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1. Executive summary

The NIDIAG (Neglected Infectious Diseases DIAGnosis) consortium aimed to translate diagnostic innovation into clinical care practice for neglected infectious diseases (NID). Clinical, biomedical, and public health scientists from 12 institutes in 12 countries as well as a diagnostics SME worked together to develop simple and cost-effective diagnosis-treatment algorithms for three NID-related clinical syndromes in Africa and Asia: neurological disorders, persistent fever and digestive disorders. NIDIAG was a very challenging endeavour as quality-assured clinical studies had to be set up in the remote tropical areas where NIDs are frequent. The investigators had to deal with context-related structural problems (a.o. difficult transport, poor internet, civil unrest), vulnerability of research subjects, poor regulatory oversight and lack of quality medical suppliers. To share the lessons learned and provide open access to the research tools that were developed, the NIDIAG consortium put together a Special Supplement to the journal *PLoS Neglected Tropical Diseases* (expected mid 2016).

The impact on end-users of NIDIAG is real and two-fold. Firstly, through the development from bench to market of two new rapid diagnostic tests (RDTs): an RDT (HAT Sero-K-Set) for second-stage human African trypanosomiasis (sleeping sickness; market authorisation has been obtained for this test in the DRC), and an RDT for visceral leishmaniasis, a severe disease of East Africa and the Indian subcontinent. This new RDT differs from existing diagnostic tests in its capacity to detect relapse and to predict progression from infection to disease. These new tests have the potential to be game changers in current HAT and VL control practice. The HAT RDT has been included already in the new HAT control policy promoted by WHO and countries.

Secondly, through the development of clinical guidance to improve quality of care in NIDs. The NIDIAG partners first investigated the causes of these syndromes. Core findings were the high frequency (35%), diversity and severity of infectious diseases among patients with neurological disorders in Bandundu, DRC. Highlights of the persistent fever syndrome study were the importance of brucellosis (4%) in Sudan, the virtual absence of malaria in Asian study sites and the high frequency of mixed infections and of unconfirmed suspicions of enteric fever in all study sites. For the digestive syndrome, the spectrum of pathogens varied widely across settings. In Mali, *Schistosoma mansoni* and *Giardia intestinalis* were highly prevalent. In Nepal, in contrast, there were very few helminthic infections but a surprisingly high prevalence of the intestinal protozoon *Cryptosporidium*. Once the causes of the three syndromes were better known, we selected clinical features, RDTs and other tests with good discriminative power and included them in diagnostic guidance tools with adapted formats. We further assessed acceptability, clinical impact and cost-savings of these new tools.

Finally, NIDIAG generated exceptional scientific output, with till date 28 publications in peer-reviewed scientific journals and 71 presentations in scientific conferences and other fora. We disseminated findings with direct relevance for public health through policy briefs and interventions in the public media. This project had a positive impact on the research capacity in all the participating institutes and on the dialogue between researchers and policy makers. At the final consortium meeting, all concurred that this network should be kept alive for the next years, to promote further the syndromatic approach to clinical management of NIDs.
2. **Summary description of project context and objectives**

Tropical diseases such as trypanosomiasis, leishmaniasis and schistosomiasis receive minimal funding for research and thus fall under the category of neglected infectious diseases (NID). Clinical management and treatment of these diseases in endemic countries is often not evidence-based and hardly ever uses state-of-the-art technology. Prior to the NIDIAG study, there were virtually no tools to guide clinical care-givers in the differential diagnosis of neurological disorders, persistent fever or persistent digestive disorders in low-income tropical countries.

Worldwide, the impact of **neurological disorders** is enormous in terms of mortality, morbidity, physical disability and psychological distress. In low-resource settings, lack of diagnosis and of treatment facilities as well as delayed management make the burden still much more considerable. The frequency of neurological disorders and the pattern of causative conditions are little documented in low-resource primary care settings, and particularly in sub-Saharan Africa. In the few observational studies conducted in African hospitals for the past 20 years, neurological disorders accounted for 7 to 24% of all admissions. Central nervous system (CNS) infections were suspected in one third of all patients admitted with neurological symptoms, with a specific microbial aetiology identified in half of these. Where it has been investigated, up to one third of neurological admissions was related to HIV infection. Neuro-infections were also the leading cause of common neurological symptoms in African HIV-positive patients, and autopsy studies have demonstrated that CNS infections accounted for 20% of the causes of death in HIV-positive individuals in sub-Saharan Africa. In contrast with other aetiologies of neurological disorders, most CNS infections may be considered as “severe and treatable diseases”. This is the case for conditions such as human African trypanosomiasis (HAT), cerebral malaria, bacterial meningitis, CNS tuberculosis, neurosyphilis, cryptococcal meningitis or toxoplasma encephalitis, to name a few. If left untreated, death or serious sequel usually occur; mortality rates of neurological admissions were as high as 30% in the abovementioned studies. However, outcome may be favourable with timely and appropriate management. In resource-constrained settings more than elsewhere, such “severe and treatable” conditions should be targeted in priority in the clinical decision-making process. Unfortunately, most neuro-infections present with non-specific symptoms in their early stages leading to important diagnostic delays. To make matters worse, neurological diagnoses frequently require relatively advanced technology to reach an accurate diagnosis, such as electroencephalography, electromyography, computed tomography (CT) or magnetic resonance imaging (MRI) of the CNS, culture or molecular workup of the cerebrospinal fluid (CSF). Since this is far beyond reach of tropical rural settings, the diagnostic approach and empirical therapies must exclusively rely on clinical judgment and first-line laboratory results (blood analysis and CSF examination). However, the confirming or excluding powers (positive or negative likelihood ratios) of most clinical and laboratory features are limited or have never been adequately quantified, in particular in settings lacking reference diagnostic methods. Several rapid diagnostic tests (RDTs) have been developed in the last decade and have considerably improved the management of conditions like malaria or HIV infection. However, their diagnostic contribution was never evaluated within a multi-disease approach.

**Tropical fevers** have been a diagnostic challenge from the antiquity. Today, despite the availability of good diagnostic capacities, undifferentiated febrile illnesses continue to be a thorny problem for travel physicians. In developing countries, the scarcity of skilled personnel and adequate laboratory facilities makes the differential diagnosis of fevers even more complex. Health care workers must
often rely on syndrome-oriented empirical approaches to treatment and might overestimate or underestimate the likelihood of certain diseases. The difficulty in establishing the cause of febrile illnesses has resulted in omission or delays in treatment, irrational prescriptions with polytherapy, increasing cost and development of drug resistance. This is particularly apparent with the management of Acute Febrile Illnesses (AFI). Health systems of most countries have, for many years, focused attention heavily on malaria, at the expense of attention to other causes of acute fever. D’Acremont and colleagues have estimated that the median proportion of fevers in sub-Saharan Africa that is associated with *P. falciparum* is 22%. Another study conducted in a rural hospital in South India showed that 88% of hospitalized adults with AFI were negative for malaria. The aetiologies of AFI are many, and in malaria endemic areas, neither clinical presentation nor the demonstration of parasitaemia are sufficient to exclude other infections. Indeed, several hospital-based studies show that viral and bacterial infections are very common among febrile patients. Dengue, leptospirosis, rickettsia and *Salmonella* infections are frequent causes of AFI in the tropics. HIV, brucellosis, relapsing fever and *Cryptococcus spp* have also been incriminated. The problem is that appropriate diagnostic facilities are often restricted to large urban centres, and in most rural health facilities these diseases remain undiagnosed. Until the revision of WHO guidelines in 2010, all febrile patients in tropical countries received anti-malarial treatment. WHO now recommends that malaria treatment should be restricted to parasitologically confirmed cases, however, this raises a concern about the appropriate management of non-malaria AFI. In the absence of adequate Point-of-Care (POC) diagnostics, the new guidelines may favour an indiscriminate use of broad-spectrum antibiotics in all malaria RDT-negative patients.

