



Rabies Diagnosis in NCVD DAH-VIET Nam



Rabies situation

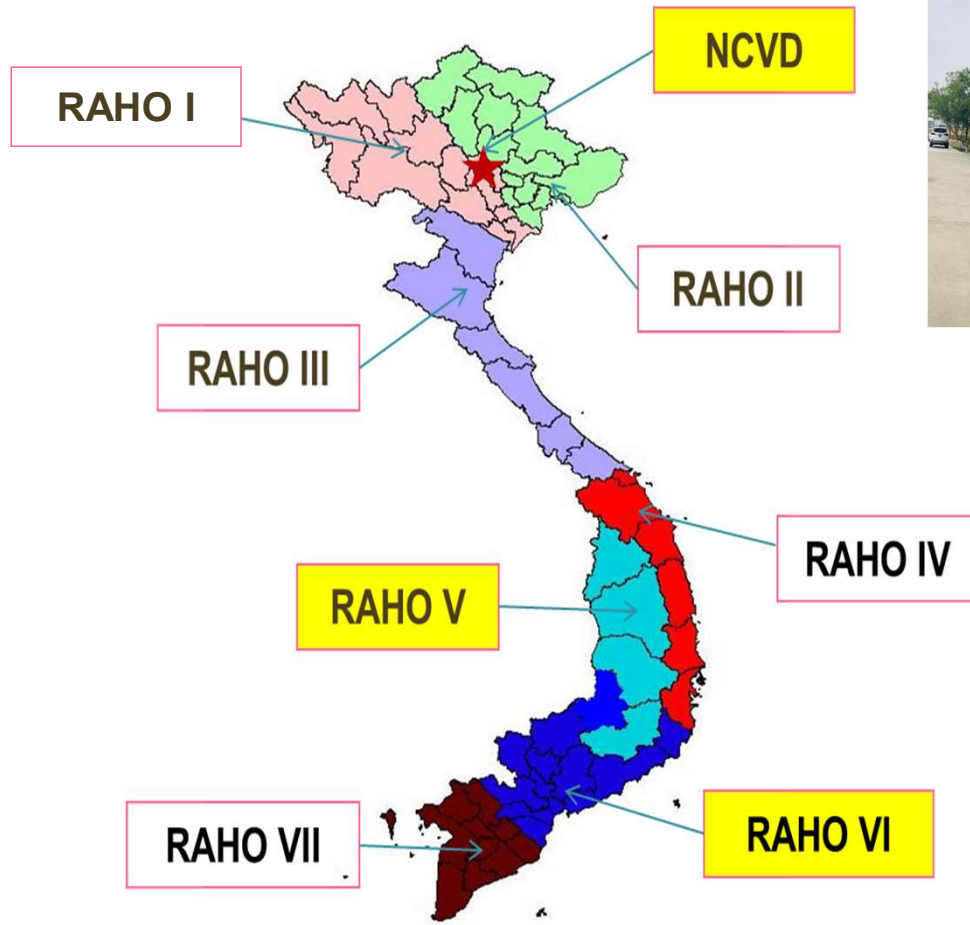
❖ Human

- To 17/08/2023: 60 deaths due to Rabies in 26 provinces and cities
- Increase of 17 cases compared to the same period in 2022

❖ Animal

- Dog population: 7 mill
- Vaccination rate of dog: low (40%). In big cities > 70%
- 2023: number of rabies cases increased
- National program for rabies prevention and control, period 2022 - 2030

