Development of serological Luminex assays for Trichinella and Salmonella in swine

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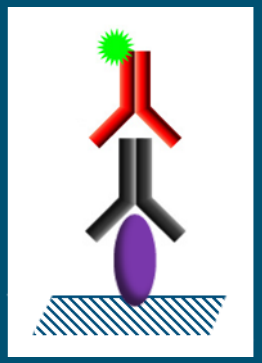
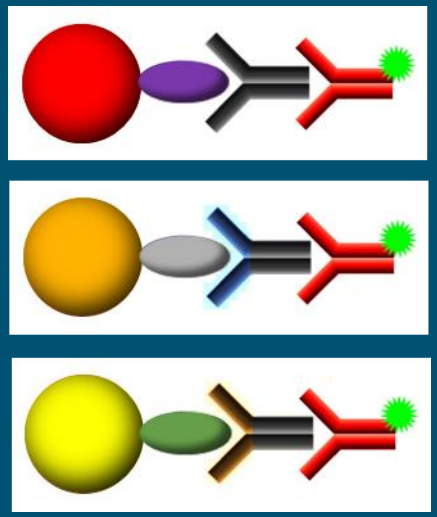
Suspension array

- Serology: mostly ELISA
  - passive coating of antigen
    - solid support (96-well plate)
    - one antigen
    - mix of antigens

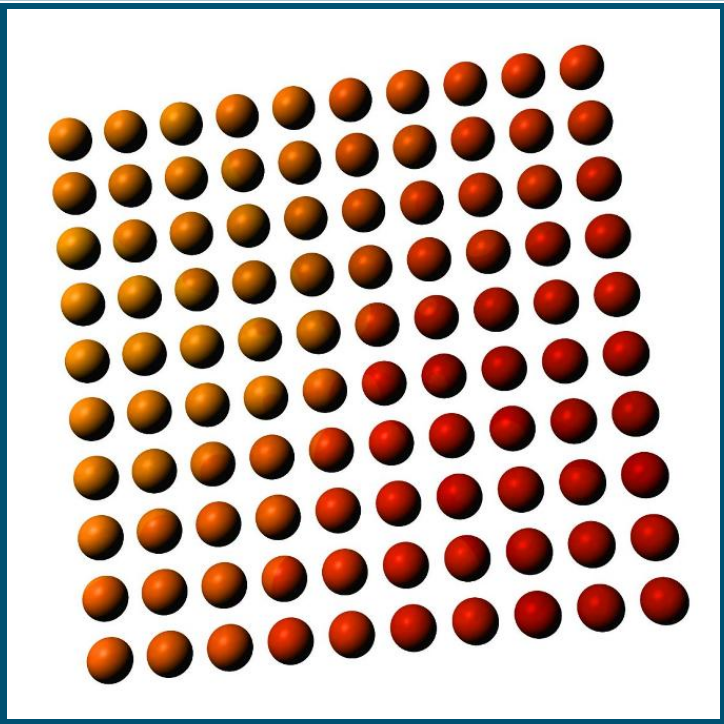


- Bead based suspension array:
  - covalent coupling of antigens
    - beads in suspension
    - one antigen per bead set
      - multiplexing

