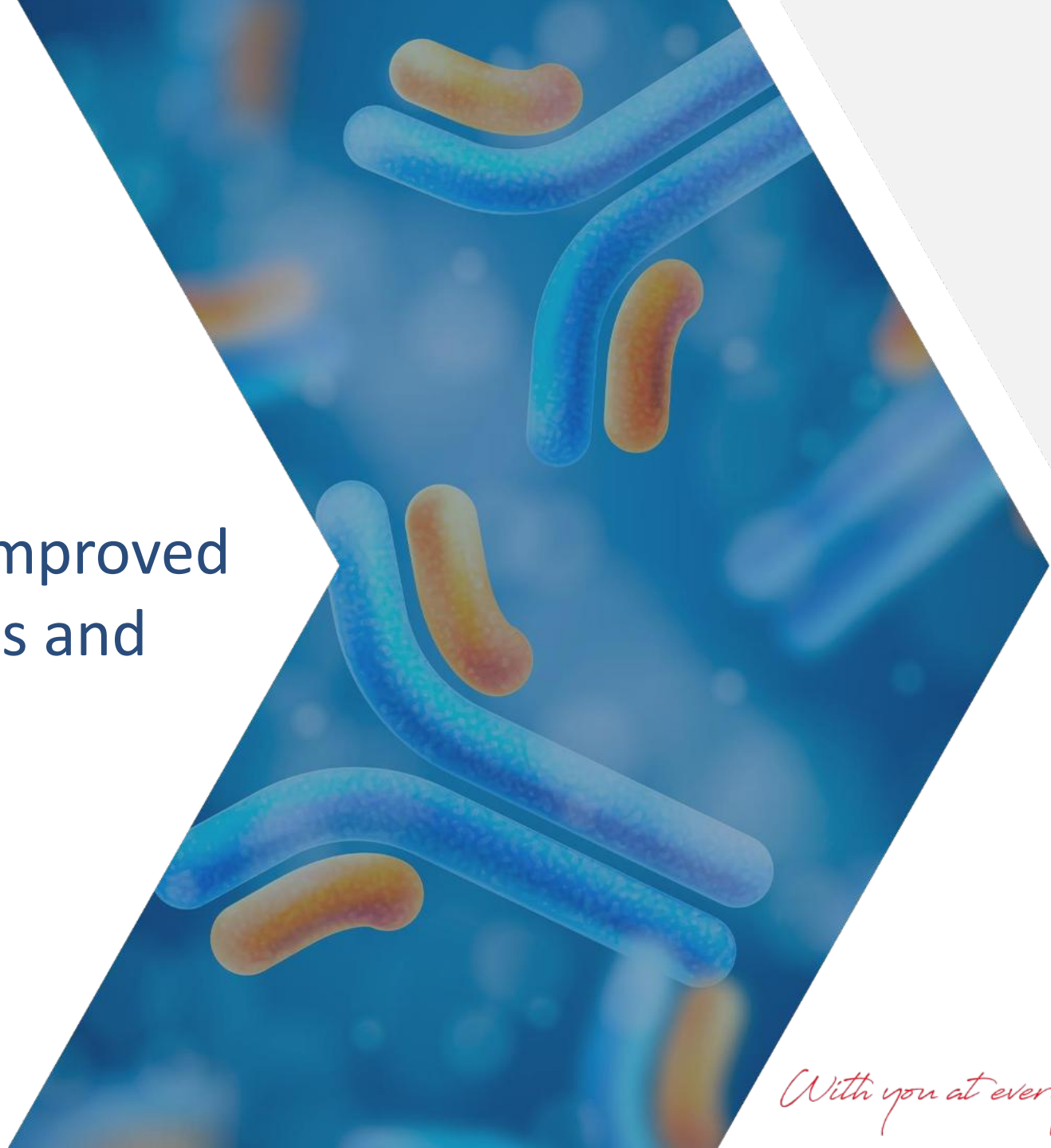
IBVARSV2

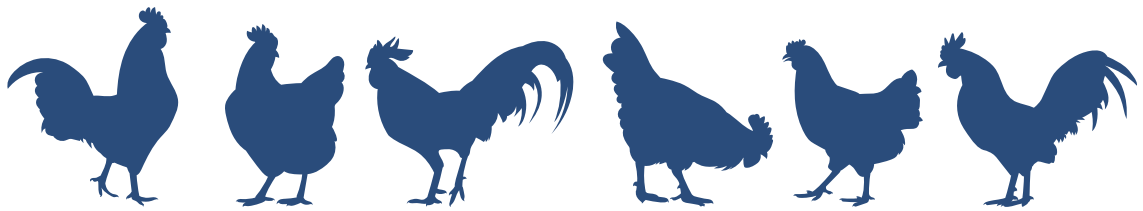
A new serological tool for improved monitoring of IB vaccines and variant detection

