THE USE OF SEKISUI DIAGNOSTICS' PENICILLINASE AND β-LACTAMASE PRODUCTS

SEKISUI DIAGNOSTICS

INTRODUCTION

The aim of this guide is to outline the general procedures for the use of Sekisui Diagnostics' Penicillinase and β-Lactamase products in pharmaceutical manufacturing facilities and to ensure that end-users are able to meet the requirements of the necessary regulatory authorities.

PRODUCT OVERVIEW

Sekisui Diagnostics' Penicillinase product is supplied as either a bulk powder with an activity of >15 U/mg powder, or as a sterile vialled product containing >3000 U/vial. The penicillinase is for use only in penicillin manufacturing facilities and its use in cephalosporin manufacturing facilities will NOT meet regulatory requirements.

Sekisui Diagnostics' β-Lactamase product is also supplied as a bulk powder containing >2 U/mg powder or as a sterile vialled product containing >50 U/vial of βII (cephalosporinase) activity as well as >500 U/vial of βI (penicillinase) activity. This product has activity against the majority of antibiotics on the market.

Sekisui Diagnostics also has a new generation of β-lactamase (β-Lactamase Extended Spectrum) which is also available in bulk or sterile vials and has activity against an even wider range of cephalosporins – in fact, against all of the antibiotics we have tested.

The β-lactamase products show both penicillinase and cephalosporinase activity, as penicillinase is produced during the manufacturing process as a by-product of the cephalosporinase manufacture.

The result is a wide range of antibiotic neutralising activity from these products that would allow them to be used in a mixed penicillin/cephalosporin manufacturing facility.

The use of β-lactamase is specified by the US Pharmacopoeia (USP) in chapter 71 for monitoring of cephalosporin manufacturing facilities and sterility testing of bulk cephalosporins. USP has been harmonised with the European and Japanese pharmacopoeias.

Both penicillinase and β-lactamase sterile vialled products have been shown to be stable for up to 4 weeks after reconstitution in water.

Product Overview

	FORMAT	CATALOGUE NUMBER	ACTIVITY
Penicillinase	Bulk Powder	PENI-70-1541	> 15 Penicillinase IU/mg
	Vials	PENI-70-1545	> 3000 Penicillinase IU/vial
β-Lactamase	Bulk Powder	BELA-70-1431	> 2 Cephalosporin U/mg
	Vials	BELA-70-1401	> 50 Cephalosporin U/vial
β-Lactamase (Extended Spectrum)	Bulk Powder	BELA-70-1491	> 2 Cephalosporin U/mg
	Vials	BELA-70-1461	> 50 Cephalosporin U/vial

UNIT DEFINITION

One Sekisui Diagnostics unit of Cephalosporinase (B-Lactamase II) activity is equivalent to one International Unit and is defined as the amount of enzyme that will catalyse the hydrolysis of 1.0 micromole of benzylpenicillin per minute at 25°C and pH 7.0.

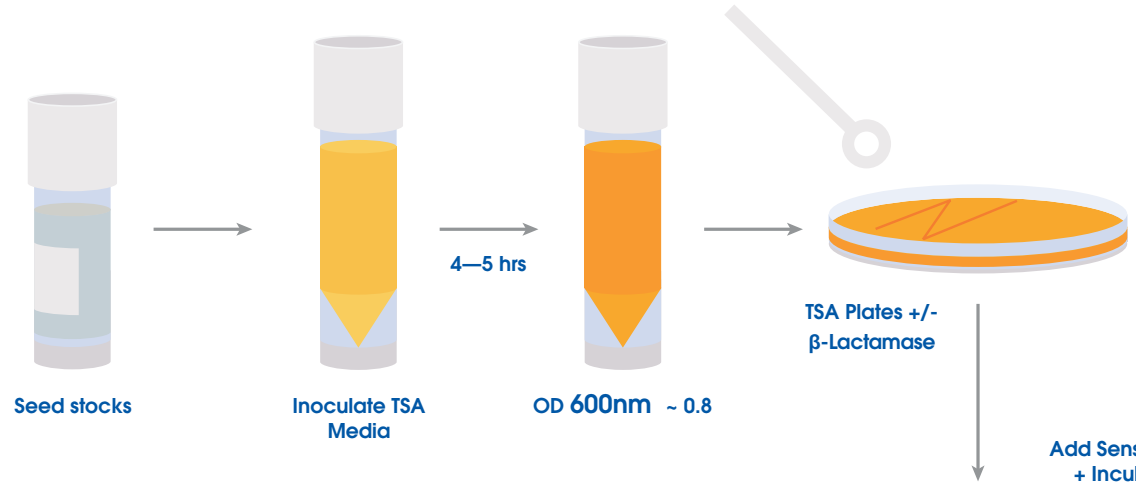
- One Sekisui Diagnostics (International) Penicillinase unit is equivalent to 600 Levy Units, 75 Pollock Units or 91200 Kersey Kinetic Units.