NCVD



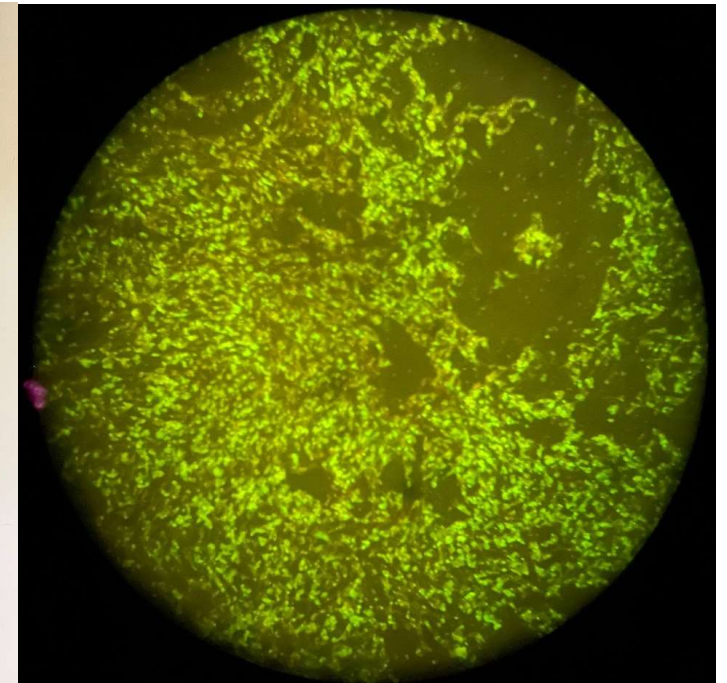
- Total: 4,3 ha
- administration
- Laboratory BSL2: 39 labs/ 2.550m²
- Laboratory BSL3: 4 labs/280 m²
- Animal experiment house (ABSL)

Testing samples in NCVD

Testing for animal disease		Testing for SARS CoV2 in human	
2021	2022	2021	2022
97.405	96.162	150.000 tests/ 782.000	12.463 / 25.540



Rabies Diagnosis in NCVD





Capacity building project in 2014 - 2015

- Rabies testing has been neglected for more than 10 years .
- NCVD, the first lab in Viet Nam was equipped with rabies diagnostic capacity under the “Rabies control and elimination program 2014” by DAH including the following items:
 - Equipment
 - Reagent and kit
 - Vaccination
 - Training

Principle equipments



Flourescent microscope



PCR machine



110 litre autoclave



Biosafety cabinet class2



Dog head immobilization equipment and surgical instruments

Vaccination



Văc-xin VEROLAB

vaccinate 3 shots within the first year

Shot 1: day 0

Shot 2 day 7

Shot 3: day 21

Repeat vaccination after 1 year (1 shot)