Mohammad Amawi
Senior Technical Manager



With you at every step

CONTENT



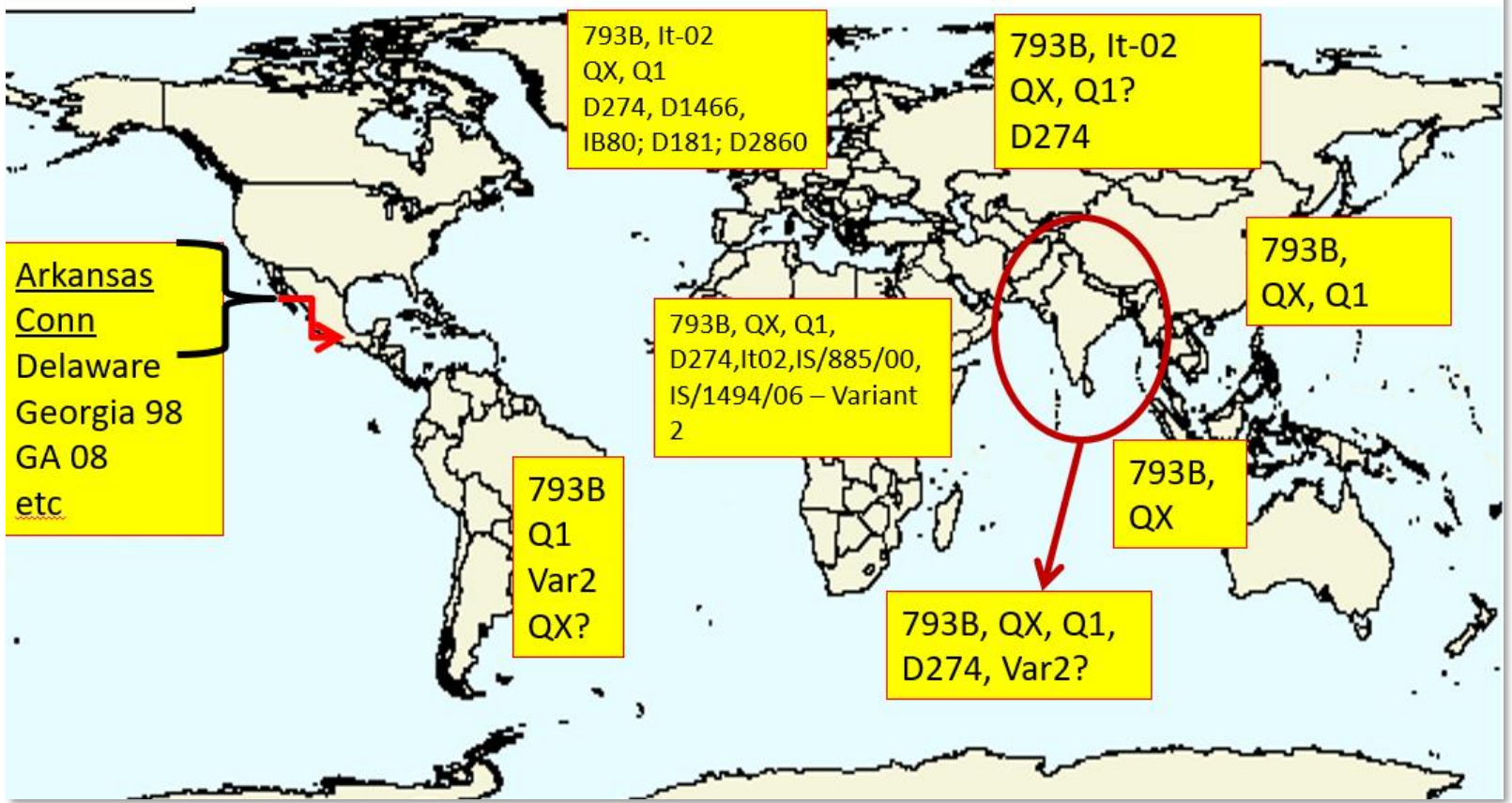
CONTEXT &
IBV ELISA PROBLEMATIC

IBV: A HIGHLY MUTATING VIRUS

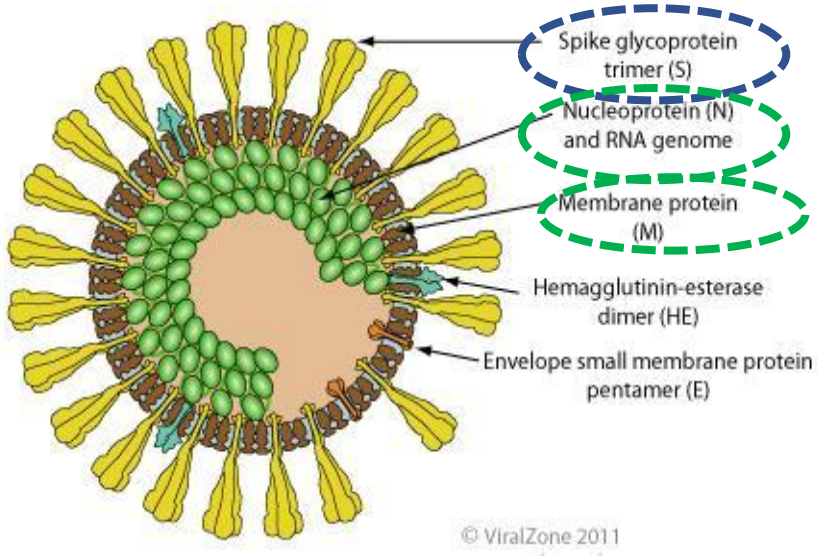
Infectious Bronchitis Virus

Virulent and important

Massachusetts worldwide



Single-stranded RNA viruses



Variability

Well conserved

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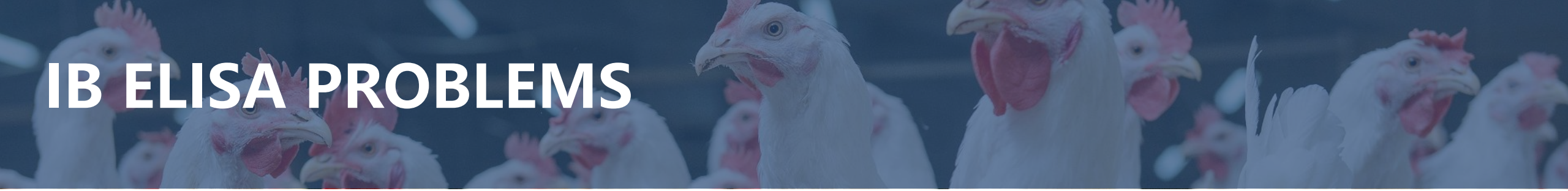
MAIN DIAGNOSTIC TOOLS FOR IB

1. VN TEST
2. HI TEST
3. PCR & SEQUENCING
4. ELISA

IB ELISA PROBLEMS



iELISA kits are used for **vaccination monitoring**.

2 majors limitations :

1

The emergence of variants regularly forces vaccine suppliers **to update vaccine strains,**

2

Under vaccination coverage, it is extremely **difficult to detect a field challenge**.

1st PROBLEMATIC: UPDATE OF VACCINE STRAINS

Infos: Broiler flock, 34 days
From Cz Republic

Vaccination program:
IB PRIMER (H120 + D274)

Státní veterinární ústav Jihlava
Rantířovská 93/20, Horní Kosov
586 01 Jihlava
20.03.2023

Case Listing

Case: VI2353-H3 - 15.03.2023-003 Š:HU089
IBV - 15.03.23 - PL - 1:500

	Well	O.D.	S/P	Titer	Group	Result
Neg	G05	0,055				
Pos	H05	0,379				
1	E08	0,085	0,093	172	0	Neg
2	F08	0,066	0,034	57	0	Neg
3	G08	0,149	0,290	594	1	Pos!
4	H08	0,101	0,142	273	0	Neg
5	A09	0,331	0,852	1924	2	Pos!
6	B09	0,094	0,120	227	0	Neg
7	C09	0,072	0,052	91	0	Neg
8	D09	0,150	0,293	601	1	Pos!
9	E09	0,124	0,213	425	1	Pos!
10	F09	0,197	0,438	932	1	Pos!

	S/P	Titer
AMn:	0,253	530
GMn:	0,169	329
SD:	0,233	532
CV:	92,1	100,4
Min:	0,034	57
Max:	0,852	1924

- **Low mean titer : 530**
- **50% NEGATIVE RESULTS**

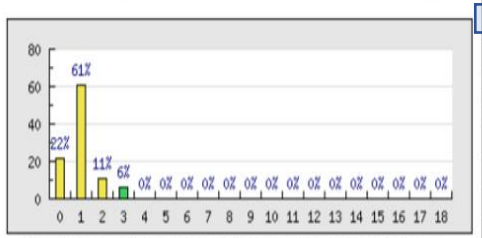
Infos: Broiler flock, 38 days
From Algeria

Vaccination program:
H120 + IBIRD (793B)

Nom	STE CEVA	Souche	NC
Elevage	STE CEVA	Age	38 Jours
Tél		Effectif	
Batiment	10112020.PC.38	Date entrée	02/12/2020
Espèce	Poulet chair	Date Sortie	03/12/2020
Kit	idexx	Lot	FS484 16-07-2021

Statistiques Echantillons 18 moyenne 740
Ecartype 522.72 Variation 70.64 %

Interprétation Sérums IBV Négatifs.



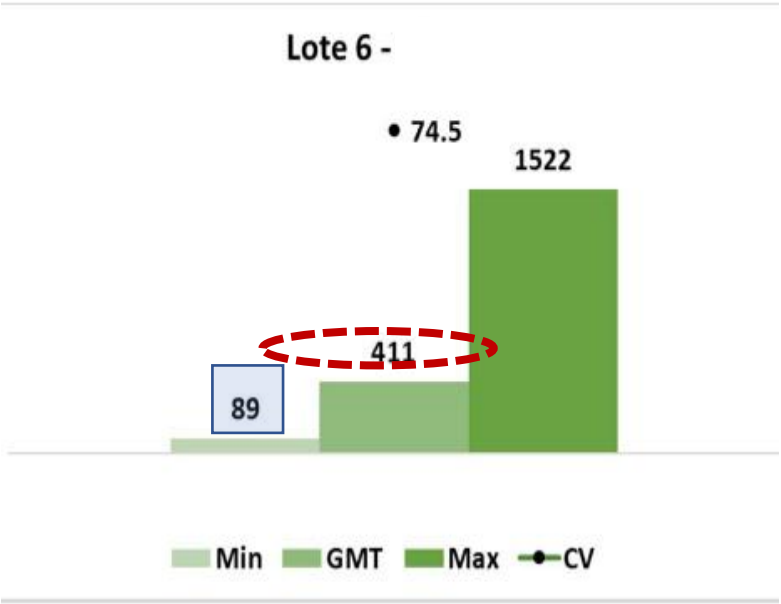
* Couleur jaune :Titres Correspondant a une prise vaccinale moyenne ou parfois insuffisante, ou séroconversion toujours en cours.

