Department of Microbiology
Superoxol Test Procedure

I. Purpose and Test Principle

The superoxol test is a useful test for the rapid, presumptive identification of *Neisseria gonorrhoeae*. *N. gonorrhoeae* strains produce immediate, brisk bubbling when the colony material is exposed to the reagent (30% hydrogen peroxide) on a glass slide. The other *Neisseria* species that grow on selective media generally produce weak, delayed bubbling, although some isolates of *N. meningitidis* may also produce a strong reaction similar to gonococcus. Isolates of oxidase-positive, gram-negative diplococci that are recovered from urogenital sites and that grow on selective media may be presumptively identified as *N. gonorrhoeae*. The superoxol test provides an additional presumptive test for identifying these isolates, particularly when isolated from non-genital sites in adults or from any body site children. Confirmatory identification tests are necessary for all isolates.

II. Specimen Information

Test isolates should be 18 to 24 h old unless additional incubation is required for growth.

III. Reagents & Equipment

- 30% hydrogen peroxide (H_2O_2) – store in cool (< 35°C), well-ventilated dark area.
  Caution! 30% H_2O_2 is extremely caustic to skin. Gloves should be worn when performing this test. If skin contact occurs flush immediately with water for at least 15 min. See Hydrogen peroxide 30% MSDS for additional first aid information.
- Glass slides
- Wooden applicator sticks
- Transfer pipettes

IV. Procedure

A. Under a biosafety cabinet, using a wooden applicator, touch the center of an 18 to 24 h, well-isolated colony to a clean glass slide. Be sure the material is visible to the naked eye on the slide.
B. Place one drop of peroxide reagent on the slide and observe immediately for effervescence.

V. Interpretation and Reporting

A. Positive Test
   A positive test shows the immediate formation of brisk bubbling.
B. Weak Test
   A weak reaction is indicated by delayed, slow bubbling.
C. Negative Test
   A negative test shows no bubbles or a few bubbles after 20 s.

VI. Quality Control

Perform QC on each new lot or shipment of reagent with the following control strains prior to use. Additionally, inexperienced users should perform QC as a comparison reference when evaluating clinical isolates.

Control strain	Expected Results
Neisseria gonorrhoeae ATCC 43069	Positive: immediate brisk bubbling
Neisseria lactamica ATCC 23970	Weak: delayed, slow bubbling
Streptococcus pyogenes ATCC 19615	Negative: no bubbling

VII. Limitations

A. Testing is ideally performed from chocolate agar. *Neisseria* species typically grow better on chocolate agar and testing from blood agar may lead to false-positive results due to the catalase in red blood cells.
B. The enzyme is only present in viable colonies. Colonies older than 24 h should not be used for testing to avoid false-negative results.
C. Do not reverse the order of adding the reagent to the colony and do not mix the reagent and the colony.

VIII. Verification of Test Method

A total of 8 test strains were used to evaluate the superoxyl test. This included *N. gonorrhoeae* ATCC 43069, a *N. gonorrhoeae* strain from the 2010 CAP survey, *N. lactamica* ATCC 23970, *N. sicca* ATCC 9913, a *N. cinerea* clinical isolate, *M. catarrhalis* ATCC 25238, and a *M. catarrhalis* clinical isolate. *Streptococcus pyogenes* ATCC 19615 was used as a negative test strain. Both *N. gonorrhoeae* strains produced strong positive reactions that appeared as an immediate mound of foaming bubbles. The other *Neisseria* species all produced relatively slower and weaker reactions. Both of the *M. catarrhalis* strains produced reactions equivalent to the gonococcus isolates. However, *M. catarrhalis* is easily distinguished from gonococcus based on other characteristics (lack of growth on selective media, colony morphology, butyrate esterase production, lack of carbohydrate utilization, etc.). The *S. pyogenes* control strain did not produce any bubbles. These reactions are all consistent with published data.

IX. References

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B. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.
C. CDC website: Identification of *N. gonorrhoeae* and Related Species

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