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(54) Title: A SYSTEM, DEVICE AND METHOD FOR IDENTIFYING AND MONITORING BREAST MILK COMPOSITION

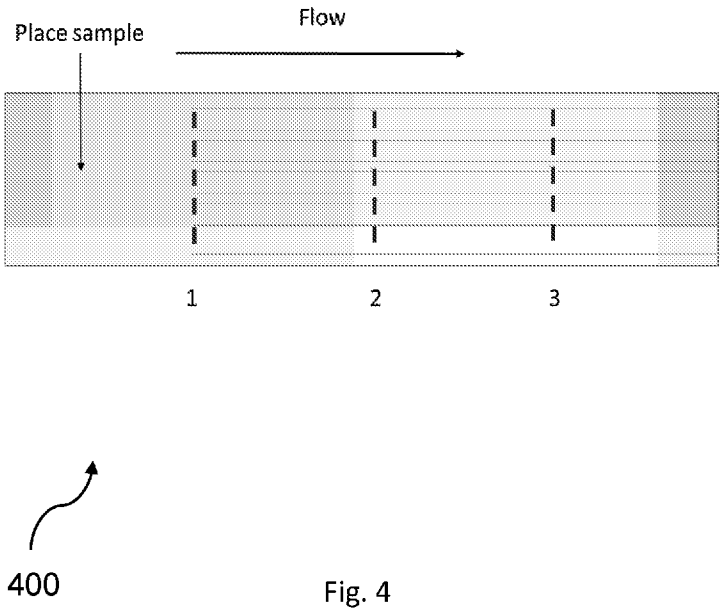

Fig. 4

(57) Abstract: There is provided herein a sampling element for the sampling and analysis of breastmilk, wherein said element comprises: a plurality of fibers to allow the flow of said breastmilk through said sampling element; a result indicator to indicate the result of said analysis; and wherein said sampling element provides a nutritional or immunological analysis of said breastmilk.

WO 2019/035121 A1

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A System, Device, and Method for Identifying and Monitoring Breast Milk Composition

Field of the Invention

The present invention relates to the field of nutrition and immunology, specifically to the detection and/or monitoring of specific elements within maternal breast milk.

Background of the Invention

Breast milk is the milk produced by the breasts (or mammary glands) of a human female to feed a child. About 40% of infants are exclusively breastfed, while over 50% of them are fed by a combination of breast milk and milk substitutes.

The various health benefits of breastfeeding have long been known. The most prominent of these are the nutritional and immunological aspect. Milk is the primary source of nutrition for newborns before they are able to consume and digest other foods; older infants and toddlers may continue to be breastfed, either exclusively or in combination with other foods from around six months of age when solid foods may be introduced. Additionally, breast milk is an essential source of immunoglobulins (i.e. antibodies), which are proteins found in the blood and function as immune defenses against infectious agents, such as viruses and bacteria. Some types of these antibodies (mainly sIgA whose function is to protect from pathogen invasion through mucosal tissues) are transferred from the plasma or the mother's blood into breast milk, or are locally produced in the mammary glands by cells that have migrated to the area, and form the primary immune defense mechanism of the nursing infant.

When breastfeeding is not possible or not desired, infant formula may be provided. Infant formula is a manufactured food designed and marketed for feeding to babies and infants, usually prepared for bottle-feeding or cup-feeding from powder (mixed with water) or liquid (with or without additional water).

Today the formulas are based on stages and the babies are moving from one stage to another according to their age. 1-6, 6-12 months and above. These stages are defined according to an average with no specific measurement of the baby's individual need.

Monitoring the baby's development is done according to growing curves and specific tests (e.g. blood tests) that are done in cases of abnormalities.

However, different babies have different nutritional requirements while providing a general formula based on average needs often fails to meet the specific requirements of an individual infant. In addition, certain diseases in the lactating mother may be transferred to her baby by the milk he consumes. In many cases, these are detected only after the baby develops the disease himself. Therefore, early identification and monitoring of components in breast milk that suggest or increase the risk of morbidity in the infant may have an important prognostic value.

Previous findings have described methods for analyzing the nutritional composition of breast milk, while others have described methods for enriching breast milk with essential nutrients.

Bellmonte et al. (Ann. 1st. Super. Sanita, Vol 26, N. 2 (1990), pp. 131-140) describes the protein and lipid consumption of human milk and infant formulas.

WO 2008/111942 discloses a method of tailoring infant formulas to individual nutritional needs prior to use.

US20080124430 teaches a human milk fortifier compositions, standardized human milk, and methods of making and using same. In one embodiment, a pasteurized human milk composition includes a human protein constituent of about 35-85 mg/mL; a human fat constituent of about 60-110 mg/mL; and a human carbohydrate constituent of about 60-140 mg/mL.

US 4,692,340 teaches a procedure for the production of a granulated infant milk food product.

US20080187619 relates to a human milk fortifier as well as to several uses and a method for the production of such a fortifier. A particularly beneficial fortifier can be realized in that at least one human component based on a product directly or indirectly derived from human mammary secretion during non-pregnant, pregnant, lactating and/or involuting periods is used, giving rise to an optimally adapted fortifying effect which is particularly useful in the context of feeding preterm infants.

US20080118615 relates to a method for analyzing and treating human milk to be fed to an infant comprising the steps of collecting own mother's milk, taking a sample of the collected own mother's milk, conducting nutritional analysis on said sample, using the collected own mother's milk as nutrition for the infant and using the collected own mother's milk in the form

of at least one of the group of: unchanged own mother's milk, fortified own mother's milk, unchanged components of own mother's milk and fortified components of own mother's milk, wherein said form is chosen depending on at least some of said results of the analysis and said infant's condition, the infants condition being chosen from at least one of the following parameters: infant's age, infant's weight, infant's health, infant's shortcomings, infant's deficiencies, time of day when the milk is fed to the infant.

In the immunological aspect, other previous findings have described methods for analyzing breast milk, and more specifically antigen and antibody levels in breast milk, but none of them has evaluated the relationship between these levels and the risk of morbidity in the nursing infant:

Hassiotou et al. (Clinical & Translational Immunology (2013) 2, e3; doi:10.1038/cti.2013.1) examined the influence of an infectious disease in usually healthy mother/infant dyads on breast milk leukocytes and immunomodulatory biomolecules compared to the baseline range of these components. Under infection state, leukocyte numbers significantly increased up to 94% leukocytes out of total cells, as compared to a baseline level in mature breastmilk of 0–2%. Upon recovery from the infection, baseline values were restored. Exclusive breastfeeding was associated with a greater baseline level of leukocytes in mature breastmilk. The authors concluded that their results suggest a strong association between the health status of the mother/infant dyad and breastmilk leukocyte levels. This could be used as a diagnostic tool for assessment of the health status of the lactating breast as well as the breastfeeding mother and infant.

Hassiotou et al. (American Society for Nutrition. Adv. Nutr. 6: 267–275, 2015) further review the effect of an infection on breast milk composition, mainly leukocytes concentrations which consistently and rapidly increase, returning to baseline concentrations upon recovery.

Field et al. (J Nutr. 2005 Jan;135(1):1-40) reviews the immunological components found in breast milk. They state that depending on the phase and stage of lactation, a variety of leukocytes are present in colostrum (4×10^9/L) and mature milk (10^8–10^9/L) that might play a role in promoting the development of the neonatal immune response. Macrophages (55–60%) and neutrophils (30–40%) dominate over lymphocytes (5–10%).

Breakey et al. (Evolution, Medicine, and Public Health [2015] pp. 21–31) examined the relationship between the presence of immune compounds in human milk and infant health.

According to one paradigm, elevated levels of such compounds suggest a protective effect to the infant, while an opposite paradigm suggests that elevated levels of such compounds in breast milk merely implies a response to infection. Milk samples and illness data were collected from 30 mother–infant dyads. Samples were assayed for two immune proteins, lactoferrin and secretory immunoglobulin A (sIgA). Generalized estimating equations were used to assess the relationship between immune composition of milk and symptoms of illness in infants. The results showed opposite relationship for the two markers (Lactoferrin was positively associated with symptoms of illness in infants while sIgA's association with such symptoms was negative).

WO2009033011A1 describes a method for measuring the level of at least one secretor antigen in a biological sample, by which it enables identifying individuals at risk for inflammatory or other type of disease (e.g. necrotizing enterocolitis). It mentions the use of secretor, lewis and sialyl antigen levels as predictors for disease. In one embodiment, the biologic fluid being tested is breast milk, while ELISA or chromatography are possible methods for such analysis.

WO2011127219A1 discloses methods and systems for characterizing a phenotype by detecting biomarkers that are indicative of disease or disease progress. The biomarkers can be circulating, including vesicles and/or microRNAs. In one embodiment, said vesicles may be found in breast milk which may serve as the biological sample. However, these findings do not teach about any possible implication to an infant being exposed to said biological sample, more specifically to breast milk.

US20030065277A1 describes method and apparatus for detecting a biological factor in a fluid sample obtained from a mammary gland, comprising the steps of: non-invasively obtaining a mammary gland fluid from a subject comprising warming the mammary gland; massaging the mammary gland; extracting the mammary fluid from the nipple by expression or aspiration; and detecting the biological factor in the mammary gland fluid. More specifically, it provides method for determining the risk of breast cancer based on breast milk composition. In this context, biological factors may include active cells, proteins, chemicals, lipids, growth factors, cytokines, nucleic acid molecules.

US5798266A discloses a non-invasive methods and kits for obtaining biological samples of mammary fluid or mammary fluid components. It describes the method of stimulating breast fluid expression, collecting it in the form of whole mammary fluid, whole cells or cellular

components, other selected liquid or solid fractions of the mammary fluid, purified or bulk proteins, glycoproteins, peptides, nucleotides or other desired constituents of mammary fluid. Methods and kits are also provided for determining the presence or amount of a breast disease marker, specifically breast cancer. Among the possible breast cancer markers are included CEA, HMFG, MCA, vasopressin, or cathepsin D. the kit includes oxytocin for intranasal administration, breast pump and immunological probes specifically designed to bind to and detect breast disease markers.

Similarly, US6471660B1 also describes a method for analyzing breast milk components for determining a risk of breast disease, more specifically risk for developing breast cancer in breastfeeding women.

CN 106226390 A describes a breast milk composition analyzer, which includes a sampling device; a temperature detection device for detecting temperature of a sample in-sucked by a sample mechanism; a detection device which uses ultrasonic detecting means for detecting composition of the sample; a cleaning device; an output device for outputting structures detected by the detection device; and a control device which controls a sample suction pump.

CN104880566 A also discloses a breast milk analyzer, which comprises a sampling unit, an analyzing unit, a waste breast milk container and cleaning units. The sampling unit is connected with the analyzing unit via a first pipeline, the analyzing unit is in one-way connection with the waste breast milk container via a second pipeline and a waste breast milk pump, each of the cleaning units comprises a cleaning container, a cleaning pipeline and a cleaning pump, and each cleaning container is in one-way connection with the first pipeline via the corresponding cleaning pipeline and the corresponding cleaning pump. The invention further discloses a using method and an automatic cleaning method of the breast milk analyzer.

US20140155281 discloses a method for assessing the infection status of a subject and in particular to a method for assessing the infection status of a subject by analyzing the cellular and/or humoral composition of breastmilk from said subject. The invention aims for detecting infection in a breastfeeding mother and/or infant. It includes analyzing breast milk composition of immunological markers, and further comparing said composition with a reference, wherein an increase in the proportion of said markers when compared to said reference indicates that said mother and/or said infant have an infection. immunological marker is selected from immune cells (IC), cytokines, and immunoglobulins.

Summary of the Invention

According to some demonstrative embodiments, there is provided herein a sampling element for sampling and/or analyzing breastmilk, for example, for providing data about the composition of the breastmilk.

According to some embodiments, data extracted from the sampling element may enable a user of the sampling element to determine and/or monitor monitoring a mother's or an infant's nutritional needs, immunological condition and predicting the likelihood for acquiring an infectious disease.

According to some embodiments, monitoring the development of the infant may include analyzing the maternal milk and accordingly adapting the proper formula for the infant based on his specific needs. For example, in cases wherein the mother is combining breastfeeding with the administration of an infant formula and/or a milk fortifier.

According to some embodiments, monitoring the development of the infant may include testing and/or analyzing the infant's individual parameters.

According to some embodiments, predicting infant's likelihood for acquiring an infectious disease may include analyzing the maternal milk and alerting against abnormal levels of milk components (i.e. antigens and / or antibodies).

According to some embodiments, there is provided herein a system for the analysis of the composition of a breastmilk, comprising at least one sampling element for sampling and analysis of said breastmilk; and an application for deciphering results of the analysis; wherein said analysis comprising a nutritional and immunological analysis.

According to some embodiments, the nutritional analysis may include a determination of the amount or concentration of macromolecules and nutrients in said breastmilk.

According to some embodiments, the immunological analysis may include determination of the amount or concentration of antibodies in the breastmilk.

According to some embodiments, the application may be installed upon a mobile device.

According to some embodiments, there is provided herein a system for determining the nutritional needs of an infant, comprising a collection and/or analysis device, also referred to herein as a "sampling element", to collect a sample of a maternal milk from a mother of said

infant and to analyze the milk to measure at least one parameter; and a result indicator to provide a result, for example, a result which corresponds to a specific infant formula.

According to some embodiments, the sampling element may include a cartridge with a funnel.

According to some embodiments, the at least one parameter may be selected from the group including whole protein concentration, oligosaccharides concentration, pH measurement, fat concentration, number of cells, Properdin, Vitamin A, Phosphorus, Iron and the like.

According to some embodiments, the at least one parameter may be whole protein concentration.

According to some embodiments, the result may be selected from the group including number indicators, color indicators, marking indicators, Latin letter indicators, and/or any other suitable electronic indicators, e.g., sound, beeping and the like.

According to some embodiments, there is provided herein a method for establishing one or more nutritional needs of an infant, wherein the method may include measuring at least one parameter in one or both of: a maternal milk and/or a blood sample of said infant and/or a saliva sample of said infant; determining said one or more nutritional needs of said infant based on said at least one parameter; and indicating an optimal nutritional infant product based on said one or more nutritional needs of said infant.

According to some embodiments, there is provided herein a sampling element for establishing one or more nutritional needs of an infant, wherein the sampling element may be adapted to receive a sample of maternal milk from a mother of the infant and perform an analysis to measure at least one parameter of the maternal milk and provide a recommendation of a specific nutritional formula for the infant based on the at least one parameter, and/or provide a mother with nutritional recommendations to consume more or less of a specific nutrient.

According to some embodiments, there is provided herein a use of the system of the present invention for the analysis of a breastmilk fed to an infant, comprising analyzing a sample of the breastmilk, determining the amount or concentration of one or more antibodies in the breastmilk and determining the chances of existence of a disease in the infant and/or in the breastfeeding mother.

According to some embodiments, there is provided herein a use of the system of the present invention for the analysis of a breastmilk fed to an infant, comprising analyzing a sample of the breastmilk, determining the amount or concentration of one or more macromolecules or

nutrients in the breastmilk and suggesting an optimal nutritional supplemental formula to the infant based on the analysis of the breastmilk.

According to some embodiments, there is provided herein a device which may perform an analysis of the sampled breastmilk contained upon the sampling element of the present invention.

According to some embodiments, the device may encompass a sample of breastmilk which may include at least one incubator to incubate the sample of the breastmilk.

According to some demonstrative embodiments, the device may be any suitable electronical device adapted to encompass the sampling element as taught herein or alternatively, be adapted to read one or more indications located on said sampling device, and provide an output, e.g., an analysis of the composition of a breastmilk sampled by the sampling element.

According to some embodiments, the device may be a smartphone, capable of reading one or more indications upon the sampling element, e.g., via the camera of the smartphone.

According to some embodiments, there is provided herein a sampling element for the sampling and analysis of breastmilk, wherein the element may include: a plurality of fibers to allow the flow of said breastmilk through the sampling element; a result indicator to indicate the result of the analysis; and wherein the sampling element may provide a nutritional or immunological analysis of said breastmilk.

According to some embodiments, the sampling element may include at least three separate zones, a first zone, a second zone and a third zone for analyzing the breastmilk, wherein the breastmilk flows through the at least three zones.

According to some embodiments, the at least three separate zones include: zone 1 comprises antibodies that bind to specific components within said breastmilk, and wherein the antibodies are conjugated to an enzyme that induces a color change; zone 2 comprises particles that induce the color release from conjugated enzymes and antibodies bound to specific components; and zone 3 comprises particles that induce the color release from conjugated enzymes and antibodies bound to specific components that did not induce a color release in zone 2.

According to some embodiments, the nutritional analysis may include a determination of the amount or concentration of macromolecules or nutrients in the breastmilk.

According to some embodiments, the macromolecules or nutrients are selected from the group including: Vitamin B, Human Milk Oligosaccharides (HMO), long chain polyunsaturated fatty acids (LCPUFA) and Total Proteins.

According to some embodiments, the immunological analysis may include the determination of the amount or concentration of at least one immunological factor in the breastmilk selected from the group including: Lactoferrin, Leukocytes and Immunoglobulins.

According to some embodiments, the at least one immunological factor may be Lymphocyte Common Antigen (CD45)

According to some embodiments, the at least one immunological factor may be sIgA.

According to some embodiments, the sampling element may be selected from the group including a test strip, a test stick, a dipstick or a vial.

According to some embodiments, the sampling element may be disposable. According to these embodiments, the use of disposable sampling elements may be beneficial since the user of the sampling elements are usually mothers and the test are preferably conducted on a daily basis or a couple of times a week.

According to some embodiments, the sampling element may be adapted to be inserted into a device for the analysis of said breastmilk.

According to some embodiments, there is provided herein a system for determining the nutritional needs of an infant, comprising: the sampling element to collect and analyze a sample of breastmilk from a mother of said infant and provide results of said analysis; an application to read said results and provide a recommendation for a specific infant formula to be fed to said infant based on said results; and wherein said sampling element analyzes at least one parameter selected from the group including whole protein concentration, separate peptides, a combination of peptides, oligosaccharides concentration, pH measurement, fat concentration, number of cells, Properdin, Vitamin A, Phosphorus and Iron.

According to some embodiments, the application may be installed upon a mobile device.

According to some embodiments, there is provided herein a use of the sampling element for determining the amount or concentration of one or more macromolecules or nutrients in said breastmilk and suggesting an optimal nutritional supplemental formula to said infant based on the analysis of said breastmilk.

According to some embodiments, there is provided herein a use of the sampling element for determining the amount or concentration of one or more immunological factor for alerting the development of an infectious disease in the infant or mother.

Brief Description of the Drawings

Figures 1A to 1H are graphs depicting the changes in different components in various infant formulas during the various stages, in accordance with some demonstrative embodiments.

Figure 2 is a graph depicting the change in Properdin in correlation to the age of the infant, in accordance with some demonstrative embodiments.

Figure 3 is an exemplary scheme for assisting in the identification of a suitable nutritional formula for an infant, in accordance with some demonstrative embodiments.

Figure 4 is an illustration of a sampling element (SE), and the movement therein of a sample liquid, according to some demonstrative embodiments.

Figures 5A to 5D are illustrations of a dipstick sampling element with a panel, according to some demonstrative embodiments.

Figure 6 illustrates a horizontal stick flow sampling element, according to some demonstrative embodiments.

Figure 7 depicts a flow chart of different possible options for sampling and analyzing breast milk using the system of the present invention, according to some demonstrative embodiments.

Figure 8 illustrates possible uses for the system of the present invention in accordance with some demonstrative embodiments.

Figure 9 is a schematic illustration of the system of the present invention and the components thereof, in accordance with some demonstrative embodiments.

Figure 10 illustrates an algorithm of activities and actions of the system of the present invention, in accordance with some demonstrative embodiments.

Figure 11 is a flow chart of the operation of the system of the present invention, in accordance with some demonstrative embodiments.

Figure 12 is a graph depicting the protein concentrations of Nutrilon® infant formula in three phases of products, as indicated by the manufacturer.

Figure 13 is a graph depicting the protein concentrations of the human milk at different age groups, using Bradford assay.

Figure 14 is a graph depicting the average relative and normalized Glycoprotein-Carbohydrates Percentage at protein concentration 2.5 mg/ml. n=24 vs. age groups.

Figure 15 is an illustration which shows how to prepare a series of dilutions, in accordance with some demonstrative embodiments disclosed herein.

Figure 16 is a graph depicting the protein concentrations of the different formulas discussed in the present application using BCA and Bradford methods, in comparison to the manufacturer's values, in accordance with some embodiments (Error bars are of standard error. n=2-3.)

Figure 17 is a graph depicting the protein concentrations of breast milk at different age groups of a baby, using BCA and Bradford methods, in accordance with some embodiments (Error bars are of standard error. n=4-8).

Figure 18 is a graph depicting the protein Concentration of breastmilk at the different infant age groups, results obtained by the Technion Proteins Lab using the Bradford Method (n=3-5).

Figure 19 is a graph depicting the comparison of protein concentration by age group as discovered by the MAO FoodTech lab team and the Technion Proteins Lab (n=3-8).

Figure 20 is a graph depicting the protein concentration results for each individual sample, organized by age [months, days], in accordance with some embodiments.

Figure 21 is a graph depicting the average protein concentration results when age groups are broken down into the general manufacturer's definitions for each formula phase (i.e phase one 0-6 months, phase 2 6-12 months etc'.)

Figure 22 is a graph depicting the average Glycoprotein Carbohydrates Concentration of all three tested formulas at 2.5mg of protein per 1ml liquid.

Figure 23 is a graph depicting the average Glycoprotein Carbohydrates Concentration of all three tested formulas at 0.25mg of protein per 1ml liquid.

Figure 24 is a graph depicting the average Glycoprotein Carbohydrates Concentration for each of the six age groups at 2.5mg of protein per 1ml liquid (n=4-5).

Figure 25 is a graph depicting the average Glycoprotein Carbohydrates Concentration for each of the six age groups at 0.25mg of protein per 1ml liquid (n=4-5).

Figures 26 and 27 are tables demonstrating various candidates that may be analyzed and/or monitored by the device of the present invention in accordance with some demonstrative embodiments.

Figure 28 is an exemplary legend in accordance with some demonstrative embodiments.

Figure 29 is an illustration of an exemplary legend, to which a sampling element result may be compared to, in accordance with some demonstrative embodiments.

Figure 30 is an illustration of an exemplary legend of average concentrations of Vitamin B1 in the breastmilk of a mother feeding an infant in correlation to the age of the infant, in accordance with some demonstrative embodiments.

Figure 30 is an illustration of an exemplary legend of average concentrations of Vitamin B12 in the breastmilk of a mother feeding an infant in correlation to the age of the infant, in accordance with some demonstrative embodiments.

Figure 32 is an illustration of a sampling element for LC-PUFA with a predetermined marked line, to indicate to the user of the sampling element the depth up to which the user should dip the element into the tested breastmilk, in accordance with some demonstrative embodiments.

Figure 33 is an illustration of an exemplary control panel, wherein sampling element is inserted into the panel, and the results indicated upon element can be compared to the reference results indicated upon the panel, in accordance with some demonstrative embodiments.

Detailed Description of the Invention

According to some demonstrative embodiments, there is provided herein a system for detecting the presence of specific components in breast milk and/or determining and/or measuring the amount and/or concentration thereof.

According to some demonstrative embodiments there is provided herein a system that enables testing and/or analyzing breast milk, for example, for detecting changes in concentrations or amounts of specific biological markers, e.g., immunoglobulins, antibodies and like.

According to some embodiments the system may alert a user of the system when changes occur in concentrations and/or amounts of one or more specific marker(s).

According to these embodiments, for example, the system may enable to detect an elevation in a concentration of a specific immunoglobulin, thereby alerting a possible development of an infection and/or a disease in the breastfeeding mother or a breastfed baby.

A possible example is the detection of antibodies against CMV, which is a virus that can cause significant illness in both nursing mothers and their infants. In case of active infection, the mother's immune system will develop IgM antibodies against the virus. Identification of these antibodies in the mother's milk will serve as an early indication of the mother's condition and may allow preventive measures to be taken to minimize the transmission of the infection to the baby (e.g. temporary halting of breastfeeding, reducing the exposure to maternal fluids, etc.).

For example, the system may include one or more devices that can monitor the concentration of one or more markers for a period of time, for example, in specific time intervals, such as on a daily basis, on an hourly basis, on a weekly basis and the like.

According to some demonstrative embodiments, the system may provide an indication of early stages of certain diseases by monitoring and/or identifying changes in specific biomarkers contained in the breast milk, for example, an elevation of a specific antibody in the breast milk of a breastfeeding mom may correlate to the existence of specific pathogens in the breastfed baby.

According to some embodiments the system may provide a detailed summary of concentrations of various markers in the breast milk and enable a care provider to effectively assess the health condition of an infant which is fed by the breast milk.

According to some embodiments, the term “assessment” and “assess” may refer to any suitable indication resulting from the analysis of the breast milk including, for example, a mere summary of concentrations of specific markers, an analysis of the concentrations, a warning regarding the change in one or more specific marker (in amount or concentration), a recommendation of treatment, estimation of a specific disease or medical condition correlating to the amount/concentration or change in one or more markers, and the like.

According to some embodiments, the term “care provider”, "user" and/or “user of the system” may refer to a breastfeeding mother and/or to any individual who provides preventive, curative, promotional or rehabilitative health care services within all branches of health care, including medicine, surgery, dentistry, midwifery, pharmacy, psychology, nursing or allied health professions, and the like.

According to some embodiments, the terms “marker”, “biomarker”, “immunological marker(s)” and/or “biological marker(s)” may refer to any measurable indicator of some biological state or condition. According to some embodiments, the marker may be used to refer

to a substance whose detection indicates the presence of a living organism and/or a disease. According to these embodiments, the marker may be measured and/or evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

According to some embodiments, the term "sampling element(s)" may include, for example, a test strip, a test stick, a dipstick, a vial and/or any suitable piece of material used for biological testing.

According to some embodiments, the sampling element may include one or more components for the analysis of the sampled breastmilk, as explained in detail below. According to some embodiments, the sampling element may further include one or more indicators to indicate the result of the analysis.

According to some embodiments, the sampling element may include one or more magnifying elements, to magnify the results. For example, results may be presented by color upon the indicator of the sampling element, however, since some of the tested elements may be in small amount and/or concentration, e.g., microns, according to these embodiments, the sampling element may include a magnifying glass or window to magnify the resulting color or the presentation of the results.

According to some embodiments, the term "application(s)" as referred to herein may include, for example, any suitable software that may enable the analysis of data and/or indication of an output based on the analysis. According to some embodiments, the application may be an internal software implemented within the system. According to other embodiments, the application may be installed on an external device, for example, a smart phone and the like.

According to some demonstrative embodiments, the sampling element may have one or more indicators that may be read by an application installed upon a smartphone. For example, the sampling element may have an indicator that may be photographed by a smartphone's camera, and analyzed by an application and/or other software to provide an analysis of the results.

According to some embodiments the system may provide a recommendation to the care provider regarding actions or measures to be taken with regard through the assessment.

According to some embodiments, the system may enable the care provider of an infant to determine the infant's immunological and/or nutritional needs, e.g., based on the detection and/or measurement described hereinabove.

According to some embodiments, the system may include one or more sampling elements adapted to collect a sample of the breast milk and analyze the breastmilk.

Specifically, the sampling element may be adapted to sample maternal milk, for example, by having a specific structure adapted to allow flow of the breastmilk with its unique viscosity, wherein the sampling element may act as a filter, having fibers in various sizes, diameter and or density to specifically allow the flow of breastmilk therethrough.

According to some embodiments, the system may optionally include one or more device adapted to encompass the one or more sampling elements and analyze the components of the sampled maternal milk.

According to some embodiments, the sampling element may enable the detection and/or analysis and/or measurement of specific components of the maternal milk, e.g., as described in detail below.

According to some embodiments, based on the detection and/or analysis and/or measurement of specific components of the maternal milk, the sampling element may enable to provide an estimate regarding the nutritional and/or immunological status of an infant consuming the maternal milk.

The maternal milk is constantly changing according to the baby's needs, nutritional and immunological alike. It is rich in immunological factors (antibodies) in the first weeks after delivery and it reduced in these factors later on. On the other hand the complement factors are increasing during lactation period. The antibodies' composition is unique to each mother and baby pair, which makes it very dynamic depending on the baby's exact needs at that time. The main component which concentrates on breast milk and is passed through it to the baby is an immunoglobulin called IgA. This antibody serves as the primary defense of the infant against infections, especially in his developing digestive and respiratory systems. IgA's level in breast milk remains high throughout several months postpartum.

According to some embodiments, there is a constant feedback between the baby and the nursing mother influencing the content of the milk. This feedback can influence the composition of the milk both in the terms of nutritional and immunological components. While being fed, the baby's mucus penetrates the breast, exposing its content to the mother's body. In return, the mother's body may react to the needs of the breastfed infant.

According to some demonstrative embodiments, if the breastfed infant is developing a disease, the body of the breastfeeding mother may produce antibodies to be transferred via the breastmilk to protect the infant.

It has been shown that (in mice) mothers that were exposed to allergens can produce antibodies that will protect their newborn becoming allergic. This may have an influence on the development of asthmas and other allergic conditions.

Accordingly, in accordance with some embodiments, in baby's mucus there may be one or more genetic factors that may penetrate the mother's body as well, e.g., via the milk.

According to some demonstrative embodiments, the one or more genetics factors may be utilized for mapping the baby's health condition and/or determining the needs of the baby on top of the immunological components.

Table 1 describes the main immunological components in breast milk, according to different time points within the first year since delivery:

Table 1

Breast milk component	Health status	Colostrum	Transitional milk	Months 1–3	Months 4–6	Months 7–9	Months 10–12
Total cells per ml milk	Healthy	110000–2250000	113492–883333	228395–255769	40000–588542	97500–433333	706667–1066667
	Infection	—	183333	50000–2867383	115278–321918	37000–504951	437 500–1000 000
% Leukocytes	Healthy	13.2–70.4	0.0–1.65	0.07–0.45	0.0–1.52	0.0–1.09	0.08–0.1
	Infection	—	18.8	0.72–90.5	1.1–33.9	1.08–93.6	> 3
Leukocytes per ml milk	Healthy	32175–784080	0–3450	160–1151	0–1025	0–1063	707–853
	Infection	—	34467	2400–2594982	2164–109130	1065–472634	> 30000
sIgA (µg ml−1)	Healthy	1428–2178	131–1096	534–1276	257–960	496–1350	401–1044
	Infection	—	922	36–1418	652–1711	611–1509	714–789
IgG (µg ml−1)	Healthy	5.3–12.2	2.8–9.7	6.4–12.4	4.6–10.8	4.0–16.4	5.0–16.1
	Infection	—	13	6.6–17.1	4.8–10.1	5.6–14.4	7.6–8.8
IgM (µg ml−1)	Healthy	16.2–56.1	8.2–29.8	10.6–14.9	6.5–11.6	4.2–23.7	8.8–23.3
	Infection	—	10.2	4.5–19.8	10.1–15.4	12.6–21.8	14.4–19.3
Lactoferrin (g l−1)	Healthy	6.3–7.7	2.1–5.2	2.5–2.9	1.9–3.7	1.3–4.0	1.2–3.9
	Infection	—	4.3	2.9–3.7	2.0–3.7	1.6–3.3	1.2–3.6

Table 2 lists several antigens which can be found in the breast milk of an infected nursing mothers, and to which the infants may be exposed:

Table 2

Antigen in breast milk	Corresponding Illness in infant
CMV IgM and CMV IgG	CMV(Cytomegalovirus)
Hepatitis B Antigen (surface antigen AKA HBsAg)	Hepatitis B Virus
Hepatitis C Antigen (surface antigen AKA HBsAg)	Hepatitis C Virus
HIV1 Antigen (p24)	Human Immunodeficiency Virus Type 1 (HIV1)
HIV2 Antigen (p24)	Human Immunodeficiency Virus Type 2 (HIV2)
HTLV-I Antigen (gp21 or p-24 Core)	Human T-lymphotropic virus Type 1 (HTLV-1)

According to some demonstrative embodiments, the system of the present invention (also referred to herein as a "kit") may include one or more sampling elements capable of testing and analyzing different nutritional factors as described below and/or immunological markers that may indicate a development of an infection\illness\disease.

