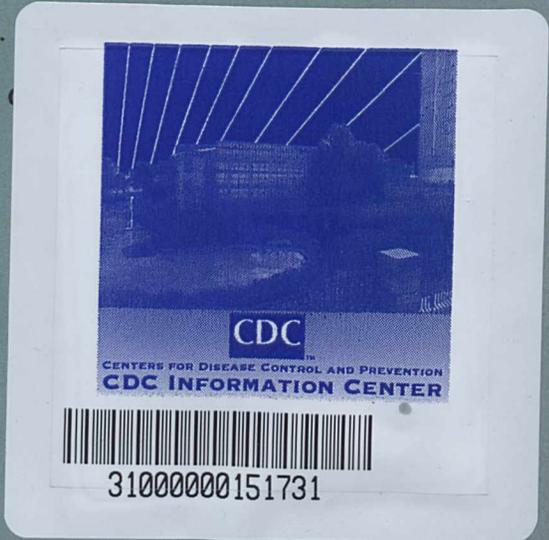


PREFACE

This summary analysis has three major purposes. First, it summarizes the performance of all participating laboratories and thus gives an indication of the general level of achievement by peer laboratories. Each laboratory is able to compare its performance with that of other peer laboratories. Second, it summarizes the performance of reference laboratories. Each participating laboratory may relate its performance to that of selected reference laboratories. Third, each summary analysis contains a critique. This critique is intended to point out possible sources of error, common pitfalls based on the experience of participating laboratories, and guidelines for improving performance.

Center for Disease Control David J. Sencer, M.D., Director
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VIROLOGY SPECIAL EVALUATION - RABIES

OCTOBER, 1973

CONTRIBUTORS:

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IN COOPERATION WITH THE

VIRAL ZOOSES BRANCH

VIROLOGY DIVISION

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AND WITH ASSISTANCE FROM THE

VIROLOGY TRAINING SECTION

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**UNITED STATES
PUBLIC HEALTH SERVICE**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
CENTERS FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333

091464

WC 550 V819 1973
Virology special evaluation

091464

CENTERS FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333

TELEPHONE: (404) 633-3311

November 4, 1974

Participants in the CDC
Proficiency Testing Program
Virology (Special Evaluation -
Rabies)

Participants:

Attached is a summary analysis of results for laboratories which participated in the Proficiency Testing Survey in Virology-Rabies evaluation. Samples VR3-001 through VR3-005 were submitted for analysis on October 9, 1973.

On November 30, 1973, a memorandum was sent to your laboratory listing the correct results for these samples and the grade achieved by your laboratory.

Generally, laboratory participation and performance was good in regard to samples VR3-001 - VR3-004. Laboratories experiencing problems with these specimens were encouraged to participate in a follow-up survey. Participation and performance in this survey was very good. Sample VR3-005 presented special problems and, therefore, was not graded in this survey.

Laboratories' responses to the questionnaire can be found at the back of the summary analysis.

Sincerely,

Charles T. Hall

Charles T. Hall, Ph.D.
Chief, Proficiency Testing Branch
Licensure & Proficiency Testing
Division

091464

105-18-87 pub Stat's Information Center



SPECIAL EVALUATION - RABIES

Number of laboratories responding - 64*

98% participation

Number of laboratories receiving specimens - 65

GRADING

Note: Sample #VR3-005 was not considered in the grading.
Therefore, each of the remaining samples was worth
25 points.

	<u>Number</u>	<u>Percent</u>
Laboratories with grade of 100	59	92%
Laboratories with grade of 75	5	8%

REFERENCE LABORATORY RESULTS

Reference Laboratory #	VR3-001	VR3-002	VR3-003	VR3-004	VR3-005
1	Neg.	Pos.	Neg.	Neg.	Neg.
2	Neg.	Pos.	Neg.	Neg.	Pos.
3	Neg.	Pos.	Neg.	Neg.	Pos.

*The remaining laboratory reported results late.

