

A Serologic Assay for Guinea Worm: Are We There Yet?

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Centers for Disease Control and Prevention

23rd International Review of Guinea Worm Eradication
Program Managers

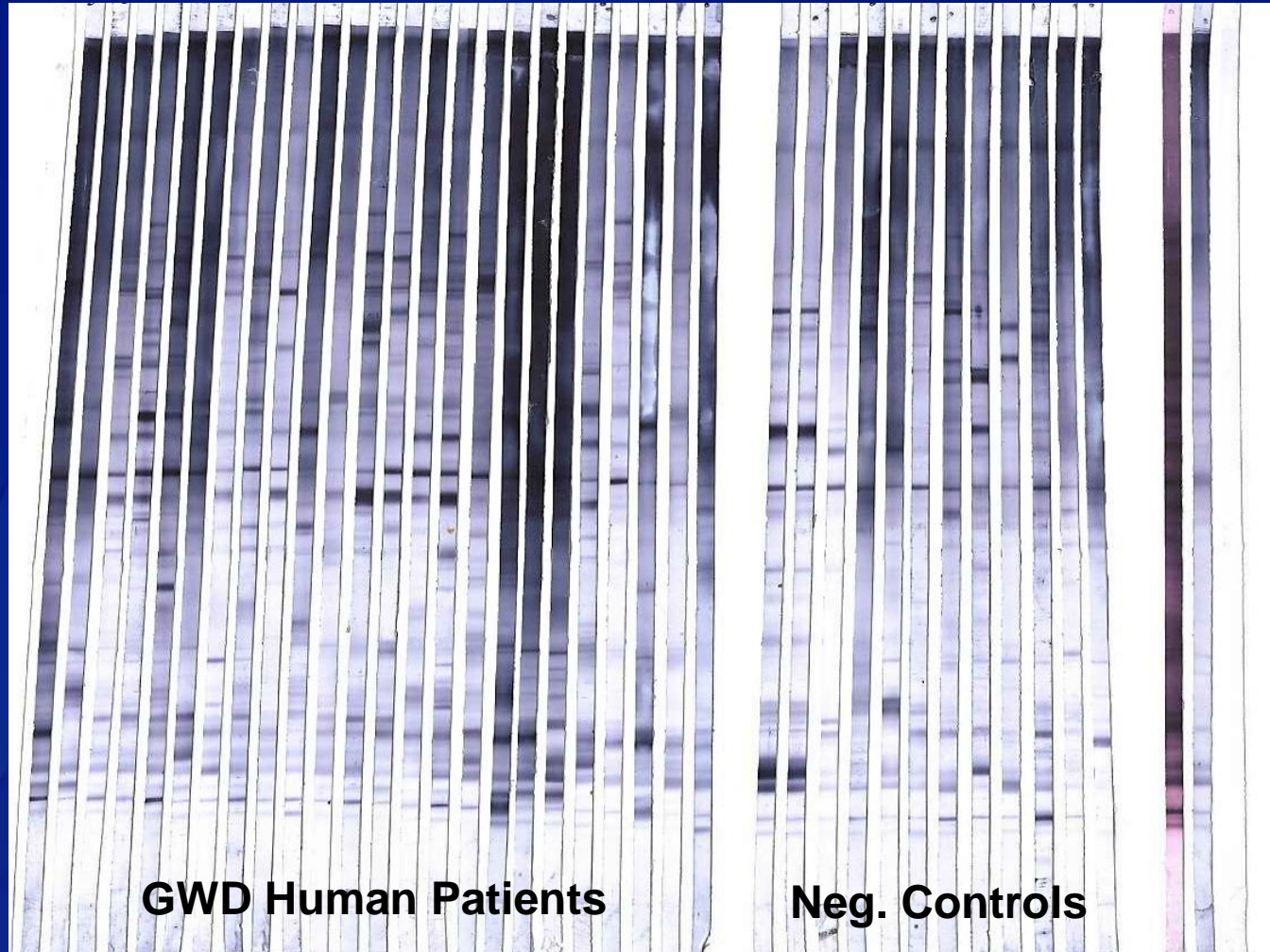
The Carter Center, Atlanta, GA

March 22, 2019

Characteristics of an ideal assay tool

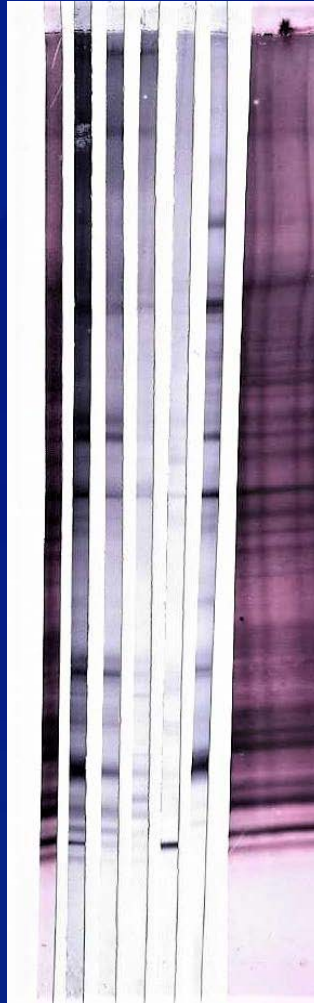
- ❑ Highly sensitive (find every case)
 - Detect exposure to L3 larvae
 - Identify individuals who will progress to patent infection
 - Detect post-emergent responses for a short time (months)
 - Would work in all potential host species (especially dogs)
- ❑ Highly specific for GWD (no cross-reactivity with other Nematodes)
- ❑ Use recombinant proteins rather than native worm material
- ❑ Suitable for use in the field

In the Beginning.... The IgG Western Blot Using Human Sera from Togo



IgG Subclass Responses to Worm Antigens

Au G 1 2 3 4 Au



GWD Patient #1 Serum

Au = Aurodye

G = IgG

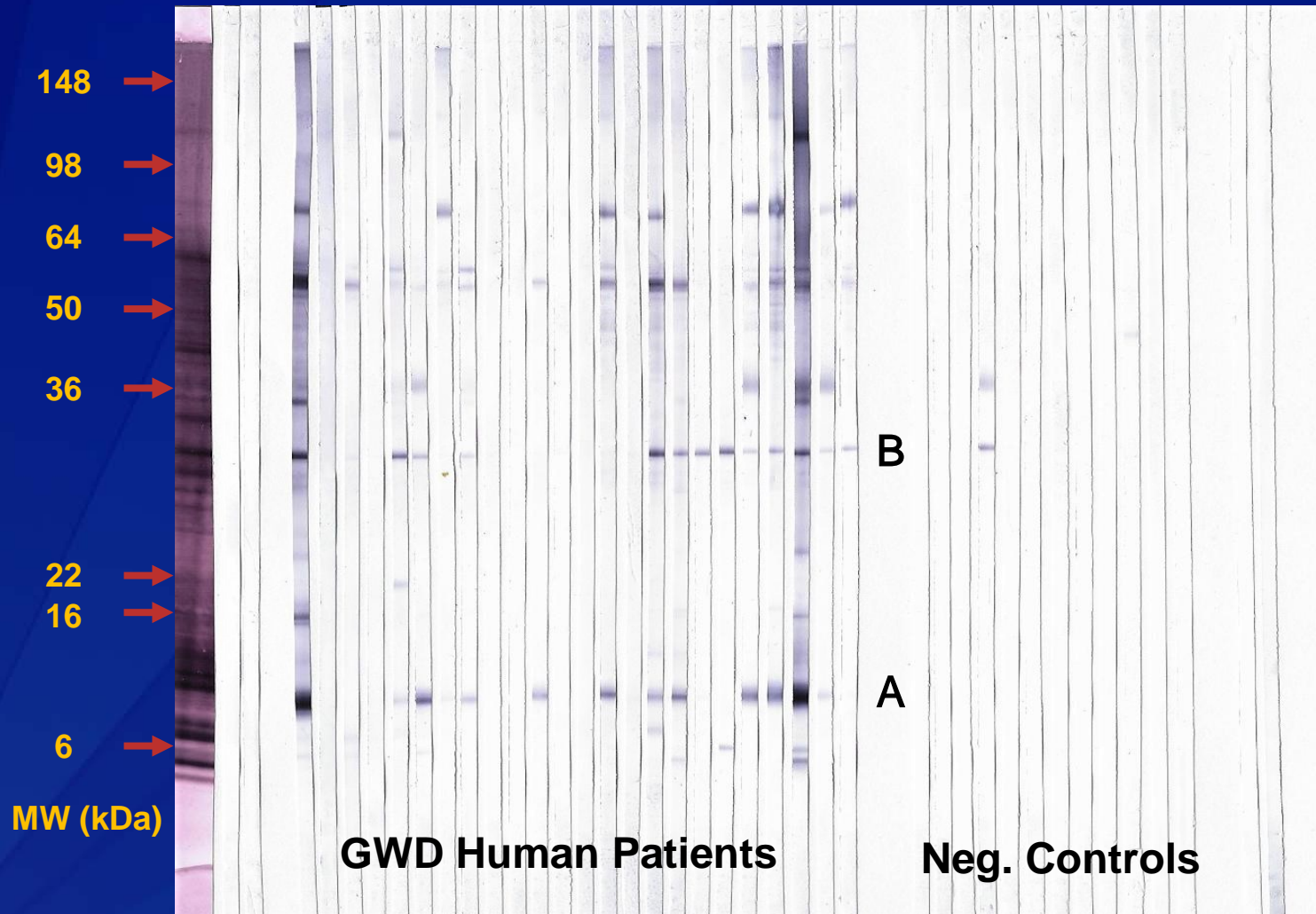
1 = IgG1

2 = IgG2

3 = IgG3

4 = IgG4

Then... the IgG₄ Western Blot Using Human Sera from Togo



Identification of GW Protein Band B

Band B is a 30 kDa Galactose binding protein (Galectin)

