Cross-Training, Collaboration & Partnership: The Path to a Comprehensive Campus Cell Sorting Biosafety Program

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Yale University

Yale Cell Sorting Biosafety Program

- Cross Training
  - Learning phase
- Collaboration
  - Setting up a program
- Partnership
  - Maintaining the program
  - Sharing responsibilities

Relationship / Partnership

- Cell Sorting Operators & Biosafety / EHS Professionals
- Biosafety/EHS Share:
  - Risk Assessment and Risk Management
  - Principles of Biosafety & training
  - Regulations and emergency response
- Cell Sorting Professionals Share:
  - Knowledge of equipment (function, operation) & training
  - Contaminated portions of equipment after spills
  - Process flow (and more…)

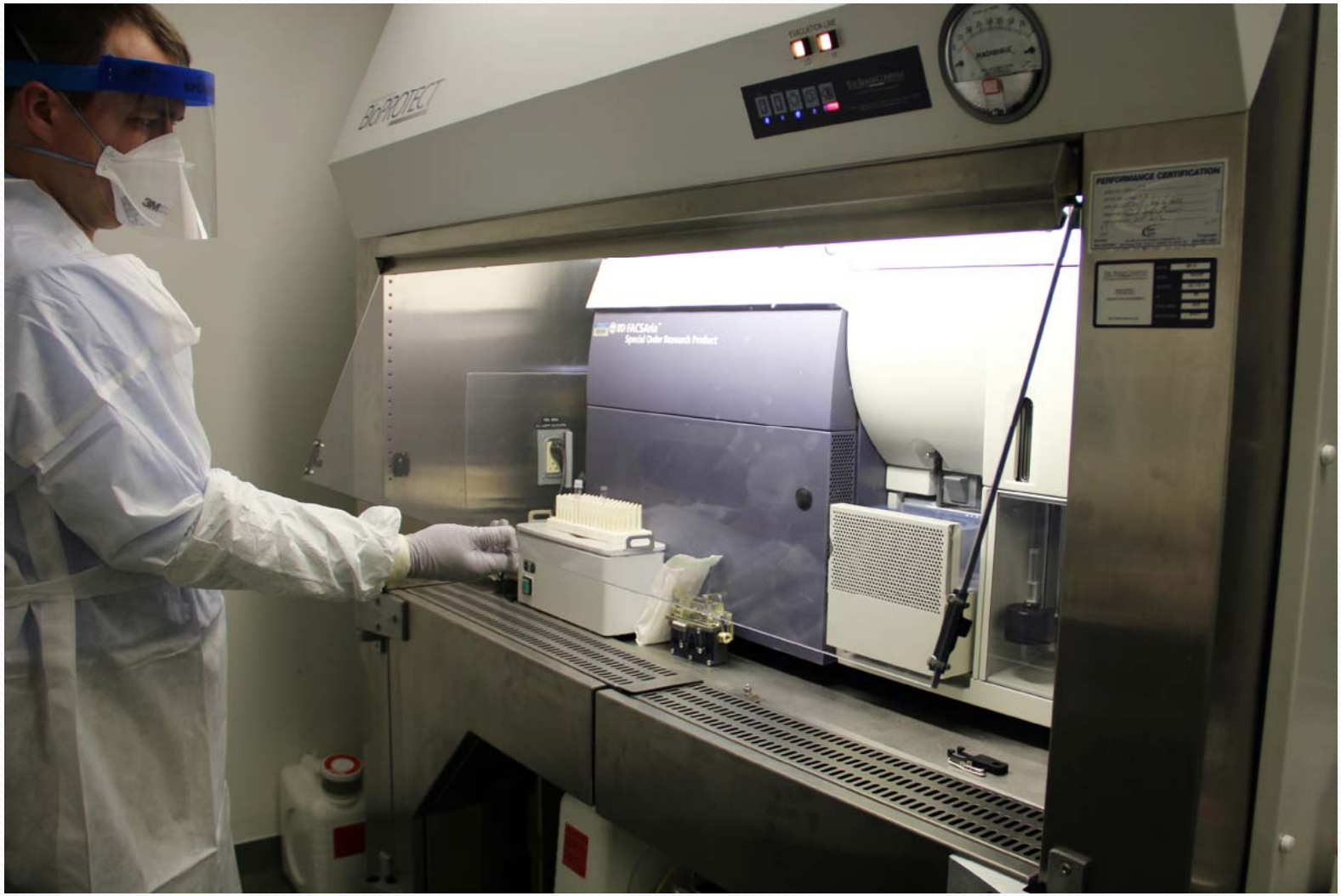
Yale Cell Sorting Biosafety Program

- Identification of all high speed cell sorters on campus
  - Interview sort operators
  - SciQuest (past, new and future purchases)
  - Core labs
  - Department specific sorters
- Equipment questions on EHS biohazard registration forms
- Training of EHS professionals (equipment recognition on inspections)

Yale Cell Sorting Biosafety Program

- Campus user group meetings
- Lab inspections
- Core Facility registration form (where applicable)
  - ISAC template
- Risk awareness of research materials (Department specific sorters)
- Authorization for high speed cell sorting procedures

