PROTOCOLS for the INTEGRITY TESTING of SYRINGES

2nd EDITION

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Protocols for the
Integrity Testing of Syringes

Introduction

Disposable syringes are regularly used as final containers for aseptic products prepared within hospital units. This protocol is intended to describe standardised and validated methods for the assessment of the integrity of individual syringe/hub combinations used in Pharmaceutical Technical Services.

The following points should be taken into account by units using disposable syringes/closure systems as containers:

- Luer-lock closures provide a more secure and integral closure than luer slip. For this reason luer-lock syringes are preferred. Where luer-slip syringes are used, a documented risk assessment to the justify use of luer slip is advised.
- Flexing or bending of the extended plunger after filling, for example during storage and transport, should be avoided. This is known to produce problems with pack integrity.

  Syringes, therefore, should not be filled to their full extent in order to help minimise the potential for leakage due to excessive sideways pressure applied the plunger during storage or transport*.

  The preparation process should include a check to ensure a firm fit of syringe and hub for each individual item.

Microbiological and Physical tests are included in this protocol.

The two **microbiological assessments** described here are the established methodology for comparison of different manufacturers of syringe and hub and can be used to establish a microbial shelf life for the container closer system. Both tests can be used in either whole or partial immersion formats. **It is necessary to test the integrity of both the syringe hub and plunger**; therefore the total immersion method may be preferable. This enables both features to be tested at the same time. Partial immersion may be useful in the event of failure of total immersion in order to identify the site of ingress (i.e. the tip or the barrel).

The **physical dye intrusion test** is a simple and rapid test to evaluate batch to batch syringe performance. This test will enable greater numbers of syringes to be tested if the batch size is large and evidence for the release of syringes for use will not rely on an incubation period.

The most complete assessment of the syringe and hub as a container and closure is evaluated by application of both a microbiological and a physical integrity test when a new or altered syringe and hub combination is considered.

A physical integrity test is acceptable for subsequent, routine approval of batches of syringes and hubs of the same combination.

*A useful "rule-of-thumb" is that the syringe, when used as a storage container, should not be filled to more that 85% of its nominal capacity.

e.g. 50ml for a 60ml syringe; 25ml for a 30ml syringe; 8.5ml for a 10ml syringe; 4ml for a 5ml syringe; 1.7ml for a 2ml syringe or 0.85ml for 1ml syringe

Protocols for the
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Method 1:

Microbiological Integrity Method using *Brevundimonas diminuta*

The principle of the test is to fill the syringe system with sterile nutritive media. The outside of the syringe container is then challenged with an actively growing small sized motile monotrichous microorganism in order to assess container integrity.

1.1 Preparation of broth filled syringes

1.1.1 A suitable number (e.g. a batch or at least 20 syringes) are prepared in the aseptic unit to contain sterile Tryptone Soya Broth (TSB) in place of the product. The syringes are prepared according to normal procedures and sealed with the appropriate hub.

1.1.2 The details of the syringe and hub manufacturer and batch number are recorded.

1.1.3 These broth filled syringes are pre-incubated at 20-25°C for 7 days, then 30 - 35°C for 7 days to ensure that the aseptic fill has been carried out correctly and the contents are sterile. Discard any syringes showing turbidity or microbial growth.

1.2. Preparation of challenge micro-organism

1.2.1 *Brevundimonas diminuta* is a suitable micro-organism since it is a small, motile rod shaped bacterium, and not known to be pathogenic, but caution should be exercised in use.

Brevundimonas diminuta is a standard organism routinely used for validation of sterilising-grade membrane filters. Previously known as *Pseudomonas diminuta,* this is a gram-negative organism whose motility is provided by a single flagellum.

1.2.2 Prepare a pure culture of the micro-organism and inoculate into a 100ml of TSB broth and incubate for 18-24 hours at 30-35°C. This is used as the inoculum in the integrity test.

1.3. Integrity Test (Whole Immersion)

1.3.1 Prepare a sterile container of suitable size to contain the syringes under test and which is also capable of being placed in an incubator. The container should be provided with a close fitting lid and be resistant to the spillage of its contents.

