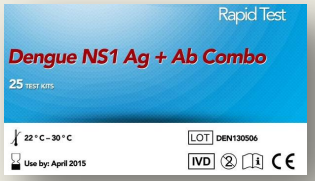
A Meta-analysis on

Accuracy of Dengue Diagnostic Tests

Used for Point-Of-Care Testing Among ASEAN patients



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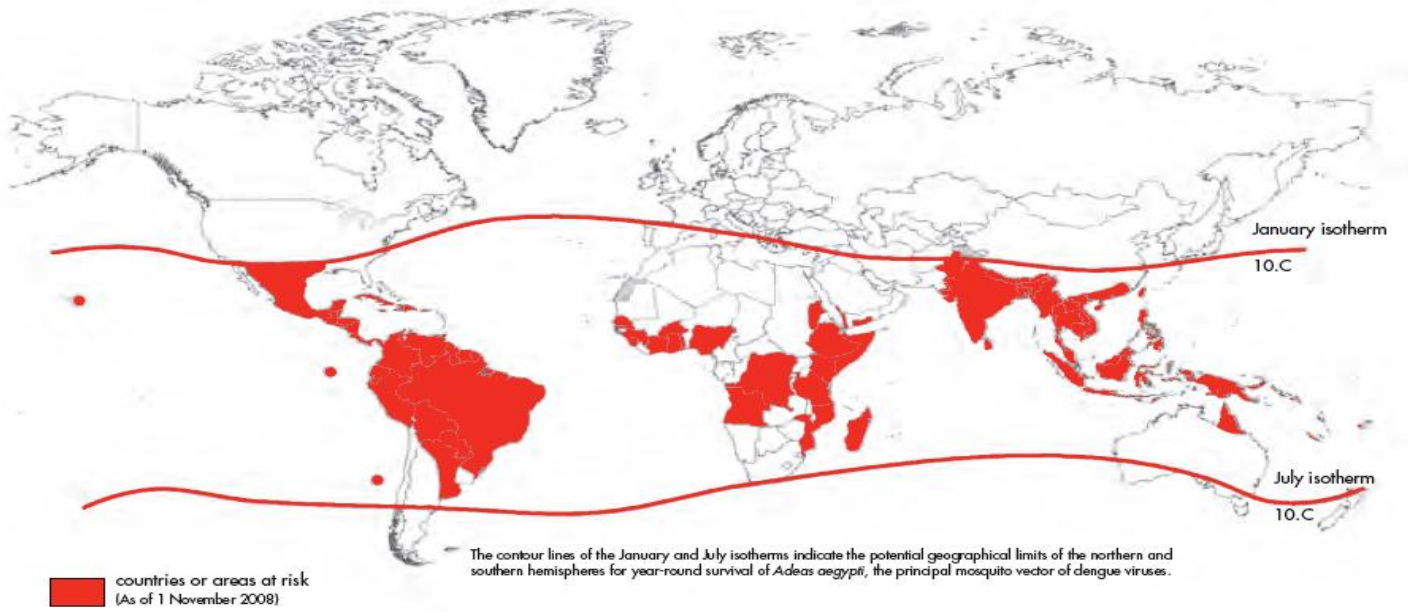
Burden of Dengue

- Dengue, a vector born disease caused by the dengue virus is a major public-health concern throughout tropical and sub-tropical regions of the world.
- Estimated that 50–100 million dengue infections occur each year
- 30-fold increase in global incidence over the past 50 years (WHO,2012).
- 75% of the global population exposed to dengue are in the Asia-Pacific region*

*Ref: * World Health Organization(WHO) "Global strategy for dengue prevention and control 2012-2020"

Countries /Area At Risk of Dengue Transmission (2008)*

Figure 1.1 Countries/areas at risk of dengue transmission, 2008



The contour lines of the January and July isotherms indicate the potential geographical limits of the northern and southern hemispheres for year-round survival of *Adeas aegypti*, the principal mosquito vector of dengue viruses.

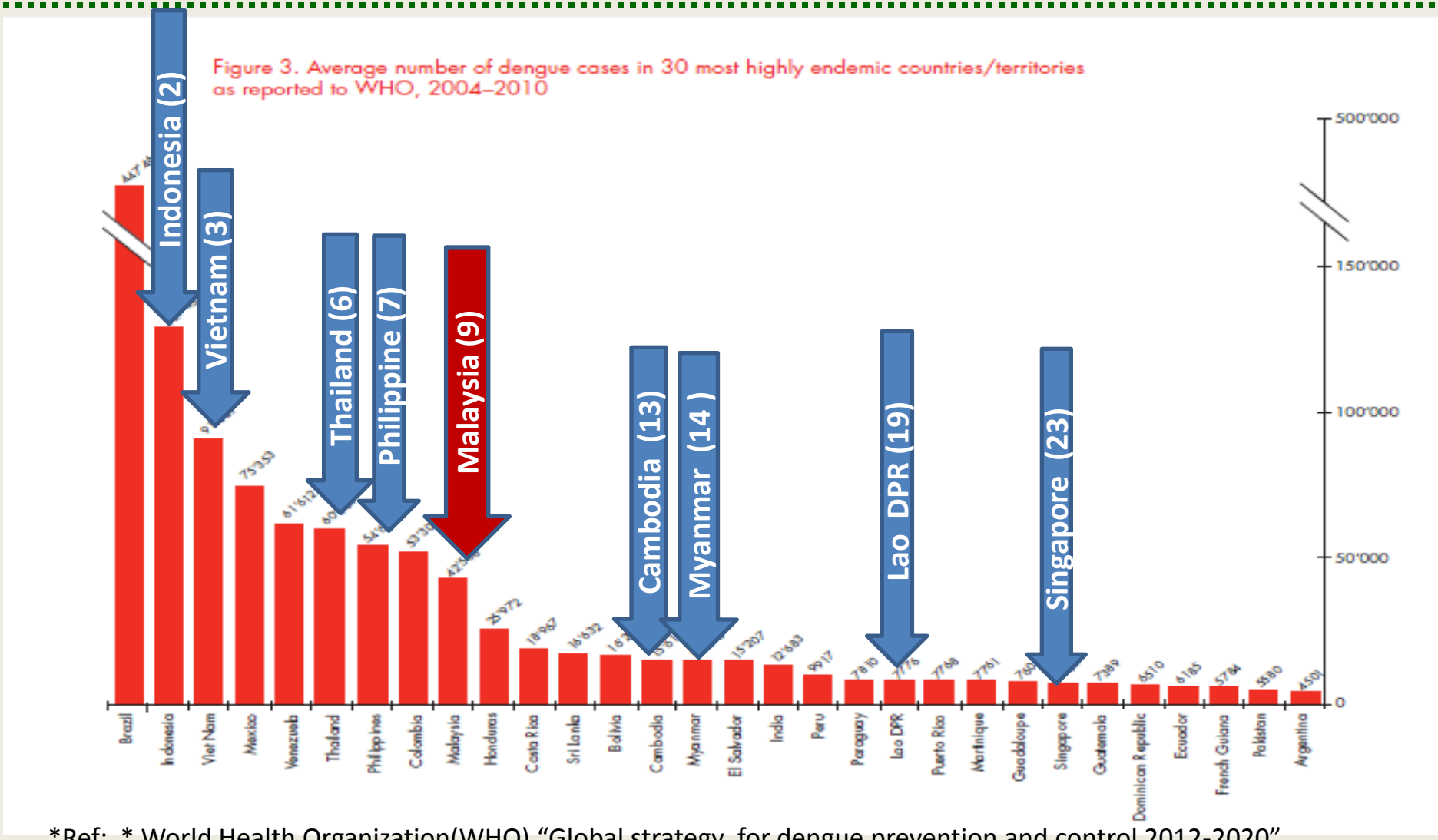
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines or maps represent approximate border lines for which there may not yest be fill agreement.

