Sven Gard/Agar

355-3430

DEFINITION

Medium for demonstrating the inapparent phase of biphasic *Salmonella* (Sven Gard method).

PRINCIPLE

The H antigens of *Salmonella* are:

- either **monophasic**: the flagellae of bacteria making up the culture all have the same specificity.
  e.g.: *S. Typhi* 9,12 (Vi): d: -.

- or **biphasic**: the bacteria can alternatively express 2 different specificities; changes in specificity generally occur with a frequency of the order of 10^{-5}.
  e.g.: *S. Paratyphi* B 1, 4, 5, 12,: b: 1,2.

To determine the serotype of a *Salmonella* strain, the O and H antigen factors are detected. The easiest way to identify these factors is by agglutination on a slide of a culture taken from an agar medium, i.e. from the lowest and most humid section of a "glucose-lactose-H_2S" medium, or better still from a soft agar which facilitates the selection of the most mobile bacteria, those with the most developed H antigen.
If a culture of a strain of *Salmonella* with a biphasic H antigen is composed of bacteria with antigens in phase 1 and bacteria with antigens in phase 2 in roughly equal proportions,
it can be agglutinated by anti-H phase 1 and anti-H phase 2 sera; diagnosis can be immediate.

If, on the other hand, one of the two phases is markedly predominant over the other, only the "majority" phase can be identified.
The immobilising properties of the anti-H serum are used to detect the second phase. If the anti-H serum corresponding to the specificity of the phase already identified is added to the soft agar, and if this agar is inoculated **in one point**, all the bacteria with a flagellate antigen corresponding to the specificity of the added serum will be **immobilised**.

The others, however, can invade the agar and the H factors corresponding to the second specificity can be identified. This method of selection by immobilisation is currently referred to as **"phase inversion"**.
e.g.: *S. Paratyphi B* and *S. Typhimurium* both belong to Group B of the Kauffmann White (0:4) schema.

Phase 2 of the H antigen: 1,2 is identical in both these serotypes. Their cultures, mainly in phase 2, cannot be distinguished.
Bacteria with H antigen: 1,2 must be immobilized to obtain a bacterial culture with the H antigen below the other phase. This will be agglutinated by the anti-b serum in the case of *S. paratyphi B*, and by the anti-i serum in the case of *S. typhimurium*.

PRESENTATION

Ready-to-use
25 ml x 25 tubes **code 355-3430**

STORAGE

- Ready-to-use: + 2°C to 8°C in the dark.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Glucose	1 g
Yeast extract	1 g
Meat extract	5 g
Powdered Trypto-casein-soy broth	30 g
Pastagar A	4.6 g
Distilled water	1,000 ml

Final pH (25°C) = 7.4 ± 0.2

Sterilization in autoclave for 20 minutes at 110°C.

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- SG 1 = agglutinines anti a + b + c + z10 **code 356-1011**
- SG 2 = agglutinines anti d + i + e, h **code 356-1021**
- SG 3 = agglutinines anti k + y + l, v + l, w + l, z13 + l, z28 **code 356-1031**
- SG 4 = agglutinines anti r + z **code 356-1041**
- SG 5 = agglutinines anti e, n, x + e, n, z15 **code 356-1051**
- SG 6 = agglutinines anti 1,2 + 1,5 + 1,6 + 1,7 + z6 **code 356-1061**

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EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Sterile Petri dishes (Ø = 90 mm)
- Blades
- Inoculating loop or sterile Pasteur pipettes
- Water-bath, precise to + 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

PROTOCOL

To the melted medium (25 ml) maintained under supercooled conditions at 44°C - 47°C, add 1 drop of one of the 6 anti-H *Salmonella* serums for phase inversion, corresponding to the previously-determined phase (the composition of the 6 SGI serums having been identified beforehand).

Mix with circular movements and pour onto Petri dishes. After solidification, check that there is no condensation on the surface, then inoculate abundantly at the center of the plate (an inoculating loop filled from the agar medium).
Incubate the plates, with covers, for 18 hours at 37°C.

The H antigen is identified by slide agglutination, by collecting the culture from the periphery of the medium invasion zone.

PRECAUTIONS

Comply with Good Laboratory Practice.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	**Results after 24h culture at 37°C**
Sven-Gard agar without serum	
Salmonella enteritidis	Invasion
Salmonella typhimurium	Invasion
Salmonella anatum	Invasion
Salmonella abortus equi	Invasion
Sven-Gard agar with serum for phase inversion	
Salmonella typhimurium	Inapparent phase appearance
Salmonella anatum	

KEY WORDS

Sven Gard / *Salmonella* / H antigen / Medium.

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