



13th Workshop for Rabies

Warsaw, Poland
15-16 June 2022

Human rabies: the diagnostic challenge



Laurent Dacheux
laurent.dacheux@pasteur.fr



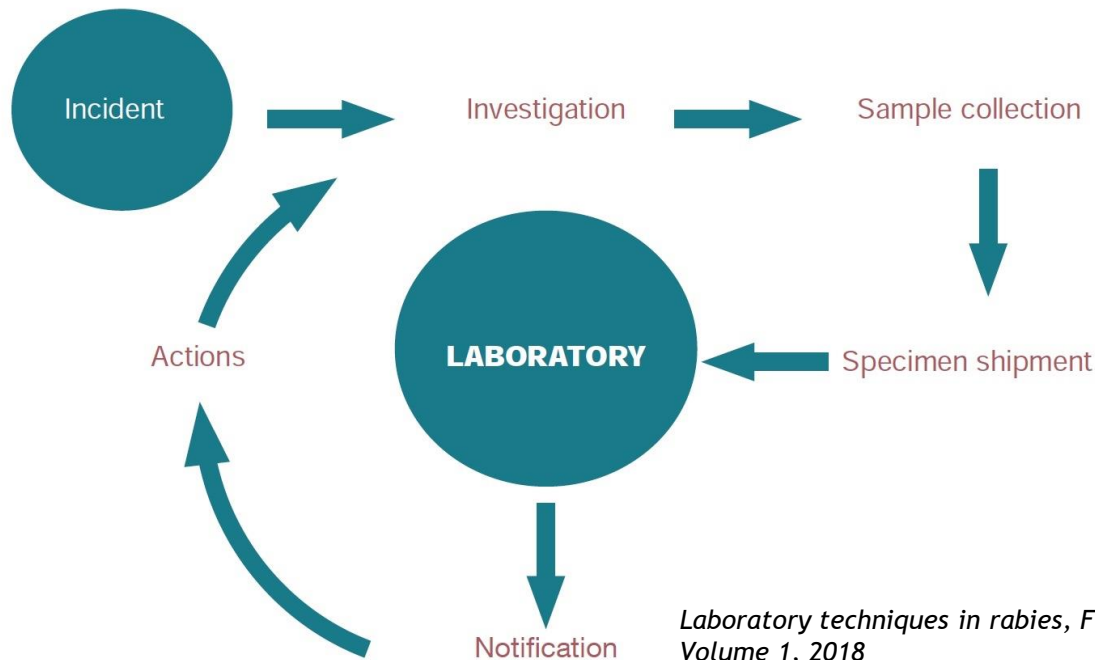
National reference center for rabies cnrrage@pasteur.fr
WHO Collaborative centre for reference and research on rabies
Unit Lyssavirus Epidemiology and Neuropathology
Institut Pasteur, Paris, France



The major objectives of laboratory techniques

- A key step in the framework of rabies surveillance

- ⇒ Demonstrate the presence and distribution of disease in humans (and animals)
- ⇒ Aid in control efforts
- ⇒ Determine the course of medical care for exposed individuals
- ⇒ Document absence of disease during elimination programmes






The need for accurate and reliable data

- Underestimation of the burden of human rabies

2017, 92, 77–88 No 7

 **World Health Organization**
Organisation mondiale de la Santé

Weekly epidemiological record
Relevé épidémiologique hebdomadaire

17 FEBRUARY 2017, 92th YEAR / 17 FÉVRIER 2017, 92^e ANNÉE
No 7, 2017, 92, 77–88
<http://www.who.int/wer>

Contents

77 Human rabies: 2016 updates and call for data

Human rabies: 2016 updates and call for data

Rage humaine: mise à jour de 2016 et appel à la communication de données

- ⇒ Weak and official reporting of disease incidence in humans and animals remains inadequate and incomplete
- ⇒ In many cases the true quantitative burden of rabies is best displayed using estimates



Why a biological diagnosis ?

- **Limitation of the clinical diagnosis**

⇒ **Few specific signs (except hydrophobia/aerophobia)**

As a reminder: encephalitis with 2 major forms (spastic and paralytic) with minor atypical or non-classical forms

⇒ **Various clinical syndrome overlapping with rabies**

Example (for human): malaria, Guillain-Barré syndrome, post-vaccinal encephalitis, psychiatric disorders, etc.

⇒ **Difficulties to suspect rabies in case of encephalitis without any history of exposure**

Demonstrate the presence and distribution of disease in humans and animals

⇒ **Some predictive elements with neuroimaging (FLAIR magnetic resonance image, but not CT)**

Confirmed case: biological confirmation of cases



Why a biological diagnosis ?

- Definition of rabies cases (WHO)

⇒ Suspect case

Human case definition

A person presenting with an acute neurological syndrome (i.e. encephalitis) that progressively worsens towards coma and death, typically within 7–10 days of onset, if no intensive care is instituted. May include any of the following signs and symptoms:

- aerophobia
- hydrophobia
- paraesthesia or localized pain
- dysphagia
- localized weakness
- nausea or vomiting



Why a biological diagnosis ?

- Definition of rabies cases (WHO)

⇒ Probable case

Human case definition

A suspect human with a reliable history of contact with a (suspect/probable/confirmed) rabid animal

⇒ Confirmed case

Human case definition

A suspect or probable human that is laboratory-confirmed using a standard diagnostic test



Why a biological diagnosis ?

- Definition of rabies cases (WHO)

⇒ Excluded case

Human case definition

A suspect or probable human that is ruled out by appropriate laboratory testing


or

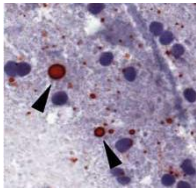
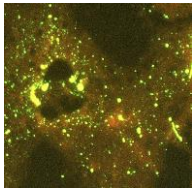
A suspect or probable human with no reasonable risk of animal contact in the past 6 months



How a biological diagnosis ?

- Identification of one or more of the following criteria:

- Viral antigen (protein) 
- Techniques: **FAT, DRIT**, RIDT




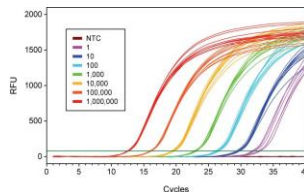
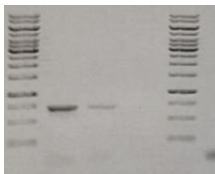
- Specific antibodies 
- Techniques: RFFIT, FAVN, ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	0.010	0.011	0.012
2	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024
3	0.025	0.026	0.027	0.028	0.029	0.030	0.031	0.032	0.033	0.034	0.035	0.036
4	0.037	0.038	0.039	0.040	0.041	0.042	0.043	0.044	0.045	0.046	0.047	0.048
5	0.049	0.050	0.051	0.052	0.053	0.054	0.055	0.056	0.057	0.058	0.059	0.060
6	0.061	0.062	0.063	0.064	0.065	0.066	0.067	0.068	0.069	0.070	0.071	0.072
7	0.073	0.074	0.075	0.076	0.077	0.078	0.079	0.080	0.081	0.082	0.083	0.084
8	0.085	0.086	0.087	0.088	0.089	0.090	0.091	0.092	0.093	0.094	0.095	0.096
9	0.097	0.098	0.099	0.100	0.101	0.102	0.103	0.104	0.105	0.106	0.107	0.108
10	0.109	0.110	0.111	0.112	0.113	0.114	0.115	0.116	0.117	0.118	0.119	0.120
11	0.121	0.122	0.123	0.124	0.125	0.126	0.127	0.128	0.129	0.130	0.131	0.132
12	0.133	0.134	0.135	0.136	0.137	0.138	0.139	0.140	0.141	0.142	0.143	0.144
13	0.145	0.146	0.147	0.148	0.149	0.150	0.151	0.152	0.153	0.154	0.155	0.156
14	0.157	0.158	0.159	0.160	0.161	0.162	0.163	0.164	0.165	0.166	0.167	0.168
15	0.169	0.170	0.171	0.172	0.173	0.174	0.175	0.176	0.177	0.178	0.179	0.180
16	0.181	0.182	0.183	0.184	0.185	0.186	0.187	0.188	0.189	0.190	0.191	0.192
17	0.193	0.194	0.195	0.196	0.197	0.198	0.199	0.200	0.201	0.202	0.203	0.204
18	0.205	0.206	0.207	0.208	0.209	0.210	0.211	0.212	0.213	0.214	0.215	0.216
19	0.217	0.218	0.219	0.220	0.221	0.222	0.223	0.224	0.225	0.226	0.227	0.228
20	0.229	0.230	0.231	0.232	0.233	0.234	0.235	0.236	0.237	0.238	0.239	0.240
21	0.241	0.242	0.243	0.244	0.245	0.246	0.247	0.248	0.249	0.250	0.251	0.252
22	0.253	0.254	0.255	0.256	0.257	0.258	0.259	0.260	0.261	0.262	0.263	0.264
23	0.265	0.266	0.267	0.268	0.269	0.270	0.271	0.272	0.273	0.274	0.275	0.276
24	0.277	0.278	0.279	0.280	0.281	0.282	0.283	0.284	0.285	0.286	0.287	0.288
25	0.289	0.290	0.291	0.292	0.293	0.294	0.295	0.296	0.297	0.298	0.299	0.300
26	0.301	0.302	0.303	0.304	0.305	0.306	0.307	0.308	0.309	0.310	0.311	0.312
27	0.313	0.314	0.315	0.316	0.317	0.318	0.319	0.320	0.321	0.322	0.323	0.324
28	0.325	0.326	0.327	0.328	0.329	0.330	0.331	0.332	0.333	0.334	0.335	0.336
29	0.337	0.338	0.339	0.340	0.341	0.342	0.343	0.344	0.345	0.346	0.347	0.348
30	0.349	0.350	0.351	0.352	0.353	0.354	0.355	0.356	0.357	0.358	0.359	0.360
31	0.361	0.362	0.363	0.364	0.365	0.366	0.367	0.368	0.369	0.370	0.371	0.372
32	0.373	0.374	0.375	0.376	0.377	0.378	0.379	0.380	0.381	0.382	0.383	0.384
33	0.385	0.386	0.387	0.388	0.389	0.390	0.391	0.392	0.393	0.394	0.395	0.396
34	0.397	0.398	0.399	0.400	0.401	0.402	0.403	0.404	0.405	0.406	0.407	0.408
35	0.409	0.410	0.411	0.412	0.413	0.414	0.415	0.416	0.417	0.418	0.419	0.420
36	0.421	0.422	0.423	0.424	0.425	0.426	0.427	0.428	0.429	0.430	0.431	0.432
37	0.433	0.434	0.435	0.436	0.437	0.438	0.439	0.440	0.441	0.442	0.443	0.444
38	0.445	0.446	0.447	0.448	0.449	0.450	0.451	0.452	0.453	0.454	0.455	0.456
39	0.457	0.458	0.459	0.460	0.461	0.462	0.463	0.464	0.465	0.466	0.467	0.468
40	0.469	0.470	0.471	0.472	0.473	0.474	0.475	0.476	0.477	0.478	0.479	0.480
41	0.481	0.482	0.483	0.484	0.485	0.486	0.487	0.488	0.489	0.490	0.491	0.492
42	0.493	0.494	0.495	0.496	0.497	0.498	0.499	0.500	0.501	0.502	0.503	0.504
43	0.505	0.506	0.507	0.508	0.509	0.510	0.511	0.512	0.513	0.514	0.515	0.516
44	0.517	0.518	0.519	0.520	0.521	0.522	0.523	0.524	0.525	0.526	0.527	0.528
45	0.529	0.530	0.531	0.532	0.533	0.534	0.535	0.536	0.537	0.538	0.539	0.540
46	0.541	0.542	0.543	0.544	0.545	0.546	0.547	0.548	0.549	0.550	0.551	0.552
47	0.553	0.554	0.555	0.556	0.557	0.558	0.559	0.560	0.561	0.562	0.563	0.564
48	0.565	0.566	0.567	0.568	0.569	0.570	0.571	0.572	0.573	0.574	0.575	0.576
49	0.577	0.578	0.579	0.580	0.581	0.582	0.583	0.584	0.585	0.586	0.587	0.588
50	0.589	0.590	0.591	0.592	0.593	0.594	0.595	0.596	0.597	0.598	0.599	0.600

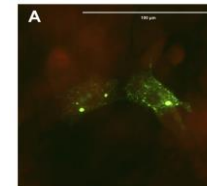


Most interesting approaches

- Viral RNA 
- Techniques: **RT-PCR, RT-qPCR**, others



- Virus 
- Techniques: RTCIT (MIT)



Reference techniques



When a biological diagnosis ?

- Only when clinical signs are already present

Main clinical signs

2 main forms:

Spastic (furious) 70%

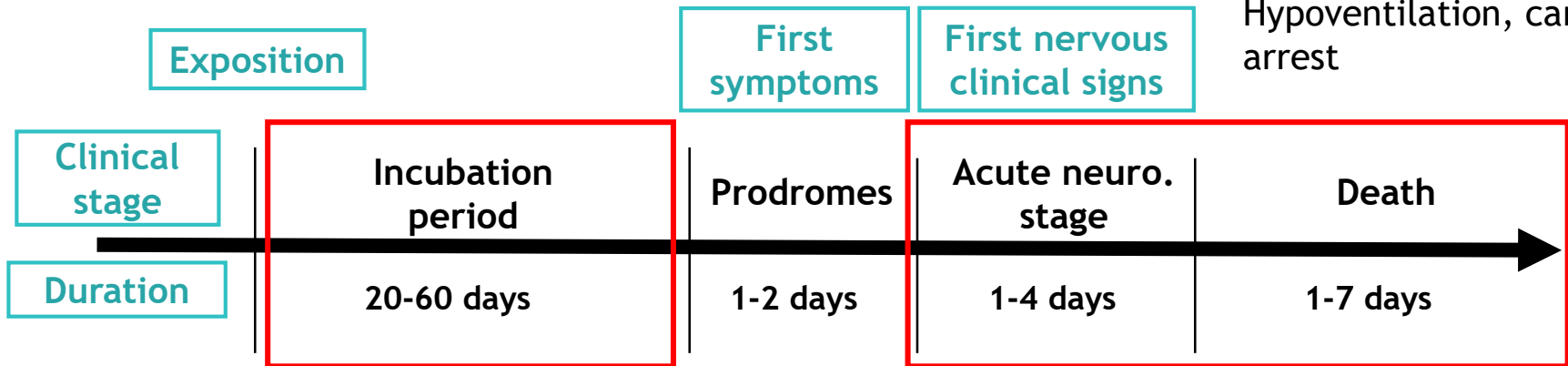
Paralytic (dumb) 30%

Fever, anorexia, nausea, vomiting
headache, lethargy, pain at the bite site

Anxiety, agitation, depression

Aphasia, incoordination
paresis, paralysis, **hydrophobia**, **aerophobia**,
Spasm of the larynx, confusion,
delirium, hallucinations, hyperactivity

