TDR For research on diseases of poverty

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Pooled Fund to support Research and Development Demonstration Projects

Technical Report

Overview

The report of the Consultative Expert Working Group on Research and Development (2012) highlighted the continuing failure of existing market and public mechanisms to support research and develop technologies to combat diseases that predominantly affect low- and middle-income countries (LMICs).[1] Its recommendations included a call to improve the identification of health priorities, coordinate global efforts around these priorities and to establish new, innovative and sustainable sources of funding to support R&D for product development in these areas.

Prior to the establishment of any proposed R&D financing mechanism, as an interim measure World Health Assembly Resolution WHA66.22 called for the implementation of a few health research and development demonstration projects for which immediate action could be taken. These projects were aimed at developing health technologies (medicines, diagnostics, medical devices, vaccines, etc.) for diseases that disproportionately affect LMICs, and for which identified R&D gaps remain unaddressed due to market failures. The projects needed to demonstrate effectiveness of alternative, innovative and sustainable financing and coordination approaches to address identified R&D gaps.

WHO Member States were invited to support these demonstration projects through the establishment of a WHO pooled fund that would accept voluntary contributions. The WHO Secretariat organized a call for project proposals through its regional offices and, based on resolution WHA66.22, six demonstration projects were selected. TDR was requested by the WHO Director-General to undertake financial administration of the fund.[2] The fund was opened in 2013 and became operational in 2014. After three years, no further contributions from Member States were received by the fund. Consequently, in a statement made by the Assistant Director-General for Health and Information Systems during the World Health Assembly in May 2017 in response to a discussion on agenda item 13.5 (A70/22), the fund was closed to receipt of new funds. The pooled fund continued to operate with the funds that had already been received and finished operating on 31 December 2019. This technical report has been prepared by TDR for review by JCB as agreed in the workplan approved by WHO. The JCB will review and approve the Final Financial Certified Statement at its next meeting pending completion of the final financial report by the African Network for Drugs and Diagnostics Innovation (ANDI), which remains pending.

Table 2 shows a summary of income and expenditure of the pooled fund during its operational life, from January 2014 to December 2019.

The pooled fund turned over more than US$ 11 million in contributions from Member States and supported six demonstration projects, as well as contributing to the development of the WHO Global Observatory on Health R&D.

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1 http://www.who.int/phi/documents/CEWG-WP/en/

2 CEWG demonstration projects: background and process http://www.who.int/phi/implementation/cewg_background_process/en/

Contributions received from WHO Member States

Contributions to the pooled fund were received from Brazil, Germany, India, Norway, South Africa and Switzerland. In addition to direct contributions, Norway and Switzerland established an incentive mechanism for donations received from low- and middle-income countries to provide an additional US$ 1 for every US$ 2 received from those countries. The total income received into the pooled fund through this mechanism was US$ 11 008 532.

Expenditure of funding provided to the demonstration projects

Member States chose six health R&D demonstration projects to support. Project plans and budgets were reviewed by a specially convened expert ad hoc committee under the chairmanship of Professor Maged Al-Sherbiny.[3] The project titles and the total awards made in this first round by the ad hoc committee are summarized below in Table 1. In 2015, the ad hoc committee reviewed the workplans and budget proposals for five demonstration projects (DEMOs 1-5), as well as those of the Global Observatory on Health Research and Development, and recommended allocation of funding for the first year of implementation of the five projects and the Observatory. Letters of agreement were signed and funds disbursed for three of the demonstration projects (DEMOs 1-3) in 2015 and one (DEMO 4) in September 2016, using all of the funds available at that time. The fifth project (DEMO 5) was approved for funding by the ad hoc committee, the letter of agreement signed and the award disbursed in February 2017. The sixth demonstration project (DEMO 6) was approved in April 2016. A meeting of stakeholders to develop the final proposal for this demonstration project took place in February 2017 in Geneva. Funding was awarded following review and approval by the ad hoc committee.

Progress of demonstration projects and the WHO Global Observatory on Health R&D

All six demonstration projects reported progress to WHO at an open-ended meeting of Member States in May 2016.[4] Subsequently, each project and the R&D Observatory have provided a final report, which are attached under Annex 2.

The total financial requirement for implementation of the strategic workplan (specifically the demonstration projects and the WHO Global Observatory on Health R&D) was estimated at US$ 85 million for 2014-2017.[5] During that period WHO received US$ 11 008 535, with no additional funds committed after February 2017. Accordingly, the Secretariat communicated to project proponents in May 2017 that no additional funding would be available (see Annex 1). In June 2017, the Committee decided to allocate the remaining funding as follows: US$ 335 000 to each DND*i*, MMV and ANDI. This left US$ 397 891 in the pooled fund which was allocated to the WHO Global Observatory on Health R&D by decision of the ad hoc committee. Note that the original amount approved by the committee for the Observatory in June 2015 was US$ 1 million. In addition to this final report, the outputs generated by the R&D Observatory are available on its website.[6]

[3] The full committee membership and biographies are available here: http://www.who.int/phi/news/adhoc_committee/en/

[4] http://www.who.int/phi/cewg/en/

[5] EB138/39. "The estimated total financial requirement for implementation of these two activities for four years (2014–2017) is US$ 85 million."

[6] https://www.who.int/research-observatory/en/

Table 1. Funds allocated to the demonstration projects.

Project	First Tranche (US$)	Second Tranche (US$)
DEMO 1: Drugs for Neglected Diseases Initiative (DND*i*). The visceral leishmaniasis global research and development access initiative	2 582 889	335 000
DEMO 2: Medicines for Malaria Venture (MMV). Exploiting the pathogen box: an international open-source collaboration to accelerate drug development in addressing diseases of poverty	1 360 000	335 000
DEMO 3: African Network for Drugs and Diagnostics Innovation (ANDI). Development of easy to use and affordable biomarkers as diagnostics for types II and III diseases	1 672 556	335 000
DEMO 4: Oswaldo Cruz Foundation, Brazil. Development of a vaccine against schistosomiasis based on the recombinant Sm14, a member of the fatty acid-binding protein family: controlling transmission of a disease of poverty	400 000	0
DEMO 5: Translational Health Science and Technology Institute (THSTI), India. Multiplexed point-of-care test for acute febrile illness	965 147	0
DEMO 6: Council for Science and Industrial Research, South Africa. Demonstration of the potential of a single-dose malaria cure of artemether-lumefantrine through reformulation in a nano-based drug delivery system	993 378	0
WHO Global Observatory on Health R&D	-	397 891

Table 2. Summary of income and expenditure as at 31 December 2019 in US dollars

R&D Demonstration Projects

Financial review as at 31 December 2019 Award 63809	2014-2015 Actual	2016-2017 Actual	2018-2019 Actual	Total
Income received				
Brazil 1	200 000			200 000
Brazil 2	946 074			946 074
Germany		2 123 142		2 123 142
India		1 000 000		1 000 000
Norway	588 512			588 512
Norway -matching against India		595 167		595 168
South Africa		685 315		685 315
Switzerland (SDC)	4 090 325			4 090 325
Switzerland (SDC) - matching against South Africa		343 104		343 105
Switzerland (SDC) -matching	436 893			436 894
Total Income	**6 261 804**	**4 746 728**		**11 008 535**
Expenditures				
Council for Scientific and Industrial Research (CSIR)		894 040	99 338	993 378
Drugs for Neglected Diseases Initiative (DNDI)	2 301 639	582 750	33 500	2 917 889
Global Observatory			397 891	397 891
Instituto Nacional de Infectologia Evandro Chagas (FIOCRUZ)		400 000		400 000
Medicines for Malaria Ventures (MMV)	1 224 000	437 500	33 500	1 695 000
Translational health Science and Technology Institute (THSTI)		898 172	66 975	965 147
United Nations Office for Project Services (UNOPS)	1 672 556	335 000		2 007 556
Staff Costs	419 413	605 589	140 999	1 166 001
Consultants	1 800	20 101		21 901
Travel	12 064	6 017		18 081
General Op. Costs	1 166	637		1 803
Meetings and courtesy expenses	483			483
WHO Admin Charges (4%)	225 325	167 192	30 888	423 405
Total Expenditures	**5 858 446**	**4 346 998**	**803 091**	**11 008 535**
Balance				-

Note: The award to the WHO Global Observatory includes $385 181 staff costs. This gives a total for WHO staff costs in 2018-2019 of $526 181, as reported to the ICFS.

Annex 1

Statement made by the Assistant Director-General for Health and Information Systems during the World Health Assembly in May 2017, in response to a discussion on Agenda item 13.5 (A70/22) Follow-up of the report of the Consultative Expert Working Group on Research and Development: Financing and Coordination. Report by the Director-General.

The Secretariat would like to acknowledge the many Member States and NGOs in official relations who have taken the floor, for their strong interest in this important agenda, which unfortunately has remained critically underfunded year after year.

The Global Observatory was released on 19 January 2017. Dear Member States, should you find the time, I encourage you to visit the Observatory. I myself find it a very interesting resource, which includes many new elements to help analyse the R&D landscape for Type II and Type III diseases and for elements of Type I diseases which affect most particularly developing countries. The CEWG strategic workplan has been on the agenda of the World Health Assembly for so long that we do not all remember the scope of work decided by Member States.

The Observatory will continue to be expanded and updated as resources and information become available. We will proceed with establishing the Expert Committee on Health R&D.

On demonstration projects, I take note of the fact that no new resources have been pledged beyond the $11 million raised in the past 3 years, and we will therefore regretfully inform the proponents of the 6 projects that they should not expect to receive more financial support before their projects officially close later this year, unfortunately unfinished.

With regards to the proposed pooled fund, we will inform TDR's Board that the WHA does not want to pursue further this proposal.

Finally, I would like to thank those Member States who have provided financial and moral support to the Secretariat's work on the CEWG strategic workplan, including for the Global R&D Observatory.

Thank you for your attention.

May 2017.

Annex 2. Technical reports

DEMO 1: DND*i*

DEMO 2: MMV

DEMO 3: ANDI

DEMO 4: FIOCRUZ

DEMO 5: THSTI

DEMO 6: CSIR

WHO Global Observatory on Health R&D

Reporting Template R&D Demonstration Project 12 month workplan

This document will report on the first 12 month workplan for a R&D demonstration project selected as per the Executive Board decision EB134(5) following review by the former Chair and Vice-Chair of the CEWG. This report should align precisely with the budget template in line with the award recommended by the Ad Hoc Committee on 19th June 2015 as set out in the Letter of Agreement.

1. **Title of the project:** ***The Leishmaniasis Global R&D & Access Initiative Phase 2***

2. **Sponsor/s of the project:**

Report Prepared by	**Rachel Tisseuil/Bijoya Banerjea**	**Date Submitted**	**19th March 2018**
Phone	***+41 22 906 9230***		
E-mail	**rtisseuil@dndi.org** **bbanerjea@dndi.org**		

Date LoA* Awarded	***5th July 2017***	**LoA Amount**	***USD 335,000***
Project Start Date	***5th August 2015 (original project)***	**Date this report covers**	***1st July 2017 – 31st December 2017***

*LoA – Letter of Agreement

5. **Project summary:**

Describe the overall project proposal, outline the objective and ultimate impact this project is designed to achieve – (can be taken from Annex 1 of the Letter of Agreement).

The objective of the Leishmaniasis Global R&D & Access Initiative is to demonstrate that visceral leishmaniasis (VL), Post Kala Azar Dermal Leishmaniasis (PKDL) and Cutaneous Leishmaniasis (CL) R&D projects can be optimized through guiding principles such as cross-regionals collaboration of existing networks, open-innovation and sharing knowledge, equitable access to new products, and sustainable funding secured through existing and new funding mechanisms.

Objective 1: The development of new safe and effective oral treatments developed as monotherapy and as early as possible as combination treatment (medical product) to prevent the risk of resistance development and a very safe, short-course one for asymptomatic carriers once their role in disease transmission has been better established.

Objective 2: the evaluation of the role of asymptomatic and PKDL patients (xenodiagnosis)

Objective 3: the development of treatment for PKDL (medical product) and completion of preclinical and clinical development of a selected immune response modifier which could be used in combination with chemotherapy for CL

Objective 4: the support to development of a shared, open-access data base that will allow to identify determinants of treatment effectiveness

This second award proposed to strengthen the overall project of developing new safe and effective oral treatments by completing preclinical development of two promising preclinical candidates DNDI-0690, DNDI-6148 (*Objective 1 Activity 1*). We are envisioning at least one candidate to progress to phase I studies beginning of Q1 2018.

We also propose to continue the *Activity 2 of Objective 3 Complete preclinical development of an immune response modifier*: testing CpG D35 in peripheral blood mononuclear cell (PBMC) samples from patients with PKDL and CL.

6. Description of progress towards activities and tasks.
As outlined in Annex 1 of the LoA please describe:

a. *Progress made on each planned activity (milestone, output and/or outcome).*
b. *Any results that were not achieved according to the proposed milestones, the reasons they were not completed and the plans for carrying them out.*
c. *Describe any unexpected results.*

Please refer to the activities described in the accompanying DNDi NCE Work Plan Mid 2015-December 2017_FINAL

7. Outputs
Please list significant outputs. For example publications, reports, press releases, online resources. Provide copies or url links where appropriate.

During the period covered, DNDi published several press releases and scientific publications as well as attending international events.

You will find below a selection of press releases and scientific publications:

Press/Media releases

- Health Analytics India: "How India can help eliminate neglected diseases"
  Published 17 October 2017
  https://www.dndi.org/2017/media-centre/in-the-media/health-analytics-india-how-india-can-help-eliminate-neglected-diseases/

- The Huffington Post: "In rural Kenya, escaping a deadly disease sometimes takes a little luck"
  Published 6 September 2017
  https://www.dndi.org/2017/media-centre/in-the-media/huffington-post-in-rural-kenya-escaping-a-deadly-disease-sometimes-takes-a-little-luck/

- Business Daily: "Neglected diseases that wreak havoc on northern Kenya"
  Published 17 July 2017
  https://www.dndi.org/2017/media-centre/in-the-media/business-daily-neglected-diseases-that-wreak-havoc-on-northern-kenya/

Scientific publications

- **Antileishmanial and antitrypanosomal drug identification**
  Published 22 December 2017
  by Croft SL, Chatelain E, Barrett MP.
  Emerging Topics in Life Sciences, December 2017

https://www.dndi.org/2017/media-centre/scientific-articles/scientific-articles-neglected-diseases/antileishmanial-antitrypanosomal-drug-identification/

- **Cytokines and chemokines measured in dried SLA-stimulated whole blood spots for asymptomatic *Leishmania infantum* and *Leishmania donovani* infection.**
  Published 8 December 2017
  by Ibarra-Meneses AV, Mondal D, Alvar J, Moreno J, Carrillo E.
  Scientific Reports, December 2017
  https://www.dndi.org/2017/media-centre/scientific-articles/scientific-articles-vl/cytokines-chemokines-sla-asymptomatic-vl/

- **Post-kala-azar dermal leishmaniasis in the Indian subcontinent: A threat to the South-East Asia region kala-azar elimination programme**
  Published 30 November 2017
  by Zijlstra EE, Alves F, Rijal S, Arana B, Alvar J
  PLOS Neglected Tropical Diseases, November 2017
  https://www.dndi.org/2017/media-centre/scientific-articles/scientific-articles-vl/post-kala-azar-dermal-leishmaniasis-indian-subcontinent/

- **Towards elimination of visceral leishmaniasis in the Indian subcontinent – Translating research to practice to public health**
  Published 20 October 2017
  by Hirve S, Kroeger A, Matlashewski G, Mondal D, Banjara MR, Das, P, Be-Nazir A, Arana B, Olliaro P.
  PLOS Neglected Tropical Diseases, October 2017
  https://www.dndi.org/2017/media-centre/scientific-articles/scientific-articles-vl/towards-elimination-of-vl-in-indian-subcontinent/

- **Visceral leishmaniasis relapse hazard is linked to reduced miltefosine exposure in patients from Eastern Africa: A population pharmacokinetic/pharmacodynamic study**
  Published 18 September 2017
  by Dorlo TPC, Kip AE, Younis BM, Ellis SJ, Alves F, Beijnen JH, Njenga S, Kirigi G, Hailu H, Olobo J, Musa AM, Balasegaram M, Wasunna M, Karlsson MO and Khalil EAG.
  Journal of Antimicrobial Chemotherapy, September 2017
  https://www.dndi.org/2017/media-centre/scientific-articles/scientific-articles-vl/vl-relapse-pharmacokineticpharmacodynamic-study/

- **Systematic review of clinical trials assessing the therapeutic efficacy of visceral leishmaniasis treatments: A first step to assess the feasibility of establishing an individual patient data sharing platform**
  Published 18 September 2017
  by Bush JT, Wasunna M, Alves F, Alvar J, Olliaro PL, Otieno M, Hopkins Sibley C, Strub Wourgaft N, Guerin PJ.
  PLOS Neglected Tropical Diseases, September 2017
  https://www.dndi.org/2017/media-centre/scientific-articles/scientific-articles-vl/systematic-review-of-clinical-trials-assessing-therapeutic-efficacy-of-vl-treatments/

- **Efficacy and safety of available treatments for visceral leishmaniasis in Brazil: A multicenter, randomized, open label trial**
  Published 4 July 2017

by Romero GAS, Costa DL, Costa CHN, de Almeida RP, de Melo EV, de Carvalho SFG, Rabello A, de Carvalho AL, de Queiroz Sousa A, Leite RD, Soares Lima S, Alves Amaral T, Piovesan Alves F, Rode J, the Collaborative LVBrasil Group.
PLOS Neglected Tropical Diseases, June 2017
https://www.dndi.org/2017/media-centre/scientific-articles/scientific-articles-vl/efficacy-and-safety-of-available-treatments-vl-brazil/

- **In vitro and in vivo pharmacodynamics of three novel antileishmanial lead series**
  By M.Van den Kerkhof, D.Mabille, E.Chatelain, C.E.Mowbray, S.Braillard, S.Hendrickx, L.Maes, G.Caljon International Journal for Parasitology: Drugs and Drug Resistance Volume 8, Issue 1, April 2018, Pages 81-86
  https://doi.org/10.1016/j.ijpddr.2018.01.006

Given below are the events DNDi participated in:

- **ASTMH 2017**
  November 5-9, 2017
  Baltimore, Maryland, USA
  DND*i* took part in symposia, poster and oral presentation sessions at the meeting.
  https://www.dndi.org/2017/media-centre/events/astmh-2017/

- **SEMTSI 2017**
  X National Congress of Tropical Medicine and International Health
  October 23-25, 2017
  Bilbao, Spain
  Representatives from DND*i* and GARDP took part in the event.
  https://www.dndi.org/2017/media-centre/events/semtsi-2017/

- **ECTMIH 2017**
  October 16-20, 2017
  Antwerp, Belgium
  DND*i* presented on sleeping sickness, visceral leishmaniasis, mycetoma, and onchocerciasis.
  https://www.dndi.org/2017/media-centre/events/ectmih-2017/

- **World Health Summit 2017**
  October 15-17, 2017
  Berlin, Germany
  Monique Wasunna, Director of DND*i* Africa, presented on strengthening capacity for visceral leishmaniasis clinical research in Africa.
  https://www.dndi.org/2017/media-centre/events/world-health-summit-2017/

- **MedTrop 2017**
  Congress of the Brazilian Society of Tropical Medicine
  August 27-30, 2017
  Mato Grosso, Cuiabá, Brazil
  DND*i* participated in the 53rd Congress of the Brazilian Society of Tropical Medicine and in ChagasLeish 2017, a meeting that was held within the congress.
  https://www.dndi.org/2017/media-centre/events/medtrop_2017_chagasleish_2017/

8. Budget variance
Please explain any difference between the original proposal and the actual expenditure.

There have been no significant differences (more than 10%) between the original proposal and actual expenditure.

9. Other Sources of Project Support:
If the project received support from other donors please use the following chart to provide the name of the donor, the amount received, the percentage of the project funded by the donation, and whether the funds are committed or potential. If the support is in-kind, describe the type of support below the chart.

Report all amounts in U.S. dollars.

Donor	**Amount**	**Received or Potential**
DDC Switzerland	319,930	Received
DFID – UK	777,686	Received
DGIS - Netherlands	47,694	Received
KfW – Gemany	269,767	Received
GHIT– Japan	406,756	Received
WHO-Demo project	335,000	Received
Description of in-kind support, if any:	0	
TOTAL	2,156,834	

10. Future developments
Please describe any significant developments, changes or adaptations to the original project you would like to make accepting these are in line with the original proposal.

The goal of this programme is to transform leishmaniasis treatment from the use of toxic, painful injectable antimonial therapies to a simple, orally acting and well tolerated treatment which can be used in resource poor countries.

Overall, DNDi's Leishmaniasis portfolio has continued to make good progress since the submission of the Demonstration project to WHO in 2014. Three new chemical entities entered the pre-clinical pipeline, bringing to seven the number of new compounds progressing in the leishmaniasis portfolio, with two already nominated as clinical candidates to progress to Phase I. The oxaborole DNDI-6148 and nitroimidazole DNDI-0690 have both been nominated clinical candidates, and Phase I program (SAD study) will start in 2018. For post kala-azar dermal leishmaniasis (PKDL), the first patient was recruited in India in the Phase II studies to test new treatments for PKDL while clinical sites were being prepared in Bangladesh and Sudan. In February 2018 a site was initiated in Sudan for the Phase III study to test a new combination treatment for leishmaniasis in the African region. In Latin America, the Phase II study on new combination treatments for cutaneous leishmaniasis progressed well.

The recommendation for progression into preclinical development for DNDI-6148 was endorsed by DNDi's independent Scientific Advisory Committee (SAC) in April 2016. Preclinical development data generated from the pharmaceutical development campaign, safety pharmacology and Good Laboratory Practice (GLP) regulatory toxicology studies were reviewed by the project team and expert consultants in the last quarter of 2017 and a recommendation was made to proceed to human single ascending dose studies in healthy volunteers and to commence Phase I work. The single ascending dose (SAD) phase preparation is underway with dosing planned for the second quarter of 2018. Additional investigational toxicology studies will be completed in parallel to the SAD study to support multiple ascending dose studies if safety and pharmacokinetic profile are acceptable in the single ascending dose part of Phase I.

For DNDI-0690, a 14-day toxicity evaluation carried out in 2015 led to its nomination as a preclinical candidate in September 2015. DNDI-0690 is a nitroimidazooxazine for the treatment of visceral leishmaniasis (VL) and possibly cutaneous leishmaniasis (CL). The full preclinical toxicology and safety studies package was completed in 2017. A decision to progress to Phase I Single Ascending Dose in healthy volunteers was made in January 2018. The production of Good Manufacturing Practices (GMP) DNDI-0690 API batch was completed in September 2017 to support clinical supplies manufacture for Phase I planned in Q2 2018.

CpG D35 is being developed as a combination therapy for the treatment of complicated CL and PKDL. Two studies, one *in vitro* and one *in vivo*, were initiated in 2016. Final results of the preclinical *in vivo* efficacy study showed an improved clinical outcome of CL lesions in animal model after administration of CpG-D35, either alone or in combination with pentavalent antimonial (glucantime). These results supported the continuation of CpG D35's preclinical development and hopefully reach its clinical development nomination by Q4-2019.

Results of the study to analyse the response of PBMC samples from CL and PKDL patients when stimulated with CpG D35 is delayed initially due to problems to get ethical approvals from countries involved in the study (7) and subsequently to import the CpG D35 and other reagents into the countries. Collection of samples is expected to be completed by Q2-2018 and results available by the end of Q3-2018.

We are grateful for the funding from WHO TDR that has not only played a key role in enabling DNDi to move forward with these critical projects in our Leishmaniasis portfolio, but also allowed us to demonstrate that these R&D projects can be optimized through appropriate cross-regional collaboration of existing networks, open-innovation and sharing knowledge, as well as equitable access to new products. The projects financed by this grant will continue beyond the completion of this grant following the same principles.

MMV

Reporting Template R&D Demonstration Project 6 month workplan

This document will report on 6 month workplan for a R&D demonstration project selected as per the Executive Board decision EB134(5) following review by the former Chair and Vice-Chair of the CEWG. This report should align precisely with the budget template in line with the award made by the Ad Hoc Committee on 19th June 2015 as set out in the Letter of Agreement.

1. Title of the project:

"Exploiting the Pathogen Box: an International Open Source collaboration to accelerate drug development in addressing diseases of poverty"

2. Sponsor/s of the project:

Medicines for Malaria Venture

Report Prepared by **Dr Jeremy Burrows**

Phone **+41 22 555 03 28**

E-mail **burrowsj@mmv.org**

Date Submitted **11/10/2018**

Date LoA* Awarded ***5th July 2017***

LoA Amount ***US$ 335,000***

Project Start Date ***8th August 2017***

Date this report covers ***August 2017 – Oct. 2018***

*LoA – Letter of Agreement

5. Project summary:
Describe the overall project proposal, outline the objective and ultimate impact this project is designed to achieve.

The "Pathogen Box" is a MMV led project that was funded by the Bill & Melinda Gates Foundation and launched in December 2015. MMV received WHO funding for a follow-up project "Exploiting the Pathogen Box" with the aim to deliver novel hit-to-lead start points for neglected and tropical disease pathogen projects (objective 1), identify mechanisms of resistance (objective 2) and support endemic region Challenge Grants (objective 3). As reported in the 1st LoA workplan report, substantial progress towards these objectives was achieved, meeting all of the goals in Year 1.

These included:

- Projects were initiated including multiple pathogens such as malaria and kinetoplastids.
- Over 700 compounds (>20 series within the PB) were synthesized/acquired based on hits against tuberculosis, malaria, kinetoplastids & onchocerciasis. ADME data on >150 compounds were obtained. These compounds and data remain freely available to all researchers.
- Mechanism of action and resistance work has identified inhibitors of Pf PI4K, ATP4, bc1 and compounds acting rapidly and with full life-cycle stage profiles.
- A Challenge Grant 'Call for Proposals' from endemic region scientists was run and after triaging 75 applications, 7 challenge grants were awarded to scientists in Brazil, Cameroon, Peru, Ethiopia and Kenya working in the full range of pathogens.

The **second phase** listed below continued the originally planned work stream of the project and was tailored to the available budget and time period (USD 335,000). The focus was to continue synthesis, acquisition, ADME and testing of novel analogues of pathogen box compounds particularly targeting tuberculosis, kinetoplastids and malaria. This aligned with the overarching goals as initially described and contributes to the delivery of robust chemical start points for new drug discovery projects.

