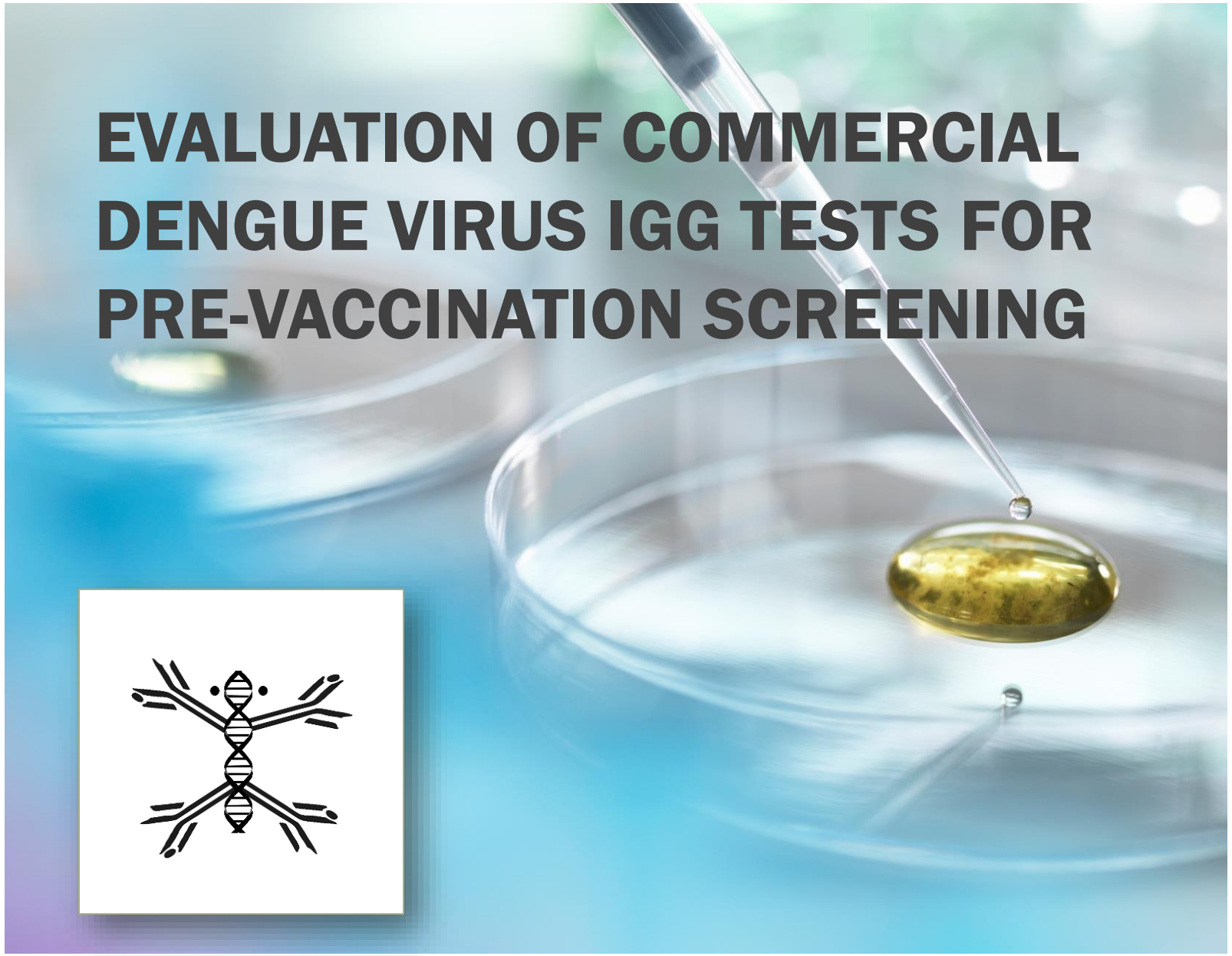
EVALUATION OF COMMERCIAL DENGUE VIRUS IGG TESTS FOR PRE-VACCINATION SCREENING



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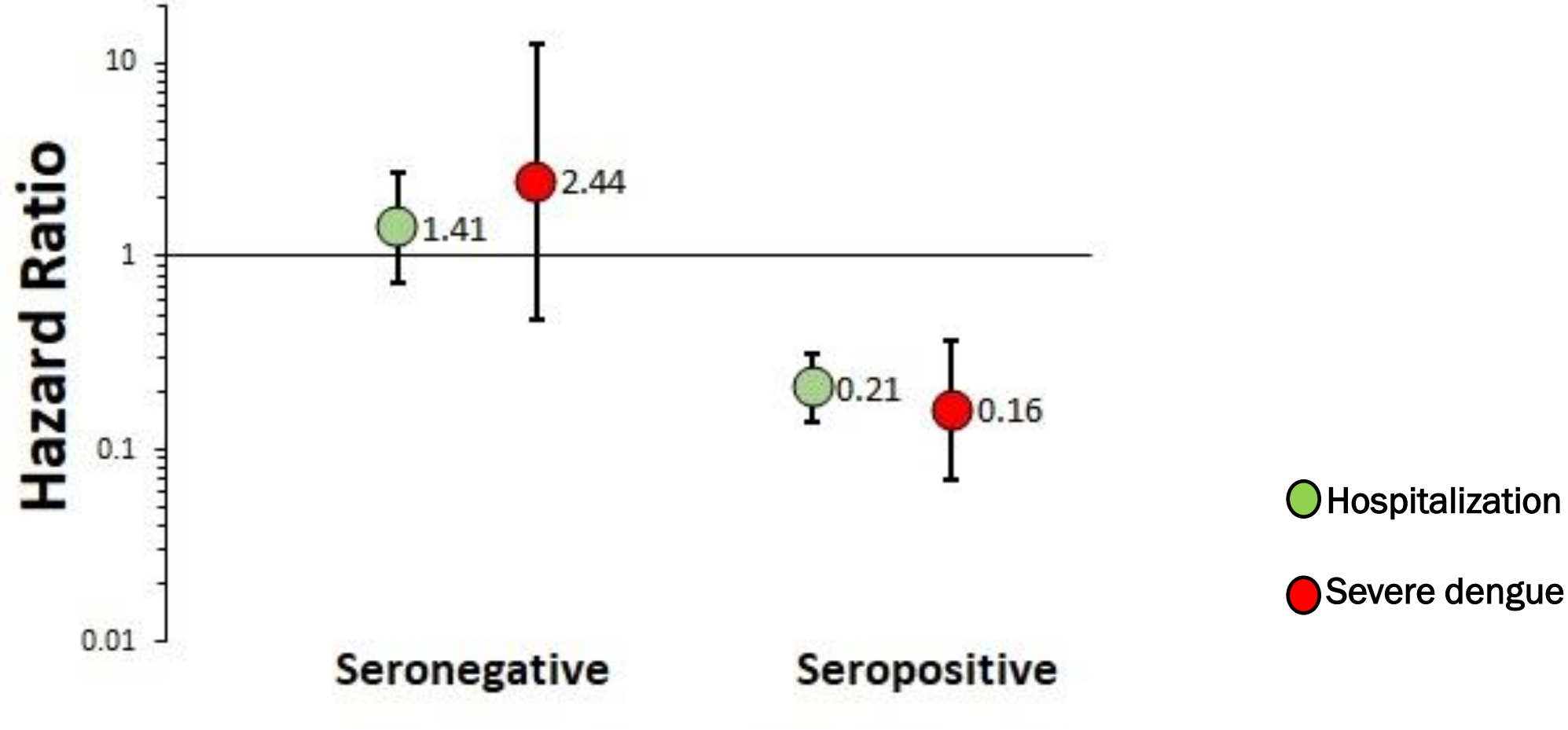
ACIP Meeting
February 24, 2021

DENGUE VACCINE PROTECTS ONLY A SUB-GROUP OF THE POPULATION



Sridhar, S, et al. N Engl J Med. 2018 Jul 26; 379(4):327-340

FDA LICENSING OF FIRST DENGUE VACCINE

sanofi pasteur
323 – DENGVAXIA®

FULL PRESCRIBING INFORMATION

- Dengvaxia is approved for use in individuals 9 through 16 years of age with laboratory-confirmed previous dengue infection and living in endemic areas.
- Previous dengue infection can be assessed through (a) medical records of a previous laboratory-confirmed dengue infection or **(b) serological testing prior to vaccination**.

