微生物試驗方法適用性

台灣東洋藥品工業股份有限公司六堵廠 微生物課

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Agenda

- ✓ 檢品配製
- ✓ 檢測方法
- ✓ 抑菌性的處理
- ✓ 方法適用性驗證

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檢品配製流程

- 水溶性物質
  - Dissolve sample by using a 1 in 10 dilution of the product in Peptone-NaCl solution pH 7.0, Phosphate Buffer Solution pH7.2 (USP<62>), or SCDB as diluent.
    - Normally prepare 10 g or 10 mL of the product in 100 mL diluent.
    - 10 g of the product for *Salmonella spp*. Examination
  - Adjust pH to 6 – 8

檢品配製流程

- 非脂肪非水溶性物質
  - Suspend sample by using a 1 in 10 dilution of the product in Peptone-NaCl solution pH 7.0, Phosphate Buffer Solution pH7.2 (USP<62>), or SCDB as diluent.
    - Normally 10 g of the product in 100 mL diluent.
    - 10 g of the product for *Salmonella spp*. examination
  - Diluent with 1% w/v surface-active agent (such as Tween 80) if necessary.
  - Adjust pH to 6 - 8

檢品配製流程

- 脂肪類物質
  - Dissolve sample in minimum quantity of Isopropyl Myristate which is sterilized by filtration, or mix sample in sterile Tween 80, or another sterile non-inhibitory surface-active agent.
  - Dissolve / Mix at 40 - 45 ºC if necessary.
  - Add pre-warmed diluent such as Phosphate Buffer Solution pH7.2 (USP<62>), or SCDB to make 1 in 10 dilution and keep temperature for mixing to form an emulsion.
    - Normally 10 g of the product in 100 mL diluent.
    - 10 g of the product for *Salmonella spp*. examination
  - Further 10-fold dilution by diluent with suitable sterile surface-active if necessary.

檢品配製流程

- 液體或固體噴霧劑
  - Transfer total contents or defined number of metered dose from each in suitable diluent.
  - Normally 10 containers to be tested.

- 經皮貼劑
  - Transfer suitable volume of diluent containing inactivators (Tween 80 and/or lecithin) on to each patch. Shake for 30 mins.
  - Normally 10 patches of the product to be tested.

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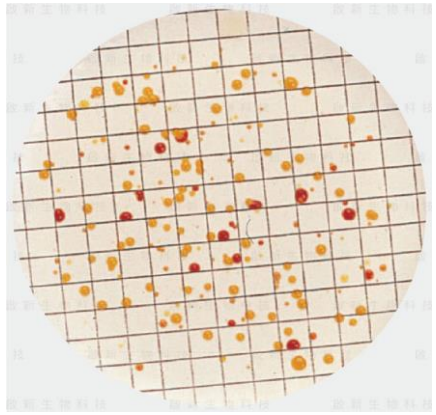
總菌數試驗 (USP<61>)

- 濾膜法
  - Pore size of membrane not great than 0.45 um.
  - Membrane material : MCE, PES, PVDF, PTFE, etc
  - Preferably representing 1 g of the product
  - Suitable wash solution and wash volume (less than 100 mL x 5 times)
  - Filter blocked by insoluble materials