Coma, Cardiac arrhythmia
Hypoventilation, cardiac
arrest



Vaccination

Biological diagnosis

⇒ At *ante-mortem* stage

⇒ At *post-mortem* stage

Which samples for biological diagnosis ?

• Main specificities of human rabies

Particular physiopathology

Quasi-exclusively neurotropic

No viremia

Long incubation period

Possible detection starts only 10 (15) days before the onset of symptoms

Detection at the late stage of the disease in nervous tissues or specific fluids

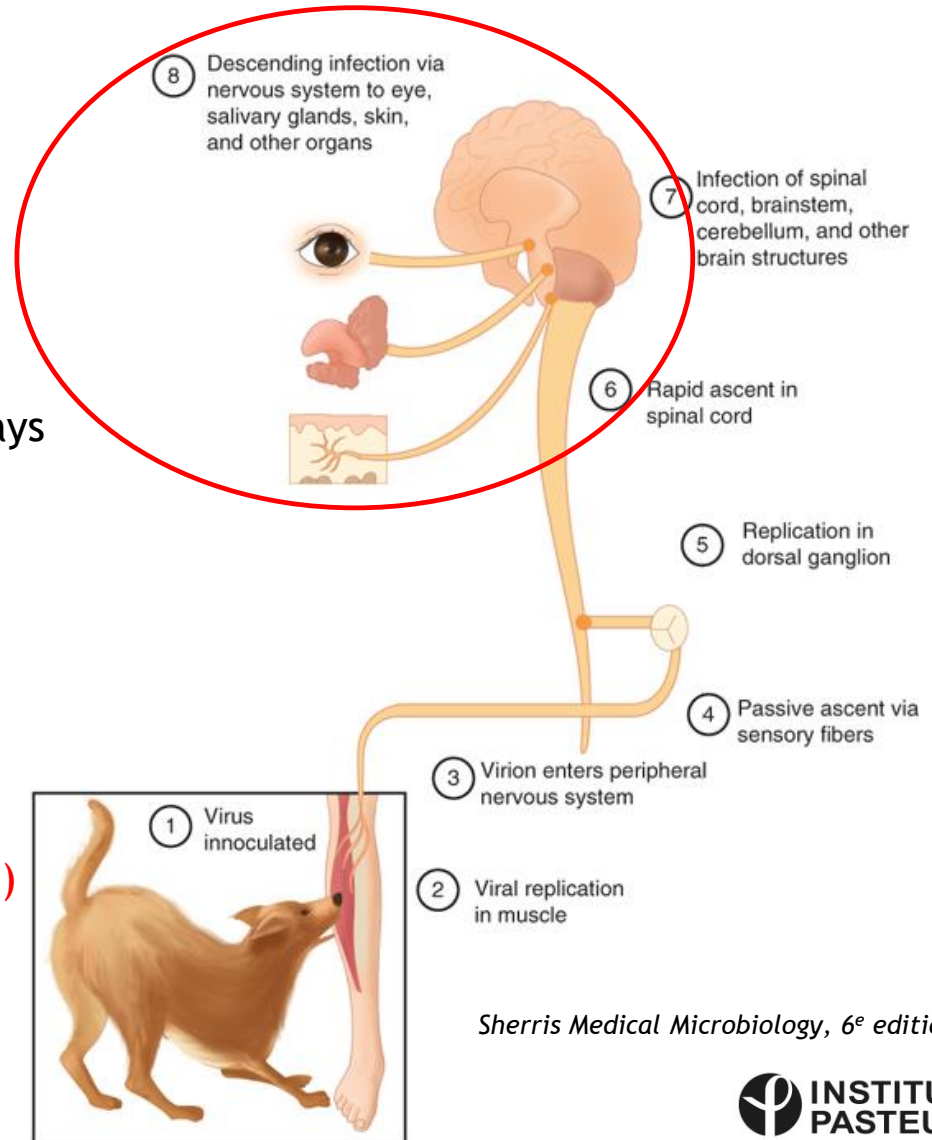
Central nervous system and CSF

Skin biopsy (nerves around the follicles)

Saliva (salivary glands)

Eye (cornea, tears)

Nerves of all innervated tissues





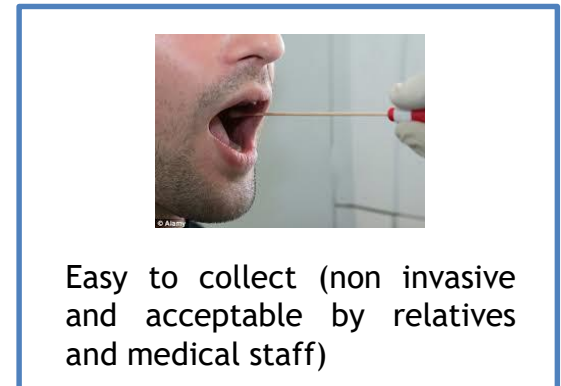
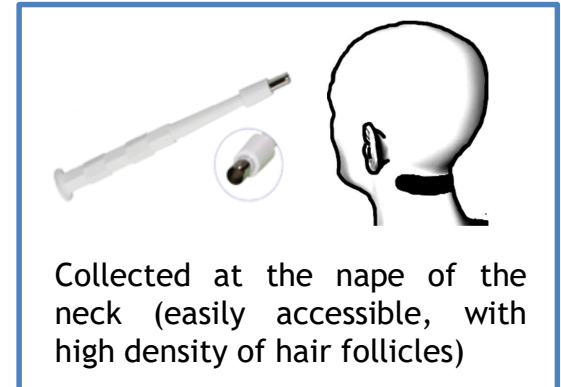
Which samples for biological diagnosis ?

- Depending of the stage of the diagnosis

- **Post-mortem**

- **Ante-mortem**

- Brain tissue *****
- Skin biopsy and hair follicles *****
- Saliva *****
- Tears ****
- CSF ****
- Blood ***
- *Nerves of all innervated tissues* **
- *Cornea* *
- *Urines* *



Dacheux L et al., Clin Infect Dis. 2008 Dec 1;47(11):1410-7. doi: 10.1086/592969.

