

Immunological applications for DNA dendrimers

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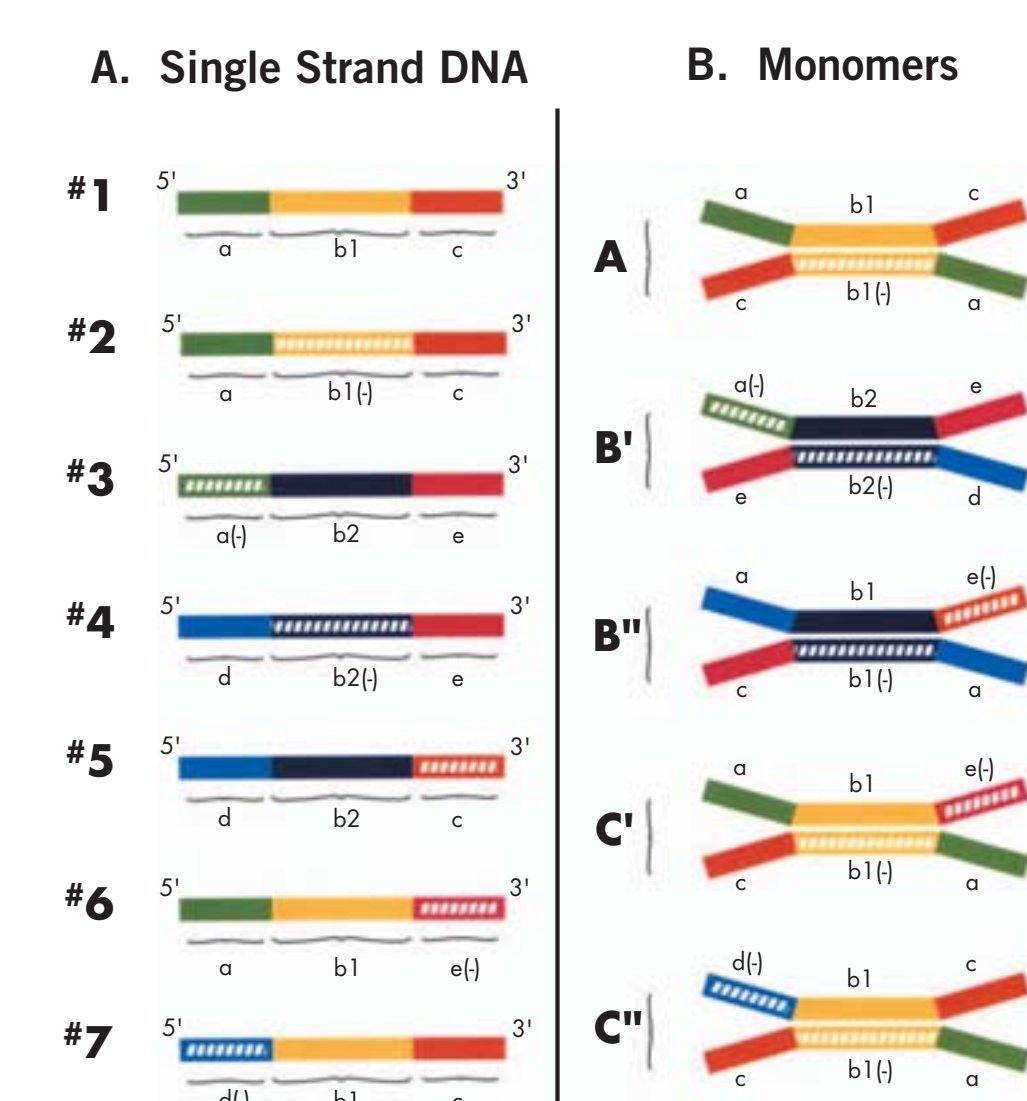
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Abstract

DNA dendrimers are branched structures of varying diameters (90-270 nm) made of DNA. Their central unit is a monomer with a double stranded waist and single stranded arms that serve as hybridization points to other monomers or oligonucleotides. Among the first applications for DNA dendrimers are DNA/RNA detection in microarrays and in situ hybridization assays. In order to apply this technology to protein detection, we have developed a new generation of DNA dendrimers (UltraAmp reagents) conjugated to a variety of antibodies, enzymes and haptens. Here we present an assortment of UltraAmp tested applications: ELISAs, Luminex, and flow cytometry. In ELISAs UltraAmp was evaluated in both sandwich and direct assays for detection of cytokines, and other disease-related antigens or antibodies (HIV, hepatitis, H. pylori). Cytokines and phospho-proteins were tested with the Luminex bead-based multiplexing assay platform with more than a 200 fold improvement of sensitivity. Overall, UltraAmp reagents provided signal amplification as high as 200-fold compared to the standard method of detection across the different platforms and would facilitate the required higher sensitivity for the analysis of biological samples.

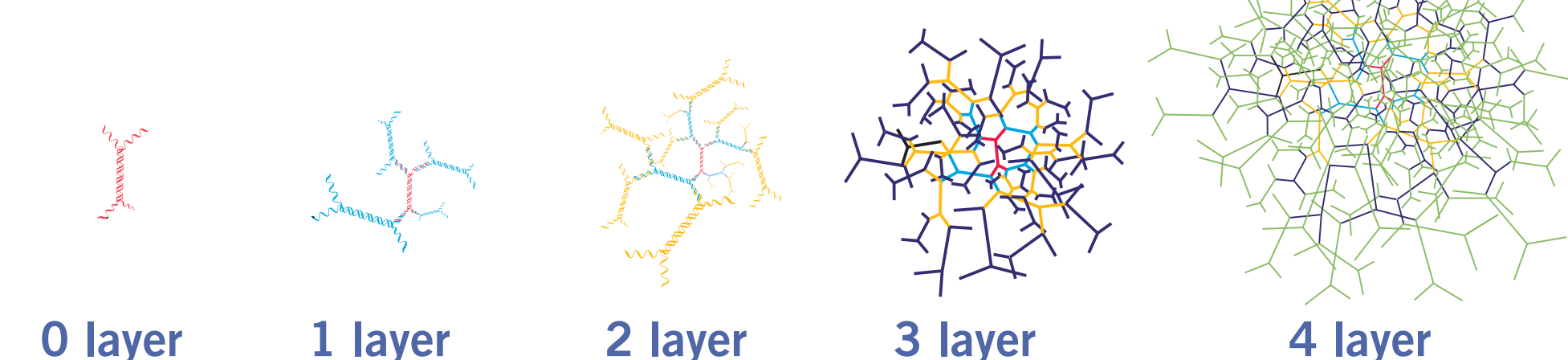
Dendrimer Components

Dendrimers are made from 7 single stranded DNAs. The strands are hybridized pair-wise to produced building block units called monomers.