The diagnosis of prolonged (≥ 1 week) fevers is even more challenging as it has only rarely been studied in the Tropics. Only a few studies have been conducted on Fever of Unknown Origin (FUO). FUO are defined as fever lasting ≥ 3 weeks with temperature ≥ 38.3°C that remained unexplained after a week of intensive hospital testing. As with AFI, malaria is frequently incriminated in prolonged fevers, but other communicable and non-communicable diseases are also prominent. NIDs contribute substantially to the burden of prolonged fevers in the Tropics, causing considerable mortality and major disability, including diseases such as Visceral Leishmaniasis (VL), Human African Trypanosomiasis (HAT), Chagas disease, Buruli ulcer and leprosy. As febrile illnesses are diagnosed clinically in most rural centres of developing countries, clinical algorithms could be a valuable aid to health workers, as they facilitate the therapeutic decision in the absence of good laboratory capacities. Such algorithms have been successfully developed for sexually transmitted infections (STIs), and for common childhood diseases through the integrated management of childhood illness (IMCI). Several attempts have been made to develop algorithms for febrile patients and to identify clinical features specifically hinting at a particular cause of fever. Results have been mixed and the utility of existing algorithms seems to depend on the geographical location. In addition, these algorithms are often based on expert opinion only, few have been properly evaluated in the field and none incorporate recently developed rapid diagnostic tests (RDT). In fact, despite the development of several relevant POC diagnostic tools, very few are readily available at primary care level. While RDT for malaria are being progressively introduced across malaria endemic countries, reliable assays are missing for almost all of its differential diagnosis, including typhoid fever and TB. Similarly, although rk39-based RDTs are widely used across VL endemic areas of Nepal and India, the challenge posed by febrile patients who are rk39 RDT-negative or with suspected relapse has yet to be solved.

The decision to specifically concentrate on persistent digestive disorders (lasting for at least 2 weeks
according to the WHO definition – WHO, 2009) was based on several considerations. First, acute diarrhoea (< 2 weeks) has already been investigated in multiple studies on all continents and the etiological spectrum is rather well known (see systematic review Shah et al., 2009). In addition, a large global study is ongoing (the Global Enteric Multicenter Study –GEMS) that thoroughly investigates the causes of diarrhoeal disease (≤7 days) in infants and young children in developing countries (Levine et al., 2012; Kotloff et al., 2012). In contrast, persistent diarrhoea and/or abdominal pain have hardly been investigated in the tropics. Moreover, most of these studies were conducted only in children and included less than 100 participants. Few had a case-control design to investigate the true relationship between digestive symptoms and the presence of pathogens. A recent systematic review revealed that diarrheagenic Escherichia coli was found in 30-40% of children with persistent diarrhoea and intestinal protozoa in 15-20% of them (Abba et al., 2009). The potential contribution of helminths in this syndrome has not been studied so far. The authors concluded that further high quality studies were required to elaborate appropriate clinical guidelines (Abba et al., 2009).

The EU-funded http://www.nidiag.org/ (NIDIAG) (Syndromic approach to neglected infectious diseases (NID) at primary health care level: an international collaboration on integrated diagnostic-treatment platforms) project aimed to introduce technological innovations in diagnostics for NID-related clinical care. The primary goal was to develop evidence-based diagnostic guidance on the NID-related persistent fever, neurological and digestive syndromes. Bringing in experts in clinical epidemiology and diagnostics development, the consortium focuses on NIDs of Africa and South Asia.

The long-term goal of NIDIAG was to contribute to rationalising drug use, and avoid disease progression, thereby reducing NID-associated morbidity and mortality. The different NIDIAG research activities aimed to provide clinicians with important tools towards better NID clinical management. A patient-centred syndrome-based approach tries to overcome the current vertical approaches in NID management and ultimately – to improve the quality of clinical care. For this purpose, local personnel underwent extensive training on diagnosis and disease management. Improving diagnostic accuracy in NIDs is of crucial importance not only to offer the most effective treatment to individual patients and therefore optimise outcome, but also as a powerful mean to restrict overuse of antibiotics and antimalarials and therefore to slow down or revert the spread of antimicrobial resistance, a global public health threat.

In summary, the main objectives (aims) of the NIDIAG consortium were:

**Aim 1** To develop and validate an integrated syndromic approach based on diagnosis/treatment algorithms for three clinical syndromes that include both neglected and non-neglected diseases frequently encountered in primary care settings.

**Aim 2** To develop novel diagnostic platforms/assays tailored to specific epidemiological contexts at primary care level in NID-endemic settings.

**Aim 3** To document the cost-savings and increased efficacy of this integrated syndromic approach for the clinical management of NID, and produce recommendations to policy makers for its broad implementation.
3. **Description of main S&T results/foregrounds**

**Aim 1 Development of diagnostic guidance tools**

The overall aim was to develop evidence-based diagnosis-treatment algorithms for clinical practice, later redefined as diagnostic guidance tools, in order to improve the integrated management of several Neglected Infectious Diseases (NIDs) at the primary care level.

The **specific objectives** in this body of work were:

1. To determine the prevalence of NIDs and other diseases in patients who present to primary health care facilities with three specific clinical syndromes (digestive syndrome, persistent fever and neurological syndrome) in several Asian and African countries.
2. To identify clinical symptoms and signs that are diagnostic predictors of NIDs and other relevant conditions in patients presenting with one of the three clinical syndromes.
3. To assess the diagnostic performance of readily available diagnostic tools and novel assays in the field in Phase III prospective designs.
4. To incorporate the acquired epidemiological and clinical knowledge and field-designed diagnostic tools into diagnosis-treatment algorithms, and to evaluate the performance of these algorithms by conducting prospective studies in primary health care settings in several Asian and African countries.

The initial task was to review existing epidemiological data of NIDs targeted by the project, describe existing diagnostic and treatment practices and ongoing disease control activities in Cambodia, Côte d’Ivoire, the Democratic Republic of Congo (DRC), Indonesia, Mali, Nepal, and Sudan. In summary, this in-depth analysis revealed that (i) epidemiological data was scarce for many NIDs targeted by the project (e.g. rickettsiosis, intestinal protozoan infections, most neurological disorders) and (ii) no diagnostic guidance tools existed for the clinical syndromes studied in NIDIAG, i.e. persistent digestive disorders, persistent fever and neurological disorders, except for a few “ordinogrammes” prepared by the DRC MoH.

During this initial phase, site assessment forms were completed for every health structures that were potential candidates for being selected as study sites. The capacities of laboratory (HR, equipment, storage capacities, availability of tests, quality assurance procedures, statistics on pathogens of interest) and clinical (HR, medical and imaging facilities, statistics of patients consulting with targeted syndromes and first-line treatment in use) facilities were assessed, which contributed to the final selection of study sites.

In order to obtain a more in-depth understanding of the clinical management practices of healthcare providers operating in several of the NIDIAG field sites, a combination of qualitative research methods (observations of consultations, in-depth interviews, focus groups discussions) was used. Studies were implemented in the NIDIAG field sites situated in DR Congo, Nepal and Indonesia, where we focused on the neurological syndrome, persistent fever and the digestive syndrome respectively. Data collection took place from January to May 2012. Over the three study sites we performed 118 observations of clinical consultations, 35 in-depth interviews with healthcare providers and 11 focus group discussions with clinical staff. Overall, knowledge regarding each of the
three clinical syndromes of interest seemed to be very good amongst healthcare providers working in the study areas. However, a range of barriers regarding the clinical management of patients was documented. Although the particularities of these issues varied from study site to study site, by and large they could be distilled into three major groups of determinants that affect the quality of care in these low-resource settings: (i) technical limitations (such as a lack of practical point-of-care diagnostic tests), (ii) health system challenges (such as issues arising from a breakdown in communication between health facilities and health policy institutions) and (iii) socio-cultural factors (such as patient expectations regarding the role of healthcare providers and their capacity to diagnose and treat). The specific outcomes of these studies were used to inform the processes around the development of new diagnostic guidance tools within NIDIAG.

The core activities of this part of the project was the conduct of a series of 9 GCP/GCLP-compliant epidemiological and phase 3 diagnostic studies to define the differential diagnosis of persistent digestive disorders, persistent fever and neurological disorders, with a focus on potentially severe and treatable NIDs and other infectious conditions. The preparation phase included the development of study protocols, the writing of 95 Standard Operating Procedures (SOPs), the submission to ethical review boards and the training of local investigators in GCP/GCLP and laboratory techniques. Patients’ recruitment and follow-up lasted from September 2012 to December 2015. The predictive weights of clinical features and point-of-care rapid diagnostic tests (RDTs) were assessed, which fed the development of diagnostic guidance tools during several dedicated workshops between October 2014 and March 2016.

