



# Triangulation ID for Genetic Evaluation of Biological Risks (TIGER)

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Approach: ID presence of individual pathogen by finding presence of specific nucleic acid sequence unique to that pathogen.

## Sensor Design

1. Determine sequence of pathogen DNA

A G G G T A G G A T G C A G T A T G C T A A A A C T G T A A T C G C G A T A T G A T G C

2. Select “probe” sequence:
  - unique to pathogen;
  - suited to sensor use.

3. Select PCR primers bordering “probe” sequence.

## Sensor Hardware Implications

- Pathogen-specific probe sequence must be present in sensor

A G G G T A G G A T G C A G T A T G C T A A A A C T G T A A T C G C G A T A T G A T G C

- Pathogen-specific PCR primers must be present in sensor

A G G G T A G G

A T G A T G C

Sample

Extract DNA

PCR: Replicate copies of selected DNA

ID: Test for match to probe sequence

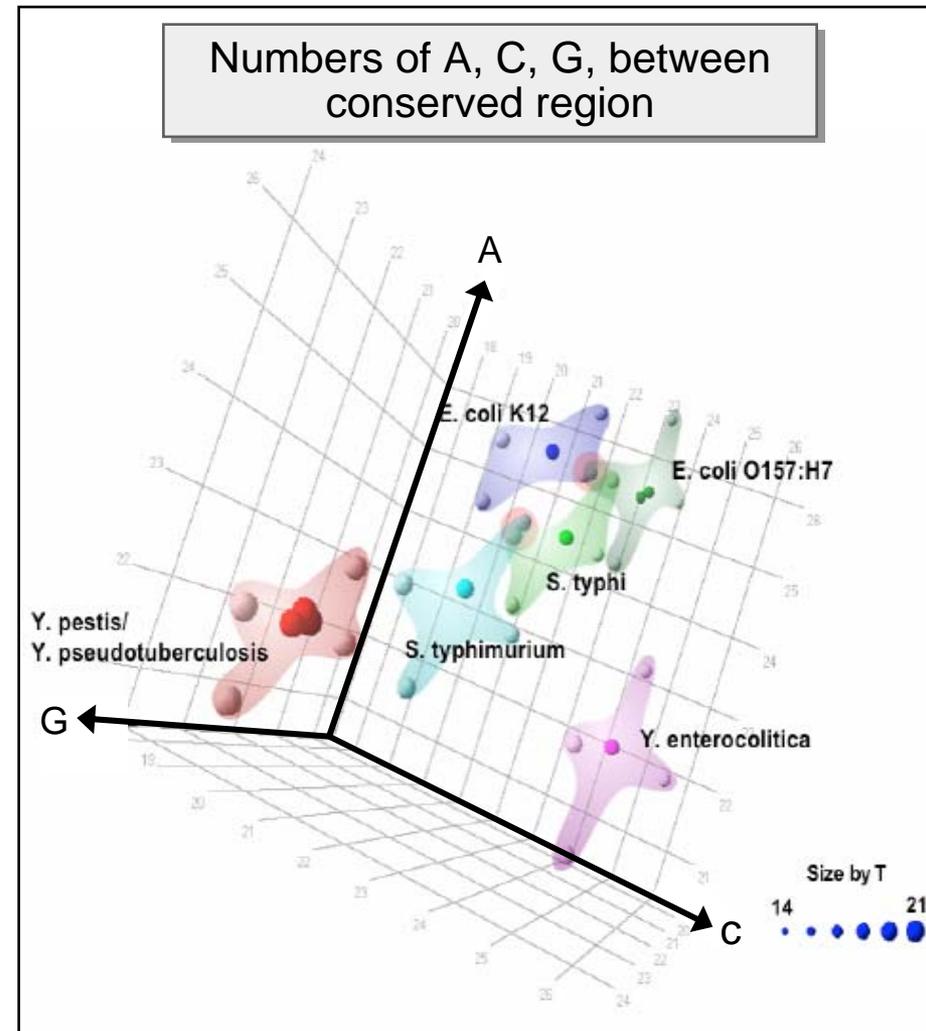
Pathogen present?  
(yes/no)

