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(54) Title: BOVINE PREGNANCY TESTING

(57) Abstract

An antibody to a bovine early pregnancy factor is produced and isolated. It is used as a diagnostic assay to determine pregnancy in cattle. The pregnancy test is performed using a test device which contains immobilized antibody to the bovine early pregnancy factor. In addition, a liquid sampling device which allows only a predetermined amount of biological liquid sample to contact the test device is provided.

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BOVINE PREGNANCY TESTING

FIELD OF THE INVENTION

The present invention relates to a bovine early pregnancy factor antibody, process for producing and isolating the same, and its use in a diagnostic assay for the detection of pregnancy in cattle.

BACKGROUND OF THE INVENTION

In today's ever increasingly competitive markets, the productivity of a cattle herd in terms of calves per cows must be optimized for maximum profitability. In order to achieve this the time a cow is not bearing a calf must be minimized. Beef and Dairy farming is an important part of the US and world food production industry in providing both milk and beef. There are approximately 34 million beef cows and 7 million dairy cows in the USA alone which contribute to the total world-wide number of 2 billion animals.

One of the major problem in cattle breeding is the reduction of calves born from the failure of the heifer or cow to follow through the gestation cycle. Another major problem in cattle breeding is the time it takes to determine the actual conception/pregnancy. The primary cause of this delay is due to the lack of a diagnostic method to determine the actual conception and pregnancy results within a very short time after breeding. The current technologies such as ultrasound, heat or invasive

examination by experienced veterinarians only work with any degree of confidence after 40 days of conception.

Several factors affecting net calf crop in a disease-free beef herd that was naturally mated over 14 years were summarized by Bellows et al. (1979). Four factors reducing net calf crop (71.0%) were non-pregnancy of females (17.4%), perinatal calf death (6.4%), calf deaths between birth and weaning (2.9%), and fetal deaths during gestation (2.3%). The primary cause of the delay in conception and pregnancy identification in the cow is lack of a diagnostic method to determine the actual results within a very short time after breeding.

The length of gestation in the cow, from the time of fertilization until actual birthing, of a calf ranges between 279-285 days with an average of 283 days. Therefore, 60 percent of the reduction in calf may could be attributed to failure to mate, fertilization failure and/or embryonic mortality. This is a very conservative estimate because beef cows could be mated two to three times during the 45 to 60 day exposure to bulls. Dairy cattle or artificially inseminated cattle can be "short cycled" to allow for maximum breeding opportunities.

Development of an easily used test offers an economic benefit for those in the breeding and beef industry in several ways. For example, it allows the beef ranchers to rotate their cows to high quality bulls, or in the artificial insemination industry (70% of

cows in USA are artificially inseminated especially in the dairy industry) a rapid test provides early results on cows who had been inseminated. This would allow the rancher to present his non-pregnant cows to the bull 22 days later or allow the rapid test to pick up non-pregnant cows 6-21 days after breeding, which can then be injected with prostaglandin to create a short cycle and be bred again 3 days later.

Maternal recognition must take place about day 16 to 17, or the uterus will produce prostaglandin F2a which will regress the corpus luteum and allow progesterone levels to drop with the result that the embryo can not implant. Significant embryo losses can occur during this period due to either failure of the embryo to produce the signal or failure of the mother to recognize the signal from the embryo. In either one of these situations, the cow can be short cycled or allowed to naturally return to estrous in about 21 days.

A current method for determining pregnancy in cow involves a rectal palpitation which requires an experienced veterinarian or technician to detect pregnancy as early as 30 days after breeding or insemination. This method requires great care, much practice and a well-developed sense of touch. Some experienced vets can detect a pregnancy by 30 days but very few persons can reliably determine pregnancy using this method. The risk factor of Bovine abortions is extremely high until day 20. Rectal palpitation is an

invasive manual veterinary diagnostic method that is costly, requires a specialist and carries risk of peritonitis and death of the cow and calf.

Early detection of pregnancy in bovines will significantly improve and provide greater efficiency in the production of bovines resulting in a great economic benefit.

Therefore, there is a need for a simpler, rapid and accurate means for detecting pregnancy in bovines. There is also a need for a method for early detection of pregnncy in bovines.

SUMMARY OF THE INVENTION

The present inventors have developed an early immunochromatographic assay which detects specific markers (*i.e.*, factors) present in saliva, sera or blood which indicate whether the conception (*i.e.*, pregnancy), has occurred. By using the assay method of the present invention, pregancy detection as early as 30 to 48 hours (the fertile life span of bovine sperm) after insemination (as opposed to the currently forty days) is feasible.

The present invention provides a method for producing an antibody to a factor indicating conception and pregnancy (*i.e.*, early pregnancy factor) and its use in detecting pregnancy in any animal producing the early pregnancy factor after pregnancy. While not wishing to be bound by any such representation, it is believed that, in addition to bovines, animals which produce early pregnancy factor include without limitation humans; domestic animals such as

swine, sheep and goats; and other mammals such as rabbits, cats and dogs. Although the present invention will now be described in reference to detecting pregnancy in a bovine, it should be appreciated that the invention is not limited to early pregnancy detection in a bovine. As used in this invention, the term "antibody" refers to an immunoglobulin which binds to a specific antigen or hapten. Specifically, the term "antibody" refers to an antibody to a bovine's early pregnancy factor, unless otherwise stated. The term "a factor indicating conception and pregnancy" or "an early pregnancy factor" refers to a compound, *for example*, a protein or a hormone, that is present in the blood stream and is produced by a pregnant cow in the early stages of the pregnancy and is not produced in any significant amount, if at all, by a non-pregnant cow. It should be understood that the early pregnancy factor may include more than one type of compound. However, for the sake of brevity and clarity, both plural and singular forms of the early pregnancy factor and the corresponding antibodies will be referred to in a singular form. Thus, the antibody of the present invention can be a polyclonal antibody or a monoclonal antibody, unless otherwise stated. Without being bound by any theory, it is believed that the early pregnancy factor is produced in cow within about 5 days after becoming pregnant, preferably within about 2 days, and more preferably within about 1 day. Moreover, the early pregnancy factor is believed to decrease to undetectable level

after about 40 days.