Antibody check after vaccination

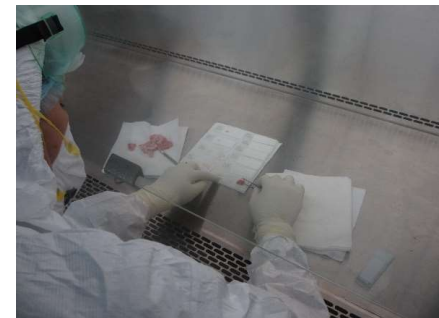
Training



Training by Viet nam NIHE experts

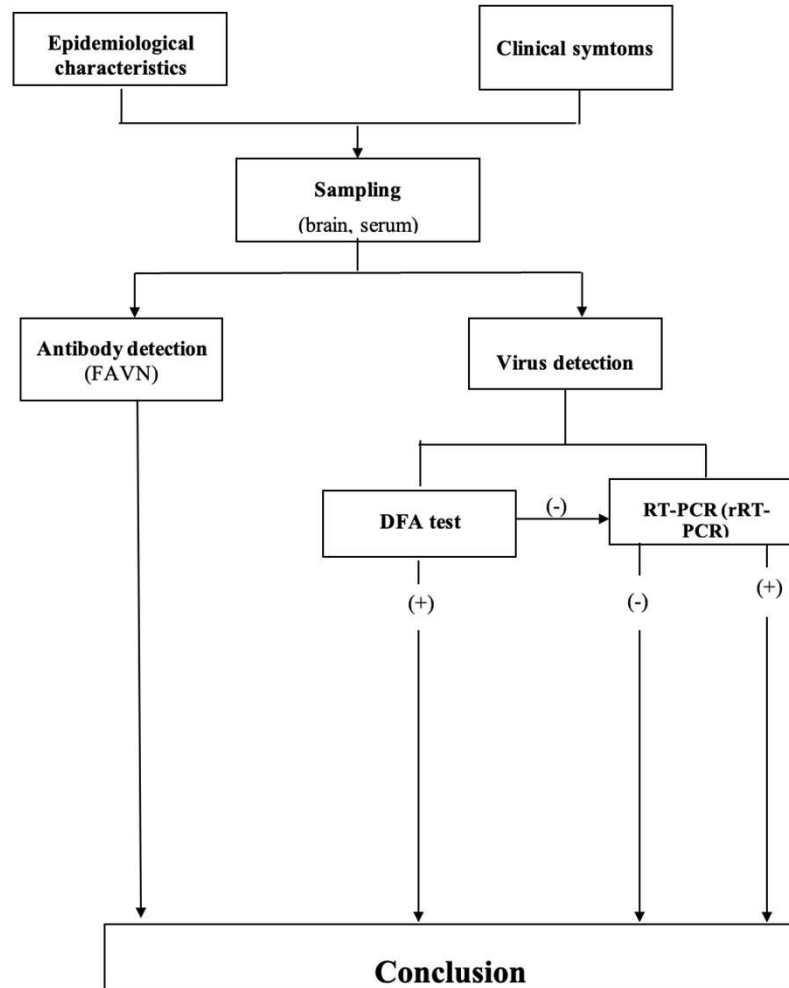


Training by US CDC experts



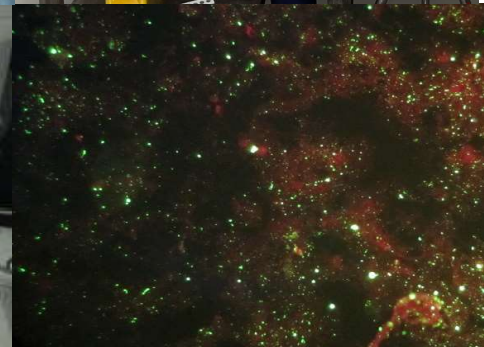
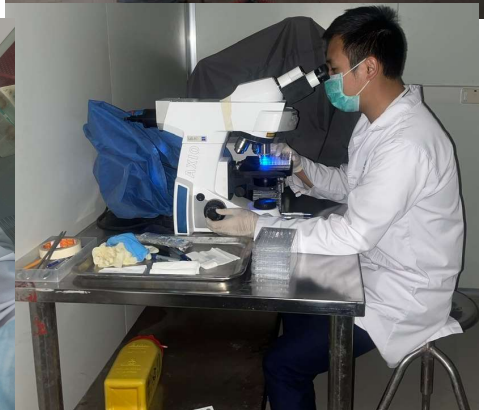
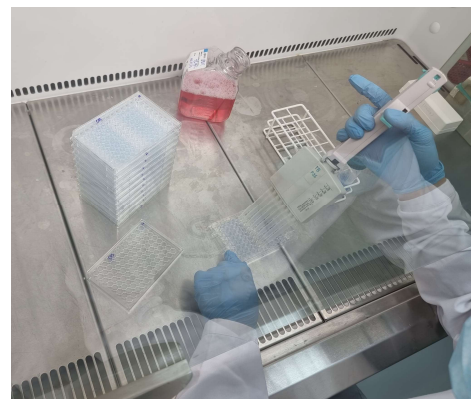
Practicing dog head opening to collect brain tissue for FAT test

Diagnosis diagram for Rabies



Testing Methods

- **DFAT - OIE**
- **Realtime RT-PCR (CDC protocol)**
 - Virus detection
 - Diagnosis & surveillance
- **FAVN test on cell culture**
 - Antibody testing
 - Safety zone certification
- **Virus isolation**



