

# Preparation and standardisation of *Plasmodium falciparum* nosode

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## Abstract

**Background:** *Malaria officinalis*, a Homoeopathic preparation used against malaria, was prepared from decomposed vegetable matter from a marshy area by G. W. Bowen in 1862. No scientific published material is available on the source of any malaria-related nosode. There is a need to develop the new nosode using the organisms. The author has developed the nosode from *Plasmodium falciparum* parasites, and the detailed method of preparation is described in this manuscript. **Objective:** Preparation and standardisation of Homoeopathic nosode prepared from *P. falciparum* parasites, which could be explored for further research and therapeutic efficacy. **Materials and Methods:** The most widely cultivated strain of malaria parasite *P. falciparum* was collected from Indian Institute of Technology-B (Mumbai). This parasite was triturated with nine parts of *Saccharum lactis* powder to achieve 1X potency. One part of 1X potency and nine parts of *Saccharum lactis* powder were taken to make subsequent 2X potency. Likewise, potencies till 6X were prepared by trituration method, prescribed in Homoeopathy pharmacopeia of India (HPI). **Results:** Nosode from *P. falciparum* strain was prepared by trituration method as prescribed in HPI and standardised for future research and clinical exploration. **Conclusion:** The nosode sourced from *P. falciparum* parasites will be one such addition, which will call for further exploration for its effect against malaria (and other diseases) as a prophylactic and therapeutic measure.

**Keywords:** Homoeopathy, Malaria, Nosode, Parasites, *Plasmodium falciparum*, Potentization

## INTRODUCTION

The nosodes are Homoeopathic preparations made from biological sources including organisms, diseased material or tissues, and prepared by the process of serial dilutions and rigorous succussions/trituration, called as potentization. The nosodes may resemble vaccines but they differ by the virtue of the preparation technique as well as by their therapeutic application.

The nosodes have demonstrated prophylactic role in Swine Flu,<sup>[1,2]</sup> *Helicobacter pylori* ulcers<sup>[3]</sup> and Leptospirosis.<sup>[4]</sup> Certain nosodes have also shown anti-disease activities *in vitro*,<sup>[5-7]</sup> animal models<sup>[8]</sup> as well as against HIV<sup>[9]</sup> and Hepatitis C<sup>[10]</sup> infected individuals. The anti-*Plasmodium* activity of a Homoeopathic medicine in Bagg Albino (inbred research mouse strain)/c mice in an *in vivo* model against *Plasmodium berghei* infection has been documented.<sup>[8]</sup>

Interestingly, the nosodes have also exhibited anti-disease efficacy against the conditions other than the diseases the source organisms primarily cause. For example, Hepatitis C

nosode and HIV nosode have demonstrated the anti-cancer effect in *in vitro* study.<sup>[11,12]</sup>

Further therapeutic and prophylactic potential of nosodes needs to be scientifically investigated as this space has remained unexplored even after the use of nosodes for almost two centuries.

The older nosodes such as *Psorinum*<sup>[13]</sup> (1826–30), *Tuberculinum*<sup>[14]</sup> (1879), *Medorrhinum*<sup>[15]</sup> (1880–85), *Syphilinum*<sup>[16]</sup> (1880) and others were prepared in the era when microbiological techniques were not well established. No major development or updates happened in the area of nosodes for a long time. However, recent research by the author has already revamped and standardised the method of nosode

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**Received:** 20.07.2017; **Accepted:** 08.08.2019; **Published:** 27.12.2019.

### Access this article online

Quick Response Code:



Website:  
[www.ijrh.org](http://www.ijrh.org)

DOI:  
10.4103/ijrh.ijrh\_38\_17

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**How to cite this article:** Shah R. Preparation and standardisation of *Plasmodium falciparum* nosode. Indian J Res Homoeopathy 2019;13:229-35.

preparation using the current technology<sup>[17]</sup> and has presented HIV nosode,<sup>[9]</sup> Hepatitis C nosode,<sup>[18]</sup> and *Mycobacterium tuberculosis* nosode.<sup>[19]</sup> The nosode category is certainly undergoing a revolution.

