LATEST DEVELOPMENT IN QUALITY CONTROL TEST FOR LIQUID DOSAGE FORMS

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Abstract:
Some of the factor that are important in preparing a good liquid dosage form have been reviewed. Various type were classified and method of preparation were discussed. The importance of satisfactory wetting & the influences of particle shape & size were also covered details. An extended discussion of the techniques & the modification in the particle size enhances has been provide.

Introduction:

Liquid dosage forms are prepared by dissolving active ingredients or by suspending the drug (if drug is insoluble) or by incorporating the drug into one of the two phases of oil and water systems.

Liquid dosage form comprises of solution, suspension & emulsion & a variety of preparation can be considered under each category. These dosage form are categorized by their homogene, promote action & easy of administration. Liquid dosage forms are suitable for both internal & external use. (Jain & Sharma, 2005)

They can be taken orally, applied on the surface or into any of the body cavity. Hence liquid dosage forms are more versatile than the solid & semisolid dosage form. Internal problems in the formulation, stabilization, preservation, packaging, and presentation of liquid dosage forms are easily solved by pharmacist. (Jain & Sharma, 2005)

Advantages of liquid dosage forms:

1) Drug is rapidly available for absorption.
2) They are the most suitable dosage form for infants, children, geriatric and mentally disturbed patients.

3) They are suitable for those drugs which create irritation in GIT if given in dry form, e.g.; bromides and iodides.

4) Bitter and obnoxious drugs can be given in sweetened colored and flavored forms.

5) Hygroscopic and deliquescent medicaments which are easily given in the liquid dosage forms.

6) Because of their aesthetic properties they create good appeal and beneficial psychological effect on patients.

**Disadvantages of liquid dosage forms:**

1) They are bulky therefore inconvenient to carry, transport and store.

2) Chances of variation in the doses since patients use the measure at the time of taking dose. Sometimes they might be in hurry and do not bother the accuracy of the dose.

3) Liquid provide the suitable media for the growth of the microorganisms and therefore require suitable preservatives.

4) In case of accidental breakage of the container, it may result in loss of whole dosage form.

5) Liquid coarse dispersions e.g. suspensions and emulsion require additional dosage form adjuvants like suspending agent, flocculating agent etc to maintain the stability of the product. Thus, a pharmacist has to handle many ingredients at a time.

6) Sweetened liquids containing sugar as a sweetening agent can not be recommended for diabetic patients.

(Lieberman et al, 2005)

**CLASSIFICATION:**

1) **Quality Control Test for other than parenterals:**

**1.1 Oral Drops :**

Uniformity Of Content:-Unless Otherwise, prescribed or justified and authorized, single dose preparation that are suspensions comply with the following test after shaking each container as completely as possible as carry out the test on the individual contents they comply with the test B for uniformity of content of single dose preparation.
Uniformity of Mass: Single dose preparations that are solutions or emulsions comply with the following test:

Weigh individually that contains of 20 containers emptied as completely as possible and determined the average mass. Not more than 2 of the individual masses deviated by more than 10% from average mass and none deviates by more than 20%

Dose And Uniformity Of dose of oral drops:- In to a suitable graduated cylinder introduce by means of the dropping device , the no. of drops usually prescribed for 1 dose and introduced by means of measuring device the usually prescribed quantity. The dropping speed does not exceed 2 drops per second. Weigh the liquid repeat the edition, weigh again and carry on repeating the adding and weighing until a total of 10 masses are obtained, no single mass deviates by more than 10% from the average mass the total of 10 masses does not differ by more than 15% from the nominal mass 10 doses. if necessary measure the total volume of 10 doses the volume does not differ by 15% from the nominal value of 10 doses. (E.P.-2004, B.P.-1999,)

1.2 Nasal Drops:

Unless otherwise prescribed or justified and authorized nasal drops supply in single dose containers and single dose is of nasal sprays intended for systemic action comply with the following test;-

Uniformity of Mass: Nasal drops that are solutions comply with the following test:

Weigh individually that contains of 10 containers emptied as completely as possible and determined the average mass. Not more than 2 of the individual masses deviated by more than 10% from average mass and none deviates by more than 20%.