The antibody of the present invention is produced by obtaining a biological fluid of a cow in its early pregnancy, injecting the biological fluid to another animal which is capable of producing antibody to the biological fluid, isolating antibodies raised against the biological fluid and substantially removing antibodies that are negative to the early pregnancy factor.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a test device for use in an assay test in accordance with the invention;

Figure 2 is an illustration of the test device of Figure 1 showing the direction of liquid sample flow in accordance with the invention;

Figure 3 illustrates a variety of detection zone markers for a possible positive pregnancy test indication;

Figure 4 shows one embodiment of the top portion of a liquid sampling tube of the present invention for use with a test device;

Figure 5 shows one embodiment of a body portion of a liquid sampling tube of the present invention; and

Figure 6 shows one embodiment of a bottom portion of a liquid sampling tube of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The production of an antibody in response to a given antigen or hapten is common occurrence in humans and animals. The antibody

is polyclonal in mammals; however, monoclonal antibodies can also be produced, for example, by using carcinoma cell lines.

The antibody in the present invention can be prepared by taking a biological fluid sample from a bovine that is less than 100 days pregnant, preferably from about 20 days to about 40 days pregnant. The term "biological fluid" refers to any fluid that can be obtained from the bovine. Such fluids include blood, saliva, urine, milk, perspiration and chorionic fluid. Preferably, the biological fluid is blood. For the production of the antibody, acellular fraction is separated from the cellular fraction by any known method such as by centrifuge, settling, or filtration. The acellular fraction, *i.e.*, serum, is then injected into a non-bovine animal following standard immunization procedures. Exemplary non-bovine animals useful in generating antibodies to the early pregnancy factor include mammals such as sheep, goats, equines, swine, mice, rabbits and poultry. In addition, an egg can also be used to produce an antibody, *e.g.*, IgY, for the early pregnancy factor. After an appropriate incubation period, the antibody is isolated from the non-bovine animal. Typically, the incubation period is from about 10 days to about 45 days, preferably from about 20 days to about 30 days, and more preferably from about 25 days to about 30 days. Isolation of the antibody of the present invention generally involves obtaining antibodies present in the blood or the lymph node system of immunized animal and isolating

the antibody to the bovine early pregnancy factor. For example, in cases where a goat is used to generate the antibody, the goat is incubated with the serum containing bovine early pregnancy factor for about 6 weeks to about 2 months. The blood of the goat is then obtained and antibodies, *e.g.*, immunoglobulin γ (*i.e.*, Igγ), is purified by Protein A affinity chromatography. Igγ is further purified to remove the majority of the non-early pregnancy factor antibodies by passing through a column that contains immobilized normal, *i.e.*, non-pregnant, bovine serum. The purity of the antibody can be further increased by passing the resulting antibody through another column containing immobilized non-pregnant bovine serum. The antibody thus obtained is negative against, *i.e.*, does not bind to, proteins present in normal cow serum. The purity of the antibody of the present is at least about 30 % by weight (by wt.), preferably at least about 60 % by wt., and more preferably at least about 90 % by wt.

Monoclonal antibody to the bovine early pregnancy factor can also be prepared using a process similar to that discussed by Milstein and Kohler as reported in Nature, **1975**, *256*, 495-497. Generally, the preparation of a monoclonal antibody involves injecting a mouse (or other suitable animal) with partially or completely purified bovine early pregnancy factor. The immunized animals are sacrificed and the cells from their spleens are fused, *e.g.*, with mouse myeloma cells. The result is a hybrid cell, known

as a "hybridoma" that is capable of reproducing *in vitro*. The population of hybridomas is then screened for immunoglobulin production using any of the known methods, for example, as described in U.S. Patent No. 4,016,043. The immunoglobulins present in the cell culture fluids are then further examined for their ability to react with the bovine early pregnancy factor used for immunization.

The antibody of the present invention may be used in a variety of manners, *e.g.*, in a solid phase or in a solution, to determine pregnancy in a female bovine. In one particular embodiment, a test device includes a dry porous solid phase with immobilized antibody in a detection zone. A use of solid phase assay with visual readout to determine the presence or the absence of a given substrate is generally described in U.S. Patent Nos. 4,703,017, and 5,656,503 which are incorporated herein by reference in their entirety.

The porous solid phase comprises any material or combination of materials having a high permeability to liquids. Exemplary porous solid phase materials include porous plastics material, such as polypropylene, polyethylene (preferably of very high molecular weight), polyvinylidene fluoride, ethylene vinyl acetate, acrylonitrile and polytetrafluoroethylene; glass fibers; paper; cellulosic materials such as nitrocellulose; cellulose esters such as cellulose acetate; Nylon®; Rayon®; polyester; polyethersulfone;

and other suitable materials known in the art or combinations thereof. It can be advantageous to pre-treat the porous solid phase material with a surface-active agent during manufacture, as this can reduce any inherent hydrophobicity in the porous solid phase material and therefore enhance its ability to take up and deliver a moist sample efficiently.

Preferably, the porous solid phase material includes nitrocellulose sheet having a pore size of between 0.1 micron and 20 micron, more preferably between 3 micron and 12 micron, and most preferably 5 micron and 8 micron.

The antibody may be bound to the matrix of the detection zone by any one of a number of methods known to the art. For example, the antibody may be absorbed onto various water insoluble matrices such as microtiter plates, Dextran beads, nylon web, glass, cellulose, polyacrylamide, charcoal, urethane, ceramic, or mixtures thereof; chemically bonded to the porous solid phase material, *i.e.*, by the formation of ionic or covalent bonds, including disulfide bond formation, hydrogen bonding, Van der Waals bonding and charge attraction; or physically attached to the porous solid phase material, *i.e.*, by absorption, entrapment in an insoluble matrix; and the like. The bound antibody can then be provided in a kit wherein body fluids from female bovine animals would be added and activity of the antibody with the fluids could be measured.