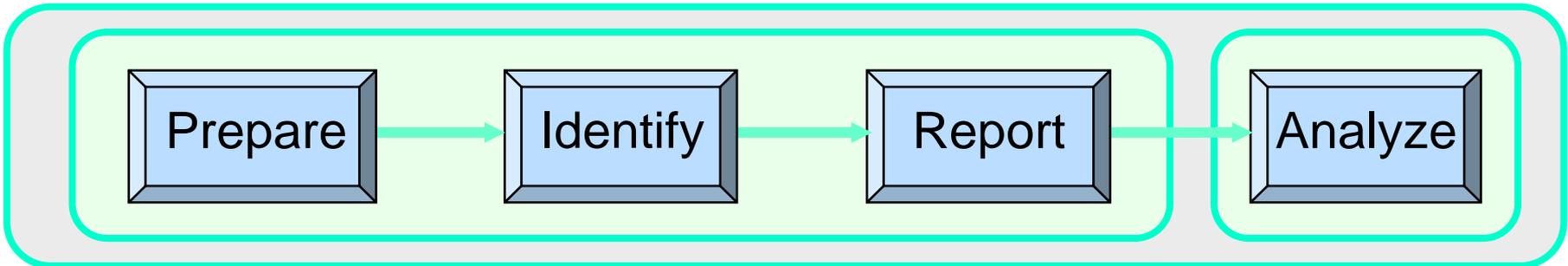
### Observations

1. Some gene functions are universally required for life:
  - e.g. translation, transcription, replication, recombination, repair, nucleotide and amino acid metabolism, energy generation, uptake, secretion, ...
2. Parts of those gene sequences are highly conserved (the same DNA sequence occurs across “all” species).
3. Between those regions are species-specific variable regions.

### Hypothesis

- Sufficient information exists in the number of (A, T, C, G) contained in these variable regions to allow unique identification of pathogens (and everything else).





### DNA extraction

### PCR

### Mass Spectrometry

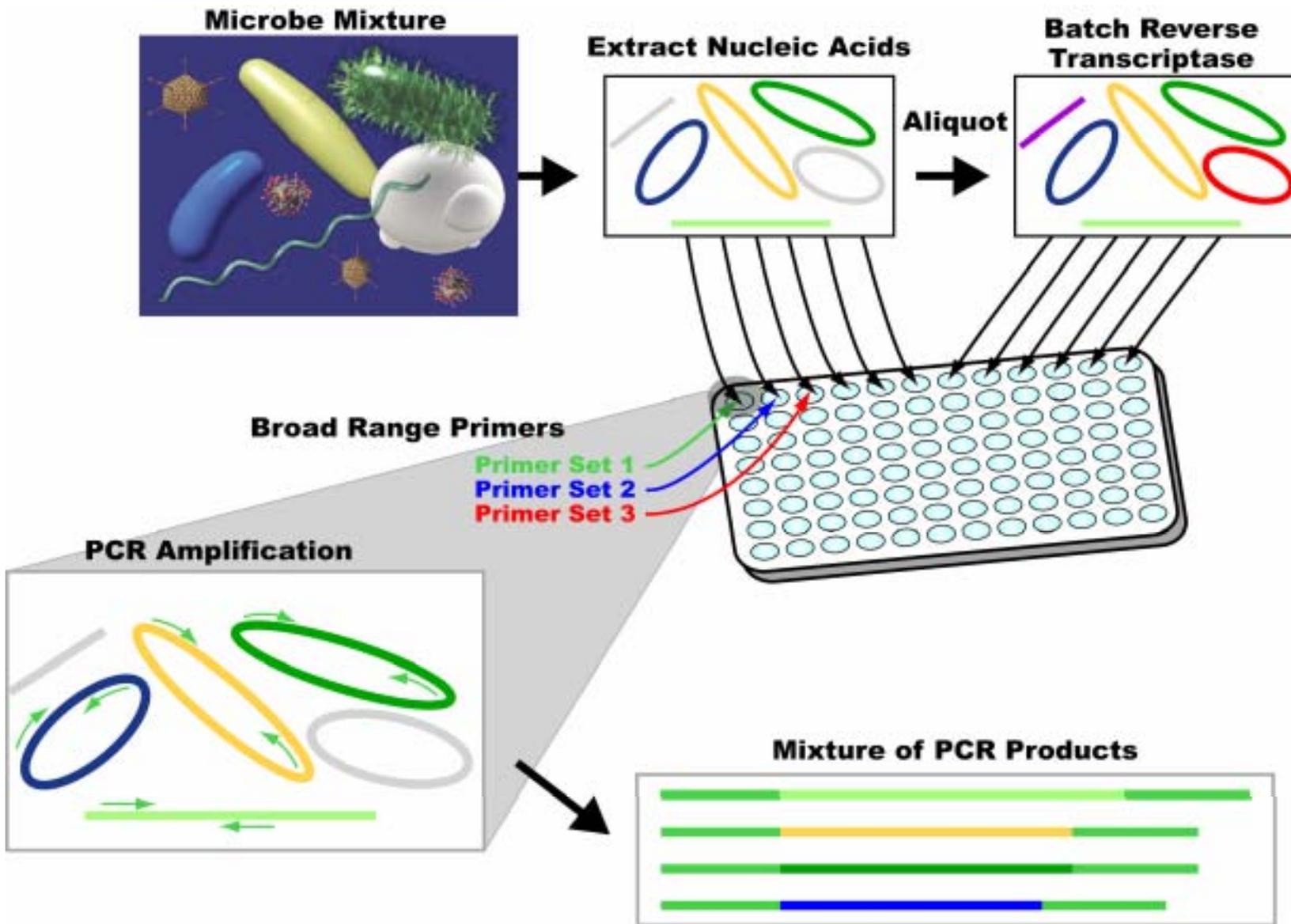
ESI needle → Capillary → Ion Storage Hexapole → Reflectron