1.3.2 In a laminar flow cabinet spray and wipe the outer surface of the syringes under test with sterile 70% IMS and allow to dry. Place syringes in the container(s) and cover with single strength TSB ensuring that the syringes remain submerged.

1.3.3 Inoculate the surrounding broth with a volume of the 18-24 hour culture of *Brevundimonas diminuta.* Use 1ml of the culture per 100ml of single strength TSB. Mix well and incubate the containers for 14 days at 30-35°C. A sample of the suspension may be taken for total viable count to establish the challenge inoculum (expected to be 10^5 to 10^6 cfu/ml).

1.3.4 Following incubation remove syringes from the broth culture (CAUTION -use PPE when handling contaminated syringes and ensure that a risk assessment has been carried out) and examine each syringe for turbidity growth showing *Brevundimonas diminuta* access into the syringe.

1.3.5 The integrity of the syringe/hub system is confirmed providing that the broth in all syringes remains free from growth.

1.4 Integrity Test (Partial Immersion)

This is a specific test to challenge areas where micro-organisms may gain access to the contents of the syringe, i.e. at the plunger barrel interface or at the hub luer fitting. Use 1ml of the culture per 100ml of single strength TSB to challenge the plunger or hub.

1.4.1 Prepare a suitable sized holder in order that the syringe(s) under test are held upright.

1.4.2 Place the syringe upright, with the plunger uppermost, in a holder and fill the barrel of the syringe above the plunger with the seeded broth culture of *Brevundimonas diminuta;*

OR

Place the hub end of the syringe in a bottle of TSB broth sufficient to cover the hub and inoculate with *Brevundimonas diminuta.*

1.4.3 Incubate the containers for 14 days at 30-35°C

1.4.4 Following incubation remove syringes, rinse off the *Brevundimonas* and check for turbidity indicating the penetration of *Brevundimonas diminuta* into the syringe contents.

1.4.5 The integrity of the syringes plunger or hub system is confirmed providing that the broth in all syringes remains free from microbial growth.

1.5 Validation of media (positive challenge)

1.5.1 If the syringes pass the test, two should be inoculated with less than 100cfu *Brevundimonas diminuta* and incubated for 3 days at 30-35°C.

1.5.2 The test is satisfactory if both syringes show signs of growth. If growth is not observed the test is invalid and the viability of the media should be investigated. The test will need to be repeated.

Method 2:

Microbiological Integrity Method using *Escherichia coli (E. coli)*

The principle of the test is to fill the syringe system with sterile nutritive media, the outside of the syringe container is then challenged with an actively growing a motile peritrichous micro-organism in order to assess container integrity.

2.1 Preparation of broth filled syringes

2.1.1 A suitable number (e.g. a batch or at least 20 syringes) are prepared in the aseptic unit to contain sterile Tryptone Soya Broth (TSB) in place of the product. The syringes are prepared according to normal procedures and sealed with the appropriate hub.

2.1.2 The details of the syringe and hub manufacturer and batch number are recorded.

2.1.3 These broth filled syringes are pre-incubated at 20-25°C for 7 days, then 30 - 35°C for 7 days to ensure that the aseptic fill has been carried out correctly and the contents are sterile. Discard any syringes showing turbidity or microbial growth.

2.2. Preparation of challenge micro-organism

2.2.1 *E-coli* is a suitable micro-organism since it is a highly motile, gram-negative, rod shaped bacterium, and seeks areas of nutrition. It is a normal human commensal found in the GI tract. The test strains do not usually cause infection, but caution should be exercised in use.

2.2.2 Prepare a pure culture of the micro-organism and inoculate into sufficient 100ml of TSB broth for one bottle per container and incubate for 18-24 hours at 30-35°C. This is used as the inoculum in the integrity test.

2.3. Integrity Test (Whole Immersion)

2.3.1 Prepare a sterile container of suitable size to contain the syringes under test. The container should be provided with a close fitting lid and be resistant to the spillage of its contents.

2.3.2 In a laminar flow cabinet spray and wipe the outer surface of the syringes under test with sterile 70% IMS and allow to dry. Place syringes in the container(s) and cover with single strength TSB ensuring that the syringes remain submerged.

