Method 1623 IMS Procedure - Guidance Information

March 20, 2006

To ensure high and consistent recoveries of organisms, the techniques used in the immunomagnetic separation (IMS) procedure of Method 1623 are critical. The procedure is designed to separate the bead-organism complexes from the debris in the sample and eliminate the transfer of the debris to the microscope slide. Because of the variation in amount and type of debris in samples, IMS is a very dynamic processing step. There are techniques which analysts may use to increase the recovery (and precision) of the bead-organism complexes and reduce the amount of transferred debris. This guidance is provided to supplement the tips and instructions in Method 1623, Section 13.3.4 and the tips in the product information for beads and magnets available at www.invitrogen.com/dynal.

A laboratory may consider implementing additional rinses if slides are covered with debris hindering the visualization of *Cryptosporidium* and/or *Giardia*. The goal is to maximize the recovery of target organisms while minimizing the amount of debris on the slide. It is suggested that a rinse be incorporated at the Leighton tube separation step or the microcentrifuge tube separation step but not both steps to prevent over-rinsing and loss of organisms. Laboratory experience analyzing samples from a particular source and experience with different types of pellets should be used to determine which samples would benefit most from an additional rinse and at which step a rinse would be most effective. Specific factors to evaluate may include how various sediments react during IMS, the amount of debris carried over to the slide, and organism recovery rates. It is suggested that a laboratory develop criteria to determine when an additional rinse is used on a particular sample. If a laboratory chooses to incorporate rinses, then the rinse also needs to be incorporated into the appropriate QC samples, i.e. the associated MS/MSD samples and an equivalent percentage of ongoing precision and recovery samples.

Guidance on IMS tips and techniques, including additional rinses, is provided below based on the Sections currently found in Method 1623, 2005 version. This document does not replace Method 1622/1623 and only provides suggestions that laboratories may implement to improve method recovery and precision.

Transfer to Leighton tube - Method 1623, Section 13.3.2.1

- Completely homogenize resuspended pellet immediately prior to transfer. Vortex vigorously for 10-15 seconds and visually inspect to ensure complete homogenization.
- The volume of the transferred sample plus 2 rinses should total 10 mL. The addition of 2 mL of buffers brings the total in the Leighton tube to 12 mL.
- Use reagent water for the rinses.
- Do not rinse the pellet to remove eluting solution prior to transfer to avoid loss of cysts and oocysts.

Leighton tube/MPC®-1 or MPC®-6 separation step - Method 1623, Sections 13.3.2.7 - 13.3.2.11

- Do not let the Leighton tubes sit motionless before decanting to prevent binding of low-mass, magnetic, or magnetizable material. Be sure to decant the supernatant immediately at the end of the 2 minute rocking period. To aid in the removal of supernatant and debris during decanting, the cap of the Leighton tube may be carefully removed when the Leighton tube is pointing down at the end of the 2 minute rocking period.
- Depending on the type and amount of debris in the sample, either of the two optional rinse procedures at the Leighton tube separation step may increase removal of debris from dirty samples. In order to reduce the risk of organism loss, it is recommended that only one of the following rinses be performed for a given sample. Alternatively, the optional rinse procedures may be performed at the microcentrifuge tube separation step instead of the Leighton tube separation step.
    - Remove remaining debris left behind in the Leighton tube after decanting. Rinse the sides of the tube with 1 mL PBS while the Leighton tube is still in the MPC®-1 (MPC®-6). Do not resuspend beads or allow rinse to flow over beads. Hold the Leighton tube horizontally with the magnet/bead side up to protect the bead pellet from the rinse.

- Remove debris from the beads by rinsing the bead pellet. Remove the Leighton tube from the MPC®-1 (MPC®-6) and add 10 mL of PBS (or PBST). Very gently resuspend the bead pellet (with the bead-organism complexes) - do not vortex or mix vigorously. Replace the tube in the magnet and repeat rock and decant steps (Method 1623 Sections 13.3.2.8 - 13.3.2.11). This rinse will help remove debris that may be trapped with the beads.

- Aspirate residual liquid and debris after decanting. Allow tube to sit undisturbed for 30-60 seconds before aspirating residual liquid.