Rabies test - 2020

Province	No of Sample	Positive	% positive	program
Bac Ninh	1	0	0.00	Routine
Dien Bien	2	1	50.00	Routine
Ha Noi	29	3	10.34	Surveillance
Ha Tinh	1	0	0.00	Routine
Hai Duong	1	0	0.00	Routine
Lang Son	46	20	43.48	Surveillance
Lao Cai	3	1	33.33	Routine
Nghe An	100	0	0.00	Surveillance
Ninh Binh	1	0	0.00	Routine
Phu Tho	1	0	0.00	Routine
Quang Binh	1	0	0.00	Routine
Quang Ninh	1	1	100.00	Routine
Son La	50	0	0.00	Surveillance
Thai Nguyen	2	0	0.00	Routine
Thanh Hoa	1	0	0.00	Routine
Tuyen Quang	1	0	0.00	Routine
Total	241	26	10.79	

- **Samples: suspected dog, cat and cattle from North and Center VN, slaughterhouse**

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Rabies test - 2021

Province	No of Sample	Positive	% positive	program
Dien Bien	5	3	60.00	Routine
Ha Nội	258	2	0.78	Surveillance
Hai Phong	1	0	0.00	Routine
Hung Yen	1	0	0.00	Routine
Lang Son	28	25	89.29	Surveillance
Lao Cai	1	0	0.00	Routine
Nam Dinh	1	0	0.00	Routine
Nghe An	67	0	0.00	Surveillance
Phu Tho	1	0	0.00	Routine
Quang Nam	3	3	100.00	Routine
Quang Ninh	6	4	66.67	Routine
Son La	66	4	6.06	Surveillance
Thai Nguyen	4	0	0.00	Routine
Tuyen Quang	1	1	100.00	Routine
Vinh Phuc	1	0	0.00	Routine
Total	444	42	9.46	

- **Samples: suspected dog, cat and cattle from North and Center VN, slaughterhouse**

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Rabies test - 2022

Province	No of Sample	Positive	% positive	program
Bac Giang	38	1	2.63	Surveillance
Bac Kan	1	1	100.00	Routine
Bac Ninh	1	0	0.00	Routine
Dien Bien	52	11	21.15	Surveillance
Ha Giang	1	1	100.00	Routine
Ha Noi	200	2	1.00	Surveillance
Hai Phong	1	0	0.00	Routine
Hung Yen	1	0	0.00	Routine
Lai Chau	69	5	7.25	Surveillance
Lang Son	68	5	7.35	Surveillance
Lao Cai	39	1	2.56	Surveillance
Nghe An	67	2	2.99	Surveillance
Ninh Binh	1	0	0.00	Routine
Phu Tho	63	35	55.56	Surveillance
Quang Nam	1	1	100.00	Routine
Quang Ninh	1	1	100.00	Routine
Son La	137	3	2.19	Surveillance
Thai Nguyen	60	0	0.00	Surveillance
Thanh Hoa	61	0	0.00	Surveillance
Vinh Phuc	24	0	0.00	Surveillance
Total	886	69	7.79	

- **Samples: suspected dog, cat and cattle from North and Center VN, slaughterhouse**

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Rabies test – 8/2023

Province	No of Sample	Positive	% positive	program
Bac Kan	2	2	100.00	Routine
Bac Ninh	2	0	0.00	Routine
Dien Bien	11	6	54.55	Surveillance
Ha Noi	32	2	6.25	Routine
Hung Yen	3	0	0.00	Routine
Lai Chau	39	7	17.95	Surveillance
Lao Cai	2	2	100.00	Routine
Nam Dinh	1	0	0.00	Routine
Nghe An	1	1	100.00	Routine
Ninh Binh	1	0	0.00	Routine
Phu Tho	33	25	75.76	Surveillance
Quang Ninh	94	2	2.13	Surveillance
Quang Tri	1	0	0.00	Routine
Son La	3	1	33.33	Routine
Thai Nguyen	2	0	0.00	Routine
Thanh Hoa	6	3	50.00	Routine
Tuyen Quang	1	0	0.00	Routine
Vinh Phuc	28	0	0.00	Surveillance
Total	262	51	19.47	

- **Samples: suspected dog, cat and cattle from North and Center VN, slaughterhouse**

