

Diagnostic trends in Scotland and Ireland 2010 - 2015

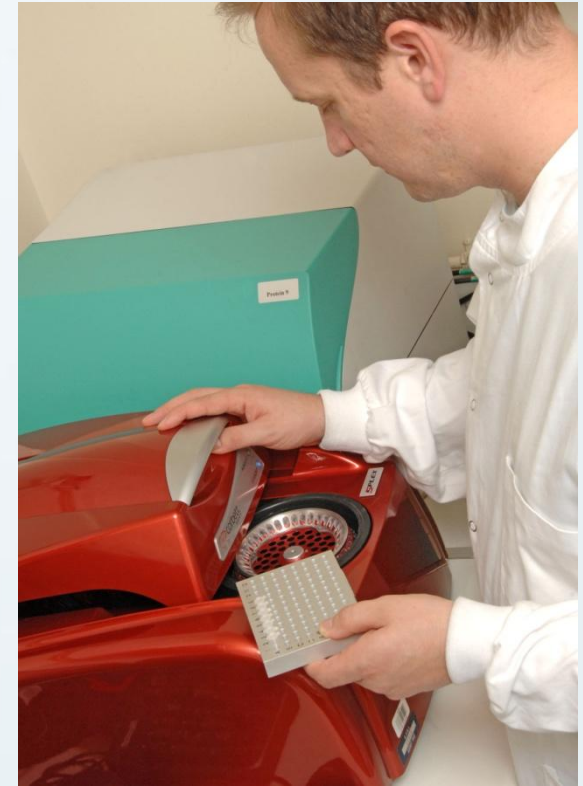
Filipe Nunes/Paul Savage

TriNation Meeting 2015

Dublin, Ireland

SAV RT-qPCR

- ISO 17025 Accredited
- Taqman One step RT-qPCR
- Primers target conserved region of nsP1 gene (SAV1 – SAV6)
- Published methodology (Hodneland & Endresen 2006)
- Duplex (SAV nsP1 and ELF α gene)
- Viral subtyping by sequencing of nsP3 or E2 genes



SAV Serology vs. Virus Isolation

SAV Serology

Objective:

Detection of neutralizing antibodies against SAV

Procedure

Add serum sample to 100 TCID₅₀ of SAV for 2 hours and incubate with CHSE cells for 3 days. Stain cell monolayers to detect SAV.

Results

Positive SAV Staining:

NeuAB **NOT PRESENT** in serum sample

Negative SAV Staining:

NeuAB **PRESENT** in serum sample

SAV Serology vs. Virus Isolation

SAV Serology

Objective:

Detection of neutralizing antibodies against SAV

Procedure

Add serum sample to 100 TCID₅₀ of SAV for 2 hours and incubate with CHSE cells for 3 days. Stain cell monolayers to detect SAV.

Results

Positive SAV Staining:

NeuAB **NOT PRESENT** in serum sample

Negative SAV Staining:

NeuAB **PRESENT** in serum sample

SAV Virus isolation

Objective:

Detect the presence of replicating SAV virus

Procedure

Add serum sample to CHSE cells and incubate for 3 days. Stain cell monolayers to detect SAV.

Results

Positive SAV Staining or CPE:

Live virus **PRESENT** in serum sample

SAV Negative Staining or CPE:

Live Virus **NOT PRESENT** in serum sample

SAV Sample Submission

	Serum	Heart	Kidney	Gill	Pancreas	Muscle
Virus Isolation	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
SAV RT-qPCR	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/> ¹				
SAV Serology	<input checked="" type="checkbox"/> ²					
Histology		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

¹ SAV RT-qPCR may be positive up to 1 year post-infection.

² Post-infection SAV antibodies persist until harvest.

SAV Diagnostic Scenarios

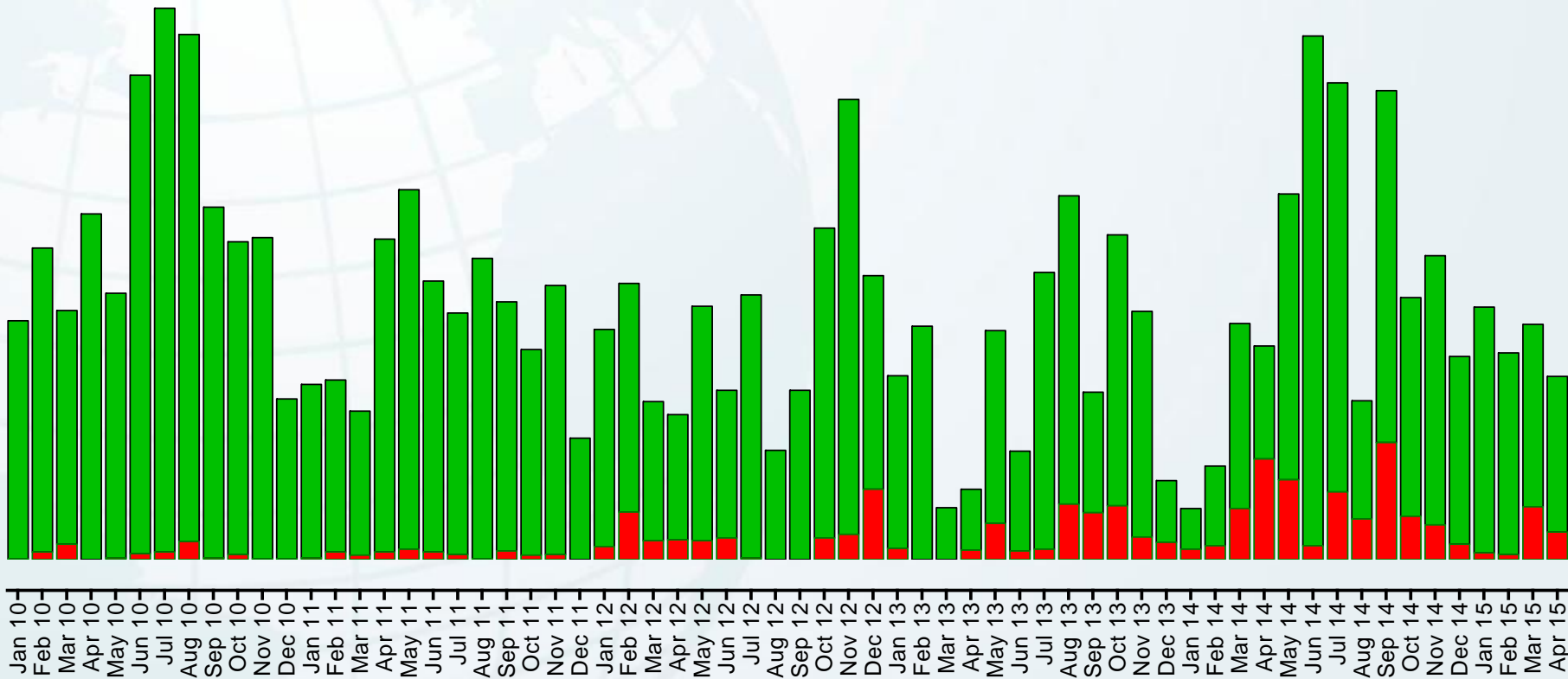
	SAV Neutralizing Antibodies (serum)	SAV Virus Isolation (serum)	SAV RT-qPCR (serum)	SAV RT-qPCR (tissue)	Comment
Scenario 1	-	-	-	-	Most likely not SAV Infection
Scenario 2	+	-	-	-	Previous SAV infection
Scenario 3	+	+	+	+	Current SAV infection
Scenario 4	+	-	-/+	+	Late stages of SAV infection
Scenario 5	-	+	+	-/+	Early Stages of SAV infection