**** Interest for diagnosis



Which samples for biological diagnosis ?

- A critical point to obtain relevant and reliable results

⇒ Importance of sampling and storage conditions

Wrong sampling or conditions of storage: risk of false negative results

Main difficulties in enzootic areas in tropical countries

Diagnosis performed exclusively in central laboratories

⇒ For all samples

Collection in dry tubes

Storage at low temperature: short storage or transport at +4°C (few hours only) or frozen (best)



Post-mortem diagnosis of human rabies

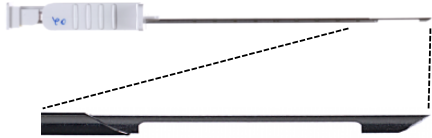
- **Diagnosis using brain biopsy**

● **Post-mortem**

⇒ **The reference sample**

Collected after autopsy or rapid sampling

Occipital foramen route



A biopsy needle or spinal needle



Courtesy of B.M. Diop, CHU Fann, Dakar

Retro-orbital route

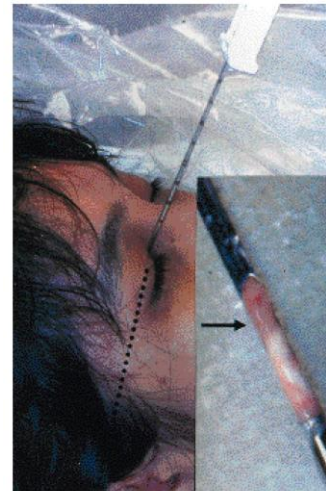


Figure 1: Introduction of trucut needle (rabid case)

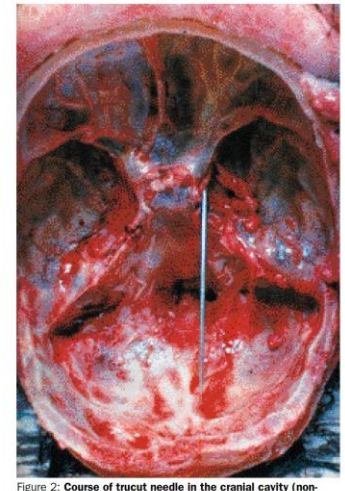


Figure 2: Course of trucut needle in the cranial cavity (non-rabid case)

Tong et al., *Lancet*. 1999 Dec 18-25;354(9196):2137-8

⇒ **Difficult (if not impossible)** to obtain (in low-, middle-, or high-income countries)



Post-mortem diagnosis of human rabies

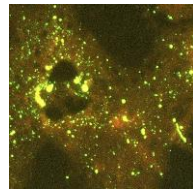
- **Diagnosis using brain biopsy**

● **Post-mortem**

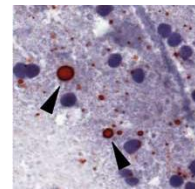
⇒ **Techniques of diagnosis**

- **Viral antigen (protein)** 

Techniques: FAT (reference)



DRIT (not really tested for human)



RIDT (need additional validation)

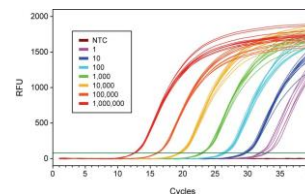


- **Viral RNA** 

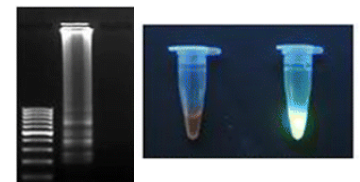
Techniques: RT-PCR (reference)



RT-qPCR (reference)



others (LAMP, RPA, etc.) (need validation)





Post-mortem diagnosis of human rabies

- Diagnosis using brain biopsy

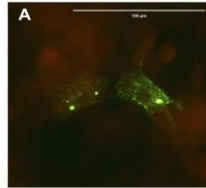
● Post-mortem

⇒ Techniques of diagnosis

- Virus



Techniques: RTCIT (confirmation) MIT (not recommended anymore)





Post-mortem diagnosis of human rabies

- Diagnosis using skin biopsy

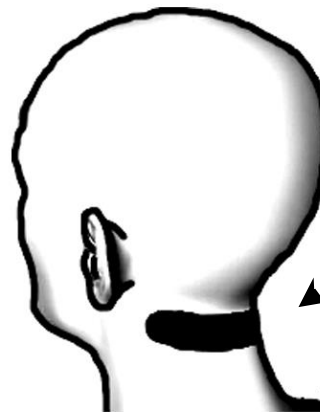
● Post-mortem

⇒ The alternative sample

Easy to collect

Biopsy punch (4 mm diameter)

Total volume: 20 mm³



Collected at the nape of the neck
(easily accessible, with high density of hair follicles)

Bryceson et al., 1975, Blendon et al., 1986, Crepin et al., 1998, Dacheux et al., 2008



Post-mortem diagnosis of human rabies

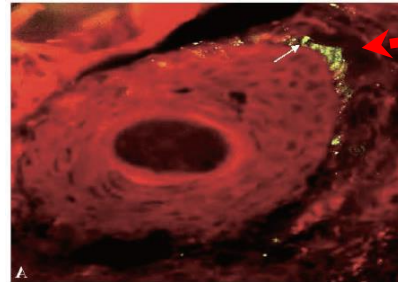
- Diagnosis using skin biopsy

● Post-mortem

⇒ Techniques of diagnosis

- Viral antigen (protein) 

Techniques: FAT
(very little used now because difficult to implement)

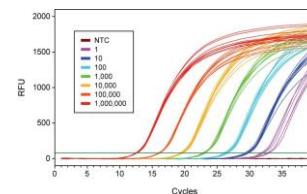


Detection of viral antigens in the nerves surrounding the hair follicle

Soun et al., J. Int J Biomed Sci. 2006 Dec;2(4):434-45

- Viral RNA 

Techniques: RT-PCR (reference) RT-qPCR (reference)





Post-mortem diagnosis of human rabies

- Diagnosis using skin biopsy

● Post-mortem

⇒ Sensitivity at the post-mortem stage (RT-PCR)

Type of samples	Number of samples			Rate of positivity*	
	Total	Per patient		At discharge	
		Median range		Per sample	Per patient
Skin biopsy	60	1,5	0-3	97,2% (36)	96,7% (31)

* Per sample: number of positive samples / total number of the considered sample; per patient: number of patients with at least one positive sample / number of total patients from whom the considered sample was collected.

Dacheux L et al., Clin Infect Dis. 2008 Dec 1;47(11):1410-7. doi: 10.1086/592969.



Ante-mortem diagnosis of human rabies

- Diagnosis using skin biopsy

● Ante-mortem

⇒ Sensitivity at the ante-mortem stage (RT-PCR)

Type of samples	Number of samples				Rate of positivity*		
	Total	Per patient		Total		At admission	
		Median	range	Per sample	Per patient	Per sample	Per patient
Skin biopsy	60	1,5	0-3	98,33% (60)	96,7% (31)	100% (33)	100% (29)

* Per sample: number of positive samples / total number of the considered sample; per patient: number of patients with at least one positive sample / number of total patients from whom the considered sample was collected.

