Assay optimization for molecular detection of Zika virus

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Objective To examine the diagnostic performance of real-time reverse transcription (RT)-polymerase chain reaction (PCR) assays for Zika virus detection.

Methods We compared seven published real-time RT–PCR assays and two new assays that we have developed. To determine the analytical sensitivity of each assay, we constructed a synthetic universal control ribonucleic acid (uncRNA) containing all of the assays’ target regions on one RNA strand and spiked human blood or urine with known quantities of African or Asian Zika virus strains. Viral loads in 33 samples from Zika virus-infected patients were determined by using one of the new assays.

Findings Oligonucleotides of the published real-time RT–PCR assays, showed up to 10 potential mismatches with the Asian lineage causing the current outbreak, compared with 0 to 4 mismatches for the new assays. The 95% lower detection limit of the seven most sensitive assays ranged from 2.1 to 12.1 uncRNA copies/reaction. Two assays had lower sensitivities of 17.0 and 1373.3 uncRNA copies/reaction and showed a similar sensitivity when using spiked samples. The mean viral loads in samples from Zika virus-infected patients were 5 × 10^4 RNA copies/mL of blood and 2 × 10^4 RNA copies/mL of urine.

Conclusion We provide reagents and updated protocols for Zika virus detection suitable for the current outbreak strains. Some published assays might be unsuitable for Zika virus detection, due to the limited sensitivity and potential incompatibility with some strains. Viral concentrations in the clinical samples were close to the technical detection limit, suggesting that the use of insensitive assays will cause false-negative results.

Introduction

The Zika virus is a mosquito-borne flavivirus with an approximately 11 kilobase ribonucleic acid (RNA) genome.1 The virus usually causes a mild infection in adults, symptoms include fever, arthralgia and rash.2–5 However, severe complications can occur, such as Guillain–Barré syndrome,6 meningoencephalitis,7 hearing loss and uveitis.8,9 In the current Zika virus outbreak, intrauterine infections have been associated with fetal malformations.10–13

Reliable detection of the Zika virus in infected people is key to understanding the epidemiology, the pathogenesis and alternative transmission routes of the virus, such as sexual intercourse and blood transfusions.12 However, in areas where the Zika virus is co-circulating with dengue and chikungunya viruses, physicians cannot reliably diagnose the Zika virus infection by clinical presentation, because the viruses cause similar symptoms. Using serology for Zika virus detection, based on the genetic variability permitted within the Asian lineage of the virus, highlights the genetic variability as a potential limiting factor of the sensitivity of Zika virus real-time RT–PCR-based diagnostics.

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Abstracts in العربية, 中文, Français, Русский and Español at the end of each article.
**Methods**

**Assays**

We included all Zika virus real-time RT–PCR assays published until 1 April 2016. These assays target the membrane (M), envelope (E), nonstructural protein (NS) 1, NS2b, NS3 and NS5 genomic domains. We designed two new assays covering the currently known Zika virus genetic variability in the E and NS1 genomic domains (Table 1). These novel assays showed up to four potential mismatches per assay (Fig. 2) and were designed to avoid mismatches in the most critical 3′-terminal regions of oligonucleotides that affect primer binding the most.

The new NS1 assay was additionally designed to allow cross-detection of the Spondweni virus – the closest relative of the Zika virus – because regions conserved between related virus taxa are expected to have less variation than other genomic regions.

**Controls**

All controls are based on a current Zika virus outbreak strain (GenBank accession number KU321639). As positive controls, we generated five assay-specific quantified in vitro transcripts (IVT) for the respective genomic target regions. Data on analytical sensitivity, including the standardized measure lower limit of detection, are not available for most of the published assays. To enable stoichiometrically exact analyses of the lower limit of detection for all of the assays, we joined all target domains into a quantitative universal control ribonucleic acid (uncRNA) containing all of the assays’ target regions on one RNA strand (Fig. 3; Table 1). All controls can be acquired via the European Virus Archive at the following links: Zika virus IVT I available at: http://www.europen-virus-archive.com/Portal/produit.php?ref=1598&kid_rubrique=9; Zika virus IVT II available at: http://www.european-virus-archive.com/Portal/produit.php?ref=1599&kid_rubrique=9; Zika virus IVT III available at: http://www.european-virus-archive.com/Portal/produit.php?ref=1600&kid_rubrique=9; Zika virus IVT IV available at: http://www.european-virus-archive.com/Portal/produit.php?ref=1601&kid_rubrique=9; Zika virus IVT V available at: http://www.european-virus-archive.com/Portal/produit.php?ref=1602&kid_rubrique=9; and uncRNA 1.0 available at: http://www.european-virus-archive.com/Portal/produit.php?ref=1603&kid_rubrique=9.

