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# XLD AGAR

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## DETECTION OF *SALMONELLA*

### 1 INTENDED USE

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XLD (Xylose Lysine Desoxycholate) Agar is used for the isolation of *Salmonella* in pharmaceutical products. The typical composition corresponds to that defined in the American and European Pharmacopeia.

The agar can also be used as a second media of choice in the normalized methods for the detection of *Salmonella* in food products and water.

A second formulation of XLD agar exists and corresponds to the composition in the standards, in food microbiology and in water microbiology under the references BK168 & BM087.

### 2 HISTORY

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The medium was formulated by Taylor in order to increase the efficiency of recovery of pathogenic enterobacteria, particularly *Shigella* and other fastidious species which do not develop in other formulations containing highly toxic inhibitors.

### 3 PRINCIPLES

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Sodium desoxycholate inhibits contaminating Gram-positive flora.

Xylose is fermented by practically all enteropathogenic bacteria, except for *Shigella* which are thus differentiated from the other species. After exhausting xylose, *Salmonella* decarboxylate lysine (via lysine decarboxylase) to cadaverine, causing the pH to rise. Colonies of salmonellae resemble those of shigellae in the medium having become basic.

The colonies formed are red in the presence of the indicator, phenol red.

The addition of lactose and sucrose to the medium enable coliform bacteria to decarboxylate lysine and thereby produce excess acidity, making the indicator turn yellow, favoring their differentiation.

In basic medium, pathogenic H<sub>2</sub>S-producers reduce ferric ammonium citrate and cause a blackening due to the production of iron sulfide at the center of the colonies. Non-pathogenic bacteria which do not decarboxylate lysine acidify the medium, a result of sugar fermentation. The pH decrease prevents the colonies from blackening.

### 4 TYPICAL COMPOSITION

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The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Yeast extract .....	3,0 g
- L-Lysine .....	5,0 g
- Lactose .....	7,5 g
- Sucrose .....	7,5 g
- Xylose.....	3,5 g
- Sodium desoxycholate .....	2,5 g
- Sodium chloride .....	5,0 g
- Sodium thiosulfate.....	6,8 g
- Ferric ammonium citrate .....	0,8 g
- Phenol red.....	80,0 mg
- Bacteriological agar.....	13,5 g

pH of the ready-to-use media at 25 °C : 7,4 ± 0,2.

## 4 PREPARATION

- Dissolve 55,2 g of dehydrated media (BK058) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Cool rapidly to 44-47 °C.
- Pour rapidly into Petri plates and let solidify on a cold, flat surface.
- Dry the plates in an incubator, covers partially removed.

✓ **Reconstitution :**  
55,2 g/L

✓ **Sterilization :**  
Do not autoclave, pour rapidly into Petri plates.

### Notes

Excessive heating or prolonged holding at 47°C may cause precipitation, so the colonies may furnish less clear-cut reactions.

The medium should be clear and orange-red.

## 5 INSTRUCTIONS FOR USE

- Re-inoculate a loop of enrichment media onto XLD agar plates prepared as above.
- Incubate at 30-35 °C for 18 to 48 hours.

✓ **Inoculation :**  
A loop of enrichment broth

### Note

For using XLD agar as the second *Salmonella* isolation media, incubate the plates 24 h at 37 °C.

✓ **Incubation :**  
18 to 48 h at 30-35°C

## 6 RESULTS

*Salmonella* present red colonies, with or without black centers.

The aspect of the colonies is as follows :

Characteristics	Microorganisms
Yellow colonies, with or without a black center	<i>Escherichia coli</i> , <i>Citrobacter</i> , <i>Enterobacter</i> , <i>Proteus</i> , <i>Serratia</i> , <i>Klebsiella</i>
Red colonies without black center	<i>Shigella</i> , <i>Providencia</i> , <i>Salmonella</i> Paratyphi A
Red colonies with black center	<i>Salmonella</i> , <i>Edwardsiella</i>

See ANNEX 1 : PHOTO SUPPORT.

## 8 QUALITY CONTROL

**Dehydrated media :** pinkish powder, free-flowing and homogeneous.

**Prepared media :** red-orange agar.

Typical culture response after 18 hours of incubation at 30-35 °C, inoculum < 10<sup>2</sup> microorganisms

Microorganisms	Growth (Productivity Ratio : P <sub>R</sub> )
<i>Salmonella</i> Typhimurium <i>Salmonella</i> Abony	WDCM 00031 WDCM 00029
	P <sub>R</sub> ≥ 50 % P <sub>R</sub> ≥ 50 %

## 9 STORAGE / SHELF LIFE

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**Dehydrated media** : 2-30 °C.

The expiration date is indicated on the label.

**Prepared media in plates (\*)** : 8 days at 2-8 °C.

(\*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

## 10 PACKAGING

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**Dehydrated media** :

500 g bottle ..... BK058HA

## 11 BIBLIOGRAPHY

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Taylor, W.I.. 1965. Isolation of *Shigellae*. I. Xylose lysine agars: new media for isolation of enteric pathogens. American Journal of Clinical Pathology, **44** : 471-475.

Pharmacopée Européenne. Chapitre 2.6.13. Contrôle microbiologique des produits non stériles : Recherche de microorganismes spécifiés.

## 12 ADDITIONAL INFORMATION

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The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : XLD\_ENv8.

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## ANNEX 1 : PHOTO SUPPORT

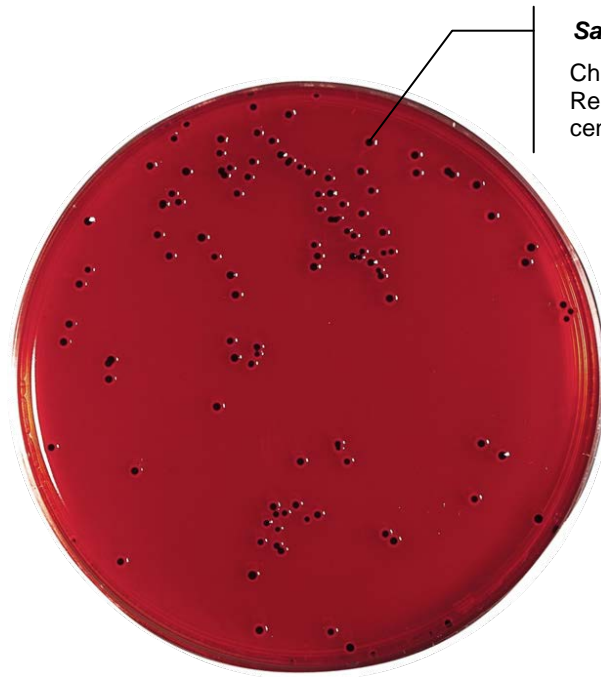
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### XLD Agar

Detection of *Salmonella*, *Shigella* and other pathogenic enterobacteria.

#### Results :

Growth obtained after 24 hours of incubation at 32,5 °C.



#### ***Salmonella* Enteritidis**

Characteristic colony :  
Red color with a black precipitate in the center of iron sulfide ( H<sub>2</sub>S [+]).