NR	DO	TITRE	GROUPE
Négatif	0.0530	0	0
Positif	0.3480	2291	3
1	0.161	768	1
2	0.170	839	1
3	0.094	260	0
4	0.138	585	1
5	0.338	2214	3
6	0.148	668	1
7	0.207	1133	2
8	0.072	109	0
9	0.147	662	1
10	0.090	240	0
11	0.169	832	1
12	0.121	457	1
13	0.118	442	1
14	0.163	784	1
15	0.277	1703	2
16	0.166	800	1
17	0.138	585	1
18	0.089	231	0

- **Low mean titer : 740**
- **22% NEGATIVE RESULTS**

Infos: Broiler flock, 40 days
From Brazil

Vaccination program:
H120 + IBVAR206)



- **Low GMT : 411**
- **NEGATIVE SAMPLES : MIN 89**

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1st PROBLEMATIC: UPDATE OF VACCINE STRAINS

1- A very low mean titers (<1000)

2- A high % of negative samples

HOW TO INTERPRET SUCH RESULTS?

- As a vaccination failure?

- As a lack of detection of ELISA?

2nd PROBLEM: DIFFICULTY TO IDENTIFY IB CHALLENGE UNDER VACCINATION

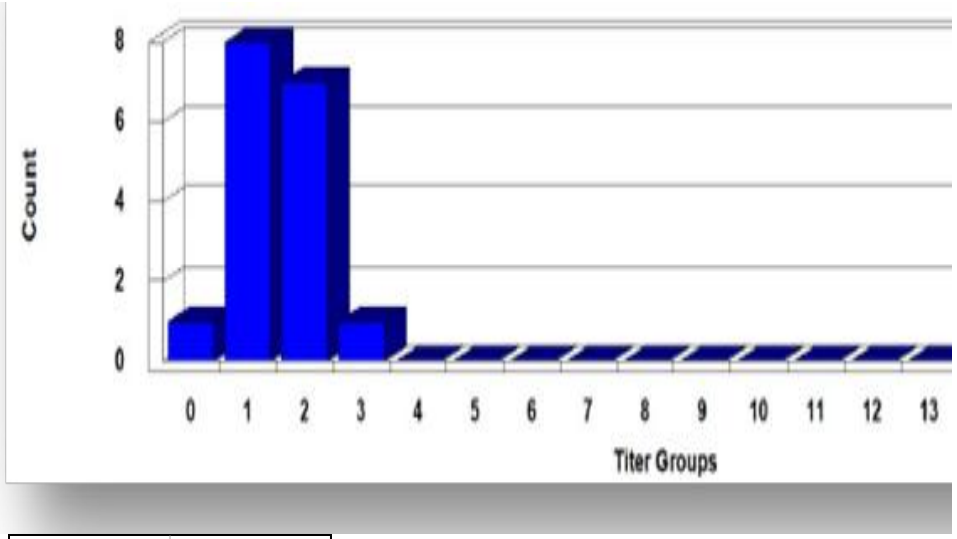
Infos: Broiler flock, 36 days
From Jordan

Vaccination program:
2-3 Live vaccines (Mass +4/91)

➢ **ACCORDING TO THE DATA, EVERYTHING IS OK!**

➢ **BUT PRESENCE OF CLINICAL SIGNS!**

KIT I



Mean	1144
CV%	74%
Min	262
Max	3254

2nd PROBLEMATIC:
DIFFICULTY TO IDENTIFY IB CHALLENGE UNDER VACCINATION

Infos: Broiler flock, 36 days
From Jordan

Vaccination program:
2-3 Live vaccines (Mass +4/91)

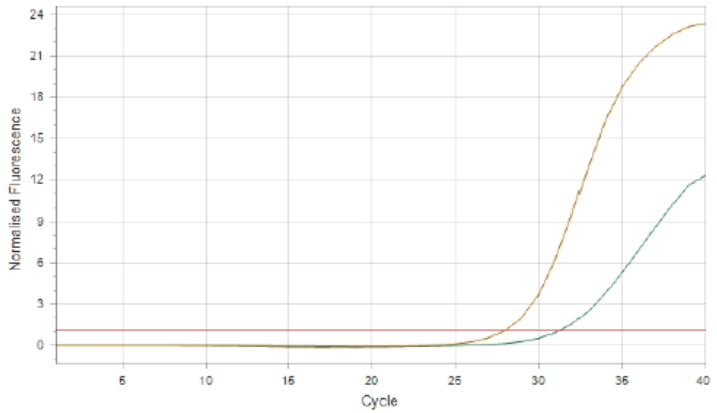
PCR ON TRACHEA ORGANS

IDENTIFICATION OF VAR02 STRAIN!!!!
DESPITE A « NORMAL » SEROLOGY!!!!!!!!

TOTAL ACov

Cycling: IBV

Target	IBV → IBV
Normalisation	Dynamic
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	1.068 (Automatic) starting at cycle 1

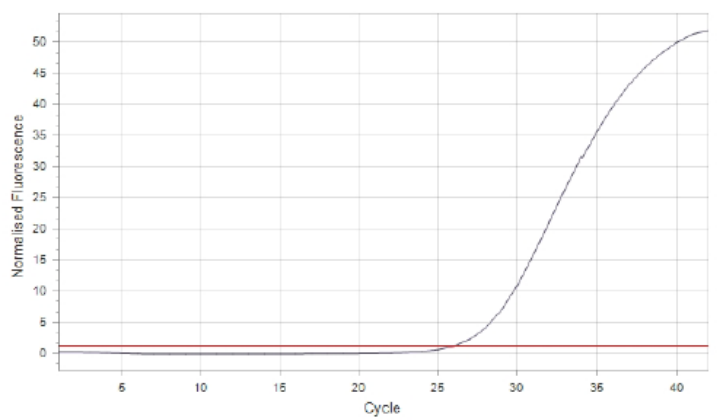


Well	Colour	Cq	Efficiency	Efficiency R²	Result
NEC					
13		-	-	-	Excluded
PAC					x̄ = 27.98 σ = 0.00
17		27.98	0.94	0.99973	
Trachea					x̄ = 31.22 σ = 0.00
15		31.22	0.94	0.99975	

VACCINE STRAIN : 4/91

Cycling: IBV-Variant 4/91

Target	IBV-Variant 4-91 → IBV-Variant 4/91
Normalisation	Dynamic
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	1.175 (Automatic) starting at cycle 1