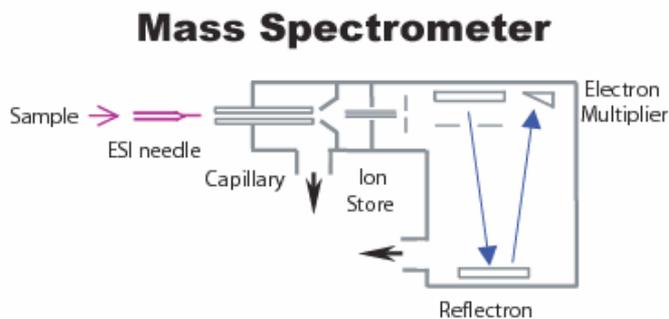
### Signal Processing

Max Likelihood Processor

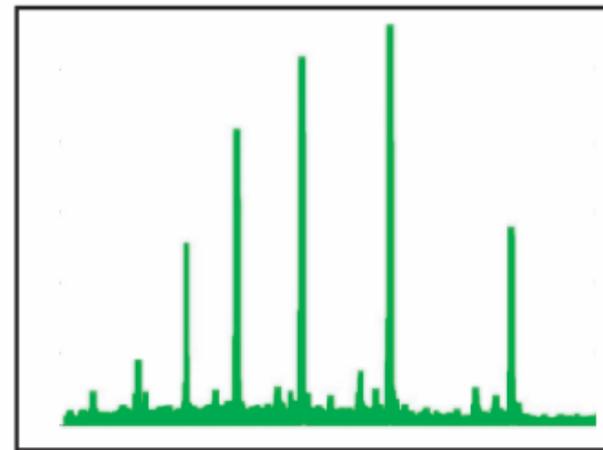
Primer Set	Quantity	A	C	T	G
Primer Set 1	1,000	15	10	6	5
	450	22	14	10	15
	12	10	8	22	14
Primer Set 2	...	...	...	...	...
Primer Set N	...	...	...	...	...