Risk Assessment:
Qualified/Trained Staff is Vital to Biosafety



Yale Cell Sorting Biosafety Program

- Testing & certification of HEPA filtered control devices (AMS', Class I and II BSCs)
- Equipment validation testing
- Negative pressure verification (room, sorter)
  - BSL3 Room Validation
- Minimum PPE requirements for biohazards
- Equipment-specific SOPs
  - (not all high speed cell sorters created equally)
- Transport SOPs
- Emergency response (spill/release) SOPs

Filter Evaluation

Filter Evaluation

Test HEPA filters before use (after shipment, storage, moving)

Leaks can occur at gasketing, glued seams, and through the glass fiber paper filter

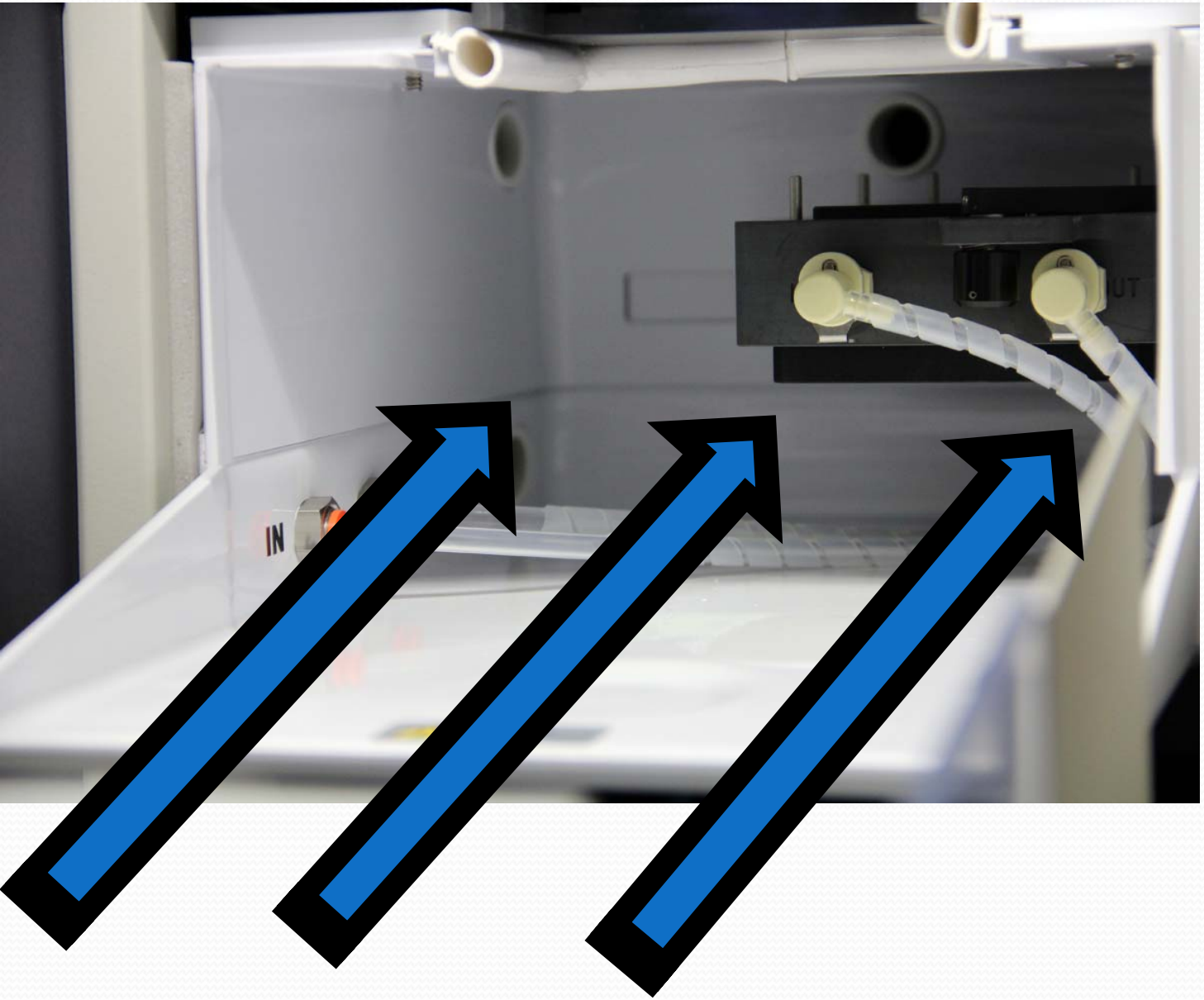
Yale Cell Sorting Biosafety Program

- Added Core Facility Manager to Yale BSL3 Subcommittee (review high risk agent research)
  - Share best practices with all groups
  - Approved SOPs used as templates for new locations (starting point)
  - Demo particle challenge testing and assessment
    - More frequent with elevated risk
  - Assist with authorization for biohazard use with new sorters
- SOP observation assessments for individuals sorting human pathogens