SAV Diagnostic Trends at AFBI Fish Disease Unit

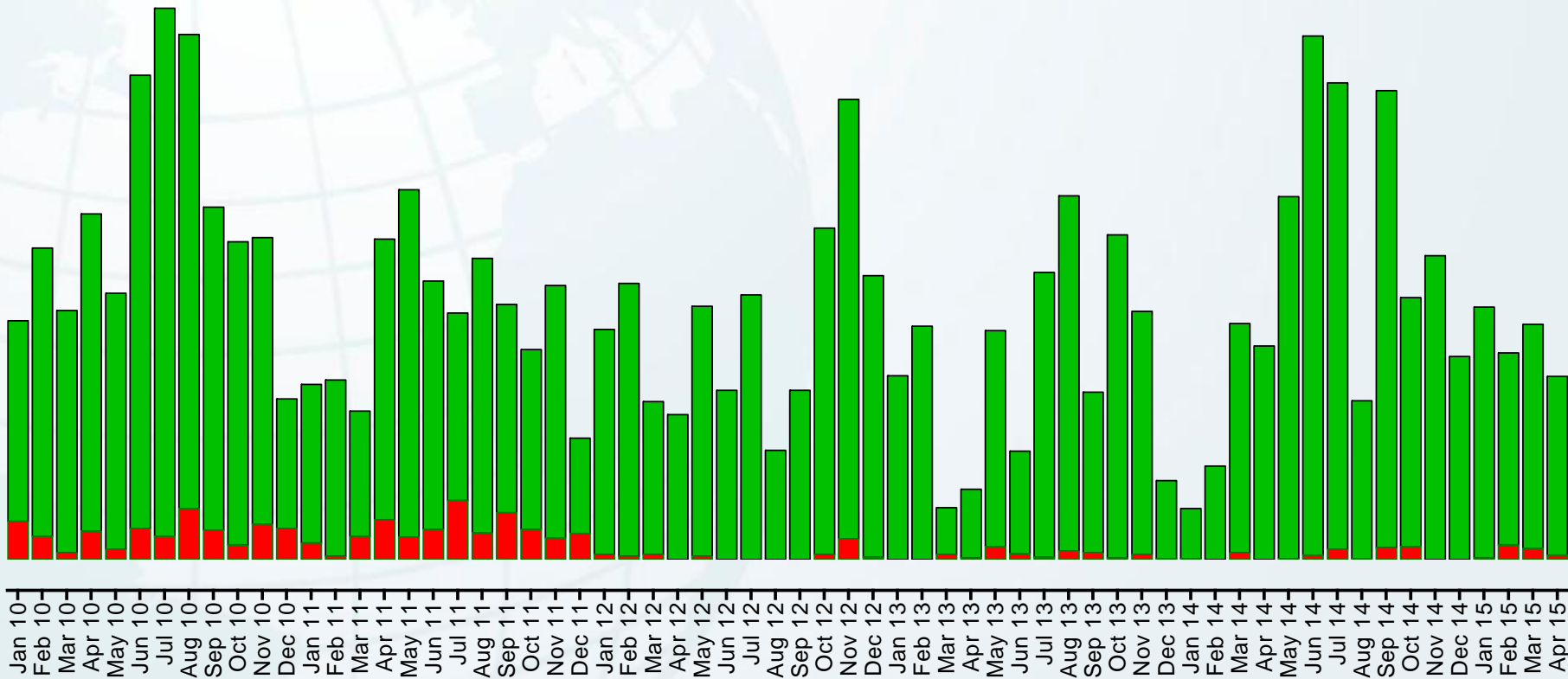
SAV Serology

Neutralizing Antibodies in Serum Samples



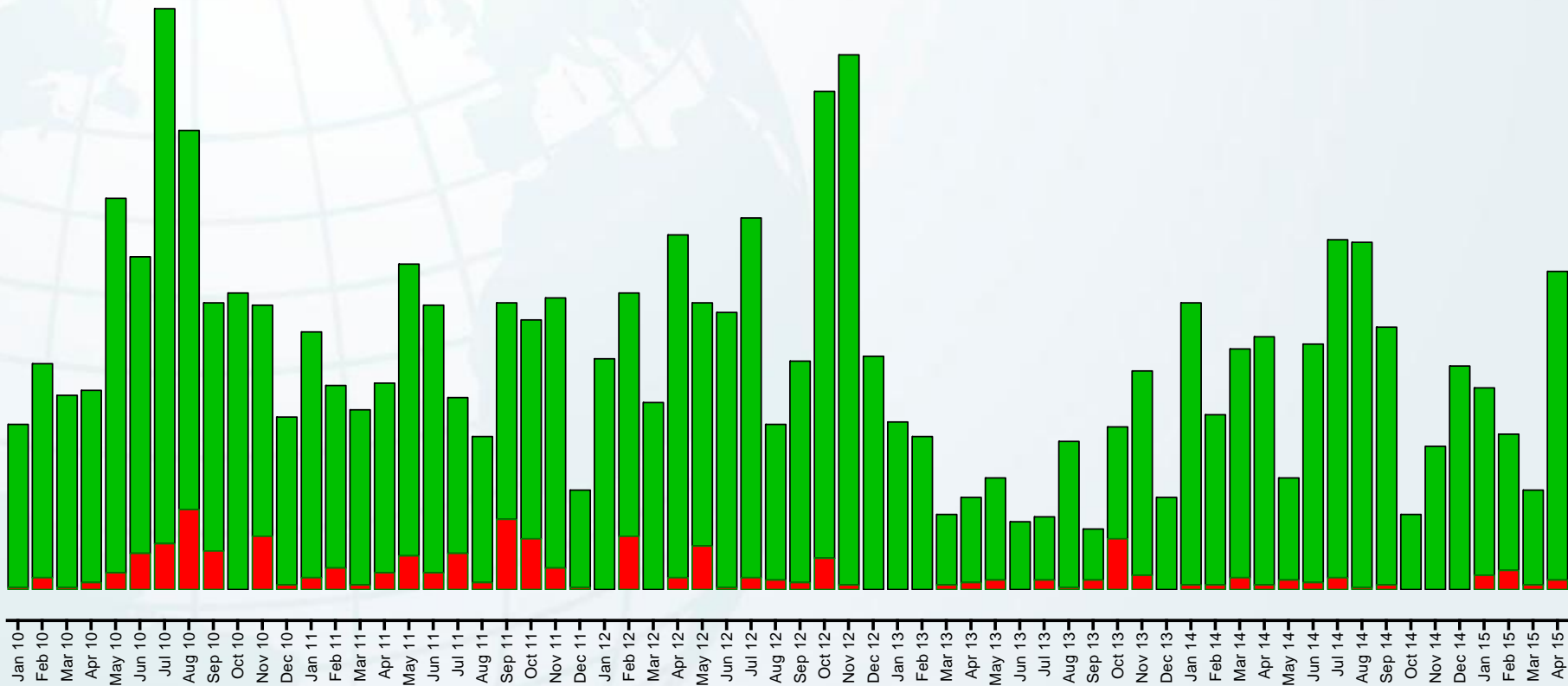
SAV Virus Isolation in Serum

SAV Virus Isolation



SAV Diagnostic RT-qPCR

SAV PCR (Serum and Tissue)