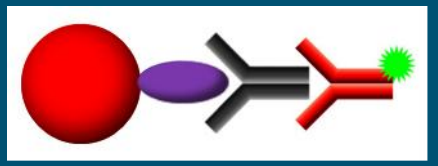


Suspension array

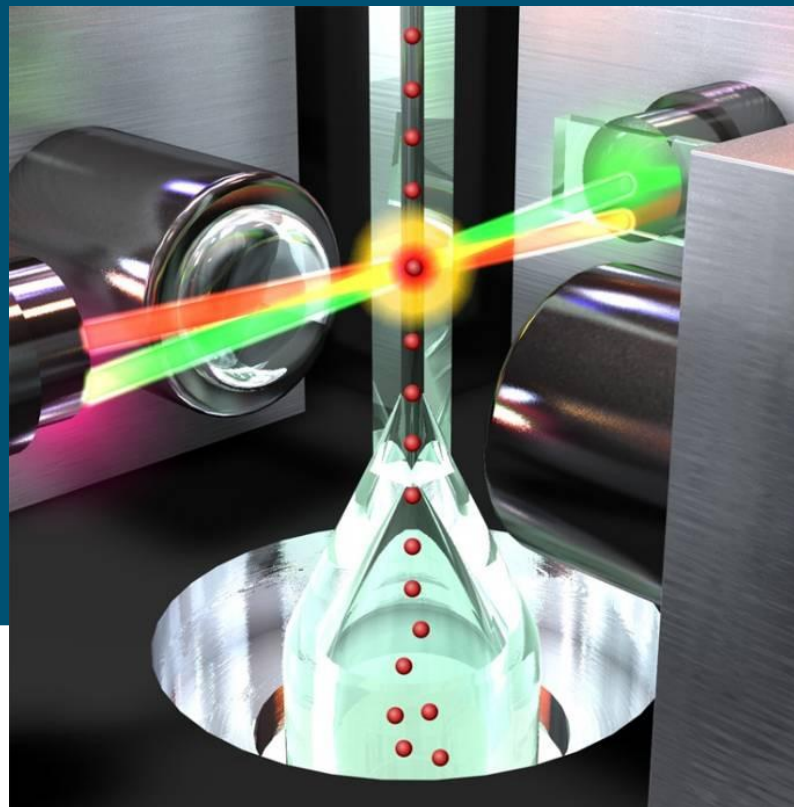
- Luminex
  - polystyrene beads
    - paramagnetic
      - handling
    - functionalized surface
      - carboxylated
    - sets with distinctive colors
      - multiplexing
  - detection platform
    - flow cytometry (100plex / 500plex)
    - magnet / CCD imaging (50plex)



Suspension array

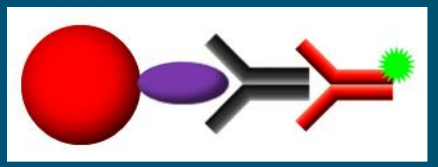


- Serology
  - conjugate antigen to beads
  - catch antibody from serum sample
  - create sandwich with a fluorescent antibody
    - phycoerythrin labeled
    - biotinylated / reporter
      - streptavidin-phycoerythrin
  - perform flow cytometry
    - red laser identifies beads
    - green laser detects presence of fluorescence

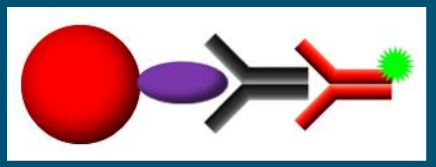


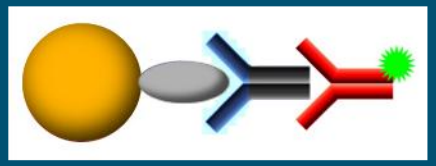
Goal: multiplex serology for swine

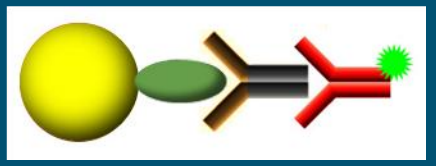
- Suspension array for Trichinella
  - test Luminex platform for serology in pigs



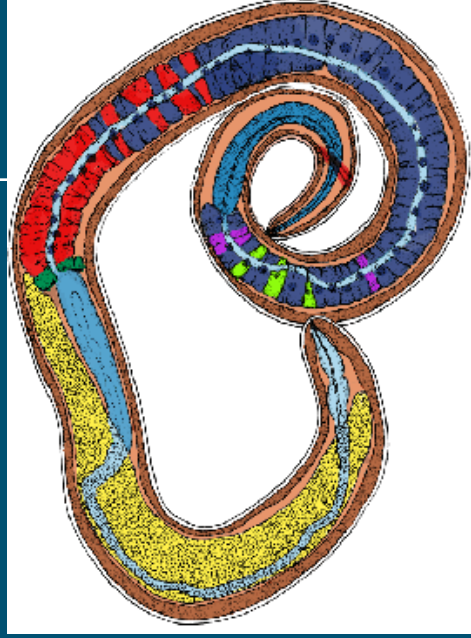
- Suspension array for Salmonella
  - multiplex serology in pigs
  - LPS-based