Dendrimer Technology

Monomers are hybridized together to assemble various size DNA dendrimers. The "core" structure is crosslinked during assembly to form a completely covalent structure.

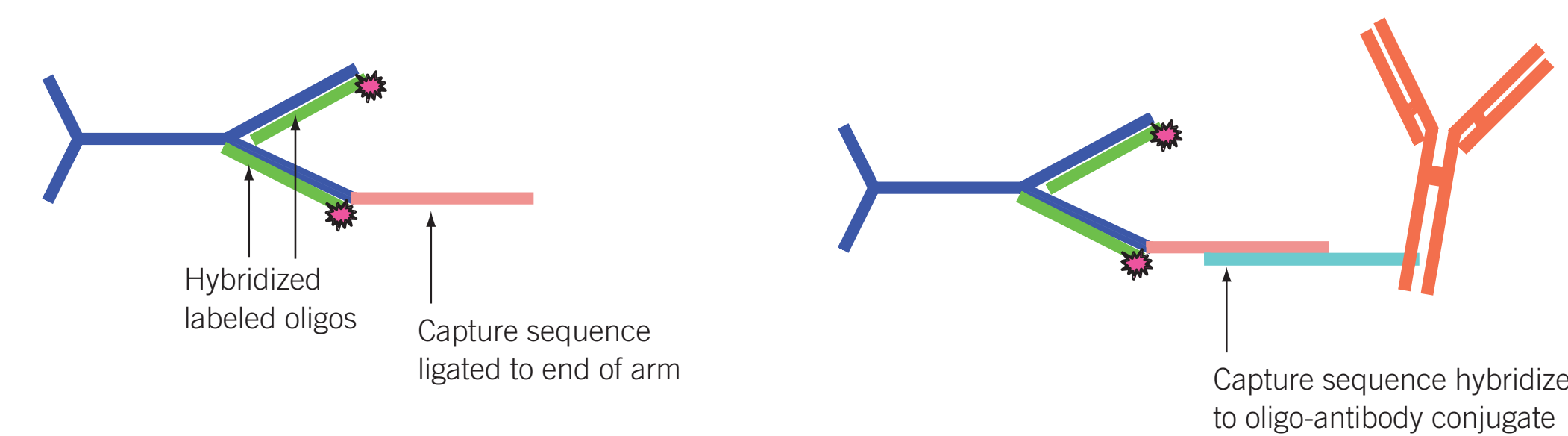


Methods

Preparation of UltraAmp[™] reagents

Enzyme and Antibody labeling

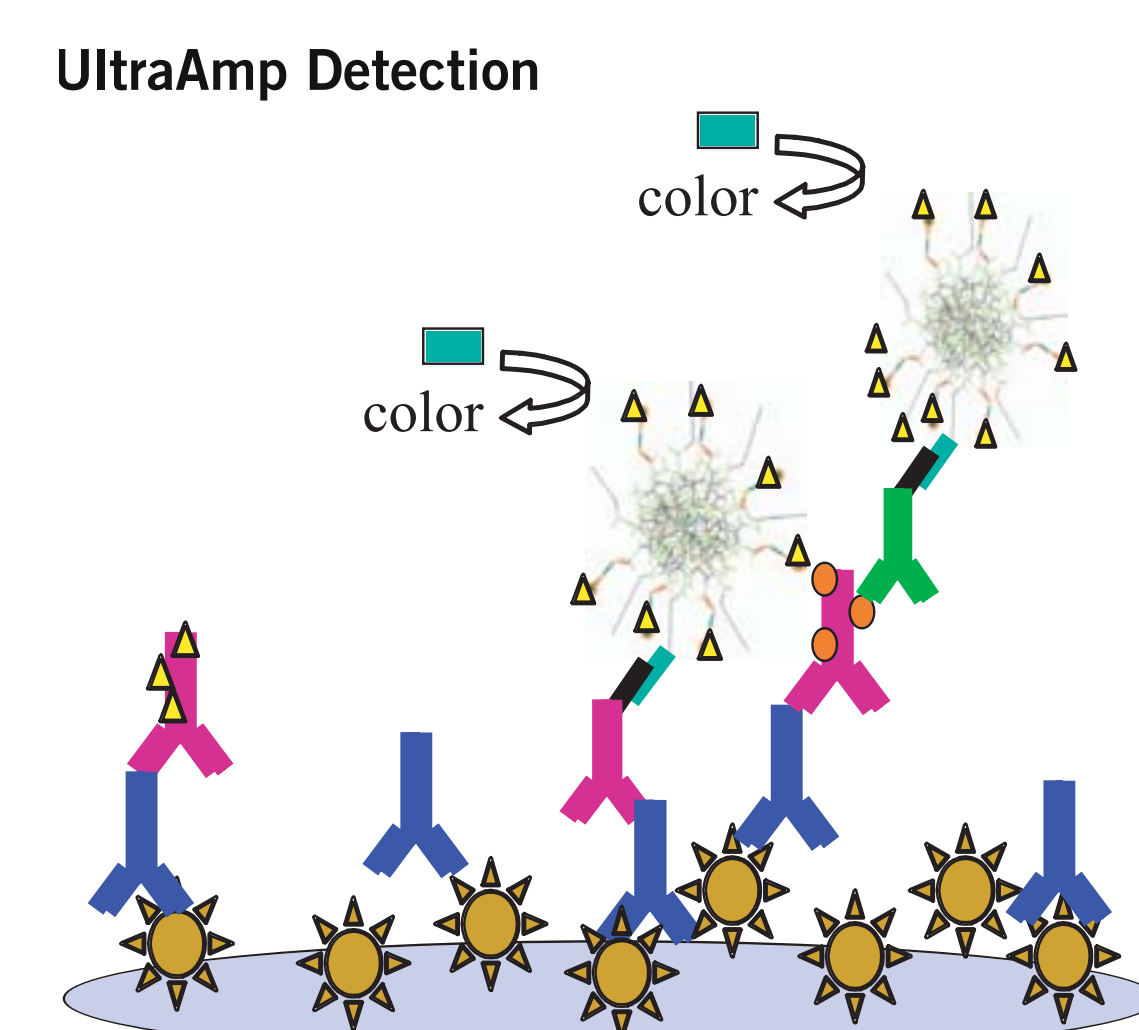
The dendrimers and HRP and anti-biotin antibodies labeled oligonucleotides complementary to the outer core of the dendrimer are incubated at room temperature for 30 minutes in a buffer that would allow hybridization. The end product is a dendrimer conjugated to the desired antibody and labeled with HRP enzymes



