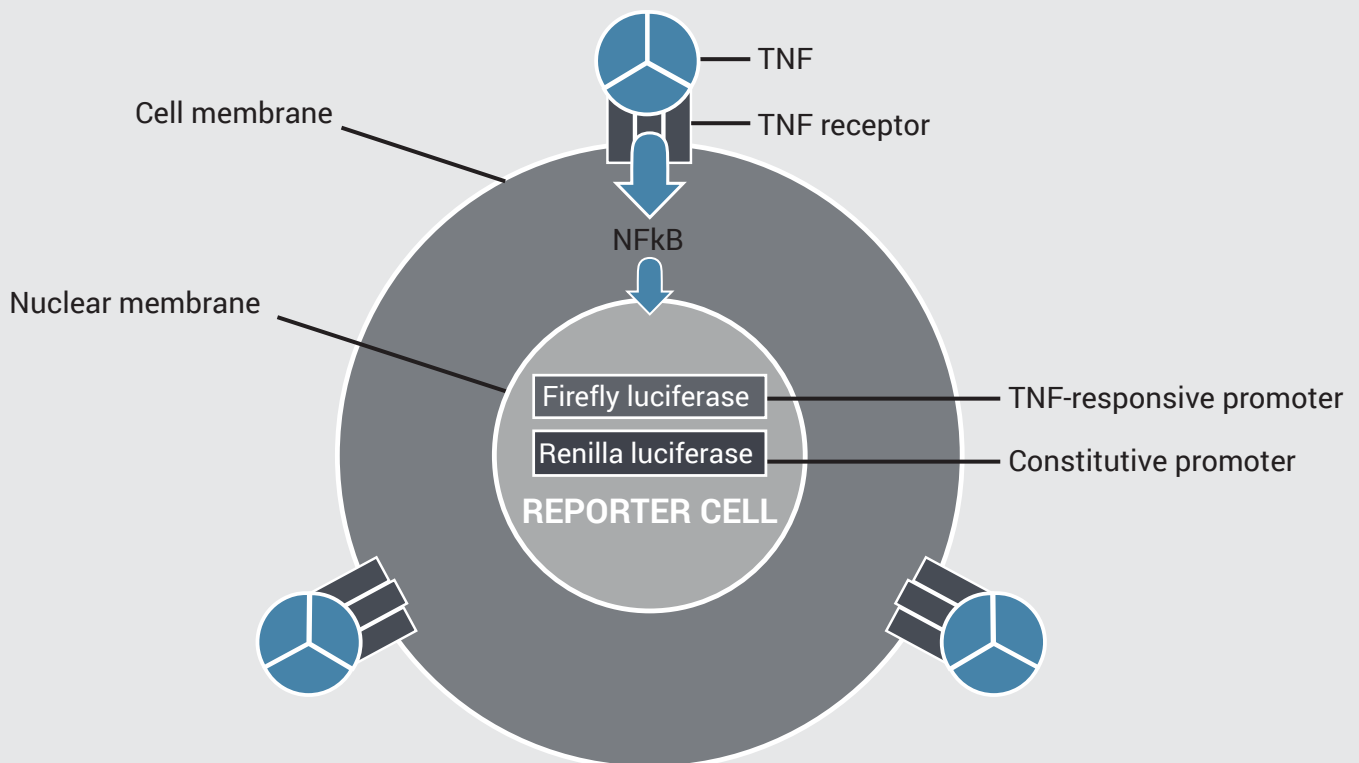


TNF Antagonists

Activity and Neutralizing Antibody Tests

TESTING METHODOLOGY

The functional reporter gene assay uses the principles of iLite technology (licensed by Euro Diagnostica). Reporter cells carry a TNF-inducible, NFκB-regulated firefly luciferase reporter gene construct. When TNF is added to the cells, the reporter gene turns on and generates firefly luciferase, which is measured by a luminometer. Results of firefly luciferase expression are normalized relative to the expression of the renilla luciferase gene, which is carried by the same reporter cell under the control of a constitutive promoter.



