handling the microvolume solution and ensuring precise incubation/washing time and volume for each well. The immunoassay process usually takes 3–8 hours depending on detailed steps. The good point of microplate immunoassay format is that many samples can be analyzed in parallel. However, in many circumstances, a small number of samples may need to be analyzed with the demand of quick results. In addition, in many areas of the world, skilled medical personnel are not available to conduct such a complicated test. More automated immunoassay systems where the volume and time are controlled by using a constant flow rate pump to introduce and to draw solutions from the reaction cell have been reported in various formats [42].

To change the format from conventional static immunoassay system to dynamic flow formats, the reaction cell has to be changed from being the wall of a microwell plate to other forms of solid surfaces that can be accommodated easily in the flow of solution. Microbeads and capillary are the main types reported. An example for the application in biomarkers research is sequential injection-glass capillary immunoassay [26, 43]. The sequential injection system was used to precisely control the incubation time and small volume of solution in the range of 10–80 μL which is even smaller than some of those used in conventional microwell plate format. A glass capillary was easily connected to the system as part of the tubing that the solution conveniently flows through without any back pressure which may occur when using beads. The wall of the glass capillary was used as the solid surface for immobilization of biomolecules to be employed in subsequent steps of competitive immunoassay.

Microbeads are used in immunoassay with the capability to increase surface area to improve sensitivity. However, beads that are packed or trapped in the reactor can cause back pressure inside the flow system, so a slow flow rate should be used. Example of sequential injection bead-based immunoassay system was reported for determination of hyaluronan, a possible biomarker for liver diseases [44]. The lab-on-valve (LOV) unit with on-valve fiber optic spectrometer can also be used in conjunction with functionalized beads that are trapped in the LOV unit to obtain direct, real time detection [45]. Although, there is no work reported on its application for study of liver disease biomarkers, the possibility exists for employing LOV bead immunoassay for such a task. Nevertheless, employing beads in the flow system would require more sophisticated control systems than most low-budget laboratories could devise unless preexisting equipment were to be adapted.

3.3. Flow-Based Analysis System with Preconcentration Capability. The use of microbeads in another aspect, other than using them as a solid surface for immobilization of biomolecules as used in immunoassay, is reported as a preconcentration surface to accumulate analyte of interest before detection. The flow of solution within an easily designed cell for trapping beads and releasing beads when needed is called a flow injection-bead injection system. An example of such a system for alkaline phosphatase in human serum was demonstrated [32]. Even though the work used beads coated with wheat germ lectin for specific binding for bone-alkaline phosphatase, it should be able to combine with total alkaline phosphatase test to estimate for liver alkaline phosphatase. The use of membrane for the same purpose of improvement of sensitivity was also reported. With-state-of-the art development in nanotechnology, nanopore membrane used with electrokinetic fluid flow as a nanofluidic protein accumulator was claimed to offer a much higher sensitivity in the analysis of human serum albumin than other methods [30].

By using a detection method that can measure the differences of surface properties before and after binding to the target analyte, such as surface plasmon resonance (SPR) technique, the amount of bound analyte on the surface could be quantitated directly without the need to add any reagents. Aoki and Toyama [46] demonstrated this system by determination of human serum albumin in urine using gold as an adsorption surface. A flow injection system was employed for continuously feeding the sample containing uric protein onto the gold surface.

4. Remarks

As can be observed from Table 1, most published works that demonstrated flow-based systems for liver diseases biomarkers used the same limited types of target analytes, that is, serum albumin and total protein. There are many substances in body fluids that have been reported as potential biomarkers for liver diseases. Determination of these different biomarkers at the same time may provide better conclusion about the existence of the diseases. Therefore, future studies using flow injection/sequential injection systems should focus on various other possible biomarkers as well as applications for conducting simultaneous detection of multibiomarkers from one shot of sample. Trends in development of analytical devices have also been gearing toward a point of care purpose. The main challenges are to develop the system for solution introduction with controllable flow rates, effective reagent mixing, and detection unit in downscaled format that can be integrated into compact stand-alone devices.

5. Conclusion

Various formats of flow injection/sequential injection analysis and micro-/nanofluidic systems can be set up to study biomarkers. Even though few works have reported on the study of liver disease biomarkers using flow-based systems, works related to determination of protein and enzymes are numerous and should be adaptable for studies of liver disease biomarkers. These flow-based systems are versatile and can be used as an alternative method for rapid screening of biomarkers to aid in disease diagnosis. Their low-volume consumption is particularly suitable for the study of a biomarker, in which samples may be divided for many other tests, either to evaluate different biomarkers or to accompany the initial biomarker test.