401A190

INTRODUCTION

Rabies is an acute, fatal, infectious disease of the central nervous system (CNS). It is caused by a neurotropic virus which is naturally transmitted through the salivary gland of carnivorous animals and propagates along nerve pathways to the CNS. Rabies is one of the zoonotic diseases related to man's association with dogs and other animals.

In 1972, a total of 4,427 laboratory confirmed cases of rabies were reported from the U.S., an increase of 14% above the average for the preceding 5 years.¹⁴

The laboratory diagnosis of rabies often determines whether a person bitten by the examined animal will receive specific rabies prophylaxis. Because rabies prophylaxis involves an extended series of inoculations which usually are accompanied by moderate discomfort and infrequently cause severe reactions, unnecessary treatment is costly and arduous. However, if a person is bitten by a rabid animal and not treated, the risk is grave.

The laboratory techniques used to diagnose rabies include the fluorescent antibody test (FA), histochemical tests, and mouse inoculation. The FA test is rapid and the most accurate microscopic test available for the diagnosis of rabies. It should be employed by all laboratories undertaking such work.²

The purpose of this survey was to determine the proficiency of laboratories in identifying rabies by the FA technique. Specimens were sent to all central state laboratories and some other selected laboratories that, routinely or occasionally, receive specimens for rabies diagnosis.

COMMENTS REGARDING SPECIMENS

Five frozen specimens were sent to each participant laboratory. Each specimen consisted of one hemisphere of a mouse brain. The remainder of each brain was identified and retained at the Center for Disease Control (CDC). If a participant laboratory reported erroneous results, the remaining tissue from the brain that was the source of the specimen was checked by FA at the CDC.

COMMENTS ON SOURCES OF ERROR

Each of three specimens (VR3-001, VR3-003, VR3-004) consisted of a negative mouse brain. Negative results were correctly reported by 62 laboratories (97%) for specimen VR3-001; by 63 laboratories (98%) for specimen VR3-004; and by 64 laboratories (100%) for VR3-003. In each instance in which a

laboratory reported one of these specimens as positive, FA examination of the remaining brain tissue at CDC corroborated the initial negative results.

Specimens VR3-002 and VR3-005 were both positive for rabies virus, but they evoked different responses from participant laboratories. VR3-002 was a rabies infected mouse brain which produced strongly positive reactions. Sixty-two laboratories (97%) correctly identified this specimen. In each case where a laboratory reported negative results for this specimen, FA examination at the CDC demonstrated positive results.

VR3-005 (not considered in the grading) was reported as positive by 48 laboratories (75%). When negative results were reported and the corresponding specimens were examined at the CDC, FA examination showed a variation of fluorescence among these VR3-005 specimens. Some of these brains produced strongly positive FA results, but others produced very weak staining that was often difficult to interpret. Results of a second examination (at CDC) of VR3-005 specimens indicated: 60% FA positive, and 40% FA negative, based on the examination of one slide per specimen.

The source of the virus in VR3-005 was skunk salivary tissue inoculated into mice via the footpad. This isolate was expected to produce long incubation periods with little demonstrable antigen when this route of inoculation was used. Since most of the rabies antigen in these specimens may have combined with specific antibodies produced by the mice during the long incubation period, diagnosis by FA or by mouse inoculation could have been difficult.

A similar situation may be encountered in a diagnostic laboratory when vaccinated animals are submitted for rabies diagnosis. Difficulty in demonstrating antigen has also been reported with human cases when long incubation periods and endogenous antibody production were involved.

METHODOLOGY

The accurate diagnosis of rabies by the FA technique is dependent on several factors, such as satisfactory reagents, adequate equipment, and experienced personnel. Several factors were reviewed (See Charts I-VI) to determine why errors occurred in this survey. However, statistical analysis of the data revealed no significant difference in the techniques used by laboratories reporting correct results and those used by laboratories reporting incorrect results.

Although it was not possible to pinpoint the source of errors, the information in Charts I-VII may prove beneficial.

