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External quality assessment studies for laboratory performance of molecular and serological diagnosis of Chikungunya virus infection

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ABSTRACT

Background: Since the re-emergence of Chikungunya virus (CHIKV) in Reunion in 2005 and the recent outbreak in the Caribbean islands with an expansion to the Americas the CHIK diagnostic became very important.
Objectives: We evaluate the performance of laboratories regarding molecular and serological diagnostic of CHIK worldwide.
Study design: A panel of 12 samples for molecular and 13 samples for serology were provided to 60 laboratories in 40 countries for evaluating the sensitivity and specificity of molecular and serology testing.
Results: The panel for molecular diagnostic testing was analysed by 56 laboratories returning 60 data sets of results whereas the 56 and 60 data sets were returned for IgG and IgM diagnostic from the participating laboratories. Twenty-three from 60 data sets performed optimal, 7 acceptable and 30 sets of results require improvement. From 50 data sets only one laboratory shows an optimal performance for IgM detection, followed by 9 data sets with acceptable and the rest need for improvement. From 46 IgG serology data sets 20 provide an optimal, 2 an acceptable and 24 require improvement performance. The evaluation of some of the diagnostic performances allows linking the quality of results to the in-house methods or commercial assays used.
Conclusion: The external quality assurance for CHIK diagnostics provides a good overview on the laboratory performance regarding sensitivity and specificity for the molecular and serology diagnostic required for the quick and reliable analysis of suspected CHIK patients. Nearly half of the laboratories have to improve their diagnostic profile to achieve a better performance.

1. Background

Chikungunya virus (CHIKV) is a mosquito-transmitted single stranded, positive-sense RNA *Alphavirus* first isolated in Tanzania in 1953. Sporadic outbreaks were reported from African and Asian countries between 1950s and 2004. The last epidemic was recorded from 2004 to 2011 spreading from Kenya across the south-western Indian Ocean region, India, South East Asia to New Caledonia in the Pacific region [1,2]. In 2013 a first local transmission of CHIKV on Caribbean island Saint Martin was reported and to date CHIKV caused infections in more than 20 countries of the Caribbean, Central and South America and the US in 2014 [3,4,21]. The main reason for increasing emergence of CHIK worldwide is the international travel of viremic persons returning to countries where competent vectors are indigenous. Under this conditions autochthonous CHIKV outbreaks can occur as was seen in Italy and France caused by imported CHIKV from Asia [5,6].

The diagnosis of acute infections is based on the molecular detection of virus genome or by serological test systems detecting specific IgM antibodies [7–14]. Commercial detection systems (ELISA, immunofluorescence assay [IFA] and rapid tests) for detection of immunoglobulins are available and have been evaluated [15–19]. Virus culture and isolation of CHIKV is a very sensitive method but not practicable for routine diagnostic [20]. Since other virus infections occur in the region like Dengue these have to be considered for differential diagnosis. A quick and reliable diagnos-

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tic of a CHIK infection is of high importance for the patient. In a previous study PCR methods showed a good overall performance whereas serological assays were less sensitive and specific [23–25]. An evaluation of the laboratories' performance for CHIK diagnostic seems an efficient procedure to increase the awareness for this recent emergence of CHIK in the Americas and Pacific region

2. Objectives

The sensitivity and specificity of CHIKV nucleic assay testing was evaluated with a panel of 12 samples and serology with 13 samples by diagnostic laboratories worldwide.

3. Study design

3.1. Participants

Fifty-six laboratories from 40 countries worldwide (Europe (23), Middle-East (1), Asia (4), Africa (4), Oceania (3) the Americas (3) and the Caribbean (2)) participated in the free of charge EQAs organized by the European Network for diagnostics of 'Imported' Viral Diseases (ENIVD) (www.enivd.de).

3.2. Molecular diagnostic

12 samples of cell culture supernatants infected with different CHIKV strains were used. The EQA panel represented two out of three circulating CHIKV genotypes worldwide: CHIKV strain H20235 Saint Martin/2013 (Asian genotype), both strain 236 origin Seychelles (2×10^8 genome equivalents/ml) and strain 3162 origin India (1.1×10^8 genome equivalents/ml) represent the East-Central-South-Africa (ESCA) genotype (the genotype of the West African strain was not available). To analyse the sensitivity of molecular diagnostic, CHIKV strain H20235 Saint Martin/2013 was prepared in a tenfold dilution series (1.8×10^8 to 2.2×10^4 copies/ml) in dest. water and lyophilisation reagent (OPS Diagnostics, Lebanon, US). Assay specificity was monitored by related alphaviruses like O'nyong-nyong virus (ONNV) strain Ahero (1.9×10^4 copies/ml), Sindbis virus (SINV) strain Edgar 339 (7.5×10^6 copies/ml) and Dengue virus (DENV-2) strain VR-345 (8.3×10^4 copies/ml). Two samples of human plasma were used for negative controls.