One Sekisui Diagnostics unit of Penicillinase (B-Lactamase I) activity is equivalent to one International Unit and is defined as the amount of enzyme that will catalyse the hydrolysis of 1.0 micromole of cephalosporin C per minute at 25°C and pH 7.0.

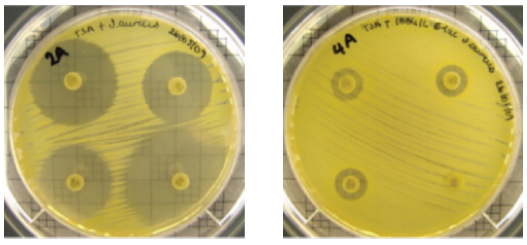
Sekisui Diagnostics (UK) Ltd. Quality Control department tests these products by titration methods, based on the release of hydrogen ion during the hydrolysis of the β-Lactam ring. For penicillinase units the substrate tested is penicillin G and for cephalosporinase testing the substrate is cephalosporin C. The rates of activity for hydrolysis of other substrates will vary and we cannot give relative rates for each of the many variations of penicillin or cephalosporins available.

In addition, we have carried out comparative studies against a wide variety of antibiotic substrates using Becton Dickinson (BD) Sensi-discs to test the effectiveness of the products against the available antibiotic range.

Figure 1: Effectiveness Testing Method



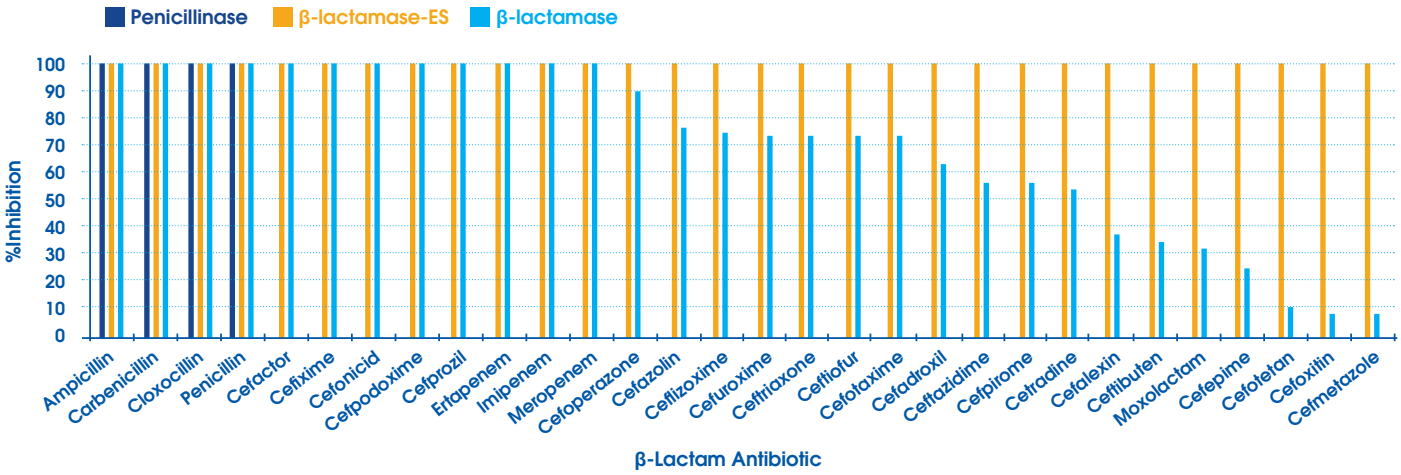
- Kirby-Bauer method.
- Antibiotic impregnated wafers are used to test whether a particular bacteria is susceptible to specific antibiotics.
- Comparison of inhbition zone with and without β-Lactamase in plates.