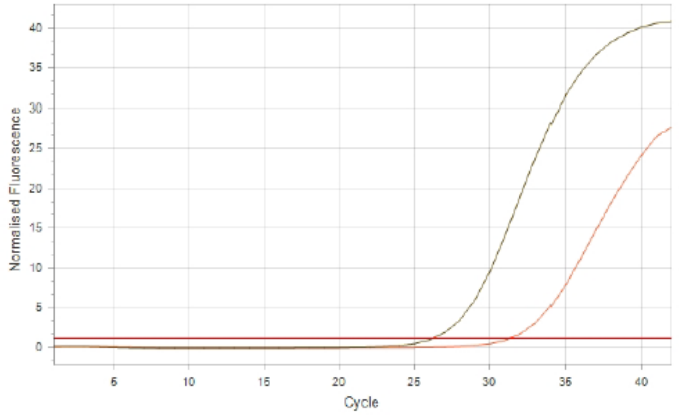


Well	Colour	Cq	Efficiency	Efficiency R²	Result
NEC					
7		-	-	-	Excluded
PAC					x̄ = 26.00 σ = 0.00
11		26.00	0.98	0.99972	
Trachea					
9		-	-	-	Excluded

FIELD STRAIN SUSPICION : VAR02

Cycling: IBV-Variant 02

Target	IBV-Variant 02 → IBV-Variant 02
Normalisation	Dynamic
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	1.047 (Automatic) starting at cycle 1



Well	Colour	Cq	Efficiency	Efficiency R²	Result
NEC					
1		-	-	-	Excluded
PAC					x̄ = 26.09 σ = 0.00
5		26.09	1.01	0.99938	
Trachea					x̄ = 31.21 σ = 0.00
3		31.21	0.99	0.99996	

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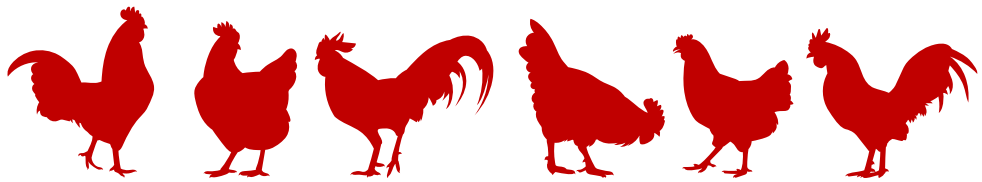
IBVARSV2
AS A SOLUTION



SOLUTION
IBVARSV2: AN IMPROVED VERSION OF KIT

ID Screen® Infectious Bronchitis Indirect (cat. IBVS)

Insert n°: 0416
Last batch : J56

Since February 2023

NEW KIT: ID Screen® Infectious Bronchitis Indirect 2.0 (cat. IBVARSV2)

Insert n°: 0223
Batch: P136

- Recombinant protein : **WELL CONSERVED**
- Same protocol

BUT….

- New Cat. Ref : **IBVARSV2**
- New titers formula

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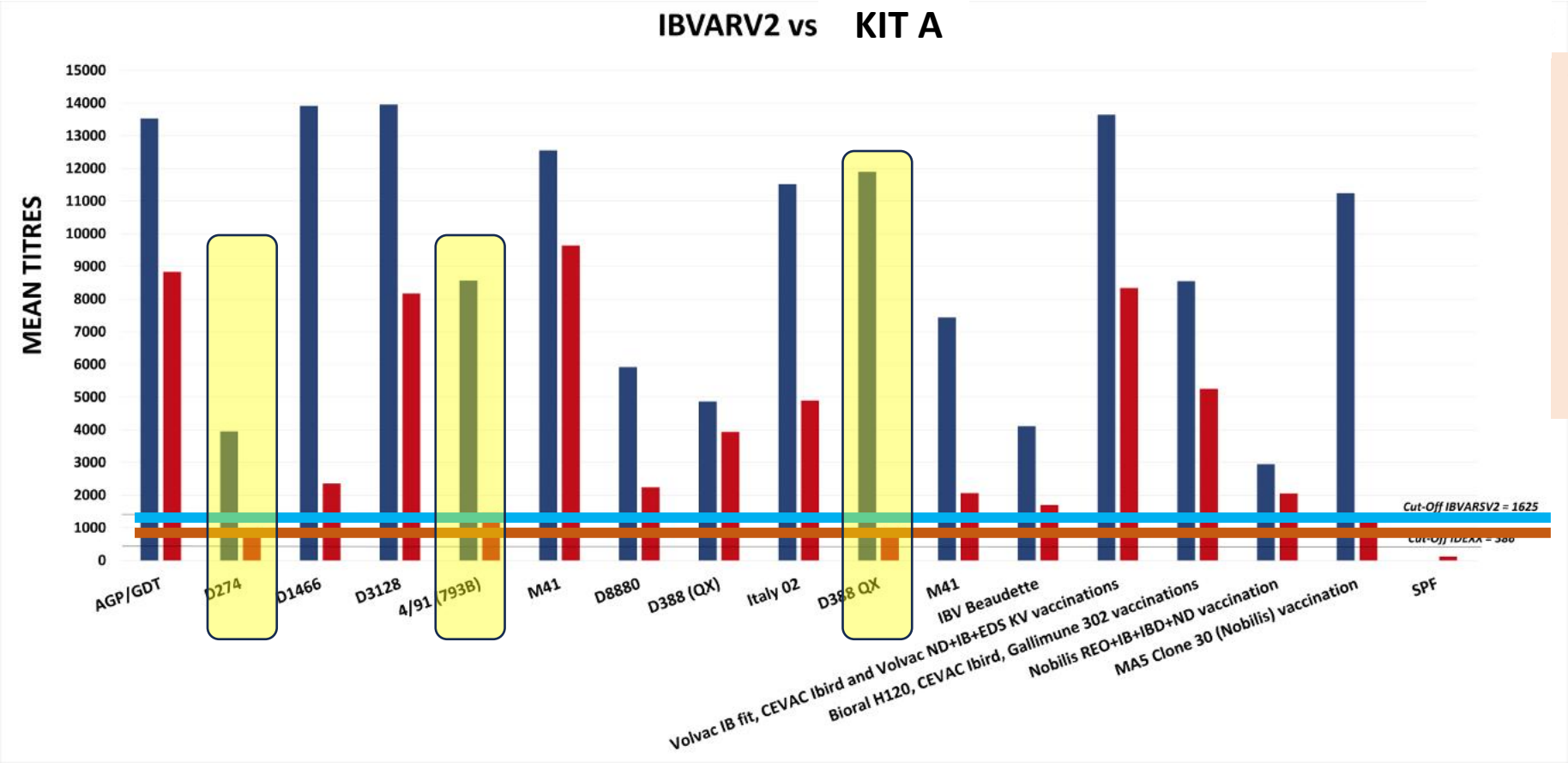
SOLUTION
IBVARSV2: A BETTER DETECTION OF VARIANTS

NEW KIT: ID Screen® Infectious Bronchitis Indirect 2.0 (cat. IBVARSV2)

Insert n°: 0223, Batch: P136



This IBVARSV2 kit is more sensitive than kit A to detect all variants

THE DETECTION OF VARIANTS IS STRONGER (from 3000 to 14 000): EASIER TO DETECT UNDER VACCINATION

IBVARSV2

KIT A

SOLUTION
IBVARSV2: A BETTER DETECTION OF VARIANTS

WHAT IS THE CONSEQUENCE OF THE IMPROVEMENT OF VARIANT DETECTION ON THE 2 MAIN ELISA PROBLEMS IDENTIFIED PREVIOUSLY?