All virus samples were inactivated by heat (56 °C, 1 h) and gamma irradiation 25–30 kgray (Synergy Health Radeberg GmbH, Radeberg, Germany). Samples of 200 μl were coded before freeze drying in glass vials with plugs (SP Industries, US) in a freeze dryer (Epsilon 2-6D, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) monitored by process recording software (CHRIST LPC-32 (LSC) SCADA) and stored at 4 °C in the dark. Sets of freeze-dried samples were pretested by two expert laboratories.

3.3. Serological diagnostic

CHIK sera were either IgM/IgG positive or IgG positive only. For analysing assay sensitivity a serial dilution series (1:2–1:16) with serum CHIK sero IgM/IgG 23366, 23717 in water (samples #3, #11, #7,#4) was done. In order to get sufficient volume CHIK sero IgG/IgM 322014 (#9) and sample #5 were diluted. Samples #1 and #10 represented serum of an acute phase (IgM) and of the convalescence phase (IgG) of the CHIKV infection. To investigate the proficiency of laboratories concerning cross reaction we chose antibodies to West Nile virus (WNV), Ross River virus (RRV) and DENV-2 (samples #6, #12, #8). Two negative human plasma samples #13, #2 were included for control purposes.

All samples of infected patients (CHIKV, RRV) used were taken for routine diagnostic purpose under the national ethical regulation with patient's consent. Dengue and WNV antibody positive sera were obtained from SeraCare (Life Sciences, Milford, MA, USA). After aliquoting, coding and freeze-drying sets of EQA samples were pretested by two expert laboratories. Control panels of samples were stored at 4 °C (>4 month) and 20 °C (4 weeks) and analysed to monitor the sample integrity.

In September 2014 the EQA panels and instructions (reconstitution of samples, reporting form) were distributed by regular post. Laboratories were informed on the anonymous process regarding the distribution of the summary table of returned results. Laboratories providing more than one dataset were listed as (a, b).

3.4. Scoring

For the molecular panel we applied the following scoring: 2 points for correct results, 1 point equivocal or borderline results, 0 point for false-positive or false negative results. In the case that the RT-PCR was false-positive but the associated sequencing result was correct, two points were given. The maximal score was 24 points. Quantitative data were not considered for the scoring procedure. For giving a brief recommendation on the laboratory performance participants were informed of an "optimal" (all results were correct), "acceptable" (one false-negative result), or "need for improvement" (one or more than one false-positive result and/or more than one false negative result) performance.

The scoring and the classification of the serological panel followed the EQA for the molecular diagnostic with a maximum score of 26 points for correct results of 13 samples (Tables 5 and 6). The laboratory performance analysing the sensitivity and, specificity of IgM and IgG antibodies directed against CHIK was analysed separately.

4. Results

From a total of 72 laboratories receiving samples 56 laboratories sent back results (60 data sets) for the CHIKV molecular EQA, corresponding to a response rate of 84.8% compared to 80.8% (42 laboratories) for the serological CHIKV EQA (Table 1). Response rates of European laboratories with 92.9% for the CHIKV PCR EQA and 91.4% for the serology EQA were rather high.

The molecular panel for the detection of CHIKV comprised different aspects of quality standards: sensitivity, specificity, and genotyping of different CHIKV strains. From 60 data sets, 23 data sets (38.3%) were classified as "optimal" with the maximum score of 24. The classification "acceptable" (data sets with only one false-negative result) was reached by seven (11.7%; scores 22–23). All data sets had a false-negative or equivocal result for the highest diluted CHIKV sample. Thirty data sets with scores between 14–22 were classified as "need for improvement" (50%). Some participants reported false-positive or equivocal results for other arboviruses mainly ONNV and DENV-2. Others had one or more false-positive result and/or two and more false-negative results for the sensitivity test. One laboratory (#53) did not detect samples of CHIKV Asian genotype while one laboratory (#55) revealed a problem of contamination in one of two negative samples.

