Agrivet Research & Advisory Private Limited
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CIN: U01403WB2012PTC184502

PCR Sample Dispatch Procedures

Guidance for veterinarians and field personnel — poultry molecular diagnostics

The quality of PCR-based diagnosis is determined principally by the quality of the sample received. The following guidance specifies the sample type, quantity, container, transport condition and acceptable transit time for samples submitted to the Agrivet Central Laboratory. Field veterinarians and submitting clinicians are requested to follow these procedures strictly. Samples that do not meet the criteria below may be rejected at the receiving counter; the reason for rejection will be communicated to the submitting party.

1. Sample specification, container and transport

Sample	Specification	Quantity per sample	Collected in	Transport condition	Maximum transit time to the lab
Whole blood	Anticoagulated blood without clot or haemolysis	1–2 ml	EDTA (K2 / K3) vacutainer or vial	Ice gel pack (cold chain)	24 hours
Faeces	Fresh collection only; no urates or litter contamination	500 mg – 1 g	Sterile 2–5 ml plastic vial or sealed zip-lock pouch	Frozen ice gel pack (cold chain)	24 hours
Tissue / organ	Freshly collected; no signs of decomposition or putrefaction	≈500 mg in 1–2 ml RNAlater, *or* 5–10 g in 10–20 ml sterile glycerol PBS (pH 7.4)	Sterile 2 ml microcentrifuge tube (Tarsons or equivalent) for RNAlater; sterile screw-cap container for glycerol PBS	Frozen ice gel pack (cold chain)	24–48 hours
Swab (tracheal / cloacal / choanal)	Paired or pooled per bird as indicated	Minimum 10 swabs per pooled sample	Sterile cotton-tipped swab preserved in viral transport medium (HiViral™ Transport Medium, HiMedia AL167 — or equivalent)	Frozen ice gel pack (cold chain)	48–72 hours
Impression smear	Impression from a freshly cut surface of the target organ, tissue or blood	4 impression circles per sample	FTA® Classic Card (air-dried, see Section 6)	Sealed in zip-lock pouch with silica desiccant; courier at ambient temperature	3–7 days

Notes:

- Always include a frozen ice gel pack in an insulated box (thermocol or equivalent) for samples requiring cold chain. Direct contact between samples and the ice pack should be avoided by using bubble wrap.
- Samples must be packed in leak-proof primary containers, placed inside a sealed secondary plastic bag with absorbent material, and an outer rigid container labelled with the biohazard symbol and the sender's contact details.
- Whole blood must not be frozen. If dispatch is delayed beyond 48 hrs, plasma should be separated & frozen.

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2. Sample identification and submission

Each sample container must be individually labelled — using a water-resistant marker for vials and tubes and pencil only for FTA cards — with the following minimum information:

- Farm / flock identification and house or shed number
- Bird identification (band number or pooled sample reference)
- Species, breed, age in days, sex (where relevant)
- Date and time of collection
- Suspected disease(s) and tissue / organ source
- Name and contact number of collecting veterinarian

A duly completed ARAPL **Sample Submission Requisition Form** must accompany the consignment.

3. Blood sample collection

- Use a commercially available EDTA (K2 or K3) vacutainer or anticoagulant vial of the correct draw volume.
- Select clinically affected birds — preferably febrile or showing early signs of disease — for representative diagnosis.
- Restrain the bird and disinfect the wing (brachial) vein site with 70 % isopropyl alcohol; allow to air-dry.
- Aspirate blood using a sterile 2 ml or 5 ml disposable syringe with a 21–23 G needle; transfer to the EDTA vial up to the marked fill line. Avoid frothing.
- Invert the vial gently 6–8 times immediately after filling to mix the anticoagulant. Do not shake.
- Store and transport at 2–8 °C with cold chain maintained. Do not freeze whole blood.
- Whole blood may also be applied to FTA Classic Cards as an alternative for selected pathogens; apply a few drops to each application circle and air-dry.

4. Swab sample collection

- Use sterile, individually packed cotton or polyester-tipped swabs (HiMedia or equivalent).
- Insert the swab gently into the anatomical site (trachea, cloaca, choanal cleft or sinus, as specified in Sections 7 and 8). Avoid contact with the surrounding skin or feathers.
- Rotate the swab against the mucosal surface for 5–10 seconds to obtain adequate cellular material.
- Immediately immerse the swab in 2–3 ml of viral transport medium (HiViral™ Transport Medium, HiMedia AL167, or equivalent); break or cut off the upper portion of the shaft and tightly close the tube.
- Store and transport with frozen ice gel pack to maintain cold chain. Up to 10 swabs from epidemiologically related birds may be pooled in a single VTM tube.