According to some embodiments, the system may further include one or more tubes for the dipping of the sampling element into the breastmilk, and a color calibration board.

According to some embodiments, the system of the present invention is designated for women who breast feed (even partially). According to some embodiments, a nursing mother may test her breast milk at the same time every day, mainly before/after a meal. For example, a mother using the system of the present invention may be asked to pump at least 15 ml of breast milk into a bottle or another sealable container and transfer the milk into a designated tube and will dip a sampling element (alternatively, the sampling element may be placed in the tube beforehand) into the tube, making sure the breastmilk is covering the sampling element up to a marked line.

According to some embodiments, after a short incubation period, the sampling element may be placed in a designated slot on a calibration panel of the system, and the mother may be able to use an application to scan the entire panel with her phone camera.

According to some embodiments, the sampling element may be coated with different antibodies and enzymes for the specific test targets. The tests are for nutritional factors and/or for immunological components. The reaction may be colorimetric and can be quantitated using a color panel.

According to some demonstrative embodiments, the sampling element may include one or more antibodies, for example, to detect and/or monitor the levels of different nutritional factors such as vitamins, e.g., vitamin A, B1, B6, B12, C, D and the like.

According to some demonstrative embodiments, the sampling element may include one or more antibodies, for example, to detect and/or monitor the levels of different immunological factors such as CD45, sIgA and the like, and/or other antibodies that may act against common antigens that are present as a result of pathogen, e.g., such as Streptococcus pneumoniae.

According to some embodiments, color indication may be the preferred option to demonstrate changes and/or results analyzed by the system of the present invention.

According to some embodiments, the color test may vary, and optionally be dependent on the final substance to be tested.

For example, protein concentration is tested by Bradford, BCA etc. –true, the color change is better visualized by a designated equipment but is quite clear also to the naked eye.

pH test can be done with pH strips that changes their color according to the pH.

Number of cells can be estimated according to some enzymatic assay such as CCK.

Total Phosphorus can be measured by different enzymatic assays such as Molybdenum blue method by adding a reducing agent. This can take place as follows:

Orthophosphate reacts with ammonium molybdate to a slightly yellow molybdenum phosphoric acid. By adding a reducing agent, the molybdenum blue is formed, which has a blue color:

$$PO_4^{3-} + 12\ (NH_4)6Mo_7O_{24} + 3H^+ \rightarrow\ H_3P(Mo_3O_{10})^4$$

$$H_3P(Mo_3O_{10})^4 + 2\ SnCl_2 + 8\ HCl \rightarrow\ H_7P(Mo_3O_{10})^4 + 2\ H_2(SnCl_6)$$

pH is a measure of the acidity or alkalinity of a solution. The pH value states the relative quantity of hydrogen ions (H+) contained in a solution. The greater the concentration of H+

the more acidic the solution and the lower the pH. In this relationship, pH is defined as the negative logarithm of hydrogen activity.

The Bradford assay, a colorimetric protein assay, is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250. Under acidic conditions the red form of the dye is converted into its bluer form, binding to the protein being assayed. The dye forms a strong, noncovalent complex with the protein's carboxyl group by Van der Waals force and amino group through electrostatic interactions. During the formation of this complex, the red form of Coomassie dye first donates its free electron to the ionizable groups on the protein, which causes a disruption of the protein's native state, consequently exposing its hydrophobic pockets. These pockets in the protein's tertiary structure bind non-covalently to the non-polar region of the dye via the first bond interaction (van der Waals forces) which position the positive amine groups in proximity with the negative charge of the dye. The bond is further strengthened by the second bond interaction between the two, the ionic interaction. The binding of the protein stabilizes the blue form of the Coomassie dye; thus the amount of the complex present in solution is a measure for the protein concentration, and can be estimated by use of an absorbance reading.

The BCA assay primarily relies on two reactions.

First, the peptide bonds in protein reduce Cu^{2+} ions from the copper (II) sulfate to Cu^{+} (a temperature dependent reaction). The amount of Cu^{2+} reduced is proportional to the amount of protein present in the solution. Next, two molecules of bicinchoninic acid chelate with each Cu^{+} ion, forming a purple-colored complex that strongly absorbs light at a wavelength of 562 nm.

The bicinchoninic acid Cu^{+} complex is influenced in protein samples by the presence of cysteine/cystine, tyrosine, and tryptophan side chains.

The amount of protein present in a solution can be quantified by measuring the absorption spectra and comparing with protein solutions of known concentration.

Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing Dojindo's highly water-soluble tetrazolium salt. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)- 5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] produces a water-soluble formazan dye upon reduction in the presence of an electron mediator. CCK-8 allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. WST-8 is reduced by dehydrogenases in cells to give an orange colored product

(formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells.

According to some embodiments, a phone application may enable scanning of the control panel to compare the colors on the panel (which resemble known concentration of the analyte) to the test color on the sampling element.

According to some embodiments, the application may have an algorithm that may convert the signal into numbers which can indicate the concentration of the analyte, thus enabling the monitoring of the concentrations of various analytes and/or markers over time.

According to some demonstrative embodiments, the application may recognize fluctuations in concentrations /decrease-increase in concentrations /sub-optimal concentration of a marker and/or a nutritional component, thus sending recommendations, for example, for treatment and/or for the supplementation of a component through diet. According to some embodiments, the nursing mother can use the application in order to monitor the nutritional composition of her breastmilk and/or follow possible recommendations provided by the application.

According to some embodiments, the application may enable direct connection to a care provider, for example, the application may send a notification to a physician in case any abnormal results are indicted, e.g., an extreme rise in one or more markers.

According to some embodiments, the application may enable a mother to input data relating to her infant, for example, such as weight and head circumference, in order to improve the complete monitoring of her baby's health and development.

If the mother chooses to combine formula in her infant's diet in addition to breast feeding, the application may recommend which formula (which stage) best fits the infant and complements the mother's milk's composition.

According to some embodiments, the system may include specific sampling elements for different purposes, for example, a nutritional sampling element including, for example, one or more components adapted to identify amounts and/or concentrations of specific nutritional factors in the breast milk; an immunological sampling element including, for example, one or more components adapted to identify amounts and/or concentrations of specific immunological markers in the breast milk; a combined sampling element, including, for example, one or more components adapted to identify amounts and/or concentrations of specific nutritional factors and specific immunological markers in the breast milk.

According to some embodiments, the immunological sampling element may include one to three tests that bind immunological factors such as sIgA, CD-45, IL-10, and/or TNF-alpha. These immunological factors may serve as an alert/red flag for the development of an illness in the mother, the baby or both.

According to some embodiments, the application of the system may recognize an increase in one or more immunological factors (for example, sIgA, CD-45, IL-10, and/or TNF), for example, following the use of a nutritional sampling element, and consequentially may recommend using the immunological sampling element.

According to some embodiments, the system may include a package of sampling elements, e.g., testing sticks, for example, every such package may contain 30 nutritional sticks and at least one immunological strip.

According to some embodiments, the sampling element may contain a plurality of test, e.g., ten to fifteen different tests, for common or dangerous infant diseases, including, for example,: Diphtheria: Hand, foot and mouth disease, Infective Diarrhea, Jaundice - Viral Hepatitis, Measles, Meningitis, Molluscum contagiosum, Mumps, Otitis, Pneumonia, Respiratory Syncytial Virus, Scarlatina, Varicella / Chickenpox, Whooping cough, Pertussis and the like.

According to some embodiments, the application of the system may provide different recommendations to the nursing mother using the system of the present invention. For example, the application may recommend monitoring the infant's fever as well as behavioral abnormalities that might indicate the development of a disease. The application may also recommend going to a physician and will allow for an immediate online connection to a physician for consulting.

According to some embodiments of the invention, the system may include at least one device made up of: A designated area for the nutritional/immunological stick, light/color sensors, a Bluetooth component, a battery pack, a memory chip and optionally an LCD screen, a USB port and a temperature sensor.

According to some embodiments, the system may include more than one options or alternatives for the sampling element to be inserted into the - device of the system and consequentially enabling the analysis of the breast milk.

For example, one option of the system would be to use a sampling stick onto which a sample of the breast milk is put, and the stick is inserted into a device of the system for analysis. A

second option may include a dipstick, which is dipped into a sample of a breastmilk, wherein the dipstick includes a panel.

According to one of the options, the nursing mother may be asked to take a dropper that may be in the box of strips (probably in a bottle with ethanol for sterilization) and take a sufficient amount, for example, 1 ml from the pumped milk onto the designated place on the strip. Then the strip may be placed in the designated place in the device and after a few minutes the device will indicate the results and broadcast them to the application, for example, installed on a smartphone.

According to some embodiments, the difference between the strip that goes into the device and the dipstick with the panel lies in the reading of the results: instead of using a color panel and her phone camera, the mother may be asked to insert the stick into the device in the designated area (and to turn the device on). The light sensors will read the color absorbance at the specific wavelengths for each reaction and the results may be transferred to the dedicated application on the mother's phone through Bluetooth.

The advantages of the device over the panel are: (1) Higher sensitivity to color changes/differences through the sensors – more accurate results. (2) Choosing specific wavelengths for the sensors to read for each test; these would be the optimal wavelength to reach the analyte's concentration, which would yield more accurate results. (3) Greater sensitivity would mean the reaction areas could be comparatively small – saves space on the stick and allows for more tests on one stick. (4) The possibility of incubations/shaking.

Alternatively, the device could also include a slot for cuvettes and magnetic sensors. The kit may also include single use cuvettes which come with all the fluids for the reaction already in them. These cuvettes would be used for specific immunological tests. The reactions within the cuvettes may be colorimetric or result in a charged particle which would be drawn to the magnetic sensors.

According to some demonstrative embodiments, the system may also enable the monitoring and/or analysis of the nutritional composition of the breast milk.

With time, the nutritional factors contained within the breast milk dynamically change in concentration and/or amounts.

Total concentration of oligosaccharides declines over the course of lactation; the mean concentration at 1 year was less than half that in the first few weeks postpartum; the

concentration of some oligosaccharides does not change during lactation. However, the total protein concentration is also increasing over the course of lactation. In addition, the amount of fat and the number of cells varies between mothers and fluctuate during the day with a maximum 30 minutes after breastfeeding.

When compared to maternal milk, infant formula at different stages include different concentrations of components, as shown in Table 3.

Table 3

	Materna			Nutrilon			Similac			
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	
Energy	69	69	66	65	62	63	68	73.8	73.8	Kcal
Protein (Albomin/Casein 60/40)	1.46	1.58	1.66	1.3	1.4	1.5	1.4	2.8	2.8	gr
Carbohydrates	7.7	7.8	8.4	7.3	8.6	8	7.4	7.5	7.5	gr
Dietary (scGOS/ICFOS)				0.8	0.8	1.2				
Lactose		7.8	8.2				7.4	7.5	7.34	gr
Fat	3.5	3.5	2.9	3.4	3	2.8	3.71	3.6	3.62	gr
Saturated Fat	1.56	1.5	1.2	1.4	1.2	0.7	1.2	1.28	1.28	gr
Trans Fat	0.5>	0.5>	0.5>				0.5>	0.5>	0.5>	gr
Cholesterol	4	4	1.9				0.7	1.7	1.7	Mg
Linoleic Acid (LA)	0.478	0.63	0.531	0.45	0.4	0.383	0.64	0.68	0.68	gr
Alpha-Linolenic (ALA)	0.05	0.066	0.049	0.08	0.07	0.68	0.05	0.06	0.06	gr
Arachidonic (ARA)	13			11	8.6	1				Mg
Docosahexaenoic Acid (DHA)	13			10	8.6	12				Mg
Sodium	23	27	34.5	17	23	26	18	36.4	36.4	Mg
Vitamin A	82	86	87	54	66	65	57	79	79	Mcg RE
Vitamin D	1.33	1.2	1.2	1.2	1.4	1.7	0.86	1.25	1.25	Mcg
Vitamin E	1.3	1.78	1.56	1.1	1.2	1.1	1.9	1.3	1.3	Mg-TE
Vitamin K	6	8.4	7.9	4.4	5	4.9	6.7	6.1	6.1	Mcg
Vitamin B1	0.1	0.11	0.1	51	54	50	0.08	0.097	0.097	Mg
Vitamin B2	0.12	0.12	0.15	121	120	124	0.14	0.12	0.12	Mg
Niacin (B3)	0.58	0.73	0.73	0.43	0.43	0.44	0.71	1.25	1.25	Mg

Table 3										
	Materna			Nutrilon			Similac			
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	
										NE
Vitamin B6	0.08	0.08	0.09	38	93	40	0.04	0.14	0.14	Mg
Vitamin B12	0.23	0.27	0.3	0.21	0.18	0.14	0.19	0.291	0.29	Mcg
Folic Acid	13	18	14	13	16	13	9.5	13.1	13.1	Mcg
Vitamin C	12	13	13	9.2	9.9	15	10	10.9	10.9	Mg
Biotin	2.6	2.9	3.3	1.4	1.4	1.6	2.5	3.3	3.3	Mcg
Pantothenic acid (B5)	0.4	0.5	0.6	343	344	339	0.4	0.43	0.43	Mg
Calcium	58	81	79	57	65	91	53	115	115	Mg
Phosphorus	37	48	56.6	32	36	50	28	66.2	66.2	Mg
Magnesium	6.6	8.5	6.9	5.1	4.8	5.6	5.1	8.7	8.7	Mg
Iodine	9.5	14	14	12	13	13	13	22.4	22.4	Mcg
Iron	0.82	1.23	1.24	0.53	1	1.2	1.2	1.2	1.2	Mg
Zinc	0.61	0.55	0.73	0.52	0.51	0.89	0.5	0.55	0.55	Mg
Selenium	2.8	4.1	4.1	1.7	1.7	1.5	1.1	1.1		Mcg
Potassium	71	67	90	68	74	70	83	128	128	Mg
Copper	49	48	44	40	43	41	51	66	0.066	Mcg
Manganese	6.6	7.5	5.8	7.7	7	7.6	13	9.5	9.5	Mcg
Chloride	63	44	52.4	42	47	41	44	84.3	84.3	Mg
Choline	16	10.4	9	12	10	10	10	17.64	17.64	Mg
Taurine	6	6.9	4.6	5.3	5.2	5.4	4.5	5	5	Mg
Inositol	4	8.2	7.7	3.9	3.6		4	3.3		Mg
Carnitine	0.9			1.6	0.95		1.1			Mg
Nucleotides	3.1	3	3	3.2	3.2		7.2		7.2	Mg
Humidity			2.5							%

Reference is now made to Figure 1, which illustrates graphs 1A-1H depicting the changes in different components in various infant formulas (Materna®, Similac® and Nutrilon®) during stages 1, 2 and 3 of the formulas, in accordance with some demonstrative embodiments.

According to some embodiments, graph 1A depicts the changes in Energy levels (Kcal) in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

According to some embodiments, graph 1B depicts the changes in Protein concentrations (grams/100ml) in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

According to some embodiments, graph 1C depicts the changes in Carbohydrate concentrations in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

According to some embodiments, graph 1D depicts the changes in Fat concentrations in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

According to some embodiments, graph 1E depicts the changes in Vitamin A concentrations in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

According to some embodiments, graph 1F depicts the changes in Vitamin B12 concentrations in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

According to some embodiments, graph 1G depicts the changes in Phosphorus concentrations in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

According to some embodiments, graph 1H depicts the changes in Iron concentrations in the various phases 1, 2 and 3 of exemplary leading infant formula brands - Materna®, Similac® and Nutrilon®.

Reference is now made to Figure 2 which illustrates a graph depicting the change in properdin in correlation to the age of the infant, in accordance with some demonstrative embodiments.

Complement factors, such as: Clq, C2, C3, C4, C5, C6, Factor B, Properdin, C3b inactivator (C3Bina) and β1H are increasing during lactation when Properdin levels in the infant serum are changing significantly during the first 3 years.

As shown in Figure 2, Properdin levels in infant's ages 1-35 months. The solid dark line represents the mean and the shaded areas ±1SD. The mean adult properdin level and ±1SD are given by the horizontal solid and dotted lines, respectively. The number of subjects in each age group is listed at the bottom of Figure 2.

The following are embodiments of possible structures and modes of operation of the sampling element:

According to some demonstrative embodiments, the mechanism of the sampling element may be based on Lateral flow tests as are frequently applied in quick pregnancy tests on absorbing fibers/ pad/membrane. For example, based on antibodies and an enzymatic reaction for color.

According to some embodiments, the sampling element may operate based on chemical reactions, enzymatic reactions etc. with or without antibodies.

According to some embodiments, the sampling element may have an edge that may be placed in a sample liquid, e.g., breastmilk. According to some embodiments, the sampling element may include fibers that absorb the liquids forcing it to move from higher concentration (where the sample is originally placed) to the lower concentration. This directional flow can be achieved by capillaries as well.

Reference is now made to Figure 3. Which illustrates an exemplary scheme for assisting in the identification of a suitable nutritional formula for an infant, in accordance with some demonstrative embodiments as shown in Figure 3, the strength of the color may indicate the stages of the formula that the baby need.

According to some embodiments, the formation of the color in the different enzymatic reactions is dependent on the amount of the tested material. For protein concentration the Coomassie dye forms a strong, noncovalent complex with the protein's carboxyl group by Van der Waals force and amino group through electrostatic interactions. According to some embodiments, the higher the protein concentration is, the stronger the color indication is.

According to some demonstrative embodiments of the present invention the system may include the identification of a suitable nutritional formula for an infant via at least one of the following parameters: number indicators, color indicators, marking indicators, Latin letter indicators, and/or any other suitable electronic indicators, e.g., sound, beeping and the like.

Reference is now made to Figure 4 which is an illustration of a sampling element (SE) 400, and the movement therein of a sample liquid, in accordance with some demonstrative embodiments.

As shown in Figure 4, SE 400 may include a sampling area, to collect the sample and at least three separate zones, zone 1, zone 2 and zone 3, through which a sampled liquid may pass.

According to some embodiments, zone 1 may include antibodies for the tested targets (e.g. vitamin A, CD45 etc.). These antibodies are conjugated to an enzyme (or other component- e.g. HRP) that can induce a color change.

According to some embodiments, when an analyte flows through its corresponding antibody (zone 1) the antibody may bind to the analyte and carry on to zone 2. At this station, there are corresponding antibodies (these may include the same protein but different epitope) that may be bound to the layer.

Zone 2 may also include particles that may induce the color release from the conjugated enzyme (e.g. Cumaric +Luminol + H_2O_2). When a specific analyte is present, the antibodies are binding to the analyte and the color change will occur at zone 2. When the analyte is not found in the tested example, or - is present in a low concentration, the color may be formed in zone 3. The intensity of the color is dependent on the amount of analytes in the sample which can be quantify by the device.

According to some demonstrative embodiments, Zone 3, is at the end of the flow, therefore the antibodies from zone 1 that are conjugated to an enzyme (e.g. HRP) and did not bind to any antigens along zone 1 or zone 2 will arrive to zone 3, e.g., by flow.

According to some embodiments, zone 3 may also include particles that induce the color release from the conjugated enzyme (e.g. Cumaric +Luminol + H_2O_2).

According to some embodiments, SE 400 may include additional zones having the same or other functionalities as zones 1, 2 and/or 3.

According to some embodiments, the sampling element of the present invention may include a dipstick, as explained in detail below with regard to Figure 5.

According to some embodiments, in contrast to a strip sampling element having a lateral flow, a dipstick sampling element may be built with layers. At the first layers (outer station), there may be antibodies in a dehydrated form and they may be conjugated to an enzyme or a chromophore (such as colored polystyrene particle) or to colloidal metals. According to some embodiments, this may also be referred to as the "reaction zone" wherein the antibodies are conjugated to the liberation pad in a leachable (easily detachable) manner.

According to some embodiments, when an antigen/analyte binds to the antibody, the complex (e.g. antibody-antigens) moves forward to the second layer, also referred to herein as "the test zone" (deeper layer). At this station there are corresponding antibodies (same protein but different epitope) that may be bound to the layer. When the complex reaches the second station, it may bond to the antibody that is bound to place, thus stopping the flow of the complex. At the second station, the enzyme, the chromophore or the colloidal metal that was bound to the

antibodies/enzymes is either released or otherwise causes a reaction which results in a color change. The intensity of the signal (color) is in relation to the concentration of antigen/analyte in the sample.

For an alternative in which the strip is inserted into the device before introducing it to the breast milk, the same principles would apply, but the lateral flow would usually be vertical and not horizontal due to the fact that there is a designated place for the milk (e.g., since entire stick is not wetted).

The antibodies based assay is an indirect ("sandwich") enzyme-linked immunosorbent assay (ELISA) when there are two antibodies that are corresponding for the same protein targeting different epitops. Alternatively, the first antibody is a monoclonal (highly specific) and the second antibody is a polyclonal antibody.

Reference is now made to Figures 5A-5D which illustrate a dipstick sampling element 500 with a panel, according to some demonstrative embodiments.

As shown in Figure 5A, sampling element 500 may include a holding area 502 and a dipstick body 503. According to some embodiments, a user of element 500 may hold element 500 by holding area 502 an immerse body 503 into a sample of breastmilk to be tested.

According to some embodiments, body 503 may include one or more testing area 504.

Figure 5B further describes testing area 504 in more detail: according to some embodiments, area 504 includes a liberation pad 506 (also referred to herein as "reaction zone 506").

According to some embodiments, reaction zone 506 includes a plurality of antibodies 508, wherein antibodies 508 may be conjugated to enzymes or chromophores.

According to some embodiments, area 504 may include a porous membrane 510.

According to some embodiments, membrane 510 may have at least two functions, wherein a first function of membrane 510 may include filtering macromolecules that are in the sample and may reduce the performance of the antibodies, and wherein the second function of membrane 510 may include stalling of the bound antigens (bound to the antibodies), e.g. for improving the chances of a stronger adherence.

According to some embodiments, area 504 includes a second layer 512 (also referred to herein as "test zone 512"), which contains bound antibodies 514 for specific components in the analyte being tested.

According to some embodiments, area 504 includes a hydrophobic center 516 comprising the dipstick. According to some embodiments, in contrast to the lateral flow stick, wherein the sample is placed in a designated area, which in turn determines the direction of the flow, the dipstick may be exposed and circumvent by the milk. Therefore, a partition (hydrophobic) blocks the milk from one end forcing it to be absorbed in a directional manner. Every square on the dipstick may be an individual unit, separated by the hydrophobic partition.

Reference is made to Figure 5C which illustrates sampling element 500 immersed in a tested sample (breast milk 518).

Reference is made to Figure 5D which depicts a used sampling element 522 inserted into a panel 520.

According to some embodiments, after a sampling element 522 has been brought into contact with a sampled breastmilk, a chemical reaction has occurred, and is reflected upon sampling element 522 in terms of a color change.

According to some embodiments, after such a reaction has occurred, sampling element 522 may be inserted into panel 520 which has a variety of detection colors for reference.

According to some embodiments, the colors of element 522 may be compared to the reference colors of panel 520 to determine the existence and/or estimated concentration of a specific analyte or a group of analytes in the tested breastmilk.

Reference is now made to Figure 6, which depicts a horizontal stick flow sampling element 600, according to some demonstrative embodiments.

Horizontal element 600 may comprise an edge for placing a breastmilk sample 602, and a body which may contain absorbing surfaces as previously described, for example, the absorbing surfaces may include any suitable fibers, pads and/or membranes.

According to some embodiments, the absorbing surfaces may be arranged in 5 lanes, separated by a hydrophobic strip (604).Reference is now made to Figure 7, which depicts a flow chart of different possible analysis options 700 for sampling and analyzing breast milk using the system of the present invention, according to some demonstrative embodiments.

According to some demonstrative embodiments, the system of the present invention may operate in 3 modes of operation:

1. Immunological- allows the parents immunological monitoring of the baby. Significant tool in predicting a developing/ disease in the baby before it shows clinical symptoms whether it is

viral/ bacterial or a specific illness that an early detection of it can really improve the baby condition and health (like: meningitis, asthma, Pneumonia etc.) or even enabling the parent to make an appointment with the physician (instead of going to work).

2. Nutritional- allowing any mothers, anywhere in the world, monitoring and improving their specific composition of breastmilk in terms of significant macromolecules and vitamins and nutrients. The mother will be able to save the data of each test on here phone and see how simple changes in her nutrition can impact her milk over time.

3. Breastfeeding combined with Baby Formula- How best to combine breastfeeding with supplementary nutrition (AKA infant food supplements, 53% of the market). All baby food supplements look the same on the supermarket shelves. The choice of product to purchase relies mostly on recommendation.

According to some embodiments, the system of the present invention enables to determine the baby development stage not by age but according to what best matches the current status of the mother breast milk.

According to some embodiments, analysis options 700 may include option 702 including transfer of data from the device, to sensors, via Bluetooth and yield a result in the application. According to some embodiments, option 702 route is applicable whether the sampling element is a horizontal stick or a dipstick.

According to some embodiments, once the breastmilk sample is placed on the stick and/or the stick is dipped in the sample (breastmilk testing actions differ depending on the stick's mechanism), the stick may then be inserted into its allotted slot in the device. According to some embodiments, coating the inner surface of this slot are light/color sensors that can analyze the results of the colorimetric tests on the stick. The device may report these findings via Bluetooth to a smartphone application. The application may translate the results into easy-to-understand data, and provide the user with conclusions of the analysis and/or recommendations.

According to some embodiments, analysis options 700 may include option 704 may be applicable for both the horizontal stick and the dipstick. According to some embodiments, once the breastmilk sample is placed on the stick/the stick is dipped in the sample (breastmilk testing actions differ depending on the stick's mechanism), the stick is placed in the middle of a color control panel.

According to some embodiments, a user of the device of the present invention may use a smartphone camera to scan the panel with the stick. An application installed upon the smartphone, may then compare the colored results on the stick to the panel and provide an easy to understand numeric result for each test. The application may then provide the user with possible recommendations, for example, recommended changes in diet or habits if necessary.

Reference is now made to Figure 8 which illustrates possible uses for the system 800 of the present invention (also referred to herein as "MAOKIT Medical Device").

According to some embodiments, as shown in fig. 8, system 800 may include a device 802 (which is a sampling element and is also referred to herein as MAOKIT medical device 802), which is adapted to sample and analyze the nutritional aspect 806 of breastmilk, adapted to provide recommendations with regard to the desired nutritional aspects of an infant receiving combined breastfeeding and formula nutrition 838 and is also adapted to analyze the immunological composition of the breastmilk 804, and optionally provide alerts with relation thereto.

According to some embodiments, device 802 may be adapted to analyze the composition of breastmilk providing information on the nutritional aspect 806 of the milk.

According to some embodiments, device 802 may include one or more elements that may enable the sampling of the milk and the analysis of the composition thereof, for example, via a chemical reaction.

According to some embodiments, nutritional aspect 806 may include the analysis of macromolecules 822 and/or nutrients 820.

According to some embodiments, the analysis of macromolecules 822 may include the analysis of protein 824, carbohydrates 826, glycoproteins 828, lipids 830, glycolipids 832 and pH level 834.

According to some embodiments, the preferred molecules to be tested include, for example,: sIgA, IgG, IgM, CD-45 (Leaukocytes), Lactoferrin, IL-10, TNF-α, INFγ, HMOs, Macrophages, Lymphocytes, IL-6, TGF-β1, pIgR, and α-Lactalbumin.

According to some embodiments, device 802 may analyze and/or determine the amount and/or concentration of each of the macromolecules 822 and provide an output detailing the amount and/or concentration of each of macromolecules 822 in the examined breast milk.

According to some embodiments, routine examination of the breast milk using device 802, for example, on a daily basis, may enable monitoring of macromolecules 822. According to some embodiments, device 802 may alert a user of device 802 when an abnormal elevation and or decrease occurs in one or more of macromolecules 822.

According to some embodiments, the analysis of nutrients 820 may include the analysis of special molecules 836 which include, for example Omega 3, Omega 6, Folic acid, Biotin, Choline, Niacin, and/or vitamins/minerals 840, including for example, Vitamin A, Thiamin (B1), Riboflavin (B2), Pyridoxine (B6), B12, Vitamin C, Vitamin D, Vitamin E, and Vitamin K.

According to some embodiments, device 802 may analyze and/or determine the amount and/or concentration of each of the nutrients 820 and provide an output detailing the amount and/or concentration of each of nutrients 820 in the examined breast milk.

According to some embodiments, routine examination of the breast milk using device 802, for example, on a daily basis, may enable monitoring of nutrients 820. According to some embodiments, device 802 may alert a user of device 802 when an abnormal elevation and or decrease occurs in one or more of nutrients 820.

According to some embodiments, device 802 may be adapted to provide recommendations with regard to the desired nutritional aspects of an infant receiving combined breastfeeding and formula nutrition 838.

According to some demonstrative embodiments, and as described in detail hereinabove, many mothers choose to supplement breastfeeding with a baby formula in order to provide for the complete nutritional needs of their child.

According to some embodiments, at present, an infant is fed with a suitable formula according to the age of the infant. For example, a 3 month old baby will be receiving a stage 1 formula. However, the composition of each baby formula does not necessarily correspond to the actual nutritional needs of the infant.

According to some embodiments, device 802 may analyze the breast milk of a mother providing combined nutrition to the infant (both breastfeeding and a baby formula), and determine the actual breastmilk composition.

According to some embodiments, based on the actual concentration and/or amount of components found in the analyzed breastmilk device 802 may provide a recommendation 842 on how to combine breastfeeding with different brands and/or stages of baby formula.

According to some embodiments, device 802 may determine the development stage of the breastfed baby not according to the age of the baby, but according to what formula best matches the current status and/or composition of the mother's breastmilk.

According to some embodiments, device 802 may provide a recommendation of the specific brand and/or stage of the baby formula which best suits the actual needs of the baby, for example, device 802 may analyze the breastmilk of a mother breastfeeding a 5 month old baby and determine that the baby actually needs a stage 2 formula and not a stage 1 formula, based on the actual composition of the breast milk analyzed.

According to some demonstrative embodiments, device 802 may analyze the immunological aspect 804 of a breastmilk.

According to some embodiments, device 802 may determine the actual percentage of different antibodies in breastmilk 808, for example, as detailed in Table 1 above.

According to some embodiments, device 802, when used on a regular basis, for example, on a daily basis, may enable the ongoing monitoring of the concentration and/or amount of different antibodies in the analyzed breastmilk.

According to some embodiments, device 802 may alert when the level of one or more antibodies may rise or decrease, for example, above or below a certain predefined level, e.g., an abnormal level.

Table 4 below details average breastmilk concentration of certain elements when the breastfed baby is healthy and when the breastfed baby is sick.