The antibody in the detection zone is relatively permanently

immobilized in the detection zone on the porous solid phase material and is therefore not mobile in the moist state. The relative positioning of the labelled antibody, if present within the test device, and the detection zone are such that a liquid sample which is applied to the device can pick up a labelled antibody (either from the liquid sample or the labelled antibody zone) and thereafter permeate into the detection zone. The antibody in the detection zone can be immobilized firmly with or without prior chemical treatment. For example, if the material comprising the porous solid phase is nitrocellulose, which is preferred, then the antibody can be immobilized firmly by applying a liquid solution containing the antibody and allowing it to dry. It is to be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, and in particular aliphatic carboxylic acids having from one to seven carbon atoms, with acetic acid being preferred. Such solid supports which are formed from cellulose esterified with nitric acid alone, or a mixture of nitric acid, are often referred to as nitrocellulose paper.

Although nitrocellulose is a preferred porous solid phase material, it is to be understood that other materials, having a surface area sufficient for supporting the antibody in a concentration as hereinafter described may also be employed for

producing such porous solid phase.

In general, the porous solid phase which is used in the assay has a surface area such that it is capable of supporting antibody in a concentration of at least about 0.2 $\mu M/cm^2$, and preferably in a concentration of at least about 1 $\mu M/cm^2$.

Preferably, the immobilized antibody in the detection zone is impregnated throughout the thickness of the porous solid phase material in the detection zone (*e.g.*, throughout the thickness of the sheet or strip if the porous solid phase material is in this form). Such impregnation can enhance the extent to which the immobilized antibody can capture any labelled antibody-early pregnancy factor complex present in the migrating sample.

The liquid solution useful in applying the antibody to the detection zone for immobilization can include a buffer solution. Useful buffer solutions include any buffer solution having a pH from about pH 6.5 to about pH 9.5. Exemplary useful buffer solutions include phosphate buffers with a phosphate level in the range of from about 10 mM to about 100 mM, phosphate buffered saline buffers, TRIS buffer pH 8.2 in the range of from about 10 mM to about 50 mM, TRIS buffered saline pH 8.2, acetate buffer, carbonate buffer, bicarbonate buffer, MOPS piperazine buffer, BIS-TRIS buffer, tricine buffer, HEPES buffer and the like.

The liquid solution can also include a surfactant. Any general surfactant can be used. Exemplary surfactants useful for

the present invention include Tween 20®, Triton X-100®. The amount of surfactant can be from about 0.01% to about 10%, preferably about 0.5%.

If the porous solid phase material comprises paper, the immobilization of the antibody in the detection zone needs to be performed by covalent linkage which can be achieved through chemical coupling using, for example, aldehydes, azo compounds, carboxylic acids, isothiocyanates, cyano compounds, CNBr, carbonyldiimidazole, or tresyl chloride.

Following the application of the antibody to the detection zone, the remainder of the porous solid phase material may be treated to block any remaining binding sites. Blocking can be achieved by treatment with protein (e.g., bovine serum albumin or milk protein), with polyvinyl alcohol or ethanolamine, surfactant, polymers (such as PVA, PVP, PEG), or combinations thereof.

The test device can also include a sample receiving zone for applying a sample, *e.g.*, a liquid biological sample such as urine, serum, or saliva. The porous solid phase material in the sample receiving zone can include any bibulous, porous or fibrous material capable of absorbing liquid rapidly. The porosity of the material can be unidirectional (*i.e.*, with pores or fibers running wholly or predominantly parallel to an axis of the member) or multidirectional (omnidirectional, so that the member has an amorphous sponge-like structure). Preferably the porous solid

phase material of the sample receiving zone should be chosen such that the sample receiving zone can be saturated with aqueous liquid within a matter of seconds. Preferably the material remains robust when moist. The liquid must thereafter permeate freely from the porous sample receiving zone into the remaining porous solid phase material of the test device. Although any of the above listed porous solid phase material can be used in the sample receiving zone, use of glass fiber in the sample receiving zone is preferred.

The test device can also include a labelled bovine early pregnancy factor antibody zone ("labelled antibody zone") within the porous solid phase located downstream relative to the sample receiving zone but upstream relative to the detection zone. As used in this invention, the term "labelled antibody" refers to antibody which is bound to a label. The label can be any entity the presence of which can be readily detected by any of the known methods in the art including by visual inspection, using an instrument, conducting a chemical reaction and combinations thereof. The term "visual" as used herein means that the results of the test can be seen without the use of instrumentation, *i.e.*, with the naked eye. Preferably the label is a direct label, *i.e.*, an entity which, in its natural state, is readily visible either to the naked eye, or with the aid of an optical filter and/or applied stimulation, *e.g.*, UV light to promote fluorescence. For example, minute colored particles, such as dye sols; metallic sols such as

gold, platinum, palladium, iron, copper; colored latex particles; and carbon sols are all suitable. Of these metallic sol particles are preferred with gold sol particles being particularly preferred. The term "sol particle" refers to particles of a sol. And the term "metal sol particle" refers to particle of a sol, consisting of a metal, a metal compound or polymer nuclei coated with a metal or metal compound. The useful size of a sol particle depends on the particular sol particle being used, for example, for gold sols useful size ranges from about 15 nm to about 100 nm, with about 40 nm being particularly preferred. Using metallic and non-metallic sol particles for immunoassay is generally described in U.S. Patent Nos. 4,313,734 and 4,954,452, respectively, which are incorporated by reference herein in their entirety. Concentration of the label into a small zone or volume should give rise to a readily detectable signal, *e.g.* a strongly-colored area. This can be evaluated by eye, or by instruments if desired.

Indirect labels, such as enzymes (*e.g.*, alkaline phosphatase and horseradish peroxidase), radio isotopes, fluorescent compounds or other compound, which can be detected using appropriate instruments, can also be used but these usually require an instrument or the addition of one or more developing reagents such as substrates before a visible signal can be detected. Such additional reagents can be incorporated in the porous solid phase material or in the sample receiving zone, such that they dissolve

or disperse in the aqueous liquid sample. Alternatively, the developing reagents can be added to the sample before contact with the porous solid phase material or the porous solid phase material can be exposed to the developing reagents after the binding reaction has taken place.

