

**FOR INTERNAL USE ONLY**

**innovax<sup>®</sup>**

**&  VIRAL  
FLEX-SEQ<sup>®</sup>**

***SUGGESTED SAMPLING  
APPROACHES***

***ALWAYS PRODUCING MORE***

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 **MERCK**  
Animal Health

# INNOVAX<sup>®</sup>-ND-IBD

In addition to bird uniformity, weight gain, FCR, days to market and other relevant performance parameters for your market, Viral Flex-Seq<sup>®</sup> can be used as an important differentiating tool for field evaluations of Innovax<sup>®</sup>-ND-IBD with your customers.

This protocol is meant to capture IBD virus population changes over time while using Innovax<sup>®</sup>-ND-IBD and to confirm proper product handling and application at the hatchery.

## STEP 1: ESTABLISH A BASELINE OF FIELD VIRUS

It can be useful to capture baseline field virus identity prior to the use of Innovax<sup>®</sup>-ND-IBD so that we can evaluate how this population changes over time. Don't forget to take photos of any mortality, bursal size comparisons and performance information and include these in your app submission.

<b>SAMPLE TYPE:</b>	Bursal smear
<b>SAMPLING AGE:</b>	Peak shedding: 18 days of age if age at challenge is unknown
<b>SAMPLE NUMBER:</b>	5 samples per house (minimum)
<b>SAMPLING FREQUENCY:</b>	Initial determination of company challenge: 1 sampling per house on 4 different farms over 1 month

\*Any unused circles on the FTA card should be crossed out to demarcate that there is no sample to analyze in these circles.

\*\* When collecting bursas for conducting smears, please delicately divide the bursa in half and place one half of the bursa in formalin if future examination by histopathology is desired. Please do not exceed 1:10 ratio of tissue to formalin and label jars clearly to allow pairing with sample submissions.

\*\*\* If virus isolation is desired, the ½ bursa that was used to smear on the FTA card may be labeled, frozen and retained.

\*\*\*\* Note, in conventionally housed laying hens, 5 samples may be submitted per cage row if there are any known differences in bird vaccination history or bird source.

## STEP 2: VACCINE TAKE VERIFICATION

Vaccine take verification has the benefit of not only confirming accurate vaccination, but also of confirming that indeed Innovax<sup>®</sup>-ND-IBD was used and not a competitor's product.

- A)** Once customers have decided to start using Innovax<sup>®</sup>-ND-IBD, complete a hatchery audit with the Convenience Evaluation to ensure proper vaccine handling and application.
- B)** Next, decide how many hatcheries you would like to evaluate. It is recommended that a minimum of one sample set is taken per hatchery being evaluated. A sample set is made up of 20 individual bird samples (1 bird per circle on FTA card = 5 FTA cards per hatchery submission). Alternatively, if we want to compare hatchery shifts, hatch days or different hatcheries within a company, a sample set of 20 individual bird samples (1 submission of 5 FTA cards) could be collected from each one of these as well.

<b>SAMPLE TYPE:</b>	Feather Pulp (10 axillary feathers) <b>or</b> Smear of Spleen
<b>SAMPLING AGE:</b>	15 days post-administration (feather pulp) <b>or</b> 8 days post-administration (spleen)
<b>SAMPLE NUMBER:</b>	20 samples per submission (5 FTA cards)
<b>SAMPLING FREQUENCY:</b>	• 1x per month per hatchery until they reach green status • Quarterly routine monitoring for loyal Innovax <sup>®</sup> -ND-IBD users, if desired

## **STEP 2 ctd...**

**PRO TIP 1:** To make sample collection easier, use 1 Whirl-pak bag (or Ziploc bag) to hold the feathers of each bird sampled as you move through the house. Once you have finished sampling 20 birds, simply bring your 20 bags into the anteroom of the house or to a clean area to transfer pulp to the FTA cards.

