Highlight:

- The novel gravity sedimentation cartridge eliminates the need for centrifugation and slide preparation steps for urinalysis, which are typically required in the gold standard method.

- The cartridge brings forth a one-step workflow, and AI100 provides automation in image acquisition and analysis using AI-based tools.

- The sample's shelf life within the cartridge has been extended to 90 minutes, a significant improvement compared to the 10-15 minutes of the gold standard method.

- The cartridge allows for multisampling, enabling the simultaneous analysis of up to three samples.
AI Driven Lab-on-chip Cartridge for Automated Urinalysis

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Abstract

After haematology, urinalysis is the most common biological test performed in clinical settings. Hence, simplified workflow and automated analysis of urine elements are of absolute necessities. In the present work, a novel lab-on-chip cartridge (Gravity Sedimentation Cartridge) for the auto analysis of urine elements is developed. The GSC consists of a capillary chamber that uptakes a raw urine sample by capillary force and performs particles and cells enrichment within 5 min through a gravity sedimentation process for the microscopic examination. Centrifugation, which is necessary for enrichment in the conventional method, was circumvented in this approach. The AI100 device (Image based autoanalyzer) captures microscopic images from the cartridge at 40x magnification and uploads them into the cloud. Further, these images were auto-analyzed using an AI-based object detection model, which delivers the reports. These reports were available for expert review on a web-based platform that enables evidence-based tele reporting. A comparative analysis was carried out for various analytical parameters of the data generated through GSC (manual microscopy, tele reporting, and AI model) with the gold standard method. The presented approach makes it a viable product for automated urinalysis in point-of-care and large-scale settings.

Keywords

Urinalysis, Centrifugation, Gravity Sedimentation, Wet Mount, Artificial Intelligence, Microfluidics

1. Introduction

Microfluidics has attracted considerable attention due to its advantages associated with miniaturisation, integration and automation of various unit operation processes [1,2]. It is capable of outperforming classical techniques in biomedical and chemical research [3]. Microfluidics also has advantages like low reagent consumption, high surface area to volume ratio and high parallelization. The use of microfluidics has made it a highly versatile tool for investigating single or group of cells. This versatility stems from the platform's ability to achieve high spatial and
temporal resolution, allowing for the trapping or fixation of individual cells or small subpopulations. This feature enables the examination of a single cell or a small group of cells’ physiology over a period of time, making it an invaluable tool for cellular research. Hence, it can play an important role in the lab-on-chip analysis of biological samples [4,5].

Urinalysis is one of the most common and major inspections of medical diagnosis. It helps in the diagnosis of some organ dysfunction or pathologies, or metabolic disorders [5–9]. Clinically, urine is analysed using physical, biochemical and microscopic examination [10]. Traditionally, the microscopic examination of centrifuge urine sediment is time-consuming, labour-intensive and has a wide variation in observation [11,12]. Besides, there is also a need for trained technicians for accurate interpretation. This method is subjected to error due to degradation of formed elements during centrifugation or sample storage or manual error on detection, decanting supernatant and mixing sediments. Consequently, there is a need for the automation of urine microscopic analysis in large laboratories and point-of-care settings.

Currently, in the market, the auto analyzers available are Sysmex UF 1000i (Sysmex), Iris iQ 200 (Beckman Coulter), SediMax (Menarini Diagnostics), Roche Cobas u701 (Roche Diagnostics), Siemens UAS800 (Siemens Healthineers) and SediVue Dx [13–15]. Sysmax instrument works on the flow cytometry principle where cells are fluorescently labelled to identify the type of cells. Iris iQ200 analyzes urine elements by passing raw urine samples to flow cells using digital imaging. Sedimax, Roche Cobas u701 and Siemens UAS800 perform centrifugation to settle the urine sediments at the surface, which is later examined using digital imaging. SediVue Dx uses an inbuilt centrifuge mechanism to make the urine sediment monolayer at the cartridge bottom surface, where imaging of cells and particles occurs. Mostly, the autoanalyzer focuses on the enrichment of urine cells and particles and the auto-detection of cells. However, these instruments are very sophisticated and expensive. Hence, the wet mount method is widespread in developing countries.

