Microbiology Focus

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Fluka® Analytical

Salmonella Infection and Detection

Chicken is a potential reservoir for *Salmonella*. The meat and the eggs are possible links to human infection. On chromogenic media, *Salmonella* can be detected quite quickly and easily as pink colonies.

Salmonella Infection and Detection

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

Salmonella have the necessary tools and mechanisms to spread in the body. Monitoring is quite important as outbreaks of *Salmonella* are frequent.

Salmonella has the ability to manipulate the host cell's metabolism and during infection, it is found within cells of the immune system where some types of *Salmonella* can grow and thrive. A study detected a bacterial sorting platform (bacterial type III protein secretion system), which organizes the needed proteins. A key function of this cytoplasmic sorting platform is to line up the secretion of proteins in a specific order to be able to overtake control of the host cell's function. The platform uses customized chaperones to organize the type III protein secretion system (T3SS). It organizes the building of a needle complex through which the effector proteins can later be injected into the host cells.[1]

On the *Salmonella* pathogenicity island 2 (SPI-2), the T3SS is encoded and it was seen that mutants with defect SPI-2 cannot replicate efficiently intracellularly under *in vitro* conditions. In the *in vivo* model, it was observed that SPI-2 T3SS mutants can replicate to high intracellular densities in phagocytes in the organs of infected animals, but appear unable to leave the infected cells.[2]

Salmonella contamination is the second leading cause of food-borne illness worldwide. Controlling outbreaks of *Salmonella* is an important task for food regulators, restaurants, and the food industry in general.

Did you know…

amphibians, such as lizards, frogs and turtles, are potential carriers of *Salmonella*?

There are several cases where a *Salmonella* infection is associated with amphibians kept as pets. It is highly recommended that you wash your hands after touching the animals and that you keep small children away from them.

Figure 1: Pet lizard

The *Salmonella* family includes over 2,300 serotypes of bacteria, but two types, *Salmonella enteritidis* and *Salmonella typhimurium*, are responsible for about half of all human infections. Most outbreaks of *Salmonella* are traced back to dairy, poultry and meat products, but *Salmonella* can grow on nearly any food. Chicken, eggs, and their derivative products are particularly high risk.

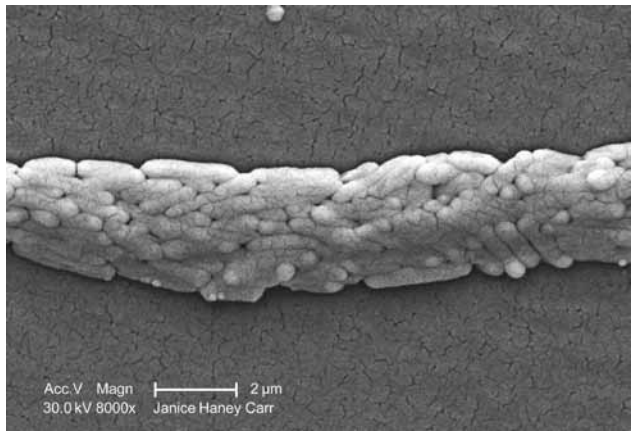

Figure 2: Under a moderately-high magnification of 8,000X, this scanning electron micrograph (SEM) revealed the presence of a grouping of Gram-negative *Salmonella typhimurium* bacteria that had been isolated from a pure culture. (Source: CDC/ Bette Jensen)

Carbohydrate	Fermentation Acid	Fermentation Gas	Cat. No.
Adonitol	–	–	55876
Arabinose	+/–	+/–	80372
Cellobiose	–	–	56481
Dextrose	+	+/–	63367
Dulcitol	+/–	+/–	73044
Fructose	+/–	+/–	53901
Galactose	+	+/–	89608
Inositol	+/–	+/–	89614
Lactose*	–	–	28816
Maltose	+	+/–	77653
Mannitol	+	+/–	94438
Mannose	+/–	+/–	94445
Melibiose	+	+	93196
Raffinose	–	–	94226
Rhamnose	+/–	+/–	93999
Salicin	–	–	92971
Sorbitol	+	+/–	93998
Sucrose	–	–	94309
Trehalose	+	+/–	92961
Xylose	+	+/–	07411

Table 1: Typical carbohydrate fermentation ability of *Salmonella*

*not available in U.S.A.