1st PROBLEMATIC UPDATE OF VACCINE STRAINS

Expected titers:

5 000-8 000
Suspicion > 9 000
Max > 12000

Infos: Broiler flock, 38 days
From Algeria
Vaccination program:
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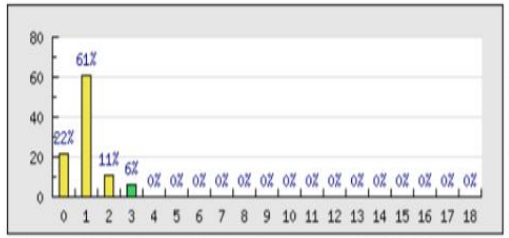

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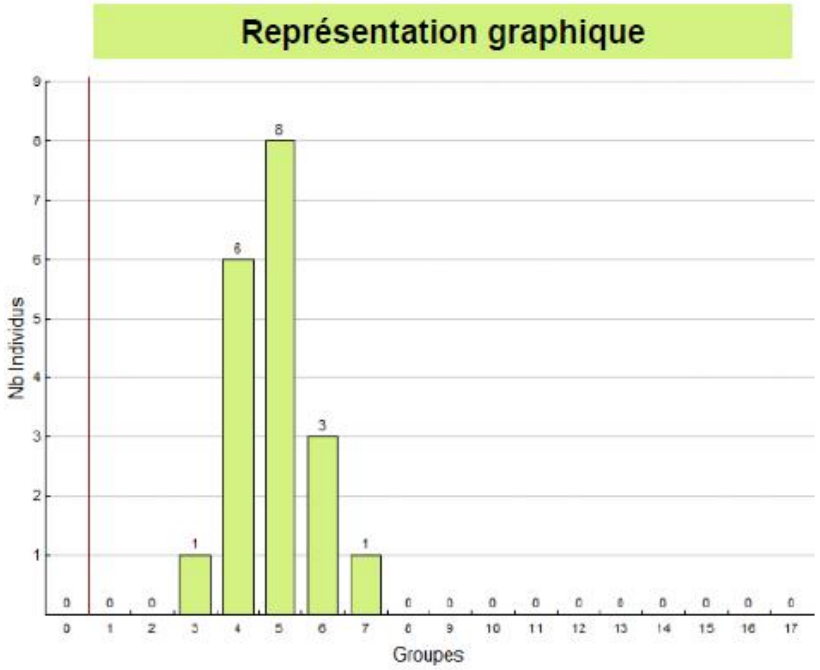
- **22% NEGATIVE RESULTS**

IBVARSV2



Moyenne	6 575
Minimum	3 152
Maximum	11 142
G.M.T.	6 328
% CV	28

- **Expected titers : 5000 - 8000**
- **100% positivity expected**

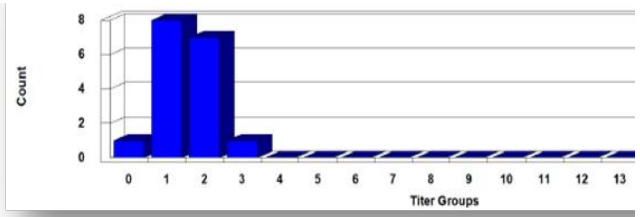
IBVARSV2: A POSSIBLE DETECTION OF CHALLENGE UNDER VACCINATION

Expected titers:

8 000-12 000
Suspicion > 13 000
Max >16 000

Infos: Broiler flock, 36 days
From Jordan

Vaccination program:
2-3 Live vaccines (Mass +4/91)

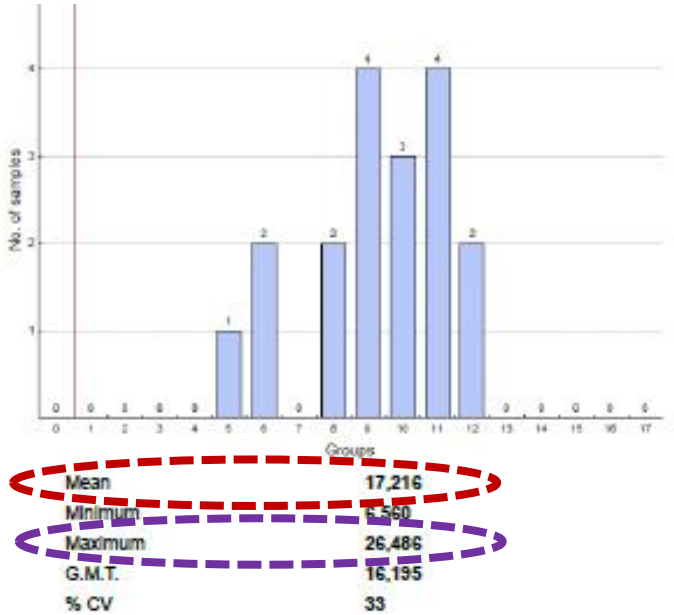


Mean	1144
CV%	74%
Min	262
Max	3254

➢ **ACCORDING TO THE DATA, EVERYTHING IS OK!**

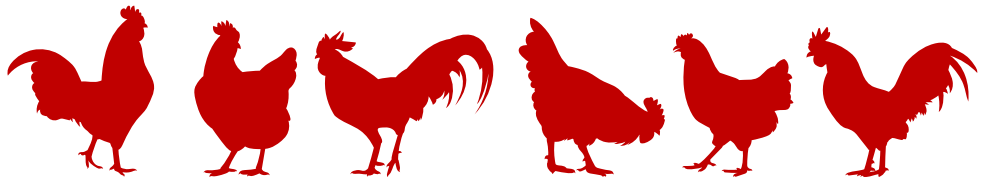
VAR02 DETECTION BY RT-PCR

KIT IBVARSV2



A CLEAR CHALLENGE IDENTIFIED!

A NEW BASELINE



GENERAL BASELINE FOR IBVARSV2

Vaccination Program	Time	Expected Mean	CV	Suspicion
1x Mass Live	D35-45	1 000-3 000	40-80%	Mean > 4000
2x Mass Live	D35-45	3 000-5 000	40-80%	Mean > 6000, max > 10 000
1x Live (class + variant) Ibird, 793B	D35-45	5 000 – 8 000	40-80%	Mean > 9 000 ; max > 12 000
1x Live (class + variant) IB Primer	D30-40	4 000 – 8 000	40-80%	Mean > 10 000; max > 12 000
2x Mass Live + 1x Variant Live	D35-45	6 000-9 000	40-60%	Mean > 10 000, max > 15 000
3-4x Live vaccine (class + variant)	D35-45	8 000-12 000	30-60%	Mean > 13 000; max > 16 000
1x Mass Killed + Multiple Live (Classical/Variant)	5-8 WPV	8 000-13000	25-50%	Mean > 13000; Max > 17 000
2x Mass Killed + Multiple Live (Classical/Variant)	5-8 WPV	9 000-13 000	20-40%	Mean > 13000; Max > 17 000
3x Mass Killed + Multiple Live (Classical/Variant)	5-8 WPV	10 000-13 000	20-40%	Mean > 13000; Max > 17 000

PRINCIPLE OF INTERPRETATION: 3 CASES

Type Flock: Broiler

Vaccination program IBV

(Classical + variant)

Bleeding: 41 days

Baseline expected:

