LECTURE 7

Standardisation and analysis of parenteral dosage forms



Sterility, freedom from pyrogens and particles, isotonicity and the manufacturing environment of injections and infusions

Standardisation of Medicines and Raw Materials
Educational programme 6B10102 – Pharmacy · 4th year · Department of Fundamental Medicine, Al-Farabi Kazakh National University

OUTLINE

Lecture plan

1. Types of parenteral preparations (Ph. Eur. 0520)
2. Critical quality attributes
3. Sterility testing
4. Bacterial endotoxins and pyrogens
5. Particulate contamination, extractable volume, osmolality
6. Sterilisation and clean rooms (GMP Annex 1)

Types of parenteral preparations



Injections

Solutions, emulsions or suspensions; single-dose or multidose



Infusions

Large-volume aqueous solutions or o/w emulsions, usually isotonic, preservative-free



Concentrates

Diluted to a prescribed volume before administration



Powders for injection or infusion

Sterile solids (often freeze-dried) reconstituted before use



Gels for injection

Viscous gels giving modified release at the site



Implants

Sterile solid forms implanted to release over a long period

All must be: sterile, free from pyrogens, practically free from particles, and prepared with materials and methods that ensure this

Tests for parenteral preparations

Attribute	Test (Ph. Eur.)	Typical requirement
Sterility	2.6.1 Sterility	No growth after 14 days
Pyrogens	2.6.14 Bacterial endotoxins; 2.6.30 MAT	Below the endotoxin limit K/M
Visible particles	2.9.20	Practically free from particles
Sub-visible particles	2.9.19	Within limits (light obscuration or microscopy)
Extractable volume	2.9.17	Not less than nominal volume
Osmolality	2.2.35	Infusions approximately isotonic (≈ 290 mOsm/kg)
pH	2.2.3	Close to physiological if possible
Assay, impurities	HPLC, UV, titration	As in the specification

Testing for sterility

Membrane filtration (preferred)

- Sample filtered through a 0.45 µm membrane
- Membrane rinsed to remove antimicrobial activity
- Membrane transferred to media
- Used for aqueous, filterable products

Direct inoculation

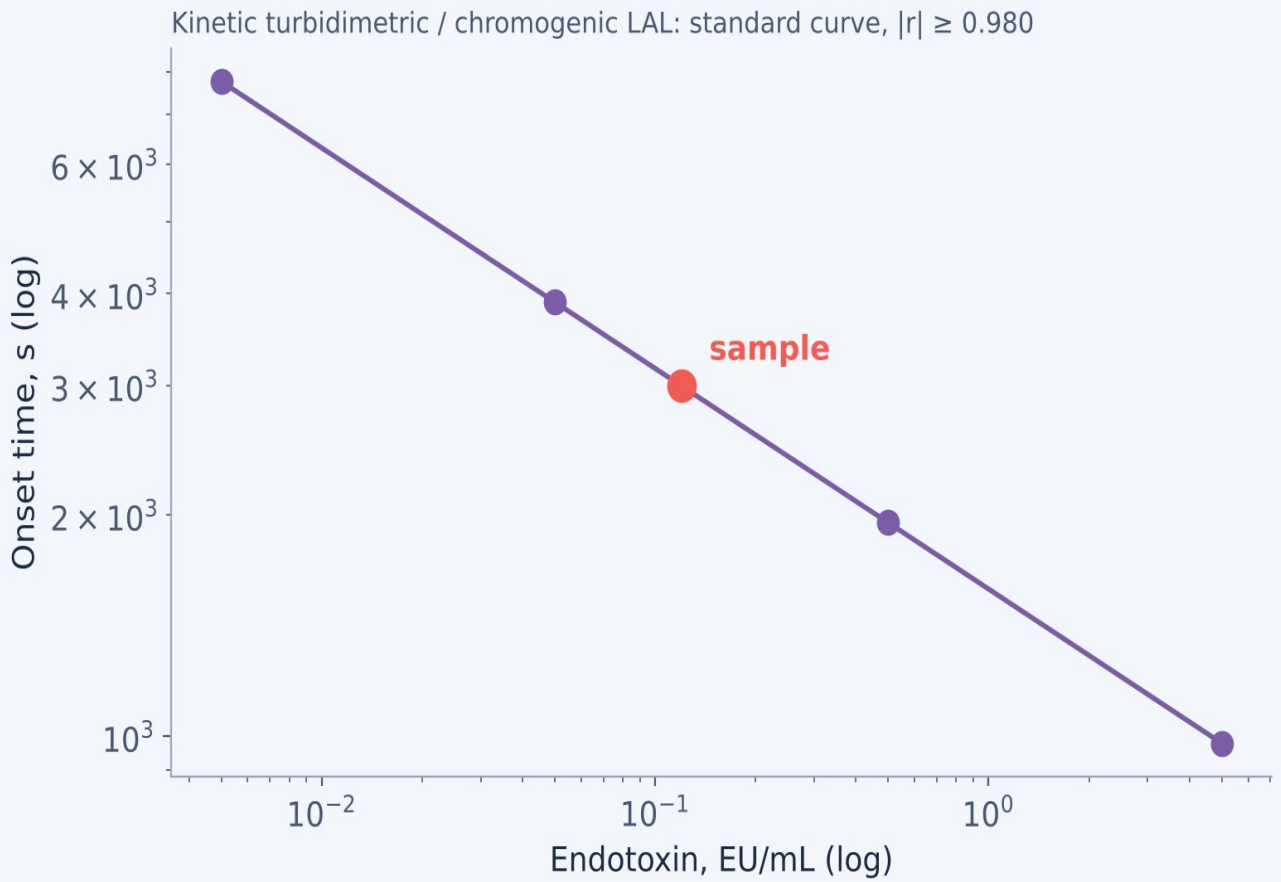
- Sample added directly to media
- For non-filterable products (suspensions, oils)
- Volume ≤ 10% of medium volume
- Method suitability test with microorganisms