# Sample Preparation and Broad Range PCR





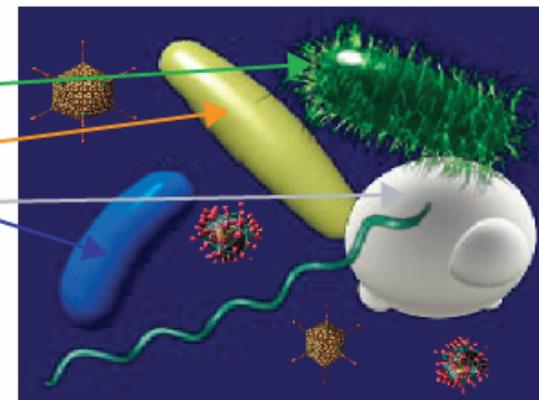
### Spectral Signal



### Signal Processing Masses to Base Compositions

Organism	Mass	Base Count
E. coli	18234.970	A <sub>12</sub> G <sub>17</sub> C <sub>17</sub> T <sub>13</sub>
S. milleri	17948.926	A <sub>14</sub> G <sub>14</sub> C <sub>12</sub> T <sub>18</sub>
M. leprae	18610.017	A <sub>11</sub> G <sub>19</sub> C <sub>15</sub> T <sub>15</sub>
Unknown brucella	17936.912	A <sub>11</sub> G <sub>17</sub> C <sub>16</sub> T <sub>14</sub>

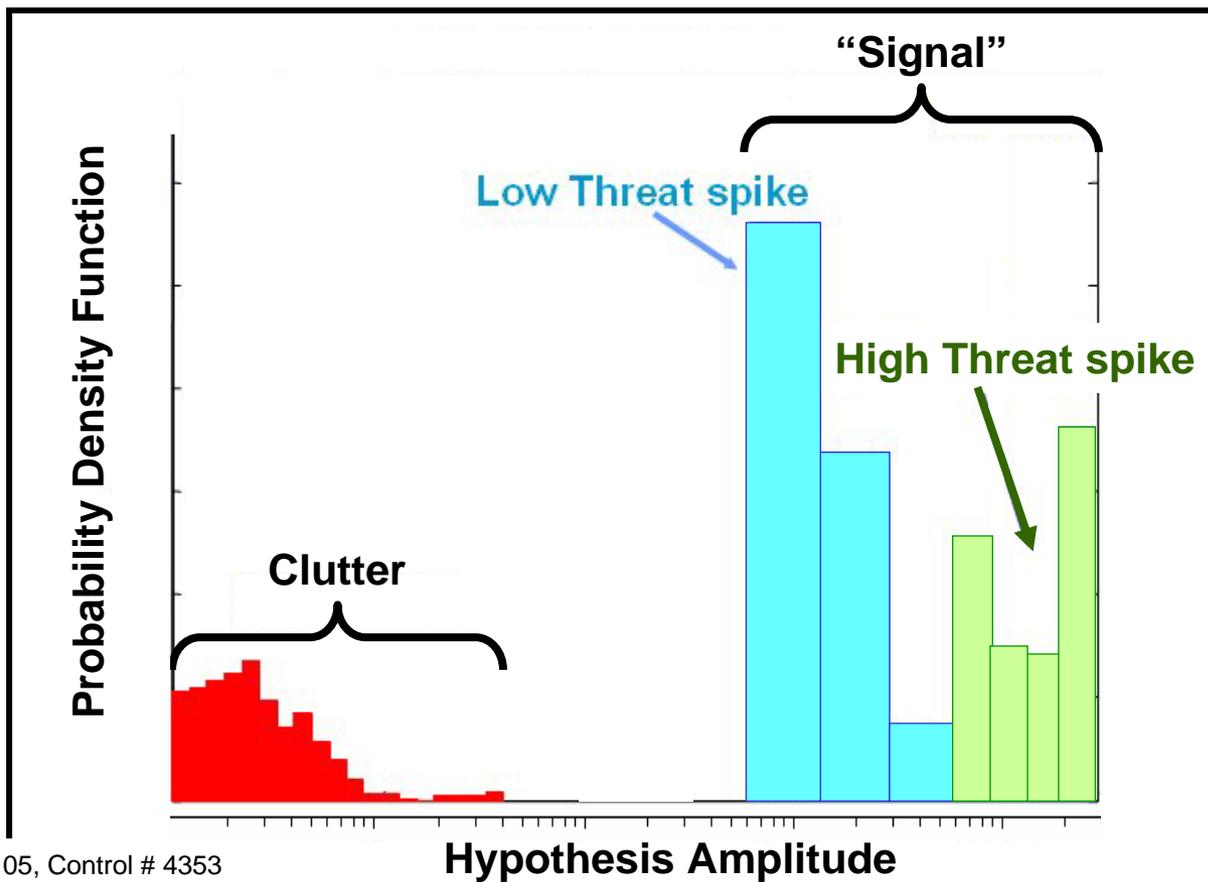
### Base Compositions Map to Microbes



# Testing for Rejection of Clutter Challenges



- Collect “worst case” environments
  - North City Sewage Treatment Plant
  - Tijuana River
  - New River (El Centro) cattle feedlot
- Evaluate separation between agent “signal” and clutter

