charles river | avian vaccine services

Technical Sheet

Technical Guidelines - SPF Eggs

Thank you for purchasing SPF eggs from Charles River Avian Vaccine Services. We have prepared these technical guidelines to assist our customers. The guidelines apply to all applications of these eggs, whether you are using them for developmental studies, vaccine production in allantoic fluid or hatching them for QC testing in birds. This information may be a review for some, but we hope it is helpful to all in maximizing your experience with Charles River Avian Vaccine Services.

History

We have been producing SPF eggs for over fifty years. In 1961, Specific Pathogen Free Avian Supplies, or SPAFAS, Inc. – a small specific pathogen free (SPF) egg production and testing laboratory – was founded by Ray Davis, a Connecticut hatcheryman, and Dr. Roy Luginbuhl, Ph.D., from the University of Connecticut. From a small number of flocks, SPAFAS grew its range of products and services to include egg production in Roanoke, Illinois and testing services in Connecticut. The business continued to grow with joint ventures around the globe, expanding into avian diagnostic reagents and more testing services. In 1992, SPAFAS was acquired by Charles River and is now known as Charles River Avian Vaccine Services.

Testing Program/Quality Assurance

At Charles River Avian Vaccine Services, our quality program is designed to assure that our SPF eggs meet or exceed customer expectations and that our quality initiatives and goals are upheld. A quality manual is maintained and is designed to comply with corporate policies that include, but are not limited to, quality system audits, change control, customer complaint handling, documentation programs, calibration/validation, CAPA, and production and process controls. The format of the manual was designed to be similar to GMP's 21 CFR Part 820. The quality system is reviewed (along with the status of CAPA and quality goals) with senior management at defined intervals. Flock rearing and testing is performed per PhEur 5.2.2 and USDA memorandum 800.65 using validated test methods. This means weekly testing of every flock starting at four weeks of age. Additionally, weekly fertility and viability testing is conducted on every flock to ensure the highest quality SPF egg.

Which Egg Quality Level is Right for you?

Our SPF white leghorn chicken flocks are tested for over 30 specific pathogens as per USDA and PhEur requirements. Flocks that are clean for all specific pathogens except for chicken anemia virus (CAV) are acceptable, per USDA requirements, and are designated as the flocks for our Premium SPF eggs. Flocks that are also CAV negative, meeting PhEur requirements, are our Premium Plus quality eggs and these are used for CAV work and for shipments to Europe and/or for the production of any material that may be registered in Europe. We also have a genetic line which is C/O and not resistant to any of the Avian Leukosis Virus subgroups, which is important for Leukosis studies. These CAV-negative and endogenous group specific antigen free eggs are our highest quality and are designated as our Research quality SPF eggs. The following chart, Table 1, lists which pathogens we test for and which test method(s) we use in determining our SPF egg quality grades.

106 Route 32 North Franklin, CT 06254
Tel: +1 (860) 889-1389 • Fax: +1 (860) 889-1991
Toll free: +1 (800) 772-3271 • Email: csdspf@crl.com
www.criver.com

Egg Quality Comparison Guide (Table 1)