5. Organ and tissue sample collection

- Sample from clinically affected live birds (after humane euthanasia) or from freshly dead birds without any sign of decomposition. Do not submit autolysed carcasses.
- Use sterile gloves, scissors and forceps. Change instruments between birds and between organs to avoid cross-contamination.
- Collect a representative piece showing both grossly affected and adjacent apparently normal tissue.
- Place approximately 500 mg of tissue in a sterile 2 ml microcentrifuge tube containing 1–2 ml RNAlater *or* 5–10 g of tissue in 10–20 ml sterile glycerol phosphate-buffered saline (pH 7.4) in a sterile screw-cap container.
- Label the container clearly (see Section 2) and store at 2–8 °C until dispatch. Use ice gel packs in transit; use dry ice if the sample is frozen.

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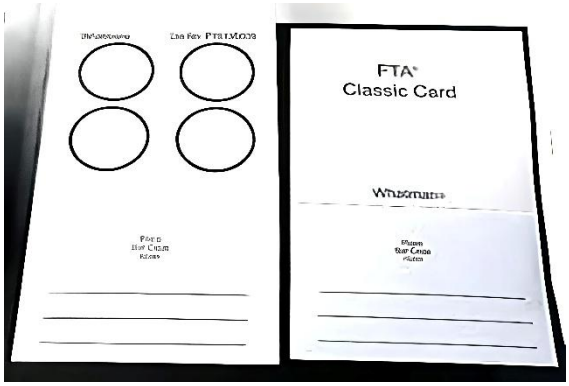
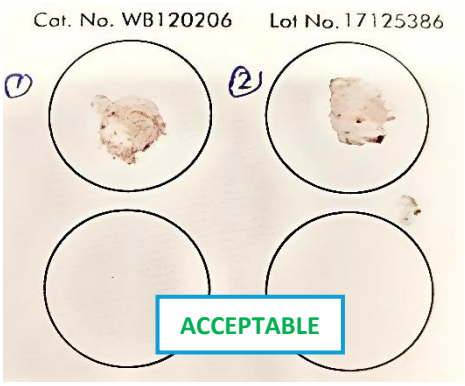
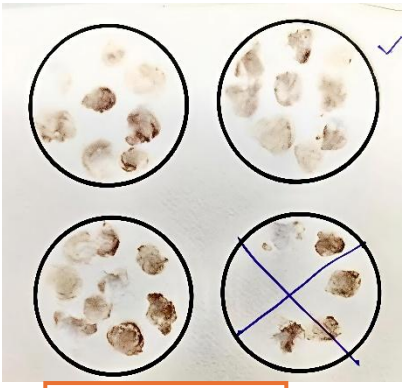
6. Impression smear on FTA card

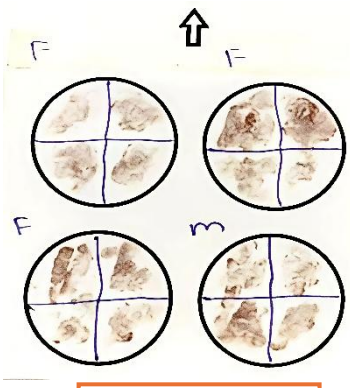
- Use freshly collected tissue from clinically affected or freshly euthanised birds; do not use autolysed tissue.
- Cut the organ to expose the mucosal or internal surface.
- Using a sterile scalpel and forceps, lift a small piece of tissue and press the cut surface gently onto each application circle of the FTA Classic Card. Apply four separate impressions per sample.
- For trachea: harvest the entire trachea, slit lengthwise, and scrape the mucosal surface with a sterile scalpel blade. Apply the scrapings to the FTA card — this yields a higher target load for respiratory pathogen detection.
- Allow the impressions to air-dry on the card for at least 30 minutes in a clean, shaded environment. Avoid direct sunlight, high humidity and heat.
- Label the FTA card and each application circle using a pencil only. Ballpoint, marker or gel ink inhibit downstream PCR.
- Place the dried card in a zip-lock pouch with a silica gel sachet, then in a secondary envelope, and dispatch by courier at ambient temperature.







NOT ACCEPTABLE



NOT ACCEPTABLE

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7. Sampling guide — viral diseases

The following table indicates the preferred tissue or organ, swab type, and FTA-card impression source for each viral disease of poultry routinely tested by PCR at ARAPL. "—" indicates that the sample type is not recommended for that disease.