Data Source: World Health Organization Map Production: Public Health Information and Geographic Information Systems (GIS) World Health Organization

World Health Organization

*Ref: * World Health Organization(WHO) “Global strategy for dengue prevention and control 2012-2020”

Average Numbers of Dengue Cases in "30 Most highly endemic Countries (2004-2010)*"



*Ref: * World Health Organization(WHO) "Global strategy for dengue prevention and control 2012-2020"

Strategy to mitigate Dengue Morbidity & Mortality vs Primary Care Physicians

- Implementing improved outbreak prediction which is determined by "early detection of the cases" through **coordination** of:

  - Clinical management,
  - Epidemiological investigation & response and
  - Entomological surveillance

- **Primary care physicians (GPs/FPs)** working at both public and private primary care clinics are crucial for early detection of dengue cases

WHO Recommendation in Dengue Diagnosis

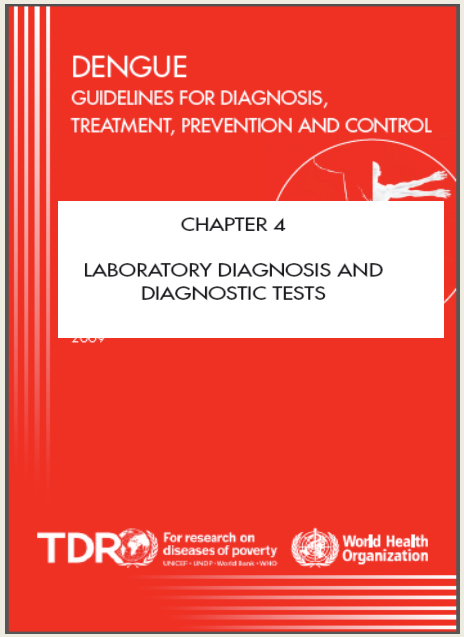


Figure 4.1 Approximate time-line of primary and secondary dengue virus infections and the diagnostic methods that can be used to detect infection

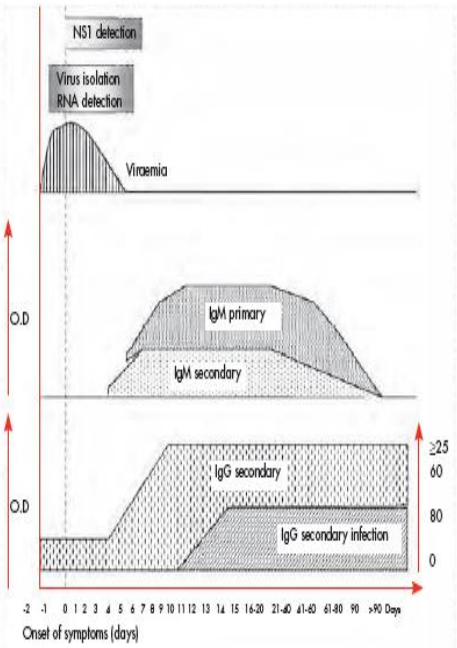
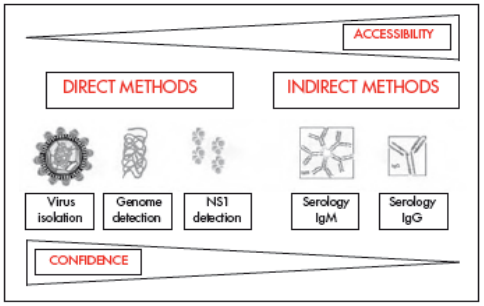


Figure 4.2 Comparison of diagnostic tests according to their accessibility and confidence



4.7 ORGANIZATION OF LABORATORY SERVICES

In a disease-endemic country, it is important to organize laboratory services in the context of patients' needs and disease control strategies. Appropriate resources should be allocated and training provided. A model is proposed in Table 4.4. Examples of good and bad practice can be found in Table 4.5.

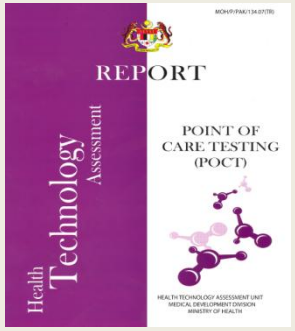
Primary Health Centers

Table 4.4 Proposed model for organization of laboratory services

Dengue diagnostic tests	Primary health centres	District centres	Reference centre
- Virus culture			+
- Nucleic acid detection			+
- Antigen detection		+	+
• ELISA		+	+
• Rapid tests	+		
- Serology			
• ELISA			
• Rapid tests	+		
Functions			
- Training and supervision		+	+
- Quality assurance	+	+	+
- Surveillance activities		+	+
- Outbreak investigations			+
- Referral of problem specimens	+	+	+
- Investigation of problem specimens			+

Rapid tests

Confirmed Dengue cases by laboratory tests



Accuracy of Dengue Diagnostic tests for "Point-Of-Care Testing"

- Primary care physicians (GPs/FPs) from both public & private should adhere WHO recommendation in dengue case management*
- Thus application of dengue diagnostic tests (RDTs) used for POCT by PCPs with a sound knowledge on "**accuracy" of these test kits** is desirable in **early detection of confirmed (ruling in/ ruling out) dengue cases** in primary care clinics

*Ref: * World Health Organization(WHO) "Global strategy for dengue prevention and control 2012-2020"*
*** Start D B (2012); " Commercial Dengue Rapid Diagnostic tests for point-of-care Application: Recent evaluation & future needs?*

Aim & Objectives

Aim

To review the **"accuracy"** of dengue diagnostic tests used for POCT by applying meta-analysis

Objectives

1. To find out range and pooled **"sensitivity"** of the dengue diagnosis tests (RDTs and ELISA)
2. To find out range and pooled "specificity" of the dengue diagnosis tests (RDT and ELISA)

Methods

- The accuracy results of **31 dengue diagnosis tests** in **5308 ASEAN patients** except Brunei were extracted from **14** published articles published within **2007 and 2014**.
- Rapid diagnosis tests **(RDT)** and **WHO-Clinical criteria** were considered for POCT.
- **ELISA tests** with/without WHO-Clinical criteria were included in meta-analysis.
- **Pooled sensitivity** (SN) and specificity (SP) were computed.
- Egger-bias was analysed for publication bias.
- StatDirect Statistical software was used for Meta-analysis

Selection of 31 Dengue Diagnostic tests for Meta- analysis

"36" Articles with dengue diagnostic tests" published 2005 onwards retrieved using keys words

(*Dengue ,Diagnostic Tests, sensitivity, specificity, name of Asian countries*)

via Google scholar & Pub-Med

Excluded – **10** articles with non-Asian subjects/patients and
4 articles without SN or SP value of the dengue diagnostic test

"22" Articles with Dengue diagnostic tests **with SN &SP value** and subjects/patients were from **Asian countries** except Brunei

Excluded – **8** articles without **95% CI value of SN &SP**

"14" Articles with Dengue diagnostic tests from ASEAN countries (except Brunei) with SN & SP value together with respective 95% CI value

Included – **the tests based on detection of antigens & Serological tests (RDT, ELISA and WHO clinical Criteria)**

Excluded- Virus Isolation, Nucleic acid detection, PCR/ R-TPCR, Isothermal amplification methods, Haem-agglutination-inhibition test

SN/SP with (95%CI) value of **"20"** tests (RDT & WHO Clinical Criteria) which are applicable in POCT, " **10"** ELISA Tests **"1"** ELISA + WHO Clinical Criteria

14 Published articles included in Meta-analysis

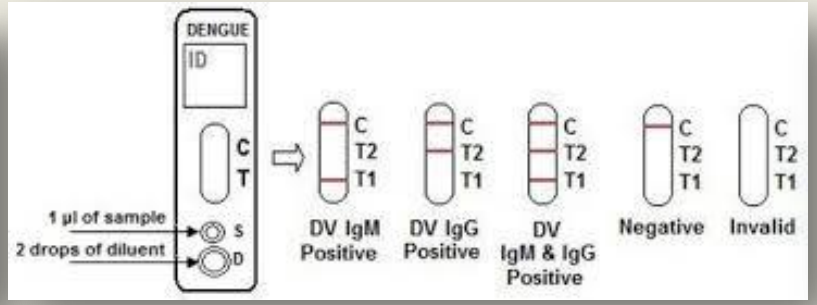
Authors	Year	Study site	Subjects (n)	Diagnosis tests
1. Fry, S. R., Meyer, M., Semple, M. G., Simmons, C. P., Sekaran, S. D., Huang, J. X., ... & Cooper, M. A	2011	Vietnam Malaysia	Vietnam (n= 298) Malaysian (n= 293)	NS1 Rapid tests Combined NS1/ IgM, Ig G
2. Guzman, M. G., Jaenisch, T., Gaczkowski, R., Ty Hang, V. T., Sekaran, S. D., Kroeger, A., ... & Simmons, C. P.	2010	Asia (Malaysia, Thailand, Philippines, Vietnam) and Americas (Nicaragua and Venezuela)	n= 1385 Malaysia (n=124) Thailand (n= 161) Philippines (n= 369) Vietnam (n= 407+214) Nicaragua (n= 36) Venezuela (n= 74)	Pan- E Dengue Early ELISA Dengue NS1 Ag Assays
3. Wang, S. M., & Sekaran, S. D.	2010	Malaysia	(n= 420)	SD Dengue Duo NS1 Ag and IgG/ IgM test Virus isolation In-house hemagglutination inhibition assay In-house IgM capture ELISA Real-Time RT-PCR
4. Gan, V. C., Tan, L. K., Lye, D. C., Pok, K. Y., Mok, S. Q., Chua, R. C., ... & Ng, L. C.	2014	Singapore	(n= 246) Singapore patients	Dengue Duo (NS1/ IgM/IgG) Dengue Duo (NS1/ IgM) Dengue Duo (NS1 only) WHO 1997, 2009 WHO 97+ Dengue Duo (NS1/IgM/IgG) WHO 2009 +Dengue Duo (NS1/ IgM/IgG) Confirmation: virus isolation, RT-PCR, NS1-IgM and IgG ELISA
5.Chaterji, S., Allen, J. C., Chow, A., Leo, Y. S., & Ooi, E. E.	2011	Singapore	(n=354) Adult 18years and above Singapore patients	Dengue NS1 Strip 15 minutes Dengue NS1 Strip 30 minutes WHO 1997, WHO 2009 Confirmation: RT-PCR, Virus isolation, IgM/IgG ELISA
6. Tricou, V., Vu, H. T., Quynh, N. V., Nguyen, C. V., Tran, H. T., Farrar, J., ... & Simmons, C. P.	2010	Vietnam	(n= 245+ 47) Vietnamese children	BioRad NS1 SD NS1 alone SD NS1 or IgM ELISA SDNS1 or IGM or IgG Confirmation: RT-PCR, IgM/IgG ELISA