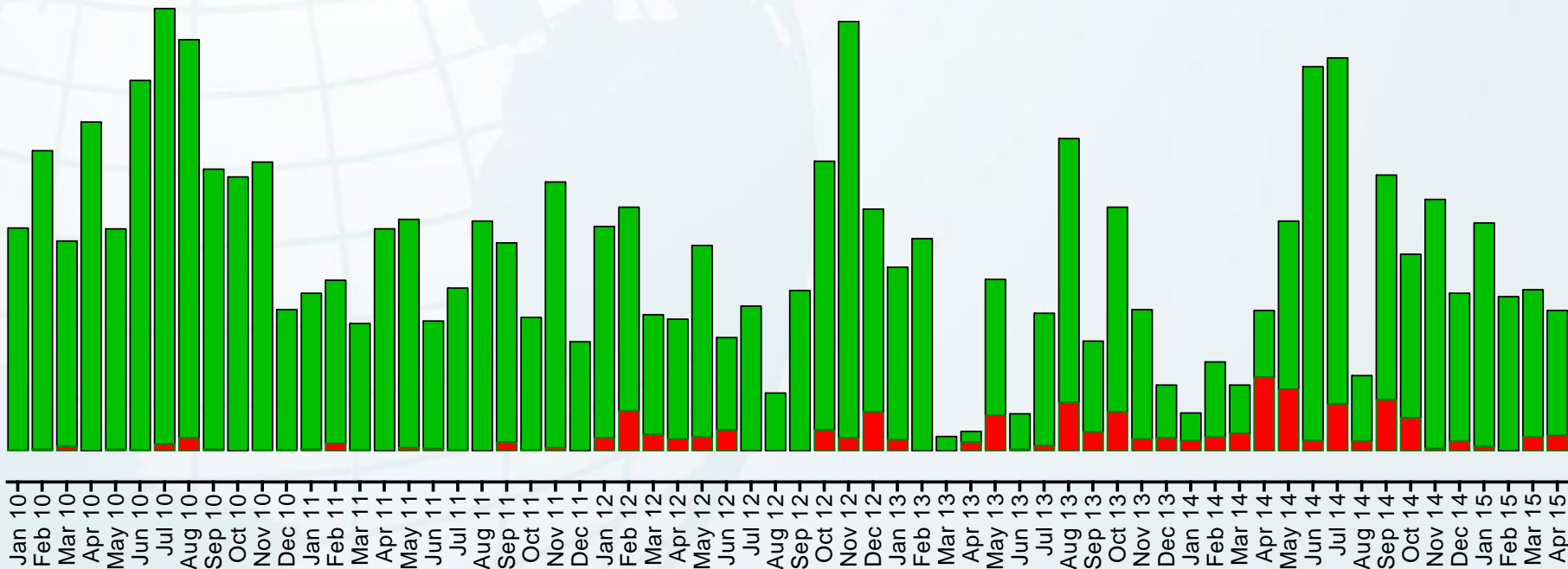




SAV Diagnostic Trends in Samples from Scotland

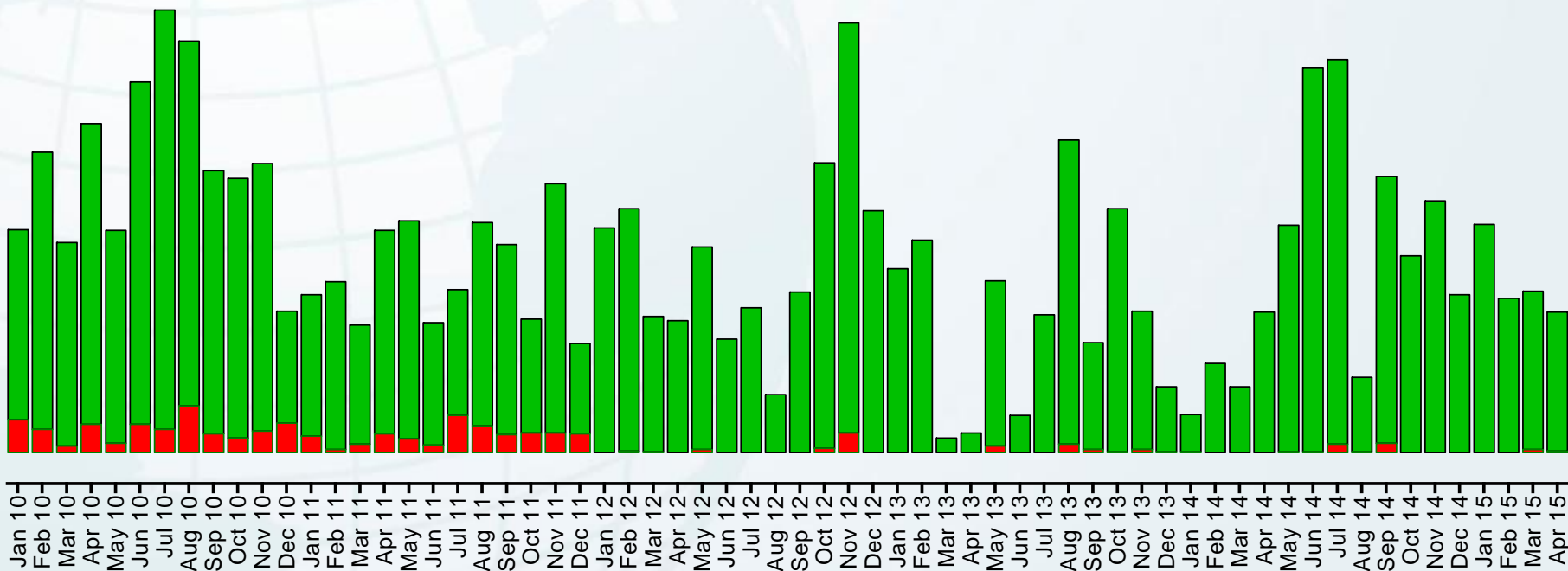
Scotland – SAV Serology

Neutralizing Antibodies in Serum Samples