*Plasmodium falciparum* is a protozoan parasite causing malaria in humans. *Plasmodium* was first identified when Charles Louis Alphonse Laveran described parasites in the blood of malaria patients in 1880.<sup>[20]</sup> *Malaria officinalis*, a Homoeopathic medicine (not a nosode) used against malaria, was prepared from decomposed vegetable matter from a marshy area by G. W. Bowen in 1862.<sup>[21]</sup> It was based on the assumption that the decomposed matter might have contained some disease-causing vector or diseased mass, years before the discovery of the parasites. No scientific published material is available on the source of any malaria-related nosode. The author has communicated with three of the leading suppliers of *Malaria officinalis* and *Malaria nosodes* in Europe and has concluded that the source of the said nosodes is obscure and not scientifically defined.

Malaria parasites are microorganisms belonging to the genus *Plasmodium*. Out of more than 100 species of *Plasmodium*, four species (*P. falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*) have been reported to infect the humans. Fifth species *Plasmodium knowlesi* has recently been shown to be a significant cause of Zoonotic malaria, particularly in Malaysia.<sup>[22]</sup>

*P. falciparum* is found worldwide in tropical and subtropical areas and responsible for most human deaths. It has the highest complication and mortality rate. *P. falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for most malaria-related deaths globally.<sup>[23]</sup>

The 2015 World Health Organization report found 212 million new cases of malaria worldwide resulting in an estimated 429,000 deaths (range 235,000–639,000).<sup>[24]</sup> In 2016, there were an estimated 216 million cases of malaria in India, an increase of about 5 million cases over 2015. Deaths reached up to 445,000. As per world malaria report 2017, weak malaria surveillance systems are reported in India.<sup>[25]</sup>

The most undeveloped, poor and remote parts of the country contribute mainly to the burden of malaria in India, with >90%–95% cases reported from rural areas and <5%–10% from urban areas. The state of Orissa contributes nearly 20%–30% of deaths caused by malaria in India, followed by Meghalaya, Mizoram, Maharashtra, Rajasthan, Gujarat, Karnataka, Goa, southern Madhya Pradesh, Chhattisgarh and Jharkhand.<sup>[26]</sup>

Since *P. falciparum* parasites multiply rapidly in blood, malaria can cause severe blood loss leading to anaemia. They make the surface of the cell sticky, which could form clots within small blood vessels, thus causing severe organ damage. Complications can be fatal in cases of cerebral malaria.<sup>[27]</sup>

Cultivation of the *Plasmodium* species *in vitro* has been achieved successfully. Life cycle stages of the four species have been established *in vitro*, of which *P. falciparum* remains

the only species for which all stages have been cultured *in vitro*.<sup>[28]</sup> Hence, the author has chosen to prepare a nosode using *P. falciparum* strain.

## Objectives

Preparation and standardisation of Homoeopathic nosode prepared from *P. falciparum* parasites, which could be explored for further research and therapeutic efficacy.

## MATERIALS AND METHODS

The most widely cultivated strain of malaria parasite *P. falciparum* was collected from Bioscience and Bio-engineering Department of Indian Institute of Technology, Mumbai (IIT-B). *Saccharum lactis* powder, which was used as a vehicle, procured from a commercially available source.

*P. falciparum* (3D7 strain) was grown on culture (red blood cells [RBCs]) as per the *in vitro* cultivation parasite protocol (Trager and Jensen protocol). RBCs (O positive) for culturing were collected from healthy volunteers using 10% citrate-phosphate-dextrose-adenine at final concentration.