https://kytac1.ypu.edu.tw/p/405-1017-41253,c4913.php?Lang=zh-tw



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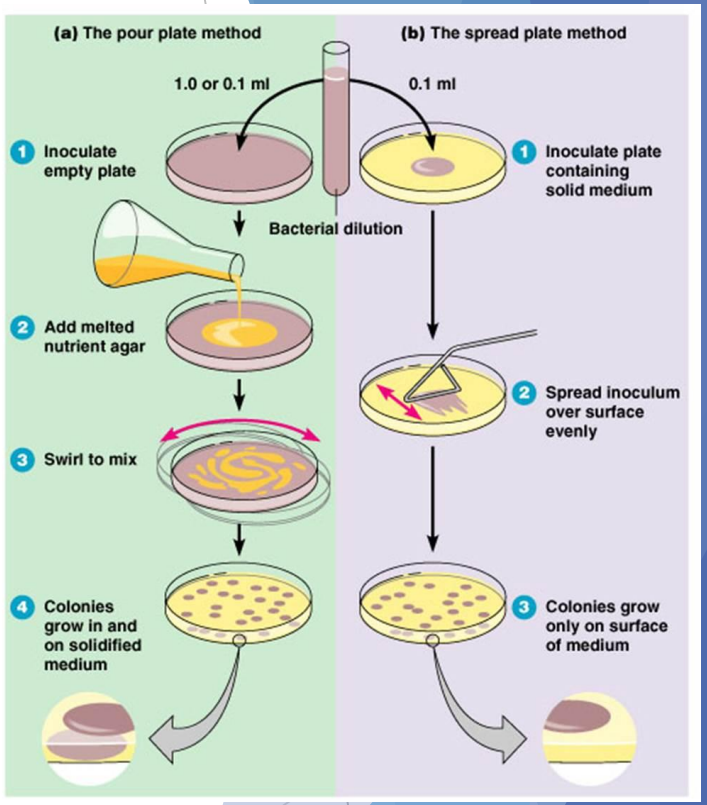
總菌數試驗 (USP<61>)

- 平板計數法
  - 傾注法
    - 1 mL of prepared sample for each plate.
    - At least 2 plates to be used.
    - Take the mean of counts and calculate the cfu in original sample.
    - Interfered by insoluble materials
  - 表面塗抹法
    - Not less than 0.1 mL of prepared sample for each plate.
    - At least 2 plates to be used.
    - Take the mean of counts and calculate the cfu in original sample.



http://wap.sciencenet.cn/blog-565899-1099544.html?mobile=1

總菌數試驗 (USP<61>)

- 最大可能數法
  - Less accuracy and unreliable for molds
  - Only use for TAMC which no other method available
  - 10 fold dilution from 0.1 to 0.001
  - 1 mL each diluted sample into 9-10 mL SCDB in triplicated

Table 3. Most-Probable-Number Values of Microorganisms

Observed Combinations of Numbers of Tubes Showing Growth in Each Set			MPN per g or per mL of Product	95% Confidence Limits
Number of g or mL of Product per Tube				
0.1	0.01	0.001		
0	0	0	<3	0–9.4
0	0	1	3	0.1–9.5
0	1	0	3	0.1–10
0	1	1	6.1	1.2–17
0	2	0	6.2	1.2–17
0	3	0	9.4	3.5–35
1	0	0	3.6	0.2–17

特殊菌試驗 (USP<62>)

- Bile-Tolerant Gram Negative Bacteria
  - Test for Absence
    - Not less than 1g sample for 1 in 10 diluted
    - EEB at 30-35 °C for 24-48 hours
    - VRBGA at 30-35 °C for 18-24 hours
  - Quantitative Test
    - Prepare 0.1, 0.01, 0.001g sample
    - EEB at 30-35 °C for 24-48 hours
    - VRBGA at 30-35 °C for 18-24 hou

Table 2. Interpretation of Results

Results for Each Quantity of Product			
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	Probable Number of Bacteria per g or mL of Product
+	+	+	more than 10^3
+	+	–	less than 10^3 and more than 10^2
+	–	–	less than 10^2 and more than 10
–	–	–	less than 10

特殊菌試驗 (USP<62>)

- *E. coli*
  - Test for Absence
    - Not less than 1g sample for 1 in 10 diluted
    - SCDB at 30-35 °C for 18-24 hours
    - MCB at 42-44 °C for 24-48 hours
    - MCA at 30-35 °C for 18-72 hours

- *Salmonella spp,*
  - Test for Absence
    - Not less than 10g sample
    - SCDB at 30-35 °C for 18-24 hours
    - RPB at 30-35 °C for 18-24 hours
    - XLDA at 30-35 °C for 18-48 hours

特殊菌試驗 (USP<62>)

- *P. aeruginosa*
  - Test for Absence
    - Not less than 1g sample for 1 in 10 diluted
    - SCDB at 30-35 ^{o}C for 18-24 hours
    - CA at 30-35 ^{o}C for 18-72 hours

- *S. aureus*
  - Test for Absence
    - Not less than 1g sample for 1 in 10 diluted
    - SCDB at 30-35 ^{o}C for 18-24 hours
    - MSA at 30-35 ^{o}C for 18-72 hours

特殊菌試驗 (USP<62> <60>)

