REPORT TO THE GOVERNMENT

OF

SYRIA

TRAINING AND INVESTIGATION

ON

THE POULTRY DISEASE

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UNDER
TECHNICAL COOPERATION PLAN
FOR
THE NEAR & MIDDLE EAST AND AFRICA

THE OVERSEAS TECHNICAL COOPERATION AGENCY

TO : Ministry of Agriculture, Syrian Arab Republic

FROM : Dr. I. Yoshida, Poultry Disease Expert

SUBJECT : Report of Research and Training on the Poultry Disease at the Damascus Veterinary Laboratory

DATE : September 20, 1966.

COPIES TO : Embassy of Japan

: Dr. K. Sugimura, FAO Veterinary Biologist

: Overseas Technical Cooperation Agency of Japan

C O N T E N T S

INTRODUCTION

The expert assigned to the Damascus Veterinary Laboratory in Syrian Arab Republic in order to train Syrian staff of the Laboratory on the poultry disease. The period of his assignment was 1 year - from the 4th of October 1965 to the 1st of October 1966.

The Veterinary Laboratory provided Mr. M. Hawary as the expert's assistant. Mr. J. Yousfi and Mr. S. Jabi were trained on the routine diagnosis of poultry disease and production work of poultry biological products by the expert respectively.

In the process of training, the expert conducted technical guidance of poultry disease, laboratory examination, tissue culture technique and some production of some poultry biological products. In addition, the expert made some serological research and a field investigation of poultry disease.

The expert wishes to thank Dr. K. Sugimura, FAO Veterinary Biologist, deeply for the thoughtful supervision and advices regarding the expert's work, and also Dr. F. Ladkani, Director of Animal Health, for his kindness, In addition, the expert would like to express his appreciation to Dr. M. Baghdady. Chief of Veterinary Laboratory, and all the Laboratory's staff for their thoughtful arrangements, support and sincere hospitality.

RECOMMENDATIONS

The following recommendations were made by the expert.

I. On the Prevention of Infectious Diseases

From the results of routine diagnosis in the Laboratory and investigation on the antibody distribution among chickens in Syria, it has been recognized that many serious poultry infectious diseases - Pullorum disease, CRD, infectious coryza, Newcastle disease, fowl-pox, leukosis, ascaridiasis and coccidiosis etc. - are spread widely in the country.

The following items should be performed for the control of these infectious diseases.

1. Promotion of vaccination (Newcastle disease and fowl-pox)
2. Promotion of rapid agglutination test (pullorum disease and CRD)
3. Promotion of weed-out of infectious chickens (pullorum disease and leukosis)
4. Improvement of environmental conditions (ventilation and avoidance of crowded breeding etc.)

5. Application of preventive medicines
   Antibiotics : for CRD and coryza
   Furazolidone : for pullorum disease and coccidiosis
   Amprolium, glycamide, zoalene and sulfamine : for coccidiosis
   Phenothiazine, piperazine : for ascaridiasis
6. Enforcement of disinfection (chicken-house, instruments, dead chickens, hands, clothes and shoes etc.)
7. Limitation of visitors
8. Eradication of insects

II. On the Prevention of Nutritional Disorders

On the other hand, it was noticed significantly that the damages caused by deficiency disease due to lack of vitamins, minerals or other nutriments are not able to neglect on the poultry industry in this country. Consequently, it is very necessary to conduct about the management by proper feeding as well as hygienic consideration against the infectious diseases.

III. Extension of Knowledge of Poultry Disease and Training of Field Technicians

Many technicians trained well should be supplied through the country to complete the preventive program of poultry disease as mentioned above. The extension of knowledges and improvement of techniques concerning poultry disease for the field technicians should be attempted taking occasions.

IV. Establishment of Central Poultry Disease Laboratory

The poultry disease section in the Veterinary Laboratory is being improved gradually both of facilities and techniques. It is, however, necessary to develop the studies constantly, specially qualified veterinarian who has a responsibility about poultry disease exclusively should be arranged urgently. Furthermore, it seems that the acceptance of technical cooperation by foreign experts is very necessary for the present.

PROGRESS OF WORK

A. GENERAL GUIDANCE OF POULTRY DISEASE

The expert was able to recognize that the routine diagnosis on poultry disease are made by staff of the Laboratory without serious difficulty. However, several points which should be improved so soon in regarding to more accurate diagnosis on various samples were noticed. So, the expert devoted his effort on improving diagnostic procedures.

I. Establishment of the Manual of Poultry Diseases

The expert made the manual of poultry diseases based upon his observations and experiments in the past as well as many reports concerned with poultry diseases (APPENDIX I). The manual was classified into 4 groups : viral diseases, bacterial diseases, parasitic diseases and non-infectious diseases, and described the main point of individual disease compactly. It is very convenient to recognize the diseases from the wide view point and to compare the characteristics of each disease. The expert is expecting to be used it practically by many technicians concerned.

II. Settlement of Diagnostic Procedure

It is very important to investigate a disease systematically from the history to laboratory examination in order to diagnose accurately, specially in the case of epizootic outbreak. The expert made a style of diagnostic working sheet to make the procedure easily and customarily (APPENDIX II).

III. Routine Diagnostic Guidance

Test samples about five to six cases daily were brought in the Veterinary Laboratory. The expert conducted the various general guidance on diagnosis on those test samples for training laboratory staffs in charge of poultry disease who have been willingly on being trained. Also, the expert made a general guidance occasionally regarding the poultry disease control by using colour slide photography.

The staffs are improving themselves gradually on their diagnostic works.

B. TECHNICAL GUIDANCE OF LABORATORY EXAMINATION

The method of isolation and identification of bacteria on main poultry bacterial diseases, cultural characteristics of virus on general poultry viral diseases and some serological reactions were explained as stated in the APPENDIX III.

BACTERIAL EXAMINATION

I. Salmonella pullorum (Pullorum disease)
(TABLES B-1, B-2 and Fig. B-1)

II. Mycoplasma gallisepticum (CRD - Avian Respiratory Mycoplasmosis)
(TABLES B-3, B-4 and Fig. B-2)

III. Haemophilus gallinarum (Infectious coryza)
(TABLE B-5 and Fig. B-3)

VIROLOGICAL EXAMINATION

IV. Susceptibility of Cell Culture to Selected Avian Viruses
(TABLE B-6)

V. Differencial Response of Chicken Embryos to Selected Avian Viruses
(TABLE B-7)

VI. Differencial Response of Susceptible Chickens to Selected Avian Viruses
(TABLE B-8)

SEROLOGICAL EXAMINATION

VII. Hemagglutination-Inhibition Test
(TABLE B-9, B-10 and B-11)

VIII. Agar Gel Precipitin Test

XI. Neutralization Test

C. TECHNICAL GUIDANCE OF TISSUE CULTURE METHOD

The establishment of tissue culture technique in the Laboratory should be of the most necessary ones in regarding to the improvement of production of virus vaccines as well as the diagnosis of viral disease. Even though, various difficulties on its practice laid in the way in the beginning - especially, lack of apparatuses, way of cleaning glasswares and preparation of chemically pure distilled water etc. - the expert attempted a trial on tissue culture. The difficulties are being removed gradually and now, the preparation of primary cell culture is nearly possible in weekly.

Laboratory staffs are being improved gradually on the tissue culture technique which is to be introduced in various virological works.

The tissue culture technique is being utilized in the isolation, identification or investigation of antibody of viruses after achieving the enough

activity on the maintenance of cell culture.

I. Method of Tissue Culture

As stated in the APPENDIX IV.

II. Application of Tissue Culture

1. Viral titration and neutralization test by the plaque technique

The freeze dried Newcastle disease living vaccine (Komorov strain) has been produced in the Laboratory based by the manufacturing standard that was established by Dr. Sugimura and the staffs in the Laboratory.

The virus titers of the vaccine were calculated by the plaque method and compared its virus titers with the embryonated egg technique. The virus passed through tissue culture one time and avirulent Ishii strain of Newcastle disease virus were also employed simultaneously.

Neutralization test (See APPENDIX III-B-IX) was performed using immune serum of chicken of strain Ishii.

As shown in the TABLE C-1, it was recognized, even though in a small trial, that there was considerable corelationship between the results of plaque and embryonated egg techniques. It is considered, therefore, that the titration of the vaccine may be available to apply to the plaque technique. The titers of virus multiplied in the tissue culture were obtained rather high values, therefore, there is a possibility of the production of vaccine by means of tissue culture if several conditions are improved.

Neutralization test by plaque technique seems to be also effective for the identification of virus or the potency test of the vaccine.

2. Isolation and identification of virus

By means of tissue culture technique, several viruses were isolated from chickens brought in the Laboratory for the diagnosis.

TABLE C-1. Virus titration by plaque and embryonated egg technique

			ND vaccine lot 8	ND vaccine lot 8 passed TC 1 time	ND virus strain Ishii passed TC 5 times
Plaque technique (log PFU/0.1 ml)	Exp. I	Virus titer	6.4		
	Exp.II	Virus titer	5.0	5.1	6.0
		N-T index	3.0		3.4
Embryonated egg technique (log EID_{50}/0.1 ml)	Exp. I	Virus titer	6.7		
	Exp.II	Virus titer	6.7	5.7	

ND : Newcastle disease

TC : Tissue culture

N-T : Neutralization test

TABLE C-2. Isolation and identification of virus

Reference No.	Date received	Died/ killed	HI titer	Materials	Isolation TC (CPE)	Isolation EE Death	Isolation EE HA	N-T (log-index)
13-48-S	Nov.16 '65	Killed	1:10>	Brain				
				Trachea	-	+	1:3200	
				Lung				
				Intestine	+	+	1:3200	2.8
13-48-D	"	Died		Brain		+	1:1600	
				Trachea	+	+	1:3200	
				Lung				
				Intestine	+	+	1:3200	
26-49	Jan.13 '66	Killed	1:10>	Brain	+			3.5
				Trachea	+			
26-49-Ot	"	Killed		Trachea	+			
41-49	Jan.27 '66	"	1:160	Brain	+			
				Trachea	+			
				Intestine	+			
43-49	Jan.29 '66	"		Brain	+			
				Trachea	+			
				Lung	+			
				Intestine	+			
55-49-D	Feb. 5 '66	Died		Brain	+			
				Intestine	+			
55-49-K	"	Killed		Brain	+			3.1
				Trachea	+			
				Lung	+			
				Intestine	+			
47-1	Feb. 8 '66	Killed	1:10>	Brain	-			
				Trachea	-			
				Lung	-			
				Intestine	-			
47-2	"	"	1:10>	Brain	+			2.4
				Trachea	+			
				Lung	+			
47-3	"	"	1:40	Brain	+			3.0
				Trachea	+			
				Lung	+			
				Intestine	+			
47-4	"	"	1:10	Brain	+			
				Trachea	+			
				Lung	+			
				Intestine	+			
Vaccine Lot 8								4.1

HI : Hemagglutination inhibition test
TC : Tissue culture
CPE : Cytopathic effect
HA : Hemagglutination test
EE : Embryonated egg
N-T : Neutralization test

The expert conducted the identification of those viruses isolated applying tissue culture method with training staff of Laboratory concerned.

As shown in TABLE C-2, all the virus was ones isolated in brain, trachea, lung and intestinal contents of chickens suspected Newcastle disease.
The isolated viruses showed the cyncytial cytopathic effect. The neutralization test by the plaque method using a positive serum of Newcastle disease virus were carried out for identifying viruses.

All the virus strains tested were identified as a Newcastle disease virus by the specific neutralization (see APPENDIX-III-B-IX) with the positive serum.

3. Investigation of antibody against chicken susceptible viruses by using neutralization test

A few diseases such as Newcastle disease, fowl-pox and avian leukosis complex have been recognized as the most frequent viral disease of chickens in Syria up to the present time.

Then, the investigation of antibody against several chicken susceptible viruses were carried out by utilizing tissue culture technique in order to learn the distribution of other viral disease in poultry. The results are reported in the next paragraph.

D. INVESTIGATION ON THE ANTIBODY DISTRIBUTION AMONG CHICKENS AGAINST SOME BACTERIA AND VIRUS OF THE CHICKEN DISEASES

Investigations on the antibody distribution among chickens against some bacteria and virus of the chicken diseases were carried out by the serological test of sera collected from various areas in Syria.

I. Place and Number Sample Collected

103 chicken sera were collected from Damascus, Hama and Deraa during eight months from November 1965 to June 1966 (TABLE D-1). These serum samples were collected from healthy chickens clinically except samples on routine diagnosis and those of Gazali Poultry Farm.

II. Method

Agglutination Test (Serum Plate Test)

The following three bacterial suspensions were used as the antigen.

Pullorum disease antigen (Salmonella pullorum, strain Nakamura and L 60131, Lot 3)

CRD antigen (Mycoplasma gallisepticum, strain KP-13, Lot 2)

Coryza antigen (Haemophilus gallinarum, strain 221)

TABLE D-1. Place and number sample collected

Places		Date	Number of samples
Damascus,	Materials from routine diagnosis	Nov. '65 - May '66	23
	Gazali Poultry Farm	June 2 '66	6
	Veterinary Laboratory	Oct. '65 - May '66	17
Hama	Gov. Poultry Farm	May 17 '66	37
Deraa	Gov. Poultry Farm	June 16 '66	20
Total			103

TABLE D-2. Positive rate of antibodies against chicken susceptible organisms

Places		CRD	Pull-orum	Coryza	Celo	Reo	ILT	IB	F-P	ND
Damascus	Material Routine diagnosis	16/16* (100)**	3/16 (18.0)	5/16 (31.3)	3/19 (15.8)	21/22 (95.5)	0/9 (0)	0/17 (0)	8/17 (47.0)	9/16 (56.3)
	Gazali Poultry Farm	5/5 (100)	4/5 (80.0)	4/5 (100)	3/3 (100)	6/6 (100)	0/2 (0)	0/5 (0)	3/5 (60.0)	4/6 (66.7)
	Veterinary Laboratory	6/17 (35.3)	0/17 (0)	7/17 (41.2)	0/10 (0)	10/11 (90.9)	0/11 (0)	0/11 (0)	0/11 (0)	0/11 (0)
	Total	27/38 (70.1)	7/38 (18.4)	16/38 (40.2)	6/32 (18.8)	37/39 (94.9)	0/22 (0)	0/33 (0)	11/33 (33.3)	13/33 (39.5)
Hama		36/36 (100)	9/35 (25.7)	8/35 (22.8)	13/26 (50.0)	35/35 (100)	0/35 (0)	0/33 (0)	10/33 (30.3)	21/22*** (95.5)
Deraa		20/20 (100)	3/20 (15.0)	3/17 (17.6)	0/15 (0)	20/20 (100)	0/15 (0)	0/17 (0)	4/13 (30.8)	9/12 (75.0)
TOTAL		83/94 (88.3)	19/93 (20.4)	27/80 (33.7)	19/73 (27.4)	92/94 (97.9)	0/72 (0)	0/83 (0)	25/79 (31.6)	43/67 (64.2)

* Number of positive / Number of samples tested

** Percentage

*** Vaccinated flock

The tests were carried out employing undiluted serum and following the way described in the manufacturing standard of CRD antigen (APPENDIX VI).

Neutralization Test (Tissue Culture Technique)

Next four viral materials passaged on chicken kidney cell culture were used as the antigen.

Celo (Chicken embyro lethal orphan virus, strain Ote)

Reo (Respiratory enteric orphan virus, strain Uchida)

ILT (Infectious laryngotracheitis virus, strain NS-175)

IB (Infectious bronchitis virus, strain KH-T44)

The method of neutralization test was employed the way of screening test (APPENDIX III-B-IX).

Hemagglutination-Inhibition Test

Newcastle disease virus (Freeze-dried living vaccine, strain Komorov) was used as the antigen. The method of test is described in APPENDIX III-B-VII.

Agar Gel Precipitin Test

A chorio-allantoic membrane infected with Hungarian fowl-pox virus was used as the antigen. The method of test is described in APPENDIX III-B-VIII.

III. Test Results and Discussions

As shown in TABLE D-2, the positive rates of antibody were calculated.

The antibody against CRD was found 100 per cent except Veterinary Laboratory. Pullorum disease was found 20.4 per cent in average in all places except Vet. Lab. where was entirely negative. The positive reaction against coryza was found in the all places including Vet. Lab., and the average was 33.7 per cent. From the above results, it was recognized that the infections of these three kind of bacteria were spread widely.

The antibodies against Celo virus were found at about 15 - 100 per cent (average, 27.4 per cent), while the Vet. Lab. and Deraa were all negative. In the case of Reo virus, the antibodies were found approximately 100 per cent in the all places. These viruses have hardly virulence against chickens, but they are able to grow very well in the respiratory organ of chickens, so that it may be to have some influence with other respiratory infectious diseases.

The outbreak of infectious laryngotracheitis and infectious bronchitis have not been reported in Syria and the antibody also could not to be found at all.

The antibodies against fowl-pox virus were found in the all places except Vet. Lab. and the average was 31.6 per cent. As the fowl-pox vaccine has not been applied widely, most of these antibodies are presumed to be due to natural infection.

The antibodies against Newcastle disease virus were found at high percentage (average, 64.2 per cent) specially in Hama and Deraa. The antibodies in Hama and Deraa are evident to be due to vaccination, but ones in chickens from routine diagnosis are supposed to be caused by natural infection mainly.

Serum sample tested were collected mainly at the middle and south parts of country, and no samples of Lattakia (west coast), Aleppo (northern area) and Dei-ez-Zor (coastal region of Euphrates River) were tested. It is, therefore, rather difficult to learn the general picture on the distribution of infection among chicken of whole country. However, results obtained in the trial as mentioned above are effective enough to presume a general situation regarding chicken infectious diseases.

E. ROUTINE DIAGNOSIS OF POULTRY DISEASE IN THE VETERINARY LABORATORY IN OCT. 1965 - AUG. 1966

The total results of routine diagnosis of poultry disease in the Veterinary Laboratory during eleven months from Oct. 1965 to Aug. 1966 were showed in TABLE E-1. The results of Agglutination test of 1,004 chicken sera on Salmonella pullorum infection which were sent from Gov. Poultry Farm at Lattakia was included in the total results.

Total cases tested were 2,351 and 1,188 cases of them were positive. From the results, it was recognized that most serious poultry diseases in Syria were pullorum disease, CRD, coryza, Newcastle disease, fowl-pox, leukosis, coccidiosis and nutritional disorder etc.

TABLE E-1. *Routine diagnosis of poultry disease (Oct.1965 - Aug.1966)*

Disease	Months											Total (%)
	10	11	12	1	2	3	4	5	6	7	8	
Pullorum disease	2	13	1			21	28	6	6 (T-1)	6	6	89 (7.5)
CRD	15	16 (Pe-1)	12	10 (C-1)	4	16	9	5	10	44 (Pi-2)	4	145(12.2)
Inf.coryza	4	8	10	13	3	12	2	8	9		2	71 (6.0)
Staphylococcosis			3									3 (0.3)
Dis. caused by Esch.							1		1			2 (0.2)
Streptococcosis							1					1 (0.1)
Inf. synovitis							6	1	1	3	4	15 (1.3)
Fowl-pox	12	16	8	6	6	22	7	1			2	80 (6.7)
Pigeon-pox	Pi-4		Pi-2								Pi-3	9 (0.8)
Newcastle disease			2	20	28	4	10	15	63	12	9	163(13.7)
Visceral lymph.	4	4	2	6		2	2	1	1	4	10	36 (3.0)
External parasitosis	3	2	5	1	7		7	4	9	29	5	72 (6.0)
Ascaridiasis	22	5	10	23	7	2	2	6	6	11	14	108 (9.1)
Strongylosis									1			1 (0.1)
Taeniasis	5	2		3	2					3	3	18 (1.5)
Coccidiosis	11	4	5		27	2	7	6	6	7	12	87 (7.3)
Spirochaetosis	2								1			3 (0.3)
Nutritional disordor	9	6 (Pe-1)	7 (Pa-1)	12	10 (T-1)	10	20	36	36 (Pe-1)	37	27	210(17.7)
Uremic poisoning	3											3 (0.3)
Inflamation of crop	2		1		2						1	6 (0.5)
Neural lymphomatosis		1			1							2 (0.2)
Others	15	15 (Pe-2 Sp-1)	5 (Pi-1)	2			1	4	8 (Pi-1)	8	5	63 (5.3)
Total	113	92	73	96	97	91	103	93	158	164	108	1,188
Negative*	47	159 (Pe-2)	12 (Pe-1)	7		380	373	48 (T-2)	42		14	1,082
Suspected**	13					17	50	T-1				81
TOTAL	160	264	85	103	97	488	526	142	200	164	122	2,351

* Pullorum disease, CRD, Internal parasitosis
** Pullorum disease, CRD, Boturism

T : Tuekey, Pe : Peacock, Pi : Pigeon, Pa : Parrot, Sp : Sparrow, C : Canary

F. ESTABLISHMENT OF MANUFACTURING STANDARD OF FOWL-POX VACCINE AND CRD ANTIGEN, AND NECESSARY BASIC EXPERIMENTS

Dr. Sugimura has established the manufacturing standard of fowl-pox vaccine and CRD antigen standards and the expert cooperated for the establishment of standard and carried out some trials necessary on the establishment of standards.

