ABSTRACT:

The present invention provides an improved method for preparation of nosodes, which takes advantage of the advancements in microbiology, immunology and cellular biology, to standardize the therapeutic source material for nosodes, while enhancing the potency of nosodes, produced thereof. Further, the method provides: a clear guideline for nosode preparation in future, can be used to reproduce the already known nosodes with scientific standardization, enhanced therapeutic efficacy for known and new nosodes,

13 MAY 2011

FIELD OF INVENTION

The present invention relates to a method for preparation of nosodes.

DEFINITIONS OF TERMS USED IN THE SPECIFICATION

The term "homeopathic" or "homeopathy" used in the specification means a system for treating disease based on the administration of minute doses of a drug that in massive amounts produces symptoms in healthy individuals similar to those of the disease itself.

The term "nosodes" used in the specification means homeopathic preparations of organic material derived from disease products, cultures of micro organisms (e.g. bacteria, fungi, viruses or parasites), infected or pathologically changed material or decomposition products from humans or animals, rendered safe during the homeopathic manufacturing process.

The term "homeopathic potentization" used in the specification means serial dilution of the original source substance with neutral vehicle (water or alcohol) in a ratio of 1:99, usually mixed in a sterile glass bottle, exposed to about 10 powerful strokes. The procedure is called succussion. The homeopathic pharmacopeia suggest 1 drop versus 99 drops ratio.

The term "mother nosode preparation" used in the specification means a solution organic animal substance and alcohol/water made according to standards set by the Homeopathic Pharmacopeia and having a potency of 1 centesimal (c) or 2c.

13 MAY 2011

BACKGROUND

Homeopathy is a form of alternative medicine, first proposed by German physician Samuel Hahnemann in 1796, in which, practitioners use preparations which cause certain symptoms in healthy individuals, for patients exhibiting similar symptoms, in highly diluted form. Homeopathic remedies are prepared by serial dilution, until none of the original substance remains, with shaking by forceful striking, which is termed as *succussion,* after each dilution, under the assumption that this increases the effect, this process is termed as *potentization.* There is a similar method potentization called trituration which entails a process of grinding the source material with a vehicle in 1:10 ratio.

Homeopathic remedies use animal, plant, mineral, and synthetic substances such as arsenicum album (arsenic oxide), natrum muriaticum (sodium chloride or table salt), lachesis muta (the venom of the bushmaster snake), opium, thyroidinum (thyroid gland extract), etc., in their preparations. Also, homeopathic remedies use preparations called nosodes (taken from the Greek word 'nosos', which means disease) which are made from diseased or pathological products such as fecal, urinary, and respiratory discharges, blood, and tissue. Further, homeopathic preparations made from healthy specimens are called sarcodes. Nosodes, the homeopathic preparations sourced from diseased materials (secretions, discharges, and tissues) have been in use since a few years after the birth of homeopathy in 1796. Nosodes have attracted the attention of many medical scientists, and is responsible for the birth of sciences of organisms such as bacteriology, virology, parasitology, mycology, etc., and eventually the well defined practice of vaccinations.

13 MAY 2011

Interestingly, the use of organisms from the diseased material was in practice before Dr. Edward Janer propounded the concept of modern vaccines. For instance, medicinal preparations from tubercular material (bacteria), gonorrheal discharge (bacteria), small pox vesicles (virus), and scabies (parasites), were in use in homeopathy as medicines namely tuberculinum, medorrhinum, variolinum and psorinum, respectively; long before the discovery of mycobacretium tuberculosis, neisseria gonorrhoeae, variola major and sarcoptes scabiei, respectively. Other examples include lyssin (rabies virus, lyssavirus genus), pyrogen (mixed organisms), anthraxinum (bacillus anthracis), diptherinum (corynebacterium diphtheriae), syphilinum (treponema pallidum), etc.

Nosodes have been in use for the treatment of diseases, from where they are sourced, e.g. tuberculinum for tuberculosis, as well as for other disease conditions. For Example: syphillinum for vitiligo, lyssin for emotional disorders, etc. In fact, it has been proved that, nosodes are more effective for ailments other than the source diseases.