Conventionally, the microscopic analysis of urine samples is carried out using a wet mount (WM) method [16] . The WM procedure majorly involves centrifugation and slide preparation. The 10 ml of raw urine sample is centrifuged at 3000 rpm for 5 min for urine cells and particles to concentrate. The supernatant is discarded, and the pellet (urine sediment) is recovered. Finally, the slide is prepared for microscopic analysis by placing the coverslip over the 10 μl of urine sediment on the glass slide. The sample stability and the drift are the main issues encountered in wet mount during microscopy evaluation. Due to evaporation from the edges of the coverslip, the sample is stable for only 10–15 min. Hence, cells and particles evaluation has to be completed within the stipulated time. Evaporation also leads to the drift of cells and particles, which makes inaccurate counting of cells and particles. Moreover, manual microscopy necessitates extensive time and effort due to its labour-intensive nature and exhibits considerable variation in observations. Hence, a microfluidic cartridge that addresses the above issues with the advantage of low cost and simplified workflow is proposed. This microfluidic cartridge is loaded into an inbuilt autoanalyzer (AI100). AI100 is an IOT (Internet of Things) based device that automatically captures microscopic images at 40x magnification from a sample loaded in the microfluidic cartridge and uploads them into the cloud. Further, these microscopic images are analyzed by Artificial Intelligence (AI) models to detect and count the elements present in the specimen to generate a report. A crucial point to note is that the same AI100 device has been previously used for automated peripheral blood smear (PBS) [17–19] analysis and the measurement of Hemoglobin.
The current study introduces a novel lab-on-chip cartridge (GSC) that facilitates the passive concentration of cells and particles through the gravity sedimentation process. Subsequently, the concentrated samples are analyzed using an AI 100 device. The comparative analysis of results between the GSC (manual microscopy, tele reporting and AI model) and gold standard method (wet mount) is presented. The shelf life of urine samples is studied in the microfluidic cartridge. Further, the staining of cells using a pre-stained cartridge, multisampling, settling velocity and settling time are studied. Finally, the results are summarized by highlighting the important conclusion.

2. Material and Methods

2.1. AI100 digital scanner

The in-house built digital scanner was used to acquire / record images of the GSC filled with the raw urine samples. The digital scanner consisted of the following hardware components:

- Computer system: A mini-ATX motherboard with Intel i5 quad-core processor, 8 GB RAM, NVIDIA GPU with 4G GPU memory, running Ubuntu Linux (v20.04).
- Optics system: An optical tube (40× Plan Achromat objective and 10× eyepiece) and an Abbe condenser with a white LED source. A 13 MP USB 3.0 colour camera procured from e-con Systems, Inc., USA, was used. The camera model See3CAM_CU135 contained a 1/3.2 AR1335 CMOS image sensor from ON Semiconductor.
- XYZ slide stage: The XYZ platform was built using commercially available low-cost ball screws, stepper motors, and some machined parts.

2.2. Gravity sedimentation cartridge (GSC)

The GSC comprises a polymethyl methacrylate (PMMA) slide, spacer tape and cover slip. PMMA slides were manufactured by the injection molding method. These slides are optically transparent and have a mirror finish surface with a dimension of $76 \times 26 \times 1 \text{ mm}^3$ (L x W x T). Spacer tapes of thickness ranging from 50 μm to 570 μm were procured from Nitto Denko Corporation (Japan), Teraoka Tapes (Japan), and Avery Dennison (USA) to make different chamber depths. Two coverslips (Microcil Manufacturers, India) of dimensions $22 \times 22 \text{ mm}^2$ and $22 \times 50 \text{ mm}^2$ were used to cover the chambers.

2.3. Pre-stained slide dye composition

The dye solution consists of 10% crystal violet in methanol (Qualigen, India) and 0.1% tween 20 (SD fine, India). Crystal violet (Nice Chemicals, India) is a dye that stains the cell wall and its nucleus. When dissolved in water, the dye dissociates in the solution to acquire a positive charge that binds to the negative charge in the nucleus and cell wall. The purpose of adding
methanol (SD fine, India) to the solution during dip coating was to expedite the evaporation process. Tween 20 was required for the evenly spreading of solution over the slide.

2.4. Sample distribution

The midstream urine sample was collected in a sterile container. The sample was processed within 4 hr after the collection and transported at room temperature. A total of 413 fresh abnormal urine samples were collected for the analysis from the Department of Pathology, Father Muller Medical College Hospital, Mangalore, India. The required consent was taken from the patients before collecting samples. The lab-on-chip cartridge analysis employed 413 samples, whereas the AI model and tele reporting analysis utilized 240 samples. Figure 1 demonstrates the urine sample distribution. Figure 1(a) presents the distribution of RBC and WBC in different grades, which are NS (Non-significant) (0 - 5), 1+ (6 - 10), 2+ (11 - 20), 3+ (21 - 50) and 4+ (>50). The number of RBC samples belonging to different grades are 326, 18, 12, 32 and 25, whereas, for WBC, these are 283, 36, 29, 42 and 23. Figure 1(b) shows the sample distribution of bacteria in urine samples collected. The grade-wise grading for bacteria is NS (0 - 1), 1+ (1 - 2), 2+ (3 - 5), 3+ (6 - 10), and 4+ (>10). The number of bacteria samples in this category are 27, 63, 70, 41 and 212. The remaining urine elements epithelial cells, crystals, cast and yeasts are classified as detected or not detected. Figure 1(c) depicts the sample distribution of epithelial cells, crystals, cast and yeasts in the urine sample collected. In the not detected category, their numbers are 15, 319, 350 and 359, whereas in the detected category, numbers are 398, 94, 63 and 54. In routine, the normal urine samples are very high in number which leads to high samples of NS (WBC, RBC) and not detected category of crystals, cast and yeast. The epithelial cells are normally present in urine sample as they are exfoliated from the tissues during urinating. The bacteria doubling time is low. Hence, we see the above urine sample distribution.