Yale Cell Sorting Biosafety Program

- Clear "bands" established (CT biohazard regulations influence work location)
  - Low Risk (BSL1)
  - Moderate Risk (BSL2)
    - Primary human materials, other human cells, defective RG2 pathogen vectors, non-human primate cells
  - High Risk (BSL2-enhanced and BSL3)
    - All Risk Group 2 and 3 human pathogens
    - Samples known to harbor human pathogens if used for research (CT State DPH registration required)

High Air Changes per Hour (ACH) in Chamber



Area = 0.3 ft3

Avg. Airflow velocity = 147 f/m
Area Opening = 0.34 ft2
CFM = 147 f/m x 0.34 ft2
CFM = 49.98

$$\text{ACH} = \frac{\text{CFM} \times 60}{\text{Area}}$$

$$\text{ACH} = \frac{147 \text{ f/m} \times 60 \text{ m/hr}}{0.3 \text{ ft3}}$$

ACH = 9,996

Note: Cell Sorter BSL3

Lab = 17 ACH

Yale Cell Sorting Biosafety Program

- Negative pressure rooms for BSL2 (and higher)
- AMS at BSL2 (and higher)
  - Containment aerosol challenge verification
- Trained, experienced operators (all levels)
- PPE (lab coat, gloves and full face protection) at BSL2
  - Gowns, 2 pair gloves, face shield, sleeves at BSL3 with respirator consideration based on risk
- Primary containment enclosure and AMS (BSL2-enhanced and BSL3)

Tell Tale Airflow Monitor

Monitoring pressure differential between lab and anteroom

Digital Pressure Monitors

Color Band Magnehelic Gauge

Power of Professional and Regional Sharing

- Journal articles, List serve groups
- Online posts (extra pulmonary TB)
- Rockefeller University Cell Sorting Biosafety Summit (Part 1 and Part 2)
  - 2 Full Days on cell sorting biosafety hazards
  - Best practices shared
  - Many different approaches
  - Q&A Forum

Central Guidance Document

- International Society for the Advancement of Cytometry (ISAC) Cell Sorter Biosafety Standards
  - Cytometry, Part A, Volume 85, Issue 5, pages 434-453, May 2014
    - Kevin Holmes et al
- Pioneers (Schmid, Perfetto, Holmes)
  - Pletcher, Lyon, Lopez….
    - Your institution's pioneer?

2014 ISAC Cell Sorting Biosafety Standards

- Updates and augmentation of:
  - Lab Design
  - PPE
  - Equipment specific SOPs
  - Validation of aerosol containment
    - Bacteriophage
    - Glo-Germ
    - Also:
      - Technetium-99 (UCONN – Wallace et al)
      - Fluorescent beads (Pletcher & Lyon)
      - NIH “next” (Holmes and continuous improvement)

Donning PPE for Training Session

Verifying PAPR Performance

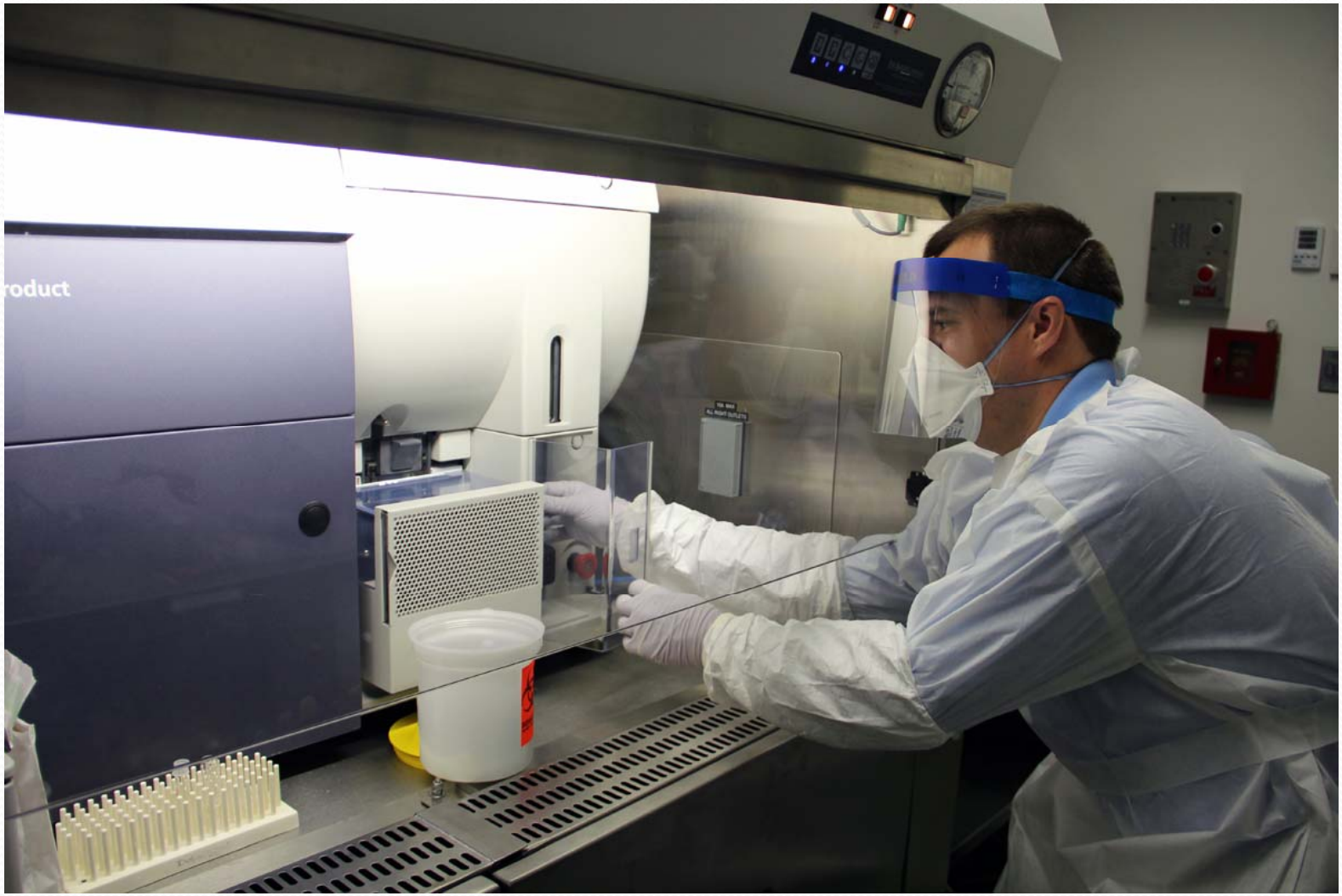
2014 ISAC Cell Sorting Biosafety Standards

