MICHIGAN DEPARTMENT OF HEALTH & HUMAN SERVICES

Automated Library Preparation with TECAN

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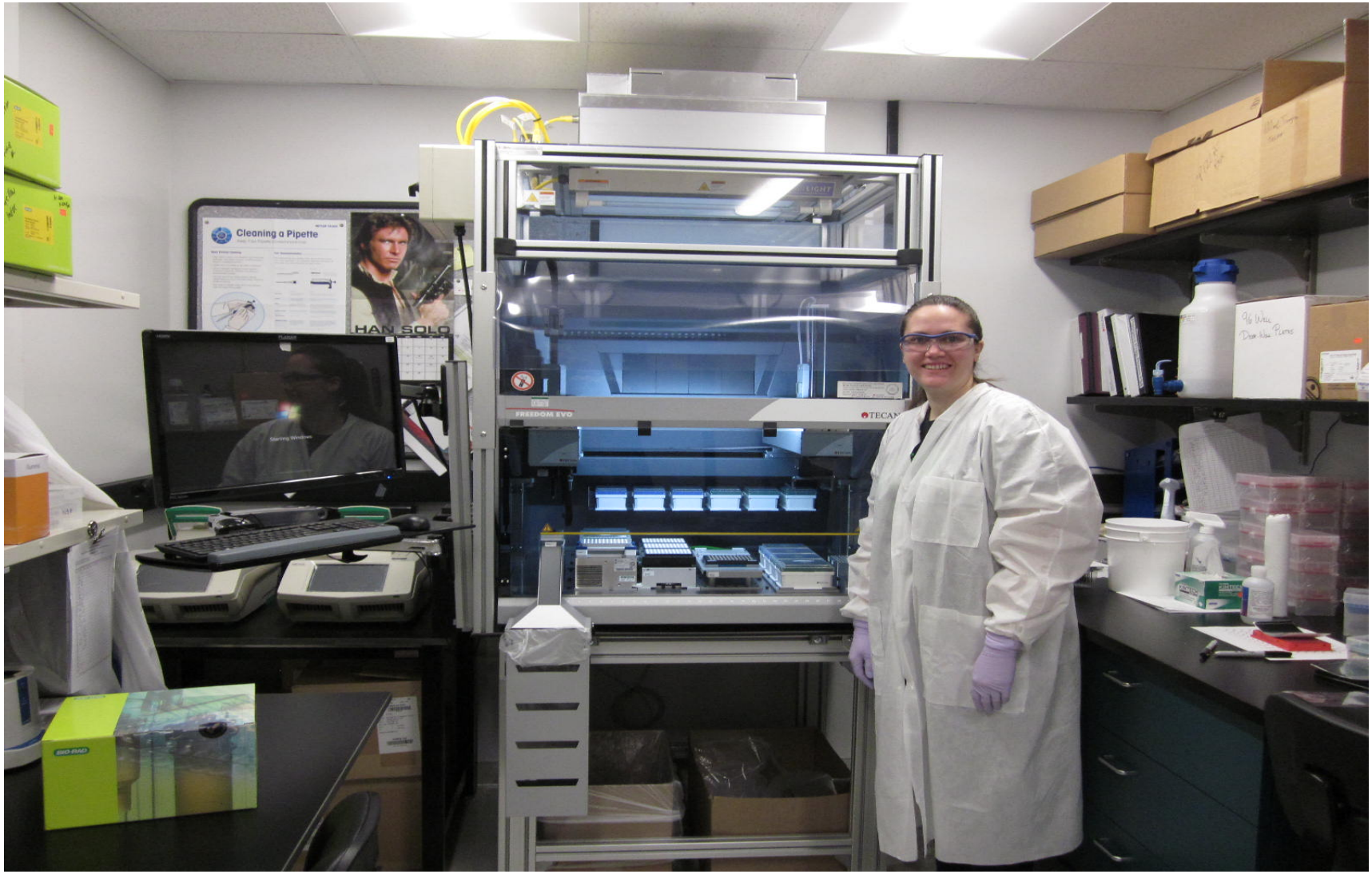
Work Load

