



The University of Georgia

College of Agricultural and Environmental Sciences
Center for Food Safety

Final Report

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Project Title

Effect of KLS-1/2 and heating on the inactivation of *Escherichia coli* O157:H7 in ground beef: Determination of D-values of *E. coli* O157:H7 in ground beef

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Objective: To determine in refrigerated and frozen ground beef with and without KLS-1/2 rates of thermal inactivation in ground beef of a strain of *E. coli* O157:H7 isolated from ground beef. A comparison of D-values will be made to determine if there is a difference in the thermal inactivation of *E. coli* O157:H7 in ground beef treated with (treated) or without (control) KLS-1/2. A comparison will also be made of the effect of storage temperature prior to heat treatment on rates of thermal inactivation.

Experimental approach

Ground beef: Ground beef samples with and without treatment by KLS-1/2 were prepared by a ground beef processor under the supervision of Mionix. Two packages of ground beef (one with KLS-1/2 and the other with no KLS-1/2) of approximately 10 pounds each were received at 7-8°C. The packages were stored at -86°C until

inoculation with *E. coli* O157:H7. The ground beef was prepared as follows. One hundred twenty-five pounds of beef were placed in a Hobart ribbon blender at 30°F. The meat beef was blended for 3.5 minutes; KLS-1/2 was added during the final one minute and was uniformly distributed during blending for the entire minute. The ground beef was then passed through a 3/32 inch plate and formed into 4 ounce patties using Formas forming technology. The patties were packaged and shipped to the UGA Center for Food Safety. Mionix solution KLS-1/2 was approximately half the strength used in a previous similar study entitled: Effect of KLS and heating on the inactivation of *E. coli* O157:H7 in ground beef: Determination of D-values of *E. coli* O157:H7 in ground beef, conducted by the University of Georgia and completed in May, 2001.

Bacteria used for evaluation: *E. coli* O157:H7 (OH1395, ground beef isolate) was grown in 10 ml of tryptic soy broth at 37°C for 18 h with agitation (100 rpm). Bacteria were sedimented by centrifugation at 4,000 x g for 20 min and washed in 0.1 M phosphate buffer, pH 7.2 for three times by the same method. Sedimented bacteria were suspended in PBS and adjusted to an OD reading of 0.5 at 630 nm (10^8 CFU/ml). Actual *E. coli* O157:H7 count was confirmed by enumeration on tryptic soy agar.

Inoculation of ground beef: Washed cells at a ratio of 1 ml of 10^8 CFU *E. coli* O157:H7 per 100 g of ground beef was inoculated into ground beef (ca. 26% fat). Bacteria were mixed into the meat by massaging meat with gloved hands for 2 min in a laminar flow hood. After mixing, the inoculated ground beef samples (KLS-1/2-treated and untreated) were separated into 12 to 14 Whirl-Pak (4 OZ) bags (about 30 g per bag), and held at either 5°C or -20°C before evaluation of the thermal inactivation of *E. coli* O157:H7. Bags containing KLS-1/2-treated and untreated ground beef and held at 5°C were used within 10 days and the bags containing KLS-1/2-treated and untreated ground beef and held at -20°C were used within 3 weeks.

Thermal inactivation: One-gram portions of refrigerated treated or untreated (control) ground beef samples were weighed and lightly packed in a laminar flow hood into each of 24 Pyrex test (10 x 75 mm) tubes capped with rubber stoppers. Frozen ground beef samples were thawed at 21°C in a laminar hood for 20 to 30 min, then lightly packed in 1-g portions into test tubes as described above. Temperature was monitored by thermocouples placed in the center of several meat samples. All tubes were submerged in a water circulator (VWR Scientific, Model 1265PC) pre-adjusted to the appropriate temperature (2°C greater than the desired temperature of the study). Once the meat reached the desired temperature (57, 60, 62.8, or 64.3°C), two tubes were immediately removed and cooled at iced water (5°C). The number of *E. coli* O157:H7 surviving in these samples was the number present at zero time (base line). Duplicate samples were taken at appropriate intervals and enumerated for *E. coli* O157:H7. Duplicate tests were done for each temperature treatment. Sampling times for KLS-1/2-treated and untreated, refrigerated ground beef were: at 57°C for 0, 5, 10, 15 and 20 min; at 60°C for 0, 2, 5, 10, and 15 min; at 62.8°C for 0, 1, 3, 5, 7, and 9 min; at 64.3°C for 0, 0.5, 0.75, 1, and 1.25 min. Sampling times for KLS-1/2-treated and untreated, frozen ground beef were: at 57°C for 0, 1, 3, 5, 10 and 15 min; at 60°C for 0, 0.5, 1, 2, 5 and 10 min; at 62.8°C for 0, 0.17, 0.33, 0.5, 1 and 1.5 min; at 64.3°C for 0, 1.7, 0.33, 0.5, 0.67 and 1 min.

Enumeration of E. coli O157:H7: Following heat treatment, the tubes were immediately submerged in ice water. The ground beef was transferred to 9 ml of 0.1% peptone water. A volume of 1 ml was serially (1:10) diluted in 0.1% peptone to 10^{-3} . A portion of 0.1 ml from each dilution was plated on tryptic soy agar plates in duplicate. The plates were incubated at 37°C for 24 h. After determining *E. coli* O157:H7 counts, selected colonies (up to 5 colonies on each plate) were randomly picked from the plate and confirmed as O157 by *E. coli* O157 latex agglutination assay (Oxoid). The D-value at each temperature was determined.

Results

1. *E. coli* O157:H7 counts after heat treatment in ground beef with and without KLS-1/2 after being held at 5°C for up to 10 days (Tables 1 and 2) or at -20°C for 3 weeks are presented in Tables 3 and 4.
2. D-values of *E. coli* O157:H7 KLS-1/2-treated and untreated ground beef held refrigerated or frozen before heat treatment are shown in Table 5.

Results revealed that *E. coli* O157:H7 (OH1395) in ground beef containing KLS-1/2 was more rapidly inactivated at an equivalent temperature than *E. coli* O157:H7 in the control ground beef (without KLS-1/2). Furthermore, holding *E. coli* O157:H7-contaminated ground beef frozen (at -20°C) for 3 weeks prior to heat treatment increased the pathogen's sensitivity to thermal inactivation resulting in lower D-values compared with studies using ground beef held refrigerated prior to heat treatment.

Table 1: Thermal inactivation of *E. coli* O157:H7 (OH1395) inoculated into ground beef (25.8% fat) and held at 5°C prior to heat treatment. *E. coli* O157:H7 were enumerated on tryptic soy agar plates.