2.3.3 Inoculate the surrounding broth with 100ml of the single strength TSB containing *E-coli* and leave for at least 30 minutes. A sample of the suspension may be taken for total viable count to establish the challenge inoculum (expected to be 10^5 to 10^6 cfu/ml). Remove the syringes and rinse away the E-coli and broth (CAUTION -use PPE when handling contaminated syringes and ensure that a risk assessment has been carried out). Dry and incubate the syringes for 14 days at 30-35°C.

2.3.4 Following incubation examine each syringe for turbidity growth showing *E-coli* access into the syringe.

2.3.5 The integrity of the syringe/hub system is confirmed providing that the broth in all syringes remains free from growth.

2.4 Integrity Test (partial immersion)

This is a specific test to challenge areas where micro-organisms may gain access to the contents of the syringe, i.e. at the plunger barrel interface or at the hub luer fitting.

2.4.1 Prepare a suitable sized holder in order that the syringe(s) under test are held upright.

2.4.2 Place the syringe upright, with the plunger uppermost, in a holder and fill the barrel of the syringe above the plunger with the seeded broth culture of *E-coli;*

OR

Place the hub end of the syringe in a bottle of TSB broth sufficient to cover the hub and inoculate with *E-coli.*

2.4.3 After at least 30 minutes Incubate the containers for 14 days at 30-35°C

2.4.4 Following incubation remove syringes and check for turbidity indicating the penetration of *E-coli* into the syringe contents.

2.4.5 The integrity of the syringes plunger or hub system is confirmed providing that the broth in all syringes remains free from microbial growth.

2.5 Validation of media (positive challenge)

2.5.1 If the syringes pass the test, two should be inoculated with less than 100cfu *E-coli* and incubated for 3 days at 30-35°C.

2.5.2 The test is satisfactory if both syringes show signs of growth. If growth is not observed the test is invalid and the viability of the media should be investigated. The test will need to be repeated.

Method 3:

Physical integrity: Dye Intrusion Test

Note: Variations or adaptions to this test may be acceptable if appropriately validated.

3.1. Apparatus:

Dye Bath – a cylindrical watertight container of suitable dimensions and construction.

A BDH bottle carrier is suitable: having a height of 240 mm and diameter of 105mm. This device can be sealed with Parafilm prior to fitting of a screw tight lid.

The device can be used to test up to 10 x 20 ml syringes in a single run.

Larger volume syringes may require separate test runs.

Roller Mixer – with variable rotation speed

Stuart SRT 9D is suitable.

3.2 Method

3.2.1 Add a volume of dye solution sufficient to fill the container to approximately 1/2 of the total volume.

3.2.2 Sample 20 syringes from a single manufacturer's batch.

- Prepare the plungers by marking the distance corresponding to the 0 to 100% scale length on the barrel.
- For 5 to 50 ml syringes the drilled hole should be 3 mm in diameter to hold a retaining screw of corresponding size.
- For 1 and 2 ml syringes the drilled hole should be 2 mm in diameter and the plunger retained with a suitably sized screw or pin of corresponding diameter (figure 1).

Refer to Figure 1 on next page

3.2.3 Fill each syringe with water or drug solution under test to 75 % of full scale on syringe.

- Securely apply the hub.
- Apply an internal vacuum by drawing back the plunger to the graduation representing 100% of syringe volume in position to maintain the internal vacuum.
- Secure the barrel in place with a retaining pin or screw placed though a pre-drilled hole in the plunger.