Participating laboratories used conventional in-house RT-PCR/in-house real-time RT-PCR (78.3%) or commercial real-time RT-PCR tests (21.7%) (Table 2). Conventional in-house RT-PCR tests had the highest rate of fully correct results. For the lowest virus load of the dilution series, the conventional Gel-RT-PCR had a low detection rate of 20%. Commercial real-time RT-PCRs had problems with detecting virus loads of 1.3×10^5 copies/ml (77%) and 2.2×10^4 copies/ml (85%). Concerning the specificity of the ana-

Table 1
Overall laboratory performance of molecular CHIKV EQA.

Sample Laboratory/data set N°	#2 CHIK Carib 1,8E + 08	#9 CHIK Carib 2,4E + 07	#4 CHIK Carib 1,8E + 06	#12 CHIK Carib 1,3E + 05	#10 CHIK Carib 2,2E + 04	#6 CHIK India 1,1E + 08	#7 CHIK Seych 2,0E + 08	#1 SINDV 7,5E + 06	#11 ONNV 1,9E + 04	#8 DENV-2 8,3E + 04	#3 neg. plasma n.d.	#5 neg. plasma n.d.	Score	Class
6#, 8#, 10#, 12#, 14#, 26a#, 26b#, 27#, 28b#, 29#, 30#, 31#, 33#, 34#, 37#, 38#, 40#, 41a#, 41b#, 42#, 46#, 49$, 56$	2	2	2	2	2	2	2	2	2	2	2	2	**24**	A
36§	2	2	2	2	1	2	2	2	2	2	2	2	**23**	B
7$, 19#, 25#, 28a§, 45§, 47§	2	2	2	2	0	2	2	2	2	2	2	2	**22**	
1$, 3$, 17$, 23$, 32#, 39$, 48$, 24#	2	2	2	2	2	2	2	2	0	2	2	2	**22**	C
4$, 9#, 18#, 21#, 22#	2	2	2	2	2	2	2	2	2	0	2	2	**22**	C
2$	2	2	2	2	2	2	2	2	0	0	2	2	**20**	C
5#	2	2	2	2	2	2	2	2	1	1	2	2	**22**	C
11#	2	2	2	2	2	2	2	2	0	1	2	2	**21**	C
13#	2	2	2	2	2	2	2	0	0	2	2	2	**20**	C
20#, 35#, 43#, 51$, 52#, 54#	2	2	2	0	0	2	2	2	2	2	2	2	**20**	C
55b#	2	2	0	2	0	2	2	2	2	2	2	2	**20**	C
16#	2	2	2	2	2	2	2	0	0	0	2	2	**18**	C
50#	2	2	0	0	0	2	2	2	2	2	2	2	**18**	C
55a$	2	2	2	0	0	2	2	2	2	2	0	2	**18**	C
15#	2	2	2	0	0	2	2	2	0	0	2	2	**16**	C
44#	2	0	0	0	0	2	2	2	2	2	2	2	**16**	C
53#	0	0	0	0	0	2	2	2	2	2	2	2	**14**	C

Correct positive result = 2 points, correct negative result = 2 points, false positive result = 0 points, false negative result = 0 points, equivocal result = 1 point; detection systems: # in-house PCR (Real time PCR); $ commercial PCR (Real time PCR); § in-house PCR (Gel-PCR); Classification: A = optimal; B = acceptable; C = need for improvement.

Table 2
CHIKV detection with different PCR technologies.

Sample code	Sample identification	Copy N°/ml	All data sets $n = 60$		In-house conventional PCR $n = 5$		In-house real-time PCR $n = 42$		Commercial real-time PCR $n = 13$	
			Correct results		Correct results		Correct results		Correct results	
			n	%	*n*	%	*n*	%	*n*	%
#2	CHIK Caribbean	1.8E + 08	59	98.3	5	100	41	97.6	13	100
#9	CHIK Caribbean	2.4E + 07	58	96.7	5	100	40	95.2	13	100
#4	CHIK Caribbean	1.8E + 06	56	93.3	5	100	38	90.5	13	100
#12	CHIK Caribbean	1.3E + 05	49	81.7	5	100	33	78.6	11	84.6
#10	CHIK Caribbean	2.2E + 04	41	68.3	1	20	30	71.4	10	76.9
#6	CHIK India	1.1E + 08	60	100	5	100	42	100	13	100
#7	CHIK Seychelles	2.0E + 08	60	100	5	100	42	100	13	100
#1	SINDV	7.5E + 06	58	96.7	5	100	40	95.2	13	100
#11	ONNV	1.8E + 04	46	76.7	5	100	35	83.3	6	46.2
#8	DENV-2	8.3E + 04	50	83.3	5	100	34	81.0	11	84.6
#3	Negative (plasma)	–	59	98.3	5	100	42	100	12	92.3
#5	Negative (plasma)	–	60	100	5	100	42	100	13	100

Table 3
Laboratory performance versus in-house PCR methods (published or adapted).