SUGGESTED "QUALITY CONTROL" MEASURES

Specimen Handling

In any laboratory a mix-up in the reporting of findings can occasionally occur, e.g., the results for two specimens might be switched or one specimen might be processed twice and another not at all. This may have occurred in those laboratories that reported erroneous results for specimens VR3-001 - VR3-004. Errors would be produced if a mix-up either of the specimens or of the specimen results occurred.

Any specimen received in the laboratory should be processed promptly and individually. It should be entered into a log book and identified by either a name or a number. Any subsequent laboratory work performed on this specimen should bear this identification. In a rabies laboratory, all slides prepared from any one specimen should be labeled with the name or number. If mice are inoculated, their animal cages should be similarly labeled. Results of FA, Seller's, and mouse inoculation tests should be clearly recorded and not written on scraps of paper that could be easily lost. These results should be recorded directly on the report form supplied.

Smear Preparation

When suspected rabies specimens are submitted to a laboratory, a number of impression smears should be made. (See Chart I.A. for the number of impression smears prepared by participant laboratories.)

Properly prepared smears can facilitate the final interpretation. Smears should be prepared from Ammon's horn of the hippocampus, usually the best portion of the brain for demonstration of Negri bodies and of rabies antigen by the FA test. Thin smears are desirable for the FA test because thick smears may show a dull grey or bright blue-grey to white background fluorescence.

At least six smears should be made and examined. Additional smears can be made, in case the specimen should later prove difficult to diagnose.

Smears should be processed according to the procedure outlined in Classical Technique.^{3,4} Although shorter methods^{6,10,11} are available, CDC recommends that laboratories follow classical procedures.

Conjugate

For best results in the FA test, a high quality conjugate should be used. Conjugate may either be prepared in the laboratory, which may be tedious and time consuming, or it may be purchased from a reliable manufacturer. Information on conjugate used by participant laboratories can be found in Chart II. Each lot of conjugate, even from the same manufacturer, must be evaluated before it is used. To evaluate a new lot, serial two-fold dilutions should be made beginning with the undilute conjugate and going through a 1:16 dilution. One portion of undilute conjugate and of each dilution should be mixed with 4 portions of 20% normal mouse brain (NMB) suspension and another portion should be mixed with 4 portions of infected mouse brain (CVS) suspension (See Chart VII). Beginning with the final conjugate dilution of 1:5, test each dilution with a positive control smear. (Be sure positive control smears exhibit a 4+ reaction with a conjugate lot previously proven to be satisfactory; staining characteristics of these smears can deteriorate upon prolonged storage.)

When conjugate is evaluated, the following criteria should be considered: (1) intensity of specific staining, (2) presence and quantity of non-specific background fluorescence, (3) quantity of Negri body and antigenic dust staining.

The intensity of fluorescent reactions is often reported as follows:

Glaring Apple Green	4+
Bright Apple Green	3+
Dull Apple Green	2+
Very Dim Apple Green	1+

Ideally, the conjugate dilution used should be the highest dilution producing 4+ specific staining.

Nonspecific staining (staining that occurs for reasons other than an immunologically specific antigen-antibody reaction) is sometimes seen. It can be due to: (1) granular precipitation of the stain, (2) diffuse staining of the tissue and (3) focal staining of normal tissue components. The first two factors are easily differentiated morphologically from specific reactions. The third can be reduced or eliminated by one or two absorptions with acetone-dried or lyophilized tissue powder (preferably from the species being stained). Another common cause of nonspecific staining is too high a fluorescein -to-protein ratio of the conjugate.

Additional information on the evaluation of conjugate is available.^{1,2,5}

Positive Control Slides

These should always be included in the FA test. Chart V-C shows number of participant laboratories using positive controls. Positive controls can be prepared from brain tissue of positive specimens or of mice and young hamsters sacrificed when moribund following inoculation with rabies street virus.

The control slide consists of two impression smears from a brain known to be positive for rabies. The impression smear stained with rabies conjugate + NMB suspension should exhibit specific apple-green fluorescence. This fluorescence occurs because the rabies conjugate did not react with the normal mouse brain suspension (NMB) and therefore was free to react with the rabies antigen in the smear.

