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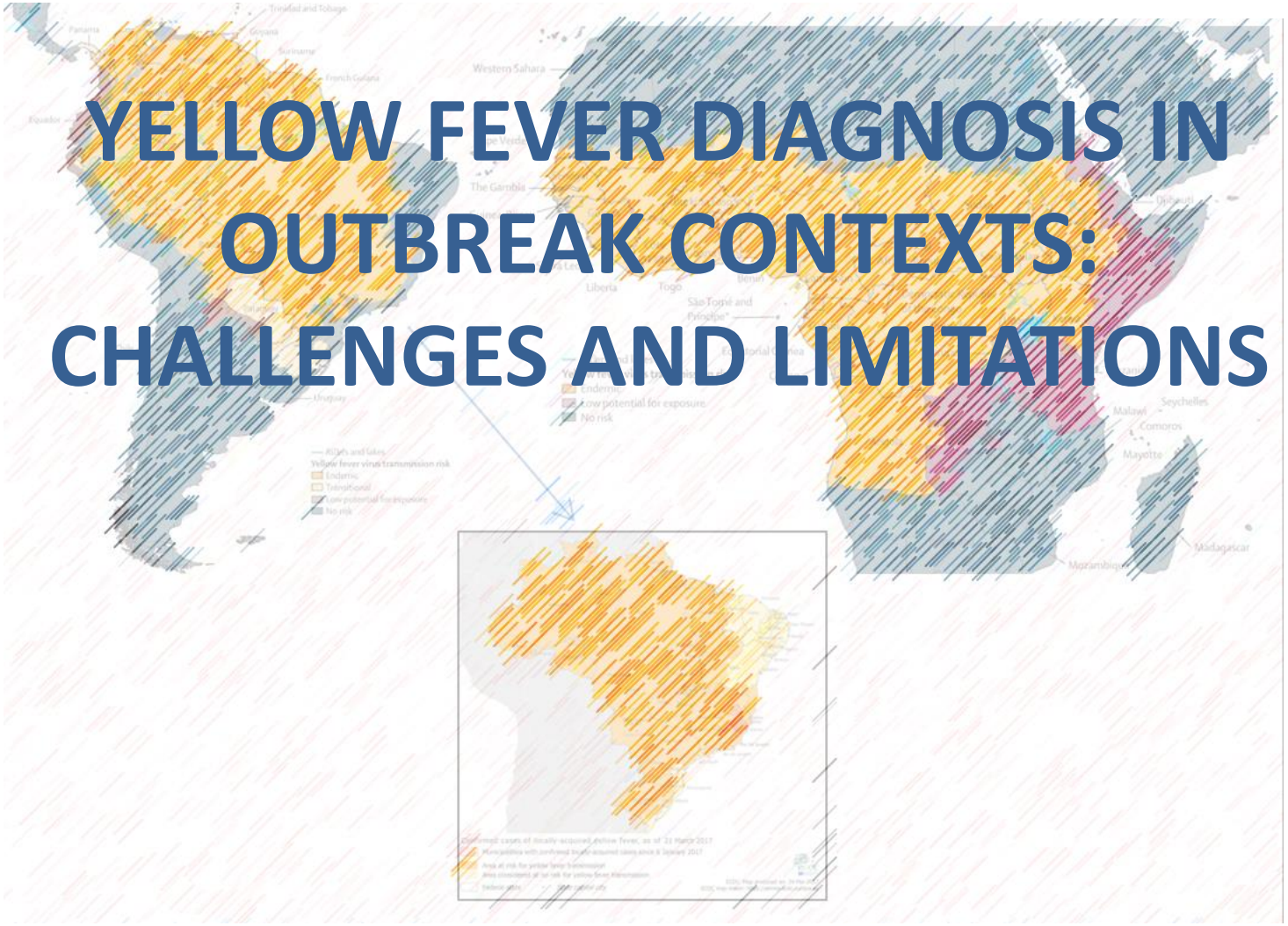
YELLOW FEVER DIAGNOSIS IN OUTBREAK CONTEXTS: CHALLENGES AND LIMITATIONS