The preparation of nosodes, used as therapeutic measures in homeopathy, were developed and used before or around the time of evolution of modern microbiology, because of which the methods for preparation of nosodes, and thus nosodes, have some practical shortcomings, including: the primitive nosodes, developed from an infected material, were presumed to have only that specific organism, and were not checked by detailed microscopic study, to verify the composition thereof. Therefore, it can be said that nosodes were generally sourced from materials consisting of numerous other organisms and tissues; e.g. tuberculinum prepared from the sputum of a tuberculosis patient,

13 MAY 2011

must have contained plenty of other bacteria, proteins, debris, cells, and the like; as a result, although a specific nosode was believed to be from a specified organism, it was highly likely that the nosode was a mix of numerous unknown organisms. The presently known methods for obtaining nosodes use such obscure assumptions. Also, the presently known methods for preparation of nosodes are not standardized in terms of quality, quantity, size, weight, composition, type and subtype of organism, and the like; resulting in discrepancies in nosodes, giving nosodes not in accordance with the ones described in the homeopathic materia medica.

Therefore, there is felt a need for an improved method for preparation of nosodes, which takes advantage of the advancements in microbiology, immunology and cellular biology, to standardize the therapeutic source material for nosodes, while enhancing the potency of nosodes, produced thereof. Further, there is need for a method which provides: a clear guideline for nosode preparation, can be used to reproduce the already known nosodes with scientific standardization, enhanced therapeutic efficacy for known and new nosodes, nosodes that can be safely used, and development of novel nosodes from any microorganism including virus, bacteria, fungi, and the like.

OBJECTS OF THE INVENTION

An object of the present invention is to provide a method for preparation of nosodes.

Another object of the present invention is to provide a method for preparation of nosodes from any microorganism including virus, bacteria, fungi, and the like.

13 MAY 2011

Yet another object of the present invention is to provide a method for preparation of nosodes having high therapeutic potency.

Still another object of the present invention is to provide a method for preparation of nosodes with scientific standardization of the therapeutic source material.

One more object of the present invention is to provide a method for preparation of nosodes that can be safely used.

Still one more object of the present invention is to provide a method for preparation of already known nosodes with scientific standardization and enhanced therapeutic potency.

Yet one more object of the present invention is to provide a method which sets a guideline for preparation of nosodes in future.

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention there is provided a method for the preparation of nosodes, said method comprising the steps of:

- identifying a donor by screening the patients and collecting a sample which is qualitatively positive for a specific infection selected from the group consisting of HIV, hepatitis (A,B,C,D,E), CMV and herpes.

- screening the selected sample to rule out any co-infection selected

13 MAY 2011

from the group consisting of Syphilis, Gonorrhea, (VDRL), Tuberculosis, CMV, Human T-lymphotropic virus 1 and 2 (HTLV);

- geno-typing or subtyping of the virus present in the sample and optionally characterizing functionally active genetic components;

- quantifying the organism in the sample;

- separating serum from the sample by serum expression said serum expression comprising keeping the samples tubes on serum expression rack and keeping the rack under a table lamp;

- subjecting the sample to centrifugation at a speed 16

- to 2000 rpm for a period ranging between 15 to 30 minutes to separate serum blood cells and clotting proteins;

- filtering the sample by using a filtration device to filter out the unwanted particles including bacteria from the sample to obtain a mother serum;

- reconfirming the presence of the virus in the serum and standardizing using RT-PCR;

- subjecting the serum to PAGE pattern-SDS protein profile (sodium dodecyl sulfate polyacrylamide gel electrophoresis) to determine

13 MAY 2011

protein profile of the serum;

- diluting the mother serum with water for injection in a proportion ranging between 1:1 to 1:99, to obtain a pre-1c preparation;

- quantifying the number of viruses in a pre-1c preparation;

- potentizing the pre-1c preparation by a method selected from the group consisting of succusion and trituration;

- diluting the potentized pre-1c preparation with water for injection in a proportion ranging between 1:1 to 1:99 to obtain a preparation with 1 c potency;

- serially diluting and potentizing the preparation with 1 c potency with water for injection to obtain preparation with potencies ranging between 2c to 2 million c;

- checking for the absence of the virus in the preparations by spiking the preparations with a mother serum followed by subjecting the spiked preparations to at least one method selected from the group consisting of RT-PCR, ultra electro-microscopy, cell-infectivity test and pro-viral DNA study.