I. Fowl-Pox Vaccine

Main point in question on the standard depend on the final decision of virus strain used for the vaccine production, and experiments on suitability of virus source of Syrian domestic and some foreign strains were carried out. In regarding to the standarization of manufacturing standard of fowl-pox vaccine, a test production and some examinations by using two strains of fowl-pox were performed.

1. Test production

Seed virus : Two strains of different production virus, Syrian domestic and Japanese strain, were taken up in the trial. The Japanese virus strain is the avianized pigeon-pox virus of "Nakano strain" which has been used for the vaccine production in Japan by a "follicle method", and passed 160 times on chorio-allantoic membrane (CAM), 5 times through pigeons and 18 times on the CAM. The Syrian domestic strain (Hungarian strain is to be a virus of fowl-pox origin, which is used as the vaccine of "stick method". The source and history of the strain are rather indistinct except it has been passed recentry through four passages on CAM.

Production: Test production was made by same means as described in manufacturing standard (APPENDIX V). The infected CAM showed muddy, thickening and swelling both Syrian and Japanese strains. In the lower dilution (10^{-5} or 10^{-6}), Japanese strain formed small round pocks, while Syrian strain formed difuse one which is found generally in virulent strains.

2. Titration

0.1 ml of 10^{-5} -diluted suspension of each products were inoculated on CAM of seven embryonated eggs of 10-day-old. The pock-forming units (PFU) were calculated on CAM harvested after five days. The details of method are described in manufacturing standard (APPENDIX V).

As shown in TABLE F-1, there were no notable decrease of viable virus content in the products of before and after freeze-drying.

Besides, the refinning of vaccine material by means of centrifugation or filtration shall be advisable as an improvement of vaccine standard in connection with a convenience of re-suspending at the field use. Also the utility of pock-counting technique for the titration of fowl-pox vaccine was confirmed.

TABLE F-1. Tiration of test products of fowl-pox vaccine

Strains	Materials		PFU / 0.1 ml
Syrian strain (Fowl-pox virus)	Suspension of CAM (x 10)		1.1×10^6
	Supernatant*	Before freeze-drying	1.3×10^6
		After freeze-drying	1.5×10^6
Japanese strain (Avianized Pigeon-pox virus)	Suspension of CAM (x 10)		5.4×10^5
	Supernatant	Before freeze-drying	4.3×10^5
		After freeze-drying	4.7×10^5

* *Centrifuged at 2,000 rpm for 5 minutes*

3. Trials on safetibility, pock-forming ability and immunogenicity

Method

Vaccine : Two kind of vaccines which were prepared by Syrian strain and Japanese strain were used. The PFU per ml in log on the CAM were 6.18 and 5.67 respectively.

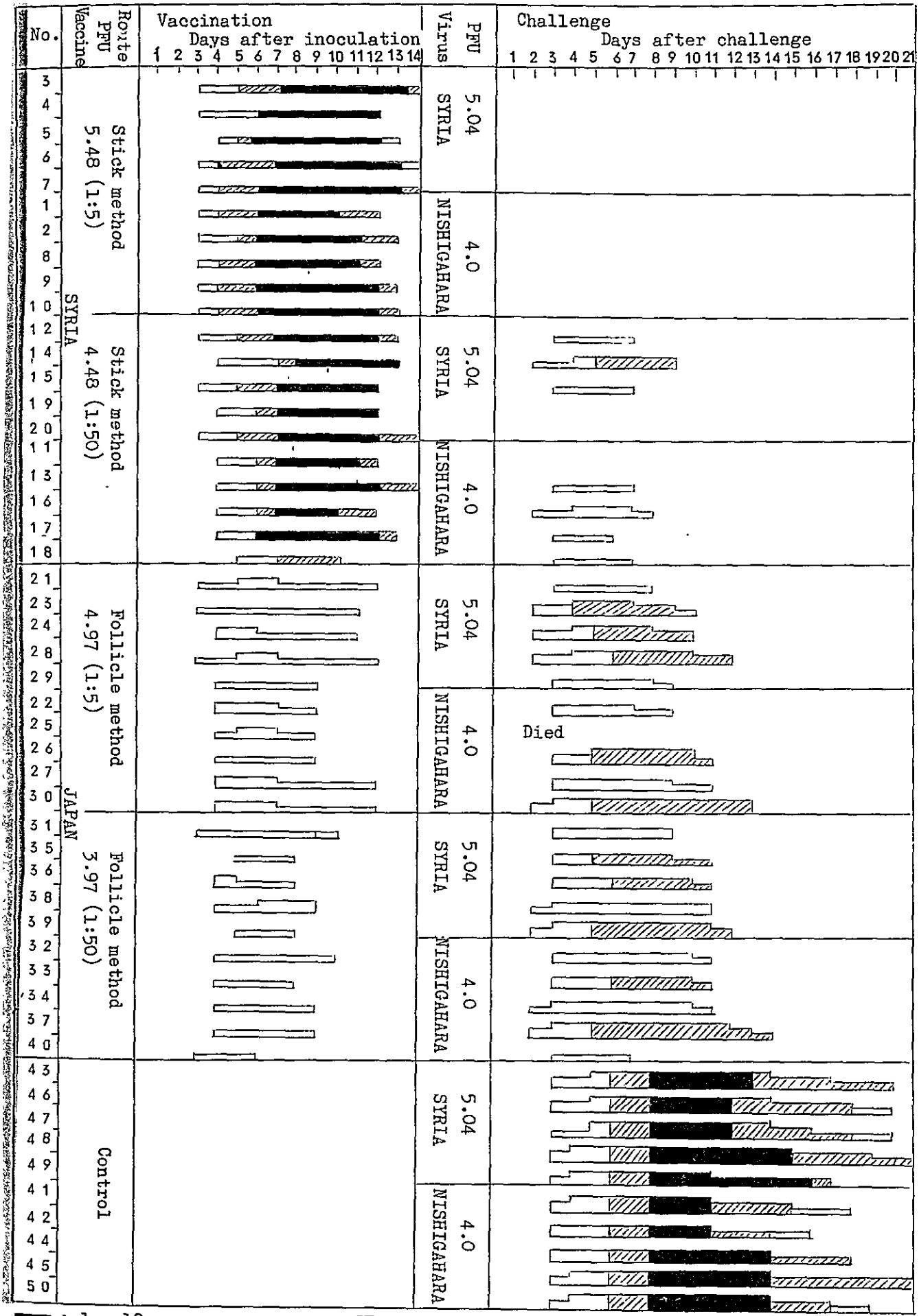
Chickens : Fifty 6-week-old Newhampshire chickens bred in the Veterinary Laboratory breeding under the sanitary management were used in tests.

Vaccination : Fifty chickens were divided into 5 separate groups of same number including control, and vaccinated with 2 kind of vaccines diluted to 1:5 and 1:50 respectively.

Syrian strain (about 0.001 ml, 3.48 and 2.48 PFU in log) was inoculated to 4 point of right wing-web by the "stick method" at space of 3-4 mm using moistened needle with the vaccine.

Japanese strain (about 0.1 ml, 4.97 and 3.97 PFU in log) was inoculated to the plucked right thigh by the "follicle method".

Fig. F-1. Pock-forming ability and immunogenicity



Five chickens of control group were sticked by the needle without vaccine and other 5 chickens were only plucked.

The general and local reaction of the chickens were observed for 2 weeks after inoculation.

Challenge : All 5 groups were sub-divided each 2 groups at random and challenged with Syrian strain (5.04 PFU in log) and Nishigahara strain (4.0 PFU in log) of fowl-pox viruses on the plucked left thigh at 38th day after vaccination.

Results

Safetibility and pock-forming ability : As shown in the left side of Fig. F-1, in chickens vaccinated with Syrian strain both high and low dilutions, the reactions began to appear at 4-5th day, reached maximum with forming crusts in size of about 7-8 mm at 7-10th day, and then the crusts dried gradually and eventually fell off until 12-14th day.
The reactions were located, generalization of infection did not occur and there was no appreciable loss of condition. Consequently, the rather strong local reaction and safetibility were confirmed in this group.

In the chickens vaccinated with Japanese strain, the follicles appeared in both dilutions at 4-5th day, reached maximum at 6-7th day and all follicles disappeared until 10-12th day without forming crusts. The reaction of chickens vaccinated with 1:50 dilution were less and shorter, compared with 1:5 dilution. There was no loss of condition during the observational period.

The chickens of control group were not noticed any changes.

Immunogenicity : The results are shown in the right side of Fig. F-1. In the chickens vaccinated with Syrian strain, the group of 1:5 dilution did not show any reactions against the challenges by both Syrian and Nishigahara strains. Some chickens of the group of 1:50 dilution also did not show any reactions, but others showed slight reaction and it disappeared quickly within 10 days after challenge.

In the chickens vaccinated with Japanese strain, the reactions began to appear in both dilutions, reached maximum with fusion of each follicles and moderate crusts at 6-8th day, and fell off until 12-13th day.

The control chickens showed same reaction against both challenge viruses. The reactions began to appear late than the vaccinated chickens, and reached maximum with extensive lesions and severe crusts at 8-11th day. The lesions remained for long time and it took more than 3 weeks to recover all chickens.

The control chickens as well as vaccinated ones did not show any appreciable loss of condition, and did not occur any metastasis of lesions throughout the observational period.

From the results mentioned above, it was able to summarize as follows:

i). The vaccine prepared from Syrian strain of fowl-pox virus showed rather severe reaction at the vaccinating point, but there was no appreciable loss of condition.

ii). The vaccine prepared from Japanese strain of pigeon-pox virus showed mild follicle reaction and safetibility.

iii). The immunogenicity was effective both Syrian and Japanese strains, but the former was more effective than the latter.

Consequently, it is considered that the application of fowl-pox vaccine in this country will be suitable to use Syrian strain of fowl-pox virus with the "stick method" from the reason as follows : obtains high immunity; uses less virus; permits more rapid vaccination by eliminating feather plucking.

In this case, the vaccine should be used carefully and should not be used in the region where fowl-pox has not outbroken because of that the vaccine prepared with Syrian strain is able to be a cause of the disease as well as the vaccinated chickens due to a natural virus. Besides, in the laying hen, it may be reduced or stopped to lay eggs due to strong vaccinating reaction, so that the vaccination should be completed until 1-2 months before beginning of egg laying.

For the vaccine production in future, it is more convenient to apply the tissue culture technique for the improvement of vaccine. Furthermore, it is desirable to use other animal material as the vaccine in order to avoid the risk of transmitting adventitious infections. Because, it has generally been made evident that many aetiological viruses against chickens are able to transmit into the vaccine through the eggs which are used as material.

The manufacturing standard of fowl-pox vaccine was established upon these basic experiments (APPENDIX V).

II. CRD Agglutination Antigen

According to the partial survey results on CRD in the past, it has already been recognized that Mycoplasma infection in chickens spread in the various parts of the country. The positive rate of 25 per cent of sample of routine diagnosis in the Laboratory and 39.5 per cent of field surveyed cases were already estimated during March 1964 to February 1965. Consequently, the need of an antigen for the field detection of this disease

was extremely urgent. The test production of CRD antigen was commenced in order to perform the basic experiment for antigen production.

1. Test production

Strain used : Lyophilized stock of the Mycoplasma gallisepticum KP-13 strain was used. The original KP-13 strain was isolated from the natural infected chickens in Japan.

Procedure of production : The actual step of 3 lots of test production was carried out following the way described in the manufacturing standard (APPENDIX VI) which was originally based upon the way of National Institute of Animal Health in Japan. Record of test production is also described in APPENDIX VIII.

2. Potency test by serum agglutination

Preparation of immune serum : Three about 6-month-old chickens were partially bled for preimmune serum. They were inoculated intranasally with 0.5 ml of undiluted 5-day-cultured broth of KP-13 strain and 12 days later received booster inoculation with 1.0 ml of the same material by the intravenous route. Exsanguination was carried out 4 weeks after first inoculation.

Serum plate agglutination test : The method of reaction is described in the manufacturing standard (APPENDIX VI). The results are shown in TABLE F-2.

Tube agglutination test : The method of reaction is described in the manufacturing standard (APPENDIX VI). The results are shown in TABLE F-3.

From the results of potency test by serum agglutination, it is evaluated that three lots of antigens produced in the Laboratory have sufficient antigenicity and specificity as compared with Japanese reference antigen. There was no particular variation in sensitivity among antigens prepared by different medium and at different time.

3. Field trial by whole blood plate test

The final assay test should be made by to be confirmed the agglutinability in a whole blood plate test. Fortunately, occasions of field trial by using whole blood plate test were given at Deraa Poultry Farm and Hama.

Place : Gov. Poultry Farm at Deraa
Gov. Poultry Farm at Hama

Date : Deraa - March 22, 1966
Hama - May 17, 1966

Number of chickens : Deraa - 100
Hama - 61

TABLE F-2. Serum plate agglutination test

Serum			Antigen Lot 1	Lot 2	Lot 3	Japan 68
Immune chickens	1	Before inoculation	+	+	+	+
		12 days after	++	+++	++	+++
		4 weeks after	+++	+++	+++	+++
	2	Before inoculation	+	+	+	+
		12 days after	++	++	++	++
		4 weeks after	+++	+++	+++	+++
	3	Before inoculation	+	+	+	+
		12 days after	++	++	++	++
		4 weeks after	+++	+++	+++	+++
Natural cases	1		+	+	+	+
	2		+	+	+	+
	3		-	-	-	-
	4		-	-	-	-
	6		++	++	++	++
	T-3		+	++	+	++
	T-6		++	++	++	++
	T-15		-	-	-	-

Date examined : Apr. 12 1966

+++ : Reacted at 30 seconds >

++ : " 1 minute >

+ : " 2 minutes >

- : Non reactor

TABLE F-3. Tube agglutination test

Serum	Antigen		Serum dilution 1:5	1:10	1:20	1:40	1:80	1:160
Immune chickens*	1	Lot 1	+++	+++	+++	+	+	-
		Lot 2	+++	+++	+++	++	+	-
		Lot 3	+++	+++	+++	+	+	-
		Japan 68	+++	+++	+++	++	+	-
	2	Lot 1	+++	+++	+++	+	+	-
		Lot 2	+++	+++	++	+	+	-
		Lot 3	+++	+++	+++	++	+	-
		Japan 68	+++	+++	+++	++	+	-
Natural cases	T-2	Lot 1	++	+	-	-	-	-
		Lot 2	++	+	-	-	-	-
		Lot 3	+	+	-	-	-	-
		Japan 68	++	+	-	-	-	-
	T-3	Lot 1	++	+	-	-	-	-
		Lot 2	++	+	-	-	-	-
		Lot 3	++	+	-	-	-	-
		Japan 68	+	+	-	-	-	-

Date examined : Apr. 13 1966

* 4 weeks after inoculation

-, +, ++ and +++ : *Degree of reaction*

TABLE F-4. Agglutinability of antigen by whole blood plate test

Poultry farm	No.of chickens tested	Reaction	Lot 1	Lot 2	Lot 3
Deraa	100	-	86		86
		+	6		4
		++	8		8
		+++	2		2
		Positive rate	14 %		14 %
Hama	61	-		50	50
		+		4	3
		++		4	5
		+++		3	3
		Positive rate		18 %	18 %

+++ : Reacted at 30 seconds ++ : Reacted at 1 minute

\+ : Reacted at 2 minutes - : Non reactor

TABLE F-5. Comparison of agglutinability between whole blood test and serum agglutination tests

		Serum plate test				Tube agglutination test					
		-	+	++	+++	1:5	1:10	1:20	1:40	1:80	1:160
Whole blood Plate test	-		6	41	3			10	39	1	
	+			2	2				1	3	
	++				4					3	1
	+++				3					1	2

Antigens : Deraa - Lot 1 and Japan 68

Hama - Lot 2 and Japan 68

Whole blood plate test : The method of reaction is described in the manufacturing standard (APPENDIX VI).

Agglutinability of antigen by whole blood plate test

As shown in TABLE F-4, cases showed positive reaction within 2 minutes were 14 cases (14 per cent) out of 100 samples in Deraa Poultry Farm and 11 cases (18 per cent) out of 61 samples in Hama. There was no particular difference in rapidity and degree of reaction appearance as compared with Japanese reference antigen.

Consequently, from the results of field test, it was confirmed that the agglutination antigen for CRD produced in the Laboratory is available to be used for rapid diagnosis practically.

Comparison of agglutinability between whole blood test and serum agglutination tests

The serum samples collected from Hama Poultry Farm were examined for serum plate and tube agglutination tests in order to compare with the results of whole blood test. The results examined by using antigen of Lot 2 are shown in TABLE F-5. At the same time, the test by Japanese reference antigen was also carried out and the results were almost corresponded with Lot 2. From the results, it is recognized that the sensitivity of serum plate test and the agglutination test is rather higher than that of whole blood plate test. Therefore, the confirmation of diagnosis of cases of negative as well as doubtful results shall be made by the re-test with serum agglutination, if necessary.

The manufacturing standard of CRD antigen was established upon these basic experiments (APPENDIX VI).

G. PRODUCTION AND ASSAY RECORD OF FOWL-POX VACCINE AND CRD ANTIGEN

I. Fowl-Pox Vaccine

The routine production of one lot of fowl-pox vaccine was made by following the established manufacturing standard. The process of production and results of assay test are reported in APPENDIX VII.

From the results of assay test, it is evaluated that the fowl-pox vaccine has sufficient potency, safetibility and other necessary characteristics.

II. CRD Agglutination Antigen

The productions of 4 lots of CRD agglutination antigen including test productions were carried out. The results of assay test about test productions were described previously. The process of production of all lots and results of assay test about Lot 4 produced by following the established manufacturing standard, are reported in APPENDIX VIII.

From the results of assay test, all lots of antigen were evaluated to have sufficient potency and other necessary characteristics.

H. FIELD INVESTIGATION OF A POULTRY DISEASE OUTBREAKEN AT HASSAKEY GOVERNMENT POULTRY FARM

The expert was requested to investigate a serious chicken disease outbroken at Hassakey by the Government. The expert proceeded the field accompanied Mr. Hawary, Veterinary Assistant who are being trained about poultry disease by the expert, from 9 to 12 Aug., 1966.

On the investigation of this chicken disease in the field, the expert contacted with Mr. M. Souyouty, Director of Aggriculture Dept. in Hassakey Prefecture, and other officials concerned, and necessary arrangement in connection with his work were given to the expert.

The process and results of the investigation, treatments carried out and recommendations are reported here.