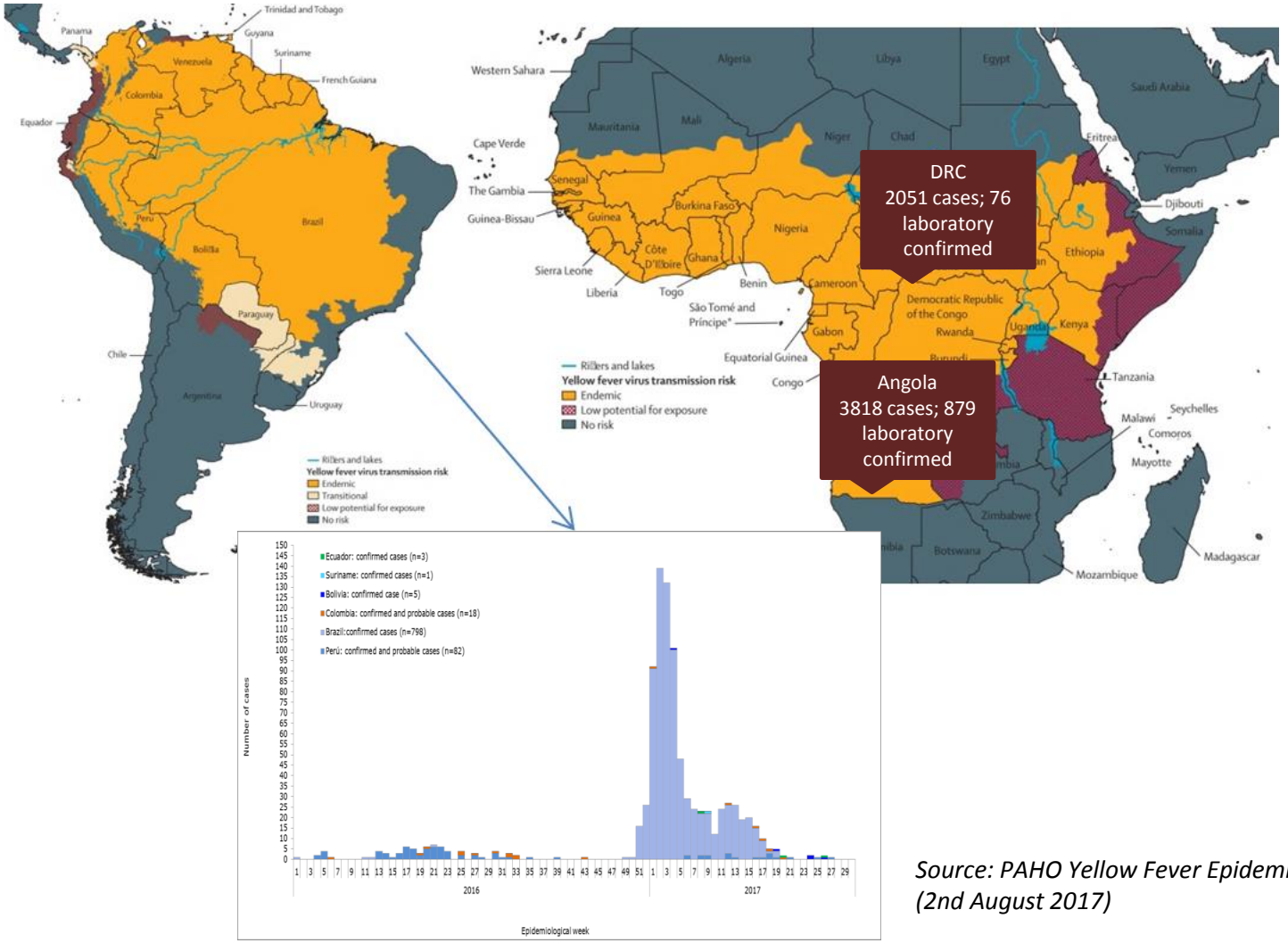


Dr. Cristina Domingo, Robert Koch Institute,
Berlin, Germany

YELLOW FEVER DIAGNOSIS IN OUTBREAK CONTEXTS: CHALLENGES AND LIMITATIONS

- ✓ **SAMPLES** (i.e. burden to the reference lab, quality, transport and storage…)
- ✓ **METHODS** (lack of validated commercial methods)
- ✓ **INTERPRETATION** (cross-reactivities, vaccination status…)

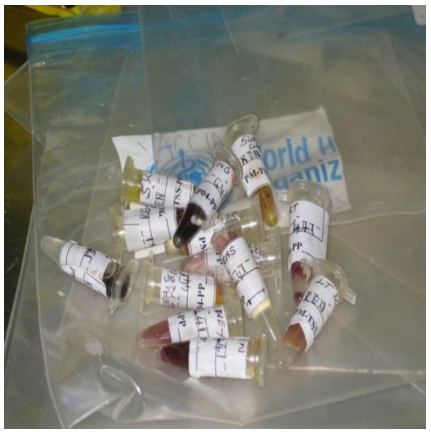
YELLOW FEVER DIAGNOSIS IN OUTBREAK CONTEXTS: CHALLENGES AND LIMITATIONS



Source: PAHO Yellow Fever Epidemiological Alert (2nd August 2017)

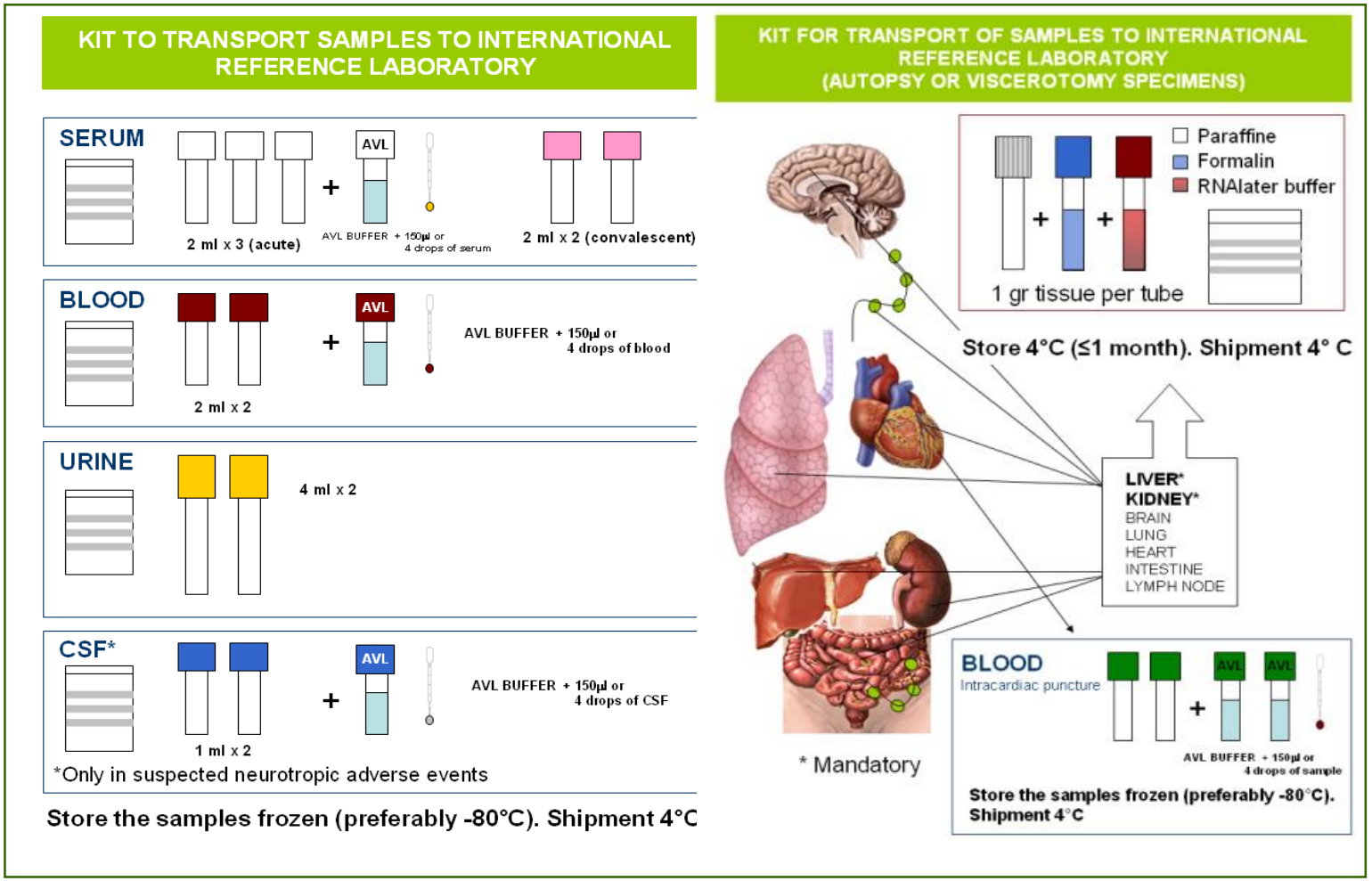
YELLOW FEVER DIAGNOSIS IN OUTBREAK CONTEXTS: CHALLENGES AND LIMITATIONS

ADEQUACY of sample collection and transport concerning both biosafety and sample integrity



YELLOW FEVER DIAGNOSIS IN OUTBREAK CONTEXTS: CHALLENGES AND LIMITATIONS

- How to improve the quality of the samples?



YELLOW FEVER DIAGNOSIS IN OUTBREAK CONTEXTS: CHALLENGES AND LIMITATIONS

- How to improve the quality of the samples?

BUT…WHERE?