Without β-Lactamase | With β-Lactamase

A reduction in the diameter of the zone of inhibition indicates that the penicillinase/β-lactamase in the media is effective in breaking down the antibiotic that diffuses out from the disc. The concentration of a particular antibiotic in each disc can vary from 1 µg to 100 µg, so this factor also needs to be taken into account. If you have a particular antibiotic that you need to ascertain the effectiveness against, please contact us to see what information we have. Figure 2 shows an overview of Sekisui Diagnostics' Penicillinase and β-lactamase products against a range of antibiotics.

Figure 2: Activity Spectrum



Agar diffusion test on Tryptone soy agar (TSA) plates containing 500IU Cephalosporinase activity/L

ENVIRONMENTAL MONITORING OF ANTIBIOTIC MANUFACTURING AREAS

For the testing of the microbiological cleanliness of antibiotic manufacturing facilities, contact plates, flow plates, or settle plates containing penicillinase or β-lactamase are used. These allow the destruction of any residual antibiotic present in the sample and the subsequent growth of any microbial contaminants that may be present.

Generally, these plates are manufactured using the bulk powder penicillinase and β-lactamase products. The bulk powder has been filtered through a 0.45 µm filter during the latter stages of manufacture, but are not sterile. However, the final plates do need to be sterile, so standard plate manufacture adds the product prior to pouring the plates and then uses an irradiation step to sterilise the final product. We believe that an irradiation dose of 9-20 kGy of either beta or gamma-irradiation is commonly used on triple-bagged plates as the final step in the manufacturing process.

Initially the penicillinase or β-lactamase can be dissolved in water or any other appropriate buffer (it is already buffered in Tris) and then added to liquefied media at approximately 50°C. The β-lactamase material also contains approximately 1mM zinc sulphate and it is known that the addition of a small amount of zinc sulphate to the media can help to increase the stability of plates made with the β-lactamase. However, as each manufacturing facility is different (range of antibiotics manufactured, room size, specific cleaning procedures, frequency of testing, etc.), Sekisui Diagnostics (UK) Ltd. is not able to give a standard methodology for the level of enzyme required in the manufacture of environmental monitoring plates.

The environmental testing by each manufacturer has to be validated for the conditions within the manufacturing facilities and the amount of penicillinase/β-lactamase titrated to the appropriate level to ensure complete inactivation of the antibiotics present.

A method for the validation of environmental monitoring plates was outlined in the European Journal of Parenteral Sciences (1996 1(3); 79-81). This paper used the Sekisui Diagnostics (UK) Ltd. (Genzyme Diagnostics UK Ltd.) sterile vialled β-lactamase product to manufacture cephalosporinase monitoring plates, because they did not include an irradiation step.

1. Various levels of β-lactamase product were incorporated into the plates to determine the amount of enzyme that would be required to inactivate various levels of cephalosporin that might be found in a cleaned antibiotic manufacturing facility.

2. The plates were then spread with a suspension of various concentrations of the relevant antibiotic to mimic these antibiotic contamination levels.

3. Finally, the plates were inoculated with a suspension of approximately 100 colony forming units of a number of microbiological test organisms to ensure that the cephalosporin added to the plates was sufficient to inactivate these levels of microbial contamination.

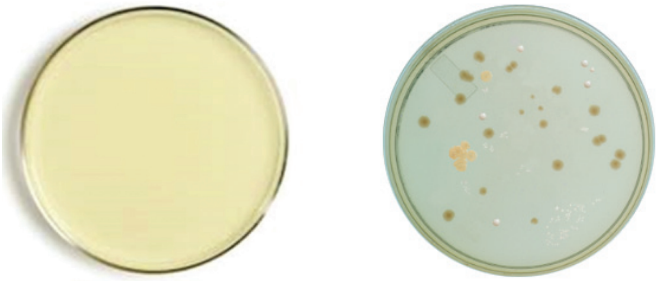
4. Positive growth of the microbial test strains indicates that the added cephalosporin has been effectively inactivated and that the level of β-lactamase added was appropriate for the level of antibiotic added to each plate.

In this way it was possible for this group to validate the level of β-lactamase required for the environmental monitoring of their manufacturing facility.

