG antibodies (22) to form IgG-IgG complex (28). The color indication at the control line (14) indicates the travelling of the sample (29) across the nitrocellulose membrane (7). The excess sample (29) and gold nanoparticles conjugated detector antibodies (23,24) are collected at the absorbent pad (8) made up of cellulose filters.

In an embodiment, as illustrated in the Fig. 13. A.; the color indication at the test lines T1 and T2 (12,13) confirms the presence of DHA (15) and EPA (16) molecules within the blood sample (29) (positive test); whereas the color indication at the control line (14) indicates the travelling of the sample (29) across the nitrocellulose membrane (7). If no color indication is observed at test lines T1 and T2 (12,13) and a color indication at control line (14) confirms the deficiency of DHA (15) and EPA (16) molecules within the blood sample (negative test). The said color detection test using the lateral flow immunoassay is completed within a range of 0-20 minutes; preferably within 1 minute.

In an embodiment, as illustrated in the Fig, 13. B.; a visible color indication at the test line T1 (12), no color indication at test line T2 (13) and color indication at control line (14) confirms the presence of DHA only in the blood sample; whereas no color indication at the test line T1 (12), visible color indication at test line T2 (13) and color indication at control line (14) confirms the presence of EPA only in the blood sample. It is to be noted that the color indication at T1 (12) and T2 (13) simultaneously may be observed if the both DHA (15) and EPA (16) molecules are present in the sample (29) above the specific threshold level.

In a further embodiment, the rapid dual test kit (1) can detect the DHA and EPA levels upto a minimum detection level ranging between 12.5 to 15 pg/ml; such that a color indication is visible (positive test) at T1 and/or T2 when the sample (29) contains Omega-3 fatty acid (including DHA and EPA) molecules (15,16) up to the said threshold level; whereas Omega-3 fatty acid (DHA and EPA) level in a sample (29) lower than the threshold level does not show color indication (negative test) at test lines T1 (12) and T2 (13); which confirms the deficiency of DHA and/or EPA and ultimately Omega-3 levels within the sample (29).

It is to be noted that a single rapid dual test kit is used for detection of DHA and EPA molecules (15,16) simultaneously; wherein the conjugate pad (6) of the strip is coated with both, the anti-DHA polyclonal conjugated detector antibodies (20) that bind to DHA molecules (15) and the anti-EPA polyclonal conjugated detector antibodies (21) that bind to EPA molecules (16) and the test line T1 (12) on nitrocellulose pad (7) is coated with polyclonal anti-DHA capture antibodies (18) which are highly specific and

are able to bind specifically to regions of DHA molecules (15);. and the test line T2 (13) on nitrocellulose membrane (7) is coated with polyclonal anti-EPA capture antibodies (19) which are highly specific and are able to bind specifically to regions of EPA molecules (16).

Assembly of immunochromatographic strip (3):

In an embodiment, the sample release pad is made up of glass fibre membrane. The preferable thickness of the sample release pad is 300 µm and the effective length and breadth is 20 mm x 3.5 mm. The conjugate pad is made up of synthetic release matrix. The preferable thickness of the conjugate pad is 250 µm and the effective length and breadth is 5mm x 3.5 mm. The nitrocellulose membrane has the dimensions of 25mm x 3.5 mm with a thickness of 100 µm. The distance between the control line and test line T2 is 3mm and distance between test line T2 and test line T1 is 3mm. The preferrable thickness of absorbent pad is 1000 µm and the effective length and breadth is 20mm x 3.5mm. All the above components of the strip are assembled on a backing material such as but not limited to polystyrene or any other plastic material coated with a medium to high tack adhesive. All the components are laminated to the backing material to provide rigidity and easy handling of the strip. The backing material is coated with a pressure-sensitive adhesive to hold the various components in place.

