## Formation and Removal of Precipitates in Alcohol Stations

One of frequently asked questions we have received about maintenance of Sakura's tissue processors is what is fat-like precipitate formed in the first and second alcohol stations. As it is very difficult to remove, we are also often asked about how to remove it and clean reagent bottles placed at these stations.

To answer such requests, we have integrated internal data into this document that explains about our presumption of and studies on the substances, referred to as "fat-like" in the question, which can accumulate on the bottom of the reagent bottles at the alcohol stations. The document also describes how to reduce the precipitation and how to remove it from the bottles.

**<Presumption>**

There are three kinds of components presumed to form precipitates in the first alcohol station in a tissue processor; (1) crystals produced from phosphates used in a fixative as a buffer, (2) insoluble components such as fat from specimens and (3) tiny tissue fragments and/or blood apart from specimens.

If tissues are directly placed into a tissue processor from a formalin fixative containing a phosphate buffer solution, they bring the buffer solution into high concentration alcohol stations, which can cause precipitation of phosphate from the buffer solution. Substances presumed to originate from tissues are fat, which is much included in mammary glands, tiny tissue fragments and blood cells apart from specimens during tissue processing.

**<Studies>**

Through cooperation from some customers in Japan, we have determined if the above three substances are present in the precipitates formed in the first alcohol station.

1. Phosphate salt

   The precipitates were dried and rinsed in distilled water. The rinse water was qualitatively analyzed with a rapid qualitative/semi-quantitative test kit for phosphate salt. The result was that a positive reaction was shown on all test subjects obtained from customers (A, B and C) who were using neutral buffered formalin. This proved that phosphate was present in precipitates. (See the photo 1.)

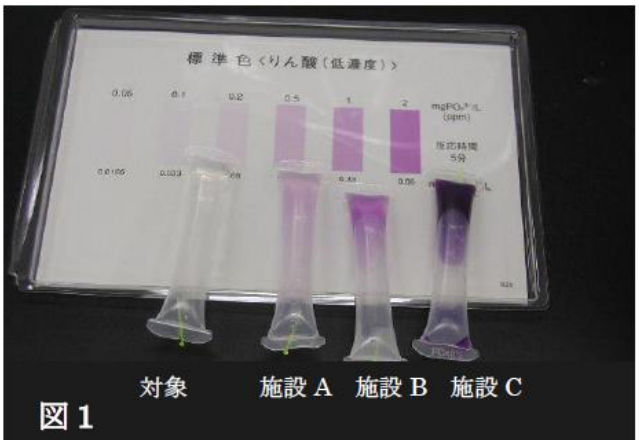
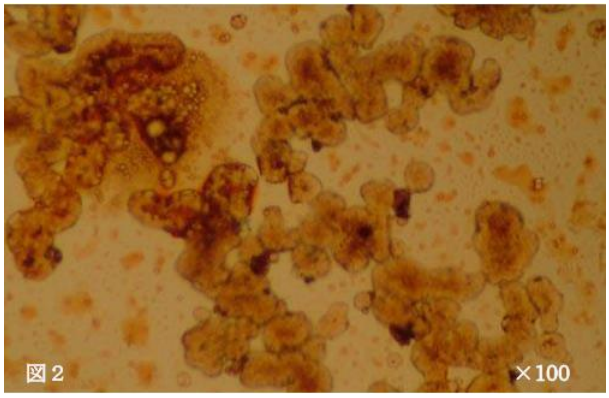



Photo 1: (from the left) Control, Customer A, Customer B and Customer C

2. Fat

A Fourier transform infrared spectroscopic technique (FT-IR) was used to analyze an intramolecular bonding in the precipitates under infrared radiation. The samples we had obtained from some customers showed all the same infrared absorption spectrum. This indicates that they were containing tissue-derived fatty acids. In addition, the precipitates were stained with the Sudan III. Although the color tone was somewhat different, they appeared orange-yellow to orange-red. This also indicates that the precipitates consist of fatty materials. (See the photo 2.)




3. Tissues and blood

The precipitates were smeared onto glass slides and stained with the H&E protocol. Red blood cells and structurally-changed tissue cells were observed under microscope.

**<Reduction of precipitation and cleaning of reagent bottles>**

1. Phosphate salt

Rinse fixed tissues with water to decrease carryover of excess phosphate salt into alcohol solutions on the instrument. To prevent forming phosphate salt as much as possible, it is also recommended to replace alcohol solutions more frequently. If phosphate salt precipitates and accumulates in the reagent bottles, even with frequent solution replacement, rinse the inside of the bottles with warm water. In addition, run the Warm Water Flush on a regular basis to remove precipitates formed inside the tubes going to the relative stations.

2. Fat

As alcohol solutions are heated in the retort during tissue processing, fat is dissolved in alcohol. Alcoholic solutions become oversaturated with fat and then the dissolved fat turns to a solid in the bottles as the solution temperature changes after the end of tissue processing.

The most preferable way to remove fatty precipitates from the bottles is to emulsify the precipitates with warm water and a neutral detergent. If they still remain in the bottles, try the way of dissolving them with an organic solvent such as chloroform. Note that both of the two ways require rinsing the inside of the bottles thoroughly with water afterward, giving consideration to using the bottles next time. In addition, replacing alcohol solutions more frequently is effective as a measure for precipitation prevention.

3. Tissues and blood

Tissue fragments and blood can be removed in a popular way. If they are left in the bottles for a long time, scrape them away with a brush because they become difficult to be removed.

In general, it is said about the cleaning (1) to prevent a contaminated bottle from being dried, (2) to wash a bottle as frequently as possible, (3) to employ physical force such as a brush to increase a cleaning effect, (4) to put a high concentration of an effective detergent in a bottle for a longer time, and (5) to use a physical cleaning method such as an ultrasonic cleaning device.

End