The other impression smear is stained with rabies conjugate + CVS-infected mouse brain suspension. There should be an inhibition of fluorescence on this smear. No fluorescence occurs because the conjugated rabies antibody was absorbed by the rabies virus in the CVS-infected mouse brain suspension and therefore was not available to react with the rabies antigen in the smear. If green staining occurs in this smear, the test is not specific and an attempt should be made to determine the problem and eliminate it.

Negative Control Slides

These controls consist of two impression smears prepared from normal brain. One smear should be stained with rabies conjugate + NMB suspension and the other with rabies conjugate + CVS-infected mouse brain suspension. No green fluorescence should be observed in either smear. (Chart V-C indicates number of participant laboratories using negative controls.)

Optical System

A standard microscope equipped with a source of UV light and a dark field condenser is satisfactory for rabies FA work. Excitation light as provided by a high pressure HBO 200 mercury vapor lamp is often used successfully. Filters that are specifically designed for rabies FA work contribute significantly to correct interpretation of the smears. The primary (excitor) filter should transmit light in the near UV range. The secondary (barrier) filter should be almost colorless, yet opaque to UV. Filters used by participant laboratories are listed in Chart IV. For rabies FA work the following filter combinations are recommended:

Primary (Excitor) filter

5840 (Corning) or UGI (Schott) or BG-12 (Schott)

Secondary (Barrier) filter

Wratten 2A or 2B (Kodak) or Zeiss 41

Recent studies, involving the use of interference filter and ordinary light sources, have produced interesting and encouraging results.^{9,13}

FA smears should be examined in a darkened room. The microscope lamp should be monitored periodically to ensure that it is emitting sufficient light. If the UV light is in constant use, there may be a gradual and unrecognized loss of intensity because of a deteriorating bulb. Excitor and barrier filters should be checked periodically to see that they are free of dust and oil and are not scratched or cracked.

When the smears are observed brightly fluorescing particles - white, yellow, pink - may be seen. This particulate fluorescence should not be confused with the distinctive green fluorescence of labeled antibody.

Mounting Media

Buffered glycerol or buffered glycerol saline is usually used as mounting medium for rabies FA preparations. This mounting medium becomes acidic with time. Because fluorescein does not fluoresce well at pH values below 7, false negative results may be obtained. Therefore, the pH of the mounting medium should be checked periodically, and acidic mountant should not be used.

Histochemical Stains

Rabies can often be diagnosed by demonstrating the presence of Negri bodies in brain specimens. Before the advent of FA, histochemical techniques such as Seller's stain were the standard test used to demonstrate the presence of rabies virus in brain specimens. Smears for Seller's stain should be made at the same time as those for FA testing. The smears for histochemical stains should be slightly thicker. In preparing histochemical stains only high quality reagents should be used. The staining technique itself should be carefully performed according to instructions.^{5,7} Known positive control slides should be included each time the staining procedure is performed.

In microscopic examination of smears, note that the Negri bodies may be outside the neurons because the neuron has been ruptured. The position of the Negri body is not important since the staining reaction with Seller's is so characteristic.

Although histochemical techniques are still used in many laboratories, various studies have shown that specimens positive for rabies virus by FA

often do not contain demonstrable Negri bodies.^{8,12} Consequently, if histochemical methods are used, they should be used in conjunction with the FA technique.

Mouse Inoculation

This survey primarily concerned the FA test performance of participant laboratories. Although mouse inoculation was performed by 45 laboratories (30 laboratories that correctly identified 5 specimens and 15 that incorrectly identified 1 or more specimens), the results of mouse inoculation may not have been available by the due date for the specimen results.