**Digestive syndrome**

For the investigation and development of diagnostic guidance for persistent digestive disorders defined as either persistent diarrhoea (≥14 days; all age groups) or persistent abdominal pain (≥14 days; individuals aged 1-18 years), a multi-country case-control study was carried out in two West African countries (Côte d’Ivoire and Mali) and two Asian countries (Indonesia and Nepal). As more than 40 different infectious pathogens are capable of causing intestinal infections, a broad range of diagnostic tests (stool microscopy, culture, RDTs and post-hoc PCR) was employed to investigate the presence of bacterial, helminthic and protozoal pathogens in stool samples. Patient recruitment started in July 2014 and ended in December 2015. In Indonesia, the study design had to be adapted due to a low number of patients presenting with persistent digestive disorders to the study sites; a cross-sectional community-based study was carried out instead. In Côte d’Ivoire, Mali and Nepal, a total of 260, 553 and 128 patients were recruited, respectively, owing to a total study cohort of 941 individuals. Additionally, similar numbers of matched controls were recruited. The clinical manifestations of persistent digestive disorders and the detected pathogen spectrum showed considerable setting-specificity. In Mali, the trematode *Schistosoma mansoni* and the intestinal protozoon *Giardia intestinalis* were highly prevalent in patients and controls, but showed higher frequencies and infection intensities in symptomatic patients. In Nepal, in contrast, very few helminthic infections were detected, but surprisingly high rates of infections due to the intestinal protozoon *Cryptosporidium* spp. were found. The post-hoc molecular examinations are currently ongoing. An algorithm development workshop was held in Bamako, Mali on 12 and 13 November 2015. The final algorithm (Figure 1) considered the study findings from Mali and is meant to be
applicable in peripheral health care centres with no or very limited laboratory infrastructure.

**Figure 1: Diagnosis-treatment algorithm for persistent abdominal pain in Mali**

A subsequent validation study was carried out in Niono, Mali in March and April 2016, during which the algorithm was employed on approximately 120 individuals, with favourable clinical treatment response in the vast majority of study participants. In the other study countries, data analysis is still ongoing and will lead to a similar development of diagnostic guidance tools.

**Persistent fever syndrome**

The main purpose was to define the differential diagnosis of persistent (≥ 1 week) fever and validate RDTs in ≥ 5 year patients in four NID-endemic countries, with the final objective to develop new diagnostic guidance tools. Between January 2013 and October 2014, patients were recruited in 5 hospitals in Sudan (Tabarakallah, Gedaref province), Nepal (Dharan and Dhankuta, eastern region), Cambodia (Phnom Penh), and DRC (Mosango, Bandundu Province). Blood and urine reference diagnostic tests were performed on site and in referral laboratories for malaria, HIV, brucellosis, leptospirosis, relapsing and enteric fevers, HAT (DRC), melioidosis (Cambodia) and VL (Nepal and Sudan). Index RDTs were performed on site (for VL, typhoid fever and leptospirosis) or on stored sera (brucellosis). In Sudan, out of 667 patients, the most prominent diagnosis were UTI, (10.6%), VL (9.4%), malaria (8.2%), RTI (8.2%), PID (4.9%) and brucellosis (4.2%). In Cambodia, out of 378 patients, pneumonia (27%), tuberculosis (19.8%), UTI (9.5%), skin/soft tissue infection (6.1%), liver abscess (5.6%), melioidosis (4.2%) and leptospirosis (4%) were the most frequent diagnosis. In Nepal, out of 425 patients investigated in Dharan, the differential diagnosis was led by VL (12.7%), RTI (8.7%), UTI (7.3%), tuberculosis (5.4%) and rickettsiosis (4%). Enteric fever was a frequent clinical diagnosis in all field sites but confirmed in only few patients, whereas malaria was rarely confirmed in Asian sites. Data from DRC (n=301 patients) remains to be analysed. Mixed infections were frequent and the etiological cause remained undefined in 14-48%. RDTs for brucellosis and VL showed good diagnostic performance. We developed country-specific guidelines recommending an initial
systematic clinical and RDT-based workup and guidance tools based on a panoramic format similar to the one developed for the neurological syndrome (see below). These guidance tools will be implemented and evaluated in Nepal and Sudan with alternative funding in 2016 and beyond.

**Neurological syndrome**

The main objective was to generate evidence-based guidance to improve the management of neurological disorders in low-resource rural hospitals of Central Africa. The study was launched in September 2012 in the 400-bed “Hôpital Général de Référence” (HGR) of Mosango, Province of Bandundu, DRC. All patients older than 5 years presenting at the study hospital with any neurological symptom were screened by a neurologist investigator and consecutively enrolled if criteria of inclusion reflecting ongoing disease were fulfilled. They were clinically assessed and systematically subjected to a set of pre-established laboratory investigations including RDTs and reference assays (either on site or in reference laboratories in Kinshasa or Antwerp). Response to specific treatment was also assessed and patients were followed-up until 6 months after discharge.

The field study was completed in January 2015. In total 351 patients were included. Patients were often severely ill with a mortality rate of about 10% and serious post-disease disability in 22%. Infections were confirmed or highly likely in about 35% of the cases with various etiologies found at frequencies ranging from 1 to 5%: unspecified meningo-encephalitis (5%); bacterial meningitis (4%); malaria (4%), human African trypanosomiasis (HAT; 3%), Pott’s disease (3%), HIV/AIDS (3%), central nervous system tuberculosis (1%) and tetanus (1%). Confronted with the variety of etiological diagnoses (with a rather low frequency for each single disease), we opted to develop guidelines recommending an initial systematic RDT-based workup (for HAT, HIV and malaria) and a panoramic/synoptic tool that allow a “not-to-miss” approach of the severe and treatable infections (Figure 2). This study results and the new guidance were presented to the medical staff of several rural hospitals in Bandundu in October 2015. The proposed guidelines were enthusiastically welcomed by the caregivers who made also constructive comments for improvement. The field evaluation of the new tool (acceptability, clinical impact, cost-savings) is ongoing since January 2016 through an alternative funding.

*Figure 2: Guidelines for the management of neurological disorders in Bandundu province, DRC*
Neurological disorders

Initial workup for patients older than 5 years

1. Perform systematically Rapid Diagnostic Tests (RDT) for:
   - Malaria
   - HIV
   - Human African Trypanosomiasis (HAT)

2. Look for the predictive arguments under each diagnosis:
   - Arguments IN FAVOUR OF diagnosis
   - Arguments AGAINST diagnosis
   - Strong argument
   - Moderate argument
   - Weak argument

3. Perform cerebrospinal fluid (CSF) examination in patients with any of these features:
   - Altered consciousness
   - Fever
   - Confirmed HIV
   - Positive CATT / RDT for HAT

   Conditions for lumbar puncture:
   1. Hemodynamic / respiratory instability
   2. Rapid deterioration of consciousness
   3. Recent convulsions (< 30 minutes ago) or repeated convulsions
   4. Altered consciousness and papilledema at fundoscopy
   5. Infections at the puncture site
   6. Bleeding disorders

Diagnostic panorama

Bacterial meningitis
- Gram staining in CSF
- Polymorphonuclear leukocytes
- Fever 1 week
- Vomiting
- CBC: 10,000/mm³
- Lumbar puncture

Normal CSF

Neurosyphilis
- Positive RPR / RDT
- Negative RPR / RDT

(Cerebral) malaria
- Positive pLDH-based RDT
- Positive RPR / RDT
- Negative RDT

Spinal tuberculosis
- Kypheus / scoliosis
- Paravertebral abscesses
- Abnormal vertebral imaging
- Fever 1 week

Tetanus
- Muscle spasm / opisthotonus
- Adequate vaccination

Neurological disorders
- Altered consciousness
- Severe daily headache
- New onset seizures
- Focal neurological deficit
- Behavioural abnormalities
- Walking disturbances

Stage 2 HAT
- Positive CATT / RDT
- Mononuclear pleocytosis
- Abnormal chest X-ray
- Fever 1 week
- Focal neurological deficit
- Lumbar puncture

HIV/AIDS
- Positive RDT
- Eosinemia
- Cognitive decline

Central nervous system tuberculosis
- Mononuclear pleocytosis
- Abnormal chest X-ray
- Fever 1 week
- Focal neurological deficit

Other diagnoses (non-exhaustive):
- Cerebrovascular accident
- Epilepsy
- Psychiatric disorders
- ...
Aim 2. Development of Rapid Diagnostic Tests

The specific objectives were, in summary:

1. To determine the target profiles of rapid diagnostic tests (RDTs) for the three clinical syndromes (fever, digestive, neurological); the advantages and disadvantages of different RDT formats; the characteristics of existing diagnostic tests, and the availability of such tests.