Method	Publication	N° of labs using method	N° of labs with correct results/all results		
			Sensitivity[a]	Specificity[b]	Geno-typing[c]
Real-time PCR	Panning et al. [26]	8	38/40	21/24	24/24
	Laurent et al. [27]	1	5/5	3/3	3/3
	Pastorino et al. [12]	9	42/54	26/27	27/27
	Lanciotti et al. [28]	3	13/15	9/9	9/9
	Lim et al. [29]	1	5/5	1/3	3/3
	Edwards et al. [30]	3	7/15	9/9	8/9
	Pongsiri et al. [31]	1	5/5	3/3	3/3
Conventional (Gel) PCR	Reddy et al. [32]	1	5/5	3/3	3/3
	Hasebe et al. [7]	1	3/5	1/3	3/3
	Sanchez Seco et al. [33]	2	17/20	11/12	12/12

[a] Include samples: #2; #9; #4; #12; #10.
[b] Include samples: #1; #11; #8.
[c] Include samples: #2; #6; #7.

lysed detection systems ONNV was frequently found positive by the commercial assays (#11, 46.2%). SINDV was never detected but 15.4% of the laboratories had positive reactions for DENV-2. In-house real-time RT-PCR systems showed the most heterogenic pattern, 100% correct results were reported for the genotype ESCA and for the negative control sample. False positive results were reported for SINDV, DENV-2 and ONNV between 4.8–19%. False negative results were revealed for all dilutions in the sensitivity test.

42 laboratories referred to in-house qRT-PCR protocols while 30 participants used published or modified RT-PCR protocols (Table 3). The real-time RT-PCRs from Panning et al. and Pastorino et al. were the most widely used methods in this study [12,26]. However laboratories using the same protocol reported heterogeneous results concerning sensitivity and specificity but correct genotyping, which indicated problems associated with laboratory procedures.

Forty-six and 50 data sets were returned for CHIKV IgG and IgM serology respectively (Tables 4 and 5). For the detection of IgM antibodies 52% of the routine diagnosis was done by IFA, 40% of the laboratories used ELISA and only 8% of the results performed an NT or multiplex immunoassay. For the detection of IgG antibodies most laboratories (58.7%) used IFA, 26.1% used ELISA systems and 15.2% of the results were generated by virus neutralisation test [VNT], HIA or multiplex immunoassay (MIA).

The highest score (26, “optimal”) in CHIKV IgM detection was achieved only by one laboratory while nine data sets scored 24 points with the classification “acceptable”. One laboratory achieved 24 points but had one false-positive result for cross reactive antibodies to RRV and thus the attribution “need for improvement”. All other laboratories received lower scores (12–22 points) with the classification “need for improvement”. For IgG serology 20 from 46 data sets show an optimal performance, 2 were acceptable and 24 require improvement regarding sensitivity and specificity (Table 5). The sensitivity of this serological EQA was associated with the assay (in-house and commercial) which was used by the laboratory (Tables 6 and 7,). The mostly used type of technology was the commercial IFA (21) followed by in-house ELISA (11), commercial ELISA (9) and in-house IFA (5), respectively. Other techniques like VNT (3), HIA (2) and multiplex immunoassay (1) were rarely used. IgG antibodies were detected 100% correctly in sample #1 (IgM/IgG) by all assay types whereas the correct detection of CHIKV IgM antibodies was lower: 0–76% in all tests (Table 5).

The most frequently used commercial IFA had problems with the detection of low CHIKV IgM antibody titres while CHIKV IgG was detected correctly in this dilution series. Whereas in-house ELISA tests were more sensitive than the commercial ELISA tests – which had the lowest percentage of correct results for CHIKV IgM and IgG sensitivity – they were less sensitive than IFA and VNT. The VNT had problems to detect IgM/IgG in the dilution of 1:16. The lack of specificity was a minor problem for IgM detection but for the IgG detection false positive results were seen for all tested arboviruses in commercial IFA systems (6) and in-house ELISA IgG (1). The specificity was good, only a few laboratories reported false-positive results for human plasma which were analysed with commercial ELISA tests.