Coupling of the label to the specific binding reagent can be by covalent bonding, if desired, or by hydrophobic or electrostatic bonding. Such techniques are well known to one of ordinary skill in the art. In general, the porous solid phase material which is used in the assay has a surface area such that it is capable of supporting labelled antibody in a concentration of at least about 0.2 $\mu M/cm^2$, and preferably in a concentration of at least about 0.5 $\mu M/cm^2$.

The selection of one or more suitable labelled antibodies on the porous solid phase material is deemed to be within the scope of those skilled in the art from the teachings herein.

To assist the free mobility of the labelled antibody when the porous solid phase is moistened with the sample, it is preferable for the labelled antibody to be applied to the porous solid phase as a surface layer, rather than being impregnated in the thickness of the porous solid phase material. This can minimize interaction between the porous solid phase material and the labelled antibody. In one particular embodiment of the present invention, the porous solid phase material is pre-treated with a glazing material in the

region to which the labelled antibody is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, *e.g.*, of sucrose or lactose, on the porous solid phase at the relevant portion, and drying. The labelled antibody can then be applied to the glazed portion. The remainder of the porous solid phase should not be glazed.

Alternatively, the labelled antibody can be provided separately from the test device and admixed with the sample prior to being applied to the sample receiving zone of the test device. In this manner, any bovine early pregnancy factor which may be present in the sample is allowed to bind with the labelled antibody prior to or during application of the sample to the sample receiving zone. Such labelled antibody can be provided as a solution or a solid which is then admixed with the sample. When the labelled antibody is in a solution, the solution generally comprises a buffer solution which stabilizes the labelled antibody, *i.e.*, prevents a significant decrease in the antibody activity by, *e.g.*, preventing decomposition or denaturing of the antibody. Useful buffer solutions include those described above. The solution may also include a surfactant described above.

Whether the labelled antigen is an integral part of the test device or is provided separately, it is essential that the labelled antibody migrates with the liquid sample as this progresses to the detection zone. Preferably, the flow of the sample continues

beyond the detection zone and sufficient sample is applied to the porous solid phase material in order that this may occur and that any excess labelled antibody which does not participate in any binding reaction in the detection zone is flushed away from the detection zone by this continuing flow. If desired, an absorbent "sink" can be provided at the distal end of the porous solid phase material. The absorbent sink may comprise, for example, Whatman 3MM chromatography paper, and should provide sufficient absorptive capacity to allow any unbound labelled antibody, *i.e*, conjugate, to wash out of the detection zone. As an alternative to such a sink it can be sufficient to have a length of porous solid phase material which extends beyond the detection zone.

The test device employed in the assay is preferably in sheet form, generally being in the form of a card, a test strip, a flow through device or dipstick, etc. It should be appreciated, however, that other forms of test devices are also within the spirit and scope of the invention. The antibody and the labelled antibody, if present, are applied in spatially distinct zones of the test device, and the liquid sample is allowed to permeate through the test device from one side or end to another. Generally, the porous solid phase of the test device is capable of conveying a liquid sample in a fluid flow direction parallel to the length of the test device.

The spatial separation between the zones (*i.e.*, sample

receiving zone, labelled antibody zone, if used, and the detection zone), and the flow rate characteristics of the porous solid phase material can be selected to allow adequate reaction times during which the necessary specific binding can occur, and to allow the labelled antibody in the labelled antibody zone to dissolve or disperse in the liquid sample and migrate through the porous solid phase material. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (*e.g.*, sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the porous solid phase material is "backed", *e.g.*, with plastic sheet, to increase its handling strength. This can be manufactured easily by forming a thin layer of porous solid phase material such as nitrocellulose on a sheet of backing material. The actual pore size of the porous solid phase material when backed in this manner may be lower than that of the corresponding unbacked material. Alternatively, a pre-formed sheet of porous solid phase material can be tightly sandwiched between two supporting sheets of solid materials, *e.g.*, between two supporting plastic sheets.

The antibody and/or the labelled antibody can be applied to the porous solid phase material in a variety of ways. Various "printing" techniques have previously been proposed for application of liquid solution of such reagents to porous solid phase materials, *e.g.*, micro-syringes, pens using metered pumps, direct

printing and ink-jet printing, and any of these techniques can be used in the present invention. To facilitate manufacture, the porous solid phase material (*e.g.*, sheet) can be treated with these reagents and then subdivided into smaller portions (*e.g.*, small narrow strips each embodying the required reagent containing zones) to provide a plurality of identical porous solid phase material units.

The sensitivity of the assay, *i.e.*, bovine pregnancy testing, can be increased by increasing the concentration of the labelled antibody and/or the antibodies on the detection zone. Accordingly, porous solid phase materials having high surface areas are particularly preferred in that the labelled antibody and/or the antibodies in the detection zone may be supported on such material in a high concentration. It should be appreciated, however, that the concentration of the labelled antibody and/or the antibodies in detection zone which is actually used is dependent in part on the binding affinity of the labelled antibody and/or the antibodies in the detection zone. Therefore, the scope of the present invention is not limited to a particular concentration of labelled antibody and/or the antibodies on the detection zone.

[Testing method]

Pregnancy testing of a female bovine generally involves contacting the test device as set forth above with an aqueous

liquid sample (*e.g.*, liquid biological sample such as serum, saliva, urine or chorionic fluid) of the female bovine. The sample is allowed to permeate by capillary action through the porous solid phase material via the labelled antibody zone, if present, migrates from the labelled antibody zone to the detection zone and the labelled antibody migrates therewith from the solution or from the labelled antibody zone, depending on how the labelled antibody is presented in the test. Thus, the labelled antibodies are carried downstream as the liquid sample front is moved along the test device by a capillary action. As the liquid sample moves along the length of the test device, any early pregnancy factor that may present in the liquid sample binds to the labelled antibody and the resulting complex is carried along the length of the test device to the detection zone. The presence of an early pregnancy factor in the sample can be determined by observing the extent (if any) to which the complex becomes bound in the detection zone. The labelled antibody zone, if present in the test device, is located at a height sufficiently high enough in the test device such that when the liquid sample is applied, the labelled antibody zone is above the liquid sample level to avoid having the labelled antibodies diffusing into the liquid sample itself.