6. Description of progress towards activities and tasks.
As outlined in Annex 1 of the LoA please describe:

Activity 1)
Objective 1: CRO chemistry: up to 10 chemists for synthesis and SAR exploration

Chemistry was conducted at two CROs in India: GVK Biosciences and Advinus. Between the two organizations, 570 compounds were synthesized from 25 chemical series representing excellent productivity and value. These compounds have been publicized in presentations and on the Pathogen Box website as being available for partners to test.

Activity 2)
Objective 1: In vitro ADME to support evaluation of novel compounds
DMPK studies were conducted at TCGLS in Kolkata on 125 compounds and these data were annotated to the structures. Partners requesting information on these open access hits have access to these data as well as the structures.

Objective 2: no new activities due to budget and time restrictions

Objective 3: no new activities, only management of ongoing Challenge Grants as per Year 1 no cost extension

7. Outputs
Please list significant outputs. For example publications, reports, press releases, online resources. Provide copies or url links where appropriate.

MMV has detailed information on the Exploiting the pathogen box project on its website: https://www.mmv.org/research-development/open-source-research/exploiting-pathogen-box. Furthermore, information on the compounds in the pathogen box having library compounds available through this project are available on the pathogen box website: https://www.pathogenbox.org/

The project was presented at the International Congress on Tropical Medicines in Brisbane and at numerous other parasitology meetings to raise the profile amongst the community.
A range of publications are planned but have not yet been completed.

8. Budget variance
Please explain any difference between the original proposal and the actual expenditure.
All funds have been disbursed and activities have been completed.

CEWG Exploiting the Pathogen Box				
Year 2 (2nd phase project) US$				
Budget				
		Budget	Expenditures	Details
Activity 1	CRO chemistry 6 month up to 10 chemists	275,000	269,887	(137,500 GVK + 137,500 Advinus)
Activity 2	ADME to support compound evaluation	21,000	31,613	(TCGLS)
Indirect costs	13%	38,480	32,980	
	Total budget	334,480	334,480	

9. Other Sources of Project Support:
If the project received support from other donors please use the following chart to provide the name of the donor, the amount received, the percentage of the project funded by the donation, and whether the funds are committed or potential. If the support is in-kind, describe the type of support below the chart.

Not applicable.

Report all amounts in U.S. dollars.

Donor	Amount	Received or Potential

Description of in-kind support, if any:

Not applicable.

10. Future developments
Please describe any significant developments, changes or adaptations to the original project you would like to make accepting these are in line with the original proposal.

Subject to further funding the project would continue along the lines of the original proposal.

ANDI

R&D Demonstration Project[1]

R&D demonstration projects were selected as per the Executive Board decision EB134 (5) following review by the former Chair and Vice-Chair of the CEWG

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1. Title of the project

Development of Easy to Use and Affordable Biomarkers as Diagnostics for Types II and III Diseases

2. Proponent/s of the project

ANDI - African Network for Drugs and Diagnostics Innovation with China NDI and African partners

3. Project executive summary

The overall objective of the project is to demonstrate specific innovative aspects of R&D coordination and financing towards the development of quality assured diagnostic tools for neglected tropical diseases that are easy to use and affordable in rural communities of disease endemic countries.

Specific aims of the project are

i) Development of protein microarrays containing 8000-10,000 selected antigens for individual diseases;
ii) Probe well-characterized infected human sera from China, Africa and identify serodiagnostic antigens;
iii) Develop, evaluate, validate and optimize field deployable tests for each agent and incorporate m/eHealth; and-
iv) Seek regulatory approval and promote use of products in endemic area.

The key deliverables of the project in first five years include:

i) 8000-10,000 gene clones of 4 parasitic diseases;

ii) 8000-10,000 recombinant proteins of 4 parasitic diseases;
iii) Four kinds of biochips of parasitic diseases;
iv) Four diagnostic kits which can meet the current challenges in diseases control and prevention;
v) Three registered diagnostic products based on multicentre evaluation and registration.

This project is based on an innovative South-South and South-North partnership that aims to address the diagnostics development and access challenges of types II and III diseases. The project leverages: i) high-throughput biomarker screening platform developed by China NDI research groups towards the development of suitable diagnostic kits, ii) available capacity for diagnostics development and evaluation in Africa through the ANDI Centres of Excellence and other global partners, and iii) the strength of ANDI in the coordination of R&D, open innovation, technology transfer/licensing, capacity building and access.

The project initially focuses on schistosomiasis, malaria (falciparum and vivax), and sleeping sickness. An m/eHealth component aims to integrate data from the project and available tests into a cell phone based diagnostic platform in Africa. This potentially revolutionary cell phone diagnostics is being implemented in collaboration with EASE-Medtrend Biotech.

4. Innovative aspects of the project

- Collaborative approaches: the project is a novel South South and South North partnerships coupled with open innovation and knowledge sharing mechanism in support of diagnostics development;
- Strengthen R&D and production capacity including through technology transfer in/to developing countries;

- Effective & Efficient coordination: the project promotes coordination among global players;
- De-linkage: cost of final products will be delinked from the cost of R&D;
- Licensing and IP: utilize licensing approaches that secure access to R&D outputs and final product; and,

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- Financing: demonstration of pooled financing for R&D.

5. The current status of the project

The project is in the implementation phase. Although the implementation started in mid-2015 with ANDI and China NDI resources, the project agreement was signed between UNOPS/ANDI and WHO in October 2015, and funding was received in November 2015. Therefore the project formally started on November 1, 2015.

As indicated above, the award for the first year of the project from WHO has already been received.

6. Progress towards activities since the start of the project

a) ANDI and partners formally launched the project at the 5th ANDI Stakeholders' Meeting held in November 2015 in Nairobi Kenya. WHO/PHI and TDR were in attendance. (http://andi-africa.org/index.php/media-corner/press-release/item/189-2015-andi-stakeholders-meeting-concludes)

b) The project had a head start prior to funding from WHO and the following activities have been initiated:
   - Screening of antigens for schistosomiasis, malaria (*falciparum & vivax*);
   - Continued development of a schistosomiasis diagnostic kit at the Theodor Bilharz Institute (TBRI), Egypt based on monoclonal antibodies; and
   - Project leveraging existing malaria Bio-bank at partner institutions, e.g. University of Lagos, Nigeria.

c) Implemented a formal kick off and planning meeting with partners in Lagos, Nigeria in February 2016. About 20 participants from partner institutions in China (NIPD, Fudan University, Ease Medtrend), University of Lagos Nigeria, TBRI Egypt, Kenya Medical Research Institute, and others. The meeting achieved the following: i) Partners shared progress on project implementation to date including result of screens in China, status of schistosomiasis diagnostics development in Egypt and capacity building etc., ii) Optimized action plans for year one activities for every partner, iii) Updated the budget for every partner and initiated finalization of agreements and transfer of funds to partners, iv) a cell phone technology for integrating datasets and diagnostic tests was discussed and prioritized by the team. The meeting report is being placed on ANDI's website.

7. The first-round award received/expected from WHO based on the recommendation of the Ad Hoc Committee

A sum of US$1,672,556 has been received from WHO covering the period November 2015 to October 2016.

8. The first-round financial support requested (for those projects having not received the recommendation of the Ad Hoc Committee)

Not Applicable

9. Future developments and challenges

Some future significant milestones and timelines include:

i) Development or updating of project SOPs for sample collection, ethical review guidelines, updating and integration of existing datasets and databases with associated capacity building (August 2016);
ii) Development of open knowledge platform for information sharing including data, protocols (November 2016);
iii) Four bio-banks of different parasite samples (October 2016 – December 2018);
iv) Twenty African scientists and institutions trained in diagnostics R&D including sample collection, biobank development, developing microarray and other omics-based capabilities etc. (June 2015 - December 2020);
v) Four diagnostic kits which can meet the current challenges in diseases control and prevention by 2020:
   - Evaluation/validity of schistosomiasis diagnostic kit using serum and urine samples from Africa (2016 –2018)
   - Development and evaluation of integrated malaria (falciparum and vivax) RDT (2017 – 2019)
   - Cell phone based diagnosis of neglected diseases (May 2016 – December 2019); and
vi) Two registered diagnostic products based on multicentre evaluation and registration 2020.

A major challenge for this project is financing. The first year of the project was funded by WHO at about 60% of what the project requested. Based on our initial five year funding needs, the project has a funding gap of about US$18,000,000.00 (Eighteen Million US Dollars) over the next five years. ANDI is hopeful that the WHO member states will scale up the funding for this and other demonstration projects.

10. Other sources of support

None at the moment.

11. Additional comments

We are excited about the potential of this project to support health innovation in developing countries. We hope to be able to extend this project to emergent infections such as Ebola and Zika Virus. The cell phone part of the project promises to integrate results from this project and available RDTs into a single tool that will support rapid diagnosis of disease. The project also promises to deliver an integrated malaria falciparum and vivax to support malaria control and eventual elimination.

12. Report provided to the ANDi Board 8-9 November 2017

Termination of WHO Demonstration Project

The Board is aware that the WHO demonstration project awarded to ANDI was supposed to be for a period of 5 years (at a total cost of about USD20 Million). Unfortunately the project was only funded for one year and half at a much reduced annual budget than projected due to limited funding commitment from WHO Member States.

The first year of the funding was from November 2015 till December 2016 (USD1,672,556). We subsequently did a no cost extension (NCE) for the funds that extended the project to 2017. A technical and interim financial reports were prepared and submitted to WHO and TDR for the first year. The Board was regularly updated also. What is pending is a certified financial report from UNOPS to WHO/TDR which UNOPS is finalising as part of the transition activities of ANDI.

The next funding from WHO was for 6 month from July 2017 – December 2017 (USD335,000). We did another NCE of this fund till June 2018 with the hope that new funding will come for the WHO demonstration project and/or from other sources before the end of this period. Interim technical and financial reports as well as justification for the NCE were presented to WHO/TDR to enable approval of the NCE (following ANDI Board's approval). A certified financial with technical deliverables is being finalized as part of the transition with UNOPS. I attach a recent email communication between TDR, UNOPS and myself on the matter (refer also to the message from Issa). I will follow up with UNOPS on the matter to ensure that certified reports are finalized and delivered to WHO/TDR.

The Board will recall that progress and challenges with funding for the demonstration project (and for ANDI's operation in general) were discussed at the 6th Board meeting hosted by ASRT in Cairo in late 2016, and also at the 7th Board meeting hosted by the AfDB in late 2017 (Board's Resolutions for the two meetings are attached). The ANDI Secretariat was in constant communication with the Executive Committee of the Board about all progress and challenges after the 7th Board meeting.

Finally, the Board will recall that prior to the demonstration project funding, we had funding from the European Union that came through WHO/TDR which briefly overlapped with the demonstration project funding, and technical/financial reports on those were delivered to TDR. Certified financial report has been prepared by UNOPS for WHO.

Action required by the Board: Board to take note of the report, and to formally notify WHO of the exit of ANDI out of UNOPS as appropriate. Executive Director to continue to liaises with UNOPS on the pending certified financial report linked to deliverables till the matter is resolved.

FIOCRUZ

R&D Demonstration Project[1]

R&D demonstration projects were selected as per the Executive Board decision EB134(5) following review by the former Chair and Vice-Chair of the CEWG

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1. Title of the project: "Development of a vaccine against schistosomiasis based on recombinant Sm14 , a member of the Fatty Acid Binding Protein family, to control the transmission of a disease of poverty"

2. Proponent/s of the project: Oswaldo Cruz Foundation, MoH, Brazil and Orygen Biotechnology, Brazil; Project Coordinator: Miriam Tendler

3. Project executive summary: Schistosomiasis, the second-most socioeconomically devastating parasitic disease after malaria, is chronic and debilitating , affecting 800 million people in poor countries living at risk of the disease and estimated 200 million infected in 74 countries (WHO, 2010) .Morbidity due to schistosomiasis is particularly pronounced in school-age children, the part of the population whose physical health and intellectual capacity are fundamental to nation development and sustainability. Control programs based on chemotherapy failed to control transmission for more than three decades .Vaccination with safe and effective vaccine, can contribute to a long term reduction of egg-excretion from the host , truly control transmission and result in a positive trade -off regarding the rebound morbidity observed following reinfection in children living in high endemic areas. The Brazilian Sm14 Schistosomiasis Vaccine Project is the result of scientific developments carried under the coordination of Fiocruz for the last 30 years . It was launched and strongly pushed in the context of a previous WHO program, towards the Development of an Anti Schistosomiasis Vaccine that selected priority antigens, out of which Sm14,t that emerged from an endemic country, continued to be successfully developed. Over the last years it was possible to overcome important bottlenecks in the process of new product/vaccine development: scalling up production process from laboratory bench to a production scale and successful conclusion of two Phase I human trials in healthy adults (both man and woman)living in a Brazilian non endemic area(2011-2014) In 2015- 2016 , already under the scope of CEWG Demonstration Project, it was concluded the first Phase II trial in 30 male adults living in highly endemic area for both Schistosoma mansoni and Schistosoma haematobium of Senegal River Basin ,vaccinated with Sm14+GLA-SE vaccine and conducted by the organization Espoir Pour La Santé(declaration of closure of the trial here enclosed)Main objectives were assessment of safety and immunogenicity achieved in Phase IIA trial .Process development , master cell bank generation and GMP manufacture of new Sm14 lot has started at IDRI

Describe the overall project, outline the objective and ultimate impact this project is designed to achieve. (The length of this section should not exceed half a page.)

4. Innovative aspects of the project

Explain in few bullet points the innovative aspects of the project in line with the recommendations of the CEWG report.

Transmission control of infectious/transmissible diseases has only been achieved through vaccination. Sanitation, chemotherapy and health education are not sufficient to eliminate parasitic diseases that affect disproportionally exclusively people living in endemic areas of poor countries. So far, there are no vaccines against parasites that afflict countries desperately fighting to emerge from poverty and reach better conditions of health and overall development .The major innovation is to address the endemic schistosome infection with up to date technology meant to interrupt transmission of a major endemic disease , based in

[1] The total length of this report should not exceed two pages.

the recombinant vaccine Sm14, formulated with synthetic GLA adjuvant from IDR!. Sm14 was the sole vaccine candidate selected by TDR/WHO, emerging from an endemic country, developed as vaccine towards the Schistosome endemy thus providing an unique and innovative basis to invert the paradigm that up to date technology is developed and provided exclusively by rich countries from Northern Hemisphere and supplied to those in need , mostly in poor underdeveloped countries .Sm14 vaccine, was essentially funded by governmental sources during experimental phase and entered a private-public format with a Brazilian Biotechnology company to guarantee continuation under CEWG guidelines of accessibility and affordability to endemic countries under a South-South collaboration path . Innovative methods were adopted since experimental phase in outbred animal models, that provided an unique opportunity to develop alternative strategy for the protection assessment, based on the measurement of frequencies of worm burden distribution of vaccinated –challenged animal population /non vaccinated infected controls , as opposed to restricted evaluation of mean values of parasite loads, as usually adopted. Sm14 demonstrated to be also protective against Fasciola hepatica, which is the main parasite of livestock worldwide and is being developed in parallel, as a veterinary vaccine

5. The current status of the project

Briefly describe if the project 1) is in the implementation process (provide the project start date) or before the implementation process; 2) received an award or is waiting for an award from WHO based on the recommendation of the Ad Hoc Committee, or submitted or plans to submit the financial request to WHO.

In 2015 , upon the selection of Sm14 Schistosomiasis Vaccine Project as one Demonstration Project under CEWG/WHO, it was subjected to Ad Hoc Committee that recommended financial support from pooled funds for the Process Development / GMP manufacture of Sm14 for the implementation of Phase II clinical trials in endemic areas of Senegal. . It is important to highlight that the Phase II A clinical trial in adults, as recommended by CEWG Ad Hoc committee , was possible to be implemented in such period , due the availability of first Sm14 GMP lot , that was analyzed under an extended stability panel, by IDRI (doc. enclosed) .In parallel Process Development /Master Cell bank Generation and GMP manufacture of Sm14 started part in doors and in part at a subcontracted CMO under IDRI 's supervision and guidance.

In addition, it was requested to Senegalese MoH Regulatory Agency an extension of Phase II A trial , specifically for additional serologic evaluation and duration of immune response induced by Sm14 vaccine in same subjects included in the Phase II A trial, over a 12 month period .

6. Progress towards activities since the start of the project The Sm14 anti Schistosomiasis Vaccine is a long term project developed originally at a Brazilian public institution Fiocruz,MoH, as referred above, with highly innovative aspects and strong adherence to CEWG principles ,as outlined before. Experimental Phase in two different animal models lasted 20 years until clinical phase . 2011-2014, after approval of data derived from an extensive experimental animal phase by the Brazilian Regulatory Agency, ANVISA and Ethics Committee, two Phase I cl**inical trials were developed** using the Sm14 vaccine formulated with GLA-SE (from Infectious Disease Research Institute, IDRI) in 20 healthy male and 10 women volunteers living in a Brazilian non endemic area for schistosomiasis in Rio de Janeiro city . Both Phase I studies were developed in the context of an unique operational design, a network of key partners Main objectives and outcomes of safety and immunogenicity, evaluated in a platform developed by IDRI, were fully achieved .In 2015-2016 , already under the scope of CEWG Demonstration Project, it was concluded the first Phase II trial in 30 male adults living in highly endemic area for both Schistosoma mansoni and Schistosoma haematobium of Senegal River Basin ,vaccinated IM with 3 doses of Sm14+GLA-SE vaccine and conducted by the organization Espoir Pour La Santé(declaration of closure of the trial here enclosed)Main objectives were assessment of safety and immunogenicity (seroconversion of 92% of individuals after second dose) and were fully achieved in Phase IIA trial .Statistic Report is ongoing by Marcia Ciol at Washington University and detailed immunogenicity study will be performed at IDRI on cells and sera derived from the 5 time points included in the trial **Phase I clinical Trials are reported in Clinical Trials.Gov ; Vaccine. 2016 Jan**

20;34(4):586-94. ; Http://agenciabrasil.ebc.com.br/pesquisa-e-inovacao/noticia/2016 among others In 2016 the Oswaldo Cruz Institute/Fiocruz ,organized a press conferencehttps://agencia.fiocruz.br/fiocruz-.
Describe progress made on each planned activity (milestone/output), list significant outputs (for example, publications, reports, press releases, online resources) and provide URL links where appropriate, and explain any unexpected results.

(The length of this section should not exceed half a page.)

7. The first-round award received/expected from WHO based on the recommendation of the *Ad Hoc* Committee
Please provide the amount and indicate the timeframe covered.

On 14 September 2016 , a LOA from WHO, was issued to Sm14 project /Fiocruz granting usd 400.000 for a 10 month period that ended August 31st as part of the presented initial budget for the Phase II a clinical trial and GMP production of a new Sm14 lot .At this point and as depicted in the presented initial budget(copy enclosed) funds shall support activities that are being developed at three institutions :Fiocruz (Coordination Laboratory) ; NGO Espoir Pour la Santé(EPLS), Senegal and Infectious Disease Research Institute , Seattle, USA.

8. **Future developments and challenges**
Describe future significant milestones and timeline, and estimate the total finance gap in the next 5 years. (The length of this section should not exceed half a page.)
- **2017** Development of extended evaluation of serologic immune response in subjects from Phase II A clinical Trial in additional 03 time points over a12 month period; Finalization of Phase II A trial as regards the Statistic Report and immunogenicity study at IDRI; Closure of extension study ref. Phase II A trial (EPLS)
-**2018 –** Process Development and Manufacture of Sm14 lot (IDRI)

Estimated Costs for :

- Immunogenicity studies of subjects from Phase II A trial and extension study (IDRI) $465.700,00
- Process Development and Manufacture of Sm14 lot $1.124,500

-2018 Planning and organization of Phase II b clinical trial in Senegal River Basin Region (EPLS) Estimated $ 900.000,00(Phase II B)

-2018 SAB Meeting and Follow up of activities at IDRI and EPLS (FIOCRUZ)

$140.000,00

9. Other sources of support
Please provide the names of other donors (existing and/or potential) if there are.
Orygen Biotechnology is providing extensive financial support for all activities related to the implementation of Phase IIA clinical trial by EPLS in Senegal River Basin region and Process Development of Sm14 at IDRI
Additional funds are being provided by Fiocruz

10. Any additional comments

1) I (Miriam Tendler) had the opportunity to be present in Senegal (endemic site and EPLS facility) to follow

the first two vaccination doses of Phase II A trial

2)A Scientific Advisory Board(SAB) was organized to follow and advise for Phase II trials , .**Members**: Cláudio Tadeu Daniel-Ribeiro - Oswaldo Cruz Institute, Fiocruz (BR) **Head of the SAB** ;Steven Reed - Infectious Diseases Research Institute, IDRI (USA); Socrates Herrera – Latin American Center for Malaria Research and Control, Cali (Colombia) ;Miriam Tendler – General Coordinator of the Sm14 Initiative,Fiocruz (BR) ; Nathalie Mielcarek, Pasteur Institute of Lille (FR) ; Santiago Mas-Coma, Universidad de Valencia (SP), ; Wilson Savino, Fiocruz(BR)

from the Data Safety Monitoring Board Dr Idrissa Talla – DSMB President (observer) (SN) ; Gilles Riveau – Senegal Coordination – BRC EPLS CEO (SN) DDoudou Diop – Principal Investigator Phase IIA – CRB; ;EPLS (SN) The 1st Meeting of the Sm14 Phase II A Scientific Advisory Board(SAB) took place at Senegal, January 2016 ; 2nd Meeting of SAB was held at Fiocruz/Brazil, April 2016 and next meeting of SAB is planned for beginning 2018

Reporting Template R&D Demonstration Project 12 month workplan

This document will report on the first 12 month workplan for a R&D demonstration project selected as per the Executive Board decision EB134(5) following review by the former Chair and Vice-Chair of the CEWG. This report should align precisely with the budget template in line with the award recommended by the Ad Hoc Committee on XX/XX/2015 as set out in the Letter of Agreement.

1. Title of the project:

Multiplexed Point-of-Care test for acute febrile illness (mPOCT)

2. Sponsor/s of the project:

Report Prepared by	**Gaurav Batra**	**Date Submitted**	
Phone	**+91-129-2876357**		
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Date LoA* Awarded		**LoA Amount**	*$997,969*
Project Start Date	*15 Feb 2017*	**Date this report covers**	*15 Feb 2017 to 31 Oct 2019*

*LoA – Letter of Agreement

5. Project summary:
Describe the overall project proposal, outline the objective and ultimate impact this project is designed to achieve.

Acute febrile illness (AFI) is common ailment of the tropics and sub-tropics caused by a very diverse set of pathogens. Differential diagnosis of these etiologies based on clinical criteria alone is nonviable as clinical signs and symptoms of most of these infections are very similar. Hence accurate diagnosis is only possible using pathogen specific diagnostic tests. In low income countries, many preventable deaths occur because of delay in or lack of correct diagnosis. Based on these facts, the availability of multiplex test which can quickly identify the causative agent from a group of pathogens causing similar symptoms, is of paramount importance.

Infectious diseases which are mainly responsible for AFI and also amenable for multiplexing include Malaria, Dengue, Typhoid/Paratyphoid, Leptospirosis, Scrub Typhus and Chikungunya. Despite a strong need, a multiplex Point of Care Test (POCT) which can be used in a resource limited setting for the detection of multiple etiologies of AFI is not available. Although, individual (singleplex) POCTs for these infections are commercially available, most

of these tests suffer from quality issues.

Major reasons for poor performance of available singleplex tests are: 1) Insufficient financial incentive to develop high quality and rigorously evaluated tests for LMIC; 2) Use of poor quality antigens/antibodies; 3) lack of knowledge on pathogen-specific target(s) culminating in cross-reactivity; 4) Lack of evaluation on local clinical specimen resulting in inappropriate cutoffs.

There are many state-of-the-art diagnostic platforms available which can be used for multiple target screening in a specimen e.g. advanced multiplex nucleic acid tests, array-based immunoassays and bead/flow-based assays. However, these platforms are resource intensive and hence not suitable under resource limiting conditions. Based on these facts, we decided to use a simple field deployable lateral flow format (LF), which with some innovation (multi-channel approach and change in tracer), can be used for the generation of a multiplex test for at least 6 major high-burden pathogens responsible for AFI. As the prevalence of different etiologies of AFI varies between regions, a practical multiplex test should be modular, enabling addition or removal of any target from the test device based on the region of implementation without the need of re-optimization of whole assay. Thus, to enhance the multiplexing potential and to have flexibility, our approach is to have parallel assay zones. As the colloidal gold tracer used in routine LFAs is generally not very sensitive, we proposed in this project to use upconverting phosphor nanoparticles (UCPs) coupled with simple handheld reader device to bring assay sensitivity close to central lab tests in the workflow of rapid test. In this approach, parallel detection of pathogen-specific IgM antibodies and pathogen-derived antigen in whole blood or serum was proposed. For *Plasmodium falciparum* and *P. vivax*, antigens were proposed to be detected. For Dengue, both antigen (NS1) and IgM were planned to be detected. For *S. Typhi/ Paratyphi A, Leptospira spp. , Orientia tsutsugamushi* and Chikungunya virus only IgM antibodies were planned to be detected. However, due to the problems in available singleplex tests, we proposed to generate first the high-quality diagnostic intermediates/reagents for each pathogen.

Objectives:

The goal of the larger project (we were seeking funding ~ USD 20 million) was to develop high quality and vigorously evaluated multiplexed point-of-care test for acute febrile illness. Because of the limited funds availability, the goals for this phase of the project were limited to:

1. Operational/techno-legal planning and initiation of project
2. Generation of UCP based LFA for Pf-HRP-2
3. Generation of UCP based LFA for Pan-LDH
4. Dengue NS1 assay reagents finalization
5. Scrub Typhus Reagent generation for IgM assay
6. Leptospira reagent generation for IgM assay
7. Production of additional antigens for S. typhi IgM detection
8. Generation of sera panel for AFI

Impact: This diagnostic innovation aims to fill the gap in rapid diagnosis of etiology of acute febrile illness caused by a panel of highly prevalent pathogens. Target disease/health condition it aims to cater is Acute Febrile Illnesses (Dengue, Malaria, Typhoid/Paratyphoid, Chikungunya, Scrub Typhus, Leptospirosis) which mainly fall under Type II and III disease category. This innovation aims to help in the prudent use of antibiotics and anti-malarial and ensure rapid clinical decision-making suited specifically for particular pathogen. Also, the technology innovation can be utilized further across any disease scenario.