5000 – 9000

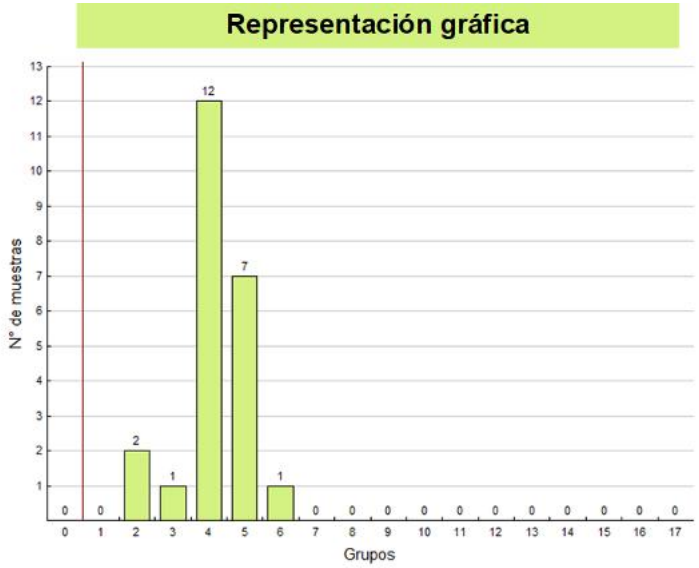
25-50%

challenge >10 000

PRINCIPLE OF INTERPRETATION: 3 CASES

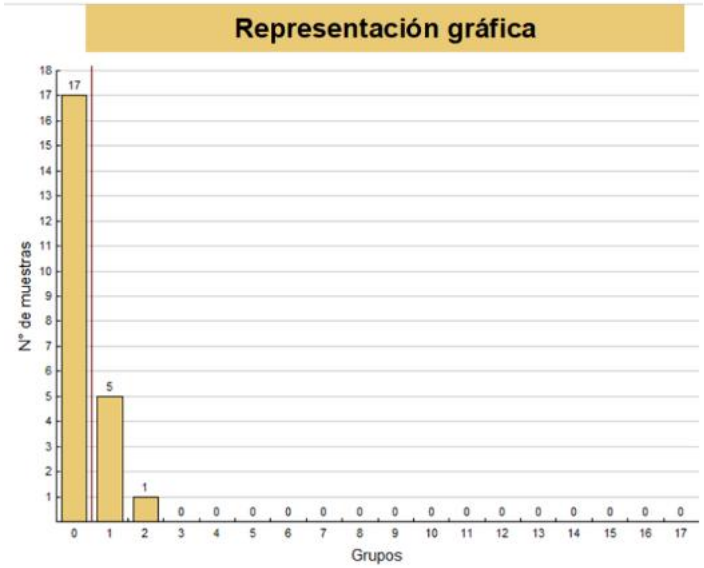
Type Flock: Boiler
Vaccination program IBV (Classical + variant)
Bleeding: 41 days
Baseline expected: 5000 – 9000 - 25-50%
challenge >10 000

CASE 1: Good vaccination



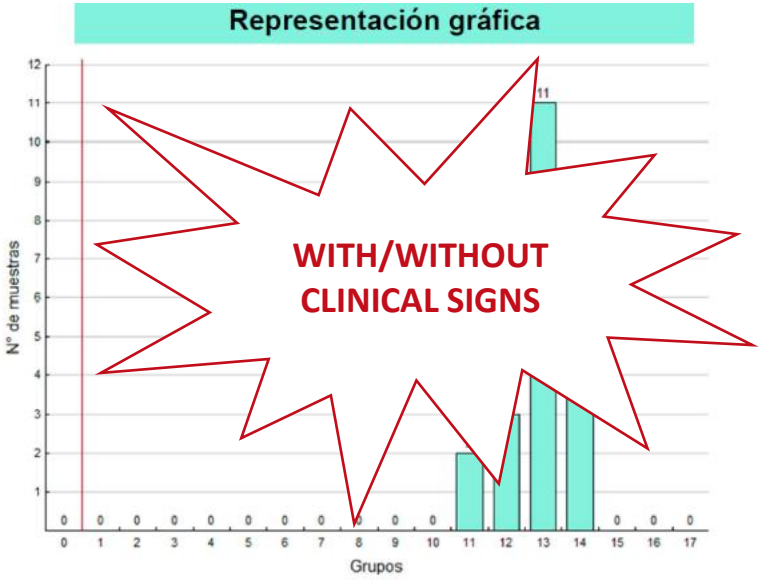
Media	5.631
Mínimo	2.776
Máximo	8.615
G.M.T.	5.405
% CV	28

CASE 2: Vaccination failure



Media	613
Mínimo	1
Máximo	2.311
G.M.T.	357
% CV	89

CASE 3: Viral circulation

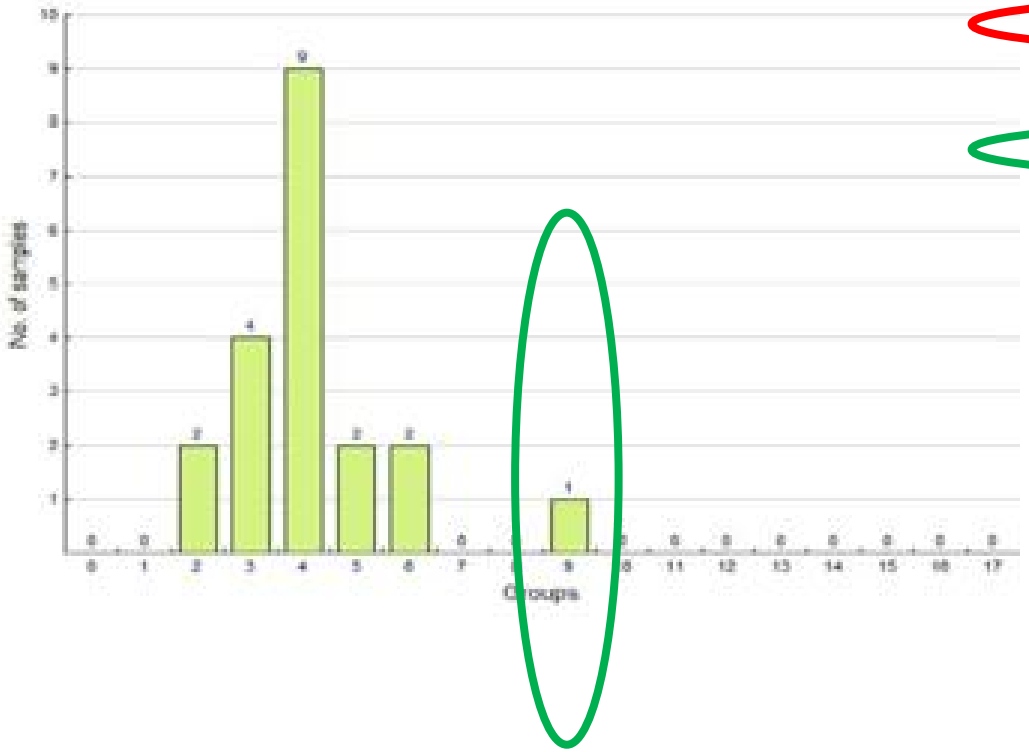


Media	31.559
Mínimo	21.813
Máximo	35.807
G.M.T.	31.245
% CV	14

PRINCIPLE OF INTERPRETATION

Type Flock: Boiler
Vaccination program IBV (Classical + variant)
Bleeding: 41 days
Baseline expected: 5000 – 9000 - 25-50%
challenge >10 000 or max > 12 000

SUSPICION OF RECENT CHALLENGE



Mean	5,496
Minimum	2,306
Maximum	14,813
G.M.T.	5,004
% CV	50

GOOD VACCINATION!!!!!

MAX TITER = A GOOD INDICATOR OF A RECENT CHALLENGE !!!!!