- Industry Guidance
- Does not take the place of Risk Assessment!
  - Page 7, bottom of Table 2, footnote a
    - **"This list represents examples of biosafety level determination for cell sorting of specific agents. The final determination of the biosafety level is dependent upon the risk assessment conducted in collaboration with safety specialists, subject matter experts, and the IBC or equivalent."**

2014 ISAC Cell Sorting Biosafety Standards

- Flexibility Based on Risk Assessment
- Page 13 – 1st full paragraph
  - PPE for BSL3
  - **"Note that given the combination of engineering controls, aerosol evacuation system and instrument located within a certified biosafety cabinet, may, dependent upon a risk assessment, allow for alternate combination of PPE. This requires approval by the cell sorting operator/facility director, biosafety professional and the Institution's biosafety committee."**

Personnel - Work with Staff to Develop Rational Procedures



2014 ISAC Cell Sorting Biosafety Standards

- Flexibility Based on Risk Assessment
- Page 13, Last Paragraph before Appendix A:
  - **Alternate combinations of engineering controls, personal protective equipment and biosafety procedures that do not perfectly match the recommended BSLs may be selected. The final risk assessment SOP should be selected based on risk assessment and endorsed by the cell sorting facility manager, biosafety professionals and the IBC."**

2014 ISAC Cell Sorting Biosafety Standards

- Flexibility Based on Risk Assessment
- Page 12 3.1.1.2 Cell Sorters in biological safety cabinets
  - **…Class I BSCs enclosing cell sorters must be manufactured to meet functional certification criteria for personal protection as defined by the BMBL or EN 12469, although it is recommended that the inward airflow velocity be 100 linear feet per minute or greater; HEPA filters must be tested for leakage annually.”**

Key Messages in ISAC 2014

- Introduction
  - Work with manufacturers
  - LAIs and aerosols
  - Pertinent regulations
- Biosafety Principles and Cell Sorting
  - "Identify and evaluate agent hazards"
    - BSPs best at this
  - "Identify laboratory procedure biohazards"
    - Cell sorter operators best at this