1.3 Ear Drops:

Uniformity of volume. Comply with the test for contents of packaged dosage for.

Particle size. This test is applicable only to Ear Drops that are suspensions. Introduce a suitable volume of the Ear Drops into a counting cell or onto a microscope slide, as appropriate. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more than 20 particles have a
maximum dimension greater than 25 µm, not more than 10 particles have a maximum dimension greater than 50 µm and none has a maximum dimension greater than 100µm.(I.P.-2007)

Sterility, Where the label indicates that the Ear Drops are sterile, it complies with the test for sterility. Droppers supplied separately also comply with these tests. Remove the dropper out of the package using aseptic precautions and transfer it to a tube containing suitable culture medium so that it is completely immersed. Incubate and carry out the tests for sterility on the medium.(E.P-2004.,1)

1.4 Eye drops:

Uniformity of volume. Comply with the test for contents of packaged dosage forms.

Particle size. This test is applicable only to Eye Drops that are suspensions. Introduce a suitable volume of the Eye Drops into a counting cell or onto a microscope slide, as appropriate. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more than 20 particles have a maximum dimension greater than 25 µm, not more than 10 particles have a maximum dimension greater than 50 µm and none has a maximum dimension greater than 100 µm.(E.P-2004.)

1.5 Suspensions:

Appearance:
The appearance of the suspension is noted and determined the uniformity of sedimentation and also determined the breaks or air pockets in the sediment.

Method:
The appearance is noted in a graduated glass cylinder or transparent glass container.

Photomicroscopic Examination:
The microscope can be used to distinguish between flocculated and non-flocculated particles and to determine changes in the physical properties and stability. Sufficient fields and samples should be examined to make these determinations.(Lechmann Leon-1991,James Swarbrick)

Method: Microscopic can be used to estimate and detect change in particle size distribution and crystal shape of suspension. The dilution of suspension for microscopic examination should be made with supernatant external
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phase rather than with purified water. Individual particle size distributions can be accurately determined, using suitable electron instrumentation, for example, a Coulter Multisizer II (an electrical sensing zone instrument from Coulter Scientific Instruments, Hialeah, FL) or the Elzone 280 PC systems. General methods for particle size analysis are given as in the Figure

![Figure 1- particle size in microns.](image)

**Color, Odor, and Taste:**

These characteristics are especially important in orally administered suspensions. Variation in color often Fig. 4 Photomicrograph of a flocculated steroid suspension indicates poor distribution and/or differences in particle size. Variations in taste, especially of active constituents, can often be attributed to changes in particle size, crystal habit, and subsequent particle dissolution. Changes in color, odor, and taste can also indicate chemical instability.(I.P.-2007,Ram I.Mahato)

Method:-

By visualisation as product and by testing the drug products.( James Swarbrick,2002)

pH Value

pH defined as the presence of H\(^+\) ions in the suspension preparation.

The pH value of aqueous suspensions should be taken at a given temperature and only
after settling equilibrium has been reached, to minimize ‘‘pH drift’’ and electrode surface coating with suspended particles.

pH defined as the presence of H\(^+\) ions in the suspension preparation.

Method:-

Immerse the electrodes in the solution under examination and measure the pH at 25° ± 2°(pH range :-4.5-7.2) [I.P.-2007]

Viscosity

A Brookfield viscometer with a helipath attachment (Stoughton, MA) is a useful rheological instrument for measuring the settling behavior and structure of pharmaceutical suspensions and for characterizing the properties and stability of flocculated suspensions. The viscometer should be properly calibrated to measure the apparent viscosity of the suspension at equilibrium at a given temperature to establish suspension reproducibility.[ James Swarbrick,2002]

Density

Specific gravity or density of the suspension is an important parameter. A decrease in density often indicates the presence of entrapped air within the structure of the suspension.