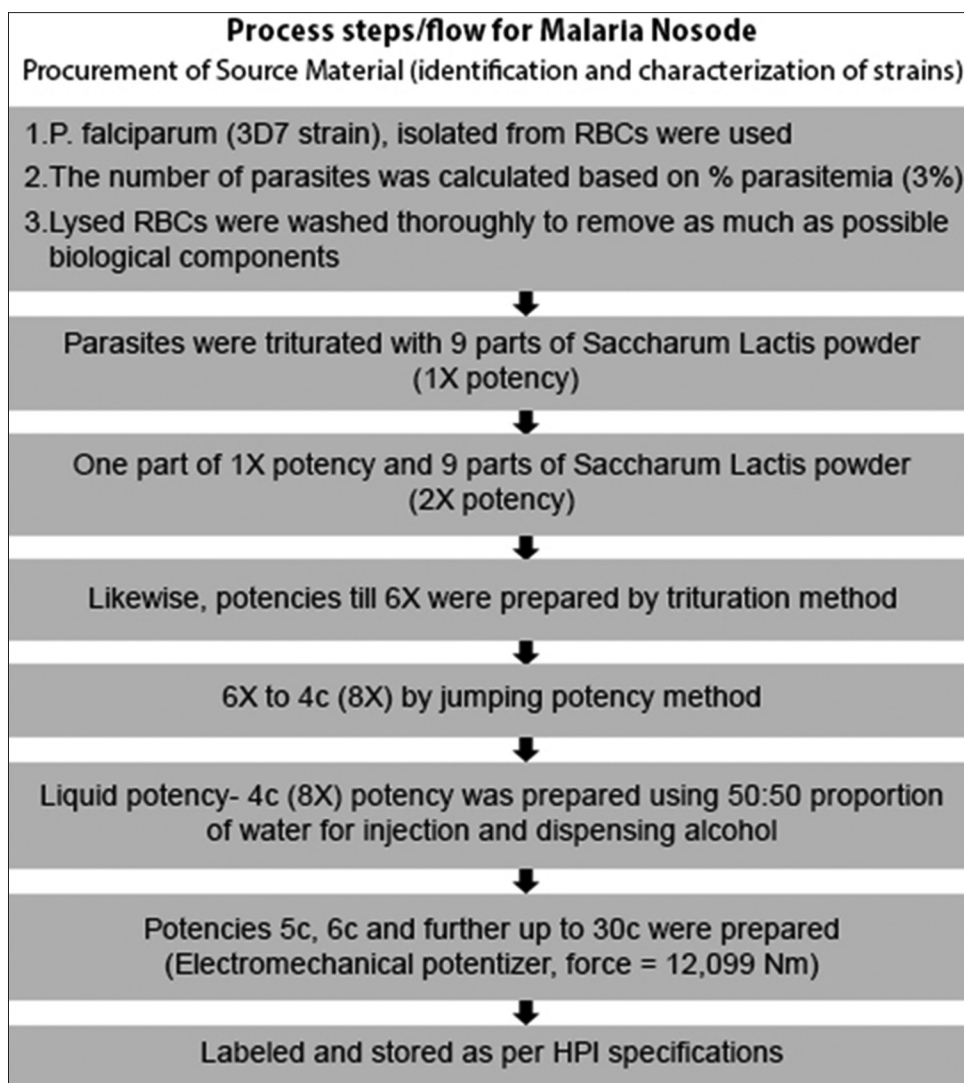
*P. falciparum* parasites were cultured in Roswell Park Memorial Institute 1640 (Gibco®) with an additional 2 mg/ml sodium bicarbonate (Sigma®), supplemented with 0.5% Albumax (Gibco®), 48 mg/L hypoxanthine (Sigma®), 2 mg/ml glucose (Sigma®) and 50 µg/ml gentamicin (Abbott). A haematocrit of 3% was maintained using human O-positive RBCs.

Parasites, predominantly in late rings and trophozoite stage [Figure 1 - parasite image observed under oil immersion lens at 100x (total magnification 1000x)], were released from the RBCs by treatment with 0.02% saponin which lyses the RBCs leaving the parasites intact. This was followed by three washes with phosphate-buffered saline to remove the debris and other chemical components from the preparation.

Parasites were isolated from 40 ml culture containing 3% haematocrit (1.2 ml packed blood cells). Parasitemia was 3% (means 3 parasites in 100 RBCs). The number of parasites



Figure 1: Parasite image



**Figure 2:** Flow chart of Malaria nosode preparation

was calculated based on percentage of parasitemia and the total volume of packed blood cells, which is  $1.8 \times 10^8$ .

Concentration 3% parasitaemia (4% is indicator of high risk)<sup>[29]</sup> was practically possible to achieve at the laboratory, and hence, it was taken at start. The parasites were isolated using centrifuge Rota 4R/VFm made by Plasto Craft. The conditions for pelleting the parasites are 10,000 rpm (9000 g) for 5 min.

