



## Instructions for Use

# CAMP SPOT TEST REAGENT

Cat. no. <a href="#">Z206</a>	CAMP Spot Test Reagent, 2ml Cryogenic Vial, 1ml Fill	20 vials/box
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## INTENDED USE

Hardy Diagnostics CAMP Spot Test Reagent is used to rapidly determine an organism's ability to produce the protein CAMP factor. CAMP factor is a diffusible, extracellular protein which acts synergistically with staphylococcal beta-hemolysin (beta-lysin). The test is particularly useful as part of the identification of *Streptococcus agalactiae* (group B streptococci), and *Listeria monocytogenes*.<sup>(1,7,8)</sup>

## SUMMARY

The CAMP test (named for the original authors: Christie, Atkins, and Munch-Petersen) was first used in the identification of group B streptococci (GBS). Group B streptococci secrete a protein called CAMP factor or "protein B" that interacts with the beta-hemolysin produced and secreted by *Staphylococcus aureus*, this results in enhanced or synergistic hemolysis.<sup>(4,13)</sup>

A variety of methods are currently available to identify GBS isolated from clinical specimens. These methods can be time consuming and/or expensive compared to the CAMP spot test. Hardy Diagnostics CAMP Spot Test Reagent is used as a rapid CAMP test method. The reagent, containing staphylococcal beta-lysin (also called beta-toxin, beta-hemolysin, or beta-staphylolysin), acts directly with the CAMP factor that is diffused into the medium around the suspect colony. The beta-lysin has a synergistic effect in the presence of CAMP factor, producing enhanced hemolysis of sheep erythrocytes. Enhanced hemolysis is visible within 30 minutes to one hour of placing a drop of CAMP Spot Test Reagent next to an isolated beta-hemolytic colony.

Group B streptococci (GBS) are associated with a broad spectrum of clinical syndromes, including neonatal sepsis and meningitis. It is the current CDC recommendation that laboratories culture for the organism during the latter part of the prenatal period, as part of an effort to prevent neonatal GBS infections.<sup>(14)</sup>

Of the *Listeria* species, *L. monocytogenes* is the only one that has been clearly documented as a human pathogen and is one of the few species of *Listeria* that is CAMP factor positive. *Listeria monocytogenes* has been associated with sepsis and meningitis in elderly and other immunocompromised persons. It can cause a flu-like illness in pregnant females, and, if untreated, can infect the fetus with serious sequelae.<sup>(1)</sup> Contaminated foods, such as dairy products, coleslaw, meat, etc. are the primary vehicle of infections.<sup>(3,10)</sup>

Production of the CAMP factor is indicative of group B streptococci and *Listeria monocytogenes*, when combined with the appropriate colony morphology, gram reaction and catalase test result.<sup>(1,7)</sup> In addition to performing the CAMP spot tests on hemolytic colonies on sheep blood agar, the test can be performed on non-hemolytic strains of GBS and on GBS growing on Columbia CNA with sheep blood agar. The CAMP spot test can also be performed on the same blood agar plate with the Bacitracin and SXT disk tests.<sup>(4)</sup> Since the GBS colony is not disturbed during the spot test, the colony can be used for additional testing.

## FORMULA

CAMP Spot Test Reagent contains a portion of filtrate from a culture of beta-lysin producing *Staphylococcus* species.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at  $\leq -20^{\circ}\text{C}$ ., away from direct light. Defrosted reagent can be stored in the refrigerator at  $4-6^{\circ}\text{C}$ . for up to two weeks. Do not refreeze.<sup>(7)</sup> Reagent should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed. Product is light and heat sensitive; protect from light and excessive heat.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at [www.cdc.gov/ncidod/dhqp/gl\\_isolation.html](http://www.cdc.gov/ncidod/dhqp/gl_isolation.html).

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

## PROCEDURE

The CAMP spot test can be performed on a single colony from a primary isolation sheep blood agar plate or Columbia CNA with sheep blood. The test can be done as soon as a colony becomes visible on the plate, and as long as the colony has been growing for at least 18 hours.<sup>(10)</sup>

1. Remove one vial from the freezer and allow it to thaw.
2. Using a pipette, place one drop of CAMP Spot Test Reagent next to a characteristic colony grown for 18-24 hours on a Blood Agar plate (Cat. no. A10) or Columbia CNA plate (Cat. no. A50). The liquid may touch or even engulf the colony.