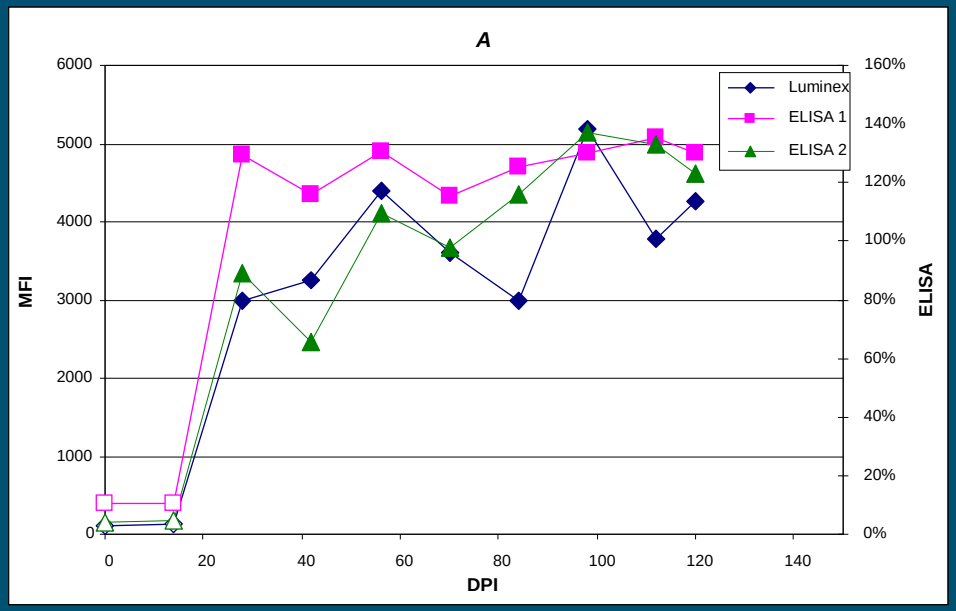
Trichinella test drive

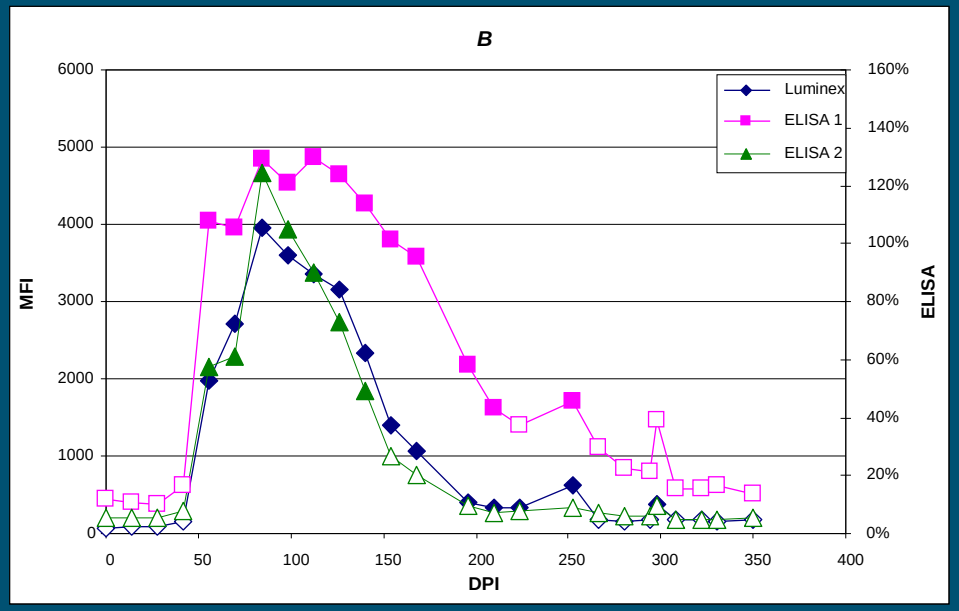


- Trichinella serology in pigs
  - zoonotic pathogen
    - parasite, travels through tissue
    - in meat of pigs and other mammals
    - Netherlands/NW Europe is free (intensive farming)
  - coupling of antigen
    - extracellular/secreted proteins (OIE manual)
    - sulfo-NHS/EDC (generic protocol)
    - free amines to carboxylated bead surface
  - assay protocol (comparable to ELISA)
    - beads + serum / anti-swine (biotinylated) / reporter
  - optimization
    - serum dilution
    - assay buffer
    - concentration secondary antibodies / reporter
    - incubation times

Trichinella test drive

- Serum from Trichinella infected pigs





- bead-based Luminex assay follows commercial ELISA's

Trichinella test drive

- Field sera
  - Dutch pigs are negative
  - sera from Argentina

		ELISA 1		
		pos	neg	total
Luminex	pos	43	1	44
	neg	12	183	195
	total	55	184	239

se 78% o.a. 95%
sp 99% c.k. 0.83