Suspected viral disease	Tissue / organ / faeces	Swab	FTA card impression
Newcastle disease (ND / Ranikhet)	Trachea, lungs, kidney, caecal tonsil, faeces	Tracheal, cloacal	Trachea, caecal tonsil, spleen, kidney
Infectious bronchitis (IB)	Trachea, lungs, kidney	Tracheal, cloacal	Trachea, kidney, cloacal content impression
Infectious laryngotracheitis (ILT)	Trachea, lungs	Tracheal	Trachea, eyelid scraping, lungs
Avian influenza (AI)	Trachea, lungs, kidney, pancreas, faeces	Tracheal, cloacal	Trachea, lungs, gut scraping, faeces
Infectious bursal disease (IBD / Gumboro)	Bursa of Fabricius	Bursa of Fabricius	Bursa of Fabricius
Avian metapneumovirus (aMPV) / Swollen head syndrome	—	Choanal cleft / oro-pharyngeal swab / infraorbital sinus exudate	Choanal cleft / oro-pharyngeal / infraorbital sinus exudate impression
Inclusion body hepatitis (IBH)	Liver, kidney	—	Liver, kidney
Avian nephritis virus / Astrovirus	Kidney, gut content	Gut content	Kidney
Chicken infectious anaemia (CAV)	Thymus, bone marrow, spleen	—	Thymus, bone marrow, spleen
Marek's disease (MD)	Spleen, tumour-bearing organs	—	Spleen, feather follicle
Avian leukosis virus (ALV)	Liver, spleen	_	Liver, spleen
Reticuloendotheliosis virus (REV)	Spleen, thymus	—	Spleen, thymus
Avian reovirus	Pancreas, proventriculus, intestinal content	Hock joint synovial fluid; intestinal mucosal (jejunal + ileal) swab	Pancreas, hock joint exudate, intestinal mucosal scraping
Avian encephalomyelitis (AE)	Brain	—	Brain impression
Egg drop syndrome (EDS)	Reproductive tract (uterus, oviduct); shell-less egg	Cloacal swab, oviduct swab	Cloacal and oviduct content impression
Fowl adenovirus (FAdV)	Liver, kidney, caecal tonsils	—	Liver, kidney, caecal tonsils
Fowl pox	Skin lesions, trachea, epithelial scrapings	Lesion site	Skin lesion, trachea, epithelial cell impression

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8. Sampling guide — bacterial diseases

The following table summarises sample preferences for the major bacterial pathogens screened by PCR. Where species-level differentiation is required (for example Mycoplasma gallisepticum vs. M. synoviae, or Salmonella serovar typing), please indicate this on the requisition form.

Suspected bacterial disease	Tissue / organ / faeces	Swab	FTA card impression
Mycoplasmosis (*Mycoplasma gallisepticum*, *M. synoviae*)	Trachea, lungs, sinus exudate; joint exudate for M. synoviae	Tracheal, choanal	Tracheal, choanal; semen smear in breeder males
Salmonellosis (*Salmonella* spp.)	Caecal content, liver, spleen	Cloacal swab	Liver, cloacal and caecal content impression
Necrotic enteritis (*Clostridium perfringens*)	Liver, intestinal content (jejunum, ileum)	Intestinal mucosal (jejunal + ileal) swab	Liver and intestinal mucosal scraping impression
Staphylococcosis (*Staphylococcus aureus*)	Affected joint / synovial tissue, liver in septicaemia	Bumble-foot or hock joint synovial fluid swab	Bumble-foot and hock joint synovial fluid impression
Enterococcal spondylitis (*Enterococcus cecorum*)	Liver, spleen, spinal abscess material	Hock joint synovial fluid swab, spinal abscess swab	Hock joint synovial fluid impression, spinal abscess impression
Fowl cholera (*Pasteurella multocida*)	Liver, lungs, spleen, heart	Lesion site	Liver, lungs, spleen, heart impression
Infectious coryza (*Avibacterium paragallinarum*)	Upper trachea, nasal turbinate	Infraorbital sinus exudate, choanal cleft, nasal swab	Infraorbital sinus exudate, choanal cleft, nasal discharge impression
Ornithobacteriosis (*Ornithobacterium rhinotracheale*, ORT)	Trachea, lungs	Infraorbital sinus exudate, choanal cleft, tracheal swab	Infraorbital sinus exudate, choanal cleft, tracheal mucosal scraping impression
Colibacillosis (*Escherichia coli*, APEC)	Pericardium, perihepatic exudate, air sac lesions, yolk sac in chicks	Lesion site	Pericardial, hepatic, air sac lesion impression

9. Sample rejection criteria

Samples meeting any of the following conditions will be rejected at the receiving counter. The submitting party will be notified and a fresh sample requested, where feasible.

- Inadequate or missing labelling, or absence of the Sample Submission Requisition Form.
- Visibly autolysed, decomposed or putrefied tissue.
- Whole blood received clotted, haemolysed or frozen.
- Container leakage, breakage or contamination of secondary packaging.
- Cold-chain failure — samples received warm when cold chain was required, or beyond the maximum permitted transit time in Section 1.

- Volume or weight below the minimum specified in Section 1.
- FTA cards received wet, soiled, or labelled in ink that interferes with PCR.

10. Sample dispatch and laboratory contact

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- *Sample receiving hours: Monday to Saturday, 10:00 to 18:00 IST.*
- *Samples received outside these hours will be logged in on the next working day*
- *For emergency dispatches please call the mobile number above in advance.*

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