Isolated parasites *P. falciparum* were taken as original stock to start with making the Homoeopathy dilutions.  $1.8 \times 10^8$  parasites (calculated based on % parasitemia) were triturated with nine parts of *Saccharum lactis* powder to achieve 1X potency. One part of 1X potency and nine parts of *Saccharum lactis* powder were taken to make subsequent 2X potency. Likewise, potencies till 6X were prepared by trituration method, prescribed in Homoeopathy pharmacopeia of India (HPI).<sup>[30]</sup> In HPI, the method of preparation of *nosode* sourced from organisms is described under Group IV category using triturations up to 6X potency.<sup>[31]</sup> Further, 8X potency (equivalent to 4c) was prepared using 50:50 proportion

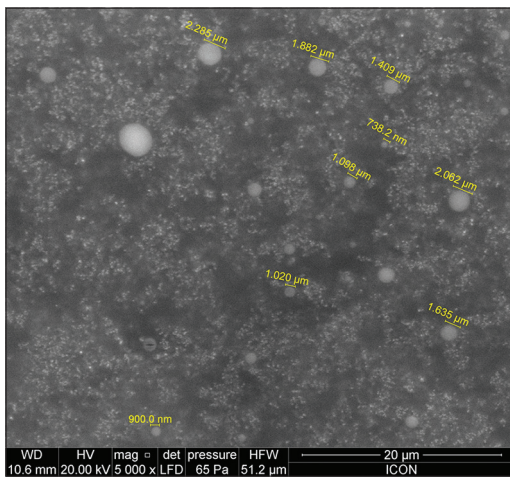
of water for injection and dispensing alcohol (92% sourced from Merck). Potencies 5c, 6c and further up to 30c were prepared using electromechanical potentiometer developed by the author with well-defined force parameters 12,099 Nm.<sup>[32]</sup> All the potencies were labelled and stored appropriately [Figure 2].

The sterility testing for 30c potency was done by direct inoculation method to detect the presence of aerobic, anaerobic bacteria and fungi as per the Indian pharmacopeia method. Fluid thioglycollate medium was used for the culture of anaerobic bacteria, and Soyabean casein digest medium was used for the culture of both fungi and aerobic bacteria.

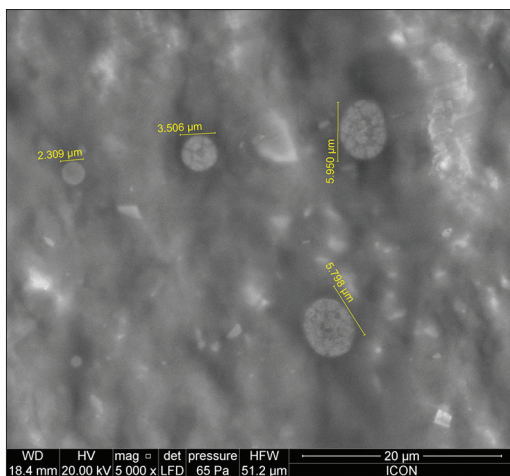
Biosafety laboratory guidance was followed during the cultivation and preparation. Clearance from the Institutional Biosafety Committee (IIT-B) was obtained. All the potencies were processed in a private laboratory setup using a laminar flow.

## RESULTS

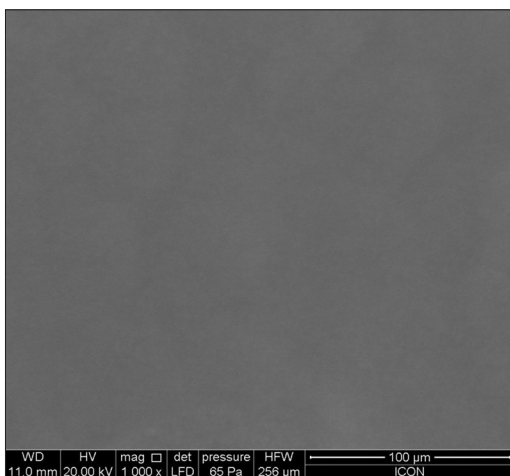
*Nosode* from *P. falciparum* (coded as PFA) strain was prepared



**Figure 3:** Scanning electron microscope image: *nosode* made from *Plasmodia falciparum* (*Malaria nosode*) 1x. Particles of size measuring from 700 nm to 3 μm seen for 1x potency



**Figure 4:** Scanning electron microscope image: *nosode* made from *Plasmodia falciparum* (*Malaria nosode*) 6c. Particles of size measuring from 2 to 8 μm seen for 6c potency



**Figure 5:** Scanning electron microscope image: *nosode* made from *Plasmodia falciparum* (*Malaria nosode*) 30c. No particulate matter seen in potency 30c

and standardised for future research and clinical exploration. The *nosode* was tested negative for sterility as per HPI guidelines for *nosodes*. The sterility testing of *P. falciparum nosode* 30c potency was done as per the Indian Pharmacopoeia monograph.<sup>[33]</sup>