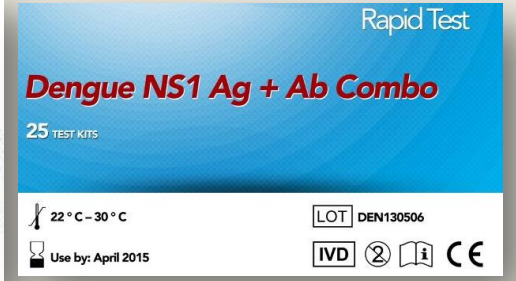
14 Published articles included in Meta-analysis

Authors	Year	Study site	Subjects (n)	Diagnosis tests
7. Pok, K. Y., Lai, Y. L., Sng, J., & Ng, L. C.	2010	Singapore	(n= 433)	NS1 rapid test, NS1 ELISA, IgM/IgG Rapid/ IgM/IgG ELISA Confirmation : RT-PCR
8. Wang, S. M., & Sekaran, S. D.	2010	Malaysia	(n=399) sera Malay Patients	SD dengue NS1 Ag ELISA confirmation: Virus isolation In-house hemagglutination inhibition assay, In-house IgM capture ELISA Real-Time RT-PCR
9. Blacksell, S. D., Bell, D., Kelley, J., Mammen, M. P., Gibbons, R. V., Jarman, R. G., & Newton, P. N.	2007	Laos	(n= 87 patients Paired samples- 174)	Ig M Rapid test, IgM/IgG Rapid test confirmation: RT-PCR IgM/ Ig G capture ELISA
10. Watthanaworawit, W., Turner, P., Turner, C. L., Tanganuchitcharnchai, A., Jarman, R. G., Blacksell, S. D., & Nosten, F. H.	2011	Thai-Myanmar Border	(n= 162) Myanmar Patients (age 15 years and above)	NS1, rRT-PCR IgM/ IgG, Combined: NS1 + IgM NS1+rRT-PCR, IgM+rRT-PCR, NS1 +rRT-PCR+IgM
11. Andries, A. C., Duong, V., Ngan, C., Ong, S., Huy, R., Sroin, K. K., ... & Buchy, P.	2012	Cambodia	(n= 157) Children	NS1 Rapid test NS1 Rapid +IgM+ IgG confirmation: RT-PCR, Virus isolation In-house hemagglutination inhibition assay, In-house IgM capture ELISA
12. Duong, V., Ly, S., Ong, S., Chroeung, N., Try, P. L., Deubel, V., ... & Buchy, P.	2011	Cambodia	(n= 260) confirmed dengue patients	NS1 Rapid test NS1 rapid + IgM antibody ELISA
13. Blacksell, S. D., Jarman, R. G., Gibbons,R.V.,Tanganuchitcharnchai ,A.,Mammen, M. P., Nisalak, A., & Lalloo, D. G.	2012	Thailand and Sri Lanka	(n= 239)Thai patients (dengue positive) paired sample total= 478 n= 98 Sri Lanka patients Paired sample total= 626	NS1 ELISA IgM/IgG ELISA NS1 (ELISA)+ IgM (ELISA) confirmation: RT-PCR
14. Kosasih, H., Alisjahbana, B., Widjaja, S., de Mast, Q., Parwati, I., Blair, P. J., ... & Williams, M.	2013	Indonesia	(n= 220) confirmed dengue + (n= 55) non- dengue febrile illness	NS1 ELISA confirmation: RT-PCR , (HI) assay

Findings


Mytest
Dengue NS1

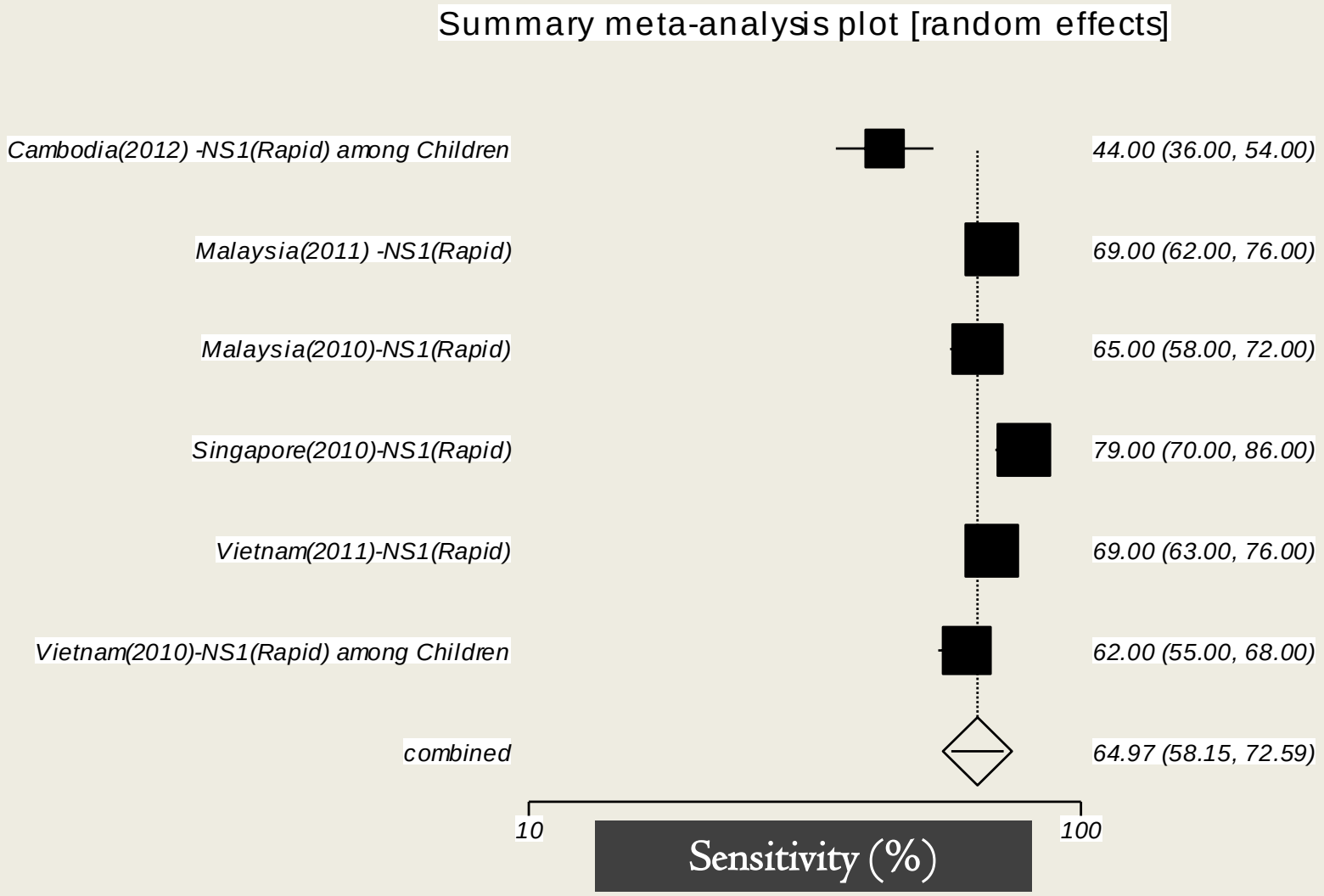

DENGUE
ID
C
T
S
D
1 µl of sample
2 drops of diluent
C
T2
T1
DV IgM Positive
DV IgG Positive
DV IgM & IgG Positive
Negative
Invalid