- ❑ Galectin 1

 - **96% Conserved, 89% Identical to Bm analog**

- ❑ Galectin 2

- ❑ Galectin 3

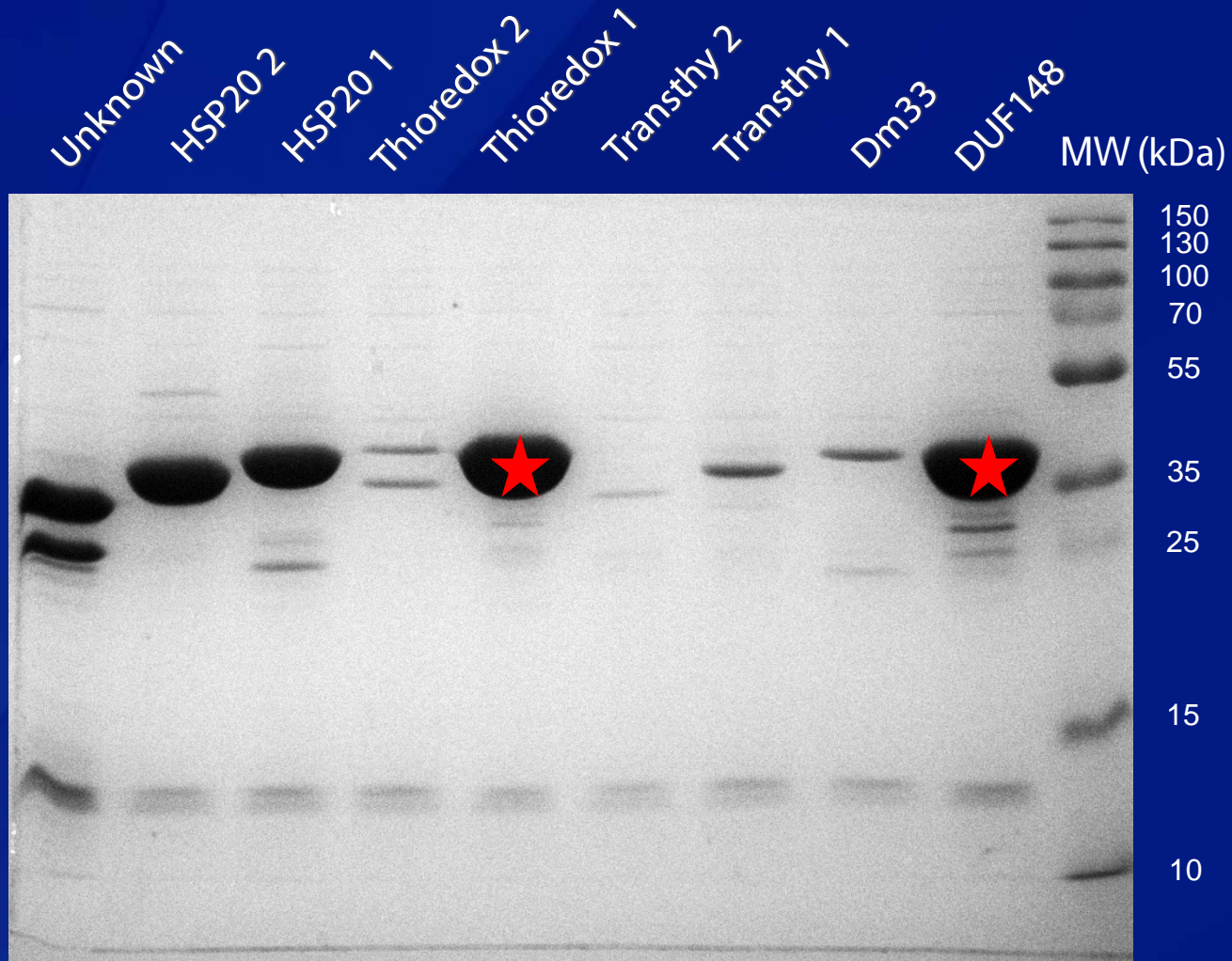
 - **70% Conserved, 56% Identical to Bm analog**

Significant cross reactivity detected in sera from *O. volvulus* patients

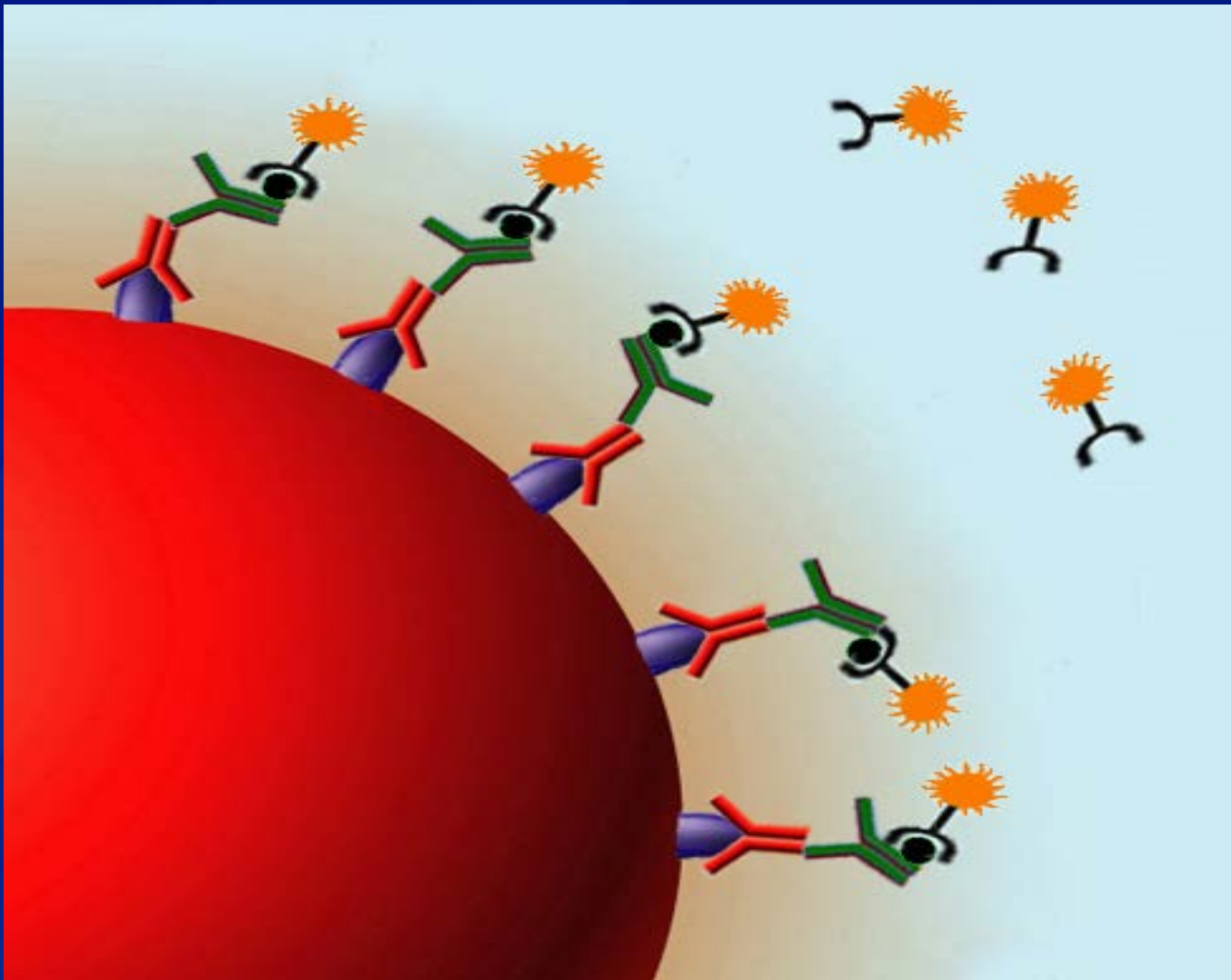
Identification of GW Protein Band A

- ❑ Thioredoxin-like proteins: Conserved proteins used in NADPH-dependent redox reactions (2 versions)
- ❑ Heat shock protein 20 (2 versions)
- ❑ DUF148 (Dm14): Homologue of Nematode SXP diagnostic antigens including *B. malayi* Bm14 and *O. volvulus* Ov17
- ❑ Transthyretin-like proteins: Conserved proteins found in the ES of multiple nematode worms/ function unknown (2 versions)
- ❑ 'Unknown' DME_0000028101: Protein of unknown function