ELISA Assays

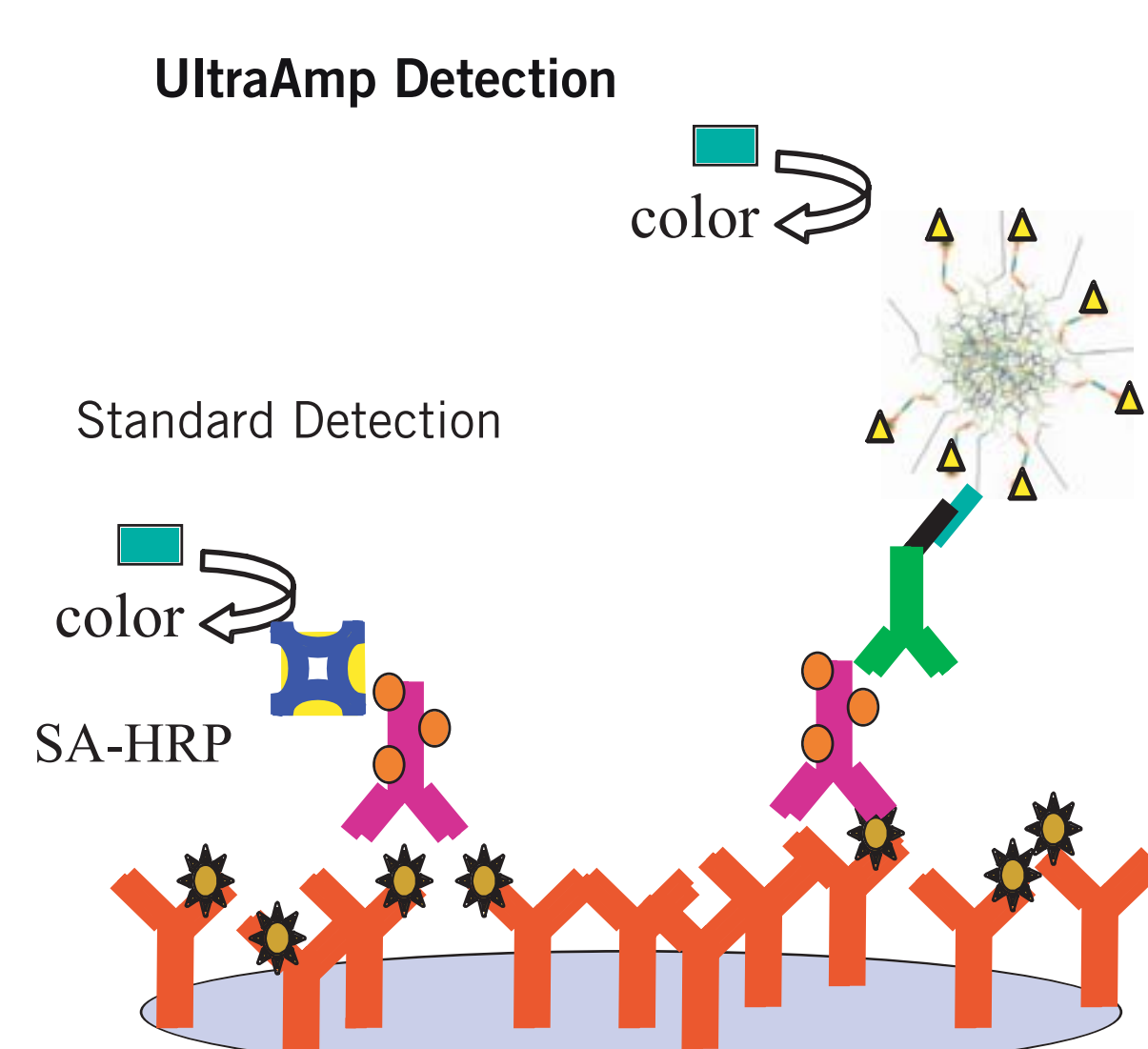
Indirect ELISA

- Antigen-coated plates from various manufacturers were used to test indirect ELISAs.
- For UltraAmp detection, incubation with the HRP labeled anti-human antibody was substituted by anti-human UltraAmp HRP or biotinylated anti-human antibody.
- For detection with biotinylated anti-human antibodies, unbound antibodies were washed off and detected with anti-biotin UltraAmp HRP



