Gamma N-HANCE®

Low-Ionic-Strength Additive Solution for Antibody Detection Tests

IVD Rx ONLY

1°C 10°C

Harmful, Preservative: 0.1% Sodium Azide

Do not use if turbid. No U.S. Standard of Potency

CAUTION: THE PACKAGING OF THIS PRODUCT (DROPPER BULB) MAY CONTAIN DRY NATURAL RUBBER

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Low-Ionic-Strength Additive Solution for Antibody Detection Tests

IMMUCOR

INTENDED USE: Gamma N-HANCE is intended for use as a low-ionic-strength additive solution for antibody detection tests.

SUMMARY OF THE TEST: It is well established that incubation of serum or plasma samples and red blood cells in a reduced ionic environment increases the rate at which blood group antibodies bind to their specific antigen receptor sites on the red blood cells [1,2,3,4,5]. The first practical application of this principle in routine antibody detection was reported in 1974 by Löw and Messeter [6], who proposed the use of a low-ionic-strength solution at an optimum sodium chloride molarity of 0.03, instead of physiologic saline, as the suspending medium for red blood cells in antibody test systems. Several studies [7,8,9,10,11,12] have confirmed that the procedure enhances antibody reactions, especially at the indirect antiglobulin phase of testing, without a significant increase in the incidence of non-specific reactions.

A low-ionic-strength test environment can be achieved either by suspending the red blood cells in an isotonic low-ionic-strength solution as originally reported in Löw and Messeter [6], or by using an additive reagent with a conventional mixture of serum and red blood cells in physiologic saline or some other normal ionic strength suspending medium. Some low-ionic-strength additive reagents (of which Gamma LO-ION™ is an example) contain macromolecular additives to potentiate direct agglutination of antigen-positive red blood cells by some immune antibodies, while others rely solely on the low-ionic-strength conditions to enhance uptake of antibody and thereby to facilitate detection at the antiglobulin phase of testing. This product belongs to the latter category of reagent, as macromolecular potentiators are not present.

PRINCIPLE OF THE TEST: The use of Gamma N-HANCE, as an additive for tests to detect blood group antibodies, creates a low-ionic-strength test environment that increases the rate of antibody uptake during incubation, thereby enabling incubation time to be shortened without loss of sensitivity.

REAGENT: Gamma N-HANCE is a modified low-ionic-strength solution containing glycine at an iso-osmotic concentration and a surfactant to improve resuspension of the red blood cells after centrifugation. When the product is used by the recommended test procedure, the ionic strength of the test system is approximately equivalent to that achieved by mixing equal proportions of serum and red blood cells suspended in the low-ionic-strength solution (LISS) proposed by Löw and Messeter [6]. Contains 0.1% sodium azide as a preservative.

PRECAUTIONS:
For in vitro diagnostic use. Store at 1° to 10°C when not in use. Do not freeze. Do not dilute. Do not use beyond the expiration date. Effort should be made to minimize contamination during use of the product. Do not use if turbid.

This reagent contains 0.1% sodium azide.
Warning: H302 Harmful if swallowed.

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.
CAUTION: The packaging of this product (dropper bulb) may contain dry natural rubber.

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

SPECIMEN COLLECTION AND PREPARATION: No special preparation of the patient is required prior to specimen collection. Blood should be drawn by aseptic technique and the serum or plasma should be tested as soon as possible. If delay in testing should occur, specimens for compatibility testing and antibody detection should be stored at 1° to 10°C. In the case of potential blood transfusion recipients, the specimen should be stored for no longer than is permitted by the relevant regulatory agencies.

PROCEDURE:
Materials Provided: Gamma N-HANCE
Additional Materials Required: Test tubes (12×75 mm or 10×75 mm), pipettes, isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5, 37ºC incubator or waterbath, timer, centrifuge, an optical aid such as a hand lens, a concave mirror or a microscope, Anti-Human Globulin containing anti-IgG, and IgG-sensitized red blood cells.

TEST METHOD:
The use of Gamma N-HANCE by the following test procedure yields an incubating mixture having an ionic strength of approximately 0.1M, assuming all drops to be of equal volume. This is equivalent to the ionic strength achieved in the LISS procedure described by Löw and Messeter [6]. A blood bank dropper or a calibrated pipette device may be used. If the volume of serum is increased in order to improve the serum-to-cell ratio, then the ionic strength of the test mixture will be somewhat greater than 0.1M, and sensitivity may be impaired, unless the volume of Gamma N-HANCE added at step 4 is adjusted to correspond to the volume of serum placed in the tube at step 2. If the drops of serum (step 2) are smaller in volume than drops of Gamma N-HANCE (step 4), the number of serum drops may be increased to compensate for the discrepancy. For example, if a vial dropper delivers approximately 20 drops to the milliliter, and serum is added to the test system using a pipette that delivers approximately 30 drops to the milliliter, three drops of serum will be required to achieve an equivalent volume to two drops of Gamma N-HANCE.