Medium	Cat. No.
Nonselective Pre-enrichment Broth	
Buffered Peptone Water (ISO)	08105
Peptone Water, phosphate-buffered	94217
Peptone Water, phosphate-buffered	77187
Peptone Water, phosphate-buffered with Ferrioxamine E*	67331
Peptone Water, phosphate-buffered, Vegitone	40893
Universal Pre-enrichment Broth	91366
Selective Enrichment Broth	
Muller-Kauffmann Tetrathionate Broth, Base (ISO)	69965
Muller-Kauffmann Tetrathionate Novobiocin Broth	89176
Rappaport Vassiliadis Broth acc. to DIN EN ISO 6579:2002	04584
Rappaport Vassiliadis Broth, modified	17173
Rappaport Vassiliadis Medium	R0773
Rappaport Vassiliadis Medium (Base), modified, semi-solid*	92322
Salmonella Enrichment Broth*	84370
Selenite Broth (Base)	70153
Selenite Cystine Broth*	84922
TBG Broth*	86352
Tetrathionate Broth*	88151
Selective Differential Media (See also **Table 3**: Chromogenic Media)	
Bismuth Sulfite Agar	95388
Brilliant Green Agar, modified	B1801
Brilliant Green Agar, modified*	70134
Brilliant Green Phenol Red Lactose Sucrose Agar*	16026
DCLS Agar*	70135
DCLS Agar No. 2	90035
Deoxycholate Citrate Agar	D7809
Hektoen Enteric Agar	51490
Leifson Agar	61792
Salmonella Agar according to Önöz	84368
SS-Agar	85640
XLD Agar	95586
XLD Agar ISO 6579:2002	14781
XLT4 Agar (Base)	76721
Confirmation Media	
Andrade Peptone Water	A0715
Andrade Peptone Water, Vegitone	28943
Bromcresol Purple Broth	36408
Decarboxylase Broth Base, Moeller	D2935
HiCrome™ MM Agar	00563
Kligler Agar	60787
Lysine Decarboxylase Broth	66304
Lysine Iron Agar	62915
Motility Test Medium	M1053
Nitrate Broth	72548
OF Test Nutrient Agar*	75315
Semi-solid Nutrient Agar ISO 6579:2002	79890
SIM Medium*	85438
Simmons Citrate Agar	85463
Triple Sugar Iron Agar*	44940
Triple Sugar Iron Agar (acc. to ISO)*	92499
Urea Broth	51463

Table 2: *Salmonella* selective and differential media

*not available in U.S.A.

Microbiological control in the food industry plays a critical role in preventing *Salmonella* outbreaks and is probably an important reason for decreasing incidences in recent years. Tests and media used for identification of *Salmonella* take advantage of unique aspects of *Salmonella* physiology or biochemistry relative to other genera within the family Enterobacteriaceae. For example, bacteria from the genus *Salmonella* are mostly facultative anaerobes, oxidase-negative, catalase-positive and Gram-negative rods. Most strains are motile and ferment glucose with production of both acid and gas.

The media currently used for the differentiation and identification of *Salmonella* are still based on the detection of carbohydrate fermentation indicated by a pH indicator (see also **Table 1** for carbohydrate fermentation ability), the detection of proteolytic activity, hydrogen sulphide production and selectivity. Most modern media also combine some of this detection system to make the media more reliable. As selective agents, diverse components are taken such as bile salts, Tergitol-4, high concentrations of magnesium chloride, thiosulfate (inhibits coliforms), selenite (inhibits Gram-positives, coliforms and enterococci), brilliant green (suppresses coliforms), malachite green (inhibits the growth of naturally present intestinal flora but not *Salmonella*), Crystal violet (inhibits most Gram-positives, especially staphylococci) and novobiocin (inhibits Gram-positive bacteria). A listing of the most common enrichment, confirmation, and differential media appears in **Table 2**. For a typical example of microbiological control of food or water, please see the ISO methods illustrated in **Figures 3 and 4**. The ISO methods have a non-selective enrichment step with Buffered Peptone Water (BPW) followed by a selective enrichment in two different broths, and then the samples are plated out on selective differential agars. At the end, the result must be confirmed by biochemical and serological tests.