(May 21, 2019)

HIGH IGG TEST PERFORMANCE IS REQUIRED IN AREAS WITH MODERATE ENDEMICITY

Test performance example (n=1000)

Prevalence at 9 years	Sensitivity	Specificity	PPV	NPV	True Positive Vaccine benefit	False Positive Vaccine risk	True Negative Not vaccinate	False Negative Denied vaccine
50%	70%	98%	97%	77%	350	10	490	150
	80%	98%	98%	83%	400	10	490	100
	90%	98%	98%	91%	450	10	490	50

Rossana Peeling – personal communication

Dengue Pre-vaccination screening workshop;
Les Pensières Center for Global Health, Annecy, France – 2020

DENGUE VIRUS (DENV) IGG TESTING ISSUES

- Current commercial kits were developed for detection of high IgG antibody levels (typically found in recent secondary infections)
- Few studies have evaluated IgG test performance from remote (>1 yr) primary and secondary infections
- Most DENV IgG tests have not been evaluated for cross-reactivity with Zika virus (ZIKV) in endemic areas

Challenge:
RDTs with high sensitivity for low levels of dengue IgG and with high specificity (no cross-reactivity with ZIKV antibodies)

PREVIOUS EVALUATIONS OF DENV IGG TESTS COULD HAVE INTRODUCED BIAS BY EXCLUDING SAMPLES

PRNT confirmed flavivirus samples West Nile (WNV) Virus, Japanese Encephalitis (JEV), Yellow Fever Virus (YFV), ZIKV

DENV NS1 IgG test

Exclusion of DENV IgG positives may result in a reduction of cross-reactive flavivirus specimens

Evaluate seronegative specimens in DENV IgG Immunoassays for cross-reactivity

OBJECTIVE

- Perform an independent evaluation of sensitivity and specificity of selected DENV IgG tests for their potential use in pre-vaccination screening, with the following emphasis:
  - Detection of monotypic DENV infections long after exposure
  - Cross-reactivity of anti-ZIKV antibodies

This study was limited in scope and size and was not intended as a large-scale evaluation

METHODS

- Reviewed manufacturers and peer-reviewed independent evaluations of more than 30 anti-DENV IgG tests made for the detection of recent infections
- Evaluated 7 tests with the best performance using samples from recent DENV or ZIKV infection (7-30 days after symptom onset)
- Further evaluated 5 tests with low ZIKV cross-reactivity and moderate-to-high sensitivity for the detection of remote DENV infections (>1 year after infection)
- Added to the evaluation one newly available rapid test in two versions (rapid test 3a and 3b) made for the specific detection of anti-DENV IgG in remote infections

METHODS

- Evaluated the best performing tests with challenging samples from early convalescence and with high ZIKV IgG and neutralizing antibodies
- Compared evaluations from the CDC and the manufacturer of the new rapid test
- All tests were purchased by CDC without established agreements with manufacturers; sample selection was made independently and confidentially by CDC

COMPOSITION OF INITIAL SAMPLE PANEL FOR REMOTE INFECTIONS

- Unexposed (n=8) – PRNT50 negative (titer≤4) specimens from Puerto Rico and Alaska
- Remote infections (> 1 year after infection)
  - DENV primary (n=13) – PRNT50 neutralization of a single serotype
  - DENV secondary (n=9) – PRNT50 neutralization of two or more serotypes
  - ZIKV primary (n=14)
    - PRNT50 neutralization of ZIKV >80 and no DENV serotypes (n=7)
    - ZIKV RT-PCR positive case, DENV and ZIKV IgG negative (days post onset [DPO] 0-5), specimen collected 3-4 years after infection very limited DENV transmission in between (n=7)

PRNT50=Plaque reduction neutralization test, neutralization titer that reduced virus by 50%

TESTS WITH BEST PERFORMANCE EVALUATED WITH ADDITIONAL SPECIMENS

- The top 3 tests with best performance were evaluated with additional samples:
  - Unexposed – PRNT50 negative (titer≤4) specimens from Puerto Rico (n=21) and Alaska (n=20)
  - Remote ZIKV primary specimens (Nicaragua cohort, n=22) - ZIKV RT-PCR positive case, DENV and ZIKV inhibition ELISA negative specimen collected ~1.5-2.5 years after infection