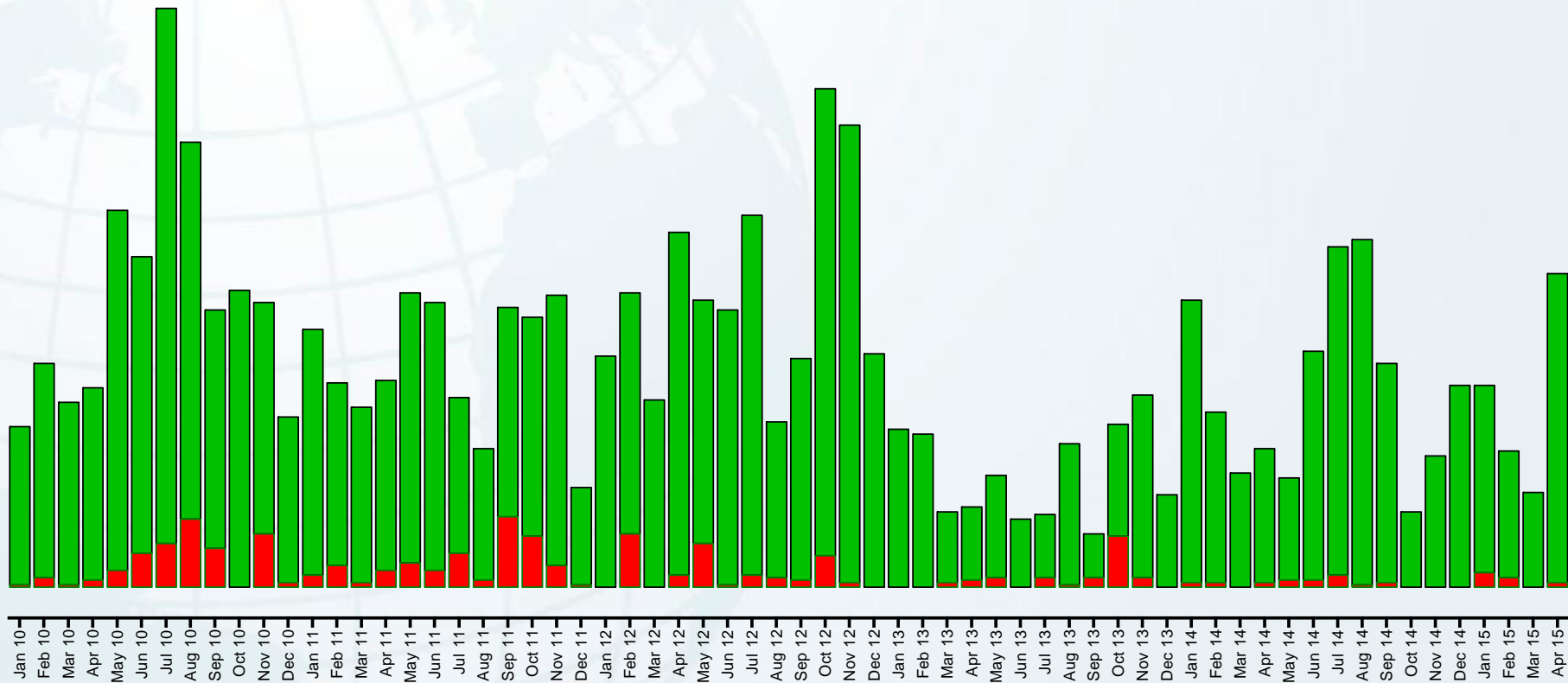
Scotland – SAV Virus Isolation in Serum


SAV Virus Isolation



Scotland – SAV Diagnostic RT-qPCR

SAV PCR (Serum and Tissue)