In addition to traditional media and tests there exist the chromogenic media, which make identification even more reliable and faster as they detect a characteristic enzyme of the *Salmonella*. These reactions are based on the cleavage of a chromogenic substrate which results in a visible color change. *Salmonella*, for example, is known to cleave indoxyl-α-galactoside by the α-galactosidase and indoxyl-fatty acid ester by the lipase (see **Table 3**).

Media	Cat. No.
HiCrome MM Agar*	00563
HiCrome RajHans Medium, Modified*	90918
HiCrome *Salmonella* Agar*	78419
HiCrome *Salmonella* Agar, Improved*	05538
Salmonella Chromogen Agar	84369
Salmonella Chromogen Agar Set	01993

Table 3: Chromogenic media for *Salmonella*

*not available in U.S.A.

Non selective Pre-enrichment	Sample → BPW 37 °C, 18 hrs (Annex D)	Buffered Peptone Water (ISO)	08105
Selective Enrichment	RVS Broth 41.5 °C, 24 hrs; MKTTn 37 °C, 24 hrs; MSRV 41.5 °C, 24 hrs (48 hrs if negative)	Rappaport Vassiliadis Broth, modified	17173
		Muller-Kauffmann Tetrathionate Broth, Base (ISO)	43052
		Rappaport Vassiliadis Medium (Base), modified, semi-solid	92322
Selective Isolation	XLD 37 °C, 24 hrs; A second media of choice *Salmonella* Chromogen Agar, BGA, XLT4, Hektoen, SS, etc.	XLD Agar	95586
		Salmonella Chromogen Agar Set	01993
		XLT4 Agar (Base)	76721
Confirmation	Nutrient Agar 37 °C, 24 hrs → Biochemical Test (e.g. TSI); Serological	Nutrient Agar No. 2	70116
		Triple Sugar Iron Agar (acc. to ISO)	92499
		Christensen's Urea Agar	27048
		Lysine Decarboxylase Broth	66304
		Lysine Iron Agar	62915

Figure 3: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp; ISO 6579:2002/A1:2007

Non selective Pre-enrichment	Sample (For volumes greater than 10 mL the water has to be filtered or alternatively use double strength BWP.) → 50 mi BPW 36 °C, 18 hrs	Buffered Peptone Water (ISO)	08105
Selective Enrichment	RVS Broth 41.5 °C, 24 hrs (+48 hrs if slow growing *Salmonella*); MKTTn 36 °C, 24 hrs	Rappaport Vassiliadis Broth, modified	17173
		Muller-Kauffmann Tetrathionate Broth, Base (ISO)	43052
Selective Isolation	XLD 36 °C, 24 hrs; A second media of choice *Salmonella* Chromogen Agar, BGA, XLT4, Hektoen, SS, etc.	XLD Agar	95586
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		Triple Sugar Iron Agar (acc. to ISO)	92499
		Christensen's Urea Agar	27048
		Lysine Decarboxylase Broth	66304

Figure 4: Water quality – Detection of *Salmonella* spp.; ISO 19250:2010

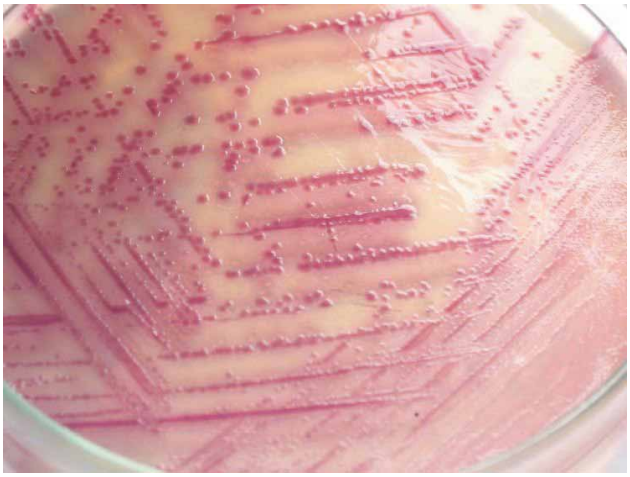

Figure 5: *Salmonella entericia* serotype Typhimurium on HiCrome™ *Salmonella* Agar, Improved