Method:-

Density measurements at a given temperature should be made using well-mixed, uniform suspensions; precision hydrometers facilitate such measurements. It is determined by using pyknometer or density bottle.

\[
\text{Density (D)} = \frac{\text{Mass}}{\text{Volume}}[E.P.2004]
\]

Particle Size Measurement

Recently with respect to the importance of particle size distribution in terms of particle characterization and product physical stability testing,

Method:-
(1) light-scattering methods for particle detection called photon correlation spectroscopy (PCS).

(2) By Using single particle optical sensing (SPOS)

(3) With the help of laser diffraction (LD)


Drug Content Uniformity

This important testing procedure is best performed using either “unit of use” volume (e.g., 5mL of oral liquid or a spray actuation of an oral inhalation product) or sampling from a well-mixed dispensing container from the top, middle, and bottom of the suspension.

Sedimentation Rate, Sediment Volume, and Resuspendability

Simple, inexpensive, graduated cylinders (100–1000 mL) are useful for determining the physical stability of suspensions. They can be used to determine the settling rates of flocculated and non-flocculated suspensions and the sediment height at equilibrium. The falling height of the liquid—sediment interface of the suspension is determined as a function of time, and the sedimentation rate test is repeated periodically during storage. The sediment volume at equilibrium should be sufficiently large to support uniform resuspension with gentle agitation.

The equilibrium sediment volume should be similar and reproducible batch after batch. [Nash]

\[
\text{Sedimentation volume} \ (F) = \frac{\text{Ultimate height(H4)of sediment}}{\text{Initial height (Ho)of total suspension}}
\]

Volumetric graduated cylinders are used to determine the “F” or flocculation ratio, a value that represents the ratio of the sediment volume to the original suspension volume at a given time. It is used to measure the relative degree of flocculation and physical stability of suspensions.

[Lieberman et al, 2005; James Swarbrick,2002]

1.6 Emulsion:-

Appearance: The appearance of the emulsion is noted and determined the uniformity of emulsion. The appearance is noted in transparent glass container.
pH value:

Immerse the electrodes in the solution under examination and measure the pH at 25° ± 2°

Colour, odour and taste method:

These characteristics are especially for orally administered emulsion. Variation in colour indicates the poor distribution in particle size. Colour by direct visualisation method and taste by in vivo method.

Conductivity evaluation:

System with an aqueous continuous phase will conduct electricity, while systems with an oily continuous phase will not.

Method: By dipping a pair of electrodes or Pt. electrodes (diameter 0.4mm distance 4mm), connected to lamp and an electrical source. If o/w type emulsion the lamp lights because of the passes of current b/w the two electrodes. If lamp does not light, it is assume that the system is w/o.

Miscibility emulsion:

An emulsion will only mix with a liquid that is miscible with its continuous phase therefore an o/w type emulsion is miscible with water and w/o emulsion with an oil.

Staining evaluation method:

For evaluation of emulsion the staining test is performed. A dry filter paper impregnated with cobalt chloride turns from blue to pink on exposure to stable o/w emulsion.

Limit

May fail if emulsion is unstable & breaks in presence of electrolyte.

Dye Test:

Method: An oil-soluble dye is used. o/w emulsion are pales in colour than w/o emulsion and vice-versa. If examined microscopically, an o/w emulsion will appears as coloured globules on a colourness background while a w/o emulsion will appear as colourness globules against a coloured background.
Limit

May fail if ionic emulsifier are present.[Ram I.Mahato-2007]

Saponification Value:-

Not more than 2.0[I.P. 2007]

(2) Quality Control test for parenterals :-

2.1 Particulate matter. Injections that are solutions, when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by the unaided eye. Injections that are supplied in containers with a nominal content of 100 ml or more comply with the test for particulate contamination[Yadav-2004, Remington, I.P.-2007]

2.2 Uniformity of content. Unless otherwise stated in the individual monograph, suspensions for injection that are presented in single dose containers and that contain less than 10 mg or less than 10 per cent of active ingredient comply with the following test. For suspensions for injection containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within accepted limits of the stated content. Determine the content of active ingredient(s) of each of 10 containers taken at random, using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The preparation under examination complies with the test if the individual values thus obtained are all between 85 and 115 per cent of the average value. The preparation under examination fails to comply with the test if more than one individual value is outside the limits 85 to 115 per cent of the average value or if any one individual value is outside the limits 75 to 125 per cent of the average value. If one individual value is outside the limits 85 to 115 per cent but within the limits 75 to 125 per cent of the average value, repeat the determination using another 20 containers taken at random. The preparation under examination complies with the test if in the total sample of 30 containers not more than one
individual value is outside the limits 85 to 115 per cent and none is outside the limits 75 to 125 per cent of the average value. [Akers M. J., -1987, Lachman Leon-1999]