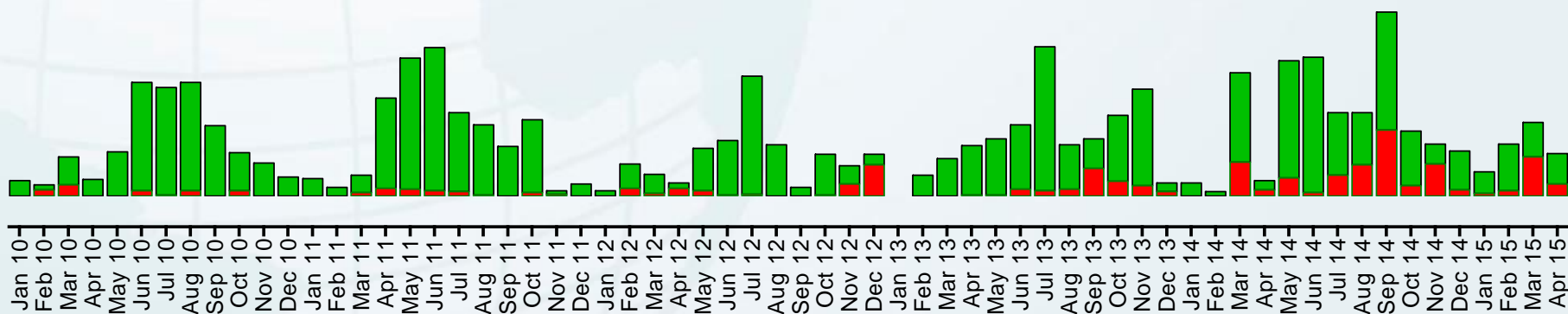




SAV Diagnostic Trends in Samples from Ireland

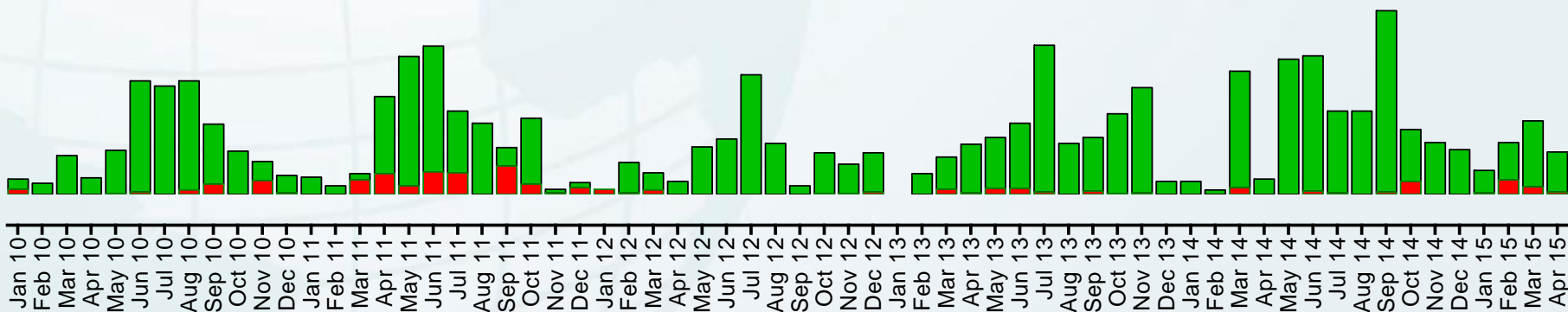
Ireland – SAV Serology

Neutralizing Antibodies in Serum Samples



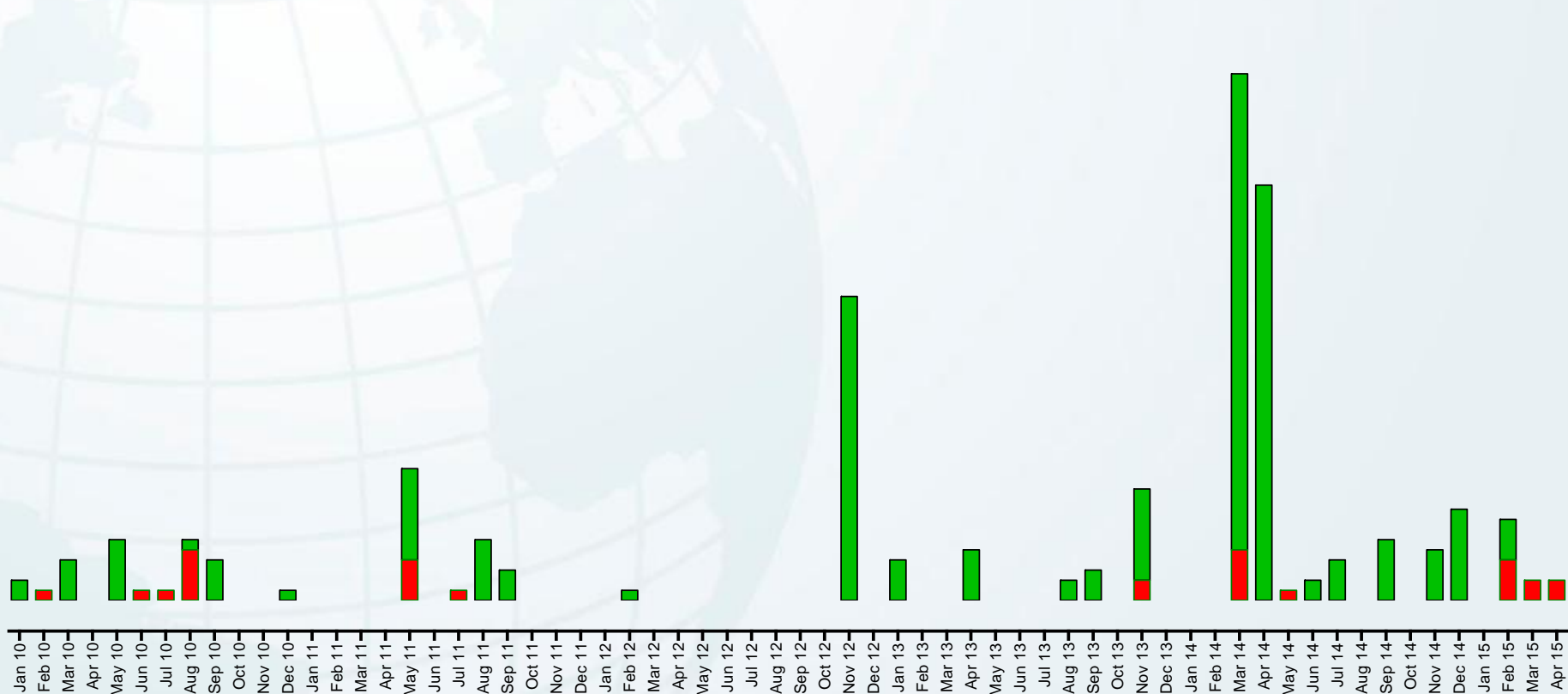
Ireland – SAV Virus Isolation in Serum

SAV Virus Isolation



Ireland – SAV Diagnostic RT-qPCR

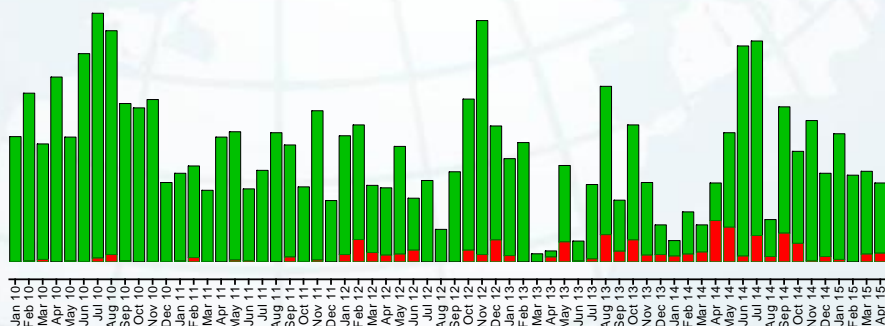
SAV PCR (Serum and Tissue)