Determination of the arrangement of the lateral flow immunoassay components especially the partial extending (overlapping junctions) over of the components on each other is critical to the success and reproducibility of the test strip. It is important that the components extending over each other are correctly defined in a way compatible with the dimensions of the strip and that it takes into consideration the limits of tolerance at the same time. In the present invention, the sample release pad partly extends for 2mm over the conjugate pad. Similarly, 2mm of the proximal end of the nitrocellulose membrane is placed under the conjugate pad. The absorbent pad is placed at the distal end of the strip. The absorbent pad extends over the nitrocellulose membrane for 2mm.

Method of preparation of the rapid dual test kit (1) of the present invention:

A. Cutting of sample release pad:

In an embodiment, the sample release pad is made from glass fiber matrix. The glass fiber matrix is cut into small pieces of 20mm x 3.5mm and stored at RT less than 30 Degree Celsius in a locking bag until further use.

B. Cutting of blood separation pad:

In an embodiment, an additional small pad named ‘blood separation pad’ is located near the sample window. The blood separation pad is made from cellulose. The cellulose is cut into small pieces of 5mm x 3.5mm and stored in a locking bag until further use.

C. Cutting of conjugate pad:

In an embodiment, the conjugate pad is made from synthetic release matrix. The synthetic release matrix is cut into small pieces of 5mm x 3.5 and stored in a locking bag until further use.

D. Cutting of nitrocellulose membrane:

In an embodiment, the nitrocellulose membrane is cut in to small pieces of 25mm x 3.5mm and stored in a locking bag until further use.

E. Cutting of absorbent pad:

In an embodiment, the absorbent pad is made from cellulose. The cellulose is cut into small pieces of 20 mm x 3.5 mm and stored in a locking bag until further use.

F. Coating of sample release pad:

In an embodiment, the cut sample release pad(s) is kept on a mesh tray. Each (if multiple) cut sample release pad is coated with 2.5 mL of sample release pad buffer. The tray

containing the cut sample release pad(s) is kept for drying in an incubator at 37 ± 2℃ for 2 hours. The dried sample release pad is stored in a locking bag with silica gel until further use.

G. Preparation of test and control (Capture) antibody:

In an embodiment, in order to prepare the test antibody, the anti-DHA rabbit anti- DHA polyclonal antibody and anti-EPA rabbit anti- EPA polyclonal antibody is diluted in buffer with a ratio of 2-3mg/ml along with stabilizer and methanol. In order to prepare the control antibody, the anti-mouse IgG is diluted in buffer along with stabilizer in ratio of 1-2mg/ml. Both the prepared test and control antibodies are separately dispensed in aliquots of 1 mL in tubes and stored at -20- 50 degree Celsius until further use.

H. Preparation of gold conjugated detector antibodies:

In an embodiment, a conjugate diluent is first prepared in order to prepare the gold conjugated detector antibodies. The conjugate diluent is prepared by dissolving 10% of Sucrose and 5% of Trehalose in Tris buffer and made up in volume upto 50-100 ml. The gold conjugated detector antibody, including anti-DHA rabbit anti-general DHA polyclonal antibody and anti-EPA rabbit anti-general EPA polyclonal antibody, is diluted in the conjugate diluent of optical density ranging between 5 OD to 10 OD; preferably at 5 OD. The gold conjugated detector antibodies so prepared are stored at 2 to 8 Degree Celsius until further use.

I. Coating of conjugate pad:

In an embodiment, the required number of cut conjugate pads are placed on a mesh tray. 2mg/ml to 6mg/ml; preferentially 5mg/ml of the gold conjugated detector antibodies for DHA and EPA are coated on each of the conjugate pads. The tray containing the conjugate pads is kept for drying in an incubator at 37 ± 2℃ for 1 hour. The dried conjugate pad is removed from the incubator and is again dried in a vacuum over at 37 ± 2℃ for 15 minutes. The dried conjugate pad so obtained is stored in a locking bag

with silica gel until further use. The locking bag is kept in an aluminium foil pouch and is sealed, until further use.