6. Description of progress towards activities and tasks.
As outlined in Annex 1 of the LoA please describe:

a. ***Progress made on each planned activity (milestone, output and/or outcome).***

Below are the specific objectives of the project and the progress made on each objective with outcome:

1. Operational/techno-legal planning and initiation of project

After signing of the LoA, all the project partners were informed about the start of the project and separate agreements with project partners were signed keeping the LoA (between the WHO and THSTI) as reference document.

Meetings were conducted with project partners and also with other stake holders like people with knowledge of diagnostic product development, regulatory requirements, specimen panel generation, disease experts, instrumentation, implementation, diagnostic market, etc. The inputs were used to design the work strategy.

Principle Investigator also attended the Advanced Course on Diagnostics (ACDx) that is organized by the Mérieux Foundation and the London School of Hygiene & Tropical Medicine. The faculty of the course included different stake holders relevant to the diagnostics development, market, donors, and policy. Our project was discussed with all the stake holders in the field of diagnostics and policy during the course. The suggestion given by the stakeholders were used to streamline the strategy. The comments on the target product profile were also used to refine the TPP. Also, suggestions/information were sought about the regulatory component in this meeting as there was a whole session on the regulations in the different parts of the world.

PI also attended a training on the WHO prequalification and the quality systems keeping in mind the future needs. Extensive discussion were done with WHO PQ team, Indian regulator, Central Central Drugs Standard Control Organization, that covers approval of diagnostics products in Indian market. Also 3 days meeting was attended on the device and IVDs regulation in India, EU and US, at Venture Centre, with interaction with experts who have handled IVD clearance in India, US and the EU. Based on the meetings with different stakeholders in the diagnostics field, regulatory requirements were identified.

Based on the discussion with different stake holders including diagnostics product distributors, ex-employees of some of the leading RDT companies, business plan was prepared. Based on the inputs from the stakeholders, we have prepared a very simple one page information brochure for this project which can be shown to the prospective donors.

The Principle investigator also attended 2 courses/conferences on the R&D to manufacturing to market for lateral flow assay (rapid diagnostic test), to understand the strength and limitations of technology in manufacturing perspective and economics of low-cost high-volume diagnostics. The information related to the lateral flow test manufacturing was used to design the R&D stage strategy that keeps the manufacturing limitations in mind. In these meetings information was also obtained about the availability of the raw martial for the lateral flow test on industrial scale.

Milestones: Achieved

2. Generation of UCP based LFA for Pf-HRP-2

This activity involved acquisition of anti-HRP-2 monoclonal antibodies (MAbs), native reference material, recombinant standard antigens, and other required reagents for assay development, conjugation optimization of MAb(s) with UCP particles and the development of ultrasensitive UCP based *plasmodium falciparum* HRP-2 antigen detection LFA. Below are the details of work done and the achievements:

We have developed a highly sensitive POC test for the detection of *P. falciparum* infection using up-converting phosphor nanoparticles based lateral flow (UCP-LF) immunoassay. The developed UCP-LF test was validated using whole blood reference panels containing samples at different parasite densities covering eight strains of *P. falciparum* from different geographical areas. The limit of detection was compared to a WHO prequalified rapid diagnostic test. The UCP-LF achieved a detection limit of 0.2 parasite/µl to 2 parasites/µl depending on the strain, which is a 50 to 250-fold improvement over the conventional malaria RDTs. The improvement in sensitivity is due to the unique label properties of the photoluminescent up-converting phosphors. In fact, the UCP-LF test is

as simple to use as the currently used conventional RDTs and the results are read after 20 minutes with a simple photoluminescence reader. The developed UCP-LF is highly stable even at 40$_{o}$C for at least 5 months (and counting). Moreover, the UCP-LF strips can either be read after 20 minutes at the testing site or in surveillance studies sent to a more central location to be read or archived. There is no loss of signal after running the strip and reading it at later time point (even 10 months after run, no loss of signal). The UCP-LF has potential both for diagnostic testing of symptomatic as well as asymptomatic individuals (transmission reservoir).

The concept:

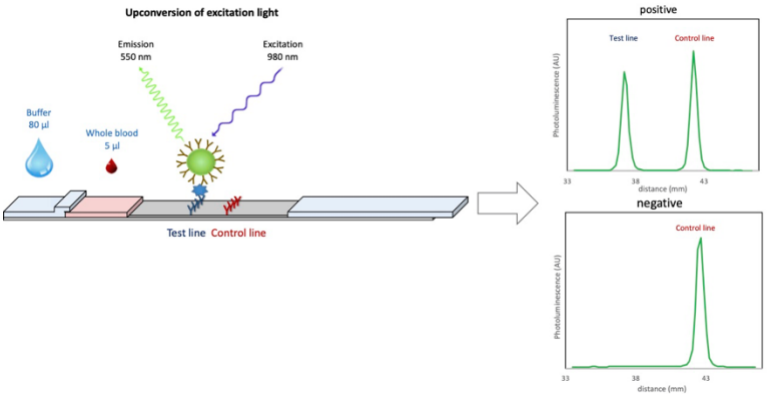


Figure 1. Principle of the UCP-LF immunoassay for HRP2 antigen detection. The photoluminescence signal of the UCPs is measured with an instrument by exciting the particles with an infrared laser at a wavelength of 980 nm and measuring the resulting emission at 550 nm. The area containing the test and control lines is scanned and the line signals are quantified with the instrument.

Comparison of UCP-LF HRP2 test with a commercial WHO Prequalified RDT for HRP2 Ag:

The performance of the UCP-LF test was compared with a WHO prequalified conventional RDT that is using visual label. Two whole blood panels containing cultured *P. falciparum* from eight different strains were used for evaluation. The panels are available from WHO/CDC and FIND/Zeptometrix, and can be used for standardized evaluation of new HRP2 immunoassays. The WHO manufacturer's panel had five strains and the Zeptometrix/FIND culture panel had six strains, with overlapping strains (Table 1). These panels cover 3 types of HRP2 sequences i.e. type A, B and C.

For the Zeptometrix/FIND *P. falciparum* panel, the detection limit with the UCP-LF was found to be 12 pg/ml for five strains and 40 pg/ml for the W2 strain. The detection limit of the conventional RDT was found to be 800 pg/ml for four strains and 400 pg/ml for FCQ79 and Borneo strains. The lowest HRP2 concentrations detected by the tests from dilution series of each sample are shown in Table 1.

Table 1. Analytical sensitivities of UCP-LF and First Response HRP2 RDT in pg/ml of HRP2 in Zeptometrix/FIND culture panel.

Sample type	Sample	Type	Test analytical sensitivity, pg/ml HRP2		
			UCP-LF	First Response HRP2 [a]	
				Visual inspection	mobile-based reader
Zeptometrix/FIND culture panel	FCQ79	A	**12[b] (400)**	400[b] (800)	400 [b] (8000)
	Benin I	A	**12**	800	800
	Borneo	C	**12**	400	400
	Santa Lucia	B	**12**	800	800
	W2	B	**40**	800	800
	PH1	C	**12**	800	800

[a] Results for the First Response HRP2 RDT are shown from both visual inspection and as read using mobile-based reader.
[b] In-house dilutions, official dilutions in parentheses

The WHO manufacturer's panel was provided in two dilutions of 2000 parasites/ µl and 200 parasites /µl. Further dilutions were made in-house in whole blood using 2000 parasite/µl samples as starting material. The UCP-LF achieved a detection limit of 0.2 to 2 parasites / µl, depending on the strain (Table 2). The detection limit of the conventional RDT varied from 10 to 100 parasites / µl, when the strips were assessed both by eye and the mobile-based reader (Table 2). A representative strip profile of UCP-LF with different parasite density is shown in Figure 2.

Table 2. Analytical sensitivities of UCP-LF and First Response HRP2 RDT in terms of parasites/µl in WHO manufacturer's panel.

Sample type	Sample	Type	**Test analytical sensitivity, parasites/µl**		
			UCP-LF	First Response HRP2 [a]	
				Visual inspection	Mobile-based reader
WHO manufacturer's panel	US05F Benin I	A	**0.2**	50	50
	US05F Santa Lucia	B	**2**	100	100
	US08F Nigeria XII	B	**0.2**	20	20
	US05F FC27/A3	B	**0.5**	10	20
	US05F PH1	C	**1**	50	50

[a] Results for the First Response HRP2 RDT are shown from both visual inspection and as read using mobile-based reader.

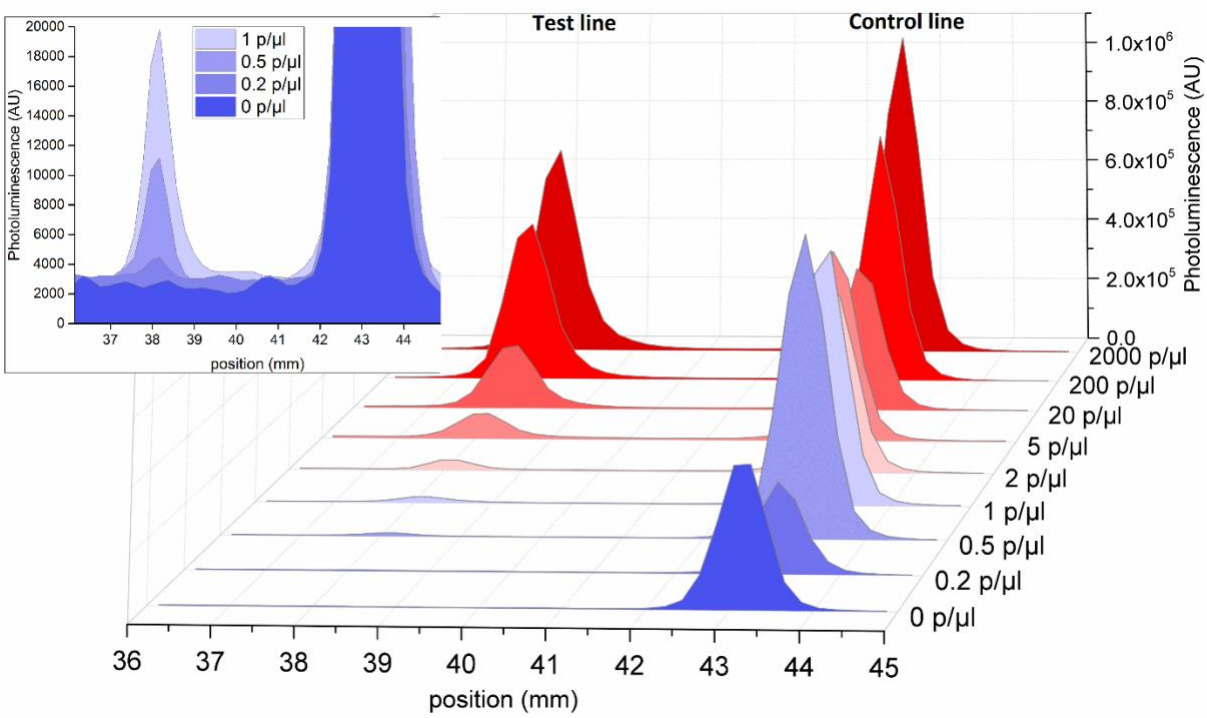


Figure 2. The photoluminescence intensity strip profiles of the UCP-LF strips, with the WHO manufacturer's panel US05F Benin I sample dilutions as an example. The low concentrations tested are shown in the inset.

The number of positive replicates for all samples are shown in Table 3. These results shows the positivity rate in 3 replicates.

Table 3. Positive replicates of the UCP-LF with two *P. falciparum* panels: Zeptometrix/FIND culture panel (A) and WHO manufacturer's panel (B).

A

HRP2 (pg/ml)	FCQ79	Benin I	Borneo	Santa Lucia	W2	PH1
8000	3/3	3/3	3/3	3/3	3/3	3/3
800	3/3	3/3	3/3	3/3	3/3	3/3
400	3/3	3/3	3/3	3/3	3/3	3/3
120	3/3	3/3	3/3	3/3	3/3	3/3
40	3/3	3/3	3/3	3/3	3/3	3/3
12	3/3	3/3	3/3	3/3	2/3	3/3
4	2/3	2/3	1/3	2/3	1/3	0/3

B

parasites / µl	US05F Benin I	US05F Santa Lucia	US08F Nigeria XII	US05F FC27/A3	US05F PH1

2000	3/3	3/3	3/3	3/3	3/3
200	3/3	3/3	3/3	3/3	3/3
20	3/3	3/3	3/3	3/3	3/3
5	3/3	3/3	3/3	3/3	3/3
2	3/3	3/3	2/2	3/3	3/3
1	3/3	1/3	3/3	3/3	3/3
0.5	3/3	0/3	3/3	3/3	2/3
0.2	3/3	0/3	3/3	1/3	1/3

The prepared UCP-LF strips remained stable for at least 21 weeks at room temperature (22 °C), +40 °C and +50 °C protected from humidity (Figure 3). However, the failure rate of the strips stored at +50 °C was 19 % (5/27 strips) at the 21-week test point. In the failed strips, the liquids did not flow through the strip and thus no control line was measurable. No failure was observed for the strips stored at 40 °C at 21-week test point. Possible hook-effect, or prozone effect of the UCP-LF was tested with recombinant HRP2 up to a concentration of 1 mg/ml and the test remained clearly positive (Figure 4).

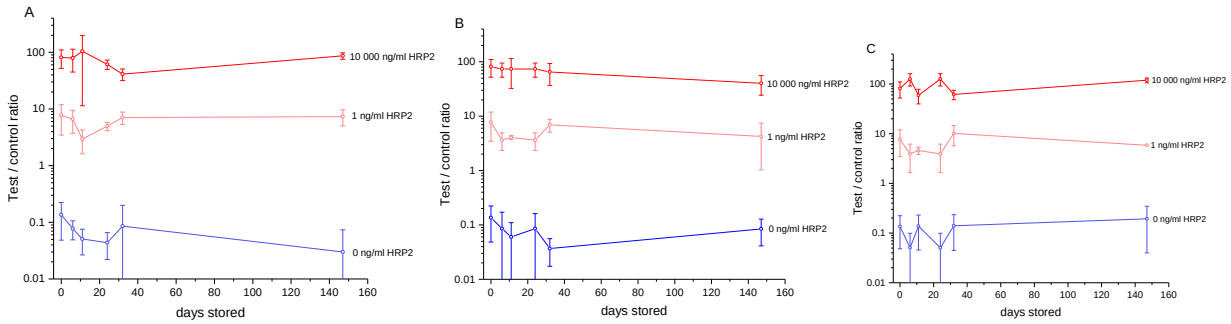


Figure 3. Stability of UCP-LF strips stored at (A) room temperature (22 °C), (B) +40 °C and (C) +50 °C, tested with different concentrations of recombinant HRP2.

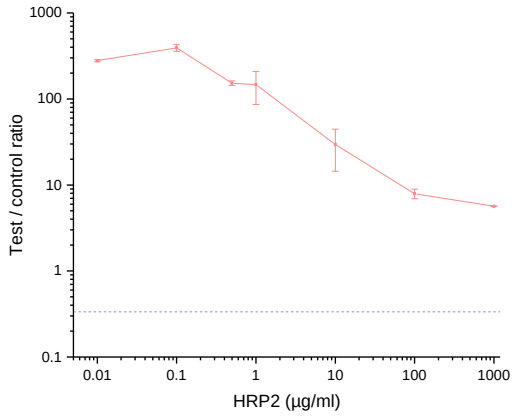


Figure 4. Evaluation of the hook effect in the UCP-LF test at high concentrations of recombinant HRP2. The test remains clearly positive even at 1000 µg/ml concentration of HRP2. The cutoff is shown as dashed line (--).

Since the UCP-labels are very photostable due to the inorganic crystal lattice of the photoluminescent particle core, the UCP-LF strips can easily be stored after running the test and measured after long-term storage. The UCP-LF test can be read at any time after 20 min, also when dried. There was no degradation in the sensitivity or specificity of the test when strips were re-measured 10 months after running the test (Figure 5). This may be useful for verification, but more importantly a barcoded test can also be run in a location without access to a reader instrument and shipped to be read and archived in a local or regional center with access to the reader. Dried blood spots (DBS) are often used to transport the sample to a laboratory for analysis. In contrast to dried blood spots, the dried UCP-LF strip do not need any preparation or assay steps but can simply be read at the central location with access of reader instrument.

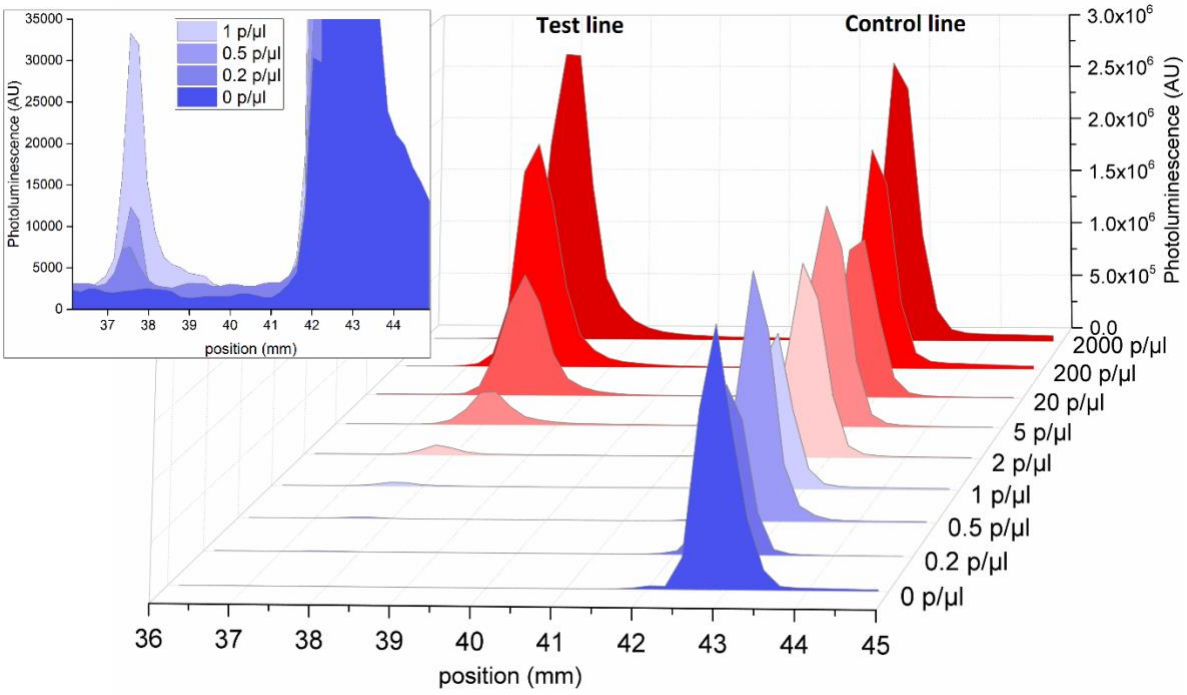


Figure 5. The photoluminescence intensity strip profiles of the UCP-LF strips measured 10 months after running the test, with the WHO manufacturer's panel US05F Benin I sample dilutions. The lowest concentrations are also shown in the inset.

The resulting assay is as rapid and simple to use as the conventional RDTs, and with the addition of a portable UCP-reader, it could be suitable for detection of low parasitemia asymptomatic patients in elimination or eradication settings. A fully robust test would still need to include plasmodium lactate dehydrogenase antigen for the detection of HRP2 deficient strains of *P. falciparum* and other human *Plasmodium* species, especially *P. vivax*.

Key features of the developed test:

- Similar to the conventional malaria RDTs (whole blood, sample volume, time) but the result is read using a battery powered reader device
- Detect *P. falciparum* HRP2 antigen
- Can detect as low as 0.2 parasite/µl (250-times higher analytical sensitivity compared to conventional RDTs)
- Test stable at + 40$_0$C for at least 5 months (stability studies are ongoing)
- Very wide detection range from 12 pg/ml to 1 mg/ml (1mg /ml is the max conc tested)
- Suitable for both the symptomatic patient and asymptomatic transmission reservoir
- Connectivity and data transfer through reader

Milestone: Generation of ultrasensitive lateral flow assay for the detection of PfHRP-2 Ag.

Achieved

3. Generation of UCP based LFA for Pan-LDH

This activity involved acquisition of anti-pLDH MAbs, native reference material, and other required reagents for assay development. The activity also involved the generation of native like (tetrameric) pLDH antigen recombinantly for 5 plasmodium species (*Plasmodium falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*) and the generation of hybridoma clones secreting the anti-pLDH antibodies. The objective includes the generation of UCP-LF for Pan-pLDH detection.

Synthetic genes for the expression of pLDH from five *Plasmodium* species were obtained and cloned in *E.coli* expression vector. The generated constructs were transformed in *E. coli* and the protein expression was induced. All the constructs found to be correct and expressed the desired recombinant protein.

Expression and purification: The expression conditions were optimized to get the recombinant protein in soluble form followed by the optimization of cell lysis conditions that gives maximum enzymatic activity from the expressed pLDH. The optimal conditions particularly the pH was slightly different for each pLDH. After optimization of lysis conditions, proteins were expressed on large scale and separately purified on Ni-affinity columns. The purified fractions were pooled based on the purity on the coomassie stained gel and the enzymatic activity of the pLDH. The pooled fractions were further purified on a gel filtration column to remove the monomer and dimer of pLDH as we were only interested in the tetrameric form as present in the infected human blood.

The activity of purified proteins were compared with the commercially available options. It was observed that the in-house generated antigens have much higher enzymatic activity then the commercially available versions. Below figure show the purification and characterization of the recombinant PLDH from five plasmodium species.

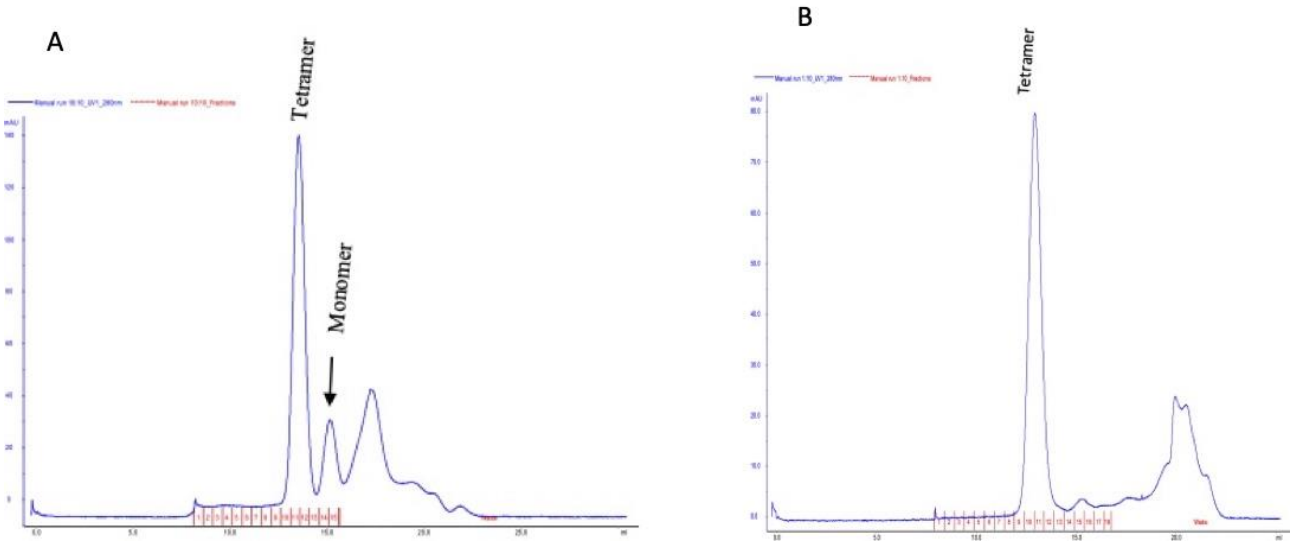


Figure 6. After the purification on Ni-column, the purified proteins were further purified on gel filtration column to separate the tetramer from monomer and dimer of pLDH. A) Gel filtration chromatography profile of PfLDH where tetramer is separated from other forms of pLDH and impurities. B) Gel filtration chromatography profile of PvLDH.

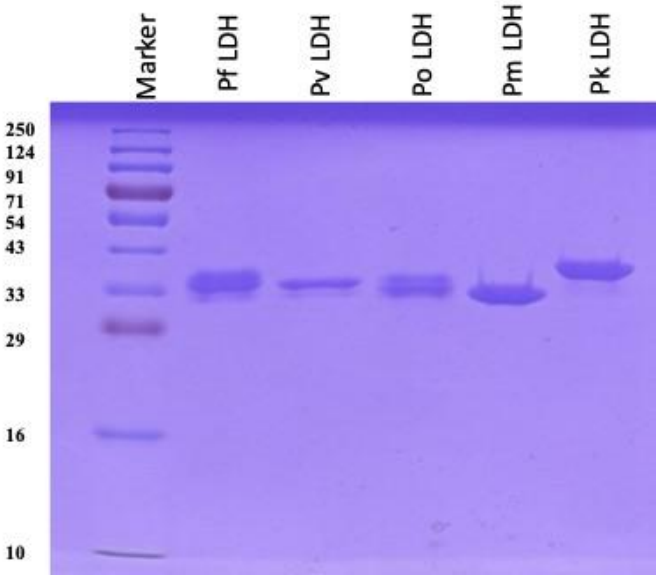


Figure 7. Coomassie stained SDS-PAGE showing the purified pLDH from Pf, Pv, Po, Pm and Pk.

The purified proteins were used for the immunization of Balb/C mice. Standard hybridoma technique was used for the generation of hybridoma clones to generate anti-pLDH antibodies. Antibody secreted by the stabilized hybridoma clones were screened for their binding with five recombinant tetrameric pLDH from five *Plasmodium* species. Based on the binding characteristics clones were divided into different categories e.g. pan binders, Pf specific binders and Pv specific binders. It is known in the literature that the antibodies which binds to either Pf or Pv often cross react with with Pk LDH. We have also observed this cross-reactivity with our Pf and Pv binders. Figure. 8 shows the reactivity of some of the promising antibodies which bind to either the pan epitope or or species specific epitope.