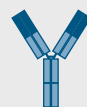
Reporter cell



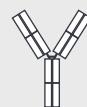
TNF receptor



TNF



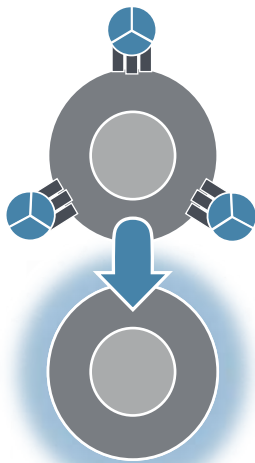
TNF antagonists



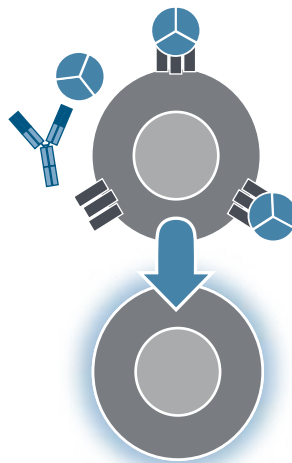
Neutralizing antibodies

ACTIVITY LEVEL

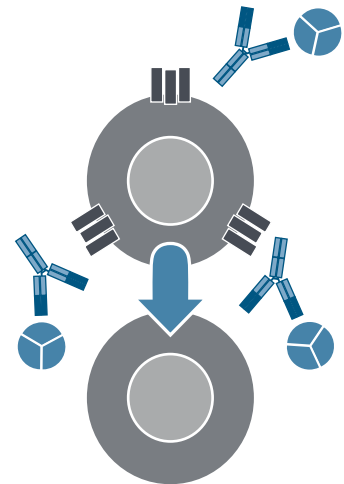
Serum of a patient taking a TNF antagonist drug is mixed with TNF and added to the cells. If the drug is present, it will block the activity of TNF, decreasing luminescence. Serum concentration of a biologically active TNF antagonist drug can be calculated using a calibration curve.