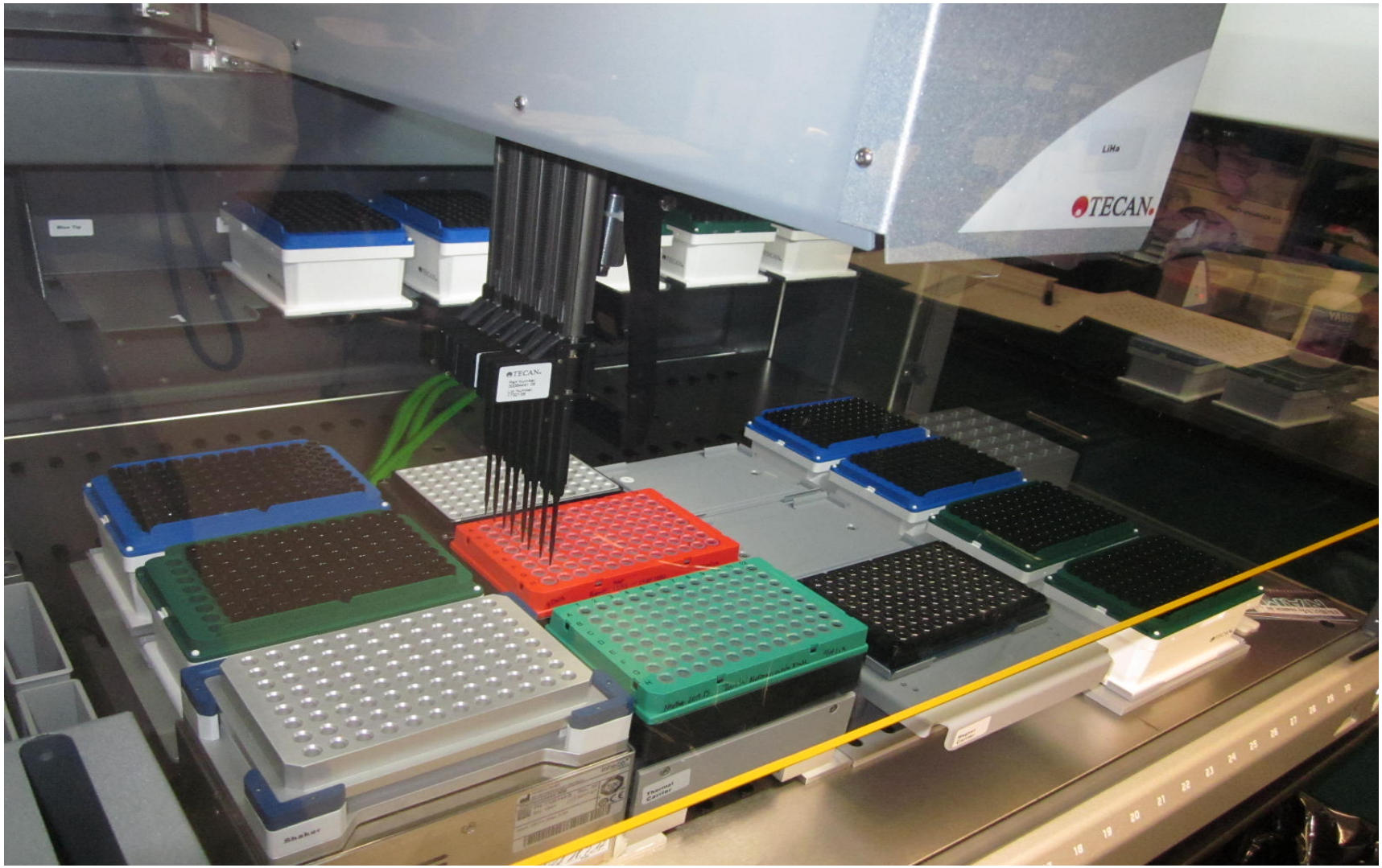
-TB genotyping in Michigan: National Tuberculosis Molecular Surveillance Center does 9,000 isolates per year. Want to minimize hands on time.

- TB uses NexteraXT assay, NextSeq 550 instrument
- TECAN model Freedom EVO
- Uses multiple plates, some aliquoted by hand
- All steps from initial dilutions through pooling are done on instrument
- Runs take about 6 hours

TECAN



TECAN



Workflow

1. Aliquot DNA by hand into DNA plate

2. Add DNA plate, dilution plate, and trough of water to TECAN

3. Spreadsheet with DNA concentrations is loaded into TECAN, TECAN dilutes DNA, proper mixing is performed

4. Add tagmentation reagents (TD and ATM) to TECAN. Reagents are aliquoted from the tubes that come with kit. TECAN adds to reaction plate: TD-DNA-ATM

5. Reaction plate is moved to 55 degree heating block on TECAN, then to 10 degree block

Workflow

6. NT stop buffer is added. This varies from normal procedure:

   - No ramping down of temperature

   - Much slower addition of NT than by hand.

   - Each column is added one at a time, mixing is performed before going to next column

   - Takes about 20 minutes to do all for a full plate

   - Does this cause over-tagmentation?

7. Aliquot indices by hand to index plate. Indices and NPM are added to reaction plate by TECAN

8. Reaction plate is put in separate thermocycler for amplification

Workflow

Bead-ethanol clean up, normalization, pooling

1. Reaction plate, beads, ethanol, elution buffer are put on TECAN. Reagents are in troughs.

2. TECAN has shaker/vortex and magnetic stand and performs the steps involved. Magnet is a ring, beads form a ring just above the bottom of the wells, allowing tips to better avoid pellet.

