accurate reporting of toxigenic *C. difficile* is essential for improving patient outcomes and minimizing hospital-acquired disease. The PCR-based in-house and commercial methods available now to detect toxigenic *C. difficile* are expensive or require nucleic acid purification. We describe a multiplex real-time loop-mediated isothermal amplification method (LAMP) to detect toxigenic *C. difficile* without DNA purification and to presumptively identify the NAP1 strain directly from diarrheal stools.

Methods & Materials: Five-hundred and eighteen stools submitted for routine PCR testing of *C. difficile* was used. The DNA was extracted by mixing and boiling 100 μl of 1:10 dilution of stools with 100 μl of lysis solution for 15 min. Two microliters of the clear supernatant was used for LAMP reactions. LAMP method amplified and detected *tcdC*, *cdtA*, and λ DNA (IC). Primers used for amplification were designed using Genbank and PrimerExplorer V4 (Eiken Chemical Co., Ltd. Japan). DNA amplification was done at 59 ^{0}C for 60 min using Rotorgene 6500 (Qiagen) and a standard LAMP reaction. Amplification was detected by displacement of a fluorescent probe annealed to the quencher-labeled primer: Detection of Amplification using Reduced Quenching (DARQ). The limit of detection (LOD) was determined by using a *C. difficile* negative stool specimen spiked with known number of colony forming units of *C. difficile* ATCC43255.

Results: Out of 518 specimens 200 tested were positive and 307 were negative for *tcdC* by both methods. There were 11 discrepant specimens that were all negative by LAMP but positive by PCR with high CT values (>35). The test performance characteristics of LAMP method as compared to the PCR was as follows: sensitivity, 95%; specificity, 100%; NPV, 97%; PPV, 100%. The LOD was estimated to be 750 genome equivalents. The presence of both *tcdC* and *cdtA* presumptively identified 65 specimens to have the NAP1 strain by both methods.

Conclusion: The turn-around-time for LAMP-DARQ was 90 min as compared to 3.5 hours for the in-house PCR. The estimated cost per test for LAMP-DARQ was Cdn $4.00 and 60% cheaper than the PCR. The LAMP-DARQ is a cost-effective, sensitive, faster method than PCR to detect toxigenic *C. difficile*.

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Cross-species multiplex microarray for serological detection of flavi-, phlebo- and alphaviruses

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Background: New animal and human diseases continue to emerge across the world, influenced by human and animal population densities, climate and globalization in travel and trade. Arboviruses form a specific group within these (re-)emerging threats and, due to their vector-borne and zoonotic nature, require extensive, complex and expensive surveillance and control schemes. They cause clinical diseases in both humans and animals, ranging from life threatening meningoencephalomyelitis and hemorrhagic fever to rash and crippling arthralgia. Diagnosis is based mostly on serology, as viremia is often short-lived. Further complicating diagnostics is the fact that clinical syndromes and geographical distribution overlap, and antibodies cross-react extensively within virus families in common serological tests. Our objective is to be able to detect, diagnose and monitor clinically significant arboviruses simultaneously in multiple species and with an approach easily adaptable to constantly changing demographics and syndromes. Therefore we develop a novel cross-species protein microarray for profiling of antibodies to six flaviviruses (DENV1-4, WNV, JEV, TBEV, USUV, YFV), three alphaviruses (CHIKV, ONNV, SINV) and one phlebovirus (RVFV).

Methods & Materials: Target antigens were selected and spotted onto nitrocellulose pads using a non-contact array spotter. Serum samples from humans (180), horses (80), sheep (160), chickens (10) and other bird species (15) with virologically and/or serologically confirmed arboviral infections and control sera of non-exposed individuals were incubated in serial 2-fold dilutions followed by incubation with a species specific IgG, IgM or IgY specific Cy5-labeled conjugate. After quantifying signals using a scanarray scanner, data were analyzed in 'R'.

