

## ***Frequently asked questions about Hyaluronic Acid (HA) Test Kit:***

### **1. How long does it take to perform the assay?**

The assay can be completed and results calculated within 2 hours and 30 minutes (hands on time approximately 15 minutes). Total incubation time is 2 hours (60', 30', 30').

### **2. Is any special equipment required to run the assay?**

Other than routine laboratory equipment (including pipets capable of accurately delivering volumes of 30 – 300  $\mu$ L), a microplate spectrophotometer capable of reading absorbance at 450 nm is the only special equipment required to perform the HA test. A multichannel pipet is recommended but not essential.

### **3. Can the HA assay be automated on microplate processing instruments?**

Yes. The assay has been successfully automated on the Grifols Triturus® and the Dynex Technologies DSX™ Automated System. Assay incubation times and kit reagent volumes facilitate automation on most, if not all, open microplate processing equipment.

### **4. How is the HA assay standardized?**

Commercially available, purified HA is used to standardize the HA assay. The material is weighed out gravimetrically and carefully diluted to prepare a series of standards recovering within the detection range of the assay.

### **5. What is the detection range of the assay?**

The assay is able to measure HA levels between 10 – 800 ng/mL. Samples recovering above the upper level of the detection range can be diluted and retested for more accurate results.

### **6. What is the recommended sample matrix?**

Serum and heparinized plasma have been validated for use in the HA assay.

### **7. What is the minimum volume of sample required in the assay?**

The assay can be run on 30 microliters of serum or plasma.

### **8. What is the maximum number of samples that can be tested with one kit?**

When tested in duplicate, a total of 41 samples and/or controls can be tested with HA each kit.

### **9. Can urine samples be tested with the Corgenix HA kit?**

HA molecules must consist of a minimum of 20 repeating units (or dimers) of  $\beta$ -(1-4)-glucuronic acid and  $\beta$ -(1-3)-N-acetylglucosamine moieties to be detected by the HA kit. The HA molecules excreted in urine are of a smaller molecular weight than serum HA, and contain less than 20 dimers. These are not detected by the Corgenix assay. Since less than 1% of HA is excreted in urine, this is not a significant issue. The vast majority of HA is cleared from the blood by the liver.

### **10. Can synovial fluid samples be tested with the HA kit?**

In several research laboratories, synovial fluid has been successfully used as a sample matrix with our HA kit. Since HA levels in synovial fluid are typically much

higher than in serum, a greater dilution of synovial fluid samples must be used for the samples to recover within the detection range of the assay

**11. Will the assay detect HA levels in non-human samples?**

Yes. The HA molecule is universal, rather than species-specific.

**12. Do any substances cross-react with the HA assay?**

None have been identified. The hyaluronic acid binding protein (HABP) molecule used to capture and detect HA in the assay is highly specific for HA. Other compounds with a similar molecular structure have been tested with the kit; no interference was found.

**13. Does age affect HA levels?**

HA levels have been reported to increase with age in normal individuals, although in our studies, the effect was minimal. The rate of increase was shown to be approximately 0.5 ng/mL per year in healthy individuals.

**14. Can blood samples be collected at any time during the day for HA testing or does the patient need to be fasting?**

A diurnal variation in HA levels has been reported in the literature. In one study, the highest HA levels were seen between 30 and 60 minutes after arising in both normal subjects and RA patients, due to normal morning activities. Thereafter, HA levels decreased to those measured at rest. An increase in HA levels was also observed after moderate – heavy exercise.

In another study, the ingestion of food markedly increased plasma HA in patients with compensated but not decompensated cirrhosis, suggesting that the use of fasting plasma HA levels in patients with liver disease improves the discriminating value of the HA test in separating normal from abnormal.

**15. Is the HA test specific for liver disease, or are there other conditions that can cause elevated HA levels?**

Elevated HA levels can be seen in patients with chronic liver disease due to decreased removal of HA from the blood by damaged sinusoidal endothelial cells in the liver. Serum HA levels can also be increased as the result of increased production and passage into circulation during synovial inflammation and cartilage destruction in rheumatoid arthritis (RA).<sup>7</sup> Elevated HA levels have also been reported in some patients with advanced osteoarthritis (OA),<sup>7</sup> progressive systemic sclerosis (PSS)<sup>8</sup> and systemic lupus erythematosus (SLE),<sup>9</sup> and are believed to result from growth factor activity in connective tissue cells and synovial involvement.

A few reports of elevated serum HA levels in other medical conditions such as sepsis,<sup>10,11</sup> acute pancreatitis,<sup>12</sup> and severe pre-eclampsia and eclampsia.<sup>13</sup> have been published in the literature. Certain types of cancer have been associated with elevated serum HA levels, including non-small cell lung cancer,<sup>14</sup> pleural mesothelioma,<sup>15</sup> breast cancer,<sup>16</sup> and bladder cancer.<sup>17</sup> Elevated HA levels have also been reported in various myeloproliferative disorders such as malignant lymphoma,<sup>18</sup> myelofibrosis,<sup>19</sup> and multiple myeloma.<sup>20</sup> In addition, patients with chronic renal failure on long-term dialysis have been shown to have elevated

serum concentrations of HA,<sup>21</sup> which was significantly related to an increased mortality rate.<sup>22</sup>

The simultaneous presence of any these conditions should be taken into consideration in the interpretation of HA results in patients with chronic liver disease.