Many workers^{8,12} have demonstrated almost complete agreement between mouse inoculation results and FA results. Although the FA test is a quick, reliable method for the detection of rabies virus, errors can occur because of faulty equipment, unsatisfactory conjugate, lack of experience in reading slides, and failure to examine infected brain areas. Consequently, although routine confirmation of FA results by mouse inoculation is expensive and unnecessary, occasional inoculation especially of specimens that are relatively difficult to diagnose by FA (for example, brains of cattle, horses, vaccinated animals) can provide a laboratory with a periodic assurance of the accuracy of its FA diagnosis. Inoculated mice should be observed for 28 days. If more rapid results are needed, several suckling mice should be used. Since rabies antigen can be detected by FA as early as 3 days after inoculation in suckling mice (before symptoms appear), one mouse per day can be sacrificed beginning at day 3.²

*Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health, Education, and Welfare.

CHART I

Preparation And Fixation Of Smears As
Reported By Participant Laboratories

A. Number of Impression Smears Prepared

	1	2	3	4	5	6	8	10
Labs Correctly Identifying Specimens (44)	1	17	11	7	2	5	0	1
Labs Incorrectly Identifying Specimens (20)	0	4	②	①	0	①	①	0

B. Method of Fixation

	<u>Acetone Fixation</u>	<u>Other</u>	<u>Not Stated</u>
Labs Correctly Identifying Specimens (44)	42	1-Methyl alcohol	1
Labs Incorrectly Identifying Specimens (20)	20		

C. Time of Fixation

	5-15mins.	20-30mins.	1hr.	2hrs.	4hrs.	6hrs.	12-24hrs.	Not Stated
Labs Correctly Identifying Specimens (44)	4	3	2	9	8	1	14	0
Labs Incorrectly Identifying Specimens (20)	1	1	0	0	③	0	①	①

D. Temperature of Fixation

	-20°F	0°F	R°	N + 50°F	0 to 50°C	-10°C	-18°C	-20°C	-25°C	-30°C to-40°C	-50°C to-70°C	N.S*
Labs Correctly Identifying Specimens (44)	1	1	2	1	5	1	1	23	1	2	4	2
Labs Incorrectly Identifying Specimens (20)	0	1	1	0	2	0	0	④	0	0	1	①

① Incorrectly Identified Specimen
Other Than #5.

*Not Specified

CHART II

Conjugate
(Used By Participant Laboratories)

Manufacturer: BBL

Lot Numbers

Dilution Used:	40604	9041906	2121621	OOEYBN	6101907	8021903	2021614	Unknown	302750	111615	09004	202161	0101924
1:4		1		1									
1:5							1*						
1:10				1			1						
1:15	1	1		1	1		1						
1:20		1	1	1111	①		11	1		1			
1:25							1 1*1*1*						
1:29				1									
1:30	1*	1*		1*		1	111			1		1*	
1:32													
1:40				1*(1)									
1:50		1						1					
1:60													①

Manufacturer: CDC

Lot Numbers

Miscellaneous Manufacturers - Lot Numbers

Dilution Used:	11	12	13	Unknown	Ariz. SHL ¹ #5	CSHL ² 219/322	GS ³ Unknown	NYSHD ⁴ #35	SYCCO 0712x8-A	Pasteur Inst 13-73	TS ⁵ Unknown	Markham Unknown
Undiluted										①*		
1:2												1
1:5	①	1*1	1*11				1					1
1:12									1*			
1:20				1*111								
1:30								1			1*1	
1:32					1		1					
1:40						1	1					
1:80				1								
1:120		1										

One lab
used 2
dilutions

Misidentified
samples #1 & #5

One lab
used 2
dilutions

- 1 Correct Response
- 1* Incorrect Response
- ① Incorrectly identified specimen other than #5

- ¹ Arizona State Health Laboratory
- ² California State Health Laboratory
- ³ Georgia State Health Department
- ⁴ New York State Health Department
- ⁵ Texas State Health Department

CHART III

Participant Laboratory Results -
FA Stain Titration

	Performed FA Stain Titration	Followed Manufacturer's Instructions	Other
Laboratories Correctly Identifying Specimens (44)	35	7	1-Dilution determined by experience 1-Dilution determined by microfluorimetry (Note: 4 laboratories used FA stain titration and manufacturer's instructions)
Laboratories Incorrectly Identifying Specimens (20)	18 ③	2 ②	(Note: 4 laboratories used FA stain titration and manufacturer's instructions)