2. To produce improved serology for visceral leishmaniasis (fever syndrome, VL), explore stool antigen detection tests (digestive syndrome) and develop a serological RDT for human African trypanosomiasis (neurological syndrome, HAT).

To achieve objective 1 a highly successful international workshop was hosted in London by the London School of Hygiene and Tropical Medicine (LSHTM) and co-organised with the SME Coris Bioconcept (Coris). Participants included laboratory scientists, researchers and clinicians from endemic regions, representatives of commercial companies and funding organisations. A resultant wide-ranging and in depth survey resulted, of the criteria required for RDTs at primary point-of-care (POC) and of the extent to which appropriate RDTs were already available. The participants subsequently published several authoritative reviews, largely stimulated by outputs of this workshop. Ideally, and subject to supplementary funding, a follow up workshop is highly desirable beyond the NIDIAG project, to assess the latest technological developments, new diagnostic tests and future priorities.

Visceral leishmaniasis (related to persistent fever syndrome)

The NIDIAG partnerships between laboratory and field were crucial to objective 2, to develop improved serology for VL (Sudan, India) and HAT (Democratic Republic of Congo).

The demanding field research on VL in Sudan, under the leadership of Professor Sayda El Safi (University of Khartoum - UNKA), involved many visits to the endemic region in Gedaref State and to hospital outpatient clinics. Examination of cases of VL included clinical evaluation, parasitological diagnosis, direct agglutination (DAT), rK39 rapid tests, and collection of serum, plasma and urine from active VL cases, treated patients, relapse, post kala-azar dermal leishmaniasis (PKDL) and healthy controls having negative or positive DAT, with appropriate consents and ethical approvals. Samples were transported in liquid nitrogen and stored at -80°C or -20°C. Importantly, 50 samples from active VL cases, 56 from treated patients, 1 relapse, 23 PKDL and 94 EHCs including 14 DAT positive EHCs, were transferred to the laboratory of LSHTM for research on development of improved diagnostics. Two London/Sudan exchange visits took place for subclass serological profiling the antibody responses of different clinical groups by enzyme linked immunosorbent assay (ELISA) and for optimising production of DAT antigen.

Similarly the Sita Ram Memorial Trust (KAMRC) led by Professor Shyam Sundar, provided large numbers of samples of blood, plasma and urine from patients with different clinical status, conditional upon informed consent, as summarised below in Table 1. Samples were transported from the field with cold chain storage, archived at 80°C, and presence of anti-Leishmania antibodies determined by ELISA, rK39 or DAT. Vital research with these samples was performed in Varanasi,
India, during annual visits by research teams from LSHTM to the laboratory of KAMRC.

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>Whole Blood</th>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active VL</td>
<td>210</td>
<td>195</td>
<td>110</td>
</tr>
<tr>
<td>Post Treatment</td>
<td>122</td>
<td>112</td>
<td>80</td>
</tr>
<tr>
<td>Cured VL (6 months follow up)</td>
<td>88</td>
<td>110</td>
<td>70</td>
</tr>
<tr>
<td>Relapsed</td>
<td>15</td>
<td>45</td>
<td>00</td>
</tr>
<tr>
<td>Endemic Healthy Control (EHC)</td>
<td>75</td>
<td>150</td>
<td>78</td>
</tr>
<tr>
<td>Non Endemic Healthy Control (NEHC)</td>
<td>50</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>HIV</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Other diseases</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>

LSHTM undertook three fundamental studies relating to regional differences between the performance of rK39 serology in India and East Africa, and the development of serology to discriminate between the clinical status of VL patients. Firstly, it was demonstrated that there was substantial regional genetic diversity in the antigens that comprised the rK39 and modified rK28 diagnostic tests. Secondly, it was shown that anti-Leishmania antibody titres in VL patients in India were significantly higher than in Sudan. Thirdly, anti-Leishmania subclass profiling showed that an IgG1 ELISA assay could discriminate between clinical groups. Elevated IgG1 antibody levels had the capacity to detect relapse in treated patients (6 months after treatment) and to predict progression from asymptomatic to active VL. Three key publications (Bhattacharyya et al.) describe this important research progress. Simple discriminative diagnostic algorithms have been defined (in preparation).

The IgG1 assays provided the basis for development of corresponding prototype RDTs, the VL Sero K-Set, by Coris. The first two prototypes performed well, prototype 2 with estimated sensitivity of 96.4% and specificity 87.2%. These tests were carried out during visits to Varanasi, and also to the BP Koirala Institute of Health Sciences, Dharan, Nepal. Prototypes were stable during at least 12 months at 30°C and 3 months at 45°C. However, manufacturing complexity prevented large scale production. Accordingly, Coris developed a new prototype (prototype 3) with a much more robust strip design; instructions for use remain exactly the same but the test is much easier to manufacture due to fewer fibre pads. The prototype was validated in the laboratory, and field evaluation is taking place in Varanasi, India in July-August 2016.

To identify specific antigens to replace lysate in the VL Sero K-Set RDT western blot strips of L. donovani lysate or extracted membrane protein antigens were probed with sera from different VL disease states and candidate antigens were analysed by mass spectrometry.

Differential EHC and VL affinity chromatography and mass spectrometry were applied to search urine for Leishmania antigens suitable for an improved non-invasive capture assay.

The discriminative point-of-care RDT is an outstanding achievement.

**Amoebiasis and other digestive pathogens (related to digestive syndrome)**

The objective for the digestive syndrome was to explore development of stool antigen RDTs.
To improve diagnosis of amoebiasis, Coris explored development of an RDT to distinguish pathogenic *Entamoeba histolytica* from non-pathogenic *Entamoeba dispar*. A strip prototype showed 60 times greater differential sensitivity for cultures of *E. histolytica* vs *E. disparate* but with clinical samples it was not adequately specific for *E. histolytica*. In a similar approach, the potential of 9 different antibodies to *Campylobacter* spp. for development of a *Campylobacter* RDT was assessed: results with pure cultures were very promising but not satisfactory with clinical stool samples.

The performance of the Clostridium K-SeT test of Coris to detect *Clostridium difficile* antigen (glutamate dehydrogenase) in stool, launched on February 1st 2012, was shown to equal or surpass that of competitors. A distinct prototype to detect *C. difficile* toxins (A&B) previously developed by Coris was improved as part of the NIDIAG project but still requires further development.

Effort in the last period of the project has been focused on a comparative genomics pathway to identify *S. stercoralis*-specific proteins for development of a faecal antigen detection test, and this will continue as an international collaboration beyond the life of the project.

### Human African trypanosomiasis (related to neurological syndrome)

Two HAT RDT formats, using native antigens, were developed (HAT Sero-K-SeT and HAT Sero-Strip) in collaboration with the Belgium Institute of Tropical Medicine (ITM). These two prototypes were very positively evaluated in a phase I study, as published (Büscher P. et al., 2013. New England Journal of Medicine 368:1069).

In June 2012, with ethical approvals, a phase 2 study was initiated by Coris and ITM in Masi Manimba, Bandundu Province, D.R. Congo to evaluate the HAT Sero-K-SeT under field conditions in a case-control study (Table 2). Additional financial support was obtained from WHO (10,000 USD) for deployment activities in the field.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (n=134)</th>
<th>Specificity (n=356)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATT whole blood</td>
<td>0.955 (95% CI: 0.906-0.979)</td>
<td>0.972 (95% CI: 0.949-0.985)</td>
</tr>
<tr>
<td>HAT Sero-K-SeT</td>
<td><strong>0.985 (95% CI: 0.947-0.996)</strong></td>
<td><strong>0.986 (95% CI: 0.968-0.994)</strong></td>
</tr>
<tr>
<td>Immune trypanolysis</td>
<td>0.985 (95% CI: 0.947-0.996)</td>
<td>0.980 (95% CI: 0.960-0.990)</td>
</tr>
</tbody>
</table>

From this phase II study, we conclude that the diagnostic performance of HAT Sero-K-SeT is adequate for *Trypanosoma brucei gambiense* antibody detection in health centres and for active screening whenever a cold chain and electricity are lacking. The final results have also been published (Büscher P. et al. 2014. The Lancet Global Health 2:e359-63). A real-time long-term stability study has shown that HAT Sero K-SeT remains stable for 25 months at 4°C, 30°C and 40°C and for at least 9 months storage at 45°C.
As the production cost of the HAT Sero-K-SeT is considered too high (>1€/unit) for large-scale deployment in central Africa, Coris worked to reduce the cost of antigen and other components. Decreasing quantity of native antigens or replacing native antigens with peptides reduced performance. Replacing native antigens with recombinant VSG antigen in E. coli and the development of a dipstick test (recHAT Sero-Strip) available in bulk (<10kg and <50L for 3000 tests) reduced cost, and laboratory evaluation showed sustained good performance (sensitivity 97% and specificity 96%), to be confirmed by evaluation at an endemic field site. In real-time long-term stability trials the recHAT Sero-Strip was stable for 3 months at 37°C and 40°C, for at least 9 months at 4°C and 30°C (ongoing) and for 2 months storage at 45°C conditions (ongoing).