J. Coating of nitrocellulose membrane:

In an embodiment, in order to coat the nitrocellulose membrane with test and control antibodies, the receptacles in the machine ISO Flow Dispenser for test and control antibodies are filled with 1% Sodium Hypochlorite. They are initially washed thrice. Later, they are washed with purified water 20 times following which the water is de-loaded from them. The receptacles for test antibody are filled with test antibody and the receptacle for control antibody are filled with 1 ml of control antibody.
The nitrocellulose membrane is coated with 20-30 µL of test and control antibodies. The coated nitrocellulose membranes are placed in a steel tray and kept for drying at 37 $\pm$ 2°C for 1 hour in an incubator. The membranes are stored at room temperature less than 30 degrees until further use.

K. Lamination of plastic pad:

In an embodiment, a plastic pad laminated with 55 mm double sided Polyester tape is placed on the working table. Marking a distance of 1.9 cm from the operator side, the coated membrane (nitrocellulose) is pasted on the upper edge of the pad marking. The absorbent pad is pasted on the upper edge of the membrane forming an overlapping junction of 2 mm. The conjugate pad is pasted on the lower edge of the membrane forming an overlapping junction of 2 mm. The cut blood separation pad is pasted on the lower edge of the conjugate pad. The sample release pad is pasted on top of the blood separation pad. A 12.5 mm tape is pasted on top of the sample release pad forming an overlapping junction of 2 mm on the coated nitrocellulose membrane. All the laminated pads are kept in a locking bag and stored at room temperature less than 30 Degree until further use.

L. Cutting of laminated plastic pad:

In an embodiment, the laminated plastic pad is mounted on the strip cutter machine with pre-programmed cutting dimensions. The cutting process is initiated and the first

two pieces are discarded. The third piece is checked for correctness of dimensions and if any burrs are found it is stored in process rejection tray. Similar process is adopted for further pieces being cut as well.

M. Assembly of cut laminates in cassettes:

In an embodiment, the bottom of the Lateral Flow cassettes is placed on the working table. The sample release pad of the cut laminate is places in the closed bracket of the cassette. The visual sample display board is referred for identification of the devices. The top of the cassette with the circular opening of the sample port resting on the sample release pad. The cassette is pressed using the pressing machine to seal the unit. All the cassettes so prepared are placed in a tray.

N. Packing of Aluminium foil pouch:

In an embodiment, silica gel is dried before use in a hot air over at 90 +5℃ for 90 minutes. One cassette is placed inside a pouch followed by silica gel sachet and a dropper.

O. Sealing packed Aluminium foil pouch:

In an embodiment, the sealing machine is set at 200-300 degree Celsius. After the said temperature is achieved, the open side of the pouch is sealed using the sealing machine. The sealed pouch is stored in a box.

P. Preparation of test diluent:

In an embodiment, to prepare the test diluent, 0.1 to 0.5% of detergent trition is dissolved in buffer 100 ml and the test diluent so prepared is stored at room temperature.

Q. Dispensing of test diluent into dropper bottle:

In an embodiment, the test diluent is dispensed into dropper bottles and a sticker for the same is pasted on the bottle.

R. Packing sealed pouches and diluent bottles in boxes:

In an embodiment, coded boxes are folded and the sealed aluminium pouches are placed in the boxes. One product pack (sealed aluminium pouch) and one diluent bottle are placed in each box.

While considerable emphasis has been placed herein on the specific elements of the preferred embodiment, it will be appreciated that many alterations can be made and that many modifications can be made in preferred embodiment without departing from the principles of the invention. These and other changes in the preferred embodiments of the invention will be apparent to those skilled in the art from the disclosure herein, whereby it is to be distinctly understood that the foregoing descriptive matter is to be interpreted merely as illustrative of the invention and not as a limitation.

