KPL TECHNICAL SERVICE REPORT

Stability of *Campylobacter* Positive Control
(Catalog No. 50-92-93)

PURPOSE

The purpose is to measure the stability of the *Campylobacter* positive control (KPL Catalog No. 50-92-93) over a 16-year period when stored at 4°C.

MATERIALS AND METHODS

The following lots of material were tested via ELISA. Representative vials from each lot were stored at the recommended temperature of 4°C in lyophilized form. Vials were rehydrated by adding 1 mL of 50% glycerol on the dates listed below:

Reagent	Lot No.	Date of Manufacture	Date of Rehydration
Campylobacter Positive Control, Cat. No. 50-92-93	PD36	04/29/1992	06/12/1995
	040344	02/04/2004	06/20/2008
	080261	06/16/2008	06/20/2008

The samples were evaluated via an amplified ELISA utilizing a polyclonal antibody to the positive control as a primary, and a biotinylated monoclonal antibody as a secondary. HRP-labeled streptavidin was used to detect the biotinylated secondary. ABTS substrate was reacted with the HRP, and subsequent O.D. readings were measured at 405 nm. The O.D. values were then compared to one another in a quantitative manner to determine their similarity to one another. These values allowed us to ascertain the stability of each lot.

ELISA Reagents	KPL Lot No.
Unlabeled Goat anti-*Campylobacter* Antibody	WE061
10X BSA Diluent Block/Solution	070519
20X Wash Buffer	080340
Biotinylated anti-Goat IgG Antibody	21-M-265-01
HRP Streptavidin	060897
ABTS 1-Component	080322
HRP Stop Solution	070961

The assay was performed as follows at room temperature:

1. Each positive control was diluted 1:100 in PBS and coated at 100 µL/well in duplicate onto a clear NUNC Maxisorp 96-well plate. Columns 1 - 2 contained Lot PD36, 3 – 4 contained Lot 040344 and 5 – 6 contained Lot 080261. The plate was incubated for one hour.
2. 200 µL of 1X BSA Diluent/Block Solution was added directly to each well and allowed to incubate for 3 minutes. The solution was removed from all wells.
3. The plate was allowed to dry.
4. Unlabeled Goat anti-*Campylobacter* antibody was diluted to a concentration of 2 µg/mL in 1X BSA Diluent/Block. It was then serially diluted 1:2 eight times. 100 µL of each dilution was added in duplicate to the corresponding wells in the ELISA plate. The plate was then allowed to incubate for 30 minutes.
5. The plate was washed three times, allowed to soak for 3 minutes, and rinsed an additional 3 times in 1X Wash Solution.
6. Biotinylated antibody was diluted 1:1800 in 1X BSA Diluent/Block and added to the plate at a volume of 100 µL/well and allowed to incubate for 30 minutes. The plate was washed according to Step 5.
7. HRP Streptavidin was diluted 1:4000 in 1X BSA Diluent Block and allowed to incubate for 30 minutes at a volume of 100 µL/well. The plate was washed according to Step 5.
8. 100 µL/well of ABTS was added to each well and allowed to incubate for 15 minutes. The plate was stopped with 100 µL/well of 1X HRP Stop Solution.
9. The plate was read at a wavelength of 405 nm.

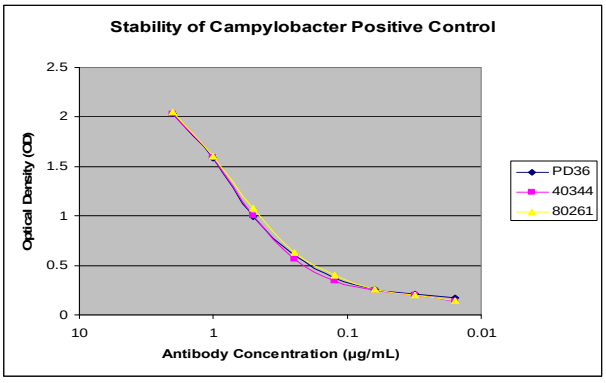
RESULTS AND CONCLUSIONS

All positive control lots were equivalent, as shown in Figures 1 and 2. Even after rehydration with 50% glycerol, product tested 13 years after rehydration showed equivalent performance to lots freshly rehydrated. Therefore, the data demonstrates that when stored at the recommended temperature of 4°C, KPL's *Campylobacter* Positive Control is extremely stable over time. In addition, this study indicates that multi-year storage of the lyophilized product does not impact product stability.

Figure 1.
Average O.D. readings of serial diluted Positive controls

	PD36	40344	80261
A	2.035	2.035	2.048
B	1.58	1.592	1.607
C	0.991	1.001	1.081
D	0.6	0.563	0.634
E	0.369	0.339	0.4
F	0.261	0.252	0.261
G	0.207	0.197	0.202
H	0.174	0.133	0.147

Figure 2.
Graph of data presented in Figure 1.



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