○ Misidentified specimen
other than #5

CHART IV

Microscope Filters - As Used By Participant Laboratories

Excitor Filters+

	BG12	BG23	BG38 ^Δ	UG1	UG2	UG5	UG20	#41	#490	#693	#695	5840	5970	OG1	K430	Leitz
GG4	1															
GG9	1															
BG12 ^Δ								①						1		
BG23	1															
BG38				1111 [◇]					1							
OG1	1*1111			1					1							
#2										1						
#41				1*111												
#44				1		1										
#723											1*					
#5113													1			
EK 2A										1		1				
K430				1*11												
K460	1															
K470	1		1													
K510								1								
Wratten 2A			1*	1111*	1*	1		1			1	1*1	1*1			
				1*1*1*1												
Wratten 2B													1*			
Euphos				1 [◇]												
UV Abs			1	1111												
				1*00												
UV Speer	1															
Leitz																1
No Barrier filter given				1*1							1			1		

Barrier Filters+

1* Incorrectly reported 1 or more of 5 specimen.

① Incorrectly identified specimen other than #5.

Δ Although these filters are listed as they were reported by participant laboratories, the BG-38 is really a red absorbing filter and BG-12 is an excitor filter.

+Note: If more than one (excitor or barrier) filter was used, the response was logged in both places.

□ Signifies the two excitor filters used in combination with one barrier filter.

◇ Signifies the two barrier filters used ...

CHART V

Proof Of Specificity

A. Conjugate Absorbed With:

	Normal Tissue	Rabies Infected Mouse Brain Suspension
Laboratories Correctly Identifying 5 Specimens (44)	40	34
Laboratories Incorrectly Identifying Specimens (20)	19	16

B. Control Slides

	Used Normal Controls	Used Positive Controls
Laboratories Correctly Identifying Specimens (44)	30	43
Laboratories Incorrectly Identifying Specimens (20)	16	20

CHART VI

Elapsed Time Between Staining
And Examination

	None	5-10mins.	15-30mins.	45-1hr.	1-2hrs.	4hrs.
Laboratories Correctly Identifying Specimens (44)	4	6	16	10	5	
Laboratories Incorrectly Identifying Specimens (20)	6 ②	3 ①	6 ①	3	3 ①	1

○ Misidentified specimen
other than #5

CHART VII
 RESULT FORM
 CONJUGATE EVALUATION

Initial Conjugate Dilutions	Undilute		1:2		1:4		1:8		1:16		Control Conjugate	
	Conj. + NMB	Conj. + CVS	Conj. + NMB	Conj. + CVS								
1:5 Conjugate Dilution in Absorbing Suspension												
Final Conjugate Dilution	1:5	1:5	1:10	1:10	1:20	1:20	1:40	1:40	1:80	1:80	Optimal	Optimal
Specific Staining Intensity												
Nonspecific Background Staining												
Negri Body and Antigenic Dust Staining												