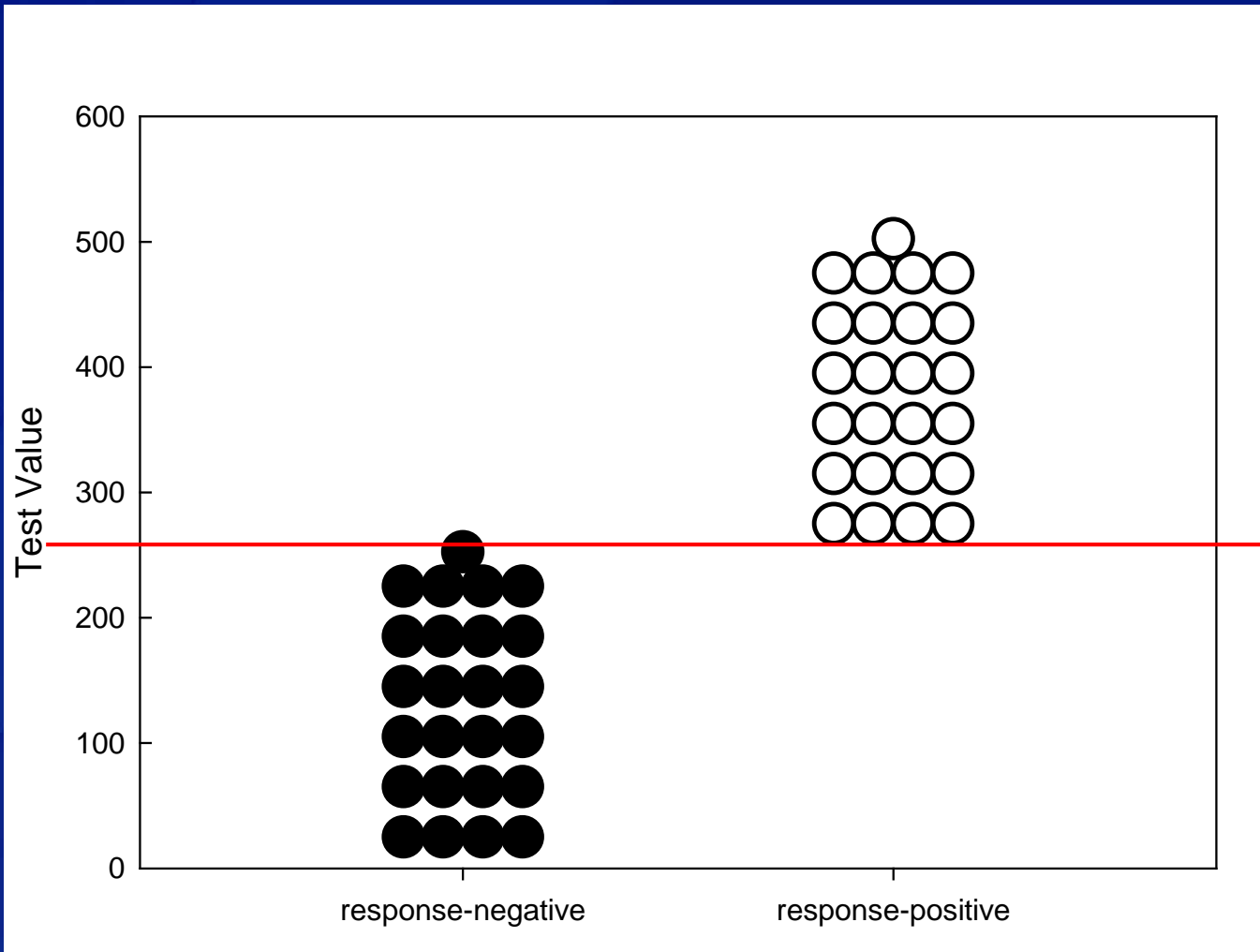
Band A Protein Expression



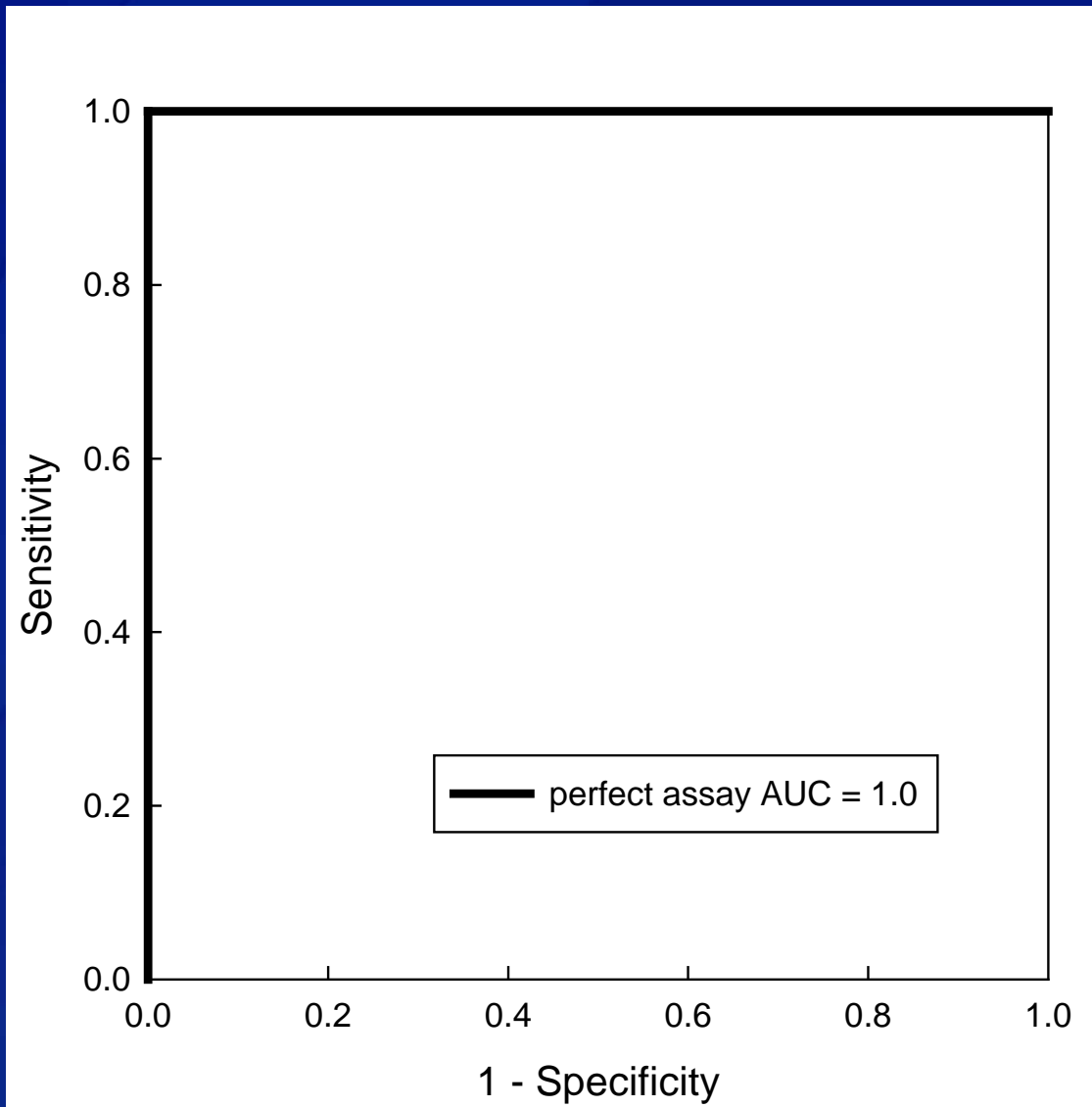
Luminex Assay Basics



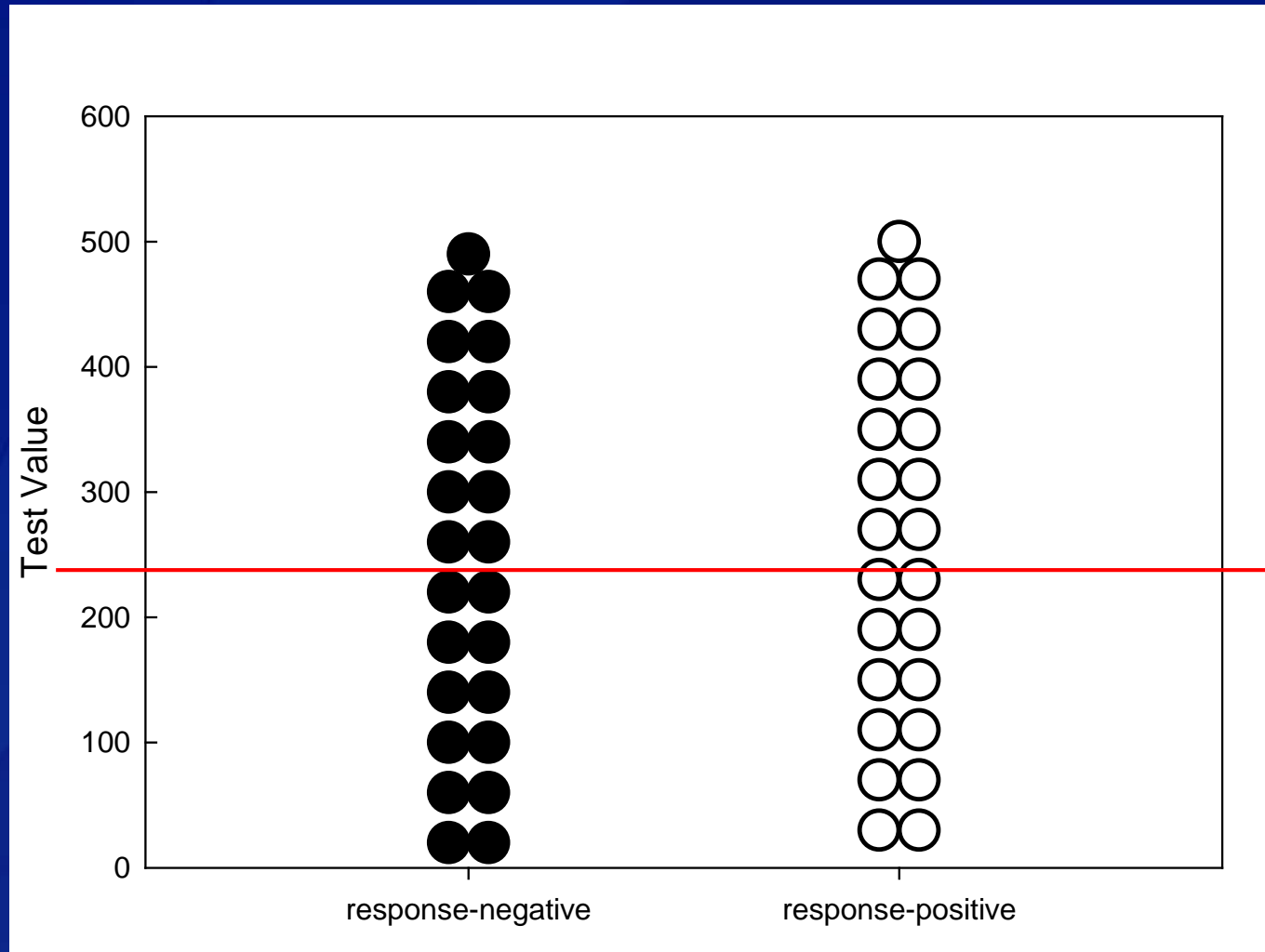
ROC Curve Basics



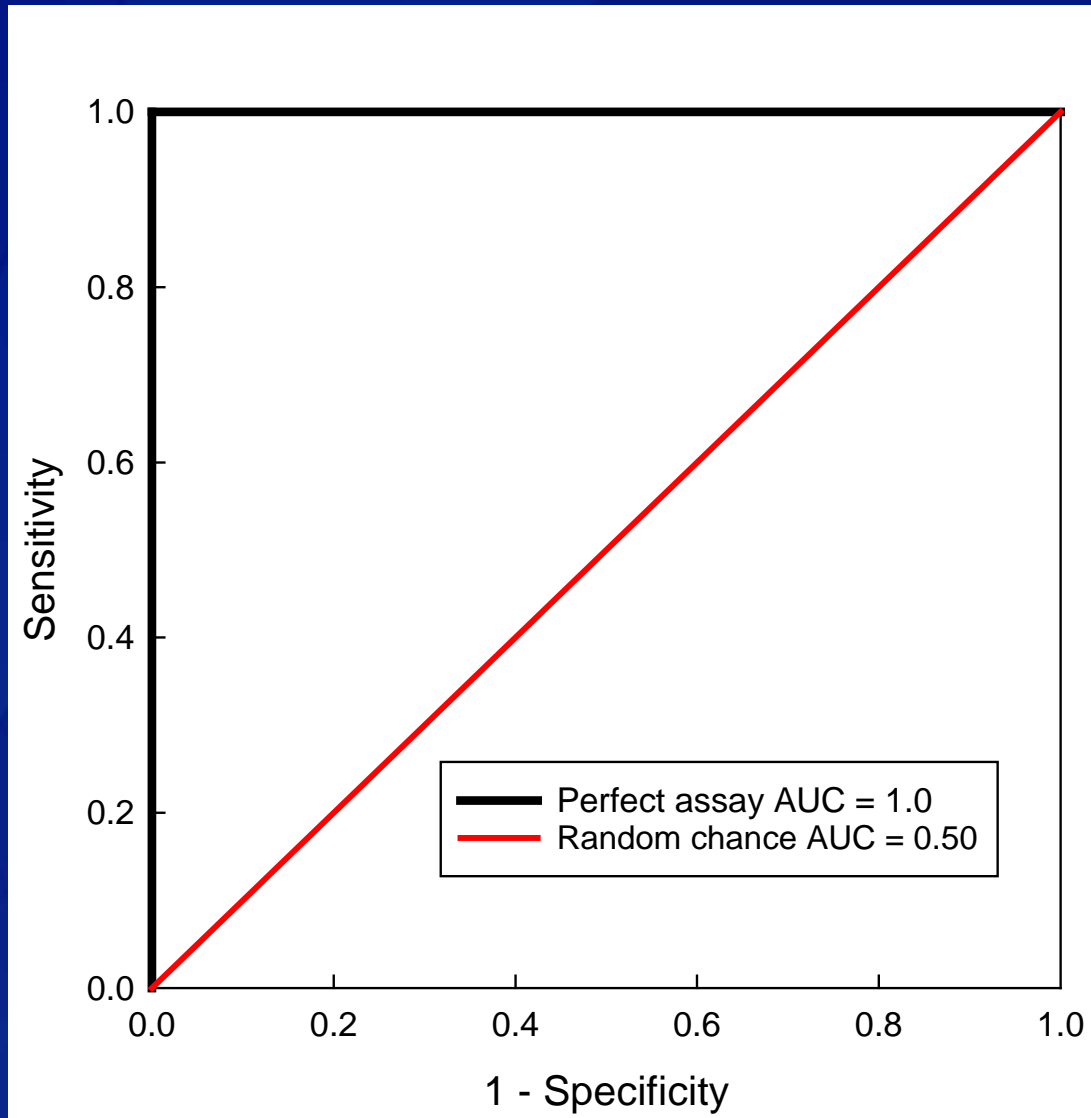
ROC Curve Basics



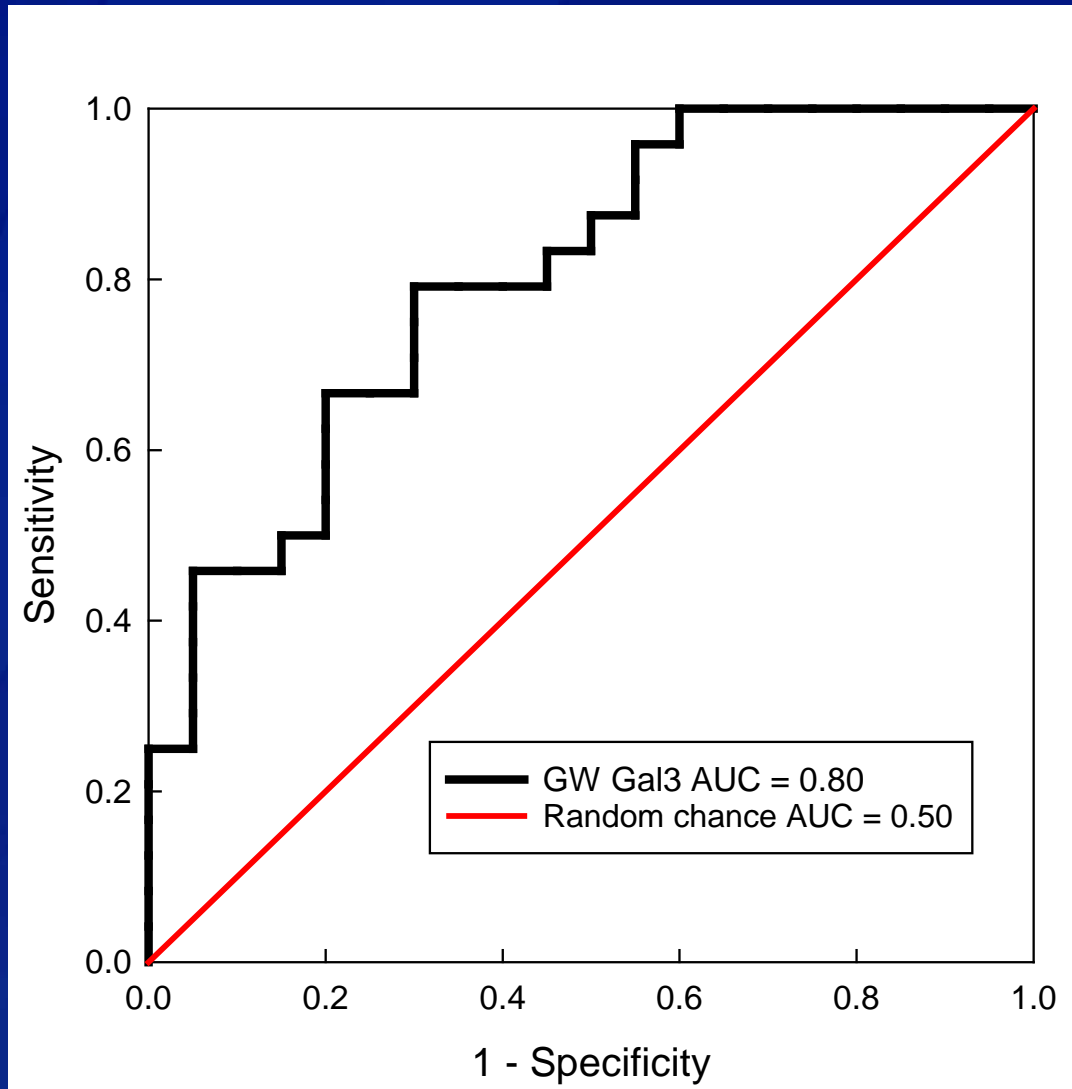
ROC Curve Basics



