

# Antigenic structure of PVY

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# Why serology of PVY is important?

- Overwhelming majority of PVY isolates inducing tuber necrosis have N serotype
- In North America, most of field isolates with N serotype are NTN isolates
- Hence, an N-serotype is a logical and straightforward marker of a possible PTNRD induction

# Sources of N-specific antibodies

- 1F5 – produced by Peter Ellis
  - Marketed by Agdia, Inc.
- Bioreba-N – produced by Paul Gugerli
  - Marketed by Bioreba AG
- SASA-N – produced by Robert Burns
  - Marketed by SASA
- Neogen-N – same as SASA?
  - Marketed by Neogen Ltd.

# Antibodies specific to N serotype

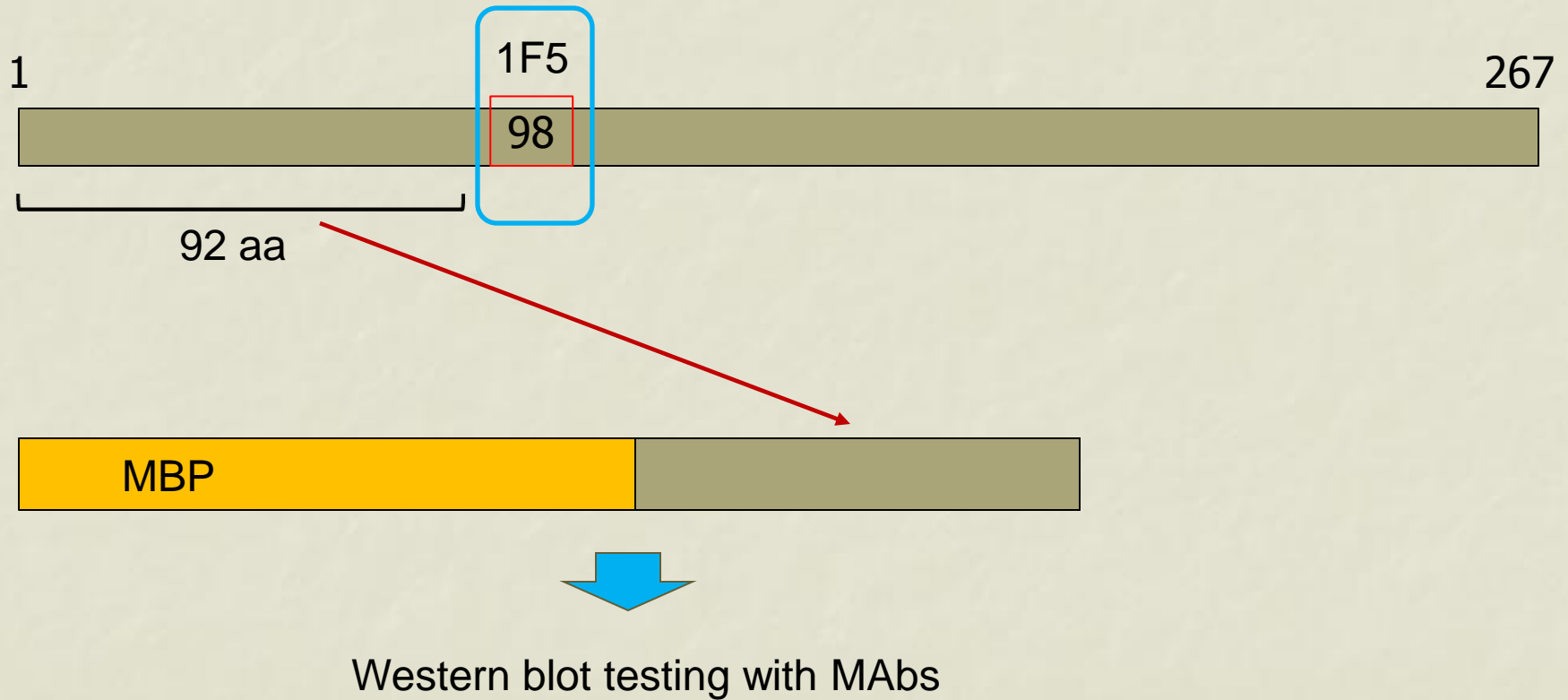
Strain group	1F5	Bioreba-N	SASA-N	Neogen-N
PVY <sup>O</sup>	-	-	-	-
PVY <sup>O</sup> -O5	+	-	-	-
PVY <sup>N:O</sup>	-	-	-	-
PVY <sup>N</sup>	+	+	+	+
PVY <sup>NTN</sup>	+	+	+	+
PVY <sup>NA-N</sup>	+	+	+	+
PVY-NE-11	+	+	+	+
PVY <sup>NTN</sup> -AST	+	+	-	-