Control Conjugate: Optimal Titer _____ Source _____

Lot # _____ Date Titered _____

Absorbing Suspension

CVS: Source _____ Lot # _____ Date _____

NMB: Source _____ Lot # _____ Date _____

Test Conjugate: Optimal Titer _____ Source _____

Lot # _____ Date Titered _____

REFERENCES

1. Dean, D.J. - 1966, The Fluorescent Antibody Test - Laboratory Techniques in Rabies - 2nd Edition - WHO, Geneva.
2. Dean, D.J., Abelseth, M.K. - 1973, The Fluorescent Antibody Test - Laboratory Techniques in Rabies - 3rd Edition - WHO, Geneva.
3. Goldwasser, R.A., Kissling, R.E., 1958, Fluorescent Antibody Staining of Street and Fixed Rabies Virus Antigens. Proc. Soc. Exp. Bio. Med. 98:219.
4. Goldwasser, R.A., Kissling, R.E. and Carski, T.R., 1959, Fluorescent Antibody Staining of Rabies Virus Antigens in the Salivary Glands of Rabid Animals. Bull WHO 20, 579.
5. Laboratory Diagnosis of Viral Diseases, 1973, DHEW, PHS, CDC, Atlanta, Ga.
6. Larghi, O.P., Jimenez, C.E. Metodo Para Acelerar La Technica De Immunofluorescencia Para El Diagnostico De Lx Rabia, 1971, Biol. San. Panamerica LXXI, #1, 36-40.
7. Lennette, E.H., Schmidt, N.J. (Ed) 1969, Diagnostic Procedures for Viral and Rickettsial Diseases, 4th Edition, APHA, 321-353.
8. Lennette, E.H., Woodie, J. D., Nakamura, K. and Magoffin, R.L., 1965, The Diagnosis of Rabies by Fluorescent Antibody Method (FRA) Employing Immune Hamster Serum. Health Lab Science, 2 #1, 24.
9. Lewis, V.J., Thacker, W.L. and Engelman, H.E., 1973. Evaluation of the Interference Filter for Use in Rabies Diagnosis by the Fluorescent Antibody Test. App. Micro. 26 #3, 429.
10. Lewis, V.J., 1974, Personal Communication.
11. Martell, M.A., Batalla, D., Baer, G.M., 1970, Redduccion Del Tiempo Del Metodo Clasico Para El Diagnostico De Rabia Par Immunofluorescencia. Technia Pecuaria en Mex, #14, 48.
12. McQueen, J.L., Lewis, A.L. and Schneider, N.J., 1960, Rabies Diagnosis by Fluorescent Antibody 1. Its Evaluation in a Public Health Laboratory. AJPH 50, #11, 1743.
13. Rygaard, J. and Olsen, W. 1969, Interference Filters for Improved Immunofluoresce Microscopy. Acta Path. Microbiol. Scand. 76, 146.
14. Annual Rabies Summary, Zoonoses Surveillance, 1972, DHEW, PHS, CDC, Atlanta, Georgia.

RABIES EVALUATION QUESTIONNAIRE

1) What is (are) the source(s) of specimens tested in your laboratory?

Private Individuals	<u>60</u>
Physicians and Veterinarians	<u>61</u>
Local Health Departments	<u>57</u>
Regional Laboratory	<u>16</u>
Others	<u>16</u>

2) Does your State confirm the diagnosis in cases of human exposure?

Comments

Yes	<u>34</u>	1 - If PHL tests uncertain or problems specimen arises. 1 - By mouse inoculation (MI). 1 - MI on all animals except rodents in cases of human exposure.
No	<u>26</u>	1 - MI when FRA equivocal. 1 - FRA negative tissues inoculated into mice.
N.A.	<u>2</u>	

Is there any other diagnostic confirmation or quality control in your laboratory?

Yes - CDC: 21

State Health Laboratory: 3

Local Federal Laboratory: 2

Local University: 1 (Pathobiology Lab.)

MI if human bite from bat, coyote, skunk, fox: 1

MI if FRA equivocal: 1

MI of brain suspension of large animals with human exposure: 1

Sellers and MI when FRA inconclusive: 1

No - 19

Quality Control - By microfluorimetry: 1

In Laboratory Quality Control: 3

Positive tissue from outside laboratory inoculated own mice: 1

3) How are specimens from animals involved in human exposures tested in your laboratory?

1 - No human exposure accepted.

FA (Fluorescent Antibody): 63

MI (Mouse Inoculation): 49*

- *MI - If FRA equivocal or problem specimen: 1
- Major animals only: 1
- Cases involving bats, foxes, raccoons, skunks, and all positives regardless of specimen: 1
- All bats, positive FA, any with questionable results: 1
- On large animal but not pet mice, guinea pigs, hamsters, etc.: 1

Sellers (Histochemical Stain): 27

Other: Williams: 1
Van Gieson's: 1
Histopathology: 3
Mann's: 1

4) How are specimens from animals not involved in human exposures tested in your laboratory?