5. Discussion

This study represents the second CHIKV EQA distributed to laboratories worldwide providing a deeper insight in the inter-laboratory performance and the techniques used for the diagnosis of CHIKV infections. In conclusion the data indicate that the lab performance on CHIKV diagnosis was good with room for improvement for some laboratories. The major problem – the lack of sensitivity and specificity – remained as found in the previous EQA (2007) with 31 participating laboratories [23]. The diagnosis of CHIKV infection is influenced by several parameters requiring qualitative test systems with high sensitivity and specificity. So far the diagnostic of an acute CHIKV infection is done by molecular detection of virus genome or by serological IgM detection most often by commercial IFA tests and/or in-house PCR.

It is of utmost importance that laboratories realise limits of the test systems which were revealed in this EQA. While rapid antigen tests may be problematic for the diagnosis of CHIKV infections [17,19], PCR and serological tests such as ELISA and IFA are often used in routine diagnostics. Magurano et al. revealed that different methods for CHIKV diagnosis give heterogeneous results depending on sampling at different times after onset of the disease which reflects the complex antigen-antibody response in patients making high quality diagnosis of CHIKV infections challenging. [34]. In CHIKV infected humans the viral loads in blood range from 1×10^5 to 1×10^9 viral RNA copies/ml in the viremic phase lasting 5–7 days [22]. Laboratories should be aware that a negative CHIKV PCR result in a clinical sample might be a problem of low assay sensitivity caused by a low viral load or improper handling of the sample.

Virus specific IgM antibodies were detectable 3–8 days after onset of symptoms immediately followed by IgG antibodies detectable from the 4th day post onset. Whereas IgG generally persists for years, also IgM may persist in few patients for a longer period. Therefore it is important to analyse the IgM/IgG status early in patient samples to detect acute primary infections [18].

Beside the commonly used ELISA and IFA the VNT seems to be the most specific and sensitive assay. However it requires time-consuming procedures, trained staff working under BSL3 conditions, and does not allow differentiation between IgM and IgG reactivity. Those few laboratories using this in-house assay showed good performance.

The specificity of CHIKV diagnosis remains still challenging. Other arboviruses as well as sera from non-CHIKV arbovirus infections to be considered for differential diagnostic prove to be a major problem for cross-reactivity in the molecular as well as in the serological panel. Although alphaviruses like Ross River virus (RRV) were not included in this EQA laboratories should be aware of a first reported case of transfusion-transmitted RRV infection in Australia 2014 and Mayaro virus infections in a Dengue outbreak in Brazil 2011–2012 [35,36].

Comparing the results of this CHIKV EQA with the previous one from 2007 the detection limit of 2×10^4 copies/ml of CHIKV (Reunion genotype ESCA) found then positive by 16 laboratories (66.6%) corresponds quite well to the highest CHIKV dilution in 2014 (2.2×10^4 copies/ml, Asian genotype) detected by 68.3% of laboratories [25]. The detection of false positive samples was a minor problem in both EQA studies. The specificity of PCR techniques was recognized by the positive finding for the ONNV which was an unexpected but important issue. Since this reactivity was mostly caused by the commercial molecular assay the manufacturer was informed. This will result in an improvement of the assay or an explanation in the assay description. Especially notable is the false positive detection of DENV-2 which was noticed in 2007 and 2014.

One major issue identified in the EQAs in 2007 and 2014 was the lack of sensitivity for IgM, which was consistent [24]. Also in 2007 the most frequently and successfully used technology was IFA (18), followed by ELISA (9), HAI (3) and few VNT and dot blot.

Table 4
Overall laboratory performance on CHIKV IgM detection.