Salmonella is also a pathogen which sometimes counts as "**viable but nonculturable**" (VNC) bacteria. That means they cannot normally be cultured, but in the case of *Salmonella*, we talk about injured cells which are not immediately culturable and not with the standard media. According to the latest VNC definition, VNC cells are regarded as viable and potentially replicative, but the methods required for resuscitation are beyond our current knowledge. In certain food products, *Salmonella* have undergone treatments such as heating, drying, setting under high osmotic pressure (high salt content) or contact with inhibiting chemicals. The end result of the treatment is sensitive cells or sub-lethally damaged cells, which can mean the loss of some ribosomes, damaged enzymes, cell membranes and other problems causing malfunctions in cells.

For *Salmonella*, it has been shown in recent years that supplementing the pre-enrichment and enrichment broths with an iron complex, called ferrioxamine E, significantly improves the recovery of *Salmonella* from artificially or naturally contaminated foods.[3-4] A concentration of 75 ng/mL ferrioxamine E (Cat. No. **38266**) or Peptone Water, phosphate-buffered with ferrioxamine E (see **Table 2**) improves the recovery rate and supports growth. Ferrioxamine E provides the essential micro-nutrient iron (III) to the organisms. This leads to a reduced lag-phase in the medium and reactivates damaged bacteria. The motility of *Salmonella* is also improved, which helps to improve the identification by semisolid selective motility media like MRSV, DIASSALM or SMS. It is recommended when isolating small quantities of cells from dried powders like tea, spices, dried fruits, etc.

References

1. María Lara-Tejero, Junya Kato, Samuel Wagner, Xiaoyun Liu and Jorge E. Galán. A Sorting Platform Determines the Order of Protein Secretion in Bacterial Type III Systems. Science, 3 February 2011.
2. A.J. Grant, F.J.E. Morgan et al., Attenuated *Salmonella* Typhimurium Lacking the Pathogenicity Island-2 Type 3 Secretion System Grow to High Bacterial Numbers inside Phagocytes in Mice, PLoS Pathog., 8(12), December 2012.
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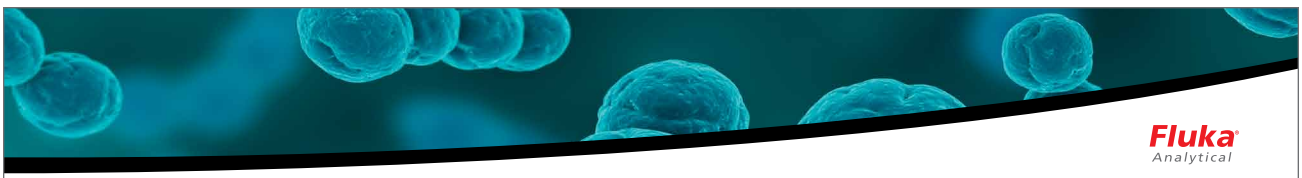
4. J.C. Choa, S.J. Kim, Viable, but Non-culturable, State of a Green Fluorescence Protein-tagged Environmental Isolate of *Salmonella typhi* in Groundwater and Pond Water, FEMS Microbiol. Lett., 170:257–264 (1999).



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RTC is a Sigma-Aldrich company producing certified reference materials (CRM) of microorganisms. The Vitroid technology stabilizes the microorganism to an extent never achieved by commercial suppliers. This technology utilizes an innovative disk that enables certification of the cfu level.