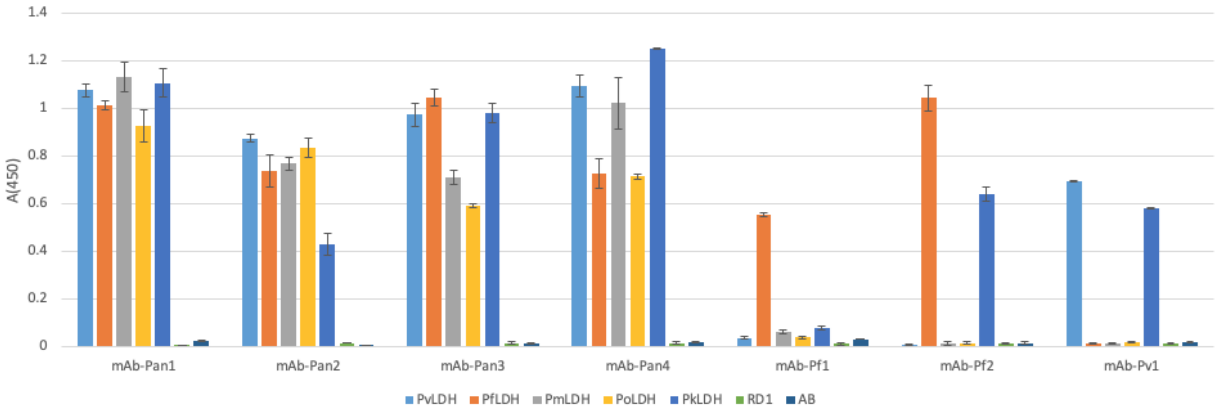


Figure 8. Cross-reactivity profile of some of the anti-pLDH mAbs using ELISA. X-axis shows the name of the mAb and the absorbance is depicted on y-axis. Only 2 ng pLDH antigens from different plasmodium species was used in this assay.

The selected antibodies were further characterized for their affinity with PLDH using Octet Biolayer interferometery system. It was observed that some of the antibodies generated by us have extremely high

affinity for the PLDH. Results are shown in the figure 9.

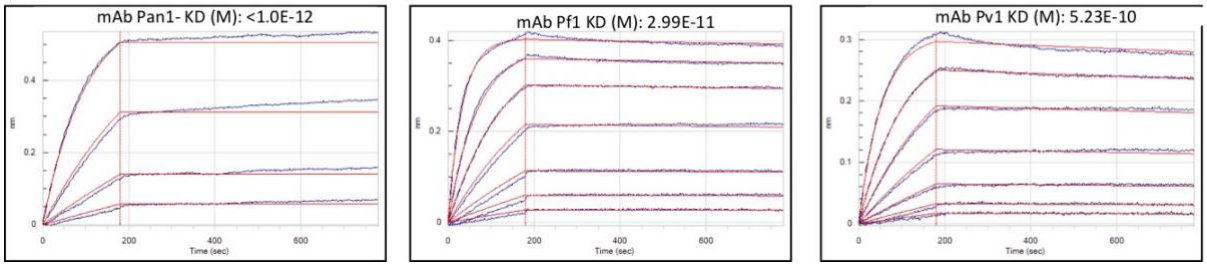


Figure 9. A representative affinity measurement data of some of the anti-pLDH antibodies using Octet BLI system.

The antibodies were further characterized in the lateral flow format and the suitable pairs were identified. A prototype dipstick assay was developed using antibodies generated in-house. Figure 10 shows the comparison between in-house UCP LFA and a commercial WHO prequalified RDT for pLDH. It can be seen that the commercial assay cannot detect 10ng/ml of pLDH whereas the UCP-dipstick can easily detect this amount of pLDH.

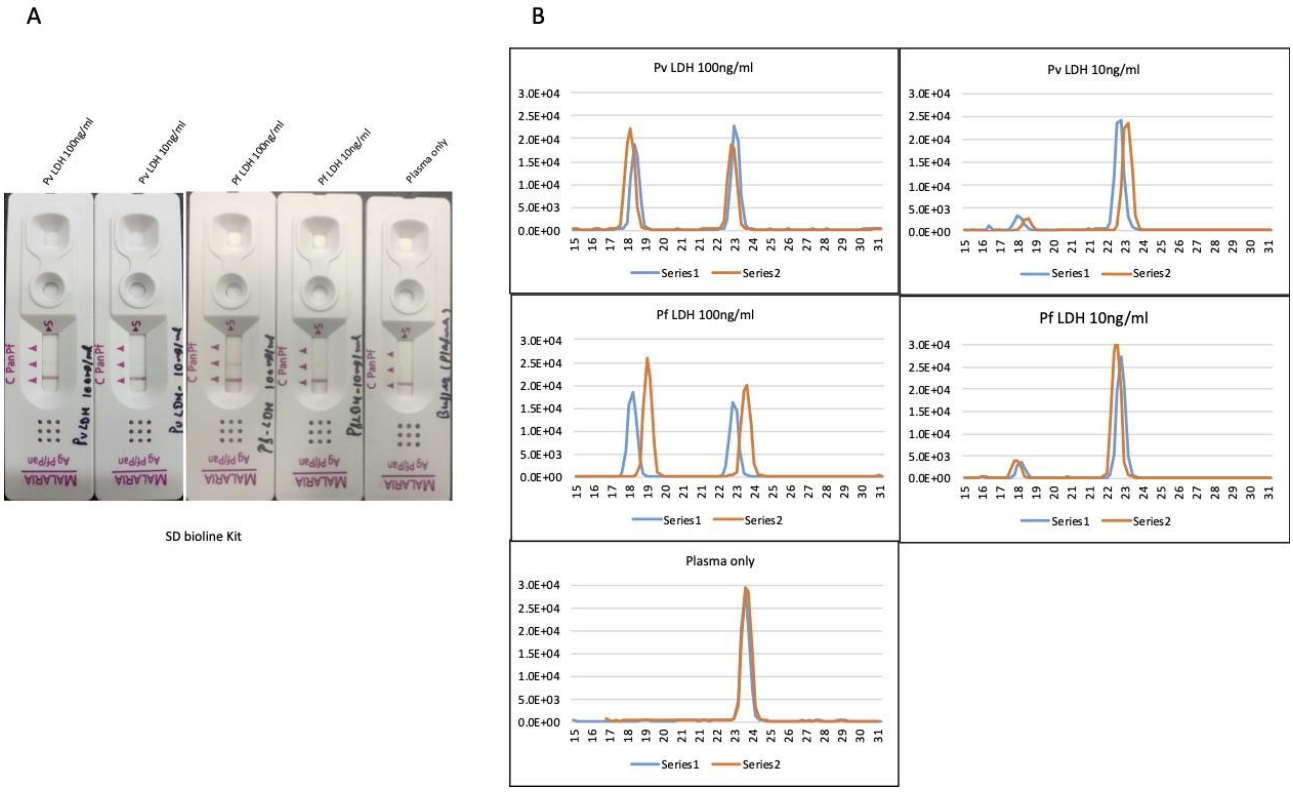


Figure 10. Comparison of UCP-dipstick with a WHO prequalified RDT for pLDH. A) images conventional RDT with different amount of pLDH as analyte; B) strip profile of UCP-dipstick for pLDH.

Summary: For this Objective we have generated mouse monoclonal antibodies specifically binding pan and species specific epitopes of pLDH antigen. The in-house antibodies were used for the development of UCP – dipstick prototype assay that is more sensitive than the commercially aware available pLDH assay. However, we could not reach to the final stage of the assay development like what has been done for PfHRP2.
In the initial stage, high sensitivity assay could not be developed using commercially available antibodies that were earlier identified. This situation forced us to develop our own monoclonal antibodies for assay development. Several Pan-LDH antibodies were developed by us and appropriate pair was identified from the antibodies generated in-house. The prototype assay with liquid tracer was developed till the end of the project. A project proposal on further development of an ultrasensitive RDT for the detection of multispecies malaria infection is in the final stage of evaluation by Indian funding agency, BIRAC. We are hopeful to get the funding for the further development of the assay targeting the pLDH antigen.

Key outputs:

- The tetrameric form of the pLDH antigen from 5 plasmodium species were recombinantly generated
- Large repertoire of mouse mAbs against pLDH antigen were generated
- Some of the antibodies found to be true pan-antibodies as they recognize pLDH from 5 plasmodium species
- Using these antibodies, a prototype assay was developed based on UCP platform
- The prototype assay (with liquid tracer) found to be more sensitive than the commercial RDTs for pLDH

Milestone: Generation of ultrasensitive lateral flow assay for the detection of LDH antigen of major plasmodium species.
Partially achieved.

4. Dengue NS1 assay reagents finalization

Earlier we had generated anti-NS1 binders derived from phage display synthetic antibody libraries. In the project activity, these library-derived recombinant antibodies were further studied to great details for their applicability for diagnostic purpose. We have also generated new anti-NS1 monoclonal hybridoma clones and screened the suitable MAbs, which can be used in diagnostic assay. Also generated the PAbs against NS1. The antibodies were screened using both well based assay and label-free real-time binding measurement system (octet K2). The shortlisted antibodies were further studied in their applicability in Lateral flow assay format and the final pairs were identified.

Different Phage clones provided different recognition profile for different NS1 serotypes as seen in the figure 11. The phage clones that were able to detect all the four serotypes were considered as potential pan clones and the one which were detecting only one of the four serotypes specifically were considered as serotype specific phage clones.

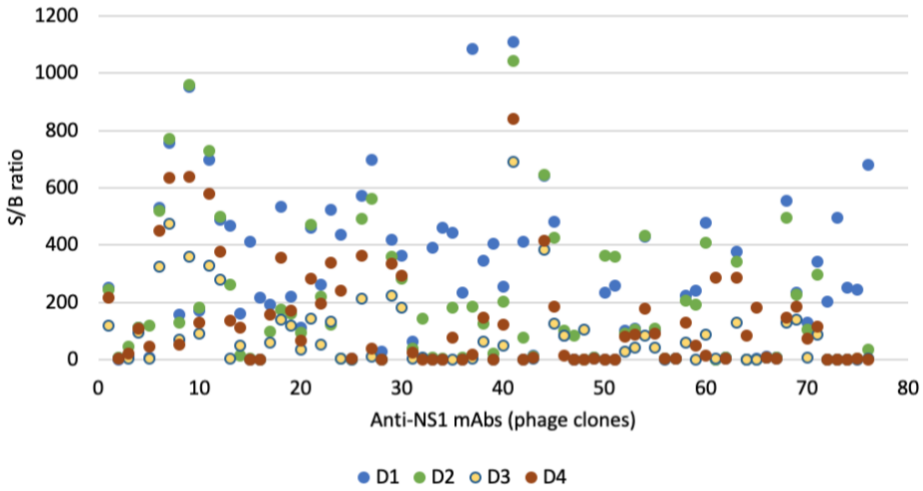


Figure 11: Comparative reactivity profile of 76 mAbs (phage clones) with different DENV NS1 serotypes. The x-axis represents the phage clones and the y-axis represents the Signal to background ration obtained for each clones Each dot is representing the binding of phage clone to respective NS1 serotype. Blue dots represent binding to D1 NS1, green dots to D2 NS1, yellow dots to D3 NS1& brown dots to D4 NS1.

The serotype specific anti-NS1 pair were evaluated for their specificity of NS1 detection by analysing the level of their cross reactivity with other NS1 serotype. For this NS1 antigen from each serotype was captured on a Pan fab and traced by all the four-serotype specific phage clones. Results suggest that each serotype specific anti-NS1 pair is highly specific for respective NS1 and devoid of any cross reactivity with NS1 antigen of other serotype (Figure 12).

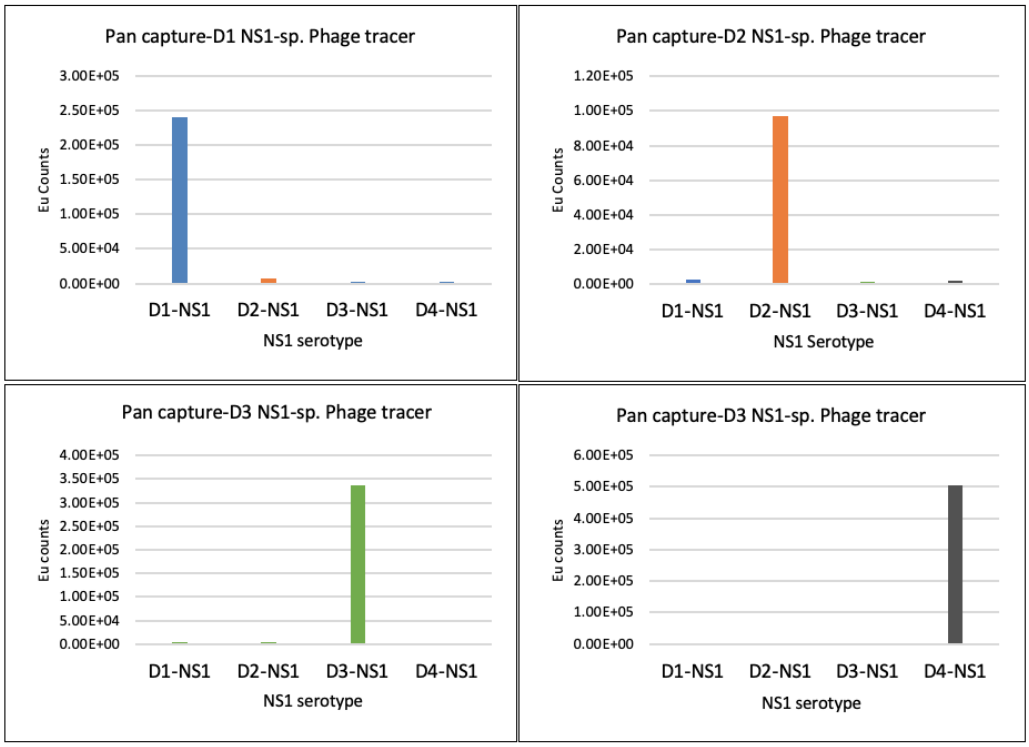


Figure 12: Immunoassay for evaluation of specificity of serotype specific anti-NS1 pair: Capture immunoassay for Vero-derived dengue 1-4 culture supernatant captured on pan clone and traced with (a) D1-specific (b) D2-specific (c) D3-specific (d) D4-specific phage clone as tracer. The x-axis represents native dengue NS1 antigen of all 4 serotypes and y-axis represents Eu counts as a measure of signal obtained from NS1 antigen.

Hybridoma technology was utilized for the generation of mouse anti-NS1 Mabs. The immunizations were performed on 6-8 weeks old female BALB/c mice. Priming of the mice was performed subcutaneously at two sites with the antigen-adjuvant emulsion. Three sets of boosters were administered subcutaneously or intraperitoneally at interval of 21 days each. For the final booster, the mouse was chosen on the basis of highest immune response observed during the titre test of immunized sera and a final booster of antigen was administered intravenously. On the 3rd day after the IV booster, mouse was sacrificed, and spleen was collected for fusion process. Fusion was mediated by polyethylene glycol and after the fusion cells were resuspended in HAT media. After 10 days of undisturbed incubation in HAT selection media, plates were examined for the presence of positive clones through ELISA.

Monoclonal antibodies were analysed for their ability to capture the native form of NS1 antigen in one step assay format. In order to imitate the real scenario, mAbs were subjected to assess their efficiency of NS1 detection in human plasma. The inactivated DENV infected Vero culture supernatant was spiked in two different dilutions of human Heparinized Plasma. All the capture antibodies were efficiently able to detect the respective NS1 serotype plasma according to their specificity (Fig 13). No signal was observed with the addition of Mock Vero culture supernatant on the anti-NS1 capture antibodies.

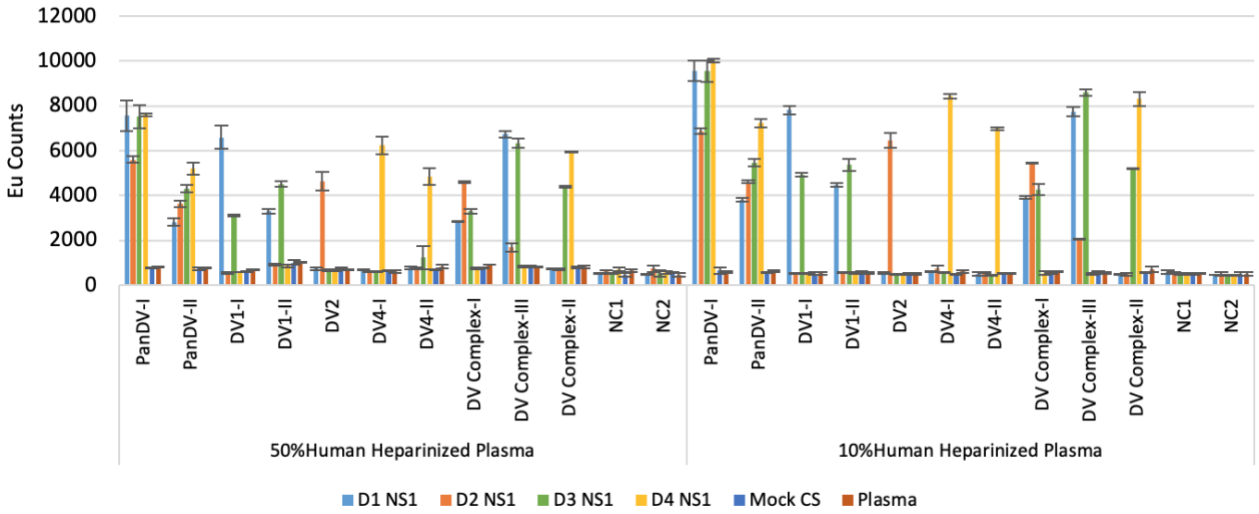


Figure 13 : Immunoassay for evaluation of final Mabs with Native NS1: X-axis represents the individual Capture Mabs and Y-axis represents the measured Eu counts. Each Mab was observed for its binding to native hexameric NS1 (1-4) spiked in 50% or 10% human Plasma in one step capture immunoassay format. NC1; Negative control 1 (anti-LDH Mab1), NC2; Negative control 2 (anti-LDH Mab2)

Affinity measurement of all the Mabs was done through Octet BLI system. Screening of the antibodies at a kinetic analysis platform confers the advantage of real time monitoring of the antigen- antibody interaction This can be better understood by taking into consideration that two antibodies may possess a similar affinity to its target but their difference in kinetic rate constant of association and dissociation can be used to estimate which of these antibodies will be more useful in assay development. A comparative analysis of the kinetic and binding constants of two Mabs (Figure 14 and Table1) clearly shows that despite having same value for the affinity constant K_D of both the antibodies, Pan2 has slow dissociation rate as compared to the Pan1 antibody. However, the Association rate of first antibody (Pan1) is slightly higher than the second antibody (pan2). This kind of critical kinetic analysis helps in selecting optimal antibodies for lateral flow assays where there is requirement of antibodies having very high on-rate for capture Mab.

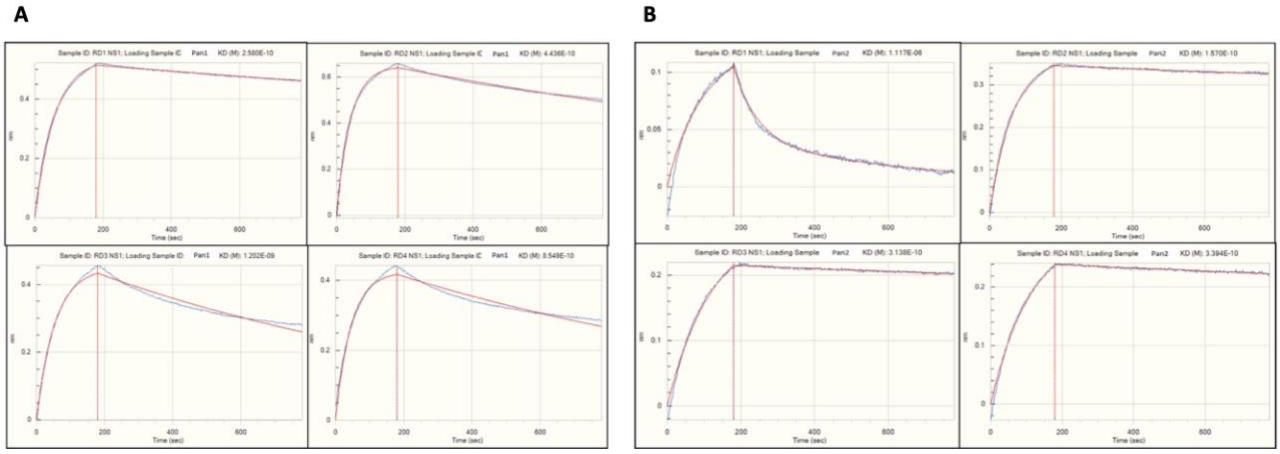


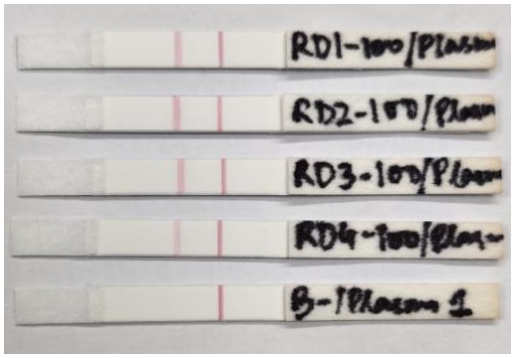
Figure 14. Kinetics analysis of two Pan monoclonal antibody against NS1 antigen using Octet system. A) Pan1; B) Pan 2.

Table1: Kinetic constant values obtained after fitting the observed data in 1:1 binding model with Octet analysis software; KD-affinity constant (ratio of K_{dis} to K_{on}); K_{dis}- Dissociation constant (fraction of complex that Decay/sec); K_{on}-Association constant (No. of complexes formed per second in 1molar solution of antigen and antibody); X^2-Chi square value for goodness of fit.

Sample ID	Loading Sample ID	Conc. (nM)	Response	KD (M)	KD Error	kon(1/Ms)	kon Error	Full X^2	Full R^2
RD1 NS1	Pan 1	25	0.5111	**2.58E-10**	2.31E-12	7.04E+05	2.15E+03	0.0095	0.9982
RD2 NS1	Pan 1	25	0.6559	**4.44E-10**	3.59E-12	9.85E+05	4.94E+03	0.044	0.9931
RD3 NS1	Pan 1	25	0.4499	**1.20E-09**	1.64E-11	7.10E+05	7.98E+03	0.084	0.9794
RD4 NS1	Pan 1	25	0.4401	**8.55E-10**	1.26E-11	8.61E+05	9.94E+03	0.0924	0.9704
RD1 NS1	Pan 2	25	0.1038	**1.30E-08**	9.49E-10	3.75E+05	2.67E+04	0.0475	0.9252
RD2 NS1	Pan 2	25	0.343	**1.57E-10**	3.11E-12	6.16E+05	2.45E+03	0.0067	0.9976
RD3 NS1	Pan 2	25	0.2094	**3.14E-10**	1.26E-11	3.32E+05	4.07E+03	0.0112	0.9931
RD4 NS1	Pan 2	25	0.2328	**3.39E-10**	1.09E-11	3.44E+05	3.63E+03	0.0111	0.9944

Preliminary Lateral Flow data

Antibodies were further characterized for their applicability/suitability in the lateral flow format. Ten antibodies that were shortlisted based on the Octet results were further tested on lateral flow in 10 x 10 design of capture and conjugate. One identified pair was used to optimized the dipstick assay. Initial studies on optimization of Lateral flow assay for Pan DENV NS1 detection involved the utilization of pan anti-NS1 antibodies as capture and tracer pair. The pan capture antibody is derived from synthetic human phage display antibody library and pan tracer is a mouse monoclonal. This particular pan anti-NS1 pair was able to detect 100ng/ml of Recombinant hexameric NS1 of all 4 serotypes spiked in Human heparinized plasma Figure 15.



Strip1	D1 NS1;100ng/ml in human Plasma
Strip2	D2 NS1;100ng/ml in human Plasma
Strip3	D3 NS1;100ng/ml in human Plasma
Strip4	D4 NS1;100ng/ml in human Plasma
Strip5	Human Plasma

Figure 15. Dipstick format for Pan anti-NS1 detection assay.

Further assay development work is supported by a research grant (Translational Research Program) supported by the Department of Biotechnology, Govt of India.

Key output:

- Generated large repertoire of antibodies against dengue NS1
- Identified antibodies that bind to all the four DENV serotypes equally well
- Identified the mAb pair based on studies done using Octet system and direct screening in the lateral flow assay

Milestone: Generation/ identification of suitable pair of antibodies for the detection of dengue NS1 of all 4 serotypes.: Achieved

5. Scrub Typhus Reagent generation for IgM assay

The 56-kDa major outer membrane protein of *Orientia tsutsugamushi* (causative agent of scrub typhus) is well known as the most immunodominant antigen of *O. tsutsugamushi* and used in currently available commercial tests for IgM detection. However, the 56-kDa antigen is also highly variable, accounting for strain differentiation in *O. tsutsugamushi*. Currently available tests use 56-Kda antigen from either one strain or maximum 3 strains which do not cover the diversity and result in variability in the performance of the test in different regions. Our approach, to overcome this issue by using 56-Kda antigens from several carefully chosen strains of *O. tsutsugamushi* (sequences covering major diversity) to cover global diversity. In this activity, we have generated recombinant clones for the production of 56-Kda antigen of 5 different strains.

Construction of clones: We have identified five strains of O. *tsutsugamushi* where the sequence of 56-Kda Antigen is different from each other and if mixed together can cover the epitopes from large number of different isolates reported in the literature. The identified strains from where we have taken the amino acid sequence of 56-Kda antigen are: Karp, Kato, Gilliam, Boryong, and T763. Synthetic gene for the expression of these antigens were obtained and cloned in the *E.coli* expression vector. The generated constructs were transformed in *E.coli* expression host and the protein expression was induced. All the constructs found to be correct and expressed the desired recombinant protein.

Expression and purification: The expression conditions were optimized to get the recombinant protein in soluble form. However, we could not get the protein in soluble form. Next, the conditions were optimized to solubilize the insoluble fraction with Urea or with a cocktail of detergents. The protein solubilized using both the methods were purified by affinity chromatography on small scale. The optimal solubilization buffer pH found to be different by up to 2 pH units for the antigen derived from different O. *tsutsugamushi* strains.

Large scale (5 Liter) expression was performed and the protein were purified, dialyzed and stored at -80$_{o}$C in aliquots. The yield of purified protein varied from 7 mg/L culture to 30 mg/ml culture for antigen derived from

different strains. In the initial IgM immunoassay the protein purified using detergent solubilization method gave higher signal.

