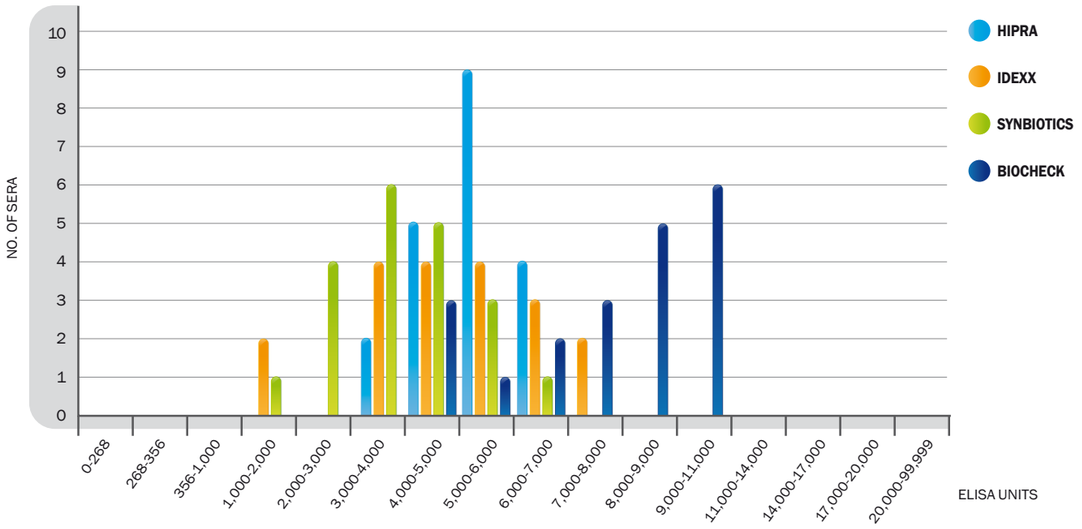
Day 35

Graph 3: Histogram of the serological results of chickens at age of slaughter analysed with different commercial ELISA kits grouped by ranges



Response to the vaccine has been rapid and consistent since the most suitable vaccination time was chosen. However, if at the time of vaccination the birds have a high enough level of passive immunity to block the vaccine, this response will not be so uniform or so rapid.
ELISA is a very useful tool for evaluating vaccination against Gumboro disease, but all the information required for an accurate understanding of what has occurred in a batch of animals is not always available. Most of the time the only available information consists of serological results obtained in the slaughterhouse. Only in specific situations is serological monitoring performed on a batch of animals when they are one day old, at the time of vaccination and at the time of slaughter.

4 CONCLUSION

ELISA kits provide quantitative but not qualitative data on the immune status of birds. Blood levels of antibodies can be measured, but the specificity of these antibodies cannot be determined.
When referring to ELISA titres, it is important to specify which kit has been used to analyse the sera.

The manufacturers of the kit should provide baseline serum levels as a guide for interpreting results. A single analysis provides information about what is happening at a particular time, but a log is needed to better interpret results.
It is vital to create a database for your farm that contains baseline serological levels so that you can distinguish between titres caused only by vaccination and titres that may be attributed to contact with a wild virus.

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Area Gumboro

UNDERSTANDING ELISA IN VACCINATION AGAINST GUMBORO DISEASE*

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In Gumboro disease control, the importance of humoral immunity is fully understood. But what are the true practical implications in the field? What purpose do ELISA kits serve?

1 SPECIFIC IMMUNITY

This is the first point to consider. In simple terms, from an antigen perspective (studies on cross-Virus Neutralisation) serotype 1 Gumboro viruses can be divided into classic and variant strains. When applying the protectotype concept and carrying out cross-challenge studies, the result is the same: serotype 1 viruses are divided into classic and variant strains. And what about highly virulent viruses? From an antigen or protectotype perspective, the immunity generated by the classic strains protects against such viruses.
This specificity is crucial when we are dealing with the challenges presented by variant strains. These can escape the passive immunity produced by vaccines containing classic strains, and infect your birds. In some countries the presence of these variant strains is well-recognised, and inactivated vaccines including such strains are used in the breeders vaccination plans. While it is true that in most countries the variant strains are not the poultry sector's biggest concern with regard to Gumboro disease, this does not mean that these strains should not be promptly isolated on farms.
Commercial ELISA kits available on the market can provide quantitative but not qualitative information on these antibodies. This means that when you measure the immunity levels of your birds, you cannot know whether their antibodies are specific to classic strains or variant strains (Table 1).

Table 1: Example of titres obtained with the CIVTEST® AVI IBD ELISA kit for SPF birds and birds challenged with different strains of IBDV.