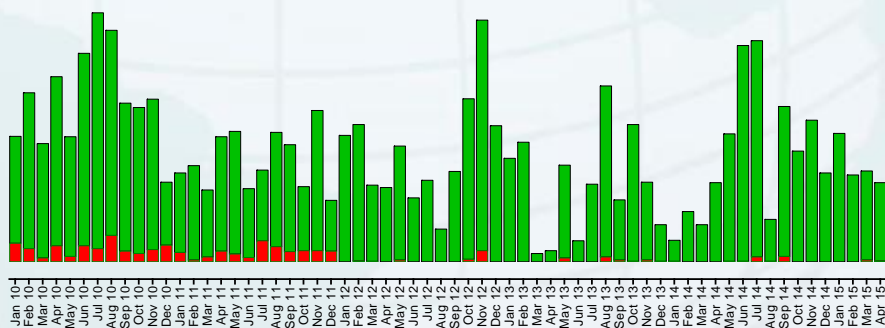
SAV Trends

Scotland

Neutralizing Antibodies in Serum Samples

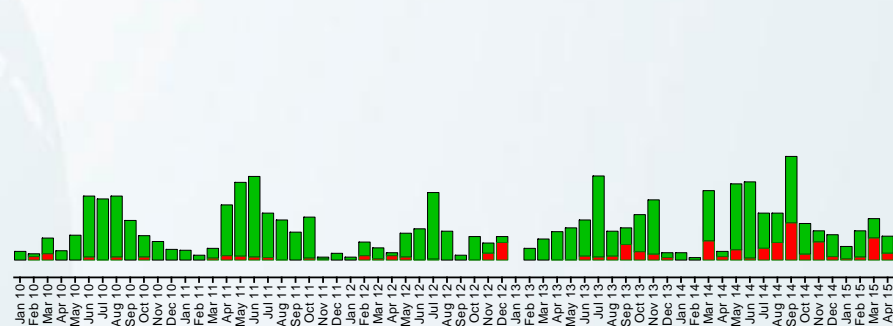


SAV Virus Isolation

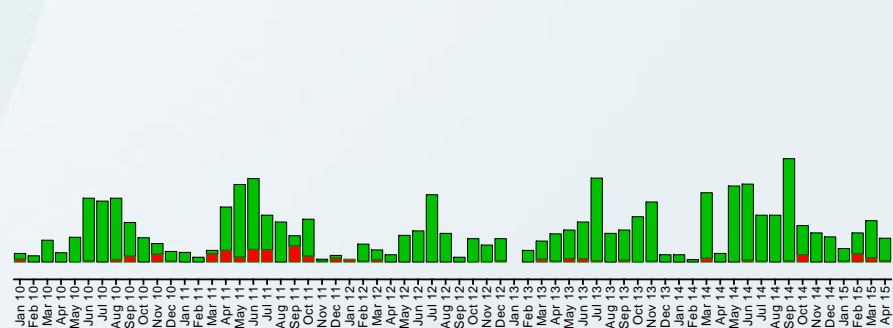


Ireland

Neutralizing Antibodies in Serum Samples



SAV Virus Isolation



SAV Subtypes

Scotland

SAV 1, 2, 4, 5 **2010**

SAV 1, 2, 4, 5 **2011**

SAV 1, 2, 4, 5 **2012**

