

enbio[®] MICROJET

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RESEARCH REPORT. EFFECTIVENESS ASSESSMENT OF THE STERILIZATION PROCESS USING MICROJET MICROWAVE AUTOCLAVE.

ASSESSMENT OF PHYSICAL, MICROBIOLOGICAL AND STERILITY PROPERTIES OF THE MEDIA STERILIZED USING THE MICROWAVE MICROJET AUTOCLAVE.

Customer: Enbio Technology Sp. z o.o.; ul. Rumska 18, 81-198 Dębogórze

Collection and/or provision of the sample: Autoclave MICROJET ML2-0365 has been delivered by the ordering party. Samples were prepared by the provider and stored in appropriate conditions.

Research start date: 18.07.2016

Research end date: 12.08.2016

Date of status report: 16.08.2016

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Research executed / Research approved: www.pro-lab.pl

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INTRODUCTION

To confirm the correctness of the sterilization process in MICROJET microwave autoclave, Prolab sp. z o.o. has performed series of validation researches. Microbiological validation was performed according to the PN-EN 556-1:2002 norm: „Sterilization of medical products – Requirements for the products considered as STERILE – PART 1: Requirements of the finally sterilized medical products. Conducted research was purposed to deliver objective evidence that the sterilization process is effective whilst preserving the microbiological preferences of the media.

About PROLAB

Prolab Laboratories have introduced the certified quality management system according to the PN-EN ISO-IEC 17025:2005 norm, confirmed by the quality certificate given by the Polish Accreditation Center.

AIM

Purpose of the research was to assess the correctness of sterilization process using MICROJET autoclave.

Each medium was thrice checked, simultaneously with the positive and negative control, solutions were checked in one go. Each analysis was performed in Prolab Laboratory according to the methodology mentioned in:

- PN-EN ISO 11133:2014 – „Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media”
- Pharmacopoeia EDITION X (2014) TOME I

MATERIALS AND METHODS

- Reference strains

All strains used in the research are from the ATCC (American Type Culture Collection) collection.

Table 1 Reference strains used in research

LP	Name of the strain	Number ATCC
1	Staphylococcus aureus	ATCC 25923
2	Staphylococcus epidermidis	ATCC 14990
3	Escherichia coli	ATCC 25922
4	Pseudomonas aeruginosa	ATCC 27853
5	Salmonella typhimurium	ATCC 14028
6	Bacillus subtilis	ATCC 6633
7	Candida albicans	ATCC 10231
8	Haemophilus influenzae	ATCC 9334
9	Listeria monocytogenes	ATCC 13932
10	Clostridium sporogenes	ATCC 19404
11	Lactobacillus plantarum	ATCC 8014

- **Substrate**

The following mediums and solutions were examined by PROLAB:

Table 2 Agar used for analysis

LP	Name of the bacterial medium	Company
1	VRBG Agar	BTL
2	TBX Agar	BTL
3	YGC Agar	BTL
4	MRS Agar	OXOID
5	Endo Agar	BTL
6	Chocolate Agar	OXOID
7	TTC Agar	BTL
8	Columbia Agar	BIOCORP
9	Sabourand Agar	BIOCORP
10	MacConkey Agar	BIOCORP
11	Chapman's Agar	BIOCORP

Table 3 Liquid substrates used for analysis

LP	Name of the bacterial medium	Company
1	Semi-Fraser Broth	BTL
2	Broth with Selenite	BTL
3	TSB Broth	BD
4	Shaedler Broth	BIOCORP

Table 4 The solutions used for analysis

LP	Nazwa roztworu	Firma
1	Peptone water	BIOCORP
2	Buffer solution PBS	CHEMPUR, Avantor Performance Materials Poland
3	Buffer solution Tris	SIGMA
4	The solution of sugar: 20% glucose	CHEMPUR
5	The solution of sugar: 20% saccharose	Avantor Performance Materials Poland
6	A solution of glycerol	CHEMPUR
7	Water	PROLAB

• **Methodology**

In order to confirm the effectiveness of the sterilization process in microvawe MICROJET autoclave the prepared mediums and solutions were injected in the adequate model strain of 106, after which the sterilization process was performed (using the PN-EN 556-1:2002 norm). The following step was to rate the sterility of the sample:

- agar and liquid medium according to PN-EN ISO 11133:2014 norm
- solutions according to Pharmakopoeia EDITION X (2014) TOME 1

• **Assessment of sterility:**

- **Agar and liquid**

Study was carried out in accordance with the PN-EN ISO 11133:2014 norm.