Scanning electron microscopy was performed for 1c, 6c and 30c using SEM-QUANTA 200 with EDS version 3.0.13 at ×300,000 magnification. Few particles of size measuring from 700 nm to 3 μm for 1x potency and 2–8 μm for 6c potency were seen. [Figures 3 and 4], which could be the particles of the vehicle (*Saccharum lactis*). There were no particulate matters seen in 30c potency at ×1000 magnification [Figure 5]. The Electron microscopy is a modern technique that can detect far smaller particles such as viruses, molecules and even individual atoms.<sup>[34]</sup>

Polymerase chain reaction (PCR) (PCR test-malaria differentiation Multiplex) for 30c was performed by ABI 7500 Dx real-time PCR. The testing was performed with an objective to trace whether any nucleic material is present in this dilution. This PCR test can detect parasitemia as low as one gene copy and allows differentiation of the common four *Plasmodium* species (*P. falciparum*, *P. vivax* and *P. malariae* and *P. ovale*). Test result shows that gene copy was not detected for *P. falciparum*, *P. vivax* and *P. malariae* and *P. ovale*.

## DISCUSSION

The old *nosodes* in Homoeopathy are undergoing a revival, revamping; and new ones are getting introduced using current scientific techniques. *Nosodes* have already unveiled significant therapeutic potential. The anti-malarial potential of *P. falciparum nosode* has been demonstrated in the *in vitro* study,<sup>[35]</sup> the study was experimental study and more *in vivo* (and other) studies need to be explored in future evaluating the efficacy of *P. falciparum nosode* against malaria and other diseases.

Many of the *nosodes* in the past were prepared from diseased tissues as the techniques for isolation and culture of organisms were not well developed. It is difficult to standardise and reproduce *nosodes* prepared from diseased tissues.

Ideally, the *P. falciparum nosode* should have been prepared from the cell-free culture. However, it is not possible at this point to procure such a culture. The limitation noted during this preparation is total blood packed cells with 3% parasitemia. The *nosode* could be suitably updated in future overcoming the limitations. Due to technical limitations, it was not possible to prepare *nosodes* from other strains of *Plasmodium* such as *P. vivax*, *P. ovale* and *P. malariae*, which could be explored in future while developing the modified, next version of separate or polyvalent *Malaria nosode*, with higher parasite count.

## CONCLUSION

The *P. falciparum nosode* sourced from *P. falciparum* parasites is an addition to the existing *nosodes* list, which will call for

further exploration for its efficacy against malaria (and other diseases) as prophylactic and therapeutic measures.

### Acknowledgement

Dr. Swati Patankar, Ph.D., at the Department of Bio-sciences and Bio-engineering, at IIT, Mumbai, India, for providing the material (Malaria Strain) to prepare the *nosode*. Dr. N.O. Goel, MD, Head of the Department, Pharmacy, CMPH Medical College for guidance on conversion of potency from decimal to centesimal scale. Ankur Path-lab for allowing to use aseptic chamber.

### Financial support and sponsorship

Life Force, Mumbai.

### Conflicts of interest

The author has a pending patent for the *nosode*.

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### प्लास्मोडियम फाल्सीपेरम नोसोड का निर्माण और मानकीकरण

**पृष्ठभूमि :** मलेरिया के विरुद्ध दी जाने वाली एक होम्योपैथिक दवा, मलेरिया ओफिसिनैलिस को 1862 में जी. डब्ल्यू. बोवेन द्वारा दलदली क्षेत्र से विघटित वनस्पति पदार्थ से तैयार किया गया था। किसी भी मलेरिया से संबंधित नासोड के स्रोत पर कोई भी विज्ञान सम्मत प्रकाशित सामग्री उपलब्ध नहीं है। जीवों का उपयोग करके नए नोसोड को विकसित करने की आवश्यकता है। लेखक ने प्लास्मोडियम फाल्सीपेरम परजीवी से नोसोड विकसित किया है, और तैयारी की विस्तृत विधि इस पांडुलिपि में वर्णित है।