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Active surveillance supported by CDC

Year	Province	Sample	Positive	%
2020	2	90	12	13.33
2021	2	126	31	24.60
2022	5	127	46	36.22
8/2023	11	131	69	52.67

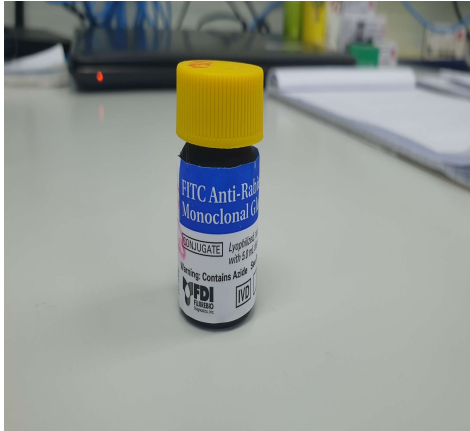
- Supported by CDC
- NCVD: 2020 - 2021 (Phu Tho, Nghe An); 2022 (Phú Thọ, Điện Biên, Lào Cai, Nghệ An, Quảng Trị): suspected and slaughter samples
- 2023 (Bắc Kạn, Điện Biên, Hà Giang, Lai Châu, Lạng Sơn, Lào Cai, Nghệ An, Phú Thọ, Quảng Ninh, Quảng Trị, Vĩnh Phúc) **suspected samples**

FAVN for Antibody testing

Year	No. Province	Sample	Positive	%	% protection
2020	5	610	452	74.09	70-100
2021	5	352	293	83.23	70-100
2022	8	1087	960	88.31	70-100
2023	8	1494	1419	94.97	60-100

- Ab evaluation after vaccination: 0.5 IU/ml: protected

FAVN for Antibody testing



Result reading

1. The reading method is "all or nothing" only : no fluorescent cell – a minus score is recorded for the well; fluorescent cells (one cell or more) – a plus score is recorded for the well.
2. Cell and virus controls are read first.

* Formula to convert the log D₅₀ value in IU/mL titer

$$\text{Serum titre (IU/ml)} = \frac{[(10^{\text{serum log D}_{50} \text{ value}}) \times \text{theoretical titre of positive standard serum 0.5 IU/ml}]}{(10^{\text{log D}_{50} \text{ of positive standard serum 0.5 IU/ml}})}$$

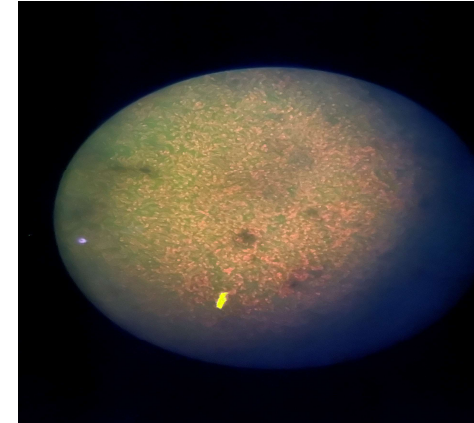
Example of conversion:

- log D₅₀ of the serum = 2.27
- theoretical titre of positive standard serum 0.5 IU/ml = 0.5 IU/ml
- log D₅₀ of positive standard serum = 1.43
(for the log D₅₀ of positive standard, the value of the day or the mean value can be considered)

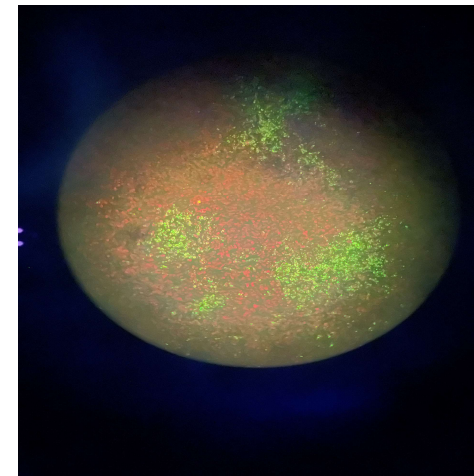
$$\text{Serum titre (IU/ml)} = \frac{10^{2.27} \times 0.5}{(10^{1.43})} = 3.46 \text{ IU/ml}$$

