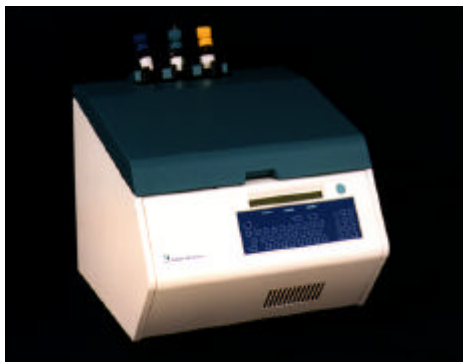


Multi-Center Validation of Rapid Bioluminescence Techniques for Microbiological Testing of Non-Sterile Pharmaceutical End-Products

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Abstract

Greater than 95% of non-sterile end-products do not contain any microbial contamination. Rapid release of these products from manufacturing provides a significant financial advantage. A rapid faster test also releases laboratory resource. Utilising ATP bioluminescence, this technique provides a rapid screen in 24 hours. The majority of products can be released at this stage. Any contamination detected can be enumerated and identified by the familiar Microbial Limits test. Pharmaceutical manufacturing sites in the USA and Europe have been involved in a multi-centre validation of this bioluminescence technique. The trial has proved the method is specific and demonstrated the limit of detection. Hence this qualitative method has been validated as defined in USP monograph 1225. The instrument based test allowed the introduction of automation into microbiological testing and has the capability to link into LIMS.

Introduction

The majority of non-sterile pharmaceutical end-products give a microbial count of zero at quality control. This zero count takes up to 7 days to obtain by conventional methods. ATP bioluminescence can give

the same answer in 24-48 hours. SteriScreen has been validated at a number of pharmaceutical manufacturing sites according to Monograph 1225, Validation of Compendial Methods, fourth supplement of USP 23.

Method

The enrichment was carried out using Lethen Broth supplied by Difco Laboratories. This broth contains preservative neutralisers and has a low ATP content.

General Protocol

1. Prepare a 1% suspension of product in broth in sterile disposable containers.
2. Incubate at 32°C, shaking at 250rpm, for 24 hours.
3. Pipette 50µl of incubated sample into a luminometer tube.
4. Load tubes into the luminometer and start the following programme:
200µl ATP Releasing Agent
10 second delay
100µl Bioluminescence Reagent
10 second integration
5. The result is printed in relative light units (RLU).

Results and Discussion

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Within a microbiological method this has been interpreted as the equivalence to the established test. The data in shown in table 1 is detection of naturally contaminated products, following the general protocol.

Table 1 : Accuracy of SteriScreen

Manufacturer	Code	Product		Detection by SteriScreen	Detection by Current Method
		Physical Form	Indications	24 hours	≤ 7 days
1	A	Syrup	Coughs	X	X
	B	Syrup	Coughs	X	X
	C	Syrup	Coughs	X	X
	D	Syrup	Coughs	X	X
	E	Syrup	Coughs	X	X
	F	Syrup	Coughs	X	X
	G	Ointment	Haemorrhoids	X	X
	H	Suppositories	Haemorrhoids	X	X
	I	Gel	Hernia	X	X
	J	Suspension	Sedative	X	X
2	A	Wipes	Haemorrhoids	X	X
	B	Toothpaste	Oral hygiene	X	X
	C	Liquid	Antihistamine	X	X
	D	Cream	Skin moisturiser	X	X
	E	Cream	Skin protection	✓	✓
3	A	Tablet	Pain relief	X	X
	B	Tablet	Anti-viral	X	X
	C	Tablet	Anti-viral	✓	✓

Specificity

The specificity of an analytical method is its ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix. This definition serves the science of chemistry very well but most microbiologists think of specificity in terms of identification. For the purposes of this rapid screening technique, specificity has been taken as the ability of the system to detect the organisms of importance, that is inoculated pharmacopeial organisms. The data is presented in table 2.

Limit of Detection

The limit of detection of an analytical method is the lowest concentration of analyte in a sample that can be detected but not quantitated. Determining the limit of quantitation for a microbiological method has practical problems. Due to the difficulty in knowing exactly how many cells are in a low inocula, the lowest detection level is reported as 1-5 cells. Where higher inoculums were the lowest level tested, this is reported at ≤. The data is presented in table 2 as the lowest number of inoculated organisms detected in products.

Summary

- Accuracy : 100% agreement between SteriScreen and the current method for naturally contaminated products
- Specificity : *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhimurium* and *Candida albicans* detected following 24 hours incubation. *Aspergillus niger* requires 48 hours incubation for reliable detection.
- Limit of detection : Low numbers of micro-organisms have been detected in products

References

US Pharmacopoeia 23, fourth supplement <1225>, Validation of Compendial Methods.

Table 2: Specificity and Limit of Detection of SteriScreen

Product Code	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Candida albicans</i>	<i>Aspergillus niger*</i>	<i>Salmonella typhimurium</i>
1A	≤2	≤2	≤2	4	≤3	4	4
1B	≤2	≤2	≤2	4	≤3	4	4
1C	≤2	5	5	≤1	≤3	4	≤3
1D	≤2	5	5	≤1	≤3	4	≤3
1E	≤2	≤2	≤2	4	≤3	4	4
1F	≤1	2	3	≤2	≤1	6	≤2
1G	≤1	NT	3	≤2	≤1	12	≤2
1H	≤1	NT	3	≤2	≤1	NT	≤2
1I	2	≤1	≤2	≤2	≤1	NT	3
1J	2	≤1	≤2	≤2	≤1	12	3
2A	≤5	≤8	≤9	NT	NT	NT	NT
2B	≤5	≤8	≤9	NT	NT	NT	NT
2C	≤5	≤8	≤9	NT	NT	NT	NT
2D	≤5	≤8	≤9	NT	NT	NT	NT
2E	≤5	≤8	≤9	NT	NT	NT	NT
3A	5	≤5	≤5	5	≤5	10	NT
3B	NT	NT	≤5	5	NT	≤5	NT
3C	5	≤5	≤5	5	≤5	≤5	NT

* Result after 48 hours incubation

NT = not tested

≤ = lowest inoculum tested