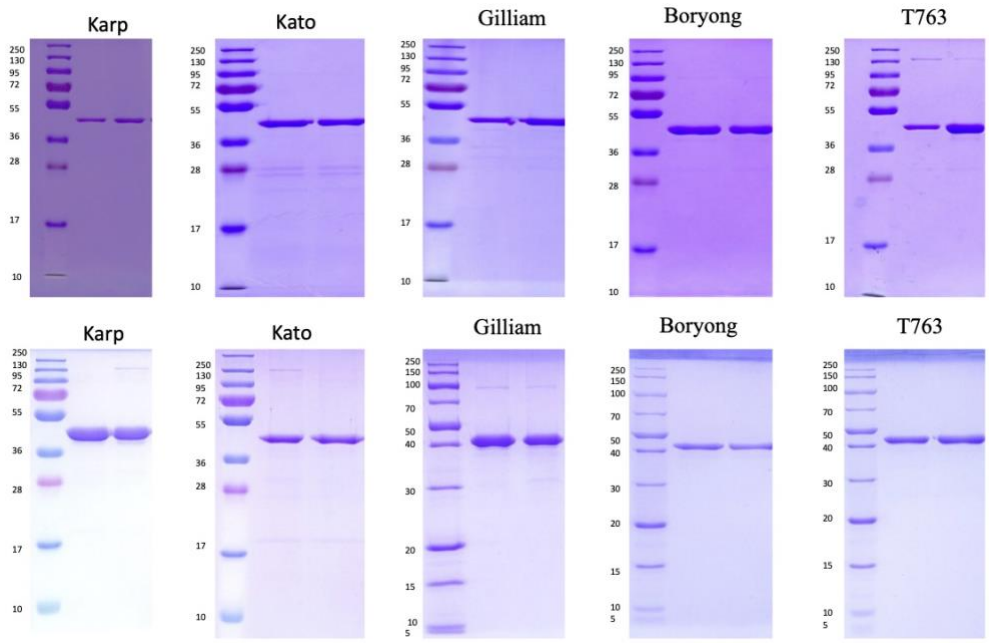


Figure 16: Figure shows the purified recombinant 56-Kda Antigen derived O. *tsutsugamushi* strain Karp, Keto, Gilliam, Boryong and T763. The top images show the purified protein from Urea extracted membrane fraction and the bottom gel images show the purified protein from detergent extracted membrane fractions.

Further assay development work is supported by THSTI own resources. We are also applying for a grant with CMC, Vellore to develop the test further.

Output:

- Generated recombinant clones for the production of 56-Kda antigen derived from 5 diverse strains of *O. tsutsugamushi*
- Purified 56-Kda antigen derived from 5 strains
- Generated 2 strategies for the purification of the antigen in soluble form (one strategy more suitable for application in lateral flow assay)

Milestones: : Achieved

Generation of recombinant clones for the production of 56-Kda antigens from several *O. tsutsugamushi* strains to cover diversity and process generation for the high yield production of these antigens.

6. Leptospira reagent generation for IgM assay

For the detection of Leptospira specific-IgM, highly specific and immunodominant antigens are required. Based on the current understanding, the most promising antigens for this purpose are recombinant LipL32 and LigA antigens. Recombinant clones for these antigens were generated and method(s) for high level expression and purification were developed.

As explained in the previous section, similar process was used for the expression and purification of the LipL32 antigen and fragment of LigA antigen. The sequence for these antigens were from Leptospira interrogans serovar Copenhageni str. Fiocruz.

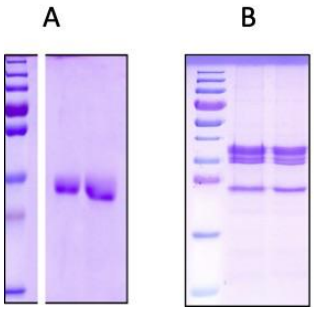


Figure 17: A) Purified LipL32 B) Purified fragments of LigA

Output:

We have generated high yielding clones for recombinant LipL32 and LigA fragment and process for the production of these antigens.

Milestones: Achieved

Generation of recombinant clones for the production LipL32 and LigA fragment and process for the high yield production of these antigens.

7. Production of additional antigens for *S. typhi* IgM detection

For the detection of IgM antibodies against *S. typhi* and *S. paratyphi A*, we have generated several putative target antigens. Recombinant *S. typhi* TolC, HlyE and OmpF antigens were expressed in *E. coli.* Expression and purification process were optimized. TolC and OmpF were extracted from membrane fraction whereas the HlyE protein was purified from soluble fraction. On large scale (5 L), we have achieved yield of 30 mg/L for HlyE, 10 mg/L for TolC and 17 mg/L for OmpF. The purified proteins were stored at -80$_{o}$C.

To remove the cross-reactivity of *S. typhi* LPS with other *Enterobacteriaceae* members, we have optimized the conditions for the removal of lipid A part of LPS and the biotinylation of polysaccharide (PS) part. The biotinylated PS was checked for it's binding on streptavidin wells and recognition by the anti-O9

MAb. The biotinylated PS found to be recognized by anti-O9 MAb.

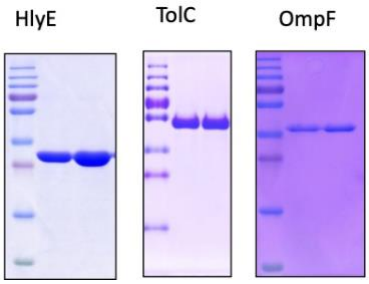


Figure 18: Purified S. typhi recombinant antigens.

We will use these antigens for the generation of UCP based lateral flow assay for *S. typhi* IgM detection using internal resources. There is also some discussion with FIND on taking the leads further in a collaborative project.

Output:

- Optimized the process for the production of recombinant TolC, HlyE and OmpF protein antigens
- Optimized the process for the production of S. typhi PS antigen that can be used in LFA

Milestone: Achieved

Improvement in putative antigen properties which can be used for the IgM assay development.

8. Generation of sera panel for AFI (this activity will be continued during the whole project duration)

To achieve the final goal of the project, one of the main tasks is to generate high quality sample/sera panel for Dengue, Chikungunya Typhoid, Malaria, Leptospirosis and Scrub Typhus. Development and evaluation of test with clinical samples from different locations are prerequisite as regional background antibody titers must be determined to tune the cut-off value.

In our project we have taken 2 clinical sites that cater large and diverse population from north and south India. The sites were:

- Christian Medical College, Vellore, India: Medicine and Infectious disease OPD and IPD, Pediatrics OPD and IPD, Microbiology departments. Task includes collection of and evaluation of samples with reference test and correlation with symptoms. The staff also collected paired samples whenever possible by making home visits.
- All India Institute of Medical Sciences, New Delhi, India: OPD and IPD patients. Task includes collection of samples from pediatric population and evaluation of samples with reference test and correlation with symptoms.

All the samples as per the inclusion criteria were sent to THSTI for repeat testing (independent testing),

aliquoting and storage at -80$_{o}$C.

Sample volume from AIIMS was ~2 serum whereas from CMC, it varied from 0.4 ml to 1.2 ml/ sample and the matrix was serum/plasma or frozen whole blood (whole blood for malaria).

Below is the summery of samples collected and characterized

Sample Type	**No. of samples**
Dengue positive samples	235
Scrub typhus positive samples	214
Chikungunya positive	12
Malaria Pv	45
Malaria Pf	30
S. typhi positive samples	60
Lepto	2
Presumed negative	329
To be characterised	289

Total 1216

There are around 286 samples that are characterized at the clinical site but not yet fully tested at THSTI as these samples from this year's monsoon season. The testing is ongoing and aliquoting, labeling and storage work is in progress. We have procured the commercial test kits for this work. Staff working for the characterization, aliquoting, labeling, and storage of these remaining samples is being supported by THSTI's TRP grant. Unfortunately, we could only get 2 samples +ve for Leptospirosis and 12 Chikungunya IgM positive samples that are not confirmed by the NAT test. For other pathogens the number found to be significant. This is likely to be because of the less no. of overall cases of Leptospira and chikungunya during the study period.

THSTI is making larger sera panel that is being supported by the TRP grant of THSTI.

Outcome:

We have generated AFI sample panel from fever patients from north and south India including adult and pediatric population.

Milestones: Achieved

Generation of well-characterized sera panel for major AFI causing pathogens (Dengue, Typhoid, Malaria, and Scrub Typhus).

b. Any results that were not achieved according to the proposed milestones, the reasons they were not completed and the plans for carrying them out.

- We could not reach to the final stage pLDH UCP-LF assay development like what has been done for PfHRP2. In the initial stage of the project, high sensitivity assay could not be developed using commercially available antibodies that were earlier identified. This situation forced us to develop our own monoclonal antibodies for assay development. Several Pan-LDH antibodies were developed by us and appropriate pair was identified from the antibodies generated in-house. As the generation of new mAbs took lot of time, we could only develop the prototype pan-LDH UCP-dipstick with liquid tracer till the end of the project. A project proposal on further development of an ultrasensitive RDT targeting pLDH for the detection of multispecies malaria infection is in the final stage of evaluation by Indian funding agency, BIRAC. We are hopeful to get the funding for the further development of the assay targeting the pLDH antigen.
- We could not collect many samples for Chikungunya and leptospirosis during the project . This is mainly due to less cases during the study period. THSTI is making larger sera panel that is being supported by the TRP grant of THSTI. We expect to collect the Chikungunya and Lepto samples in coming seasons.

c. Describe any unexpected results.

- We were able to achieve analytical sensitivity of 0.2 parasite/µl for our HRP RDT that is equal or better than nucleic acid LAMP assay. We have not expected before the start of the project that we will be able to achieve such high sensitivity on lateral flow platform.

7. Outputs

Please list significant outputs. For example publications, reports, press releases, online resources. Provide copies or url links where appropriate.

- Developed an ultra-sensitive RDT for Pf malaria with analytical sensitivity of 0.2 parasite/µl whole blood that is 50 to 250 times more sensitive compared to conventional RDTs. A manuscript on this work is ready and will be submitted to a reputed journal in less than a month time.
- Generated very high affinity true Pan-LDH antibodies that are suitable for the development of ultrasensitive RDT. This work will be published with the antigen preparation work.
- Generated Pan-DENV-NS1 antibody with high affinity that will be used of the generation of true-Pan serotype NS1 assay. The structure of the manuscript is ready and we expect that in 4-5 months we will be able to submit the manuscript in a reputed journal.
- We have critical reagents ready for most of the listed pathogens, we expect that in coming 2-3 years,

we should have the modular multiplexed test ready with support from different funding sources.

8. Budget variance
Please explain any difference between the original proposal and the actual expenditure.

We have got the approval twice for the re-appropriation of funds (from one budget head to another). The first time change was at the time of No additional cost extension (NCE) of the project in Oct-Nov 2018 as we planned the funds utilization as per the pending tasks. Second time re-appropriation was done from HR head to consumables head to buy the kits for the characterization of samples received from 2019 monsoon season and also to characterise the mAbs. These adjustments are clearly marked in the expenditure report.

Apart from these changes we have savings in the travel and HR head that is also reflected in the spent report. The main reason for this saving is less than anticipated cost for the flight tickets and large number of stakeholder were met at the ACDx conference and 2 LFA meetings so the travel cost is saved. Also more participation from Indian experts in discussion resulted in the savings of the travel cost. The HR cost savings is primarily because the PI didn't use the money for his own salary from the project.

9. Other Sources of Project Support:
If the project received support from other donors please use the following chart to provide the name of the donor, the amount received, the percentage of the project funded by the donation, and whether the funds are committed or potential. If the support is in-kind, describe the type of support below the chart.

Report all amounts in U.S. dollars.

Donor	**Amount**	**Received or Potential**
DBT, India Translational Research Program (TRP) (This grant has multiple components including diagnostics, repository, bioassays etc. A part of this grant is for fever sera panel, dengue LFA work)	USD 3 million (A part of this grant will be used for fever diagnostics)	Received
BIRAC (for further development of pLDH assay)	USD 100,000	Potential (proposal in the final stage of evaluation)
DBT, India (for scrub typhus assay, panel generation, and surveillance. This will include other rickettsial fever like spotted fever)	USD 0.5 million	Potential (proposal in writing stage with CMC, Vellore)
Wellcome trust (for typhoid assay. This will be in collaboration with FIND)	USD 1 million	Potential (discussion with FIND are ongoing)
ICMR, India (for leptospirosis assay)	USD 200,000	Potential (THSTI with partners from JIPMER, CMC)

10. Future developments

Please describe any significant developments, changes or adaptations to the original project you would like to make accepting these are in line with the original proposal.

- Bringing the ultrasensitive singleplex RDTs to the market without waiting for the final multiplexed product
- Multiplexed RDT for antigen detection for DENV, malaria and ChickV on single strip
- Multiplexed RDT for IgM detection for DENV, CHICKV and scrub typhus.
- Final multiplexed test for listed pathogens for both antigen and antibodies combined.

Development of a Single Dose Malaria Cure of Artemether-Lumefantrine through a Nano-based Drug Delivery System

FINAL REPORT

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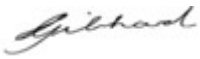
	Dr Liezl Gibhard	
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Table of contents

Executive summary

Artemether (ART) co-formulated with lumefantrine (LUM) is one of the most widely used artemisinin-based combination therapy (ACT) in the treatment of uncomplicated malaria. This ACT therapy continues to be efficacious; however, limitations due to poor oral bioavailability and the need for multiple dosing have led to poor patient compliance. The limited absorption and poor bioavailability necessitates the administration of larger doses of the drugs and the need for fat intake during drug administration, as well as a complex-dosing regimen; all to ensure efficacious plasma concentrations.

The main strategic objective of this study was to demonstrate the nanoencapsulation platform for reformulating antimalarial drugs and to show its relevance for diseases of poverty. In particular, the project focussed on developing new oral formulations for the antimalarials lumefantrine, artemether, and UCT944 (a novel antimalarial drug candidate under development at the University of Cape Town),. Furthermore, the focus was to demonstrate proof of concept for improving drug properties such as dissolution rate, absorption, bioavailability and uptake. A second objective was to design and develop an intravenous (IV) formulation of lumefantrine as a partner drug therapy for artesunate, for treatment of complicated malaria. The overall aim was to reduce the pill burden and food effect of the antimalarials to improve the therapeutic outcome. The project involved a partnership between the CSIR and the University of Cape Town (UCT)'s Drug Discovery and Development Centre (H3D).

Novel formulations were developed for all three antimalarials using one of the following technologies i.e. polymer-drug conjugates, emulsion/spray-drying, and/or supercritical fluid encapsulation. For many of the reformulations, the nanosized drugs displayed suitable particle size distributions between 300-600 nm while for others micron sized particles, particle size >5µm was observed indicating formation of aggregates. Encapsulation efficiencies >70%, and drug loading >20% was observed. Two LUM formulation displayed very high zetapotential's (-28mV and -55mV) indicating high stability. IC_{50} studies showed at least seven samples were highly active with antimalarial activity ranging from 15 ng/ml to 2220 ng/ml demonstrating that the new formulations did not hamper activity of the drug.

Pharmacokinetic (PK) studies for LUM in mice showed three lumefantrine formulations (i.e. PN35, LK45 and AS2.48), displaying significant improvement in drug absorption as compared to the reference lumefantrine drug. The maximum exposure over 24 hours seen in the PK studies for after a single dose was recorded as **seven fold greater** than that of the reference (PN35), displaying significant improvement in drug absorption compared to the reference. These results are very promising and are thought to be due to improved solubilisation of lumefantrine; importantly, it was not necessary to increase the fatty food intake of the mice to increase the plasma concentration of the drug. The PN35 formulation was tested in a mouse infection model and showed an increase in efficacy (at least two times) when compared to parent LUM.

For the UCT944 antimalarial lead, we have developed two novel formulations demonstrated improved solubilisation of the active. During PK studies in healthy mice, a significant increase in exposure was observed for the two formulations (PN32 & LK37), with a maximum exposure of **six** times observed (LK 37) compared to the parent. During studies in a mouse infection model with LK37 dosed orally, it was noted that the drug was

indeed released from the formulation and the efficacy compared very well to the parent drug (UCT944). Increase in efficacy was observed with the 5 $mg.kg^{-1}$ dose.

A novel IV formulation for LUM has also been developed that showed promising results *in vivo* in healthy mice during the PK studies. This IV formulation was also tested in the mouse infection model at UCT and unfortunately the results were inconclusive.

The new formulation of ART had challenges due to the sensitivity of the drug to external factors such as pH, redox potential and heat, to name a few. Although ART was detected by means of liquid chromatography, in high concentrations in the reformulations, the *in vivo* PK results did not show any drug exposure in the blood with two formulations that have been assessed thus far. However, the *in vitro* IC_{50} analysis showed that the ART formulations were very active indicating drug stability. Further investigation is required to understand the low drug exposures for the ART formulations in mice.

Achievements at a Glance

Target	**Achievements**
Reduce multiple daily oral dose for Coartem	LUM was successfully reformulated and a single dose retains significant (PK) after 24 hrs; up to 7x improvement in uptake compared to control drug. The efficacy dose was more than halved in infected mice compared to control drug, showing the potential for a reduced dose. Further optimisation is required for the combination formulation with ART.
Reduce fatty meal requirement for significant plasma concentration	No particular fatty meal intake required for significantly higher plasma concentration for LUM
Increase lumefantrine solubility in water	Solubility increased over a thousand times
Develop an IV formulation for LUM	Promising pharmacokinetic data was achieved with this formulation. Pharmacodynamic results were unfortunately inconclusive and should ideally be repeated when funds are available.
Develop an optimised formulation for UCT944	UCT944 was reformulated and a single dose retains significant PK after 24 hrs; up to 6x improvement in uptake compared to control drug. Drug was also released from the formulation during pharmacodynamics studies
Produce high-quality outputs	A provisional patent was filed at the UK patent office for "Chemical Synthesis of Water Soluble Lumefantrine-Polymer Conjugate" , and a second invention disclosure was filed at the CSIR and provisional filling in process for aqueous solubilisation and in vivo absorption of hydrophobic active compounds. 1 peer reviewed publication and 1 conference proceeding was produced.

1 Introduction

Malaria remains a major health problem in many countries and the disease continues to have a devastating impact on people's health, lives, and livelihoods. In 2016, there were 212 million reported cases of malaria and 429 000 deaths worldwide[1]. The WHO African region carries the largest burden of the disease in the world accounting for 92% of all reported deaths, with the most vulnerable being children under the age of five (accounting for 70% of all deaths). Currently 91 countries still have on-going malaria transmission. The WHO Global Technical Strategy for Malaria sets out a target of reducing both malaria mortality rates and malaria case incidences globally by ≥90% by 2030 as compared to 2015[1]. A further target is to eliminate malaria by 2030 in at least 35 countries that had on-going transmission in 2015. While substantial progress has already has been made towards this target, a lot more is required from all sectors of society including government, policy makers, pharmaceutical companies, research & development institutions and citizens.

It is well-known that almost all malaria deaths are due to the *Plasmodium falciparum* parasite. For over a decade now, the WHO has strongly recommended that artemisinin-based combination therapy (ACT) be used to replace monotherapies for treatment in *P. falciparum* endemic countries[2]. The artemether (ART)–lumefantrine (LUM) fixed-dose combination appears to be the most preferred oral therapy for uncomplicated *P. falciparum* malaria. This combination is marketed as Coartem®, offering a 28-day curing rate of more than 95%[1]. In this combination, ART displays a rapid onset of action but with a short half-life whereas LUM is a slow-acting with a relatively longer elimination half-life. The LUM mechanism of action consists mainly in preventing the detoxification of haem, which acts together with free radicals as parasites destroyers; whereas ART features multiple mechanisms of action including the interference with the parasite transport proteins, disruption of parasite mitochondrial function, modulation of host immune function and inhibition of angiogenesis[3].

The ART-LUM combination treatment has had a significant positive impact on malaria mortality and morbidity. However, the gains are at risk of being reversed as artemisinin resistance is now prevalent in many far eastern Asian countries and treatment failures are beginning to emerge. Re-admission of patients for severe malaria after ACT treatment has also been reported in the Democratic Republic of Congo[4]. The exact reasons for these treatment failures have not been confirmed, however poor adherences to the ACT treatment regimen have been reported in many of these regions.

While ART-LUM is a highly effective and lifesaving treatment for non-complicated malaria, it consists of a lengthy treatment regimen requiring multiple daily doses. In the current WHO-approved therapy, LUM and ART are combined in a mass ratio of 1:6, packed in 24 tablets to be taken as 6 doses for a 3-day treatment course, thus a 4 tablet-dose orally administered twice a day. Complicated malaria, or

instances where the patient is incapable of orally ingesting the ACT drugs, is first treated with artesunate, an injectable analogue of artemisinin. Artesunate, the hemisuccinate derivative prodrug form of dihydroartemisinin (DHA), is injected intravenously. This treatment is accompanied by a full oral ACT treatment regimen.

Completion of the oral ACT therapy is usually the responsibility of the patient or family members to be carried out at home. Malaria-patients are burdened by the number of doses and in most cases interrupt the treatment as they start feeling better. Research has shown that there is an inverse relationship between patient adherence to treatment and the length of treatment and frequency of dosing[5]. Lessons from other diseases like tuberculosis show that patients tend to show much lower compliance after recovering from the initial severe clinical state, which is problematic both for achieving complete eradication of the pathogen and for staving off the development of resistance. Furthermore, due to the lipophilicity of the drugs, the ART/LUM combination has a significant food effect increasing drug bioavailability as large as 2-16 fold as recorded in clinical studies[6]. Alas, oftentimes malaria patients have loss of appetite and difficultly holding down food. Hence, the value of improving bioavailability without a meal is well-recognized.

1.1 Project aim and objectives

The CSIR has developed a nanoencapsulation platform based on encapsulation of drugs in nanoparticles using an emulsion based system, whereby during oral delivery, the drug is protected against premature degradation in the gastric juices by the nanoparticle carrier, while allowing absorption via the gastrointestinal mucosa for absorption into the bloodstream. In addition, the CSIR is establishing a polymer-therapeutics research area that involves conjugating drugs onto polymer backbones to solubilise the drug such that during delivery, the labile linker is broken and the drug is released to the target site. The CSIR used its nanoencapsulation and polymer therapeutic platforms to reformulate the antimalarial drugs. The overall aim of this project was to demonstrate proof-of-concept of the nano-drug delivery technology with respect to improving important drug properties (such as dissolution rate, absorption, and bioavailability) of selected antimalarials drugs with the main focus being on ART and LUM. The specific objectives of this project were as follows:

1. Development of novel formulations for **Coartem (ART-LUM)**: Oral dose form with proof-of-concept demonstrating improved drug solubility/solubilisation and oral bioavailability toward eliminating the food effect and reducing dose/dose frequency.

2. Development of an **injectable LUM** formulation: IV dosage form of LUM with proof-of-concept demonstrating improved drug solubility for dosing and efficacy in a mouse infection model.

3. Development of an optimized formulation for UCT's **antimalarial drug candidate UCT944**: Oral dosage form of UCT944 with proof-of-concept demonstrating improved drug dissolution rate, oral bioavailability and efficacy in a mouse infection model.

2 Overview of Project Progress

The project was planned according to four work packages, i.e.

1) Project/Stakeholder management,

2) Formulations,

3) Preclinical,

4) Route to market (not pursued)

This report describes the progress made for the work packages 1-3. Due to the project funding not continuing within the CEWG framework, it was decided that the route to market work package will not pursued within Phase 1 of the project. Instead funds for this work package was redirected based on approval by WHO to the formulation and preclinical work package in order to improve the evidence base for the proof of concept studies.

A high-level overview of progress made in the project is tabulated in **Table 1**. More detailed information regarding work packages 1-3 is described in Sections 3-5 respectively.

Table 1: High-level overview of progress to date.

Work package	Activities	Planned Deliverables	%Complete	Progress to date
1) PROJECT /STAKEHOLDER MANAGEMENT WORKPACKAGE	**Project & Stakeholder management**	• Detailed work plan completed • Agreements in place with all collaborators • Project on track and within budget	100	• LOA signed between WHO & CSIR • Research collaboration agreement signed between CSIR and UCT • Weekly project meetings held at CSIR to monitor progress and track deliverables. • Progress meetings held with CSIR/UCT (April 2017,August 2017, March 2018, June 2018, August 2018, October 2018 & December 2018) • Two Steerco meetings were held (representatives from CSIR, UCT, MRC), final steerco meeting planned for May 2019.
2) FORMULATION WORKPACKAGE	**API**	• Quality control for API & raw materials *(Thermal, physico-chemical properties)*	100	• Task completed – All three API's were purchased and QC done
	Formulations	• Formulations of lumefantrine, artemether and UCT944 prepared and characterised: Desirable size: < 500 nm, drug loading > 20% (for oral formulation), high solubility: 3-fold improvement over the experiment	100	• Novel formulations developed for all three antimalarials. Formulations were characterised for size, charge, drug loading. • Drug release studies were done.

		time course, Stability: > 6 months		
	Samples for animal studies	• Most promising formulations produced up to 2 g quantities and properties maintained	100	• LUM, ART and UCT944 formulations were repeatable and prepared in large quantities and sent for animal studies as well as IC_{50} determinations
3) PRECLINICAL WORKPACKAGE	**IC_{50} in sexual and asexual (Biosciences)**	1. Technical Report	100	• IC_{50} determinations showed at least 7 samples were highly active with antimalarial activity ranging from 15 ng/ml to 2220 ng/ml.
	PK Studies 1. Mouse: oral artemether, lumefantrine and Coartem (Controls) 2. Mouse: oral Coartem fomlation 3. Mouse: IV lumefantrine 4. Mouse: oral UCT944	2. IV & oral exposure parameters 3. Demonstrated improvement of exposure over standard formulations & demonstration of food independent exposure 4. Sustained exposure & reproducibility 5. Oral exposure, reproducibility, & demonstrated improvement for exposure over previous formulations	100	• Mouse PK completed for oral and IV LUM, oral ART and oral UCT944. • Significant improvement in exposure seen for three LUM formulations, and UCT 944 • Difficulty detecting ART
	Efficacy in Mouse NSG model 1. Oral lumefantrine 2. IV lumefantrine 3. Oral UCT944	1. Demonstration of efficacy for IV LUM 2. Demonstration of *in vivo* efficacy commensurate with exposure 3. Data to support decreasing dosing interval and/or decreasing dose 4. Demonstration of improved efficacy relative to previous formulations	100	• PD studies were completed for oral LUM, IV LUM and oral UCT944 formulations with demonstrated efficacy

3 WORK PACKAGE 1: PROJECT MANAGEMENT & STAKEHOLDER ENGAGEMENT

- **Project management:** The project was managed according to the CSIR project management best practices, and involved use of the following project management tools: PeopleSoft, eProcurement and Workflow. For project document management, the Micro Focus (previously Novell) Vibe system was used. A virtual open-access workspace was created for the project with organisational folders for sharing of all project related documentation (reports, publications, minutes of meetings, results, proposals, etc.). Team members at the CSIR and UCT were granted access at different levels of access control, to ensure optimal management of project files. Using the VIBE system, the project team was able to manage, share, locate, and access all project related documents, calendars, discussion forums, wikis, and blogs. Weekly project meetings were held at the CSIR for the formulation work package. For the pre-clinical work package joint progress meetings between the CSIR and UCT teams were conducted using GoToMeeting and formal project minutes were also recorded.