Rapid Test
Dengue NS1 Ag + Ab Combo
25 TEST KITS
22 °C – 30 °C
LOT DEN130506
Use by: April 2015
IVD

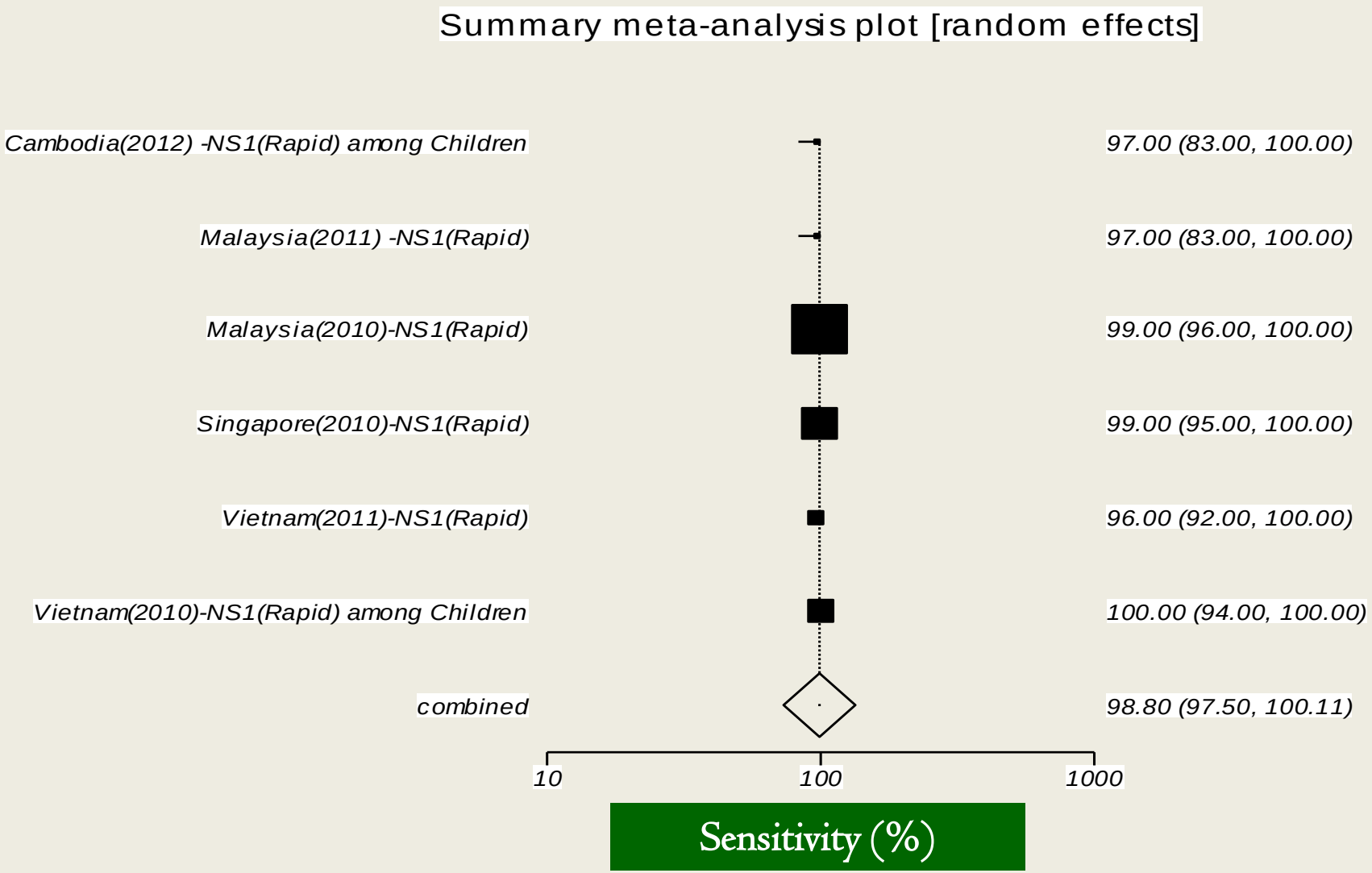
Accuracy Description of "31" Dengue Diagnostic Tests

Types	Accuracy	No of tests	Minimum	Maximum	Mean(SD)
NSI (RDT)	SN (%) SP (%)	6	44 96	79 100	64.7(11.6) 98(1.5)
NSI (ELISA)	SN (%) SP (%)	8	45 93	82 100	64.8(14) 98.2(2.4)
IgM/IgG (RDT)	SN (%) SP (%)	7	3 93	50 99	17.3(15.8) 96.1(2.3)
IgM/IgG (ELISA)*	SN (%) SP (%)	1	50 100	50 100	No Meta-Analysis
NSI+ IgM/IgG(RDT)	SN (%) SP (%)	3	69 84	86 98	79.7(9.3) 93(7.8)
NSI IgM/IgG (ELISA)*	SN (%) SP (%)	1	94 92	94 92	No Meta-Analysis
WHO-Clinical Criteria(1997 /2009)	SN (%) SP (%)	4	80 20	97 57	92(8) 34.8(16.2)
NSI +IgM/IgG(ELISA) + WHO –Clinical Criteria (2009)*	SN (%) SP (%)	1	91 94	91 94	No Meta-Analysis

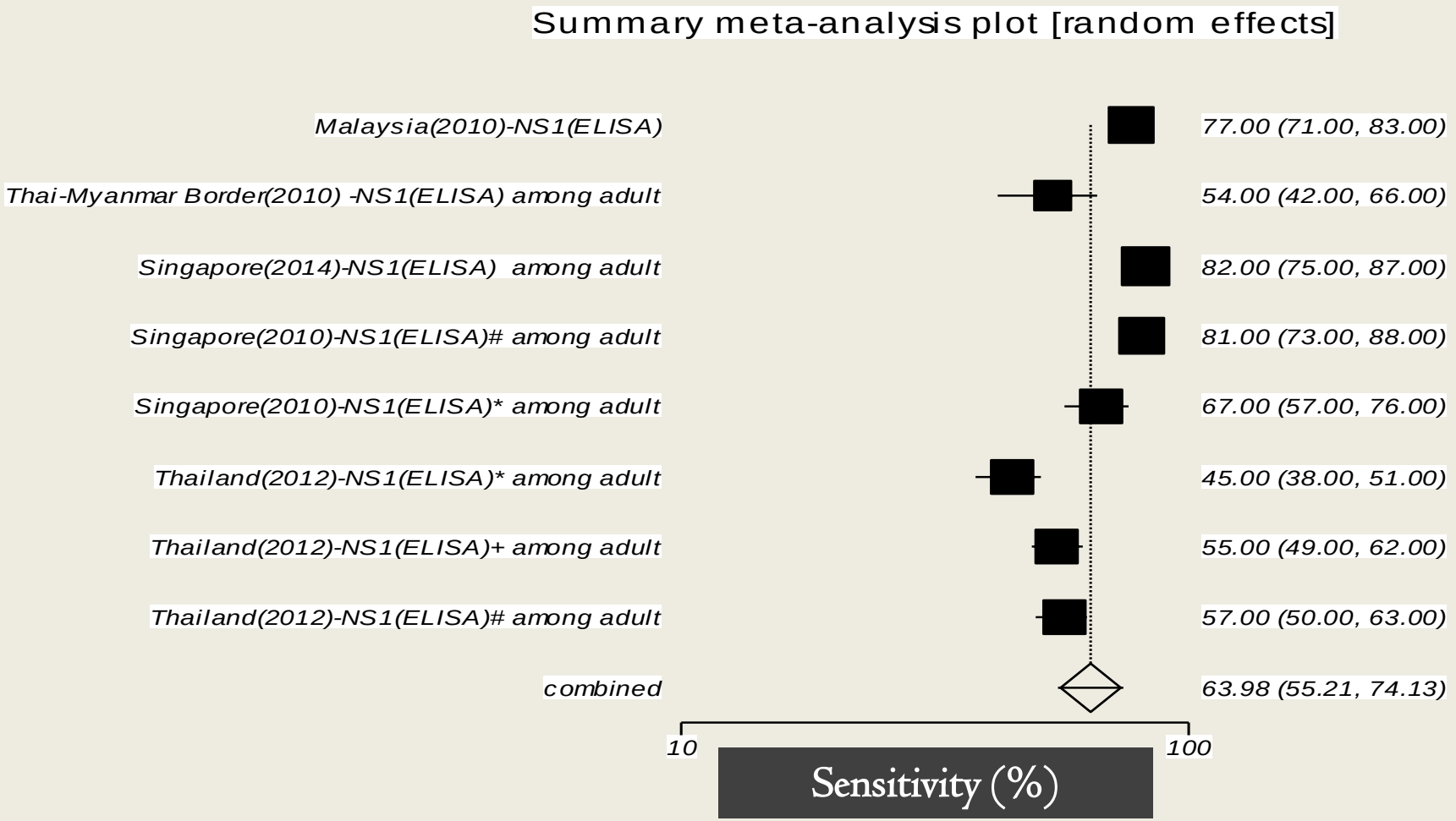
Meta-analysis Plot of " Sensitivity" of NSI(RDT)" (n=6)