When the test device includes both the labelled antibody zone and the detection zone, the distance between these two zones should be sufficiently large enough to allow the formation of labelled

antibody-early pregnancy factor complex prior to the capture of the complex by the antibodies present in the detection zone. Preferably such a distance is at least about 1 cm, and more preferably at least about 2 cm.

The detection zone can be any shape which aids in visualizing the bovine pregnancy test result. For example, as shown in Figure 1, the detection zone can be a strip of line across the partial or entire width of the test device, an "X", "+", a check mark, a circle, or a ring.

Preferably, the observation is made visually by the presence of a particular color within the detection zone where the antibody has been immobilized. For example, with gold sols as labels the color red indicates the positive result, with carbon or palladium sols the positive pregnancy is indicated by black color, and a wide variety of color can be used as indicator for latex particles depending on the dye that is used.

[Liquid sampling device]

Another embodiment of the present invention provides a liquid sampling tube which may be used in conjunction with the test device discussed above. As used in this invention, the term "tube" refers to any conduit with a hollow interior. The cross section of the hollow interior of the tube can be a circle, rectangle, hexagon, triangle, oval or any similarly shaped symmetrical or non-

symmetrical area. The liquid sampling tube includes a transparent region for reading the result of a female bovine pregnancy test using the test device described above. Preferably the entire length of the tube is transparent. Such a liquid sampling tube can be made from any variety of materials which is transparent or allows incorporation of a transparent material on or near the detection zone of the above test device. Exemplary materials suitable for manufacturing the liquid sampling tube include polycarbonate, tetrafluoroethylene polymer, polyacetate, plastics, glass and combinations thereof.

The tube includes a bottom portion having a means for allowing a liquid to enter into the tube. Such a means includes any device which allows a liquid sample to enter into the tube. Exemplary devices include an opening in the bottom portion of the tube, a filter which may or may not be removable, a porous membrane, and combinations thereof. When a filter is used to allow liquid to enter into the tube while preventing any solids from entering the tube, the porosity of the filter should be sufficiently large enough to allow any dissolved material, in particular the early pregnancy factor, within the liquid to also enter into the tube. Thus, the filter should have porosity of no greater than 200 micron with a flow rate greater than 10 liters per minute per square meter of material, at a differential pressure of 1 bar.

The liquid sampling tube of the present invention also

includes a body portion having at least one opening or pressure equalizing means. A "pressure equalizing means" refers to a device or a means for allowing the pressure within the tube to equalize with the pressure outside the tube. Such means includes an opening (*e.g.*, a hole), and a porous membrane. The presence of means for equalizing pressure allows a relative control as to how much the liquid is sampled by the tube.

The liquid sampling tube of the present invention also includes a top portion for enclosing the tube. In this manner, the gas that is displaced within the tube by the liquid sample is released primarily through the pressure equalizing means.

The amount of liquid sampling by the tube is substantially proportional to the height of the opening on the body portion of the tube relative to the bottom of the tube.

The liquid sampling tube of the present invention eliminates the requirement for a small sample to be removed from the body of a liquid in order to be tested. The process of correct use of the liquid sampling tube also ensures that sufficient liquid material is presented to the testing area, provided that sufficient material is available. The requirement for accurate placement of the sample is also eliminated as the design of the liquid sampling device ensures that with correct operation, material is only presented to the correct sampling location.

Those skilled in the art will appreciate that numerous changes

and modifications may be made to the preferred embodiments of the invention and that such changes and modifications may be made without departing from the spirit of the invention. It is therefore intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A composition for testing pregnancy of an animal comprising:

(a) a label; and

(b) an antibody to an early pregnancy factor of an animal conjugated thereto.

2. The composition of Claim 1, wherein said animal is a bovine.

3. The composition of claim 1, wherein said label is a sol particle.

4. The composition of claim 3, wherein said sol particle is selected from the group consisting of gold, silver, platinum, latex, carbon, palladium, iron, copper, zinc and mixtures thereof.

5. The composition of Claim 3, wherein said sol particle is gold.

6. The composition of Claim 5, wherein said gold sol particle has a particle size of from about 15 nm to about 100 nm.

7. The composition of Claim 6, wherein said gold sol particle has a particle size of about 40 nm.

8. The composition of Claim 1, wherein said antibody is prepared by the process comprising:

(a) injecting a liquid biological sample of said animal to a second animal, wherein said liquid biological sample comprises said early pregnancy factor, and wherein said second animal is

capable of producing said antibody to said early pregnancy factor of said pregnant animal;

(b) isolating antibodies from said second animal; and

(c) substantially removing antibodies that are negative to said early pregnancy factor of said animal.

9. The composition of Claim 8, wherein said pregnant animal is a bovine which is pregnant for less than about 100 days.

10. The composition of Claim 9, wherein said bovine is pregnant for from about 20 days to about 40 days.

11. The composition of Claim 8, wherein said liquid biological sample is selected from the group consisting of serum, urine, saliva, milk, perspiration and combinations thereof.

12. The composition of Claim 11, wherein said liquid biological sample is serum.

13. The composition of Claim 9, wherein said pregnant bovine is a cow.

14. A test device for detecting an animal early pregnancy factor, said test device comprising:

(a) a porous solid phase material capable of conveying a liquid sample in a fluid flow direction generally parallel to the length of said test device;

(b) a sample receiving zone within said porous solid phase material whereat the liquid sample and other assay reagents may be contacted with said device; and

(c) an antibody zone within said porous solid phase material comprising an immobilized antibody to said early pregnancy factor of said animal disposed at a downstream location from said sample receiving zone.