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7count (log ₁₀ CFU/g)				
		0	5	10	15	20
57	1	5.7	5.2	4.8	4.7	4.1
	2	6.3	6.0	5.6	5.2	4.6

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7count (log ₁₀ CFU/g)				
		0	2	5	10	15
60	1	5.5	5.0	3.6	1.9	1.7
	2	6.2	5.6	4.3	<1.7	<1.7

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7count (log ₁₀ CFU/g)					
		0	1	3	5	7	9
62.8	1	5.3	3.9	2.0	<1.7	<1.7	<1.7
	2	5.9	4.2	<1.7	<1.7	<1.7	<1.7

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7count (log ₁₀ CFU/g)				
		0	0.5	0.75	1	1.25
64.3	1	4.9	3.2	2.4	1.7	<1.7
	2	5.1	3.2	<1.7	<1.7	<1.7

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Table 2: Thermal inactivation of *E. coli* O157:H7 (OH1395) inoculated into ground beef treated with KLS-1/2 (25.8% fat) and held at 5°C prior to heat treatment. *E. coli* O157 were enumerated on tryptic soy agar plates

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)				
		0	5	10	15	20
57	1	6.4	6.0	4.0	2.5	2.4
	2	6.5	6.2	4.5	3.1	2.2

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)				
		0	2	5	10	15
60	1	6.2	3.7	3.0	<1.7	<1.7
	2	6.2	4.9	<1.7	<1.7	<1.7

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)					
		0	1	3	5	7	9
62.8	1	6.0	3.3	<1.7	<1.7	<1.7	<1.7
	2	5.7	3.1	<1.7	<1.7	<1.7	<1.7

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)				
		0	0.5	0.75	1	1.25
64.3	1	3.9	2.8	<1.7	<1.7	<1.7
	2	5.7	2.9	<1.7	<1.7	<1.7

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Table 3: Thermal inactivation of *E. coli* O157:H7 (OH1395) inoculated into ground beef (25.8% fat) and held at -20°C for 3 weeks prior to heat treatment. *E. coli* O157 were enumerated on tryptic soy agar plates

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)					
		0	1	3	5	10	15
		(minutes)					
57	1	6.4	6.3	6.2	6.1	5.0	3.7
	2	6.4	6.1	6.0	6.0	4.9	4.0

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)					
		0	0.5	1	2	5	10
		(minutes)					
60	1	6.3	6.2	6.1	5.9	4.2	1.8
	2	6.4	6.3	6.2	6.0	4.3	1.7

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)					
		0	0.17	0.33	0.5	1	1.5
		(minutes)					
62.8	1	5.9	5.3	4.9	3.9	1.7	<1.7
	2	5.9	5.6	5.6	5.1	<1.7	<1.7

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)					
		0	1.7	0.33	0.5	0.67	1
		(minutes)					
64.3	1	3.9	2.4	<1.7	<1.7	<1.7	<1.7
	2	3.5	2.1	<1.7	<1.7	<1.7	<1.7

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Table 4: Thermal inactivation of *E. coli* O157:H7 (OH1395) inoculated into ground beef (25.8% fat) and held at -20°C for 3 weeks prior to heat treatment. *E. coli* O157 were enumerated on tryptic soy agar plates

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)					
		0	1	3	5	10	15
57	1	6.0	5.9	5.7	5.5	3.5	2.0
	2	6.0	5.8	5.6	5.2	3.5	2.7

Temperature (°C)	Trial No.	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)					
		0	0.5	1	2	5	10
60	1	5.9	5.5	5.2	4.1	2.3	<1.7
	2	5.7	5.4	4.0	4.5	2.9	<1.7

Temperature (°C)	Trial No.	0	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)				
			0.17	0.33	0.5	1	1.5
62.8	1	3.7	2.1	1.7	<1.7	<1.7	<1.7
	2	3.3	2.4	<1.7	<1.7	<1.7	<1.7

Temperature (°C)	Trial No.	0	<i>E. coli</i> O157:H7 count (log ₁₀ CFU/g)				
			1.7	0.33	0.5	0.67	1
64.3	1	2.0	<1.7	<1.7	<1.7	<1.7	<1.7
	2	<1.7	<1.7	<1.7	<1.7	<1.7	<1.7

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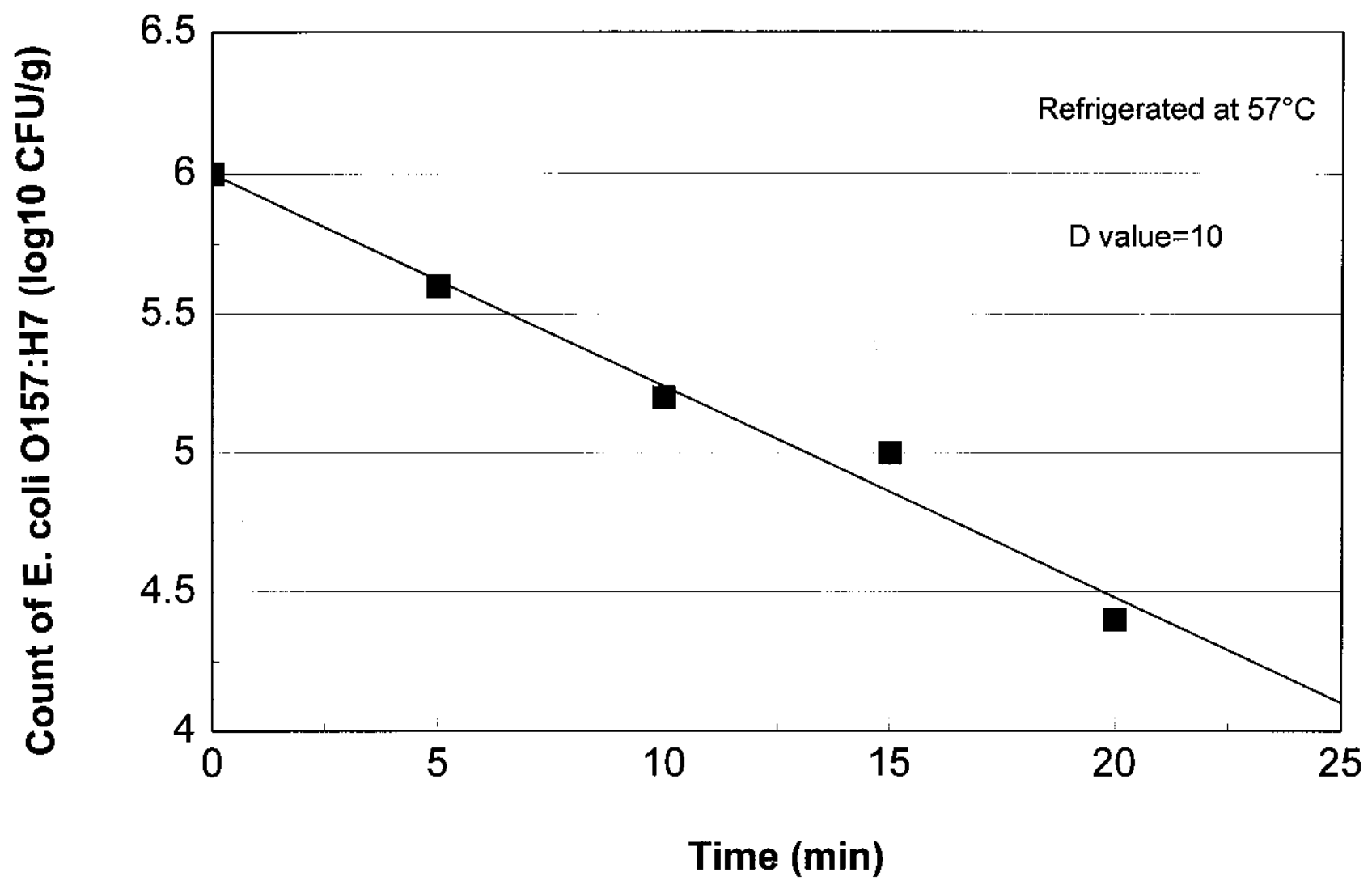
Table 5: D-values of *E. coli* O157:H7 (OH1395) in ground beef and with and without KLS-1/2 and held at 5°C for up to 10 days or at -20°C for 3 weeks before receiving heat treatment.