The uncRNA was generated as described previously. In brief, the uncRNA was custom designed as a gBlocks® fragment with a T7 promoter sequence (Integrated DNA Technologies, Leuven, Belgium) and in vitro transcribed.

A disadvantage of using a test control with a high concentration of viral RNA (e.g. from cell culture) is the potential for laboratory contamination, potentially causing false-positive test results. In contrast to natural viral RNA, potential cases of laboratory contamination with the uncRNA can be proven by two real-time RT–PCR marker assays we designed specifically to detect the uncRNA (Table 1). These two marker assays contain detection probes that target the overlap of two joined genomic target domains, which do not naturally occur in the Zika virus genome (Fig. 3).

**Quantification and characterization**

We purified viral RNA using the Qia-gen Viral RNA Mini Kit (Qian, Hilden, Germany) or the MagNA Pure 96 Viral NA Small Volume Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Dengue virus RNA quantification and flavivirus typing were done as described previously.

For all of the experiments, except when assessing threshold cycle variation using different reaction conditions and thermocyclers, we quantified Zika virus RNA using the LightCycler® 480 Instrument II (Roche, Basel, Switzerland). Generally, 25 µl reactions were set up with 5 µl of RNA; 12.5 µl of 2 × reaction buffer from the Superscript® III one step RT–PCR system with Platinum® Taq polymerase (Thermo Fisher Scientific, Darmstadt, Germany); 0.4 µl of a 50 mM magnesium sulfate solution (Thermo Fisher Scientific); 1 µg of nonacetylated bovine serum albumin (Roche), 600 nM of each primer and 280 nM of each probe and 1 µl of SuperScript® III RT/Ptimium® Taq mix. Amplification involved 50 °C for 15 minutes, followed by 95 °C for 3 minutes and 45 cycles of 95 °C for 15 seconds, 56 °C for 20 seconds and 72 °C for 15 seconds. For comparison of C₅₀ values using different PCR cyclers and chemistry, we used the Bonn E- and NS1-based assays using either the Superscript III One-Step RT–PCR kit (Thermo Fischer) or the Qiagen® One-Step RT–PCR kit (Qiagen) on a Roche LightCycler® 480 and LightCycler 2.0, a Qiagen Rotor Gene HQ and an Applied Biosystems 7500 thermocycler. Reference conditions refer to the usage of Life Technologies SuperScript III One-Step enzyme mix and a Roche LightCycler® 480 thermocycler as described above.
Probit regression analyses, to determine the lower limit of detection for all real-time RT–PCR assays, were done using SPSS V22 (IBM, Ehningen, Germany) and eight parallel test replicates.

**Clinical specimens**

We obtained clinical specimens from travellers for which routine medical investigation of either Zika or dengue virus had been requested due to compatible clinical symptoms or a travel history to affected countries. The travellers had acquired their infections in Brazil, the Dominican Republic or Suriname during 2015 and 2016.

We spiked a Zika virus-negative human plasma and urine sample with defined quantities of the African Zika virus strain MR766 and an Asian lineage outbreak strain H/PF/2013. Spiked samples were serially diluted and two replicates of each dilution were individually purified using the MagNA Pure Viral NA Small Volume Kit with an input volume of 200 μL and an extraction volume of 100 μL.

