Lipoarabinomannan point-of-care tests: evaluation with fresh samples needed

Tobias Broger and colleagues[1] reported an evaluation of a urine point-of-care test for tuberculosis targeting lipoarabinomannan. The authors tested frozen specimens retrieved from a biorepository and acknowledged that a limitation of their study was the potential difference in analytical performance between frozen and fresh urine samples.

We investigated the effect of freezing on lipoarabinomannan concentrations of unprocessed patient urine. We measured the concentration of lipoarabinomannan in urine samples (n=15) before and after freezing to –70°C in polyethylene terephthalate tubes using an in-house ELISA. Samples were frozen for a minimum of 1 day before being retested. The mean lipoarabinomannan concentration decreased from 138 pg/mL (SD 99) to 76 pg/mL (SD 77), while the average loss was 50%. These results indicate that fresh samples contain more detectable lipoarabinomannan, which might improve the sensitivity of the described test. The use of fresh samples might be most crucial in patients expected to have low lipoarabinomannan concentrations, such as those with a CD4 count higher than 200 cells per µL.

Depending on the handling of urine before and after freezing, differences in protein content and pH can be dramatic—urine alkalises over time,[2] and protein content can drop after a freeze–thaw.[3] These factors could affect performance of lateral flow tests by eliminating some of the variability between urine samples, leading the assay developer to produce a less robust test, unable to handle the spectrum of fresh urine. This could yield a high rate of false positivity.[4,5]

On the basis of our results and the crucial changes in urine with freezing,[2,3] we conclude that the difference between fresh and frozen urine samples is a non-trivial matter.

We declare no competing interests.

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Vaccine against Middle East respiratory syndrome coronavirus

In *The Lancet Infectious Diseases*, Kayvon Modjarrad and colleagues[1] reported results of the first in-human clinical trial of the GLS-5300 vaccine candidate against Middle East respiratory syndrome (MERS) coronavirus. The vaccine induced both humoral and cellular MERS coronavirus-specific immune responses. These data suggest that GLS-5300 has potential value in protecting humans from MERS coronavirus infections. However, who should be vaccinated?

Epidemiological surveys[2] concluded that camel contacts, healthcare workers, and patient household contacts are high-risk groups. Therefore, they should be the target groups for the vaccine. However, there are many infection cases for which the source of infection could not be identified. The unpredictability of these infections makes it hard to prevent human infections through vaccination for postexposure prophylaxis.

For zoonotic disease prevention and control, one-sided disease prevention (either human or animal) is often inefficient. Severe acute respiratory syndrome (SARS) is a successful precedent for containment of emerging coronaviruses based on elimination of the primary reservoir. Although the pathogens of SARS and MERS are both coronaviruses, two major factors lead to different control results. First, although the number of human SARS cases is greater than for MERS, most were due to a so-called super-spreader strain. By contrast, all clades of MERS coronavirus are shared by camels and humans, indicating that MERS coronavirus can easily spill-over from camels to infect humans.[3] Second, palm civet, the intermediate amplifying host of SARS coronavirus, is an exotic animal that could easily be controlled by banning wild animal trading. However, the intermediate host of MERS coronavirus (dromedary camel) is an important livestock for the Middle East, with key roles in transportation, food, and fabric (wool); thus, it is impossible to eliminate all camels.

To control this disease in camels two possibilities exist: mass slaughtering of infected animals or vaccination. Unfortunately, serological surveys showed a very high prevalence (up to 100%) of MERS coronavirus-neutralising antibodies in dromedary camels.[4] Therefore, it seems impossible to eliminate this disease by mass slaughtering of positive animals. Vaccination of camels seems to be the only choice, but no licensed vaccine for camels is currently available, although several vaccines are in development.[5] Additionally, GLS-5300 has been reported to also be immunogenic in camels.[6] Camels are not used routinely in research and, for most laboratories, it would be hard to attain enough animals and work with them. Moreover, they

are too big to study in most biosafety facilities. These limitations restrict vaccination tests in camels.

It is increasingly recognised that a One Health approach is needed for effective investigation, prevention, and control of emerging zoonotic diseases. In the context of emerging zoonoses, human and veterinary medicines must work together. The eradication of MERS coronavirus in dromedary camels is the primary condition for the control of this disease in the Arabian Peninsula. If the virus continues to circulate in camels, it might attain new mutations that enable human-to-human transmission, resulting in the generation of super-spreader strains. A comprehensive MERS prevention and control effort should focus not only on a human vaccine but also on camel vaccination.

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The phase 1, open-label, single-arm, first-in-human evaluation of the Middle East respiratory syndrome (MERS) coronavirus DNA vaccine by Kayvon Modjarrad and colleagues[1] is an important step forward for achieving one of the WHO R&D Blueprint for MERS aims, which calls for development of two types of human MERS vaccines[2] for long-term protection of people at high exposure risk and for reactive use in outbreak settings. Modjarrad and colleagues' results should be viewed with cautious optimism. Apart from overcoming the operational challenges stated in the accompanying Comment by In-Kyu Yoon and Jerome Kim,[3] advancement of this DNA vaccine to a second phase 1 or 2a trial will need to overcome other operational and logistical challenges and must target those most at risk of succumbing to the disease.

The high mortality and severe disease seen in MERS are positively correlated with age and presence of comorbidities, including chronic liver, kidney, and heart disease, diabetes, and immunosuppressive conditions.[4] Furthermore, host immune responses to MERS coronavirus could contribute to disease severity and outcomes. Thus, vaccine-induced immune responses in populations with these high-risk characteristics could potentially have harmful effects. These barriers were encountered in severe acute respiratory syndrome coronavirus vaccine development over 15 years ago, and might also hold true for MERS coronavirus.[5] Therefore, any MERS coronavirus vaccine must specifically target the most vulnerable populations and assess safety and generation of robust, long-lasting protective immune responses.[6] At week 60, the MERS DNA vaccine induced humoral and cellular responses in only 51 (77%) of 66 participants and 42 (64%) of 66 participants, respectively, and only two (3%) of 66 participants maintained neutralising antibodies until the end of the study. Thus, generation of humoral and cellular immune responses might not equate with long-term protection.

The phase 1 DNA vaccine developed for the US military aptly illustrates that advances in technology, vaccine platforms, clinical trial designs, and bioinformatics, together with serious investment by stakeholders, provide opportunities for rapid vaccine development and evaluation. Countries where MERS is endemic must invest more seriously in both human and camel vaccine development. With the continuing outbreaks of MERS coronavirus 7 years after it was first discovered, effective human vaccines could be the ideal way to prevent spread and evolution of the virus. Logistical issues of the small and sporadic number of new MERS cases at different geographical locations need to be overcome by a more coordinated approach for research, something that needs to be advanced more rapidly than the current pace of research and development.[7] Being a DNA vaccine candidate, the GLS-5300 MERS coronavirus vaccine allowed for rapid design and production and was advanced into the clinic within 9 months of preclinical candidate vaccine selection. The encouraging results of the phase 1 MERS DNA vaccine study[1] should be advanced quickly to include studies with adequate numbers of elderly and comorbid populations, with careful consideration of safety and of the longevity of the protective response, thereby mitigating future outbreaks and alleviating disease burden from the most susceptible populations—elderly people, immunosuppressed people, and health-care workers.