Leighton tube to microcentrifuge tube transfer - Method 1623, Section 13.3.2.12 - 13.3.2.13

- When resuspending the beads prior to transfer, add the 1X SL Buffer A directly to the flat side of the tube. Resuspend the beads and material located on the flat side only. Try to avoid any debris still present on the round sides of the Leighton tube.
- Pipette mix during resuspension of the beads and during each rinse in the Leighton tube to ensure resuspension of all beads. During each pipette mix, release the liquid down the flat side of the tube to further rinse the tube. Remove all liquid and beads from the Leighton tube.
- Completely rinse the flat side of the tube by using 0.5 mL of 1X SL buffer A to perform each of the two rinses.
- Remove all the liquid. Allow the Leighton tube to sit undisturbed for 60 seconds after last rinse to collect residual liquid, then transfer to the microcentrifuge tube.

Microcentrifuge tube/MPC®-S separation step and dissociation - Method 1623, Sections 13.3.2.14 - 13.3.3.6

- MPC®-M magnet is no longer available for purchase. The magnets in circulation can still be used.
- The MPC®-S magnet has two notch positions for the placement of the magnet (Figure 1), each holds the magnet against a different wall of the housing. The result is that the magnet is held in either a vertical position (Figure 2) or a slanted or tilted position (Figure 3).
- The vertical position is used with larger volumes (1.5 - 2.0 mL) and/or round bottom microcentrifuge tubes. Use the vertical position (Figure 2) when producing the bead pellet prior to dissociation. This produces a compact, consolidated bead pellet. The location of the pellet is higher on the back wall of the tube as compared to the location of the pellet if the slanted magnet position is used.
- In Section 13.3.2.16 after waste liquid volume has been aspirated to below the bead pellet, discard the aspirated waste and gently pipette mix the remaining liquid and debris, without disturbing the pellet, to resuspend the debris and improve the removal of the debris.
- If rinses were not performed at the Leighton tube separation step, two optional rinse procedures at the microcentrifuge separation step may reduce debris in dirty samples. In order to reduce the risk of organism loss, it is recommended that only one of the following rinses be performed for a given sample.
    - Remove debris remaining after aspiration. If debris remains after aspirating the waste liquid from the tube, the microcentrifuge tube can be rinsed with 0.5 mL of PBS added to the front side of the tube while still positioned in the MPC®-S. Do not resuspend the bead pellet or allow rinse to flow over beads. Again, pipette mix and aspirate the rinse.
    - Remove debris from the beads by rinsing the bead pellet. Remove the tube from the magnet and add 1 mL of PBS (do not use PBST as the Tween® may interfere with adhesion to the slide). Resuspend the beads (with bead-organism complexes) by gentle inversion, do not vortex or mix vigorously. Repeat Sections 13.3.2.14 - 13.3.2.16 of Method 1623.
- After aspirating rinse, allow microcentrifuge tube to sit undisturbed for 30-60 seconds before aspirating any remaining liquid.
- The slanted or tilted position (Figure 3) is used with smaller volumes (0.05 - 1.0 mL) in step 13.3.3.6. Use the slanted position for the collection of the bead pellet during acid dissociation. The slanted position will place the magnet closer to the conical microcentrifuge tube and produce a compact and consolidated bead pellet. The location of the pellet is lower on the back wall of the tube as compared to the location if the vertical magnet position is used.