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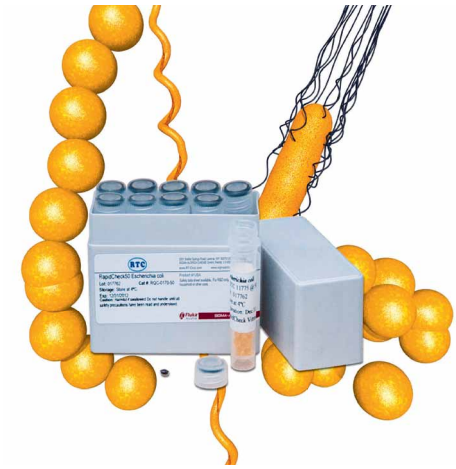

For more information and a complete listing of the certified test strains see
sigma-aldrich.com/vitroids

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The Filamentous Alga *Zygnema* – An Indicator for Clean Water

By R. Bachofen, H. Brandl, F. Schanz, Mikroskopisch klein, aber doch sichtbar! Ein Feldführer für Mikroorganismen. Neujahrsblatt der Naturforschenden Gesellschaft in Zürich, Bd. 209, 148 pp. — *helmut.brandl@ieu.uzh.ch*

Microscopic but Visible, Algae are a Natural Indicator for Water Quality.

In many villages of the Swiss Alps you might observe, mainly in spring or early summer, algae living in freshwater fountains (**Figure 6**). What, at first glance, seems to be an algal bloom due to water pollution is in reality an indicator of clear and nutrient-poor water. Green algae of the genus *Zygnema* are growing as large, light green, free-floating, fluffy flocks or balls. These flocks are slimy mass aggregations of unbranched algal filaments consisting of several thousand cylindrical cells with a diameter of approximately 20 µm (**Figure 7**). In every cell, two star-shaped chloroplasts flanking the nucleus are visible. Due to their morphology, the chloroplasts have a highly specific surface (**Figure 8**). Small oxygen bubbles resulting from photosynthesis get entangled in the slimy algal network and are responsible for the flotation of the biomass. Cell divisions follow a day/night cycle: Division of cell nuclei occurs during the night, whereas longitudinal growth of the cells and filaments takes place during the day. *Zygnema* needs quiet, clear, cold and nutrient-poor freshwater (Bachofen et al. 2006).

Figure 6: Fountain in the Village of Zuoz (Engadin Valley, Canton of the Grisons, Switzerland).

Figure 7: Fluffy Filaments of the Green Alga *Zygnema* (100 fold magnification).

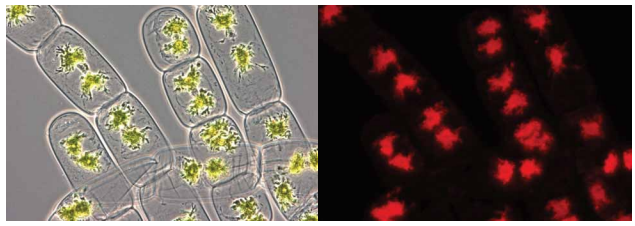


Figure 8: Each cell of *Zygnema* contains two star-shaped chloroplasts flanking the cell nucleus (left, 400 fold magnification). Chloroplasts show red autofluorescence under UV illumination (right).

Media for Algae and Cyanobacteria

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

Who is not familiar with the nice green or turquoise color of a lake or fountain? Depending on other parameters and the species found, it is either a sign of intact ecosystem or it can signal over eutrophication. There are diverse studies of cyanobacterial toxicity, and the cyanotoxins have been identified and their mechanisms of toxicity established. In contrast, toxic metabolites from freshwater algae have scarcely been investigated, but toxicity has been shown for freshwater species of Dinophyceae and also the brackish water Prymnesiophyceae and an ichthyotoxic species, *Peridinium polonicum*, has been detected in European lakes (Pazos et al., in press; Oshima et al., 1989). As marine species of these genera often contain toxins, there is a chance of finding toxic species among these groups in fresh water as well.