- *C. albicans*
  - Test for Absence
    - Not less than 1g sample
    - SDB at 30-35 ^{o}C for 3-5 days
    - SDA at 30-35 ^{o}C for 24-48 hours

- *B. cepacia* complex
  - Test for Absence
    - Not less than 1g sample
    - SCDB at 30-35 ^{o}C for 48-72 hours
    - BCSA at 30-35 ^{o}C for 48-72 hours

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中和抑菌性的方法

- Recovery less than 50 % means antimicrobial activity exist
- Neutralization method
  - Increase diluent volume
  - Wash via membrane filtration
  - Add suitable neutralizing agent and concentration
    - Common agent as USP<61> Table 2
    - Specific agent such as β-lactamases
  - Combination above method

Common neutralizing agent

Table 2. Common Neutralizing Agents/Methods for Interfering Substances

Interfering Substance	Potential Neutralizing Agents/Method
Glutaraldehyde, mercurials	Sodium hydrogen sulfite (Sodium bisulfite)
Phenolics, alcohol, aldehydes, sorbate	Dilution
Aldehydes	Glycine
Quaternary ammonium compounds (QACs), parahydroxybenzoates (parabens), bis-biguanides	Lecithin
QACs, iodine, parabens	Polysorbate
Mercurials	Thioglycollate
Mercurials, halogens, aldehydes	Thiosulfate
EDTA (edetate)	Mg or Ca ions

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總生菌數方法適用性驗證

- 負控制組: 沒有產品，僅有稀釋液的空白組
- 產品組: 用於確認產品本身含菌狀況
- 產品加挑戰菌組: 確認檢驗方法適用性
  - 依生長促進試驗要求，所有菌種皆需挑戰
  - 挑戰菌數 < 100 cfu
  - 挑戰菌液不超過稀釋產品的1 %
  - 挑戰菌於消除抑菌性後再添加
  - 合格標準: 和正控制組相比 recovery 50 -200 %
- 正控制組: 沒有產品，僅有挑戰菌，用於確認配製的挑戰菌數

舉例 – 無抑菌性產品

- 產品加挑戰菌組
  - 10 g 水溶性檢品溶於 100 mL SCDB (1:10) →檢品溶液濃度 0.1 g/mL
  - 取10 mL (相當於1 g 檢品量)以濾膜法法進行方法適用性驗證

    → 取符合規格 (例如10^3 cfu/g) 要求的量
  - 挑戰菌液體積需小於 10 mL x 1% = 0.1 mL

    → 挑戰菌液濃度: < 100 cfu x 0.1 mL → < 1000 cfu/mL
  - 過濾後計數 (結果1)

- 正控制組
  - 取10 mL SCDB以濾膜法進行方法適用性驗證
  - 挑戰菌體積需小於 10 mL x 1% = 0.1 mL
  - 過濾後計數 (結果2)

舉例 – 具有抑菌性產品

- 產品加挑戰菌組
  - 10 g 水溶性檢品溶於 100 mL SCDB (1:10)
  - 取10 mL (1 g/10 mL)以濾膜法進行方法適用性驗證
  - 中和抑菌性(例如檢品溶液過濾後再潤洗濾膜數次)
  - 於最後一次潤洗時加入挑戰菌 < 100 cfu (0.1 mL of < 1000 cfu/mL)
  - 過濾後計數 (結果1)

- 正控制組
  - 取10 mL SCDB以濾膜法進行方法適用性驗證
  - 中和抑菌性(例如潤洗濾膜)
  - 於最後一次潤洗時加入挑戰菌 < 100 cfu (0.1 mL of < 1000 cfu/mL)
  - 過濾後計數 (結果2)

特殊菌檢測方法適用性驗證

- 負控制組: 沒有產品，僅有稀釋液的空白組
- 產品組: 用於確認產品本身含菌狀況
- 產品加挑戰菌組: 確認檢驗方法適用性
  - 依生長促進試驗要求，所有菌種皆需挑戰
  - 挑戰菌數 < 100 cfu
  - 挑戰菌於消除抑菌性後再添加
  - 每個培養基階段皆以最短時間培養
  - 合格標準: 挑戰菌生長，指示試驗符合要求
- 正控制組: 沒有產品，僅有挑戰菌，用於確認配製的挑戰菌

~ Thanks for your attention ~