  Financial management and accounting for the project was conducted according to the Public Finance Management Act (PFMA), 1999 (Act No. 1 of 1999) (as amended by Act No. 29 of 1999) of SA. A financial report for the project period was generated and it is attached in the **Appendix A1**.

- **Contracting:** A Letter of Agreement was signed between WHO and CSIR (17 July 2017), for release of US$ 993 378 for phase 1 of the project (01 April 2017 - September 2018). The CSIR requested a four month no-cost extension from 1 October 2018 – 31 January 2019 due to delays with the preclinical work package, which was approved in November 2018. For the partnership between CSIR and UCT, a research collaboration agreement was signed (GrpWise Pta Projects GWDS #991147).
- **Steering committee:** To ensure proper governance, a steering committee (Steerco) was set-up and comprised representatives from CSIR (Martin Sanne and Dr Biotumelo Semete, Dr Avashnee Chetty), MRC (Dr Richard Gordon), and UCT (Dr Greg Basarab). The mid-term project progress was presented to the Steerco in July 2018, and invited committee member included malaria clinician Prof Bernard (KEMRI). A final steerco meeting is planned for 17 May 2019.
- **Reporting:** Mid-term project progress reports have been compiled and submitted to WHO in July and November 2018. Additionally a mid-term financial report was submitted in October 2019.

- **Stakeholder engagement**: A number of stakeholders have been engaged during the course of this project and include MRC, MMV, Novartis, Right-to-Care, and other local pharmaceutical SMME's. A number of collaborations have also been established and these have been captured in **Table 2** below.

Table 2: Collaborations as a result of the project.

Collaborators	**Nature of collaboration**	**Output**
Local collaborations		
Prof Greg Basarab UCT, Drug Discovery and Development Centre H3D, Department of Chemistry, University of Cape Town, South Africa	- Collaboration on PK and PD investigation of the reformulated antimalarials, and provision of UCT 944 antimalarial candidate	- Publication to follow. (Delays are due to the patent filing.)
Prof Bert Klumperman, Stellenbosch University, South Africa	- Collaborating on development of polymer therapeutics for Malaria	- Currently collaborating on LUM conjugates for a PhD study at Stellenbosch University
Dr Lynne Pilcher, Department of Chemistry, University of Pretoria, South Africa	- Collaborating on synthesis of polymer conjugates for malaria treatment	- Currently co-supervising 2 PhD students, published 1 joint publication
Prof Duncan Cromarty, Department of Pharmacology, Currently collaborating on LUM conjugates for a PhD study at Stellenbosch University	- Collaborating on pharmacology of polymer therapeutics	- Currently co-supervising one Master student

International Collaborations		
Dr Maria Vicent Department and institution: Polymer Therapeutics laboratory, Centro de Investigacion Principe Felipe (CIPF), Valencia, Spain	Technical expertise support on design of polymer therapeutics	Co-authored joint conference presentation on Polymer therapeutics for malaria presented at the 12th International Symposium on Polymer Therapeutics in Valencia, Spain, in 2018
Dr Joaquin Sanchis Department and institution: School of Pharmacy, Monash University, Australia	Technical expertise on polymer therapeutics and generous provision of PNAM polymer	
Dr Bernhards Ogutu and Dr Lucy Ochola Kenyan Medical Research Institute (KEMRI), Kenya	- Consulting on drug dosage and clinical trials - Collaborating currently on further animal studies for the antimalarials to be conducted at KEMRI	- Finalising proposal for further animal studies on primate model for the reformulated antimalarials

4. WORK PACKAGE 2: FORMULATIONS

Significant progress was achieved with respect to reformulating LUM, ART, and UCT944 and the main achievements are described in more details below.

4.1 API Source and Validation

Artemether and lumefantrine were purchased from the reputable local South African supplier DB Fine Chemicals who sourced it from LeapChem and Hangzhou Neway Chemicals internationally. Both APIs were supplied with full certificates of analyses. Before use, we also conducted confirmatory analyses using thin layer chromatography (TLC) and nuclear magnetic resonance (NMR), which confirmed that the actives (ART and LUM) were pure and identical to Coartem. UCT944 was manufactured by Syngene and characterised by NMR and LC-MS. All other reagents were purchased from reputable suppliers and details are available in the full technical report[8].

4.2 Encapsulation and nanonization of antimalarials

4.2.1 The design process for the encapsulated and nanonized formulations

This strategy aimed to improve the aqueous dissolution rates of these hydrophobic antimalarial APIs with the objective of enhancing their bioavailability when administered orally. Moreover, formulations were designed to intentionally minimise the intake of fatty foods required for the conventional therapy. Novel formulations of three APIs were investigated: UCT944, LUM and ART.

For the evaluations of designed formulations, it was of paramount importance to investigate the PK parameters of the APIs using identical protocols to serve as comparators to demonstrate improve drug exposures.

Three key actions were undertaken to significantly improve targeted PK parameters to achieve the ultimate goal of a more efficacious antimalarial treatment:

1. Reducing the lag-time of LUM while increasing its bioavailability by improving its GIT absorption via nanonization and/or incorporation into lipid matrices
2. Prolonging the residence time of ART and increasing its C_{max} and AUC through a core-shell encapsulation
3. Enhancing the bioavailability of UCT944 by increasing its aqueous solubilisation either via nanonization or via its incorporation into microemulsions.

All these strategies were extensively investigated and the formulations to take forward to the next level of *in vivo* trials in rodents were shortlisted based on matching the set of targets in terms of physico-chemical properties including:

- Particle size range of circa 500 nm or below to ensure enhanced GIT uptake,
- Drug loading > 20% (for oral formulation)
- Zeta potential in absolute value above 10 mV to ensure good stability of colloidal particles in suspension
- Physico-chemical and thermal stability of the drug to maintain the integrity of the active compound and particles
- Shelf-life stability to maintain the integrity of the active compound and particles
- Re-dispersibility in water to ensure acceptable IV administration and oral absorption
- All ingredients must be GRAS, approved by FDA and their ultimate residues content complying with the regulatory framework.

- Process scalability: the selected cost-effective process must contain fewer steps and be easy to scale up
- Lipid as encapsulating matrix to minimise the recommended fatty food intake and to induce the release of the drug
- Final powder product must be free flowing for acceptable packaging processes and contain a significant amorphous fraction for a speedy solubilisation

4.2.2 Results and Discussion

Several formulations were designed and developed in the framework of the above guidelines and the following section describes some of them.

1. Novel formulation of LUM via supercritical fluid (SCF) technique (Sample AS2.48)
   This novel formulation was produced via Particles from Gas-Saturated Solution process (PGSS) by means of a supercritical CO_2 pilot reactor at the CSIR. The drug was premixed with excipients and then introduced into the SCF reactor where the blend was processed. Free flowing powder containing the drug in an amorphous state was obtained by means of a SCF process, featuring relatively very small particle size (~50 nm) as depicted in **Figure 1** when imaged with a scanning electron microscope (SEM). However, particles size of circa 5000 nm was obtained when using dynamic laser scattering (DLS) method, owing probably to the agglomeration. Drug loadings as high as 30% w/w were obtained.

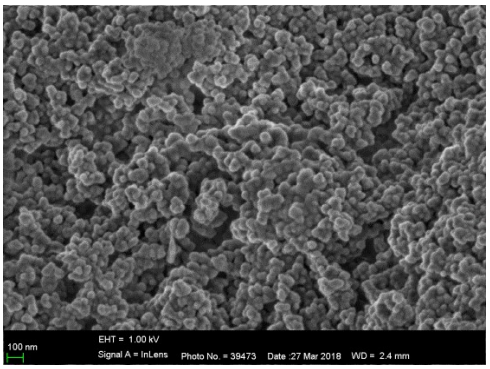

Figure 1: SEM image of LUM-loaded lipid nanoformulation (AS2.48) obtained via SCF technique (Scale bar: 100 nm).

2. Novel formulation of UCT944 (PN37) and LUM (PN35) via spray drying of a microemulsion
   A novel technique was developed resulting in free flowing and water-dispersible nanoparticles with narrow size distribution. Microemulsions of the drug in a lipid solution dispersed in an aqueous

cocktail of surfactants were produced with an average droplet size of 100 nm and thereafter spray dried to yield powder of nanoparticles suitable for IV or oral administration, with particle size averaging 500 nm[10].

3. Nanonization of UCT944 (Sample LK32)

Pristine UCT944 powder features sharp longitudinal or fractured crystals under the microscope (**Figure 2A**). These crystals were converted into amorphous nanoparticles by homogenisation then spray dried at a moderate temperature to produce free flowing powder. These nanoparticles were intentionally wrapped into muco-adhesive and enteric polymers during the reformulation process (**Figure 2B**). The wrapping was done to achieve a "Trojan horse" strategy whereby the drug nanoparticles are protected during the gastric transit and only released in the intestinal region following the dissolution of the enteric polymer. The muco-adhesive envelop is expected, owing to its sticky nature, to play the role of slowing down the movement of the dose, thus prolonging its intestinal transit time; a subsequent release of significant amount of drug particles is expected for absorption to the blood stream either through rapid dissolution and uptake via the portal vein or either as lipid nanoparticles through the lymphatic system[10].

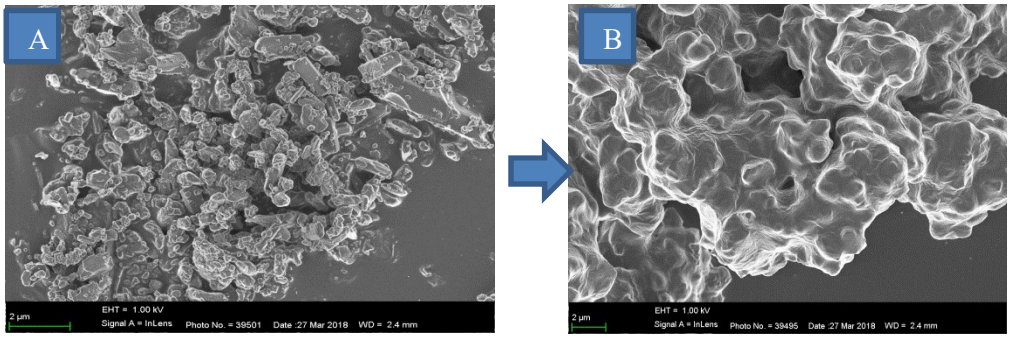

Figure 2: SEM images of: A. highly crystalline UCT944 and B. Formulated UCT944 in a Trojan horse design (LK32).

4. Supersaturable self-emulsifying drug delivery system (S-SEDDS) for LUM (Sample LK45)

This strategy stems from the natural occurrence of an emulsification process during absorption of fatty food through the GI. Excipients in the formulation was selected to spontaneously produce an emulsion when in contact with water. A polymeric precipitation inhibitor was additionally added to minimise the extent of precipitation or recrystallisation of the drug -loaded SEDDS resulting in a high concentration of free drug in the GI. As stated above, a Trojan horse strategy was utilised aiming at wrapping LUM nanoparticles into a muco-adhesive and enteric hydrophilic polymer to slow down transit through the GI track and enable a subsequent release of nanoparticles from microparticles triggered by the higher enteric pH. When re-dispersed in water, nanoparticles with

size averaging 500 nm were obtained. The free flowing powder was produced by spray drying an aqueous hydrotropic nanosuspension obtained from an oily solution of LUM when introduced dropwise into an aqueous saline solution.

5. Adsorption of micellar solution of ART onto colloidal silica (Sample LK43)

   ART-based solid formulation was developed by adsorbing a micellar solution of ART onto colloidal fume silica in a ratio of 1:1 resulting in a drug loading close to 45% w/w.

6. Liquid state reformulation of LUM/ART (Sample LK44)

   A liquid formulation containing both LUM and ART in a mass ratio of 6:1 was also developed. The ultimate goal is to develop a paediatric formulation of the two potent antimalarial drugs combined. As it stands currently, the formulation is quite stable for several months when stored in a refrigerator (4 °C).

Table 3: Summary of physioco-chemical characterisation of formulated drugs

Formulation Identifier	PS (nm) via DLS	PDI	ZP (mV)	F yield (% w/w)	EE (%)	DL (%)
AS2.48 (LUM)	> 5000	1	^	~90	~90	31.5
PN35 (LUM)	300.2 ± 22.5	0.12 ± 0.02	-28.2 ± 5.3	90.2 ± 16.9	70.5 ± 12.4	20.5 ± 2.1
LK32 (UCT-944)	598±32	0.30±0.03	-5.0±0.4	65±12	-	19.3
LK45 (LUM)	475±21	0.3±0.02	-55±2	67±5	-	33.7
LK43 (ART)	>5000	1	^	99±1	100	45±5
LK44 (LUM//AR)	*	*	*	100	100	**166.7//27.8

^: ZP not determined because of size out of range; *Viscous liquid solution of LUM and AR; **: The DL unit for LUM//AR in this liquid formulation is expressed as [mg/ml]; PS: Particle size using Zetasizer; PDI: Polydispersity Index; ZP: Zeta potential; F Yield: Formulation yield; EE: Encapsulation efficiency; DL: Drug loading; NB. EE of LK32 and LK45 to be determined;

4.3 Development of Water-Soluble Lumefantrine Conjugates

4.3.1 The design process for the drug conjugate

This strategy involved development of a polymer-lumefantrine conjugate which would enable solubilisation of the drug. LUM has a very low aqueous solubility of 3.1 x 10^{-5} mg/ml and a partition coefficient (logP) of between 8 and 9. Its ACT counterpart, ART, has a relative low aqueous solubility of 4.6 x 10^{-1} mg/ml and a logP of about 3. A derivative of ART, artesunate, is readily soluble in bicarbonate solution and is currently the only artemisinin derivative that can be administered intravenously. A rational design approach was adopted to design a delivery system for LUM and provide adequate aqueous solubilisation for IV administration[11,12].

1. Polymer selection: For the polymer selection, polyethyleneglycol (PEG) as the initial carrier backbone and a second polymer, p-NAM-stat-p-AA (PNAM), were selected. PEG was chosen since it has been extremely well-researched in the field of drug delivery systems and nanomedicine, it is non-biodegradable, safe, water soluble and approved by the Food and Drug Administration (FDA). Additionally it is amenable to drug conjugation due it its functional group-terminated forms. PNAM was also selected for its multivalence as high as ten, i.e. it can have as many as ten points for drug attachment. Indeed, we used PNAM with a valence of ten. PNAM is not commercially available, but the method of its synthesis is available in the scientific literature. We obtained our supply as a generous gift from our Monash University Australia collaborator Dr Joaquin Sanchis.
2. Linker: The only hydrolytically scissile linkage possible between LUM and PEG-OH or PEG-NH is through an ester bond. To obtain this a homobifunctional linker was needed. We selected the linker succinic acid, the functional adduct that was used to derivatize dihydroartemisinin to artesunate. Hence, it has precedence in malaria chemotherapy.
3. Conjugation: The conjugation of LUM to either PEG-OH or PEG-NH was achieved in two reaction steps. The first step involved the conjugation of LUM to succinic acid. This reaction was simple and high-yielding. The lumefantrine-succinic acid (LUM-Suc) product was purified and conjugated to either PEG-OH or PEG-NH using carbodiimide coupling chemistry. The PEG-LUM conjugate was purified from the crude reaction mixture after removal of the DMF reaction solvent by extracting it into a phosphate-buffered saline (PBS) of pH 7.4 at 25 °C. An intense yellow colour of the solution strongly indicated that the conjugation was successful. Conjugation of LUM to PNAM was achieved in only one reaction step. Using carbodiimide coupling chemistry PNAM was activated and reacted with LUM. The PNAM-LUM conjugate was obtained from the crude reaction mixture by a similar process to PEG-LUM.

4.3.2 Results and discussion

Conjugation of the LUM to the polymers was confirmed by NMR and Fourier-transform infrared spectroscopy (FTIR). We are currently making arrangements with our collaborators to further characterize the conjugates with gel permeation chromatography (GPC) and matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectrometry.

The main findings are as follows:

1. The amount of drug loaded in the conjugates was determined by ultraviolet (UV) spectroscopy and NMR. PEG-LUM conjugate had a drug loading of 19 $\mu g.mg^{-1}$ of conjugate (determined by UV) while the PNAM-LUM conjugate had a drug loading of 8 mol % (determined by NMR).
2. Drug release kinetics studies were conducted, however the results were inconclusive and is not reported here since the study is still ongoing for the two different drugs to track the release from the formulations.
3. Solubility studies were carried out using UV spectroscopy. The polymer-lumefantrine conjugates were dissolved in PBS pH 7.4 solution at 25 °C and centrifuged. The absorbances of supernatants were used to determine the amount of LUM in solution from a standard curve of LUM. The values obtained were between 200 times (PEG-LUM conjugate) and a thousand times (PNAM-LUM conjugate) greater solubility than free LUM. We will be carrying out experiments to determine the maximum solubilities of the conjugates (**Figure 3**).

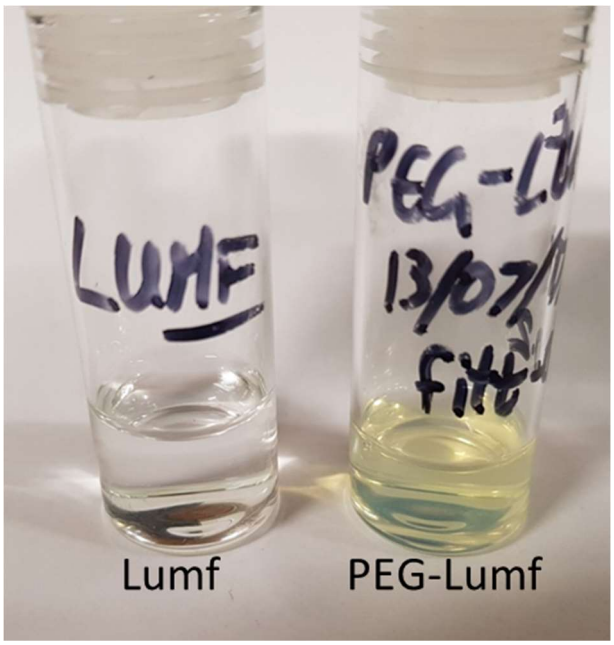

Figure 3: The tubes represent the supernatants obtained after centrifuging equal amounts of LUM as free drug (left tube) and PEG-conjugated LUM (right tube) in PBS buffer (pH 7.4). The concentration

of LUM dissolved in PBS as a conjugate was determined by UV spectroscopy to be 200 times greater than the free drug.

4. The sizes of the polymer-lumefantrine conjugates in water (known as the hydrodynamic size) were determined by dynamic light scattering (DLS). The average sizes obtained was 190 nm for the PEG-LUM conjugate and 90 nm for PNAM-LUM conjugate.

Table 4: Summary of physioco-chemical characterisation of polymer-lumefantrine conjugates

Conjugate	Particle Size (nm)	Zeta potential (mV)	PDI	Drug loading
PEG-LUM	190	NA	0.17±0.02	19 µg.mg^{-1}
PNAM-LUM	90	-28.5±1.59	0.25±0.01	8 mol %

5. WORK PACKAGE 3: PRECLINCIAL

The preclinical work package which will be reported here entails the following aspects:

- Antimalarial IC_{50} determination using both sexual (gametocytes) and asexual red blood cell parasite stages was performed at CSIR Biosciences. Only the asexual stage data is reported on since there was no activity (as expected) of the formulations against the sexual parasite stage. Current treatment as well as our formulations targets the asexual blood stage of the parasites.
- PK studies in mice were conducted at UCT-H3D Labs.
- PD studies were conducted at UCT-H3D Labs.

5.1 *In vitro* anti-malarial assay (IC_{50} determination)

The test samples *in vitro* antimalarial activity against the 3D7 strain of the malaria parasite, *Plasmodium falciparum,* was measured by parasite survival using the parasite lactate dehydrogenase (pLDH) assay. The percentage parasite survival was plotted against the logarithm of the concentration to obtain dose-response curves. IC_{50} data were calculated graphically by interpolation from these curves. From the

dose-response curves, test compounds that show *in vitro* antimalarial activity of ≤ 10 µg/ml are considered active. The following IC_{50} results as indicated in **Table 5** were used to gauge activity level[13].

Table 5: Explanation of IC_{50} results related to activity level

IC_{50} Values	**Activity level**
> 10 µg/ml	Inactive
7 µg/ml ≤ IC_{50} ≤ 10 µg/ml	Marginal activity
3 µg/ml ≤ IC_{50} ≤ 6.9 µg/ml	Moderate activity
IC_{50} < 3 µg/ml	Highly active

Table 6: IC_{50} and Z'-factors of active test samples against 3D7 strain of *P. falciparum.*

Test sample	**IC_{50} (ng/ml)**	**Activity**	**Z'-factor**
LUM-Suc (B4)	230	Very active	0.72
CS_{3kDa}-LUM (B8)	2220	Very active	0.84
PNAM-LUM (B9)	210	Very active	0.89
LUM SCF (AS2.48)	260	Very active	0.92
LUM-ART (LK44)	15.56	Very active	0.77
UCT944 (LK32)	14.54	Very active	0.76
UCT944 (PN37)	16.04	Very active	0.76
ART	1.07	Control	0.65
944	3.74	Control	0.73
CQ	8.29	Control	0.85

*LUM control is being repeated. From literature the value is ~7-8 ng/ml[14].

The quality of the screening assay is determined by the Z'-factor which is used to quantify how well the assay works. A Z'-factor between 0.5 and 1.0 is an excellent assay while the one between 0 and 0.5 is a marginal assay. Therefore, a prerequisite for all experiments is to have a Z'-factor > 0.5.

Twenty seven samples were screened for *in vitro* antimalarial activity at full dose concentrations against the chloroquine-sensitive strain, 3D7 of *Plasmodium falciparum*. Seven samples were highly active with antimalarial activity ranging from 15 ng/ml to 2220 ng/ml (**Table 6**).

5.2 *In vitro* release studies

The *in vitro* release studies of LUM from the nano-formulations PN 35, LK 45 and AS 2.48 were performed by incubating the drug-loaded particles (1 mg/ml) in PBS pH 7.4 at 37℃ in a shaking water bath at 300 rpm. Samples were collected at 0, 2, 4, 6, 8, 12 and 24 hours. The samples were then freeze-dried to collect the precipitated LUM released at each time point[10,15].

The drug released from the particles was separated from the particle debris via a silica-column clean-up to prepare the samples for quantification using HPLC. The release patterns obtained from the novel formulations PN35, LK45 and AS2.48 **(**

Figure 4) show that the delivery systems used in the formulations are dispersed or solubilised in aqueous medium to enable release of the LUM entrapped in the system upon treatment. The different delivery systems release drug at different rates based on the material used to entrap the drug, with the LK45 formulation showing superior release rates. A true representation of the *in vitro* release behaviour of the various formulations will be obtained by performing the release studies in blood plasma.

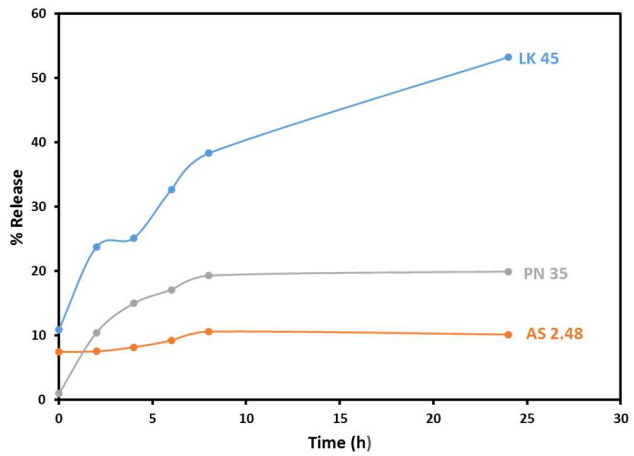

Figure 4: *In vitro* release profiles of PN 35, LK 45 and AS 2.48.

5.3 *In vivo* PK studies

An evaluation of the PK obtained using several formulations of LUM were conducted in Balb/c mice, using a reference preparation for comparisons. The experimental formulations were prepared as per indicated in **Table 7**, immediately prior to dosing, with amounts adjusted for the relative concentration of LUM or UCT 944 in each preparation to dose at an equivalent 20mg/kg for oral administration (PO) experiments in approximately 200µL total volume adjusted for each animal's individual weight[16].

For oral dosing, the reference compound was suspended in a 0.5% (w/v) aqueous solution of hydroxypropyl-methylcellulose (HPMC) which would deliver 20 mg/kg in approximately 200µL. The dosages were prepared 60 minutes prior to administration to male Balb/c mice (n=3 for each group).

Table 7: Formulations of LUM and UCT944 used in this study

Formulation	Route	Description	Drug loading	Preparation
LK45	PO	Oral formulation of LUM nanoparticles	33.7% w/w	Suspend in water
PN35	IV and PO	Oral formulation – LUM - SLN	19.0% w/w	Suspend in water (oral) or PBS buffer pH 7.4 (IV)
AS 2.48	PO	Oral formulation of LUM	31.5 % w/w	Suspend in water
LK44	PO	Oral formulation of LUM/Art in a liquid lipid dosage form	166.7 mg/ml (Lum)	Diluted to dose 20mg/kg in polyethylene glycol
B7 BM-T108	PO	Polymer therapeutic of LUM.01	5.73% w/w	Solution in PBS buffer pH 6.5
PN37	PO	Oral formulation of UCT944	20.08% w/w	Suspend in water or buffer
B8B-BM-T108	PO	Polymer therapeutic of LUM	12.82% w/w	Suspend in water
B9 BM-T108	IV	Polytherapeutic of LUM.02	3.12% w/w	Solution in PBS buffer pH 7.4
Lumefantrine	IV and PO	API	100% w/w	0.5% (w/v) aqueous solution of HPMC

The area under the curve (AUC) for parent LUM was 12711 h.µmol/L (**Table 8**). **The novel** formulations of PN35 **(Figure 5**), LK45 (**Figure 6**), and AS2.48 (**Figure 7**), all showed improved exposure relative to parent LUM suspended in a standard delivery solution. The overall exposure as reported from PK studies in mice after a single dose was approximately **seven, four, and three-fold greater** respectively than that of the reference (**Table 8**), over the observed 24 hr period. Formulations B7-BT108 and LK44 both showed lowered LUM exposure in comparison with the parent compound, possibly related to technical issues during preparation of the dose. These formulations are being optimised[16].