Incorporation of multiple barriers

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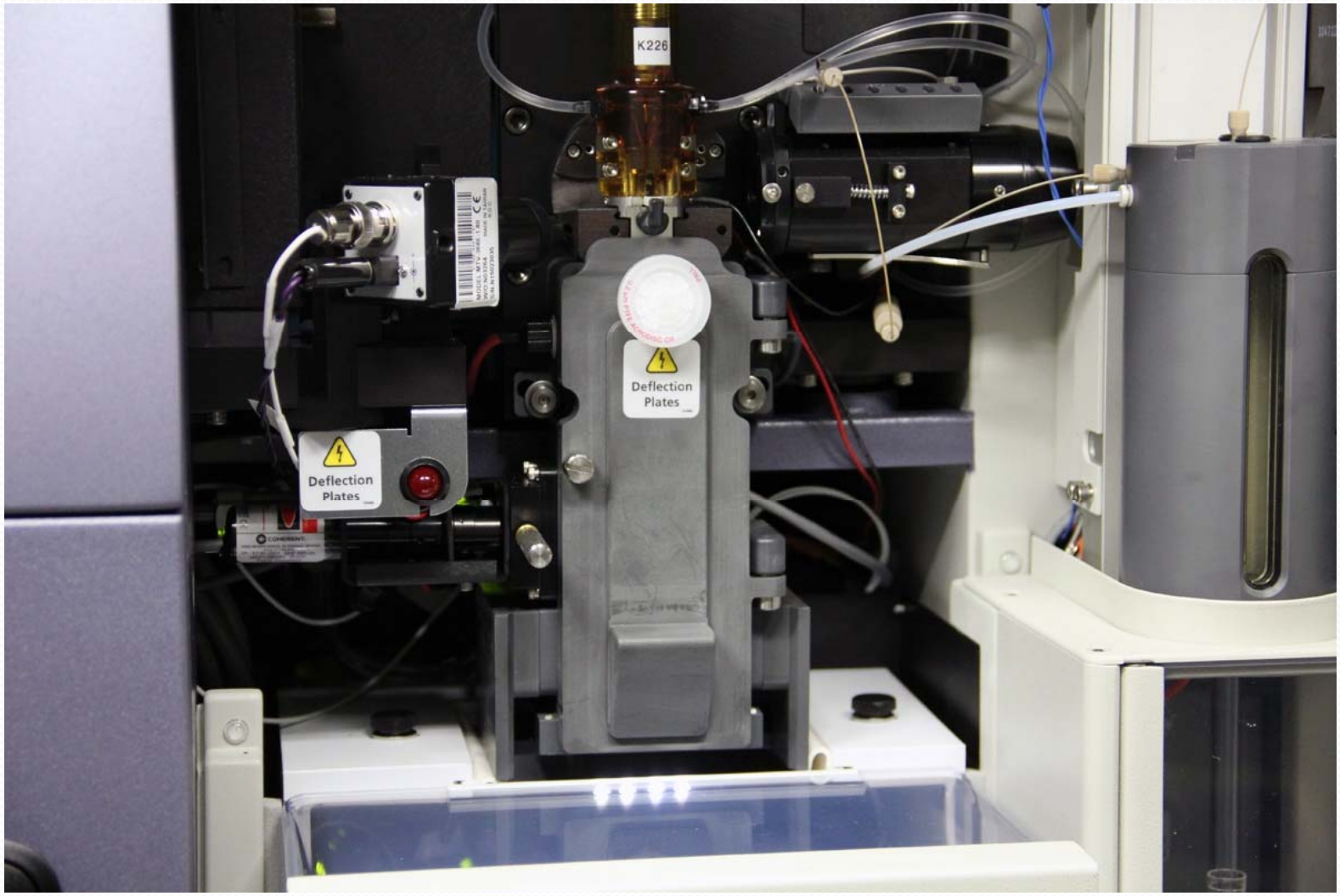
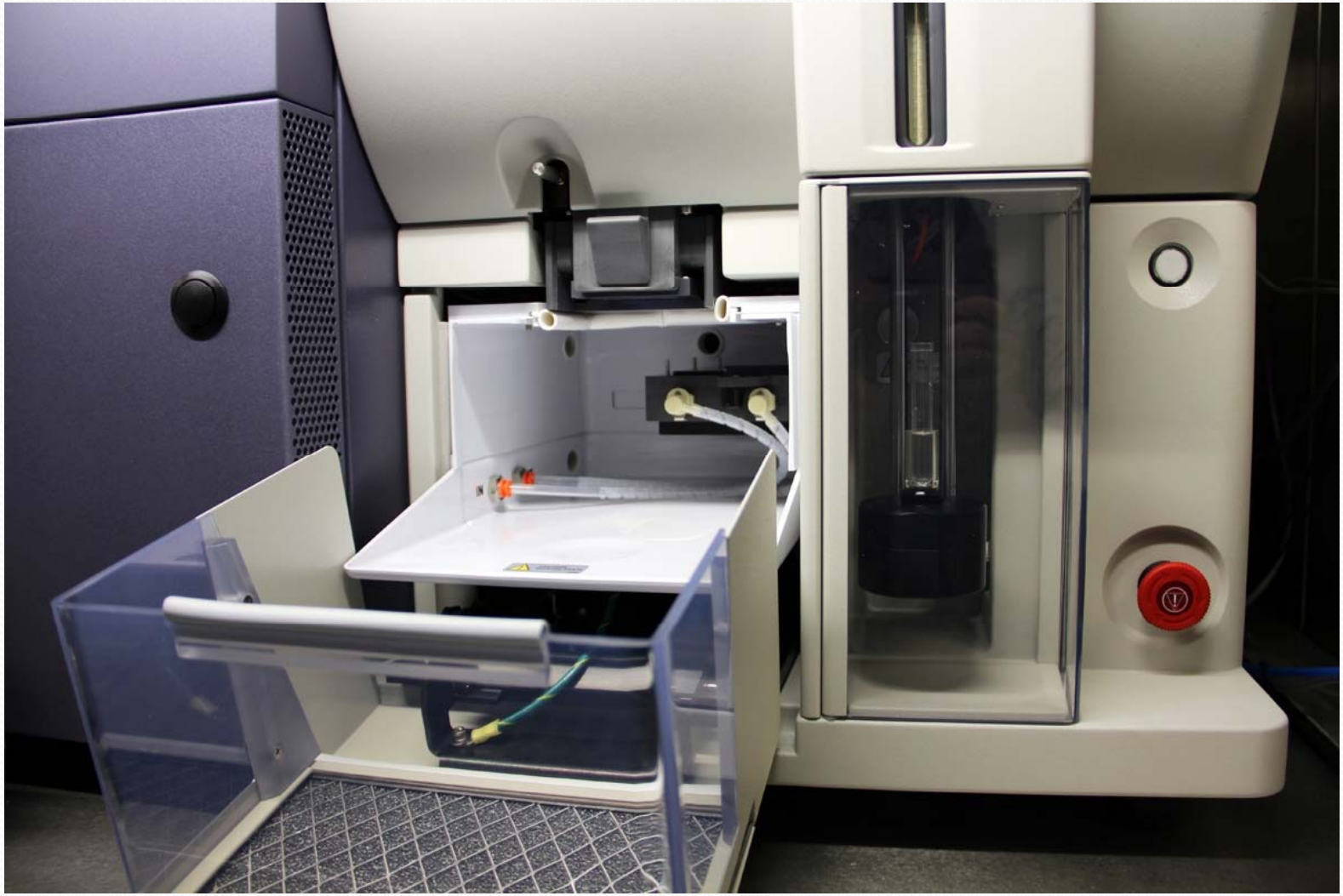