		ELISA 2		
		pos	neg	total
Luminex	pos	41	3	44
	neg	2	198	200
	total	43	201	244

se 95% o.a. 98%
sp 99% c.k. 0.93

		ELISA 1		
		pos	neg	total
ELISA2	pos	44	0	44
	neg	12	185	197
	total	56	185	241

se 79% o.a. 95%
sp 100% c.k. 0.85

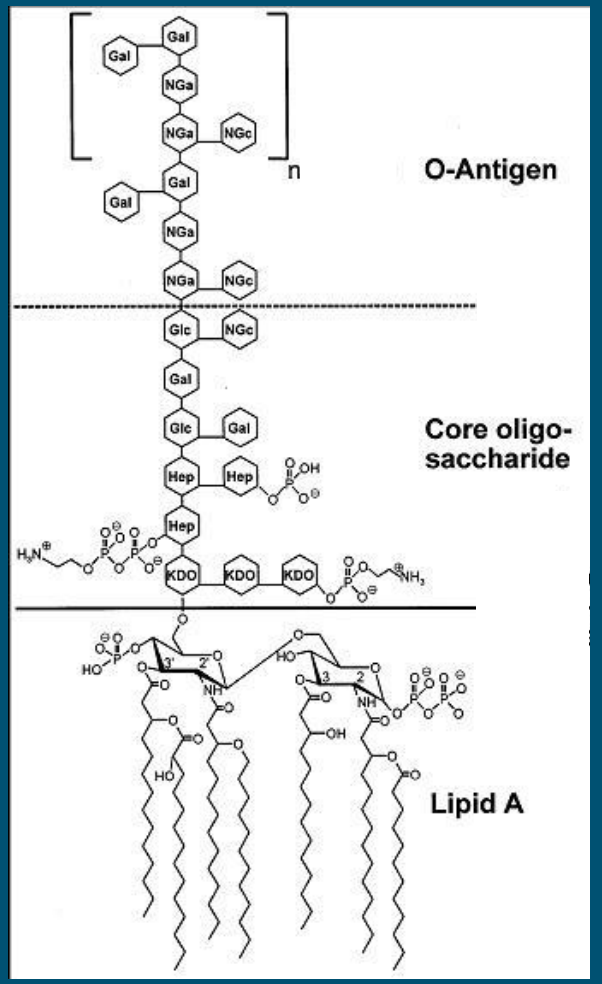
- bead-based suspension array is in agreement with ELISA's
- Luminex platform is suitable for veterinary serology

Salmonella serological suspension array

- Salmonella
  - foodborne pathogen
    - a.o. animal products
    - gastroenteritis
  - Denmark: Salmonella surveillance programme since 1995
    - routine testing / classification / revision
      - bacteriology at various levels
      - serology (random sampling) of herds (1.000.000 samples/year)
      - classification / actions to reduce Salmonella
    - Salmonella prevalence decreases
  - The Netherlands
    - farm: 31 pigs or more: 12 serum samples
    - slaughterhouse: bacteriology on carcasses
    - classification in category 1-3 / cat. 3 advise to take actions to reduce Salmonella