Dacheux L et al., *Clin Infect Dis.* 2008 Dec 1;47(11):1410-7. doi: 10.1086/592969.



Ante-mortem diagnosis of human rabies

- **Diagnosis using saliva sample**

● Ante-mortem

⇒ **Another suitable sample (in addition to skin biopsy)**

Easy to collect

Directly collected (liquid) or with swabs





Post-mortem diagnosis of human rabies

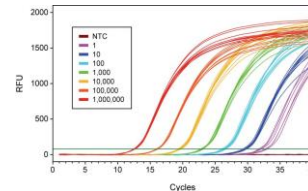
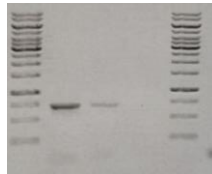
- Diagnosis using saliva sample

● Ante-mortem

⇒ Techniques of diagnosis

- Viral RNA 

Techniques: RT-PCR (reference) RT-qPCR (reference)





Ante-mortem diagnosis of human rabies

- Diagnosis using saliva sample

● Ante-mortem

⇒ Sensitivity at the ante-mortem stage (RT-PCR)

Type of samples	Number of samples		Rate of positivity*		
	Total	Per patient	Total		
		Median range	Per sample	Per patient	
Saliva	84	1	0-6	70,2% (84)	70,7% (41)

* Per sample: number of positive samples / total number of the considered sample; per patient: number of patients with at least one positive sample / number of total patients from whom the considered sample was collected.

Dacheux L et al., Clin Infect Dis. 2008 Dec 1;47(11):1410-7. doi: 10.1086/592969.



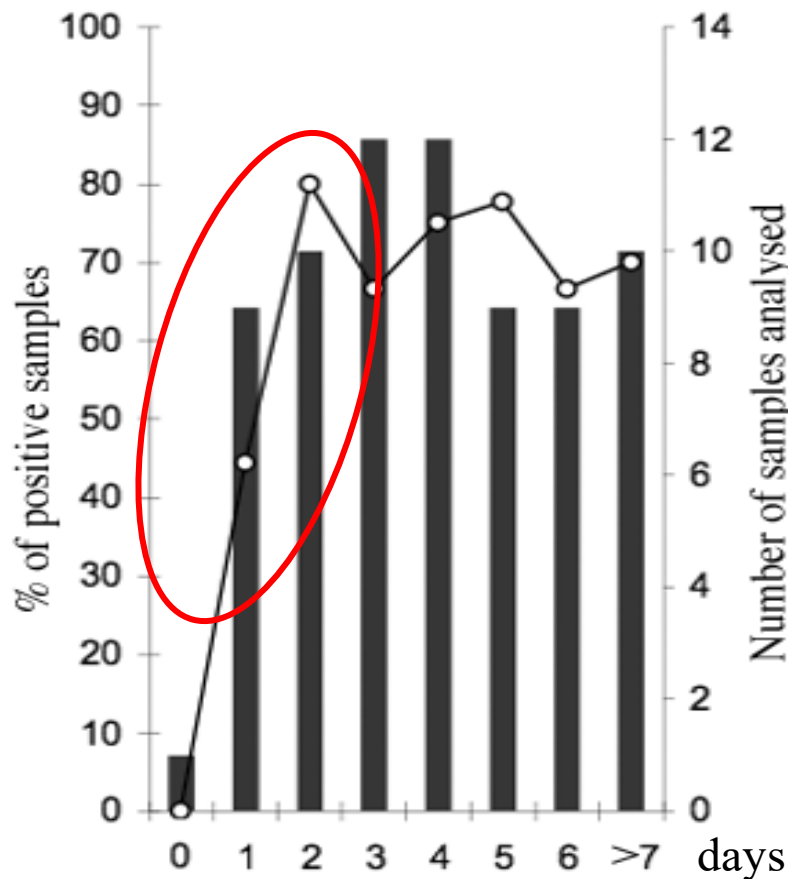
Ante-mortem diagnosis of human rabies

- Diagnosis using saliva sample

● Ante-mortem

⇒ Sensitivity at the ante-mortem stage (RT-PCR)

Onset of symptoms



Increase of sensitivity during the two first days after onset of symptoms

High sensitivity

With 3 sequential salivas (intermittent viral excretion):

100% per patient



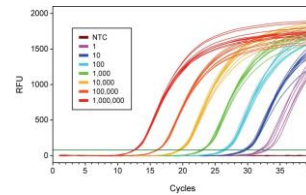
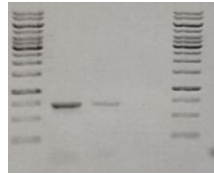
Ante-mortem diagnosis of human rabies

• Diagnosis using CSF sample

● Ante-mortem

• Viral RNA

Techniques: RT-PCR (reference) RT-qPCR (reference)



Moderate sensitivity

• Specific antibodies

Techniques: RFFIT, FAVN ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
B	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
C	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
D	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
E	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
F	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
G	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
H	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
I	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600
J	NTC	0.025	0.050	0.100	0.200	0.400	0.800	1.600	3.200	6.400	12.800	25.600



Low sensitivity
(at least >8 days post infection)



Ante-mortem diagnosis of human rabies

- Diagnosis using blood/serum sample

- Ante-mortem

- Specific antibodies



Techniques:

RFFIT, FAVN

ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00



Low sensitivity
(at least >8 days post infection)