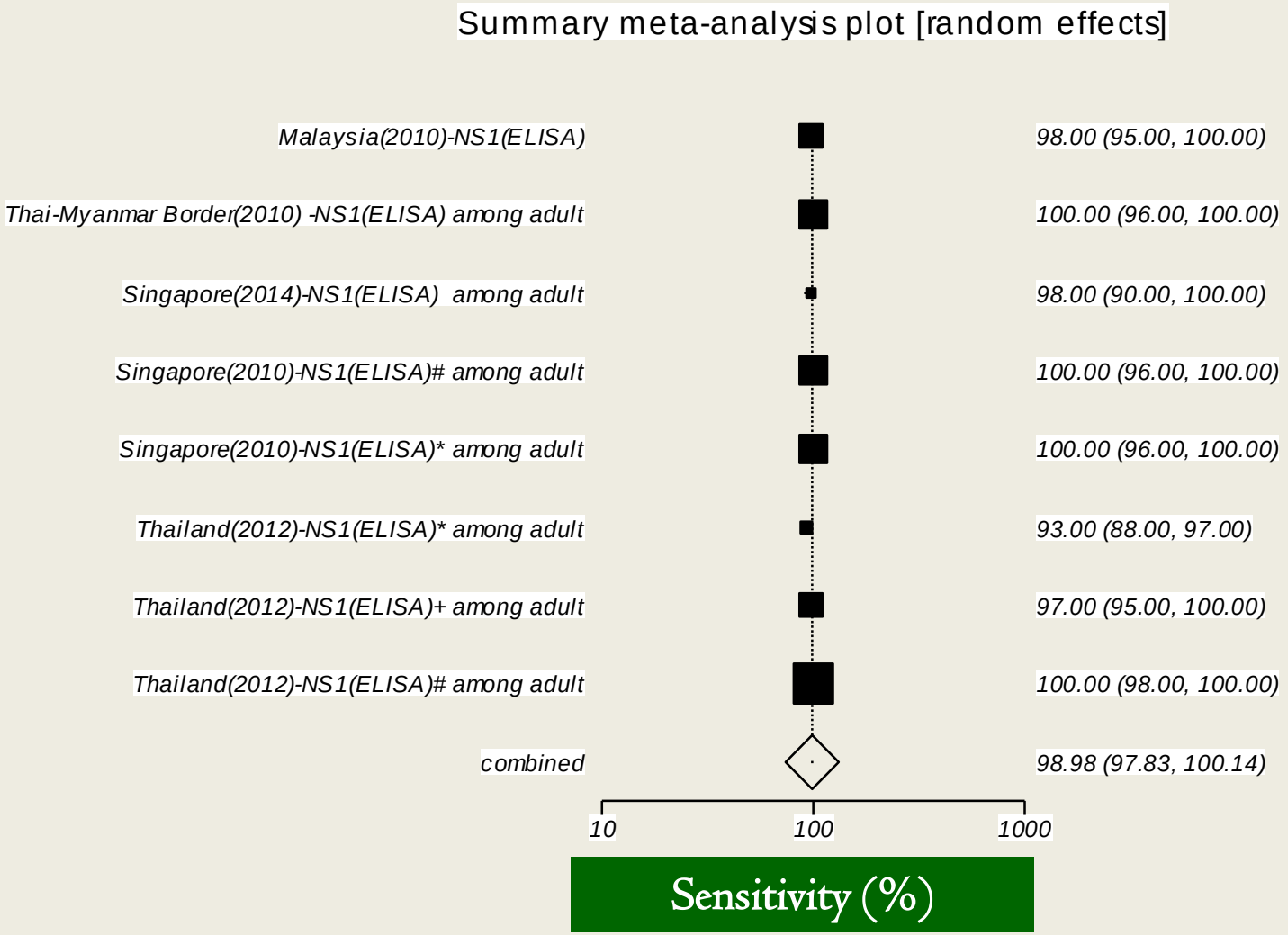
Meta-analysis Plot of " Specificity" of NSI(RDT) (n=6)



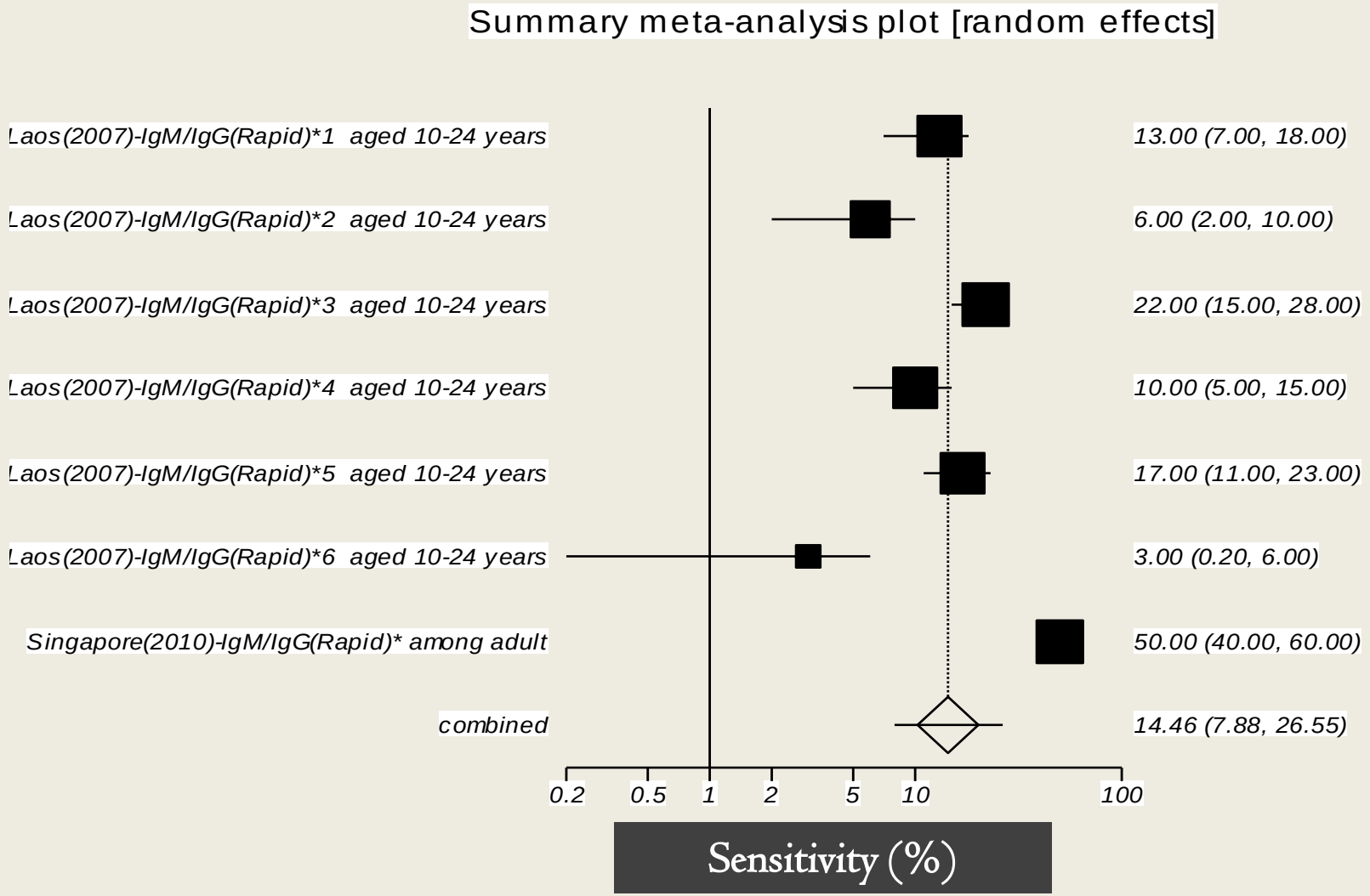
Meta-analysis Plot of " Sensitivity of NSI(ELISA)" (n=8)