Ultraelectromicroscopy is able to magnify the sample by 1,00,000 time, capable of viewing viral particles in the potentized preparation.

13 MAY 2011

In pro-viral count study, HIV inside a cell that has been converted to DNA is counted. The HIV DNA can either be in the integrated or un-integrated with the DNA within a cell.

In accordance with one embodiment of the present invention, the method of the present invention further comprises a method step of lyophilizing part of the serum for future use optionally.

Typically, the serum comprises more than one genotype of the virus.

Typically, the potentization is carried out in an electromechanical machine having an arm with a length of 55cm and mass of 7.5kg; said machine being adjusted to drop the arm through an angle of 90^0.

Typically, the organism is a virus selected from the group consisting of HIV, hepatitis and herpes.

DETAILED DESCRIPTION OF THE INVENTION

The description provided is purely by way of examples and illustrations which do not limit the scope and ambit of the invention.

The present invention envisages an improved method for preparation of nosodes, which takes advantage of the advancements in microbiology, immunology and cellular biology, to standardize the therapeutic source material for nosodes, while enhancing the potency of nosodes, produced thereof. Further, the method provides: a clear guideline for nosode preparation in future,

13 MAY 2011

can be used to reproduce the already known nosodes with scientific standardization, enhanced therapeutic efficacy for known and new nosodes, nosodes that can be safely used, and development of novel nosodes from any microorganism including virus.

In accordance with a first aspect of the present invention there is provided a method for the preparation of nosodes.
The method of the present invention begins with the identification of a donor by screening the patients and collecting a sample which is qualitatively positive for a specific infection. Typically, the specific infection is selected from the group consisting of HIV, Hepatitis, CMV and Herpes.

The sample thus selected is screened using various standard tests and assays to eliminate the possibility of any co-infection. Typically, the screening is carried out to eliminate the occurrence of following co-infections in the sample: Syphilis, Gonorrhea, (VDRL), Tuberculosis, CMV, Human T-lymphotropic virus 1 and 2 (HTLV), HIV, Hepatitis. To ensure high potency and purity, it is ensured that the selected sample is essentially devoid of any co-infection.

After the identification of a suitable sample, it is genotyped or subtyped to indentify specific genetic components in the viruses. Optionally, the functionally active components are characterized. Typically, the genotyping is carried out by employing a RT-PCR method. Alternatively, depending on the nature of the virus, different specific markers are employed for genotyping. If the patient selected for collecting a sample is HIV positive, then genotypes HIV type I and type II are identified. Similarly, in case of Hepatitis C genotypes I to VI are indentified. Typically, the sample contains only one genotype of the

13 MAY 2011

virus. Alternatively, the sample contains more than one type of genotypes for the virus rendering the sample polyvalent.

The method of nosode preparation in accordance with the present invention also involves quantification of viral particles in the sample. Typically, the microbial count per milli litre (ml) is found by microbial count processes known in the art.

After quantification, the serum is separated from the sample by serum expression. Serum expression carried in accordance with the process of the present invention comprises keeping the samples tubes on serum expression rack. Typically, the racks are kept under a table lamp. The heat of the lamp is preferred because it helps in serum separation without affecting the serum components.

The sample is then subjected to centrifugation at a speed 1500 to 2000 rpm for a period ranging between 15 to 30 minutes to separate serum blood cells and clotting proteins. A centrifuge separates out blood components such as RBC's, WBC's. The centrifuged samples are further subjected to filtration using a filtration device to filter out the unwanted particles including bacteria from the sample to obtain a mother serum. Typically, the filtration device that is employed is such that it filters off unwanted bacteria but retains the virus and /or viral particles in the serum.