Agent	Antigen	Test	Premium	Premium Plus	Research
Avian Adenovirus Group I	CELO-Phelps	AGP	X	X	X
Avian Adenovirus Group II (HEV)	Domermuth	MFIA	X	X	X
Avian Adenovirus Group III (EDS)	CLKK115D	HI	X	X	X
Avian Encephalomyelitis	van Roekel	MFIA	X	X	X
Avian Influenza (Type A)	T/W/66	MFIA, AGP	X	X	X
Avian Nephritis Virus	G4260	MFIA	X	X	X
Avian Paramyxovirus Type 2	Yucaipa	MFIA	X	X	X
Avian Reovirus	S1133	AGP, MFIA, IFA	X	X	X
Avian Rhinotracheitis Virus	UK	ELISA	X	X	X
Avian Rotavirus	Ch-2	AGP	X	X	X
Avian Tuberculosis	M. avium	CO, PM	X	X	X
Chicken Anemia Virus	DelRose	IFA		X	X
Endogenous GS Antigen	p27	ELISA			X
Fowl Pox	Conn	MFIA, CO	X	X	X
Hemophilus paragallinarum	Serovars A,B,C	CO	X	X	X
Infectious Bronchitis - Ark.	Ark 99	MFIA	X	X	X
Infectious Bronchitis - Conn.	Conn A5968	MFIA	X	X	X
Infectious Bronchitis - JMK	JMK	MFIA	X	X	X
Infectious Bronchitis - Mass.	Mass 66579	MFIA	X	X	X
Infectious Bursal Disease Type 1	M4040(2512)	MFIA, AGP	X	X	X
Infectious Bursal Disease Type 2	M4040(2512)	AGP	X	X	X
Infectious Laryngotracheitis	UC A92430	MFIA, AGP	X	X	X
Lymphoid Leukosis A, B	RSV-RAV A,B	MFIA	X	X	X
Avian Lymphoid Leukosis Virus J (ALV J)	Hc-1	MFIA	X	X	X
Lymphoid Leukosis Viruses	A,B,C,D,E,J	ELISA	X	X	X
Marek's Disease (Serotypes 1,2, 3)	SB-1	AGP	X	X	X
Mycoplasma gallisepticum	A5969	SPA	X	X	X
Mycoplasma synoviae	WVU 1853	SPA	X	X	X
Newcastle Disease	LaSota	MFIA	X	X	X
Reticuloendotheliosis Virus	ATCC 770 T	IFA, AGP	X	X	X
Salmonella pullorum-gallinarum	K Polyvalent	SPA	X	X	X
Salmonella species (Every 4 weeks)		IA	X	X	X

Test abbreviation key:

AGP=Agar Gel Precipitin
MFIA=Multiplexed Fluorometric Immunoassay
HI=Hemagglutination Inhibition
IFA=Indirect Fluorescent Antibody
ELISA=Enzyme-Linked Immunosorbent Assay
SPA=Serum Plate Agglutination
CO=Clinical Observation
PM=Postmortem
IA=Isolation of Agent

Collection and disinfection

Charles River collects eggs regularly throughout each day, 365 days a year. Eggs are sanitized, inspected and stamped with the flock ID shortly after collection. The eggs are then transported to regional egg processing centers where they are candled for cracks, sorted by weight and visually inspected one egg at a time. Unless pre-incubated eggs are requested, the eggs you receive are at day 0 in the incubation process.

Pre-Incubated egg orders

Pre-incubated eggs, 1 to 17 days of age, are available to customers along the Eastern Seaboard of the United States and in select areas of Europe. All pre-incubated eggs are set specifically for each order, so it is important to request shipments in advance. Once the eggs are set, cancellations are not accepted. Prior to shipment, pre-incubated eggs are candled to remove infertile eggs and dead embryos. Pre-incubated eggs ship in specially designed heated vehicles for optimal delivery conditions.

Packing

Eggs are placed in new cardboard egg flats and are packed in custom egg cases designed for optimum handling and shipping (both domestically and internationally). We have two TSA-certified screening facilities where our eggs are held in temperature-controlled rooms that meet or exceed TSA security screening standards. This secure chain of custody allows for smoother domestic and international air freight shipments. This eliminates or reduces the need for further TSA inspection of the eggs, ensuring safe and secure transit from our facility to yours.

Shipping

We ship our SPF eggs worldwide from three locations (CT, USA; IL, USA; and Hungary). Eggs are shipped domestically using overnight freight companies, and we have a contracted freight company that delivers on the East Coast using temperature-controlled vehicles. Eggs are shipped overnight where possible. It is important that the eggs are handled properly from our door to yours. Freight forwarders and loading dock personnel should be instructed to process the eggs quickly and to keep eggs away from extreme temperatures. Please notify our customer service immediately if the shipping container and or eggs are damaged.

Receipt and Storage

Upon receipt, inspect the shipping container for damage. For best results, set the eggs on the day of receipt, but first let them sit for at least 6–8 hours at room temperature prior to setting. By setting on the day of receipt, you can expect 85–90% viability and we guarantee 85% fertility.

For storage, keep the eggs at 55–70°F (13–21°C) at 70–80% relative humidity. Under these conditions, you can expect 80–85% viability when setting 3–5 days post receipt. For each additional storage day, expect a 3–5% drop in viability (with a maximum suggested holding period of 7 days). DO NOT PUT THE EGGS IN A REFRIGERATOR AND DO KEEP THEM AWAY FROM HEAT SOURCES. Fertile eggs that are kept above 70°F (21°C) for an extended period of time may begin to develop, resulting in embryo mortality and or stunting.