Depends on animal - rodents-sellers;
Other-FA and Sellers. -1

a. Same as human exposure - 33

- If pets or livestock exposed - 1
- Certain ones - 1
- Bats, not involved in human exposure, not put in mice - 1
- Also forwarded to Vet. Diag. Lab. - 1

b. FA - 47

- MI - 1
- Negri body - 1
- Rodents, skunks
bats, raccoons - 1

c. Only those with human exposure tested - 2

By FA - 1

d. MI - 6 - Test domestic animals without human exposure for Dept. of Agriculture.

e. Sellers Stain - 16

Histopathology - 1

5) What do you estimate as the percentage of your specimens positive by one diagnostic test - negative by another?

NA - 4

a. FA positive
MI negative _____%

MI done only if -
- FRA questionable or
FRA + unique animal = 3
- FRA-bats with human
exposure 1

<u>0%</u>	<u>0.46%</u>	<u>0.5%</u>	<u>< 1%</u>	<u>1%</u>
18	1	1	5	2
<u>3%</u>	<u>4%</u>	<u>60%</u>	<u>95-98%</u>	
1	2	1	1	

b. FA negative MI positive _____% MI only on FA negative specimen - 1

<u>0%</u>	<u>.004%</u>	<u>.05%</u>	<u>.01%</u>	<u>0.11%</u>	<u>0.1%</u>
17	1	1	2	1	1
<u><0.1%</u>	<u>0.3%</u>	<u>0.57%</u>	<u><1%</u>	<u>5.2%*</u>	<u>Unknown</u>
2	1	1	5	1	1

*In dogs
38% based on total + only

c. Sellers stain negative FA positive _____%

<u>0%</u>	<u><1%</u>	<u>1%</u>	<u>8%</u>	<u><10%</u>	<u>10%</u>	<u>20%</u>	<u>25%</u>
3	1	3	1	1	1	2	2
<u>30%</u>	<u>14-47%</u>	<u>50%</u>	<u>62%</u>	<u>95-98%</u>	<u>100%</u>		
1	1	4	1	1	2		
<u>Unknown</u>							

2

6) What do you consider the chief diagnostic problem(s) in your laboratory (e.g., poor equipment, lack of mice, unsatisfactory specimens, inadequate conjugate, etc.)?

Problems with: Unsatisfactory Specimens
 Specimens (decomposed, etc.) - 41

 Delay in receiving specimens - 1

 Specimens in glycerine - 1

 Unnecessary specimens - 3

 Excessive # of specimens - 1

 Poor histories - 2

 Problem specimens (such as cats) - 1

Mice Lack of mice - 6

Conjugate/
 Reagents Unsatisfactory conjugate - 11

 Inadequate supply of conjugate - 11

 Availability of good reagents - 1

Equipment,
 Facilities,
 Personnel Poor equipment or work area - 3

 Inadequate facilities to handle
 specimens - 1

 Inadequate animal facilities and
 animal caretakers - 2

 Lack of personnel to work in rabies
 diagnosis - 1

Procedural Problems	Incompatibility of rabies procedures - <u>1</u>
	Technical difficulty with FA scope - <u>2</u>
	Non specific fluorescence - <u>6</u>
	Problems with tissue fixation - <u>1</u>
Problem with Controls	Lack of good positive control material - <u>2</u>
	Lack of positive results - <u>1</u>
Other Problems	Public pressure for immediate animal examination regardless of laboratory hours - <u>1</u>

7) Have the persons in your laboratory who handle rabies specimens received pre-exposure immunization?

Yes - 60 - all not sensitive to DEV - 1
 - 50% - 3

No - 3

Has their titer been confirmed?

Yes - 50

No - 9

- Insufficient time to detect rise - 2

- No demonstrable titer - 1

- Reaction to vaccine - 1

Additional Comments:

Because additional comments were, in many instances, quite lengthy, it was not feasible to respond to these at the present time. However, every effort will be made to supply necessary information on an individual basis.