**Results**

All assay–specific IVT and the unRNA allowed comparable quantification of Zika virus RNA with a mean twofold deviation of results (maximum deviation: sixfold), suggesting the ability to use these controls to generate comparable results even when different real-time RT–PCR assays are used in different laboratories. The two marker assays showed no detection of Zika virus RNA even upon using RNA from high-titre cell culture isolates (10⁶–10⁸).
Table 1. Oligonucleotides used in Zika virus real-time reverse transcription polymerase chain reaction assays and potential nucleotide mismatches with Zika virus strains

| Assay reference | All Zika virus (5′ to 3′) | Asian lineage (5′ to 3′) | Target genomic domain (base)(a) | No. potential nucleotide mismatches | Forward primer sequence (5′ to 3′) | Reverse primer sequence (5′ to 3′) |
|-----------------|--------------------------|------------------------|--------------------------------|------------------------------------|----------------------------------|----------------------------------|
| Lanciotti M(b)  | AAGTTTGCATGCTCCAAGAAAAT | AAGTTTGCATGCTCCAAGAAAAT | P: 906–922                      | 12                                 | AGTGGTGCTGYAGRGARTGCACAATGC     | CAATAACGGCTGGATCACACTC          |
| Bonn E(c)       | AAGTTTGCATGCTCCAAGAAAAT | AAGTTTGCATGCTCCAAGAAAAT | P: 906–922                      | 12                                 | AGTGGTGCTGYAGRGARTGCACAATGC     | CAATAACGGCTGGATCACACTC          |
| Pyke E(b)       | AAGTTTGCATGCTCCAAGAAAAT | AAGTTTGCATGCTCCAAGAAAAT | P: 906–922                      | 12                                 | AGTGGTGCTGYAGRGARTGCACAATGC     | CAATAACGGCTGGATCACACTC          |
| PAHO NS2b       | AAGTTTGCATGCTCCAAGAAAAT | AAGTTTGCATGCTCCAAGAAAAT | P: 906–922                      | 12                                 | AGTGGTGCTGYAGRGARTGCACAATGC     | CAATAACGGCTGGATCACACTC          |