1. Label an appropriate number of test tubes for each red blood cell suspension to be tested.
2. Place 2 drops of the serum or plasma to be tested into each of the tubes.
3. Add 1 drop of the appropriate red blood cell suspension, previously washed and resuspended in saline to approximately 3-4%, to each appropriate tube. Reagent Red Blood Cells may be used directly from the vial, or in accordance with the manufacturer's directions. *NOTE: Do not suspend the red blood cells directly in Gamma N-HANCE, as the product is designed to yield the required ionic strength when added to serum or plasma and red blood cells in the proportions noted in these directions.*
4. Add 2 drops of Gamma N-HANCE and mix well. *NOTE: The addition of Gamma N-HANCE may be postponed until immediately before the incubation phase at step 8 of this procedure. This is recommended in the context of crossmatching, as the detectability of anti-A and anti-B may be impaired by incubation at 37ºC in a low-ionic-strength medium [13].*
5. Centrifuge for:
   (a) 1 minute at 1,000 rpm (rcf 100 to 125) or
   (b) 15 seconds at 3,400 rpm (rcf 900 to 1000) or
   (c) a time appropriate to the calibration of the centrifuge.
6. Examine for hemolysis and record if present.
7. Resuspend the red blood cells by gently shaking and examine for agglutination. Record results.
8. Incubate the tubes at 37º±1ºC for 10 minutes. The incubation time may be extended up to 30 minutes. Incubating for the upper end of the time range may enhance reactivity.

Key:
Underline = Addition or significant change ▲ = Deletion of text

9. Repeat steps 5 through 7.
10. Wash the red blood cells in the tube at least 3 times with the tubes full of saline, being careful to decant the saline between washes and to resuspend the red blood cells thoroughly when adding saline for the next wash. Decant the saline completely following the last wash.
11. Add 1 or 2 drops of Gamma-clone® Anti-Human Globulin to each 'dry button' of red blood cells or follow the directions of the Anti-Human Globulin manufacturer. Adding 2 drops of AHG may enhance reactivity.
12. Mix well and centrifuge as described in step 5.
13. Resuspend the red blood cells by gentle shaking and examine for agglutination. Negative reactions may be examined with an optical aid. Record results.

Stability of Reaction: The washing phase of the antiglobulin test must be carried out without interruption, and test results must be interpreted immediately upon completion of the test.

QUALITY CONTROL:

1. All negative antiglobulin tests should be confirmed by adding IgG-sensitized red blood cells, such as Checkcell®, then repeating centrifugation and reading. A positive test result at this point confirms that active antiglobulin (anti-IgG) was added to the test system and was present when the original antiglobulin test was interpreted as negative.
2. To control for the efficacy of the product in enhancing reactivity, it is suggested that a weak IgG antibody should be selected (or prepared by diluting a stronger one in inert human serum or equivalent) and tested at suitably regular intervals. corQC® may be used for quality control testing of this product.
3. An autologous control (patient serum or plasma plus own red blood cells) is recommended for compatibility and antibody identification tests.

INTERPRETATION OF TEST RESULTS: Agglutination and/or hemolysis of the red blood cells in the immediate-spin or 37°C incubation phases of testing, or agglutination occurring at the antiglobulin phase, constitutes a positive test result and indicates that the specimen being tested contains antibodies directed at an antigen or antigens present on the red blood cells.

No agglutination or hemolysis at any phase of testing constitutes a negative test result and indicates that the specimen being tested does not contain antibodies directed at antigens present on the red blood cells, as determined by this test method.

A positive autologous control test indicates the presence of an autoantibody. Further studies will be required to assure that alloantibodies are not also present in the serum.

LIMITATIONS: As in all serological procedures, such factors as contaminated materials, improper incubation time, temperature, centrifugation, examination for agglutination, and deviation from the recommended test procedures may give rise to false results. In addition:

1. Some cold antibodies requiring room temperature incubation may not be optimally reactive under the conditions of the recommended test procedure.
2. The recommended test procedure for this product requires that the volume of Gamma N-HANCE used in each test should be equal to the volume of serum being tested, in order to achieve the desired final ionic strength of 0.1M. This molarity was determined by Löw and Messeter [6] to yield acceptable sensitivity when incubation time was shortened to five minutes. Deviation from these proportions, either by using drops of unequal volume, or by increasing the number of drops of serum without a corresponding increase in the drops of Gamma N-HANCE, may alter the sensitivity of the test.
3. Unlike some low-ionic-strength solutions (LISS), Gamma N-HANCE is not intended as a red blood cell-suspending medium, but should be used as an additive to mixtures of serum and red blood cells as detailed in the directions for use.
4. The use of aged serum or plasma may result in failure to detect complement-dependent antibodies.
5. In using Gamma N-HANCE for crossmatching, an immediate-spin phase before adding the reagent is recommended to avoid missing some examples of anti-A and anti-B.

SPECIFIC PERFORMANCE CHARACTERISTICS: When used in accordance with the recommended test procedure, Gamma N-HANCE improves the rate of association between many blood group antibodies and their corresponding antigens, thus enabling incubation times to be shortened. Since this product contains no macromolecular substances, it does not generally potentiate direct agglutination by IgG antibodies. Each lot is tested serologically with selected antibodies and with inert sera to assure optimal performance; and pH, electrical conductivity and osmolarity are measured to maintain consistency from lot to lot.

The performance of this product is dependent on adhering to the recommended methods found in this insert. For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

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