Strain	Refrigerated (5°C) or frozen (-20°C)	D value (min) at:			
		57°C	60°C	62.8°C	64.3°C
OH1395	refrigerated	10	2.4	1.1	0.26
OH1395	refrigerated with KLS-1/2	5.1	2.1	IS ^a	IS
OH1395	frozen	6.3	2.0	0.25	IS
OH1395	frozen with KLS-1/2	3.8	1.6	IS	NS ^b

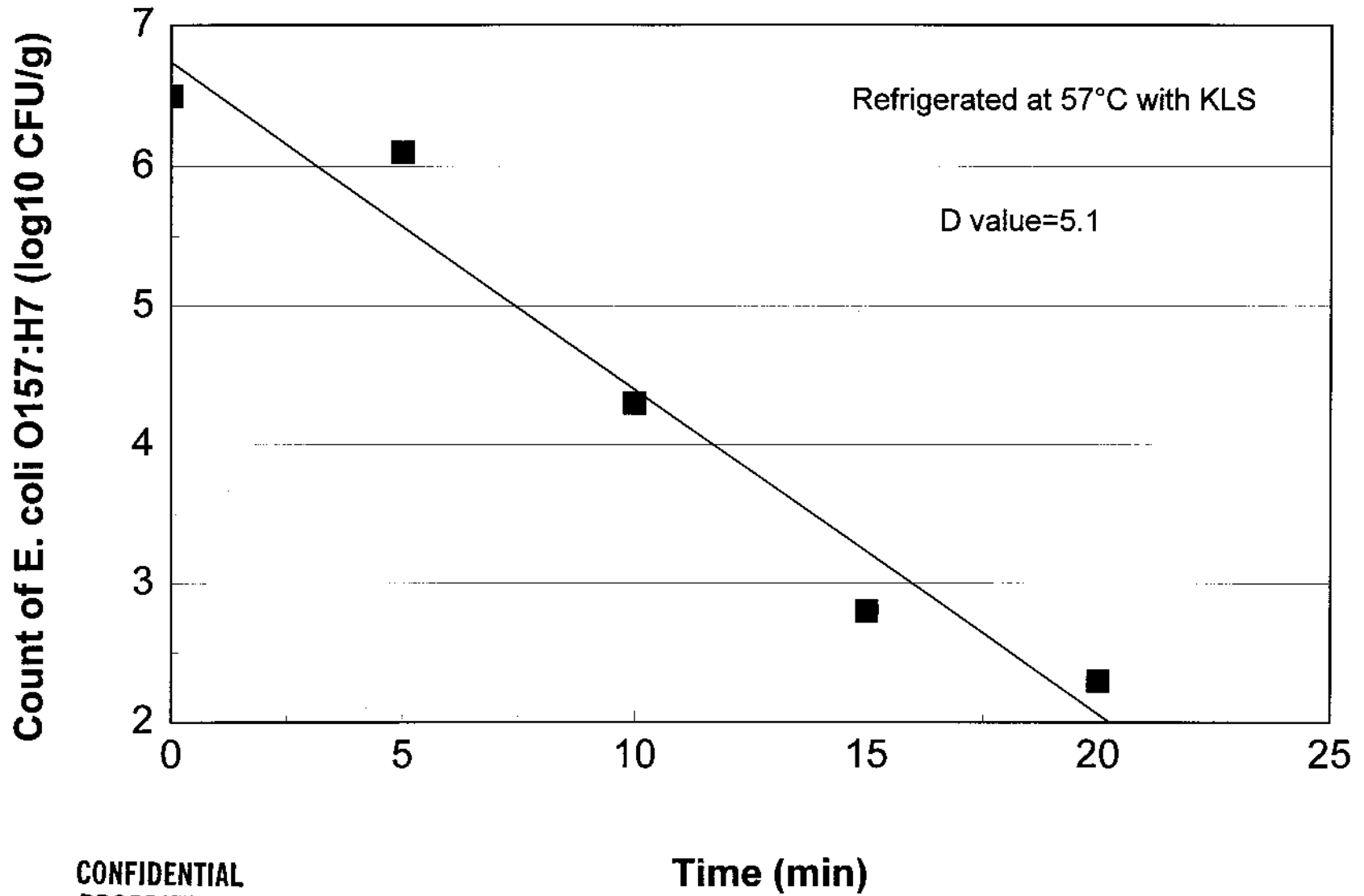
^a IS, insufficient number of data points to calculate D-value.

^b NS, no survivors; no detectable cells at zero time (initial cell counts were ca. 10^7 CFU/g before heating). Ca. 10^5 *E. coli* O157:H7/g were inactivated during the come-up time.