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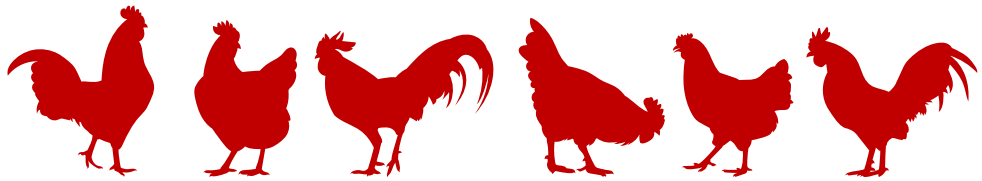
CASE STUDIES

BRAZILIAN CASE : 39 d broiler flocks



CASE 1 : BRAZILIAN BROILER FLOCKS

Broiler flocks, 39 days old

Vaccination program: Mass vaccine (1x)

with respiratory destress

Expected baseline:

1000-3000
40-80%
Suspicion > 4000
Max > 6000

➢ **SUSPICION OF CHALLENGE**

Mean titer < 1000
HIGH PERCENTAGE OF NEGATIVE SAMPLES
NO FIELD CHALLENGE!

COMPETITOR

IBVARSV2

IN ALL CASES :
BR1 FIELD STRAIN
DETECTED BY PCR !!!!!

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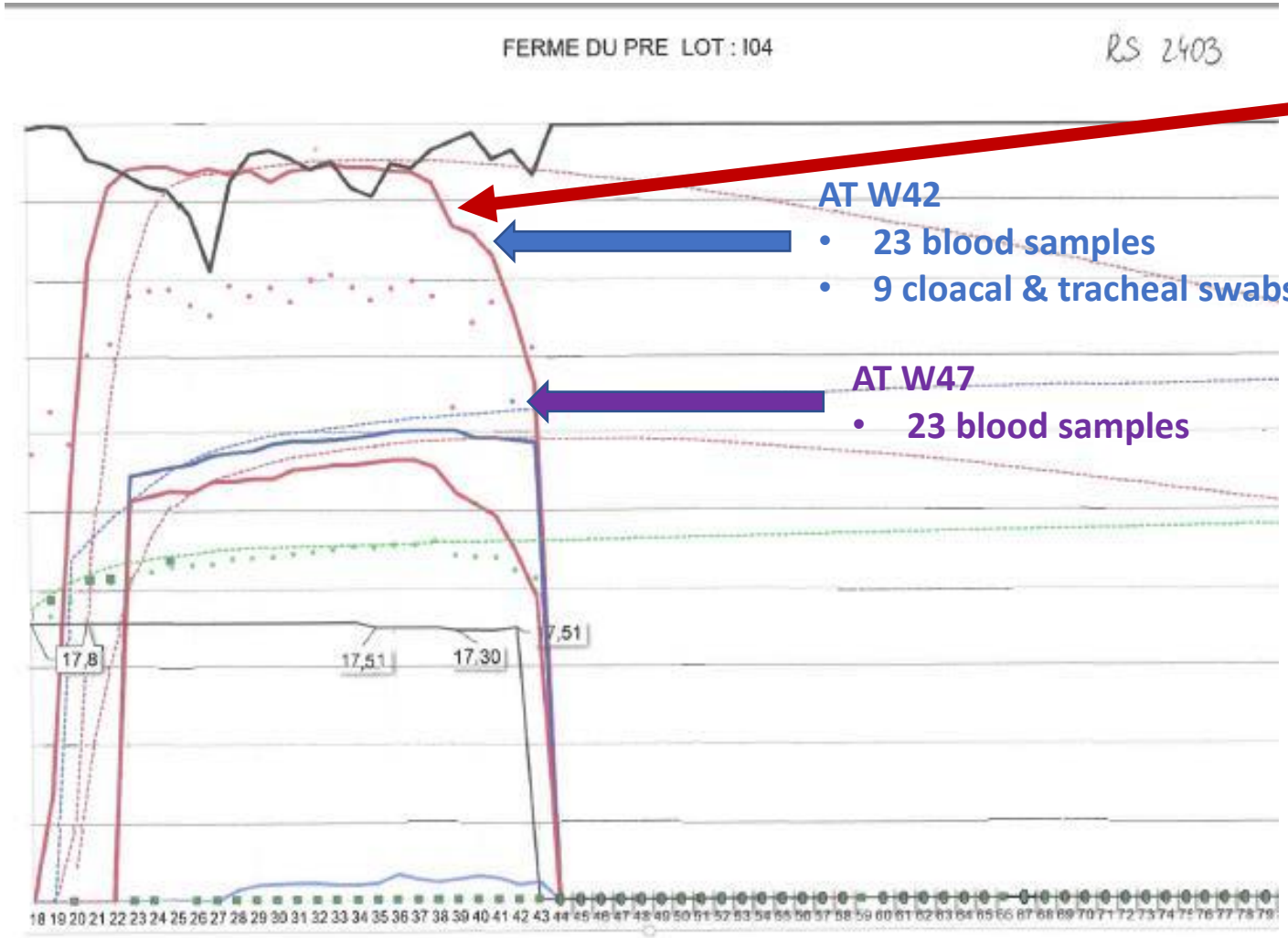
FRENCH CASE : 42w layer flocks

CASE 2 : LAYER FLOCK I04 FRANCE



EGG DROP AROUND W40

AT W42

- 23 blood samples
- 9 cloacal & tracheal swabs

AT W47

- 23 blood samples

CASE 2 – IB DIAGNOSTIC
W42 and W47 BLEEDING

Expected titers:

9000-13000

Suspicion max > 17 000

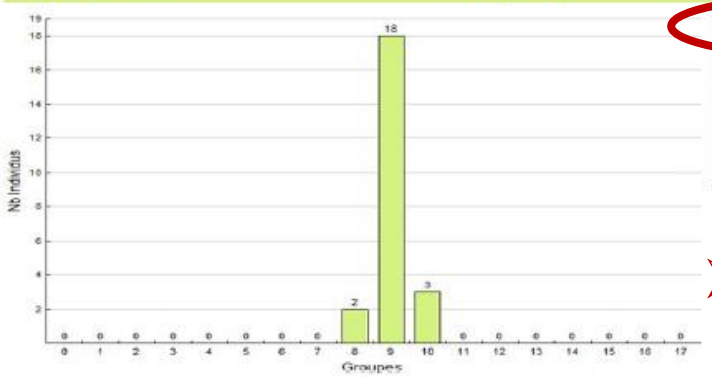
Vaccination program:

Killed vaccine : W16

2x live BI (Ma5 + 4.91) : W26 and W34

➢**SUSPICION OF IB CHALLENGE**

IBVARSV2-0223 Ferme du pré - 20230529-37 - 29/05/2023 12:57:18

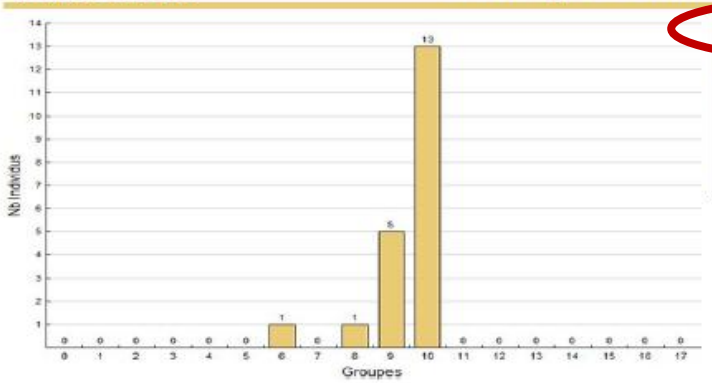


Moyenne	15 890
Minimum	13 161
Maximum	18 171
G.M.T.	15 833
% CV	9

Exploitation	Ferme du pré
Troupeau N°	Lot I04
Type de lot	Pondeuses
Age	42 Semaine(s)
Nb Individus	23
Positif	23 (100%)