CLAIMS:

We claim,

1. A rapid dual test kit (1) for DHA and EPA detection within 0-20 minutes, preferably one minute; comprising of a test device (cassette) (2), a test diluent (29), a dropper (32), a pack insert (instruction sheet) (33) and a silica gel (34) where an immuno-chromatographic strip (3) encased in a cassette (2) with dimensions of 70 mm x 20 mm x 5mm, consisting of three open areas namely a sample port (11), test line viewing area (30), and control line viewing area (31), wherein the Omega-3 fatty acid comprises of both, the docosahexaenoic acid (DHA) molecules (15) and the eicosapentaenoic acid (EPA) molecules (16);

characterized in that:
the rapid dual test kit (1) detects the DHA level and the EPA level in the sample (29) simultaneously;

the silica gel (34) maintains ideal storage condition for the cassette (2);

the immuno-chromatographic strip (3) in the cassette (2) of the test kit (1) comprises of:

a) a sample release pad (5) made of a glass fibre membrane, for loading whole blood, serum or plasma as test sample, having dimensions 20 mm x 3.5 mm and thickness 300 µm;
b) a conjugate pad (6) made of synthetic release matrix, coated with detector antibodies (20,21) conjugated with gold nanoparticles (17), having dimensions 5 mm x 3.5 mm and thickness 250 µm;
c) a nitrocellulose membrane (7) with three indicator lines- a control line (14) is coated with anti-mouse IgG antibody (22) and two test lines T1 (12) and T2 (13) coated with polyclonal anti-DHA capture antibodies (18) to enable detection of DHA molecules (15) and polyclonal anti-EPA capture antibodies (19) for detection of EPA molecules (16) respectively, having dimensions 25 mm x 3.5 mm; and

d) an absorbent pad (8) made of cellulose filters, acting as a collector of excess sample (29) and gold nanoparticle conjugated detector antibodies (23,24), having dimensions 20 mm x 3.5 mm and thickness 1000 µm;

such that all the components of the immune-chromatographic strip (3) are assembled on a backing material (4) coated with pressure sensitive medium to high tack adhesive and are arranged in a sequential manner from a proximal end (9) to the distal end (10) of the strip (3) in such a manner that each component pad is partially overlapping over other up to a length of 2mm; allowing lateral flow of sample (29) from sample release pad (5) to absorbent pad (8) via capillary action.

2. The rapid dual test kit (1) as claimed in claim 1, wherein the arrangement of the components of the strip (3) in sequence of the pads from the proximal end (9) of the strip (3) is a sample release pad (5), followed by conjugate pad (6), followed by the nitrocellulose membrane (7), followed by the absorbent pad (8) placed at the distal end (10) of the strip (3); such that all the components are laminated to the backing material (4) to provide rigidity and easy handling of the strip (3).

3. The rapid dual test kit (1) as claimed in claim 1, wherein the sample release pad (5) is placed exactly below the sample port (11) such that when a sample (29) is loaded in the sample port (11), it directly travels and comes in contact with the sample release pad (5) so as to allow the lateral flow of the sample (29) from the sample release pad (5) to the absorbent pad (8) via capillary action.

4. The rapid dual test kit (1) as claimed in claim 1, wherein distance between the control line (14) and test line T2 (13) is 3mm and the distance between test line T2 (13) and Test line T1 (12) is 3mm.

5. The rapid dual test kit (1) as claimed in claim 1, wherein the anti-mouse IgG antibody (22) coating the control line (14) are raised in mouse at a concentration of 2-5mg/mL; and the polyclonal anti-DHA capture antibodies (18) and polyclonal anti- EPA capture antibodies (19), coated/immobilized on the test lines T1 (12) and T2 (13) are IgG antibodies raised in rabbit at a concentration ranging from 1 mg/ml to 2 mg/ml with a preferential concentration of 1 mg/ml.

6. The rapid dual test kit (1) as claimed in claim 1, wherein the size of gold nanoparticles (17) conjugated with the detector antibodies is 14 nm.

7. The rapid dual test kit (1) as claimed in claim 1, wherein the concentration of DHA-conjugated detector antibodies (23) and EPA-conjugated detector antibodies (24) coated/immobilized on the conjugate pad (6) ranges from2mg/ml to 6mg/ml with a preferential concentration of 5mg/ml.

8. The rapid dual test kit (1) as claimed in claim 1, wherein all the component pads of the strip (3) are laminated to the backing material (4) made of polystyrene or any other plastic material coated which is with a pressure-sensitive medium to high tack adhesive thereby providing rigidity and easy handling of the strip (3).

