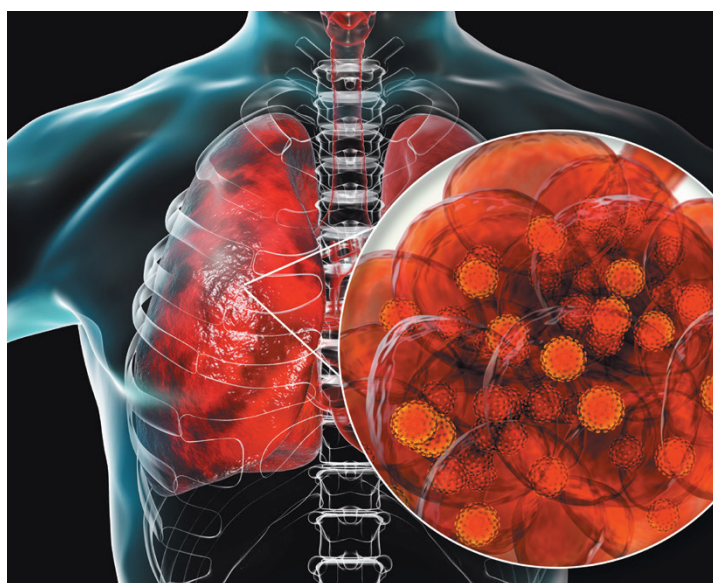


High quality ELISA for measuring

HMGB1 in COVID-19 samples.

UNDERSTANDING DISEASE AETIOLOGY

It is well established that HMGB1 plays a crucial role in many diseases, including COVID-19. This article demonstrates the value of HMGB1 as a clinical biomarker in various sample types and diseases, and how Tecan's HMGB1 Express ELISA kit can accurately measure levels in different sample types. This kit has been used in over 1,400 publications to date, with its accuracy and reproducibility making it an ideal choice for measuring HMGB1 levels in COVID-19 patients.



Measuring HMGB1 in the immune response to SARS-CoV-2

TECAN HMGB1 EXPRESS ELISA KIT

Accurately measuring HMGB1 antibodies is fraught with difficulties, mainly due to non-specific activity or cross-reactivity associated with available HMGB1 antibodies. However, Tecan's HMGB1 Express ELISA kit shows virtually no cross-reactivity to HMGB2, which is normally a serious confounding factor. The kit can therefore readily measure changes in the normal level of HMGB1, once the baseline level has been determined through careful analysis of a relevant population.

The kit can be used with a wide range of sample types, including serum, plasma, synovial fluid, cerebrospinal fluid and cell culture extracts. It has also been used in many in vitro studies, and in a range of mammalian species. It is CE marked for in vitro diagnostic use in Europe, as well as being validated for research use in the USA.

Professor Helena Erlandsson Harris, a pioneer in HMGB1 research, found Tecan's HMGB1 Express ELISA kit to be the most reproducible of all those assessed by her teams, noting: "Perhaps what is most important is that this kit has become the gold standard in the field, meaning you can accurately compare your results with what other groups are publishing."

The following case studies illustrate how it has been used to demonstrate the utility of HMGB1 as a biomarker.



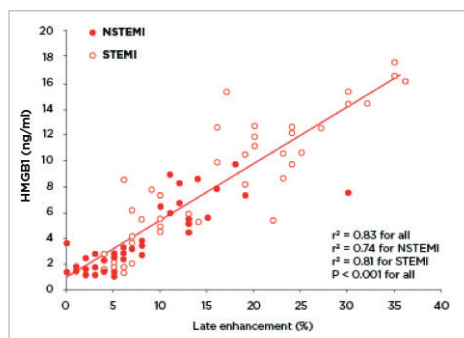


Figure 1: HMGB1 expression was related to infarct extent in patients with ST-elevation myocardial infarction (STEMI) or non-ST-elevation myocardial infarction (NSTEMI). Redrawn from Figure 1(a) in Andrassy *et al.* 2011.