Production and deployment of two versions of a new RDT for HAT has been an outstanding success.

Aim 2 (cnt). Evaluation of new diagnostics tools and set up of reference laboratory

The specific objectives were (i) the conduct of phase-II laboratory evaluations of new diagnostic tools developed both internally and externally, (ii) the constitution of a panel of reference samples for NIDs to facilitate diagnostic test development, (iii) to provide to partners involved in the development on Diagnostic guidance tools state-of-the-art laboratory diagnostic tools to achieve an exhaustive differential diagnosis for the three clinical syndromes, and (iv) to strengthen human and institutional capacity for laboratory NID diagnosis in endemic countries.

The first objective was largely achieved by the development of 4 prototypes of new diagnostic platforms: one for human African trypanosomiasis (HAT), two for visceral leishmaniasis and one for Strongyloides stercoralis. The HAT sero-K-set passed phase-II evaluation and it was concluded that the test performance was adequate for Trypanosoma brucei gambiense antibody detection for diagnostic purposes and for active screening, results have been published. The other prototypes will need more fine-tuning before being able to pass phase-II evaluation, and adaptations are on-going and will be continued after the NIDIAG period.

The second objective, the installation of biobanks on site and in coordination sites in Europe, represents a major achievement. All study sites installed temporary and/or long-term biobanks for the NIDIAG samples and gained experience in the management of biobanks and shipment of biological samples. The well characterized NIDIAG samples are very valuable for current and future research (e.g. diagnostic test validation) and the valorization of these biobanks will remain a very important activity in the future.

The third objective, the reference diagnostics for the three syndromes, proved to be a challenging activity (high sample numbers to be matched with staff availability and time-consuming reference tests). The workload for this activity was high and some testing will be finalized only after the NIDIAG term has finished. Nevertheless, the reference diagnostic testing was an indispensable component of the NIDIAG work since it contributed to the final case ascertainment for the development of diagnostic guidance tools. A delay for this objective however also delayed the differential diagnosis for the three clinical syndromes.

The fourth objective, the laboratory capacity strengthening, was a main goal of the NIDIAG study. Due to the implementation of the clinical studies in the study sites on-the-job trainings, GCP/GCLP
and technical trainings have been carried out, next to the regular monitoring visits took place (See section Quality Insurance system). Also the availability of new equipment has improved the level of the laboratories involved. In addition, a 6 week training in PCR techniques of a Malian biologist involved in the NIDIAG study was done in Homburg and facilitated by Swiss TPH, resulting in the installation of PCR techniques in the laboratory of the Institute of Public Health (INRSP) in Mali. Lastly, the organization of External Quality Assessments sessions for diagnostic bacteriology by ITM proved to be an important way of self-assessment for the participating laboratories.

**Aim 3. Economic Evaluation of diagnostic treatment strategies**

Extensive costing studies were carried out in three NIDIAG study sites, each for a separate syndrome: D.R. Congo for the neurological syndrome, Nepal for the persistent fever syndrome and Mali for the digestive syndrome. A consistent methodology was used across study sites based on a combination of top-down and micro-costing techniques to make the most optimal use of available data in study settings. Thirty different laboratory tests used in the NIDIAG research consortium across the three sites were costed with unit costs ranging from US$0.3 for a urine dipstick test (for urinary tract infection) to US$4.7 for blood culture (for salmonellae/all bacteraemia).

The unit cost data were subsequently used as inputs for a number of ongoing cost-effectiveness studies. In Mali, a cost and cost-effectiveness study comparing the duplicate Kato-Katz thick smear and the POC-CCA test for the diagnosis of *Schistosoma mansoni* (*S. mansoni*) among patients presenting with persistent diarrhoea and/or abdominal pain was carried out at a peripheral health care centre in Mali. The Kato-Katz thick smear technique is currently the most widely used method in Africa for the diagnosis of intestinal schistosomiasis due to *S. mansoni*. The technique is highly specific but its sensitivity may vary with prevalence and intensity of infection. A possible alternative to the Kato-Katz is a commercially available point-of-care assay to detect circulating cathodic antigen (POC-CCA). The POC-CCA test has been shown to be more or equally sensitive than multiple Kato-Katz smears from one stool, though less specific. The POC-CCA gives results within 20 minutes and is based on urine, which is easier and more rapid to collect than a stool sample. While the POC-CCA has been available for many years, its use in epidemiological surveys and clinical settings is still limited probably due to the cost of the test cassette. We found that the POC-CCA was about 34% more expensive than the duplicate Kato-Katz (US$3.3 and US$2.5 respectively) primarily due to the cost of the POC-CCA test cassette and the cost of transportation from the manufacturer to Mali. The incremental cost-effectiveness ratio (i.e. incremental cost per additional case detected) was sensitive to changes in the salary of the laboratory technician performing the tests and the price of the POC-CCA test cassette. If the price of the POC-CCA test cassette were lower than US$ 1.35 (keeping all other variables constant), the POC-CCA would become more cost-effective.

A second study was developed to evaluate the cost-effectiveness of the use of a newly developed lateral flow assay test which has been developed as part of the NIDIAG work output. The IgG1 test has shown promising very early results in differentiating between patients with a history of VL who progress to relapsing with disease. However, very limited data for the sensitivity and specificity of existing parasitological techniques for the diagnosis of relapse currently exist. As such, we conducted a Delphi survey to develop a basis for evaluation of feasibility and effectiveness and of existing
techniques. Delphi methodology takes the form of a structured communication technique which relies on a panel of experts answering a set of questions on the need for parasitological confirmation at the district hospital level, and establishing based on decades of experience estimates of sensitivity and specificity of different tests and treatment strategies. All costs, contextual representation and sensitivity/specificity estimates were based on the Indian situation.

A number of interesting observations emerged from the Delphi survey. It became clear that there is an urgent need for a safe, effective and easy to use diagnostic tool in the use of management of relapse cases of VL. Although splenic aspiration at the district hospital was widely accepted of critical need, availability of surgical intervention and blood bank were also considered to be crucial. This therefore would exclude the vast majority of treatment sites in the South Asian setting. If IgG1 testing for diagnosis of VL relapse proves to be of high sensitivity and specificity, it is likely to have an important impact on the management of refractory VL. Once full results are gathered, the output of this economic exercise will demonstrate the cost and comparative cost-effectiveness of the different techniques of diagnosing patients relapsing with VL though the use of a decision tree modelled within this work package. The decision tree compares presumptive treatment, invasive parasitological diagnosis (spleen, bone marrow and lymph) with the IgG1 RDT, with cost per patient correctly diagnosed and ICERs to be expressed as cost per death averted. We will then finally apply probabilistic sensitivity analysis to assess the robustness of the cost-effectiveness results.

For the neurological syndrome, in order to understand current practice, two prospective studies were conducted to better understand analytical decision making by clinicians. The first study comprised a prospective non-participatory observational study of patient management through the health care system at a selected district hospital. The second study was an evaluation of clinician knowledge, practice and understanding of seven different common pathologies that present with neurological syndrome (all but 1 being treatable infectious diseases) through the use of clinical vignettes. A total of 20 observations were conducted. During the observations, none of the clinicians used any diagnostic guideline with much variation between patients in the clinical examination. For 60% of cases, the provisional diagnosis was confirmed by diagnostic tests, while for the remainder no final diagnosis was made. The vignettes were administered in 3 district hospital in Bandundu province (D.R. Congo). A total of 157 vignettes were completed, which showed that the general quality of care for patients presenting with neurological syndrome was low in all three centres. Overall scoring by pathology and hospital ranged from between 18% to 39%, with clinicians struggling with the diagnosis of HAT and cryptococcal meningitis, while were more comfortable with bacterial meningitis and cerebral malaria. Results were consistent across all three centres; two of these have subsequently had the guideline panorama introduced, while the third remains a control setting. Once repeated following 6 months of the panorama being available and used, the vignettes will be repeated, and utility scores applied to the difference.