Figure 1 Urine sample distribution over the sample population. (a) Sample distribution for RBC and WBC over the grades: NS, 1+, 2+, 3+, 4+. (b) Sample distribution of bacteria over the following grades: NS, 1+, 2+, 3+, 4+. (c) Distribution of epithelial cells, crystals, cast and yeasts samples over not detected and detected buckets.
2.5. Cartridge Design & Fabrication

Figure 2 represents the GSC. The cartridge consists of a PMMA slide, spacer tape and coverslip. The PMMA slide and coverslip are cleaned using soap solution (5% Extran (Merck, Germany)). Both are then ultrasonicated in a soap solution (5% Extran), DI water for 5 min and air-dried. Further, the PMMA slide is spray-coated with P100 (Joninn, Denmark). Finally, the coverslip and coated PMMA slide are used directly for the assembly. The PMMA slide forms the base of the cartridge. The spacer tape (25.5 × 17.8 × 0.4 mm³) is placed over the PMMA forming the lateral walls of the chamber. The spacer tape of the required dimension is cut using a laser machine (SM Laser Technology EC6.4, India). The chamber formed by the spacer tape is enclosed from the top using a coverslip in such a way that the inlet / outlet ports are open to the atmosphere. The entire fabrication is carried out in a dust-free area. After loading the raw urine sample at the inlet, it fills the chamber by capillary force. The urine cells and particles are counted / scanned within the scan zone present in the centre of the chamber.

![Diagram of GSC](image)

**Figure 2** Gravity sedimentation cartridge (GSC) (a) Top view (b) Side View (c) Isometric view

2.6. Effect of depth on particle and cell enrichment

The chamber depth is the characteristic dimension that influences the enrichment of cells and particles, and the depth is proportional to the chamber volume. The number of particles and cells per FOV either increases or decreases accordingly. Hence, optimising the necessary depth of the chamber is important to obtain accurate cells and particles per FOV that agree with the gold standard. A suitable grade of urine sample has to be chosen to understand the cells / FOV dependence over the depth of the chamber. Initially, a medium-sized grade sample was used to evaluate the chamber depth significance. The sample chosen for the study was RBC (2+). Experiments were carried out with different depths of the capillary chamber. Figure 3(a) shows the dependence of cells and particles per FOV over the depth of the capillary chamber. The plot shows a linear dependence. Hence, increasing the depth of the chamber increases the number of cells and
particles per FOV and vice versa. The 2+ grade corresponds to the 11 - 20 cells / FOV. Figure 3(a) shows that for 11 - 20 cells / FOV, the depth of the chamber is in the range of 190 - 400 μm. As the grade-wise analysis of urine cells and particles not corresponding to the absolute number of cells / FOV, the depth of the chamber has a wide range.

If a high-grade urine sample is chosen, i.e., 4+ (>50 cells / FOV, Table 3). Since, the upper end of the grade, i.e., cells / FOV, is not fixed, the one end of the grade in the graph corresponds to infinite chamber depth. Similarly, for low-grade urine samples, i.e., NS (0 - 5 cells / FOV), the lower end of the grade, i.e., 0 cell / FOV, the one end of the grade in the graph corresponds to the zero chamber depth. Hence, it is not possible to arrive at a definite chamber depth. Thus, low and high-grade samples are unsuitable for accurately determining the chamber’s depth. Hence, the medium-grade urine sample was used for the study. Further, studies were carried using grade 1+ (RBC) and 3+ (WBC) of the urine samples which are presented in Figure 3(b). For the 1+ grade, we observe a range of 6-10 cells/FOV, which correlates with a capillary chamber depth spanning from 260 to 460 μm. On the other hand, the 3+ grade corresponds to a higher cell count of 21-50 cells/FOV, aligning with a capillary chamber depth in the range of 190 to 450 μm. Consequently, it is evident that capillary chambers with a depth ranging from 260 to 400 μm encompass a spectrum of grades, including 1+, 2+, and 3+. As a result, we have chosen to focus our further studies on capillary chambers with a depth of 400 μm.

![Figure 3](image_url)

**Figure 3** Graph shows the number of cells / FOV variation with respect to the depth of the chamber (a) 2+ (RBC) (b) 1+ (RBC), 3+ (WBC)

### 2.7. Sedimentation time

When the raw urine sample flows into the chamber by capillary force, the particle distribution is divided into three regions. Figure 2(a) shows the particle distribution in the chamber. The inlet region is called the coarse region, the central region is the focus region, and the outlet region is called the sparse region. The enriched layer, defined by the plurality of the cells and particles in the chamber, includes a coarse region, defined as the group of large-size cells and particles. Further, the enriched layer, defined by the plurality of cells and particles, includes a focus region, defined by the combination of a group of small, moderate and large-size cells and particles. This region is suitable for urine cells and particles counting / scanning as it contains a mixture of all size cells and particles. The area considered for counting / scanning is called a scan zone, which is of size 6 x 8 mm². Cell counting is avoided close to the chamber's walls as more
cells and particles accumulate due to the no-slip condition. Furthermore, the enriched layer includes a sparse region defined by a group of lower-size cells and particles in the plurality of cells and particles. Hence, cells and particle enrichment are based on gravity sedimentation, whose velocity is given by particle settling velocity.