Media and incubation

- **Fluid thioglycollate medium:** 30–35 °C (anaerobes and aerobes)
- **Soya-bean casein digest medium:** 20–25 °C (fungi, aerobes)
- **Not less than 14 days**
- **Isolator or grade A in a grade B room**

Limitation: the test examines a small sample; sterility is assured by validated sterilisation and aseptic processing, not by testing

Endotoxins: from LAL to recombinant factor C



Endotoxin limit

- **Limit = K / M**
- **K:** 5 EU/kg (parenteral), 0.2 EU/kg (intrathecal), per hour
- **M:** maximum dose per kg per hour
- **Example:** M = 10 mg/kg → limit = 0.5 EU/mg
- **Methods:** gel-clot, turbidimetric, chromogenic (LAL); rFC (2.6.32)
- **MAT (2.6.30):** human cells; replaces the rabbit pyrogen test

Limits for sub-visible particles (light obscuration)

Container	Particles ≥ 10 µm	Particles ≥ 25 µm
Nominal volume ≤ 100 mL	≤ 6000 per container	≤ 600 per container
Nominal volume > 100 mL	≤ 25 per mL	≤ 3 per mL



Visible particles

- 2.9.20: inspection against black and white backgrounds
- Illumination 2000–3750 lux
- Each unit inspected in production; 100% inspection
- Particles may block capillaries or cause granulomas

Microscopic method (method 2): used when light obscuration is unsuitable; limits ≤ 3000 / 300 per container or ≤ 12 / 2 per mL

Physical requirements that protect tissues

≈ 290

mOsm/kg
osmolality of blood plasma; infusions approximately isotonic

−0.52 °C

Freezing point of plasma
basis of cryoscopic isotonicity calculations

0.9%

Sodium chloride
isotonic solution; glucose 5% is approximately isotonic

≥ nominal

Extractable volume (2.9.17)
patient must be able to withdraw the labelled dose

Hypotonic solutions cause haemolysis; strongly hypertonic ones cause pain and phlebitis, so they are given slowly into large veins

Terminal sterilisation or aseptic processing

Terminal sterilisation (preferred)

- **Steam:** reference 121 °C for 15 min
- **Dry heat:** reference 160 °C for 2 h; depyrogenation at higher temperature
- **Ionising radiation:** reference 25 kGy
- **SAL ≤ 10^{-6}:** probability of a non-sterile unit
- Product sterilised in its final container

Aseptic processing

- **Sterile filtration:** 0.22 µm, integrity tested
- Filling in grade A with grade B background
- **Media fill** (process simulation) validates the process
- Used for heat-sensitive products: proteins, many antibiotics
- Higher risk than terminal sterilisation

Clean room grades: particles ≥ 0.5 µm per m^3

Grade	At rest	In operation	Typical activity
A	3 520	3 520	Aseptic filling, open sterile product (unidirectional air flow)
B	3 520	352 000	Background for grade A in aseptic processing
C	352 000	3 520 000	Preparation of solutions to be filtered; filling of terminally sterilised products
D	3 520 000	not predetermined	Handling of components after washing

Also monitored

- Viable particles: air, surfaces, gloves
- Grade A: no growth expected
- Pressure differentials, air changes
- Contamination control strategy (CCS)

Key take-aways

1. **Parenteral preparations must be** sterile, pyrogen-free and practically free from particles
2. **Sterility test (2.6.1):** two media, ≥ 14 days; assurance comes from validated processes
3. **Endotoxin limit = K/M;** LAL, rFC and MAT replace animal tests
4. **Sub-visible particles:** ≤ 6000 / 600 per container for ≤ 100 mL
5. **Terminal sterilisation preferred** (121 °C, 15 min); aseptic processing in grade A/B

SELF-CHECK

Control questions and literature

Questions

1. Which types of parenteral preparations does Ph. Eur. 0520 describe?
2. Describe the two methods of the sterility test and the media used.
3. Why can a passing sterility test not prove sterility of a whole batch?
4. Calculate the endotoxin limit for a drug with M = 2 mg/kg/h.
5. Give the limits for sub-visible particles in a 10 mL ampoule.
6. Why must infusions be approximately isotonic?
7. Compare terminal sterilisation and aseptic processing.
8. What are the grade A and grade B limits for particles ≥ 0.5 µm?

Sources

- Ph. Eur. 0520 Parenteral preparations
- Ph. Eur. 2.6.1, 2.6.14, 2.6.30, 2.6.32, 2.9.17, 2.9.19, 2.9.20, 2.2.35, 5.1.1
- EU GMP Annex 1 (2022); EAEU GMP (Decision No. 77)
- State Pharmacopoeia RK