**Note:** Manufacturers of commercially prepared culture media are not required to verify that Columbia CNA with sheep blood will provide a positive CAMP reaction. Users should test each lot of Columbia CNA with a positive and negative control prior to using the media for the CAMP Spot Test.<sup>(12)</sup>

3. Incubate the plate aerobically or in 5-10% CO<sub>2</sub> at 35°C. (right side up so that the reagent won't run over the plate's surface) for 20 to 30 minutes.

4. Observe, using transmitted light, for an arc or circle of enhanced hemolysis next to the colony. If reaction is negative, reincubate for an additional 30 minutes.

**Note:** Refrigeration after the initial incubation period may enhance the reaction.

## INTERPRETATION OF RESULTS<sup>(7)</sup>

A positive result is indicated by the presence of a clear zone (arc or circle) of enhanced hemolysis. Enhanced hemolysis only where the diffused, slight hemolysis overlaps is considered a positive reaction.

A negative result will show no areas of enhanced hemolysis near the colony in the presence of the CAMP Spot Test Reagent.

CAMP test reactions for *Listeria* species:<sup>(8)</sup>

	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. seeligeri</i>	<i>L. welshimeri</i>	<i>L. ivanovii</i>	<i>L. grayi</i>	<i>L. murrayi</i>	<i>L. denitrificans</i>
CAMP test ( <i>S. aureus</i> )	+	-	+	-	-	-	-	-

\* *Listeria monocytogenes*, ATCC® 15313, is atypical with respect to its CAMP reaction compared to other strains and does not give a positive CAMP reaction with *S. aureus*.<sup>(8)</sup>

## LIMITATIONS

The CAMP test should not be used alone, but rather, in combination with the appropriate colony morphology, gram reaction, and other biochemical tests on colonies from pure culture for complete identification.

Extended incubation times or elevated incubation temperatures may give false-positive results.

Interpretation of the CAMP spot test can be affected by excessive agar depth. Plate depth of approximately 1.5mm has been recommended.<sup>(6)</sup>

It is recommended that CAMP spot test be used in conjunction with the Centers for Disease Control (CDC) protocol for processing clinical specimens when testing for group B streptococci.<sup>(14)</sup>

Use sheep blood agar plates only. Human, horse, rabbit, or guinea pig blood plates will not give a proper reaction.<sup>(6)</sup>

*L. ivanovii* only shows a positive CAMP reaction when using an alternative CAMP test method, in which *Rhodococcus equi* replaces *S. aureus*.<sup>(8)</sup>

A small percentage of group A streptococci will have a positive CAMP reaction.<sup>(4)</sup> The test should only be performed on colonies that have the morphology of group B streptococci (gray to translucent colonies which have a narrow zone of beta-hemolysis with a gram stain and catalase reaction indicative of streptococci). The PYR Test (Cat no. Z75) may be used to further differentiate group A streptococci from group B.

Colonies of *Listeria monocytogenes* have a narrow zone of beta-hemolysis on sheep blood agar and may be confused with group B beta-hemolytic streptococci, if catalase and gram stain are not performed.<sup>(3)</sup>

The presence of beta-antitoxin in some batches of sheep blood will inhibit staphylococcal beta-toxin production; therefore QC of CAMP Spot Test Reagent is recommended each lot of sheep blood agar. The user should verify that the manufacturer has tested each lot of media for the CAMP reaction, or alternatively, perform quality control in-

house for each lot of media.<sup>(6)</sup>

Only colonies that have been growing for at least 18 hours should be tested with the spot test. Colonies only 12 hours old can give false-negative results, presumably because the colony may not yet have produced adequate amounts of CAMP factor in the synergistic hemolysis. Also, if the test is performed on GBS colonies that are on sheep blood agar that have been incubated for more than 48 hours, the zone of synergistic hemolysis may be more difficult to interpret if darkening of the blood medium has occurred.<sup>(10)</sup>

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL (7,10)

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Reaction
<i>Streptococcus agalactiae</i> ATCC ® 13813	Enhanced hemolysis present; CAMP positive
<i>Streptococcus pyogenes</i> ATCC ® 19615	Enhanced hemolysis absent; CAMP negative
No colony (To verify that auto-hemolysis will not occur)	No hemolysis

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

## PHYSICAL APPEARANCE

CAMP Spot Test Reagent should appear clear, and light amber in color.

## REFERENCES

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