15. The test device of Claims 14, wherein said animal is a bovine.

16. The test device of Claims 14, wherein said porous solid phase material further comprises a labelled antibody zone disposed at a downstream location from said sample receiving zone and upstream from said antibody zone.

17. The test device of Claim 16, wherein said labelled antibody comprises a particle composition, wherein said particle composition comprises:

(a) a label; and

(b) an antibody to said early pregnancy factor of said animal conjugated thereto.

18. The test device of Claim 17, wherein said label is a sol particle.

19. The test device of Claims 18, wherein said sol particle is selected from the group consisting of gold, silver, platinum, latex, carbon, palladium, iron, copper, zinc and mixtures thereof.

20. The test device of Claim 19, wherein said sol particle is gold.

21. The test device of claim 20, wherein said gold sol particle has a particle size of from about 15 nm to about 100 nm.

22. The test device of Claim 20, wherein said gold sol particle has a particle size of about 40 nm.

23. The test device of Claim 14, wherein said antibody is isolated by a process comprising:

(a) injecting a liquid biological sample of said animal to a second animal, wherein said liquid biological sample comprises said early pregnancy factor, and wherein said second animal is capable of producing said antibody to said early pregnancy factor of said pregnant animal;

(b) isolating antibodies from said second animal; and

(c) substantially removing antibodies that are negative to said early pregnancy factor of said animal.

24. The test device of Claim 23, wherein said pregnant animal is a bovine which is pregnant for less than about 100 days.

25. The test device of Claim 24, wherein said bovine is

pregnant for from about 20 days to about 40 days.

26. The test device of Claim 23, wherein said liquid biological sample is selected from the group consisting of serum, urine, saliva, milk, perspiration and combinations thereof.

27. The test device of Claim 26, wherein said liquid biological sample is serum.

28. The test device of Claim 24, wherein said pregnant bovine is a cow.

29. A method for determining pregnancy of an animal comprising:

(a) obtaining a liquid biological sample of said animal; and

(b) testing for a presence of an early pregnancy factor in said liquid biological sample.

30. The method of Claim 29, wherein said animal is a bovine.

31. The method of Claim 29, wherein said test comprises contacting said liquid biological sample to a pregnancy test device, wherein said test device comprises:

(a) a porous solid phase material capable of conveying a liquid sample in a fluid flow direction generally parallel to the length of said test device;

(b) a sample receiving zone within said porous solid phase material whereat the liquid sample and other assay reagents may be contacted with said device; and

(c) an antibody zone within said porous solid phase material comprising an immobilized antibody to said early pregnancy factor of said animal disposed at a downstream location from said sample receiving zone.

32. The method of Claim 31, wherein said porous solid phase material further comprises a labelled antibody zone disposed at a downstream location from said sample receiving zone and upstream from said antibody zone.

33. The method of Claim 32, wherein said labelled antibody comprises a particle composition, wherein said particle composition comprises:

(a) a sol particle; and

(b) an antibody to said early pregnancy factor of said animal conjugated thereto.

34. The method of Claim 33, wherein said sol particle is gold.

35. The method of Claim 34, wherein said gold sol particle has a particle size of from about 15 nm to about 100 nm.

36. The method of Claim 34, wherein said gold sol particle has a particle size of about 40 nm.

37. The method of Claim 33, wherein said antibody is isolated by a process comprising:

(a) injecting a biological fluid of said animal to a second animal, wherein said second animal is capable of producing antibody to said biological fluid, and wherein said biological fluid comprises said early pregnancy factor of said animal;

(b) isolating antibodies from said second animal; and

(c) substantially removing antibodies that are negative to said early pregnancy factor of said animal.

38. The method of Claim 37, wherein said animal is a bovine which is pregnant for from about 20 days to about 40 days.

39. The method of Claim 37, wherein said biological fluid is

selected from the group consisting of serum, urine, saliva, milk, perspiration and combinations thereof.

40. The method of Claim 39, wherein said biological fluid is serum.

41. The method of Claim 29, wherein said liquid biological sample is contacted with a particle composition prior to said step (b), wherein said particle composition comprises:

(a) a sol particle; and

(b) an antibody to said early pregnancy factor of said animal conjugated thereto.

42. The method of Claim 41, wherein said sol particle is gold.

43. The method of Claim 42, wherein said gold sol particle has a particle size of from about 15 nm to about 100 nm.

44. The method of Claim 42, wherein said gold sol particle has a particle size of about 40 nm.

45. The method of Claim 42, wherein said antibody is isolated by a process comprising:

(a) injecting a biological fluid of said animal to a second animal, wherein said second animal is capable of producing antibody to said biological fluid, and wherein said biological fluid comprises said early pregnancy factor of said animal;

(b) isolating antibodies from said second animal; and

(c) substantially removing antibodies that are negative

to said early pregnancy factor of said animal.

46. The method of Claim 45, wherein said animal is a bovine which is pregnant for from about 20 days to about 40 days.

47. The method of Claim 45, wherein said biological fluid is selected from the group consisting of serum, urine, saliva, milk, perspiration and combinations thereof.

48. The method of Claim 47, wherein said biological fluid is serum.

49. A method for isolating an antibody to an early pregnancy factor of an animal comprising:

(a) injecting a biological fluid of said animal to a second animal, wherein said second animal is capable of producing antibody to said biological fluid, and wherein said biological fluid comprises said early pregnancy factor of said animal;

(b) isolating antibodies from said second animal; and

(c) substantially removing antibodies that are negative to said early pregnancy factor of said animal.

50. The method of Claim 49, wherein said animal is a bovine.

51. The method of Claim 50, wherein said bovine is pregnant for less than about 100 days.

52. The method of Claim 50, wherein said bovine is pregnant for from about 20 days to about 40 days.

53. The method of Claim 49, wherein said liquid biological sample is selected from the group consisting of serum, urine, saliva, milk, perspiration and combinations thereof.