➢**ABOVE THE BASELINE**

IBVARSV2-0223 Ferme du pré - 20230529-38 - 29/05/2023 12:58:57



Moyenne	17 163
Minimum	9 932
Maximum	19 053
G.M.T.	16 996
% CV	13

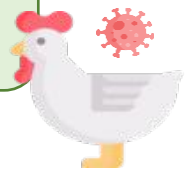
Exploitation	Ferme du pré
Troupeau N°	Lot I04
Type de lot	Pondeuses
Age	47 Semaine(s)
Nb Individus	20
Positif	20 (100%)

➢**TITER INCREASE BETWEEN W42 AND W47**

FIELD CASE – PCR RESULTS CLOACAL/TRACHEAL SWABS AT

MSH VACCINE STRAIN

NEED OF SEQUENCING



	MGMS			FLUA-MS		IBV		NDV	
Valeur de Ct	FAM - MG	VIC-MS	CY5-IC endo	FAM - FLUA	VIC - IC endo	FAM - IBV	VIC - IC exo	M - NDV	VIC - IC exo
EC Trach 1	-	26,70	22,25	-	24,78	-	24,60		
EC Trach 2	-	22,08	23,25	-	25,67	35,23	24,59	-	25,13
EC Trach 3	-	21,98	19,84	-	22,58	-	24,09	-	25,12
EC Trach 4	-	28,6	21,87	-	24,00	-	24,7	-	25,00
EC Trach 5	-	30,10	24,44	-	27,03	-	24,	-	25,19
EC Trach 6	-	24,30	22,59	-	24,94	-	24	-	25,07
EC Trach 7	-	25,59	21,82	-	23,82	-	3	-	25,08
EC Trach 8	-	23,90	23,89	-	26,10	-	4,48	-	25,35
EC Trach 9	-	22,18	21,48	-	23,88	-	24,58	-	25,15
EC Trach	-	23,16	22,65	-	24,93	-	24,75	-	25,35
EC Cloac 1	-	32,18	28,62	-	29,02	25,71	24,38	-	25,28
EC Cloac 2	-	31,80	29,95	-	31,06	31,10	24,63	-	25,10
EC Cloac 3	-	-	22,53	-	25,13	30,44	24,94		
EC Cloac 4	-	33,67	29,82	-	30,11	-	24,55		
EC Cloac 5	-	-	30,45	-	32,07	-	24,83		
EC Cloac 6	-	-	29,32	-	30,61	28,53	24,69		
EC Cloac 7	-	-	31,00	-	29,30	21,43	24,59		
EC Cloac 8	-	-	25,60	-	27,95	-	24,76		
EC Cloac 9	-	-	27,34	-	29,74	-	24,71	-	25,28
NEC eau	-	-	-	-	-	-	25,02	-	25,59
PAC	26,15	25,55	-	28,37	-	27,50	-	27,21	-
NAC	-	-	-	-	-	-	-	-	-

LAST 4/91 LIVE VACCINE APPLIED :
W34 OF AGE
8 WPV

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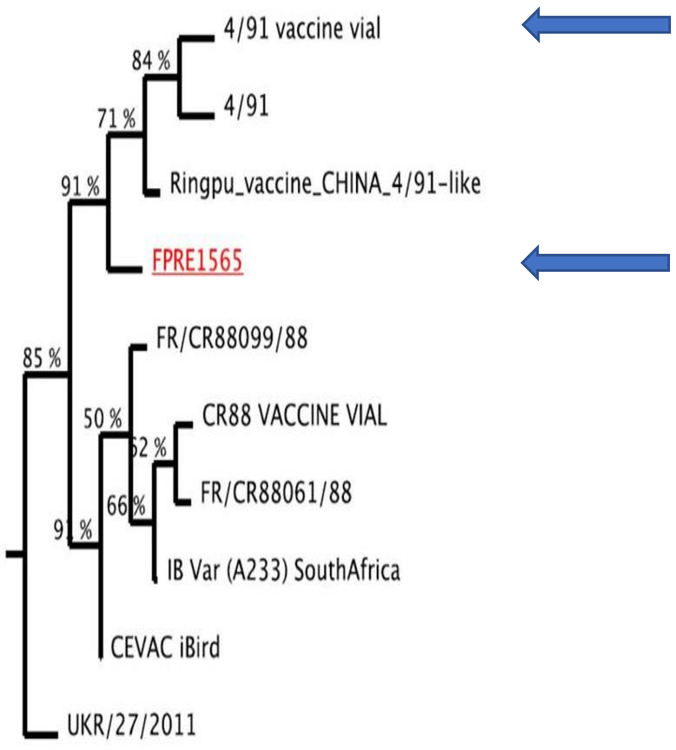
FIELD CASE – SEQUENCING RESULTS ON POSITIVE CLOACAL SWABS

Appendix 1 – Phylogenetic Tree



Predicted Epitope Alignment

```
                              61                                                         120
                              ....:....|....:....|....:....|....:....|....:....|....:....|
VIR21/8558 XOVO-2021-1550  |  ----~SVAMTVPPAGMSWSVAQFCTAHCNFSDFTVFVTHCFKNQQGSCPLTGMIPQNHIR
4/91 vaccine vial          |  --------MTVPPAGMSWSVSQFCTAHCNFSDFTVFVTHCFKSQQGSCPLTGMIPQNHIR
CR88 VACCINE VIAL          |  ---------~TVPHNGMSWSVSQFCTAHCNFSDFTVFVTHCFKNQLGSCPLTGMIPQNHIR
CEVAC iBird                |  NISAASVAMTVPLNGMSWSVSQFCTAHCNFSDFTVFVTHCFKSQQGSCPLTGKIPQHHIR
                                       ***   ****** ******************** * ******* *** ***
                              NISAASVAMTVPXXGMSWSVXQFCTAHCNFSDFTVFVTHCFKXQXGSCPLTGXIPQXHIR

                              121                                                        180
                              ....:....|....:....|....:....|....:....|....:....|....:....|
VIR21/8558 XOVO-2021-1550  |  ISAMRSGSLFYNLTVSVSKYPKFKSLQCVGNSTSVYLNGDLV-----------------
4/91 vaccine vial          |  ISAMRSGFLFYNLTVSVSKYPKFKSLQCVGNSTSVYLN~---------------------
CR88 VACCINE VIAL          |  ISAMRDGVLFYNLTVSVSKYPRFKSLQCVSNSTSV~------------------------
CEVAC iBird                |  ISAMRDRTLFYNSTVTVSKYPRFKSLQCVSNLTAVYLNGDLVFTSNETSYVTGAGVYFRS
                              *****   **** ** ***** ******* * * *
                              ISAMRXXXLFYNXTVXVSKYPXFKSLQCVXNXTXVYLNGDLVFTSNETSYVTGAGVYFRS
```

Conclusion

The analysis confirmed the presence of a 793B field virus in a cloacal FTA card sample from 42 week old layers last vaccinated with 4/91 34 weeks of age.

Analysis of the predicted epitope alignment of the field virus relative to the 4/91 vaccine vial sequence demonstrated two areas of mismatch.

CONCLUSIONS ON IBVARSV2

The new ID Screen® Infectious Bronchitis Indirect 2.0 kit (cat. **IBVARSV2**):

- ✓ The only one kit based on a **well conserved protein**
- ✓ For a **better detection of all strains**, including variant strains

AS A CONSEQUENCE

- ✓ **A MORE POWERFUL TOOL FOR THE MONITORING OF LIVE VACCINES**
- ✓ OFFERS A **POSSIBLE DETECTION OF CHALLENGE**, REGARDLESS OF THE STRAIN, EVEN UNDER HEAVY VACCINE COVERAGE

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With you at every step

THANK YOU!

For any question, please contact:

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