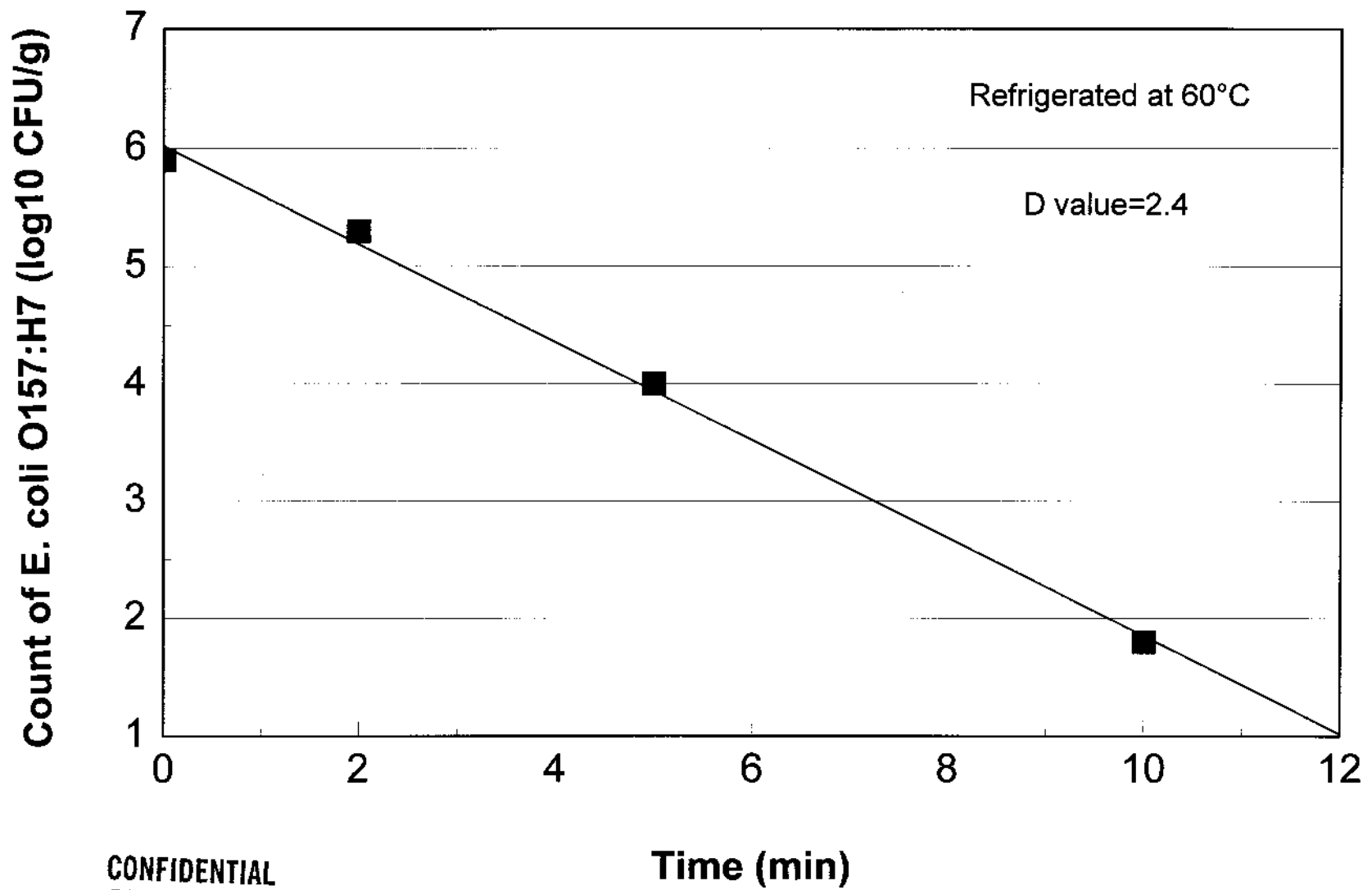
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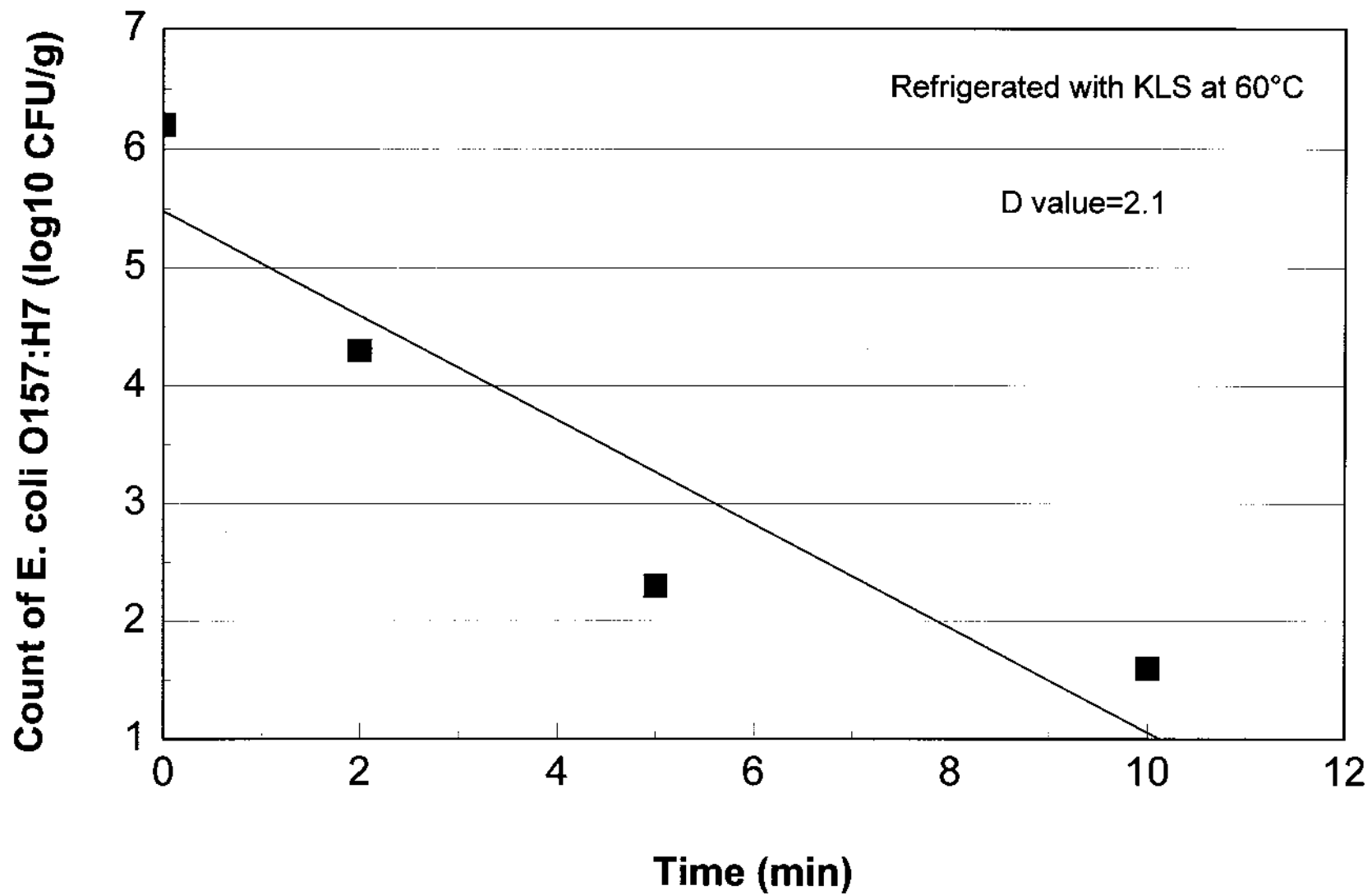
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