Table 8: Comparison of LUM exposure obtained from the formulated material, relative to the reference.

Parameter	Reference	AS2.48	B7-BMT108	PN35	LK45	LK44
AUC (h.µmol/L)	12711	35663	6509	93111	53852	3591
Fraction of reference	1	2.806	0.512	7.325	4.237	0.283

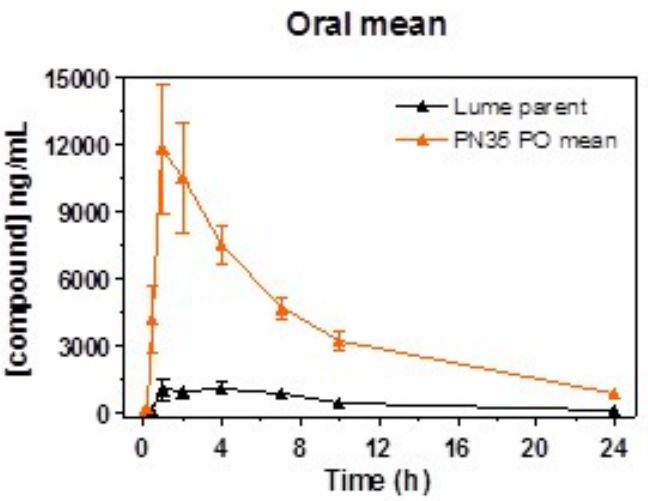

Figure 5: Direct comparison of delivery of LUM from preparation PN35 to the reference, dosed orally at an equivalent 20 mpk of LUM in male Balb/c mice.

These formulations yielded PK parameters (i.e. C_{max}) 10-fold greater compared to controls of un-formulated LUM with a distinctive prolonged tailing curve above MIC for several hours. It shows a fast absorption of LUM following oral administration depicted by a long half-life.

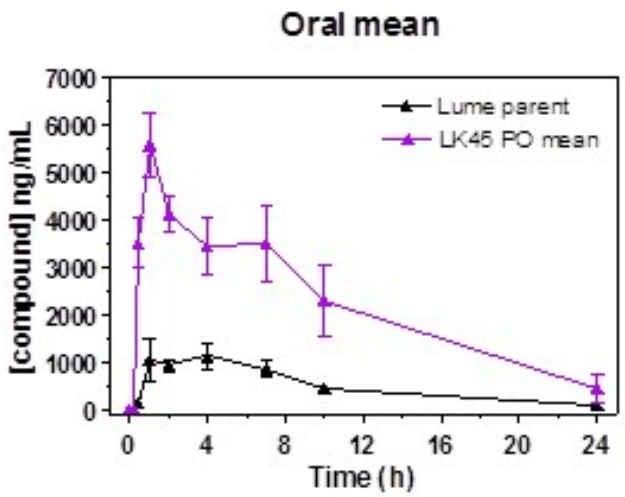

Figure 6: Direct comparison of delivery of LUM from preparation LK45 to the reference formulation, dosed orally at an equivalent 20mpk of LUM in male Balb/c mice.

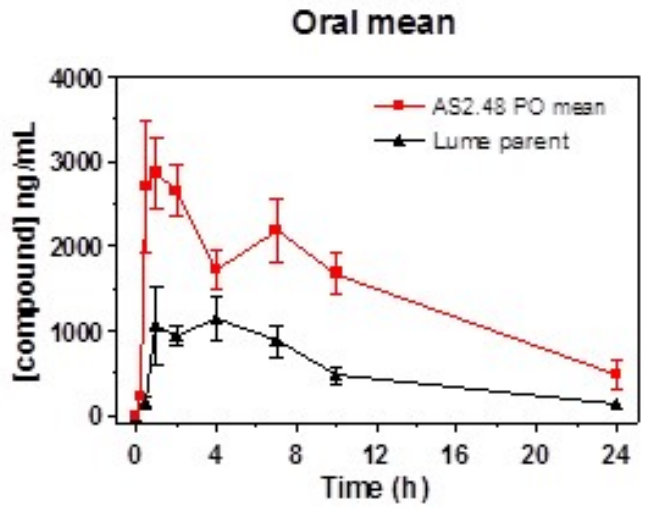

Figure 7: Direct comparison of delivery of LUM from preparation AS2.48 to the reference, dosed orally at 20mpk in male Balb/c mice.

Table 9: Comparison of IV LUM exposure obtained from the formulated material, relative to the reference.

Parameter	IV reference (parent)	B9B-BM108
AUC (h.µmol/L)	92	90
Fraction of reference	1	0.98

At present there is no IV LUM commercially available. The B9B-BM108 conjugate that performs just as well as the parent reference is indeed a very good result. The parent reference was solubilised in HPMC which is not a suitable matrix for IV administration, whereas the B9B-BM108 conjugate was solubilised in PBS[16].

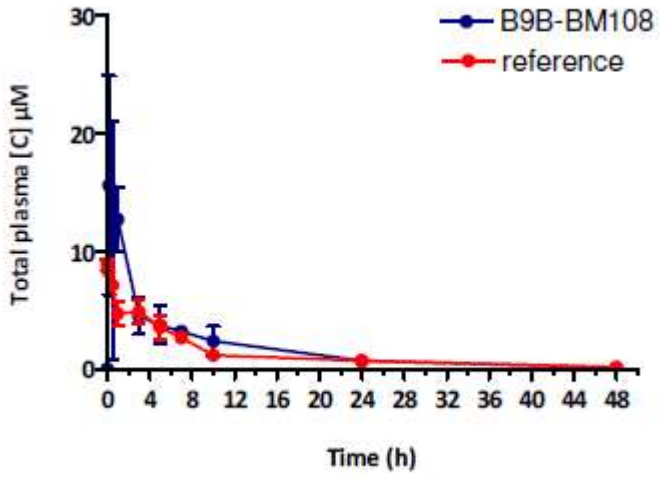

Figure 8: Circulating concentrations of LUM in the IV formulation.

The AUC for parent UCT944 was 12 h.µmol/L (normalised for dose) (**Table 11**). **The novel** formulations of PN37 and LK32 (**Figure 9**) both showed improved exposure relative to parent UCT944 suspended in a standard delivery solution. The overall exposure as reported from PK studies in mice after a single dose was approximately **six and four-fold greater** respectively than that of the reference **(Table 11),** over the observed 48 hr period. Although PN37 gave a much higher AUC compared to LK32, the exposure between animals was more consistent by using LK32[17].

Table 10: Formulations of UCT944 used in this study

Formulation	Route	Description	Drug loading	Preparation
PN37	PO	Oral formulation of UCT944	20.08% w/w	Suspend in water
LK32	PO	Oral formulation of UCT944	19.27% w/w	Suspend in water
UCT944	IV and PO	API	100% w/w	0.5% (w/v) aqueous solution of HPMC, 0.2% Tween80

Table 11: Comparison of UCT944 exposure obtained from the formulated material, relative to the reference.

Parameter	Reference	LK32	PN37
AUC (h.µmol/L) (Normalised for dose)	715	3025	4336
Fraction of reference	1	4	6

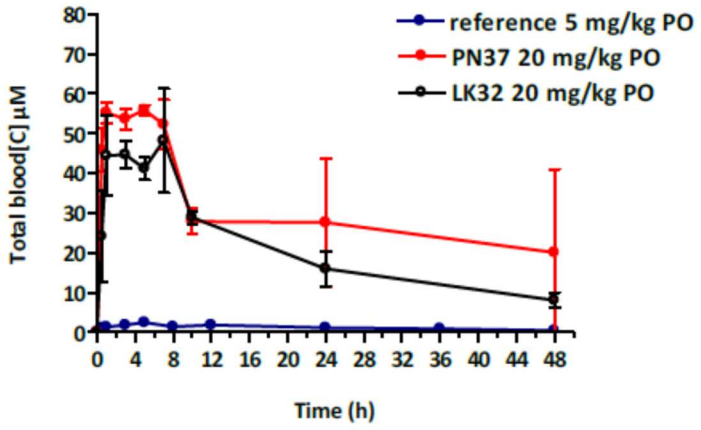

Figure 9: Direct comparison of delivery of UCT944 from preparations PN37 and LK32 to the reference, dosed orally at 20mpk in male Balb/c mice.

For the artemether formulations, however, the analysis to determine ART *in vivo* is still challenging. The preliminary results yielded negligible amounts of ART for all the *in vivo* samples of the plasma. The investigation still continues trying to understand the fate of ART *in vivo*[18].

5.4 *In vivo* PD studies

5.4.1 PN35 formulation of LUM for oral use

This study was done to evaluate the therapeutic efficacy of the novel oral PN35 formulation of LUM against *P. falciparum* P*f*3D7$^{0087/N9}$. The positive control that was used in this study was chloroquine at 10mg/kg and an empty vehicle only group was included as negative control. The parameters analysed in this study were the ED_{90} and AUC_{ED90} values, where the former value is the effective dose in mg/kg that reduces parasitemia by 90% on day 7 following infection compared to the control group (untreated) and the latter value is the estimated average daily exposure in whole blood that is necessary to reduce parasitemia by 90% on day 7 following infection compared to the untreated control group[19].

The therapeutic efficacy of PN35 formulation of LUM (dosed orally) against *in vivo P. falciparum* is illustrated in **Figure 10** and **Table 12**.

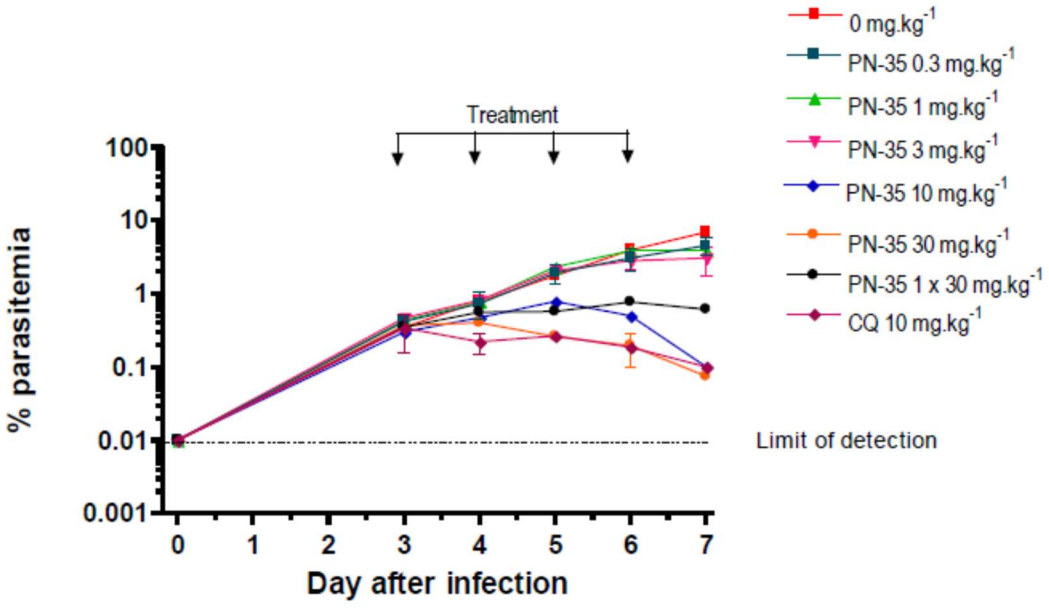

Figure 10: *In vivo* therapeutic efficacy of PN35 formulation of LUM

Table 12: Efficacy data summary

Compound	Target dose (mg/kg)	% Parasitemia (Day7)
Vehicle	0	6.88
PN35	0.3	4.55
PN35	1	3.86
PN35	3	3.05
PN35	10	0.10
PN35	30	0.07
PN35	30 x1	0.61
Chloroquine	10	0.08

To determine the ED_{90} value of the novel PN35 formulation non-linear fitting to a sigmoid dose-response curve of log_{10} of % parasitemia on day 7 following infection versus the dose that was used (**Figure 11** &

Table 13).

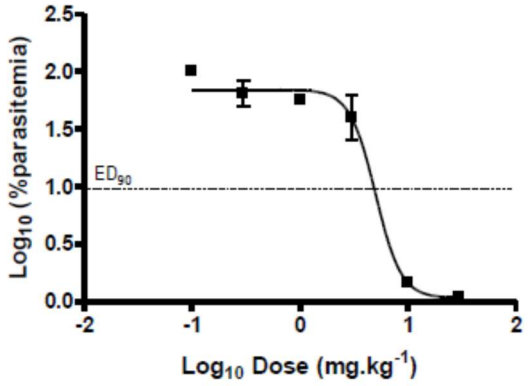

Figure 11: *In vivo* dose-response relationship of novel PN35

Table 13: Efficacy parameters for PN35 against *P. falciparum* P*f*3D7$^{0087/N9}$

Method of estimation	Goodness of fit	Parameter	Value of parameter
Log fit	0.97	ED_{90}	5.02 mg/kg

The PK studies done in healthy mice with PN35 translated well to efficacy in the NSG mouse model for malaria with and ED_{90} value of 5.02 mg/kg. The ED_{90} of parent LUM has been previously determined to be 12 mg/kg, therefore it is observed that there is an increase in efficacy for the PN35 formulation when compared to parent LUM. The single dose of 30 mg/kg also performed really well and shows promise that dose frequency could be decreased in future[19].

5.4.2 B9B_BM108 (polymer therapeutic of LUM) for IV use

The aim of this study was to evaluate the therapeutic efficacy of B9B_BM108 (BMT-108_B9) against *P. falciparum* P*f*3D7$^{0087/N9}$. An empty vehicle group was also included as a negative control and LUM as the reference. The efficacy of B9B_BM108 (BMT-108_B9) was assessed by administering a single IV dose per day for four consecutive days and measuring the effect on blood parasitemia by flow cytometry. Following the first dose on the first day the whole blood levels of LUM and desbutyl lumefantrine (DBL) were determined in order to quantify the area under the curve for the first 24 hours, denoted as $AUC_{0\text{-}24}$. The latter was then used to determine the potency of the compound[20].

The therapeutic efficacy of B9B_BM108 (BMT-108_B9) and LUM reference against *P. falciparum* is illustrated in **Figure 12** and **Figure 13** respectively.

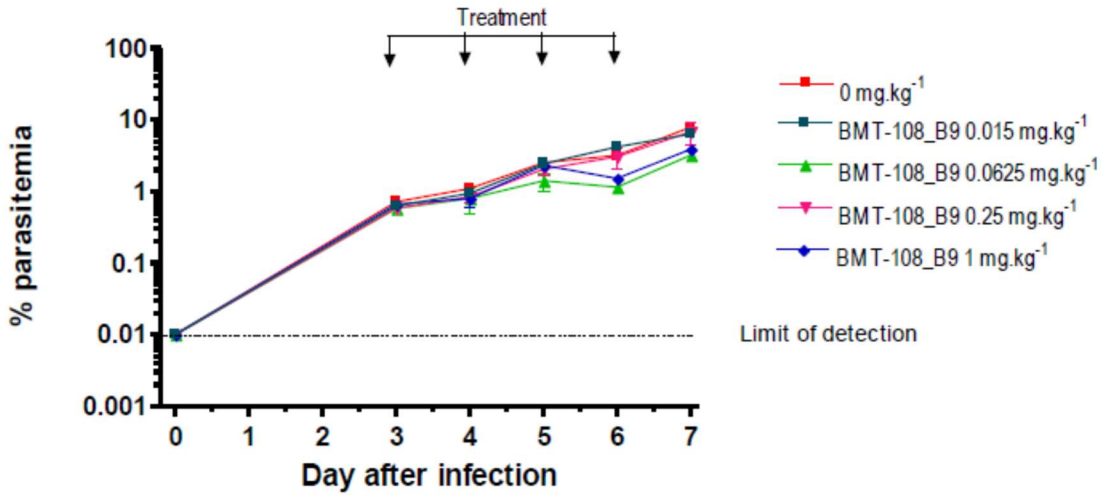

Figure 12: *In vivo* therapeutic efficacy of B9B_BM108 (BMT-108_B9).

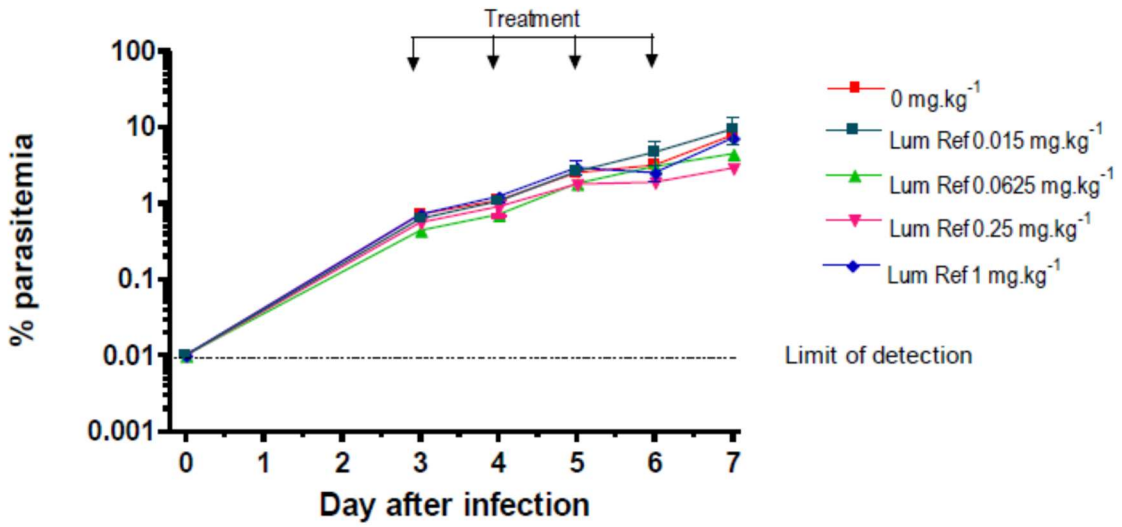

Figure 13: *In vivo* therapeutic efficacy of LUM reference.

Following the oral studies of LUM in the NSG mouse model, it was determined that the levels needed for efficacy is 3.5 µg.h.mL-1.day-1. During the PK study in healthy mice the exposure levels of B9B_BM108 (BMT-108_B9) and LUM parent following IV administration were 47 and 48 µg.h.mL-1, respectively. Based on the observed exposure levels in healthy mice the highest IV dose selected for the NSG study was 1 mg.kg-1. However, in both cases (B9B_BM108 (BMT-108_B9) and LUM reference) we only observed exposure levels of approximately 1.5 µg.h.mL-1.day-1 taking both the parent and metabolite levels into consideration. This could possibly explain why we did not observe the desired efficacy. The reason why exposure levels were lower in the NSG mouse model is unclear, however we need to keep in mind that for the NSG studies we are working in a different mouse specie that could contribute to the observed differences. However the exposure levels were comparable between the B9B_BM108 (BMT-108_B9) formulation and the LUM reference formulation at the highest dose of 1 mg.kg-1[20].

5.4.3 PN37 (reformulated UCT944) for oral use

This study was done to evaluate the therapeutic efficacy of the novel oral PN37 formulation of UCT944 against *P. falciparum* P*f*3D7$^{0087/N9}$. The positive control that was used in this study was chloroquine at 10mg/kg and an empty vehicle only group was included as negative control. The parameters analysed in this study were the ED_{90} and AUC_{ED90} values, where the former value is the effective dose in mg/kg

that reduces parasitemia by 90% on day 7 following infection compared to the control group (untreated) and the latter value is the estimated average daily exposure in whole blood that is necessary to reduce parasitemia by 90% on day 7 following infection compared to the untreated control group[21].

The therapeutic efficacy of PN37 formulation of UCT944 (dosed orally) against *in vivo P. falciparum* is illustrated in **Figure 14** and **Table 14**.

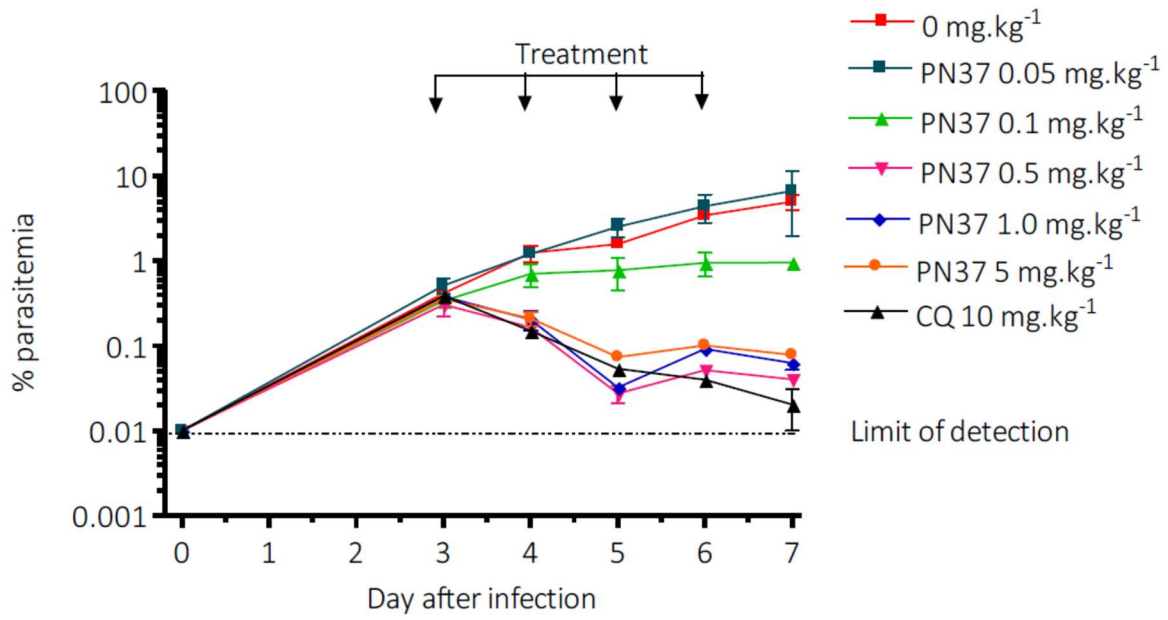

Figure 14: *In vivo* therapeutic efficacy of PN37.

To determine the ED_{90} and AUC_{ED90} values of PN37 non-linear fitting to a sigmoid dose-response curve log_{10} of % parasitemia on day 7 following infection versus the dose and $AUC_{(0\text{-}24)}$ was used, respectively (**Figure 15, Figure 16 & Table 14)**.

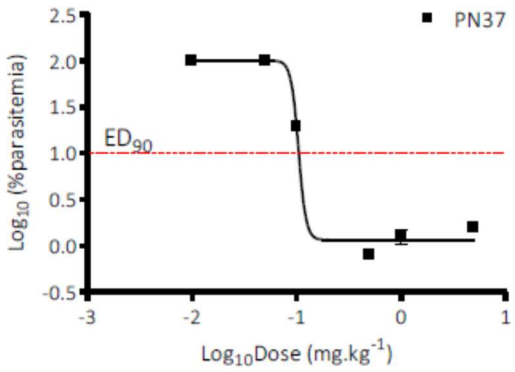

Figure 15: *In vivo* dose-response relationship of PN37.

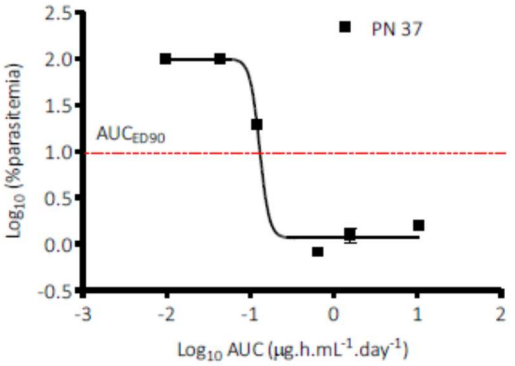

Figure 16: *In vivo* determination of the AUC_{ED90} for PN37**.**

Table 14: Efficacy parameters for PN37 against *P. falciparum* $Pf3D7^{0087/N9}$ (values in black (PN37) and values in red (UCT944 reference)).

Method of estimation	Goodness of fit	Parameter	Mean		Unit of the parameter
Log fit	0.99	ED_{90}	0.10	0.11	$mg.kg^{-1}$
Log fit	0.99	AUC_{ED90}	0.13	0.20	$\mu g.h.mL^{-1}.day^{-1}$

From the data it can be concluded that the drug, UCT944 is indeed released from the formulation. Higher exposure was observed for the 5 $mg.kg^{-1}$ dose while the exposure levels of the lower dosing groups compared well[21].

6. Outputs

A number of science engineering and technology (SET) outputs have emanated from this project and are given in **Table 15** below.

Table 15: SET outputs

Output Type	Description	Status
Patents	Patent title: Chemical Synthesis of Water-Soluble Lumefantrine-Polymer Conjugate Inventors: Mohammed Balogun, Lesego Tshweu, William Matshe Applicant(s): CSIR Country: United Kingdom Filing date: 07 December 2018 Type *(provisional, PCT, national)*: A provisional Patent Application Number: 1819985.1 2. Patent title: Enhanced aqueous solubilisation and in vivo absorption of hydrophobic active compounds using a polymer –lipid intercomplexation Inventors: Patric Nkuna, Lonji Kalombo Applicant(s): CSIR Filing date: Under filing process Type *(provisional, PCT, national)*: provisional Patent Application Number: NA Status *(under examination, granted, etc.)*: under filing	- Invention disclosure approved at CSIR - Provisional patent filed, awaiting examination report - Invention disclosure approved at CSIR - Provisional patent under filing
Publication	Nanomedicines for malaria: Encapsulation vs. Polymer Therapeutics (S. Mvango, W.M.R. Matshe, A.O. Balogun, L.A. Pilcher & M.O. Balogun.)	Published in "Pharmaceutical Research"; 2018; 35(12):237
Conference Presentation	Polymer Therapeutics for Infectious Diseases of Poverty: Extending Frontiers (M. Balogun)	Presentation at the 12th International Symposium on Polymer Therapeutics in Valencia, Spain. Oral and Poster presentations (28-30 May 2018).