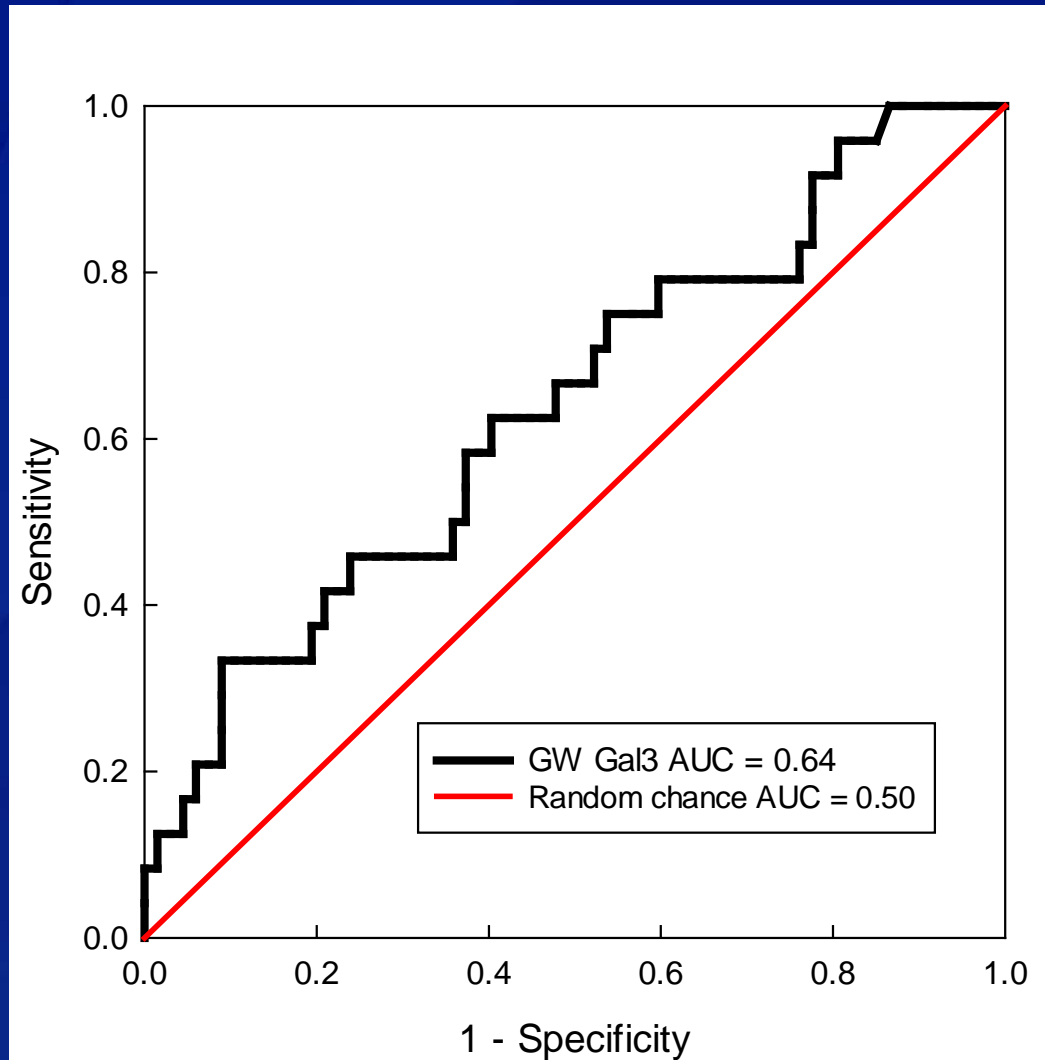
ROC Curve Basics



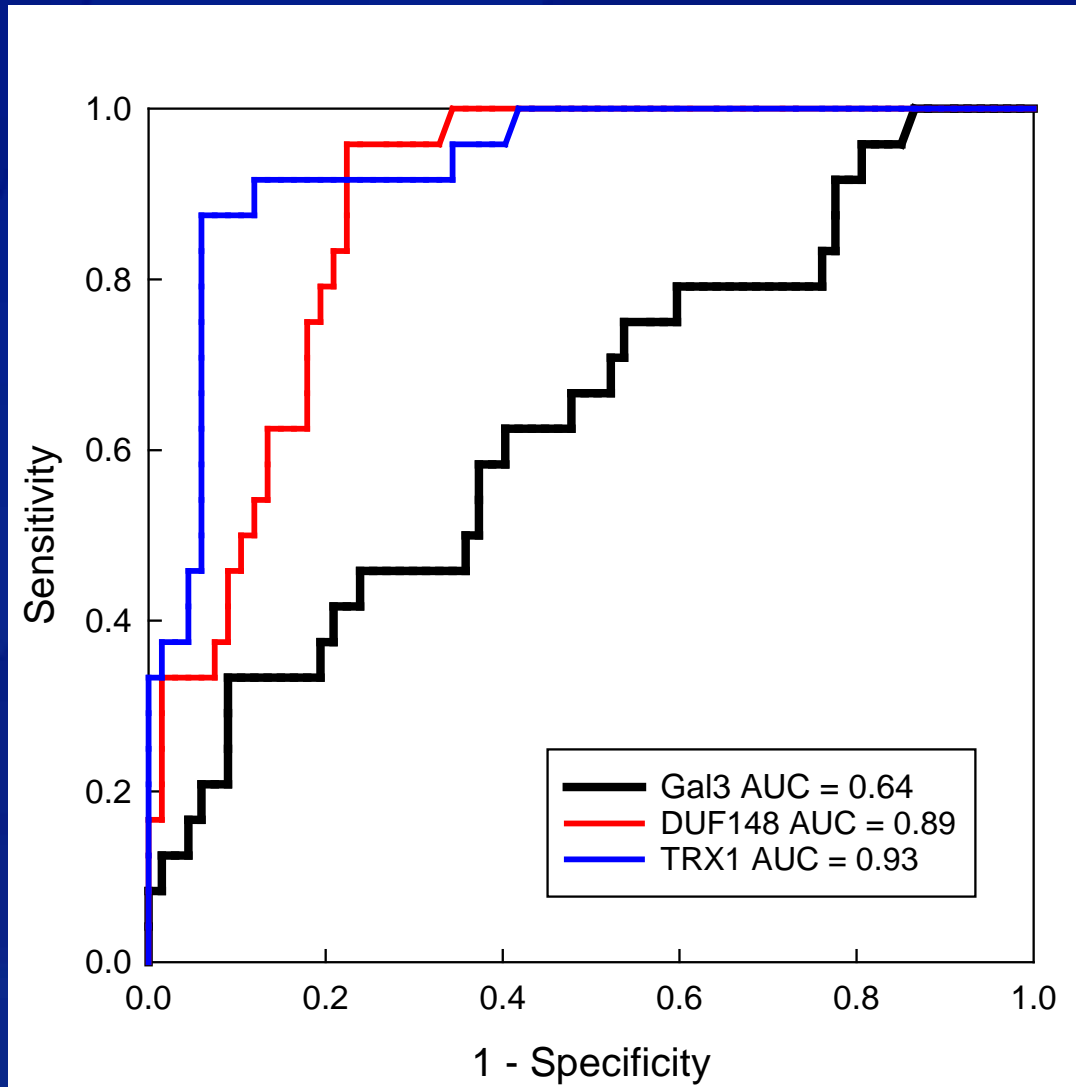
ROC Curve Human vs. Gal3 (no Oncho) Using Monoclonal anti-IgG



ROC Curve Human vs. Gal3 (with Oncho) Using Monoclonal anti-IgG



ROC Curves Human (with Oncho) vs. All Antigens Using Monoclonal anti-IgG



Human Sensitivity and Specificity (with Oncho) Using Monoclonal anti-IgG