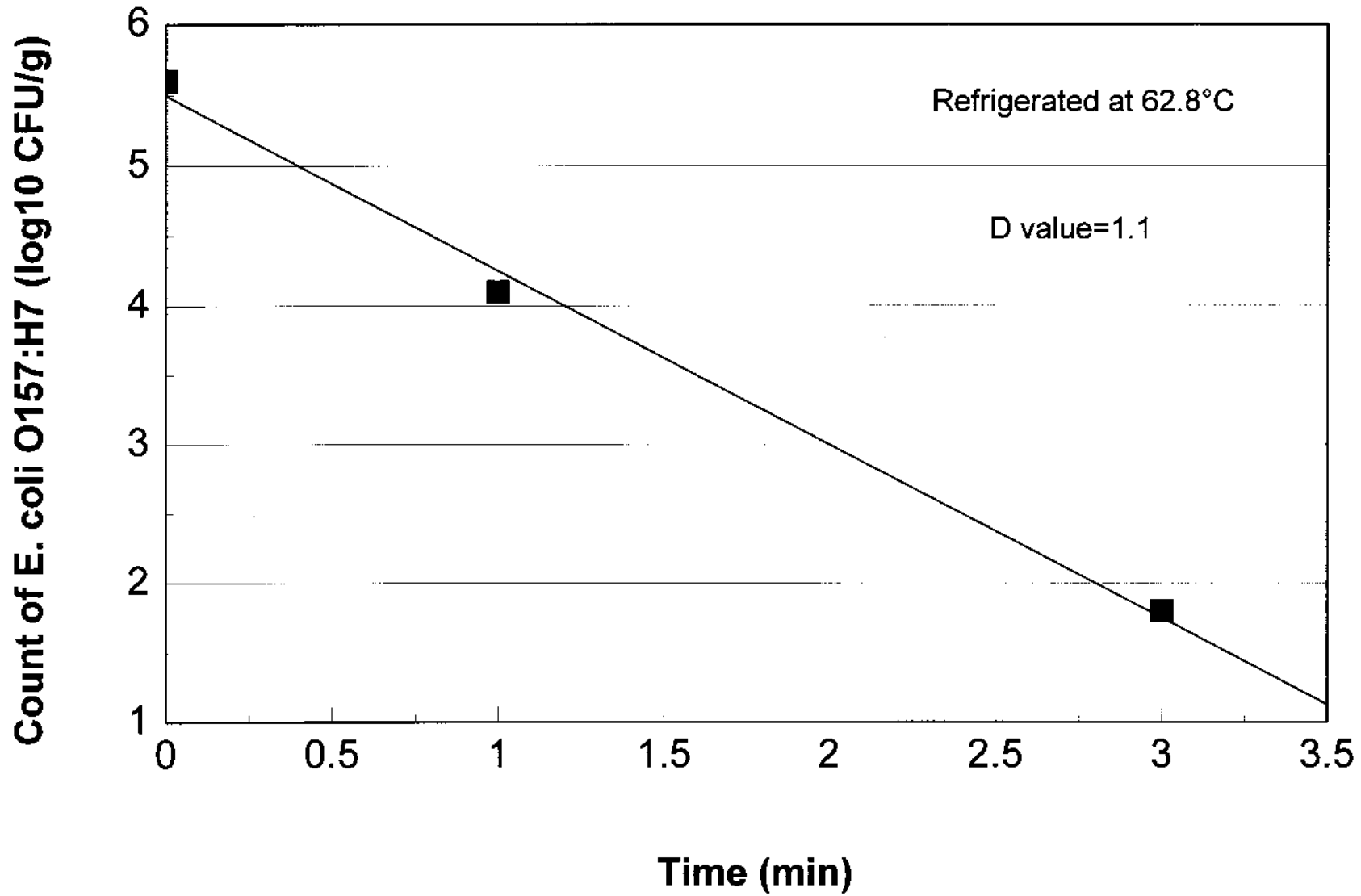
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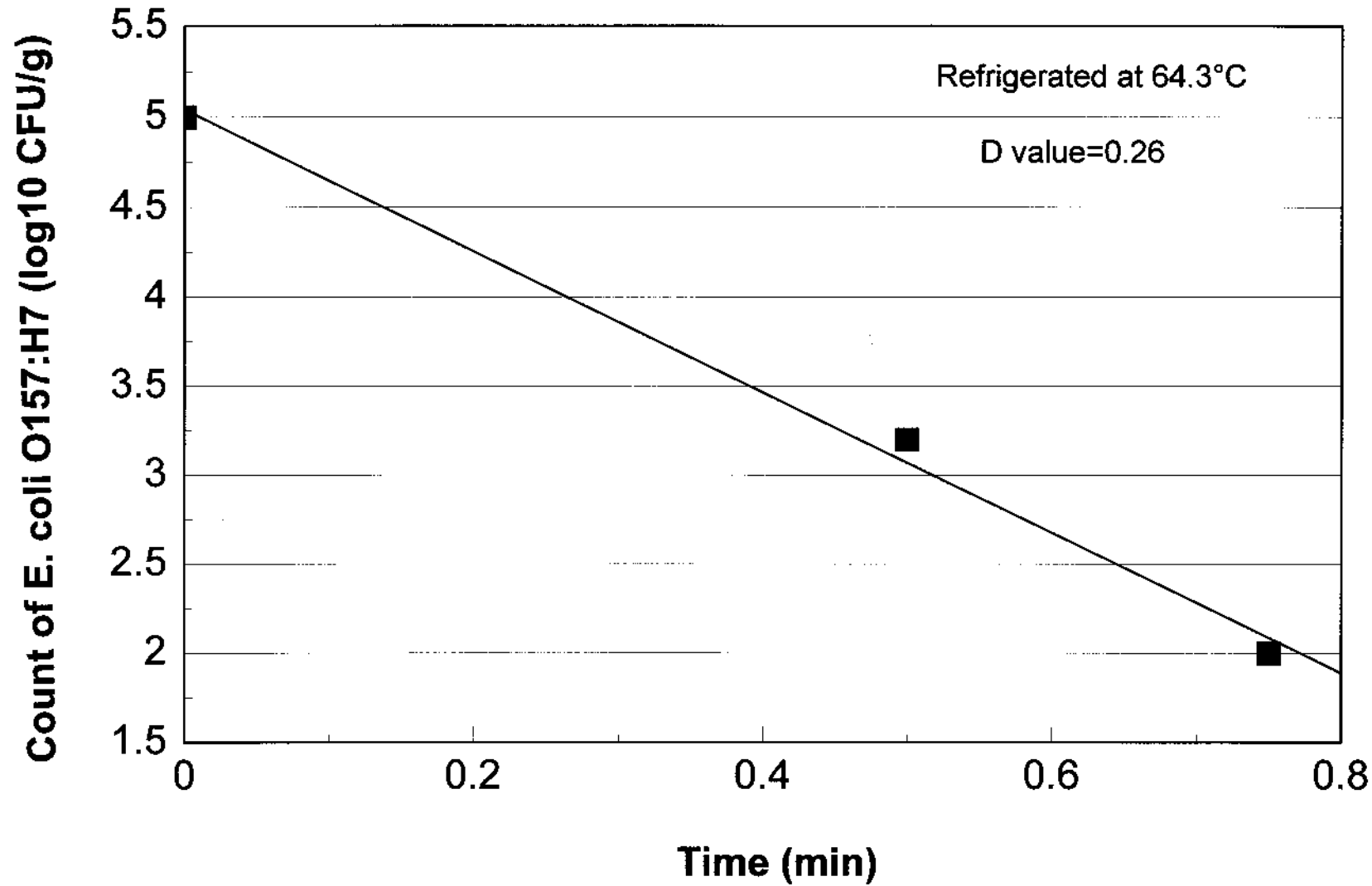