Please note that this paper tested for Cefotaxime only. Because the relative rates of activity of the Sekisui Diagnostics (UK) Ltd. products to different antibiotics (and the other variables mentioned previously) it is important that each manufacturing facility tests specifically for the antibiotics that they are manufacturing and use the appropriate enzymes for their facility.

During routine use it is also recommended that positive control plates are used to demonstrate continuing effectiveness of the plates used. The addition of microbiological test organisms to control plates, and the subsequent growth of those organisms on those control plates should validate the continuing effectiveness of the process used in each facility.

Figure 3 Typical Examples of Environmental Testing Plates



Negative test plate

Positive control plate

STERILITY TESTING OF BULK ANTIBIOTICS

The various Pharmacopoeias outline the requirement that antibiotic materials are tested for sterility at the end of manufacture. Rather than trying to remove all the antibiotics in the sample by enzymatic means alone, the preferred methodology is to use a filtration system to remove most of the antibiotic in the sample first, and then incubate the filter in a media broth containing the β-lactamase enzymes.

A system such as the Millipore Steritest system is suited for this purpose, as it is a sealed system and is designed to enable complete sterility of the entire process, using sealed filter cartridges.

An article in Contract Pharma states that "The Membrane Filtration Sterility Test is the method of choice for pharmaceutical products." See below for link to website.

The sterility testing of antibiotic products has to be carried out under aseptic conditions, the correct products to use for this testing are the Sekisui Diagnostics (UK) Ltd. sterile vials. These vials have been gamma-irradiated at 25-35 kGy during manufacture to ensure sterility. Each batch of product is tested for sterility in our QC, testing for both aerobic and anaerobic micro-organisms. The amount of sample required for testing is defined in the Pharmacopoeias (it can be as much as 10g) and needs to be reconstituted in solutions also defined in the Pharmacopoeias. At this point, it may also be a benefit to add some penicillinase/β-lactamase in the solution. This material is then filtered through a single filter contained within a sealed, sterile cartridge. Reconstituted antibiotic should pass through the filter, but any contaminating micro-organisms will be retained on the filter. Once filtration is complete, the filter cartridge is filled with the Fluid Glycollate Medium and sealed. Growth of any organisms in the medium is then monitored over a period of 2 weeks.

Please note that the procedures used need to be validated by use of a positive control which has had a microbial bioburden added to it.

When validating such a method for antibiotic sterility each laboratory should validate the number of units of antibiotic that need to be used. Because each batch of vials can contain varying amounts of enzyme the actual number of units should be used rather than the number of vials (unless the minimum stated value of 50 units is assumed). We cannot guarantee to supply vials with any particular minimum content except for the specification of >3000 U/vial penicillinase or >50 U/vial β-lactamase.

Figure 4
The Steritest system consists of sealed, sterile cartridges and a peristaltic pump to allow sterile filtration of large volumes of test solutions through two filter cartridges. These are then filled with medium to allow the growth of contaminating micro-organisms. One of the cartridges can be inoculated with a test micro-organism to act as a positive control.

For technical support relating to these products please email:

technicalmarketinguk@sekisuidiagnostics.com
or call +44 1732 220022.

Footnote

Sekisui Diagnostics does not manufacture antibiotics and neither do we carry out the same testing that would be done in one of those facilities. Therefore we do not have specific protocols that we can share that can be applied to every manufacturing facility, as every site is different and there are many factors that will affect the validation of each site's sterility testing program. We can only give guidance on how the material should be used, tested and validated in order to satisfy regulatory requirements.

References

1. US Pharmacopoeia, chapter 71: Sterility Tests.

2. Haberer, K. Schulz, H. Romeyke, Y., Sauer, D. Validation of Enzymatic Inactivation of Cephalosporins Adhering to Environmental Monitoring Samples. European Journal of Parenteral Sciences (1996 1(3); 79-81).

3. Microbial Bioburden Assay for β-Lactam Antibiotics. Ernest Asilonu, Sekisui Diagnostics UK Ltd.

Useful Links

Millipore Steritest System
http://www.millipore.com/catalogue/module/c10686#2

Pharmaceutical Sterility Testing: Essential things to know
http://www.contractpharma.com/issues/2008-03/view_features/pharmaceutical-sterility-testing/

Environmental Monitoring Equipment: The latest in cleanrooms & isolator
http://www.contractpharma.com/issues/2012-10/view_features/environmental-monitoring-equipment/