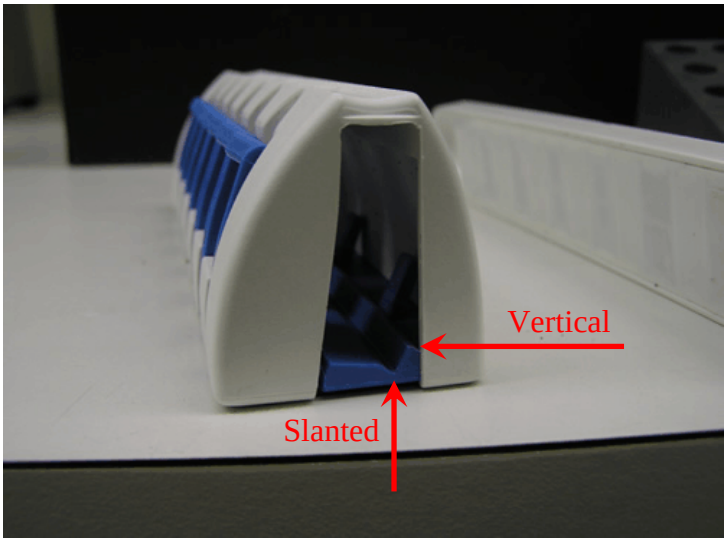

Figure 1. Vertical and slanted notch positions

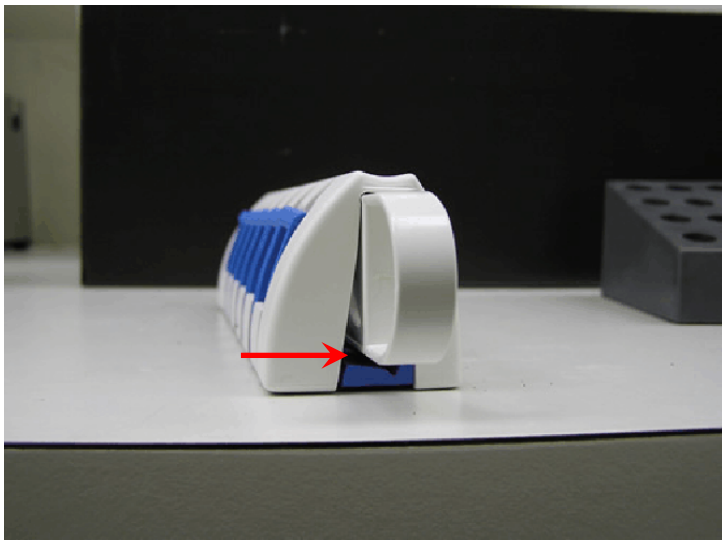

Figure 2. Vertical magnet position showing space between tube and magnet

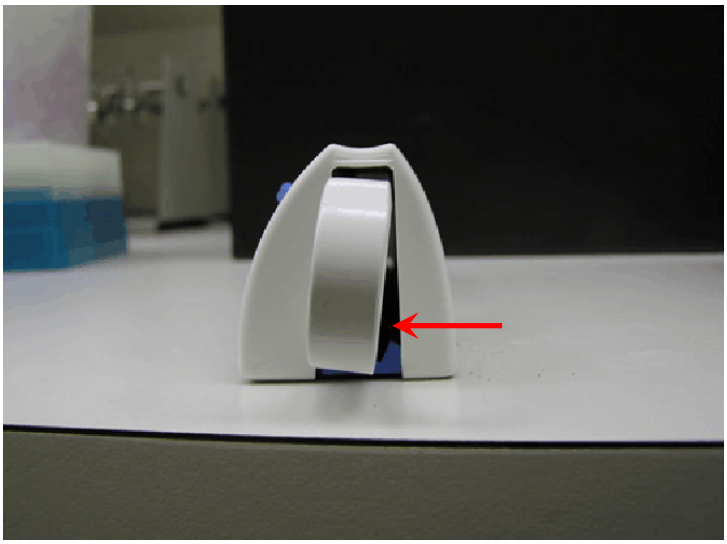

Figure 3. Slanted magnet position showing space between the magnet and the back of the holder

Application of dissociated sample to slide - Method 1623 Sections 13.3.3.7 - 13.3.3.10

- The following suggestions will help reduce the impact of debris on slide examination and may be used instead of or in combination with any additional rinses.
  - Apply the two dissociations to separate well slides. Impact of the debris on slides can be reduced by spreading the debris over two well slides instead of one. In Method 1623, Sections 13.3.3.7 and 13.3.3.8, prepare two separate well slides for each sample.
  - Use the largest-sized well slide in order to spread debris over a larger area. See Method 1623, Section 6.10.1 for possible slides. Other equivalent slides are available including 15 mm SuperStick™ slides from Waterborne™, Inc.

Quality Control

- Incorporating additional rinses is considered a "substantive" modification to Method 1623 and requires demonstration of acceptable performance through an IPR set and method blank. It is strongly recommended that a laboratory also demonstrate acceptable performance with a matrix spike/matrix spike duplicate (MS/MSD) and an unspiked matrix sample. If a laboratory uses additional rinses for some samples, a laboratory must process associated matrix spike samples using the same method variation (with or without rinses). The procedure must be used to demonstrate ongoing laboratory performance by using the different variations with the same percentage of ongoing performance samples as percentage of field samples analyzed with that variation. For example, if 25% of the field samples are processed with an additional rinse, every 4th OPR would be analyzed using the same type of additional rinse.