❑ Gal3

- 62.5% Sensitivity/ 60% Specificity

❑ DUF148

- 95.8% Sensitivity/ 77.6% Specificity

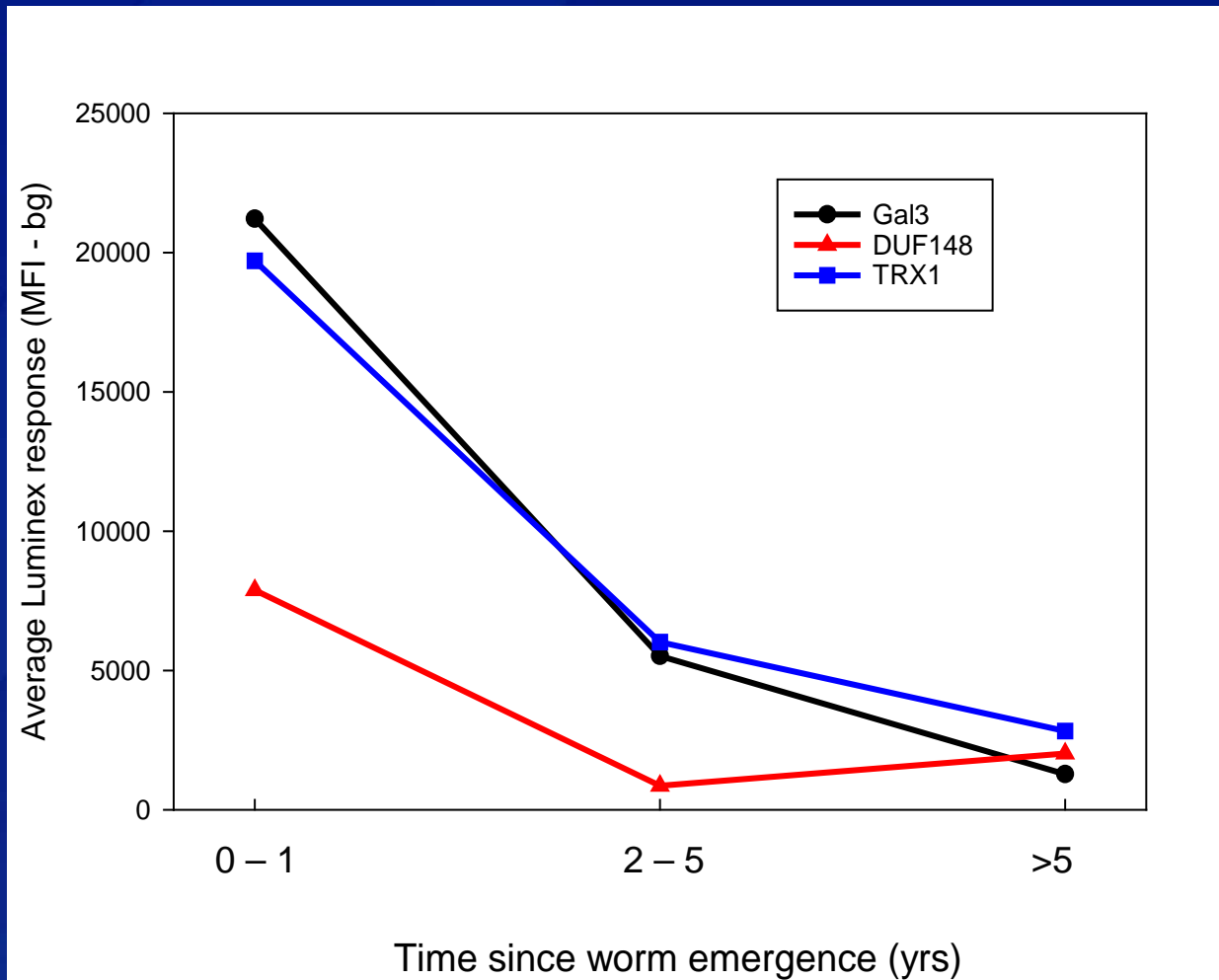
❑ TRX1

- 87.5% Sensitivity/ 94% Specificity

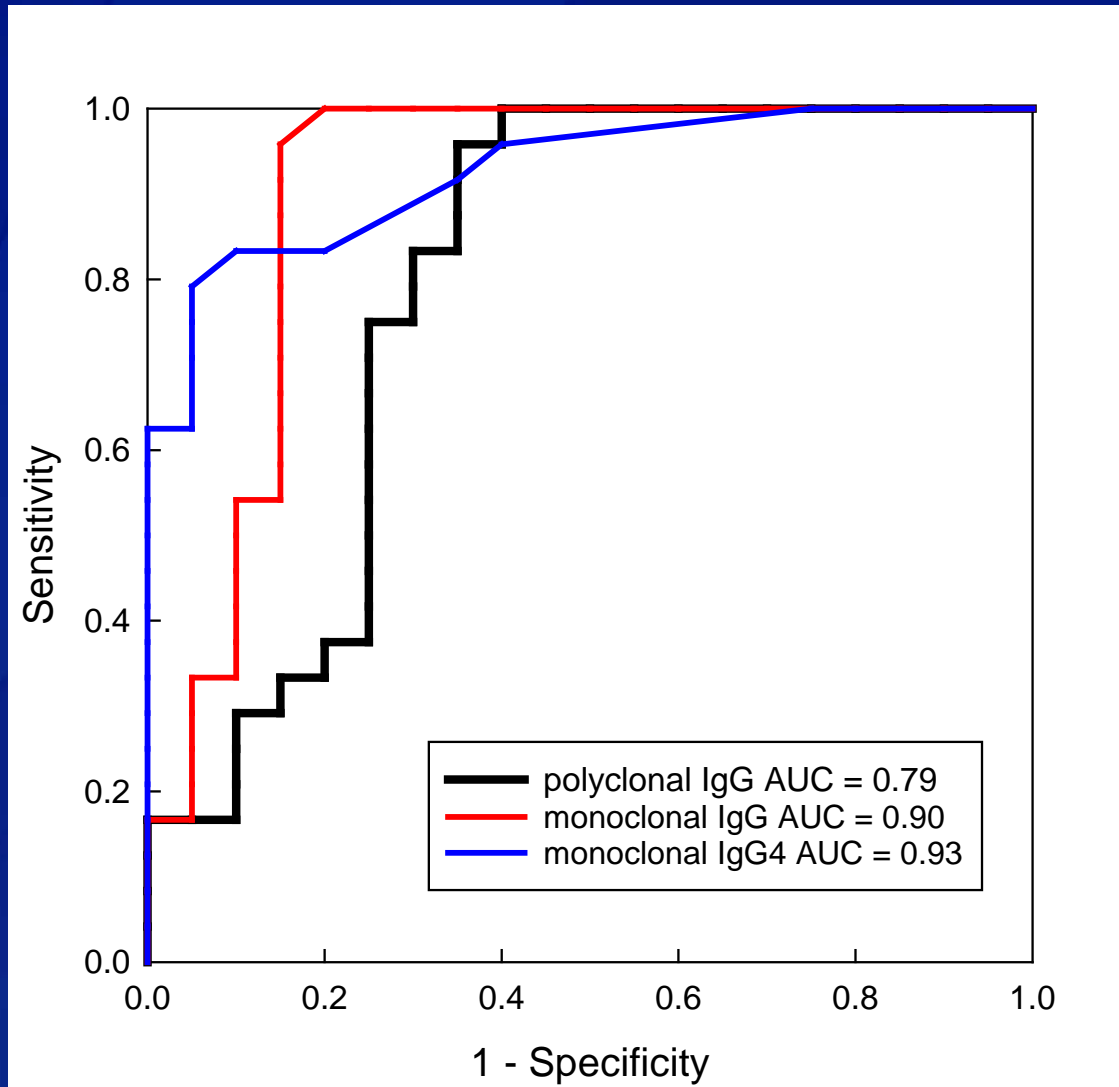
“Positive for both TRX1 and DUF148”

83% Sensitivity and 97% Specificity.