Identification of key sites and strategy development, including „train of trainers“, as part of preparedness activities

OVERVIEW ON AVAILABLE MOLECULAR DETECTION COMMERCIAL ASSAYS

KIT	Sensitivity	CE marked	IVD marked
Genekam	No data	X (only YFV)	X
Genesig	No data		
ViPrimePLUS	100 cop/rxn		
FTD Tropical Fever Africa	1000 cop/ml	X	X
LifeRiver kits	1000 cop/ml	X	X
PCRMAX	No data		

The assays must be validated with different strains and different human samples. Validation data using the YF-17D strain have relative value. At least *in silico* analyses must be done.

Press release

Genekam DNA-Test for detection of yellow fever virus (a new threat to the world)

Duisburg, 23.05.2016: Genekam Biotechnology AG, Germany has developed a new DNA kit for detection of yellow fever virus. This kit is with CE-IVD and can be used in diagnostic in Europe and other CE accepting countries.

At present, there are outbreaks of yellow fever in Africa and WHO is keeping a close eye on situation as it is holding emergency meeting to control this disease. To control the disease in the world, very important step is that this virus should be detected. To detect this virus, one needs an assay, hence Genekam has developed an assay, which can be used on different samples ranging from blood, plasma, urine and stool. Genekam DNA test is highly accurate and sensitive as it detects only yellow fever virus. This test is approved in Europe at present. It makes it also possible to detect this test in mosquitoes, which are spreading source of yellow fever virus. At present, there is no therapy available for yellow fever virus, but the vaccine is available. Yellow fever virus belongs to group of mosquito borne flaviviruses and Genekam carries a number of kits for other flaviviruses like Zika, Dengue, HCV, Westnile. St Louis encephalitis virus etc. This kit comes with internal control and it costs 599,- for 100 reactions i.e. the price per reaction is 3 euro. It means that each country can afford this kit to be used.

Genekam Biotechnology AG is focussing on virology and immunology. It carries one of largest range of kits for Influenza viruses including bird flu, swine flu, seasonal flu and other pathogens HIV, TB, Salmonella, Herpes viruses. It distributes its products in 70 countries around the world. Genekam has 29 kits with CE-IVD and Zika virus kit is being applied for EUA for FDA.

YFV molecular ans serological diagnosis External Quality Assesment results

Sample ID		#2	#9	#12	#4	#14	#10	#5	#13	#1	#6	#11	#3	#8	#7		
Viral load in sample		**$3x10^6$**	**$3x10^5$**	**$3x10^4$**	**$3x10^3$**	**$3x10^2$**	**10^4**	**10^3**	**$2x10^4$**	**$2x10^3$**	**69**	NEG	NEG	NEG	NEG		
Lab. no.	**RT-PCR technique**	17D	17D	17D	17D	17D	Brazil	Brazil	Ivory Coast	Ivory Coast	Ivory Coast	SLE/JE WN/TBE	DEN1-4	neg	neg	**Score***	**Classification**
16a	TaqMan RT-PCR[a]	+	+	+	+	+	+	+	+	+	(-)	-	-	-	-	26	OPTIMAL
8	TaqMan RT-PCR[e]	+	+	+	+	(-)	+	+	+	+	(-)	-	-	-	-	24*	OPTIMAL
17b	RT nested PCR[g]	+	+	+	+	+	+	+	+	+	+	-	(+)	-	-	24*	NON OPTIMAL
1	TaqMan RT-PCR[a]	+	+	+	+	+	+	+	+	+	+	-	(+)	-	-	24	NON OPTIMAL
27	TaqMan RT-PCR[j]	+	+	+	+	+	+	+	+	+	+	-	(+)	-	-	24	NON OPTIMAL
28	TaqMan RT-PCR[c]	+	+	+	+	+	+	+	+	+	+	-	(+)	-	-	24	NON OPTIMAL
15	TaqMan RT-PCR[a]	+	+	+	+	+	+	+	+	+	(-)	-	(+)	-	-	22	NON OPTIMAL
17a	TaqMan RT-PCR[b]	+	+	+	+	+	-	-	-	-	-	-	(+)	-	-	22	NON OPTIMAL
6	RT nested PCR[x]	+	+	(-)	(-)	(-)	-	-	-	-	-	-	-	-	-	20*	OPTIMAL
22a	RT nested PCR[f]	+	+	ND	+	-	-	-	-	-	-	-	-	-	-	20*	OPTIMAL
16b	Heminest Rt-PCR[g]	+	+	+	+	+	-	-	-	-	-	-	(+)	-	-	20*	NON OPTIMAL
2	TaqMan RT-PCR[a]	+	+	+	+	(-)	-	-	-	-	-	-	(+)	-	-	20	NON OPTIMAL
9	TaqMan RT-PCR[a]	+	+	+	+	(-)	+	+	+	+	(-)	-	(+)	-	-	20	NON OPTIMAL
14	RT nested PCR[f]	+	+	+	(-)	(-)	+	-	-	(-)	(-)	-	-	-	-	18*	OPTIMAL
4	TaqMan RT-PCR[a]	+	+	+	+	(-)	+	+	+	(-)	(-)	-	(+)	-	-	18	NON OPTIMAL
10	TaqMan RT-PCR[c]	+	+	+	+	+	(-)	(-)	(-)	(-)	(-)	-	(+)	-	-		AL
11	RT-PCR[d]	+	+	+	+	+	(-)	(-)	(-)	(-)	(-)	-	(+)	-	-		AL
20	Heminest RT-PCR[g]	+	+	+	+	+	+	+	+	+	+	(+)	(+)	-	-		AL
3b	TaqMan RT-PCR[c]	+	+	+	+	+	(-)	(-)	(-)	(-)	(-)	-	(+)	-	-		AL
5	TaqMan RT-PCR[x]	+	+	+	+	+	(-)	(-)	(-)	(-)	(-)	-	(+)	-	-		AL
13	TaqMan RT-PCR[a]	+	+	ND	+	+	(-)	(-)	+	(-)	(-)	-	(+)	-	-	14	NON OPTIMAL
18	TaqMan RT-PCR[c]	+	+	+	+	+	(-)	(-)	(-)	(-)	(-)	(+)	-	-	-	14	NON OPTIMAL
3a	RT nested PCR[b]	+	+	+	(-)	(-)	+	(-)	(-)	(-)	(-)	-	(+)	-	-	12	NON OPTIMAL
19	TaqMan RT-PCR[a]	+	+	+	(-)	(-)	+	(-)	(-)	(-)	(-)	-	(+)	-	-	12	NON OPTIMAL
22b	TaqMan RT-PCR[c]	+	+	ND	+	+	(-)	(-)	(-)	(-)	(-)	-	(+)	-	-	12	NON OPTIMAL
7	TaqMan RT-PCR[k]	+	+	+	+	+	+	(-)	(-)	(-)	+	(+)	(+)	-	(+)	10	NON OPTIMAL
21	TaqMan RT-PCR[c]	+	+	+	(-)	(-)	(-)	(-)	(-)	(-)	(-)	-	(+)	-	-	10	NON OPTIMAL
25	RT-nested PCR[x]	+	(-)	(-)	(-)	(-)	ND	(-)	(-)	ND	(-)	-	-	-	-	8	NON OPTIMAL
12	SYBR-RT-PCR[e]	+	+	+	(-)	(-)	+	(-)	(-)	+	(-)	(+)	(+)	(+)	-	6	NON OPTIMAL
23	RT-PCR[h]	+	+	+	(-)	(-)	+	(-)	(-)	(-)	(-)	-	(+)	-	-	0	NON OPTIMAL
24	RT-nested PCR[i]	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	-	-	-	-	NE	NON OPTIMAL
26	RT-nested PCR[b]	+	(-)	(-)	+	(-)	(-)	+	(-)	+	+	(+)	(+)	(+)	(+)	NE	NON OPTIMAL
CORRECT RESULTS (%)		**96.8**	**90.6**	**86.2**	**71.8**	**53.1**	**67.7**	**50**	**50**	**48.3**	**25**	**84.3**	**25**	**93.7**	**93.7**		