Salmonella serology in pigs using LPS

- Commercial assays
  - ELISA
    - three serogroups used: B, C1 & D LPS
  - surface plasmon resonance (Biacore)
    - B, C1 & D LPS
      - high background by C LPS
      - LPS mix with C difficult to couple
    - as good as ELISA
    - not in production anymore
- Suspension array
  - also based on LPS: B, 3xC, D
    - couple individual LPS types to separate beads
    - define individual cut-offs



Anspach 2001 JBBM

Salmonella serology in pigs using LPS

serogroup	subsp./serovar	O-antigens
B	S. Typhimurium	O1, O4, O5, O12
C1	S. Choleraesuis	O6, O7
C1	S. Livingstone	O6, O7
C2	S. Newport	O6, O8
D	S. Enteritidis	O1, O9, O12

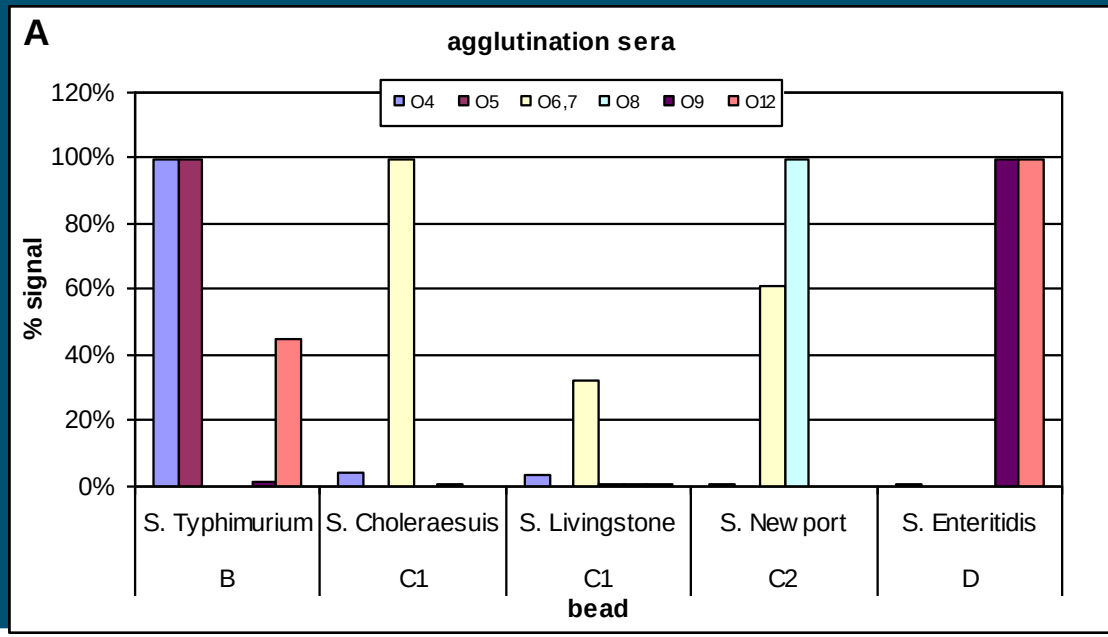
- LPS
  - coupling to 5 beads
  - test agglutination sera
    - used for serotyping Salmonella

Salmonella serology in pigs using LPS

serogroup	subsp./serovar	O-antigens
B	S. Typhimurium	O1, O4, O5, O12
C1	S. Choleraesuis	O6, O7
C1	S. Livingstone	O6, O7
C2	S. Newport	O6, O8
D	S. Enteritidis	O1, O9, O12