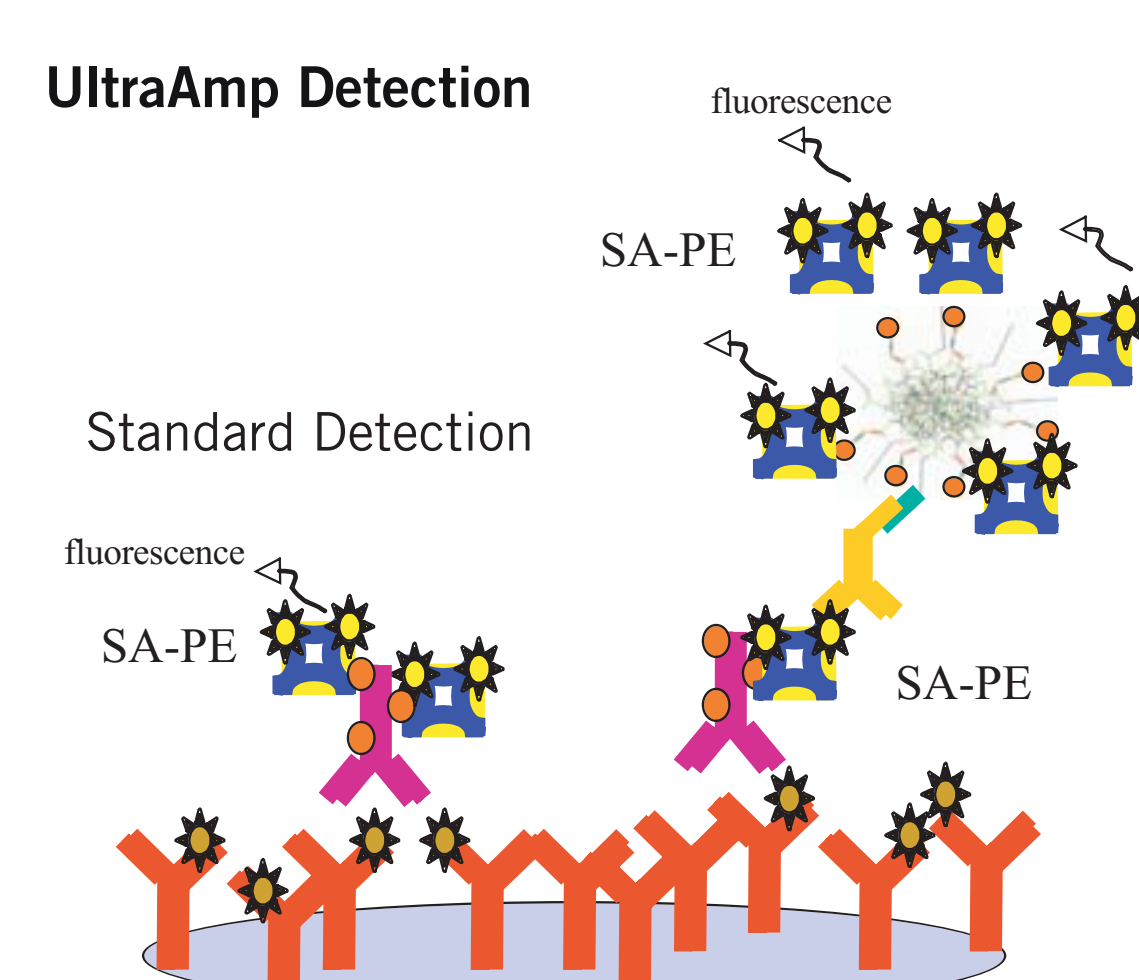
Sandwich ELISA

- Antibody pre-coated plates were used to test the sandwich approach.
- For UltraAmp detection, streptavidin-HRP was substituted by incubation with anti-biotin UltraAmp HRP



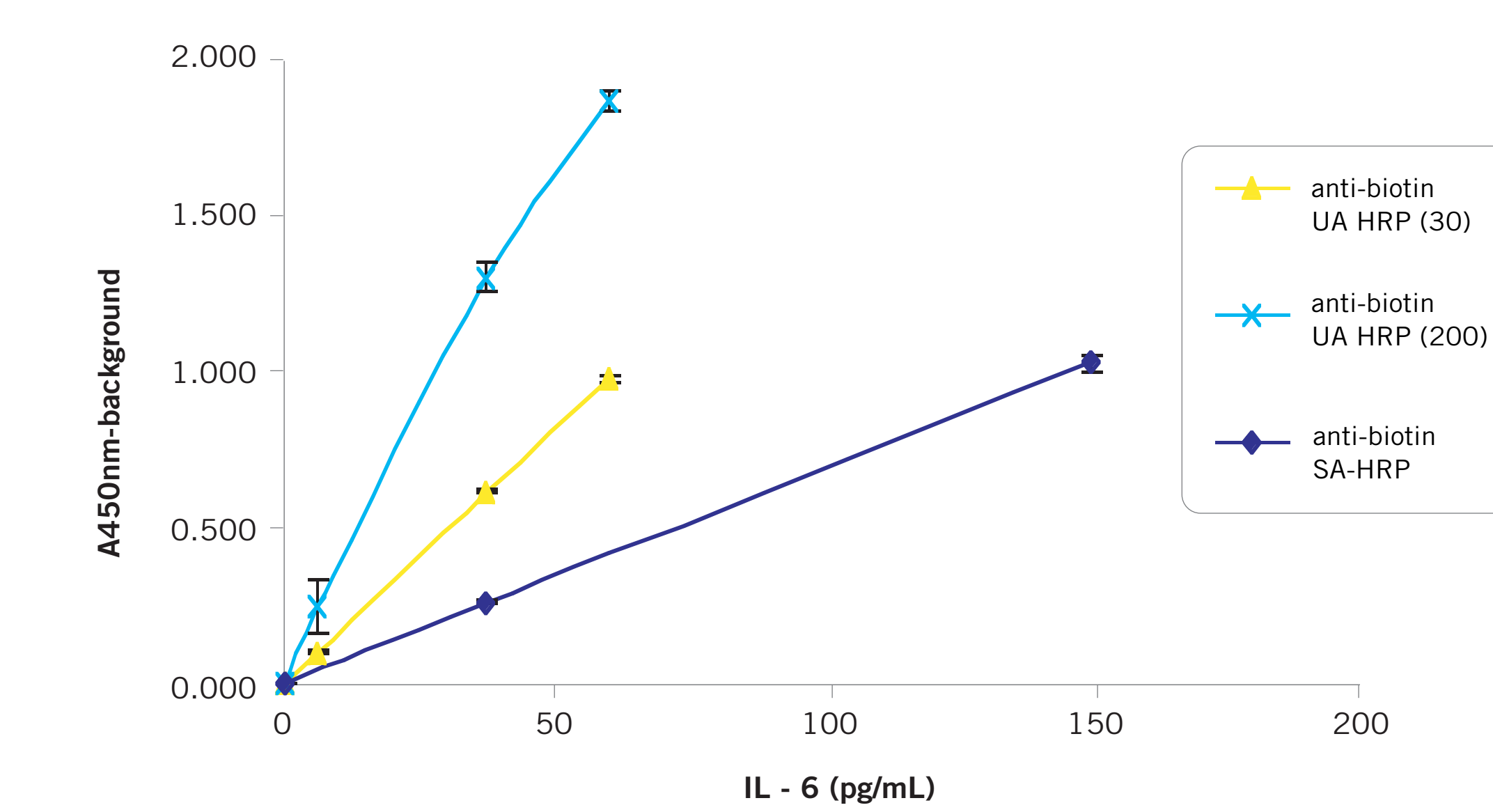
Luminex Assays

- Beads covalently modified with capture antibodies were used to test the sandwich approach.
- For UltraAmp detection, streptavidin-PE (SA-PE) was followed by incubation with biotinylated anti-PE UltraAmp and then SA-PE