False negatives

False positives

Domingo C., et al. PLoS One 2012

PAHO-YFV molecular diagnosis EQA 2017

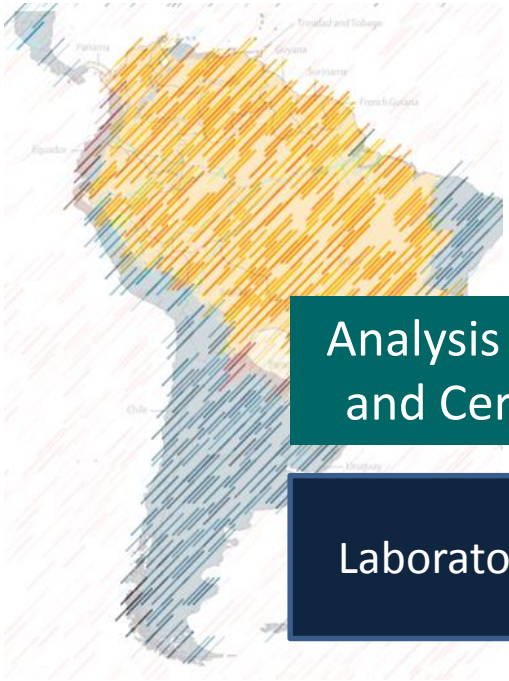
Panel Design

Sample Preparation

Inactivation

Lyophilization

RKI Validation



Analysis of Results and Certification

Laboratory Reports

CDC Validation

Distribution to regional laboratories

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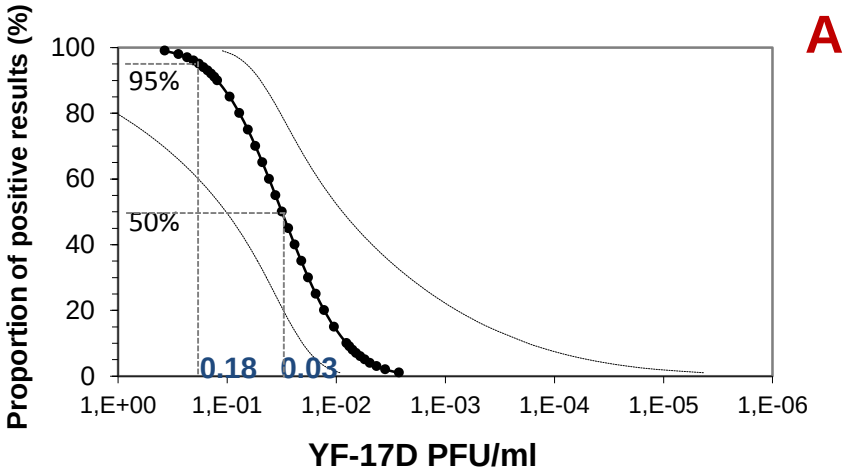
CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

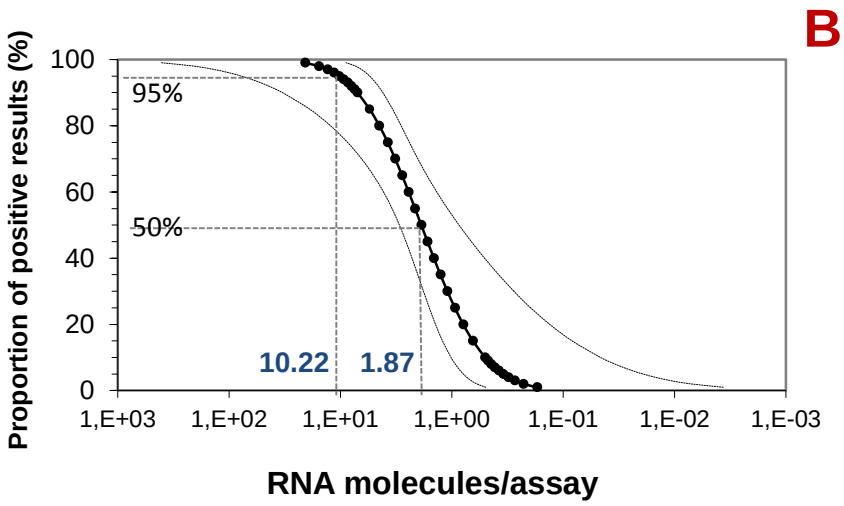
Pan American Health Organization

World Health Organization
REGIONAL OFFICE FOR THE Americas

ANALYTICAL PERFORMANCE OF THE YFall REAL-TIME RT-qPCR



Serial dilutions of titrated viral cell culture supernatant



Serial dilutions of quantified in vitro transcribed RNA

Domingo C. et al JCM 2012

SPECIFICATIONS OF THE YF RT-PCR (Domingo C., et al. 2012)

Primer or probe	Sequence[a]	Position[b]
Primers		
YFallF	5′-GCTAATTGAGGTGYATTGGTCTGC-3′	15–38
YFallR	5′-CTGCTAATCGCTCAAMGAACG-3′	83–103
YFallR-Biotin	5′-Biot-CTGCTAATCGCTCAAMGAA CG-3′	83–103
Probe		
YFallP	5′-FAM-ATCGAGTTGCTAGGCAATAAA CAC-TMR-3′	41–64

[a] Biot, biotin; TMR, 6-carboxytetramethylrhodamine; FAM, 6-carboxyfluorescein.
[b] Positions are indicated relative to GenBank sequence AY640589.1 for yellow fever virus Asibi strain.

Specificity tested:

- ✓ West Nile virus strain New York
- ✓ Japanese encephalitis virus strain SA-14-02
- ✓ St. Louis encephalitis virus strain Parton
- ✓ Tick-borne encephalitis virus strain K063; Chikungunya virus (CHIKV) strain Marseille
- ✓ and the four dengue virus serotypes DENV-1 VR344 strain Thai 1958), DENV-2 VR345 (strain TH-36), DENV-3 VR216 (strain H87), and DENV-4 VR217 (strain H241).