2.3 Extractable volume. Where the nominal volume does not exceed 5 ml, the containers comply with the requirements of Method 1 and where the nominal volume is greater than 5 ml, the containers comply with the requirements of Method 2. Suspensions should be shaken before the contents are withdrawn; oily injections may be warmed but should be cooled to 25º before carrying out the test.

Method 1 — Use 6 containers, 5 for the test and 1 for rinsing the syringe used. Inspect the 5 containers to be used in the test visually and ensure that each contains approximately the same volume of the preparation. Using a syringe with a capacity not exceeding twice the volume to be measured and fitted with a suitable needle, take up a small quantity of the liquid under examination from the container reserved for rinsing the syringe, and discharge it from the syringe whilst the needle is pointing upwards so as to expel any air. Withdraw as much as possible the contents of one of the containers reserved for the test and transfer, without emptying the needle, to a dry graduated cylinder of such capacity that the total combined volume to be measured occupies not less than 40 per cent of the nominal volume of the cylinder. Repeat the procedure until the contents of the 5 containers have been transferred and measure the volume. The average content of the 5 containers is not less than the nominal volume and not more than 115 per cent of the nominal volume.

Method 2 — Transfer the contents of not less than 3 containers separately to dry graduated cylinders such that the volume to be measured occupies not less than 40 per cent of the nominal volume of the cylinder and measure the volume transferred. The contents of each container are not less than the nominal volume and not more than 110 per cent of the nominal volume. [Akers M.J.-1987, Remington, , Niazi-2004]

2.4 Sterility Test:— The test for sterility is applied to pharmacopoeial articles that are required according to the Pharmacopoeia to be sterile. However, a satisfactory result only indicates that no contaminating viable microorganisms have been found in the sample examined in the conditions of the test. If the number of micro-organisms
present in a given amount of the article under examination is large, the probability of detecting them increases.

Very low levels of contamination cannot be detected on the basis of random sampling of a lot.

The test must be carried out under aseptic conditions designed to avoid accidental contamination of the product during testing. For achieving these conditions, a grade A laminar airflow cabinet or an isolator is recommended.

The test environment has to be adapted to the way in which the tests are performed. Precautions taken for this purpose should not adversely affect any micro-organisms, which are to be revealed in the tests.[Niazi-2004]

Method A – Membrane Filtration

Apparatus

A suitable unit consists of a closed reservoir and a receptacle between which a properly supported membrane of appropriate porosity is placed. A membrane generally suitable for sterility testing has a nominal pore size not greater than 0.45 µ and diameter of approximately 50 mm, the effectiveness of which in retaining micro-organisms has been established. Preferably assemble and sterilise the entire unit with the membrane in place prior to use.

Diluting Fluids

Fluid A. Dissolve 1 g of peptic digest of animal tissue (such as bacteriological peptone) or its equivalent in water to make 1 litre, filter or centrifuge to clarify, adjust to pH 7.1 ± 0.2, dispense into flasks in 100-ml quantities and sterilise at 121º for 20 minutes.

*NOTE* — Where fluid A is to be used in performing the test for sterility on a specimen of the penicillin or cephalosporin class of antibiotics, aseptically add a quantity of sterile penicillinase to the fluid A to be used to rinse the membrane(s) sufficient to inactivate any residual antibiotic activity of the membrane(s) after the solution of the specimen has been filtered.

Fluid B. If the test sample contains lecithin or oil, use fluid A to each litre of which has been added 1 ml of polysorbate 80, adjust to pH 7.1 ± 0.2, dispense into flasks and sterilise at 121º for 20 minutes.