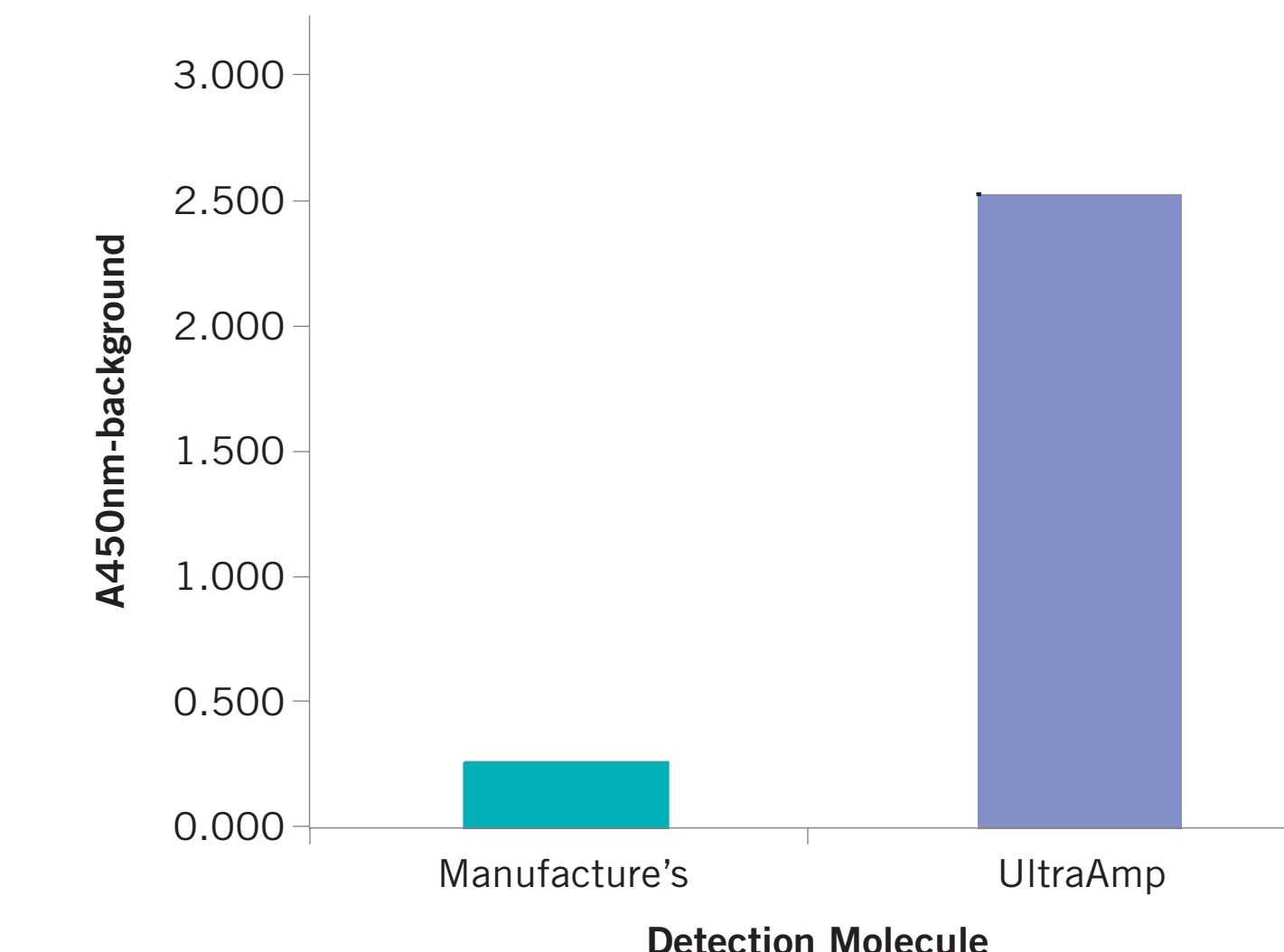
Results

IL-6 sandwich ELISA



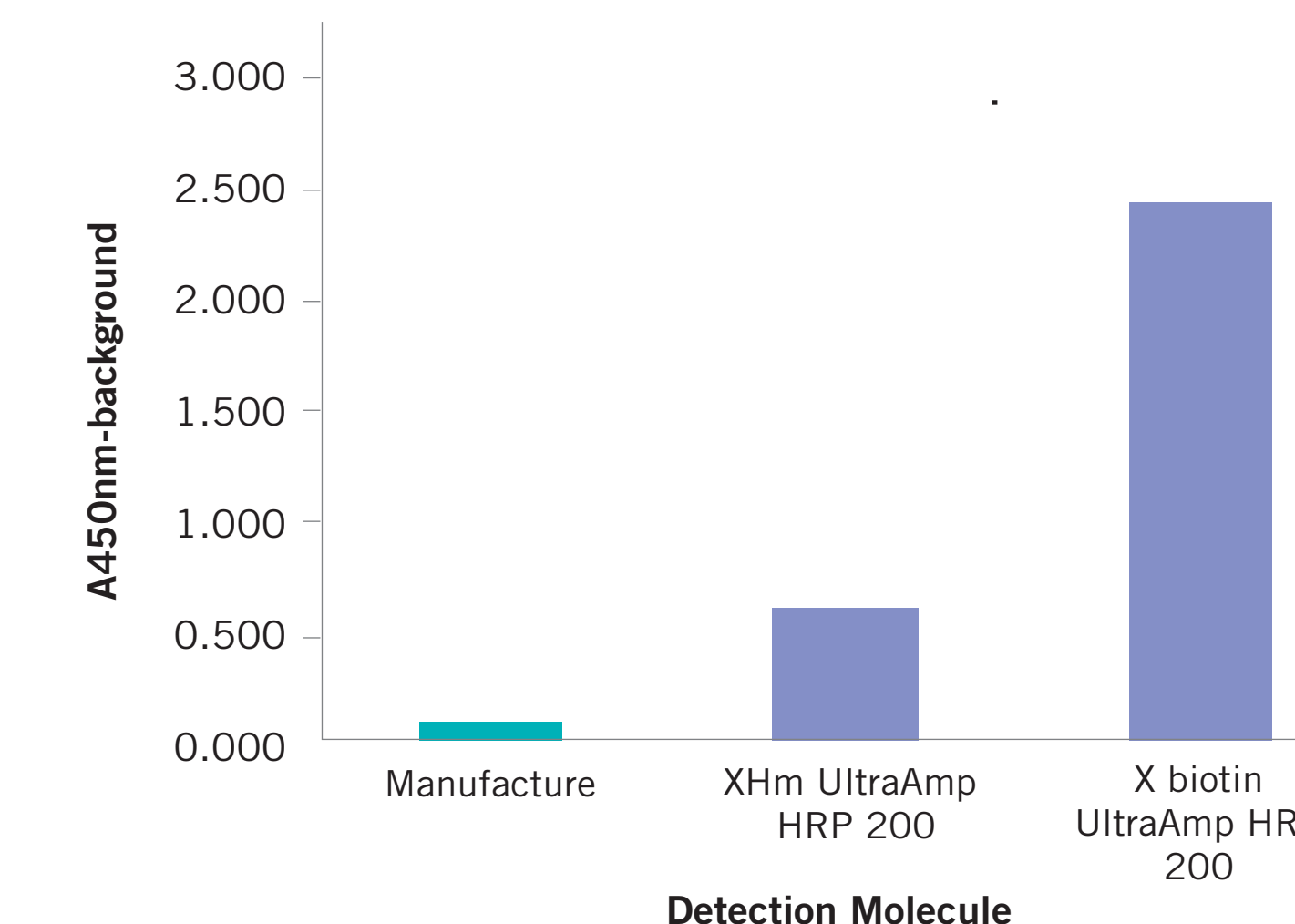
Detection of IL-6 in a sandwich ELISA: Comparison of signal response between HRP-labeled anti-biotin UltraAmp reagents and streptavidin-HRP