I. Situation and Environmental Conditions of the Poultry Farm

The Poultry Farm is situated in the direction of north-west about 2 km from Hassakey city. The Farm is sorounded by cotton field and there is no other poultry farm within 2 km.

The Farm has been settled since about 5 years before and the scale has become bigger year after year. A new main chicken house which has ability to breed on the ground directly about 5,000 chickens, was built at the beginning of this year.

Many new-hatched chickens are being distributed to this area from the Farm. A serious accident caused by infectious disease has not been noticed in the past. The general management are being done well by 5 staffs including 3 workers, but the preventive management against infectious disease seems unsatisfactory.

II. History of the Disease

Age : Laying hens, 3.5- and 2-month-old chickens

Breed : White-leghorn, Rhode-island-red, Cornish, White-plymouth-rock and Cross-breeding

Date of first occurrence : July 2, 1966.

Total No. in flock : 6,300

No. ill : About 2,300

No. dead (Mortality) : 1,632 (25.9 per cent)

The detail of process are shown in Table H-1.

Vaccinations : Newcastle disease vaccine, May 1966, only for laying hens

Previous diseases : None

Current medications : Aureomycin, only for Rhode-island-red group

III. Described Symptoms

Appetite : Poor and thirsty

Egg production : 25 July - 315 eggs
8 Aug. - 296 eggs

Faeces : Diarrhoea (white and green)

Respiratory symptoms : Difficult breathing, beak open

Nervous symptoms : None

IV. Observations

General symptoms : Lose appetite, high temperature, thirsty, depression, diarrhoea (white and green)

Respiratory symptoms : Difficult breathing, beak open

Nervous symptoms : Few (paralysis of leg)

Agglutination test : Pullorum - - - -
CRD - - - -
Coryza - - - -

Parasitic examination : Faeces - - -
Blood - - -

TABLE H-1. Number of dead chickens with the process of disease

Date	Laying-hen (1,000)*	3-month-old (1,800)	1.5-month-old (3,500)	Total (6,300)
July 2, '66	15		32	47
3	7	5	34	46
4		5	12	17
5	5		19	24
6	5		27	32
7	4	5	40	49
8	3	2	21	26
9	5	2	18	25
10	11	5	12	28
11	20	15	13	48
12	10	8	17	35
13	4	16	17	37
14	4	15	20	39
15	7	14	22	43
16	3	14	30	47
17	25	7	25	57
18	3		31	34
19	8	24	16	48
20		14	13	27
21	3	35	3	41
22	5	25	15	45
23	1	12	28	41
24	5	29		34
25	1	30	37	68
26	3	25	27	55
27	2	39	20	59
28		37	2	41
29	2	58	19	79
30	8	22	10	40
31	5	31	7	43
Aug. 1	10	25	21	56
2	2	20	10	32
3	9	19	17	45
4 & 5	20	47	14	81
6	5	31	10	46
7	5	25	6	36
8	10	61	10	81
Total	235(23.5%)**	722(40.1%)	685(19.6%)	1,632(25.9%)

* No. of chickens before outbreak. ** Mortality

V. Post-Mortem Examination

The results are shown in TABLE H-2. One died and five killed chickens were examined. The haemorrhagic and inflamatory changes were found chiefly in subcutis, muscle, heart, lung, trachea, proventriculus, small intestine, ovary and brain.

VI. Diagnosis

From the results of investigation, it was made evident that the disease showed typical pattern of outbreak, clinical symptoms and post-mortem findings as Newcastle disease. The expert decided to be Newcastle disease for the chicken disease investigated without making laboratory examination.

VII. Treatments

The expert coped with the situation in order to stop in the minimum damage as follows:

1. Disinfection of contaminated chicken-house
2. Separation of infected and healthy batches
3. Inoculation of Newcastle disease vaccine for young chickens
4. Prevention of secondary infection by antibiotics

VIII. Recommendations

The expert recommended to carry out strictly at least next items in order to keep clean the Poultry Farm from infectious disease and to keep off spreading the disease to other poultry farms in future as well as under this situation.

1. Limitation of visitors from outside and to the other poultry farms
2. Enforcement of disinfection (chicken-house, instruments, dead chickens, hands, clothes and shoes etc.)
3. Enforcement of changing clothes and shoes for workers
4. Reconstruction of chicken-house (settlement of thick walls among each batches)

TABLE H-2. Post-mortem examination

Chicken No.	1	2	3	4	5	6
Date	Aug.9	Aug.9	Aug.10	Aug.10	Aug.10	Aug.10
Age	Laying -hen	Cock	2-month -old	2-month -Old	Laying -hen	Laying -hen
Died / Killed	Killed	Killed	Killed	Killed	Died	Killed
Diarrhoea	+	+	+	+	+	+
Subcutis	+++	++	++	++	++	+
Muscle	+++	+	+	++	++	+
Heart	++	+	-	++	+++	-
Lung	+	-	-	+	-	-
Trachea	+++	+	+	+	+	++
Proventriculus	+++	+	-	-	++	-
Small intestine	++	+	-	+	+	-
Ovary	++		-	-	+++	++
Brain	+	+	++	+	+	+

-, +, ++ and +++ : Degree of lesion (Haemorrhagic or congestive inflamation)

DISEASES

ases	Cause	Distribution	Host range	Outbreak	Age	Latent period
-pox	Fowl-pox virus	Whole world	chicken, pigeon, turkey, canary, sparrow, duck, geese, grouse, guinea-fowl, jackdaw, junco, partridge, pheasant, ptarmigan	Moderate	All ages (after 1 month)	4-14 days (4-6 days)
astle ease	Newcastle disease virus	Whole world	chicken, turkey, duck, geese, pigeon, guinea fowl, pheasant, partridge, crow, sparrow, maya, martin, starling, gannet, owl, osprey, parakeet, raven, eagle, kingfisher, quail, dove, cowbird, grackle	Rapid	All ages	3-6 days
ctious chitis	Infectious bronchitis virus	North America, England, Netherland, Japan	Chicken	Rapid	All ages	1-3 days
ctious ngo- aeitis	Infectious laryngo-tracheitis virus	North America, Australia, Europe, Japan	Chicken, Pheasant	Rapid	All ages	2-12 days
halo- itis	Avian encephalo-mylitis virus	Whole world	Chicken, Pheasant, Duckling, Turkey, Young pigeon, Guinea fowl	Moderate (located a flock)	All age (carrier) specially 0-60 days (1-3 weeks)	5-40 days (9-21 days
ro- osis	Virus			Few	All ages	
- osis	Virus			Rare	All ages	
eral omatosis	Virus	North America, Europe, Australia, Japan, Syria	Chicken, Turkey, Pheasant, Duck, Pigeon, etc.	Frequent	(5-8 months)	
l omatosis	Not known			Frequent	(1.5-5 Months)	
rmal eyes	not known			Few		
petrosis	(Virus)			Few		

	Outbreak	Age	Latent period	Process (in flock)	Mortality	Transmission	General symptoms	Clinical sign: Respiratory	Nervous	Laying	Faeces	Post-mortem findings	Isolation of agent: Material	Medi
aw idge ant igan	Moderate	All ages (after 1 month)	4-14 days (4-6 days)	1 month or more	5-60%	Scratch (oral & skin) Mosquito Through egg?	Pocks Respiratory	Gasping Diphtheria	Normal	Drop	Normal	Diphtheria Mucosal swelling of sinus and trachea	Lesion	CAM (pock CK (CPE o round typ cytoplasm inclusion
n ing t y eet isher rd le	Rapid	All ages	3-6 days	1-3 weeks	0-100%	Oral Digestive Respiratory Ocular Cloacal Through egg	Respiratory Nervous	Beak open Wheezing Sneezing	Paralysis of legs and neck	Drop or stop Misshapen eggs	Diarrhoea (green)	Tracheal exudate Pneumonia Turbid air sac Abnormal ovary Haemorrhage of subcutane, muscle, digestive organ, heart, brain	Trachea Lung Faeces Brain Spleen liver	CAC (embr death) CK (cyncy cytoplasm inclusion Had, HA
	Rapid	All ages	1-3 days	1-2 weeks	0-10%	Respiratory Through eggs?	Respiratory	Beak open Wheezing Sneezing Scream Nasal exudate	Normal	Drop Thin-shelled and misshapen eggs?	Diarrhoea	Respiratory Catarrh Abnormal ovary	Trachea Lung	CAC (CK)
	Rapid	All ages	2-12 days	4-6 weeks	0-85% (13-70%)	Respiratory Ocular Cloacal	Respiratory	Gasping Wheezing Sneezing Whistling Scream Nasal exudate Tear Bloody phlegm	Normal	Drop	Sometimes diarrhoea	Mucosal swelling, blooding and caseous exudate in respiratory tract	Trachea	CAM, CAC CK, CE (c CPE, intr clear inclusion
	Moderate (located a flock)	All age (carrier) specially 0-60 days (1-3 weeks)	5-40 days (9-21 days)	2-4 weeks	0-70% (10%)	Digestive Respiratory? Through egg	Nervous	Normal	Tremor Ataxia of legs	Drop or stop	Normal	Normal	Brain	Yolk sac (dwarfing Chick
	Few	All ages		Several months Chronic	(100%)		Yellowish comb. Anaemia	Normal	Normal	Stop	Diarrhoea	Enlarged and discoloured liver, spleen		
	Rare	All ages		Several months Chronic	(100%)		Anaemia	Normal	Normal	Stop	Diarrhoea	Enlarged and discoloured liver, spleen		
	Frequent	(5-8 months)		1-5 weeks (Acute)	(100%)	Digestive Respiratory Cloacal Through egg	Depressing Dwarf comb Toutch enlarged liver from outside	Normal	Normal	Stop	Diarrhoea	Tumore and enlarged liver, spleen, kidney, ovary etc.		
	Frequent	(1.5-5 Months)		Chronic	(100%)		Nervous	Normal	Paralysis of leg, wing or neck	Drop	Normal	Inflamation of nerves in leg, wing neck Enlarged ovary		
	Few			All life	0%		Abnormal eyes	Normal	Normal	Normal	Normal	Normal		
	Few			All life	0%		Thick legs	Normal	Normal	Normal	Normal	Thick bone		

ansmission	General symptoms	Clinical sign: Respiratory	Clinical sign: Nervous	Clinical sign: Laying	Clinical sign: Faeces	Post-mortem findings	Diagnosis: Isolation of agent: Material	Diagnosis: Isolation of agent: Medium	Diagnosis: Serum reaction	Diagnosis: Microscopic examination	Prevention	Treatment
ratch ral & in) squito rough egg?	Pocks Respiratory	Gasping Diphtheria	Normal	Drop	Normal	Diphtheria Mucosal swelling of sinus and trachea	Lesion	CAM (pock) CK (CPE of round type, cytoplasmic inclusion)	AGP	Cytoplasmic inclusion	Living vaccine	None
l estive piratory lar acal ough egg	Respiratory Nervous	Beak open Wheezing Sneezing	Paralysis of legs and neck	Drop or stop Misshapen eggs	Diarrhoea (green)	Tracheal exudate Pneumonia Turbid air sac Abnormal ovary Haemorrhage of subcutane, muscle, digestive organ, heart, brain	Trachea Lung Faeces Brain Spleen liver	CAC (embryionic death) CK (cyncytial CPE cytoplasmic inclusion) Had, HA	HI N-T	Glial foci Perivascular infiltration in cerebellum	Living or inactivated vaccine	None
piratory ough s?	Respiratory	Beak open Wheezing Sneezing Scream Nasal exudate	Normal	Drop Thin-shelled and misshapen eggs?	Diarrhoea	Respiratory Catarrh Abnormal ovary	Trachea Lung	CAC (CK)	N-T AGP		Living vaccine	None
piratory lar acal	Respiratory	Gasping Wheezing Sneezing Whistling Scream Nasal exudate Tear Bloody phlegm	Normal	Drop	Sometimes diarrhoea	Mucosal swelling, blooding and caseous exudate in respiratory tract	Trachea	CAM, CAC CK, CE (cyncytial CPE, intranuclear inclusion)	N-T AGP	Intranuclear inclusion	Living vaccine	None
estive piratory? ough	Nervous	Normal	Tremor Ataxia of legs	Drop or stop	Normal	Normal	Brain	Yolk sac (dwarfing) Chick	N-T	Perivascular infiltration in cerebellum pancreas etc. Enlargement of spinal cord	Living vaccine	None
	Yellowish comb Anaemia	Normal	Normal	Stop	Diarrhoea	Enlarged and discoloured liver, spleen				Pro-erythroblast	None	None
	Anaemia	Normal	Normal	Stop	Diarrhoea	Enlarged and discoloured liver, spleen				Hetero-eosinophile	None	None
estive piratory ical ough egg	Depressing Dwarf comb Toutch enlarged liver from outside	Normal	Normal	Stop	Diarrhoea	Tumore and enlarged liver, spleen, kidney, ovary etc.				Granular cells	None	None
	Nervous	Normal	Paralysis of leg, wing or neck	Drop	Normal	Inflamation of nerves in leg, wing neck Enlarged ovary					None	None
	Abnormal eyes	Normal	Normal	Normal	Normal	Normal					None	None
	Thick legs	Normal	Normal	Normal	Normal	Thick bone					None	None

APPENDIX I. THE MANUAL OF POULTRY DISEASE

VIRAL DISEASES

Diseases	Cause	Distribution	Host range	Outbreak	Age	Latent period	Process (in flock)	Mortality	Transmission	Clinical sign: General symptoms	Clinical sign: Respiratory	Clinical sign: Nervous	Clinical sign: Laying
Fowl-pox	Fowl-pox virus	Whole world	chicken, pigeon, turkey, canary, sparrow, duck, geese, grouse, guinea-fowl, jackdaw, junco, partridge, pheasant, ptarmigan	Moderate	All ages (after 1 month)	4-14 days (4-6 days)	1 month or more	5-60%	Scratch (oral & skin) Mosquito Through egg?	Pocks Respiratory	Gasping Diphtheria	Normal	Drop
Newcastle disease	Newcastle disease virus	Whole world	chicken, turkey, duck, geese, pigeon, guinea fowl, pheasant, partridge, crow, sparrow, maya, martin, starling, gannet, owl, osprey, parakeet, raven, eagle, kingfisher, quail, dove, cowbird, grackle	Rapid	All ages	3-6 days	1-3 weeks	0-100%	Oral Digestive Respiratory Ocular Cloacal Through egg	Respiratory Nervous	Beak open Wheezing Sneezing	Paralysis of legs and neck	Drop or stop Misshapen eggs
Infectious bronchitis	Infectious bronchitis virus	North America England Netherland Japan	Chicken	Rapid	All ages	1-3 days	1-2 weeks	0-10%	Respiratory Through eggs?	Respiratory	Beak open Wheezing Sneezing Scream Nasal exudate	Normal	Drop Thin-shelled and misshapen eggs?
Infectious laryngo-tracheitis	Infectious laryngo-tracheitis virus	North America Australia Europe Japan	Chicken Pheasant	Rapid	All ages	2-12 days	4-6 weeks	0-85% (13-70%)	Respiratory Ocular Cloacal	Respiratory	Gasping Wheezing Sneezing Whistling Scream Nasal exudate Tear Bloody phlegm	Normal	Drop
Avian encephalo-myelitis	Avian encephalo-mylitis virus	Whole world	Chicken Pheasant Duckling Turkey Young pigeon Guinea fowl	Moderate (located a flock)	All age (carrier) specially 0-60 days (1-3 weeks)	5-40 days (9-21 days)	2-4 weeks	0-70% (10%)	Digestive Respiratory? Through egg	Nervous	Normal	Tremor Ataxia of legs	Drop or stop
Erythro-blastosis	Virus			Few	All ages		Several months Chronic	(100%)		Yellowish comb. Anaemia	Normal	Normal	Stop
Myelo-blastosis	Virus			Rare	All ages		Several months Chronic	(100%)		Anaemia	Normal	Normal	Stop
Visceral lymphomatosis	Virus	North America Europe Australia Japan Syria	Chicken Turkey Pheasant Duck Pigeon etc.	Frequent	(5-8 months)		1-5 weeks (Acute)	(100%)	Digestive Respiratory Cloacal Through egg	Depressing Dwarf comb Toutch enlarged liver from outside	Normal	Normal	Stop
Neural lymphomatosis	Not known			Frequent	(1.5-5 Months)		Chronic	(100%)		Nervous	Normal	Paralysis of leg, wing or neck	Drop
Abnormal eyes	not known			Few			All life	0%		Abnormal eyes Thick legs	Normal Normal	Normal Normal	Normal Normal
Osteopetrosis	(Virus)			Few			All life	0%					

ality	Transmission	General symptoms	Clinical sign: Respiratory	Clinical sign: Nervous	Clinical sign: Laying	Clinical sign: Faeces	Post-mortem findings	Diagnosis: Isolation of agent: Material	Diagnosis: Isolation of agent: Medium	Diagnosis: Serum reaction	Diagnosis: Microscopic examination	Prevention	Treatment
%	Scratch (oral & skin) Mosquito Through egg?	Pocks Respiratory	Gasping Diphtheria	Normal	Drop	Normal	Diphtheria Mucosal swelling of sinus and trachea	Lesion	CAM (pock) CK (CPE of round type, cytoplasmic inclusion)	AGP	Cytoplasmic inclusion	Living vaccine	None
0%	Oral Digestive Respiratory Ocular Cloacal Through egg	Respiratory Nervous	Beak open Wheezing Sneezing	Paralysis of legs and neck	Drop or stop Misshapen eggs	Diarrhoea (green)	Tracheal exudate Pneumonia Turbid air sac Abnormal ovary Haemorrhage of subcutane, muscle, digestive organ, heart, brain	Trachea Lung Faeces Brain Spleen liver	CAC (embryionic death) CK (cyncytial CPE cytoplasmic inclusion). Had, HA	HI N-T	Glial foci Perivascular infiltration in cerebellum	Living or inactivated vaccine	None
%	Respiratory Through eggs?	Respiratory	Beak open Wheezing Sneezing Scream Nasal exudate	Normal	Drop Thin-shelled and misshapen eggs?	Diarrhoea	Respiratory Catarrh Abnormal ovary	Trachea Lung	CAC (CK)	N-T AGP		Living vaccine	None
% (70%)	Respiratory Ocular Cloacal	Respiratory	Gasping Wheezing Sneezing Whistling Scream Nasal exudate Tear Bloody phlegm	Normal	Drop	Sometimes diarrhoea	Mucosal swelling, blooding and caseous exudate in respiratory tract	Trachea	CAM, CAC CK, CE (cyncytial CPE, intranuclear inclusion)	N-T AGP	Intranuclear inclusion	Living vaccine	None
%)	Digestive Respiratory? Through egg	Nervous	Normal	Tremor Ataxia of legs	Drop or stop	Normal	Normal	Brain	Yolk sac (dwarfing) Chick	N-T	Perivascular infiltration in cerebellum pancreas etc. Enlargement of spinal cord	Living vaccine	None
%)		Yellowish comb. Anaemia	Normal	Normal	Stop	Diarrhoea	Enlarged and discoloured liver, spleen				Pro-erythroblast	None	None
%)		Anaemia	Normal	Normal	Stop	Diarrhoea	Enlarged and discoloured liver, spleen				Hetero-eosinophile	None	None
%)	Digestive Respiratory Cloacal Through egg	Depressing Dwarf comb Toutch enlarged liver from outside	Normal	Normal	Stop	Diarrhoea	Tumore and enlarged liver, spleen, kidney, ovary etc.				Granular cells	None	None
%)		Nervous	Normal	Paralysis of leg, wing or neck	Drop	Normal	Inflamation of nerves in leg, wing neck Enlarged ovary					None	None
		Abnormal eyes	Normal	Normal	Normal	Normal	Normal					None	None
		Thick legs	Normal	Normal	Normal	Normal	Thick bone					None	None