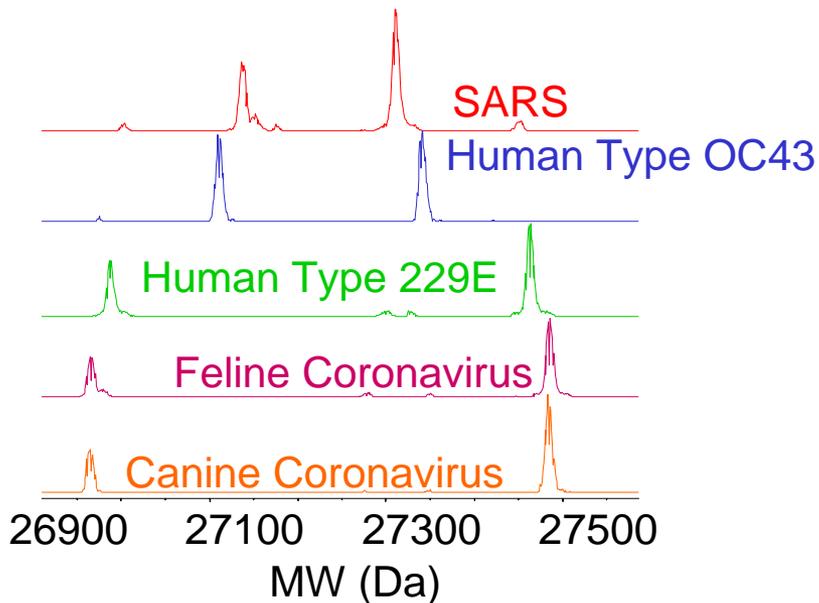


# Real-World Example

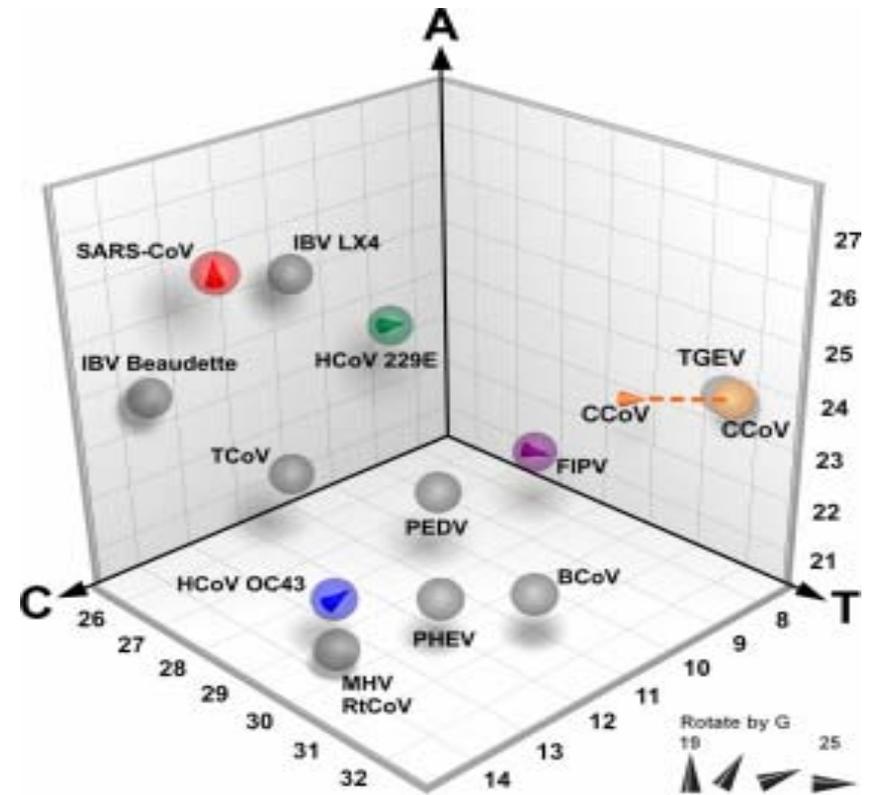
## SARS Diagnostic – Corona Virus

- All coronaviruses can be detected and identified using a single pair of TIGER primers, rapidly, without culture