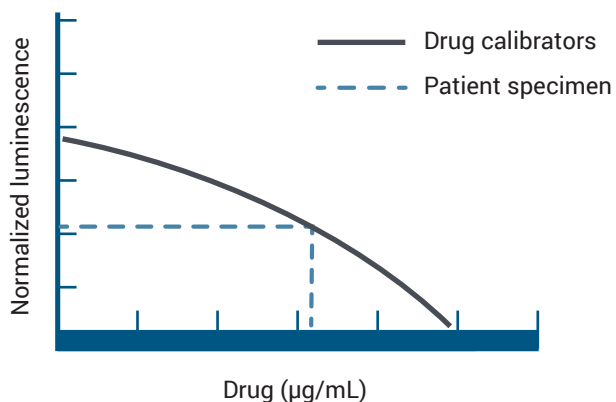
NO DRUG
HIGH LUMINESCENCE



LOW DRUG
MODERATE LUMINESCENCE



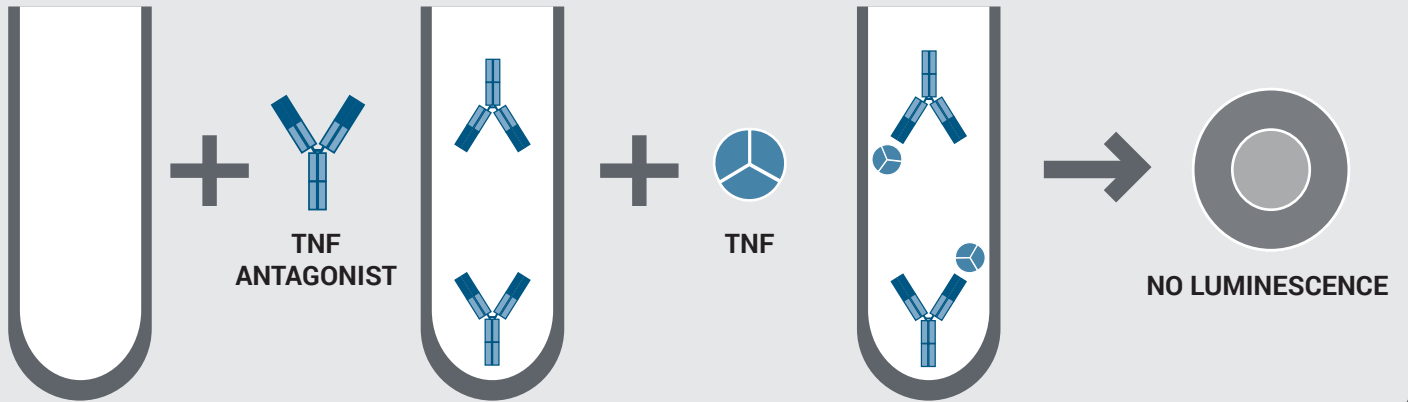
HIGH DRUG
NO LUMINESCENCE



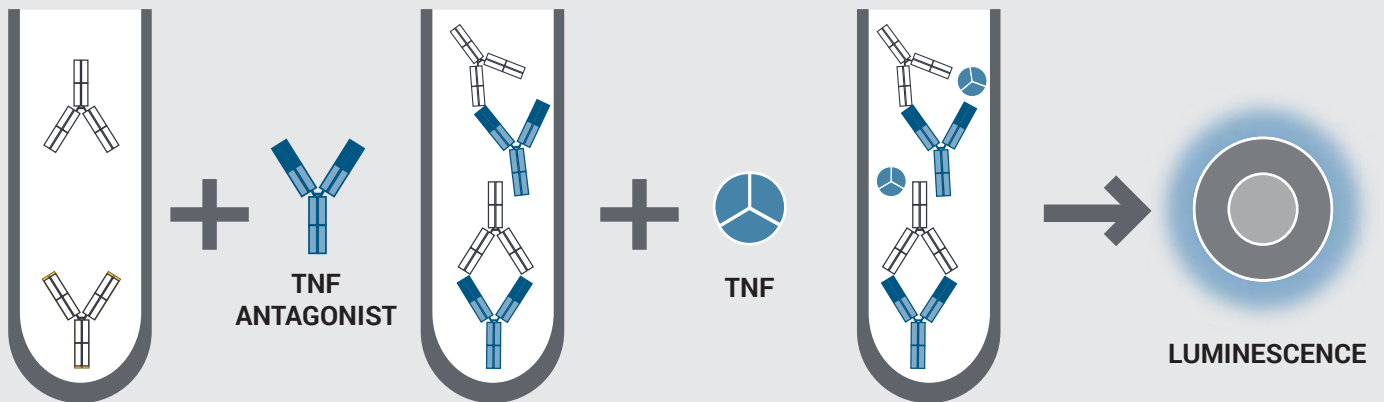
The amount of TNF antagonist in serum can be calculated by comparing the level of TNF with calibrators of known TNF antagonist concentrations.

NEUTRALIZING ANTIBODIES

PATIENT SERUM WITH NO NEUTRALIZING ANTIBODIES



PATIENT SERUM WITH NEUTRALIZING ANTIBODIES

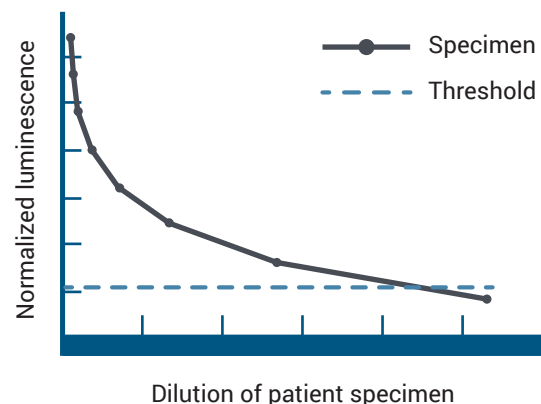


Some patients develop antibodies to TNF antagonists.

It is important to detect detrimental antibodies that neutralize the drug, rather than those that bind to the drug but do not block drug activity.

In the presence of neutralizing antibodies, the reporter gene is turned on despite the presence of exogenous drug in the assay.

Antibody titer is obtained by identifying the dilution point of patient's serum at which blocking of TNF antagonist activity is no longer observed.



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*A nonprofit enterprise of the University of Utah
and its Department of Pathology*

aruplab.com/testing/TNF



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BD-CS-061, Rev 1, July 2021