Incorporation of multiple barriers



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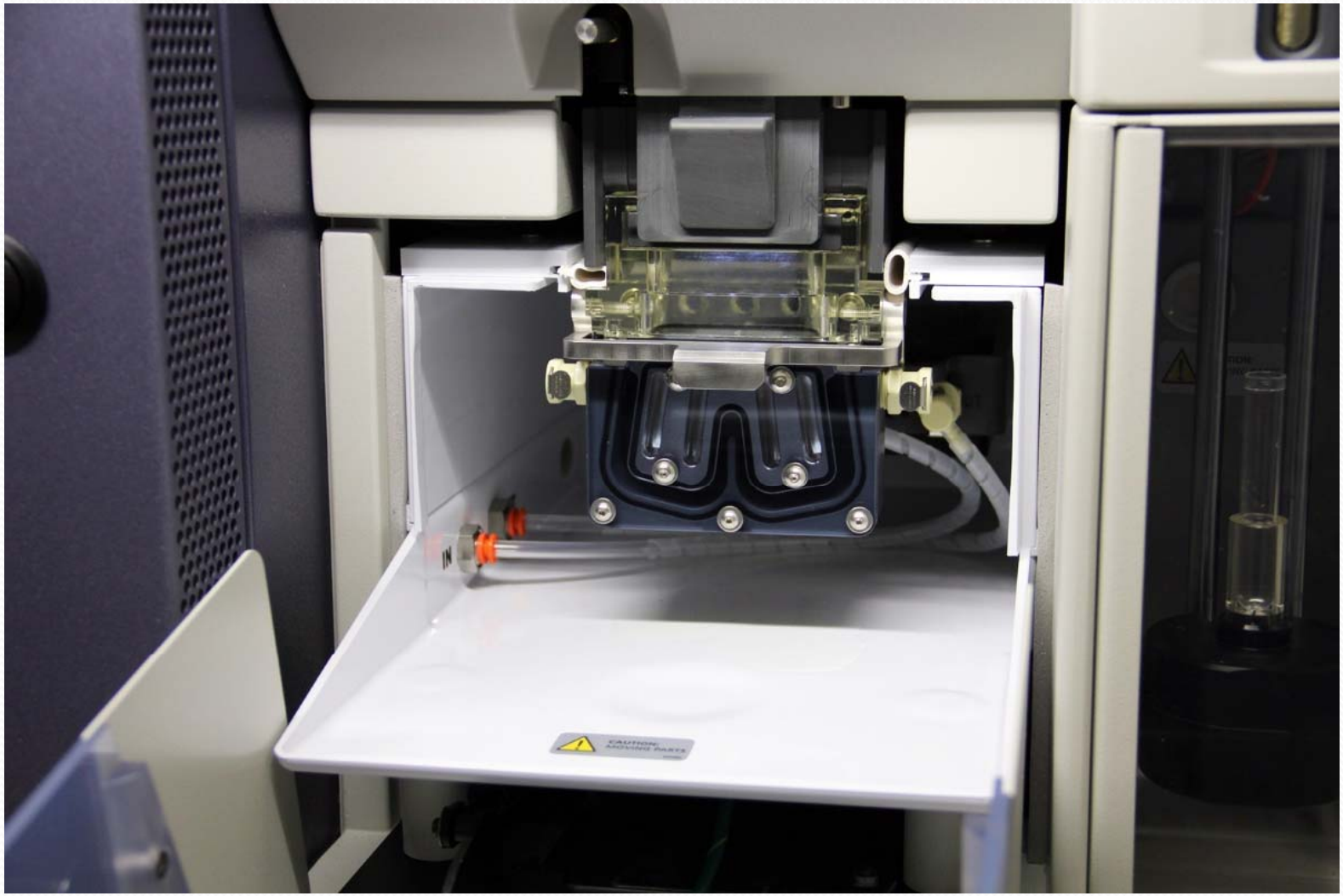