Dilution	1		2		3		4		
	Log	IU/ml	Log	IU/ml	Log	IU/ml	Log	IU/ml	
A	0.48	0.36	0.07	0.48	0.10	0.60	0.13	0.72	0.17
B	0.95	0.84	0.22	0.96	0.23	1.08	0.39	1.19	0.59
C	1.43	1.51	0.66	1.43	0.87	1.55	1.15	1.67	1.51
D	1.91	1.79	1.99	1.91	2.62	2.03	3.46	2.15	4.56
E	2.39	2.27	6.01	2.39	7.92	2.51	10.45	2.69	13.77
F	2.87	2.75	18.15	2.87	23.93	2.99	31.95	3.11	41.58
G	3.35	3.23	94.63	3.35	72.27	3.47	95.27	3.59	125.59
H	3.83	3.71	185.97	3.83	218.28	3.95	287.72	4.07	379.29

❖ All Ab negative : Log 0.24, 0.06 IU/ml
 ❖ Rabies protective level : 0.5 IU/ml = VN (8-16 fold)



Negative sample



Positive sample

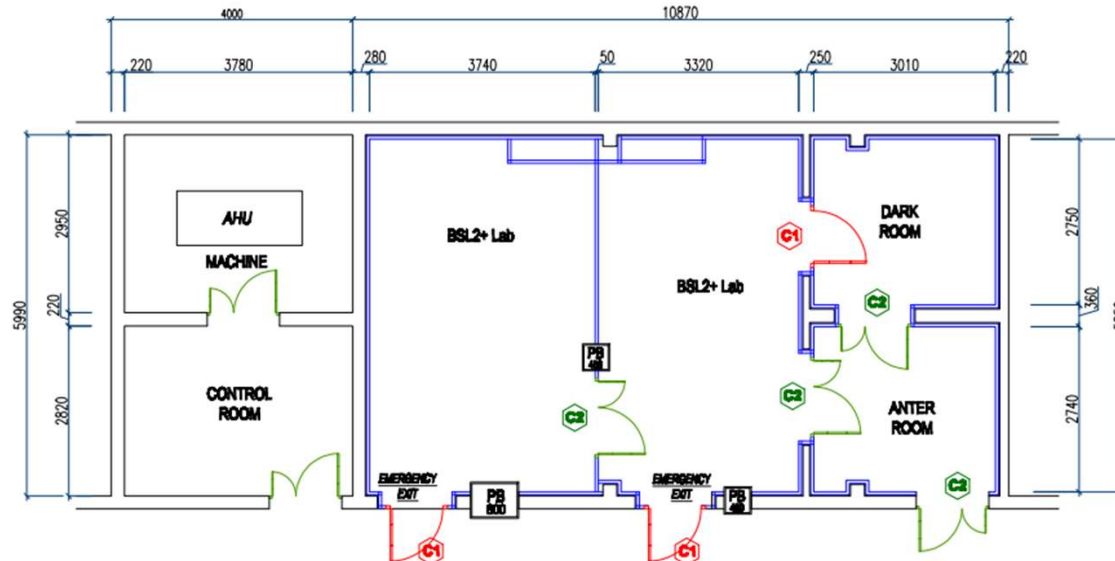
Rabies lab – BSL3

Purpose of usage: FAVN, virus isolation and culture







Design of new Rabies testing lab – BSL2+

Purpose of usage: Autopsy, FAT, Training



ROOMS PLAN

	- BRICK WALL		PASSBOX 450X450X450MM, 1M HIGH FROM FLOOR
	- WALL PANEL 50MM, HIGH 2,6 m		PASSBOX 800X600X800MM, 1M HIGH FROM FLOOR