Refer to Figure 1 on next page

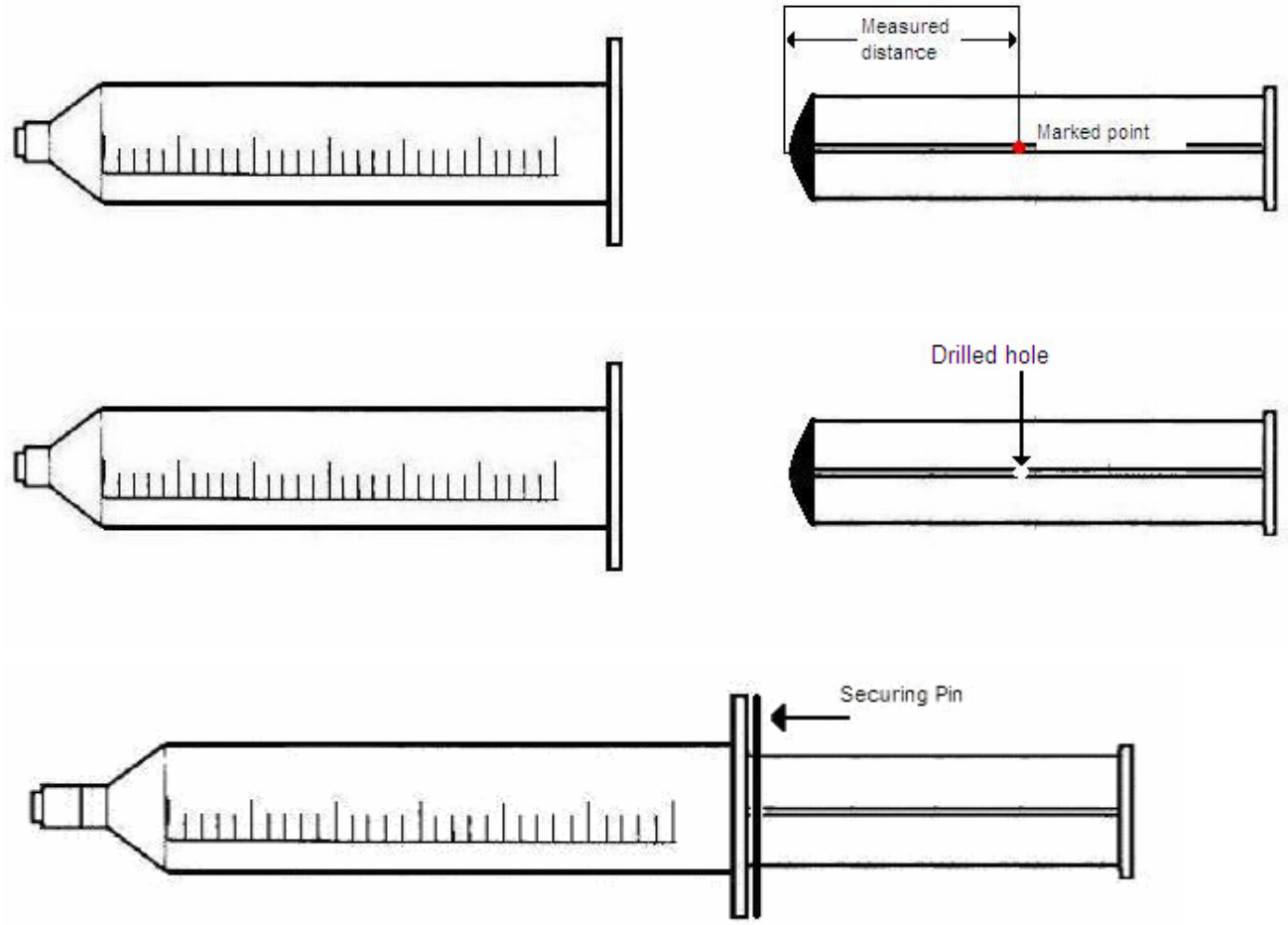

Figure 1.

3.2.4 Place the syringes into the dye bath in an upright position.

- Add a single positive control syringe (see 3.4) in the centre of the group of syringes.
- Seal the bath and rotate by 90 degrees so bath is on its side.
- Ensure all syringes are fully submerged and the lid of the bath is secure.

3.2.5 Place the dye bath onto the roller mixer and

- Rotate at 45 rpm for 2 hours.

3.2.6 Remove the dye bath from the roller mixer

- Working over a sink, remove the individual syringes and positive control.
- Release the internal vacuum by removal of the retaining devices (screw or wire)
- Thoroughly wash the external surfaces.
- Dispense a quantity of each syringe and the positive control into a suitable cuvette or matched Nessler cylinder.
- Examine visually for the presence of dye using the contents from an untested control syringe as the reference. (A validated spectrophotometric method may also be used for quantifying the ingress of external solution)

3.3 Dye solutions:

3.3.1 Amaranth (E123 / FD&C Red No. 2) 0.2% w/v and **methylthioninium chloride** (methylene blue) 0.4% are both nominated dyes for BS 1679 Eye Dropper Bottle Efficacy of Closure Test (part 5) and therefore considered suitable for this test . The colour of the dye solution chosen should be shown to be unaffected by the sample solution under test.