Table 4

Molecule	Average Breastmilk Concentration when **Healthy** baby	Average Breastmilk Concentration when **Sick** baby
CD45	2,122 cells/ml BM	5,655 cells/ml BM
macrophage	300 cells/ml BM	1,220 cells/ml BM
TNFα	2.91 ± 1.51 pg/ml BM	3.66 ± 1.68 BM

Total lymphocytes	325 cells/ml BM	2,474 cells/ml BM
Neutrophils	813 cells/ml BM	2,941 cells/ml BM

Source: *Riskin, Arieh, et al. "Changes in immunomodulatory constituents of human milk in response to active infection in the nursing infant." Pediatric research 71.2 (2011): 220-225.*

According to some embodiments, device 802 may enable, based on the analysis of immunological aspect 804 of the breastmilk, to detect the early stages of common diseases 810, in the breastfeeding mother and/or breastfed infant, for example, device 802 may enable the early detection of the most common 10 diseases often found in babies.

According to some embodiments, often, an increase in a specific antibody in the breast milk may correspond with the existence of specific pathogens, existing in the baby and/or mother.

According to some embodiments, device 802 may enable to detect a correlation between specific antibody levels in the breast milk and specific upcoming diseases related to specific viral pathogens 818 and/or specific bacterial pathogens 816, for example, as set forth in Table 2 and Table 5 below, which lists common exemplary diseases and their relative pathogens.

Table 5

	Disease	**Pathogen (example)**
1	Diphtheria	bacterium Corynebacterium diphtheriae
2	Hand foot and mouth disease	Coxsackievirus A16 & Enterovirus 71 (EV-71)
3	Infective Diarrhea	rotavirus
4	Jaundice - Viral Hepatitis	Hepatitis A, B, C, D, E
5	Measles	Measles virus
6	Meningitis	Neisseria meningitides & Streptococcus pneumoniae
7	Molluscum contagiosum	Poxvirus (MCV)
8	Mumps	Mumps virus
9	Pneumonia	Streptococcus pneumoniae
10	Respiratory Syncytial Virus	Syncytial Virus
11	Scarlatina	group A streptococcus
12	Varicella / Chickenpox	varicella zoster virus (VZV)
13	Whooping cough / Pertussis.	Bordetella pertussis.

According to some embodiments, the analysis of the immunological aspect 804, as performed by device 802 may contribute and/or improve the condition and/or health of a baby 812.

According to some embodiments, device 802 may assist in the improvement of the overall condition and/or health of a baby due to early diagnosis of possible deficiencies in the breast milk content (e.g., low levels of HMOs which might lead to discomfort or digestive tract infections, low levels of Omega 3 or Omega 6 which might lead to slower or deficient brain development) and/or early diagnosis of possible illnesses, both of which lead to early treatment.

According to some embodiments, device 802 may also uncover "hidden" diseases, such as meningitis, which may often be misdiagnosed as the common cold or a light infection at its early stages. For example, device 802 may assists in the early detection of these diseases by specifically testing for meningitis antigens/antibodies, e.g, using an immunological sampling element.Reference is now made to Figure 9 which illustrates a system 900 and the components thereof, in accordance with some demonstrative embodiments.

As shown in fig. 9, system 900 may include screen 902. According to some embodiments, screen 902 may be an LCD screen adapted to present the results analyzed by system 900.

According to some embodiments, screen 902 may include any suitable output device for presentation of information in visual or tactile form, including for example, Cathode ray tube display (CRT), Light-emitting diode display (LED), Electroluminescent display (ELD), Electronic paper, E Ink, Plasma display panel (PDP), Liquid crystal display (LCD), High-Performance Addressing display (HPA), Thin-film transistor display (TFT), Organic light-emitting diode display (OLED), Surface-conduction electron-emitter display (SED) (experimental), Field emission display (FED), Laser TV, Carbon nanotubes, Quantum dot display, Interferometric modulator display (IMOD), Digital microshutter display (DMS).

Screen 902 may also include three-dimensional displays, including for example, Swept-volume display, Varifocal mirror display, Emissive volume display, Laser display, Holographic display, Light field displays and the like.

According to some preferred embodiments, screen 902 is an LCD screen.

System 900 may also include power supply source 904. According to some embodiments, source 904 may include any power supply coming from the electric power grid, such as an electrical outlet, energy storage devices such as batteries or fuel cells, generators or alternators, solar power converters, or another power supply.

According to some preferred embodiments, source 904 is a 5 volt battery. System 900 may also have a built-in or separate battery pack storage unit 920.

System 900 may also include one or more sensors that can translate the results from the nutritional/immunological tests to easy-to-understand numerical values. According to some embodiments, these can be color/light sensors 906 and 908, and/or magnetic sensor 910.

System 900 may include one or more inputs to which the sample can be inserted, either as a stick hole 912 or a cuvette hole 914, depending on the method of use chosen by the user.

System 900 may also include a Bluetooth component 922 that communicates with a dedicated application 926. This application may be accessed via a mobile phone, laptop, or any other electronic device that supports Bluetooth and onto which the application can be installed.

According to some preferred embodiments, application 926 is accessed via mobile phone.

According to some embodiments, system 900 may include an internal memory device 924 (also referred to herein as "microchip 924") that can also be expanded and used outside the 900 system. The memory device may allow continuous data storage, its retrieval and display on the device screen or on external devices.

According to some embodiments, system 900 may also have a USB port 928 which enables additional data transfer option from and to system 900.

According to some embodiments, system 900 may include an incubator 918 having one or more incubation lights, to enable the incubation of the analysed maternal milk. According to some embodiments, incubator 918 may include any suitable device and/or array of device or components to enable optimal temperature, humidity and/or other conditions such as the carbon dioxide (CO_2) and oxygen content to allow the growing and/or development of microbiological cultures. According to some embodiments, incubator 918 may include an adjustable heater, typically going up to 60 to 65 °C , most preferably, most preferably approximately 37 °C, e.g., to provide optimal growing conditions.

Reference is now made to Figure 10 which illustrates an algorithm of activities and/or actions 1000 of the system of the present invention, in accordance with some demonstrative embodiments.

As shown in Figure 10, algorithm 1000 may be fully automatic and computerized, except for the initial stage in which the user is required to choose the method by which the sample is inserted into the device (dipstick input 1002 or cuvette input 1004).

According to some embodiments, algorithm 1000 may include a checkpoint 1006 that gives an indication about the correctness of sampling placement into the device. In a setting, an error message 1008 can be displayed on the device's screen and / or transmitted to an external device (1010).

According to some embodiments, algorithm 1000 may have predefined processes for analyzing the composition of the sample being tested. According to some embodiments, these processes 1012 enable to analyze the nutritional and/or immunological composition of the breast milk sample, and may, for example, determine the appropriate formula for the infant and/or alert regarding the possible development of a disease.

According to some embodiments, algorithm 1000 may include a checkpoint 1014 for detecting abnormal deviations in the values of the sample's components being tested. According to some preferred embodiments, these deviations may be identified by comparing the sample to some reference values.

For example, referring to the horizontal stick - Zone 3 may act as a control zone, which confirms that the test is working properly. According to some embodiments, in zone 3, the antigen which travels from Zone 1 should activate a dye, regardless of the presence of the bound molecule (i.e the target molecule). This is to ensure the antigen has been properly detached from Zone 1, has travelled across the stick, and the enzyme it carries which causes the color reaction is functioning properly.

For the dipstick, there are a few possible scenarios for the assessment of false results:

a. a control square with a generic test which should always be positive, that indicated the stick is valid and the milk sample has been properly introduced.

b. each test square could contain a two-toned reaction, one would indicate a positive result (a match with the target molecule) and the other would be a negative result (no match with the target molecule).

c. each square might have two layers, similar to the horizontal stick, with the outer layer acting like Zone 3. In addition, the outer layer might induce a different color in case of a negative result.

According to some embodiments, algorithm 1000 may enable displaying the results of the analysis on the device's screen, whether valid (1018) or non-valid (1020) results, and/or sending these data to external device (1016).

According to some demonstrative embodiments the system of the present invention may include one or more of the following: sensors that can translate the results from the nutritional/immunological tests to easy-to-understand parameters; Bluetooth component that communicates with a smartphone application; Separately-purchased box with 30 disposable nutritional stick and 1 disposable immunological stick; Nutritional stick allows for the monitoring of the nutritional composition of the breast milk (including total antibodies concentration); Immunological stick checks for the presence of specific childhood diseases; an application to help mothers monitor their infants' growth, health and development.

According to some embodiments, the application may include the following features: User Friendly; Alerts the mother when her breastmilk shows deviations from her infant's nutritional needs; Gives nutritional suggestions that can improve the breast milk quality; Alerts the mother for possible illnesses in her infant; Gives invaluable nutritional and immunological information in case of a visit to the doctor; Growth monitoring section where the mother can input data and keep track of her infant's development.

According to some embodiments, the system may integrate with Child Care Centres and/or Human Milk Banks.

According to some embodiments, a nursing mother using the system of the present invention may perform a daily nutritional test and discover that her milk lacks essential fatty acids. The application of the system may notify her and can throughout the day send her reminders to eat a variety of foods that contain these lacking ingredients.

According to some embodiments, for mothers who choose to mix formula feeding with breast feeding, relying on the nutritional information from the nutritional test performed by the mothers, the application can suggest which stage of the formula best fits their infant (stage 1, stage 2 or stage 3). This way the mother can choose a formula that best meets her infant's developmental needs.

According to some embodiments, the regular nutritional stick sampling element may also contain a test for general antibody concentration in the breastmilk. As soon as the test shows an abnormal result, the application will let the mother know she should use an immunological stick to provide a more accurate analysis of the breast milk.

According to some embodiments, an abnormal antibody concentration in the breastmilk suggests that the infant might get sick in the following days.

According to some embodiments, the immunological kit tests for common childhood diseases. If the test is positive for any of the diseases, the application will let the mother know, suggest she should check her child's fever and ask whether she wants to make a doctor's appointment or not.

According to some embodiments the system of the present invention may allow for a prediction of a baby's disease, thereby obviating the need to see a physician. For example, a baby in his first year of life visits the doctor at least nine times. The system of the present invention and the application thereof will give the mother the tools necessary to predict possible illnesses and give the doctor critical information, be it the results of the immunological kit or the fever monitoring which was recorded by the mother.

According to some embodiments, the application will have a dedicated section where the mother can input data regarding her infant's growth, development, mood/behavioral changes, and so on. The mother will be able to use this information to track her infant's growth and development, as well as pick up behavioral patterns.

For example, the mother inputs her infant's weight at every weighing with the doctor/at infant care centers. The application will save this information for her convenience and will also be able to show her in graph form her infant's growth through.

According to some embodiments, the system may integrate with Child Care Centers:

1. The mother's breastmilk's nutritional and immunological information is saved over time in the device's application, and can be used to monitor the child's growth and development. Child Care Centers can use that information to make sure that the infant is receiving the adequate nutrition for his age. If, for example, the infant is under the normal weight for his age, the nurse at the care center can look over the mother's nutritional information and make suggestions accordingly.

2. The mother can input data from the care center check-ups such as the baby's weight and head circumference in the application's growth monitoring section, so that all her infant's growth data is organized in one place, in an easy and accessible manner.

According to some embodiments, the system may integrate with Human Milk Banks.

There are Human Milk Banks in about 33 countries around the world. These are multi-million dollar establishments on which countless infants rely as their only source of human breast milk. Our device can be used by the Human Milk Bank to determine the nutritional quality of the

milk they collect or receive, see whether it meets the required criteria and classify it accordingly.

According to some embodiments, the system of the present invention may include specific sampling elements, with correlation to the specific region the breastfeeding mother is located. For example, a stick that check specifically for region-based diseases (either occurrence in the mother that may infect the child through breastmilk or occurrence in the infant). For example, a stick that checks for the Zika virus or for HIV presence in the breastmilk.

Reference is now made to Figure 11, which is a flow chart of the operation 1100 of the system of the present invention, in accordance with some demonstrative embodiments.

As described in Figure 11, the system's operation may include the following steps:

turning a device of the system (1102) by a power switch;

taking a breast milk sample 1104, for example, using either a disposable glass or a feeding pump adapter (both of which may come along with the system);

dipping the nutritional strip in the milk sample 1106;

inserting the stick into the device in its indicated place and pressing a dedicated key to run the test 1108.

According to some embodiments, the system further may perform an analytical step 1110 of nutritional and total antibody status, at the end of which it presents numerical results on the device's screen. According to some embodiments, this step includes an option to store the data in the application for future use.

According to some embodiments and as described in Figure 11, the system may identify an abnormal status 1112 of total antibody level, and accordingly send the user one or more notifications 1114 through the application. According to some preferred embodiments, these notifications may come in the form of text and may include a recommendation to use the immunological stick. In such a case, the system may test the milk sample for specific antigens (e.g. viruses, bacteria and the like). Eventually, the system may perform a correlation between the immunological test results and the risk of the infant to acquire a disease.

According to some embodiments and as described in Figure 11, the results of the immunological test may be presented to the user as a message via the application. According to some embodiments, this message may come in the form of text.

According to some preferred embodiments, in case of negative result 1116, the user may receive a message 1120 informing that there was no correlation found between total antibody test and any of the diseases test, although other medical conditions cannot be totally ruled out and a further inquiry is recommended with her physician.

According to some preferred embodiments, in case of positive result 1118, the user may receive a message 1122 informing that a correlation to a specific disease was indeed found, along with a recommendation to visit her physician for further inquiry.

According to some embodiments and as described in Figure 11, the system may identify an abnormal status 1124 of nutritional components in the milk, and accordingly send the user one or more notifications 1126 through the application.

According to some embodiments, these notifications may include nutritional and/or dietary recommendations aimed to improve breast milk composition as a function of the nutritional test results. According to some embodiments, the system may allow a comparison between the present test values and previous tests stored in the application, allowing a continuous follow-up and recommendations for specific formulas for partially breastfed infants.

According to some embodiments, there is provided herein a quick test kit enabling partially lactating mothers to monitor the development of their babies and to decide when to move to another stage formula.

According to these embodiments, upon analyzing the composition and/or concentration of several components in the mother's breast milk, the kit of the present invention allows for the determination of the development stage of the infant. According to some embodiments, the kit is designed for mothers that wants to combine maternal milk and formulas.

According to some demonstrative embodiments, there is provided a method for establishing an infant's nutritional needs and enabling a care provider to decide if the infant is ready for the next phase formula, for example, not just according to an average aged based but individually.

According to some demonstrative embodiments, the method of the present invention may include measuring the maternal milk which reflects the condition of the infant.

One simple example is the first 3 distinctive stages in maternal milk: colostrum, transitional milk, and mature milk. According to some examples, the first formula is for very young babies that need the colostrum or the transitional milk (usually between 0-1 months). The second

formula may be used for babies that need mature milk, and the third formula is intended for mature infants, usually over 1 years old.

According to some embodiments, there are provided herein infant formulas which may be divided to different content according to the developmental needs of the growing infant. For example, the formulas may include 6 different formulas that address most of the infant's needs and other formulas that are more specific to different conditions.

According to some embodiments, the kit of the present invention may include a cartridge with a funnel that ensure that a set volume of maternal milk (e.g., 50µl) will be measured.

According to some embodiments, the kit may be based on an enzymatic, biological and/or chemical reactions. The kit is based on a simple algorithm that can factor in the different tests and to recommend on the next steps that should be taken.

For example, the kit may enable to determine that the pH of a mother's milk is changing with time postpartum. According to some embodiments, different formulas may include different pH levels. According to some embodiments, a mother using the kit of the present invention may test her milk, and according to the pH levels of the milk, the kit of the present invention may indicate to the mother which of the existing formulas possesses a pH level which is closest to the actual pH of the mother's milk.

Example 1

1. The mother will put maternal milk up to a line in the cartridge (about 5 ml).
2. After 5 minutes there will be a development of a color (e.g. blue). The kit will come with a scale of the color (e.g. light to dark blue) corresponding to the different formulas or stages. The funnel will direct the milk sample to the other tests as well.

Example 2:

The mother will inject a requested volume (1 mL) into a container with copper sulfate and 1% (w/w) of sodium hydroxide. The liquid in the container will turn purple and by comparison of the observed shade to the shade scale given (maximum 5 shades), the protein concentration range is known.

Example 3:

A colorless, transparent, semi permeable, perforated tube containing phosphomolybdate and phosphotungstate, is inserted inside a sample of maternal milk (minimum 5 mL). After 3 seconds of immersion, the tube is removed from the maternal milk and a shade of the color blue is formed inside the test tube. The observed shade is compared to the shade scale provided with the kit to find the protein concentration of the maternal milk that was tested.

Example 4 -antibodies

In our case, the nutritional strip/dipstick will have an antibody for CD45 in order to monitor an increase that is in high correlation to an infection. Thus, may indicate that the baby is becoming sick, or that the mother is becoming sick. The antibodies will bind to the CD45 as described (liberation pad) and the intensity of the signal could be measured either by comparison to a color-panel as in alternative 1 or with a sensor in a designated device.

If the count is high, the application will recommend the mother to use the immunological strip/dipstick. Once the immunological strip will indicate a problem, the application will guide the mother for further actions including a recommendation to visit the physician.

Example 5 – Enzymatic response

Glucose test

The mother's milk is rich with glucose, however, monitoring its levels might be important for the baby growth especially if there are great changes on the same conditions. Clearly, there will be differences in the glucose levels before and after a meal. However, if the mother is measuring the glucose levels under the same conditions it should be more or less the same.

The detection of glucose by test strips is based on the enzymatic reaction of glucose oxidase. This enzyme catalyzes the oxidation of glucose by atmospheric oxygen to form D-glucono-δ-lactone and hydrogen peroxide. A second linked reaction, mediated by a peroxidase, catalyses the reaction between the peroxide and a chromogen (a substance that acquires colour after a chemical reaction) to form a colored compound that indicates the glucose concentration.

1) Catalyzed by glucose oxidase:

$$\text{Glucose} + O_2 \rightarrow \text{D-glucono-}\delta\text{-lactone} + H_2O_2$$

2) Catalysed by peroxidase:

$$H_2O_2 + \text{Chromogen} \rightarrow \text{Oxidised chromogen (colored)} + H_2O$$

Example 6 – color response

Protein concentration is important for the nutrition of the growing baby. Therefore, the nutritional strip will measure the protein levels. One of the ways to measure the protein level is by utilizing the principle of Bradford assay. This assay involves the binding of Coomassie Brilliant Blue dye to proteins. Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (Amax = 470 nm). However, when the dye binds to protein, it is converted to a stable unprotonated blue form (Amax = 595 nm). Changes in protein concentration can be visualized by utilizing the Coomassie by naked eye as well. The application would be able to keep record on the protein concentration and to recommend the mother on a diet or the most suitable formula for the baby.

Example 7:

Detecting CMV antibodies (CMV-IgG and CMV-IgM), especially important for pre-term babies who have not received the mother's CMV immune factors. Cytomegalovirus is usually not a deadly virus among healthy infants and adults, but can be dangerous for pre-term babies. The virus can lie dormant or appear as other common diseases. Early detection through the presence of antibodies in the breast milk can help prevent the virus from developing in the infant into a dangerous disease.

Example 8:

Human Oligosaccharides (HMOs) are lipids which feed the infants' intestinal fauna (the bacteria within the infant's digestive system). An imbalance in HMOs (a decrease or increase from the Healthy concentrations - Colostrum: 20–25 g/L and Mature Milk: 5–20 g/L) may lead to an imbalance in the infant's digestive system, which can cause cramps, diarrhea, gas etc'. Detecting an imbalance of HMOs as the cause for the infant's discomfort can help the mother take early and quick measures to ensure an increase/decrease in HMOs in her breastmilk (through changes in her eating habits) and help her infant recover.

Example 9:

Elevated levels of IL-6 have been observed when a pathogenic bacteria (such as Bacterial Meningitis) takes root. The healthy level of IL-6 in colostrum is 978.80 ± 86.80 pg/mL, in transitional milk it is 162.90±29.67 pg/mL and in mature Milk it is 86.92 ± 2.47 pg/mL according to Ustundag, Bilal, et al. By diagnosing elevated levels of IL-6, the parents of the infant can be forewarned of a possible bacterial infection at play and an early detection and prevention of the infection may be achieved.

While this invention has been described in terms of some specific examples, many modifications and variations are possible. It is therefore understood that within the scope of the appended claims, the invention may be realized otherwise than as specifically described.

Example 10:

Protein concentration and estimated the carbohydrates quantity in breast milk were determined and compared between 6 groups according to the age of the infant.

Samples collection - Breast milk samples were collected from 98 Israeli donors whose infants' ages ranged from 7 days to 843 days, about 16 donors from each age group. The infants were divided to age groups: 0-1 months, 1-3 months, 3-6 months, 6-9 months, 9-12 months and 12 months and above.

The tested infant formulas were the three main brands in the Israeli market: Materna, Similac and Nutrilon.

Protein quantification - The protein concentration was determined by two methods: Bradford assay (Bio-Rad Protein Assay) and BCA assay (Pierce™ BCA Protein Assay Kit), both are spectrophotometric methods using ELISA kits. Bradford assay was independently executed both by M.A.O FoodTech Labs and by the Technion.

Carbohydrates quantification - Thermo Scientific's Glycoprotein Carbohydrates Estimation Kit. A spectrophotometric method using ELISA kit.

Results:

First, the protein concentration of three different infant formulas in the market was assessed. These (and many other) companies divide the infant's development into 3 stages or phases: phase 1 (0-6 months), phase 2 (6-12 months) and phase 3 (1 year+). In figure 12 the protein concentrations of the three-phase products of Nutrilon® are presented. It can be noticed that

there is an increase in the protein content between phase 1 to 3, ranging from 14.5 g/ml in phase 1 to 15.1 g/ml in phase 2 and 16.6 g/ml in phase 3.

Next we wanted to quantify the protein content in human breast milk. We divided the donors into 6 groups, according to the age of the infant. Using Bradford and BCA assays, we quantified the average protein concentration in each of the groups. As can be seen in figure 13, (which shows graph depicting the protein concentrations of the human milk at different age groups, using Bradford assay. Graphs show mean and S.E values, n=99; * indicates statistical significance ($P<0.05$) to all other age groups, and ** to all other age groups except for group 2) the highest average protein concentration can be found in group 1 (14.5 mg/ml). In group 2 there is a major decrease in the protein concentration (10.5 mg/ml), and in groups 3 a further moderate decrease, which stays steady until group 5 (9.2-9.4 mg/ml). In groups 6 there is again an increase in the protein concentration (11.9 mg/ml). In addition, there was further increase after 15 months, which cannot be seen in the graph. There was no pattern within each age group.

Carbohydrates found in the breast milk were also estimated. Thus, we found the percentage of carbohydrates in glycoproteins in the breast milk, in a known concentration of protein (0.25 mg/ml). The absorbance was outside of the linear range of the calibration curve. Therefore, we normalized the absorbance into a relative percentage of the carbohydrates content in reference to the blank (0%) and to the maximal absorbance. As shown in figure 14, there is no clear pattern when comparing the age groups. In addition, most of the results are not statistically significant from one another ($P>0.05$). In addition, there is high variance within each age group between the mothers.

When comparing figure 12 and figure 13, major differences between the two trends in the protein concentration along the development of the infant is seen. Not only does the formulas roughly divide the infant's development into three stages, but it also suggests protein values that are different from those found in real breast milk. While there is a major increase in the formula, our results show that the protein in breast milk decreases until age 3-12 months, and increases afterwards. Both groups 1 and 6 are statistically significant (P-value<0.05), a result that emphasize that this trend in protein concentration is real.

The high protein concentration in the first month adheres to our assumption and can be explained by the high immunologic factors present in the milk after giving birth. The decline

after the first month also corresponds with the literature. The increase in protein after 12 months however is surprising and may suggest that there is a change in the protein composition in the milk, which is necessary for the infant.

These results were analyzed using the fast Bradford ELISA kit, as well as BCA ELISA kit. Both assays gave similar results, and also the two independent experiments with Bradford method gave similar results. This finding makes our results more robust. In addition, Bradford method is quick to execute and gives fast result. This allows us to repeat and further continue our research easily. The results reflect averages of the protein concentration and there was high variation between the donors. This finding also supports the fact that each baby is nourished by different nutrient composition, unlike the relatively uniform composition found in the formulas. The different milk composition can be either a result of the mother's or the infant's status.

In spite of the fact that the carbohydrates quantification assay did not give absolute values, it is clear from figure 14 that there is no clear pattern between the age groups. This can be derived from the high variance between the mothers. The infant formulas however suggest products with very minor changes in the carbohydrates content and do not correspond to the breast milk and subsequently to the biological age and needs of the baby.

In agreement with our assumption and the literature that the quantity of nutrients in maternal milk changes along the growth of the baby, we have seen differences in protein and carbohydrates levels in maternal milk. In correspondence to the literature, our results have indicated a decrease in total protein after the first month. However, our results have shown an increase in total protein after a year. There was a certain variation in total protein among mothers within the same age group and there was no trend within the group. In addition, there is high variance in the carbohydrate levels within each group.

These results are in conflict with the composition of the infant formulas as exist in the market today, which suggest more uniform products with little variance and no adherence to the biological age or status of the infant. Therefore, upon knowing the breastmilk composition, each mother will be able to choose a formula that fits better to her baby. Alternatively, more accurate and fine formulas can be developed, that will better fit the baby's status.

Example 11

Infants require a different combination of proteins, lipids and sugars during the different stages of their growth. The human breast milk composition changes over time to fit the needs of the infant. For example, newborns require a higher concentration of protein and immunogenic factors, as opposed to older infants who require a higher concentration of lipids and energy.

When developing an infant food supplement, the requirements of infants during their different developmental stages must be taken into account – one supplement will not fit all infants of all ages. This is why companies nowadays tend to create a three-stage model – three products in the same line that are designed for three developmental stages of the baby.

The purpose of this experiment comes in three parts: the first is to test whether there is a pattern in the amount of proteins and glycoproteins within human breast milk that corresponds to, or changes with, the age of the infant. The second is to analyze the differences in three infant food supplement brands using biological and chemical essays. And the third is to test simple kits for the identification of different developmental stage of the baby. This essay focuses on the quantification of proteins and carbohydrates (through glycoproteins quantification).

Human breast milk was collected separately from this protocol, and divided into six groups according to the infant's age: 0-3 months, 3-6 months, 6-9 months, 9-12 months and 12 months and over. 90 donors participated, 15 donors from each age group. 90 samples for the use of this experiment were collected.

The infant food supplements used in this experiment is chosen from the three main brands in the Israeli market today: Formula 1 (Materna®), Formula 2 (Nutrilon®) and Formula 3(Similac®). Each of these three has at least one line of products with a three-stage model.

The three-stage model for all three supplement brands used in this experiment follows the following: Phase 1 is for infants between the ages of 0 to 6 months, Phase 2 is for infants between the ages of 6 to 12 months, and Phase 3 is for infants over the age of 12 months.

The tests performed in this essay included:

Bradford: based on an absorbance shift of the dye Coomassie Brilliant Blue G-250. The dye changes its hue from red to blue when in contact with an acid, and binds to the proteins in the liquid it has been placed. According to the book Nutrition During Lactation, Chapter 6: Milk Composition, total protein concentration decreases rapidly during the first month post-partum, and keeps decreasing gradually for the next five months (results were not shown beyond the

first five months after birth) (Lactation, 1991). Therefore, we expect the total protein concentration to be higher in Phase 1 formula than in Phase 2 formula and so on.

BCA: An assay based on the bicinchoninic acid (BCA) for the colorimetric detection of total protein concentration. This method combines the reduction of Cu+2 to Cu+1 by protein in an alkaline medium with the colorimetric detection of the cuprous cation (Cu+1) using a reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This complex exhibits a strong absorbance at 562 nm. This reaction has a broad working range (20-2,000 μg/ml) (Sino Biological).

Glycoprotein Carbohydrate Estimation Kit: The Thermo Scientific Glycoprotein Carbohydrate Estimation Kit is used for the detection of carbohydrate content. Glycoprotein is first oxidized with sodium meta-periodate to form aldehydes that react with the proprietary Glycoprotein Detection Reagent. The resulting purple reaction may be detected at 550nm. Unknowns are compared with protein standards of known glycoprotein content. It is important to note that non-glycosylated proteins, such as lysozyme and bovine serum albumin, produce a low absorbance at 550nm.

All above molecular assays rely on the use of a standard with known concentrations of the molecule in question. With these standards it is possible to create curves where the x-axis represents the spectrophotometric readout, and the y-axis represents the concentration of the sample. In order to receive the most accurate results, it is required that the spectrophotometric result of the unknown sample falls within the linear part of the standard curve.

Objectives:

1. To test whether there is a pattern within human breast milk that corresponds to the age of the infant / changes with the age of the infant.

2. To quantify and graph the pattern in human breastmilk which corresponds to/changes with the age of the infant. The parameters tested for this pattern are: total protein concentration and total carbohydrates concentration.

3. To test whether there is a pattern within brand baby food supplements that matches the changes in human breast milk with correspondence to the age of the infant.

The human breast milk composition changes as the infant grows in age. These changes may be noted in decreased levels of proteins and increased carbohydrates (especially in the form of

lactose and oligosaccharides). The composition data available on the sites of the brand baby food supplements suggest there exists a small pattern in increased levels of protein as the phases progress and increased levels of carbohydrates as the phases progress (with the notable difference of Similac®, that remains almost unchanged within the phases).

5

The projected concentrations of the values tested in this experiment are depicted in table 6 below:

	Materna Phase 1 (0-6 MO)	Materna Phase 2 (6-12 MO)	Materna Phase 3 (12+ MO)	Nutrilon Phase 1 (0-6 MO)	Nutrilon Phase 2 (6-12 MO)	Nutrilon Phase 3 (12+ MO)	Similac Phase 1 (0-6 MO)	Similac Phase 2 (6-12 MO)	Similac Phase 3 (12+ MO)
Protein (Albomin/Casein 60/40) [mg/ml]	16.50	17.25	18.00	14.55	15.16	16.64	15.58	29.41	31.96
Carbohydrates [gr/100ml]	7.7	7.8	8.4	7.3	8.6	8	7.4	7.5	7.5

10 Table 6: Concentrations of proteins and carbohydrates and fat within the nine-brand product, as disclosed on each of the three brand's websites and packaging, according to the manufacturer's instructions of preparation, using DDI water.

15

	Range Phase 1 (0-6 MO)	Range Phase 2 (6-12 MO)	Range Phase 3 (12+ MO)
Protein (Albomin/Casein 60/40) [mg/ml]	14.55-16.5	15.16-29.41	16.64-31.96
Carbohydrates [gr/100ml]	7.3 - 7.7	7.5 – 8.6	7.5 - 8.4

Table 7: Projected range of values for proteins and carbohydrates and fats in the infant food supplements. Based on information from table 1.