Sensitivity (LOD):

95% LOD : **10.22 RNA copies/reaction (QIAGEN reagents)**.

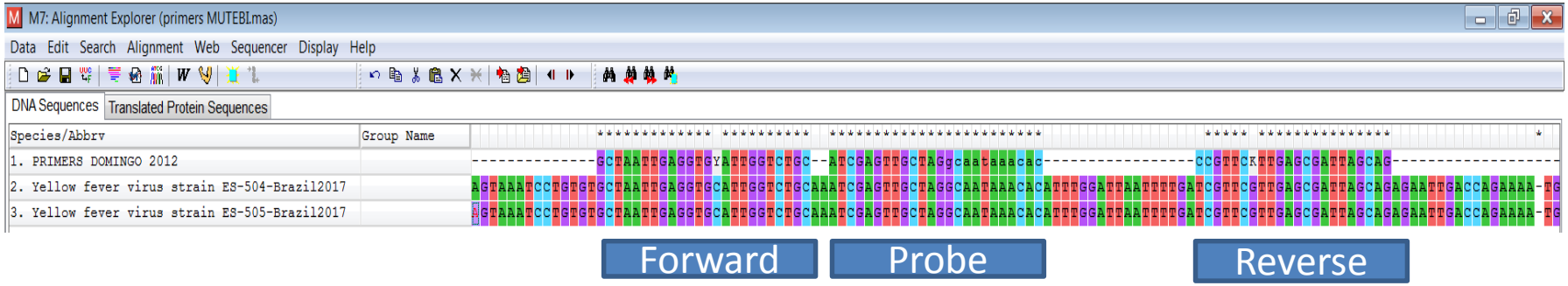
0.4µM primer (each)
0.2µM probe
2-5 µl sample

Thermal profile.
50°C 20 min
95°C 5 min
95°C 15 sec } 40 cycles
60°C 45 sec

Different batches of reagents, primers or probe must be validated prior to use for diagnostics

CHALLENGES FOR MOLECULAR DIAGNOSIS: VIRAL VARIABILITY

IN SILICO ANALYSIS OF PUBLISHED REAL-TIME RT-PCR DETECTION ASSAYS



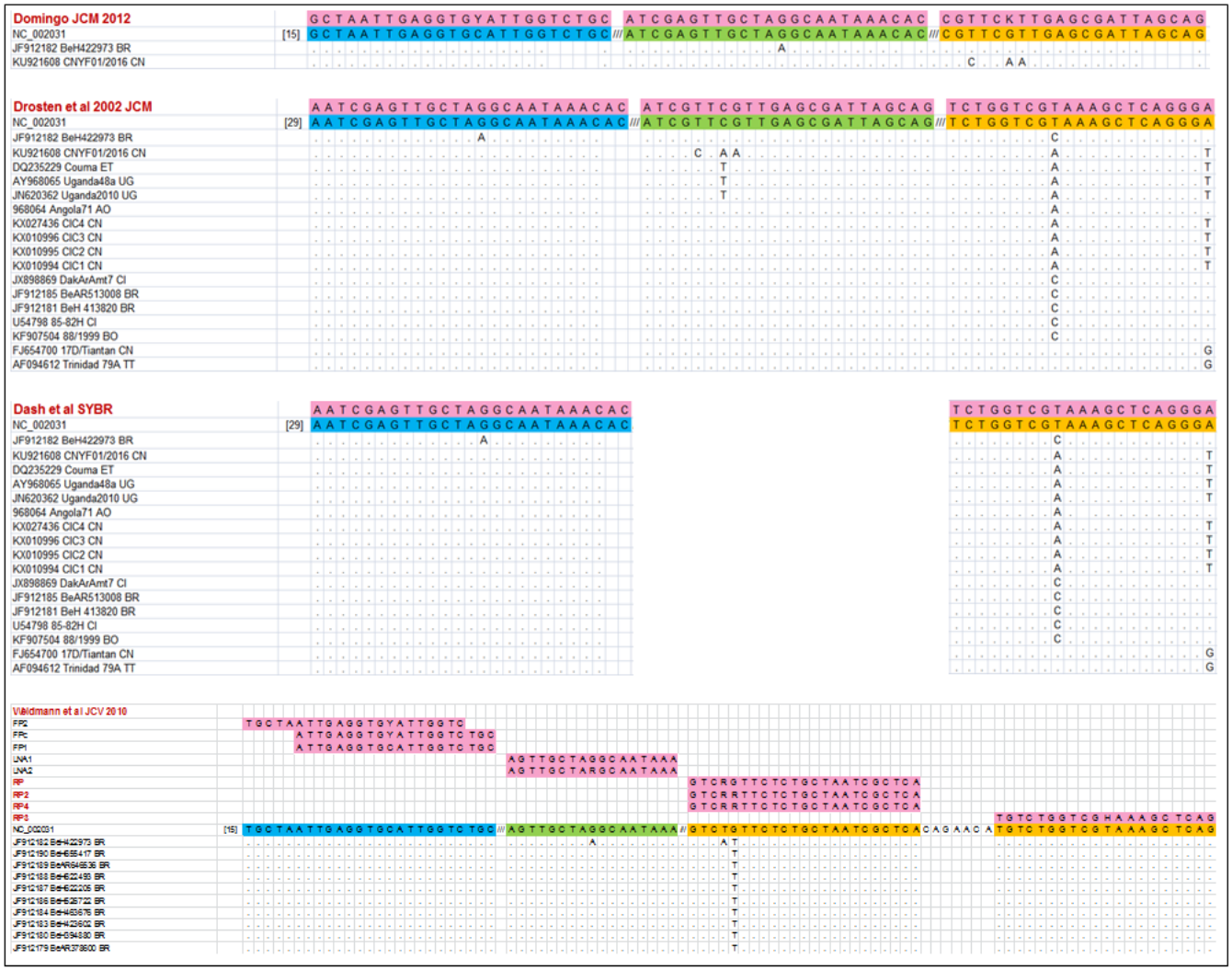
It is neccessary to check periodically that the presence of missmatches does not compromise the detection profile

In an outbreak context we need to have available the sequence of the circulating viruses to predict our capacity to detect it.

Domingo C. Charrel R. et al. Submitted.

CHALLENGES FOR MOLECULAR DIAGNOSIS: VIRAL VARIABILITY

IN SILICO ANALYSIS OF PUBLISHED REAL-TIME RT-PCR DETECTION ASSAYS



Domingo C. Charrel R. et al. Submitted.