The particle settling that occurs under the influence of gravity can be predicted by Stokes’ law [21,22]. Stokes’ law predicts the settling velocity of small spherical parts in a fluid where particles are well separated, i.e., dilute solution, and the fluid behaves like a continuous medium. When a particle falls through a fluid, the interplay between gravity and drag force decides the settling behaviour of the particle. The gravity acting on the particle accelerates, and simultaneously, the drag force deaccelerates the particle. The terminal velocity is given as

$$V = \frac{(2(\rho_p - \rho_f)g r^2)}{(9\mu)}$$  \hspace{1cm} (1)

Where, $V$ represents the settling velocity of the particle in micrometre per second ($\mu$m / s). $\rho_p$ denotes the density of the particle in grams per millilitre (g / ml), while $\rho_f$ represents the density of the fluid in grams per millilitre (g / ml). The variable $r$ represents the particle’s radius in micrometre ($\mu$m), and $\mu$ signifies the viscosity of the fluid in centipoise (cP).

**Table 1** Settling velocity of cells present in the raw urine samples after loading in GSC.

<table>
<thead>
<tr>
<th>Urine elements</th>
<th>Density (g / ml)</th>
<th>Average density (g / ml)</th>
<th>Size ($\mu$m)</th>
<th>Average size ($\mu$m)</th>
<th>Viscosity (cP)</th>
<th>Settling Velocity in a raw urine sample (V $\mu$m / s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>1.11</td>
<td>6-8</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2.47</td>
</tr>
<tr>
<td>WBC</td>
<td>1.08</td>
<td>12-20</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>8.71</td>
</tr>
<tr>
<td>Bacteria</td>
<td>1.11</td>
<td>1-2</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>0.11</td>
</tr>
<tr>
<td>Yeast</td>
<td>1.11</td>
<td>3-4</td>
<td>3.5</td>
<td>-</td>
<td>-</td>
<td>0.63</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>1.84-2.08</td>
<td>1.96</td>
<td>20</td>
<td>20</td>
<td>-</td>
<td>47.86</td>
</tr>
<tr>
<td>Raw urine sample</td>
<td>1.005-1.030</td>
<td>1.02</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1 represents the settling velocity of cells and particles present in raw urine samples after filling inside the chamber by capillary force. The settling velocity indicates that all the cells and particles settle at the bottom surface of the chamber. The density of all the cells and particles is higher than the raw urine, which gives a positive settling velocity thereby, cells and particles settle at the bottom surface of the chamber and form an enriched layer. The degree of settling depends on the viscosity of the raw urine sample, gravity acting on cells and particles and its size.

Here, the sedimentation time for urine cells and particles was determined for the GSC by analysing it under the microscope at 40x objective. To analyse the sedimentation time, the GSC was visualized under the microscope for a period of 10 min immediately after loading the raw urine sample. A total of 5 samples were studied. Figure 4 shows the images of urine cells and
particles in GSC from the time period of 1 - 10 min. The majority of cells are not in focus till 4 min. However, after 5 min, most of the cells are in focus as they settle down at the bottom surface and form an enriched layer. The GSC cells and particles count data after 5 min agrees with the wet mount shown in Table 2. Thus, the sedimentation time required for enrichment in GSC is 5 min. This optimizes the scanning process by allowing the scanner to precisely locate the designated regions within the chamber. Performing scans before the optimized sedimentation time, introduces complexities in imaging due to the urine cells and particles are disperse throughout the chamber’s depth. This, in turn require the capture of multiple images and the development of an additional algorithm for image stacking. Ultimately prolonging the scanning duration and having a detrimental impact on the device’s overall throughput.

**Table 2** Sedimentation time study for the urine cells and particles in GSC.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Cartridge</th>
<th>Time (min)</th>
<th>Observation (layer)</th>
<th>RBC</th>
<th>WBC</th>
<th>Bacteria</th>
<th>Cast</th>
<th>Crystals</th>
<th>Yeast</th>
<th>Epithelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>0</td>
<td>Single</td>
<td>4+</td>
<td>NS</td>
<td>NS</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSC</td>
<td>1</td>
<td>Multi</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Multi</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Multi</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Multi</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Enriched</td>
<td>4+</td>
<td>NS</td>
<td>NS</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Enriched</td>
<td>4+</td>
<td>NS</td>
<td>NS</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Enriched</td>
<td>4+</td>
<td>NS</td>
<td>NS</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>D</td>
</tr>
</tbody>
</table>
In GSC, the large cells and particles like RBC, WBC, epithelial cells, crystals etc. have relatively higher settling velocity which settles down at the bottom of the chamber within 5 min. While, the small size cells, like bacteria and yeast, have a smaller magnitude of settling velocity and hence, take ~60 min and ~10 min to settle down at the bottom surface. This occurs only when all the small cells and particles are at the top of the chamber. However, when the well-mixed urine sample is filled in the chamber by capillary force, the cells and particles are distributed throughout the depth of the chamber. Hence, the layer of bacteria and yeast present close to the bottom surface approaches the settled larger urine elements layer within 5 min and forms an enriched layer. To capture urine elements, the AI100 scanner was programmed to acquire images at multiple depths, which are later stacked together to form a single image. The scanner initially focuses and captures the larger particles close to the bottom surface. Then the images of bacteria and yeast above and below the larger particles at a distance of 2 μm are acquired. These three images are stacked together to form a single image for the tele reporting and AI model analysis. Similarly, during the manual microscopy of GSC, fine focusing was used to capture bacteria and yeast after the larger particles are focused.