54. The method of Claim 49, wherein said liquid biological sample is serum.

55. The method of Claim 50, wherein said pregnant bovine is a cow.

56. The method of Claim 50, wherein said second animal is selected from the group consisting of goat, rabbit, a poultry, sheep, swine, feline, horse, donkey, ass, rodents and dog.

57. The method of Claim 50, wherein said second animal is goat.

58. A liquid sampling tube comprising:

(a) a bottom portion having a means for allowing a liquid sample to enter into said tube;

(b) a body portion having at least one opening; and

(c) a top portion for enclosing said tube,

wherein the amount of liquid sampling is substantially proportional to the height of said opening on said body portion relative to the bottom of said tube.

59. The liquid sampling tube of Claim 58, wherein said means comprises at least one opening in said bottom portion.

60. The liquid sampling tube of Claim 58, wherein said means comprises a filter, wherein said filter allows a liquid to enter to said tube but prevents solids from entering said tube.

61. The liquid sampling tube of Claim 60, wherein said filter has a porosity of less than about 100 nm.

62. The liquid sampling tube of Claim 60, wherein said opening on said body portion is about 30 cm above the bottom of said tube.

63. The liquid sampling tube of Claim 58, wherein said body portion is removably mounted partially inside said top portion.

64. The liquid sampling tube of Claim 58, wherein said bottom portion is removably mounted partially inside said body portion.

65. A bovine pregnancy testing kit comprising:

(a) a bovine pregnancy test device; and

(b) a liquid sampling tube.

66. The bovine pregnancy testing kit of Claim 65, wherein said test device comprises:

(a) a porous solid phase material capable of conveying a liquid sample in a fluid flow direction generally parallel to the length of said test device;

(b) a sample receiving zone within said porous solid phase material whereat the liquid sample and other assay reagents may be contacted with said device; and

(c) an antibody zone within said porous solid phase material comprising an immobilized antibody to a bovine early pregnancy factor disposed at a downstream location from said sample receiving zone.

67. The bovine pregnancy testing kit of Claim 66, wherein said porous solid phase material further comprises a labelled antibody zone disposed at a downstream location from said sample receiving zone and upstream from said antibody zone.

68. The bovine pregnancy testing kit of Claim 66, wherein said labelled antibody comprises a particle composition, wherein said particle composition comprises:

(a) a sol particle; and

(b) an antibody to said bovine early pregnancy factor

conjugated thereto.

69. The bovine pregnancy testing kit of Claim 68, wherein said sol particle is gold.

70. The bovine pregnancy testing kit of Claim 69, wherein said gold sol particle has a particle size of from about 15 nm to about 100 nm.

71. The bovine pregnancy testing kit of Claim 69, wherein said gold sol particle has a particle size of about 40 nm.

72. The bovine pregnancy testing kit of Claim 68, wherein said antibody is isolated by a process comprising:

(a) injecting a biological fluid of a pregnant bovine to a non-bovine animal;

(b) isolating antibodies from said non-bovine animal; and

(c) removing non-early pregnancy factor antibodies.

73. The bovine pregnancy testing kit of Claim 72, wherein said bovine is pregnant for from about 20 days to about 40 days.

74. The bovine pregnancy testing kit of Claim 72, wherein said biological fluid is selected from the group consisting of serum, urine, saliva, milk, perspiration and combinations thereof.

75. The bovine pregnancy testing kit of Claim 72, wherein said biological fluid is serum.

76. The bovine pregnancy testing kit of Claim 66, further comprising a particle composition comprising:

(a) a sol particle; and

(b) an antibody to said bovine early pregnancy factor conjugated thereto.

77. The bovine pregnancy testing kit of Claim 76, wherein said sol particle is gold.

78. The bovine pregnancy testing kit of Claim 77, wherein said gold sol particle has a particle size of from about 15 nm to about 100 nm.

79. The bovine pregnancy testing kit of Claim 77, wherein said gold sol particle has a particle size of about 40 nm.

80. The bovine pregnancy testing kit of Claim 66, wherein said antibody is isolated by a process comprising:

(a) injecting a biological fluid of a pregnant bovine to a non-bovine animal;

(b) isolating antibodies from said non-bovine animal; and

(c) removing non-early pregnancy factor antibodies.

81. The bovine pregnancy testing kit of Claim 80, wherein said biological fluid is serum.

82. The bovine pregnancy testing kit of claim 66, wherein said pregnant bovine is a cow.

83. The bovine pregnancy testing kit of claim 66, wherein said liquid sampling tube comprise:

(a) a bottom portion having a means for allowing a liquid

to enter into said tube;

(b) a body portion having at least one opening; and

(c) a top portion for enclosing said tube,

wherein the amount of liquid sampling is substantially proportional to the height of said opening on said body portion relative to the bottom of said tube.

84. The bovine pregnancy testing kit of claim 83, wherein said means comprises at least one opening in said bottom portion.

85. The bovine pregnancy testing kit of claim 83, wherein said means comprises a filter, wherein said filter allows a liquid to enter to said tube but prevents solids from entering said tube.

86. The bovine pregnancy testing kit of claim 85, wherein said filter has a porosity of less than about 100 nm.

87. The bovine pregnancy testing kit of claim 83, wherein said opening on said body portion is about 30 cm above the bottom of said tube.

88. The bovine pregnancy testing kit of claim 83, wherein said body portion is removably mounted partially inside said top portion.

89. The bovine pregnancy testing kit of claim 83, wherein said bottom portion is removably mounted partially inside said body portion.

