	Doc. Name	Evaluating the Polyvac-70 adjuvant
	TITLE	Evaluating the ability of Polyvac-70 (oil adjuvant) in a chicken model

### Study Objective:

1. Evaluating Polyvac-70 as an adjuvant for inactivated Newcastle disease virus vaccine in chickens

### Birds

SPF eggs were procured from PDRC, Venky's (India) Limited, SPF Division, Pune and hatched. Birds were examined for general health after hatch. The birds were acclimatized to study room (for 48hrs) after hatch. The birds were individually identified by wing tags and sorted into groups following vaccination. Additionally the cages were also identified by study number, bird number, group name etc. The birds were provided 23 hrs. light per day.

### Vaccine and Virus Used:

**NDV Vaccine (live):** The commercially available live lentogenic NDV vaccine (Lasota strain; manufactured from Hester laboratories) was used in this study as a primary vaccine.



**NDV Vaccine (inactivated):** Lasota strain of NDV was grown in SPF eggs (procured from PDRC, Venky's (India) Limited, SPF Division, Pune) and the allantoic fluid was collected. The EID<sub>50</sub> of the allantoic fluid was determined in SPF eggs as per standard protocol. Precisely 3 x 10<sup>6</sup> EID<sub>50</sub> /dose (equivalent to the commercial vaccine) was inactivated using 0.5% formaldehyde, blended with the oil adjuvant (Polyvac70 from Mukta Industries, Pune) and used for vaccination.


### Details of groups

Vaccination	Groups and details
<b>Primary Vaccine</b>	Live ND vaccine (Lasota) Occulo-nasal delivery 3 x 10 <sup>6</sup> EID <sub>50</sub> at day 5 post hatch for all the groups except control *
<b>Secondary Vaccine</b>	<ul style="list-style-type: none"> <li>• Vaccine delivered intramuscularly in the vaccinated groups</li> <li>• Vaccination performed three weeks after the primary vaccine</li> </ul> NDV Inactivated Vaccine + Oil adjuvant (Polyvac 70) - 10 birds per group Control: Unvaccinated - 10 birds per group

\* *All chickens were primed with live NDV vaccine on day 5 post hatching. Serum antibody titres by HI and ELISA tests were determined on days 9 & 19 post vaccination and post-killed vaccination on days (after the secondary vaccine) as indicated in the experimental design*

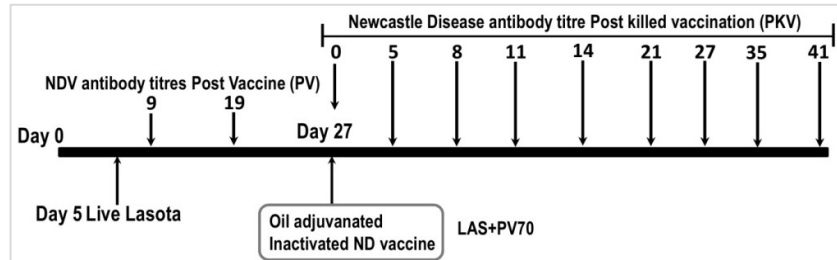
### Collection of animal samples

- (i) Serum samples: The blood samples were collected from the wing vein using a sterile 24G needle. Approximately 100 – 200 µl of blood was collected each time in a sterile micro centrifuge tube. The collected blood was allowed to clot for 18 hours at 4°C, serum separated by centrifugation, sorted as per the date of collection and stored at -20°C until assayed.

	Doc. Name	Evaluating the Polyvac-70 adjuvant
	TITLE	Evaluating the ability of Polyvac-70 (oil adjuvant) in a chicken model

### Measurement of antibody titres

The NDV antibody titres were measured pre-vaccination and also on different days post killed vaccination (as listed in below) by Haemagglutination inhibition test and also by ELISA.



#### (i) Haemagglutination inhibition (HI) antibody titres to measure NDV antibodies

The HI antibody titres were measured using the protocol as described in the OIE Terrestrial Manual 2012 (Chapter 2.3.14 - Newcastle disease; pages 7 & 8).

#### (ii) ELISA to determine NDV antibody titres

To determine the ELISA antibody titres the Newcastle Disease Virus Antibody Test Kit (CK116 NDV, BioChek, ME) was used. This kit has been certified by OIE in May 2014 as fit for detecting Newcastle disease virus specific IgG antibodies in chicken sera for the following purposes

- ✓ To demonstrate historical freedom from infection in a defined population (country/zone/compartments/flock)
- ✓ To determine immune status in individual animals or populations (post-vaccination)
- ✓ To monitor infection or disease in unvaccinated populations
- ✓ To estimate prevalence of infection to facilitate risk analysis in non-vaccinated populations (surveys/flock health schemes/disease control).

The serum samples collected in this study were tested using this kit following the manufacturer's instructions at a dilution of 1:500. The OD value obtained for each sample was used to calculate the S/P ratio

$$S/P \text{ ratio} = \frac{(\text{Mean of Test Sample} - \text{Mean of negative control})}{(\text{Mean of Positive control} - \text{Mean of negative control})}$$


The following equation was used to convert the S/P ratio into titre

$$\text{Log}_{10} \text{ Titre} = 1.0 * \text{Log} (SP) + 3.52 ; \quad \text{Antilog} = \text{Titre}$$

**Titres of 1158 or less is considered Negative and greater than 1159 as Positive**

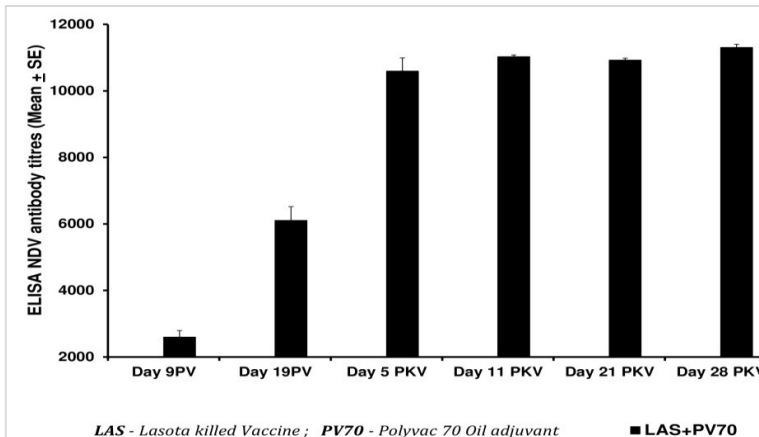
### RESULTS

Post hatch the birds were screened for the pre-vaccinal NDV antibody titres and were primed with live Lasota vaccine on day 5 post hatch. On Day 27 post hatch (day 21 PV), the birds were immunized with Inactivated Lasota vaccine blended with PV70 (oil adjuvant) or DIP-HIP and the serum antibody titres were determined as per the experimental plan below:

	Doc. Name	Evaluating the Polyvac-70 adjuvant
	TITLE	Evaluating the ability of Polyvac-70 (oil adjuvant) in a chicken model

### Total serum NDV antibody responses

The ELISA method measures the total antibody titres. The antibody titres were monitored on days 9 and 19 PV and on days 5, 11, 21 and 28 days post killed vaccination (PKV). The primary vaccination results in positive antibody titres (>1158 titre is considered positive) as early as day 9. Secondary vaccination with PV70 adjuvanted inactivated NDV resulted in significantly higher serum antibody response as early as day 5 post killed vaccination. The responses reached peak levels by day 11 PKV and sustained until day 28 of screening.



**Figure 1: Total serum NDV antibody titres assessed by ELISA**

The ELISA antibody titers are expressed as Mean  $\pm$  SE within each day across the groups.

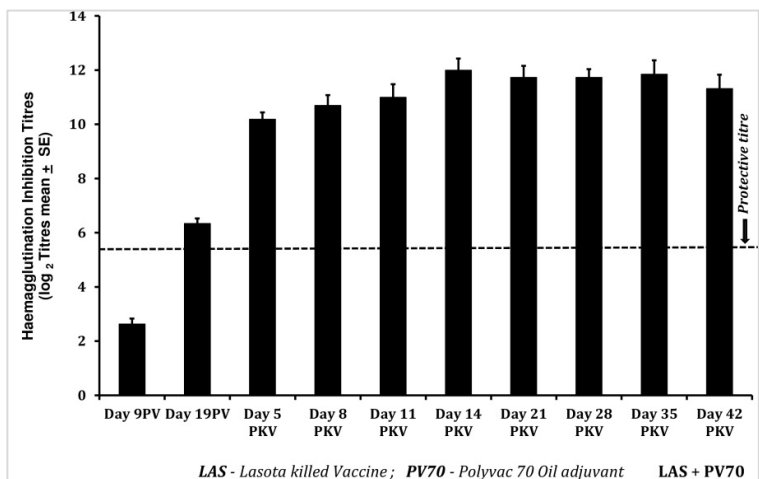
Note: Positive titres as early as day 9 PV, significantly higher ELISA titres on day 5 PKV and sustained levels from Day 11 PKV. n=10 per group.

PV- Post vaccination

PKV – Post killed vaccination

### Protective antibody response to NDV

The HI method measures the protective antibody titres with  $2^5$  (HA titre of 32) being protective. The primary vaccination resulted in protective antibody titres by day 19 PV. The oil adjuvanted inactivated NDV (LAS+PV70) resulted in significantly higher protective antibody titres by day 5 PKV and reached significantly higher peak antibody titres on day 21 PKV which sustained until day 42 PKV of screening.



**Figure 2: NDV protective antibody titre assessed by HI test**

The HI antibody titers are expressed as Mean  $\pm$  SE within each day across the groups.

Note: Significantly higher antibody titres in the Oil adjuvanted vaccine group on days 5 and peaked on day 21 PKV. Peak antibody titres sustained until day 42 PKV of screening. n=10 per group

PV- Post vaccination

PKV – Post killed vaccination

### Summary:

**Polyvac-70 adjuvanted inactivated ND vaccine induced significantly higher levels of protective antibody titres in primed birds. The antibody levels sustained until day 42 PKV of screening ( $\sim 2^{11.3}$ )**