**उद्देश्य :** पी. फाल्सीपेरम परजीवियों से तैयार होम्योपैथिक नोसोड की तैयारी और मानकीकरण, जिसे आगे के अनुसंधान और चिकित्सीय प्रभावकारिता के लिए खोजा जा सकता है।

**सामग्री और विधि :** सबसे व्यापक रूप से उपलब्ध मलेरिया परजीवी पी. फाल्सीपेरम को भारतीय प्रौद्योगिकी संस्थान (मुंबई) से एकत्र किया गया था। इस परजीवी को 1X पोटेंसी प्राप्त करने के लिए सेक्रम लैक्टिस पाउडर के नौ भागों के साथ मिलवाया गया। 1X पोटेंसी का एक हिस्सा और सेक्रम लैक्टिस पाउडर के नौ हिस्से बाद के 2X पोटेंसी बनाने के लिए लिये गए थे। इसी तरह, होम्योपैथी फार्माकोपिया ऑफ इंडिया (एचपीआई) में निर्धारित ट्राइचुरेशन विधि द्वारा 6X तक की शक्ति तैयार की गई।

**परिणाम :** पी. फाल्सीपेरम स्ट्रेन से नोसोड को ट्राइचुरेशन विधि द्वारा एचपीआई में निर्धारित तरीके से तैयार किया गया और भविष्य के अनुसंधान और नैदानिक अन्वेषण के लिए मानकीकृत किया गया।

**निष्कर्ष :** पी. फाल्सीपेरम परजीवी से मिलने वाला नोसेड, एक ऐसा योगदान होगा, जो रोगनिरोधी और उपचारात्मक उपाय के रूप में मलेरिया (और अन्य बीमारियों) के खिलाफ जिसके प्रभाव को याद किया जाएगा।

### Préparation et normalisation du Plasmodium falciparum Nosode

**Contexte :** Le Malaria officinalis, une préparation homéopathique utilisée contre le paludisme, a été préparé à partir de matière végétale décomposée provenant d'une zone marécageuse par G. W. Bowen en 1862. Aucun document scientifique publié n'est disponible sur la source d'un quelconque nosode lié au paludisme. Il convient de développer le nouveau nosode en utilisant les organismes. L'auteur a développé le nosode à partir de parasites Plasmodium falciparum et la méthode de préparation détaillée est décrite dans ce manuscrit. **Objectif :** Préparation et standardisation du nosode homéopathique préparé à partir de parasites P. falciparum, qui pourrait être exploré pour de nouvelles recherches et son efficacité thérapeutique. **Matériaux et méthodes :** La souche la plus cultivée du parasite responsable du paludisme, le P. falciparum, a été collectée auprès de l'Institut indien de technologie B (Mumbai). Ce parasite a été trituré avec neuf mesures de poudre de Saccharum lactis pour atteindre une dilution de 1x. Une mesure de la dilution 1x et neuf mesures de poudre de Saccharum lactis ont été prises pour obtenir une dilution ultérieure de 2x. De même, des dilutions jusqu'à 6x ont été préparées par la méthode de trituration, prescrite dans Homeopathy Pharmacopeia of India (HPI). **Résultats :** Le nosode de la souche de P. falciparum a été préparé par la méthode de trituration prescrite dans l'HPI et normalisé pour la recherche future et l'exploration clinique. **Conclusion :** Le nosode provenant de parasites P. falciparum sera l'un de ces ajouts, ce qui nécessitera une exploration plus approfondie de ses effets contre le paludisme (et d'autres maladies) en tant que mesure prophylactique et thérapeutique.

### Preparación y estandarización del nosode de Plasmodium falciparum

**Fundamentos:** Malaria officinalis, un preparado homeopático utilizado contra la malaria, fue preparado por G. W. Bowen en 1862 a partir de material vegetal descompuesto de un área pantanosa. No se dispone de material científico publicado sobre la fuente de ningún nosode relacionado con la malaria (paludismo). Es necesario desarrollar un nosode nuevo utilizando los correspondientes microorganismos. El autor ha desarrollado el nosode a partir de parásitos de Plasmodium falciparum. En su manuscrito, describe el método detallado de preparación. **Objetivo:** Preparación y estandarización del nosode homeopático preparado a partir de parásitos de P. falciparum, que podrían explorarse para futuras investigaciones y eficacia terapéutica.