Sample	#3 CHIK sero, IgM/IgG	#11 CHIK sero, IgM/IgG	#7 CHIK sero, IgM/IgG	#4 CHIK sero, IgM/IgG	#9 CHIK sero, IgM/IgG	#10 CHIK sero, IgG	#1 CHIK sero, IgM	#5 CHIK sero, IgG/IgM	#6 RR Virus, IgG/IgM	#12 WN Virus, IgG/IgM	#8 DEN Virus-2, IgG	#13 Neg. human plasma	#2 Neg. human plasma	Score	Class
Laboratory/data set N°	1:2	1:4	1:8	1:16	1:2	1:5	1:4	1:2.5	1:4	1:4	1:4	Undiluted	Undiluted		
29a[#X]	2	2	2	2	2	2	2	2	2	2	2	2	2	26	A
6[⋆], 25[$], 28[#], 35[⋆], 31a[⋆]	2	2	2	0	2	2	2	2	2	2	2	2	2	24	B
11[#], 30b[§]	2	2	2	2	2	0	2	2	2	2	2	2	2	24	B
34a[#X], 34b[#Y]	2	2	2	2	2	2	0	2	2	2	2	2	2	24	B
18a[#]	2	2	2	2	2	2	2	2	0	2	2	2	2	24	C
2[⋆], 4[⋆], 15[⋆], 23[⋆], 32[⋆], 38[⋆]	2	2	0	0	2	2	2	2	2	2	2	2	2	22	C
18b[♦]	2	2	2	1	2	2	2	1	0	2	2	2	2	22	C
31b[§]	2	2	2	0	2	0	2	2	2	2	2	2	2	22	C
42[●]	2	2	0	0	1	2	2	2	2	2	2	2	2	21	C
29b[#Y]	2	0	1	0	2	2	2	2	2	2	2	2	2	21	C
3[⋆], 8[⋆], 22[$], 26[⋆]	2	0	0	0	2	2	2	2	2	2	2	2	2	20	C
20[⋆]	2	2	0	0	2	2	2	0	2	2	2	2	2	20	C
33[#]	2	1	0	0	2	2	2	2	2	2	1	2	2	20	C
40[⋆]	2	2	0	0	2	2	2	0	2	2	2	2	2	20	C
27a[#Y]	2	1	1	0	1	2	1	2	2	2	2	2	2	20	C
30a[⋆]	2	2	2	0	0	2	0	2	2	2	2	2	2	20	C
36[●]	1	2	0	0	2	2	2	0	2	2	2	2	2	19	C
5[●]	2	2	0	0	2	2	0	0	2	2	2	2	2	18	C
7[⋆]	2	2	2	0	0	0	0	2	2	2	2	2	2	18	C
9[⋆]	0	2	0	0	2	2	2	0	2	2	2	2	2	18	C
12[⋆]	2	0	0	0	0	2	2	2	2	2	2	2	2	18	C
13[●]	2	0	0	0	2	2	2	0	2	2	2	2	2	18	C
43[#]	0	0	0	0	2	2	2	2	2	2	2	2	2	18	C
27b[#X]	2	1	0	0	1	2	0	2	2	2	2	2	2	18	C
37a[§]	2	2	2	0	2	0	0	0	2	2	2	2	2	18	C
41[$]	2	0	0	0	2	2	0	1	2	2	2	2	2	17	C
1[⋆], 14[⋆]	2	0	0	0	2	2	0	0	2	2	2	2	2	16	C
21[⋆]	2	0	0	0	2	2	1	0	2	2	2	2	1	16	C
37c[$X]	1	1	0	0	0	2	0	2	2	2	2	2	2	16	C
10[$], 24[$], 37b[$Y]	0	0	0	0	0	2	0	2	2	2	2	2	2	14	C
19[●]	1	0	0	0	1	2	0	0	2	2	2	2	2	14	C
16[$]	1	0	0	0	0	0	0	2	2	2	2	2	2	13	C
17[$]	0	0	0	0	0	2	0	0	2	2	2	2	2	12	C

Correct positive result = 2 points, correct negative result = 2 points, false positive result = 0 points, false negative result = 0 points, equivocal result = 1 point; 1:50 starting dilution of sample, 1:100 starting dilution of sample. Detection system: [#] in-house ELISA; [#X] in-house ELISA (1:50); [#Y] in-house ELISA (1:100); [$] commercial ELISA; [$X] commercial ELISA (1:50); [$Y] commercial ELISA (1:100); [●] in-house IFA; [⋆] commercial IFA; [§] in-house VNT; [♦] in-house MIA; classification: A = optimal; B = acceptable; C = need for improvement

Table 5
Overall laboratory performance on CHIKV IgG detection.