# Reactivity of four Mabs against PVY isolates with N serotype

<i>Isolate</i>	<i>PAb</i>	<i>SASA-N</i>	<i>Neogen-N</i>	<i>Bioreba-N</i>	<i>1F5</i>
PVY <sup>N</sup> -Mont	+	+	+	+	+
PVY <sup>Z</sup> -L26	+	+	+	+	+
PVY <sup>NTN</sup> -N4	+	+	+	+	+
PVY <sup>NTN</sup> -423.3	+	+	+	-	+
PVY <sup>NTN</sup> -AST	+	-	-	+	+
PVY <sup>NTN</sup> -SGS-MO	+	-	<i>NT</i>	<i>NT</i>	+
PVY-NE-11	+	+	+	+	+
PVY <sup>E</sup> -MON	+	+	+	+	+
PVY <sup>E</sup> -AGA	+	-	-	+	+

Assay: TAS-ELISA

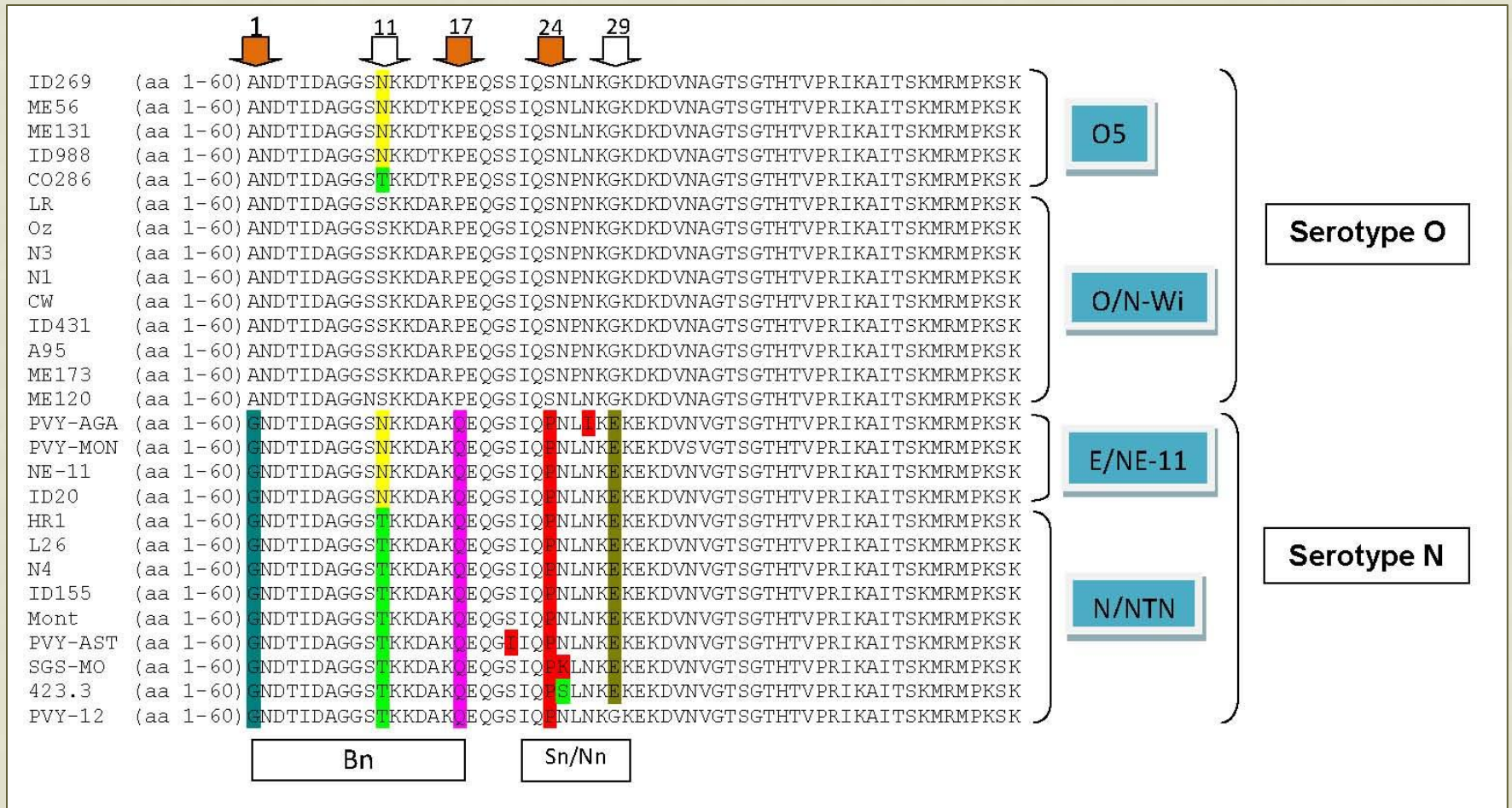
# Positioning of N-specific epitopes



# Reactivity of PVY<sup>N</sup> antigens with different MAbs

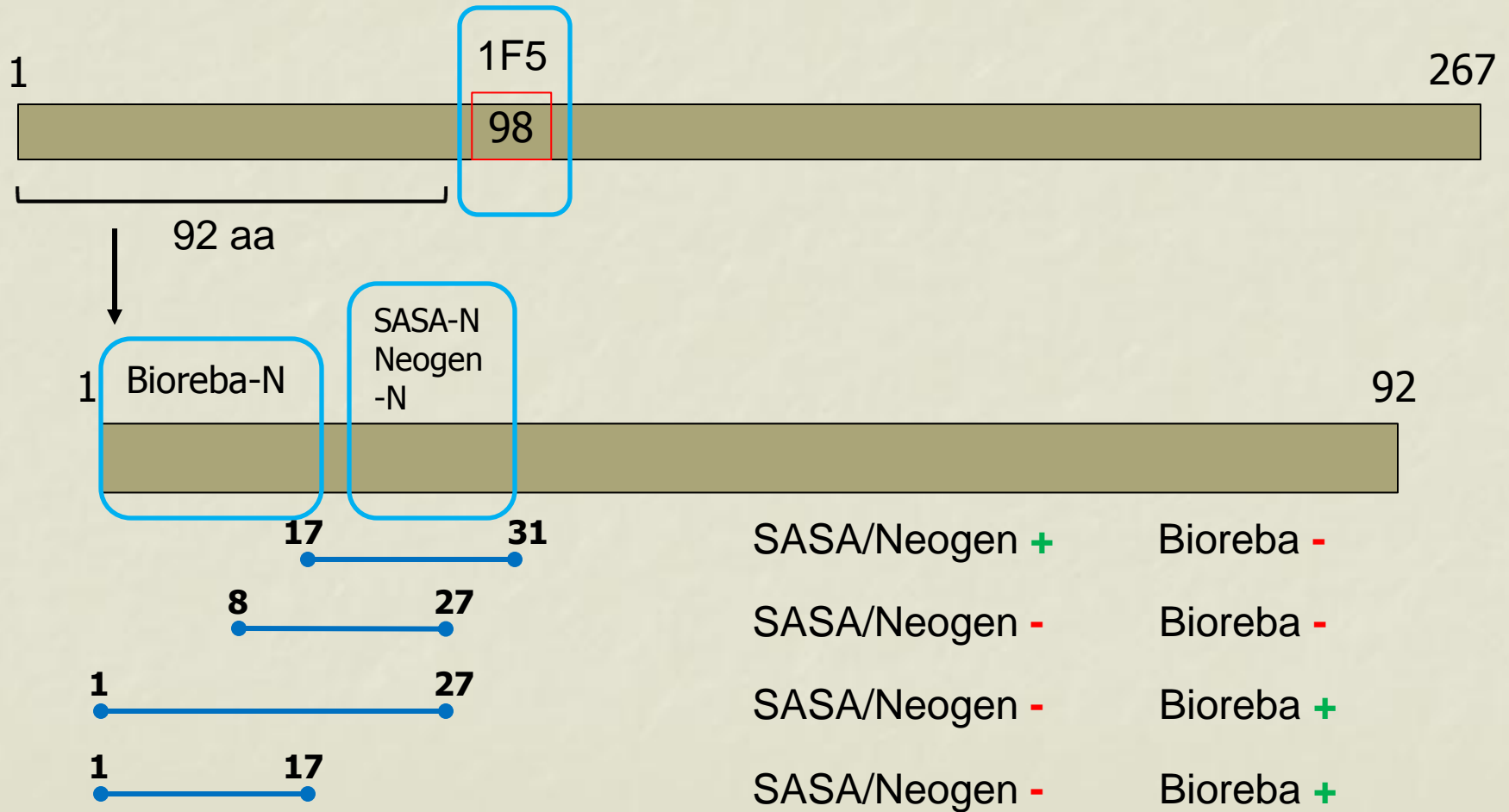
	Whole CP		N-terminus	Peptides
	<i>TAS-ELISA</i>	<i>Western</i>	<i>Western</i>	<i>ELISA</i>
1F5	+	-	-	-
Bioreba-N	+	+	+	+
SASA-N	+	+	+	+
Neogen-N	+	+	+	+