SAV 2 **2013**

SAV 2, 4, 5 **2014**

SAV 1, 5 **2015**

Ireland

SAV 1, 4

SAV 1

SAV 1, 4

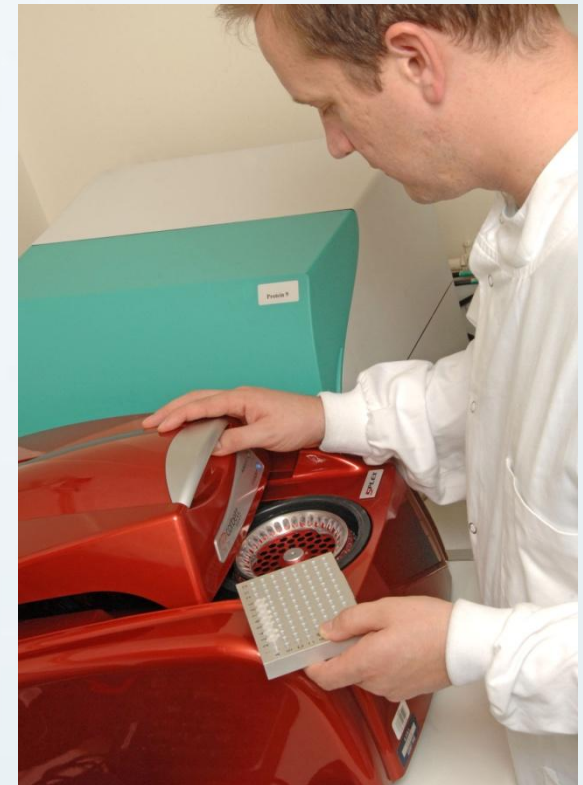
SAV 1, 4

SAV 1, 4

-/-

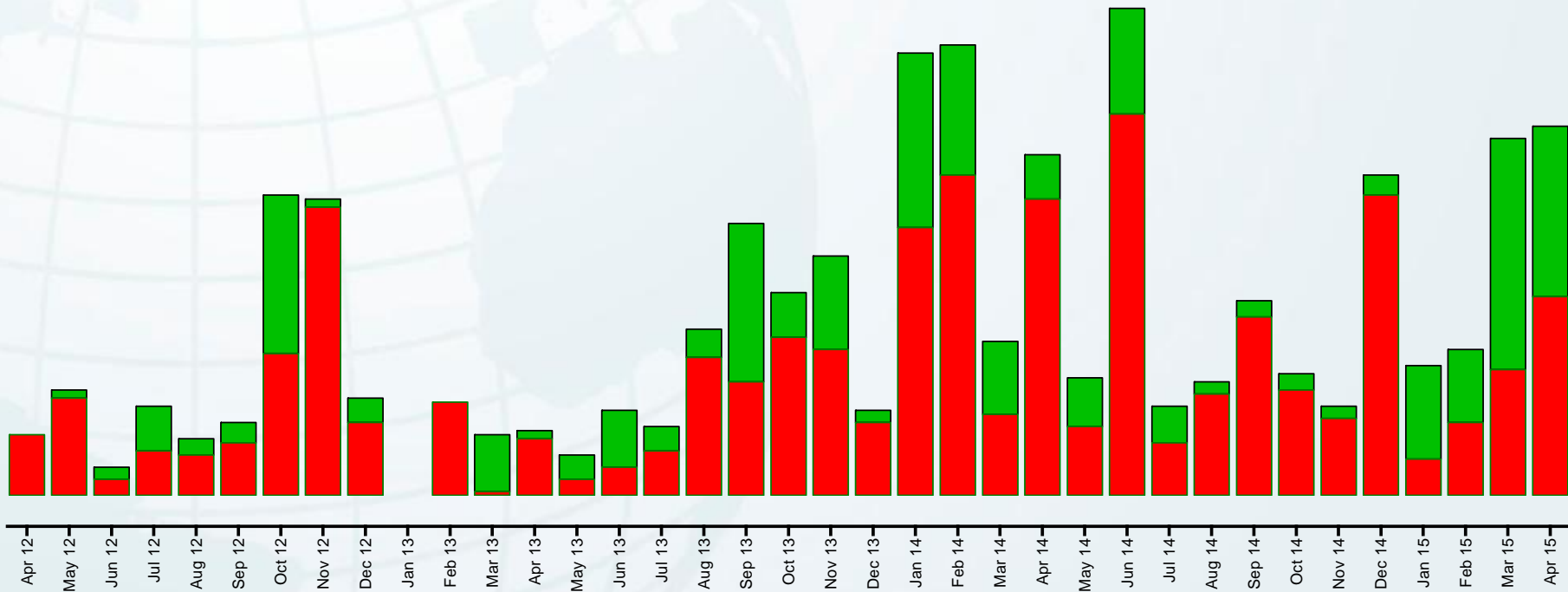
PRV RT-qPCR

- ISO 9001
- One step RT-qPCR Taqman Assay
- Primers target conserved region of L1 segment
- Published methodology (Palacios *et al.* 2010)
- Duplex (PRV L1 and ELF α gene)