CHALLENGES FOR MOLECULAR DIAGNOSIS: REAGENTS AVAILABILITY AND VALIDATION

It is not always easy for the laboratories to obtain the primers or probes **timely**

In some countries and/or in remote areas it can be difficult to mantain the **cold chain**

Field laboratories may not count with indepedent **PCR facilities with differentiated areas for premix preparation, sample extraction, PCR setting and thermocycling**

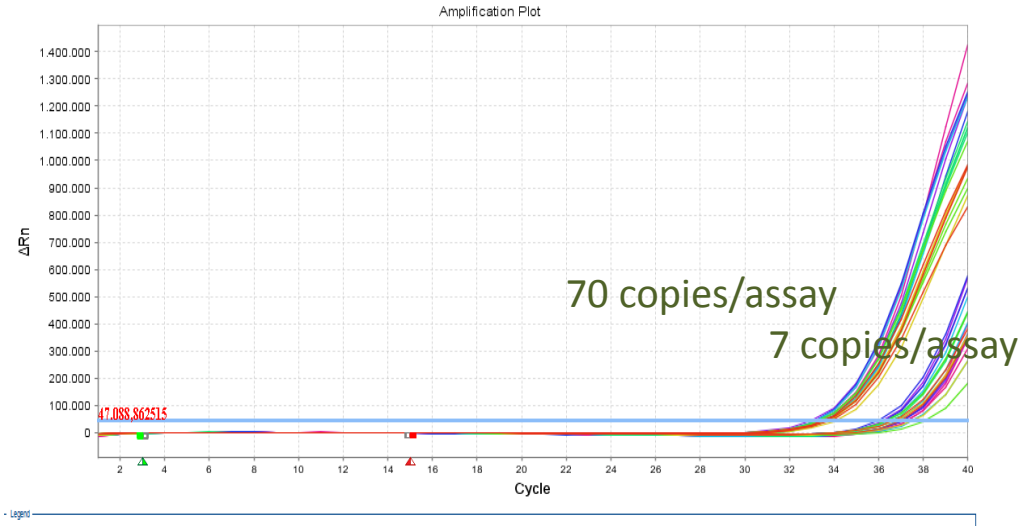
Expertise in molecular diagnostics is not easy to transfer in short time

OUTBREAK INTERVENTION OR PREPAREDNESS ACTIVITIES: LYOPHILIZED ASSAYS

Primer or probe	Sequence[a]	Position[b]
Primers		
YFallF	5′-GCTAATTGAGGTGYATTGGTCTGC-3′	15–38
YFallR	5′-CTGCTAATCGCTCAAMGAACG-3′	83–103
YFallR-Biotin	5′-Biot-CTGCTAATCGCTCAAMGAA CG-3′	83–103
Probe		
YFallP	5′-FAM-ATCGAGTTGCTAGGCAATAAA CAC-TMR-3′	41–64

[a] Biot, biotin; TMR, 6-carboxytetramethylrhodamine; FAM, 6-carboxyfluorescein.
[b] Positions are indicated relative to GenBank sequence AY640589.1 for yellow fever virus Asibi strain.

- ***Ready to use*** *YF-RT-PCR thermal tubes*
- *Minimal pipetting: 25 microliters RNA sample*
- *Includes an internal control*
- ***Standardized*** *master mix reagents: enables comparison between different runs and between different locations*
- *Independence of primer and probes supply*
- ***NO COLD CHAIN!!!***



Multicenter international evaluation

INSTITUTO EVANDRO CHAGAS
MS-SECRETARIA DE VIGILÂNCIA EM SAÚDE

Ministério da Saúde
FIOCRUZ - PARANÁ
Instituto Carlos Chagas

LABORATORIO CENTRAL DE SALUD PÚBLICA
MINISTERIO DE SALUD PÚBLICA Y BIENESTAR SOCIAL

Ministerio de Salud Pública
Instituto Nacional de Investigación en Salud Pública - INSPI

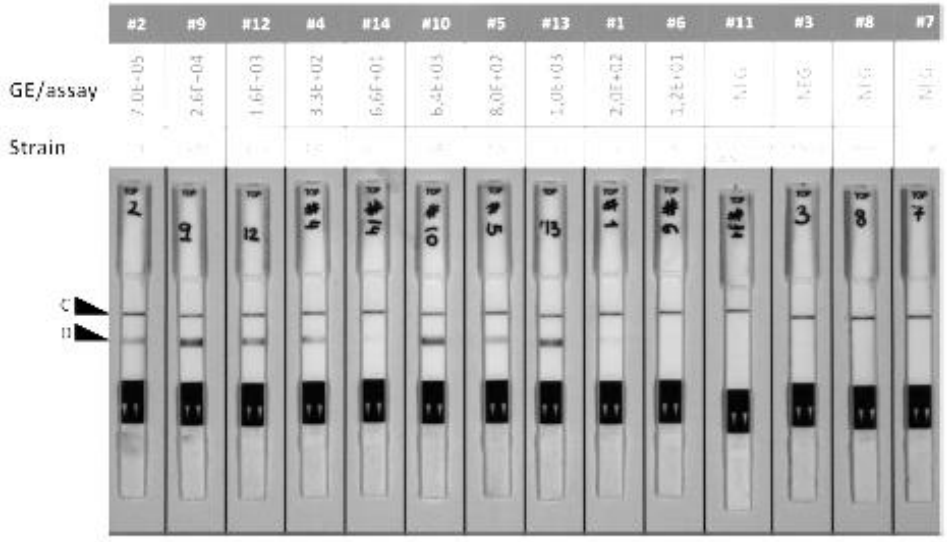
INRB

OUTBREAK INTERVENTION OR PREPAREDNESS ACTIVITIES: ISOTHERMAL ASSAYS

Primer or probe	Sequence[a]	Position[b]
Primers		
YFallF	5′-GCTAATTGAGGTGYATTGGTCTGC-3′	15–38
YFallR	5′-CTGCTAATCGCTCAAMGAACG-3′	83–103
YFallR-Biotin	5′-Biot-CTGCTAATCGCTCAAMGAA CG-3′	83–103
Probe		
YFallP	5′-FAM-ATCGAGTTGCTAGGCAATAAA CAC-TMR-3′	41–64

[a] Biot, biotin; TMR, 6-carboxytetramethylrhodamine; FAM, 6-carboxyfluorescein.
[b] Positions are indicated relative to GenBank sequence AY640589.1 for yellow fever virus Asibi strain.

- ***NO THERMOCYCLER!!***
- ***Easy interpretation***



Multicenter international evaluation

UNIVERSITÄTSMEDIZIN GÖTTINGEN : UMG

INSTITUTO NACIONAL DE SALUD

Institut Pasteur de Bangui

CHALLENGES AND LIMITATIONS OF THE SEROLOGICAL DIAGNOSIS OF YELLOW FEVER INFECTIONS

1. Presence of cross reactivities among flaviviruses
2. Lack of commercially available tests with good profile of sensitivity/especifity and **easy** to implement
3. Possible persistence of IgM antibodies (still to be confirmed in the general population)
4. PRNT as gold-standard (PRNT50 versus PRNT90)
5. Correct attribution of severe symptoms to either natural infection or the adverse effects of vaccination is particularly difficult in outbreak contexts.

YFV serological diagnosis External Quality Assesment results

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Table 2. EQA results of the 29 participant labs in the serological diagnosis panel.