It has been observed that Heat inactivation of serum leads to loss of antigenicity in the serum. It is for this reason; the inventors of the present invention have not employed the heat inactivation procedure.

13 MAY 2011

The presence of the virus in the mother serum is reconfirmed in the serum using RT-PCR and the mother serum is subjected to PAGE pattern-SDS protein profile (sodium dodecyl sulfate polyacrylamide gel electrophoresis) for determining the protein profile of the serum. This provides the nature of soluble proteins in the sample. Typical, serum profiles suggestive of specific positive or negative viral profile are checked on the basis of standard assays.

A pre-1c preparation is prepared from the mother serum after protein profiling. Typically, the mother serum is diluted with water for injection in a proportion ranging between 1:1 to 1:99. The inventors of the present invention have specifically avoided the use of other vehicles such as alcohol. Its been observed by the inventors of the present invention that alcohol affects the antigenicity of the viral particles and thereby affecting the efficacy of the preparations.

The number of viral particles present in the pre-1 c preparation is quantified again. At this stage, the pre-1c preparations are standardized with reference to the number of particles present in them. Typically, viral count ranges between 500 to about 1 million copies per millimeter, preferably greater than 10,000 copies/ml.

The standardized pre 1c preparations are potentized. Typically, the potentization is carried out in an electromechanical machine having an arm with a length of 55cm and mass of 7.5kg; said machine being adjusted to drop the arm through an angle of about 90^0. The potentized pre-1c preparation thus obtained is diluted with water for injection in a proportion ranging between 1:1 to 1:99 to obtain a preparation with 1 c potency.

13 MAY 2011

Depending on the requirement, the preparation with 1c potency is serially diluted with water for injection to obtain preparations with potencies ranging between 2c to 2 million c.

The preparations with various potencies are finally checked for the absence of the virus. Typically, the method for checking the absence of the viral particles in the preparations comprises spiking the preparations with a mother serum followed by subjecting the spiked preparations to at least one method selected from the group consisting of RT-PCR, ultra electro-microscopy, cell-infectivity test and pro-viral DNA study.

In pro-viral count study, HIV inside a cell that has been converted to DNA is counted. The HIV DNA can either be in the integrated or un-integrated with the DNA within a cell.

Ultraelectromicroscopy is able to magnify the sample by 1,00,000 time, capable of viewing viral particles in the potentized preparation.

The method of present invention is herein explained with the help of following non-limiting examples related to HIV nosodes. Generally, all the known possible co-infections were ruled out from the source serum, therefore, assuring that the source serum / tissues was positive only for the specific infection; and negative for all the known other infections.

Other nosodes can be prepared using a similar method, with a few variations in terms of viral load (or microbe quantification), types of virus (or microbes), elimination of co-infections, use or omission of filtration, etc. Primarily, the

13 MAY 2011

blood donor who was verified to be qualitatively positive for a specific infection, from which the nosode is to be developed, was identified.

For developing nosodes for HIV, two separate donors were identified qualitatively positive for the HIV type I and type II infections, respectively, and blood samples were collected separately from these donors. For developing nosodes for Hepatitis C, blood samples were collected from patients positive for various geno-types such as Geno-type I, II, III, IV, V, VI, etc.

The blood samples were then checked for common co-infections including: hepatitis C, HSsAg (hepatitis B), human T-lymphotropic virus 1 & 2 (HTLV), syphilis, Gonorrhea (VDRL), and cytomegalovirus. In future, other newly discovered possible co-infections can be ruled out from the source serum. In case of HIV, the blood sample is mainly negative for the other co-infections.

Nosode was developed with one of the geno-types. The blood sample was geno-typed for the disease causing agent (a virus in this case). By geno-typing of the virus the genetic nature of the exact virus, was determined.

The blood samples were quantified to calculate the viral load. The blood samples were investigated to find. A sample containing HIV viral count of more than 20,000 per milli litre (ml) was used for the experiments.