Number of samples

Rate of positivity*

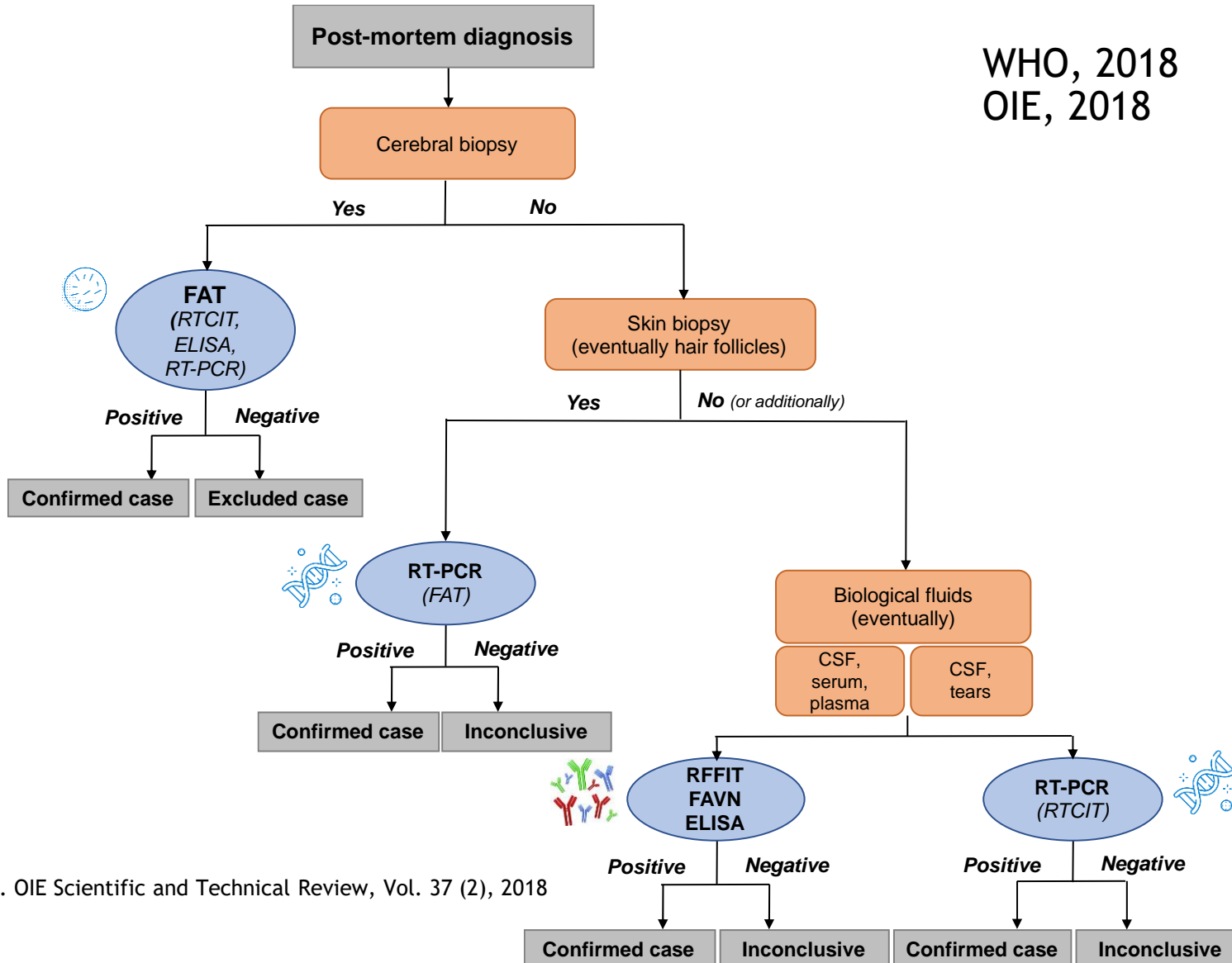
Type of samples	Total	Per patient		Total		At admission		At discharge	
		Median	range	Per sample	Per patient	Per sample	Per patient	Per sample	Per patient
Serum	46	1	1-3	0% (46)	0% (43)	0% (33)	0% (32)	0% (20)	0% (18)

Dacheux L et al., Clin Infect Dis. 2008 Dec 1;47(11):1410-7. doi: 10.1086/592969.

The biological diagnosis of human rabies: Algorithm

- At post-mortem stage

WHO, 2018
OIE, 2018

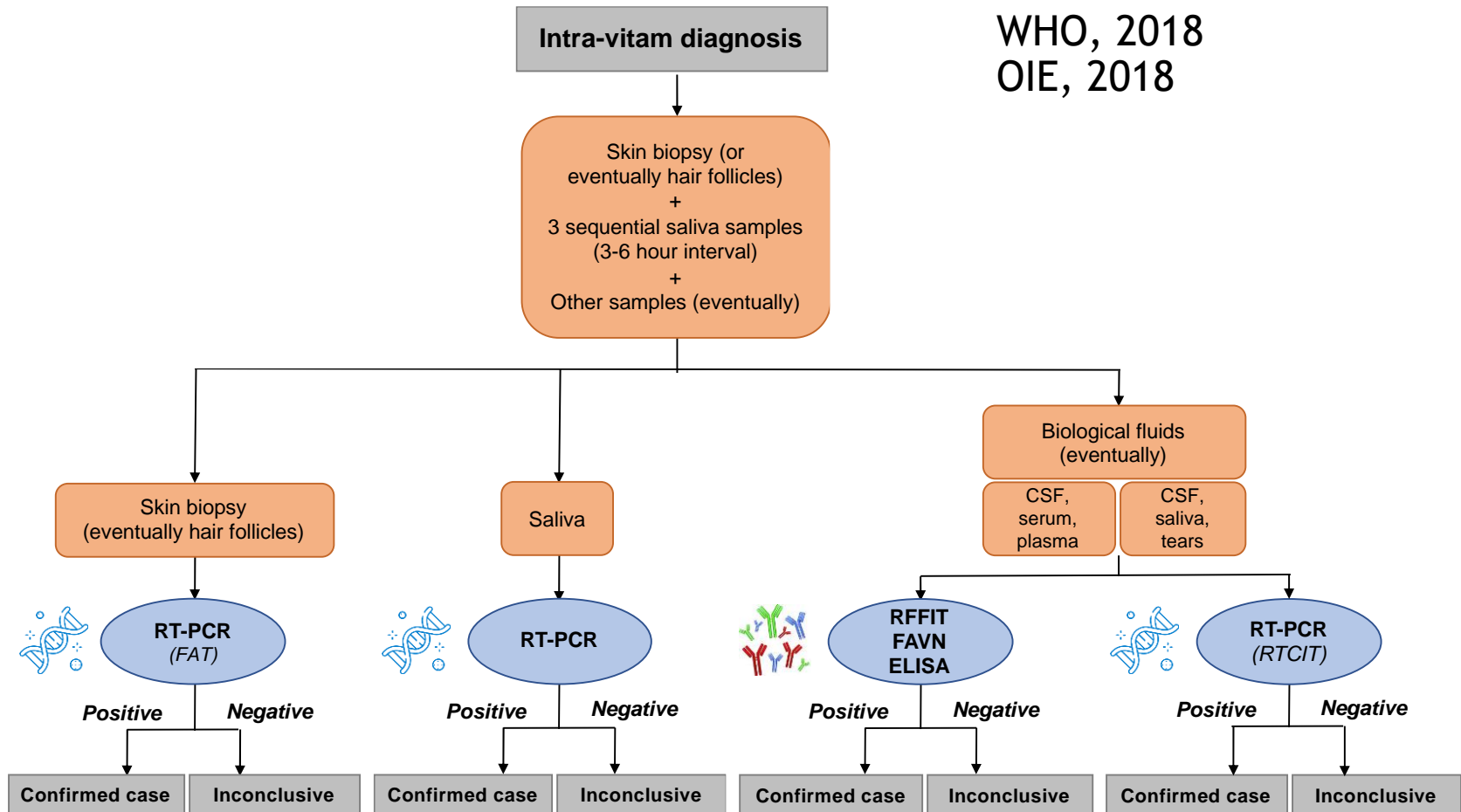




The biological diagnosis of human rabies: Algorithm

- At ante-mortem stage

WHO, 2018
OIE, 2018



Rabies. OIE Scientific and Technical Review, Vol. 37 (2), 2018



But real life can be more complicated...

- **First example**

⇒ **Difficulty of the ante-mortem diagnosis**

Patient: Male, 41 years

Hospitalization in mid-June 2016 (Paris)

>> Febrile confusional syndrome

Onset of symptoms: early June (non specific)

Worsening of the clinical condition despite resuscitation

Death: mid-July 2016

>> **40 days** after onset of symptoms

>> **26 days** after hospitalization

Antecedent of dog bite in Bangladesh **1 year and 4 months** before hospitalization



But real life can be more complicated...