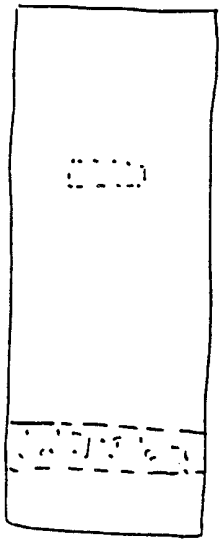


Fig. 1

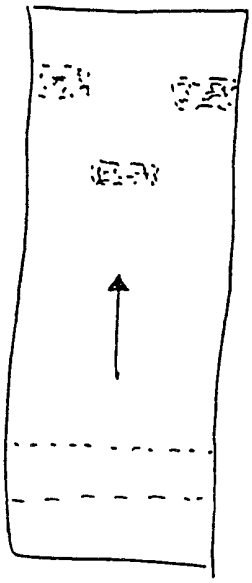


Fig 2

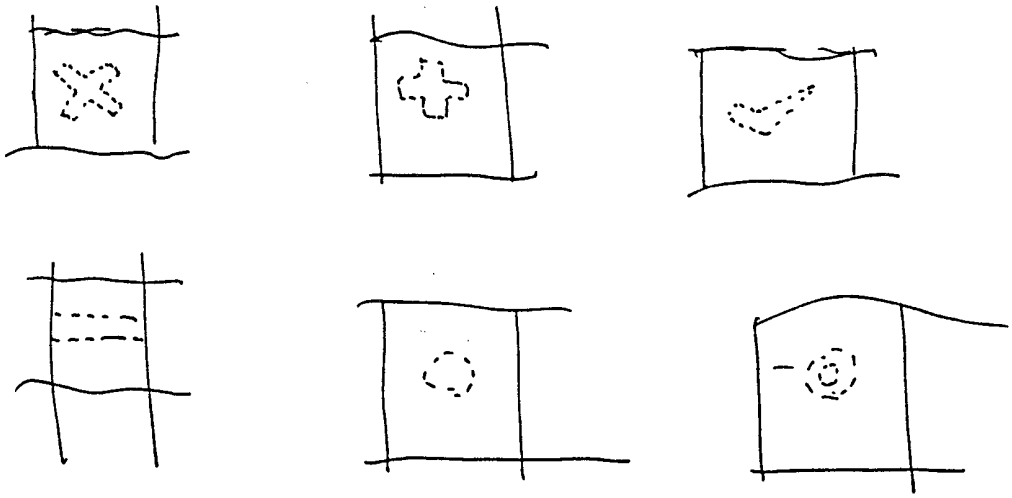


Fig. 3

Title: Body Part no P1

DO NOT SCALE

Material: Polycarbonate; clear

Tolerances: +/- 0.1 per 10mm. Unless otherwise stated

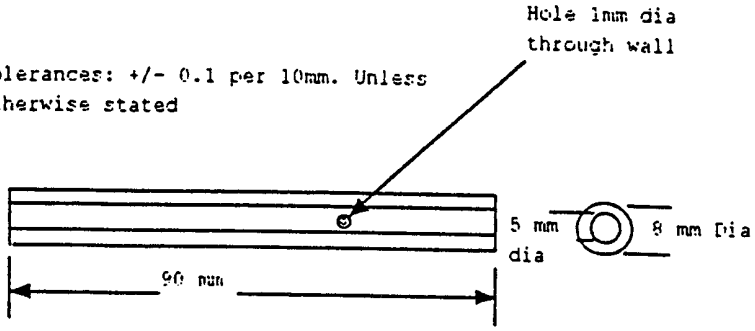


Fig 5

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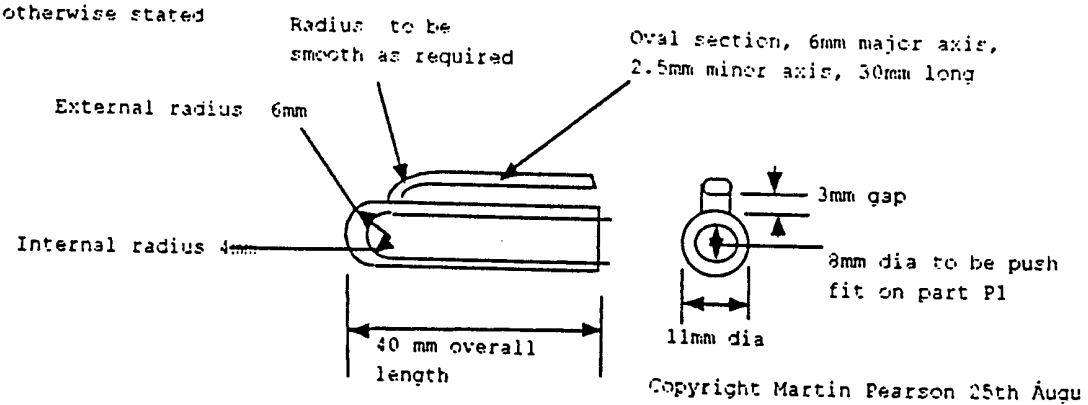
Title: End Part no P2

DO NOT SCALE

Material: Polycarbonate; clear

All edges to be smooth and blended as required.

Tolerances: +/- 0.1 per 10mm. Unless otherwise stated



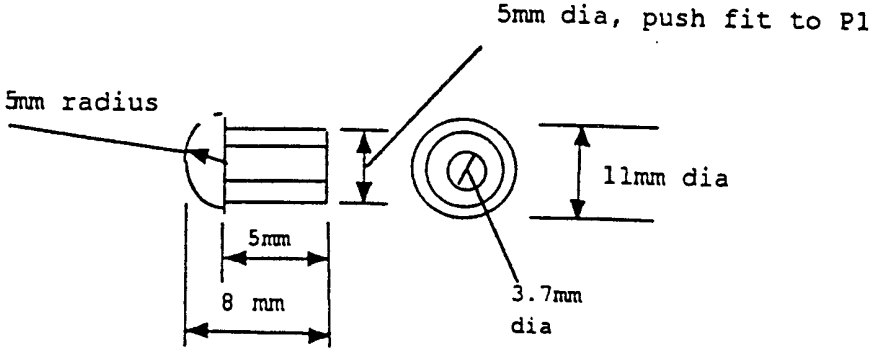
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Fig 4

Title: Bung Part no B1

Material: Nylon 66; black

Tolerances: +/- 0.1 per 10mm. Unless otherwise stated



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Fig 6

DO NOT SCALE