**PRO TIP 2:** Use the shaft of one sterile swab to push the feather pulp from the 10 feathers of one bird onto an FTA card circle. Discard the swab and use a fresh sterile swab for each bird sampled to prevent cross-contamination.

## **STEP 3: QUARTERLY FIELD VIRUS EVALUATION**

Regular quarterly sampling of the same house(s) as initially sampled for determination of baseline. Additional sampling may be conducted if desired. Don't forget to take photos of any mortality, bursal size comparisons and performance information and include these in your app submission.

**SAMPLE TYPE:** Bursal smear

**SAMPLING AGE:** Peak shedding: 18 days of age if age at challenge is unknown

**SAMPLE NUMBER:** 5 samples per house

**SAMPLING FREQUENCY:** 1 sampling per house on 4 farms per quarter (minimum)

\*Any unused circles on the FTA card should be crossed out to demarcate that there is no sample to analyze in these circles.

\*\* When collecting bursas for conducting smears, please delicately divide the bursa in half and place one half of the bursa in formalin if future examination by histopathology is desired. Please do not exceed 1:10 ratio of tissue to formalin and label jars clearly to allow pairing with sample submissions.

\*\*\* If virus isolation is desired, the ½ bursa that was used to smear on the FTA card may be labelled, frozen and retained.

\*\*\*\* Note, in conventionally housed laying hens, 5 samples may be submitted per cage row if there are any known differences in bird vaccination history or bird source.

## **STEP 4: CORRELATION OF PERFORMANCE RESULTS WITH VIRAL FLEX-SEQ® RESULTS**

One of the most important parts of this exercise is to correlate what we see in terms of virus population changes with performance. Bringing information on predicted changes in performance to our customers BEFORE they happen is our goal. We will only be able to establish these types of trends if we take the time to correlate our survey results with bird performance.

Compile your captured data from each collection time point (found on app), as well as additional data provided by your customer, and look for trends. The greater number of submissions, the better we understand our product's behavior in the field, and the more information we can feed back to our customers. In addition, showing the customer how poor application at the hatchery can result in suboptimal performance in the field, particularly when it costs them money, encourages them to strive for excellence in hatchery vaccine application.

# INNOVAX®-ILT

This protocol is meant to be used to capture ILT virus population changes over time while using Innovax®-ILT and to confirm proper product handling and application at the hatchery.

## STEP 1: ESTABLISH A BASELINE OF FIELD VIRUS

It can be useful to capture baseline field virus identity prior to the use of Innovax®-ILT so that we can evaluate how this population changes over time. Don't forget to take photos of any mortality charts, water intake, any field necropsies & reports and include these in your app submission.

- SAMPLE TYPE:** Tracheal smear or oropharyngeal swab
- SAMPLING AGE:** Peak shedding, during outbreak, during stressful period (35-40 days for broilers; at peak lay for hens) or anytime for routine surveillance.
- SAMPLE NUMBER:** 5 samples per barn, most severely affected area of barn\*
- SAMPLING FREQUENCY:**
- Sites in Transition: 1x per month per affected site until reach green status for viral load for sites in transition with Innovax®-ILT
  - Initial determination of company challenge: 1 Sampling per barn on 4 different farms over 1 month
  - Routine Surveillance: 1 Sampling per barn on 4 farms per quarter

\*Note, in conventionally housed hens, 5 samples may be submitted per cage row if there are any known differences in bird vaccination history or bird source.

\*\* Any unused circles on the FTA card should be crossed out to demarcate that there is no sample to analyze in these circles.

\*\*\* If further characterization of the virus is desired for unique local ILT challenge viruses, please see protocol for sample collection and submission to CDS Boxmeer.

\*\*\*\* If troubleshooting an Innovax ILT case, it is strongly recommended that Field Virus Evaluation is coupled with Vaccine Take Verification sampling to confirm the use of Innovax (and not competitor products) and to score vaccination at the hatchery in question.