**Materiales y métodos:** La cepa de parásito de la malaria, P. falciparum, más ampliamente cultivado se obtuvo en el Indian Institute of Technology-B (Mumbai, India). Este parásito fue triturado con nueve partes de polvo de lactosa para conseguir la primera potencia X. Una parte de esta potencia 1X se mezcló con nueve partes de polvo de lactosa para obtener la potencia 2X. De forma similar, se fueron aplicando lo método de trituration para llegar a la potencia 6X, prescrita en la farmacopea homeopática de la India (HPI).

**Resultados:** El nosode de la cepa de P. falciparum fue preparado con el método de trituration, según lo prescrito en la HPI y estandarizado para su futura investigación científica y utilización clínica.

**Conclusiones:** El nosode de los parásitos de P. falciparum será una de estas adiciones que exigirá un posterior aprovechamiento debido a su efecto frente al paludismo (y otras enfermedades) como medida profiláctica y terapéutica.

## Herstellung und Standardisierung von *Plasmodium falciparum* Nosode

**Hintergrund:** Malaria officinalis, ein homöopathisches Präparat gegen Malaria, wurde 1862 von G. W. Bowen aus zersetztem pflanzlichen Material aus einem sumpfigen Gebiet hergestellt. Über die Quelle von malariabedingten Nosoden ist kein wissenschaftlich veröffentlichtes Material verfügbar. Es besteht die Notwendigkeit, die neue Nosode unter Verwendung der Organismen zu entwickeln. Der Autor hat die Nosode aus *Plasmodium falciparum*-Parasiten entwickelt, und die detaillierte Methode der Herstellung ist in diesem Manuskript beschrieben. Ziel: Herstellung und Standardisierung von homöopathischen Nosoden, die aus *P. falciparum*-Parasiten hergestellt wurden und für weitere Untersuchungen und therapeutische Wirksamkeiten untersucht werden konnten.

**Material und Methoden:** Der am häufigsten kultivierte Stamm des Malariaparasiten *P. falciparum* wurde vom Indian Institute of Technology-B (Mumbai) gesammelt. Dieser Parasit wurde mit neun Teilen Saccharum lactis-Pulver verrieben, um eine 1-fache Wirksamkeit zu erzielen. Ein Teil 1 × Potenz und neun Teile Saccharum lactis-Pulver wurden genommen, um die nachfolgende 2 × Potenz herzustellen. Ebenso wurden Potenzen bis zum 6-fachen durch eine Verreibungsmethode hergestellt, die in Homöopathie Pharmacopeia of India (HPI) vorgeschrieben ist. Ergebnisse: Die Nosode aus dem *P. falciparum*-Stamm wurde nach dem in HPI vorgeschriebenen Verreibungsverfahren hergestellt und für zukünftige Forschungen und klinische Untersuchungen standardisiert.

**Schlussfolgerung:** Die von *P. falciparum*-Parasiten stammende Nosode wird eine solche Ergänzung sein, die eine weitere Erforschung ihrer Wirkung erforderlich macht gegen Malaria (und andere Krankheiten) als prophylaktische und therapeutische Maßnahme.

## 惡性瘧原蟲病質藥的製備和標準化

**背景:** 瘧疾，是一種用於治療瘧疾的順勢療法製劑，由G.W.Bowen於1862年從沼澤地區，透過分解植物製備而來。沒有關於任何與瘧疾來源有關的病質藥的科學出版物。因此需要使用有機體研發新的病質藥。作者研發了惡性瘧原蟲寄生蟲的病質藥，詳細的製備方法在本手稿中有所描述。

**目的:** 由惡性瘧原蟲製備而成的順勢療法病質藥之製備與標準化，其項目為進一步的研究和治療提供依據。

**用料和方法:** 從孟買印度科技學院採集最廣泛栽培的惡性瘧原蟲寄生蟲。將這種寄生蟲與九份乳糖粉一起磨碎，以達到1X層級。取1份1X層級和9份乳糖粉製成隨後的2X層級。如此類推，直至透過研磨法達到 6X層級，這是《印度順勢療法藥典》(HPI)中規定的。

**結果:** 由惡性瘧原蟲菌株作來源的順勢療法，透過採用HPI規定的研磨方法製備和標準化，用於未來的研究和臨床探索。

**結論:** 源自惡性瘧原蟲寄生蟲的病質藥將是一種附加治療，這將需要進一步探索其對抗瘧疾（和其他疾病）的作用作為預防和治療措施。