		#6	#11	#7	4#	#5	#10	#14	#13	#1	#2	#9	#8	#12		
		αYF 17D	αYF17D	αYF17D	αYF 17D	αYF-AFR	αYF-AFR	αYF-SA	αYF-SA	αYF-SA	αWNV	αDEN	neg	neg		
		Expected results for YFV serology													Score*	
Lab. N°	Assay	IgM /IgG +/+	IgM /IgG +/+	IgM /IgG +/+	IgM /IgG +/-	IgM /IgG -/+	IgM /IgG -/-	IgM /IgG +/+	IgM /IgG +/+	IgM /IgG +/+	IgM /IgG -/-	IgM /IgG -/-	IgM /IgG -/-	IgM /IgG -/-	IgG	IgM
18	IIF [a]	-/+	-/+	-/-	-/-	-/-	-/-	+/+	+/+	-/+	-/-	-/-	-/-	-/-	22	16
12b	IIF [a]	-/+	-/+	-/-	-/-	-/-	-/-	+/+	+/+	+/+	-/-	-/-	-/-	-/-	22	18
27	IIF	+/+	-/+	-/-	-/-	-/-	-/-	+/+	+/+	+/+	-/-	-/-	-/-	-/-	22	20
4	IIF [a]	-/+	-/+	-/+	-/-	-/-	-/-	+/+	+/+	+/+	-/-	-/+	-/-	-/-	20	18
6	IIF [a]	-/+	-/-	-/eq	-/-	-/-	-/-	+/+	+/+	eq/+	-/eq	-/-	-/-	-/-	20	18
7	IIF [c]	-/+	-/+	-/-	-/-	-/-	-/-	+/+	+/+	eq/+	-/-	-/-	-/-	-/+	20	18
8	IIF [a]	+/+	-/+	-/-	-/-	-/-	-/-	+/+	-/+	+/-	-/-	-/-	-/-	-/-	20	18
29	IIF [a]	+/+	-/+	-/-	-/-	-/-	-/-	+/+	+/+	+/-	-/-	-/-	-/-	-/-	20	20
10	IIF [b]	+/+	+/+	+/+	-/+	-/-	-/-	+/+	+/-	-/-	-/-	-/-	-/-	-/-	20	22
5	IIF [b]	+/+	+/-	-/-	-/-	-/-	-/-	+/+	+/+	+/+	-/-	-/-	+/-	+/+	18	18
20a	IIF	+/+	-/-	-/-	-/-	-/-	-/-	+/+	+/+	-/-	-/-	-/-	-/-	-/-	18	18
3	IIF [a]	+/-	+/-	+/-	-/-	-/-	-/-	+/+	+/+	+/+	-/-	-/-	+/-	-/-	18	22
2	IIF [b]	nd/+	nd/+	nd/-	nd/-	nd/-	nd/-	nd/+	nd/+	nd/+	nd/+	nd/+	nd/-	nd/-	18	nd
25	IIF [a]	+/+	eq/-	-/-	-/eq	-/-	-/-	+/+	+/+	eq/-	eq/-	-/-	eq/-	-/-	16	18
12a	IIF [b]	nd/-	nd/-	nd/-	nd/-	nd/-	nd/-	nd/+	nd/+	nd/-	nd/-	nd/-	nd/-	nd/-	16	nd
13	IIF [d]	nd/-	nd/-	nd/-	nd/-	nd/-	nd/-	nd/+	nd/+	nd/-	nd/-	nd/-	nd/-	nd/-	16	nd
28	IIF [a]	-/-	-/-	-/-	-/-	-/-	-/-	+/+	+/-	-/-	-/-	-/-	-/-	-/-	14	16
14a	IIF [a]	+/-	-/-	-/-	-/-	-/-	-/-	+/+	+/-	+/-	-/-	-/-	-/-	-/-	14	20
11	IIF [b]	nd/-	nd/-	nd/-	nd/-	nd/-	nd/-	nd/+	nd/-	nd/-	nd/-	nd/-	nd/-	nd/-	14	nd
1	IIF [a]	-/-	-/-	-/-	-/-	-/-	-/-	+/-	+/-	-/-	-/-	-/-	-/-	-/-	12	16
16	ELISA [f,g]	-/-	-/-	-/-	-/-	-/-	-/-	+/-	+/-	+/-	-/-	-/-	-/-	-/-	12	18
19	ELISA [b]	+/eq	+/-	+/+	eq/+	-/-	-/-	+/-	+/-	-/+	+/+	-/+	+/+	-/+	10	20
26	ELISA [b]	-/nd	-/nd	-/nd	nd/nd	-/nd	-/nd	nd/nd	+/nd	-/nd	-/nd	-/nd	-/nd	-/nd	nd	14
23	ELISA	-/nd	-/nd	-/nd	-/nd	-/nd	-/nd	+/nd	+/nd	+/nd	-/nd	-/nd	-/nd	-/nd	nd	18
22	ELISA [e]	eq/nd	eq/nd	-/nd	-/nd	-/nd	-/nd	+/nd	+/nd	+/nd	-/nd	-/nd	-/nd	-/nd	nd	22
24	NT	+	+	+	+	-	-	+	+	+	-	-	-	-	24	
30	NT	+	+	+	+	+	-	+	+	+	-	-	-	+	24	
9	NT	+	+	+	-	-	-	+	-	-	-	-	-	-	18	
15	NT	+	+	+	-	+	-	-	+	+	-	-	+	+	18	
14b	NT	+	+	-	-	-	-	+	-	-	-	-	-	-	16	
20b	HAI	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	6	

IgM is the preferred test for the identification of acute cases!!!

CHALLENGES AND LIMITATIONS OF THE SEROLOGICAL DIAGNOSIS OF YELLOW FEVER INFECTIONS

1. Presence of cross reactivities among flaviviruses

2. Lack of commercially available test with a good (and **validated**) profile of sensitivity/especifity and ***easy*** to implement

3. Possible persistence of IgM antibodies (still to be confirmed in the general population)

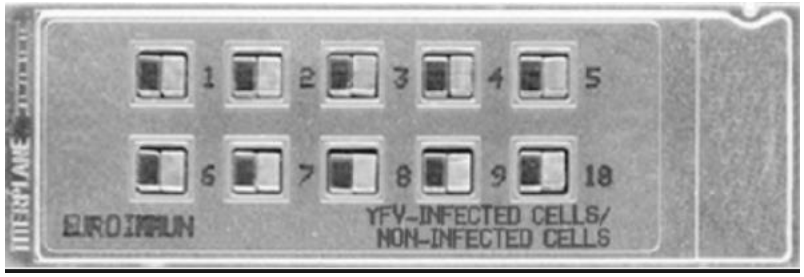
4. PRNT as gold-standard (PRNT50 versus PRNT90)

5. Correct attribution of severe symptoms to either natural infection or the adverse effects of vaccination is particularly difficult in outbreak contexts.