# Alignment of the PVY CP sequences





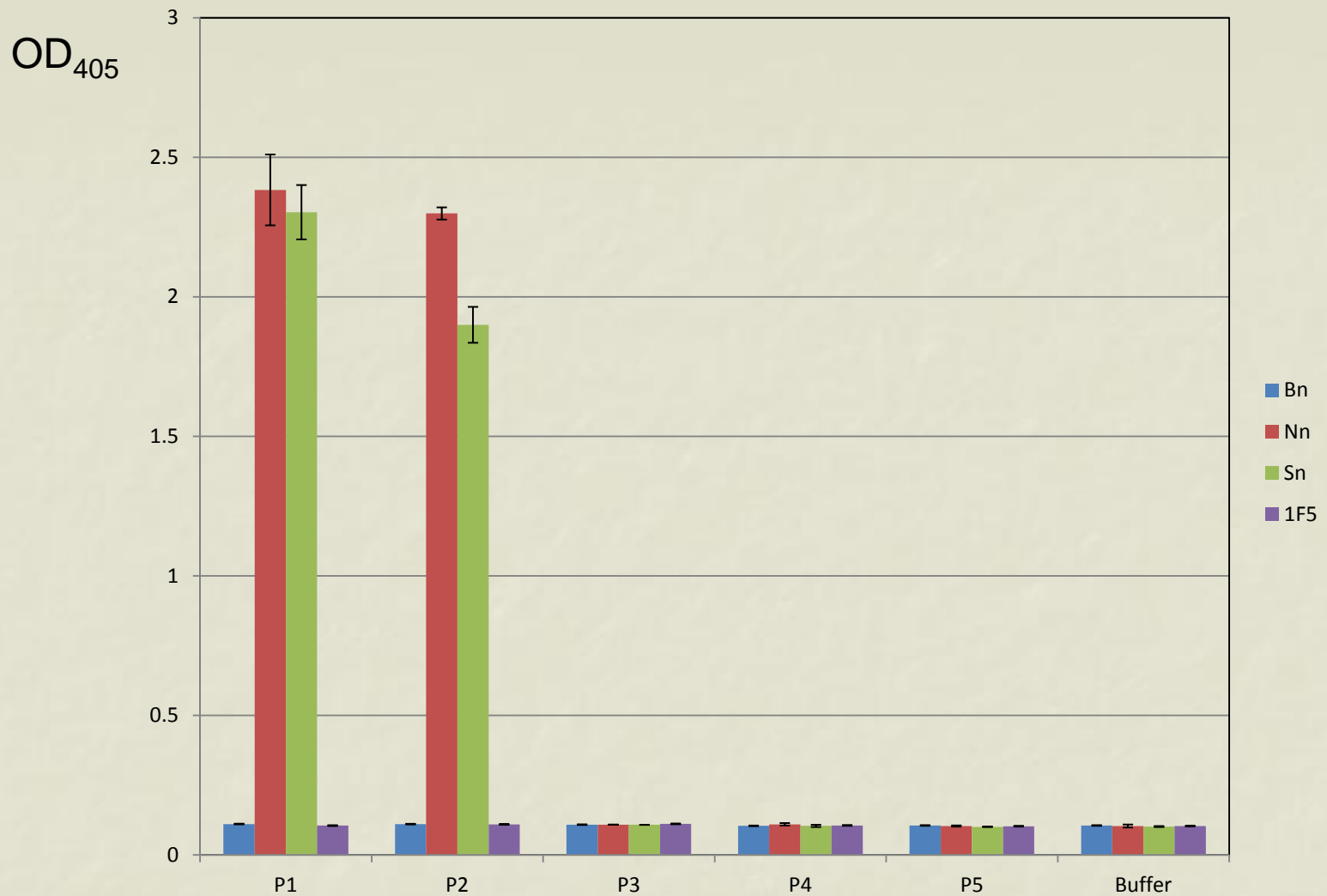
# Positioning of N-specific epitopes



# Summary of peptide testing

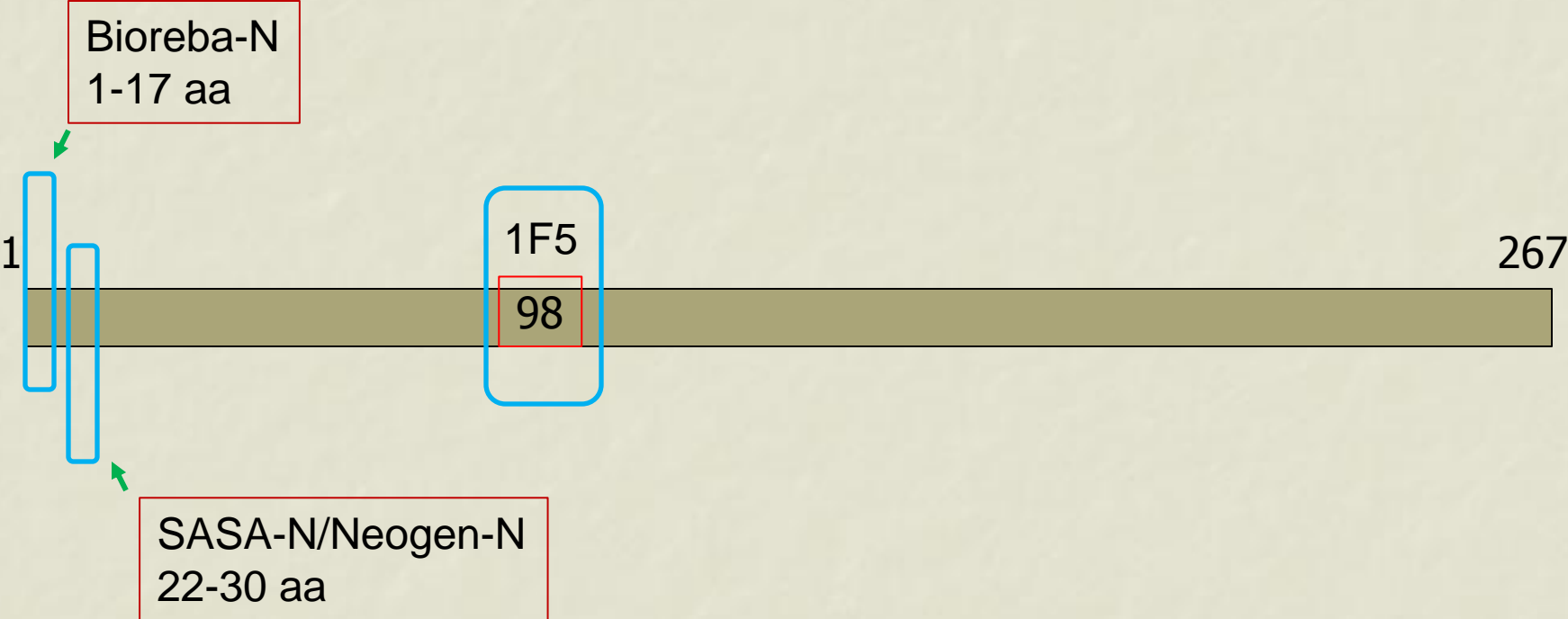
	Peptide ID	Peptide length, aa	Sequence	Bioreba-N		SASA-N		Neogen-N	
				D	C	D	C	D	C
1.	mon17_31	15	QEQGSIQP <sup>N</sup> LNKEKE	-	-	+++	++	+++	++
2.	ast17_31	15	QEQG <sup>I</sup> IQP <sup>N</sup> LNKEKE	-	-	+++	++	+++	++
3.	aga17_31	15	QEQGSIQP <sup>N</sup> L <sup>I</sup> KEKE	-	-	-	-	-	-
4.	423_17_31	15	QEQGSIQP <sup>N</sup> L <sup>S</sup> KEKE	-	-	-	-	-	-
5.	pvy12_17_31	15	QEQGSIQP <sup>N</sup> L <sup>N</sup> KGKE	-	-	-	-	-	-
6.	unk17_31	15	QEQGSIQP <sup>N</sup> LNKEK <sup>D</sup>	-	-	+++	++	+++	++
7.	hr1_1-17	17	GNDTIDAGG <sup>S</sup> TKKDAKQ	+++	++	-	-	-	-
8.	id20_1-17	17	GNDTIDAGG <sup>S</sup> NKKDAKQ	-	+	-	-	-	-