## STEP 2: VACCINE TAKE VERIFICATION

Vaccine take verification has the benefit of not only confirming accurate vaccination, but also of confirming that indeed Innovax®-ILT was used and not a competitor's product.

- A)** Once customers have decided to start using Innovax®-ILT, complete a hatchery audit with the Convenience Evaluation to ensure proper vaccine handling and application.
- B)** Next, decide how many hatcheries you would like to evaluate. It is recommended that a minimum of one sample set is taken per hatchery being evaluated. A sample set is made up of 20 individual bird samples (1 bird per circle on FTA card = 5 FTA cards per hatchery submission). Alternatively, if we want to compare hatchery shifts, hatch days or different hatcheries within a company, a sample set of 20 individual bird samples (1 submission of 5 FTA cards) could be collected from each one of these as well.

- SAMPLE TYPE:** Feather Pulp (10 axillary feathers) **or** Smear of Spleen
- SAMPLING AGE:** 15 days post-administration (feather pulp) **or** 8 days post-administration (spleen)
- SAMPLE NUMBER:** 20 samples per submission (5 FTA cards)
- SAMPLING FREQUENCY:**
- 1x per month per hatchery until they reach green status
  - Quarterly routine monitoring for loyal Innovax®-ILT users, if desired

## **STEP 2 ctd...**

**PRO TIP 1:** To make sample collection easier, use 1 Whirl-pak bag (or Ziploc bag) to hold the feathers of each bird sampled as you move through the house. Once you have finished sampling 20 birds, simply bring your 20 bags into the anteroom of the house or to a clean area to transfer pulp to the FTA cards.

**PRO TIP 2:** Use the shaft of one sterile swab to push the feather pulp from the 10 feathers of one bird onto an FTA card circle. Discard the swab and use a fresh sterile swab for each bird sampled to prevent cross-contamination.

## **STEP 3: QUARTERLY FIELD VIRUS EVALUATION**

Regular quarterly sampling of the same house(s) as initially sampled for determination of baseline. Additional sampling may be conducted if desired. Don't forget to take photos of any mortality and performance information and include these in your app submission.

**SAMPLE TYPE:** Tracheal Smear or Oropharyngeal Swab

**SAMPLE NUMBER:** 5 samples per barn

**SAMPLING FREQUENCY:**

- Sites in Transition: 1x per month per affected site until reach green status for viral load for sites in transition with Innovax®-ILT
- Routine Surveillance: 1 Sampling per barn on 4 farms per quarter

\*Note, in conventionally housed hens, 5 samples may be submitted per cage row if there is any known differences in bird vaccination history or bird source.

\*\* Any unused circles on the FTA card should be crossed out to demarcate that there is no sample to analyze in these circles.

\*\*\* If further characterization of the virus is desired for unique local ILT challenge viruses, please see protocol for sample collection and submission to CDS Boxmeer.

## **STEP 4: CORRELATION OF PERFORMANCE RESULTS WITH VIRAL FLEX-SEQ® RESULTS**

One of the most important things that we can give our customers is peace of mind. Bringing information on accuracy of hatchery vaccine application, providing them with information on predicted changes in virus populations and advising them on vaccination program makes us a trusted partner.

Compile your captured data from each collection time point (found on app), as well as additional data provided by your customer, and look for trends. The greater number of submissions, the better we understand our product's behaviour in the field, and the more information we can feed back to our customers. In addition, showing the customer how poor application at the hatchery can result in suboptimal performance in the field, particularly when it costs them money, encourages them to strive for excellence in hatchery vaccine application.