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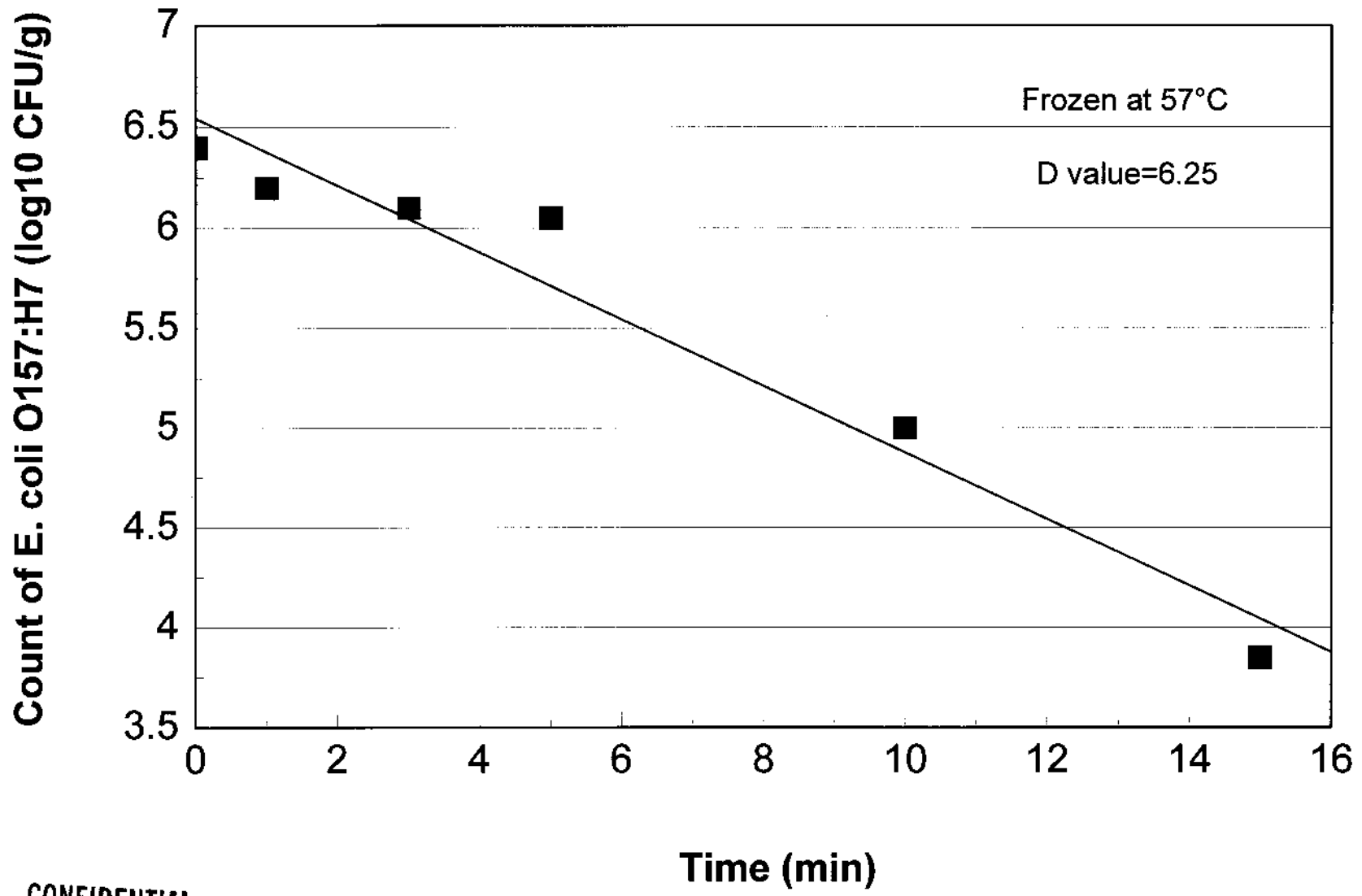
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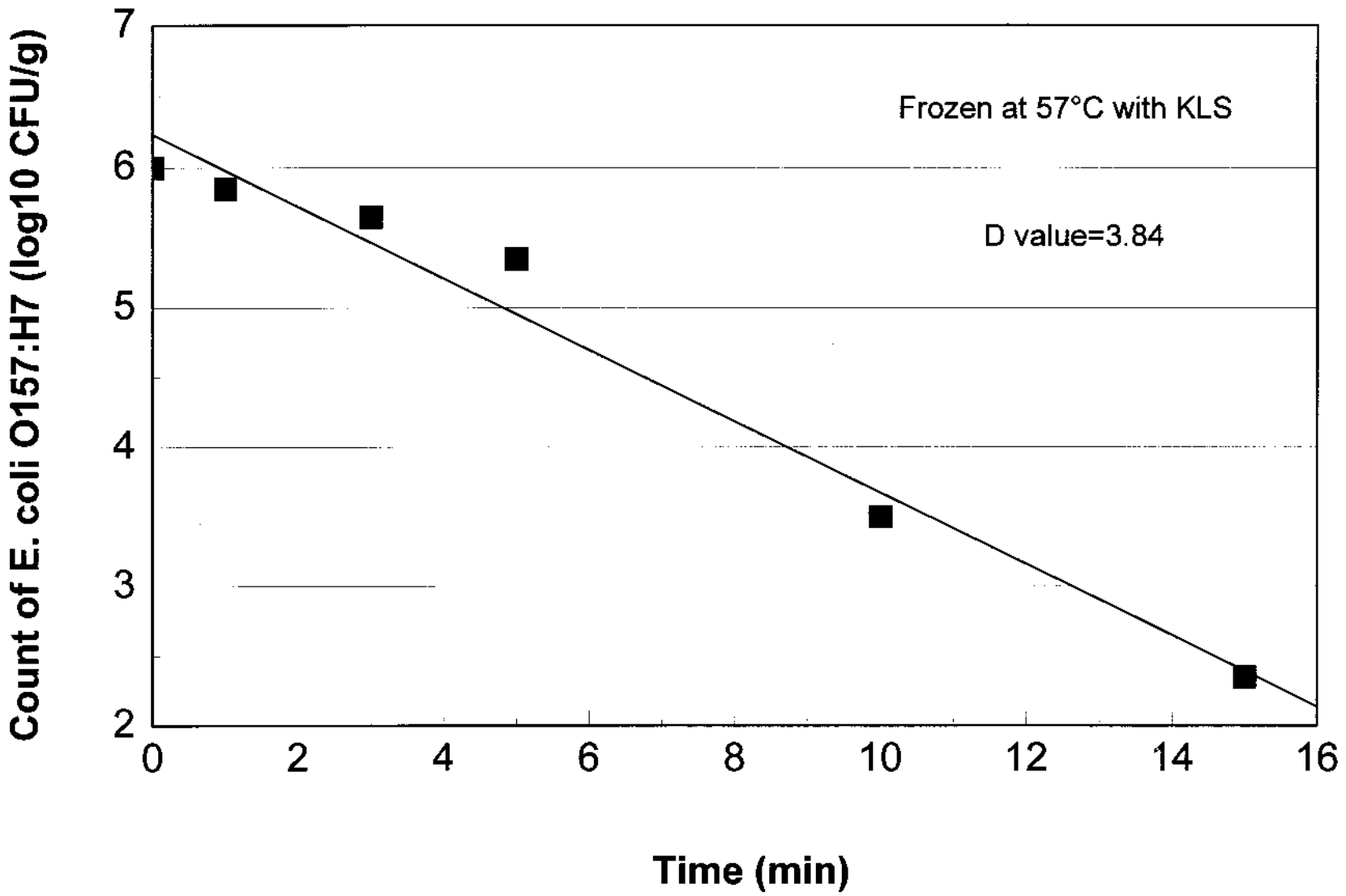