3. DNA is quantified off TECAN

4. Normalization: Spreadsheet with DNA concentrations is loaded into instrument and dilutions are performed

5. Pooling: DNA from each well is combined in a microfuge tube on TECAN

Workflow

DNA concentration readings are also semi-automated using a plate reader. Some hands on time is saved.

1. Perkin-Elmer Victor Nivo plate reader

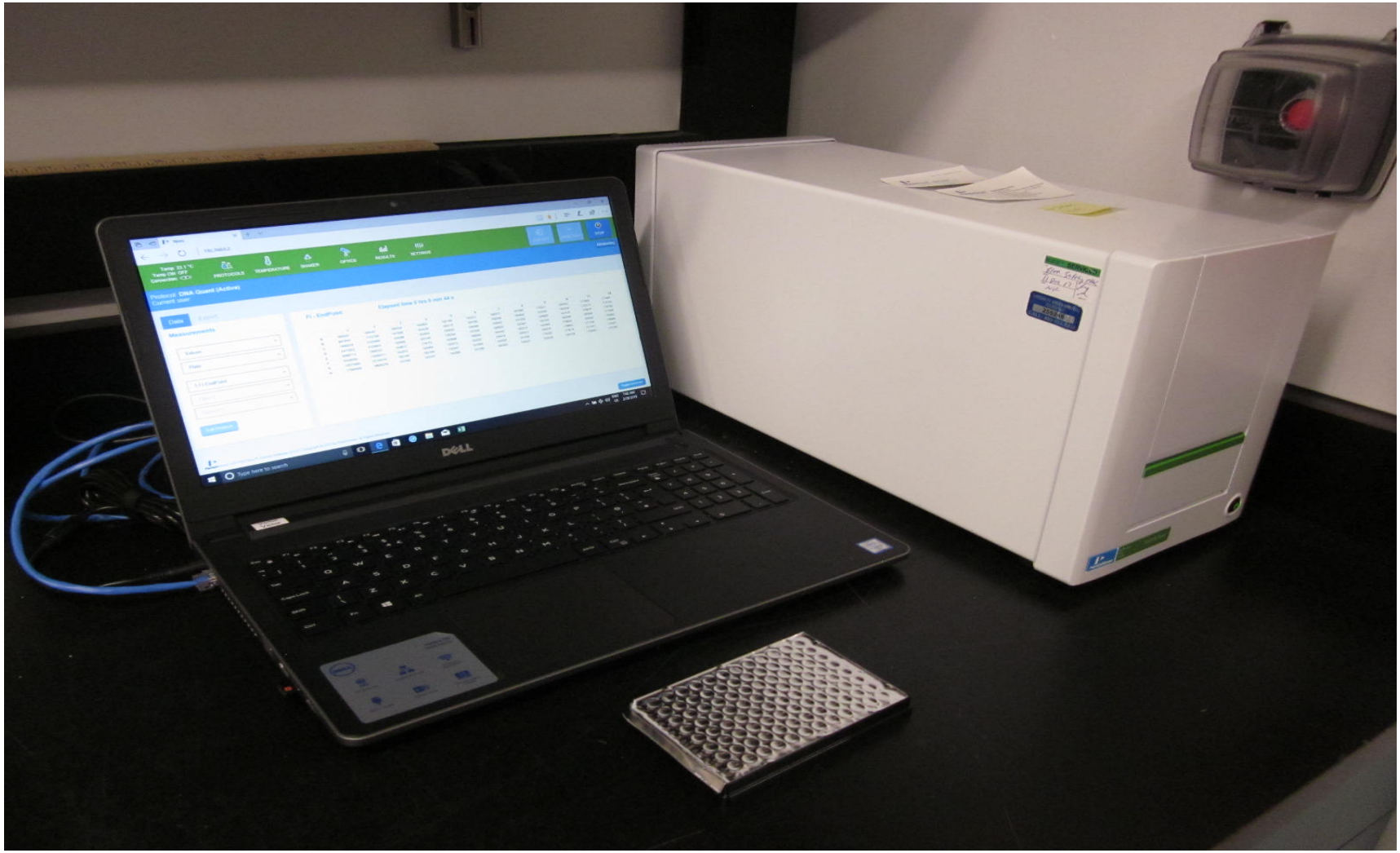
2. DNA is aliquoted into 96 well plate. Dye and reagent are added to trough and multi-channeled into plate

3. Standards are aliquoted into one column of separate plate and read.

4. All wells are read at same time, about one minute total.

5. Readings are added to Excel worksheet and concentrations calculated and printed. File is added to TECAN to determine dilutions for normalization.

Workflow



Problems encountered

-Each robot has unique technique, like people

-Settings had to be adjusted for accurate pipetting. Settings used by TECAN in CDC TB lab were not fine tuned for our instrument. Had to experiment with settings to get right volumes.

-Cost is higher, tips and plates are expensive, but cost is balanced by less personnel time.

- Pipetting is slower than by hand