2.8. Automated digital analyzer (AI100)

The AI100 is an imaging-based autoanalyzer that accepts the GSC and scans the sample. The scan was done by capturing twenty-five microscopic images at different locations along the length and width of the scan zone in the cartridge. Thereafter, these images are pushed to the cloud, where a deep learning-based object detection model is invoked. Object detection models are deep models based on convolutional neural networks. The deep layers help in learning from simple to complex features present in the data. Usually, object detection is done via two types of models, single-stage detection or two-stage detection. In our case, the YoloX model [23] is implemented which is a single-stage detector. This suits the inference time feasibility and compute resource bandwidth. The YoloX model is mainly divided into three sections. Backbone, neck and head. The
backbone acts as a feature extractor, the neck connecting the backbone aggregates the features at different scales and provides them to the head. Finally, the head predicts the bounding box coordinates and object probabilities. For training this architecture, labelled data was prepared by getting it annotated via a pathologist. Subsequently, the model was trained using annotated data and prediction was performed. The model has learned various features for locating the medically significant cells. Figure 5 shows the input images on the left and corresponding feature maps learned by the model on the right for the WBC and RBC. Feature maps like background, foreground, cell shapes etc. are learnt. The model at the output generates potential bounding boxes along with respective confidence scores. This helps in locating medically significant cells in the image. Figure 6 shows the cells located by the AI model over the microscopic image acquired by the AI100. The AI model locates and identifies the various urine cells and particles.

![Figure 5](image-url) Input image to the left and various feature maps learned by model on the right (a) WBC (b) RBC
A consolidated report is published once the model is run on all the images captured for a sample. The model currently detects white blood cells (WBC), red blood cells (RBC), epithelial cells, yeast, crystal, cast and bacteria. It gives a quantitative assessment for RBC, WBC and bacteria and a qualitative assessment for epithelial, yeast, cast and crystal. The reporting methodology for AI100 for these cell types is according to the Table 3. Hence, for RBC, WBC and bacteria, it computes the average cell counts over all the FOV images and grades it as per Table 3. For qualitative assessment, epithelial, cast, crystal and yeast are marked as detected if detected by the AI model otherwise marked as not detected. Apart from the report generation at the web-based application, the pathologist can even view the microscopic image of urine elements to do tele reporting studies.

**Qualitative assessment**

| Epithelial, Crystal, Cast & Yeast | Detected (D) or Not Detected (ND) |

**Quantitative assessment**

**Table 3 Bucket-wise grading of urine cells and particles**

<table>
<thead>
<tr>
<th>Grade</th>
<th>RBC/WBC (cells per FOV)</th>
<th>Bacteria (cells per FOV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Significant (NS)</td>
<td>0-5</td>
<td>0-1</td>
</tr>
<tr>
<td>1+</td>
<td>6-10</td>
<td>1-2</td>
</tr>
<tr>
<td>2+</td>
<td>11-20</td>
<td>3-5</td>
</tr>
<tr>
<td>3+</td>
<td>21-50</td>
<td>6-10</td>
</tr>
<tr>
<td>4+</td>
<td>&gt;50</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

**Figure 6** Urine cells and particles detected and located by AI model.
2.9. Workflow

Figure 7 shows the workflow of GSC. Initially, 100 μl of raw urine sample is loaded into the chamber using a pipette allowing the cells and particles to settle down within 5 min. Here, the cells and particles settle down due to the low viscosity and density of the sample, enriching them at the bottom chamber surface. Finally, the cartridge is loaded into the AI100 device for automated image processing and analysis. Here, the images captured by AI100 is uploaded in the cloud where AI based analysis occurs and the report is generated.

![Figure 7 Workflow of GSC](image-url)
3. Results and Discussion

3.1. Comparative study

A comparative analysis of the GSC data (manual microscopy, tele reporting and AI model) with the gold standard (wet mount) is carried out. The cartridge performance was investigated using a range of analytical parameters: sensitivity, specificity, precision and accuracy. The definition of analytical parameters are described in the supplementary material. This evaluation enables a thorough understanding of the cartridge's performance and its ability to provide reliable and precise results. A total of 413 raw urine samples were analyzed in GSC and validated with the wet mount. The samples were loaded as mentioned in the workflow. The cells and particles were counted within the scan zone (6 x 8 mm²), which is present at the centre of the chamber. The urine sediment results were considered in agreement if they are within 1 grading difference [24].