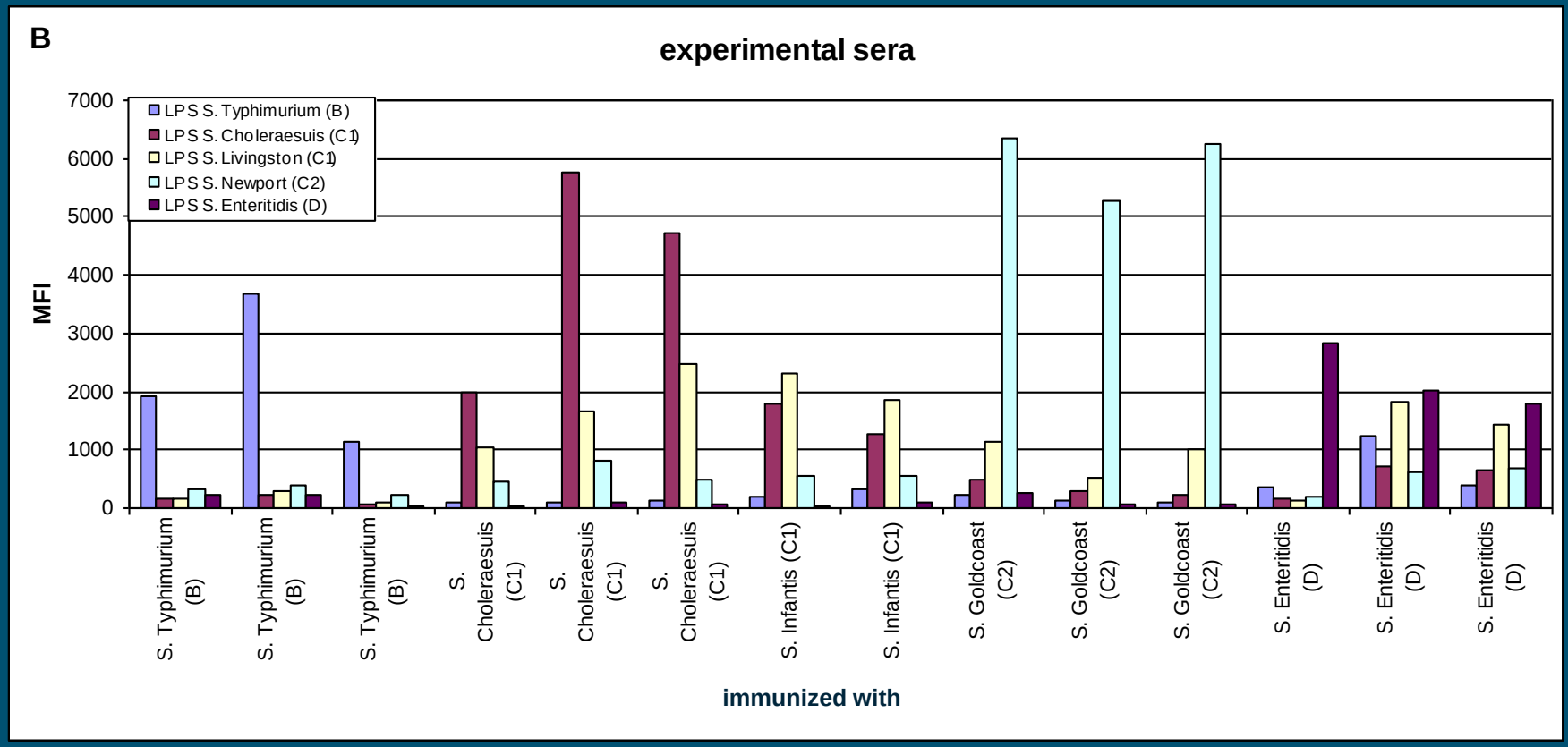
- LPS

LPS beads react with the proper agglutination sera



Salmonella serology in pigs using LPS

- Experimental pig sera



- two S. Goldcoast (C2) samples negative in commercial ELISA

Salmonella serology in pigs using LPS

- Field sera
  - separate cut-offs for each bead
    - based on experimental sera and expectancy