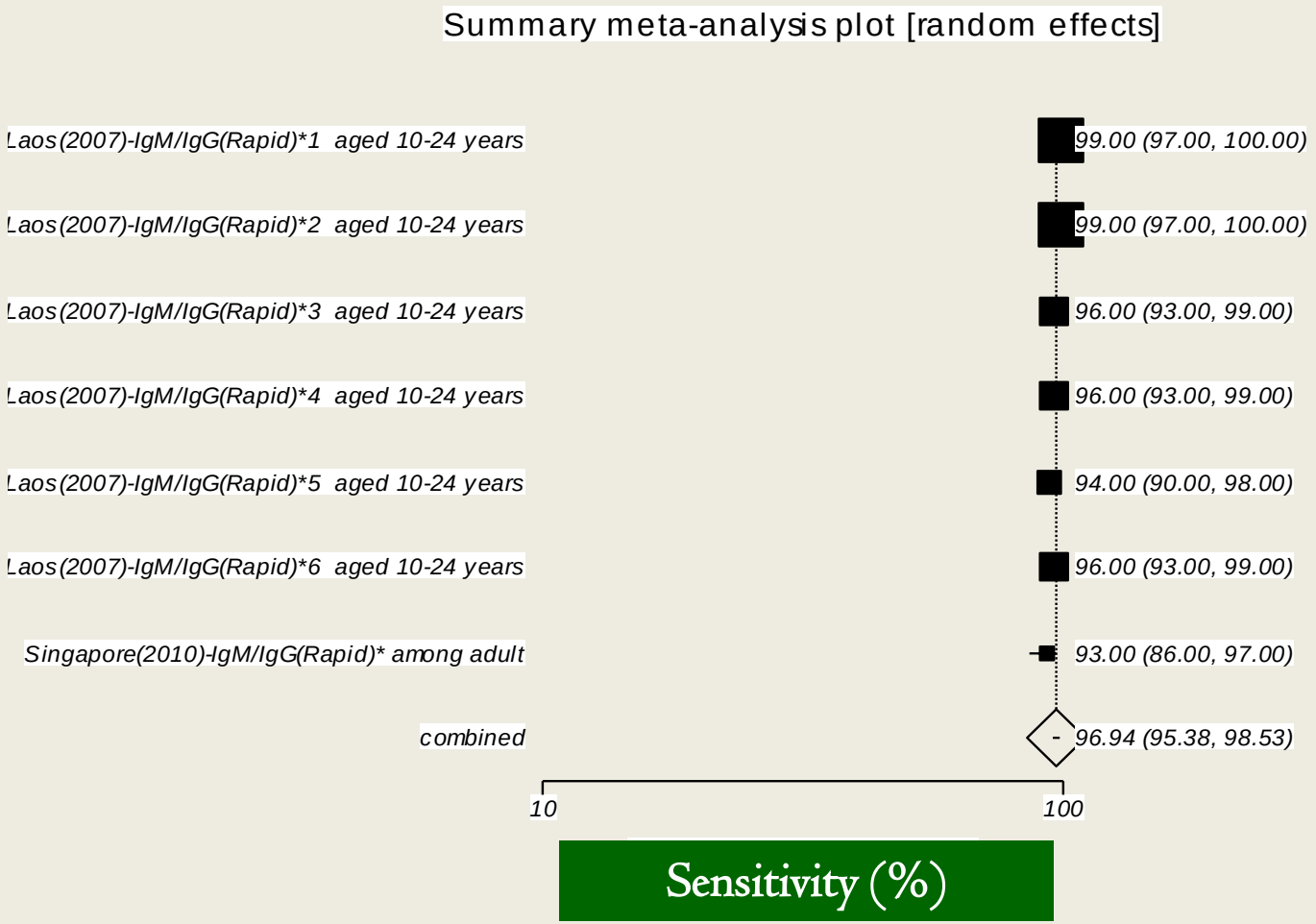
Meta-analysis Plot of " Specificity" of NSI(ELISA)" (n=8)



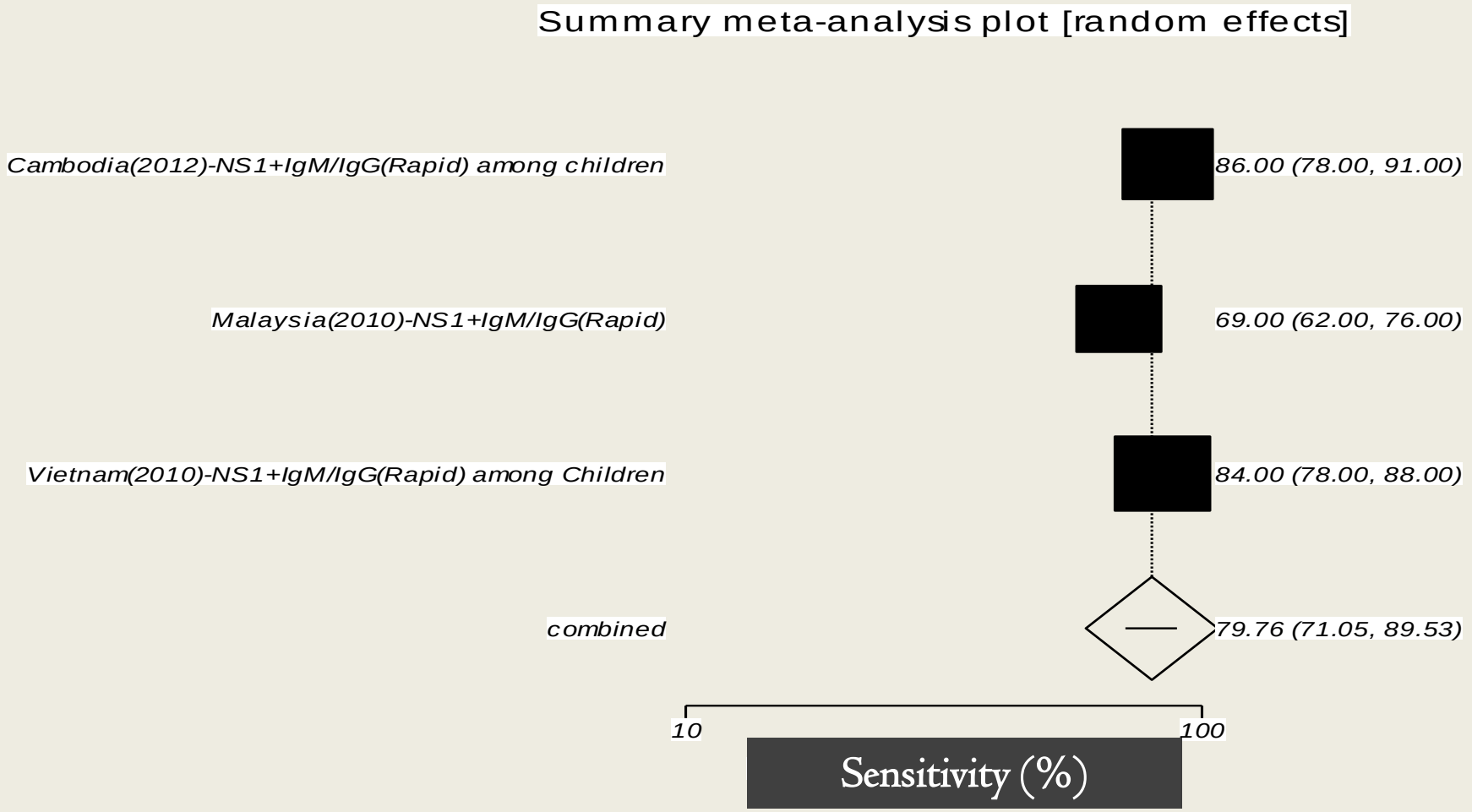
Meta-analysis Plot of " Sensitivity" of IgM/IgG (RDT)" (n=7)