3.1.1. Comparative study between GSC and wet mount data

Here, the comparative study of urine cells and particles count between the GSC (manual count) and wet mount (WM) was carried out with the help of pathologists. The cell count data for WM and GSC for three urine samples are shown in Table 4 (Only 3 samples data is shown. However, the study was carried out for 413 samples). This manual count data of GSC is used to calculate the analytical parameters by comparing with wet mount manual microscopy. Table 5(a) lists the sensitivity, specificity, precision, and accuracy value for the GSC cartridge by comparing it with WM data. The sensitivity is more than 94% for all urine cells and particles and has a maximum of 100% for RBC and cast. The specificity is more than 93% for all urine cells and particles and a maximum of 100% for RBC, WBC, bacteria, crystal, and cast. The precision is more than 96% for all urine cells and particles and has a maximum of 100% for WBC, bacteria, cast, crystals, and epithelial cells. The accuracy in the case of cast & crystal is 100%, and for the other elements, it is 99%. The data shows that gravity sedimentation cartridge mimics a conventional wet mount method by overcoming the centrifugation and slide preparation.

Table 4 Comparative study of cell count between GSC and wet mount

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Cartridge</th>
<th>RBC</th>
<th>WBC</th>
<th>Bacteria</th>
<th>Cast</th>
<th>Crystals</th>
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<th>Epithelial Cells</th>
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<td>ND</td>
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</table>
In Table 5(b), the analytical parameters for the tele reporting are presented. Tele reporting refers to the process of pathologists generating a report on the basis of microscopic images captured by the AI100 of a given urine sample. This data generated by tele reporting is compared with wet mount data. The images of various urine cells and particles like WBC, RBC, bacteria, epithelial cells, yeast, uric acid crystal, and calcium oxalate crystals captured by AI 100 during analysis is shown in Figure 8. The sensitivity is greater than 84% for all the elements except for cast is 63%. The WBC and cast have a high specificity of 97% and 98 % respectively. The epithelial have a low specificity of 80%. All other elements have a specificity greater than 90%. The precision is more than 84% for all the elements except for the yeast. The yeast has a precision value of 57%. The epithelial and bacteria have the least accuracy of 85% & 86%, and the other elements have more than 90% accuracy.

Table 5 Comparative analytical parameters between (a) GSC and WM, (b) Tele reporting and WM (c) AI model and WM and (d) AI model and Tele reporting results.

3.1.2. Comparative study between tele reporting and wet mount data

In Table 5(b), the analytical parameters for the tele reporting are presented. Tele reporting refers to the process of pathologists generating a report on the basis of microscopic images captured by the AI100 of a given urine sample. This data generated by tele reporting is compared with wet mount data. The images of various urine cells and particles like WBC, RBC, bacteria, epithelial cells, yeast, uric acid crystal, and calcium oxalate crystals captured by AI 100 during analysis is shown in Figure 8. The sensitivity is greater than 84% for all the elements except for cast is 63%. The WBC and cast have a high specificity of 97% and 98 % respectively. The epithelial have a low specificity of 80%. All other elements have a specificity greater than 90%. The precision is more than 84% for all the elements except for the yeast. The yeast has a precision value of 57%. The epithelial and bacteria have the least accuracy of 85% & 86%, and the other elements have more than 90% accuracy.
3.1.3. Comparative study between AI-model and wet mount data

The AI-based analysis is tabulated in Table 5(c). Here, the AI model generates sample report data using the images captured by AI100. This is compared with the wet mount data. Apart from bacteria and cast, the sensitivity of other elements is more than 81%. The cast report the least sensitivity of 71%, while the bacteria demonstrate a sensitivity of 76%. The epithelial, yeast and cast have the same specificity of 88%; for the other elements, the specificity is greater than 89%. The yeast and cast show the least precision of 52% and 56%, and epithelial has the highest 99%. All other elements have a precision value ranging from 76% to 98%. The accuracy of all urine elements is greater than 80%. The comparisons between the AI model and WM, as well as tele-reporting and WM, indicate that the AI model and tele-reporting exhibit slightly lower performance when contrasted with WM. This divergence can be attributed to the approach employed in WM, where pathologists typically analyse 6-8 field of view (FOV) by traversing a larger area of the slide. In contrast, AI and tele-reporting involve the examination of 25 FOV within a fixed area of the slide. Consequently, WM is subject to subjectivity, whereas the AI model follows an objective approach. This disparity can sometimes result in differences in cell count, particularly in the case of low-density samples. Therefore, the use of the AI model represents a significant step towards standardizing urine microscopy.