*NOTE* — A sterile fluid shall not have antibacterial or antifungal properties if it is to be considered suitable for dissolving, diluting or rinsing a preparation being examined for sterility.
Quantities of Sample to be used

For parenteral preparations. Whenever possible use the whole contents of the container, but in any case not less than the quantities prescribed in Table 3, diluting where necessary to about 100 ml with a suitable diluent such as fluid A.

For ophthalmic and other non-parenteral preparations. Take an amount within the range prescribed in column (A) of Table 4, if necessary, using the contents of more than one container, and mix thoroughly. For each medium use the amount specified in column (B) of Table 4, taken from the mixed sample.[niazi-2004,E.p.-2004]

Method of Test

For aqueous solutions:- Prepare each membrane by aseptically transferring a small quantity (sufficient to moisten the membrane) of fluid A on to the membrane and filter it. For each medium to be used, transfer aseptically into two separate membrane filter funnels or to separate sterile pooling vessels prior to transfer not less than the quantity of the preparation under examination that is prescribed in Table 3 or Table 4. After filtration, aseptically remove the membrane(s) from the holder, transfer the whole membrane or cut it aseptically into 2 equal parts. Transfer one half to each of two suitable media.

Use the same volume of each medium as in the procedure[Liebermann et al.,I.P.-2007]

Validation of Tests:- Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days. Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilised by a validated moist heat process, incubate the test specimen for not less than 7 days.

For liquids immiscible with aqueous vehicles, and suspension:- Carry out the test described under For aqueous solutions but add a sufficient quantity of fluid A to the pooled (I.P-2007.,U.S.P.-2000)

**Method B – Direct Inoculation**

Quantities of Sample to be used
The quantity of the substance or preparation under examination to be used for inoculation in the culture media varies according to the quantity in each container.

Method of Test

*For aqueous solutions and suspensions.* Remove the liquid from the test containers with a sterile pipette or with a sterile syringe or a needle. Transfer the quantity of the preparation under examination prescribed in Table 4 directly into the culture medium so that the volume of the preparation under examination is not more than 10 per cent of the volume of the medium, unless otherwise prescribed. When the quantity in a single container is insufficient to carry out the tests, the combined contents of two or more containers are to be used to inoculate the media.

If the preparation under examination has antimicrobial activity, carry out the test after neutralising this with a suitable neutralising substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate, the concentrated medium may be added directly to the product units container.

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilised by a validated moist heat process, incubate the test specimen for not less than 7 days.[E.P.-2004]

*For oils and oily solutions.* Use media to which has been added a suitable emulsifying agent at a concentration shown to be appropriate in the validation of the test, for example, polysorbate 80 at a concentration of 10g/l and which has been shown not to have any antimicrobial properties under the conditions of the test. Carry out the test as described under for aqueous solutions and suspensions.
During the incubation period shake the cultures gently each day. However, when thioglycollate medium or other similar medium is used for the detection of anaerobic micro-organisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.[E.P.-2004]

2.5 Pyrogen Test:-

Test For Pyrogens

The test involves measurement of the rise in body temperature of rabbits following the intravenous injection of a sterile solution of the substance being examined. It is designed for products that can be tolerated by the test rabbit in a dose not exceeding 10ml per kg injected intravenously within a period of not more than 10 minutes.

Test Animals

Use healthy, adult rabbits of either sex, preferably of the same variety, weighing not less than 1.5kg, fed on a complete and balanced diet and not showing loss of body weight during the week preceding the test. House the animals individually in an area of uniform temperature (± 2° ), preferably with uniform humidity, and free from disturbances likely to excite them.

Do not use animals for pyrogen tests more frequently than once every 48 hours. After a pyrogen test in the course of which a rabbit’s temperature has risen by 0.6° or more, or after a rabbit has been given a test substance that was adjudged pyrogenic, at least 2 weeks must be allowed to elapse the animals is used again.

Materials

All glassware, syringes and needles must be thoroughly washed with water for injection and heated in a hot air oven at 250° for 30 minutes or at 200° for 1 hour. Treat all diluents and solutions for washing and rinsing of devices in a manner that will assure that will assure that they are sterile and pyrogen-free.