Materials & Equipment:

	1	Materna Dairy Phase 1	
	2	Materna Dairy Phase 2	
	3	Materna Dairy Phase 3	
	4	Similac Top Phase 1	
	5	Similac Top Phase 2	
	6	Similac Top Phase 3	
	7	Nutrilon Phase 1	
	8	Nutrilon Phase 2	
	9	Nutrilon Phase 3	
	10	90 breast milk samples, aliquoted.	
	11	Eppendorf's tubes	
	12	Pipettes (1mL to 5mL, 5µl to 100µl, 100µl to 1000µl)	
	13	Pipette Tips for 1mL to 5mL, 5µl to 100µl and 100µl to 1000µl pipettes	
	14	Elisa reader (Spectrophotometer with a plate reader)	
	15	96 well plates	
	16	10 250ml glass beakers	
	17	10 100ml glass beakers	
	18	Distilled water	
	19	Bovine Serum Abumin (BSA)	
	20	Bradford Dye Reagent Concentrate	
	21	Markers (for marking Eppendorf's Tubes)	
	22	Digital Scales	
	23	Measuring spoon	
	25	BSA (protein Standard)	
	24	Stopper	
	25	Rhenium BCA Protein Assay Kit -> BCA Reagent A BCA Reagent B -> Albumin Standard Ampules, 2mg/mL	
	26	Multichannel pipette	
	27	Thermo Fisher Scientific's Glycoprotein Carbohydrates Estimation Kit Sodium meta-periodate, 500mg Glycoprotein Detection Reagent, 500mg Glycoprotein Assay Buffer, 250mL, contains 0.1% sodium azide Negative Controls: lysozyme, 2.5mg; bovine serum albumin, 2.5mg Positive Controls: ovalbumin, 2.5mg; human apotransferrin, 2.5mg; fetuin, 0.25mg; α1-acid glycoprotein, 0.25mg	
	28	-20°C Storage space	
	29	-80°C Storage space	
	30	Incubator (37°C)	
	31	96 well plate Shaker	

	32	Vortex	
	33	Bucket	
	34	Ice	
	35	Stands for Eppendorf's tubes	
	36	Electric dispenser	
	37	Stirrer	
	38	Magnets	
	39	Breast Milk Samples	
	40	Heat Plate	
	41	Label Printer for Eppendorf's tubes labels	

Table 8: Complete materials and equipment list for all experiments.

Methods:

Part I: Sample Preparation, Dilutions and testing BCA incubation time

Materials & Equipment:

	1	Materna Dairy Phase 1
	2	Materna Dairy Phase 2
	3	Materna Dairy Phase 3
	4	Similac Top Phase 1
	5	Similac Top Phase 2
	6	Similac Top Phase 3
	7	Nutrilon Phase 1
	8	Nutrilon Phase 2
	9	Nutrilon Phase 3
	10	Breast Milk Samples
	11	Eppendorf's tubes
	12	Pipettes (1mL to 5mL, 5µl to 100µl, 100µl to 1000µl)
	13	Pipette Tips for 1mL to 5mL, 5µl to 100µl and 100µl to 1000µl pipettes
	14	Markers (for marking Eppendorf's Tubes)
	15	Digital Scales
	16	Measuring spoon
	17	10 250ml glass beakers
	18	10 100ml glass beakers
	19	3 96 wells plate
	20	Elisa reader (Spectrophotometer with a plate reader)
	21	Incubator (37°C)

Table 9: Materials and Equipment needed for sample preparation and dilutions.

Step 1: Discovering the correct dilutions:

For the Powder Supplements:

The dilutions used: 1:50, 1:100, 1:200, 1:400, 1:800

Tests were done in TRIPLICATES

For Materna® Dairy Phase 2, prepare a batch of the product according to the manufacturer's instructions (Table 5) using DDW water (60 ml). this is the 1:1 batch. Use magnetic stirrer until mixture is homogenous

Formula + Phase	gram	ml
Nutrilon Phase 1	9	60
Nutrilon Phase 2	4.9	30
Nutrilon Phase 3	4.8	30
Materna Phase 1	9	60
Materna Phase 2	9	60
Materna Phase 3	9	60
Similac Phase 1	8.8	60
Similac Phase 2	10.4	60
Similac Phase 3	10.2	60

Table 10: The manufacturer's instructions for the preparation of the formulas, made with DDl water.

Step 2: Create the following dilutions: 1:1, 1:10, 1:100, 1:1000, 1:10000.

For 1ml of the 1:10 dilution, take 100µl of the 1:1 batch, and add 900µl of distilled water. Vortex thoroughly. Do the same to create the next dilution.

See as referenced in Figure 15.

2. Label each sample as such:

Materna Dairy Phase 1	1.A XX
Materna Dairy Phase 2	1.B XX
Materna Dairy Phase 3	1.C XX
Similac Top Stage 1	2.A XX
Similac Top Stage 2	2.B XX
Similac Top Stage 3	2.C XX
Nutrilon Stage 1	3.A XX
Nutrilon Stage 2	3.B XX
Nutrilon Stage 3	3.C XX

Table 11: Sample labeling guidelines, where XX is the dilution number (i.e 1:1, 1:1000).

3. Preform the following assays using all seven dilutions:

Bradford Assay

Carbohydrates Assay

4. To choose the dilutions: the dilutions that fall into the linear part of the concentration graph are those chosen. Five dilutions in total. If needed, repeat step 1 with different dilutions in order to find the best fit for the assays.

For the Human Breastmilk: Make the dilutions on the basis of the dilutions found for the formula. Otherwise, the following dilutions are used: 1:1, 1:10, 1:100, 1:1000, 1:10,000. Tests are done in triplicates

1. The samples "as is" will serve for the 1:1 dilutions.
2. For the 1:100 dilution, take 10µl of the 1:1 sample (the sample "as is") and add 990µl of distilled water. For the 1:1000 dilutions, take 100µl of the 1:100 dilution and ass 900µl of distilled water.
3. Label the human breast milk dilutions by using the code on the sample's Eppendorf's tube and add the dilution (i.e 1:1, 1:100 or 1:1000).
4. Preform the following assays for one sample from each of the six breast milk groups (0-3 months, 3-6 months, 6-9 months, 9-12 months and 12 months and over):

Bradford Assay

Carbohydrates Assay.

5. From the results, determine which dilutions might fall into the linear part of the protein concentration graph. Five or four dilutions in total should be chosen.

Part II: Tests/Assays

The following tests and assays were be performed as follows:

1. Each test is done in triplicates.
2. For each test, there are Blanks and Standards (also in triplicates).
3. For each test there are the number of dilutions set forth in the Quick and Dirty test.
4. Note on Breast Milk:

a. Number of Eppendorf's tubes used: Two Eppendorf's tubes per sample (tubes #4 and #10) were mixed. For some samples, tube #5 was used as well.
b. Number of samples: Each "batch" to be tested will contain one (1) sample per age group, six (6) age groups, total of six (6) samples per "batch". Number of batches tested is determined per day by the discretion of the team leader of the day.
c. One sample from each of the six age groups is tested at a time, so that in total we have six samples being tested together, on the same 96-well plate (at least one "batch" per 96-well plate).
d. To thaw the breast milk: Place Eppendorf's tubes on ice and let thaw. You may use a vortex or the heat from your fingers to quicken the process (only if the process for thawing is repeated exactly the same way for each of the 60 test tubes).

Bradford Assay

The purpose of this assay is to measure the total concentration of proteins in the samples and deduce whether there is a correlation between the changes in total protein concentration and the developmental stage of the infant. It is expected that the protein content of each products will increase as the developmental stages progress. The protein concentration for phase 1 will remain about the same for all three brand products. In phases 2 and 3 the protein concentration in Similac is the highest among the three brand products, while Materna will have the second highest concentration.

*This test is performed twice – once on the powder infant food supplements and once on the breast milk samples.

The projected values and ranges for the total concentration of proteins in each of the samples are:

	Range Phase 1 (0-6 MO)	Range Phase 2 (6-12 MO)	Range Phase 3 (12+ MO)
Protein (Albomin/Casein 60/40) [mg/ml]	14.55-16.5	15.16-29.41	16.64-31.96

Table 12: based on Table 2 **These ranges refer to the 1:1 dilution.

Materials & Equipment:

	1	infant formula samples in their dilutions (9 products x 5 dilutions)
	2	Breast Milk Samples
	3	BSA (protein Standard)
	4	Elisa reader (Spectrophotometer with a plate reader)
	5	7 96-well plates
	6	Eppendorf's tubes
	7	Bradford Dye Reagent Concentrate
	8	Multichannel pipette
	9	Pipettes (1mL to 5mL, 5µl to 100µl, 100µl to 1000µl)
	10	Pipette Tips for 1mL to 5mL, 5µl to 100µl and 100µl to 1000µl pipettes
	11	Dark bottle or 250ml glass beaker and aluminum paper
	12	Stopper
	13	Markers

Table 13: Materials and Equipment needed for Bradford Assay.

Procedure

Type of product	Volume of product	Replications?	Total volume of product needed
Protein Standard	10 µl	3	30 µl
Sample (breastmilk/supplement)	10 µl	3	30 µl
Diluted Reagent (1:5)	200 µl	3	600 µl
Total (in each well)	210 µl		

Table 14: Amount of samples/product needed for this part.

Total number of standards: 5 dilutions in triplicates (5x3=15)

Total number of blanks: 1 blank in triplicates (1x3=3)

Total number of Powder Samples: 9 products in 5 dilutions in triplicates (9x5x3=135)

Total number of Breastmilk Samples: 5 samples from each of the six age groups, in five dilutions, in triplicates (5x6x5x3=450)

1. Prepare dye reagent by diluting 1 part Dye Reagent Concentrate with 4 parts DDI water. This diluted reagent may be used for about 2 weeks when kept at room temperature.
2. Prepare three to five dilutions of a protein standard, which is representative of the protein solution to be tested. The linear range of this microtiter plate assay is 0.05 mg/ml to approximately 0.5 mg/ml. Protein solutions are normally assayed in duplicate or triplicate.
   Dilutions for Standard (BSA): 0.75mg/ml, 0.5mg/ml, 0.4 mg/ml, 0.25 mg/ml, 0.1 mg/ml
   Dilutions for Powder Supplements: 1:20, 1:40, 1:80, 1:100
   Dilutions for Human Breast Milk: 1:20, 1:40, 1:80, 1:100
3. Pipet 10 µl of each standard and sample solution into separate microtiter plate wells.
4. Add 200 µl of diluted dye reagent to each well. Mix the sample and reagent thoroughly (30 seconds, medium speed) using a microplate mixer. Alternatively, use a multi-channel pipet to dispense the reagent. Depress the plunger repeatedly to mix the sample and reagent in the well. Replace with clean tips and add reagent to the next set of wells.
5. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.
6. Mix for 5 seconds using the microplate mixer. Measure absorbance at 595 nm.

BCA Assay

An assay based on the bicinchoninic acid (BCA) for the colorimetric detection of total protein concentration (~562nm). The purpose of this assay is to measure the total concentration of proteins in the samples and deduce whether there is a correlation between the changes in total protein concentration and the developmental stage of the infant. It is expected that the protein content of each products will increase as the developmental stages progress. The protein concentration for phase 1 will remain about the same for all three brand products. In phases 2 and 3 the protein concentration in Similac is the highest among the three brand products, while Materna will have the second highest concentration.

*This test is performed twice – once on the powder infant food supplements and once on the breast milk samples.

The projected values and ranges for the total concentration of proteins in each of the samples are:

	Range Phase 1 (0-6 MO)	Range Phase 2 (6-12 MO)	Range Phase 3 (12+ MO)
Protein (Albomin/Casein 60/40) [mg/ml]	14.55-16.5	15.16-29.41	16.64-31.96

Table 15: based on Table 2 **These ranges refer to the 1:1 dilution.

Materials & Equipment:

	1	infant formula samples in their dilutions (9 products x 5 dilutions)
	2	Breast Milk Samples
	3	Rhenium BCA Protein Assay Kit -> BCA Reagent A -> BCA Reagent B -> Albumin Standard Ampules, 2mg/mL
	4	7 96 well plates
	5	Multichannel pipette
	6	Pipettes (1mL to 5mL, 5µl to 100µl, 100µl to 1000µl)
	7	Pipette Tips for 1mL to 5mL, 5µl to 100µl and 100µl to 1000µl pipettes
	8	Incubator (37°C)
	9	Stopper
	10	Elisa reader (Spectrophotometer with a plate reader)
	11	Eppendorf's tubes
	12	Markers

Table 16: Materials and Equipment needed for BCA assay.

Procedures:

Type of product	Volume of product	Replications?	Total volume of product needed
Protein Standard	25 µl	3	75 µl
Sample (breastmilk/supplement)	25 µl	3	75 µl
Working solution	200 µl	3	600 µl
Total (in each well)	225 µl		

Table 17: Amount of samples/product needed for this part.

Total number of standards: 5 dilutions in triplicates (5x3=15)

Total number of blanks: 1 blank in triplicates (1x3=3)

Total number of Powder Samples: 9 products in 5 dilutions in triplicates (9x5x3=135)

Total number of Breastmilk Samples: 5 samples from each of the six age groups, in five dilutions, in triplicates (5x6x5x3=450)

1. Standard assay procedure
   Dilutions for Standard (BSA): 1mg/ml, 0.75mg/ml, 0.5mg/ml, 0.25mg/ml, 0.1 mg/ml
   Dilutions for Powder Supplements: 1:20, 1:40, 1:80, 1:100
   Dilutions for Human Breast Milk: 1:20, 1:40, 1:80, 1:100
   Prepare working solution

   Mix reagent A (in general the blue bottle in a BCA kit) and reagent B with ratio of A:B=50:1 for enough volume of using.

   Note: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).
2. Pipette 25µL of each standard or unknown sample replicate into a microplate well (working range = 20-2000µg/mL).
3. Add 200µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
4. Cover plate and incubate at 37°C for 30 minutes.
5. Cool plate to RT. Mix for 5 seconds using the microplate mixer. Measure the absorbance at or near 562nm on a plate reader.

Carbohydrates Assay

The purpose of this assay is to measure the total carbohydrates concentration in a liquid sample using Thermo Scientific's Glycoprotein Carbohydrates Estimation Kit and deduce whether there is a correlation between the changes in total Carbohydrates concentration and the developmental stage of the infant. It is estimated that the Carbohydrates concentration will increase for the Materna formulas as the developmental stages progress, while the Similac carbohydrates concentration will remain the same and the Nutrilon concentration will increase between stages 1 and 2 but decrease between stages 2 and 3. Materna will have the highest concentration, and Nutrilon the second highest, for phases 1 and 3, while for phase 2, Nutrilon will have the highest concentration, with Materna coming second.

*This test is performed twice – once on the powder infant food supplements and once on the breast milk samples.

The projected values and ranges for the total concentration of carbohydrates in each of the samples are:

	Range Phase 1 (0-6 MO)	Range Phase 2 (6-12 MO)	Range Phase 3 (12+ MO)
Carbohydrates [gr/100ml]	7.3 - 7.7	7.5 – 8.6	7.5 - 8.4

Table 18: based on Table 2 **These ranges refer to the 1:1 dilution.

Materials & Equipment:

	1	Infant formula samples in their dilutions (9 products x 5 dilutions)
	2	Breast Milk Samples
	3	Thermo Fisher Scientific's Glycoprotein Carbohydrates Estimation Kit Sodium meta-periodate, 500mg Glycoprotein Detection Reagent, 500mg Glycoprotein Assay Buffer, 250mL, contains 0.1% sodium azide Negative Controls: lysozyme, 2.5mg; bovine serum albumin, 2.5mg Positive Controls: ovalbumin, 2.5mg; human apotransferrin, 2.5mg; fetuin, 0.25mg; α1-acid glycoprotein, 0.25mg
	4	7 96 well plates
	5	Multichannel pipette
	6	Pipettes (1mL to 5mL, 5µl to 100µl, 100µl to 1000µl)
	7	Pipette Tips for 1mL to 5mL, 5µl to 100µl and 100µl to 1000µl pipettes
	8	Incubator (37°C)
	9	Stopper
	10	Elisa reader (Spectrophotometer with a plate reader)
	11	Eppendorf's tubes
	12	Markers
	13	Microplate shaker

Table 19: Materials and Equipment needed for carbohydrates assay.

Procedures:

Type of product	Volume of product	Replications?	Total volume of product needed
Standard	50 µl	3	150 µl
Sample (breastmilk/supplement)	50 µl	3	150 µl
Glycoprotein Assay Buffer (for blank)	50 µl	3	150 µl
Sodium meta-periodate solution	25 µl	3	75 µl
Glycoprotein Detection Reagent	150 µl	3	450 µl
Total (in each well)	225 µl		

Table 20: Amount of samples/product needed for this part.

Total number of standards: six standards in triplicates (6x3=18)

Total number of blanks: 1 blank in triplicates (1x3=3)

Total number of Powder Samples: 9 products in 2 dilutions in triplicates (9x2x3=54)

Total number of Breastmilk Samples: 5 samples from each of the six age groups, in 2 dilutions, in triplicates (5x6x2x3=180)

Based on Thermo Fisher Scientific's Glycoprotein Carbohydrates Estimation Kit instructions manual.

Material Preparation

Note: Equilibrate the Glycoprotein Carbohydrate Estimation Kit components to room temperature before use.

1. Sodium meta-periodate Solution: Immediately before use, prepare 10mM sodium meta-periodate by dissolving 21.4mg of sodium meta-periodate in 10mL of Glycoprotein Assay Buffer.
2. Glycoprotein Detection Reagent: Immediately before use, prepare 0.5% Glycoprotein Detection Reagent by dissolving 50mg of the reagent in 10mL of 1N NaOH.
3. Glycoprotein sample: Dissolve sample in Glycoprotein Assay Buffer at 0.25 and 2.5mg/mL. If the sample is already in solution, dilute sample in Glycoprotein Assay Buffer at 0.25 and 2.5mg/mL.
4. Protein standards During shipment, lyophilized proteins have may come in contact with the septa. Before opening, verify that protein is settled to the bottom of each vial. If necessary, gently tap the vial sides to settle protein. Carefully remove septa to avoid disturbing any protein that may have settled on its underside. Add 1mL of Glycoprotein

Assay Buffer to each protein standard vial. Replace septa and gently rock vial so that the buffer contacts all inside surfaces. Store reconstituted standard solutions for up to one month at 4°C.

*After Protein Standards are reconstituted, aliquote each as such: 170µl of Standard into each marked Eppendorf's Tubes (leftovers in a final Eppendorf's tube, marked with an approximation of the liquid's volume)

Procedure

1. Place 50µL of each standard and the sample in the plate wells. For the blank, use 50µL of Glycoprotein Assay Buffer. Test each sample and standard in triplicate.
   Standards: Come with the kit, use as is.
   Dilutions for Powder Supplements: As dictated by the protocol: one sample dilution with 2.5mg protein per ml and a second sample dilution with 0.25mg protein per ml.
   Dilutions for Human Breast Milk: As dictated by the protocol: one sample dilution with 2.5mg protein per ml and a second sample dilution with 0.25mg protein per ml.
2. Add 25µL of the sodium meta-periodate solution to each well.
3. Mix plate for 30 seconds in a microplate shaker.
4. Cover and incubate plate at room temperature for 10 minutes.
5. Add 150µL of the Glycoprotein Detection Reagent to each well.
6. Mix plate for 30 seconds in a microplate shaker.
7. Cover and incubate plate at room temperature for 1 hour.
8. Mix plate for 5 seconds in a microplate shaker. Measure absorbance at 550nm in a microplate reader and plot a standard curve.

Results:

We noticed that the results of the Bradford test give protein values that are generally lower than the manufacturer's values, whereas the results of the BCA test give values that are higher than the manufacturer's (see figure 16).

In Both the Bradford and the BCA tests, a unique trend is observed: the highest protein concentration is in age group 1 (BCA: 18.85±1.63 mg/ml, Bradford: 11.88±0.29 mg/ml), with a decline until stage 4 (BCA: 11.57±0.74 mg/ml, Bradford: 8.00±1.03 mg/ml, then again incline until stage 6 (BCA: 18.65±1.42 mg/ml, Bradford: 11.24±1.02 mg/ml) (See Figure 17). However, it is not clear whether the highest concentration is at stage 1 or 6, as well as whether the lowest

concentration is at stage 2, 3, 4 or 5. with some mothers this trend was not clear. Similar to the tests of the formulas, the Bradford assay always showed lower concentrations than the BCA assay.

Discussion:

Proteins

It is clear from Figure 16 that both BCA and Bradford assays don't give identical results to the manufacturer's values, nor identical to one another. In addition, there is no single mathematical factor that can be calculated for the conversion the assays' values to the manufacturer's, not to mention that two results showed higher values for the Bradford assay compared to the manufacturer's, in contrast to the general opposite behavior. Nevertheless, with further repeats and calibration such factor may be found, since both methods are credible for protein quantification. It's surprising that the Bradford method was allegedly inaccurate, since the literature claims that Bradford method is the most accurate for total protein quantification in milk, regardless of the presence of lipids. In order to better determine the true accuracy of this method, we can use standards of Albumin and Casein. It is here to mention that the milk samples were frozen at -80°C and then defrosted two times, a fact that may have an impact on the protein concentration in each test.

In figure 17 it can be observed that the hypothesis regarding the protein concentration was partially approved: there is a decline of the protein concentration as the baby grows older. The colostrum and the transitional milk are known to have the highest concentration of proteins, for they include a concentrated dose of immune factors that are necessary for the baby's immune system. However, there is an increase in the protein concentration in the fifth and sixth age groups. It is reported in the literature that while there is a decrease in the protein concentration from 4-6 months to 12-20 months in milk of full-lactating women (Volume > 500 ml/day), when the volume of milk is unknown (only known that >= 4 feedings/day) there is an increase in the protein concentration. It is also reported that protein concentration increases as volume of lactated milk decreases. Older babies start to wean from breast milk and feed on other food, a fact that can explain the above results.

As part of a second experiment taking place at the Technion Institute's Protein Lab, a set of five breast milk samples from each age group were tested for protein concentration using the Bradford method. Some of these samples were from the same mother of the samples in this experiment, and some were from different mothers. It is possible to observe in Figure 18 the same protein concentration trend as in Figure 17. The values obtained by the Technion group were lower by comparison than those obtained by our results (referred to herein as "MAO") see Figure 19), but the decrease in protein concentration observed by the MAO team from age group 1 to age group 4, and subsequent increase in protein concentration in the latter age groups, can also be seen in the Technion's findings. This similar trend can be observed even if the lactation age is divided by the usual commercial division of phases – phase 1 is for infants aged 0-6 months, phase 2, for infants

aged 6-12 months and phase 3 for infants over 12 months old. In Figure 21, a similar decrease between the first months of lactation up to the twelfth month of lactation can be observed, although the definition for infants aged 9 and above is lost, therefore the increase in protein is only observed at phase 3 and not at group 5 as before.

Although between each age group there is a noticeable difference in the group's average protein concentration, within each group individually there is no significant correlation between the age of the infant and the protein concentration of the breastmilk sample (figure 20). This means that there is variance in the protein concentration between mothers, which can also be observed in the standard errors of the results. This could be a result of various factors: mother's nutrition, mother's age and history of pregnancy, physical and health status of the mother or the infant, time in the day of the collection of the samples, etc.

Carbohydrates

The test used to determine the glycoprotein carbohydrates concentration within each breast milk and formula sample was the Thermo Scientific Glycoprotein Carbohydrate Estimation Kit, which depends on a set protein concentration for the creation of a standard curve. The two protein concentrations required by the kit were 0.25mg of protein per 1ml of liquid or 2.5mg of protein per 1 ml of liquid. However both the formulas and the breastmilk samples used in this experiment had a higher glycoprotein concentration than the kit could detect. At 2.5mg protein per ml, the samples were overexposed and at 0.25mg protein per ml, the OD results were far outside the range of the standard curve.

Although the numbers cannot be claimed as accurate, there is no observable trend in Figures 22 and 23 among the three infant food formulas. Each formula follows its own pattern of increase and decrease in carbohydrates percentage and glycoprotein concentration, and that trend is for the most part consistent between the results from the 2.5mg protein per ml and the 0.25mg protein per ml samples (with the exception of Materna, where a steady decrease seen in Figure 22 cannot be observed in Figure 23). On the formula's labels, Materna boasted of a steady increase in carbohydrates content from phase 1 to phase 2 and finally to phase 3, while Similac showed a more steady concentration through the three phases and Nutrilon showed an increase between phases 1 and 2 and a decrease between phases 2 and 3. Only Nutrilon's results using this kit somewhat matched the trend displayed on the products' labels. Since this kit tests for glycoprotein carbohydrates percentage, and the products' label do not make a distinction between carbohydrates that are found in glycoproteins and free carbohydrates, it was not expected that the results would match.

For the breastmilk samples, an interesting trend can be seen in Figure 24. Although these results were obtained using samples that were overexposed, a similar trend as that of the protein concentrations can be observed in the different age groups' glycoprotein carbohydrates percentage. Age group 1 shows the highest percentage of carbohydrates, with a steady decrease until age group

4. Age group 5 shows an increase in the carbohydrates percentage, which carries on to age group 6. These results were expected, since the kit used in this experiment ultimately tests for glycoproteins, which are part of the total protein concentration estimated using BCA and Bradford. The results further suggest that there is a higher concentration of glycoproteins in the first month of lactation (group 1) than after a year of lactation (group 6), even though Figure 17 shows that the total protein concentrations for these two groups is similar. Figure 25 does not corroborate the trend found in Figure 24.

According to some demonstrative embodiments, the device of the present invention may allow for the analysis and/or monitoring of one or more components in the breastmilk, and optionally provide an indication regarding the nutritional and/or immunological needs of a breast fed infant which is at least partially fed by the breastmilk.

According to some embodiments, the components in the breastmilk may include one or more of the candidates appearing in the tables depicted in figures 26 and 27.

According to some embodiments, the device may be pre-configured with normal ranges, e.g., concentrations, for each of the components, and optionally the device may also be pre-configured with non-normal ranges od the components, e.g., indicating and infection.

According to some embodiments, upon analysis of one or more components the device may indicate whether the one or more components are within or outside the normal range, and optionally provide a recommendation for the user of the device.

Example 12

For concentration results, which vary over time, the best testing method is using a legend with a color gradient. The stick will not give a numerical result which can be directly compared to that expected concentration, but rather a hue which directly correlates to the amount of protein found in the sample (the stronger the hue, the more protein in the sample). The user can compare the clue stain which will appear on the stick directly with the following legend, and extrapolate the results of the test from there.

Age	0 to 1 Months	1 to 3 Months	3 to 12 Months	12 Months Over
Protein Concentration (mg/ml)	14.50 ± 0.2	11.1 ± 0.05	9.15 ± 0.1	11.85 ± 0.2

Table 21: Total Protein Concentration using a colorimetric result

1. The user will dip the Total Proteins Concentration stick (sampling element) in the breastmilk to the marked line
2. The user will wait the indicated amount of time for the reaction to occur, preferably less than 10 minutes, more preferably, less than 5 minutes.
3. The user will compare the reaction to the legend.
4. The user will read the instructions on the legend: if the color on the stick matches or is above the total protein concentration for the infant's age group, the user does not have to make any changes, e.g., because the protein concentration in the breastmilk corresponds or is higher than the desired concentration for the infant in accordance with its age. If the color on the stick matches a protein concentration which is lower than the infant's age group, it will be recommended for the mother (whose breastmilk was tested) to increase her protein intake throughout the day.

For example, for a mother of a 2.5months old infant, the total protein concentration average is expected to be around 11.1 ± 0.05 (mg/ml). If the total protein concentration in the tested milk is 9.00mg/ml, the colorimetric result on the stick will match a concentration outside the range of the infant's age group. In such a case, the mother is recommended to increase her protein intake through her daily nutrition or through supplements.

Reference is made to Figure 28 which shows an exemplary legend in accordance with some demonstrative embodiments, according to which a user of a sampling element of the present invention may get an indication regarding the levels of protein concentrations in the breastmilk.

According to some embodiments, a user of the sampling element will compare the results indicated upon the sampling element to the legend's colors, and may verify a correspondence with the expected protein concentration.

Fig. 28 shows the legend's colors which correspond to the protein concentration according to the expected age of the infant. If the tested sampling element shows colors which are brighter than those of the expected age of the infant, this may mean the mother's breastmilk contains protein concentration which is lower than the expected and/or desired for her infant at that specific age.

The expected protein concentrations represented by the legend shown in Fig. 28 are visual representations of the information of table 21 and are based on known data derived from the literature.

Example 13

	Formula 1 (Materna)			Formula 2 (Nutrilon)			Formula 3 (Similac)		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Protein mg/ml	14.6	15.8	16.6	13	14	15	14	28	28

Table 22: Total Protein Concentration in different formulas

As shown in table 22, three different infant formulas were examined for their protein concentration at each phase.

Age by Phases	0-6 Months (Phase 1)	6-12 Months (Phase 2)	12 Months Over (Phase 3)
Protein Concentration (mg/ml)	11.4877 ± 0.1	9.3013 ± 0.1	11.8353.2

Table 23: Average Total Protein Concentration according to the age of an infant

1. The user will dip the sampling element in the breastmilk up to a predetermined marked line
2. The user will wait the indicated amount of time for the reaction to occur, preferably less than 10 minutes, more preferably, less than 5 minutes.
3. The user will compare the results appearing on the sampling element to the legend's indicators
4. The user will read the instructions on the legend: The color on the sampling element will tell the user which baby supplement phase best suits the infant, according to the total protein concentration in the milk.

Reference is made for example to figure 29 which demonstrates an exemplary legend 2900, to which a sampling element result may be compared to. According to figure 29, if the sampling element shows a color which corresponds to the colors 2904, this may indicate that the infant should be consuming a formula phase 2, which corresponds to the protein concentration in the breastmilk of his mother. However, if the sampling element shows a color which corresponds to the colors 2902, this may indicate that the infant should be consuming a formula phase 1 (if the infant is under the age of 6 months), or formula phase 3 (if the infant is over the age of 6 months).

Example 14

Vitamins B:	Days 6-10	Days 11-20	Days 21-89	Days 90-180	Days 181-365
Vitamin B1 (µg/100ml)	6.6±3.7	7.6±4.8	12.0±2.2	13.2±2.4	13.4±2.5
Vitamin B2 (µg/100ml)	37.7±15.6	34.0±9.7	38.0±12.6	39.7±12.6	38.5±13.3
Vitamin B2 (FAD) (µg/100ml)	74.7±29.2	67.1 ± 19.9	68.0 ± 19.8	69.3 ± 24.7	66.8 ± 22.9
Vitamin B6 (µg/100ml)	1.9±1.0	5.5 ± 3.8	4.6 ± 2.1	7.3 ± 2.3	6.4 ± 1.8
Vitamin B12 (µg/100ml)	0.07±0.05	0.06±0.02	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02

Table 24: Average Vitamin B Concentration in breastmilk according to the age of a breastfed infant

*The Vitamin B (B1, B2, B6 or B12) in question will be referred to as Vitamin Bx in this example

1. The user will dip the Vitamin Bx Concentration stick (sampling element) in the breastmilk up to a predetermined marked line
2. The user will wait the indicated amount of time for the reaction to occur, preferably less than 10 minutes, more preferably, less than 5 minutes.
3. The user will compare the reaction to the legend
4. The user will read the instructions on the legend: if the color on the stick matches or is above the concentration of the Vitamin Bx for the infant's age group, the user does not have to make any changes. If the color on the stick matches a Vitamin Bx concentration which is lower than the infant's age group, it will be recommended for the mother (whose breastmilk was tested) to increase her Vitamin Bx intake throughout the day

According to some demonstrative embodiments, the invention may include a pack of Vitamin B sampling elements which may include test sticks, a legend and a list of foods recommended to increase the Vitamin Bx levels in the breastmilk

Reference is made to figure 30, which illustrates an exemplary legend of desired concentration of Vitamin B1 in the breastmilk of a mother feeding an infant in correlation to the age of the infant.

According to some embodiments, and as referred to in Figure 30, if the Vitamin B1 concentration in the breastmilk for an infant at the age of 2 months is about 8µg/100ml, the color indicator on

the sampling element will not match the respective color on the legend for infants between 1-3 months of age, which will tell the user that the vitamin B1 concentration in the tested breastmilk is lower than the average, e.g., the desired concentration for an infant aged 2 months, and the mother will be recommended to ingest more Vitamin B1 in her daily nutritional intake.

Reference is made to figure 31, which illustrates an exemplary legend of desired concentration of Vitamin B12 in the breastmilk of a mother feeding an infant in correlation to the age of the infant.