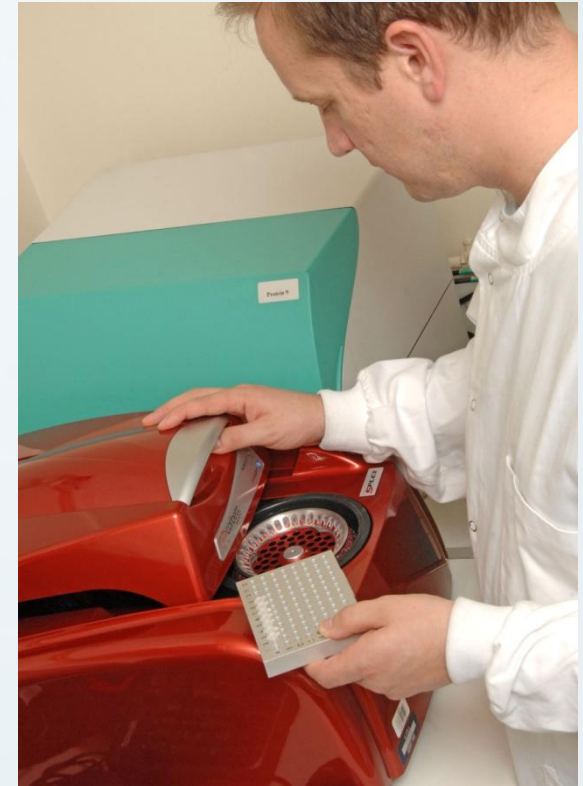
PRV Diagnostic RT-qPCR

PRV PCR (Serum and Tissue)



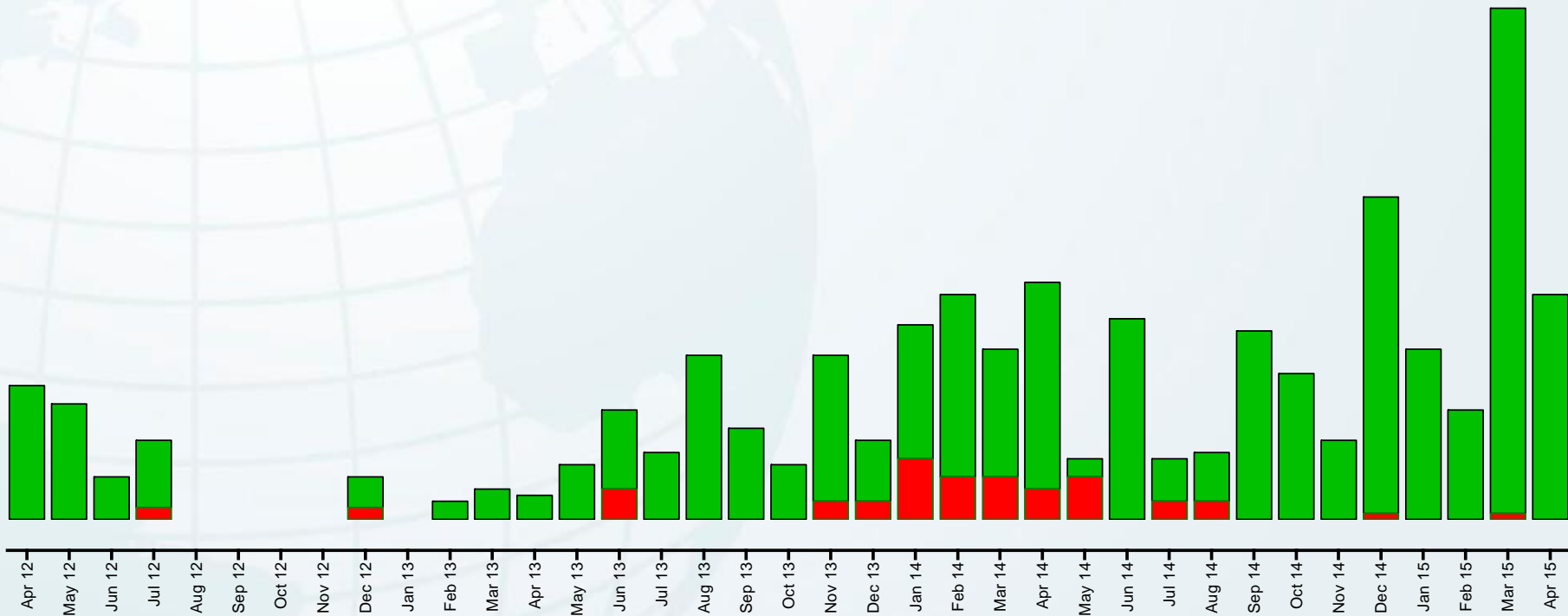
PMCV RT-qPCR

- ISO 9001 Accreditation
- One step RT-qPCR Taqman Assay
- Primers target region of RNA-Dependant RNA Polymerase gene
- Published methodology (Lovoll *et al.* 2010)
- Duplex (PMCV and ELF α gene)



PMCV Diagnostic RT-qPCR

CMS PCR (Serum and Tissue)



Acknowledgments

- FDU Staff Members
- Marian McLoughlin
- David Graham
- Commercial Partners
- Research Collaborators