BACTERIAL DISEASES

Diesease	Cause	Distribution	Hose range	Outbreak	Age	Latent period	Process (in flock)	Mortality	Transmission	General symptoms	Clinical sign: Respiratory	Nervous	La
Pullorum disease	Salmonella pullorum	Whole worls	Chicken Sparrow Turkey Finch Duck Pigeon Guinea-fowl Dove Pheasant Canary Quail Gosling Geese Bittern	Rapid	All ages (carrier) specially 1-14 days	2-3 days	1-2 weeks (carrier, all life)	0-100%	Respiratory Digestive Ocular Through egg Cloacal	Depression Diarrhoea	Difficult breathing (young)	Normal	Dr
Infectious coryza	Haemophilus gallinarum	Whole world	Chicken	Rapid	All ages specially 3-5 months	1-4 days	10-14 days	0 %	Oral specially with water	Respiratory Facial aedema	Wheezing Scream Mucosal exudate Tear	Normal	Dr
CRD (Avian respiratory mycoplasmosis)	Mycoplasma gallisepti-cum	Whole world	Chicken Partridge Turkey Guinea fowl Pheasant Pigeon	Moderate	All ages Specially 3-5 months	10-20 days	Several months	0-10%	Respiratory Through egg	Respiratory	Beak open Wheezing Sneezing Scream Nasal exudate Tear	Normal	Dr
Infectious synovitis	Mycoplasma synoviae (and others)	USA Europe Japan Syria	Chicken Turkey Pheasant	Moderate Located a flock	All ages specially 3-5 months	24-80 days (3-5 days experimen-tally)	Several months	3-10 %	Contact Through egg	Swelling of joing and breast brister	Normal	Normal	Dr
Staphylo-cocosis	Staphylo-coccus	Europe USA Japan Syria	Chicken Geese Duck Pigeon Pheasant	Moderate located a flock	Generally 1-2 months	1-2 days	4-5 days or more	40-50 % (acute)	Scratch	Septicaemia Depression Swelling of joint	Normal	Normal	Dr
Strepto-cocoosis	Strepto-coccus	USA Europe Australia Soviet union Japan	Chicken Pigeon Turkey Duck Geese Canary	Moderate located a flock	All ages	1-14 days	1 day - 4 months	5-90%	(respiratory) (Scratch) Through egg	Anaemia Septicaemia Depression	Difficult breathing Nasal exudate	Normal	Dr
Fowl cholera	Pasteurella multocida	East & south Asia North America Europe (Japan in past)	Do mestic fowls of all species Game bird Small feral bird	Rapid	All ages	2-9 days	Several hrs-several days (carrier, several months)	60-75% (acute)	Respiratory	Septicaemia Depression Diarrhoea Respiratory Lose appetite	Difficult breathing	Normal	St
Avian tuberculosis	Mycobacte-rium avium	North America East Asia Europe Japan	Chicken Turkey Sparrow Duck Pigeon Crow Geese Parrot Owl Swan Canary Cowbird Peacock Pheasant Blackbird Hawk Swallow	Moderate	All ages specially after 1 year	Several months - all life	Several months - all life	Rather high	Oral	Depression Anaemia Thinness	Normal	Normal	Dr
Avian vibrionic Hepatitis	Vibrio	USA Japan	Chicken Turkey	Moderate	All ages specially 30-40 days	2-15 days	Several weeks	5-15%	(Oral)	Depression Dwarf comb	Normal	Normal	Dr
Disease caused by Escherichia	Escherichia coli	Europe North America Japan	Chicken Turkey	Moderate	All ages special 6-10 weeks	1 day or more (experimen-tally)	1-3 weeks	5%	(Oral)	Depression oral haemorrhage	Difficult breathing	Normal	Dr
Disease caused by Arizona	Arizona	USA	Chicken Turkey Duck Canary etc.	Rapid	0-3 weeks	Less than 1 week	1-2 weeks	0.5-50%	Oral Ocular Cloacal Through egg	Depression Diarrhoea	Normal	Normal	
Aspergillosis	Aspergillus (fumigatus)	Whole world	Chicken Ostrich Pigeon Penguin Hawk Captivo wild bird	Rapid	Embryonated egg, 5 weeks or more	2-4 days	Several months	10-50%	Respiratory Through egg shell	Respiratory Depression	Difficult breathing Gasping	(Convul-sion)	Dr

Process (in flock)	Mortality	Transmission	Clinical sign: General symptoms	Clinical sign: Respiratory	Clinical sign: Nervous	Clinical sign: Laying	Clinical sign: Faeces	Diagnosis: Post-mortem findings	Diagnosis: Isolation of agent: Material	Diagnosis: Isolation of agent: Medium	Diagnosis: Immune reaction	Diagnosis: Microscopic examination	Prevention	Treatment
1-2 weeks (carrier, all life)	0-100%	Respiratory Digestive Ocular Through egg Cloacal	Depression Diarrhoea	Difficult breathing (young)	Normal	Drop	Diarrhoea white (young)	Liver:swelling and typhom Pericarditis Abnormal ovary	Liver Spleen Pancreas Ovary intestine	Selenite MacConkey YCC Kligler SIM	Aggl. AGP	Liver: necrosis	Weed out (Furazolidone)	(Furazolidone)
10-14 days	0 %	Oral specially with water	Respiratory Facial aedema	Wheezing Scream Mucosal exudate Tear	Normal	Drop	Diarrhoea	Sinus: inflamation Abnormal ovary	Exudate of sinus infraorbital	Haemophilus medium	Aggl (C-F)	Sinus: catarrh	Streptomycin Sulfamine	"
Several months	0-10%	Respiratory Through egg	Respiratory	Beak open Wheezing Sneezing Scream Nasal exudate Tear	Normal	Drop	Normal	Inflamation of respiratory mucosa.Caseous exdate of air sac	Exudate of sinus, trachea, air sac	PPLO medium CKC (CPE,Had)	Aggl. HI	Lympho-follicular reaction of respiratory mucosa	Aureomycin Streptomycin Terramycin Tylocin	"
Several months	3-10 %	Contact Through egg	Swelling of joing and breast brister	Normal	Normal	Drop	Normal	Lesion: exudate like cream or cheese Swelling of liver, spleen or kidney	Lesion	PPLO medium York sac	Aggl.		Aureomycin Terramycin	Aureomycin Terramycin
4-5 days or more	40-50 % (acute)	Scratch	Septicaemia Depression Swelling of joint	Normal	Normal	Drop	Sometimes diarrhoea	Subcutaneous haemorrhagic infiltration and inflamatory exudate	Lesion Blood	Blood agar		Staphylo-coccus	Penicillin Terramycin Aureomycin Sulfamine	"
1 day - 4 months	5-90%	(respiratory) (Scratch) Through egg	Anaemia Septicaemia Depression	Difficult breathing Nasal exudate	Normal	Drop	Diarrhoea	Congestion and enlargement of parenchymental organs, Enteritis	Blood Exudate of abdominal cavity	Blood agar		Strepto-coccus	Penicillin Terramycin sulfamine	"
Several hrs-several days (carrier, several months)	60-75% (acute)	Respiratory	Septicaemia Depression Diarrhoea Respiratory Lose appetite	Difficult breathing	Normal	Stop	Diarrhoea	Haemorrhages in S/C heart, liver and intestine	Blood Parenchy-mental organs	Blood agar	(Aggl.)	Bipolar staining Necrosis of liver	Inactivated vaccine Sulfamine Penicillin Tetracyclin Chloromycetin	None
Several months - all life	Rather high	Oral	Depression Anaemia Thinness	Normal	Normal	Drop	Diarrhoea (intest-inal TB)	Tuberculous legion of liver, spleen lung, intestine and bone marrow etc.	Lesions	Ogawa's medium	Tuberculin reaction	Epitherioid and giant cells Tubercle bacilli	Weed out	
Several weeks	5-15%	(Oral)	Depression Dwarf comb	Normal	Normal	Drop	Normal	Liver:swelling, necrosis and haemorrhages	Liver Bile	Vibrio medium Yolk sac		Liver: haemorrohages heterophiles lymphocytes	Streptomycin Aureomycin Terramycin Furazolidone	"
1-3 weeks	5%	(Oral)	Depression oral haemorrhage	Difficult breathing	Normal	Drop	(Diarrhoea)	Duodenum: haemorrhagic granuloma Liver:swelling necrosis, Pericarditis	Blood Liver Spleen	Nutrient agar	(Aggl.)		Furazolidone Chloromycetin Tetracyclin Colistin	"
1-2 weeks	0.5-50%	Oral Ocular Cloacal Through egg	Depression Diarrhoea	Normal	Normal		(Diarrhoea)	(Liver: swelling pericarditis)	Liver Lung Kindney Intestine	MacConkey	(Aggl.)		Furazolidone Aureomycin Terramycin	
Several months	10-50%	Respiratory Through egg shell	Respiratory Depression	Difficult breathing Gasping	(Convul-sion)	Drop	Normal	Lung, trachea and air sac: Yellowish white miliary nodules (liver, spleen, kidney, ovary)	Lesion	Sabouraud agar		Fungi	Potassium iodine	"

SIS

	Cause	Distribution	Host range	Outbreak	Age	Latent period	Process (in flock)	Mortality	Transmission	General symptoms	Clinical sign: Respiratory	Nervous	Laying	Faeces
	Eimeria tenella	Whole world	Chicken, Pelican, Cormorant, Geese, Duck, Grouse, Pheasant, Quail, Crain, Coot, Moor-hen, Plover, Sandpiper	Rapid	1-3 months	3-5 days	Several days	25-100%	Oral (digestive)	Depression Diarrhoea	Normal	Normal	Drop or Delay	Bloody diarrh
	E.necatrix			Rapid	2-5.5 months	5-6 days								Bloody sticky
sis	E.maxima			Moderate	2.5-7 months	5-6 days								Liquid diarrh
	E. acervulina			Moderate	2.5-7 months	5-6 days	1 month	10-18%						Liquid diarrh
o-	Leucocytozoon caulleryi (L.sabrazesi) (L. andrewsi)	East & south Asia	Chicken, Duck, Turkey, Guinea fowl, Owl, Crow, Grouse	Rapid	All ages	(12-13 days)	Several months	Several-80% (20-30%)	Sandfly	Anaemia Hemoptysis	Normal	Normal	Drop or Stop	Greeni diarrh
e-	Treponema gallinarum etc.	Europe, Middle & west Asia, Africa, South America	Geese, Chicken, Duck, Turkey, Partridge, Crow, Sparrow (Pigeon)	Moderate	All ages	2-6 days	3 days or more	80%(geese) 60-90% (Chicken)	Fowl ticks or "Blue bug"	Fever Thirst Somnolena Anaemia	Normal	Normal	Drop	Diarrh
ad ous s)	Histomonous meleagridis	Whole world	Chicken, Peacock, Turkey, Pheasant, Guinea fowl, Quali	Moderate	1-3 months	4-6 days	2 days-several weeks (1-2 weeks)	90-100% (Peacock) 50-100% (Turkey) 5-25% (Chicken)	Cloacal Oral by Heterakis gallinae	Depression Anaemia Diarrhoea	Normal	Normal	Delay	Diarrh
asis	Ascaridia galli	Whole world	Chicken, Turkey	Moderate	All ages		Chronic	Several	Oral	Droopiness Emaciation Diarrhoea	Normal	Normal	Drop	Diarrh
idae)	Heterakis gallinae	Whole world	Chicken, Turkey		All ages				Oral	(Carrier of the Black head organism)				
idae)	Capillaria obsignata	Europe North America Japan	Pheasant, Pigeon, Chicken, Turkey	Moderate	(All ages)		Chronic	Several	Oral	Droopiness Emaciation Diarrhoea Anaemia	Normal	Normal	Drop	Diarrh
osis	Strongy-loides avium	Europe Syria	Chicken, Turkey	Moderate	(Young age)		Acute or Chronic	Several	Oral	Droopiness Emaciation Diarrhoea Anaemia	Normal	Normal		Thin & diarrh
s	Raillietina tetragona	Whole world	Chicken, Turkey, Guinea fowl, Peafowl, Quail	Moderate	(Young age)		Chronic		Oral by Ant	Droopiness Emaciation Diarrhoea	Normal	Normal		Diarrh
	R. echino-bothrida		Chicken, Turkey						Ant					
	R.cesticillus		Chicken, Quinea fowl, Turkey Quail, Gray jungle fowl						Housefly Beetle					
s	Chicken Lice, Mites or Ticks	Whole world	Chicken	Moderate	All ages		Chronic	Several	Contact	Emaciation Anaemia (carrier of spiro-chaetosis, leucosis: mite, tick)	Normal	Normal	Drop	

k)	Mortality	Transmission	General symptoms	Clinical sign: Respiratory	Nervous	Laying	Faeces	Post-mortem findings	Diagnosis: Isolation of agent: Material	Medium	Serum reaction	Microscopic examination	Prevention	Treatment
days	25-100%	Oral (digestive)	Depression Diarrhoea	Normal	Normal	Drop or Delay	Bloody diarrhoea	Haemorrhages of caeca	Faeces Bloody faeces			Oocyst Schizont Merozoite	Amprolium Glycamide Zoalene	Sulfamine
							Bloody, sticky	Haemorrhages of ileum						
	10-18%						Liquid diarrhoea	Haemorrhages of ileum						
							Liquid diarrhoea	Haemorrhages of duodenum						
	Several-80% (20-30%)	Sandfly	Anaemia Hemoptysis	Normal	Normal	Drop or Stop	Greenish diarrhoea	Haemorrhages of muscle and parenchymental organs Spleen: swelling	Lesion Blood			Gametogony (Giemsa) Schizont (unstained)	Eradication of sandfly Furazolidone Sulfamine Pyrimethamin	
or	80% (geese) 60-90% (Chicken)	Fowl ticks or "Blue bug"	Fever Thirst Somnolena Anaemia	Normal	Normal	Drop	Diarrhoea	Swallen spleen Enlarged liver	Blood			Spirochaeta	Penicillin	Penicillin
	90-100% (Peacock) 50-100% (Turkey) 5-25% (Chicken)	Cloacal Oral by Heterakis gallinae	Depression Anaemia Diarrhoea	Normal	Normal	Delay	Diarrhoea	Caeca: thick & hard Liver: swelling & necrosis	Caecal mucosa Liver			Histomonas	Eradication of Heterakis gallinae Nithiazide Furazolidone	Furazolidone
eks)	Several	Oral	Droopiness Emaciation Diarrhoea	Normal	Normal	Drop	Diarrhoea	Ascaris in small intestine 50-76 mm 61-116 mm	Faeces			Eggs		Phenothiazine Piperazine
		Oral	(Carrier of the Black head organism)					Caeca: inflamation & thickening 7-13mm 15 mm	Faeces			Eggs		Phenothiazine Piperazine
	Several	Oral	Droopiness Emaciation Diarrhoea Anaemia	Normal	Normal	Drop	Diarrhoea	Small intestine: inflamation 10-25 mm 16-25 mm	Faeces			Eggs		Phenothiazine Piperazine
r	Several	Oral	Droopiness Emaciation Diarrhoea Anaemia	Normal	Normal		Thin & bloody diarrhoea	Caeca: thickening 2.2 mm	(Faeces)			(Eggs)		(Phenothiazine) (Piperazine)
		Oral by Ant Ant Housefly Beetle	Droopiness Emaciation Diarrhoea	Normal	Normal		Diarrhoea	Ileum, 250-300 mm Ileum, 200-250 mm Jejunum, 50-130 mm	Faeces (segment)				Eradication of ant	Bithionol
	Several	Contact	Emaciation Anaemia (carrier of spirochaetosis, leucosis: mite, tick)	Normal	Normal	Drop								Nicotin sulfate Malathion

FECTIOUS DISEASES

Cause	Distribution	Host range	Outbreak	Age	Latent Period	Process	Mortality	Transmission	Clinical sign: General symptoms	Respiratory	Nervous or confusing	Laying	Faeces
Lack of Vitamin-A			Located a flock	All ages (specially young)			5-10%		Depression Emaciation Leg weekness Respiratory	Gasping Nasal exudate	Ataxia of leg	Drop	Normal
Lack of Vitamin-B1 (Thiamine) Vitamin-B2 (Rivoflavin)			Located a flock	All ages (young)					Leg weekness Retarded growth Leg weekness	Normal	Star gazing pose Curled toes in ward Resting pose	Drop	Normal
Lack of Vitamine-D			Located a flock	All ages (Young)					Leg weekness Retarded growth Rickets	Normal	Unsteady steps	Drop Thin-shelled egg	(Diarrh
Lack of Vitamin-E			Located a flock (less than 20% in a flock)	10-65 days (20-40 days)		10 days	100%		Nervous Ataxia Leg weekness	Normal	Backward or downward retruction of lateral twisting of the head Contraction and relaxation of legs		Normal
Lack of calcium			Located a flock	All ages (laying hen)					Leg weekness	Normal	Unsteady steps	Drop	(Diarrh
Lack of Manganese			Located a flock	All ages (Growing chick)			Several		Nervous (perosis)	Normal	Convulsions Twisting or bending of distal end of tibia	Drop	
Poor feeding (Lack of vitamine-B)			Few	All ages (young)			Several		Enlargement of crop	Beak open	Normal		
Unknown (Miscella-neous)			Few	All ages			10-20%		Gout White-grey urine	Normal	Normal	Drop Misshapen egg	White urine Diarrho
Autointoxications Bacterial toxins Molds & Fungi Drugs & Chemicals Phytotoxins Insects Miscellaneous food poisons				All ages			High		Lose appetite Thirst Depression Emaciation Nervous Vomiting Cyanose	Difficult breathing	Convulsions Paralysis		(Diarrh

ality	Transmission	Clinical sign: General symptoms	Clinical sign: Respiratory	Clinical sign: Nervous or confusing	Clinical sign: Laying	Clinical sign: Faeces	Post-mortem findings	Diagnosis: Isolation of agent: Material	Diagnosis: Isolation of agent: Medium	Diagnosis: Immune reaction	Diagnosis: Microscopic examination	Prevention	Treatment
%		Depression Emaciation Leg weekness Respiratory	Gasping Nasal exudate	Ataxia of leg	Drop	Normal	Eyes, Trachea: caseous exudate Pharynx, Esophagus: pustle-like lesions				Reformation of epithelium in pharynx & esophagus	Proper feeding	Vitamin-A
		Leg weekness Retarded growth	Normal	Star gazing pose	Drop	Normal						Proper feeding	Thiamine
		Leg weekness		Curled toes in ward Resting pose								Proper feeding	Riboflavin
		Leg weekness Retarded growth Rickets	Normal	Unsteady steps	Drop Thin-shelled egg	(Diarrhoea)	Beading and curveture of ribs Tibia: short and rough edge					Proper feeding	Vitamin-D
		Nervous Ataxia Leg weekness	Normal	Backward or downward retruction of lateral twisting of the head Contraction and relaxation of legs		Normal	Cerebellum: haemorrhage, soft & swelling				Edema in Purkinje cell zone	Proper feeding	
		Leg weekness	Normal	Unsteady steps	Drop	(Diarrhoea)	Fructure (shank)					Proper feeding	Calcium
al		Nervous (perosis)	Normal	Convulsions Twisting or bending of distal end of tibia	Drop		Englargement of tibial-metatarsal joint Curved and elastic bone Testes: dwarfing Embryo: parrot beak shortend limbs					Proper feeding	Manganese
al		Enlargement of crop	Beak open	Normal			Inflamation in crop					Proper feeding	Removal of contents
%		Gout White-grey urine	Normal	Normal	Drop Misshapen egg	White urine Diarrhoea	Kidney & Ureter: swelling Parenchymental organs: deposition of uremic acid					Proper feeding	
		Lose appetite Thirst Depression Emaciation Nervous Vomiting Cyanose	Difficult breathing	Convulsions Paralysis		(Diarrhoea)	Lung: congestion and haemorrhage Heart: Haemorrhage Kidney: swelling Haemorrhagic gastroenteritis					Proper feeding	

Note

CAM : Chorioallantoic membrane

CK : Chicken kidney cell

CPE : Cytopathic effect

CAC : Chorioallantoic chamber

N-T : Neutralization test

Had : Haemoadsorption test

HA : Hemagglutination test

HI : Hemagglutination inhibition test

AGP : Agar gel precipitin test

Aggl. : Agglutination test

C-F : Complement fixation test

APPENDIX II. DIAGNOSTIC WORKING SHEET FOR POULTRY DISEASE

REFERENCE NO .....