According to some embodiments, and as referred to in Figure 31, if the Vitamin B12 concentration in the breastmilk for an infant at the age of 2 months is about 0.03µg/100ml, the color indicator on the sampling element will not match the respective color on the legend for infants between 1-3 months of age, which will tell the user that the vitamin B12 concentration in the tested breastmilk is lower than the average, e.g., the desired concentration for an infant aged 2 months, and the breastfeeding mother will be recommended to ingest more Vitamin B12 in her daily nutritional intake.

Example 15

Long Chain- Polyunsaturated Fatty Acids (LC-PUFA): Using a panel and smartphone application to decipher the results of the sampling element

This sampling element will test two LC-PUFA molecules, Omega 3 and Omega 6, wherein the recommended ratio of Omega 3 to Omega 6 should be 1:4 at most, with the optimal ratio being 1:1

1. The user will dip the LC-PUFA stick (sampling element) in the breastmilk up to a predetermined marked line
2. The user will wait the indicated amount of time for the reaction to occur, preferably less than 10 minutes, more preferably, less than 5 minutes
3. The user will place the stick on the accompanying color panel at the indicated location
4. The user will use her smartphone, open a dedicated app and scan the color panel with the stick using the application.
5. The application will calculate the ratio of Omega 3 to Omega 6 and tell the user the results and recommendations. If the ratio is above a 1:4 ratio, the app will recommend the mother

whose breastmilk was tested to increase the omega 3 intake in her diet by, for example, consuming more fatty fish.

Figure 32 is an illustration of a sampling element for LC-PUFA with a predetermined marked line, to indicate to the user of the sampling element the depth up to which the user should dip the element into the tested breastmilk.

Figure 33 is an illustration of an exemplary control panel 3300, wherein sampling element 3306 is inserted into panel 3300, and the results indicated upon element 3306 can be compared to the reference results indicated upon panel 3300.

According to some embodiments, sampling element 3306 may be dipped into or contacted by a suitable amount of breast milk, e.g., 5 ml, and a reaction may occur to indicate the concentration of Omega 3 and/or Omega 6 in the breastmilk. According to some embodiments, this indication of the results may be presented by indicator 3310 for Omega 3 and indicator 3308 for Omega 6.

According to some embodiments, panel 3300 may have reference indicators, e.g, indicators 3314 for reference of various concentrations of Omega 3, and indicators 3312 for reference of various concentrations of Omega 6.

According to some embodiments, after insertion of sampling element 3306 into panel 3300 the result indicators 3310 and 3308 may be compared to reference indicators 3314 and 3312, respectively.

According to some embodiments, this comparison may be done by a user using naked eye or alternatively, using any suitable method that may allow an easy comparison of the result indicators 3310 and 3308 to reference indicators 3314 and 3312, including, for example, a smart phone application which may enable a user to take a picture of the sampling element 3306 within panel 3300 and provide an immediate feedback regarding the concentration of Omega 3 and/or Omega 6 in the tested breastmilk.

Example 16

		Colostrum	Transitional milk	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactation
Leukocytes (CD 45) per ml milk	Healthy	32,175–784,080	0–3,450	160–1,151	0–1,025	0–1,063	707–853	0–288	0–13,750
	Infection	—	34,467	2,400–2,594,982	2,164–109,130	1,065–472,634	> 30,000	1,293–759,834	3,127–49,817

Table 25: Average Leukocytes count in a healthy or infected breastmilk

According to this example, there is provided a sampling element for detecting the presence and/or amount of Leukocytes (CD 45) in the tested breastmilk.

According to this example the sampling element may include a control indicator and a positive or negative indicator (also referred to as Yes/No indicator), however, it is to be understood that the sampling element may also have a range indicator, in addition or instead of the positive or negative indicator.

1. The user will dip the sampling element in the breastmilk up to a predetermined marked line
2. The user will wait the indicated amount of time for the reaction to occur, preferably less than 10 minutes, more preferably, less than 5 minutes
3. The user will interpret the results based on the guide on the sticks pack
4. A control mark must always appear on the stick. If the control mark does not appear, the reaction did not occur properly.

If the infant or mother is developing an infection, CD-45 levels in the tested breastmilk would be elevated. For example, for an infant 2 months old that is beginning to develop an infection, CD-45 levels would be over 4000 particles per ml of milk. In such a case, the sampling element would have a positive control indicator, indicating that the sampling element is working properly, and a positive indication in the Yes/No indicator.

Example 17

According to this example, there is provided a sampling element (Lactoferrin stick) for detecting the presence and/or amount of Lactoferrin in the tested breastmilk.

According to this example the sampling element may include a control indicator and a positive or negative indicator (also referred to as Yes/No indicator), however, it is to be understood that the sampling element may also have a range indicator, in addition or instead of the positive or negative indicator.

		Colostrum	Transitional milk	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year +
Lactoferrin (g/ l)	Healthy	6.3–7.7	2.1–5.2	2.5–2.9	1.9–3.7	1.3–4.0	1.2–3.9	2.3–4.5
	Infection	—	4.3	2.9–3.7	2.0–3.7	1.6–3.3	1.2–3.6	2.1–4.6

Table 25: Average Lactoferrin concentration in a healthy or infected breastmilk

1. The user will dip the Lactoferrin stick in the breastmilk to the marked line
2. The user will wait the indicated amount of time for the reaction to occur
3. The user will interpret the results based on the guide on the sticks pack

If the infant or mother is developing an infection, Lactoferrin levels in the tested breastmilk should be elevated. For example, for an infant 2 months that is beginning to develop an infection Lactoferrin levels should be over 3g/L. In such a case, the sampling element would have a positive control indicator, indicating that the sampling element is working properly, and a positive indication in the Yes/No indicator.

Example 18

		Colostrum	Transitional milk	Months 1–3	Months 4–6	Months 7–9	Months 10–12	1 Year +
sIgA (µg/ml)	Healthy	1428–2178	131–1096	534–1276	257–960	496–1350	401–1044	137–1243
	Infection	—	922	36–1418	652–1711	611–1509	714–789	173–2002

Table 27: Average sIgA concentration in a healthy or infected breastmilk

According to this example, there is provided a sampling element for detecting the presence and/or amount of Immunoglobulins, e.g., sIgA in the tested breastmilk.

According to this example the sampling element may include a control indicator and a positive or negative indicator (also referred to as Yes/No indicator), however, it is to be understood that the sampling element may also have a range indicator, in addition or instead of the positive or negative indicator, and a control indicator to indicate whether the element is working properly.

1. The user will dip the sampling element in the breastmilk to the marked line
2. The user will wait the indicated amount of time for the reaction to occur
3. The user will interpret the results based on the guide on the sticks pack
4. A control mark must always appear on the stick. If the control mark does not appear, the reaction did not occur properly.

If the infant or mother is developing an infection, sIgA levels in the tested breastmilk would be elevated. For example, for an infant 2 months that is beginning to develop an infection sIgA levels should be over 1300µg/ml. In such a case, the sampling element would have a positive control indicator, indicating that the sampling element is working properly, and a positive indication in the Yes/No indicator.

According to some embodiments, there is provided a sampling element, also referred to herein as an immunological stick, which may contain more than one immunological test. According to these embodiments, the immunological stick may include a test for Leukocytes, Lactoferrin and an Antibody test.

According to these embodiments, the immunological stick may provide a more comprehensive indication about the infant's and/or mother's health condition, in comparison to a single test.

While this invention has been described in terms of some specific examples, many modifications and variations are possible. It is therefore understood that within the scope of the appended claims, the invention may be realized otherwise than as specifically described.

Claims

1. A sampling element for the sampling and analysis of breastmilk, wherein said element comprises:

   a plurality of fibers to allow the flow of said breastmilk through said sampling element;

   a result indicator to indicate the result of said analysis; and

   wherein said sampling element provides a nutritional or immunological analysis of said breastmilk.

2. The sampling element of claim 1, wherein said sampling element comprises at least three separate zones, a first zone, a second zone and a third zone for analyzing the breastmilk, wherein said breastmilk flows through said at least three zones.

3. The sampling element of claim 1, wherein said at least three separate zones comprise:

   zone 1 comprises antibodies that bind to specific components within said breastmilk, and wherein said antibodies are conjugated to an enzyme that induces a color change;

   zone 2 comprises particles that induce the color release from conjugated enzymes and antibodies bound to specific components;

   zone 3 comprises particles that induce the color release from conjugated enzymes and antibodies bound to specific components that did not induce a color release in zone 2.

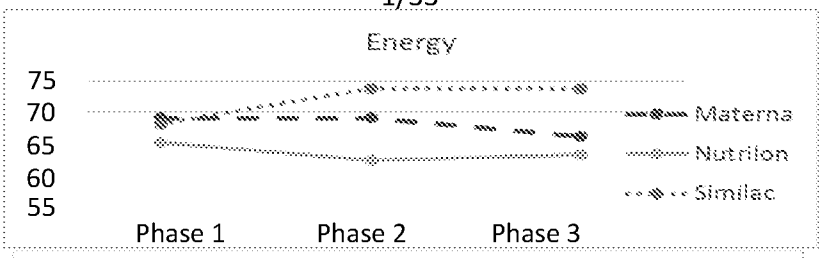
4. The sampling element of claim 1, wherein said nutritional analysis comprises a determination of the amount or concentration of macromolecules or nutrients in said breastmilk.

5. The sampling element of claim 1, wherein said macromolecules or nutrients are selected from the group including: Vitamin B, Human Milk Oligosaccharides (HMO), long chain polyunsaturated fatty acids (LCPUFA) and Total Proteins.

6. The sampling element of claim 1, wherein said immunological analysis comprises determination of the amount or concentration of at least one immunological factor in said breastmilk selected from the group including: Lactoferrin, Leukocytes and Immunoglobulins.

7. The sampling element of claim 1, wherein said at least one immunological factor is Lymphocyte Common Antigen (CD45)
8. The sampling element of claim 1, wherein said at least one immunological factor is sIgA.
9. The sampling element of claim 1, selected from the group including a test strip, a test stick, a dipstick or a vial.
10. The sampling element of claim 1, wherein said sampling element is disposable.
11. The sampling element of claim 1, wherein said sampling element is adapted to be inserted into a device for the analysis of said breastmilk.
12. A system for determining the nutritional needs of an infant, comprising:
the sampling element of claim 1 to collect and analyze a sample of breastmilk from a mother of said infant and provide results of said analysis;
an application to read said results and provide a recommendation for a specific infant formula to be fed to said infant based on said results; and
wherein said sampling element analyzes at least one parameter selected from the group including whole protein concentration, separate peptides, a combination of peptides, oligosaccharides concentration, pH measurement, fat concentration, number of cells, Properdin, Vitamin A, Phosphorus and Iron.
13. The system of claim 1, wherein said application is installed upon a mobile device.
14. Use of the sampling element of claim 1, for determining the amount or concentration of one or more macromolecules or nutrients in said breastmilk and suggesting an optimal nutritional supplemental formula to said infant based on the analysis of said breastmilk.
15. Use of the sampling element of claim 1, for determining the amount or concentration of one or more immunological factor for alerting the development of an infectious disease in said infant or mother.

1A



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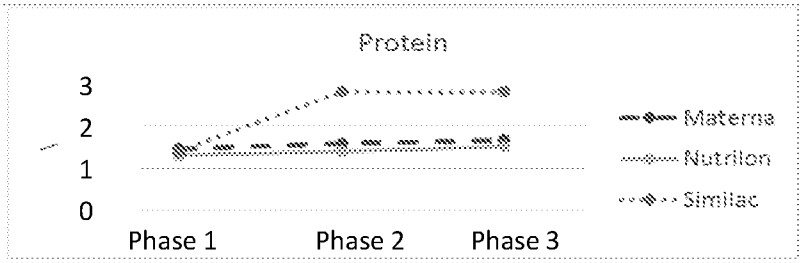

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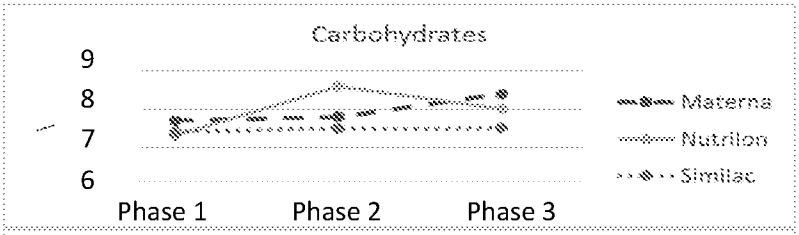

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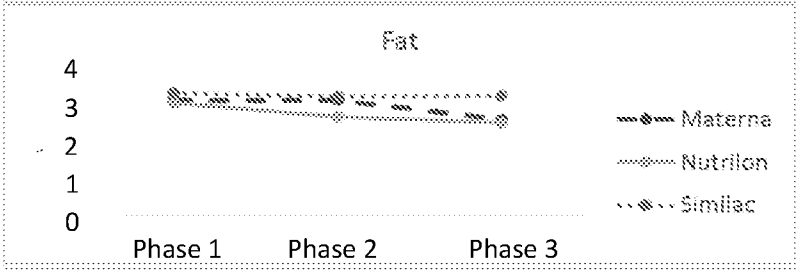

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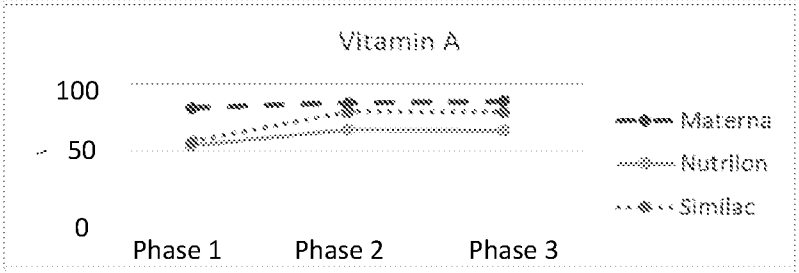

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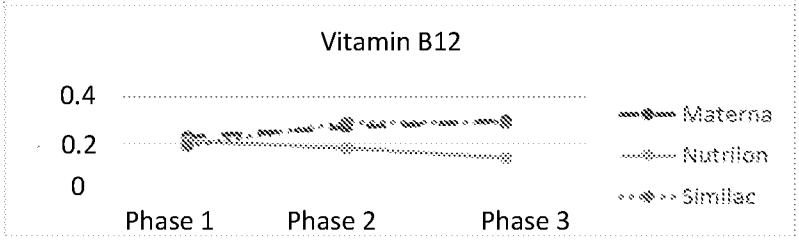
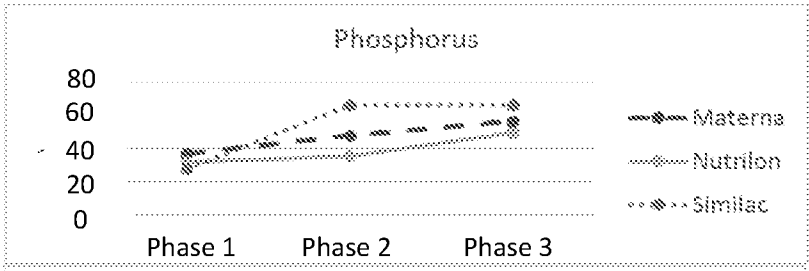


1G



1H

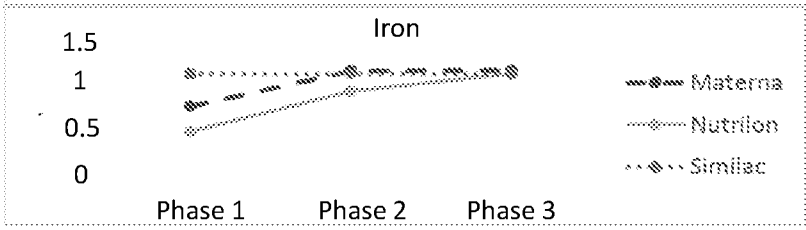


Fig. 1

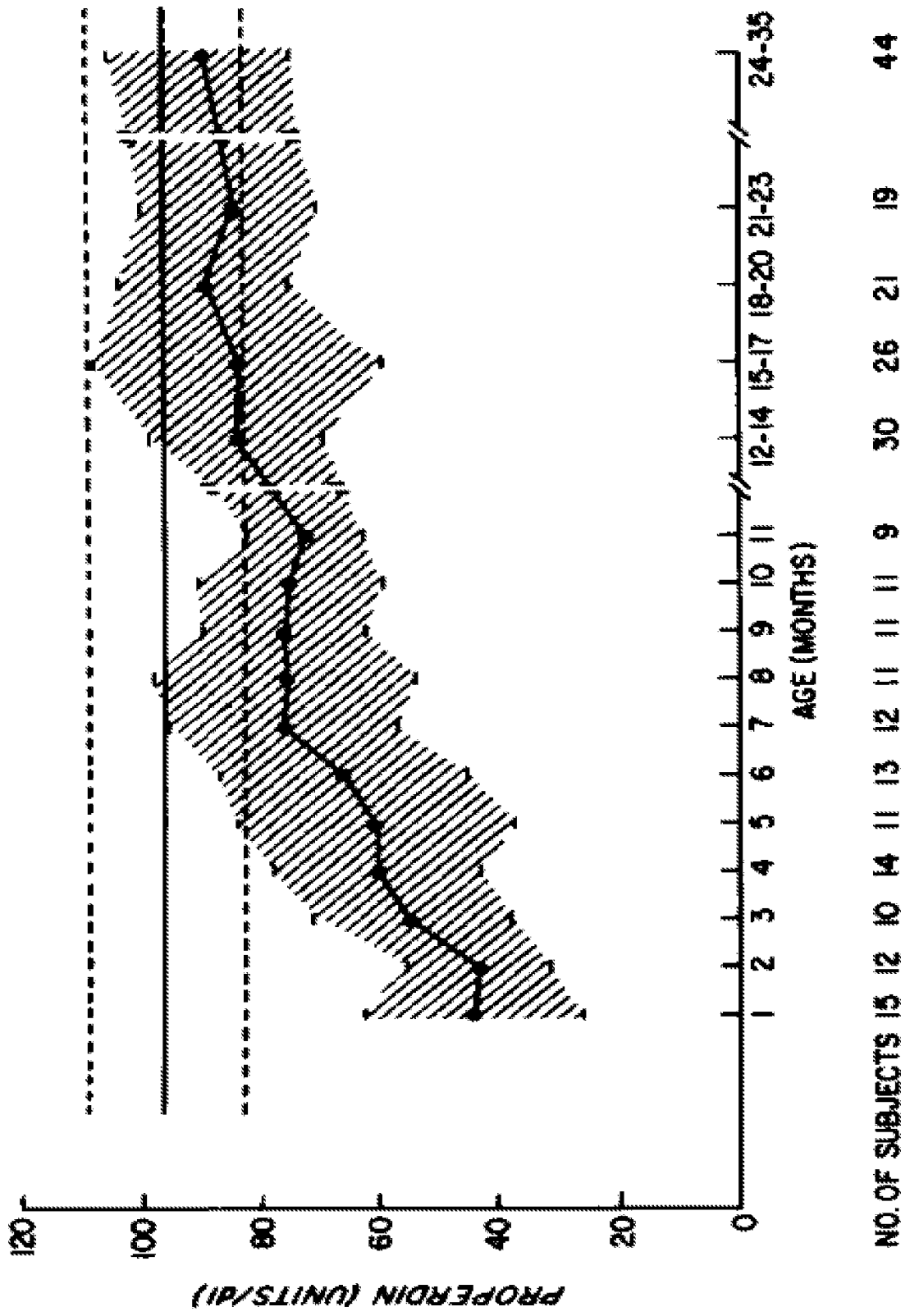

PROPERDIN (UNITS/ml)
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12-14 15-17 18-20 21-23 24-35
AGE (MONTHS)
NO. OF SUBJECTS 15 12 10 14 11 13 12 11 11 11 9 30 26 21 19 44