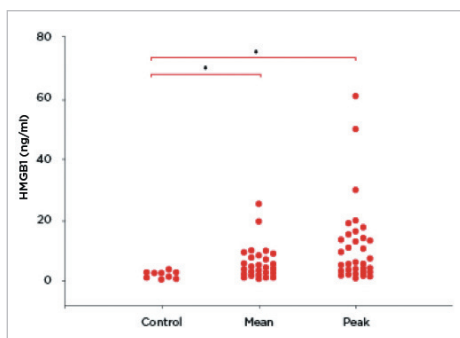


Figure 2: Mean and peak levels of HMGB1 in CSF from patients with TBI were raised compared with controls (**p* < 0.05). Redrawn from Figure 1B, Au *et al.* 2012.

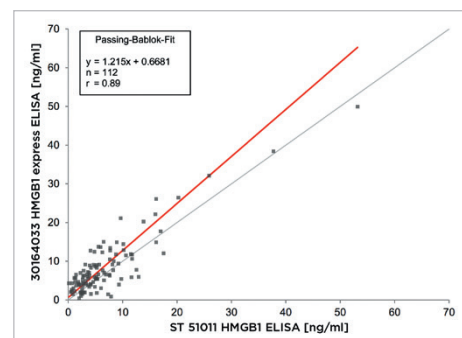


Figure 3: Correlation of results between Tecan HMGB1 Express ELISA kit and existing HMGB1 ELISA kit.

Case study 1: HMGB1 in serum can help diagnose myocardial infarction

Myocardial inflammation is one of many causes of chronic heart failure. Professor Martin Andrassy and his colleagues at the University of Heidelberg in Germany were first to show that increased HMGB1 levels are closely related to infarct extent in patients with acute myocardial infarction (Figure 1).

Case study 2: HMGB1 in cerebrospinal fluid is a predictive marker for traumatic brain injury

Traumatic brain injury (TBI) is a significant cause of morbidity and mortality in children in the United States. Dr Alicia Au and her colleagues at the Safar Center for Resuscitation Research and the UPMC Children's Hospital of Pittsburgh, Pittsburgh, USA, investigated the value of HMGB1 as a possible predictive marker that could direct effective treatment. They found that raised levels of HMGB1 were indeed associated with poor outcomes in infants and children with TBI, signaling tissue necrosis (Figure 2).

Case study 3: HMGB1 in synovial fluid is an independent factor for radiographic severity of osteoarthritis

Osteoarthritis is a leading cause of disability in the elderly, with the knee being a primary site for its development and progression. Zhan-Chun Li and colleagues at the Department of Orthopedic Surgery, Renji Hospital, Shanghai, China, compared HMGB1 levels between osteoarthritis patients and a control group to determine if HMGB1 is a marker for osteoarthritis. They concluded that osteoarthritis patients had higher levels of HMGB1 in the knee synovial fluid than the control group, but not in their serum. These levels also correlated positively with scores based on the Kellgren-Lawrence grading

system for osteoarthritis, and HMGB1 levels were an independent factor for radiographic severity of osteoarthritis.

Adding the Tecan HMGB1 Express ELISA kit to our COVID-19 armory

Developing suitable antibodies has, until now, been a major obstacle to more widespread use of HMGB1 ELISA testing. More specific antibodies will decrease assay times, improve sensitivity and increase the dynamic range of detection. A significant reduction in incubation times is especially advantageous for clinical research and diagnostics applications – particularly in diseases such as COVID-19, where progression of severity can be extremely rapid.

With these needs in mind, researchers at Tecan have developed a new generation of HMGB1 ELISA, featuring:

- New monoclonal anti-HMGB1 pre-coated onto microplate wells
- Significantly reduced assay time: 4 hours (previously ~24h)
- Larger dynamic range: up to ~2.0 OD (previously 1-1.5 OD)
- Wide measurement range: 0.89-80 ng/ml
- Good correlation to current HMGB1 ELISA data (Figure 3)

We hope this article has provided some fresh insights into the crucial role of HMGB1 in the immune response to disease and how Tecan's new generation ELISA kit has alleviated diagnostic obstacles, especially as we learn more about HMGB1's involvement in SARS-CoV-2 infection and progression in severe cases of COVID-19.

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