Response in Humans Is Impacted by Time Since Worm Emergence



Detection Reagents Impact Response: Human (no Oncho) vs. DUF148 (Band A)



Summary of Human GW Assays

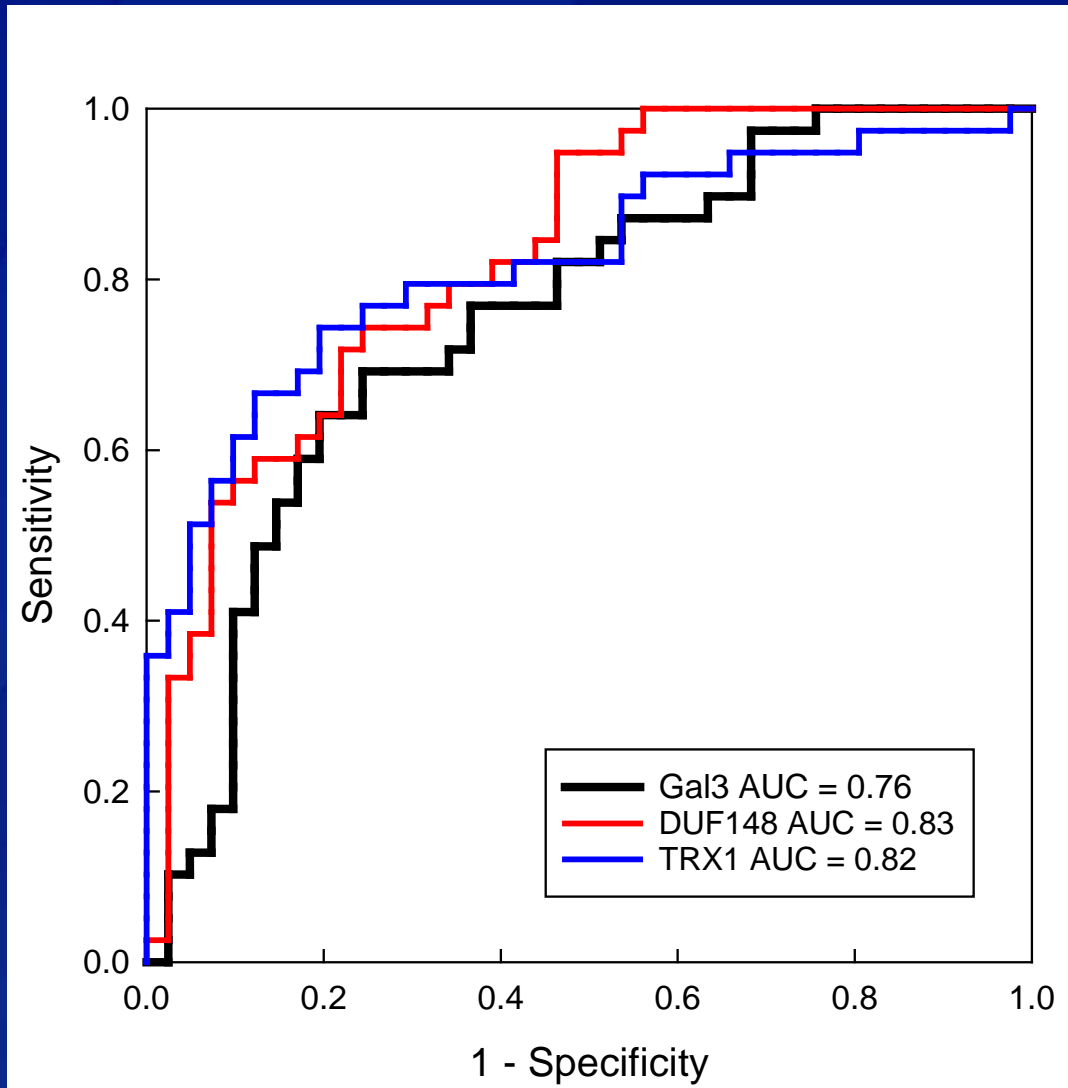
- ❑ “Band B” Gal3 antigen exhibits significant cross reactivity with sera from donors with other Nematode infections (72% of Oncho)
- ❑ The new “Band A” DUF148 and TRX1 antigens are more sensitive and more specific than Gal3 (TRX1, 16% of Oncho)
- ❑ Average IgG responses decline after worm emergence
- ❑ Monoclonal secondary antibody detection reagents yield higher sensitivity and specificity compared to polyclonal reagents
- ❑ Anti-IgG4 secondary antibody improves DUF148 assay

**GWD Dogs
(1-14 worms)**

Neg. Controls

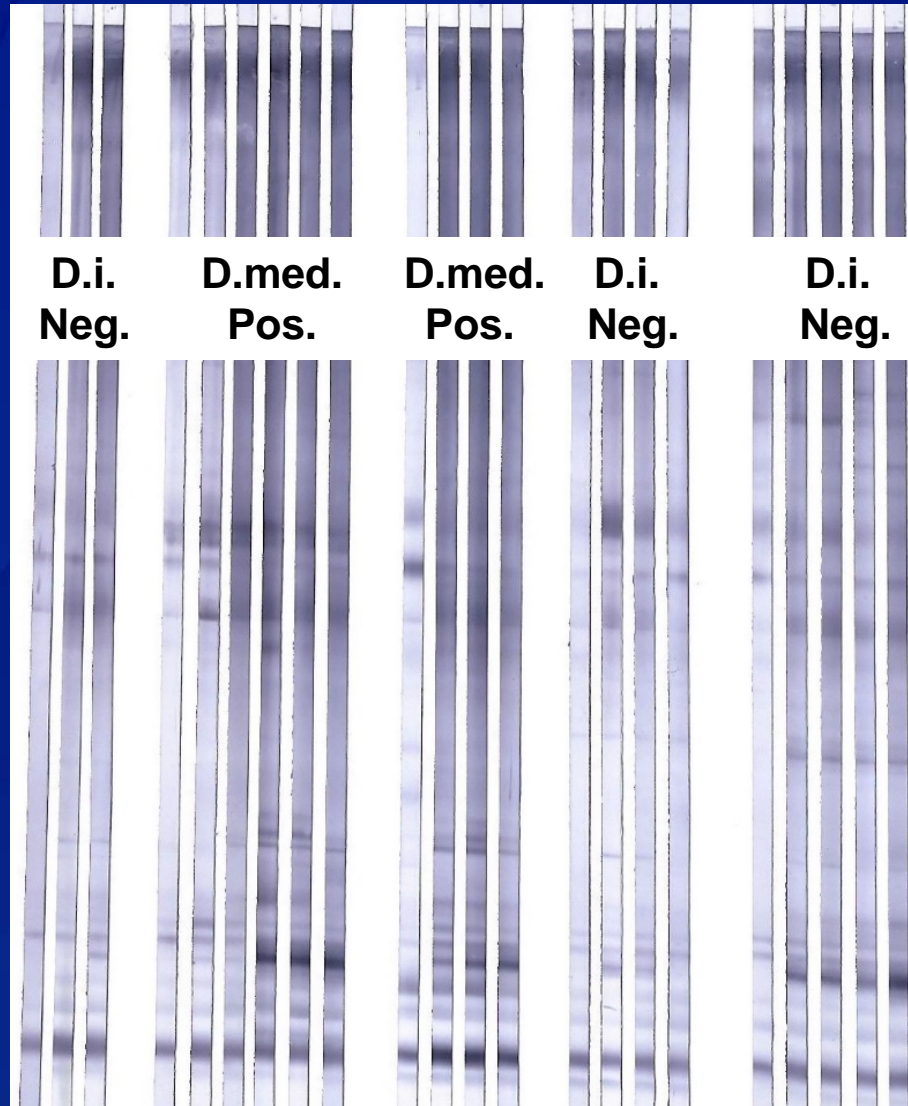
Neg. Controls

ROC Curves Chad Dogs vs. All Antigens Using Polyclonal anti-Dog IgG



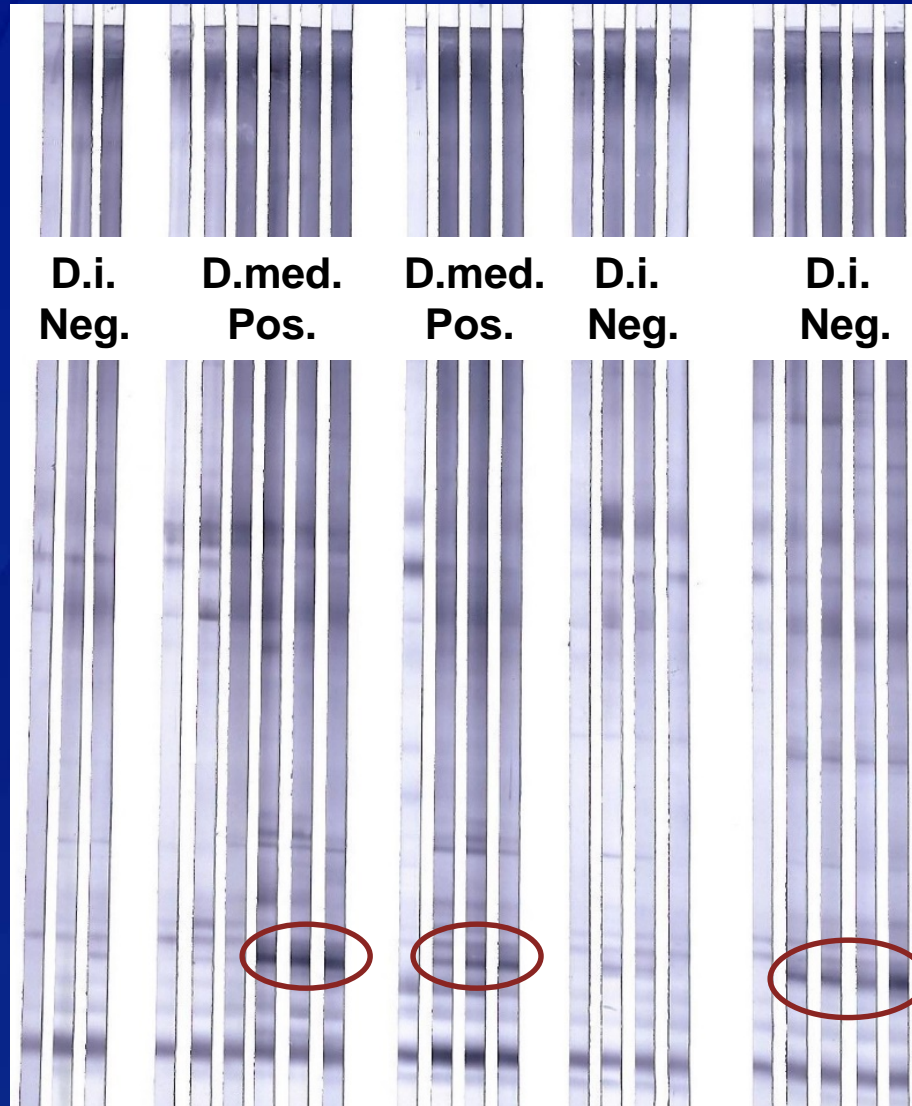
Western Blots of Longitudinal UGA Dog Sera Using Polyclonal anti-Dog IgG