When 3 ml of total sample was used for potentization, in a regular glass bottle, a proportion of 1 : 99 (for preparing the first potency), the serum to vehicle ratio was 2.97 ml : 0.03 ml in the 3 ml total sample. Here, the quantity of organisms in 0.03ml was calculated. The viral count in the HIV serum was

13 MAY 2011

5,00,000 per ml, and therefore 0.03ml of the sample contained 5000 copies of virus.

The blood sample tubes with serum separated therein were centrifuged at 1500 – 2000 rpm for 15 to 30 minutes, to better separate the serum and the filtrate, thereof obtain a mainly bacteria-free preparation which was further filtered off by using a Seitz filter, generating a bacteria-free virus containing preparation.

The presence of the virus in the serum was reconfirmed by standardization with respect to the viral copies (e.g. HIV copies) by using a poly chain reaction (RT-PCR). This process confirmed whether the serum had a decided number of viral copies thereby ensuring the desired anti-genicity. Further, by using a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) the protein profile of the virus preparation was defined. In the present case, the PAGE profile reflected a typical serum profile suggestive of a specific positive or negative viral profile checked on the basis of standard assays. In virus preparations from a HIV positive mother, the serum was confirmed to be positive for anti-HIV and negative for anti-hepatitis C and hepatitis B (HBsAg).

The virus preparations were next passed through the potency making procedure, i.e. homeopathic potentization. The potentization procedure of the present invention provides freedom for variations in dilutions from 1 : 1 to 1 : 99, by using suitable micropipettes. The dilution ratio used for the first potency, was typically maintained throughout the entire range of higher potencies. The potentization procedure in accordance with the present employed "water for injection" (WFI) as a vehicle. A 5ml glass vial was used. 3ml of the total

13 MAY 2011

sample was prepared using 0.03 ml (mother serum) and 2.97 ml of water for injection. (1 : 99 ratio).

The vial containing the dilution was placed on an electro-mechanical stroking machine and given 10 vertical downward uniform strokes. The mechanical potentizer device was standardized as follows: A bottle was placed in the bottle holder space. A lid was fitted and the arm was locked. The machine was started to give 10 strokes. The arm was unlocked to remove the bottles. Machine specifications: arm length- 55cm, angle at which arm drops from base level- approximately 90 degree, weight of the arm- approximately 7.5 gm.

Thus, a HIV nosode preparation with 1c (centesimal) potency was obtained. For subsequent potencies, after the above step, in the water for injection the 1c potency nosode preparation was added. The 2.97 ml of water for injection was taken in a fresh evacuated vial with the help of an auto pipette and 0.03ml of 1 c potency nosode was added to the 2.97 ml of water for injection, the resulting solution was then stroked as before in the electro-mechanical stroking machine. Various subsequent potencies of nosodes were prepared in a similar manner, by using 0.03 ml of the 1c nosode to arrive at next higher potency nosode.

Nosodes with 29 c potency was obtained using the aforementioned method. Nosodes having 30 c potency was obtained, in two separate vials by dispensing alcohol and purified water, respectively; wherein 30 c potency nosodes was prepared in alcohol for the purpose of dispensing.

In the second step, safety check was performed on the potentized preparations of multiple samples having a potency of 30c, 50c, etc. by spiking with a mother

13 MAY 2011

nosode preparation (depending on the viral or bacterial copies per ml). The positive control sample along with negative control sample, in water as medium, and 30c potency medicine were sent for RT-PCR to verify the safety of the preparation.

The safety check method helped in ruling out any infectivity of 30c (and higher potencies) by using the RT-PCR method. The final potencies of nosodes (30c, 50c, and beyond) were subjected to a cell infectivity test on a cell line to rule out the infectivity of the product and to ensure safe use in humans.

30 potency sample was sent for checking Pro-viral DNA study as well as for ultraelectromicroscopy for examining and ruling our any viral particles; for safety purpose.