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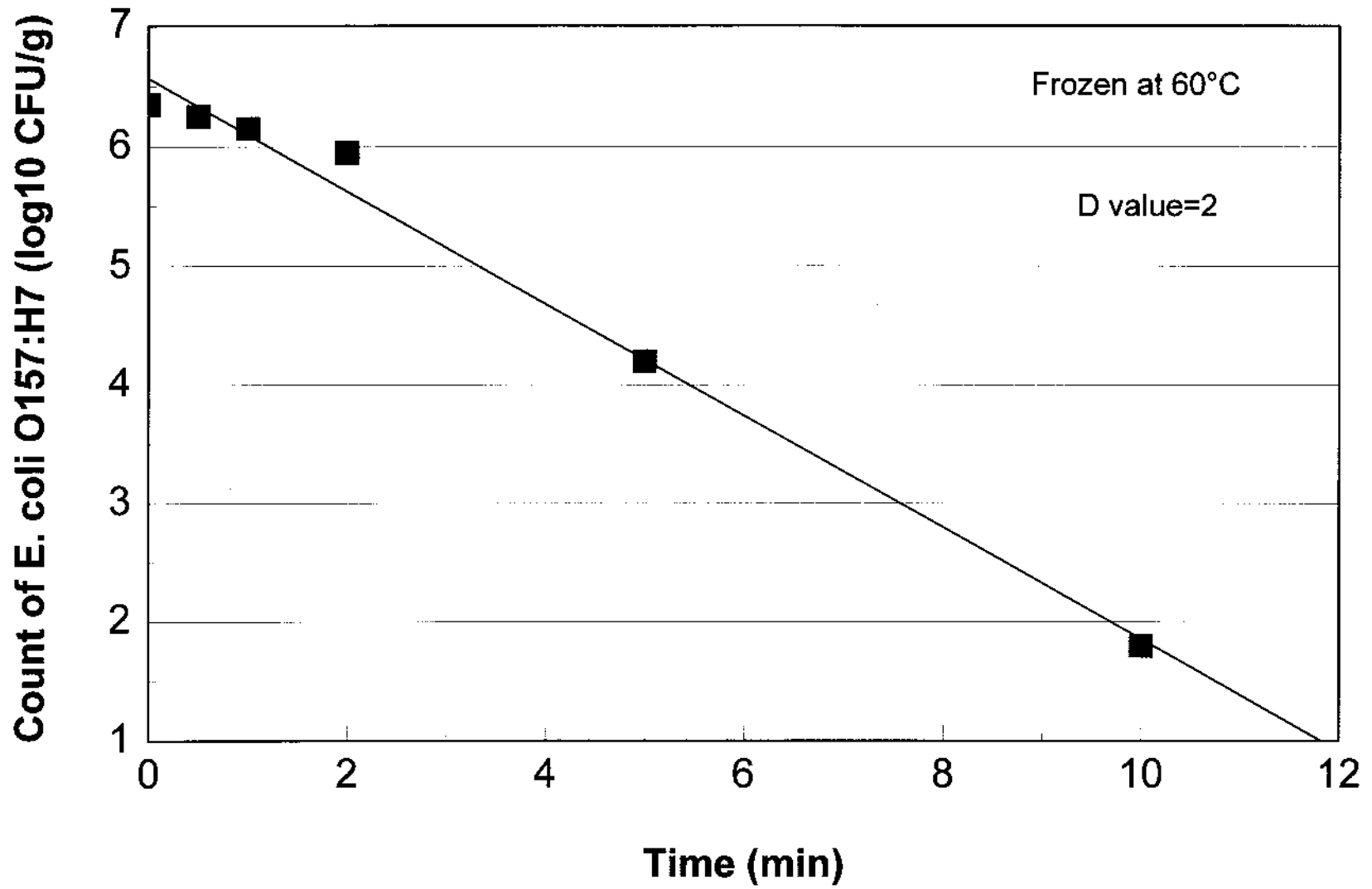
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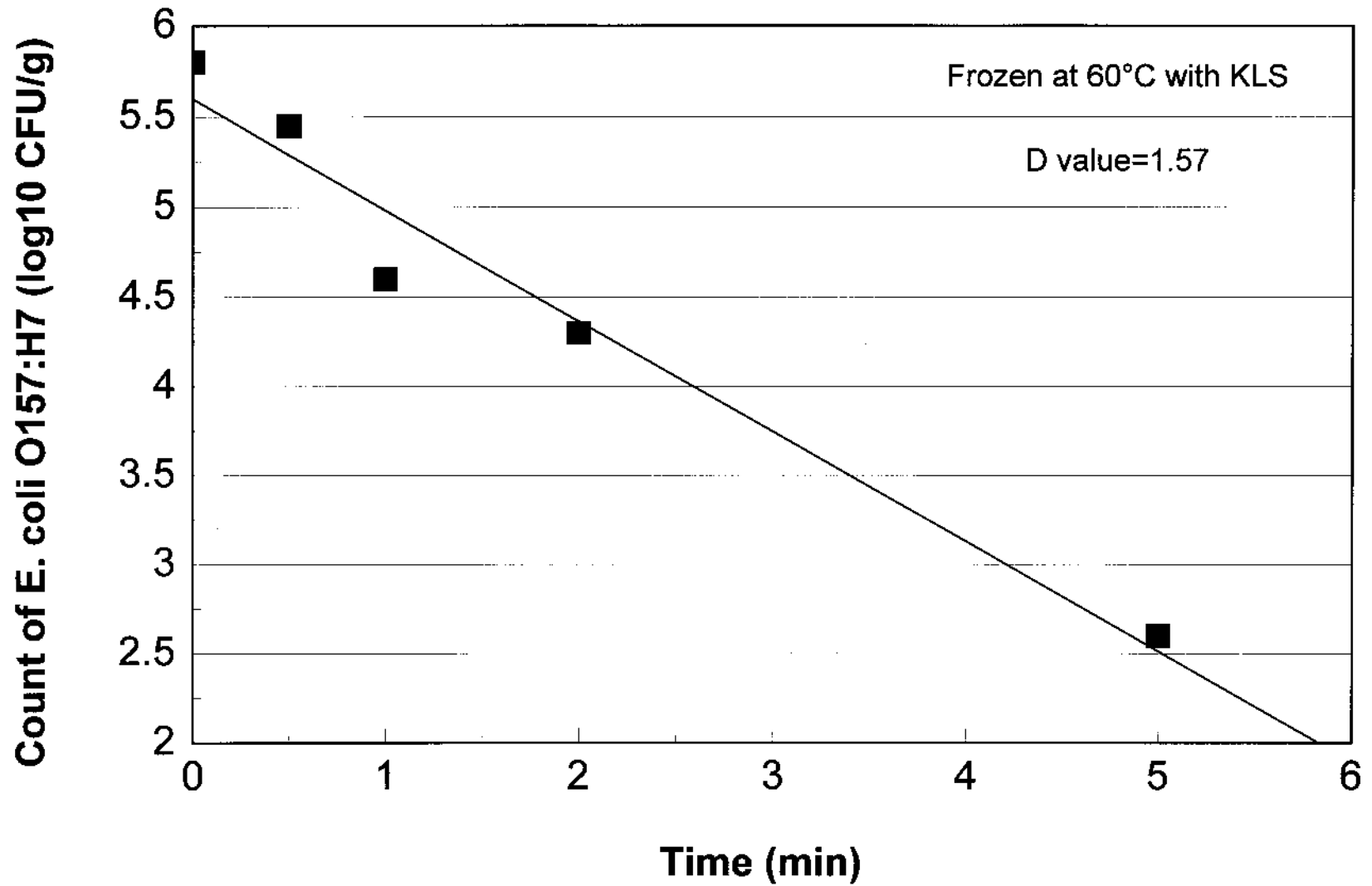
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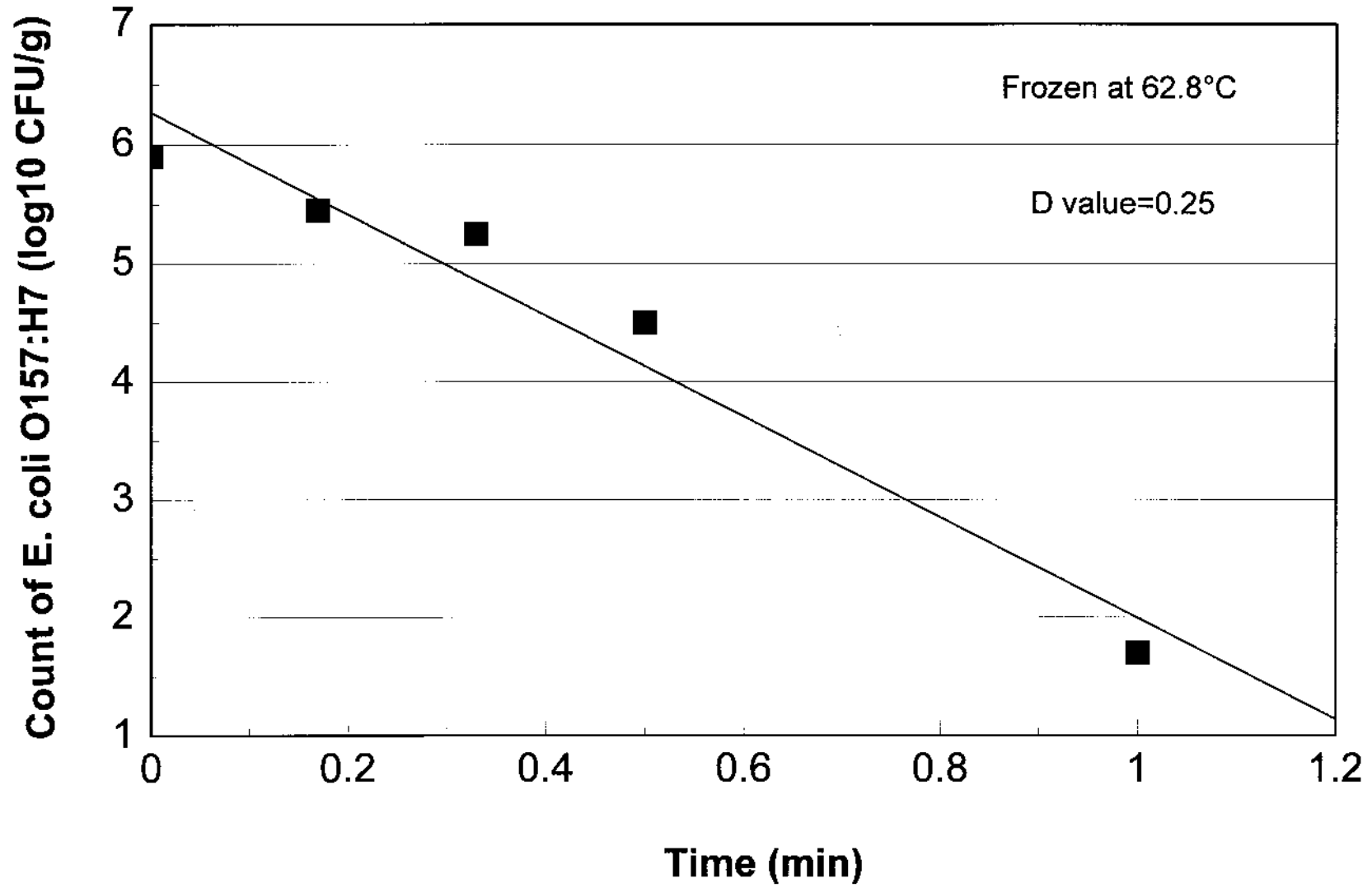
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