9.	l26_8-27	20	GGSTKKDAKQEQGSIQP <sup>N</sup> LN	-	-	-	-	-	-
10.	423_8-27	20	GGSTKKDAKQEQGSIQP <sup>S</sup> LN	-	-	-	-	-	-
11.	hr1_1-27	27	GNDTIDAGG <sup>S</sup> TKKDAKQEQGSIQP <sup>N</sup> LN	+++	++	-	-	-	-
12.	423_1-27	27	GNDTIDAGG <sup>S</sup> TKKDAKQEQGSIQP <sup>S</sup> LN	+++	++	-	-	-	-
13.	id20_1-27	27	GNDTIDAGG <sup>S</sup> NKKDAKQEQGSIQP <sup>N</sup> LN	+++	+	-	-	-	-

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Reactivity of peptides P1-P5 with Mabs Bioreba-N (Bn), Neogen-N (Nn), SASA-N (Sn), and 1F5 (control) in plate-trapped, indirect ELISA.

# Locations of N-specific epitopes



PVY capsid protein, aa 1-267

# Conclusions

- There are 3 distinct epitopes defining the N serotype: 1F5, Bioreba-N, and SASA-N
- MAb 1F5 binds a conformational, SDS-sensitive epitope
- Epitopes for MAbs Bioreba-N, SASA-N, and Neogen-N are linear, SDS-insensitive epitopes

# Conclusions - 2

- Epitope for the MAb 1F5 includes position 98 in the CP sequence
- Epitope for MAb Bioreba-N spans aa 1-17
- Epitopes for MAbs SASA-N and Neogen-N are identical or overlapping, and span aa 22-30

# Definition of the PVY N-serotype

- To be considered an N-serotype, a PVY isolate must be negative for an O-specific MAb
- It must also react with at least 1 of the N-specific MAbs, like 1F5, Bioreba-N, SASA-N, or Neogen-N
- Keep in mind that MAbs SASA-N and Neogen-N are likely identical

# Take home messages:

- Not all N-specific MAbs react to all PVY isolates with N-serotype
- If you use more than one N-specific MAb, reliability of your typing is much better
- But remember that SASA-N and Neogen-N have identical specificities!



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