Quality Assurance

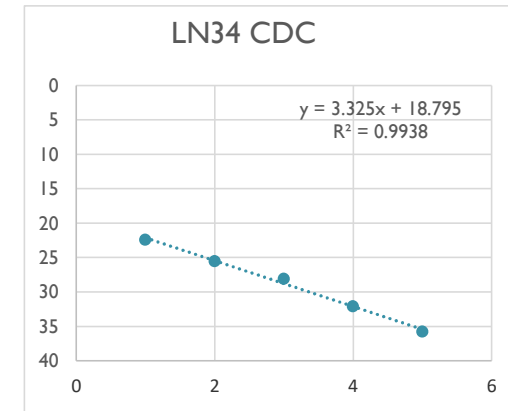
- Validation Real time RT PCR
- National Diagnostic Procedure for rabies
- ISO 17025 accreditation
- Biosafety SOP
- Set up separated lab for rabies

Review & validate qRT PCR

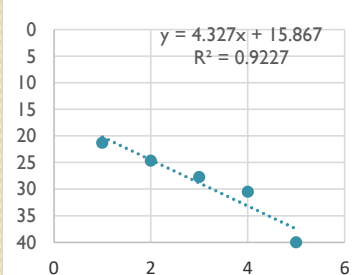
No	PPP Name	Target	Source/ Used by	Primer/Probe	Sequences (5'-3')	Reference
1	RABVD 1	N gene	AAHL	RABVD1F	ATGTAACACCYCTACAATG	Nadin-Davis et al. (2009) J. Medical Virology 81: 1484-1497.
				RABVD1R	GCMGGRTAYTTRTAYTCATA	
				RABVD1P	FAM-CCGAYAAGATTGTATTYAARGTCAAKAATCAGGT-BHQ1	
2	RABVD 2	N gene	AAHL/RAHO6	RABVD2F	TRATGACAACYCACAARATGT	
				RABVD2R	TGARCAGTCYTCRTARGC	
				RABVD2P	FAM-TAYGACATGTTTTTCTCYCGGATTGARCATC-BHQ1	
3	RABVD 3	N gene	AAHL	RABVD3F	AYTTCTTCCAYAARAACCTTYGA	
				RABVD3R	CATCCRACAAAGTGRATGAG	
				RABVD3P	FAM-TGYCCYGGCTCRAACATYCTYCTTAT-BHQ1	
4	LN34	N gene	CDC/NCVD	LN34 F. primer 1	ACGCTTAACAACCAGATCAAAGAA	
				LN34 F. Primer 2	ACGCTTAACAACAAAATCADAGAAG	
				LN34 Re Primer	CMGGGTAYTTRTAYTCATAYTGRTC	
				LN34 Probe	(FAM) AA+C+ACCY+C+T+ACA+A+TGGA (BHQ1)	
	B-actin (internal control)	B-actin mRNA	CDC	LN34 Lagos Probe	(FAM) AA +C +ACTA +C +T +ACA +A +TGGA (BHQ1)	
				β-Actin Fr Primer	CGATGAAGATCAAGATCATTGC	
				β-Actin Re Primer	AAGCATTGCGGTGGAC	
				β-Actin Probe	(HEX)-TCC ACC TTC CAG CAG ATG TGG ATC A-(BHQ1)	
5	RVFQ	N gene	CVRI	RVFQ For	ATGTAACACCYCTACAATG	China
				RVFQ Rev	GCAGGG TAYTTRTACTCATA	
				RVFQ Probe	FAM-ACAAGATTGTATTCAAAGTCAATAATCAG-TAMRA	
6	Lyssa N1	N gene	NCVD	Forward	CACMGSNAAYTAYAARACNAA	Coertse, J (2010) J. C. Microbiology
				Reverse	GTRCTCCARTTAGCRCACAT	
				Probe	FAM-CATCACACCTTGATGACAACCTCACAA-BHQ1	
7	Rabies N2	N gene	NCVD	Forward	GATCCTGATGAYGTATGTTCCCTA	Hoffmann, B. (2010) J. Cl. Microbiology
				Reverse	RGATTCCGTAGCTRGTTCCA	
				Probe	FAM-CAGCAATGCAGTYYTTTTGAGGGGAC-BHQ1	