Incubation

For best results, follow cleaning and operational guidelines provided by your incubator manufacturer. Regular thermometer and humidity calibrations are encouraged.

After storage, stage your eggs prior to setting by warming to 72–82°F (22–28°C) for a minimum of 6–8 hours, or overnight if possible. This will prevent a quick temperature change that may cause condensation on the eggs, which could lead to bacterial growth. Disinfecting of eggs with 70% isopropyl alcohol (IPA) or a similar disinfectant prior to incubation is recommended.

Proper temperature and humidity levels in the incubator are important to healthy embryo development (Table 2). Incubators should be calibrated for proper temperature and humidity on a regular basis (at least once a year), and regular cleaning and disinfection is needed. An incubator contaminated with bacteria or fungus can cause severe problems with egg viability, hatching and/or affect the quality of harvested allantoic fluid. The water line and/or water pan should be cleaned and tested regularly, ensuring that no bacterial contamination is present. Proper air flow around the egg, as well as regular turning (at least every four hours), is also required for optimal development. It is recommended to candle the eggs prior to use to ascertain their general health.

Suggested Incubation Temperatures and Humidity (Table 2).

Consult your incubator manual for more specific information on proper incubation.

Days Incubated	Temp Set Point/Range (°F)	Temp Set Point/Range (°C)	Humidity Set Point (RH)	Humidity Set Point (Wet Bulb °F)	Comments
0–transfer/ (day 16–18)	99.5 +/- 0.5	37.5 +/- 0.3	48–55	80–85	Try 55–65% RH for allantoic fluid virus preps
Transfer–18	99.5 +/- 0.5	37.5 +/- 0.3	52–58	84–86	
19–20	99.0 +/- 0.5	37.2 +/- 0.3	55–62	84–88	
21	98.5 +/- 0.5	36.9 +/- 0.3	56–63	84–88	
Day of hatch	98.0 +/- 0.5	36.7 +/- 0.3	Off	Off	

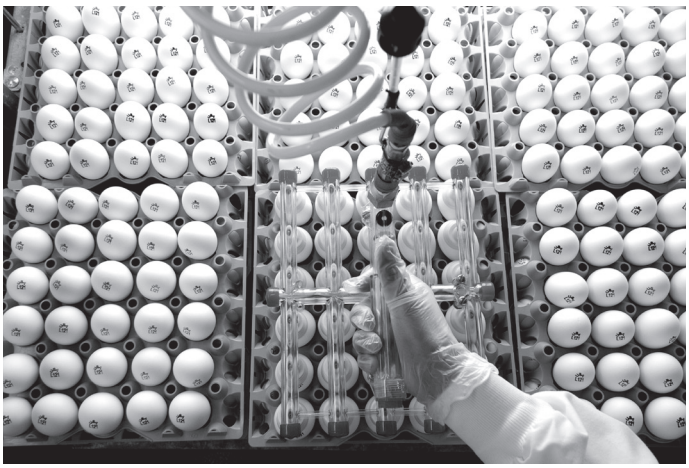
Egg Incubation Troubleshooting Chart

Symptom	Probable Cause	Solution
Clear Eggs/no blood	Infertility	Check flock
	Poor handling/storage	Check shipping and or storage conditions
Early dead (0–4 days)	Temperature	Calibrate incubator temperature
	Turning	Check turning frequency
	Ventilation	Check airflow/air exchange
Pipped eggs not hatching	Humidity	Check humidity levels
Blood rings	Temperature	Calibrate incubator temperature
	Fumigation	Air out incubator after fumigation
	Poor handling/storage	Check storage room conditions

Contact info

Please contact us with questions about our SPF eggs and/or other products and services at:

Charles River Avian Vaccine Services
Phone: 1-800-772-3271 (USA toll-free) or 1-860-889-1389
Fax: 1-860-889-1991
E-mail: csdspf@crl.com
Address: 106 Route 32, North Franklin, CT 06254, USA



charles river | avian vaccine services

106 Route 32 North Franklin, CT 06254
Tel: +1 (860) 889-1389 • Fax: +1 (860) 889-1991
Toll free: +1 (800) 772-3271 • Email: csdspf@crl.com
www.criver.com