TECHNICAL ADVANTAGES

A method for preparation of nosodes as described in the present invention has several technical advantages including but not limited to the realization of:

- a method for preparation of nosodes from any microorganism including virus, bacteria, fungi and the like;
- a method for preparation of nosodes having high therapeutic potency;
- a method for preparation of nosodes with scientific standardization of the therapeutic source material;
- a method for preparation of nosodes that can be safely used;

13 MAY 2011

- Reproducibility of the medicinal product becomes possible with this method.

The numerical values mentioned for the various physical parameters, dimensions or quantities are only approximations and it is envisaged that the values higher/lower than the numerical values assigned to the parameters, dimensions or quantities fall within the scope of the invention, unless there is a statement in the specification specific to the contrary.

In view of the wide variety of embodiments to which the principles of the present invention can be applied, it should be understood that the illustrated embodiments are exemplary only. While considerable emphasis has been placed herein on the particular features of this invention, it will be appreciated that various modifications can be made, and that many changes can be made in the preferred embodiments without departing from the principle of the invention. These and other modifications in the nature of the invention or the preferred embodiments will be apparent to those skilled in the art from the disclosure herein, whereby it is to be distinctly understood that the foregoing descriptive matter is to be interpreted merely as illustrative of the invention and not as a limitation.

13 MAY 2011

I Claim:

1. A method for the preparation of nosodes, said method comprising the steps of:

- identifying a donor by screening the patients and collecting a sample which is qualitatively positive for a specific infection selected from the group consisting of HIV, hepatitis(A,B,C,D E), CMV and herpes.

- screening the selected sample to rule out any co-infection selected from the group consisting of Syphilis, Gonorrhea, (VDRL), Tuberculosis, CMV, Human T-lymphotropic virus 1 and 2 (HTLV);

- geno-typing or subtyping of the virus present in the sample and optionally characterizing functionally active genetic components;

- quantifying the organism in the sample;

- separating serum from the sample by serum expression said serum expression comprising keeping the samples tubes on serum expression rack and keeping the rack under a table lamp;

- subjecting the sample to centrifugation at a speed 1500 to 2000 rpm for a period ranging between 15 to 30 minutes to separate serum blood cells and clotting proteins;

- filtering the sample by using a filtration device to filter out the unwanted particles including bacteria from the sample to obtain a

13 MAY 2011

mother serum;

- reconfirming the presence of the virus in the serum and standardizing using RT-PCR;

- subjecting the serum to PAGE patter–SDS Protein profile (sodium dodecyl sulfate polyacrylamide gel electrophoresis) to determine protein profile of the serum;

- diluting the mother serum with water for injection in a proportion ranging between 1:1 to 1:99, to obtain a pre-1c preparation;

- quantifying the number of viruses in a pre-1c preparation;

- potentizing the pre-1c preparation by a method selected from the group consisting of succusion and trituration;

- diluting the potentized pre-1c preparation with water for injection in a proportion ranging between 1:1 to 1:99 to obtain a preparation with 1 c potency;

- serially diluting and potentizing the preparation with 1 c potency with water for injection to obtain preparation with potencies ranging between 2c to 2 million c;

- checking for the absence of the virus or viral particles in the

13 MAY 2011

preparations by spiking the preparations with a mother serum followed by subjecting the spiked preparations to at least one method selected from the group consisting of RT-PCR, ultra electro-microscopy, cell-infectivity test and pro-viral DNA study.

2. The method as claimed claim 1, which further comprises a method step of lyophilizing part of the serum for future use.

3. The method as claimed claim 1, wherein the serum comprises more than one genotype of the virus.

4. The method as claimed in claim 1, wherein, the potentization is carried out in an electromechanical machine having an arm with a length of 55cm and mass of 7.5kg; said machine being adjusted to drop the arm through an angle of about 90^0.

5. A nosode preparation as claimed in claim 1 to 4, wherein the organism is a virus selected from the group consisting of HIV, hepatitis and herpes.

6. A nosode preparation prepared in accordance with the method as claimed in the preceding claims as herein above.

Dated this 13th day of May, 2011

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APPLICANT'S PATENT ATTORNEY

13 MAY 2011