Date received : .....

Specimens : ..... Owner : .....

Submitted by : ..... Address : .....

HISTORY

Age : ..... Sex : .....

Breed : ..... Hatchery : .....

Date of first occurrence : .....

Total No. in flock : ..... No. in affected batch : .....

No. ill : ..... No. dead : .....

Vaccinations : Newcastle disease Date : .....

Fowl - Pox Date : .....

Previous disease: .....

Treatment (current medications) : .....

Feeding : .....

DESCRIBED SYMPTOMS

Appetite : good / not / thirsty

Egg production : Before illness ..... Now .....

Thinshelled / rough / misshapen

Faeces: Normal / soft / diarrhoea / white / green / sticky / Liquid / bloody

Respiratory symptoms : Normal / difficult breathing / gasping / whistling Wheezing / sneezing / coughing / beak open / scream

Nervous symptoms : Legs (left / right)

Wings (left / right)

Neck

Others : .....

Registered by .....

DIAGNOSTIC WORKING SHEET FOR POULTRY DISEASE

REFERENCE NO. ....

CLINICAL OBSERVATIONS

Size for age : biger / normal / smaller
Nutrition : good / normal / poor
Head : comb & watlle/ pocks / swelling / dwarfing / red / anaemia
Eyes : tear / exudata (liquid / pus / cheese)/abnormal pupil / swelling of eyelid (upside / down side)
Nose : exudate (liquid / pus / cheese)
Beak & mouth : pocks / diphtheria
Neck : abnormal pose (bend / stretch / convulsion)
Crop : contents (much / less / empty / foreign substances)
Wings : hung down (lfet / right)
Skin & feathers : external parasites (lice / mites / ticks)/rough / ruffled
Breast brister : swelling
Abdomen : expansion / enlarged liver
Cloaca : swelling / protocele / dirty
Bursa of fabricius : swelling
Legs : swelling (left / right)
abnormal pose (left / right)
bend / stretch / convulsion / paralysis
Feet : swelling (left / right)
bend (left / right)
Others

CLINICAL EXAMINATIONS

Blood : whole blood agglutinnation tests
Pullorum
C R D
Smear
Parasites
Bacteria
Blood cells
Faeces :
Parasite
Lesions :

REMARKS

DIAGNOSIS

TREATMENT

Examined by .....

Haemo.: haemorrhage
Cong. : Congestion

DIAGNOSTIC WORKING SHEET FOR POULTRY DISEASE

Reference No. .....
Date : .....
Hours after killed / died

POST - MORTEM OBSERVATIONS

Subcutis	: edema/haemo./necrosis/exudate/fat(much/less)
Muscle	: Haemo./dwarfing/colour(dark/light)
Peritoneum	: haemo./cong./healing/nodules/colour(dark/light)/healing
Sinus	: swelling/exdate(liquid/pus/cheese)
Larynx	: swelling/nodules/diphtheria/exdate(pus/cheese)
Trachea	: haemo./cong./exdate(much/less/liquid/pus/cheese)
Air sacs	: turbid/thick/nodules/healing/exdate(much/less/liquid/pus/cheese)
Heart	: blood(thick/thin)/haemo.
Pericardium	: liquid(much/turbid)/thick
Lungs	: haemo./cong./nodules
Pharynx	: swelling/nodules/diphtheria/exudate(pus/cheese)
Oesophage	: pustle - like lesion (much/less)
Crop	: expansion/contents(much/less)
Proventriculus	: haemo./cong./catarrh
Gizzard	: necrosis
Duodenum	: thick/caterrh/maemo./cong./nodules/parasites
Jejunum	: thick/caterrh/haemo./cong./nodules/parasites
Ileum	: thick/caterrh/haemo./cong./nodules/parasites
Caeca	: thick/caterrh/haemo./cong./nodules/parasites
Rectum	: uremic acid
Bursa of fabricius	: swelling
Liver	: swelling/haemo./cong./nodules/elastic/fragile/colour(dark/light) (dar k/light)
Gall	:
Pancreas	: swelling/haemo./cong./nodules/colour(dark/light)
Spleen	: swelling/haemo./cong./nodules/colour(dark/light)
Testes	: dwarfing
Ovary	: drop/haemo./misshapen/dwarfing/nodules/colour(green/dark)
Oviduct	: dwarfing
Kidney	: swelling/haemo./cong./nodules/fragile(dark/light)/uremic acid
Ureters	: swelling/uremic acid
Hock joint	: swelling/exudate(pus/cheese)
Hock tendon	: swelling
Feet	: swelling/exudate(pus/cheese)
Wing joint	: swelling/exudate(pus/cheese)
Cerebrum	: haemo./fragile
Cerebellum	: haemo./fragile
Nerves	: swelling
Bone texture	: rough/fragile/beading
Bone marrow	: haemo.
Others	

REMARKS

Observed by .....

DIAGNOSTIC WORKING SHEET FOR POULTRY DISEASE

DEFERENCE NO.

LABORATORY EXAMINATIONS

Serological Exam.

Virological Exam.

Bacteriological Exam.

Parasitological Exam.

Histological Exam.

Chemical Exam.

Others

Remarks

Examineed by .....

APPENDIX III. METHOD OF LABORATORY EXAMINATION

BACTERIOLOGICAL EXAMINATION

B-1. Pullerum Disease (Salmonella pullorum)

1. Materials of sampling

Pericardium
Liver
Spleen
Pancreas
Kidney
Lung
Bile
Peritoneum
Ovaries or testes
Oviduct
Bone marrow
Intestinal contents
Unabsorbed yolk, etc.

2. Culture media

YCC (yeast-extract, cystein and casein hydrolysate) agar: - nutrient
YCC broth: - enrichment
Selenite broth: - selective and enrichment
MacConkey agar: - selective and differencial
Kligler semi slant agar: - differencial (for lactose, glucose and H_2S)
SIM semisolid agar: - differencial (for H_2S, indol and motility)

3. Isolation and cultural procedure

Streak materials of sampling on YCC and MacConkey agar plates and culture in YCC or in Selenite broth (only intestinal contents or contaminated materials). Examine the agar plate after 24-48 hours of incubation at 37°C. Streak the broth culture on YCC and MacConkey agar plate after 48 hours of incubation, and examine the plate 48 hours later.

4. Identification

Selection of colonies from selective media:

Observe MacConkey agar plates for colonies (colourless) of non lactose fermenting bacteria. Pick and transfer suspected colonies to Kligler semislant agar and SIM semisolid unslanted agar. Store plates from which transfers are made, as well as any additional plate

revealing typical colonies, at refrigeration temperature until identification procedures on selected culture are complete.

Klingler semislant agar:

Inoculate by stabbing and streaking and incubate 24h.
Select slant which have the typical acid butt (yellow: - positive fermentation of glucose) with or without gas and alkaline slant (red: - negative fermentation of lactose).
Hydrogen sulfide (H_2S) production is indicated by blackening in the depth of the medium.

SIM semisolid unslanted agar:

Inoculate by stabbing through the center of the medium, which is incubated at 37° C and examine at 8, 24 and 48 hours. Motile organisms will manifest a dense zone of growth spreading from the line of inoculation.
Samomella pullorum will show no spreading from the line of inoculation.

TABLE B-1. Typical reactions on differencial media

Type (Genus)	Klıgler			SIM		
	Butt		Slant			
	Glucose	H_2S	Lactose	Indol	Motility	H_2S
Salmonella pullorum	Y G	B d	R	-	-	d
Other Salmonella	Y G	B	R	-	+	+
Arizona	Y G	B	R	-	+	+
Shigella	Y		R	d	-	-
Escherichia (Typical	Y G		Y	+	+	-
Escherichia (Non typical	Y G		d	d	d	-
Proteus	Y G	B	R	+ or -	+	+

R : red, Y : yellow, B : black, G : gas, + : positive, - : negative

d : positive or negative

Serological confirmation:

The rapid serological spot test; using a salmonella D group antiserum is very useful in the immediate confirmation of a culture as S.pullorum.

Divide the serotypes into standard type ($9,12_1,12_3$) variant type ($9, 12_1, 12_2$) or intermediate type ($9, 12_1, 12_2, 12_3$) with monospecific sera, if necessary.

Other characteristics of S. pullorum:

S.pullorum is gram-negative, non sporing bacilli, 0.4 - 0.6 μ by 1 - 3 μ, existing as single cells.

TABLE B-2. Biochemical Characteristics of S. pullorum

	S.pullorum	Other salmonella (paratyphoids)	Arizona
Indol	-	-	(-)
Methyl red	+	+	+
Voges-proskauer	-	-	-
Simmon's citrate	-	+	+
Motility	-	+	+
H_2S	weak	+	+
Urea	-	-	-
Tartrate	+ or -	+	(+)
K C N	-	-	-
Lysine	+	+	+
Dextrose	A (G)*	A G	A G
Lactose	-	-	A G(L)
Sucrose	-	-	-
Mannitol	A (G)	A G	A G
Maltose	(-)**	A G	A G
Dulcitol	-	A G	-
Salicin	-	-	-
Malonate	-	-	+
Gelatin	-	-	+(L)

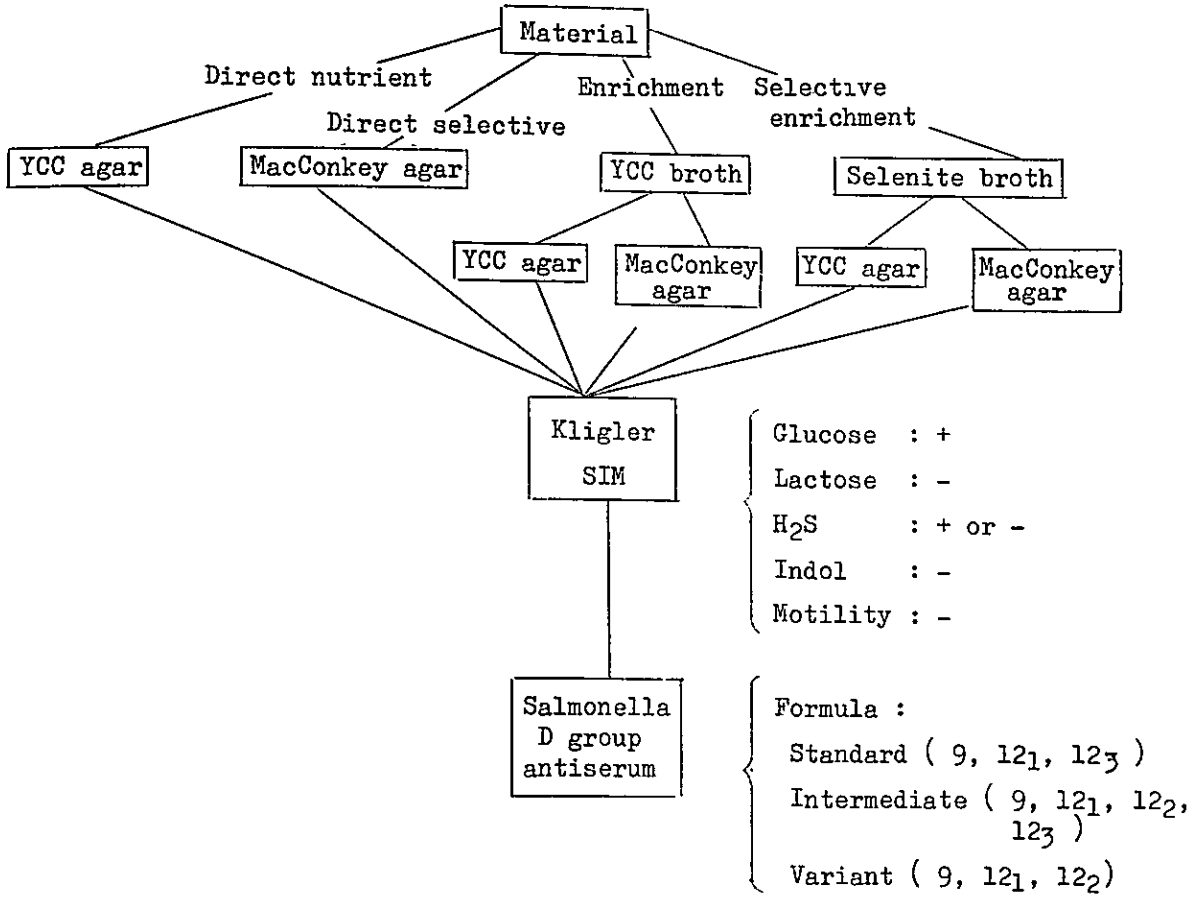
A : Acid produced, G : Gas produced, - : Negative, () : Variable

L : Late, usually 7 - 10 days, + : positive

* : Most S.pullorum cultures produce gas; however, anaerogenic strains may be encountered and others produce a small volium of gas only after extended incubation.

**: Most S.pullorum cultures do not ferment maltose.

Fig. B-1. General outline of the procedure for the isolation and identification of salmonella pullorum



B - II. CRD - Avian respiratory mycoplasmosis (Mycoplasma gallisepticum)

1. Materials of sampling

Nasal exudate (Infraorbital sinuses)

Tracheal exudate

Air sac

Lung

2. Culture media

PPLO broth:

Basal broth medium

Soy peptone(or Bacto - peptone "Difco")	10 g
Heart infusion broth	1 g
NaCl	5 g
Thalium acetate	0.25 g
Sodium carbonate	0.8 g

Distilled water 1.000 ml

Sterilize by autoclaving at 120° C for 15 minutes and cool at less than 37° C.

Horse serum (or PPLO serum fraction "Difco") 10%

Penicilin 500 U/ml

Phenol red (1% solution) 0.2 %

PPLO agar:

An agar medium may be prepared from the above by omitting the phenol red and adding 15 g of Bacto - agar per litter of broth.

Basal broth medium 1.000 ml

Bacto - agar 15 g

Sterilize by autoclaving at 120° C for 15 minutes and cool at about 50° C.

Horse serum (or PPLO serum fraction "Difco") 10 %

Penicilin 500 U/ml

Distribute in petri - dishs as about 15 ml each one

3. Isolation and cultural procedures

Inoculate portions of the test sample into tubes (5 ml each) of the broth media and incubate at 37° C. Examine cultures daily and transfer a 0.1 ml portion to an agar plate and 0.2 ml to a broth tube as soon as fermentation has taken place or after 72 hours incubation.

Carefully spread the inoculum over the surface of the agar and incubate plates at 37° C. Examine the plates after 5 days incubation and daily thereafter up to 7 days for evidence of colony formation.

If there is no fermentation in the broth subcultures or if mycoplasma colonies do not appear on the second passage, repeat for the third time before the test sample determined negative.

4. Identification

Characteristics of Mycoplasma gallisepticum:

i) M.gallisepticum will grow only in a medium containing enrichments such as serum - like proteins.

ii) Growth is usually fine and uniform in broth culture.

iii) Numerous cocooid bodies (125 - 500mμ) visible in Giemsa - stained preparations and not observed by the usual aniline stain are found in broth culture.

iv) Small (not exceeding 0.5 mm) circular colonies are produced which grow into the agar; these colonies, when stained by the procedure of Dienes or klieneberger - Nobel (Giemsa), consist of fine coccoid forms at the periphery, usually with central dense papillae.

v) The same small typical colonies are maintained unchanged through many subcultures.

TABLE B-3. Identification of M.gallisepticum

PPLO	Colonies			Haemo-adsorption	Flocculation (Ammonium phosphate) 15% 20% 25%
	Appearance	Size	Peripheral texture		
M.gallisepticum	3-5 days	0.1 - 0.5 mm	Granular	+	+ ++ +++
Others	1-2 days	0.5 - 1.0 mm	Lacy net-work Vacuole	- (some species +)	- - -

Colony staining (dienes):

i) Preparation of stain solution

Methylene blue	2.50 g
Azur II	1.25 g
Maltose	10.00 g
Na_2CO_3	0.25 g
Benzoic acid	0.20 g
Distilled water	100.00 ml

ii) Application of stain solution

Apply the stain solution in a thin film over the surface of crean micro cover glasses by means of a partially saturated cotton swab. Dry in air. The stain on the cover glass will not be affected by storage at room temperature for at least tow months. Store stained over glasses in a petri dish or other covered container.

iii) Colony staining procedure

Cut agar blocks containing colonies that appear to have the general characteristics of Mycoplasma from the petri dish and place them,

colony side up, on a microscope slide. Place a stained cover glass, stain side down, over the colony. Allow five to ten minutes for the colonies to take up the stain from the cover glass, and then examine at 50 times magnifications. Other bacterial colonies, with the exception of haemophilus gallinarum, usually decolorize the stain.

Broth staining (Giemsa):

M. gallisepticum produces acid from glucose as indicated by a change in color of the phenol red indicator. Some strains may fail to colonize yet will grow in broth. When acid production in broth is observed in the absence of mycoplasma or bacterial colonies on agar, the broth cultures should be examined for mycoplasma. Some strains of avian mycoplasma, although not M. gallisepticum, do not ferment sugars; however, they grow well in broth and generally colonize.

Mycoplasma may be identified in broth as follows:

i) Prepare a smear and gently heat - fix by flaming the slide.
ii) Flood the slide with methyl alcohol for five minutes.
iii) Wash off thoroughly in tap water.
iv) Stain by flooding slide with Giemsa stain for 30 minutes.
v) Rinse with tap water, air - dry, and examine under oil immersion. Mycoplasma are difficult to demonstrate in stained smears, but the Giemsa procedure described is satisfactory for broth cultures. The organism is not easily seen by gram's method of staining; however, it is gram - negative.

Chicken red blood cell adsorption test:

Add approximately 15 ml of 0.3% chicken red blood cell suspension to an agar plate where Mycoplasma is growing. Allow the plate to stand at room temperature for 15 minutes and then shake gently to make the sedimented red blood cells float on the surface. Discard the red blood cell suspension. Add approximately 10 ml of saline to the agar plate and wash off the blood cells which has not been adsorbed to the colony in such manner as mentioned above. Wash again and then examine under the dissecting microscope at a 50 times magnification to identify colonies of Mycoplasma.

Flocculation test:

Prepare a bacterial suspension by using phosphate buffered saline at a concentration of 50 times of the No.1 tube of McFarland nephrometer in turbidity. Make 15%, 20% and 25% solution of ammonium phosphate in amount of 3.2 ml each. Add the bacterial suspension into the tubes as 0.2 ml each and then shake well. Observe the flocculation after 10 minutes incubation at 55°C.