H. pylori indirect ELISA



Detection of antibodies against *H. pylori* by indirect ELISA: Comparison of signal response between HRP labeled anti-human (manufacture's) and biotinylated anti-human chased with anti-biotin UltraAmp HRP detection

Amplification in HIV test



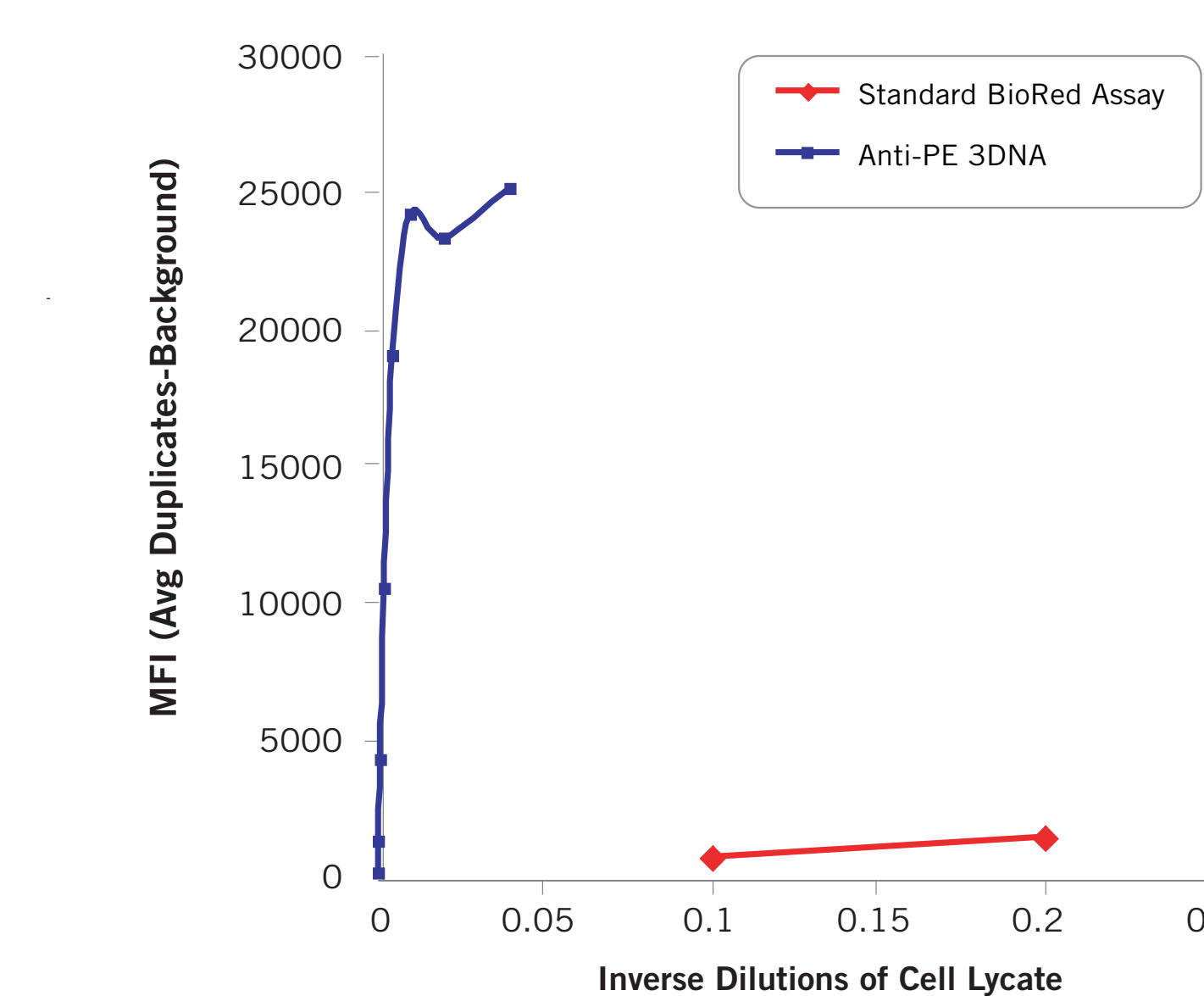
Detection of antibodies against HIV by indirect ELISA: Signal comparison between detection with manufacturer's molecule, anti-human HRP labeled UltraAmp reagents, and biotinylated anti-human chased with anti-biotin UltraAmp.

