Authors' reply: diagnostic challenges to be considered regarding Zika virus in the context of the presence of the vector *Aedes albopictus* in Europe

G Venturi [1], L Zammarchi [2 3], C Fortuna [1], ME Remoli [1], E Benedetti [1], C Fiorentini [1], M Trotta [3], C Rizzo [4], A Mantella [2], G Rezza [1], A Bartoloni [2 3]

1. Department of Infectious, Parasitic and Immune-Mediate Diseases, Istituto Superiore di Sanità, Rome, Italy
2. Clinica Malattie Infettive, Dipartimento di Medicina Sperimentale e Clinica, Università Degli Studi di Firenze, Florence, Italy
3. SOD Malattie Infettive e Tropicali, Azienda Ospedaliero-Universitaria Caeggi, Florence, Italy
4. National Center for Epidemiology and Health Promotion, Istituto Superiore di Sanità, Rome, Italy.

Correspondence: Alessandro Bartoloni (alessandro.bartoloni@unifi.it)

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To the editor: We do agree, with Dr Rengina Vorou [1], that knowledge gaps in the field of Zika virus (ZIKV) diagnostics urgently need to be addressed. Indeed, the use of molecular tests is limited by the short duration of viraemia; moreover, flavivirus serology is complex, due to extensive cross-reactivity between antibodies triggered by different flavivirus infections or vaccination. More generally, the reliance on the use of molecular and serological diagnostics to rule out or confirm infections requires careful consideration, as the experience of clinicians and diagnostic laboratories is limited by default for emerging diseases. At now, few available tests have been only, marginally, validated, and the laboratory community is in an urgent need for validation and evaluation of serology tests in the field.

However, we would like to make some clarifications on specific points in the letter: the most important one is that it is not accurate to state that we 'concluded that the two patients were confirmed cases of Zika virus infection, on grounds of a positive PRNT': indeed, we also, and most importantly, observed for both patients a sharp increase in the neutralising antibody titre between the first and second serum sample (from 1:10 to ≥ 1:160), which is also considered in general a helpful diagnostic criterion (for example, see the European Centre for Disease Prevention and Control health professional factsheet [2]).

The patients were tested retrospectively: however, we think that we could not detect viral nucleic acids in serum samples because the viraemic phase was already at its end at the time of samples collection (5 days after the onset of symptoms). We have subsequently demonstrated through the use of positive plaque reduction neutralisation test (PRNT) that ZIKV specific neutralising antibodies were already present at that time (even if at a low titre). In our experience with dengue virus (DENV) and chikungunya, neutralising antibody positive serum samples are hardly polymerase chain reaction positive. The main limitation in our study is that urine samples were not collected.

We surely agree that PRNT should include any flavivirus that might be found in a given geographical area where a patient had previously been: however, although some cross-reactivity can still occur, virus neutralisation tests, particularly PRNTs, are considered the most specific serology for flaviviruses, and a 'gold standard' also for the evaluation of different serological tests. Indeed we obtained a 'borderline' result (inhibition of only 50% of plaques with a 1:10 serum dilution) with DENV PRNT in the second sample of both patients, so the criterion of a ratio of Zika to dengue virus PRNT titres less than four was met. However, it must be considered that this criterion can be useful for travellers, but much less for people residing in areas with circulation of several different flaviviruses. As the National Reference Laboratory for Arboviruses, we have often observed PRNT 'borderline' (more rarely positive) results for closely related flaviviruses in cases of confirmed infection with a flavivirus (since we mainly confirm infections among travellers). However, we agree that a more accurate assessment of the degree of cross-reactivity in PRNT between different flaviviruses is needed.

Finally, there are several reasons for ruling out West Nile virus (WNV) infection in our patients: (i) symptoms like conjunctivitis (patient 1), and wrists and fingers oedema (patient 2) [3] are not typical of WNV infection [4]; (ii) no cases of WNV have been diagnosed

in Tuscany in the period 2008–2014 [5]; (iii) there is no evidence about recent active WNV circulation in Thailand [6], even if seropositivity for WNV was also noted in the past [7].

In conclusion, it should be stressed that, in our opinion, at this stage, PRNT increasing titres are sufficiently specific to confirm Zika virus infection in presence of consistent clinical and epidemiological criteria. Of course, caution is needed in the interpretation of laboratory results in the absence of other criteria. It is important to consider the need for more specific tests and appropriate guidelines.

Conflict of interest

None declared.

Authors' contributions

LZ, GR, GV, AB; performed laboratory investigations: AM, CF, MER, EB, CF, GV; revised the manuscript: GR, MT, CR; managed the patients: LZ, MT.

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