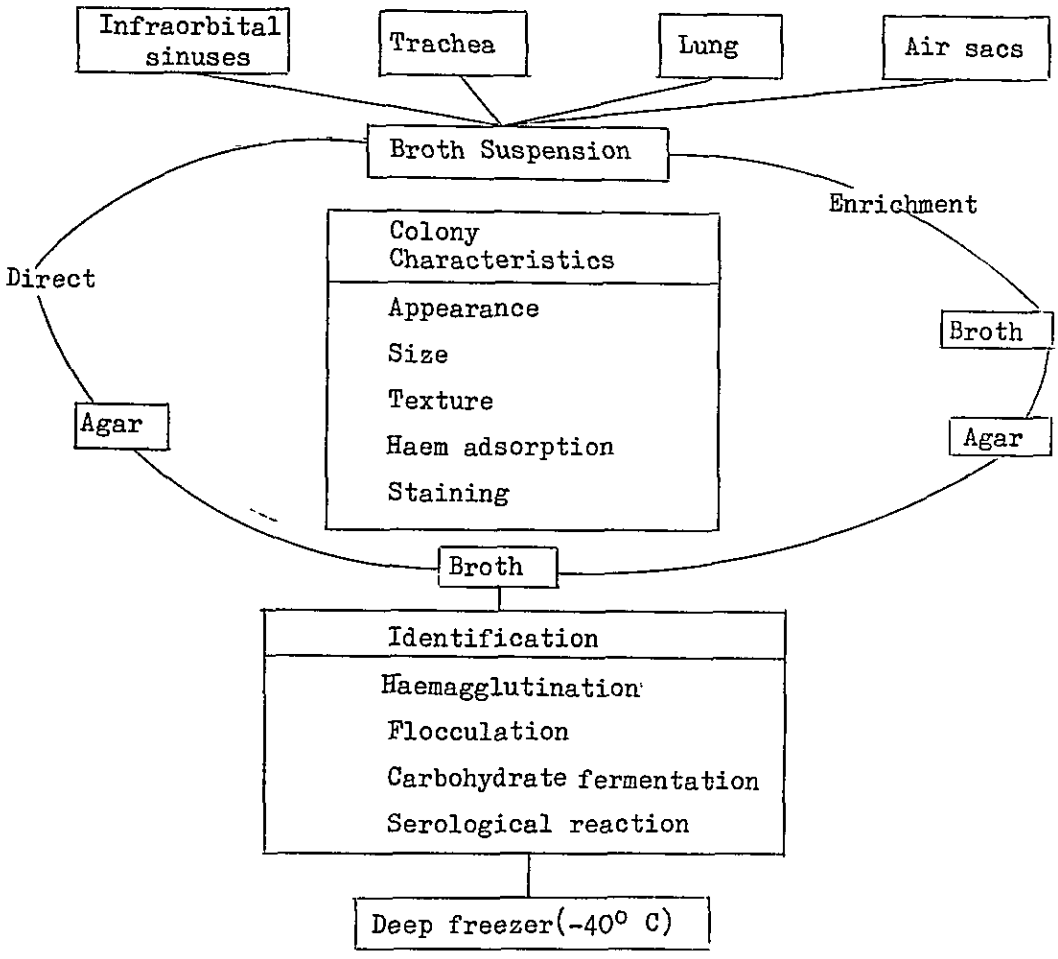
TABLE B-4. Carbohydrate Fermentation:

PPLO	Lact-ose	Mal-tose	Suc-rose	Glu-cose	Galac-tose	Treha-lose	Man-nose	Dext-rin	Sta-rch
M.gallisepticum	-	+	-	+	-	-	+-	+	+
Other Mycoplasma	-	+	-	+	-	-	+	+	+
	-	+	+	+	-	-	-	+	+
	-	+	-	+	-	-	-	+	+
	-	-	-	-	-	-	-	-	-

Serologic Confirmation:

The haemagglutination inhibition, tube agglutination and serum plate agglutination tests can be applied to identify. To simplify procedures, a single antigen may be used for all of the tests.
(See the manufacturing standard of CRD antigen).

Fig. B - 2 General outline of the procedure for the isolation and identification of Mycoplasma gallisepticum



B-III. Infectious Coryza (Haemophilus gallinarum)

1. Material of sampling

Exudate of infraorbital sinuses

2. Culture media

Blood agar:

Nutrient agar	100 ml

Sterilize by autoclaving and cool at about 50° C

Horse blood	10 %

Distribute in petri dishes as about 15 ml each one dish.

Enrichment broth (chicken meat - chicken serum broth):

Chicken meat extract	1000 ml
Minced chicken meat	500 g
Distilled water	1000 ml

Boil the mixture at 100° C for 20 minutes.

Cool and filtrate.

NaCl	5 g
Soy peptone	10 g
Poly peptone	1 g

Adjust the pH at 7.0.

Sterilize the mixture at 120° C for 15 minutes and cool at less than 37° C. (Final pH will be 6.6 - 6.8)

Chicken serum (inactivated)	5 %

3. Isolation and cultural procedure

Streak material of sampling on blood agar plate and culture in enrichment broth. Incubate the blood agar at 37° C in an atmosphere containing 10 % CO_2 for 24 hours, and the enrichment broth at same temperature and for same period in an ordinary incubator.

Streak the broth culture on a blood agar plate, if there is no suspected colonies on the blood agar. Incubate with same way as mentioned above and examine the plate 24 hours later.

4. Identification

Colony and tinctorial characteristics:

H. gallinarum form tiny, dewdrop-like colonies on the surface of a blood agar. This organism is a haemophilic, Gram-negative, pleomorphic, non-motile bacterium and it has bipolar staining characteristics. In young cultures it occurs as a short coccoid rod, but after 24 to 48 hours many long forms are found. For optimum growth an atmosphere containing 10% CO_2 is desirable.

Biochemical Characteristics

TABLE B-5. Biochemical characteristics of H. gallinorum

Glucose	+	Dextrin	+
Arabinose	-	Inulin	-
Rhamnose	-	Adonit	-
Xylose	-	Dulcitol	-
Galactose	+	Mannitol	+ or -
Mannose	+	Sorbit	-
Levulose	+	Inosit	-
Saccharose	+	Salicin	-
Maltose	+	Nitrate reduction	+
Lactose	-	Indol	-
Trehalose	-	H_2S	-
Raffinose	-	Gelatin	-
		Katalase	-

Serological confirmation:

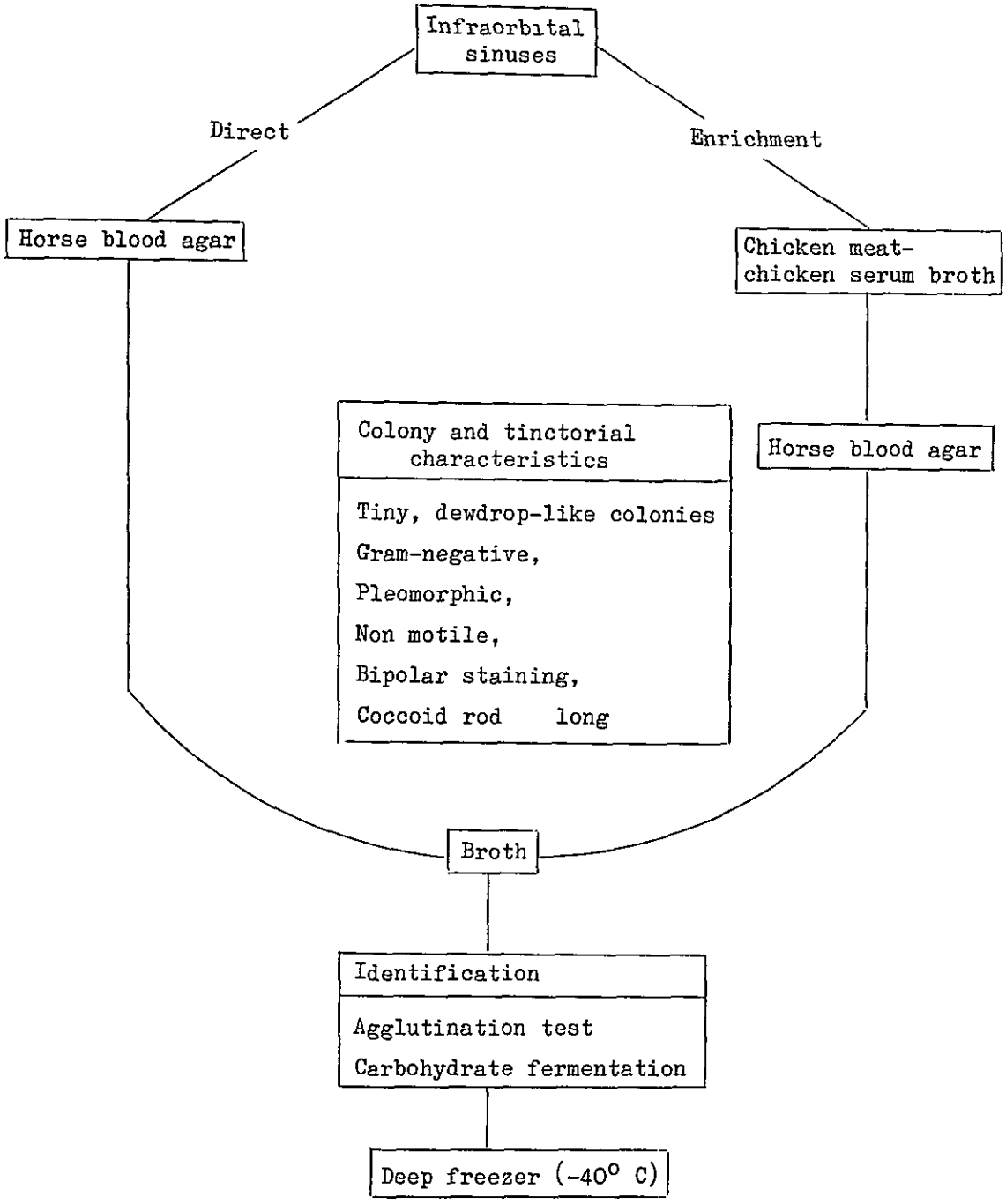
The tube agglutination test can be applied to identify.

i) Antigen: Centrifuge the enrichment broth culture after 24 hours of incubation. After washing 2 times, resuspend the packed cells in phosphate buffered saline (pH 7.0) containing 1/10,000 of merthiolate to make a concentration of the No.2 tube of McFarland nephrometer in turbidity.

ii) Immune serum: Inoculate the enrichment broth culture of H. gallinarum (strain 221) to 2-3 month old chickens intra-nasally, and take blood 2-3 weeks after inoculation.

iii) Tube agglutination test: Apply the same way as used in CRD.

Fig. B-3. General outline of the procedure for the isolation and identification of Haemophilus gallinarum



VIROLOGICAL EXAMINATION

B-IV. Susceptibility of cell Cultures to selected Avian Viruses

TABLE B-6

Cell Culture	Fowl plag.	ND	Myxo-Y-age-ent	CELO	IB	ILT	Fowl-pox	Pige-on pox	Cana-ry pox	Orni-tho-sis	AE	GAL	REO	Leuk-osis
Chicken embryo	+++	+++		+++	+	+++	++r	-	++r c				+++	+
" heart	++	++			-									
" intestine		++												
" kidney		+++		+++	(+++)	(++)					+++	+++		
" liver		++			-		+					+++		
" lung		+++				++s i								
" amnion	++	++												
" chorioallan -tois		+			+	++								
Chicken kidney		+++s c		+++r i	* +++s	+++s i	++r	++r	++r		++	+++s	+++s c	
" leucocyte	+	++												
Duck kidney														
Pigeon embryo	++													
" kidney		+++s				-	++r	++r	++r					
Bovine leuco -cyte		++												
Porcine kidney		++												
" leucocyte		++								+				
Monkey kidney					+									
Human H		++	++											
Chang L										+				
Hela		++	++											

Virus : ND = Newcastle disease
CELO = Chicken embyro lethal orphan
IB = Infectious bronchitis
LIT = Infectious laryngotracheitis
AE = Avian encephalomyelitis
GAL = Gallus adeno - like
REO = Respiratory enteric orphan

Lesion : +++ = Induces plaques and is usually cytopathic
++ = Cytopathic

+ = Other evidence of activity, such as inclusion bodies, hemadsorption or interference with a cytopathic agent.

( ) = Contradictory reports cited

* = After passed in chicken embryo

\- = Indicate negative

s = CPE of syncytial type

r = CPE of round type

c = Cytoplasmic inclusions

i = Intranuclear inclusions

B-V. Differencial Response of Chicken Embryos to Selected Avian Viruses

TABLE B-7

Virus	Route	Morta-lity (%)	Time death (day)	Lesions					Log10 titer LD50 (ID50)	Log10 Neut. titer	Log 2 HA titer
				Emb	Liv	Kid	Other	CAM			
Fowl plague	Any	90-100	2-4	C					9	?	7-10
Newcastle (velo)	Any	90-100	2-4	C				(P)	9	6-8	3-10
(meso)	Any	90-100	3-5	C				(P)	9	6-8	7-10
Disease (lento)	Any	50-100	4-8	CS		EN		(P)	1-(9)	6-8	7-10
Myxo-Y-agent	CAC	50-100	4-10	CS					8		3-10?
CELO	CAC	90-100	3-10	CS	EM	EN	AT		9	6-8	
	YS	0-100	2-H	CS						?	
	CAM	80-100	3-6	CS				P	8-9	?	
Infectious bronchitis	CAC	0-100	2-10	CS	EM	EN	AT		0-(8)	6-8	
	YS	0-10	2-6	CS		EN			-	?	
	CAM	0-10	2-6	CS		EN			0-(8)	?	
Infectious laryngo-tracheitis	CAC	50-100	4-8	-	EM	-		P	0-(8)	0-4	
	YS	0-10	H	-	-	-			?	?	
	CAM	50-100	4-8	-	EM	-		P	0-(8)	0-4	
Fowl pox	CAC	0-50	4-8	-	EM	-	ES	P	2-(2)	0-4	?
	YS	0-10	H	-	-	-	ES	P	?	?	?
	CAM	0-50	4-8	-	EM	-	ES	P	2-(7)	?	?
Pigeon pox	CAC	0-50	4-8	-	EM	-	?	P	0-(7)	0-4	
	YS	0-10	H	-	-	-	?	P	?	?	
	CAM	0-50	4-8	-	EM	-	?	P	0-(7)	0-4	
Canary pox	CAC										
	YS										
	CAM		4-8					P	(5.6-8.5)		
Ornithosis	CAC	0-100	2-8	C	?	?		P	0-8		
	YS	0-100	3-8	C	?	?		P	0-8		
	CAM	-	-	-	-	-	-	P	-	-	
Avian Encephalomyelitis	YS		H	S			DE		(4) (5)	(3) ?	
	OC		H	S			DE				
GAL	CAC	-	-								
	CAM							P			
REO	CAC	100	4-13								
	YS	100	3-8								
	CAM	100	4-7					P			
Lymphomatosis	CAC		H								

Route : CAC = Intra-allantoic chamber, 9-11 days

CAM = Suprachorioallantoic membrane, 10-12 days

YS = Intrayolk sac 7-9 days

OC = Intraocular, 9-11 days

Time death : H = hatching

Lesions : C = Petechiae and congestion

S = Retardation of growth

P = Plaque

EM = Enlarged mottled liver

EN = Enlarged kidney or spleen, necrotic foci

AT = Amniotic membrane thickened

ES = Splenomegaly

DE = Muscular dystorophy and encephalomalacia

( )= Inconsistent

- = Negative report

B-VI. Differencial Response of Susceptible Chickens to Selected Avian Viruses Causing Responses in 21 Days

TABLE B-8

Virus	Age of chicken and route of inoculation							
	Day old		6 - 12 week old					
	IC	IT	IC	IT	Sinus	FF	Cloaca	Pad
Fowl plague	D7	D7	D7	D7	D7	D7	D7	D7
Newcastle (velo) disease (meso)	D7 D7	D7 (D14)	D7 D14	D7 S7	D7 S7	D7 -	D7 -	D7 -
Myxo - Y - agent	(D)	S5		S5				
CELO (QBV)	(D14)	-21	(D14)	-21	-21	-21	-21	-21
Infectious bronchitis	-	(D7)	-	S7	S7	?	?	-
Infectious laryngo-tracheitis	-	S7	-	S7	S7	-	S7	-
Fahey - Crawley virus	-	-	-	-	-	-	-	-
Fowl pox	(D14)	?	-	(S7)	S7	S7	S7	S7*
Pigeon pox	?	?	-	-	S7	S7	?	-
Ornithosis	D7	D7	?	?	?	-	-	-?
Avian encephalomylitis (resistant strain)	D21	?	(D21)	-	-	-	-	-
GAL	-	-	-	-				
REO								
Avian lymphomatosis	-	-	-	-	-	-	-	-

Results : D = Fatal to 50% in the number of days indicated

S = Characteristic signs or lesions in 50% in the number of days indicated

- = No signs, lesions, or deaths

( )= Result inconsistent or rare

Route : IC = Intracerebral injection

IT = Intratracheal

sinus = Intrasinusoidal

FF = Rubbed into feather follicle

Cloaca= Rubbed into cloaca

Pad = Inoculated deep into foot pad

* = Swelling in foot pad only

SEROLOGICAL EXAMINATION

B-VII. Hemagglutination - Inhibition (HI) Test

1. Antigen

Newcastle disease virus (freeze-dried living vaccine)

2. Preliminary titration of antigen

Make titration of antigen as in the Table B-9.

TABLE B-9. Preliminary titration of antigen

Tube	Dilution of virus	Quantity (ml)				Sample results
		Saline	Virus	Transferred	0.75% Chicken RBC	
1	5	0.8	0.2	0.5	0.5	+ **
2	10	0.5		0.5	0.5	+
3	20	0.5		0.5	0.5	+
4	40	0.5		0.5	0.5	+
5	80	0.5		0.5	0.5	+
6	160	0.5		0.5	0.5	+
7	320	0.5		0.5	0.5	+
8	640	0.5		0.5	0.5	-
9	1280	0.5		0.5	0.5	-
10	5260	0.5		0.5 *	0.5	-
11	0	0.5			0.5	-

Note: * Material discarded

**- No agglutination, + agglutination, titer of sample is 1:640

i). Read the result after half an hour at room temperature (20-25°C).

ii). Take the blood from hens which have not received Newcastle disease vaccine. Take it in a tube with half amount of Alsver solution (Glucose 2.05 g, NaCl 0.42 g, Sodium citrate 0.8 g Citric acid 0.055 g, Distilled water 100 ml, 120°C, 10 minutes autoclaving) and wash 3 times with saline.

In the table, HA titer exhibited positive at 1:320 as the end - point dilution. Then, take the using unit/0.5 ml antigen at the dilution reversed 4 times. A 1:30 dilution should be taken as the adequate dose of antigen in this trial.

3. HI test

After diluting test serum serially, add 4 units/0.5 ml of antigen and 0.75% chicken blood cell suspension into diluted sera as shown in Table B-10. Make the reading of test result after half an hour at room temperature and take up the tubes which exhibited complete HI at the dilution at least 1:40 as a positive reaction.

TABLE B-10. HI test

Tube	Dilution of serum	Quantity (ml)					Sample result
		Saline	Serum	Transferred	4 units/0.5 antigen	0.75% Chicken RBC	
1	5	0.4	0.1	0.25	0.25	0.5	-
2	10	0.25		0.25	0.25	0.5	-
3	20	0.25		0.25	0.25	0.5	-
4	40	0.25		0.25	0.25	0.5	-
5	80	0.25		0.25	0.25	0.5	+
6	160	0.25		0.25	0.25	0.5	+
7	320	0.25		0.25	0.25	0.5	+
8	640	0.25		0.25*	0.25	0.5	+

* Material discarded

TABLE B-11. Antigen control

Tube	Units of antigen	Quantity (ml)				Sample result
		Saline	4 units/0.5 ml of antigen	Transferred	0.75% of chicken RBC	
1	4		0.1	0.5	0.5	+
2	2	0.5		0.5	0.5	+
3	1	0.5		0.5	0.5	+
4	0.5	0.5		0.5	0.5	±
5	0.25	0.5		0.5	0.5	-
6	0.125	0.5		0.5 *	0.5	-

* Material discarded

B-VIII. Agar Gel precipitin test

1. Antigen

Salmonella pullorum —Pullorum antigen for rapid agglutination

Fowl-pox virus -- Infected chorio-allantoic membrane

Infectious bronchitis virus -- Infected chorio-allantoic membrane

Infectious laryngotracheitis virus -- Infected chorio - allantoic membrane

Infected tracheal exudate

Avian adeno virus -- Infected fluid of tissue culture

Avian reo virus -- Infected fluid of tissue culture

2. Preparation of agar gel medium

Agar gel medium

Bacto-ager (Difco)	1.5 g
Phenol	0.5 ml
Methyl - orange or methylene blue	3 ml
Phosphate buffered saline solution (pH 7.2)	100 ml

Pipette 7 ml of molten agar in a 60 mm Petri - dish, where Penicillin cups, 8 mm in diameter, are set 3 mm apart in advance to make holes in the agar plate. After coagulation of agar, remove the cups carefully and put a drop of molten agar into a hole to seal its bottom. Keep the plates overnight at room temperature before use.