(a) Nucleotide position according to GenBank® accession number: KX124389. (b) Nucleotide position according to GenBank® accession number: KX124389. (c) Nucleotide position according to GenBank® accession number: KX124389. (d) Locked nucleic acid bases are underlined. (e) Probes are labelled with fluorescein amidite (FAM) at the 5′-end. (f) Marker assay 1 (this study). (g) Marker assay 2 (this study). (h) Marker assay 3 (this study). (i) Marker assay 4 (this study). (j) Marker assay 5 (this study). (k) Marker assay 6 (this study). (l) Marker assay 7 (this study). (m) Marker assay 8 (this study). (n) Marker assay 9 (this study). (o) Marker assay 10 (this study). (p) Marker assay 11 (this study). (q) Marker assay 12 (this study). (r) Marker assay 13 (this study). (s) Marker assay 14 (this study). (t) Marker assay 15 (this study). (u) Marker assay 16 (this study). (v) Marker assay 17 (this study). (w) Marker assay 18 (this study). (x) Marker assay 19 (this study). (y) Marker assay 20 (this study). (z) Marker assay 21 (this study). (aa) Marker assay 22 (this study). (bb) Marker assay 23 (this study). (cc) Marker assay 24 (this study). (dd) Marker assay 25 (this study). (ee) Marker assay 26 (this study). (ff) Marker assay 27 (this study). (gg) Marker assay 28 (this study). (hh) Marker assay 29 (this study). (ii) Marker assay 30 (this study). (jj) Marker assay 31 (this study). (kk) Marker assay 32 (this study). (ll) Marker assay 33 (this study). (mm) Marker assay 34 (this study). (nn) Marker assay 35 (this study). (oo) Marker assay 36 (this study). (pp) Marker assay 37 (this study). (qq) Marker assay 38 (this study). (rr) Marker assay 39 (this study). (ss) Marker assay 40 (this study). (tt) Marker assay 41 (this study). (uu) Marker assay 42 (this study). (vv) Marker assay 43 (this study). (ww) Marker assay 44 (this study). (xx) Marker assay 45 (this study). (yy) Marker assay 46 (this study). (zz) Marker assay 47 (this study). (aa1) Marker assay 48 (this study). (bb1) Marker assay 49 (this study). (cc1) Marker assay 50 (this study). (dd1) Marker assay 51 (this study). (ee1) Marker assay 52 (this study). (ff1) Marker assay 53 (this study). (gg1) Marker assay 54 (this study). (hh1) Marker assay 55 (this study). (ii1) Marker assay 56 (this study). (jj1) Marker assay 57 (this study). (kk1) Marker assay 58 (this study). (ll1) Marker assay 59 (this study). (mm1) Marker assay 60 (this study). (nn1) Marker assay 61 (this study). (oo1) Marker assay 62 (this study). (pp1) Marker assay 63 (this study). (qq1) Marker assay 64 (this study). (rr1) Marker assay 65 (this study). (ss1) Marker assay 66 (this study). (tt1) Marker assay 67 (this study). (uu1) Marker assay 68 (this study). (vv1) Marker assay 69 (this study). (ww1) Marker assay 70 (this study). (xx1) Marker assay 71 (this study). (yy1) Marker assay 72 (this study). (zz1) Marker assay 73 (this study). (aa2) Marker assay 74 (this study). (bb2) Marker assay 75 (this study). (cc2) Marker assay 76 (this study). (dd2) Marker assay 77 (this study). (ee2) Marker assay 78 (this study). (ff2) Marker assay 79 (this study). (gg2) Marker assay 80 (this study). (hh2) Marker assay 81 (this study). (ii2) Marker assay 82 (this study). (jj2) Marker assay 83 (this study). (kk2) Marker assay 84 (this study). (ll2) Marker assay 85 (this study). (mm2) Marker assay 86 (this study). (nn2) Marker assay 87 (this study). (oo2) Marker assay 88 (this study). (pp2) Marker assay 89 (this study). (qq2) Marker assay 90 (this study). (rr2) Marker assay 91 (this study). (ss2) Marker assay 92 (this study). (tt2) Marker assay 93 (this study). (uu2) Marker assay 94 (this study). (vv2) Marker assay 95 (this study). (ww2) Marker assay 96 (this study). (xx2) Marker assay 97 (this study). (yy2) Marker assay 98 (this study). (zz2) Marker assay 99 (this study). (aa3) Marker assay 100 (this study). (bb3) Marker assay 101 (this study). (cc3) Marker assay 102 (this study). (dd3) Marker assay 103 (this study). (ee3) Marker assay 104 (this study). (ff3) Marker assay 105 (this study). (gg3) Marker assay 106 (this study). (hh3) Marker assay 107 (this study). (ii3) Marker assay 108 (this study). (jj3) Marker assay 109 (this study). (kk3) Marker assay 110 (this study). (ll3) Marker assay 111 (this study). (mm3) Marker assay 112 (this study). (nn3) Marker assay 113 (this study). (oo3) Marker assay 114 (this study). (pp3) Marker assay 115 (this study). (qq3) Marker assay 116 (this study). (rr3) Marker assay 117 (this study). (ss3) Marker assay 118 (this study). (tt3) Marker assay 119 (this study). (uu3) Marker assay 120 (this study). (vv3) Marker assay 121 (this study). (ww3) Marker assay 122 (this study). (xx3) Marker assay 123 (this study). (yy3) Marker assay 124 (this study). (zz3) Marker assay 125 (this study). |
Fig. 3. Genomic locations of oligonucleotides and controls used in real-time reverse transcription polymerase chain reaction assays for Zika virus detection

Table 2. Analytical sensitivity of Zika virus real-time reverse transcription polymerase chain reaction assays