Fig. 2

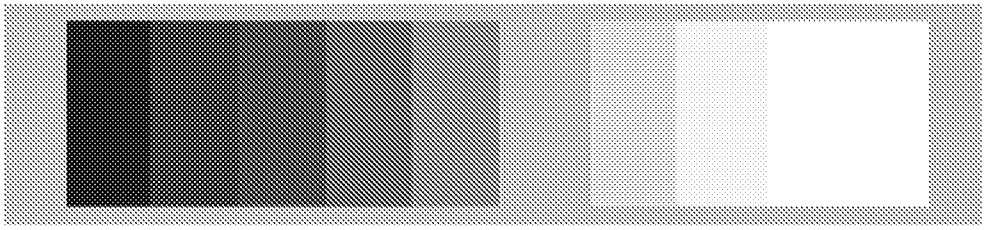


Fig. 3

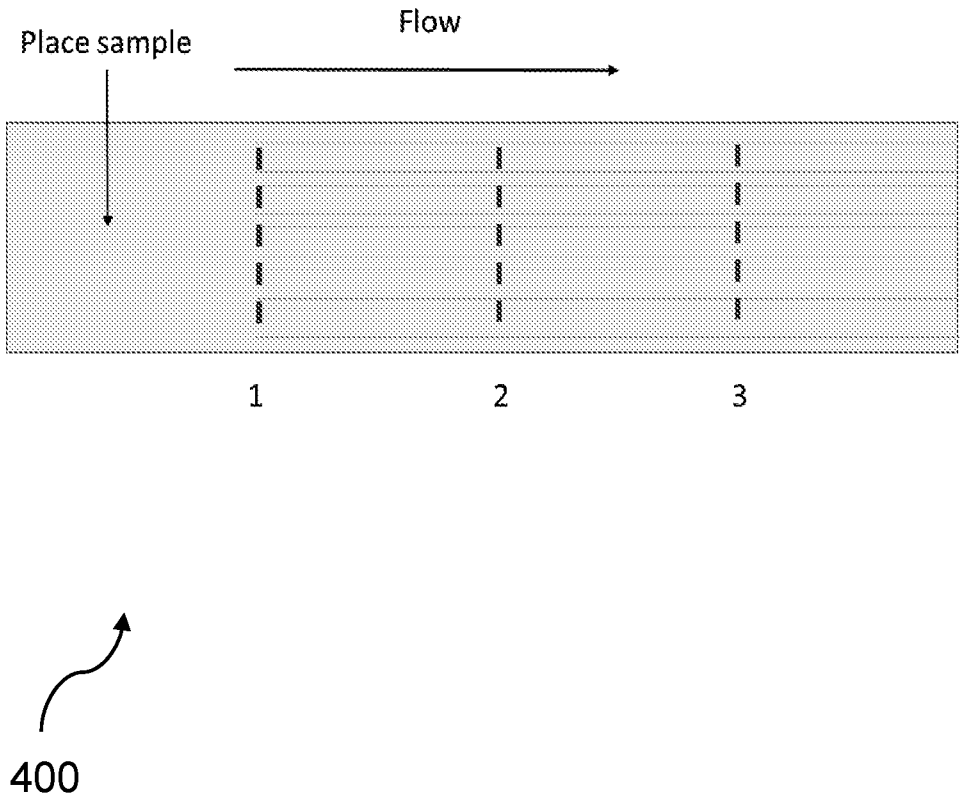


Fig. 4

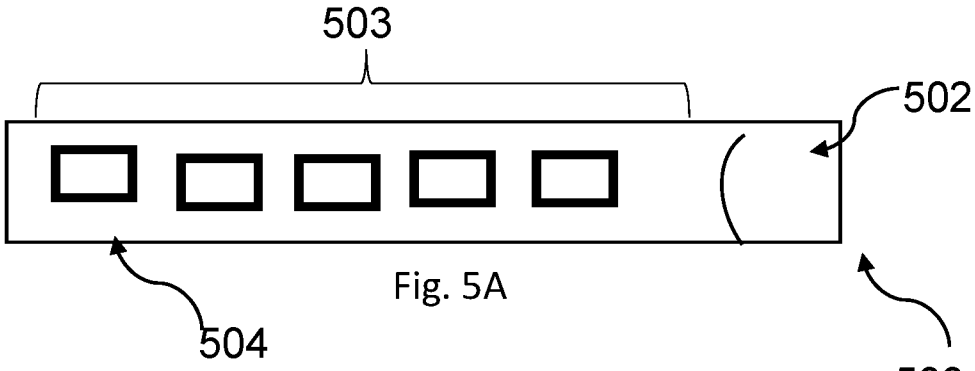


Fig. 5A

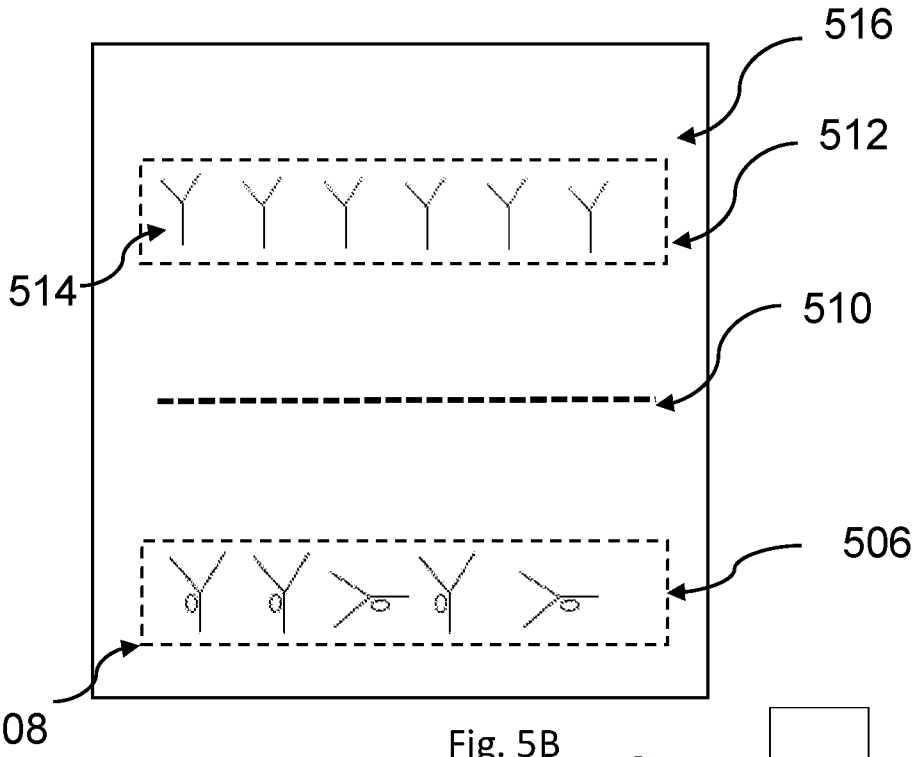


Fig. 5B

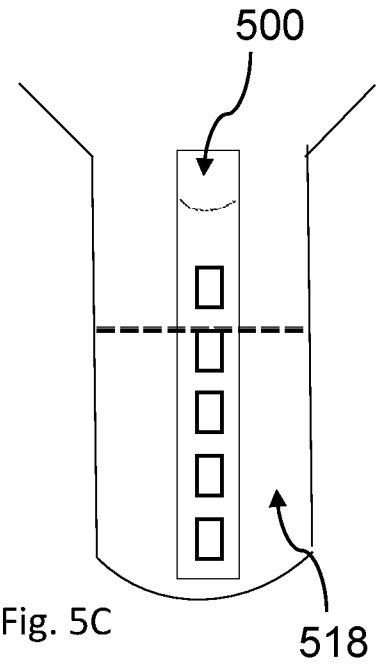


Fig. 5C

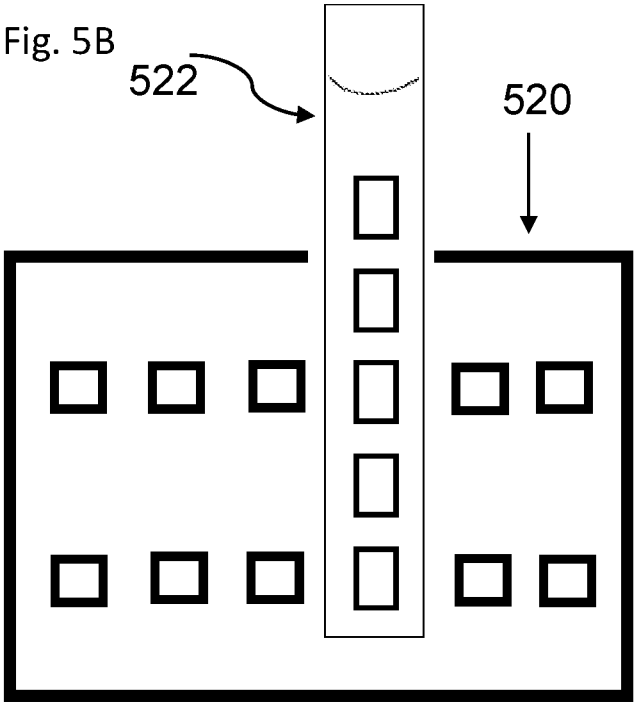


Fig. 5D

Fig. 5

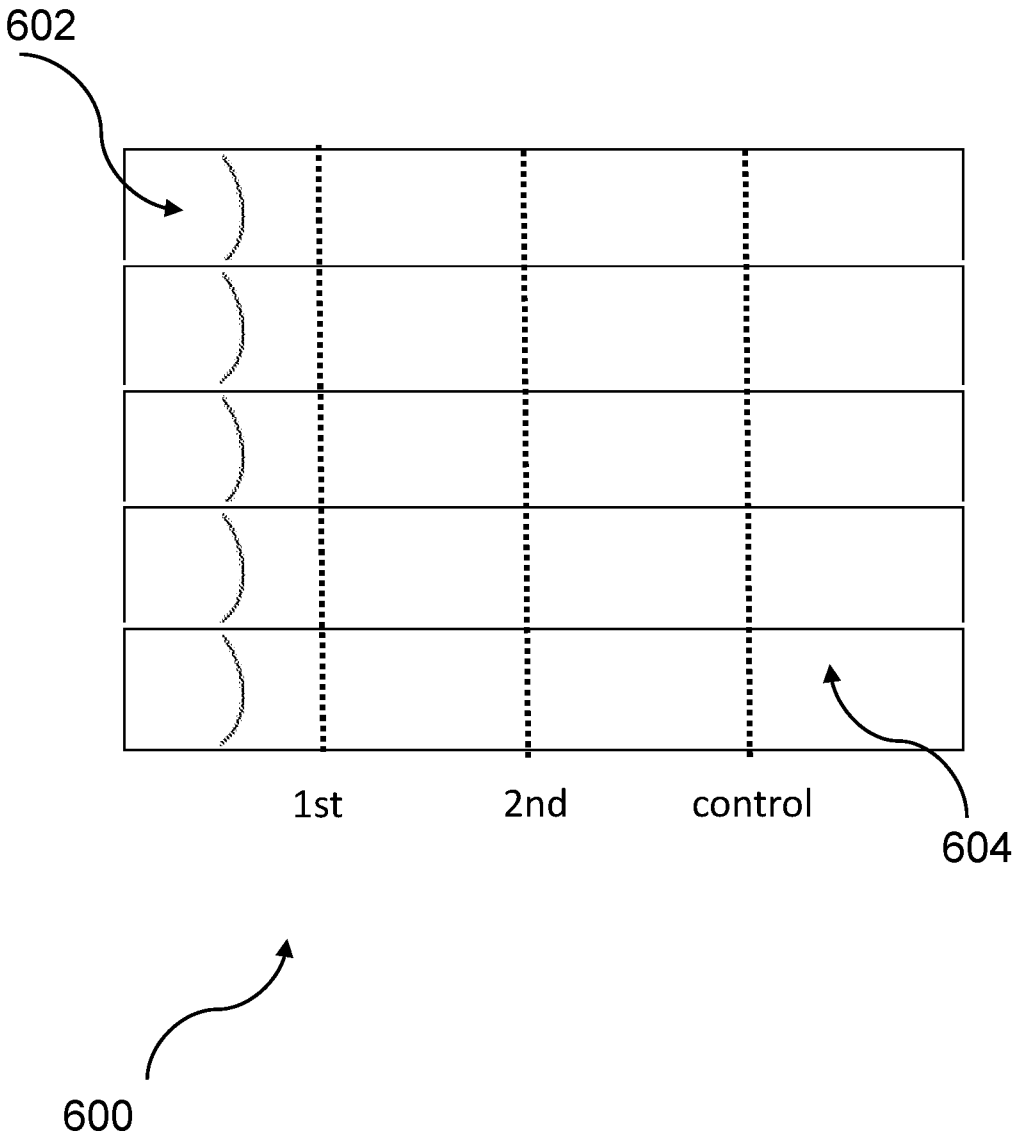


Fig. 6

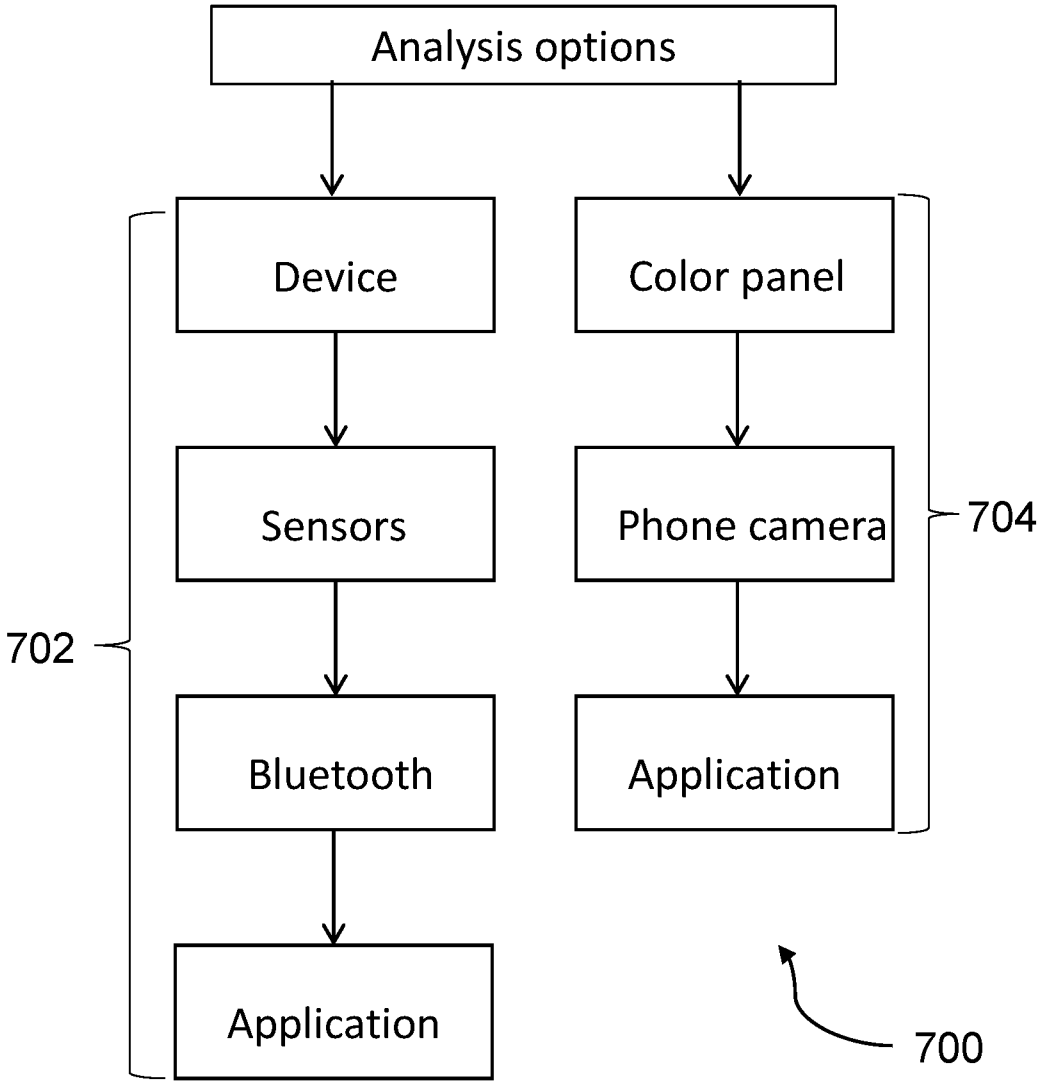

Analysis options
Device
Color panel
Sensors
Phone camera
Bluetooth
Application
Application
702
704
700

Fig. 7

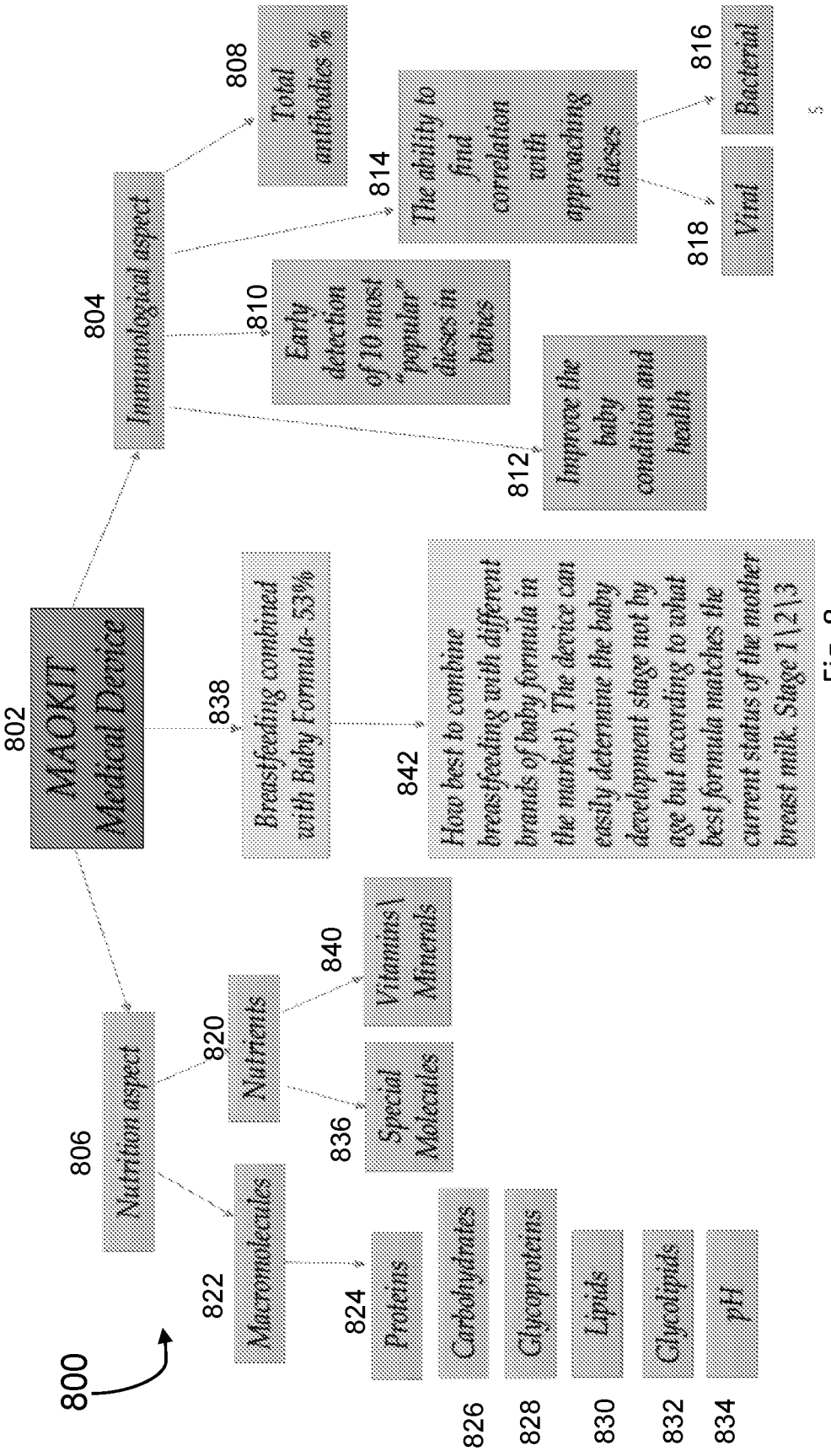

800
802
MAOKIT Medical Device
804
Immunological aspect
806
Nutrition aspect
808
Total antibodies %
810
Early detection of 10 most "popular" deses in babies
812
Improve the baby condition and health
814
The ability to find correlation with approaching dises
816
Bacterial
818
Viral
820
Nutrients
822
Macromolecules
824
Proteins
826
Carbohydrates
828
Glycoproteins
830
Lipids
832
Glucolipids
834
pH
836
Special Molecules
838
Breastfeeding combined with Baby Formula- 53%
840
Vitamins\ Minerals
842
How best to combine breastfeeding with different brands of baby formula in the market). The device can easily determine the baby development stage not by age but according to what best formula matches the current status of the mother breast milk. Stage 1\2\3

Fig. 8

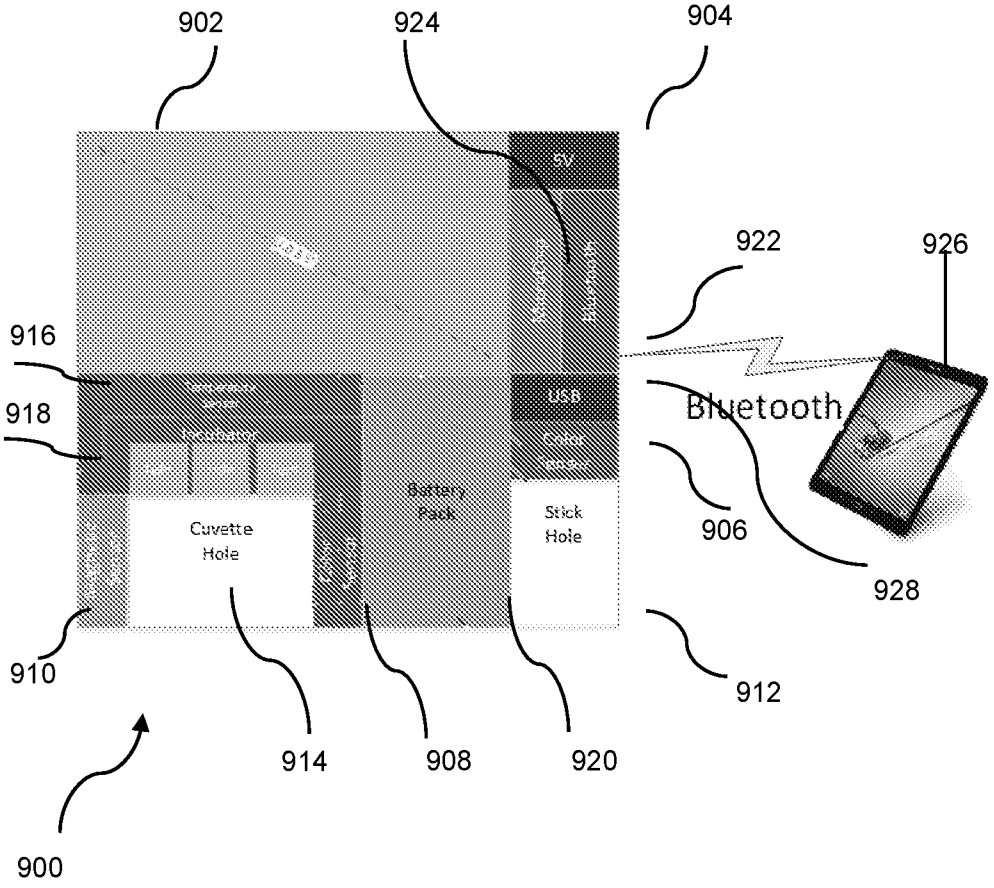

902
924
904
5V
922
926
916
USB
Bluetooth
918
Buttery Pack
Cuvette Hole
Stick Hole
906
928
910
912
914
908
920
900

Fig. 9

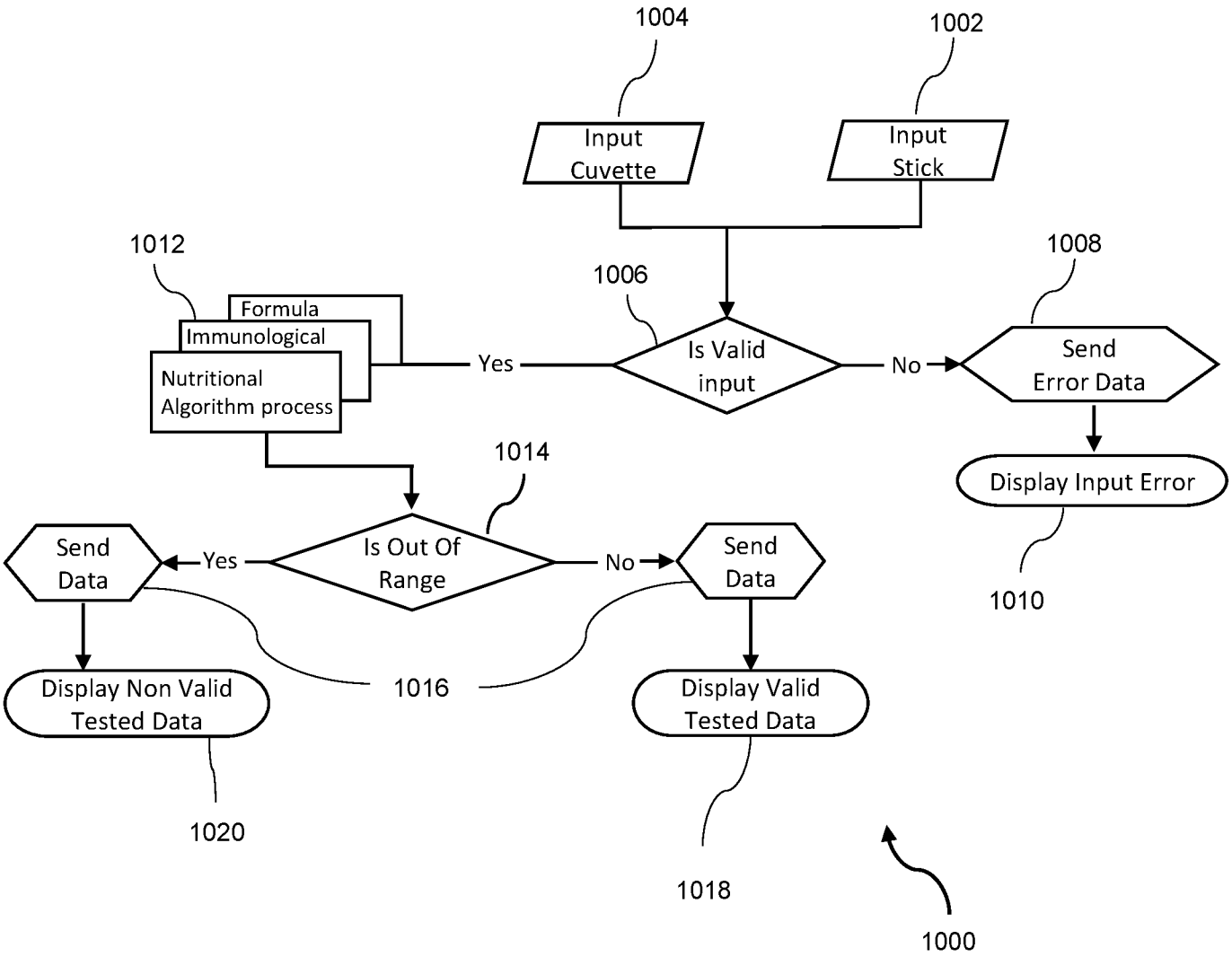

1004
1002
Input Cuvette
Input Stick
1012
1006
1008
Formula
Immunological
Nutritional Algorithm process
Yes
Is Valid input
No
Send Error Data
Display Input Error
1010
1014
Send Data
Yes
Is Out Of Range
No
Send Data
1016
Display Non Valid Tested Data
Display Valid Tested Data
1020
1018
1000

Fig. 10

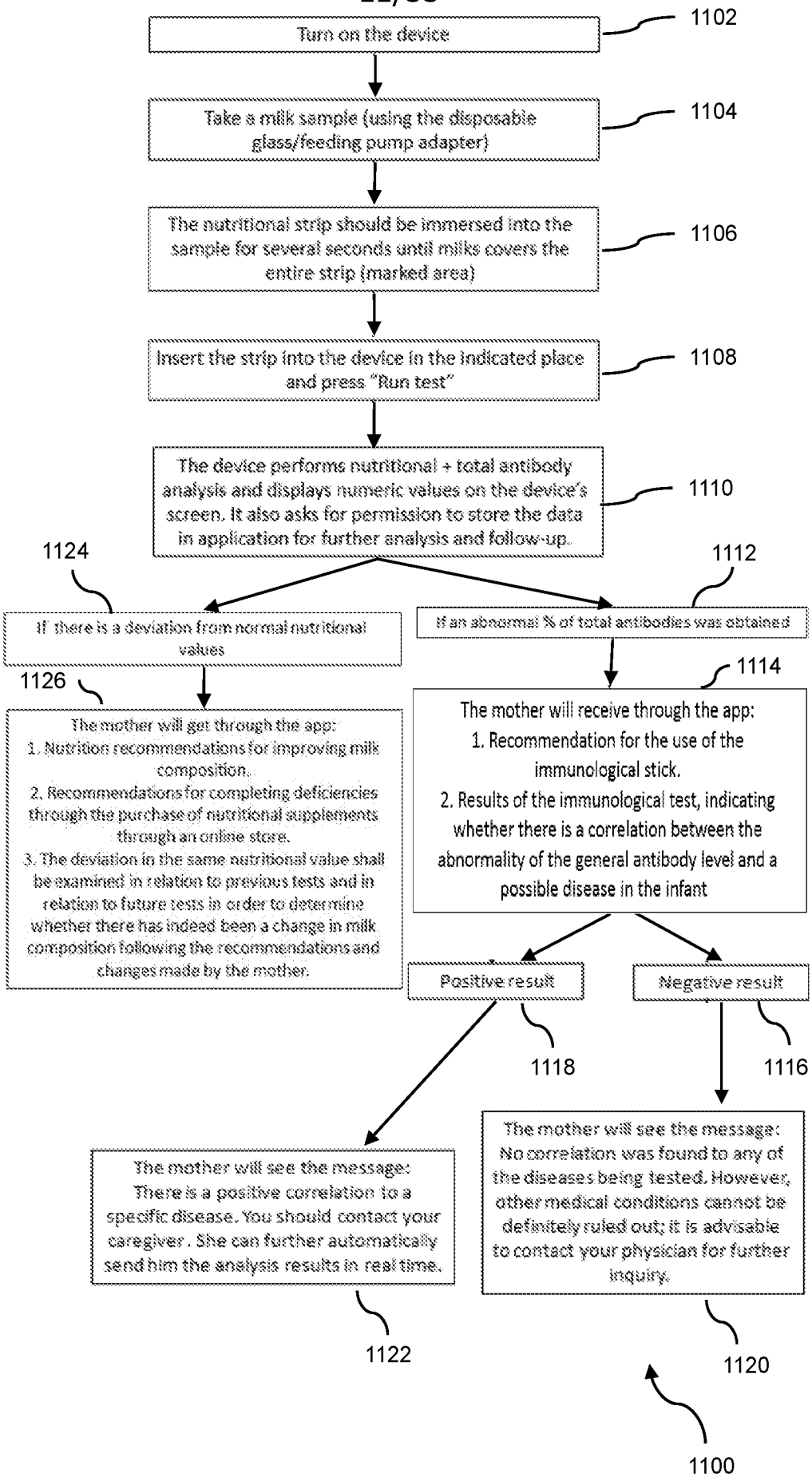

Turn on the device
1102
Take a milk sample (using the disposable glass/feeding pump adapter)
1104
The nutritional strip should be immersed into the sample for several seconds until milks covers the entire strip (marked area)
1106
Insert the strip into the device in the indicated place and press "Run test"
1108
The device performs nutritional + total antibody analysis and displays numeric values on the device's screen. It also asks for permission to store the data in application for further analysis and follow-up.
1110
1124
1112
If there is a deviation from normal nutritional values
If an abnormal % of total antibodies was obtained
1126
1114
The mother will get through the app:
1. Nutrition recommendations for improving milk composition.
2. Recommendations for completing deficiencies through the purchase of nutritional supplements through an online store.
3. The deviation in the same nutritional value shall be examined in relation to previous tests and in relation to future tests in order to determine whether there has indeed been a change in milk composition following the recommendations and changes made by the mother.
The mother will receive through the app:
1. Recommendation for the use of the immunological stick.
2. Results of the immunological test, indicating whether there is a correlation between the abnormality of the general antibody level and a possible disease in the infant
Positive result
Negative result
1118
1116
The mother will see the message: There is a positive correlation to a specific disease. You should contact your caregiver. She can further automatically send him the analysis results in real time.
The mother will see the message: No correlation was found to any of the diseases being tested. However, other medical conditions cannot be definitely ruled out; it is advisable to contact your physician for further inquiry.
1122
1120
1100

Fig. 11

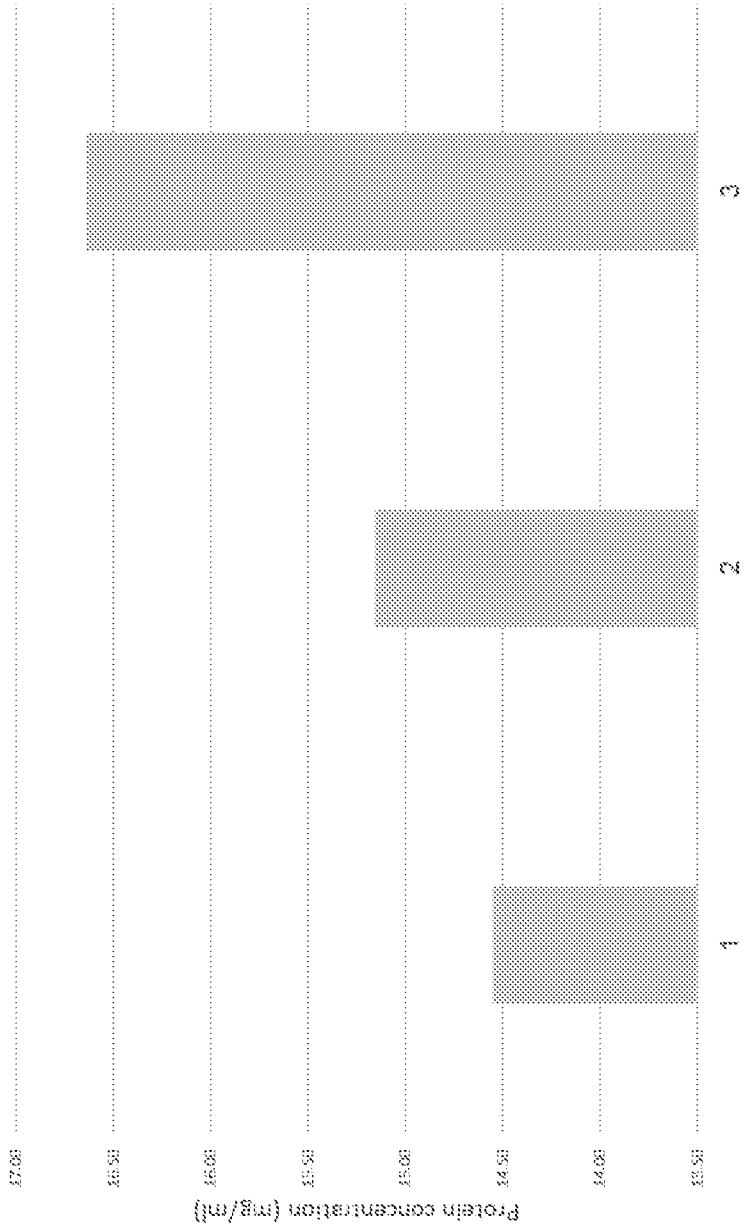

Protein concentration (mg/ml)
18.27
18.01
17.91
17.81
17.71
17.61
17.51
17.41
Phase
1
2
3

Fig. 12

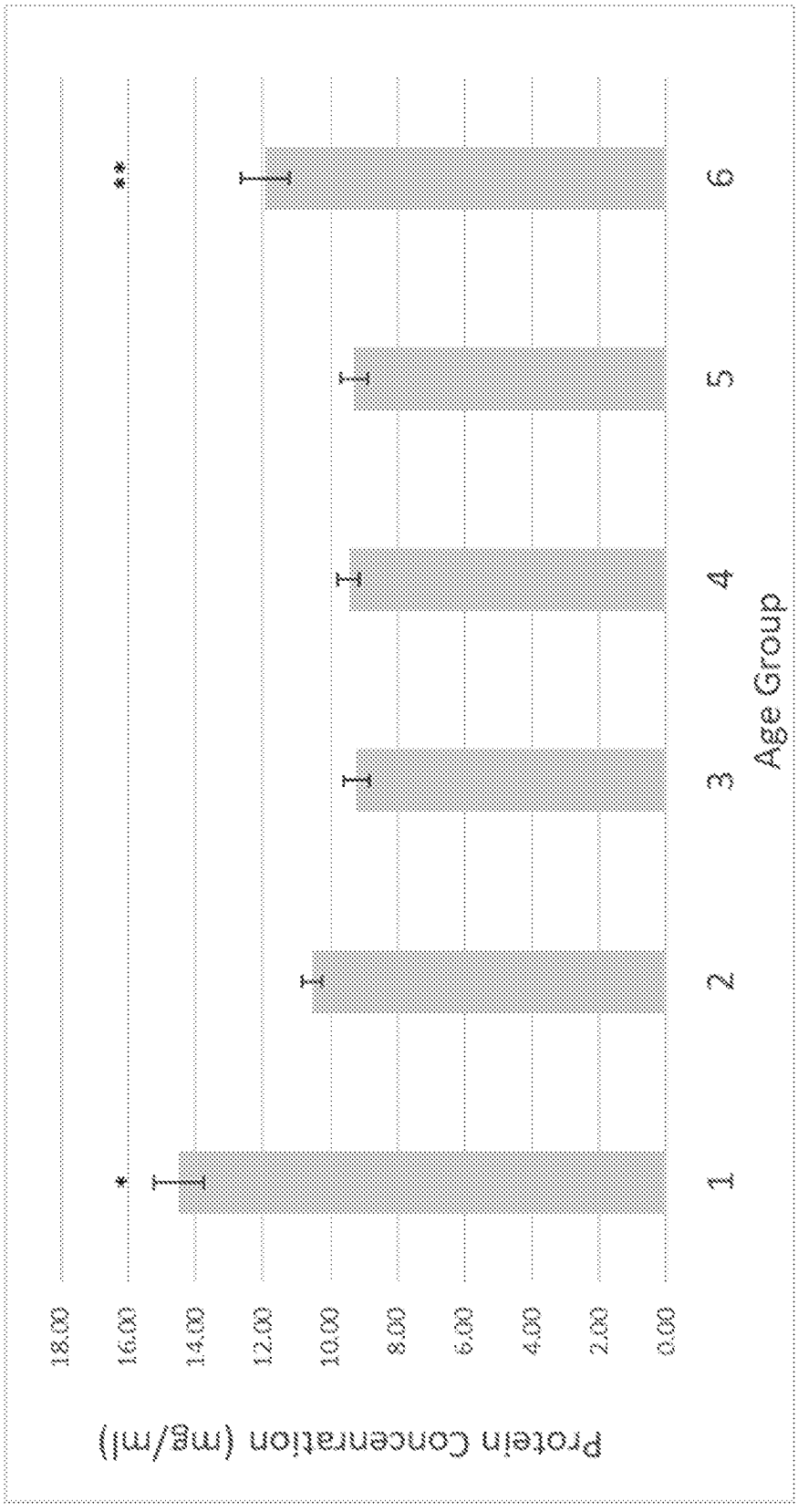

Protein Concentration (mg/ml)
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
*
**
1
2
3
4
5
6
Age Group

Fig. 13

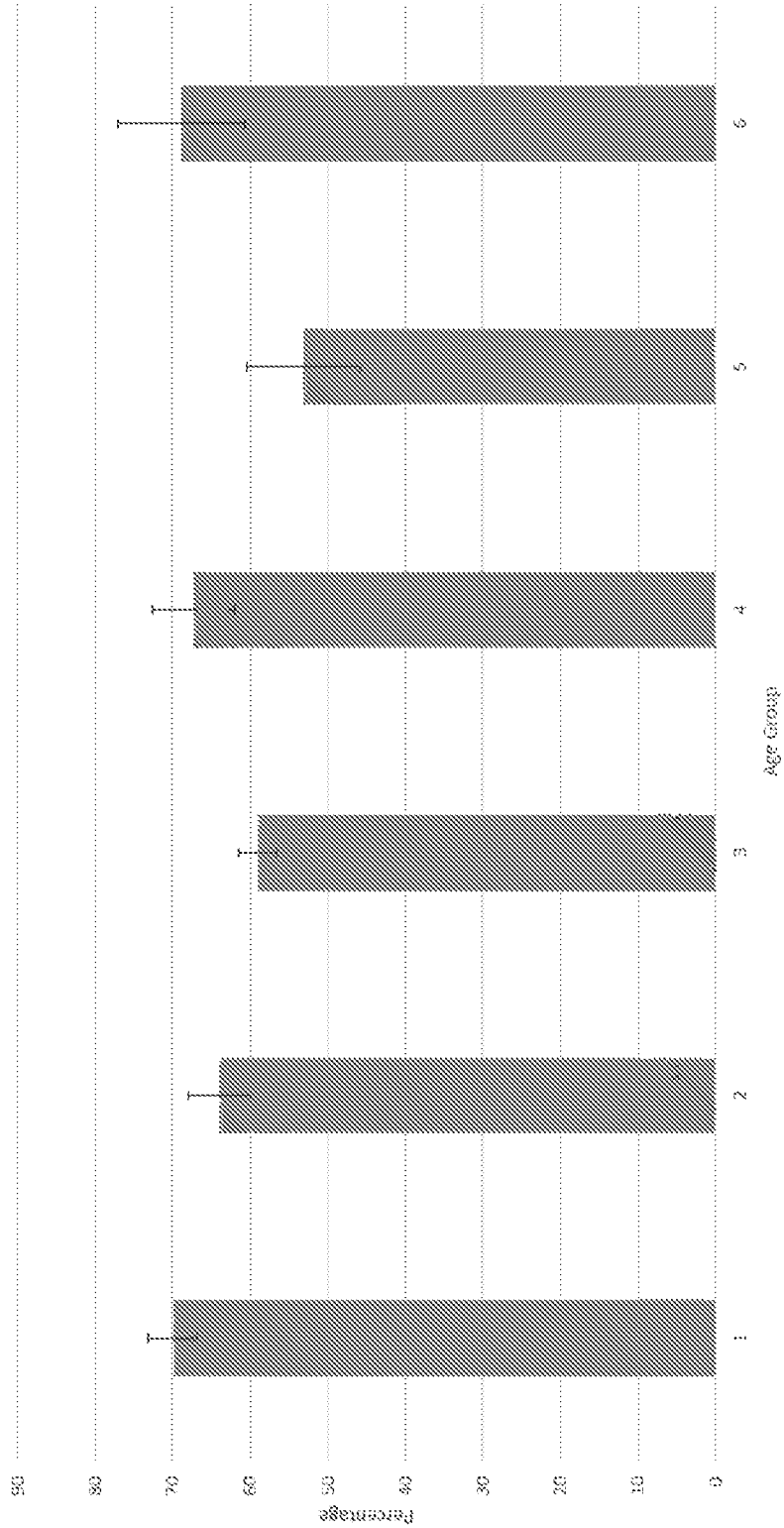


Fig. 14

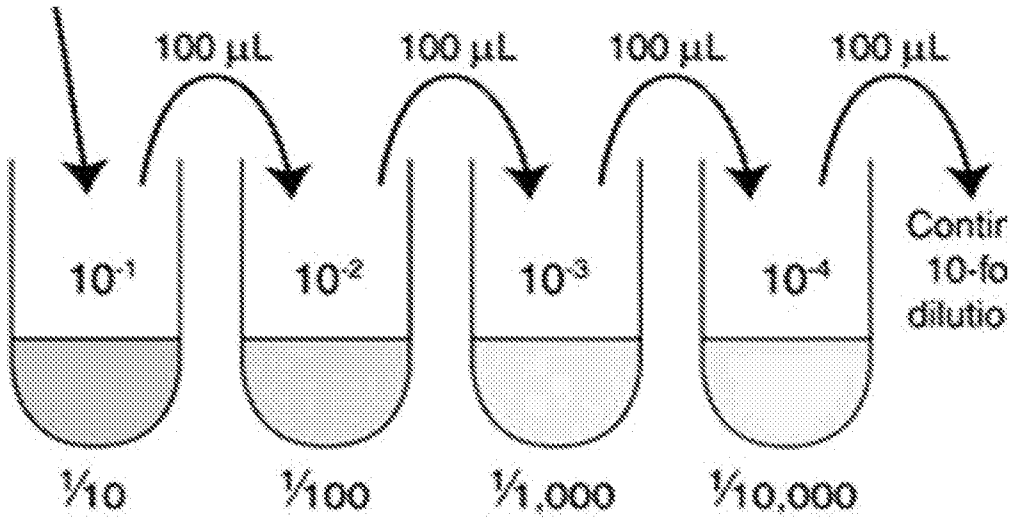

100 µL
100 µL
100 µL
100 µL
10^{-1}
10^{-2}
10^{-3}
10^{-4}
Contir
10-fo
dilutio
1/10
1/100
1/1,000
1/10,000