3. Precipitin test

Put antigen into center hole and place sera into surrounding holes on the plate. Allow to diffuse at room temperature. Make the final reading of result 7 days after diffusion, though precipitin lines become visible within 2 days.

B-IX. Neutralization test (Tissue Culture Method)

1. Antigen

Infected fluid of tissue culture

Newcastle disease virus

Infectious bronchitis virus

Infectious laryngo tracheitis virus

Avian adeno virus

Avian reo virus etc.

2. Serum

Dilute serum 1:5 or 1:10 with the viral diluent (Earle's balanced salt solution containing lactalbumin hydrolysate at 0.5 per cent) and inactivate at 56° C for 30 minutes.

3. Neutralization test

Mix serial ten - flod dilutions in the viral dilution of the virus with equal amount of the serum dilution, and inoculate 0.2 ml of mixture to the dishes or bottles for virus titration after incubated at room temperature for 1 hour and at refrigerator over night. After incubation at 37° C for 1 to 2 hours, overlay 5 ml of first agar medium cooled at 38° to 39° C to each dish. After the agar medium fixed, place the dishes in the incubator at 37° C. After 3 to 4 days, overlay same amounts of second agar medium to each dish and then place again in the incubator. After 1 to 2 days, observe the plaques. As a control, include virus dilution mixed with the viral diluent instead of a serum dilution. Take the difference in log plaque forming unit (PFU) between the serum and control as neutralization log index. In the screening test, mix the virus dilution of about 10^5 titer with equal amount of the serum dilution. Inoculate the mixture to 3 to 5 tubes. Observe the tubes during three days after inoculation, and take up the tubes which did not appear any CPE as a positive reaction. (See C-1. Method of Tissue Culture)

APPENDIX IV. METHOD OF TISSUE CULTURE

C-I. Method of Tissue Culture

1. Primary Cell Culture (Chicken Kidney)

i) Harvest a kidney aseptically from 2-to-3 month-old chicken, mince sufficiently with surgical scissors and wash 3 times with a phosphate buffered saline solution (PBS).

ii) Add 10 ml of 0.25% trypsin in PBS to about 1 g of minced tissue. Stir the mixture with a magnetic stirrer at 34° to 37° C for 15 minutes. Repeat the trypsinization 3 to 4 times with a renewed trypsin solution, and throw off the first one.

iii) Filtrate the resulting suspension through sterile gauze to remove large-masses, centrifuge at 1,000 rpm for 5 minutes, and decant.

iv) Wash the precipitated cells 3 times with a growth medium by repeated centrifugation and resuspension.

v) After washing, make a 0.5% cell suspension in a growth medium and examine for the number of cells with a hemocytometer to ascertain that it contains about 2 x 10^6 cells per ml.

vi) Dispense this suspension, in 0.5 ml amount, in roller tubes 11 mm in diameter and 100 mm in depth: in 5.0 ml amounts, in bottles 20 x 40 x 70 mm: and in 5.0 ml amounts, in petri - dishes 60 mm in diameter and 20 mm in depth. Seal the tubes and bottles with rubber stoppers.

vii) Place the tubes in a slanted position in racks, and lay the bottles and the dishes flat in an incubator which is kept a high percentage of humidity at 37°C

2. Inoculation

The cultures form a solid sheet in 3 to 4 days. After the solid sheet completed, remove the growth medium and inoculate viral material. After incubation at 37° C for 1 to 2 hours, add a maintenance medium to each culture. Then place the tubes in a racks, and lay the bottles and dishes flat in the incubator. The amount of the inoculum and maintenance medium are 0.1 and 0.9 ml in a tube, 0.2 and 5.0 ml a bottle and dish, respectively.

3. Observation of Cytopathic Effect (CPE)

Examine the cultures under the microscope for 7 days for the appearance of CPE.

4. Plaque method

Inoculate 0.2 ml of ten - fold serial dilution of viral material in dishes or bottles after incubation at 37° C for 1 to 2 hours, overlay 5

ml of first agar medium cooled at 38° C to each dish. After the agar medium fixed, place the dishes in the incubator at 37° C. After 3 to 4 days, overlay same amounts of second agar medium to each dish and then place again in the incubator. After 1 to 2 days, observe the plaques.

Note:

Phosphate buffered saline solution (PBS)

NaCl	8.0 g
KCl	0.2 g
Na_2HPO_4	1.15 g
KH_2PO_4	0.2 g
Double distilled water	1,000 ml

Modified Earle's balanced salt solution (Earle's BSS)

NaCl	7.4 g
KCl	0.4 g
$CaCl_2$	0.2 g
$MgSO_4 \cdot 7H_2O$	0.22 g
$NaH_2PO_4 \cdot 2H_2O$	0.1625 g
Glucose	1.0 g
Phenol red	0.02 g

Growth medium

Earle's BSS	1,000 ml
Lactalbumin hydrolysate	5.0 g
Bovin serum (inactivated at 56°C for 30 minutes)	10 %
Penicillin	100 units/ml
Streptomycin	100 µg/ml

Maintenance medium

Earle's BSS	1,000 ml
Lactalbumin hydrolysate	5.0 g
Bovin serum (inactivated)	3 - 5 %
Sodium bicarbonate (2%)	2 - 3 %
Penicillin	100 units/ml
Streptomycin	100 µg/ml

First agr medium

Maintenance medium	1,000 ml
Bact-agar	10 g
Mycostatin	50 units/ml

Second agar medium:

First agar medium	1,000 ml
Neutral red (0.1%)	7 %

Sterilize the media by autoclaving at 120° C for 15 minutes.
Cool to about 40°C, then add the bovin serum and antibiotics.

APPENDIX V. TECHNICAL GUIDE NO.11 10 July, 1966.

Subject: THE MANUFACTURING STANDARD OF FOWL-POX VACCINE

I. Definition

This standard shall be for the production of fowl-pox vaccine in the Damascus Veterinary Laboratory. It presents a living vaccine freeze-dried and is obtained from virus containing tissue material of chorio-allantoic membrane of embryonated eggs which has resulted from the artificial inoculation of the seed virus.

II. Production of Vaccine

II - 1. Seed Virus

II - 1 - 1. Original Seed Virus

The seed virus "HUNGARIAN FOWL-POX VIRUS" is to be used. The original seed virus should be stored in a freeze-dried ampoule and to be kept in a deep freezer.

The passage of seed virus should be performed on the chorio-allantoic membrane once every year.

II - 1 - 2. Practical Seed Virus

The practical seed virus for the production of vaccine should be harvested freshly of chorio-allantoic membranes which show the evidence of growth of virus indicated by the production of pocks on the chorio-allantoic membrane as the result of inoculation of the original seed virus.

The practical seed virus material for the production of vaccine should contain no bacteria or virus contaminant, as indicated by appropriated tests.

II - 2. Eggs for the Production of Vaccine

The fertilized eggs to be used should be collected in a flock of hens which are bred separately in a farm controlled under rigid sanitary conditions or the Poultry Section of Damascus Veterinary Laboratory where it does not come in contact with other flock.

The eggs should be taken from hens that have not been inoculated with a fowl-pox vaccine previously.

II - 3. Incubation of Egg prior the Inoculation of Seed Culture

Eggs should be incubated in the brooder at 38°C for 10-11 days prior to the inoculation of seed virus material.

II - 3 - 1. Candling

Eggs under incubation shall be inspected daily by means of candling to confirm the regular growth of embryo and eggs which contain vital embryo shall be continued further incubation.

II - 4. Inoculation of Seed Virus

II - 4 - 1. Preparation of Inoculum

Chorio-allantoic membrane to be used shall be emulsified with adding of five times Earle's balanced salt solution (Appendix I) containing 10 per cent of bovine serum, 0.5 per cent of lactal bumin hydrolysate, 100 U of penicillin G sodium salt and 0.1 mg of dihydro-streptomycin per ml, and filtered through a cotton gauze.

II - 4 - 2. Preparation of Eggs

The portion of air space of eggs shall be marked with pencil on the spherical line. Also, the point for inoculation of virus material shall be marked on the egg shell, near the head of embryo and far from vessels.

II - 4 - 3. Drilling and other Manipulations

The marked point on egg shell shall be sterilized widely with iodine tincture. Operating hand-drill, the marked point on an egg shell where corresponding the top of air-space should be punctured gently with rigid attention and regard. Then, only the egg shell at the other marked point shall be removed to make a hole of about 3 x 5 mm by using a dental file electrically operated.

One drop of saline shall be dropped on the hole of the egg shell and punctured carefully in the only shell membrane with a needle of fine gauge in order to sink saline into space between shell membrane and chorio-allantoic membrane. Gentle action of handling a rubber teat is applied to groove over the air-space, and the chorio-allantoic membrane fall away from the shell membrane, drawing in the saline dropped.

II - 4 - 4. Inoculation of Seed Virus

The inoculation of seed virus material shall be made with a syringe with needle of fine gauge. The inoculum of 0.1 ml shall be deposited on the chorio-allantoic membrane. Soon after that, the rubber teat is applied again in order to make the pulling of the inoculum into the air-space sure.

II - 4 - 5. Sealing the Hole in the Shell

The hole in the shell shall be sealed with a mixture of paraffin-vaseline (4 : 1) which is melt at 50°C.

After inoculation, the eggs must not be turned in any way that will disturb the position of artificial air-space.

II - 5. Incubation after the Inoculation of Seed Virus

Eggs inoculated seed virus material shall be placed in an incubator of 37°C for 5 days.

II - 6. Harvesting

II - 6 - 1. Inspection of Eggs prior to Harvesting

Eggs taken out from the incubator after 5 days shall be inspected by candling and eggs containing dead embryo shall be discarded.

II - 6 - 2. Collection of Chorio-allantoic Membrane

Eggs dipped in 70 per cent alcohol for 5 minutes shall be placed on a suitably molded pad of cotton wool moistened with antiseptic solution and the part of artificial air-space shall be sterilized widely with iodine tincture. The sealing material shall be removed, and the shell is flamed rapidly with a Bunsen burner. Then, the shell is cut away with a scissor within the boundary of artificial air-space, and the whole air-space of membrane which shows a formation of characteristic foci are collected in a Petri-dish, as five membranes each one dish.

II - 7. Preliminary Sterility Test

0.1 - 0.2 ml of liquid of membranes collected at the bottom of dish is planted in one tube of agar slant medium. The planted agar slant medium shall be incubated at 37°C for 24 - 48 hours. During this period, the dishes containing membranes shall be kept in a deep-freezer at -40°C. The membrane of sterile and ones containing few contaminant of harmless bacteria can be taken to the next step of production process.

II - 8. Homogenizing

The total weight of one batch of collected membrane shall be measured. The measured material is removed in the cup of homogenizer or grinding machine. The homogenizing shall be started with a slow speed at the first stage and speeded up gradually. The Earle's solution (Appendix I) containing lactalbumin, bovine serum and antibiotics at the same concentration as II - 4 - 1 shall be added in material under homogenizing as amount of 5 times of material.

II - 9. Filtration

The vaccine material homogenized should be filtered through one sheet of cotton attached to a funnel.

II - 10. Dispensing

The final vaccine material should be dispensed immediately into ampoule using serial injection syringe or an automatic filling machine. A 0.5 ml of vaccine material is to be dispensed into ampoule and it should correspond generally to 1,000 doses as the final product dried.

II - 11. Freeze-Drying

II - 11 - 1. Primary Drying

The vaccine material dispensed in ampoules should be placed immediately

in the primary drying dram chambre of centrifugal freeze-drying machine of Edward type. Centrifugation and exhausting should be operated at first for about half an hour to create a vacuum in the chambre, and then the operation of primary drying shall be continued for about 24 hrs.

II - 11 - 2. Final Condition of Primary Drying

After approximately 24 hours of successive operation of primary drying, it must be checked that the temperature in the drying chambre restores up to the same rate as the outside of the chambre and that the maintenance of vacuum in the drying chambre is at 0.2 - 0.1 mm Hg constantly.

II - 11 - 3. Constriction of Ampoules

Ampoules taken out from the primary drying chambre shall be stoppered with a cotton plug or covered completely with a sterilized cotton cloth, and the constriction of ampoules should be made by operating a construction machine of Edward type or by the hand manipulation with Bunsen burner.

II - II - 4. Secondary Drying

The primary-dried vaccine in constricted ampoules shall be attached to the secondary drying system of Edward type and be operated for at least 24 hours. It must be checked that the final vacuum extent in the secondary drying system is to be indicated at approximately 0.05 - 0.04 mm Hg.

II - 11 - 5. Cutting Ampoules Secondary Dried

After approximately 24 hours of secondary drying, each ampoules attached to the secondary drying system should be cut off by using a triangle burner under rigid vacuum conditions.

II - 11 - 6. Vacuum Extent Test of Final Product

Each final product in the ampoule should be tested for vacuum extent by using a Tesla coil. Electric discharge of fluorescent colour shall be observed in each ampoule. It is preferable that vacuum extent of the product shall be checked at the final stage of production and again after one month or the time of issue of product.

II - 12. Printing of Ampoules

The ampoule containing freeze-dried vaccine shall be printed the following items.

i. The name of product
ii. Lot number
iii. Date released from the assay
iv. Expiration date

Also, the label indicating the following items shall be sticked on the container of suspending fluid which shall be used only for the freeze-dried fowl-pox vaccine.

i. Suspending Fluid for Fowl-pox Vaccine

ii. Date of production.

III. Suspending Fluid for the Freeze-dried Fowl-pox Vaccine

A 50 per cent glycerine-saline shall be used as the suspending fluid for the freeze-dried fowl-pox vaccine. The manufacturing procedure of suspending fluid shall be as follows:

III - 1. A 8.5 g of natrium chloride, chemically pure, is melt into 1.000 ml of distilled water. Filtered once through a chemical filter paper.

III - 2. A 500 ml of glycerine, chemical pure, is mixed with 500 ml of physiological saline made as above.

III - 3. Dispensed 5 ml each in bottle of 5 or 10 ml capacity. Stoppered and sealed as usual way.

III - 4. Sterilized at 120°C for 30 minutes.

IV. Storage and Expiration Date

IV - 1. Storage

The product should be stored continuously at 0° - 5°C.

IV - 2. Expiration Date

The expiration date is two years after the assay passed.

V. Field Use

V - 1. Re-suspending of Dried Vaccine

One ampoule of freeze-dried fowl-pox vaccine consists of about 1,000 doses and it should be suspended in 5 ml of suspending fluid attached with vaccine. It should be used after mixing thoroughly for making a homogenous suspension without lumps or sediment.

The re-suspended vaccine shall be inoculated within 5 hours after re-suspending.

V - 2. Vaccination

V - 2 - 1. Special cautions

V - 2 - 1 - 1. The vaccination shall be completed before the beginning of egg laying, and should not inoculate in the area where the fowl-pox has not outbroken.

V - 2 - 1 - 2. The chicken vaccinated shall be observed its reaction of vaccination about one week. Chicken without local response of vaccination shall receive re-vaccination.

V - 2 - 1 - 3. The remainder of re-suspended vaccine in bottle should be thrown off after sterilized by adequate means.

V - 2 - 2. Method of Inoculation

The vaccination should be performed by means of the "STICK METHOD". The vaccine shall be introduced into the skin by sticking two points of wing-web at space of 3 - 4 mm with a sharp eyed needle moistened with the vaccine.

V - 3. Immunity

The immunity in chickens vaccinated will be completed after 2 - 4 weeks and maintains for approximately one year.

VI. Assay Standard for Fowl-pox Vaccine

The standard shall be for the assay of fowl-pox vaccine manufactured in the Damascus Veterinary Laboratory. The tests shall be performed with the samples in one lot which is collected in accordance with the sampling method in Appendix II attached herewith.

The vaccine under test in ampoules should be in conformity with the tests specialized as below:

1. Property Test
2. Vacuum Extent Test
3. Sterility Test
4. Safety Test and Pock-forming Test
5. Potency Test

VI - 1. Property Test

VI - 1 - 1. Property Test for Freeze-dried Vaccine

The fowl-pox vaccine under test should be of a spongely appearance and of fainty grayish-brown tint in the mass at the bottom of ampoule.

VI - 1 - 2. Property Test for Suspending Fluid

The suspending fluid should be colourless and transparent. It shall not contain any foreign substances.

VI - 1 - 3. Property Test for Re-suspended Vaccine

The re-suspended vaccine should be a fainty gray turbid homogenous suspension. It shall not contain lumps, sediment or foreign bodies.

VI - 2. Vacuum Extent Test

Before cutting ampoules for the other tests, each samples should be tested for vacuum extent by using a Tesla coil. Electric discharge of fluorescent colour shall be observed in each samples.

VI - 3. Sterility Test

VI - 3 - 1. Preparation of Test Sample

Each ampoules sampled shall be re-suspended with suspending fluid. The test shall be performed conformably in items of Appendix III attached herewith.

VI - 3 - 2. Judgement of Test Result

The product under test shall be acceptable to be checked as free from any viable micro-organism demonstrable by this test or not be found bacterial colonies more than 100 per ml of test sample.

VI - 4. Safety Test and Pock-forming Test

VI - 4 - 1. Test Method

Each samples re-suspended shall be poured in one container with the same amount of each sample. It shall be used for potency test.

Three of 3 - 4 month-old chickens of quite healthy shall be inoculated the test sample by the same way of field use as chapter V - 2 - 2 in the manufacturing standard.

VI - 4 - 2. Observation and Judgement of Result

The inoculated chickens shall be under observation for 3 weeks. During this period, the chicken should not exhibit any sign of disorder such as loss of appetite, low-spirit and others. The pock-forming shall start at 2 - 5th day and shall be on the peak at 7 - 10th day with a crust formation in size of about 7 - 8 mm, then the crust dry gradually and fall off eventually in 2 - 3 weeks after the inoculation. The reaction should be localized and the result shall never be acceptable if the pock-formation is generalized or severe general reactions are accompanied.

VI - 5. Potency Test by Estimating Viable Virus Content

VI - 5 - 1. Test Method

The test sample shall be arranged in serial 10 - fold dilution with Earle's balanced salt solution. Twelve in total of embryonated eggs of 10 - 11 incubation-days old are prepared for the test. Each 0.1 ml of 10^{-4}, 10^{-5} and 10^{-6} dilution shall be inoculated on the chorio-allantoic membrances of 4 eggs in one group.

VI - 5 - 2. Observation and Reading Results

The product under test shall be acceptable if the pock-forming unit (PFU/ml) is calculated as 10^6 or more.

After 5 days of post-inoculation of eggs, chorio-allantoic membranes shall be taken out and spread in Petri-dish filled with saline containing formaline in 1 per cent. The number of pocks formed on the chorio-allantoic membrane shall be counted on a black background. The pock-forming unit per ml should be shown more than 10^6, i.e. at least one pock shall be found on an average in 10^{-5} dilution.

The calculated unit of virus content by the number of pocks counted is to be represented as PFU (Pock forming unit). It shall be calculated by the following formula:

$$10^a \times b \times \frac{1}{0.1\ (ml)} = PFU/ml$$

a = Dilution orde--, b = Average number of pock counted.

APPENDIX (THE MANUFACTURING STANDARD OF FOWL-POX VACCINE)

I. Ingredient of Earle's Balanced Salt Solution

NaCl	7.4 g
KCl	0.4 g
$CaCl_2$	0.2 g
$MgSO_4 \cdot 7H_2O$	0.22 g
$NaH_2PO_4 \cdot 2H_2O$	0.1 625 g
Glucose	1.0 g
Phenol Red	0.02 g
Double Distilled water	1,000 ml.