The animals must be put in the boxes 1 hour before the test and remain in them throughout the test. Ensure that the room temperature where the test is carried out is within 3° of that of the rabbits living quarters or in which the rabbits have been kept for at least 18 hours before the test. Withhold food from the animals overnight and until the test is completed; withhold water during the test.[Akers M.J.,I.P-2007., E.P.]
Recording of Temperature: Use an accurate temperature-sensing device such as a clinical thermometer or thermistor or thermo couple[ as in figure 2] or other suitable probes that have been calibrated to assure an accuracy of 0.1° and have been tested to determine that a maximum reading is reached in less than 5 minutes. Insert the thermometer or temperature sensing probe into the rectum of the test rabbit to a depth of about 5-cm.

![Thermocouple](image)

**Figure 2- thermo couple.**

Preliminary Test (Sham Test): If animals are used for the first time in a pyrogen test or have not been used during the 2 previous weeks, condition them 1 to 3 days before testing the substance being examined by injecting intravenously into them 10 ml per kg of body weight of a pyrogen-free saline solution warmed to about 38.5°.

Record the temperatures of the animals, beginning at least 90 minutes before injection and continuing for 3 hours after injection of the solution being examined. Any animal showing a temperature variation of 0.6° or more must not be used in the main test.[pyrogen test –link]

Main Test: Carry out the test using a group of three rabbits.

*Preparation of the sample:* Dissolve the substance being examined in, or dilute with, pyrogen-free saline solution or other solution prescribed in the monograph. Warm the liquid being examined to approximately 38.5° before injection.

*Procedure:* Record the temperature of each animal at intervals of not more than 30 minutes, beginning at least 90 minutes before the injection of the solution being examined and continuing for 3 hours after the injection. Not more than 40 minutes immediately preceding the injection of the test dose, record the "initial temperature " of each rabbit, which is the mean of two temperatures recorded for that rabbit at an interval of 30 minutes in the 40 minute period. Rabbits showing a temperature variation greater than 0.2° between two successive readings in the
determination of "initial temperature " should not be used for the test. In any one group of test animals, use only those animals whose " initial temperatures" do not vary by more than 1° from each other ,and do not use any rabbit having a temperature higher than 39.8° and lower than 38°.

Inject the solution being examined slowly into the marginal vein of the ear of each rabbit over a period not exceeding 4 minutes, unless otherwise prescribed in the monograph. The amount of sample to be injected varies according to the preparation being examined and is prescribed in the individual monograph. The volume of injection is not less than 0.5 ml per kg and not more than 10 ml per kg of body weight. Records the temperature of each animal at half-hourly intervals for 3 hours after the injection. The difference between the "initial temperature " and the "maximum temperature" which is the highest temperature recorded for a rabbit is taken to be its response. When this difference is negative, the results is counted as a zero response.

**Interpretation of results:** if the sum of the responses of the group of three rabbits is less than 0.6°, the preparation being examined passes the test. If the response of any rabbit is 0.6° or more, or if the sum of the response of the three rabbits exceeds 1.4°, continue the test using five other rabbits. If not more than three of the eight rabbits show individual responses of 0.6° or more, and if the sum of responses of the group of eight rabbits does not exceed 3.7°, the preparation being examined passes the test.[Akers M.J.-1987,USP-2000].
Test Interpretation—USP

According to the July/August 1991 issue of *Pharmacopeial Forum*, the solution may be judged nonpyrogenic if no single rabbit shows a rise in temperature of 0.5°C or greater above its control temperature. If this condition is not met, the test must proceed to a second stage. There is no longer a second condition involving the sum of individual temperatures. In the second stage, five additional rabbits are given a new preparation of the same test sample as the original three rabbits. The solution may be judged nonpyrogenic if not more than three of the eight rabbits show individual temperature rises of 0.5°C or more.

The U.S. Public Health Requirements for Biological Products, Part 73, judge a solution to be pyrogenic if at least half of the rabbits tested show a temperature rise of 0.6°C or more, or if the average temperature rise of all rabbits is 0.5°C or more.