3.4 Positive control

Prepare a syringe from the batch tested and incorporate a fine thread of stainless steel wire (diameter 0.12mm) running parallel to the barrel between the plunger seal and the inner barrel wall (figure 2)

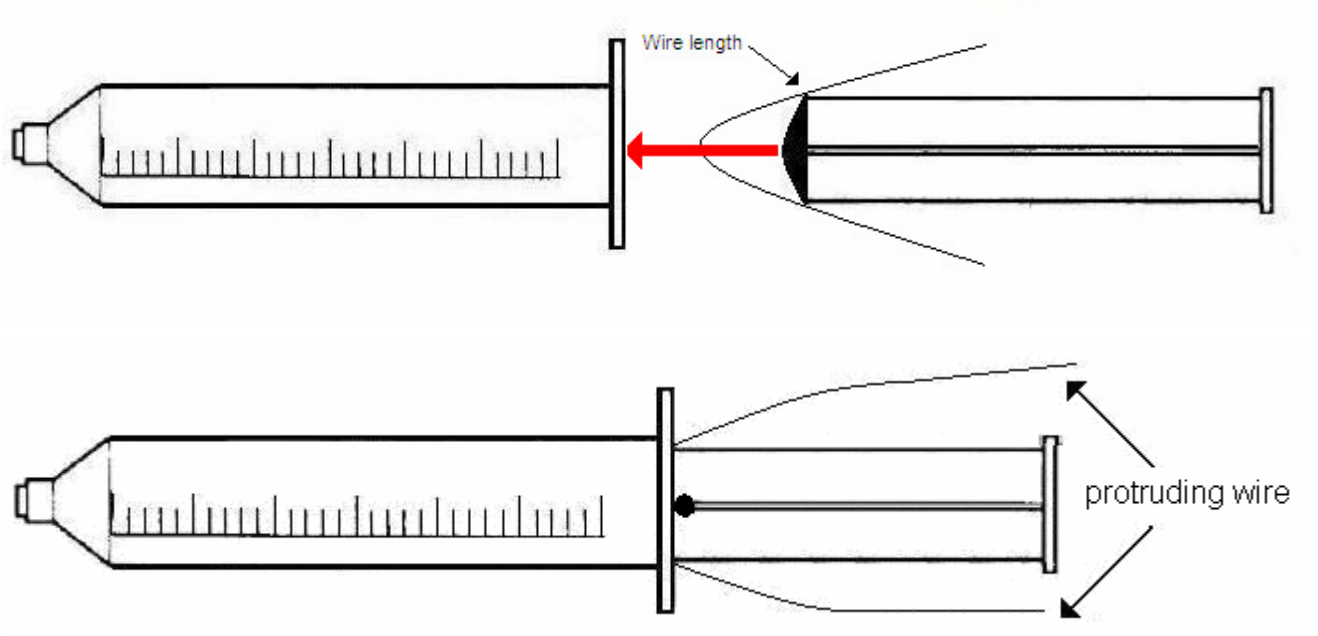

Figure 2.

3.5 Assessment of results:

- The test is valid only if the positive control syringe contents are coloured with the dye.
- Where syringes are tested in contact with specific drug solutions, potential changes in colour caused by drug/ diluent interaction with dye should be tested and eliminated.
- Syringes comply with the test if contents of all units show no evidence of dye ingress.

4 REFERENCES

"Quality Assurance of Aseptic Preparation Services" 4th Edition Ed. A. M. Beaney, Pharmaceutical Press 2006 ISBN 0 85369 615 2.

Document History	Issue date and reason for change
Version 1	Issued Feb 2006
Version 2	Issued April 2013 updated to incorporate physical test method and new microbiological method
Version 3	
Version 4	