The solution is sterilized in an autoclave at 120°C for 15 minutes.

II. Sampling from the Lot of Final Product and Supplementary Caution in regard to the Performance of Assay

II - 1. Sampling method

The sampling from final containers in one lot and the handling of test ampoule shall be carried out with the following steps.

Samples to be assayed should be collected in compliance with the number of ampoules in one lot, as shown in the following Table. The test ampoules are collected by means of a random sampling method.

No. of ampoule in one lot	200 or less	201-300	301-400	401-500	501-600	601-700	701-800	801 or more
No. of ampoule to be sampled	4	5	6	7	8	9	10	11

II - 2. Handling of Test Ampoule

II - 2 - 1. Sampling from the test ampoule and re-suspending shall be carried out aseptically.

II - 2 - 2. The sterilized room for testing ampoules shall always be kept throughly clean. Prior to working, the room shall be lighted with ultra-violet ray sterilization lamp and 5 percent phenol splayed in it.

II - 2 - 3. Any person engaged in test in the sterile room shall wear a disinfected cap, mask and gown for exclusive use in the room.

II - 3. Opening Test Ampoules

II - 3 - 1. Protective goggles shall be worn while opening ampoules in the sterile room.

II - 3 - 2. Ampoules shall be wiped well with a gauze with 5 per cent phenol solution.

II - 3 - 3. Ampoules shall be scratched with a small file at a spot near the extremity of ampoule where it is to be broken, passed through the flame, and beaten by a triangular file on the portion to be cut down.

II - 3 - 4. After the opening of the ampoule, they were passed through the flame, their content shall be drawn into a sterilized syringe with adding a small amount of the suspending fluid for fowl-pox vaccine (Chapter III in the manufacturing standard) and returned into the remaining part of the suspending fluid in the bottle.

III. Test method of Sterility

III - 1. Method to be used

Ordinary agar, ordinary broth and liver-liver bouillon are to be used. Prior to the planting, each 10 ml of agar shall be fixed at 48°C after thoroughly melted. Also, liver-liver bouillon in test tube shall be cooled in the running water after 15 - 20 minutes boiling.

III - 2. Planting

III - 2 - 1. In planting, more than 1.8 ml of re-suspended vaccine of each ampoules under test shall be drawn at a time into a sterility test pipette.

III - 2 - 2. 1.8 ml of test vaccine re-suspended from one ampoule should be planted in 3 tubes of agar melted, 3 tubes of broth and 3 tubes of liver-liver bouillon in equality of inoculum - that is, 0.2 ml to each tube.

III - 2 - 3. Plate culture of agar shall be made. Agar media received inoculum shall be decanted immediately into Petri-dishes.

III - 3. Culture

III - 3 - 1. Planted media shall be incubated for 7 days at 35 - 37°C.

III - 3 - 2. Cultures except agar plates shall be examined on the 2nd, 4th and 7th day after planting.

III - 3 - 3. Examination on the agar plate cultures shall be made after 2 days of incubation by using a magnifying lens or illuminated reading device. The isolation of colony or further examination are to be made, if necessary.

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With co-operation of

M. Baghdady, the Veterinary Laboratory

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APPENDIX VI. TECHNICAL GUIDE NO. 12

SUBJECT: THE MANUFACTURING STANDARD OF C R D (Avian Respiratory Mycoplasmosis) ANTIGEN

I. Definition

This standard shall be for the production of CRD antigen in the Damascus Veterinary Laboratory. The CRD antigen represented in this standard is a product of inactivated and crystal violet stained suspension of Mycoplasma gallisepticum artificially cultured.

II. Production of CRD Antigen

II - 1. Original Seed Strain

The seed strain of "Mycoplasma gallisepticum KP-13" [1] is to be used. The original seed strain should be stored in a deep-freezer as freeze-dried organism in ampoule.

II - 2. Maintenance of Seed Strain

The passage of seed strain should be performed once a year in the PPLO broth media (Appendix 1) containing one-hundredth volume of 2 per cent phenol red. An adequate amount of saline is added into the dried seed in an ampoule cut. After a slight shaking of ampoule added saline, the suspension in ampoule shall be taken out by a sterilized syringe and be planted in PPLO broth. After the cultivation at 37°C for about 3 days (as soon as fermentation of glucose has taken place), the organisms in culture shall be dispensed in ampoules and freeze-dried for the next seed strain. The seed strain freeze-dried in ampoules shall be kept in the refrigerator below than -5°C.

II - 3. Preparation of Seed Culture

An adequate amount of saline shall be added into the ampoule of seed strain. After a slight shaking of ampoule, the saline suspension in the ampoule shall be taken out by a sterilized syringe and planted on the PPLO agar plate (Appendix 2).

It shall be incubated at 37°C for 3 - 4 days. A single colony in the growth on PPLO agar shall be cut off squarely with the base of media and re-planted into the PPLO broth (Appendix 1).

The culture passed through several passage in PPLO broth for each about 3 days incubation period until the organism is familialized enough to

1) Isolated in Japan in 1963.

develop plentifully shall be used for the practical seed culture of the mass culture. The final culture as the practical seed shall be made of PPLO broth in an adequate size of glass container.

II - 4. Planting in the Production Media

II - 4 - 1. Production Media

The PPLO broth (Appendix 1) shall be used. A 1,500 ml of media are to be dispensed in glass containers of 3,000 ml capacities.

II - 4 - 2. Planting and Cultivation

The broth culture of seed which is measured as one tenth amount of the production media prepared shall be planted in the production media and incubated at 37°C for 4 - 6 days.

II - 5. Harvesting

II - 5 - 1. Checking of Contamination before Harvesting

The stained smear of mass cultured broth which shows a slight turbidity shall be observed by the microscope and the culture under test are to be released to the next step of production if a pure growth of mycoplasma without any contaminant is recognized under microscope. The staining procedures are described in Appendix 4 attached herewith.

II - 5 - 2. Inactivation of Organism in Mass Culture

The merthiolate (Sodium Ethyl Mercuri Thiosalicylate, C_9H_9 Hg. Na. O_2S) shall be added into the mass culture at the rate of 1:10,000 in order to kill the living Mycoplasma in the culture. Then, the material of original culture shall be stirred for 30 minutes by a stirring machine and put overnight in the refrigerator.

II - 5 - 3. Collecting of Inactivated Organisms by Centrifugation

The organism inactivated shall be collected by means of high speed centrifugation (5,000 rpm, 30 minutes). The organism inactivated as a precipitated mass in the bottom of centrifugal tubes shall be collected and re-suspended in calculated amount of phosphate buffered saline (Appendix 3) which contains merthiolate at the rate of 1:10,000. The amount of phosphate buffered saline for re-suspending shall be 1/200 amount to the original amount of culture.

II - 5 - 4. Filtration

The suspension shall be filtered through cotton wool of one cm thickness. It may be convenient to use a 50 ml or 100 ml glass syringe attached to a seat of cotton wool at the bottom of cylinder of syringe for pressing the material by the piston tube.

II - 6. Adjustment of Density of the Suspension filtered

The density of suspension filtered shall be adjusted as 25 times dilution of No. 1 of Mc Farland turbidity standard by adding phosphate buffered saline containing merthiolate.

II - 7. Staining of Suspension by Crystal Violet

For staining organisms in suspension, the crystal violet solution shall be added into suspension adjusted density at the rate of 1:10,000, i.e. one per cent crystal violet water solution is added in the suspension at the rate of one per cent.

II - 8. Stabilization

The crystal violet stained suspension shall be put at $4^{\circ}C$ for one week in regarding to the stabilization of the product.

III. Dispensing of Final Product

The final product shall be dispensed in a sterilized glass container of 5 ml or 10 ml with rubber stopper enclosed by metal tamper-proof seal.

A 5 ml of product shall be corresponded as 100 doses for diagnosis in the field.

IV. Label

The label printed and stamped the following items shall be sticked on each container.

1. The Name of Product (CRD Antigen or Avian Respiratory Mycoplasmosis Antigen)
2. Lot Number
3. Date released from Assay
4. Expiration Date
5. The Name of Manufactory (Damascus Veterinary Laboratory)

V. Storage and Expiration Date

V - 1. Storage

The product should be stored continuously at 0° - $10^{\circ}C$ in refrigerator.

V - 2. Expiration Date

The expiration date shall be the day after one year of asssy passed.

VI. Field Use

VI - 1. Cautions

In regarding to the checking for the grade of invasion of disease among the flock under question, the test will usually be carried out on individual of hens sampled at least 10 per cent of the whole number in the flock.

The antigen in container shall be shaken frequently before and during the test in order to make antigen thoroughly homogenous.

A cleaned and dried glass plate shall be used for the test.

A syringe for taking blood shall be once sterilized. Or the syringe may be allowed to re-use after washing in water as the primary step and in phenol solution of 3 per cent as the next step and in the sterilized saline as the final washing step. The test blood taken from hens shall be mixed immediately with antigen on the glass, otherwise the blood may coagulate partially. The test shall be carried out at 20 - 25°C under avoiding direct sunshine and over drying of blood-antigen mixture.

VI - 2. Test Method

Two drops of antigen taken out through rubber stopper by a syringe attached a needle of 1/1 gauge shall be dropped on the square of the glass plate. Then, one drop of blood taken from the wing vein by the syringe attached 1/1 needle shall be dropped immediately beside the drop of antigen and mixed thoroughly by using a bent needle or a glass stick.

VI - 3. Reading the Result

It shall be recognized as the positive reaction when the agglutination occur within two minutes from the beginning of mixing of blood and antigen.

And it shall also be a negative reaction where no agglutination occurs after two minutes or a slight reaction performs after two minutes.

VII. Assay Standard for CRD (Avian Respiratory Mycoplasmosis) Antigen

VII - 1. Definition

This standard shall be for the assay of CRD antigen manufactured in the Damascus Veterinary Laboratory. The tests shall be performed with the sample in one lot which are collected in accordance with the sampling method in Appendix 5 attached herewith.

The product under test should be in conformity with the tests specialized as below:

1. Property Test
2. Purity Test
3. Identity Test
4. Potency Test

VII - 2. Property Test

The test shall be performed on each content of containers sampled. The CRD antigen under test should be a distinct violet coloured suspension of deep turbidity. It shall be free from strange smell and contain no foreign bodies. The contents of the final containers sampled shall be of homologous property.

VII - 3. Purity Test

The test shall be performed on each content of containers sampled. No bacteria other than Mycoplasma shall be observed in smear prepared from the product under test. The staining method of smear prepared shall be as in Appendix 4 attached herewith.

VII - 4. Identity Test

VII - 4 - 1. Test Method

The test shall be conducted with a mixture of equal volumes of the contents of containers sampled.

It shall be conducted by the tube agglutination test of a known positive and negative chicken sera with antigens under test and a known product in valid period as a control. To each 0.25 ml of serial two fold dilution of these sera which starts from 1:5 dilution, 0.25 ml of 1:12.5 fold diluted products in saline shall be added as antigens. Tubes contain saline and antigens shall be arranged as controls. After being throughly shaken, the mixture shall be placed in incubator of 37°C for two hours and then put overnight in ice chamber of 0° - 4°C.

At the end of this time, the result of agglutination shall be read.

VII - 4 - 2. Reading of Results

The equal positive results of agglutination shall be obtained in the known positive serum with the both antigens of product under test and the control product.

And no agglutination shall be confirmed in tubes containing negative sera and control tubes of saline with the both antigens.

VII - 5. Potency Test

VII - 5 - 1. Test Method

The test shall be conducted with a mixture of equal volumes of the containers sampled.

The serum plate agglutination test shall be performed as the potency test. The test shall be made at room temperature of 20° - 25°C. The product under test and a known CRD antigen, which is in valid period, shall be dropped separately on the same glass plate in two drops by 1/1 needle. One drop of serum collected from at least three carrier chickens exhibiting

strong and weak positive reactions respectively, shall be added to drops of antigen under test and the control product already placed on the glass plate, and mixed immediately with a bent needle or a glass stick. The observation period of reaction shall be two minutes. The control test shall be performed in the same way with several specimen of healthy chicken sera.

VII - 5 - 2. Reading of Results

If the positive reaction, which represents a formation of violet coloured granules in the mixture of positive sera and the product under test and the control product in equal extent the product under test shall be acceptable.

At the same time, no positive reactions shall be confirmed in the mixtures of healthy sera and saline only with the product under test.

APPENDIX (THE MANUFACTURING STANDARD OF C R D ANTIGEN)

Appendix 1. The Ingredient of PPLO Broth

1. Basic Medium:	Soy Peptone (or Bacto-Peptone "Difco")	10 g
	Heart Infusion Broth	1 g
	NaCl	5 g
	Glucose	1 g
	Thalium Acetate	0.25 g
	Sodium Carbonate	0.8 g
	Distilled water	1,000 ml

2. After mixing ingredients, sterilized by autoclaving at 120°C for 15 minutes, cooled at 37°C.

3. Add horse serum at the rate of 10 per cent, penicillin 250 U/ml.

Appendix 2. The Ingredient of PPLO Agar

1.	PPLO Broth as Basic Medium (Appendix 1)	100 ml
	Bacto-Agar	1.5 g

2. After mixing ingredients, sterilized by autoclaving at 120°C for 15 minutes, cooled at 50°C.

3. Add horse serum at the rate of 10 per cent, penicillin 250 U/ml.

4. Immediately dispensed into Petri-dish each 15 ml of media.

Appendix 3. The Ingredient of Phosphate Buffered Saline

NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4	1.15 g
KH_2PO_4	0.20 g
Distilled Water	1,000 ml

Appendix 4. The Staining Procedure for Checking Purity

1. Prepare a smear and gently heat-fixed by flaming.
2. Flood the slide with methyl alcohol for 5 minutes.
3. Wash off thoroughly in tap water.
4. Stain by flooding slide with Giemsa stain for 30 minutes.
5. Rinse with tap water, air-dry, and examine under oil immersion.

Note: The stain is composed of one drop of stock solution per ml of buffered distilled water at pH 7.0. A fresh stain should be prepared each time and filtered before use.

Appendix 5. The Sampling Method of Specimens for Assay

The sampling from final containers in one lot shall be carried out with the following steps. Samples to be assayed should be collected in compliance with the number of bottles in one lot, as shown in the following Table. The test bottles are collected by means of a random sampling method.

No. of bottle in one lot	200 or less	201-300	301-400	401-500	501-600	601-700	701-800	801- or more
No. of bottle to be sampled	4	5	6	7	8	9	10	11

11 September 1966

K. Sugimura, FAO Veterinary Biologist

I. Yoshida, Poultry Disease Expert

APPENDIX VII. PRODUCTION AND ASSAY RECORD OF FOWL-POX VACCINE

Serial Lot No. 5

Production

		Sub lot 1	Sub lot 2	Sub lot 3
Prior inoculation	Date	Apr. 26	May 1	May 19
	No. of eggs	40	58	60
Inoculation	Date	May 6	May 11	May 30
	No. of eggs	30	52	49
Harvesting	Date	May 11	May 16	June 4
	No. of eggs	21	43	23
Sterility test	Date	May 11	May 16	June 4
	No.of dishes	4	8	4
	Results	- - - -	- - - -	+ + + +
			- - - -	
Homogenizing	Date	July 4		
	Total weight	61 g		
	Suspension	300 ml		
Dispensing	Date	July 4		
	No. of ampoules	600		
Freeze-drying	Date	July 4		
	No. of ampoules	600		
Sealing	Date	July 10		
	No. of ampoules	550		

Assay tests Judgement : Accepted

Property test	Date Results	July 10 Good
Vacuum test	Date Results	July 10 Good
Sterility test	Date No. of ampoules sampled Results	July 14 7 Agar slant - - - Bouillon - - - L.L.Bouillon - - -
Potency test	Date of inoculation Date of observation Results	July 14 July 18 2×10^6 / ml (PFU)
Pock-forming and safety tests	Date Observational period Results	July 14 3 weeks As follows

	Days after inoculation 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chicken 1	− + + + [++ ++ ++ ++ ++ ++ ++ ++ ++ ++ + + +] + + − −
Chicken 2	− + + + [++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ + +] + − − −
Chicken 3	− + + + [+ ++ ++ ++ ++ ++ ++ ++ ++ ++ + +] ± ± − − −

−, +, ++ and +++ : Degree of reaction

[ ] : Crust-formation

Three chickens did not exhibit any loss of general condition during observational period

APPENDIX VIII. PRODUCTION AND ASSAY RECORD OF CRD AGGLUTINATION ANTIGEN

Production

Lot No. 1, 2, 3, and 4

		Lot 1*	Lot 2*	Lot 3*	Lot 4
Medium used		"Eiken"	"Eiken"	Prepared in the Lab.	"Eiken"
Seed culture	Date	Mar. 6	Mar. 16	Mar. 26	June 1
	Amount (ml)	300	300	300	600
Production culture	Date	Mar. 9	Mar. 19	Mar. 29	June 5
	Amount (ml)	3,500	6,000	4,000	6,000
Inactivation	Date	Mar. 13	Mar. 23	Apr. 4	June 9
	Amount (ml)	3,500	6,000	4,000	6,000
Harvesting	Date	Mar. 15	Mar. 24	Apr. 5	June 11
	Amount (ml)	3,500	6,000	4,000	6,000
Adjustment of density and staining	Date	Mar. 15	Mar. 24	Apr. 5	June 12
	Amount (ml)	45	90	35	90
Harvesting rate** (%)		1.28	1.50	0.88	1.50

* Test production

** Amount of antigen / Amount of medium used

Assay tests

Lot No. 4

Judgement : Accepted

Date : June 20, 1966

Property test : Good

Purity test : Good

Potency test : As following TABLE

Serum		Antigen	Serum* plate test	Tube test**					
				1:5	1:10	1:20	1:40	1:80	1:160
High-titer	CRD imm. - 1	Lot 4	+++	+	++	++	++	+	+
		Control***	+++	++	+++	+++	++	++	+
	CRD imm. - 2	Lot 4	++	+	++	++	+	+	+
		Control	++	+	+++	+++	++	++	+
	No. 8	Lot 4	++	+	++	++	++	+	±
		Control	++	+	++	++	++	++	+
Low-titer	T - 6	Lot 4	++	+	+	+	±		
		Control	++	+	+	+	±		
	T - 8	Lot 4	++	+	+	+	-		
		Control	+	+	+	+	±		
	T - 12	Lot 4	+	+	+	±	-		
		Control	+	+	+	+	-		
Negative	T - 15	Lot 4	-	-	-	-			
		Control	-	-	-	-			
	T - 16	Lot 4	-	-	-	-			
		Control	-	-	-	-			
	T - 17	Lot 4	-	-	-	-			
		Control	-	-	-	-			

* +++ : Reacted at 30 seconds >, ++ : Reacted at 1 minute >,

+ : Reacted at 2 minutes >, - : Non reactor

** -, +, ++ and +++ : Degree of reaction

*** Japan 68

APPENDIX IX. LIST OF PRESENTED EQUIPMENT, INSTRUMENTS AND STRAINS OF MICROORGANISM

I. Refrigerated centrifuge — 1 set

Compact model S - 60

Made by TOMINAGA WORK LTD.

II. Heater (Part of speed clave) — 2 pcs.

III. Rubber stoppers for tissue culture

For test tube (Roller tube)	1,000 pcs.
For test tube (Ordinary tube)	1,000 pcs.
For Roux bottle	50 pcs.

IV. Strains of microorganism

Salmonella pullorum	Nakamura (standard)
	9-25 (standard)
	L-60131 (variant)
Mycoplasma gallisepticum	KP-13
Haemophilus gallinarum	221
Fowl-pox virus	Nishigahara
Pigeon-pox virus (avianized)	Nakano
Infectious laryngotracheitis virus	NS-175
Infectious bronchitis	KH-T44
Newcastle disease virus	Ishii (avirulent)
Avian adeno (Celo) virus	Ote
Avian Reo virus	Uchida