E: envelope; M: membrane; NS: nonstructural protein; UTR: untranscribed region.
Notes: Zika virus genomic representation (GenBank® accession no. KU321639), with real-time RT–PCR assays identified below by respective first authors or location and corresponding control in vitro transcripts (IVT) and parts of the universal control ribonucleic acid (uncRNA) identified above. Genomic regions not containing published assays so far, but potentially useful for future assay design due to genomic conservation within the Asian Zika virus lineage were also included in the uncRNA. Lower panel, representation of uncRNA and the marker assays, which can detect potential cases of laboratory contamination. The lower limit of detections of the two marker assay variants (both can be used interchangeably for confirmatory analyses) were comparable to Zika virus-specific assays with 4.3 (95% confidence interval, CI: 2.9–10.9) and 3.3 (95% CI: 2.4–6.5) copies/reaction, respectively. Already published assays are Lanciotti M,[7] Lanciotti E,[8] Pyke E,[9] Pyke NS1,[10] PAHO NS2b,[11] Tappe NS3,[12] and Faye NS5.[13]
this study (24 samples) resulted in mean viral loads of $1 \times 10^4$ RNA copies/mL of blood (range: $1 \times 10^2$–$4 \times 10^5$) and $5 \times 10^3$ RNA copies/mL of urine (range: $4 \times 10^2$–$6 \times 10^4$). The samples were taken during comparable intervals: within 11 days after symptom onset for urine and within 12 days after symptom onset for serum samples. Two of these 41 samples contained viral loads of less than $5 \times 10^2$ RNA copies/mL, leading to an estimated risk of false-negative test results of 5% even when using highly sensitive assays with a technical lower limit of detection of 5 copies/reaction (equivalent to $5 \times 10^2$ copies/mL; Fig. 9 and Table 2). Using an insensitive assay with a lower limit of detection of $1 \times 10^4$ copies/reaction, the proportion of estimated false-negative test results is about 50% (Fig. 10).
Many of the laboratories in countries affected by the current outbreak that are conducting Zika virus testing also have experience in detecting and quantifying dengue virus. To compare the risk of false-negative test results between these two viruses, we quantified 38 clinical samples positive for dengue virus, that had been sent to our laboratories for medical diagnostics previously. The mean viral load of dengue was $5 \times 10^5$ RNA copies/mL (range: $5 \times 10^2$–$5 \times 10^8$; Fig. 11), meaning that viral loads of dengue in blood were generally about 100-fold higher than viral loads of Zika virus ($t$-test, $P = 0.03$). Accordingly, the estimated risk of false-negative results was several-fold lower for dengue than for the Zika virus (Fig. 10 and Fig. 12).

Discussion

This study provides guidance on the choice of method for diagnosing Zika virus infections and a molecular control that enables the comparison of results between different laboratories and studies. The low viral loads presented in this and a previous study suggest a possibility of false-negative test results when using real-time RT–PCR assays in diagnostics. The results presented here suggest that some published real-time RT–PCR assays may be of limited use for clinical diagnostics during the current Zika virus outbreak. One NS3-based assay that was intended for virus typing should not be used for diagnostics because of its low sensitivity.

Some other assays have features that potentially limit their use in the current outbreak, including limited access to specific probe formats (e.g. the Faye NS5-based assay), relatively lower analytical sensitivity and high numbers of potential mismatches with members of the Asian Zika virus lineage. Our novel assays may be more robust against genetic variation, but real-time monitoring of all assays’ oligonucleotide binding regions is required during the current situation. According to our data, the Lanciotti E-, the Pyke E- and NS1-, the Bonn E- and the Bonn NS1-based assays are highly sensitive for the Asian Zika virus lineage and show few mismatches within genomic domains targeted by these assays. With the present knowledge of Zika virus variability, we sug-
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suggest that laboratories can use these five assays for diagnostics during the current outbreak and preferably combine at least two assays to increase clinical sensitivity. 

The low viral loads we detected in urine and blood samples are in agreement with two previous studies reporting quantitative data. In contrast to data from six patients from French Polynesia, we did not observe a significant difference in the viral loads between the urine and blood samples. Hence, our data do not support urine as a generally more suitable clinical specimen to detect the Zika virus. However, since Zika virus RNA seems to remain detectable in urine and semen longer than in blood, we suggest that both blood and urine samples should be used for reliable Zika virus diagnostics. The Zika virus has also been detected in saliva, but we could not evaluate the suitability of saliva samples because we did not have access to such samples.

Although commercial diagnostic real-time RT–PCR reagents for Zika virus detection are available, laboratories in areas affected by the Zika virus outbreak often use non-commercial formulations because of resource constraints. The non-commercial assays are difficult to standardize and compare. The transfer of essential reagents with coordinated implementation of laboratory protocols and practical training for staff can strengthen accurate real-time RT–PCR diagnostics in resource-constrained settings. Among the most essential contributions to technology transfer is the provision of standardized control material that can be shipped internationally without biosafety concerns. Research consortia and public health structures can use these reagents to establish a technical basis for test implementation, as demonstrated for severe acute respiratory syndrome and Middle East respiratory syndrome-coronaviruses. When these viruses emerged, they were novel and new diagnostic tests had to be developed along with the provision of reagents to laboratories. However, for the detection of the Zika virus, where test formulations are already available, assay standardization can only work with the provision of a reference reagent that is universally applicable in

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**Fig. 7. Viral loads of Zika virus in clinical specimens**

![Graph showing viral loads of Zika virus in clinical specimens](image)

RNA: ribonucleic acid.