The British Pharmacopoeia (BP) (24) pyrogen test employs a sliding scale based on 3 rabbits and additional groups of 3 rabbits, if required, for a total of 12 rabbits.

Table 1-Comparision between usp and bp interpettion.

<table>
<thead>
<tr>
<th>Number of rabbits</th>
<th>Maximum total peak response (°C) to pass the test</th>
<th>Minimum total peak response (°C) to fail the test</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>USP</td>
<td>BP</td>
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<td>1.4</td>
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<td>6</td>
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<td>8</td>
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<tr>
<td>9</td>
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<td>12</td>
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<td>6.60</td>
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BACTERIAL ENDOTOXINS TEST (BET)

A Limulus amebocyte lysate (LAL) reagent is the basis for an in vitro pyrogen test method that is specific for bacterial endotoxin pyrogen. For this reason, it is now referred to as the bacterial endotoxins test (BET), although
BET and LAL testing are used interchangeably. When it was first introduced, there was concern by the industry and regulators that its specificity would limit its application in the parenteral industry. However, experience gained in this industry over the past 25 years confirms that endotoxin is the principal pyrogen of concern to pharmaceutical and medical device manufacturers. The BET has steadily gained acceptance globally as a replacement for the rabbit pyrogen test.

Discovery of Limulus Amebocyte Lysate (LAL) Reagent

The LAL test reagent is prepared by lysing amebocyte blood cells obtained from the American horseshoe crab, Limulus polyphemus. The origin of LAL reagent is traced to Frederick Bang, who first recognized the association between bacterial endotoxin and Limulus blood coagulation in studies of marine organism lysate. A lysate of amebocytes from either of the species of the horseshoe crab, Limulus polyphemus, Tachypleus gigas, Tachypleus tridentatus or Carcinoscorpius rotundicauda reconstituted as stated on the label. The species from which the lysate is obtained is stated on the label.

Method. Carry out the following procedure in receptacles such as tubes, vials or wells of micro-titre plates. Into each of the chosen receptacle, add an appropriate volume of negative control (NC), standard CSE solutions in water BET, test solution and positive product control (PPC). At intervals that will permit the reading of each result, add to each receptacle an equal volume of the appropriately constituted lysate unless single test vials are used. Mix the sample-lysate mixture gently and place in an incubating device such as a water-bath or a heating block, accurately recording the time at which the receptacles are so placed. Incubate each receptacle at 37º ± 1º undisturbed for 60 ± 2 minutes. Remove the receptacles and examine the contents carefully. A positive reaction is characterised by the formation of a firm gel that retains its integrity when inverted through 180º in one smooth motion. Record this result as positive (+). A negative result is characterised by the absence of such a gel or by the formation of a viscous gel that does not maintain its integrity. [lal test link]

Calculation and interpretation of results.

The test for interfering factors is valid if
(a) solutions of series A and D give negative results;

(b) the results obtained with solutions of series C confirm the labelled sensitivity of the lysate;

(c) the geometric mean of the end-point concentration of solutions of series B is not more than 2l or not less than 0.5l.

If the result obtained is outside the specified limit, the test preparation under examination is acting as an inhibitor or activator. The interfering factors may be eliminated by further dilution (not greater than MVD), filtration, neutralisation, inactivation or by removal of the interfering substances. The use of a more sensitive lysate permits the use of greater dilution of the preparation under examination.

Ultrafiltration may be used, if necessary, when the interfering factor passes through a filter with a nominal separation limit corresponding to a molecular weight of 10,000 to 20,000, such as asymmetrical membrane filters of cellulose triacetate. Such filters should be checked for the presence of any factors causing false positive results. The material retained on the filter, which contains the endotoxins, is rinsed with water BET or tris-chloride buffer pH 7.4 BET. The endotoxins are recovered in the water BET or the buffer. The endotoxin concentration in the test volume and the final volume are determined for each preparation under examination.

Establish that the chosen treatment effectively eliminates interference without removing endotoxins by repeating the test for interfering factors using the preparation under examination to which the CSE has been added and which has been submitted to the chosen treatment.

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