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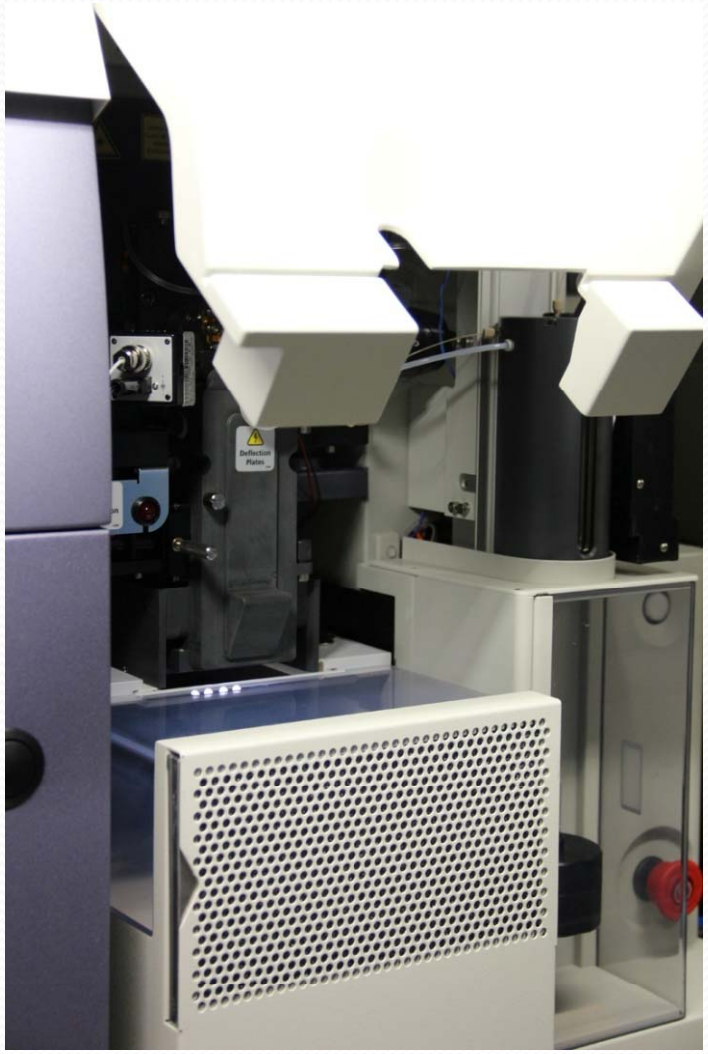
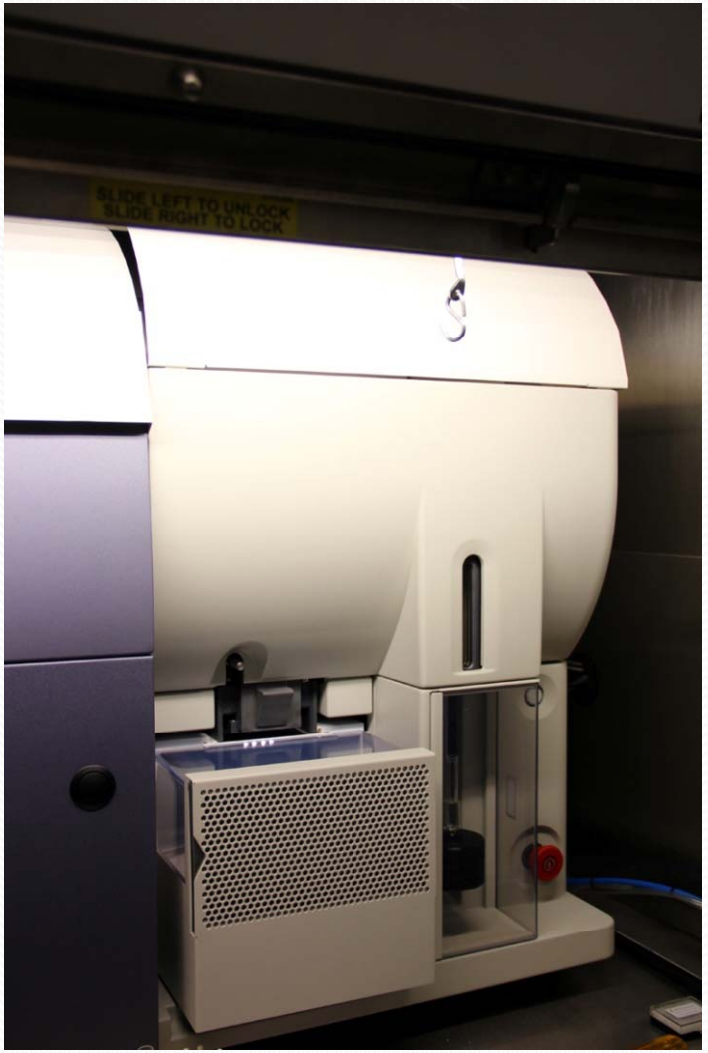