- **Solutions**

Effectiveness of the sterilization process was based on the tests conducted on solutions according to the Pharmacopoeia methodology.

THE RESEARCH RESULTS

• **MICROBIOLOGICAL PROPERTIES OF MEDIUM**

- Medium Agar

Name of the medium	Test strain	The study feature	Result [PR>0,7]	Rate
VRBG Medium	E.coli	Fertility	PR = 1,2	positive
	S.aureus	Selectivity	No growth	positive
TBX Agar	E.coli	Fertility	PR = 1,1	positive
	S.aureus	Selectivity	No growth	positive
	S.typhimurium	Specificity	PR = 0,89	positive
YGC Medium	C.albicans	Fertility	PR = 1,08	positive
	S.aureus	Selectivity	No growth	positive
MRS Agar	L.plantarum	Fertility	PR = 1,12	positive
	S.aureus	Selectivity	No growth	positive
Endo Medium	E.coli	Fertility	PR = 1,22	positive
	S.aureus	Selectivity	No growth	positive
	S.typhimurium	Specificity	PR = 0,98	positive
Chocolate Agar	H.influenzae	Fertility	PR = 1,0	positive
	E.coli	Selectivity	No growth	positive
Lactose TTC Agar with Tregitol 7	E.coli	Fertility	PR = 1,06	positive
	S.aureus	Selectivity	No growth	positive
	S.typhimurium	Specificity	PR = 1,0	positive

Columbia Agar	S.aureus	Fertility	PR = 1,18	positive
	H.influenzae	Selectivity	No growth	positive
	S.epidermidis	Specificity	PR = 0,8	positive
Sabourand Agar	C.albicans	Fertility	PR = 1,14	positive
	S.aureus	Selectivity	No growth	positive
MacConkey Agar	E.coli	Fertility	PR = 1,24	positive
	S.aureus	Selectivity	No growth	positive
	P.aeruginosa	Specificity	PR = 1,0	positive
Chapman's Agar	S.aureus	Fertility	PR = 1,3	positive
	E.coli	Selectivity	No growth	positive
	S.epidermidis	Specificity	PR = 0,96	positive

- Liquid medium

Name of the medium	Test strain	The study feature	Result	Rate
Semi-Fraser Broth	E.coli	Fertility	2	positive
	H.influenzae	Selectivity	0	positive
Broth with Selenite	S.typhimurium	Fertility	2	positive
	E.coli	Selectivity	0	positive
TSB Broth	E.coli	Fertility	2	positive
	H.influenzae	Selectivity	0	positive
Shaedler Broth	L.plantarum	Fertility	2	positive
	H.influenzae	Selectivity	0	positive

- Solutions

Name of the solution	Assessment of sterility
Peptone water	positive
Buffer solution PBS	positive
Buffer solution Tris	positive
20% glucose	positive
20% saccharose	positive
The solution of glycerol	positive
Water	positive

CONCLUSION

Microbiological validation of the sterilization process using microwawe MICROJET autoclave was the purpose of analysis. Also performed was the control of physical and microbiological preferences of undergone medium.

All tests were done in repetitions to emphasize the reliability and repeatability of the whole process. According to plan 15 substrates and 7 solutions were tested in the period 18.07.2016 - 12.08.2016.

Results for the tested substrates confirmed preservation of the microbiological and physical preferences once the sterilization process is complete.

- Examined samples meet the expectation of fertility, selectivity and specificity.
- Also the physical attributes such as: pH value, substrate presentation, agar thickness, artifact presence, gel consistency, are matching the manufacturer's guidelines.
- On top of the before mentioned also the sterility of all samples after incubation was checked. This was performed in routine conditions and confirmed the sterilization effectiveness for substrates (agar, liquid) and also for solutions.
- None of the cases showed growth of the microorganisms.
- Research confirmed decrease by 6 logarithms for the number of microorganisms, therefore the SAL sterility level for substrates and solutions was reached.

SUMMING UP, SUBSTRATES AND SOLUTIONS STERILIZED USING MICROWAVE MICROJET AUTOCLAVE, MANUFACTURED BY ENBIO, MEET ALL THE REQUIREMENTS (PHYSICAL AND MICROBIOLOGICAL). MICROBIOLOGICAL VALIDATION ALSO CONFIRMED THE EFFICIENCY OF THE PROCESS.