9. The rapid dual test kit (1) as claimed in claim 1, wherein the antibodies used include but are not limited to IgG, IgY, IgA, IgD, IgE and IgM, and/or recombinantly expressed antibodies that may be single chain antibodies or double chain antibodies.

10. The rapid dual test kit (1) as claimed in claim1, wherein the sample (29) comprises either 10µl (approximately one drop) of serum or plasma sample (29); or 20µl (approximately two drops) of whole blood sample (29) can be loaded in the sample port (11) of the kit (1).

11. The rapid dual test kit (1) as claimed in claim 1, wherein the kit (1) detects the DHA and EPA levels upto a minimum detection level ranging between 12.5 to 15 pg/ml; such that a color indication is visible (positive test) when the sample (29) contains DHA (15) and/or EPA (16) molecules up to the specified threshold levels respectively; whereas a DHA and/or EPA level in a sample (29); lower than the threshold level does not show color indication (negative test) at test lines T1 (12) and/or T2 (13); which confirms the deficiency of DHA and/or EPA and ultimately Omega-3 levels within the sample (29).

12. The rapid dual test kit (1) as claimed in claim 1, wherein the lateral flow of sample (29) includes the following steps:

i. Loading a sample (29) in the sample port (11) where it directly travels and comes in contact with the sample release pad (5),
ii. allowing the lateral flow of the sample (29) from the sample release pad (5) to the absorbent pad (8) via capillary action,
iii. binding of gold nanoparticle (17) conjugated detector antibody complex (23, 24) to DHA or EPA molecules (15,16) to form a conjugate-antigen-antibody complex-I (25) and complex-II (26) ,
iv. moving of conjugate-antigen-antibody complexes I and II (25,26) from the conjugate pad (6) to the nitrocellulose membrane (7),
v. binding of anti-DHA capture antibodies (18) and anti-EPA capture antibodies (19) to the DHA (15) and EPA (16) molecule already bound to the respective gold nanoparticle-Detector antibody complex (23,24),
vi. capturing all the DHA and EPA bound gold nanoparticle -Detector antibody complexes (25, 26) on the test lines T1 (12) and T2 (13) respectively, such that the DHA or EPA molecules (15,16) are sandwiched between the gold nanoparticle-detector antibody complex (23,24) and anti-DHA capture antibodies (18) or anti-EPA capture antibodies (19),

vii. capturing unbound conjugated detector antibodies (27) traveling further by anti-mouse IgG antibodies (22) to form IgG- IgG complex (28) at the control line (14),

viii. viewing color indication at the control line (14) that indicates the travelling of the sample (29) across the nitrocellulose membrane (7),

ix. collecting the excess sample (29) and gold nanoparticles conjugated detector antibodies (23,24) at the absorbent pad (8).

13. A method of preparation of rapid dual test kit (1) comprises of the following steps:

a. cutting of sample release pad;
where a glass fibre matrix material is cut into small pieces preferably of 20 mm x 3.5 mm and stored at RT less than 30 degree Celsius in a locking bag until further use;

b. cutting of blood separation pad;
where cellulose is cut into small pieces preferably of 5mm x 3.5mm and stored in a locking bag until further use;

c. cutting of conjugate pad;
where synthetic release matrix is cut into small pieces of 5mm x 3.5 and stored in a locking bag until further use;

d. cutting of nitrocellulose membrane;
where the nitrocellulose membrane is cut in to small pieces of 25mm x 3.5mm and stored in a locking bag until further use;

e. cutting of absorbent pad,
where the cellulose is cut into small pieces of 20 mm x 3.5 mm and stored in a locking bag until further use;

f. coating of sample release pad, including the steps of;

keeping the cut sample release pad(s) on a mesh tray,
coating each pad with 2.5 mL of sample release pad buffer,
keeping the tray containing the cut sample release pad(s) for drying in an incubator at 37 ± 2℃ for 2 hours,
storing the dried sample release pad in a locking bag with silica gel until further use;