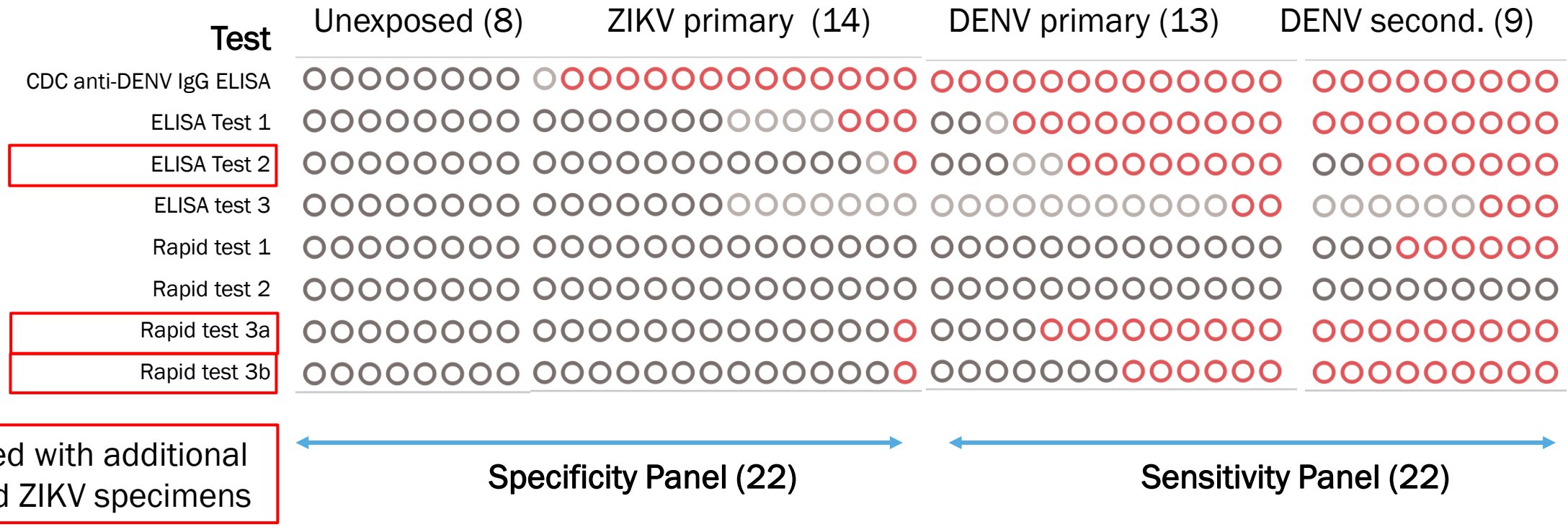
CHALLENGING SPECIMENS USED TO FURTHER EVALUATE BEST PERFORMING TESTS

- ZIKV primary (n=12)
  - ZIKV RT-PCR positive case
  - DENV and ZIKV IgG negative (DPO 0-5)
  - High ZIKV IgG ELISA
  - High ZIKV neutralization titers
  - Specimens collected 3-4 months after infection

Note: These samples are not included in the performance panel for evaluation of specificity

CDC PERFORMANCE EVALUATION RESULTS

VISUAL SUMMARY OF DENV IGG TEST EVALUATION BY IMMUNE STATUS



Dengue IgG Interpretation

- negative/non-reactive
- equivocal/borderline/weakly reactive
- positive/reactive

N=44

PERFORMANCE OF DENV IGG TESTS

Test	Sensitivity % (95% CI) DENV N=22	Specificity % (95% CI) NEG + ZIKV N=22 and 85
CDC DENV IgG ELISA	100 (85, 100)	41 (21, 64)
ELISA test 1	86 (65, 97)	86 (65, 97)
ELISA test 2	68 (45, 86)	97 (90, 99)
ELISA test 3	23 (8, 45)	100 (85, 100)
Rapid test 1	27 (11, 50)	100 (85, 100)
Rapid test 2	0 (0, 15)	100 (85, 100)
Rapid test 3a	82 (60, 95)	98 (92, 100)
Rapid test 3b	68 (45, 86)	98 (92, 100)

ELISA

RDT

Evaluated with additional NEG and ZIKV specimens for a total n=85

CROSS-REACTIVITY OF DENV IGG TESTS WITH ZIKV

Test	n	Cross-reactivity % (95% CI) ZIKV only
CDC DENV IgG ELISA	14	93 (66, 100)
ELISA test 1	14	21 (5, 51)
ELISA test 2	36	8 (2, 22)
ELISA test 3	14	0 (0, 23)
Rapid test 1	14	0 (0, 23)
Rapid test 2	14	0 (0, 23)
Rapid test 3a	36	6 (1, 19)
Rapid test 3b	36	6 (1, 19)

ELISA RDT

Evaluated with additional NEG and ZIKV specimens for total n=36

CHALLENGING SAMPLES FROM EARLY CONVALESCENCE AND WITH HIGH ZIKV IGG AND NEUTRALIZING ANTIBODIES

Test	Cross-reactivity
ELISA test 2	4/12 (33%)
Rapid test 3a	1/12 (8%)
Rapid test 3b	1/12 (8%)

Note: These samples are not included in the specificity panel

COMPARISON OF CDC AND MANUFACTURER EVALUATIONS FOR RAPID TEST 3A AND 3B

CDC AND MANUFACTURER EVALUATION OF RAPID TEST 3A AND 3B

Evaluation	RDT Version	Sensitivity		Specificity	
		n	% (95% CI)	n	% (95% CI)
Manufacturer	Rapid test 3a	233	95 (92, 98)	346	98.0 (96, 99)
CDC	Rapid test 3a	22	82 (60, 95)	85	98 (92, 100)
Manufacturer	Rapid test 3b	233	87 (82, 91)	340	99 (98, 100)
CDC	Rapid test 3b	22	68 (45, 86)	85	98 (92, 100)

Note: Manufacturer evaluation included 35% of samples from monotypic infections and 65% multitypic. The CDC samples were 59% monotypic and 31% multitypic. Manufacturer evaluation of specificity includes only negative samples; CDC evaluation of specificity includes negative and ZIKV specimens.

EVALUATION OF ZIKV CROSS-REACTIVITY OF RAPID TEST 3A AND 3B BY CDC AND MANUFACTURER

Evaluation	RDT Version	ZIKV Cross-reactivity	
		n	% positive (95% CI)
Manufacturer	Rapid test 3a	35	0 (0, 10)
CDC	Rapid test 3a	36	6 (1, 19)
Manufacturer	Rapid test 3b	--	not done[1]
CDC	Rapid test 3b	36	6 (1, 19)

[1] Cross-reactivity will be performed as part of analytical validation to support US FDA filing

EVALUATION OF FLAVIVIRUS CROSS-REACTIVITY OF RAPID TEST 3A BY MANUFACTURER

Minimal to no cross-reactivity observed to related flaviviruses with RDT 3a version

Flavivirus[1]	n	Cross-reactivity, % (no. positive)	
		Rapid test 3a	Rapid test 3b
ZIKV	35	0 (0)	To be performed with consistency lots
Yellow fever virus (YFV)	42	2.4 (1)	
JEV	36	2.8 (1)	
WNV	32	0 (0)	

[1] Flavivirus (FV) cross-reactivity was assessed in DENV reference seronegative samples with prior FV exposure documented by neutralization tests (Zika, YFV, JEV), IgG ELISA (WNV), or known history of prior vaccination (YFV, JEV). Dengue serostatus was determined according to the reference algorithm, except WNV samples which were obtained from US and Israeli residents and only tested negative in dengue NS1 IgG ELISA (i.e., dengue PRNT not done).

LIMITATIONS

- The number of specimens in this evaluations was small, particularly for sensitivity
- Sensitivity of DENV IgG tests may be underestimated due to emphasis in remote primary DENV infections
- High proportion of ZIKV samples included in the specificity panel are greater than the prevalence in the target population
- Cross-reactivity with ZIKV has been addressed in the context of past infections but may need additional testing of early convalescent specimens

CONCLUSIONS

- There are commercial tests currently available that could potentially be used for pre-vaccination screening
- Three anti-DENV IgG tests performed with high specificity (97%-98%) and moderate sensitivity (68%-82%) with low Zika cross-reactivity (6%-8%)
- Half of the commercial tests evaluated performed poorly (sensitivity <30%) for the detection of anti-DENV IgG antibodies long after initial exposure despite their demonstrated use to diagnose recent infections.
- Test sensitivity was higher for multitypic DENV infections than monotypic DENV infections.

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HIGH IGG TEST PERFORMANCE IS REQUIRED IN AREAS WITH MODERATE ENDEMICITY

Test performance example (n=1000)

Prevalence at 9 years	Sensitivity	Specificity	PPV	NPV	True Positive **Vaccine Benefit**	False Positive **Vaccine risk**	True Negative **Not vaccinate**	False Negative **Denied vaccine**
30%	70%	98%	94%	88%	210	14	686	90
	80%	98%	94%	92%	240	14	686	60
	90%	98%	95%	96%	270	14	686	30
50%	70%	98%	97%	77%	350	10	490	150
	80%	98%	98%	83%	400	10	490	100
	90%	98%	98%	91%	450	10	490	50

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