Sample N°	#3 CHIK sero IgM/IgG	#11 CHIK sero IgM/IgG	#7 CHIK sero, IgM/IgG	#4 CHIK sero IgM/IgG	#9 CHIK sero IgM/IgG	#10 CHIK sero, IgG	#1 CHIK sero, IgM/IgG	#5 CHIK sero, IgM/IgG	#6 RR Virus, IgM/IgG	#12 WN Virus, IgM,/IgG	#8 DEN Virus-2, IgG	#13 Neg. human plasma	#2 Neg. human plasma	Score	Class
Data set	1:2	1:4	1:8	1:16	1:2	1:5	1:4	1:2.5	1:4	1:4	1:4	Undiluted	Undiluted		
1⋆, 3★, 4⋆, 6⋆, 9⋆, 11§, 12⋆, 13●, 14⋆, 15⋆, 17⋆, 23★, 32★, 35⋆, 38★, 40⋆, 42●, 30a⋆, 30b§, 31a⋆	2	2	2	2	2	2	2	2	2	2	2	2	2	26	A
18a #, 18b♦	2	2	2	2	2	2	2	1	2	2	2	2	2	25	B
21⋆	2	2	2	2	2	2	2	2	2	2	2	2	1	25	C
5●, 31b§	2	2	2	0	2	2	2	2	2	2	2	2	2	24	C
8⋆, 20⋆	2	2	2	2	2	2	2	2	2	2	0	2	2	24	C
16$	2	2	2	2	1	2	2	1	2	2	2	2	2	24	C
24$, 36●	2	2	2	2	2	2	2	0	2	2	2	2	2	24	C
26⋆	2	2	2	2	2	2	2	2	2	2	2	1	0	23	C
7⋆	2	2	2	2	0	2	2	2	2	2	0	2	2	22	C
19●	2	2	1	0	2	2	2	1	2	2	2	2	2	22	C
27a #Y, 27b #X	2	2	2	2	0	2	2	0	2	2	2	2	2	22	C
37a§	2	2	2	0	2	2	2	0	2	2	2	2	2	22	C
2⋆	2	2	2	2	2	2	2	2	0	0	0	2	2	20	C
41■	2	2	0	0	2	0	2	2	2	2	2	2	2	20	C
25$	2	2	0	0	0	2	2	0	2	2	2	2	2	18	C
28 #	2	2	0	0	2	0	2	2	0	2	2	2	2	18	C
22$	2	0	0	0	0	0	2	0	2	2	2	2	2	14	C
10$, 34■, 37b$Y, 37c$X	0	0	0	0	0	0	2	0	2	2	2	2	2	12	C
43 #	0	0	0	0	0	0	2	0	2	2	2	2	2	10	C

Correct positive result = 2 points, correct negative result = 2 points, false positive result = 0 points, false negative result = 0 points, equivocal result = 1 point; 1:50 starting dilution of sample, 1:100 starting dilution of sample. Detection system: # in-house ELISA; #X in-house ELISA (1:50); $Y in-house ELISA (1:100); $ commercial ELISA; $X commercial ELISA (1:50); $Y commercial ELISA (1:100); ● in-house IFA; ⋆ commercial IFA; § in-house VNT; ♦ in-house MIA; ■ in-house HIA; Classification: A = optimal; B = acceptable; C = need for improvement

Table 6
CHIKV IgM detection with different technology types.

Sample code	Sample identification	Dilution	Total data sets $n = 50$		ELISA				IFA				In-house VNT $n = 3$		In-house MIA $n = 1$	
				In-house $n = 11$	Commercial $n = 9$		In-house $n = 5$		Commercial $n = 21$							
			n	%	n	%	n	%	n	%	n	%	n	%	n	%
#3	CHIK sero, IgM/IgG, 23366, 23717	1:2	40	80.0	10	90.9	3	33.3	3	60.0	20	95.2	3	100.0	1	100.0
#11	CHIK sero, IgM/IgG, 23366, 23717	1:4	28	56.0	6	54.5	1	11.1	3	60.0	14	66.7	3	100.0	1	100.0
#7	CHIK sero, IgM/IgG, 23366, 23717	1:8	16	32.0	6	54.5	1	11.1	0	0.0	5	23.8	3	100.0	1	100.0
#4	CHIK sero, IgM/IgG, 23366, 23717	1:16	6	12.0	5	45.5	0	0.0	0	0.0	0	0.0	1	33.3	0	0.0
#9	CHIK sero, IgG/IgM, 322014	1:2	37	74.0	9	81.8	3	33.3	3	60.0	18	85.7	3	100.0	1	100.0
#10	CHIK sero, IgG, 16459	1:5	44	88.0	10	90.9	8	88.9	5	100.0	20	95.2	0	0.0	1	100.0
#1	CHIK sero, IgG/IgM, 17089	1:4	31	62.0	7	63.6	2	22.2	3	60.0	16	76.2	2	66.7	0	0.0
#5	CHIK sero, IgG/IgM, 322014	1:2.5	36	72.0	11	100.0	7	77.8	1	20.0	15	71.4	2	66.7	0	0.0
#6	Ross River Virus, IgM, IgG	1:4	49	98.0	10	90.9	9	100.0	5	100.0	21	100.0	3	100.0	1	100.0
#12	WNV, IgG, IgM	1:4	50	100.0	11	100.0	9	100.0	5	100.0	21	100.0	3	100.0	1	100.0
#8	DENV-2, IgG	–	49	98.0	10	90.9	9	100.0	5	100.0	21	100.0	3	100.0	1	100.0
#13	Negative human plasma	–	50	100.0	11	100.0	9	100.0	5	100.0	21	100.0	3	100.0	1	100.0
#2	Negative human plasma	–	49	98.0	11	100.0	9	100.0	5	100.0	20	95.2	3	100.0	1	100.0