Results: Profiling of antibodies in human patients exposed to flaviviruses and alphaviruses showed highly discriminatory patterns of reactivity with sensitivities and specificities ranging from 87%-100%. Additionally, vaccinated individuals could be distinguished from non-vaccinated individuals. Initial results also showed high sensitivity and specificity of 100% for sheep infected with RVFV and horses infected with JEV, while WNV showed some cross-reactivity with JEV and USUV antigens in horses. Further testing is ongoing to determine the usefulness of this system for multiple bird species.

Conclusion: In conclusion, preliminary results show that this method may provide an easily adaptable high throughput alternative for multiplex detection and monitoring of arboviruses in multiple species.

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Evaluation of *Lactobacillus rahmnosus* for its anti-inflammatory and analgesic properties

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Background: Inflammation is the cells and body tissues response against an injury. Injury can be caused due to any reasons like infections, chemicals and thermal and mechanical. Though, inflammation is body's own defense mechanism but sometimes these complex events and mediators involved in the inflammatory response can stimulate or intensify many reactions which lead to damage to body's tissues. Non-steroidal anti-inflammatory drugs (NSAID's) are mostly used for the treatment of inflammation and other related diseases.However, constant use of NSAID may lead to gastro-intestinal ulcers, bleedingand renal disorders. Immunomodulatory and anti-arthritic activity of *Lactobacillus casei* and *Lactobacillus acidophilus* are well known. Therefore present investigations were carried out to evaluate analgesic and anti-inflammatory activity *L. rahmnosus* in female wistar rats.

Methods & Materials: Diclofenac sodium was used as standard drug for comparison. *L. rahmnosus*, drugs and vehicle were administered orally with feeding cannula. Analgesic activity was evaluated by acetic acid-induced writhing test while anti-inflammatory activity was tested by using carrageenan induced paw edema model.

Results: Results showed that *L. rahmnosus* significantly decreased the paw thickness at t = 24 hours in female wistar rats at $P < 0.05$. Also, it protected the females rats from writhing induced by acetic acid. Protection provided by *L. rahmnosus* was more pronounced in comparison to standard drug diclofenac sodium.

Conclusion: Present study clearly suggests that *L. rahmnosus* suppress the first phase of carrageenan induced paw edema and decreased the acetic acid induced writhings in wistar rats. Thus it can be used as a natural NSAID.

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Comparison of two isothermal amplification methods: Thermophilic helicase dependent amplification (tHDA) and loop mediated isothermal amplification (LAMP) for detection of *Plasmodium falciparum*

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Background: As malaria incidence declines and transmission becomes more heterogeneous, large numbers of samples need to be screened to target intervention measures appropriately. Recently, the World Health Organisation (WHO) launched a global initiative called *T3: Test. Treat. Track*, urging the global malaria community to scale up diagnostic testing, treatment and surveillance for malaria. Current malaria diagnostic methods used in most endemic countries do not rapidly and accurately detect asymptomatic infections which also contribute significantly to transmission. Polymerase chain reaction (PCR) methods accurately diagnose sub-microscopic infections but are not field-deployable. To address this handicap, we report the development, evaluation and comparison of two isothermal amplification method for detection of *Plasmodium falciparum.*

Methods & Materials: Primers were designed form a highly conserved region of approximately 1.5 kb containing genes coding for several ribosomal proteins (including LSU rRNA and SufB), on the complete apicoplast genome sequences of *P. falciparum* from 15 Gambian isolates and 8 laboratory clones, aligned against the PlasmoDB reference sequence (ID: emb|X95275.2|). The detection limit of each method was determined using ten-fold serial dilution of DNA from *P. falciparum* 3D7 clone. Assay sensitivity and specificity will be determined by screening 300 archived DNA samples with varying parasite densities from both laboratory and field isolates, using PCR as the 'gold standard' or reference method.

Results: Preliminary results showed that the detection limit of both tHDA and LAMP were comparable to the reference method, consistently detecting < 2parasites/μl.

Conclusion: Based on the preliminary results, the novel isothermal amplification techniques (LAMP and tHDA) showed comparable detection limits to standard PCR, detecting < 2parasites/μl. Being easily field-adaptable, without need for thermocycling equipment, these assays could facilitate targeted interventions towards malaria control and elimination

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