Note: Viral loads in different types of clinical specimens plotted per day after symptom onset. The Bonn NS1 assay was used for quantification.

**Fig. 8. Viral loads of Zika virus in paired urine and blood samples**

![Graph showing viral loads of Zika virus in paired urine and blood samples](image)

RNA: ribonucleic acid.

Notes: Paired urine and blood samples from three patients taken on the same day within the first 10 days after symptom onset. The Bonn NS1 assay was used for quantification.
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all assays, such as our uncRNA reagent or a multicentre validated natural virus standard. Quantitative comparability between studies will enable relative estimates of the transmission risks associated with blood transfusion and solid organ transplantations as well as the transmission risks from body fluids such as semen or saliva. Quantitative data may also shed light on Zika virus pathogenesis, since a higher viral load may be associated with more severe clinical complications, as shown in other arboviral infections, such as those caused by dengue, chikungunya and Crimean-Congo haemorrhagic fever viruses.36–38

Low viral loads in patients imply a high risk of false-negative test results. Until 7 April 2016, only 3% of 199 922 suspected Zika virus cases could be laboratory-confirmed in the PAHO Region.39 The low number of confirmed cases could be due to the difficulty in processing high numbers of diagnostic requests in resource-constrained settings. A study from Puerto Rico, showed that 30 (19%) of 155 patients with suspected Zika virus disease could be laboratory-confirmed using molecular and serologic tools.40 In addition, a study from Brazil demonstrated that 119 (45%) of 262 patients with suspected Zika virus infection had a positive real-time RT–PCR result.41 The higher proportion of laboratory-confirmed cases in those studies and our data suggest that a considerable proportion of patients with low viral loads may have gone undiagnosed by molecular testing during the current outbreak.

Endemic countries also need highly sensitive molecular Zika virus detection methods to ensure safe blood transfusions. The Zika virus has been detected in 3% of blood donors in previous outbreaks42 and transfusion-associated transmission has been reported in Brazil.43 Our comparison of blood viral loads and real-time RT–PCR sensitivity suggest a risk of false-negative results during pooled and even individual blood donor screening. Such risk has been reported for the West Nile virus, where several people have acquired the virus through blood transfusion or solid organ transplantation, because of false-negative real-time RT–PCR results.44

Notes: Data from Lanciotti et al. were reported in11 and data from the Bonn NS1 assay were generated in this study. Lines in plots show mean viral loads.

Fig. 9. Comparison of viral loads of Zika virus in blood and urine from different studies

Fig. 10. Risk of false-negative Zika virus test results

Notes: Projection of false-negative test results according to different lower limits of detection for Zika virus, according to a 2:1 input versus elution volume (e.g. 140 µL blood eluted in 70 µL) as in11 and assuming a 100% extraction efficacy.
The association of Zika virus infection and fetal malformations demand reliable Zika virus diagnostics for pregnant women. The current sensitivity of real-time RT–PCR assays suggests that molecular testing during pregnancy may preferentially diagnose highly viremic pregnant women. If intrauterine Zika virus infections and the congenital malformations correlate positively with high Zika virus concentrations, the limited test sensitivity might influence estimates of the manifestation index of congenital disease. The low viral loads in many patients suggest a limited capacity for molecular protocols to exclude Zika virus infection in highly affected areas. Hence, cohort studies investigating Zika virus pathogenesis in pregnant women need to do additional serological testing.

In conclusion, our data emphasize the need for highly sensitive assays in molecular Zika virus diagnostics. In addition to an appropriate choice of method, clinical sensitivity can be increased by testing several specimens per patient, by using more than one real-time RT–PCR target, optimizing RNA purification from clinical samples and by combining molecular and serological testing. The unRNA reagent – used as a universal quantitative positive control – can ensure high sensitivity and good comparability of qualitative and quantitative results in diagnostic laboratories and clinical studies.

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Competing interests: None declared.
Optimisation des tests pour la détection moléculaire du virus Zika

Objectif
Étudier la performance diagnostique des tests basés sur l’amplification en chaîne par polymérase (PCR) d’un acide ribonucléique synthétique de contrôle universel (ARNcun) pour détecter le virus Zika.