Table 7
CHIKV IgG detection with different technology types.

Sample code	Sample identification	Dilution	Total data sets $n = 46$		ELISA				IFA				In-house VNT $n = 4$		Other in-house test systems[a] $n = 3$	
					In-house $n = 5$		Commercial $n = 7$		In-house $n = 5$		Commercial $n = 22$					
			n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
#3	CHIK sero, IgM/IgG, 23366, 23717	1:2	41	89.1	4	80.0	4	57.1	5	100.0	22	100.0	4	100.0	2	66.7
#11	CHIK sero, IgM/IgG, 23366, 23717	1:4	40	87.0	4	80.0	3	42.9	5	100.0	22	100.0	4	100.0	2	33.3
#7	CHIK sero, IgM/IgG, 23366, 23717	1:8	36	78.3	3	60.0	2	28.6	4	80.0	22	100.0	4	100.0	1	33.3
#4	CHIK sero, IgM/IgG, 23366, 23717	1:16	33	71.7	3	60.0	2	28.6	3	60.0	22	100.0	2	50.0	1	33.3
#9	CHIK sero, IgG/IgM, 322014	1:2	35	76.1	2	40.0	1	14.3	5	100.0	21	95.5	4	100.0	2	66.7
#10	CHIK sero, IgG, 16459	1:5	38	82.6	3	60.0	3	42.9	5	100.0	22	100.0	4	100.0	1	33.3
#1	CHIK sero, IgG/IgM, 17089	1:4	46	100.0	5	100.0	7	100.0	5	100.0	22	100.0	4	100.0	3	100.0
#5	CHIK sero, IgG/IgM, 322014	1:2.5	30	65.2	1	20.0	0	0.0	3	60.0	22	100.0	3	75.0	1	33.3
#6	Ross River Virus, IgM, IgG	1:4	44	95.7	4	80.0	7	100.0	5	100.0	21	95.5	4	100.0	3	100.0
#12	WNV, IgG, IgM	1:4	45	97.8	5	100.0	7	100.0	5	100.0	21	95.5	4	100.0	3	100.0
#8	DENV-2, IgG	1:4	42	91.3	5	100.0	7	100.0	5	100.0	18	81.8	4	100.0	3	100.0
#13	Negative human plasma	–	45	97.8	5	100.0	7	100.0	5	100.0	21	95.5	4	100.0	3	100.0
#2	Negative human plasma	–	44	95.7	5	100.0	7	100.0	5	100.0	20	90.9	4	100.0	3	100.0

[a] Other test systems included in-house HI test (2x) and in-house multiplex immune assay (1x).

Since the number of samples is limited such EQA studies are not applicable to evaluate commercial- or in-house assays as this requires more specific and negative sera, preferable also from endemic areas. However, for the participating laboratories such EQAs give a clear grading of their laboratory performance in comparison to several other expert laboratories. Problems with low sensitivity and unspecific reactivity became obvious allowing the participating laboratory to improve its methods and/or train the personal. Only the regular performance of EQAs for CHIKV diagnostic will help to evaluate the laboratories' performance according to transparent quality criteria for keeping a high standard. Without these assessments reliable data on confirmed cases of an emerging disease in any outbreak scenario are an uncertain parameter.

Competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2016.01.008.

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