Exposed to:
Adult worm:



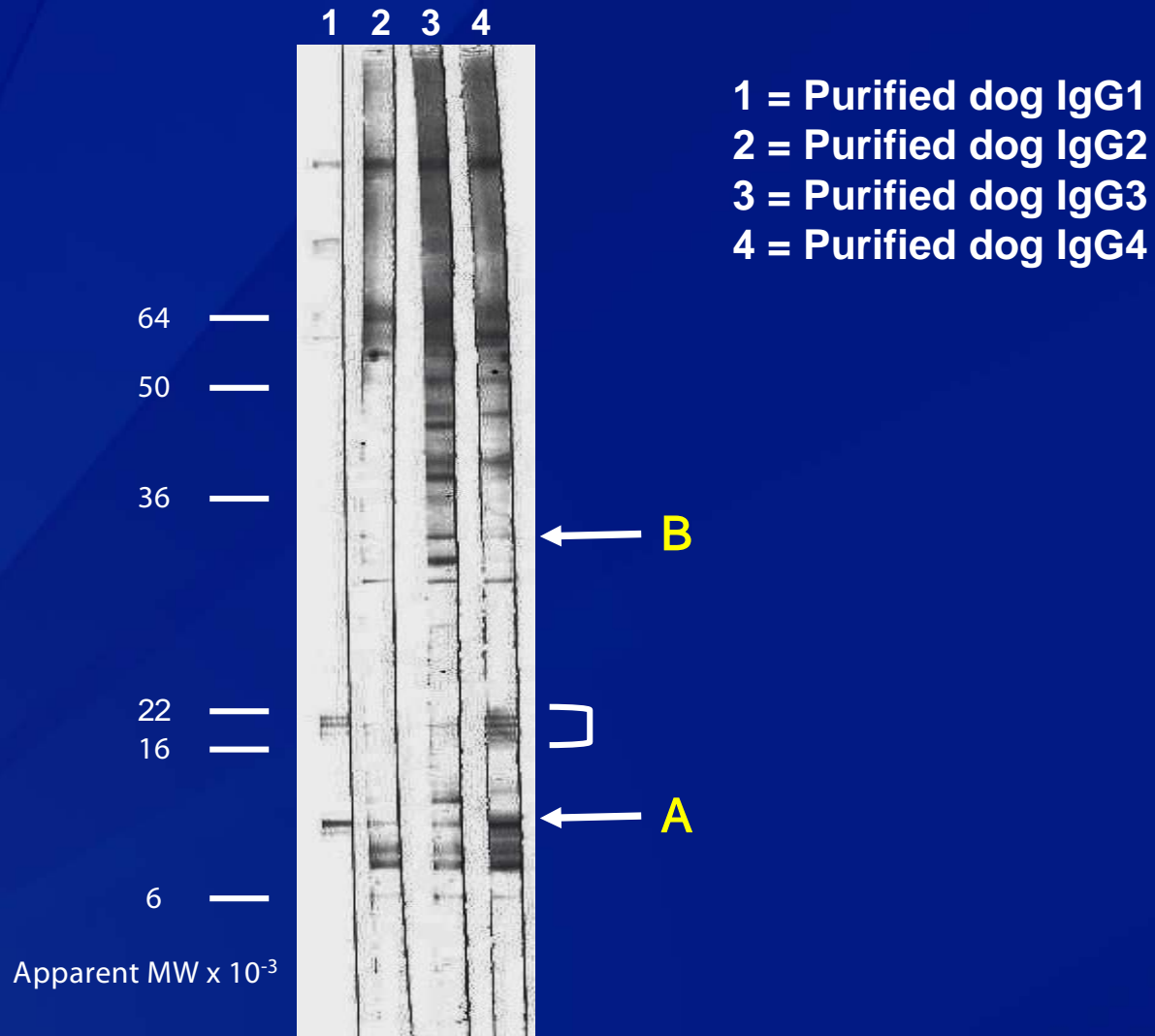
Western Blots of Longitudinal UGA Dog Sera Using Polyclonal anti-Dog IgG

Exposed to:
Adult worm:



Band A Region

Western Blots of Purified Dog IgG Subclasses Using Polyclonal anti-Dog IgG



Generation of Mouse anti-Dog IgG Monoclonal

Order No. 233499						
19-Mar-19	Mouse No. 8504		Mouse No. 8505		Mouse No. 8506	
Serum Dilution	Pre-Immune	Immunized	Pre-Immune	Immunized	Pre-Immune	Immunized
1:500	-0.002	1.018	0.001	1.108	0.002	1.014
1:1500	-0.005	0.996	-0.001	0.922	-0.001	1.004
1:4500	-0.001	0.910	-0.006	0.744	-0.004	0.852
1:13500	-0.007	0.687	0.001	0.611	-0.003	0.810
1:40500	-0.008	0.490	-0.004	0.267	-0.004	0.583
1:121500	-0.003	0.270	-0.002	0.154	-0.004	0.272
1:364500	-0.004	0.096	-0.001	0.072	-0.001	0.182
Blank	0.000	-0.001	0.002	-0.001	0.000	-0.001

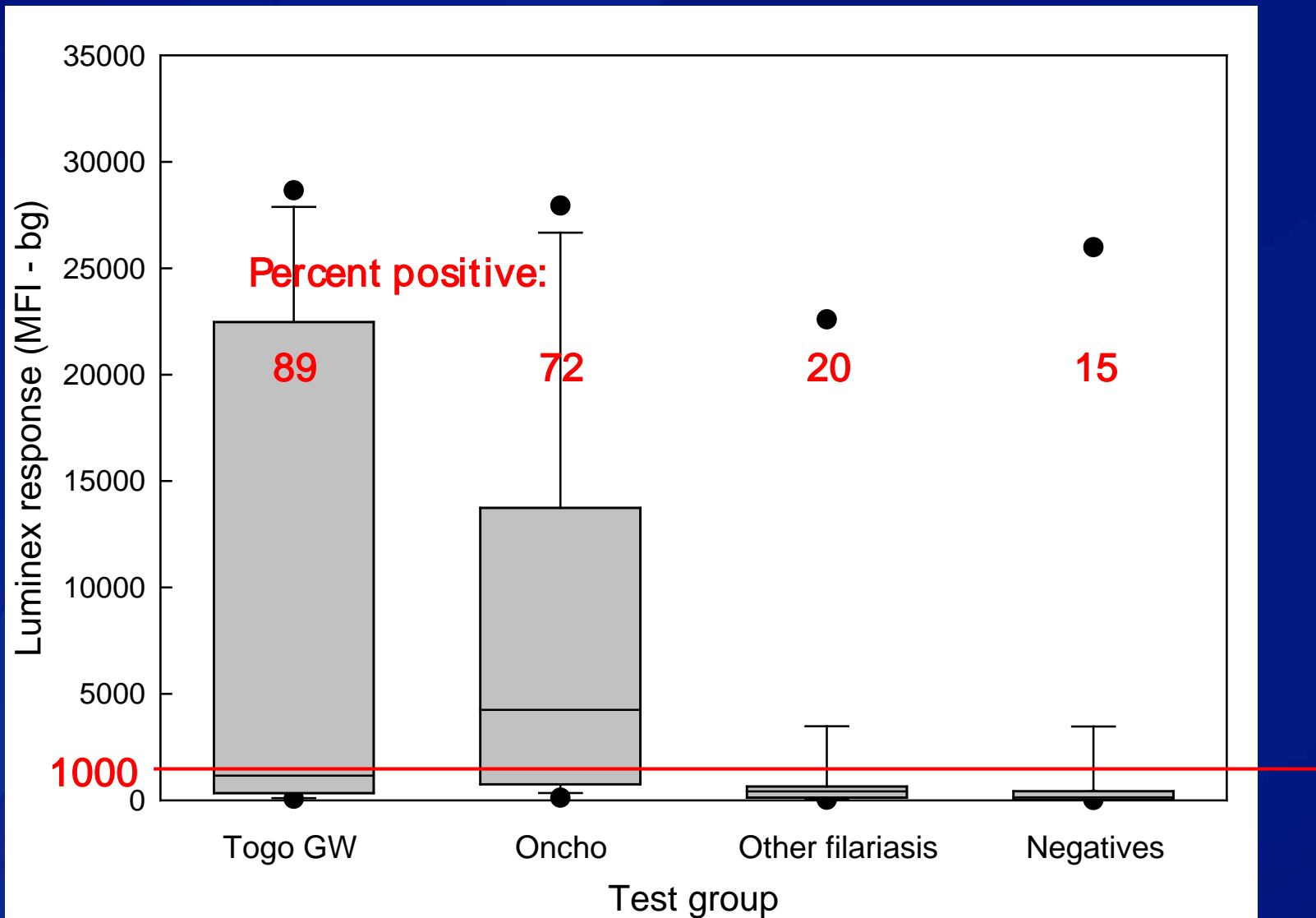
Next Steps with “Band A” Assays

- ❑ Can one or a combination of antigens be sensitive enough in dogs? TRX2 (not discussed) has interesting assay characteristics, but is difficult to produce.
- ❑ Will improved monoclonal reagents increase the sensitivity and specificity of the dog assay?
- ❑ Will the serologic assay detect responses in baboon sera?
- ❑ Can we move to a more user friendly assay format (ELISA?) and away from Luminex?