For the fever syndrome, a patient survey was conducted in Sudan to examine the health seeking behaviour and costs of illness of visceral leishmaniasis (VL). VL was chosen as a tracer condition given the public health importance of the disease in Sudan, and the very limited knowledge on the socio-economic aspects of the disease. We found that over 75% of households incurred catastrophic out-of-pocket expenditures (defined as expenditures exceeding 10% of annual median income) when considering only direct costs, whereas 83% of households exceeded this threshold when also
including indirect costs. In Nepal, a retrospective analysis of patient files at the BPKIHS who had been admitted for fever syndrome showed that the mean in-hospital cost of diagnosis and management of pathologies within persistent fever (excluding drug cost) ranged from US$30.26 for malaria to US$66.44 for tuberculosis. Once the guideline is introduced, the observations can be repeated, with any change attributable to the tool being introduced.

The NIDIAG Quality Assurance System

The main objectives were to provide overall scientific and technical oversight to the three clinical studies carried out, i.e. on neurological, febrile and digestive syndromes, and to implement a quality assurance (QA) system guaranteeing that they were conducted in line with the appropriate ethical standards as defined in the Declaration of Helsinki (World Medical Association, 2013), with the requirements of the ICH Good Clinical Practices (GCP) (1996), and with the ethical and regulatory requirements in the study countries. The full list of applicable guidelines and regulations can be found in the NIDIAG Ethical Charter.

Compliance with appropriate ethical, GCP and regulatory standards provide public accountability that the rights and wellbeing of research participants and their communities are protected, and that the research results are accurate and reliable and - will appropriately inform diagnostic and treatment policies. Nonetheless, full compliance with the GCP requirements may be challenging in low- and middle-income countries (LMIC), due to a mix of poor regulatory oversight, special vulnerability of research subjects and structural lack of resources, e.g. difficult transports, poor internet connection, electricity shortages, lack of research-friendly laboratory infrastructure, lack of trained personnel, lack of on-site quality suppliers etc. Therefore, a special effort was needed to address and overcome such challenges, by means of tailored procedures and with a special investment on ongoing training/retraining for all the study staff.

During the study period, the following results were achieved:

- **Scientific Advisory board** This board consisted of three distinguished scientists with expertise in tropical diseases and clinical trials. They were Dr. Zeno Bisoffi, Verona, Italy, Professor Simon Croft, London School of Hygiene & Tropical Medicine, United Kingdom and Dr. Steven Reed, Infectious Diseases Research Institute, Seattle, USA. The board members provided scientific and technical advice for the three trials starting with giving feedback on the trial protocols. They also attended NIDIAG meetings and workshops where possible to provide continuing guidance on the studies. Special subject experts were also consulted for their guidance. Where necessary, subject matter experts (Dr. Antoine Hadengue and Dr. Jaques Pepin) were also consulted for advice on specific topics on an ad hoc basis.

- **Ethical board** The Ethics Review Board consisted of Dr. Francis Crawley from Good Clinical Practice Alliance, Europe and Dr. Fazia Osman from Sudan. An Ethical Charter was developed for the implementation of NIDAG. The NIDIAG Governing Council endorsed the Charter which was then also adopted by each of NIDAG country investigators and institutions. The main issues referred to the Ethics Board concerned the obligation to provide treatment to study subjects at each site, insurance for NIDIAG staff and adolescent assent/parental consent for
testing for HIV. While Dr. Osman addressed these issues for NIDIAG, Dr. Crawley was not able
to find time to respond. Since Professor Peeling was Chair of the WHO Ethics Review
Committee, she took over the responsibility from Dr. Crawley for the remainder of the
project.

- **Standard Operating Procedures (SOPs)** A complex set of SOPs was developed, to ensure that
clinical, laboratory and GCP activities were carried out accurately and consistently across
sites and countries. The full list of developed SOPs has been provided to the EC. They were
written in English and/or French for the febrile and digestive syndromes and in French for the
neurological syndrome.

- **External monitoring** All clinical sites have been monitored according to the ICH-GCP criteria,
e.g. they have been regularly supervised by external qualified experts (‘clinical monitors’) who
checked compliance of the research with the protocol, ethical guidelines, study SOPs and
GCP, and reported their findings in formal reports submitted to the sponsor of the
studies. Such visits were carried out either by members of the Quality Insurance system core
team (Raffaella Ravinetto, Ninon Horié, Céline Schurmans, Sören L. Becker), or by external
experts selected by the core team and reporting to them. To ensure ongoing Quality Control
(QC) in-between external visits, one or two individuals per site were trained as ‘quality
manager’. They provided and documented further ongoing verification of the compliance of
the research with the protocol, ethical guidelines, study SOPs and GCP (in the study site in
the Democratic Republic of the Congo, a ‘quality manager’ could not be identified, but
internal verifications were carried out by the local principal investigator and lab coordinator).

- **Lab supervision** Even if *ad hoc* laboratory supervision is not explicitly requested by the ICH-
GCP, a major challenge for research in LMIC is represented by the need to upgrade the local
laboratories, making them shift from a ‘routine mode’ to a ‘research mode’, which requires
much more in terms of internal and external QC, staff training, documentation of source
data, long term storage of data, etc. Therefore, lab training and supervision from external
experts were provided for all the sites, with different schedules depending on the sites’
specific features and needs. Such visits were carried out either by members of the Quality
Insurance system core team (Barbara Barbé, Basudha Khanal), or by external experts
selected by the core team and reporting to them.

- **Training** Clinical and laboratory staff in LMIC, and especially in remote locations where
neglected tropical diseases are highly prevalent, are usually not familiar with research rules
and requirements. Therefore, a significant training effort was achieved. All staff at clinical
sites, including investigators, nurses, lab personnel, data entry clerks, data managers etc.,
were trained in research ethics and GCP and Good Clinical Laboratory Practice (GCLP) at the
beginning of the trial, and then retrained on an ongoing basis according to specific needs.
Most training was carried out by the external monitors and by the lab supervisors during
their scheduled pre-study visits and monitoring visits, and documented in such reports. For
some specific activities, e.g. data management, *ad hoc* meetings or small workshops were
organized with the concerned staff, separately for each of the three clinical studies.

**Clinical data management** Harry van Loen (ITM) organized and supervised the data
management for the clinical studies in close collaboration with the relevant teams. Study-
specific paper case report forms (CRFs) and databases were designed for each of the 3
clinical studies, with the support of Paritosh Malaviya. Extensive testing or validation of the
databases took place before the start of data entry at the sites. To ensure data quality, a
system was put in place of data cleaning, resolving of queries and lastly database lock.
Capacity building activities focused on efficient organization, timely communication, good documenting and data management practices.

Translation to Policy

Given the aim of NIDIAG, to make a difference on clinical practice in NID, we attached a lot of importance to activities facilitating the Translation of our research findings into policy. The main goal of NIDIAG was to translate existing and future diagnostic innovation into demonstrable impact on the quality of care for NID-related syndromes in DEC. To achieve this impact, clinical practice and health policy in this domain will need to change, and such change requires an efficient dialogue between scientists and policy makers. Publications in peer-reviewed scientific journals are not sufficient to reach these policy makers, and therefore, we also concentrated on the dialogue with policy makers. The dialogue with policy makers should be two-way, as policy makers and expert clinicians should have substantial input in decisions on best standard of care for local health systems. Both goals of scientific dissemination and policy dialogue are of course closely linked as the dissemination efforts promote an enabling environment in which the evidence generated by NIDIAG and related policy recommendations can be more easily be exploited.

The specific objectives of this work were

1. To make the knowledge gained by NIDIAG accessible to the scientific community and a number of distinct target audiences (e.g. general public, local communities, health workers, doctors, patients, policy makers, international bodies, NGOs, industry).

2. To develop a policy dialogue with DEC health systems allowing for project results to be influencing clinical management policy at primary health care level and contributing to more efficient diagnosis and treatment of NIDs in a diverse range of endemic settings.

