

General & use information

For the demonstration of intracytoplasmic reduction of nitroblue tetrazoleum (NBT) in neutrophils.

Principle of test

Normal activated phagocytes ingest and reduce NBT to a blue-black intracytoplasmic precipitate using the O₂-generated in the respiratory burst. As respiratory burst activity is necessary for this reaction, cases with conditions in which the respiratory burst is lacking (e.g: CGD) are unable to reduce NBT.

Heparin blood is incubated with NBT reagent. After incubation smears are prepared, counterstained and examined microscopically for intracytoplasmic deposits of blue-black formazan (reduced NBT).

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Reagents (for a minimum of 25 tests)

- 1 - NBT Vials x 5 (each makes 1 ml of reagents (5 tests minimum per vial)
- 2 - Heparin vials x 25
- 3 - Siliconized vials x 25
- 4 - Stimulant preparation x 2
- 5 - Counterstain 100ml

Reagent components in this test are for 'In-vitro' diagnostic use only. No liability will be accepted for misuse of these products. Standard precautions in the handling of laboratory reagents should be observed.

Storage & stability

Store NBT Vials in refrigerator in darkness 2-6°C.

Store stimulant in refrigerator 2-6°C.

Store all other items at room temperature in darkness.

Reagent preparation and set-up

- 1) Add 1.0ml of distilled water to one NBT vial allow to stand for 1-2 minutes then shake to dissolve completely. Store in refrigerator and use within 24 hours.
- 2) Add 1.0ml of distilled water to stimulant vial, shake to dissolve. Use immediately or freeze to store and use later. Reagent may be thawed and refrozen many times.

Specimen collection & storage

Use first samples (<2 hours). Store untested samples in refrigerator. Use plastic syringe for blood collection, remove needle and dispense 1ml of blood into one of the heparin vials provided. Replace cap and mix well by tilting and rolling for 30 seconds.

All blood samples should be considered infectious take all appropriate precautions.

Technique

- 1 - Use a plastic pipette to transfer 0.12ml of NBT reagent to a clean vial provided with the kit.
- 2 - Add 0.2ml heparin blood prepared earlier, replace cap, tilt vial and mix well by rolling between the fingers. Do not invert to mix. Take care to avoid mechanical damage to the m cells.
- 3 - Incubate at 37°C for 10-12 minutes. *Remove, roll the vial gently to mix. Allow to stand at room temperature (18-26°C) for a further 10-12 minutes.
- 4 - Gently resuspend the mixture by *fitting the vial and rolling between the fingers for 10 seconds.
- 5 - Transfer 50-70 μ l of mixture onto a clean glass slide. Care: to avoid mechanical damage to cells at this stage.
- 6 - Carefully prepare fairly thick smear. Avoiding mechanical damage is crucial at this stage as cells containing formazan deposits are fragile.
Allow smear to dry completely in air before proceeding.
- 7 - To dry smear add 1ml counterstain, ensure smear is covered.
After 1 minute add 1ml deionised water mix gently by tilting slide, allow to stand for 30-60 seconds to achieve light staining. [Adjust this staining time to suit requirement].
- 8 - Rinse with tap or deionised water and blot or air dry and examine.

Examination

Use oil immersion objective and count a minimum of 100 neutrophils. Record as positive those cells staining formazan deposits.

Formazan deposits occur as:

- Dark blue/purple to black irregularly shaped intracellular deposits, occasionally seen as diffusely granular.
- Guidelines for the count [After R.D.Feigin] Count only neutrophils and include in the count only those cells that are:
 - Whole with cell membrane intact.
 - Solitary – do not count neutrophils found in a clump or with cellular material in contact except red cells.
 - Positive neutrophils shall contain formazan deposits as large irregular shaped, *discrete masses.
 - Having counted a minimum of 100 neutrophils calculate the percentage positive.

[For the most accurate determination of NBT % positive figure conduct this test concurrently with total white cell count and differential].

Technique – Stimulated

This test may be carried out concurrent with or after the normal test.

- 1 - Use a plastic pipette to transfer 0.1ml of NBT reagent to a clean vial provided with the kit.
- 2 - Add 0.05ml heparin blood and 5 μ l stimulant reagent. Replace the cap, tilt vial and mix by rolling gently between the fingers. Do not invert to mix. Take care to avoid mechanical damage to the white cells.
- 3 - Continue through stages 3-8 for the technique for 'normal' blood above. Follow by examination and counting as discussed above.

Quality Control

Carry out both a 'normal' and 'stimulated' test to certify that the reagent system is functioning as expected. The 'stimulated' test should demonstrate an elevated level of formazan containing cells. If this is not the case the reagent should be considered deteriorated and a fresh lot made up and tested again.

Each laboratory should establish its own normal range.

Expected observations

Normal range	2-17% Formazan positive cells
Mean value	9% Formazan positive cells

Most report typical mean value of 10% or less for formazan containing neutrophils in healthy individuals providing normal metabolic function.

It is generally accepted that percentage of positive neutrophils is increased during bacterial infection.

Further information in this reagent along with references can be provided by request.

Material safety data section

Reagents in this kit are irritating to eyes and sensitive skin and by inhalation. The counterstain is FLAMMABLE and TOXIC contains Methanol. Keep away from sources of ignition. Nitroblue Tetrazoleum is HARMFUL / TOXIC.

Do not consume the reagents. Wear suitable protective clothing i.e. skin, eyes and face protection. Wear appropriate dust / vapour mask. In all cases take off contaminated clothing and wash with plenty of water. In case of contact with skin and eyes, rinse with large volumes of water or treatment from an eye bath station for several minutes and seek medical attention. Show this sheet.

If any substance is taken internally rinse out mouth with plenty of water and seek medical advice on what treatment to administer.

Show this sheet.

1 - NBT (Nitroblue Tetrazoleum salt) is harmful in the kit quantities provided or toxic in larger amounts if taken internally or by breathing dust. Will irritate eyes and internal organs. Powder may irritate eyes skin and internal organs. Do not breathe dust. Wear face mask when using.

2 - Stimulant contains lyophilised bacterial cells – TAKE CARE. Do not breathe dust. Take appropriate precautions when handling.

3 - Counterstain is and TOXIC FLAMMABLE. (Contains methanol) Harmful/Toxic by inhalation. Do not breathe vapour. May cause irritation to eyes, skin and internal organs. Danger of irreversible effects. Maybe absorbed through skin. May cause harm to the unborn child. Wear appropriate protection when in use.

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Accidental spillage & waste disposal

In all cases for the compounds listed in this kit – mop up spillage with damp cloth, rinsing cloth under tap water diluting to public sewer.

Waste reagents should be diluted to public sewer with a large amount of water.

Consult local regulations as there may be restrictions on the disposal of some of the reagents in this kit.

Unsatisfactory performance

As part of our duty to monitor product performance and our policy of continual improvement. Please report to us any unsatisfactory performance you may experience with this product. If any reagent degrades before expiry date of shelf life we will replace that reagent free of charge.

GCC Diagnostics guarantees that the highest quality reagents are supplied with this product and that the product conforms to the information contained in this leaflet.

The user should however, determine the suitability of this product for their particular use.

If you wish to report any findings to us or if you require help or further information on the use of this product please contact us.

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