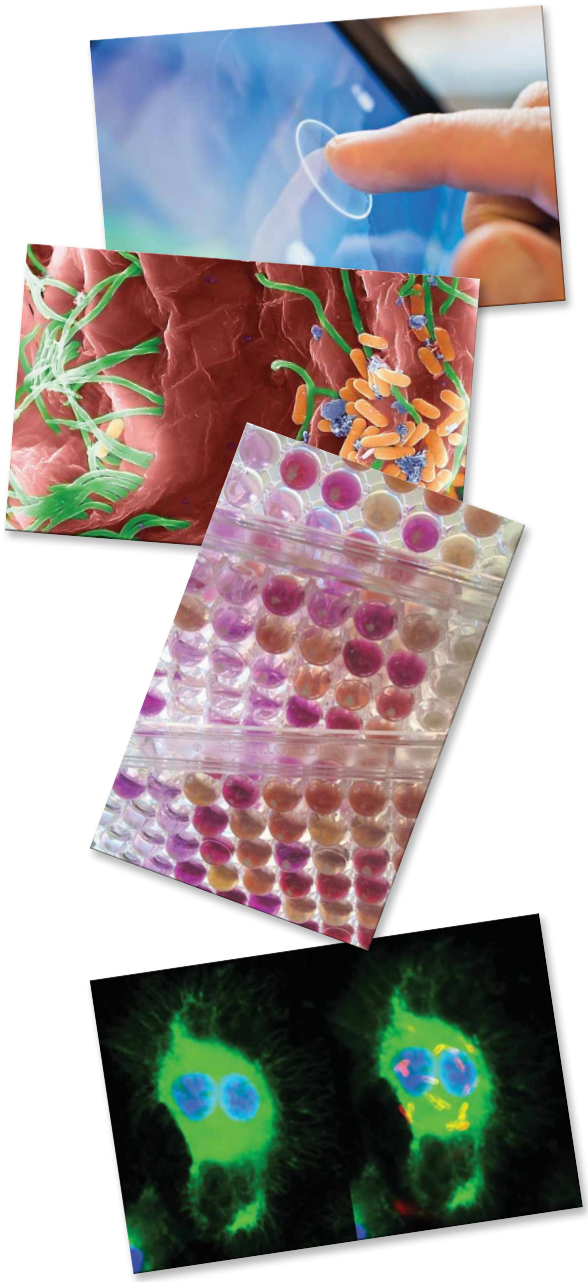
For analysis and study of algae and cyanobacteria, Sigma-Aldrich® produces media especially suited for these organisms.

Media	Description	Cat. No.
Algae Culture Broth	For the isolation and cultivation of algae from soil, water and sewage.	**17124**
BG11 Broth	Universal medium for the culture and maintenance of cyanobacteria.	**73816**
Trace Metal Mix A5 with Co	A supplement recommended for the culture and maintenance of cyanobacteria	**92949**

Table 4: Media for Algae and Cyanobacteria

Win an Android™ Tablet in the 2013 Fluka® Microbiology Photo Competition

This photography competition is sponsored by Sigma-Aldrich with the aim of encouraging microbiologists to promote some aspect of their work or their field of research. The best photographic entries with the best description of the photograph's subject will win prizes such as a tablet PC, MP3 player, a USB flashdrive, a laser pointer, and a mini Swiss army knife. The winning images will be published in Microbiology Focus and the best one will have the distinction of being featured on the cover.



Rules of the Competition and Conditions of Entry

1. The competition is open to all residents worldwide.
2. Entries should illustrate any microorganisms (living or dead) or a microbiologist in action at work.
3. Picture size should be at least 400 dpi and 90 x 120 mm (max 5 MB). The file format **must** be in jpg, tiff or pdf.
4. The entries will be judged on:
   - clarity of presentation
   - composition
   - illumination and contrast
   - congruency of subject matter and title of photograph
   - scientific interest and relevance
   - originality
5. Winning entries will be retained by Sigma-Aldrich, who will have sole rights of publication, reproduction and display.
6. Closing date for contest entries will be August 15th, 2013.
7. Entries received after the closing date will not be considered. Entries received incomplete, illegible, mutilated, altered or not complying exactly with the instructions and theme may be disqualified.
8. Decisions of the judges in all matters affecting the competition will be final and legally binding.

The competition will be judged by:

Dr. Lars Fieseler, Zurich University of Applied Sciences – ZHAW, Supervisor, Department Microbiology

Prof. Mohammad Manafi, Medical University of Vienna, Head of the Department for Food Hygiene

Jvo Siegrist, Sigma-Aldrich, Product Manager, Microbiology

Method of Entry

There is no entry fee, but an entry form must be completed for each entry (a maximum of two entries may be submitted).

Entry forms are available at
www.sigma-aldrich.com/fluka-mibi-competition

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