		ELISA 1		
		pos	**neg**	**total**
Luminex	**pos**	54	7	61
	neg	6	83	89
	total	60	90	150

se 90% o.a. 91%
sp 92% c.k. 0.82

  - Assay is in agreement with the commercial ELISA

Concluding remarks

- Trichinella assay and Salmonella fiveplex suspension array are comparable with the commercial ELISAs

→ Luminex platform suitable for (multiplex) serology in swine

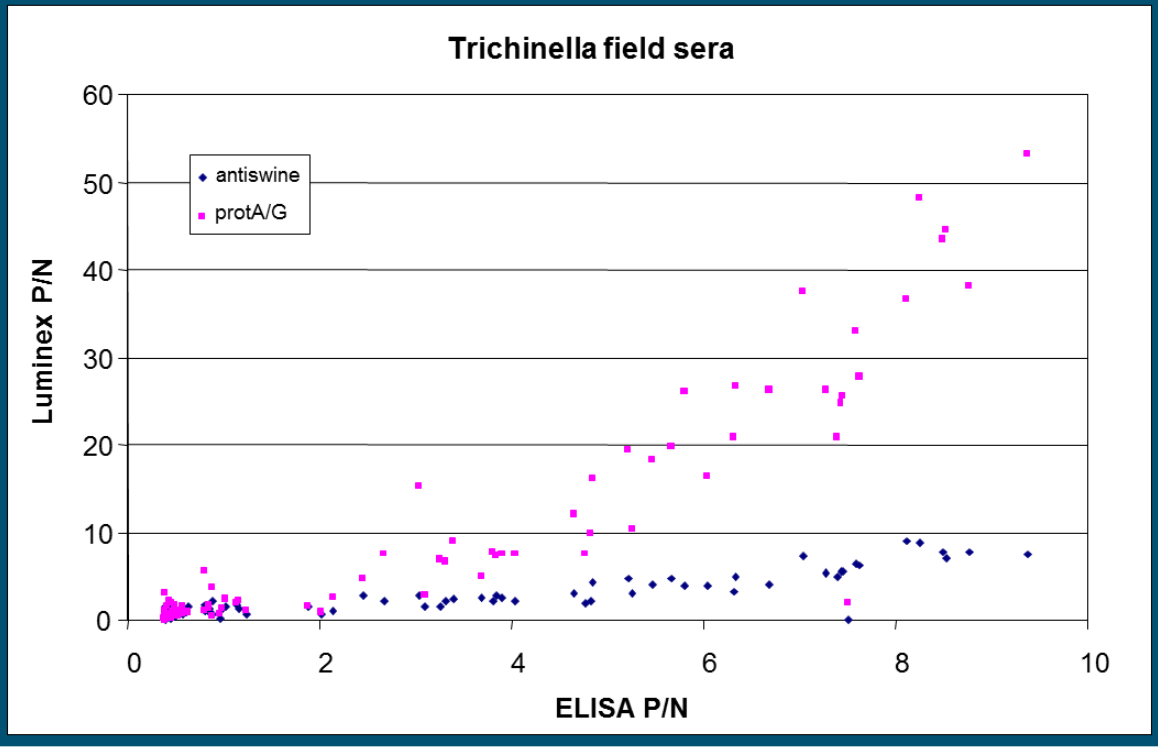
- Improve cut-offs?
  - Protein A/G
    - higher P/N ratio's
    - other species
    - wildlife assays

Concluding remarks

- Trichinella assay and Salmonella fiveplex suspension array can compete with commercial ELISAs

→ Luminex platform suitable for (multiplex) serology in swine

- Improved cut-off?
  - Protein A/G
    - higher P/N ratio's
    - other species
    - wildlife assays



Concluding remarks

- Assays can be expanded with other LPS / antigens
  - Pilot with Trichinella/Salmonella 6-plex
    - looks good
  - Other pathogens
    - Toxoplasma
    - Mycobacterium avium subsp. avium
    - SVDV
    - Aujeszky disease virus
    - etcetera
- Use with marker vaccines
  - Subunit vaccine / deletion mutant
    - RFVF in lambs
- Automation is possible

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