# SUBMITTING IBDV ISOLATES TO CDS

In certain special cases, there may be an interest in collecting IBDV isolates from the field for use in future internal challenge trials. Please discuss with Global Poultry Technical directors the suitability of your submission prior to shipment to CDS Boxmeer

## SAMPLING FOR VIRUS ISOLATION

**When:** In acute phase of infection.

**What:** Bursa material of 5 clinical affected animals.

**Transport:** Frozen.

## IDENTIFICATION OF THE ISOLATES

- Submit at least the following information about the isolates:
  - Date of isolation
  - Type of bird
  - If applicable: vaccination schedule
  - Clinical Signs
  - Place of isolation
  - Age of bird

Note: this information can be written on the submission form

## PAPERWORK FOR SHIPMENT

- See the CDS intranet page for the latest information.  
(<https://collaboration.merck.com/sites/SyncPub/ah/GloFun/AHRDBio/RDBox/RDSerLab/Pages/default.aspx>)
- Add a filled submission form inside the package (make sure this form is not in direct contact with the samples itself)
- Add a filled commercial document outside the package
- Address to ship to:  
**MSD Animal Health  
R&D Service Lab PP063 (Portiersloge)  
Exportstraat 39c  
5831 AK Boxmeer  
The Netherlands**

## SHIPMENT FROM OUTSIDE EU

- Besides the items mentioned before please make sure that you:
  - Send an e-mail (at least two days before the samples will be shipped) to:  
*DistributionCoordination.inb@merck.com* (cc *R&D Service Lab Sample Processing*)
  - Mention in the subject line of this e-mail the following information:  
Import (and your country name) and invoice number, for example:  
*Import Australia* and invoice number *123456789*.
- Add a copy of the import permit at the outside of the package.

## PACKAGING INSTRUCTIONS

- The consignment must be adequately, break proof and leak free packed, (e.g. biopack P600 or 650) or double leak proof package with absorbing material.
- Indicate on the outside of the outer packaging the name, address and telephone number of the consignor and the full name, address and telephone number of the addressee, the nature of the consignment and the quantity and type of the material concerned.
- The Commercial document should be available on the outside of the package.

## ACTIONS AT CDS AFTER ARRIVAL

- A unique number (SLXXAYYYY) will be assigned to the samples and the samples will be registered in our LIMS system.
- The samples will be processed (homogenisation)
- The homogenised samples will be aliquoted and stored at -70°C.
- Homogenates will be tested:
  - IBDV PCR
  - NDV PCR
  - AI PCR

Note: to be able to produce challenge virus the homogenates must be free of AIV and NDV; IBDV should be present
- Optional: NGS (at CRO)
- After consultation with PBU: Egg passage of homogenate to produce a workingstock of challenge virus (A unique Antigen code will be assigned to this material)
- Egg passage will be tested in:
  - AI PCR
  - NDV PCR
  - IBDV PCR
  - Egg titration (three times)  
Note: to be able to produce challenge virus the homogenates must be free of AIV and NDV; IBDV should be present;
- After consultation with PBU: Pathogenicity study in chicken of challenge virus
- After consultation with PBU: Vaccination challenge study in chicken

# SUBMITTING ILT ISOLATES TO CDS

In certain special cases, there may be an interest in collecting ILT isolates from the field for use in future internal challenge trials. Please discuss with Global Poultry Technical directors the suitability of your submission prior to shipment to CDS Boxmeer

## SAMPLING FOR VIRUS ISOLATION

- When:** In acute phase of infection.
- What:** Trachea or trachea swab. preferably don't use cervical dislocation to kill the bird.
- Transport:** In virus transport medium, preferably frozen or at least cooled.

## IDENTIFICATION OF THE ISOLATES

- Submit at least the following information about the isolates:
  - Date of isolation
  - Type of bird
  - If applicable: vaccination schedule
  - Clinical Signs
  - Place of isolation
  - Age of bird

Note: this information can be written on the submission form

## PAPERWORK FOR SHIPMENT

- See the CDS intranet page for the latest information. (<https://collaboration.merck.com/sites/SyncPub/ah/GloFun/AHRDBio/RDBox/RDSerLab/Pages/default.aspx>)
- Add a filled submission form inside the package (make sure this form is not in direct contact with the samples itself)
- Add a filled commercial document outside the package
- Address to ship to:  
**MSD Animal Health  
R&D Service Lab PP063 (Portiersloge)  
Exportstraat 39c  
5831 AK Boxmeer  
The Netherlands**

## SHIPMENT FROM OUTSIDE EU

- Besides the items mentioned before please make sure that you:
  - Send an e-mail (at least two days before the samples will be shipped) to: *DistributionCoordination.inb@merck.com* (cc *R&D Service Lab Sample Processing*)
  - Mention in the subject line of this e-mail the following information: Import (and your country name) and invoice number, for example: *Import Australia* and invoice number *123456789*.
- Add a copy of the import permit at the outside of the package.