Key Messages in ISAC 2014

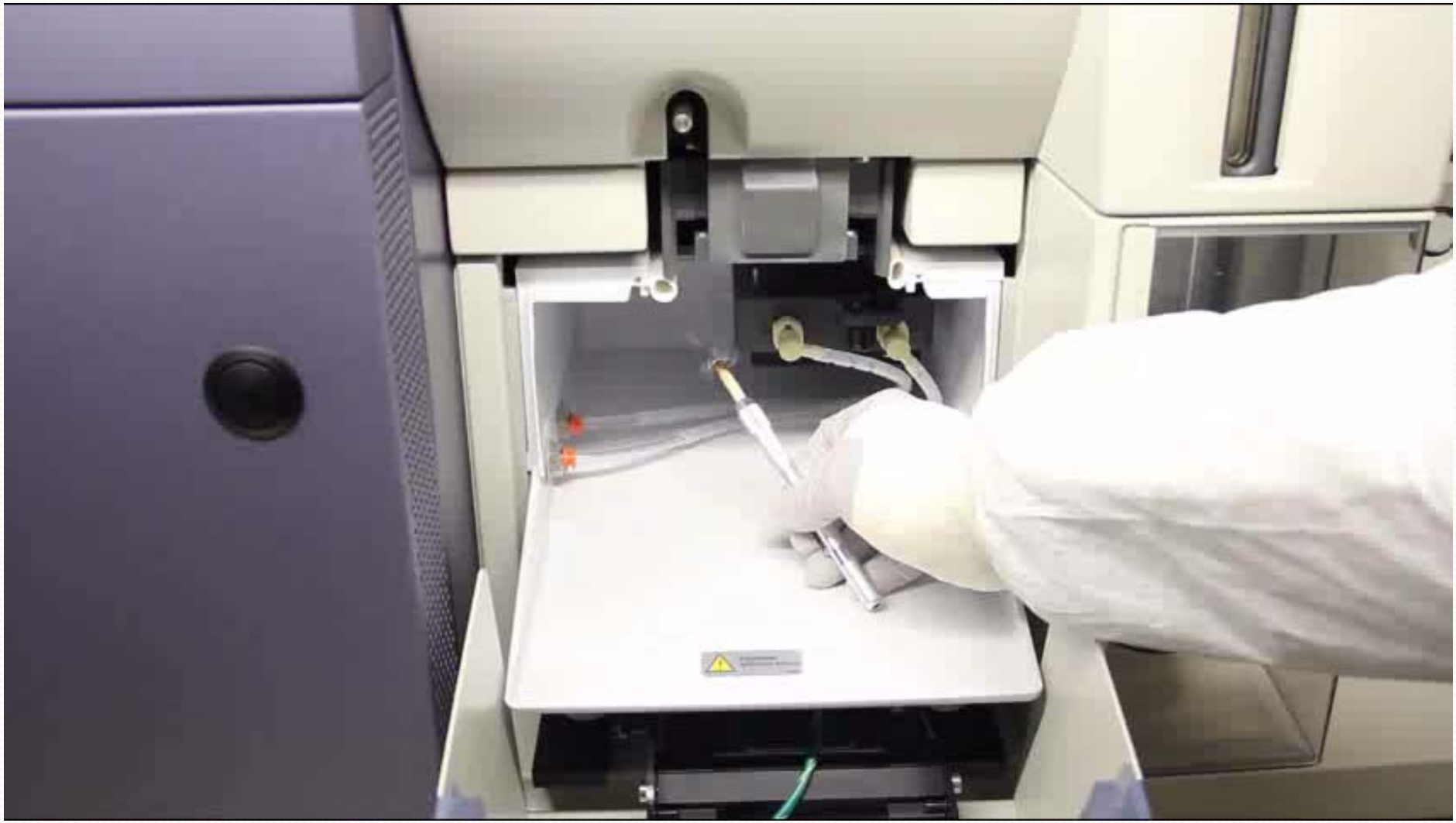
- Biosafety Principles and Cell Sorting
- Cell sorter operator serves as PI/Lab Director
  - Must conduct risk assessment
  - Most knowledgeable of the work in their labs
  - Each “operator” knows “their” instrument
- Teach Biosafety and work with operators
- Learn “Cell Sorting” and work with operators
  - See procedure from start to finish
    - Transport to/from and all steps in between

Key Messages in ISAC 2014

- Biosafety Principles and Cell Sorting
  - Have SOPs in place that provide a foundation for selection of controls at each BSL (BSL1, BSL2, BSL2-enhanced, BSL3)
  - Evaluate sorters selected for biohazard use
    - Not all sorters created equal
  - Minimize # of operators sorting biohazards (where applicable)
  - Hands-on training and proficiency documentation

Daily Smoke Verification Test

Smoke check



Key Messages in ISAC 2014

- Emergency procedures
  - Know what a spill is for cell sorters (stream deviation)
  - Use biosafety "common" sense
    - Evacuate, allow aerosols to settle, be removed
  - Know contamination zone of the sorter before sorting biohazards
    - Develop decontamination SOPs

Questions?