

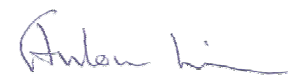
Protocol

Stability study of buffers for heparin analysis

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Date


Signature

Aim

The aim of the study is to determine the shelf life of buffers for heparin analysis (No. A12-0016, A12-0017 and A12-0018) after dissolution in purified water.

Background

Contents

Buffer:	A No: A12-0016	B No: A12-0017	C No: A12-0018
Sodium Chloride	175 mM	175 mM	175 mM
Tris(hydroxymethyl)aminomethane	50 mM	50 mM	50 mM
EDTA	7.5 mM	7.5 mM	7.5 mM
Polyethylene glycol 6000	1 %	0 %	0.1 %
Bovine serum albumin	0,2 %	0,2 %	0
pH	8.4	8.4	8.4

Theoretically possible carbon sources for microbial growth are tris (hydroxymethyl) aminomethane (Tris), Polyethylene glycol (PEG) and bovine serum albumin (BSA). Furthermore, the possibility exists for photosynthesizing organisms that use carbon dioxide as a carbon source. EDTA should act as a preservative as it has an ability to form complexes with metal ions contained in oxidative enzymes. Some Pseudomonas strains have been shown to be able to use PEG as the sole carbon source hence the choice of Pseudomonas aeruginosa in front of the also available Bacillus subtilis.

PRINCIPLE

The three different buffer solutions are analyzed in terms of pH, microbial growth and visual appearance. Samples are taken at time 0, 1, 2, 4 and 7 days at 5-8 ° C and 0, 2, 4, 6 and 24 hours at room temperature (20-25 ° C). At room temperature, a test in which a known amount of Pseudomonas aeruginosa (ATCC 6633) are added to measure the speed of growth in the buffer. Duplicates are taken of each buffer but the duplicate is analyzed only at the beginning and end of the test. All vessels and the water used (milli-Q) is autoclaved at 121 ° C before use. Sterile spreaders are used in streaks and TSA plates were ordered from SVA. All handling of the buffers and the plates are in LAF bench. 100 µl sample volume was smeared on each plate. The pH meter is calibrated before each measurement exercise and new calibration solutions are used every day. The plates are incubated at 30-35 ° C and read preliminary after 2-3 days and finally after 5-7 days.

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Buffer No:A12-0016 is named “A”, A12-0017 is named “B” and A12-0018 is named “C”.

Refrigerated ”A11”, ”B11”, ”C11” and duplicate ”A12”, ”B12”, ”C12”

Samples stored at room temperature: “A21”, “B21”, “C21” and duplicate “A22”, “B22”, “C22”

Samples with added microorganism ”A3”, ”B3”, ”C3”

The measured pH value with two decimals is noted in the ”pH” field. The visual appearance (clear or turbid) is noted in the ”Vis” field. In the field ”CFU” ,the number of colony forming units per 100 µl buffer is noted.

Analysis of buffers stored in 5-8°C

	A11				B11				C11			
	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final
0 Days	8.43	ok	0	0	8,41	ok	0	0	8.39	ok	0	0
1 Days	8.44	ok	1	1	8,40	ok	1	1	8.41	ok	0	0
2 Days	8.42	ok	0	0	8,40	ok	0	0	8.40	ok	0	0
4 Days	8.44	ok	0	0	8,42	ok	0	0	8.41	ok	0	0
7 Days	8.44	ok	0	0	8,41	ok	0	0	8.41	ok	0	0
14 Days	8,43	ok	0	0	8,42	turbid	4	4	8.41	ok	0	0

Analysis of buffers stored in 5-8°C (duplicate)

	A12				B12				C12			
	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final
0 Days	8.44	ok	1	1	8.42	ok	0	0	8.43	ok	ok	ok
1 Days												
2 Days												
4 Days												
7 Days	8.44	ok	0	0	8.40	ok	1	1	8.42	ok	ok	ok
14 Days												

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Analysis of buffers stored in 20-25°C

	A21				B21				C21			
	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final
0 h	8.43	ok	0	0	8.40	ok	0	0	8.40	ok	0	0
2 h	8.43	ok	1	1	8.42	ok	0	0	8.42	ok	0	0
4 h	8.43	ok	0	0	8.40	ok	0	0	8.43	ok	49	49
6 h	8.44	ok	0	0	8.41	ok	0	0	8.43	ok	0	0
24 h	8.44	ok	0	0	8.42	turbid	8	8	8.44	ok	0	0
48 h	8.44	turbid	5	5	8.40	turbid	n.a	n.a	8.42	ok	0	0

Analysis of buffers stored in 20-25°C (duplicate)

	A22				B22				C22			
	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final
0 Days	8.44	ok	1	1	8.42	Ok	0	0	8.43	ok	0	0
1 Days												
2 Days												
4 Days												
7 Days	8.43	ok	0	0	8.40	turbid	2	2	8.42	ok	0	0
14 Days												

Analysis of inoculated buffers stored in 20-25°C

	A3				B3				C3			
	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final	pH	Vis	CFU Prel	CFU Final
0 h	8.44	ok	1	1	8.40	ok	1	1	8.43	ok	0	0
2 h	8.43	ok	1	1	8.39	ok	1	1	8.42	ok	1	1
4 h	8.42	ok	0	0	8.41	ok	1	1	8.43	ok	1	1
6 h	8.44	ok	1	1	8.42	ok	0	0	8.41	ok	1	1
24 h	8.43	ok	0	0	8.41	turbid	15	15	8.42	ok	0	0
48 h	8.43	turbid	0	0	8.40	turbid	n.a	n.a	8.44	ok	0	0

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Evaluation

Evaluation is performed based on the results of the study

Conclusions: Buffer "B" showed to be the most sensitive buffer for microbial growth, followed by buffer "A",. Microbial growth could not be detected in buffer "C" during test period. The pH did not change significant during the test period. The chosen test organism did not seem to grow well in either of the buffers. Conclusions: By practical reasons the shelf life of all buffers are set as the same as for the most sensitive buffer ("B") 1 day at room temperature and 1 week at 5-8 °C.