Human Capital Development: A number of students were trained on the project (see **Table 16**). These young researchers are obtaining experience in the development of nanomedicines and will expend the competency in this technology available in the country.

Table 16: Progress with HCD

Student Name	University	Degree & Year of study	Status
Patric Nkuna	Tshwane University of Technology	Hons BTech	Completed in June 2018
William Matshe	University of Pretoria	HonsBSc (Pharmacology)	Completed Dec 2018 (currently enrolled for Master degree)
Sindi Mvango	University of Pretoria	PhD (Chemistry)	Expected to complete Dec 2019
Lesego Tshweu	University of Pretoria	PhD (Chemistry)	Thesis submitted for examination, completion expected in June 2019

Infrastructure: Some of the equipment which were critical to ensure delivery of the planned tasks, and which were not available at the CSIR's laboratories were identified and purchased as indicated in **Table 17**. Students were also trained on the equipment.

Table 17: List of equipment purchased for project in order to meet project deliverables

Equipment Name	Use	Trained Users
Rotary Evaporator	Efficient and gentle removal of solvents by evaporation.	W. Matshe, S. Mvango, M. Balogun, Z. Cele, P. Nkuna, A. Swanepoel, L. Tsheweu.
Freeze dryer	Low temperature dehydration process	W. Matshe, S. Mvango, M. Balogun, Z. Cele, P. Nkuna, A. Swanepoel, L. Tsheweu.
Biosafety Cabinet	Protection from biological hazards.	I. du Preez, A. Swanepoel.

7. Conclusion

Novel formulations for all three antimalarials: Lumefantrine, UCT 944 and Artemether were produced using polymer-drug conjugates, emulsion/spray-drying, and supercritical fluid technologies. The reformulated lumefantrine displayed increased water solubility, which yielded **~7-fold increase in lumefantrine absorption** in mice (Area under the Curve (AUC)), with a shortened Tmax and extended tailing of PK curve above the Minimum inhibitory concentration (MIC). This translated in halving the efficacy dose (ED) parameter when tested in infected mice with a high curing rate. The formulation was based on a lipid matrix, and showed that an additional fatty food intake was not required for improved exposure. For the reformulated artemether, the improvement in absorption was however not reproducible owing to the drug instability. The team is currently investigating ways of stabilising the drug during reformulation. **A novel IV formulation for LUM has also been developed that showed promising results *in vivo*** in healthy mice during the PK studies. In the infected mouse model, however the results were inconclusive due to lack of a proper protocol of administering LUM intravenously. Another formulation made through microemulsions is also earmarked to perform as an IV dose. UCT 944, was equally rendered hydrophilic and shown improved PK parameters when orally administered in mice, with the **AUC for reformulated UCT 944 increasing to ~6 fold compared to the reference**. Its ED was similar to the reference drug due to its relatively high potency.

The results demonstrate that the nanoencapsulation platform can be successfully used to reformulated poorly soluble drugs, with improvements in solubility, absorption, bioavailability and uptake demonstrated. The platform has the potential to reduce the dose and dose frequency for these antimalarials. This innovation is expected to make a significant impact in terms of enhancing the aqueous solubility of highly hydrophobic drugs and thus oral exposure, increasing their biopharmaceutical performance and reducing the dose and dose frequency. The same innovation can be applied for infectious diseases such as TB where DOTs strategy will be minimized as well as reducing the frequency for chronic treatment such as ARVs and drugs for high blood pressure.

Outputs achieved in the project includes 1 provisional patent, a second provisional patent is in filing stage, 1 peer-reviewed publication, and 1 conference proceedings. Furthermore a number of students receiving skills and training on the project.

8. Future work

The team has demonstrated proof of concept of the platform for reformulation of antimalarial drugs. Future development of the project includes securing funding for Phase 2 which will enable testing of the new antimalarials in higher order animals and finally bioequivalence studies in human clinical trials.

For phase 2 of the project will include more rigorous *in vivo* testing with potentially a dog and/or baboon malaria model where the bioequivalence at a similar or reduced dosage will be demonstrated, and potentially more optimisation of the novel formulations and retesting. From this data the most promising candidate/s will be scaled-up and taken forward for GMP manufacturing. After this process has been established, the dossier will be ready and phase 1 human clinical trials can commence.

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APPENDIX A1: Financial report

Financial report for the WHO project:

Development of a Single Dose Malaria Cure of Artemether-Lumefantrine through a Nano-based Drug Delivery System

Document Reference Number: Pta Projects GrpWise Doc. No.
Document Version 01: 30 April 2019

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Please see table 1 for the official financial report for the period 1 March 2017 – 31 March 2019 as issued by the Council for Scientific and Industrial Research's (CSIR) finance team.

Table 1: Financial report for the period 1 March 2017 – 31 March 2019

CSIR: MATERIALS SCIENCE AND MANUFACTURING

WHO - CEWG Demonstration

INCOME AND EXPENDITURE REPORT

PERIOD: 1 MAR 2017 TO 31 March 2019

	$
Balance of funds as at 1 Jan 2017	**894 040**
Income	**894 040**
Funds received	894 040
Less: Project Expenditure	**990 208**
Formulations	722 937
Preclinical	129 434
Project Management	137 837
Balance of funds as at 31 March 2019	**(96 168)**
Interest capitalised	7 774
Balance of funds including Interest as at 31 March 2019	**(88 394)**

Note: 10% retention not yet invoiced

Based on the "Addendum to CEWG proposal – "Development of a single dose malaria cure of artemether-lumefantrine through a nano-based drug delivery system" – revised work plan and budget" – the following budget was approved (Table 2):

Table 2: Approved budget (US$)

Work package	**Budget amount**	**Plus 8% contingency**	**Plus 14% VAT**
Formulations	603 164	651 417.12	742 615.52
Preclinical	133 980	144 698.40	164 956.18
Project Management	131 271	141 772.68	161 620.86
		Total:	1 069 192.55
		CSIR contribution:	75 851
		Grand total:	993 377.55
		Amount still to be invoiced:	99 337.55

Table 3 shows the actual spend amount per work package against the approved budget amount (Table 3):

Table 3: Comparison between actual spend and approved budget.

Work package	**Budget amount**	**CSIR contribution**	**WHO Actual budget**	**Spend amount**	**Difference**
Formulations	742 616	25 284	717 332	722 937	(5 605)
Preclinical	164 956	25 284	139 673	129 434	10 239
Project Management	161 621	25 284	136 337	137 837	(1 500)
Total	1 069 193	75 852	993 342	990 208	3 134

There was no significant deviation from the budget for any of the work packages.

The CSIR is requesting permission to make use of the interest capitalised in order to fund some of the phase 2 activities.

Global Observatory

World Health Organization

Global Observatory on Health R&D

Research for Health Department, Science Division

FINAL REPORT ON FUNDING ALLOCATION FROM THE POOLED FUNDS FOR DEMONSTRATION PROJECTS

TO BE PRESENTED TO THE TDR JOINT COORDINATING BOARD
Dec 04, 2019

Background

As part of the follow-up to the report of the Consultative Expert Working Group on Health Research and Development: Financing and Coordination (CEWG), the World Health Organization (WHO) facilitated the implementation of selected health R&D demonstration projects in collaboration with the WHO Special Programme for Research and Training in Tropical Diseases (TDR), and established a Global Observatory on Health R&D. An Ad-Hoc Committee was established to evaluate technical progress of the R&D projects and the Observatory and to provide recommendations on the allocation of resources in line with the objectives and achievements of each project.

Following the 70th World Health Assembly decision to officially close the demonstration projects and based on the decision of the Ad-Hoc Committee, the Observatory received the funds remaining in the pooled fund, which amounted to US$350,000 to cover a 12 month period from 30 November 2017 – 30 November 2018 in support of a revised proposal to the Committee (Annex 1). The Committee also authorized any funds remaining in the pooled fund after accounting for the administration charges required by TDR to be transferred to the Observatory on closure of the pooled fund. This amounted to an extra $47,888 to support the Observatory in 2019; hence a total of $397,888.

The following report describes the progress to date with the Observatory, which greatly benefited from the generous contributions of the pooled fund, direct contributions from four Member States and the European Commission to enable its conception and establishment.

Key achievements to date

Strategic objective
The Observatory managed to establish itself as a **comprehensive and authoritative "one-stop-shop"** for up to date information and analysis on health R&D to serve **health R&D monitoring and decision-making needs** globally and within WHO.

Opportunity
An expanding amount of **untapped data sources and information is being exploited** to enhance knowledge and information sharing.
For the first time, there is a **coherent approach for tracking and analyzing health R&D**, with WHO's assessment of unmet public health needs at the core.

Added value
1. **Consistent analytical approach across** diseases/conditions and across the whole R&D space including implementation and health systems questions.

2. **Enables coordination of R&D** investments, decision-making, capacity strengthening and monitoring at all levels.

Overall reach and expansion to date

The Observatory was launched on 19 January 2017 (http://www.who.int/research-observatory/en/). It currently includes **24 data sources** covering various aspects of health R&D monitoring including: health products in the pipeline, funding flows by disease and by country, health researchers by country, publications, clinical trials, target product profiles, research grants by major funders and other relevant global indicators such as ODA for medical research and global domestic expenditures on health R&D. The Observatory continues to expand since its launch both in terms of data sources and functionality, employing state of the art analysis techniques including data mining and interactive data visualizations.

The analyses and data provided by the Observatory constitutes a major resource for WHO's overall research coordination efforts and its evidence-based decision-making processes on global priorities for health R&D based on public health needs. These can be used by global stakeholders and WHO to coordinate and prioritize new investments as well as coordinating efforts to strengthen national capacity in health research.

Overall, the Observatory is very well received since its launch by a wide range of global stakeholders and users. It has doubled its web traffic in its second year of operation with more than **250 000 hits** to date. Several other parameters such as **average pages per session (7.5) and average session duration (4:30 minutes)** are **twice or triple the average for the WHO website** as per google analytics reports, see Annex 2. 78% of the Observatory's users find the Observatory website through web search for the information they need[1] and 56% of its user are new users at any point in time (Figure 1); both are indications that the Observatory is constantly being used by new users interested in this type of information.

[1] see Annex 2, google analytic report p 11, visits by traffic type and see % of users from organic search

Distribution of users by geographical region is also encouraging, see Figure 2.

Figure 1. Returning vs new users

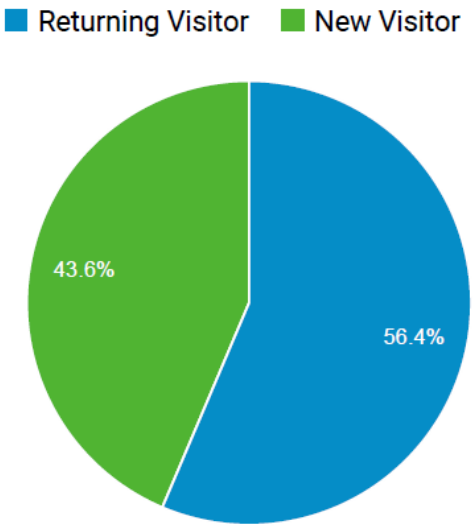
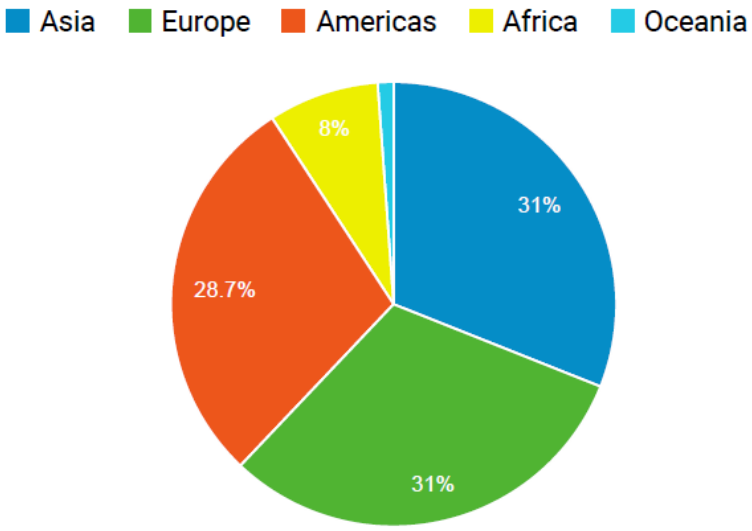


Figure 2: Users by continent



Since its inception, the Observatory Secretariat continues to be actively involved in various international R&D stakeholders fora and groups, such as the steering group of the world's major funders of health R&D (the World RePORT platform steering group); the Global Antimicrobial resistance R&D Hub; and the ESSENCE Working Group on Reviewing Investments (WGRI). This includes leading sub groups on data harmonization and analysis and efforts to develop common metrics for data collection among countries such as indicators for local capacity for health research.

In addition, various dissemination activities have been undertaken throughout including regular **email announcements** of updates sent to various relevant listserves and almost **30 presentations** to date by WHO staff in various international fora to share the objectives and

content of the Observatory. The use of **interactive analysis and data visualisations** is the most appreciated feature of the Observatory so far due to its informative, exploratory and customizable approach and the possibility to examine different combinations of questions together based on users interests and needs.

Developing solutions for problems related to data standardization and analysis

The Observatory used automated data mining techniques to classify clinical trials and R&D grants data by disease since this categorization was not readily available from the data. This was done through the development and curation of over 7000 disease synonyms complied and curated by the Observatory from various sources. These include: The Unified Medical Language System (UMLS) and the 10th version of the International Classification of Diseases, complemented by synonyms drawn from the data, mostly to account for errors in data entry such as spelling errors or use of abbreviations.

The result was significantly enhanced and informative data visualizations on clinical trials (WHO ICTRP data) and research grants by major funders (World RePORT data) providing pertinent information on the distribution of clinical trials and grants distribution by health category that was not available before. The work and experience on approaches for **data classification and standardization were shared** with WHO regional offices for further supporting their country offices in collecting and analyzing their R&D data.

In addition, an automated tool to classify "text" data into health categories and the standard classification of health categories and list of synonyms are available online through the Observatory[2]; as a step towards the Observatory's goals and aspirations to encourage exploitation of available R&D data and to help improve the quality and harmonization of future data collection. It is anticipated that the automated tool will particularly enhance the user's ability to analyze and share their own R&D data in the future. This area of work, employing data mining techniques to solve data problems, is expected to continue to expand in the coming years.

Analysis of R&D strategic priorities and gaps

The Observatory continues to work closely with WHO technical departments to develop detailed disease-specific analysis of R&D needs, gaps and priorities. The overall objective is to set the foundation for the required analyses and processes to set priorities for new R&D investments, as reflected in the WHA resolution.

Analysis of the strategic directions and R&D needs and priorities has been developed for malaria, tuberculosis, HIV/AIDS, neglected tropical diseases, R&D blueprint pathogens, anti-microbial resistance, mental health, and digital health research. A section on target product profiles is also included as a cross cutting theme. These analyses are updated yearly with input from WHO technical departments. New analyses for additional health topics is being pursued covering non-communicable diseases and other GPW13 priorities.

[2] https://www.who.int/research-observatory/classifications/disease/en/

Funding situation

In 2018-19 the Observatory received funding support from the Government of France, Switzerland, the pooled fund for the demonstration projects and the European Commission (EC DEVCO). In 2020, the only anticipated resources are from the European Commission (EC DEVCO) covering the period till the end of 2020.

Key priorities for 2020/2021

1. **Maintain a regular update current interactive analysis** on the Observatory website with more recent data to be published once or twice a year according to the frequency of update by the source.

2. **Add new analysis** (e.g., funding amounts on health R&D by diseases from key funders) **and new data sources** (e.g., purchased data from web of science for analysis of publications and funders of research data; and WHED for distribution of institutions for higher education and their number of students, specialties etc. by country).

3. Continue to progressively **expand the scope** of the observatory to other priorities emphasized in GPW13 such as Non-communicable diseases, maternal and reproductive health, mental health and others.

4. **Develop and share tools and templates to strengthen the capacity** to collect and analyse health research information at country and regional level, especially on domestically funded health research and indicators to assess national health research systems capacity (resource permitting).

5. **Work with WHO regional research focal points** to determine country needs to strengthen capacity in this area and together develop plans to fulfil these needs.

Conclusion

The Observatory grew significantly since its inception and is receiving increasing recognition in global discussions around R&D priorities, capacity and investments, as a credible global tracking and analysis source of information.
With its funding support being uncertain beyond 2020, it is critical that the resources to cover its current operating level and opportunities for expansion are secured and sustained to continue to capitalize on the investments, progress and experience gained so far.

For more information contact: Taghreed Adam at adamt@who.int (RFH/SCD)

Annex 1

World Health Organization

Global Observatory on Health R&D

REK/IER/HIS

UPDATE ON PROGRESS & BUDGET PROPOSAL FOR US$ 500'000

TO BE PRESENTED TO THE ADHOC COMMITTEE FOR THE DEMONSTRATION PROJECTS/GLOBAL OBSERVATORY ON HEALTH R&D
June 06, 2017

Background and key achievements to date

The Global Observatory on Health Research and Development (R&D) functions as a centralized and comprehensive source of information and analyses on global health R&D activities. It builds on existing data and reports from a wide range of data sources and gathers new information, where needed and feasible, with the aim of enabling decisions on priorities in R&D. The analyses and data provided by the Observatory will constitute a major resource for the work of the newly established WHO Expert Committee on Health Research and Development through WHA resolution 69.23, which aims at providing technical advice to the Director-General on priorities for health R&D based on public health needs.

In January 2016, a demonstration version of the Observatory was published online, which incorporated data on: funding flows for product-related health R&D for neglected diseases (from Policy Cures Research's Grant Finder survey); health products that are under development (from four data sources); research publications (from PubMed); clinical trials (from the International Clinical Trials registry Platform) and other relevant country-level macroeconomic data such as total health expenditures (from WHO's global health expenditures database) and burden of disease (from the Global Health Observatory).

Following consideration of feedback from users, work in 2016 focussed on developing and including new elements and functionalities, such as:

1. Interactive analysis and data visualisations that can be tailored to user's needs (at global, regional or country-level),

2. Additional indicators for monitoring resources for health R&D (such as the health-related R&D indicators of the SDG goals and targets and benchmarking R&D activities such as comparisons of funding flows to burden of disease),

3. Developing comprehensive analyses of disease-specific R&D data that incorporates quantitative and qualitative information and data needed for priority setting, done in collaboration with the WHO disease departments to guide the work of the Expert Committee on health R&D.

4. Documenting and sharing the approach for data classification and standardization used by the Observatory as a first step towards wider consensus building towards harmonization of future data collection efforts.

The Observatory was launched on 19 January 2017. It included 12 data sources covering additional data and analysis on health products in the pipeline, funding flows by disease and by country, health researchers by country, publications and other relevant global indicators for comparisons such as gross domestic product (GDP) data and burden of disease. It continues to be expanded since its launch with now 14 data sources included in total.

Priorities for 2017/2018

Key priorities for 2017/2018 include the following:

6. Continue to update and expand the analysis provided by the Observatory to be published twice a year, the next update to be published before the World Health Assembly in May 2017.

7. Develop solutions for problems related to data standardization and analysis and further exploit the information that can be obtained from the data by developing data mining solutions initially developed and tested for clinical trials (ICTRP) to enable the analyse of trials by disease, previously not possible, and obtain comments from the clinical trials registry network to improve the data analysis and visualisation (face to face meeting in Geneva, 3-4 May 2017)

8. Include data on research grants from the 12 biggest funders of health R&D through data obtained from the World RePORT data source https://worldreport.nih.gov/app/#!/ and collaborate with these funders on the development of more harmonization and common formats for data sharing for future data collection efforts (face to face meeting in UK, April 27, 2017).

9. Continue to validate and curate available data with technical experts and jointly develop detailed disease specific analysis of needs, gaps and priorities to present to the Expert Committee on health R&D.

10. Prepare for and hold the first meeting of the Expert Committee on health R&D in 2017/18

Overview of income and planned expenditure from January 2017-December 2019

1. The estimated costs for continued development of the Global Observatory on Health Research and Development for 2016-2019 are shown in **Error! Reference source not found.**. They were presented and approved by the 140th Executive Board of WHO in January 2017.

shows available funds for 2016-17 and the funding gap.

The estimated total net cost for 2016–2019 is US$ 6.3 million. The total net earmarked funds received or pledged (as of 20 January 2017) are US$ 1.77 million (net of programme support costs) for 2016–2017. Taking into account the allocated WHO programme budget funds from assessed contributions, the total gross funding gap for 2016–2017 is US$ 0.32 million. Without additional financial contributions, the total funding gap is estimated at US$ 2.05 million for 2018–2019.

Table 1. Summary of planned activities and costs for the period 1 January 2016 to 31 December 2019 (in US$)

Budget item	**Budget 2016–2017**	**Budget 2018–2019**
Global Observatory on Health Research and Development portal	585 000	675 000
Research and development knowledge generation and dissemination	400 000	575 000
Total activity costs	1 085 000	1 250 000
Total staff costs	1 961 133	1 961 133
Total net* biennial costs	3 046 133	3 211 133

* Net of programme support costs at 13%.

Table 2. Available funding and funding gap for the years 2016–2019 (in US$)

Source	**Total 2016–2017**	**Total 2018–2019**
Total earmarked funds* for the Observatory (net of programme support costs**)	1 765 985	394 715
Programme budget: assessed contributions	1 000 000	1 000 000
Grand total net of programme support costs**	2 765 985	1 394 715
Funding gap net of programme support costs**	280 148	1 816 418
Total gross funding gap (including programme support costs)**	**316 567**	**2 052 553**
Total gross funding gap (in million US$)	0.32	2.05

* Funds received or pledged from France, Germany, Switzerland and the European Commission as of 20 January 2017.

** Programme support costs at 13%.

Budget proposal for the amount of US$ 500,000

The allocated budget amount of USD 500,000 will contribute to the staff and activity costs to develop the following objectives of the overall workplan and priorities for 2017-18:

1. Continue to update and expand the analysis provided by the Observatory to be published twice a year
2. Develop solutions for problems related to data standardization and analysis and further exploit the information that can be obtained from the data by developing data mining solutions initially developed and tested for clinical trials (ICTRP) to enable the analyse of trials by disease.
3. Continue to validate and curate available data with technical experts and jointly develop detailed disease specific analysis of needs, gaps and priorities to present to the Expert Committee on health R&D

Annex 2

Global Observatory on Health R&D Jan 15, 2017 - Dec 2, 2019

All Users
100.00% Sessions

Visits

37,100
% of Total: **0.01%** (386,343,661)

Hits

251,254
% of Total: **0.02%** (1,391,469,562)

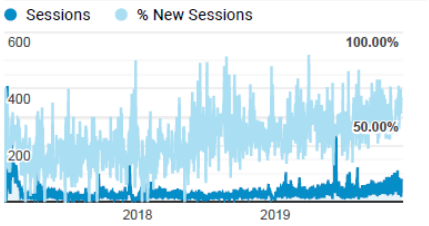
Pages / visit

7.30
Avg for View: **2.06** (253.73%)

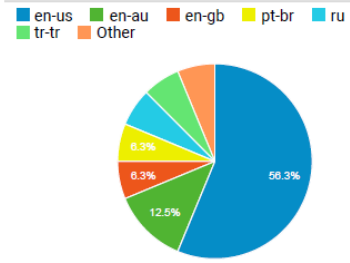
Visitor loyalty

Count of Sessions	Users
1	26,897
2	11,808
3	9,184
4	5,904
5	1,968
6	1,968
11	1,312
13	1,312
14	1,312
70	1,312

Visits and % New Visits



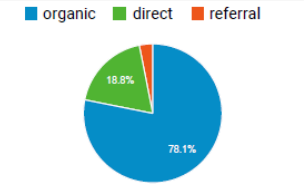
Visits by language



Visits by Social Network

Social Network	Sessions
Facebook	656

Visits by Traffic Type



Total Events by Page Title

Page Title	Total Events
WHO | Global Observatory on Health Research and Development (R&D)	26,241
WHO | R&D indicators	4,592
WHO | Antibacterial products in clinical development for priority pathogens	3,936
WHO | Gross domestic R&D expenditure on health (health GERD) as a % of gross domestic product (GDP)	3,936
WHO | R&D funding flows for neglected diseases (G-FINDER), by disease, year and funding category	3,936
WHO | Analyses and syntheses	3,280
WHO | Health researchers (in full-time equivalent) per million inhabitants, by income group (second set of charts)	3,280
WHO | Target product profiles	3,280
WHO | World Health Organization	3,280
WHO | Databases with a focus on health R&D	2,624

R&D Observatory Jan 15, 2017 - Dec 2, 2019

All Users
100.00% Sessions

Total Hits
251,254
% of Total: **0.02%** (1,391,469,562)

Total Sessions
37,101
% of Total: **0.01%** (386,343,661)

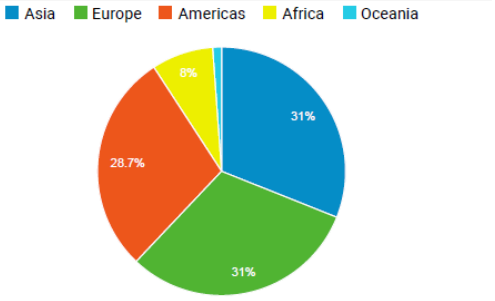
Pages / Session
7.30
Avg for View: **2.06** (253.73%)

Avg. Session Duration
00:04:19
Avg for View: **00:02:30** (72.29%)

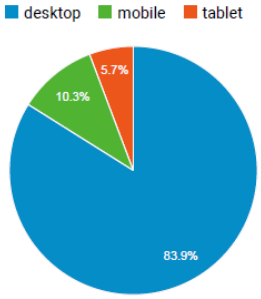
Hits and Sessions by Country

Country	Hits	Sessions
Russia	33,457	1,312
China	24,929	0
United States	24,929	4,592
United Kingdom	19,024	656
India	15,088	1,968
Germany	13,776	1,968
Indonesia	12,464	0
Turkey	11,808	1,312
Australia	7,216	2,624
Iran	7,216	656

Users by Continent



Users by device type



Returning vs New Users