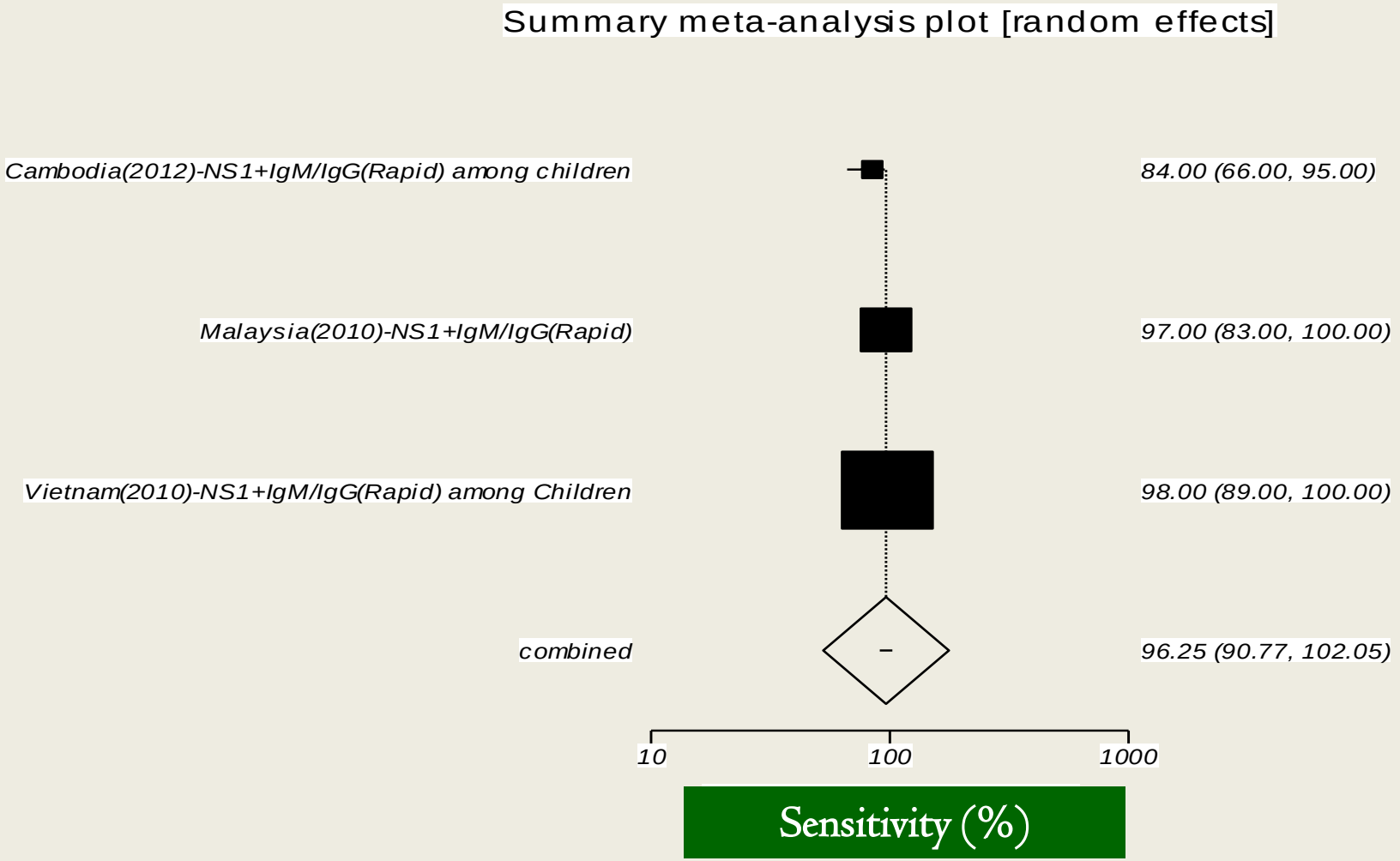
Meta-analysis Plot of " Specificity" of IgM/IgG (RDT)" (n=7)



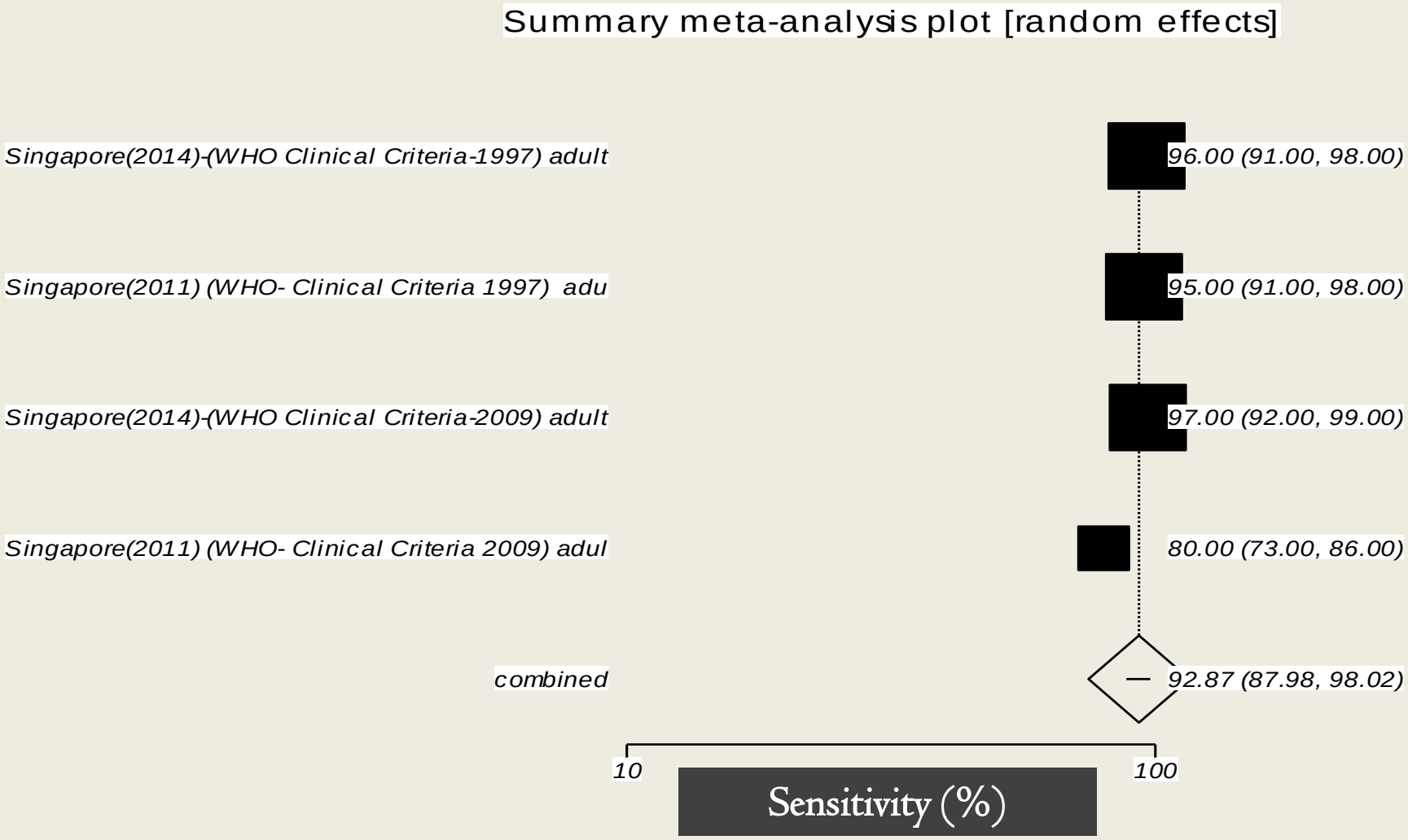
Meta-analysis Plot of " Sensitivity of NSI+ IgM/IgG (RDT)" (n=3)



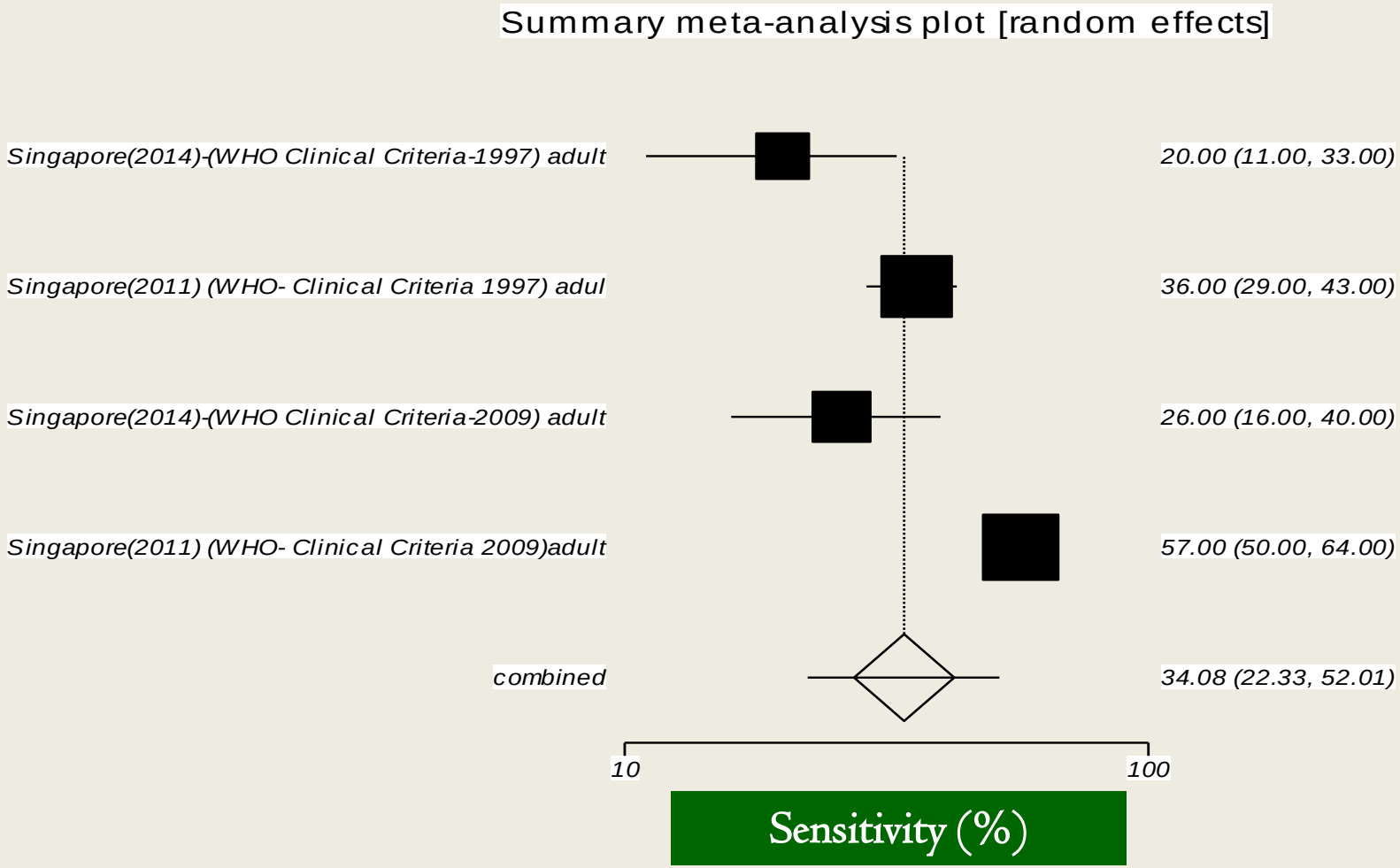
Meta-analysis Plot of " Specificity" of NSI+IgM/IgG (RDT)" (n=3)



