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## BACKGROUND

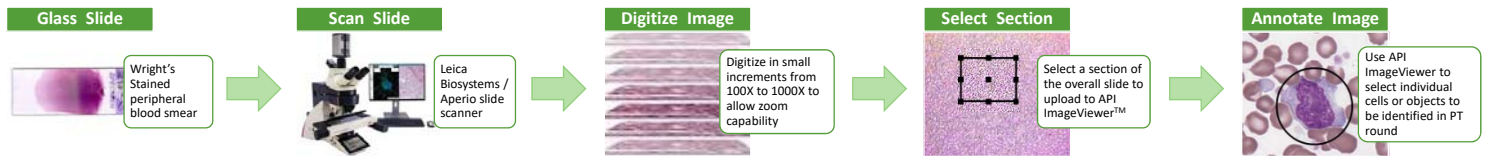
In order to provide a valid assessment of performance, a proficiency testing (PT) program must provide comparable proficiency test items to all participants. In addition, when possible, proficiency samples should mimic actual patient specimens and be tested in the same manner. However, the provision of PT samples that meet these criteria has proven to be a challenge in some areas of the laboratory, such as clinical microscopy. API identified three such situations where traditional PT samples do not fully meet the needs for PT assessment.

1. Blood cell identification and assessment of morphology is performed by microscopically viewing a Wright's Stain blood smear. While it is possible to produce multiple blood smears for use in a PT program, it is not possible to mark the same cell for identification.
2. Along with Gram Stain reaction, an important component of sputum Gram Stain review is the determination of sputum quality. While it is possible to obtain sputum in volumes necessary for a PT program, homogeneity across PT samples would be difficult to achieve.
3. Sperm Motility testing may only be performed on fresh (<1 hour after collection) semen specimens. Due to this extremely short window of viability, PT samples that mimic patient specimens are not available for this test.

## DEVELOPMENT / METHODS

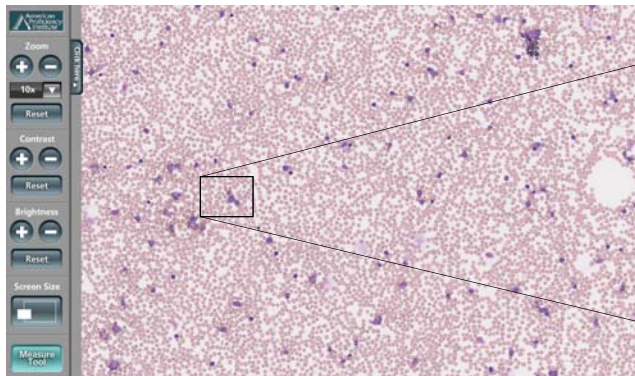
The solution to the limitations inherent in these three microscopy situations was to create virtual PT challenges.

The process used to create the virtual PT for the blood cell identification and the direct Gram Stain was similar. We wanted to create an on-line simulation of the microscopy that is used to perform these tests. The blood cell smears and direct Gram Stain slides were scanned and digitized by an outside provider of these services. A web-based application was developed to present these images to participants on the API website. This custom application was used by API to add annotations to cells or objects to be identified, and by participants to perform manipulations to the image that simulate viewing under a microscope.



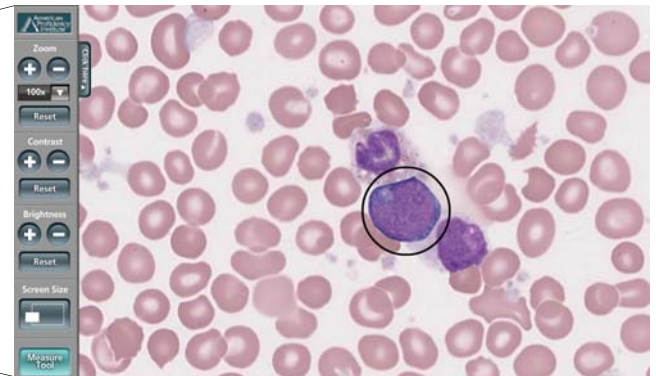
In a sperm motility test, laboratories assess live sperm for motility and progressive motility. For virtual PT, the movement of the sperm was recorded and then made available as a PT challenge in a video format.

## BLOOD CELL IDENTIFICATION / MORPHOLOGY



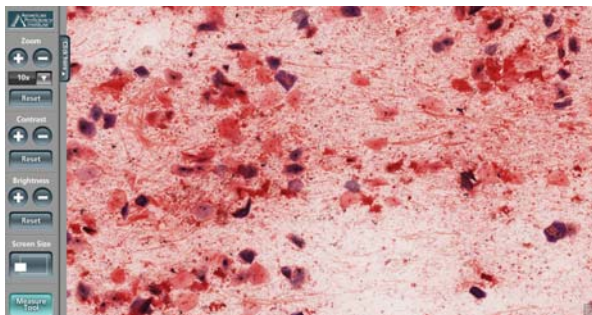
Wright's Stained slide at 100X magnification, viewed with the API ImageViewer

- Slide may be reviewed at low power (100X magnification) to assess overall smear quality and patterns.
- Use toggle switch to zoom in to 1000X magnification to examine individual cells.
- Adjust contrast and brightness as needed.



Portion of the slide at 1000X magnification, with a specific cell marked for identification

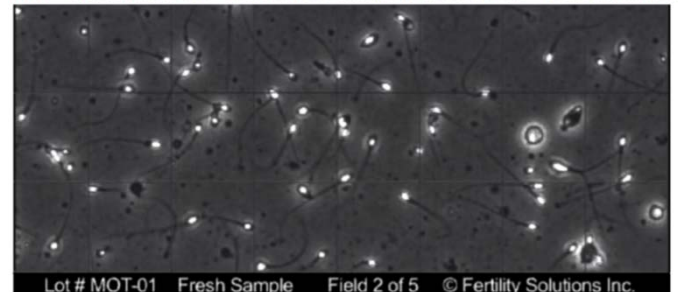
## DIRECT GRAM STAIN



Gram stained sputum specimen at 100X magnification. Slide shows presence of >10 squamous epithelial cells per low power field. This sputum specimen was not adequate for culture and should have been rejected as a clinical specimen. As a PT challenge, 20 of 20 participants did reject this specimen.

## SPERM MOTILITY

Press to start the video. Press to view the video full-screen. Press the Esc key or the to exit full-screen mode.



Still frame from a video of a fresh semen specimen taken using phase microscopy at 200X magnification. The ten-minute video shows five fields of fresh semen and five fields with sperm immobilized, from which participants are to determine the percentage of motile and progressively motile sperm.

## CONCLUSION

While the three situations described above do not completely recreate the entire testing process with a patient specimen, virtual PT provides opportunities for assessment of laboratory performance in areas not previously possible. Viewing whole slide images for blood cell identification allows PT providers to mark individual cells for identification, as well as allowing laboratorians to assess morphology within the context of the entire smear. Virtual PT for direct Gram Stain on sputum specimens provides external evaluation of a laboratory's ability to determine suitability of a sputum specimen for diagnosis of pneumonia. Semen specimens for sperm motility are extremely labile; a video image captures the motility so that it becomes possible to assess performance across testing sites. It is likely that virtual PT can be used with similar success in other areas of the clinical laboratory where the use of traditional samples is not feasible.

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