g. preparation of test and control antibody, including steps of;
preparing a test antibody, where the either the anti-DHA rabbit anti-general DHA polyclonal antibody or the anti-EPA rabbit anti-general EPA polyclonal antibody, is diluted in buffer with a ratio of 2-3mg/ml along with stabilizer and methanol;
preparing the control antibody, where the anti-mouse IgG is diluted in buffer along with stabilizer in ratio of 1-2mg/ml,
separately dispensing both the prepared test and control antibodies in aliquots of 1 mL in tubes and stored at -20 to - 50 degree Celsius until further use;

h. preparation of gold conjugated detector antibodies, including the steps of;
preparing a conjugate diluent by dissolving 10% of Sucrose and 5% of Trehalose in Tris buffer and making the volume upto 50-100 mL,
diluting gold conjugated detector antibody; either anti-DHA rabbit anti-general DHA polyclonal antibody or anti-EPA rabbit anti-general EPA polyclonal antibody in the conjugate diluent of optical density ranging between 5 OD to 10 OD, preferably 5 OD to form gold conjugated detector antibodies,
storing at 2 to 8 Degree Celsius until further use;

i. coating of conjugate pad, including the steps of;
placing the required number of cut conjugate pads on a mesh tray, such that 2mg/ml to 6mg/ml; preferentially 5mg/ml of the gold conjugated detector antibodies is coated on each of the conjugate pads,
keeping the tray for drying in an incubator at 37 ± 2℃ for 1 hour,
removing the dried conjugate pad from the incubator,
drying in a vacuum over at 37 ± 2℃ for 15 minutes,

finally storing the dried conjugate pad in a locking bag with silica gel; which is kept in an aluminium foil pouch and sealed, until further use;

j. coating of nitrocellulose membrane, including the steps of;

filling the receptacles in the machine ISO Flow Dispenser for test and control antibodies with 1% Sodium Hypochlorite,

washing the receptacles thrice,

washing with purified water 20 times and then de-loading the water from them;

filling the receptacles for test antibody with test antibody and the receptacle for control antibody with 1 ml of control antibody; such that the nitrocellulose membrane is coated with 20-30 µL of test and control antibodies,

placing the coated nitrocellulose membranes in a steel tray and kept for drying at 37 $\pm$ 2°C for 1 hour in an incubator,

storing at room temperature less than 30 degrees until further use;

k. lamination of plastic pad, including the steps of;

placing a plastic pad laminated with 55 mm double sided Polyester tape on the working table,

marking a distance of 1.9 cm from the operator side,

pasting the coated nitrocellulose membrane on the upper edge of the pad marking,

pasting the absorbent pad on the upper edge of the membrane forming an overlapping junction of 2 mm,

pasting the conjugate pad on the lower edge of the membrane forming an overlapping junction of 2 mm,

pasting the cut blood separation pad on the lower edge of the conjugate pad.

pasting the sample release pad on top of the blood separation pad,

pasting a 12.5 mm tape on top of the sample release pad forming an overlapping junction of 2 mm on the coated nitrocellulose membrane,

keeping all the laminated pads in a locking bag, and

storing at room temperature less than 30 degree Celsius until further use;

l. cutting of laminated plastic pad,

where the laminated plastic pad is mounted on the strip cutter machine with pre-programmed cutting dimensions such that after initiating, the first two pieces are discarded and the third piece is checked for correctness of dimensions where on finding any burrs the piece is stored in process rejection tray;

m. assembly of cut laminates in cassettes, including the steps;
placing the bottom of the lateral flow cassettes on the working table,
placing the sample release pad of the cut laminate in the closed bracket of the cassette,
referring the visual sample display board for identification of the devices, the top of the cassette with the circular opening of the sample port resting on the sample release pad, pressing the cassette using the pressing machine to seal the unit,
placing all the cassettes so prepared in a tray;

n. packing of aluminium foil pouch,

where drying silica gel before use in a hot air over at 90 +5°C for 90 minutes, is followed by placing one cassette inside a pouch and further followed by silica gel sachet and a dropper;