Meta-analysis Plot of “ Sensitivity” of WHO-Clinical Criteria (1997/2009) ” (n=4)

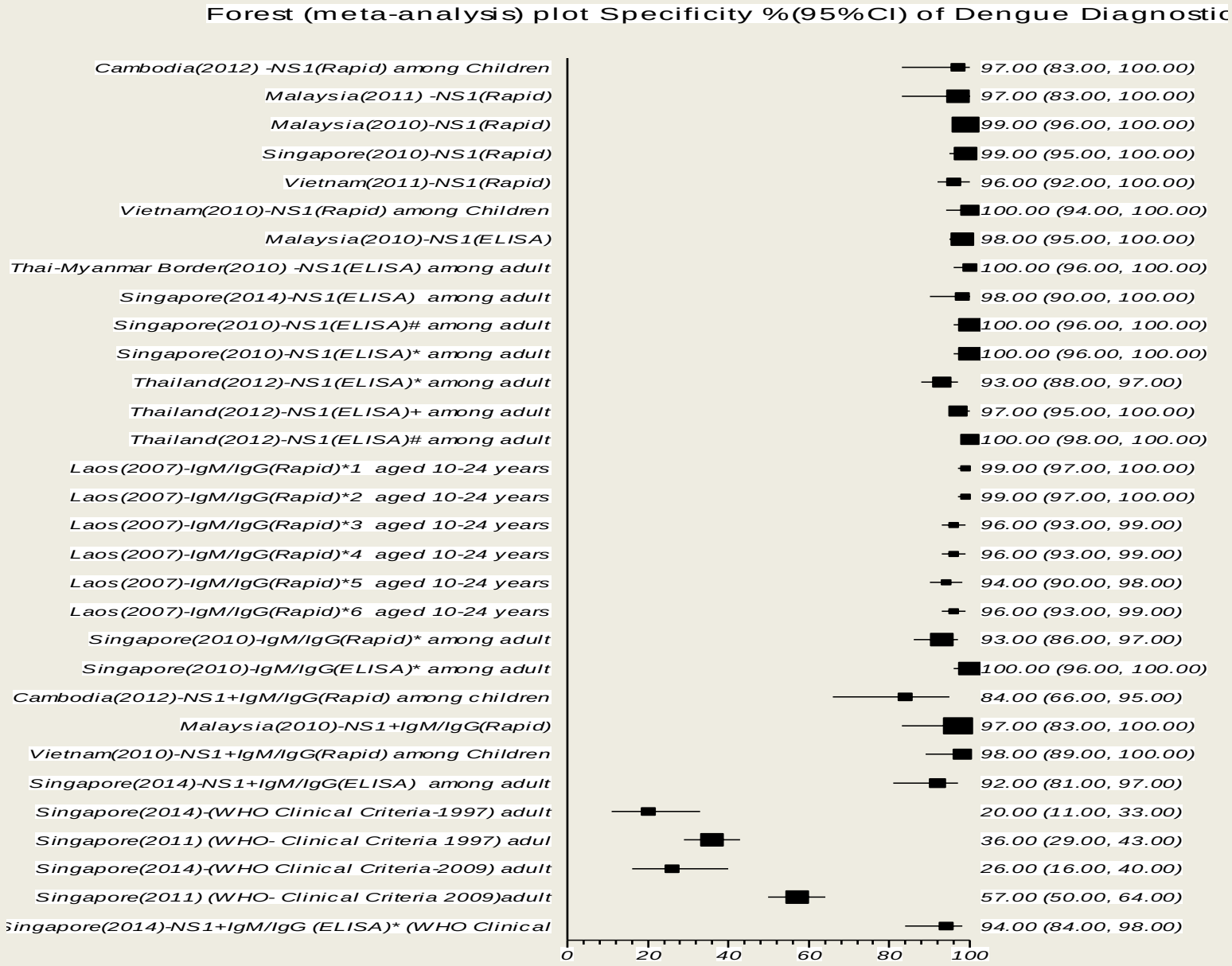


Meta-analysis Plot of "Specificity" of WHO-Clinical Criteria (1997/2009) " (n=4)



Summary Meta-analysis Plot of " Sensitivity" of 31 Tests

Summary Meta-analysis Plot of " Specificity" of 31 Tests



Bias Assessment

Bias indicators ((**Sensitivity**)

Begg-Mazumdar: Kendall's tau b = -0.678919 P < 0.0001

Egger: bias = -8.667958 (95% CI = -9.783378 to -7.552538) **P < 0.0001**

Bias indicators (**Specificity**)

Begg-Mazumdar: Kendall's tau b = -0.576383 P < 0.0001

Egger: bias = -3.747528 (95% CI = -5.082488 to -2.412568) **P < 0.0001**

Discussion and Conclusion (Specificity)

- Specificity (SP) of Dengue diagnostic tests (RDT) except WHO-Clinical Criteria -2009(20%) was as high (84%-100%) as those of ELISA tests .
- Thus, the Dengue Rapid Tests can be used as POCT in primary care clinics to **rule in dengue diagnosis** for **early detection of confirmed dengue cases** for clinical management, control , prevention and efficacy of dengue vaccine (in future)

Discussion and Conclusion (Sensitivity)

- Very low sensitivity (3%) of the dengue diagnostic test (IgM/IgG-RDT) and high sensitivity (97%) in WHO-Clinical Criteria were noticed.

- Wide range of **SN** value particularly in RDT highlighted to improve SN of the tests for **ruling out** the dengue cases in primary care clinics

Discussion & Conclusion (Epidemic information)

- For confirmation of dengue diagnosis , **2 out 8 WHO-Clinical Criteria** (Acute febrile illness ≥38°, Generalized rashes, Headache, Retro-orbital pain, Myalgia, Arthralgia, Bleeding manifestation and Leucopenia) and **Positive result** of either NS1Ag or IgM /IgG are usually used in primary care clinics.
- However, **ONE out of 8** WHO-Clinical Criteria is applied for confirmation of the dengue suspected cases from **defined epidemic area during outbreak.**
- **Thus, added value of "dengue epidemic information" in the accuracy of the dengue tests should be verified.**

Recommendation

- **A multi-centred study** with **a standardized protocol** (considering geographical/prevalence variation, standardization of diagnostic assessment (paired samples, timing of sample collection), primary or secondary infections, age of subjects and confirmation tests applied (RT-PCR, r-RT PCR, virus isolation) **should be conducted for diagnostic test accuracy variation for POCT among ASEAN patients.**

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Thank You !

WHO Recommendation in Dengue Diagnosis

Efficient and accurate diagnosis of dengue is of primary importance for...

- **Clinical care** (i.e. early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases)
- **Surveillance** activities, Outbreak control, pathogenesis,
- **Academic research**, vaccine development & clinical trials*

- **Recommended to use dengue diagnostic tests**

*Ref: * World Health Organization(WHO) ; Dengue Guidelines for diagnosis, treatment, prevention and control ,2009