Méthodes
Nous avons comparé 7 tests publics utilisant la RT-PCR en temps réel et deux nouveaux tests développés par nos soins. Afin de déterminer la sensibilité analytique de chaque test, nous avons conçu un acide ribonucléique synthétique de contrôle universel (ARNcun) contenant toutes les régions ciblées par les tests sur une hélice ARN de quantités connues de souches africaines ou asiatiques du virus Zika. Les charges virales dans 33 échantillons provenant de patients infectés par le virus Zika ont été déterminées à l’aide de l’un des nouveaux tests.

Résultats
Les oligonucléotides des tests publiés utilisant la RT-PCR en temps réel ont présenté jusqu’à 10 mauvais appariements potentiels avec la lignée asiatique provoquant l’épidémie actuelle, alors qu’on a constaté de 0 à 4 mauvais appariements dans les cas des nouveaux tests. Les charges virales dans les échantillons infectés par le virus Zika ont varié de 0 à 4 mauvais appariements dans les tests les plus sensibles.

Conclusion
Nous proposons pour déterminer le virus Zika des réactifs et des protocoles actualisés, adaptés aux souches responsables de la flambée actuelle. Tous les tests publiés ne permettent pas de déterminer le virus Zika en raison d’une sensibilité limitée et d’une incompatibilité potentielle avec certaines souches. Les concentrations virales dans les échantillons cliniques étaient proches de la limite de détection technique, ce qui laisse penser que l’utilisation de tests insensibles donnera des résultats faussement négatifs.

La limite de détection inférieure à 95% des sept tests les plus sensibles variait de 2,1 à 12,1 copies d’ARNcun/reaction. Deux tests présentaient des sensibilités plus basses de 17,0 et 1373,3 copies d’ARNcun/reaction et montraient une sensibilité similaire lorsque des échantillons enrichis pour le virus Zika étaient de 5 × 10^4 copies d’ARNcun/mL de sang et de 2 × 10^4 copies d’ARNcun/mL d’urine.

Conclusion
Nous proposons pour déterminer le virus Zika des réactifs et des protocoles actualisés, adaptés aux souches responsables de la flambée actuelle. Tous les tests publiés ne permettent pas de déterminer le virus Zika en raison d’une sensibilité limitée et d’une incompatibilité potentielle avec certaines souches. Les concentrations virales dans les échantillons cliniques étaient proches de la limite de détection technique, ce qui laisse penser que l’utilisation de tests insensibles donnera des résultats faussement négatifs.
Резюме
Оптимизация молекулярных методов выявления вируса Зика

Цель
Изучить диагностические возможности выявления вируса Зика с помощью полимеразной цепной реакции (ПЦР) с обратной транскриптазой (ОТ) в режиме реального времени.

Методы
Авторы сравнили семь методов ОТ-ПЦР, сведения о которых были опубликованы в литературе, и два новых метода, разработанные авторами. Для выявления аналитической чувствительности каждого метода были сконструированы синтетические универсальные контрольные рибонуклеозидные кислоты (ункРНК), содержащие все целевые участки на одной из цепей РНК.

Результаты
Олигонуклеотиды для опубликованных методов ОТ-ПЦР показали до 10 потенциальных несовпадений при определении вируса азиатского происхождения, вызванного нынешнюю вспышку заболеваемости, тогда как новые методы показали от 0 до 4 несовпадений.

Вывод
Авторы предлагают реактивы и обновленные протоколы для выявления вируса Зика, вызванную нынешнюю вспышку заболевания. Некоторые из методов, по которым имеются опубликованные литературные данные, могут быть неподходящими для текущей ситуации из-за ограниченной чувствительности и потенциальной несовместимости с некоторыми штаммами вируса.

Conclusión
Se proporcionan reactivos y protocolos actualizados para la detección adecuada del virus de Zika en el caso de las cepas de brotes actuales. Algunas pruebas publicadas pueden no ser adecuadas para la detección del virus de Zika, debido a la limitada sensibilidad y a la posible incompatibilidad con algunas cepas. Las concentraciones viricas en las muestras clínicas se acercaron al límite de detección técnico, lo que sugería que el uso de pruebas intensivas causaría resultados falsos negativos.

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