The proposed workflow enhances the productivity of pathologists by allowing them to concentrate on abnormal urine samples and also reduces the time required for sample preparation by reducing the time required for sample preparation by reducing the time required for sample preparation by...
technicians, thereby increasing overall throughput. Furthermore, the data obtained from GSC/WM should be complemented with biochemistry reports and patient-reported symptoms to aid in the diagnosis.

### 3.1.4. Comparative study between AI-model and Tele reporting

Finally, in Table 5(d), we present the comparative data for the AI model and tele-reporting. The sensitivity exceeds 90% for all urine elements except for casts, which have a sensitivity of 81%. In terms of specificity, all urine elements exhibit values of over 92%, except for epithelial and casts, which have specificities of 88% and 87%, respectively. Casts have the lowest precision at 48%, while the other elements maintain a precision of greater than 82%. White blood cells (WBC) and red blood cells (RBC) achieve the highest accuracy at 99%, while casts have the lowest accuracy at 86%. The accuracy of all other urine elements exceeds 90%. These results demonstrate that the AI model's performance closely aligns with the manual verification of images as reported by pathologists during tele-reporting.

Once the comparative studies demonstrated significant consistency, we proceeded with repeatability and reproducibility experiments. Repeatability studies involved six replicates within each run, while reproducibility studies spanned across different days and devices. Overall, all the urine elements exhibited over 90% mean agreement among the replicates. Additional information can be found in the sections 2 & 3 of supplementary material.

### 3.2. Sample stability in GSC

In this study, we conducted the evaporation of raw urine samples and counted the cells and particles in GSC at various time intervals. A total of 6 samples with a cartridge having three chambers are evaluated. To begin with, the sample is loaded in all three chambers of a cartridge. Further, at various time intervals of 0, 30, 60 and 90 min, the position of the gas-liquid interface is tracked at the inlet and outlet of each chamber and cells and particles are counted at the scan zone. The same experiments are repeated for the other five samples. Figure 9 (b) - (e) shows the change in the gas-liquid interface of raw urine samples in GSC at different time intervals. Even for the highest time of 90 min, it is observed that the evaporation is minimal, mostly occurring at the inlet side due to a broader inlet opening of 4 x 2 mm². The gas-liquid interface travelled a maximum of 2.9 ± 0.4 mm toward the scan zone without affecting it. The evaporation is minimal at the outlet as the opening is 1 x 0.5 mm². The gas-liquid interface moved toward the scan zone at the outlet is 2 ± 1.2 mm. Hence, it is observed that after loading a sample in GSC, it can be analyzed up to 90 min as the evaporation loss is not affecting the scan zone (central region) for imaging and analysis.

Similarly, Table 6 depicts the urine cells and particles count data of the sample loaded in all three chambers for different time intervals. Cells and particle count data agree with wet mount prediction. Hence, till 90 min, there is no change in morphology and count. This indicates there is no leakage between the chambers. The bond between the coverslip, spacer tape and PMMA slide is leak proof. The sample stability is increased to 90 min keeping the cells and particles count unaffected compared to wet mount where the sample stability is 10 - 15 min. Since, evaporation is very minimal, the drift is avoided. This was visually inspected by checking the movement of cells and particles under the microscope.
Table 6 Cells and particle count of raw urine sample in GSC at a different time interval

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cartridge</th>
<th>Chamber</th>
<th>RBC</th>
<th>WBC</th>
<th>Bacteria</th>
<th>Cast</th>
<th>Crystals</th>
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</tbody>
</table>

Figure 9 Shelf-life study of raw urine sample in GSC at (a) Empty three chamber cartridge (b) 0 min (c) 30 min (d) 60 min (e) 90 min
3.3. Multisampling of chamber

The wet mount is a conventionally employed technique for microscopic analysis of urine. The cost of consumables for the wet mount (BOM price) is $0.05. The price of GSC should be lower or equal to that of the wet mount consumables. However, a single chamber GSC is priced at $0.18. GSC optimization of the chamber area on the slide becomes necessary to achieve a cost-competitive. Since, the sample volume required is 100 µl to encompass all urine cells and particles, the corresponding dimension of the chamber is 13.8 x 22.5 mm². This dimension allows the inclusion of three chambers in a single cartridge, enabling the simultaneous analysis of three urine samples. Figure 9(a) illustrates the GSC with three chambers. The total cost for the three-chamber GSC amounts to $0.20, resulting in a price of $0.06 per test. Thus, by incorporating multiple sampling within a single cartridge, the cost becomes comparable to wet mount consumables and simultaneously facilitates the analysis of multiple samples.