	SPF	Genetic Variant	Highly Virulent IBDV	Serotype 2
CIVTEST® AVI IBD	15	6,271	5,688	27

2 PASSIVE IMMUNITY

Passive immunity is a key element in the discussion on Gumboro disease. The antibodies acquired through the egg yolk will protect the offspring from a challenge, provided that these antibodies are specific. But the immunity that protects the animals from the challenge of a field virus also takes effect in the presence of a vaccine virus.
It is very important to know your birds' level of passive immunity of so that you can design a suitable Gumboro disease control plan.
When determining the date of vaccination against Gumboro disease, using the Deventer formula for example, you take into account the level of antibodies of the one-day-old birds, the metabolism of these antibodies and the characteristics of the vaccine itself. But the first thing you have to

ask yourself is which ELISA kit you are using and how it behaves with different levels of immunity.

To get an idea of how the different kits behave, we monitored the poultry on a farm at different ages with four commercial kits (Table 2 and Graph 1).

When analysing the same serum with various kits, different results are produced. That is why, when you look at the maternal immunity level of one-day-old chicks, you must check which ELISA kit has been used.

Table 2:
Serological results of one-day-old-chicks analysed with different commercial ELISA kits

VN FARM ONE-DAY-OLD-CHICKS				
SERUM NO.	HIPRA	IDEXX	SYNBIOTICS	BIOCHECK
1	2717	3419	3678	4155
2	5692	7389	5866	8000
3	5987	7906	6942	8355
4	5788	7504	7351	7912
5	6546	7810	8439	9523
6	6265	7944	8859	10001
7	3373	3407	2767	5384
8	5935	8393	8902	10137
9	3968	3671	2955	6136
10	2877	3520	2138	4832
11	3320	2860	2517	6238
12	4959	6351	4963	8572
13	5197	5271	5165	9607
14	3509	3526	3239	7345
15	4670	4568	3994	8100
16	6122	7326	9348	9394
17	5638	5898	5529	8000
18	4972	4683	4182	5850
19	2072	2977	1686	3792
20	5164	5728	4618	8862
ST. DEV.	1313	1899	2365	1908
AVERAGE	4739	5508	5157	7510
CV	28%	34%	46%	25%

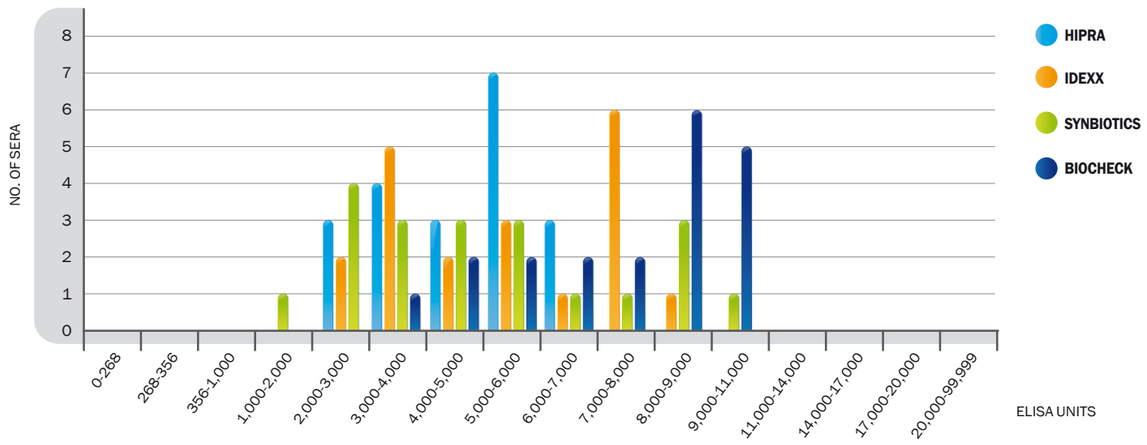
But how has this maternal immunity been metabolised? What is the immunity status of these same animals on the day they are vaccinated? It could be concluded from the previous table that the Idexx, Synbiotics and HIPRA (Civtest®) kits will produce similar values and that Biocheck will always produce higher values. Let's see what happens in reality. (Table 3 and Graph 2)

Table 3:
Serological results of chickens at age of vaccination analysed with different commercial ELISA kits