- **First example**

⇒ **Difficulty of the ante-mortem diagnosis**

Suspicion of rabies: mid-June 2016

4 skin biopsies

6 saliva samples

3 serum samples

1 CSF sample

Technique: **Combo RT-qPCR with pan-RABV RT-qPCR (TaqMan) and pan-lyssa RT-qPCR (SYBR Green)**

Previoulsy validated on ante-mortem / post-mortem diagnosis:

- **211 biological samples** (positive n = 76 and negative n = 135) including saliva, skin and brain biopsies.
- detection all **41 human cases** of rabies
- sensitivity of skin biopsy: **91.5%**, saliva: **54%**

Dacheux et al., PLoS Negl Trop Dis. 2016 Jul 5;10(7):e0004812. doi: 10.1371/journal.pntd.0004812.

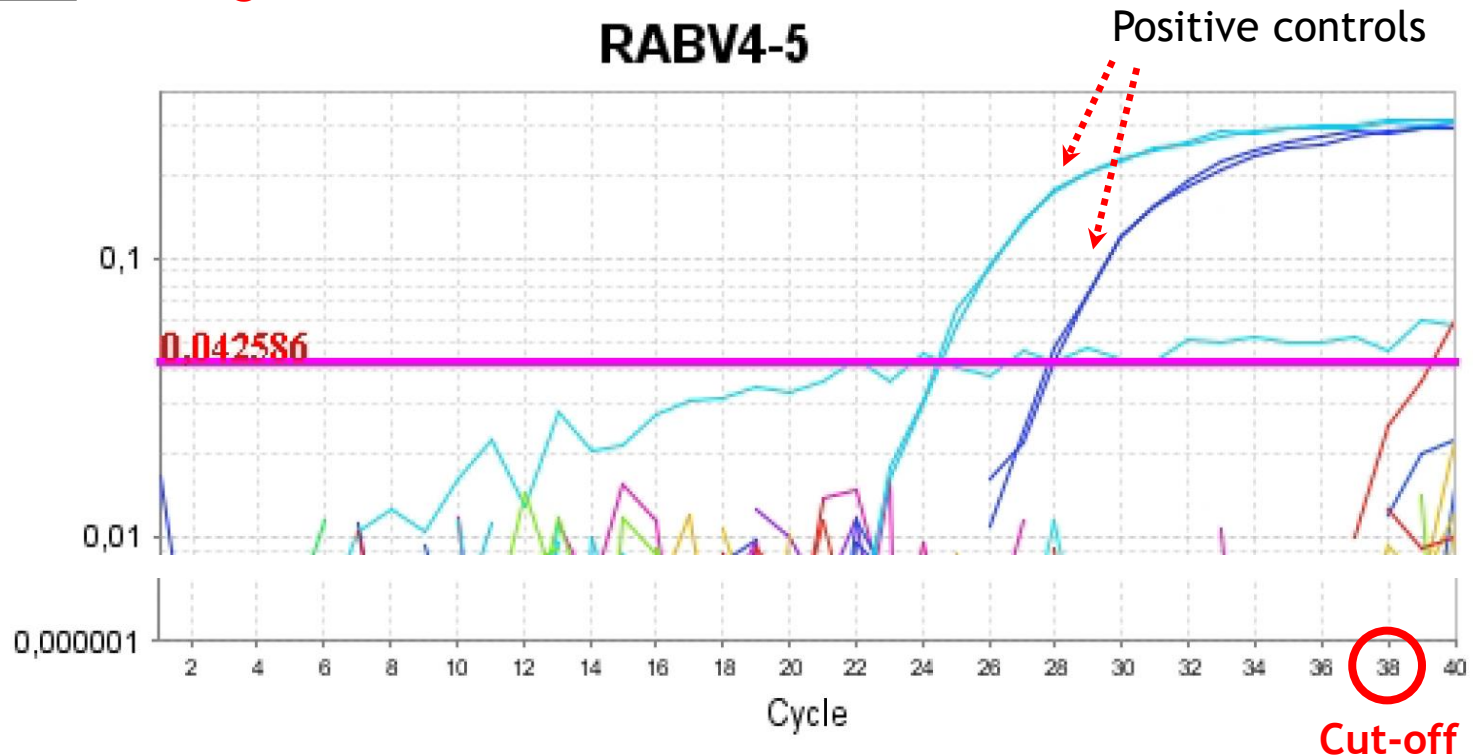


But real life can be more complicated...

- **First example**

⇒ **Difficulty of the ante-mortem diagnosis**

Results: all **negative**



Example of pan-RABV RT-qPCR (TaqMan) results



But real life can be more complicated...

- **First example**

⇒ **Difficulty of the ante-mortem diagnosis**

Retrospective diagnosis: mid-September 2017

1 brain biopsy (frontal cortex)

(as part of a cohort of patients who died of unknown encephalitis)

Technique: **FAT**

Combo RT-qPCR with pan-RABV RT-qPCR (TaqMan) and pan-lyssa RT-qPCR (SYBR Green)

