

ISO 4768:2023-07 (E)

Measurement method of anti-biofilm activity on plastic and other non-porous surfaces

Contents		Page
Foreword		iv
Introduction		v
1	Scope	1
2	Normative references	1
3	Terms and definitions	1
4	Materials	1
4.1	Bacterial strain to be used for the tests	1
4.2	Reagents	2
4.3	Culture media and solution	2
4.3.1	General	2
4.3.2	Nutrient agar (NA)	2
4.3.3	Suspension medium-1/5 Tryptic soy broth (1/5TSB)	2
4.3.4	Plate count agar	2
4.3.5	Slant culture medium	2
4.3.6	0,1 % Crystal violet aqueous solution	2
4.3.7	1,0 % Sodium dodecyl sulfate aqueous solution	3
5	Apparatus	3
6	Sterilization of apparatus and storage of stock cultures	4
6.1	Dry-heat sterilization	4
6.2	High-pressure steam sterilization	4
6.3	Preparation of glassware	4
6.4	Maintenance of stock cultures	4
7	Procedure	4
7.1	Biofilm formation on the surface of the test specimens	4
7.1.1	Pre-culture of bacteria	4
7.1.2	Preparation of test specimens	4
7.1.3	Preparation of the inoculated incubating medium	5
7.1.4	Inoculation of test specimens	5
7.1.5	Incubation of the inoculated test specimens	5
7.2	Determination of the amount of biofilm	6
7.2.1	First washing of test specimens	6
7.2.2	Staining of test specimens with crystal violet	6
7.2.3	Second washing of test specimens	6
7.2.4	Biofilm recovery from test specimens	7
7.2.5	Absorbance measurement	7
7.3	Determining the viable bacteria count	7
8	Expression of results	8
8.1	Conditions for valid test	8
8.2	Calculation of anti-biofilm activity on the basis of absorption spectrum method for anti-biofilm materials	8
8.3	Effectiveness of the anti-biofilm agent	8
9	Repeatability and reproducibility	8
10	Test report	8
Annex A (informative)	Repeatability and reproducibility	10
Bibliography		12