Hepatitis E Virus (HEV) detection with UltraAmp

Sample	Dilution	Detection Molecule	S/N
control	40	Manufacturer's	3.4
control	80	Manufacturer's	2.1
control	40	anti-biotin UA HRP (200)	21.0
control	80	anti-biotin UA HRP (200)	11.7

Detection of antibodies against HEV by indirect ELISA: Positive controls were incubated in HEV antigen coated plates, and detected with biotinylated anti-human chased with HRP labeled anti-biotin UltraAmp reagents

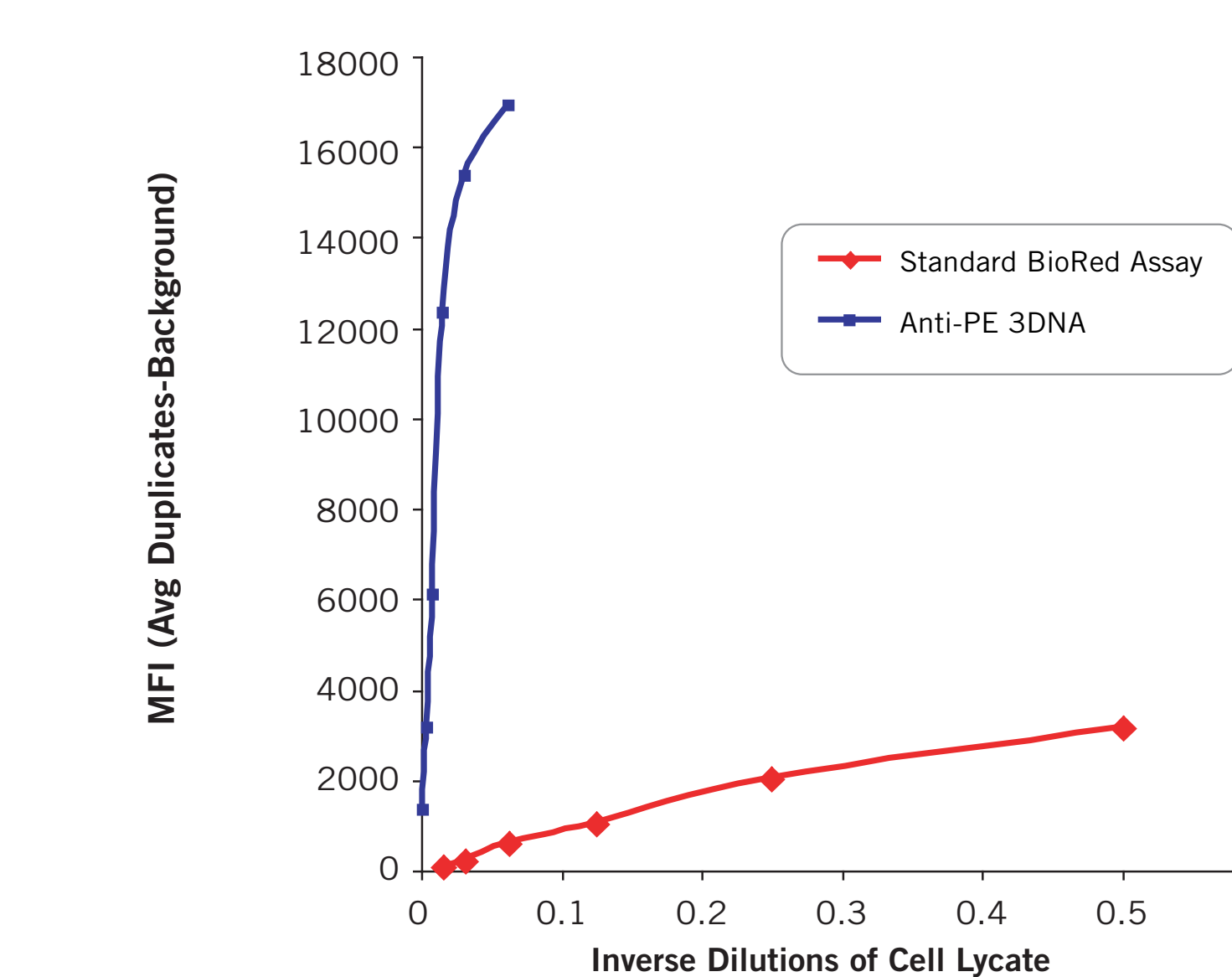
Luminex Results



Comparison of Standard and 3DNA Bio-Rad p38 MAPK Luminex Bead Based Assays Using an Anti-PE Dendrimer with 960 Biotins and Chased with SA-PE

Raw MFI Data

Cell Lysate Dilution	Standard Assay	With 3DNA
None	42	176
1:2.5	1546	ND
1:5	772	ND
1:10	407	ND
1:25	ND	25217
1:50	ND	23403
1:100	ND	24273
1:200	ND	19113
1:400	ND	10698
1:800	ND	4528
1:1600	ND	1527
1:3200	ND	421