CHALLENGES AND LIMITATIONS OF THE SEROLOGICAL DIAGNOSIS OF YELLOW FEVER INFECTIONS

EUROIMMUN IIF YELLOW FEVER

- Uses whole YF antigen as substrate
- 96% IgM sensitivity; 94.7% IgG sensitivity (tested in yellow fever vaccinees)
- Specificity tested in German blood donors (4% IgM positive; 6% IgG positive)
- Tested with positive sera for JEV, DENV, and TBEV gives a 20% positive results in YF IgM.
- Positive IgG DENV sera presented a positive IgG YFV result in 100% cases; IgM 22.2% cases
- Positive IgG JEV sera presented a positive IgG result in 100% cases; IgM 33.3% cases
- Positive IgG WNV sera presented a positive IgG result in 91.7% cases; 33.3%
- **No data on Zika cross-reactivities!!!**
- It has a lower sensitivty for the immune response to the vaccine
- Advantage of testing in parallel no-antigen preparation.



DEMANDING REGARDING:

Microscope maintenance (LED???)

Technical expertise of the person interpreting the results (sujective)

CHALLENGES AND LIMITATIONS OF THE SEROLOGICAL DIAGNOSIS OF YELLOW FEVER INFECTIONS

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CDC YFV MAC-ELISA

- Whole-virus antigen propagated in mouse brain
- Over two days to perform
- Reagents exhibit lot-to-lot variation (not all reagents are supplied by US CDC)
- Variation in storage conditions may also influence the quality of results.
- Prior standardization is required in each practicing locale, which confines the test to well-trained laboratories.
- Despite these limitations, the availability of these reagents has for years afforded access to IgM testing of laboratories in endemic regions.
- An **improved MAC-ELISA** kit provided by US CDC, employing antigen produced in Vero cells and reagents supplied lyophilized and stabilized. This test, which can be run in one day, is intended for use in standard laboratories.

Abbexa Ltd. (Cambridge, UK) (Human yellow fever virus IgM/IgG ELISA kit)

96-well plate format. **No data** on performance

MyBiosource Inc. (San Diego, CA, USA) Human Yellow Fever Antibody IgM (YFV-IgM)/IgG (YFV-IgG) ELISA kit

48 and 96 samples format. **No data** on specificity/sensitivity

TARIKI Fiebre Amarilla IgM capture ELISA

Sensitivity of 95% (CI 95%: 87-100) and specificity of 98% (CI 95%: 87-100)

A **validation** of these reagents is urgently needed

CHALLENGES AND LIMITATIONS OF THE SEROLOGICAL DIAGNOSIS OF YELLOW FEVER INFECTIONS

1. Presence of cross reactivities among flaviviruses
2. Lack of commercially available test with good profile of sensitivity/especifity and easy to implement
3. **Possible persistence of IgM antibodies** still to be confirmed in the general population
4. PRNT as **gold-standard** : only in Reference laboratories, difficult to transfer, time and work intensive
5. Correct attribution of severe symptoms to either natural infection or the adverse effects of vaccination is particularly difficult in outbreak contexts.

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YELLOW FEVER VACCINATION CAMPAIGNS IN OUTBREAK CONTEXTS: CHALLENGES FOR DIAGNOSIS

The **antigenic similarity** between wild-type and YFV vaccine strains does not permit clear serological identification in such cases, and the distinction is only possible at present through the molecular characterization of the strain involved by strain-specific PCR amplification or by sequence analysis.

HOWEVER: the detection of the yellow fever vaccine strain in an individual shortly after vaccination does not imply that this is the causative agent of the disease

It is important to accurately apply the Brighton criteria for the definition and classification of YFAE and to perform an exhaustive **differential diagnosis**

STUDY OF ADVERSE EVENTS AFTER YELLOW FEVER VACCINATION IN AFRICAN MASS CAMPAIGNS: SUMMARY OF CASES STUDIED FROM 2009 TO 2012

CLASSIFCATION RECEIVED AT RKI

	SUSPECTED SEVERE YFAE/RKI EVALUATED (fatal cases)	SUSPECTED/CONFIRMED YF-AVD	SUSPECTED/CONFIRMED YF-AND	SUSPECTED/CONFIRMED HYPERS. REAC.	NON CLASSIFIED	MALARIA	DENGUE	HEPATITIS	OTHER INFECTIONS	PROGRAMATIC ERROR
Ivory Coast	18/8 (1 fatal)	5	3/1	0	0	-	2 (1 fatal)	-	-	-
Burkina Faso	NA/23	3	17	1	2	2*	2	-	•Bacterial meningitis*	1*
Liberia	52/17 (4 fatal)	6	7	0/1	4	4	1	1	•Herpes Zoster*	-
Benin	NA/29 (2 fatal)	9	9	8/**7**	3	4	2	1	•Bacterial meningitis* plus EBV	-
Sierra Leone-2	399/4 (1 fatal)	1	2	1/1	0	-	1	-	•Sickle cell disease* •Thyphoid*	-
Cameroon	45/8 (1 fatal)	1	3	2/**1**	2	3	-	-	-	1*
Total	89 cases RKI	25	32/**1**	12/**10**	11	**13**	**8**	**2**	**5**	**2**

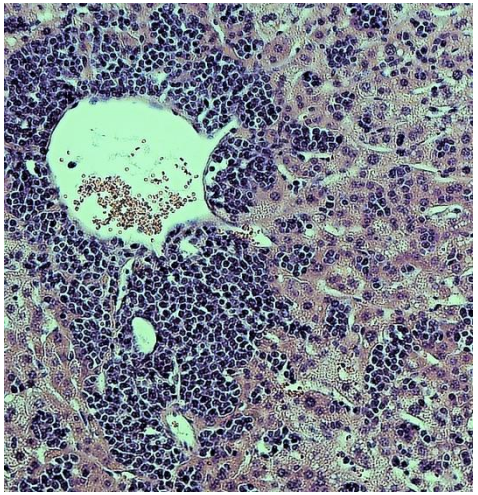
*** Diagnosis at Reference Hospital**

YELLOW FEVER DIAGNOSIS IN OUTBREAK CONTEXTS: CHALLENGES AND LIMITATIONS

YELLOW FEVER VIRAL ISOLATION

Epithelial and kidney fibroblasts from monkey (MA-104, Vero, LLC-MK2), rabbit (MA-111), baby hamster (BHK), and mosquito derived cells like *Aedes pseudoscutellaris* (AP-61), and *Aedes albopictus* (C6/36) cell lines

Intracerebral inoculation of suckling mice or hamsters or intrathoracic inoculation of mosquitoes



CHALLENGES

- Requires well stablished cell culture and **BSL-3** facilities and vaccinated personnel
- **Not a rapid result**, not for first line diagnosis
- **Influenced** by antibodies against the virus, the conditions of sample maintenance, the isolation system used, and the presence of metabolic products
- Increases dramatically the burden of the Reference Lab. Strategy definiton in outbreak context.

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