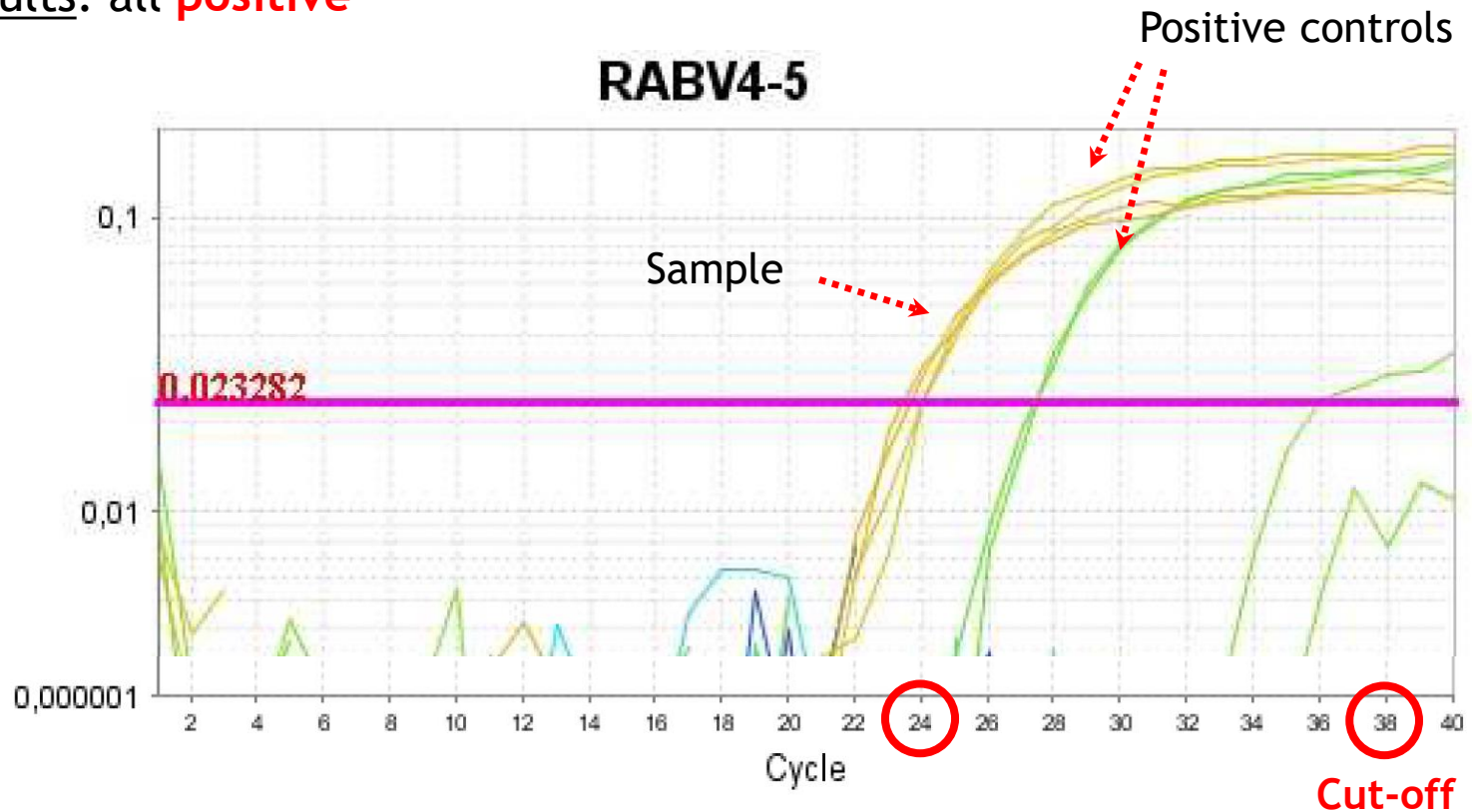


But real life can be more complicated...

- **First example**

⇒ **Difficulty of the ante-mortem diagnosis**

Results: all **positive**



Example of pan-RABV RT-qPCR (TaqMan) results

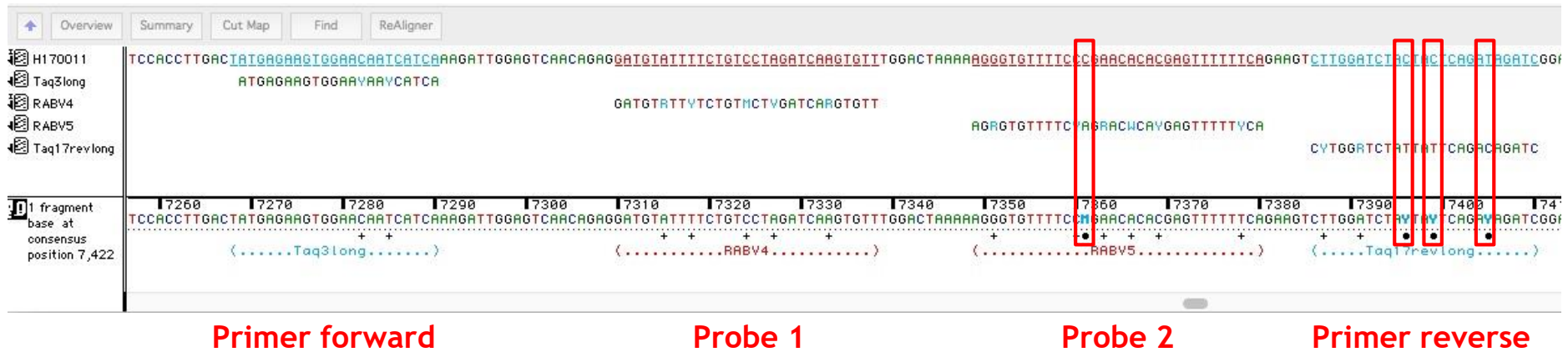


But real life can be more complicated...

- **First example**

⇒ **Difficulty of the ante-mortem diagnosis**

Rational: Decrease of sensitivity due to 4 mutations



Polymerase (L) sequences of RABV from Arctic-like 1 (AL-1) lineage (Bangladesh) not available at the time of the primers/probes design and validation

Lower sensitivity (lower viral load) in ante-mortem samples vs brain samples (commonly used for PCR validation)



But real life can be more complicated...

- **Second example**

⇒ **Unexpected results with the post-mortem diagnosis**

Patient: Male, 59 years

Hospitalization in June 2019

>> encephalitis

Onset of symptoms: 3 days before (non specific)

Worsening of the clinical condition despite resuscitation

Death: **51 days** after hospitalization, without aetiology

No specific antecedent (at this time)



But real life can be more complicated...

- **Second example**

⇒ **Difficulty of the post-mortem diagnosis**

Retrospective diagnosis: October 2020

1 brain biopsy (frontal cortex)

(as part of a cohort of patients who died of unknown encephalitis)

Metagenomic analysis (NGS)

Detection and sequencing of **EBLV-1a strain genome**

Clinical Infectious Diseases

MAJOR ARTICLE



First Case of Lethal Encephalitis in Western Europe Due to European Bat Lyssavirus Type 1

Béatrice Regnault,^{1,2} Bruno Evrard,^{3,4} Isabelle Plu,^{5,6} Laurent Dacheux,⁷ Eric Troadec,^{3,2} Pascal Cozette,⁷ Delphine Chrétien,^{1,2} Mathilde Duchesne,⁸ Jean-Michel Vallat,⁹ Anne Jamet,¹⁰ Marianne Leruez,¹⁰ Philippe Pérot,^{1,2} Hervé Bourhy,⁷ Marc Eloit,^{1,2,11,a} and Danielle Seilhean^{5,7,a}

Regnault et al. *Clinical Infectious Diseases*, Volume 74, Issue 3, 1 February 2022, Pages 461-466.





But real life can be more complicated...

- **Second example**

⇒ **Difficulty of the post-mortem diagnosis**

Techniques (classical): **FAT** **Negative** (different conjugates used)
RTCIT/MIT **Negative** (different conjugates used)
Specific RT-qPCR **Positive**

Other tests/samples: **Serum** **Positive (1/100)**
(at the day of admission)
mRFFIT (EBLV-1) **Negative**
CSF
(at the day of admission and 34 later)

Rational: Puzzling!

EBLV-1 antigens and virus isolation from naturally infected sheep and stone martens also difficult

Possibly in relation to the type (meninge and cortex), size or conservation of brain samples, or to sample processing to dilute inhibitors.



Take home messages

- **Biological diagnosis:** the only way to confirm rabies
- Selection of the **right samples**, with the **right conditions** of conservation and with the **right validated techniques**
- Availability of **reliable ante-mortem diagnostic techniques** / protocols
- **Skin biopsy / 3 saliva samples:** keys samples for molecular diagnosis
- **Variable and lower viral loads** in ante-mortem samples vs brain samples
- Can be complicated / unexpected! **Multiplication of the samples and retesting** in case of negative results



NO BITES = NO RABIES 

Learn dog body language

ALERT



Design: AM Labouche, original drawings by © Lié Chin



Thanks for your attention !!!