The research output of the NIDIAG consortium was disseminated to the scientific community through peer-reviewed journals as the main channel. A specific dissemination plan was developed and monitored by a publication tracking tool developed in EXCEL. Publications in peer reviewed journals were stimulated and yielded an impressive return (see below section on dissemination for more details). To promote and validate the syndromic approach amongst peers, we presented the NIDIAG project at several international scientific meetings. The NIDIAG consortium secured a session slot on the European Congress on Tropical Medicine and International Health (ECTMIH) 2012, Barcelona conference programme. The session aimed to introduce the NIDIAG project, its syndromic approach and its key goals to an international audience of health researchers. Several NIDIAG researchers presented their research plan and an update of their activities.

Engagement with the policy makers was the topic of a separate strategic plan that was co-developed from the first consortium meeting onwards. All NID endemic country partners actively contributed to this task. First they identified the relevant policy makers, and raised awareness amongst them about the problem addressed by NIDIAG and its approach to address this. Stakeholders in this policy dialogue were the local, regional, national and international institutions involved in health policy decision making and implementation. In Mali, for example, we included at the national level: the Ministry of Health, the National Direction of Health, the Division for Disease Prevention and Control and Other Divisions for Health promotion, the Division for Health Information Education and Communication, the Regional Direction of Health, the District Health Centre, the Community Health Centre, international non-governmental organization (NGOs; Helen Keller International, Safe the Children, USAID, a.o.). At the international level we distinguish WHO, international public-private partnerships and partner academic institutions.

Each DEC partner informed key decision makers in the health system from a very early stage onwards
about the general aim of NIDIAG. In this way we raised awareness amongst decision makers and health professionals of regional, district and community level on the need for quality assured diagnosis and treatment for NIDs at primary health care level. Local stakeholders were kept regularly updated on NIDIAGs results and their feedback and input was sought.

The Institute of Public Health from Mali (INRSP) guided this policy work, and the other partners adapted this approach to their specific settings. Over the course of the project each partner implemented these activities on a country-by-country basis, and reported this in the annual reports of the consortium.

In the final year of the project, we presented and discussed the research results with stakeholders.

E.g. in Mali, a meeting was held late 2015 in Niono following the workshop for development of the clinical algorithm in Bamako. The purpose of the meeting was to inform the health workers of Niono District Medical Center about the preliminary results prior to the validation process. Another meeting was held in Niono (end of April 2016) to share the results of the validated algorithm. A policy brief entitled “Diagnosis and treatment of neglected tropical diseases in patients presenting with persistent digestive disorders (≥14 days) in the health district centre of Niono, Mali” has been translated into French and shared.

A number of other policy briefs have been developed and shared with relevant stakeholders in each country as listed below (available on request).

1. Note explicative autorités sanitaires concernant les résultats de l’étude NIDIAG
NIDIAG did also engage at international levels in the policy debate. In 2012 we co-organized at the request of the Commission a workshop in Johannesburg, South Africa in 2012 on research priorities for zoonoses & neglected infectious diseases of Africa. The aim of this stakeholder consultation workshop was to provide a platform for exchange between scientists, health workers, policy makers and funding agencies on the subject of NIDs and zoonoses which are disproportionately affecting Africa. Participants were asked to assist in formulating recommendations to help the European Commission and other international agencies to establish research priorities for these groups of infectious diseases.

4. Potential impact and the main dissemination activities and exploitation of results

Potential impact

NIDIAG project delivered several key results with significant potential impact on the diagnosis and management of the persistent fever, digestive and neurological syndromes, as detailed below:

Key result 1: Development of diagnostic guidance tools with adapted formats, i.e. classical diagnosis-treatment algorithm or panoramic/synoptic approach.

There were virtually no existing tools to guide physicians and other care-givers in the clinical management of persistent abdominal disorders, persistent fever or neurological disorders in low-income tropical countries. Existing diagnostic algorithms were mono-disease specific, for example focusing on the diagnosis of HAT or VL, and ignoring other conditions.

Potential Impact: Improving diagnostic accuracy is of crucial importance not only to offer the most effective treatment to individual patients and therefore optimize outcome, but also as a powerful mean to restrict overuse of antibiotics and antimalarials and therefore to slow down or revert the spread of antimicrobial resistance, a global public health threat.

Related to this, the clinical over-diagnosis of enteric fever by physicians and the limited diagnostic accuracy of typhoid fever RDTs observed in NIDIAG studies and elsewhere highlight the needs for pursuing research for better diagnostic tools for this condition and for properly educating care-givers. Indeed, diagnostic guidance tools such as those developed in this project are not only important at the bed side, but also for improved teaching and training of medical and paramedical professionals in pre-graduate, post-graduate and continuous education settings.
Key result 2: The development of 2 new rapid diagnostic tests (RDTs):

1) An RDT for point-of-care serodiagnosis of VL, with the capacity to detect relapse and to predict progression from asymptomatic to active VL (VL Sero K-seT RDT).

**Potential impact:** Visceral leishmaniasis is a global public health problem, with approximately 400,000 cases per year. Detection of VL relapse following chemotherapy is vital, because without effective follow up or alternative treatment symptomatic VL is almost invariably fatal. The VL Sero K-SeT RDT has shown capacity for life-saving point-of-care detection of relapse after treatment. Furthermore, it has potential to predict progression of asymptomatic infection to active disease, which may enable prompt, preemptive implementation of vector control to prevent spread of infection. The new VL Sero K-SeT RDT (prototype 3) has a robust strip design for large scale lower cost manufacture, and further improvement is likely through the identification of individual antigenic markers.

![IgG1 RDT for distinguishing VL relapse from cure](image)

**Fig.1** Relapsed samples  
Cured samples

Bhattacharyya et al. (2014) *PLoS NTD*

2) An RDT for serodiagnosis of *T. b. gambiense* sleeping sickness patients (HAT Sero K-SeT and rHAT Sero-Strip)

The evidence generated within NIDIAG on the new Rapid Diagnostic Test for Sleeping sickness was shared with the relevant authorities in DRC. A full dossier was put together and submitted. A market authorization was obtained.

**Potential impact:** As a result of NIDIAG the HAT Sero K-SeT is now a marketed product that is being used for sleeping sickness control in central Africa. The test is registered at the Public Health Ministry of the Democratic Republic of the Congo. The second version of the test, which is currently under evaluation (rHAT Sero-Strip) may become much more widely distributed due to its lower cost.
production price, and much reduced weight and volume per test, compared to the HAT Sero K-SeT test. The (rHAT Sero-Strip) should have substantial, life-saving impact on the control and prospective elimination of HAT due to *T. b. gambiense* as a public health and socio-economic problem in Africa.

**Key result 3: Set up of panels of well-characterised biological samples**

**Potential impact:** The well characterized NIDIAG sample panels archived in the biobanks in the partner institutes will be a desirable resource for future research projects, *e.g.* for validation studies of new diagnostic tools. Exploitation of these panels will be submitted to stringent agreements including restricted research goals and a defined ownership of the results. The management of these biobanks will remain an intensive activity for the future.

**Key result 4: On-the-job training of local laboratories in NID diagnostics**

**Potential impact:** Through on-the-job training, individual technical training, monitoring activities and the organization of EQAs, the capacities of the local laboratories and their staff have increased which can only prove to be fruitful for the local communities and can possibly create future research opportunities.

**Key result 5: Costing of diagnostic tests**

**Potential impact:** Given the limited exposure to costing of project collaborators and research staff in the study countries, efforts were made to conduct the study in a participatory manner and build capacity of local staff and partners in health economics research. Many of these diagnostic tests were costed for the first time in this study, a particularly challenging exercise in the rural settings where NIDIAG was based. Additionally, we attempted to develop a ‘triangulation’ methodology of identifying baseline costing of clinician decision making, which also included a novel vignette approach to demonstrate clinician knowledge and practice, which has potential in both future economic and capacity building impact evaluation initiatives.

**Key result 6: Quality assurance package**

Development of a tailored approach to GCP/GCLP implementation in developing countries taking into account the contextual weaknesses (social vulnerability of the communities, structural lack of resources, sometimes poor regulatory oversight etc.), with special focus on training of local staff for transferring research capacities to endemic countries.

This approach includes, among other things, a tailored and ongoing training for all the different cadres of staff involved in research; the implementation of a system of external monitoring for local
labs, which goes beyond the standard GCP requirements; and the implementation of standardised supply policies across the countries and sites (Ravinetto R, Alirol E, Mahendradhata Y et al. Clinical research in neglected tropical diseases: the challenge of implementing Good Clinical (Laboratory) Practices. PloS Negl Trop Dis 2016; in press).