Fig. 15

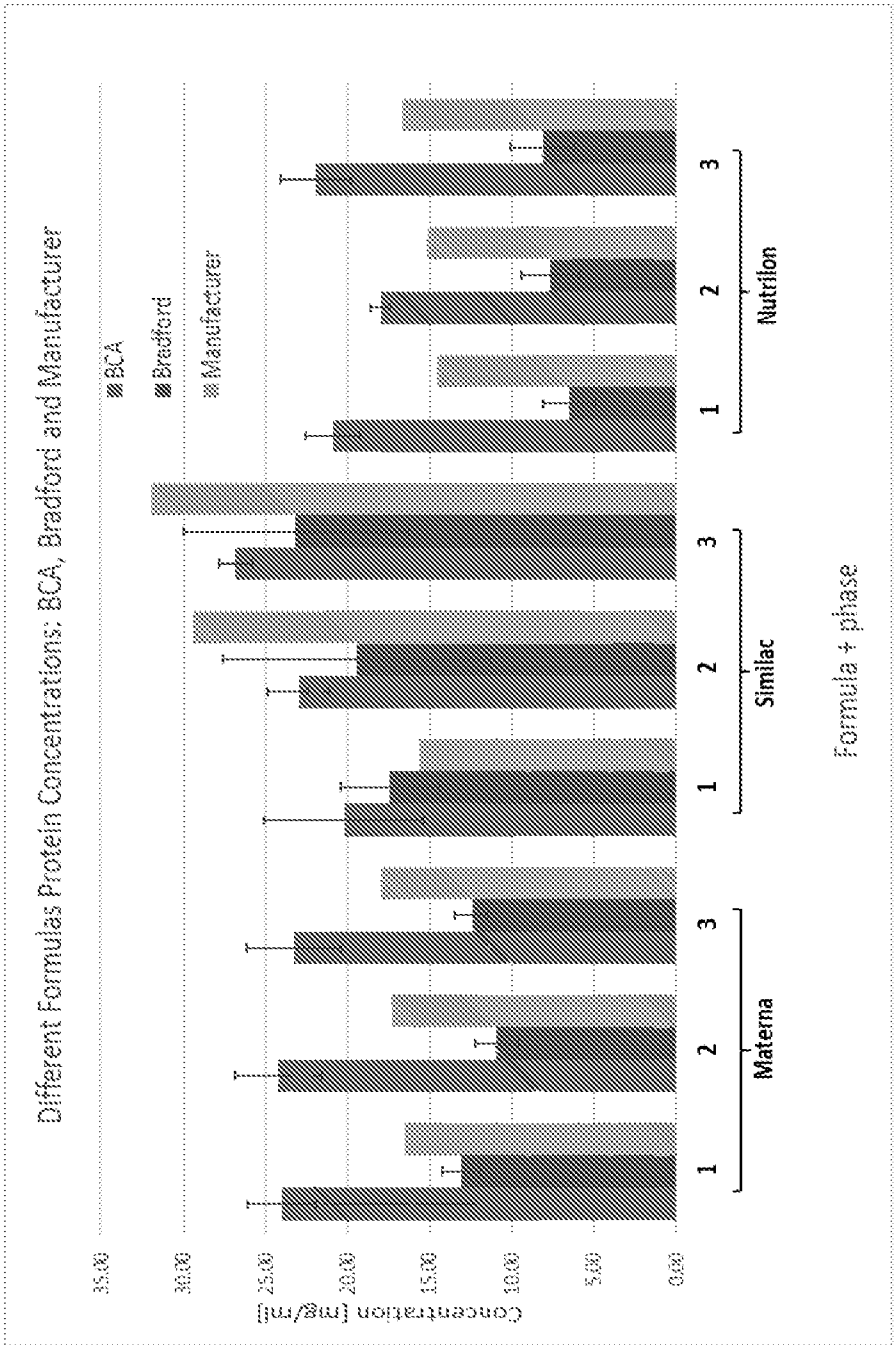

Different Formulas Protein Concentrations: BCA, Bradford and Manufacturer
BCA
Bradford
Manufacturer
Concentration [mg/ml]
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
1
2
3
Materna
Similac
Nutrilon
Formula + phase

Fig. 16

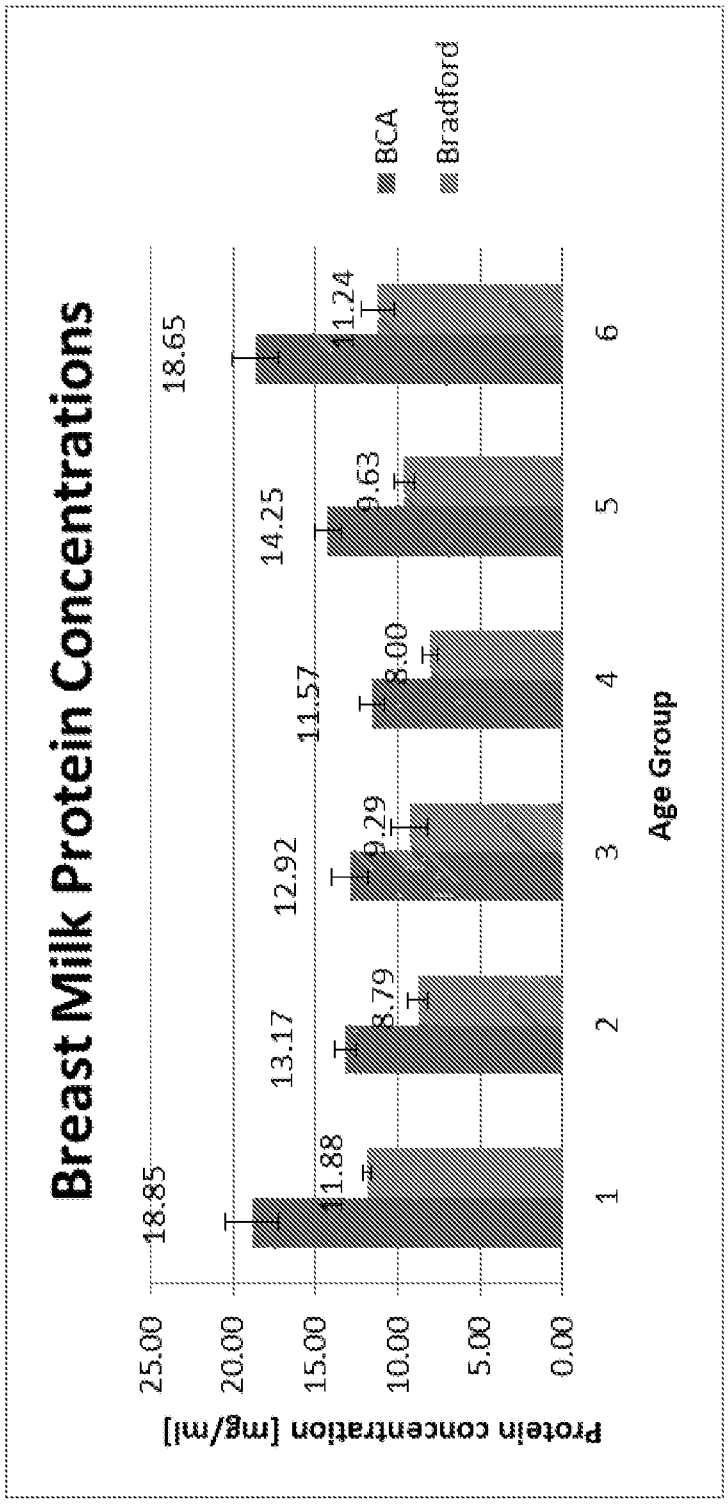

Breast Milk Protein Concentrations
BCA
Bradford
Protein concentration [mg/ml]
25.00
20.00
15.00
10.00
5.00
0.00
18.85
11.88
13.17
8.79
12.92
9.29
11.57
8.00
14.25
9.63
18.65
11.24
1
2
3
4
5
6
Age Group

Fig. 17

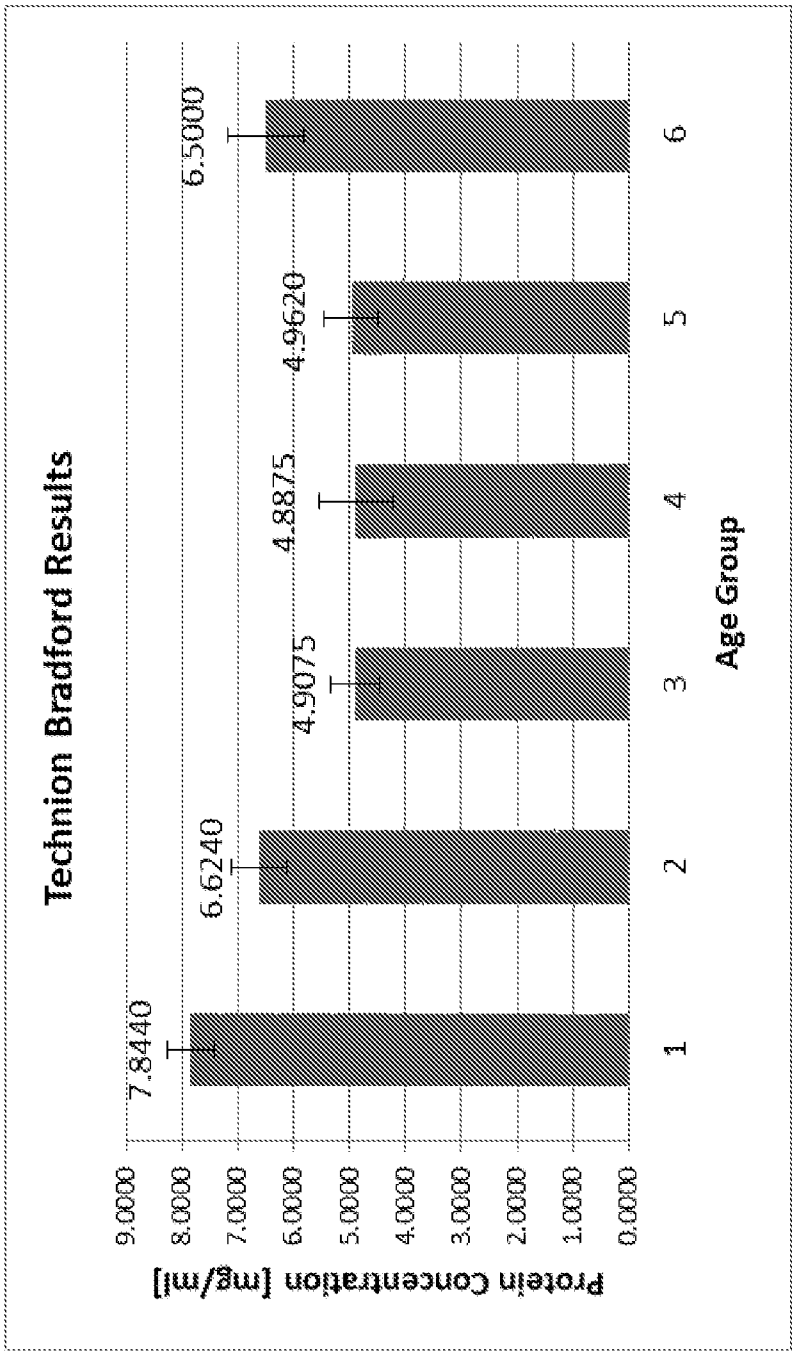

Technion Bradford Results
Protein Concentration [mg/ml]
9.0000
8.0000
7.0000
6.0000
5.0000
4.0000
3.0000
2.0000
1.0000
0.0000
7.8440
6.6240
4.9075
4.8875
4.9620
6.5000
1
2
3
4
5
6
Age Group

Fig. 18

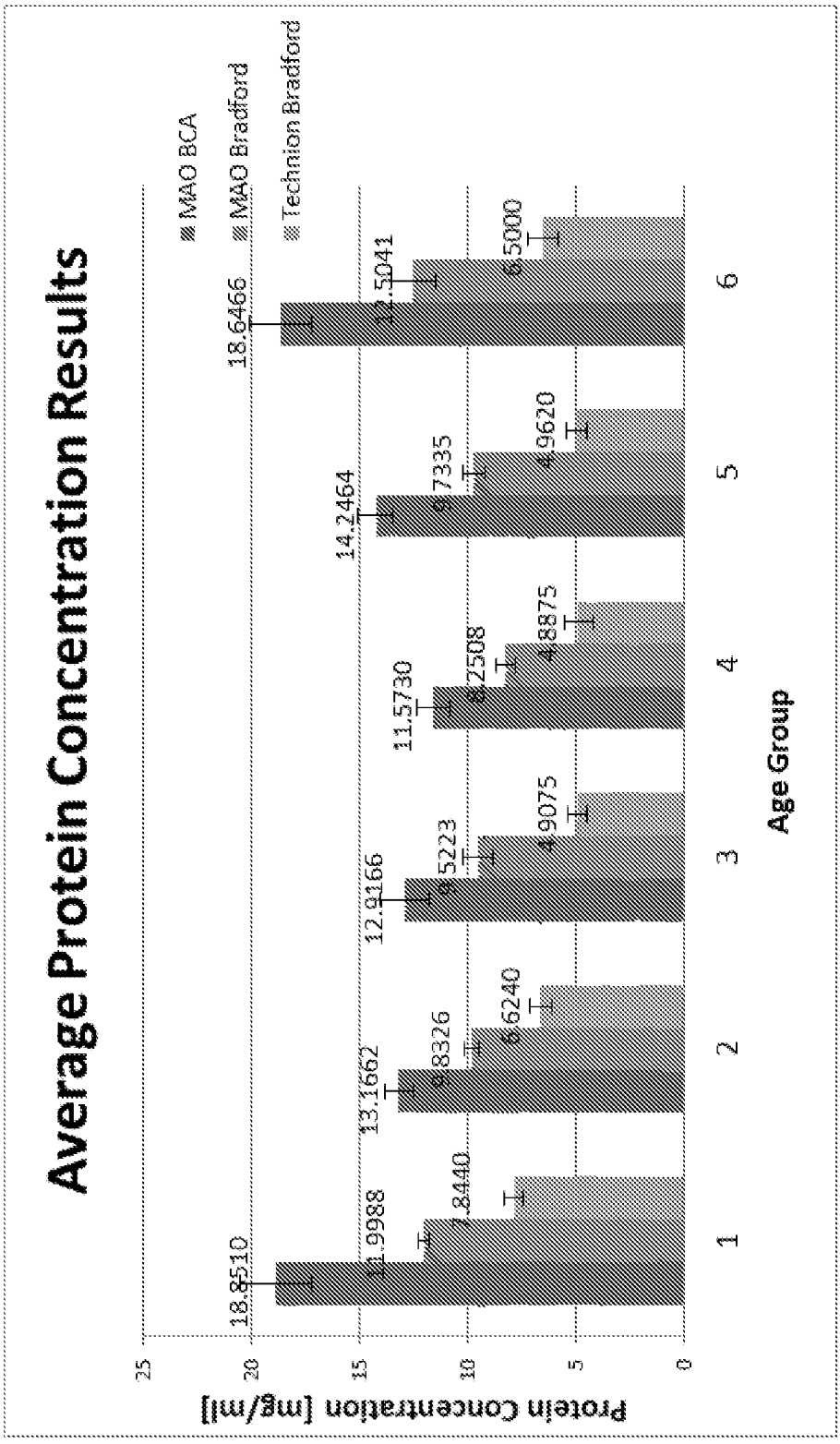

Average Protein Concentration Results
MAO BCA
MAO Bradford
Technion Bradford
Protein Concentration [mg/ml]
Age Group
0
5
10
15
20
25
1
2
3
4
5
6
18.8510
11.9988
7.8440
13.1662
9.8326
6.6240
12.9166
9.5223
6.9075
11.5730
8.2508
4.8875
14.2464
9.7335
4.9620
18.6466
12.5041
6.5000

Fig. 19

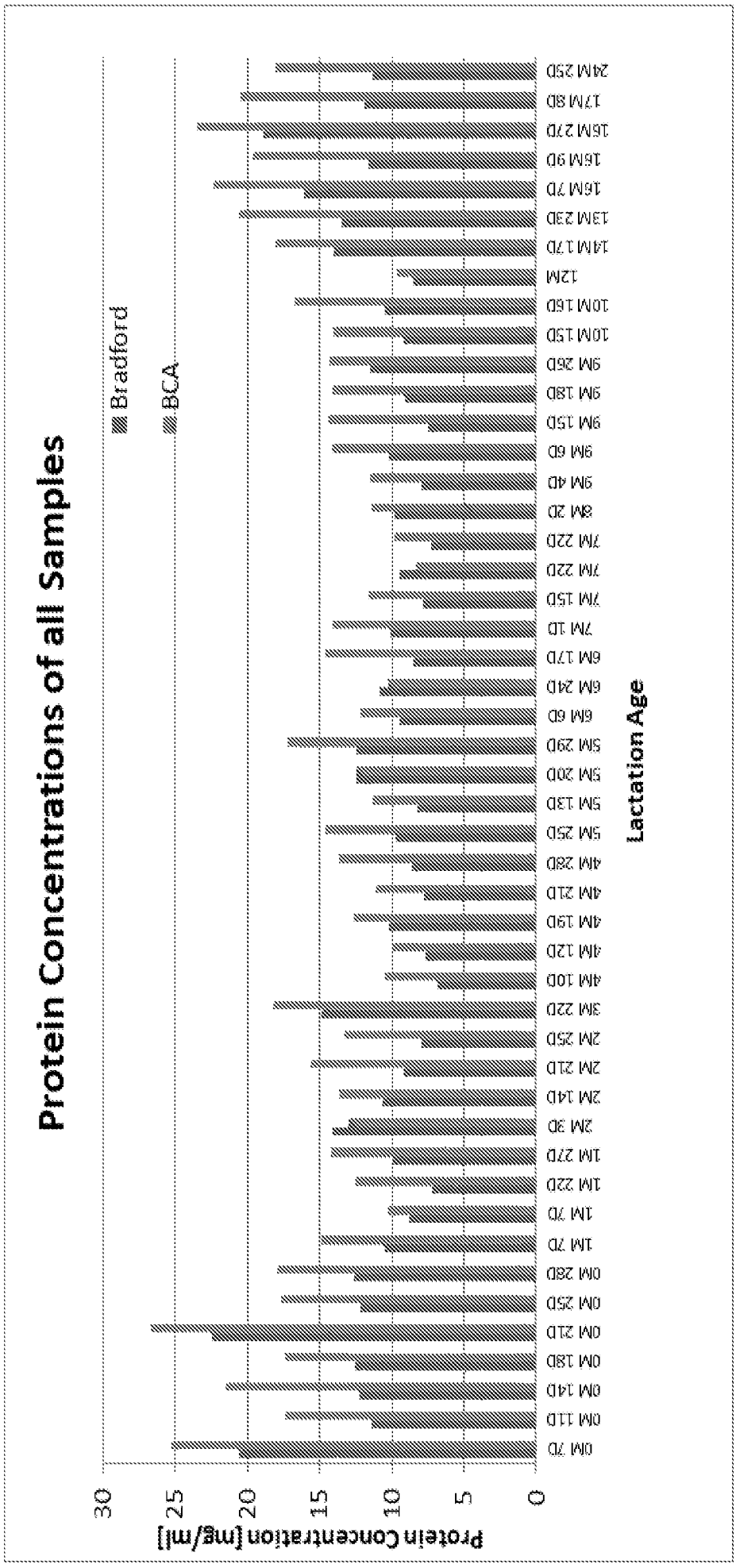

Protein Concentrations of all Samples
Bradford
BCA
Protein Concentration [mg/ml]
0
5
10
15
20
25
30
Lactation Age
0M 7D
0M 11D
0M 14D
0M 18D
0M 21D
0M 25D
0M 28D
1M 7D
1M 7D
1M 22D
1M 27D
2M 3D
2M 14D
2M 21D
2M 25D
3M 22D
4M 10D
4M 12D
4M 19D
4M 21D
4M 28D
5M 25D
5M 13D
5M 20D
5M 29D
6M 6D
6M 24D
6M 17D
7M 1D
7M 15D
7M 22D
7M 22D
8M 2D
9M 4D
9M 6D
9M 15D
9M 18D
9M 26D
10M 15D
10M 16D
12M
14M 17D
13M 23D
16M 7D
16M 9D
16M 27D
17M 8D
24M 25D

Fig. 20

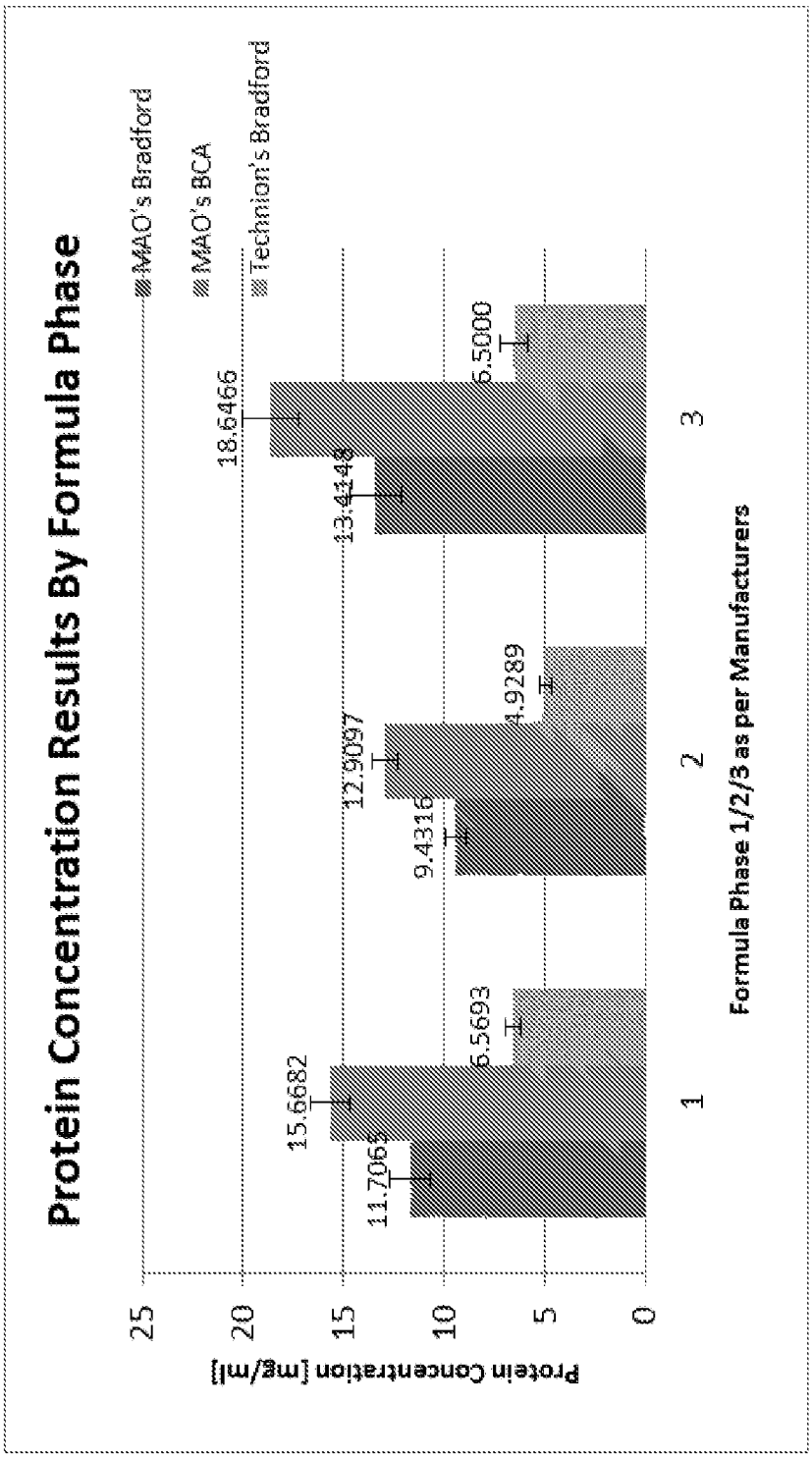

Protein Concentration Results By Formula Phase
MAO's Bradford
MAO's BCA
Technion's Bradford
Protein Concentration [mg/ml]
25
20
15
10
5
0
11.7065
15.6682
6.5693
9.4316
12.5097
4.9289
18.6466
13.4148
6.5000
1
2
3
Formula Phase 1/2/3 as per Manufacturers

Fig. 21

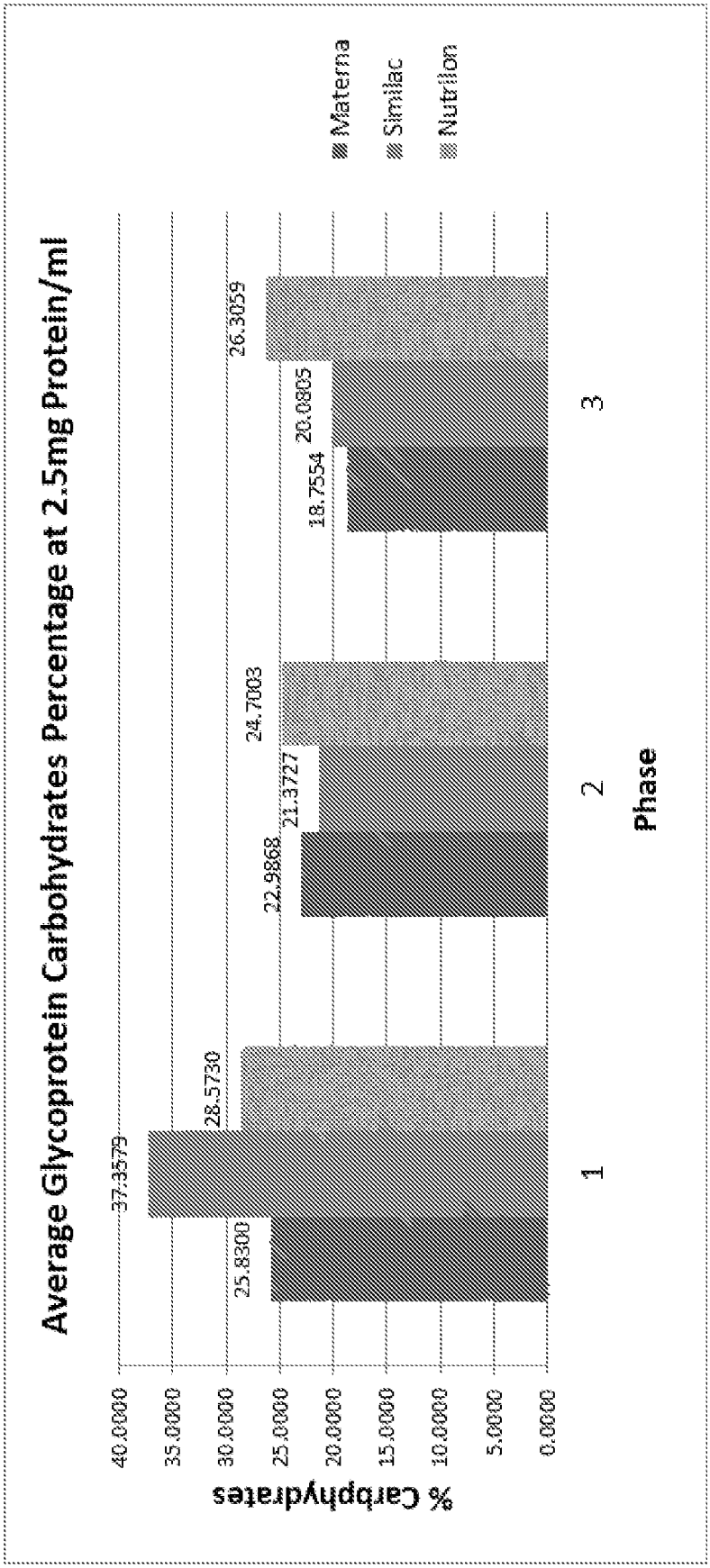

Average Glycoprotein Carbohydrates Percentage at 2.5mg Protein/ml
% Carbohydrates
40.0000
35.0000
30.0000
25.0000
20.0000
15.0000
10.0000
5.0000
0.0000
37.3579
28.5730
25.8300
26.3059
20.0805
18.7554
24.7003
21.3727
22.9868
1
2
3
Phase
Materna
Similac
Nutrilon

Fig. 22

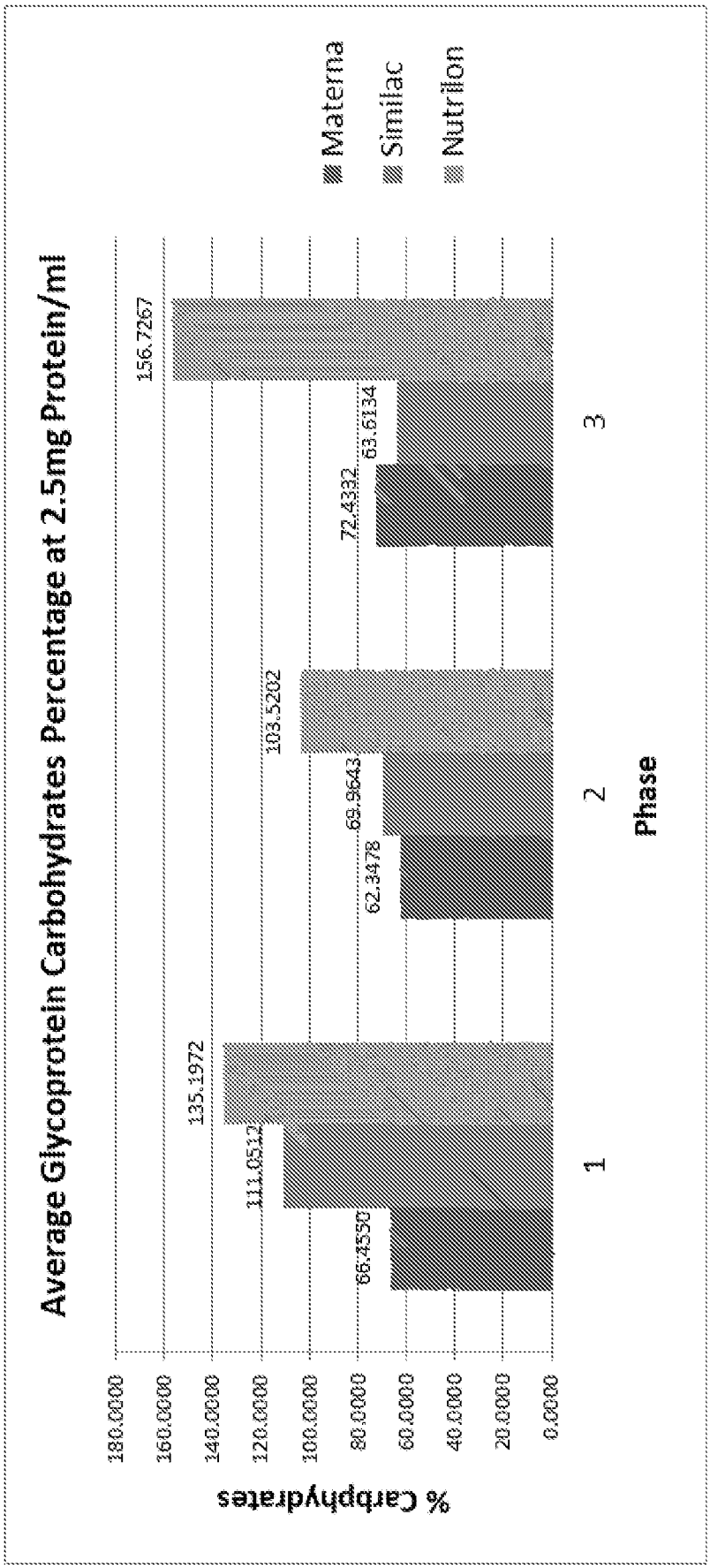

Average Glycoprotein Carbohydrates Percentage at 2.5mg Protein/ml
% Carbhydrates
180.0000
160.0000
140.0000
120.0000
100.0000
80.0000
60.0000
40.0000
20.0000
0.0000
135.1972
111.0512
66.4550
156.7267
103.5202
69.9643
62.3478
72.4332
63.6134
1
2
3
Phase
Materna
Similac
Nutrilon

Fig. 23

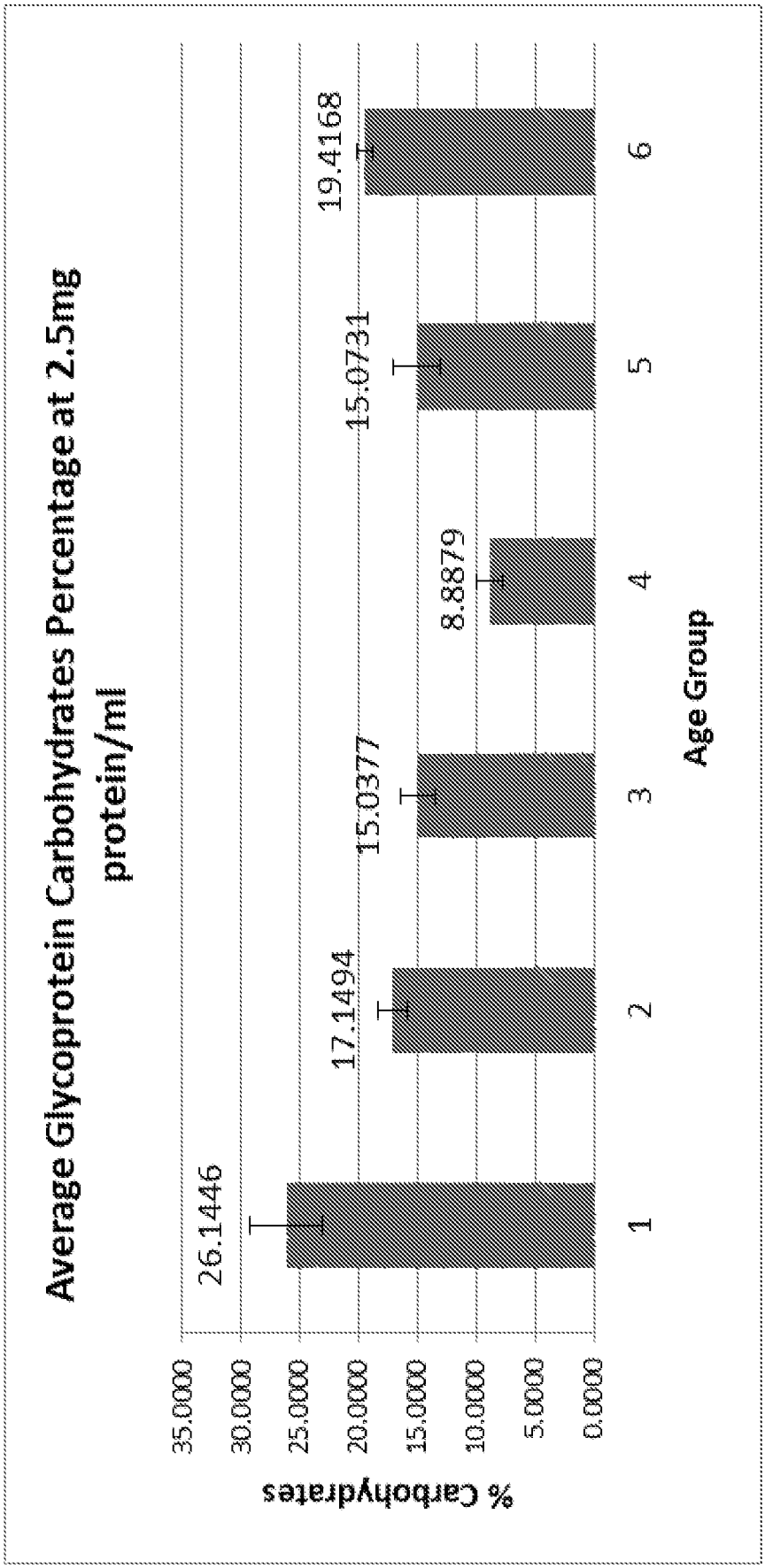

Average Glycoprotein Carbohydrates Percentage at 2.5mg protein/ml
% Carbohydrates
35.0000
30.0000
25.0000
20.0000
15.0000
10.0000
5.0000
0.0000
26.1446
17.1494
15.0377
8.8879
15.0731
19.4168
1
2
3
4
5
6
Age Group

Fig. 24

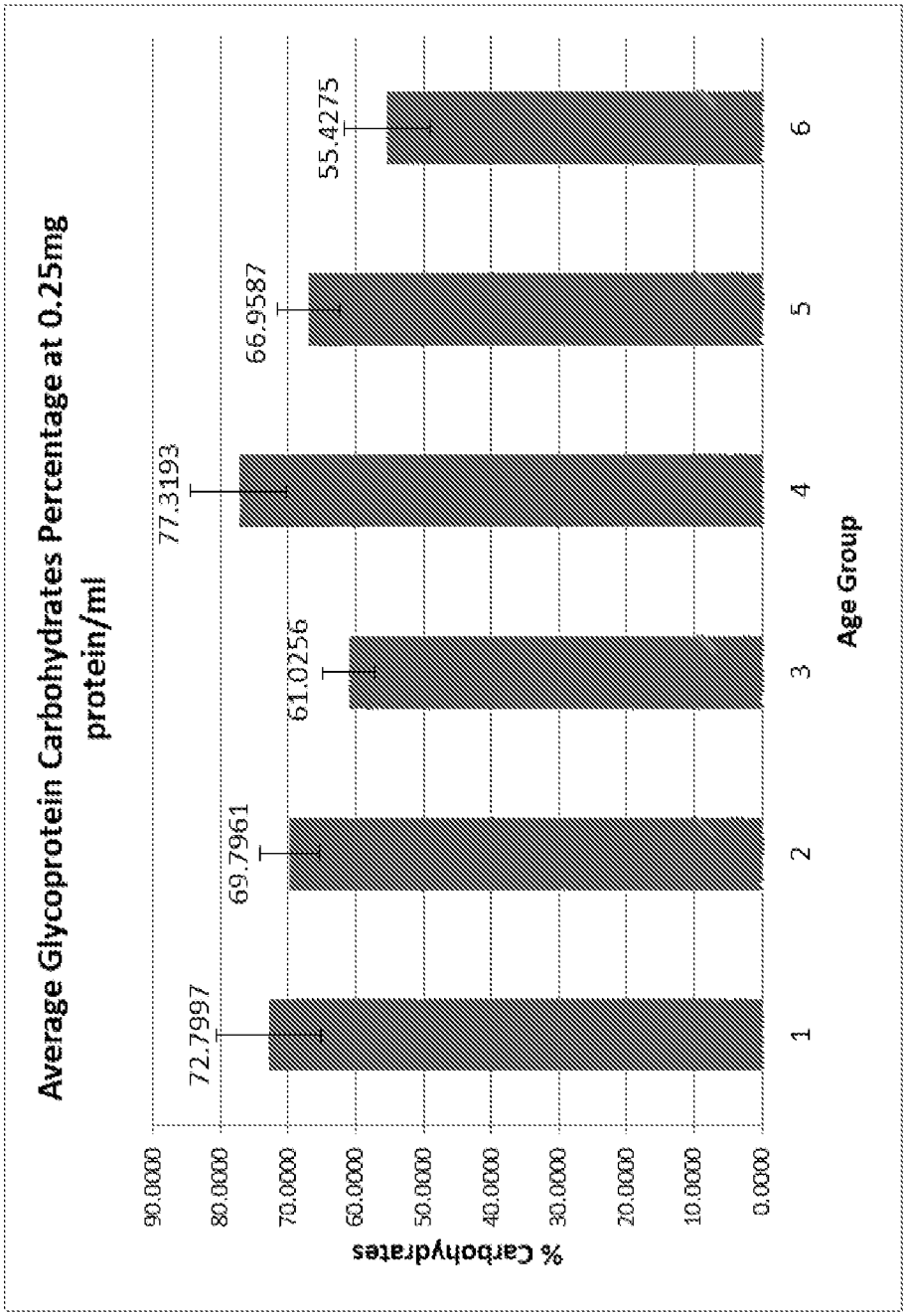

Average Glycoprotein Carbohydrates Percentage at 0.25mg protein/ml
% Carbohydrates
90.0000
80.0000
70.0000
60.0000
50.0000
40.0000
30.0000
20.0000
10.0000
0.0000
72.7997
69.7961
61.0256
77.3193
66.9587
55.4275
1
2
3
4
5
6
Age Group

Fig. 25

Leaukocytes (CD45)		Colostruma	Transitional milkb	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactationc
	Healthy	13.2–70.4	0.0–1.65	0.07–0.45	0.0–1.52	0.0–1.09	0.08–0.1	0.0–0.06	0.0–0.55
	Infection	—	18.8	0.72–90.5	1.1–33.9	1.08–93.6	> 3	2.13–71.7	4.27–10.8
Leukocytes per ml milk		Colostruma	Transitional milkb	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactationc
	Healthy	32 175–784 080	0–3450	160–1151	0–1025	0–1063	707–853	0–288	0–13 750
	Infection	—	34 467	2400–2 594 982	2164–109 130	1065–472 634	> 30 000	1293–759 834	3127–49 817
sIgA (µg ml−1)		Colostruma	Transitional milkb	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactationc
	Healthy	1428–2178	131–1096	534–1276	257–960	496–1350	401–1044	137–1243	976–1991
	Infection	—	922	36–1418	652–1711	611–1509	714–789	173–2002	1657–1906
IgG (µg ml−1)		Colostruma	Transitional milkb	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactationc
	Healthy	5.3–12.2	2.8–9.7	6.4–12.4	4.6–10.8	4.0–16.4	5.0–16.1	4.0–10.4	4.6–17.8
	Infection	—	13	6.6–17.1	4.8–10.1	5.6–14.4	7.6–8.8	2.3–25.9	9.4–13.4
IgM (µg ml−1)		Colostruma	Transitional milkb	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactationc
	Healthy	16.2–56.1	8.2–29.8	10.6–14.9	6.5–11.6	4.2–23.7	8.8–23.3	2.9–13.0	7.1–23.1
	Infection	—	10.2	4.5–19.8	10.1–15.4	12.6–21.8	14.4–19.3	5.9–31.1	10.5–23.5
Lactoferrin (g l−1)		Colostruma	Transitional milkb	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactationc
	Healthy	6.3–7.7	2.1–5.2	2.5–2.9	1.9–3.7	1.3–4.0	1.2–3.9	2.3–4.5	3.3–5.8
	Infection	—	4.3	2.9–3.7	2.0–3.7	1.6–3.3	1.2–3.6	2.1–4.6	4.6–5.6
IL-10	Infection	>50pg/ml							
TNFα	Healthy	2.91 ± 1.51 pg/ml BM							
	Infection	3.66 ± 1.68 pg/ml BM							
Total lymphocytes	Healthy	325 cells/ml BM							
	Infection	2,474 cells/ml BM							
Neutrophils	Healthy	813 cells/ml BM							
	Infection	2,941 cells/ml BM							
lysozyme		Colostrum	transitional milk	Days 15 to 28	Days 29 to 56	Days 57 to 84			
	Healthy	0.37 g/L	0.27g/L	0.24 g/L	0.33 g/L	0.89 g/L			
HMOs		Colostrum	Mature Milk						
	Healthy	20–25 g/L	5–20 g/L						
IL-6		Colostrum	Transitional Milk	Mature Milk					
	Healthy	978.80 ± 86.80 pg/mL	162.90±29.67 pg/mL	86.92 ± 2.47 pg/mL					

Fig. 26

		Colostruma	Transitional milkb	Months 1–3	Months 4–6	Months 7–9	Months 10–12	Year 2	Late lactationc
DHA (omega 3)	Healthy	0.32% ± 0.22% of Breas	****According to the NIH (National Institute of Health) the proper dose for breastfeeding moms should be 300mg of DHA (Omega 3 fatty acid strand) per day						
	Optimal	1% of breastmilk							
AA (omega 6)	Healthy	0.47± 0.13%							
	Optimal	No higher than 1:4 DHA:AA ratio							
		Days 6-10	Days 11-20	Days 21-89	Days 90-180	Days 181-365			
Vitamin B1 (µg/100ml)		6.6±3.7	7.6±4.8	12.0±2.2	13.2±2.4	13.4±2.5			
		Days 6-10	Days 11-20	Days 21-89	Days 90-180	Days 181-365			
Vitamin B2 (µg/100ml)		37.7±15.6	34.0±9.7	38.0±12.6	39.7±12.6	38.5±13.3			
		Days 6-10	Days 11-20	Days 21-89	Days 90-180	Days 181-365			
Vitamin B2 (FAD) (µg/100ml)		74.7±29.2	67.1 ± 19.9	68.0 ± 19.8	69.3 ± 24.7	66.8 ± 22.9			
		Days 6-10	Days 11-20	Days 21-89	Days 90-180	Days 181-365			
Vitamin B6 (µg/100ml)		1.9±1.0	5.5 ± 3.8	4.6 ± 2.1	7.3 ± 2.3	6.4 ± 1.8			
		Days 6-10	Days 11-20	Days 21-89	Days 90-180	Days 181-365			
Vitamin B12 (µg/100ml)		0.07±0.05	0.06±0.02	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02			
		Days 6-10	Days 11-20	Days 21-89	Days 90-180	Days 181-365			
Vitamin C (µg/100ml)		5.4 ± 2.8	6.9 ± 2.7	6.5 ± 1.4	4.7 ± 1.2	3.7 ± 1.3			
		5–11 Days	12–30 Days	1–2 Months	2–4 Months	4–8 Months			
TGF-β1 (ng/L), Median (IQR)		1258 (1305)	685 a (482)	600 (356)	598 (379)	659 (410)			
		5–11 Days	12–30 Days	1–2 Months	2–4 Months	4–8 Months			
TGF-β2 (ng/L), Median (IQR)		5286 (10,444)	2322 a (3100)	1877 a (1890)	1920 a (2112)	2311 b (2868)			
		5–11 Days	12–30 Days	1–2 Months	2–4 Months	4–8 Months			
α-lactalbumin (g/L), Median (IQR)		3.27 (0.60)	3.16 (0.55)	2.84 a (0.55)	2.53 a (0.47)	2.28 a (0.63)			
		5–11 Days	12–30 Days	1–2 Months	2–4 Months	4–8 Months			
Serum albumin (g/L), Median (IQR)		0.48 (0.14)	0.48 (0.14)	0.42 (0.09)	0.44 (0.10)	0.42 (0.08)			
		5–11 Days	12–30 Days	1–2 Months	2–4 Months	4–8 Months			
Total caseins (g/L), Median (IQR)		5.84 (3.17)	6.57 a (2.15)	6.24 (2.25)	5.79 a (1.69)	5.60 (1.73)			
		1 Month	2-3 Months	3-6 Months	6-9 Months	9-12 Months	12+ Months		
Total Protein (ug/ul) (TECHNION BRADFORD)		14.5274	11.0818	8.8536	9.408	9.1947	11.8353		

Fig. 27

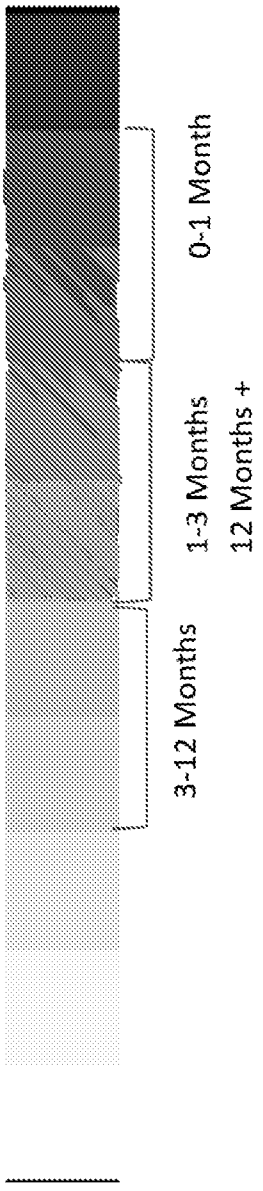


Fig. 28

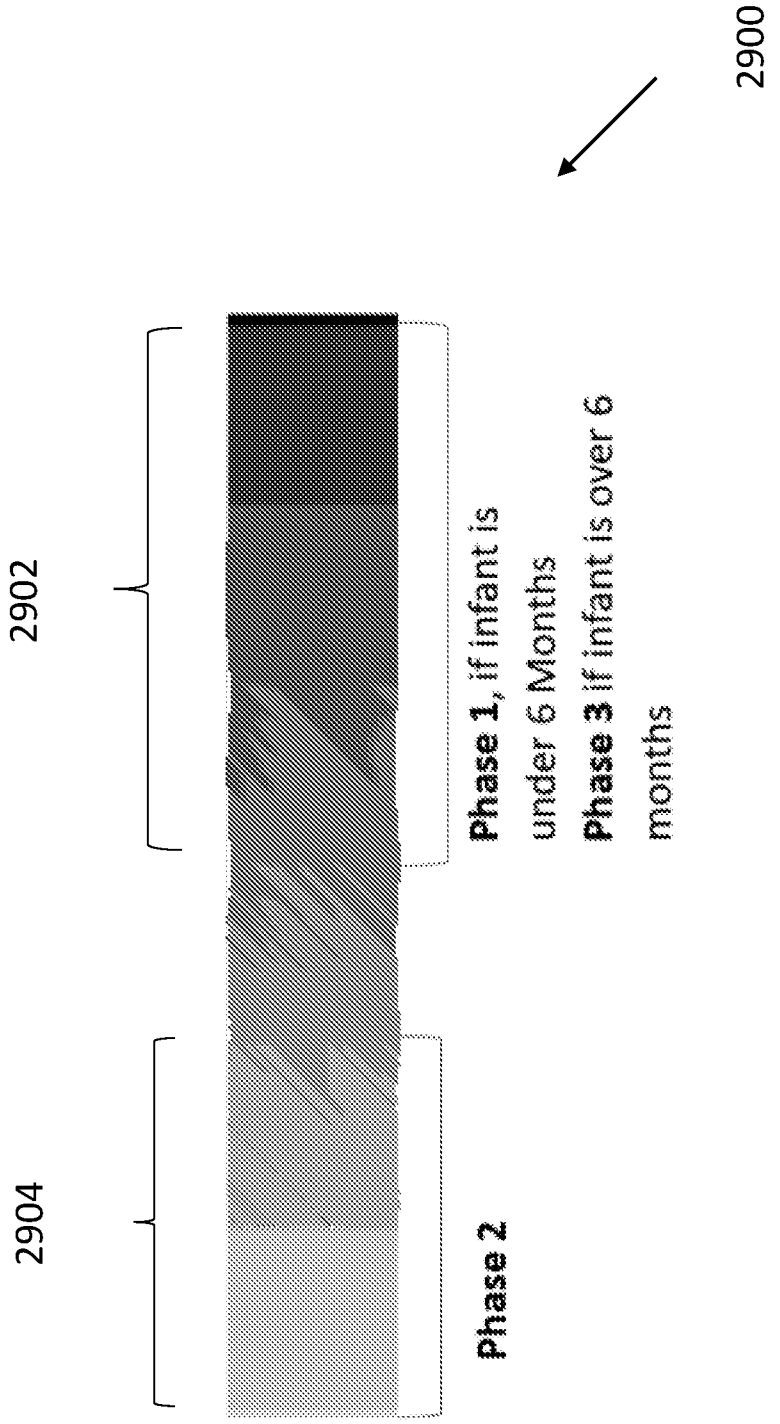

2900
2902
2904
Phase 1, if infant is under 6 Months
Phase 3 if infant is over 6 months
Phase 2

Fig. 29

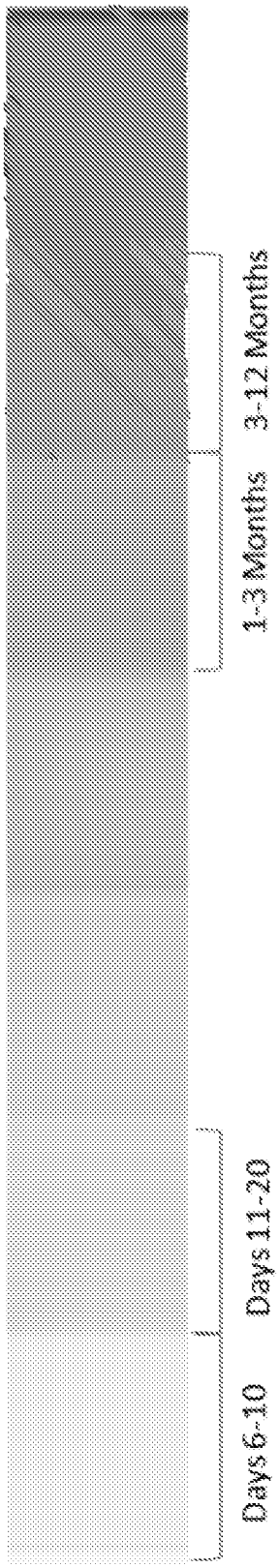


Fig. 30

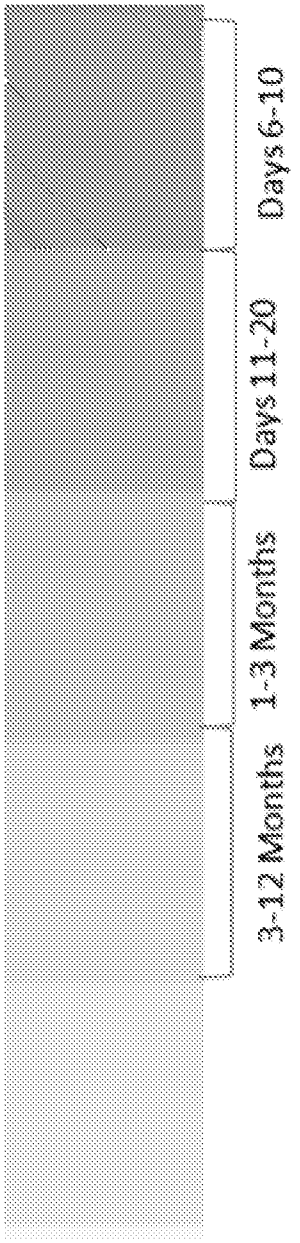


Fig. 31

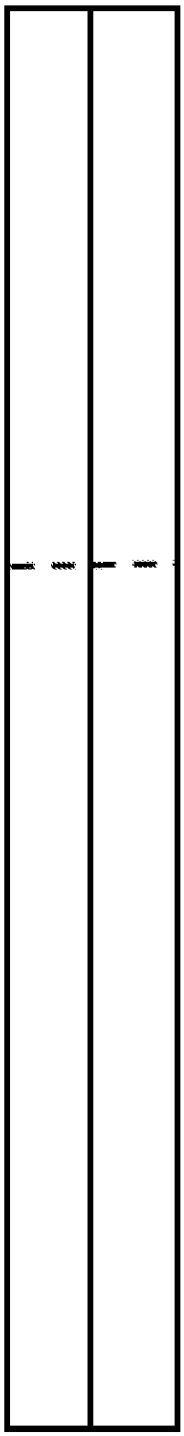


Fig. 32

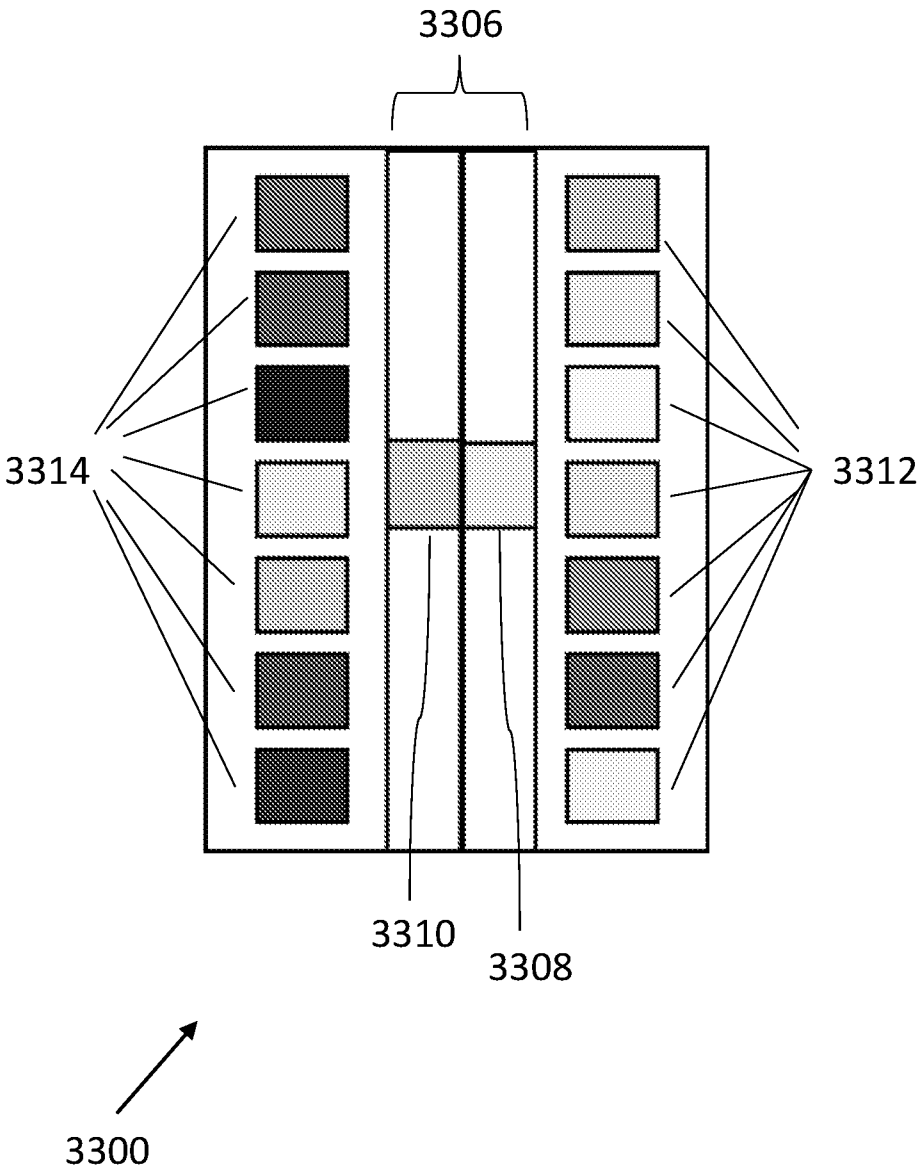


Fig. 33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2018/050893

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2018.01) A61B 5/00, G01N 29/02, C12Q 1/68, G01N 33/566, G01N 33/53, G01N 33/50, G01N 33/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2018.01) A61B 5/00, G01N 29/02, C12Q 1/68, G01N 33/566, G01N 33/53, G01N 33/50, G01N 33/48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: Google Patents, Google Scholar, Orbit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US US2013040289 A1 UPSPRING LTD 13 Feb 2013 (2013/02/13) the whole document	1-15
A	DELL, Nicola Lee, et al. Towards a point-of-care diagnostic system: automated analysis of immunoassay test data on a cell phone. In: Proceedings of the 5th ACM workshop on Networked systems for developing regions. ACM, 2011. p. 3-8. DELL, Nicola Lee, et al 31 Dec 2011 (2011/12/31) abstract	12,13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 Nov 2018	25 Nov 2018
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer ORENSHTEIN-VILENSKY Liya Telephone No. 972-2-5651662

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2018/050893

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
US US2013040289 A1	13 Feb 2013	NONE	

专利名称(译)	用于识别和监测母乳成分的系统，装置和方法		
公开(公告)号	EP3664699A1	公开(公告)日	2020-06-17
申请号	EP2018845849	申请日	2018-08-12
[标]发明人	ORBACH ARIEL ASHKENAZI MAYA		
发明人	ORBACH, ARIEL ASHKENAZI, MAYA		
IPC分类号	A61B5/00 G01N29/02 C12Q1/68 G01N33/566 G01N33/53 G01N33/50 G01N33/48		
CPC分类号	A61B5/00 G01N29/02 G01N33/558 G01N2291/022 G01N2291/02466 G01N33/48		
优先权	62/544892 2017-08-13 US 62/638908 2018-03-05 US		
外部链接	Espacenet		

摘要(译)

本文提供了一种用于对母乳进行采样和分析的采样元件,其中,所述元件包括:多个纤维,以允许所述母乳流过所述采样元件;以及 结果指示器,指示所述分析的结果; 其中所述采样元件提供了所述母乳的营养或免疫学分析。