o. sealing packed aluminium foil pouch,
where setting the sealing machine at 200-300 degree Celsius is followed by sealing the open side of the pouch, and storing the sealed pouch is stored in a box;

p. preparing test diluent,
by dissolving 0.1 to 0.5% of detergent trition in 100 ml and storing at room temperature;

q. dispensing test diluent into dropper bottle, followed by pasting a sticker on the bottle;

r. packing sealed pouches and diluent bottles in boxes, where folding the coded boxes is followed by placing the sealed aluminium pouches in the boxes such

that one product pack (sealed aluminium pouch) and one diluent bottle are placed in each box.

Dated this 22nd day of July, 2024.

For the applicants,

Mrs. Allison Katariya

(Patent agent of the applicant IN/PA 2190)

ABSTRACT

Title: A RAPID DUAL TEST KIT FOR DHA AND EPA DETECTION AND METHOD OF PREPARATION THEREOF.

A rapid dual test kit (1) for DHA and EPA detection and method of preparation thereof; that detects DHA and EPA within 0-20 minutes, preferably 1 min; comprising of-a test device (2), a test diluent (29), a dropper (32), a pack insert (instruction sheet) (33) and a silica gel (34), an immuno-chromatographic strip (3) further comprising of a sample release pad(5), a conjugate pad(6), a nitrocellulose membrane (7), and an absorbent pad (8) arranged in a sequential overlapping manner over a backing material (4); encased in a test device (2) consisting of three open areas namely a sample port (11), test line viewing area (30), and control line viewing area (31); allowing lateral flow of sample (29) from sample release pad (5) to absorbent pad (8) via capillary action; and the method of preparation of kit includes steps of; cutting and coating of various pads and nitrocellulose membrane, preparation of capture and detector antibodies, lamination of plastic pad, assembly of cut laminates in cassettes, packing and sealing, preparing and dispensing test diluent into dropper bottle, packing sealed pouches and diluent bottles in boxes.

ABSTRACT FIGURE

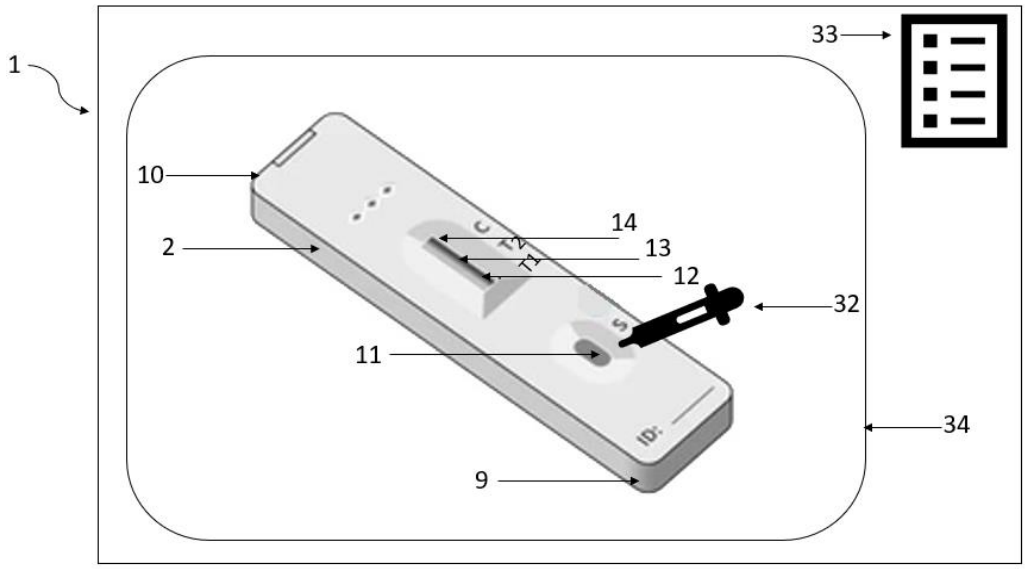

Fig. 3.