Efficiency Validation

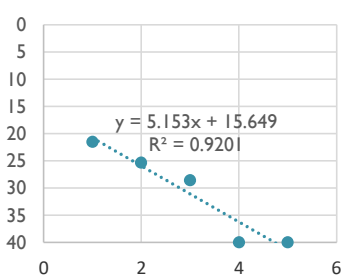
Môi/Nguồn	Slope	R ²	Thời gian gốc (phút)
RabiesN2 - NCVD	4.327	0.9227	132
LyssaN1 - NCVD	5.153	0.9201	132
RABVD1 - AAHL	3.438	0.9997	85
RABVD2 - AAHL	3.171	0.976	85
RABVD3 - AAHL	3.369	0.9948	85
RVFQ - CVRI	3.339	0.9964	112
LN34/B-actin - CDC	3.325	0.9938	110 (chạy được multiplex)



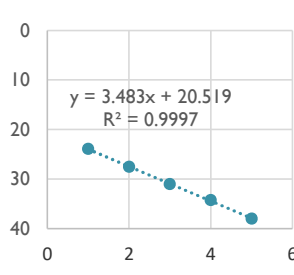
NCVD Rabies N2



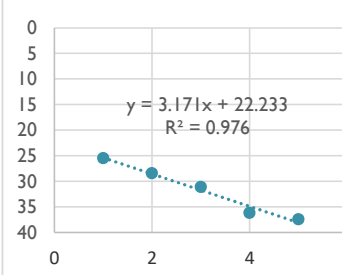
NCVD Lyssa NI



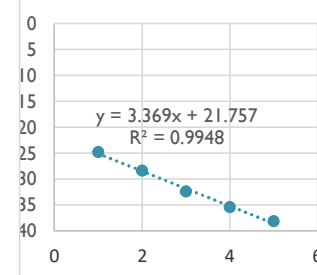
AAHL RABVD I



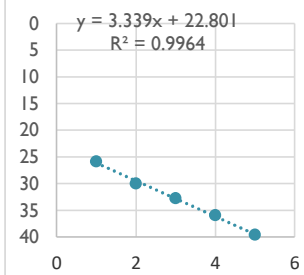
AAHL RABVD 2



AAHL RABVD 3



CVRI RVFQ



Training and Networking

- Trainings provided
 - RAHO 5: sampling, transportation, diagnostic method (DFAT and realtime RT-PCR)
 - Sub-DAH (LaoCai, Thai Nguyen, Phu Tho): surveillance, sampling, transportation
- Supply of reagents to RAHO 5: DFAT reagents & Realtime RT-PCR primers/probe
- Collaboration with Human Health Sector
- Meeting

Twining project with WOAH lab preparation

- Meeting with NCEZID representative of US CDC in NCVD on 18/9/2023
 - Update rabies diagnosis activity in NCVD
 - Discuss on objectives, goals, expected outcomes, application process of the project



Work Plan

- ❖ **Apply for twinning project with WOAHA lab**
 - **To enhance capacity of NCVD in rabies diagnosis and research**
- ❖ **Validate Real-time TaqMan RT PCR**
 - **To Recommend to WOAHA Manual of Diagnostic Tests**
- ❖ **Upgrade rabies diagnosis laboratory to BSL2+**
- ❖ **Organize Rabies PT program**
- ❖ **Perform sequencing and phylogenetic analysis**

Challenges

- **Lack of inverted fluorescent microscope for FAVN test.**
- **Some serum samples were submitted in hemolytic condition causing difficulty for FAVN testing.**
- **Some brain samples submitted in rotted condition causing difficulty for FAT testing**
- **No PT programs for rabies available in the region**
- **Some reagents for rabies testing are not available in Viet Nam such rabies conjugate, probe with special lock**

THANK YOU FOR YOUR ATTENTION