3.4. Pre-stained capillary slides

The predominant bacteria found in urine sample are E.coli and Bacilli which can also resemble dust particle. Hence, to overall improve the imaging quality and AI model performance staining is employed. The staining of urine cells is also required for urine cytology, especially for better imaging and classification of cancerous cells. Here, the objective is simultaneously performing cell staining and gravity sedimentation of cells and particles. Figure 10(a) shows the GSC with the pre-stained slide. Pre-stain slides are prepared by cleaning PMMA slides and coverslips using a soap solution (5% Extran). Both are then sonicated for 5 min with a soap solution & DI water and air-dried. The slide is dip coated with P100 and air-dried to make the surface hydrophilic. Again, it is dip coated in the dye solution and air-dried at room temperature. The spacer tape cut in the required dimension using a laser machine is placed over the pre-stain slide, and the coverslip is placed over the spacer tape to seal the chamber by leaving inlet and outlet ports. After the pre-stain slide is fabricated, the raw urine sample (100 µl) is loaded. Apart from the cells and particle settling, the dye coated at the bottom of the chamber diffuses into the raw urine solution, which stains the cells. Figure 10 (b) the GSC is shown with unstained cells on uncoated slides while Figure 10 (c) illustrates the GSC with stained cells on pre-stained slides. Here, it shows that WBC and epithelial cells are stained in the presence of pre-stain slides. Traditionally, the staining of urine cells involves mixing the stains with the urine sediments (centrifuged) for 1 - 2 min and the wet mount is prepared for microscopic examination. In the present case, the GSC manufactured using pre-stain slides reduces the steps required for staining the cells. The overall workflow involves only loading raw urine samples in the GSC. Further, studies are in progress to enhance the staining performance of individual urine cells and particles.
4. Conclusion

In urinalysis, simple workflow and automation are the need of the hour. The GSC brings forth an easy workflow, and AI100 provides automation in image acquisition and analysis using AI-based tools. The novelty of the cartridge is the unique design and workflow in which urine cells and particles are concentrated using gravity-based sedimentation at a specified depth of the chamber. Here, a raw urine sample is loaded into the capillary chamber. This is a passive process where no external pump or centrifugation is required for enrichment. The entire process of sedimentation completes within 5 min. This process acts as a substitute for the traditional process for enrichment using centrifugation and sample preparation over the glass slide.

The chamber’s depth is critical to match the cells and particle count with the gold standard. The number of cells / FOV increases as depth increases and vice versa. Hence, an optimum depth range has been found to agree with the gold standard. Also, the chamber is designed in such a way that the evaporation is reduced drastically, and no drift of the particles and cells in the chamber occurs. In a wet mount, the sample is exposed to the atmosphere at the periphery of the coverslips, accelerating the evaporation and, in turn, the drift of cells and particles. This reduces the sample
shelf life to 10 - 15 min. While in the GSC, only the inlet and outlet are exposed to the atmosphere, drastically reducing evaporation. This increases the shelf life of the sample in the chamber by 90 min and prevents the drift of cells and particles, which assists in the counting.

To optimally utilize the cartridge space, multisampling of the chamber is realised. Three chambers were incorporated into the cartridge. This helps analyse three different urine samples simultaneously, bringing down the cartridge's cost by one-third. Along with enrichment, staining of cells also occurs within 5 min using pre-stain slides. Hence, this is a unique design and method where enrichment and staining of cells co-occur within 5 min with a simplified workflow, drastically improving the inducer experience.

Once urine elements are enriched, the GSC is loaded in AI100, where auto-imaging occurs at a high-power field. The images are uploaded into the cloud, which is analyzed using an AI-based model. The AI model works using deep learning. It’s formulated as an object detection method that helps in the accurate detection of RBC, WBC, bacteria, yeast, epithelial cells, crystals and cast. The RBC, WBC and bacteria are estimated quantitatively, and yeast, epithelial cells, crystals, and cast are estimated qualitatively. The analytical parameters like sensitivity, specificity, precision and accuracy are calculated for WM vs GSC (manual microscopy, tele reporting and AI Models). The GSC vs WM investigation demonstrates that GSC effectively emulates a traditional wet mount method eliminating the need for centrifugation and slide preparation. In the case of the AI vs tele reporting, the results indicate that the model is performing good. Hence, a novel GSC with a simplified workflow designed to auto analyse using AI100 would drastically improve the inducer experience by reducing the sample preparation time, being less labour-intensive and minimising the manual error by employing AI tools for urine cells and particles detection and counting.

Supplementary Material

The supplementary material includes the definition of analytical parameters, repeatability and reproducibility data.

Acknowledgement

The authors express their gratitude to Anil Modali and Apurv Anand (Product Department, SigTuple Technologies Pvt. Ltd, Bengaluru) and Department of Pathology, Father Muller Medical College Hospital in Mangalore, India, for providing the valuable samples. Special thanks are extended to Ms Saranya for her invaluable effort in manufacturing the cartridges.
References


Conflict of interest

The authors assert that they do not possess any identifiable conflicting financial interests or personal relationships that might have given the appearance of influencing the research presented in this paper.

Data availability

The information backing the conclusions of this study can be obtained from the corresponding author upon a reasonable request.
Graphical Abstract

STEP 1
Loading 100 µl of raw urine sample into the cartridge

STEP 2
Automated scanning of cartridge in A1100
Loading the cartridge in A1100
Wait for 5 min

Urine cells and particle settles under the influence of gravity to form enriched layer.
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: