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LABORATORY SERVICES
IMPORTANT TEST ANNOUNCEMENTS

- **Functional Innate Immune Panel for Inflammasome and Canonical NFkB Pathways**
- **Functional Assessment of Canonical NFkB Pathway and TLR4**

Test Codes: **FINNPC and FINNPN** **Live Date:** **6/10/2024**

- **Methodology:** Cell Culture and PCR-based immunoassay
- **Performed:** Monday -- Thursday
- **Turnaround Time:** Seven (7) Days
- **Specimen Required:**
  - **Collect:** Sodium Heparin
  - **Specimen Volume:** Typically, 8 mL (but based on patient WBC. Please contact the Lab Director to discuss volume based on clinical context and age)
  - **Specimen Preparation:** Mix the tube well immediately following collection
  - **Storage/Transport/Temperature:** Store at room (ambient) temperature, Test should be drawn Monday-Wednesday and samples should arrive no later than noon (12:00 pm). For urgent cases, please contact the Lab Director of DIL at (800)934-6575 to organize exceptions to this shipping policy.
  - **Unacceptable Conditions:** Improper tube type or storage; significant immunosuppression, anti-cytokine therapy against any of the cytokine targets in this assay, steroids (dose and duration-dependent).
  - **Stability:** 48 Hours

FINNPC

- **Clinical Utility:** (This is not a comprehensive list of all the clinical contexts this assay can be used for):
  - Inflammasome complexes (which are multi-protein subunits) are critical in regulating the innate immune response and inflammation (post-translational level).
  - Aberrant activation of inflammasomes and gain-of-function (GOF) pathogenic variants (mutations) in the component inflammasome genes contribute to the development and progression of autoimmune and autoinflammatory disorders, called inflammasomopathies or systemic autoinflammatory disorders (SAIDs) and sometimes, pyrinopathies or Relopathies, in certain contexts.
  - These autoinflammatory diseases are characterized by aberrant IL-1beta (IL-1b) and IL-18 production with excessive pyroptosis. Examples of inflammasomopathies include familial cold autoinflammatory syndrome (FCAS, Muckle-Wells syndrome, Neonatal-onset multisystem autoinflammatory disease (NOMID), all related to *NLRP3* mutations, complex or acquired inflammasomopathies (gout, pseudogout, silicosis, asbestosis, type II diabetes mellitus, NLRP3-extrinsic inflammasomopathies, e.g. Familial Mediterranean Fever (FMF and Pyrin-associated autoinflammation, *MEFV* mutations), *TNFRSF1* mutations causing TNF-receptor-associated periodic fever syndrome (TRAPS), Pyogenic arthritis with pyoderma gangrenosum (PAPA syndrome, *PSTPIP1, NLRP3, CD2BP1* mutations), Hyper immunoglobulinemia D with periodic fever syndrome (HIDS, *MVK* mutations), Schnitzler's syndrome, NLRP1-associated disorders (mutations in *NLRP1*), NOD2-associated disorders, including Crohn's

FINNPC (cont.)

disease, Blau syndrome (mutations in *CARD15* encoding NOD2), NLRP12-associated disorder (Guadeloupe variant periodic fever syndrome, FCAS2, NLRP12-related autoinflammatory disease, mutations in *NLRP12*), NLRC4-associated disorders, including autoinflammation with infantile enterocolitis (AIFEC), NOMID and FCAS4 (mutations in *NLRC4*), DIRA (deficiency of IL-1 receptor antagonist, *IL1RN* mutations), DITRA (deficiency of IL-36 receptor antagonist, *IL36RN* mutations) recurrent fever syndromes, hyperinflammatory contexts, suspected to be autoinflammatory, among others.

- **Comments:**
  - This assay is designed to assess the inflammasome activation by stimulating PBMCs (specifically monocytes) with LPS alone or LPS+ ATP (inflammasome activation - NLRP3, NLRC4, NLRP1, NLRP12, Pyrin etc.) for 2h or 24h.
  - The assay read-out is the measurement of 4 cytokines after stimulation: IL-1b, IL-18, TNFa, and IL-6, at each time-point (2h and 24h) for each stimulation (LPS and LPS + ATP) and the unstimulated condition.
  - The NFkB pathway is important in the activation of the inflammasome. Results are reported in pg/mL after normalization to the absolute monocyte count (AMC) and are interpreted in the context of a reference interval, generated from adult or pediatric healthy controls.
  - An interpretive report will be provided. A patient information form for external patients (not internal to NCH) should be completed when ordering the test to facilitate interpretation of results

FINNPN

- **Clinical Utility:** (This is not a comprehensive list of potential clinical utility for this assay).
  - LPS stimulation can be used to assess a patient with sepsis, and TLR4 signaling pathway defects, including TLR4, IRAK4, and MyD88 deficiencies, since it binds to an LPS receptor complex comprised of TLR4, CD14, and MD-2.
  - The canonical pathway of NFkB can be activated through diverse stimuli, including pathogen recognition receptors (PRRs), such as TLR4, TNF receptors (TNFR), and the T cell and B cell receptors (TCR and BCR).
  - The canonical pathway is involved in almost all aspects of the immune response, this assay can be useful to assess defects in the components of the canonical pathway, NEMO (*IKBKG* or IKK-g), *IKBKB*, *NFKBI/NFKBIA, NFKB1, RELA* (p65), etc. In fact, the latter, RELA haploinsufficiency belongs to a group of disorders called the Relopathies, and includes, A20 haploinsufficiency (*TNFAIP3* mutations), ORAS – Otulin-related autoinflammatory syndrome/Otuilpenia (*OTULIN* mutations), HOIL-1/HOIP deficiencies (due to mutations in HOIL1 and HOIP), biallelic RIPK1 deficiency (mutations in RIPK1, and NEMO deficiency. The assay could also be utilized to assess diseases, like ADA2 deficiency (DADA2), which is often treated with anti-TNFa inhibitors, including in patients experiencing breakthrough symptoms on therapy
- **Comments:**
  - This test only utilizes stimulation with LPS of monocytes for 24h and should not be used for assessment of inflammasomopathies and autoinflammatory disorders (order FINNPC for this purpose).
  - The assay read-out is the measurement of 4 cytokines after stimulation and for unstimulated conditions: IL-1b, IL-18, TNFa, and IL-6.
  - Results are reported in pg/mL after normalization to the absolute monocyte count (AMC) and are interpreted in the context of a reference interval, generated from adult or pediatric healthy controls. An interpretive report will be provided. A patient information form for external patients (not internal to NCH) should be completed when ordering the test to facilitate the interpretation of results.

If you have any additional questions about **FINNPC** or **FINNPN**
please refer to the Laboratory Test Directory or call
Client Services at 614-722-5477