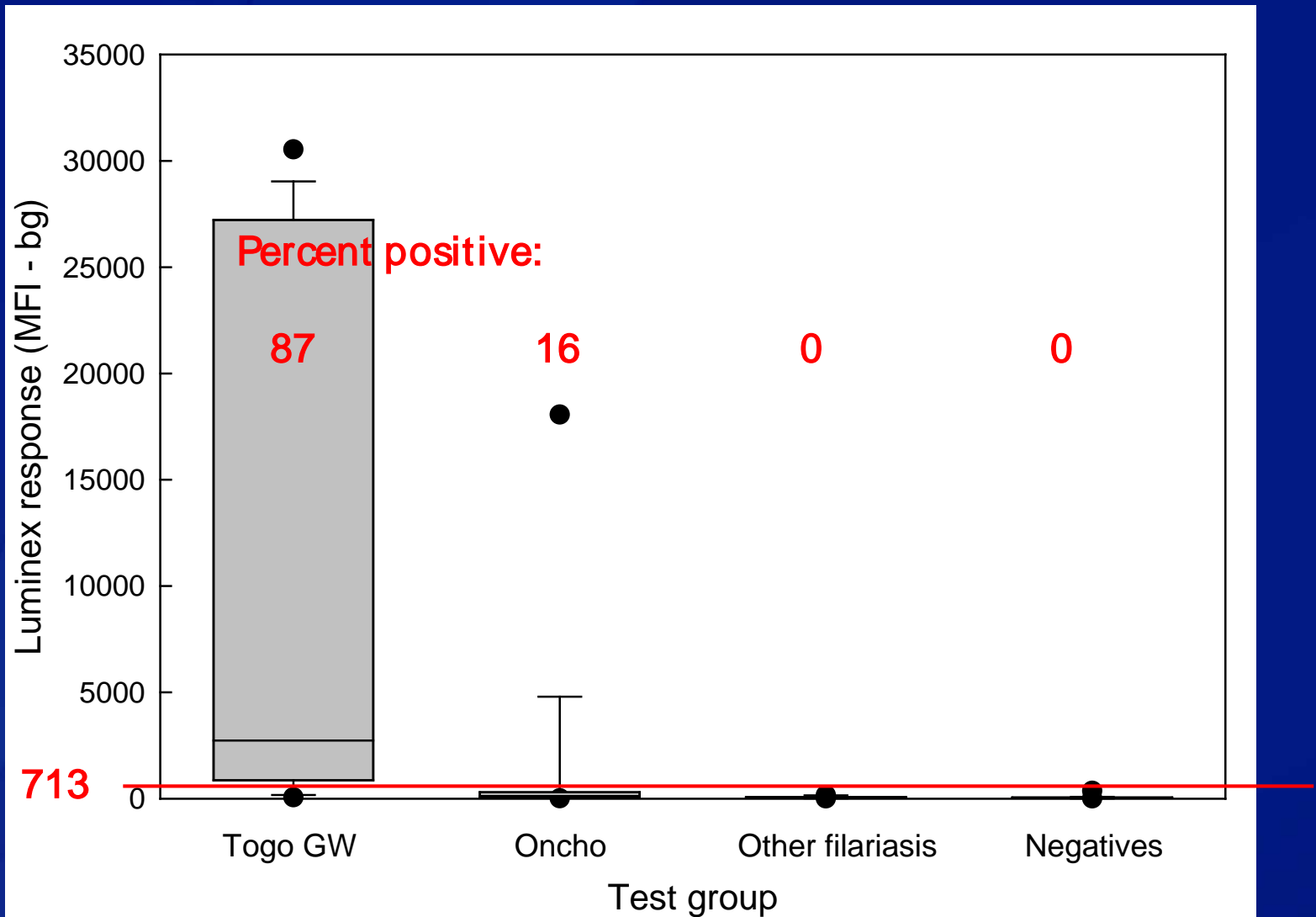
Thank You!

We Look Forward to Your Comments...

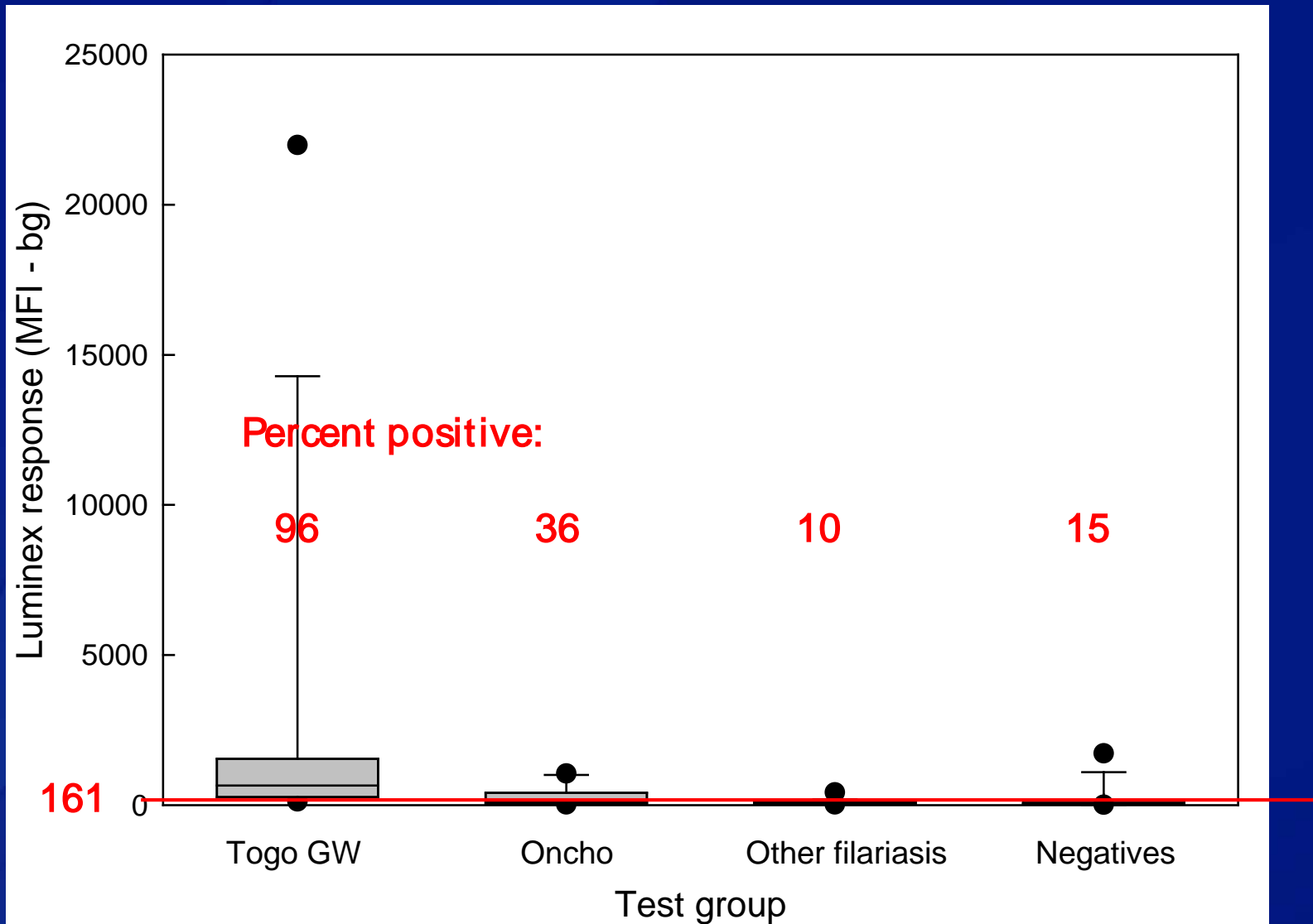
Human IgG Responses to GW Gal3



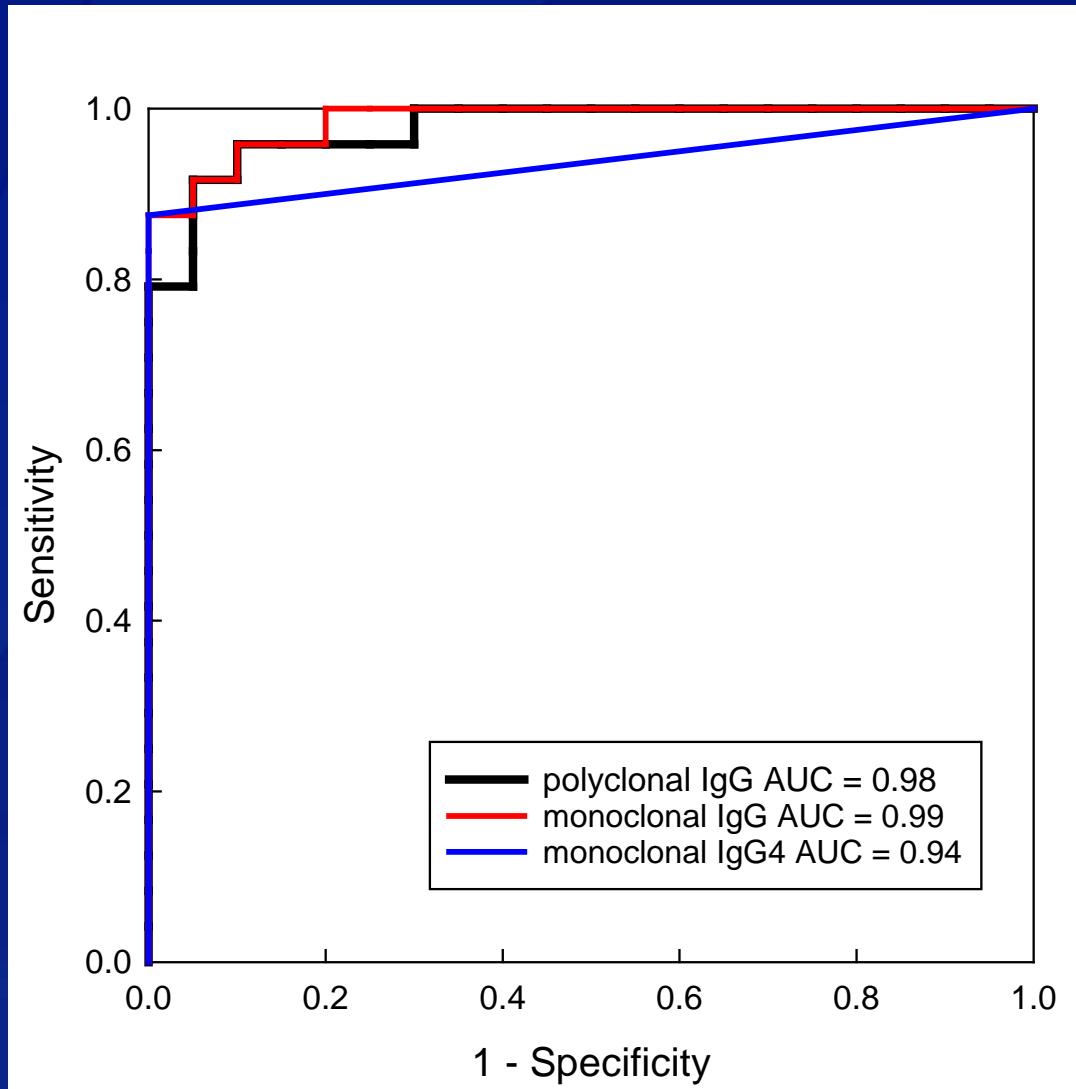
Human IgG Responses to GW TRX1



Human IgG Responses to GW DUF148



Detection Reagents Impact Response: Human (no Oncho) vs. TRX1 (Band A)



ROC Curves Human (with Oncho) vs. All Antigens Using Monoclonal anti-IgG4