**Potential impact:** The training activities have resulted in the ‘availability’ of highly motivated local teams, who have the capacity to carry out further research in the field on neglected tropical diseases. The clinical data managers from individual sites also belong to an informal platform that allows peer discussion and mutual learning/support\(^1\)—something of great value for a group that is traditionally neglected in research groups. All these acquired technical skills, coupled to the high motivation and positive ‘team spirit’, will allow future research, that could be locally co-led or led, and will allow addressing more specific health needs in these countries.

In other terms, there is now an existing network of clinical research teams able to carry out research in compliance with adequate ethical and GCP/GCLP standards, and that may be quickly deployed for further research. All the protocols and SOPs are being published in open access journals. The availability of all NIDIAG study protocols and SOPs will enable other researchers to conduct similar studies in other regions of the world without having to duplicate efforts, and as diagnostic technologies improve for NTDs, the impact of novel technologies on the management of these syndromes can be measured with the same parameters.

**Dissemination**

**Development, implementation and evaluation of the NIDIAG dissemination plan**

NIDIAG has developed a dissemination plan which has been implemented by all partners throughout the project timeframe. For example, KAMRC in India disseminated its research findings through means of oral presentations and publications in open access journals. Priority targets are physicians and medical staff who face a major challenge in endemic areas as they do not deal with a single disease issue but with patients presenting with a wide spectrum of complaints. ITM organized a specific session on NIDIAG at the second international conference of the African Society of Laboratory Medicine 2014, which took place 30 November - 4 December 2014 at the Cape Town International Convention Centre in Cape Town, South Africa. Partners chaired and/or presented at the session. Of note, the EC has selected and highlighted NIDIAG on the European Year of Development 2015 webpage. The NIDIAG deputy scientific coordinator gave an interview on the project for a special feature about NIDIAG to be published on the DG R&I and the Horizon 2020 websites showcasing investment in Africa. Specific NIDIAG sessions were also organised at the European Congress on Tropical Medicine and International Health in Barcelona (2011).

More recently, several partners have also presented their research findings from Congo, Cambodia, Nepal, Sudan, Mali, and Switzerland through means of oral presentations and poster sessions at the 9th European Congress on Tropical Medicine and International Health from 6-10 September 2015 in

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\(^1\) See also Association for Data Management in the Tropics at [www.admitnetwork.org](http://www.admitnetwork.org)
Basel, Switzerland. NIDIAG also participated in the development of a guide to spotting forgotten diseases as part of EU-Africa cooperation in science, technology and innovation. The document is available in English, French and Portuguese version and can be downloaded from internet. Final consortium meeting of NIDIAG has been held in 14-16 March 2016 in Yogyakarta. This meeting was dedicated to finalize all potential publications that can be share from NIDIAG projects to various stakeholders. The meeting was also integrated in the Annual Scientific Meeting Program commemorating 70th anniversary of the Faculty of Medicine, GMU in a joint symposium where partners have opportunity to showcase their research findings to policy makers, students and tropical diseases researchers from Indonesia.

NIDIAG website and related tools

The NIDIAG website (www.nidiag.org) is fully operational. The full list of scientific publications related to NIDIAG within the current reporting period is made available for publication on the website, and a pdf file is provided in case of Open Access Journals. The Partners INRB and ITM provided video material from the television interviews given about NIDIAG to the RTNC2 channel of the DRC (Radio et Télévision Nationale du Congo) broadcasted in Kinshasa respectively on 25 April 2014 and 19 September 2014. All partners are in the stage of finishing up their research project and many more findings are expected to be published in various format in near future.

Organisation of an international dissemination workshop at the end of the project

The final general meeting of NIDIAG was held on 14-15-16 March 2016 in Yogyakarta, Indonesia at Phoenix Hotel and Faculty of Medicine, Gadjah Mada University. A total of 29 participants from ITM Belgium, UNIGE Switzerland, London School of Tropical Hygiene UK, INRSP Mali, Swiss TPH Switzerland, BPKIHS Nepal, UNKA Sudan, SHCH Cambodia, CORIS Belgium and FM GMU Indonesia
have participated to this meeting. Partner teams were also invited to developed action points by country to trigger further research initiatives. Special guests who represent Indonesian stakeholders (Ministry of Health, Tulehu Hospital, District Health Office of Maluku Tengah) were invited to attend the joint symposium on tropical diseases which was held as part of the Annual Symposium Meeting to commemorate the 70th anniversary of the Faculty of Medicine, GMU. NIDIAG team contributed sessions on syndromic approach to NIDs at primary health care level, NIDIAG Diagnostic developments, lessons from implementing GCP/GCLP and Panel Session on clinical syndrome. The scientific coordinator of NIDIAG presented an overview of NIDIAG project and importance of NTDs control in a press conference attended by journalists from several major Indonesian mass media.

The full list of NIDIAG dissemination and publications can be consulted on the website www.nidiag.org. Below is a summary table.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number Scientific papers</th>
<th>Number of Dissemination activity) (Poster, MSc thesis ; PhD thesis, Oral session</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2012</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2013</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>2014</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>2015</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>2016</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>71</td>
</tr>
</tbody>
</table>

Plans to disseminate results of the project after the end of project.

- Organization of National dissemination meeting in endemic following validation of algorithms
- Implementation of the algorithm for the neurological syndrome in DR Congo
- Implementation of the algorithm for digestive syndrome in Mali
- Validation of clinical guidance on febrile syndrome in endemic countries
- Presentation of results to international scientific communities will be continued
- Maintenance of the NIDIAG network beyond the project’s life cycle
- Specific satellite session to be organized at the ECTMIH congress in Antwerp in 2017

5. Address of the project public website and relevant contact details

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

Please contact Prof. Marleen Boelaert (mboelaert@itg.be) or Dr K.Verdonck, (tverdonck@itg.be) for further queries related to NIDIAG.
6. **ANNEX: List of NIDIAG contributors**

The NIDIAG Consortium is composed of the following investigators from various institutions:

1. for the **Institute of Tropical Medicine, Antwerp, Belgium**: Marleen Boelaert, Barbara Barbé, Emmanuel Bottieau, Christophe Burm, Philippe Büscher, Jozefien Buyze, Stijn Deborggrave, Koen De Winne, Philippe Gillet, David Hendrickx, Arabella Huys, Jan Jacobs, Veerle Lejon, Filip Meheus, Joris Menten, Evelien Paessens, Katja Polman, Raffaella Ravinetto, Stijn Rogé, Céline Schurmans, Achilleas Tsoumanis, Johan Van Griensven, Harry van Loen, Kristien Verdonck, and Cédric Yansouni;

2. for the **Geneva University Hospitals, Geneva, Switzerland**: François Chappuis, Emilie Alirol, and Ninon S. Horié;


4. for the **Swiss Tropical and Public Health Institute, Basel, Switzerland**: Jürg Utzinger, Sören L. Becker, Martin W. Bratschi, Justin K. Chatigre, Jean T. Coulibaly, Jean-Paul Gohou, Mathias Herrmann, Stefanie Knopp, Hanspeter Marti, Eliézer K.N’Goran, Beatrice Nickel, Pierre H.H. Schneeberger, Kigbafori D. Silué, Peter Steinmann, Lutz von Müller, Penelope Vounatsou, Joel A. Yao, Patrick K. Yao, and Peiling Yap;

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8. for the **London School of Hygiene and Tropical Medicine, London, United Kingdom**: Michael Miles, Tapan Bhattacharyya, Sakib Burza, Graham Clark, Andrew Falconar, Tegwen Marlais, Adelaide Michaels, Rosanna Peeling, and Matthew Yeo;
9. for the **Kala-Azar Medical Research Centre, Muzaffarpur, India**: Shyam Sundar, Shahnawaj Alam, Jaya Chakravarty, Poonam Kumari, Madhukar Rai, and Deepak K. Verma;

10. for **Coris BioConcept, Gembloux, Belgium**: Pascal Mertens, Stéphane Degallaix, Laurence Denorme, Quentin Gilleman, Thierry Leclipteux, Thomas Simon, and Caroline Thunissen;

11. for the **Institut National de Recherche en Santé Publique, Bamako, Mali**: Moussa Sacko, Cheik O. Coulibaly, Birama D. Diakité, Mama N. Doumbia, Aly Landouré, Rénion Saye, Mamadou S. Traoré, and Hassan K.M. Fofana;


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