## PACKAGING INSTRUCTIONS

- The consignment must be adequately, break proof and leak free packed, (e.g. biopack P600 or 650) or double leak proof package with absorbing material.
- Indicate on the outside of the outer packaging the name, address and telephone number of the consignor and the full name, address and telephone number of the addressee, the nature of the consignment and the quantity and type of the material concerned.
- The Commercial document should be available on the outside of the package.

## ACTIONS AT CDS AFTER ARRIVAL

- A unique number (SLXXAYYYY) will be assigned to the samples and the samples will be registered in our LIMS system.
- The samples will be processed (homogenisation)
- The homogenised samples will be aliquoted and stored at -70°C.
- Homogenates will be tested:
  - AIV PCR
  - NDV PCR
  - ILT PCR

Note: to be able to produce challenge virus the homogenates must be free of AIV and NDV; ILT should be present
- Optional: NGS (at CRO)
- After consultation with PBU: Egg passage of homogenate to produce a workingstock of challenge virus (A unique Antigen code will be assigned to this material)
- Egg passage will be tested in:
  - AI PCR
  - NDV PCR
  - ILT PCR
  - Egg titration (three times)  
Note: to be able to produce challenge virus the homogenates must be free of AIV and NDV; ILT should be present;
- After consultation with PBU: Pathogenicity study in chicken of challenge virus
- After consultation with PBU: Vaccination challenge study in chicken

# HOW DO I GET STARTED?

1. Set up Rapid Genomics as a vendor in your country or region.
2. Open a purchase order for a minimum of 500 tests locally.
3. Provide purchase order number and shipment address for shipment of FTA cards Orin McCormick [omccormick@rapid-genomics.com](mailto:omccormick@rapid-genomics.com). Cards will only be shipped in 500 card quantities to a single address, so please distribute them locally once they arrive to additional country colleagues if required.
4. Get sampling!

## FAQ

- Q. What if I have already collected samples on non-barcoded FTA cards?
- A. If you are confident that sample integrity has been maintained and that they have been stored appropriately (in individual bags), please reach out to Orin McCormick so that he can provide you with barcode numbers. These numbers can then be entered manually in the app during submission.
- Q. Can I take fewer than 20 samples to determine vaccine take?
- A. Ideally, no. Part of our contract with Rapid Genomics requires that we meet a certain sample threshold not only from a cost perspective, but also to optimize the use of their diagnostic equipment. This number was selected to balance the quality of the information that we can extract from each sampling with cost.
- Q. What if I send samples in that have not been submitted through the app?
- A. Samples will only be processed if they were submitted through the app. They will be discarded upon receipt.
- Q. Will I receive a confirmation that my submission has been successful?
- A. Yes, you will receive an email. Please retain for your records.
- Q. What if I submit samples through the app and ship them for processing, but Rapid Genomics has not yet received a PO from my country?
- A. Your samples will not be processed. After a 3 month holding period, they will be discarded.
- Q. When can I submit samples to CDS Boxmeer?
- A. Samples will only be submitted to CDS Boxmeer for virus isolation in very specific, rare cases. There must be prior agreement and approval by Global Poultry Technical Directors prior to submission.

**FOR ANY ASSISTANCE OR QUESTIONS SURROUNDING VIRAL FLEX-SEQ®, PLEASE CONTACT YOUR POULTRY TEAM TECHNICAL DIRECTORS.**

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