Mass spectra of 5 different Coronaviruses



Observed base compositions (solid cones) and expected compositions (hollow spheres)

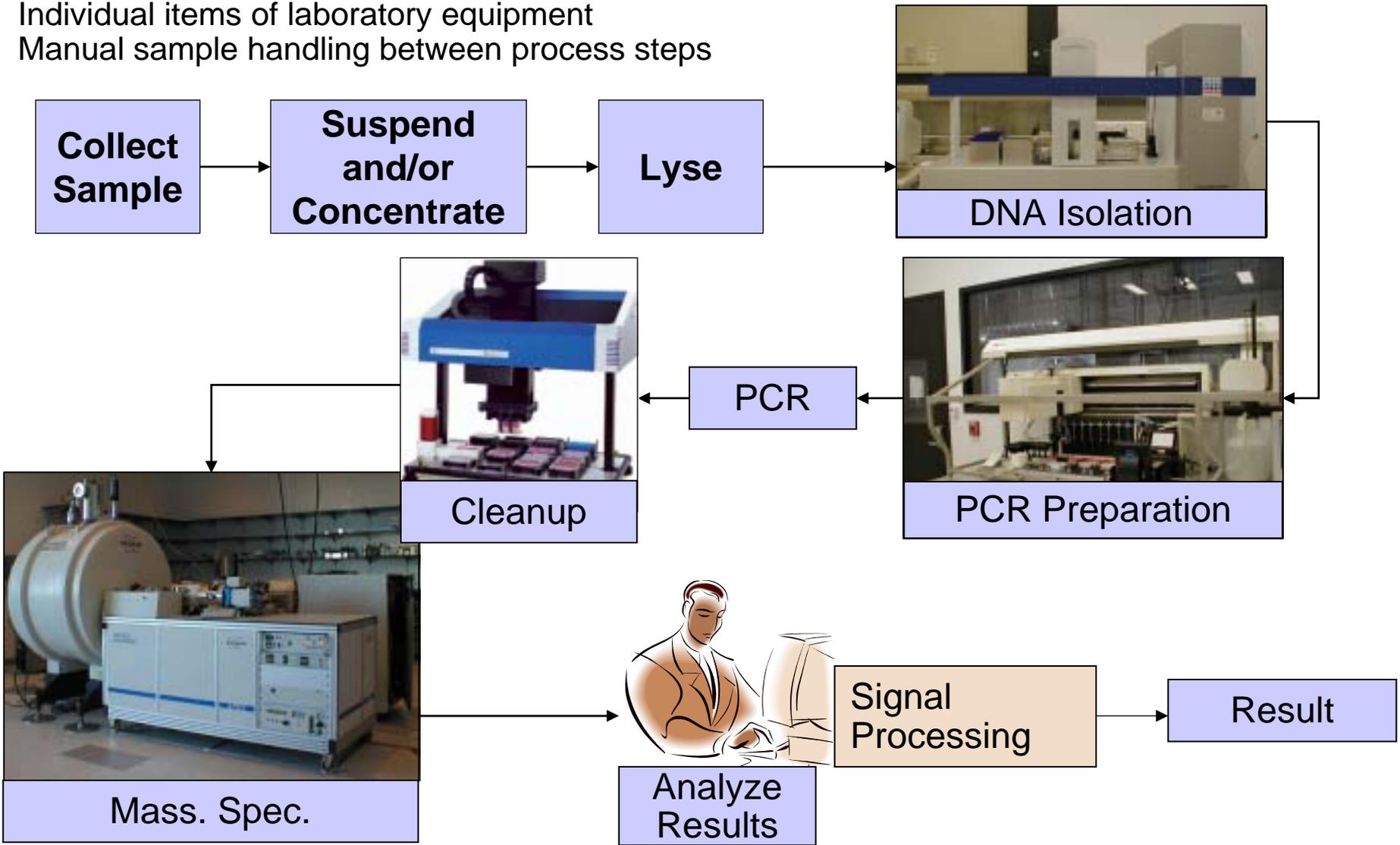


- Manuscript under review in PNAS

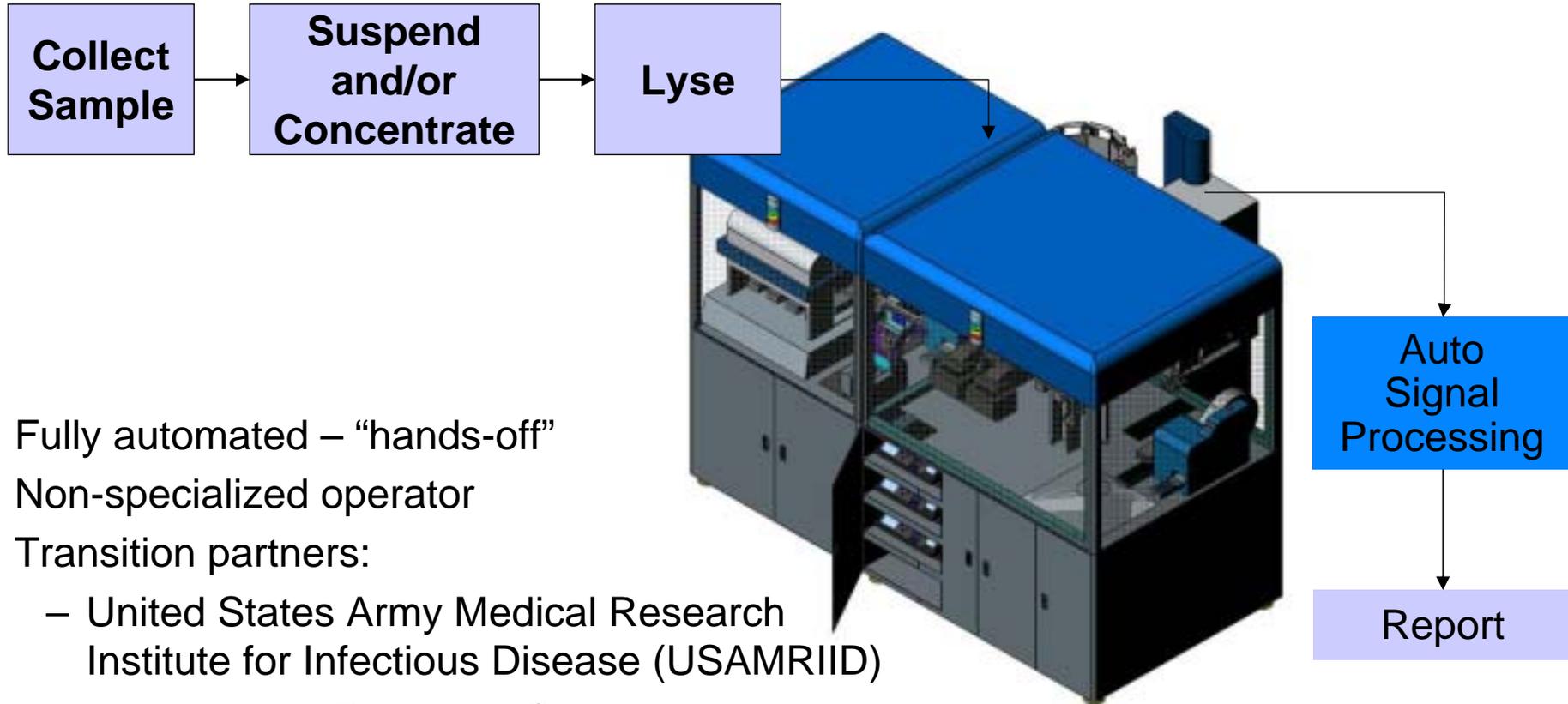
# TIGER 1.0

## Semi-Automated Process

Individual items of laboratory equipment  
Manual sample handling between process steps



Integrated items of laboratory equipment  
Automated sample handling between process steps



- Fully automated – “hands-off”
- Non-specialized operator
- Transition partners:
  - United States Army Medical Research Institute for Infectious Disease (USAMRIID)
  - Naval Health Research Center
  - National Bioforensic Analysis Center
  - Centers for Disease Control and Prevention

**Animal Reservoirs of Infectious Agents**

**Environmental Surveillance of Public Places**

**Clinical Diagnostics/Biosurveillance**

**Agricultural Diagnostics/Biosurveillance**

