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BETTER DIAGNOSTICS FOR BETTER RESULTS

Good Sampling and Serological Monitoring

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The value of serological monitoring depends largely on good sampling to give valid results. To be more precise, interpretation of flock titers is only meaningful , when flock titers are, in fact, reliable estimates of the flocks that you are looking at. In order to obtain reliable, and representative flock titer results, one has to:

1) Use statistically valid sampling methods to ensure that samples are truly representative.
2) Use proper sampling handling to ensure a uniform quality of serum samples, and
3) Use proper ELISA assay techniques to ensure reproducibility and reliability of the test results.

This paper will mainly focus on the importance of proper sampling methods, sample handling and sample size.

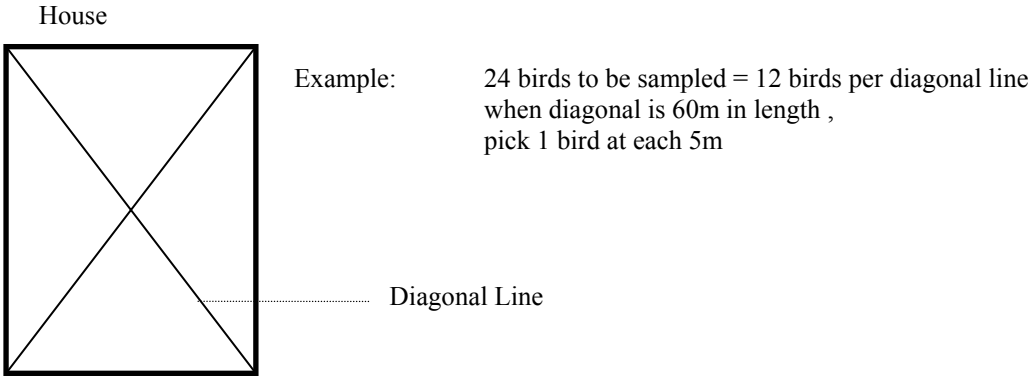
Sample Methods

The application of statistically valid sampling methods is of vital importance to obtain reproducible and reliable estimates of mean flock titers. For sampling to be statistically valid there are 2 basic conditions that will have to be met:

1) Random selection of birds for sampling.
2) Proper sample size (for further discussion see “Sample size” page 2)

Random sampling of birds basically means that every bird in a flock has an equal chance of being selected from a flock. This is of course easier said then done, but there are in practice various different ways to approach random selection of birds in a house and an example is given in Figure 1. (below). Randomization of sampling is very important, as it tends to average out effects of what in statistics is called , “uncontrollable variation”. (i.e. uneven vaccine uptake through drinking water, uneven feed uptake, effects of uneven temperature, moisture etc.). To illustrate this point, suppose that in a house the drinking water system is defect. In the front half of the house the drinking water system is not working and in the second half of the house the system works properly. The birds are vaccinated through the drinking water system. If you would take samples from the first half of the house, you would conclude that the entire flock has not been vaccinated at all. If you would take only samples from the second half of the house, you would conclude that the entire flock has been correctly vaccinated. If you would take random samples trough out the whole house, you would notice that the vaccination has only been partially successful, which is the correct reflection of what really happened to the flock.

Figure 1. Random sampling of birds in a free-range house.



When sampling from cages or boxes, one can number each box, let’s say number 1 to 102. Then with a random number table, draw a number and take 1 bird from the box with the corresponding number.

Sample Handling and Storage

During and after sampling , the next important step is to make sure that the blood samples are correctly handled and stored, to ensure a uniform and good quality of the samples. The following are some guidelines for the proper handling and storage of your samples:

- Collect 2-3 ml blood , this will yield 0.5 - 0.75 ml serum, which is more then sufficient for ELISA's
- Use disposable syringes
- Separate serum from blood by centrifugation or natural coagulation (1-2 hrs at room temp)
- ALWAYS LABEL SAMPLES PROPERLY (Company ID, Flock ID, Age, Type Bird etc.)
- Short term storage samples (< 48 hrs): 4- 8 ^{o}C
- Long term storage : -20 ^{o}C in plastic storage vials with airtight cap (microcentrifuge tubes 1.5 ml) and in labeled zip-lock bag
- Avoid many freeze/thaw cycles of serum samples
- Diluted serum samples (in sample diluent) more stable than non-diluted samples.
- Do not use spoiled serum samples (bad smell). Spoiled samples contain proteolytic enzymes which may affect ELISA results.

Sample size

Next to randomization of sample taking, the number of blood samples taken from a flock is of vital importance to get reliable
estimates of the "true mean flock titer". The sample size varies with the level of uniformity/non-uniformity of titers found within a flock. In general, the larger the titer variability (non-uniformity) in a flock, the larger your sample size has to be to correctly estimate the "true mean titer". Therefore, the best indication of proper sample size is to examine historical titer data within flocks.

In practice, the number of samples taken from flocks is often a compromise between the statistically correct number and the costs of sample taking and analysis. However, compromising too much on sample size to save money, may have a negative effect on the reliability of the Calculated means. Table 1. shows what happens to the mean titer of a group of 100 samples taken from day-old Broilers, when one splits them up (at random) into smaller group of samples.

The 100 individual samples were run on a BioChek IBD ELISA. The mean maternal antibody titer for these day-old chicks was 4057 with a %CV of 48 %. The predicted Days To Vaccination (DTV) with the LOG2 method and the Deventer Log2 method was 19 days and 21 days, respectively. The individual samples were then randomly divided into groups of various sample sizes and the mean titers, together with the DTV were calculated. This was repeated 16 times.

Table 1. Relationship of Sample Size on estimation of Mean Flock Titer and Predicted Days to Vaccination (DTV) (Log2 method).

No.of Samples/group	Mean Titer		%CV		DTV	
	Min	Max	Min	Max	Min	Max
46	3712	4449	42	58	20	22
30	3333	4779	37	57	20	22
23	3388	4753	40	64	20	22
15	3390	4973	33	62	19	22
10	2374	5260	25	71	17	23
5	2221	5371	23	71	16	23

The above table clearly shows that with decreasing sample size, the deviation of the mean titer, % CV, and Predicted DTV increases. This means for instance that for this flock, a sample size of 23 samples, will give a mean titer in between 3388 and 4753, with a CV in between 40% and 64%, and with a vaccination date prediction varying in between 20 and 22 days. For 10 samples, the estimated mean titer will fluctuate in between 2374 and 5260, with a CV in between 25 and
71 %!

The data also shows that a sample size from 46 to 23 samples, seem to give similar results in terms of deviations from the mean titer, but that below a sample size of 23 samples the deviations for estimating the mean titer, significantly increase. The increasing variability with decreasing sample size, also affects the reliability of calculated mean titers and vaccination date prediction.

Table 2 shows how many % of predicted mean titers per sample size, fall within a 20% range of the real mean titer of the 100 samples. Also the % predicted DTV's per sample size, which fall within 1 day variation of the real mean DTV is shown below.

Table 2. Relationship of sample size and % reliable prediction of the real population mean.

No.of Samples/group	Mean Titer % within range of 4057 +/- 20%	DTV % within range of Mean +/- 1 day	
		LOG2	DEV LOG2
46	100%	100%	100%
30	100%	100%	100%
23	100%	88%	100%
15	88%	88%	94%
10	50%	43%	57%

- True Population Mean titer (TMT) was 4057, estimated mean titers within TMT +/- 20% variation range were defined as acceptable estimations.
- True Mean DTV LOG2 method was 19 days and 21 days for Deventer Log2 method, estimated mean DTV's within 1 day variation range of true mean DTV were defined as acceptable estimations.

Conclusions sample size:

The sample size "cutoff", in terms of reliability and reproducibility, seems to be around 23 samples.
Below 23 samples, there is a marked decline of reliability of mean titer and vaccination date estimations.

Based on the above data, practical experience and statistical information a sample size of 23 samples seems to be a reasonable number for establishing baselines or profiles within a flock, or for vaccination date prediction. Once baselines are established, 15 samples can show trends, by detecting the most common titer groups, but this number is insufficient for quantitative analysis.

Compromising on sample size to save money, may have an adverse effect on reliability of calculated mean titers and may lead to misleading results and conclusions.

For disease monitoring , sample size varies according to % infection rate and sampling frequency.
One may have to increase sample size beyond 23 samples, when titer detection at low infection or incidence rates (< 2-5 %) is desired (i.e. Salmonella monitoring). Further guidelines are given in Table 3.

Table 3. Disease Monitoring: Relation of sample size, flock size, and infection rate.

NUMBER OF SAMPLES NEEDED TO BE 95% CONFIDENT THAT THE DISEASE WILL BE DETECTED IF PRESENT AT VARIOUS LEVELS OF PREVALENCE OR CONTAMINATION

FLOCK SIZE = N	CONTAMINATION RATE						
	20%	10%	5%	2%	1%	0.5%	0.1%
	Sample size = n						
N	n	n	n	n	n	n	n
20	10	15	19	48	20	20	20
50	12	22	34	77	50	50	50
100	13	25	44	94	96	100	100
200	13	26	48	105	155	190	200
500	14	28	55	128	225	349	500
1000	14	28	56	138	258	450	950
5000	14	28	58	146	290	564	2253
10000	14	28	58	147	294	581	2588
100000	14	28	58	148	299	596	2995

To pick up 10% infection rate in a flock of 5000 - 10000 birds, one needs to take 28 random samples

To pick up a 2% infection rate in a flock of 5000 - 10000 birds, one needs to take 147 random samples