VN FARM CHICKENS AT AGE OF VACCINATION				
SERUM NO.	HIPRA	IDEXX	SYNBIOTICS	BIOCHECK
1	1296	1787	1252	2432
2	19	19	0	166
3	844	789	1023	1757
4	89	220	0	497
5	120	127	0	545
6	98	146	0	412
7	7	0	0	117
8	112	0	0	177
9	161	3	0	275
10	0	11	0	51
11	356	463	0	963
12	118	156	0	341
13	1220	1592	1100	2469
14	0	0	0	219
15	0	3	0	53
16	0	15	0	73
17	55	35	0	208
18	1003	1179	1173	2414
19	251	342	0	673
20	75	235	0	628
ST. DEV.	417	534	456	816
AVERAGE	291	356	227	724
CV	143%	150%	201%	113%

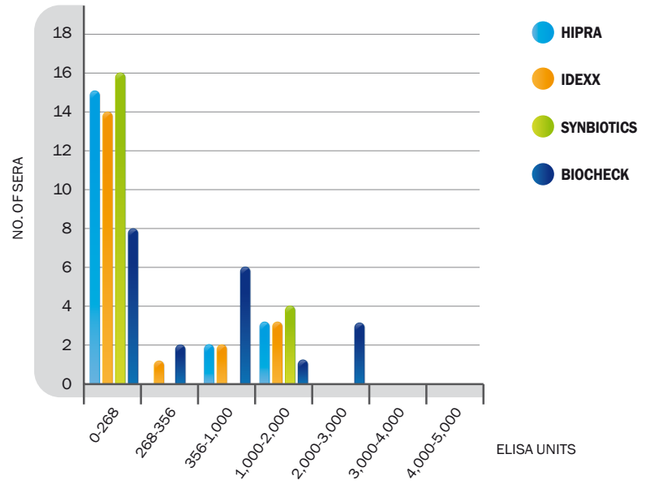
Day 1

Graph 1: Histogram of the serological results of one-day-old chicks analysed with different commercial ELISA kits grouped by ranges



Day 16

Graph 2: Histogram of the serological results of chickens at age of vaccination analysed with different commercial ELISA kits grouped by ranges



What can be seen in these analyses is that when the immune level decreases, not all the kits we used provide useful information. If these animals were vaccinated with an intermediate vaccine (breakthrough titre of 125 ELISA units in Idexx) and you wanted to evaluate whether the vaccination day chosen was suitable, the Synbiotics kit would provide you with confusing information. You would see that only three animals were resistant to the vaccine while the rest were completely sensitive. However, examination of the HIPRA (Civtest®) and Idexx results reveal that, in some animals within the 200-400 ELISA unit range, the vaccine would in fact be blocked.

3 ACTIVE IMMUNITY

Once their maternal antibodies are metabolised, or if these antibodies are not specific, the birds are unprotected. Before this happens, a suitable vaccination programme should be established to replace this passive immunity.

Vaccination exposes the immune system to an antigen so that the former develops antibodies against the latter. When administrating of a live Gumboro vaccine, a measurable humoral response can be expected to occur in two or three weeks. The amount of time will vary depending on the degree of attenuation of the vaccine strain and the level of residual passive immunity at the time of vaccination. Next, let's look at how the birds we analysed responded when they were vaccinated with HIPRAGUMBORO® GM97 at 16 days of age.

The breakthrough titre of HIPRAGUMBORO® GM97 is 500 ELISA units with Idexx, and at the time of vaccination (16 days of age), 80% of the birds were sensitive to the vaccine. With these favourable conditions, a rapid response following vaccination could be expected. The birds on this farm were sent to the slaughterhouse at 35 days of age, 19 days post vaccination. Now let's look at the seroconversion of these animals (Table 4 and Graph 3).

Table 4: Serological results of chickens at age of slaughter analysed using different commercial ELISA kits

VN FARM CHICKENS AT AGE OF SLAUGHTER				
SERUM NO.	HIPRA	IDEXX	SYNBIOTICS	BIOCHECK
1	4794	3008	3045	7263
2	6334	6563	5579	9514
3	4310	3533	2385	4422
4	5734	5643	4922	8412
5	5947	4735	4406	10152
6	4493	4235	3220	6559
7	3914	1621	1895	4697
8	6300	6589	6018	9778
9	4999	5486	3913	4843
10	5600	7213	5095	7833
11	5620	6920	4176	9989
12	5489	5453	2910	8714
13	3404	1306	2554	5855
14	5610	4748	3548	8518
15	5275	3583	3521	8661
16	6221	5467	4735	9766
17	6145	5930	5869	9821
18	4424	4293	2878	6739
19	5749	7000	3475	8033
20	5378	3759	4007	7856
ST. DEV.	807	1659	1151	1790
AVERAGE	5287	4854	3908	7871
CV	15%	34%	29%	23%