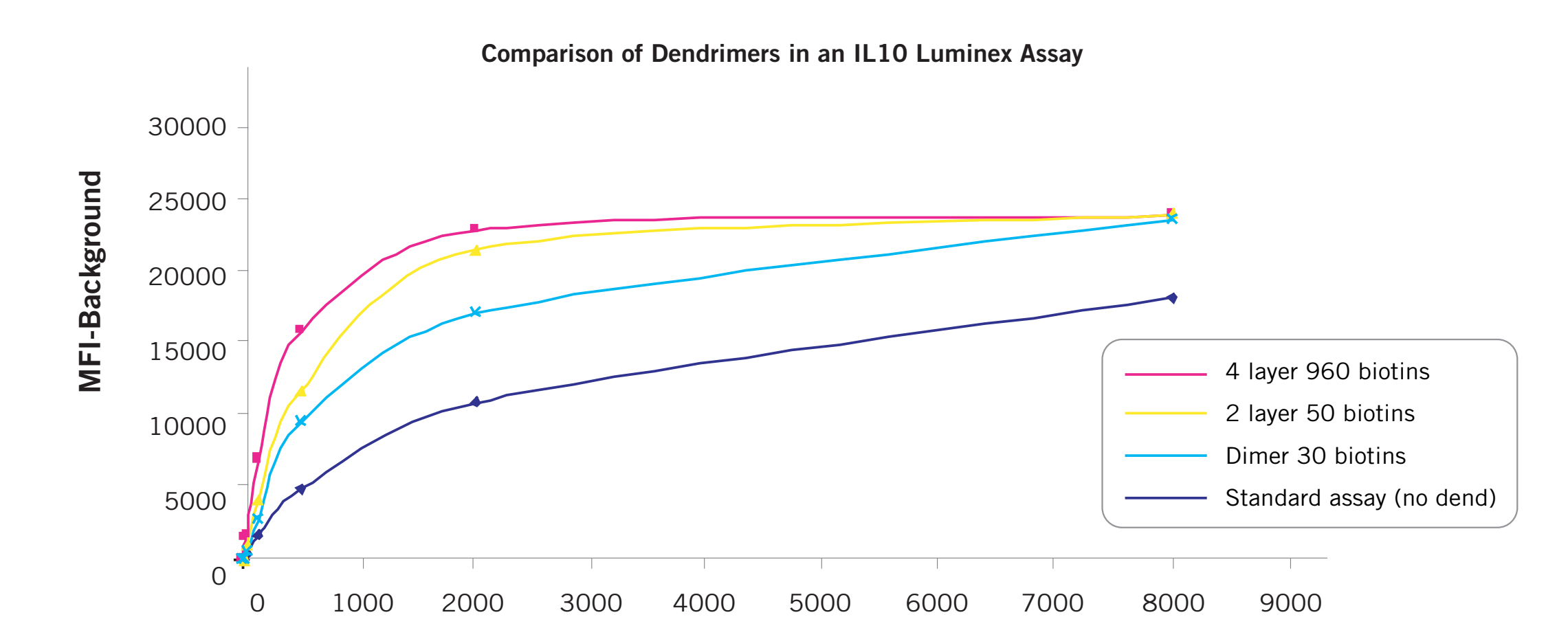
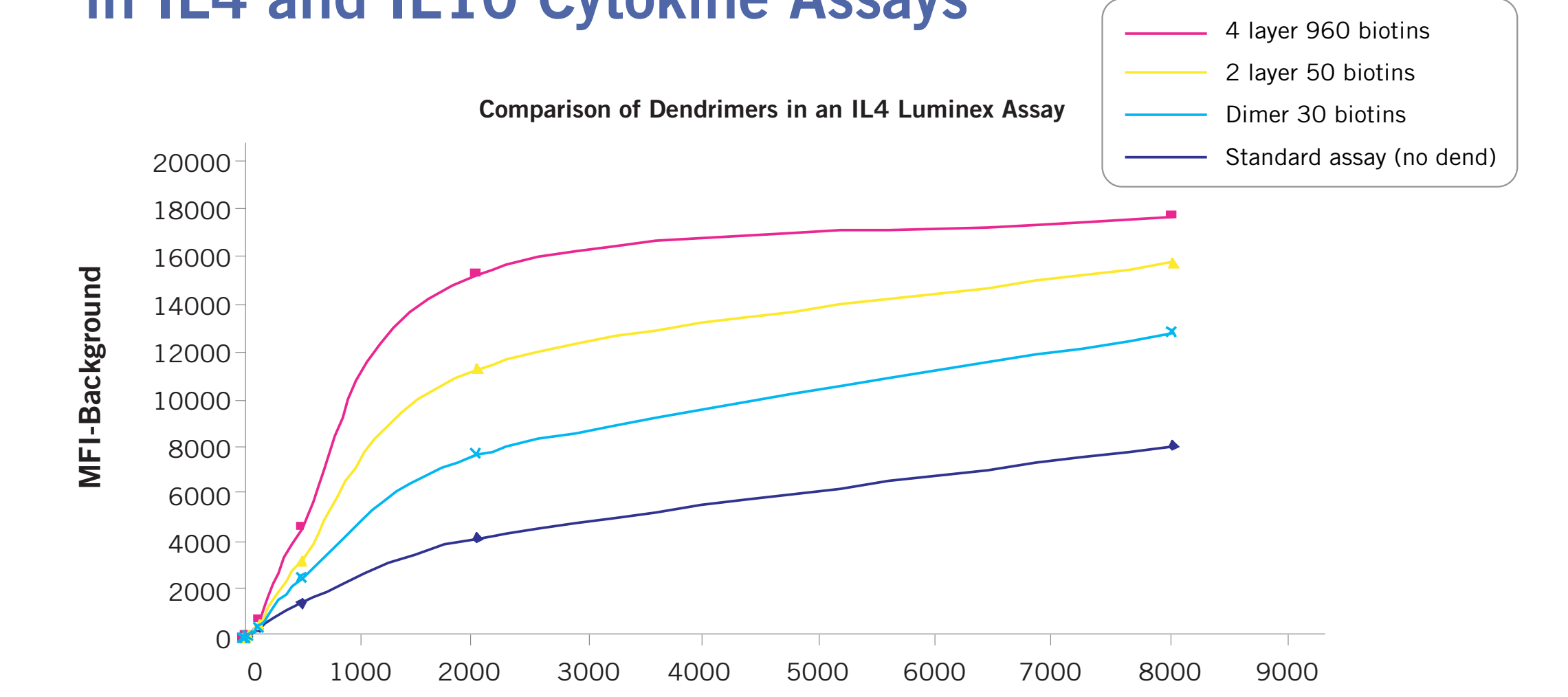


Comparison of Standard and 3DNA Bio-Rad jnk MAPK Luminex Bead Based Assays Using an Anti-PE Dendrimer with 960 Biotins and Chased with SA-PE

Raw MFI Data

Cell Lysate Dilution	Standard Assay	With 3DNA
None	20	17
1:2	3178	ND
1:4	2044	ND
1:8	1021	ND
1:16	614	16926
1:32	218	15367
1:64	92	12340
1:128	ND	6114
1:256	ND	3156
1:512	ND	1320

Comparison of Different Size anti-PE Dendrimers in IL4 and IL10 Cytokine Assays



Estimated Fold Improvement of Sensitivity for Phospho-protein and Cytokine Assays

Antigen Target	Estimated Increase of sensitivity
p38	>500 fold
JNK	~128 fold
egfr	~4 fold
erk2	~64 fold
IL2	~10 fold
IL4	~40 fold
IL6	~2 fold
IL10	~10 fold

Conclusions

- Anti-biotin HRP UltraAmp reagents provided up to or more than 200-fold signal amplification in sandwich as well as indirect ELISAs and Luminex bead based assays
- In sandwich ELISAs anti-biotin UltraAmp reagents labeled with 200 HRP's provide greater signal amplification than those labeled with 30 HRP's
- In Luminex bead based assays phospho-proteins tend to perform better than cytokines, with larger dendrimers (with more biotins) providing better signal amplification
- In ELISAs, antibody stacking results in greater signal amplification.
- There is a great improvement in signal when human antibodies are detected first with anti-human biotinylated followed by anti-biotin HRP labeled UltraAmp